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The role of the *teashirt* gene in trunk segmental identity in *Drosophila*

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Summary

The phenotypes of different mutant combinations of *teashirt* (*tsh*) and homeotic genes together with their regulatory interactions are described in order to gain insight into *tsh* gene function. We show that when *tsh*, *Scr*, *Antp* and BX-C genes are missing, the ventral part of the trunk (or thorax and abdomen) is transformed to anterior head identity showing that *tsh* is a homeotic gene. These genes act synergistically to suppress the expression of the procephalic gene *labial* (*lab*) in subsets of cells in each segment of the trunk. Transcripts from the *tsh* gene always accumulate in segments destined to acquire trunk identities. *tsh* gene activity is required for the normal function of the *Antp* and BX-C genes, which modulate in part the expression of *tsh*. As a whole, our results suggest that *tsh* plays an essential dual role, during embryogenesis, for determining segmental identity of the trunk. First, *tsh* is required critically for the identity of the anterior prothorax. Second, *tsh* is required globally for segmental identity throughout the entire trunk whereas the "classical" homeotic genes have more specific roles. Our results are consistent with the idea that *tsh* is defining the ground state of the *Drosophila* trunk region seen in the absence of the *Antp* and BX-C genes.

Key words: *Drosophila*, homeotic genes, *teashirt* gene, segmental identity.

Introduction

The body plan of higher animals, to some extent, has developed along a common theme. The head region, the trunk region and the terminal parts are morphologically distinct in fully differentiated animals. For insects (Fig. 1), these regions or tagma are defined by the head (acron, procephalon and gnathocephalon), trunk (thoracic and abdominal) and tail (abdominal segments posterior to A8 and telson) domains.

In *Drosophila*, the identity of segments in these regions depends on the function of a group of selector genes (Garcia-Bellido, 1975) called the homeotic loci (Lewis, 1978; Wakimoto and Kaufman, 1981; Sanchez-Herrero et al., 1985; Duncan, 1987; Kaufman et al., 1990). In this insect, two clusters of genes, the Antennapedia (ANT-C) and Bithorax (BX-C) complexes (Lewis, 1978; Kaufman et al., 1990), control sets of genes necessary for the identity of certain, but not all known, segments of the head (procephalic and gnathocephalic) and trunk (thorax and abdomen) of the larva and adult fly. For example, in embryos, absence of the *Antennapedia* (*Antp*) gene causes a replacement of the posterior prothorax, mesothorax and anterior metathorax (i.e. parasegments or PS 4 and 5; see Fig. 1 and Martinez-Arias and Lawrence, 1985, for the definition of parasegments and segments) with posterior labial, prothoracic and anterior mesothoracic (or PS 3 and 4) ones respectively (Kaufman et al., 1990; Schneuwly and Gehring, 1985; Martinez-Arias, 1986).

The ANT-C and BX-C genes have been cloned and their transcription patterns (see Fig. 1) and protein distribution patterns determined (reviewed in Kaufman et al., 1990; Akam, 1987). Each gene possesses a homeobox (McGinnis et al., 1984; Scott and Weiner, 1984), coding for a 60 amino acid domain required for regulation of downstream genes. A similar clustering of homeobox genes exists in vertebrates (Duboule and Dolle 1989; Graham et al., 1989), including man (Boncinelli et al., 1988), indicating that these clusters may be controlled in part by a common gene network, and may have common targets (McGinnis et al., 1990) across a wide spectrum of animals.

If the *Scr*, *Antp* and BX-C (or trunk) genes are removed, all trunk segments exhibit both prothoracic and gnathal identities (Struhl, 1983; Sato et al., 1985) and consequently there is an unknown function that determines the prothoracic identity of the trunk. With respect to function, this prothoracic-gnathal identity defines a ground state, a term initially proposed by Lewis (1963), which is the prototype upon which certain ANT-C and the BX-C genes act to modify segment identity.

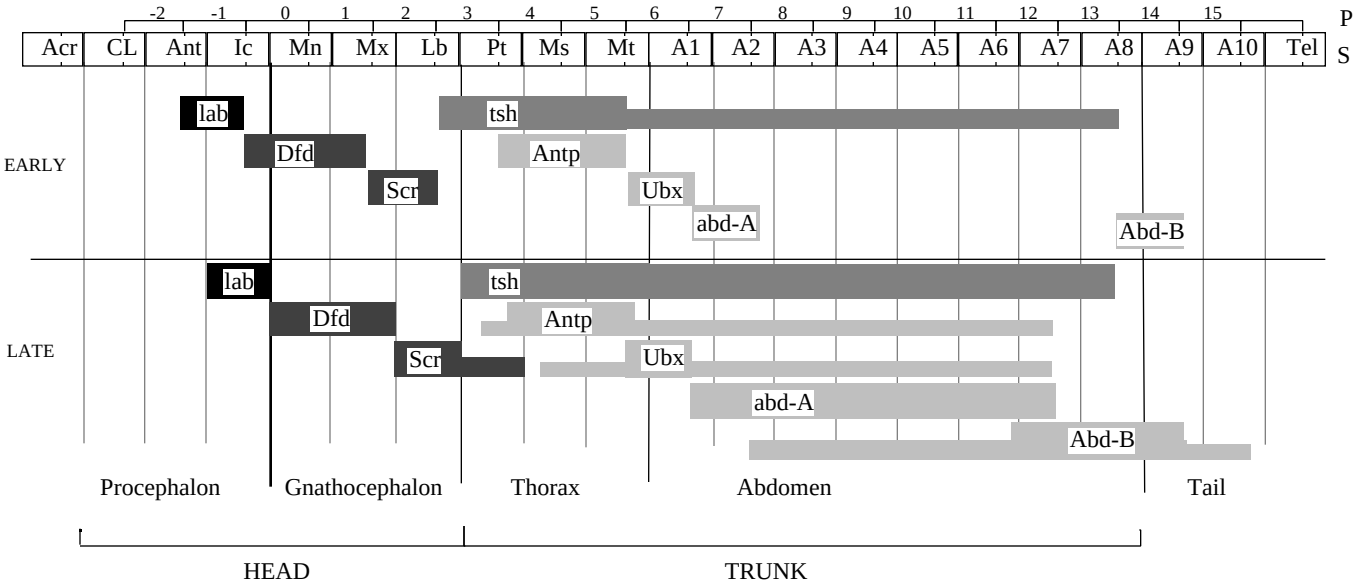


Fig. 1. Summary of the regions where RNA accumulates during embryogenesis from the homeotic genes of the ANT-C, BX-C and the *tsh* gene. At the top the segmental (S) and parasegmental (P) metameric divisions are indicated. At the bottom the principle morphological parts, or tagmata, of the insect body are indicated. The accumulation of transcripts at the blastoderm (Early) and germ band retraction (Late) stages are shown for each of the genes. Only epidermal expression patterns are given. Abbreviations and references: *labial* (*lab*, Diederich et al., 1989), *Deformed* (*Dfd*, Chadwick and McGinnis, 1987; Martinez-Arias et al., 1987), *Sex combs reduced* (*Scr*, Kuroiwa et al., 1985; Mahaffey and Kaufman, 1987; Martinez-Arias et al., 1987), *Antennapedia* (*Antp*, Wirz et al., 1986; Martinez-Arias, 1986), *Ultrabithorax* (*Ubx*, Akam and Martinez-Arias, 1985), *abdominal-A* (*abd-A*, Harding et al., 1985), *Abdominal-B* (*Abd-B*, Kuziora and McGinnis, 1988; Sanchez-Herrero and Crosby, 1988) and *teashirt* (*tsh*, Fasano et al., 1991; this study). The ANT-C genes *lab*, *Dfd*, *Scr* and *Antp* are required for the identity of intercalary, mandibular-maxillary, labial-prothoracic and anterior thoracic segments respectively; the BX-C genes *Ubx*, *abd-A* and *Abd-B* identify posterior thoracic and abdominal segments (Kaufman et al., 1990; Lewis; 1978; Sanchez-Herrero et al., 1985) and *tsh* is required throughout the trunk region (Fasano et al., 1991).

Earlier we described a gene that we called *teashirt* (*tsh*), which belongs to the homeotic class of genes; it is expressed in the trunk region of the embryo in a pattern similar, but not identical, to the known homeotic genes. However, it is clearly different from the classical homeotic genes: it encodes a zinc finger protein and mutations disrupt the entire trunk domain (Fasano et al., 1991). The phenotype of *tsh* and different homeotic mutations is described in addition to their regulatory interactions to gain insight into the role of *tsh* in trunk development. We show that the tsh^{+} gene: (1) is essential for the specific identity of the anterior prothorax, (2) acts independently of the trunk homeotic genes for promoting trunk identity, (3) acts synergistically with the *Antp* and BX-C genes, to repress anterior head development and head gene activity in the trunk, (4) is modulated and required by the *Antp* and BX-C genes for their complete function. Together our results show that *tsh* is a unique homeotic gene essential for global trunk identity. In particular, *tsh* seems to define the basal segmental identity (or ground state) of the trunk upon which the trunk homeotic genes act to modify segmental identity.

Materials and methods

Drosophila *stocks, egg collections and cuticle preparations*

The alleles of the homeotic genes used were Dfd^{RX1} (Hazelrigg and Kaufman, 1983), lab^{f8} (Merrill et al., 1989), $Antp^{W10}$ (Riley et al., 1987), Scr^{W17} (Wakimoto and Kaufman, 1981), tsh^{8} (Fasano et al., 1991), *Df(2L)TW161* (Wright et al., 1976), *Df(2L)305* (the kind gift of B. Wakimoto) which removes only the *tsh* gene in combination with tsh^{8} (Coré and Kerridge, unpublished data), *Df(3R)P9* (Lewis, 1978), $Ubx^{9.22}$ (Kerridge and Morata, 1982), and sal^{IIA55} (Jürgens, 1988). The Scr^{C1} $Antp^{Ns+RC3}$ *Df(3R)P9* chromosome is described by Struhl, (1983) and the $Antp^{W10}$ *Df(3R)P9* one by Riley et al. (1987). Egg lays were made from *trans* heterozygotes over a wild-type chromosome where possible. Since *Df(3R)P9* requires two copies of the *Abd-B* gene for fertility, *Dp(3;3)P5* (Lewis, 1978), which is a tandem duplication of the BX-C, was used in the relevant crosses. Cuticle preparations were done as in Fasano et al. (1991).

Probes and in situ hybridisation to whole embryos

The in situ hybridisation technique is described in Fasano et al., (1991). The probes used for in situ hybridisation to whole embryos were: *lab*: a 1.5 kb *Xho*I- *Xba*I cDNA fragment (Diederich et al., 1989); *Dfd*: a 5 kb *Eco*RI genomic fragment; *Scr*: a 5 kb *Hind*III genomic fragment (Martinez-Arias et al., 1987); *tsh*: a 2.5 kb *Eco*RI cDNA fragment (Fasano et al.,1991); *Antp P1*: a 3.6 kb *Eco*RI genomic fragment; *Antp P2*: a 1.2 kb *Hin*dIII- *Eco*RI, from the 4.6 kb *Eco*RI, genomic fragment (Bermingham et al., 1990); *Ubx*: a 1.44 kb *Hin*dIII-*Eco*RI genomic fragment from the 5′ end of the gene (Irish et al., 1989); *abd-A*: a 2 kb *Ava*I fragment (a gift from P. Ingham); and *Abd-B*: a 2.8 kb *Pst*I fragment common to all three *Abd-B* transcripts (Kuziora and McGinnis, 1988). The different fragments were PCR amplified and marked with digoxygenin (DIG)-labelled nucleotides (Boehringer-Mannheim).

Double labelling by whole mount *in situ* and antibody staining was performed essentially as described by Cohen (1990) with the following modifications: devitillinized eggs were treated for 15

minutes with 3% H_2O_2 in methanol before rehydration. Proteinase K treatment was for 2 minutes 30 seconds (a suggestion from Dougan and DiNardo). Embryos were then hybridized with DIG probes (see above), washed and blocked with 0.1% bovine BSA, 0.1% Tween in PBS. The eggs were incubated overnight at 4°C with dilutions of anti-invected (the gift of M. Wilcox) and anti-DIG antibodies simultaneously. Following standard washes, embryos were incubated with biotinylated anti-mouse IgG at a dilution of 1:400. All other steps were as described by Cohen (1990).

Heat shock experiments

Embryos, with P elements carrying the *Scr* (HSS), *Ubx* (HSU) or *Antp* (HSA) mini genes under the control of a heat shock promoter, were collected following 2 hour egg lay periods. The HSU P element is that described in Gonzalez-Reyes et al. (1990), the HSA one by Gibson and Gehring (1988) and the HSS one was a gift from M. Scott. All are localized on chromosome 3. After aging for 2 or 4 hours, eggs were heat shocked in a water bath at 36°C using one of the following regimes: 1 hour, 2 hours or three 25 minute heat shocks. For multiple heat shocks, eggs were left to recover at 25°C for $1\frac{1}{2}$ hours between each heat shock. For *in situ* hybridization, eggs were aged for 2 or 4 hours and treated as described above; half the embryos were labelled with *tsh* probe and, in order to verify efficient expression of the respective homeotic gene following heat shock, the other half were probed with *Scr*, *Antp* or *Ubx* probes. For cuticle preparations, eggs were allowed to develop at 25°C for 48 hours and the larvae mounted as described above.

Results

A closer look at the mutant phenotype and transcription pattern of the tsh *gene*

Fasano et al. (1991) showed that *tsh* null mutations disrupt all of the ventral trunk region and especially result in the loss of patterns of PS3 or the posterior labium and anterior prothorax of the differentiated larva. Every trunk (thoracic and abdominal) segment is smaller and sclerotic cuticle differentiates in ventro-lateral positions located between the denticle belts (Fig. 2A and B). Internally, a striking phenotype observed in *tsh* null mutations is that the trunk-specific ventral set of neuron clusters is disrupted suggesting, but not proving, a partial transformation to a gnathal-like arrangement (Fasano et al., 1991). For 10-20% of tsh^- individuals the prothoracic clusters resemble those found in the labial segment (Figs. 3A and B). Mutations in the *tsh* gene can therefore be interpreted in two ways; either they partially transform the trunk segments into a gnathal-like identity, and in particular the prothoracic segment into a labial one, or they cause a non-specific change in segmental identity perhaps due to cell death.

The evolution of *tsh* transcription during embryogenesis suggests that the gene is similar to the classical homeotic ones (Fasano et al., 1991). It is initiated at the blastoderm stage and then is expressed in all trunk segments. Here we analyse in more detail the evolution of *tsh* transcription during the transition from parasegmental to segmental morphology of the wild-type embryo. Normal embryos were doubly labelled to detect *tsh* transcripts as well as the *invected* protein, which provides a marker for the posterior compartments of each segment similar to the *engrailed* protein (Coleman et al., 1987). In the fully extended germ band (Fig. 4A), the anterior border of *tsh* transcripts occupies cell for cell the *invected* stripe localized in PS 3. During retraction of the germ band, transcripts from the *tsh* gene become segmental in the epidermis, being restricted to the thorax and first eight abdominal segments (Fig. 4B). However, in the central nervous system, *tsh* mRNAs are found in the posterior part of the labial segment (Figs. 4B and C). Thus in the epidermal cells *tsh* is expressed in a parasegmental and then a segmental register whereas in the central nervous system its expression remains parasegmental.

tsh *and the homeotic genes act at the same level in the gene hierarchy*

The homeotic-like nature of the *tsh* gene prompted us to analyse its cross-regulatory interactions with the classical homeotic genes. If *tsh* is a bona fide homeotic gene then it should have three properties with respect to this regulation: first, that its transcription is initiated independently of other homeotic genes, second that maintenance of its transcription depends on the activity of other homeotic genes and third that tsh^+ function is required for the maintenance of transcription from other homeotic genes.

For the transcript distributions of the different genes in different mutant situations described below, we focus on the epidermal patterns of expression. No deviation from the wild-type patterns of expression of the homeotic genes or the *tsh* one (see Fig. 1) have been found before the fully extended germ band stage in any mutant situation analysed. Thus transcription of *tsh* and the homeotic genes are initiated and evolve independently of each other from the blastoderm through to the early stages of gastrulation. During later stages of embryogenesis however, specific regulatory interactions have been discovered.

Maintenance of tsh *transcription is modulated by the trunk but not the head homeotic genes*

First, the expression pattern of *tsh* in *Sex combs reduced* (*Scr*), *Deformed* (*Dfd*) and *labial* (*lab*) mutant embryos has been followed. No change in the spatial distribution of *tsh* mRNAs can be detected (data not shown). We have also examined the expression of *tsh* in embryos carrying the Scr^+ gene under the control of a heat shock promoter (HSS). In these embryos, following 1 hour or multiple heat shocks during embryogenesis, *tsh* expression is unchanged in comparison to the wild-type pattern (not shown). These results show that the activity of these head genes are irrelevant for *tsh* expression.

In *Antennapedia* (*Antp*) null mutants at the extended germ band stage, significantly lower levels of *tsh* transcripts are seen in PS 4 and 5 (Fig. 5C) compared to wild-type embryos. During germ band retraction (Fig. 5D), *tsh* transcripts are not found in the cells which are transformed to labial identity (the posterior prothorax). These observations show that $Antp^+$ gene activity is required to maintain a normal level of *tsh* transcription throughout PS 4 and 5, the epidermal domains of $Antp^+$ gene function (Schneuwly and Gehring, 1985; Martinez-Arias, 1986), and especially for maintaining *tsh* transcription in the posterior compartment of the prothorax.

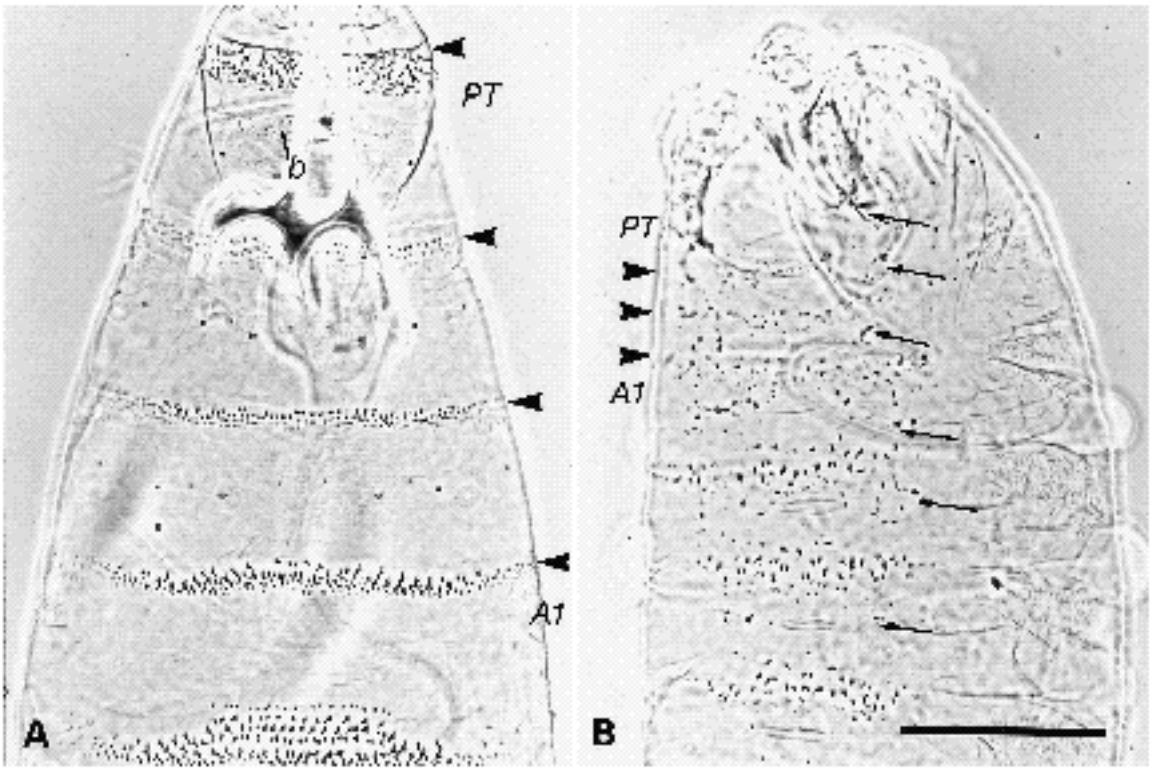


Fig. 2. A comparison of the larval cuticular phenotypes of wild-type (A) and a *tsh* (B) null mutation. For a description of the wild-type cuticular phenotype see Lohs-Schardin et al. (1979). In A, note the typical array of ventral denticle belts and distinguishing features of the individual thoracic and abdominal segments. In B, the denticle belts throughout the thorax and abdomen are disrupted, the prothoracic denticle belt is missing and the presence of sclerotic cuticle in each trunk segment (arrows). Abbreviations: b: prothoracic beard; PT: prothorax; A1: first abdominal segment. The anterior limits of the prothoracic, mesothoracic, metathoracic and first abdominal segments are indicated with large arrowheads. Bar represents 50μm.

In *Ultrabithorax* (*Ubx*) mutations at the germ band elongation stage, *tsh* transcription is similar to wild type but higher levels of transcripts are detected in PS 6 (Fig. 5E), the principle site of Ubx^+ gene function (Lewis, 1978). In the absence of the three BX-C genes, *Ubx*, *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*), *tsh* is expressed more strongly throughout the abdominal region (PS 6-13; Fig. 5F), as in PS 4 of wild-type embryos. The strong expression of *tsh* observed in these cases is probably mediated by $Antp^+$ gene activity which is expressed strongly in these parasegments (Hafen et al., 1984; Carroll et al., 1986).

In the absence of the *Antp* and BX-C or *Scr*, *Antp* and BX-C gene activities, *tsh* expression is normal until the end of the elongated germ band stage. During retraction of the germ band, *tsh* transcripts are no longer detected in the posterior compartments of each trunk segment and in the anterior compartments *tsh* is strongly expressed (Fig. 5G and H). Therefore, the evolution of *tsh* transcript distribution in this mutant resembles that of PS 3 in the epidermis of wild-type embryos (Figs 4, 5A and B); in the posterior compartment, the absence of *tsh* mRNA accumulation correlates with labial identity (Struhl, 1983; Sato et al., 1985). These observations indicate that the maintenance of *tsh* expression, in the posterior compartments of trunk segments is dependent on a common function of the $Antp^+$ and $BX\text{-}C^+$ genes, whereas in the anterior compartments it is independent and correlates with prothoracic identity.

Transcription of some head but not trunk homeotic genes are modulated by the tsh *gene*

Transcripts from the the *lab* gene are located in PS-1 at the blastoderm and the germ band elongation stages in wild-type embryos (Fig. 6A; Diederich et al., 1989). Once segments are formed, during retraction of the germ band, *lab* transcripts occupy the intercalary segment, the optic lobe and dorsal ridge (Fig. 6B; Diederich et al., 1989). Below we describe the patterns of *lab* mRNAs in different loss of function mutations for *tsh*, ANT-C and BX-C genes. None affect the wild-type pattern of *lab* in the head and none have a novel accumulation pattern of *lab* transcripts before the extended germ band stage.

In *tsh* null mutations at the extended germ band stage, the *lab* gene is expressed ectopically in PS 4 to 13 in a small number of cells in each segment close to the primordia of the tracheal placodes of the trunk (Fig. 6C; see Glaser and Shilo, 1991) and in some cells at the ventral midline (not shown see Fig. 9C).

None of the homeotic mutations of the ANT and BX complexes, alone or in combination, alter the pattern of *lab* mRNA distributions compared to wild type in embryos until after the extended germ band stage. In $Antp^-$ embryos during retraction of the germ band, *lab* expression deviates from the wild-type pattern for the first time; it is expressed ectopically in the posterior dorsal part of the prothoracic

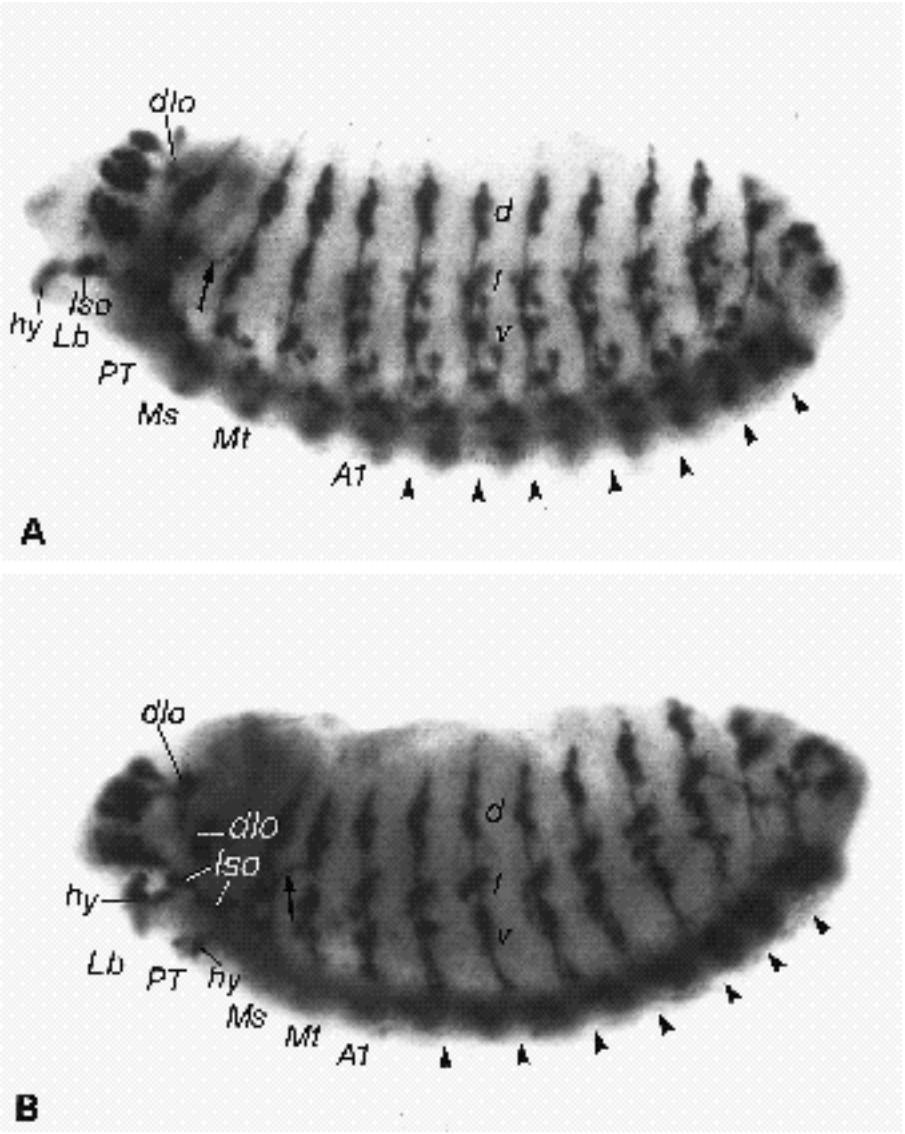


Fig. 3. The peripheral nervous system of wild-type (A) and a *tsh*^{8}*/Df(2L)TW161* (B) embryos at germ band retraction. The trunk segments of wild-type larvae are made up of three thoracic segments, the prothorax (Pt), mesothorax (Ms) and metathorax (Mt), and 8 abdominal segments (marked with arrowheads: first abdominal segment: A1). The segment immediately anterior to the trunk is called the labial (Lb) segment. The neuron clusters of the peripheral nervous system have been revealed using the antibody 22C10. Each trunk segment has three groups (dorsal, d; lateral, l; and ventral, v; labelled here between the third and fourth abdominal segments only) of neuron clusters which are variations on the same theme. The labial segment has neuron clusters of only dorsal and lateral origin (see Fig. 6 in Ghysen and O'Kane, 1989), which give rise to the hypophysis (hy), the labial sense organ (lso) and a previously undiscovered cluster we call the dorsal labial organ (dlo). The clusters deriving from more head segments are normal and their names can be found in Ghysen et al., (1986). In B, note that the clusters in the prothorax (PT) resemble those of the labial segment (Lb) and have lost their typical trunk characteristics; a small hy, an lso and a dlo differentiate in the prothoracic position (labelled in white). Only two thoracic and the usual 8 abdominal segments remain. Note also that the posterior mesothoracic compartment has a posterior prothoracic identity since a prothoracic specific neuron cluster, *Ich 3*, sends an axon towards the metathoracic segment (large white arrow in B; a black one in A). Anterior is left and dorsal at the top for each photograph. Bar represents 50μm.

segment (Fig. 6D). In BX-C^{-} embryos, *lab* expression is indistinguishable from the wild-type pattern at all stages (not shown). In *Antp*$^{-}$ BX-C^{-} embryos however, *lab* transcripts are found in the dorsal parts of each trunk segment (not shown); similarly for *Scr*, *Antp* and BX-C null mutants we found the same ectopic pattern of *lab* gene expression and in addition *lab* is expressed ectopically in the labial lobe (Fig. 6E). Thus the *tsh* gene acts early and *Scr*, *Antp* and BX-C genes act late to suppress *lab* gene expression in different subsets of cells within the trunk region.

The expression patterns of the *Dfd* and *Scr* genes in *tsh*$^{-}$ and other homeotic mutations has been described previously (Jack et al., 1988; Riley et al., 1987; Fasano et al., 1991). Only the expression of *Scr* is altered compared to wild type in *tsh* mutations.

We have examined the patterns of expression of the P1 and P2 transcripts of the *Antp* gene, plus the patterns of the *Ubx*, *abd-A* and *Abd-B* genes in *tsh* null embryos to ask whether or not these genes are expressed normally. The spatial distribution of all of these transcripts is as in wild type (data not shown; for wild-type expression patterns of these genes see Bermingham et al., 1990; Akam and Martinez-Arias, 1985; Harding et al., 1985; Kuziora and McGinnis, 1988; Sanchez-Herrero and Crosby, 1988) showing that the expression of these genes is independent of *tsh*$^{+}$ gene activity.

In summary, our results show that transcriptional activation of the *tsh* gene occurs independently of the homeotic genes at the blastoderm stage and that initiation of transcription of the homeotic genes is independent of *tsh* and other homeotic gene activities. In addition, we show that *tsh* is at the same level as the homeotic genes in the hierarchy of gene interaction; i.e. *tsh* as well as the homeotic gene mutations may alter the maintenance of expression of those genes that are initiated in more anterior positions but never those initiated in more posterior domains. The phenotype of *tsh* mutations can be considered to be a mixture of normal (*Antp*, *Ubx*, *abd-A* and *Abd-B*) and ectopic (*lab*) homeotic gene products in the trunk.

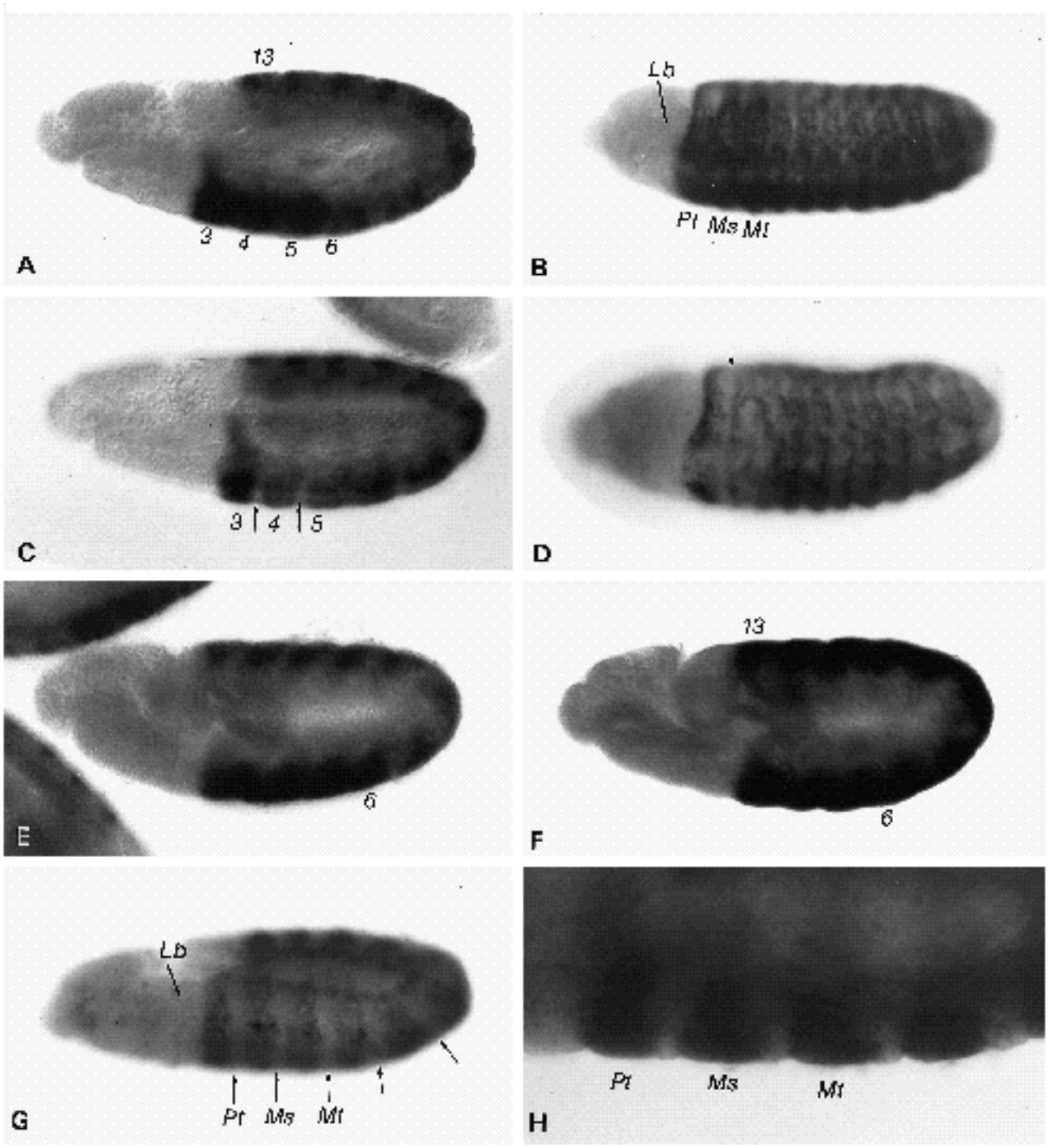


Fig. 5. The distribution of *tsh* transcripts in different homeotic gene mutations. Genotypes are: wild type (A, B), and homozygotes for $Antp^{W10}$ (C, D), $Ubx^{9.22}$ (E), *Df(3R)P9* (F) and Scr^{13A} $Antp^{Ns+RC3}$ *Df(3R)P9* (G, H). In homozygotes for the *Antp* null allele (C, D), *tsh* transcripts are weakly expressed, compared to wild type (A) in PS 4 and 5, where ANTPprotein is abundant, especially in the posterior compartments (little arrows). In the abdomen, levels of *tsh* messages are comparable in wild type (A) and *Antp* mutants (C). By germ band retraction (B, D), a line of cells in the posterior prothorax lack *tsh* transcripts in $Antp^{W10}$ homozygotes (D, arrow). In Ubx^{-} embryos (E), *tsh* transcripts are more abundant in PS 6 compared to wild type (A). In the absence of BX-C genes (F), *tsh* messages are abundantly expressed in PS 6-13. In embryos lacking products of *Scr Antp* and BX-C genes (G, H), *tsh* is not detected in the posterior part of each segment (arrows in G). In H, note that about four cells do not transcribe *tsh* in each segment; this is the expected size of the posterior compartment. Numbers indicate the identity of parasegments; otherwise segmental designations are shown: labial (Lb), prothoracic (Pt), mesothoracic (Ms) and metathoracic (Mt) segments are labelled. Embryos, in E and F, are at the germ band extension stage, whereas A, C, G and H are during early germ band retraction and B and D at the end of germ band retraction. See Campos-Ortega and Hartenstein (1985) for developmental stages.

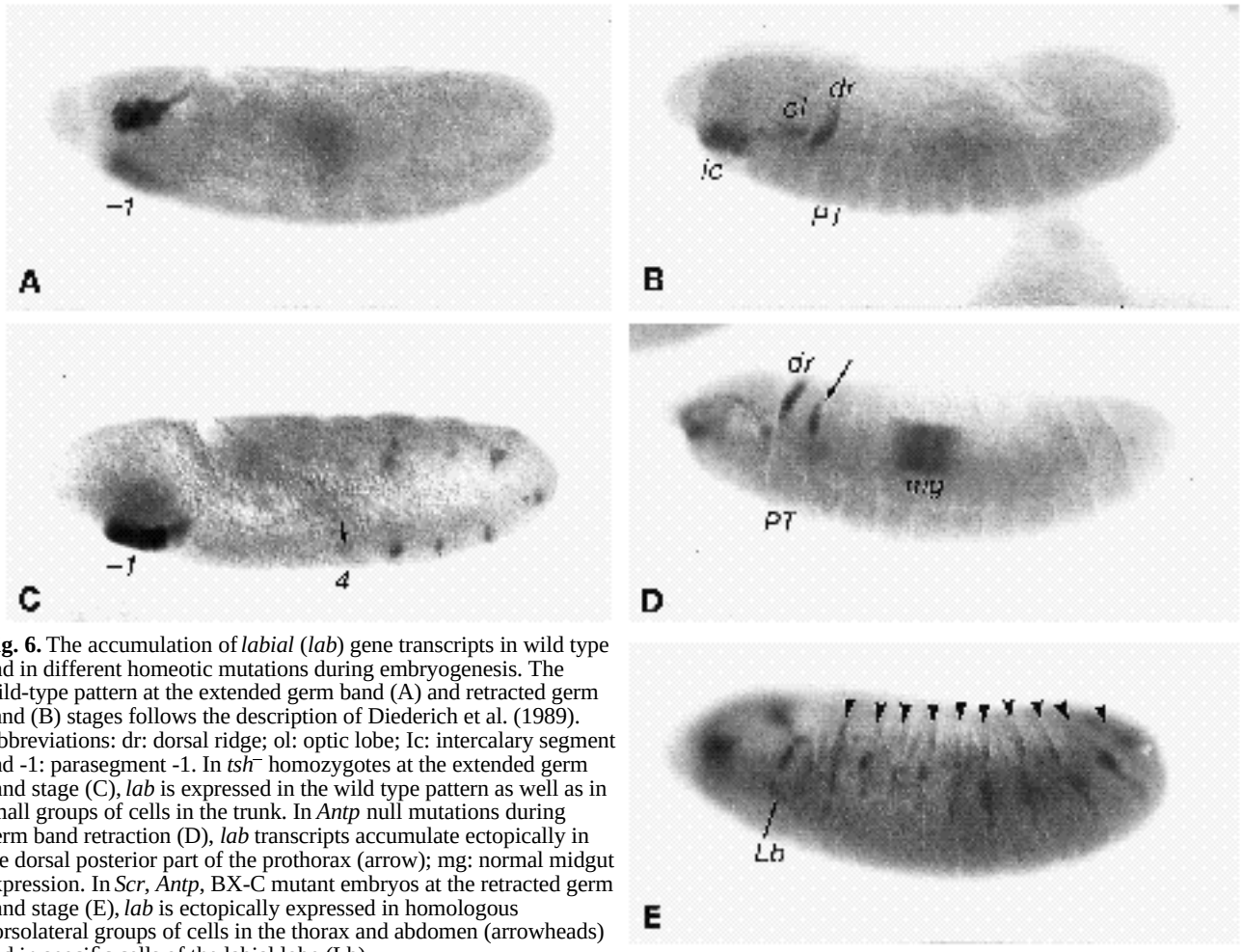


Fig. 6. The accumulation of *labial* (*lab*) gene transcripts in wild type and in different homeotic mutations during embryogenesis. The wild-type pattern at the extended germ band (A) and retracted germ band (B) stages follows the description of Diederich et al. (1989). Abbreviations: dr: dorsal ridge; ol: optic lobe; Ic: intercalary segment and -1: parasegment -1. In *tsh*$^-$ homozygotes at the extended germ band stage (C), *lab* is expressed in the wild type pattern as well as in small groups of cells in the trunk. In *Antp* null mutations during germ band retraction (D), *lab* transcripts accumulate ectopically in the dorsal posterior part of the prothorax (arrow); mg: normal midgut expression. In *Scr*, *Antp*, BX-C mutant embryos at the retracted germ band stage (E), *lab* is ectopically expressed in homologous dorsolateral groups of cells in the thorax and abdomen (arrowheads) and in specific cells of the labial lobe (Lb).

The tsh *gene is essential for anterior prothoracic identity*

From the phenotypic analysis of *tsh* mutations (Figs 2 and 3) and the analysis of its cross regulatory interactions with homeotic genes (Figs 5 and 6) it seems that *tsh*$^+$ gene activity is essential for the development of the prothorax. For example, in the absence of all homeotic gene activity, where all trunk segments have a prothoracic-like identity (Struhl, 1983; Sato et al., 1985), the *tsh* gene is transcribed (Fig. 5G and H). An apparent paradox arising with this idea is that *Scr*$^+$ gene activity is known to be essential for identity of the anterior prothorax. Null mutations at the *Scr* locus cause an incomplete transformation of the prothorax to a mesothoracic segment (Wakimoto and Kaufman 1981; Pattatucci et al., 1991) as well as a homeotic transformation of part of the labial segment to a maxillary one. Furthermore, ectopic expression of the *Scr* protein under the control of a heat shock promoter (HSS) induces the transformation of the second and third thoracic segments to prothoracic ones (Gibson et al., 1990).

To analyse whether *tsh* has a critical role for the identity of the anterior prothorax, the cuticular phenotype of *tsh*$^-$ larvae developing with high levels of *Scr* protein, were examined. Following heat shock treatments of HSS *tsh*$^-$ embryos during embryogenesis, two phenotypic differences are observed compared to HSS *tsh*$^+$ or *tsh*$^-$ controls. First, no prothoracic patterns develop and second the numbers of denticles in the trunk segments are reduced (see Figs 7A and 2B). Therefore, in the absence of *tsh*$^+$ activity, the *Scr*$^+$ gene cannot promote prothoracic identity and represses trunk (denticle belt) development instead.

We have also compared the cuticular phenotypes of *tsh* and *tsh Scr* double mutations. The only phenotypic difference in the double compared to single mutants is that the dorsal part of the prothoracic trunk segment is made whereas it is missing in *tsh* mutations (compare Figs 7B and 2B). This shows that *Scr*$^+$ gene activity suppresses dorsal trunk development in the prothorax in the absence of *tsh*$^+$ gene activity.

Mutations in the *spalt* (*sal*) gene (Jürgens, 1988) cause anterior prothoracic patterns to differentiate in the labial segment, providing an independent test to ask whether *tsh*$^+$ activity is linked to prothoracic identity. In *sal* mutant homozygotes, *tsh* is expressed ectopically, for the first time at the germ band extension stage as well as at later stages, in the position of the labium (Fig. 8). This same mutant also replaces two tail segments with trunk segments (Jürgens, 1988) and *tsh* is transcribed ectopically in these segments (Fig. 8) showing that one normal maintenance func-

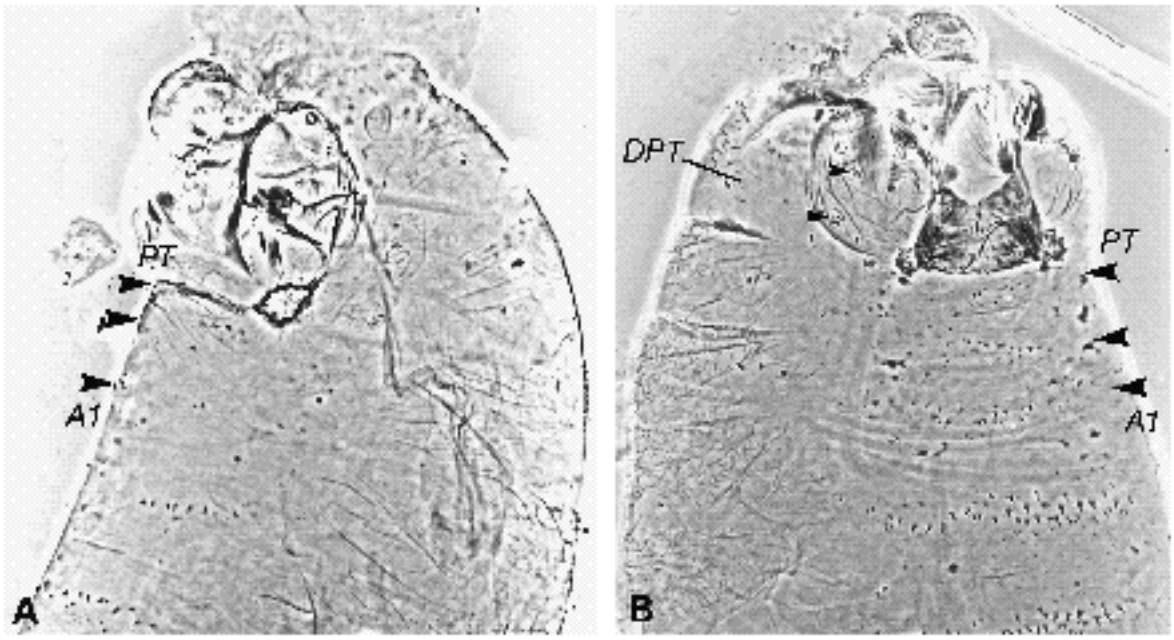


Fig. 7. The cuticular phenotypes of *tsh*$^{-}$ larvae that developed with high levels (A) or in the absence (B) of *Scr*$^{+}$ gene activity. Note that when the *Scr* gene is expressed ectopically, prothoracic denticles do not form in the thorax, as is the case in *tsh*$^{+}$ larvae (Gibson et al., 1990). The number of denticles is reduced in each segment compared to *tsh*$^{-}$ larvae (see Fig. 2B). In more extreme examples, the thoracic denticles may be absent whereas in more posterior segments, a reduced number of abdominal denticles always differentiate. Control larvae (not shown) carrying a *tsh*$^{+}$ allele caused the transformation of second and third thoracic segments to prothoracic identity as described by Gibson et al., (1990). In the absence of *Scr* and *tsh* gene products (B), the prothoracic denticle belt is missing and maxillary sense organs (small arrowheads) are found in the labium due to the loss of *Scr* gene activity, as well as in the normal position. Note that the dorsal part of the prothorax differentiates (DPT) whereas in *tsh*$^{-}$ larvae it does not (compare with Fig. 2B).

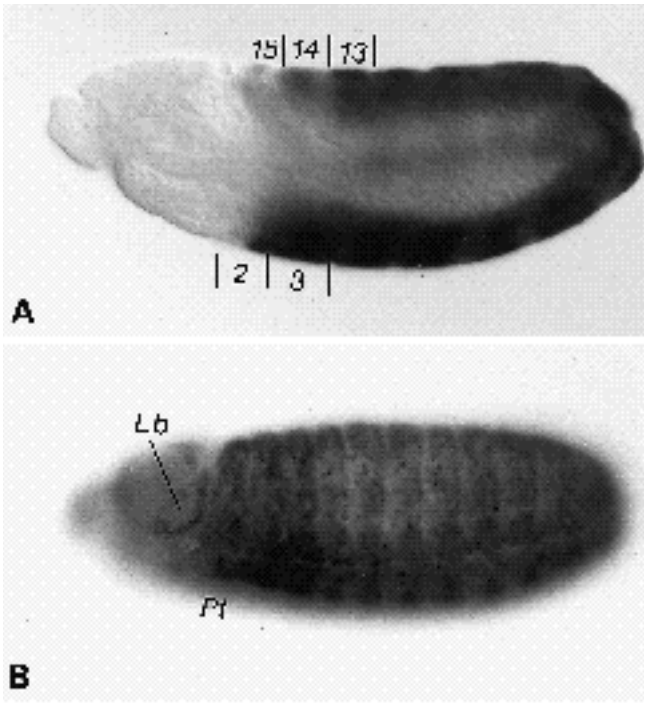


Fig. 8. Ectopic expression of *tsh* transcripts in *sal* homozygotes. The *tsh* transcription pattern at germ band extension (A) and at the retracted germ band (B) stages is shown. Compared to wild type (see Figs. 5 A and B), *tsh* is expressed ectopically in PS 2 (2) and the labial (Lb) as well as the tail regions (14 and 15). Abbreviations are the same as in the previous Figs.

tion of the *sal*$^{+}$ gene is to repress, directly or indirectly, *tsh* in the labial and tail domains. These observations are consistent with the idea that *tsh*$^{+}$ gene activity is always correlated with prothoracic and more generally with trunk identity. Together these results show that *tsh*$^{+}$ and *Scr*$^{+}$ activities are indispensable for the identity of the anterior prothorax.

tsh *and trunk homeotic genes together promote trunk and suppress head segmental identity*

Our results suggest that *tsh*$^{+}$ activity is associated with segments destined to differentiate denticle belts (i.e. trunk identity; Figs 4, 5, 6, 8) and never associated with segments destined to make head or other identities. However, the *Antp* and BX-C genes seem to have the same function since they are transcribed normally in *tsh*$^{-}$ embryos and in this genotype, denticles (although abnormal ones) are made (Fig. 2B). To test whether *tsh*$^{+}$ and the trunk homeotic genes act independently for trunk (i.e. denticle belt) identity, we have examined the cuticular phenotypes of *tsh*$^{-}$ larvae in the absence of trunk homeotic gene activities. To test whether these same genes also repress head development, we have monitored the expression of head genes in the same genotypes.

Amorphic *Antp* mutations cause replacement of the mesothoracic and metathoracic denticle belts with mixed prothoracic-mesothoracic and prothoracic-first abdominal ones respectively (Wakimoto and Kaufman, 1981; Mar-

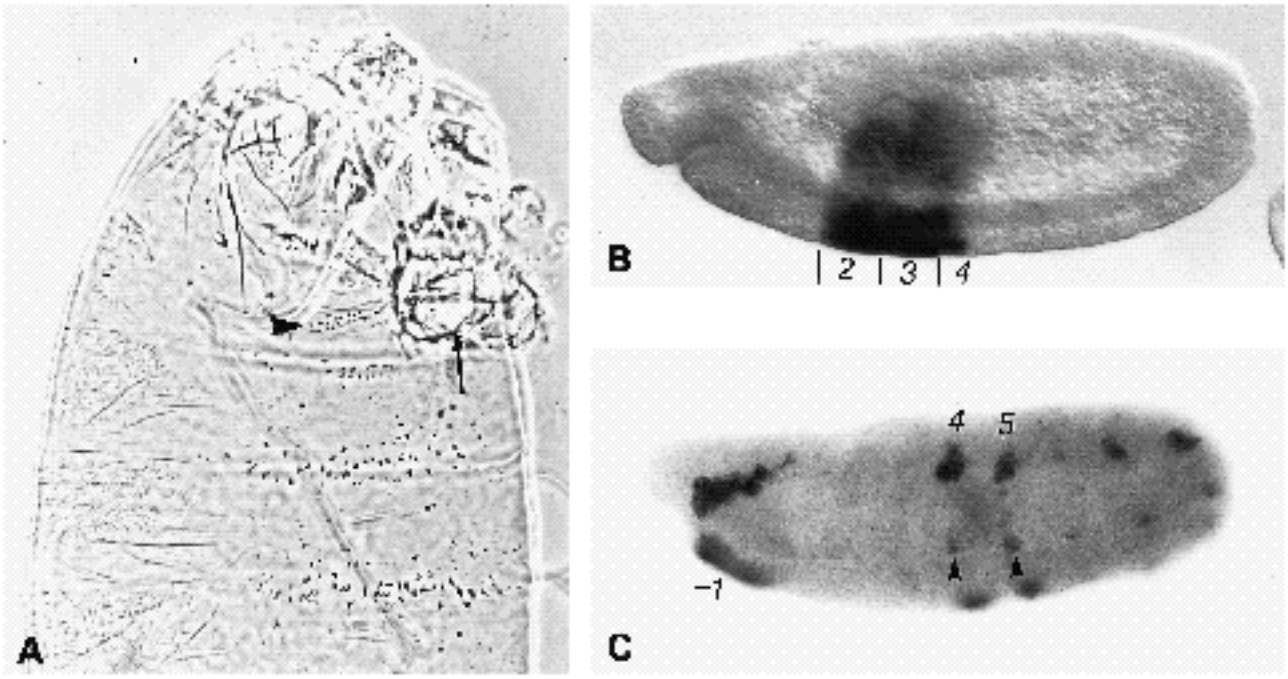


Fig. 9. The effects of removing *tsh*$^+$ and *Antp*$^+$ gene activities on larval cuticular patterns (A) as well as on the *Scr* (B) and *lab* (C) gene expression patterns. In *Antp tsh* double mutants, prothoracic and mesothoracic denticle belts are absent and replaced with head cuticle (arrow in A); a hemi denticle belt differentiates in the position metathorax (arrowhead). In the absence of *Antp* and *tsh* functions (B), *Scr* is expressed ectopically in the anterior part of PS 4. At the same developmental stage, *Scr* messages in wild type or *Antp*$^-$ mutations are restricted to PS 2 and to a small patch of dorsal cells in PS 3 (Riley et al., 1987; data not shown). In *tsh* mutant homozygotes, *Scr* shows the wild-type distribution pattern and is ectopically expressed in the ventral part of PS 3 (Fasano et al., 1991). There is no staining of the posterior midgut in this figure. The 2nd, 3rd and 4th parasegments are indicated. In the same genotype, *lab* is expressed ectopically as in *tsh*$^-$ (Fig. 6C) embryos except transcripts are expressed in more cells and more abundantly in parasegments 4 and 5, the principle sites of *Antp*$^+$ function. Note in this ventro-lateral view that cells in the ventral midline (small arrowheads) accumulate *lab* transcripts; a similar pattern is seen in *tsh* homozygotes.

tinez-Arias, 1986). In *tsh Antp* double mutant embryos the first two thoracic denticle belts are completely absent and replaced with cuticle typical of the head skeleton. In addition, the third thoracic belt is partially or completely deleted (Fig. 9A). Therefore, on the basis of the differentiation of denticle belts, the normal function of the *tsh*$^+$ and *Antp*$^+$ genes is to suppress head development and to promote normal thoracic identity. We have examined the expression of the *lab*, *Dfd* and *Scr* genes in this genotype. Compared to the expression pattern of these genes in *tsh* mutations alone, only the *Scr* (Fig. 9B) and *lab* (Fig. 9C) genes are expressed differently when *Antp*$^+$ activity is missing: *Scr* is expressed ectopically in PS 3 due to loss of *tsh*$^+$ activity (Fasano et al., 1991) and in the anterior part of PS 4 due to the combined loss of *tsh*$^+$ and *Antp*$^+$ products (Fig. 9B); the *lab* gene is expressed ectopically in PS 4-13 due to loss of *tsh*$^+$ gene activity (Fig. 6C) and more extensively in PS 4 and 5 (Fig. 9C) due to the absence of *Antp*$^+$ and *tsh*$^+$ gene activities.

When *Scr*, *Antp* and the three BX-C gene activities are missing, all the segments of the trunk have similar identities: in the anterior compartment a mixture of prothoracic and mesothoracic denticles (that we call prothoracic-like) develop, and in the posterior compartment labial sense organs differentiate (Struhl, 1983; Sato et al., 1985). If *tsh*$^+$ activity is removed in addition to these genes, the prothoracic-like denticle belts are missing (Fig. 10A) and replaced ventrally with cuticle typically found in the head skeleton; according to Jürgens et al. (1986), this cuticle derives from the procephalon and/or acron (or anterior head; compare Figs 10B and C). On the dorsal side of larvae of this genotype, trunk elements still differentiate indicating that the specification of dorsal trunk patterns is independent of *tsh* and the trunk homeotic gene functions tested here. In larvae mutant for *Antp*, BX-C and *tsh* genes, the *Scr*$^+$ gene activity suppresses the formation of this head cuticle in the most anterior thoracic region (not shown). No readily recognizable patterns (e.g. denticle belt) differentiate in this position, confirming that the *Scr*$^+$ gene cannot promote trunk (or denticle) identity alone but plays a role in suppressing anterior head identity. In conclusion, the *tsh* gene is critical for identity of the prothorax and, together with the homeotic genes *Antp*, *Ubx*, *abd-A* and *Abd-B*, is required for global trunk identity of the larvae.

In embryos lacking the *tsh*, *Antp* and the three BX-C gene functions, the expression of the *Scr* gene is identical to that described for *tsh*$^-$ *Antp*$^-$ embryos (Fig. 9B). On the other hand in the same genotype, *lab* is expressed as in *tsh*$^-$ mutations alone (Fig. 6C) except that this ectopic expression within PS 4-13 is more extensive (Fig. 10D). When the *Scr*$^+$ gene function is removed in addition to *tsh*, *Antp* and BX-C genes, the same deviation from the wild-

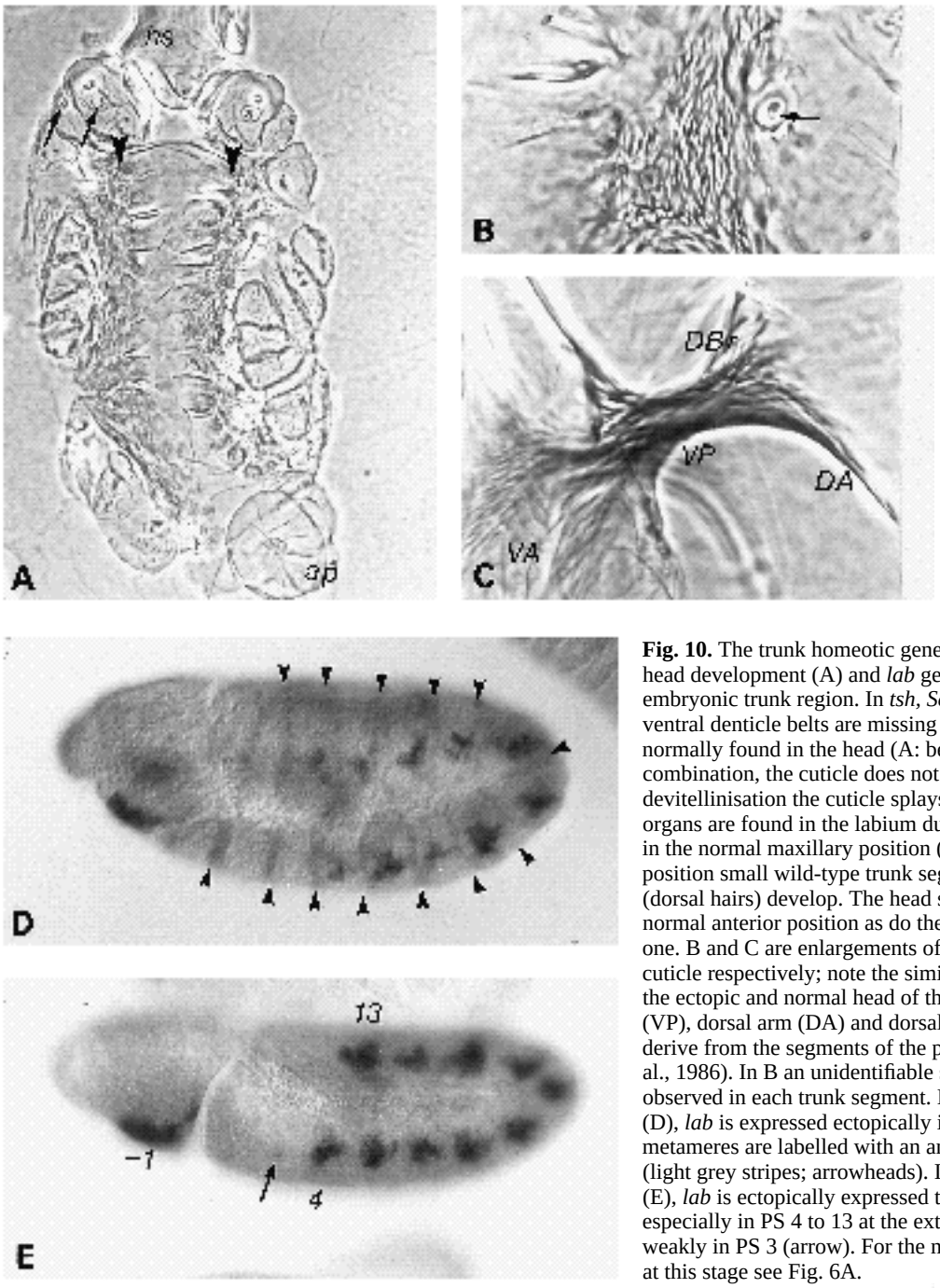


Fig. 10. The trunk homeotic genes act co-operatively to repress head development (A) and *lab* gene expression (D, E) in the embryonic trunk region. In *tsh, Scr, Antp*, BX-C mutations, the ventral denticle belts are missing and replaced with cuticle normally found in the head (A: below arrowheads). In this mutant combination, the cuticle does not fuse dorsally; upon devitellinisation the cuticle splays out as a sheet. Maxillary sense organs are found in the labium due to the *Scr* mutation as well as in the normal maxillary position (small arrowheads). In the dorsal position small wild-type trunk segments and pattern elements (dorsal hairs) develop. The head skeleton (hs) differentiates in its normal anterior position as do the anal pads (ap) in their posterior one. B and C are enlargements of the ectopic and normal head cuticle respectively; note the similarity of rippled cuticle found in the ectopic and normal head of the ventral arm (VA), ventral plate (VP), dorsal arm (DA) and dorsal bridge (DBr), all of which derive from the segments of the procephalon or acron (Jürgens et al., 1986). In B an unidentifiable sense organ (arrow) is often observed in each trunk segment. In *tsh, Antp*, BX-C mutations (D), *lab* is expressed ectopically in parasegments 4 to 13; here the metameres are labelled with an antibody to the *invected* protein (light grey stripes; arrowheads). In *tsh, Scr, Antp*, BX-C mutations (E), *lab* is ectopically expressed throughout the trunk region especially in PS 4 to 13 at the extended germ band stage and weakly in PS 3 (arrow). For the normal expression of the *lab* gene at this stage see Fig. 6A.

type pattern is observed, plus the presence of a small patch of *lab* expression in PS3 (Fig. 10E). Therefore, even though tsh^+ activity alone represses *lab* expression in homologous subsets of cells within the trunk, tsh^+ and Scr^+ activities repress *lab* in PS3; tsh^+ and $Antp^+$ in PS 4 and 5 and tsh^+, $Antp^+$ and BX-C^+ genes repress *lab* in PS4-13. In conclusion, tsh^+ activity in combination with that of the other trunk homeotic genes represses the expression of at least one head gene as well as head development in a synergistic manner.

We have also examined the expression of the *Dfd* gene in the absence of different combinations of homeotic mutations. *Dfd* expression resembles that of wild type in all cases except when the Scr^+ and tsh^+ genes are missing. By the extended germ band stage, *Dfd* transcripts are detected in a small patch of cells of the posterior part of the labial segment and, in some cases, in the posterior part of the prothoracic segment (Fig. 11A-C). Thus Scr^+ and tsh^+ genes suppress *Dfd* gene activity in subsets of cells of the labium and prothorax, whereas the $Antp^+$ and BX-C^+ genes have no detectable role in suppressing *Dfd* in the trunk.

In conclusion, *tsh* as well as the *Scr*, *Antp* and BX-C genes have common and independent functions to repress head gene activity and head development in the embryonic

trunk. Within this domain in mutants of the trunk homeotic genes, the derepression of the procephalic gene *lab* is more extensive than that observed for the gnathocephalic genes *Scr* and *Dfd*.

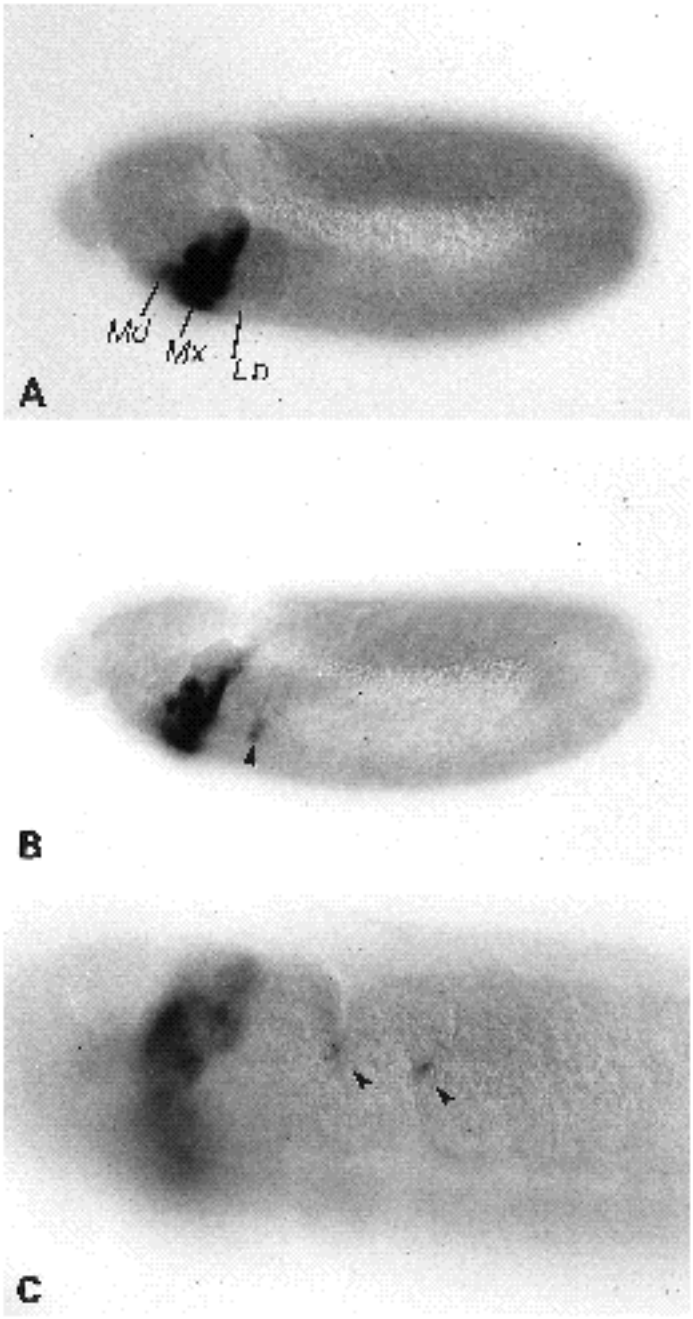


Fig. 11. *Scr*$^+$ and *tsh*$^+$ gene functions act in combination to suppress the gnathal gene *Dfd*. The expression of *Dfd* in wild type (A) and *tsh*, *Scr*, *Antp* BX-C mutant (B, C) embryos, at extended germ band stage, is shown. The *Antp* and BX-C mutations do not affect *Dfd* expression patterns. *Dfd* is normally (A) expressed in the maxillary (Mx) and mandibular (Md) segments at this stage as described by Jack et al. (1988). In *tsh*, *Scr*, *Antp*, BX-C embryos (B, C), a small patch of cells (arrowheads) express *Dfd* in the posterior part of the labium (B, C) and sometimes a group of cells in the posterior prothorax (C), in addition to the normal regions.

Expression of the lab *gene in trunk segments of* tsh$^-$ *embryos is functionally significant*

A feature of *tsh* mutations is that the head gene *lab* is expressed ectopically in the trunk region (Fig. 6C); therefore in this region a mixture of head and trunk homeotic gene activities exists. Is the ectopic expression of *lab* functionally significant? To test this idea the cuticular phenotypes of *tsh lab* double mutants were compared to those of *tsh* and *lab* mutants alone. Null mutations in the *lab* gene delete specific patterns of the anterior head thought to be part of the intercalary segment (Merrill et al., 1989). Null mutations of the *lab* gene act as partial suppressors of the *tsh*$^-$ phenotype (compare Figs 12 and 2B); the number of denticles in each belt of the trunk domain is increased and the sclerotic cuticle, found in the trunk segments of *tsh*$^-$ mutations, no longer differentiates. Therefore in the trunk region of *tsh* mutations, *lab*$^+$ gene expression is functionally significant. In addition, these observations suggest that the *lab*$^+$ gene functions to promote head (sclerotic cuticle) and repress trunk (denticle belt) development.

Normal Antp *and BX-C gene function requires* tsh$^+$ *gene activity*

The cross regulatory interactions described above suggest that the *Antp* and BX-C genes are modulating the transcription of the *tsh* gene (Fig. 5C-H) and therefore raise the possibility that *tsh*$^+$ activity is a requisit for normal trunk homeotic gene function. To analyse this point we have examined *tsh* transcription in embryos carrying the struc-

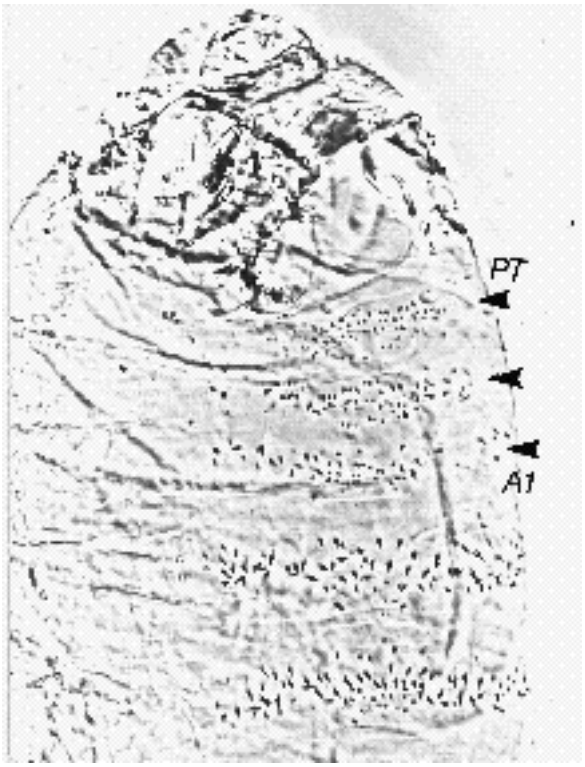


Fig. 12. The cuticular phenotype of a *tsh*$^-$ *lab*$^-$ larvae. Compared to *tsh*$^-$ *lab*$^+$ cuticular patterns (Fig. 2B), the numbers of individual denticles is increased in each segment and the dark coloured sclerotic cuticle is missing.

tural genes coding for the *Antp* or *Ubx* proteins fused to the control elements of a heat shock promoter (HSA and HSU respectively). Following heat shock of such embryos, trunk segments differentiate in specific head metameres; the labial, the maxillary and at least one procephalic head segment are transformed to trunk (Gibson and Gehring, 1988; Gonzalez-Reyes and Morata, 1991). HSA and HSU embryos, have been heat shocked during embryogenesis and then tested for *tsh* transcript distribution (see Materials and methods).

After multiple heat shock treatments, *tsh* messages can be detected in the normal position as well as ectopically in particular domains of the head in HSA and HSU embryos (compare Figs 5A and 13). *tsh* transcripts were found more frequently in the procephalic domain than in the maxillary one, which in turn was more frequently labelled than the labial segment (see Table 1). These results correlate with the larval phenotypes observed by Gonzalez-Reyes and Morata (1991) following heat shock treatment of HSU embryos. The head segments transformed are the labial, the maxillary and at least one anterior procephalic head segment including part of the mandibular one. In terms of frequency, the procephalic segment(s) is (are) transformed more frequently than the maxillary one, which in turn, is affected more frequently than the labial one. It is noteworthy that, whereas *Ubx* or *Antp* are expressed all over the embryo following heat shock (data not shown), *tsh* does not respond in the same way in all cells. Ectopic *tsh* expression is never observed in the tail region. Non-heat shocked HSU or HSA embryos or heat shocked wild type controls always give a normal pattern of *tsh* expression. These results show that *tsh* expression is correlated with trunk identity and support the idea that the *Antp* and *Ubx* genes act, directly or indirectly, as positive regulators of *tsh* transcription.

Table 1. *Ectopic* tsh *expression in different segmental positions of the head in HSA and HSU embryos following heat shock*

	Percentage of embryos with *tsh* transcripts in:		
Genotype	Procephalon	Maxilla	Labial
HSA	100	58	22
HSU	100	64	29

Embryos were given three 25 minute heat shocks at 36°C. Transcript accumulation from the *tsh* gene was detected and from the population of embryos, those with ectopic *tsh* transcription were scored.

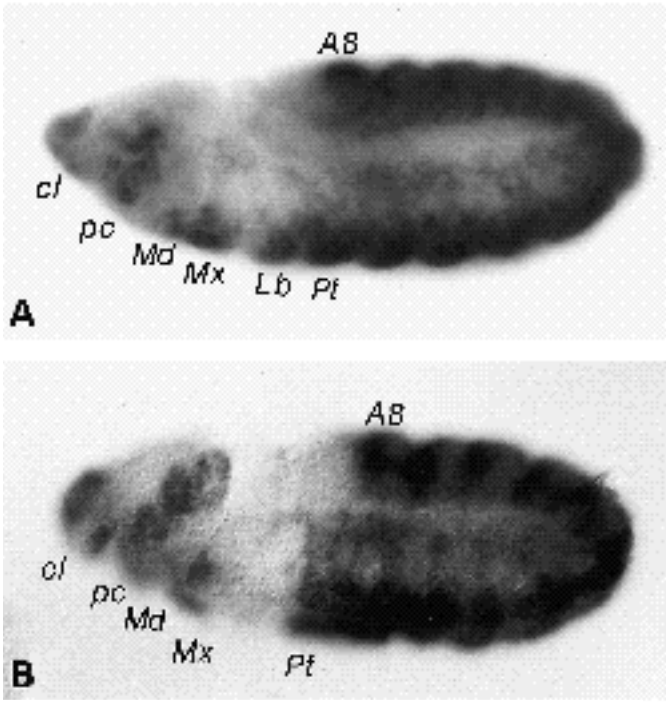
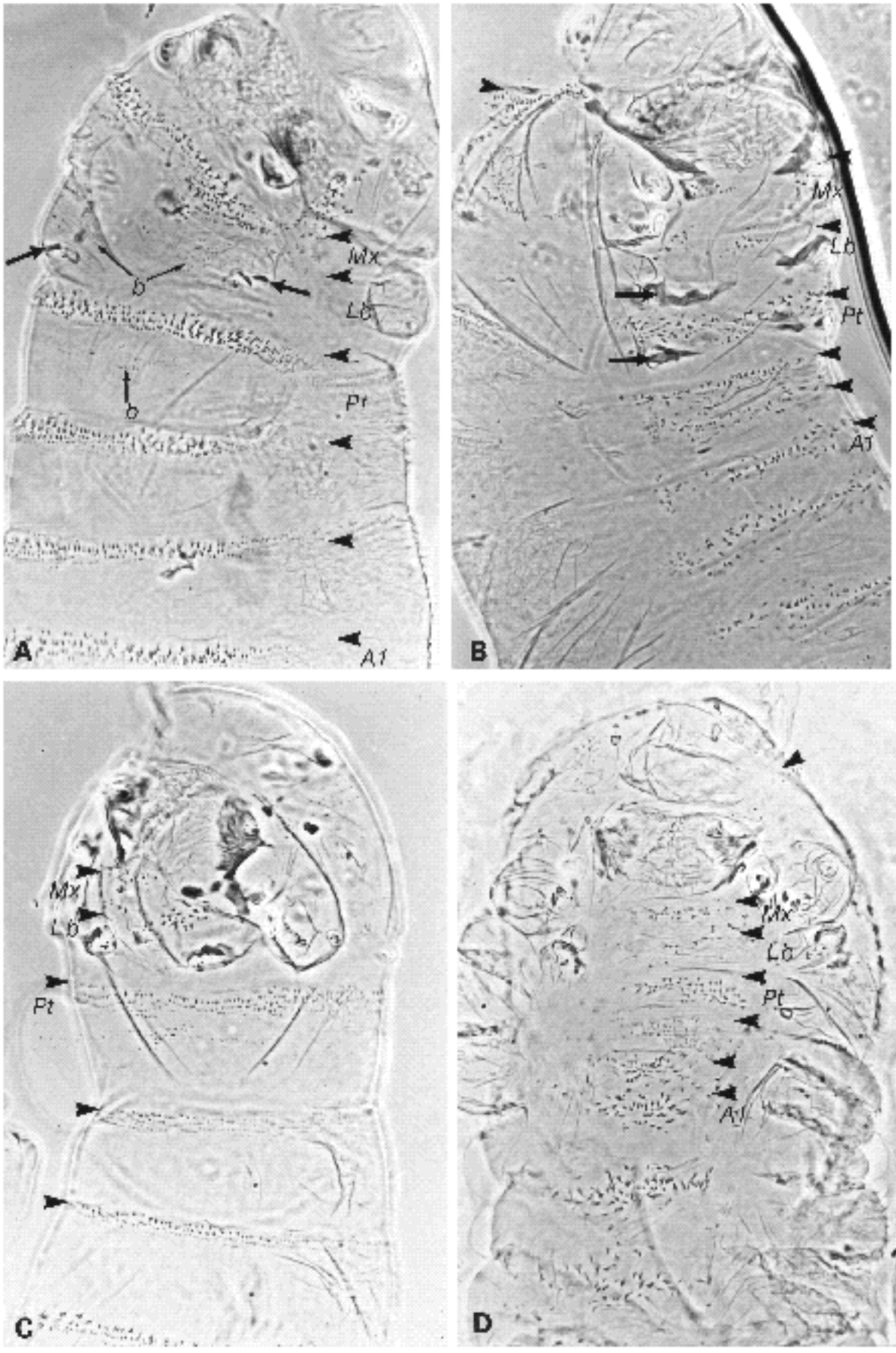


Fig. 13. Transcript accumulation of *tsh* in HSU (A) and HSA (B) embryos, following three heat shock pulses during embryogenesis. Embryos are at the extended germ band stage despite being aged for 10 hours. As well as the normal distribution of transcripts in the thoracic and abdominal segments (compare to Fig. 4), *tsh* is expressed ectopically in the head region. In A, *tsh* expression is detected in parts of the procephalon (pc) including the clypeolabrum (cl), mandibular (Md), maxillary (Mx) and labial (Lb) segments. Similar ectopic *tsh* mRNA accumulations are observed in HSA embryos; in B expression is absent in the labial and maxillary segments. Similar patterns of *tsh* expression are also observed in HSU embryos. In the populations of HSA or HSU embryos the numbers of cells expressing *tsh* in the head is variable. For 1 hour heat shock treatments, *tsh* is expressed almost exclusively in the procephalon of both HSA and HSU embryos. Abbreviations as in Figs 3, 4 and 5 except for the eighth abdominal segment (A8).

It is clear that *tsh* transcription depends in part on the normal function of the $Antp^+$ and Ubx^+ genes but is this functionally significant? The *Antp* and BX-C genes are expressed normally in *tsh* mutations and they can make denticle belts which have abnormal morphology (Fig. 2B). Thus the classical trunk homeotic genes are capable of directing cells into the trunk developmental pathway but require tsh^+ activity to do this correctly. We have examined the cuticles of HSU and HSA embryos, following heat shock during embryogenesis, in the presence and absence of tsh^+ activity to analyse this point. If tsh^+ activity is

Fig. 14. The effect of ubiquitous *Ubx* and *Antp* expression in the presence and absence of *tsh* function. Cuticular phenotypes of wild type (A and C) and a *tsh* null mutation (B and D) carrying a construct with the structural genes for the *Antp* (HSA) or *Ubx* (HSU) proteins fused to the control region of a heat shock promoter, following three heat shocks of 25 minutes each during embryogenesis. As described previously, ectopic *Ubx* protein (A) may replace the thoracic and three head segments with A1 patterns (Gonzalez-Reyes et al., 1990). In addition, prothoracic patterns, as seen here by the ectopic beard (b), may differentiate in the labial segment. Note the presence of sclerotic cuticle in the posterior part of the labium (arrows). Ectopic *Antp* protein (C) induces mesothoracic denticle belts in the prothorax and in the head positions (see Gibson and Gehring, 1988). In the absence of *tsh* activity and in the presence of ubiquitous *Ubx* or *Antp* protein (B and D), ectopic denticle belts differentiate in the same ectopic positions as wild type and in the prothorax; no denticle belt differentiates normally in *tsh* mutations in this position (compare to Fig. 2B). In B, note that the prothorax resembles the labial segment in that head cuticle forms in both segments posterior to the denticle belts (arrows). Also note that sclerotic cuticle in the trunk region has been eliminated in the *tsh* mutations (B and D) and that segments are larger and better defined, compared to *tsh* mutations (Fig. 2B).


A
Mx
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B
Mx
Lb
Pt
A1
C
Mx
Lb
Pt
D
Mx
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Pt
A1

essential for homeotic gene function, abnormal denticle belts should form in the head in the absence of *tsh*$^+$ gene activity following heat shock.

In *tsh*$^+$ larvae, our results are in accordance with those described previously except that occasionally, prothoracic (PS 3) patterns can be distinguished in the labial segment of HSU embryos (see Fig. 14A). In the absence of *tsh*$^+$ activity, ubiquitous expression of *Ubx* or *Antp* proteins cause the differentiation of denticle belts in ectopic positions. Following a one hour heat shock treatment, a denticle belt appears in the position of the prothorax with about the same frequency as the denticle belt deriving from the procephalic domain (Gonzalez-Reyes and Morata, 1990), in both *tsh*$^-$ HSA and *tsh*$^-$ HSU larvae (Fig. 14B, D). More extreme heat shock treatments give rise to additional denticle belts in the maxillary and less frequently the labial segmental positions, as described by Gonzalez-Reyes and Morata (1990) for wild-type larvae. These ectopic denticle belts resemble those in the trunk domain of *tsh* null mutations (Fig. 2B), confirming that *Antp*$^+$ and *Ubx*$^+$ genes require *tsh*$^+$ activity for normal function but can direct the development of a trunk segment, independently of *tsh*$^+$ activity.

Another observation is that high levels of the *Antp*$^+$ and *Ubx*$^+$ genes can partially rescue the *tsh*$^-$ phenotype in the trunk; the sclerotic cuticle observed in the trunk of *tsh* mutations is not observed and the size of segments seems to be more normal than in *tsh* mutations alone (compare Figs 2A, B with 14B, D). Surprisingly, following overexpression of the *Ubx* gene, this rescue can occur even posteriorly to the first abdominal segment, whereas in wild-type embryos, high levels of *Ubx* protein have no effect in these positions (Gonzalez-Reyes et al., 1990). This observation suggests that, in part, *tsh* has a function in common with *Ubx* in the trunk.

In conclusion, these results show that the *Antp* and *Ubx* genes act independently of *tsh* for the determination of trunk identity (denticle belts) although *tsh*$^+$ function is indispensable for the complete function of the trunk homeotic genes (normal denticle belts).

Discussion

In *Drosophila*, specific trunk segmental identity is determined by homeotic genes of the Antennapedia (*Scr* and *Antp*) and Bithorax complexes (Kaufman et al., 1990; Lewis, 1978); each of these trunk homeotic genes is expressed and functional within restricted domains of the embryonic trunk (reviewed by Akam, 1987; Ingham, 1988). The pattern of ventral denticle belts of the larval cuticle are morphological markers that allow the specific identities of most segments to be distinguished. The absence of any single homeotic gene results in a change in the identity of a specific segment or segments into another type, giving rise to a typical homeotic transformation. Trunk morphology in the *Drosophila* embryo also depends on the normal function of the *tsh* gene, a member of the homeotic class (Fasano et al., 1991). However, the *tsh* gene is different from the classical homeotic genes in at least two respects: it codes for a zinc finger protein and the analysis of the phenotype of mutations at this locus showed that the entire trunk is affected. In this paper, we present evidence that the *tsh*$^+$ gene has a specific role in determining the identity of the anterior prothorax (PS 3) as well as a more general one in all trunk segments. We also show that *tsh* acts in combination with the trunk homeotic genes, *Scr*, *Antp*, *Ubx*, *abd-A*, and *Abd-B*, to promote specific trunk identity and to repress anterior head development in the trunk. Since *tsh*$^+$ activity is necessary for segment identity of the entire trunk region it has characteristics in common with the "region specific" homeotic genes *spalt* and *fork head* (Jürgens, 1988; Jürgens and Weigel, 1988).

The tsh$^+$ *gene determines the specificity of function of the Scr*$^+$ *gene for anterior prothoracic identity*

Our results argue that *tsh*$^+$ function is critically required for the establishment of the specific identity of the anterior prothorax and probably for PS 3. First, of the homeotic genes expressed in the prothorax, neither *Scr*$^+$ nor *Antp*$^+$ gene activities can account fully for this identity; absence of the *Scr*$^+$ and *Antp*$^+$ gene functions still give rise to a denticle belt with prothoracic-like characteristics (Struhl, 1983; Sato et al., 1985). Second, we show that *tsh* expression always correlates with prothoracic identity (Figs 5 and 8). Third, in *tsh* mutants, the pattern elements specific to the anterior prothoracic segment are absent (Fig. 2B).

The *Scr*$^+$ gene plays a role in anterior prothoracic identity since mutations cause a partial and weak transformation of this compartment to an anterior mesothoracic one (Wakimoto and Kaufman, 1981; Pattatucci et al., 1991). In *tsh*$^-$ embryos, the *Scr* gene is expressed ectopically in the cells in the position of the anterior prothorax (Fasano et al., 1991) but no prothoracic patterns develop. When the *Scr* gene is expressed under the control of heat shock promoter, all thoracic segments resemble the prothorax (Gibson et al., 1990); however, in the absence of *tsh*$^+$ activity, high levels of *Scr* cannot induce the formation of anterior prothoracic structures (Fig. 7A). These results are consistent with the idea that the *tsh*$^+$ gene has a critical role for promoting the anterior prothoracic pathway.

The specific identity of the anterior prothorax is probably determined by the simultaneous activities of the *tsh* and *Scr* gene products. Whenever *tsh*$^+$ and *Scr*$^+$ products coexist in a segment prothoracic identity ensues. For example, we show that in HSU embryos prothoracic patterns are sometimes observed in the labial position (Fig. 14A); similarly, in *spalt* (*sal*) mutations, the labial segment is transformed to anterior prothorax (Jürgens, 1988). In the labial segment for both of these cases, *Scr* expression is unaffected (Gonzales-Reyes and Morata, 1990; Casanova, 1989) and *tsh* transcripts coexist with it (Figs 8 and 13). When an *Scr* protein is expressed ectopically it has no affect on *tsh* transcription (this work) and therefore it coexists with *tsh* products forming prothoracic patterns in each thoracic segment (Gibson et al., 1990).

In conclusion, the *tsh* gene seems to play a key role in determining the identity of the anterior prothorax independently of the trunk homeotic activities. The specificity of action of the *Scr* gene seems to be determined by the presence or absence of *tsh* gene products; when *tsh* activity is

missing, a labial segment is made and when *tsh* is present a prothoracic segment forms (see Fig. 1).

Independent and similar roles for tsh, Antp *and BX-C genes for trunk identity*

Our results show that the *tsh*, *Antp* and BX-C genes have common and independent functions for trunk identity, which can account for the lack of a clear homeotic transformation in *tsh* mutations. First, homeotic genes together with *tsh* are required for trunk identity since in their absence (Fig. 10A) ventral trunk identity disappears (i.e. no denticle belts). Second, if any one of these genes is active (Figs 2 and 13; Struhl, 1983), denticle belts differentiate showing that this global aspect of trunk identity is under the control of each of these genes independently. Third, high levels of Ubx^+ or $Antp^+$ products can partially rescue the tsh^- phenotype in the trunk (Fig. 14B and D). Finally, any one of these genes repress the *lab* gene in the trunk (Fig. 6) again indicating a common and overlapping function for these genes.

Normal Antp *and BX-C gene function modulates and requires* tsh^+ *activity*

It is obvious that, although *tsh* and homeotic gene products have common functions, they also have unique ones. For example, tsh^+ function is required throughout the trunk domain whereas the trunk homeotic genes have restricted functions within it. This difference is linked to our observations that *tsh* activity is required for normal homeotic gene function throughout the trunk.

The simultaneous presence of tsh^+ and homeotic gene activities is always correlated with a specific trunk segmental identity (Fig. 1). We show that the *Antp* and *Ubx* proteins, when expressed ectopically and at high levels, lead to activation of *tsh* transcription in the head, in regions corresponding to the segments thought to be transformed to trunk (Gonzalez-Reyes and Morata, 1991). In the absence of tsh^+ activity the ventral denticle belts, even in ectopic positions, are abnormal (Figs 2B, 14B and D), showing that tsh^+ activity is required for normal trunk development. In specific domains of the trunk, *tsh* and particular homeotic genes define unique segmental identities; as described above, *tsh* and *Scr* combine for the identity of the prothorax, *tsh* and *Antp* for the mesothorax and so on throughout the posterior trunk (see Fig. 1).

We show that the *Antp* and *Ubx* gene products could act as direct or indirect activators of the *tsh* gene. Very few bona fide targets of these trunk homeotic genes are known (reviewed by Andrew and Scott, 1992). For example, Gould et al. (1990) have described two targets of the *Ubx* gene and Wagner-Bernholz et al. (1991), have analysed several putative target genes of *Antp*, one of which may be the *spalt* (*sal*) gene. Furthermore, we note that *Antp* and *Ubx* proteins are unable to induce ectopic *tsh* expression in all parts of the head (Fig. 13) suggesting that other factors determine whether or not the *tsh* gene will be regulated by the homeotic genes. A similar type of differential behaviour has been noted for repression of the *sal* gene, following ectopic expression of the *Antp* gene (Wagner-Bernholz et al., 1991); repression of *sal* expression occurs in the antennal discs but not in the brain or wing discs.

It seems likely that the $abdA^+$ and $AbdB^+$ gene activities are also required for the maintenance of *tsh* transcription; in the absence of the *Antp* and BX-C genes at the beginning of germ band retraction, *tsh* transcription is not detected in the posterior compartments of segments in the entire trunk domain (Fig. 5G and H); since these cells differentiate head identities (Struhl, 1981; Sato et al., 1985), this indicates that the regulatory effects observed between the *Antp*, the BX-C and *tsh* genes are functionally relevant.

In conclusion, *Antp* and BX-C genes regulate and require *tsh* products for determining the specific identities of the different trunk segments from the posterior prothorax to the eighth abdominal segment.

Co-operative repression of head genes by the trunk homeotic genes

We argue above that *tsh*, *Antp* and BX-C genes have independent roles for trunk identity. Clearly when all these gene functions are missing ventral trunk patterns are replaced with structures deriving normally from the procephalon and/or acron (Fig. 10), indicating that *tsh*, in combination with these homeotic genes, is required for repressing head formation in the trunk tagmata.

Mutations in the *tsh* gene disrupt the trunk of the embryo and our results suggest that this in part is due to the failure of repression of at least one procephalic gene in this domain, the *lab* gene (Fig. 6C). As shown in Figs 2B and 12 the ectopic expression of *lab* in tsh^- embryos is functionally significant and therefore, a mixture of trunk (*Antp* and BX-C) and procephalic (*lab*) homeotic gene products coexist in the trunk, accounting in part for the disrupted thorax and abdomen of *tsh* mutations. The expression of *lab* is also repressed by the trunk homeotic genes in homologous dorsal-lateral groups of cells in the trunk (Fig. 6D and E). Sato et al. (1985) showed that *Antp* BX-C mutants develop head cuticle in dorsal positions. Taken together these observations suggest that the ectopic expression of *lab* directs these cells into this head developmental pathway in these mutant embryos.

Although the combination of *tsh*, *Antp* and BX-C mutations results in a clear transformation of ventral trunk to head (Fig. 10A), the precise segmental origin of this cuticle is in doubt, but it derives from the anterior head. The derepression of the *lab* gene (Fig. 10D and E) is limited to epidermal cells located laterally in the extended germ band embryo whereas the head cuticle is located ventrally in the larva. In our opinion, the combination of trunk homeotic genes is therefore critical for the repression of other head genes in the trunk region. Consistent with this idea is that *lab* mutations cannot totally rescue the tsh^- phenotype in the trunk (Figs 2B and 12). Not all of the trunk region is replaced with head patterns (Fig. 10A) in the absence of *tsh*, *Antp* and BX-C genes since in the dorsal position trunk patterns develop. It seems probable that in the dorsal position other genes are responsable for trunk development.

Surprisingly, we find that the effect of the absence of *tsh* and the trunk homeotic genes on the expression of the *lab*, as well as that of the *Dfd* and *Scr*, genes is not simply additive but synergistic (Figs 9-11). These results suggest that *tsh* and the homeotic genes have the same function in a common set of cells to repress these head genes. The *tsh*

gene codes for a zinc finger protein which therefore is a potential DNA binding factor (Fasano et al., 1991). Thus, it is tempting to speculate that *tsh* regulates directly, together with the homeotic genes, an overlapping set of genes. Further experiments are required to confirm this idea at the molecular level.

The co-operative effects of *tsh* with some of the classical trunk homeotic genes is also described for restricting the expression of the *Scr* and *Dfd* genes to the gnathal segments. However, the ectopic expression of these genes is not found in all trunk segments (Figs 9B and 11), as is the case for the procephalic gene *lab*. From a morphological standpoint, the gnathal segments appear to be modified trunk segments. Furthermore, on the basis of the distinct genetic mechanisms involved in establishing the head and trunk domains, several authors have speculated that the gnathocephalon is part of the trunk (Finkelstein and Perrimon, 1991; Cohen and Jürgens, 1991; Gonzalez-Reyes and Morata, 1991). We show that the gnathal genes seem to be distinct, compared to the procephalic genes, in terms of their regulation by the trunk genes (see also Jack et al., 1988). Therefore, the gnathocephalic region can be considered as a distinct morphological unit, with particular genetic properties, compared to the procephalic and the trunk domains.

What is the evolutionary significance of tsh*?*

Several authors have speculated that a primitive homeobox gene may be implicated in the distinction of trunk from the ends of the embryo and that via duplication and divergence of this primitive function, segmental specialisation occurred (Stuart et al., 1991; Akam et al., 1988). If this hypothesis is true, what might be function of *tsh*? In Diptera, Snodgrass (1935) has suggested that there was considerable selective pressure on the development of larval features and therefore *tsh* could have evolved for the development of dipteran larval trunk. In this respect it is noteworthy that deletion of homeotic genes in the more primitive insect *Tribolium*, cause trunk to antennal transformations (Stuart et al., 1991). However, we would like to raise the point that thoracic to antennal transformations in *Tribolium* are observed in the leg appendages and possibly not in the proximal parts to which the legs are attached. In *Drosophila*, null mutations of the *Antp* gene cause a similar transformation of parts of the leg discs but not apparently in the most proximal (or distal) regions (Struhl, 1981). Since *tsh* is active only in the proximal parts of the leg discs (data not shown), perhaps in evolutionary terms *tsh* has been conserved for identifying this particular proximal trunk identity in *Drosophila* as well as in *Tribolium*.

An alternative hypothesis is that a set of genes distinct from the homeotic ones are defining a ground state (Lewis, 1963), i.e. the identity of segments in the absence of all homeotic gene activities. In this hypothesis, the homeotic genes act to modify the segmental ground state and specialize segmental identities. For the trunk region in *Drosophila*, this basal identity is prothoracic-like (Struhl, 1983; Sato et al., 1985). We show that tsh^+ function is critical for this identity and is modulated by, and required for the normal function of, the *Antp* and BX-C genes. Our results support the hypothesis that tsh^+ (and presumably other gene activities) are contributing to the ground state identity of segments of the *Drosophila* embryo.

Elements of both these hypotheses could be true. A primitive homeobox gene similar to *Antp* and a zinc finger protein similar to *tsh* may have evolved in conjunction in the primitive ancestor. In *Drosophila*, we show that *tsh* and specific homeotic genes act in concert to determine trunk identity. Evolutionary studies are necessary to distinguish between these possibilities.

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DEV1874 FIGURE 4 COLOUR CAPTION

Fig. 4. Wild-type embryos doubly labelled with a probe to the *tsh* coding sequence and an antibody to the *invected* protein. Embryos are at the extended germ band stage (A) or during germ band retraction (B, C) when segments are forming. The *invected* protein (brown) marks the posterior compartments of each segment giving a reference for the position of *tsh* transcript (blue) accumulation. Overlap between the two gene products is dark brown whereas *invected* alone is light brown. In A, note that the *invected* stripe in PS 3 coincides with the anterior border of *tsh* transcript accumulation, and in B and C that this stripe does not overlap that of *tsh* messages (arrow) in the epidermis of PS 3 (3). Contrarily, in the central nervous system (C, between arrows), *tsh* and *invected* overlap in PS 3. A and B are lateral views and C is a ventral one. Anterior is to the left. Bar represents 50μm

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