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

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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; Gorfinkiel 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; Gorfinkiel et al., 1999). Moreover, the genital disc also expresses *Dll*, similar to leg or antennal discs (Gorfinkiel et al.,

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 of *Dll* 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; Gorfinkiel 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 of *Dll* 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: $Abd\text{-}B^{M1}$ (Casanova et al., 1986) and $Abd\text{-}B^{D18}$, which is a small deficiency that removes the homeobox and adjacent sequences (Hopmann et al., 1995). Other mutants used were Dll^{SA1} (Cohen and Jürgens, 1989a), $Antp^{Ns+RC3}$ (Struhl, 1981), hth^{P2} (Pai et al., 1998), put^{135} (Letsou et al., 1995) and the allele DCO^{B3}, 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 (BS3.0; 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 of Dll^{-} flies in combination with UAS-*Dll* – therefore reproducing the normal *Dll* expression pattern (Gorfinkiel et al., 1997). The other Gal4 line used was *dpp*-Gal4 (Morimura et al., 1996). The UAS stocks used were UAS-*Dll* (Gorfinkiel 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 25ºC, except the cross to obtain *dpp*-GAL4/UAS-*Dll* larvae, which was kept at 17ºC for 7 days and transferred then to 25ºC. 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 *FRT82B hs-piMyc Sb* y^{+} (Xu and Rubin, 1993); *FRT82B hs-CD2* y^{+} *M(3)w* (Abu-Shaar and Mann, 1998); *FRT82B arm-lacZ Dp* (f^{+}) *M(3)W123* (Weigmann and Cohen, 1999); *FRT40A arm-lacZ* and *FRT42D arm-lacZ* (Chen and Struhl, 1996); and *FRT82B* hth^{P2} (Pai et al., 1998) and *FRT42D* Dll^{SA1} (Gorfinkiel et al., 1997). The *FRT82B* $Abd\text{-}B^{M1}$, *FRT82B* $Abd\text{-}B^{D18}$, *FRT82B* $Antp^{Ns+RC3}$ $Abd\text{-}B^{D18}$ and *FRT82B* put^{135} $Abd\text{-}B^{D18}$ chromosomes were obtained by standard genetic recombination. *hs-flp122* is described by Struhl and Basler (Struhl and Basler, 1993).

Clones were induced, in general, during second and third larval instars by a 37ºC 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 $Abd\text{-}B^{-}$ 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 $Abd\text{-}B^{M1}$ and $Abd\text{-}B^{D18}$ alleles gave comparable results. The genotypes of the larvae in which the clones were induced were as follows.

$Abd\text{-}B^{-}$ clones: *y hs-flp122; FRT82B* $Abd\text{-}B^{M1}$ or *FRT82B* $Abd\text{-}B^{D18}$/*FRT82B hs-CD2* y^{+} *M(3)w* or *FRT82B hs-piMyc Sb* y^{+} or *FRT82B arm-lacZ Dp*(f^{+}) *M(3)W123*

$Antp^{-}$ $Abd\text{-}B^{-}$ *clones*: *y hs-flp122; FRT82B* $Antp^{Ns+RC3}$ $Abd\text{-}B^{D18}$/*FRT82B hs-CD2* y^{+} *M(3)w*

put^{-} $Abd\text{-}B^{-}$ clones: *y hs-flp122; FRT82B* put^{135} $Abd\text{-}B^{M1}$/FRT82B *arm-lacZ Dp*(f^{+}) *M(3)W123* or *FRT82B hs-CD2 M(3)w* y^{+}

$Abd\text{-}B^{-}$ clones in the *Dll* domain: *em212 (Dll-GAL4) UAS-flp/+; FRT82B,* $Abd\text{-}B^{D18}$/*FRT82B hs-CD2* y^{+} *M(3)w*

$Abd\text{-}B^{-}$ clones, *dpp* expression: *y hs-flp122; dpp-lacZ/+; FRT82B* $Abd\text{-}B^{M1}$/*FRT82B hs-piMyc Sb* y^{+}

hth^{-} clones: *y hs-flp122; FRT82B* hth^{P2}/*FRT82B arm-lacZ Dp*(f^{+}) *M(3)W123* or *FRT82B hs-CD2* y^{+} *M(3)w*

Dll^{-} clones: *y hs-flp122; FRT42D* Dll^{SA1}/*FRT42D arm-lacZ*

PKA^{-} clones: *y hs-flp122;* DCO^{B3} *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 4ºC 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 37ºC; 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 (Promega) and mouse anti-Myc (Babco). Secondary antibodies were coupled to Red-X and FITC fluorochroms (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 60ºC 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 $Abd\text{-}B^{-}$ 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

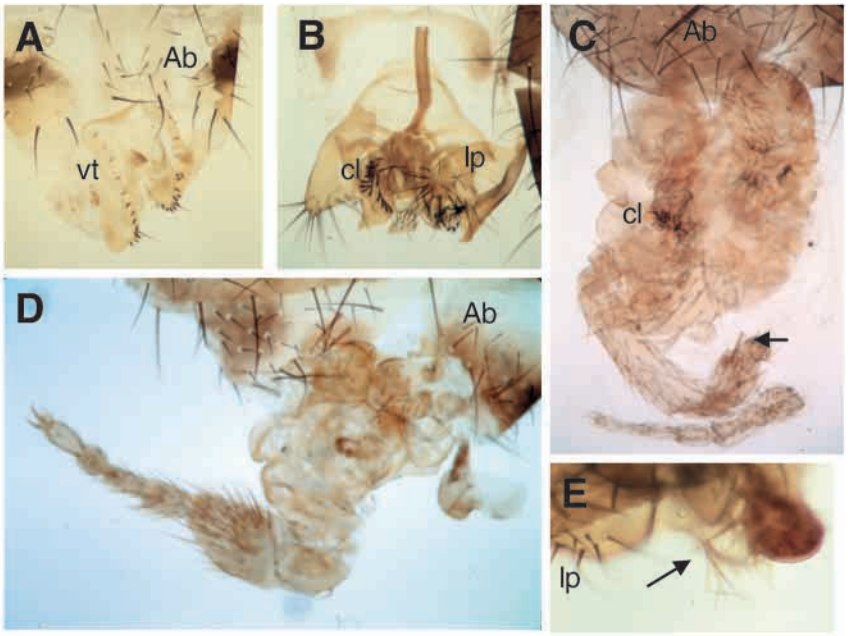

Fig. 1. Transformations produced by *Abd-B*$^{-}$ clones in the genitalia. (A) Wild-type female genitalia. (B) Wild-type male genitalia. (C,D) *Abd-B*$^{-}$ clones (marked with *yellow*) transforming male (C) and female (D) genitalia into leg. Note unidentified tissue in the upper part of the legs. The arrow in C marks the apical bristle, characteristic of mesothoracic leg. In females, these legs substitute for much of the vulva and part of the vaginal plates, some vaginal teeth frequently remaining. In males, the legs substitute for the hypandrium, penis apparatus and part of the clasper. The elements of the genitalia that remain (all y^{+}), however, are deformed by the transformation and difficult to identify. (E) *Abd-B*$^{-}$ clone (marked with *yellow*) showing transformation into arista (arrow) in a male genitalia. Ab, Abdomen; cl, clasper teeth; lp, lateral plate; vt, vaginal teeth.

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 *Abd-B*$^{-}$ clones induced leg development in the genitalia. The formation of legs requires the activity of genes such as *homothorax* (*hth*), *dac* and *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; Gorfinkiel 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 *Abd-B* on the expression of these genes, we describe their expression and function in mature wild-type genital discs.

Dll expression is regulated by the combined activities of *wingless* (*wg*) and *decapentaplegic* (*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 Dll protein (Gorfinkiel et al., 1999). Since *Abd-B* is transcribed in the entire genital primordia of the two sexes, some cells co-express *Abd-B* and *Dll* (Fig. 2C,D). In the male disc, *hth* is not expressed in the *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 *hth* domain of expression occupies the whole primordium. Lower levels of Hth are detected in a region encompassing the *Dll*-expressing cells, whereas higher levels are observed in the male repressed primordium (Fig. 2F). In both sexes, *hth* expression is absent from the anal primordium.

dac is expressed differently in male and female genital primordia (Gorfinkiel, 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 *wg*-expressing region (Fig. 2K). Therefore, the expression patterns of *hth*, *dac* and *Dll* differ substantially from those observed in legs.

Role of *homothorax* in the genital primordia

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

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

Ectopic expression of *Dll* and *dac* is observed in the genital disc in the absence of *Abd-B*

We have detailed above that *Abd-B*$^{-}$ clones transform genitalia into leg or antennal tissue. To characterize this transformation further we studied the expression of genes

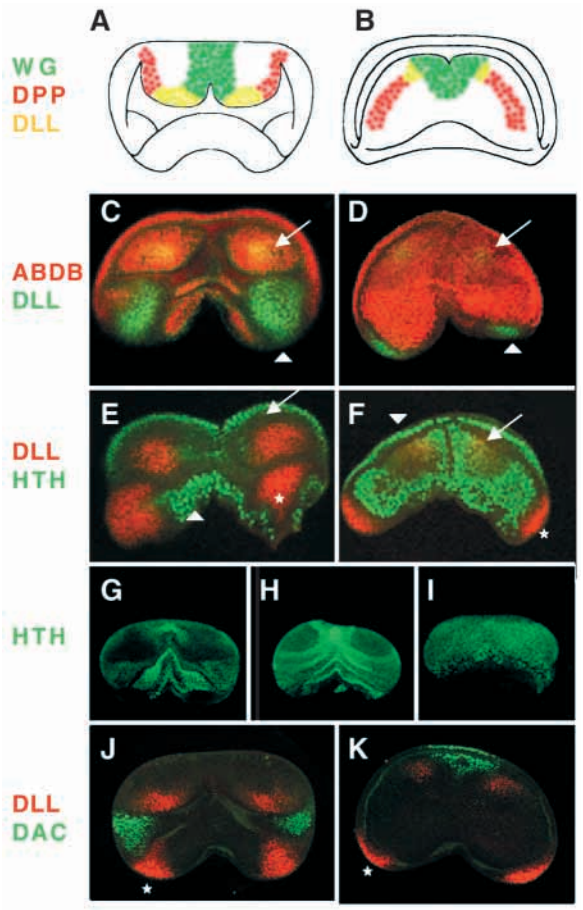

Fig. 2. Expression of *Abd-B*, *Dll*, *hth* and *dac* in the wild-type genital disc. The orientation of the different discs in this and following figures is not exactly the same: only the anal primordium is observed in some. Since the genital disc is a bilateral disc, we indicate the expression of genes only in the left or right sides. (A,B) *dpp*, *wg* and *Dll* expression in male (A) and female (B) genital primordia (Freeland and Kuhn, 1996; Chen and Baker, 1997; Casares et al., 1997; Gorfinkiel et al., 1999). (C,D) Co-expression (in yellow, arrows) of *Abd-B* (red) and *Dll* (green) in male (C) and female (D) primordia of male and female genital discs. Arrowheads show *Dll* expression in the anal primordium. (E,F) Male (E) and female (F) genital discs showing expression of *hth* (green) and *Dll* (red). *hth* and *Dll* are co-expressed in females (in yellow; arrow in F) but not in males. Note absence of *hth* signal in cells surrounding the *Dll* domain in the male disc (arrow in E) and low levels in cells close to this domain in the female disc. See also higher levels of *hth* expression in the female and male repressed primordia (arrowheads in E and F). Asterisks indicates *Dll* expression in the analia. (G-I) Three different optical sections in a dorsal-ventral axis of a male genital disc marked with anti-*hth* antibody. (J,K) Male (J) and female (K) genital discs showing expression of *dac* (in green) and *Dll* (in red). Asterisks indicate expression of *Dll* in the analia.

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).

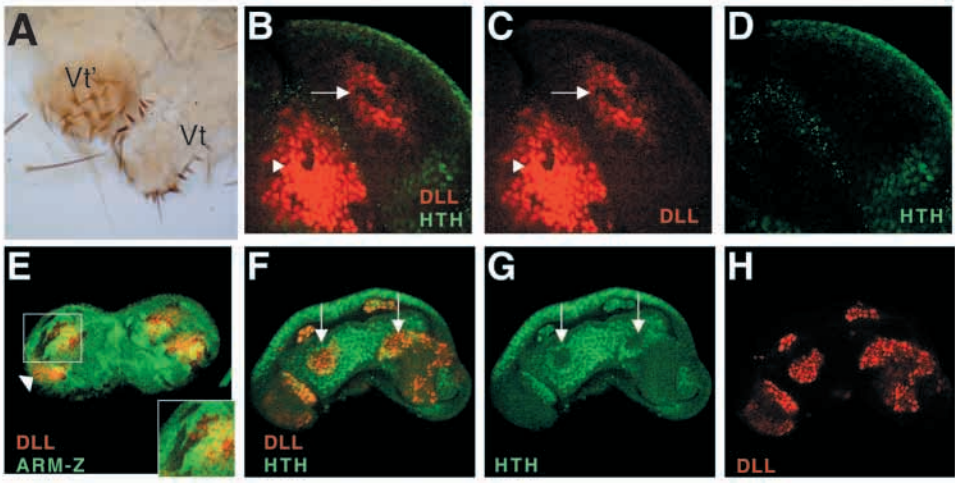

Fig. 3. Function of *hth* in the female genitalia and interactions between *Dll* and *hth* in the genital disc. (A) *hth*$^{-}$ clone in the female genitalia, marked with *yellow*. The clone forms ectopic, disorganized, vaginal teeth (Vt′), close to the wild-type ones (Vt). (B-D) Male genital disc with *Dll*$^{-}$ clones (marked by the absence of *Dll* expression, red in C) showing that there is no derepression of *hth* expression (in green, D). B shows merged image. The arrow points to a clone in the genital primordium and the arrowhead indicates *Dll* expression in the analia. (E) Male genital disc with *hth* clones (in black, owing to the absence of the β-galactosidase marker, which is in green elsewhere) showing there is no ectopic *Dll* expression (in red). Detail of the region within the square is shown in the inset below. Arrowhead shows *Dll* expression in the analia. (F-H) Female genital disc with *Dll*$^{+}$ clones (*Dll* in red, F,H). Some of these clones (arrows) repress *hth* expression (in green, F,G), although some cells co-express *Dll* and *hth*, resulting in a yellow color (F, merged image).

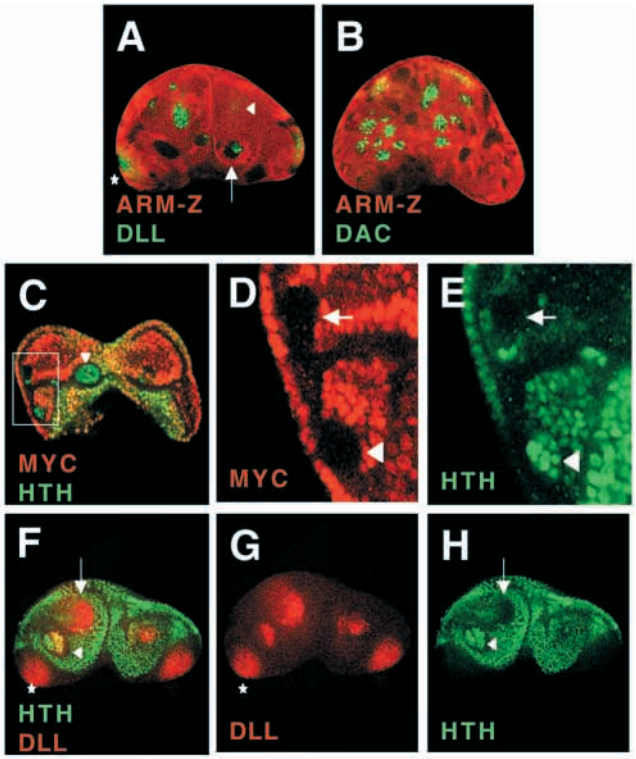

Fig. 4. Expression of *Dll*, *dac* and *hth* in *Abd-B*$^{-}$ clones in the genital disc. (A) Female genital disc showing *Abd-B*$^{-}$ clones (marked by the absence of the β-galactosidase marker, which is in red elsewhere) stained with anti-*Dll* antibody (green). Some clones do not show ectopic *Dll* 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 (in 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*.

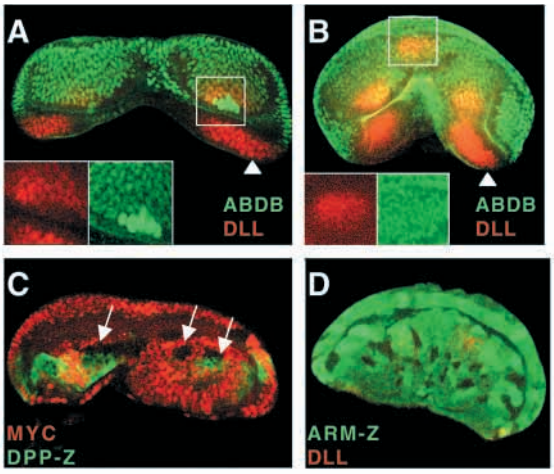

Fig. 5. *Abd-B*, *wg* and *dpp* determine *Dll* expression in the genital primordia. (A) Male genital disc with an *Abd-B*$^{+}$ clone, marked by the higher levels of green fluorescence, showing repression of *Dll* (in red). Higher magnification views of the clone in the insets below. Arrowhead shows expression in the analia. (B) Female genital disc with a *PKA*$^{-}$ clone (within the square) that presents ectopic *Dll* expression (in red). *Abd-B* staining (green) is not changed in the clone, so the merged image appears yellow. Higher magnification views of the clone in the insets below. Arrowhead shows *Dll* expression in the analia. (C) Female genital disc of a *dpp-lacZ* larva with *Abd-B*$^{-}$ clones (arrows; marked by the absence of the *Myc* marker, in red) and stained with anti-β-galactosidase antibody (green, revealing the *dpp* wild-type expression). *Abd-B*$^{-}$ clones do not activate *dpp* ectopically. (D) Female genital disc showing *put*$^{-}$ *Abd-B*$^{-}$ clones (black, owing to the absence of the β-galactosidase marker, which is in green) and stained with anti-*Dll* antibody (red). There is no ectopic *Dll* in these clones.

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;

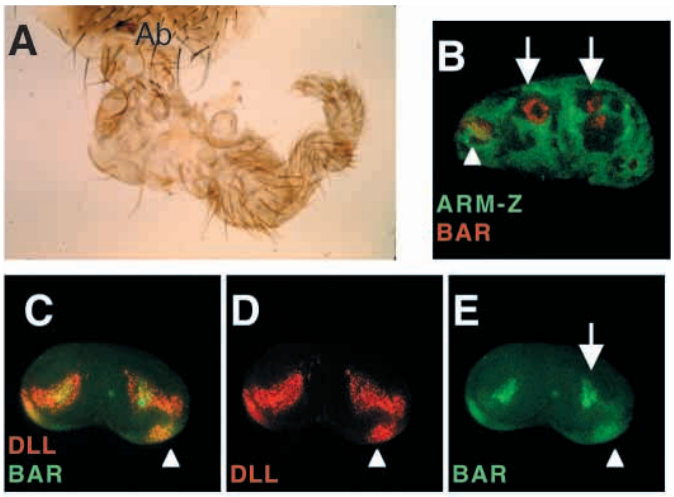

Fig. 6. *Abd-B* expression and *Dll* activity. (A) *Abd-B*$^{-}$ clone (marked with *yellow*) induced in *Dll*-expressing cells in the female genitalia, showing transformation to leg. (B) Female genital disc showing ectopic expression of *Bar* (red, arrows) in an *Abd-B*$^{-}$ clone (black, because of the absence of the β-galactosidase marker, which is green elsewhere). Arrowhead indicates *Bar* wild-type expression in the analia. (C-E*) dpp*-GAL4/UAS-*Dll* female genital disc, showing ectopic *Dll* expression (red, D) coincident in some cells with ectopic *Bar* signal (arrow; green, E). Merged image in C. Arrowheads indicate expression in the analia.

Lecuit and Cohen, 1997) and in the anal primordium (Moreno and Morata, 1999; Gorfinkiel et al., 1999).

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).

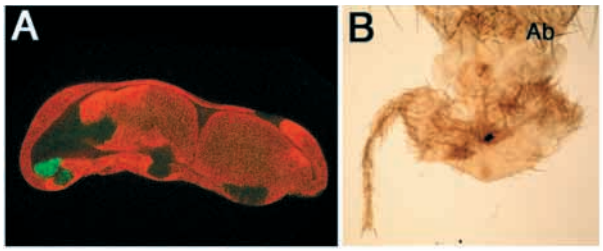

Fig. 7. *Abd-B* interaction with *Antp* in the genitalia. (A) Female genital disc with *Abd-B*$^{-}$ clones (black, owing to the absence of the β-galactosidase marker, which is in red elsewhere). One clone shows *Antp* expression (green). (B) *Antp*$^{-}$ *Abd-B*$^{-}$ clone (marked with *yellow*) in a female, transforming the genitalia into a leg. Ab, Abdomen.

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

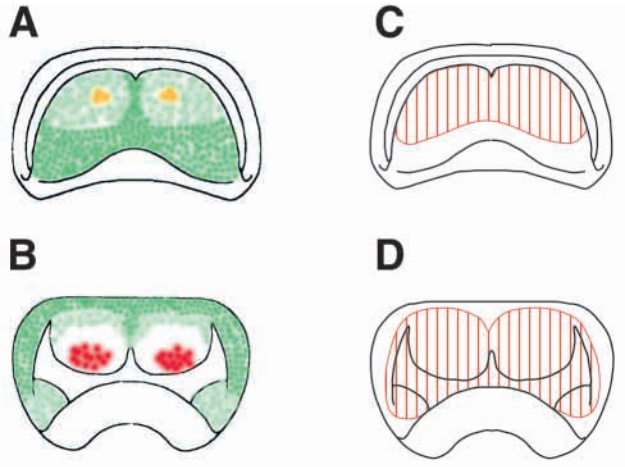

Fig. 8. Summary of *Dll* and *hth* expression in the genital primordia and definition of the 'appendage-competent' region. (A,B) Drawings of female (A) and male (B) discs showing the expression of *Dll* (red) and *hth* (green). Co-expression in yellow. (C,D) Drawings of female (C) and male (D) genital discs showing the 'appendage-competent' region (red). Since *Abd-B*$^{-}$ clones segregate from the tissue, the limits of this region are approximate. Some *Abd-B*$^{-}$ clones within this region (those far apart from *Dll*-expressing cells) co-express *Dll* and *hth*, and possibly cause transformation to antennae.

the embryonic abdomen, *Dll* is repressed by BX-C genes (Simcox et al., 1991; Cohen et al., 1991; Vachon et al., 1992), whereas in the maxilla *Deformed* maintains *Dll* activity (O'Hara et al., 1993). While *Ubx* represses *Dll* only in early embryos (Castelli-Gair and Akam, 1995), the repressive effect of *Abd-B* on *Dll* is maintained throughout embryonic and larval stages.

In the genital disc, *Dll* is activated by *wg* and *dpp* (Gorfinkiel et al., 1999) and repressed by *Abd-B*. Some cells, however, co-express *Dll* and *Abd-B*, suggesting that in those cells the combined activity of *dpp* and *wg* reaches maximum levels and overcomes *Abd-B* repression. This competition depends on the levels of *Abd-B* and *dpp*/*wg* activity, since increasing the signalling of *dpp*/*wg* (in *PKA*$^{-}$ clones) activates *Dll*, and increasing *Abd-B* transcription represses *Dll* expression. We have shown that this competition is not mediated through the control of either *Abd-B* or *dpp*/*wg* expression. Rather, since the *Ubx* and *abd-A* proteins repress *Dll* by direct binding to the *Dll* regulatory region (Vachon et al., 1992) it is likely that the *Abd-B* product does it as well. The *Dll* regulatory region would integrate the activating and repressing signals to control *Dll* transcription. If so, *Abd-B* would specify genitalia by modifying the response to the positional information provided by Dpp/Wg signalling. In the absence of *Abd-B*, *Dll* would be activated according to the range of diffusion of *dpp* and *wg* products. However, the fact that clones of *PKA* only activate *Dll* in certain places (Gorfinkiel et al, 1999) suggests that factors other than *Abd-B* limit the extent of Dpp and Wg activity, or that *Abd-B* activity is higher in certain positions.

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 of the terminal segment (Panganiban et al., 1994; Zheng et al., 1999), but 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 chelicerate *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).

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