Effect of Wing Scalloping Mutations on *cut* Expression and Sense Organ Differentiation in the Drosophila Wing Margin

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ABSTRACT

A number of wing scalloping mutations have been examined to determine their effects on the mutant phenotype of *cut* mutations and on the expression of the Cut protein. The mutations fall into two broad classes, those which interact synergistically with weak *cut wing* mutations to produce a more extreme wing phenotype than either mutation alone and those that have a simple additive effect with weak *cut wing* mutations. The synergistically interacting mutations are alleles of the *Notch*, *Serrate* and *scalloped* genes. These mutations affect development of the wing margin in a manner similar to the *cut wing* mutations. The mutations inactivate the *cut* transcriptional enhancer for the wing margin mechanoreceptors and noninnervated bristles and prevent differentiation of the organs. Surprisingly, reduction of *Notch* activity in the wing margin does not have the effect of converting epidermal cells to a neural fate as it does in other tissues of ectodermal origin. Rather, it prevents the differentiation of the wing margin mechanoreceptors and noninnervated bristles.

THE Drosophila wing margin is composed entirely of sense organs and noninnervated bristles. Wild-type wings have a triple row of mechanosensory organs and chemosensory organs on the anterior margin and a double row on the tip. The posterior margin is populated by noninnervated bristles. The *cut wing* mutations of the *cut* locus are a group of tissue specific mutations that inactivate an enhancer (cut wing enhancer) that drives *cut* transcription specifically in the mechanoreceptors and noninnervated bristles of the wing margin but has no effect on expression in other external sense organs, including the chemoreceptors of the margin. The absence of *cut* expression in the wing margin mechanoreceptors and noninnervated bristles prevents their differentiation into sensory cells. Within 3–4 hr of the time that the cells would normally express neural antigens, they die, causing the margin of the wing to erode (JACK *et al.* 1991). This produces a characteristic scalloping of the adult wing margin.

The product of the *cut* locus is a homeobox protein that is present in the nuclei of all external sensory organs as well as a number of other tissues (BLOCHLINGER *et al.* 1988). In this regard the mechanoreceptors of the wing margin are like all other external sensory organs. However, the epigenetic program that governs the development of the wing margin mechanoreceptors differs from that of other external sensory organs. The organs outside the wing margin require activity of the *achaete* (*ac*) and *scute* (*sc*) genes for development. In ac^- sc^- mutants, these organs do not differentiate (GARCIA-BELLIDO 1979; GARCIA-BELLIDO and SANTA MARIA 1978; GHYSEN and DAMBLY-CHAUDIERE 1988). In the nonmargin sense organs of the wings, ac^- sc^- flies do not express *cut* (JACK *et al.* 1991). So, *achaete* and *scute* are apparently upstream of *cut* in the developmental regulatory pathway leading to external sensory organ development. These sensory organs are transformed by ct^- mutations into internal stretch receptors called chordotonal organs (BODMER *et al.* 1987). By contrast, the wing margin mechanoreceptors do not require the activity of *achaete* and *scute* (GARCIA-BELLIDO and SANTA MARIA 1978), and the loss of *cut* does not alter the type of the sense organs but rather prevents their development altogether, producing a cut wing phenotype.

Mutations of other genes involved in the determination and differentiation of the wing margin mechanoreceptors and noninnervated bristles may also cause gaps in the wing margin, enhance the phenotype of weak *cut wing* mutations, or both. The analysis of *Notch*, one gene known to regulate neural *vs* epidermal development, reveals a function in the wing margin different from what has been observed in other parts of the ectoderm. The *Notch* locus controls the determination of external sensory organs (HARTENSTEIN and POSAKONY 1990), and loss of function mutations dominantly cause nicking of the wing margin (LINDSLEY and ZIMM 1990). *Notch* is one of a group of genes, called neurogenic genes, whose loss of function mutations cause neural hypertrophy (LEHMANN *et al.* 1981). In adult development, *Notch* is instrumental in controlling both the entry of cells into sensillum development and the fates of the four cells that compose

the sense organ (HARTENSTEIN and POSAKONY 1990). In most sense organs in both larvae and adults, the neuron or neurons and accessory cells are derived from a single sensory mother cell by a series of cell divisions (BATE 1978; HARTENSTEIN 1988). For typical external sensory organs, three accessory cells and a neuron differentiate. Two of the accessory cells, the trichogen and tormogen, secrete the bristle and socket, respectively, of the external cuticular part of the organ, and the third accessory cell, the thecogen, secretes a cap over the dendrite near the base of the bristle shaft. On the adult notum, loss of N^+ function causes the cuticular structure of the sensory organs to be missing (DIETRICH and CAMPOS-ORTEGA 1984; SHELLENBARGER and MOHLER 1978) because all four progeny of the sensory organ mother cells differentiate into neurons (HARTENSTEIN and POSAKONY 1990). Most bristles on the wing margin are also absent when *Notch* function is lost (HARTENSTEIN and POSAKONY 1990), and reduction of Notch in heterozygous null mutants causes gaps in the double bristle row of the wing tip. The gaps in heterozygotes could reasonably be expected to be due to transformation of bristle forming cells to neurons. However, this is not the case. Rather, the wing margin gaps in *Notch* heterozygotes result from the failure of margin cells to differentiate into sense organs at all, much like the phenotype observed in *cut wing* mutants. Thus, the reduction of *Notch* activity in the ectoderm can have a nonneurogenic effect, suggesting that *Notch* has another function in the ectoderm in addition to being required for many cells to take an epidermal rather than neural fate.

Mutations of a number of other genes cause loss of all or parts of the wing margin. Some of these mutations could also be involved in the cell type specification of the sense organs on the wing margin. We report the examination of phenotypic interactions with weak *cut wing* alleles, the altered wing development, and the effects on *cut* activity of mutations of *Notch* and a number of other genes that cause tissue loss from the wing margin. On a phenotypic level, mutations that were examined interact either additively or synergistically with *cut*. Those that interact synergistically were found to decrease *cut* expression in the margin mechanoreceptors and noninnervated bristles. That effect alone would be sufficient to explain the adult wing scalloping of the mutations.

MATERIALS AND METHODS

Drosophila stocks used: The *cut* mutations (1–20.0) used are described in JACK (1985). The ct^{46l} mutation is an insertion of a B104 element near the cut wing enhancer, and ct^{53d} is a 0.5-kb deletion that probably partially deletes the enhancer (JACK *et al.* 1991; JACK 1985). The *Notch* (1–3.0) mutations, $N^{264\text{-}40}$ and *nd*, are described in LINDSLEY and ZIMM (1990). The mutation *Ser* (3–92.5) is described in LINDSLEY and ZIMM (1990) with further information available in FLEMING *et al.* (1991). The *sd* and sd^2 stocks were obtained from the Mid-America Stock Center at Bowling Green and are described in LINDSLEY and ZIMM (1990) with other information in CAMPBELL *et al.* (1991). The mutations Ly (3–40.5), Bx^J (1–59.4), and $T(2;3)ap^{Xa}$ = T(2;3)41F;89E8-F1 are described in LINDSLEY and ZIMM (1990). The stocks carrying the *Ser*, *Ly*, $T(2;3)ap^{Xa}$ and Bx^J alleles were obtained from the Bloomington, Indiana, Stock Center.

Stocks used for analysis of Cut protein expression in mutants: Analysis of *cut* expression in pupal wings was done by dissecting wings at 7 hr after pupariation (AP) for staining with an anti-Cut antibody and at 20 hr AP for staining with mAb 22C10. For analysis of heterozygous *Notch*, pupae from the cross $y\ w\ N^{264\text{-}40}\ rb/FM7 \times FM7/Y$ were used. For analysis of homozygous *Ser*, pupae were obtained from the stock, *Ser/TM3, Ser*. Homozygous *scalloped* pupae were from the stock sd^1/sd^1.

Crosses to analyze the effect of mutations on the wing margin expression of ctwHZ-2 β-galactosidase expression: *ctwHZ-2/CyO* is a strain which carries the construct ctwHZ integrated into the second chromosome. ctwHZ is a derivative of HZ50 (HIROMI and GEHRING 1987), which has a restriction fragment containing the cut wing enhancer inserted upstream of the *hsp70-lacZ* fusion gene present in HZ50 (JACK *et al.* 1991). For the analysis of *X*-linked mutations, crosses were made as follows, using *nd* as an example: $nd/nd;\ +/+\ X\ nd^+/Y;\ ctwHZ\text{-}2/CyO$. From this cross the $nd/Y;\ ctwHZ\text{-}2/+$ males were mated to *nd/nd* females. All of the resulting progeny are homozygous or hemizygous for *nd*, and half carry the *ctwHZ-2* chromosome.

To test *ctwHZ-2* in a *Ser/+* background the cross *Ser/TM3, Ser* × *ctwHZ-2/CyO* was made. All progeny are *Ser/+* and half carry *ctwHZ-2*.

Immunohistochemistry: Staining with antibodies was as described in (JACK *et al.* 1991). The anti-Cut antibody was clp2 (BLOCHLINGER *et al.* 1988) and the anti-β-galactosidase antibody was a polyclonal antibody obtained from Cappel. Monoclonal antibody 22C10 was kindly furnished by DENNIS BALLINGER.

RESULTS

Wing margin mutations that interact synergistically with *cut*: The strongest *cut wing* mutations remove tissue from the entire wing margin resulting in the loss of approximately 16% of the total wing (SANTAMARIA and GARCIA-BELLIDO 1975) (Figure 1, A and B). The $ct^{L\text{-}32}$ mutation is typical of the strong *cut wing* mutations and is the strong allele used in the experiments reported here. The loss of tissue is caused by the lack of *cut* expression in the mechanoreceptors and noninnervated bristles of the wing margin and the resulting failure of those cells to differentiate followed by their death (JACK *et al.* 1991). However, a small number of *cut wing* mutations have a weak phenotype, and only a limited amount of the margin is lost in mutants of these alleles. Two of these weak mutations are ct^{46l} and ct^{53d} (Figure 1, C and D). Flies homozygous for ct^{46l} are typically missing a small amount of tissue at the tip of the wing, often only a few bristles. Flies homozygous for ct^{53d} are missing more tissue from the wing tip and often some tissue from the posterior margin, but the anterior margin is

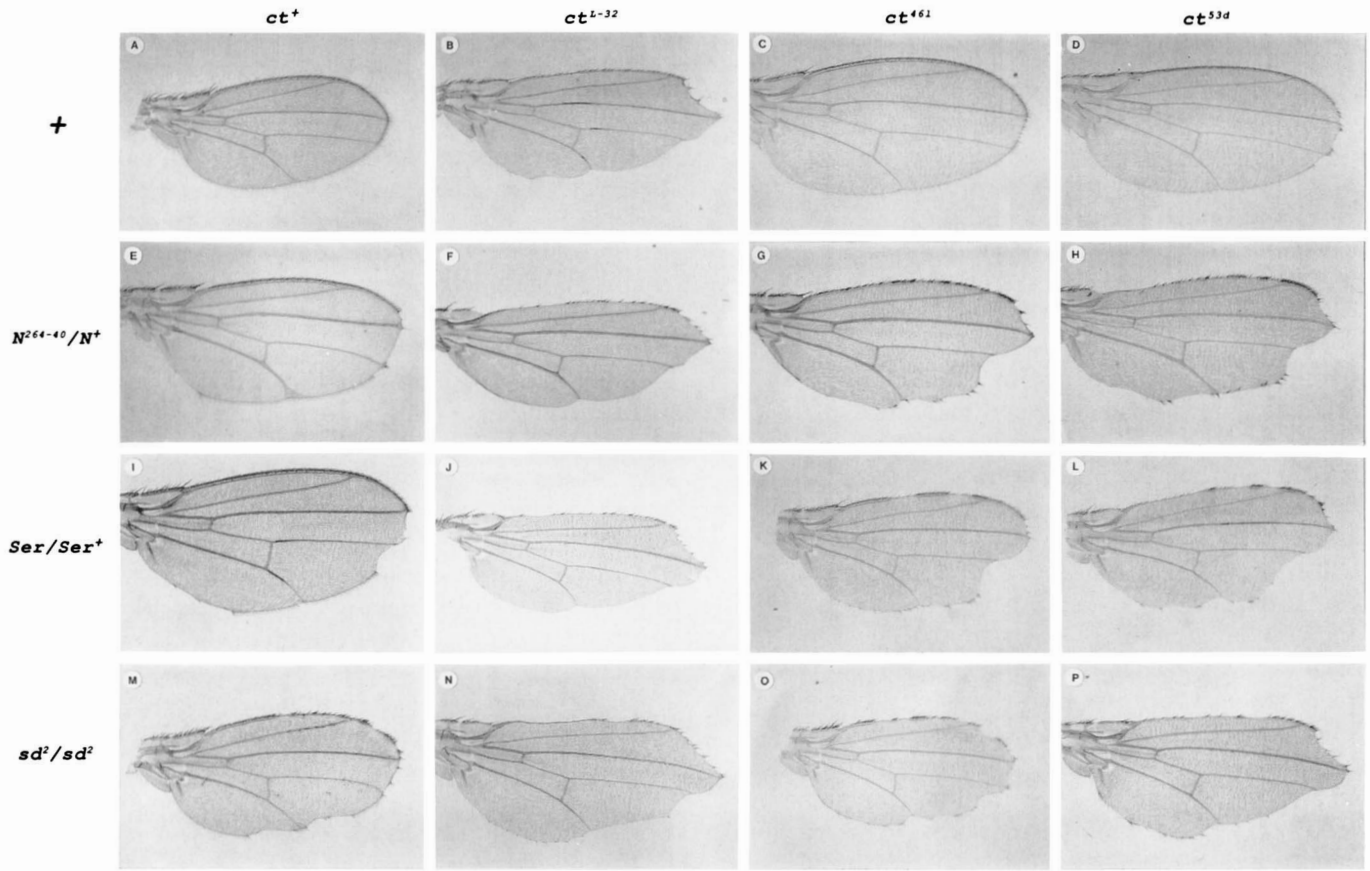

FIGURE 1.—Phenotypic interactions between weak *cut* alleles and mutations of *Notch*, *Serrate* and *scalloped*. The gene designations at the top show the *cut* genotype of the wings in the column below, and the designations at the left of the figure show the additional mutant phenotypes of the wings in the row to the right. The exact genotypes are: (A) Wild-type wing, (B) $ct^{L\text{-}32}/ct^{L\text{-}32}$ wing, (C) $ct^{46l}/ct^{L\text{-}32}$wing and (D) $ct^{53d}/ct^{L\text{-}32}$ wing. The heterozygotes of the weak *cut wing* mutations ct^{46l} and ct^{53d} with the strong *cut wing* mutation $ct^{L\text{-}32}$ are nearly identical to the homozygotes or hemizygotes of the weak mutations. (E-P) Adult wings of genotypes: (E) $N^{264\text{-}40}/N^+$, (F) $N^{264\text{-}40}$ $ct^{L\text{-}32}/N^+$ $ct^{L\text{-}32}$, (G) $N^{264\text{-}40}$ ct^{46l}/N^+ ct^{46l}, (H) $N^{264\text{-}40}$ ct^{53d}/N^+ ct^{53d}, (I) *Ser/Ser*$^+$, (J) $ct^{L\text{-}32}/Y$; *Ser/Ser*$^+$, (K) ct^{46l}/Y; *Ser/Ser*$^+$(L) ct^{53d}/Y; *Ser/Ser*$^+$, (M) sd^2/sd^2, (N) $ct^{L\text{-}32}$ $sd^2/ct^{L\text{-}32}$ sd^2, (O) ct^{46l} sd^2/ct^{46l} sd^2 and (P) ct^{53d} sd^2/ct^{53d} sd^2.

usually intact. Heterozygotes of weak cut wing mutations with strong ones, such as $ct^{L\text{-}32}$, have the phenotype typical of the weak allele. Mutations at a number of other genes either enhance these weak cut wing phenotypes or have a strong synergistic effect.

Loss of function Notch alleles have strong synergistic effects with weak cut alleles: Notch (*N*) null or loss of function mutations, typified by $N^{264\text{-}40}$, recessively cause a hypertrophy of the nervous system and dominantly cause gaps in the tip of the wing margin (LINDSLEY and ZIMM 1990) (Figure 1E). The interaction of heterozygous $N^{264\text{-}40}$ with *cut* is typical of other null alleles. While neither heterozygous $N^{264\text{-}40}$ nor homozygous ct^{46l} affect either the anterior or posterior portion of the wing margin, double mutant females have gaps in their wings around the entire margin (Figure 1G). The $N^{264\text{-}40}$ allele has a similar effect with ct^{53d} (Figure 1H). The loss of tissue around the entire wing margin indicates that both the weak *cut* alleles and the heterozygous *N* mutations reduce expression of their genes along the entire margin even though the phenotypic effect of either mutation alone is only observed at the tip. However, the phenotype of a strong *cut wing* mutation is not made more extreme by a heterozygous *Notch* mutation, indicating that the dominant *Notch* effect is limited to cells that are already missing in strong *cut wing* mutants.

Interaction of cut with Serrate: The dominant *Ser* mutation causes a gap in the posterior wing tip that includes the margin and a small portion of the blade (Figure 1I). *Ser/Ser* flies have a much more extreme but variable phenotype. Some *Ser/Ser* wings have deep incisions of the wing involving considerably more tissue than the wing margin, while others have a phenotype that is identical to the extreme cut wing phenotype, involving the margin almost exclusively (Figure 2G). Double mutants, heterozygous for *Ser* and hemizygous for either ct^{53d} or ct^{46l}, were examined to determine whether the *Ser* mutation enhances the phenotypes of the weak *cut wing* mutations. The double mutants have much more extreme phenotypes, suffering loss of tissue that is not missing in either of

the single mutants (Figure 1, K and L). The gap at the wing tip is larger than the single mutants, and tissue is lost on both the anterior and posterior margins, whereas none of the three mutants alone cause loss other than at the wing tip.

The loss of wing blade tissue in *Ser/Ser* demonstrates that more cells than just the bristles of the margin are affected by the *Ser* mutations. However, the enhancement of the weak *cut wing* phenotypes is probably caused by the combined effects of the *cut* and *Serrate* mutations on the mechanoreceptors and noninnervated bristles of the margin because even in the most extreme *cut wing* mutants only the wing margin mechanoreceptors and noninnervated bristles are affected. Furthermore, heterozygous *Ser* has no effect in $ct^{L\text{-}32}$ mutants (Figure 1, B and J), which already lack those organs.

Interaction of cut with scalloped: Mutations of the gene *scalloped* (*sd*) can remove a large portion of the wing, but a number of mutations affect only the margin (SIMPSON, LAWRENCE and MASCHAT 1981). The sd^2 allele, which produces gaps in the bristle rows along most of the margin but does not cause loss of other wing tissue, was tested in combination with ct^{46l} and ct^{53d} to examine their interaction. Double homozygotes of ct^{46l} and sd^2 have a somewhat more extreme phenotype than the sd^2 homozygotes, but the ct^{53d} sd^2 double homozygotes have a phenotype that is identical to the most extreme *cut wing* phenotype (Figure 1, M to P). This is considerably more extreme than either of the two alleles alone and is typical of the phenotype that is produced by the death in $ct^{L\text{-}32}$ mutants of all the mechanoreceptors and noninnervated bristles on the wing margin (JACK *et al.* 1991) (Figure 1B). sd^2, unlike *Ser* and $N^{264\text{-}40}$, enhances the wing margin phenotype of $ct^{L\text{-}32}$ (Figure 1N), indicating that sd^2 affects other cells in addition to the wing margin mechanoreceptors and noninnervated bristles that $ct^{L\text{-}32}$ affects.

scute mutations enhance the phenotype of strong cut wing mutations: Mutations of *achaete* and *scute* together block development of most external sensory organs, including wing margin chemoreceptors, but they do not affect the wing margin mechanoreceptors or noninnervated bristles (CAMPUZANO *et al.* 1985; GARCIA-BELLIDO and SANTA MARIA 1978; LEYNS, DAMBLY-CHAUDIERE and GHYSEN 1989; VILLARES and CABRERA 1987). Because strong *cut wing* mutations prevent development of the mechanoreceptors without affecting the chemoreceptors, *scute* mutations may cause more margin loss in even the most extreme *cut wing* mutations by removing the chemoreceptors in addition to the mechanoreceptors, which are already missing. Indeed, the combination of the weak *scute* allele sc^1, which alone causes the loss of about ⅓ to ½ of the wing margin chemoreceptors, with a strong *cut wing* allele produces an extreme wing phenotype lacking almost all chemoreceptors and mechanoreceptors. The tissue loss in the double mutants is restricted to the wing margin but is considerably more extreme than the strong cut wing phenotype alone, with much of the anterior wing vein missing. The enhancement of the $ct^{L\text{-}32}$ wing nicking phenotype would not necessarily be expected since even the loss of all the wing margin chemoreceptors caused by $sc^{10}\text{-}^1$, a strong mutation of both achaete and scute (CAMPUZANO *et al.* 1985; JACK *et al.* 1991; VILLARES and CABRERA 1987), does not itself cause wing nicking.

Wing margin mutations that interact additively with *cut*: Other mutations that cause loss of part of the wing were tested and found to have additive effects as double mutants with weak *cut wing* mutations. The mutation ap^{Xa} is a dominant mutation that causes the loss of a large portion of the distal half of the wing. A large incision into the blade is typical of this mutation, and the margin of the remaining wing is left intact. ap^{Xa} removes the tip of the wing, which is the tissue that is affected by ct^{46l} and ct^{53d}. The phenotype of ct^{46l}/Y; $ap^{Xa}/+$ is the same as ap^{Xa} alone. The same is true of the double mutant combinations with ct^{53d}. The phenotype of the flies homozygous for ct^{53d} and heterozygous for ap^{Xa} is the same as the heterozygous ap^{Xa} alone.

The interaction of dominant mutant alleles of the genes *Beadex* (*Bx*) and *Lyra* (*Ly*) with weak *cut wing* alleles is also additive. Bx^J causes tissue loss from the entire wing margin and blistering of the wing blade. Flies homozygous or hemizygous for ct^{53d} Bx^J have the same phenotype as flies homozygous or hemizygous for Bx^J alone. *Ly* causes loss of tissue from the anterior and posterior margins of the wing, but does not affect the tip. Two long strips of cells along the anterior and posterior edges of the wing are lost. These strips of cells include both the margin bristles and the adjacent cells of the wing blade. In double mutants of *Ly* and weak *cut wing* alleles, no part of the margin is lost that is not lost in one or the other mutant alone. Males of the genotype *cm* ct^{53d}/Y; Ly/Ly^+ suffer loss of anterior and posterior margin typical of *Ly* and loss of margin at the tip typical of ct^{53d}. Therefore, the *Beadex* and *Lyra* genes affect some of the same cells as *cut*, but they apparently affect a different process in wing development, resulting in loss of particular regions of the wing disc without regard to cell type. This is in contrast to *cut wing* mutations, which affect specific cell types on the wing margin.

Mutations with synergistic interactions block *cut* expression and sense organ differentiation in the wing margin: Because the wing margin mutants have phenotypes similar to *cut wing* mutants and some of the mutations have strong synergistic effects on *cut* mutants, the effect of the mutations on wild type *cut* expression in the wing margin was investigated. An

anti-Cut antibody clp 2 (BLOCHLINGER *et al.* 1988) was used to detect wing expression of Cut protein in pupal wings at 7 hr after pupariation (AP). At this time, the sensory mother cells have begun to divide (HARTENSTEIN and POSAKONY 1989), and *cut* is expressed in the developing mechanoreceptors, chemoreceptors and noninnervated bristles of the wing margin (JACK *et al.* 1991). At this stage, the wing has everted, and neural antigens are normally expressed on the margin in the chemoreceptor precursors but not in mechanoreceptors or noninnervated bristles. Morphological differentiation has not begun in any of the sense organs (HARTENSTEIN and POSAKONY 1989).

The monoclonal antibody 22C10 was used to examine the effect of the mutations on the differentiation of the sense organs and uninnervated bristles of the wing margin at 20 hr AP. 22C10 is a marker for the differentiation of neurons of the peripheral nervous system and other cells of sense organs (CANAL and FERRUS 1986; HARTENSTEIN 1988; ZIPURSKY *et al.* 1984). It is normally expressed in all cells of the mechanoreceptors and noninnervated bristles beginning at around 16–20 hr AP in wild type (HARTENSTEIN and POSAKONY 1989). In *cut wing* mutants, 22C10 fails to be expressed in the wing margin mechanoreceptors and noninnervated bristles at 20 hr AP, indicating their failure to differentiate (JACK *et al.* 1991).

Heterozygous Notch mutations block cut expression in wing margin mechanoreceptors at the wing tip: The effect of heterozygous *Notch* mutations on *cut* expression is very similar to the effect of weak *cut wing* mutations. A strong *Notch* mutation dominantly blocks *cut* expression in the mechanoreceptors at the tip of the wing, where the loss of margin tissue is observed in adults. Cut protein expression in the chemoreceptors in the same region is unaltered by the mutation. In wild-type 7-hr wings, a stripe of *cut* expression three cells wide is continuous around the entire margin (Figure 2B). These cells are the precursors of the wing margin mechanoreceptors anteriorly and of the noninnervated bristles posteriorly. On the anterior margin, the stripe is flanked by clusters of cells that are the precursors of the margin chemoreceptors (JACK *et al.* 1991) (Figure 2B). The 7-hr wings of *Notch* heterozygotes are similar to wild type except that at the tip of the wing, a gap in the margin stripe occurs where the cells that would normally form mechanoreceptors fail to express *cut* (Figure 2E). Along the anterior and posterior margin, the intensity of the staining in the margin stripe of mechanoreceptor and noninnervated bristle precursors is reduced relative to the staining of the chemoreceptor precursors. The shape of the wing appears normal at this time, and the flanking clusters of cells, the chemoreceptor precursors, express *cut* at levels comparable to wild type.

At 20 hr AP the cells of both mechanoreceptors and chemoreceptors of normal wings express the neural specific antigen 22C10 (HARTENSTEIN and POSAKONY 1989) (Figure 2C). However, the presumptive mechanoreceptors at the tip of heterozygous *Notch* wings fail to express the antigen even though the shape of the wing is normal (Figure 2F). Thus, at 20 hr AP the cell death that leads to the Notch phenotype has not begun, but the cells have not differentiated into sense organs. The staining of the mechanoreceptors and noninnervated bristles on the anterior and posterior margins appears normal. This phenotype is very similar to the effect of *cut wing* mutations, which also cause both the absence of 22C10 expression in the wing margin mechanoreceptors and the death of those cells soon after their normal time of differentiation. Thus, the loss of *cut* expression alone could account for the notching of the wing tip in *Notch* heterozygotes.

Ser blocks cut expression and prevents differentiation of wing margin mechanoreceptors and noninnervated bristles: Like *Notch*, the mutation *Ser* prevents *cut* expression in the mechanoreceptors and noninnervated bristles of the wing margin without affecting the chemoreceptors. The phenotype of *Ser/Ser*$^{+}$ wings at 7 hr AP is more extreme than the dominant *Notch* phenotype, lacking Cut expression in a larger portion of the margin. The tip and posterior margin are still the primary areas affected (not shown). At the same time in development, homozygous *Ser* wings lack Cut expression in almost all of the presumptive mechanoreceptors and noninnervated bristles, without affecting the chemoreceptors (Figure 2H). This is identical to expression in wings of the extreme *cut wing* mutant *ct*$^{L\text{-}32}$. At this stage the shape of the wing is normal. In some wings a few cells in the margin express Cut protein, albeit at reduced levels. At 20 hr AP the *Ser/Ser* wing margin mechanoreceptors and noninnervated bristles fail to express the neural antigen 22C10 (Figure 2I), as is the case in *cut wing* mutations. However, the shape of the wing is still normal, and the chemoreceptors on the margin express 22C10 as in wild type.

By these measures the phenotype of the wing up to 20 hr AP is identical to that of the extreme *cut wing* mutations. Both mutations block the differentiation of the wing margin mechanoreceptors and noninnervated bristles. The failure of *cut* to be expressed in the margin bristles could account for the extreme cut wing-like phenotype exhibited by many *Ser/Ser* flies. However, *Ser/Ser* wings often lack a large portion of the wing blade that is unaffected by *cut wing* mutations (FLEMING *et al.* 1991; THOMAS, SPEICHER and KNUST 1991). Therefore, *Serrate* must function in wing cells outside the margin, and the pattern of expression of Serrate protein in the wing disc bears this out

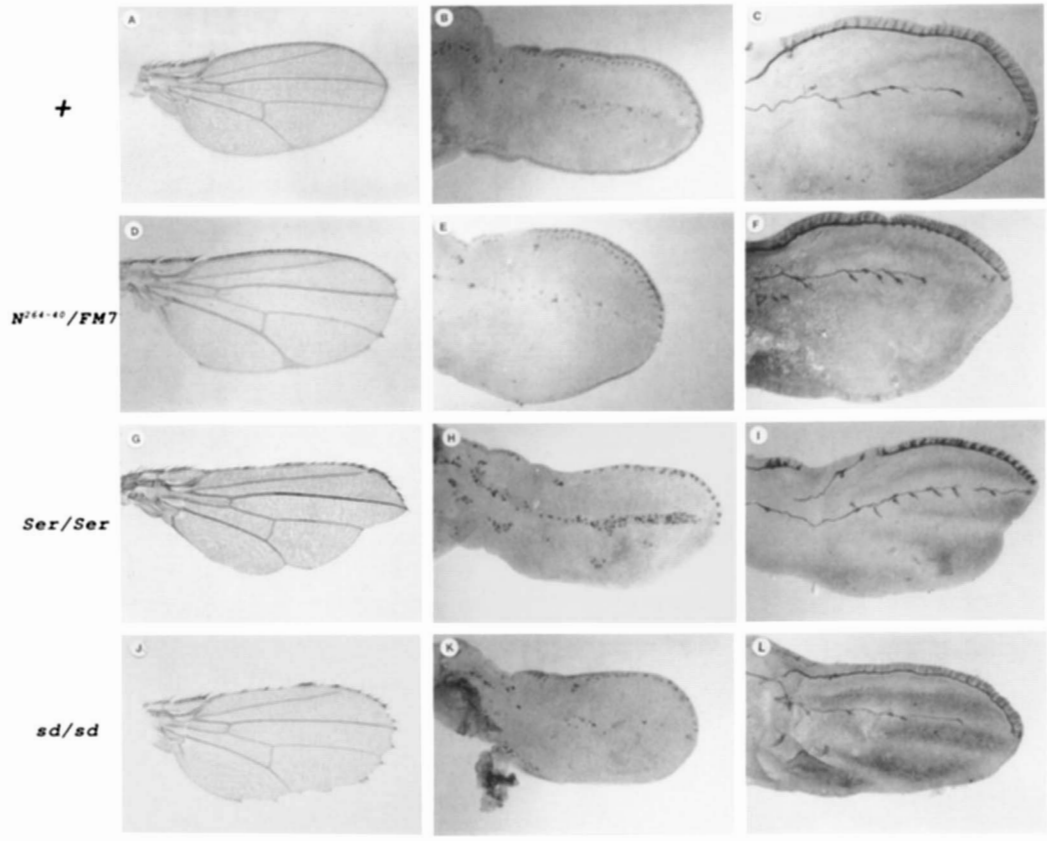

FIGURE 2.—Effect of *Notch*, *Serrate* and *scalloped* mutations of Cut protein expression and differentiation of the mechanoreceptors and noninnervated bristles of the wing margin. (A, D, G, J) Adult wings; (B, E, H, K) 7-hr AP pupal wings stained with the anti-Cut polyclonal antibody clp2; and (C, F, I, L) 20-hr AP pupal wings stained with monoclonal antibody 22C10. Genotypes are (A, B, C) wild type; (D, E, F) *y w* $N^{264\text{-}40}$ *rb/FM7*, N^{+}; (G, H, I) *Ser/TM3*, *Ser* and (J, K, L) *sd/sd*. E shows a gap in Cut expression in the mechanoreceptors, and F shows the gap in antigen 22C10 expression. H and I show a few cells that continue to express Cut (H) and antigen 22C10 (I) in *Ser/TM3*, *Ser* wings.

(THOMAS, SPEICHER and KNUST 1991). In addition to being expressed in the location of the wing margin mechanoreceptors and noninnervated bristles, the protein is expressed in a stripe that corresponds roughly to the L3 wing vein, on which the campaniform sensillae are located.

scalloped mutations block cut expression and differentiation of all wing margin bristles: Some mutations of *scalloped* cause gaps specifically in the wing margin. However, the *scalloped* mutations differ from the margin gapping mutations of *cut*, *Notch* and *Serrate* by decreasing *cut* expression in the chemoreceptors as well as the mechanoreceptors and noninnervated bristles. Mutations of *scalloped* vary widely in the extent of their mutant phenotype. The most extreme mutations are lethal (CAMPBELL *et al.* 1991), while viable mutations range in severity from the loss of most of the wing to loss of only parts of the wing margin (SIMPSON, LAWRENCE and MASCHAT 1981).

Cut protein expression was examined in flies homozygous for sd^1, the effects of which are limited to the wing margin. In adult wings of sd^1/sd^1 mutants, tissue is lost around most of the margin, although the most proximal part of the posterior margin is usually intact (Figure 2J). Wings of 7-hr sd^1/sd^1 pupae have reduced *cut* expression in all of the sense organs and noninnervated bristles of the margin (Figure 2K). At 20 hr AP the wing margins have not begun to decay. However, the number of margin cells expressing 22C10 is reduced, and the organs that fail to stain are of all three types (Figure 2L). The wing blade is not noticeably affected by sd^1. The wing margin is apparently the tissue that is the most sensitive to a reduction in the level of *scalloped* activity since it is affected even when no other phenotype is detectable.

Wing scalloping mutations that interact additively with *cut* do not preferentially affect sense organs: Cut protein expression was examined in flies heterozygous for the mutations *Ly*, Bx^J and ap^{Xa}. These mutations cause the loss of wing tissue which is not restricted to the margin, and as described above, they interact additively with *cut wing* mutations. In *Ly* heterozygotes the adult wings are missing tissue along the anterior and posterior margins including part of the blade in addition to margin sense organs (Figure 3A). Consistent with the reported loss of the tissue in imaginal discs as early as third instar (ABBOTT and SPREY 1990), Cut protein in *Ly*/+ at 0 hr AP is not

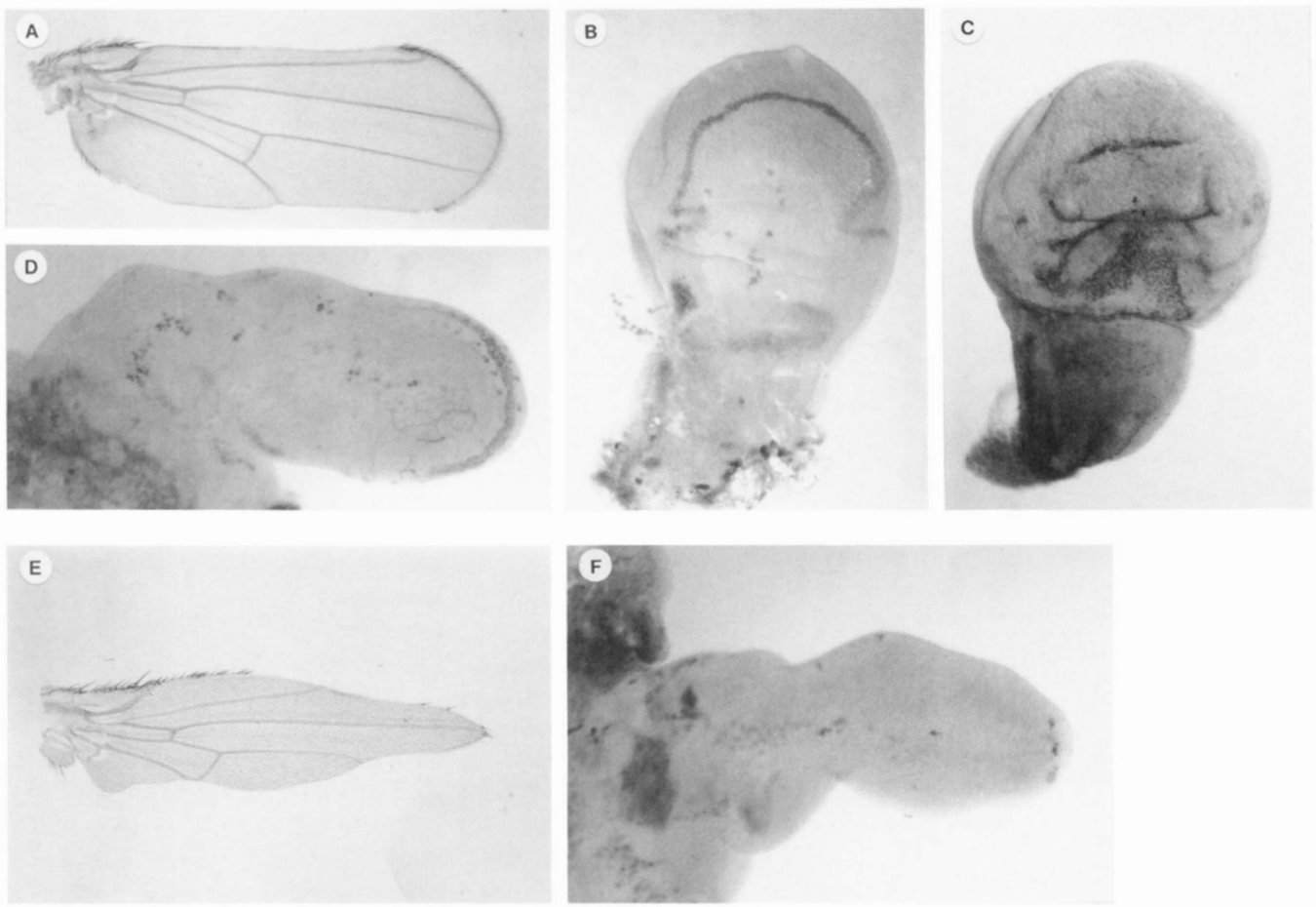

FIGURE 3.—Alterations in morphology and *cut* expression in *Ly* and Bx^{J} mutant wings. (A) *Ly* adult, (B) wild-type disc 0 hr AP, (C) *Ly* disc 0 hr AP, (D) *Ly* wing 7 hr AP, (E) Bx^{J} adult wing, (F) Bx^{J} wing 7 hr AP. The 0- and 7-hr pupal wings are stained with the anti-Cut antibody clp2.

expressed in the presumptive anterior and posterior margins (Figure 3C). At 7 hr AP *Ly*/+ wings are more narrow than wild type, and they lack *cut* expression entirely on the anterior and posterior margins (Figure 3D). The shape of the 7-hr AP *Ly* wing is very similar to *Ly* adult wings, and the 0-hr AP discs are smaller than normal and misshapen (ABBOTT and SPREY 1990) and (Figure 3C). Therefore, the lack of Cut expression on the anterior and posterior margins apparently reflects the fact that the anterior and posterior parts of the wing are absent even in 0-hr prepupae. Cut protein expression at the tip of the wing appears to be normal at 0 and 7 hr AP. Thus, *Ly* affects the formation of particular areas of the wing but does not have a general effect on the sensory elements of the margin. Cut expression is simply absent where the cells that would normally express it are absent, consistent with the additive interaction of *cut* and *Lyra* mutations.

Adult Bx^{J} wings display gaps in the entire wing margin and blisters in the wing blade (Figure 3E). At 7 hr AP most Cut expression in the margin is absent in mechanoreceptor, noninnervated bristle, as well as chemoreceptor precursors (Figure 3F). However, in the Cut expressing cells that remain, the level of expression appears normal. The simplest explanation for these observations and the additive interaction of Bx^{J} with weak *cut wing* mutations is that Bx^{J} affects the development of the wing margins and possibly the veins without specifically blocking the differentiation of the sense organs or noninnervated bristles. This is similar to the effect of *Ly* except that the effect is not restricted to the anterior and posterior margins.

The mutation ap^{Xa} removes a large part of the distal portion of the wing (Figure 4A). Prepupal wings heterozygous for the dominant mutation ap^{Xa} were examined for *cut* expression at 7 hr AP. As in adults, approximately the distal half of the 7-hr pupal wing is missing. However, *cut* expression is normal on the anterior and posterior margin (Figure 4B). The distal edge of the mutant wings has no *cut* expression, as if the distal half of normal 7-hr wings has been cut off. This is consistent with the truncated appearance of the wing at this stage. The normal level of *cut* expression in the remaining part of the margin is consistent with the observation that ap^{Xa} does not enhance the adult phenotype of weak *cut wing* mutants in the

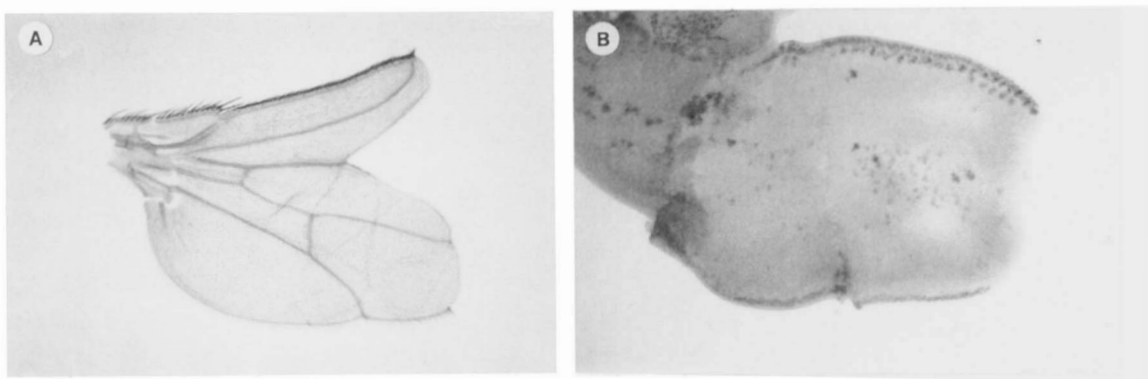

FIGURE 4.—Effect of ap^{Xa} on the expression of *cut* in the developing wing. (A) ap^{Xa} adult wing, (B) ap^{Xa} pupal wing 7 hr AP.

anterior and posterior margins. The cells of the distal wing die in early to mid third instar in ap^{Xa} larvae (FRISTROM 1969). Thus, the loss of cells earlier in third instar prevents the expression of *cut* in ap^{Xa} mutants. The genes are necessary earlier in development for the formation of a large portion of the wing, but they do not have specific effects on the development of the sense organs on the margin.

Mutations of *Notch*, *Serrate* and *scalloped* block the activity of the *cut* wing margin enhancer: An enhancer, positioned 80 kb upstream of the probable start of *cut* transcription, drives *cut* transcription in the mechanoreceptors and noninnervated bristles of the wing margin (JACK *et al.* 1991). Since some or all of these cells fail to accumulate detectable Cut protein in *Notch*, *Serrate* and *scalloped* mutants, the *cut* wing margin enhancer may be inactive in the absence of the product of any of the three genes. This hypothesis can be tested using a strain of flies carrying a construct, *ctwHZ*, in which the *cut* wing margin enhancer drives expression of the *lacZ* gene from an *hsp70* promoter. The *ctwHZ-2/CyO* strain carries the cut wing enhancer construct integrated into the second chromosome. In wild type strains carrying the construct, β-galactosidase is synthesized in the wing margins in the presumptive mechanoreceptors and noninnervated bristles (JACK *et al.* 1991).

The *notchoid* (nd^1) mutation of the *Notch* locus was used to test the effect of reducing the level of *Notch* activity on the activity of the cut wing enhancer. The nd^1 mutation is a missense mutation that alters one amino acid and inserts another in the intracellular domain of the *Notch* protein near the carboxy terminus (XU *et al.* 1990). At 25° the phenotype of homozygotes is similar to $N^{264\text{-}40}$/+ heterozygotes, having notching at the tip of the wing (Figure 5A). nd^1 was chosen because the homozygous viability allows appropriate crosses to be made so that all of the offspring analyzed were homozygous for the mutation. Because nd^1 causes an alteration in the *Notch* protein product, producing in homozygotes a phenotype similar to heterozygous *N* mutations, nd^1 homozygotes probably suffer a reduction in *Notch* activity similar to N^-/N^+ heterozygotes. At 7 hr AP homozygous nd^1 pupae have gaps in ctwHZ-2 expression of β-galactosidase in the distal wing margin, where notching is evident in adult wings (Figure 5B).

The dominant *Ser* mutation has a similar effect on β-galactosidase expression from *ctwHZ-2*. *Ser*/+ pupae also have a distal gap in wing margin β-galactosidase expression from *ctwHZ-2* (Figure 5C). The gap in expression is in the position where mechanoreceptors and noninnervated bristles lack Cut protein expression in *Ser*/+ pupae and where tissue is missing in adults. In both nd^1 homozygotes and *Ser* heterozygotes, the wing margin is unperturbed where β-galactosidase is not expressed, demonstrating that the nonexpressing cells are still present. Thus, both *nd* and *Ser* have their effect on *cut* expression by inactivating the *cut* wing margin enhancer.

The sd^1 mutation was used to examine the activity of the cut wing enhancer in a *scalloped* mutant background. Homozygous sd^1 pupae display gaps around the entire wing margin in β-galactosidase expression by *ctwHZ-2* (Figure 5D). This is consistent with the wing margin phenotype of *sd* mutants and the reduction of Cut protein detected in the mechanoreceptors and noninnervated bristles of *scalloped* mutants (Figure 2, M and N). Therefore, although *scalloped* mutations reduce the amount of Cut protein that accumulates in the chemoreceptors as well as the mechanoreceptors and noninnervated bristles, the reduction that occurs in the mechanoreceptors and noninnervated bristles is caused by the inactivity of enhancer for these cells in the absence of the *sd* product. Thus, the activity of the cut wing enhancer requires the products of all three genes whose mutations affect primarily the margin of the wing. Because *sd* mutations reduce protein expression in the chemoreceptors of the margin as well as the mechanoreceptors and noninnervated bristles, the *sd* product is apparently required for the function of a second *cut* enhancer that drives transcription in all or part of the peripheral nervous system outside the mechanoreceptors and noninnervated bristles of the wing margin.

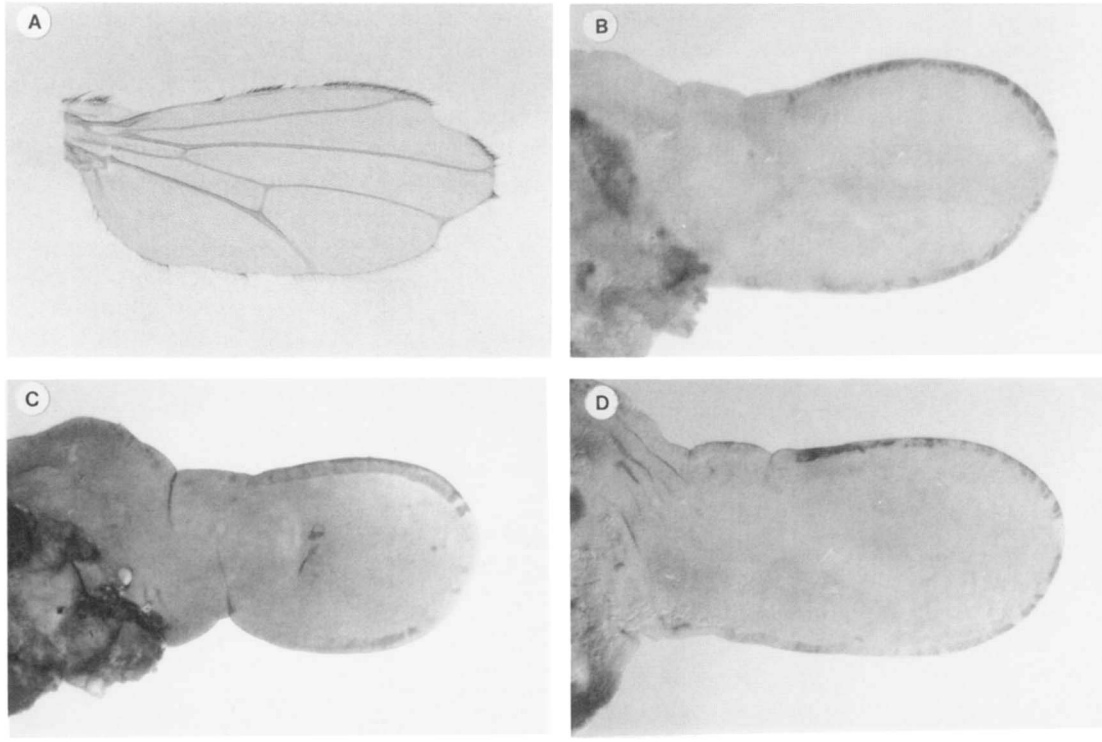

FIGURE 5.—Mutations of *nd*, *Ser* and *sd* block the activity of the *cut* wing margin enhancer. The construct ctwHZ-2 places the *lacZ* gene under the control of the enhancer that drives *cut* expression in the mechanoreceptors and noninnervated bristles of the wing margin. The figure shows the expression of the construct in several mutant backgrounds. (A) *nd* adult wings, (B) *nd/nd* or *nd/Y; ctwHZ-2/+* 7-hr pupal wings, (C) *ctwHZ-2/+; Ser/Ser*$^{+}$ 7-hr pupal wings and (D) *sd/Y; ctwHZ-2/+* 7-hr pupal wings. The pupal wings are stained with anti-β-galactosidase. β-Galactosidase is not expressed at the tip of the *nd* and *Ser* wings (B and C), although the cells appear certainly to be present. β-Galactosidase expression is reduced around the entire margin in the *sd* mutant wings.

DISCUSSION

The wing scalloping phenotype of a number of mutations is caused by the effects of the mutations on the development of the external sensory organs and noninnervated bristles of the wing margin. The primary effect of the *cut wing* mutations of the *cut* locus is to prevent the differentiation of exclusively the wing margin mechanoreceptors and noninnervated bristles, leading to their death (JACK *et al.* 1991). Strong *cut wing* mutations result in loss of all wing margin mechanoreceptors and noninnervated bristles.

A number of mutations of other genes that cause loss of part of the wing margin were sampled and three were found that have synergistic effects on the *cut wing* phenotype of weak *cut wing* mutations. Of the mutations tested, all three that cause scalloping restricted to the wing margin do so by blocking the differentiation of the mechanoreceptors and the noninnervated bristles. Of the mutations that were tested for a phenotypic interaction with weak *cut wing* mutations, $N^{264\text{-}40}$, *Ser* and sd^{2} interact synergistically, while ap^{Xa}, Bx^{J} and *Ly* have simply additive effects. We examined the development of the pupal wing and the expression of Cut protein in all of the mutations and found similarities to *cut wing* mutations in the mutations that interact synergistically. For the mutations that interact additively, the wings already appear, by 7 hr AP, to have the pattern deficiencies that are manifested in adult mutant wings. Further, the mutations remove these portions of the wings without favoring any particular cell type within the area removed. These genes may be important at an early stage to form the broad pattern of the wing disc, as has been hypothesized for *Ly* (ABBOTT and SPREY 1990).

The mutations that interact synergistically with *cut* are similar to *cut* in blocking differentiation of wing margin sense organs. Loss of the margin cells occurs after the cells normally begin to differentiate. Mutations of the three synergistically interacting genes reduce or completely block *cut* expression specifically in the sense organs and noninnervated bristles of the wing margin. This effect alone could block differentiation of the wing margin mechanoreceptors and noninnervated bristles and cause gaps in the bristle rows since the *cut wing* mutations accomplish the same thing by reducing expression of *cut*. Heterozygous *Notch* and *Serrate* mutations, as well as weak *cut wing* mutations, reduce *cut* expression in mechanoreceptors and noninnervated bristles on the anterior and posterior margins, but expression is completely lost only at the wing tip, where tissue is lost in adults. The loss

of cells of the bristle rows along the anterior and posterior margins in the double mutants may be the result of the cumulative loss of *cut* expression there.

A nonneurogenic effect of *Notch* in the ectoderm: The effect of the heterozygous null mutation $N^{264\text{-}40}$ in preventing the mechanoreceptors and noninnervated bristles from differentiating into neural cells is unique to these wing margin cells. In other cells that have been studied, mutations of *Notch* cause the opposite effect. That is, cells that would normally be epidermal take a neural fate in mutants, producing a hyperplasia of the central and peripheral nervous systems (LEHMANN *et al.* 1981, 1983). In adult bristle development, *Notch* functions both in controlling the number of cells that develop as sense organs and in determining the cell type of the cells in the sensillae (HARTENSTEIN and POSAKONY 1990). When early pupae homozygous for the temperature sensitive allele N^{ts1} are raised at the restrictive temperature before the sensory mother cell divides, many extra sensory bristles develop. However, if N^{ts1} pupae are shifted to the restrictive temperature for a period beginning after the sensory mother cell divides, many bristles are missing in adults because the accessory cells of individual sense organs are transformed into neurons. Gaps in the wing margin were observed by the same authors when pupae were exposed to the restrictive temperature at the later time. These gaps would be observed if the same type of transformation of the accessory cells to neurons occurs in the wing margin. However, the wing margin gaps that we see when *Notch* function is reduced are due, not to transformation of accessory cells to neurons, but to the failure of any of the cells to differentiate as sensory cells. The N^{ts1} wing margin gaps may also be caused by failure of the margin sensory cells to differentiate, rather than by the transformation of accessory cells to neurons. In any case, the prevention of neural differentiation in the wing margin by reduced levels of *Notch* expression is contrary to what is observed for reduction of *Notch* function in other ectodermal derivatives. The wing margin mechanoreceptors behave differently from other external sense organs in other regards as well. The lack of *cut* activity in these cells prevents the cells from differentiating (JACK *et al.* 1991) rather than transforming them into chordotonal organs as it does other external sensory organs (BODMER *et al.* 1987). In addition, the *achaete* and *scute* genes are not required for development of the wing margin mechanoreceptors as they are for all other external sensory organs (GARCIA-BELLIDO and SANTA MARIA 1978; LEYNS, DAMBLY-CHAUDIERE and GHYSEN 1989). In this epigenetic background, the Notch protein may still be part of the mechanism for determining cell fate in the wing margin, but the cell fate decision must not be between neural and epidermal fates.

The *Ser* mutation behaves similarly to *Notch* in the wing margin of heterozygotes. Heterozygotes of either mutation display margin gaps, loss of expression of *cut*, loss of expression of the neural antigen 22C10 in the mechanoreceptors and noninnervated bristles, and enhancement of weak *cut wing* mutations. *Notch* mutations also have a strong synergistic interaction with the *Ser* mutation, and *Ser* is suppressed by duplications of N^{+} (FLEMING *et al.* 1991), suggesting that the two genes may be functionally related. The sequences of the protein products are also similar. Both have sequences characteristic of membrane spanning proteins and have epidermal growth factor (EGF)-like repeats on the putative extracellular domains (FLEMING *et al.* 1991; KIDD, KELLEY and YOUNG 1986; THOMAS, SPEICHER and KNUST 1991; WHARTON *et al.* 1985). However, *Serrate* is not a neurogenic gene in embryos, although null mutations disrupt the embryonic central and peripheral nervous systems (FLEMING *et al.* 1991). Furthermore, the *Ser* allele is not a null mutation of the *Serrate* gene. Nor is it likely to cause a simple loss of *Serrate* function restricted to the wing margin because *Ser* is dominant, and deletion of the gene does not produce a dominant phenotype (FLEMING *et al.* 1991).

We have shown that mutations of *Notch*, *Serrate* and *scalloped* block *cut* expression in the wing margin mechanoreceptors and noninnervated bristles by inactivating the enhancer for *cut* transcription in these cells. Since *Notch* and *Serrate* encode membrane spanning proteins, their products are unlikely to interact directly with the cut wing enhancer but more likely act further upstream, possibly in determination of the fate of the cells. A description of the product of the *scalloped* gene, on the other hand, has not been reported; so, the *scalloped* product could potentially act either directly or indirectly on the cut wing enhancer. Interestingly, all three of the genes that have mutations that interact synergistically with *cut* to cause loss of margin bristles, also are involved in neural development outside the wing margin. Among the genes examined, three cause gapping of the wing margin and interact synergistically with *cut wing* mutations. All three of the genes, *Notch*, *Serrate* and *scalloped*, are also involved in neural development outside the wing margin. *Notch* is well known as a neurogenic gene, whose mutations cause hypertrophy of the central and peripheral nervous systems in embryos (LEHMANN *et al.* 1981) and adults (HARTENSTEIN and POSAKONY 1990). Null mutations of *Serrate* cause disorganization of the embryonic nervous system (FLEMING *et al.* 1991). And some alleles of *scalloped* cause the development of numerous extra bristles distributed on the wing blade (CAMPBELL *et al.* 1991) and some-

times extra campaniform sensillae on the L3 vein (Figure 2M). sd^2 has in common with the neurogenic genes the capacity to transform normally epidermal cells into sense organs. Thus, the synergistic interaction with weak *cut* alleles is capable of identifying at least some mutations that are involved in neural development.

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