CMLS, Cell. Mol. Life Sci. 54 (1998) 60–70
1420-682X/98/010060-11 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Chromatin domain boundaries in the Bithorax complex

J. Mihaly[a], I. Hogga[a], S. Barges[a], M. Galloni[b], R. K. Mishra[a], K. Hagstrom[c], M. Müller[c], P. Schedl[c], L. Sipos[d], J. Gausz[d], H. Gyurkovics[d] and F. Karch[a,*]

[a]Department of Zoology and Animal Biology, University of Geneva, CH-1211 Geneva 4 (Switzerland), Fax +41 22 702 64 39
[b]Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle (Washington 98109, USA)
[c]Department of Molecular Biology, Princeton University, Princeton (New Jersey 08544, USA)
[d]Institute of Genetics, Biological Research Center, Hungarian Academy, H-6701 Szeged (Hungary)

Abstract. Eukaryotic chromosomes are thought to be organized into a series of discrete higher-order chromatin domains. This organization is believed to be important not only in the compaction of the chromatin fibre, but also in the utilization of genetic information. Critical to this model are the domain boundaries that delimit and segregate the chromosomes into units of independent gene activity. In *Drosophila*, such domain boundaries have been identified through two different approaches. On the one hand, elements like scs/scs′ and the reiterated binding site for the SU(HW) protein have been characterized through their activity of impeding enhancer-promoter interactions when intercalated between them. Their role of chromatin insulators can protect transgenes from genomic position effects, thereby establishing independent functional domains within the chromosome. On the other hand, domain boundaries of the Bithorax complex (BX-C) like *Fab-7* and *Mcp* have been identified through mutational analysis. *Mcp* and *Fab-7*, however, may represent a specific class of boundary elements; instead of separating adjacent domains that contain separate structural genes, *Mcp* and *Fab-7* delimit adjacent *cis*-regulatory domains, each of which interacts independently with their target promoters. In this article, we review the genetic and molecular characteristics of the domain boundaries of the BX-C. We describe how *Fab-7* functions to confine activating as well as repressive signals to the flanking regulatory domains. Although the mechanisms by which *Fab-7* works as a domain boundary remain an open issue, we provide preliminary evidence that *Fab-7* is not a mere insulator like scs or the reiterated binding site for the SU(HW) protein.

Key words. Bithorax complex; chromatin domain boundary; insulator; *Fab-7*; *Mcp*; scs; scs′; suppressor of Hairy-wing.

Introduction

Many eukaryotic genes are regulated by extensive and complex regulatory regions that often act from a considerable distance. This raises the question of how particular enhancer elements find their proper target promoters. What are the mechanisms that prevent inappropriate interactions with adjacent genes? One attractive hypothesis which currently dominates the field is the notion that chromatin is subdivided into largely independent functional units called chromatin domains. Regulatory elements in one domain are postulated to exert their positive or negative effects only on promoters located within the same domain. A corollary to this is the requirement of the so-called domain boundary elements that are supposed to mark, separate and insulate adjacent domains (for reviews, see

* Corresponding author.

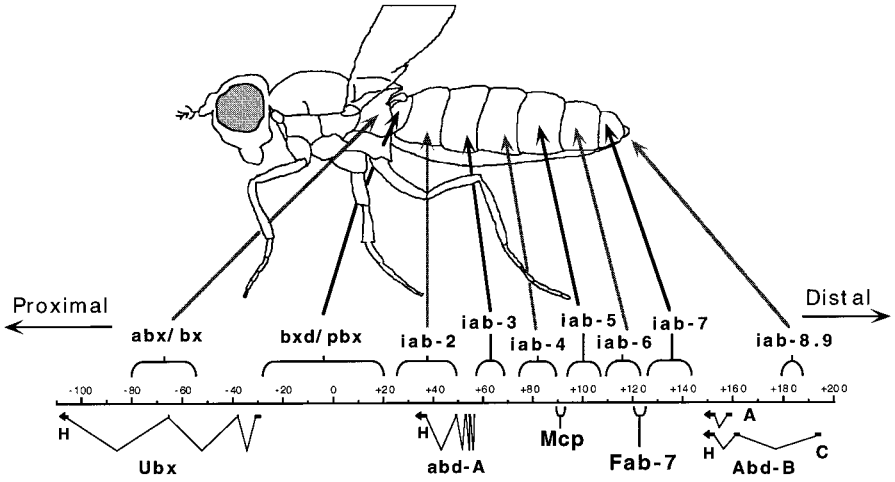

Figure 1. Molecular genetics of the BX-C. The thin horizontal line represents the 300 kb of genomic DNA marked off in kilobases. Proximal points toward the centromere; distal indicates the direction of the telomere. The *Ubx*, *abd-A* and *Abd-B* transcription units are drawn below the genomic DNA line, the H indicating the positions of the homeoboxes. The 5′–3′ polarity is from right to left. Horizontal brackets above the genomic DNA lines represent the extent of the parasegment-specific *cis*-regulatory domains. The arrows point toward the parasegments of a fly in which the *cis*-regulatory domains are the most active.

refs 1–3). Significant progress toward the definition of boundary elements was achieved in *Drosophila* through the discovery of two special chromatin structures (scs and scs′) that flank the *hsp70* genes at 87A7 [4–8], and through the finding of reiterated binding sites for the suppressor of Hairy wing [su(HW)] protein in the *gypsy* retrotransposon [9, 10]. The scs and scs′ elements are localized at the puff boundaries of the 87A7 heat-shock locus and may play a role in limiting the spread of open and closed chromatin states. In transgenic flies, the scs/scs′ boundary element (as well as the reiterated binding sites of the [su(HW)] protein) is capable of insulating a reporter gene at most sites of insertion from the effects of the local chromatin environment. Furthermore, as expected from a generic domain boundary, these elements can prevent an enhancer from activating transcription when interposed between the enhancer and the promoter.

Although these elements have properties that fulfill the requirements of a domain boundary, no mutation has been recovered so far which would suggest that scs/scs′ or the reiterated binding sites for the SU(HW) protein do play the role of boundary elements in their original chromosomal context. A prediction of a domain boundary element would be the fusion of two adjacent independent domains into one single functional unit. Two such mutations, *Mcp* and *Fab-7*, exist in the Bithorax complex (BX-C) [11–14]. *Mcp* and *Fab-7*, however, may represent a specific class of boundary elements; instead of separating adjacent domains that contain separate structural genes, *Mcp* and *Fab-7* delimit adjacent *cis*-regulatory domains, each of which interacts independently with its target promoters. The purpose of this article is to review the current knowledge of the domain boundaries in the BX-C and then to compare them with SCS/SCS′ and the reiterated binding site for the SU(HW) protein.

Molecular genetics of the BX-C

The BX-C contains three homeotic genes, *Ubx*, *abd-A* and *Abd-B*. These three genes are responsible for the identities of parasegments 5–14 (PS5-14), which form the posterior half of the thorax and the abdomen of the fly [15–17]. Precise parasegmental expression patterns of these homeotic genes are crucial for generating a normal body plan, and misregulation of these genes results in dramatic transformation of one body segment into another. The PS-specific expression patterns of *Ubx*, *abd-A* and *Abd-B* are generated by a complex *cis*-regulatory region that spreads over 300 kb of DNA (fig. 1). Genetic and molecular analysis has shown that this large regulatory region is subdivided into nine PS-specific *cis*-regulatory subregions (*abx/bx*, *bxd/pbx*, *iab-2*, *iab-3*, *iab-4*, *iab-5*, *iab-6*, *iab-7* and *iab-8,9*). The *abx/bx* and *bxd/pbx cis*-regulatory subregions are responsible for proper Ubx expression in PS5 and PS6, respectively [18]. Similarly, the *iab-2*, *iab-3* and *iab-4 cis*-regulatory subregions direct *abd-A* expression in

PS7, PS8 and PS9 [19]. Finally, the *iab-5* through the *iab-8,9* subregions direct *Abd-B* expression in PS10-14. [20, 21]. As shown in figure 1, the proximal-distal order of the *cis*-regulatory subregions along the chromosome corresponds to the anteroposterior order of the parasegments they specify (for reviews, see refs 22, 23).

BX-C gene regulation can be divided into two phases: initiation and maintenance. During the early phases of embryogenesis, when segmental identity is initially selected, the PS-specific *cis*-regulatory subregions are the targets of the gap and pair-rules gene products [24–27]. These gap and pair-rule proteins activate the *cis*-regulatory subregions in successively more posterior parasegments. For example, the set of regulatory proteins present in PS11 activates *iab-6* in this parasegment, but not *iab-7*, whereas the regulatory proteins present in PS12 activate *iab-7*. The gap and pair-rule gene products are only transiently present during early development. The fact that homeotic genes are expressed throughout development implies the existence of a mechanism that maintains the activity state of each of the *cis*-regulatory subregions. This maintenance system requires the Polycomb-group (Pc-G) and trithorax-group (trx-G) genes [28–30]. While the products of the Pc-G function as negative regulators, the products of the trx-G act as positive regulators. The products of the Pc-G exert their regulatory effects by interacting with specific elements in each of the *cis*-regulatory domains called *Polycomb*-response elements (PREs) [30–37]. There may be equivalent or overlapping *trx* response elements (TREs) for the trx-G proteins [34]. Though their precise mode of action is unknown, the products of the Pc-G and trx-G genes are thought to stabilize the expression patterns in each parasegment by imprinting inactive or active chromatin conformation of the PS-specific *cis*-regulatory subregions (for reviews, see refs 29, 38, 39).

Loss-of-function mutations in any one of the nine PS-specific *cis*-regulatory subregions transform the corresponding parasegment into a copy of the parasegment immediately anterior. For example, *iab-7* is responsible for the specific expression pattern of the short *Abd-B* transcription unit in PS12. A mutation that removes much of *iab-7* (*iab-7*^{Sz}; fig. 2A) results in the transformation of PS12 into PS11. Consistent with this, the normal *Abd-B* expression in PS12 is replaced by a PS11-like pattern, which is generated by *iab-6* [13]. This is illustrated by the *Abd-B* patterns of expression in the embryonic central nervous system (CNS) shown in figure 3. In wild-type CNS (middle panel), *Abd-B* expression is very low in

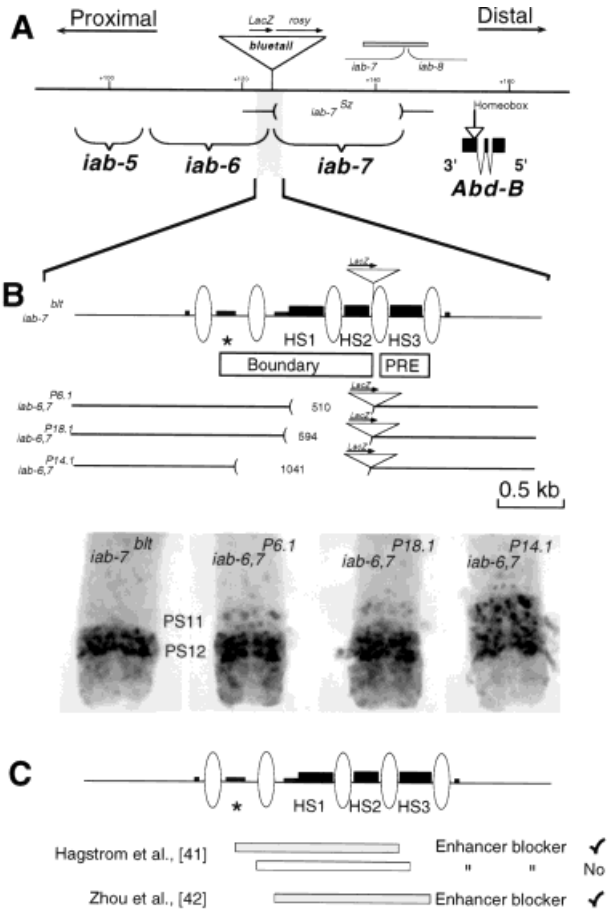

Figure 2. (*A*) Distal part of the BX-C. The thin horizontal line represents the genomic DNA of the distal part of the BX-C marked off in kilobases. Proximal points toward centromere; distal indicates the direction of the telomere. The *Abd-B* homeobox is indicated by a vertical arrow. For simplicity, only the class A *Abd-B* transcript which is required for morphogenic functions of PS10 to PS13 is shown below the DNA line [20, 55–57]. The horizontal brackets below the genomic DNA indicate the extent of the *iab-5*, *iab-6 and iab-7 cis*-regulatory domains which regulate the class A *Abd-B* transcript in PS10, PS11 and PS12, respectively. The proximal and distal deficiency endpoints of the *iab-7*^{Sz} deletion are also indicated below the DNA line. The position of the P[lacZ] transposon in the *blt* line (drawn at the same scale) is indicated above the genomic DNA. Also shown above the genomic DNA is the position of the 8-kb DNA fragment that contains the putative domain boundary that separates *iab-7* from *iab-8 cis*-regulatory domains (see other domain boundary in the BX-C section). The gray rectangle under the genomic DNA indicates the position of the *Fab-7* region which is magnified in B and C. (*B*) Chromatin structure of the *Fab-7* domain boundary and *iab-7*PRE. The thin line represents the DNA of the *Fab-7* region drawn at the scale indicated. The nuclease hypersensitive regions (HS) are shown by black rectangles (while thick black rectangles indicate strong hypersensitive sites, weak sites are shown by thin black rectangles). HS nomenclature is according to Karch et al. ([12]; asterisk indicates the weak hypersensitive site). The ovals indicate putative nucleosome core particles. The triangle at the distal edge of HS2 indicates the position of the *blt* transposon. Below the molecular map of *Fab-7* are shown the extents of the three deficiency derivatives in which the *blt* transposon remains in place. The lacZ expression pattern in the embryonic CNS is shown below. While lacZ expression is restricted to PS12 in the *iab-7*^{blt} line, lacZ expression is also visible in PS11 in the three derivatives *iab-6,7*$^{P6.1}$, *iab-6,7*$^{P18.1}$ and *iab-6,7*$^{P14.1}$. (*C*) Extents of the DNA fragments tested in the enhancer-blocker assay. The chromatin structure of the *Fab-7* region is shown as in (*B*). The extent of three fragments that have been tested in enhancer-blocker experiments by Hagstrom et al. [41] and Zhou et al. [42] are shown below. Fragments shaded in gray block interactions when inserted between an enhancer and a promoter; the fragment not shaded has no blocking activity.

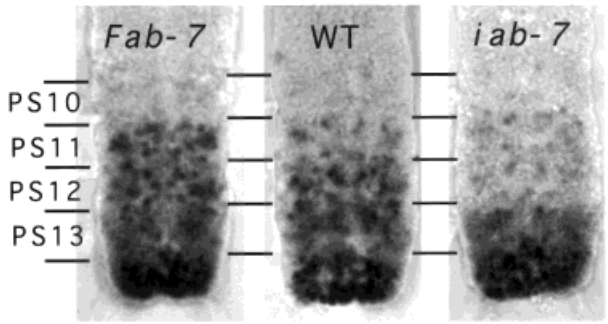

Figure 3. *Abd-B* expression in the CNS of *Fab-7*^{1} and *iab-7*^{Sz} mutant embryos. Wild-type *Fab-7*^{1} and *iab-7*^{Sz} embryos were stained with anti-Abd-B antibodies, and the CNS was dissected from embryos at the stage of CNS shortening. (*A*) *Fab-7*^{1}; (*B*) wild type; (*C*) *iab-7*^{Sz}. The position of PS11 and 12 is indicated.

PS10, higher in PS11, still higher in PS12 and reaches a very high level in PS13 and PS14. In the right panel, the CNS of an embryo lacking the *iab-7* regulatory subregion, the PS12-specific pattern of *Abd-B* expression is replaced by the PS11 pattern.

Regulatory domains and domain boundaries in the BX-C

Many observations suggest that the PS-specific *cis*-regulatory subregions are organized into functionally independent domains [22]. For example, removal of the whole *iab-7 cis*-regulatory subregion in the *iab-7*^{Sz} mutation (discussed above) affects only PS12 identity and leaves the *iab-6 cis*-regulatory subregion fully functional. Organization of the PS-specific *cis*-regulatory subregions into domains is best depicted by the expression patterns of 'enhancer trap' transposons integrated in different *cis*-regulatory subregions of the complex [13, 40]. These enhancer traps are subject to regulatory elements located within the same domain, but are insensitive to regulatory elements in adjacent domains (see also the *bluetail* transposon, below). Implicit in the idea of organization of the *cis*-regulatory elements into PS-specific autonomous domains is the existence of domain boundaries. Two such boundaries have been revealed by the *Mcp* and *Fab-7* mutations [11, 12]. *Mcp* is located between the *iab-4* and *iab-5 cis*-regulatory subregions, while *Fab-7* is located between *iab-6* and *iab-7* (figs 1 and 2A). Unlike loss-of-function mutations in the *cis*-regulatory subregions, which show a transformation of the affected parasegment into the parasegment immediately anterior, deletions that remove either of the boundary elements have an opposite gain-of-function phenotype; they transform the affected parasegment into a copy of the parasegment immediately posterior. For example, *Fab-7*^{1} deletion results in the development of the sixth abdominal segment (A6, which corresponds to PS11) into a copy of A7 (PS12). The transformation appears to be due to the inappropriate activation of the *iab-7 cis*-regulatory subregion in PS11, where *Abd-B* is normally controlled by *iab-6*. Consistent with this, in *Fab-7*^{1} mutant embryos, the pattern of *Abd-B* protein expression in the CNS of PS11 is identical to that found in PS12 (fig. 3) [13].

Genetics of *Fab-7*

The original *Fab-7*^{1} mutation is due to a 4.3-kb deletion that maps between *iab-6* and *iab-7*. There are a few alternative hypotheses to explain how a deletion in a regulatory region can result in a dominant gain-of-function phenotype. Perhaps the simplest is to assume that the deletion removes binding sites for a repressor or silencer that normally keep *iab-7* inactive in PS anterior to PS12 (perhaps PREs, see also below). However, for the reasons detailed below, we do not favour this hypothesis. A classical approach to gain more insights into the mechanisms underlying a dominant mutation consists of inducing and mapping second-site mutations that revert the dominant phenotype. This approach has provided much valuable information and remains perhaps the best argument supporting the proposal that the *Fab-7* deletion removes a domain boundary between the *iab-6* and *iab-7 cis*-regulatory domains [11]. In one such experiment, *Fab-7*^{1} homozygous males were treated with X rays and crossed to wild-type females. Nearly all the progeny of this cross are heterozygotes (*Fab-7*^{1}/+) and should show the dominant transformation of PS11/A6 into PS12/A7. If the X-ray treatment hits a region necessary for the *Fab-7* phenotype, the fly will be easily recognizable, because of the wild-type appearance of PS11/A6. Figure 4 summarizes the three classes of revertants that were recovered following this scheme. The top row represents the distal part of the wild-type BX-C (WT) and a schematic representation of *Abd-B* expression in the embryonic CNS. The second row represents the *Fab-7* chromosome and the corresponding *Abd-B* expression in the CNS (as already mentioned in figure 3, the PS11 pattern is replaced by the PS12 pattern). The first class of revertants is represented in the third row; they carry a mutation in the *Abd-B* structural gene. This result suggested that the *Fab-7* mutation affects *Abd-B* regulation, an observation which was confirmed when antibodies directed against ABD-B became available (fig. 3). The second class of revertants carry chromosomal rearrangements that disrupt the *iab-7* domain, so that the *Fab-7* deletion as well as *iab-6* and *iab-5* are no longer in *cis* with the *Abd-B* gene. Such rearrangements cause the loss of *Abd-B* expression in PS10 to PS12, and in homozygotes these parasegments are transformed into PS9(A4) (see also Celniker et al., 1990). This class of revertants also

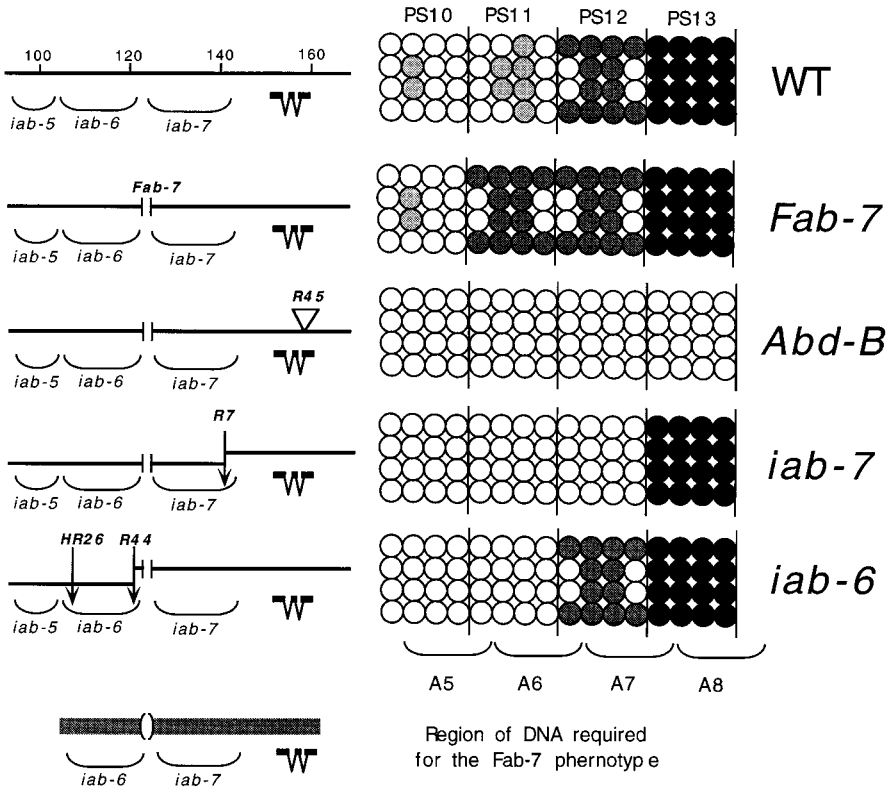

Figure 4. Three classes of *Fab-7* revertants. The first row shows the distal part of the BX-C as in figure 2A and a schematic representation of *Abd-B* expression in PS10 to PS13. In the second row the 4.3-kb deletion that occurred in the original *Fab-7*^{1} deletion is indicated, as well as the resulting pattern of *Abd-B* expression. The third row shows the *Abd-B* class of revertants of *Fab-7*^{1}. In the revertant shown (R45), an insertion of 5 kb of foreign DNA occurred within the *Abd-B* transcription unit. The *iab-7* class of *Fab-7*^{1} revertants is shown in the fourth row. In this case, a chromosomal rearrangement break disrupts continuity of the *iab-7* domain. Since all the proximal regulatory domains are separated from the *Abd-B* transcription unit, *Abd-B* expression is lost in PS10 to PS12. The *iab-6* class of revertants is shown in the fifth row. Two chromosomal rearrangement breaks are indicated. Both disrupt the *iab-6* domain and result in the loss of *Abd-B* expression in PS10 and PS11. Note that PS12 *Abd-B* expression is normal in PS12. The last row indicates the region of DNA that has to be intact to obtain the *Fab-7* dominant gain-of-function phenotype.

confirm that in order to obtain the dominant *Fab-7* phenotype, *iab-7* and the *Fab-7* deletion must be in *cis* with the *Abd-B* gene. The third class of revertants disrupt the *iab-6* domain indicating that the effect of *Fab-7* depends upon the presence of *iab-6* in *cis*. This finding is critical, because it allows us to rule out the hypothesis that the *Fab-7* region contains binding sites for factors that negatively regulate *iab-7* in PS11. Indeed, if this hypothesis were true, we would not expect to revert *Fab-7* phenotype by inactivating *iab-6*. Revertants mapping in *iab-5* or further to the left in *iab-4* were not recovered, indicating that *iab-6* functions autonomously with respect to the *Fab-7* phenotype. Moreover, the fact that, in the *iab-6* revertants, *Abd-B* expression appears normal in PS12 indicates that *Fab-7*^{1} deletion does not remove sequences essential for *iab-7* activity. Based on this reversion experiment, we concluded that in PS11 of *Fab-7*^{1} flies, the *iab-6* and *iab-7* domains are fused into a single functional unit with mixed characteristics: parasegment specificity is provided by *iab-6*, and parasegment identity is provided by *iab-7*. These findings have led us to propose that *Fab-7* corresponds to a domain boundary between the *iab-6* and *iab-7* *cis*-regulatory domains. When *Fab-7* is deleted, adventitious interactions between the *iab-6* and *iab-7* domains become possible.

Another, somewhat more sophisticated model was proposed suggesting that the *Fab-7* DNA segment contains a complex array of binding sites for multiple negative and positive regulatory factors that act during the initiation phase of activation of the PS-specific regulatory domains, like the gap and pair-rule gene products *knirps* and *evenskipped* [33]. The removal of these key regulatory targets by *Fab-7* deletions is thought to shift the balance between negative and positive regulatory signals, leading to the inappropriate activation of *Abd-B*. Once again, this model does not explain why mutations in *iab-6* revert the *Fab-7* phenotype. In addition, this model is not easily reconciled with either the phenotypic transformations or the patterns of *Abd-B* expression that are actually observed in the affected parasegments. Thus, it is difficult to understand why eliminating key

regulatory targets should have no effect on PS12, while it has such a profound effect in the more anterior parasegment PS11. This model also fails to explain why the *Abd-B* expression pattern should precisely mimic the expression pattern in the immediately posterior parasegments (PS12). Rather, one would expect that deletion of key regulatory elements, altering the delicate balance between positive and negative signals, should result in entirely novel patterns of *Abd-B* expression in the affected parasegments. Finally, this model does not account for the fact that the temporal pattern of *Abd-B* activation in PS11 and PS12 in *Fab-7* embryos follows the same course as in wild-type, in PS12 first, then PS11 [13]. By contrast, shifting the balance between positive and negative regulatory elements would be expected to dramatically alter the activation time course in the affected parasegment.

Molecular genetics of *Fab-7*

The *bluetail* transposon

A P-element transposon carrying a *Ubx-lacZ* reporter gene was recovered close to the *Fab-7* boundary, at the proximal edge of the *iab-7 cis*-regulatory region (the *bluetail* transposon; [13]). As visible in figure 2A, the *lacZ* reporter gene is equidistant from both *iab-6* and *iab-7* and could, in principle, be regulated by both *cis*-regulatory domains. Yet, lacZ expression is detected only from PS12, indicating that the *lacZ* gene is subject to the control of the *iab-7 cis*-regulatory domain alone (figure 2B). This finding led to the prediction that the *Fab-7* domain boundary resides on the left of the insertion site of the *bluetail* transposon.

The *bluetail* transposon allowed us to induce many deletion derivatives. Analysis of these derivatives confirmed that the domain boundary lies on the left of the insertion site. This is best illustrated in $iab\text{-}6,7^{P6.1}$ $iab\text{-}6,7^{P18.1}$ and $iab\text{-}6,7^{P14.1}$, in which deletion of 515, 594 and 1041 bp, respectively, occurred on the proximal side of the transposon without excision of the *blt* element (fig. 2B) [14]. The pattern of expression of lacZ observed in these lines is very instructive. As shown in figure 2B, lacZ expression in the CNS of the original *blt* line is restricted to PS12 because the *iab-7 cis*-regulatory region alone is acting on the reporter gene [13]. In $iab\text{-}6,7^{P6.1}$, $iab\text{-}6,7^{P18.1}$ and $iab\text{-}6,7^{P14.1}$, the anterior border of lacZ expression is shifted in PS11, indicating that the reporter gene can also be activated by the *iab-6 cis*-regulatory subregion. In the line carrying the larger deletion ($iab\text{-}6,7^{P14.1}$), the pattern of lacZ expression is similar in PS11 and PS12, implying that all constraints have been removed, allowing the *Ubx* promoter of the reporter gene to be equally regulated by both the *iab-6* and *iab-7 cis*-regulatory domains. In the smaller deletions, however, some residual activity of the boundary remains as revealed by the pattern of lacZ expression in PS11, which is much weaker than in PS12.

A PRE adjoins the *Fab-7* domain boundary

Three classes of deletions in which the *blt* transposon has been excised completely were recovered. Analysis of these deficiencies confirmed that the *Fab-7* boundary lies to the left of the *blt* transposon and revealed the presence of a PRE just to the right of it. For reasons that will become more clear below, this PRE belongs to the *iab-7* domain and is called *iab-7*PRE. Class I deletions remove both the boundary and the *iab-7* PRE. These deficiencies appear to correspond to the $Fab\text{-}7^{1}$ mutation described above in which *iab-7* is ectopically activated in all cells of PS11. Class II deletions remove the boundary alone. These deletions cause a complex gain- and loss-of-function phenotype in PS11, which reveals a new aspect of the *Fab-7* boundary function. In some groups of cells, both *iab-6* and *iab-7* are active (like in $Fab\text{-}7^{1}$), while in others both *iab-6* and *iab-7* are inactive [14]. Thus, the *Fab-7* domain boundary has two activities in PS11: first, it constrains activating signals to the *iab-6* domain, and second the boundary confines the repressive state (mediated by the Pc-G repressive complex, see below) to the *iab-7* domain.

Class III deletions remove DNA on the right of the *blt* transposon. These deletions have no phenotypes as hetero- or homozygotes, indicating that they do not affect the boundary function. The fact that these deficiencies uncover the *iab-7* PRE is in part suggested by the comparisons of the *Fab-7* phenotypes in class I and II deletions. As mentioned above, deletion of the boundary alone can lead, occasionally, to *iab-6* inactivation in groups of cells of PS11 (where *iab-6* should normally be active). Inactivation of regulatory domains in the BX-C is usually mediated by PREs, which provide sites for the assembly of a repressive complex of Pc-G proteins. In agreement with this, *iab-6* inactivation in PS11 of class II deletions is suppressed in Pc-G mutant background. Moreover, *iab-6* inactivation in boundary deletions originates from *iab-7*, because we do not observe it in larger *Fab-7* boundary deletions which extend to the right of the *blt* transposon, toward *iab-7*. This places the PRE responsible for *iab-6* inactivation just distal to the *blt* transposon, in the region uncovered by the class I deletions [14].

The conclusions we have drawn from this in situ analysis of the *Fab-7* region are supported by the work of Hagstrom et al. [41]. These authors used conventional 'enhancer-blocking' assays (in which putative boundary elements are interposed between an enhancer and a promoter in a transgene reporter construct) to demonstrate that sequences from the *Fab-7* region have boundary function. In these experiments, the sequences required for blocking activity correspond (within 62 bp)

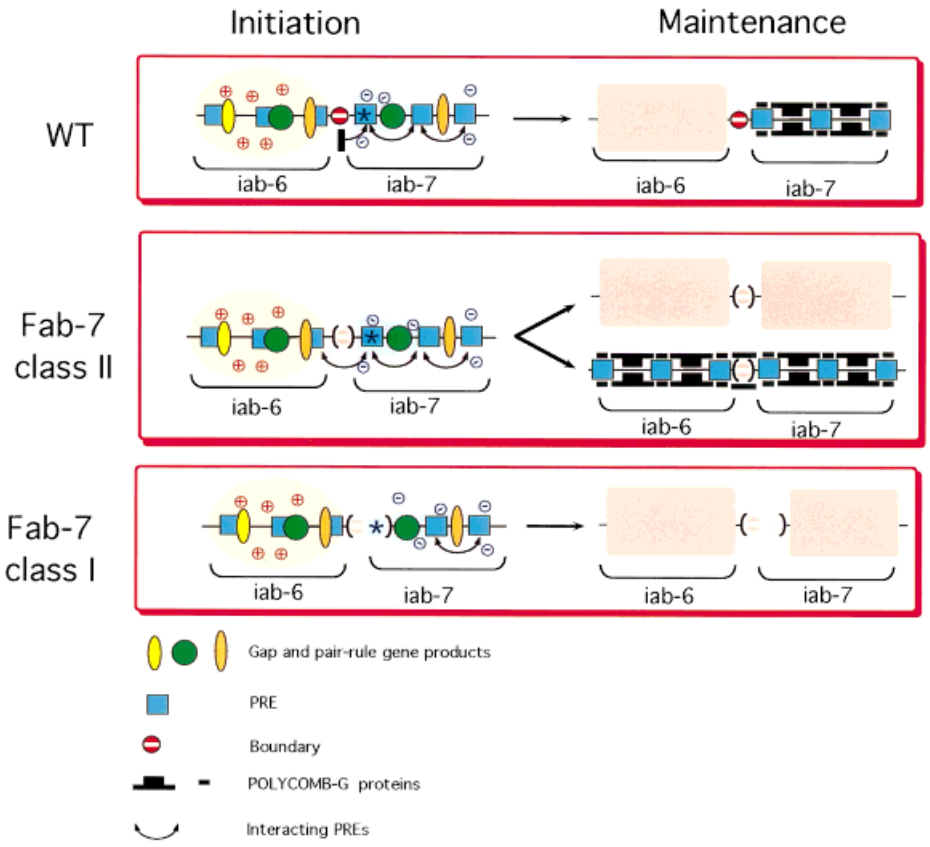

Figure 5. Schematic representation of the function of the *Fab-7* domain boundary in insulating *iab-6* and *iab-7*. (Reprinted with permission from: Mihaly J., Hogga I., Gausz J., Gyurkovics H. and Karch F. (1997) In situ dissection of the *Fab-7* region of the bithorax complex into a chromatin domain boundary and a *polycomb*-response element. Development **124:** 1809–1820, © 1997 Company of Biologists.) See text.

to the sequence which when deleted in the $iab\text{-}6,7^{P14.1}$ line abolishes completely boundary activity (fig. 2C). This is also in almost complete agreement (see below) with similar experiments performed by Zhou et al. [42].

Hagstrom et al. [41, 43] also brought further evidence of the presence of the *iab-7* PRE. They showed that a DNA fragment covering class III deletions is able to induce pairing sensitive repression of a *mini-white* reporter construct, a property which is characteristic of other well-documented PREs [38, 44, 45]. Fragments spanning the boundary do not show any silencing activity.

Chromatin structure of the *Fab-7* region

Like other chromatin domain boundaries [4, 5, 46], the *Fab-7* region is organized into a special chromatin structure that contains four discrete nuclease hypersensitive sites. From the staining pattern of lacZ in PS11 given by the three deletion derivatives that retain the *blt* transposon, it appears that HS1, HS2 and at least part of the upstream minor hypersensitive site HS* (fig. 2B) are required for boundary function. This is in perfect agreement with the enhancer-blocking experiments of Hagstrom et al. [41], which show that the minor hypersensitive site HS* is required for blocking activity (significantly, a fragment that does not cover the minor HS* loses the enhancer-blocking activity; fig. 2C). In this respect, there is a slight discrepancy with the results of Zhou et al. [42], because their fragment, which shows enhancer-blocking activity, does not contain the minor HS*. Inasmuch as their element extends further to the right into HS3, it is possible that these sequences can complement the lack of the upstream minor HS site.

Role of the *Fab-7* boundary

Our results indicate that the *Fab-7* boundary plays a critical role in ensuring the functional autonomy of the *iab-6* and *iab-7* *cis*-regulatory domains (fig. 5). Early in development, when segmental identity is initially selected, the combination of gap and pair-rule gene pro-

ducts expressed in PS11 (shown in yellow, green and orange) results in the activation of the *iab-6* domain (represented by + on a pink background). The *iab-7* domain, however, remains inactive (represented by − in a bluish background). One of the functions of the boundary element is to prevent spreading of the active sate of *iab-6* into *iab-7*. Because *iab-7* is inactive, the PREs (shown by blue rectangles) are accessible to the Pc-G silencing complex, which will then assemble on the *iab-7* domain (shown by black arrows). Another function of the boundary element is to prevent the *iab-7* PRE (shown by a star in a blue rectangle) from interacting with adjacent PREs in *iab-6*. Note that the number and position of PREs drawn in *iab-6* and *iab-7* are arbitrary except for the *iab-7* PRE immediately distal to the boundary. In the maintenance phase, after gap and pair-rule gene products have decayed, the active state of *iab-6* (shown by a reddish rectangle) is sustained by the activity of the products of the trx-G. Meanwhile, the *iab-7* domain is permanently repressed by the Pc-G silencing complex that has assembled on the PREs. The *Fab-7* boundary (the one-way sign) is responsible for keeping the limit between active and inactive states.

In *Fab-7* class II deletions, removal of the boundary alone (shown by brackets) allows activating as well as repressing signals to travel between *iab-6* and *iab-7*. On one hand, the active state of *iab-6* can invade the *iab-7* domain leading to ectopic activation of *iab-7* in PS11. On the other hand, we envision that the most proximal PRE of *iab-7* (the *iab-7* PRE) can interact with the most distal PRE of *iab-6*, leading to inactivation of *iab-6* by the Pc-G silencing complex. Thus, cells in PS11 have either both *iab-6* and *iab-7* active, or both repressed.

In *Fab-7* class I deletions, the boundary as well as the *iab-7* PRE are deleted (shown by brackets). The fact that *iab-7* is always ectopically activated in class I alleles can be explained by the assumption that the lack of a PRE in *iab-7* weakens the assembly of the Pc-G silencing complex on *iab-7*, allowing the active state of *iab-6* to invade *iab-7*. It is possible that the remaining PREs in *iab-7* are too remote to interact with PREs in *iab-6*.

Identification of factors that bind to *Fab-7*

In order to gain insights into the molecular organization of the *Fab-7* domain boundary, we have decided to identify proteins that might interact with it. Our strategy is to subdivide the region spanning the nuclease hypersensitive sites HS1, HS2 as well as the minor site HS* into overlapping fragments and to use them as probes to look for interacting proteins in embryonic nuclear extracts. As some of the sequences recognized by these putative factors may be conserved through evolution, we have also cloned and sequenced the *Fab-7* region from *D. virilis*. So far, we have identified two relatively high abundance proteins that bind to the *Fab-7* domain boundary in a sequence-specific manner. Interestingly these two proteins appear to bind at multiple sites, and the sequences recognized by these proteins are conserved in the *D. virilis* sequence.

Because of the presence of many binding sites in the nuclease-hypersensitive regions HS1 and HS3, the GAGA factor is likely to play a role into the organization of the *Fab-7* region. GAGA was originally isolated as a transcription factor [47]. Further studies on the *hsp-26 and hsp-70* promoters have shown that GAGA does not stimulate transcription in vitro like a standard transcription factor but rather enhances transcription by generating nucleosome free regions at promoter regions [48, 49] (for a review, see ref. 50). In this respect, it is tempting to speculate that the presence of many GAGA-binding sites in the *Fab-7* region is at the origin of the chromatin structure of the boundary element and of the *iab-7* PRE. In agreement with this possibility, Hagstrom et al. [43] have shown that the pairing-sensitive repression mediated by the *iab-7* PRE spanning HS3 is suppressed in mutants of *Trithorax-like*, the gene which encodes GAGA [51].

The *Mcp* domain boundary

The *Mcp* domain boundary is identified by three overlapping deficiencies that map between *iab-4* and *iab-5*. They all result in the same dominant gain-of-function phenotype in which PS9/A4 is transformed into PS10/A5. The dominant gain-of-function *Mcp* phenotype appears to be due to the inappropriate activation of the *iab-5 cis*-regulatory domain in PS9, where normally only *iab-4* is active. This is supported by the fact that induction of second-site mutations in *iab-5* reverts the dominant gain-of-function phenotype [52]. Also consistent with this observation, *Abd-B*, which is not normally transcribed in PS9, is expressed in PS9 in a PS10-like pattern in *Mcp* embryos [20, 21]. The second-site reversion analysis also revealed that the dominant gain-of-function *Mcp* phenotype can be reverted by mutations in *iab-4* [52]. As in the case of *Fab-7*, these results argue strongly in favour of the interpretation that a domain boundary between the *iab-4* and *iab-5 cis*-regulatory domains is deleted in *Mcp* mutations.

The overlap between the three deletions associated with *Mcp* phenotype defines a DNA segment of ~0.4 kb. These essential sequences closely coincide with a single major nuclease hypersensitive region in chromatin (fig. 6). Attempts to further refine the *Mcp* domain boundary activity have been performed in ectopic constructs with the *mini-white* gene as reporter. All these experiments have shown that the region spanning the major nuclease-hypersensitive site contains a PRE which causes strong pairing-sensitive repression of the

white mini-gene (M. Müller and P. Schedl, unpublished). The silencing activity of the PRE on mini-white expression makes it difficult to determine whether the element behaves as an enhancer blocker. So far, constructs with a *lacZ* reporter gene which would allow testing for enhancer blocking during early embryogenesis have not been attempted.

Other domain boundaries in the BX-C

We would like to propose a model for the organization of BX-C chromatin in which each of the PS-specific domains of regulation is flanked by domain boundary elements. However, only *Mcp* and *Fab-7* have been identified so far. *Mcp* and *Fab-7* mutations affect the identities of abdominal segments that are easy to recognize in adult males. Since the remaining abdominal segments are very much alike, mutations in additional putative boundaries are probably too subtle to be recognized in genetic screens. We have, however, collected indirect evidence suggesting the existence of a domain boundary that separates *iab-7* from *iab-8 cis*-regulatory domains.

To gain insight into the organization of the *iab-7 cis*-regulatory region, we cloned a fragment spanning *iab-7* in front of a *Ubx-lacZ* reporter gene and introduced these constructs into flies by P-mediated germ line transformation. A fragment of ~8 kb gave us unexpected results. When the proximal side of the fragment was next to the *Ubx* promoter, we observed weak expression of β-galactosidase in PS12 during early development. This result identified a PS12-specific enhancer and was anticipated from a fragment derived from *iab-7* which is normally active in PS12. To our surprise, we observed a different pattern of expression when the fragment was cloned in the opposite orientation: β-galactosidase was expressed in PS13 and PS14 during early development, and this pattern was maintained throughout embryogenesis. Thus, it appears that the 8-kb fragment can be separated into two autonomous regulatory regions: a proximal region specific for PS12 and a distal region specific for PS13. The proximo-distal arrangement of the two elements on the fragment corresponds to the order seen in the context of the BX-C. While the proximal part is toward *iab-7*, the PS12-specific regulatory domain, the distal part of the fragment is toward *iab-8*, the PS13-specific regulatory domain. This result suggests the existence of a boundary element in between (*Fab-8*), allowing autonomy of the two regulatory domains. Significantly, two major nuclease-hypersensitive sites are found in the middle of the 8-kb fragment. In order to determine if they correspond to the putative *Fab-8* boundary, we have deleted from the construct a fragment of 2 kb that spans the two hypersensitive sites. Preliminary results indicate that the autonomy of the PS12- and -13-specific regulatory elements is lost. It seems also significant that a fragment that spans part of the two hypersensitive sites behaves as an enhancer blocker (M. Müller and P. Schedl, unpublished). We recently received from A. Spradling's laboratory an enhancer trap line with a *lacZ* reporter gene inserted near the first nuclease-hypersensitive site. The PS12-specific β-galactosidase expression pattern indicates that the element belongs to the *iab-7* domain. We are in the process of generating deletions within the context of the BX-C to address directly whether the element we have identified corresponds to the *Fab-8* domain boundary.

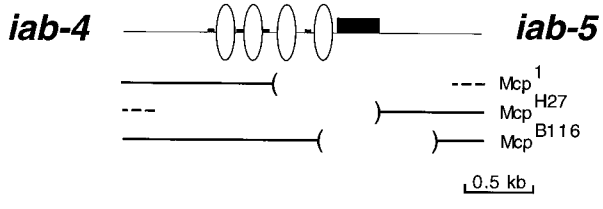

Figure 6. Chromatin structure of the *Mcp* domain boundary. The thin top line represents the *Mcp* genomic DNA drawn at the scale indicated below. The black rectangles indicate the extent of the hypersensitive regions described in Karch et al. [12]. Strong hypersensitive sites are shown by thick black rectangles, while weak hypersensitive regions are indicated by thin black rectangles. The ovals indicate nucleosome core particles. The different *Mcp* boundary deletions are shown below the DNA line.

Insulators, attenuators or domain boundaries?

The ability of *Fab-7* to block enhancer-promoter communication when interposed between an enhancer and a promoter raises the following question. Why does this enhancer-blocking activity not prevent the more proximal *iab-5* and *iab-6 cis*-regulatory domains from controlling the expression of the distal *Abd-B* gene in PS10 and PS11? One possibility may be the strength of the *Fab-7* boundary. Using constructs which differ in the number of reiterated binding sites for the SU(HW)-binding protein, Hagstrom et al. [41] have devised a system to evaluate the strength of enhancer-blocker elements. While the presence of 12 reiterated binding sites leads to full blocking activity, they showed that *Fab-7* has a moderate blocking activity which corresponds to the presence of 5 reiterated binding sites for the SU(HW) protein. The intermediate enhancer-blocking activity of the *Fab-7* element might be sufficient to prevent cross-talks between *iab-6* and *iab-7 cis*-regulatory domains, but insufficient to obstruct proximal domains from interacting with *Abd-B*. Zhou et al. [42] have proposed that *Fab-7* may function as an attenuator that moderates interactions between the *iab-6* distal

enhancers and the *Abd-B* target promoter. According to this model, the loss of the attenuator in *Fab-7* deletions would cause hyperactivity of *iab-6*, leading to the PS12-like pattern of *Abd-B* expression in PS11. Although we cannot formally exclude the possibility, this interpretation does not explain why *Fab-7* deletions allow repressing signals mediated by the POLYCOMB repressing complex to inactivate *iab-6*. The attenuator hypothesis is also difficult to reconcile with the effects of *Mcp* mutations. It does not explain, for instance, how hyperactivation of the *iab-4 cis*-regulatory domain by the *Mcp* deletion would deviate this domain to interact with *Abd-B* instead of its regular *abd-A* target promoter.

It is possible that there are special elements within each of the *iab cis*-regulatory domains that somehow function to promote 'pairing' with *Abd-B* promoters, allowing enhancer elements in more proximal *cis*-regulatory domains to circumvent the effects of intervening enhancer blockers. In this respect it may be of interest that most of the *gypsy* transposon-induced mutations in the BX-C are inserted in *cis*-regulatory domains controlling *Ubx* expression, while there are no gypsy transposon-induced mutations in the regulatory domains controlling *Abd-B* expression. While there may be a variety of reasons why such mutations have not been recovered, it is possible that the enhancer-blocking activity of the reiterated *su*(*Hw*) binding sites is unable to prevent interactions between the *iab cis*-regulatory domains and *Abd-B*.

In order to test this hypothesis, we replaced the *Fab-7* boundary element by the scs or the reiterated binding site for the SU(HW) within the context of the BX-C using the gene conversion method described in refs 53 and 54. Our results show that both scs and the reiterated binding sites for the SU(HW) protein revert the dominant gain-of-function *Fab-7* phenotype, indicating that these elements can prevent adventitious interactions between *iab-6* and *iab-7 cis*-regulatory domains. However, the enhancer-blocking activity of these elements has a deleterious effect on the proximal *cis*-regulatory domains, which are prevented from interacting with the *Abd-B* promoter (I. Hogga and F. Karch, unpublished observations). These new results lead us to consider that even if the *Fab-7* boundary element has properties of an enhancer blocker, it is not its major activity within the context of the BX-C.

Concluding remarks

Domain boundaries have been identified using two different approaches. On the one hand, elements like scs/scs′, the reiterated binding site for the SU(HW) protein, and the 5′ element of the chicken β-globin locus have been characterized through their activity of impeding enhancer-promoter interactions when intercalated between them. Their role of insulators can protect transgenes from genomic position effects, thereby establishing independent functional domains within the chromosome. On the other hand, domain boundaries like *Fab-7* and *Mcp* have been identified through mutational analysis. Deletions of *Mcp* or *Fab-7* lead to the fusion of otherwise independent *cis*-regulatory domains. *Fab-7* functions to constrain activating signals to the *iab-6* domain, and to confine repressive states mediated by the POLYCOMB repressive complex to the *iab-7* domain. The presence of PREs next to *Fab-7* and within *Mcp* suggests that they act as borders that could delineate the extent of particular chromatin conformations mediated in part by the POLYCOMB repressor complex. Although the mechanisms by which *Fab-7* functions as a domain boundary remain an open issue, our boundary-swapping experiment in the context of the BX-C indicates that *Fab-7* is not a mere insulator.

Acknowledgements. We especially thank the Human Frontier Science Program organization, whose support made this collaborative research project possible. The research was also supported by grants from the Swiss National Research Foundation, the State of Geneva, the Hungarian National Science and Research Fund (OTKA) and the National Institutes of Health. We thank F. Cléard for her comments on the manuscript.

1 Eissenberg J. C. and Elgin S. C. (1991) Boundary functions in the control of gene expression. Trends Genet. **7:** 335–340
2 Gerasimova T. I. and Corces V. G. (1996) Boundary and insulator elements in chromosomes. Curr. Opin. Genet. Dev. **6:** 185–192
3 Geyer P. K. (1997) The role of insulator elements in defining domains of gene expression. Curr. Opin. Genet. Dev. **7:** 242–248
4 Udvardy A., Maine E. and Schedl P. (1985) The 87A7 chromomere: identification of novel chromatin structures flanking the heat shock locus that may define the boundaries of higher order domains. J. Mol. Biol. **185:** 341–358
5 Kellum R. and Schedl P. (1991) A position-effect assay for boundaries of higher order chromosomal domains. Cell **64:** 941–50
6 Kellum R. and Schedl P. (1992) A group of scs elements function as domain boundaries in an enhancer-blocking assay. Mol. Cell. Biol. **12:** 2424–2231
7 Vazquez J. and Schedl P. (1994) Sequences required for enhancer blocking activity of scs are located within two nuclease-hypersensitive regions. EMBO J. **13:** 5984–5993
8 Vazquez J., Farkas G., Gaszner M., Udvardy A., Muller M., Hagstrom et al. (1993) Genetics and molecular analysis of chromatin domains. Cold Spring Harb. Symp. Quant. Biol. **58:** 45–54
9 Geyer P. K. and Corces V. G. (1992) DNA position-specific repression of transcription by a *Drosophila* zinc finger protein. Genes LDev. **6:** 1865–1873
10 Roseman R. R., Pirrotta V. and Geyer P. K. (1993) The su(Hw) protein insulates expression of the *Drosophila melanogaster* white gene from chromosomal position-effects. EMBO J. **12:** 435–442
11 Gyurkovics H., Gausz J., Kummer J. and Karch F. (1990) A new homeotic mutation in the *Drosophila* bithorax complex removes a boundary separating two domains of regulation. EMBO J. **9:** 2579–2585
12 Karch F., Galloni M., Sipos L., Gausz J., Gyurkkovics H. and Schedl P. (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
13 Galloni M., Gyurkovics H., Schedl P. and Karch F. (1993) The bluetail transposon: evidence for independent *cis*-regulatory domains and domain boundaries in the bithorax complex. EMBO J. **12:** 1087–1097
14 Mihaly J., Hogga I., Gausz J., Gyurkovics H. and Karch F. (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
15 Lewis E. B. (1978) A gene complex controlling segmentation in *Drosophila*. Nature **276:** 565–570
16 Sanchez-Herrero E., Vernos I., Marco R. and Morata G. (1985) Genetic organization of *Drosophila* bithorax complex. Nature **313:** 108–113
17 Casanova J., Sanchez-Herrero E., Busturia A. and Morata G. (1987) Double and triple mutant combinations of the bithorax complex of *Drosophila*. EMBO J. **6:** 3103–3109
18 White R. A. and Wilcox M. (1984) Protein products of the bithorax complex in *Drosophila*. Cell **39:** 163–171
19 Karch F., Bender W. and Weiffenbach B. (1990) abdA expression in *Drosophila* embryos. Genes Dev. **4:** 1573–1587
20 Celniker S. E., Sharma S., Keelan D. J. and Lewis E. B. (1990) The molecular genetics of the bithorax complex of *Drosophila*: *cis*-regulation in the Abdominal-B domain. EMBO J. **9:** 4277–4286
21 Sanchez-Herrero E. (1991) Control of the expression of the bithorax complex genes abdominal-A and abdominal-B by *cis*-regulatory regions in *Drosophila* embryos. Development **111:** 437–449
22 Peifer M., Karch F. and Bender W. (1987) The bithorax complex: control of segmental identity. Genes Dev. **1:** 891–898
23 Duncan I. (1987) The bithorax complex. Annu. Rev. Genet. **21:** 285–319
24 Qian S., Capovilla M. and Pirrotta V. (1991) The bx region enhancer, a distant *cis*-control element of the *Drosophila* Ubx gene and its regulation by *hunchback* and other segmentation genes. EMBO J. **10:** 1415–1425
25 Muller J. and Bienz M. (1992) Sharp anterior boundary of homeotic gene expression conferred by the fushi tarazu protein. EMBO J. **11:** 3653–3661
26 Shimell M. J., Simon J., Bender W. and O'Connor M. B. (1994) Enhancer point mutation results in a homeotic transformation in *Drosophila*. Science **264:** 968–971
27 Casares F. and Sanchez Herrero E. (1995) Regulation of the infraabdominal regions of the bithorax complex of *Drosophila* by gap genes. Development **121:** 1855–1566
28 Shearn A. (1989) The ash-1, ash-2 and trithorax genes of *Drosophila melanogaster* are functionally related. Genetics **121:** 517–525
29 Paro R. (1990) Imprinting a determined state into the chromatin of *Drosophila*. Trends Genet. **6:** 416–421
30 Simon J., Chiang A., Bender W., Shimell M. J. and O'Connor M. (1993) Elements of the *Drosophila* bithorax complex that mediate repression by Polycomb group products. Dev. Biol. **158:** 131–144
31 Simon J., Peifer M., Bender W. and O'Connor M. (1990) Regulatory elements of the bithorax complex that control expression along the anterior-posterior axis. EMBO J. **9:** 3945–3956
32 Zhang C. C. and Bienz M. (1992) Segmental determination in *Drosophila* conferred by hunchback (hb), a repressor of the homeotic gene Ultrabithorax (Ubx). Proc. Natl. Acad. Sci. USA **89:** 7511–7515
33 Busturia A. and Bienz M. (1993) Silencers in abdominal-B, a homeotic *Drosophila* gene. EMBO J. **12:** 1415–1425
34 Chan C. S., Rastelli L. and Pirrotta V. (1994) A Polycomb response element in the Ubx gene that determines an epigenetically inherited state of repression. EMBO J. **13:** 2553–2564
35 Christen B. and Bienz M. (1994) Imaginal disc silencers from Ultrabithorax: evidence for Polycomb response elements. Mech. Dev. **48:** 255–266
36 Chiang A., O'Connor M. B., Paro R., Simon J. and Bender W. (1995) Discrete Polycomb-binding sites in each parasegmental domain of the bithorax complex. Development **121:** 1681–1689
37 Poux S., Kostic C. and Pirrotta V. (1996) Hunchback-independent silencing of late Ubx enhancers by a Polycomb Group Response Element. EMBO J. **15:** 4713–4722
38 Pirrotta V. and Rastelli L. (1994) White gene expression, repressive chromatin domains and homeotic gene regulation in *Drosophila*. Bioessays **16:** 549–556
39 Simon J. (1995) Locking in stable states of gene expression: transcriptional control during *Drosophila* development. Curr. Opin. Cell. Biol. **7:** 376–385
40 McCall K., O'Connor M. B. and Bender W. (1994) Enhancer traps in the *Drosophila* bithorax complex mark parasegmental domains. Genetics **138:** 387–399
41 Hagstrom K., Muller M. and Schedl P. (1996) *Fab-7* functions as a chromatin domain boundary to ensure proper segment specification by the *Drosophila* bithorax complex. Genes Dev. **10:** 3202–3215
42 Zhou J., Barolo S., Szymanski P. and Levine M. (1996) The *Fab-7* element of the bithorax complex attenuates enhancer-promoter interactions in the *Drosophila* embryo. Genes Dev. **10:** 3195–3201
43 Hagstrom K., Muller M. and Schedl P. (1997) A Polycomb and GAGA dependent silencer adjoins the *Fab-7* boundary in the *Drosophila* bithorax comple. Genetics **146:** 1365–1380
44 Kassis J. A. (1994) Unusual properties of regulatory DNA from the *Drosophila* engrailed gene: three 'pairing-sensitive' sites within a 1.6-kb region. Genetics **136:** 1025–1038
45 Gindhart J. G. Jr. and Kaufman T. C. (1995) Identification of Polycomb and trithorax group responsive elements in the regulatory region of the *Drosophila* homeotic gene Sex combs reduced. Genetics **139:** 797–814
46 Chung J. H., Whiteley M. and Felsenfeld G. (1993) A 5′ element of the chicken beta-globin domain serves as an insulator in human erythroid cells and protects against position effect in *Drosophila*, Cell **74:** 505–514
47 Biggin M. D. and Tjian R. (1988) Transcription factors that activate the Ultrabithorax promoter in developmentally staged extracts. Cell **53:** 699–711
48 Lu Q., Wallrath L. L., Granok H. and Elgin S. C. (1993) (CT)n (GA)n repeats and heat shock elements have distinct roles in chromatin structure and transcriptional activation of the *Drosophila* hsp26 gene, Mol. Cell. Biol. **13:** 2802–2814
49 Tsukiyama T., Becker P. B. and Wu C. (1994) ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor, Nature **367:** 525–532
50 Granok H., Leibovitch B. A., Shaffer C. D. and Elgin S. C. (1995) Chromatin: Ga-ga over GAGA factor. Curr. Biol. **5:** 238–241
51 Farkas G., Gausz J., Galloni M., Reuter G., Gyurkovics H. and Karch F. (1994) The Trithorax-like gene encodes the *Drosophila* GAGA factor. Nature **371:** 806–808
52 Karch F., Weiffenbach B., Peifer M., Bender W., Duncan I., Celniker S. et al. (1985) The abdominal region of the bithorax complex. Cell **43:** 81–96
53 Gloor G. B., Nassif N. A., Johnson-Schlitz D. M., Preston C. R. and Engels W. R. (1991) Targeted gene replacement in *Drosophila* via P element-induced gap repair. Science **253:** 1110–1117
54 Hogga I. and Karch F. (1995) Targeting a specific deletion, a la Engels, in the *Fab-7* boundary of the bithorax-complex. DIS **76:** 157–158
55 Celniker S. E., Keelan D. J. and Lewis E. B. (1989) The molecular genetics of the bithorax complex of *Drosophila*: characterization of the products of the Abdominal-B domain. Genes Dev. **3:** 1424–1436
56 Zavortink M. and Sakonju S. (1989) The morphogenetic and regulatory functions of the *Drosophila* Abdominal-B gene are encoded in overlapping RNAs transcribed from separate promoters. Genes Dev. **3:** 1969–1981
57 Boulet A., Lloyd A. and Sakonju S. (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