

## New Properties of *Drosophila* Fab-7 Insulator

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### ABSTRACT

In the *Abd-B* 3' cis-regulatory region, which is subdivided into a series of *iab* domains, boundary elements have previously been detected, including the Fab-7 element providing for the autonomous functioning of the *iab-6* and *iab-7* cis-regulatory domains. Here, it has been shown that a single copy of the 860-bp Fab-7 insulator effectively blocks the *yellow* and *white* enhancers. The eye and testis enhancers can stimulate the *white* promoter across the pair of Fab-7, which is indicative of a functional interaction between the insulators. Unexpectedly, Fab-7 has proved to lose the enhancer-blocking activity when placed near the *white* promoter. It seems likely that Fab-7 strengthens the relatively weak *white* promoter, which leads to the efficient enhancer–promoter interaction and insulator bypass.

AS enhancers exert long-distance effects, a question arises as to how an enhancer can specifically activate its target gene without affecting adjacent genes (DORSETT 1999; BONDARENKO *et al.* 2003; WEST and FRASER 2005; FRASER 2006; GASZNER and FELSENFELD 2006). In this relation, of interest is the class of DNA sequence elements, named insulators, that contribute to the organization of independent gene function domains by restricting the functions of enhancers and silencers (SUN and ELGIN 1999; KUHN and GEYER 2003; CAPELSON and CORCES 2004; BRASSET and VAURY 2005; WEST and FRASER 2005; GASZNER and FELSENFELD 2006; VALENZUELA and KAMAKAKA 2006). Insulators are characterized by two properties. First, they operate in a position-dependent manner, preventing the functioning of enhancers and silencers when inserted between these regulatory elements and a promoter but not when located upstream or downstream from them (GYURKOVICS *et al.* 1990; HOLDRIDGE and DORSETT 1991; GEYER and CORCES 1992; KELLUM and SCHEDL 1992; SIGRIST and PIRROTTA 1997; MALLIN *et al.* 1998; COMET *et al.* 2006). However, insulators do not inactivate enhancers, silencers, or promoters, which indicates that they interfere with signaling between these classes of control elements (GEYER and CORCES 1992; CAI and LEVINE 1995; SCOTT and GEYER 1995). Second, insulators protect gene expression from the positive and negative effects of chro-

matin surrounding a gene (ISHII *et al.* 2002; MUTSKOV *et al.* 2002) and confer the capacity for position-independent transcription to transgenes reliably integrated into the genome (BONIFER *et al.* 1990; KELLUM and SCHEDL 1991; ROSEMAN *et al.* 1993, 1995; BURGESS-BEUSSE *et al.* 2002). Most of the insulators that have been identified in multicellular organisms appear to be constitutive and remain active irrespective of developmental stage or tissue type.

Several constitutively active insulators/boundaries that have been found in the *Drosophila Antennapedia* (ANT-C) and *bithorax* (BX-C) complexes are crucial for the developmental functions of homeotic genes in each complex (GYURKOVICS *et al.* 1990; HAGSTROM *et al.* 1996; ZHOU *et al.* 1996, 1999; MIHALY *et al.* 1998; ZHOU and LEVINE 1999; BARGES *et al.* 2000; BELOZEROV *et al.* 2003; GRUZDEVA *et al.* 2005). The three homeotic genes of the *bithorax* complex—*Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*), and *Abdominal-B* (*Abd-B*)—are responsible for specifying the identity of parasegments 5–14 (PS5–PS14), which form the posterior half of the thorax and all abdominal segments of an adult fly (LEWIS 1978; SANCHEZ-HERRERO *et al.* 1985; MIHALY *et al.* 1998; MAEDA and KARCH 2006). The PS-specific expression patterns of *Ubx*, *abd-A*, and *Abd-B* are determined by a complex cis-regulatory region that spans a 300-kb DNA segment (SIPOS and GYURKOVICS 2005; MAEDA and KARCH 2006). For example, *Abd-B* expression in PS10, PS11, PS12, and PS13 is controlled by the *iab-5*, *iab-6*, *iab-7*, and *iab-8* cis-regulatory domains, respectively (LEWIS 1978; KARCH *et al.* 1985; DUNCAN 1987; CELNIKER *et al.* 1990; BOULET *et al.* 1991; SANCHEZ-HERRERO 1991). The current model suggests that boundaries flank each *iab* region and organize the *Abd-B* regulatory DNA into a series of separate chromatin loop domains (GYURKOVICS *et al.* 1990;

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GALLONI *et al.* 1993; MIHALY *et al.* 1998; SIPOS and GYURKOVICS 2005; MAEDA and KARCH 2006).

Among *Abd-B* boundaries, the best characterized is the Fab-7 element located between the *iab-6* and *iab-7* cis-regulatory domains. Mutations that inactivate Fab-7 lead to the fusion of the *iab-6* and *iab-7* domains, and this disrupts the specification of PS11 (GYURKOVICS *et al.* 1990; GALLONI *et al.* 1993; KARCH *et al.* 1994; MIHALY *et al.* 1997). As with other known insulators, the insulating activity of the Fab-7 element is neither restricted to specific enhancer–promoter combinations nor stage or tissue specific (GALLONI *et al.* 1993; HAGSTROM *et al.* 1996; ZHOU *et al.* 1996; SCHWEINSBERG and SCHEDL 2004). The minimal Fab-7 boundary defined in different enhancer blocking assays is 1.2 kb long (HAGSTROM *et al.* 1996; ZHOU *et al.* 1996).

In this study, we have found that the Fab-7 insulator and its 0.86-kb subfragment block the *yellow* and *white* enhancers with similar efficiency. The 0.86-kb insulator has the same activity when placed at different sites between the *yellow* enhancers and promoter. Unexpectedly, the insulator has proved to lose the enhancer-blocking activity when inserted near the *white* promoter. As previously shown for several other insulators (CAI and SHEN 2001; MURAVYOVA *et al.* 2001; CONTE *et al.* 2002; KUHN *et al.* 2003; GRUZDEVA *et al.* 2005; KYRCHANOVA *et al.* 2007), the interaction between the Fab-7 insulators leads to mutual neutralization of their enhancer-blocking activity.

## MATERIALS AND METHODS

**Transgenic constructs:** The 3-kb *Sall*–*Bam*HI fragment containing the *yellow* regulatory region (*yr*) with the body and wing enhancers (–2873 to –1266 bp relative to the transcription start; GEYER and CORCES 1987) was subcloned into the pGEM7 plasmid digested with *Bam*HI and *Xho*I. The *white* eye enhancer (*Ee*, fragment –1465 to –1084 bp relative to the *white* transcription start; QIAN *et al.* 1992) was then inserted at position –1868 from the *yellow* transcription start site (*yr-Ee*). The 5-kb *Bam*HI–*Bgl*II fragment containing the *yellow* coding region (*yc*) was subcloned into CaSpeR2 (*yc-C2*). The *I-Sce*I+126x2-*Eye-yr* plasmid containing the *yellow* and *white* enhancers between the *I-Sce*I sites was described by RODIN and GEORGIEV (2005).

The 1.252-kb Fab-7 fragment (*F7*<sup>1-2</sup>) was cloned by PCR amplification of the genomic DNA between primers 5'-ACTGCAGTGAAGACGACGAACC-3' and 5'-CGTGAGCGACCGAACTC-3'; the 0.858-kb Fab-7 fragment was cloned by PCR amplification between primers 5'-GATTTCAAGCTGTGTGCGGGG-3' and 5'-CGTGAGCGACCGAACTC-3'. Thereafter, these Fab-7 fragments were sequenced to confirm their identity and subcloned between lox [lox(*F7*) and lox(*F7*<sup>1-2</sup>) plasmids] and *frt* [*frt*(*F7*) plasmid] sites. Sequences from *e(y)3* cDNA were used as spacers. In particular, the 2.7-kb *Bam*HI–*Not*I and the 1.4-kb *Pvu*II–*Bgl*II fragments were cut from the coding region of the *e(y)3* gene and cloned, respectively, between lox [lox(2.7)] and *frt* [*frt*(1.4)] sites.

To construct Eye(*F7*<sup>1-2</sup>)YW and Eye(*F7*)YW, the lox(*F7*<sup>1-2</sup>) and lox(*F7*) fragments were inserted into the *yr-Ee* plasmid digested with *Eco*47III at –893 from the *yellow* transcription start site [*yr-Ee-lox*(*F7*<sup>1-2</sup>) and *yr-Ee-lox*(*F7*)]. Next, the *yr-Ee-*

lox(*F7*<sup>1-2</sup>) and *yr-Ee-lox*(*F7*) fragments were cloned into the *yc-C2* plasmid digested with *Xba*I and *Bam*HI.

To construct (Eyw)(*F7*)Y(*F7*)W and (Eyw)(*F7*<sup>R</sup>)Y(*F7*)W, the lox(*F7*) fragment was inserted into *C2-yc* between the *yellow* and *white* genes [*C2-lox*(*F7*)-*yc*]. Here and below, the orientation of the Fab-7 fragment relative to the direction of the *yellow* and *white* genes was verified by PCR. The *frt*(*F7*) fragment was inserted in the direct or reverse orientation into the *I-Sce*I+126x2-*Eye-yr* plasmid digested with *Eco*47III [*I-Sce*I+126x2-*Eye-yr-frt*(*F7*)]. The resulting fragment was subcloned into *C2-yc-lox*(*F7*) digested with *Xba*I and *Bam*HI.

To construct (Eyw)Y(*F7*)W, the lox(*F7*) fragment was inserted in the direct orientation between the *yellow* and *white* genes [*C2-yc-lox*(*F7*)]. The *I-Sce*I+126x2-*Eye-yr* fragment was subcloned into *C2-yc-lox*(*F7*) digested with *Xba*I and *Bam*HI.

To construct (Eye)(2.7)(*F7*)YW, the *frt*(*F7*) fragment was inserted into the lox(2.7) plasmid digested with *Bam*HI [lox(2.7)-*frt*(*F7*)]. The lox(2.7)-*frt*(*F7*) fragment was inserted into the *I-Sce*I+126x2-*Eye-yr* plasmid digested with *Eco*47III [*I-Sce*I+126x2-*Eye-yr-lox*(2.7)-*frt*(*F7*)]. The resulting fragment was subcloned into *C2-yc* cleaved with *Xba*I and *Bam*HI.

To construct (Eyw)Y(*F7*<sup>R</sup>)(1.4)W, the *frt*(1.4) fragment was inserted into the lox(*F7*) plasmid digested with *Bam*HI [lox(*F7*)-*frt*(1.4)]. The lox(*F7*)-*frt*(1.4) fragment was subcloned into the *C2-yc* digested with *Bgl*II [*C2-yc-lox*(*F7*)-*frt*(1.4)]. The *I-Sce*I+126x2-*Eye-yr* fragment was subcloned into *C2-yc-lox*(*F7*)-*frt*(1.4) digested with *Xba*I and *Bam*HI.

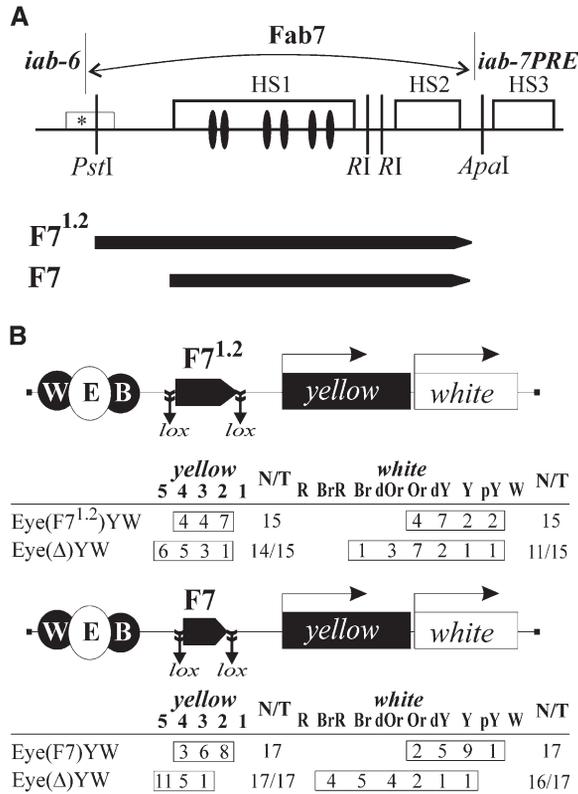
To construct EywF7Y(*F7*<sup>R</sup>)(1.4)W, the *I-Sce*I+126x2-*Eye-yr* fragment was subcloned into *C2-yc-lox*(*F7*)-*frt*(1.4) digested with *Xba*I and *Bam*HI.

To construct (Eye)*F7*<sub>–172</sub>YW, the 500-bp fragment was obtained by PCR amplification of the *yr* plasmid between primers 5'-CGCAAAGTTGGCCGATCTATGG-3' and 5'-CAGGAAACAGCTATGAC-3'. After sequencing, the 500-bp fragment was cloned into *F7* digested with *Pst*I and *Spe*I (*F7*-500). The 770-bp fragment was obtained by PCR amplification of the *yr* plasmid between primers 3'-ATCCAGTTGATTTTCAGGGACCA-5' and 5'-TGTCTTCCATGATTGATTTTCACGC-3'. After sequencing, the 770-bp fragment was cloned into *F7*-500 plasmid digested with *Hind*III and *Xho*I (*F7*-500-770). The *F7*-500-770 fragment was cloned into the pSK-*I-Sce*I+126x2-*Eye* plasmid digested with *Hinc*II [(Eye)-*F7*<sub>–172</sub>]. Finally, the resulting DNA fragment was cloned into *C2-yc* digested with *Xba*I and *Bam*HI.

To construct (Eye)(*F7*<sub>–343</sub>)YW the lox(*F7*) fragment was inserted in direct orientation into the *yr* pGEM7 plasmid digested with *Kpn*I [*yr-lox*(*F7*<sub>–343</sub>)]. *I-Sce*I+126x2-*Eye-yr* fragment was subcloned into *yr-lox*(*F7*<sub>–343</sub>) plasmid digested with *Xba*I-*Eco*47III [*I-Sce*I+126x2-*Eye-yr-lox*(*F7*<sub>–343</sub>)]. The resulting fragment was subcloned into *C2-yc* cleaved with *Xba*I and *Bam*HI.

**Generation and analysis of transgenic lines:** All flies were maintained on the standard yeast medium at 25°. The mutant alleles and chromosomes used in this study and the balancer chromosomes are described elsewhere (LINDSLEY and ZIMM 1992). The construct, together with P25.7wc, a *P* element having defective inverted repeats used as a transposase source (KARESS and RUBIN 1984), was injected into *y ac w*<sup>118</sup> preblastoderm embryos as described (RUBIN and SPRADLING 1982; SPRADLING and RUBIN 1982). The resulting flies were crossed with *y ac w*<sup>118</sup> flies, and the transgenic progeny were identified by the color of their eyes and cuticle structures. The transformed lines were tested for transposon integrity and copy number by Southern blot hybridization.

The lines with excisions were obtained by crossing the flies bearing the transposons with flies of *Flp* (*w*<sup>118</sup>; *S*<sup>2</sup> *CyO*, *hsFLP*, *ISA/Sco*; +) or *Cre* (*y*<sup>1</sup>, *w*<sup>1</sup>; *CyO*, *P[w+, cre]/Sco*; +) recombinase-expressing lines. A high level of FLP recombinase was produced by exposing late embryos and second or third instar larvae to heat shock at 37° for 2 hr. A high level of *I-Sce*I



**FIGURE 1.**—Comparison of the enhancer-blocking activities of 1.2-kb (F7<sup>1.2</sup>) and 0.86-kb (F7) fragments from the Fab-7 insulator. (A) Diagram shows Fab-7 region that includes one minor (marked with an \*) and three major nuclease hypersensitive sites (HS1, HS2, and HS3) whose extent is indicated by open boxes (KARCH *et al.* 1994) and the 1.2-kb and 0.86-kb Fab-7 fragments tested in the enhancer-blocking assay. GAF binding sites are shown as solid ovals. (B) Reductive schemes of transgenic constructs and analysis of F7<sup>1.2</sup> and F7 in an enhancer blocking assay. In schemes of constructs (not drawn to scale), Fab-7 fragments are shown as pentagons with apexes indicating their orientation. The *yellow* wing (W) and body (B) enhancers are shown as solid ovals, and the eye enhancer (E) inserted between them is shown as an open oval. The *yellow* (Y) and *white* (W) genes are shown as boxes with arrows indicating the direction of their transcription. Downward arrows indicate the target sites of F1p recombinase (frt) or Cre recombinase (lox); the same sites in construct names are denoted by parentheses. The “yellow” column shows the numbers of transgenic lines with the *yellow* pigmentation level in the abdominal cuticle (reflecting the activity of the body enhancer); in most of the lines, pigmentation levels in wing blades (reflecting the activity of the wing enhancer) closely correlated with these scores. The “white” column shows the numbers of transgenic lines with the *white* pigmentation level in eyes (reflecting the activity of the eye enhancer). The level of pigmentation (*i.e.*, of *y* expression) was estimated on an arbitrary five-grade scale, with wild-type expression and the absence of expression assigned scores 5 and 1, respectively. The “white” column shows the numbers of transgenic lines with different levels of eye pigmentation. Wild-type *white* expression determined the bright red eye color (R); in the absence of *white* expression, the eyes were white (W). Intermediate levels of pigmentation with the eye color ranging from pale yellow (pY), through yellow (Y), dark yellow (dY), orange (Or), dark orange (dOr), and brown (Br), to brownish red (BrR) reflect the increasing levels of

endonuclease was achieved by heat-shock treatment for 2 hr on day 3 after hatching, as described (RODIN and GEORGIEV 2005). The excisions were confirmed by PCR analysis. The details of the crosses used for genetic analysis and for excision of functional elements are available upon request.

The yellow phenotype was determined from the level of pigmentation of the abdominal cuticle and wings in 3- to 5-day-old males developing at 25°. As a reference group, we used flies in which the *y* allele had been characterized previously. The level of pigmentation (*i.e.*, of *y* expression) was estimated on an arbitrary five-grade scale: wild-type expression was assigned score 5, and the absence of expression, score 1. The white phenotype was determined from eye pigmentation and testis pigmentation in adult flies. Wild-type *white* expression in eyes determined bright-red eye color (R); in the absence of *white* expression, the eyes were white (W). Intermediate levels of *white* expression (in increasing order) were reflected in the eye color, ranging from pale yellow (pY) through yellow (Y), dark yellow (dY), orange (Or), and dark orange (dOr), to brown (Br) or brown-red (BrR). Males from different transgenic lines were allowed to age 10 days before dissection and visual inspection of the testes.

## RESULTS

**The 1.2-kb Fab-7 insulator and its 0.86-kb subfragment block the *yellow* and *white* enhancers with similar efficiency:** To test the enhancer-blocking activity of the Fab-7 insulators and functional interaction between them, we employed the test system with the *yellow* and *white* genes that have been extensively used in insulator studies (GEYER and CORCES 1992; KELLUM and SCHEDL 1992; ROSEMAN *et al.* 1993; MURAVYOVA *et al.* 2001; KUHN *et al.* 2003; SCHWEINSBERG *et al.* 2004; SCHWEINSBERG and SCHEDL 2004; GRUZDEVA *et al.* 2005; SAVITSKAYA *et al.* 2006). The *yellow* gene (designated Y) is required for dark pigmentation of larval and adult cuticle and its derivatives. Two upstream enhancers (designated Ey) are responsible for *yellow* activation in the body cuticle and wing blades, while the enhancer responsible for *yellow* activation in bristles resides in the intron of the *yellow* gene (GEYER and CORCES 1987; MARTIN *et al.* 1989). The *white* gene (designated W) is required for eye and testis pigmentation and is regulated by the eye- and testis-specific enhancers (designated Ew; QIAN *et al.* 1992). These enhancers were inserted between the *yellow* wing and body enhancers (Eyw).

Previously, the minimal Fab-7 insulator was mapped in the 1.2-kb fragment between *PstI* and *ApaI* (Figure 1A). In our assay, we used the same 1.2-kb fragment (F7<sup>1.2</sup>) and its 0.86-kb subfragment (F7) in which the 5' sequences of F7<sup>1.2</sup> were deleted. The F7 subfragment contains all GAF binding sites related to the enhancer-blocking activity of the insulator (SCHWEINSBERG *et al.* 2004).

*white* expression. N is the number of lines in which flies acquired a new *y* phenotype upon deletion (Δ) of the specified DNA fragment; T is the total number of lines examined for each particular construct.

The F7 [Eyw(F7)YW] and F7<sup>1.2</sup> [Eyw(F7<sup>1.2</sup>)YW] flanked by lox sites were inserted between the enhancers and promoters at  $-893$  relative to the *yellow* transcription start site (Figure 1B). We obtained 15 transgenic lines carrying a single copy of Eyw(F7<sup>1.2</sup>)YW and 17 Eyw(F7)YW transgenic lines. Both F7<sup>1.2</sup> and F7 partially blocked the *yellow* and *white* enhancers with comparable efficiency (Figure 1B). Deletion of the insulators resulted in recovery of *yellow* and *white* expression in most of transgenic lines, confirming the role of these elements in insulation. These results suggest that the 0.86-kb subfragment contains the sequences required for the Fab-7 insulator activity in adult flies.

**Functional interaction between two copies of Fab-7 insulator leads to neutralization of their enhancer-blocking activity:** Considering that two consecutive Mcp insulators (KYRCHANOVA *et al.* 2007) or *gypsy* insulators (CAI and SHEN 2001; MURAVYOVA *et al.* 2001; KUHN *et al.* 2003) between an enhancer and the target gene promoter fail to block gene activation, we decided to find out whether two Fab-7 insulators would neutralize each other's enhancer-blocking activity. In all further experiments, we used the minimal 0.86-kb Fab-7 insulator (F7).

In the (Eyw)(F7)Y(F7<sup>R</sup>)W construct (Figure 2), one Fab-7 insulator (F7) flanked by *frt* sites was inserted at  $-893$  and the second Fab-7 copy flanked by lox sites was inserted between the *yellow* and *white* genes in the inverted orientation (designated F7<sup>R</sup>). To check the contributions of the enhancers to *yellow* and *white* expression in the presence or absence of Fab-7 insulators, the fragment containing the enhancers was flanked by 126-bp direct repeats and sites for the rare-cleaving I-*SceI* endonuclease that permits excision of enhancers as described in MATERIALS AND METHODS (RODIN and GEORGIEV 2005).

Throughout the text, parentheses in construct designations enclose the elements flanked by the *frt* (GOLIC and LINDQUIST 1989), lox (SIEGAL and HARTL 2000), or I-*SceI* sites at which those elements can be excised in crosses with flies expressing Flp or Cre recombinase or I-*SceI* endonuclease (as noted in MATERIALS AND METHODS). By comparing *yellow* and *white* expression in transgenic lines and their derivatives with two, one, or no Fab-7 elements, and in the presence or absence of the enhancers, it is possible to estimate the levels of enhancer blocking by different combinations of the elements at the same genomic site.

Twelve transgenic lines carrying a single insertions of the (Eyw)(F7)Y(F7<sup>R</sup>)W were obtained (Figure 2). By comparing *yellow* phenotypes in the original and derivative transgenic lines with one Fab-7 element inserted at  $-893$  or no Fab-7 insulators, we found that Fab-7 blocked the *yellow* enhancers effectively, while the second Fab-7 copy inserted downstream of the *yellow* gene had no notable effect on insulation. Deletion of the *yellow* enhancers resulted in decreasing of pigmentation to  $y^2$ -like level in all transgenic lines, suggesting that the *yellow* enhancers

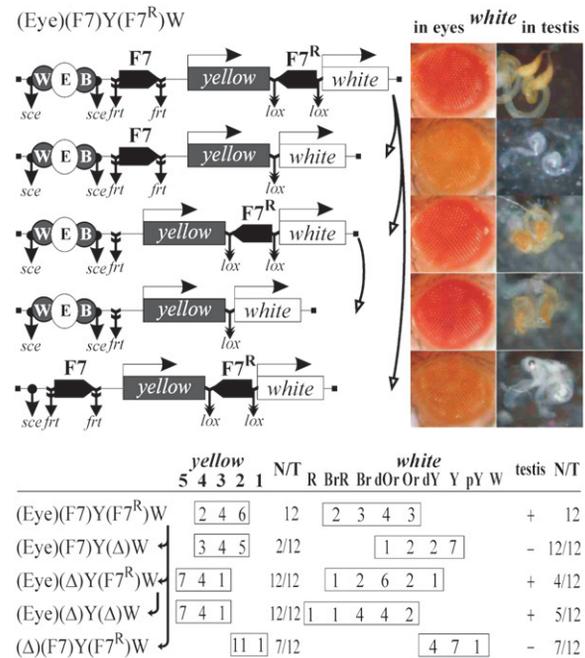


FIGURE 2.—Tests for the functional interaction between Fab-7 insulators based on the ability of the eye and testis enhancers to stimulate transcription across the pair of the Fab-7 elements. In schemes of constructs, downward arrows indicate the target sites of Flp recombinase (*frt*), or Cre recombinase (*lox*), or I-*SceI* endonuclease (*sce*). Arrows near the names of the construct show the order in which the N/T values were calculated. The “testis” column shows *white* expression in testis: +, extensive pigmentation; –, weak pigmentation or its absence. Other designations are as in Figure 1. Photographs show the eyes and testes of males heterozygous for the construct (P/+) from one transgenic line and its derivatives.

partially stimulate *yellow* expression across the Fab-7 insulator in half of the transgenic lines tested.

Comparing eye and testis pigmentation in the (Eyw)(F7)Y(F7<sup>R</sup>)W lines and their derivatives with the deleted enhancers showed that the eye and testis enhancers can effectively stimulate *white* expression across two Fab-7 insulators (Figure 2). At the same time, the deletion of the Fab-7 insulator located downstream of the *yellow* gene led to a strong reduction of eye and testis pigmentation, indicating that one Fab-7 copy at  $-893$  effectively blocked the *white* enhancers. Unexpectedly, we found that a single Fab-7 insulator located close to the *white* promoter failed to effectively block the eye and testis enhancers (Figure 2). Thus, the Fab-7 insulator located close to the *white* promoter proved to lose its enhancer-blocking activity.

**Orientation of Fab-7 insulators relative to genes and to each other is not crucial for their insulating activity and the outcome of their functional interaction:** The proximal and distal Fab-7 copies in the (Eyw)(F7)Y(F7<sup>R</sup>)W construct were in opposite orientations, and this factor might have an effect on the observed enhancer-blocking activity. To test the enhancer-blocking activity of the proximal (relative to the enhancers)

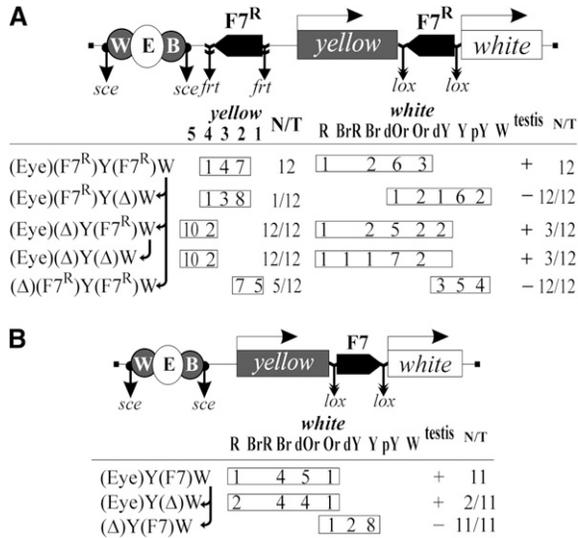


FIGURE 3.—Tests for orientation dependence of the Fab-7 insulator. (A) The (Eyw)(F7<sup>R</sup>)Y(F7<sup>R</sup>)W and (B) (Eyw)Y(F7)W constructs and phenotypic analysis of the corresponding transgenic lines carrying these constructs and their derivatives. Other designations are as in Figures 1 and 2.

Fab-7 insulator inserted in the opposite orientation and to reveal the outcome of pairing between collinear Fab-7 insulators, we made the (Eyw)(F7<sup>R</sup>)Y(F7<sup>R</sup>)W construct in which both Fab-7 elements were in the same orientation, opposite to the direction of the *yellow* and *white* genes (Figure 3A).

The levels of *yellow* pigmentation in flies of all 12 transgenic (Eyw)(F7<sup>R</sup>)Y(F7<sup>R</sup>)W lines (Figure 3A) were similar to those in flies of the initial (Eyw)(F7)Y(F7<sup>R</sup>)W lines (Figure 2). Thus, the orientation of the proximal Fab-7 insulator is not crucial for enhancer blocking. Again, deletion of the distal (relative to the enhancers) Fab-7 insulator had no influence on *yellow* pigmentation.

Comparing eye and testis pigmentation in the (Eyw)(F7)Y(F7<sup>R</sup>)W (Figure 2) and (Eyw)(F7<sup>R</sup>)Y(F7<sup>R</sup>)W (Figure 3A) transgenic lines, we concluded that the insulator bypass by the *white* enhancers in heterozygous flies did not depend on the relative orientation of Fab-7 insulators. One Fab-7 copy (F7<sup>R</sup>) inserted at -893 in reverse orientation [in (Eyw)(F7<sup>R</sup>)Y(Δ)W derivative lines, Figure 3] strongly blocked the *white* enhancers, confirming that the orientation of the proximal Fab-7 copy is not crucial for insulating activity.

Thereafter, we tested if the activity of Fab-7 located near by the *white* promoter was also orientation independent. In the (Eyw)Y(F7)W construct, we inserted the Fab-7 insulator flanked by lox sites between the *yellow* and *white* genes in the direct orientation (Figure 3B). In all 11 (Eyw)Y(F7)W lines tested, the enhancers effectively stimulated *white* expression, with the deletion of Fab-7 having no significant effect on it. Thus, the Fab-7 insulator inserted in the direct orientation also failed to block the *white* enhancers. Taken together, these results

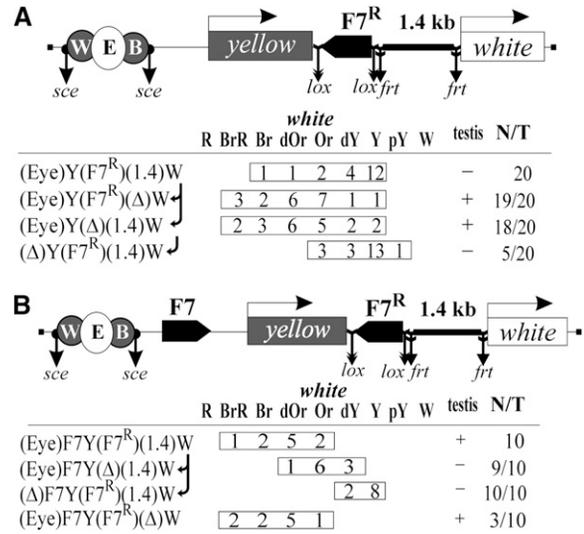


FIGURE 4.—Tests for role of the distance between Fab-7 and the *white* promoter in insulator bypass by the enhancers: (A) (Eye)Y(F7<sup>R</sup>)(1.4)W and (B) (Eye)(F7)Y(F7<sup>R</sup>)(1.4)W transgenes and analysis of *yellow* and *white* expression in original transgenic lines and their derivatives obtained by deleting either Fab-7, or the enhancers, or the DNA spacer (1.4 kb) inserted between Fab-7 and the *white* promoter. Other designations are as in Figures 1 and 2.

suggest that the orientation of Fab-7 relative to the enhancer–promoter pair is not crucial for its enhancer-blocking activity.

**Distance between Fab-7 and the *white* promoter is crucial for enhancer blocking:** The insulator bypass by the enhancers in the case of Fab-7 placed close to the *white* promoter suggested that the distance between the promoter and the insulator could have a role in enhancer blocking. To test this possibility, we inserted a 1.4-kb spacer flanked by *frit* sites between *white* and Fab-7 in the (Eyw)Y(F7<sup>R</sup>)(1.4)W construct (Figure 4A). As in the previous constructs, Fab-7 flanked by lox sites was inserted in the reverse orientation on the 3' side of the *yellow* gene. Flies in 16 of 20 transgenic (Eyw)Y(F7<sup>R</sup>)(1.4)W lines had low levels of eye and testis pigmentation, which indicated that the *white* enhancers were blocked (Figure 4A). Deletion of either Fab-7 insulator or the 1.4-kb spacer restored activity of the enhancers. Thus, the 1.4-kb DNA fragment did not block the enhancers but increased the distance between Fab-7 and the *white* promoter, which allowed Fab-7 to insulate the enhancers.

Next, we checked whether Fab-7 pairing allows enhancer–promoter communication when the distal Fab-7 and the *white* promoter are separated by the 1.4-kb spacer. In the (Eyw)F7Y(F7<sup>R</sup>)(1.4)W construct, both Fab-7 insulators were inserted in the same positions as in the original (Eyw)(F7)Y(F7<sup>R</sup>)W construct, but the *white* promoter was separated from the distal Fab-7 insulator by the 1.4-kb spacer (Figure 4B). In 10 transgenic (Eyw)F7Y(F7<sup>R</sup>)(1.4)W lines, the eye and testis enhancers stimulated *white* expression. Deletion of either

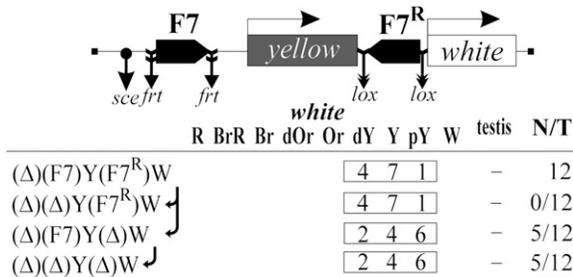


FIGURE 5.—Tests for the influence of Fab-7 on the activity of *white* promoter: analysis of eye pigmentation in flies from enhancerless derivatives of (Eyw)(F7)Y(F7<sup>R</sup>)W lines. Other designations are as in Figures 1 and 2.

the enhancers or the distal Fab-7 insulator led to strong reduction of eye pigmentation in flies, while deletion of the 1.4-kb spacer did not significantly change *white* expression (Figure 4B). Thus, an increase in the distance between the Fab-7 insulator and the *white* promoter does not affect the ability of the enhancers to stimulate *white* expression across the pair of Fab-7 insulators.

**The near-by Fab-7 insulator improves the basal activity of the *white* promoter:** To explain the inability of the Fab-7 insulator to block enhancers in position near the *white* promoter, we suggest that Fab-7 can strengthen the relatively weak promoter. To test this assumption, we compared eye pigmentation in the enhancerless ( $\Delta$ )(F7)Y(F7<sup>R</sup>)W derivatives in the presence of Fab-7 insulators and after their deletion (Figure 5). The deletion of the Fab-7 insulator at -893 had no influence on *white* expression, while that of the distal Fab-7 insulator markedly decreased eye pigmentation in 5 of 12 transgenic lines, suggesting that the Fab-7 insulator located near the *white* promoter improves its activity.

**Fab-7 displays insulating activity in different positions near the *yellow* promoter:** The next question was whether Fab-7 fails to block the *yellow* enhancers when it is located close to the promoter. To study this position dependence, we inserted the Fab-7 insulator flanked by lox sites at -343 bp relative to the *yellow* transcription start site [(Eyw)(F7<sub>-343</sub>)YW, Figure 6A]. The distance between Fab-7 and the *yellow* promoter was approximately the same as between Fab-7 and the *white* promoter in the (Eyw)Y(F7<sup>R</sup>)W construct. In all 14 (Eyw)(F7<sub>-343</sub>)YW transgenic lines, the *yellow* and *white* enhancers were blocked with similar efficiency as in the transgenic lines in which the Fab-7 insulator was inserted at -893 (Figures 1B and 2).

In the next (Eyw)F7<sub>-172</sub>YW construct, we did not flank the Fab-7 insulator by lox sites, thereby reducing the distance between Fab-7 and the transcription start site to 172 bp (Figure 6B). Once again, the Fab-7 insulator effectively blocked the *yellow* and *white* enhancers. These results suggest that the distance between Fab-7 and the *yellow* promoter is not crucial for insulation.

Finally, we checked whether the distance between the enhancers and the Fab-7 insulator is crucial for en-

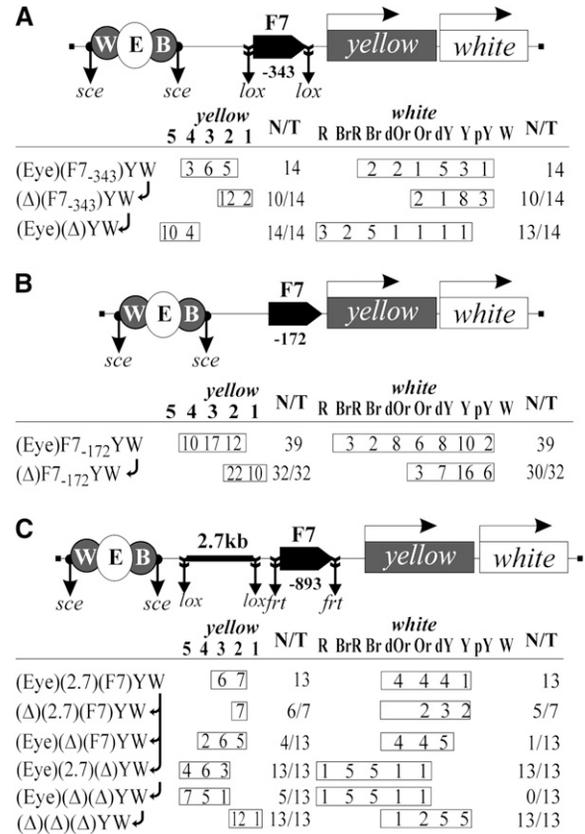


FIGURE 6.—Tests for the insulating activity of Fab-7 located at different distances from the *yellow* promoter or enhancers: (A) (Eyw)(F7<sub>-343</sub>)YW, (B) (Eyw)F7<sub>-172</sub>YW, and (C) (Eyw)(2.7)(F7)YW transgenes and analysis of *yellow* and *white* expression in original transgenic lines and their derivatives obtained by deletion of Fab-7, or the enhancers, or the DNA spacer (2.7 kb) inserted between the enhancers and Fab-7. Other designations are as in Figures 1 and 2.

hancer blocking. In the (Eyw)(2.7)(F7)YW construct, the 2.7-kb spacer flanked by lox sites was inserted between the enhancers and Fab-7 at -893 (Figure 6C). Comparing *yellow* pigmentation in transgenic lines with or without Fab-7 before and after the deletion of the spacer showed that the presence of the 2.7-kb spacer decreased the activity of the *yellow* enhancers but had no influence on the insulating properties of Fab-7.

## DISCUSSION

Previously, the minimal Fab-7 insulator was mapped with the aid of the eye enhancer-*mini-white* gene model (HAGSTROM *et al.* 1996). The test fragments of the Fab-7 insulator were inserted in close proximity to the *white* promoter. As a result, it was found that only the 1.2-kb fragment corresponding to our Fab-7<sup>1,2</sup> (rather than its 0.86-kb subfragment) blocked the eye enhancer in ~50% of transgenic lines. Here, we found that both 0.86- and 1.2-kb fragments insulated the enhancers with similar efficiency when placed at a distance from the *white*

promoter. The simplest explanation of these contradictory results is that the 1.2-kb Fab-7 insulator contains the regulatory element that inhibits the activity of the *white* promoter, which is deleted from the smaller 0.86-kb Fab-7 element. Indeed, our preliminary data show that the 0.4-kb fragment overlapping the proximal part of the 1.2-kb Fab-7 insulator often recruits the transposon in genome regions that negatively influence *white* expression (O. KYRCHANOVA, unpublished data). Interestingly, the 1.2-kb Fab-7 insulator contains separable regions that function at different stages of development (SCHWEINSBERG and SCHEDL 2004). The elements responsible for the insulating activity in embryos and adult flies (the eye enhancer–*white* promoter) are present in the 0.86-kb Fab-7 element. Thus, these results are in agreement with our finding that the 0.86-kb fragment contains all sequences required for blocking the adult enhancers.

As the 0.86-kb Fab-7 insulator inserted close to the *white* but not the *yellow* promoter fails to block the enhancers, we suggest that the Fab-7 insulator stimulates binding of the basic transcription factors to the relatively weak *white* promoter. The 0.86-kb Fab-7 insulator contains nine consensus-binding sites for the GAGA factor (SCHWEINSBERG *et al.* 2004). The GAGA factor functions to antagonize the repressive effects of chromatin by promoting the formation of nucleosome-free regions over promoters and other regulatory elements (LEIBOVITICH *et al.* 2002; LEHMANN 2004). It seems likely that GAGA helps in the binding of proteins to the *white* promoter that helps to overcome the insulating activity of Fab-7. While further study is required to prove this model, it is apparent that the DNA fragment tested for the insulating activity should be placed at a distance from an enhancer and a promoter to avoid effects complicating interpretation of the results.

Recently, we found that relative orientation of the Mcp elements defines the mode of loop formation that either allows or blocks stimulation of the *white* promoter by the eye enhancer (KYRCHANOVA *et al.* 2007). In contrast to previous observations (ZHOU *et al.* 1996; MAJUMDER and CAI 2003), we demonstrated here that the Fab-7 insulators can functionally interact with each other. In contrast to Mcp, however, the relative orientation of Fab-7 does not affect communication between the eye enhancer and the *white* promoter across the pair of Fab-7 insulators. The Mcp insulator located downstream of the *yellow* gene significantly improves the enhancer-blocking activity of the insulator located between the enhancers and promoter of the *yellow* gene (KYRCHANOVA *et al.* 2007). It appears that the interaction between the Mcp insulators results in the formation of a loop that restricts communication between the enhancers located outside the loop and the promoters located inside it. We found that Fab-7 inserted downstream of the *yellow* gene did not contribute to insulation by Fab-7 located between the enhancers and the promoters. If the Fab-7 insulators

interact in all tissues, their presence on both sides of a gene does not ultimately improve the blocking of enhancers located outside the chromatin loop formed due to their interaction.

The role of Fab-7 and other boundary elements in transcriptional regulation of *Abd-B* is as yet uncertain. According to the accepted model, the insulator/boundary element functions as a barrier separating the *iab* domains differing in the status of chromatin (MIHALY *et al.* 1998; SIPOS and GYURKOVICS 2005; MAEDA and KARCH 2006). Recently, CLEARD *et al.* (2006) directly demonstrated the interaction between Fab-7 and the *Abd-B* promoter, concluding that Fab-7 and other boundary elements appear to be involved in regulating long-distance interactions between the *iab* enhancers and the *Abd-B* promoter. It is noteworthy that the interaction of Fab-7 with the promoter was effective in the tissues where *Abd-B* is not expressed, *e.g.*, in the eyes. These results are in agreement with our observation that the Fab-7 insulators functionally interact in supporting the interaction between the enhancers and promoters of the *white* gene. An important task now is to find out whether the Fab-7 insulators are capable of interaction in all tissues and at all developmental stages.

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#### LITERATURE CITED

- BARGES, S., J. MIHALY, M. GALLONI, K. HAGSTROM, M. MULLER *et al.*, 2000 The *Fab-8* boundary defines the distal limit of the *bithorax* complex *iab-7* domain and insulates *iab-7* from initiation elements and a PRE in the adjacent *iab-8* domain. *Development* **127**: 779–790.
- BELOZEROV, V. E., P. MAJUMDER, P. SHEN and H. N. CAI, 2003 A novel boundary element may facilitate independent gene regulation in the *Antennapedia* complex of *Drosophila*. *EMBO J.* **22**: 3113–3121.
- BONDARENKO, V. A., Y. V. LIU, Y. I. JIANG and V. M. STUDITSKY, 2003 Communication over a large distance: Enhancers and insulators. *Biochem. Cell. Biol.* **81**: 241–251.
- BONIFER, C., M. VIDAL, F. GROSVELD and A. E. SIPPEL, 1990 Tissue specific and position independent expression of the complete gene domain for chicken lysozyme in transgenic mice. *EMBO J.* **9**: 2843–2848.
- BOULET, A. M., A. LLOYD and S. SAKONJU, 1991 Molecular definition of the morphogenetic and regulatory functions and the *cis*-regulatory elements of the *Drosophila Abd-B* homeotic gene. *Development* **111**: 393–405.
- BRASSET, E., and C. VAURY, 2005 Insulators are fundamental components of the eukaryotic genomes. *Heredity* **94**: 571–576.
- BURGESS-BEUSSE, B., C. FARRELL, M. GASZNER, M. LITT, V. MUTSKOV *et al.*, 2002 The insulation of genes from external enhancers and silencing chromatin. *Proc. Natl. Acad. Sci. USA* **99**: 16433–16437.
- CAI, H., and M. LEVINE, 1995 Modulation of enhancer–promoter interactions by insulators in the *Drosophila* embryo. *Nature* **376**: 533–536.
- CAI, H. N., and P. SHEN, 2001 Effects of *cis* arrangement of chromatin insulators on enhancer-blocking activity. *Science* **291**: 493–495.
- CAPELSON, M., and V. G. CORCES, 2004 Boundary elements and nuclear organization. *Biol. Cell* **96**: 617–629.
- CELNIKER, S. E., S. SHARMA, D. J. KEELAN and E. B. LEWIS, 1990 The molecular genetics of the *bithorax* complex of *Drosophila*. *cis*-regulation in the Abdominal-B domain. *EMBO J.* **9**: 4227–4286.

- CLEARD, F., Y. MOSHKIN, F. KARCH and R. K. MAEDA, 2006 Probing long-distance regulatory interactions in the *Drosophila melanogaster* *bithorax* complex using Dam identification. *Nat. Genet.* **38**: 931–935.
- COMET, I., E. SAVITSKAYA, B. SCHUETTENGRUBER, N. NEGRE, S. LAVROV *et al.*, 2006 PRE-mediated bypass of two Su(Hw) insulators targets PcG proteins to a downstream promoter. *Dev. Cell* **11**: 1–8.
- CONTE, C., B. DASTUGUE and C. VAURY, 2002 Coupling of enhancer and insulator properties identified in two retrotransposons modulates their mutagenic impact on nearby genes. *Mol. Cell. Biol.* **22**: 1767–1777.
- DORSETT, D., 1999 Distant liaisons: Long range enhancer–promoter interactions in *Drosophila*. *Curr. Opin. Genet. Dev.* **9**: 505–514.
- DUNCAN, I., 1987 The *bithorax* complex. *Annu. Rev. Genet.* **21**: 285–319.
- FRASER, P., 2006 Transcriptional control thrown for a loop. *Curr. Opin. Genet. Dev.* **16**: 490–495.
- GALLONI, M., H. GYURKOVICS, P. SCHEDL and F. KARCH, 1993 The *bluetail* transposon: Evidence for independent *cis*-regulatory domains and domain boundaries in the *bithorax* complex. *EMBO J.* **12**: 1087–1097.
- GASZNER, M., and G. FELSENFELD, 2006 Insulators: Exploiting transcriptional and epigenetic mechanisms. *Nat. Rev. Genet.* **7**: 703–713.
- GEYER, P. K., and V. G. CORCES, 1987 Separate regulatory elements are responsible for the complex pattern of tissue-specific and developmental transcription of the *yellow* locus in *Drosophila melanogaster*. *Genes Dev.* **1**: 996–1004.
- GEYER, P. K., and V. G. CORCES, 1992 DNA position-specific repression of transcription by a *Drosophila* zinc finger protein. *Genes Dev.* **6**: 1865–1873.
- GOLIC, K. G., and S. LINDQUIST, 1989 The FLP recombinase of yeast catalyzes site-specific recombination in the *Drosophila* genome. *Cell* **59**: 499–509.
- GRUZDEVA, N., O. KYRCHANOVA, A. PARSHIKOV, A. KULLYEV and P. GEORGIEV, 2005 The Mcp element from the *bithorax* complex contains an insulator that is capable of pairwise interactions and can facilitate enhancer–promoter communication. *Mol. Cell. Biol.* **25**: 3682–3689.
- GYURKOVICS, H., J. GAUSZ, J. KUMMER and F. KARCH, 1990 A new homeotic mutation in the *Drosophila bithorax* complex removes a boundary separating two domains of regulation. *EMBO J.* **9**: 2579–2585.
- HAGSTROM, K., M. MULLER and P. SCHEDL, 1996 Fab-7 functions as a chromatin domain boundary to ensure proper segment specification by the *Drosophila bithorax* complex. *Genes Dev.* **10**: 3202–3215.
- HOLDRIDGE, C., and D. DORSETT, 1991 Repression of *hsp70* heat shock gene transcription by the suppressor of hairy-wing protein of *Drosophila melanogaster*. *Mol. Cell. Biol.* **11**: 1894–1900.
- ISHII, K., G. ARIB, C. LIN, G. VAN HOUWE and U. K. LAEMMLI, 2002 Chromatin boundaries in budding yeast: The nuclear pore connection. *Cell* **109**: 551–562.
- KARCH, F., B. WEIFFENBACH, M. PEIFER, W. BENDER, I. DUNCAN *et al.*, 1985 The abdominal region of the *bithorax* complex. *Cell* **43**: 81–96.
- KARCH, F., M. GALLONI, L. SIPOS, J. GAUSZ, H. GYURKOVICS *et al.*, 1994 *Mcp* and *Fab-7*: Molecular analysis of putative boundaries of *cis*-regulatory domains in the *bithorax* complex of *Drosophila melanogaster*. *Nucleic Acids Res.* **22**: 3138–3146.
- KARESS, R. E., and G. M. RUBIN, 1984 Analysis of P transposable element functions in *Drosophila*. *Cell* **38**: 135–146.
- KELLUM, R., and P. SCHEDL, 1991 A position-effect assay for boundaries of higher order chromosomal domains. *Cell* **64**: 941–950.
- KELLUM, R., and P. SCHEDL, 1992 A group of scs elements function as domain boundaries in an enhancer-blocking assay. *Mol. Cell. Biol.* **12**: 2424–2431.
- KUHN, E. J., and P. K. GEYER, 2003 Genomic insulators: Connecting properties to mechanism. *Curr. Opin. Cell Biol.* **15**: 259–265.
- KUHN, E. J., M. M. VIERING, K. M. RHODES and P. K. GEYER, 2003 A test of insulator interactions in *Drosophila*. *EMBO J.* **22**: 2463–2471.
- KYRCHANOVA, O., S. TOSHCHAKOV, A. PARSHIKOV and P. GEORGIEV, 2007 Study of the functional interaction between Mcp insulators from the *Drosophila bithorax* complex: effects of insulator pairing on the enhancer–promoter communication. *Mol. Cell. Biol.* **27**: 3035–3043.
- LEHMANN, M., 2004 Anything else but GAGA, a nonhistone protein complex reshapes chromatin structure. *Trends Genet.* **20**: 15–22.
- LEIBOVITICH, B. A., Q. LU, L. R. BENJAMIN, Y. LIU, D. C. GILMOUR *et al.*, 2002 GAGA factor and the TFIID complex collaborate in generating an open chromatin structure at the *Drosophila melanogaster* *hsp26* promoter. *Mol. Cell. Biol.* **22**: 6148–6157.
- LEWIS, E. B., 1978 A gene complex controlling segmentation in *Drosophila*. *Nature* **276**: 565–570.
- LINDSLEY, D. L., and G. G. ZIMM, 1992 *The genome of Drosophila melanogaster*. Academic Press, New York.
- MAEDA, R. K., and F. KARCH, 2006 The ABC of the BX-C: the *bithorax* complex explained. *Development* **133**: 1413–1422.
- MAJUMDER, P., and H. N. CAL, 2003 The functional analysis of insulator interactions in the *Drosophila* embryo. *Proc. Natl. Acad. Sci. USA* **100**: 5223–5228.
- MALLIN, D. R., J. S. MYUNG, J. S. PATTON and P. K. GEYER, 1998 Polycomb group repression is blocked by the *Drosophila* suppressor of Hairy-wing [su(Hw)] insulator. *Genetics* **148**: 331–339.
- MARTIN, M., Y. B. MENG and W. CHIA, 1989 Regulatory elements involved in the tissue-specific expression of the *yellow* gene of *Drosophila*. *Mol. Gen. Genet.* **218**: 118–126.
- MIHALY, J., I. HOGGA, J. GAUSZ, H. GYURKOVICS and F. KARCH, 1997 In situ dissection of the Fab-7 region of the *bithorax* complex into a chromatin domain boundary and a *Polycomb*-response element. *Development* **124**: 1809–1820.
- MIHALY, J., I. HOGGA, S. BARGES, M. GALLONI, R. K. MISHRA *et al.*, 1998 Chromatin domain boundaries in the *bithorax* complex. *Cell. Mol. Life Sci.* **54**: 60–70.
- MURAVYOVA, E., A. GOLOVNI, E. GRACHEVA, A. PARSHIKOV, T. BELENKAYA *et al.*, 2001 Loss of insulator activity by paired Su(Hw) chromatin insulators. *Science* **291**: 495–498.
- MUTSKOV, V. J., C. M. FARRELL, P. A. WADE, A. P. WOLFFE and G. FELSENFELD, 2002 The barrier function of an insulator couples high histone acetylation levels with specific protection of promoter DNA from methylation. *Genes Dev.* **16**: 1540–1554.
- QIAN, S., B. VARJAVAND and V. PIRROTTA, 1992 Molecular analysis of the zeste–white interaction reveals a promoter-proximal element essential for distant enhancer–promoter communication. *Genetics* **131**: 79–90.
- RODIN, S., and P. GEORGIEV, 2005 Handling three regulatory elements in one transgene: Combined use of *cre-lox*, FLP-FRT and *I-SceI* recombination systems. *Biotechniques* **39**: 871–875.
- ROSEMAN, R. R., V. PIRROTTA and P. K. GEYER, 1993 The su(Hw) protein insulates expression of the *Drosophila melanogaster* *white* gene from chromosomal position-effects. *EMBO J.* **12**: 435–442.
- ROSEMAN, R. R., E. A. JOHNSON, C. K. RODESCH, M. BJERKE, R. N. NAGOSHI *et al.*, 1995 A P element containing suppressor of hairy-wing binding regions has novel properties for mutagenesis in *Drosophila melanogaster*. *Genetics* **141**: 1061–1074.
- RUBIN, G. M., and A. C. SPRADLING, 1982 Genetic transformation of *Drosophila* with transposable element vectors. *Science* **218**: 348–353.
- SANCHEZ-HERRERO, E., 1991 Control of the expression of the *bithorax* complex *abdominal-A* and *Abdominal-B* by *cis*-regulatory regions in *Drosophila* embryos. *Development* **111**: 437–448.
- SANCHEZ-HERRERO, E., I. VERNOS, R. MARCO and G. MORATO, 1985 Genetic organization of *Drosophila bithorax* complex. *Nature* **313**: 108–113.
- SAVITSKAYA, E., L. MELNIKOVA, M. KOSTUCHENKO, E. KRAVCHENKO, E. POMERANTSEVA *et al.*, 2006 Study of Long-Distance Functional Interactions between Su(Hw) Insulators that Can Regulate Enhancer–Promoter Communication in *Drosophila melanogaster*. *Mol. Cell. Biol.* **26**: 754–761.
- SCHWEINSBERG, S., and P. SCHEDL, 2004 Developmental modulation of Fab-7 boundary function. *Development* **131**: 4743–4749.
- SCHWEINSBERG, S., K. HAGSTROM, D. GOHL, P. SCHEDL, R. P. KUMAR *et al.*, 2004 The enhancer-blocking activity of the Fab-7 boundary from the *Drosophila bithorax* complex requires GAGA-factor-binding sites. *Genetics* **168**: 1371–1384.
- SCOTT, K. S., and P. K. GEYER, 1995 Effects of the su(Hw) insulator protein on the expression of the divergently transcribed *Drosophila* *yolk* protein genes. *EMBO J.* **14**: 6258–6267.

- SIEGAL, M. L., and D. L. HARTL, 2000 Application of Cre/loxP in *Drosophila*. Site-specific recombination and transgene co-placement. *Methods Mol. Biol.* **136**: 487–495.
- SIGRIST, C. J., and V. PIRROTTA, 1997 Chromatin insulator elements block the silencing of a target gene by the *Drosophila* polycomb response element (PRE) but allow *trans* interactions between PREs on different chromosomes. *Genetics* **147**: 209–221.
- SIPOS, L., and H. GYURKOVICS, 2005 Long-distance interactions between enhancers and promoters. The case of the *Abd-B* domain of the *Drosophila bithorax* complex. *FEBS J.* **272**: 3253–3259.
- SPRADLING, A. C., and G. M. RUBIN, 1982 Transposition of cloned *P* elements into *Drosophila* germ line chromosomes. *Science* **218**: 341–347.
- SUN, F. L., and S. C. ELGIN, 1999 Putting boundaries on silence. *Cell* **99**: 459–462.
- VALENZUELA, L., and R. T. KAMAKAKA, 2006 Chromatin insulators. *Annu. Rev. Genet.* **40**: 107–138.
- WEST, A. G., and P. FRASER, 2005 Remote control of gene transcription. *Hum. Mol. Genet.* **14**: R101–R111.
- ZHOU, J., and M. LEVINE, 1999 A novel *cis*-regulatory element, the PTS, mediates an anti-insulator activity in the *Drosophila* embryo. *Cell* **99**: 567–575.
- ZHOU, J., S. BAROLO, P. SZYMANSKI and M. LEVINE, 1996 The Fab-7 element of the *bithorax* complex attenuates enhancer–promoter interactions in the *Drosophila* embryo. *Genes Dev.* **10**: 3195–3201.
- ZHOU, J., H. ASHE, C. BURKS and M. LEVINE, 1999 Characterization of the transvection mediating region of the *Abdominal-B* locus in *Drosophila*. *Development* **126**: 3057–3065.

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