Cell Fates in the Adult Abdomen of Drosophila Are Determined by wingless during Pupal Development

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wingless activity is required for the patterning of tergites and sternites in the adult abdomen of Drosophila melanogaster. In the absence of wg, tergite and sternite cuticular differentiation is replaced by that of pleura. Temperature shift analysis of a wg temperature-sensitive allele has shown that sensory bristles and tergite/sternite histotype are determined independently and that wg is required between 15 and 20 hr after pupariation for bristle formation. The determination of sensory mother cells at this stage of development was confirmed by expression of the neuralized gene in a subset of the proliferating histoblasts. Ectopic expression of wg leads to the appearance of ectopic bristles and expanded tergite and sternite, indicating that wg expression is sufficient to promote both bristle formation and tergite/sternite differentiation. wg is expressed in the dividing and spreading histoblast population in a restricted pattern which may determine the spatial arrangement of cuticular elements. © 1996 Academic Press, Inc.

INTRODUCTION

The dorsal surface of a typical abdominal segment of an adult Drosophila melanogaster (segments A2 to A6 in the male and A2 to A7 in the female) is composed of segmentally arranged plates of tanned cuticle called tergites. The tergites occupy the anterior half of the segment and bear micro- and macrochaetae as well as trichomes. Regularly spaced microchaetae are borne on the posterior two-thirds of the tergite with a single row of equally spaced macrochaetae at the posterior edge of the tergite. A dark pigment band is present towards the posterior of the tergite which is broader in posterior segments in male flies. Between the tergites, and generally folded beneath them in the posterior half of the segment, is a flexible, bristle-free cuticle.

The ventral surface of the abdomen is composed, along the midline, of segmentally arranged plates of bristle-bearing cuticle called sternites. Around the sternites and between the sternites and the tergites is a membranous cuticle bearing regular rows of trichomes called the pleura. The spiracular openings are found at the lateral edge of each tergite (Fig. 1). The anterior-most and posterior-most abdominal segments show segment-specific variations of this basic pattern. For example, the first abdominal segment has no macrochaetae or pigment in the tergite and no sternite whereas the cuticular structures of the terminal segment (A7 in males and A8 in females) are derived from the genital imaginal disc and do not differentiate trichomes.

The adult abdominal epidermis of segments A1–A6 in males (A1–A7 in females) develops from groups of cells, the abdominal histoblast nests, which proliferate and spread during metamorphosis, replacing the larval polytene epidermal cells (Roseland and Reinhardt, 1982). In the larva there are four pairs of histoblast nests per segment; anterior dorsal (12–19 cells per nest), posterior dorsal (6–7 cells per nest), ventral (12–13 cells per nest), and spiracular (2–3 cells per nest) (Fig. 1). Thermocautery studies have shown that each nest gives rise to a defined part of the adult cuticle (Roseland and Schneiderman, 1979). Thus, the tergite is derived from the anterior dorsal nest, the intertergal membrane is derived from the posterior dorsal nest, the sternite and pleura are derived from the ventral nest and the spiracle is derived from the spiracular nest (Fig. 1). The cells of the histoblast nests are contiguous with the larval epidermis and secrete larval cuticle (Madhavan and Schneiderman, 1977). They do not divide during larval stages but shortly after pupariation they begin a series of divisions with an average doubling time of 3.6 hr (García-Bellido and Merriam, 1971; Roseland and Schneiderman, 1979). During the initial phase of cell division there is no increase in cell size so that, at 15 hr after puparium formation (APF), large numbers of small, columnar cells occupy the original area of each nest. At this stage cells at the periphery of the nests flatten and begin to
migrate outwards, gradually displacing the larval polytene epidermal cells which are phagocytosed in the haemolymph (Roseland and Schneiderman, 1979). At 18 hr APF the anterior and posterior dorsal nests fuse to form a single hemitergite nest. At 18–22 hr APF the hemitergite nest and the ventral nest fuse with the spiracular nest. Replacement of the larval cells at the intersegmental boundaries is delayed, beginning at around 24 hr APF laterally and progressing medially. The last cells to be replaced, at approximately 36 hr APF, are those at the dorsal and ventral midlines. Adult/pupal apolysis then occurs and differentiation of the epidermis begins with cuticle deposition and bristle formation.

The Wingless (Wg) protein is a member of the Wnt family of secreted glycoproteins and has been shown to act as a signal during patterning of the larval epidermis and imaginal discs (for reviews see Martinez Arias, 1993; Perrimon, 1994). The wingless (wg) gene is required for different processes throughout wing development, early for the generation of ventral cell fates (Couso et al., 1993) and late for the patterning of bristles in the wing margin (Couso et al., 1994) and notum (Phillips and Whittle, 1993). These activities of Wg are implemented in the target cells by the activation of the genes armadillo (arm) and dishevelled (dsh) and by the inactivation of the Shaggy (Sgg) protein kinase (Couso et al., 1994; reviewed in Perrimon, 1994).

Very little, however, is known about pattern formation in the adult abdomen. It has been suggested that the larval epidermal cells play a role in this process (Santamaría and García-Bellido, 1972; M adhavan and M adhavan, 1984, 1990; Smith, 1989). However, the grafting experiments of M adhavan et al. (1988) and Pearson (1977) suggest that pattern is established autonomously in the histoblast nests of Diptera.

Studies of the tergite bristle pattern show that it is autonomously determined in histoblast cells by the activity of the proneural genes of the achaete–scute complex (AS–C) (García-Bellido and Santamaría, 1978), in the same manner as in other epidermal tissues of the fly (reviewed in Campuzano and Modolell, 1992).

The gene engrailed has been implicated in the control of posterior identity of the intertergal membrane in a fashion similar to its role in controlling posterior patterning in other imaginal epidermal derivatives (Kornberg, 1981). This may suggest that other segment polarity genes are involved in adult abdominal patterning. We have studied the role of wg in this process, since it has a prominent role in both larval and imaginal epidermal patterning. Our results suggest that wg acts during development to determine the formation of bristles, tergite and sternite plates and pigment. The restricted anterior-medial gradient of wg expression throughout development within the growing histoblast population generates the final spatial arrangement of these elements.

**MATERIALS AND METHODS**

**Fly Strains**

The wg" mutation was described in van den Heuvel et al. (1993). wg" homologotes were obtained as Tb" individuals from a cross between wg"- + cn bw sp/SM 6aT M 6b and wg"- pr/SM 6aT M 6b (Couso et al., 1994). At 17°C these homologotes survive to adulthood. At 25°C the mutation behaves as a null. The mutation is known to prevent Wg protein secretion at the restrictive temperature (van den Heuvel et al., 1993).

CyO, wg IacZ is a chromosome which has a P element insertion which has caused a loss of wg function and expresses β-galactosidase under control of the wg gene (Kassis et al., 1992). This strain reproduces faithfully the embryonic and imaginal patterns of wg expression (Couso et al., 1993).

The heat shock-wg strain used in this study was described in Noordermeer et al. (1992).

Clones of cells expressing wg from the Actin5C promoter were generated using strains and protocols described in Struhl and Basler (1993). An average of one clone per segment was found when clones were generated in third instar larvae. Clones generated after puparium formation almost completely covered the adult abdomen and the resulting flies were indistinguishable from hs-wg flies which had been given a repeated heat shock regime during metamorphosis.

sgg clones were generated by X-irradiating either sgg"11-1/y f36a or y sgg"+/+ larvae with 1000 rad using an A1 filter 48–72 hr after egg laying. In the first case, following X-ray induced recombination, a sgg clone and a y f twin are generated. In the second case, a y sgg clone is generated.

Adult or pharate adult abdomens were dissected and the body walls soaked in 10% KOH at 60°C for 10 min and then mounted in Hoyer's or dehydrated through an ethanol series, cleared in Histoclear (National Diagnostics), and mounted in Histomount (National Diagnostics).
FIG. 2. Phenotype of the adult abdomen of \( \text{wg}^4 \) homozygotes. (A) Oregon R female. (B) \( \text{wg}^4 \) homozygous female, raised at 17°C. (C) \( \text{wg}^4 \) homozygous female, raised at 17°C and then shifted to 25°C as a late third instar larva. The tergites have been reduced to small lateral patches. (D) Scanning electron micrograph of the lateral portion of the third tergite of a \( \text{wg}^4 \) homozygote which had been transferred from 17 to 25°C as a late third instar larva. (E,F,G) Comparison of wild-type pleural (E) and tergal (F) trichomes with the trichomes found around the tergite patch in D (G). (H) Ventral surface of a \( \text{wg}^4 \) homozygote which had been transferred from 17 to 25°C as a late third instar larva. There are no sternites and the entire ventral surface is composed of pleura.
Histological Techniques

Larval body walls or pupal cuticles with adhering epidermis were stained for β-galactosidase activity using X-gal and standard procedures (Ashburner, 1989). Haematoxylin staining following detection of β-galactosidase activity was carried out as described in Hayashi et al. (1993).

Immunocytochemistry

To detect Wg protein, puparia of the genotype wgIL/SM6aTM6B which had been raised at 25°C were dissected in PBS 24 hr APF. These animals are wg but, because of the wgIL mutation, some protein fails to be secreted, increasing its intracellular concentration and improving the signal after antibody detection (van den Heuvel et al., 1993; Couso et al., 1994). The abdominal pupal cuticle was carefully removed and flushed clear of debris, leaving the epidermis attached to the cuticle. The pupal cuticles were fixed in 4% paraformaldehyde in PBS for 20 min, rinsed three times in PBS + 0.3% Triton X-100 and blocked for at least 1 hr in PBS, 0.3% Triton X-100, 0.5% BSA, 10% normal goat serum (PBT) at 4°C before incubation with an anti-Wg antibody (1:200 dilution in PBT) for 16 hr at 4°C. Preparations were washed 3 × 30 min in PBT at room temperature before incubating with HRP conjugated secondary antibody (1:200 dilution) for 1 hr at room temperature. Three further washes in PBT (30 min per wash) were carried out followed by a 1-min wash in PBS. Peroxidase activity was detected by incubation in 0.5 mg/ml diaminobenzidine, 0.01% H2O2.

RESULTS

Loss of wingless Function

Comparison of wgIL/wgIL homozygotes which have been maintained at 17°C throughout development with wild-type flies (Figs. 2A and B) reveals an almost wild-type phenotype. However, wgIL flies tend to have fewer bristles on their tergites (an average of 87 ± 11.4 on a female wgIL 4th tergite vs 117 ± 5.6 on the same tergite of an ORR female). This is partly due to a slight reduction in the width of the tergite. In wild-type flies the edge of the tergite coincides with the position of the spiracle whereas in wgIL at 17°C the spiracle is separated from the edge of the tergite by pleura. The sternites are also reduced or missing in these flies with occasional bristles being found misplaced to one side of the sternite in the pleura. Similar phenotypes are observed in viable mutant conditions of dsh and arm (not shown).

To investigate further whether the wingless gene product is required for development of the adult abdomen wgIL/wgIL third instar larvae were transferred from the permissive temperature of 17°C to the restrictive temperature of 25°C and the appearance of the adult abdomen observed (Fig. 2C). Such individuals fail to eclose but examination of pharate and then shifted back down to 17°C at the times shown to complete development (●). (B) Oregon R left third hemitergite. (C) Left third hemitergite of a wgIL fly which had been raised at 17°C and then transferred to 25°C at 18.5 hr APF. Bristles are missing but pigment is still present.

FIG. 3. Effects of temperature shifts during metamorphosis on adult abdominal development in wgIL homozygotes. (A) Bristle formation in the fourth tergite. Animals were raised at 17°C and then transferred at the times shown to 25°C to complete development (●) or raised at 17°C, transferred to 25°C as late third instar larvae, and then shifted back down to 17°C at the times shown to complete development (○). (B) Oregon R left third hemitergite. (C) Left third hemitergite of a wgIL fly which had been raised at 17°C and then transferred to 25°C at 18.5 hr APF. Bristles are missing but pigment is still present.
FIG. 4. Expression of neuralized in the anterior dorsal histoblast nest at 18 hr APF. Anterior to the top. Expression was determined by X-gal staining of a neu enhancer trap strain. Strong $\beta$-galactosidase expression was observed in a row of cells (between arrows) towards the posterior of the anterior dorsal nest. Some weaker staining was observed in more anterior cells (arrowheads). The broken line indicates the posterior margin of the anterior dorsal nest (ad) where it is fusing with the posterior dorsal nest (pd).

tion of each hemi-segment. The dorso-lateral-posterior region of each segment, instead of being naked as in the wild type, is covered with trichomes which are reminiscent of those found on the pleura (Fig. 2G). These pleura-like trichomes extend into the cuticle medial to the patches of tergite. Towards the dorsal midline, both anterior and posterior halves of each segment become progressively more naked, resembling the intertergal membrane of wild-type flies. Ventrally there is a complete loss of sternites which are replaced by pleura; the remainder of the pleura appears to be normal (Fig 2H). Haematoxylin staining of mutant epidermis revealed that these pattern defects are not due to gross failures in histoblast proliferation or spreading as dividing cells were observed in all nests and, at 40 hr APF the abdomen was seen to consist entirely of adult cells (data not shown).

To determine the temperature sensitive phase for these defects wg$^+\text{homozygotes}$ were either maintained at 17°C

FIG. 5. wg expression in the developing adult abdomen. Expression was determined by X-gal staining of a wg enhancer trap strain. In A–F larval epidermal cells (large cells) and histoblasts (small cells) were revealed by staining with haematoxylin. In A–D the blue-staining larval epidermal cells correspond to the dorsal and ventral stripes of wg expression indicated in Fig. 1A. Anterior is to the top, dorsal to the right. (A) Ventral nest, 15 hr APF (arrow). Most cells of the nest are expressing $\beta$-galactosidase; however, a few cells at the anterior and posterior margins appear not to have stained. The spiracular nest also shows wg expression (arrowhead). (B) Dorsal nests, 15 hr APF. Expression is seen in a dorsal-posterior sector of the anterior nest, abutting the stripe of expressing larval cells (arrow). There is no expression in the posterior nest (arrowhead). (C) Ventral nest 24 hr APF. Expression is seen in a small patch of cells adjacent to the stripe of expressing larval cells, towards the ventral edge of the nest (arrow). wg expression is present in the spiracle (arrowhead). (D) Dorsal nest 24 hr APF. The anterior and posterior nests have fused to make a single dorsal nest. $\beta$-Galactosidase expression is seen in a zone, occupying the anterior-dorsal region of the nest, which has a sharp posterior margin but a diffuse anterior margin. (E) Ventral view of two segments 36 hr APF. $\beta$-Galactosidase expression is seen in a patch at the ventral midline corresponding to the future position of the sternite (arrow). (F) Dorsal view of three segments 36 hr APF. Bristle organs (arrowheads) have begun to form. $\beta$-Galactosidase expression occupies around two-thirds of the width of the tergite, the lateral margins showing no apparent staining. Expression is strongest at the posterior edge of the tergite and the posterior boundary of expression is sharply defined whereas the anterior margin is fuzzy. (G) Sternite and pleura of a newly eclosed adult. $\beta$-Galactosidase expression is observed in the posterior half of the sternite (arrow) and in the spiracle (arrowhead). (H) Dorsal view of a newly eclosed adult. $\beta$-Galactosidase expression is seen in a large patch occupying the dorsal two-thirds of the tergite. The posterior margin of expression coincides with the posterior margin of the tergite. The spiracle can be seen to the left (arrowhead).
FIG. 6. Effects of heat shock treatments on hs-wg flies. Lateral tergites, pleura, and sternites of flies subjected to a regime of 1 hr pulses at 37°C followed by 1.5 hr at 25°C during the indicated periods. (A) hs-wg without heat treatment. There are no bristles in the pleura. The position of the spiracles is indicated by the small arrowheads. (B) Heat pulses 10–20 hr APF. Ectopic bristles (small arrows) are found in the pleura (including the first abdominal segment) although there is little expansion of the tergites or sternites. Extra bristles are apparent in the lateral tergite of segment 2 (large arrowhead). The small arrowheads indicate the position of the spiracles. (C) Heat pulses 20–30 hr APF. There is expansion of both tergite and sternite. Extra bristles are apparent in the lateral tergite of segment 2 (large arrowhead). The small arrowheads indicate the position of the spiracles. (D) Heat pulses 15–40 hr APF. There is strong expansion of both tergite and sternite and dark pigmentation of the more posterior sternites.

and then shifted to 25°C at various times APF or they were transferred to 25°C as third instar larvae and then shifted back to 17°C at various times APF to complete development. Figure 3A shows the number of bristles on the fourth abdominal tergite. Both sets of experiments reveal a temperature-sensitive period of approximately 15–20 hr APF for the appearance of bristles. At this time the histoblasts have just begun to migrate after their initial period of cell division. When the dorsal cuticle of flies which had been shifted from 17 to 25°C at 18.5 hr APF was examined, in occasional segments, bristles were found to be missing but pigmentation and tergite-like trichomes were present (Fig. 3C), indicating that the requirements for wg in bristle and cuticle formation are separable.

To ascertain whether the sensitive phase for bristle formation, observed in the temperature shift experiments, coincides with the period during which bristles are normally determined, expression of the neuralized gene was examined in pupae 10–20 hr APF using the neuA101-lacZ strain. Expression of neuralized is found in sensory mother cells (SMCs), including bristle precursors, and is a marker for neural fate (see Campuzano and Modolell, 1992). β-galactosidase expression was observed in a single anterior dorsal nest cell at 10 hr APF and by 18 hr APF strong expression was observed in a row of cells along the posterior edge of the nest (Fig. 4). The number of these cells is approximately the same as the number of macrochaetae found along the posterior edge of the adult hemitergite. A few, more weakly staining cells were observed anteriorly which presumably correspond to the microchaetae precursors which are determined after the macrochaetae and appear in those positions (García-Bellido and Santamaría, 1978; Mari-Beffa et al., 1991). Thus, the timing of the appearance of bristle precursors correlates with the sensitive period for wg, suggesting...
TABLE 1
Effects of 1-hr 37°C Pulses on Ectopic Bristle Formation and Tergite Expansion in hs-wg Flies

<table>
<thead>
<tr>
<th>Hours APF</th>
<th>Bristles on pleura</th>
<th>Expanded tergite</th>
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<tbody>
<tr>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>–</td>
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<tr>
<td>14</td>
<td>++</td>
<td>–</td>
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<td>16</td>
<td>++</td>
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<td>18</td>
<td>+</td>
<td>++</td>
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<tr>
<td>20</td>
<td>+</td>
<td>++</td>
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<tr>
<td>22</td>
<td>–</td>
<td>++</td>
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<tr>
<td>24</td>
<td>–</td>
<td>++</td>
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<tr>
<td>26</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>28</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>30</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Note. Heat pulses were given at the times indicated. (−) Experimental animals were indistinguishable from controls. (+++) Phenotype similar to Figs. 6A and 6B. (++) Phenotype intermediate between controls and that shown in Figs. 6A and 6B.

that wg is required for the determination of sensory organ precursor cells.

Pattern of wingless Expression
The expression pattern of the wg gene was determined by X-gal staining of a P element insertion strain which expresses β-galactosidase under the control of wg (see Materials and Methods). At pupariation (0 hr APF) staining is observed in a band of larval epidermal cells, three to four cells wide, situated in the middle of each segment (Fig. 1A). Staining is weaker at the lateral margins. These bands of staining pass through the ventral histoblast nests, with most cells expressing β-galactosidase, and between the anterior and posterior dorsal nests, with only two or three cells at the posterior end of the anterior nests staining. At 15 hr APF, X-gal staining is still apparent in the larval epidermal cells but is absent from the larval cells which lie on the lateral side of the ventral histoblast nest. Staining is present in the ventral nest in the cells which abut the β-galactosidase-expressing larval cells. These cells form a zone on the ventral side of the nest, flanked anteriorly, posteriorly, and laterally by histoblasts which do not express β-galactosidase (Fig. 5A). In the anterior dorsal nest β-galactosidase expression is found in a sector abutting the β-galactosidase-expressing larval cells (the most posterior dorsal part of the nest). Staining is weak or absent from the larval cells and histoblasts closest to the lateral margin and no staining is observed in the posterior dorsal nest (Fig. 5B). At 24 hr APF β-galactosidase expression in the ventral nest has become restricted to a zone of cells which abut the β-galactosidase-expressing larval cells (Fig. 5C). The zone of β-galactosidase expression in the hemitergite nest has expanded as the histoblasts have spread. The posterior margin of this zone of expression is sharp, coinciding approximately with the future position of the posterior margin of the tergite. The anterior margin is ragged and diffuse (Fig. 5D). At 36 hr APF replacement of the larval epidermal cells is complete. Ventrally, the left and right ventral nests have fused and form a single zone of expression which corresponds with the future position of the sternite (Fig. 5E). The left and right presumptive hemitergites have fused so that there is single zone of expression comprising approximately two-thirds of the dorsal surface of the segment, but expression is absent from the lateral margins. As before, the posterior margin of expression is sharp with the anterior margin being more diffuse. Expression extends anteriorly to the first row of bristles and is strongest posteriorly where the posterior limit of expression can be seen to coincide with the posterior margin of the tergite (Fig. 5F). A similar pattern of β-galactosidase expression is found in newly eclosed adult flies (Figs. 5G and 5H).

To demonstrate that the pattern of β-galactosidase expression reflects the expression pattern of the endogenous wg gene, an anti-Wingless antibody was used to detect Wg protein at 24 hr APF. Antibody staining was found, in the dorsal nest, in a pattern which closely resembled that of the X-gal staining (data not shown).

Strong β-galactosidase expression was observed at 15, 24, and 36 hr APF in the descendants of the spiracular nest though this expression was absent from the perispiracular epidermis, being restricted to the spiracular chamber (Fig. 5).

The expression observed in the dorsal and ventral histoblast nests is dependent on wg function when development occurs at 25°C, although proliferation of the nests still occurred (data not shown). Staining in the imaginal discs was used as a control in all experiments in which β-galactosidase expression was monitored.

Ectopic Expression of wingless
To investigate the effects of ectopic wg expression, pupae carrying a heat shock–wingless fusion gene were given 1 hr pulses at 37°C at various times APF. Two main effects of this treatment were observed which are reciprocal to the gene lacking function phenotypes. The first was the appearance of ectopic microchaetae on the pleura, including A1, which does not normally have a bristle-bearing sternite (Fig. 6B). The second effect was a lateral expansion of the tergites and sternites (Fig. 6C). Table 1 shows that the time period during which these effects were observed was 10–22 hr APF for the ectopic bristles on the pleura and 18–28 hr for the expansion of the tergites. Applying a regime of repeated heat pulses separated by 90 min at 25°C from 10–30 hr...
FIG. 7. Clonal phenotypes of excess of wg function. Orientation as in Figs. 5G and 5H. (A) Wild-type tergite. The spiracle is indicated by an arrow at the edge of the tergite. (B) y Ac5wg clone in a tergite showing the phenotype of extra bristles. The yellow bristles of the clone (arrow) appear in a dorso-lateral position with a higher density than normal. Ectopic wild-type bristles (y') are also formed around the clone (compare the density of bristles in and around the yellow clone with the normal density at the edges of the image). (C) y Ac5wg clone in the pleura showing ectopic tergite/sternite differentiation and bristles. Around the y bristles, the trichomes are typical of tergite and not pleura. The ectopic tergite/sternite patch appears close to the spiracle (arrow) but not connected with the normal tergite (to the right). (D) y Ac5wg clone showing ectopic tergite/sternite differentiation and pigmentation, with recruitment of wild-type cells. The y bristles mark the clone which has differentiated ectopic sternite/tergite trichome and tergite pigmentation (arrow) in the pleura region close to the normal sternite (to the left). A wild-type bristle (arrowhead) is found within the ectopic patch of tergite/sternite, showing that wild-type cells can be influenced by the neighbouring y Ac5wg clone, as in (B). (E) Small y sgg clone (arrow and arrowhead)
TABLE 2
Phenotypes of Clones Expressing wg Constitutively

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Number of clones</th>
<th>Extra bristles</th>
<th>Extra tergite/sternite tissue</th>
<th>Total mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clones in wg-expressing regions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central tergite</td>
<td>49</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Posterior sternite</td>
<td>25</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total in wg-expressing regions</td>
<td>74</td>
<td>3</td>
<td>0 (25)</td>
<td>3</td>
</tr>
<tr>
<td>Clones outside wg-expressing regions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral tergite</td>
<td>35</td>
<td>25</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Perispiracular cuticle</td>
<td>19</td>
<td>7</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Anterior sternite</td>
<td>13</td>
<td>10</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Total outside wg-expressing regions</td>
<td>67</td>
<td>42</td>
<td>24 (31)</td>
<td>54</td>
</tr>
</tbody>
</table>

Note. Only clones induced following a 40-min heat shock during the third larval instar are shown. Clones induced later, although showing even stronger phenotypes, are not suitable for quantification (see Materials and Methods).

* Defined by X-gal staining of wg-lacZ adult cuticles as in Figs. 5G and 5H. For example, lateral tergite means the tergite area delimited by the four lateral-most macrochaetae of the tergite and central tergite is the rest of the tergite between these lateral patches.

* These clones are not adjacent to pleura and therefore cannot induce extra tergite/sternite tissue differentiation.

* The numbers in parentheses show the total number of clones capable of producing such a phenotype because of their position (posterior sternite, anterior sternite, or perispiracular epidermis).

* Some of these clones appear to be of pleural origin.

led to a marked expansion, laterally, of both tergites and sternites so that they became fused (Fig. 6D). Haematoxylin staining of these animals following heat treatment revealed that, although proliferation of all histoblast nests was delayed, there were no observable changes in the pattern of cell divisions or migration (data not shown).

Ectopic wg expression was also induced in clones of cells using the site-specific recombination system of Struhl and Basler (1993), which generates clones of cells carrying a construct that expresses the wg gene constitutively. Clones were induced at random positions within the histoblast nests in third instar larvae (Fig. 7) and studied in adult abdomens where they were identified by their yellow (y) phenotype (see Material and Methods and Table 2). Clones in regions that normally express wg produced a normal phenotype. However, wherever clones were found in regions that do not express wg in the wild type, they produced phenotypes similar to those generated by ectopic expression of wg in hs-wg flies. Thus, the clones differentiate extra bristles (Fig. 7B) and expanded tergite and sternite at the expense of pleura. However, although clones are, in principle, generated in any histoblast, isolated patches of tergite/sternite-like cuticle imbedded in the pleura were rarely found (Fig. 7C); in most cases the ectopic patches appeared fused to the wild-type tergite/sternite regions (Fig. 7D). In these flies, both yellow and neighbouring y tissue were seen to differentiate ectopic structures, as would be expected if the Wg product, produced by yellow-marked cells, is diffusing and instructing neighbouring cells to produce pattern elements typical of wg-expressing regions (Figs. 7B and 7D). Mild excess of wg phenotypes were seen when clones of cells mutant for the sgg gene were produced by X-ray induced mitotic recombination in sgg/+ animals (see Materials and Methods). The sgg patches showed ectopic differentiation of bristles and dark pigmentation, as well as limited expansion of the tergites/sternites (Figs. 7E and 7F). These phenotypes, although similar to those described above for Ac5wg and hs-wg flies, represent a weaker expressivity which is probably due to a long perdurance of the Sgg product (Ripoll et al., 1988; Couso et al., 1994).

DISCUSSION

We have shown here that the wg gene is expressed in the presumptive tissues of the adult abdomen during their differentiation. The density of bristles within the yellow patch is higher than normal (arrowhead) and ectopic pigment is formed near the spiracle, where it is not seen in the wild type. The tergite is slightly expanded such as it encompasses the spiracle (arrow). (F) sgg<sup>Ac5</sup> clone with y f control twin clone. The oval patch of ectopic pigment also shows extra bristles (arrowhead). Its sgg genotype is assured by the neighbouring y f bristles (arrows), which are part of the y f clone formed in a recombination event that simultaneously forms a sgg<sup>Ac5</sup> clone. In this case, because the sgg cells are y<sup>+</sup>, the phenotype of ectopic pigmentation can be more readily appreciated than in (D) and (E), where the paler y cuticle seldom differentiates darker than wild-type sternite/tergite cuticle.
been shown to be the case for the wing imaginal disc (Phillips and Whittle, 1993; Couso et al., 1994). Ectopic bristles appear only where wg is not normally expressed—in the pleura and the antero-lateral patches of the tergites—suggesting that, as far as bristle induction is concerned, the wg-expressing zones have saturating levels of Wg protein, compared to the levels generated in our experiments. We did not observe ectopic bristles on the intertergal cuticle. This may be due to relatively low levels of Wg protein being synthesised in response to heat shock. Alternatively, the lack of ectopic bristles in the intertergal membrane may reflect the repressive activity of the Engrailed protein (Santamaría and García-Bellido, 1972; Hidalgo, 1995) which has been shown to override the effects of Wingless signalling created by sgg mutations (Ripoll et al., 1988).

**Wingless Determines a Choice of Histotype**

The results of temperature shift experiments using the wg\textsuperscript{IL} temperature-sensitive allele show that the Wingless protein is required for the allocation and differentiation of tergite and sternite territories. In agreement with this hypothesis, the results of the ectopic expression experiments show that wg expression promotes the formation of tergite and sternite at the expense of pleura. Our experiments indicate that the critical time for wg activity in tergite differentiation is 18–28 hr APF when the pattern of wg expression corresponds closely to the areas affected by loss of wg function.

The intertergal membrane of each segment is displaced during development and it is involved in several patterning processes which are summarized in Fig. 8.

**Wingless Promotes Bristle Formation**

One role for Wingless in the development of the adult abdomen appears to be to promote the determination of sensory bristles. This proneural function of Wg is suggested by the loss of bristles in wg\textsuperscript{IL} flies which have been maintained at 17°C or which have been exposed to the restrictive temperature prior to 20 hr APF. The occurrence of ectopic bristles in the pleura and in the lateral tergites of hs-wg flies after heat shock treatment shows that wg expression is sufficient to induce bristle formation and resembles the effects of hs-sc (Rodríguez et al., 1990). The ectopic bristles in the pleura of hs-wg flies are surrounded by typical pleural trichomes. Thus, it is unlikely that the appearance of these bristles is a secondary consequence of a transformation towards tergite or sternite as this would entail tergite/sternite trichome differentiation. Moreover, the sensitive period for this proneural effect begins earlier than that of the ectopic tergite/sternite formation effect and coincides with the time during which sensory mother cell determination occurs in wild-type flies (García-Bellido and Merriam, 1971; Poodry, 1975; this paper). We would propose that the proneural effect of wg is mediated by positive control of the expression of genes of the achaete–scute complex, as has been shown to be the case for the wing imaginal disc (Phillips and Whittle, 1993; Couso et al., 1994). Ectopic bristles appear only where wg is not normally expressed—in the pleura and the antero-lateral patches of the tergites—suggesting that, as far as bristle induction is concerned, the wg-expressing zones have saturating levels of Wg protein, compared to the levels generated in our experiments. We did not observe ectopic bristles on the intertergal cuticle. This may be due to relatively low levels of Wg protein being synthesised in response to heat shock. Alternatively, the lack of ectopic bristles in the intertergal membrane may reflect the repressive activity of the Engrailed protein (Santamaría and García-Bellido, 1972; Hidalgo, 1995) which has been shown to override the effects of Wingless signalling created by sgg mutations (Ripoll et al., 1988).

**Different Instructions Are Integrated in the Final Pattern**

We propose that wg determines the spatial organization of the adult abdominal pattern by a combination of its spa-
ationally restricted expression and its changing effects upon target cells. In the case of the anterior dorsal nest, it is possible that the gradient of wg expression also creates a graded prepattern of proneural activity that might coincide with the one revealed by mutations in proneural genes (García-Bellido and Santamaria, 1978; Mari-Beffa et al., 1991; Rodríguez et al., 1990). A correlation between time of determination of bristle precursors, the onset of their differentiation and the final bristle size has been repeatedly found in the fly abdomen (Poodry, 1975; García-Bellido and Santamaria, 1978; Rodríguez et al., 1990; Mari-Beffa et al., 1991). We propose that cells have to register a certain amount of wg signalling to achieve competence to become bristle precursors. Cells close to the posterior region of the developing tergite are exposed to high levels of Wg per unit of time and therefore are determined earlier and so give rise to macrochaetae. Cells located more anteriorly within the wg expressing region are determined later and give rise to microchaetae, and finally those cells furthest from the posterior edge of the tergite cannot acquire proneural competence. The bristle precursors are then spaced amongst the bands of competent cells by the action of the lateral inhibition mechanism mediated by the ‘‘neurogenic’’ genes (Mari-Beffa et al., 1991).

Wg, by promoting tergite and sternite and suppressing pleura, helps to organise the final abdominal pattern in plates of coherent histotypes as presumptive pleural cells exclude other cell types from amongst them during histoblast migration. Although wg appears to promote its own expression, this must be lost once the cells are outside a domain with high levels of wg expression. This means that only a restricted stripe of the histoblast population around the original wg-expressing sites will keep expressing wg. This would ensure that the bristles are allocated within tergite/sternite territories and that those of weak pigment coincides with the wg expressing cells in the posterior dorsal part of the tergite.

One question that will require further study is the molecular mechanisms whereby Wg triggers different responses in the histoblasts as development proceeds. Since all abdominal wg phenotypes are also produced by mutations in known elements of the wg-signalling pathway, the explanation might be found at other levels of wg function. Whereas this is a common theme in development, the adult Drosophila abdomen might be an excellent model system to study the use of a common signal in the integration of cell determination, migration, and differentiation.

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