Patterns of expression of Cut, a protein required for external sensory organ development in wild-type and *cut* mutant *Drosophila* embryos

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The loss of *cut* activity in *Drosophila* results in the transformation of the neurons and support cells of external sensory (es) organs into those of chordotonal (ch) organs. The *cut* locus encodes a homeo domain-containing protein, which is expressed in the cells of es, but not in ch, organs. We show by Western analyses the presence of two embryonic protein species whose approximate relative molecular masses of 280 and 320 kD are compatible with that predicted from the primary sequence. We also describe the development of the Cut protein expression pattern and show that Cut is expressed in sensory precursor cells that divide to give rise to es organs. Finally, we analyze the changes in the Cut expression pattern of several mutant alleles of the complex *cut* locus and show that the mutations affecting es organ development are associated with either altered protein distribution in the PNS or incorrect subcellular Cut protein localization.

[Key Words: Sensory organ development; *cut* locus]

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The neurons of the embryonic peripheral nervous system (PNS) of *Drosophila melanogaster* are arranged in an invariant, segmentally repeated pattern (Campos-Ortega and Hartenstein 1985; Dambly-Chaudière and Ghysen 1986; Bodmer and Jan 1987; Hartenstein 1988). The majority of sensory neurons have single dendrites and are associated with sensory structures formed by non-neuronal support cells (Hertweck 1931; Kankel et al. 1980; Zacharuk 1985). These sensory structures, together with the innervating neuron(s), constitute a sensory organ.

There are two groups of sensory organs: The external sensory (es) organs have mechanosensory or chemosensory properties, whereas the internal chordotonal (ch) organs are thought to be stretch receptors (McIver 1985; Zacharuk 1985; Dambly-Chaudière and Ghysen 1986; Bodmer et al. 1987; Hartenstein 1988). Embryonic lethal mutations in the *cut* locus cause the transformation of es organs into ectopic ch organs, without changing the overall number or position of sensory organs (Bodmer et al. 1987). We have shown previously that the *cut* locus encodes a large nuclear protein containing a homeo domain (Blochlinger et al. 1988). Analogy with other homeo domain-containing proteins suggests that Cut protein may function as a transcription regulator, conceivably inducing the expression of genes involved in es organ differentiation. We have now examined the embryonic Cut expression profile by Western analysis and

show the presence of two Cut protein species of apparent relative molecular masses of ~280 and 320 kD, whose abundance increases with embryonic age.

A typical sensory organ consists of one neuron and a set of three support cells that form concentric sheaths around the dendrite and produce the external sensory structure (Hertweck 1931; McIver 1985; Zacharuk 1985; Hartenstein 1988). It has been proposed that es and ch organs originate close to their final position through divisions of ectodermal sensory precursor cells (Bate 1978; Bodmer et al. 1989) and that all of the cells within a sensory organ are related by lineage (Bodmer et al. 1989; R. Bodmer and G. Technau, unpubl.). Recently, we demonstrated that Cut protein is expressed in cells within differentiated es organs but not in cells of ch organs (Blochlinger et al. 1988). Here, we show further that the Cut protein(s) can be detected in precursor cells that subsequently divide to give rise to the cells forming es organs.

The *cut* locus is large and genetically complex (Johnson and Judd 1979; Jack 1985). All embryonic lethal mutations fall into two complementation groups, *lethal I* and *lethal II*, and result in the transformation of es organs into ch organs. In addition, there are several complementation groups consisting of mutations that are lethal at later stages of development and do not affect es organ identity. Finally, two groups of *cut* mutations are viable and cause altered morphology of the wing (*cut wing*) or the leg (*kinked femur*). Here, we examine the

embryonic pattern of Cut expression in mutant alleles of five complementation groups of the *cut* locus and contrast the effects thereon of mutations that either do or do not affect es organ development. Thus, we show that the incorrect specification of es organ identity in *cut* mutants results from either the absence of Cut protein in some or all es organ cells or lack of nuclear localization of Cut protein.

Results

Late embryonic pattern of Cut staining

We have previously published a preliminary description of the distribution of Cut protein in late embryogenesis, as seen with a peptide antiserum (Blochliger et al. 1988). An additional antiserum was subsequently obtained by immunizing rats with a partially purified fusion protein consisting of a carboxy-terminal region (including the homeo domain) (F2) of the predicted Cut protein and several amino acids of the T7 *gene 10* protein expressed in *Escherichia coli* (Rosenberg et al. 1987). The resulting antiserum, as well as the antiserum used previously (clp2) directed against a synthetic peptide corresponding to amino-terminal Cut sequences (Blochliger et al. 1988), was affinity-purified by use of *E. coli*-produced fusion proteins.

We now present a detailed analysis of the Cut protein expression pattern in the thoracic and abdominal segments of the embryonic PNS, where a complete characterization of neurons and their support cells is available (Campos-Ortega and Hartenstein 1985; Dambly-Chaudiere and Ghysen 1986; Bodmer and Jan 1987; Hartenstein 1988) (Fig. 1A,B). Each es organ contains at least one neuron and three support cells, of which the thecogen forms a sheath around the tip of the dendrite, the trichogen produces the sensory process (such as a sensory bristle), and the tormogen forms a circular socket at the base of the sensory process. The neuron(s) and thecogen cell are subepidermally located, and the trichogen and tormogen lie within the epidermal layer. The most numerous es organs in the thoracic and abdominal segments are the campaniform sensilla (strain receptors) and trichoid sensilla (touch receptors), two of which, *des2* and *v'es2*, are innervated by two neurons each. Two types of complex es organs are unique to the thoracic segments: basiconical sensilla (probably chemoreceptors) and Keilin's organs (humidity receptors), which are innervated by three and five neurons, respectively.

In all es organs innervated by a single neuron, four cells are labeled by Cut antibodies: The trichogen and tormogen are intensely labeled, and the neuron and thecogen are moderately labeled (see Fig. 5A, below). In doubly innervated campaniform es organs, five cells label: two intensely (trichogen, tormogen), and three moderately (neurons, thecogen). In most basiconical sensilla, six cells are labeled; again, the trichogen and tormogen are darkly stained, whereas the three neurons and the thecogen show less intense labeling. However, it appears that in some basiconical sensilla, particularly in older embryos, there is only one darkly stained cell at

the surface. Finally, in the Keilin's organs, which are innervated by five neurons, there are 12–16 α -Cut-labeled cells, of which 6–8 are intensely labeled.

In addition to the neurons with single dendrites innervating sensory structures, there are sensory neurons that have multiple dendrites (md) and are not associated with defined sensory structures (Ghysen et al. 1986; Bodmer and Jan 1987). The most abundant subset of md neurons in the PNS are those with dendritic arborizations (da neurons). Many of these neurons are also labeled by α -Cut antibodies. Other cells in the periphery also label, notably presumptive glial cells along the nerve trunks and cells around primary tracheal branches, as well as several unidentified subectodermal cells.

In addition to peripheral cell staining, many cells in the central nervous system (CNS) are labeled. As described previously (Blochliger et al. 1988), α -Cut staining is also seen in the cells lining the Malpighian tubules and cells surrounding both the anterior and posterior spiracles.

The spatial pattern of Cut mRNA expression appears to be identical to that of Cut protein, both late in embryogenesis (Fig. 1C) and during earlier development (data not shown). It was not possible to determine whether there are differences in the abundance of *cut* transcripts between cells within an es organ.

Developmental profile of the embryonic Cut-expression pattern

Lineage analyses and studies of DNA replication patterns of insect sensory organs suggest that each es organ originates from a first-order ectodermal precursor cell that divides to produce two second-order precursor cells: one pair of siblings thereof forms the tormogen and the trichogen, whereas the other pair differentiates into a neuron and the thecogen (Bate 1978; Bodmer et al. 1989). In the case of multiply innervated es organs, additional divisions occur in the neuron/thecogen lineage. The four cells in a ch organ are also presumed to be lineally related; however, the division pattern is probably serial, unlike the symmetrical pattern observed in es organs (Bodmer et al. 1989).

The sensitivity of the affinity-purified antibodies permits the detection of Cut expression early in embryonic development. At 5–6 hr of development at 25°C (stage 10; Campos-Ortega and Hartenstein 1985), clusters of cells corresponding to the progenitors of the Malpighian tubules, as well as cells in the vicinity of the future anterior and posterior spiracles, are labeled with these antibodies (Fig. 2A). In the head region, presumptive precursors for the antenna–maxillary organ and other external sensory organs label. In each thoracic and abdominal hemisegment of the developing PNS, labeling is seen in one or two nearby cells, followed shortly by labeling of a third, more ventral, cell (Fig. 3A, 1 and 2). Somewhat later, small clusters of about three Cut-expressing cells are seen at each of these positions (Fig. 3B). As little cell migration is found to occur during PNS development, it is possible to tentatively assign the two α -Cut-labeled clusters to the position of the basiconical

Figure 1. (A) Schematic representation of α -Cut-stained cells in the PNS in a thoracic (T) and an abdominal (A) hemisegment. Moderately α -Cut-stained cells are depicted as stippled red figures; darkly stained cells, as filled red symbols. Circles indicate es organ cells; diamonds, da neurons; ovals, ch organ cells; squares, neurons with bipolar dendrites (Bodmer and Jan 1987); triangles, neurons with dendrites that arborize around tracheal branches (Bodmer and Jan 1987). Nomenclature is according to Dambly-Chaudière and Ghysen (1986). (B) Late-stage embryo stained with F2 antibodies. (C) In situ hybridization of late-stage embryo with a *cut* cDNA probe. Anterior is left; dorsal is up. (B and C) (T1) First thoracic segment; (A1) first abdominal segment; (mt) Malpighian tubules; (asp) anterior spiracle; (psp) posterior spiracle. Arrowheads indicate underlying CNS.

sensilla (les3, v'es3) in the thoracic segments and to the position of the doubly innervated trichoid sensillum and campaniform sensillum (des2, v'es2) in the abdominal segments, respectively. The early Cut expression in presumptive precursors for these es organs would be consistent with their earlier morphological differentiation (Hartenstein 1988).

As development proceeds, progressively more cells in each segment are labeled with Cut antibodies (Figs. 2 and 3B–D). Some of the labeled cells evidently arise through division of Cut-expressing cells: Labeled elongated figures resembling dividing cells are often observed, and the final number of staining cells is severely reduced in *string* embryos (data not shown), in which cell division is arrested after the blastoderm stage (Edgar and O'Farrell 1989). Other α -Cut-labeled cells are likely to be first-order precursor cells for es organs, by virtue of their position and spatial isolation from other Cut-expressing cells. It has been shown previously that embryonic sensory organs differentiate in two dorsal-to-ventral waves in each segment (Hartenstein 1988). The first wave includes the chordotonal organs, the basiconic sensilla, and the doubly innervated trichoid/cam-

paniform sensilla. The singly innervated trichoid and campaniform sensilla are generated in the second wave. A similar dorsoventral sequence of DNA replication of sensory precursors has been observed (Bodmer et al. 1989). Although it is not possible to definitively identify individual Cut-expressing cells, similar temporal restrictions seem to apply to their sequence of appearance.

To further substantiate the claim that the Cut-expressing cells observed early in development are precursors for es organs, we performed double-labeling experiments in *P[lac,ry⁺]*A37 embryos. This strain of flies contains the *lacZ* gene inserted into a P element and has been shown recently to express β -galactosidase in (probably) all cells of the PNS (Ghysen and O'Kane 1989). At early stages of embryonic development, the β -galactosidase-expressing cells in *P[lac,ry⁺]*A37 have been proposed to be precursors for sensory organs on the basis of genetic evidence. Accordingly, the first labeled cell to appear in each segment, P, is thought to be a ch organ precursor, and the second labeled cell, A, which is anterior to P, an es organ precursor. Shortly thereafter, a pair of labeled cells is seen at each of the positions of the A and P cells, and additional labeled cells appear both dor-

Figure 2. Developmental profile of the embryonic Cut expression pattern with F2 antibodies. Anterior is *left*; dorsal is *up*. (T1) First thoracic segment; (A1) first abdominal segment; (mt) Malpighian tubules; (asp) anterior spiracle; (psp) posterior spiracle. Times of development are measured at 25°C. (A) Lateral view of a 5- to 6-hr embryo; (B) lateral view of a 6- to 7-hr embryo; (C) ventral view of a 6- to 7-hr embryo; (D) ventral view of a 7- to 8-hr embryo; (E) lateral view of a 7- to 8-hr embryo; (F) lateral view of a 10- to 11-hr embryo.

sally and ventrally. Double-labeling of *P[lac,ry⁺]*A37 embryos with Cut antibodies (Fig. 4A) and β -galactosidase antibodies (Fig. 4B) demonstrates both the coincidence of Cut and β -galactosidase expression in the pair of A cells and a more ventral cell (Fig. 4C) and the absence of Cut expression in the pair of P cells.

At the stage at which about three to seven cells are labeled by Cut antibodies in the periphery of each hemisegment, α -Cut staining of CNS cells first becomes apparent along the midline (Fig. 2B,C). The domain of expressing cells in the CNS subsequently expands. Similarly, the number of stained cells increases in the area surrounding the anterior and posterior spiracles, as well as in the anlagen for the Malpighian tubules.

Western analysis

The Cut protein predicted from embryonic cDNAs con-

tains 2175 amino acids. Protein extracts of staged embryos were subjected to Western analysis with F2 antibodies. Two bands of apparent molecular masses (on the basis of their relative mobility through SDS gels) of ~ 280 and ~ 320 kD are present in wild-type embryos. These bands first appear in 6- to 9-hr embryo extracts and become progressively more intense in extracts of older embryos (Fig. 5A). The same developmental pattern of expression is seen with *clp2* antibodies (data not shown). Both bands are absent in extracts of homozygous embryos of the *lethal II* mutant *ct^{C145}* (see below; Fig. 5B, lane 5).

Cut expression profile of cut mutants

The pattern of Cut protein expression in mutant embryos from several complementation groups was ana-

Figure 3. (A–D) Developmental profile of the embryonic Cut expression pattern in an abdominal hemisegment with F2 antibodies. (A) Lateral view of 5- to 6-hr embryo. The first two labeled cells to appear (1) may correspond to precursors for des2; the third (2), to v'es2. (B) View of a 6- to 7-hr embryo. Note that there are now clusters of three to four Cut-expressing cells at positions 1 and 2. The single Cut-expressing cells at positions 3 and 4 may be precursors for desC and desD, respectively. (C) View of 8-hr embryo. α -Cut-labeled cells at positions 5–9 probably give rise to desB, lesC, lesB, lesA, and v'esA/v'esC, respectively. (D) View of 10-hr embryo. Cut-expressing cells at positions 9–12 correspond to cells of v'esA, v'esB, v'esC, and vesB, respectively. Cut-expressing cells belonging to vesA are more ventral and are not shown. (E) Schematic representation of the α -Cut-stained cells in the PNS of an abdominal hemisegment. Our assignments of individual or groups of Cut-expressing cells to particular es organs/es organ precursors up to 10 hr of embryonic development are speculative but consistent with the position and presumptive time of appearance of the latter. Anterior is left; dorsal is up.

Figure 4. Abdominal hemisegment of a 5- to 6-hr A37 embryo double-labeled with Cut and β -galactosidase antibodies. (A) View of the Cut-expression pattern. Numbers refer to the same cells numbered in Fig. 3A. (B) View of the β -galactosidase expression pattern. A and P refer to the same cells described by Ghysen and O'Kane (1989). (C) Superimposition of views A and B to show coincidence of Cut and β -galactosidase expression in cells labeled 1 in A and A in B. Anterior is left; dorsal is up.

Figure 5. (A–E) Cut-expression pattern in the dorsal cluster in an abdominal hemisegment of a late-stage, wild-type (A), homozygous *ct^{db7}* (B), homozygous *ct^{Ls}* (C), homozygous *ct^{L65}* (D and E), and homozygous *ct^{Ls2}* (F) embryo with clp2 antibodies. (A) Cells within *desD* are labeled at left; cells within *desC* are labeled at right. (to) Tormogen; (tr) trichogen; (th) thecogen; (n) neuron. (B and C) Note cytoplasmic staining; (D and E) arrowheads indicate unstained cells within transformed sensory organs; (F) note partially cytoplasmic staining. Anterior is left; dorsal is up.

lyzed immunocytochemically by use of antibodies recognizing either a region of the amino (clp2) or the carboxyl (F2) terminus of the predicted Cut protein (Table 1). Also, protein extracts from a mixture of homozygous and heterozygous mutant embryos were examined on Western blots with clp2 antibodies.

lethal II mutants Mutations in the *lethal II* group are genetically null for *cut* function because they do not complement any mutations in the *cut* locus (Jack 1985), and they cause the same extent of es organ transformation as a large deficiency of the locus (Bodmer et al. 1987). The entire Cut protein-coding sequence predicted from cDNAs maps to the *lethal II* region (Blochliger et al. 1988).

Most homozygous *lethal II* mutants show no staining with either of these antibodies (data not shown). The exceptions are *ct^{db7}*, *ct^{Ls}* and *ct^{db10}* embryos, in which cytoplasmic staining is seen, and no staining is discernible in the nuclei with clp2 antibodies (Fig. 5B,C). The conspicuous punctate cytoplasmic staining observed in *ct^{Ls}* embryos (Fig. 5C) could be due to Cut protein aggregation or its sequestration into cytoplasmic vesicles.

By Western analysis, the two bands observed in wild-type embryos corresponding to relative molecular masses of ~280 and 320 kD are absent in extracts of homozygous embryos of the *lethal II* mutant *ct^{C145}* (Fig. 6B, lane 5), consistent with the lack of staining immunocytochemically for this mutant (Table 1). Two additional bands are seen in extracts of a mixture of homozygous and heterozygous embryos from each of the *lethal II* mutants *ct^{db10}*, *ct^{Ls}*, and *ct^{db7}* (Fig. 6B, lanes 6–8), all three of which are associated with cytoplasmic staining with clp2 (see above). *ct^{db7}* has a genomic deletion of ~1

kb in the carboxy-terminal region of the Cut-coding sequence (Blochliger et al. 1988), which is compatible with the apparent molecular weights of ~180 and 155 kD of the additional bands. No alterations were observed in genomic DNA from either *ct^{db10}* or *ct^{Ls}* mutants (K. Blochliger, unpubl.). The reduction in size of the Cut proteins to ~170 and 145 kD in *ct^{db10}* and to 185 and 165 kD in *ct^{Ls}* in these embryos may be attributable to premature termination codons. Despite the carboxy-terminal truncations, two species of Cut proteins are clearly present in each of these three mutants, and the size difference between them appears to be comparable to that between the two species of wild-type Cut protein.

lethal I mutants *lethal I* mutations are also embryonic lethal (Jack 1985) and result in es organ transformation, albeit to a slightly lesser degree than *lethal II* mutations (Bodmer et al. 1987). Many of the mutations map molecularly within several kilobases upstream of the embryonic cDNAs obtained (the site of transcription initiation has not been determined).

In the two *lethal I* mutants examined, *ct^{L1}* and *ct^{L65}*, the number of α -Cut-labeled cells in the PNS is reduced (Fig. 5D,E). There is considerable variability in the amount of PNS staining observed in different mutant embryos. Within most transformed sensory organs, several cells express Cut, although often at reduced levels, the expression in the remainder of the cells is either very weak or absent. Similarly, the number and intensity of α -Cut-stained cells surrounding the spiracles is often diminished, and the Malpighian tubules are absent (data not shown). At earlier stages of development, fewer and less intensely stained es organ precursors are observed in

Table 1. Embryonic phenotype and Cut expression pattern of alleles of five complementation groups of cut mutations

Cut mutation	Embryonic phenotype	Embryonic Cut Expression Pattern	
		whole mounts	protein extracts
<i>lethal II</i>			
C145	es to ch transformation, Malpighian tubules missing, lethal	no staining	no Cut-specific bands
db1-7,8,11-13		no staining	not tested
Ls, db7,10		clp2:cytoplasmic, F2: no staining	clp2: truncated Cut proteins
<i>lethal I</i>			
L65, L1	es to ch transformation, Malpighian tubules missing, lethal	PNS, spiracles: reduced number of staining cells, Malpighian tubules: no staining	wild-type Western profile ^a
<i>lethal III</i>			
L59, L39	no embryonic phenotype	wild-type staining pattern	not tested
<i>lethal IV</i>			
L188	no embryonic phenotype	reduced staining intensity ^b	change in relative intensities of bands
L221	no embryonic phenotype	reduced staining intensity ^b	wild-type Western profile ^a
Ls2	no embryonic phenotype	clp2: nuclear and cytoplasmic	wild-type Western profile ^a
<i>cut wing</i>			
6	no embryonic phenotype	wild-type staining pattern	not tested

^aWestern analysis was performed with an extract of a mixture of homozygous and heterozygous embryos; therefore, differences in the abundance of Cut proteins in homozygous mutants would probably not have been detected.

^bNo independent marker was available to confirm the identity of mutant embryos.

the PNS and around the future spiracles and there is no staining in the regions corresponding to the precursors for the Malpighian tubules (data not shown).

No apparent change in the pattern of Cut expression was observed on Western analysis of protein extracts of *lethal I* mutants *ct^{L65}* or *ct^{L1}* (Fig. 6B, lane 4).

lethal III/lethal IV/cut wing mutants lethal III, lethal IV, and cut wing mutations all do not affect es organ identity or cause any other detectable phenotype in embryos (Bodmer et al. 1987; R. Bodmer, unpubl.). Although the stage of lethality of *lethal III* and *lethal IV* mutations has not yet been determined, none is embryonic lethal (J. Jack, pers. comm.). Mutations in *cut wing*, a viable complementation group, result in the scalloping of the posterior wing margin.

In *ct^{L39}* and *ct^{L59}*, two *lethal III* mutants, and in *ct⁶*, a viable mutant of the *cut wing* complementation group, the pattern of embryonic Cut expression is immunocytochemically indistinguishable from that of wild type (Table 1).

In the *lethal IV* mutant *ct^{Ls2}* cytoplasmic localization of Cut protein is observed by use of clp2 antibodies; however, in contrast to *ct^{db7}*, *ct^{db10}*, and *ct^{Ls}*, the nuclei seem not to be excluded from staining in homozygous mutant embryos (Fig. 4F). No difference in the size of Cut proteins in extracts of *ct^{Ls2}* embryos is detectable on Western blots (Fig. 6B, lane 3). In *ct^{L188}* and *ct^{L221}*, two other *lethal IV* mutants, the overall level of staining appears to be reduced with either clp2 or F2 antibodies (Table 1); however, no independent marker was available to confirm the identity of mutant embryos. On Western blots, no apparent change is observed in the pattern of Cut expression in protein extracts of *lethal IV*

mutant *ct^{L211}* (Fig. 6B, lane 1). However, in protein extracts of mixed homozygous and heterozygous embryos from *lethal IV* mutant *ct^{L188}* embryos, a significant change in the relative intensities of the two Cut-specific bands is observed, with the faster migrating species predominating (Fig. 6B, lane 2).

Discussion

Expression of Cut in es organ precursors

We have reported previously that Cut protein is expressed in at least some of the cells constituting an es organ but not in any cells of ch organs (Blochliger et al. 1988). We now show that it is clearly expressed in all cells within each es organ. In addition, we are now able to demonstrate expression earlier in development in sensory precursor cells, which subsequently divide to give rise to es organs. A number of studies have suggested that sensory organs arise through divisions of ectodermal sensory precursor cells, so that the cells constituting a sensory organ are clonally related (Bate 1978; Bodmer et al. 1989). For es organs, the lineage is thought to be symmetric; in contrast, recent evidence suggests that the division pattern of ch organ precursors is serial (Bodmer et al. 1989). It will be interesting to determine the division patterns of the ectopic ch organs formed in the absence of *cut* activity and, thus, to evaluate the importance of cell division patterns in the determination of sensory organ identity.

The earliest Cut expression in the PNS of the thoracic and abdominal segments is in a pair of nearby cells within each hemisegment. We have shown by double-labeling that these cells are identical to the two cells (A pair) observed to express β -galactosidase early in devel-

Figure 6. (A) Western analysis of staged embryonic protein extracts with F2 antibodies. (Lane 1) 0–3 hr; (lane 2) 3–6 hr; (lane 3) 6–9 hr; (lane 4) 9–12 hr; (lane 5) 12–15 hr; (lane 6) 15–18 hr; (lane 7) 18–21 hr. (B) (Lanes 1–4) Western analysis with clp2 antibodies of *cut* mutant protein extracts from a mixture of homozygous and heterozygous mutant embryos: (lane 1) *ct^{L221}*; (lane 2) *ct^{L188}*; (lane 3) *ct^{Ls2}*; (lane 4) *ct^{L1}*. (Lane 5) *cut* mutant protein extracts from homozygous *ct^{C145}* mutant embryos. (Lanes 6–8) *cut* mutant protein extracts from a mixture of homozygous and heterozygous mutant embryos. (lane 6) *ct^{db10}*; (lane 7) *ct^{Ls}*; (lane 8) *ct^{db7}*. Dots mark positions of migration of truncated Cut proteins. The band migrating below the 97-kD marker is recognized only by clp2 antibodies and may represent a Cut cleavage/degradation product.

opment in a strain containing the *lacZ* gene inserted into a P element (Ghyssen and O’Kane 1989). In this strain, P[*lac,ry⁺*]A37, β -galactosidase is probably expressed in all cells of the PNS. The two A cells are speculated to be es organ precursors on the basis of genetic evidence. At the same developmental stage, another, more posterior pair of cells (P pair) expresses β -galactosidase, and genetic evidence suggests that these two cells are ch organ precursors. No cells in the position of the P pair are stained with Cut antibodies, consistent with the lack of Cut staining in ch organs.

What is the relevance of the different levels of Cut expression in cells within es organs? Although Cut is expressed in all cells of an es organ, the level of expression in the tormogen and trichogen cells is considerably higher than in the neuron and thecogen cells. We were unable to determine whether this difference is already present in the precursors for these pairs of siblings. The pattern of Cut protein expression appears to closely resemble that of *cut* RNA expression, as determined by whole-mount in situ hybridization with a probe corresponding to the Cut-coding region, but it could not be

unequivocally established if the differential levels of Cut protein expression in es organ cells are reflected at the level of transcription. It is interesting to note that in *numb* mutant embryos, where the precursor for the thecogen and neuron is apparently transformed into a trichogen/tormogen precursor, four intensely α -Cut-stained cells are seen in each singly innervated mutant es organ (Uemura et al. 1989). Conversely, the *oversensitive* mutation may cause the transformation of trichogen/tormogen precursors into thecogen/neuron precursors, and all of the cells in a mutant es organ are lightly stained with Cut antibodies (E. Bier et al., in prep.). It thus appears that the level of Cut expression is correlated with the identity of cells composing es organs. It has been shown that profound changes in patterns of gene expression can result from relatively small alterations in the concentrations of sequence-specific transcription factors (Driever and Nüsslein-Volhard 1988; Struhl et al. 1989).

Western analysis of Cut expression

Two protein species of relative molecular masses of ~320 and 280 kD were recognized by antibodies directed toward either amino- or carboxy-terminal sequences of the predicted Cut protein. These sizes are compatible with a content of 2175 amino acids predicted from cDNA sequences. In protein extracts from heat-shocked embryos of a transformant line of flies containing the entire Cut-coding sequences under the regulatory control of a heat shock promoter, the same two bands are seen, albeit at a significantly increased level (K. Blochliger, unpubl.). Also, examination of embryonic cDNAs yielded no evidence for alternative splicing within coding sequences. This suggests that the presence of two Cut-specific bands is a consequence of either alternative translational initiation or post-translational processing.

Cut expression in cut mutants

The prevailing hypothesis suggests that the *lethal II* region represents the protein-coding potential of the *cut* locus and that the upstream region encompassing the viable and all the other lethal complementation groups is involved in regulating its spatial and temporal pattern of expression (Johnson and Judd 1979; Jack 1985). *lethal I* and *II* are the only embryonic lethal groups, and only mutations belonging to these groups result in the transformation of es organs into ch organs.

All *lethal II* mutants examined show either an absence of Cut protein, as assayed with antibodies directed toward two different regions of the predicted protein, or cytoplasmic localization of truncated Cut proteins, consistent with the lack-of-function phenotype attributed to the *lethal II* group of mutations. Because none of these truncated Cut proteins is recognized by antibodies directed toward a carboxy-terminal portion of the predicted protein (F2), we assume that this region overlaps sequences necessary for nuclear localization of the Cut protein.

The two *lethal I* mutants examined both exhibit a significant reduction of α -Cut staining in the PNS. Within

some of the transformed sensory organs, several cells appear to express Cut at wild-type levels, whereas others stain lightly or not at all. The overall frequency of transformation detected is slightly lower in *lethal I* mutants than in *lethal II* mutants (Bodmer et al. 1987). It is possible that the correct specification of es organ identity does not depend on the expression of Cut in every cell within an es organ at the time of fixation. Therefore, further analyses may be inconclusive without knowledge of the α -Cut-staining history of individual cells prior to fixation. Earlier in development, the number and/or intensity of α -Cut-labeled precursor cells in mutant embryos are diminished, but because the number of cells expressing Cut in the PNS varies considerably between mutant embryos, it is not possible to correlate the staining patterns of early and late embryos. In addition to the altered α -Cut-staining pattern in the PNS, the staining in cells surrounding the spiracles is frequently reduced, and the Malpighian tubules are absent in the *lethal I* mutants examined, consistent with the lack of α -Cut staining in Malpighian tubule precursors. The aberrant expression patterns in the two *lethal I* mutants suggest that *lethal I* mutations perturb regulatory elements controlling Cut expression in the PNS, as well as in the Malpighian tubules and the cells surrounding the spiracles.

cut wing, *lethal III*, and *lethal IV* mutants do not affect es organ identity and are not associated with any embryonic phenotype (R. Bodmer, unpubl.). Predictably, no change in the embryonic profile of Cut expression was observed in two *lethal III* mutants and a viable allele of the *cut wing* group. However, the *lethal IV* mutant *ct^{Ls2}* shows partially cytoplasmic α -Cut staining (although there is no detectable change in Cut protein expression seen by Western analysis). A plausible explanation for this observation would be a small mutation in the coding sequences affecting (but not completely abolishing) nuclear localization without impeding the function of correctly localized protein. Mutant embryos of two other alleles of the *lethal IV* group appear to display an overall reduction in the intensity of α -Cut staining. Protein extracts from one of these mutant lines, *ct^{L221}*, show no change in the late embryonic profile of Cut expression as seen on Western blots, whereas in protein extracts from the other, *ct^{L188}*, the abundance of the more slowly migrating Cut protein species is clearly decreased, relative to the faster migrating species. Most likely, the mutation in *ct^{L188}* perturbs either the post-translational modification or the initiation of translation of Cut proteins. Thus, both *ct^{Ls2}* and *ct^{L188}*, which were included in the *lethal IV* group on the basis of their complementation behavior, apparently have mutations in the protein-coding sequence. The resulting reduced level of functional nuclear Cut protein is presumably sufficient for es organ specification during embryogenesis and only becomes limiting during later development.

Expression of Cut in cells not associated with es organs

In addition to es organ cells, most peripheral neurons

with dendritic arborizations (da neurons), many cells within the CNS, cells surrounding anterior and posterior spiracles, and the cells of Malpighian tubules express Cut proteins. What happens to these tissues in the absence of Cut expression? It is not clear in what way, if any, the α -Cut-stained da neurons and CNS cells are affected by *cut* mutations. However, a spiracular defect may be associated with the lack of complete air-filling of the trachea observed in embryonic lethal mutants (Bodmer et al. 1987), and the Malpighian tubules appear to be missing in all *lethal II* and *lethal I* mutants (K. Blochlinger, unpubl.). These observations suggest that Cut proteins are involved in the specification of cell fate in several different tissues.

Materials and methods

Drosophila stocks

The following fly strains were maintained at 25°C and used for immunocytochemistry and Western analyses: Oregon R, *ywct^{C145}/FM7c*, *ywct^{db1-13}/FM7c*, *yzsplcmct^{Ls3}/FM7*, *ywct^{L1}/FM7c*, *ct^{L65m}/FM7c*, *yzsplct^{Ls2}/FM7c*, *ct^{L221}/FM7c*, *ct^{L188}/FM7c*, *cmct^{L39sn3m}/FM6*, *ct^{L59}/FM7c*, *cmct^{sn3}*, *P[lac,ry⁺]A37*.

Antibodies

clp2 The rabbit polyclonal antiserum and the synthetic peptide corresponding to amino acids 567–581 of the predicted Cut protein were described previously (Blochlinger et al. 1988). A fusion protein containing the first 11 amino acids of the T7 *gene 10* protein and amino acids 180–614 of the predicted Cut protein was overexpressed in *E. coli*. The cells were lysed in lysis buffer [0.05 M Tris-Cl (pH 8.0), 0.002 M EDTA, 5% glycerol, 1 mM dithiothreitol, 0.001 M phenylmethylsulfonyl fluoride] at 4°C, sonicated, and centrifuged at 10,000g for 10 min. The supernatant was coupled to Affigel 15 (Bio-Rad), according to manufacturer's recommendations, and subsequently incubated with the *clp2* antiserum. Bound antibodies were eluted with 1.5% glycine (pH 2.3).

F2 A rat polyclonal antiserum was elicited against a gel-purified fusion protein containing the first 11 amino acids of the T7 *gene 10* protein and amino acids 1616–1836 of the predicted Cut protein overexpressed in *E. coli*. For affinity purification, the cells expressing the fusion protein were lysed in lysis buffer at 4°C, sonicated, and centrifuged at 10,000g for 10 min. The pellet was resolubilized in lysis buffer containing 8 M urea and dialyzed against lysis buffer containing 4 M urea, followed by 2 M urea, 1 M urea, and no urea. Finally, the fusion protein was coupled to Affigel 10 (Bio-Rad), according to manufacturer's recommendations, and subsequently incubated with the F2 antiserum. Bound antibodies were eluted with 20 mM glycine (pH 2.3).

Immunocytochemistry

The procedure for whole-mount staining of embryos was described previously (Bodmer et al. 1987). Affinity-purified F2 and *clp2* antibodies were diluted 1 : 100. Purified rabbit β -galactosidase antibodies (Cappel) were diluted 1 : 5000. For β -galactosidase/Cut double-labeling, Texas Red-conjugated α -rabbit (Protos) and biotinylated α -rat IgGs, followed by FITC-conjugated avidin (Vector), were used to label β -galactosidase and Cut, respectively, and the embryos were viewed, analyzed,

and photographed with an MRC-600 confocal imaging system (Bio-Rad) and a Zeiss Axioplan microscope.

Whole-mount *in situ* hybridization

Whole-mount *in situ* hybridization to Oregon-R embryos was carried out with a digoxigenin-labeled probe corresponding to Cut protein-coding sequences [K. Blochlinger, unpubl.] according to a previously published procedures (Tautz and Pfeiffle 1989; Bier et al. 1990).

Western analysis

Homozygous *ywct^{C145}* embryos were selected by the absence of Malpighian tubules and incomplete air-filling of the trachea in late embryos. For all other mutants, protein extracts were made from a mixture of homozygous and heterozygous mutant embryos. Embryos were dechorionated in 50% bleach, washed with 0.7% NaCl and 0.3% Triton X-100 and homogenized in equal volumes of 0.05 M Tris-Cl (pH 8.0), 0.002 M EDTA, 5% glycerol, 0.001 M phenylmethylsulfonyl fluoride, 20 μ M benzamidine HCl, 2 μ g/ml phenanthroline, 20 μ g/ml aprotinin, 20 μ g/ml leupeptin, 20 μ g/ml pepstatin A, and 20 μ g/ml TLCK.

Protein extracts were resolved by electrophoresis through 5% SDS-polyacrylamide gels (Laemmli 1970) and transferred to nitrocellulose in running buffer containing 20% methanol. Filters were blocked by incubation in 10 mM Tris (pH 8.0), 30 mM NaCl, 0.05% Tween-20 (TBST), containing 5% nonfat dry milk, for 30–60 min at 37°C. Incubations with affinity-purified F2 and clp2 antibodies diluted 1 : 200 in TBST were performed for 3–5 hr at 37°C. Washes, incubations with alkaline phosphatase-conjugated secondary antibodies (Promega), and alkaline phosphatase reactions were performed according to manufacturers' recommendations.

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