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Postembryonic patterns of expression of *cut*, a locus regulating sensory organ identity in *Drosophila*

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SUMMARY

The *cut* locus is both necessary and sufficient to specify the identity of a class of sensory organs in *Drosophila* embryos. It is also expressed in and required for the development of a number of other embryonic tissues, such as the central nervous system, the Malpighian tubules and the tracheal system. We here describe the expression of *cut* in the precursors of adult sensory organs. We also show that *cut* is expressed in cells of the prospective wing margin and correlate the wing margin phenotype caused by two *cut* mutations with altered *cut* expression patterns. Finally, we observe *cut*-expressing cells in other adult tissues, including Malpighian tubules, muscles, the central nervous system and ovarian follicle cells.

Key words: *cut* locus, expression patterns, imaginal discs

INTRODUCTION

Two lines of evidence demonstrate that the *cut* locus is responsible for specifying the identity of sensory organs in the peripheral nervous system (PNS) of *Drosophila* (1) embryonic lethal *cut* mutations result in the antigenic and morphological transformation of external sensory (es) organs, such as chemoreceptors or mechanoreceptors, into internal chordotonal (ch) organs, which are thought to sense stretch (Bodmer et al., 1987) and (2) ectopic *cut* expression in embryos containing *cut* coding sequences under inducible regulatory control results specifically in the transformation of ch organs into es organs (Blochlinger et al., 1991).

The *cut* locus encodes a nuclear homeodomain-protein (Cut) which is expressed in es organs, but not in the cells of ch organs (Blochlinger et al., 1988). Each sensory organ consists of one or more neurons and three support cells, all of which derive from a common precursor (Bate, 1978; Bodmer et al., 1989). We have previously shown that the expression of Cut is activated in the precursors of embryonic es organs and is subsequently maintained in the differentiated cells (Blochlinger et al., 1990).

During metamorphosis most larval sensory organs degenerate and the adult sensory organs are specified in imaginal discs. Similar transformations of es organs into ch organs are observed in marked patches of *cut* mutant tissue in adult mosaic flies (Bodmer et al., 1987), suggesting that *cut* may have parallel roles in embryonic and adult sensory organ development. We demonstrate here that, as expected, *cut* is expressed in precursors of adult es organs and their progeny in imaginal discs.

In embryos, Cut expression is observed in many tissues in addition to the PNS (Blochlinger et al., 1990). Many cells in the central nervous system (CNS) are labelled by Cut antibodies and staining is also seen in cells of the Malpighian tubules, cells tentatively identified as tracheal histoblasts and cells surrounding both the anterior and posterior spiracles. All these different tissues require *cut* expression for their normal differentiation: in the absence of *cut* expression, the Malpighian tubules fail to form (Blochlinger et al., 1990; Liu et al., 1991), the commissural bundles in the CNS are loosened (C. Klämbt, personal communication) and the airways are not completely air-filled, perhaps due to the malformation of the spiracles (Wieschaus et al., 1984). These observations indicate that *cut* activity is required for the development of other embryonic tissues besides sensory organs.

The adult phenotypes caused by viable *cut* mutations, which result in scalloped wing margins (Lindsley and Zimm, 1992), suggest that this conclusion could be extended to include adult development. These mutations affect the entire wing periphery; however, only the anterior wing margin contains es organs. It thus appears that the effects of loss of *cut* function on the differentiation of es organs and on the development of the wing margin are distinct. In this study, we compare the pattern of *cut* expression of wild-type and *cut* mutant imaginal discs and correlate the phenotype of two different *cut* mutations associated with wing margin phenotypes with corresponding changes

in the *cut* expression pattern in the mutant wing discs. Our results suggest that *cut* is necessary for the development of the wing margin in addition to its requirement for the differentiation of adult es organs.

Finally, we also document the *cut* expression in other cell types including ovarian follicle cells, adepithelial cells and adult muscles, cells in the CNS, Malpighian tubules, tracheal cells and the cone cells in the eye. This description will provide a basis for a future analysis of *cut* function in adult development.

MATERIALS AND METHODS

Fly strains

Wild-type *Drosophila melanogaster* (Oregon R), *yw*, the 'enhancer trap' line A101/TM3 (Bellen et al., 1989; Huang et al., 1991), *ywct*^{6} and *yct*^{k} flies were raised at 18-25°C on standard cornmeal-yeast agar medium. A101/TM3 was provided by H. Bellen.

Dissections

For middle to late third instar larval discs, wandering-stage larvae were collected off bottle walls; for pupal discs, white prepupae (0-1 hour after pupariation) were collected and aged for controlled time periods on moist filter paper at 18°C, or 25°C. Alternatively, the position of white prepupae was marked on the bottles and the pupae collected after aging for appropriate time periods at 25°C. Discs were dissected in PBS and fixed for 10 minutes in 4% formaldehyde/PBS.

Ovaries were dissected in modified Robbs medium and fixed for 20 minutes in 4% formaldehyde/PBS.

Adult fly sections

10 μm sections of adult flies were cut using a SLEE Cryostat, transferred to gelatin-coated slides and fixed in 4% formaldehyde/PBS for 20 minutes.

Immunocytochemistry

Immunocytochemistry was performed according to a previously published procedure (Bodmer et al., 1987). Affinity-purified rat anti-Cut antibodies (F2, Blochlinger et al., 1990) were diluted 1:250. Purified rabbit β-galactosidase antibodies (Cappel) were diluted 1:5000. Rabbit anti-*twist* antibodies (provided by F. Perrin-Schmitt) were diluted 1:5000. For β-galactosidase/*cut* and *twist*/*cut* double-labelling, FITC-conjugated anti-rabbit and biotinylated anti-rat IgGs followed by Texas Red-conjugated avidin (Vector) were used to label β-galactosidase and *cut*, respectively. The discs were viewed, analysed and photographed using an MRC-600 confocal imaging system (Biorad) and a Nikon Optiphot microscope. All other samples were viewed and photographed using a Zeiss Axioplan microscope.

RESULTS

We have investigated the pattern of *cut* expression in imaginal discs and adult flies by staining fixed preparations with affinity-purified Cut-specific antibodies (Blochlinger et al., 1990). The antibodies we used are directed towards a carboxyterminal region of the Cut protein predicted from embryonic cDNAs and specifically recognize two protein species of approximately 280 and 320×10^{3} M_r, which appear to be derived from the same cDNA sequences (Blochlinger et al., 1991). To date, there is no evidence for the expression of alternative Cut coding sequences in embryos, however, developmental northern analysis shows that additional *cut* mRNA species are present at later stages of development that are not observed in embryos (Blochlinger et al., 1988).

cut expression in adult es organs

We have focussed our studies on the developing wing imaginal disc because the pattern of sensory organs on the wing and their development in the wing disc has been characterized in detail (Murray et al., 1984; Hartenstein and Posakony, 1989; Huang et al., 1991; Blair et al., 1992), and is summarized below.

cut expression in es organs of the wing disc

On the anterior wing margin (excluding the wing hinge region), there are rows of multiply innervated, recurved (chemosensory) bristles (Fig. 1A, arrowhead) and singly innervated, stout or slender (mechanosensory) bristles. At the distal tip of the wing, there is a double row of long, non-innervated hairs. Ten campaniform es organs have been identified on the wing blade, and both the dorsal and ventral wing blade surfaces, as well as the notum, are covered almost uniformly by fine, non-innervated trichomes. In addition, the notum carries numerous regularly arranged microchaetes and characteristically located macrochaetes.

The appearance of the precursors and the neurons of the es organs has been well characterized. It has been shown that the neurons of the identified campaniform es organ appear in two groups: one between late larval life and 4 hours after puparium formation (APF), and the other between 9 and 14 hours APF. Similarly, there are two phases in which the es organs of the notum and the wing margin appear. The precursors of the macrochaetes on the notum and the chemosensory bristles on the wing margin start dividing around the time of puparium formation, whereas those of the microchaetes on the notum and the mechanosensory bristles on the wing margin start to divide in the second phase (9-18 hours APF).

cut expression is observed in isolated cells, or groups of cells, on the prospective wing blade, wing hinge region, and notum (for example, white arrowhead Fig. 2A) of third larval instar wing discs. The patterns of expression of the *achaete-scute* genes and a *lacZ* marker that is expressed in all sensory organs (A101) have been used to define the position, and time of appearance and divisions of es organ precursors (Cubas et al., 1991; Huang et al., 1991; Skeath and Carroll, 1991). The temporal and spatial aspects of the *cut* expression in the cells described above suggest that these cells correspond to precursors of campaniform sensilla of the wing blade and macrochaetes of the wing hinge region and the notum.

Between 0 and 2 hours APF, *cut* expression is first apparent in cells at the position of the chemosensory precursors in the most distal portion of the prospective anterior wing margin (see below). Fig. 2C shows a wing disc at 6 hours APF. By this time *cut* expression is observed in the cells of the chemosensory organs of the anterior wing margin (white arrowhead).

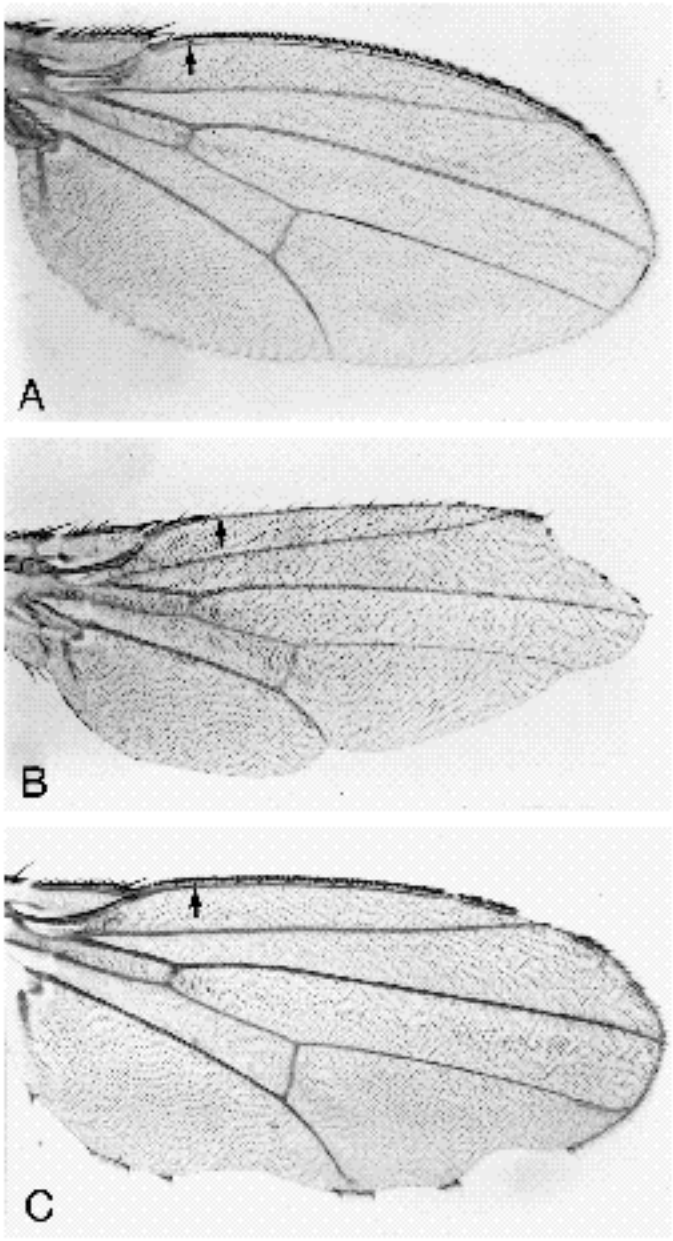

Fig. 1. Wing of (A) *yw*, (B) *ywct*^{6} and (C) *yct*^{k} flies. Arrows point to recurved (chemosensory) bristles.

We confirmed the identity of *cut*-expressing cells observed in the positions of es organs by labelling wing discs of the enhancer trap line A101, in which all sensory organ precursors and progeny express *lacZ*, simultaneously with anti-Cut (Fig. 3A) and anti-β-galactosidase (Fig. 3B) antibodies. Superimposition of the images by confocal microscopy from a wing disc 2-3 hours APF illustrates the coincident expression of both antigens in campaniform es organ precursors of the wing blade and the chemosensory precursors of the anterior wing margin (Fig. 3C). The expression of *cut* in sensory precursors appears to be activated after that of β-galactosidase in A101 (data not shown), which is already detectable in chemosensory precursors before puparium formation (Huang et al., 1991).

At 24 hour APF a distinctive pattern of clusters of four anti-Cut-stained nuclei is seen on the notum (Fig. 4F). These correspond to the cells of the microchaetes and macrochaetes. Within each cluster, the two large nuclei apparently belong to the outer support cells (tormogen and trichogen), the smaller nuclei to the inner support cell and neuron. The outer support cells of the regularly spaced microchaetes appear to be smaller than those of the macrochaetes, which occupy fixed positions and can be individually identified.

cut expression in es organs of the leg and eye-antenna disc

The eye-antenna disc gives rise to almost all adult head structures, including the compound eye and the antennae (Bryant, 1978). Each eye consists of about 800 subunits called ommatidia, between which there are mechanosensory bristles. An eye disc dissected 24 hour APF shows the regularly spaced *cut*-expressing cells in the positions of the interommatidial bristle precursors (arrowhead, Fig. 4D).

The leg discs of a third-instar larva are essentially flat and concentrically organized, so that the future most distal segment is at the center (Schubiger, 1968). During pupariation, the leg discs unfold and extend like a telescope. In third-instar larval leg discs, *cut* expression is observed in two rows of cells in the most distal segment, which appear to be in the position of the future claw organ (solid arrowhead, Fig. 5A) and small clusters of cells in the position of larval pioneer neurons (Jan et al., 1985) (e.g. outlined arrowhead, Fig. 5A). These cells, and additional clusters of cells in the position of other pioneer neurons (e.g. outlined arrowhead), continue to express *cut* in leg discs 2-3 hours APF (Fig. 5C-F).

cut expression along the prospective wing margin

The most prominent feature of the pattern of *cut* expression in the wing disc is a band about 4 cells wide, spanning the entire prospective wing margin, which is the boundary between the dorsal and ventral wing surfaces. The presence of this band of staining was documented from the third instar larval stage until 24 hours APF (data not shown). Several lines of evidence suggest that this expression pattern is not associated with es organs (see Discussion). We took advantage of the existence of viable *cut* mutants that show an altered morphology of the wing to examine the significance of *cut* expression along the wing margin.

The *cut* locus is genetically complex (Johnson and Judd, 1979; Jack, 1985). There are two classes of embryonic lethal mutations, *lethal I* and *lethal II*, that result in the transformation of es into ch organs (Bodmer et al., 1987). *lethal II* mutations are genetically null for *cut* function and affect the most proximal region of the locus, which contains the Cut coding sequences derived from embryonic cDNAs (Blochlinger et al., 1988). Several *lethal I* mutations have been shown to alter the embryonic *cut* expression pattern and have therefore been proposed to perturb regulatory regions (Blochlinger et al., 1990; Liu et al., 1991). In addition, there are several groups of mutations that are lethal at postembryonic stages of development and two groups of mutations that are associated with altered wing (*cut wing*) and leg (*kinked femur*) morphology. We have

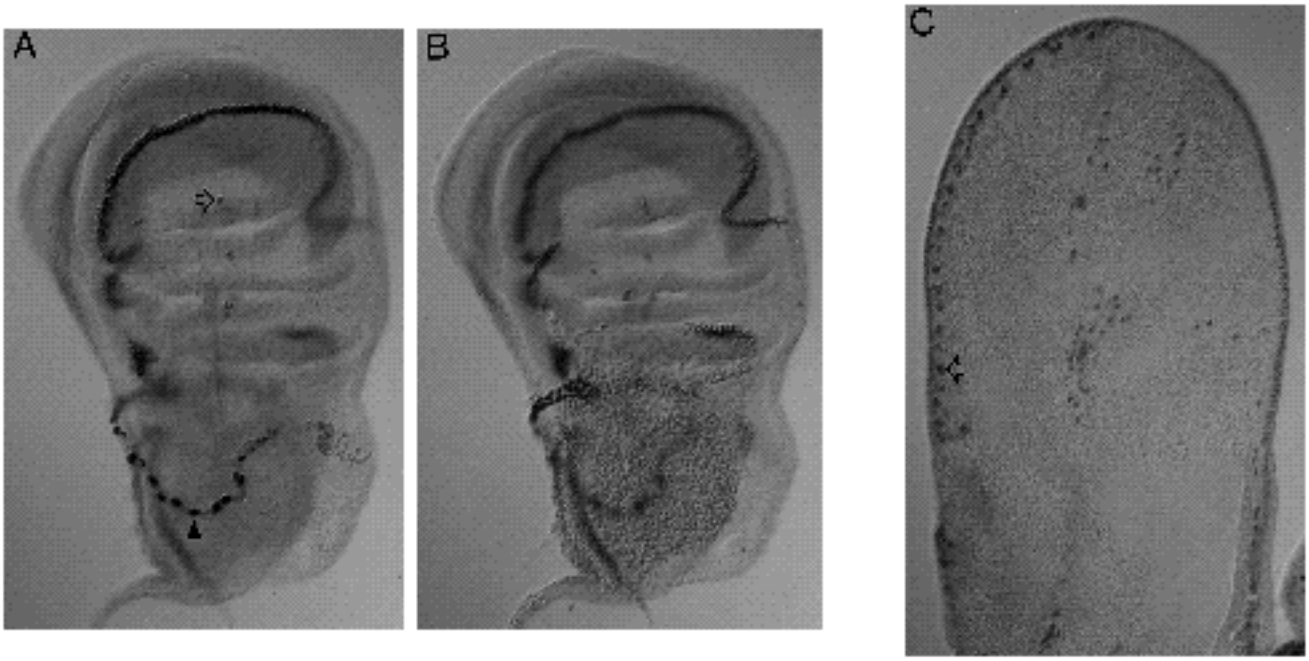

Fig. 2. Wing discs labelled with Cut antibodies. (A,B) Two focal planes of third instar larval disc; (C) pupal disc dissected 6 hours APF. Solid arrowhead points to trachea, outlined arrowhead points to chemosensory organs.

examined the pattern of *cut* expression in wing discs of two mutants, ct^k and ct^6, which show characteristic patterns of scalloping of the wing margin.

ct^6 belongs to the *cut wing* group of mutations, which are adult viable. Mutant flies exhibit scalloping along the entire wing margin (Fig. 1B). Concomitantly, some of the chemosensory es organs and most of the mechanosensory es organs are missing from the anterior margin and all non-innervated hairs are absent in the posterior wing margin (Fig. 1B). Fig. 6A and B show two focal planes of a third-instar wing disc of a ct^6 mutant. The normal pattern of *cut* expression is observed on the prospective wing blade, notum and trachea; however, the band of staining around the prospective wing margin is absent. At around 2 hours APF, the precursors of the chemosensory organs start dividing and expressing *cut* (Fig. 6C), and at 6 hours APF the cells of all the chemosensory organs are labelled (Fig. 6E). These results suggest that loss of the band of *cut* expression along the prospective wing margin is the basis for the wing scalloping phenotype.

ct^k has been classified as a *lethal I* mutation because of the reduced viability of homozygous flies. The pattern of Cut expression is apparently normal in ct^k mutant embryos (K. B., unpublished). Adult survivors have small gaps or nicks in the wing margin (Fig. 1C). These gaps can occur all along the wing margin and are variable in number and size. In affected regions within the anterior wing margin, both the chemosensory and the mechanosensory bristles are

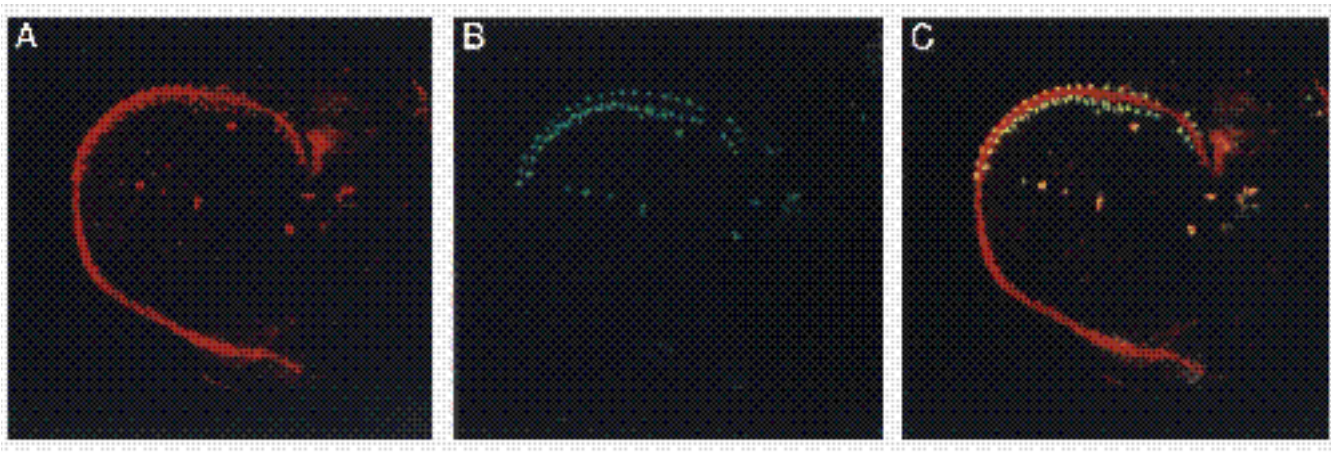

Fig. 3. Confocal images of A101 wing disc labelled 2-3 hours APF with Cut and β-galactosidase antibodies. (A) Image of Cut labelling; (B) image of β-galactosidase labelling; (C) superimposition of A and B.

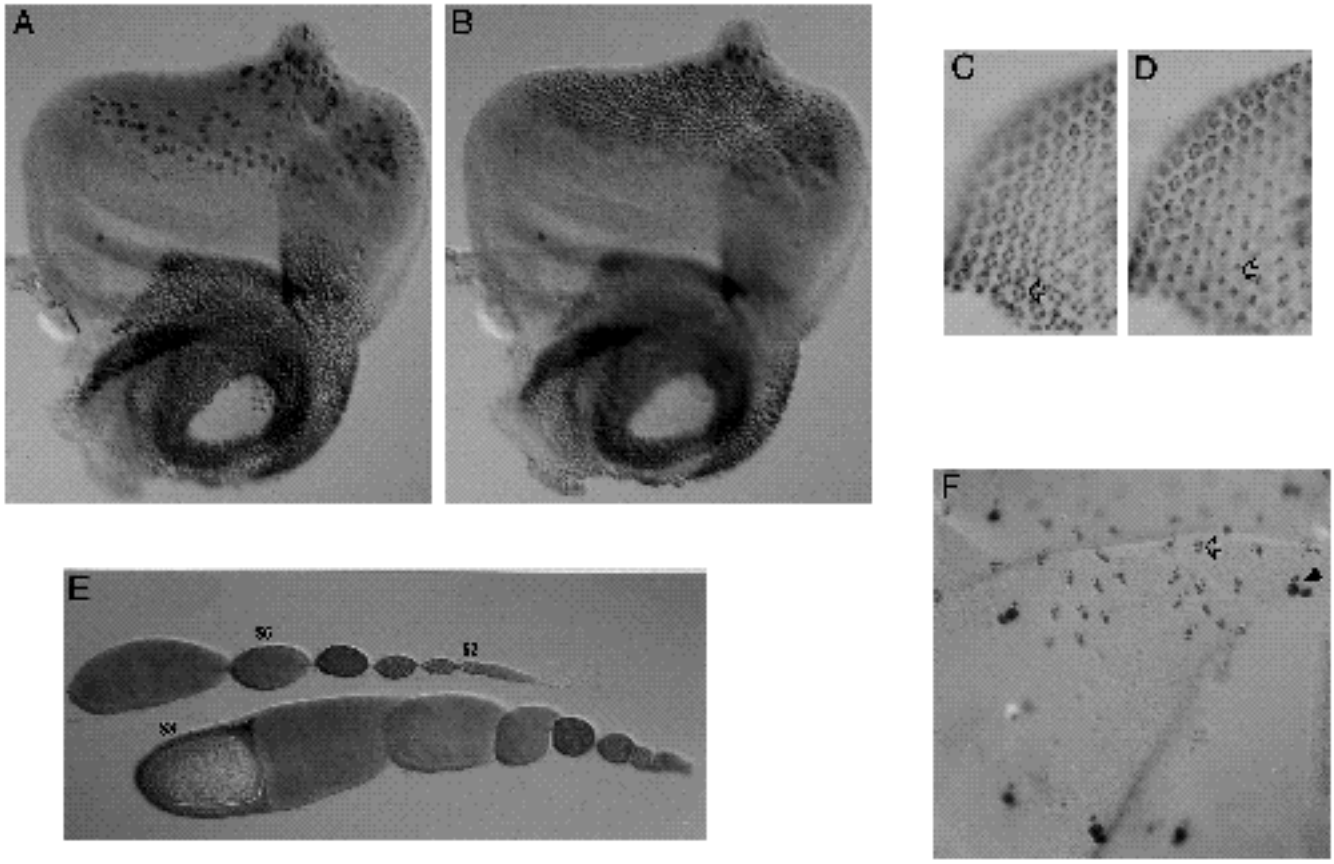

Fig. 4. (A,B) Two focal planes of a third instar larval eye-antenna disc labelled with Cut antibodies. (C,D) Two focal planes of a pupal eye-antenna disc dissected 24 hours APF and labelled with Cut antibodies, outlined arrowhead in C points to group of 4 cone cells, outlined arrowhead in D points to precursor of interommatidial bristle. (E) Two ovarioles labelled with Cut antibodies, follicles at stages 2,6, and 8 are labelled S2, S6 and S8, respectively. (F) Notal region of a pupal wing disc dissected 24 hours APF and labelled with Cut antibodies, solid arrowhead points to macrochaete, outlined arrowhead points to microchaete.

usually absent. Similary, along the posterior wing margin the non-innervated hairs are missing in the scalloped regions. We have found that there are discontinuities in the band of *cut* expression along the prospective wing margin of the third-instar larval wing disc of ct^k mutants (Fig. 6F), suggesting that local loss of *cut* expression is causally related to the gaps in the margin.

cut expression in adepithelial cells

The prospective notal region of the wing disc displays a regular pattern of staining cells (Fig. 2B). The nuclei of these cells have a characteristic morphology with prominent nucleoli. A swirling pattern of labelled cells with a similar morphology is observed both in third-instar larval leg discs and at later stages (Fig. 5B,C). The pattern of *cut* expression on the prospective notal region of the third-instar larval wing disc is remarkably similar to the pattern of *twist* expression (Bate et al., 1991). *twist* is expressed in adepithelial cells, which are closely associated with the disc epithelium and represent the precursors of the adult muscles. To correlate the patterns of *cut* and *twist* expression, we simultaneously labelled third-instar discs with antibodies against Cut (Fig. 7A) and *twist* (Fig. 7B) and found that the cells expressing *cut* on the prospective notal region of the larval wing disc also express *twist* (Fig. 7C), as do the morphologically similar cells in the leg discs (data not shown). Thus, both *cut* and *twist* are expressed in precursors of adult muscles.

cut expression is also observed in adult muscles of the thorax (Fig. 8A), head and abdomen (data not shown).

cut expression in the ovary

Each ovariole within the ovary consists of a succession of developmental stages (Mahowald and Kambysellis, 1978). The stem cells of the germ line divide in the germarium to produce a 16-cell cyst, which is surrounded by somatic follicle cells and pinches off to progress through 14 stages in the vitellarium until oviposition. The follicle cells divide four times between stages 2 to 5. *cut* expression is first observed in the follicle cells at stage 2 (Fig. 4E). The expression appears to decline at around stage 6, except in a couple of cells at both ends of the follicle (not shown), resumes at about stage 8 in anterior dorsal follicle cells (Fig. 4E) and persists until the follicle cells degenerate at stage 14. The expression of *cut* in follicle cells can also be observed in anti-Cut-labelled sections of the abdomen of adult female flies (Fig. 8B).

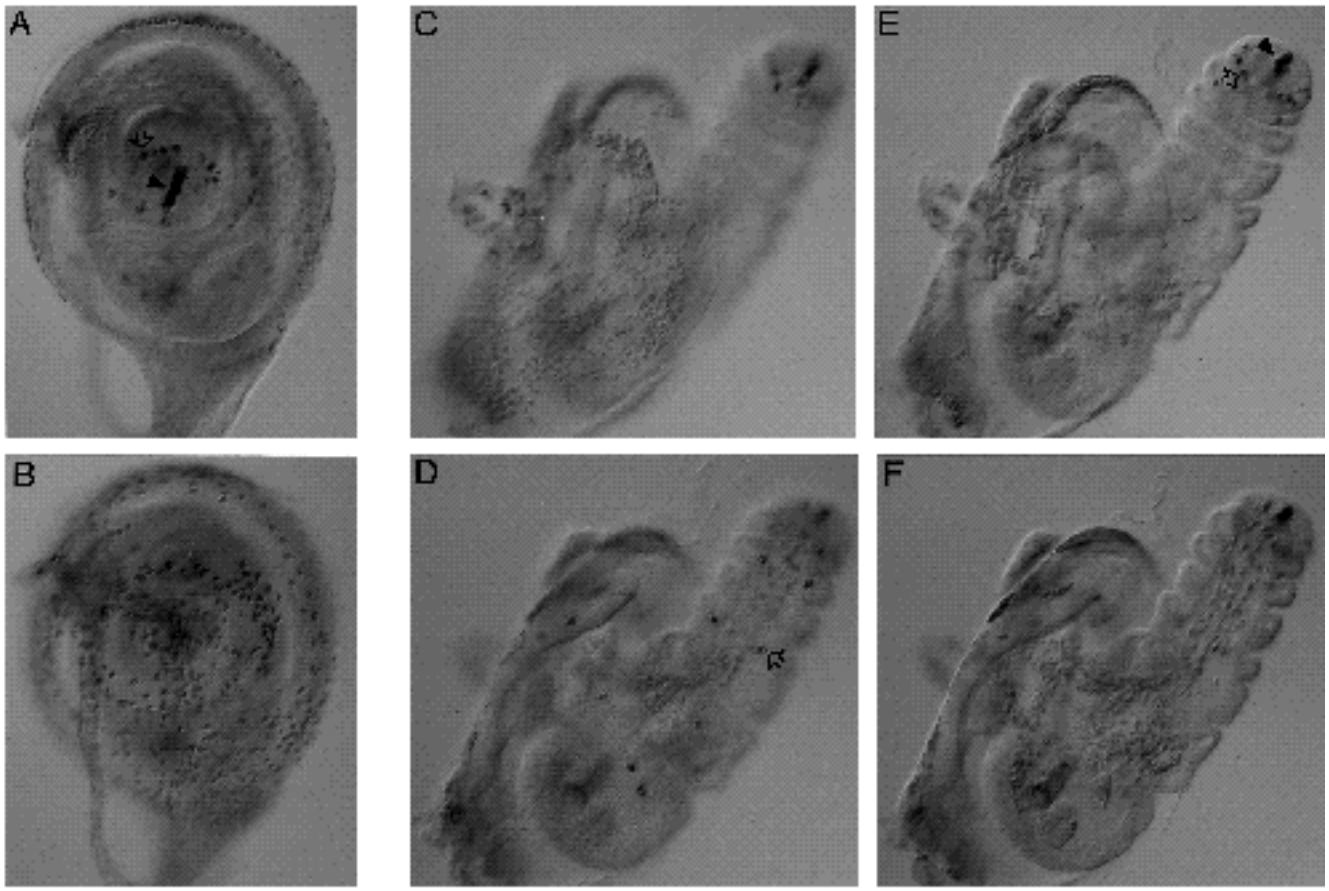

Fig. 5. Leg discs labelled with Cut antibodies. (A,B) Two focal planes of third instar larval disc; (C-F) Four focal planes of pupal disc dissected 2-3 hours APF. Outlined arrowheads point to position of pioneer neurons, solid arrowhead points to position of claw organ.

cut expression in the CNS

We examined the *cut* expression pattern in the head by labelling horizontal sections of adult flies with Cut antibodies. In the head, *cut* expression is observed in cells of all cortical areas. Strong labelling is seen in the cortices of the medulla and lobula complex, less intense staining in cells scattered within the neuropil, which presumably are glial cells, and in putative glial cells within the lamina neuropil. No expression is observed in the photoreceptor cells (Fig. 8A). Many cells in the thoracic ganglion also express *cut*, in both the cortex and the neuropil (Fig. 8A).

cut expression in other cells

Each ommatidium of the compound eye contains four cone cells which lie above the photoreceptors and secrete two of the lens elements (Ready, 1989). *cut* expression is observed in the cone cells of the third-instar larval eye disc (Fig. 4B). An eye disc dissected 24 hour APF shows the expression of *cut* in the regular array of four cone cells per ommatidium (arrowhead, Fig. 4C). *cut* continues to be expressed in adult cone cells (Fig. 8A).

cut expression is also apparent in the cells lining the trachea associated with the imaginal discs (e.g. Fig. 2A, solid white arrowhead), and in cells of the Malpighian tubules in the abdomen (Fig. 8B).

We also observe *cut* expression in many as yet unidentified cells; for example in cells apposed to the eye disc and in many cells in the antennal region of the disc of third-instar larvae (Fig. 4A), some of which also express *twist* (data not shown).

DISCUSSION

We examined the expression pattern of *cut* in imaginal discs and adult tissues in order to extend our previous analysis of the embryo (Blochlinger et al., 1990), and to provide a basis for investigating the role of *cut* in adult development.

Our results endorse a function for *cut* in specifying the identity of all es organs: as in embryos, *cut* is expressed in the positions of precursors of adult es organs and its expression continues in their progeny. We verified the proposed identity of these *cut*-expressing cells through double-labelling experiments in an enhancer trap line (A101) expressing *lacZ* in all sensory precursors (Huang et al., 1991). Concomitantly, we observed that the onset of *lacZ* expression appeared to precede *cut* protein (data not shown). This might suggest that there is a significant delay between the time at which a cell is determined to become a sensory organ precursor and the time at which its identity as an es organ precursor is specified.

Apart from es organs, we also observed *cut* expression in a number of other tissues, including a band of cells along the prospective wing margin, muscle cells, CNS cells, the

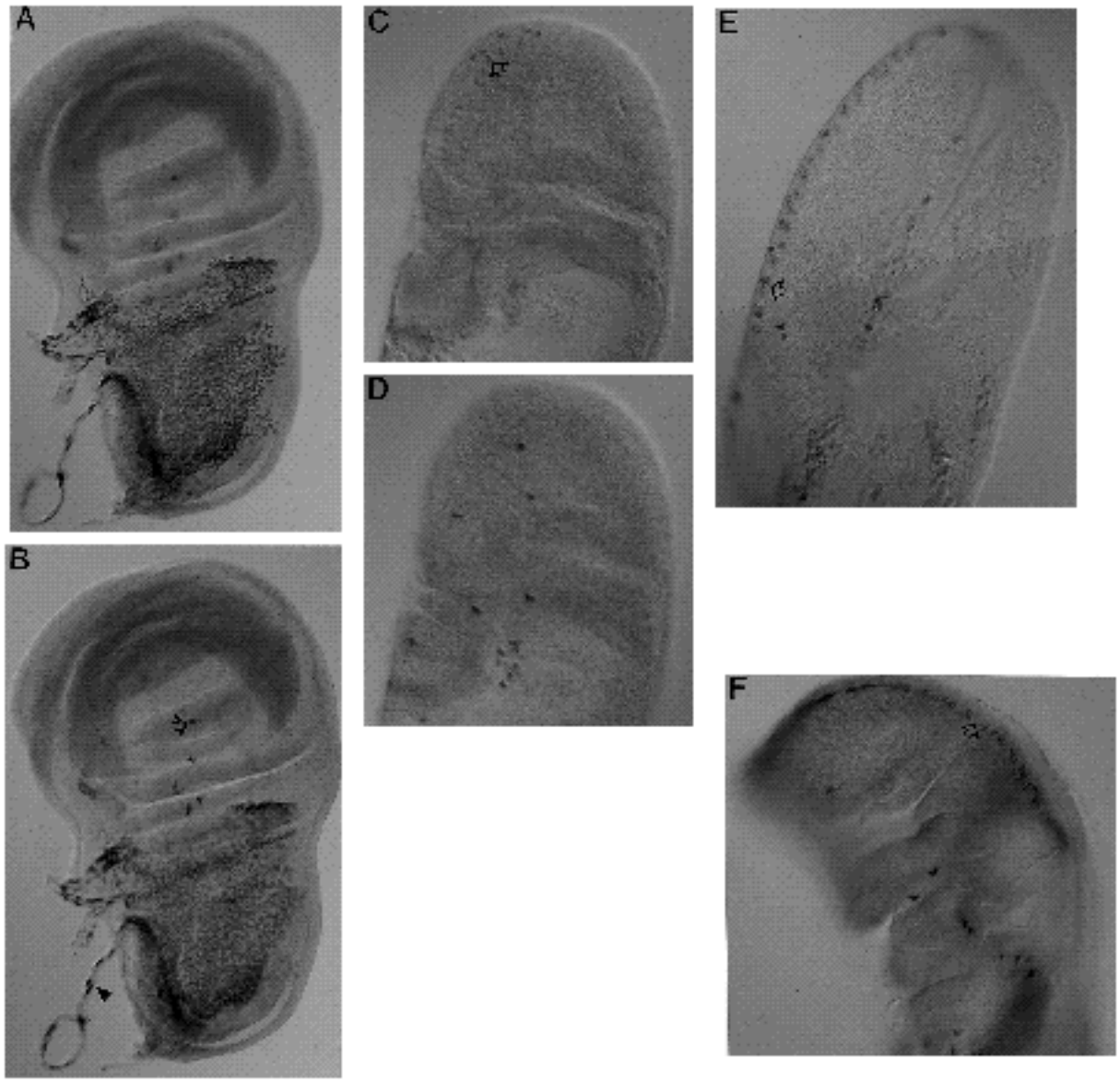

Fig. 6. *cut* mutant wing discs labelled with Cut antibodies. (A,B) Two focal planes of third instar larval disc of ct^6 mutant, solid arrowhead points to trachea, outlined arrowhead points to campaniform organ on wing blade. (C,D) Two focal planes of pupal wing disc of ct^6 mutant dissected 2 hours APF, outlined arrowhead points to precursors of chemosensory organ. (E) Pupal wing disc of ct^6 mutant dissected 6 hours APF, outlined arrowhead points to chemosensory organ. (F) Third instar larval wing disc of ct^k mutant, outlined arrowhead points to gap in Cut labelling of prospective wing margin.

follicle cells of the ovary and cells associated with trachea and Malpighian tubules. Below, we summarize these findings and speculate on their significance.

A prominent band of staining 4-5 cells wide is found along the prospective wing margin of the third instar larval wing discs, which persist at least up to 24 hours APF. This staining has been previously concluded to represent the precursors of the mechanosensory bristles of the anterior wing margin and the non-innervated hairs of the posterior wing margin (Jack et al., 1991). We disagree with this conclusion for several reasons. Firstly, this would be temporally inconsistent with the *cut* expression in all other es organ precursors examined because precursors for es organs in the embryo and precursors for the chemosensory organs of the wing margin, macrochaetes of the notum and the campaniform sensilla of the wing blade express *cut* immediately prior to their first division. The precursors of the mechanosensory bristles of the wing margin divide between 9 and 14 hours APF (Hartenstein and Posakony, 1989), long after *cut* expression is observed along the prospective wing margin. The microchaetes of the notum also divide around 10 hours APF, but there is no evidence of *cut* expression in presumptive microchaete precursors in third-instar larval discs. Also, both in embryos and in discs, the *lacZ* marker lines A101 and A37 generally label es organ precursors shortly before *cut* expression is detectable, and none of

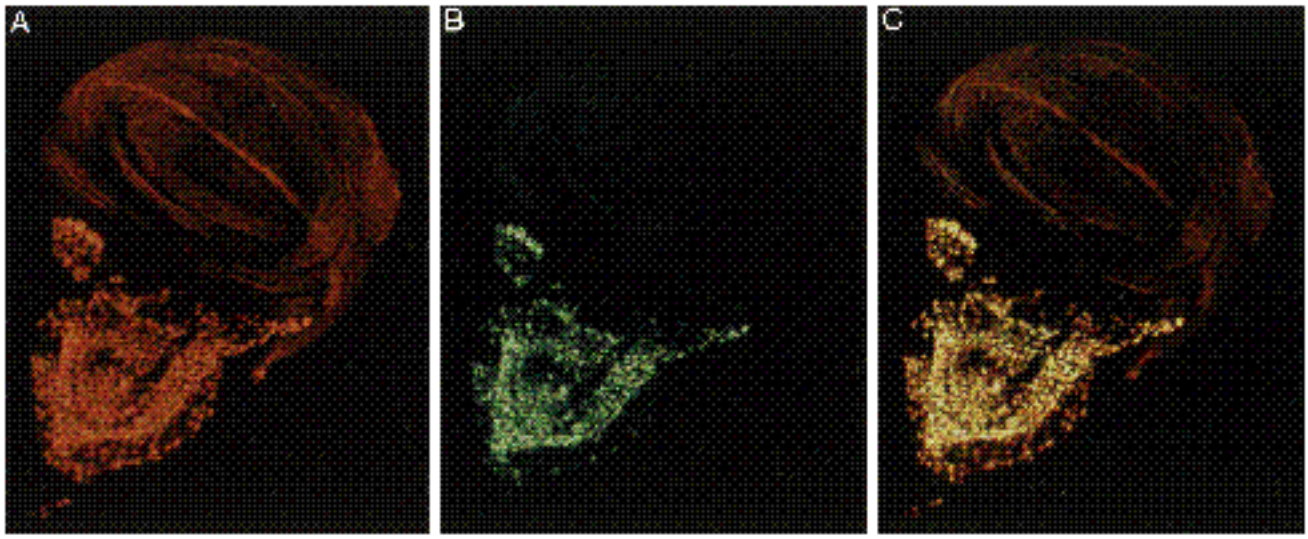

Fig. 7. Confocal images of third instar larval wing disc labelled with Cut and *twist* antibodies. (A) Image of Cut labelling; (B) image of *twist* labelling; (C) superimposition of A and B.

these label the prospective wing margin of the third-instar larval wing disc (Huang et al., 1991). In fact, at the time when the precursors of mechanosensory bristles and non-innervated hairs start expressing *lacZ* in the A101 line, they do not bind Cut antibodies (S. Blair, personal communication). Moreover, the anterior wing margin of a wild-type fly contains about 200 mechanosensory bristles and, although the exact number of anti-Cut-labelled cells along the prospective wing margin cannot be easily determined, there appear to be far more than 200 in the anterior region.

We have further addressed this issue by examining *cut* expression in two *cut* mutants with scallopped wing margins. In both, the band of *cut* expression along the prospective wing margin of developing wing discs is specifically affected: in one of them, ct^6, this staining is absent, in the other, ct^K, the staining is irregular with small gaps. These expression patterns suggest that the wing phenotypes are caused by interfering with regulatory sequences that direct *cut* expression along the prospective wing margin. A similar conclusion was reached through the analysis of another *cut wing* mutation and the identification of an enhancer element in the *cut* locus that controls expression in wing discs (Jack et al., 1991). As the altered *cut* expression patterns in ct^6 and ct^K wing discs occur along the entire prospective wing margin and well before the specification of the mechanosensory bristles along the anterior wing margin, we propose that *cut* has a function along the wing margin distinct from its role in specifying sensory organ identity. Although the pattern of es organs along the wing margin is disrupted in ct^6 and ct^K mutants, we believe that the primary defect is in the cells forming the wing margin and that the effect on the marginal bristles is a consequence of their position. Earlier studies noted a correlation between the wing shape of ct^6 mutants and an abnormal pattern of cell death in the wing imaginal disc during metamorphosis (Goldschmidt, 1935; Blanc, 1942; Braun, 1942; Fristrom, 1969). This suggests that *cut* may be necessary for the survival of cells along the prospective wing margin. Alternatively, it has been proposed that abnormal folding of the wing disc is responsible for the scalloping phenotype (Waddington, 1940). Clonal analysis and mosaics have shown that wing margin gaps caused by ct^6 affect both dorsal and ventral wing surfaces, although clones are restricted to one surface, indicating non-autonomous behaviour of the margin cells (Santamaria and Garcia-Bellido, 1975). These results have led to the suggestion that ct^6 and other wing scalloping mutants may affect cellular interactions, for example, cohesion between the dorsal and ventral wing surfaces (Santamaria and Garcia-Bellido, 1975). Consistent with this hypothesis, we have found that *cut* is expressed in both the dorsal and the ventral cells along the wing margin. The proposed non-autonomy for *cut* function along the wing margin further argues that *cut* has a role in the formation of the wing margin that is distinct from its activity in specifying sensory organ identity, because the latter has previously been shown to be autonomous (Bodmer et al., 1987).

We also observe *cut* expression in a set of cells in close apposition to the disc epithelium called adepithelial cells (Poodry and Schneiderman, 1970). These cells give rise to the thoracic muscles of the adult fly. We confirmed the identity of these *cut*-expressing cells in double-labelling experiments with *twist* antibodies, which had been previously shown to be expressed in adepithelial cells (Bate et al., 1991). *cut* expression in these cells appears to persist throughout development, because most, if not all, of the adult thoracic muscle nuclei are labelled by Cut antibodies. Similarly, adult head and abdominal muscle nuclei express *cut*. The abdominal muscles have been shown to derive from *twist*-expressing cells present in the abdominal segments of the embryo (Bate et al., 1991). We have found that these embryonic precursors are also labelled with Cut antibodies (data not shown).

In the adult CNS, anti-Cut-labelling is observed in many, but not all cortical cells. Some of the cells expressing *cut* appear to be glial cells, for example in the cell layer prox-

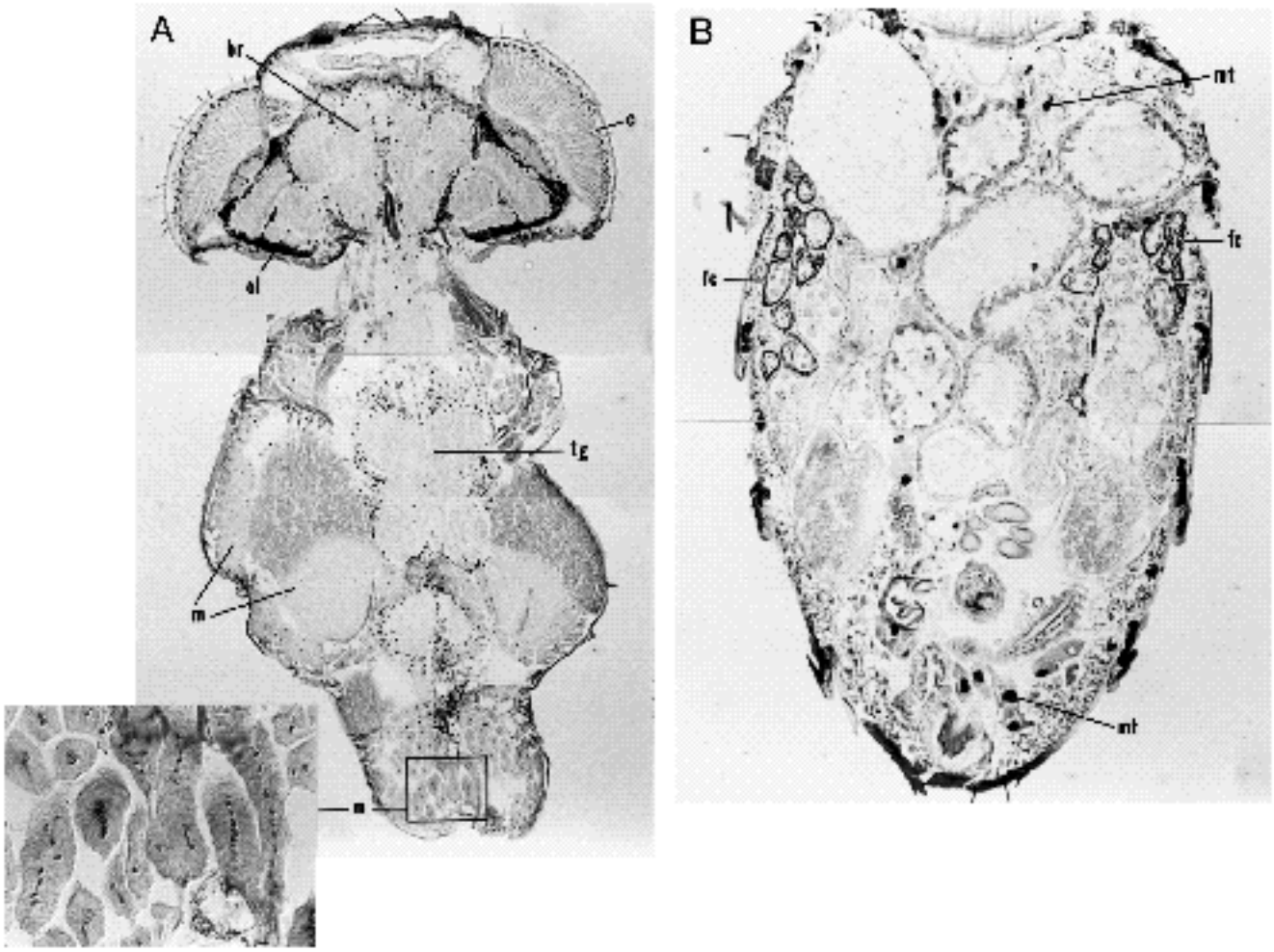

Fig. 8. Adult fly sections labelled with Cut antibodies. (A) Head and thorax section, inset shows a higher magnification of labelled muscle cells; (B) abdominal section: br, brain; ol, optic lobe; tg, thoracic ganglion; m, muscle; c, cone cells; mt, Malpighian tubule; fc, follicle cells of ovarioles.

imal to the laminar neurons and in the neuropils of the central brain and the thoracic ganglion. In the eye, *cut* is expressed in the cone cells, but not in photoreceptor cells and probably not in the neurons of the lamina. The staining intensity is not equivalent for all anti-Cut-labelled nuclei in the CNS. This difference is particularly conspicuous between the cells in the cortices of the medulla or lobula complex and the putative glial cells proximal to the laminar neurons. This could be explained by different levels of *cut* expression or possibly protein species derived from alternatively spliced mRNAs that are not as efficiently bound by the Cut antibodies. Northern analysis has indicated the existence of additional mRNA species in pupae and adult heads, which are not represented in embryos (Blochlinger et al., 1988), and the antibodies used in this study are directed towards embryonic protein sequences.

We have also observed *cut* expression in cells associated with the trachea, in Malpighian tubules, in ovarian follicle cells and in other as yet unidentified cells. Both the tracheal system and the Malpighian tubules are affected by embryonic lethal *cut* mutations (Blochlinger et al., 1990; Liu et al., 1991). It has been observed recently that numerous genes involved in neural development are expressed in subsets of follicle cells and are involved in the establishment of both the anterior-posterior and the dorsal-ventral axis during oogenesis (*Notch*, *Delta*, and other neurogenics, Ruohola et al., 1991; *rhomboid*, H. Ruohola, personal communication). The expression of *cut* in dorsal anterior follicle cells at the stage when the oocyte axes are being specified raises the possibility that *cut* participates in this process.

Are there any properties that are shared by the tissues in which *cut* is expressed? One common attribute is that expression appears to be activated at the precursor stage and then persist in the differentiated progeny. Also, *cut* is generally expressed in only a subset of cells within a tissue, for example in the central and peripheral nervous systems or the follicle cells, which may indicate that *cut* has a role in specifying cell fate of subsets of cells in many tissues. Apart from es organs and wing margin, it has not been tested whether *cut* function is needed for the development of the adult tissues in which it is expressed. Nonetheless, we predict that this is the case because we have found that the development of all the embryonic *cut*-expressing tissues examined depends on *cut* activity (Blochlinger et al., 1991).

We plan to address this issue in adults using mosaic analysis.

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