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Silencers in *Abdominal-B*, a homeotic *Drosophila* gene

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Homeotic genes determine the developmental fates of cells. Restriction of their expression along the body axis is of prime importance for normal development. We searched for *cis*-regulatory sequences within *Abdominal-B* (*Abd-B*), a homeotic *Drosophila* gene, by testing genomic *Abd-B* fragments for their ability to confer β-galactosidase (β-gal) expression in transformed embryos. One of the *Abd-B* fragments, called IAB5, mediates a β-gal pattern restricted along the body axis to the *Abd-B* expression domain. Alterations of the IAB5 pattern in gap mutants provide evidence that the protein products of the gap genes *hunchback*, *Krüppel* and *knirps* act as repressors through IAB5. The anterior *Abd-B* expression limit is apparently determined by *Krüppel* repression, whereas the *knirps* repressor may be responsible for the graded *Abd-B* expression within the *Abd-B* domain. IAB5 and two other fragments called MCP and FAB show region-specific silencing activity: they suppress at a distance β-gal expression mediated by a linked heterologous enhancer. Silencing requires *hunchback* as well as *Polycomb* function and evidently provides maintenance of *Abd-B* expression limits throughout embryogenesis. We conclude that transcriptional repression is a key mechanism operating at multiple levels to control *Abd-B* expression. The striking similarities between the control of *Abd-B* and of *Ultrabithorax*, another homeotic *Drosophila* gene, may point to a universal principle underlying homeotic gene regulation.

Key words: *Drosophila*/expression boundaries/homeotic gene/silencers/transcriptional regulation

Introduction

Homeotic genes determine developmental pathways along the body axis of the *Drosophila* embryo (Lewis, 1963, 1978; Kaufman *et al.*, 1980). Their function is required until late stages of development (Morata and García-Bellido, 1976). Expression of homeotic genes is confined to unique domains along the body axis whose anterior and posterior boundaries are maintained throughout development (reviewed by Akam, 1987). The process of restricting homeotic gene expression along the body axis is crucial for normal development as the presence of homeotic gene products in cells is sufficient in many cases to determine their developmental fate (e.g. Schneuwly *et al.*, 1987; Gibson and Gehring, 1988; González-Reyes *et al.*, 1990). This process initially relies on positional information which, in the early embryo, is transiently provided by segmentation gene products (Nüsslein-Volhard and Wieschaus, 1980). Somehow, this positional information is converted into stable states of homeotic gene activity or inactivity which are propagated throughout development.

Recent work on transcriptional control regions of *Ultrabithorax* (*Ubx*), one of the homeotic genes from the bithorax complex (Sánchez-Herrero *et al.*, 1985), led to a picture as to how this may occur. First of all, *Ubx* expression boundaries are due to repression, in other words, the *Ubx* gene is potentially active along the entire body axis, but its activity is repressed outside the *Ubx* domain (Müller and Bienz, 1991). Repression is initially conferred by *hunchback* (*hb*) protein (Qian *et al.*, 1991; Zhang *et al.*, 1991), a segmentation gene product providing positional information in the early embryo by virtue of its uneven distribution in regions that are roughly complementary to the *Ubx* domain (Tautz, 1988). *hb* protein acts as a direct repressor of *Ubx* by competing with early embryonic activators, such as *fushi tarazu* (*ftz*) protein, for DNA-binding and/or for transcriptional activation (Müller and Bienz, 1992). Repression at this early stage is based on short-range competition between localized activators and repressors (Small *et al.*, 1991), a mechanism that appears to be universally used to position anteroposterior limits of gene expression in the early embryo (Stanojevic *et al.*, 1991; Hoch *et al.*, 1992). Later, *Ubx* expression boundaries are due to a mechanism of long-range repression or silencing which, unlike short-range repression, requires the function of *Polycomb* (*Pc*) (Müller and Bienz, 1991). Apparently, silencing at late embryonic stages is also mediated by *hb* protein even though *hb* protein is no longer detectable at these stages (Zhang and Bienz, 1992). We proposed that *hb* protein bound to a *Ubx* silencer prompts the formation of a stable silencing complex between *Ubx* silencer and proximal promoter by tethering to the *Ubx* gene other proteins, perhaps *Pc*-like proteins, that are ubiquitously expressed (Zhang and Bienz, 1992). Silencing complexes are expected to be propagated through cell divisions and to bestow on a homeotic gene permanent and irreversible repression; their heritability may be based on their putative multimeric structure (reviewed by Bienz, 1992).

Is the activity of other homeotic genes controlled by the same underlying principle of repression? To answer this, we decided to study the properties of transcriptional control regions within *Abdominal-B* (*Abd-B*), another homeotic gene located within the bithorax complex (Sánchez-Herrero *et al.*, 1985). This gene is unique among *Drosophila* homeotic genes as it contains two separable genetic subfunctions, called m and r, which control morphogenesis in different posterior domains of the larva and the adult (Casanova *et al.*, 1986): m function is required in parasegments (ps) 10–13, whereas r function is required in ps14 (Casanova *et al.*, 1986; see also Karch *et al.*, 1985; Tiong *et al.*, 1985; Celniker and Lewis, 1987). The two functions are provided by two distinct

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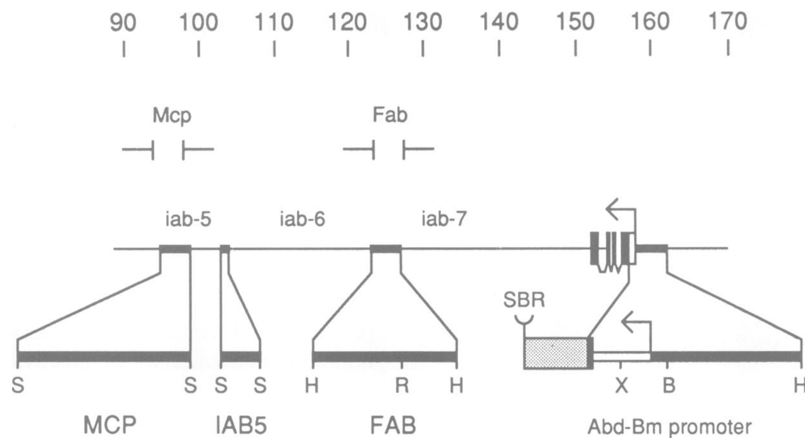

Fig. 1. Maps of *Abd-B* control regions. Map of 3′ flanking region (solid line) and coding region (vertical black boxes) of the *Abd-B* gene (transcription start site of *Abd-B* m marked by arrow; open box, mRNA leader sequence). Above, genomic positions of the *Mcp* and *Fab* deletions as well as the approximate regions within which *iab-5*, *iab-6* and *iab-7* mutations map (Karch *et al.*, 1985; Gyurkovics *et al.*, 1990). Underneath, maps and genomic positions of MCP, IAB5 and FAB fragments as well as a map of the proximal *Abd-B* m promoter (including transcription start site) fused to β-gal coding sequences (hatched box). B, *Bam*HI; H, *Hin*dIII; P, *Pst*I; R, *Eco*RI; S, *Sal*I; X, *Xho*I (all sites shown).

proteins which share the same homeodomain, but whose expression is controlled by different promoters (Sánchez-Herrero and Crosby, 1988; Celniker *et al.*, 1989; DeLorenzi *et al.*, 1988; Kuziora and McGinnis, 1988; Zavortink and Sakonju, 1989).

Another unique aspect of *Abd-B* is that *Abd-B* m protein expression appears gradually from more posterior to more anterior parasegments and that the levels of *Abd-B* m expression, graded throughout embryogenesis, increase from ps10 to ps13 in parasegmental steps (Celniker *et al.*, 1989; DeLorenzi and Bienz, 1990; Boulet *et al.*, 1991). Close examination of *Abd-B* m protein expression revealed that the patterns in individual parasegments within ps10−12 are probably the same and that they differ mostly (or only) in their relative intensities (DeLorenzi and Bienz, 1990). Genetic analysis suggested that ps-specific patterns of *Abd-B* expression within ps10−12 are controlled by multiple *cis*-regulatory elements residing in the large 3′ flanking region of the *Abd-B* gene (Karch *et al.*, 1985; Gyurkovics *et al.*, 1990). Indeed, breakpoints and deletions within this region eliminate *Abd-B* m expression in individual parasegments from ps10 to ps12 (Celniker *et al.*, 1990; Sánchez-Herrero, 1991; these mutations have no effect on *Abd-B* expression in ps13 and ps14 which therefore must be controlled by upstream *Abd-B* sequences).

We were interested in two specific questions concerning *Abd-B* expression. Firstly, we asked how the anterior limit of *Abd-B* expression at ps10 is determined. Secondly, we wondered how the graded levels of *Abd-B* expression throughout ps10−12 might be generated. Both these aspects of *Abd-B* expression are presumed to be controlled by elements residing in the 3′ flanking region of the gene (see above). We therefore searched through this region for *cis*-regulatory sequences which, linked to a β-galactosidase (β-gal) gene, confer *Abd-B*-like β-gal expression patterns in transformed embryos. Here, we describe the regulatory properties of three *Abd-B* fragments and their functional dependence on segmentation gene products.

Results

We made a basic construct (see Figure 1) containing the *Abd-B* m proximal promoter (4.1 kb of 5′ flanking and 1.2 kb of mRNA leader sequence; Zavortink and Sakonju, 1989) as well as the β-gal coding region and we joined to this genomic fragments from the 3′ flanking region of the *Abd-B* gene. We tested 17 different fragments covering >50 kb of 3′ flanking genomic sequence for their ability to confer *Abd-B*-like β-gal expression patterns in transformed embryos.

This basic *Abd-B* construct directs a considerable amount of β-gal expression in a pattern mostly restricted to the dorsal ectoderm (not shown; this 'basal' pattern is distinct from the 'basal pattern' due to the proximal *Ubx* promoter; Bienz *et al.*, 1988). It also directs β-gal expression in the posterior spiracles, apparently reflecting one aspect of *Abd-B* expression (Kuhn *et al.*, 1992), but apart from this, the 'basal' pattern bears no resemblance to *Abd-B* expression.

Among the 17 constructs with added distal *Abd-B* sequences, only two confer additional β-gal staining patterns (Figure 1). This suggests that, contrary to expectations, enhancer sequences within the 3′ flanking region of the *Abd-B* gene are sparse. It is possible that we missed several such sequences, due to the 'basal' pattern; however, any enhancer sequence conferring β-gal expression in the ventral half of the embryo should have been detectable by our assay.

The two fragments with enhancer activity are located adjacent to one another in a very remote region of the *Abd-B* gene. The more proximal of these mediates β-gal staining in ventral regions of the embryo in a head-to-tail pattern which we did not further analyse (to be described elsewhere). The more distal one, 0.9 kb in length, is located in a genomic region within which *iab-5* mutations map (Karch *et al.*, 1985), 54 kb away from the *Abd-B* m transcription start site (Zavortink and Sakonju, 1989; see Figure 1). We shall call it IAB5 fragment. It confers a striking β-gal staining pattern, the IAB5 pattern (Figure 2) as described below, whose anterior limit coincides with the anterior *Abd-B* expression boundary at ps10.

The IAB5 pattern

We first detect β-gal staining in IAB5 transformants at the blastoderm stage in a band with somewhat blurred edges (Figure 2A), positioned roughly between 30 and 12% egg length (0% egg length corresponds to the posterior pole). This band is wider anteriorly (by ~2 ps) than the band of

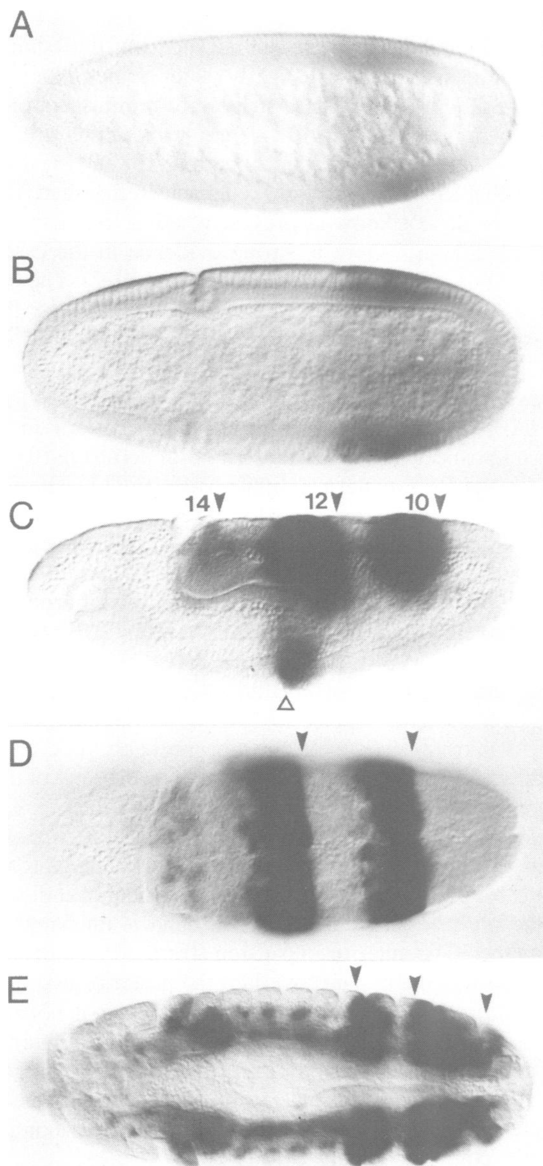

Fig. 2. The IAB5 pattern. IAB5 transformant embryos were stained with β-gal antibody. (**A**) Side view of blastoderm embryo, showing early β-gal band. (**B**) Early gastrula viewed from top; sharpening of β-gal band visible. (**C** and **D**) Extended germ band embryos (C, side view; D, ventral view of tail end), showing *ftz*-like β-gal stripes (three stripes in *Abd-B* domain ps10–14, and additional weak stripe, marked by triangle, near the head region in ps4); sharp anterior limits of stripes coincide with ps limits (arrowheads). (**E**) Top view of embryo after shortening of germ band; *ftz*-like stripes in the ectoderm still visible (arrowheads pointing to ps10, ps12 and ps14 stripes), in addition to low levels of late appearing β-gal staining throughout the mesoderm. Anterior ends and heads to the left.

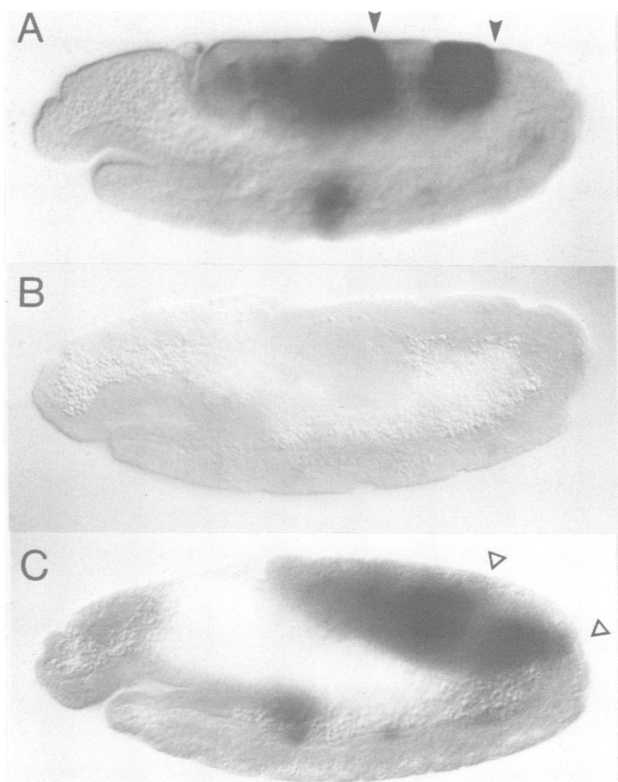

Fig. 3. IAB5 pattern in *ftz* and *eve* mutants. Side views of extended germ band embryos stained with β-gal antibody. (**A**) Wild type (ps10 and ps12 stripe marked by arrowheads). (**B**) *ftz*⁻, β-gal staining virtually eliminated. (**C**) *eve*⁻, β-gal stripes visible, slightly weaker and wider than in the wild type; anterior limits of stripes not very sharp (ps10 and ps12 stripe marked by triangles). Heads to the left.

Abd-B RNA expression that is detectable at about the same time with probes containing the *Abd-B* homeobox (Harding and Levine, 1988; Sánchez-Herrero and Crosby, 1988). It corresponds approximately to the band of early RNA expression detected with genomic probes derived from the *iab-5* and *iab-6* region (Sánchez-Herrero and Akam, 1989). This early RNA is thought to reflect immature nuclear RNA initiated at 'pseudopromoters' that are activated by nearby enhancers located within the *iab-5*/*iab-6* region (Sánchez-Herrero and Akam, 1989), a notion supported by our result.

As gastrulation begins, β-gal expression increases, the anterior boundary of the β-gal band sharpens and shifts slightly towards anterior (Figure 2B). Eventually, the band resolves into three stripes; the middle one of these is the most prominent one, the posterior one is very faint (Figure 2C; an additional weak stripe arises at this stage in an anterior position near the head). β-gal staining within these stripes is strong anteriorly, but fades towards posterior (Figure 2D), and the stripes resemble those produced by *ftz*–β-gal fusion genes (Hiromi *et al.*, 1985; Lawrence *et al.*, 1987). Double-staining experiments with β-gal and *engrailed* (*en*) antibody show that the three posterior stripes are located within ps10, 12 and 14 (and the late appearing anterior one in ps4) and that their sharp anterior limits coincide with ps boundaries. β-gal staining in these stripes remains detectable throughout embryogenesis, though more advanced embryos also show staining in the dorsolateral ectoderm and in the mesoderm of most segments (Figure 2E) as well as in a restricted region within the midgut.

The IAB5 pattern shows striking resemblance to the pattern conferred by the PBX control region from the *Ubx* gene in most respects (Müller and Bienz, 1991): both patterns arise at about the same time as a band of expression which later resolves into *ftz*-like stripes. The appearance of these *ftz*-like stripes (IAB5- or PBX-mediated) is almost identical, suggesting that they are due to the same embryonic activator(s). In the case of PBX, this activator is the *ftz* protein (Müller and Bienz, 1992). We therefore asked whether the IAB5 stripes are also due to activation by *ftz*.

We crossed transformants bearing an IAB5 construct with

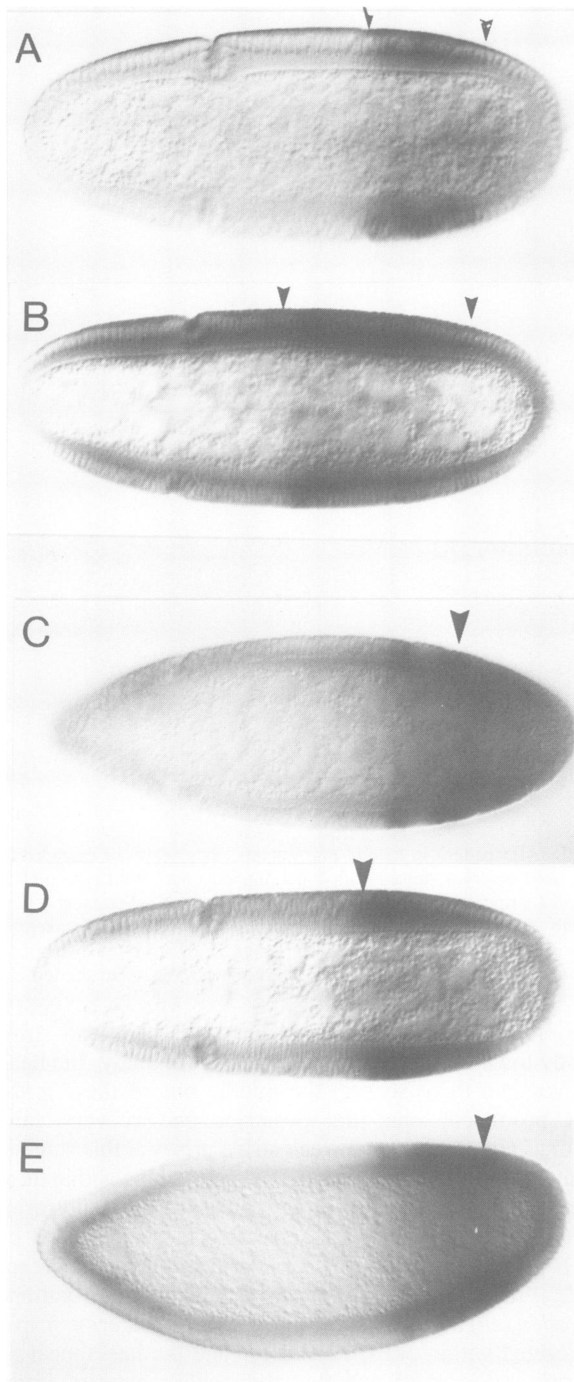

Fig. 4. Early IAB5 pattern in gap mutants. Early gastrulas showing band of β-gal staining on the verge of resolving into stripes (A–D, side views; E, viewed from top). (**A** and **C**) Wild type (embryo in C slightly older than embryo in A), showing anteroposterior extent of band (between small arrowheads in A) and maximal β-gal staining in incipient ps12 stripe (arrowhead in C). (**B**) *Kr*⁻, anteriorly expanded band (between small arrowheads). (**D**) *kni*⁻, maximal β-gal staining anteriorly within band (arrowhead). (**E**) *hb*⁻, maximal β-gal staining posteriorly within band (arrowhead). The following mutant alleles were used: hb^{XT15}, Kr^{1}, kni^{IID}. Anterior to the left.

ftz or *even-skipped* (*eve*) mutant strains and analysed homozygous mutant embryos for β-gal expression (see Materials and methods). We find that virtually all β-gal staining is eliminated in *ftz*⁻ embryos (Figure 3B; note that the late appearing ps4 stripe is also completely *ftz*-dependent). In *eve*⁻ embryos, we see a considerable amount of β-gal staining, though mostly in the mesoderm; this staining resolves into similar, albeit slightly wider stripes than in the wild type (Figure 3C). These stripes are reminiscent of the stripes of *ftz* expression in *eve*⁻ embryos which are also slightly widened (Lawrence and Johnston, 1989). Our results suggest that the IAB5 stripes exclusively reflect activation by *ftz*. We do not know at present whether this activation is direct, although there is strong evidence in the case of PBX that this is the case (Müller and Bienz, 1992). The early band of IAB5 expression, like the early band of PBX expression, is not affected by *ftz* or *eve* mutation.

Repressors acting through IAB5

The IAB5 pattern differs from the PBX pattern in one important aspect: the IAB5 pattern is limited to ps10–14, whereas the PBX pattern is limited to ps6–12. The PBX expression boundaries are due to repression by *hb* protein, expressed outside ps6–12 (Tautz, 1988), which acts as a direct repressor of PBX (Zhang *et al.*, 1991; Müller and Bienz, 1992). Because of its spatial distribution, it is unlikely that *hb* protein also determines the limits of the IAB5 pattern. Indeed, genetic analysis suggests that *Abd-B* expression limits are dependent on *knirps* (*kni*) and *Krüppel* (*Kr*), two gap genes expressed more posteriorly than *hb* (Gaul and Jäckle, 1989; Pankratz *et al.*, 1990), but probably not on *hb* (Harding and Levine, 1988). We asked whether any of these gap genes affect the IAB5 pattern and its limits.

In *Kr* mutants, we observe a striking anterior expansion of the IAB5 band (Figure 4A and B). The mutant band extends roughly between 50 and 12% egg length, indicating that the posterior limit of the IAB5 band is unchanged in *Kr* mutants. The anterior expansion (from 30% in the wild type to 50% in the mutant) overlaps the posterior two-thirds of the embryonic zone within which *Kr* protein is normally expressed (60–33%; Gaul and Jäckle, 1989; see Figure 8). This suggests that *Kr* acts as a repressor through the IAB5 fragment to determine the anterior IAB5 boundary. Note that the anterior IAB5 limit and the posterior *Kr* expression limit virtually coincide (Figure 8).

In *kni* mutants, the anteroposterior extent of the early IAB5 band appears unchanged. However, in gastrulating embryos, we observe two differences between the wild type and the mutant β-gal pattern. Firstly, the IAB5 band, although not the mutant band, begins to resolve into stripes at this stage. The failure of the mutant band to resolve into stripes probably reflects *ftz* protein expression which also fails to resolve into stripes in this embryonic region (Carroll and Scott, 1986). Secondly, β-gal staining in the region of the nascent ps10 stripe, normally weaker than that in the region of the nascent ps12 stripe (Figure 4C), is enhanced in *kni* mutant embryos: in these mutants, β-gal staining is strongest within the anteriormost portion of the IAB5 band (Figure 4D). This substantial increase in anterior staining coincides with the embryonic zone which, in the wild type, contains highest levels of *kni* protein (Pankratz *et al.*, 1990; see Figure 8). The mutant staining pattern suggests that *kni* might be responsible for the comparatively low level of β-gal expression within the nascent ps10 stripe in the wild type and, thus, may act as a weak repressor through the IAB5 fragment.

Finally, in *hb* mutants, the extent of the IAB5 band appears to be normal too, though again, we see differences of β-gal

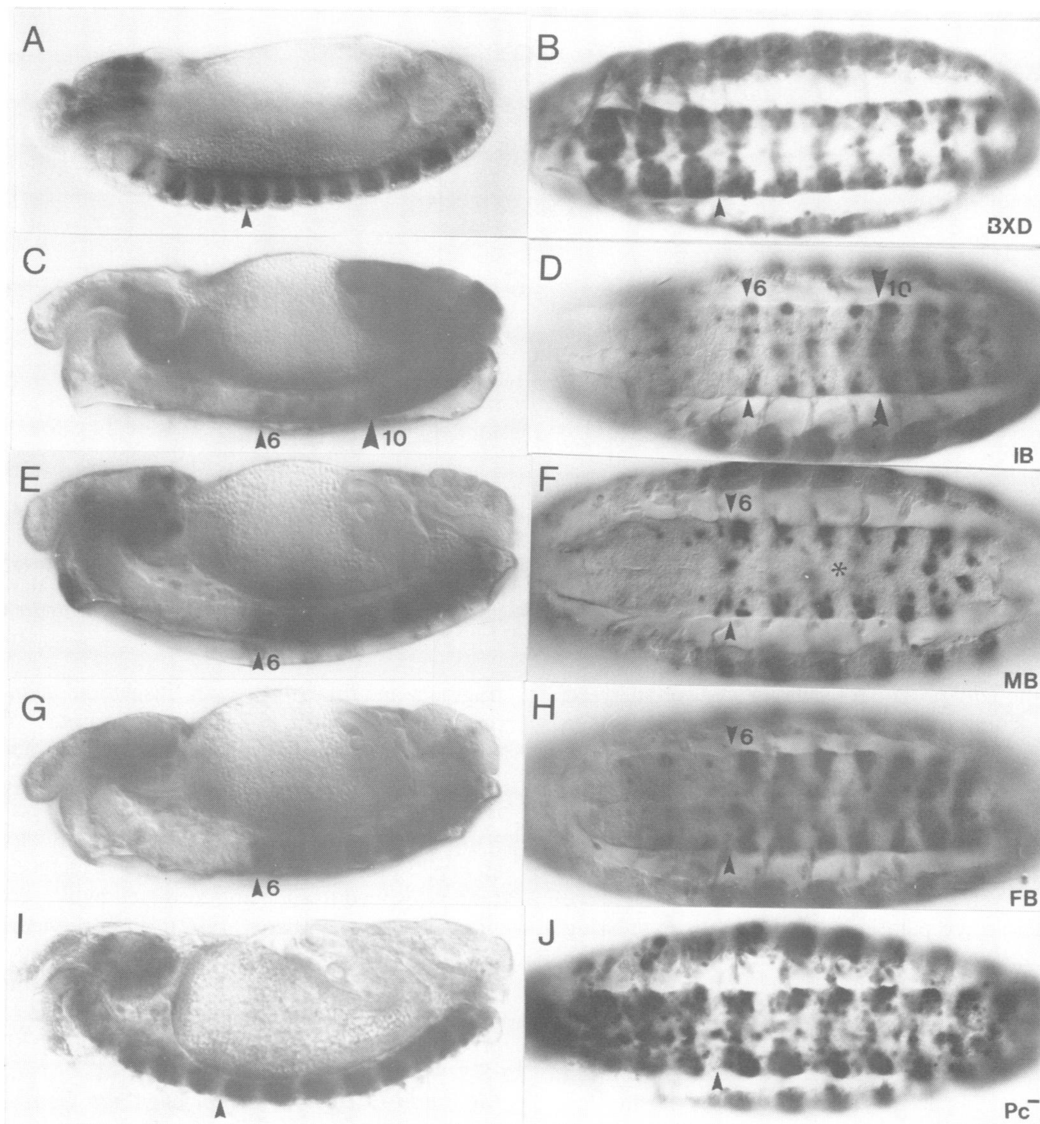

Fig. 5. Silencing mediated by IAB5, MCP and FAB. Side views (left hand column) and ventral views showing ventral nerve cord (right hand column) of 10–14 h old embryos; small arrowheads point to anterior limit of ps6. (**A** and **B**) Head-to-tail pattern conferred by the BXD enhancer from *Ubx*; staining extends from ps2 to ps14 (Müller and Bienz, 1991). (**C** and **D**) Silencing mediated by IAB5 (IB transformants); β-gal staining in ventral regions almost fully suppressed outside ps6–13, and partially suppressed within ps6–9: full BXD pattern (V-shapes in ventral nerve cord) only visible within ps10–13 (large arrowhead points to anterior limit of ps10). (**E–H**) Silencing mediated by MCP (E and F, MB transformants) and FAB (G and H, FB transformants); β-gal staining in ventral regions suppressed outside ps6–13 (note partial suppression of staining in medial regions of the ventral nerve cord, visible in focal plane of F and marked by asterisk, reminiscent of a similar effect in HB transformants; see Figure 6A and B). (**I** and **J**) *Pc*⁻ MB transformant embryos; BXD pattern is evenly derepressed from head to tail, demonstrating that silencing requires *Pc* function (the same *Pc*⁻ patterns are seen in FB and IB transformants). Heads to the left, dorsal up.

staining between wild type and mutant gastrulating embryos. In the wild type, the nascent ps14 stripe stains very weakly compared with the other two (Figure 4C). In contrast, in *hb* mutants, β-gal staining is strongest in the posteriormost portion of the IAB5 band, in the region where the ps14 stripe normally arises (Figure 4E; this stripe does not seem to resolve from the ps12 stripe, again probably reflecting altered *ftz* expression in these mutants; Carroll and Scott, 1986). The striking increase of posterior staining indicates that *hb* protein acts as a repressor through the IAB5 fragment. This repressor function seems to manifest itself only in ps14, the posterior and slightly delayed *hb* expression domain (Tautz, 1988), since we do not detect any anterior derepression in *hb* mutants. However, an anterior repression function of *hb* may be obscured by *Kr* protein whose distribution in *hb* mutants reaches abnormally anterior positions (Gaul and Jäckle, 1987) as well as by maternal *hb* protein perduring anteriorly in early *hb* mutant embryos (Tautz, 1988; Hülskamp *et al.*, 1990; Hülskamp, 1991).

IAB5 acts as a silencer

The IAB5 equivalent from the *Ubx* gene, the PBX fragment, acts as a silencer (i.e. confers repression at large distances): when this fragment is linked to the BXD fragment, a head-to-tail enhancer from the *Ubx* gene, it suppresses BXD-mediated expression outside ps6–12 (Müller and Bienz, 1991). PBX-mediated silencing is observed throughout embryogenesis and requires *Pc* function (Müller and Bienz, 1991). We wondered whether the IAB5 fragment might have silencing activity. We linked this fragment to the BXD

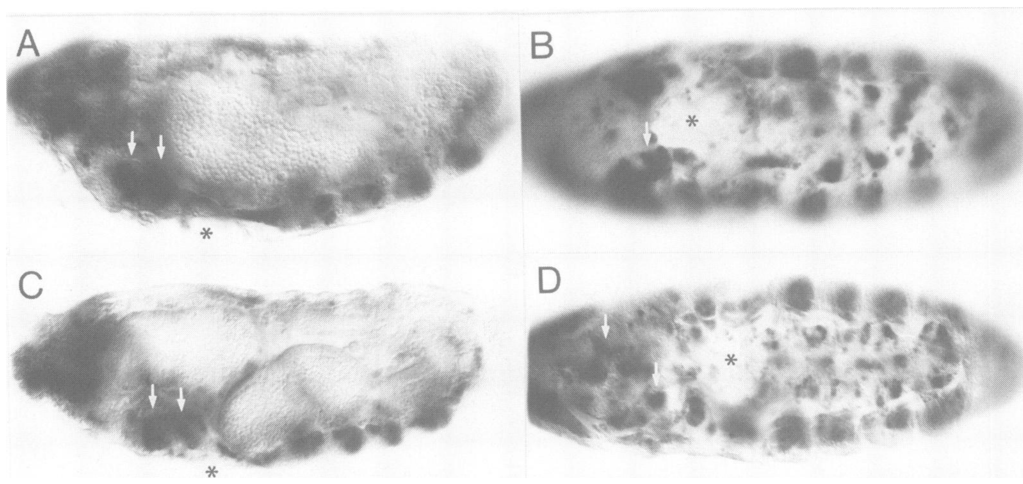

Fig. 6. Dependence of silencing on *hb* function. Side views (A and C) or ventral views (B and D) of *hb* mutant embryos, 10–14 h old, stained with β-gal antibody; gap due to *hb* mutation visible in ventral nerve cord (asterisk). (**A** and **B**) HB transformants in which silencing outside ps6–13 in the wild type is due to *hb* protein binding sites (Zhang and Bienz, 1992). (**C** and **D**) MB transformants. HB and MB transformants, virtually indistinguishable, showing derepressed β-gal staining anterior to gap (arrows) equally strong as staining posterior to gap. To maximize the effect, the antimorphic allele hb^{9K57} was chosen (see text). Heads to the left, dorsal up.

enhancer and the *Ubx* proximal promoter and we analysed transformants of this IAB5/BXD construct (called IB) for β-gal staining.

In two of the IB transformant lines, we find that the BXD pattern (Figure 5A and B) is strikingly restricted along the body axis (Figure 5C and D). A strong BXD pattern can only be discerned in ps10–13 where we observe strong β-gal staining in the ventrolateral ectoderm. In the ventral nerve cord within these three parasegments, the cells expressing β-gal are arranged in V-shapes that are characteristic for the BXD pattern. In ps6–9, we can discern a partial and patchy BXD pattern; however, outside ps6–13, β-gal staining is mostly eliminated (apart from some staining in the dorsolateral ectoderm and in the head). Evidently, the IAB5 fragment has silencing activity as it suppresses most of the BXD pattern outside ps6–13 and some of the BXD pattern within ps6–9. It is possible that silencing is inefficient within ps6–9 due to some degree of incompatibility between the *Abd-B* silencer and the responding *Ubx* promoter (and/or the BXD enhancer): the *Ubx* sequences may not be fully responsive to silencing in the regions in which they are normally active. No silencing was observed in two other IB lines, indicating a variation of silencing efficiency between transformant lines, maybe attributable to interference from flanking chromosomal sequences, as previously observed (Müller and Bienz, 1991).

The silencing capacity of the IAB5 fragment can also affect the promoter (and enhancers) of the *rosy* (ry^+) gene, for the following reasons. We were not able to isolate any ry^+ transformants with a construct in which IAB5 is linked to the hsp70 TATA-box (see Materials and methods), but we did find embryos showing the IAB5 pattern among the second generation of progeny from ry^- flies that hatched after injection with this construct. This implies that the ry^+ gene (within the transposon) in these transformants is not active enough to rescue the *ry* eye colour of the host flies. This suggested to us that the IAB5 fragment potently silences the adjacent ry^+ gene in this construct. Indeed, we were able to isolate ry^+ transformants at the normal frequency, if we inserted into the same construct between the IAB5 fragment and the ry^+ gene an 'insulation' sequence known to interfere with enhancer–promoter interactions (*suppressor-of-Hairy-wing* protein binding sites; Dorsett, 1990; Holdridge and Dorsett, 1991). We were also able to isolate transformants if, instead of the *ry* vector, we used a *white* (*w*) vector in which the w^+ gene is expressed from an hsp70 promoter (see Materials and methods): the latter cannot respond to silencing mediated by *hb* protein binding sites (Zhang and Bienz, 1992), and is evidently also not capable of responding to IAB5-mediated silencing as we observed no silencing whatsoever in any of the eight lines transformed with an IB construct in which the *Ubx* proximal promoter was replaced with a minimal hsp70 promoter.

MCP and FAB, two further silencers from Abd-B

Among the mutations located within the 3′ flanking region of the *Abd-B* gene, two are due to small deletions, called *Mcp* and *Fab* (Lewis, 1978; Karch *et al.*, 1985; Gyurkovics *et al.*, 1990; see Figure 1). These are gain-of-function mutations: *Mcp* causes ectopic expression of *Abd-B* protein in ps9 in a pattern and at a level similar to that normally found in ps10 (Celniker *et al.*, 1990; Sánchez-Herrero, 1991), whereas the phenotype of *Fab* implies that *Abd-B* protein is expressed more strongly in ps11, at the level normally found in ps12 (Gyurkovics *et al.*, 1990). These phenotypes could reflect partial anterior derepression of *Abd-B* protein, and the genomic sequences around the *Mcp* and *Fab* lesions might therefore contain target sites for repressors. Since fragments covering the two deletions (a 4.3 kb fragment called MCP and a 3.3 kb fragment called FAB; see Figure 1) did not show any enhancer activity in transformed embryos (see above), we wondered whether they may have silencing activity. We linked either of these fragments to the BXD enhancer and the *Ubx* proximal promoter and tested the combination constructs (called MB and FB) for β-gal expression in transformed embryos.

In both cases, we observe striking silencing effects in the majority of transformant lines. Surprisingly, we found that the MCP and FAB fragments suppress BXD-mediated β-gal staining outside ps6–13 (Figure 5E–H). In some lines,

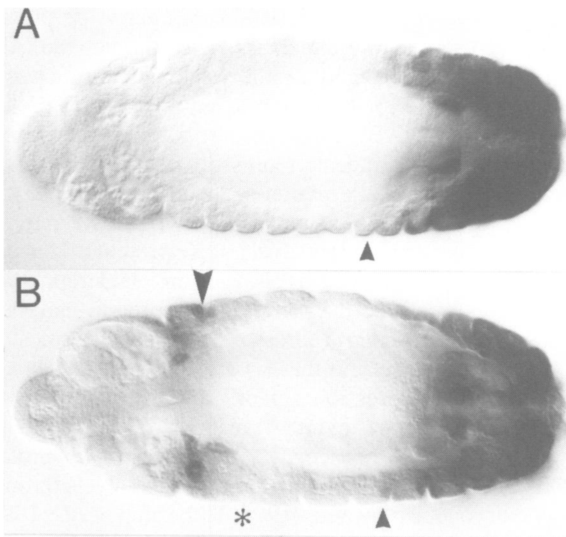

Fig. 7. *Abd-B* derepression in *hb* mutants. 10 h old embryos, viewed from dorsal side, stained with *Abd-B* antibody. (**A**) Wild type; (**B**) hb^{9K57} mutant embryo. The anterior limit of *Abd-B* expression at ps10 (small arrowhead) is unaltered in the mutant; however, there is strong ectopic expression in the epidermis of the mutant embryo (large arrowhead in B) anteriorly to the gap due to the *hb* mutation (above asterisk). Note also internal *Abd-B* derepression. Anterior is to the left.

β-gal staining is to a large extent eliminated outside this domain, particularly in ventral regions. Within ps6–13, we observe the BXD pattern evenly spread throughout, with no difference of staining intensity in ps6–9 compared with ps10–13. MCP and FAB silencing is reminiscent of PBX silencing, though different from silencing mediated by ABX, another *Ubx* silencer (Müller and Bienz, 1991), and different from silencing mediated by IAB5 which is detectable not only outside ps6–13, but also within ps6–9.

Silencing requires hb and Pc function

MCP and FAB mediate silencing in the same embryonic regions as *hb* protein binding sites (outside ps6–13). IAB5 silencing is also most efficient in these regions, and our results provided evidence that IAB5 is a target of early *hb* repression. This indicates that *hb* protein may be the repressor acting through all three fragments to promote silencing.

We tested this by analysing the MB, FB and IB patterns in *hb* mutant embryos. We chose hb^{9K57}, an *hb* null allele exhibiting loss of zygotic *hb* function as well as an antimorphic effect with respect to maternal *hb* function (Lehmann and Nüsslein-Volhard, 1987; Hülskamp, 1991), in order to minimize the functional contribution of maternal *hb* protein which perdures anteriorly in early hb^- embryos. For comparison, we analysed the β-gal pattern of HB transformants (bearing a construct in which the BXD pattern is silenced by *hb* protein binding sites; Zhang and Bienz, 1992) in the same mutants. Indeed, all four patterns appear to be anteriorly expanded in these *hb* mutant embryos (Figure 6). This expansion is best visible in the ventral nerve cord where we observe substantial β-gal staining anteriorly to the gap due to lack of *hb* function (white arrows in Figure 6); staining anteriorly to the gap appears equally strong as staining posteriorly to the gap. This apparent anterior expansion is virtually indistinguishable in all types of transformants, MB, FB, IB and HB. As the gap encompasses ~ps3–6 (White and Lehmann, 1986; Lehmann and Nüsslein-Volhard, 1987), this means that anterior β-gal staining is located in and/or anteriorly to ps3, i.e. in a region where β-gal staining is normally efficiently suppressed by the MCP, FAB and IAB5 silencers (Figure 5E–H). These results provide evidence for a requirement of *hb* for silencing and suggest that *hb* protein may act as a repressor to promote silencing through MCP, FAB and IAB5.

Finally, we tested the IB, MB and FB patterns in *Pc* mutant embryos and found, as expected (Müller and Bienz, 1991; Zhang and Bienz, 1992), that the BXD pattern is fully derepressed in these mutants: β-gal staining extends evenly from head to tail (Figure 5I and J; the derepressed patterns appear indistinguishable in all three cases). Thus, silencing mediated by IAB5, MCP and FAB requires *Pc* function.

hb functions as a repressor of Abd-B

The possible role of *hb* as an early repressor of IAB5 and in silencing came as rather a surprise since previous analyses did not reveal a function of *hb* in repressing *Abd-B*. There was only one piece of indirect evidence, from a phenotypic analysis of an antimorphic *hb* mutant, suggesting that *hb* may repress *Abd-B* in an anterior region of the embryo (Lehmann and Nüsslein-Volhard, 1987). We therefore wondered whether we could detect any ectopic *Abd-B* expression in this antimorphic mutant by staining mutant embryos with an *Abd-B* antibody.

We find that the anterior limit of *Abd-B* expression at ps10 is unaffected in these hb^{9K57} mutants, consistent with our notion (see above) that this limit is due to *Kr* repression. However, there is indeed conspicuous ectopic *Abd-B* staining in a region anterior to that (Figure 7). This staining is clearly visible in the epidermis anteriorly to the gap due to *hb* mutation (i.e. in or anterior to ps3). There is also ectopic staining inside the embryo, probably in mesodermal tissues including the tracheal tree (Figure 7B). This result provides direct evidence for a role of *hb* in repressing *Abd-B* expression in anterior regions of the embryo.

Discussion

Our work on *Abd-B* control sequences provides strong evidence that the regulatory mechanisms and components controlling *Abd-B* expression in the embryo are remarkably similar to those controlling *Ubx* expression. As in the case of *Ubx*, the primary confinement of *Abd-B* expression to a restricted domain along the body axis is achieved by repression. In both cases, repression is mediated by gap gene products which act as carriers of early embryonic positional information. From the comparative analysis of the two genes, a principle begins to emerge which may be used universally for ruling the expression of homeotic *Drosophila* genes. According to this, early transient repression mediated by gap gene products is converted into silencing, amounting to stable and permanent repression of a homeotic gene.

Early transient repression

Apart from its limits (see below), the IAB5 pattern is strikingly similar in virtually all aspects to the PBX pattern mediated by a *Ubx* control region. In both cases, the stripes of β-gal expression are strictly dependent on *ftz* function. From this, and by extrapolating from the results obtained

with the PBX control region (Müller and Bienz, 1992), we expect that *ftz* protein directly binds as an activator to the IAB5 fragment.

In the case of PBX, activity of *ftz* protein is only manifest in some parasegments, but is prevented in others by *hb* repressor (Müller and Bienz, 1992). Similarly, *ftz* protein mediates efficient transcriptional activation through the IAB5 fragment only in ps10 and 12 and weak activation in ps14 (and in ps4; Figure 8). A major role in preventing *ftz* protein activity through IAB5 must be attributed to *Kr* which functions as an efficient repressor of IAB5-mediated expression in the middle region of the embryo (in ps6 and 8, see Figure 8; in ps4, repression of *ftz* by *Kr* may be somewhat leaky, due to low levels of *Kr* protein in this ps). The weakness of the IAB5 ps14 stripe and its derepression in *hb* mutants indicate that, in ps14, *hb* protein may be a (somewhat less efficient) repressor of the posteriormost *ftz* activity. It is conceivable that *hb* also plays a crucial role in anteriormost repression (in ps2 and perhaps in ps4): although the *hb* mutants did not reveal this repressor activity, the latter may have been masked by maternal *hb* protein which perdures anteriorly at moderately high levels through early embryogenesis (Tautz, 1988; Hülskamp *et al.*, 1990; Hülskamp, 1991). We consider it very likely, in analogy to the situation with PBX (Müller and Bienz, 1992), that *Kr* and probably *hb* protein compete as repressors with *ftz* protein for binding to IAB5 and/or for transcriptional activation, thereby preventing *ftz* protein activity in those parasegments in which their own levels are sufficiently high. We presume that *Kr*- and *hb*-mediated repression acts at short range (Small *et al.*, 1991; i.e. the repressor only affects activators bound to DNA nearby). The role of *ftz* in this process may be to define precisely the anterior limit of IAB5 (and *Abd-B*) expression and to align it with a parasegmental boundary (Müller and Bienz, 1992), but also to prevent silencing (see below) in order to keep nearby control sequences accessible for activators (e.g. those marked by L in Figure 9) that are not available till later in development (Bienz, 1992).

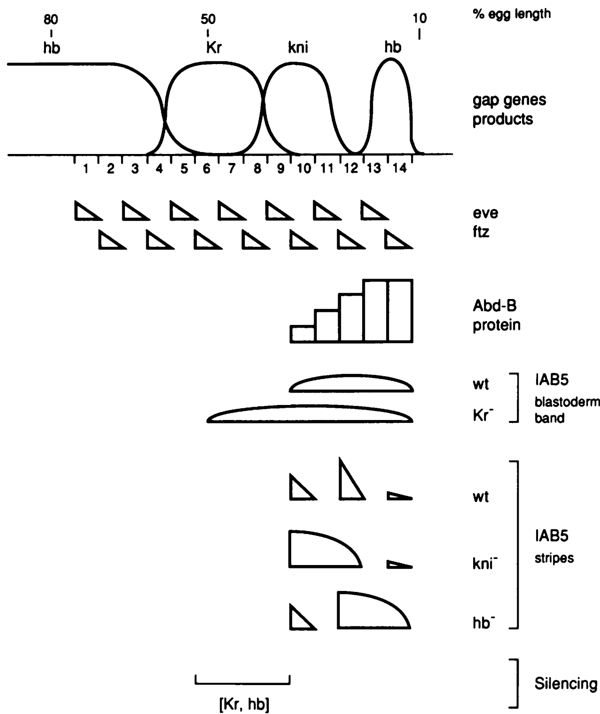

Fig. 8. Positional information determining the IAB5 pattern. **Above**, bell-shaped distributions of gap gene products (*hb*, *Kr* and *kni* proteins), according to Tautz (1988), Gaul and Jäckle (1989) and Pankratz *et al.* (1990), and stripes of *ftz* and *eve* protein expression with relation to egg length (in %) and parasegments (ps). **Below**, *Abd-B* domain (ps10–14; graded expression of *Abd-B* m protein indicated schematically by steps) and early band of IAB5 pattern as well as subsequent *ftz*-like IAB5 stripes in the wild type (all schematically drawn). Mutant IAB5 patterns (in Kr^-, kni^- and hb^-) showing that *Kr* repressor determines the anterior IAB5 limit, whereas *kni* and *hb* repressors modify IAB5 expression within the IAB5 (or *Abd-B*) domain. The bracket at the bottom indicates the region (ps6–9) within which *hb* repressor may be assisted by *Kr* repressor in order to prompt silencing (see text).

Our results also hint at a possible role of *kni* as a repressor of early IAB5-mediated expression (Figure 8). *kni* protein too may compete at short range with *ftz* protein (or with other early activators, see below) for transcriptional activation. The weak repression by *kni* as observed is most effective in the embryonic region containing maximal *kni* protein levels, in ps10 (Pankratz *et al.*, 1990).

Thus there is evidence that all three gap gene products act as repressors through IAB5, *Kr* protein in the middle

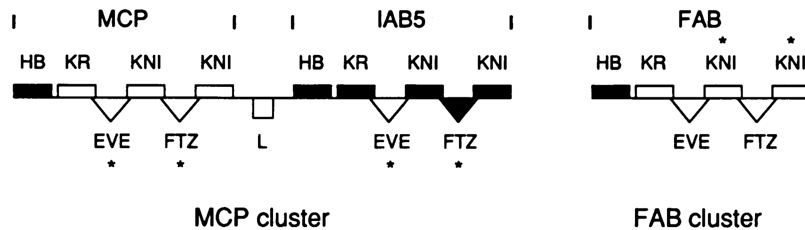

Fig. 9. Hypothetical structures of MCP and FAB clusters. We propose that the MCP and FAB clusters represent large 'Levinian elements' (Small *et al.*, 1991), consisting of multiple binding sites, adjacent to and overlapping one another, at which repressors (shapes above line) and activators (shapes below line) compete for transcriptional activation of a linked and distant promoter. Both clusters may be constructed in a similar fashion as indicated in the figure (filled shapes: binding sites proposed on the basis of experimental evidence; open shapes, purely hypothetical binding sites; extent of MCP, IAB5 and FAB fragments indicated above clusters); for simplicity, only one or two binding sites are indicated for each type of regulator. We propose that *ftz* and *eve* individually compete as activators with *kni* (and probably with *Kr*) as repressors at these clusters and that this competition, after gastrulation, is first won at the FAB cluster by *ftz* protein (in ps12), later at the same cluster by *eve* protein (in ps11), and finally at the MCP cluster by *ftz* protein (in ps10). The outcome of these competitions may be determined by relative affinities of sites, by number of sites and/or by cooperativity between proteins binding to these sites; asterisks signify a balance in favour of repressor binding sites at the FAB cluster, and a balance in favour of activator binding sites at the MCP cluster. The square marked with L signifies one of several putative late embryonic activators (as found in the corresponding genomic *Abd-B* fragment) which may be responsible for the particular parasegmental pattern of *Abd-B* expression in ps10–12. See also main text.

region of the embryo (determining the anterior IAB5 limit at ps10), *hb* protein in posteriormost and perhaps in anterior embryonic regions, and *kni* protein modifying the levels of IAB5 expression within the IAB5 expression domain.

Harding and Levine (1988) analysed the expression patterns of early *Abd-B* transcripts in gap mutants and found an anterior expansion of the band of early *Abd-B* expression to a new anterior limit at ~ps10 in *kni* mutants, but no expansion of this band in *Kr* mutants. Their analysis implied that *kni* acts as a strong repressor of early *Abd-B* transcription in a region spanning ~ps10−12, lending strong support to our notion of *kni* acting as a repressor of IAB5 in the same region. This strong repressor function of *kni* on *Abd-B* expression might be expected to obscure a repressor function of *Kr* since *kni* protein remains expressed in its normal domain in *Kr* mutants (Pankratz *et al.*, 1989): indeed, in *Kr kni* double mutants, early *Abd-B* transcription is strikingly derepressed towards anterior, far beyond ps10 and spreading throughout most of the embryo (Harding and Levine, 1988). Thus, the results by Harding and Levine (1988) largely agree with our results on IAB5, identifying *Kr* and *kni* protein as repressors of *Abd-B* (note that expression of *Abd-B* appears to be more sensitive to *kni* repression than that mediated by IAB5). In addition, we found strong evidence, analysing an antimorphic *hb* mutant, for *hb* being a repressor of *Abd-B* in anterior embryonic regions (Figure 7), a function that was not revealed in the study of *hb* null mutants (Harding and Levine, 1988), probably for reasons mentioned. In summary, the three gap gene proteins evidently act as repressors not only of IAB5, but also of *Abd-B* expression during early embryonic development.

Silencing

Each of the three *Abd-B* control fragments described here confers repression. We refer to this as silencing (Brand *et al.*, 1985) since repression occurs at a distance and since it affects a heterologous enhancer and promoter from *Ubx*. Another heterologous promoter affected by IAB5-mediated silencing is the *ry* promoter, though the hsp70 promoter proved refractory to silencing. Silencing mediated by *Abd-B* control regions requires *Pc* function.

Pc-dependent silencing is also conferred by *Ubx* control fragments (Müller and Bienz, 1991). In this case, the embryonic regions in which silencing is effective coincide with the regions expressing *hb* protein, and *hb* protein binding sites are indeed sufficient to confer this silencing activity (Zhang and Bienz, 1992). The MCP and FAB fragments display a silencing activity which, in every respect (including its dependence on *hb* function), is indistinguishable from that of *hb* protein binding sites. The IAB5 fragment also shows *hb*-dependent silencing and, additionally, appears to be subject to *hb* repression in the early embryo. Therefore, it seems possible that *hb* protein acts through MCP, FAB and IAB5 to promote silencing outside ps6−12.

Which is the repressor promoting the partial IAB5 silencing activity in the embryonic region of ps6−9? *hb* protein levels are hardly detectable in this region (Tautz, 1988), and it therefore seems unlikely that this protein is capable of binding to IAB5 by itself in ps6−9. However, *Kr* protein apparently acts as an early repressor of IAB5 in this region. This suggests that *Kr* may play a crucial role in promoting subsequent silencing in the same embryonic region. *Kr* protein might effect this function by itself, by interacting, directly or indirectly, with one (or several) of the *Pc*-like proteins to prompt the formation of a stable silencing complex (Zhang and Bienz, 1992; Bienz, 1992). Alternatively, *Kr* protein might act more indirectly, tethering *hb* protein to IAB5: there is recent evidence for cooperative interactions between *Kr* and *hb* protein (H.Jäckle, personal communication). Note that *hb* acts as a repressor of early gap gene expression in posterior embryonic regions in which there is no detectable *hb* protein (Struhl *et al.*, 1992). Tethering of *hb* protein by other gap gene products to control regions of *Abd-B* and perhaps of other homeotic genes would be necessary, if *hb* repressor had a universal role in the establishment of silencing complexes, e.g. by virtue of its capability of interacting with members of the *Pc* group proteins.

Control of the Abd-B gene

The embryonic patterns of *Abd-B* protein expression in ps10−12 seem to vary only with respect to expression levels (DeLorenzi and Bienz, 1990), suggesting that the same kind of enhancer sequences are active, albeit to a different extent, in these parasegments. Factors contributing to the differential activity of these enhancers could be their distance from the *Abd-B* promoter, their relative strength of interaction with this promoter via 'adaptor proteins' (Bienz, 1992) as well as the time at which they first become occupied with activators.

The fruit of our search for *Abd-B* enhancers was surprisingly meagre: we only found two fragments with enhancer activity within >50 kb of the *Abd-B* 3′ flanking region. It is possible that there are more of these, and that we missed them for a variety of reasons. Nevertheless, it seems striking that the four fragments we found with enhancer or silencer activity are derived from one of two locations within these >50 kb, coinciding with the locations of the *Mcp* and *Fab* deletions (Karch *et al.*, 1985; Gyurkovics *et al.*, 1990). We would like to propose that these two regions constitute the two main clusters (called the MCP and FAB clusters) of downstream *cis*-regulatory sequences controlling *Abd-B* expression in ps10−12 (Figure 9).

It has been proposed by Gyurkovics *et al.* (1990) that the *Mcp* and *Fab* sequences in the wild type represent insulating sequences or 'boundaries' between ps-specific *cis*-regulatory regions of *Abd-B*. These authors explain the effects of the *Mcp* and *Fab* mutations by suggesting that a set of ps-specific enhancers becomes activated in a more anterior parasegment because of its new intimate vicinity, uninterrupted by a 'boundary', to a new set of ps-specific enhancers active in a more anterior parasegment. We would like to propose an alternative view, based on our finding that the MCP and FAB fragments contain target sites for repressors, as follows.

This view is based on the hypothesis that the repressor function of *kni* within ps10−12 (see Harding and Levine, 1988, and Figure 4D) confers the different ps-specific levels of *Abd-B* expression within this region. According to this hypothesis, the two regulatory clusters, MCP and FAB, might initially be fully occupied by *kni* repressor; this would result in a restriction of *Abd-B* expression to ps13 and 14, as observed by Sánchez-Herrero and Crosby (1988), Kuziora and McGinnis (1988) and Harding and Levine (1988). The apparent anterior spreading of *Abd-B* expression, as observed subsequently, may reflect gradual relief of *kni* repression

as the levels of *kni* proteins start to decay. From the bell-shaped expression of *kni* protein, maximal in ps10 (Pankratz *et al.*, 1990), we expect that *kni* protein sinks below a critical threshold first in ps12 and last in ps10. We further propose that the relief of *kni* repression is stepwise and mediated by *ftz* and, by analogy with *Ubx* (Müller and Bienz, 1992), by *eve* protein. Either of these proteins may compete at short range (Small *et al.*, 1991) with *kni* repressor, and perhaps also with *Kr* repressor, for transcriptional activation of the *Abd-B* gene. There should be three discrete time points where the balance between activators and repressors becomes tipped at one of the two regulatory clusters, FAB or MCP, so that the short-range competition is won by one of the activators, *eve* or *ftz*. Central to the hypothesis is that winning of the short-range competition at a particular cluster is intimately linked to developmental time and position along the body axis (see legend of Figure 9 for further explanations). We would like to emphasize that although *ftz* and *eve* can be considered ps-specific activators, it is not their role to convey unique positions or 'coordinates' along the body axis; instead, these 'coordinates', in our hypothesis, are conferred by a decaying repressor, by *kni* protein.

The two types of anterior derepression due to *Mcp* and *Fab* deletion can be explained if we assume that, in each of the two mutations, a majority of the repressor binding sites are deleted at a particular cluster, whereas sufficient *eve* activator binding sites are left intact at the same cluster. This would favour the competition exerted by *eve* protein and might thus lead to anterior derepression in ps9 (*Mcp*) and ps11 (*Fab*), respectively, as observed in these mutants. A third mutation, called *Sab*, causes ectopic expression of *Abd-B* in ps8, but not in ps9 (Celniker *et al.*, 1990), suggesting that *Sab* might consist of deletion of repressor binding sites at the MCP cluster which in this case would favour *ftz* competition at this cluster. We expect that all three mutations result in the removal of repressor binding sites (of *kni* and probably of *Kr* repressor), although we would like to emphasize that their target sites in the MCP and FAB fragments currently are purely hypothetical (repression through these fragments would have gone unnoticed as the latter do not confer any β-gal expression).

Conclusions

Above, we have stressed the view that the *Abd-B* gene is controlled by the same principal mechanisms and by many of the same regulatory proteins as the *Ubx* gene. The chief difference evidently concerns the repressing role of gap gene products. In the case of *Ubx*, *hb* protein appears to be the only repressor responsible for establishing the anterior limit of *Ubx* expression and for maintaining this limit by promoting silencing. In the case of *Abd-B*, *hb* protein may also have a role in early anterior *Abd-B* repression and is required to promote silencing, but, in addition, *Kr* repressor is necessary to define the anterior limit of *Abd-B* expression and to promote silencing in the middle region of the embryo. Finally, *kni* repressor may be responsible for the graded expression of *Abd-B* protein within the embryonic *Abd-B* domain.

The *Abd-B* m protein is unique among *Drosophila* homeotic products as its expression apparently spreads across several parasegments from posterior towards anterior during early embryogenesis, maturing into an expression pattern with a distinct anteroposterior gradation. Interestingly, expression of *Abd-B* in the locust *Schistocerca*, a short germ band insect, displays similar anterior spreading as well as anteroposterior gradation (Kelsh *et al.*, 1993). Furthermore, in vertebrate species, there appear to be multiple adjacent *Abd-B*-like genes whose expression in growing limb buds differs from one another with respect to time as well as axial limits (Dollé *et al.*, 1989; Izpisúa-Belmonte *et al.*, 1991; reviewed by Duboule, 1992), a phenomenon vaguely reminiscent of insect *Abd-B* expression spreading in space and time. Cell divisions, gain of cell mass as well as lapse of time are inherent parameters of budding insect segments and growing vertebrate limb buds. It is conceivable that these two cellular systems also rely on the principle of decaying (or diluted) repressors, such as proposed here for *Drosophila Abd-B*, for the temporal and spatial control of their homeotic genes.

Materials and methods

Fly strains

Host flies for P-element transformation were *cn*; ry^{42} or w^{1118}. The following mutant alleles of segmentation genes were used: hb^{XT15} and hb^{9K57} (the latter is an antimorphic allele belonging to the class of 'homeotic' *hb* alleles; Lehmann and Nüsslein-Volhard, 1987; Hülskamp, 1991), Kr^{1}, kni^{IIE72}, kni^{IID}, ftz^{w20}, $eve^{3.77}$ (Tearle and Nüsslein-Volhard, 1987), Pc^{3} (Lewis, 1978). Most of the segmentation gene alleles were balanced with *CyO* and *TM3* balancer chromosomes bearing *hb*/β-gal transposons (obtained from G.Struhl), for unambiguous identification of homozygous mutant embryos.

Plasmids and transformation

Three different transformation vectors were used: HZ50PL (Hiromi and Gehring, 1987) and pRCm1, both containing the ry^{+} gene, and pWZ50PL (obtained from T. Gutjahr) containing the w^{+} gene (for identification of transformants). HZ50PL and pWZ50PL are related vectors containing a minimal hsp70 promoter and mRNA leader sequence fused to a bacterial β-gal gene as well as a similar polylinker, upstream adjacent to the hsp70 TATA-box. pRCm1 is similar to HZ50PL, except that the hsp70 sequences are substituted by the corresponding sequences from the *Abd-B* m gene (Zavortink and Sakonju, 1989); the latter are contained within a *Hin*dIII–*Pst*I fragment (from +157 to +163; Karch *et al.*, 1985) spanning 4.1 kb of 5′ flanking, 1.2 kb of mRNA leader and some coding sequence. The fusion between *Abd-B* and β-gal sequences is after the 5th amino acid of the *Abd-B* m protein (Zavortink and Sakonju, 1989). pRCm1 contains unique *Xba*I, *Not*I and *Kpn*I sites (5′ → 3′) upstream of the proximal *Abd-B* promoter into which further fragments from the 3′ flanking sequence of *Abd-B* were cloned (see below).

*Sal*I and *Hin*dIII fragments from the lambda phages R8019, L8034, R8053, L8060 and L8077 (Karch *et al.*, 1985) were subcloned into Bluescript and subsequently introduced as *Kpn*I–*Xba*I or *Kpn*I–*Not*I fragments into the polylinkers of transformation vectors (orientation with respect to transcription the same as in the genome). Several independent transformant lines were isolated in each case as described; many of these were made homozygous for the transposon insertion (Bienz *et al.*, 1988). A total of 17 fragments were tested as pRCm1 constructs (see Results; some of these fragments, or subfragments thereof, were further tested as pWZ50PL or as HZ50PL constructs). Two of these (IAB5 and FAB) were also tested as pWZ50PL or as HZ50PL constructs. The genomic positions of fragments described in the text, MCP, IAB5 and FAB, are 95–99, 104–105 and 123–127, respectively (Karch *et al.*, 1985). We failed to get ry^{+} transformants with an HZ50PL construct containing IAB5 (see Results), but we were able to isolate transformants after insertion into this construct (between the IAB5 fragment and the ry^{+} gene) of a 350 bp BaBx fragment containing *suppressor-of-Hairy-wing* protein binding sites (Holdridge and Dorsett, 1991). The IAB5 pattern was found to be essentially the same if the *Abd-B* proximal promoter was replaced with an hsp70 TATA-box. Although the latter construct showed virtually no background staining, we did not use this type of construct for our initial screen as the hsp70 TATA-box is liable to fail to respond to enhancers in large constructs (Müller and Bienz, 1991).

For silencing tests, the IAB5, MCP and FAB fragments were inserted into a silencing vector containing the minimal BXD enhancer joined to the *Ubx* proximal promoter (Zhang and Bienz, 1992). The three fragments were

inserted as *Not*I/*Kpn*I fragments into unique *Not*I and *Kpn*I sites between BXD enhancer and proximal promoter (IB, MB and FB constructs). Silencing was observed in many, but not all independent transformant lines: two out of four (IB), four out of six (MB) and three out of five (FB); silencing efficiency was comparable in the different isolates that showed silencing (see Figure 5). Silencing was, however, not observed in any of the eight independent transformants from a construct in which the proximal *Ubx* promoter was substituted with an hsp70 TATA-box.

Detailed maps of the plasmids used are available on request.

Antibody staining

Transformed embryos were fixed and stained with antibody as described by Lawrence and Johnston (1989) and Tremml and Bienz (1989). A polyclonal rabbit serum (Cappell) or a monoclonal antibody (Vector Laboratories) against β-gal as well as a monoclonal antibody against *engrailed* (Patel *et al.*, 1989; provided by M.Wilcox) or against *Abd-B* (Celniker *et al.*, 1989) were used. We found that, in the case of the monoclonal β-gal antibody, the protocol used by Lawrence and Johnston (1989) gave the best results (though formaldehyde instead of paraformaldehyde was used for fixation).

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