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Homeotic complex and *teashirt* genes co-operate to establish trunk segmental identities in *Drosophila*

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SUMMARY

Homeotic genes determine the identities of metameres in *Drosophila*. We have examined functional aspects of the homeotic gene *teashirt* by ectopically expressing its product under the control of a heat-shock promoter during embryogenesis. Our results confirm that the gene is critical for segmental identity of the larva. Under mild heat-shock conditions, the Teashirt protein induces an almost complete transformation of the labial to prothoracic segmental identity, when expressed before 8 hours of development. Positive autoregulation of the endogenous *teashirt* gene and the presence of *Sex combs reduced* protein in the labium explain this homeosis. Patterns in the maxillary and a more anterior head segment are partly replaced with trunk ones. Additional Teashirt protein has no effect on the identity of the trunk segments where the gene is normally expressed; *teashirt* function is overriden by some homeotic complex acting in the posterior trunk. Strong heat-shock regimes provoke novel defects: ectopic sense organs differentiate in posterior abdominal segments and trunk pattern elements differentiate in the ninth abdominal segment. Teashirt acts in a partially redundant way with certain homeotic complex proteins but co-operates with them for the establishment of specific segment types. We suggest that Teashirt and HOM-C proteins regulate common sets of downstream target genes.

Key words: *Drosophila*, ectopic *teashirt* expression, heat-shock promoter, autoregulation

INTRODUCTION

Segment identity in *Drosophila* is under the control of homeotic genes: the region-specific gene *forkhead* (*fkh*) is necessary for the extreme ends of the embryo (Jürgens and Weigel, 1988) and *spalt* (*sal*) for subterminal (certain gnathal and tail) segments (Jürgens, 1988). A novel homeotic gene, *teashirt* (*tsh*), defines the identity of the trunk (or thorax and abdomen (Röder et al., 1992) in between. The homeotic complex genes (HOM-C) define the identities of larval segments within these domains and fall into two clusters called the Antennapedia (ANTP-C) and Bithorax (BX-C) complexes. The first is made up of the *labial* (*lab*), *Deformed* (*Dfd*), *Sex combs reduced* (*Scr*) and *Antennapedia* (*Antp*) genes, necessary for intercalary, mandibular-maxillary, labial-prothoracic and mesothoracic-metathoracic identities, respectively (reviewed in Kaufman et al., 1990). The second consists of the *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*) genes required respectively for metathoracic-first abdominal, second through seventh and fifth to ninth, abdominal segmental identities (Lewis, 1978; Sanchez-Herrero et al., 1985).

All homeotic genes code for proteins that bind (Scott et al., 1989; Affolter et al., 1990) or probably bind to DNA and in some cases are known to regulate the transcriptional activities of downstream genes (Andrew and Scott, 1992; Gould et al., 1990; Graba et al., 1992; Vachon et al., 1992). *tsh* and *sal* code for zinc finger proteins (Fasano et al., 1991; Kühnlein et al., 1994) and *fkh* for a DNA-binding protein of the Fkh/HNF-3 class (Weigel et al., 1989). The HOM-C genes all code for homeodomain proteins (Scott et al., 1989; Affolter et al., 1990; Kaufman et al., 1990).

Several mechanisms have been proposed for the determination of segmental identities by the homeotic genes including: restricted spatial expression of products (reviewed in Akam, 1987; Ingham, 1988), cross-regulatory interactions (Hafen et al., 1984; Röder et al., 1992), functional hierarchies (Gonzalez-Reyes et al., 1990), competition (Lamka et al., 1992) and co-operativity with novel proteins on common target genes (Peifer and Wieschaus, 1989; Röder et al., 1992; Rauskolb et al., 1993).

The *tsh* gene is expressed and required for trunk segmental identity; in particular, it has a critical function for the identity of the first (or prothoracic) trunk segment. Previously we suggested that the Tsh protein is instrumental for establishing the type of segment made by the *Scr* HOM-C gene. In the presence of Tsh protein, *Scr* directs cells into a prothoracic and, without it, into a labial pathway (Röder et al., 1992). In addition, we showed that the trunk homeotic genes, *tsh*, *Antp*, *Ubx* and *abd-A* have partially redundant functions since they all independently promote trunk and repress head developmental pathways.

A useful way to study homeotic gene function is to express them ectopically at different phases of development and examine the defects caused upon differentiation. One phenomenon that can be studied following ectopic expression is called phenotypic suppression (Gonzalez-Reyes et al., 1990) or posterior prevalence (Lufkin et al., 1991); more posteriorly acting HOM-C genes often function epistatically to those active in more anterior positions. *Ubx*, for example, exerts its function dominantly to *Antp* but not to *abd-A*. A second is that autoregulation can be examined (Kuziora and McGinnis, 1988).

We have made transgenic flies that express the *tsh* gene under the control of a heat-shock promoter in order to analyse its function. Ubiquitous expression of the Tsh protein promotes trunk development instead of certain head segments. Prothoracic identity results when Tsh coexists with Scr. Within the trunk region, where *tsh* is functional during wild-type embryogenesis, increasing the levels of protein have no effect on identity; posteriorly acting HOM-C genes override *tsh* in these positions. Higher levels of Tsh protein affect the identity of the ninth abdominal segment and cause the differentiation of novel sense organs in posterior abdominal segments. Our results support the notion that Scr, Antp, Ubx, Abd-A and Abd-B proteins co-operate with Tsh for trunk identity, probably by regulating common target genes.

MATERIALS AND METHODS

Construction of pHS-teashirt and generation of transgenic flies

A 3097 base pair *Dra*I fragment, containing the entire coding region from the *tsh* cDNA (Fasano et al., 1991), was cloned in the *Stu*I polylinker site of the PCaSpeR-hs (Thummel and Pirotta, 1992) vector. This HS-*tsh* construct was coinjected with pπ25.7wc DNA (Spradling and Rubin, 1982) into *y* w^{1118} embryos. Three HS-*tsh* lines were obtained; two were completely lethal when homozygous and the third produces a few homozygous escapers. The insertion used predominantly here is localised on chromosome 2 and kept with the balancer *CyO*; the other two were localised on chromosome 3 and balanced with *TM3*.

Fly stocks

Transgenic HS-*Scr*, HS-*Antp* and HS-*Ubx* stocks as well as *tsh*, *Scr*, *Antp* and BX-C alleles were those described in Röder et al. (1992). A strong EMS-induced allele, tsh^9, was also employed. For other stocks, see Lindsley and Zimm (1992).

Heat-shock procedure and cuticle preparation

The effect of heat shock on cuticle pattern was studied in embryos of specific ages. Eggs were collected for 6 hour periods from female *y* w^{1118} and male HS-*tsh* / *CyO* parents, washed in PBS 0.1% Triton (X-100), transferred to slides, dried partially with blotting paper and covered with 3-5 oil. Groups of embryos of the desired stage were picked out (Wieschaus and Nüsslein-Volhard 1986), transferred to Eppendorf tubes and submerged in a water bath at 36°C for 20 minutes. The larvae that developed were dechorionated, devitellinized by hand and mounted in Hoyer's medium to study the cuticle phenotype. Heat-shocked *y* w^{1118} embryos were used as a control for general effects of heat shock on embryonic development; these embryos were mostly wild type.

For heat shocks carrying HS-*tsh* and different HOM-C mutations, eggs were collected for 6 hour periods. Mild heat-shock treatments were for 20 minutes, stronger treatments were for 1 or 2 hours or following multiple heat-shock regimes. For multiple heat-shock regimes (see text), recovery times of 11/2 hours were used between pulses.

Tsh antibody

Anti-*teashirt* antibodies were made in rats by injecting a glutathion-S-transferase-Tsh fusion protein made in bacteria (Alexandre, Graba, de Zulueta, Fasano, Pradel, Kerridge and Jacq, unpublished data); the *tsh* sequence codes for 556 amino acids from the C-terminal part of the Tsh protein.

Detection of homeotic gene products

For in situ antibody staining and mRNA detection, embryos were collected for 6 hour periods, aged for 1-2 hours and heat shocked for 20 minutes, 1 or 2 hour periods at 36°C. Eggs were then incubated at 22°C for 2, 4 or 8 hours when they were dechorionated and fixed in 4% formaldehyde. mRNA in situ hybridisation on whole-mount embryos using digoxygenin-labelled DNA probes and antibody detection was as described by Röder et al. (1992). β-galactosidase (β-gal) activity in tsh^1, that carries an enhancer trap insertion, was detected in embryos as described in Fasano et al. (1991). The different fragments used for in situ hybridisation are described in Röder et al., (1992).

RESULTS

HS-*tsh* transgenes express functional Tsh protein

Transgenic flies were made, in which the coding sequence of the *tsh* gene is placed under the control of a heat-shock promoter, in order to study tsh^+ function. Three HS-*tsh* lines were recovered that were virtually homozygous lethal. Eggs were collected from the different transgenic lines and heat shocked at 36°C for 20 minutes. Two striking phenotypes were observed in the resulting larval cuticles. First, denticle belts (trunk segments) differentiated in place of certain head features and second, the identities of the trunk and tail regions were not affected (Fig. 1B). Non heat-shocked control embryos resembled wild-type embryos.

Anti-Tsh antibody was used on treated transgenic embryos to examine if the protein was expressed ectopically. From stage 8 and later, Tsh protein is detected throughout the trunk region in parasegments 3 to 13 in non treated HS-*tsh* (Fig. 1C) and wild-type embryos (Alexandre, Graba, de Zulueta, Fasano, Pradel, Kerridge and Jacq, unpublished data). HS-*tsh* embryos that were heat shocked and aged for 1, 2, 4 or 8 hours showed evenly distributed protein patterns throughout the embryo (Fig. 1D). Since development was severely affected in these embryos, we conclude that the HS-*tsh* transgenes produce functional Tsh protein.

Transformation of head to trunk identity following treatment of HS-*tsh* embryos

We next determined the precise period(s) during embryogenesis when 20 minute pulses of heat shock induced Tsh protein could disrupt larval morphology. Throughout we define trunk metameres as those bearing denticle belts and use C3, C2 and C1 to indicate respectively the labial, maxillary and procephalic head segments (Gonzalez-Reyes and Morata, 1990). Individual pattern elements and their segmental provenance are described by Jürgens et al. (1986). Developmental stages are those of Campos-Ortega and Hartenstein (1985).

Following treatment at the blastoderm stage (stages 4-5),

larvae exhibited defects in the segmentation pattern of the head, trunk and tail parts (Figs 2B, 3). Since wild-type embryos at this stage do not present any detectable Tsh protein (Alexandre, Graba, de Zulueta, Fasano, Pradel, Kerridge and Jacq, unpublished data), these defects are probably due to ectopic Tsh products upsetting the function of the segmentation genes (Nüsslein-Volhard and Wieschaus, 1980). Segmentation defects were not observed in a significant proportion of larvae that were treated at stage 6 or later (Fig. 3).

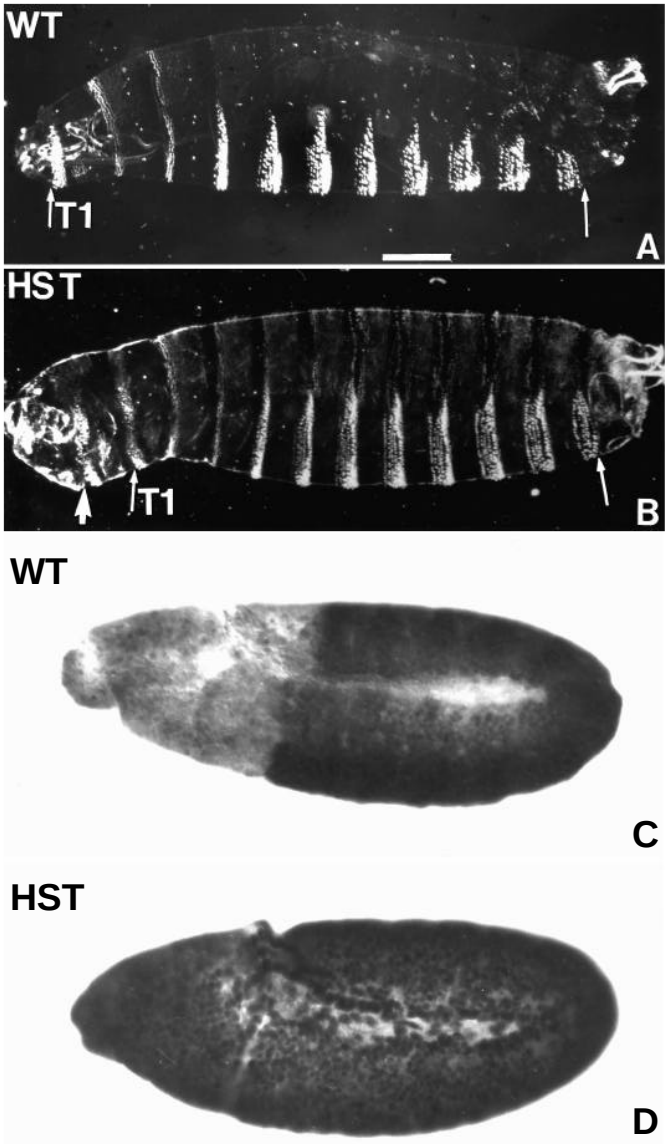

Fig. 1. HS-*tsh* transgenes express functional Tsh protein. Cuticle preparations of wild-type (A) and HS-*tsh* (B) larvae derived from 2-8 hour old embryos heat shocked for 20 minutes at 36°C. The trunk domain is between the arrows; the prothoracic (T1) segment is indicated. Head involution and identity are affected in B. An ectopic trunk segment differentiates in the position of the C3 or labial segment (large arrow). Note that the trunk segments and tail region are untouched by heat-shock-induced Tsh protein. Tsh protein distribution is shown in C and D which are non-treated and treated HS-*tsh* embryos, respectively. D received a heat shock before 8 hours of development and was left to develop for 6 hours before detection of Tsh protein, which is present in all cells. Note in C, the protein is largely restricted to the trunk region as in wild-type larvae (data not shown), but isolated cells express Tsh in ectopic positions indicating a leaky promoter. Anterior to left and dorsal uppermost. Bar represents 50 μm for all figures.

38% of HS-*tsh* larvae exhibit homeotic transformations of head to trunk identity if treated at or before stage 10 (Fig. 3). The most striking homeosis is observed in the C3 position, where patterns are replaced by a prothoracic denticle belt, naked cuticle and beard (Figs 1, 2C,D,E). At a lower frequency, two other denticle belts are seen in the head region but these are always less well defined compared to the C3 belt. Not all head structures are suppressed when Tsh is expressed in the head allowing the segmental origin of denticle belts to be inferred. Maxillary sense organs, cirri (Fig. 2D,E) and mouth hooks differentiate between the C3 and adjacent ectopic belt. Since patterns of mandibular origin form anteriorly to the latter, we believe this ectopic belt derives from the C2 position. The most anterior belt is located dorsally; its precise segmental origin is not known but we refer to it as the C1 belt by analogy to previous work (Gonzalez-Reyes and Morata, 1990). Head-to-trunk homeosis is not limited to ventral denticle belts; a set of dorsal hairs, found normally in the dorsal trunk, differentiate in the head (Fig. 2D,E).

For larvae treated at stage 10, a scattering of trunk denticles could be discerned in the C3 and C1 head segments (data not shown). No evident head-to-trunk homeosis was found in larvae that were treated at later stages although a significant proportion of embryos exhibited defects in head involution and development (Figs 2F, 3).

We conclude that ectopic Tsh protein, present before 8 hours of development, provokes homeotic transformations of segments anterior to its normal domain of action in the trunk and that C3 patterns are replaced with prothoracic ones.

Higher levels of Tsh protein affect the morphology of posterior trunk and tail segments

Multiple and 1 or 2 hour heat-shock treatments were performed to see if we could increase the expressivity of homeosis in HS-*tsh* larvae. Embryos were collected for 6 hour periods, heat shocked and cuticle morphology was examined. In the head, more complete transformations of the C2 and C1 to prothoracic-like segmental identity is observed (Fig. 4) but transformation was still not as complete as in the C3 position.

Two novel phenotypes are observed: first, small groups of ectopic denticles, as well as naked cuticle, developed between the eighth abdominal denticle belt and the anal pads (Fig. 4). The ninth abdominal patterns are replaced with abdominal segmental ones. Second, although identity is globally unchanged, pairs of papilla sense organs differentiate ectopically in ventral positions in the naked region of posterior abdominal segments (Fig. 4B).

Autoregulation of the *tsh* gene

Some HOM-C genes are thought to autoregulate their own transcription (Kuziora and McGinnis, 1988; Beachy et al., 1988; Müller et al., 1989; Regulski et al., 1991); does *tsh*?

As a monitor for *tsh* transcription, we used an enhancer trap line inserted into the *tsh* gene (Fasano et al., 1991). Spatial expression of the reporter gene *lac Z* was examined in embryos in the presence and absence of ectopic Tsh protein. Controls exhibit β-gal activity in the trunk and weakly in the head region (Fig. 5A). β-gal activity is observed in the C3 segment in 20%

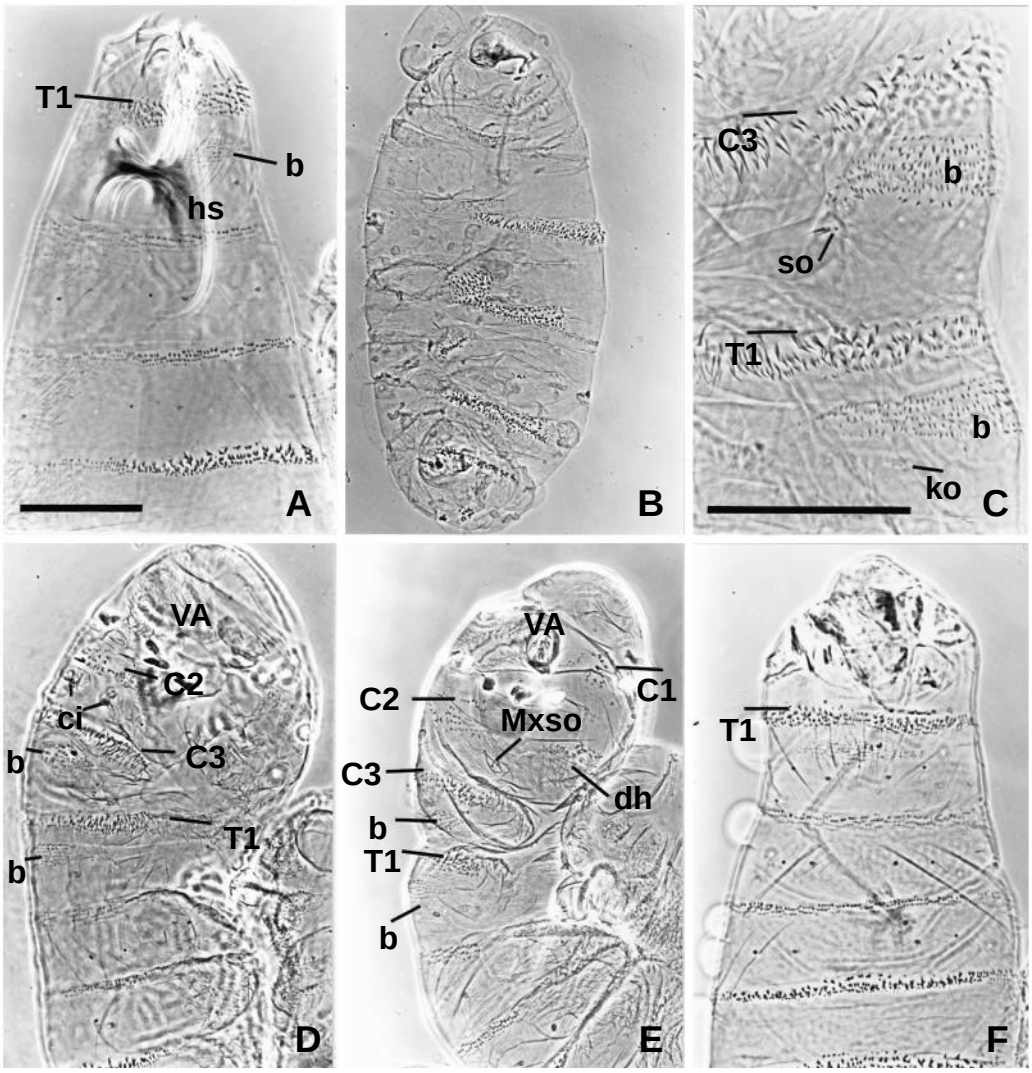

Fig. 2. Cuticle phenotypes derived from HS-*tsh* embryos heat shocked at 36°C at precise developmental periods. The anterior boundaries of prothoracic (T1), labial (C3), maxillary (C2) and procephalic (C1) head segments are shown. (A) Non-heat-shocked control resembles a wild-type larva with a T1-specific beard (b) and denticle belt. (B) Heat shocked at blastoderm stage: the segmental patterning, including head, trunk and tail regions, has been disrupted. (C) High power magnification of prothoracic and labial segments following treatment at stage 7: note the duplication of typical prothoracic denticle patterns including the beard in the C3 metamere; abnormal sense organs (so) differentiate here that partially resemble Keilins organs (ko) seen in thoracic segments. Labial sense organs are missing. (D,E) Treated at stage 7: an ectopic prothoracic denticle belt, naked cuticle and beard differentiates in the C3 position; denticle belts with naked cuticle differentiate in the C2 and C1 positions; markers allowing identification of the C2 metamere include the maxillary sense organ (Mxso) and cirri (ci), which develop between the C3 and C2 denticle belts. Note the ventral arm and t-ribs (VA) that develop from the mandibular segment (Jürgens et al., 1986). Dorsal hairs (dh), typical of the mesothoracic or metathoracic trunk segments, differentiate ectopically in the head; their segmental origin is unsure. Note the reduced head skeleton (hs in A) and failure of head involution. **(**F) Treated at stage 10: note the failure of head involution and development.

of treated HS-*tsh* embryos (Fig. 5B), if treatment is applied before 8 hours of development; if left to develop, all larvae with an ectopic trunk segment show ectopic β-gal activity in this position (not shown). In 1-2% of embryos, ectopic β-gal activity is also observed in other head segments (Fig. 5C,D). These results suggest that the tsh^+ gene positively regulates its own transcription within the C3 metamere and can explain the clear prothoracic transformation observed in this position (Fig. 2C,D,E).

In a second test, the HS-*tsh* insertion was recombined with two different *tsh* alleles on the same chromosome to ask whether the absence of the endogenous tsh^+ gene affects the HS-*tsh* phenotype. 2-6 hour old embryos were heat shocked for one or two 20 minutes pulses. Their larval cuticles were compared to non-treated controls. A quarter of control larvae exhibit a *tsh* null phenotype: absence of prothoracic patterns and smaller disrupted ventral denticle belts in the trunk domain (Fig. 6A; Röder et al., 1992). For experimental cuticles, the frequency of *tsh* null individuals was reduced; however, a quarter of the progeny had weaker phenotypes and, as such, *tsh* homozygotes could be unambiguously identified (Table 1; Fig. 6). The degree of rescue is dependent on position; abdominal morphology was normalized more frequently than thoracic ones. Mesothoracic and metathoracic morphology could be rescued as well, especially with more extreme treatments (Table 1). The size and morphology of the prothoracic metamere was never identical to the wild-type one. In most embryos, when denticles formed in the T1 position, a rudimentary belt also formed in the C3 one (Fig. 6B,C,D). We conclude that a high level of Tsh protein, attained via positive autoregulation of the endogenous *tsh* gene, is required for prothoracic identity.

Scr^+ and tsh^+ function for prothoracic identity

The *Scr* gene is expressed and functions for the identities of the prothoracic and labial segments (Pattatucci et al., 1991). In ectopic positions, Scr protein is incapable of inducing trunk identity in the head (Gibson et al., 1990; Zeng et al., 1993) but

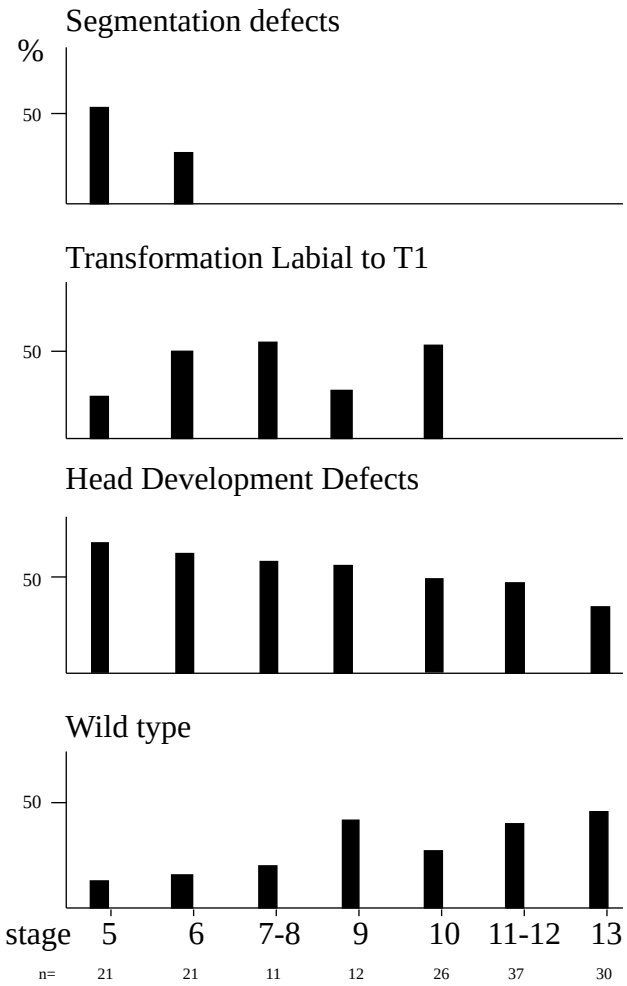

Fig. 3. Graphic representation of developmental abnormalities produced following 20 minute heat-shock treatment at 36°C at precise times during embryonic development. *y w* females were crossed to HS-*tsh*/CyO males; 50% of the progeny carry an HS-*tsh* chromosome. The number of larvae scored and corresponding developmental stage appears at the base of the diagram.

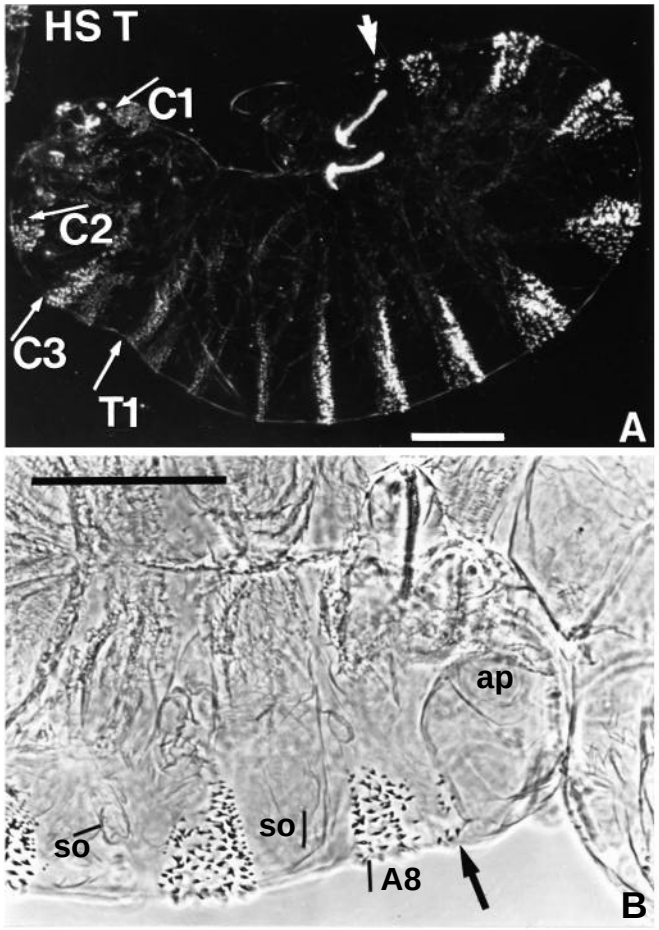

Fig. 4. The effects of high levels of ubiquitous Tsh protein during early embryogenesis. (A, dark field; B, phase contrast) Larvae that received three 20 minute heat-shock treatments at 36°C before 8 hours of development. (A) Note the ectopic trunk segments in the C1, C2 and C3 head segments; the prothoracic (T1) segment is indicated. An ectopic denticle belt differentiates in the tail domain (large arrow) between the eighth abdominal segment (A8) and anal pads (ap). (B) Note the papilla-like sense organs (so) in the abdominal segments between the denticle belts. Germ band retraction has not occured normally in these larvae.

Table 1. Rescue of *tsh*^{8} phenotype following ectopic expression of HS-*tsh* transgene

Heat-shock regime	% of *tsh*$^{-}$		Number of larvae scored
	Strong	Partial rescue	
Non-heat-shock control	21	0	95
Single pulse	8	15	123
Double pulse	5	16	148

replaces posterior thoracic with prothoracic segmental patterns. Since the *Scr* gene is expressed in the labial and prothoracic segments, a hypothesis proposed by Röder et al. (1992) is that when Tsh co-exists with Scr, a prothoracic segment differentiates. Three experiments were designed to analyse this idea.

First, we examined *Scr* expression in HS-*tsh* embryos. *Scr* transcripts were detected by in situ hybridisation to whole embryos, following recovery times of up to 8 hours. *Scr* transcription patterns were identical to the wild-type one irrespective of the heat-shock regime (data not shown).

We next examined the phenotype of larvae in which both Tsh and Scr proteins were ectopically expressed. HS-*tsh*/HS-*Scr* embryos were collected and heat shocked for 20 minute periods before 8 hours of development. Their cuticles were examined and compared to HS-*tsh* and HS-*Scr* controls. The combination of ectopic Scr and Tsh proteins produces more extreme prothoracic transformations in the C2 (Fig. 7A) and C1 positions compared to HS-*tsh* controls (Fig. 2D,E). No other novel defects were noted.

Finally, the phenotype of HS-*tsh* larvae were analysed in the absence of *Scr* gene product. Denticle belts differentiate in the C3 position (Fig. 7B) but the prothoracic nature of this (and the T1) belt is less well defined compared to *Scr*$^{+}$ controls.

Together these results show that Scr requires Tsh products for prothoracic identity and that Tsh (but not Scr) can provoke trunk identity when alone.

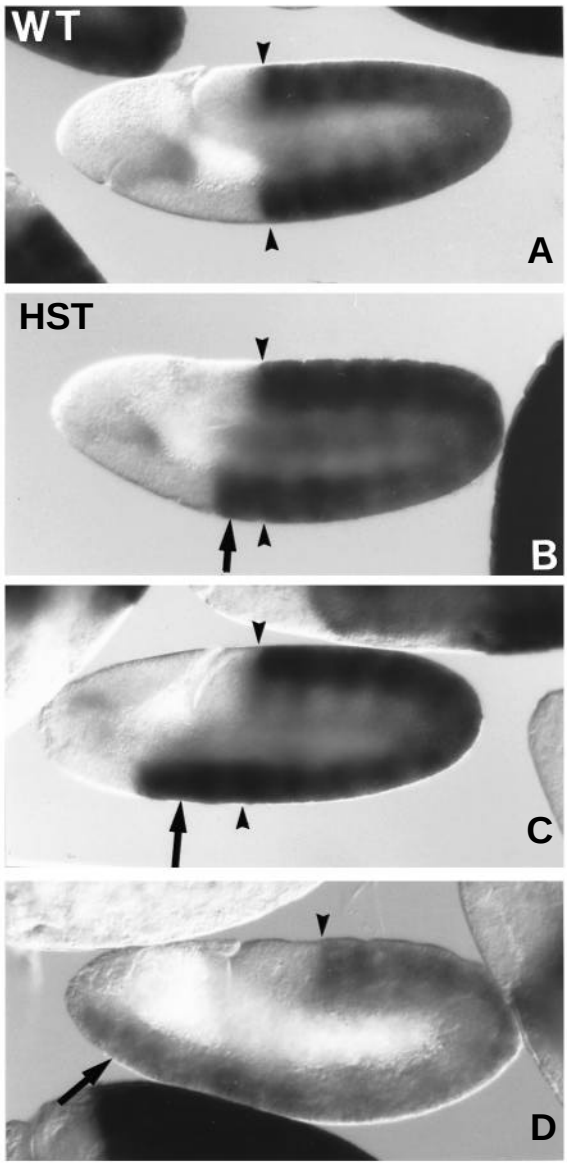

Fig. 5. Autoregulation of the *tsh* gene. β-gal activities from the *tsh*^{1} enhancer trap insertion (Fasano et al., 1991) have been detected in non-treated (A) and treated HS-*tsh* (B-D) embryos. B and C were heat shocked for 1 hour and D twice for 20 minutes at 36°C before 8 hours of development; these were left to recover for 7 hours before histochemical enzymatic detection. Expression is detected in the trunk of all embryos and 20% exhibit ectopic expression in the labial (C3) segment (B arrow). 1-2% of embryos show ectopic β-gal activity in C2 (C arrow) and other head segments (D arrow). At the time of the first heat shock, embryos were 2-6 hour old.

Common properties of *tsh* and HOM-C genes

Ectopic expression of the Tsh protein has little effect within the domain of the trunk where the gene is expressed in wild-type embryos. The protein predominantly disrupts identity anteriorly to its normal domain. These phenomena are similar to phenotypic suppression described by Gonzalez-Reyes et al. (1990) for HOM-C genes. Here we analyse this aspect further by expressing Tsh under heat-shock control with and without specific HOM-C gene activities.

Antp or Ubx proteins were ectopically induced simultaneously with Tsh in the same embryos and compared to HS-*Antp* and HS-*Ubx* controls. The expressivity of the homeotic transformations (mesothoracic for *Antp* and first abdominal for *Ubx*) in head segments (Fig. 8A,B) is increased in larvae carrying both *tsh* and HOM-C transgenes. In the thorax, where *tsh* is normally expressed (Fasano et al., 1991; Fig. 4D), perfect HOM-C induced homeotic transformations are observed as described previously (Gibson and Gehring, 1988; Gonzalez-Reyes and Morata, 1990). We conclude that *Antp* and *Ubx* genes override *tsh* for establishing specific posterior trunk segmental identities. No other obvious effects were seen in these larvae.

High levels of Tsh were then induced in 2-8 hour old embryos lacking *Scr*, *Antp* and BX-C gene activities. Without treatment, such embryos exhibit similar identities in every trunk segment (Struhl, 1983); a novel denticle belt and rudimentary beard differentiates anteriorly and the naked, posterior cuticle has head patterns within it (Fig. 8C). In these embryos, *tsh* transcription is initiated correctly but is not detected in the posterior part of the trunk segments by stage 10 or in older embryos (Röder et al., 1992). Ectopic induction of Tsh has one novel effect in this genotype in all trunk segments: the head cuticle in homologous posterior parts is eliminated (Fig. 8D). Ectopic activation of Antp or Ubx in this genotype has the same effect (Chan and Mann, 1993), showing that HOM-C and *tsh* genes have a partially redundant function to repress head identity.

DISCUSSION

We have expressed the Tsh protein ubiquitously at different stages during embryonic development and examined the effects on larval morphology. Heat-shock-induced Tsh promotes trunk identity in certain head segments of larvae, if present early in embryonic development. The C3 metamere is transformed into a prothoracic one, which depends on positive autoregulation of the *tsh* gene and the presence of the Scr HOM-C protein. We show that *tsh* has partially redundant functions in common with certain HOM-C genes. The similarity of *tsh* and HOM-C gene functions suggest that they may co-operate to regulate a set of downstream genes that determine larval trunk identities.

Common properties of *tsh* and some HOM-C genes

The *Drosophila* embryo can be divided into three regions on the basis of morphology; the head, the tail and the trunk in between. Here we define the trunk (or thorax and abdomen) as those segments that bear the denticle belts, the common larval pattern element that the two other regions do not possess. The genes *Antp*, *Ubx*, *abd-A* and *tsh* function independently for this trunk decision; only when all of these gene activities are removed are trunk patterns replaced with head ones (Röder et al., 1992).

Ectopic expression of the *tsh* gene induces trunk segmental identity in the head (Figs 1, 2). Only three of the head segments are transformed and these appear to be the same ones affected following ectopic expression of some trunk-specific HOM-C proteins. For example, following ectopic expression, Ubx and

Antp transform three head segments to first abdominal or metathoracic identities respectively (Mann and Hogness, 1990; Gonzalez-Reyes and Morata, 1990; Gibson and Gehring, 1988). In their normal domains of action, ectopic expression of Tsh (Fig. 8D), Antp or Ubx (Chan and Mann, 1993) repress head development in a HOM-C mutant background showing a common function of these genes. These features as a whole suggest that *Ubx*, *Antp* and *tsh* genes exhibit similar functions in that they promote trunk identities at the expense of head (Röder et al., 1992).

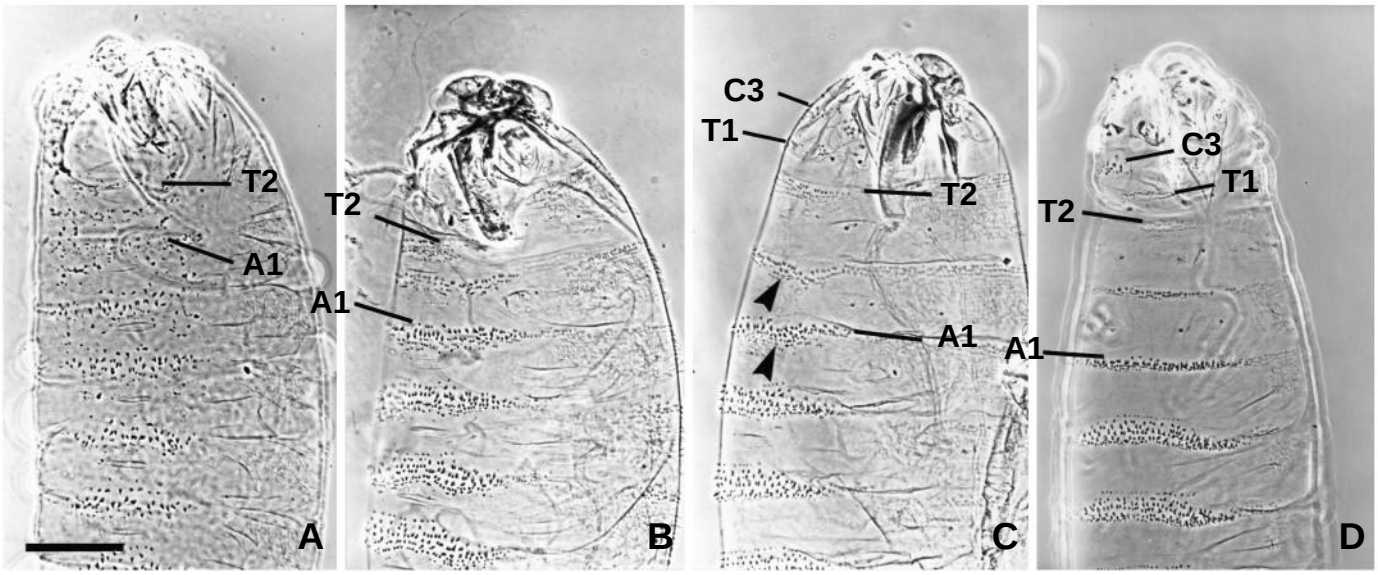

Fig. 6. Rescue of *tsh*$^{-}$ larvae with HS-*tsh* transgenes. Anterior boundaries of mesothoracic (T2) and first abdominal (A1) metameres are indicated. (A) A non-treated *tsh*^{8} HS-*tsh* homozygous larvae: the prothoracic denticle belt is missing, the size of trunk segments is reduced and denticle belt morphology is abnormal. Following ectopic expression of Tsh (B,C,D), the *tsh*^{8} null phenotype is partially rescued following a 20 minute pulse (B); more naked cuticle develops and denticle morphology is improved. (C,D) Treated twice for 20 minutes at 36°C, abdominal morphology is almost identical to wild type (compare to Fig. 2A). (C) The first abdominal and metathoracic segments exhibit duplications of denticles (arrow heads) in the naked cuticular region. (D) Only the prothoracic trunk segment is abnormal. In the T1 and C3 positions, segment size is reduced compared to wild type irrespective of the treatment employed. Similar results were obtained with the strong EMS-induced allele *tsh*^{9} (not shown).

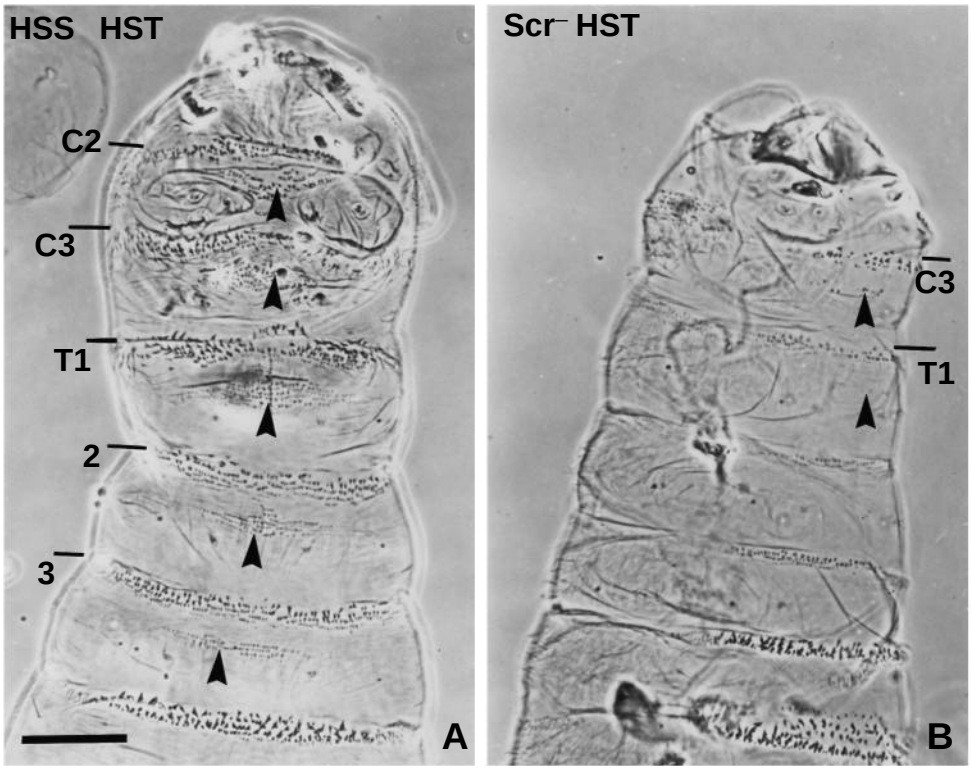

Fig. 7. *Scr*$^{+}$ and *tsh*$^{+}$ gene activities are required for prothoracic identity. (A) An HS-*tsh*/HS-*Scr* larva that received a heat shock at 36°C for 20 minutes before 8 hours of development. Note the presence of five prothoracic segments in the positions of the three thoracic (1, 2, 3), C3 and C2 segments. Compared to HS-*tsh* controls, the prothoracic identity in C2 is more complete with typical denticles and beard (arrowheads). (B) An HS-*tsh* *Scr*$^{-}$ larva treated for 20 minutes before 8 hours of development. Note the trunk segment in the position of the labial segment. In the T1 and C3 positions, a rudimentary beard (arrowheads) differentiates; the identity of these dentical belts is unlike any wild-type one.

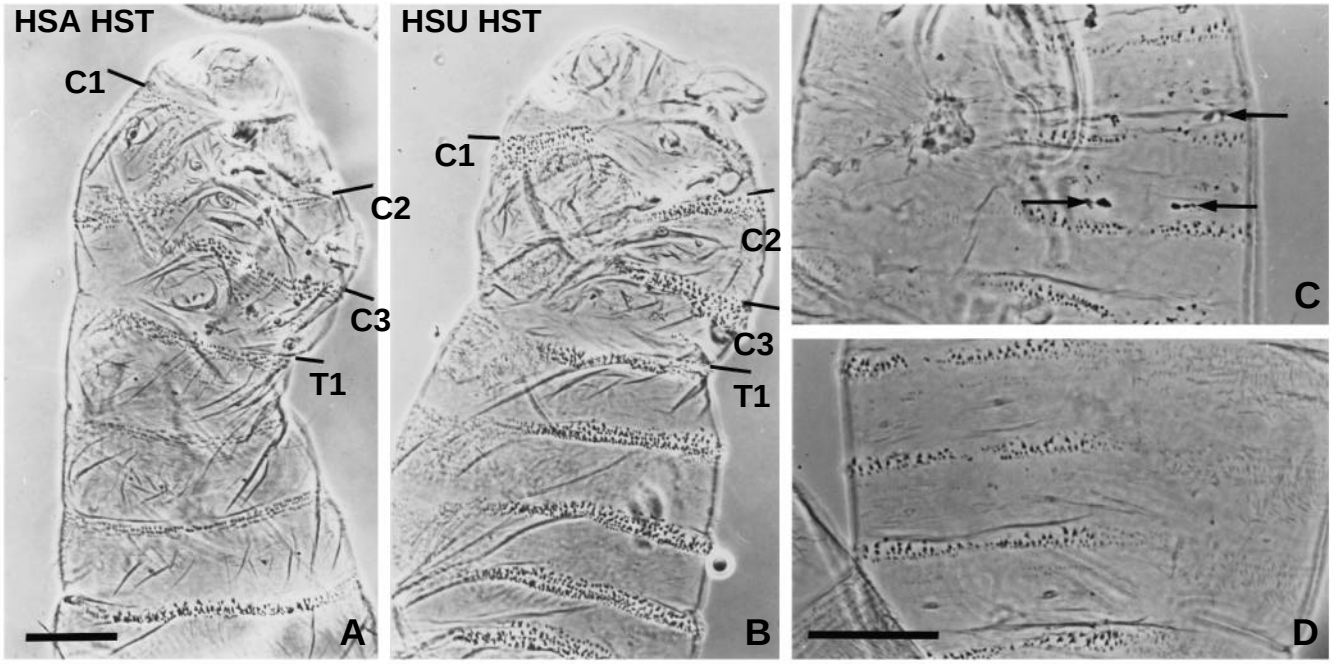

Fig. 8. Functional relationships between *tsh*, *Antp* and *Ubx* genes. Antp (A) or Ubx (B) was induced together with Tsh. Heat shocks were for 20 minutes at 36°C in 2-6 hour old embryos. (A) Note that T1, C3, C2 and C1 denticle belts resemble those of the wild-type mesothorax. The same segments, as well as the mesothoracic and metathoracic ones, resemble the first abdominal (A1) segment in B. (D) Tsh was produced ectopically in the absence of *Scr*, *Antp* and BX-C genes and compared to non-heat-shocked controls (C). In both cases, the denticle belts are unlike any wild-type one. In the posterior part of each segment, head cuticle differentiates (C) which is missing following induction of the HS-*tsh* transgene (D).

Ectopic tsh^+ activity primarily affects the identity of the C3 segment; C2 and C1 segments show weaker head-to-trunk transformations (Fig. 2C). Contrarily, ectopic *Ubx* or *Antp* products preferentially alter the identity of the C1 head segment and less frequently the C2 and especially the C3 gnathal segments (Gonzalez-Reyes and Morata, 1990; Gibson and Gehring, 1988). The preferential effect of Tsh on the C3 segment is due to positive autoregulation of the endogenous tsh^+ gene (Fig. 5B). Position-specific autoregulation has been reported for other homeotic genes; ectopic Dfd, for example, induces endogenous Dfd^+ activity only in specific cells of the trunk segments (Kuziora and McGinnis, 1988).

An intriguing observation is that HS-*tsh* cannot totally rescue the tsh^- phenotype (Fig. 6). Rescue is complete in most of the trunk except for the prothoracic segment. Either a higher, more continuous level of Tsh protein, or a novel one, is required in the prothorax. In more posterior segments, a lower level of Tsh may be required since Antp and BX-C genes have instructed cells to make trunk. Our results suggest that Tsh autoregulates its own transcription for complete function.

Phenotypic suppression and Tsh

Ectopic expression experiments demonstrate that even when a HOM-C protein is produced in all cells, only some cells respond. The presence or absence of other proteins may affect the influence of the ectopically expressed protein (Gibson and Gehring, 1988; Kuziora and McGinnis, 1988; Mann and Hogness, 1990; Gonzalez-Reyes et al., 1990). This phenomenon has been called phenotypic suppression (Gonzalez-Reyes et al., 1990, 1992); posteriorly acting HOM-C genes are in general 'dominant' to more anteriorly acting ones (Gonzalez-Reyes et al., 1990, 1992). A molecular explanation of phenotypic suppression could be that there is competition and differential affinity for similar binding sites (Gibson and Gehring, 1988; Gonzalez-Reyes et al., 1990), or differential affinities for similar cofactors (Zeng et al., 1993), changing the activities of downstream target genes.

The *tsh* gene exhibits the properties of phenotypic suppression; ectopic expression in the head, overrides and imposes its function (prothoracic-like pathway) over that of *Scr* (labial pathway; Fig. 2) and, albeit to a lesser extent, that of *Dfd*. Contrarily, it cannot override *Antp* or *Ubx* (Fig. 8) functions in more posterior trunk segments.

tsh^+ function is not similar in all ways to that of the HOM-C genes. First, tsh^+ activity is essential throughout the trunk domain (Fasano et al., 1991; Röder et al., 1992) whereas individual HOM-C gene activities are not. Second, outside of its functional domain, only Tsh can partially transform the ninth (tail) abdominal to trunk segmental identity (Fig. 4; Gonzalez-Reyes et al., 1992); one interpretation is that Tsh may compete with Abd-B since this phenotype is shared by $Abd\text{-}B^r$ mutations (Karch et al., 1985; Casanova et al., 1986). Third, ectopic sense organs differentiate in the posterior abdomen (Fig. 4) following high level Tsh expression, indicating a novel *tsh*-specific decision that is not reported for HOM-C genes. Finally, the *tsh* gene codes for a zinc finger protein that binds and recognises DNA sequences that are distinct (Fasano et al., 1991; Alexandre, Graba, de Zulueta, Fasano, Pradel, Kerridge and Jacq, unpublished data) from those of the HOM-C proteins (Scott et al., 1989; Affolter et al., 1990; Laughon, 1991). These

differences suggest that, although *tsh* shares common properties with HOM-C genes, it exhibits unique ones as well.

Co-operative functions of *tsh* and HOM-C genes

Although the trunk segments of the larva are similar, they show distinct pattern elements. The thoracic larval segments, for example, bear unique sense organs that abdominal segments do not. Differences can also be discerned between individual thoracic and abdominal segments. Certain HOM-C gene activities are critical for these differences (Lewis, 1978; Sanchez-Herrero et al., 1985; Kaufman et al., 1990) in combination with that of the *tsh* gene (Röder et al., 1992).

Co-operation between Tsh and Scr proteins is critical for prothoracic identity. Ectopic expression of Scr in the thorax changes the identity of the mesothorax and metathorax to prothorax (Gibson et al., 1990; Zeng et al., 1993) because Tsh is present (Röder et al., 1992). When *tsh* is expressed ectopically, the C3 metamere is homeotically transformed to prothorax because Scr is there (Figs 1, 2, 7). Other trunk identities seem to depend on the presence of Tsh with other specific HOM-C partners (Fig. 8). Tsh and Antp promote mesothorax, Tsh and Ubx promote first abdominal identity and so on.

An important requisite for co-operation is that genes are functional in the same cells and at similar times of development. For head-to-trunk homeosis, *tsh* must be active before 8 hours of development (Fig. 3). Several authors (Gonzalez-Reyes and Morata, 1990; Mann and Hogness, 1990; Lamka et al., 1992) noticed that the head segments were resistant to transformation by ectopic Ubx protein after 8 hours of development. Tsh and these HOM-C proteins may therefore be active in common cells during similar times of embryogenesis.

Previously, we have proposed that Tsh protein establishes a ground state trunk prepattern, by controlling the activity of a set of target genes (Röder et al., 1992). HOM-C proteins, such as Scr and Antp modulate the activity of a subset of these same downstream genes resulting in a change in segmental identity. It is noteworthy in this hypothesis that the Tsh protein can be detected before (Alexandre, Graba, de Zulueta, Fasano, Pradel, Kerridge and Jacq, unpublished data) the Scr and Antp ones (Wirz et al., 1986; Carroll et al., 1986; Mahaffey and Kaufman, 1987; Riley et al., 1987). As a whole, our results support the idea that the HOM-C genes *Scr*, *Antp* and *Ubx* are acting as modifiers, refining co-operatively the more general (regional) decision of trunk made by the *tsh* gene.

Another view of Tsh function is that it determines the specificity of action of HOM-C proteins. Specificity of these proteins is determined by the amino acids within or close to the homeodomain (Gibson et al., 1990; Mann and Hogness, 1990; Lin and McGinnis, 1992; Zeng et al., 1993). For the Scr and Antp homeoproteins (Gibson et al., 1990; Zeng et al., 1993), four amino acids, which lie in the N-terminal part of the homeodomain, are crucial; the Scr-type residues determine prothoracic, and the Antp ones mesothoracic, identity. It is important to note that Tsh does seem to be involved in this specificity decision although it is required co-operatively with both of these HOM-C proteins for these trunk identities; Tsh is critical for deciding whether Scr contributes to the prothoracic rather than the labial pathway. To our knowledge, the portion(s) of the Scr protein that are critical for this head-trunk distinction have not been determined. If Tsh determines how HOM-C genes act, it would share functional similarities with the *Drosophila* gene *extradenticle*, that may be involved in normal HOM-C gene function (Peifer and Wieschaus, 1989; Rauskolb et al., 1993).

The properties of partial redundancy, co-operativity and competition suggest that Tsh may share common target genes with certain HOM-C proteins. Our preliminary, as well as recent (Andrew et al., 1994), results support this working hypothesis. One attractive idea is that since Tsh shares common structural features with a protein thought to be necessary for normal chromatin structure (Fasano et al., 1991), it may assist HOM-C proteins to find their targets by altering the conformation of DNA near downstream target genes. The characterisation of common target genes should clarify how Tsh and HOM-C proteins co-operate at the molecular level.

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