The formation of commissures in the *Drosophila* CNS depends on the midline cells and on the *Notch* gene

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**SUMMARY**

The commissures of the *Drosophila* central nervous system (CNS) are formed in close relation to the ventral midline cells, a morphologically distinct set of cells located at the midline of the developing CNS. To analyze the function of these cells during commissure formation, we looked for mutations that result in the absence of commissures. One example of a gene that can give rise to such a phenotype is the neurogenic gene *Notch*. Here we show that mutant *Notch* embryos are devoid of commissural connections and have an abnormal midline. The midline cells of the embryonic *Drosophila* CNS are specified during the blastoderm stage about two hours before the first neuroblasts start to delaminate from the neurogenic region. To analyze *Notch* function for commissure development further, we took advantage of the *Notch*ts1 allele. Temperature-shift experiments demonstrated that the lack of commissures in mutant *Notch* embryos results from defects in the anlage of the CNS midline cells. Here maternal as well as zygotic *Notch* function are required for the correct activation of the gene single-minded, since mutant *Notch* embryos derived from germ-line clones lack most of the single-minded-positive midline cells.

Key words: *Drosophila* CNS, midline, commissure formation, *Notch*

**INTRODUCTION**

A common feature of the organization of higher nervous systems is bilateral symmetry. The two lateral halves of the CNS are generally connected by a large number of contralaterally projecting neurons, the axons of which form the commissures. Typically these axons first head towards the midline, and only after crossing it do they dramatically change their behavior turning to follow specific pathways, which they had ignored on the ipsilateral side (for review see Dodd and Jessel, 1988; Goodman and Shatz, 1993).

The formation of commissures in the embryonic *Drosophila* CNS has been described recently (Klämbt et al., 1991). As in vertebrates, commissural growth cones initially grow towards the midline and change their behavior after crossing it, as they now follow specific longitudinal tracts. Based on these observations, the midline of the embryonic *Drosophila* CNS is believed to exert important functions during commissure formation. It comprises a small number of neuronal and glial cells with unique morphological properties (Poulson, 1950; Jacobs and Goodman, 1989; Klämbt et al., 1991). Similar to their vertebrate counterparts, the floorplate cells, the midline cells of the *Drosophila* CNS are among the first CNS cells to be specified, which is reflected by the expression of the gene single-minded (Crews et al., 1988; Thomas et al., 1988; Schoenwolf and Smith, 1990). This gene encodes a bHLH-type transcription factor and regulates the expression of genes that are subsequently activated in the midline (Nambu et al., 1990, 1991). Mutations in single-minded lead to a selective death of all midline cells after gastrulation (Klämbt et al., 1991; Nambu et al., 1991). The final CNS axon phenotype of homozygous single-minded embryos is a fusion of the longitudinal axon tracts at the midline; however, commissures do form initially (Thomas et al., 1988; Klämbt et al., 1991).

The bulk of the neurons in the *Drosophila* CNS develops from a set of stereotypically arranged neuroblasts (Hartenstein and Campos-Ortega, 1984; Doe, 1992). The first neuroblasts delaminate into the interior of the embryo during stage 9, about 2-3 hours after the midline cells have been specified. Commissural connections are pioneered at the beginning of the second third of embryonic development during stage 12 (Klämbt et al., 1991).

The segregation of neuroblasts from the neurogenic region is under the control of the proneural and the neurogenic genes (Lehmann et al., 1983; Brand and Campos-Ortega, 1988, for review, see Campos-Ortega, 1993). Mutations in the neurogenic genes lead to a hyperplasia of the nervous system with concomitant loss of the underlying epidermis, because all cells of the neurogenic region adopt a neural fate. A long known member of the group of the neurogenic genes is *Notch* (Poulson, 1940; Lehmann et al., 1983). It encodes a large transmembrane protein containing 36 EGF-like repeats and is involved in the lateral inhibition mechanism used to single out neuronal precursor cells during nervous system development (Wharton et al., 1985; Kidd et al., 1986; Heitzler and Simpson, 1991; for review, see Simpson, 1990; Artavanis-Tsakonas and Simpson 1991). *Notch* appears to act as the receptor for an embryonicizing signal that is probably encoded by the neurogenic gene Delta (Vässin et al., 1987; Kopczynski et al., 1988; Fehon et al., 1990; Heitzler and Simpson, 1991, 1993; Rebay et al.,...
1991; 1993; Struhl et al., 1993). Besides its function during the development of the embryonic nervous system, Notch is required for a large number of developmental processes including oogenesis and mesoderm, eye, bristle and wing development (Shellenberger and Mohler, 1978; Cagan and Ready, 1989; Hartenstein and Posakony, 1990; Ruohola et al., 1991; Corbin et al., 1991; Xu et al., 1992; Hartenstein et al., 1992). Maternally expressed Notch has been shown to be required for embryonic viability. Embryos lacking only the maternal Notch function die as fully developed embryos; this lethality cannot be rescued by increasing zygotic Notch activity (Jiménez and Campos-Ortega, 1982).

Here we show that, in addition to its function during neuroblast segregation, Notch is required for midline development. Mutant Notch embryos show a hyperplastic CNS with no commissures and disrupted longitudinal connectives. The analysis of germ-line clones indicates that maternal Notch function is required for correct activation of single-minded. To analyze the role of the midline in the formation of commissures, we have pursued a genetic approach. Using the Notch<sup>ts1</sup> allele and appropriate temperature-shift experiments, we were able to uncouple the requirement for Notch in the midline from its function during neuroblast segregation. From our results, we conclude that the midline is required to allow commissure formation in the embryonic Drosophila CNS.

**MATERIALS AND METHODS**

**Fly strains**

The following fly stocks were used. Notch<sup>55e11</sup>, Notch<sup>264-47</sup> and Notch<sup>101</sup> (Lindsley and Zimm, 1992) were obtained from the Köln stock collection. Notch<sup>55e11</sup>, although classified as a weak allele, shows a similar embryonic phenotype to Notch<sup>264-47</sup> which shows a deficiency phenotype (Lindsley and Zimm, 1992). Embryos from both Notch alleles yielded the same results. Notch<sup>101</sup> carries a missense mutation in the extracellular domain of Notch. At the restrictive temperature, the subcellular distribution of the Notch<sup>101</sup> protein is altered (Xu et al., 1992). The enhancer trap lines AA142 and 1277 specifically label midline glial cells, the line AE60 specifically labels the neuronal midline cells, the six ventral unpaired median neurons (VUMs) and the two MPI neurons; X55 labels the neuronal cells, the median neuroblast and the posterior pair of midline glial (MGP) cells and Aul labels the MGP cells (Klämbt et al., 1991; Scholz et al., 1993). The enhancer trap lines rC56 (=3109), M84 and P101 were used to analyze the various CNS glial cells (Klämbt and Goodman, 1991). FRT<sup>101</sup> and ovo<sup>D1</sup>, FRT<sup>101</sup>, hsFLP<sup>38</sup> flies (Chou and Perrimon, 1992) were kindly provided by T.-B. Chou and N. Perrimon.

**Antibody staining and sectioning**

Immunohistochemistry was carried out as described previously (Klämbt et al., 1991). For cryostat sections, embryos of the desired developmental stage were embedded in OTC Tissue Tec (Miles Scientific). Sections of 8 μm were cut on a Reichert-Jung cryotome. Photographs were taken on a Zeiss Axioshot.

**Temperature-shift experiments**

Notch<sup>101</sup> flies were collected on apple juice 3% agar plates (=1 mm thick). The restrictive temperature was 31°C, the permissive temperature was 18°C. Oregon R wild-type flies were kept under identical conditions as a control.

**Germ-line clones**

To generate germ-line clones, a FRT<sup>101</sup> element (Chou and Perrimon, 1992) was recombined onto the Notch<sup>55e11</sup> chromosome. Virgins carrying the recombined chromosome were crossed to ovo<sup>D1</sup>, FRT<sup>101</sup>, hsFLP<sup>38</sup> males. The offspring (2-3 days old) were subjected to a 2 hour heat pulse at 37°C in a water bath. Eclosed Notch<sup>55e11</sup>, FRT<sup>101</sup>/ovo<sup>D1</sup>, FRT<sup>101</sup>; +/hsFLP<sup>38</sup> virgins were then crossed to males carrying a P[w<sup>1</sup>, sim-lacZ]<sup>2</sup> construct on the third chromosome (Nambu et al., 1990).

**RESULTS**

Fig. 1A shows a schematic summary of the embryonic CNS development. At the blastoderm stage, the embryo is divided into the major anlagen, mesoderm, neurogenic region and dorsal epidermis by the action of the dorsal group genes (St Johnston and Nüsslein-Volhard, 1992). The mesodermal anlage is separated from the neurogenic region by a single-cell-wide
Commissure formation depends on the midline row of mesectodermal progenitor cells. These cells specifically express the gene *single-minded* and some of the genes of the *Enhancer of split* complex (Crews et al., 1988; Knust et al., 1987; 1992). They also express the neurogenic genes *Delta* and *Notch* (Viissin et al., 1987; Hartley et al., 1987). As the mesoderm invaginates into the interior of the embryo during gastrulation, the two cell rows expressing the *single-minded* gene are brought together at the midline, where they intermingle to form the single row of midline progenitor cells. At the time when the first neuroblasts delaminate from the neurogenic region, all midline progenitor cells also invaginate into the interior of the embryo (Hartenstein and Campos-Ortega, 1984; Nambu et al., 1991). Hence, the delamination of neuroblasts occurs about 2-3 hours after the midline cells are specified.

**CNS axon phenotype of Notch embryos**

In mutant *Notch* embryos, all cells of the neurogenic region adopt the neural cell fate resulting in a hyperplastic nervous

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**Fig. 2.** Development of the CNS axon pattern in wild-type and mutant *Notch* embryos. CNS axons are visualized using the monoclonal antibody BP102 and subsequent HRP immuno-histochemistry. Dissected CNS preparations are shown with anterior up. Scale bar is 10 μm.

(A-C) The development of the wild-type axon pattern, (D-F) the development of the *Notch*<sup>55e11</sup> axon pattern. (A) The formation of the posterior commissure occurs at the beginning of stage 12. Commissural axons grow towards the midline and cross it in a characteristic pattern. (B) At the end of stage 12 both anterior and posterior commissures have formed but still appear fused. Note that the intersegmental connectives have not yet formed. (C) The final CNS axon pattern of a stage 15 embryo. Anterior commissure (ac) and posterior commissure (pc) have separated and the longitudinal connectives (lc) are established. (D-F) The development of the axon pattern in mutant *Notch* embryos. (D) In an early stage 12 embryo BP102-positive cells appear at the same time as they do in wild-type embryos; however, they fail to extend axons that grow towards the midline and instead form intensely staining structures (arrowheads). (E) Embryo of a similar age to that shown in B. In *Notch* embryos axons accumulate lateral to the midline at positions where commissures usually originate (arrowheads). Note the BP102-positive cells in the midline. (F) The axon pattern of a stage 15 *Notch* embryo. No commissures and no longitudinal connectives have formed. In some segments one can still detect BP102 staining in the midline.
system (Poulson, 1940; Lehmann et al., 1983). The supernumerary neuroblasts all produce ganglion mother cells and subsequently axon-projecting neurons. However, despite the fact that neurons in Notch embryos extend growth cones, they fail to become organized in the correct fashion. To analyze the CNS axon pattern of Notch embryos, we performed whole-mount staining either with the mAb BP102, which labels most CNS axon tracts (Fig. 2), or anti-HRP antibodies, which visualize all neuronal membranes (data not shown). Mutant Notch^{5scl1} (or Notch^{267-47}) embryos rarely form commissural

![Fig. 3. Distribution of glial cells in a Notch CNS. CNS glial cells are labeled by anti-β-galactosidase staining of the enhancer trap line rC56. Antibodies against β-galactosidase are visualized using alkaline phosphatase. CNS axon tracts are visualized using the mAb BP102 and HRP immunochemistry. Scale bar is 10 μm. (A) A wild-type stage 16 CNS. (B) Schematic drawings of the glial cells close to the neuropile. At this developmental stage, the rC56 enhancer trap allows the detection of eight longitudinal glial cells (numbered in green in B), two A and two B glial cells (red dots in B), the segment boundary cell (SBC, blue dots in B), the VUM support cells (not in focus) and the exit glial cells (eg). The A and B glial cells appear to express the lacZ reporter construct slightly less intensely, whereas the segment boundary cell expresses the lacZ gene more intensely as the longitudinal glial cells. In some segments two A glial cells are found in a hemisegment (indicated by the red circle). Longitudinal glial cells are represented by the green numbers from 1 to 8. Note the regular and stereotyped appearance of the individual glial cells in the CNS. Numbering of glial cell starts arbitrarily. (C) A Notch^{5scl1} CNS at stage 15. No commissure form in the hyperplasic CNS. The longitudinal connectives lie further apart than in wild-type. The glial cells labeled by the enhancer trap line are not all in focus. (D) A summary drawing combining several focal planes depicting the glial cells associated with the neuropile. Note that one hemisegment lacks most glial cells, whereas another hemisegment contains twice as many glial cells as normal. No distinction between A, B, cells segment boundary cells or longitudinal glial cells can be made. Counts of the glial cells reveals, in most hemisegments, 11 glial cells associated with the neuropile, the same number as in wild-type.
Commissure formation depends on the midline (Fig. 2F). Identical results were obtained. In wild-type embryos, formation of the CNS axon pattern starts during stage 12 (staging of embryos is according to Campos-Ortega and Hartenstein, 1985). In mutant Notch^{55e11} embryos, the first axons grow out at the same time as the wild-type axons; however, they are never oriented.

**Fig. 4.** Notch affects midline development. CNS midline cells are labeled by antibody staining of the enhancer trap line X55. Antibodies against β-galactosidase are visualized using alkaline phosphatase. CNS axon tracts are visualized using the mAb BP102 and HRP immunochemistry. Dorsal is up in C-F. Anterior is to the left in A,B,E and F. (A,B) Dorsal views of dissected CNS preparations from wild-type and mutant Notch stage 15 embryos, respectively. Note that, in mutant Notch embryos, all labeled midline cells appear on the dorsal surface of the CNS (D,F), whereas they are found throughout the width of the CNS in the wild-type CNS (C). In mutant Notch embryos (B,D,F), we find 16-20 cells per segment expressing the X55 enhancer trap marker. (E) In sagittal cryostat sections through a wild-type CNS, 13-15 X55-positive cells can be counted. The labeled cells are found at ventral positions in the CNS (VUM neurons) or at dorsal positions (posterior pair of midline glial cells). (F) In sagittal cryostat sections through a mutant Notch CNS, all X55-positive midline cells appear at the dorsal surface of the CNS. Similar results are seen in cross sections through wild-type (C) and Notch embryos (D).
towards the midline as they are in wild-type (compare Fig. 2A,D). Instead, they remain where they originated, even failing to form connections on their ipsilateral side (Fig. 2E, arrows). No other serious abnormalities are obvious in mutant embryos at this early developmental stage. In wild-type embryos, the anterior commissure forms immediately after the posterior commissure is established. Again in mutant Notch<sup>55e11</sup> embryos, no axons cross the midline and neither the anterior nor the posterior commissures form. During stage 15, longitudinal connectives are visible in wild-type embryos; in Notch<sup>55e11</sup> embryos, these axon tracts are also not established. During this developmental stage, a mutant Notch CNS can be easily recognized by its hyperplastic appearance (Fig. 2F).

Neuronal and glial CNS lineages in Notch embryos

To investigate the cellular basis of the observed axon pattern defect, we analyzed the influence of Notch on various identified neurons and glial cells in the CNS. The MP2 precursor and its progeny can be followed using a ftz-lacZ construct or the enhancer trap line A96 (Doe et al., 1988; C. K. and C. S. Goodman, unpublished data; see Goodman and Doe, 1993 for nomenclature of CNS cells). Using these markers the number of MP2 neurons appears unchanged in mutant Notch<sup>55e11</sup> or Notch<sup>264-47</sup> embryos (data not shown). The Sp and some lateral neurons, which are labeled by the enhancer trap line rF112 (C. K. and C. S. G., unpublished data), however, are present in multiple number (data not shown).

The embryonic CNS contains a number of glial cells that can be individually identified by their position and by lacZ expression in a number of enhancer trap lines (Klämbt and Goodman, 1991). In a wild-type stage 16 CNS abdominal hemisegment, the enhancer trap line rCS6 labels four exit glial cells, the eight longitudinal glial cells, one segment boundary cell, the A and B glial cells and one VUM support cell. In some segments, additional A,B cells are visible (arrowheads in Fig. 3A; see also Udolph et al., 1993). In mutant Notch embryos, we find about three to four times as many exit glial cells as in wild-type (data not shown; see also Hartenstein et al., 1992). The number of longitudinal glial cells, A and B cells and VUM support cells, however, appears unchanged in mutant Notch<sup>55e11</sup> (Fig. 3) as well as in Notch<sup>264-47</sup> (data not shown). The most striking defect seen in these cells is that their precise and stereotyped segmental arrangement is lost in Notch embryos (compare Fig. 3A and C). Some segments are devoid of any longitudinal glial cells, whereas neighboring segments contain twice the number of glial cells (see Fig. 3). The cells labeled by an asterisk in Fig. 3C correspond to VUM support cells, which lie in a different focal plane relative to wild type (see also below).

The midline phenotype of Notch embryos

Of the 26 wild-type midline cells per segment only a few are believed to perform important functions during commissure formation (Jacobs and Goodman, 1989; Klämbt et al., 1991). These are the three pairs of midline glial cells, the VUM neurons and the two MP1 interneurons. The midline glial cells ensheath the commissures and occupy dorsal positions in the CNS, whereas the neuronal midline cells are typically located ventral to the commissures (Jacobs and Goodman, 1989). All midline cell lineages are affected in mutant Notch embryos. Both the anterior and the middle pair of midline glial cells seem to be missing based on observations using the enhancer trap lines AA142 and 1277. However, the posterior pair of midline glial cells, which is labeled by the enhancer trap line AU1, is duplicated but is found at ectopic positions in mutant Notch embryos (data not shown). The neuronal midline cells are labeled by the enhancer trap lines X55 and AE60 can be detected. In Notch embryos 4–8 additional cells per segment express X55 (or AE60) driven β-galactosidase activity (Fig. 4B,D,F). In addition all X55-positive cells are now found at ectopic positions within the CNS since all midline cells remain at the dorsal surface of the CNS, a position that is normally occupied by the midline glial cells (Fig. 4B). These results indicate that Notch is required for determination and differentiation of neuronal and glial midline cells.

Temperature-shift experiments distinguish between the requirement for Notch in midline and in neuroblast development

To define the function of Notch during midline development, we took advantage of the Notch<sup>ts1</sup> allele. The fact that the determination of midline cells occurs well before the neuroblast segregation (Fig. 1A) allowed us to remove Notch function during the anlage of the midline, but reactivate it during neuroblast segregation. We performed two series of experiments to establish the critical period (Fig. 1B). First we kept Notch<sup>ts1</sup> flies at the restrictive temperature of 30°C and collected eggs in 30 minute intervals. These egg collections were kept for up to 9 hours at 30°C and then transferred to the permissive temperature of 18°C and allowed to develop until stage 15. For the second set of experiments, we collected embryos at the permissive temperature at 30 minute intervals and transferred them at various time points to the restrictive temperature, where they were allowed to develop until stage 15. Subsequently, we analyzed the axon pattern with the monoclonal antibody BP102.

90% of the embryos that were exposed to the restrictive temperature only during the interval from 2 to 3 hours after egg laying lacked most of the midline cells and longitudinal connections were fused. This phenotype resembles the single-minded CNS axon pattern phenotype (compare Fig. 5B and D,F). Since Notch function is normal during the period of neuroblast segregation, epidermis develops normally in these embryos (Fig. 5F, arrowhead). When we shifted the embryos to the restrictive temperature three hours after egg laying, disruption of Notch function interfered with the normal segregation of neuroblasts. These embryos developed a hyperplastic nervous system and lacked epidermis (Fig. 5C,E). However, these embryos possessed midline cells and formed separate anterior and posterior commissures (compare Fig. 5A and C). Interestingly longitudinal connectives were still not formed properly in these embryos (Fig. 5C, arrows). The results indicate that Notch is required only during early stages of midline development, since late midline functions seem unaffected by the later temperature shift (see Discussion). They furthermore show that a normal midline is required for commissure development.

Notch is required upstream of single-minded

To analyze further the early Notch function, we generated germ-line clones using the FRT, ovo<sup>D1</sup>/hsFLP system (Chou and Perrimon, 1992). ovo<sup>D1</sup> is a dominant female-sterile mutation on the X-chromosome, which blocks oogenesis
Commissure formation depends on the midline (Busson et al., 1983). To generate Notch germ-line clones, we induced FLP activity by administering a mild heat shock (2 hours at 37˚C) to Notch FRT1 ovoD1 FRT; +/hsFLP larvae 24-48 hours after hatching. The larvae were allowed to develop into adults and virgins were mated to appropriate males. In embryos lacking the maternal Notch contribution, we detected four different axonal phenotypes (Fig. 6). About 50% of the embryos showed a severely hyperplasic nervous system with no recognizable CNS axon phenotype (Fig. 6D). About 7% of the embryos showed no obvious developmental defects and had a normal CNS axon pattern (Fig. 6A). 30% of the embryos developed a spitz-like CNS axon pattern phenotype with partially fused commissures and missing connectives (Fig. 6B). The remaining embryos showed a phenotype more like that of single-minded, with fusion of the longitudinal connectives (Fig. 6C). It should be noted that the differences between these last two phenotypic classes are not very pronounced, since some embryos showed characteristics of both classes.

All male embryos derived from germ-line clones lack maternal as well as zygotic Notch function, whereas female embryos lack only the maternal Notch contribution since zygotic Notch activity is supplied by the paternal X-chromosome. To determine the genotype of the embryos, we used a monoclonal antibody against the Sex lethal protein, which is expressed only in female embryos (Bopp et al., 1991). To monitor the fate of the midline cells, we used a P[sim-lacZ] promoter fusion construct on the third chromosome, which drives lacZ expression in all midline cells (Nambu et al., 1990). Double staining of embryos with mAb BP102 and anti-Sex-lethal MAb revealed that the severe hyperplasic CNS phenotype developed only in male embryos, in which maternal and zygotic Notch function is lacking. To assay for expression of the sim-lacZ reporter construct, we analyzed stage 8 to 10 embryos doubly stained for Sex-lethal and β-galactosidase. In embryos that lack only the maternal Notch contribution (e.g. female embryos), activation of single-minded can be affected to different degrees (Fig. 6E-G). In about 10% of the female embryos, expression of the sim-lacZ construct appears normal (Fig. 6E). These embryos probably correspond to the 7% of embryos that show no obvious developmental defects. In the remaining female embryos, the number of sim-lacZ-expressing cells is reduced to differing extents (Fig. 6F,G). In male embryos, the number of the sim-lacZ-expressing cells is always greatly reduced (Fig. 6H). Similar results were obtained from analyzing younger embryos (stages 5 to 6), indicating that no cell death reduces the number of single-minded-positive cells in stage 8-10 embryos. From these results, we conclude that maternal as well as zygotic Notch activity is required for the correct activation of the single-minded promoter.

**DISCUSSION**

Commissural connections are formed in similar ways in the CNS of different species. Commissural growth cones first grow...
towards the midline and, after crossing it, grow along tracts that they had ignored on the ipsilateral side. Based on such observations, the cells located at the ventral midline of the vertebrate spinal cord, the floor plate cells, were implicated in axon guidance already early this century (Kingsbury, 1930). In rat and chick spinal cord, in vitro experiments demonstrated that the floor plate cells can chemotropically attract commissural growth cones over a distance (Tessier-Lavigne et al., 1988; Placzek et al., 1990; Yaginuma and Oppenheim, 1991). The importance of floor plate cells for axonal patterning is further substantiated by data obtained from in vivo studies. Elimination of the floor plate cells by mutations such as cyclops in zebrafish (Bernhardt et al., 1992; Hatta, 1992) or Danforth’s short-tail in mice (Bovolenta and Dodd, 1991) leads to a perturbation of axon guidance in the spinal cord.

Similar phenotypes are observed after ablating floor plate cells in Xenopus or zebrafish (Clarke et al., 1991; Bernhardt et al., 1992; Hatta, 1992). However, in all these cases, the initial growth of commissural axons towards the midline seems unaffected by the loss of floor plate cells. In light of the chemotactic capabilities of the floor plate cells, these results point towards the existence of redundant pathways involving multiple mechanisms that serve to direct commissural growth cones towards the floor plate. The specificity of the directed growth of commissural growth cones towards the floor plate cells might be in part an autonomous property of the commissural neurons themselves.

The Drosophila mutation single-minded leads to specific cell death of all midline cells just before commissure formation and causes a phenotype comparable to those associated with

Fig. 6. Axon pattern phenotypes of embryos derived from Notch germ-line clones. (A-D) Dissected CNS preparations of stage 15 embryos. CNS axon tracts are visualized using the mAb BP102 and HRP immunochemistry. (E-H) Dissected CNS preparations of stage 10 embryos carrying a sim-lacZ reporter construct stained for β-galactosidase expression. Anterior is up. Scale bar is 10 μm in A to D and 5 μm in E to H. (A–C) Range of possible axon phenotypes in embryos lacking only the maternal Notch contribution. (A) About 7% of the embryos developed an apparently normal CNS. 43% developed variable CNS axon pattern phenotypes ranging from spitz-like (B) to single-minded-like (C). Interestingly, the longitudinal connectives did not develop in either case. (D) All embryos that lacked maternal as well as zygotic Notch activity developed a severely hyperplasic nervous system with no recognizable axon pattern. E-H sim-lacZ driven β-galactosidase expression in embryos derived from Notch germ-line clones. (E-G) β-galactosidase expression in female embryos. (H) β-galactosidase expression in male embryos lacking maternal as well as zygotic Notch expression.
mutations in the vertebrate genes cyclops or Danforth’s short-tail: in both of these mutants, axons also cross the midline although the midline cells are genetically ablated (Crews et al., 1988; Thomas et al., 1988; Klämbt et al., 1991). Does this indicate that similarly redundant mechanisms direct commissural fibers towards the midline in Drosophila? The results of studies on the fasI/ablI double mutant phenotype favor this idea (Elkins et al., 1990). Here only the elimination of both the cell adhesion molecule FasI and the tyrosine kinase AblI leads to the striking no-commissure phenotype (Elkins et al., 1990). If redundant pathways direct the formation of commissures in Drosophila, no single mutation would be expected to cause a ‘commissureless’ phenotype like that seen in the fasI/ablI double mutant. However, in a near saturation screen for mutations that affect the embryonic CNS axon pattern, a mutant that indeed lacks commissures (commissureless) has been identified (Seeger et al., 1993). In both the fasI/ablI 1 double mutant and the commissureless mutant, all midline cells are present and no cell fate changes in CNS neurons can be detected (Elkins et al., 1990; Seeger et al., 1993). This mutant phenotype, and the axon pattern phenotype that we describe for Notch embryos, would argue against the notion that redundant mechanisms direct commissural growth cones.

In mutant single-minded embryos, commissural axons cross the midline despite the fact that all midline cells have died. The question thus arises whether the midline functions to attract commissural growth cones at all? Or are the midline cells long enough in place in mutant single-minded embryos to attract the first commissural growth cones? To discriminate between these two possibilities, we took advantage of mutations in the neurogenic gene Notch. In mutant Notch embryos, no commissures form and the development of the midline is disrupted. To determine whether the midline defect alone is responsible for the axon phenotype, we used the Notch10 allele and temperature-shift experiments. Keeping the Notch10 embryos at the permissive temperature until gastrulation is sufficient to rescue the commissureless axon phenotype fully. Shifting the embryos to the restrictive temperature after gastrulation resulted in a hyperplastic nervous system with no underlying epidermis. However, commissures were formed in the CNS of these embryos. These results show that Notch is required early during midline development at the cellular blastoderm stage. If the midline is not specified properly in Notch embryos, no commissures form. Thus in Drosophila, unlike vertebrates, the midline seems essential for guiding commissural growth across the midline. In single-minded embryos, the midline cells are probably long enough in place to allow the initial formation of commissures. After gastrulation Notch appears dispensable for midline cell function since the separation of the two segmental commissures, which is achieved by a migration of the midline glial cells along cell processes of the VUM neurons (Jimenez and Campos-Ortega, 1982). These genes have been shown to act epistatically during neuroblast segregation (Brand and Campos-Ortega, 1988; DeLa Concha et al., 1988; see Campos-Ortega, 1993 for review). The midline anlage could therefore represent an equivalence group within which neuronal and glial cell fates have to be assigned with the help of Notch pathway. In contrast, Notch function seems to be required to specify the ventrolateral stripe of blastoderm cells flanking the mesodermal anlage to a midline fate by activation of the single-minded gene.

Notch does not affect all CNS glial cells in a similar manner. A previous report has shown that excess glioblasts and, subsequently, excess longitudinal glial cells are generated in Notch embryos (Jacobs et al., 1989). In our analysis the overall number of longitudinal glial cells was not affected by mutations in Notch. Although in some segments we find twice the normal number of longitudinal glial cells, in such cases the neighboring segments lack most glial cells, thus leaving the overall number of longitudinal glial cells unchanged. The segmental differences in glial cell number could explain the differences to earlier reports (Jacobs et al., 1989). Other cell lineages also seem not to be affected by the removal of zygotic Notch function. These include the neuronal lineage that generates the MP2 neurons as well as the proneural gene lethal of scute and Delta as well as the proneural gene. Other neurogenic genes like E(spl) and Delta are expressed specifically in the midline anlage (Knust et al., 1987, 1992; Vässin et al., 1987; Kosman et al., 1991). These genes have been shown to act epistatically during neuroblast segregation (Brand and Campos-Ortega, 1988; DeLa Concha et al., 1988; see Campos-Ortega, 1993 for review). The midline anlage could therefore represent an equivalence group within which neuronal and glial cell fates have to be assigned with the help of Notch pathway. In contrast, Notch function seems to be required to specify the ventrolateral stripe of blastoderm cells flanking the mesodermal anlage to a midline fate by activation of the single-minded gene.

Commissure formation depends on the midline
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