Development 116, 841-854 (1992)
Printed in Great Britain © The Company of Biologists Limited 1992

Ectopic expression of UBX and ABD-B proteins during *Drosophila* embryogenesis: competition, not a functional hierarchy, explains phenotypic suppression

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Summary

The *Abdominal-B* (*Abd-B*) gene, a member of the bithorax complex (BX-C), specifies the identities of parasegments (PS) 10-14 in *Drosophila*. *Abd-B* codes for two structurally related homeodomain proteins, ABD-B m and ABD-B r, that are expressed in PS10-13 and PS14-15, respectively. Although ABD-B m and r proteins have distinct developmental functions, ectopic expression of either protein during embryogenesis induces the development of filzkörper and associated spiracular hairs, structures normally located in PS13, at ectopic sites in the larval thorax and abdomen. These results suggest that other parasegmental differences contribute to the phenotype specified by ABD-B r activity in PS14.

Both ABD-B m and r repress the expression of other homeotic genes, such as *Ubx* and *abd-A*, in PS10-14. However, the importance of these and other cross-regulatory interactions among homeotic genes has been questioned. Since ectopic UBX protein apparently failed to transform abdominal segments, González-Reyes et al. (González-Reyes, A., Urquía, N., Gehring, W. J., Struhl, G. and Morata, G. (1990). *Nature* 344, 78-80) proposed a functional hierarchy in which ABD-A and ABD-B activities override UBX activity. We tested this model by expressing UBX and ABD-B m proteins ectopically in wild-type and BX-C-deficient embryos. Ectopic ABD-B m does not prevent transformations induced by ectopic UBX. Instead, ectopic UBX and ABD-B m proteins compete for the specification of segmental identities in a dose-dependent fashion. Our results support a quantitative competition among the homeotic proteins rather than the existence of a strict functional hierarchy. Therefore, we suggest that cross-regulatory interactions are not irrelevant but are important for repressing the expression of competing homeotic proteins.

To explain the apparent failure of ectopic UBX to transform the abdominal segments, we expressed UBX at different times during embryonic development. Our results show that ectopic UBX affects abdominal cuticular identities if expressed during early stages of embryogenesis. In later embryonic stages, abdominal segments become resistant to transformation by ectopic UBX while thoracic segments remain susceptible. Head segments also show a similar stage-dependent susceptibility to transformation by ectopic UBX in early embryogenesis but become resistant in later stages. These results suggest that abdominal and head identities are determined earlier than are thoracic identities.

Key words: *Drosophila*, bithorax complex, *Abdominal-B*, *Ultrabithorax*, ectopic expression, functional hierarchy, phenotypic suppression.

Introduction

Different segmental identities in *Drosophila* are established by the action of the homeotic genes. The homeotic genes of the bithorax complex (BX-C), *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*), determine the identities of the third thoracic (T3) and first through ninth abdominal (A1-A9) segments (Lewis, 1978), corresponding to parasegments (PS) 5 through 14. *Ubx* is required for the proper development of T3 and A1 (PS5, 6); *Ubx* and *abd-A* specify A2-A4 (PS7-9) identities; and *Ubx*, *abd-A* and *Abd-B* determine A5-A8 (PS10-13) identities (reviewed in Duncan, 1987; Morata et al., 1990). The domains of BX-C gene function correspond to domains of BX-C protein expression: UBX is expressed in PS5-13 (White and Wilcox, 1985; Beachy et al., 1985), ABD-A in PS7-13 (Karch et al., 1990; Macías et al., 1990) and ABD-B in PS10-15 (Celniker et al., 1989; DeLorenzi and Bienz, 1990; Boulet et al., 1991) (Fig. 1A).

Two genetic functions have been distinguished for *Abd-*

B (Casanova et al., 1986): a morphogenetic (m) function is required in A5-A8 (PS10-13) and a regulatory (r) function is necessary in A9 (PS14). A genetic function for *Abd-B r* in A10 (PS15) has not been described. In *Abd-B* m^-r^+ larvae, filzkörper, spiracular hairs and posterior spiracles, normally located in A8 (Fig. 1C,D), are absent (Karch et al., 1985; Casanova et al., 1986; Sato and Denell, 1986; Whittle et al., 1986). In *Abd-B* m^+r^- larvae, the posterior spiracles are reduced in size, but the filzkörper and spiracular hairs are present (Karch et al., 1985; Casanova et al., 1986). These cuticular phenotypes suggest that *Abd-B m* function, but not *Abd-B r* function, is required for the formation of filzkörper and spiracular hairs in A8. Similarly, *Abd-B m* function, but not *Abd-B r* function, alters the denticle, or setal, belt in A8: the A8 denticle belt in *Abd-B* m^-r^+ larvae is transformed to an anterior abdominal identity but is unaffected in *Abd-B* m^+r^- larvae. In *Abd-B* m^+r^- larvae, a narrow region of naked cuticle and a rudimentary denticle belt are formed posterior to the A8 denticle belt (Casanova et al., 1986). This phenotype requires *Abd-B m* function (Casanova et al., 1986). Additionally, *Abd-B m* expression is derepressed in PS14 of *Abd-B* m^+r^- larvae (Boulet et al., 1991). Therefore, the primary function of *Abd-B r* appears to be the repression of *Abd-B m* in A9. However, *Abd-B r* function is not exclusively regulatory since weak morphogenetic activity can be detected when *Abd-B r* is derepressed in the absence of all other BX-C functions (Casanova et al., 1986). When both *m* and *r* functions are eliminated (*Abd-B* m^-r^-), larvae resemble *Abd-B* m^-r^+ larvae except that sclerotic plates develop in A9 (Karch et al., 1985; Casanova et al., 1986; Whittle et al., 1986).

The domains of *Abd-B m* and *r* genetic functions correlate with the expression patterns of two different ABD-B protein products. The ABD-B m (55×10^3 M_r) and r (30×10^3 M_r) proteins are translated from mRNAs that are transcribed from different promoters (Kuziora and McGinnis, 1988; Sánchez-Herrero and Crosby, 1988; Celniker et al., 1989; Zavortink and Sakonju, 1989; Celniker et al., 1990; Boulet et al., 1991). ABD-B m and r proteins share a carboxy-terminal domain that contains the ABD-B homeodomain, but only the ABD-B m protein has a glutamine-rich amino-terminal domain. An antibody specific for ABD-B m detects protein in PS10-13 (Boulet et al., 1991) while antibodies that recognize both ABD-B m and r proteins detect protein in PS10-15 (Celniker et al., 1989; DeLorenzi and Bienz, 1990; Boulet et al., 1991). Analyses of ABD-B protein distribution in *Abd-B* mutants show a correlation of the morphogenetic function with ABD-B m protein distribution and of the regulatory function with the ABD-B r protein distribution (Celniker et al., 1990; DeLorenzi and Bienz, 1990; Boulet et al., 1991; Sánchez-Herrero, 1991).

ABD-B m and r proteins participate in a network of interactions that regulates the expression of other homeotic genes. ABD-B r represses *Abd-B m* expression in PS14 and 15 while high levels of ABD-B m repress the expression of *Ubx* and *abd-A* in PS13 (Struhl and White, 1985; Casanova et al., 1986; Casanova and White, 1987; Karch et al., 1990; Macías et al., 1990; M. Lamka, unpublished observations). In PS7-12, ABD-A down-regulates *Ubx* expression in posterior compartments (Struhl and White, 1985; Karch et al., 1990; Macías et al., 1990). Therefore, although BX-C expression patterns overlap in PS7-13, cross-regulatory interactions establish distinct, reciprocal patterns of BX-C gene expression in each parasegment (Fig. 1A).

Although cross-regulatory interactions among many homeotic genes have been demonstrated (reviewed in Levine and Harding, 1989), their functional significance has recently been questioned (González-Reyes et al., 1990). Ectopic, embryonic expression of UBX protein, which disrupted normal, reciprocal patterns of BX-C expression, transformed larval cephalic and thoracic segments toward A1, a UBX-specified identity, but had no apparent phenotypic effect on larval abdominal segments (González-Reyes et al., 1990; González-Reyes and Morata, 1990; Mann and Hogness, 1990). Therefore, with respect to cuticular morphology, high levels of UBX seemed to be inconsequential in regions of the abdomen where cross-regulatory interactions keep UBX levels low. Since endogenous ABD-A and ABD-B apparently suppressed the phenotypic effects of ectopic UBX, this phenomenon was termed 'phenotypic suppression' (González-Reyes and Morata, 1990). As an explanation for phenotypic suppression, González-Reyes et al. (1990) proposed a hierarchy of homeotic protein functions, independent of transcriptional regulation, in which ABD-A and ABD-B protein activities override UBX protein activity.

In this paper, we have addressed two questions concerning ABD-B protein function in vivo: (1) do ABD-B m and r proteins have similar or different intrinsic activities? and (2) is ABD-B m protein functionally dominant to UBX protein? To analyze the activities of ABD-B m and r proteins, the effects of ectopic ABD-B m or r expression on larval segmental identities were examined. Our results indicate that ABD-B m and r proteins possess similar activities and suggest that the presence or absence of another factor(s) in PS14 and 15 affects ABD-B r activity. To test the homeotic protein hierarchical model, we ectopically expressed ABD-B m alone or in combination with UBX. Our results suggest that, contrary to a strict functional hierachy, ectopic UBX and ABD-B m proteins compete for the specification of segmental identities. Furthermore, we show, in contrast to previous reports (González-Reyes et al., 1990; González-Reyes and Morata, 1990; Mann and Hogness, 1990), that ectopic UBX is able to affect abdominal as well as head identities if expressed early in embryogenesis and suggest that abdominal and head segments are determined earlier in development than are thoracic segments.

Materials and methods

Construction of hs-m *and* hs-r *transformant lines*

To construct the *hs-m* fusion gene (referred to as *hsp70*-M in Boulet et al., 1991), a 1.9 kb *Ssp*I-*Bgl*II fragment from the Class A cDNA clone B3 (Zavortink and Sakonju, 1989) was cloned into the pCaSpeR-hs P element vector (Thummel and Pirotta, 1992) cut at the *Bgl*II and *Hpa*I sites. The polylinker in the pCaSpeR-hs vector lies between the *hsp70* promoter and sequences from the 3′ end of the *hsp70* gene which include the polyadenylation site. The *hs-r* fusion gene (referred to as *hsp70*-R in Boulet et al.,

1991) was similarly constructed by cloning a 1.4 kb *Nru*I-*Bgl*II fragment from the Class B cDNA clone E19 (Zavortink and Sakonju, 1989) into the pCaSpeR-hs P element vector cut at the *Bgl*II and *Hpa*I sites. The *hs-m* and *hs-r* DNAs were coinjected with pπ25.7wc (Rubin and Spradling, 1982) into *Df(1)w67c23y* embryos. Six *hs-m* lines and one *hs-r* line were recovered. Four of the *hs-m* lines were homozygous viable. The *hs-m(F1.2)* and *hs-m(M2)* insertions were located on the first chromosome, the *hs-m(M4)* insertion on the second chromosome and the *hs-m(F4)* insertion on the third chromosome. The two homozygous lethal insertions *hs-m(F1.1)* and *hs-m(M11)*, located on the second chromosome, were maintained as *CyO*-balanced stocks. The single *hs-r* line, designated *hs-r(M2)*, carried a homozygous viable insertion on the third chromosome. Using a genomic source of transposase (Robertson et al., 1988), an additional *hs-r* line was obtained by mobilizing the original *hs-r* P element in the *hs-r(M2)* transformant. This homozygous viable *hs-r(7A)* line carried a new insertion on the second chromosome.

Drosophila *strains and genetic crosses*

We constructed flies of the following genotypes to analyze the effects of ectopic ABD-B m or r protein in *BX-C*$^{-}$ embryos: w^{1118}*; hs-m(M4)/CyO; Dp(3;3)P5, Sb/Df(3R)P9* and w^{1118}*; hs-r(7A)/hs-r(7A); Dp(3;3)P5, Sb/Df(3R)P9*. *Df(3R)P9* is deficient for the entire BX-C (Lewis, 1978) whereas *Dp(3;3)P5* contains a tandem duplication for the BX-C (Duncan and Lewis, 1982). *hs-m, BX-C*$^{-}$ or *hs-r, BX-C*$^{-}$ larvae were readily identified by their *BX-C*$^{-}$ phenotypes (transformations of T3 and A1-A8 toward T2) and the development of filzkörper, which are normally absent from *BX-C*$^{-}$ larvae.

The *hs-Ubx-1a-22* line, a gift from Richard Mann and David Hogness, was used to express UBX ectopically (Mann and Hogness, 1990). This heterozygous line carries a third chromosome insertion of a *Ubx-1a* cDNA fused to the *hsp70* promoter. To express UBX and ABD-B m proteins together throughout *BX-C*$^{-}$ embryos, we constructed a *hs-Ubx, DfP9* recombinant (maintained over *Dp(3;3)P146* + $Mc^{RS1\text{-}10}$, a multiply inverted third chromosome carrying a duplication of the BX-C, obtained from Ian Duncan) and examined heat-shocked progeny from the following crosses: w^{1118}*/Y; hs-m(M4)/hs-m(M4); Dp(3;3)P5, Sb/Df(3R)P9* × *Dp(3;3)P146* + $Mc^{RS1\text{-}10}$*/Df(3R)P9, hs-Ubx* (for 1 copy of *hs-m* with or without 1 copy of *hs-Ubx*) and *+/Y; hs-m(M4)/+; Dp(3;3)P5, Sb/Df(3R)P9, hs-Ubx* × w^{1118}*; hs-m(M4)/hs-m(M4); Dp(3;3)P5, Sb/Df(3R)P9* (for 1 or 2 copies of *hs-m* with or without 1 copy of *hs-Ubx*). *BX-C*$^{-}$ larvae carrying 1 copy of *hs-Ubx* and at least 1 copy of *hs-m* were identified by the transformations of head and thoracic segments toward A1 (González-Reyes et al., 1990; González-Reyes and Morata, 1990; Mann and Hogness, 1990).

We generated chromosomes carrying two copies of *hs-m* by recombining second chromosome insertions: *hs-m(M4)* with *hs-m(F1.1)* or *hs-m(M11)*. These flies were maintained as *CyO*-balanced stocks.

To analyze the phenotypes of larvae carrying 1-4 copies of *hs-m* with or without 1 copy of *hs-Ubx*, we heat shocked progeny from the following crosses: (1) w^{1118}*/Y; hs-Ubx/+* × w^{1118}*; hs-m(F4)/hs-m(F4)* (for 1 copy of *hs-m* with or without 1 copy of *hs-Ubx*), (2) w^{1118}*/Y; hs-m(M4)/+; hs-Ubx/+* × w^{1118}*; hs-m(F4)/hs-m(F4)* (for 1 or 2 copies of *hs-m* with or without 1 copy of *hs-Ubx*), (3) w^{1118}*/Y; hs-m(M4)/+; hs-Ubx/+* × w^{1118}*; hs-m(M11) hs-m(M4)* or *hs-m(F1.1) hs-m(M4)/CyO* (for 1, 2 or 3 copies of *hs-m* with or without 1 copy of *hs-Ubx*) and (4) w^{1118}*/Y; hs-m(M11) hs-m(M4)* or *hs-m(F1.1) hs-m(M4)/+; hs-Ubx/+* × w^{1118}*; hs-m(M11) hs-m(M4)* or *hs-m(F1.1) hs-m(M4)/CyO* (for 2 or 4 copies of *hs-m* with or without 1 copy of *hs-Ubx*). Progeny from these crosses were genotypically classified on the basis of phenotypic transformations. Larvae carrying *hs-Ubx* were identified by the consistent head and thoracic transformations toward an A1 identity. Larvae carrying *hs-m* were scored by the appearance of ventral pits in abdominal segments and/or non-involuted heads. Crosses yielded progeny with different copy numbers of *hs-m*, which we grouped together for analysis. In all cases, the observed frequencies of the phenotypic classes were in agreement with the predicted frequencies of the corresponding genotypic classes.

Heat-shock treatment of embryos

To express UBX and ABD-B proteins ectopically at specific times in development, embryos were staged by examination under Voltalef 3S oil. Stage 6 (2:50-3 hours) embryos (Campos-Ortega and Hartenstein, 1985) were selected, transferred to coverslips and aged in a humidified 25°C incubator. Heat shocks were administered for the specified times described below by placing the coverslips on a glass plate in a humid chamber of a gravity convection incubator. Embryos were returned to the humidified 25°C incubator until larvae had hatched or embryos had aged for greater than 24 hours. The chorion and vitelline membranes were removed from unhatched larvae using a tungsten needle. Larvae were fixed in acetic acid:glycerin (4:1) for 15-30 minutes at 60°C and then left at room temperature for at least 24 hours. Larvae were mounted in CMCP-10 Mounting Media (Master's Chemical Company):lactic acid (3:1) and placed on a 45°C slide warmer to clear overnight. Cuticles were analyzed using phase-contrast and dark-field microscopy.

For most of the experiments, embryos were heat shocked at 36°C for 30 minute periods at both 4 hours and 6 hours of embryogenesis. This method produced more severe ABD-B-induced phenotypic transformations than single 30 minute (36°C) heat shocks at either 4 or 6 hours or than two 30 minute (36°C) heat shocks at 5 and 7 hours. The double-pulse heat treatment at 4 and 6 hours did not significantly affect w^{1118} control embryos: 95% of w^{1118} embryos hatched as first instar larvae with cuticles indistinguishable from non-heat-shocked wild-type larvae. Under the same conditions, heat-shocked *hs-m* and *hs-r* embryos laid down larval cuticle but did not hatch.

To test for stage-related effects of ectopic UBX on the abdominal segments, we heat shocked embryos from w^{1118} control and w^{1118}*; hs-Ubx-1a-22/TM3* stocks at 4 and 6 hours, 6 and 8 hours, or 8 and 10 hours of embryogenesis. Each heat shock was 30 minutes at 36°C using the heat-shock protocol described above.

Results

To determine the phenotypic consequences of ectopic expression of ABD-B m or ABD-B r protein, we created several transformant lines carrying *Abd-B m* or *r* cDNAs fused to the heat-inducible *hsp70* promoter (Fig. 1B; Materials and methods). These lines were designated *hs-m* and *hs-r*. Anti-ABD-B antibodies detected the ABD-B m and ABD-B r protein forms on western blots of extracts from heat-shocked *hs-m* and *hs-r* embryos (Boulet et al., 1991). Antibody staining of embryos indicated that most cells expressed ABD-B m or r protein at a level similar to that of endogenous ABD-B in PS13 and 14 after heat shock of *hs-m* or *hs-r* embryos (data not shown). Antibody staining detected ectopic ABD-B protein in *hs-m* embryos at 1 hour and 2 hours, but not 3 hours, following a 30 minute heat shock at 36°C (data not shown). We induced ectopic ABD-B m or r protein in at least two independent *hs-m* or *hs-r*

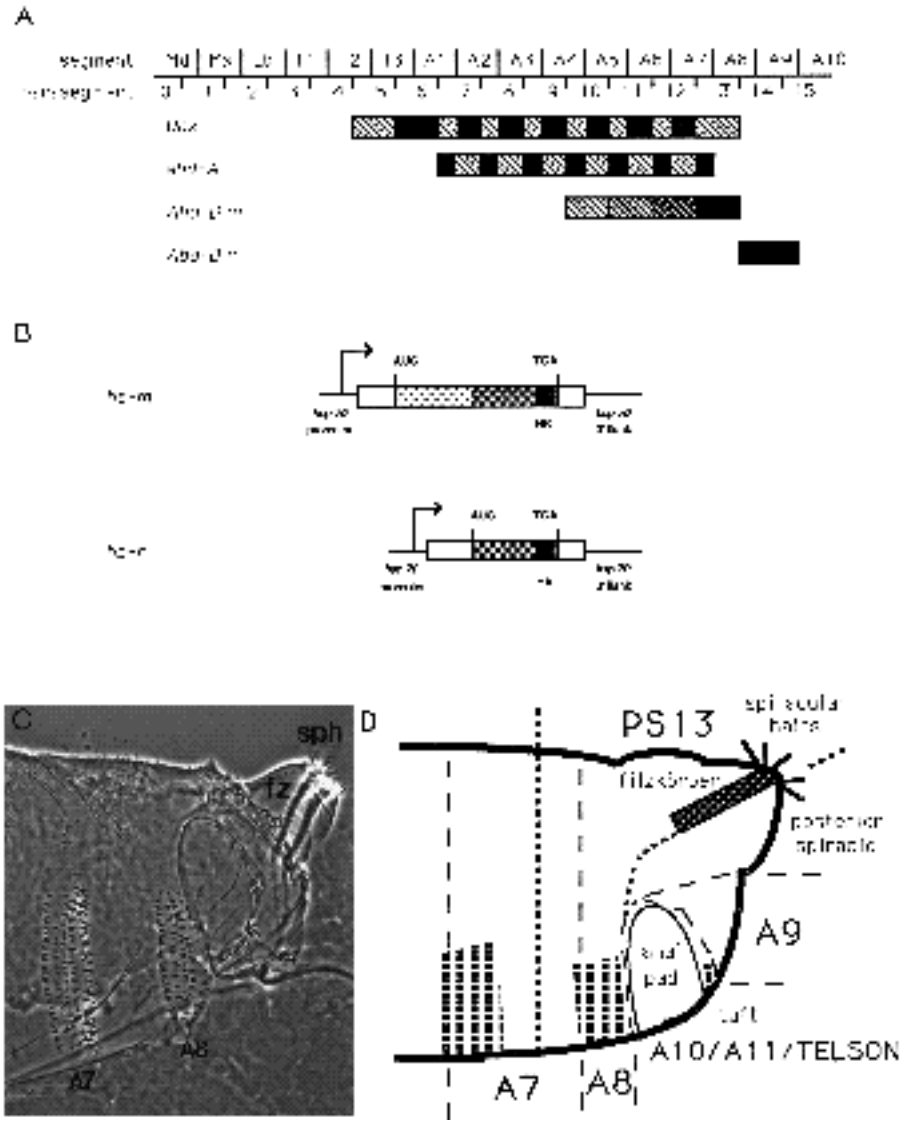

Fig. 1. (A) Summary of *Ultrabithorax (Ubx)*, *abdominal-A (abd-A)* and *Abdominal-B (Abd-B) m* and *r* expression patterns in the ectoderm at germband extension. Black boxes represent high levels of expression. Unshaded hatched boxes represent low levels of expression. The light to dark shading of the hatched boxes for *Abd-B m* represents its graded expression. (B) Structure of the *hs-m* and *hs-r* fusion constructs used to express ABD-B m and r proteins ectopically. Unshaded boxes represent non-coding *Abd-B* sequences. Translational start and stop codons delimit the open reading frames (ORF). The lightly stippled box represents amino-terminal sequences unique to the *Abd-B m* ORF. Darkly stippled boxes represent sequences shared by *Abd-B m* and *r* ORFs. Black boxes denote the identical homeoboxes (HB). The thin lines flanking the 5′ and 3′ *Abd-B* non-coding regions respectively represent *hsp70* promoter and 3′ untranslated sequences which include the polyadenylation signal. Arrows indicate transcriptional start sites. (C) Photomicrograph showing a lateral view of the tail region of a first instar larva (dorsal surface at top). The normal locations of two structures (fz, filzkörper; sph, spiracular hairs) scored in this study are indicated. (D) Schematic drawing of the tail region of a first instar larva adapted from Sato and Denell (1986) and Jürgens (1987). Thin dashed lines indicate segmental boundaries. Thick dashed lines indicate parasegment (PS) 13 boundaries. Rows of black squares denote ventral denticle belts.

transformant lines with 30 minute, 36°C heat-shock pulses at 4 and 6 hours of embryogenesis, unless otherwise indicated. Under these heat-shock conditions, the cuticles of untransformed control larvae were indistinguishable from wild-type larval cuticles. Although many heat-shocked *hs-m* and *hs-r* embryos failed to undergo head involution and complete germ band retraction, in the subsequent text we only describe the phenotypic transformations that can be scored by the appearance or disappearance of larval cuticle markers.

Ectopic expression of ABD-B m protein induces A8 structures in thoracic and abdominal segments

Heat-shock induction of ABD-B m protein in embryos carrying one to four copies of *hs-m* produced major alterations in larval morphology. The most distinctive phenotype was the presence of filzkörper pairs, structures normally derived only from A8, in the thoracic and abdominal segments and occasionally as far anterior as the head (Figs 2B,C, 7). Spiracular hairs, which normally surround the openings of the posterior spiracles, were also found in thoracic and abdominal segments (Fig. 2C inset). Therefore, ABD-B m protein is necessary, as predicted by *Abd-B* mutant analyses and also sufficient to promote the development of filzkörper and spiracular hairs.

The number and thickness of the ectopic filzkörper depended on the level of ABD-B m protein. Generally, a single copy of *hs-m* induced fewer additional filzkörper than were induced by two or four copies of *hs-m* (Table 1). The ectopic filzkörper produced by a single copy of *hs-m* appeared thinner than those formed in the normal A8 position (Fig. 2B) and than those in larvae with two or four copies of *hs-m* (Fig. 2C).

Several other phenotypes were produced by ectopic expression of ABD-B m. First, ventral pits, normally confined to the three thoracic segments (T1-T3), appeared in the abdomen (Fig. 2C). Because ventral pits develop in the abdominal segments of *Ubx* mutants, the formation of these structures is thought to be suppressed by UBX in wild-type larvae (Lewis, 1978; Mann and Hogness, 1990). Therefore, ABD-B m protein may interfere with either the expression or function of UBX protein in cells forming the ventral pits.

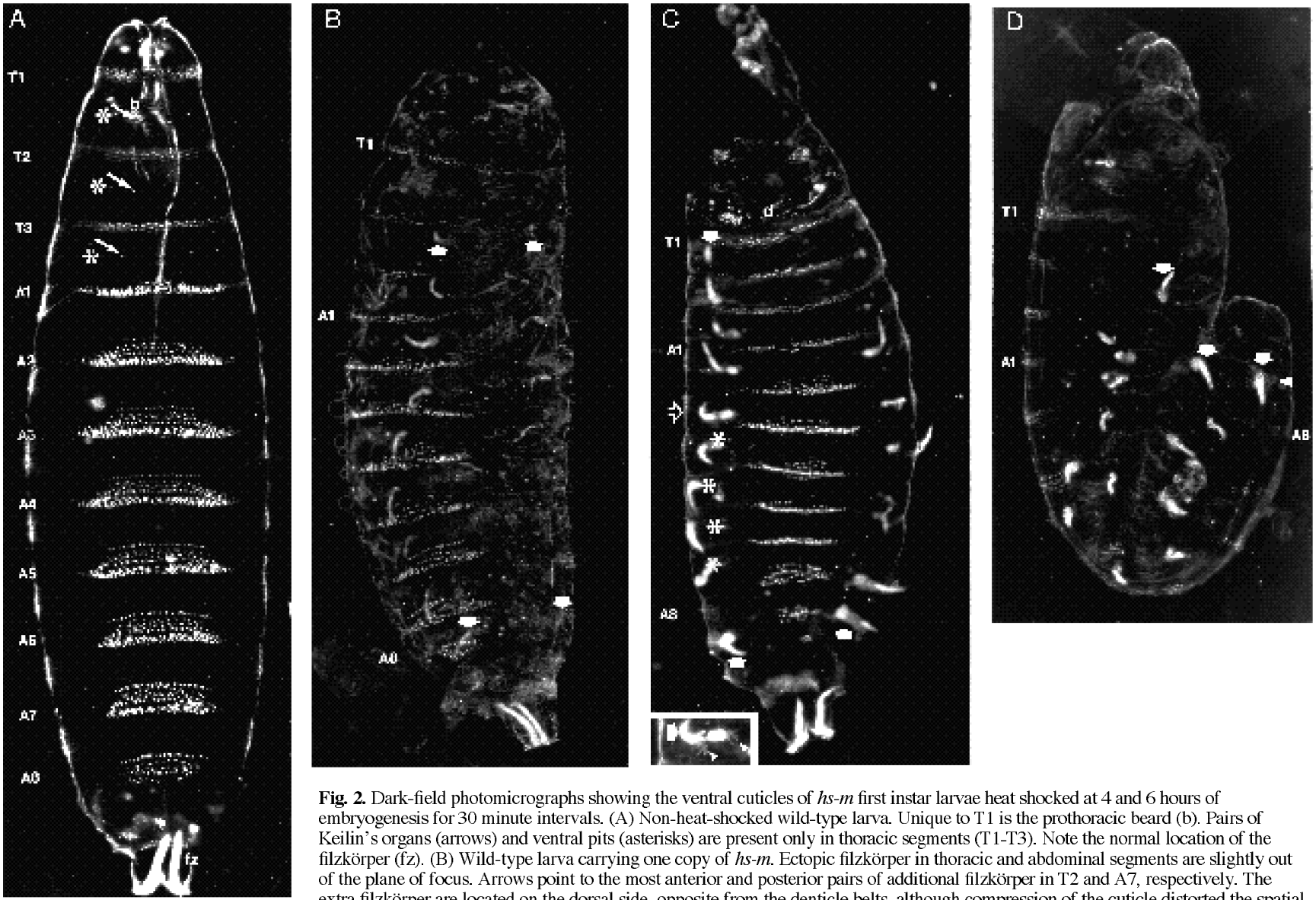

Fig. 2. Dark-field photomicrographs showing the ventral cuticles of *hs-m* first instar larvae heat shocked at 4 and 6 hours of embryogenesis for 30 minute intervals. (A) Non-heat-shocked wild-type larva. Unique to T1 is the prothoracic beard (b). Pairs of Keilin's organs (arrows) and ventral pits (asterisks) are present only in thoracic segments (T1-T3). Note the normal location of the filzkörper (fz). (B) Wild-type larva carrying one copy of *hs-m*. Ectopic filzkörper in thoracic and abdominal segments are slightly out of the plane of focus. Arrows point to the most anterior and posterior pairs of additional filzkörper in T2 and A7, respectively. The extra filzkörper are located on the dorsal side, opposite from the denticle belts, although compression of the cuticle distorted the spatial relationships in this photograph. (C) Wild-type larva carrying either two or four copies of *hs-m*. This larva shows a reduction in the size of the abdominal denticle belts. Note that the shape of the A1 belt resembles that of the T3 belt. Ectopic denticles (d) are seen inthe head. A few of the ventral pits present in the abdominal segments are denoted by asterisks. Ectopic filzkörper and spiracular hairs are out of the ventral plane of focus. Only the most anterior and posterior extra filzkörper are marked (arrows). The inset shows an enlarged view of the region marked by the open arrow to illustrate the ectopic filzkörper (arrow) and spiracular hairs (arrowheads). (D) *BX-C*$^{-}$ larva carrying one or two copies of *hs-m*. The germ band failed to retract in this larva. Denticle belts resemble those of a *BX-C*$^{-}$ larva. Arrows point to the most anterior and posterior ectopic filzkörper. Arrowhead indicates the spiracular hairs in association with the filzkörper.

Table 1. *Quantitation of filzkörper induced by increasing doses of* hs-m *with or without one copy of* hs-Ubx

Copy number*		Total number of larvae	Larvae with extra filzkörper	Mean† (number of extra filzkörper)
hs-m	*hs-Ubx*			
1	0	53	10 (19%)	5±5
1	1	61	1 (2%)	2
1 or 2	0	69	26 (38%)	13±7
1 or 2	1	73	14 (19%)	4±3
1, 2 or 3	0	46	39 (84%)	16±6
1, 2 or 3	1	34	28 (82%)	4±4
2 or 4	0	75	75 (100%)	14±5
2 or 4	1	68	62 (91%)	7±6

*See Materials and methods for crosses and genotypic details.
†Mean±standard deviation.

Second, Keilin's organs, also normally in T1-T3, were absent from *hs-m* larvae. Finally, ectopic ABD-B m protein affected thoracic and abdominal denticle belts. The size of the T1 belt was reduced and the T1 beard, a small patch of denticles below the T1 belt, was absent (Fig. 2B,C). Since these alterations in T1 resembled the transformation of T1 toward T2 in *Sex combs reduced* (*Scr*) mutant larvae (Wakimoto et al., 1984; Sato et al., 1985; Pattatucci et al., 1991), the transformation of T1 in *hs-m* larvae may result from the repression of the endogenous *Scr* gene. Alternatively, endogenous *Antennapedia* (*Antp*) could be ectopically activated since T1 transforms toward T2 when ANTP is expressed throughout embryos (Gibson and Gehring, 1988). Individual thick denticles were observed in the thoracic belts and the head region (Fig. 2C). In larvae carrying two to four copies of *hs-m*, the abdominal denticle belts of A2-A8 were reduced in size, with varying numbers of missing denticles, suggesting a transformation toward A1 as in *abd-A* mutants (Fig. 2C; Sánchez-Herrero et al., 1985). The clearest effect of ABD-B m on the denticle belts was the transformation of the denticle belt in A1 toward a T3 denticle belt (Fig. 2C). This transformation of A1 is reminiscent of the *Ubx* mutant phenotype in which T3 and A1 are transformed toward T2 (Lewis, 1978). The similarity of abdominal transformations induced by ectopic ABD-B m to abdominal transformations in *Ubx* and *abd-A* mutants suggests that *Ubx* and/or *abd-A* mediate ABD-B m-induced effects on abdominal denticle belts. This interpretation is supported by the results of ectopic ABD-B m expression in the embryos deficient for the entire BX-C (see below).

Ectopic expression of ABD-B r protein also induces structures derived from segment A8

The primary function previously attributed to ABD-B r was the repression of *Abd-B m* expression in PS14 (Casanova et al., 1986). Surprisingly, heat induction of ABD-B r protein in homozygous *hs-r* embryos produced larval phenotypes very similar to those seen in *hs-m* larvae. Segmentally repeated filzkörper pairs and spiracular hairs were present in the thorax and abdomen, and ventral pits appeared occasionally in abdominal segments A1-A7 (Figs 3A,B, 7). Keilin's organs were suppressed in *hs-r* larvae. Like ectopic ABD-B m, ectopic ABD-B r protein affected the denticle belts of the thoracic and abdominal segments (compare Figs 2C and 3A). Thoracic denticle belts maintained thoracic shapes, but T1 transformed toward T2, and some large denticles appeared in the thoracic belts and head region (Fig. 3A,B). The A1 belt weakly transformed toward a T3 denticle belt (Fig. 3A,B). The number of denticle rows in the A2-A8 belts of *hs-r* larvae were reduced, suggesting a transformation toward an A1 morphology (Fig. 3A). This effect is consistent with the repression of endogenous BX-C gene expression by ABD-B r in denticle precursor cells of the abdomen as described above. The similar phenotypes of *hs-r* and *hs-m* larvae indicate that ABD-B r protein is able to carry out the same activities as ABD-B m protein.

Induction of filzkörper and spiracular hairs by ectopic ABD-B m or r does not require endogenous BX-C genes

The ability of ectopically expressed ABD-B m and r proteins to induce filzkörper and spiracular hairs in segments as far anterior as the head, outside the domain specified by the BX-C genes, suggests that *Ubx*, *abd-A* and endogenous *Abd-B* genes are not required for the formation of these structures. To rule out the possibility that the phenotypic changes induced by ectopic ABD-B m or r are due to their effects on endogenous BX-C gene expression, *hs-m* or *hs-r* was induced in embryos deficient for the entire BX-C (*BX-C*$^{-}$). In *BX-C*$^{-}$ larvae, segments T3 and A1-A8 (PS5-13) all assume T2 (PS4)-like identities, showing the characteristic narrow denticle belts and ventral pits (Lewis, 1978). Expression of ABD-B m throughout *BX-C*$^{-}$ embryos promoted the development of filzkörper and spiracular hairs in the larval thorax and abdomen (Figs 2D, 7), similar to ectopic structures induced in larvae of a wild-type genetic background (Figs 2B,C, 7). ABD-B r also induced the formation of filzkörper and spiracular hairs in *BX-C*$^{-}$ larvae (Figs 3C, 7), but the transformations were much weaker than those induced by ectopic ABD-B r in a wild-type genetic background (Fig. 3A,B) or than those induced by ectopic ABD-B m in a *BX-C*$^{-}$ genetic background (Fig. 2D). Like *BX-C*$^{-}$ larvae, ventral pits were present in all thoracic and abdominal segments of *hs-m* or *hs-r* larvae of a *BX-C*$^{-}$ genetic background. The denticle belts resembled those of *BX-C*$^{-}$ larvae, but T1 transformed toward T2, and some thick denticles appeared in the denticle belts and the head region. These results indicate that ABD-B m or r protein is the only BX-C protein necessary to commit cells to filzkörper and spiracular hair fates. Although ABD-B proteins affected the thickness of individual denticles, they did not transform the identities of denticle belts in T2, T3 or A1-A8 of *BX-C*$^{-}$ larvae. These results support the conclusion that ectopic ABD-B proteins in wild-type embryos modify abdominal denticle belt shapes by affecting endogenous *Ubx* and/or *abd-A* expression or function.

ABD-B and UBX proteins compete for the specification of segmental identity when coexpressed throughout embryos

Ectopic expression of *Ubx* from a heat-inducible promoter transformed larval head and thoracic segments toward A1, but had no reported effect on larval abdominal segments (Figs 4A, 7; González-Reyes et al., 1990; González-Reyes and Morata, 1990; Mann and Hogness, 1990). A hierarchy of homeotic protein functions was proposed to account for the failure of ectopic UBX protein to transform the

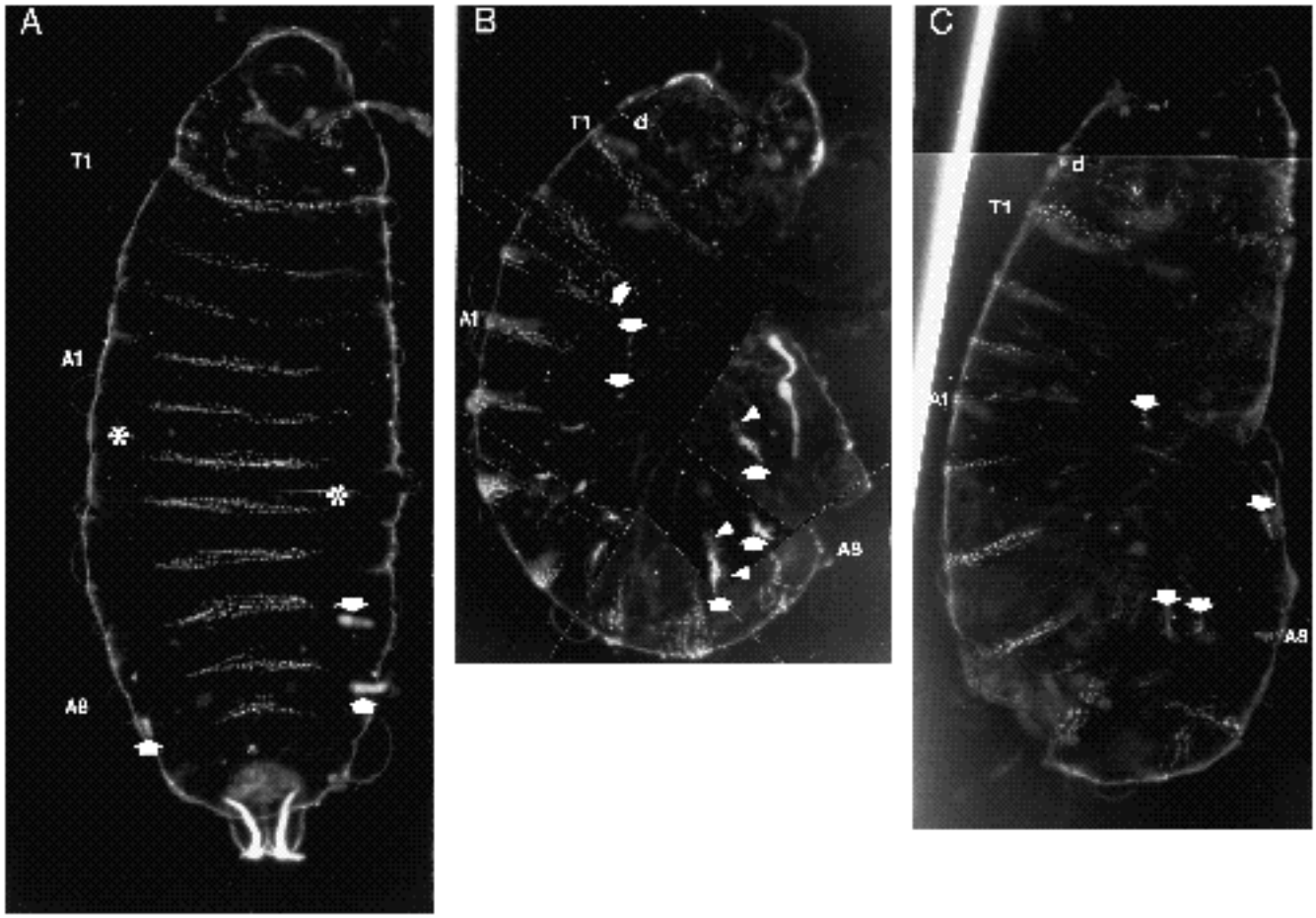

Fig. 3. *hs-r* first instar larvae heat shocked for 30 minutes at 4 and 6 hours of development. Cuticles shown in A and B are from wild-type larvae carrying two copies of *hs-r*. (A) Ventral view showing the reduced size of the abdominal belts. Ectopic filzkörper (arrows) are out of the ventral plane of focus. Asterisks mark a few of the ventral pits present in the abdominal segments. (B) Lateral view showing the ectopic filzkörper. Arrows indicate six of the extra filzkörper. Arrowheads point to the ectopic spiracular hairs associated with the filzkörper. Ectopic denticles (d) are seen in the head. (C) Lateral view of a *BX-C*$^{-}$ larvae carrying two copies of *hs-r*. Arrows mark ectopic filzkörper. Ectopic denticles (d) are seen in the head. The germ bands of the larvae shown in B and C failed to retract.

abdomen (González-Reyes et al., 1990). This model predicts that ABD-B function will override UBX function when both proteins are ectopically expressed. To test this hypothesis, UBX and ABD-B m proteins were simultaneously expressed throughout wild-type embryos. Wild-type larvae carrying single copies of *hs-Ubx* and *hs-m* (designated *hs-Ubx* + *hs-m*) exhibited the characteristic *Ubx* transformation of larval head and thoracic segments toward A1 identities (data not shown). Only 2% of *hs-Ubx* + *hs-m* larvae developed ectopic filzkörper compared to 19% of larvae carrying a single copy of *hs-m* without *hs-Ubx* (Table 1). Furthermore, ventral pits were not seen in the thorax or abdomen of these larvae, indicating that UBX was, in fact, active in these segments. Therefore, contrary to the expectations of the functional hierarchy model, the observed phenotypes suggest that ABD-B m does not prevent UBX from acting in the thorax and abdomen. Rather, UBX protein appears to interfere with the ability of ABD-B m to form ectopic A8 structures.

To test if the interference of UBX can be overcome by increasing the relative level of ABD-B m protein, we heat shocked groups of embryos, each with increasing copies of *hs-m* but only a single copy of *hs-Ubx*. When a single copy of *hs-m* was present, only 2% of larvae showed ectopic filzkörper (Table 1). A second copy of *hs-m* increased the percentage of larvae with ectopic filzkörper to 19% (Table 1). In the group of larvae with one to three copies of *hs-m*, the percentage of transformed larvae was even greater (82%) and, finally, 91% of larvae with two or four copies of *hs-m* showed ectopic filzkörper (Table 1; Figs 4B, 7). Since additional copies of *hs-m* overcome the interference of UBX, our results indicate that UBX and ABD-B m compete for their respective developmental programs in a dose-dependent manner.

To eliminate possible contributions of the endogenous BX-C genes to the phenotypes observed in larvae carrying a single copy of *hs-Ubx* and one to four copies of *hs-m*, UBX and ABD-B m proteins were coexpressed throughout embryos deficient for the BX-C. In homozygous *BX-C*$^{-}$ larvae carrying one copy of *hs-Ubx* and either one or two copies of *hs-m*, the three cephalic, three thoracic and nine abdominal segments showed a transformation toward A1

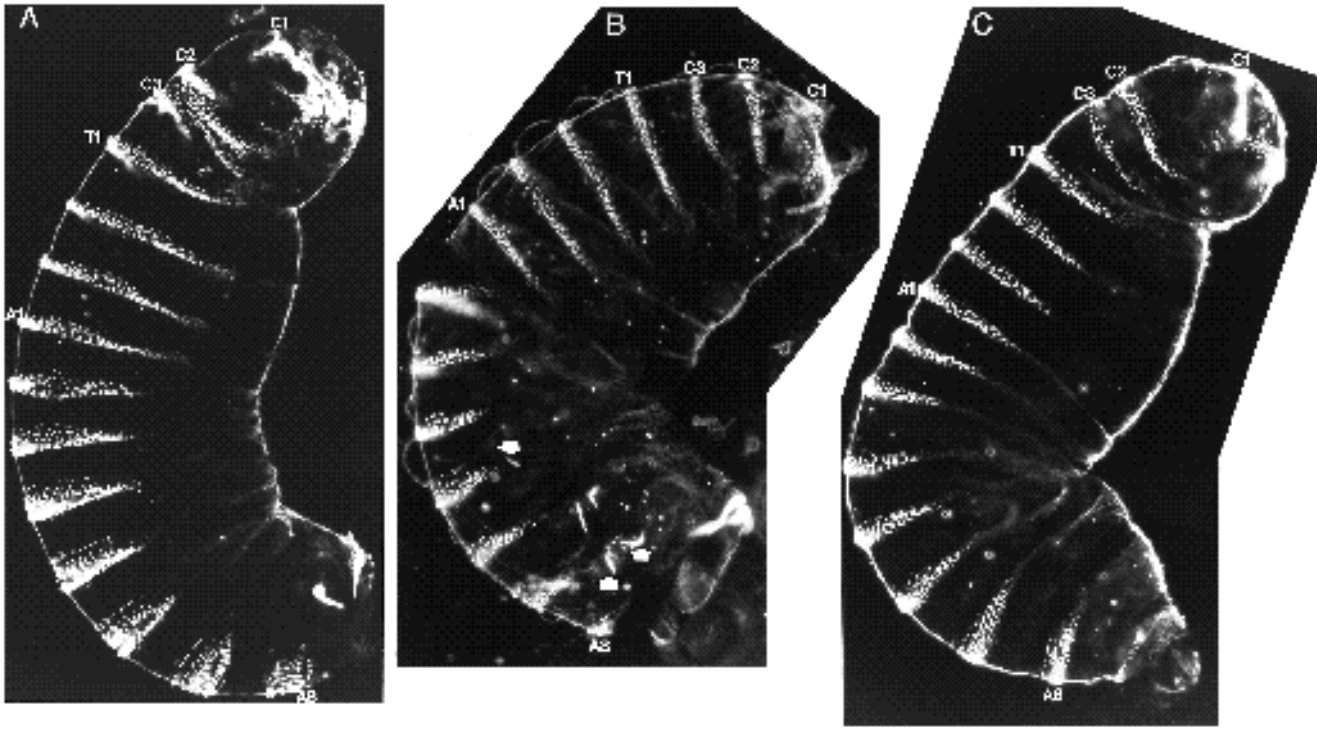

Fig. 4. Cuticles from first instar larvae in which *hs-Ubx* or *hs-Ubx* + *hs-m* were ectopically induced by 30 minute heat shocks at 4 and 6 hours of embryonic development. (A) Lateral view of a *hs-Ubx* larva showing the transformation of the cephalic and thoracic segments toward A1 identities, as described by González-Reyes et al. (1990) and Mann and Hogness (1990). (B) Lateral view of a wild-type larva carrying one copy of *hs-Ubx* and two or four copies of *hs-m*. Note the transformation of the head and thoracic segments toward A1 identities and the additional filzkörper. Arrows indicate the most anterior and posterior ectopic filzkörper. Filzkörper are not always limited to segments A5-A7 as shown in this figure, but can be seen as far anterior as the thoracic segments. (C) Lateral view of a *BX-C*$^{-}$ larva carrying one copy of *hs-Ubx* and one copy of *hs-m*. Note that all segments are transformed toward A1 and filzkörper are absent. The same phenotype was observed when an additional copy of *hs-m* was present.

(Figs 4C, 7). No filzkörper were seen in these larvae, even at the normal A8 position. Therefore, in the absence of endogenous BX-C genes, the identities of all segments were specified by ectopic UBX. The presence of extra filzkörper in wild-type larvae with a single copy of *hs-Ubx* and one or two copies of *hs-m*, but not in *BX-C*$^{-}$ larvae with a single copy of *hs-Ubx* and one or two copies of *hs-m*, suggests that endogenous *Abd-B* contributed to the formation of filzkörper. In A5-A8 (PS10-13) of wild-type embryos, endogenous ABD-B m protein could supplement ectopic ABD-B m protein so that ABD-B m levels are sufficiently high to overcome suppression by ectopic UBX. Additionally, ectopic ABD-B m protein could activate endogenous *Abd-B* expression as was observed when a DEFORMED (DFD)/ABD-B chimeric protein was ectopically expressed (Kuziora and McGinnis, 1991). Taken together, these results argue that specification of segmental identity by ectopic UBX and ABD-B m is determined by a quantitative competition between UBX and ABD-B m proteins for the regulation of downstream genes, but do not support the presence of a hierarchy in which ABD-B activity dominates.

Ectopic expression of UBX protein at early embryonic stages transforms identities of abdominal segments

The ability of ectopic UBX to compete with ectopic ABD-B m appears to contradict the inability of UBX to transform abdominal segments of *hs-Ubx* embryos. This paradox could be explained if abdominal segment identities are determined earlier in development than thoracic segment identities. At the time of heat shock in previous experiments (González-Reyes et al., 1990; González-Reyes and Morata, 1990; Mann and Hogness, 1990), proper identities of the abdominal segments, but not of the thoracic segments, may have already been specified. To test this hypothesis, UBX protein was induced in accurately staged embryos by two 30 minute heat-shock pulses, with a 90 minute recovery, beginning at either 4, 6 or 8 hours of embryonic development.

Heat induction of *hs-Ubx* beginning at 4 hours or 6 hours of development weakly transformed abdominal segments toward A1 (Fig. 5B). We quantified the degree of abdominal transformation toward A1 using the number of setae in the first row of the denticle belts in A2-A8. This row derives from the posterior compartments of A1-A7 (Szabad et al., 1979; Jürgens, 1987), the region corresponding to high levels of ABD-A in wild-type embryos (Karch et al., 1990; Macías et al., 1990). This row of setae is absent from the A1 belt where ABD-A is not expressed (Fig. 5C). In *abd-A* mutants, the first denticle row is missing from the belts in A2-A8 (Sánchez-Herrero et al., 1985), further substantiating its use as a marker for the segmental transformations of A2-A8 toward A1. As a control, the setae in the second

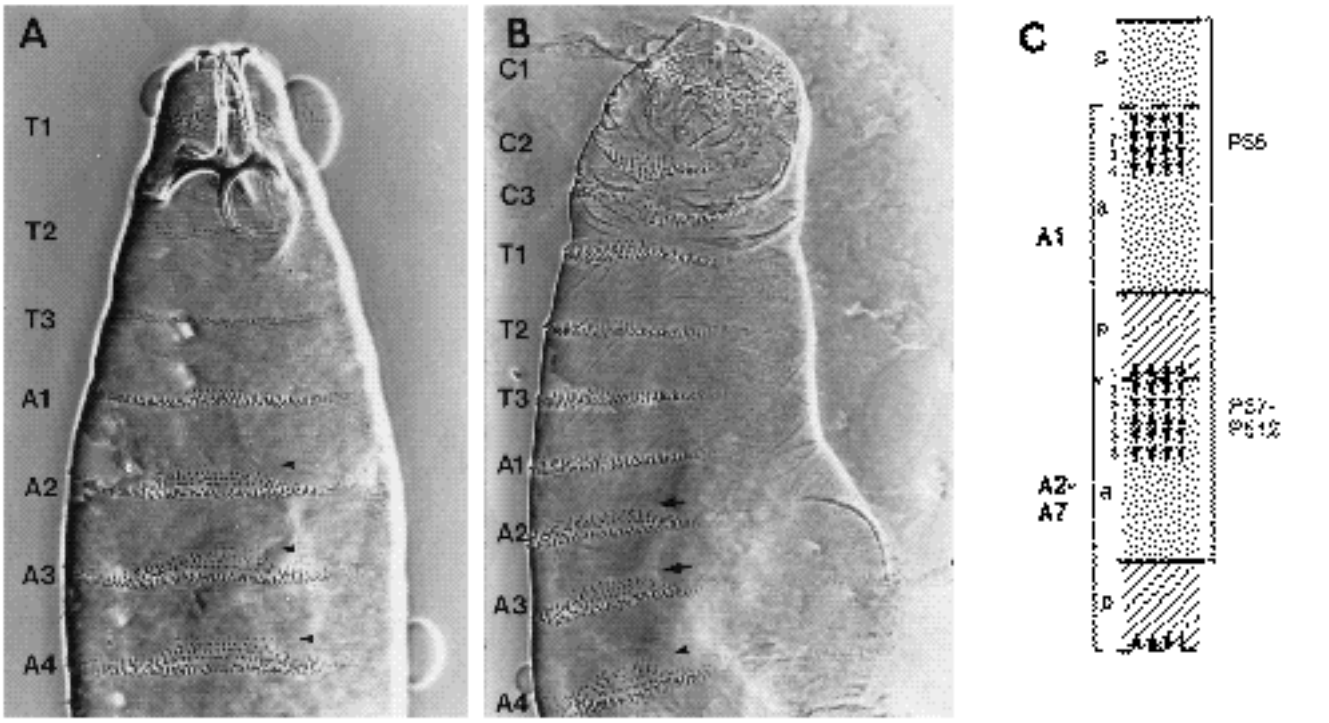

Fig. 5. (A-B) Bright-field photomicrographs showing the anterior halves of cuticles from w^{1118} control and *hs-Ubx* larvae heat shocked for 30 minutes at 4 and 6 hours of embryogenesis. (A) Cuticle from a w^{1118} control larva. Arrowheads point to the first row of setae in A2-A4 denticle belts. (B) Cuticle from a *hs-Ubx* larva. Note that the first row of setae are absent from denticle belts of A2 and A3 (arrows), and a reduced number of setae are seen in the first row of the A4 denticle belt (arrowhead). (C) Schematic diagram illustrating the segmental compartments. Note that a high level of UBX (stippled shading) is expressed in the anterior compartments (a) of segments A1-A7, corresponding to parasegments (PS) 6 through 12, while a high level of ABD-A (hatched shading) is expressed in the posterior compartments (p) of segments A1-A7, corresponding to PS7-12. Arrowheads represent denticles. Arrowhead direction indicates the polarity of denticles. The denticle row number is adjacent to each denticle row. Solid thick lines represent parasegmental boundaries. Dashed thick lines denote segmental boundaries.

row of the denticle belts in A2-A8 were counted since this row derives from the anterior compartments of A2-A8 (Szabad et al., 1979; Jürgens, 1987), the region which corresponds to high levels of UBX in wild-type embryos (Fig. 5C; White and Wilcox, 1985). When embryos were heat shocked beginning at 4 hours of development, the number of setae in the first row of the denticle belts in A2-A8 was reduced by as much as 70% in *hs-Ubx* larvae relative to control larvae (Fig. 6A,E). Induction of *hs-Ubx* starting at 6 hours of development reduced the number of denticles in the first row of A2-A8 belts by 10% to 35% (Fig. 6B,F). However, the number of setae in the first row of the A2-A8 denticle belts was similar in *hs-Ubx* and control embryos when heat shocks were initiated at 8 hours of development (Fig. 6C,G).

The cephalic segments also showed a stage-dependent susceptibility to transformation by ectopic UBX as previously described (González-Reyes and Morata, 1991). When *hs-Ubx* embryos were heat shocked at 4 hours of embryogenesis, three cephalic segments (C1, C2, C3) developed A1-type denticle belts (Figs 5B, 6E). However, heat shocks begun at 6 hours of development transformed only two cephalic segments, C1 and C2, toward A1 (data not shown; Fig. 6F) and heat shocks initiated at 8 hours of development did not transform the cephalic segments except for the development of a few denticles in the head region (Fig. 6G). In contrast, heat induction of *hs-Ubx* beginning at 4, 6 or 8 hours transformed the thoracic denticle belts toward A1 denticle belts (Fig. 6E-G). These results suggest that the identities of the head segments as well as the abdominal segments are determined earlier in development than those of the thoracic segments. We suggest that the previous failure to transform the abdominal segments after heat shock of *hs-Ubx* embryos (González-Reyes et al., 1990; González-Reyes and Morata, 1990; Mann and Hogness, 1990) was due to differences in the timing or the level of ectopic UBX expression.

Discussion

Both ABD-B m and r proteins possess morphogenetic functions

Genetic and molecular analyses of *Abd-B* have shown that it is a complex gene. Four overlapping *Abd-B* transcripts encode two homeodomain proteins with independent functions (Casanova et al., 1986; DeLorenzi et al., 1988; Kuziora and McGinnis, 1988; Sanchez-Herrero and Crosby, 1988; Celniker et al., 1989; Zavortink and Sakonju, 1989; Boulet et al., 1991). The *m* function, provided by a 55×10^3 M_r protein, specifies the proper identities of PS10-13 and the *r* function, contributed by a 30×10^3 M_r protein, is

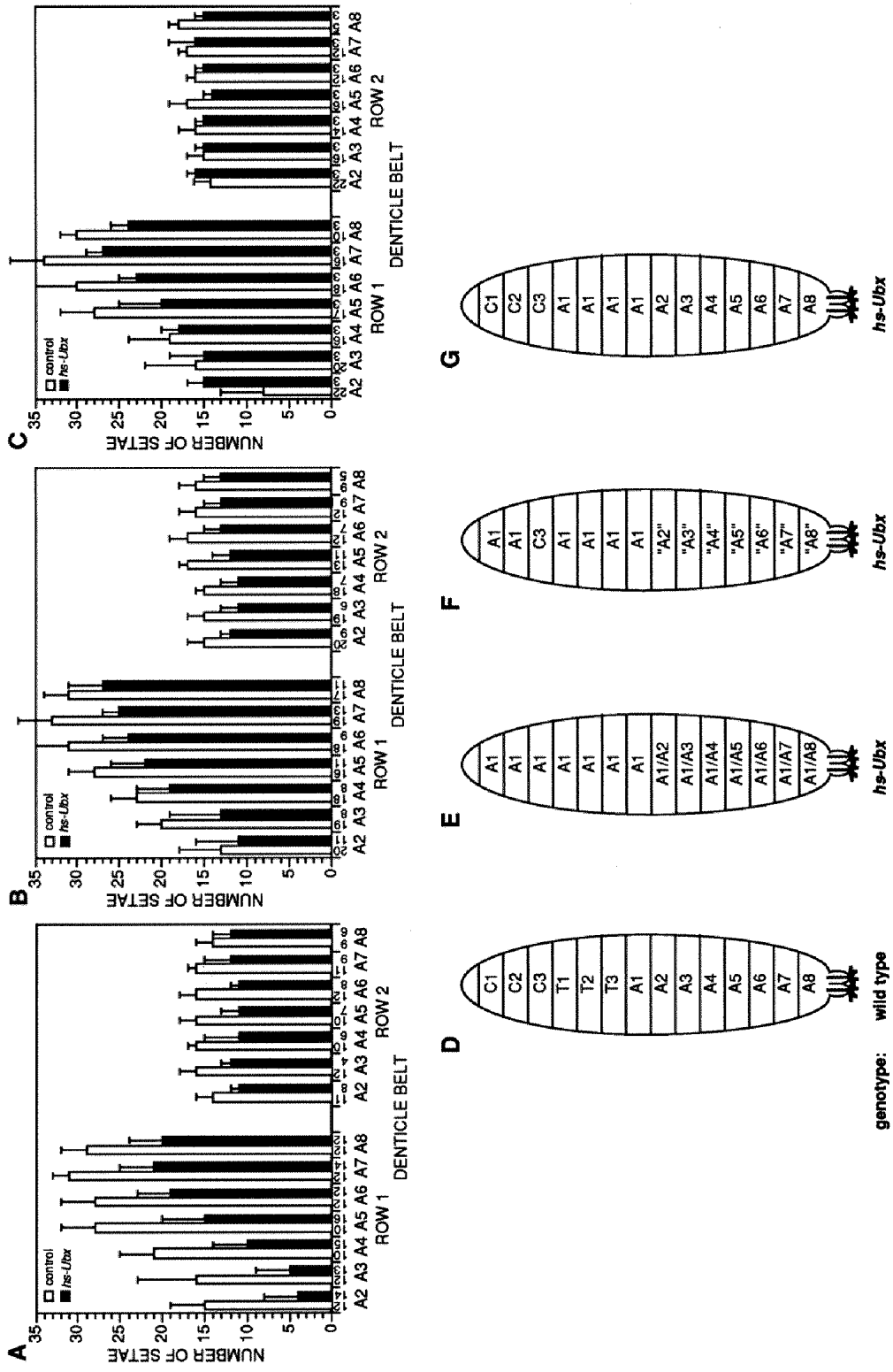

Fig. 6. (A-C) Quantitation of setae in the first and second rows of the denticle belts in the second through eighth abdominal segments (A2-A8) of heat-shocked w^{1118} control and *hs-Ubx* larvae. Thirty minute heat shocks were given at (A) 4 and 6 hours of embryogenesis, (B) 6 and 8 hours of embryogenesis or (C) 8 and 10 hours of embryogenesis. The number of denticle belts (*n*) examined is represented below the bars. The plotted values represent the average number of setae from *n* denticle belts. Error bars indicate the standard deviation. Denticle belts in which setal rows 1 or 2 were difficult to identify were not included in the analysis. (D-G) Schematic summary of the phenotypic transformations induced by ectopic UBX: 4 and 6 hours of embryogenesis (E), 6 and 8 hours of embryogenesis (F) or 8 and 10 hours of embryogenesis (G). Different abdominal designations were used in E and F to indicate that the reduction in the number of row 1 setae in A2-A8 denticle belts, indicative of a weak transformation to A1, is more extreme when *hs-Ubx* is induced at 4 and 6 hours (as shown in A) than at 6 and 8 hours (as shown in B).

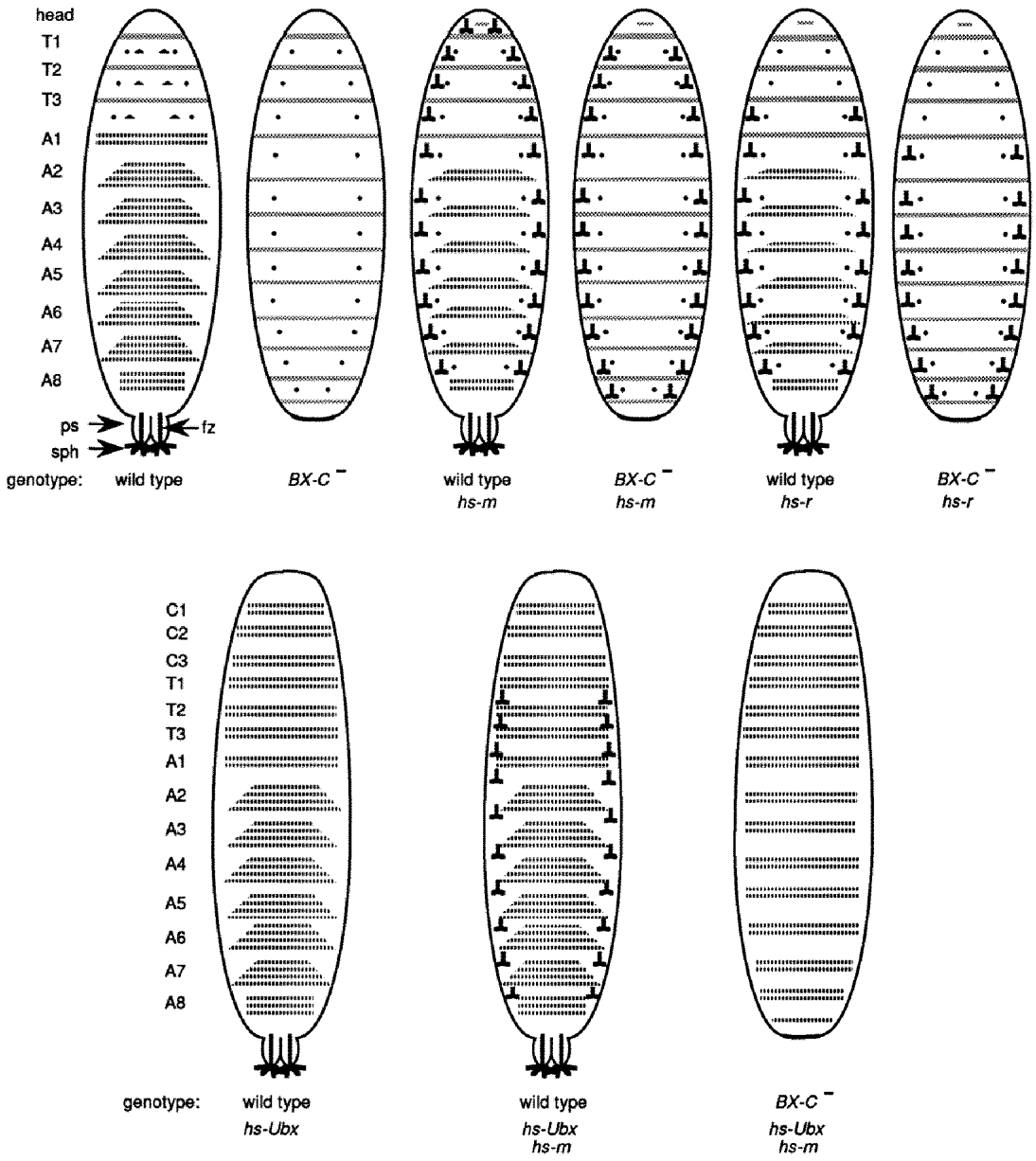

Fig. 7. Schematic summary of larval phenotypes resulting from ectopic ABD-B and/or UBX proteins in wild-type or *BX-C*$^{-}$ genetic backgrounds. Abbreviations and symbols: fz, filzkörper; ps, posterior spiracles; sph, spiracular hairs; ⊥, ectopic filzkörper and spiracular hairs; ⁞⁞⁞, abdominal denticles; ⁓, thoracic denticles; ⌒, Keilin's organ; •, ventral pit.

required for PS14 identity (Celniker et al., 1990; Boulet et al., 1991). The ABD-B r protein corresponds to a truncated ABD-B m protein which lacks the additional 25×10^3 M_r amino-terminal domain (Celniker et al., 1989; DeLorenzi et al., 1988; Zavortink and Sakonju, 1989). By expressing ABD-B m or r protein ectopically in embryos, we have shown that the two proteins possess similar abilities to specify segmental identities. Ectopic ABD-B m and r proteins can both induce the formation of segmentally repeated pairs of filzkörper and associated spiracular hairs, structures thought to derive from PS13 (Jürgens, 1987), in the thorax and abdomen of larvae. A summary of these phenotypes is shown in Fig. 7.

Although induction of filzkörper and spiracular hairs by ectopic ABD-B m protein is consistent with the absence of these structures from *Abd-B* $m^{-}r^{+}$ larvae (Karch et al., 1985; Casanova et al., 1986; Sato and Denell, 1986; Whittle et al., 1986), induction of PS13-derived structures by ectopic ABD-B r was unexpected. ABD-B r is normally expressed only in PS14 and 15, and the mutant phenotype of *Abd-B* $m^{+}r^{-}$ larvae suggests that *r* function is not required for specifying filzkörper and spiracular hairs (Casanova et al., 1986). It is possible, however, that filzkörper derive from cells of both PS13 and 14 and that ABD-B r function also contributes to their specification: *Abd-B m* is derepressed in PS14 of *Abd-B* $m^{+}r^{-}$ mutants (Casanova et al., 1986; Boulet et al., 1991) and may substitute for any *r* function required for the normal development of filzkörper.

The similarity of phenotypes induced by ectopic ABD-B m and ABD-B r proteins implies that the ability to promote the development of filzkörper and spiracular hairs can be primarily attributed to the domain common to both proteins. An important feature of the shared domain is the homeodomain. Homeodomain swap experiments suggest that most of the specificity of homeotic proteins lies in the homeodomain (Kuziora and McGinnis, 1989, 1991; Gibson et al., 1990; Mann and Hogness, 1990). For example, a DFD/ABD-B chimeric protein, in which the ABD-B homeodomain was substituted for DFD homeodomain, induced filzkörper and other PS13-derived structures in larval thoracic and abdominal segments, and also activated the ectopic expression of endogenous *Ubx*, *abd-A* and *Abd-B* (Kuziora and McGinnis, 1991). These experiments, however, did not distinguish whether the development of PS13-derived structures resulted from direct activation of downstream genes or from ectopic activation of endogenous *Abd-B* and/or other BX-C genes which in turn activated their target genes. Our results show that ABD-B proteins are sufficient to induce filzkörper and spiracular hairs since these PS13-derived structures developed when ectopic ABD-B m or r was expressed in *BX-C*$^{-}$ larvae. Because ectopic ABD-B m and ABD-B r induced similar structures, most, if not all, of the specificity for inducing filzkörper and spiracular hairs lies in the domain shared by both proteins. Therefore, ABD-B m and r proteins may regulate common downstream genes. The glutamine-rich amino-terminal domain of ABD-B m, not present in ABD-B r, may contribute to the ability of ectopic ABD-B m protein to induce stronger transformations toward PS13 than ectopic ABD-B r. Amino-terminal sequences of the ANTP protein have also been implicated in stronger transformations of the larval head and prothorax by ectopic ANTP (Gibson et al., 1990).

Although ABD-B r protein can direct the development of filzkörper and spiracular hairs, these structures do not appear in PS14 and 15. Our results suggest that parasegmental differences contribute to the phenotype specified by ABD-B r in its normal domain. A factor(s) found in PS14 and 15 may affect the activity of ABD-B r protein. A candidate for such a factor is the *spalt (sal)* gene product. The *sal* expression domain (Frei et al., 1988; Wagner-Bernholz et al., 1991) overlaps the *Abd-B r* expression domain (Kuziora and McGinnis, 1988; Sánchez-Herrero and Crosby, 1988; DiLorenzi et al., 1988; Boulet et al., 1991) and *sal* mutants exhibit ectopic pairs of filzkörper and spiracular hairs in PS14 and 15 (Jürgens, 1988). Since derepression of ABD-B m in PS14 of *Abd-B m*$^{+}$*r*$^{-}$ larvae does not result in extra filzkörper and spiracular hairs (Casanova et al., 1986), the SAL protein must also be capable of suppressing *Abd-B m* function. In fact, ectopic pairs of filzkörper and spiracular hairs develop when ABD-B m is derepressed in PS14 and 15 of *sal* mutants (Jürgens, 1987). Therefore, SAL may modify ABD-B r activity (and can modify ABD-B m activity) in PS14 and 15. Alternatively, SAL activity may affect the fate of cells in PS14 and 15 prior to determination by ABD-B r. For example, SAL may act as a general repressor by altering the accessiblity of binding sites in downstream target genes to ABD-B r protein (Casanova, 1989).

A functional hierarchy does not explain the failure of ectopic UBX to transform the abdomen

In previous studies, ectopic expression of UBX transformed cephalic and thoracic segments but did not seem to affect segments A2-A9 (González-Reyes et al., 1990; González-Reyes and Morata, 1990; Mann and Hogness, 1990). The phenotypic effects of ectopic UBX activity were apparently suppressed in the abdomen by endogenous ABD-A and ABD-B functions (González-Reyes et al., 1990). When UBX was ectopically expressed in embryos lacking all BX-C functions except *abd-A* (*Ubx*$^{-}$ *abd-A*$^{+}$ *Abd-B*$^{-}$), the identities of A2-A4 were not transformed (González-Reyes and Morata, 1990). Similarly, in *Ubx*$^{-}$ *abd-A*$^{-}$ *Abd-B m*$^{-}$*r*$^{+}$ embryos, ABD-B r function prevented ectopic UBX from inducing the development of an extra A1-type denticle belt in A9 (González-Reyes and Morata, 1990). Finally, ABD-B m was able to suppress the phenotypic effects of ectopic UBX in A8 of larvae deficient for *Ubx* and *abd-A* (Mann and Hogness, 1990).

In the hierarchical model proposed by González-Reyes et al. (1990), ABD-A and ABD-B activities override ectopic UBX activity. According to the model, a functionally dominant ABD-B m protein should suppress UBX-induced transformations toward A1. Our data do not support the existence of a hierarchy of homeotic protein functions (González-Reyes et al., 1990). Instead, concurrent expression of UBX and ABD-B m proteins throughout wild-type embryos resulted in UBX-induced transformations of cephalic and thoracic segments toward A1. Moreover, UBX suppressed the development of ABD-B m-induced filzkörper in the thorax and the abdomen. The relative levels of ectopic UBX and ectopic ABD-B m determine the resultant phenotype: an increase in the dose of *hs-m* from one to four copies decreases the ability of UBX to suppress filzkörper.

The sensitivity of ABD-B m-induced transformations to the relative levels of UBX and ABD-B m proteins suggests that UBX and ABD-B m compete for the regulation of common targets. Such a competition among homeotic proteins could explain the previously observed phenomenon of phenotypic suppression (González-Reyes and Morata, 1990). The failure of these experiments to show competition among homeotic proteins may have been due to the relative levels or stabilities of ectopic proteins. UBX and ABD-B m could compete for common binding sites. In vitro binding studies have indicated that homeodomains recognize similar consensus sequences (reviewed in Hayashi and Scott, 1990; Laughon, 1991). In vivo, common binding sites are used by different homeodomain proteins to activate or repress the *Antp* promoter 2 (Appel and Sakonju, 1992). Alternatively, UBX and ABD-B m could compete for an accessory factor required for the expression of downstream genes. Another possibility is that UBX and ABD-B m direct segments into mutually exclusive developmental pathways through the regulation of unique downstream genes.

Cephalic and abdominal segments may be determined earlier than thoracic segments

Previous reports concluded that ectopic UBX had no effect

on abdominal cuticular identities (González-Reyes et al., 1990; González-Reyes and Morata, 1990; Mann and Hogness, 1990). However, in our experiments, high levels of ectopic UBX induced at 4 hours or 6 hours of development weakly transformed the denticle belts of A2-A8 toward A1 denticle belts. Ectopic UBX also transformed the cephalic and thoracic segments as previously described (González-Reyes et al., 1990; González-Reyes and Morata, 1990, 1991; Mann and Hogness, 1990). By 8 hours of development, the cephalic and abdominal segments appeared to be resistant to transformation by ectopic UBX, but the thoracic segments were still transformed toward A1.

The temporal difference in the determination of segmental identities may be a function of the sizes of homeotic gene transcription units (Gubb, 1986; Kornfeld et al., 1989; Thummel, 1992). The transcription units of *Dfd*, *Scr*, *abd-A* and *Abd-B*, homeotic genes responsible for the identities of head and abdominal segments, are shorter than the transcription units of *Antp* and *Ubx* which are required for proper thoracic patterning. Consequently, DFD and SCR may determine the identities of the cephalic segments, and ABD-A and ABD-B the identities of the abdominal segments, before ANTP and UBX determine the identities of the thoracic segments. If one postulates that the activation of the three BX-C genes occurs at the same time during development, transcription of the shorter *abd-A* (23 kb) and *Abd-B m* (8 kb) genes will be completed prior to the transcription of the 77 kb *Ubx* gene (Kornfeld et al., 1989; Zavortink and Sakonju, 1989; M. Lamka and S. Sakonju, unpublished observations). Assuming RNA polymerase transcribes at a rate of 1.4 kb/minute (Thummel et al., 1990; Shermoen and O'Farrell, 1991), it would take 16 minutes and 6 minutes to transcribe *abd-A* and *Abd-B m*, respectively, while 55 minutes would be required for *Ubx* transcription. Thus, the difference in transcription times alone might allow ABD-A and ABD-B m proteins to appear 40-50 minutes earlier in development than UBX protein. Similarly, transcription units of the *Dfd* (11 kb) and *Scr* (20 kb) genes are shorter than *Antp* (P1, 105 kb; P2, 40 kb) and *Ubx* (Kaufman et al., 1990). Therefore, *Dfd* and *Scr* would require shorter transcription times than *Antp* and *Ubx*. Although a number of studies report that UBX, ABD-A and ABD-B m proteins are detectable with antibodies at approximately 4 hours of embryogenesis (White and Lehmann, 1986; Celniker et al., 1989; DeLorenzi and Bienz, 1990; Karch et al., 1990; Macías et al., 1990; Boulet et al., 1991; Irvine et al., 1991), the relative sensitivities of these antibodies are unknown and staging by different groups may be variable. It is also difficult to assess the appearance of DFD and SCR relative to ANTP and UBX. In wild-type embryos, ABD-A and ABD-B m proteins may be present before UBX protein, and repression of *Ubx* by ABD-A and ABD-B m in posterior parasegments would ensure that UBX does not compete for segmental identities. We suggest, therefore, that transcriptional cross-regulatory interactions among the homeotic genes are important for normal development.

We are grateful to Richard Mann and David Hogness for the *hs-Ubx* flies, to Ian Duncan and Ed Lewis for other fly strains used in this study and to Joe Dickinson for the cuticle preparation protocol. We also thank Bruce Appel, Ken Burtis, Dan Cimbora, Barry Condron, Jill Hendrickson and Anthea Letsou for valuable comments on the manuscript and members of the Sakonju lab for discussion during the progress of this project. This work was supported by Howard Hughes Medical Institute. M. L. L. was supported, in part, by an NIH Genetics Predoctoral Training Grant to the University of Utah.

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(Accepted 11 September 1992)