The Hox gene Abdominal-B antagonizes appendage development in the genital disc of Drosophila

Beatriz Estrada and Ernesto Sánchez-Herrero*

Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain

*Author for correspondence (e-mail: esherrero@cbm.uam.es)

Accepted 21 November 2000; published on WWW 11 January 2001

SUMMARY

In Drosophila, the Hox gene Abdominal-B is required to specify the posterior abdomen and the genitalia. Homologues of Abdominal-B in other species are also needed to determine the posterior part of the body. We have studied the function of Abdominal-B in the formation of Drosophila genitalia, and show here that absence of Abdominal-B in the genital disc of Drosophila transforms male and female genitalia into leg or, less frequently, into antenna. These transformations are accompanied by the ectopic expression of genes such as Distal-less or dachshund, which are normally required in these appendages. The extent of wild-type and ectopic Distal-less expression depends on the antagonistic activities of the Abdominal-B gene, as a repressor, and of the decapentaplegic and wingless genes as activators. Absence of Abdominal-B also changes the expression of Homothorax, a Hox gene co-factor. Our results suggest that Abdominal-B forms genitalia by modifying an underlying positional information and repressing appendage development. We propose that the genital primordia should be subdivided into two regions, one of them competent to be transformed into an appendage in the absence of Abdominal-B.

Key words: Drosophila, Genitalia, Hox genes, Abdominal-B, Distal-less, Pattern formation

INTRODUCTION

The homeotic (Hox) genes specify different structures along the anteroposterior axis in multicellular animals. The genetic and molecular characterization of Hox genes in insects and vertebrates has established that Hox gene expression correlates with their requirements along the anteroposterior axis (Lewis, 1978; Kaufmann et al., 1990; Krumlauf, 1994). The specification of a certain structure by a Hox gene, therefore, depends more on its position in this axis than on its nature.

Most of our knowledge about Hox genes stems from studies in Drosophila. It has been shown that Hox genes alter the response to the underlying positional information (Szüts et al., 1997; Weatherbee et al., 1998). In some cases the result of Hox protein activity is the modification of similar, homologous structures. For example, the activity of the Ultrabithorax (Ubx) Hox gene of the bithorax complex (BX-C) establishes the difference between mesothoracic and metathoracic legs, two similar appendages (Lewis, 1963). Ubx activity, however, also serves to illustrate a different situation: it is responsible for specifying two consecutive metameres, the third thoracic segment and the first abdominal one (A1), which differ in many respects, including presence or absence of appendages as well as proliferation dynamics and morphology (Lewis 1963). In this case, Ubx appears to drastically change the way in which positional information is interpreted. For example, in A1, it represses the transcription of the homeobox gene Distal-less (Dll), a gene required for the formation of legs or antennae (Sunkel and Whittle, 1987; Cohen and Jürgens, 1989a; Cohen and Jürgens, 1989b; Gorlinskii et al., 1997), without changing the underlying positional information (Cohen et al., 1991; Castelli-Gair and Akam, 1995).

Abdominal-B (Abd-B) is the Hox gene of the BX-C expressed more posteriorly in the anteroposterior axis, and is also required to establish different morphologies in the embryo and adult. Abd-B mutations transform posterior abdominal segments into more anterior ones, a change between similar metameres, and Abd-B is also needed to form the genitalia (Sánchez-Herrero et al., 1985; Tiong et al., 1985), which develop by distinct developmental mechanisms.

The genitalia of Drosophila (as well as the analia) derive from a single bilateral imaginal disc, the genital disc, formed at the posterior of the embryo (Jürgens and Hartenstein, 1993). The disc is sexually dimorphic: while both sexes have an anal primordium, in males the male genital primordium develops to make adult male genitalia while the female genital primordium is repressed. Conversely, in females it is the male primordium that is repressed, whereas the female genital primordium gives rise to the adult female genitalia (Nöthiger et al., 1977). Patterning mechanisms in the genital disc resemble those of other discs, particularly of leg discs (Freeland and Kuhn, 1996; Chen and Baker, 1997; Casares et al., 1997; Sánchez et al., 1997; Gorlinskii et al., 1999). Moreover, the genital disc also expresses Dll, similar to leg or antennal discs (Gorlinskii et al., 1997).
1999; Moreno and Morata, 1999). Abd-B is also expressed in the genital primordia of the genital disc, both in males and females, but not in the anal primordium (Freeland and Kuhn, 1996; Casares et al., 1997). Although the requirement of Abd-B for genitalia development was discovered long ago (Sánchez-Herrero et al., 1985; Tiong et al., 1985; Casanova et al., 1986), its precise role has not been well established.

We have investigated in detail the role of Abd-B in the formation of the genitalia. Our results show that the absence of Abd-B transforms male or female genitalia into legs or antennae. This transformation is accompanied by the ectopic expression ofDll and dachshund (dac), two genes that are responsible for the formation of most of the leg (Cohen and Jürgens, 1989a; Cohen and Jürgens, 1989b; Mardon et al., 1994; Gorinkiel et al., 1997). We suggest that Abd-B promotes genital development by changing how positional information is interpreted, leading to repression of leg-specific genes in specific regions of the genital disc. The activation ofDll in precise positions when Abd-B activity is impaired allows the genital primordia to subdivide into two regions, one of them competent to form appendages.

MATERIALS AND METHODS

Fly stocks

Two Abd-B null mutations were used in this study: Abd-B<sup>M1</sup> (Casanova et al., 1986) and Abd-B<sup>B18</sup>, which is a small deficiency that removes the homeobox and adjacent sequences (Hopmann et al., 1995). Other mutants used were Dll<sup>SA1</sup> (Cohen and Jürgens, 1989a), Antp<sup>NoRC3</sup> (Struhl, 1981), hh<sup>PT2</sup> (Pai et al., 1998), puf<sup>125</sup> (Letsou et al., 1995) and the allele DCO<sup>B1</sup>, mutant in the principal catalytic subunit of the protein kinase A (PKA) gene (Pka-C1 – FlyBase; Li et al., 1995). The dpp-lacZ reporter line (B5.3; Blackman et al., 1991) serves to reveal dpp transcription.

The Gal4/UAS system (Brand and Perrimon, 1993) was used to express different gene products ectopically. em-212 (Calleja et al., 1996) is a Dll-Gal4 line mutant for Dll that rescues the phenotype ofDll flies in combination with UAS-Dll – therefore reproducing the normal Dll expression pattern (Gorinkiel et al., 1997). The other Gal4 line used was dpp-Gal4 (Morimura et al., 1996). The UAS stocks used were UAS-Dll (Gorinkiel et al., 1997), UAS-hth (Pai et al., 1998), UAS-flp (Campbell and Tomlinson, 1998) and a UAS-Abd-B construct that drives expression of the m (or I) protein isoform (Castelli-Gair et al., 1994). All the crosses were made at 25°C, except the cross to obtain dpp-GAL4/UAS-Dll larvae, which was kept at 17°C for 7 days and transferred then to 25°C. Larvae of the relevant genotype were identified in some crosses by the use of the Tubby and Black Cells larval markers.

Clonal analysis

Mitotic recombination clones were produced by the FLP/FRT system (Xu and Rubin, 1993), with or without the Minute technique, which confers a growth advantage (Morata and Ripoll, 1975). The different chromosomes used to make the clones were FRT828 hs-piMyc Sb y<sup>+</sup> (Xu and Rubin, 1993); FRT828 hs-CD2 y<sup>+</sup> M(3)w (Abu-Shaar and Mann, 1998); FRT828 arm-lacZ Dp(f<sup>+) M(3)w)123 (Weimann and Cohen, 1999); FRT40A arm-lacZ and FRT42D arm-lacZ (Chen and Struhl, 1996); and FRT82B hth<sup>PT2</sup> (Pai et al., 1998) and FRT42D Dll<sup>SA1</sup> (Gorinkiel et al., 1997). The FRT828 Abd-B<sup>M1</sup>, FRT828 Abd-B<sup>B18</sup>, FRT828 Antp<sup>NoRC3</sup> Abd-B<sup>18</sup> and FRT82B puf<sup>125</sup> Abd-B<sup>B18</sup> chromosomes were obtained by standard genetic recombination. hs-flp<sup>122</sup> is described by Struhl and Basler (Struhl and Basler, 1993).

Clones were induced, in general, during second and third larval instars by a 37°C heat-shock for 1 hour. In the adult they were marked by the yellow or yellow and (loss of) Stubble cuticular markers, and in the discs, by loss of markers for Myc or β-galactosidase. The Abd-B<sup>+</sup> clones were induced at different stages of larval development: 24-48, 48-72, 72-96 and 96-120 hours after egg laying. In cases of extreme transformation of the genitalia, flies had to be taken out of the pupal case. Clones made with the Abd-B<sup>M1</sup> and Abd-B<sup>B18</sup> alleles gave comparable results. The genotypes of the larvae in which the clones were induced were as follows.

**Abd-B**- clones: y hs-flp<sup>122</sup>; FRT828 Abd-B<sup>M1</sup> or FRT828 Abd-B<sup>B18</sup>FRT828 hs-CD2 y<sup>+</sup> M(3)w or FRT828 hs-piMyc Sb y<sup>+</sup> or FRT82B arm-lacZ Dp(f<sup>+) M(3)w)123

**Antp** Abd-B**-** clones: y hs-flp<sup>122</sup>; FRT828 Antp<sup>NoRC3</sup> Abd-B<sup>B18</sup>FRT828 hs-CD2 y<sup>+</sup> M(3)w

**put** Abd-B**-** clones: y hs-flp<sup>122</sup>; FRT828 put<sup>125</sup> Abd-B<sup>M1</sup>FRT828 arm-lacZ Dp(f<sup>+) M(3)w)123 or FRT828 hs-CD2 y<sup>+</sup> M(3)w

Abd-B**-** clones in the Dll domain: em<sup>121</sup> (Dll-GAL4) UAS-flp/+; FRT828, Abd-B<sup>B18</sup>FRT828 hs-CD2 y<sup>+</sup> M(3)w

Abd-B**-** clones, dpp expression: y hs-flp<sup>122</sup>; dpp-lacZ<sup>121</sup>; FRT828 Abd-B<sup>B18</sup>FRT828 hs-piMyc Sb y<sup>+</sup>

**hth**-clones: y hs-flp<sup>122</sup>; FRT828 hth<sup>PT2</sup>FRT82B arm-lacZ Dp(f<sup>+) M(3)w)123 or FRT828 hs-CD2 y<sup>+</sup> M(3)w

**Dll**-clones: y hs-flp<sup>122</sup>; FRT42D Dll<sup>SA1</sup>FRT42D arm-lacZ

**PKA**-clones: y hs-flp<sup>122</sup>; DCO<sup>B1</sup> FRT40A/FRT40A arm-lacZ

To express Abd-B and Dll ectopically, the hs-flp act >CD2> GAL4 chromosome (Pignoni and Zipursky, 1997) was crossed to an UAS-Abd-B or UAS-Dll stock. Clones were induced by heat-shock in different larval periods.

Immunohistochemistry

Imaginal discs were dissected from third instar larvae, fixed in 4% paraformaldehyde, 0.5% Triton X-100 in PBS solution for 20 minutes, washed in PBS for 5 minutes and blocked in bovine serum albumin (BSA) 1%, 0.1% Tween 20 in PBS for 1 hour. Discs were incubated overnight at 4°C with primary antibodies. Washes were performed with PBS and 0.1% Tween 20 solution for 1 hour, and the discs were incubated for 2 hours with the secondary antibodies in the blocking solution. After washing the secondary antibodies with PBS 0.1% Tween 20 for 1 hour, discs were dissected and mounted in Vectashield Mounting Medium for Fluorescence (Vector Laboratories). The Myc marker was induced by 1 hour heat-shock at 37°C; larvae were left at room temperature for 1 hour before dissecting. The primary antibodies used were mouse anti-Abd-B (Celniker et al., 1989), mouse anti-Antp (Condie et al., 1991), mouse anti-Dac (Mardon et al., 1994; Developmental Studies Hybridoma Bank), rabbit anti-Hth (Kurant et al., 1998), mouse anti-Dll (Duncan et al., 1998), rabbit anti-Bar (Higashijima et al., 1992), mouse anti-Wg (Brook and Cohen, 1996), rabbit anti-β-galactosidase (Cappel), mouse anti-β-galactosidase (Pompea) and mouse anti-Myc (Babco). Secondary antibodies were coupled to Red-X and FITC fluorochromes (Jackson Immunoresearch). Discs were analyzed under a laser-scan Zeiss microscope.

Adult cuticle analysis

Flies were kept in a mixture of ethanol:glycerol (3:1) for several days, macerated in 10% KOH at 60°C for 15 minutes, thoroughly washed with water and ethanol, and mounted in Euparal for inspection under a compound microscope.

RESULTS

**Loss of Abd-B function transforms genitalia into legs or, less frequently, into antennae**

We induced Abd-B<sup>-</sup> clones and, as previously reported (Sánchez-Herrero et al., 1985; Tiong et al., 1985), they transform posterior abdominal segments into more anterior ones but are normal in the analia. In the genitalia (see Fig. 1A,B for
wild-type female and male genitalia) we frequently observed patches of cuticle bearing trichomes and some bristles that we could not unambiguously identify. Occasionally, we saw bracted bristles or a claw, indicating transformation to leg tissue. Using the Minute method we saw more extensive transformations of genitalia into leg (Fig. 1C,D), including typical leg pattern elements such as the claws, tarsus and tibia. In one case we could identify leg structures typical of mesothoracic leg (Fig. 1C). Three clones transformed to distal antennae (second and/or third antennal segment and arista; Fig. 1E) and 19 clones (out of 72 flies) transformed into legs. Transformations to legs or antennae are cell autonomous.

Expression of Distal-less, homothorax and dachshund in the genital primordia

We describe above that \textit{Abd-B} \textsuperscript{-} clones induced leg development in the genitalia. The formation of legs requires the activity of genes such as \textit{homothorax} (\textit{hth}), \textit{dac} and \textit{Dll}, which specify their proximal, medial and distal parts, respectively (Cohen and Jürgens, 1989a; Cohen and Jürgens, 1989b; Mardon et al., 1994; Lecuit and Cohen, 1997; Gorfrinkiel et al., 1997; González-Crespo et al., 1998; Campbell and Tomlinson, 1998; Abu-Shaar and Mann, 1998; Wu and Cohen, 1999). Before analysing the effect of \textit{Abd-B} on the expression of these genes, we describe their expression and function in mature wild-type genital discs.

\textit{Dll} expression is regulated by the combined activities of \textit{wingless} (\textit{wg}) and \textit{decapentaplegic} (\textit{dpp}) in the genital primordia (Fig. 2A,B), and is confined to two groups of cells in male and female discs, the female domains being smaller and expressing lower levels of \textit{Dll} protein (Gorfrinkiel et al., 1999). Since \textit{Abd-B} is transcribed in the entire genital primordia of the two sexes, some cells co-express \textit{Abd-B} and \textit{Dll} (Fig. 2C,D). In the male disc, \textit{hth} is not expressed in the \textit{Dll}-expressing cells and is also excluded from a large group of cells surrounding them (Fig. 2E,G-I). Levels of antibody signal vary within the disc, and are higher in the female repressed primordium. In females, the \textit{hth} domain of expression occupies the whole primordium. Lower levels of \textit{Hth} are detected in a region encompassing the \textit{Dll}-expressing cells, whereas higher levels are observed in the male repressed primordium (Fig. 2F). In both sexes, \textit{hth} expression is absent from the anal primordium.

\textit{dac} is expressed differentially in male and female genital primordia (Gorfrinkiel, 1998): in male discs, Dac protein is detected in two broad lateral bands (Fig. 2J), while in female discs it is found in the central region, almost coincident with the \textit{wg}-expressing region (Fig. 2K). Therefore, the expression patterns of \textit{hth}, \textit{dac} and \textit{Dll} differ substantially from those observed in legs.

Role of homothorax in the genital primordia

It is known that expression of \textit{Dll} is not required to make male genitalia and that it has only a minor role in the formation of the female one (Gorfrinkiel et al., 1999). To ascertain the role of \textit{hth} in the genitalia, we induced \textit{hth} \textsuperscript{-} clones during the third larval period and examined them in the adult structures. In the female genitalia, \textit{hth} \textsuperscript{-} clones cause extra growths with additional vaginal teeth (Fig. 3A). In males, these clones show occasionally some abnormalities in the clasper teeth (not shown). \textit{hth} clones in the analia are wild type.

We looked for possible interactions between \textit{Dll} and \textit{hth} in the genital disc. In these and following experiments, unless stated, the results apply both to male and female genital primordia. \textit{Dll} \textsuperscript{-} clones in the \textit{Dll} domain of the male disc have no \textit{hth} expression (Fig. 3B-D). Similarly, in \textit{hth} \textsuperscript{-} clones \textit{Dll} is not ectopically expressed (Fig. 3E). We also expressed \textit{Dll} ectopically and looked for the effect on \textit{hth} expression. \textit{Dll}-expressing cells close to the wild-type \textit{Dll} domain repress \textit{hth} expression, although not all the cells do so. By contrast, clones far from the \textit{Dll} domain do not affect \textit{hth} expression (Fig. 3F-H).

Ectopic expression of \textit{Dll} and \textit{dac} is observed in the genital disc in the absence of \textit{Abd-B}

We have detailed above that \textit{Abd-B} \textsuperscript{-} clones transform genitalia into leg or antennal tissue. To characterize this transformation further we studied the expression of genes
required to form these appendages such as Dll and dac. Abd-B^- clones in the genital primordia tend to segregate from the rest of the tissue, round up and have smooth borders, suggesting they have acquired different affinities. By contrast, clones in the analia have indented borders and do not segregate. We find that Abd-B^- clones in the genital primordium close to the normal Dll domain show ectopic, cell-autonomous Dll expression (Fig. 4A), whereas those far apart do not show such expression. dac is also activated cell autonomously in many Abd-B^- clones (Fig. 4B), although we have not precisely defined the region where dac is activated. As expected, Dll target genes, such as Bar, also become activated in these clones (see below).

**Abd-B is required both to repress and to maintain hth expression in the genital disc**

Abd-B^- clones exhibit differential effects on hth, depending on their position: those close to the Dll domain show no hth expression, whereas those located away from the Dll domain show a slight increase in hth signal. Clones in intermediate positions do not significantly change hth levels (Fig. 4C-H). This distribution, however, is clearer in females, since in males there is a wide region with no hth expression (Fig. 2E,G,H).

The repression of hth observed in some Abd-B^- clones may be mediated by the ectopic Dll (See Fig. 3F-H).
Abdominal-B antagonizes leg development

Fig. 4. Expression of Dll, dac and hth in Abd-B− clones in the genital disc. (A) Female genital disc showing Abd-B− clones (marked by the absence of the β-galactosidase marker, which is in red elsewhere) stained with anti-Dll antibody (green). Some clones do not show ectopic Dll expression and others only in part of the clone (arrow). Arrowhead and asterisk mark wild-type Dll expression in the genital primordium and in the analia, respectively. (B) Female genital disc with Abd-B− clones marked as in A and showing ectopic dac expression (green). The upper part of the disc shows the dac wild-type signal (yellow). (C) Male genital disc with Abd-B− clones, marked by the absence of the Myc marker (red), showing changes in hth expression (in green). The arrowhead points to a clone in which levels of hth are not significantly changed. (D,E) Higher magnification views of the region within the square in C. The arrows in D,E mark a clone in which hth expression disappears, and the arrowhead a clone in which some cells show higher hth signal. In the upper clone, cells closer to the disc border maintain hth expression, perhaps because they are far away from the wild-type Dll domain. (F−H) Female genital disc with Abd-B− clones, showing ectopic Dll expression (red, F,G) and hth signal (green, F,H). Co-expression of hth and Dll is marked in yellow. Expression of hth is repressed in one clone (arrows; not in all the cells of the clone), but hth expression is maintained in another case (arrowheads). The asterisks in F,G show wild-type Dll expression in the anal primordium.

In summary, Abd-B− clones far from wild-type Dll-expressing have no ectopic Dll expression and slightly increased Hth levels. Clones close to the Dll domain, by contrast, have ectopic Dll expression and no hth expression. Clones in intermediate regions have ectopic Dll expression and wild-type hth expression (Fig. 4A,C-H).

Antagonistic activities of Abd-B and wg/dpp to repress and activate Dll expression

In the genital disc, the transcription of Dll depends, as in the leg disc, on dpp and wg signals (Gorfinkiel et al., 1999; Moreno and Morata, 1999). We have shown that Abd-B represses Dll expression. Moreover, increasing Abd-B levels in the Dll domain suppresses Dll transcription (Fig. 5A). We have characterized the antagonistic activities of dpp/wg and Abd-B to determine Dll distribution. Mutations in PKA ectopically activate wg and dpp expression (reviewed by Perrimon, 1995). PKA− clones in the genital primordia activate Dll, although only in some places (Gorfinkiel et al., 1999). This activation is not mediated by changes in Abd-B levels (Fig. 5B). Similarly, although Dll is derepressed in many late Abd-B− clones, we have not observed derepression of either dpp or wg (Fig. 5C and data not shown). We conclude that there is an antagonism between the activation of Dll by dpp/wg signalling and its repression by Abd-B. This is not mediated by changes in the expression of either dpp, wg or Abd-B.

To characterize this antagonism further we made Abd-B− clones that at are also unable to transduce the dpp signal. This signal requires the presence of the type II receptor encoded by the gene punt (put; Ruberte et al., 1995; Letsou et al., 1995). In put− Abd-B− double mutant clones, Dll is not activated, indicating that, in the absence of Abd-B, Dpp (and possibly Wg) are still required to activate Dll (Fig. 5D).

We also note that Abd-B− clones far from the wild-type Dll domain fail to activate Dll ectopically, suggesting that activation of Dll in the absence of Abd-B depends on the range of diffusion of Dpp and Wg, as in the leg disc (Díaz-Benjumea et al., 1994;
Abd-B prevents full Dll activity in the genital disc

Dll is required for the development of legs and antennae, and induces these structures when expressed ectopically in the wing or eye-antennal discs (Gorfinkiel et al., 1997; Dong et al., 2000). However, although Dll is also expressed in the genital primordia, this expression does not lead to the formation of any of these appendages. To test if Abd-B prevents Dll function we eliminated Abd-B in Dll-expressing cells, and found that they formed leg tissue (Fig. 6A). However, it is possible that the high levels of Dll observed in these mutant cells accounted for the leg transformation. To test this, we made use of the GAL4/UAS system to increase Dll expression in the genital disc (dpp-GAL4/UAS-Dll flies). Male and female genitalia of this genotype are abnormal, but not transformed into leg (not shown).

To extend these observations, we studied the ability of Dll to promote Bar transcription, a gene expressed in the leg disc and activated by Dll (Kojima et al., 2000). Bar is not expressed in the female genital primordium and only in a few cells within the Dll domain (not shown) in the male genital primordium; however, Abd-B clones show Bar expression in both sexes (Fig. 6B). When Dll is ectopically expressed in the genital disc, Bar expression is activated in some of the cells that express Dll (Fig. 6C-E). These results suggest that, in females, Dll levels are insufficient to activate Bar when Abd-B is present, but that increasing Dll expression or removing Abd-B activates Bar transcription. Abd-B, therefore, prevents some Dll activity in females. In males, although there is Bar transcription, leg tissue is not formed, probably because Abd-B modifies or prevents the activation of other Dll target genes. A similar case has been reported in the wing disc: ectopic Dll activates bric a brac, a gene downstream of Dll, both in the wing pouch and the body wall region of the wing disc; however, legs appear in the wing, but not in the notum (Gorfinkiel et al., 1997).

Abd-B is required to prevent Antennapedia expression in the genital disc

The Hox gene Antennapedia (Antp) is involved in leg development (Struhl, 1981). Therefore, we have examined whether Antp is derepressed in Abd-B− clones. Antp is not transcribed in the wild-type genital disc, but some Abd-B− clones show Antp signal (Fig. 7A). The presence of the Antp product, however, is not required to transform the genitalia into a leg, since Antp− Abd-B− double mutant clones still form ectopic legs (Fig. 7B). This result is consistent with the view that the role of Antp in leg specification is simply to repress hth expression (Casares and Mann, 1998). It seems that Dll alone is able to direct leg development, provided that Hox and hth genes are not transcribed. Under these conditions, leg tissue can be formed in several appendages: leg, wing, antennal and genital primordia (Gorfinkiel et al., 1997; Casares and Mann, 1998; this report).

We also looked at Ubx and abdominal A (abd-A) expression in Abd-B− clones. Ubx was not derepressed in these clones, whereas some clones presented weak ectopic abd-A expression, but only in some cells (data not shown).

DISCUSSION

We have found that in the absence of Abd-B, genital tissue is transformed into leg or, more rarely, into antennal tissue, both in males and females. Our results explain similar transformations observed in mutants for some trithorax-group genes (Ingham, 1985; Shearn et al., 1987; Breen, 1999), since mutations in these genes reduce Abd-B expression (Breen and Harte, 1993). They also account for the development of leg or antennal tissue when the posterior segments of BX-C embryos are cultured in vivo (Simcox et al., 1991). These transformations imply changes in the expression of key genes in the specification of legs and antennae, like Dll and hth, which we discuss below.

Abd-B antagonizes Dll activation in the genital disc

The relationship between Dll expression and Hox gene activity depends on the Hox gene and on the developmental stage: in
the embryonic abdomen, 

Abdominal-B antagonizes leg development

Abd-B specifies genitalia as opposed to leg or antennal development

Relationship between Abd-B, Dll and hth in the genital primordia

In the leg disc, hth and Dll are expressed in distinct domains (Abu-Shaar and Mann, 1998; Wu and Cohen, 1999). In the antennal disc, by contrast, these two genes are co-expressed (Casares and Mann, 1998; Dong et al., 2000). The situation in the genital disc is different from these two cases: hth and Dll are co-expressed in the female genitalia, while in the male genitalia they are not co-expressed, nor do their expression patterns abut (Fig. 8A,B).

Although BX-C genes repress hth expression in the embryo and eye-antennal disc (Kurant et al., 1998; Yao et al., 1999), in the genital disc Abd-B both represses and sustains hth expression. The region where Abd-B maintains hth expression is included within that in which Abd-B represses Dll. The precise limits of these regions cannot be defined, owing to the complex structure of the genital disc and to the fact that in the male disc there is a large region with no hth expression. However, careful examination of Abd-B- clones allows subdividing the genital primordia into two regions: the first region is competent to form an appendage when Abd-B is removed. Some cells within this region transcribe Dll in the wild type, while other cells activate Dll ectopically when Abd-B is not functional. The second region has no Dll expression, either in the presence or absence of Abd-B. This region, defined by cells located far from the wild-type Dll-expressing cells, would be equivalent to the ‘body-wall’ region of legs, but, contrary to what happens in these appendages, it is not delimited by hth expression. Abd-B slightly downregulates hth expression in this domain. In the region competent to form appendages, and in the absence of Abd-B, hth expression is either eliminated (in clones near wild-type Dll-expressing cells) or does not change. Therefore, there is an overlap of Dll and hth expression in a subset of the clones induced in the ‘appendage-competent’ region (see Fig. 8C,D).

Abd-B specifies genitalia as opposed to leg or antennal development

While Dll alone is able to form leg tissue (Gorfinkiel et al., 1997), the co-expression of Dll and hth forms antennae (Casares and Mann, 1998; Dong et al., 2000). Some Abd-B- clones in the genital primordia show ectopic Dll transcription and do not change levels of hth expression. We suspect that these clones could account for the occasional appearance of antennal tissue in the adult genitalia. Dong et al. report that the co-expression of Dll and hth in the genital disc forms antennae, although it is not entirely clear whether this takes place in the genital or the anal primordium (Dong et al., 2000).

The interaction between Abd-B and Dll in the formation of genital structures may not be unique to Drosophila. In the locust Schistocerca gregaria, Abd-B is expressed in the appendages of the terminal abdominal segments and modifies their development in order to form genitalia (Kelsh et al., 1993). In the butterfly Precis coenia, and the moth Manduca sexta, there is also Dll expression in the proleg primordia. However, it is not known if Abd-B plays any role in delimiting this expression.

Abd-B expression correlates with development of genital structures in Drosophila, Schistocerca (Kelsh et al., 1993), the crustacean Artemia (Averof and Akam, 1995) and the chilopod Cupiennus salei (Damen and Tautz, 1999). Genes homologous to Abd-B in the Hox complex D of mouse are also required for the formation of genital structures in mammals (Dollé et al., 1991). It seems that Abd-B is the gene required to make genitalia, and that this was its primordial function in evolution (Akam et al., 1988; Damen and Tautz, 1999).
REFERENCES


