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)

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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).
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; Gorfinikel 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-BM1 (Casanova et al., 1986) and Abd-BD18, which is a small deficiency that removes the homeobox and adjacent sequences (Hoppman et al., 1995). Other mutants used were DllSA1 (Cohen and Jürgens, 1989a), AntpNS+RC3 (Struhl, 1981), hhP2 (Pai et al., 1998), puf155 (Letsou et al., 1995) and the allele DCOB+, mutant in the principal catalytic subunit of the protein kinase A (PKA) gene (Pua-Cl – 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 (Callega 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 (Gorfinikel et al., 1997). The other Gal4 line used was dpp-Gal4 (Moriyama et al., 1996). The UAS stocks used were UAS-Dll (Pai et al., 1997), UAS-hth (Pai et al., 1998), UAS-fli (Campbell and Tomlinson, 1998) and a UAS-Abd-B construct that drives expression of the m (or I) protein isoform (Castelli-Gair et al., 1998). 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 FRT82B hs-piMyc Sb y+ (Xu and Rubin, 1993); FRT82B hs-CDD 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 hhP2(Pai et al., 1998) and FRT42D DllPA1 (Gorfinikel et al., 1997). The FRT82B Abd-BM1, FRT82B Abd-BD18, FRT82B AntpNS+RC3 Abd-BD18 and FRT82B puf155 Abd-BD18 chromosomes were obtained by standard genetic recombination. hs-fliP122 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-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-BM1 and Abd-BD18 alleles gave comparable results. The genotypes of the larvae in which the clones were induced were as follows.

Abd-B- clones: y hs-fliP122; FRT82B Abd-BM1 or FRT82B Abd-BD18/FRT82B hs-CDD y+ M(3)w or FRT82B hs-piMyc Sb y+ or FRT82B arm-lacZ Dp(f+) M(3)W123

Antp- Abd-B- clones: y hs-fliP122; FRT82B AntpNS+RC3 Abd-BD18/FRT82B hs-CDD y+ M(3)w

put- Abd-B- clones: y hs-fliP122; FRT82B put155 Abd-BM1/FRT82B arm-lacZ Dp(f+) M(3)W123 or FRT82B hs-CDD y+ M(3)w

Abd-B- clones in the Dll domain: em122 (Dll-GAL4) UAS-fliP+/FRT82B, Abd-BD18/FRT82B hs-CDD y+ M(3)w

Abd-B- clones, dpp expression: y hs-fliP122; dpp-lacZl+/FRT82B Abd-BM1/FRT82B hs-piMyc Sb y+ hth- clones: y hs-fliP122; FRT82B hthP2/FRT82B arm-lacZ Dp(f+) M(3)W123 or FRT82B hs-CDD y+ M(3)w

Dll- clones: y hs-fliP122; FRT42D DllPA1/FRT42D arm-lacZ

PKA- clones; y hs-fliP122; DCOB+ FRT40A/FRT40A arm-lacZ

To express Abd-B and Dll ectopically, the hs-fli 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 (Promega) 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- 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. (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
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. 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; Gorlinski 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. 

**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).
Antagonistic activities of Abd-B and wg/dpp to Abd-B

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 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 (Diáz-Benjumea et al., 1994;
the female genital primordium and only in a few cells within the Dll domain (not shown) in the male genital primordium; however, AbdB- 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).

**AbdB 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 tissue 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.

**AbdB 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...
Abd-B antagonizes leg development

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., 1997), 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).

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 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.

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.

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
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