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The roles of *hedgehog* and *engrailed* in patterning adult abdominal segments of *Drosophila*

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SUMMARY

We present evidence that *hedgehog* (*hh*) protein secreted by posterior compartment cells plays a key role in patterning the posterior portion of the anterior compartment in adult abdominal segments. Loss of function of *hh* in the hh^{ts2} mutant causes the loss of posterior tergite characteristics in the anterior compartment, whereas ectopic expression driven by hs-*hh* or the gain-of-function allele hh^{Mir} causes transformation of anterior structures toward the posterior. FLP-out *hh*-expressing clones in the anterior compartment induce surrounding wild-type cells to produce posterior tergite structures, establishing that *hh* functions non-autonomously. The effects of pulses of ectopic expression driven by hs-*hh* indicate that bristle type and pigmentation are patterned by *hh* at widely different times in pupal development.

We also present evidence that the primary polarization of abdominal segments is symmetric. This symmetry is strikingly revealed by ectopic expression of *engrailed* (*en*). As expected, this transforms anterior compartment cells to posterior compartment identity. In addition, however, ectopic *en* expression causes an autonomous reversal of polarity in the anterior portion of the anterior compartment, but not the posterior portion. By determining the position of polarity reversal within *en*-expressing clones, we were able to define a cryptic line of symmetry that lies within the pigment band of the normal tergite. This line appears to be retained in hh^{ts2} mutants raised at the restrictive temperature, suggesting it is not established by *hh* signaling. We argue that the primary role of *hh* in controlling polarity is to cause anterior compartment cells to reverse their interpretation of an underlying symmetric polarization. Consistent with this, we find that strong ectopic expression of *hh* causes mirror-symmetric double posterior patterning, whereas *hh* loss of function can cause mirror-symmetric double anterior patterning.

Key words: *hedgehog*, *engrailed*, pattern formation, segment polarity, gradient, compartment, *Drosophila*

INTRODUCTION

The epidermis of the embryonic segments and imaginal discs of *Drosophila* is organized into lineage compartments defined by expression of selector genes. Compartments along the anterior-posterior axis are determined by the *engrailed* (*en*) gene, which is expressed in the posterior but not the anterior compartment of each segment. Pattern formation within each segment is largely dependent on interactions between anterior and posterior cells at compartment boundaries (reviewed in: Lawrence and Struhl, 1996; Tabata et al., 1995).

An important component of signaling between compartments is the secreted protein encoded by *hedgehog* (*hh*). *hh* is expressed in posterior compartment cells under the control of *en* (Lee et al., 1992; Mohler and Vani, 1992; Tabata et al., 1992; Zecca et al., 1995), and functions as a short-range inducer that causes a stripe of adjacent anterior compartment cells to respond by activating *hh* target genes, which include the Hh receptor component *patched* (*ptc*), the transcription factor *cubitus interruptus*, and the long-range morphogens *wingless* (*wg*) and *decapentaplegic* (*dpp*) (Alexandre et al., 1996; Dominguez et al., 1996; Hepker et al., 1997; Ingham, 1993; Johnson et al., 1995).

The long-range morphogens induced by *hh* serve to pattern both anterior and posterior compartments. *wg* plays such a role in the embryonic epidermis (Dougan and DiNardo 1992; Lawrence et al., 1996) and *dpp* has been shown to pattern much of the wing disc (de Celis et al., 1996; Lecuit et al., 1996; Nellen et al., 1996). Both *dpp* and *wg* participate in patterning the leg and antennal discs (Brook and Cohen, 1996; Jiang and Struhl, 1996; Penton and Hoffmann, 1996; Theisen et al., 1996). However, some functions of *hh* in imaginal discs and the embryonic epidermis are independent of *dpp* and *wg* (Bokor and DiNardo, 1996; Jiang and Struhl, 1995; Lawrence et al., 1996; Li et al., 1995; Mullor et al., 1997).

In this report, we address the roles of *hh* and *en* in the epidermis of the adult abdomen of *Drosophila*. Transplantation experiments in other insect species suggest that pattern in abdominal segments is established by segmental morphogen gradients (Bhaskaran and Röller, 1980; Campbell and Caveney, 1989; Lawrence, 1966). A major motivation for our work is to

determine whether Hh or other signaling molecules are responsible for such gradients in the abdomen of *Drosophila*.

The epidermis of the adult abdomen in *Drosophila* develops from histoblast nests that are laid down during embryogenesis as derivatives of the embryonic epidermis (Simcox et al., 1991). Each larval abdominal hemisegment contains three major histoblast nests: an anterior dorsal nest, which produces the abdominal tergite, a posterior dorsal nest, which produces the intertergal cuticle, and a ventral nest, which produces the sternite and pleural cuticle (Madhavan and Madhavan, 1980). Unlike imaginal disc cells, abdominal histoblasts do not proliferate during the larval stages. Instead, they remain part of the larval epidermis and secrete larval cuticle through each of the larval molts. Histoblast mitoses resume 1-1.5 hours after puparium formation (APF) and continue until approximately 40 hours APF, as the histoblasts migrate and replace the degenerating larval epidermal cells (LEC) (Madhavan and Madhavan, 1980). By 40-41 hours APF, all LEC are replaced by the newly formed imaginal epidermis.

As in other segments, the epidermis of adult abdominal segments is subdivided into anterior and posterior compartments (Hama et al., 1990; Kornberg, 1981). We present evidence that *en* and *hh* specify cell fates in the posterior compartment and that *hh* also functions non-autonomously to induce posterior characteristics within the anterior compartment. Although *hh* is able to polarize anterior compartment cells, it does not appear to play a primary role in establishing polarity. Rather, we present evidence that the abdominal segment is polarized symmetrically by a mechanism that is independent of *hh* and that *hh* causes anterior compartment cells to reverse their interpretation of this underlying polarization.

MATERIALS AND METHODS

Enhancer trap lines used were: Xho-25 *en-lacZ* (Hama et al., 1990), P2023-44 *hh-lacZ* (Tabata et al., 1992), P{ry$^{+t7.2}$=PZ} *wg^{r0727}* (G. Rubin/ BDGP), P{ry$^{+t7.2}$=PZ} *dpp^{10638}* (A. Spradling/ BDGP). FLP-out *en*, *hh* and *dpp* constructs are described by Basler and Struhl (1994) and Zecca et al. (1995). The hs-FLP line used was the X-chromosomal FLP 122, provided by Konrad Basler. The hs-GAL4 driver used was P{w^{+mC}=GAL4-Hsp70.PB}89-2-1 (Brand and Perrimon, 1993). The hs-*hh* line is described by Tabata et al. (1992). The UAS-*ptc* line was a gift from Matt Scott (Johnson et al., 1995).

Adult abdominal cuticles were prepared and mounted as described by Duncan (1982).

Heat-shock pulses were administered in glass vials in an agitated water bath at 37°C. For FLP-out experiments, a single 10-minute heat shock was given.

To prepare the pupal abdominal epidermis for staining, pupae were bisected longitudinally with a razor blade and placed in an Eppendorf tube with 1 ml of ice-cold PBS+0.1% Tween-20 (PBT). The tube was gently agitated to remove most of the gut and fat body. For in situ hybridization, body walls were then fixed for 30 minutes in 5% formaldehyde in PBT. For antibody staining, fixation was for 10 minutes in 1% formaldehyde in PBT, and, for X-Gal staining, 10 minutes in 1% glutaraldehyde in PBT. After fixation, body walls were washed twice for 10 minutes in PBT, stained in 0.5% eosin-Y (Sigma) in PBT and rinsed in PBT. Internal organs and most muscles were then removed with a pair of sharp forceps, leaving the abdominal epidermis exposed.

In situ hybridization was as described by Poeck et al. (1993), with the following modifications. Pupal body walls were digested in 2 μg/ml Proteinase K for 2-3 minutes; stronger treatment destroys the abdominal epidermis. Hybridization and washes were performed at 55°C, and 0.1% Tween-20 was present in all solutions. The *hh* RNA probe used was prepared as described by Tabata et al. (1992). X-Gal staining was performed as described by Hama et al. (1990). The monoclonal antibody 4D9 of Patel et al. (1989), which recognizes both *en* and *invected* proteins, was used for En stainings. Antibody stainings were as described by Kellerman et al. (1990). Stained body walls were washed in ethanol, rehydrated, mounted flat in 80% glycerol and viewed with DIC optics.

RESULTS

Segment and compartment boundaries in the adult abdomen

Each abdominal segment produces a large dorsal cuticular plate, the tergite, and a smaller ventral plate, the sternite. Tergites of adjacent segments are separated by flexible intertergal cuticle, and sternites are separated from one another and from the tergites by pleural cuticle. Each tergite can be divided into three regions: an anterior region (the acrotergite) that contains undecorated sclerotized cuticle, a central region containing an array of microchaetes, and a posterior region that contains a dark pigment band and a row of large macrochaetes at its posterior edge (Fig. 1A). The central and posterior regions, but not the acrotergite, are covered with trichomes. For convenience, we define the posterior boundary of the tergite to be the posterior edge of the pigment band. This does not agree with other authors (Madhavan and Madhavan, 1980; Roseland and Schneiderman, 1979). The intertergal cuticle is unpigmented and composed of an anterior trichome-bearing region (the posterior hairy zone or PHZ) and a posterior region of naked cuticle (the intersegmental membrane or ISM). All trichomes and bristles in the abdomen are oriented from the anterior to the posterior.

The positions of the compartment and segment boundaries relative to cuticular structures in the abdomen can be inferred from expression of *en-lacZ* in newly emerged adults (Hama et al., 1990, and Fig. 2A). The anterior boundary of *en* expression, which marks the compartment boundary, is not fixed precisely with respect to cuticular pattern. In some cases it coincides with the tergite-PHZ boundary, while in others it lies 3-4 trichome rows posterior to it (Fig. 2B, C). This indicates that a 3- to 4-cell-wide region of the PHZ can be contributed by either compartment. The posterior edge of each *en* stripe coincides with the ISM-acrotergite border (Hama et al, 1990, and Fig. 2A).

In larvae, *en* is expressed throughout the posterior dorsal histoblast nest, but is not expressed in the anterior dorsal nest. In the single ventral nest, posterior cells express *en*, whereas anterior cells do not (Hama et al., 1990). We find that this pattern is preserved in prepupae and early pupae (not shown). Following fusion of the dorsal nests at 18-20 hours APF, *en* protein accumulates in a gradient that is highest at the anterior-posterior compartment boundary and trails off posteriorly (Fig. 3A).

The most posterior row of LEC in each segment is the last to be replaced by the expanding histoblast nests. These ‘border cells’ are easily distinguished from their neighbors by a more

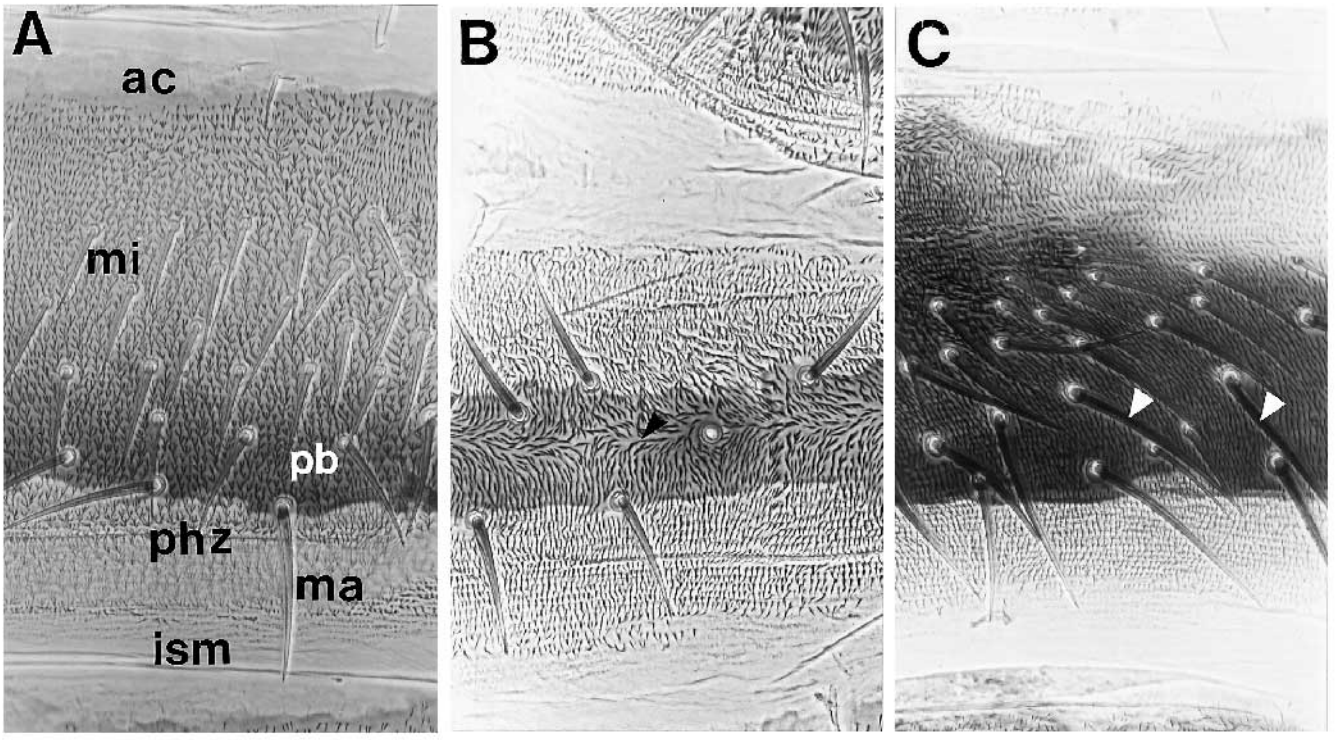

Fig. 1. Cuticular phenotypes of *hh* gain-of-function mutants. (A) Wild-type pattern of the dorsal cuticle of the third abdominal segment. ac, acrotergite; mi, microchaete; ma, macrochaete; pb, pigment band; phz, posterior hairy zone; ism, intersegmental membrane. The sharp posterior limit of the pigment band marks the tergite – PHZ boundary. Note that all bristles and trichomes are oriented posteriorly. (B) Mirror-image posterior duplication in hh^{Mir}. Most of the tergite is deleted, and replaced with ISM, PHZ and posterior tergite structures of reversed polarity. The arrowhead points to the line of polarity reversal. (C) Posterior transformation in $hh^{MirRevBx1}$. Note extension of the pigment band to the anterior and ectopic macrochaetes (arrowheads).

convex basal surface (Fig. 3B). The border cells are replaced by *en*-expressing histoblasts, indicating that adult and larval segment boundaries coincide precisely. The gradient of *en* protein persists until several hours after elimination of the border cells. Later, *en* expression in the posterior compartment becomes uniform, as seen in the adult (Fig. 2A).

hedgehog directs posterior patterning

We have recovered a gain-of-function allele of *hh*, $hh^{Mirabile}$ (hh^{Mir}), that causes a dramatic phenotype in the adult abdomen in heterozygotes: the anterior tergite is absent and replaced by a mirror-image duplication of the posterior tergite and intertergal region (Fig. 1B). The duplicated structures correspond to the posterior compartment and the posterior region of the anterior compartment. Internally, the oenocytes underlying the posterior tergite are also duplicated, and the insertion points of the adult muscles reflect the symmetric epidermal pattern (not shown). The sternites are usually unaffected. With the exception of a mid-dorsal thoracic groove seen in heterozygotes raised at 29˚C, the effects of hh^{Mir} appear to be limited to the adult abdomen.

hh^{Mir} was isolated following X-ray mutagenesis and is associated with a complex chromosomal rearrangement (new order: 1-20F|81F-64BC|94A1-81F|94A1-100 + 61-64BC|20F). To eliminate involvement of the X chromosome, a derivative of sequence 61-64BC|81F-64BC|94A1-81F|94A1-100 was recovered after further irradiation. By recombining this derivative with other inversions, we mapped the abdominal phenotype of hh^{Mir} to near the 81F|94A1 breakpoint. Allelism with *hh* was established by recovering X-ray-induced reversions. Three such revertants had chromosomal breaks at the *hh* locus (94DE) and behaved as *hh* nulls or hypomorphs. hh^{Mir} complements the recessive lethality of *hh*, suggesting that it does not compromise most normal functions of *hh*.

In wild type, *hh* expression in histoblasts closely matches the pattern of *en* expression. In prepupae and early pupae, *hh* transcript can be detected throughout the posterior dorsal nest, but is absent from the anterior dorsal nest (Fig. 3C). Following fusion of the two nests, *hh* is expressed in an anterior-posterior gradient with a sharply defined anterior border, which presumably corresponds to the compartment boundary (Fig. 3D). The enhancer trap P2023-44 (Tabata et al., 1992) is expressed in histoblasts in a pattern identical to the *hh* transcript (not shown). We also see *hh* expression in the LEC in a posterior stripe that appears to match the *en* stripe, with the notable exception that *hh* is not expressed in the larval border cells (Fig. 3C,D). *hh* transcript can be detected in the border cells in *hs-hh* pupae following heat shock (see below), indicating that the failure of these cells to stain in wild type is not due to exclusion of the *hh* probe.

The basic defect in hh^{Mir} appears to be that it drives ectopic expression of *hh* in the anterior compartment in the pupal abdomen. Consistent with the double posterior cuticular pattern seen in the adult, *hh* is expressed strongly at the anterior and posterior edges of the combined dorsal histoblast nest in hh^{Mir} heterozygotes (Fig. 3E). *hh* expression in the ventral histoblasts is usually normal (not shown).

We find that hh^{Mir} also causes ectopic activation of both the wild-type *hh* allele and *en*. *hh* activation was monitored by the expression of the *hh-lacZ* enhancer trap P2023-44 (Tabata et al., 1992). When heterozygous with hh^{Mir}, this reporter was expressed in the ectopic anterior domain, although at only a low level (not shown). We also observe an ectopic *en* stripe in the middle of the anterior compartment (Fig. 2D). Ectopic expression of *hh* in the anterior compartment of the wing also causes activation of *en* and endogenous *hh* (Guillén et al., 1995).

To test the effects of ubiquitous expression of *hh*, prepupae and pupae carrying a hs-*hh* transgene (Tabata et al., 1992) were subjected to 2-hour heat shocks at different stages. Heat-shock treatment during the first 5 hours APF has no effect in the abdominal cuticle. Heat shocks delivered between 5 and 10 hours APF result in the transformation of posterior microchaetes into macrochaetes (Fig. 4A). Flies subjected to single heat shocks between 11 and 17 hours APF are usually unaffected, although weak disturbances in bristle polarity are occasionally observed (not shown). Finally, heat-shock treatment between 18 and 35 hours APF (23-29 hours APF is the most sensitive stage) results in an anterior expansion of the pigment band of the tergite without any effect on the bristle pattern (Fig. 4B).

When flies carrying two copies of the hs-*hh* construct are subjected to three or four 2-hour heat shocks between 5 and 30 hours APF, tergites are compressed to approximately one half of their normal width, while the ISM is considerably expanded. The polarity of structures in the anterior tergite is sometimes completely reversed (Fig. 4C). We also observed mirror-image posterior duplications in many sternites (Fig. 4E). No comparable abnormalities were observed in wild-type flies subjected to similar treatment. We observed no stable ectopic activation of either *hh* or *en-lacZ* following heat-shock treatment, suggesting that the phenotypes observed were caused by the pulses of uniform *hh* expression.

The phenotypes produced by ubiquitous *hh* expression suggest that relatively low levels of ectopic *hh* are sufficient to promote posterior cell fates in the anterior of the tergite, but higher levels are required to affect polarity. This is supported by the phenotype of a partial reversion of hh^{Mir}, $hh^{Mir\text{-}RevBx1}$. Heterozygotes for this revertant show expansion of the posterior pigment band to include most of the tergite and transformation of some microchaetes to macrochaetes (Fig. 1C), but do not show any polarity reversal. Homozygotes die as embryos that show a partial *hh* loss-of-function phenotype, indicating that $hh^{MirRevBx1}$ is associated with a hypomorphic allele of *hh*. We find that the expression pattern of $hh^{MirRevBx1}$ is identical to that of hh^{Mir} (not shown). Hence, it would appear that *hh* protein produced by $hh^{MirRevBx1}$ is sufficiently active to promote posterior characteristics, but not to cause polarity reversal.

To test the autonomy of *hh*, we generated 'FLP-out' (Basler and Struhl, 1994) *hh*-expressing clones in the anterior compartment. These produce ectopic ISM, PHZ and posterior tergite structures. Since the cuticular marker used (*yellow*) cannot be scored in ISM or PHZ, we cannot determine the limits of *hh*-expressing clones within these cuticle types. Except for an occasional *yellow* bristle, the posterior tergite structures induced were produced by y^+ cells, indicating that *hh* functions non-autonomously.

The effects of *hh*-expressing clones in the anterior compartment depend strongly on their position in the tergite. Clones in the middle region, anterior to the pigment band, induced concentric patterns that consisted of a patch of PHZ cuticle surrounded by posterior tergite structures (Fig. 5C). Trichomes and bristles in the reorganized areas were oriented radially towards the center of the patch. In contrast, more anterior clones were polarized in their effects, and induced ectopic posterior tergite structures only at their posterior edges. Tergite structures located laterally and anteriorly to such clones were completely unaffected (Fig. 5A). This is not due to differential competence to respond to *hh*; the same tergite region produces posterior tergite structures when located posterior to *hh*-expressing clones, but is unaffected when located lateral or anterior to such clones (Compare Fig. 5A and B). These observations suggest that secretion of *hh* protein may be polarized in these anterior clones. Anterior clones also induced PHZ and ISM; the PHZ always had reversed polarity and was located to the posterior of the ISM. The boundary between the two was straight and perpendicular to the anterior-posterior axis of the segment (Fig. 5A). The pattern produced by large anterior *hh*-expressing clones is remarkably similar to that of hh^{Mir}. Identifiable clones in the posterior tergite were rare, but a few patches of PHZ associated with ectopic macrochaetes were found (Fig. 5D). Polarity of the PHZ and macrochaetes was normal.

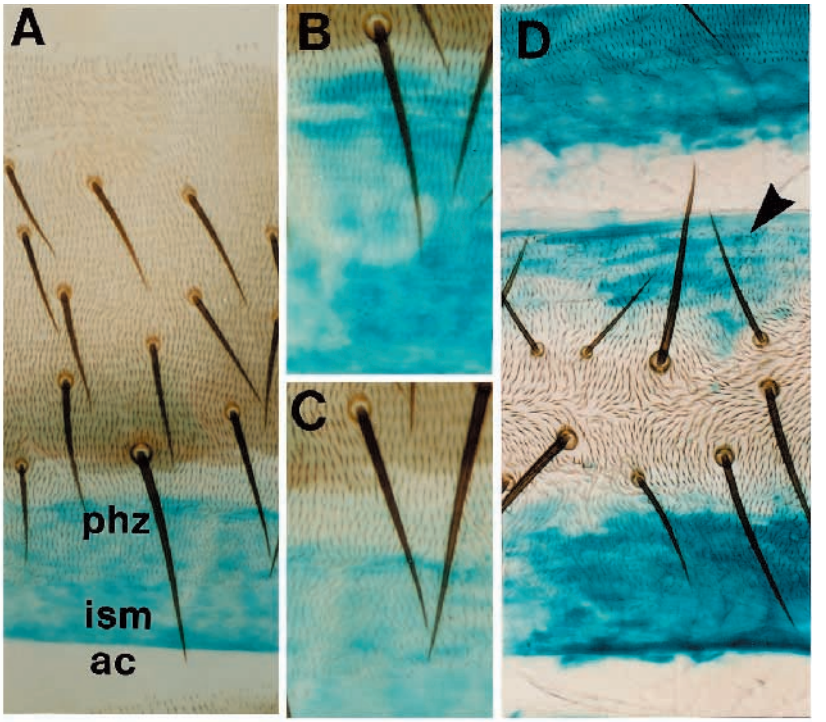

Fig. 2. Compartment and segment boundaries in the adult abdomen. (A-C) *en-lacZ* expression in the wild type. Note the variable positioning of the compartment boundary with respect to cuticular pattern (compare B and C). (D) Ectopic activation of *en-lacZ* in the anterior compartment in hh^{Mir}(arrowhead).

To remove *hh* function from the developing abdomen, homozygotes for the temperature-sensitive allele hh^{ts2} (Ma et al., 1993) were reared at 18˚C up to the late third larval instar and shifted to 29.5˚C immediately after pupariation. Among animals surviving to the late pharate adult stage, we found that many hemitergites displayed mirror-image anterior duplications. In such cases, posterior tergite, PHZ and ISM were deleted and replaced with a mirror-image duplication of the anterior tergite, creating a pattern opposite to that seen in hh^{Mir}(Fig. 6A). Other phenotypes found include complete or partial deletion of PHZ and ISM, missing posterior pigment band and transformation of macrochaetes to microchaetes (Fig. 6B). These weaker phenotypes are reciprocal to the patterns seen in $hh^{MirRevBx1}$ heterozygotes and hs-*hh* animals given single heat shocks at the pupal stage. Ventrally, the sternites were greatly reduced in size and the sternal bristles were usually absent (not shown). No comparable abnormalities were observed in wild-type flies reared at 29.5˚C.

The loss-of-function phenotype of *hh* can be mimicked when *hh* signaling is blocked by overexpression of *ptc*. We expressed *ptc* ubiquitously in the pupal abdomen by subjecting hs-GAL4/UAS-*ptc* flies (Johnson et al., 1995) to a single 2-hour heat shock at the prepupal or early pupal stage. The phenotypes produced included the loss of the pigment band, transformation of macrochaetes to microchaetes and complete or partial deletion of PHZ and ISM (Fig. 6C).

The function of *hh* in patterning the posterior portion of the tergite does not appear to be mediated by *dpp* or *wg*. *dpp* is expressed in the pupal abdomen in the pleural portion of the ventral histoblast nest and in a few cells at the dorsal midline in the dorsal nests (Fig. 7A,B). *wg* is expressed in the sternite primordia and in the medial tergite, but is excluded from cells at the dorsal midline (Shirras and Couso, 1996, and Fig. 7C,D). Neither gene is expressed in the lateral tergite (Fig.7A-D). The expression of both genes is limited to the posterior region of the anterior compartment (Shirras and Couso, 1996, and Fig. 7) and both are activated anteriorly in hh^{Mir} (not shown), suggesting that their expression patterns are controlled by *hh*. However, neither gene is responsible for anterior-posterior patterning. *wg* functions to promote bristle formation and to differentiate sternite and tergite primordia from the pleura (Shirras and Couso 1996). The function of *dpp* is less clear, but we find that large FLP-out *dpp*-expressing clones develop normally in most of the tergite (not shown).

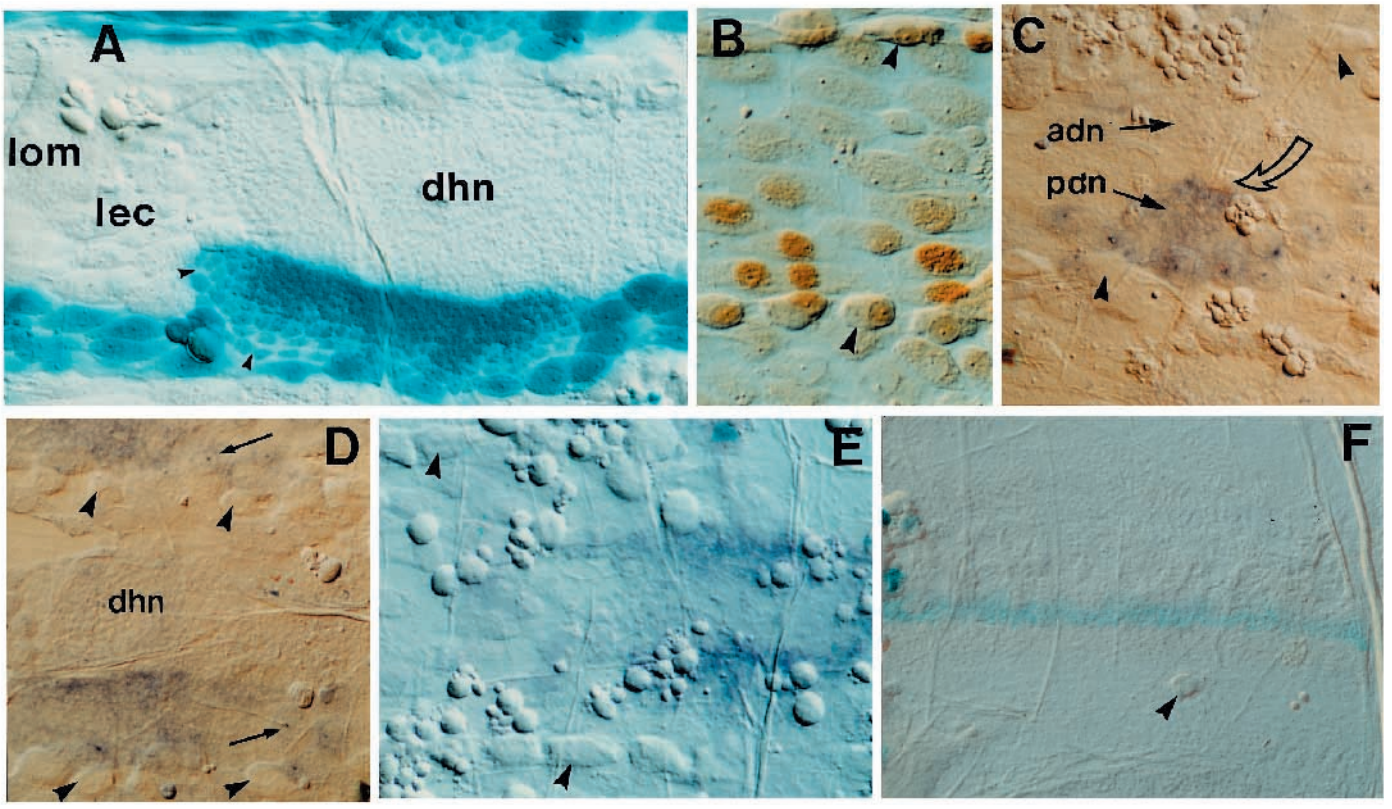

Fig. 3. Expression of *en*, *hh* and *ptc* in the pupal abdominal epidermis. (A) Expression of *en-lacZ* in the dorsal pupal abdomen. dhn, combined dorsal histoblast nest; lec, larval epidermal cells; lom, larval oblique muscle. Note that *en* expression decreases with distance from the compartment boundary (arrowheads). The anti-*en* monoclonal antibody 4D9 reveals an identical pattern. (B) Expression of the en protein in the LEC in the pupal abdomen. Note the specialized 'border cells' at the segment boundaries (arrowheads). (C) Expression of the *hh* transcript in the dorsal pupal abdomen prior to the fusion of the anterior (adn) and posterior (pdn) dorsal histoblast nests. The two nests are separated by a persisting LEC (open arrow). (D) *hh* expression in the combined dorsal nest (dhn). Note the sharp limit of expression at the compartment boundary and the diminishing level of expression toward the posterior. In C and D, note that while most posterior compartment LEC express *hh* (thin arrows in D), the specialized border cells (arrowheads) do not. (E) Expression of the *hh* transcript in the dorsal histoblast nest in hh^{Mir}. Arrowheads point to the border cells. (F) Expression of *ptc-lacZ* in the dorsal pupal abdomen in wild type at 40 hours APF. The arrowhead points to the last persisting border cell, which marks the segment boundary. Note that no *ptc-lacZ* expression is detectable at the anterior edge of the more posterior segment.

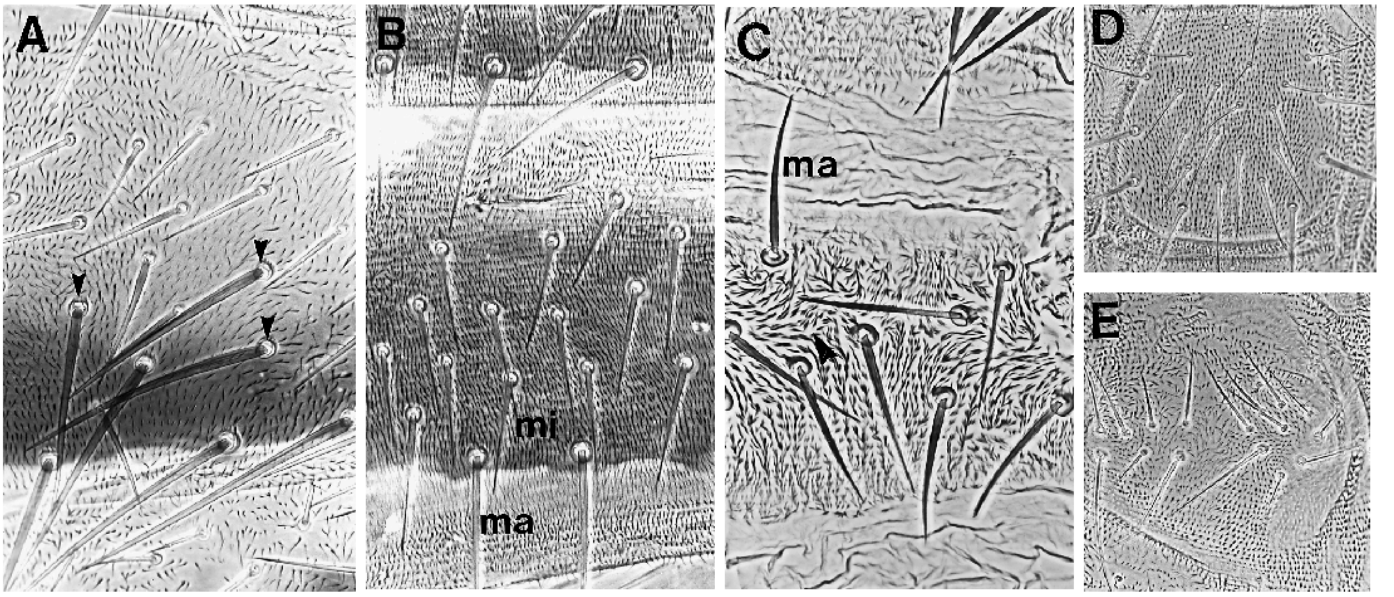

Fig. 4. Effects of ubiquitous *hh* expression in hs-*hh* pupae. (A) Early heat shock (5 hours APF). Arrowheads point to ectopic macrochaetes. Note that pigmentation is not affected. (B) Late heat shock (between 20 and 30 hours APF). Note the extension of the pigment band; the bristle pattern is unaffected. ma, macrochaete; mi, microchaete. (C) Multiple heat shocks. The tergite is compressed, while the ISM is widened. Note the reversed polarity and an ectopic macrochaete in the anterior tergite. Arrowhead points to a line of polarity reversal. (D) A wild-type sternite. (E) Mirror-image posterior duplication in the sternite resulting from ubiquitous expression of *hh*.

engrailed controls cell fates and polarity in the adult abdomen.

Two gain-of-function alleles of *en*, en^{Erased} (en^{Es}) (Lindsley et al., 1972) and $en^{Apigmented\ abdomen}$ (en^{Apa}) (E. B. Lewis, personal communication) cause ectopic expression of *en* in the pupal abdomen. Both alleles are associated with rearrangements broken at the *en* locus (48A). In heterozygotes for these mutants, *en* is expressed broadly in the anterior compartment. However, expression is always reduced in a 3- to 4-cell-wide stripe of cells anterior to the compartment boundary, and is excluded from apparently randomly distributed patches more anteriorly (Fig. 8C). *en* expression in the ventral epidermis appears normal. Both en^{Es} and en^{Apa} cause weak ectopic activation of *hh* in the anterior compartment (not shown), indicating that *hh* is positively regulated by *en* in the abdomen, as it is in imaginal discs and embryonic epidermis (Guillén et al., 1995; Tabata et al., 1992; Zecca et al, 1995). The *en-lacZ* enhancer trap element is not activated in the abdomen by en^{Es} or en^{Apa}.

The cuticular phenotypes of en^{Es} and en^{Apa} are dramatic; with the exception of interspersed islands of apparently normal tergite cuticle, each abdominal segment consists of a broad central region of PHZ cuticle flanked to the anterior and posterior by ISM. Each segment is mirror symmetric, with the PHZ trichomes in the anterior half of the segment directed anteriorly, and the trichomes in the posterior half directed posteriorly (Fig. 8A). However, polarity is always normal within the islands of tergite cuticle; in the anterior of the segment, this leads to a sharp discontinuity in polarity where PHZ and tergite meet. The patches of tergite tissue in the anterior of the segment are lightly pigmented and do not carry macrochaetes (Fig. 8A), indicating that the level of *hh* produced in the anterior compartment is not sufficient to promote posterior cell fates. en^{Es}/en^{Apa} heterozygotes have a similar phenotype, with the exception that no tergite islands are present (Fig. 8B).

To determine whether the tergite islands present in en^{Es} and en^{Apa} correspond to the patches in the anterior compartment that do not express *en*, we stained en^{Apa} heterozygotes at about 55 hours APF, when cuticular differentiation is well under way, but *en* can still be detected. We found that *en*-expressing cells secrete PHZ or ISM cuticle, whereas cells that do not express *en* produce tergite structures (Fig. 8D). *en* is also autonomous with respect to polarity reversal; in the anterior of the tergite, trichomes secreted by *en*-expressing cells are oriented to the anterior, whereas trichomes secreted by adjacent non-expressing cells are oriented to the posterior.

We have used the FLP-out system (Basler and Struhl, 1994; Zecca et al., 1995) as an additional way to examine the effects of ectopic *en* expression. The cuticular marker present within the FLP-out construct used (*forked*) cannot be scored in *en*-expressing clones in the abdomen, since these produce PHZ and ISM cuticle, and usually lack bristles. However, because the effects of ectopic *en* expression are autonomous in en^{Apa}, we assume that the limits of *en*-expressing clones correspond to the edges of the PHZ or ISM patches seen.

The effects of *en*-expressing clones depend upon their position within the segment. Clones in the posterior tergite were transformed to PHZ, whereas more anterior clones contained both PHZ and ISM (Fig. 5E-G). Larger clones also caused wild-type cells located to the posterior to produce posterior tergite structures (Fig. 5E). This non-autonomous effect is probably due to activation of *hh* within the *en*-expressing clones. As with *hh*-expressing clones, ectopic ISM cuticle was always induced anteriorly to the ectopic PHZ, and was never induced posterior to the second (from the anterior) row

of microchaetes; tergite structures located laterally and anteriorly to the clone were always unaffected (Fig. 5E).

Like en^{Es} and en^{Apa}, *en*-expressing clones reveal underlying mirror symmetry to the polarization of abdominal segments. Clones in the anterior tergite produced PHZ with reversed trichome orientation, whereas clones in the posterior tergite produced PHZ of normal polarity (Fig. 5E,G). *en*-expressing clones that straddled both regions showed an internal line of polarity reversal within the PHZ region (Fig. 5F). By plotting where this line intersects normal tergite tissue for a number of such clones, we were able to define a line of underlying polarity reversal within the tergite. Although not rigidly placed

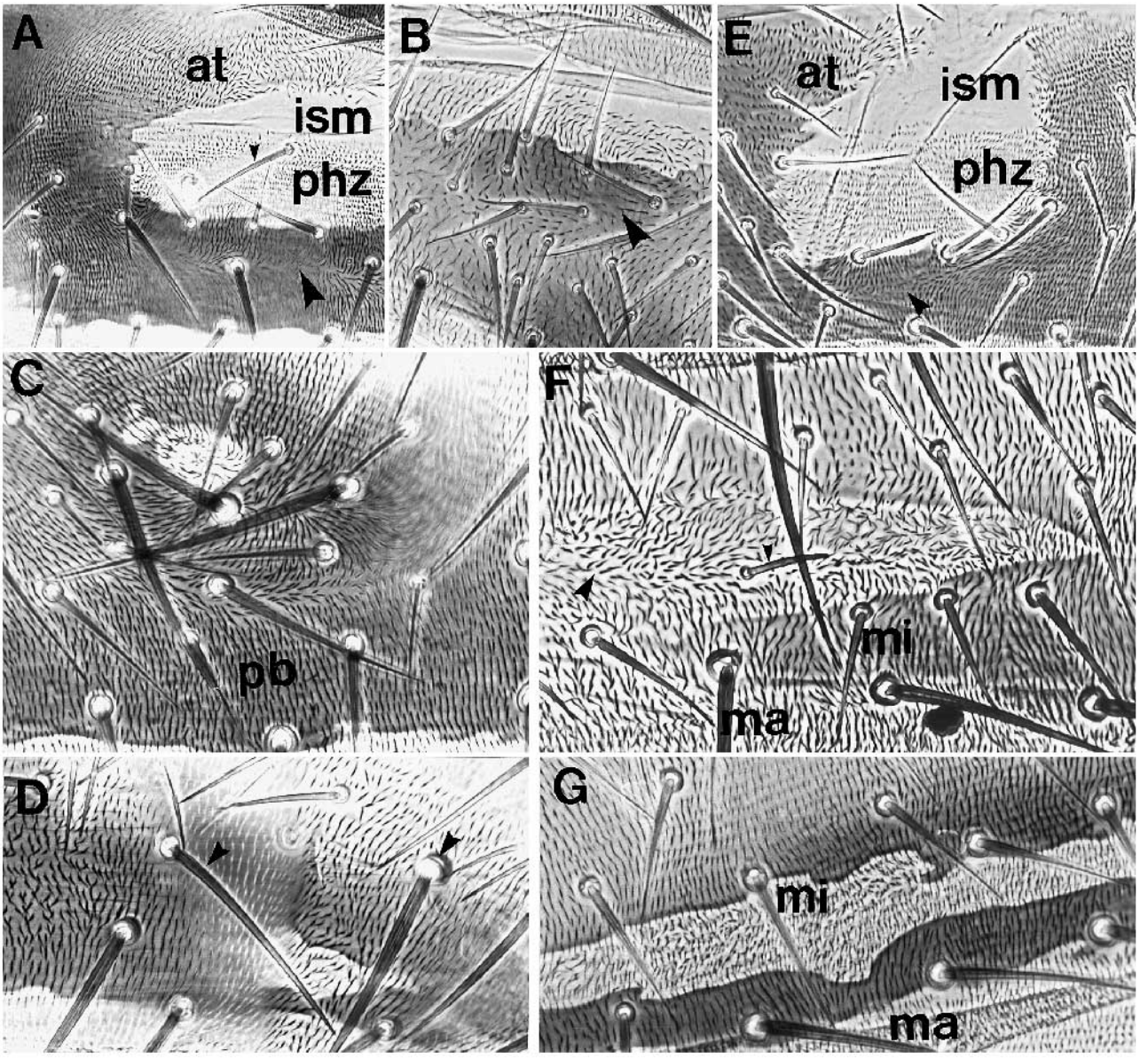

Fig. 5. Phenotypes induced by FLP-out somatic clones that ectopically express *hh* (A-D) and *en* (E-G) in the anterior compartment. Abbreviations as in Fig. 1. (A) *hh*-expressing clone in the anterior tergite. Ectopic PHZ and posterior tergite structures induced by the clone have reversed polarity. The large arrowhead points to the line of polarity reversal in the posterior tergite. The bristle located in the ectopic PHZ (small black arrowhead) is y^+, and so originated outside the clone. (B) *hh*-expressing clone at the anterior edge of the tergite. Posterior structures of reversed polarity are induced in the anterior tergite (arrowhead). Note that anterior tergite (at) structures are affected when located posterior (B), but not lateral or anterior (A), to the clone. (C) Central *hh*-expressing clone. Note concentric arrangement of structures and radial polarization of trichomes and macrochaetes. (D) Presumed posterior *hh*-expressing clone. Note PHZ and ectopic macrochaetes (arrowheads). Polarity is unaffected. (E) Anterior *en*-expressing clone. Ectopic PHZ and posterior tergite structures induced by the clone have reversed polarity. The arrowhead points to the line of polarity reversal. Note that only the structures located posteriorly to the clone are affected. (F) Central *en*-expressing clone. Note the line of polarity reversal within the clone (large arrowhead). The presence of a *f* bristle (small arrowhead) suggests that the level of *en* produced by the clone does not completely suppress bristle development. (G) Posterior *en*-expressing clone. Polarity in the ectopic PHZ is normal.

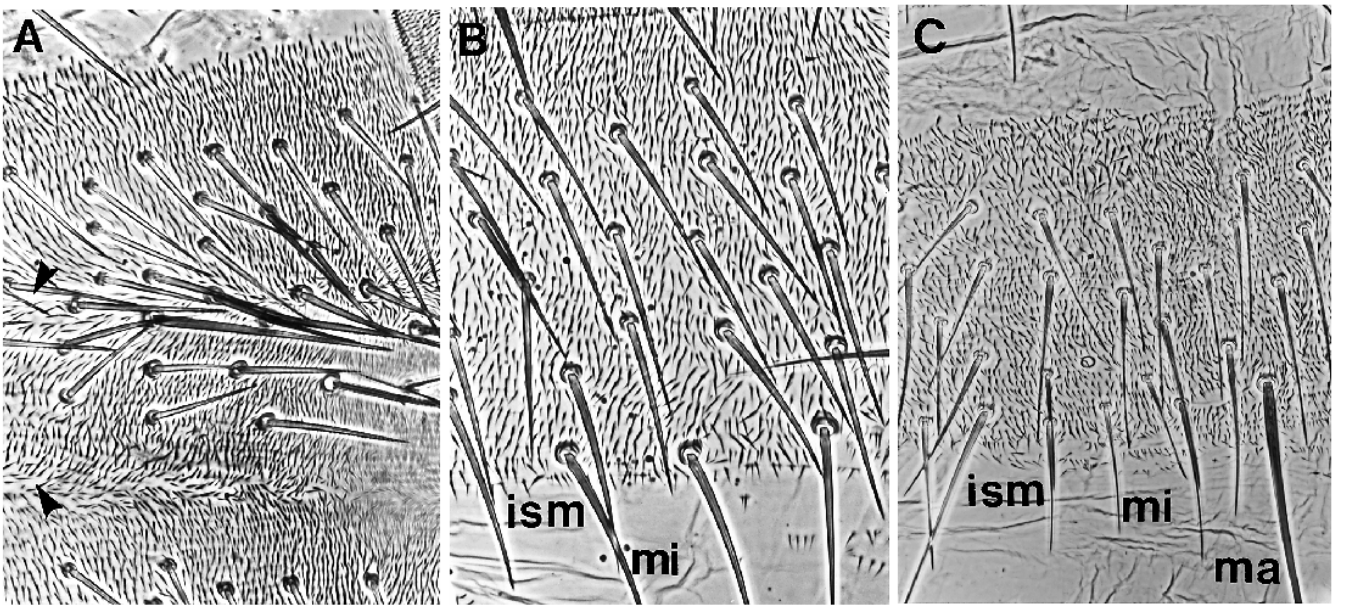

Fig. 6. Loss-of-function phenotypes of *hh*. (A,B) Phenotypes produced in hh^{ts2} homozygotes shifted to restrictive temperature after pupariation. (A) Posterior compartment and posterior tergite structures are replaced with a mirror-image duplication of the anterior tergite minus the acrotergite. The acrotergite of the following segment is also deleted. Arrowheads indicate the lines of polarity reversal. (B) Deletion of posterior compartment and posterior tergite structures. Note the absence of the PHZ, pigment band, and macrochaetes. (C) Phenotype caused by overexpression of *ptc* in the pupal abdomen. Note similarity to B.

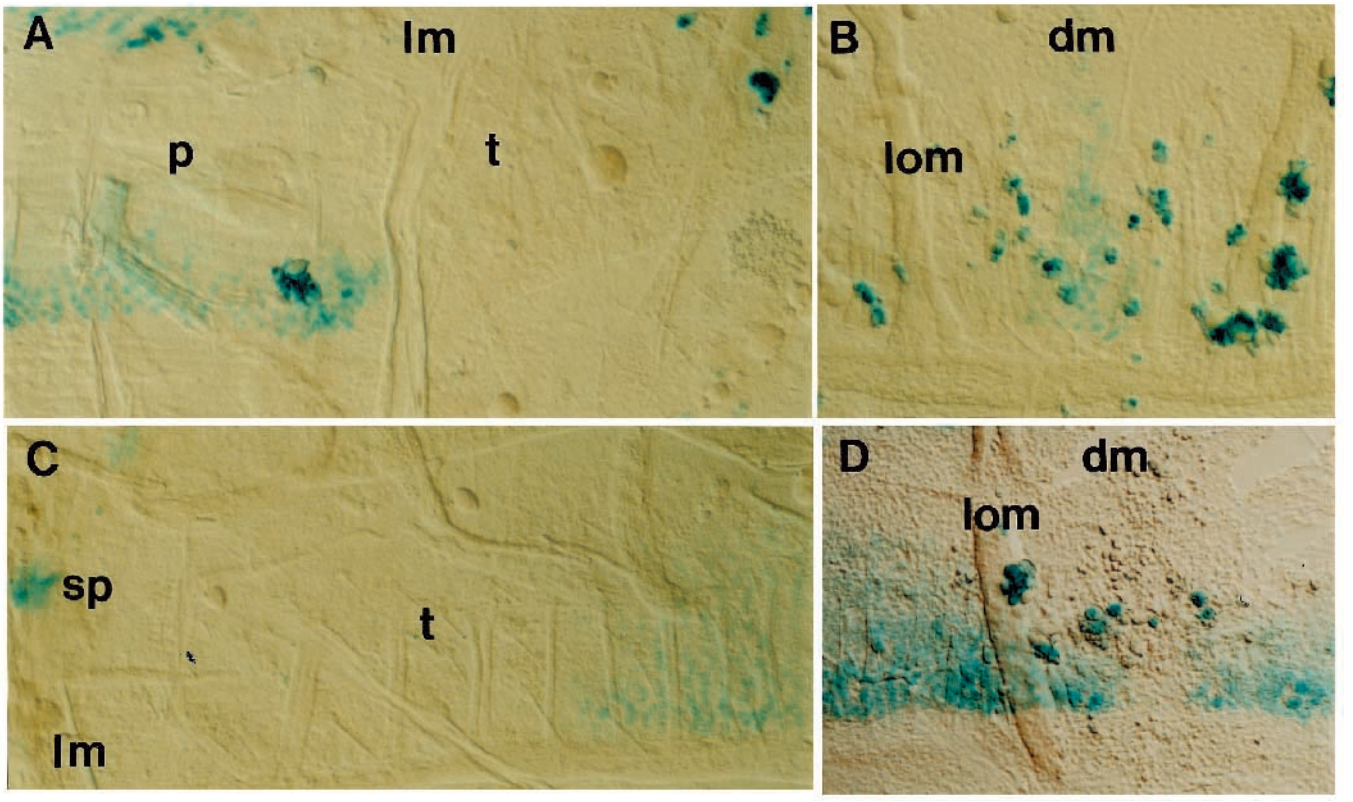

Fig. 7. Expression of *dpp-lacZ* and *wg-lacZ* in the pupal abdominal epidermis. Transcripts are expressed in patterns identical to the enhancer traps. p, pleura; t, tergite; lm, lateral midline; dm, dorsal midline; lom, larval oblique muscle; sp, spiracle. (A) *dpp* expression in the pleura. (B) *dpp* expression at the dorsal midline of the tergite. (C,D) *wg* expression in the tergite. Note exclusion from the dorsal midline and lateral tergite.

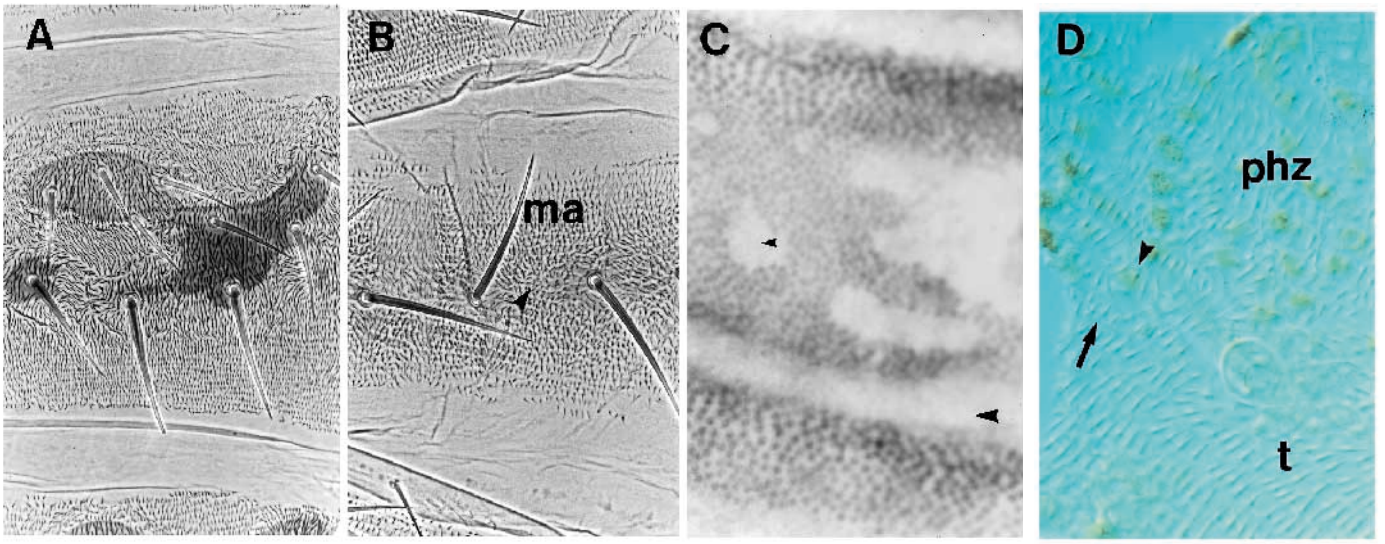

Fig. 8. Effects of ectopic *en* expression. (A) en^{Apa}/+: Ectopic PHZ has mirror-image polarity, whereas the polarity of tergite islands remains normal. Note the sharp discontinuity in polarity at PHZ-tergite boundaries. (B) en^{Apa}/en^{Es}: The arrowhead points to the line of polarity reversal. (C) *en* expression in en^{Apa}. Note that *en* expression is reduced in a zone probably located anterior to the compartment boundary (large arrowhead) and in random patches in the anterior compartment (small arrowhead). (D) Autonomous control of cell fate and polarity by *en*. phz, posterior hairy zone; t, tergite island. Note that *en*-expressing PHZ cells (arrowhead) and the tergite cells that do not express *en* (long arrow) have opposite polarity.

with respect to tergite structures, this line lies within the posterior pigment band in a narrow zone centered on the most posterior row of microchaetes (Fig. 9).

DISCUSSION

Control of anterior compartment patterning by *hedgehog*

Four lines of evidence are presented that *hh* functions to pattern the posterior portion of the anterior compartment in the adult abdomen. First, loss of *hh* function in hh^{ts2} pupae causes transformation of posterior tergite towards a more anterior fate, as indicated by the loss of the pigment band and transformation of macrochaetes to microchaetes. Second, ubiquitous expression of *hh* causes reciprocal transformations of anterior to posterior. Third, 'FLP-out' clones that express *hh* in the anterior compartment can induce surrounding wild-type cells to produce posterior tergite characters. Fourth, expression of an ectopic stripe of *hh* at the anterior edge of the segment in the gain-of-function allele hh^{Mir} causes double-posterior patterning in the tergite. Most of the anterior tergite develops normally in hh^{ts2} homozygotes, indicating that a large part of the abdominal segment is patterned independently of *hh*.

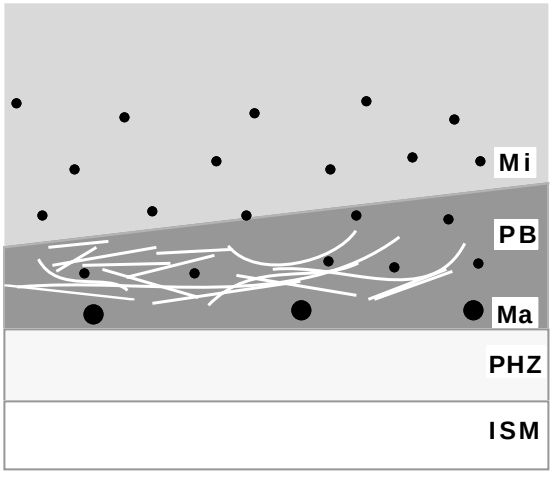

Fig. 9. The line of underlying symmetry in abdominal segments. The figure shows the left dorsal quadrant of a generalized abdominal segment. The white lines represent the lines of polarity reversal inside *en*-expressing clones. Data from both sides of A2-A5 have been pooled. The line of polarity reversal falls within the pigment band, close to the most posterior row of microchaetes.

The nonautonomy of *hh*, and the graded transformations caused by ectopic expression, suggest that *hh* may function as a morphogen in the abdomen. The properties that we have defined for *hh* match closely those of a morphogen postulated by Madhavan and Madhavan (1982) to control posterior patterning in the *Drosophila* tergite. They also correspond closely to a morphogenetic activity that controls posterior patterning in abdominal segments of the moth *Galleria* (Bhaskaran and Röller, 1980).

In imaginal discs, the long-range patterning effects of *hh* are mediated by *dpp* and *wg* (Brook and Cohen, 1996; de Celis et al., 1996; Jiang and Struhl, 1996; Lecuit et al., 1996; Nellen et al., 1996; Penton and Hoffman, 1996; Zecca et al., 1995). However, the expression patterns of *wg* and *dpp*, and direct tests of their function (Shirras and Couso, 1996; this report), indicate that these genes are not involved in anterior-posterior patterning in the abdomen. Whether *hh* acts directly, or indirectly by activation of a secondary morphogen, is not resolved by our work. In the embryonic epidermis, *wg* and *hh* mutually activate one another at the compartment boundary (Heemskerk

et al., 1991; Ingham, 1993). That a similar reinforcement may take place in the pupal abdomen is suggested by our finding that *en* and *hh* expression become graded after fusion of the anterior and posterior histoblast nests, with peak expression occurring at the compartment boundary. It seems unlikely that *wg* or *dpp* are involved in this reinforcement, however, since neither is expressed in the lateral tergite region when upregulation of *en* and *hh* is seen.

Pigmentation and bristle patterns can be dissociated

The effects of timed ubiquitous expression suggest that *hh* controls bristle patterning between 5 and 10 hours APF, whereas it controls pigmentation between 18 and 35 hours APF. Bristles appear to be patterned by *hh* before the anterior and posterior dorsal histoblast nests make contact. This suggests that anterior compartment histoblasts receive *hh* signal from posterior compartment LEC, which express *hh* at this time (not shown). Differential control of pigment and bristle patterning is also shown by gain-of-function alleles of *optomotor-blind* (*omb*), the main target of *hh* signaling in the abdomen (Kopp and Duncan, 1997), and is seen after cauterization or transplantation of pieces of abdominal epidermis (Bhaskaran, 1973; Roseland and Schneiderman, 1979). The two main functions of *wg* in the abdomen, promotion of bristle formation and allocation of the sternite and tergite primordia, also occur at different times (Shirras and Couso, 1996).

Control of posterior compartment patterning by *hh* and *en*

In addition to patterning the posterior tergite, *hh* is required within the posterior compartment. Both PHZ and ISM are often lost in hh^{ts2} animals raised at the restrictive temperature during pupal development, and ectopic expression of *hh* in the anterior compartment causes transformation of anterior tergite to PHZ and ISM. Defining the role of *hh* in posterior compartment patterning is complicated by our demonstration that *hh* and *en* mutually activate one another in the pupal abdomen. This raises the possibility that the effects of *hh* on posterior compartment patterning result from alterations in expression of *en*. However, in hh^{Mir}, *en-lacZ* is activated only in the region of ectopic PHZ, and not in the region of ectopic ISM. This suggests that *hh* can pattern some posterior compartment structures in the abdomen independently of *en*. *hh* is also required for normal patterning within the posterior compartment of the dorsal larval epidermis (Heemskerk and DiNardo, 1994).

Insulating role of the larval border cells

Segment boundaries have long been postulated to have special insulating properties that allow segments to develop as independent morphogenetic fields (Blennerhassett and Caveney 1984; Campbell and Caveney 1989; Warner and Lawrence 1982). The properties of the LEC at the posterior edge of each abdominal segment suggest they may serve such an insulating role. These 'border cells' are morphologically distinct, and are the last LEC to disappear as the histoblast nests expand and fuse. Significantly, although these cells express *en*, they do not express *hh*. These properties suggest the border cells may serve as a 'morphogen seal', preventing histoblasts of neighboring segments from interacting until most patterning has been accomplished.

Consistent with such a role, histoblasts at the anterior edge of the segment appear to be insulated from *hh* protein produced by posterior compartment histoblasts of the preceding segment. Judging from the effects of timed ectopic expression of *hh* (this report), *omb* (Kopp and Duncan, 1997) and *wg* (Shirras and Couso, 1996), most cell fates and polarity in abdominal segments are already determined by 40-42 hours APF, when the larval border cells are replaced by histoblasts. At this time, *ptc* is strongly upregulated in histoblasts anterior to the *hh* stripe, but no *ptc* expression is seen in histoblasts located posteriorly across the segment border (Fig. 3F). *hh* also induces the expression of *omb* anterior, but not posterior, to the *hh* stripe (Kopp and Duncan, 1997). Ectopic expression of *hh* in hh^{Mir} and hs-*hh* (not shown) induces high levels of *ptc* and *omb* in anterior histoblasts, indicating these cells are capable of responding to *hh*. We suggest they are normally shielded from exposure to *hh* protein by the larval border cells. Cauterization of the segment boundary often causes mirror-symmetric double posterior hemitergites to develop posterior to the operation (Madhavan and Madhavan, 1982; Roseland and Schneiderman, 1979). Our observations suggest this may occur because damage to the border cells allows *hh* to influence pattern across the segment boundary.

en and *hh* interact with an underlying symmetric patterning system

By analogy with imaginal discs, one might expect abdominal segments to be patterned by a long-range morphogen induced at the compartment boundary by *hh* (Lawrence and Struhl, 1996). This model makes two clear predictions: first, patterning should be lost when *hh* function is either removed or provided ubiquitously; second, localized ectopic expression of *hh* should produce structures organized symmetrically around the source of the *hh* signal. We find these predictions are not fulfilled, indicating that this 'compartment boundary' model is not sufficient to explain pattern formation in the adult abdomen. We present evidence that cuticular pattern in abdominal segments is established through interaction of two distinct patterning systems: a *hh*-dependent system, responsible for patterning near the compartment boundary, and a more global mirror-symmetric system that is independent of *hh*.

The underlying symmetry of the abdominal segments is revealed when *hh* is either inactivated, resulting in a mirror-image double anterior pattern (Fig. 6A), or expressed ubiquitously, creating a reciprocal, double-posterior pattern (Fig. 4C). Additional evidence for a *hh*-independent mirror-symmetric patterning system is provided by the mirror-symmetric patterning of abdominal segments caused by ectopic expression of *en*. By examining a number of FLP-out *en*-expressing clones in the anterior compartment, we were able to determine that a cryptic line of symmetry is present in the posterior tergite; *en*-expressing cells anterior to this line always orient toward the anterior, whereas *en*-expressing cells posterior to this line orient toward the posterior (Figs 5F, 9). Several lines of evidence suggest that this line of symmetry is established independently of *hh* function. Most convincing, this line appears to be unaffected by *hh* loss of function, as it coincides with the plane of symmetry in the mirror-image tergites present in hh^{ts2} animals raised at the restrictive temperature (Fig. 6A). The line of symmetry also appears to be retained when *hh* is expressed ectopically, as it coincides with the line of polarity reversal in the double posterior tergites of hh^{Mir} (Fig. 1B) and hs-*hh* animals (Fig. 4C), and with the posterior limit of polarity reversals caused by *hh*-expressing

and *en*-expressing clones (Fig. 5A,E). The line of polarity reversal is also retained in en^{Es} and en^{Apa} mutants (Fig. 8A,B), in which ectopic expression of *en* would be expected to render most or all cells of the anterior compartment insensitive to *hh* (Guillén et al., 1995; Zecca et al., 1995).

We suggest that *hh* acts to reverse a ground-state double anterior polarity in the posterior of the segment (see also the model in Fig. 9, Kopp and Duncan, 1997). The phenotypes of hh^{ts2} and *hs-hh* animals suggest that, although the requirement for *hh* in specifying posterior structures is graded, polarity is reversed in an all-or-nothing fashion by a threshold level of *hh*.

Interaction between *hh* and the inferred underlying symmetric patterning system is most clearly seen in the phenotypes induced by *hh*-expressing clones in the anterior compartment. These clones produce the concentric patterns predicted by the 'compartment boundary' model only in the central region of the tergite, which we suggest is susceptible to polarization by *hh* because it is minimally instructed by the *hh*-independent symmetric polarization (Fig. 5C). Cuticular patterns produced by *hh*-expressing clones in the anterior of the tergite are dramatically different: PHZ cuticle has reversed polarity, and always forms posterior to the ISM, and ectopic posterior tergite structures are induced only posterior to the clone (Fig. 5A). This arrangement is a perfect mirror image of the normal pattern seen in the posterior of the segment, suggesting that patterning of posterior structures by *hh* is superimposed on an underlying mirror-symmetric system. Our results suggest that one effect of this underlying system may be to polarize Hh secretion (see Results). If so, the asymmetric patterning of ectopic ISM and PHZ associated with anterior *hh*-expressing clones may result from the action of a morphogen induced by *hh* at the posterior edges of these clones.

The well-characterized limb imaginal discs are derived from only the portion of the segment straddling the compartment boundary, and develop without contact with neighboring segments. This may explain the predominance of the compartment boundary in patterning the imaginal discs. The more global symmetric patterning of the abdominal segments appears to have no equivalent in the imaginal discs and may be directed by the segment boundaries. The observations of Madhavan and Madhavan (1980) suggest that histoblasts may be symmetrically polarized by contact with the larval border cells, and Bhaskaran and Röller (1980) report that cells transplanted from either the anterior or posterior borders of abdominal segments in the moth *Galleria* can repolarize centrally located cells.

To summarize, we argue that abdominal segments are patterned by two interacting systems: a global system that specifies mirror-symmetric patterning within the entire segment, and a system dependent on *hh* that functions only near the compartment boundary. We argue that *hh*, or a gene product induced by *hh*, functions as a morphogen that directs anterior compartment cells to produce a range of posterior tergite structures, and may also pattern part of the posterior compartment. *hh* also determines polarity, apparently by two mechanisms. First, *hh* has some ability to orient cells toward points of high expression. Second, and perhaps more important, *hh* expression causes cells to reverse their interpretation of underlying polarity specified by a global mirror-symmetric system.

NOTE ADDED IN REVISION:

Coincident with acceptance of this paper, two papers by Struhl et al. (1997a,b) appeared that address the role of *hh* in patterning the adult abdomen. These authors demonstrate that *hh* specifies tergite cell fates by functioning directly as a morphogen, but specifies polarity by an indirect mechanism. In addition, these authors show that *hh* patterns the anterior as well as the posterior edge of the tergite. The anterior region affected appears to be limited to the acrotergite. Consistent with this, we find that the acrotergite is lost in hh^{ts2} animals raised at the restrictive temperature, while the remainder of the anterior tergite develops normally (Fig. 6A). Struhl et al. (1997a) also show that, in wild type, *ptc* is upregulated at both the anterior and posterior edges of the tergite. These observations would seem to contradict our proposal that the larval border cells insulate anterior cells from exposure to *hh*. However, our failure to find activation of *ptc* at the anterior of the segment up to 40 hours APF indicates that *ptc* activation here must be delayed until after the disappearance of the border cells, long after *ptc* is activated at the posterior of the tergite (Fig. 3F). Moreover, by delivering timed pulses of ectopic *hh* expression, we show that exposure of anterior tergite cells to *hh* early in pupal development causes them to develop as posterior tergite, PHZ, or ISM, but not acrotergite. These observations indicate that the response of anterior tergite cells to *hh* signaling is critically dependent on timing, and are consistent with an important role for the border cells in shielding anterior tergite cells from early Hh in pupal development.

We also differ significantly in our interpretation of the role of *hh*. Struhl et al. (1997a,b) propose that *hh* is the central determinant of patterning in the tergite, whereas we suggest that the patterning activities of *hh* in the tergite are superimposed on a global mirror-symmetric pattern. Our view is supported by a number of observations described in this report. Among these are two observations also made by Struhl et al. (1997a). First, both groups find that a large portion of the anterior tergite [regions a2 and a3 of Struhl et al. (1997a)] is patterned independently of *hh*. We suggest that these regions are patterned by the underlying symmetric system. Second, both groups find that *hh*-expressing clones in the anterior tergite are internally patterned, with ISM always developing anterior to PHZ. We suggest this patterning is also directed by the mirror-symmetric system.

Finally, our papers differ in the emphasis given to the role of *hh* in determining polarity. Both papers demonstrate that *hh* is able to polarize tergite cells. However, both papers also present evidence for a second, *hh*-independent, polarizing activity. Because of the nature of our data, we stress a *hh*-independent mechanism, whereas Struhl et al. (1997b) stress the polarization activity of *hh* itself. Which is more important, and how many polarizing mechanisms exist, remain to be determined.

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