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The function of the neurogenic genes during epithelial development in the *Drosophila* embryo

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Summary

The complex embryonic phenotype of the six neurogenic mutations *Notch, mastermind, big brain, Delta, Enhancer of split* and *neuralized* was analyzed by using different antibodies and P*lac*Z markers, which allowed us to label most of the known embryonic tissues. Our results demonstrate that all of the neurogenic mutants show abnormalities in many different organs derived from all three germ layers. Defects caused by the neurogenic mutations in ectodermally derived tissues fell into two categories. First, all cell types that delaminate from the ectoderm (neuroblasts, sensory neurons, peripheral glia cells and oenocytes) are increased in number. Secondly, ectodermal tissues that in the wild type form epithelial structures lose their epithelial phenotype and dissociate (optic lobe, stomatogastric nervous system) or show significant differentiative abnormalities (trachea, Malpighian tubules and salivary gland). Abnormalities in tissues derived from the mesoderm were observed in all six neurogenic mutations. Most importantly, somatic myoblasts do not fuse and/or form an aberrant muscle pattern. Cardioblasts (which form the embryonic heart) are increased in number and show differentiative abnormalities; other mesodermal cell types (fat body, pericardial cells) are significantly decreased. The development of the endoderm (midgut rudiments) is disrupted in most of the neurogenic mutations (*Notch*, *Delta, Enhancer of split* and *neuralized*) during at least two stages. Defects occur as early as during gastrulation when the invaginating midgut rudiments prematurely lose their epithelial characteristics. Later, the transition of the midgut rudiments to form the midgut epithelium does not occur. In addition, the number of adult midgut precursor cells that segregate from the midgut rudiments is strongly increased. We propose that, at least in the ectodermally and endodermally derived tissues, neurogenic gene function is primarily involved in interactions among cells that need to acquire or to maintain an epithelial phenotype.

Key words: neurogenic genes, *Drosophila*, epithelia, *Notch*, *mastermind, big brain, Delta, Enhancer of split, neuralized*.

Introduction

Neuronal precursors (neuroblasts) of the *Drosophila* embryo delaminate from a specialized ectodermal territory called neurogenic region. A group of genes, called neurogenic genes, were shown to control the balance between those cells of the neurogenic region that delaminate as neuroblasts and those that stay behind as epidermal precursors (Lehmann et al., 1983). Thus, embryos lacking the function of any of the neurogenic genes show an increased number of delaminating neuroblasts, at the expense of epidermal precursors. The neurogenic genes *Notch* (*N*), *big brain* (*bib*) and *Delta* (*Dl*) encode membrane proteins (Wharton et al., 1985; Kidd et al., 1986; Vaessin et al., 1987; Kopczynski et al., 1988; Rao et al., 1990). *Notch* and *Delta* have been expressed in cultured cells and mediate heterophilic adhesion between these cells (Fehon et al., 1990). *bib* shows sequence similarity to a number of different membrane proteins of both prokaryotes and eukaryotes. One of these proteins, MIP, may be involved in the formation of gap junctions. The other neurogenic loci, *mastermind* (*mam*), *neuralized* (*neu*) and *Enhancer of split* complex [*E(spl)-C*] code for nuclear proteins (Smoller et al., 1990; Boulianne et al., 1991; Knust et al., 1987; Preiss et al., 1988; Klaembt et al., 1989). There is now ample evidence for several of the different transcripts of the *E(spl)* complex to encode DNA-binding transcription factors. The predicted gene products of two of the *E(spl)-C* transcripts, m9 and m10, show sequence similarity to transducin, a G-protein involved in phototransduction (Hartley et al., 1988).

Despite the wealth of available molecular data on the neurogenic genes, the mechanism by which they act to suppress the delamination of neuronal precursors is still unclear. Experimental evidence in grasshopper neurogenesis (Doe and Goodman, 1985) suggests that neuroblasts produce an inhibitory signal that acts on their neighbours and prevents them from becoming neuroblasts as well. It has been proposed that the neurogenic genes encode proteins involved in this inhibitory cell-cell interaction pathway (for review, see Campos-Ortega, 1988; Heitzler and Simpson, 1991). Recent findings demonstrate that the requirement for *Notch* and at least several other members of the neurogenic genes during development is much more general. In both embryonic and postembryonic development, the neurogenic genes are involved in the development of sensillum precursors and sensory neurons (Hartenstein and Campos-Ortega, 1986; Hartenstein and Posakony, 1990). During eye development, *Notch* function is necessary for the photoreceptor neurons, as well as a number of different non-neural cell types (Cagan and Ready, 1989). Both *Notch* and *Delta* are involved in the development of the ovarian follicle cells of adult females (Ruohola et al., 1991). All six of the neurogenic genes play a role in the formation of the larval somatic muscles (Corbin et al., 1991). These results prompted the hypothesis that the neurogenic genes may be mediating a more universal type of interaction between cells which is required when specific signals (encoded by other genes) must pass between these cells to influence their differentiative fate (Cagan and Ready, 1989).

In order to further our understanding of the function of the neurogenic genes, a more complete picture of the developmental processes that depend on this function is necessary. We here present an analysis of the embryonic phenotype caused by all six neurogenic mutations. Most of the tissues known in the *Drosophila* embryo were investigated, using both antibodies against or P*lac*Z insertions expressed in specific embryonic organs. The rationale for this analysis was the assumption that, by comparing the different developmental processes dependent on neurogenic gene function, one might be able to define one or a few 'key characteristics' common to all of these processes. These 'key characteristics' then might lead to a more adequate definition of the cellular mechanism controlled by the neurogenic genes.

Our results demonstrate that all of the neurogenic mutants indeed show abnormalities in many developmental events, most of which had not been previously described. Tissues derived from all three germ layers were affected. Most of the defects caused by neurogenic mutations could be grouped into two categories. First, there were defects that occurred at developmental steps in which a homogeneous population of epithelial cells splits up into two subpopulations, one that remains epithelial and another one that delaminates from the epithelium and thereby loses its epithelial characteristics. If neurogenic gene function is reduced during these steps, less cells are able to remain epithelial, leading to an increased ratio of cells that delaminate. Secondly, many tissues that either transiently or permanently form epithelial structures also depend on neurogenic gene function. In these tissues, loss of neurogenic gene function leads to differentiative abnormalities of variable degree; in extreme cases (e.g., optic lobe, midgut), cells lose their epithelial characteristics entirely. Based on these findings, we propose that neurogenic gene function controls a transient morphogenetic process in which embryonic tissues, independent of their final differentiative fate, acquire or maintain an epithelial phenotype.

Material and methods

Fly stocks

Flies were cultured on standard yeast-cornmeal-molasses-agar medium. Oregon R was used as the wild-type stock. In addition, the following fly stocks were used: Df(1)N^{81K1}/FM7; N^{ts1} / FM4 (Shellenbarger and Mohler, 1975; kindly provided by Dr R. Cagan); *cn mam* ^{IB99} *bw sp* / CyO; *bib*^{ID05} *cn bw sp* / CyO; Df(3)*Dl*^{X43} / TM6b, *Hu e Tb ca*; *st e E(spl)*^{8D06} / TM3, *Sb*; *st neu*^{IIIA} *e*/ TM3, *Sb.*

Marker mutations and the balancer chromosomes are described in Lindsley and Grell (1968).

Markers used to study the phenotype of the neurogenic mutations

To study the defects in the various organs of the neurogenic mutants, specific antibodies as well transgenic fly strains that express the bacterial β-galactosidase protein in organs of interest were used. All of these strains carry enhancer detection insertions (Bier et al., 1989; Hartenstein and Jan, 1992). These insertions were introduced into the background of the different neurogenic mutations by crossing the appropriate strains to flies carrying the neurogenic mutant allele over a balancer chromosome (see above). Flies heterozygous for the insertion and the neurogenic mutation were selected from the progeny and crossed inter se. The list below indicates the combinations of insertions and neurogenic genes analyzed.

(1) A2-3-18 (peripheral glia cells (Fredieu and Mahowald, 1989) in *N, mam, bib.*

(2) A6-2-45 (optic lobe) in *N, neu, Dl, E(spl).*

(3) B2-3-20 (cardioblasts, some ventral somatic muscles, foregut and hindgut) in *N, mam, bib.*

(4) B11-2-2 (midgut, periligament cells, peritracheal cells, and midline cells) in *N, neu, Dl, E(spl).*

(5) B6-3-23 (somatic musculature) in *N.*

(6) E7-3-63 (fat body, cardioblasts, and pericardial cells) in *N, mam, bib.*

(7) E2-3-9 (oenocytes and fat body) in *N.*

(8) B12-3-3 (gonad sheath cells) in *N.*

(9) C8-3-5 (dorsomedial cells (subset of midline cells) in *N.*

In addition, a recombinant chromosome carrying the insertion B2-3-20 and *Df(3)Dl*^{X43} was generated.

The following antibody markers were used. (1) Monoclonal antibody Cq4 against the *crumbs* protein (Tepass and Knust, unpublished), which is expressed on the apical membrane of ectodermally derived epithelia, including the tracheae, foregut, hindgut, Malpighian tubules, salivary gland, stomatogastric nervous system and optic lobe (Tepass et al., 1990); (2) monoclonal antibody 22C10 (Zipursky et al., 1984), which labels sensory neurons; (3) monoclonal antibody 6D6 (Zipursky et al., 1984), which labels the visceral myoblasts, epidermal cells and accessory cells of the sensilla; (4) polyclonal antibody against the *asense* gene product (Brand et al., unpublished), which labels neuroblasts, sensillum precursors, sensory neurons and adult midgut precursor cells; (5) polyclonal antibody against muscle myosin (Kiehart and Feghali, 1986), which labels somatic muscles, visceral muscles and cardioblasts.

Heat-pulse experiments

Heat pulses were applied to flies carrying N^{ts1} over a small *N* deletion (D(1)N^{81K1}). The transheterozygotes were routinely kept at 22°C. Embryos were collected on yeasted apple agar plates at 22°C. At appropriate stages embryos were heat pulsed by placing the plates in a chamber at 31°C for 2 hours. Wild-type embryos (Oregon R) subjected to the same temperature regimens served as controls.

Antibody labeling

Embryos were collected, dechorionated and fixed for 30 minutes in a mixture of 4% formaldehyde in PEMS(0.1 M Pipes, 2 mM

$MgSO_4$, 1 mM EGTA, pH 7.0) with heptane. They were devitellinized in methanol and further prepared for antibody labeling following the standard procedure (e.g., Ashburner, 1989). Antibodies used were mAb22C10 and 6D6 (kindly provided by Dr S. Benzer) and anti-β-galactosidase (Cappel).

Electron microscopy

Embryos were dechorionated, fixed in 12.5% glutaraldehyde in PBS and heptane for 20 minutes, then placed on double-sided tape and devitellinized by hand. Embryos were further prepared for electron microscopy as described in a previous paper (Hartenstein, 1988).

Application of bromodeoxyuridine (BrdU)

The base analogue BrdU, which is incorporated into replicating DNA, was applied by permeabilizing staged, dechorionated embryos with octane (Sigma) for 3 minutes and spreading them on BrdU-containing Grace medium (1 mg/ml). Embryos were allowed to develop at 25°C for specific times, then collected and fixed for 30 minutes in a mixture of 4% formaldehyde in PEMS(0.1 M Pipes, 2 mM $MgSO_4$, 1 mM EGTA, pH 7.0) with heptane. Next they were devitellinized in methanol. After several washes in PBT, embryos were incubated for 35 minutes in 2 N HCl to denature the DNA. After this step, they were washed for 30 minutes in several changes of PBT. The preparations were then incubated for 1 hour in PBT+N, followed by an overnight incubation in a monoclonal antibody against BrdU (Beckton-Dickinson) at a dilution of 1:50. For further steps of antibody labelling, see Ashburner (1990)

Results

The requirement of N *during ectoderm development*

Within a short period of time following gastrulation, the ectoderm gives rise to several different organ primordia (Fig. 1). Some of these separate from the ectoderm by delamination, a process in which individual cells move out of the ectodermal epithelium. Ectodermally derived pre-

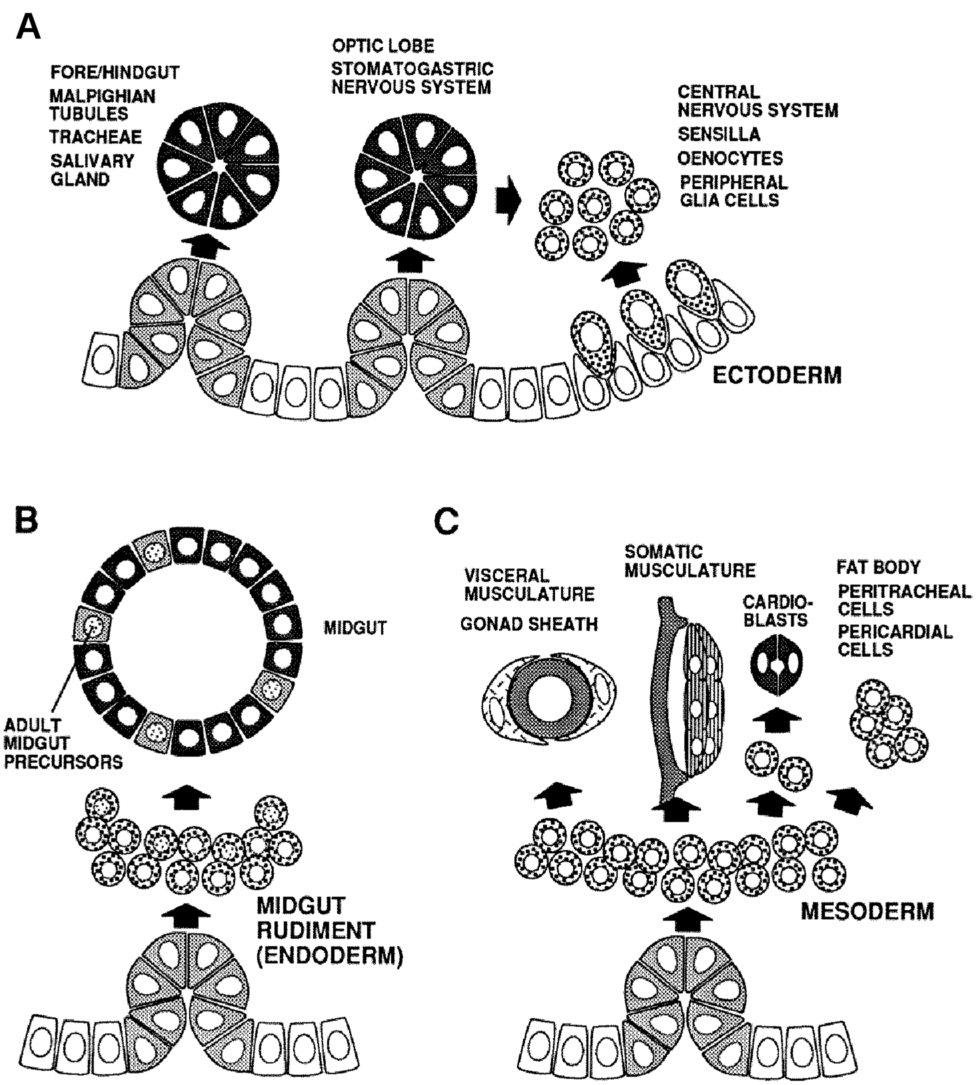
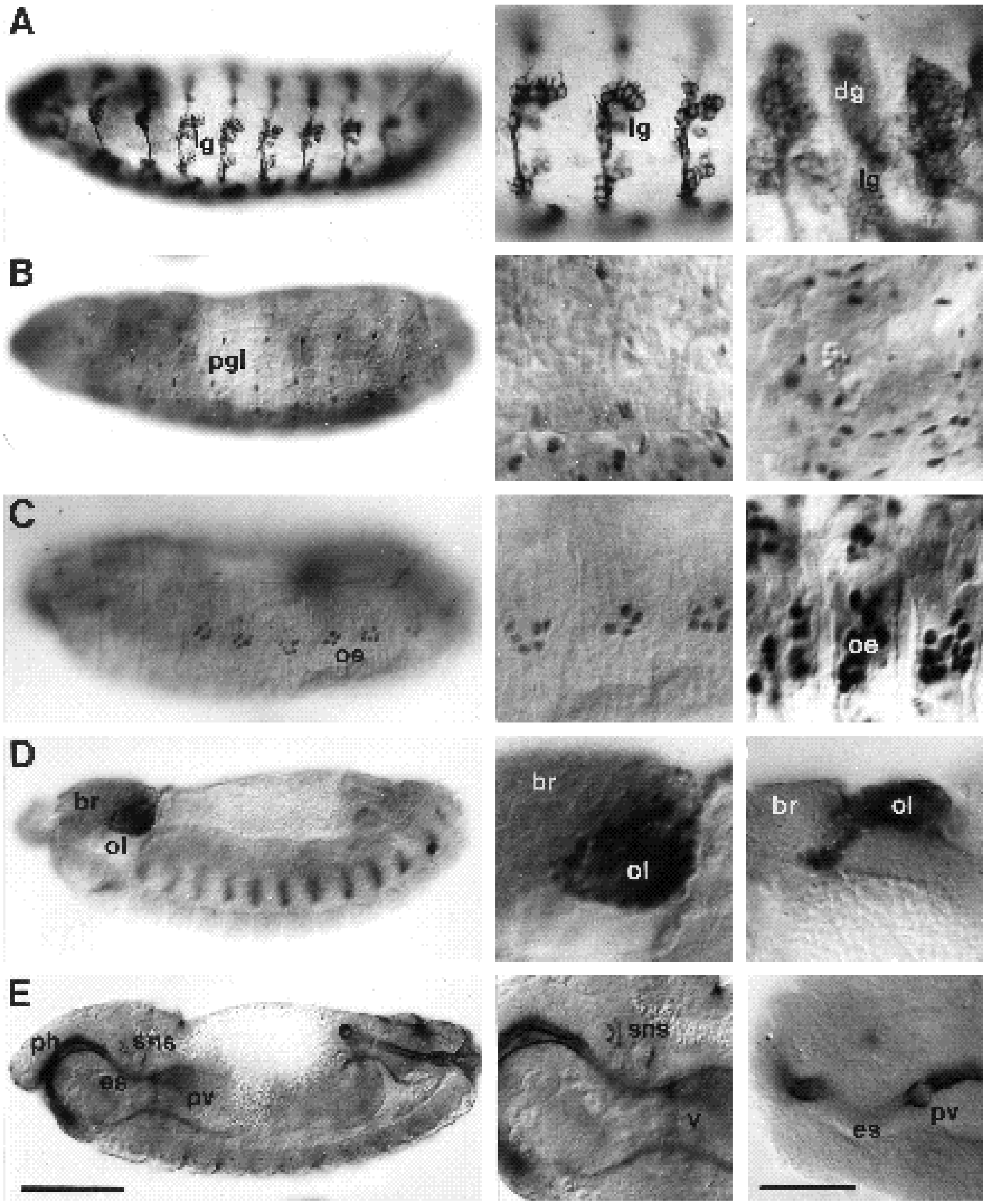


Fig. 1. Diagram illustrating the segregation of different organs from the ectoderm (A), endoderm (B) and mesoderm (C) in wild-type development. For the ectoderm, three different modes of segregation are shown: some epithelial organs (indicated by dark shading) originate by invagination (left of diagram); precursors of the optic lobe and stomatogastric nervous system (middle) also invaginate and transiently form epithelia (hatched) that later dissociate into individual neural precursors (stippled). Precursors of the CNS, oenocytes, peripheral glia cells and sensory neurons (stippled; right part of diagram) delaminate. The endoderm (B) arises by invagination. Soon after gastrulation, the endodermal cells lose their epithelial characteristics (symbolized by shading) to form the midgut rudiments (stippled). Cells of the midgut rudiments reorganize into an epthelium during stage 13. Most cells develop into larval midgut cells (dark shading). A subpopulation of cells become the adult midgut precursors (light shading), which are transiently incorporated into the larval midgut epithelium. The mesoderm also arises by invagination and later becomes reorganized into a loose layer of non-epithelial cells (stippled). This layer splits up into a variety of different tissues. For further details, see text.



cursors that delaminate are the neuroblasts, sensory neurons, oenocytes and peripheral glia cells. All of these cells originate in an invariant spatiotemporal pattern, described in previous studies in detail for the neuroblasts and sensory neurons (Hartenstein and Campos-Ortega, 1984; Ghysen et al., 1986; Hartenstein, 1988). Oenocytes and peripheral glia cells delaminate from a circumscribed region of the lateral ectoderm during embryonic stage 12 (staging according to Campos-Ortega and Hartenstein, 1985). Oenocytes, which form clusters of 4-7 cells in each abdominal hemisegment, remain attached to the basal surface of the epidermis close to the position where they delaminated (Hartenstein and Jan, 1992). The peripheral glia cells (Fredieu and Mahowald, 1989) also delaminate from the posterior-lateral ectoderm of each segment and become associated with the segmental nerve (own unpublished observation).

In *N* mutant embryos, all of the cell types that originate by delamination are increased in number (Fig. 2). This has been well documented in previous studies for the neuroblasts (Lehmann et al., 1983). Thus, the entire ventral and anterolateral part of the ectoderm (ventral neurogenic region and procephalic neurogenic region, respectively) is

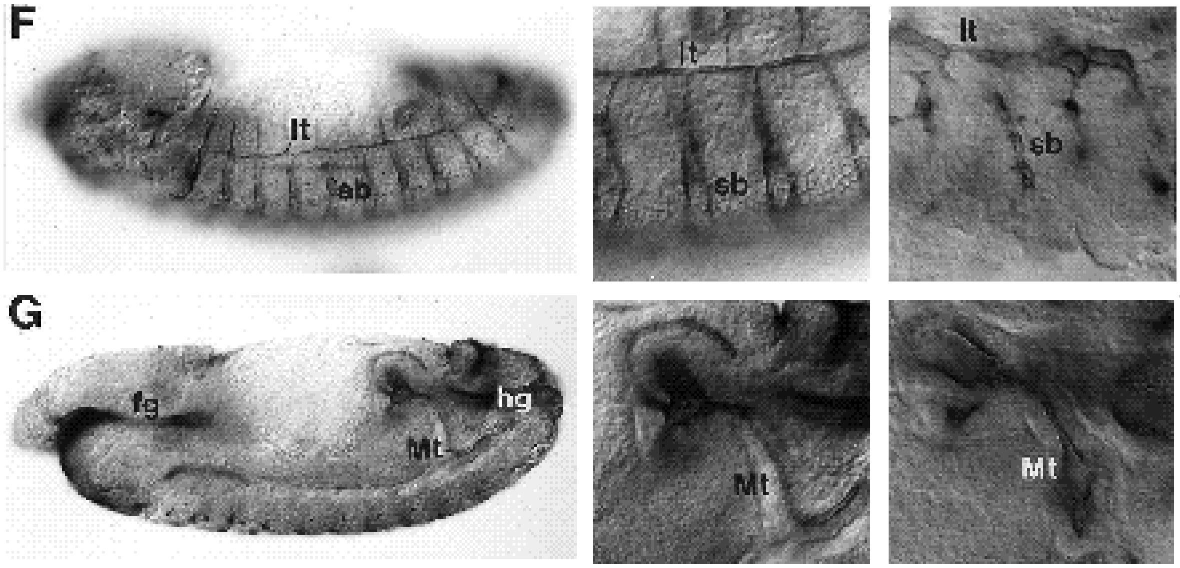


Fig. 2. *Notch*-related defects in ectoderm development. A-H show ectodermally derived organs, labeled by P*lac*Z expression or antibody markers, in wild-type (left column: low magnification view; middle column: high magnification) and hemizygous $Df(1)N^{81K1}$ mutant embryos (right column). Embryos vary in age between stage 13 (D, G), 14 (E, F) and 16 (A, B, C). All panels show lateral views; anterior is to the left, dorsal to the top. A shows sensory neurons of the lateral group (*lg*) and dorsal group (*dg*), stained with mAb22C10. In $Df(1)N^{81K1}$, this cell type is increased in number. Similarly, peripheral glia cells (*pgl*, shown in B; labeled by P*lac*Z insertion A2-3-18) and oenocytes (*oe*, shown in C; labeled by P*lac*Z insertion E2-3-9) are increased in the *N* mutant background. D shows the optic lobe (*ol*, labeled by P*lac*Z insertion A6-2-45) which in wild type forms an epithelial vesicle attached to the brain (*br*). In $Df(1)N^{81K1}$, optic lobe cells are present in approximately normal numbers, but they have lost their epithelial characteristics. E shows the foregut, consisting of pharynx (*ph*), oesophagus (*es*) and proventriculus (*pv*). Anti-*crb* antibody was used to label the apical surface of the foregut epithelium. In addition, the three vesicles constituting the primordium of the stomatogastric nervous system (*sns*) are labeled. In $Df(1)N^{81K1}$, proventriculus and oesophagus develop quite normally. Most cells of the pharynx are neuralized and contribute to the hyperplastic brain. Precursors of the stomatogastric nervous system do not form epithelial vesicles. In F, the cells forming the longitudinal trunk (*lt*) and the segmental branches (*sb*) of the tracheal tree (labeled with anti-*crb* antibody) are shown. In $Df(1)N^{81K1}$, epithelial cells are decreased in number. They form rudimentary tubes which exhibit many defects in fusion and branching. G depicts the hindgut (*hg*) and Malpighian tubules (*Mt*), stained with anti-*crb* antibody. These structures develop into epithelia in $Df(1)N^{81K1}$ mutant embryos, although they show differentiative defects (note irregularly shaped lumen of Malpighian tubule shown in right panel). Occasionally, short supernumerary branches are present. Bars: 100 μm (panels of left column); 40 μm (panels of middle and right column).

converted into neuroblasts, at the expense of the epidermal precursors, which would normally develop from these regions.

Supernumerary sensory neurons delaminate at the expense of accessory cells, similar to what has been described for sensillum development in N^{ts1} pupae raised at the restrictive temperature (Hartenstein and Posakony, 1990). In normal embryonic development, only one cell per sensillum cell cluster delaminates and becomes a sensory neuron. In *N*, all sensillum cells become sensory neurons, and no accessory cells appear. Labeling of Df(1)N^{81K1} mutant embryos with the sensory-neuron-specific mAb 22C10 shows the strongly increased number of these cells (Fig. 2A); mAb 6D6 staining (revealing the circum-dendritic sheaths formed by accessory cells) is absent (data not shown).

Peripheral glia cells (Fig. 2B, *pgl*) and oenocytes (Fig. 2C, *oe*) are increased by a factor of 2-3. This is less clear in $Df(1)N^{81K1}$ mutant embryos, where these cells are located in irregular clusters interspersed in between the hyperplastic CNS and sensory neurons, than it is in N^{ts1} /$Df(1)N^{81K1}$ embryos subjected to 2 hour heat pulses (see below).

Cells that are also strongly affected by the loss of *N* function are the precursors of the sensilla (Hartenstein and Campos-Ortega, 1986; Ghysen and O'Kane, 1989). These cells can be defined by their invariant position and division pattern in each segment of the stage 11 embryo (Ghysen and O'Kane, 1989). While dividing into the individual sensillum cells (see above), most sensillum precursors remain integrated within the ectodermal epithelium, although their nucleus shifts basally. In $Df(1)N^{81K1}$ mutant embryos, sensillum precursors are increased in number. The analysis of $Df(1)N^{81K1}$ mutant embryos in which epidermis cells were labeled with the enhancer trap line indicates that the increase of sensillum precursors happens at the expense of epidermis cells (data not shown).

Precursors of the optic lobe and the stomatogastric nervous system (SNS) segregate from the ectoderm by invagination (stage 12). Here, instead of individual cells sliding out of the ectodermal epithelium, coherent patches of cells constrict at their apical pole and fold in (Poulson, 1950). Following the invagination process, cells maintain their epithelial characteristics and form hollow vesicles. In the case of the stomatogastric nervous system, three pouches evaginate from the roof of the oesophageal primordium (Poulson, 1950). Around stage 13, these pouches separate from the oesophagus and form transient vesicles. The vesicles then dissociate into apolar cells which give rise to the neurons forming the stomatogastric ganglia (stage 14). Some of the SNS precursors delaminate from the vesicles already at an earlier stage (own unpublished results). The vesicle forming the optic lobe invaginates from the posterior head region. It remains epithelial throughout embryogenesis and early larval life (Green et al., 1992).

In *Df(1)N^{81K1}* mutant embryos, cells with the identity of SNS and optic lobe precursors develop at approximately normal numbers, but they do not form epithelial vesicles. Instead, these cells appear as solid, irregular clusters of apolar cells (Fig. 2D, *ol*; see also Green et al., 1992).

The remaining ectodermally derived organs form permanent epithelia which arise by invagination (Fig. 1). They are the foregut and hindgut, salivary glands, Malpighian tubules and trachea. The salivary glands and part of the foregut (pharynx) and trachea, which originate from within the neurogenic ectoderm, are converted into neuroblasts in *Df(1)N^{81K1}* mutant embryos. The remaining structures form epithelial tissues in the absence of zygotic *N* function, although at least the trachea (Fig. 2F, *tr*; Fig. 3C,D) and Malpighian tubules (Fig. 2G, *Mt*) exhibit severe abnormalities in their differentiation.

The requirement of N *function during endoderm development*

The midgut epithelium develops during stage 13 from the two midgut rudiments, solid clusters of apolar cells (Fig. 4A,B). Scattered among the larval midgut cells are the adult midgut precursors (AMPs). These cells become distinct from the larval midgut cells already at stage 11 (Fig. 4E; see Hartenstein and Jan, 1992). During stage 13, these cells form a layer on the apical surface of the developing midgut epithelium. During late embryogenesis, the AMPs are transiently incorporated into the larval epithelium. Later in embryogenesis, the AMPs shift to the basal surface of the larval midgut cells and resume proliferation. In the present study, we used a monoclonal antibody raised against the *asense* (T8) gene product, which in addition to the central and peripheral nervous system also stains the AMPs (Brand et al., unpublished data).

In *Df(1)N^{81K1}* mutant embryos, both larval midgut cells and AMPs appear. The small cell size and high packing density of the midgut cells in *Notch* mutant embryos made it impossible to perform accurate cell counts. It is clear, however, that these cells (including larval midgut cells and AMPs) are at least equal in number to the wild-type midgut cells (Fig. 4C,D). Furthermore, the number of AMPs is strongly increased at the expense of the larval midgut cells (Fig. 4F).

At later stages (13, 14), the midgut precursors do not undergo the transition into an epithelium. Instead, they retain the apolar phenotype typical for the wild-type midgut rudiments (Fig. 4C,D). The yolk in *Df(1)N^{81K1}* mutant embryos is only surrounded by the yolk membrane. Typically, anterior and posterior midgut rudiment do not fuse.

The requirement of N *in mesoderm development*

The mesoderm forms by the invagination of the midventral part of the blastoderm (ventral furrow). After gastrulation, mesodermal cells lose their epithelial phenotype and spread out as an irregular layer at the basal surface of the ectoderm. During stages 11 and 12, the mesoderm splits up into several different organ primordia. This process is not well understood, nor do we have a complete picture of all the cell types and organs deriving from the mesoderm. In the early stage 12 embryo, the following spatially separate mesodermal cell populations have been identified histologically and/or by specific markers (Fig. 1; see Hartenstein and Jan, 1992).

(1) Precursors of somatic musculature are represented by most cells of the outer layer of the mesoderm; these cells become organized into elongated clusters of myoblasts which fuse into syncytial muscle fibres.

(2) Precursors of visceral musculature form a narrow band of cells laterally in the inner layer of mesoderm. These myoblasts remain individual cells; they become long fibres forming a complete layer around the gut.

(3) Precursors of the fat body develop from the ventral inner mesoderm layer; later they form a loose sheet of cells in between the somatic muscles and the gut.

(4) Precursors of the heart (cardioblasts, pericardial cells) are derived from a narrow band of two to three cell rows in the lateralmost mesoderm.

(5) Peritracheal cells and periligament cells (Hartenstein and Jan, 1992) arise as individual, segmentally repeated cells in the outer mesoderm layer.

(6) Gonad sheath cells derive from the inner mesoderm layer of the posterior abdominal segments.

In *Df(1)N^{81K1}* mutant embryos, abnormalities occur in several of these cell populations (Fig. 5). As described by Corbin et al. (1991), the precursors of the somatic muscu-

Fig. 3. TEM micrographs of transverse sections showing *Notch*-related defects in the heart, trachea and midgut. A and B compare the cardioblasts (*cb*) of a stage 17 wild-type embryo (A) and *Df(1)N^{81K1}* embryo (B). Note single lumen (*lu*), lined by two cardioblasts which secrete extracellular material at their luminal (arrow) and abluminal surface. In a *Df(1)N^{81K1}* embryo of comparable age (B), cardioblast-like cells (*cb*) are increased in number and form an irregular cluster. Multiple, irregular clefts lined with extracellular material (arrows) appear in this cluster. They may represent ‘attempts’ of the cardioblast-like cells to form a lumen. C and D compare a small tracheal branch (*tr*) of a wild-type (C) and *Df(1)N^{81K1}* (D) embryo. In wild type, a single trachea cell folds around and thereby forms a lumen (*lu*; note prominent adherens junctions, marked by arrowheads, at the position where the two opposing ends of the trachea cell contact each other). In *Df(1)N^{81K1}*, the upper half of the lumen is formed by an unusually shaped trachea cell (secreting cuticle); this cell is contacted by several tracheole cells with multiple intracellular lumina (large arrows). In wild type, tracheole cells are slender tubes with a single lumen (arrow in C). E and F compare the structure of the midgut in a stage 16 wild-type (E) and *Df(1)N^{81K1}* (F) embryo. In wild type, the midgut epithelium (*mg*) is formed by a monolayer of flattened cells surrounded by a layer of visceral muscles (*vm*). Both apically and basally, the midgut cells at this stage form processes (arrows) that connect neighbouring cells. In*Df(1)N^{81K1}*, similar processes at the edges of the midgut cells can be seen (arrows); however, midgut cells are clustered in a solid mass of cells, which fails to form a monolayered epithelium. Bars: 2 μm (Bar in A applies for A-D; Bar in F applies to E, F).

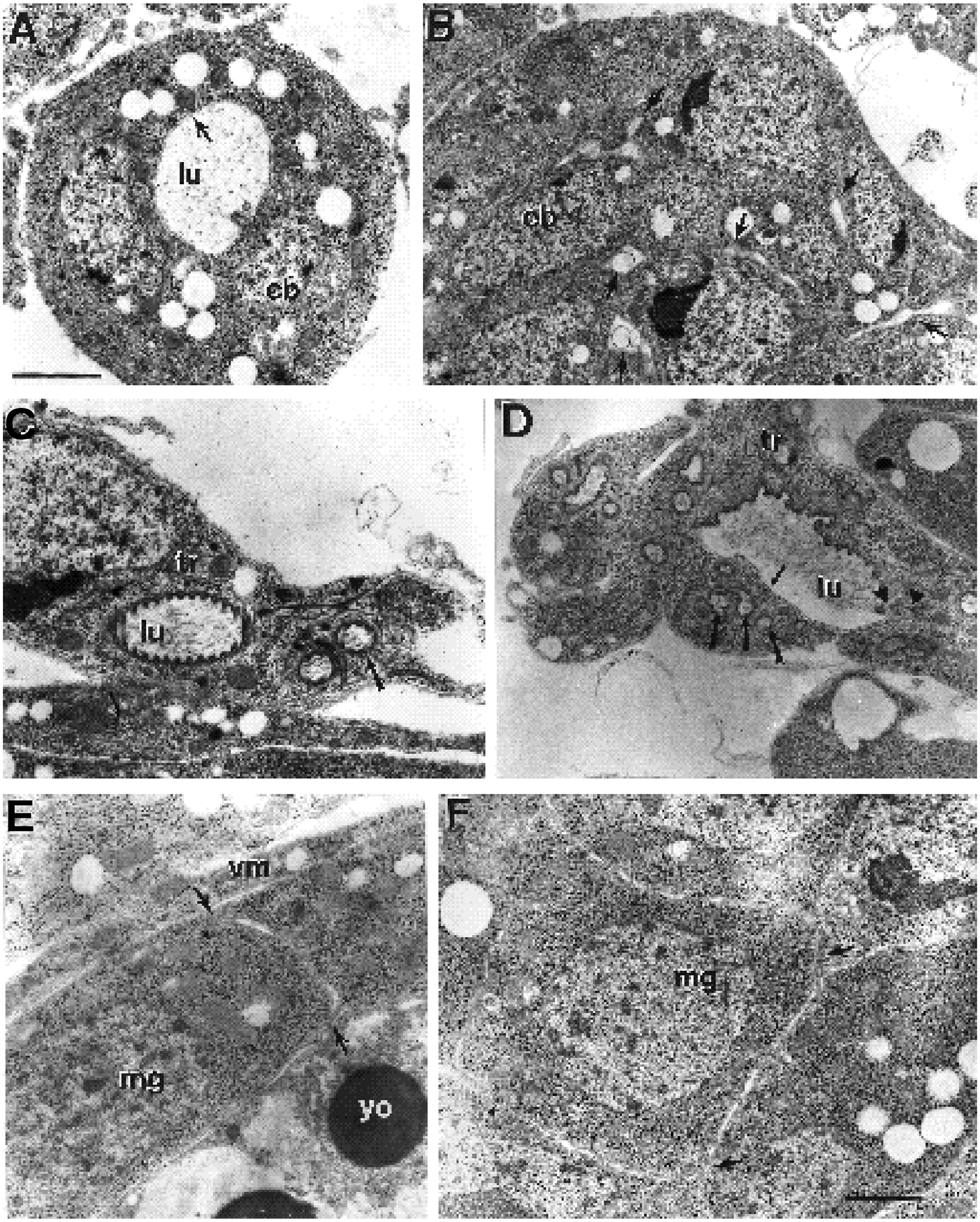


Fig. 3

lature apparently do not fuse into multinucleate fibres (Fig. 5A, *sm*). Visceral muscle cells develop at roughly normal numbers (Fig. 5B). Furthermore, in a stage 12/13 *Df(1)N^{81K1}* mutant embryo, these cells show a relatively normal arrangement, forming two longitudinal bands adjacent to the midgut rudiments. Later, visceral muscle cells located at mid-levels of the embryo show severe pattern abnormalities, possibly due to the absence of a midgut epithelium to which they normally become attached. Only occasionally, visceral muscle fibres attach to the yolk membrane. On the other hand, the visceral musculature surrounding the foregut and hindgut appears relatively normal.

Peritracheal cells and periligament cells are also increased in number. In wild-type embryos, in each hemisegment there exists one periligament cell (Fig. 5C, *pl*;

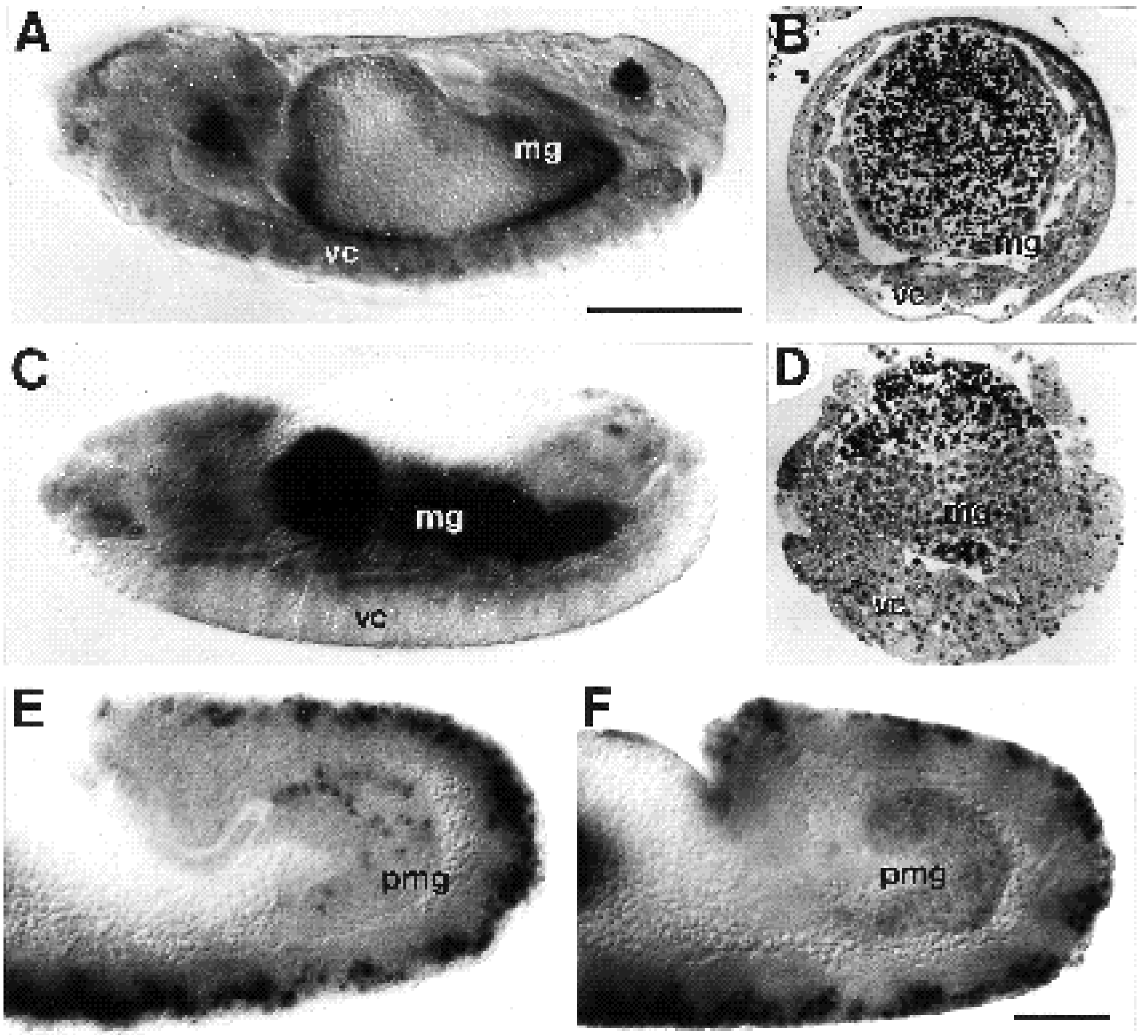


Fig. 4. *Notch*-related defects in endoderm development. A-D compare the midgut structure in a stage 15 wild-type (A: lateral view; B: transverse section) and *Df(1)N*^{81K1} embryo (C: lateral view; D: transverse section). Midgut cells (*mg*) are labeled by their expression of the P*lac*Z insertion B11-2-2. Note that in *Df(1)N*^{81K1}, midgut cells do not form an epithelium. E and F show the posterior half of a stage 12 wild-type (E) and *Df(1)N*^{81K1} (F) embryo in which the adult midgut precursors present in the posterior midgut rudiment (*pmg*) are labeled with anti-*asense* antibody. Note increased number of labeled cells in*Df(1)N*^{81K1}. Counts from whole mounts of five wild-type and the same number of *Df(1)N*^{81K1} mutant embryos yielded a 3- to 4-fold increase in the number of AMPs in the mutant. Other abbreviations: *vc*, ventral nerve cord. Bars: 100 µm (A-D); 40 µm (E,F).

Hartenstein and Jan, 1992) and three to five peritracheal cells (attached to the segmental branches of the trachea). In *Df(1)N*^{81K1} mutant embryos, the number of these cells is increased by a factor of at least 2-3.

Among the mesodermally derived tissues that are affected strongest by a lack of *N* function is the heart (dorsal vessel). In wild type, the heart forms a simple tube, lined by a regular double row of myoendothelial cells called cardioblasts (Fig. 5D, *cb*). These cells express muscle-myosin. Attached to the cardioblasts on either side is one row of pericardial cells, non-polarized cells, which do not express myosin (Fig.5D, *pc*). In a *Df(1)N*^{81K1} mutant embryo, the number of heart cells is strongly increased (approximately four-fold). All cells express the markers characteristic for cardioblasts (myosin, P*lac*Z insertion B2-3-20) and no pericardial cells develop. However, the cardioblast-like cells do not form a regular tube. Instead, they are organized into a densely packed cluster of cells. EM analysis shows the formation of multiple, irregularly scattered clefts, lined by a thin layer of extracellular material (which also appears in the lumen of the wild-type heart; see Fig. 3A,B). These clefts might represent the attempt of irregularly shaped cardioblasts to form a lumen.

The fat body of *Df(1)N*^{81K1} mutant embryos forms a loose cellular sheet underlying the body wall. Phenotypically, these cells appear like in a wild-type embryo (Fig. 5E, *fb*). However, cell counts yielded a significant reduction in cell number (approximately 400 cells on each side, compared to about 800 cells in wild type). Possibly the supernumerary cells recruited into the heart and the populations of periligament and peritracheal cells correspond to those missing from the fat body.

Cells of the gonad sheath (i.e., the precursors of the follicle cells) appear normal in number and phenotype in *Df(1)N*^{81K1} mutant embryos (Fig. 5F, *gs*).

The pattern of postblastoderm proliferation is not substantially altered in N *mutant embryos*

Df(1)N^{81K1} mutant embryos of various stages were incubated for 1 hour in BrdU-containing medium, followed by anti-BrdU antibody staining to visualize the pattern of cells that had incorporated BrdU at these particular stages. Using this approach, the pattern of postblastoderm proliferation could be reconstructed. The experiments showed that the pattern of cell proliferation in *Df(1)N*^{81K1} mutant embryos is similar to that described for wild type (Hartenstein and Campos-Ortega, 1985). For example, most epidermal precursors undergo two rounds of division during stages 8-10 (data not shown); at stage 11, there is a third wave of division affecting selected subsets of ectodermal cells, in particular the sensillum precursors (Fig. 6B,D). No supernumerary rounds of division were seen in *Df(1)N*^{81K1} mutant embryos. During stages 12-14, specific territories of the gut, salivary glands and Malpighian tubules undergo a round of endoreplication (Smith and Orr-Weaver, 1991). This is also observed in *Df(1)N*^{81K1} mutant embryos (Fig. 6A,B).

Phenocritical periods of N *function during embryogenesis*

2 hour heat pulses (31°C) were applied to *N*^{ts1} /*Df(1)N*^{81K1} embryos of different ages that also carried a variety of different P*lac*Z insertions. The results are summarized in Table 1.

The strongest hyperplasia of the CNS was achieved by heat pulses between 2 and 6 hours. Oenocytes and peripheral glia cells were increased after heat pulses between 4 and 8 hours; sensory neurons were affected between 8 and 10 hours (Fig. 7B). These phenocritical periods are consistent with the developmental stages at which the four different cell types delaminate from the ectoderm (Table 1). An effect on sensillum precursor cells could be noticed with pulses between 4 and 8 hours. The loss of epithelial structure of the optic lobe and SNS was observed with heat pulses later than 5 hours (see also Green et al., 1992).

Abnormalities of several of the remaining epithelial derivatives of the ectoderm (trachea, Malpighian tubules and salivary gland) resulted from heat pulses applied between 6 and 9 hours, the stage at which important morphogenetic movements shaping these tissues take place. The defects in the trachea and Malpighian tubules (Fig.7D, *Mt*) following such heat pulses qualitatively resembled those described for the homozygous *Df(1)N*^{81K1} mutant embryos (see above), although they were usually milder. Since heat pulses starting later than 6 hours left the ventral neurogenic ectoderm intact, the effect of *N* on salivary gland differentiation could be studied (Fig. 7C). In embryos pulsed between 6 and 9 hours approximately the normal number of cells was incorporated into the salivary gland. Salivary gland cells also adopted their normal, cuboideal-epithelial configuration. However, similar to the Malpighian tubules, irregularities in the diameter of the lumen were apparent. Most common was a constriction that subdivided the body of the salivary gland into a small, posterior segment and a larger anterior segment.

Table 1. *Phenocritical periods of N function in different embryonic organs*

	0 - 2	2 - 4	4 - 6	6 - 8	8 - 10	10 - 12
NEUROBLASTS		N▲	N▲	N▲		
SENSILLUM PRECURSORS			N▲	N▲		
SENSORY NEURONS					N▲	
PERIPHERAL GLIA CELLS			N▲	N▲		
OENOCYTES			N▲	N▲		
OPTIC LOBE				ED	ED	
STOMATOGAS. NERVOUS SYS.				ED		
SALIVARY GLAND		A	A	D		
FOREGUT		a	a			
MALPIGHIAN TUBULES				D		
TRACHEA				D		
ENDODERM	ED					
LARVAL MIDGUT				ED	ED	
SOMATIC MUSCULATURE			D	D		
CARDIOBLASTS					N▲	
PERICARDIAL CELLS				N▲	N▼	
PERILIG.&PERI-TRACH. CELLS			N▲	N▲		
DORSOMEDIAL CELLS		N▲	N▲			

Listed are the different organs for which *N* related defects were observed after applying heat pulses to *N*^{ts1} /*Df(1)N*^{81K1} mutant embryos. The horizontal axis represents time (scale gives hrs after fertilization at 22°C). Letters indicate the type of defects resulting from 2 hour heat pulses (31°C) applied at the corresponding time intervals. Letters stand for: *A*, organ totally absent; *a*, organ partially absent; *D*, differentiative defect; *E*, loss of epithelial structure; *N*, numerical defect (*N* with upward arrowhead: increase in number; *N* with downward arrowhead: decrease in number). Size of letters indicate severe defects (large size) versus mild defects (small size). Differential toning of horizontal bars indicates prominent morphogenetic events during the development of the corresponding organs. Grey shading shows the approximate developmental stage at which the corresponding organ segregates from one of the germ layers; black filling indicates that cells form an epithelium; stippling indicates that cells are non-epithelial.

The differentiation of the midgut epithelium could be affected with pulses starting later than 6 hours, which is consistent with the stage at which the midgut rudiments undergo their transition into an epithelium (8-10 hours). None of the experiments resulted in quite such a strong midgut phenotype as that presented by the *Df(1)N*^{81K1}

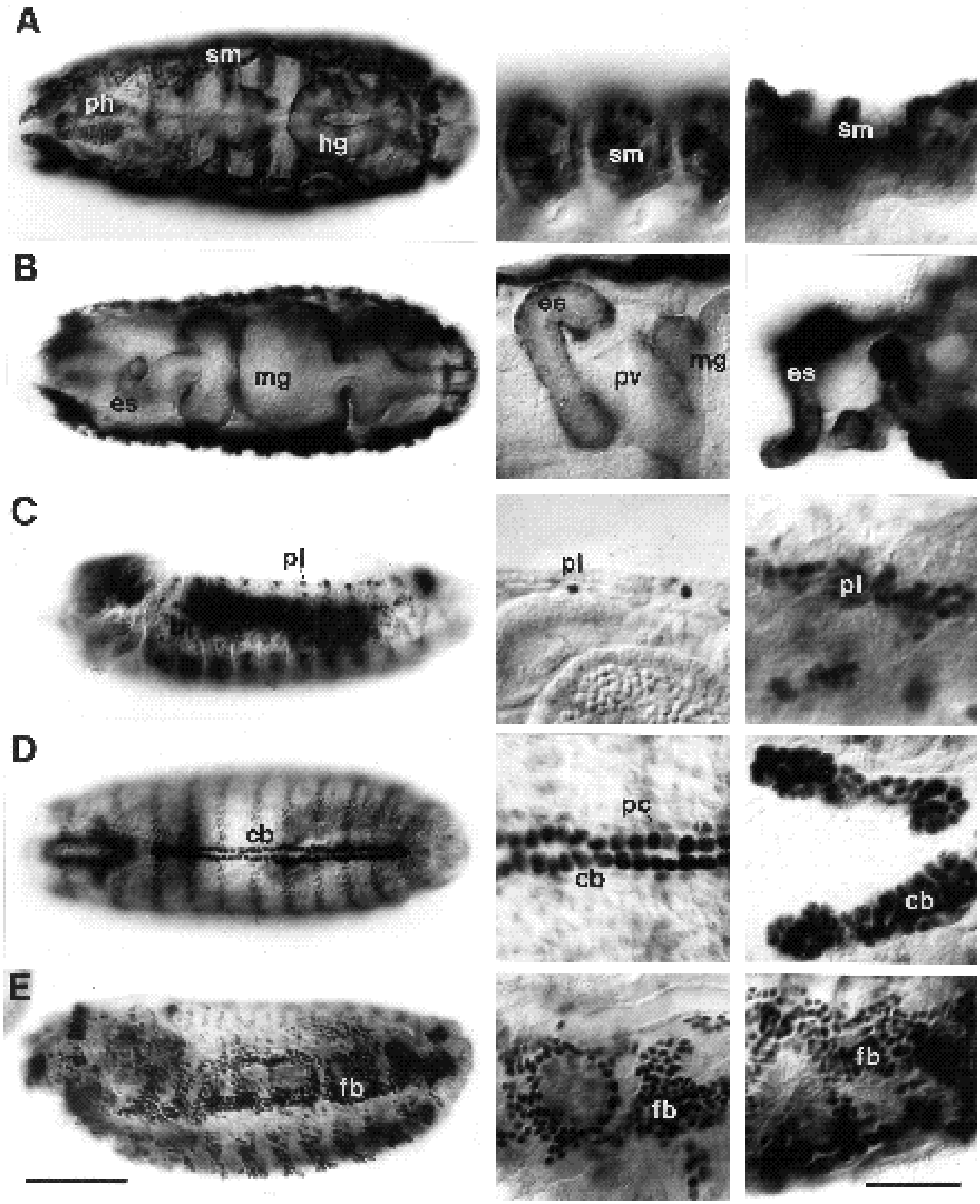


Fig. 5A-E

mutant embryos. Typically, some segments of the midgut, in particular the posterior segments, showed a multilayered, irregular epithelium. In the same embryos, gaps appeared at other positions in the midgut (data not shown). Furthermore, the normal pattern of midgut constrictions frequently showed abnormalities.

A surprising observation was that the early endoderm during its invagination from the blastoderm requires *N* function. Thus, in embryos heat pulsed between 0 and 4 hours and fixed at about 6-8 hours, the anterior midgut rudiment remained as an irregular cluster of cells in the ventral head ectoderm, posterior to the stomodeum which invaginated normally (Fig. 7F, *amg*). Also the posterior midgut rudiment lost its epithelial structure prematurely, although it seemed to invaginate from the posterior blastoderm normally (Fig. 7F, *pmg*). No significant defects in the forma-

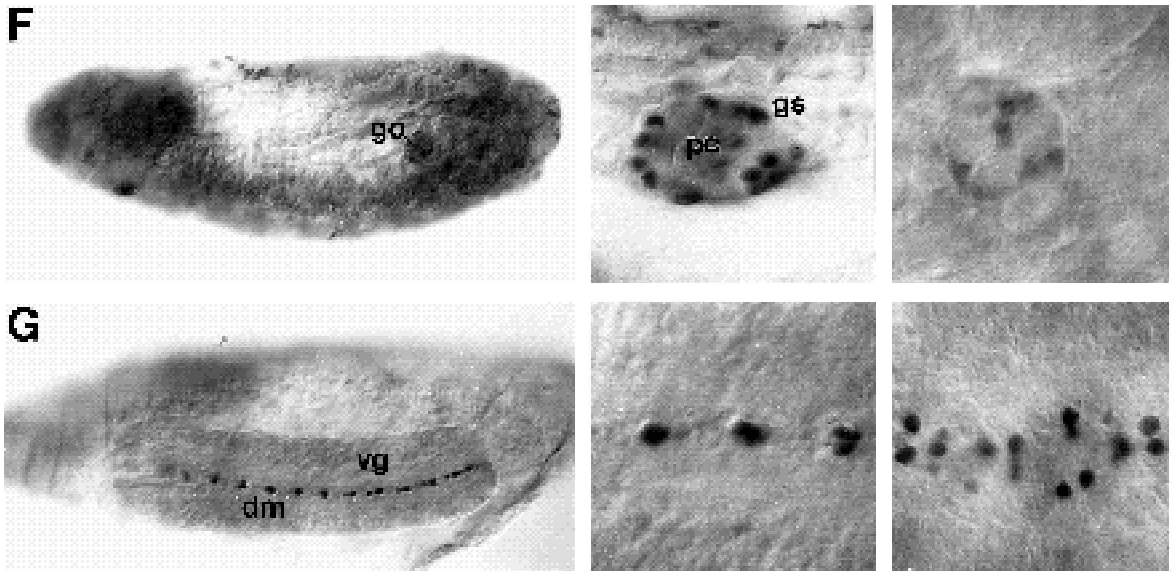


Fig. 5. *Notch*-related defects in mesoderm development. A-H show mesodermally derived organs, labeled by P*lac*Z expression or antibody markers, in wild-type (left column: low magnification view; middle column: high magnification) and hemizygous *Df(1)N*^{81K1} mutant embryos (right column). Embryos vary in age between stage 13 (C), 15 (A, B, D, E, F) and 17 (G). (A, B and D) Dorsal views; (C, E) represent embryos in lateral view and (G) ventral view. In A, the somatic musculature (*sm*) is labeled with an anti-muscle myosin antibody. In *Df(1)N*^{81K1}, most somatic muscle cells form irregular clusters of round, unfused cells. Nuclear counts, performed in embryos expressing a muscle specific P*lac*Z line (B6-3-23), yielded 100-150 nuclei per hemisegment (data not shown). Counts are extremely difficult since, for most of the embryo, repeated segmental sets of muscles cannot be distinguished in a late *Notch* embryo. Our figure indicates, however, that, compared to the wild type (Bate, 1990), there do not seem to be dramatic changes in overall number of somatic muscle cells in *Df(1)N*^{81K1} mutant embryos. (B) The visceral musculature (also stained with anti-muscle myosin antibody) which surrounds the oesophagus (*es*), part of the proventriculus (*pv*), midgut (*mg*) and hindgut. Visceral muscle cells around fore- and hindgut appear relatively normal in *Df(1)N*^{81K1}. (C) The so called periligament cells (*pl*; labeled by expression of P*lac*Z insertion B11-2-2) which, in wild type, form a single pair of cells per segment flanking the heart. In *Df(1)N*^{81K1}, these cells are strongly increased in number. (D) The cardioblasts (*cb*; labeled by expression of P*lac*Z insertion B2-3-20) which, in wild type, are flanked by pericardial cells (*pc*). In *Df(1)N*^{81K1}, cardioblasts are increased in number. However, these cells do not form a regular tube (see also Fig. 4). Pericardial cells are absent. (E) The fat body (*fb*; labeled by expression of P*lac*Z insertion E7-3-63). In both wild type and *Df(1)N*^{81K1}, these cells form an irregular sheet between the body wall and the gut. Similarly, gonad sheath cells (*gs*, shown in F; labeled by expression of P*lac*Z insertion B12-3-3) appear normal in *Df(1)N*^{81K1}. (G) The dorsomedian cells (*dm*, labeled by expression of P*lac*Z insertion C8-3-5). These cells, which derive from the mesectoderm and are arranged as a single pair per segment on the dorsal surface of the ventral nerve cord (*vg*), are increased by a factor of approximately 2 in *Df(1)N*^{81K1} mutant embryos. Other abbreviations: *go* gonads; *hg* hindgut; *ph* pharynx; *pc* pole cells. Bars: 100 μm (panels of left column); 40 μm (panels of middle and right column).

tion of the ventral furrow (early mesoderm) were observed (data not shown). The fact that the early endoderm defect was only apparent in heat-pulsed *N*^{ts1} /*Df(1)N*^{81K1} embryos, and not hemizygous *Df(1)N*^{81K1} embryos, implies that the *N* protein may have to form intact dimers or multimers in order to function normally. A similar conclusion was reached by previous investigators based on genetic and molecular studies (Foster, 1975; Hartley et al., 1987; Kelley et al., 1987).

Among the mesodermal derivatives, the periligament cells and peritracheal cells were increased in number following heat pulses applied to *N*^{ts1} /*Df(1)N*^{81K1} between 5 and 8 hours. Abnormalities in the somatic musculature were only mild; in none of the temperature-shift experiments could the severe defects in myoblast fusion typical for the *Df(1)N*^{81K1} mutant embryos be observed. The heart showed abnormalities in *N*^{ts1} /*Df(1)N*^{81K1} embryos that were pulsed between 6 and 10 hours. Two different effects were observed. Following heat pulses between 6 and 8 hours of development (stage 11-12), the number of heart cells is strongly increased, similar to what has been shown for the *Df(1)N*^{81K1} mutant embryo. However, in contrast to the condition in *Df(1)N*^{81K1}, the differentiation of two distinct cell types had taken place. One medial row of regularly arranged cardioblasts, flanked by 2-3 irregular rows of pericardial cells, develop. If *N* function is reduced at a later stage (8-10 hours), no increase in the number of heart cells is observed; however, the normal differentiation into the two distinct cell types fails to occur. In this experiment, all cells express cardioblast-like characteristics, although they fail to assemble into a regular tube (data not shown).

Embryonic phenotype caused by the neurogenic mutations bib, mam, neu, Dl *and* E(spl)-C

Antibody markers and P*lac*Z insertions expressed in specific organs were used to analyze embryonic defects in embryos carrying mutations in *bib*, *mam*, *neu*, *Dl* and *E(spl)*. The results are summarized in Table 2; for the different alleles utilized see Material and methods. The same spectrum of defects found in *N* mutant embryos can be observed in homozygous embryos carrying a null allele for *Dl*, *neu* or a deletion of the *E(spl)-C*; examples are shown

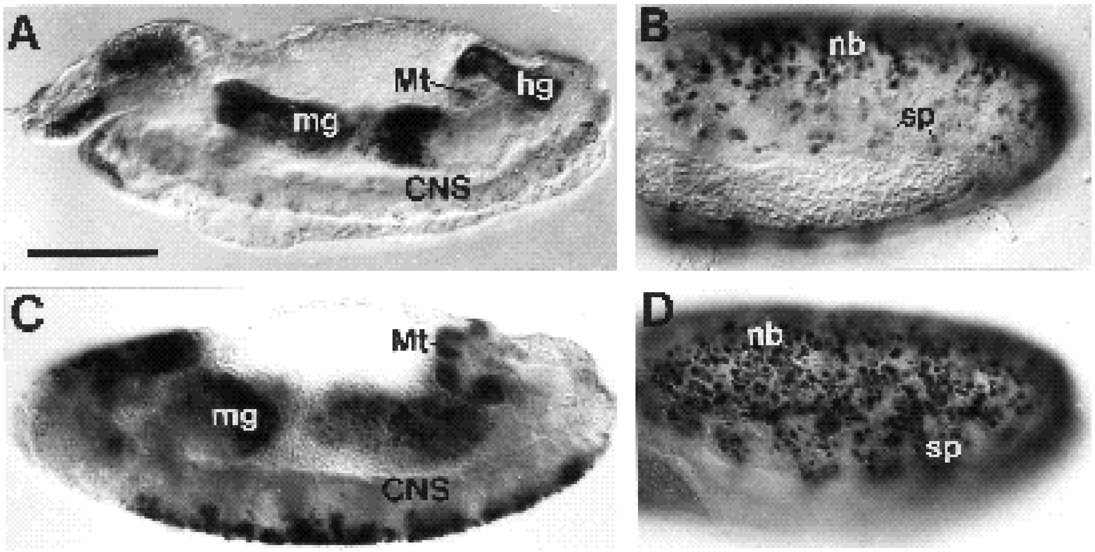


Fig. 6. Pattern of DNA replication in wild type (A, B) and *Df(1)N*^{81K1} (C, D). (A, C) Whole mounts of stage 14 embryos pulsed for 1 hour with BrdU; (B, D) Stage 11 embryos were pulsed with BrdU for 1 hour. (A, C) The wave of endoreplication which during stage 14 affects the midgut (*mg*), Malpighian tubules (*Mt*) and part of the hindgut (*hg*) of both wild-type and *Df(1)N*^{81K1} embryos. (B, D) Dorsolateral views of embryos in which, in both wild type and *Df(1)N*^{81K1}, replication is restricted to the sensillum precursor cells (*sp*) and neuroblasts (*nb*). Bar: 80 μm.

in Fig. 8. Defects in *mam* and *bib* were less severe and apparently did not include the endoderm. The comparatively mild phenotype of *mam* mutant embryos may be partly due to a maternal expression of this gene (Jimenez and Campos-Ortega, 1982); *bib*, on the other hand, does not seem to be expressed maternally (Jimenez and Campos-Ortega, 1982; Rao et al., 1990). Among the ectodermal derivatives, a hyperplasia of neuroblasts, sensillum precursor cells and peripheral glia cells was observed in both *bib* and *mam* mutant embryos. The precursor cells of the optic lobe and SNS had largely lost their epithelial phenotype (Fig. 9A,B, *ol*; Fig. 9E,F, *sns*). Preparations labeled with the anti-*crb* Cq4 antibody revealed only rudimentary patches of *crb* staining in both SNS and optic lobe. This finding implies that the SNS and optic lobe precursors in *mam* and *bib* mutant embryos may undergo an incomplete transition from their normal epithelial phenotype towards an apolar phenotype. Occasional defects in the branching pattern of the trachea and Malpighian tubules occurred in *mam*; both mam and bib showed such branching defects in the Malpighian tubules. The foregut and hindgut of both *mam* and *bib* mutant embryos did not show any notable abnormalities.

Whereas the visceral musculature in *mam* and *bib* mutant embryos developed relatively normally, somatic muscle fibres showed severe pattern defects (see also Corbin et al., 1991). The heart of both *mam* and *bib* mutant embryos resembled the heart of *N*^{ts1} /*Df(1)N*^{81K1} embryos subjected to a late (8-10 hours) heat pulse (Fig. 8B, *cb*). Thus, approximately the normal number of cells were present in the heart. However, all of these cells expressed a cardioblast-like phenotype (expression of myosin and the P*lac*Z insertion B2-3-20), although they failed to form a regular tube. No pericardial cells were present.

Discussion

Neurogenic gene function in epithelial-mesenchymal transitions

It has been proposed that the proteins encoded by the neurogenic genes are involved in one or more signaling pathway(s) controlling cell fate. *N* and *Dl*, for example, encode membrane proteins that could represent the signal and/or receptor (for recent review see Artavanis-Tsakonas, 1991; Campos-Ortega and Knust, 1991; Heitzler and Simpson, 1991). Alternatively, these proteins could represent structural molecules that promote adhesion among cells (discussed in Hoppe and Greenspan, 1986, 1990; Kidd et al., 1989). Studies in vertebrate embryos and culture systems indicate that particular types of adhesion molecules (e.g., cadherins, integrins), in combination with cytoskeletal movements that are modulated by these adhesion molecules, play a predominant role in epithelial-mesenchymal transitions and the maintenance of epithelial cells (see for review Hynes, 1987; Ruoshlati and Pierschbacher, 1987; Takeichi, 1987; Fleming and Johnson, 1988; Gumbiner et al., 1988). It is conceivable that the proteins encoded by the neurogenic genes *N* and *Dl* have a related function in *Drosophila* epithelial development.

The results presented in this manuscript do not allow us to draw any firm conclusions regarding the molecular nature of the factors encoded by the neurogenic gene. Furthermore, our results leave open the question whether the observed defects in a particular cell type are caused by the autonomous requirement of the neurogenic genes in this cell type. The expression data available for *Notch* and the other neurogenic genes are certainly compatible with this idea. According to in situ hybridization and antibody labeling experiments published in previous studies, all six of the

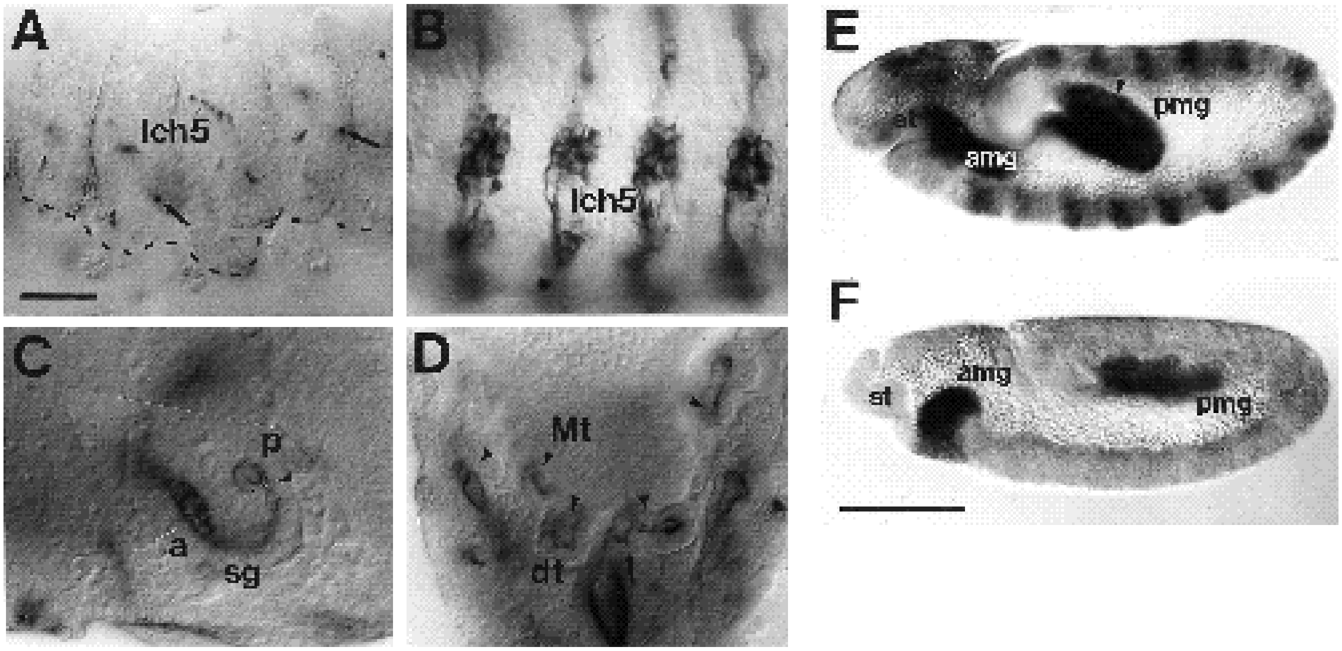


Fig. 7. *Notch*-related defects in ectodermally and endodermally derived tissues following 2 hour heat pulses applied to $N^{ts1}/Df(1)N^{81K1}$ embryos at different stages. (A) Part of anti-*crb* antibody stained embryo (lateral view; stage 16) pulsed between 2 and 4 hours. Dashed line indicates ventral boundary of the remaining epidermis. Sensilla (e.g., lateral chordotonal organ, *lch5*) develop normally. The medial part of the neurogenic region is completely neuralized. Its lateral part (inbetween dashed line and the tracheal openings which are indicated by arrows), however, develops into epidermis. This is different from the situation in hemizygous $Df(1)N^{81K1}$ mutant embryos where the entire neurogenic region is neuralized. (B) The lateral chordotonal organs (*lch5*) of four consecutive abdominal segments (mAb 22C10 labeling) in a $N^{ts1}/Df(1)N^{81K1}$ embryo heat pulsed between 8 and 10 hours. All cells of the chordotonal organs, each of which normally contains 5 sensory neurons and 15 accessory cells, are converted into sensory neurons (compare this figure to Fig.3A showing the wild-type chordotonal organs). (C) The salivary gland (*sg*; anti-*crb* antibody labeling) of a $N^{ts1}/Df(1)N^{81K1}$ embryo heat pulsed between 6 and 8 hours is shown. Note constriction (arrowhead) which partitions the lumen into a posterior (*p*) and an anterior (*a*) portion. (D) Malpighian tubules (*Mt*) of a $N^{ts1}/Df(1)N^{81K1}$ embryo heat pulsed between 6 and 8 hours stained with the anti-*crb* antibody. In focus are the distal tips (*dt*; arrowheads) of all four tubules. The arrow points to one of the small supernumerary branches which frequently develop under this temperature regimen. (E) The midgut rudiments of a stage 10 wild-type embryo (P*lac*Z insertion B11-2-2). (F) By comparison, the midgut rudiments of a stage 10 $N^{ts1}/Df(1)N^{81K1}$ embryo heat pulsed from 0-4 hours. Note that the anterior midgut rudiment (*amg*) in F is still integrated within the ectoderm posterior to the stomodeum (*st*). The posterior midgut rudiment (*pmg*) in F has lost its epithelial character which, in a wild-type embryo of this stage, is still conserved (arrowhead in E). Bars: 25 μm (A-D); 100 μm (E,F).

neurogenic genes are widely expressed during early embryogenesis in all three germ layers and their derivatives (Artavanis-Tsakonas et al., 1991, reviewing *N*; Vaessin et al., 1987, for *Dl*; Knust et al., 1987, for *E(spl)-C*; Smoller et al., 1990, for *mam*; Rao et al., 1990, for *bib*; Boulianne et al., 1991, for *neu*). However, genetic mosaic experiments are necessary to settle the issue of cell autonomy. The findings presented in this manuscript bear on the question of what is the developmental process controlled by the neurogenic genes. Thus, by analyzing the different developmental processes dependent on neurogenic gene function, we have described a few ‘key characteristics’ common to most of these processes. These ‘key characteristics’ might help to define more adequately the cellular mechanism controlled by the neurogenic genes. In previous interpretations of the role of the neurogenic genes in development, their importance for cell fate decisions was emphasized. Based on the present results, we propose that in the case of several embryonic tissues, the neurogenic genes may control a specific morphogenetic function, namely the promotion or maintenance of the epithelial state.

The neurogenic genes are necessary during numerous developmental events in tissues derived from all three germ layers. In many of these events (e.g., segregation of neuroblasts, sensory neurons, oenocytes, peripheral glia cells), a formerly homogenous population of epithelial cells splits up into two subpopulations, one that remains epithelial and another one that delaminates from the epithelium and thereby loses its epithelial characteristics. Neurogenic gene function in all of these cases is needed for the cells that remain epithelial; a reduction of neurogenic gene function leads to an increased ratio of the cells that delaminate. In vertebrate embryos, the developmental process in which epithelial tissues give rise to cells that no longer express the epithelial phenotype have been called epithelial-mesenchymal transitions. Well-known examples for such transitions are the formation of the mesoderm from the epiblast in chicken (Balinsky and Walther, 1961; Trelstad et al., 1967) and the dissociation of the bone precursor (sklerotome) cells from the somitic mesoderm (Mestres and Hinrichsen, 1976). Transitions from a mesenchymal to an epithelial state are also common; examples are the forma-

Table 2. *Embryonic defects caused by mutations of the neurogenic genes mam, bib, neu, Dl and E(spl)*

	bib	*mam*	*neu*	*Dl*	*E(spl)*
NEUROBLASTS	++	+	++	++	++
SENSILLUM PRECURSORS	+	+	++	++	++
SENSORY NEURONS	−	++	++	++	++
PERIPHERAL GLIA CELLS	+	+			
OENOCYTES	+	+			
OPTIC LOBE	+	++	++	++	++
STOMATOGAS. NERVOUS SYS.	++	+	++	++	++
SALIVARY GLAND	a	+	A	A	A
FOREGUT	−	−	a	a	a
MALPIGHIAN TUBULES	+	+	+	+	+
TRACHEA	−	+	++	++	++
ENDODERM	−	−		++	
LARVAL MIDGUT	−	−	++	++	++
SOMATIC MUSCULATURE	+	+	++	++	++
CARDIOBLASTS	+	+	++	++	++
PERILIG.&PERI-TRACH. CELLS			++	++	++

Listed are the various embryonic organs in which defects were observed (vertical axis; presence of + indicates that defect was present) and the neurogenic loci causing these defects (horizontal axis). ++ stands for severe defects, + indicates mild defects. The type of defects observed in the different mutations listed in this table (i.e., numerical increase, differentiative defect etc.) were the same as those specified for the *Notch* mutation in Tab.1. − indicates that no defects were evident. In cases in which a particular organ in one of the different mutations was not analyzed the corresponding box was left blank.

tion of the somites from the early mesoderm or the formation of kidney tubules from the metanephrogenic mesenchyme (Grobstein, 1955).

We propose that the term epithelial-mesenchymal transition should also be applied in insect embryogenesis to processes in which all or part of an epithelium transforms into cells that no longer express epithelial characteristics. Thus, the cells that delaminate from the ectoderm (e.g., neuroblasts, oenocytes), or the cells of the early mesoderm and the midgut rudiments, fulfill all criteria by which mesenchymal cells in vertebrate embryos are defined. Ultrastructurally, the delamination of mesenchymal cells from the epiblast resemble neuroblast delamination in every detail. So far, the term mesenchyme was not in wide use in insect embryology. Non-epithelial cells in most insects form tightly packed clusters, in contrast to the situation in vertebrate embryos where they are usually more loosely organized and surrounded by wide spaces filled with extracellular matrix. However, this is not an essential property of mesenchymal cells, since many populations of vertebrate mesenchymal cells undergo stages at which they are as densely packed as for example the mesoderm cells in *Drosophila*.

During the epithelial-mesenchymal transitions that occur in the *Drosophila* ectoderm, neurogenic gene function could primarily be important for interactions among ectoderm cells in order to maintain their epithelial phenotype, rather than for cell-cell communication events that decide over differentiative cell fates (see also Hoppe and Greenspan, 1986, 1990). The fact that presumptive neuroblasts, oenocytes, glia cells and other cell types delaminate from the ectoderm may not be directly related to their final differentiation. Instead, acquiring mesenchymal characteristics merely enables these cells to move around and reach certain positions in the embryo. For example, in order to reach the peripheral axons that they ultimately attach to, the peripheral glia precursors have first to move out of the ectoderm. Thus, generally speaking, the role of epithelial-mesenchymal transitions may be mainly to control the proper dispersal of embryonic cells. The fate which individual cells ultimately express may depend on other cues that are unrelated to the delamination movement per se, and which are therefore independent of the function of the neurogenic genes.

A number of cell types that depend on neurogenic gene function for their proper development, i.e., the sensillum precursor cells or the primary pigment cells also segregate from an epithelium; however, these cells do not fully lose contact to the apical surface. Thus, the sensillum precursors in the embryo constrict apically and their nuclei shift basally. In the eye disc, the segregation of pigment cells may involve a similar movement (see Tomlinson and Ready, 1987). One might view this morphogenetic movement as a ‘partial delamination’. The fact that the neurogenic genes are also involved in the ‘partial delamination’ could be explained if one assumes that the initial cellular mechanism (i.e., changes in contacts to neighbouring cells, cytoskeletal movements) leading to delamination and ‘partial delamination’ are the same.

Neurogenic gene function in the development of epithelia

A number of tissues that transiently or permanently display epithelial characteristics (e.g., early endoderm, optic lobe, SNS precursors, trachea, Malpighian tubules and salivary gland) require neurogenic gene function to express their normal epithelial phenotype. In these tissues, where no obvious decision between different cell fates is being made, the neurogenic genes may fulfill a specific morphogenetic function related to the development of the epithelial state.

A morphogenetic step that is strongly affected by a reduction in function of most neurogenic genes [*N*, *Dl*, *neu* and *E(spl)-C*] is the formation of the midgut epithelium. This event represents an example for a transition in phenotype from mesenchymal to epithelial. In embryos homozygous for a mutation in the above listed neurogenic genes, this transition does not occur. The interpretation of the function of the neurogenic genes in midgut morphogenesis is complicated by the fact that around the same stage when the midgut rudiments reorganize into epithelial structures, they give rise to at least two different cell types, namely the larval midgut cells and the adult midgut precursors. In the neurogenic mutants, the ratio of AMPs is strongly increased. How the separation of larval and adult midgut precursors takes place (i.e., whether it also involves an

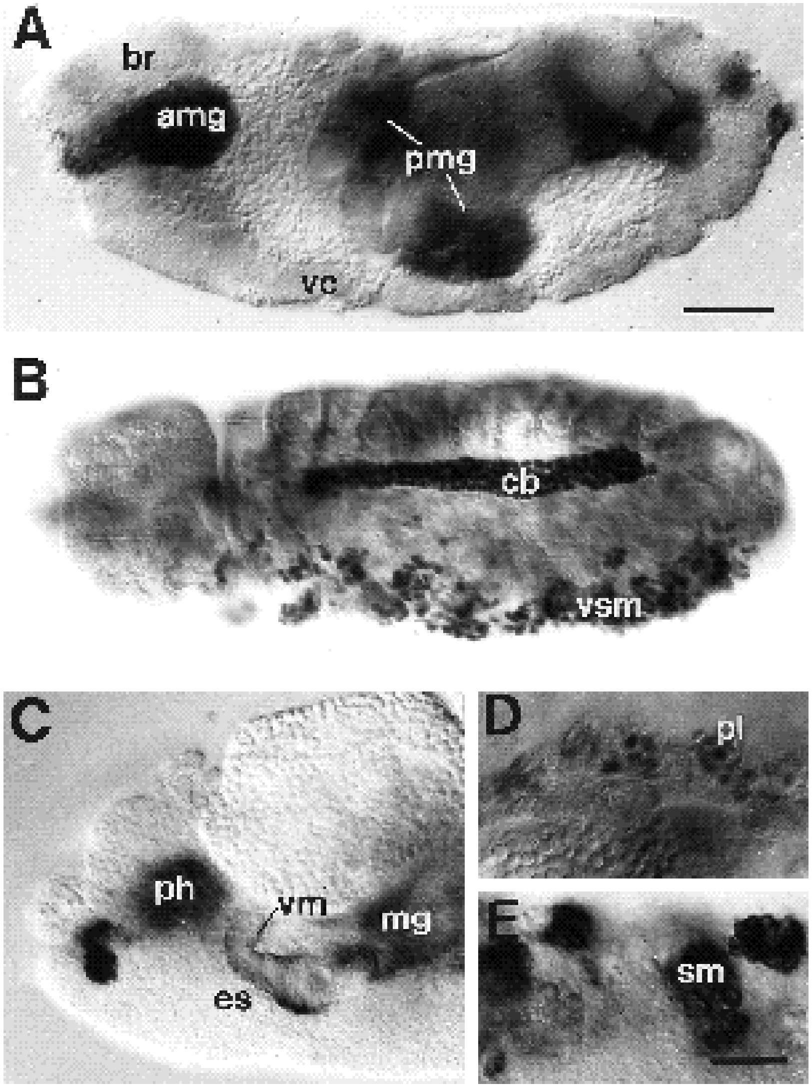


Fig. 8. Embryonic defects related to mutations in the neurogenic loci *neu* (A), *mam* (B) and *Dl* (C-E). (A) Midgut cells (labeled by expression of P*lac*Z insertion B11-2-2) in a late stage *neu* mutant embryo. In *neu* [as well as *Dl* and *E(spl)*], the endoderm does not differentiate into a midgut epithelium. Instead, cells remain as disorganized clusters of rounded cells at the posterior and anterior end of the embryo (*amg; pmg*). Frequently, the anterior midgut rudiment (*amg*) is still integrated into the ventral (neuralized) ectoderm, suggesting that *neu*, *Dl* and *E(spl)* play a role during gastrulation. (B) Increase in cardioblasts (*cb*; labeled by expression of P*lac*Z insertion B2-3-20) in a stage 16 *mam* mutant embryo. (C, E) Somatic musculature (*sm*) and visceral musculature (*vm*) of a late stage *Dl* mutant embryo labeled with anti-muscle myosin antibody. (D) Increased numbers of periligament cells (*pl*; labeled by expression of P*lac*Z insertion B11-2-2; see Fig. 5 for wild-type pattern of these cells). Other abbreviations: *br* brain; *es* oesophagus; *mg* midgut; *ph* pharynx musculature; *pmg* posterior midgut; *vc* ventral nerve cord; *vsm* ventral muscles. Bars: 50 μm (A, B); 25 μm (C-E).

epithelial-mesenchymal transition) is currently unknown, and we have initiated an analysis of this problem. The AMPs are transiently integrated within the larval midgut epithelium; they later obtain a more basal position and possibly lose their epithelial characteristics. The fact that in the neurogenic mutants the ratio of AMPs to larval midgut cells is strongly increased could be involved in causing the failure of the midgut rudiments to form an epithelium. One argument in favor of the view that the acquisition of epithelial characteristics of the midgut cells per se is the step dependent on neurogenic gene function is provided by the finding that even late heat pulses (i.e.,pulses applied clearly after the separation of larval midgut cells and AMPs has taken place; Table 1) are effective to impair strongly the epithelial arrangement of the midgut cells in N^{ts1} /$Df(1)N^{81K1}$ embryos.

Neurogenic gene function in mesoderm development

Following gastrulation, the mesoderm forms a monolayer of cells. At the late extended germband stage (stage 11/12), mesoderm cells reorganize and form 2-3 layers which then rapidly split up into different organ rudiments. The present study provides evidence that the mechanisms controlling some of these segregation events may be similar to those regulating the segregation of different ectodermal derivatives. Thus, for some of the mesodermally derived organs such as the heart, peritracheal cells and periligament cells, there seem to exist within the mesoderm ‘competent zones’ in which all cells acquire the potential to express a particular fate. The actual number of cells that finally express this fate is then restricted in a second step that requires neurogenic gene function. The heart provides the clearest example for such a mechanism.

The temperature-shift experiments with N^{ts1} /$Df(1)N^{81K1}$ suggest that, around the time when the mesoderm splits into the different organ primordia (6-8 hours), a population of about 200 cells located on either side of the embryo in the dorsal part of the mesoderm become competent to develop as heart precursor cells. We propose the name ‘cardiogenic region’ for this territory, in analogy to the ‘neurogenic region’ which gives rise to the neuroblasts. In a *N*-dependent step, the cells of the cardiogenic region that actually become heart precursors are selected. They amount to roughly 50% of the cardiogenic region. The remaining cells,

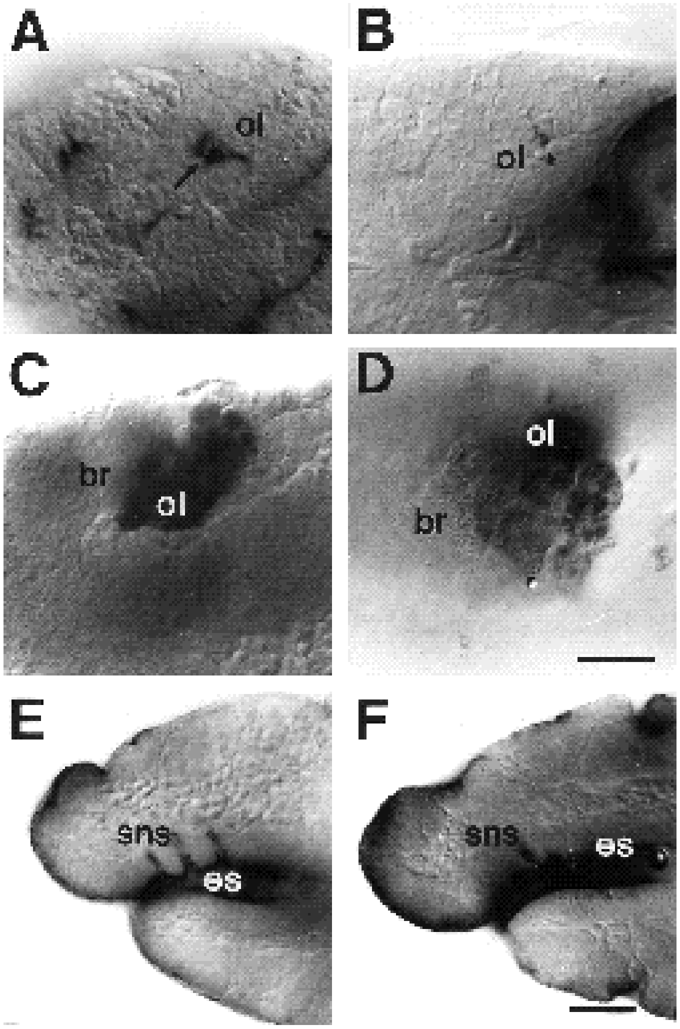


Fig. 9. Embryonic defects related to mutations in the neurogenic loci *bib* (A), *Dl* and *mam*. (A) Anti-*crb* labeled optic lobe (*ol*) of a stage 15 wild-type embryo. Arrow points to darkly labeled apical surface of optic lobe cells that form an epithelium enclosing a central lumen. In *bib* (B), optic lobe cells partially lose their epithelial character, indicated by the disintegration of anti-*crb* staining into multiple small dots (arrow). (C) Defective optic lobe invagination (labeled by expression P*lac*Z insertion A6-2-45) of a stage 13 heterozygous Dl/+ embryo (compare to wild-type optic lobe in Fig. 3). This indicates that *Dl* has a dominant effect on optic lobe development. (D) By comparison, the optic lobe of a late stage homozygous *Dl* mutant embryo. (E, F) Comparison of the developing stomatogastric nervous system (*sns*) of a stage 12 wild-type (E) and a *mam* mutant embryo (F; anti-*crb* antibody staining). In the wild type, the stomatogastric nervous system derives from three evaginations that appear in the roof of the oesophagus (*es*). In *mam*, the evaginations are irregularly reduced in size. Other abbreviations: *br*, brain. Bars: 20 μm.

which do not become heart precursors, take on a different fate. Possibly, they become part of the fat body, since this structure is reduced in cell number in *N* mutant embryos.

During heart development neurogenic gene function is also required at a later stage. Thus, all heart precursors have the potential to develop into cardioblasts. In the step that requires *Notch* function, the heart precursors themselves segregate into two distinct and spatially discrete cell types, the cardioblasts and pericardial cells. Consistent with this model, high levels of *N* are expressed in the cells involved in heart development at the appropriate times (own unpublished results).

It would be important to know how one can compare the mechanism by which neurogenic genes affect mesoderm development to the function of these genes in the development of the ectoderm and endoderm. Unlike the ectoderm, the cells of the mesoderm do not show any clear epithelial characteristics. In particular, zonulae adherentes, which surround the ectodermal cells apically, are missing in the mesoderm (own unpublished results) and the distribution of organelles does not reveal any polarization. However, it is possible that shortly before the mesoderm splits up into different organ rudiments, parts of it transiently acquire epithelial characteristics. Thus, in other insects, the lateral mesoderm clearly forms segmented vesicles (called coelomata) whose cells seem to show an epithelial phenotype. The inner walls of the coelomata (splanchnopleura) give rise to the visceral musculature, the outer walls (somatopleura) form the somatic myoblasts; the heart precursors develop from a narrow zone located where both spanchnopleura and somatopleura meet (for review see Weber, 1974). Although in *Drosophila* embryos of a comparable stage, one cannot recognize coelomic vesicles whose cells express a clear-cut epithelial phenotype, part of the lateral mesoderm may transiently express an epithelial-like structure. If that were the case, one could further assume that certain cell populations, such as the precursors of the heart and periligament cells, have to ‘delaminate’ from this transient epithelium. Neurogenic gene function in such a scenario could then be involved in stabilizing the epithelial phenotype and thereby restricting the number of cells that delaminate.

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