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Functional Analysis of *Drosophila* and Mammalian

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The *cut* locus acts as a bimodal switch controlling cell fate in the peripheral nervous system of *Drosophila* and is also required for the development of the wing margin. It encodes a protein, Cut, that contains an atypical homeodomain and three copies of a new motif which can bind DNA *in vitro.* The human protein CDP and the murine protein Cux have recently been isolated as DNA-binding activities and they are structurally related to Cut. We show that ectopic expression of Cut, CDP, or Cux similarly affects embryonic sensory organ development and can rescue a wing scalloping mutant phenotype associated with loss of *cut* expression along the prospective wing margins. This suggests that the function of Cut is evolutionarily conserved. © 1996 Academic Press, Inc.

INTRODUCTION

The loss of *cut* activity in *Drosophila* results in a discrete change of cell fate in the peripheral nervous system (PNS) (Bodmer *et al.,* 1987). There are two major types of sensory organs present in the PNS: external sensory (es) organs, which receive chemo- or mechanosensory information, and internal chordotonal (ch) organs, which are responsive to stretch. Embryonic lethal *cut* mutations cause es organs develop morphologically and antigenically as ch organs. The *cut* locus encodes a predicted 2175-amino-acid nuclear protein (Cut) that is expressed in all cells of es organs including their precursors, but not in chordotonal organs (Blochlinger *et al.,* 1988, 1990). Ectopic expression of Cut from a heat shock-inducible transgene causes ch organs to differentiate as es organs (Blochlinger *et al.,* 1991). These results suggest that Cut is acting as a bimodal switch to control sensory organ fate.

cut activity is required in multiple cell types and at all developmental stages (Blochlinger *et al.,* 1990, 1993; Bodmer *et al.,* 1987; Liu *et al.,* 1991; Jack *et al.,* 1991) and loss of cut activity is embryonic lethal (Jack, 1985). The locus was named after a class of regulatory *cut* mutations which are viable (cut wing). The wing margin includes rows of chemosensory and mechanosensory organs along the anterior portion and noninnervated hairs along the posterior portion. Cut wing mutations, which alter the pattern of Cut expression along the prospective wing margin (Jack *et al.,* 1991; Blochlinger *et al.,* 1993), result in scalloping along the entire wing margin as well as a loss of es organs of the anterior wing margin and hairs of the posterior wing margin. Based on this and other results, we speculated that *cut* may have a function in wing margin formation that is distinct from its role in sensory organ differentiation (Blochlinger *et al.,* 1993). We now present our results from ectopic expression studies to rescue the cut wing phenotype.

The Cut protein is unusual because it contains a distinct type of homeodomain and three dispersed copies of a 73-amino-acid sequence (*cut* repeat) with no apparent similarity to previously identified protein motifs (Blochlinger *et al.,* 1990). Several vertebrate proteins have recently been identified that show significant structural similarity to the Cut protein, particularly in the homeodomain, the *cut* repeats, and their arrangement within the protein. A human protein, CDP, was isolated as a DNA-binding activity in myeloid, erythroid, and placental cells (Dufort and Nepveu, 1994; Neufeld *et al.,* 1992; Superti-Furga *et al.,* 1989). Cux is a murine protein isolated from pheochromocytoma cells and is 91% identical in sequence to CDP, apart from a single internal region of divergence (Valarche *et al.,* 1993). Homologous proteins have also been identified from dog (Andres *et al.,* 1992) and rat (Yoon and Chikaraishi, 1994). All of the vertebrate proteins appear to act as regulators of gene expression (such as *gp91-phox,* γ-Globin, c-*myc,* and N-CAM) in transfection assays (for overview, see Lievens *et*

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al., 1995). In addition to the structural similarities between these vertebrate proteins and Cut, Cut appears to bind the same DNA sequence as CDP in band-shift assays (Neufeld *et al.,* 1992).

It has become apparent that vertebrate and invertebrate development use similar regulatory mechanisms. For example, the homeotic gene complexes of *Drosophila* (*HOM-C*) and vertebrates (*Hox*) specify pattern along the anterior–posterior axis of many organisms from nematodes to mammals and encode proteins containing a DNA-binding homedomain. In addition to the remarkable conservation of their genome structure and organization (for review see Kenyon, 1994), several studies have shown that the mouse and human *Hox* genes can carry out some of the functions of their *Drosophila* counterparts in flies (Malicki *et al.,* 1990; McGinnis *et al.,* 1990; Zhao *et al.,* 1993). Morever, it appears that even regulatory elements of *HOM-C/Hox* genes from *Drosophila* and mouse can work in heterologous species (Awgulewitsch and Jacobs, 1992; Malicki *et al.,* 1992; Popperl *et al.,* 1995). Another example of functional conservation of a transcription factor is provided by *Pax-6,* which codes for a protein with a homeodomain and a *paired* DNA-binding domain. This gene is mutated in human aniridia, mouse and rat *small eye,* and *Drosophila eyeless* (reviewed in Hanson and Heyningen, 1995).

In vitro studies indicate that the homeodomain and each of the three *cut* repeats can independently bind to DNA (Andres *et al.,* 1994; Aufiero *et al.,* 1994; Harada *et al.,* 1994; Valarche *et al.,* 1993, Fan and Blochlinger, unpublished). The presence of a divergent homeodomain and the *cut* repeats in Cut, CDP, Clox, and Cux suggest that they define a new family of DNA-binding proteins whose function may also be conserved from flies to mammals. Since regulatory circuitries are more difficult to study in vertebrates, the functional conservation in flies of Cut and the vertebrate Cut-like proteins would provide a model system to characterize the molecular role of this protein family. To test the hypothesis that the function of members of this family is evolutionarily conserved, we established transgenic lines with CDP and Cux coding sequences and examined the effects of expressing these proteins in flies using two assays for *cut* activity, the development of embryonic ch organs and the wing margin.

MATERIALS AND METHODS

Fly Stocks

GAL4 line 1J3 (Brand and Perrimon, 1993) was crossed into *ywct*^{db10}/FM6*y*^{31d} *w++B* (Blochlinger *et al.,* 1990) and into A1-2-29/Cyo (Hartenstein and Posakony, 1990) and maintained homozygous for 1J3.

GAL4 line C96 (kindly provided by G. Boulianne) was crossed into *wct*^{6} and maintained homozygous for both chromosomes.

Cut, Cux, and CDP Transgenic Lines

The full-length coding sequences of Cut (Blochlinger *et al.,* 1991), CDP (Neufeld *et al.,* 1992), and Cux sequences from nucleotides 442–5046 (Valarche *et al.,* 1993) were inserted into pUAST (Brand and Perrimon, 1993) and the resulting constructs were microinjected into *w*^{1118} embryos along with pπ25.7wcΔ2-3 to produce transgenic flies (Spradling, 1986). At least two independent insertion lines for each construct were used in the experiments described.

Immunocytochemistry

The procedure used for whole-mount immunocytochemistry of embryos was previously described (Bodmer *et al.,* 1987). Affinity-purified anti-Cut antibodies (F2, Blochlinger *et al.,* 1990) were diluted 1:300, MAb35D7.1 (kindly provided by K.-F. Fischbach) and MAb22C10 (kindly provided by S. Benzer) were diluted 1:10 and anti-β-galactosidase antibodies (Cappel) were diluted 1:3000. Biotinylated secondary antibodies and avidin-HRP were used according to manufacturor's specifications (Vector Labs).

RESULTS

Ubiquitous ectopic expression of Cut affects a number of tissues and the lethality associated with it presents substantial problems in generating transgenic flies and in the development of functional assays at later stages of development (C. Ludlow and K. Blochlinger, unpublished). To circumvent this problem, we used the two-part GAL4 system (Brand and Perrimon, 1993) to specifically target ectopic Cut expression. In order to assay the effects of GAL4-dependent ectopic expression of Cut, CDP, and Cux on embryonic ch organ and adult wing development, transgenic lines were created containing coding sequences for Cut, CDP, and Cux downstream from GAL4 binding sites (UCut, UCDP, and UCux).

The Embryonic PNS

There are two classes of peripheral neurons with single dendrites, the chordotonal neurons and the external sensory neurons (for review see Jan and Jan, 1993). Each of these neurons is associated with at least three support cells to form a sensory organ. The support cells of es organs produce the cuticular sensory structure (for example, the bristle and socket) and ensheath the dendrite. In ch organs, one of the support cells forms an electron-dense structure termed a scolopale surrounding the tip of the dendrite, and the other two promote the attachment of the organ to the cuticle. The arrangement of es and ch organs (and neurons with multiple dendrites) in abdominal hemisegments A1 to A7 is shown schematically in Fig. 1.

Each sensory organ, with the exception of the five ch organs in the lateral cluster (lch5), appears to form close to the position of its precursor. The precursors for lch5 are born at a dorsal position and their neuronal progeny migrate and rotate to end up in a lateral position with their dendrites pointing dorsally.

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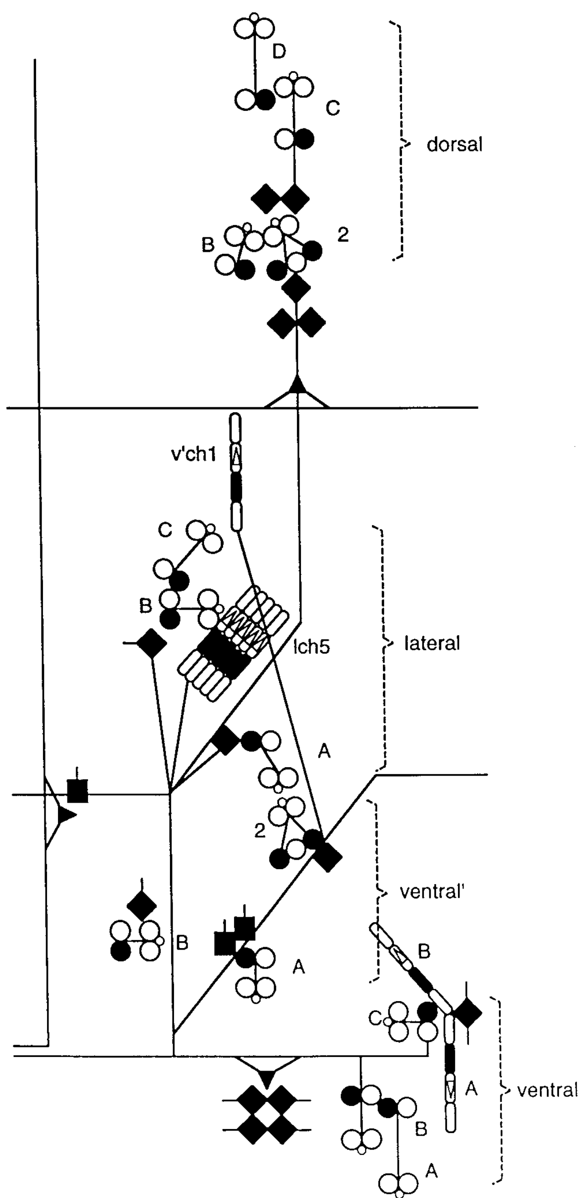

FIG. 1. Schematic representation of the neurons and support cells in the PNS of abdominal hemisegments A1 to A7. Large circle, es organ cell; small circle, dendritic specialization of an es dendrite; oval, ch organ cell; small triangle within oval, dendritic specialization of a ch dendrite (scolopale); diamond, neuron with dentritic arborisations; square, neuron with bipolar dendrites; large triangle, neuron with dendrites that arborize around tracheal branches; lines correspond to axons and dendrites. Nomenclature is according to Bodmer and Jan (1987); Dambly-Chaudiere and Ghysen (1986).

Ectopic Cut Expression Affects ch Organs in Alternate Segments

The enhancer trap line 1J3 appears to be a GAL4 insertion into the *hairy* locus (Brand and Perrimon, 1993). In the embryo, *hairy* is expressed in a pair-rule pattern of seven parasegmental stripes (posterior and adjacent anterior parts of alternate segment primordia, Carroll *et al.,* 1988). We examined the effect of 1J3-mediated Cut expression on the development of embryonic chordotonal organs in the progeny of 1J3 females and UCut males. In our analysis we focused primarily on the development of the lch5 neurons which are probably the only neurons that are located in the posterior compartments of abdominal hemisegments (Hartenstein, 1987). Since *hairy* is expressed in the posterior compartments of odd-numbered abdominal segments the effects of 1J3-induced expression were assayed by comparing the lch5 phenotype in abdominal segments A1, A3, A5, and A7 to control abdominal segments (A2, A4, and A6) within the same embryo.

In wild-type embryos, there are eight chordotonal organs in each abdominal hemisegment, the scolopales of which are labeled by MAb35D7.1 (Fig. 2A). In *cut* mutant embryos, in which es organs develop as ch organs, additional labeling is seen in positions normally occupied by es organs (Fig. 2B). This indicates that the expression of the epitope recognized by MAb35D7.1 correlates with sensory organ identity. Many of the embryos in which Cut is expressed in a 1J3-dependent pattern are morphologically abnormal and arrest development prior to the completion of germ band retraction. However, in those embryos that develop until the end of embryogenesis, loss of MAb35D7.1 labeling is observed in odd-numbered abdominal segments (Fig. 3A), suggesting that ch organs are developing as es organs. Whereas the MAb35D7.1 labeling of ventral ch organs in affected segments is variable, no labeling is typically seen in the position of the lch5. In some odd-numbered segments, labeling is observed in a position dorsal to lch5 (Fig. 3A, arrowhead). The expression of the epitope recognized by MAb35D7.1 is also repressed by 1J3-driven ectopic Cut expression in *cut* mutant embryos (data not shown), albeit not as efficiently.

These data are consistent with our previous observation that ectopic Cut expression regulated by heat shock affects the migration of ch organ neurons (Blochlinger *et al.,* 1991). We verified this by staining 1J3:UCut embryos with an antibody that recognizes all neurons (MAb22C10, Hartenstein, 1988). As expected, a cluster of neurons is found in a more dorsal position posterior to the dorsal cluster of sensory neurons in affected segments (Fig. 3C). In addition to the altered position of these neurons, their morphology resembles that of es neurons rather than ch neurons. Finally, we examined the expression of a *lacZ* enhancer trap line (A1-2-29), which is specifically expressed in two support cells of each es organ, in 1J3:UCut: A1-2-29 embryos. Figure 4A shows ectopic *lacZ* expression in odd-numbered segments in the same position as the transformed lch5 neurons, indicating that at least some of the support cells associated with these neurons have assumed an es support cell fate, as was observed previously after heat shock-regulated ectopic Cut expression (Blochlinger *et al.,* 1991).

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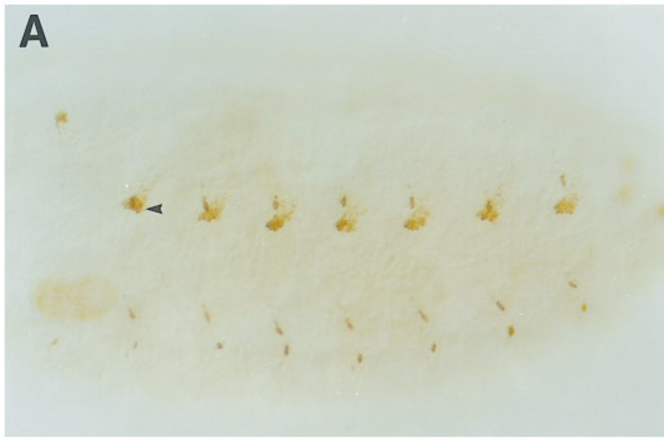
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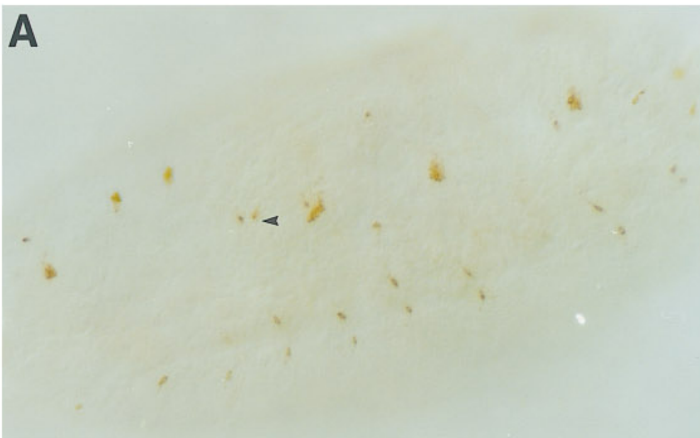
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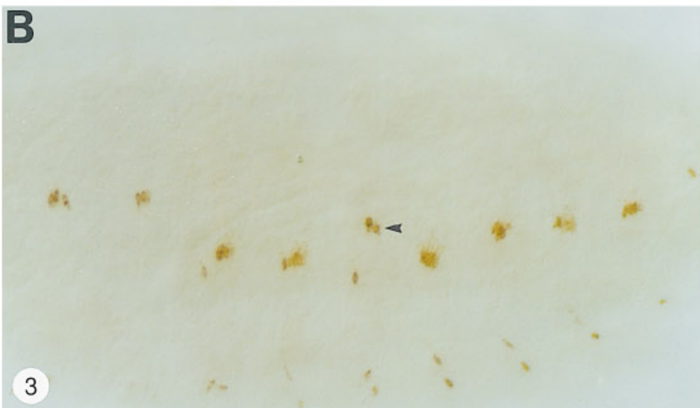
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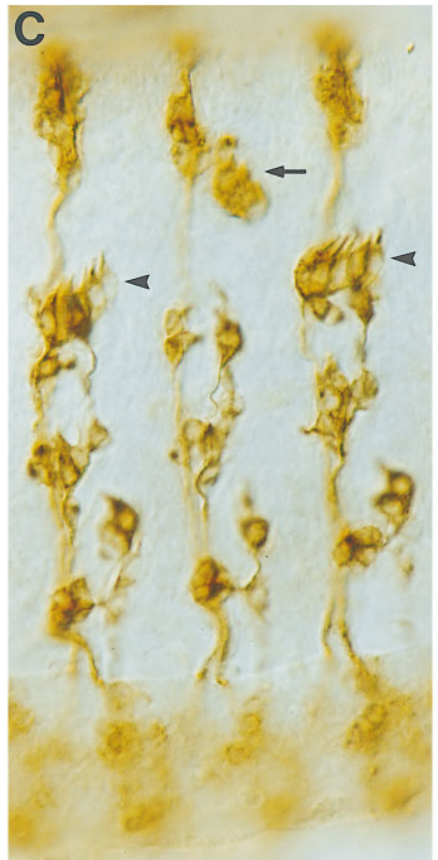
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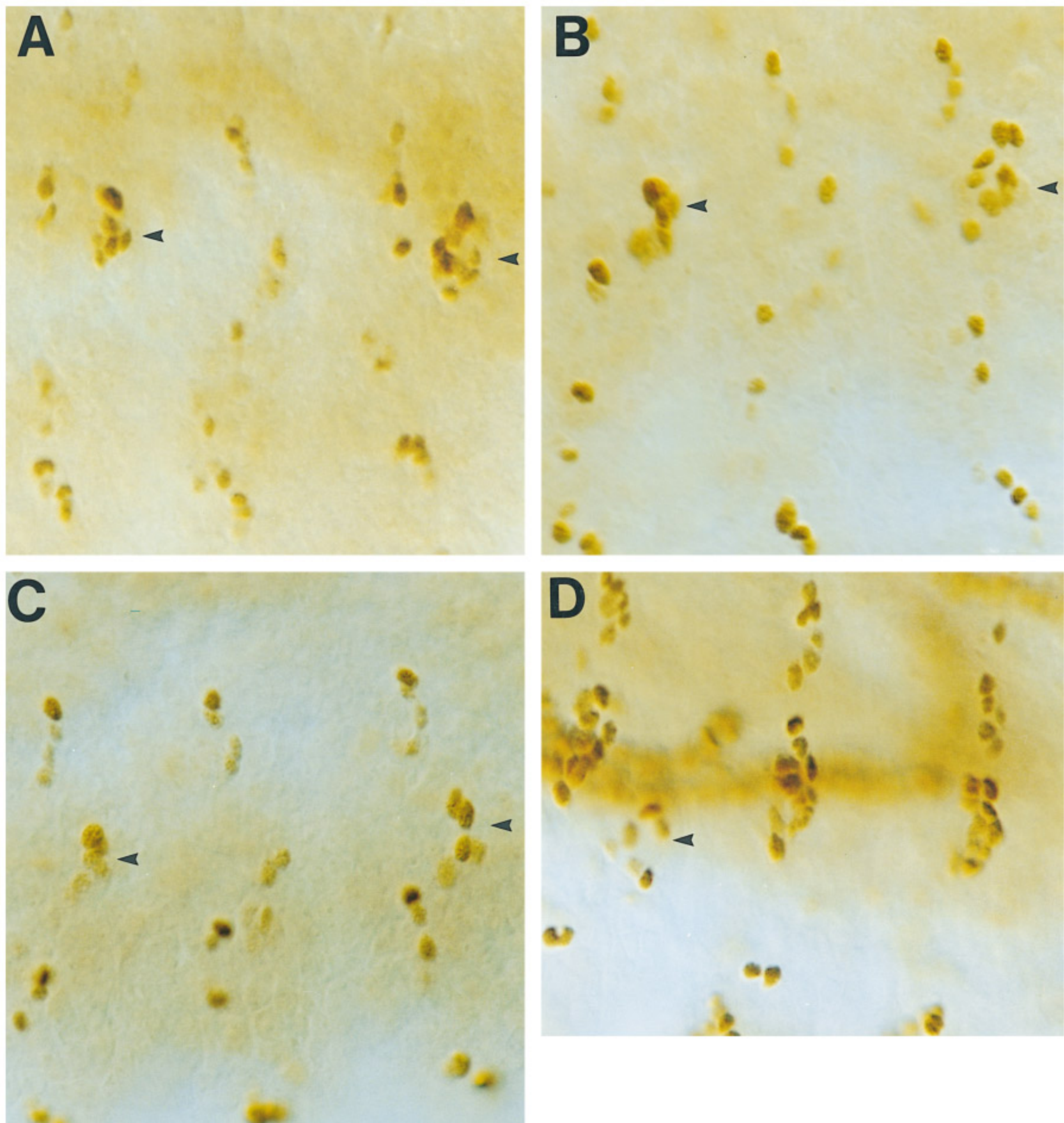

FIG. 4. (A–C) *lacZ* expression in three embryonic abdominal hemisegments in the following genetic backgrounds: (A) A1–2–29/UCut25–9; 1J3/+; (B) A1–2–29/UCux6–1; 1J3/+; and (C) A1–2–29/UCDP13–2; 1J3/+. (D) Cut expression in three abdominal hemisegments of a A1–2–29/UCux6–1; 1J3/+ embryo. Arrowheads in (A–C) point to a cluster of *lacZ* expressing cells at the position of ch organ precursors in the first and third abdominal segments; the arrowhead in (D) points to a cluster of *cut* expressing cells at the position of ch organ precursors in the third abdominal segment. Anterior is to the left, dorsal is up.

FIG. 2. MAb35D7.1 labeling in abdominal hemisegments of (A) a wild-type embryo and (B) a ct^{db10} mutant embryo. Arrowheads point to lch5 of the first abdominal hemisegments. Anterior is to the left, dorsal is up.
FIG. 3. (A and B) MAb35D7.1 labeling in abdominal hemisegments of embryos of the following genetic backgrounds: (A) *w;* UCut19-3/+; 1J3/+ and (B) *w;* UCux5-3/+; 1J3/+. (C) MAb22C10 labeling in three abdominal hemisegments of a *w;* UCut25-9/+; 1J3/+ embryo. The arrowhead in (A) points to the region of lch5 in the first abdominal segment; the arrowhead in (B) points to dorsally positioned lch5 scolopales in the third abdominal hemisegment; arrowheads in (C) point to lch5 in the second and fourth abdominal hemisegment; the arrow in (C) points to a cluster at the position of ch organ precursors. Anterior is to the left, dorsal is up.

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TABLE 1
Effects of Expression of CDP and Cux on ch Organs

Genotype	Hemisegments affected	%	Embryos affected	%
w; Cut25-9/+; 1J3/+	>1000	100%[a]	>100	100%[a]
w; Cut19-3/+; 1J3/+	>1000	100%[a]	>100	100%[a]
w; Cux5-3/+; 1J3/+	161/776	21%	63/90	70%
w; Cux6-1/+; 1J3/+	125/896	14%	52/108	48%
w; CDP13-2/+; 1J3/+	98/1666	6%	62/182	34%
w; CDP3-1; 1J3/+	114/1636	7%	71/198	36%

Note. Column 2 indicates the numbers of odd-numbered abdominal hemisegments affected of the total number examined. Column 4 indicates the number of embryos in which at least one odd-numbered abdominal hemisegment is affected of the total number of embryos examined. Percentages are noted in columns 3 and 5.

[a] Many of the embryos were morphologically abnormal and/or had arrested development prior to the completion of germ-band retraction and couldn't be scored.

Ectopic CDP and Cux Expression Affects ch Organs in Alternate Segments

To determine if CDP or Cux is able to influence larval sensory organ development in *Drosophila* we examined the effects of 1J3-mediated CDP and Cux expression in the progeny of 1J3 females and UCux or UCDP males. In contrast to our results with ectopic Cut expression, the epitope recognized by MAb35D7.1 is still present in most segments; however, the position, arrangement, and/or orientation of lch5 neurons is altered in a significant number of odd-numbered abdominal segments (Fig. 3B). This phenotype was never observed in even-numbered abdominal segments or in control embryos. For one line of UCux flies (UCux5-3), 21% of odd-numbered segments were affected and the lch5 neurons were aberrantly positioned in at least one odd-numbered segment in 70% of the embryos (Table 1). For another line of UCux (UCux6-1) and for two lines of CDP (UCDP13-2, UCDP3-1) these percentages were considerably smaller (Table 1). These results suggest that the ch neuron fate is at least partly changed as a consequence of Cux or CDP expression. We also observed ectopic activation of *lacZ* expression from the es support cell marker A1-2-29 (Figs. 4B and 4C). This was seen more frequently and in a larger number of cells for Cux than for CDP. In UCux5-3; 1J3 embryos that were double-labeled with anti-β-galactosidase and MAb35D7.1 the aberrantly positioned ch neurons were associated with *lacZ* expressing cells in 80% (24/30) of the segments (not shown). Occasionally, ectopic activation of A1-2-29 is observed in cells in close proximity of lch5 neurons which appear to be appropriately positioned in odd-numbered segments. As these groups of cells are not in the vicinity of other sensory organs, this suggests that the neurons and support cells of ch organs may be independently affected by ectopic expression.

Transient ectopic expression of Cut in ch organ precursors results in activation of the endogenous *cut* locus (Blochlinger *et al.,* 1991). 1J3-mediated Cux expression is able to activate endogenous *cut* expression in the position of ch organ cells that have failed to migrate (Fig. 4D). This was observed in at least one odd-numbered segment in 59% (33/56) of UCux5-3; 1J3 embryos and 19% (8/42) of UCux6-1; 1J3 embryos. Typically, activation of endogenous *cut* expression was seen in only 1 or 2 cells within the cluster of lch5 cells, whereas 15–20 *cut* expressing cells are often observed after ectopic expression of Cut (Blochlinger *et al.,* 1991). 1J3-induced CDP expression did not result in detectable activation of the endogenous *cut* locus. This is consistent with the fact that the ch organ phenotype caused by CDP expression has the same severity and penetrance in *cut* mutant embryos (not shown).

The Wing Margin

There are three types of es organs along the anterior portion of the wing margin: recurved chemosensory bristles and slender and stout mechanosensory bristles (Figs. 5A and 5B). Along the posterior wing margin there are noninnervated hairs. The sensory bristles of the anterior wing margin are arranged in ventral and dorsal rows, with each row having a characteristic number and type of bristle components.

The precursors of the chemosensory bristles appear along the prospective wing margin in the wing imaginal disc and divide around pupariation, and the precursors for the mechanosensory bristles divide about 10 hr later.

Expression of Cut, Cux, and CDP in ct^6 Mutants Rescues the Wing Phenotype

Viable *cut* wing mutants, such as ct^6, have scalloped wing margins (Fig. 5C) and in addition lack many of the sensory bristles of the anterior wing margin: the numbers of chemosensory and slender and stout mechanosensory bristles are reduced to 74, 3, and 23% of wild-type, respectively (Fig. 5D). In addition, all noninnervated hairs of the posterior wing margin are absent. In wild-type imaginal discs of third instar larvae *cut* is expressed in a band four to five cells

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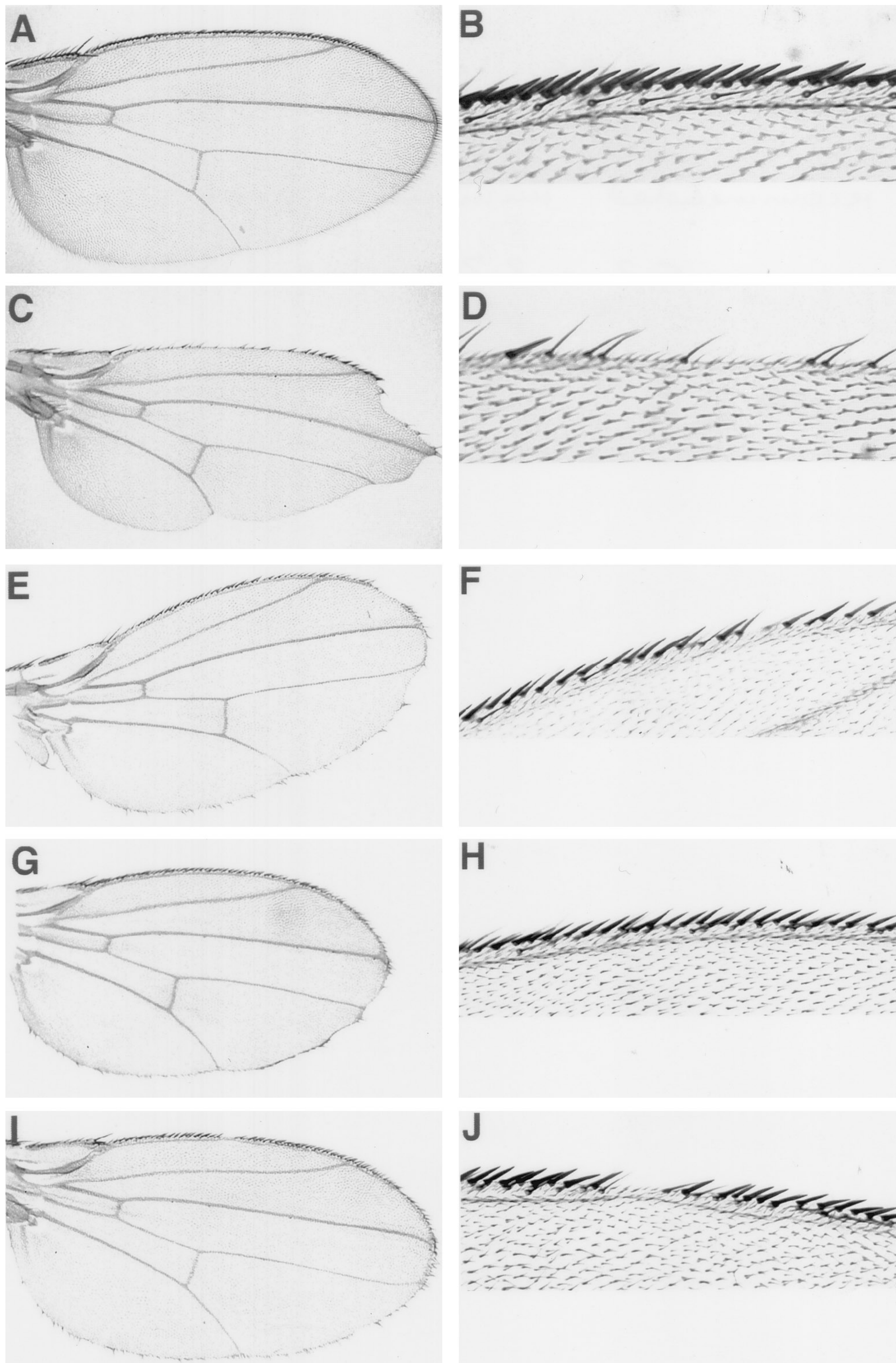

FIG. 5. Whole wings and anterior part of wing margin of flies with the following genetic constitution: (A and B) *w;* C96/+; (C and D) *wct*^{6}; C96/+; (E and F) *wct*^{6}; UCut25–9/+; C96/+; (G and H) *wct*^{6}; UCDP3–1/+; C96/+; (I and J) *wct*^{6}; UCux6–1/+; C96/+. The chemosensory bristles are recessed from the wing margin and are most clearly visible in B.

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TABLE 2
Effects on Wing Marginal Bristle Number of Ectopic Expression of Cut, CDP, and Cux in ct^6 Flies

Genotype	Recurved bristles		Slender bristles		Stout bristles	
	24°C	17°C	24°C	17°C	24°C	17°C
w; C96	42.8 (42–44)		95.3 (93–98)		78.3 (76–80)	
+	100%		100%		100%	
wct^6; C96	31.4 (23–36)		2.6 (0–6)		17.9 (10–25)	
+	74%		3%		23%	
wct^6; UCut25-9; C96	19.4 (11–25)	27.5 (26–30)	41.9 (26–64)	80.5 (76–85)	35.9 (26–56)	65.75 (55–70)
+ +	46%	64%	44%	85%	46%	84%
wct^6; UCut19-3; C96	no progeny	25.75 (22–31)	no progeny	72.75 (60–84)	no progeny	50.5 (37–58)
+ +		60%		76%		64%
wct^6; UCDP3-1; C96	25.5 (22–29)	29.3 (24–32)	54.8 (48–61)	79.7 (65–90)	58.75 (51–68)	60.7 (53–74)
+ +	60%	69%	57%	84%	75%	77%
wct^6; UCDP13-2; C96	6.5 (4–9)	22.5 (21–24)	17.5 (14–21)	33 (32–34)	13.5 (8–19)	44.5 (41–48)
+ +	15%	53%	18%	35%	17%	57%
wct^6; UCux6-1; C96	33.3 (32–36)	37 (34–40)	79.7 (76–86)	97 (96–98)	60.7 (59–62)	70.5 (69–72)
+ +	78%	86%	84%	102%	77%	90%
wct^6; UCux5-3; C96	12.25 (10–13)	29 (25–31)	17.5 (17–20)	50 (38–65)	9.75 (8–11)	58.25 (54–63)
+ +	29%	68%	18%	52%	12%	74%

Note. Average number of bristles obtained at 24° and 17°C is shown, with the variation in bristle number in parentheses. Percentage numbers were obtained by comparing the numbers of bristle to control wings (*w*; C96/+).

wide along the entire prospective wing margin. Shortly after pupariation, *cut* expression is seen in the precursors of the chemosensory bristles, which are slightly recessed from the wing margin. The band of *cut* expression spanning the prospective wing margin is absent in ct^6 mutant discs; however, *cut* expression in the chemosensory precursors is not affected (Blochlinger *et al.,* 1993).

C96 is an enhancer trap line in which GAL4 is expressed along the prospective wing margin in third instar discs (G. Boulianne, personal communication). To test if we could rescue the ct^6 mutant phenotype by C96-mediated expression of Cut, Cux, or CDP we introduced the C96 chromosome into a ct^6 background and examined the progeny of ct^6; C96 females and UCut, UCDP, and UCux males.

Most of the UCut lines tested in combination with C96 did not produce viable progeny at 24°C, possibly due to the fact that GAL4 is also expressed in the larval brain of C96 flies (G. Boulianne, personal communication). However, some survivors were obtained at 17°C, consistent with the reported cold sensitivity of the GAL4 protein in flies (Brand *et al.,* 1994). We therefore measured the effects of ectopic expression at two different temperatures, 17° and 24°C, using two independent transgenic lines for each construct (Table 2).

In ct^6; UCut25-9 flies there is a substantial reduction of scalloping along both the anterior and posterior wing margin at 24°C (Fig. 5E), such that the shape of the wing appears almost wild-type. Moreover, the numbers of slender and stout mechanosensory bristles are increased by 16- and 2-fold, respectively (Fig. 5F, Table 2). The noninnervated hairs of the posterior wing margin also are restored; however, there is a decrease, relative to ct^6, in the number of chemosensory bristles from 74% of wild-type to 46%. A greater extent of rescue of wing marginal tissue and sensory bristles was observed at 17°C (Table 2). As the GAL4 protein is a less potent transcriptional activator at 17°C, this result suggests that higher levels of Cut expression are inhibitory to wing margin and bristle development (dominant negative effect). Consistent with this, loss of bristles, wing scalloping, and other phenotypes (see below) are also observed after C96-driven Cut expression in wild-type flies (data not shown). Another UCut transgenic line, ct^6; UCut19-3, in combination with C96 produced a slightly reduced level of rescue at 17°C compared to UCut25-9, and no survivors were found at 24°C (Table 2).

C96-induced expression of CDP and Cux also ameliorates the ct^6 mutant phenotype (Figs. 5G–5J, Table 2). In fact, the scalloping appears to be rescued equally by ectopic expression of Cut, CDP, or Cux (compare Figs. 5E, 5G, and 5I). The extent of bristle rescue varies in different lines containing the same transgene and is always higher at 17°C than at 24°C for all the lines tested (Fig. 5F, 5H, and 5J, Table 2). Compared to ct^6; UCut25-9, similar, if not better, rescue is observed in ct^6; UCux6-1 and ct^6; UCDP3-1 flies. However, in ct^6; UCux5-3 and ct^6; UCDP13-2 flies the number of bristles restored is significantly lower, especially at 24°C.

Other phenotypes associated with C96-induced Cut, CDP, or Cux expression include disorganized bristle rows, curled or folded wing margins, wing vein deltas or bifurcations, and turbid or incompletely expanded wings (not shown). Also, morphological defects of wing marginal bris-

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tles are sometimes observed, such as intermediate between slender and stout, and stout bristles that are reduced in size (not shown).

DISCUSSION

The studies described here were designed to examine if expression of Cut coding sequences predicted from embryonic cDNAs could rescue wing margin development in a cut wing mutant and to test the functional equivalence of two vertebrate proteins with considerable sequence similarity to Cut.

Expression of Cut, CDP, or Cux Perturbs ch Organ Development

We show here that ectopic expression of CDP or Cux can induce antigenic and morphological properties of es organs in ch organs, however, to a lesser extent and at a markedly reduced frequency compared to Cut.

Our analysis has focused on a group of five ch organs in the lateral region (lch5) because these are most frequently affected by 1J3-mediated ectopic expression (see below). The precursors for lch5 are located in a more dorsal region (Ghysen and O'Kane, 1989). During development lch5 cells migrate to a lateral position and rotate to assume their final orientation. Ectopic expression of Cut, as well as CDP and Cux, can perturb the migration of lch5 organs. This phenotype is not dependent on endogenous *cut* activity because aberrant lch5 position is observed in a *cut* null mutant background after ectopic expression of Cut (Blochlinger *et al.,* 1991) or CDP and Cux (not shown). Three mutants, *u-turn, lola,* and *dorsotonals,* have recently been described that show similar deviations in the position of lch5 (Giniger *et al.,* 1994; Salzberg *et al.,* 1994). *u-turn* additionally alters the polarity of ch organs (Salzberg *et al.,* 1994). It is possible that Cut, CDP, and Cux exert their effect on ch organ movement by interacting with such genes.

Cut, CDP, and Cux are also able to activate the expression of the es organ-specific enhancer trap line A1-2-29, which expresses *lacZ* in two of the support cells of each es organ. Cut and Cux can additionally cause the ectopic activation of the *cut* locus in affected ch organs. This is often observed after ectopic expression of Cut, less frequently and in fewer cells following Cux expression, and it has not been seen after expression of CDP.

Cut is able to negatively regulate the expression of the ch-specific epitope recognized by MAb35D7.1. Although significant immunoreactivity is still present in ch organs after ectopic CDP and Cux expression, loss of expression of this antigen is sometimes observed using one of the UCux lines (UCux5-3). The onset of labeling with MAb35D7.1 is a relatively late event and it is possible that 1J3-induced proteins are no longer present in ch organs at this time. 1J3 embryos still possess GAL4 activity after germ-band retraction, but we have failed to find GAL4 activity in differentiated ch organs (data not shown). We have shown previously that the maintenance of es organ identity is contingent on the continued presence of *cut* activity (Blochlinger *et al.,* 1991). This is achieved after transient expression of Cut in ch organs cells because of autoregulation; however, activation of the *cut* locus by Cux is not as efficient and it is not clear if CDP possesses this activity at all. Repression of the Mab35D7.1 epitope by C96-driven Cut expression is not as strong in *cut* mutant embryos (data not shown), suggesting that autoregulation plays a role in this repression. Therefore, it is conceivable that persistent expression of CDP or Cux in ch organ cells could result in a complete absence of MAb35D7.1 labeling.

There is considerable variability in the number of segments affected by 1J3-mediated expression of CDP, Cux, and, to a lesser extent, Cut. Abnormal position of lch5 organs and ectopic expression of A1-2-29 occurs most frequently in the third abdominal segment, which is most readily explained by variations between the stripes of GAL4 expression (previously documented for *hairy,* Carroll *et al.,* 1988) and uneven GAL4 activity in cells within a stripe (data not shown). Another source of variability might be the levels of expression or the stability of the vertebrate proteins. We have noted differences in the percentage of affected segments using different UCux transgenic lines, which suggests that the level of expression of the transgenes depends on the site of insertion.

Expression of Cut, CDP, or Cux Rescues cut Mutant Wing Phenotype

cut wing mutations, such as ct^6, affect the expression of *cut* along the developing wing margin in the imaginal disc (Jack *et al.,* 1991; Blochlinger *et al.,* 1993). In ct^6 mutant discs, the stripe of Cut labeling that straddles the entire wing margin is completely absent in third instar discs, leading to a severely scalloped margin and to a loss of most mechanosensory and some chemosensory bristles. The noninnervated hairs on the posterior wing margin are also absent. We have shown here that restoring Cut expression along the wing margin using the GAL4 enhancer trap line C96 can substantially correct the ct^6 mutant phenotype. Moreover, a similar degree of rescue is obtained by C96-mediated CDP and Cux expression.

The GAL4 protein is cold-sensitive in flies (Brand *et al.,* 1994) and we observed that the extent of rescue varied with the temperature at which the flies were raised. All the lines tested provided a better restoration of wing morphology and bristle number at lower temperatures. This suggests that high levels of transgene expression are inhibitory to bristle and margin development. The amount of rescue obtained correlates better with the level of transgene expression than with the specific protein expressed. For example, using one Cux transgenic line (UCux6-1) in this assay restores the number of wing marginal bristles to almost wild-type numbers at 17°C, whereas a reduction (compared to ct^6) of chemosensory and stout mechanosensory bristles is observed

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in a second line (UCux5-3) at 24°C. In addition, there is usually a reduction in the number of chemosensory bristles after C96-mediated expression of Cut, CDP, and Cux in ct^6 flies, especially at 24°C, although the numbers of mechanosensory bristles are substantially increased. As the precursors and progeny of the chemosensory bristles still express *cut* in ct^6 mutant wing discs (Blochlinger *et al.,* 1993), chemosensory organ cells presumably express a mixture of endogenous *cut* protein and Cut, CDP, or Cux which would result in overall elevated levels of Cut-related proteins. We have also observed dominant effects on wing development after expressing Cut, CDP, or Cux in wild-type flies, such as the loss of bristles and scalloping of the wing margin producing a phenotype resembling loss of *cut* activity (not shown). This indicates that overexpression of Cut-like proteins may interfere with the activity of both the ectopically expressed proteins and the endogenous Cut proteins. Self-inhibition at high concentrations has previously been observed for the transcription factors GAL4-VP16 (Kelleher *et al.,* 1990) and AP-2 (Kannan *et al.,* 1994). Experiments to determine if the bristle loss is caused by cell death, alterations in cell fate, or some other mechanism are in progress.

Functional Similarity between Cut, CDP, and Cux

In the embryonic assay, all three proteins affect the development of ch organs but their effectiveness differs: Cut has the strongest effects and CDP has the weakest. Are these differences in potency qualitative or quantitative? The fact that Cut, CDP, and Cux are all equally capable of rescuing the ct^6 mutant phenotype may indicate a qualitative difference between the fly and vertebrate proteins in embryonic sensory development. It is possible that the differences reside in the ability to efficiently activate the endogenous *cut* locus and thus maintain persistent Cut expression. This may be more important in the embryonic assay than the wing assay if the GAL4 line 1J3 induces expression for a relatively shorter time period than C96. At this point, however, we cannot exclude that the weaker activities of the vertebrate proteins in the embryonic assay represent quantitative differences. For example, it is possible that 1J3 induces a lower level of expression than C96 and that elevating the levels of CDP and Cux expression during embryogenesis would increase their effects on ch organ development. Differential stability of the proteins may also contribute to the differences observed. Experiments to address these possibilities are in progress.

Cut, Cux, and CDP are members of a new family of DNA-binding proteins, characterized by the presence of a distinctive homeodomain and three *cut* repeats. The molecular mechanism of action of Cut is not known, but the vertebrate and sea urchin proteins have been shown to repress or activate transcription of putative target genes in tissue culture (see Lievens *et al.,* 1995). Cut is both necessary and sufficient to specify es organ cell fate in the PNS (Blochlinger *et al.,* 1991). Since CDP and Cux are able to partially convert ch organs to es organs and both proteins have activities equivalent to Cut in the wing margin assay, we speculate that the Cut family of proteins function analogously to regulate transcription during development.

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REFERENCES

Andres, V., Chiara, M. D., and Mahdavi, V. (1994). A new bipartite DNA-binding domain: Cooperative interaction between the *cut* repeat and the homeodomain of the *cut* homeo proteins. *Genes Dev.* 8, 245–257.

Andres, V., Nadal-Ginard, B., and Mahdavi, V. (1992). Clox, a mammalian homeobox gene related to *Drosophila cut,* encodes DNA-binding proteins differentially expressed during development. *Development* 116, 321–334.

Aufiero, B., Neufeld, E. J., and Orkin, S. H. (1994). Sequence-specific DNA binding of individual cut repeats of the human CCAAT displacement/cut homeodomain protein. *Proc. Natl. Acad. Sci. USA* 91, 7757–7761.

Awgulewitsch, A., and Jacobs, D. (1992). *Deformed* autoregulatory element from *Drosophila* functions in a conserved manner in transgenic mice. *Nature* 358, 341–344.

Blochlinger, K., Bodmer, R., Jack, J., Jan, L. Y., and Jan, Y. N. (1988). Primary structure and expression of a product from *cut,* a locus involved in specifying sensory organ identity in *Drosophila. Nature* 333, 629–635.

Blochlinger, K., Bodmer, R., Jan, L. Y., and Jan, Y. N. (1990). Patterns of expression of Cut, a protein required for external sensory organ development, in wild-type and *cut* mutant *Drosophila* embryos. *Genes Dev.* 4, 1322–1331.

Blochlinger, K., Jan, L. Y., and Jan, Y. N. (1993). Postembryonic patterns of expression of *cut,* a locus regulating sensory organ identity in *Drosophila. Development* 117, 441–450.

Blochlinger, K., Jan, L. Y., and Jan, Y. N. (1991). Transformation of sensory organ identity by ectopic expression of Cut in *Drosophila. Genes Dev.* 5, 1124–1135.

Bodmer, R., Barbel, S., Shepherd, S., Jack, J. W., Jan, L. Y., and Jan, Y. N. (1987). Transformation of sensory organs by mutations of the *cut* locus of *D. melanogaster. Cell* 51, 293–307.

Brand, A., Manoukian, A. S., and Perrimon, N. (1994). Ectopic expression in *Drosophila. Methods Cell Biol.* 44, 635–654.

Brand, A., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118, 401–415.

Dambly-Chaudiere, C., and Ghysen, A. (1986). The sense organs in

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the *Drosophila* larva and their relation to the embryonic pattern of sensory neurons. *Roux's Arch. Dev. Biol.* 195, 222–228.
Dufort, D., and Nepveu, A. (1994). The human Cut homeodomain protein represses transcription from the *c-myc* promoter. *Mol. Cell. Biol.* 14, 4251–4257.
Ghysen, A., and O'Kane, C. (1989). Neural enhancer-like elements as specific cell markers in *Drosophila. Development* 105, 35–52.
Giniger, E., Tietje, K., Jan, L. Y., and Jan, Y. N. (1994). *lola* encodes a putative transcription factor required for axon growth and guidance in *Drosophila. Development* 120, 1385–1398.
Harada, R., Dufort, D., Denis-Laroses, C., and Nepveu, A. (1994). Conserved *cut* repeats in the human *cut* homeodomain protein function as DNA binding domains. *J. Biol. Chem.* 269, 2062–2067.
Hartenstein, V. (1988). Development of *Drosophila* larval sensory organs: spatiotemporal pattern of sensory neurones, peripheral pathways and sensilla differentiation. *Development* 102, 869–886.
Hartenstein, V. (1987). The influence of segmental compartmentalization on the development of the larval peripheral nervous system in *Drosophila melanogaster. Roux's Arch. Dev. Biol.* 196, 101–112.
Hartenstein, V., and Posakony, J. W. (1990). Sensillum development in the absence of cell division: the sensillum phenotype of the *Drosophila* mutant string. *Dev. Biol.* 138, 147–158.
Jack, J. W. (1985). Molecular organization of the *cut* locus of *Drosophila melanogaster. Cell* 42, 869–876.
Jack, J., Dorsett, D., Delotto, Y. and Liu S. (1991). Expression of the *cut* locus in the *Drosophila* wing margin is required for cell type specification and is regulated by a distand enhancer. *Development* 113, 735–747.
Jan, Y. N., and Jan, L. Y. (1993). The peripheral nervous system. *In* "The Development of *Drosophila melanogaster,*" (M. Bate and A. Martinez-Arias, Eds.), pp. 1207–1244, Cold Spring Harbor Laboratory Press Cold Spring Harbor, NY.
Kannan, P., Buettner, R., Chiao, P. J., Yim, S. O., Sarkiss, M., and Tainsky, M. A. (1994). N-ras oncogene causes AP-2 transcriptional self-interference, which leads to transformation. *Genes Dev.* 8, 1258–1269.
Kelleher, R. J. I., Flanagan, P. M., and Kornberg, R. D. (1990). A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. *Cell* 61, 1209–1215.
Kenyon, C. (1994). If birds can fly, why can't we? Homeotic genes and evolution. *Cell* 78, 175–180.
Liu, S., McLeod, E., and Jack, J. (1991). Four distinct regulatory regions of the *cut* locus and their effect on cell type specification in *Drosophila. Genetics* 127, 151–159.
Lievens, P. M. J., Donady, J. J., Tufarelli, C., and Neufeld, E. J. (1995). Repressor activity of CCAAT displacement protein in HL-60 myeloid leukemia cells. *J. Biol. Chem.* 270, 12745–12750.
Malicki, J., Cianetti, L. C., Peschle, C., and McGinnis, W. (1992). A human *HOX4B* regulatory element provides head-specific expression in *Drosophila* embryos. *Nature* 358, 345–347.
Malicki, J., Schughart, K., and McGinnis, W. (1990). Mouse *Hox-2.2* specifies thoracic segmental identity in *Drosophila* embryos and larvae. *Cell* 63, 961–967.
McGinnis, N., Kuziora, M. A., and McGinnis, W. (1990). Human *Hox-4.2* and *Drosophila Deformed* encode similar regulatory specificities in *Drosophila* embryos and larvae. *Cell* 63, 969–976.
Neufeld, E. J., Skalnik, D. G., Lievens, P. M.-J., and Orkin, S. H. (1992). Human CCAAT displacement protein is homologous to the *Drosophila* homeoprotein, cut. *Nature Genet.* 1, 50–55.
Popperl, H., Bienz, M., Studer, M., Chan, A.-K., Aparicio, S., Brenner, S., Mann, R. S., and Krumlauf, R. (1995). Segmental expression of *Hoxb-1* is controlled by a highly conserved autoregulatory loop dependent upon *exd/pbx. Cell* 81, 1031–1042.
Salzberg, A., D'Evelyn, D., Schulze, K. L., Lee, J.-K., Strumpf, D., Tsai, L., and Bellen, H. J. (1994). Mutations affecting the pattern of the PNS in *Drosophila* reveal novel aspects of neuronal development. *Neuron* 13, 269–287.
Superti-Furga, G., Barberis, A., and Schaffner, G., Busslinger, M. (1989). The protein CDP, but not CP1, footprints on the CCAAT region of the g-globulin gene in unfractionated B-cell extracts. *Biochim. Biophys. Acta* 1007, 237–242.
Valarche, I., Tissier-Seya, J.-P., Hirsch, M.-R., Martinez, S., Goridis, C., and Brunet, J.-F. (1993). The mouse homeodomain protein Phox2 regulates *Ncam* promoter activity in concert with Cux/CDP and is a putative determinant of neurotransmitter phenotype. *Development* 119, 881–896.
Yoon, S. O., and Chikaraishi, D. M. (1994). Isolation of two E-box binding factors that interact with the rat tyrosine hydroxylase enhancer. *J. Biol. Chem.* 269, 18453–18462.
Zhao, J. J., Lazzarini, R. A., and Pick, L. (1993). The mouse *Hox-1.3* gene is functionally equivalent to the *Drosophila Sex combs reduced gene. Genes Dev.* 7, 343–354.

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