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The ePress version of this article published on 30 November 2005 contains an error.

UAS-*myc*-EGFP^{F} should read UAS-*nls*-*myc*-EGFP on pages 118 and 119.

Both the published print on final online versions of the article are correct.

The authors apologise to readers for this mistake.

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Requirement of *abdominal-A* and *Abdominal-B* in the developing genitalia of *Drosophila* breaks the posterior downregulation rule

David Foronda*, Beatriz Estrada*,†, Luis de Navas and Ernesto Sánchez-Herrero‡

The genitalia of *Drosophila* derive from the genital disc and require the activity of the *Abdominal-B* (*Abd-B*) Hox gene. This gene encodes two different proteins, Abd-B M and Abd-B R. We show here that the embryonic genital disc, like the larval genital disc, is formed by cells from the eighth (A8), ninth (A9) and tenth (A10) abdominal segments, which most likely express the Abd-B M, Abd-B R and Caudal products, respectively. *Abd-B m* is needed for the development of A8 derivatives such as the external and internal female genitalia, the latter also requiring *abdominal-A* (*abd-A*), whereas *Abd-B r* shapes male genitalia (A9 in males). Although *Abd-B r* represses *Abd-B m* in the embryo, in at least part of the male A9 such regulation does not occur. In the male A9, some *Abd-B* $m^{-}r^{-}$ or *Abd-B* r^{-} clones activate *Distal-less* and transform part of the genitalia into leg or antenna. In the female A8, many *Abd-B* $m^{-}r^{-}$ mutant clones produce similar effects, and also downregulate or eliminate *abdominal-A* expression. By contrast, although *Abd-B m* is the main or only *Abd-B* transcript present in the female A8, *Abd-B* m^{-} clones induced in this primordium do not alter *Distal-less* or *abd-A* expression, and transform the A8 segment into the A4. The relationship between *Abd-B* and *abd-A* in the female genital disc is opposite to that of the embryonic epidermis, and contravenes the rule that posteriorly expressed Hox genes downregulate more anterior ones.

KEY WORDS: Hox, *abdominal-A*, *Abdominal-B*, *Distal-less*, Genitalia, Imaginal disc

INTRODUCTION

The Hox genes specify the morphological diversity along the anteroposterior axis of most animal species (Mann and Morata, 2000; McGinnis and Krumlauf, 1992). Hox proteins contain a DNA-binding domain, the homeodomain (Gehring et al., 1994), and act as transcription factors, controlling the expression of different downstream genes (Graba et al., 1997). The outcome of Hox gene activity is the formation of different structures. In fact, one remarkable aspect of some Hox mutations is the transformation of one structure into another one (Mann and Morata, 2000; McGinnis and Krumlauf, 1992).

These transformations are particularly striking in *Drosophila* appendages. For example, the ectopic expression of the Hox gene *Antennapedia* in the antennal primordium converts the antenna into a leg (Frischer et al., 1986; Jorgensen and Garber, 1987; Schneuwly et al., 1987a; Schneuwly et al., 1987b). In some cases, homeotic transformations have revealed the hidden appendage structure of certain organs. Thus, the genitalia of *Drosophila* derive from a primordium with appendage-like characteristics (Gorfinkiel et al., 1999), and are indeed transformed into a leg or an antenna in the absence of the Hox gene *Abdominal-B* (*Abd-B*) (Estrada and Sánchez-Herrero, 2001).

The genitalia and analia (collectively known as terminalia) are ectodermic structures located at the posterior region of the adult that derive from the genital disc. This disc is the only unpaired disc of *Drosophila*, and is formed by the fusion of three primordia corresponding to the eighth (A8), ninth (A9) and tenth (A10) abdominal segments. The different development of these primordia in males or females depends on the sex-determination signals. In females, the A8 segment forms the female genitalia and the eighth tergite, the A9 forms the parovaria and part of the uterine wall (both belonging to the internal genitalia), and the A10 forms the female analia. In males, the A8 gives rise to a tiny A8 segment, the A9 to the male genitalia and the A10 to the male analia (Keisman et al., 2001; Nöthiger et al., 1977; Schüpbach et al., 1978). The morphology of these structures, however, also depends on two other groups of genes: one group is formed by genes such as *engrailed*, *hedgehog*, *decapentaplegic* (*dpp*) and *wingless*, genes that are involved in signaling pathways (Casares et al., 1997; Chen and Baker, 1997; Emerald and Roy, 1998; Freeland and Kuhn, 1996; Keisman and Baker, 2001; Sánchez et al., 1997; Sánchez et al., 2001); the other group includes the Hox gene *Abd-B*, which is needed for the formation of the genitalia (Casanova et al., 1986; Celniker et al., 1990; Estrada and Sánchez-Herrero, 2001; Karch et al., 1985; Sánchez-Herrero et al., 1985; Tiong et al., 1985), and the Hox-like gene *caudal* (*cad*), which is required for analia development (Moreno and Morata, 1999). It is the combined activity of these three sets of elements (sex-determination genes, signaling pathways and Hox genes) that shapes the terminalia of the adult fly (reviewed by Christiansen et al., 2002; Estrada et al., 2003; Sánchez and Guerrero, 2001).

Homeotic gene function in the genital primordia, however, is more complex than what we have just described. First, the *abdominal-A* (*abd-A*) Hox gene, required for the development of A1-A8 in the embryo (Sánchez-Herrero et al., 1985; Tiong et al., 1985), is expressed in the A8 of the female genital disc (Casares et al., 1997; Freeland and Kuhn, 1996). No function has been ascribed yet to such expression. Second, *Abd-B* is a complex gene: the use of four different promoters and the existence of specific exons give rise

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to several transcripts that encode two different proteins. The *A* (*m*) transcript encodes the Abd-B M (or Abd-B I) protein, and the *B*, *C* (*r*) and γ RNAs encode the Abd-B R (or Abd-B II) protein (Celniker et al., 1989; DeLorenzi et al., 1988; Kuziora and McGinnis, 1988; Zavortink and Sakonju, 1989). The Abd-B M protein has 221 amino acids more than the Abd-B R product does in its N-terminal domain but both proteins share a common C-terminal region, which includes the homeodomain (Celniker et al., 1989; Zavortink and Sakonju, 1989). In the embryonic epidermis, the Abd-B M transcript and protein are expressed in parasegments (PS) 10-13 (A5-A8 segments), whereas the Abd-B R transcript and protein are present in PS14-PS15 (A9-A10) initially, and in PS14 (A9) at late stages (Boulet et al., 1991; Celniker et al., 1989; DeLorenzi et al., 1988; DeLorenzi and Bienz, 1990; Kuziora and McGinnis, 1988; Sánchez-Herrero and Crosby, 1988). The γ RNA is transcribed in just a few cells of PS14 or PS15 (Kuziora and McGinnis, 1988).

The role of Abd-B M and Abd-B R products in genital development remains unclear. *Abd-B m* mutations transform the A5-A8 segments into the A4 segment, both in males and females; the female genitalia are lost whereas male genitalia remain intact (Casanova et al., 1986; Karch et al., 1985; Sánchez-Herrero et al., 1985; Tiong et al., 1985). By contrast, mutations in *Abd-B r* function eliminate genitalia and analia in both sexes (Casanova et al., 1986). Significantly, the transformations obtained in either *Abd-B m* or *Abd-B r* mutants clearly differ from those observed when all *Abd-B* functions are eliminated: in some of the clones mutant for *Abd-B* (*m* and *r*), part of the male or female genitalia are transformed into leg or antenna (Estrada and Sánchez-Herrero, 2001). Therefore, the precise role of *abd-A*, *Abd-B m* and *Abd-B r* in genitalia development is not well defined.

We have analyzed homeotic expression and requirement in terminalia development. We propose that in the embryonic genital disc, as in the larval discs, *Abd-B m*, *Abd-B r* and *cad* are expressed in the A8, A9 and A10, respectively. We also report that *abd-A*, *Abd-B m* and *Abd-B r* are needed for development of the internal female genitalia, *Abd-B m* for the development of female external genitalia and *Abd-B r* for the development of male genitalia. Strikingly, *abd-A* and *Abd-B* bear unexpected relationships in mature genital discs. In the A8 of the female genital disc, Abd-B M maintains *abd-A* expression. In *Abd-B m* mutant clones, however, another Abd-B protein maintains *abd-A* expression but does not prevent *abd-A* function, as these clones transform the A8 segment into the A4. In the male A9, *Abd-B r* function does not repress the *Abd-B m* transcript, at least in part of the primordium, and some *Abd-B r* mutant clones transform male genitalia into leg or antenna. These relationships between Hox genes are different from those reported in the embryonic epidermis and contravene the rule that posteriorly expressed Hox genes repress those expressed more anteriorly.

MATERIALS AND METHODS

Genetics

abd-A^{M1} is an *abd-A* null mutation (Sánchez-Herrero et al., 1985); *abd-A*$^{iab3\text{-}277}$ is a breakpoint located at +63.0-64.5 kb [coordinates according to Karch et al. (Karch et al., 1985)], which transforms abdominal segments posterior to the A2 into the A2 (Busturia et al., 1989; Karch et al., 1985). The *Df109* (or *DfUbx*^{109}) deficiency (89D1-89E1-2) eliminates the *Ubx* and *abd-A* genes (Karch et al., 1985; Lewis, 1978; Sánchez-Herrero et al., 1985), and the *DfR59* deficiency eliminates the *bxd* regulatory region of *Ubx*, the *abd-A* gene and the 3′ regulatory region of *Abd-B* (Gyurkovics et al., 1990). *Dpbxd*^{111} is a duplication on the X chromosome that includes the *abd-A* and *Abd-B* genes (Lewis, 1981). *Abd-B*^{D18}, a small deficiency (from +148.5-150.0 to +163.5-166.5 kb) removing the *Abd-B* gene (Hopmann et al., 1995), and *Abd-B*^{M1} (Casanova et al., 1986; Sánchez-Herrero et al., 1985) are *Abd-B* (m^- r^-) mutations. *Abd-B*^{M5}, *Abd-B*^{M3} (Casanova et al., 1986; Sánchez-Herrero et al., 1985), *Abd-B*^{T2N} (Estrada et al., 2002) and *Abd-B*^{D14} (Karch et al., 1985) are *Abd-B* m^- mutations: *Abd-B*^{M5} is caused by a C to T base substitution that produces a stop codon at amino acid 119, and thus resulting in an Abd-B M protein of 118 amino acids instead of the normal 493 (S. Pelaz and G. Morata, personal communication); *Abd-B*^{T2N} is a derivative of a P-element insertion located at 48,957 (Estrada et al., 2002) [coordinates according to Martin et al. (Martin et al., 1995)]; *Abd-B*^{D14} is a 411 bp deletion extending from 66 bp upstream to 345 bp downstream of the transcription initiation site of the *Abd-B m* RNA (Zavortink and Sakonju, 1989). *Abd-B*^{Uab1} and *Abd-B*$^{X23\text{-}1}$ are *Abd-B* r^- mutations (Casanova et al., 1986): *Abd-B*^{Uab1} is an inversion within the bithorax complex with a breakpoint at +185 kb (Karch et al., 1985) and *Abd-B*$^{X23\text{-}1}$ has a breakpoint at, approximately, +189 kb (Mack et al., 1997). The *abd-A-lacZ* (*HC7JA1*) (Bender and Hudson, 2000) and *hdc-lacZ* (*B5*) (Weaver and White, 1995) lines are *P-lacZ* insertions that reproduce the expression of the *abd-A* and *headcase* genes, respectively. The Gal4/UAS system (Brand and Perrimon, 1993) was used to drive the expression of different genes with the following constructs: UAS-*abd-A* (Michelson, 1994), UAS-*Abd-B m* (Castelli-Gair et al., 1994), UAS-*Dll* (Gorfinkiel et al., 1997), UAS-GFP (Ito et al., 1997), UAS-*lacZ* (Brand and Perrimon, 1993) and UAS-*myc*-EGFP^F (Allan et al., 2003). The *Abd-B*-Gal4^{LDN} line (L.d.N. and E.S.-H., unpublished) and the *cad*-Gal4 line (MD509) (Calleja et al., 1996) are insertions in the *Abd-B* and *cad* genes, respectively, which reproduce the pattern of expression of the *Abd-B m* and *cad* transcripts in the posterior embryonic abdominal segments. The *dpp*-Gal4 line has been previously described (Staehling-Hampton et al., 1994). The MKRS, *abd-A-lacZ* (Bender and Hudson, 2000), TM6B, and TM6B, *abd-A-lacZ* balancers were used to identify mutant larvae and embryos.

In situ hybridization

In situ hybridization was done basically as described (Cubas et al., 1991; Wolff, 2000), with slight modifications. The DNA probe for the *Abd-B m* (A) transcript is a *Bam*HI genomic probe from 50,702 to 48,864 [coordinates as in Martin et al. (Martin et al., 1995)]. RNA probes for the *Abd-B m* (A) or *Abd-B r* transcripts were obtained by the amplification of *Abd-B* cDNA or genomic DNA with region-specific primers followed by in vitro transcription reactions (Stoflet et al., 1988), using the Roche DIG RNA-labeling mix. The *Abd-B m* probe includes 671 bp of the *Abd-B m*-specific exon and was obtained with the following primers: 5′-CAGCAACTACAACAACAGCCGAC-3′ and 5′-ACACGCACACTGCCTAAAGAGC-3′. Two 'common' probes were used to detect all *Abd-B r* RNAs. The first one (I) includes 220 bp of two exons common to all the *Abd-B* r cDNAs and 159 bp of the exon specific for the *Abd-B r* C cDNA, and was obtained with the following primers: 5′-TGGAAAGATCAGACTTGCAGGTCACG-3′ and 5′-TGGGATGGGAACTGACGCTGGA-3′. The second one (II) includes 7 bp of an exon common to all the *Abd-B* cDNAS, 223 bp of two exons common to all the *Abd-B r* cDNAs and 66 bp of the exon specific for the *Abd-B* r γ cDNA, and was obtained with the following primers: 5′-CGGAAGATTGTATTTGTGCGGTTG-3′ and 5′-TTGATGTCTGTGGGATGGGAAC-3′.

Double X-gal staining and in situ hybridization was performed basically as described previously (Wolff, 2000). For double antibody staining and in situ hybridization in imaginal discs, in situ hybridization was carried out first (Wolff, 2000), and this was followed by four washes in PBS for 15 minutes each, incubation with the primary antibody overnight at 4°C, four washes in PBS and incubation with the appropriate biotinylated secondary antibody for two hours at room temperature; the imaginal discs where then washed and stained with the Vectastain ABC kit.

Immunohistochemistry

Immunohistochemistry was carried out as previously described (Sánchez-Herrero, 1991; Estrada and Sánchez-Herrero, 2001), with slight modifications. The antibodies used were mouse and rabbit anti-β-galactosidase (Cappel), mouse anti-Abd-B lA2E9 (Celniker et al., 1989), rabbit anti-Abd-B (DeLorenzi and Bienz, 1990), rat anti-abd-A (Macías et al., 1990), guinea-pig anti-Hth (Azpiazu and Morata, 2002), guinea-pig anti-Snail (Kosman et al., 1991), rabbit anti-Tsh (Wu and Cohen, 2000), rabbit

Anti-Dll (Panganiban et al., 1995), rat anti-Dll (Wu and Cohen, 2000) and mouse anti-Dll (Duncan et al., 1998). Secondary antibodies were coupled to Red-X, Texas Red, FITC and Cy5 fluorochromes (Jackson Immunoresearch). Staining with To-Pro-3 iodide was done as previously described (Baena-López et al., 2003).

Histochemical staining

Internal genitalia were dissected out of late pupae, pharates or adults, and the staining to detect β-galactosidase expression was carried out as described previously (Ashburner, 1989).

Clonal analysis

Mitotic recombination clones were induced during the larval period by the FLP/FRT system (Xu and Rubin, 1993), with or without using the *Minute* technique (Morata and Ripoll, 1975). Clones were identified in the adult by the *yellow* cuticular marker and in the imaginal discs by the loss of the *lacZ* or *GFP* markers. The genotypes of the larvae in which the clones were induced are the following (in females the *hs-flp* is carried as an heterozygous insertion).

abd-A^{-} clones: *y hs-flp122; FRT82B abd-A*^{M1}*/FRT82B arm-lacZ.*

Abd-B^{-} (*Abd-B m*$^{-}$*r*$^{-}$) clones: *w f*^{36a} *hs-flp122; FRT82B Abd-B*^{D18}*/FRT82B arm-lacZ Dp(f*$^{+}$*) M(3)w123, y hs-flp122; FRT82B Abd-B*^{D18}*/FRT82B hs-CD2 M(3)w y*$^{+}$ and *y hs-flp122; FRT82B Abd-B*^{D18}*/FRT82B Ubi-GFP M(3)rpS3*.

Abd-B m^{-} clones: *y hs-flp122; FRT82B Abd-B*^{M5} or *FRT82B Abd-B*^{M3}*/FRT82B arm-lacZ, y hs-flp122; FRT82B Abd-B*^{M5} or *FRT82B Abd-B*^{M3}*/FRT82B hs-CD2 M(3)w y*$^{+}$*, y hs-flp122; FRT82B Abd-B*^{M5} or *FRT82B Abd-B*^{M3}*/FRT82B Ubi-GFP M(3)rpS3, w f*^{36a} *hs-flp122; FRT82B Abd-B*^{M5} or *FRT82B Abd-B*^{M3}*/FRT82B arm-lacZ Dp(f*$^{+}$*) M(3)w123*, and *y hs-flp hs-GFP FRT18A/Dpbxd*^{111} *arm-lacZ FRT18A; Abd-B*^{D14}*/Abd-B*^{D18}.

Abd-B r^{-} clones: *y hs-flp122; FRT82B Abd-B*^{Uab1} or *FRT82B Abd-B*$^{X23\text{-}1}$*/FRT82B arm-lacZ, w f*^{36a} *hs-flp122; FRT82B Abd-B*^{Uab1} or *FRT82B Abd-B*$^{X23\text{-}1}$*/FRT82B arm-lacZ Dp(f*$^{+}$*) M(3)w123* and *y hs-flp122; FRT82B Abd-B*$^{X23\text{-}1}$*/FRT82B hs-CD2 M(3)w y*$^{+}$.

Abd-B^{-} *abd-A*$^{+}$ clones: *y tub-Gal4 UAS-GFP; FRT82B Gal80/FRT82B Abd-B*^{D18} *UAS-abd-A*.

Adult cuticle analysis

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

RESULTS

Expression and function of *Abdominal-B* in the embryonic genital disc

In the third instar genital disc of *Drosophila*, *Abd-B* is expressed in the A8 and A9 segments, and *cad* in the A10 (Casares et al., 1997; Freeland and Kuhn, 1996). To study whether these expression domains are established early in development, we have analyzed *Abd-B* and *cad* transcription in the embryonic genital disc. This disc is identified by the expression of genes like *snail*, *escargot* or *headcase* (*hdc*), and we selected the *hdc-lacZ B5* line, which reproduces the pattern of *hdc* RNA expression (Weaver and White, 1985), to mark the genital disc. At about stage 15 [stages according to Campos-Ortega and Hartenstein (Campos-Ortega and Hartenstein, 1995)], *hdc* is expressed in three clusters of cells, two anterior ones placed bilaterally, and a third one located in a more posterior and central position (Weaver and White, 1995) (Fig. 1A and Fig. 2A). The three clusters fuse later in development to form the genital disc. At stage 15, we counted six to seven cells at each of the two anterior groups, and two to three cells in the posterior one, making up a total of 14-17 cells (n=12). Double staining with anti-Abd-B and anti-β-galactosidase antibodies (in *hdc-lacZ* embryos), or with GFP and anti-β-galactosidase antibody (in *cad-Gal4*/UAS-GFP; *hdc-lacZ*/+ embryos), shows that *Abd-B* is expressed in the two anterior clusters and *cad* in the posterior one (Fig. 1A-F).

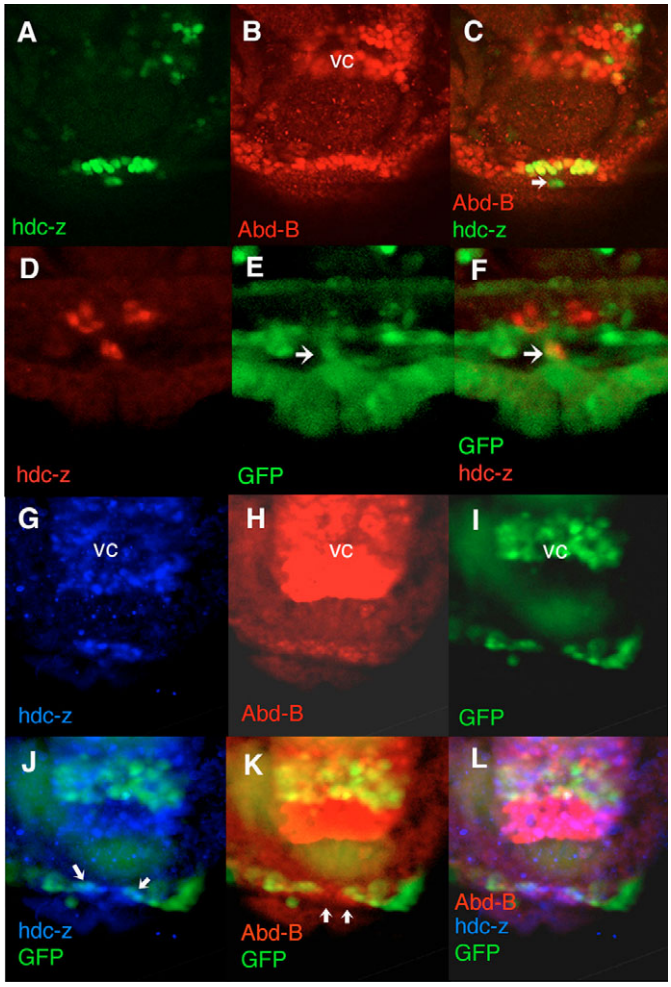

Fig. 1. Expression of *Abd-B* and *cad* in the embryonic genital disc. Anterior is to the top. (**A**-**C**) Double staining of a late stage 15 *hdc*-lacZ embryo with anti-β-galactosidase (green, A, note the three clusters) and anti-Abd-B (red, B) antibodies. A merged image is shown in C. Note that the posterior cluster of genital disc cells is not marked with *Abd-B* (arrow). (**D**-**F**) Double staining of a stage 15 *cad-Gal4*/UAS-GFP; *hdc-lacZ*/+ embryo marked with anti-β-galactosidase (red, D) and GFP (green, E). A merged image is shown in F. See that the posterior cluster (arrow) shows *cad* expression. (**G**-**L**) Embryonic genital disc cells of a UAS-*myc*-EGFP^{F}/+; *Abd-B-Gal4*^{LDN}*/hdc-lacZ* embryo, showing expression corresponding to the *Abd-B m* transcript (GFP expression, green, I-L) in the anterior lateral cells of the disc primordium, marked with an anti-β-galactosidase antibody (blue, G,J,L). GFP-marked cells are a subset of those expressing *Abd-B* (red, H,K,L). Note that about four anterior-lateral cells of the disc primordium express GFP (arrows, J) and that some *Abd-B*-expressing cells are not marked with GFP (arrows, K). A merged image is shown in L. vc, ventral cord.

To ascertain whether the two Abd-B products (Abd-B M and Abd-B R) are present in the genital disc primordium, we compared the expression driven by an *Abd-B m*-Gal4 line (see Materials and methods) with the signal detected with an antibody that recognizes both Abd-B M and Abd-B R proteins (Celniker et al., 1989). In UAS-*myc*-EGFP^{F}/+; *Abd-B*-Gal4^{LDN}*/hdc-lacZ* embryos, we saw a GFP signal in about two cells located laterally in each of the two

anterior clusters; these cells most likely express *Abd-B m*, and, therefore, are also labelled with the anti-Abd-B antibody (Fig. 1G,I-L). There are also 8-10 *Abd-B*-expressing cells not labelled with GFP, and these, probably, correspond to those expressing the Abd-B R protein (Fig. 1H,K,L). Taken together, our results suggest that the embryonic genital primordium includes three groups of cells that probably express *Abd-B m*, *Abd-B r* and *cad*, respectively.

We have studied the *Abd-B* requirement in the development of the embryonic genitalia by analyzing the disc morphology in different *Abd-B* mutants. In *Abd-B* m^- homozygous embryos, the genital primordium lacks about four *hdc*-expressing cells, two in each of the two anterior clusters (n=15; Fig. 2B, the wild type is shown in 2A). In *Abd-B*^{Uab1}/*Abd-B*^{M1} (*Abd-B* r^-) embryos, the disc primordium is disorganized and contains about eight cells (n=15; Fig. 2C). Finally, in embryos deficient for both *Abd-B* functions (henceforth, we refer to embryos or cells mutant for both functions as *Abd-B*$^-$) there are a few scattered *hdc*-expressing cells, occasionally forming lateral groups within the A8 segment (Fig. 2D). Some of the cells from these groups ectopically express *Dll* (Fig. 2E-F), a marker of distal appendage development (Cohen et al., 1989; Cohen et al., 1991). We conclude that the two *Abd-B* functions are required to form a normal embryonic genital disc, and that *Abd-B* confers identity to the A8 and A9 genital disc cells already at the late embryonic stages.

While this manuscript was under review, Chen and collaborators (Chen et al., 2005) showed that whereas *snail* (*sna*) or *escargot* are expressed in all of the cells from the embryonic genital disc (Chen et al., 2005; Fuse et al., 1996; Hartenstein and Jan, 1992; Whiteley et al., 1992), *hdc* is transcribed just in a subset of these cells. We have confirmed this result by staining *hdc-lacZ* embryos with anti-β-galactosidase and anti-Sna antibodies: three to five cells between the two anterior *hdc*-expressing groups of cells, and some cells posterior to them are stained with the anti-Sna antibody (data not shown). However, whether or not we mark the disc with either *hdc*-lacZ or Sna, *Abd-B* is expressed only in the anterior cells of the disc, and the *Abd-B m*-Gal4 line drives signal just a subset of these cells (Fig. 1A-C,I-L; data not shown). Chen et al. (Chen et al., 2005) have also analyzed homeotic gene expression and requirement in the genital disc, and conclude, as we do, that the embryonic genital disc is formed by cells that express *Abd-B m* (plus *abd-A*), *Abd-B r* and *cad*, and which probably correspond to the A8, A9 and A10 segments, respectively.

abdominal-A is required to form the internal female genitalia

To study homeotic gene function in the genital disc after embryonic stages, we analyzed *abd-A* and *Abd-B* expression and function in larvae and pupae. The mature female genital disc expresses *abd-A* (Casares et al., 1997; Freeland and Kuhn, 1996) (Fig. 3A) in a domain within the A8 segment that, according to fate maps, corresponds to the presumptive internal female genitalia (Epper, 1983; Littlefield and Bryant, 1979). We have confirmed this correspondence by studying *abd-A* expression throughout late larval

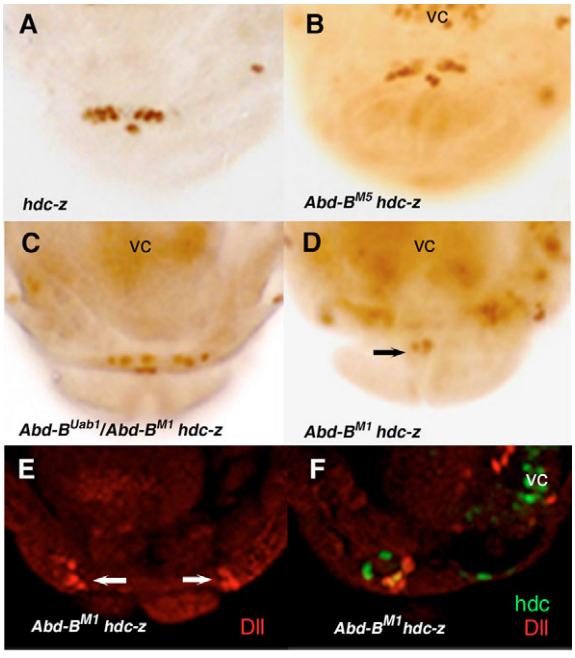

Fig. 2. *Abd-B* is needed for the formation of the embryonic genital disc. Anterior is to the top. (**A**) Late stage 15 *hdc-lacZ* embryo showing the three clusters of cells that will form the genital disc. (**B**) Stage 15 *Abd-B*^{M5} *hdc-lacZ* homozygous embryo. The embryonic genital disc is formed by fewer cells in the mutant than in the wild type. (**C**) Similar stage embryo of the genotype *Abd-B*^{Uab1}/*Abd-B*^{M1} *hdc-lacZ*. The genital disc is disorganized and includes fewer cells than the wild type does. (**D**) Stage 15 *Abd-B*^{M1} *hdc-lacZ* homozygous embryo. Some β-galactosidase-expressing cells are scattered (out of focus) and only the posterior cluster remains (arrow). (**E**,**F**) In *Abd-B*^{M1} *hdc-lacZ* homozygous embryos, *Dll* is ectopically activated in some posterior cells (arrows, E; red, F); *hdc* is marked in green in F. vc, ventral cord.

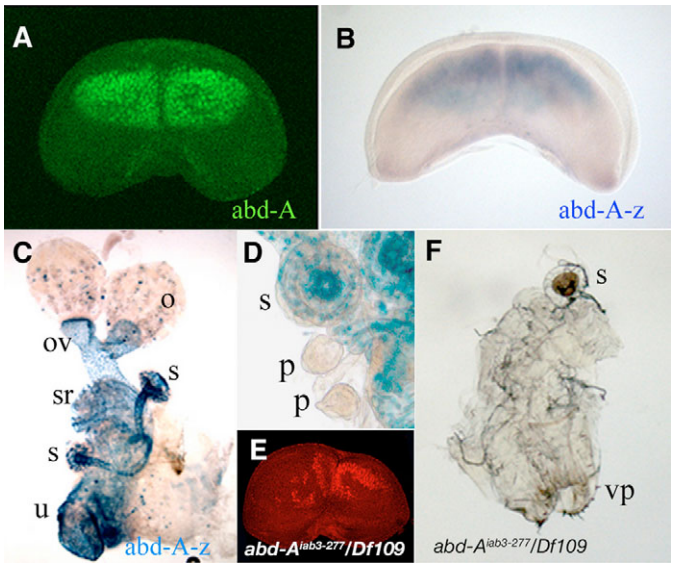

Fig. 3. Expression and requirement of *abd-A* in the female internal genitalia. (**A**) Third instar female genital disc, stained with an anti-abd-A antibody. (**B**) Similar disc of an *abd-A-lacZ* larva stained with X-Gal. The pattern of expression reproduces that of the anti-abd-A antibody. (**C**,**D**) X-Gal staining of internal female genitalia from two late pupae. The seminal receptacle (sr), spermathecae (s), uterus (u) and oviducts (ov) are stained, whereas parovaria (p) are not. o, ovaries. (**E**) Female genital disc of an *abd-A*$^{iab3-277}$/*DfR59* female larva stained with anti-abd-A antibody, showing a reduction in *abd-A* expression compared with wild type. (**F**) Female internal genitalia of an *abd-A*$^{iab3-277}$/*Df 109* female. See that most elements of the internal genitalia and the ovaries have disappeared (compare with C). vp, vaginal plates (part of the external genitalia).

and pupal development with an *abd-A-lacZ* line that reproduces *abd-A* expression in the third instar female genital disc (Bender and Hudson, 2000) (Fig. 3B, compare with 3A). In *abd-A-lacZ* pupae, the internal female genitalia are intensively stained, including the uterus, seminal receptacle, oviducts and spermatheca, but not the parovaria (Fig. 3C,D). To explore whether *abd-A* is required for female internal genitalia development, we used the *abd-A*$^{iab3\text{-}277}$ allele (see Material and methods). In *abd-A*$^{iab3\text{-}277}$/*abd-A*$^{-}$ female genital discs, *abd-A* expression is substantially reduced (Fig. 3E), and in *abd-A*$^{iab3\text{-}277}$/*abd-A*$^{-}$ females most of the internal genitalia disappear or are highly abnormal (Fig. 3F, compare with wild-type internal genitalia in 3C). As previously reported (Karch et al., 1985) these mutant females lack ovaries.

Abdominal-B m is needed for the development of the A8 and seems to maintain *abdominal-A* in the A8 of the female genital disc

We wanted to study the expression and requirement of the different *Abd-B* transcripts and proteins in the genital disc. A previous report showed that an *Abd-B m* enhancer trap labels the A8 of female and male genital discs, whereas an *Abd-B r* transcript is expressed in the A9 of the male disc (Casares et al., 1997). To confirm these results, we hybridized genital discs with probes specific for *Abd-B m* and *Abd-B r* transcripts (Fig. 4A). Our results show, within our limits of detection, that *Abd-B m* expression is strong in the A8 of male and female genital discs, and weak in the A9 of the male disc (Fig. 4B-E). This A9 signal is also observed in the embryo when similar probes are used (Sánchez-Herrero and Crosby, 1988; Kuziora and McGinnis, 1988; Boulet et al., 1991), and may represent genuine *Abd-B m* transcription or may be due to hybridization to *Abd-B r* unprocessed RNA. In support of the latter conclusion, we note that the *Abd-B*-Gal4^{LDN} insertion (although it drives expression only where *Abd-B m* levels are high) is co-expressed with two markers of the A8 segment, *teashirt* and high levels of *homothorax* (data not shown) (Estrada and Sánchez-Herrero, 2001; Gorfinkiel et al., 2003). *Abd-B r* is expressed in the A9 of male and female genital discs, with some expression in a few cells in the lateral regions of the female A8 (Fig. 4B,C,F,G).

As Abd-B M is the main or only product expressed in the A8, most *Abd-B m* mutant clones in this primordium should behave as clones that eliminate both *Abd-B* functions, that is, they should transform part of the female genitalia into leg or antenna (Estrada and Sánchez-Herrero, 2001). However, *Abd-B m* mutant clones, similar to *Abd-B m* escapers (Casanova et al., 1986; Karch et al., 1985; Sánchez-Herrero et al., 1985; Tiong et al., 1985), transform the dorsal eighth tergite into an anterior tergite, and the genitalia into structures sometimes resembling a sternite (Fig. 5A,B). In males, these clones also transform the tiny A8 into an anterior abdominal segment (Fig. 5C).

The difference between *Abd-B*$^{-}$ and *Abd-B m*$^{-}$ clones is also observed in the A8 of the female genital disc. Although *Abd-B*$^{-}$ clones present smooth borders and sometimes activate *Dll* (Estrada and Sánchez-Herrero, 2001), *Abd-B m*$^{-}$ clones are indented and do not express *Dll* (Fig. 5D-F). We note that most of the *Abd-B*$^{-}$ clones that induce *Dll* transcription are located in the region where *abd-A* is expressed. Because *abd-A* represses *Dll* in the embryo (Cohen et al., 1991; Simcox et al., 1991; Vachon et al., 1992), we decided to examine in more detail the relationship between *abd-A*, *Abd-B* and *Dll*. *abd-A* mutant clones do not activate *Dll* (Fig. 5G-I) and do not change *Abd-B* expression (Fig. 5J-L). Reciprocally, *Abd-B m*$^{-}$ clones do not alter *abd-A* transcription (Fig. 5M-O).

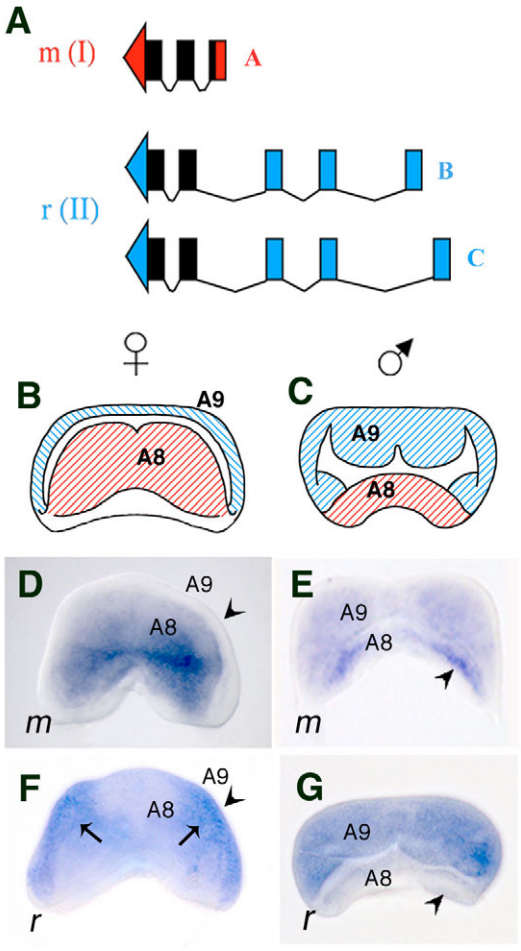

Fig. 4. *Abd-B m* and *Abd-B r* expression in mature genital discs. (**A**) Scheme representing the *Abd-B* transcription unit (not drawn to scale). The γ RNA is not represented. Red or blue rectangles represent exons of *Abd-B m* (red) or of *Abd-B r* (blue) transcripts, and black boxes represent coding regions. (**B**,**C**) Drawings of the female (B) and male (C) genital discs (ventral views), indicating the A8 and A9 primordia. The A10 primordium is on the opposite side in both discs. (**D**,**F**) Female discs hybridized with probes detecting the *Abd-B m* transcript (D) or the *Abd-B r* RNAs ('common' probe; F). (**E**,**G**) Male discs hybridized with *Abd-B m* (E) and *Abd-B r* (G) probes. Arrowheads in D and F indicate the A9, and in E and G, the A8 segment. Note that the *Abd-B m* transcript is expressed in the A8 of female and male discs, with some weak expression in the male A9, and that the *Abd-B r* transcript is present in the male and female A9, with some signal also in the female A8 (arrows; the most central expression in this primordium, out of focus, is the signal from the dorsal A9). Note also the low or absent *Abd-B r* expression in the anterior female A9.

Surprisingly, *abd-A* disappears or is strongly downregulated in *Abd-B*$^{-}$ (*Abd-B*^{D18}) clones (Fig. 5P-R). We note that *abd-A* also seems to be absent in wild-type cells around some of the large clones, but this is probably due to the bulging of the epithelium in and around mutant cells, as double staining of the clones with a nuclear ubiquitous marker, or with To-Pro-3 iodide, suggests that *abd-A* repression is cell autonomous (not shown). The absence of *abd-A* in *Abd-B*$^{-}$ clones indicates that *Abd-B* is required to maintain *abd-A* expression in the A8 of the female genital disc (perhaps through *iab* regulatory regions), which is the opposite of its role in the embryonic ectoderm, where *Abd-B* represses *abd-A* (Karch et al.,

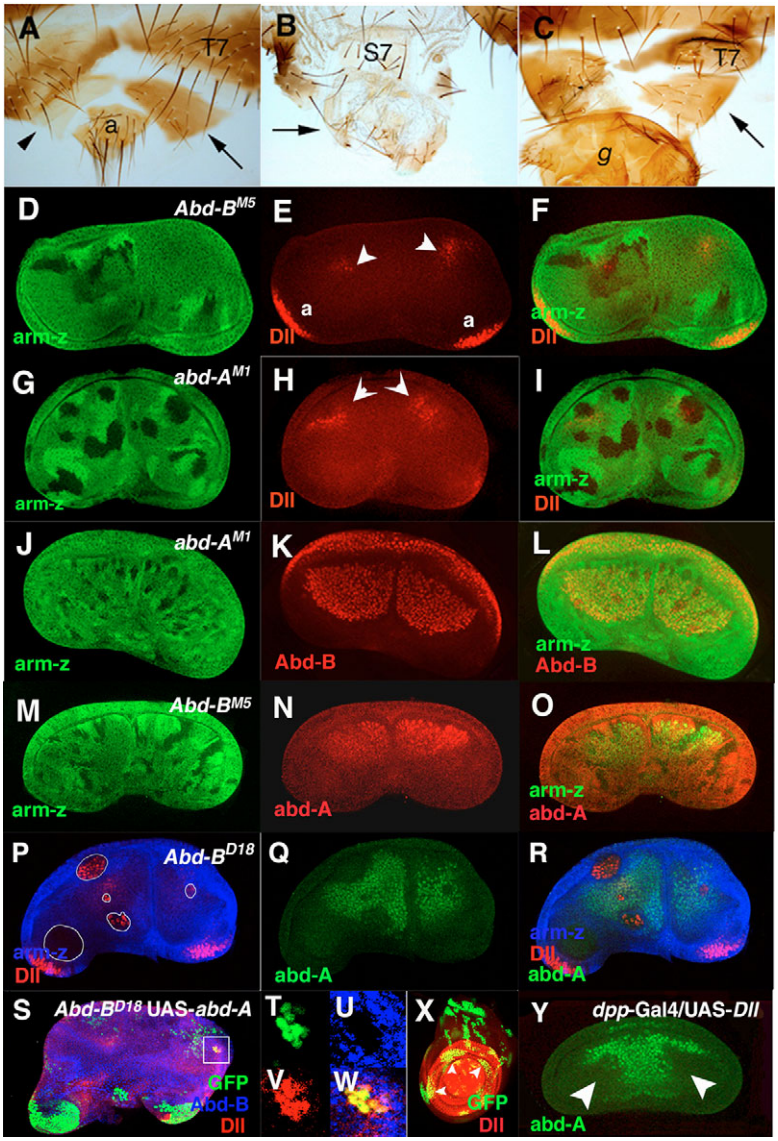

Fig. 5. *Abd-B m* function in the genitalia and the female genital disc. (**A**) An *Abd-B*^{M5} mutant clone in the female right eighth hemi-tergite, marked with *yellow*, is converted into an anterior tergite (arrow). Compare with the left, wild-type, hemi-tergite (arrowhead). (**B**) Similar mutant clone showing transformation of the genitalia into a sternite (arrow). S7, seventh sternite. (**C**) *Abd-B*^{M3} clone, marked with *yellow*, showing transformation of the male A8 tergite (normally very small) into an anterior one (arrow). T7, seventh tergite; g, genitalia. (**D-F**) *Abd-B*^{M5} clones in the A8 of the female disc, marked by the absence of the *lacZ* marker (green, D), do not activate *Dll* (red, E; arrowheads in E and H mark the wild-type *Dll* expression). a, analia. The merged image is shown in F. (**G-L**) *abd-A*^{M1} mutant clones in the female A8, marked by the absence of the β-galactosidase marker (G,J, green), do not eliminate *Dll* (H, red) or *Abd-B* (K, red) expression. Merged images are shown in I and L. (**M-O**) *Abd-B*^{M5} clones in the A8 of the female genital disc, marked by the absence of the *lacZ* marker (in green in M), do not change *abd-A* expression (red, N). A merged image is shown in O. (**P-R**) *Abd-B*^{D18} mutant clones in the A8 segment, marked by the absence of the β-galactosidase marker (blue, P,R; clones are outlined), eliminate *abd-A* expression, detected by an anti-abd-A antibody (green, Q,R), and activate Dll protein expression (red, P,R). (**S**) *Abd-B*^{D18} *abd-A*$^{+}$ clones, marked with GFP (in green), eliminate *Abd-B* (blue) and activate *Dll* (red) expression. The boxed clone is shown in detail in **T-W**. (**X**) Similar clones induced in the leg disc do not eliminate *Dll*, as shown by the co-expression of the GFP marker (green) and *Dll* (red), giving a yellow color (arrowheads). (**Y**) *dpp*-Gal4/UAS-*Dll* female disc showing elimination of *abd-A* expression in the *dpp* domain (arrowheads).

1990; Macías et al., 1990; Sánchez-Herrero, 1991). In *Abd-B*$^{-}$ clones, *Dll* is ectopically expressed (Fig. 5P,R), and this is independent of *abd-A*, as in clones that simultaneously lose *Abd-B* and gain *abd-A* expression (*Abd-B*$^{-}$ *abd-A*$^{+}$ clones) *Dll* signal is still present (Fig. 5S-W). Moreover, ectopic *abd-A* expression in *Abd-B*$^{-}$ *abd-A*$^{+}$ clones does not repress *Dll* in the male primordium of the genital disc or in the leg discs (Fig. 5X and not shown). By contrast, ectopic Abd-B m protein represses Dll in the leg disc (data not shown). Strikingly, ectopic *Dll* expression represses *abd-A* (Fig. 5Y). Two conclusions can be drawn from these results: first, that although the *Abd-B m* transcript is the main or only *Abd-B* transcript expressed in the female A8, a very different outcome is obtained if *Abd-B m* or both *Abd-B m* and *Abd-B r* functions are eliminated in this primordium; second, that the relationships between *Abd-B* and *abd-A*, and between *abd-A* and *Dll*, are the opposite of those observed in the embryo.

The results presented above prompted us to investigate *Abd-B* expression in *Abd-B*$^{-}$ and *Abd-B m*$^{-}$ clones. In *Abd-B*^{D18} (*Abd-B*$^{-}$) clones there is, as expected, no *Abd-B* expression (not shown). Surprisingly, the *Abd-B* signal does not change in *Abd-B*^{M5}, *Abd-B*^{M3} or *Abd-B*^{D14} mutant clones (Fig. 6C-E and data not shown). This result was observed with two different antibodies that recognize both Abd-B proteins (Celniker et al., 1989; DeLorenzi and Bienz, 1990) and cannot be attributed to the presence of an abnormal Abd-B M protein because the alleles used do not make Abd-B M product, and the *Abd-B*^{D14} mutation does not even make *Abd-B m* RNA (Boulet et al., 1991; Casares and Sánchez-Herrero, 1995; Celniker et al., 1990: DeLorenzi and Bienz, 1990; Zavortink and Sakonju, 1989) (Fig. 6A-B). One possible interpretation of this result is that *Abd-B r* is derepressed in *Abd-B m*$^{-}$ clones induced in the female A8. To confirm this, we looked at *Abd-B r* expression (using the 'common' probe I; see Materials and methods) in *Abd-B*^{M5} mutant clones. However, as shown in Fig. 6F-H, such derepression was not observed.

Our phenotypic analysis of *Abd-B m* function has been confined so far to the external genitalia and the eighth tergite. However, *Abd-B m* transcripts are also present, although at low levels, in the presumptive internal genitalia of third instar genital discs (Fig. 4D). To follow *Abd-B m* transcription at later stages, we examined *lacZ* expression in the internal female genitalia of *Abd-BGal4*^{LDN}/UAS-*lacZ* late pupae and pharate adults. As shown in Fig. 6I,J, and similar to the expression observed in *abd-A-lacZ* females (Fig. 3C), X-gal

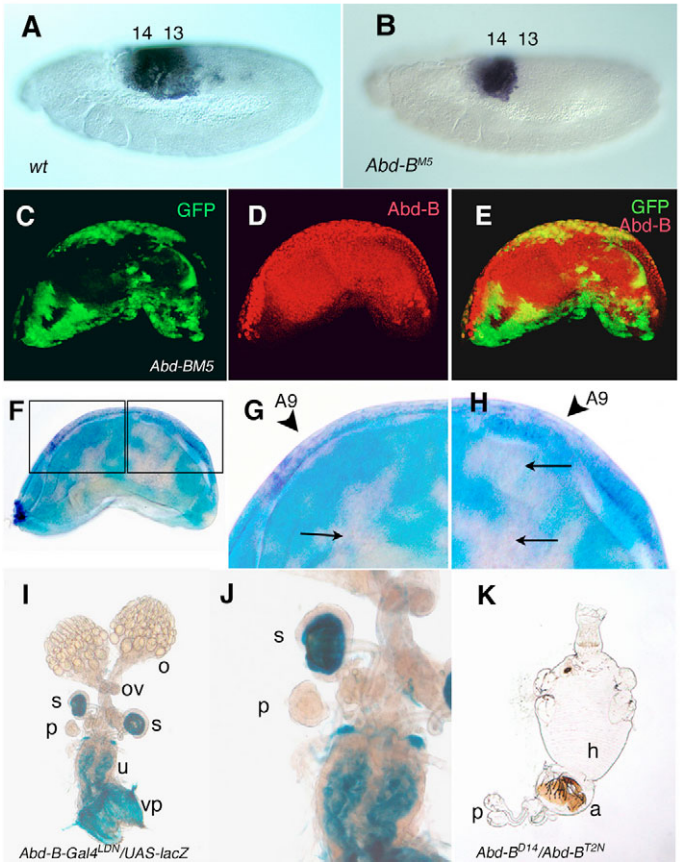

Fig. 6. *Abd-B* expression in *Abd-B m* mutant embryos and clones, and *Abd-B m* expression and function in the female internal genitalia. (**A**) Wild-type stage 11 embryo showing *Abd-B* expression, detected with the lA2E9 monoclonal antibody, in PS12-PS14 (A7-A9). (**B**) *Abd-B*^{M5} homozygous embryo at a similar stage, stained with the same antibody. The *Abd-B* expression is restricted to PS14. (**C-E**) *Abd-B*^{M5} clones induced in female A8 do not eliminate *Abd-B* expression. Clones are marked by the absence of GFP marker (green, C), and *Abd-B* expression is detected with the anti-rabbit anti-Abd-B (red, D). A merged image is shown in E. (**F**) *Abd-B*^{M5} clones induced in the same primordium, marked by the absence of X-gal staining (light blue), do not ectopically activate *Abd-B r* expression (purple). (**G**,**H**) Detail of the regions boxed in F. Note the *Abd-B r* wild-type expression in the female A9 (arrowheads) and its absence, or very low expression, in the clones (arrows). (**I**) Female internal genitalia of an UAS-*lacZ*/+; *Abd-B-Gal4*^{LDN}/+ late pupa. The spermathecae (s) and uterus (u) are stained, whereas parovaria (p) and oviducts (ov) are not. o, ovaries; vp, vaginal plates. (**J**) Detail of I, showing absence of staining in the parovaria. (**K**) *Abd-B m* mutant female showing disappearance of all the external and internal genitalia except the parovaria (p). h, hindgut; a, analia.

staining is detected in the spermathecae, the uterus and, weakly, in the seminal receptacle, but not in the parovaria; however, and in contrast to the results obtained with the *abd-A* insertion, oviducts were not stained (Fig. 6I). In females mutant for *Abd-B m*, the internal female genitalia do not develop properly and, in some cases, parovaria are the only structures remaining (Fig. 6K). In males, *Abd-B m* mutations do not affect the internal genitalia, although the testes are reduced in size and are not connected properly with the internal genitalia (data not shown).

Abdominal-B r is required for the development of male genitalia

Abd-B r transcripts are expressed mainly or only in the A9 segment of male and female genital discs (Fig. 4F,G), but *Abd-B r* mutations eliminate genitalia and analia in both sexes (Casanova et al., 1986; Celniker and Lewis, 1987; Karch et al., 1985; Kuhn et al., 1981). To define the requirement for the Abd-B R product during larval development, we induced *Abd-B r*$^{-}$ mitotic recombination clones during larval stages. These clones are normal in the analia of both sexes and in the external female structures. In the male genitalia, we observe two types of clones: some of them, probably induced in the internal genitalia, transform genital structures into distal leg or, less frequently, antenna (Fig. 7A-D), thus resembling the transformations observed in *Abd-B*$^{-}$ clones (Estrada and Sánchez-Herrero, 2001). By contrast, clones in the lateral plates or clasper teeth are wild type or cause only slight pattern alterations. In accordance with the transformations observed in the adult, some *Abd-B r*$^{-}$ clones induced in the male A9 eliminate *Abd-B* expression (Fig. 7E-G), and a few of these activate *Dll* (Fig. 7H-J); these clones have smooth borders and are located mostly in the penis apparatus presumptive region (Bryant and Hsieh, 1977; Bryant, 1978). Clones in other regions are, in general, indented, do not activate *Dll* and show normal or slightly reduced *Abd-B* expression (see summary in Fig. 7K). Some clones mutant for the *Abd-B*$^{x23\text{-}1}$ hypomorph allele also show a reduction in or elimination of Abd-B protein expression in the male A9 and transformation into leg tissue in the adult.

In *Abd-B r* mutant flies, the internal genitalia of males is absent (not shown), while that of females is abnormal. A common feature of these females is the absence of parovaria, and in about half of them we observe three and even four spermathecae instead of the normal two (Fig. 7L). Supernumerary spermathecae are also detected in females bearing *Abd-B r*$^{-}$ clones (data not shown). In a few cases, duplications of part of the internal genitalia are observed in *Abd-B r* mutant females.

DISCUSSION

In this work, we describe the expression and function of *abd-A* and *Abd-B* in *Drosophila* genitalia development. As in the embryonic cuticle, *abd-A* and *Abd-B m* are needed in the A8 whereas *Abd-B r* is required in the A9. The relationship between these homeotic products in the mature genital discs, however, clearly differs from what is observed in the embryonic epidermis. In what follows, we discuss several aspects of *abd-A* and *Abd-B* expression and function in genital development.

Abdominal-B expression and function in the embryonic genital disc

We report that the embryonic genital disc has three distinct cell populations at stages 15/16: some anterior-lateral cells transcribe *Abd-B m*, anterior-central and middle cells express *Abd-B r* and posterior cells transcribe *cad*, although the expression of these products may overlap. Because the genital disc is formed by the fusion of cells coming from the A8, A9 and A10 segments (Nöthiger et al., 1977; Schüpbach et al., 1978), and by analogy to the expression of these genes in the mature genital discs (Casares et al., 1997; Freeland and Kuhn, 1996) (this report), we conclude that *Abd-B m*, *Abd-B r* and *cad* are probably expressed in the A8, A9 and A10 segments, respectively, of the embryonic genital disc.

Abd-B is not only expressed, but also required in the embryonic genital primordium. In the absence of *Abd-B m*, the number of *hdc*-expressing cells in the disc is reduced, most likely because these

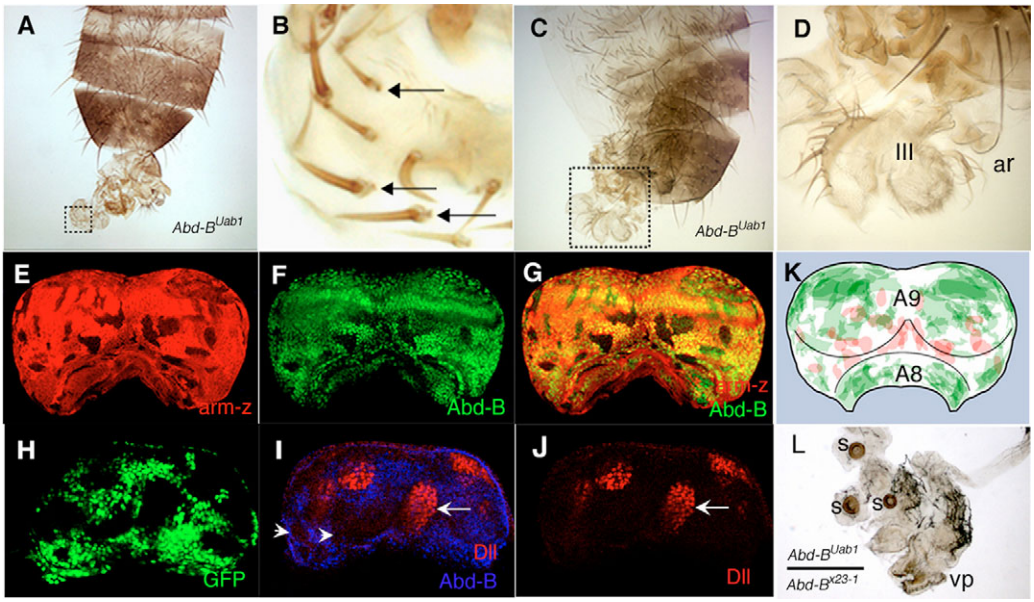

Fig. 7. The *Abd-B r* function is required for the development of the male genitalia. (**A**-**D**) In the male genitalia, some *Abd-B*^{Uab1} clones transform into leg tissue (A, boxed region is amplified in B; arrows point to bracted bristles) or into antenna (C, boxed region is amplified in D), ar, malformed arista; III, third antennal segment. (**E**-**G**) Some *Abd-B*^{Uab1} clones in the A9 of the male disc, marked by the absence of the *lacZ* marker (red, E) eliminate *Abd-B* expression (green, F). Merged image in G. (**H**-**J**) Male disc bearing similar clones, marked by the absence of GFP (green, H), eliminate *Abd-B* expression (I, blue), and in some cells activate *Dll* (I,J, red, arrow). In some clones there is no *Abd-B* expression but no *Dll* activation either (arrowheads, I). (**K**) Drawing of a male genital disc showing the distribution of *Abd-B*^{Uab1} clones that eliminate *Abd-B* expression (pink) and those that do not (green). The region where most 'pink' clones accumulate includes, mainly, the presumptive penis apparatus domain. (**L**) Internal genitalia of an *Abd-B*^{Uab1}/*Abd-B*$^{x23-1}$ female. Note the presence of three spermathecae (s) in the highly abnormal internal genitalia. vp, vaginal plates.

cells adopt now a more anterior fate, as occurs in the cuticle (Sánchez-Herrero et al., 1985; Tiong et al., 1985). When *Abd-B r* is absent, the genital primordium lacks some cells and is disorganized, and when both Abd-B products are absent, the primordium is reduced to a few, dispersed cells, some of which express *Dll* ectopically, suggesting a transformation into a leg primordium.

The A8, A9 and A10 primordia of the mature genital discs bear anterior and posterior compartments, with expression of *en* and *wg* in each of these three primordia (Casares et al., 1997; Chen and Baker, 1997; Freeland and Kuhn, 1996; Sánchez et al., 1997). Curiously, although we can define three primordia in the embryonic disc, based on the expression of *Abd-B m, Abd-B r* and *cad*, neither *en* nor *wg* is expressed in the three separate domains at this stage (Casares et al., 1997). This may suggest, as was also recently proposed (Chen et al., 2005), that new bands of *en* and *wg* expression may be formed later in development, in precise concordance with the three primordia defined previously by the *Abd-B m, Abd-B r* and *cad* genes (see Casares et al., 1997). We note that late *en* expression is also characteristic of the antennal primordium of the eye-antennal disc (Morata and Lawrence, 1978).

abdominal-A and *Abdominal-B m* requirement in internal female genitalia development

We have shown that *abd-A* is expressed in the whole internal female genitalia except for the parovaria, and this is consistent with experiments indicating that parovaria derive from the female A9 segment (Keisman et al., 2001). *abd-A* has been shown to be required for gonad development (Boyle and Dinardo, 1995; Brookman et al., 1992; Cumberledge et al., 1992; Greig and Akam, 1995; Karch et al., 1985; Karch et al., 1990; Moore et al., 1998; Warrior, 1994), and in the *abd-A*$^{iab-3}$/*Df* mutant combinations ovaries are also absent. However, the defects we observe in the female internal genitalia are not simply due to an indirect effect of the lack of gonads, as *iab-4* mutations prevent the formation of the ovaries but do not alter internal genitalia formation (Cumberledge et al., 1992).

Our results indicate that *Abd-B m* is required for the development of female external and internal genitalia, both derived from the female A8 (Nöthiger et al., 1977; Schüpbach et al., 1978). The internal genitalia of *Abd-B-Gal4*^{LDN}/UAS-*lacZ* females were stained with X-gal except in two structures, the oviducts and parovaria. The absence of oviduct staining in *Abd-B-Gal 4*^{LDN}/UAS-*lacZ* females is probably due to the particular expression driven by this reporter, and does not imply an absence of *Abd-B m* transcription in these organs, for two reasons: first, *Abd-B m* transcripts are present in the whole A8 segment of the female genital disc; and second, oviduct development is affected in *Abd-B m* mutant females. Parovaria, by contrast, are not stained in *Abd-B-Gal 4*^{LDN}/UAS-*lacZ* or *abd-A-lacZ* females, and this agrees with their A9 provenance (Keisman et al., 2001). This is supported by the observation that in some *Abd-B m* mutant females parovaria are the only structures that remain in the internal female genitalia.

abdominal-A, Abdominal-B m and *Abdominal-B r* cross-regulatory interactions in the genital disc

Abd-B M seems to be the main or only *Abd-B* product present in the female A8, so it was expected that elimination in this segment of just Abd-B M or of all Abd-B proteins would give similar results. This is not so. Some *Abd-B*$^{-}$ clones transform part of the female genitalia into leg or antenna (Estrada and Sánchez-Herrero, 2001), whereas *Abd-B m* mutant clones convert the eighth tergite, and probably the female genitalia, into an anterior abdominal segment. The differences between *Abd-B m*$^{-}$ and *AbdB*$^{-}$ clones in the A8 of the female genital disc reveal the existence of unsuspected regulatory interactions between the *abd-A* and *Abd-B* genes: whereas *Abd-B m*$^{-}$ clones do not affect *abd-A*, in *AbdB*$^{-}$ clones *abd-A* expression is eliminated. This is a surprising result, because it is contrary to what is observed in the embryo, where *Abd-B* represses *abd-A* (Karch et al., 1990; Macías et al., 1990; Sánchez-Herrero, 1991).

Abd-B m^{-} clones induced in the female A8 do not alter *abd-A* expression but do not change *Abd-B* expression levels either. This is observed with mutations that do not make Abd-B M protein, so the

DEVELOPMENT

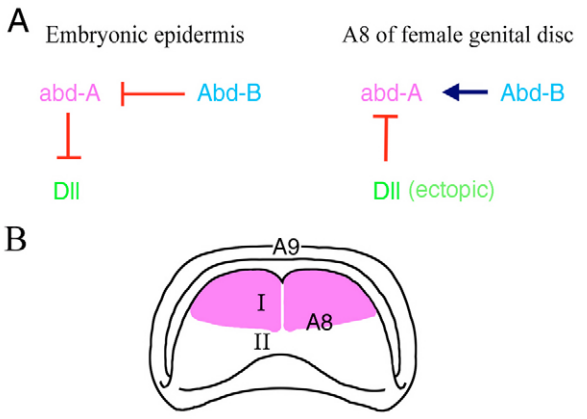

Fig. 8. Comparison of genetic interactions in embryonic epidermis and in the mature female genital disc, and organization of the A8 in the female genital disc. (**A**) Regulatory interactions between *abd-A*, *Abd-B* and *Dll* in the embryonic epidermis and in the mature genital discs. In the embryo, *Abd-B* represses *abd-A* in the A8, and *abd-A* represses *Dll* in the abdominal segments. In the third instar female genital disc, it seems that *Abd-B* maintains *abd-A* transcription, and that ectopic *Dll* can repress *abd-A*. (**B**) In the A8 of the female disc we can distinguish two regions (Estrada and Sánchez-Herrero, 2001) (this report). The anterior region (region I, purple) expresses *buttonhead*, *abd-A* and low levels of *Abd-B*, and can activate *Dll* in the absence of *Abd-B*. It represents the 'appendage' part of the genitalia and corresponds mainly or exclusively to the internal genitalia. The lower region (region II) does not express *abd-A* or *buttonhead*, but expresses high levels of *Abd-B*. It corresponds to the 'trunk' region of the female A8. In this region, *Dll* is not activated in the absence of *Abd-B*.

Abd-B protein detected is not the Abd-B M product. Surprisingly, although we detect some *Abd-B r* expression in the female A8, we do not see uniform *Abd-B r* expression throughout this primordium and *Abd-B r* transcripts seem not to be derepressed in $Abd\text{-}B^{M5}$ mutant clones. We have no explanation for this conundrum. Perhaps the probe used, although it includes sequences complementary to all of the *Abd-B r* cDNA sequences that have been published (Celniker et al., 1989; DeLorenzi et al., 1988; Kuziora and McGinnis, 1988; Zavortink and Sakonju, 1989), does not efficiently detect all of the non-*Abd-B m* transcripts.

The differences in regulatory and functional interactions among gene products in the embryo and the genital discs are not limited to those of *Abd-B* and *abd-A* that have been discussed above. First, there may be changes in phenotypic suppression: the transformation of the eighth tergite to the fourth one in $Abd\text{-}B\ m^{-}$ clones is due to *abd-A* (E.S.-H., unpublished). Because in these clones Abd-B protein is still present, this suggests that *abd-A* may phenotypically suppress *Abd-B*, differently from what is generally observed in the embryo (Castelli-Gair et al., 1994; Sánchez-Herrero et al., 1994). Second, *Abd-B r* represses *Abd-B m* in the embryo (Casanova et al., 1986; Boulet et al., 1991), but some $Abd\text{-}B\ r^{-}$ clones do not activate *Abd-B m* in the male disc (see below). Finally, *abd-A* represses *Dll* in the embryo (Cohen et al., 1991; Simcox et al., 1991; Vachon et al., 1992), but not in the female genital disc, and ectopic *Dll* can repress *abd-A* instead. *abd-A* does not repress *Dll* in the leg discs either, and this resembles *Ubx* function, which represses *Dll* only early in development (Castelli-Gair and Akam, 1995). By contrast, we have shown that *Abd-B* represses *Dll* in the embryo (this report), in the larval genital disc (Estrada and Sánchez-Herrero, 2001), and in the leg disc when ectopically expressed (this report).

The *Abdominal r* function and male genitalia development

Abd-B r expression is restricted to the A9 segment in male genital discs, but shows expression in the A9 and in some cells of the A8 in female genital discs. In spite of this, $Abd\text{-}B\ r^{-}$ clones in the external female genitalia (A8) are phenotypically wild type. In the male A9, some *Abd-B r* mutant clones eliminate *Abd-B*, activate *Dll* and transform part of the genitalia into distal leg or antenna. This is similar to the result obtained in some $Abd\text{-}B^{-}$ clones, and it implies that *Abd-B m* is not derepressed in these mutant clones. However, *Abd-B m* is perhaps derepressed in those *Abd-B r* mutant clones where *Abd-B* signal (detected by the common antibody) remains.

Although $Abd\text{-}B\ r^{-}$ clones affect, almost exclusively, male genitalia development, *Abd-B r* hemizygous or trans-heterozygous flies lack genitalia and analia in both sexes (Casanova et al., 1986). This probably reflects the absence of proper interactions between the different primordia needed for the growth of the genital disc (Gorfinkiel et al., 2003). In *Abd-B r* mutant females, the internal genitalia are abnormal, and in some of these females we observe an absence of parovaria and the presence of three or four spermathecae. This phenotype is consistent with a segment-autonomous transformation of A9 derivatives (parovaria) into A8 structures (spermathecae), similar to the embryonic cuticular transformation of A9 into A8 observed in *Abd-B r* mutations (Casanova et al., 1986). A transformation of parovaria into spermathecae has been previously described in *Polycomblike* mutants (Duncan, 1982), and may also indicate a transformation of A9 to A8.

Genetic organization of the genital disc

Our results illustrate that there are quite different Hox cross-regulatory interactions in the embryo and in the genital disc (Fig. 8A). The effects in the genital discs contradict the general rule that genes transcribed more posteriorly suppress or downregulate the expression of more anterior ones (Hafen et al., 1984; Struhl and White, 1985). This rule has, nevertheless, some exceptions in genes of the Antennapedia complex (Miller et al., 2001). Further, differences in Hox cross-regulation between the embryo and imaginal discs are not unprecedented: the *proboscipedia* (*pb*) Hox gene is positively regulated by *Sex combs reduced* in the embryo (Rusch and Kaufman, 2000), but *pb* activates *Sex combs reduced* in the labial imaginal disc (Abzhanov et al., 2001).

It has been proposed that the primordia of female and male genitalia could be subdivided into an 'appendage-like' and a 'trunk-like' region (Estrada and Sánchez-Herrero, 2001). We can now define these two regions of the female A8 more precisely. The 'appendage-like' region would be that expressing *abd-A* and low levels of *Abd-B*, and corresponds approximately to the presumptive internal female genitalia (region I in Fig. 8B). This domain is roughly coincident with the region of expression of a reporter insertion in *buttonhead*, the gene that defines ventral appendage development (C. Estella, PhD Thesis, Universidad Autónoma de Madrid, 2003) (Estella et al., 2003), and this is also, approximately, the domain where $Abd\text{-}B^{-}$ clones may activate *Dll* (Estrada and Sánchez-Herrero, 2001). If this subdivision is correct, the 'appendage' specification defined by *buttonhead* would be repressed in the wild type by *Abd-B*, which both limits *Dll* expression to a few cells of the A8 primordium and prevents *Dll* function (Estrada and Sánchez-Herrero, 2001). $Abd\text{-}B^{-}$ clones in this region eliminate *abd-A* expression and promote leg or antenna development. This subdivision may also apply to the male disc, the penis apparatus presumptive region being the main 'appendage' domain. Similar to what we have described, the labial disc possesses a large

'appendage' region that is revealed by *Dll* derepression in *pb* mutations (Abzhanov et al., 2001). This characteristic, and the changes in Hox gene cross-regulation between the embryo and the imaginal disc, are two features shared by *pb*/labial disc and *Abd-B*/genital disc.

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