

## A Dual Function of the *Notch* Gene in *Drosophila* Sensillum Development

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We have investigated the function of the neurogenic gene *Notch* (*N*) during development of the adult sensilla of *Drosophila*. Heat pulses were applied to flies carrying the temperature-sensitive *Notch* allele *N<sup>ts1</sup>* at different larval and pupal stages. We can show that the reduction of *Notch*<sup>+</sup> function during a short interval prior to the onset of sensillum precursor division, resulting from a heat pulse between 0 and 14 hr after puparium formation (apf), leads to an increase in microchaete precursors at the expense of epidermal cells. The structure and cellular composition of the sensilla produced by these supernumerary precursors are normal. Later heat pulses which include the interval immediately after sensillum precursor division (14–20 hr apf) lead, among the progeny of the sensillum precursors, to a hyperplasia of sensory neurons, at the expense of accessory cells. The resulting "sensilla" consist of neurons only and lack the external cuticular structures (i.e., shaft, socket). These results demonstrate that similar mechanisms both of which involve the function of the *Notch* gene may be operating to sort out (premitotic) sensillum precursors from epidermal precursors and (postmitotic) sensory neurons from accessory cells. They further show that in postmitotic sensillum cells the differentiative fate is not yet irreversibly fixed, but presumably requires cell-cell interaction to become established.

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### INTRODUCTION

The *Drosophila* epidermis bears numerous appendages of different structures and functions, among them the sensory organs (sensilla). Each sensillum is composed of one or more neurons and a number of nonneuronal accessory cells. All of these cells derive in a stereotyped series of divisions from sensillum precursors which appear at distinct positions in the epidermal primordium of the embryo and pupa, respectively (Campos-Ortega and Hartenstein, 1985; Bodmer *et al.*, 1989; Hartenstein and Posakony, 1989). Following the terminal precursor divisions, each developing sensillum forms a coherent group of undifferentiated cells embedded in the surrounding epidermal tissue. Sensillum cells then express their divergent structural fates in a characteristic sequence of events (Hartenstein, 1988; Hartenstein and Posakony, 1989). First, the presumptive neuron(s) segregates into the subepidermal space and sends out a dendrite and an axon. Consecutively, three accessory cells (thecogen cell, trichogen cell, and tormogen cell), remaining integrated within the epidermal layer, form sheath processes around the dendrite(s), and, during the phase of cuticle secretion, build processes around which the stimulus-receiving cuticular apparatus is formed. A fourth, subepidermal accessory cell (glia cell) encloses the cell body of the sensory neuron(s).

Evidently, a central process in sensillum development is the sorting out of different cell fates. First, the sensillum precursor cells are set apart from the remaining epidermal progenitors. Secondly, among the progeny of the sensillum precursors, the neurons are separated from the various, nonneuronal accessory cells. Finally, each individual accessory cell expresses a different phenotype. The factors controlling these cell fates are largely unknown. In the present study we have investigated the role of the neurogenic gene *Notch* during sensillum development. The neurogenic loci were originally defined as a group of genes which act during early neurogenesis in the *Drosophila* embryo (Lehmann *et al.*, 1981). The precursors of the larval central nervous system, called neuroblasts, originate from a specialized ectodermal region, the ventral neurogenic region (Lehmann *et al.*, 1983; Campos-Ortega, 1983; Hartenstein and Campos-Ortega, 1984). Within the ventral neurogenic region, neuroblasts are mixed with epidermoblasts; in normal development, only one of four cells of the ventral neurogenic region segregates as a neuroblast (Hartenstein and Campos-Ortega, 1984). In embryos bearing a loss of function mutation in any of the neurogenic loci, an increased number of cells develop as neuroblasts, leading to a hyperplasia of the CNS and a reduction of the ventral epidermis (Lehmann *et al.*, 1981; 1983). It was hypothesized that the products of the neurogenic loci form part of a signaling mechanism by which cells of the ventral neurogenic region sort out which one of them is going to become a neuroblast and which one an

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epidermoblast. Recent experimental and molecular studies have assigned putative roles for the different gene products encoded by the neurogenic loci (for review, see Campos-Ortega, 1988; Artavanis-Tsakonas, 1988). Thus, it was demonstrated that *Notch* codes for a transmembrane protein whose extracellular domain consists of 36 cysteine-rich repeats bearing sequence similarity to the mammalian EGF receptor (Wharton *et al.*, 1985) and to mammalian clotting factors (Kidd *et al.*, 1986); the intracellular domain showed sequence similarity to yeast division factors (Breeden and Nasmyth, 1987). These molecular data are compatible with the notion that the *Notch* gene product is involved in a cell-cell communication process which directs cell proliferation and the expression of distinct cellular fates.

Besides its role during neuroblast segregation, *Notch* is also involved in the development of the sensilla, as well as numerous other developmental events, such as wing vein formation (Portin, 1975) and ommatidium differentiation (Cagan and Ready, 1989). By applying heat pulses to the temperature-sensitive *Notch* allele *N<sup>ts1</sup>*, which, in trans to other *Notch* alleles, behaves like a hypomorphic allele (Shellenbarger and Mohler, 1978), it was shown that the reduction of *Notch*<sup>+</sup> at early pupal stages increased the number of adult microchaetes; slightly later occurring heat pulses reduced microchaetes. *Notch*<sup>-</sup> epidermal clones, recovered by X-ray induced mitotic recombination in a *Minute* background, did not bear any sensilla (Dietrich and Campos-Ortega, 1984). In the embryo, homozygosity for the loss of *Notch*<sup>+</sup> function leads to an increased number of larval sensilla (Hartenstein and Campos-Ortega, 1986). In these previous studies, the developmental basis for the effects of *Notch* on sensillum development was not well understood, partly because no markers were available for the different sensillum cell types, partly because the normal sequence of events defining sensillum development in *Drosophila* (e.g., time and pattern of sensillum precursor division; sensillum cell differentiation) had not been worked out yet. In the present study we have investigated the role of *Notch* during adult sensillum development. Heat pulses were applied at different stages to developing *N<sup>ts1</sup>* larvae and pupae. Following this treatment, the pattern of sensillum precursor division and sensillum cytodifferentiation was analyzed in dissected pupae. Markers for the different cell types [MAb 22C10 (Zipursky *et al.*, 1984) for neurons, thecogen cells, and trichogen cells (Hartenstein and Posakony, 1989); MAb 21A6 (Zipursky *et al.*, 1984) for thecogen cells (Hartenstein and Posakony, 1989); P-element insertion A1-2nd-29, carrying *lacZ* expressed in trichogen cells and tormogen cells (Bier *et al.*, 1989)] and bromo-deoxyuridine (BrdU) to label DNA replicating nuclei were used. We can show that the *Notch*<sup>+</sup> product has a dual

function during sensillum development. At an early stage it is required for the decision of uncommitted cells to become either sensillum precursor cells or epidermal precursors. At a later stage it is again required for the decision of postmitotic sensillum cells to become either sensory neurons or accessory cells.

## MATERIALS AND METHODS

### Heat Pulse Experiments

Stocks of *N<sup>ts1</sup>* flies (Shellenbarger and Mohler, 1975; kindly provided by R. Cagan) were kept at 22°C. For pulses during the pupal period, white prepupae were collected and put in a moist chamber kept at 25°C. At appropriate stages the chamber was incubated at 30°C. For pulses during the larval period, newly hatched larvae were put on a yeast-containing agar plate kept at 25°C. For the heat pulse the plate was incubated at 30°C. Pupae of wild-type flies (Canton S) subjected to the same temperature regimens served as controls.

### Antibody Labeling

At an appropriate stage (i.e., 24–32 hr after puparium formation) the heat-pulsed and control *N<sup>ts1</sup>* flies were dissected in phosphate-buffered saline (PBS; 0.1 M; pH 7.3) and fixed for 10 min in 4% paraformaldehyde in PBS. After several washes in 0.1 M PBS containing 0.3% Triton X-100, preparations of the nota and wings were incubated for 1 hr in 0.1 M PBS containing 10% goat serum, 0.3% Triton X-100, and the monoclonal antibody 22C10 or 21A6 (kindly provided by Dr. S. Benzer) at a dilution of 1:50. After several washes in PBS, they were then incubated for 1 hr in HRP-conjugated rabbit anti-mouse IgG (Boehringer) diluted at 1:50 in 0.1 M PBS containing 10% goat serum and 0.3% Triton X-100. Preparations were washed several times in PBS and incubated in diaminobenzidine (DAB, Sigma) at 0.1% in 0.1 M phosphate buffer (pH 7.3) containing 0.006% hydrogen peroxide. The reaction was interrupted after 5–10 min by diluting the substrate with 0.1 M phosphate buffer. Preparations were dehydrated in graded ethanol (70, 90, 95%, 5 min each; 100%, 15 min) and acetone (5 min) and left overnight in a mixture of Epon and acetone (1:1). They were then mounted in a drop of fresh Epon and coverslipped.

### Cuticle Preparations

Pupae were removed from the pupal case 5 days after puparium formation. They were fixed and dissected in 100% ethanol. Preparations were cleared in benzyl benzoate/benzyl alcohol and mounted in Canada balsam.

### $\beta$ -galactosidase Activity Staining

The second chromosomal P *lacZ* insertion A1-2nd-29 (kindly provided by Dr. Y. N. Jan) was used as a marker for trichogen and tormogen cells (Bier *et al.*, 1989; Hartenstein and Posakony, 1990). After crossing homozygous A1-2nd-29 males to homozygous *N<sup>ts1</sup>* virgin females, all male offspring are hemizygous for *N<sup>ts1</sup>* and carry one A1-2nd-29 chromosome. To label *lacZ*-expressing cells in pupal whole mounts, staged pupae were fixed and dissected in glutaraldehyde (2% in 0.1 M PBS). After several washes in PBS, preparations were incubated overnight in a staining buffer containing the chromogenic substrate X-gal (Boehringer; dissolved at 8% in dimethylformamide) at 0.2%. The staining buffer consisted of the following: 3.6 ml 0.2 M Na<sub>2</sub>HPO<sub>4</sub>; 1.9 ml 0.2 M Na<sub>2</sub>PO<sub>4</sub>; 3.0 ml 5 M NaCl; 100  $\mu$ l 1 M MgCl<sub>2</sub>; 12.2 ml 25 mM K<sub>3</sub>[Fe(CN)<sub>6</sub>]/K<sub>2</sub>[Fe(CN)<sub>6</sub>]; 0.3 ml Triton X-100; 50 ml distilled water. After incubation in the staining solution, the preparations were washed in PBS, dehydrated in graded ethanol, and embedded in Epon as described above.

### Electron Microscopy

For electron microscopy, 32-hr pupae were dissected in a solution containing 2% glutaraldehyde and 2% paraformaldehyde in PBS. Dissected nota and wings were left for 2 hr in this solution; they were washed in several changes of 0.15 M cacodylate buffer (pH 6.9) and postfixed for 30 min in 2% osmium tetroxide in 0.15 M cacodylate buffer. Specimens were washed several times in PBS and dehydrated in graded ethanol and acetone (see above). Preparations were left overnight in a 1:1 mixture of Epon and acetone and then for 5–10 hr in unpolymerized Epon. They were transferred to molds, oriented, and placed at 60°C for 12 hr to permit polymerization of the Epon. Blocks were sectioned with an LKB Ultrotome III. Sections were mounted on net grids and treated with uranyl acetate and lead citrate (Reynolds, 1963). Sections were inspected and photographed with a Philips EM 300.

### Application of Bromodeoxyuridine (BrdU)

The base analogue BrdU, which is incorporated into replicating DNA, was applied by injecting it into pupae. For the injections, staged pupae were mounted, ventral side down, in a row on double-stick Scotch tape. A micropipette (tip diameter, 10–20  $\mu$ m) was attached to a micromanipulator, filled (from the front) with approximately 0.001–0.003  $\mu$ l of 1 mM BrdU (in PBS), and inserted into the pupa from the caudal end. The pipette was pushed forward so that its tip reached the level of the thorax. Injection was by air pressure, applied man-

ually by a 1-ml syringe connected to the pipette with plastic tubing. Pupae were then placed in a moist chamber at 25°C for an appropriate interval, after which they were dissected in PBS. In our hands, BrdU application did not cause any significant delay in development during the first 2 days of the pupal period.

For visualization of incorporated BrdU, the preparations were fixed for 2 min in modified Carnoy's fixative (100% ethanol:glacial acetic acid, 6:2), washed several times in PBS, and incubated for 50 min in 2 N HCl to denature the DNA. After this step they were washed for 30 min in several changes of PBS containing 0.3% Triton X-100. The preparations were then incubated for 1 hr in 0.1 M PBS containing 10% goat serum, 0.3% Triton X-100, and a monoclonal antibody against BrdU (Becton-Dickinson) at a dilution of 1:5. In several experiments, the sensillum-specific MAAb 22C10 was added to this solution at 1:50. After several washes in PBS, preparations were incubated for 1 hr in HRP-conjugated rabbit anti-mouse IgG (Boehringer), diluted at 1:50 in 0.1 M PBS containing 10% goat serum and 0.3% Triton X-100. The histochemical reaction and further treatment of the preparations were identical to those described above ("Antibody Labeling").

### Quantitative Analysis of the Microchaete Pattern in Heat-Pulsed Flies

The microchaetes of five individuals for each heat-pulse experiment were charted with a camera lucida. A regular grid of squares, which were calculated to approximately correspond in size to the area surrounding one microchaete of a wild-type notum, was drawn on a transparency and superimposed on the individual charts (see Fig. 9C). To compensate for the variability in body size (and the resulting variability in absolute microchaete density), different grid sizes, adapted to the overall size of a particular notum preparation, were used. The distance between the posterior dorsocentral macrochaetes was taken as a measure of the size of a particular notum preparation. This distance was set to seven squares (in a wild-type fly, the distance between the dorsocentral macrochaetes approximates seven intermicrochaete distances). For some temperature regimens yielding high microchaete densities in the central notum, the squares corresponding to this region were further subdivided into two halves (see Fig. 9). Subsequently, for the five individuals of each heat-pulse experiment, the average number of microchaetes falling into each of the squares of the grid was calculated.

### RESULTS

Heat pulses at the restrictive temperature (30°C) applied to developing *N<sup>ts1</sup>* animals during the larval and

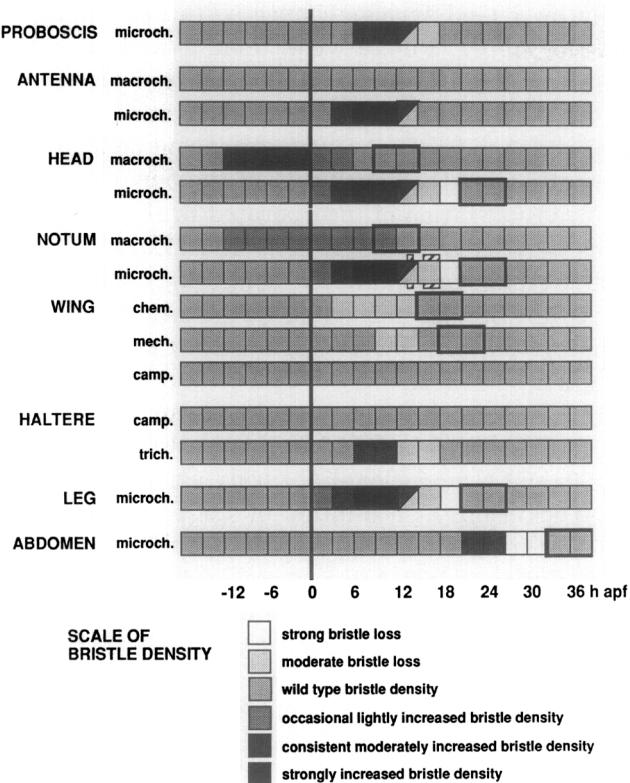


FIG. 1. Density of different classes of bristles of adult *N<sup>ts1</sup>* flies following heat pulses (30°C) applied at different stages of larval and pupal development. The time scale at the bottom indicates hours after puparium formation (apf). Each class of bristles is represented by a horizontal bar. Bars are subdivided into squares representing 3-hr time intervals each; shading intensity of a square correlates to the density of the corresponding bristles resulting from a 6 to 9-hr heat pulse which includes the time interval represented by that square. Medium grey stands for normal density, darker shades indicate increased density, and lighter shades stand for bristle loss (see scale of shading intensities at bottom of figure). Note that "bristle loss" refers to the absence of only the externally visible cuticle parts of the corresponding bristles; the underlying cells are there, yet express a neuronal phenotype (see text). Black frames around squares indicate the stage at which cells of sensilla represented by the corresponding bars are postmitotic and start differentiating. Hatched frames in the bar which represents the microchaetes of the notum show the time at which the two rounds of divisions of the microchaete precursors take place (see Hartenstein and Posakony, 1989). Some squares show two different shadings. This implies that radically different bristle densities were obtained, depending on whether the time interval represented by the corresponding square fell into the beginning or the end of the heat pulse. For example, a heat pulse from 6 to 15 hr apf resulted in a strong increase in notum microchaete density; a pulse from 12 to 20 hr caused a severe microchaete loss. Abbreviations: microch., microchaetes; macroch., macrochaetes; chem., chemosensory bristles; mech., mechanosensory bristles; camp., campaniform sensilla; trich., trichoid sensilla.

early pupal stages had different effects on the pattern and structure of the adult epidermis, depending upon the specific time at which the pulse was applied. The minimum length of pulse yielding any detectable defects was 6 hr. Short heat pulses (6–10 hr) applied during the

larval period had no detectable effect. Only occasionally, pulses immediately preceding puparium formation resulted in duplications of some of the macrochaetes on the head and thorax. Longer pulses (12–72 hr) resulted in gross defects of the imaginal body wall. Among these were the almost complete absence of the head and removal of discrete parts of the legs and wings. Preparations of wing discs shortly after puparium formation demonstrated that, by this stage, the defects caused by the heat pulse during larval life were already present (data not shown). This implies that the defects developed during the larval period, either by a defective pattern of proliferation or by increased cell death.

Sensillum development was affected by pulses between the late third larval instar and 30 hr after puparium formation (apf) (see Fig. 1; compare also results of Shellenbarger and Mohler, 1978). The microchaetes of the notum, the main subject of this study, were affected by pulses applied between 0 and 24 hr apf. This interval includes the phase when, during wild-type development, the cells constituting the microchaete organs are born and commence differentiation (Hartenstein and Posakony, 1989) (Fig. 1). Four cells of a microchaete, namely, the sensory neuron and three accessory cells, are derived from a single precursor cell (first order precursor) which divides around 14 hr apf, yielding two daughter cells (secondary precursor cells). One of these divides around 16 hr, giving rise to the two outer accessory cells (trichogen and tormogen cells); the other divides at 18 hr and produces the neuron and inner accessory cell (thecogen cell). The subepidermal glia cell which is present in most microchaetes is also born between 16 and 18 hr; however, it does not derive from the precursor of the other four sensillum cells.

#### *Reduction of N<sup>+</sup> Function prior to Microchaete Precursor Division Causes a Hyperplasia of These Cells at the Expense of Epidermal Cells*

Heat pulses spanning all or part of the interval from 0 to 15 hr apf yielded a significant increase in microchaete number and density on the notum and head (Fig. 2). This effect was evident both in cuticle preparations of 5-day-old pupae and in antibody-stained preparations of younger pupae. The increase in microchaete density achieved by heat pulsing *N<sup>ts1</sup>* pupae showed strong regional differences over the notum (see below). The highest densities observed (i.e., near the dorsal midline following shifts between 6 and 12 hr apf) were 12–15 times normal.

The cells composing the supernumerary microchaetes are born, and differentiate, at approximately the same time as normal microchaetes. In normal development, as described above, microchaete cells are born between

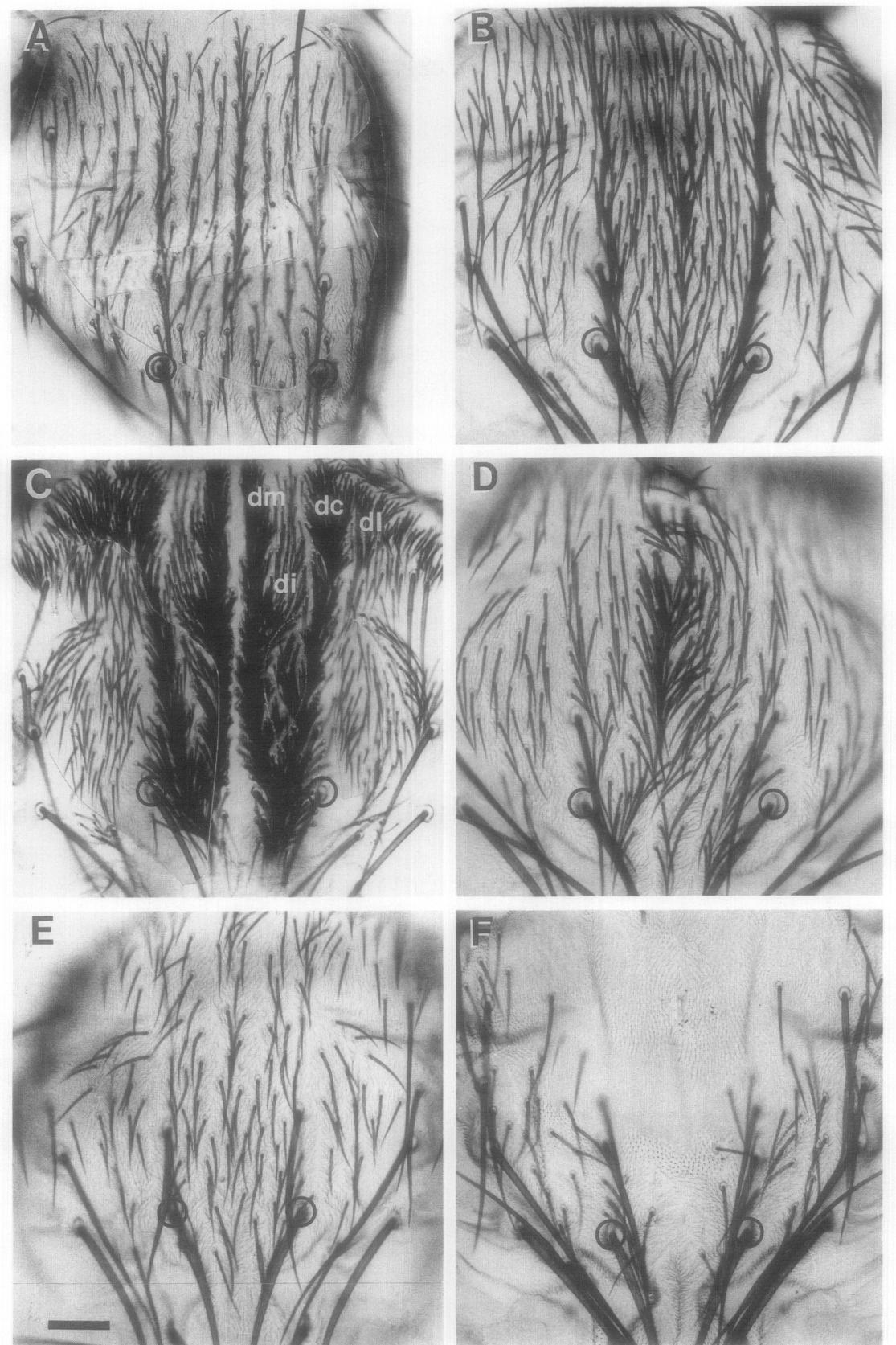
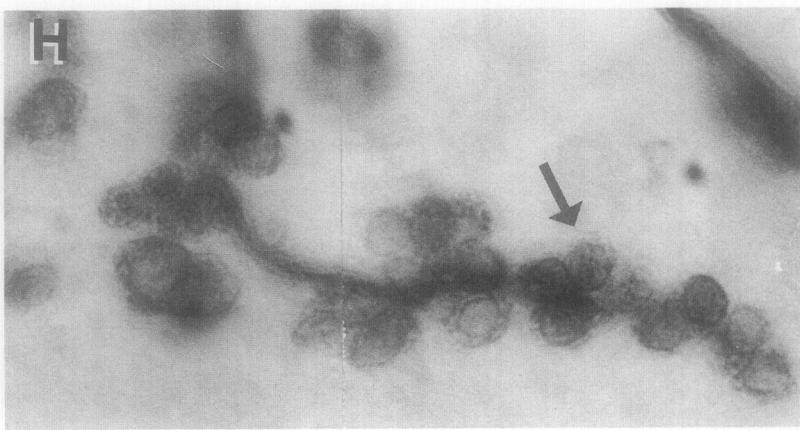
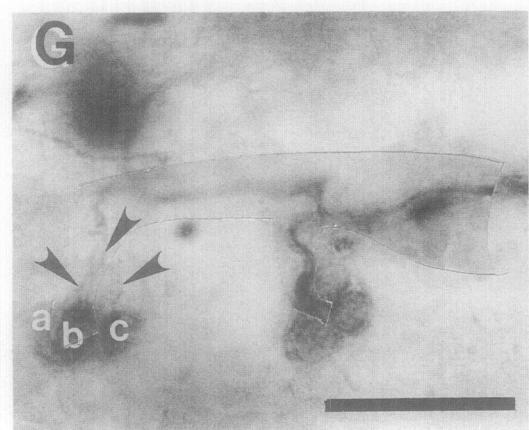
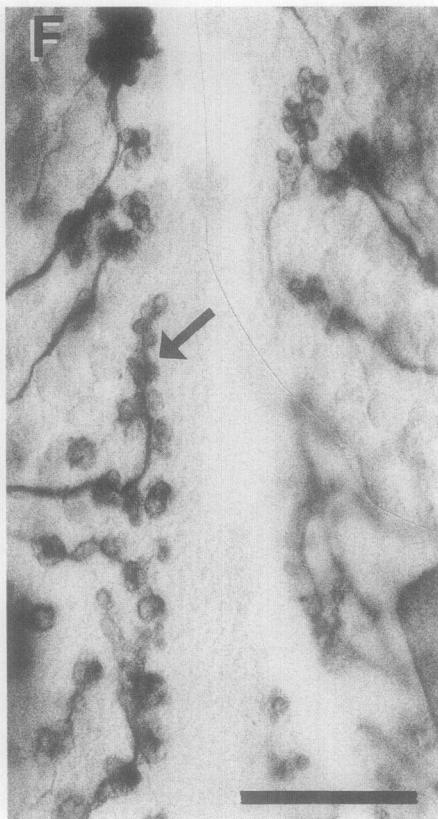
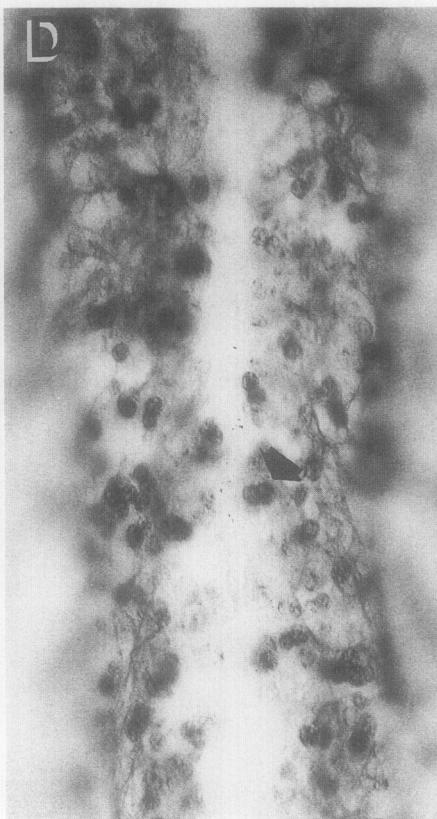
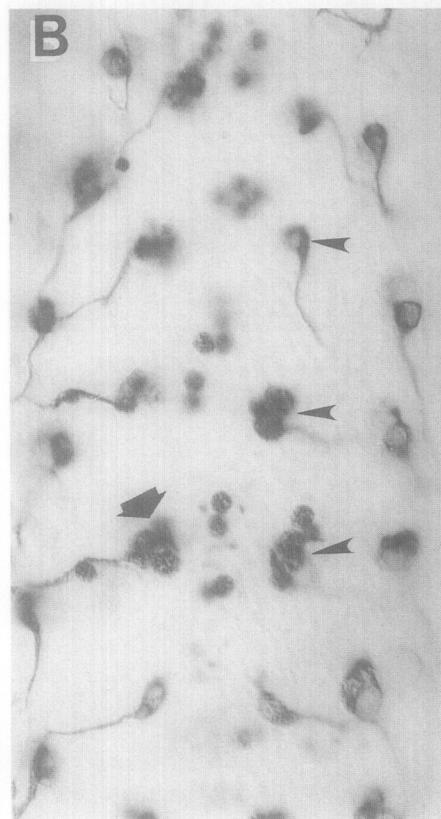
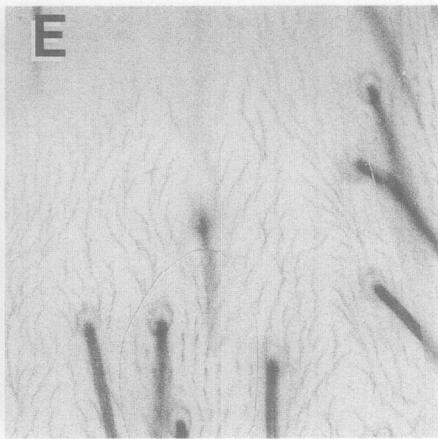
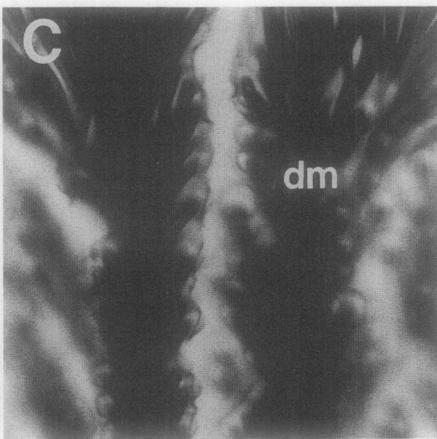
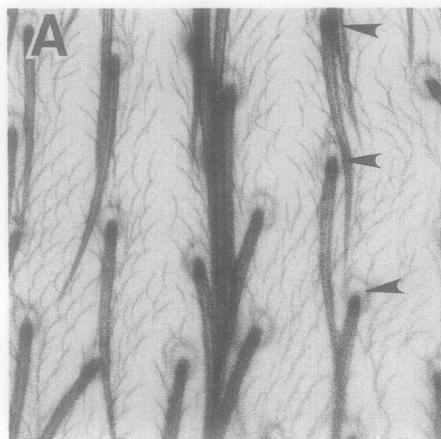


FIG. 2. Bristle pattern on the notum of a wild-type fly (A) and *N<sup>ts1</sup>* flies which were pulsed between 3 and 9 hr (B), 6 and 12 hr (C), 9 and 15 hr (D), 14 and 20 hr (E), and 16 and 24 hr apf (F). In all photographs, anterior is to the top and the dorsal midline is in the middle. Note the longitudinal zones with differential sensitivity toward the reduction in *N<sup>+</sup>* function (marked in C: dm, dorsomedial zone; di, dorsointermediate zone; dc, dorsocentral zone; dl, dorsolateral zone). For orientation purposes, the posterior dorsocentral macrochaetes are marked with circles. Bar, 75  $\mu$ m.



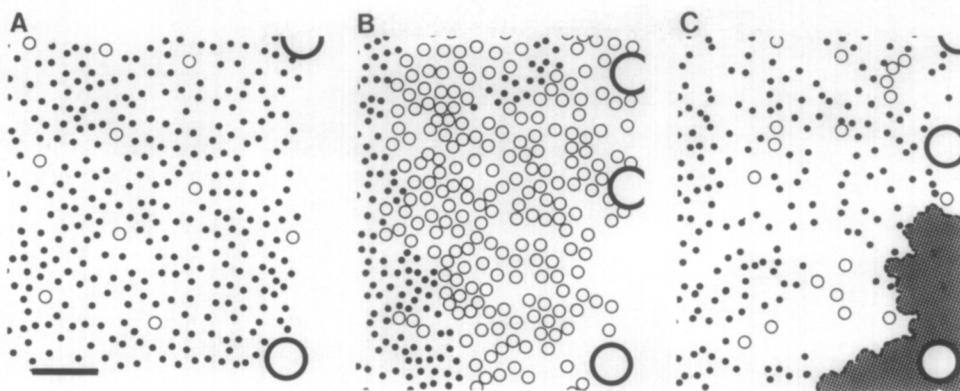


FIG. 4. Microchaete/epidermal cell ratio on the notum of an adult wild-type fly (A), a *N<sup>ts1</sup>* fly heat-pulsed between 0 and 12 hr apf (B), and a *N<sup>ts1</sup>* fly heat-pulsed between 0 and 24 hr apf (C). A, B, and C are derived from camera lucida drawings of individual animals. Part of the medial notum is shown. In each drawing, the left margin represents the dorsal midline and anterior is to the top; parts of large circles on the right indicate the dorso-central macrochaetes. Dots indicate individual trichomes; each trichome represents one epidermal cell. Microchaetes are marked by open circles. The shading in C marks a highly sclerotized region in which individual microchaetes and trichomes could not be resolved. The average numbers of epidermal cells and microchaetes in one-half of the dorsocentral notum, counted from five individuals each, are  $11 \pm 0.5$  (SD) microchaetes and  $298 \pm 27.7$  (SD) epidermal cells in wild-type flies (A);  $177 \pm 3.1$  microchaetes and  $83 \pm 13.5$  epidermal cells in *N<sup>ts1</sup>* flies pulsed between 0 and 12 hr apf (B); 2–40 microchaetes and  $150 \pm 24.2$  epidermal cells in *N<sup>ts1</sup>* pulsed between 0 and 24 hr apf (C). Bar, 20  $\mu$ m.

16 and 18 hr apf; they are first detectable with the MAb 22C10 at about 20 hr apf, when they begin differentiation (Hartenstein and Posakony, 1989). In wing imaginal discs of *N<sup>ts1</sup>* animals pulsed between 0 and 15 hr apf, MAb 22C10 detects at 20 hr apf a large number of supernumerary microchaetes just commencing differentiation. By injecting BrdU into *N<sup>ts1</sup>* pupae pulsed between 0 and 15 hr apf, it could be demonstrated that the cells of most, if not all, supernumerary microchaetes are born around the normal time (Fig. 3). Thus, prior to 16 hr in these animals, there must exist a greater than normal number of precursor cells which then divide into the individual microchaete cells. In regions with only a moderate increase in microchaete density, the shape and position of the cells contained within individual microchaetes, as well as their lineage relationship, could be reconstructed. It was found that the developing su-

pernumerary microchaetes are indistinguishable from normal microchaetes in both cellular architecture and lineage (see Hartenstein and Posakony, 1989). In regions with a high density of supernumerary microchaetes, the cells of neighboring sensilla contact each other, thereby forming a continuous "lawn" of densely packed sensillum cells (Fig. 3). This high packing density precluded reconstructing in early pupal preparations the architecture of individual sensilla and the lineage relationships of cells contained within them. Cuticle preparations of 5-day-old *N<sup>ts1</sup>* pupae pulsed between 6 and 12 hr apf show that in areas with high microchaete density, the sockets of neighboring bristles may fuse. Single sockets surrounding more than one shaft are also frequently encountered. Often, such sockets are subdivided by notches into separate sectors, indicating that they may have been produced by more than one tormo-

FIG. 3. Microchaete development in a wild-type fly (A, B) and *N<sup>ts1</sup>* flies heat-pulsed between 6 and 12 hr (C, D) and 16 and 24 hr (E–H). (A) Part of the dorsocentral region of an adult notum containing several regularly spaced microchaetes (arrowheads). (B) Dorso-central region of a 24-hr pupal notum in which sensillum cells are stained with the MAb 22C10 (arrowheads point at examples). In addition, BrdU injected at 16 hr was incorporated in all four sensillum cells of a major portion of the microchaetes (broad arrow; BrdU-containing nuclei are visualized by a monoclonal antibody), indicating that these cells are born in the interval between 16 and 24 hr. (C) The massive multiplication of microchaetes of the dorsomedial zone (dm) of an adult fly pulsed from 6–12 hr apf. (D) A 24-hr notum of a *N<sup>ts1</sup>* pupa subjected to the same temperature regimen and injected with BrdU at 16 hr; the preparation is labeled with 22C10 and anti-BrdU. Note continuous belts of 22C10-positive microchaete cells, most of which also contain anti-BrdU-positive nuclei (example shown by arrow). Injection of BrdU later than 20 hr apf did not lead to any labeling of sensillum cells. These results indicate that the cells of the supernumerary microchaetes are born at the normal stage (16–20 hr). A temperature shift between 16 and 24 hr in *N<sup>ts1</sup>* leads to the absence of most microchaetes (E). MAb 22C10-stained whole mounts of 24-hr nota of *N<sup>ts1</sup>* pupae subjected to this temperature regimen (F) show that microchaete cells appear, but develop as neurons: all 22C10-positive cells obtain a subepidermal position and emit axons (see arrowheads in G, pointing to individual axons which originate from a clusters of cells (a, b, c) representing the progeny of an individual microchaete precursor). Nuclear staining results from the injection of BrdU at 16 hr and subsequent labeling with anti-BrdU. Note that cells of many transformed microchaetes have fused along the subepidermal axonal tracts (arrows in F, H). Bars: 50  $\mu$ m (A–F), 25  $\mu$ m (G, H).

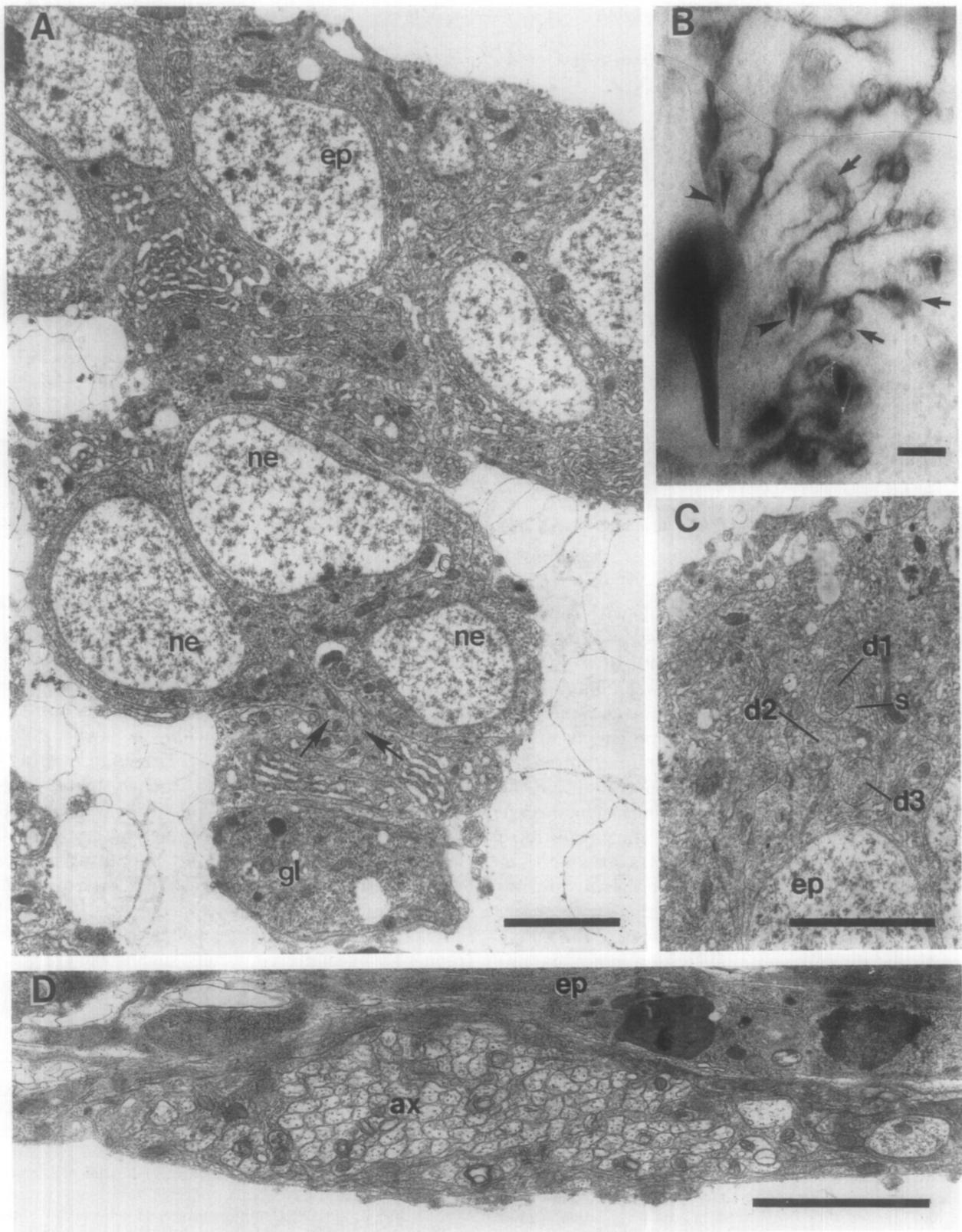
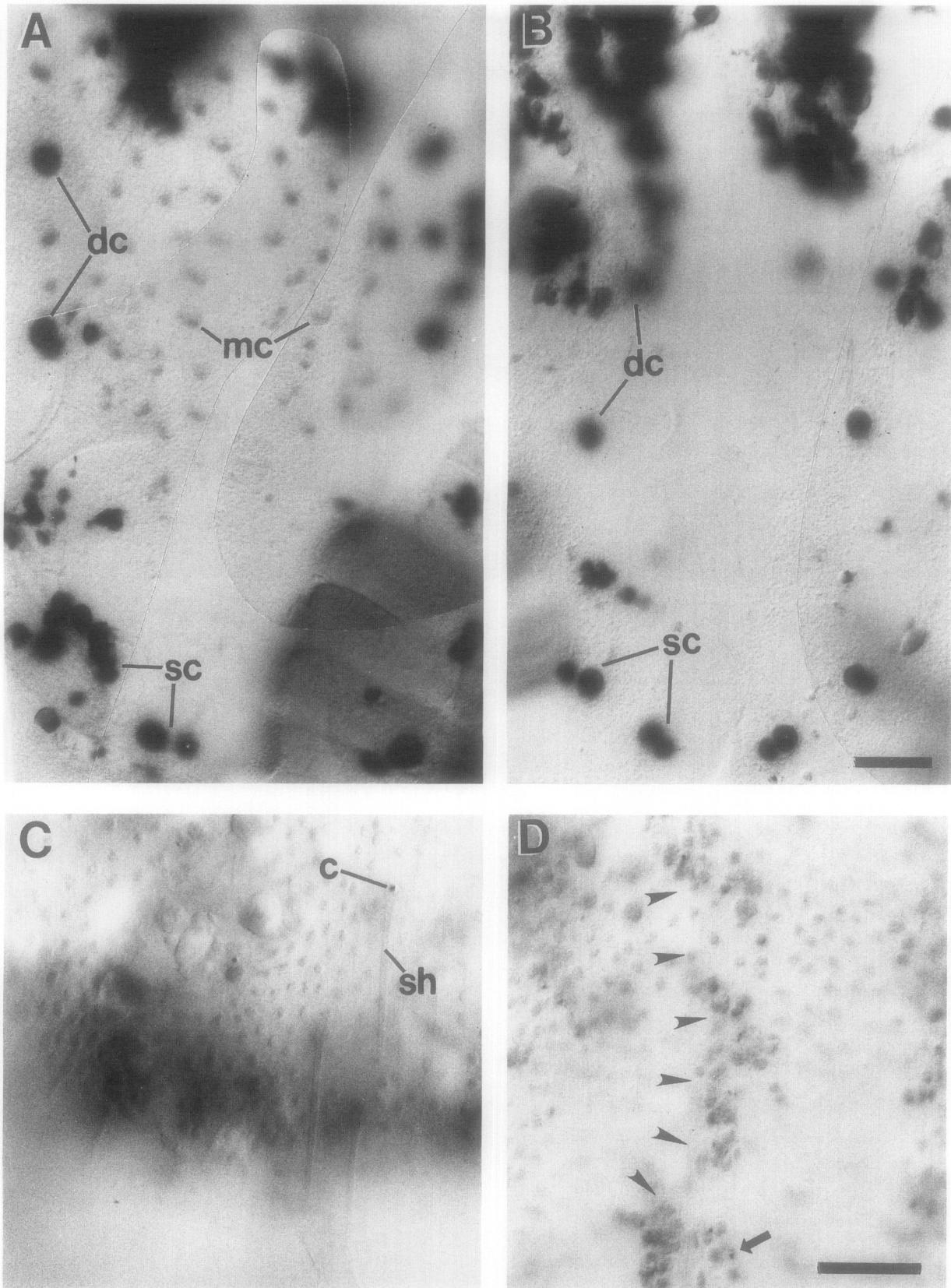
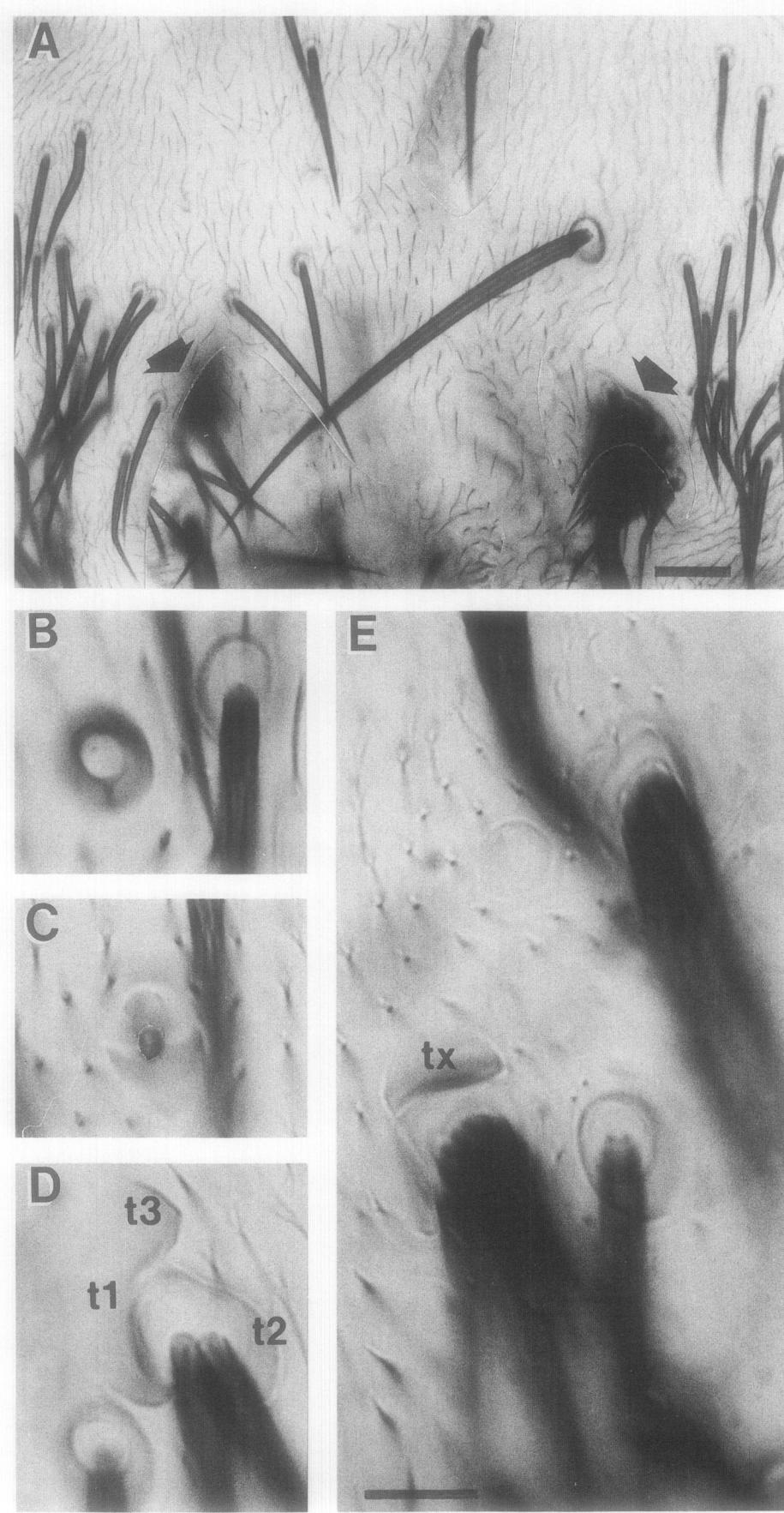


FIG. 5. Microchaete development in *N<sup>tsi</sup>* flies heat-pulsed between 12 and 24 hr apf. (A, C, D) Electron micrographs of sections of 32-hr nota (A, C) and a 5-day-old notum (D). (B) A light micrograph of a whole-mount preparation of a 32-hr notum labeled with the MAb 22C10. In flies pulsed between 12 and 24 hr, most microchaetes consist of sensory neurons only (arrows in B). (A) A section of such an abnormal microchaete. Beneath the epidermal layer (ep), there appear the cell bodies of typically four bipolar cells (ne; the fourth cell is out of the plane of this section) which extend axons at their basal surface (arrows). The cell marked gl may represent a glia cell. (C) Three dendrite-like profiles (d1-3) surrounded by the cytoplasm of an epidermal cell (ep). The dendrite d1 is surrounded by a sheath-like process (s) which, unlike the normal inner sheath of a microchaete, is mesaxon-like and not cylindrical. This process therefore is formed most probably by a neuron. (D) A massive subepidermal bundle of axons (ax) in the notum of a 5-day-old *N<sup>tsi</sup>* pupa subjected to a 12- to 24-hr heat pulse. Bars: 5  $\mu$ m (A, C, B), 30  $\mu$ m (B).



**FIG. 6.** Microchaete development in a wild-type fly (A, C) and *N<sup>ts1</sup>* flies which were heat-pulsed between 16 and 24 hr (B, D). (A, B) Micrographs of 28 hr pupal nota of flies carrying the P-*lacZ* insertion A1-2nd-29 which is expressed in the tormogen and trichogen cells of all microchaetes (mc) and macrochaetes (the dorsocentrals (dc) and scutellars (sc) are indicated). In the heat-pulsed *N<sup>ts1</sup>* pupa, A1-2nd-29 expression is not observed for most microchaetes, indicating that in these sensilla tormogen and trichogen cells do not differentiate. (C, D) Micrographs (Nomarski optics) of parts of 36-hr pupal nota stained with the MAb 21A6. This antibody labels the dendritic cap (c) which is secreted by the thecogen cell and appears as a black dot beneath the base of the shaft (sh) of each microchaete; in addition, nuclei of some microchaete cells are also variably stained in these preparations. In the transformed microchaetes of heat-pulsed *N<sup>ts1</sup>* animals (indicated by arrowheads in D), bristle shafts are absent and cap-like structures are only infrequently encountered (arrow), suggesting that thecogen cells are not differentiated. Bar, 50  $\mu$ m.



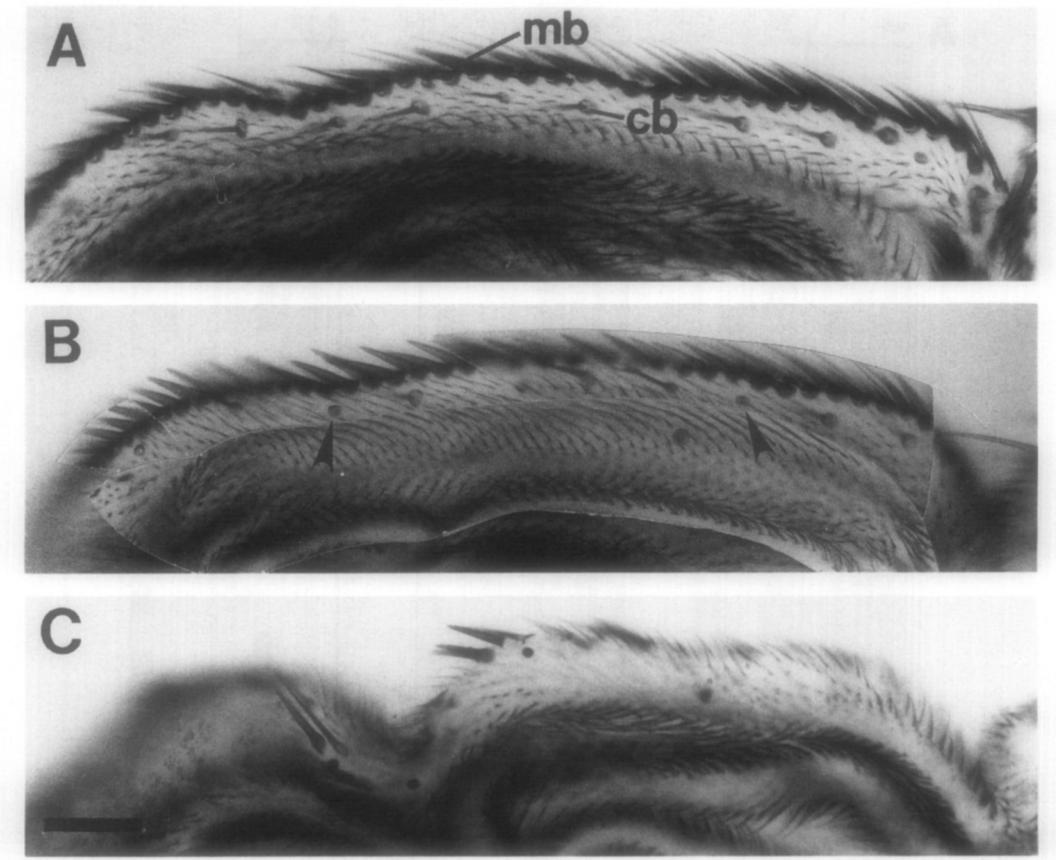


FIG. 8. Bristle pattern on the dorsal anterior margin of a late pupal left wing of a wild-type fly (A) and  $N^{ts1}$  flies heat-pulsed from 3 to 9 hr (B) and 8 to 24 hr (C). Two classes of sensilla are shown: the stout (mechanosensory) bristles of the medial triple row (mb) and the recurved (chemosensory) bristles of the dorsal triple row (cb). The 3- to 9-hr pulse leads to partial loss of chemosensory bristles (arrowheads point at cuticular "blobs" at positions normally occupied by these sensilla). The 8- to 24-hr pulse removes most mechano- and chemosensory bristles. Bar, 50  $\mu$ m.

gen cell. Thus, in a condition of greatly increased density of sensillum precursors and, consequently, of post-mitotic sensillum cells, adjacent tormogen cells and trichogen cells can form composite bristle organs. In these cases, the number and configuration of the underlying neurons, thecogen cells, and glia cells could not be determined.

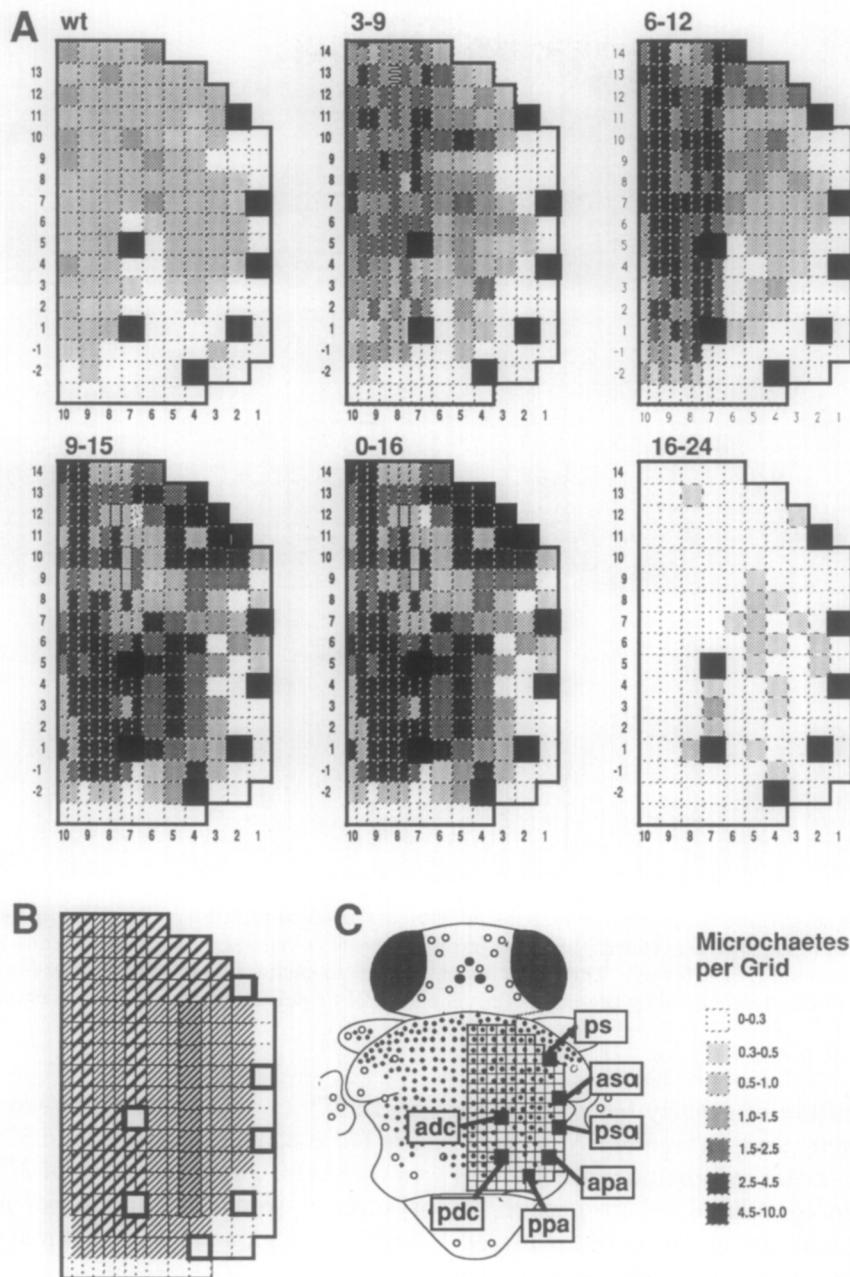
The increase in the number of microchaete precursors in  $N^{ts1}$  animals following a heat pulse between 0 and 15 hr apf is accompanied by a local loss of epidermal cells. In one-half of the dorsocentral region of the notum (the rectangular field defined by the four dorsocentral macrochaetes), there are 11–12 microchaetes and  $298 \pm 27.7$  (SD) epidermal cells in control wild-type flies ( $n = 5$ ; Fig.

4). In  $N^{ts1}$  ( $n = 5$ ) pulsed between 6 and 12 hr apf, there are  $177 \pm 3.1$  microchaetes and  $83 \pm 13.5$  epidermal cells in this field. Thus, reduction of  $N^+$  activity at this stage results in an overproduction of microchaete precursor cells at the expense of epidermal cells.

#### *Reduction of $N^+$ Activity during and after Sensillum Precursor Division Leads to the Overproduction of Sensory Neurons at the Expense of Accessory Cells*

Whereas earlier (0–12 hr) heat pulses result in the appearance of large numbers of supernumerary microchaetes, most microchaetes are absent in 5-day-old pupal cuticle preparations of  $N^{ts1}$  animals subjected to a

FIG. 7. Microchaete development in  $N^{ts1}$  flies heat-pulsed between 0 and 24 hr apf. (A–E) Micrographs of cuticle preparations of adult nota. The cuticular parts of most microchaetes (i.e., shafts and sockets) are removed. There remain, however, tufts of densely packed microchaetes (arrows in A). In addition, there frequently appear microchaetes with abnormal structure: B shows a small, abnormally shaped socket without a shaft; in C, there is a socket surrounding a rudimentary shaft. (D, E) "Composite" microchaetes. In D, apparently two tormogen cells (t1, t2) produce a socket surrounding two shafts; t3 marks what looks like the cuticular part of a third tormogen cell which is not incorporated into the socket. In E, a single socket surrounds at least seven shafts; another tormogen cell (tx) lies beside the socket. Bars, 10  $\mu$ m.



**FIG. 9.** Density of the notum microchaetes of adult *N<sup>ts1</sup>* flies following heat pulses (30°C) applied at different stages of pupal development. Each diagram in A represents a schematic chart of the right half of a fly notum. Charts are subdivided into regular squares; square size was calculated to correspond approximately to the area occupied by a single microchaete on a wild-type notum. The intensity of shading of a square correlates with the average density of microchaetes at the location represented by that square, caused by applying a heat pulse during the time interval indicated at the top of the corresponding chart. A scale of shading intensities is given at the bottom right of the figure. See Materials and Methods for how microchaete densities were calculated. Large black squares indicate positions of several macrochaetes for better orientation. For names of these macrochaetes see C (adc, anterior dorsocentral; apa, anterior post-alar; asa, anterior supra-alar; pdc, posterior dorsocentral; ppa, posterior post-alar; ps, presutural; psa, posterior supra-alar). B schematically indicates that the notum is subdivided into longitudinal zones of differential sensitivity toward the reduction of *N<sup>+</sup>* function. There are two stripes with high sensitivity (thick hatching; dorsomedial stripe, dorsocentral stripe). In the dorsolateral region, there is at least one stripe (intermediate hatching) with higher sensitivity than the surrounding region (light hatching).

heat pulse between 12 and 24 hr apf (Fig. 2) (see also Shellenbarger and Mohler, 1978). Since the external cuticular apparatus (shaft and socket) of each microchaete is the product of only two of its component ells, we investigated the cellular basis for this microchaete

“loss” by analyzing the whole-mount preparations of early pupae using markers which allowed us to follow the development of all microchaete cells. In MAb 22C10-stained notum preparations of 24-hr *N<sup>ts1</sup>* pupae pulsed between 12 and 24 hr, small clusters of about four cells

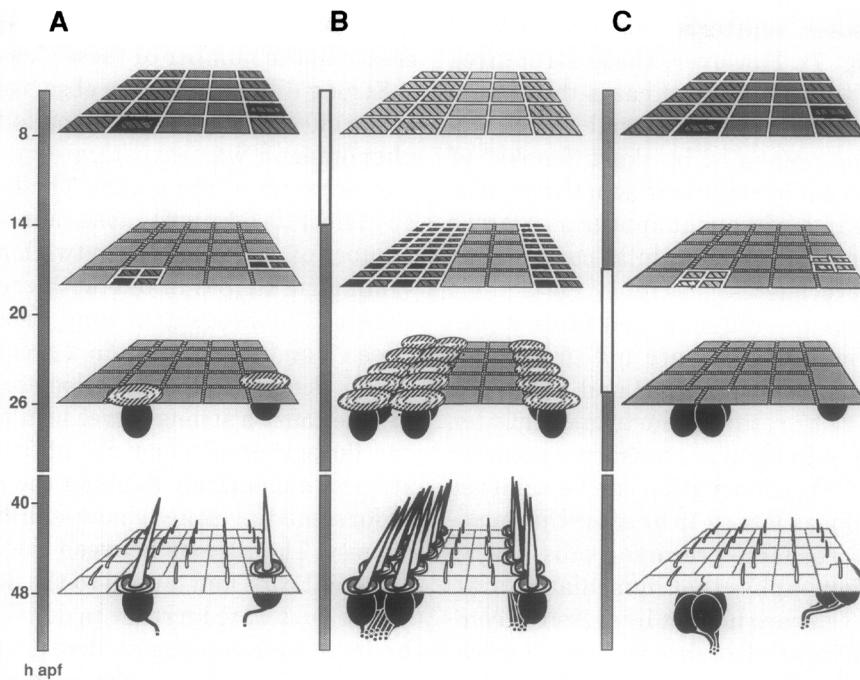


FIG. 10. Model of the role of *Notch* in microchaete development. (A) schematic illustration of four stages of microchaete development in the wild-type. Each drawing depicts a small part of the imaginal disc, where individual cells are drawn as trapezoids. The vertical bar to the left represents the time axis. Shading of this bar indicates the presence of a normal level of *N<sup>+</sup>* function throughout development. Groups of cells (hatched) become competent to develop as microchaete precursor cells (late third larval instar until 12 hr apf; upper drawing in A). The *Notch* protein is required to restrict the expression of the sensillum precursor fate to a few, selected cells (indicated by thick frames). These cells divide and give rise to four progeny, all of which are competent to develop as sensory neurons; they form miniature neurogenic regions (second drawing, hatched). In a second, *N<sup>+</sup>* dependent step the number of cells which actually express a sensory neuronal fate (black) is restricted to one. This cell segregates subepidermally, whereas the other three cells, remaining at the surface, form concentric sheaths around the neuron and develop as accessory cells (third drawing). The neuron puts out a dendrite and an axon, the accessory cells form the cuticular apparatus of the microchaete (bottom drawing). (B) Microchaete development in *N<sup>ts1</sup>* flies heat-pulsed between 0 and 12 hr apf. Due to this heat pulse, *N<sup>+</sup>* function is reduced at a stage when the number of potential sensillum precursors (upper drawing; hatched) becomes restricted to those cells which actually express this fate. As a consequence, all cells develop as microchaete precursors and divide (second drawing). *N<sup>+</sup>* function being restored after the division of the microchaete precursors, the progeny of these cells express their normal fates and develop into regular bristles (bottom drawing). In C, *N<sup>+</sup>* function is reduced at a later stage (16-24 hr) when the different cell fates become established among the progeny of the divided microchaete precursors. As a result, all of these cells develop as sensory neurons, at the expense of accessory cells and, thereby, of differentiated bristles.

appear distributed in roughly the normal microchaete pattern (Fig. 3). Frequently, cells of adjacent clusters are in contact, making it impossible to determine the exact number of cells per cluster. The clusters are composed exclusively of cells which display a neuronal phenotype: All cells are located subepidermally and possess rudimentary apical dendrites; in several instances an axon coming out of each of the cells of an individual cluster can be clearly observed (Fig. 3); in addition to MAb 22C10, all cells of an individual cluster are labeled by anti-*elav* antibody, which specifically recognizes neurons (Robinow, 1989) (data not shown). These light microscopic observations are confirmed by an electron microscopic analysis of nota of *N<sup>ts1</sup>* pupae subjected to the same 12- to 24-hr heat pulse. Ultrastructurally, the only recognizable sensillum cells are groups of subepidermally located bipolar neurons (Fig. 5). Their dendrites individually penetrate the overlying epidermal layer without reaching the apical surface of this layer. The

dendrites are not enveloped by concentric sheath processes, which in the wild-type are provided by the three accessory cells.

To confirm the absence of cells expressing an accessory cell fate, we made use of specific markers for these cell types. The P element transposon insertion A1-2nd-29, which expresses *Escherichia coli*  $\beta$ -galactosidase in both embryonic and pupal trichogen and tormogen cells (see Bier *et al.*, 1989), is not expressed in microchaete cells of 32-hr *N<sup>ts1</sup>* pupae subjected to a 12- to 24-hr heat pulse (Fig. 6). In addition, application of MAb 21A6, which is a marker for the dendritic cap secreted by the thecogen cell (Hartenstein and Posakony, 1989), shows that in 32-hr nota of *N<sup>ts1</sup>* pupae heat-pulsed between 12 and 24-hr dendritic caps (and presumably thecogen cells as well) are absent (Fig. 6).

At low frequency, we also observed what appeared to be abnormally differentiated microchaetes. Thus, in regions of the notum in which most shafts and sockets

were entirely lost, some scattered sockets without shafts were found (fig. 7). However, these structures differed from normal sockets; in most cases they had a smaller diameter and height than a normal socket. Occasionally they were accompanied by short "knobs" of cuticle material, which could represent abortive shafts. Due to their scattered and infrequent appearance it was not possible to further analyze the cellular composition of these abnormal microchaetes.

In summary, whereas the different cell fates represented in individual microchaetes are not grossly affected following heat pulses between 0 and 15 hr apf, shifts between 12 and 24 hr result in the transformation of all of the progeny of individual microchaete precursor cells into neurons. This transformation can be achieved by heat pulses beginning as late as 18 hr after the terminal division of the microchaete precursor cells has occurred. This observation implies that immediately after their birth, sensillum cells are not yet irreversibly committed to a specific differentiative fate and that a mechanism (presumably cell-cell interaction) which involves the  $N^+$  gene product is required for the decision to become a neuron versus an accessory cell.

#### *The Requirement of $N^+$ Function for Microchaete Development Is Characterized by a Distinct Spatiotemporal Pattern*

Different classes of sensilla exhibit substantial differences in their sensitivity to reduction of  $N^+$  function. For most classes of sensilla, the phenotypic response to heat pulses follows a consistent pattern: An earlier heat pulse causes an increase in sensillum number (due to supernumerary sensillum precursors), while a later pulse causes sensillum "loss" (due to the transformation of accessory cells into neurons). These early and later periods of sensitivity occur at different absolute developmental times for different classes of sensilla. Nevertheless, for those classes of adult sensilla for which data are available (bristles of the head capsule, notum, and wing margin) (Hartenstein and Posakony, 1989), it appears that these different times correspond to the same stages in their respective developmental programs. Thus, heat pulses effective in increasing sensillum number coincide with the interval immediately preceding the division of the sensillum precursor cells; heat pulses effective in causing sensillum "loss" fall in the period during and after sensillum precursor division. Some classes of sensilla do not exhibit these same two phenotypic responses to the heat pulses. For example, while pulses between 6 and 15 hr apf (i.e., during and after the terminal precursor divisions) cause a considerable "loss" of both chemosensory and mechanosensory bristles of the anterior

wing margin, no temperature regimen causes an increase in the number of these classes of bristles (Fig. 8).

Strong differences were observed in the local severity of the effect of a heat pulse applied at a given time. This phenomenon was investigated quantitatively for the microchaetes of the notum. The first pulse which yields any effect (0–6 hr apf) causes a moderate increase in the number of microchaetes in two longitudinal, bilaterally symmetric stripes in line with the dorsocentral macrochaetes ("dorsocentral zone"; see Fig. 9). Pulsing between 3 and 9 hr results in a greater increase in microchaete density in the same location. Pulses from 6 to 12 hr also cause a strong increase in microchaete density in the dorsocentral zone; in addition, two longitudinal stripes immediately flanking the midline on either side ("dorsomedial zone") now exhibit high microchaete density. The regions between the dorsocentral and dorsomedial zone and lateral to the dorsocentral zone show only a moderate increase in density. Pulses from 9 to 15 hr increase microchaete density predominantly in the dorsomedial zone. In the anterior-posterior axis, no consistent differences in the response to the reduction of  $N^+$  activity could be detected. Furthermore, it is important to note that no heat-pulse regimen resulted in the appearance of microchaetes outside the boundaries of the microchaete-containing field in wild-type flies.

Later heat pulses, leading to transformation of accessory cells into neurons and, thereby, the absence of microchaete shafts and sockets, also revealed a differential sensitivity of different parts of the notum toward reduction of  $N^+$  function at any given stage. Pulses from 12 to 18 hr apf resulted in patches of partial microchaete loss in the anterior dorsocentral zone (Fig. 9). Pulses between 14 and 20 hr yielded almost complete microchaete loss in the dorsocentral zones. Later pulses (all pulses that included the time between 16 and 24 hr) removed most microchaetes from the entire notum.

In pupae raised at the restrictive temperature throughout the first day apf, most regions of the notum were bare of microchaetes; however, there generally remained irregularly distributed "tufts" of high microchaete density (Figs. 2, 7). In addition, there were often patches of highly sclerotized, irregularly shaped cuticle close to these tufts of intact microchaetes. This observation implies that the effects of the first part of the long heat pulse (e.g., high microchaete precursor density) render some postmitotic microchaete cells insensitive to reduction of  $N^+$  activity during the second part of the pulse.

#### DISCUSSION

Reduction of  $Notch^+$  function at different stages of development has demonstrated that this locus is in-

volved in the control of at least two basic steps of sensillum development, namely, the determination of sensillum precursor cells and, subsequently, the sorting out of the neural fate versus the (nonneural) accessory cell fate. In addition, this study has revealed some interesting aspects of the cell biology of sensillum development which are discussed separately below.

#### *Notch Plays a Role during Inhibitory Cell-Cell Interaction Events among Potential Sensillum Precursors*

The somatic mosaic studies of Stern (1954, 1968) provided the first evidence for inhibitory cell-cell interaction events in *Drosophila* sensillum development. In whole flies mutant for *achaete* (*ac*), the posterior dorso-central macrochaetes do not appear. Stern found that when a mosaic patch of *ac* mutant tissue fully surrounded the normal site of these bristles, the bristle likewise failed to appear. However, when the *ac* mutant tissue included the normal bristle site but the clonal boundary passed close by, he found that a bristle often did differentiate at an ectopic site in the adjacent *ac*<sup>+</sup> tissue. Stern interpreted these observations to mean that, in the imaginal disc, bristle-forming potential is initially distributed in fields consisting of more than one cell, surrounding the normal site of each bristle. Paralleling an earlier hypothesis of Wigglesworth (1940) concerning the spacing of plaques and bristles in the hemipteran *Rhodnius*, he further suggested that a local inhibitory signal was responsible for restricting the differentiative expression of this potential to a single cell in each field.

The results presented here are consistent with an essential role for *Notch* in such a local inhibitory cell-cell interaction event. In this view, reduction of *N*<sup>+</sup> activity in the period immediately preceding the divisions of the microchaete precursor cells interferes with this inhibitory interaction and reveals an initial spatial pattern of microchaete precursor potential in the imaginal disc. This pattern can be described as follows. There exist stripe-like longitudinal zones (dorsomedial stripe, dorsocentral stripe) in which most, if not all, cells have the potential to become a microchaete precursor cell. The territory outside these stripes seems to possess a less neurogenic potential. If *N*<sup>+</sup> function is reduced, most cells in this territory still develop as epidermal cells, although the overall microchaete density, in comparison to wild-type, is increased. It should be noted, however, that the *N<sup>ts1</sup>* allele, even at the restrictive temperature, is not a null allele. Thus, it could be that the zones which we here interpret as zones of differential potential of microchaete precursor formation merely reflect zones

of differential requirement for *N*<sup>+</sup> function; according to this interpretation, the complete removal of *N*<sup>+</sup> would result in all cells of the notum developing as microchaete precursors.

#### *The Period of Notch-Dependent Cell-Cell Interaction Events among Potential Sensillum Precursors Falls into the Early Pupal Phase*

The interval in which a reduction of *N*<sup>+</sup> function causes hyperplasia of microchaete precursors at the expense of epidermal cells spans the 10–15 hr preceding the final divisions of the microchaete precursors (14–18 hr apf; Hartenstein and Posakony, 1989). Temperature pulses during the larval period have no effect on the microchaete pattern. This finding implies that the *Notch*<sup>+</sup>-dependent sorting out of microchaete precursors from epidermal precursors occurs much later than might have been expected from previous studies. In their mosaic analysis of wing imaginal disc development, Garcia-Bellido and Merriam (1971a) found that no somatic clones including both microchaetes and epidermal cells could be obtained after 40 hr before puparium formation (bpf), indicating that the last division of a cell producing both structures must take place before this time (barring the death of progeny cells). It was concluded from this observation that wing imaginal disc cells "become committed to one of these two possible pathways [microchaete precursor or epidermal precursor] by this time" (Garcia-Bellido and Merriam, 1971b). The results of the present study demonstrate, however, that a large number of cells in the disc retain the potential to be microchaete precursors much later than 40 hr bpf. Thus, the final restriction of the microchaete precursor cell fate in the notum to just those cells giving rise to wild-type microchaetes is not completed until sometime during the first 14 hr of pupal development. Furthermore, our results show that *Notch* is required for this process only after puparium formation.

#### *Notch Plays a Role during Inhibitory Cell-Cell Interaction Events among Potential Sensory Neurons*

The present findings show that heat pulses applied to *N<sup>ts1</sup>* pupae as late as 18 hr apf (i.e., after the final division of the microchaete precursors) lead to a transformation of (postmitotic) accessory cells into neurons, implying that all undifferentiated sensillum cells have the potential to become sensory neurons. One might envisage the progeny of an individual microchaete precursor as a miniature neurogenic region in which *N*<sup>+</sup> function, presumably as part of a cell-cell interaction process, is needed to establish the different cell fates expressed in a normal sensillum. In the presence of *N*<sup>+</sup> activity one cell in each miniature neurogenic region segregates as a sen-

sory neuron, while the other cells remain integrated within the epithelium and form sheaths around the emerging dendrite of the neuron.

The development of sensilla, in particular the pattern of division of sensillum precursors, has been studied on a descriptive level in numerous arthropod species (reviewed by Lawrence, 1966; Bate, 1978). It was concluded that, first, the cells of an individual sensillum form a clone (i.e., they derive from a single precursor) and, second, sensillum precursor division is "differentiative," in the sense that the various progeny of an individual precursor cell become morphologically different (in shape, position, or staining properties) during, and as a function of, sensillum precursor division. This has led to the notion that the fate of a sensillum cell is determined by its lineage. Most sensory organs in *Drosophila* do appear to derive by fixed lineages from single precursor cells (Bodmer *et al.*, 1989; Hartenstein and Posakony, 1989). There seem to be, however, exceptions. For example, no strict clonal relationship seems to exist between the cells composing the mechanosensory bristles of the anterior wing margin (Hartenstein and Posakony, 1989). Furthermore, sensillum precursor division in *Drosophila* does not seem to be differentiative in the original sense; that is, prior to sensillum differentiation, the progeny of an individual sensillum precursor appear indistinguishable in position, shape, or ultrastructure. These results suggest that cell-cell interaction between the postmitotic sensillum cells plays an essential role in sensillum development (Hartenstein and Posakony, 1989).

The findings of the present study are consistent with this hypothesis. They document that prior to onset of differentiation all sensillum cells have the potential to develop as sensory neurons. The commitment of a cell toward a (nonneuronal) accessory cell fate is either absent or "unstable" and additional, *Notch*-dependent mechanisms are required for the accessory cell fates to become firmly established.

Our results further show that the fate of a particular accessory cell can be expressed in a variety of ways. Thus, in a normal microchaete, one tormogen cell forms a complete socket surrounding the single shaft produced by the trichogen cell. In *N<sup>ts1</sup>* flies heat-pulsed between 0 and 15 hr apf, composite microchaetes were frequent in regions of high bristle density. In these bristles, the socket was evidently formed by more than one tormogen cell. In other composite microchaetes, a socket formed by a variable number of tormogen cells surrounded several shafts. These findings indicate that the differentiation of a tormogen cell is variable, in the sense that it can form a socket around a variable number of trichogen cells and can either form this socket by itself or join other tormogen cells to form a multicellular socket.

Which of these different choices a tormogen cell actually makes might depend on local conditions, such as the overall density of sensillum cells.

#### *Notch Is Involved in the Segregation of Neuroblasts and Sensory Neurons*

The segregation of postmitotic sensory neurons resembles the segregation of neuroblasts during early embryogenesis in several ways. Both processes are independent of cell division (Hartenstein and Posakony, 1989, 1990). Both neuroblasts and sensory neurons adopt an "inverted bottle" shape (Poulson, 1950) before losing contact with the apical surface of the epithelial layer from which they segregate, indicating that some kind of cytoskeletal rearrangement (e.g., constriction of an apical ring of actin filaments, as proposed for segregation of photoreceptor neurons in developing ommatidia) (Fristrom and Rickoll, 1986) might be causally involved in the segregation event. Finally, in view of the present results, it is clear that *Notch* is involved not only in restricting the number of cells expressing the neuroblast fate in the ventral neurogenic region of the embryo, but also in a similar restriction of the sensory neuron fate in the miniature neurogenic regions which appear in the imaginal disc during the early pupal period. Paralleling its phenotypic effect in the embryonic ventral ectoderm, reduction of *N<sup>+</sup>* function in the miniature neurogenic regions results in all four of their component cells adopting a neural fate at the expense of nonneural fates.

#### *Models of the Role of Notch in Sensillum Development*

The function of the *Notch* locus during *Drosophila* development has been the subject of numerous studies. Mutations at the *Notch* locus lead to a variety of phenotypes, including the originally described neurogenic effect in the embryo (i.e., transformation of epidermal precursors into neural precursors) (Poulson, 1937; Lehmann *et al.*, 1981, 1983), an increase or decrease in the number of sensory organs (Shellenbarger and Mohler, 1978; Dietrich and Campos-Ortega, 1984; Hartenstein and Campos-Ortega, 1986), defects in various other tissues [e.g., wing veins (Welshons, 1965; Portin, 1975; Shellenbarger and Mohler, 1978; this study); eye (Welshons, 1965; Shellenbarger and Mohler, 1978); legs (Shellenbarger and Mohler, 1978)], and abnormal assignment of cell fates during ommatidium development (Welshons, 1965; Cagan and Ready, 1989). No model has yet been put forward which satisfactorily integrates all of these diverse mutant effects with the available data on the molecular genetics of the *Notch* locus. However, it seems to be generally accepted that *Notch* encodes a transmembrane protein involved in cell-cell interaction and that such *Notch*-dependent interaction is involved

in regulating the fate and/or proliferatory activity of a variety of cell types throughout development.

In the present study we have analyzed the role of *Notch* during the development of adult sensilla. The results show that *Notch* is required for two important steps of this process, namely, the decision between the sensillum precursor versus the epidermal precursor fate, and the decision between the sensory neuron versus accessory cell fate. In what follows, we interpret our data in terms of two models of  $N^+$  function that, while very different in emphasis, are not necessarily mutually exclusive.

By analogy to a model proposed for the  $N^+$  function during neuroblast segregation (discussed in Campos-Ortega, 1988), it might be speculated that the extracellular portion of the *Notch* protein forms part of an inhibitory signal sent first from the presumptive sensillum precursor cells to neighboring cells with the same potential and later from the presumptive sensory neuron to the other three progeny of the precursor cell. In both cases, this signal initiates in the receiving cell a signal transduction cascade that results in the suppression of the sensillum precursor or neuronal fate, respectively. The failure of this signaling mechanism, due to reduced  $N^+$  activity, results in an excessive number of sensillum precursors and/or sensory neurons, at the expense of nonneuronal cell types.

The second model we will consider is motivated by the widespread expression of the *Notch* gene (Artavanis-Tsakonas, 1988; Kidd *et al.*, 1989) and by sequence similarities between the *Notch* protein and both platelet clotting factors (Kidd *et al.*, 1986) and cell cycle control genes (Breeden and Nasmyth, 1987). It is possible that the *Notch* protein represents part of a general cell adhesion system (Hoppe and Greenspan, 1986) required for the function of other molecules that specifically control cell fate (Cagan and Ready, 1989) and/or cell proliferation. Studies carried out primarily in vertebrate cell culture systems have shown that ubiquitous cell adhesion molecules (mediating contacts with neighboring cells or with components of the extracellular matrix) indeed play an important role in regulating cell fate and cell division (see, for example, Panayotou *et al.*, 1989). Thus, the role of *Notch* in sensillum development could be modeled in the following way (see Fig. 10). First, toward the end of larval development, a pool of cells within the imaginal disc is set apart with respect to their proliferatory behavior. These cells are capable of continuing to divide for a couple of more rounds, whereas the remaining cells stop dividing and start expressing their fate as epidermal cells. Among the cells which continue dividing are the sensillum precursor cells (which, in order to generate the four individual sensillum cells, must undergo two rounds of division). The role of the *Notch* pro-

tein might simply be to promote a type or strength of adhesiveness among cells that at this stage of imaginal disc development is capable of suppressing further mitosis in most cells. With *Notch* being expressed at a normal level, only a restricted number of cells escape the mitotic suppression and are able to divide. The pattern in which these cells are normally distributed, and which of them represent sensillum precursors, would be controlled by other, more specific factors.

The second function of *Notch* during sensillum development, namely, the restriction, among the progeny of sensillum precursors, of those cells which segregate as neurons, can be explained in similar terms. Again,  $N^+$  expression promotes adhesive cell-cell contacts among the cells of the miniature neurogenic regions and their neighbors. As a result of these cell contacts, three of the four undifferentiated sensillum cells remain an integral part of the epithelium and develop as accessory cells. Only one cell is not incorporated into the epithelium and segregates as a sensory neuron. If  $N^+$  function is reduced, all four cells fail to form appropriate contacts with each other and the surrounding epidermal cells and segregate as neurons.

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