

Homeotic genes have specific functional roles in the establishment of the *Drosophila* embryonic peripheral nervous system

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Summary

The *Drosophila* embryonic peripheral nervous system (PNS) contains segment-specific spatial patterns of sensory organs which derive from the ectoderm. Many studies have established that the homeotic genes of *Drosophila* control segment specific characteristics of the epidermis, and more recently these genes have also been shown to control gut morphogenesis through their expression in the visceral mesoderm (Tremml, G. and Bienz, M. (1989), *EMBO J.* 8, 2677-2685). We report here the roles of homeotic genes in establishing the spatial patterns of sensory organs in the embryonic PNS.

The PNS was examined in embryos homozygous for mutations in the homeotic genes *Sex combs reduced* (*Scr*), *Antennapedia* (*Antp*), *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*) with antibodies that label specific subsets of sensory organs. Our results suggest that the homeotic genes have specific roles in establishing the correct spatial patterns of sensory

organs in their normal domains of expression. In addition, we also report the effects of ectopic expression of the homeotic genes *labial* (*lab*), *Deformed* (*Dfd*), *Scr*, *Antp* or *Ubx* on the normal development of sensory organs in the embryonic PNS. Interestingly, while previous studies have concluded that ectopic expression of the homeotic genes *Dfd*, *Scr* and *Antp* has no effect on the segmental identity of the abdominal segments, our results demonstrate that this is not true. We show that ectopic expression of these genes does result in the disruption of the developing PNS in the abdomen. Our results are suggestive of a role for the homeotic gene products in regulating genes which are necessary for generating sensory progenitor cells in the developing PNS.

Key words: homeotic genes, peripheral nervous system, sensory organs, ectopic expression, *Drosophila* embryo.

Introduction

The *Drosophila* embryonic PNS develops in a stereotypical manner and contains a wide variety of sensory organs. A typical sensory organ consists of a neuron(s) and three support cells which will form the sensory structure innervated by the neuron (Hertweck, 1931; McIver, 1985; Zacharuk, 1985; Hartenstein, 1988). The entire embryonic PNS has been characterized in such detail that the positions of each sensory neuron and the associated sensory structure can be identified in both the thoracic and abdominal segments (Campos-Ortega and Hartenstein, 1985; Dambly-Chaudière and Ghysen, 1986; Bodmer and Jan, 1987; Hartenstein, 1988). There are two types of sensory organs in the embryonic PNS: external sensory (es) organs which are believed to sense mechanical and chemical stimuli and chordotonal organs (ch), proprioceptors that are proposed to sense stretch (McIver, 1985; Zacharuk, 1985; Dambly-Chaudière and Ghysen, 1986; Bodmer et al., 1987).

The es organs are composed of at least one neuron which has a single dendrite and three support cells. The support cells are the thecogen, trichogen and tormogen which respectively form the sheath around the dendrite,

the sensory process (bristle), and the circular socket of the sensory organ. The tormogen and trichogen cells are located in the epidermis, while the neuron(s) and thecogen are located beneath the epidermis. A scolopidium, the basic unit of ch organs, consists of four cells: a neuron with a single dendrite, the scolopale cell, cap cell, and ligament cell. The scolopale cell forms a sheath around the dendrite, while the cap cell and ligament cell mediate the attachment of the ch organ to the body wall. The prothoracic lateral triscolopodial (lch3) and abdominal pentascolopodial (lch5) organs have an additional pair of attachment cells at the apical end of the structure (Matthews et al., 1990; Ghysen and O'Kane, 1989; J. Heuer, unpublished observations, Fig. 1). All cells of ch organs are located beneath the epidermis.

It has been proposed that all cells comprising the es and ch sensory organs are lineally related (Bodmer et al., 1989) and arise through divisions of ectodermal precursor cells close to their final position (Bate, 1978; Bodmer et al., 1989). Despite their basic similarity, there are several characteristics which allow one to distinguish between es and ch organs including morphology, antigenic properties, division patterns and

Fig. 1. Schematic representation of the neurons and support cells in the PNS of the prothoracic (T1), meso- and metathoracic (T), and first through the seventh abdominal (A) segments. Moderately anti-Cut stained cells are colored yellow, while darkly anti-Cut stained cells are colored red. Cap and ligament cells of ch organs stained with the P12 α 85E antibody are colored blue. (Circle) es organ cell; (oval) ch organ cell; (diamond) neuron with dendritic arborization; (square) neuron with bipolar dendrites; (triangle) neuron with dendrites that arborize around tracheal branches; lines correspond to dendrites and axons (Bodmer and Jan, 1987). Much of this schematic has been redrawn from the diagram in Blochliger et al., 1990. Nomenclature is according to Dambly-Chaudière and Ghysen (1986).

Fig. 2. Detection of ch organs with the P12 α 85E antibody in 12–14 hour wild type and homeotic mutant embryos. Orientation of embryos in all figures is anterior to the left and dorsal is up. (A) Ore R embryo. In T1, the dch3 (small arrowhead) and lch3 (large arrowhead) are denoted. In T2 and T3 (T), the dch3 is marked (large arrowheads), while in A1–A7 (A) the lch1 (small arrowhead), lch5 (large arrowhead), and vchB (arrow) are denoted. (B) A homozygous *Scr*⁴ embryo. Note the transformation of the lch3 to a dch3 (arrow) in T1. (C) A homozygous *Antp*^{w10} embryo. Ectopic ch organs (arrowheads) frequently appear in the ventral parts of T2 and T3. (D) A homozygous *Ubx*¹⁰¹ embryo. The dch3 in T2 and T3 is transformed to a lch3 (small arrowheads), while a vch1 (arrow) replaces the vchA and vchB in A1. The lch5 (large arrowhead) in A1 is normal, yet the lch1 is absent. (E) A homozygous *abd-A*^{M1} embryo. Note the transformation of the lch5 to a dch3 (arrows) in the abdominal segments while the lch1 (small arrowhead) and vchB (large arrowhead) in the abdominal segments are normal. (F) A homozygous *Ubx*¹⁰⁹ embryo. The dch3 of T2 and T3 is transformed to a lch3, while the ch organs of the abdominal segments are replaced by a lch3 (arrowheads) and a vch1.

Fig. 3. Staining of es organs in 12–14 hour wild type and homeotic mutant embryos with the F2 antibody. Anterior is left and dorsal is up. (A) Ore R embryo. Note differences in the spatial arrangement of es organs in the T1, T, and A patterns. The dorsal (small arrowheads) and lateral clusters (large arrowheads) of es organs are

denoted. (B) Another Ore R embryo at a higher magnification illustrates the different spatial patterns of es organs in the thoracic and abdominal segments. The dorsal, lateral and ventral clusters are labeled in each pattern. (C) A homozygous *Scr*⁴ embryo. Note that the lateral (long arrow) and dorsal clusters (wide arrow) of the T1 pattern are altered and resemble the spatial arrangement of the T pattern, while the v' cluster (arrowhead) is normal. (D) Same embryo as in C at a higher magnification. Open arrow points to the lateral cluster and the closed arrow points to the dorsal cluster, both of which appear to be transformed toward the spatial patterns typical of the T2 and T3 segments. (E) A homozygous *Antp*^{w10} embryo. Note the loss of es organs in the lateral clusters of T2 and T3 (large arrowheads) and in the ventral clusters of T1–T3 (small arrowheads). The anterior spiracle is also malformed (open arrow). (F) Same embryo as in E at higher magnification. Both the lateral clusters (arrowheads) and ventral clusters (closed arrows) are missing es organs and the anterior spiracle (open arrow) is dramatically reduced in size.

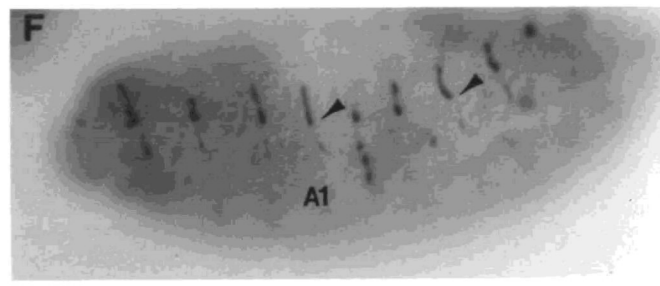
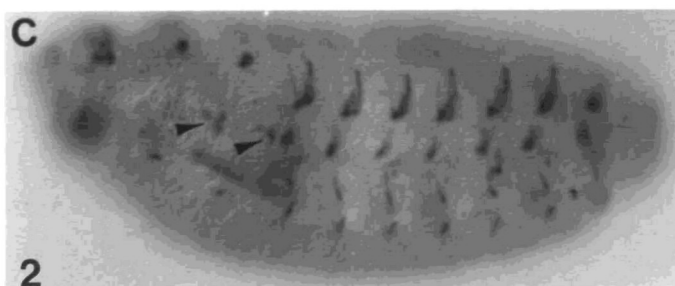
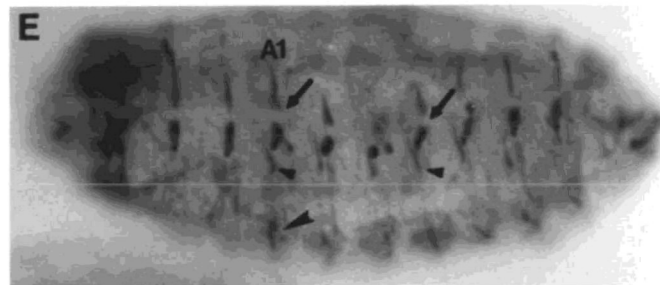
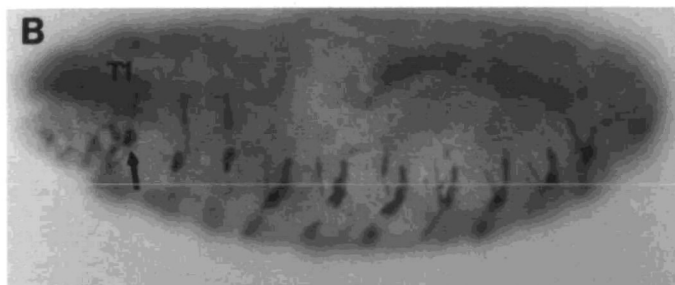
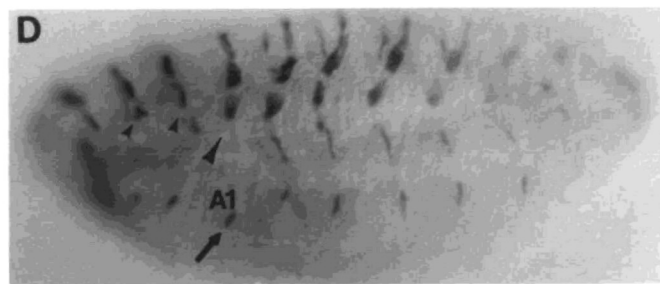
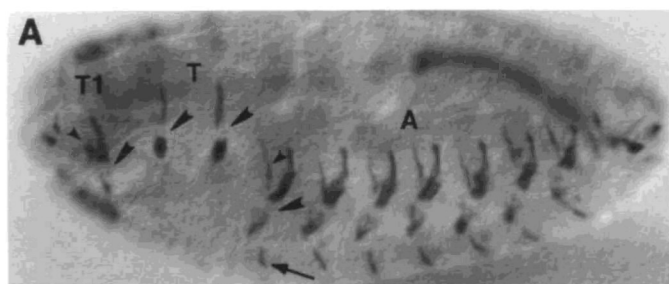
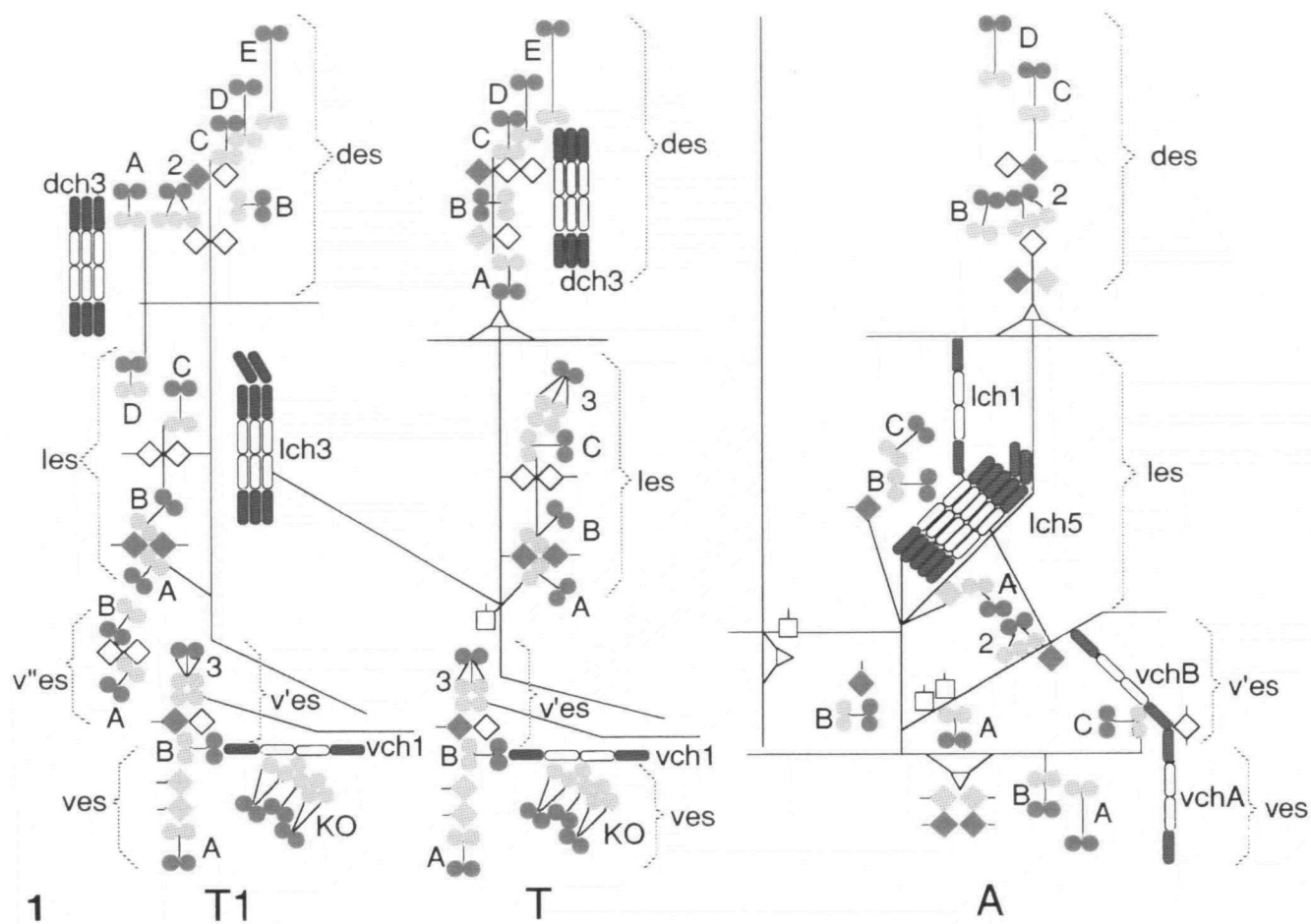
Fig. 4. Staining of es organs in 12–14 hour homeotic mutant embryos with the F2 antibody. Anterior is left, dorsal is up. (A) A homozygous *Ubx*¹⁰¹ embryo. Note the appearance of two additional anterior spiracles (short arrows) and the transformation of the spatial pattern of es organs in A1 (long arrow) into a pattern resembling that of T2 and T3. (B) A homozygous *Ubx*¹⁰⁹ embryo. There are additional anterior spiracles in the thoracic and abdominal segments (wide arrows) and the spatial arrangement of es organs in A1–A7 resembles the pattern characteristic of T2 and T3. (C) Double staining of an Ore R embryo with the F2 and P12 α 85E antibodies allows for both es and ch organs to be detected. The es organs are labeled brown and the ch organs are black. In A1–A7, the lch1 (small arrowhead), lch5 (large arrowheads), vchB (open arrow) and vchA (closed arrow) are denoted. (D) A homozygous *abd-A*^{M1} embryo double stained with the F2 and P12 α 85E antibodies. Note that the lch5 of the abdominal segments is transformed to a dch3 (large arrowheads), while the pattern of es organs in the abdominal segments (triangle) is normal. The lch1 (wide arrow), vchB (open arrow) and vchA (closed arrow) are also normal.

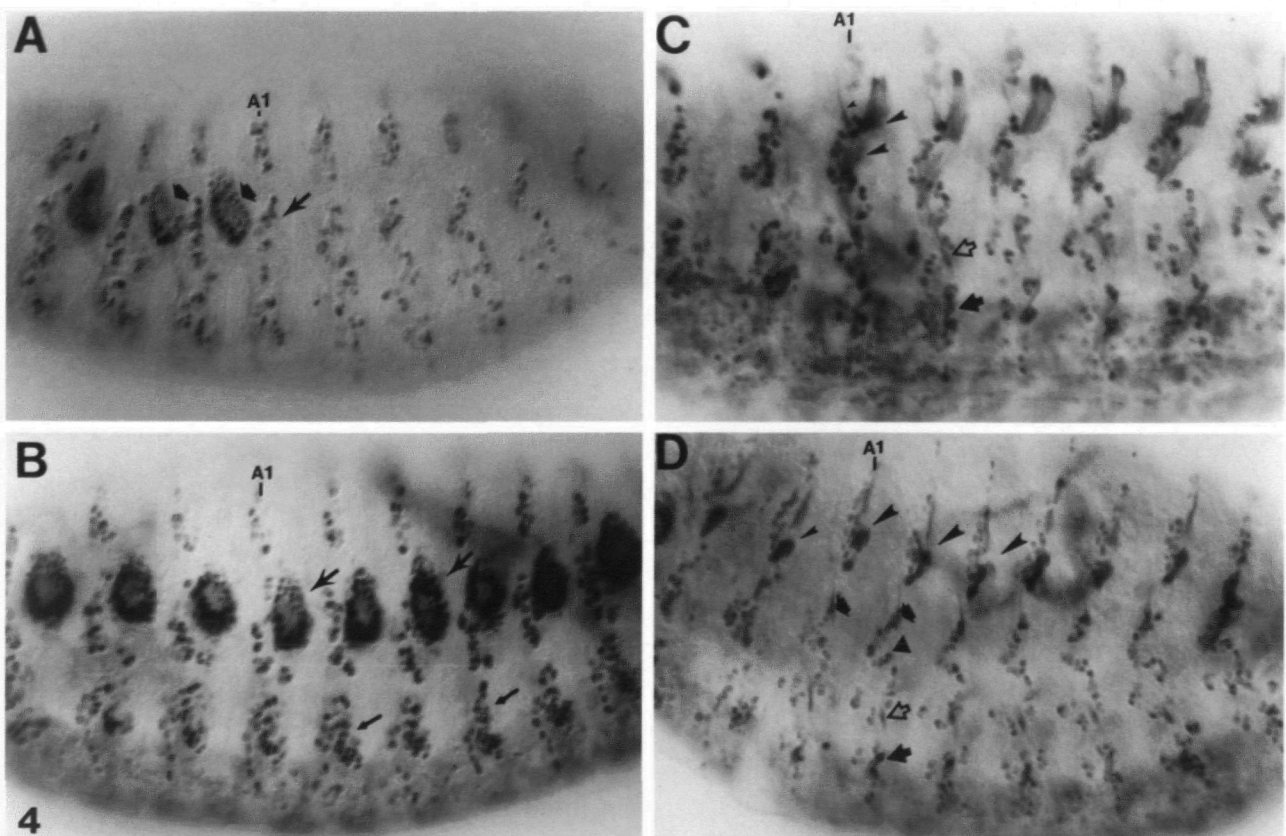
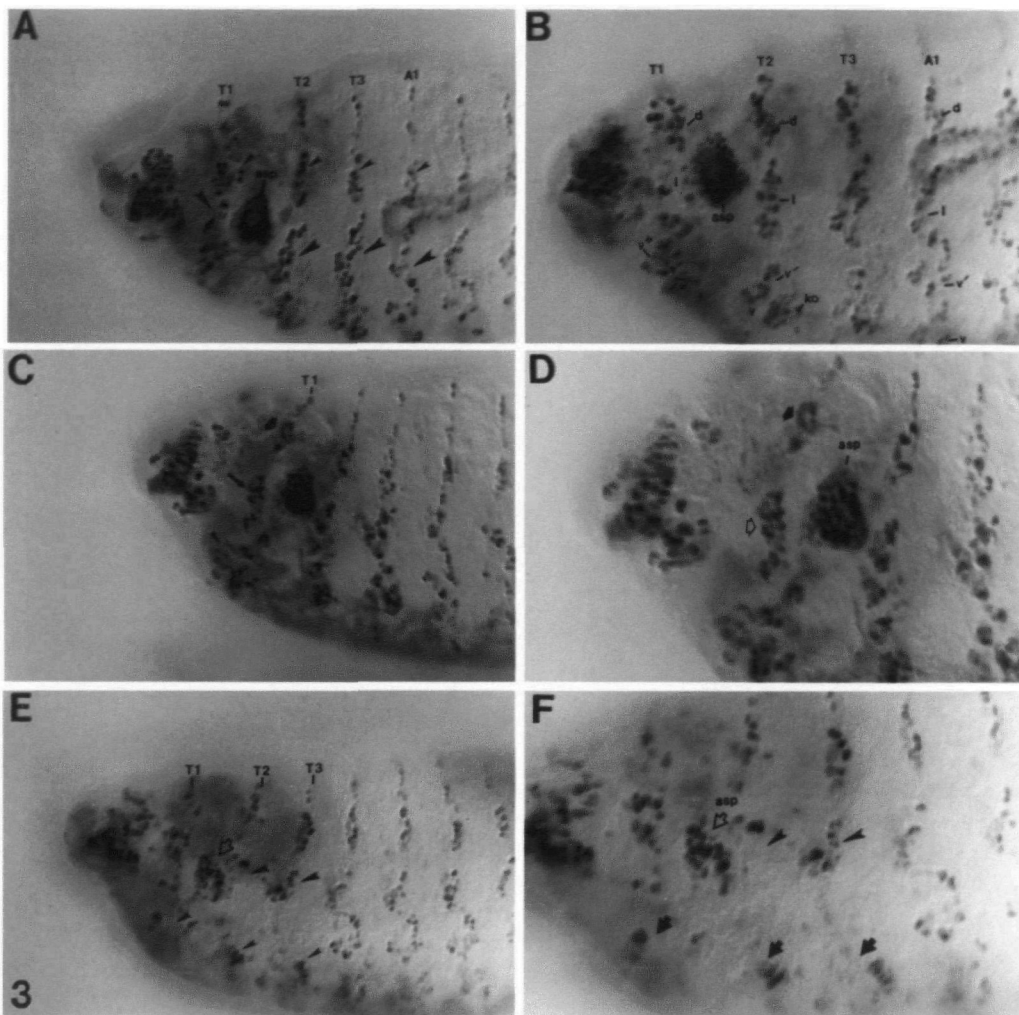
their final position with respect to the cuticle (Bodmer et al., 1987; Bodmer et al., 1989).

There are five distinct spatial patterns of sensory organs in the trunk of the segmented *Drosophila* embryo. Unique patterns are present in: (1) the prothoracic segment (T1), (2) the meso- and metathoracic segments (T), (3) the first through the seventh abdominal segments (A), (4) the eighth abdominal segment (A8) and (5) the ninth abdominal segment (A9) (Campos-Ortega and Hartenstein, 1985; Dambly-Chaudière and Ghysen, 1986). In *Drosophila*, extensive research has shown that alternate segmental identities realized in the epidermis are controlled by the homeotic genes (Lewis, 1963, 1978; Mahaffey and Kaufman, 1987a and reviews by Akam et al., 1988 and Kaufman et al., 1989). Mutations in the homeotic genes have been shown to result in improper head involution and the transformation or "homeosis" of cuticular (epidermal) structures of one or more metameric segments towards

the identity of another segment (Lewis, 1963, 1978; Sanchez-Herrero et al., 1985; Kaufman et al., 1989). The observation that there are distinct spatial patterns of sensilla in the embryonic PNS suggests that the homeotic genes may also be responsible for these differences.

There are two clusters of homeotic genes in the *Drosophila* genome: the Antennapedia complex (ANT-C) and the Bithorax complex (BX-C) (Kaufman et al., 1980, 1989; Lewis, 1978). The ANT-C contains the homeotic genes *labial* (*lab*), *proboscipedia* (*pb*), *Deformed* (*Dfd*), *Sex combs reduced* (*Scr*) and *Antennapedia* (*Antp*) which are required for head and anterior thoracic development, while the BX-C contains the homeotic genes *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*) which specify posterior thoracic and abdominal identity. All of the homeotic genes are required for both adult and larval development with the exception of *pb*, which is





Figs 3 and 4. For legends see p. 36

Fig. 6. Transformation of thoracic sensory organs caused by ectopic expression of *Ubx* protein. Anterior is left, dorsal is up. (A) A 12-14 hour Ore R embryo double immunostained with the BP104 (brown) and P12a85E (black) antibodies. Note the presence of a *dch3* in T3 (open arrow), the *lch5* (arrowhead) and *lch1* (closed arrow) in the abdominal segments. *vnc*, ventral nerve cord. (B) A 12-14 hour *hsUbx* embryo given a one hour heat shock at 4 hours AEL and double stained with the BP104 (brown) and P12a85E (black) antibodies. There are now *lch1* (open arrows) and *lch3* organs (closed arrows) in the thoracic segments instead of a *dch3*. The *ch* organs in the abdominal segments are normal. (C) A 12-14 hour *hsUbx* embryo administered a one hour heat shock at 4 hours AEL and immunostained with the F2 antibody. The spatial patterns of *es* organs in T1 (open arrow) and T2 and T3 (closed arrows) are transformed toward the pattern in A1. *vnc*, ventral nerve cord.

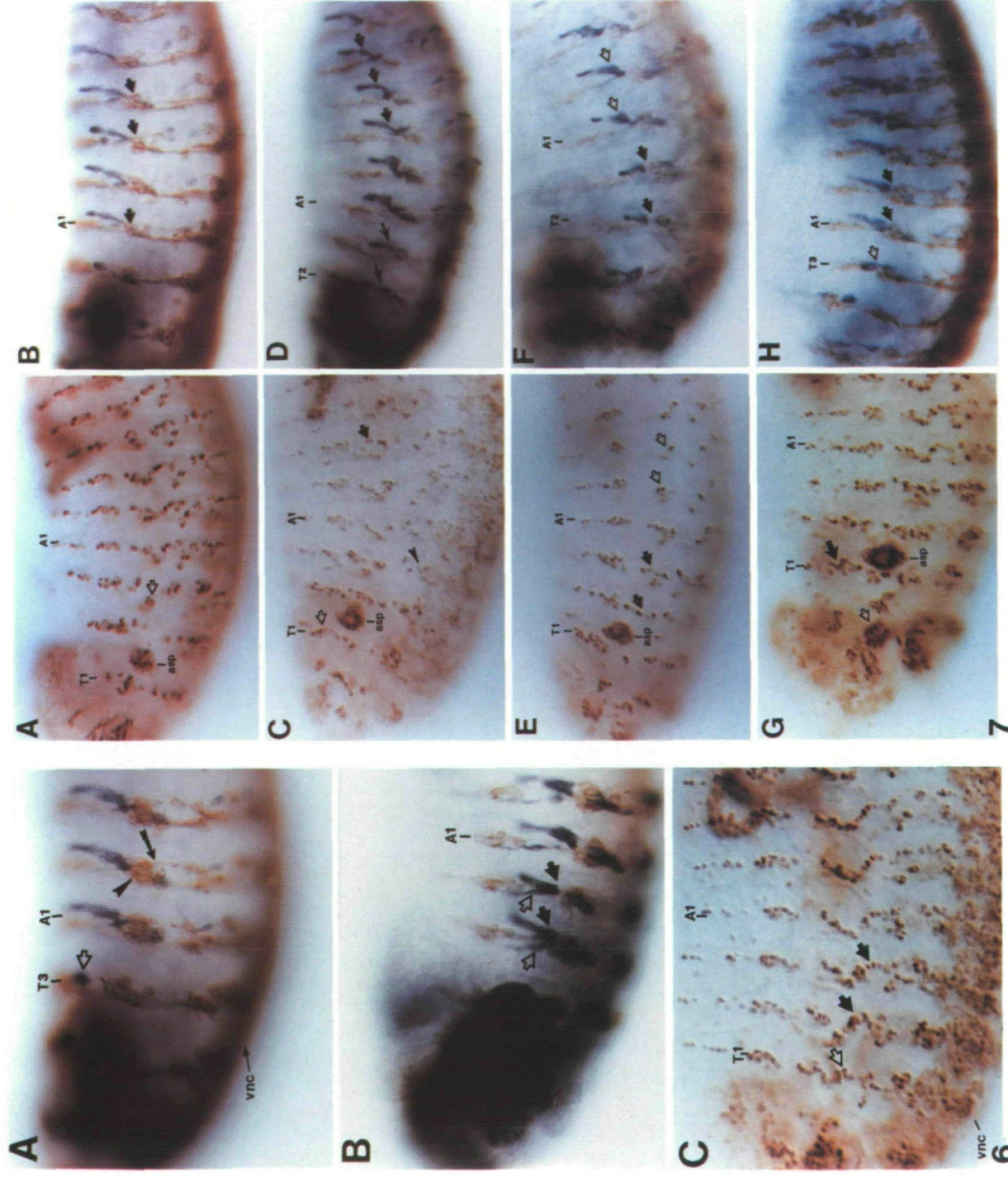


Fig. 7. Ectopic expression of homeotic proteins causes abnormalities in sensory organs. 12-14 hour embryos from the various *hs* lines were administered a 1 hour heat shock at 3 hours AEL and then stained with either the F2 antibody (A, C, E and G) or double stained with the P12a85E (black) and BP104 (brown) antibodies (B, D, F and H). Anterior is left, dorsal is up. (A and B), *hslab*; (C and D), *hsDfd*; (E and F), *hsScr* and (G and H), *hsAnip*. (A) The spatial patterns of *es* organs in the thoracic segments are abnormal. Note the presence of an ectopic *es* organ in T3 (open arrow) and the loss of *es* organs in the dorsal cluster of T1. The anterior spiracle (*asp*) is also reduced in size. (B) The *lch5* organs of the abdominal segments are transformed to mono- or discoloripodia (arrows). (C) Note the abnormalities in the spatial distribution of *es* organs particularly in T1 (open arrow), T3 (arrowhead) and A3 (closed arrow). The anterior spiracle (*asp*) is also reduced in size. (D) There are *lch3* organs in T2 and T3 instead of *dch3* organs (thin arrows) and the *lch5* organs in some of the abdominal segments are transformed to monoscolopidia (wide arrows). (E) There appear to be fewer *es* organs in T2 and T3 (closed arrows) and also a reduction of *es* organs in the abdominal segments (open arrows). The anterior spiracle (*asp*) is also malformed. (F) Note the presence of *lch3* organs in T2 and T3 (closed arrows) instead of *dch3* organs and the *lch5* organs in the

abdominal segments are transformed to mono- or discoloripodia (open arrows). (G) The dorsal cluster of T1 has an altered spatial pattern (closed arrow), while the patterns of *es* organs in T2, T3 and the abdominal segments are normal. The anterior spiracle (*asp*) is normal, yet an ectopic anterior spiracle has formed anterior to the normal one (open arrow). (H) The *dch3* of T3 is present in a more lateral position than normal (open arrow), while the *lch5* organs in A1 and A2 are transformed to tri- and tetrascopipodia (closed arrows).

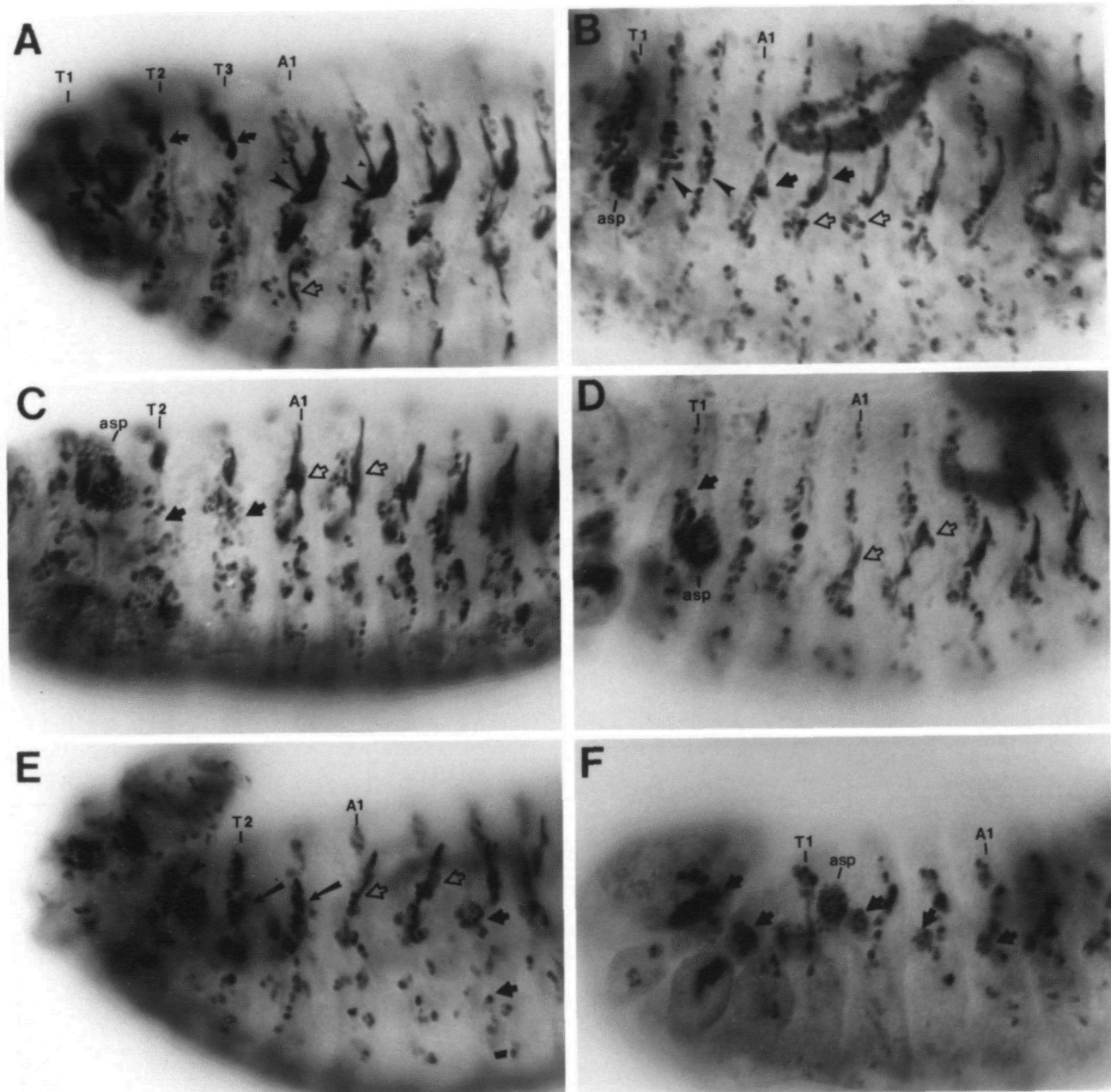


Fig. 8. Detection of both es and ch organs with the F2 and P12 α 85E antibodies in 12-14 hour embryos in which the homeotic proteins were ectopically expressed via a 1 hour heat shock administered at 4 hours AEL. Anterior is left, dorsal is up. The es organs are labeled brown and the ch organs are black. (A) An *Ore R* embryo. The curved arrows in T2 and T3 point to the dch3 organs, while the lch5 (large arrowhead), lch1 (small arrowhead) and vchB (open arrow) are denoted in the abdominal segments. (B) A *hsScr* embryo. While there are now lch3 organs in T2 or T3, there is not a concomittant decrease in the number of es organs in the lateral clusters of these segments (arrowheads). In the abdominal segments, although the lch5 consists of only 1 scolopidium instead of 5 (closed arrows), there is not an increase in the number of es organs in the lateral cluster adjacent to the lch5 (open arrows). (C) A *hsIab* embryo. Although the dch3 organs in T2 or T3 are located in a more lateral position than normal, there is not a similar decrease in the number of es organs in the lateral clusters (closed arrows). There is a reduction in the number of scolopidia in the lch5 of A1 and A2 (open arrows), but not a similar increase in the number of anti-Cut stained cells in the lateral clusters of these segments. (D) A *hsAntp* embryo. The loss of es organs in the dorsal cluster of T1 (closed arrow) does not result in the development of an additional ch organ in that position. Although the lch5 has a reduced number of scolopidia in the abdominal segments (open arrows), there are not additional es organs developing in that area. (E) A *hsDfd* embryo. There is an absence of additional es organs in the abdominal segments where the lch5 is abnormal (open arrows) while in the thoracic segments, there is not a loss of es organs in the lateral cluster where a lch3 develops (thin closed arrows). There is also not an additional ch organ developing in T1 where there is a loss of es organs (arrowhead). (F) A *hsAntp* embryo. Note the appearance of clusters of anti-Cut staining cells in segments anterior and posterior to T2 which resemble developing anterior spiracles (arrows). asp, anterior spiracle.

required only for adult development (Pultz et al., 1988). The homeotic genes of *Drosophila* are believed to encode transcription factors containing a 60 amino acid motif called the homeodomain (McGinnis et al., 1984; Scott and Weiner, 1984) that has been shown to bind DNA in vitro (Müller et al., 1988; Desplan et al., 1985; Hoey and Levine, 1988). It has been proposed that the protein products of these genes elicit segmental identity through the activation of a genetic program involving downstream "realisator" genes which impart segment-specific characteristics (García-Bellido, 1977).

If the specification of the PNS requires the action of the homeotics in a similar fashion to their role in the establishment of epidermal identity, an important question involves the time in development during which the homeotic genes influence PNS development. Moreover, the nature of the involvement of the homeotics in PNS specification is constrained by how the PNS develops relative to the known spatiotemporal patterns of homeotic gene expression. The formation of the *Drosophila* embryonic PNS from the ectoderm occurs between 5 and 9 hours of embryogenesis and is dependent on the action of several classes of genes (for a review, see Jan and Jan, 1990) such as the prepattern, proneural and neurogenic genes. The prepattern genes include the segmentation genes which provide positional information and divide the embryo into segmental units. The proneural genes include members of the *achaete-scute* complex (AS-C) and the *daughterless* (*da*) gene. These loci are expressed early in the ectoderm and control the expression of genes necessary for the commitment and differentiation of neuronal precursor cells (Ghysen and Dambly-Chaudière, 1989). Mutations in proneural genes result in a deficit of sensory organs (Dambly-Chaudière and Ghysen, 1987; Caudy et al., 1988). As a neuronal precursor cell develops, it inhibits its neighbors from becoming neuroblasts through the action of the neurogenic genes (Lehmann et al., 1983). This group includes *Notch* (*N*) and *Delta* (*DI*), which encode transmembrane proteins with EGF-like repeats (Wharton et al., 1985; Kopczynski et al., 1988), and are probably involved in a cell-cell interaction pathway. Mutations in the neurogenic genes result in a hypertrophy of the PNS and CNS at the expense of the epidermis (Hartenstein and Campos-Ortega, 1986).

Mutations in the homeotic genes have also been demonstrated to result in defective PNS development. For example, previous reports describing defects in sensilla of the embryonic PNS for the homozygous mutant *bithoraxoid* (*bxd*) (Hartenstein, 1987) and effects of homeotic mutations on the central projections of sensory neurons in adult flies (Ghysen et al., 1983) provide evidence for a role in PNS development for the homeotic genes. Furthermore, the observation that homeotic gene products accumulate in ectodermal cells prior to the appearance of the first sensory progenitor cells (Ghysen and O'Kane, 1989) suggests that the homeotic genes may influence early events in PNS development. The goal of this study was to further define the roles of the homeotic genes in embryonic PNS development. This was accomplished by examin-

ing the embryonic PNS with cell specific markers in homozygous mutants for the homeotic genes *Scr*, *Antp*, *Ubx*, *abd-A* and *Abd-B*. In addition, ectopic expression of the homeotic genes *lab*, *Dfd*, *Scr*, *Antp* or *Ubx* via an *hsp-70* promoter at various stages of development was employed to determine the effect of aberrant expression of these proteins on the normal development of sensory organs in the embryonic PNS.

Materials and methods

Fly stocks and heat shock lines

Flies were raised at 25°C on standard medium supplemented with baker's yeast. The wild-type *Drosophila melanogaster* stock used was Oregon R. The mutant stocks used were *Scr*⁴ red e/TM6B, which is a protein null for *Scr* (Wakimoto and Kaufman, 1981; Riley et al., 1987), *Antp*^{w10} red e/TM6B, which accumulates no detectable *Antp* protein (Wakimoto and Kaufman, 1981; Carroll et al., 1986), *Sb/In(3LR)Ubx*¹⁰¹, a *Ubx* breakpoint mutation (Hayes et al., 1984), *abdA*^{M1}/TM1, which is a protein null for *abd-A* (Sanchez-Herrero et al., 1985; Macias et al., 1990), *Abd-B*^{Mx2}, which produces no detectable *Abd-B* protein in parasegments 10-12 (Sanchez-Herrero et al., 1985; Celniker et al., 1990), and *Df(3R)Ubx*¹⁰⁹/Dp(3,3)P5, *Sb*, which is deficient for the *Ubx* and *abd-A* transcription units (Lewis, 1978; Karch et al., 1985). Heterozygous parents were crossed to produce populations of embryos in which, on average, 25% of the animals were homozygous for the mutation of interest. Between 25-50 homozygous mutant embryos were immunostained with each antibody for PNS analysis.

The transformant line *hsDfd58*, which carries a *Dfd* cDNA under control of the *hsp70* promoter, was kindly provided by W. McGinnis (Kuziora and McGinnis, 1988), while the transformant line *hsUbx1a22*, which contains a *Ubx* cDNA under control of the *hsp70* promoter, was kindly provided by D. Hogness (Mann and Hogness, 1990). The transformant line *hslab* was generated by placing genomic fragments of the *labial* gene containing the coding sequences and second intron behind the *hsp70* promoter in the P element vector CoSpeR *NotI* (S. Chouinard, personal communication) which contains the *white* gene as a visible marker (J. Tamkin, personal communication). The transformant lines *hsAntp* and *hsScr* were generated by cloning the *Antp* cDNA G1100 (Scott et al., 1983) and an *Scr* cDNA (Mahaffey and Kaufman, 1987b) downstream of the *hsp70* promoter in the P element vector CaSpeR (F. Randazzo, personal communication), which contains the *white* gene as a visible marker (Pirrotta, 1988). Standard P-element mediated germline transformation techniques were used for obtaining independent insertion lines of the *hslab*, *hsAntp* and *hsScr* constructs (Robertson et al., 1988). The *hslab* line used in these experiments has the P-element insertion on the X chromosome and is homozygous viable. The *hsAntp* line has the P-element insertion on the third chromosome and is balanced over TM3, while the *hsScr* line has the P-element insertion on the X chromosome and is homozygous viable.

Antibodies and immunostaining

The collection, fixation and staining of embryos was performed as described previously (Mahaffey and Kaufman, 1987b). Double staining of embryos was performed as described in Matthews et al (1990). The anti-*lab* antibody was generated as described in Diederich et al (1989). The anti-*Dfd* and anti-*Scr* antibodies were generated as described in

Mahaffey et al. (1989). The hybridoma line 8C11.1 (anti-*Antp*) (J. Condie and D. Brower, unpublished results) and the hybridoma line FP3.38 (anti-*Ubx*) (White and Wilcox, 1984) were kindly provided by D. Brower and M. Muskavitch respectively. The supernatant from the hybridoma line BP104 (anti-neuroglian) was kindly provided by C. Goodman and is described by Hortsch et al. (1990). The F2 anti-Cut antibody was kindly provided by Y. Jan and is described by Blochlinger et al. (1990). The anti- α -tubulin 85E antibody (P12 α 85-E) was kindly provided by K. Matthews and is described by Matthews et al. (1990). The antibodies 8C11.1, FP3.38 and BP104 were used as hybridoma supernatants at dilutions of 1:50, 1:100 and 1:5 respectively. The other antibodies were affinity purified polyclonals derived from rabbits, with the exception of anti-Cut (rat). The anti-*lab*, anti-*Dfd*, anti-*Scr*, P12 α -85E and F2 antibodies were used at dilutions of 1:150, 1:150, 1:100, 1:100 and 1:500 respectively. The secondary antibodies used for immunostaining were goat anti-rabbit conjugated to HRP (Sigma) and goat anti-mouse (H+L) conjugated to HRP (Sigma). Both secondary antibodies were used at a dilution of 1:200. Stained embryos were examined and photographed using Nomarski optics with a Zeiss Axiophot. Photographs were taken with Kodak Kodacolor Gold color print film with a blue filter in place for color correction and a tungsten light source.

Heat shock experiments

Embryos were collected on hard agar plates coated with a dab of yeast paste for one hour at room temperature. A precollection of embryos was obtained to ensure that adult females did not retain fertilized eggs. Two different heat shock regimes were employed. In the first, embryos were allowed to age at 25°C for either 3, 4, 6 or 7 hours after the collection period, before administering a one hour heat shock at 37°C (35°C for *hslab*) by immersing the plates in a water bath of the appropriate temperature. After the heat shock, the embryos were allowed to recover at 25°C for one hour or until they were about 12-14 hours of development. In the second heat shock regime, embryos were allowed to age for 3 hours at 25°C, given a one hour heat shock at the appropriate temperature, allowed to recover at 25°C for one hour, given a second heat shock for 30 min, allowed to recover at 25°C for one hour, given a third heat shock for 30 min, and placed at 25°C until they were about 12-14 hours of development. For each of the regimes, approximately 25-50 embryos were examined with each antibody for PNS defects and 20-50 embryos were examined for cuticular transformations.

Cuticle preparations

Following the heat shock period, embryos were allowed to age until about 24-26 hours of development and then washed several times. The embryos were dechorionated in 50% bleach, followed by several washes, and then devitellinized in heptane and methanol. The embryos were then rehydrated in PBS, placed on a microscope slide and excess PBS was blotted away. A drop of polyvinyl lactophenol was applied to the embryos and a coverslip was placed over them. The slides were then placed on a hot plate at 60°C for 24 hours. Cuticle preparations were examined and photographed under phase contrast optics on a Zeiss Axiophot.

Results

The patterns of embryonic sensory organs were examined in homozygous mutant embryos for the homeotic genes *Scr*, *Antp*, *Ubx*, *abd-A* and *Abd-B*, and in

embryos in which the homeotic genes *lab*, *Dfd*, *Scr*, *Antp* or *Ubx* were ectopically expressed via the *hsp-70* promoter. In addition, denticle belts in the cuticle were examined in embryos in which the homeotic genes were ectopically expressed. Analysis of the PNS was accomplished with antibodies which recognize the proteins Cut (F2), neuroglian (BP104) and α -tubulin 85E (P12 α -85E). These antibody markers allow for the detection of specific subsets of cells in the embryonic PNS. In order to identify definitively the PNS phenotype in protein null homeotic mutant embryos, double stains were performed with antibodies which recognize the homeotic proteins in conjunction with the antibodies which label cells in the PNS. For example, to identify the es organs in an *Scr*⁴ homozygous embryo, embryos derived from *Scr*⁴ *red e/TM6B* parents were double immunostained with anti-*Scr* and anti-Cut antibodies. The anti-Cut staining pattern was then carefully examined in embryos which did not stain with anti-*Scr* antibodies. Since antibodies which recognize *abd-A* and *Abd-B* were not available, the mutant phenotype was taken as that present in one quarter of the embryos analyzed.

The F2 antibody recognizes the protein product of the *cut* gene, which is required for es organ development (Bodmer et al., 1987), and is localized to the nucleus of both neuronal and support cells of es organs, but not in cells of ch organs (Blochlinger et al., 1990). The monoclonal antibody BP104 recognizes a nervous system-specific form of neuroglian, a member of the immunoglobulin superfamily (Bieber et al., 1989), which is localized to the cell surface of neurons in the central nervous system (CNS) and neurons and some support cells in the PNS (Hortsch et al., 1990). The P12 α -85E antibody specifically recognizes a cytoplasmic α -tubulin protein in the support cells of ch organs, but not es organs (Matthews et al., 1990). For a description of the embryonic expression patterns of *cut*, the nervous system-specific neuroglian and α -85E, see the papers by Blochlinger et al. (1990), Hortsch et al. (1990) and Matthews et al. (1990) respectively. The normal staining patterns for these antibodies are illustrated in Figs 1, 2A, 3A and 6A.

The pattern of sensory organs in a normal wild type embryo

The normal pattern of sensory organs has been fully described elsewhere (Campos-Ortega and Hartenstein, 1985; Dambly-Chaudière and Ghysen, 1986; Hartenstein, 1988), thus only a brief description will be given here. There are three major types of sensory organs in the *Drosophila* embryo: es and ch organs described previously, and multiple dendrite (md) neurons, which are probably proprioceptive sensory cells that contain a single neuron with numerous dendrites and a single support cell. As mentioned previously, the sensory organs in the trunk are segmentally arranged according to five patterns (see Introduction). The segmental patterns of the sensory organs for the prothorax (T1), meso- and metathorax (T) and abdominal (A) metameres are illustrated in Fig. 1. The majority of sensory

organs, in any segment, reside in the anterior compartment, while the posterior compartment contains only ch organs laterally and dorsally (lch3 of T1, dorsal triscolopodial organs (dch3) of T2 and T3, lch5 of A1-A7) (Hartenstein, 1987). This study will focus on the ch and es organs in the T1, T (T2,T3) and A (A1-A7) patterns only, as the sensory organs in the A8, A9 and head segments were difficult to identify. Within these segmental patterns, the cell bodies of the sensory organs are conspicuously arranged in four clusters from the most ventral to the most dorsal: v, v', v'' (T1 only), l and d. Peripheral neurons are identified by: (1) their segmental pattern (T1-A9), (2) cluster (v-d), (3) nature (es, ch or md), and (4) position within a cluster from ventral to dorsal (A, B, C, D, E).

In a normal embryo, staining with the antibody P12 α -85E reveals three sets of ch organs in the T1 pattern: a ventral monoscolopodial organ (vch1), a lch3 and a dch3 (Fig. 2A). Differences between the dch3 and lch3 in T1 include their location, axonal projections and number of attachment cells. While the axons from most ch organs in the embryo project into the segmental nerve of the segment in which they are located, the axons from the lch3 in T1 cross the segmental boundary and join with the T2 segmental nerve (Ghysen et al., 1986). There are two sets of ch organs in the T pattern: a vch1 and a dch3 (Fig. 2A). The A pattern is characterized by ventral monoscolopodial organs A and B (vchA, vchB), a lateral monoscolopodial organ (lch1) and a lch5 (Figs 2A, 4C). The es organs of the PNS are derived from neuronal precursors which are different from those giving rise to ch organs (Ghysen and O'Kane, 1989). Immunostaining of normal embryos with the F2 antibody reveals es organs present in three unique patterns in the trunk: T1, T and A (Figs 1, 3A, B). The major differences between the T1 and T patterns are the presence of a v'' cluster in T1 and the positions and types of es organs in both the lateral and dorsal clusters. The spatial distribution of es organs in the A1-A7 metameres is also easily distinguished from the patterns of es organs in the thoracic segments (Fig. 1, 3A, B).

PNS defects in embryos homozygous for mutant Scr, Antp, Ubx, abd-A and Abd-B alleles

Loss of function mutations in the homeotic genes result in the transformation of some sensilla and the development of ectopic sensilla as revealed with the P12 α 85E, BP104 and F2 antibodies. Similar to certain cuticular defects observed in homeotic mutant embryos, defects in sensilla also show variable penetrance and expressivity, thus only general descriptions are given here. The effects of homeotic mutations on the normal patterns of sensilla are summarized in Table 1.

Previous studies have indicated that in the absence of *Scr* function, T1 is transformed to a more posterior thoracic identity (Wakimoto and Kaufman, 1981; Sato et al., 1985; Pattatucci et al., 1991). The defects in sensilla of *Scr*⁴ embryos follow this type of transformation (Figs 2B, 3C,D). Interestingly, while there is 100% transformation of the lch3 in T1 to a dch3, in about 50% of *Scr*⁴ embryos the axonal projection of this transformed sensory organ still crosses the T1/T2 segmental boundary and joins the axons of the T2 segmental nerve as in the wild-type embryo (data not shown).

In *Antp*^{w10} embryos, cuticular features indicate that T2 and T3 are transformed towards a more anterior thoracic identity (Wakimoto and Kaufman, 1981). However, the defects in sensilla of *Antp*^{w10} embryos do not follow this type of transformation. Instead, there is no apparent homeotic transformation, yet there is abnormal development of many sensilla in all the thoracic segments. Specifically, the positions of the dch3 organs in T2 and T3 with respect to the cuticle are slightly abnormal in about 50% of mutants and ectopic monoscolopodia frequently appear in ventral positions in T2, T3 or both (Fig. 2C). A particularly interesting observation is that while the position of the lch3 in T1 is normal in *Antp*^{w10} embryos, double immunostaining with P12 α 85E and BP104 reveals that the axons from the lch3 do not cross over to T2, but appear to join the T1 segmental nerve (data not shown). The es organs of the thoracic segments are also affected by the loss of

Table 1. Specification of sensory organs by homeotic genes

Protein	Function in epidermis	Function in PNS
Sex combs reduced (<i>Scr</i>)	Gives PS2 and PS3 identity	Gives T1 pattern of es organs Required for lch3 morphology
Antennapedia (<i>Antp</i>)	Required for PS4 identity Contributes to PS5	Necessary for many thoracic es organs Required for lch3 axonal projection Contributes to ch organs in T2 and T3
Ultrabithorax (<i>Ubx</i>)	Confers PS6 identity upon epidermis Contributes to PS5 also	Required for A pattern of es organs in A1 Required for dch3 in T2 and T3 Required for lch1, vchA, and vchB in A1 Contributes to es and ch organs in A2-A7
abdominal-A (<i>abd-A</i>)	Required for PS7-PS13 identity	Required for lch5 in A1-A7 Contributes to es and ch organs in A2-A7
Abdominal-B (<i>Abd-B</i>)	Required for PS10-PS14 identity	No function in A5-A7

Note: A parasegment consists of the posterior compartment of one segment and the anterior compartment of the next segment. For instance, PS3 consists of the posterior compartment of the labial segment and the anterior compartment of the prothoracic segment (T1).

PS, parasegment; es, external sensory, ch, chordotonal; lch3, lateral triscolopodial organ; dch3, dorsal triscolopodial organ; lch1, lateral monoscolopodial organ; lch5, lateral pentascolopodial organ; vchA and B, ventral monoscolopodial organ A and B; T, thoracic, A, abdominal.

Antp function. The anterior spiracle is dramatically reduced and malformed, and Keilins organs are incomplete in all three segments (Fig. 3E, F). There are losses of es organs in the v', l, and d clusters of all three thoracic segments, but the T2 segment is most severely affected in all cases (Fig. 3E, F).

The loss of *Ubx* function in the embryo has been previously shown to result in a transformation of anterior T3 and A1 towards a T2 identity and posterior T2 and T3 towards a T1 identity (Hayes et al., 1984; Sanchez-Herrero et al., 1985). The defects in sensilla observed in *Ubx¹⁰¹* embryos also follow this type of transformation (Figs 2D, 4A). Curiously, as observed in *Antp^{w10}* embryos, many *Ubx¹⁰¹* embryos also exhibit ectopic monoscolopodial organs located in a ventral position in T3, A1 or both.

In *abd-A^{M1}* embryos, previous studies have indicated that the A2-A7 metameres are transformed toward an A1 identity (Sanchez-Herrero et al., 1985). PNS defects in *abd-A* loss of function embryos follow this type of transformation for most sensilla in the abdominal segments (Figs 2E, 4D). However, the lch5 organs of the abdominal segments are transformed more anteriorly to a posterior thoracic identity (Figs 2E, 4D).

The loss of *Abd-B* function in *Abd-B^{MX2}* embryos has been shown to result in a transformation of A5-A7 towards an A4 identity (Sanchez-Herrero et al., 1985). There are no detectable defects in the sensilla of these embryos (data not shown). Nevertheless, it is possible that a similar transformation occurs in the PNS, since in normal embryos the sensilla in A5-A7 are indistinguishable from those in A4. Previous studies have also shown that the identity of the abdominal segments is transformed to a thoracic identity in *Ubx¹⁰⁹* embryos (Lewis, 1978) which are deficient in the *Ubx* and *abd-A* transcription units (Karch et al., 1985). Defects in the sensilla of these embryos follow the same type of transformation (Figs 2F, 4B).

Effects of ectopic expression of lab, Dfd, Scr, Antp or Ubx on the cuticle

The homeotic genes *lab*, *Dfd*, *Scr*, *Antp* or *Ubx* were ubiquitously expressed in embryos under the control of the *hsp 70* promoter by administering a heat shock at various times in development (see Materials and methods). As a way of verifying that the fly lines carrying these constructs were not subject to leaky expression of these genes, embryos were collected over a 16 hour time period without the administration of a heat shock and immunostained with the appropriate anti-homeotic protein antibody (i.e., *hslab* with the anti-*lab* antibody, *hsDfd* with the anti-*Dfd* antibody, etc...). All the hs lines exhibited normal patterns of immunostaining with the exception of the *hslab* and *hsUbx* lines, in which a small percentage of embryos showed some ectopic immunostaining (data not shown). However, the percentage of *hslab* and *hsUbx* embryos exhibiting aberrant expression is too small to account for the phenotypes observed following heat induction. As a further control, cuticles and the PNS were examined in non-heat shocked embryos from all

the hs lines and were found to be normal (data not shown). To determine whether ubiquitous expression of the homeotic protein was induced following a heat shock, embryos were administered a one hour heat shock, and then immunostained with the appropriate anti-homeotic protein antibody 30 minutes and one hour after the heat shock. In all the hs lines, the appropriate homeotic protein was detected in all cells of the embryo at both 30 minutes and one hour post heat shock (data not shown).

In order to confirm that the hs lines employed in this study exhibit the same cuticular phenotypes following heat induction that have been previously described (*hsDfd*, Kuziora and McGinnis, 1988; *hsAntp*, Gibson and Gehring, 1988; *hsScr*, Gibson et al., 1990; *hsUbx*, González-Reyes and Morata, 1990; Mann and Hogness, 1990), embryos were administered a one hour heat shock at various stages of development and cuticles were prepared for microscopy (described in Materials and methods). The cuticular transformations resulting from overexpression of the homeotic proteins are summarized in Table 2. The cuticular phenotypes observed for *hsDfd*, *hsScr*, *hsAntp*, and *hsUbx* are in agreement with the results previously reported (Fig. 5C-F). Since the phenotype of *hslab* has not been reported as yet, a description will be presented here.

During embryogenesis, the *lab* protein is normally expressed in epidermal cells of the procephalon in the intercalary segment, dorsal ridge of the head and also in endoderm cells of the midgut (Diederich et al., 1989). The *hslab* construct is unique in that it contains an intron, thus heat shocks were administered at 35°C to ensure that proper splicing of the RNA product was efficient (Yost et al., 1990). The cuticular phenotype of an embryo in which the *lab* protein was ectopically expressed is illustrated in Fig. 5B. The effects of aberrant *lab* expression include disruption of normal head involution and defects in the cephalopharyngeal skeleton. In particular, the lateralgräte is dramatically reduced or missing entirely and the dorsal bridge, dorsal arms, ventral plate, ventral arms and T-bars are malformed. The epistomal sclerite, ectostomal sclerite, labral sense organ and labrum are sometimes missing and sclerotized material is occasionally observed around the dorsal pouch area. While these structures are either missing or malformed, there are no apparent homeotic transformations. Four different heat shock regimes were employed (see Materials and methods) to determine the stage of development in which cuticular structures are most sensitive to aberrant expression of the *lab* protein (Table 2). The multiple heat shock regime seemed to give the most severe defects, while defects in the cephalopharyngeal skeleton were most common when the heat shock was administered at 4 hours after egg lay (AEL).

Effects of ectopic expression of lab, Dfd, Scr, Antp or Ubx on the development of sensory organs

As ectopic expression of the homeotic genes *lab*, *Dfd*, *Scr*, *Antp* and *Ubx* has been demonstrated to cause abnormalities or transformations in the segmental

Table 2. Effects of heat shock induced expression of homeotic proteins in epidermis and PNS

hs line	% transformation in Cuticle*			Cuticular phenotype	% Grossly abnormal in PNS*			Typical abnormalities in PNS†
	3	4	6		3	4	6	
Ore R	0	0	0	Wild type	0	0	0	None
hslab	29	50	21	CPS defects	50	50	5	T1-T3 es organs abnormal lch5 abnormal in some A segments dch3 partially transformed to lch3 in T2, T3
hsDfd	100	23	0	MH or MC in thoracic segments	100	100	7	Severe defects in es organs in T1-A7 dch3 in T2, T3 partially transformed to lch3 lch5 in A1-A7 abnormal
hsScr	10	21	80	T1 beard of denticles in T2 and T3	100	100	12	T2-A7 es organ abnormalities T2 and T3 dch3 partially transformed to lch3 lch5 in A1-A7 abnormal
hsAntp	65	65	80	T1 beard of denticles absent	73	78	8	Most severe defects in T1 es organs T2 or T3 dch3 occasionally in abnormal position
hsUbx	ND	75	72	A1 denticle belt in T1-T3	ND	75	12	A1 like pattern of es organs in T1-T3 lch1 in T1-T3 dch3 in T2, T3 partially transformed to lch3

*Percentages of abnormalities are listed for embryos administered a one hour heat shock at either 3, 4 or 6 hours AEL and are based on 25 embryos or cuticles

†For PNS analysis, embryos were double stained with the F2 and P12a85E antibodies. Only those embryos which showed obvious defects in both es and ch organs were scored as grossly abnormal. Phenotypic abnormalities in the PNS were different from embryo to embryo and thus only generalities are listed. Abnormalities indicate differences in the position and/or number of sensory organs relative to the wild type embryo. CPS, cephalopharyngeal skeleton; MH, mouth hooks, MC, maxillary cirri, T, thoracic, A, abdominal, ND, not determined, abbreviations for sensory organs are the same as listed in Table 1

identities of the embryonic epidermis, it was of interest to determine if ubiquitous expression of these proteins would lead to the same types of transformations in the PNS. To answer this question, embryos were collected from the hs lines, administered a one hour heat shock at various times in development, allowed to recover until the embryos were about 12-14 hours old, and then the PNS was analyzed with the F2, BP104 and P12a-85E antibodies. The data from these experiments are summarized in Table 2. As there were numerous abnormalities in the PNS of all the hs lines, and the phenotypes varied from one embryo to another, only a general description of the most consistent defects will be presented here. Major defects were observed in both es and ch organs of the PNS in all the hs lines when the heat shock was administered at 3 or 4 hours AEL, while heat shocks administered at 6 or 7 hours AEL caused only minor (mostly in the thoracic ch organs) or no abnormalities (Table 2). PNS abnormalities were never observed in any of the heat shocked Oregon R embryos, which were used as a control in all experiments.

Ectopic expression of *Ubx* has been reported to transform the identity of the thoracic and head segments towards that of an A1 identity (González-Reyes and Morata, 1990; Mann and Hogness, 1990). One of these studies also demonstrated that the PNS of a *hsUbx* animal also follows this type of transformation (Mann and Hogness, 1990). We used the same *hsUbx* line in this study with similar results (Fig. 6B, C). However, in addition, we find some defects in sensilla of the thoracic segments which indicate a partial

transformation of these segments toward an anterior thoracic identity. For instance, the dch3 organs in T2 and T3 are not transformed to a lch5 typical of the abdominal segments, but are partially transformed to a prothoracic lch3 (Fig. 6B). Interestingly, while the position and morphology of this sensory organ are characteristic of a prothoracic lch3, the axonal projection is not (i.e., it does not project to the next segmental nerve).

As ectopic expression of *Ubx* protein has been shown to alter segmental identity only in segments anterior to its normal domain of expression, previous studies have also indicated that this rule applies for ectopic expression of the *Antp* protein (Schneuwly et al., 1987; Gibson and Gehring, 1988). These studies demonstrated that ubiquitous expression of the *Antp* protein causes a failure in head involution and a clear transformation of prothoracic identity towards mesothoracic identity. The PNS defects observed in *hsAntp* embryos do not show any parallel homeotic transformations. However, while most sensilla in the abdominal segments are not affected, the lch5 organs in A1 and A2 are frequently transformed to triscopolidial or tetrascopolidial organs (Fig. 7H). In addition, there is no clear transformation of sensilla in T1 towards a T2 pattern, but rather the sensilla appear in abnormal numbers and positions with respect to the cuticle (Figs 7G, H, 8D, F).

While *hsAntp* embryos show a cuticular transformation of prothoracic identity towards mesothoracic identity (Gibson and Gehring, 1988), the reciprocal transformation occurs in *hsScr* embryos, namely,

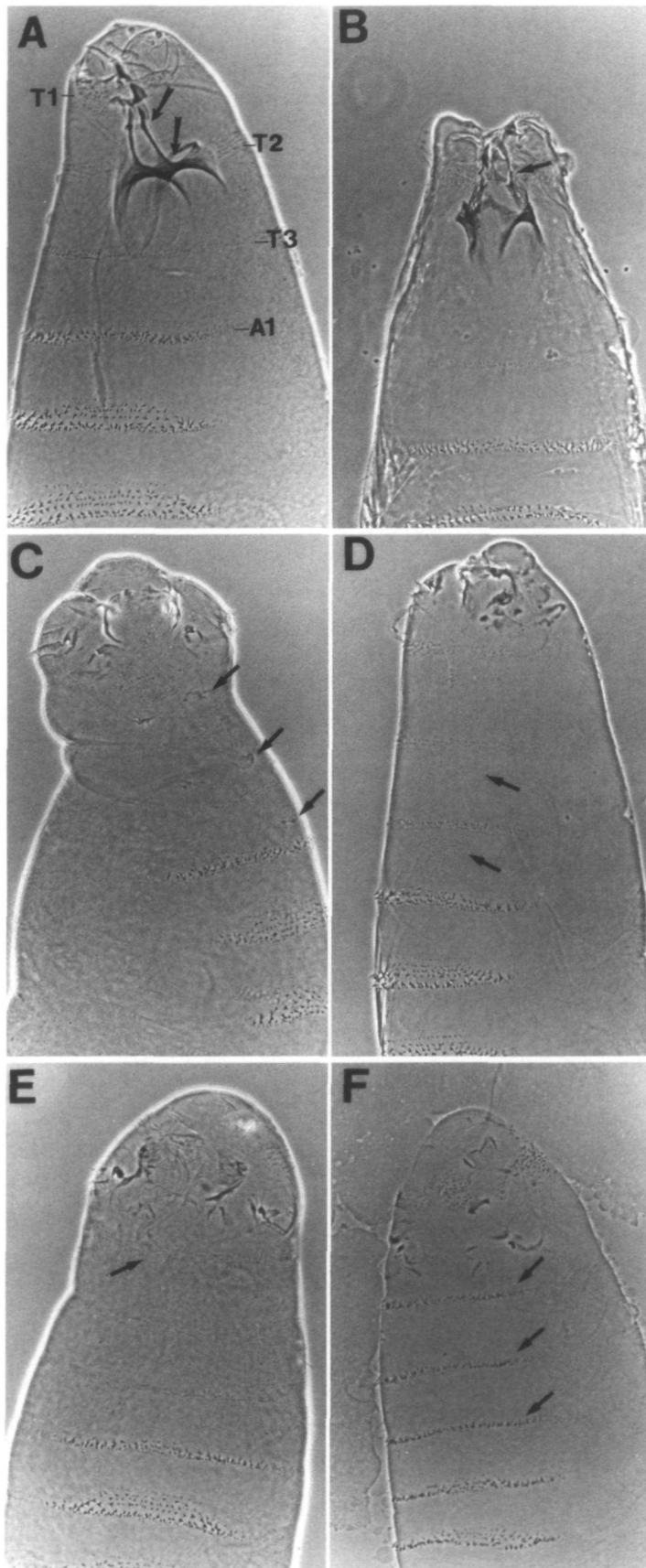


Fig. 5. Cuticular phenotypes of first instar larvae caused by ectopic expression of homeotic proteins. The ventral surfaces of the larvae are shown with anterior (head) structures located at the top of all figures. For a description of the heat shock lines employed, see Materials and methods. (A) Wild type Ore R larvae. The cephalopharyngeal skeleton (CPS) is marked with arrows and the denticle belt patterns of the T1, T2, T3, and A1 segments are labeled. (B) A *hslab* larva given a one hour heat shock at 4 hours after egg lay (AEL). Note the defects in the CPS (arrow). (C) A *hsDfd* larva administered a one hour heat shock at 3 hours AEL. There are mouth hooks (arrows) in the thoracic segments indicating a partial transformation of thorax to maxillary identity (Kuziora and McGinnis, 1988). (D) A *hsScr* larva heat shocked for one hour at 6 hours AEL. There are prothoracic denticle beards in T2 and T3 (arrows) indicating a transformation of these segments towards T1 (Gibson et al. 1990). (E) A *hsAntp* larva given a one hour heat shock at 6 hours AEL. Note the loss of the prothoracic denticle beard in T1 (arrow) indicating a transformation of this segment towards T2 (Gibson and Gehring, 1988). (F) A *hsUbx* larva given a one hour heat shock at 4 hours AEL. The denticle belts of the thoracic segments (arrows) are transformed towards a parasegment 6 identity (Mann and Hogness, 1990).

posterior thoracic identity is transformed to a prothoracic identity (Gibson et al., 1990). The PNS phenotype in *hsScr* embryos doesn't follow this type of transformation and reveals no obvious parallel homeotic transformation. Despite this fact, there are discernable defects in sensilla in all segments of the trunk (Figs 7E, F, 8B), indicating that ubiquitous expression of the *Scr* protein can alter the fates of cells in the abdominal segments, an observation at odds with previous conclusions (Gibson et al., 1990). The most dramatic transformation of sensilla occurs in the abdominal segments where the *lch5* organs are transformed into mono- or discalopodial organs (Figs 7F, 8B).

Similar to *hsScr* embryos, which show a posterior directed transformation in the cuticle, *hsDfd* embryos also exhibit a posterior directed transformation. That is, a transformation of the thoracic segments toward maxillary identity is observed (Kuziora and McGinnis, 1988). There is no apparent homeotic transformation in the PNS, as again sensory organ defects do not follow the transformation seen in the cuticle. However, PNS defects are observed in *hsDfd* embryos and these are very similar to those defects seen in *hsScr* embryos (Figs 7C, D, 8E). Sensilla in *hsDfd* embryos occur in abnormal numbers and positions with respect to the cuticle in all segments of the trunk. Curiously, the *dch3* organs in T2 and T3 are very sensitive to overexpression of the *Ubx*, *Scr* and *Dfd* products, exhibiting a transformation to a prothoracic *lch3*. However, this transformation is incomplete, as the axonal projection of these *lch3* organs is not the same as in wild-type embryos (data not shown).

While previous studies have shown that overexpression of the *Ubx*, *Antp*, *Scr* and *Dfd* proteins results in cuticular transformations in the embryo, this study has

demonstrated that this is not the case for the *lab* protein (Fig. 5B). As there is no apparent homeotic transformation in the cuticle of *hslab* embryos, the same is true for the PNS in these embryos. Overexpression of the *lab* protein does, however, cause PNS defects in all segments of the trunk, with a general reduction in the number of sensory organs and alterations in their normal spatial patterns (Figs 7A, B, 8C). In addition, *hslab* embryos frequently display a partial transformation of the dch3 organs in T2 and T3 to a prothoracic lch3 similar to that seen in embryos overexpressing the *Ubx*, *Scr* and *Dfd* products.

While there are no obvious homeotic transformations in the PNS which parallel observed cuticular changes accompanying overexpression of the *lab*, *Dfd*, *Scr* and *Antp* proteins, sensory organs are often found in abnormal numbers and locations. Could ectopic expression of homeotic proteins, in general, act as a switch in neuronal precursor development and transform es organ precursors into ch organ precursors or vice versa? In order to answer this question, heat shocked embryos were double stained with the P12 α -85E and F2 antibodies to detect both es and ch organs in the same embryo (Fig. 8). Examination of these embryos with the P12 α -85E and F2 antibodies indicates that transformations between es and ch organ precursors is not a likely cause of the abnormalities observed in the sensory organs.

Discussion

The homeotic genes *Scr*, *Antp*, *Ubx*, *abd-A* and *Abd-B* encode protein products which provide positional information in an antero-posterior fashion to cells in the epidermis, by imposing segment-specific cuticular characteristics (Akam et al., 1988; Kaufman et al., 1989). In the absence of the various homeotic gene products, the epidermal characteristics of one or more segments are transformed into the characteristics of another segment. The homeotic proteins are expressed in a complex manner during embryogenesis, in a majority of cases beginning around the time of germ band extension. In the ectoderm, the homeotic genes are, in general, expressed in parasegments (PS) which consist of the posterior compartment of one segment and the anterior compartment of the next most posterior segment. The most anteriorly accumulated homeotic proteins are *lab*, *Dfd* and *pb*, which are present in cells of the head segment primordia (Diederich et al., 1989; Regulski et al., 1987; Pultz et al., 1988). The *Scr* protein accumulates in cells of the labial and T1 segments (Riley et al., 1987; Mahaffey et al., 1989), while the *Antp* protein is localized in cells from the posterior compartment of the labial segment to the anterior compartment of A1 (PS 3-6), with highest levels in PS 4 (Carroll et al., 1986). *Ubx* protein appears in a complicated pattern from PS 5-12, with highest levels in PS 6 (White and Wilcox, 1984; Carroll et al., 1988). The expression of the *abd-A* protein overlaps with the *Ubx* pattern and is detectable in cells from PS

7-13, with the highest levels in the posterior compartments of these parasegments (Karch et al., 1990; Macias et al., 1990). The homeotic protein expressed most posteriorly is the *Abd-B* protein, which accumulates in cells of PS10-15, with highest levels of protein in PS 13-15 (Celniker et al., 1989; DeLorenzi and Bienz, 1990).

There are also studies which indicate that some of the homeotic proteins are expressed in a subset of cells of the PNS at later stages of development. For instance, the *Antp* (Carroll et al., 1986), *lab* (Diederich et al., 1989), *Ubx* and *abd-A* proteins (Karch et al., 1990) have been localized to cells of the PNS in the late embryo. However, the function of this later expression is unknown as cells in the PNS have already formed and differentiated at this time. Possibly, the homeotic proteins are involved in maintaining the differentiated state of these cells by regulating such processes as neurotransmitter synthesis. The present study was aimed at determining the effects, if any, of loss of function and gain of function mutations of the homeotic genes on the generation of the segment-specific patterns of sensilla in the PNS. Our results suggest that the homeotic genes, through their early expression in the ectoderm, do provide positional information which is necessary for the appropriate development of the segmental spatial patterns of sensilla in the trunk of the embryo.

PNS in homeotic loss of function mutants

The PNS was examined in homozygous mutant embryos carrying null alleles for the homeotic genes *Scr*, *Antp*, *Ubx*, *abd-A* and *abd-B*. We utilized antibody markers which allowed for the identification of both ch and es organs in the PNS. Our results indicate that, in general, loss of homeotic gene function results in a similar type of transformation in the PNS as observed in the epidermis. Perhaps, this result is not too surprising when one considers that both epidermal structures and sensory organs are derived from the same ectodermal tissue and may respond to the same or similar positional cues. However, there are exceptions to the rule, which suggests that the homeotic genes may act in concert to generate some sensilla, and act autonomously to generate others. On the other hand, the results point out that not all sensilla are affected by loss of function mutations in the homeotic genes, supporting the view that many sensilla are probably determined by the action of the segmentation genes (Hartenstein, 1987).

The lch3 organ in the prothoracic segment is one sensory organ which appears to be affected by the action of two different homeotic gene products, *Scr* and *Antp*. This sensory organ is a derivative of the posterior compartment of T1 (Hartenstein, 1987) and is different from other ch organs in the embryo in that it projects its axons from T1 to the T2 segmental nerve (Ghysen et al., 1986). Loss of function of *Scr* results in a transformation of this organ to a dch3, an organ found in the T2 and T3 segments. However, in about 50% of these embryos, the axons still cross the segmental boundary and join the T2 segmental nerve. Furthermore, in *Antp* loss of function embryos, the lch3 in T1 is

morphologically normal, yet the axonal projection is abnormal. Thus it would appear that *Scr* dictates the morphology and position of the prothoracic lch3, while *Antp* determines the axonal projection. Interestingly, *Scr* protein accumulates in cells of the posterior compartment of T1 at lower levels than in cells of the anterior compartment of T1 (Mahaffey and Kaufman, 1989). However, as the absence of *Scr* function leads to a change in the identity of the lch3, which is a derivative of the posterior compartment (Hartenstein, 1987), we can conclude that the low level of *Scr* expression in cells of the posterior compartment of T1 is significant, at least for the specification of this one structure.

Defects in sensilla observed in *Antp* loss of function embryos also do not follow a simple homeotic transformation. While previous studies have demonstrated a transformation of T2 and T3 towards T1 identity in *Antp*^{w10} embryos (Wakimoto and Kaufman, 1981), this type of transformation is not observed in the PNS. Instead, there is a dramatic reduction in the number of es organs in the thoracic segments. Compared to the es organs, the ch organs of the thoracic segments are relatively unaffected in most *Antp*^{w10} embryos, yet there are ectopic monoscolopidial organs which develop ventrally in T2 and T3. This phenotype is reminiscent of the patterns of sensilla characteristic of the head segments, which contain relatively few es organs and only a single monoscolopidial organ per segment (Hartenstein and Campos-Ortega, 1986). This would seem to indicate a partial transformation of the PNS in the thoracic segments towards head identity in *Antp*^{w10} embryos. This interpretation is strengthened by the observation that structures resembling mouthparts develop in the thoracic segments of embryos carrying strong loss of function alleles of the *Antp* gene (T. Kaufman, unpublished observations).

The PNS defects observed in loss of function mutants for the homeotic genes which specify the identity of the abdominal segments, again suggest that the homeotic genes function both independently and together to influence sensilla development in the abdomen. The *Abd-B*^{MX2} mutant is a protein null for PS10-12 (A5-A7) (Celniker et al., 1990) and showed no obvious defects in the sensilla of these segments. This result indicates that the expression of *Abd-B* in A5-A7 is apparently unnecessary for sensilla formation, although it has a known role in establishing the cuticular identity of these segments. While *Abd-B* has no apparent role in sensilla development of the A pattern, either *Ubx* or *abd-A* function alone appears to be sufficient for the proper development of most sensilla in A1-A7, strengthening the argument that some functions of *Ubx* and *abd-A* are redundant where their patterns of expression overlap (Akam et al., 1988). However, *Ubx* and *abd-A* are also required independently for the formation of specific sensilla in A1-A7. For instance, the formation of the lch5 organs of A1-A7 are dependent on *abd-A* function. The transformation of this organ to a dch3 in *abd-A*^{M1} embryos has been previously reported (Karch et al., 1990) and is probably due to increased expression of *Ubx* protein in the posterior compartments of the

abdominal segments of *abd-A*^{M1} embryos (Macias et al., 1990). *Ubx*, on the other hand is necessary for the formation of all sensilla in A1 which are derivatives of the anterior compartment, such as all the es organs, the lch1, vchA and vchB (Hartenstein, 1987).

Cuticular defects in *hslab* embryos

Previous results have shown that ectopic expression of *Dfd* (Kuziora and McGinnis, 1988), *Scr* (Gibson et al., 1990), *Antp* (Gibson and Gehring, 1988), and *Ubx* (González-Reyes and Morata, 1990; Mann and Hogness, 1990) via an *hsp-70* promoter causes homeotic transformations of cuticular structures in the head and thorax. However, ectopic expression of the homeotic gene *lab* doesn't result in any apparent homeotic transformation, but rather causes defective head involution and abnormal development of the cephalopharyngeal skeleton. These latter types of defects are also commonly observed in embryos in which the *Dfd*, *Scr*, *Antp* and *Ubx* genes are overexpressed, although with much more severity. The structures most affected by overexpression of *lab* are the lateralgrate, ventral arms of the mandibular segment and the dorsal bridge, dorsal arms and vertical plates of the acronal segment. Ectopic expression of *lab* can therefore influence the normal development of structures derived from segments both anterior and posterior to the normal domain of *lab* expression, similar to the effects observed in embryos overexpressing the *Dfd* and *Scr* genes (Kuziora and McGinnis, 1988; Gibson et al., 1990). However, the absence of any apparent homeotic transformation in *hslab* embryos stands in contrast to results obtained from the overexpression of other homeotic genes which cause specific homeotic transformations. This would seem to indicate that the presence of the *lab* protein is either masked in cells which express other homeotic proteins or that the *lab* protein is extremely unstable in cells outside its normal domain of expression and thus cannot exert any effect. In keeping with this latter possibility, the *lab* protein does appear to be more unstable than the other homeotic proteins when ectopically expressed, as the protein is barely detectable at one hour after heat shock (J. Heuer, unpublished observations). Alternatively, the *lab* protein may not effectively compete with other homeotic proteins for downstream genes or maybe the genetic program normally under control of the *lab* protein in the epidermis is closer to the so called "ground state" than the other more posterior acting homeotic genes. If the latter were true, then overexpression of *lab* would not dramatically interfere with the development of segmental characteristics imposed by the other homeotic genes.

PNS in homeotic gain of function embryos

While the cuticular effects of overexpressing the *lab* protein are limited to the acronal, mandibular and labial segments, defects in the PNS are observed in all the thoracic segments and in some of the abdominal segments. Major defects in sensilla are also observed in the abdominal segments in which the *Dfd* and *Scr* proteins are overexpressed. This contrasts with pre-

vious studies which have concluded that the identity of the abdominal segments is unaffected by overexpression of the *Dfd* and *Scr* proteins (Kuziora and McGinnis, 1988; Gibson et al., 1990). In addition, while ubiquitous expression of *Dfd*, *Scr* or *Antp* protein causes obvious homeotic transformations in epidermal structures, similar conclusions cannot be drawn for the PNS. The phenotypes of the PNS in embryos overexpressing the *lab*, *Dfd* and *Scr* gene products are very similar. These embryos have reduced numbers of sensilla in both the thoracic and abdominal segments relative to the wild-type embryo. In addition, the lch5 organs of the abdominal segments are transformed to mono- or discopodial organs. As both of these phenotypes are characteristic of sensilla in the head segments, we cannot exclude the possibility that overexpression of the *lab*, *Dfd* or *Scr* products results in a transformation of the PNS toward head identity, similar to that observed in *Antp^{w10}* embryos. However, an alternate interpretation is that the presence of such high levels of head specific homeotic proteins expressed in the trunk segments nonspecifically disturbs the positional information normally required by PNS precursor cells to form the proper sensilla. The observed morphological abnormalities would result from a reduction in the number of cells recruited to form sensory organs in the thorax and abdomen rather than a homeotic transformation.

Could the PNS defects which occur in these embryos simply be caused by overexpression of any homeodomain containing protein, in that high levels of these proteins in the nucleus might lead to aberrant gene regulation? This possibility seems unlikely, as the *cut* gene product, which also contains a homeodomain, causes a much different, yet unique phenotype in the PNS following ubiquitous expression upon heat induction (Blochliger et al., 1991). In addition, while overexpression of the *lab*, *Dfd* and *Scr* products causes major PNS defects in the abdominal segments, overexpression of the *Antp* and *Ubx* products has relatively little or no effect on the PNS in these segments.

The observation that both loss of function and gain of function mutations in the homeotic genes cause aberrant PNS development leads us to ask about the mechanism by which these gene products might influence this process. Clearly, the results of the heat shock experiments demonstrate that alterations in the sensilla patterns are more susceptible when the homeotic proteins are overexpressed at 3-4 hours of development compared to 6-7 hours of development. This result suggests that the homeotic genes likely function to influence sensilla patterns at an early stage of PNS development, possibly during or before the first sensory precursors arise, which is around 5 hours of development (Ghyssen and O'Kane, 1989). This time period coincides with a time in PNS development when the proneural and neurogenic genes are presumably active. As the homeotic gene products are nuclear proteins with the potential to act as transcriptional regulators, the possibility exists that the homeotic proteins influence the regulation/expression of these

genes. Our results in this study provide several lines of evidence which support this conclusion, albeit, indirectly: 1) sensory organs in homeotic loss of function or gain of function mutant embryos always consist of the proper number of neuronal and support cells, indicating that cell lineage genes are not affected; 2) sensory organs are not transformed from one type of organ into another, thus neuronal type selector genes are not affected; 3) the PNS phenotypes of loss of function or gain of function mutant embryos are similar to the PNS phenotypes observed in loss of function mutants for the proneural or neurogenic genes (i.e., too few or too many of one type of sensory organ). While we propose that the homeotic gene products affect sensilla patterns through the regulation of early events in PNS development, we cannot rule out the possibility that the homeotic genes might also affect the processes of cell death or cell division in the PNS, as we examined the PNS at only one stage in development (12-14 hours). Nevertheless, the possibility that the homeotic proteins might regulate the genes which are necessary for the generation of sensory progenitor cells, either directly or indirectly, certainly warrants further studies aimed at addressing this question.

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