Characterization of the transvection mediating region of the *Abdominal-B* locus in *Drosophila*

Jumin Zhou¹, Hilary Ashe¹, Christian Burks² and Michael Levine¹,*

¹Dept Mol. Cell Biol., Division of Genetics, University of California, Berkeley, CA 94720, USA
²Exelixis Pharmaceuticals, Inc., 260 Littlefield Avenue, South San Francisco, CA 94080, USA

*Author for correspondence (e-mail: mlevine@uclink4.berkeley.edu)

Accepted 22 April; published on WWW 21 June 1999

**SUMMARY**

Genetic studies have identified an unusual transvection process in the *Abdominal-B* (*Abd-B*) locus of *Drosophila*. In some cases distal *infraabdominal* (*iab*) regulatory domains continue to activate the *Abd-B* promoter even when translocated onto different chromosomes. Transvection depends on an approx. 10 kb genomic DNA sequence, termed the *transvection mediating region* (*tmr*), located immediately downstream of the *Abd-B* transcription unit. Here we report a detailed analysis of this region. Different DNA fragments from the *tmr* were inserted into a variety of *P*-transformation vectors. Analyses of reporter gene expression in transgenic embryos and adults identify at least three cis-regulatory elements, including two enhancers (IAB7 and IAB8) and a new insulator DNA (*Frontabdominal-8*, *Fab-8*). Evidence is also presented for a Polycomb **Response Element** (**PRE**) linked to the IAB8 enhancer, and an internal promoter in the *iab-8* domain, which transcribes the *iab-7* and *iab-8* cis-regulatory DNA, including the *Fab-8* insulator. We discuss the significance of these findings with regard to *Abd-B* transvection and long-range enhancer-promoter interactions in mammalian loci.

Key words: Enhancer, Promoter, Insulator, Transvection, *Abd-B*, *tmr*, IAB7, IAB8, *Fab-7*, *Fab-8*, *Drosophila melanogaster*

**INTRODUCTION**

Enhancers direct stripes, bands and tissue-specific patterns of gene expression in the early *Drosophila* embryo. During the past few years we have become interested in the next tier of gene regulation, namely the regulation of enhancer-promoter interactions within complex genetic loci. Recent studies suggest that insulator DNAs and core promoter elements play an important role in the orderly trafficking of enhancers within complex loci (Foley and Engel, 1992; Li et al., 1994; Ohtsuki et al., 1998; see reviews by Geyer, 1997; Mihaly et al., 1998a; Udvardy, 1999). Consider a shared enhancer located near three potential target promoters (Fig. 1). In principle, an insulator DNA located between the enhancer and leftward promoter would block the activation of *gene A* without interfering with the expression of *gene B* (Scott and Geyer, 1995; Cai and Levine, 1995). Moreover, recent studies have shown that certain core promoters possess an intrinsic enhancer blocking activity (Ohtsuki and Levine, 1998). The placement of such a promoter at *gene B* would block the activation of *gene C* (see Fig. 1).

To determine whether there are additional cis-regulatory elements that might influence the regulation of enhancer-promoter interactions we have examined the *Drosophila* bithorax complex (BX-C). The BX-C contains more than 300 kb of genomic DNA and genetic studies suggest that the bulk of this DNA corresponds to cis-regulatory information. The BX-C contains only three *Hox* transcription units, *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*); each is regulated by 60-80 kb of cis DNA (Lewis et al., 1995; Martin et al., 1995; reviewed by Morata, 1986; Duncan, 1987; Peifer et al., 1987). In the case of the *Abd-B* gene, most of this cis-regulatory DNA is located downstream of the transcription unit.

The 3′ *Abd-B* cis-regulatory region spans approx. 50 kb and contains a series of genetically defined domains, termed *infraabdominal*-5 (*iab-5*), *iab-6*, *iab-7* and *iab-8* (Boulet et al., 1991; Celniker et al., 1990; Sanchez-Herrero, 1991; Fig. 2). Mutations in a given *iab* domain disrupt the morphogenesis of the corresponding abdominal segment. For example, mutations in *iab-5* disrupt abdominal segment 5, or more accurately, parasegment 10, which includes the posterior compartment of the fourth abdominal segment and the anterior compartment of the fifth abdominal segment. Thus far, only three *Abd-B* cis-regulatory elements have been characterized, the IAB5 enhancer, the *Fab-7* insulator and the *iab-7 PRE* (Busturia and Bienz, 1993; Gyurkovics et al., 1990; Galloni et al., 1993; Karch et al., 1994; Zhou et al., 1996; Hagstrom et al., 1996, 1997; Mihaly et al., 1997; summarized in Fig. 2).

It has been suggested that insulator DNAs organize the different *iab* regions into discrete chromatin boundary domains (Galloni et al., 1993; Vazquez et al., 1993; also see Fig. 2). The *Frontabdominal-7* (*Fab-7*) DNA element has been shown to possess an enhancer blocking activity that is similar to those observed for the *scs* and *scs*′ chromatin boundary elements of the *hsp70* locus, as well as the *Su(Hw)* insulator DNA from the *gypsy*
A second potential insulator DNA, termed the insulator from the genomic DNA sequence of approx. 10 kb, is located in the indicated position. This insulator DNA, such as the 340 bp B (HindIII) fragment from phage clone #81-16 (DSO7696, L07835; Gary Karpen laboratory, The Salk Institute, La Jolla, CA). The 1.9 kb IAB7 enhancer is a PCR fragment (PII) corresponding to nucleotides (nt) 66,258-68,186 within the DSO7696 sequence of the Abd-B gene (Martin et al., 1995; Lewis et al., 1995). Similarly, the 5.3 kb IAB8 enhancer was cloned as a BamHI-HindIII fragment from nt 59,197-64,580; the 1.7 Fab-8 DNA corresponds to a PCR fragment from 63,709-65,407. The 0.7 kb IAB7 enhancer is an EcoRI-SmaI fragment within the 1.9 kb PCR fragment, whereas the 5.3 kb IAB8 enhancer is a PCR fragment (PII) corresponding to nucleotides (nt) 66,258-68,186 within the DSO7696 sequence of the Abd-B gene (Martin et al., 1995; Lewis et al., 1995).

**Fig. 1.** Summary of enhancer-promoter interactions. The diagram depicts a shared enhancer located near three potential target genes, A, B and C. In principle, an insulator DNA, such as the 340 bp B (HindIII) insulator from the gypsy retrotransposon, located in the indicated position would block the activation of gene A, but would not interfere with enhancer-gene B interactions. In addition, the activation of gene C can be blocked by promoter competition, whereby the gene B promoter is ‘stronger’ than the gene A promoter. Alternatively, the gene B promoter region might contain an enhancer blocking activity that would preclude the activation of gene C.

**Fig. 2.** Summary of the Abd-B locus. The bithorax complex is over 300 kb in length and contains three Hox transcription units, Ubx, abd-A and Abd-B. The Abd-B locus is over 80 kb in length. The leftward arrow (middle diagram) indicates the transcription start site, and the black rectangles represent exons present in the mature Abd-B mRNA. Genetic studies suggest that iab-8 cis-regulatory elements, which direct expression in parasegment (ps) 13, are located both upstream and downstream of the Abd-B transcription unit. The other iab regulatory domains that control Abd-B expression are also indicated (iab-5, iab-6, and iab-7). These latter domains regulate expression in ps10, ps11 and ps12, respectively. It has been proposed that the 3’ Abd-B cis-regulatory DNA contains a series of insulators, which are indicated by the red ovals. Only Fab-7 has been definitively identified, although there is evidence that Mcp also functions as an insulator. This study provides evidence for a Fab-8 insulator DNA. It is unclear whether Fab-6 exists. A detailed map of the approx. 10 kb tmr is shown at the bottom of the figure. The numbers refer to the DSO7696 sequence of Abd-B in kb units. This study provides evidence that the tmr also contains two enhancers, IAB7 and IAB8, and an internal promoter in the iab-8 domain, which is indicated by the leftward arrow near position 60. A potential iab-8 PRE is located between position 62 and 64.

**MATERIALS AND METHODS**

**DNA plasmid construction**

The 9.5 kb DNA spanning the tmr region was cloned as a BamHI-HindIII fragment from P1 phage clone #81-16 (DSO7696, L07835; Gary Karpen laboratory, The Salk Institute, La Jolla, CA). The 1.9 kb IAB7 enhancer is a PCR fragment (PII) corresponding to nucleotides (nt) 66,258-68,186 within the DSO7696 sequence of the Abd-B gene (Martin et al., 1995; Lewis et al., 1995). Similarly, the 5.3 kb IAB8 enhancer was cloned as a BamHI-HindIII fragment from nt 59,197-64,580; the 1.7 Fab-8 DNA corresponds to a PCR fragment from 63,709-65,407. The 0.7 kb IAB7 enhancer is an EcoRI-SmaI fragment within the 1.9 kb PCR fragment, whereas the
2.7 kb IAB8 enhancer was derived from an EcoRI fragment within the 5.3 kb DNA.

The yellow gene was recovered as a 7.7 kb SalI fragment from a plasmid provided by Gary Struhl (Struhl and Basler, 1993). Fab-7 and Fab-8 insulator cassettes were inserted at a HindIII site 200 bp proximal to the gypsy insertion site within the y2 allele (Geyer et al., 1986). Frt sites are identical to those described by Struhl and Basler (1993). The 5xglass enhancer was obtained from the Rubin laboratory (Moses and Rubin, 1991).

The 2xPE-IAB8-P-transformation vector used to identify the Fab-8 insulator (Fig. 5) is described in Zhou et al. (1996). Different tmr DNAs were cloned into a unique BamHI site located within the 2xPE and IAB8 enhancers.

To generate the construct in Fig. 7E, a 228 bp SpeI-BamHI fragment of the hairy stripe one enhancer (HI; Zhou et al., 1996) was inserted between the mini white and the transposase-lacZ promoter of the C4PLZ vector. The 9.5 tmr DNA was inserted in the Bg/II site at the 3’ end of the lacZ gene.

**RESULTS**

The approx. 10 kb tmr is located just downstream of the Abd-B transcription unit (Fig. 2). Chromosomal breakpoints within the tmr disrupt long-range interactions between iab-5, iab-6 and iab-7 enhancers and the Abd-B promoter (Hopmann et al., 1995). Such disruptions appear to cause a reduction of Abd-B function. Cis-regulatory elements were identified within the tmr by inserting different DNA fragments into P-element transformation vectors containing lacZ, white and yellow reporter genes. The regulatory activities of these DNA fragments were determined by analyzing reporter gene expression in transgenic embryos and adult flies. In situ hybridization was used to analyze reporter gene expression in embryos, while eye color and body color were used to examine reporter gene activity in adults.

**Characterization of the IAB7 enhancer**

The distal approx. 4 kb region of the tmr extends into the iab-7 domain (Fig. 2; Gyurkovics et al., 1990), which controls the morphogenesis of the tissues that comprise the seventh abdominal segment. Thus far, no enhancers, either embryonic or larval, have been identified in this region. A 1.9 kb PCR fragment, which includes nearly half of the iab-7 sequences contained within the tmr, was inserted into a P-transformation vector used to identify the Fab-8 insulator (Fig. 5) is described in Zhou et al. (1996). Different tmr DNAs were cloned into a unique BamHI site located within the 2xPE and IAB8 enhancers.

To generate the construct in Fig. 7E, a 228 bp SpeI-BamHI fragment of the hairy stripe one enhancer (HI; Zhou et al., 1996) was inserted between the mini white and the transposase-lacZ promoter of the C4PLZ vector. The 9.5 tmr DNA was inserted in the Bg/II site at the 3’ end of the lacZ gene.

**Characterization of the IAB7 enhancer**

The distal approx. 4 kb region of the tmr extends into the iab-7 domain (Fig. 2; Gyurkovics et al., 1990), which controls the morphogenesis of the tissues that comprise the seventh abdominal segment. Thus far, no enhancers, either embryonic or larval, have been identified in this region. A 1.9 kb PCR fragment, which includes nearly half of the iab-7 sequences contained within the tmr, was inserted into a P-transformation vector used to identify the Fab-8 insulator (Fig. 5) is described in Zhou et al. (1996). Different tmr DNAs were cloned into a unique BamHI site located within the 2xPE and IAB8 enhancers.

To generate the construct in Fig. 7E, a 228 bp SpeI-BamHI fragment of the hairy stripe one enhancer (HI; Zhou et al., 1996) was inserted between the mini white and the transposase-lacZ promoter of the C4PLZ vector. The 9.5 tmr DNA was inserted in the Bg/II site at the 3’ end of the lacZ gene.

**Characterization of the IAB7 enhancer**

The distal approx. 4 kb region of the tmr extends into the iab-7 domain (Fig. 2; Gyurkovics et al., 1990), which controls the morphogenesis of the tissues that comprise the seventh abdominal segment. Thus far, no enhancers, either embryonic or larval, have been identified in this region. A 1.9 kb PCR fragment, which includes nearly half of the iab-7 sequences contained within the tmr, was inserted into a P-transformation vector used to identify the Fab-8 insulator (Fig. 5) is described in Zhou et al. (1996). Different tmr DNAs were cloned into a unique BamHI site located within the 2xPE and IAB8 enhancers.

To generate the construct in Fig. 7E, a 228 bp SpeI-BamHI fragment of the hairy stripe one enhancer (HI; Zhou et al., 1996) was inserted between the mini white and the transposase-lacZ promoter of the C4PLZ vector. The 9.5 tmr DNA was inserted in the Bg/II site at the 3’ end of the lacZ gene.

**Characterization of the IAB7 enhancer**

The distal approx. 4 kb region of the tmr extends into the iab-7 domain (Fig. 2; Gyurkovics et al., 1990), which controls the morphogenesis of the tissues that comprise the seventh abdominal segment. Thus far, no enhancers, either embryonic or larval, have been identified in this region. A 1.9 kb PCR fragment, which includes nearly half of the iab-7 sequences contained within the tmr, was inserted into a P-transformation vector used to identify the Fab-8 insulator (Fig. 5) is described in Zhou et al. (1996). Different tmr DNAs were cloned into a unique BamHI site located within the 2xPE and IAB8 enhancers.

To generate the construct in Fig. 7E, a 228 bp SpeI-BamHI fragment of the hairy stripe one enhancer (HI; Zhou et al., 1996) was inserted between the mini white and the transposase-lacZ promoter of the C4PLZ vector. The 9.5 tmr DNA was inserted in the Bg/II site at the 3’ end of the lacZ gene.

**Characterization of the IAB7 enhancer**

The distal approx. 4 kb region of the tmr extends into the iab-7 domain (Fig. 2; Gyurkovics et al., 1990), which controls the morphogenesis of the tissues that comprise the seventh abdominal segment. Thus far, no enhancers, either embryonic or larval, have been identified in this region. A 1.9 kb PCR fragment, which includes nearly half of the iab-7 sequences contained within the tmr, was inserted into a P-transformation vector used to identify the Fab-8 insulator (Fig. 5) is described in Zhou et al. (1996). Different tmr DNAs were cloned into a unique BamHI site located within the 2xPE and IAB8 enhancers.

To generate the construct in Fig. 7E, a 228 bp SpeI-BamHI fragment of the hairy stripe one enhancer (HI; Zhou et al., 1996) was inserted between the mini white and the transposase-lacZ promoter of the C4PLZ vector. The 9.5 tmr DNA was inserted in the Bg/II site at the 3’ end of the lacZ gene.

**Characterization of the IAB7 enhancer**

The distal approx. 4 kb region of the tmr extends into the iab-7 domain (Fig. 2; Gyurkovics et al., 1990), which controls the morphogenesis of the tissues that comprise the seventh abdominal segment. Thus far, no enhancers, either embryonic or larval, have been identified in this region. A 1.9 kb PCR fragment, which includes nearly half of the iab-7 sequences contained within the tmr, was inserted into a P-transformation vector used to identify the Fab-8 insulator (Fig. 5) is described in Zhou et al. (1996). Different tmr DNAs were cloned into a unique BamHI site located within the 2xPE and IAB8 enhancers.

To generate the construct in Fig. 7E, a 228 bp SpeI-BamHI fragment of the hairy stripe one enhancer (HI; Zhou et al., 1996) was inserted between the mini white and the transposase-lacZ promoter of the C4PLZ vector. The 9.5 tmr DNA was inserted in the Bg/II site at the 3’ end of the lacZ gene.

**Characterization of the IAB7 enhancer**

The distal approx. 4 kb region of the tmr extends into the iab-7 domain (Fig. 2; Gyurkovics et al., 1990), which controls the morphogenesis of the tissues that comprise the seventh abdominal segment. Thus far, no enhancers, either embryonic or larval, have been identified in this region. A 1.9 kb PCR fragment, which includes nearly half of the iab-7 sequences contained within the tmr, was inserted into a P-transformation vector used to identify the Fab-8 insulator (Fig. 5) is described in Zhou et al. (1996). Different tmr DNAs were cloned into a unique BamHI site located within the 2xPE and IAB8 enhancers.

To generate the construct in Fig. 7E, a 228 bp SpeI-BamHI fragment of the hairy stripe one enhancer (HI; Zhou et al., 1996) was inserted between the mini white and the transposase-lacZ promoter of the C4PLZ vector. The 9.5 tmr DNA was inserted in the Bg/II site at the 3’ end of the lacZ gene.
vector containing divergently transcribed white and lacZ reporter genes (see diagrams in Fig. 5). This iab-7 DNA directs the expression of the white reporter gene within the limits of a posterior stripe (Fig. 3A, arrows) that resolves into two stripes in late stage 5 embryos (data not shown). Reporter gene expression was visualized after hybridizing transgenic embryos with a digoxigenin-labeled antisense RNA probe. A similar staining pattern was observed when the lacZ reporter gene was monitored via in situ hybridization (data not shown).

Different pieces of the 1.9 kb iab-7 DNA fragment were analyzed in an effort to identify the minimal IAB7 enhancer (Fig. 3G). A 700 bp fragment retains full activity (Fig. 3E). A 1.6 kb fragment does not activate the lacZ reporter gene in transgenic embryos (data not shown). PRE, Polycomb response element.

vector containing divergently transcribed white and lacZ reporter genes (see diagrams in Fig. 5). This iab-7 DNA directs the expression of the white reporter gene within the limits of a posterior stripe (Fig. 3A, arrows) that resolves into two stripes in late stage 5 embryos (data not shown). Reporter gene expression was visualized after hybridizing transgenic embryos with a digoxigenin-labeled antisense RNA probe. A similar staining pattern was observed when the lacZ reporter gene was monitored via in situ hybridization (data not shown). The early stripes of white and lacZ expression appear to fall within the limits of parasegments (ps) 12-14 (Martinez-Arias and Lawrence, 1985; Busturia and Bienz, 1993).

Different pieces of the 1.9 kb iab-7 DNA fragment were analyzed in an effort to identify the minimal IAB7 enhancer (Fig. 3G). A 700 bp fragment retains full activity (Fig. 3E). A 1.6 kb fragment does not activate the lacZ reporter gene in transgenic embryos (data not shown). PRE, Polycomb response element.
The 5' portion of the 1.9 kb fragment lacks regulatory activity (Fig. 3D), and larger fragments containing the minimal 700 bp enhancer direct the same pattern of white expression as that observed with the minimal fragment (Fig. 3B,C; compare with E). The IAB7 enhancer directs somewhat stronger expression in dorsal versus ventral regions; a similar asymmetry is observed for the IAB5 enhancer in precellular embryos (e.g. Zhou et al., 1996).

Transcriptional repressors encoded by the gap genes have been shown to be essential for establishing the initial limits of homeotic gene expression (Levine and Harding, 1987). Direct regulatory interactions have been identified for the Krüppel repressor and iab-2 cis-regulatory elements within the abd-A locus (Shimell et al., 1994). Mutations in critical Krüppel binding sites result in an anterior expansion of the normal abd-A expression pattern. Genetic studies suggest that Krüppel might also regulate Abd-B expression (Harding and Levine, 1988). Krüppel- mutants exhibit an ectopic stripe of Abd-B expression in central regions of developing embryos, within the presumptive thorax. A computational search of Krüppel recognition sequences throughout the BX-C revealed two closely linked sites within an approx. 130 bp region of the minimal 700 bp IAB7 enhancer (Fig. 3H). Nucleotide substitutions in both core sequences (consensus: AACGGGTTAA) alter the activity of the IAB7 fusion gene in transgenic embryos (Fig. 3F). The white reporter gene exhibits ectopic expression in anterior regions (arrow, Fig. 3F) as compared with the normal pattern directed by the wild-type enhancer (Fig. 3E). These results suggest that Krüppel functions as a direct repressor of Abd-B expression.

Identification of the IAB8 enhancer

The tmr includes approx. 5 kb from the iab-8 cis-regulatory domain (see summary in Fig. 2, and below). A 5.3 kb BamHI-HindIII DNA fragment spanning this region was inserted into a P-transformation vector containing divergently transcribed white and lacZ reporter genes (see diagrams in Fig. 5). This DNA directs the expression of both reporter genes within a posterior stripe in transgenic embryos (Fig. 4A). This stripe appears to span parasegments 13 and 14 (Busturia and Bienz, 1993). This expression pattern suggests that the 5.3 kb region belongs to the iab-8 regulatory domain. Smaller DNA

---

**Fig. 6.** Insulator activity in adult tissues. The Fab-7 and Fab-8 insulators were placed between divergently transcribed white and yellow marker genes. Yellow controls pigmentation of the body cuticle, bristles and wing. The insulator DNAs were inserted at a HindIII site between the distal wing and body enhancers and the yellow transcription start site, just next to the insertion site of the gypsy retrotransposon in the y2 mutation (see summary in G). The insulator DNAs were flanked by frt sites; five copies of the eye-specific glass enhancer were placed in a distal position relative to the white reporter gene. (A) Wild-type adult head. The eyes have a bright red pigmentation. (B) Transgenic strain carrying the Fab-7 insulator DNA positioned between the white and yellow marker genes. The eyes have a pale yellow, slightly orange pigmentation. (C) Same as B except that the yeast Flip recombinase was randomly expressed via heat shock (see Materials and methods). Mosaic patches of Flip-expressing cells result in the rearrangement of the frt cassette so that the Fab-7 insulator is excised in these cells. The distal glass enhancer can now activate white, resulting in patches of red pigmented cells in the eyes. (D) Same as C except that the P-transposon contains the Fab-8 insulator DNA. In the absence of the Flip recombinase, the eyes are yellowish. Mosaic expression of the recombinase results in patches of red pigmentation, presumably due to the removal of the Fab-8 insulator. (E) Portion of the abdominal cuticle from a yellow, white (yw) mutant male. There is a substantial reduction in pigmentation as compared with wild-type flies. (F) Abdominal cuticle from the same transgenic strain as C. The Fab-7 insulator blocks the interaction of the distal body enhancer with the yellow promoter, so that the body cuticle is only slightly pigmented. Mosaic patches of Flip-expressing cells result in clones of pigmented tissue, presumably due to the loss of the Fab-7 insulator. (G) Summary of the white/yellow P-transposons. (H) Summary of Fab-7 insulator DNAs used in previous studies. The experiments shown in C and F involved the use of the Fab-7 DNA described by Zhou et al. (1996).
fragments were also examined to identify the minimal IAB8 enhancer (Fig. 4E); a distal 1.6 kb fragment failed to direct lacZ expression, while the proximal 2.7 kb fragment retains full activity (Fig. 4C).

Both the 5.3 kb, and to a lesser extent, the 2.7 kb DNA fragments continue to direct reporter gene expression in the posterior germ band of stage 10 (Fig. 4B,D) to stage 13 embryos (data not shown). In contrast, the iab-7 cis-regulatory DNAs direct transient stripes of expression only during early periods of embryogenesis (data not shown). It is therefore conceivable that the iab-8 region includes a maintenance element, perhaps a PRE, which sustains expression during development (reviewed by Hagstrom and Schedl, 1997). Additional evidence for a PRE stems from the analysis of eye color in the transgenic strains. Adults carrying the 5.3 kb iab-8 DNA exhibit strong position effect variegation (PEV) of mini white expression (data not shown), which has been shown to be diagnostic for other PREs (Chan et al., 1994). The 2.7 kb DNA fragment causes substantially weaker variegation. It is interesting to note that a conserved sequence motif (CRGCCATYDTRG) found in other PREs is present in the 5.3 kb, but not the 2.7 kb, iab-8 DNA (Mihaly et al., 1998b; Brown et al., 1998).

**Identification of an insulator DNA**

It has been proposed that the different iab regulatory regions are organized into separate chromatin loop domains by a series of insulator DNAs (Galloni et al., 1993; Vázquez et al., 1993; see Fig. 2 summary). If so, the most likely location of an insulator within the tmr is between the IAB7 and IAB8 enhancers identified in the preceding analyses. Three different DNA fragments spanning this interval, each about 2 kb in length, were tested for insulator activity (summarized in Fig. 5E). The assay involved the use of a P-transposon that contains two different enhancers, 2xPE and IAB5, positioned 5’ of divergently transcribed white and lacZ reporter genes (see diagrams below A,B and C,D in Fig. 5). The 2xPE enhancer corresponds to two tandem copies of the 180 bp proximal enhancer (PE) from the twist promoter region, which directs expression in the ventral mesoderm (Jiang et al., 1991; Thissle et al., 1991; Pan et al., 1991). The IAB5 enhancer directs a broad band of expression in the presumptive abdomen (Busturia and Bienz, 1993). When a neutral spacer DNA is inserted between the two enhancers, both white and lacZ exhibit composite patterns of expression in the mesoderm and abdomen (Fig. 5A,B). Only one of the DNA fragments that was tested in this assay exhibits an insulator activity (Fig. 5C,D). Insertion of this fragment, hereafter called Fab-8, in place of the spacer sequence alters both the white and lacZ staining patterns. The white reporter gene is now expressed exclusively in the mesoderm; staining is essentially eliminated in the presumptive abdomen (Fig. 5C). The opposite staining pattern is observed for the lacZ reporter gene (Fig. 5D). In this case, expression is detected primarily in the presumptive abdomen, while staining in the mesoderm is markedly reduced. These results suggest that the Fab-8 DNA functions as an insulator, and selectively blocks the interaction of a distal enhancer with a target promoter. It blocks interactions between IAB5 and white, as well as 2xPE and lacZ. Fab-8 is located approximately 2.5 kb 3′ of the IAB7 enhancer and 2 kb 5′ of IAB8 within the tmr (see Fig. 2, summary).

Fab-8 and the previously identified Fab-7 insulator were tested for their ability to block enhancer-promoter interactions in heterologous tissues (Fig. 6). The P-transformation vector used for these experiments contains the white and yellow genes; the latter reporter gene is normally expressed in the body cuticle, wings and bristles of adult flies (summarized in Fig. 6G). The yellow promoter region contains separate enhancers that regulate expression in the wings and body cuticle (Geyer and Corces, 1987). Previous studies have
identified the $y^2$ mutation as an insertion of the gypsy retrotransposon (Geyer et al., 1986). The gypsy insulator specifically blocks the activities of the distal wing and body enhancers, but not the intronic bristle enhancer (see review by Geyer, 1997).

$P$-transposons were prepared that contain either a Fab-7 or Fab-8 cassette (Fig. 6G). Each cassette contains five copies of an eye-specific enhancer from the glass gene, and an insulator DNA (Fab-7 or Fab-8) flanked by binding sites (frt) for the yeast Flip recombinase. Transgenic flies carrying either insulator cassette possess only yellow to light orange eyes (Fig. 6B, compare with A), presumably due to a block in glass-white interactions. These transgenic strains were mated with flies containing the Flip recombinase under the control of the hsp70 promoter. Heat-induction of the recombinase results in patches of pigmented body cuticle (Fig. 6F; compare with E), due to the mosaic activation of Fab-7 and Fab-8 insulators, and not the result of position effects resulting from differing sites of transgene integration. When the insulators are removed by the Flip recombinase, the glass enhancer leads to robust expression of the white gene. The insulator cassettes also block the activation of the yellow gene by the wing and body enhancers but not the intronic bristle enhancer (data not shown). Heat-induced expression of the Flip recombinase results in patches of pigmented body cuticle (Fig. 6F; compare with E), due to the mosaic activation of yellow by the distal body enhancer.

The Fab-7 insulator DNA that was used in the preceding analysis is summarized in Fig. 6H. It is distinct from Fab-7 sequences examined in other studies, although all three Fab-7 DNAs contain a cluster of six linked GAGA sites. GAGA binds to a zinc finger protein, Trithorax-like (Trl), and Trl-GAGA interactions have been shown to be important for the enhancer blocking activity of the eve promoter (Farkas et al., 1994; Bhat et al., 1996; Ohtsuki and Levine, 1998). It is conceivable that GAGA is also important for the insulator activity of Fab-7.

Identification of an internal promoter

Previous studies provide evidence that the Abd-B 3′ cis-regulatory DNA is transcribed (Sanchez-Herrero and Akam, 1989; Casares and Sanchez-Herrero, 1995). To investigate this issue, different DNA fragments from the tmr were used for in situ hybridization assays (Fig. 7). All three cis-regulatory elements, IAB7, Fab-8 and IAB8, detect specific RNAs (Fig. 7A-C). Staining is observed in a posterior stripe, similar to the pattern directed by the IAB8 enhancer when attached to a reporter gene (see Fig. 4). The use of small DNA fragments spanning the iab-8 regulatory region suggests that there may be one major internal promoter, located just downstream of the Abd-B transcription unit (data not shown; Fig. 7E). This promoter can respond to heterologous enhancers (Fig. 7D). A $P$-transposon was prepared that contains the 9.5 kb tmr and the heterologous 200 bp hairy stripe 1 (H1) enhancer (Fig. 7E). Transgenic embryos were then hybridized with the IAB7 enhancer as a probe. Normally, the enhancer is expressed in a posterior stripe (Fig. 7C). However, the enhancer is expressed in both a posterior stripe and an anterior band in transgenic embryos (Fig. 7D). The latter pattern is presumably due to activation of the iab-8 promoter by the H1 enhancer.

DISCUSSION

Previous studies have shown that the tmr is important for an unusual transvection process, whereby distal Abd-B enhancers continue to activate the Abd-B promoter even when translocated onto different chromosomes (Hendrickson and Sakonju, 1995; Hopmann et al., 1995; Sipos et al., 1998). In an effort to determine the basis for this phenomenon, we have attempted to identify all of the cis-regulatory elements within the tmr. Several disparate elements were identified, including two embryonic enhancers, IAB7 and IAB8, an insulator DNA (Fab-8), an internal promoter, and possibly a PRE. Thus, it would appear that the tmr contains a densely packed assortment of cis-regulatory elements. Previous studies identified only three elements in the entire 50 kb 3′ regulatory region, the IAB5 enhancer, the Fab-7 insulator and iab-7 PRE (Busturia and Bienz, 1993; Gyurkovics et al., 1990; Galloni et al., 1993; Karch et al., 1994; Zhou et al., 1996; Hagstrom et al., 1996, 1997; Mihaly et al., 1997).

The Abd-B regulatory region contains multiple insulators

The identification of the Fab-8 insulator is consistent with the previous proposal that each Abd-B iab domain may be flanked by insulators (Galloni et al., 1993; Vazquez et al., 1993, see Fig. 2, summary). For example, it now appears that the iab-7 region is flanked by Fab-7 and Fab-8. Future studies will determine whether Mbp functions as a bona fide insulator, and whether there is an insulator, Fab-6, located between the iab-5 and iab-6 regulatory regions.

It has been proposed that insulator DNAs organize the 3′ Abd-B cis regulatory regions into a series of separate chromatin loop domains (Galloni et al., 1993; Vazquez et al., 1993). For example, it is possible that proteins bound to the Fab-7 and Fab-8 insulators interact and organize iab-7 cis-elements within a discrete chromatin loop. Such loops might help account for the Abd-B transvection process. Chromatin loops might interact with proteins that recognize the Abd-B promoter region, thereby facilitating long-range interactions of distal enhancers with the Abd-B promoter.

The iab-8 internal promoter

Previous studies have shown that cis-regulatory regions of the BX-C are transcribed (Sanchez-Herrero and Akam, 1989; Casares and Sanchez-Herrero, 1995). In the case of the Abd-B 3′ cis DNA, it would appear that there is a major internal promoter located just downstream of the Abd-B transcription unit (summarized in Fig. 2). The identification of an iab-8 promoter is reminiscent of the situation seen in mammalian immunoglobulin genes, whereby unrearranged, germline genes contain internal promoters that are thought to maintain neighboring intronic enhancers in an open conformation for interactions with appropriate transcriptional regulatory proteins (e.g. Xu and Stavnezer, 1992). It is conceivable that the iab-8 promoter plays a similar role in Abd-B regulation. The maintenance of an open chromatin conformation might help account for Abd-B transvection. Perhaps proteins bound to the 3′ region serve as ‘signposts’ for the distal Abd-B enhancers.

The iab-8 promoter might also play a more specific role in ensuring peak expression of Abd-B in parasegment 13 (and
perhaps ps 14). Transcription of the 3′ region might inactivate the Fab-7 and Fab-8 insulators within parasegment 13 so that all distal regulatory elements can activate the Abd-B promoter in this posterior region of the embryo. It is also conceivable that the iab-8 promoter, as well as other internal promoters in the 3′ region, might direct long-range enhancer-promoter interactions. Perhaps such promoters help ‘reel-in’ distal enhancers to the Abd-B transcription unit.

There are parallels between the organization of the Abd-B locus in Drosophila and mammalian globin loci. Both contain insulator DNAs and exhibit extensive transcription of the cis-regulatory DNA (Chung et al., 1993; Ashe et al., 1997). It has been shown that exogenous DNAs from the globin locus, either the globin transcription units or the neighboring cis DNAs, physically associate with the endogenous globin locus in transfected tissue culture cells. This physical interaction of exogenous DNA with the endogenous gene leads to the activation of the internal promoters in the endogenous locus. There may be a similar long-range physical interaction between distal cis-regulatory elements and the Abd-B locus in the transcription phenomenon. Future studies will determine whether the cis-elements identified in this study are sufficient to account for transvection, or whether the tmr contains additional elements that facilitate long-range enhancer-promoter interactions.

We thank Jennifer Gin, Brian Castro and Lanyin Chwang for excellent technical help. This work was funded by a grant from the NIH (GM34431) and done in part under the auspices of the DOE with funds from the UCL/LANL CULAR program (UCDRD #9624). J. Z. is supported by an NIH postdoctoral fellowship (GM19329).

REFERENCES


Mihaly, J., Hogga, I., Gausz, J., Gyrkoviczs, H. and Karch, F. (1997). In situ dissection of the Fab-7 region of the bithorax complex into a chromatin


