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Regulation of the *infraabdominal* regions of the bithorax complex of *Drosophila* by gap genes

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SUMMARY

The expression of the *abdominal-A* and *Abdominal-B* genes of the bithorax complex of *Drosophila* is controlled by *cis*-regulatory *infraabdominal* regions. The activation of these regions along the anteroposterior axis of the embryo determines where *abdominal-A* and *Abdominal-B* are transcribed. There is spatially restricted transcription of the *infraabdominal* regions (*infraabdominal* transcripts) that may reflect this specific activation. We show that the gap genes *hunchback, Krüppel, tailless* and *knirps* control *abdominal-A* and *Abdominal-B* expression early in development. The restriction of *abdominal-A* and *Abdominal-B* transcription is preceded by (and requires) the spatially localized activation of regulatory regions, which can be detected by the distribution of *infraabdominal* transcripts. The activation of these regions (except the *infraabdominal-8* one) could require no specific gap gene. Instead, a general mechanism of activation, combined with repression by gap genes in the anteroposterior axis, seems to be responsible for delimiting *infraabdominal* active domains. The gradients of the *hunchback* and *Krüppel* products seem to be key elements in this restricted activation.

Key words: bithorax complex, gap genes, hunchback gradient, *Drosophila*

INTRODUCTION

The homeotic genes of *Drosophila*, which are clustered in the *Antennapedia* and *bithorax* (*BX-C*) complexes, are required for segment specification along the anteroposterior (A/P) axis of the embryo and adult (Lewis, 1978; Duncan, 1987; Kaufman et al., 1990). The crucial role of these genes demands a precise mechanism to restrict their expression along the A/P axis. When this control fails, homeotic transformations occur, frequently leading to a lethal phenotype (e.g. Lewis, 1978; Struhl, 1981; Schneuwly et al., 1987; Kuziora and McGinnis, 1988a).

The gap genes are required for the development of contiguous regions of the body (Nüsslein-Volhard and Wieschaus, 1980; reviewed in Hülskamp and Tautz, 1991; Pankratz and Jäckle, 1993). It has been shown that the gap genes are primary determinants in delimiting the expression of homeotic genes along the A/P axis (White and Lehmann, 1986; Ingham et al., 1986; Harding and Levine, 1988; Irish et al., 1989; Reinitz and Levine, 1990; Jack and McGinnis, 1990; Riley et al., 1991; Busturia and Bienz, 1993). Within this context, the control of the *Ultrabithorax* (*Ubx*) gene of the BX-C by the gap gene *hunchback* (*hb*) has been studied in detail. *hb* establishes the *Ubx* anterior limit of expression (White and Lehmann, 1986; Irish et al., 1989) through the interaction of the *hunchback* protein with the *abx*/*bx* regulatory region of the *Ubx* gene (Simon et al., 1990; Qian et al., 1991; Zhang et al., 1991; Müller and Bienz, 1992; Zhang and Bienz, 1992; Qian et al., 1993). Therefore, the spatial restriction of the *Ubx* transcription is a consequence of the spatial limited activation of their regulatory regions. The way gap genes regulate the spatial activation of the regulatory regions of the *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*) genes of the BX-C, however, has not been studied in detail.

The *abd-A* and *Abd-B* genes specify the parasegments (PS) 7-14 (abdominal segments A2-A9; Sánchez-Herrero et al., 1985; Tiong et al., 1985). The transcription of *abd-A* and *Abd-B* in different parasegments is controlled by regulatory regions called *infraabdominal* (*iab*) regions (*iab-2* - *iab8*) (Sánchez-Herrero and Akam, 1989; Karch et al., 1990; Macías et al., 1990; Celniker et al., 1990; Gyurkovics et al., 1990; Boulet et al., 1991; Sánchez-Herrero, 1991; Crosby et al., 1993). These regions contain regulatory elements that direct, early in development, the transcription of a *lac-Z* reporter gene with a precise parasegmental anterior limit of expression (Simon et al., 1990, 1993; Busturia and Bienz, 1993; Shimell et al., 1994). This implies an ability of the *iab* domains to receive and confer positional information along the A/P axis.

A particular property of most BX-C regulatory DNA is that it is transcribed at blastoderm (Lipshitz et al., 1987; Sánchez-Herrero and Akam, 1989; Cumberledge et al., 1990). The anterior limit of expression of transcripts from different regions bears a relationship with the order of these DNA regions on the chromosome (Sánchez-Herrero and Akam, 1989). This is similar to the relation between the chromosomal order of *iab*

mutations and the order of segments transformed (Lewis, 1978). However, the role of these transcripts, if any, is unknown. It has been proposed that the transcription in the *iab* regions reflects their differential activation along the A/P axis at the blastoderm stage (Sánchez-Herrero and Akam, 1989). Thus, the transcription would indicate that the region is 'open' or active (Peifer et al., 1987; Gyurkovics et al., 1990), that is, able to contact the *abd-A* or *Abd-B* promoters and make them transcribe. Therefore, the final boundaries of *iab* regulation could be already defined at blastoderm. A similar explanation was previously proposed for the transcription in the *bithoraxoid* regulatory region of the *Ubx* gene (Lipshitz et al., 1987).

We have investigated the genes that promote this differential 'opening' of *iab* sequences. To this aim, we have studied the changes in *iab* transcription in different gap mutants, and correlate them with changes in *abd-A* and *Abd-B* expression. In the case of *Abd-B* we have studied the separate effect of gap genes on the different *Abd-B* promoters. We find that mutations in some gap genes alter the activity of *iab* regulatory elements at the blastoderm stage, as monitored by the distribution of *iab* transcripts, while other mutations leave them unchanged. Many of these effects (or lack of them) correlate with changes on *abd-A* and *Abd-B* expression. The *iab* regions (except *iab-8*) may be activated by a general unknown activator and be repressed anteriorly by the *hunchback* and *Krüppel* (*Kr*) genes. The gap genes *tailless* (*tll*) and *knirps* (*kni*) delimit the activation of the *iab-8* domain. The graded distributions of the *hb* and *Kr* products seem to be key elements that control the activation of the *iab* domains.

MATERIAL AND METHODS

Genetics

The mutations in the gap genes used are: $Df(3)p^{xT15}$ (Lehmann and Nüsslein-Volhard, 1987), kni^{2D48} (Jürgens et al., 1984), Kr^{1} (Wieschaus et al., 1984), tll^{G} (Strecker et al., 1988) and gt^{X11} (Petschek et al., 1987). The BX-C mutations are described in Lewis (1978), Sánchez-Herrero et al. (1985), Karch et al. (1985) and Casanova et al. (1986).

In situ hybridization

Whole-mount in situs were done as described in Tautz and Pfeifle (1989). Mutations in the gap genes were balanced over *CyO* or *TM3* chromosomes carrying an insertion of the *lac-Z* gene under the control of the *hb*-promoter (gifts of Gary Struhl). This allows the identification of the mutant embryos by staining with an antibody against the β-galactosidase protein. The limits of expression at blastoderm were measured in percentages of egg length (0% corresponding to the posterior pole). The genomic probes used for the transcripts in the *iab* region are described in Sánchez-Herrero and Akam (1989). The *abd-A* probe is a 1.7 kb *Eco*RI fragment from an *abd-A* cDNA (kindly provided by François Karch). The probes for the *m* and *r* transcript correspond to the probes B and D described in Sánchez-Herrero and Crosby (1988).

Antibody staining

It was done as described in Sánchez-Herrero (1991). Antibodies were polyclonal anti-*abd-A* (Macías et al., 1990) and monoclonal anti-*Abd-B* (Celniker et al., 1989), kindly provided by Jordi Casanova and Susan Celniker, respectively. Mutant embryos were recognized by balancing the mutations over the *CyO* and *TM3* balancer chromosomes described above.

Embryonic cuticle

Embryonic cuticles were mounted as described in Wieschaus and Nüsslein-Volhard (1986).

RESULTS

The *iab-2*, *iab-3* and *iab-4 cis*-regulatory regions control the parasegmental expression of *abd-A*, and the *iab-5* to *iab-8* regions that of *Abd-B* (Sánchez-Herrero and Akam, 1989; Celniker et al., 1990; Karch et al., 1990; Macías et al., 1990; Boulet et al., 1991; Sánchez-Herrero, 1991). The *iab-3* to *iab-7* regions are transcribed early in embryogenesis (Sánchez-Herrero and Akam, 1989; Cumberledge et al., 1990). Transcripts corresponding to the *iab-3*/*iab-4* region (type I transcripts), *iab-5*/*iab-6* region (type II) and *iab-7* region (type III transcripts) are expressed with anterior limits at about 38%, 28% and 20% egg length, (EL) respectively, and have a common posterior limit of expression around 10% EL (Sánchez-Herrero and Akam, 1989; Cumberledge et al., 1990; Fig. 1). Since the transcription of these RNAs along the A/P axis may be indicative of the 'opening' of *iab* domains (Sánchez-Herrero and Akam, 1989; Cumberledge et al., 1990), we have compared the transcription of type I RNAs in gap mutants with the expression of the *abd-A* product, and of type II and III transcripts with that of *Abd-B*. Changes in *iab* transcription at blastoderm would reflect changes in enhancer activity in situ and this would alter *abd-A* and *Abd-B* transcription. In accordance, *iab* transcription precedes *abd-A* or *Abd-B* expression in wild-type (Fig. 2F-G) or mutant embryos. We have also studied *abd-A* and *Abd-B* expression in embryos double mutant for gap genes and *iab* regions to ascertain the activation of the *iab-2* region (which shows no transcription at blastoderm) and other ones. Fig. 1B summarizes the results obtained.

hunchback and *Krüppel* establish the anterior limit of *abd-A* expression by spatially restricting the activation of the *iab-2* to *iab-4* regions

Transcription of *abd-A* in wild-type embryos begins at the end of the cellular blastoderm, in a domain from about 44% to 20% EL (Fig. 2A; see also Macías et al., 1994), fading somewhat at the edges. ABD-A protein is first detected at germ band extension in PS7-13 (Karch et al., 1990; Macías et al., 1990; Fig. 3A).

At blastoderm, expression of *abd-A* is observed more anteriorly in *hb* embryos, from around 52% to 20% EL (Fig. 2B), than in the wild type. In *Kr* blastoderms there is no such a clear extension, although some embryos seem to show a more anterior transcription of *abd-A* to about 48% EL (Fig. 2C). When the protein is first detected, it is also observed more anteriorly in *hb* or *Kr* mutants (Fig. 3C,E), with an irregular anterior limit of expression. The strongest signal within these irregular bands coincides with *en* expression, as in wild-type or other mutant combinations (Karch et al., 1990; Macías et al., 1990, 1994; unpublished results).

We have studied if there is abnormal activation of *iab-3*/*iab-4* sequences in *hb* and *Kr* mutants by monitoring *iab* transcription. *iab-3*/*iab-4* (type I) transcripts either seem not to change their limits of expression or are expressed slightly more anteriorly in *hb* embryos (to about 42% EL; Fig. 4D).

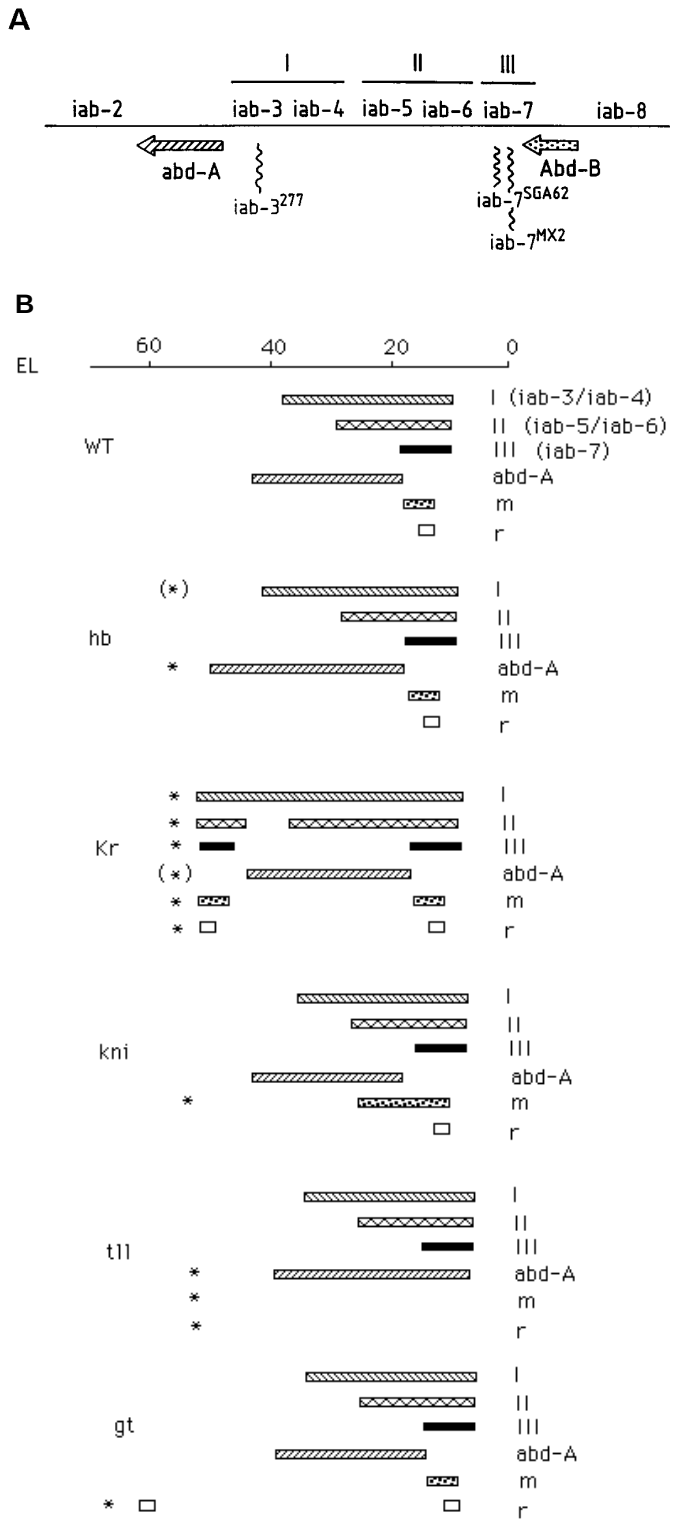

Fig. 1. (A) Map of the BX-C DNA showing the *iab* regions, the DNA regions where the three classes of *iab* RNAs are detected (Sánchez-Herrero and Akam, 1989), and the position of the *iab-3* and *iab-7* breakpoints used. (B) Summary of the expression of *abd-A*, *Abd-B* and *iab* RNAs at blastoderm in wild-type and mutant embryos. At the top, the percentage of egg length (EL). The asterisks show the patterns that change. Asterisks in brackets indicate that the changes in expression are not clear in all the embryos. Type III transcripts are sometimes absent in *tll* embryos.

Enhancers of the *iab-3* region, when fused to the *lac-Z* gene, show changes in their anterior limit of β-galactosidase expression in *hb* mutants (Simon et al., 1990). There is also a clear more anterior activation of *iab3*/*iab4* sequences in *Kr* embryos (to around 54% EL; Fig. 4G).

We note that the anterior limit of expression of *abd-A* in *hb* embryos (52% EL) differs from the anterior limit of *iab-3/iab-4* activation (42% EL). A difference is also observed in *Kr* embryos. To see if the *iab-2* region could be responsible for this discrepancies, we have looked at ABD-A protein distribution in *hb iab-3^{277}* and *Kr iab-3^{277}* embryos. We reasoned that, by looking at the *abd-A* expression when *iab-3/4* sequences are separated from the *abd-A* promoter by the *iab-3^{277}* breakpoint (Karch et al., 1985; Fig 1A), we could study the effect of the gap genes on the *iab -2* region. In *hb iab-3^{277}* embryos (Fig. 3D), the expression of ABD-A is extended more anteriorly than in the wild type, as in *hb* embryos (Fig. 3C). Similarly, in *Kr iab-3^{277}* embryos (Fig. 3F), the more anterior expression of ABD-A protein is also like in *Kr* embryos (Fig. 3E). Thus, in the absence of *hb* or *Kr*, the *iab-2* region is also activated more anteriorly, being responsible for the *abd-A* expression in *hb iab-3* or *Kr iab-3* embryos. Enhancers of the *iab-2* region, when fused to the *lacZ* gene, also show an anterior expression of β-galactosidase signal in *hb* and *Kr* mutants (Simon et al., 1990; Shimell et al., 1994). Although *hb* and *Kr* may also act directly on the *abd-A* promoter and not through the *iab-2* region, an effect of *Kr* on *iab-2* sequences has been demonstrated by Shimell et al. (1994). We conclude that *iab-2*, *iab-3* and *iab-4* sequences are active more anteriorly in *hb* and *Kr* embryos. However, initial *abd-A* transcription in *Kr* embryos does not extend anteriorly as type I transcripts do, suggesting that, in *Kr* blastoderms, the *abd-A* promoter probably integrates different regulatory inputs to establish its initial anterior limit of expression.

tailless delimits posteriorly the *abd-A* expression, while *knirps* and *giant* do not affect its anterior or posterior limits

In *tll* embryos, the transcription of *abd-A* extends posteriorly to about 13% EL at blastoderm (Fig. 2D). In germ band extended embryos, ABD-A expression extends also posteriorly (Fig. 3B). Region I transcripts have normal limits of expression in *tll* embryos (Fig. 4J). Since in wild-type embryos, the *iab-3*/*iab-4* sequences are active in the 10%-20% EL region, where there is no *abd-A* transcription, perhaps the effect of *tll* on *abd-A* could be mediated by repression through the *iab-2* region or directly through the *abd-A* promoter.

In *knirps* and *giant* embryos *abd-A* transcription is normal at blastoderm (Fig 2E; data not shown for *gt*), although in *kni* blastoderms the signal seems to be weaker. The distribution of type I transcripts does not change in *kni* or *gt* embryos either (not shown). The limits of ABD-A protein expression in *kni* or *gt* mutants are also as in wild-type embryos, although the distribution within the *abd-A* domain is abnormal. In *kni* embryos, the anterior border of expression is sharp when the protein is first detected but turns fuzzy later on. As in *hb* and *Kr* embryos, an (irregular) limit of expression is maintained, even though in *kni* embryos there is no *en* band to delimit this transcription.

Ectopic activation of *iab-5* to *iab-7* regions in *Kr* blastoderms correlates with later abnormal expression of ABD-B protein

The *Abd-B* gene contains two sets of transcripts and two different proteins (*m* or type I and *r* or type II) that specify PS10-13 and 14, respectively (Casanova et al., 1986; Sánchez-Herrero and Crosby, 1988; DeLorenzi et al., 1988; Kuziora and McGinnis, 1988b; Celniker et al., 1989; DeLorenzi and Bienz, 1990; Boulet et al., 1991). The *m* RNA is first transcribed at late cellular blastoderm, in the primordium of PS13 (Harding and Levine, 1988; Sánchez-Herrero and Crosby, 1988; Kuziora and McGinnis, 1988b; Boulet et al., 1991; Fig. 5A), probably under the control of the *iab-8* region. This region, which should be responsible for *Abd-B* expression in PS13, is probably located upstream the *m* transcription unit (unpublished results). The ABD-B m protein is initially detected, as the germ band extends, in PS13. Then, it extends stepwise from PS12 to PS10, in the same way that the *m* transcript does (Sánchez-Herrero and Crosby, 1988; Kuziora and McGinnis, 1988b; Celniker et al., 1989; DeLorenzi and Bienz, 1990; Boulet et al., 1991), under the control of *iab-5* to *iab-7* sequences. The *r* element transcript (class C RNA, Boulet et al., 1991) and protein are expressed in

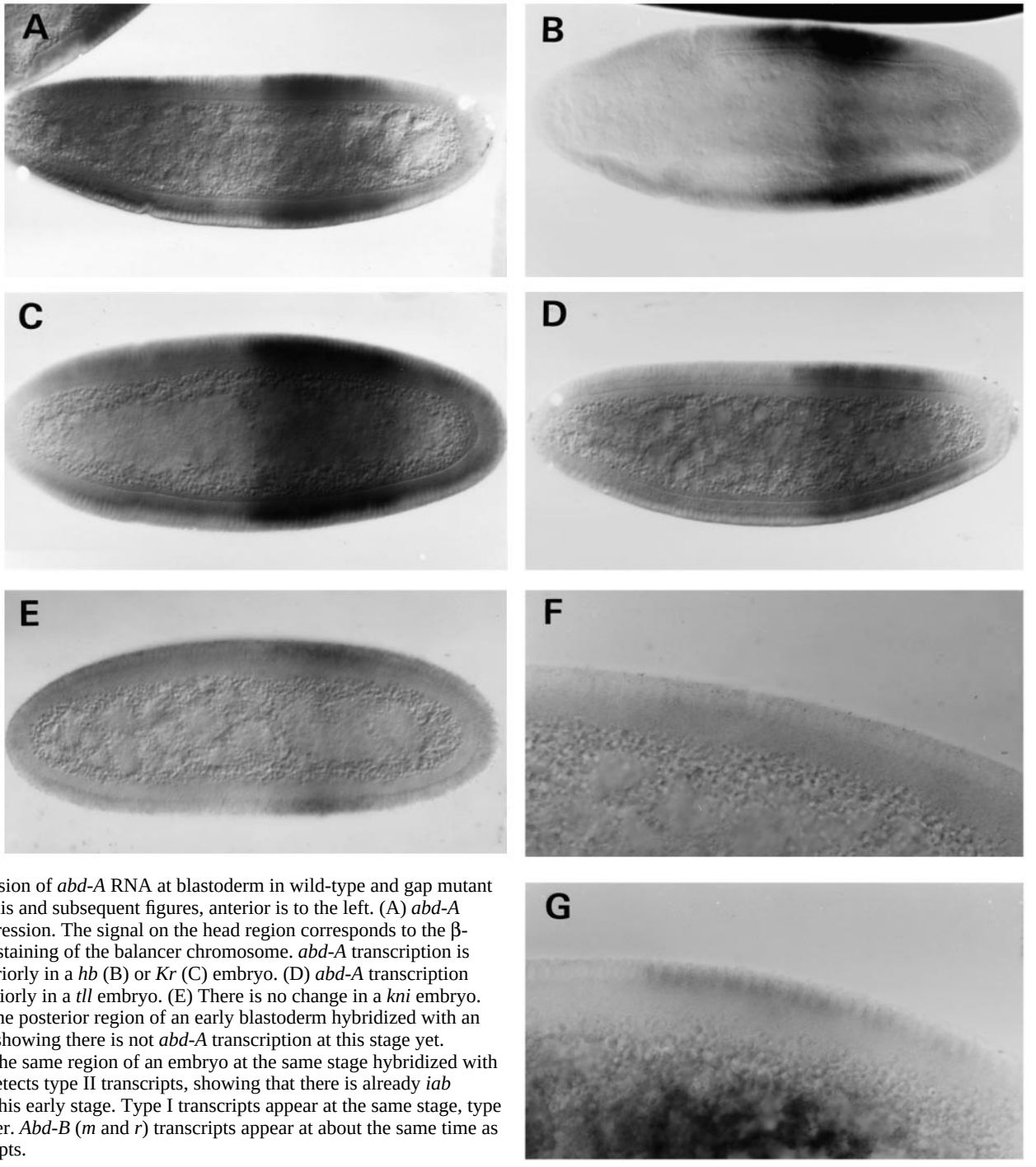

Fig. 2. Expression of *abd-A* RNA at blastoderm in wild-type and gap mutant embryos. In this and subsequent figures, anterior is to the left. (A) *abd-A* wild-type expression. The signal on the head region corresponds to the β-galactosidase staining of the balancer chromosome. *abd-A* transcription is extended anteriorly in a *hb* (B) or *Kr* (C) embryo. (D) *abd-A* transcription extends posteriorly in a *tll* embryo. (E) There is no change in a *kni* embryo. (F) Detail of the posterior region of an early blastoderm hybridized with an *abd-A* probe, showing there is not *abd-A* transcription at this stage yet. (G) Detail of the same region of an embryo at the same stage hybridized with a probe that detects type II transcripts, showing that there is already *iab* transcripts at this early stage. Type I transcripts appear at the same stage, type III slightly later. *Abd-B* (*m* and *r*) transcripts appear at about the same time as *abd-A* transcripts.

the primordium of PS14-15 (Sánchez-Herrero and Crosby, 1988; DeLorenzi et al., 1988; Kuziora and McGinnis, 1988b; Celniker et al., 1989; DeLorenzi and Bienz, 1990; Boulet et al., 1991; Figs. 5B, 6B). We have looked to the specific expression of the *m* and *r* products in gap mutants by two methods. First, we have studied the expression of the *r* protein in embryos double mutant for gap genes and for *Abd-B*^{M5}, since the *Abd-B* antibody used recognizes the two *Abd-B* proteins (Celniker et al., 1989), and since this mutation produces only *r* protein (DeLorenzi and Bienz, 1990; Sánchez-Herrero, 1991). Second, we have used in situ hybridization with specific probes for the *m* and *r* transcripts. Previous works that analyzed the expression of *Abd-B* in gap mutants (Harding and Levine, 1988; Reinitz and Levine, 1990), did not compare the expression of different *Abd-B* products. We describe first the effects on the *m* promoter.

There is early ectopic transcription of *Abd-B* in *Kr* embryos (Harding and Levine, 1988). The *m* promoter is activated ectopically in the primordium of PS4 (Figs. 5C). When the ABD-B protein is first detected there is normal expression in PS13-15 and ectopic signal posterior to the labial segment (Fig. 6C). Later on, ABD-B extends from PS13 anteriorly and from the ectopic region of expression posteriorly, so that the *Abd-B* signal from both sites meet in the middle region, forming an incomplete mirror image (Fig. 7B), which is reflected later in the transformed cuticle of *Kr* embryos (Nüsslein-Volhard and Wieschaus, 1980; Wieschaus et al., 1984; Fig. 7C).

The changes in the expression of *iab* transcripts at early cellular blastoderm allow us to predict, to some extent, the later changes in the *Abd-B* (*m*) protein distribution. However, since the transcription of the *Abd-B* (*m*) RNA at blastoderm (in PS13) is probably directed by *iab-8* sequences, only the late spatial distribution of *Abd-B* can be anticipated by the blastoderm transcription of *iab5/iab-6* and *iab-7* RNAs. In *Kr* embryos *iab-5/iab-6* transcripts show an anterior expansion to about 40% EL instead of the normal 28%. Besides, they are ectopically expressed in a region from about 54% to 45% EL (Figs. 4H,7A), with a gap of variable length between the two regions of transcription. An enhancer of the *iab-5* domain, when fused to the *lac-Z* gene, also shows an anterior extension to about 50% EL (Busturia and Bienz, 1993). *iab-7* (type III) transcripts are expressed normally in their domain, but they are also weakly expressed at about 54% EL (Fig. 4I). These abnormal patterns of transcription suggest that *iab-5* to *iab-7* regions are active at two different positions in the *Kr* blastoderms. These changes anticipate those of ABD-B expression (Fig. 7B).

We have explored the correlation between the early activation of *iab-5* to *iab-7* sequences and the late expression of ABD-B controlled by these regions (Fig. 7A,B) by comparing

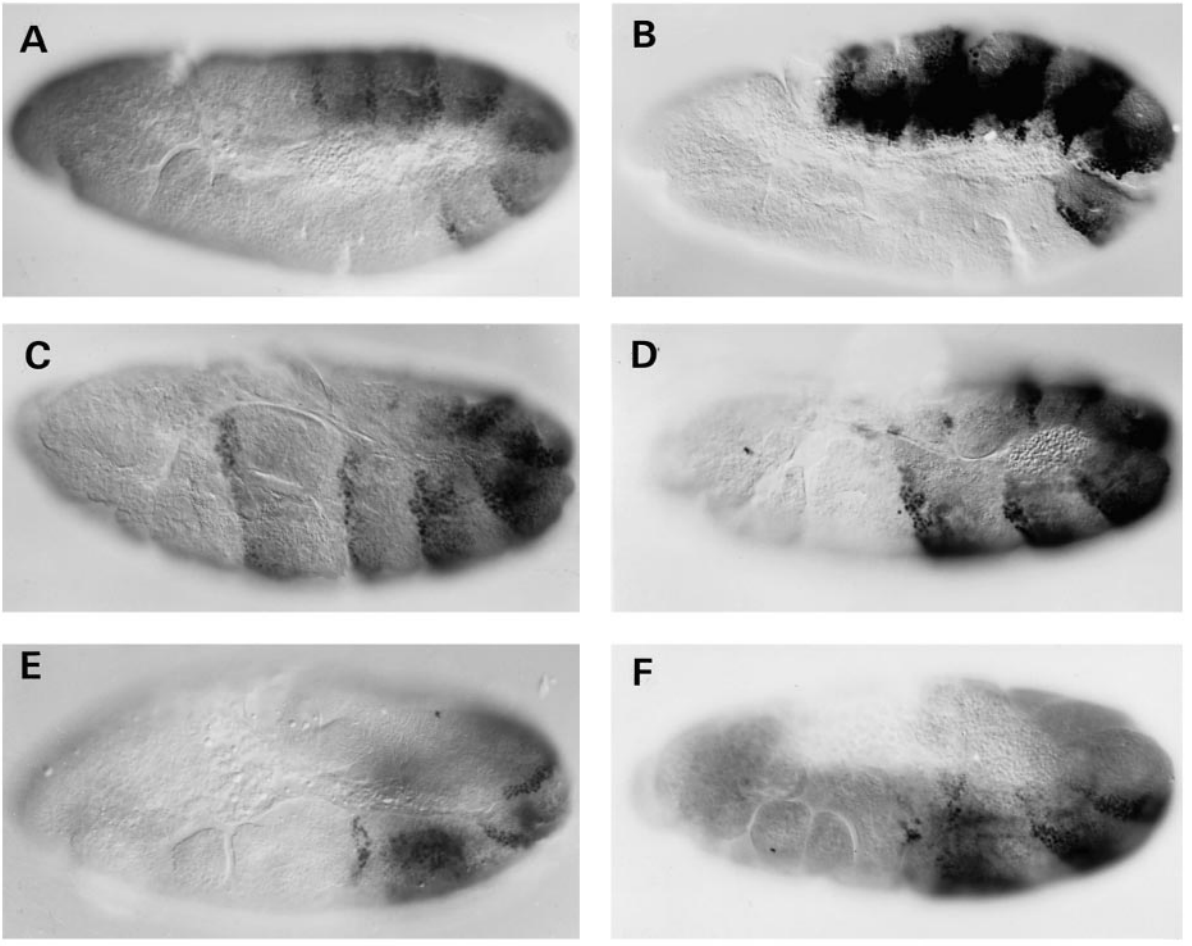

Fig. 3. Expression of ABD-A in gap mutants. (A) Wild-type *abd-A* protein expression in PS7-13. (B) In *tll* embryos *abd-A* is expressed almost to the end of the germ band. In *hb* (C) and *Kr* (E) embryos, protein expression is observed more anteriorly. In *hb iab-3*^{277} (D) and *Kr iab-3*^{277} embryos (F), there is ectopic *abd-A* expression as in *hb* (see C) and *Kr* embryos (see E).

the *Abd-B* expression in *Kr* and *Kr iab-7^{MX2}* embryos. In *iab-7^{MX2}* embryos (see Fig. 1A), the expression of *Abd-B* (*m*) is just driven by the *iab-8* region and it is limited to PS13 throughout development (Sánchez-Herrero and Akam, 1989; Boulet et al., 1991; Sánchez-Herrero, 1991; Crosby et al., 1993). Therefore, in *Kr iab-7^{MX2}* embryos, since the *iab-5* to *iab-7* sequences cannot activate the *Abd-B* promoter, ABD-B is expressed only in the normal and ectopic initial domains under the control of the *iab-8* region (compare Fig. 7B and D; the *r* protein also contributes to the signal). The distribution of type II transcripts does not change in *iab-7^{MX2}* blastoderms (Sánchez-Herrero and Akam, 1989), and we assume these RNAs are probably present in the normal and ectopic positions in *Kr iab-7^{MX2}* embryos. Thus, although there is ectopic, mirror-image activation of *iab-5* to *iab-7* sequences (as in *Kr* blastoderms), since only the *iab-8* region can contact the *Abd-B* promoter, the symmetric stepwise *Abd-B* expression typical of *Kr* embryos is absent (Fig. 7D).

knirps and *tailless* affect *Abd-B* (*m*) expression probably through the effect on the *iab-8* region

knirps and *tailless* have opposite effects on the transcription of the *m* RNA. It was shown, with a probe detecting both *m* and *r* transcripts, that *Abd-B* transcription extends anteriorly in *kni* blastoderms (Harding and Levine, 1988), whereas it is reduced in *tll* embryos (Reinitz and Levine, 1990). By using specific probes, we see that the effect of *kni* is exclusive to the *m* transcript (Fig. 5E,F), and that the expression of the *m* element is absent or reduced in *tll* blastoderms (not shown). At germ band extension stage, the ABD-B m protein distribution shows an anterior expansion in *kni* embryos (Fig. 7E), and a weak and variable protein expression in *tll* embryos (Fig. 6E).

These effects could be mediated by the action of *kni* and *tll* on the *iab-8* regulatory region or directly on the *Abd-B* promoter. The distribution of type II and III transcripts does not change in either *kni* or *tll* embryos (Fig. 4K,L; not shown for *kni* embryos), suggesting a normal spatial activation of *iab-5* to *iab-7* regions, although in some *tll* embryos type III transcripts are absent. The limits of activation of an *iab-5* enhancer do not change in *kni* embryos either (Busturia and Bienz, 1993). Consistently, the protein distribution in PS10-12 of *tll* embryos appears normally in time and position. In *kni* embryos, this is more difficult to ascertain, due to the fusion of parasegments 6 to 12 (Nüsslein-Volhard and Wieschaus,

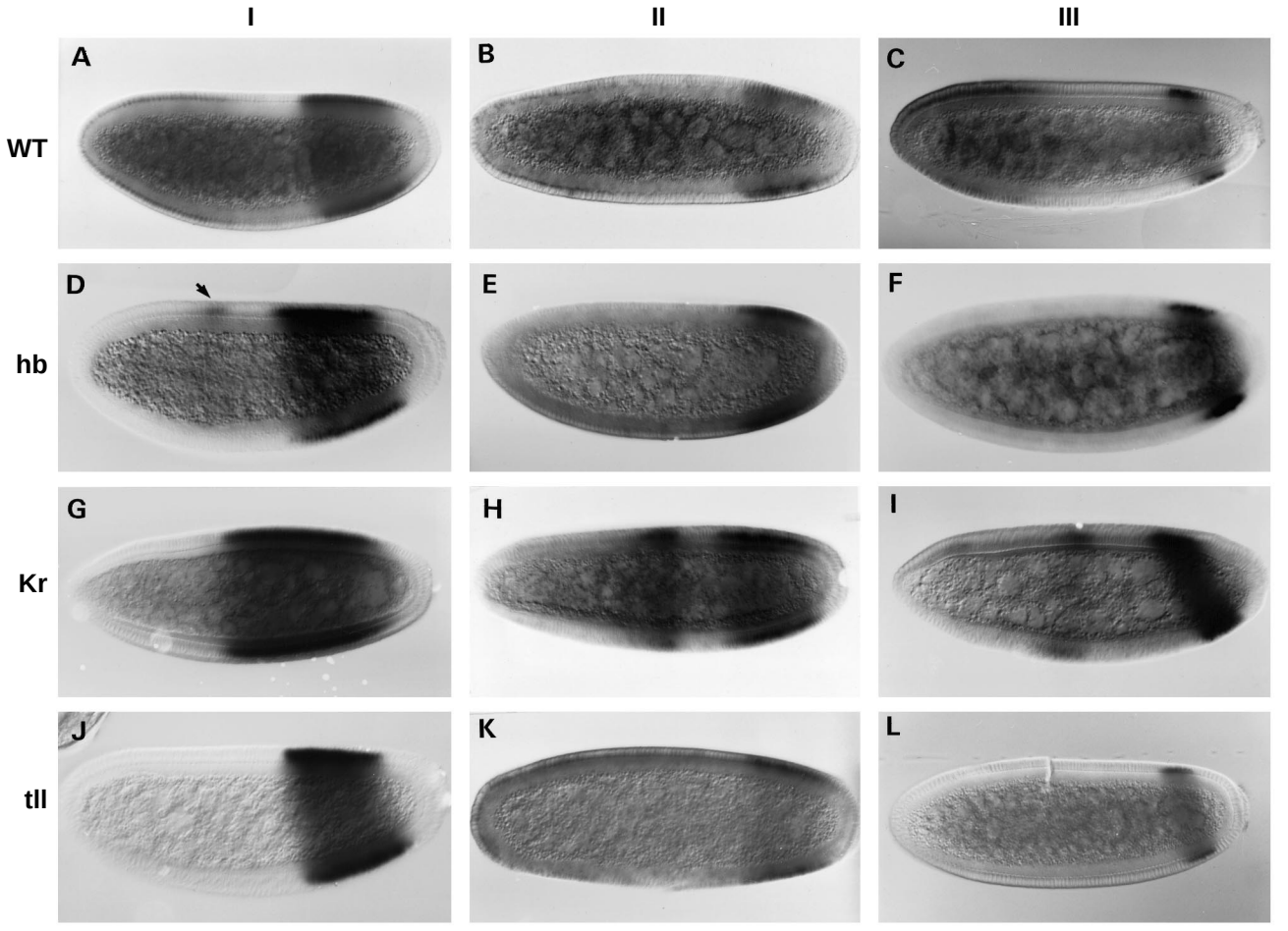

Fig. 4. Transcription of *iab* RNAs at blastoderm in wild-type (A-C), *hb* (D-F), *Kr* (G-I) and *tll* (J-L) embryos. In *hb* embryos, there is a slight more anterior expression of type I RNAs (D) and also some signal in a dorsal region around 65% EL (arrow). The expression of type II (E) and type III (F) transcripts does not change. Type I (G) and II (H) RNAs are detected more anteriorly in *Kr* embryos. There is also ectopic expression of type II (H) and type III (I) transcripts in more anterior regions. The expression of type I (J), type II (K) or type III (L) transcripts does not change in *tll* embryos compared to wild-type ones. There is no change in *kni* or *gt* embryos either.

1980). However, we observe that some cells accumulate ABD-B in the posterior region of this fused metamere at later stages, with an irregular anterior border of expression. This late expression is probably driven by the *iab-5* to *iab-7* regions.

To confirm that these effects are mediated by the *iab-8* region, we have looked at ABD-B expression in *kni* $iab7^{SGA62}$ and *tll* *iab-*7^{SGA62} embryos. The *iab-*7^{SGA62} mutation (Fig. 1A) eliminates ABD-B in PS10-12 (Boulet et al., 1991; Crosby et al., 1993). In these mutant combinations, therefore, ABD-B expression is responding just to *iab-8* sequences. In *kni* $iab7^{SGA62}$ and *tll* *iab-*7^{SAG62} embryos, the abnormal *Abd-B* signal throughout development is basically the same as the early one in *kni* (Fig. 7F, compare with E) and *tll* mutants. By contrast, they lack the late *Abd-B* expression observed in *kni* or *tll* . Thus, the *iab-5* to *iab-7* sequences are probably not responsible for the major changes in ABD-B expression observed in *kni* or *tll* embryos.

hunchback and *giant* do not modify the limits of expression of *Abd-B(m)*

The early transcription of *Abd-B*, detected with a common probe, is normal in *hb* mutants (Harding and Levine, 1988). The *m* transcript also shows normal (perhaps weaker) expression during gastrulation (not shown). The protein staining is weak and irregular in PS13-14, but with normal limits of expression. In *giant* embryos, the *Abd-B* common probe detects ectopic *Abd-B* transcription in some lateral cells of PS2 or PS3 (Reinitz and Levine, 1990). This ectopic signal is due only to the *r* transcript (see below), since the expression of the *Abd-B m* transcript does not change (Fig. 5G). When the

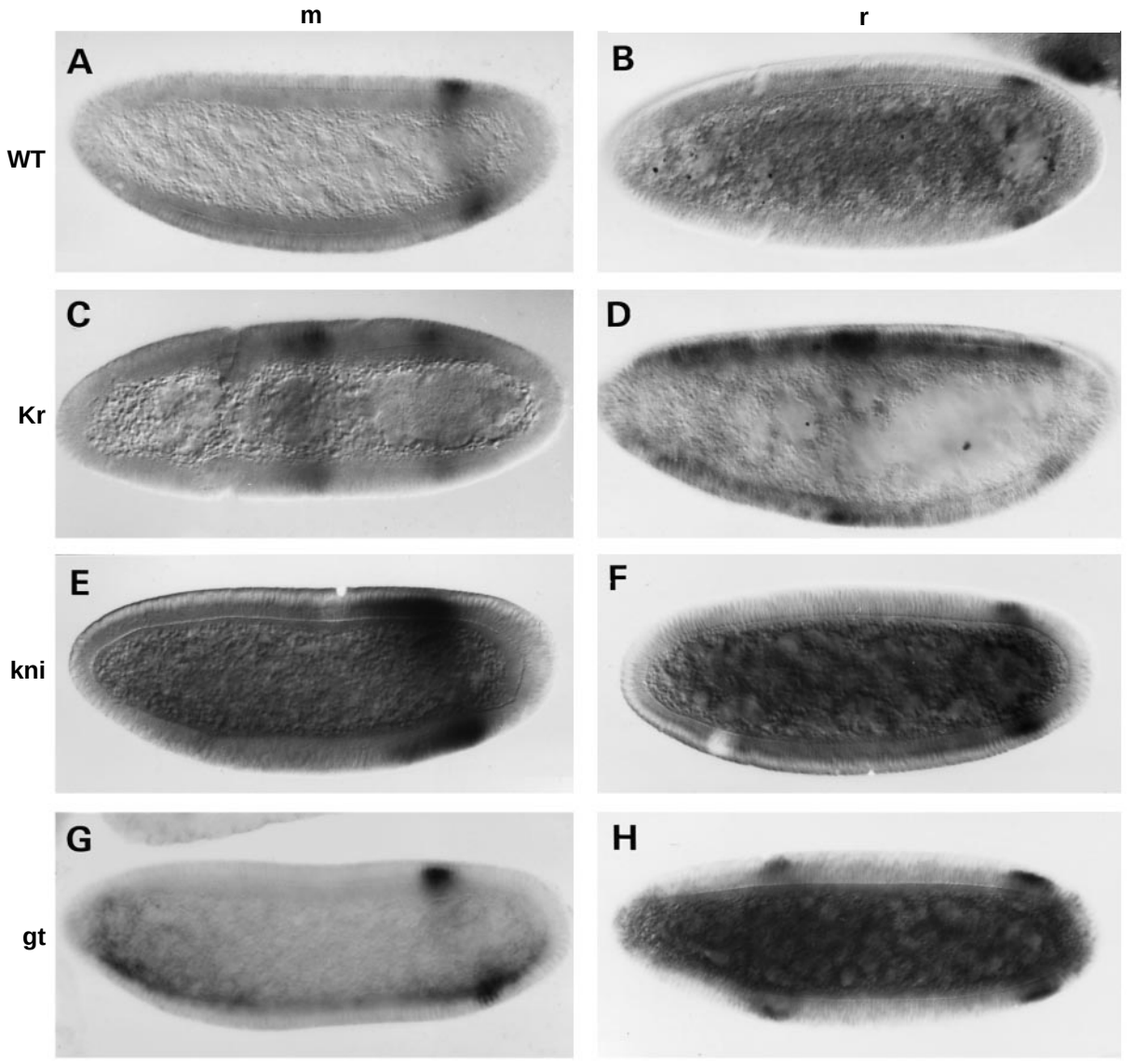

Fig. 5. Effect of gap mutations on early *Abd-B* transcription. To the left (A,C,E,G), expression of *m* RNA and to the right (B,D,F,H), of the *r* transcript. A,B) Expression of the *m* and *r* transcripts in wild-type embryos. (C,D) In *Kr* embryos, these RNAs are ectopically expressed. (E) There is more anterior expression of *m* transcripts in *kni* embryos, whereas there is no change in *r* transcription (F). (G) In *gt* embryos, the *m* transcription is not altered, but there is ectopic *r* transcription (H).

germ band is extended, we observe ectopic protein (*r* protein, see below) in the labial segment and an irregular and variable signal in parasegments 10-12, which are frequently fused (Fig. 6G). In accordance with the normal limits of transcription of *Abd-B* (*m*) in *hb* or *gt* embryos, the expression of *iab-5/iab-6* and *iab-7* transcripts does not change in *hb* (Fig. 4E,F) or *gt* mutants (not shown), and an *iab-5* enhancer does not modify its limits of activation in *hb* mutants (Busturia and Bienz, 1993).

Regulation of the *r* products of *Abd-B*

Some gap genes control similarly the *m* and *r* promoters. For example, *hb* does not affect the distribution of either *m* or *r* transcripts, while *tll* is required for the expression of the *m* (this work) and *r* products (Casanova, 1990; Fig. 6E,F). Similarly, the absence of *Kr* results in ectopic expression of both *m* and *r* products (Figs 5C,D, 6C,D). By contrast, *kni* and *gt* control exclusively one of the promoters. *kni* is not needed for *r* RNA expression (Fig. 5F), whereas it is required for

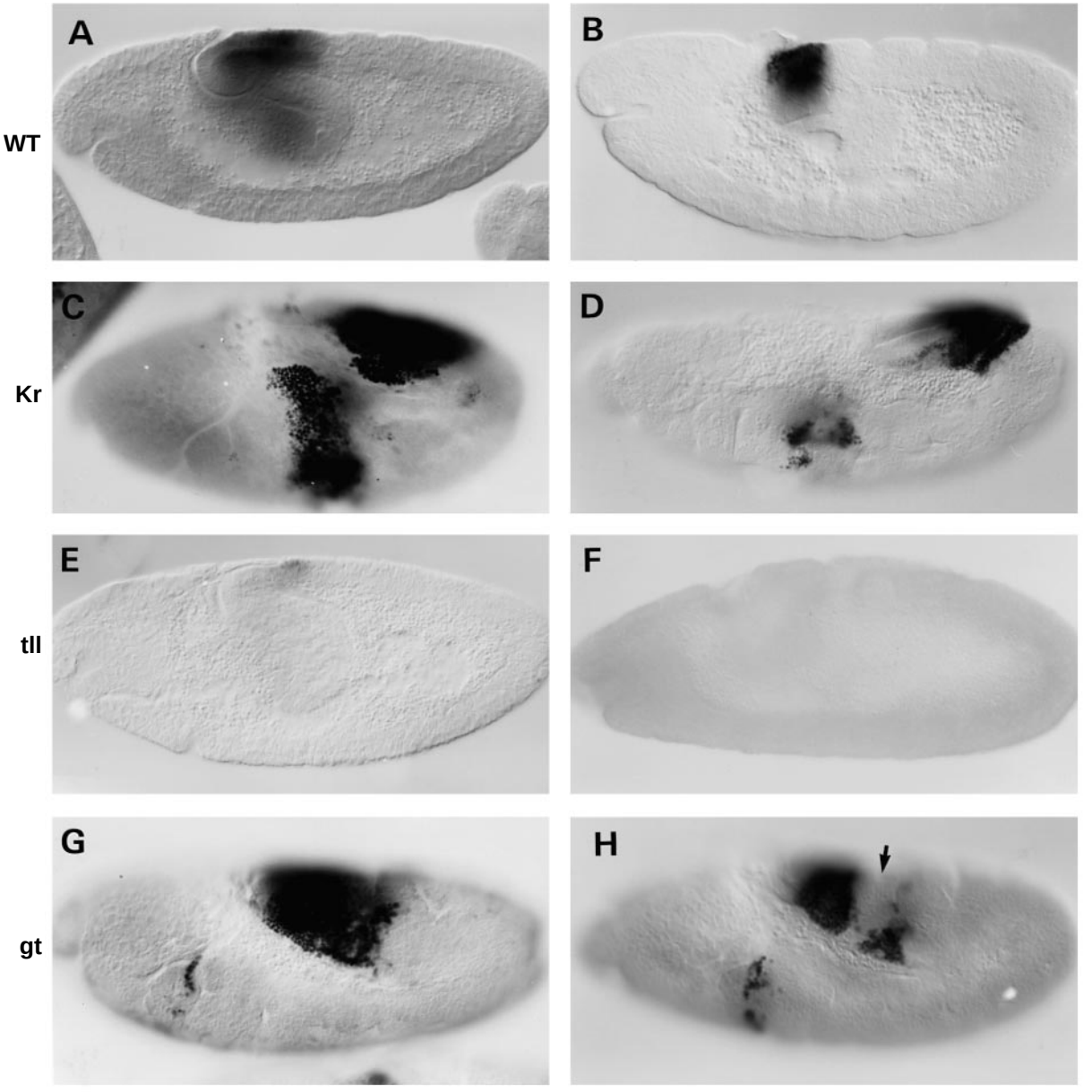

Fig. 6 Expression of ABD-B in wild-type and gap mutants during germ band extension stage. To the left (A,C,E,G), the expression of ABD-B (*m* and *r* proteins), detected with an antibody that recognizes both products, and to the right (B,D,F,H), the expression of the *r* product (in embryos that are also mutant for *Abd-B*^{M5}, see Material and Methods). (A,B) Wild-type embryos. *Abd-B* is expressed in PS13-15, and the *r* protein (B) is present just in PS14 and 15 (*Abd-B*^{M5} embryo). (C) *Kr* embryo. There is ectopic *Abd-B* expression in the PS4 region at this stage. (D) *Kr Abd-B*^{M5} embryo. The *r* protein is present in the same ectopic region, but it is detected only in a small group of cells. (E) *tll* embryo showing a reduced signal of *Abd-B* protein. (F) There is no *Abd-B r* product in *tll Abd-B*^{M5} embryos. (G) *gt* embryo. There is irregular staining in the PS10-12 region and ectopic expression in the labial segment. (H) *gt Abd-B*^{M5} embryo. The signal in PS14 is normal. Besides, ectopic *r* protein is observed in the PS10-12 region and in the labial segment. Note the gap of expression in PS13 (arrow) where the *m* protein is normally present at this stage.

proper *m* transcription (Fig. 5E). *giant* seems to repress specifically the *r* transcripts (Fig. 5G,H). Besides, in *gt Abd-B*^{M5} embryos, the ABD-B signal in PS12 and 11 and on the labium is like that of *gt* embryos (Fig. 6H, compare with G), indicating that the ectopic expression is due to the *r* protein. The expression in PS12 and 11 may be responsible for the posterior spiracles that appear in that region of the cuticle of *gt* embryos (Gergen and Wieschaus, 1986; Petschek et al., 1987; Mohler et al., 1989). It has been previously shown that, when the ABD-B (r) protein is ubiquitously expressed, it induces the formation of posterior spiracles (Lamka et al., 1992; Kuziora, 1993).

DISCUSSION

In this work, we have studied the control of *abd-A* and *Abd-B* expression by gap genes. In what follows, we discuss how this is achieved through the activation of *iab* regulatory regions along the A/P axis.

Activation of *iab* domains and the control of *abd-A* expression

The effects of gap genes on *abd-A* and *Abd-B* expression are a consequence of how they control *iab* activation (summarized in Fig. 8). The genes *kni* and *gt*, although transcribed in a central region where the *iab* RNAs are present (Rothe et al., 1989; Pankratz et al., 1989; Mohler et al., 1989; Eldon and Pirrotta, 1991; Kraut and Levine, 1991), do not seem to affect their transcription, suggesting that *kni* and *gt* may not be required to initiate or restrict the 'opening' of *iab-3* to *iab-7* regions. Furthermore, the initial transcription of *kni* and *gt* themselves does not appear to require a specific activator. As proposed for these two genes (Eldon and Pirrotta, 1991; Kraut and Levine, 1991; Pankratz et al., 1992; Struhl et al., 1992; Pankratz and Jäckle, 1993), the *iab-2* to *iab-7* sequences may be initially activated ('open') by an unknown activator, and its limits of transcription be set up by repression (Busturia and Bienz, 1993). These repressors may be *Kr* and *hb* anteriorly (see below) and perhaps the gap gene *huckebein* (Weigel et al., 1990) posteriorly. Thus, the *iab* regions are not active in a

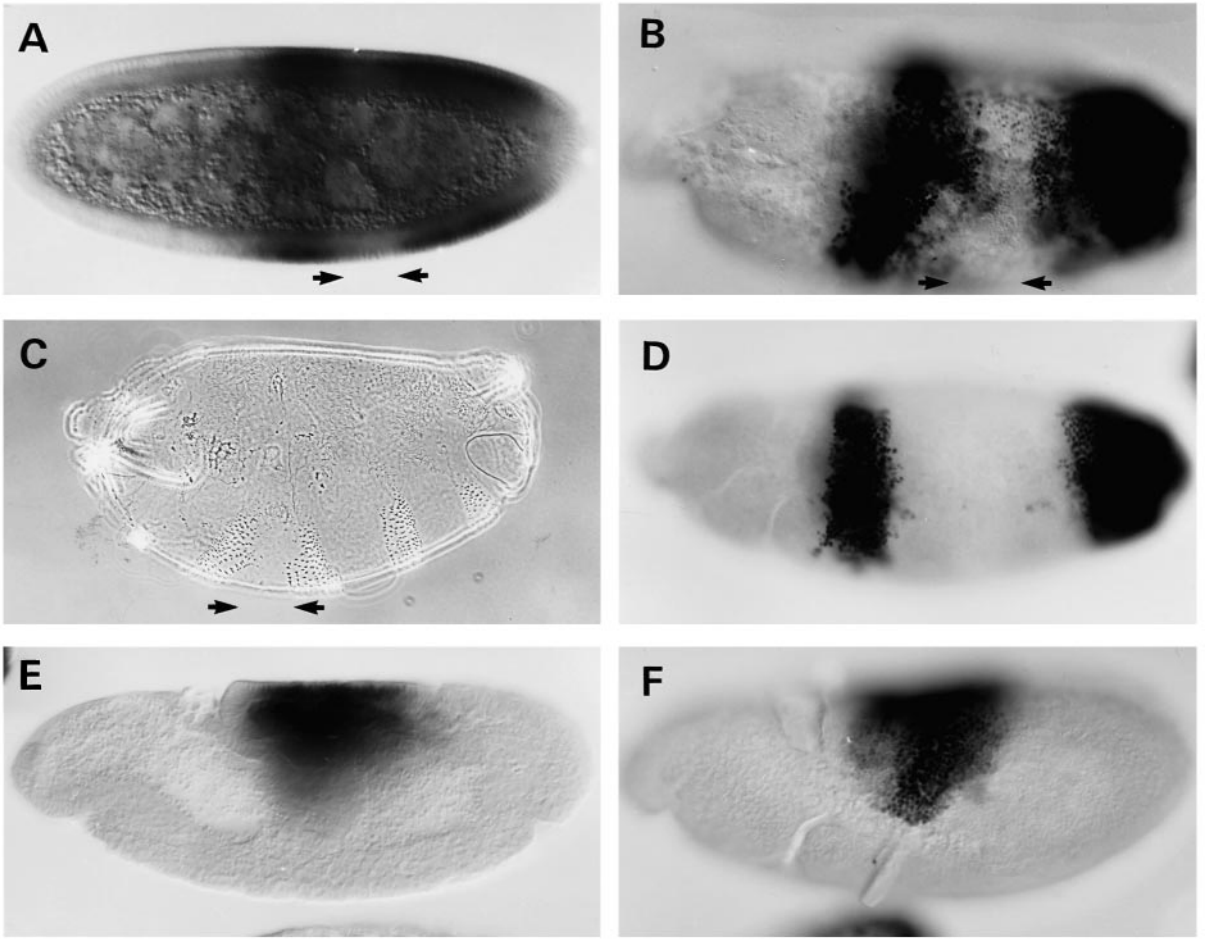

Fig. 7. Correlation between activation of regulatory sequences and *Abd-B* expression. (A) Transcription of type II RNAs in a *Kr* blastoderm, showing ectopic signal. (B) *Abd-B* protein expression in a germ band-retracted *Kr* embryo. Note that *Abd-B* signal forms an imperfect mirror image symmetry. (C) Cuticular phenotype of a *Kr* embryo. Arrows indicate the mirror-image symmetry in *Abd-B* expression, cuticular phenotype, and the abnormal transcription of type II RNAs at blastoderm. (D) Late expression of *Abd-B* in a *Kr iab-7*^{MX2} embryo. *Abd-B* is not expressed in the central region of the embryo, as it is in B. (E) Early protein expression in a *kni* embryo. (F) *kni iab-7*^{SGA62} embryo. The anterior extension of *Abd-B* expression is similar to that of *kni* embryos at this stage.

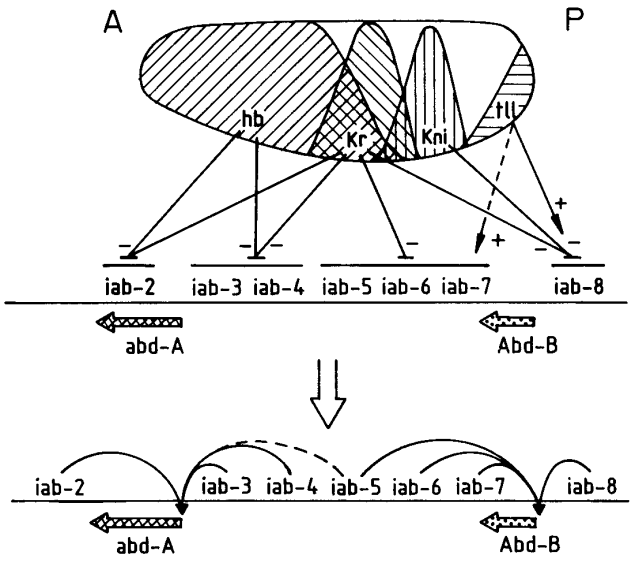

Fig. 8. Summary of the control of *abd-A* and *Abd-B* by gap genes. At the top, drawing of an embryo showing the expression of gap genes (reviewed in Hülskamp and Tautz, 1991; Pankratz and Jäckle, 1993). *gt* is not included, since there is no effect of *gt* mutations on the activation of *iab* regions, only on *r* transcription. Arrows indicate activation (+) or repression (–) of *iab* regions by gap genes. The broken line indicates a possible effect of *tll* on *iab-7* activation. There is perhaps a repressing effect of *hb* on *iab-5* to *iab-8* not indicated. Below is a map of the BX-C. Only the effect of *Kr* on *iab-2* sequences has been shown to be direct (Shimell et al., 1994).

certain parasegment (except perhaps the *iab-8* region), but within a broad region with precise anterior limits.

The initial expression of *abd-A* follows the specific activation of its regulatory sequences, *iab-2* to *iab-4*. The *iab-2* region shows no transcription at blastoderm (Sánchez-Herrero and Akam, 1989). However, we can infer the effects of gap genes on *iab-2* activation by looking at the expression of *abd-A* in double mutant combinations where the *abd-A* promoter is responding just to the *iab-2* region. These studies, and the changes in *iab* transcription observed in gap mutants, show that the activation of *iab-2*, *iab-3* and *iab-4* domains is limited anteriorly by *hb* and *Kr* products (Simon et al., 1990; Shimell et al., 1994; this work). In accordance with these results, the anterior limit of *abd-A* transcription seems to be dictated by the products of the *hb* and *Kr* genes (Shimell et al., 1994; this work). The effect of *hb* is mostly or exclusively achieved through the *iab-2* region, since the distribution of type I transcripts does not (or only slightly) change. By contrast, type I transcripts extend more anteriorly than *abd-A* transcripts in *Kr* blastoderms, perhaps suggesting that in *Kr* embryos the early *abd-A* anterior limit of transcription is controlled through the *iab-2* region or directly through the *abd-A* promoter.

The posterior limit of *abd-A* activation may be established by *tll* at about 20% egg length, probably through the *iab-2* region or directly on the *abd-A* promoter. Finally, *kni* and *gt* are not needed for a normal distribution of *iab* or *abd-A* transcripts. The activation of *iab-2* to *iab-4* sequences, and therefore the initial *abd-A* transcription, does not depend on any of these two gap genes.

The temporal sequence of *Abd-B* expression

The *m* transcript of *Abd-B* is initially expressed in PS13, probably under the control of the *iab-8* region. Changes in this initial transcription, therefore, may be indicative of the effects of gap genes on *iab-8* activation. *Abd-B* (*m*) early transcription is absent or reduced in *tll* embryos, suggesting that, in this case, the *iab-8* region is not (or barely) active. By contrast, it is negatively controlled by *kni* (in PS10-12) and *Kr* (in PS4).

The *iab-5* to *iab-7* regions control the later expression of *Abd-B* (*m*) in PS12-10 (Sánchez-Herrero and Akam, 1989; Celniker et al., 1990; Boulet et al., 1991; Sánchez-Herrero, 1991; Crosby et al., 1993). The gene *Kr* represses the activation of these region in the central part of the embryo, since there are type II and III transcripts at that position in *Kr* blastoderms. In accordance, the ABD-B protein is observed in that region at later stages. By contrast, the *iab-5* to *iab-7* regions are normally active in *tll, gt, kni* or *hb* (but see below) embryos (normal class II and III transcription) and, consistently, the expression of ABD-B (m) present normal limits of expression in PS12-10 of *tll, gt, hb* and possibly *kni* embryos.

This *Abd-B* expression in PS12, 11 and 10 takes place with decreasing intensity and following a temporal sequence (Harding and Levine, 1988; Sánchez-Herrero and Crosby, 1988; Kuziora and McGinnis, 1988b; Celniker et al., 1989; DeLorenzi and Bienz, 1990; Boulet et al., 1991; Crosby et al., 1993). The expression in PS10-12 depends on the *iab-5*, *iab-6* and *iab-7* regions, which are ordered distal to proximal downstream of the *Abd-B* transcription unit (Karch et al., 1985). The temporal effect in PS12-10 may be simply due to the fact that *Abd-B* transcripts are less abundant in more anterior parasegments and it may require more time to accumulate to the levels required for detection. Alternatively, it may represent a genuine mechanism of temporal activation (see Crosby et al., 1993 and Busturia and Bienz, 1993, for discussion of this point). The presence of *iab* -5/*iab-6* and *iab-7* RNAs at blastoderm (Sánchez-Herrero and Akam, 1989) and the early activity of an *iab-5* enhancer (Busturia and Bienz, 1993) suggest that the *iab-5* to *iab-7* regions are active at blastoderm. The distance to the promoter (the *iab-5* region being farther, the *iab-7* being closer) could determine the temporal sequence (or the levels) of *Abd-B* transcription. Therefore, although the three regions could be 'open' at the same time, they would contact the promoter sequentially. A mechanism whereby the distance to the promoter determines temporal activation has also been described in the globin genes (Hanscombe et al., 1991) and may also operate in other *Drosophila* genes (Grossniklaus et al., 1992). Although *kni* regulates the anterior levels of activation of the *iab-5* region, and seems to be instrumental in this temporal activation (Busturia and Bienz, 1993), the early expression of ABD-B protein is similar in *kni* and *kni iab-7* embryos, while the late expression is not, suggesting that the temporal sequence is maintained, at least in part, in the absence of *kni* and that *kni* may also act to repress the activation of the *iab-8* region.

A gradient of *hb* may delimit anteriorly the activation of regulatory sequences in the BX-C

The *hb* product establishes the anterior limit of *Ubx* (White and Lehmann, 1986; Irish et al., 1989) directly through the *abx*/*bx* and *bxd*/*pbx* regulatory sequences (Simon et al., 1990; Qian et

al., 1991; Zhang et al., 1991; Zhang and Bienz, 1992; Müller and Bienz, 1992; Qian et al., 1993). The control of *abd-A* transcription by *hb* may also take place directly through the *iab-2*, *iab-3* and *iab-4* domains since their anterior limits of activation change in *hb* mutant embryos. If so, *hb* would establish the anterior limit of activation of the BX-C regulatory sequences that specify identity in parasegments 5 to 9. Since the *hb* protein is distributed as a gradient in the embryo (Tautz, 1988), it suggests that the *abx/bx*, *bxd*, *iab-2*, *iab-3* and *iab-4* regions may respond to different concentrations of the *hb* gradient (see also Struhl et al., 1992).

Although these results have been obtained in the absence of the *hb* zygotic product, *hunchback* has also a maternal contribution (Lehmann and Nüsslein-Volhard, 1987). Embryos lacking maternal and zygotic *hb* products show posterior abdominal segments in the anterior region of the embryo (Lehmann and Nüsslein-Volhard, 1987). Moreover, in embryos mutant for an antimorphic *hb* allele that inactivates the zygotic and in part the maternal *hb* protein, ABD-B shows up in the anterior region of the embryo (Busturia and Bienz, 1993). These results suggest that the maternal product, which is also distributed as a gradient (Tautz et al., 1987; Tautz, 1988), represses, in the anterior region of the embryo, sequences controlling *Abd-B*. Furthermore, the anterior limits of activation of *abx*/*bx* through *iab-3*/*iab-4* regions in the absence of zygotic *hb* follow a striking sequence: 75% for *abx/bx* (Simon et al., 1990; Qian et al., 1991), 62% (Irish et al., 1989) or 58% (Zhang et al., 1991) for *bxd/pbx*, 52% for *iab-2* (based on *abd-A* distribution), 42% for *iab-3*/*iab-4*. It seems therefore that the BX-C regulatory domains may respond to the *hb* maternal gradient in the absence of the zygotic one. Although the maternal gradient is dispensable if the zygotic one is present (Lehmann and Nüsslein-Volhard, 1987), the former could contribute to this differential activation in the absence of the latter (see also Hülskamp et al., 1990; Struhl et al., 1992).

The phenotype of embryos lacking *bicoid* (*bcd*) and maternal and zygotic *hb* (Hülskamp et al., 1990; Simpson-Brose et al., 1994), compared with those just lacking *hb* (Lehmann and Nüsslein-Volhard, 1987; Simpson-Brose et al., 1994) suggests that *bcd* may be another repressor of *iab* activation in the anterior region of the embryo, although *hb* is the major component in this repression. Part of it is achieved probably through the direct effect of *hb* on *iab* sequences, but *bcd* and *hb* are also responsible for establishing secondary gradients of gap genes, like *Kr* and *kni* (Jäckle et al., 1986; Hülskamp et al., 1990; Kraut and Levine, 1991; Eldon and Pirrotta, 1991; Struhl et al., 1992; Pankratz and Jackle, 1993), that further repress *iab* activation. The use of the *bcd* and *hb* gradients to couple segmentation and segment identity (Struhl et al., 1992; Qian et al., 1993; Pelegri and Lehmann, 1994), in part through their contribution to the formation of secondary gradients, may serve for the early development of the abdominal region in long germ band insects like *Drosophila*.

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