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Drosophila Single-Minded Gene and the Molecular Genetics of CNS Midline Development

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ABSTRACT Our goal is to understand the molecular mechanisms that govern the formation of the central nervous system. In particular, we have focused on the development of a small group of neurons and glia that lie along the midline of the *Drosophila* CNS. These midline cells possess a number of unique attributes which make them particularly amenable to molecular, cellular, and genetic examinations of nervous system formation and function. In addition, the midline cells exhibit distinctive ontogeny, morphology, anatomical position, and patterns of gene expression which suggest that they may provide unique functions to the developing CNS. The *single-minded* gene encodes a nuclear protein which is specifically expressed in the midline cells and has been shown to play a crucial role in midline cell development and CNS formation. Genetic experiments reveal that *sim* is required for the expression of many CNS midline genes which are thought to be involved in the proper differentiation of these cells.

In order to identify additional genes which are expressed in some or all of the midline cells at different developmental stages, a technique known as enhancer trap screening was employed. This screen led to the identification of a large number of potential genes which exhibit various midline expression patterns and may be involved in discrete aspects of midline cell development. Further molecular, genetic, and biochemical analyses of *sim* and several of the enhancer trap lines are being pursued. This should permit elucidation of the genetic hierarchy which acts in the specification, differentiation, and function of these CNS midline cells.

The development of the nervous system involves an intricate sequence of cellular and molecular events. Historically, knowledge of nervous system formation was derived primarily from descriptive morphological and cellular analyses. However, recent molecular genetic investigations of several invertebrate and vertebrate systems has allowed the identification of genes which are involved in nervous system development. Such studies have been particularly informative in *Drosophila* where much effort has focused on developmental studies of the embryonic CNS, embryonic and adult PNS, and adult eye using combined molecular, genetic, cellular, and behavioral approaches (for recent reviews, see Thomas and Crews, '90; Banerjee and Zipursky, '90). In *Drosophila,* sensory organs generally consist of a small number of identified cell types, a feature that has greatly facilitated developmental analysis of the PNS (Bodmer and Jan, '87) and the eye (Tomlinson and Ready, '87). However, recent advances on several fronts have accelerated the developmental analysis of the more complex CNS.

Several laboratories, including our own, have begun to concentrate their studies on a discrete subset of CNS cells which lie along the midline of the *Drosophila* embryonic ventral nerve cord. These midline cells (whose development was first described in detail by Bate and Grunewald, '81, in the grasshopper embryo), have characteristic developmental, anatomical, and functional features that distinguish them from the lateral CNS. The midline of the *Drosophila* embryonic CNS consists of only 25 to 30 cells per segment, and includes both neurons and glia (Thomas et al., '88; Klambt et al., '90). Elegant studies using cell-specific markers have begun to elucidate the entire cell lineage of this group of cells (Klambt et al., '90). Analysis of the CNS midline cells using genetic and molecular approaches promises to provide answers to questions regarding CNS cell fate, axon guidance, synaptic connectivity, and neural and glial function.

MIDLINE CELL DEVELOPMENT

The CNS midline cells derive from a small set of blastoderm precursor cells that form two stripes along the anterior/posterior axis of the embryo. These stripes lie between the cells that will give rise to the mesoderm and the cells that will form

the lateral CNS and ventrolateral epidermis (Fig. 1A-St 5). During gastrulation these cells come together at the ventral midline (St 6), divide synchronously, and then form a set of precursor cells whose nuclei extend into the neuroblast (NB) cell layer (St 10). These cells go on to differentiate into mature cells that populate the midline of the ventral nerve cord (VNC) (St 15). The mature midline (Fig. 1B) consists of six glial cells (Jacobs and Goodman, '89a), eight neurons (MP1s and the VUMs), one median neuroblast that gives rise to a large set of neuronal progeny (Thomas et al., '84; Klambt et al., '90), and several other non-neuronal cells (Klambt et al., '90). These cells represent typical CNS cell types: the midline glia wrap the anterior and posterior commissural axon tracts and the VUMs are motorneurons.

DEVELOPMENTAL ISSUES

Several specific questions can be asked concerning the development of the CNS midline. For example, what are the mechanisms that specify a set of blastoderm cells to become the specific progenitor cells of the CNS midline? This question ultimately relates to how positional information is imparted along the dorsal/ventral and anterior/posterior axes early in embryogenesis. Positional information along the dorsal/ventral axis specifies a particular set of cells to become CNS midline and not mesoderm or lateral cells of the CNS. During gastrulation, the four cells/segment on each side of the embryo come together, resulting in eight progenitor cells/segment. Cell lineage studies suggest that the eight progenitor cells possess unique identities (Klambt et al., '90), and it is possible that both cell-cell interactions among these cells and positional information along the anterior/posterior axis may contribute to their individual cell fates.

The eight midline progenitor cells undergo a synchronous cell division after gastrulation (Foe, '89), and soon after their nuclei migrate into the neuroblast cell layer. The cells then begin to differentiate into mature midline glia and neurons. This is followed by considerable cell migration as the cells attain their final CNS positions. Since these processes can be studied in great detail, the molecu-

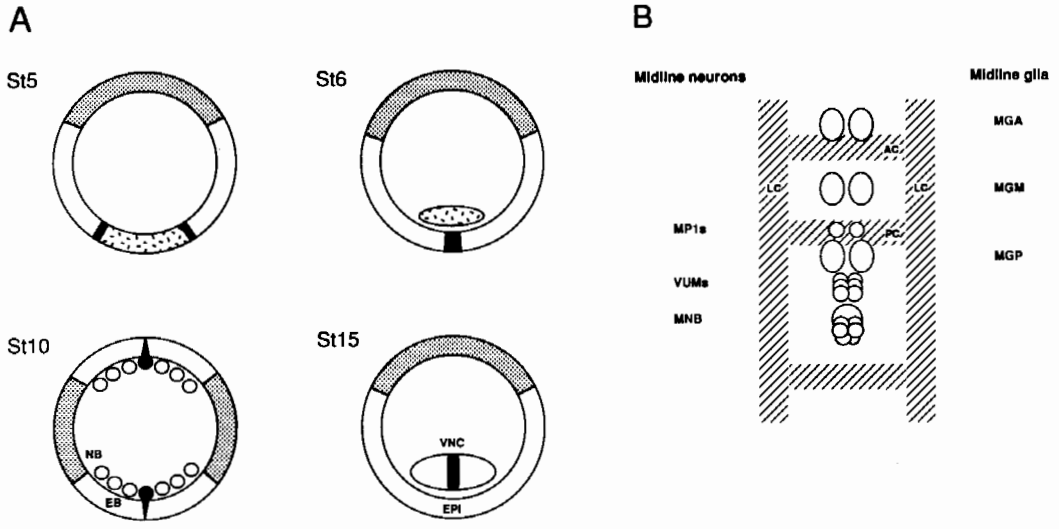

Fig. 1. Summary of CNS midline cells and their development. **A:** Four schematic cross-sections of a *Drosophila* embryo illustrate the development of the midline cells of the CNS. All four stages (Campos-Ortega and Hartenstein, '85) are dorsal side up. Stage 5: The cellular blastoderm—the dashed cells will give rise to the mesoderm, the two black regions to the midline cells of the CNS, the white cells to the lateral cells of the CNS and the ventral epidermis, and the stippled cells to the dorsal epidermis and amnioserosa. Stage 6: Gastrulation results in the invagination of the mesoderm and the two single cell-wide layers that give rise to the CNS midline join together at the ventral surface of the ectoderm. Stage 10: The embryo has undergone germ band extension and neuroblast segregation from the ventral ectoderm. The neuroblasts (NB) form their own cell layer internal to the epidermoblasts (EB). The midline progenitors have their nuclei internal and extend a cytoplasmic process to the surface of the embryo. Stage 15: The neuroblasts and midline progenitors have divided and differentiated into the neurons, glia, and other non-neuronal cells that form the mature embryonic ventral nerve cord (VNC). The VNC lies just above the ventral epidermis (EPI). The midline CNS cells are flanked by the lateral cells of the CNS. **B:** The identified CNS midline cells are illustrated in a horizontal view showing a single ganglion of the embryonic CNS. The anterior direction of the embryo is up. The hatched regions represent axon bundles: LC, longitudinal axon bundles; AC, anterior commissural bundles; PC, posterior commissural bundles. There are three pairs of midline glia (MG): MGA, anterior; MGM, median; and MGP, posterior. There are three recognizable sets of midline neurons: the two MP1 cells, the six VUM cells, and the median neuroblast and its progeny (from Nambu et al., '90).

lar genetic analysis of the midline cells has the potential to significantly contribute to our understanding of the control of cell division, cell fate, and cell migration during CNS development.

The midline cells later participate in axonogenesis. There are at least two aspects of axon guidance to be considered and both may provide useful insights into axonogenesis upon experimental examination. The first concerns the mechanisms that control the pathway choices made by axons of the midline neurons, the MP1s and VUMs. Their axon paths have been described using monoclonal antibody staining and electron microscopic analysis, and their growth cones have been shown to interact with other axons and glia (Jacobs and Goodman, '89b). The second aspect of axonogenesis concerns the formation of the CNS commissural tracts. These axons are predominantly derived from lateral neurons as they cross the midline. They move toward the midline, cross, and make certain pathway choices after reaching the contralateral side of the CNS. In the rat spinal cord, the floor plate cells, which lie at the ventral midline, attract commissural axons by a chemotactic mechanism; a similar process could operate in invertebrates (Tessier-Lavigne et al., '88). Work in both vertebrates and invertebrates has shown that axons exhibit altered expression of surface proteins upon crossing the midline (Dodd et al., '88; Bastiani et al., '87), and this change in protein expression may explain why axons make different contralateral versus ipsilateral pathway choices when the cellular environments are the same. Genetic experiments have implicated both the midline neurons and glia in controlling commissure formation and the identification of genes involved in this process has begun (Klambt et al., '90). In the following sections, we will describe work indicating the importance of the *single-minded (sim)* gene in controlling the differentiation of the midline cells, and our identification of novel midline genes by enhancer trap screening. Finally, we will summarize current knowledge of the hierarchy of genetic interactions that influence CNS midline development.

FUNCTION OF *SIM* IN CONTROLLING CNS MIDLINE DEVELOPMENT AND GENE EXPRESSION

CNS phenotype of sim *mutants and expression of the* sim *gene*

Initial genetic approaches to identify genes that are involved in the development of the CNS involved staining mutant embryos with neuron-specific antibodies or transgenic β-galactosidase markers and looking for CNS defects. This method has proven useful for identifying genes involved in the development of the CNS midline. The *sim* gene was identified in such a screen (Thomas et al., '88), and additional genes with midline defects have been subsequently identified by this method (e.g., Mayer and Nüssllein-Volhard, '88; Finklestein et al., '90; Klambt et al., '90). Mutations in the *sim* locus result in the collapse of the CNS along the midline (Fig. 2), and it was shown that while the midline cells are strongly affected, the lateral nerve cells appear relatively normal (Thomas et al., '88). Thus, *sim* appears to specifically affect the fate of the midline cells.

Subsequent cloning of the gene allowed the expression of *sim* to be investigated by in situ hybridization and immunocytochemical techniques (Thomas et al., '88; Crews et al., '88). These results revealed that *sim* transcripts are initially detected in the cellular blastoderm in the two stripes of CNS midline progenitor cells. Later expression occurs in all of the midline progenitors during germband extension, and then is restricted to the midline glia in the mature CNS. Antibody staining of embryos also revealed that *sim* is a nuclear protein, and sequence analysis indicates that *sim* has significant similarity to the *Drosophila period (per)* gene (Crews et al., '88). *per* controls the periodicity of biological rhythms, is found in the nuclei of some cell types, and has been postulated to be a transcriptional regulatory protein (Siwicki et al., '88; Hardin et al., '90). However, *sim* and *per* do not contain any known DNA binding motif, and it is unknown how they function at the biochemical level. Overall, these results suggest a model in which *sim* regulates the expression of genes which play a role in CNS midline formation.

sim *is required for the differentiation of the CNS midline cells*

Further evidence indicating that *sim* functions in the differentiation of the CNS midline cells was obtained by following the fate of the midline cells in *sim* mutants (Nambu et al., '90). This was accomplished by constructing a fly strain that contains a P-element transposon with a *sim* promoter element fused to the *Escherichia coli* β-galactosidase gene. This strain expresses β-galactosidase in the midline cells of wild-type embryos similar to the native *sim* gene product (Fig. 3A,C,E). When used to follow the midline cells in *sim* mutant embryos (Fig. 3B,D,F), the cells appear relatively normal until differentiation of the progenitor cells into neurons and glia. At this time, the midline cells do not differentiate properly and take their appropriate

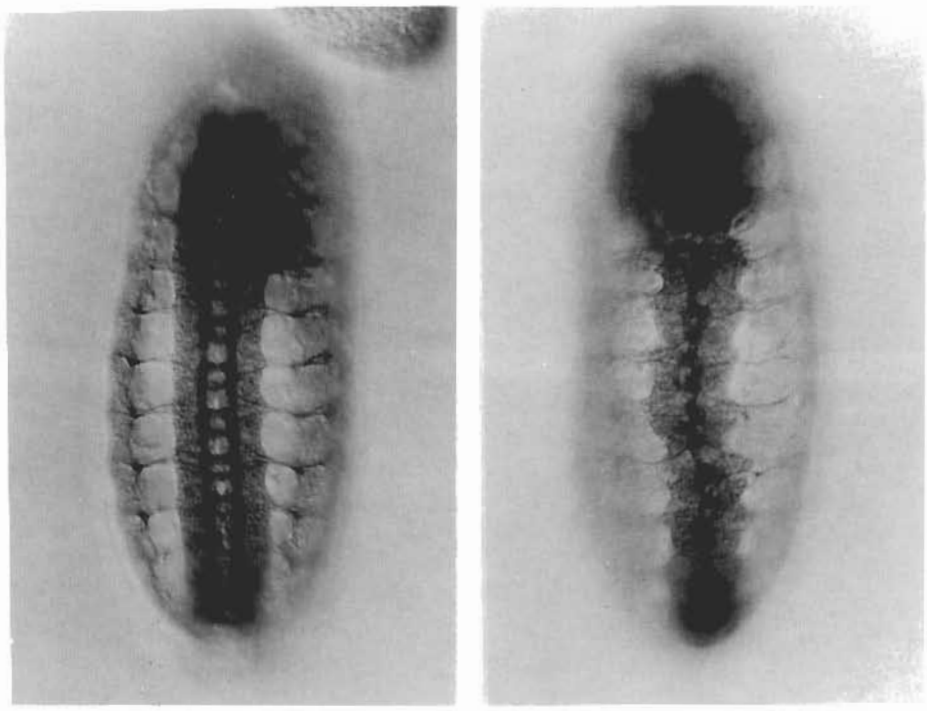

Fig. 2. CNS phenotype of *sim* mutant embryos. Embryonic CNS of **(A)** wild-type, and **(B)** *sim* mutant (*Df(3R)126d*). Whole-mount stage 15 embryos were stained with anti-HRP antisera, which reacts with all nerve cells and their axons. Ventral views are shown with anterior pointing to the top of the page. Cells along the midline of the CNS are misplaced in *sim* (Thomas et al., '88) resulting in a collapse of the CNS, and a fusion of the longitudinal connectives (adapted from Nambu et al., '90).

place in the CNS, but fall out of the CNS and appear to die. These experiments indicated that minimally, *sim* is necessary for the proper differentiation of the midline cells.

sim *is required for midline gene expression*

Since *sim* is a nuclear protein expressed in the midline cells, it is possible that *sim* is required for the expression of one or more genes that are expressed in the midline cells and play a role in their development. A number of potential target genes have been identified that are expressed along the CNS midline, and their requirement for *sim* function was addressed by examining the expression of these genes in *sim* mutant embryos. The results shown in Figure 4 clearly indicate that *sim* is required for the midline expression of many CNS midline genes, including *Toll, rhomboid, slit, engrailed,* and additional less characterized genes that reside at chromosomal positions 32E, 38F, 58D, 91F, and 94F (Nambu et al., '90 and unpublished). The lack of expression is not due simply to the loss of the midline cells since the cells are still present in germband extended embryos when expression is assayed. The transcription of these genes has been shown to require *sim* function, since no midline β-galactosidase expression is observed in *sim* mutant embryos that contain β-galactosidase enhancer trap insertions into the flanking regions of the *Toll, slit,* and 91F genes.

The expression of many CNS midline genes requires *sim* function. *sim* may act directly or indirectly to control the transcription of these genes. Most of the genes analyzed so far show midline expression soon after *sim* protein can be detected in cell nuclei. Thus, *sim* may act as a transcription factor and directly control the transcription of those genes. There is currently no evidence from sequence analysis or biochemical experiments to indicate that *sim* binds DNA. However, *sim* does contain stretches of glutamine-rich and proline-rich regions which resemble sequences shown to function as transcriptional activation domains (Mitchell and Tjian, '89). Further understanding of the biochemical role of *sim* in controlling midline gene expression will require the identification of the midline-conferring *cis*-control elements of the putative target genes using germline transformation and

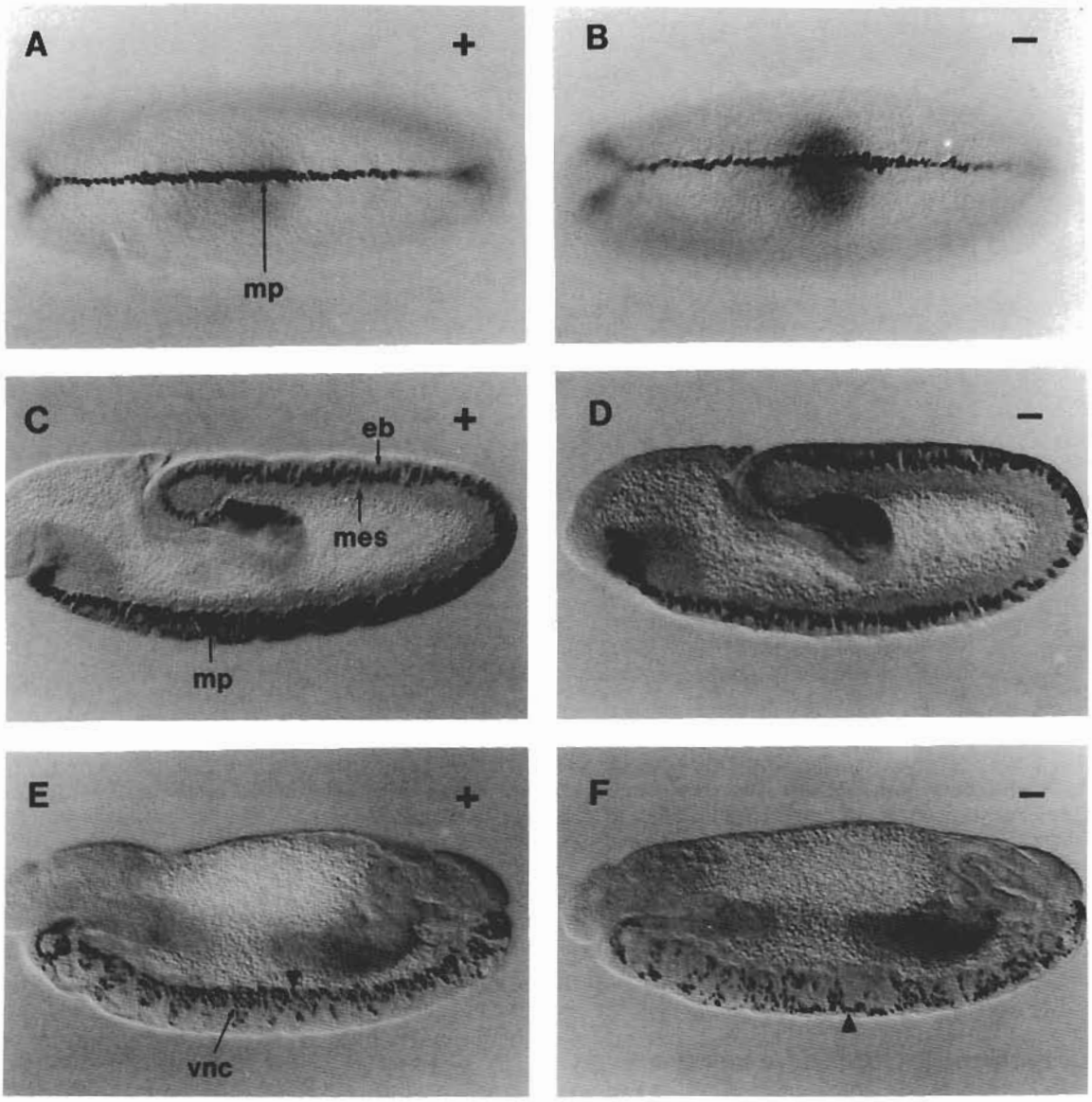

Fig. 3. Fate of the midline cells in wild-type and *sim* mutant embryos. Whole-mount embryos containing a P[*sim/lacZ*] chromosome were stained with an antibody against β-galactosidase to visualize the midline cells. **A,C,E:** *sim*$^+$ embryos. **B,D,F:** *sim*$^-$ embryos. The midline progenitor staining due to the P[*sim/lacZ*] element is similar in both the *sim*$^+$ and *sim*$^-$ stage 10 embryos: (A,B) stage 10 ventral view; (C,D) stage 10 sagittal view. By stage 13, the *sim*$^+$ embryos (E) show organized CNS midline cells, primarily in the dorsal half of the CNS (arrowhead), whereas the midline cells in the *sim*$^-$ embryos (F) are disorganized and predominantly found at the ventral surface of the embryo (arrowhead). All frames have anterior to the left and in the sagittal views, dorsal is up. eb, epidermoblasts; mp, midline progenitors; mes, mesodermal precursors; vnc, ventral nerve cord. Scale bar: 50 mm (adapted from Nambu et al., '90).

subsequent use of biochemical and cell culture transformation experiments.

IDENTIFICATION AND FUNCTION OF NOVEL CNS MIDLINE GENES

Enhancer trap screens

One major goal is to systematically identify genes that are required for the development of the CNS midline. Purely genetic approaches to screen for mutations which alter the formation of the midline CNS remain possible. However, they are labor intensive and many genes may be missed because the mutations may be 1) pleiotropic and affect other cells or tissues thus obscuring the midline defect, 2) phenotypically too subtle to detect, or 3) masked by genetic redundancy. Another approach is to identify genes based on their expression within the developing CNS midline cells. Enhancer trap screens

TABLE 1. CNS midline enhancer trap lines

Insertion	Chromosomal location	Gene
AA69	62A	*rhomboid*
BA01, (87, 242-Hiromi)	91F	
BG17	38F	
BL82, b1	58CD	
BL97	32E	
BP26	87D	*sim*
BP68	52D	*slit*
LJ08	97D	*Toll*
LN01	36A	

have proven an effective way to identify genes in this manner. This technique, developed by W. Gehring and his colleagues (O'Kane and Gehring, '87), utilizes a P-element transposon that contains the *E. coli* β-galactosidase gene fused to a weak promoter element. The transposon is allowed to randomly insert throughout the *Drosophila* genome, and a large number of stocks that contain a single insertion are created. Transposons which have landed near genes may be sensitive to genomic control elements that direct spatially and temporally specific expression patterns, thus β-galactosidase expression can mimic that of the endogenous gene. Embryos from each single line are stained for β-galactosidase expression using X-gal histochemistry and assayed for expression in the cells of interest.

Enhancer trap screening has been successfully used to identify genes expressed in many *Drosophila* tissues including the embryonic peripheral and central nervous system (Ghysen and O'Kane '89; Bier et al., '89; Bellen et al., '89). We have carried out a screen searching for genes expressed in the CNS midline cells. We have screened a total of 2,500 single insert lines and have found 52 that exhibit strong expression in either all of the midline cells or subsets of midline cells. These lines can be roughly divided into three categories: 1) genes that are expressed in all of the midline progenitors early in development (11 lines) (Fig. 5A), 2) genes expressed during differentiation, usually in subsets of cells (25 lines) (Fig. 5B), and 3) genes expressed in subsets of cells after CNS formation (16 lines). The first two classes are likely to play roles in midline cell differentiation and axon guidance, while the last class may participate in CNS terminal differentiation and function. It is worth mentioning that all of the lines are expressed in cells outside of the CNS midline. This is not surprising since utilization of developmentally important genes in multiple developmental processes is a common theme in embryogenesis.

Identification of novel midline genes involved in differentiation

We have focused our attention initially on genes expressed early in development immediately after *sim* expression. The expression of these genes is most likely to be under direct control of *sim*, and also play a role in midline cell differentiation. Nine loci (representing 11 lines plus two lines located at 91F isolated by Yasushi Hiromi and Corey Goodman) have been identified which express β-galactosidase strongly in the midline progenitors in a germband extended embryo (Table 1). It is encouraging that several of these insertions map to genes that were previously known to be expressed in the midline progenitors: *Toll, rhomboid, sim,* and *slit.*

We have begun genetic and molecular analysis of four novel genes identified by enhancer trap screening that show early midline expression (Fig. 5A). They reside at 32E, 38F, 58D, and 91F. The genes have been cloned and mutations have been generated by imprecise P-element excision. Phenotypic analysis of these mutations will be carried out in single and double mutant combinations looking for specific defects on midline cell development and CNS function.

Numerous lines show expression in subsets of midline cells. Figure 5B provides examples of lines that expresses β-galactosidase in all of the midline cells (BL82), the midline glia (BP48 and AA41), or a subset of midline neurons (BJ90).

Cell lineage analysis and formation of commissures

Enhancer trap lines have the potential to provide markers for diverse cell types at different times of development. These can be used to trace cell lineages during normal development, and assess the effects of mutations on development. Initial insight into the lineage of the midline cells came from descriptions of *sim* expression during development (Thomas et al., '88; Crews et al., '88), and use of the P[*sim/lacZ*] element allowed cell fate to be followed in mutants such as *sim* and *slit* (Nambu et al., '90). Recent work by Klambt et al. ('90) has begun to describe in great detail the lineage of the midline glia and neurons from the progenitor cells using a set of midline enhancer trap lines that stain subsets of cells. This work provides the basis for detailed genetic and cellular work similar to development of *Caenorhabditis* (Deppe et al., '78; Sulston et al., '83) and the *Drosophila* retina (Tomlinson and Ready, '87), for example. The enhancer trap lines included markers specific for the VUM neurons, all midline glia, the posterior midline glia, and a combination of the VUM neurons, the median neuro-

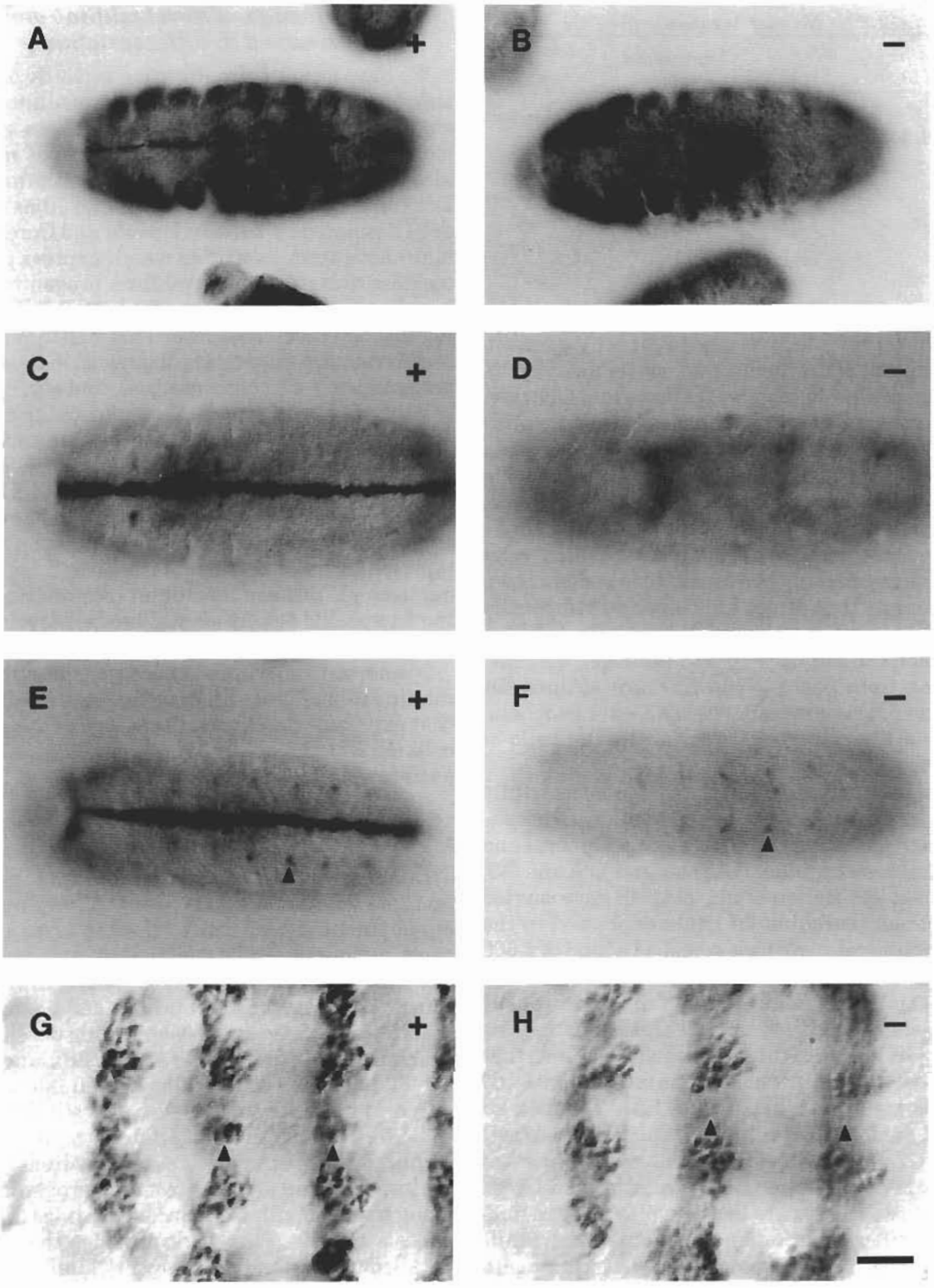

Fig. 4. Requirement of *sim* for midline gene expression. Absence of midline staining of a group of midline genes is observed in mutant embryos homozygous for *sim* deficiencies. **A,C,E,G:** wild-type (+) stage 11 embryos (ventral views). **B,D,F,H:** *sim* mutant (−) stage 11 embryos (ventral views). Genes examined: (A,B) *slit;* (*C,D*) *Toll*; (E,F) 91F, arrowheads denote non-midline glioblasts that stain in both *sim*$^+$ and *sim*$^-$ embryos; (G,H) *engrailed*, notice the absence of *engrailed* staining along the midline in *sim* mutant embryos in contrast to the staining observed in the *sim*$^+$ embryos (arrowheads). Scale bar: (A–F) 50 mm, (G–H) 20 mm (from Nambu et al., '90).

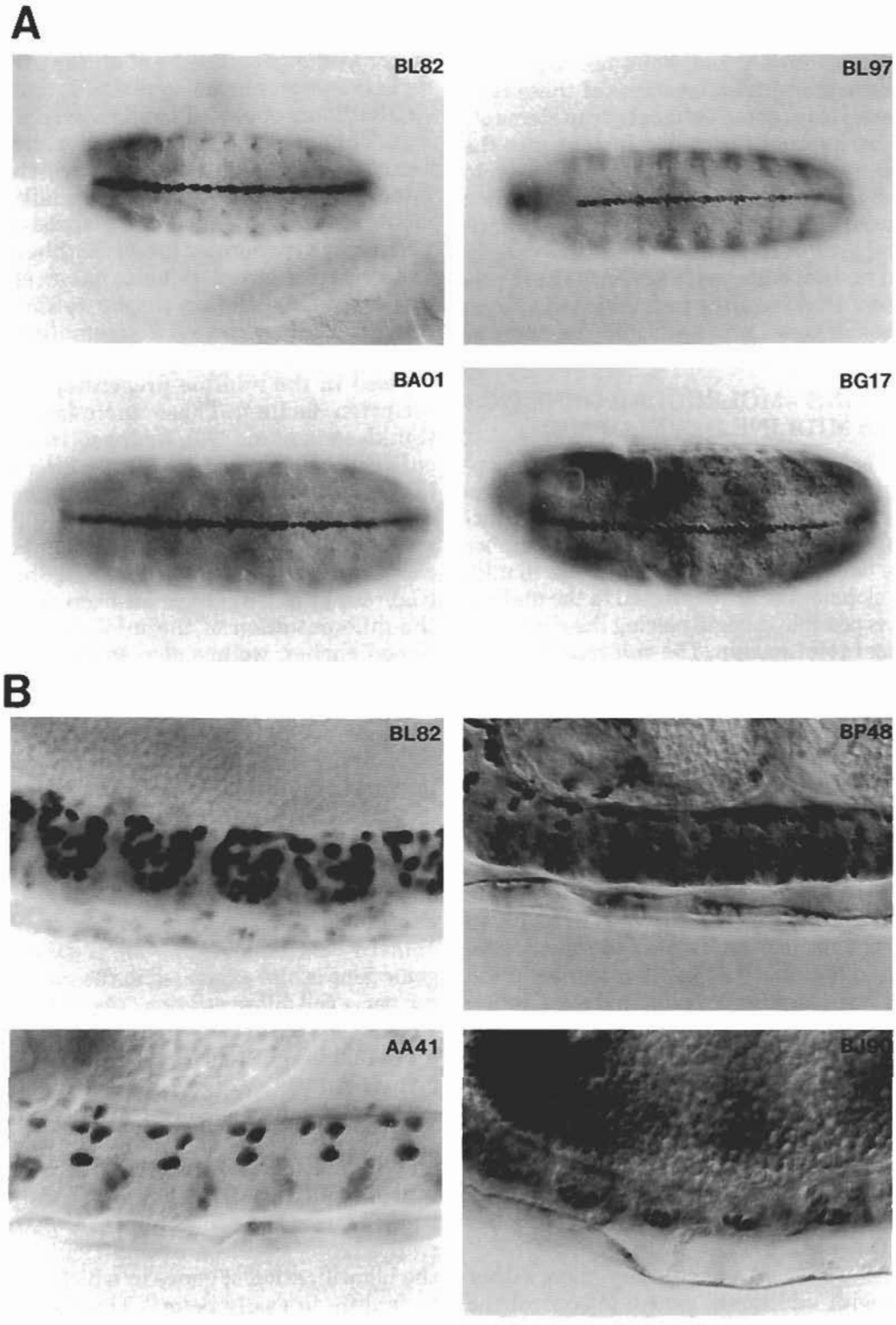

Fig. 5. Enhancer trap lines that stain CNS midline cells. Staining is with a monoclonal antibody that recognizes β-galactosidase followed by HRP histochemistry. **A:** Ventral views of germband extended embryos. Four lines are indicated by name that stain all of the midline progenitors. **B:** Sagittal views of germband retracted ventral nerve cords. Four lines are indicated by name that show staining in all of the midline nerve cells (BL82), the midline glia (BP48, AA41), and a subset of midline neurons (BJ90).

blast, the posterior midline glia, and their support cells. These enhancer trap lines stain the midline precursors early in development and β-galactosidase expression continues throughout neurogenesis, allowing the origins and migrations of these cells to be followed. Use of these enhancer trap elements in mutant backgrounds provided insight into the midline defects of several mutant strains known to have CNS abnormalities. This information was then creatively used to analyze the relationship between commissural growth cones and the midline cells in normal and mutant embryos. The results have provided a model that requires interactions between crossing growth cones and both midline neurons and glia for proper commissure formation.

CONCLUSIONS—MOLECULAR GENETICS OF CNS MIDLINE DEVELOPMENT

Previous sections have described genetic approaches to identify genes involved in CNS midline development and explored in some detail the function of the *sim* gene. However, an increasing number of genes are being identified that play roles in midline cell development or are expressed in the midline cells, and it is possible to begin placing these genes in a hierarchical relationship. The *sim* gene is one of the first genes expressed specifically in the blastoderm cells that will give rise to the CNS midline. As such, it indicates that these cells are already distinct from neighboring cells by the cellular blastoderm stage. The mechanism is not yet clear, but it has been shown that the zygotic genes *snail* and *twist* influence the spatial distribution of *sim* (Nambu et al., '90; Rushlow and Arora, '90). These genes are both likely to be transcription factors (Boulay et al., '87; Thisse et al., '88) that are activated ventrally in response to the nuclear gradient of the *dorsal* protein; *snail* and *twist* (as well as other transcription factors) may work together to result in the activation of the *sim* gene specifically in the blastoderm cells destined to give rise to CNS midline. Two other genes are also expressed in the midline blastoderm cells at the same time as *sim: rhomboid*, which is a transmembrane protein that is involved in midline development (Bier et al., '90), and *Enhancer of split,* a transcription factor that is transiently expressed in these cells, and whose midline function is unknown (Knust et al., '87).

sim itself may act as a regulatory gene, either by itself or with additional genes, to control the transcription of a large battery of genes involved in the differentiation of the CNS midline cells (Nambu et al., '90). These genes can be classified into groups based on when they are expressed and their function: some may be directly controlled by *sim*, others may appear later in the regulatory hierarchy. One set of genes that is expressed in all of the midline progenitors includes *Toll* (Gerttula et al., '88), *amalgam* (Seeger et al., '88), the *Drosophila* EGF receptor homologue (DER) (Zak et al., '90), *slit* (Rothberg et al., '88), and *rhomboid* (Bier et al., '90). This class of genes encodes membrane or secreted proteins which may participate in cell-cell interactions important for CNS midline development. *slit* mutants have a collapsed commissure CNS phenotype similar to *sim* (Rothberg et al., '88), and we have shown that it also has an effect on midline cell differentiation similar to *sim* (Nambu et al., '90). The functions of *Toll, amalgam,* and DER are unknown. Another class of genes that is expressed in the midline progenitors encode transcription factors. These include *orthodenticle* (Finklestein et al., '90), *Krüppel* (Hoch et al., '90), *cut* (Blochlinger et al., '90), *cubitis interruptus* (Eaton and Kornberg, '90), and some *achaete-scute* complex (AS-C) genes (Cabrera et al., '87). *orthodenticle* (Finklestein et al., '90; Klambt et al., '90), *cubitis interruptus* (Patel et al., '89b), and AS-C (Cabrera et al., '87) have all been shown to affect the differentiation of the midline cells. As mentioned earlier, we are also characterizing at the genetic and molecular level several additional genes in these classes (32E, 38F, 58CD, and 91F). How *sim* controls their expression, and how they interact together to influence midline differentiation are important questions to be addressed.

Additional genes act in the establishment of neuronal and glial cell identity, and their migration within the CNS. *Star, spitz,* and *rhomboid* appear to affect the formation or migration of the midline glia, but the neurons appear normal (Mayer and Nüssslein-Volhard '88; Klambt et al., '90), and the *Delta* neurogenic gene is also expressed in the midline cells during nerve cell differentiation (Vässin et al., '87). Both *sim* and *slit* are also restricted late in development to the midline glia and may play a role in their development or function (Crews et al., '88; Rothberg et al., '88). Other genes, such as NTF-1 (Dynlacht et al., '89), *Ultrabithorax* (Peifer and Wieschaus, '90), and *engrailed* (Patel et al, '89a) are expressed in subsets of midline cells, as are many of the enhancer trap lines (see Fig. 5B; Klambt et al., '90). Enhancer trap screens should prove particularly useful in the identification of genes in which mutations are too subtle to easily detect. This may also be important for genes that are part of redundant pathways or in which other genes can functionally compensate. Additional genetic screens that look for suppressors and enhancers of the known midline mutants should allow the further identification

of developmentally important genes. Ultimately, by identifying and analyzing a large number of genes involved in midline cell development, important insights will be revealed at the molecular level in understanding CNS cell fate determination, cell migration, axon guidance, connectivity, and nerve cell function.

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