

The role of homeotic genes in determining the segmental pattern of chordotonal organs in *Drosophila*

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ABSTRACT The homeotic genes are instrumental in establishing segment-specific characteristics. In *Drosophila* embryos there is ample evidence that the homeotic genes are involved in establishing the differences in the pattern of sense organs between segments. The chordotonal organs are compound sense organs made up of several stretch receptive sensilla. A set of serially homologous chordotonal organs, *lch3* in the 1st thoracic segment, *dch3* in the 2nd and 3rd thoracic segments and *lch5* in abdominal segments 1 to 7, is composed of different numbers of sensilla with different positions and orientations. Here we examine this set of sense organs and a companion set, *vchA/B* and *vch1*, in the wild type and mutants for *Sex combs reduced*, *Antennapedia*, *Ultrabithorax*, and *abdominal-A*, using immunostaining. Mutant phenotypes indicate that *Ultrabithorax* and *abdominal-A* in particular influence the formation of these sense organs. Differential expression of *abdominal-A* and *Ultrabithorax* within compartments of individual parasegments can precisely modulate the types of sense organs that will arise from a segment.

KEY WORDS: *sense organs, PNS, homeosis, Drosophila, sense organs, bithorax complex, antennapedia complex*

Introduction

In *Drosophila*, the homeotic genes are responsible for specifying the diversity of segments. Most of the homeotic genes are found in two clusters, the Antennapedia Complex (ANT-C) and the Bithorax Complex (BX-C). The ANT-C contains the homeotic genes *labial*, *proboscipedia*, *Deformed*, *Sex combs reduced*, and *Antennapedia* (*Antp*). The BX-C contains the genes *Ultrabithorax* (*Ubx*), *abdominal A* (*abd-A*), and *Abdominal B* (*Abd-B*) (Lewis, 1978; Sanchez-Herrero *et al.*, 1985). The homeotic genes are expressed in patterns that differ from segment to segment within a domain of activity covering multiple segments. The domains and patterns within domains are usually delimited by parasegmental rather than segmental boundaries (Martinez Arias and Lawrence, 1985). Parasegments are metameric units that are out of register with the conventionally defined segments of larvae and adults. The overlap of parasegments and segments defines smaller units, the anterior and posterior compartments. A feature of the homeotic genes is that they cross-regulate each other's expression (McGinnis and Krumlauf, 1992). Generally the genes with the more posterior expression domains down-regulate expression of those with more anterior expression domains. The result of these interactions is that each parasegment has a unique "homeotic code" responsible for its individual identity. Mutations affect the distribution of homeotic proteins, altering the homeotic code, and producing posterior-ward transformations of identity.

The peripheral nervous system (PNS) of *Drosophila* embryos provides a useful set of landmarks for investigating homeosis. Visible cuticular structures are associated with some sense organs and a number of specific antibody probes are available. The sense organ precursor cells are ectodermal in origin and thus would be expected to respond to the homeotic code in the same way as cuticular features such as denticle and spiracle patterns (Lewis, 1978). Heuer and Kaufman (1992) investigated the effects of null alleles of *Scr*, *Antp*, *Ubx*, *abd-A* and *Abd-B* on a range of sense organs and found that segmental transformations were approximately in accord with the types of transformation seen in the epidermis. The sense organs were considered in discrete segmental units by Heuer and Kaufmann (1992) rather than in compartmental units. Here we consider a set of serially homologous chordotonal sense organs and discuss the role of homeotic genes in establishing the differences between compartments within segments.

The *lch3/dch3/lch5* set of serial homologs is composed of 3 or 5 sensilla: *lch3* in T1, *dch3* in T1/2 and *lch5* in A1-7. All are derived from the posterior compartment of each segment (Hartenstein, 1987). An additional set of primarily mono-chordotonal organs is derived from the anterior compartments of the thorax (*vch1*) and abdomen (*vchA* and *vchB*). They too show segmental differences

Abbreviations used in this paper: ch, chordotonal; dbd, dorsal bipolar dendrite.

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so that together with the lch/dch organs homeosis can be comprehensively assessed in mutants.

In this study we review and reexamine the distribution of sense organs in homeotic mutant embryos, concentrating on the chordotonal organs. First we examine the phenotypes of null mutations of *Scr*, *Antp*, *Ubx* and *abd-A*. Second we examine the effects on sense organ patterns of sub-function alleles of *Ubx* in which the distribution of homeotic protein is altered. The sense organ phenotype of this class of mutations has not previously been examined in detail in embryos. Finally we integrate the expression patterns of homeotic genes and their role in specifying parasegmental identity to clarify their role in the specification of the chordotonal sense organs of the *Drosophila* larva.

Results

Parasegmental Pattern of Sense Organs in Wild Type Embryos

The sense organs of the thoracic and abdominal segments are shown diagrammatically in Fig. 1, after 22C10 immuno-staining for sensory neurons in Fig. 2 A,B and after anti-fishhook immuno-staining for chordotonal (ch) organ scolopale cells in Fig. 3 A,B. The prothoracic (T1) and mesothoracic (T2) segments show minor differences. T2 and T3 are identical. Abdominal segments A1-7 also have an identical pattern of sense organs that is markedly different to the thoracic segments. Chordotonal (ch) organs are composed of from 1

to 5 sensilla. Each sensillum is a clonal unit (i.e. the cells are derived from a common precursor) comprised of 5 cells, a bipolar neuron, an enwrapping scolopale cell, a ligament cell, a cap cell, and an ectodermal cell (Brewster and Bodmer, 1995; Orgogozo *et al.*, 2001). One pole of the bipolar neuron gives rise to the axon that extends to the central nervous system (CNS). A single, unbranched, stretch-sensitive dendrite arises at the opposite pole.

Additional sensilla examined include the keilin's organs, which are external sense organs composed of three cuticular hairs projecting from a common socket. Each keilin's organ spans parasegmental borders in the thorax. Two anterior hairs sit in the anterior compartment of a parasegment and a posterior hair sits in the posterior compartment of a parasegment (Struhl, 1984). The dorsal bipolar dendrite neuron (dbd) is a multiple dendrite neuron derived from the anterior compartment of T2, T3 and A1-8.

Homeotic Gene Expression Patterns

The expression patterns of the homeotic genes during embryogenesis have been examined in detail using antibody probes. Expression is strongest in the CNS where the pattern generally matches epidermal expression. The expression regions of the homeotic genes in the wild type are shown in diagrammatic form in Fig. 4. The altered expression patterns in the homeotic mutants are described by various authors (see Merritt and Whittington, this issue) and are taken here as a general guide only because fine-

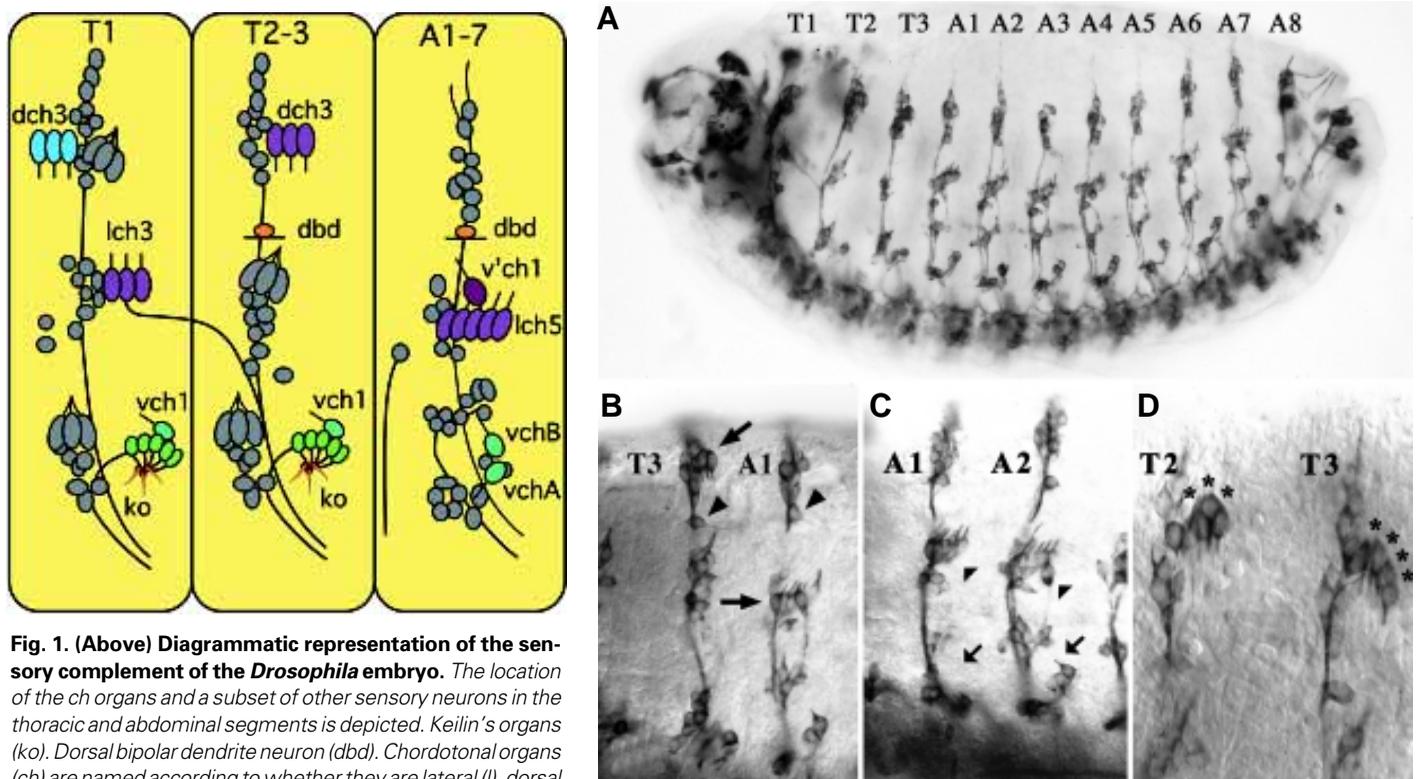


Fig. 1. (Above) Diagrammatic representation of the sensory complement of the *Drosophila* embryo. The location of the ch organs and a subset of other sensory neurons in the thoracic and abdominal segments is depicted. Keilin's organs (ko). Dorsal bipolar dendrite neuron (dbd). Chordotonal organs (ch) are named according to whether they are lateral (l), dorsal (d), ventral (v) or ventrally originating (v'), and to how many sensilla comprise the sense organ (1, 3 or 5).

Fig. 2. (Right) Sense organs of wild type, *bxd* and *abx* embryos stained with antibody 22C10. (A) Wild type larva showing the full complement of sensory neurons on one side of the body. In this and all other figures, anterior is to the left and dorsal is up. **(B)** Segments T3 and A1 of a wild type larva showing the *dch3* of T3 (oblique arrow) and *lch5* of A1 (horizontal arrow). The *dbd* neurons are indicated with oblique arrowheads. **(C)** Segments A1 and A2 of a *bxd* mutant embryo. Typically in *bxd* embryos *vchA/B* (arrow) and *v'ch1* (arrowhead) are missing in A1. **(D)** Segments T2 and T3 of an *abx* mutant embryo showing an additional ch sensillum comprising *dch3* in T3. T2 *dch3* is normal. Individual ch sensilla are indicated with asterisks.

scale temporal and spatial regulation of homeotic gene expression within parasegments does occur (Duncan, 1996).

abd-A

Loss of *abd-A* activity transforms *lch5* of segments A1-7 into *dch3* (Karch *et al.*, 1990; Heuer and Kaufman, 1992). A keilin's organ mono-hair is present in the ventral region of each abdominal segment (Sanchez-Herrero *et al.*, 1985). *V'ch1*, *vchA* and *vchB* are present in the abdominal segments (Heuer and Kaufman, 1992). We have confirmed these observations using the null mutation, *abd-A^{MX1}* (data not shown, summarized in Fig. 4B). The phenotype is consistent with transformation of the abdominal segments PS 7-12 to reiteration of T3p/A1a (PS 6) (Sanchez-Herrero *et al.*, 1985; Heuer and Kaufmann, 1992; Kurant *et al.*, 1998). T3p gives rise to *dch3* (Hartenstein 1987) and the keilin's organ mono-hair, thus the thorax-like *dch3* is present in PS7-12.

Ubx

The *Ubx* null mutation affects the sense organs of segments T2, T3 and A1. The *dch3* organs of T2 and T3 become transformed to the likeness of *lch3* (Subramaniam *et al.*, 1994; Heuer and Kaufman, 1992; this study). *V'ch1*, *vchA* and *vchB* are missing from A1, although they are present in A2-7. *Vch1*, usually present in the thoracic segments only, is seen in A1. Keilin's organ twin hairs are present in A1. The sensory complement of A2-7 is normal (data not shown, summarized in Fig. 4C).

The sense organ phenotype is in accord with a transformation of PS5 and 6 to PS4 identity. On a compartmental basis, the phenotype corresponds with transformation of T2 and T3 to T2a/T1p identity, and A1 to a T2a/A1p identity.

Ubx Subfunction Alleles

The subfunction alleles of *Ubx* have been shown by White and Wilcox (1985a,b) to alter the distribution of the *Ubx* gene product in subregions of its normal expression. The four subfunction alleles that cover the *Ubx* region are *bx*, *abx*, *bx^d*, and *pbx*. Mutants are viable and their segmental transformations have been recorded in adults. The effect of the mutations on the embryonic PNS has not been investigated in detail.

bx^{d55i} In adults *bx^d* causes transformation of PS6 toward PS5 (Duncan, 1987). In larvae the mutation is reported to affect the sense organ complement of A1 (Lewis, 1978). Homozygous *bx^d* embryos stained with 22C10 show a loss of *vchA*, *vchB* and *v'ch1* from A1a (Fig. 2C). An ectopic *vch1*, usually characteristic of T1a, T2a and T3a, is present in A1a (Fig. 4D). There is no keilin's organ twin-hair in A1 of the mutant. The sensory complement of T3p is normal. The sense organ phenotype is consistent with a transformation of PS6 (T3p/A1a) to a PS5 identity.

abx¹ *abx* homozygotes have been shown in adult flies to cause a transformation of PS 5 to a PS 4 identity (Duncan, 1987). In

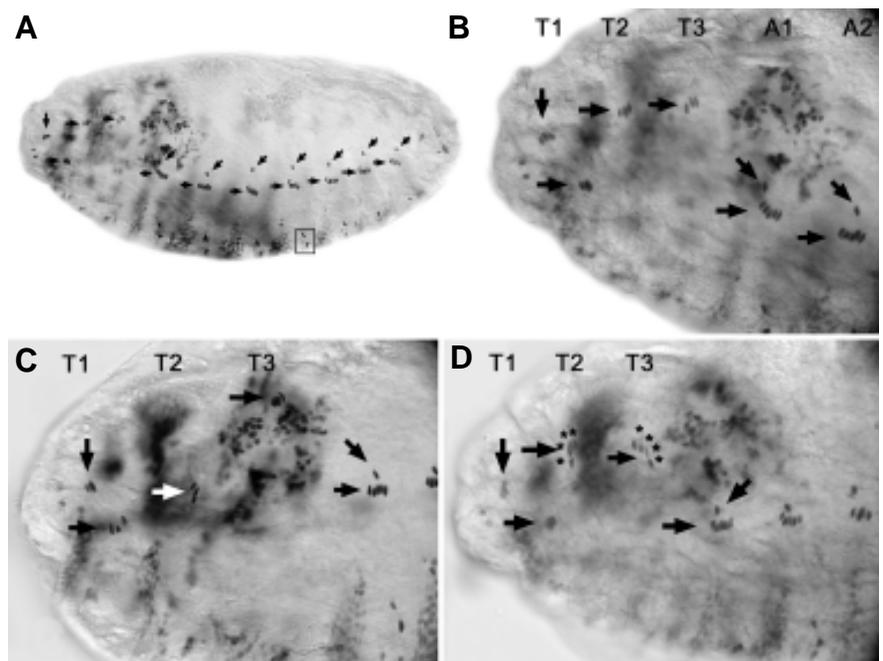


Fig. 3. Chordotonal organ phenotypes of *Antp* embryos. (A) Wild type embryo stained with anti-fishhook. The antibody binds to the scolopale cells of ch organs. Ch organs of the *lch3/dch3/lch5* class of serial homologs are indicated with horizontal arrow in each segment. *Dch3* of T1 is indicated with a vertical arrow. *V'ch1* of the abdominal segments is indicated with an oblique arrow. (B) T1-3 and A1-2 of a wild type embryo. (C,D) *Antp* mutant embryos showing transformation of the T2 *dch3* to an *lch3* (white arrow in panel C) and transformation of *dch3* of T2 and T3 to *dch4* (individual sensilla indicated with asterisks).

embryos the stripe of strong *Ubx* expression in anterior PS5 is lost (White and Wilcox, 1985a). In the PNS a transformation of the *dch3* in T2 to a T1-like *lch3* was expected, however homozygous *abx¹* mutant embryos showed no phenotype in the PNS. When *abx¹* was placed over the deficiency *Df(3R)Ubx¹⁰⁹* an additional ch sensillum was added to the *dch3* organ in either T2 or T3. 57% had an extra sensillum incorporated into the *dch3* in T2 while 43% had an extra in T3 (Fig. 2D). Examination of cuticle showed no defects in the keilin's organs. Orientation of the ch organs was normal.

bx* and *pbx In *bx* adults, PS5 is partially transformed to PS4, in particular the T3a compartment becomes transformed to T2a (Peifer and Bender, 1986), correlating with a reduction of *Ubx* expression in T3a. In adults, *pbx* mutations are reported to transform T3p to T2p (Bender *et al.*, 1983). No change is expected in the sensory complement of either *bx* or *pbx* embryos because the sense organs of the posterior compartments of T2 and T3 are identical. Both 22C10 staining and observations under DIC optics showed no changes in the PNS of embryos.

Antp

Antp is normally expressed in PS4 to 12 and is most strongly expressed in PS4 (Carroll *et al.*, 1986). Investigations of epidermal phenotypes indicate that loss of function of *Antp* produces a transformation of T2/T3 to a T1 identity however the transformation is not clear-cut (Wakimoto and Kaufman, 1981). The PNS of embryos indicated a possible transformation of T2 and T3 toward a more anterior head segment identity, based on a general

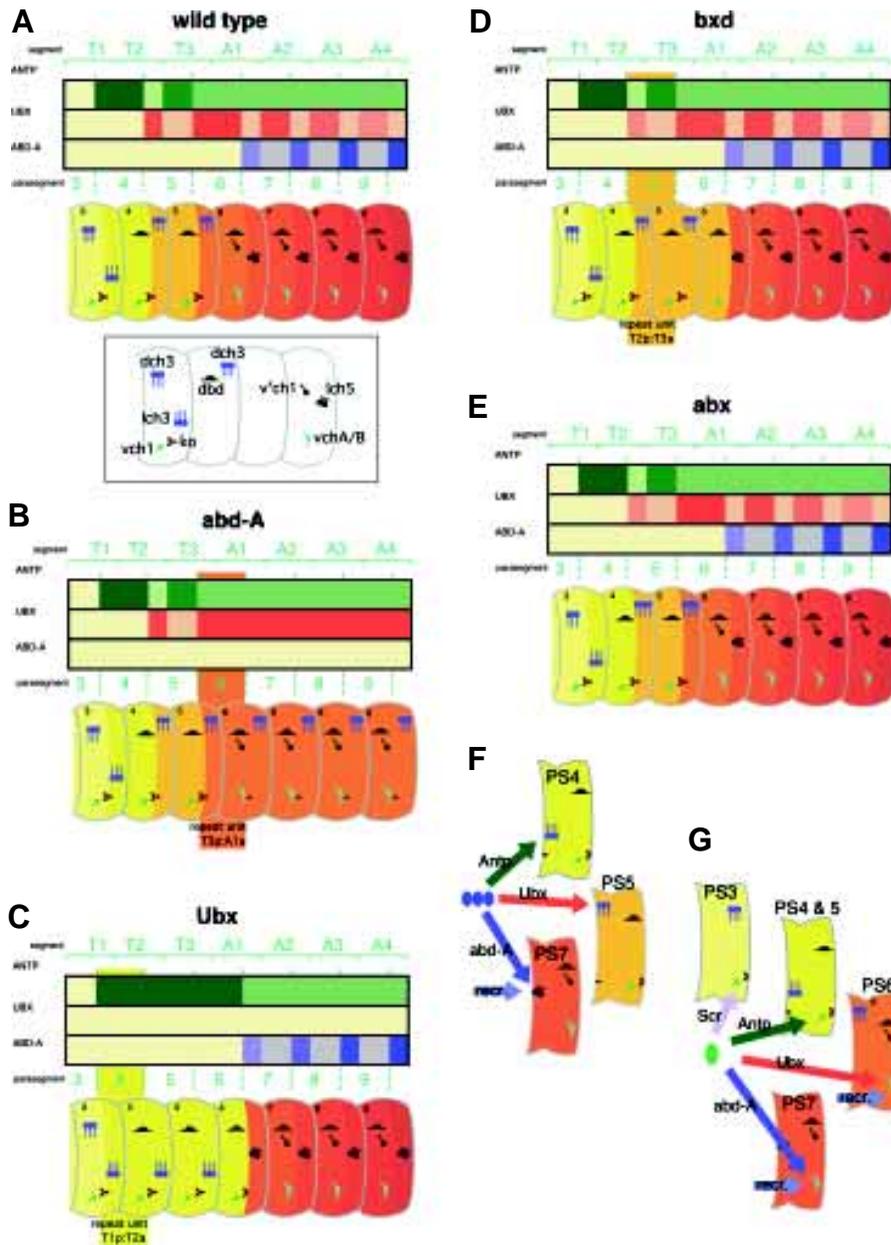


Fig. 4. Diagrammatic representations of homeotic protein distributions and phenotypes of a subset of sense organs in homeotic mutants. (A) Distribution of a subset of sense organs in wild type. **(B)** *abd-A* mutant. **(C)** *Ubx* mutant. **(D)** *bxd* mutant. **(E)** *abx* mutant. **(F)** Summary diagram of homeotic gene influence on formation of the different types of ch organs occurring in the posterior compartments. **(G)** Summary diagram showing formation of ch organ types in anterior compartments. In F and G, the arrow labeled "recr." indicates that recruitment of secondary chordotonal precursors from a primary precursor occurs.

reduction in the number of sense organs and presence of ectopic ch organs in T2 and T3 (Heuer and Kaufman, 1992).

A re-examination of *Antp* embryos using 22C10 immunostaining for neurons and dendrites showed that the *dch3* organs in T1 and T2 were frequently misoriented or malformed, in T2, T3 or both (67% of embryos). Anti-fishhook immunostaining for scolopale cell nuclei (Ma *et al.*, 1998; D. Merritt, personal observations) showed that the *dch3* of T2 is transformed to an *lch3* in 54% of embryos (Fig. 3C). In 8% of embryos the *dch3* in both T2 and T3

had an additional sensillum, becoming transformed to *dch4* (Fig. 3D). In 78% of embryos the axonal projection of *lch3* in T1 is abnormal, running into the T1 segmental nerve rather than T2, also shown by Heuer and Kaufman (1992). The pattern of the remaining sense organs, especially in the lateral and dorsal clusters of T2 and T3 is abnormal, frequently showing loss of many sense organs. The *dbd* sense organ, normally present in T2, T3 and A1-7 (Fig. 5A), was missing from T2 in 50% of embryos (Fig. 5B).

Scr

Scr is normally expressed in PS 2 and 3. Investigations of *Scr* mutants indicate that T1 is transformed to a more posterior thoracic identity (Wakimoto and Kaufman, 1981; Pattatucci *et al.*, 1991). Heuer and Kaufman (1992) investigated the effects of *Scr* loss of function on the sense organs and found that *lch3* of T1 (derived from the PS 4 component of the segment) was transformed to *dch3*. No effect upon the pre-existing *dch3* of T1 (derived from the anterior compartment) was mentioned. We reexamined the defects in *Scr* embryos using anti-fishhook staining and found that *dch3* is frequently in an abnormal position with fewer than three component sensilla (50%) and that *lch3* (derived from the posterior compartment of T1) is sometimes missing (14%) (Fig. 5D). The *dbd* neurons show their normal distribution in T2, T3 and the abdominal segments (Fig. 5C).

Discussion

The subset of ch organs, *lch3* (T1), *dch3* (T2/3), *lch5* (A1-7) and *lch3/lch1* (A8) show a number of features indicating that they are serially homologous. First, they are among the few sense organs that arise in posterior compartments (Hartenstein, 1987). Second, the precursor cells for this subset of ch organs, C1-3, originate in a similar location in each segment (Salzberg *et al.*, 1994; Younossi-Hartenstein and Hartenstein, 1997; Rusten *et al.*, 2001). The genetic interactions that give rise to the different numbers and orientation of sensilla comprising *lch3* and *lch5* are also relevant. In the thorax, C1-3 each divide to give rise to a neuron, a cap cell, ligament cell, sheath cell and an accessory cell (Brewster and Bodmer, 1996). In the abdominal segments, C3 recruits two secondary precursors from the adjacent ectoderm via epidermal growth factor receptor (EGFR) signaling (Elstob *et al.*, 2001). All five precursors divide to form sensilla. In the abdominal segments only, precursor C1 also recruits a cluster of cells from the ectoderm that will differentiate to become oenocytes, a type of secretory cell (Elstob *et al.*, 2001; Gould *et al.*, 2001). A suite of genes is involved in the EGFR pathway, including *argos*, *pointed*,

spitz, *rhomboid* and the proneural gene, *atonal* (reviewed in Gould *et al.*, 2001).

The different position and orientation of the *lch5* and *dch3* organs is attributed to migration and rotation of the developing sense organs, most likely due to ventral-ward migration of the basal attachment point, the ligament cells (Hartenstein, 1987). The apical cap cells of the *lch3*, *dch3* and *lch5* remain attached to the epidermis where they originate at similar dorso-ventral positions in the abdomen and thorax (Matthews *et al.*, 1990). A number of mutations affecting the ventral migration of *lch5* have been described (Salzberg *et al.*, 1994; Kurant *et al.*, 1998).

The homeotic genes are the obvious candidates for regulation of the segmental differences in the orientation, position, and numbers of chordotonal organs. First, considering *lch3* (T1), *dch3* (T2/3) and *lch5* (A1-7) (Fig. 4), the working model is: (1) that the ground state for this class of sense organs (*lch3*, normally found in PS4) occurs in the absence of both *Ubx* and *abd-A* expression, (2) that *Ubx* expression results in the formation of *dch3* (Heuer and Kaufman, 1992), normally found in PS5 and 6, and (3) that *abd-A* expression results in the formation of *lch5* (Karch *et al.*, 1990), normally found in PS7-12. The model, summarized in Fig. 4F, is supported by the phenotypes of homeotic mutants. In *Ubx* mutants, *Antp* expression is up-regulated throughout PS5 and 6 resulting in the formation of *lch3* (Fig. 4C). Loss of *abd-A* function results in up-regulation of *Ubx* in PS7-12 resulting in the formation of *dch3* organs in the posterior compartments of these segments (Fig. 4B). The *abx* mutation frequently produces *dch4* in T2 and T3. This transformation is not consistent with a simple homeotic transformation and could be interpreted as a partial posteriorward transformation.

While *Antp* is the predominant homeotic gene expressed in the ground state PS4, it does not appear to play a major role in the formation of this class of sense organ. Loss of *Antp* function affects the sense organs in PS4 and 5 however there is no highly penetrant homeotic transformation, rather in some cases a sensillum is added to the *dch3* so that it is comprised of 4 sensilla, or in some cases there is partial rotation of the *dch3* to take on some features of *lch3*, both features indicative of a posteriorward transformation. *Antp* is not required for formation of the *lch5* because these organs are normal in *Antp* mutants. *Scr* mutants show various degrees of disruption of the normal segmental pattern in PS4 and 5. As pointed out by Heuer and Kaufman (1992) *Scr* and *Antp* may together play a role in the formation of the ground state *lch3*. A precise determination requires an examination of double mutants for *Scr* and *Antp*.

Oenocytes form only in the abdominal segments (Gould *et al.*, 2001) and their formation is linked with the formation of the *dch3*/*lch3*/*lch5* class of sense organs because oenocytes are induced via signaling from C1 (Gould *et al.*, 2001). EGFR-mediated recruitment of oenocytes or additional ch precursors does not appear to take place in the thoracic segments (zur Lage *et al.*, 1997) so it would not be surprising if homeotic genes played a role in EGFR-mediated recruitment of oenocytes as well as recruitment of secondary ch precursors. Gould *et al.* (2001) cite preliminary evidence that the bithorax complex is involved in oenocyte formation because the cells are absent in mutants lacking *Ubx*, *abd-A* and *Abd-B*. We propose that, more specifically, oenocyte formation would be under control of *abd-A* because *abd-A* is required for formation of *lch5*. However recruitment of *vchA* from C5 (see below) occurs in the absence of *abd-A* expression so *Ubx* may play a role as well (Fig. 4G).

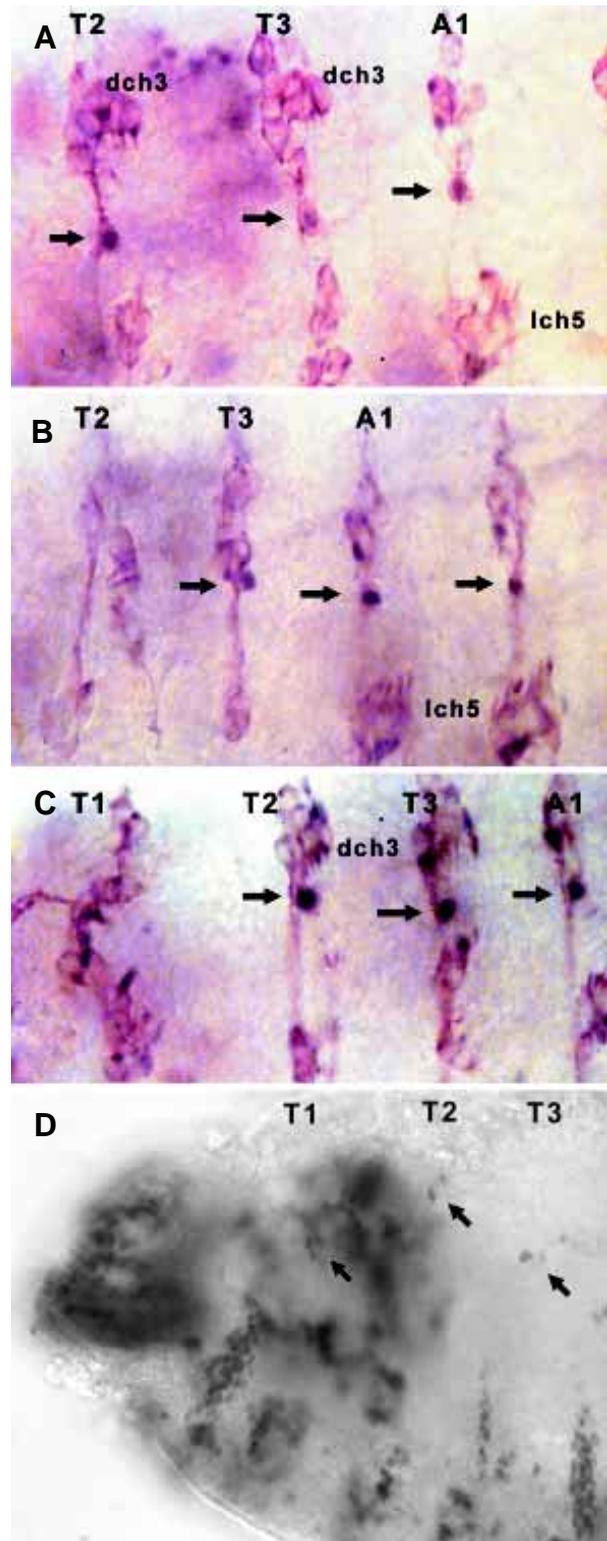


Fig. 5. Location of the *dbd* sense organ in wild type, *Scr* and *Antp* embryos. (A) Wild type embryo stained with 22C10 (brown) and with *pdm1* (blue/black) to highlight the *dbd* neuron. The *dbd* neuron (arrow) is normally present in T2-3 and A1-8. (B) In this *Antp* embryo *dbd* is missing from T2. (C) An *Scr* embryo shows *dbd* is present in T2, T3 and A1. (D) *Scr* mutant embryo stained with anti fishhook has a *dch3* in each of T1, T2 and T3. The *lch3* is missing from T1.

The remaining sets of ch organs arise from the anterior compartments. The vch1 organ found in each thoracic segment is likely to be the serial homolog of vchB. As mentioned above, the three precursors from the posterior compartment (C1-3) give rise to lch3, dch3 and lch5. Two additional precursors (C4, 5) arise in the anterior compartment and give rise to v'ch1 (C4) and vchB (C5) in the abdomen (Okabe and Okano, 1997; zur Lage *et al.*, 1997). The vchA precursor is then recruited from adjacent ectoderm by EGFR signaling from C5, in the same way that C3 recruits additional precursors. While the thoracic segments have not been investigated in detail it appears that recruitment does not operate in PS3-5 (zur Lage *et al.*, 1997). The C5 precursor most likely gives rise to vch1 in T1-3 and does not recruit a secondary precursor, hence the thorax has no serial homolog of vchA. The homology, if any, between lch3 of T1 and the anterior compartment-derived ch of the abdomen remains unknown.

VchA and vchB form in parasegments where either *Ubx* or *abd-A* is expressed and vch1 forms where *Antp* or *Scr* is expressed (Fig. 4G). Once again the ground state appears to occur in PS4. *Ubx* and *abd-A* are able to substitute for each other because vchA and vchB form normally in PS6-12 of the *abd-A* mutant and PS7-12 of the *Ubx* mutant. Abdominal segment 1 of *Ubx* mutants contains a vch1 instead of vchA/B due to the absence of expression of both *Ubx* and *abd-A* in this compartment. The *bx*d mutation shows the same phenotype as *Ubx* in the anterior compartment of PS6.

Transformations in the PNS of homeotic mutants of *Drosophila* are consistent with homeotic regulation of sense organ type. Multiple genetic interactions including proneural gene expression, precursor selection, precursor recruitment, lineage determination, and migration are involved in specifying the differences in the ch organs between segments. Precisely which processes come under homeotic control is not yet certain, although a regulation of the recruitment process could explain a number of the differences between the abdomen and thorax (Gould *et al.*, 2001).

The relatively few studies of ch organ distribution outside of *Drosophila* indicate that sense organs considered here are strikingly conserved across distantly related insects suggesting that the chordotonal organs may be fundamental elements of a ground plan sensory system common to all insects. The grasshopper embryo has a set of ch organs in T2 and T3 that are highly likely to be homologs of *Drosophila* dch3, while the sternal and pleural ch organs of the grasshopper abdomen are likely homologs of the lch5 and vchA/B of *Drosophila* (Meier *et al.*, 1991). In the late stage embryo of the moth *Manduca sexta* the distribution of ch organs in the abdominal segments shows a striking similarity to *Drosophila*, with a pair of ventral ch, a single lch and a parallel cluster of 4 ch in the lateral region (Grueber and Truman, 1999) which positionally correspond to *Drosophila* vchA and B, v'ch1 and lch5, respectively. It is interesting that the lch is composed of four sensilla in *M. sexta* compared with the five of *Drosophila*. Adult moths preserve a three sensillum dch in the thoracic segments, except noctuid moths, which have evolved a hearing organ around the sense organ and reduced the number of associated sensilla (Hasenfuss, 1997). As in *M. sexta* larvae, the lch5 homolog in the first abdominal segment is composed of four sensilla (Hasenfuss, 1997). The wealth of knowledge concerning the development of the *Drosophila* sensory nervous system and the role of homeotic genes in the regulation of segmental identity holds promise that a parallel investigation of sense organ development and homeotic genes in other arthropods could contribute greatly to a phylogenetic perspective on nervous system development.

Materials and Methods

Fly Stocks and Mutant Lines

Two classes of mutant embryos were analyzed, the null mutants and the sub-function allele mutants. Mutant lines include the following: *Antp*25 red¹ e¹/TM3, Sb¹ (*Antp*), *Scr*⁴ red¹ e¹/TM6B, Tb¹ (*Scr*), *Ubx*^{bx}d-55i/TM1 (*bx*d⁵⁵ⁱ), T(2;3) *abd-A*^{MX1}, mwh¹, jv¹, st¹, red¹, Sb^{bx}d-2, *abd-A*^{MX1} (*abd-A*^{MX1}), *Df*(3R) *Ubx*¹⁰⁹, Sb¹/Dp(3;3)P5 (*Df*(3R) *Ubx*¹⁰⁹), *Ubx*^{abx-1}/TM1 (*abx*¹) and Sb¹/1n(3LR) *Ubx*¹⁰¹ (*Ubx*¹⁰¹). Lines *Df*(3R) *Ubx*¹⁰⁹ and *abx*¹ were crossed to obtain hemizygous *abx*¹ embryos.

Immunostaining

Embryos were dechorionated in 50% household bleach (sodium hypochlorite), rinsed with double distilled water, and fixed in a 1:1 heptane:fixative (3.7% formaldehyde) solution. Embryos were devitellinized in a 1:1 solution of heptane and methanol. The MAb22C10 (Developmental Studies Hybridoma Bank, Iowa City, USA) antibody was used at a concentration of 1:7. The antibody, anti-pdm-1, used at a concentration of 1:100 and anti-fishhook at a concentration of 1:100 (Ma *et al.*, 1998). For a brown staining antibody reaction, a 1:1 solution of 100 µl 3 mg/ml diaminobenzidine (DAB) in 100 µl 1% PBS was used in the final step. For a blue staining antibody reaction 0.2% nickel chloride was added to the DAB solution. The staining reaction was developed by the addition of 1 µl of 30% hydrogen peroxide. A Zeiss Axioskop microscope with Nomarski optics equipped with a Dage DC330 3 CCD camera (Dage-MTI, Michigan City, USA) was used to image embryos and larvae. A series of focal planes were projected onto a single plane using the program Adobe Photoshop (Adobe Systems Inc., San Jose, USA).

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