Comp. Biochem. Physiol. Vol. 104A, No. 3, pp. 399–409, 1993
Printed in Great Britain

0300-9629/93 $6.00 + 0.00
Pergamon Press Ltd

MINI REVIEW

THE DEVELOPMENT AND FUNCTION OF THE *DROSOPHILA* CNS MIDLINE CELLS

J. R. NAMBU, J. O. LEWIS and S. T. CREWS*

Molecular Biology Institute, Department of Biology, University of California, Los Angeles, CA 90024, U.S.A. (Tel. 310 206-3598; Fax 310 206-7286)

(*Received* 1 *September* 1992; *accepted* 30 *September* 1992)

Abstract—1. The midline cells of the *Drosophila* embryonic CNS comprise a discrete neuroanatomical structure consisting of a small subset of neurons and glia.

2. Developmental commitment of the CNS midline cells requires the action of dorsal/ventral patterning genes.

3. The *single-minded* gene encodes a basic–helix–loop–helix transcription factor and acts as a master regulator for the CNS midline lineage.

4. A number of different transcription factors and proteins involved in cell–cell interactions are necessary for the differentiation of midline neurons and glia.

5. CNS midline cells have important functions in the formation of the ventral epidermis and axon commissures.

INTRODUCTION

Formation of the central nervous system (CNS) involves an intricate array of coordinated cellular events. The initial step is the formation of nerve cell precursors from ectodermal cells. The developing nerve cells go on to acquire unique identities and carry out specialized functions. Neurons then extend axonal processes which must locate and synapse with their appropriate cellular targets. Ultimately the cells of the nervous system become organized in a highly complex yet precise fashion. One experimental system which has become increasingly useful for studies of neural development is the fruit fly, *Drosophila melanogaster*. The value of *Drosophila* lies in the availability of a battery of sophisticated genetic, cellular and molecular techniques which can be used to illuminate specific neurodevelopmental events.

The *Drosophila* embryonic CNS consists of over 10,000 neurons and glia organized into two paired brain lobes connected to a ventral nerve cord which is comprised of a series of segmentally repeated and fused ganglia. Each ganglion consists of around 500 nerve cells and associated glia. The neurons extend axons which form a ladder-like scaffold of two parallel longitudinal axon connectives which run along the length of the nerve cord connected by two segmentally repeated commissural tracts.

Within the CNS, the cells that lie along the midline of the ventral nerve cord comprise a well defined subset of CNS cells, approximately 30 neurons and glia per segment (Thomas *et al.*, 1988; Klämbt *et al.*, 1991). Figure 1A outlines CNS midline cell development. The midline cells are derived from two single-cell wide strips of 4 cells per parasegment, referred to as the mesectoderm, which run along the anterior/posterior length of the blastoderm embryo and lie between the mesodermal and neuroectodermal anlages. During gastrulation the mesoderm invaginates and the mesectodermal cells join at the ventral midline. These cells subsequently undergo a synchronous cell division which doubles their number from 8 to 16 cells per parasegment (Foe, 1989). They extend their nuclei into the nerve cell precursor layer, while maintaining cytoplasmic extensions to the underlying epidermis. The midline nerve cell precursors differentiate into neurons and glia which, through a series of cell migrations, take their appropriate places within the mature CNS (Fig. 1B). Midline neurons include: six ventral unpaired median (VUM) cells, two midline precursor 1 (MP1) cells, and the median neuroblast (MNB) and its progeny. These cells include both motoneurons and interneurons. The VUM and MNB neurons also have additional support cells associated with them. There are three pairs of midline glia which ensheath the commissural axons and assist in commissure formation (Jacobs and Goodman, 1989; Klämbt *et al.*, 1991).

The cellular simplicity of the CNS midline and the presence of numerous reagents which allow for cytological visualization of the midline cells provide an excellent system for genetic, molecular and cellular analysis of many aspects of neural development and function. In this review, we discuss a number of

*Author to whom all correspondence should be addressed.

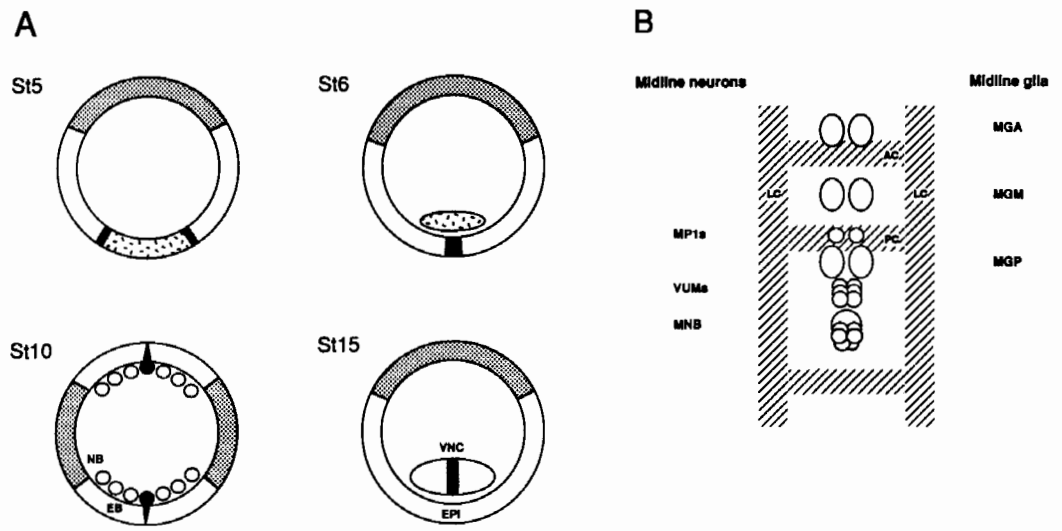

Fig. 1. Summary of CNS midline development. (A) Cross sections of a *Drosophila* embryo illustrate the development of the CNS midline. The four stages depicted are as defined by Campos-Ortega and Hartenstein (1985) and all are dorsal side up. (St5) The cellular blastoderm. The dashed cells give rise to the mesoderm, the two black regions to the CNS midline, the white cells to the lateral CNS and ventral epidermis, and the stippled region to the dorsal epidermis and amnioserosa. (St6) Gastrulation results in the invagination of the mesoderm and the mesectodermal cells join at the ventral midline of the embryo. (St10) The embryo has undergone germband extension and neuroblast segregation from the ventral ectoderm. The neuroblasts (NB) form a layer internal to the epidermoblasts (EB). The midline nerve cell precursors extend their nuclei into the nerve cell precursor layer and maintain cytoplasmic processes to the surface of the embryo. (St15) The germband has retracted and neurons and glia have differentiated to form the mature ventral nerve cord (VNC). The VNC lies just above the ventral epidermis (EPI). St = stage. (B) Identified CNS midline cells are illustrated in a horizontal view of a single ganglion of the embryonic CNS. Anterior 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; MGP, posterior. There are three sets of midline neurons: the two midline precursor 1 (MP1) cells, the six ventral unpaired median (VUM) cells, and the median neuroblast (MNB) and its progeny. (From Nambu *et al.*, 1990.)

developmental issues as they relate to the CNS midline, including the initial commitment of the mesectoderm, the role of lineage-specific regulators of development, interactions between CNS and epidermis, differentiation of neurons and glia, and axonal pathfinding. In addition, we will draw analogies to specialized midline cells in the nervous systems of other species.

DEVELOPMENTAL COMMITMENT OF THE CNS MIDLINE CELLS

Genetic analysis of dorsal/ventral patterning

The *Drosophila* embryo is initially subdivided by dorsal/ventral patterning (D/V) genes into several discrete domains (reviewed in Anderson, 1987; Ferguson and Anderson, 1991). These include the mesoderm, mesectoderm ventral/lateral neuroectoderm, dorsal ectoderm, and amnioserosa (Fig. 2). The first issue in the development of the mesectoderm concerns how D/V genes commit this group of blastoderm cells to become CNS midline. This can be approached genetically by examining the effects of mutations in D/V genes on the patterns of mesectodermal gene expression. In particular, the *single-minded* (*sim*), *lethal of scute*, *Enhancer of split*, and *veinlet* (previously referred to as *rhomboid*) genes have proven to be useful markers of mesectodermal cell fate. Three D/V genes have been shown to play a role in formation of ventrally-derived tissues. They are *dorsal* (*dl*), *snail* (*sna*), and *twist* (*twi*) (reviewed in Rushlow and Arora, 1990) (Fig. 2). All three encode transcription factors (Steward, 1987; Boulay *et al.*, 1987; Thisse *et al.*, 1988). The *dl* gene product forms a nuclear gradient with highest nuclear concentrations ventrally (Roth *et al.*, 1989; Rushlow *et al.*, 1989; Steward, 1989). It is required for the expression of *twi*, which also forms a gradient with highest concentrations ventrally; both *dl* and *twi* are expressed in the mesectoderm. Expression of *sna* also requires *dl* function though *sna* is expressed only in the mesodermal cells, not in the mesectoderm (Alberga *et al.*, 1991; Kosman *et al.*, 1991; Leptin, 1991).

The requirement of *dl*, *sna* and *twi* function for mesectodermal transcription is revealed by the results that *dl* mutants or *sna*/*twi* double mutants completely lack mesectodermal transcription (Rushlow and Arora, 1990). However, since *dl* function is required for expression of *sna* and *twi*, it is unclear whether *dl* directly regulates mesectodermal gene expression. Mutations in *sna* result in ectopic expression of

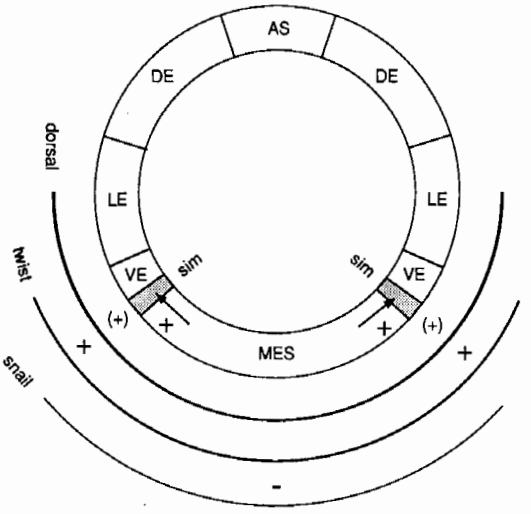

Fig. 2. Dorsal ventral patterning genes regulate *sim* expression. Cross section through a blastoderm stage embryo depicting anlages of: mesoderm (MES), mesectoderm (shaded), ventral ectoderm (VE), lateral ectoderm (LE), dorsal ectoderm (DE), and amnioserosa (AS). *Sim* mRNA is detected in two single cell wide strips corresponding to mesectoderm. The *dorsal* gene is expressed in a nuclear gradient with highest nuclear concentrations ventrally. The *twist* gene is expressed in a steeper gradient. Expression of both *dorsal* and *twist* extends into the mesectodermal cells; *twi* and possibly *dl* are likely to positively regulate (+) *sim* expression. The *snail* gene is expressed only in the mesoderm where it represses (−) *sim* expression. Proper activation of *sim* expression is also thought to depend upon an inductive pathway from the mesoderm, which is depicted by arrows.

mesectodermal genes in the mesodermal anlage (Nambu *et al.*, 1990; Rushlow and Arora, 1990). Mutations of *twi* reveal weak mesectodermal gene transcription in two strips that are shifted ventrally (Rushlow and Arora, 1990; Leptin, 1991; Rao *et al.*, 1991). These results can be explained by the existence of two partially redundant pathways for mesectodermal gene activation. First, *twi* (and possibly *dl*) acts as a ventral activator that includes the mesectodermal and mesodermal anlages, while *sna* acts as a repressor in the mesoderm. Second, a distinct activation signal is sent by the mesodermal cells to the adjacent mesectodermal cells. The requirement for an inductive signal emanating from the mesoderm is consistent with the complete absence of *sim* expression in *twi*/*sna* double mutants where both the *twi* activator and a functional mesoderm are lacking. These two pathways serve to activate mesectodermal gene expression in precise one cell wide domains and are crucial for the developmental commitment of a discrete subset of CNS cells.

Molecular analysis of single-minded *expression*

In order to obtain a more mechanistic understanding of mesectodermal gene activation, our laboratory has begun to examine transcriptional regulation of the *sim* gene using biochemical and germline transformation approaches. Because of its early and specific expression in the mesectoderm, analysis of *sim* transcription is particularly instructive. The gene consists of eight exons spread over 20 kilobases (kb) of DNA and has two promoters (Nambu *et al.*, 1990). The early promoter drives the initial transcription of *sim* in the blastoderm cells. Germline transformation experiments have shown that when fused to the *E. coli lacZ* gene, a 2.8 kb DNA fragment from the *sim* gene directs expression of β-galactosidase in an identical fashion to early *sim* expression (Kasai *et al.*, 1992). Recent work has focused on identifying binding sites for regulatory proteins thought to control the *sim* gene. The *snail* gene (*sna*) encodes a zinc finger transcription factor (Boulay *et al.*, 1987) which has been shown to bind five different sites within the 2.8 kb *sim* fragment (Kasai *et al.*, 1992). Interestingly, the consensus binding site for *sna* contains the E box sequence commonly bound by members of the basic–helix–loop–helix family of transcription factors (Murre *et al.*, 1989a; Blackwell and Weintraub, 1990). Since *twi* encodes a member of this family, this result suggests a mechanism of how *sna* might repress *twi* activation of *sim* in the mesodermal anlage; bound *sna* would prevent *twi* from binding and activating *sim* expression. Efforts are in progress to define the *twi* and *dl* binding sites within *sim* regulatory DNA and to identify the genes involved in the inductive activation signal from the mesoderm. The technique of *in vitro* mutagenesis coupled with germline transformation will be used to test the *in vivo* relevance of these binding sites.

ROLE OF THE *SINGLE-MINDED* GENE

The D/V genes activate the transcription of a set of control genes specifically in the mesectoderm. It is the role of these latter genes to direct the developmental program of the CNS midline lineage. Genetic studies have clearly revealed that the *sim* gene plays a critical role in this pathway. Originally, the *sim* locus was identified on the basis of a mutation which exhibits a severe CNS defect, characterized by the absence of commissural axon tracts and fusion of the longitudinal axon tracts along the midline (Thomas *et al.*, 1988) (Fig. 3). The expression pattern of *sim* has been analysed in detail by *in situ* hybridization, immunohistochemistry using a *sim* antibody, and *sim*/*lac*Z gene reporter constructs (Thomas *et al.*, 1988; Crews *et al.*, 1988; Nambu *et al.*, 1990). Transcripts of *sim* are initially detected in the mesectoderm at the cellular blastoderm stage. As gastrulation takes place, *sim* protein is detected in all of the CNS midline nerve cell precursors. After these cells differentiate into neurons and glia, *sim* expression becomes restricted to the three pairs of midline glia. Antibody staining experiments reveal that *sim* protein is at highest concentrations in cell nuclei.

Initial experiments suggested that the CNS defect in *sim* mutants is due to a specific defect in the formation or positioning of the CNS midline cells; the lateral CNS appeared relatively unaffected (Thomas

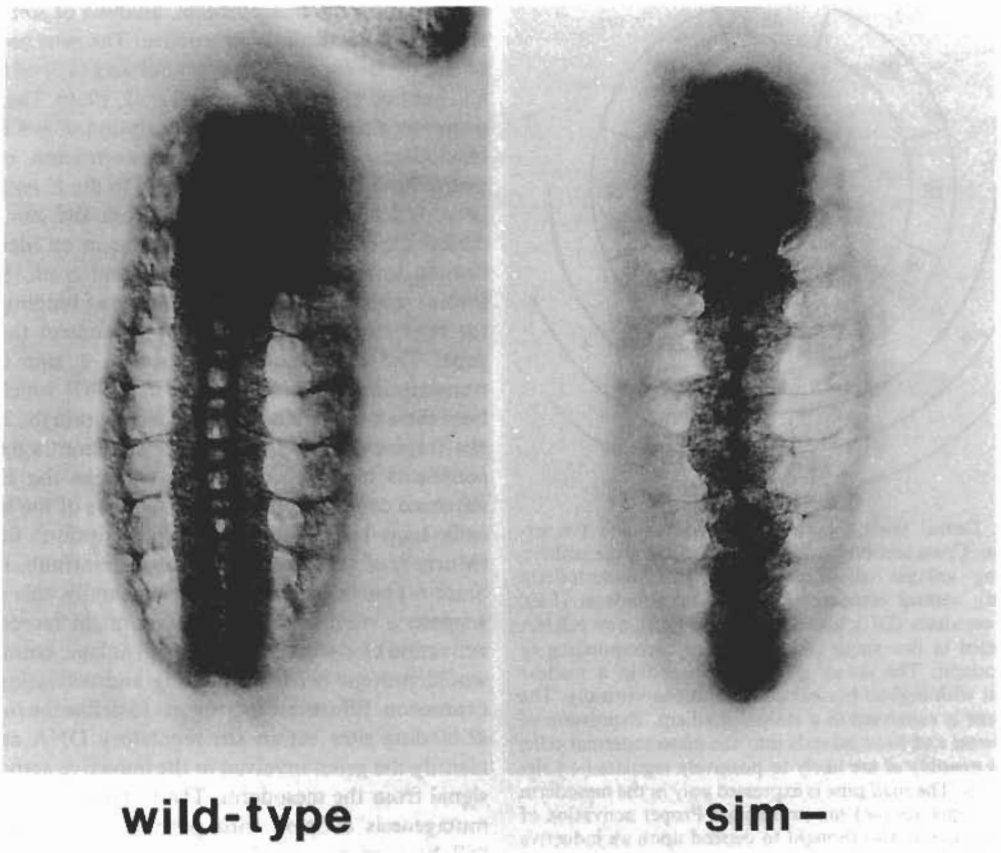

Fig. 3. CNS phenotype of *sim* mutant embryos. Wild type and *sim* mutant embryos were stained with anti-horseradish peroxidase, which recognizes all nerve cells and their axons. In *sim* mutants, there is a lack of commissural axon tracts and a fusion of the longitudinal axon connectives at the midline. Ventral views are shown with anterior pointing to the top of the page. (From Crews *et al.*, 1992.)

et al., 1988). More detailed experiments using a P[*sim*/*lac*Z] reporter construct allowed the fate of the midline cells to be directly followed in *sim* null mutants (Nambu *et al.*, 1991). They revealed that the mutant cells do meet at the ventral midline at gastrulation. However, none of the subsequent events associated with CNS midline development take place. The mutant midline cells fail to undergo their synchronized division and fail to properly extend their nuclei into the nerve cell precursor layer (Fig. 4A). The mutant cells retain the rounded shape of undifferentiated ectodermal cells rather than adopting their normal morphology, and fail to differentiate into neurons and glia and take their appropriate positions in the developing CNS (Fig. 4B).

Since *sim* encodes a nuclear protein and *sim* mutants have a dramatic effect on CNS midline development, it seems likely that *sim* controls gene expression in CNS midline cells. This has been tested genetically by examining the midline expression of 15 genes in *sim* mutant backgrounds. The results indicate that all of these genes require *sim* function for proper transcription in the CNS midline (Nambu *et al.*, 1990; Crews *et al.*, 1992) (Fig. 4C). This includes the *sim* gene itself, which is autoregulatory.

These results indicate that *sim* behaves as a master regulator of midline cell development. This role is reinforced by experiments in which *sim* is ectopically expressed in the embryo (Nambu *et al.*, 1991). Using a *Drosophila* transgenic strain containing the *sim* coding sequence fused to the heat-shock protein 70 (*hsp*70) promoter, *sim* was ectopically expressed in all cells of the embryo. These heat-shocked embryos were examined for midline gene transcription and CNS morphology. The results indicate that when *sim* is ectopically expressed around gastrulation, it has the dramatic ability to transform the entire CNS into CNS midline cells (Fig. 4D). This suggests that *sim* acts as a developmental switch which directs a specific subset of the neuroectoderm to develop into CNS midline. In summary, *sim* is expressed early in neurogenesis at the beginning of CNS midline development and is required for all known developmental events of the CNS midline lineage, including the transcription of all genes expressed in the CNS midline.

The single-minded *gene encodes a basic–helix–loop–helix regulatory protein*

Insight into the biochemical role of *sim* comes from analysis of its protein sequence. Beginning with the

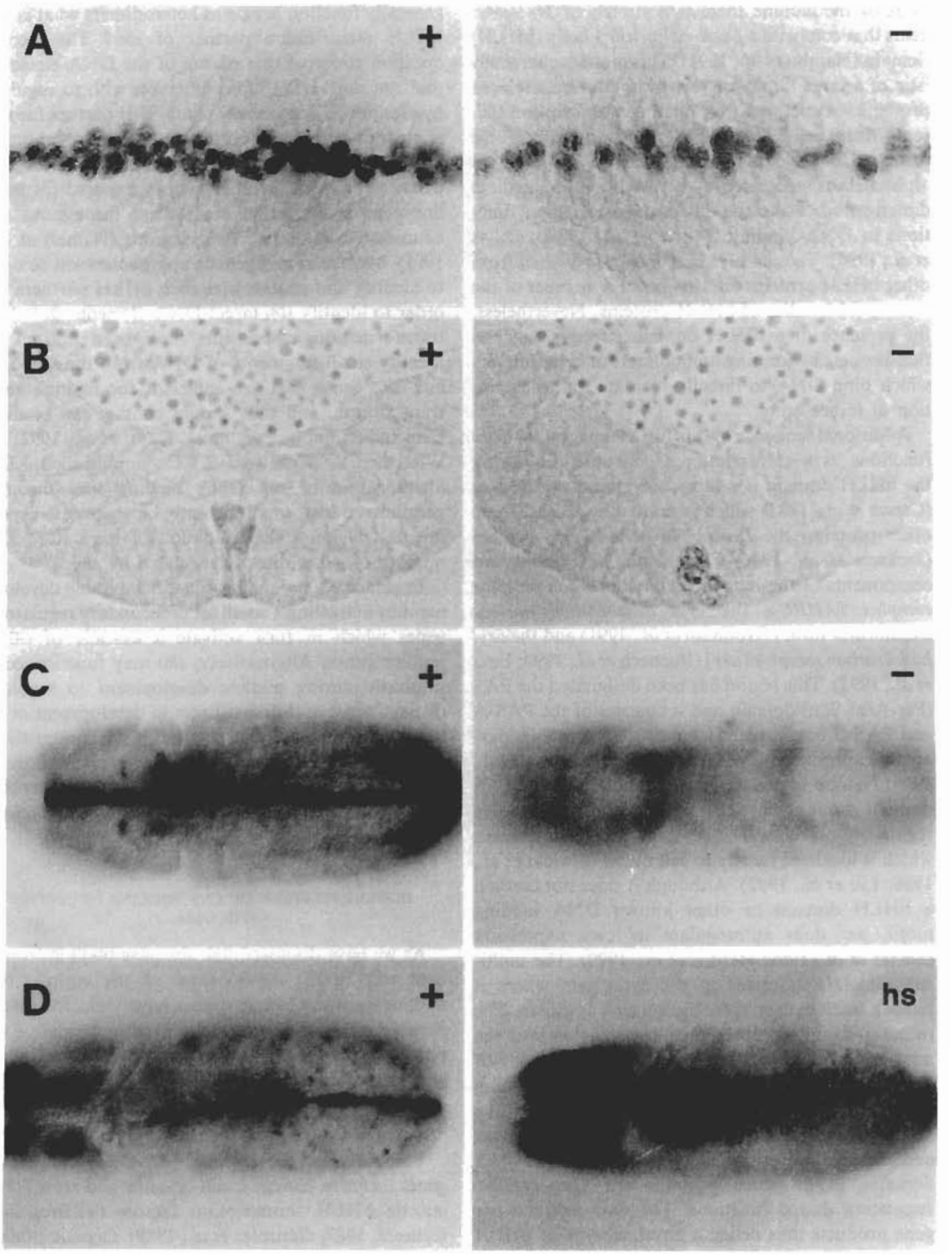

Fig. 4. Function of the *sim* gene. (A) *Sim* is required for the synchronous CNS midline cell division. Expression of a *sim*/*lac*Z reporter construct indicates that there are only half as many midline cells present in *sim* mutant (−) embryos as in wild type (+) after the cell division has normally occurred. Ventral view shown. (B) *Sim* mutant midline cells fail to form nerve cell precursors. Cross sections through wild type and *sim* mutant embryos carrying a *sim*/*lac*Z reporter construct indicate that the mutant midline cells (−) fail to extend nuclei into the nerve cell precursor layer as in wild type (+), and instead retain the rounded shape of undifferentiated ectodermal cells. Dorsal is up. (C) *Sim* is required for midline gene expression. Midline expression of a *Toll*/*lac*Z enhancer trap marker in wild type (+) and in *sim* mutant (−) embryos. Ventral view shown, anterior is to left. (D) Ectopic *sim* expression transforms the lateral CNS and cephalic region into CNS midline cells. Expression of a *veinlet*/*lac*Z enhancer trap marker in wild type (+) and *hsp70*/*sim* (hs) embryos. Ectopic expression of *sim* protein results in activation of the *veinlet* gene in most or all cells of the lateral CNS and cephalic region. Dorsal view shown, anterior is to left. (Adapted from Nambu *et al.*, 1990, 1991.)

initiator methionine there is a stretch of 56 amino acids that comprise a basic–helix–loop–helix (bHLH) domain (Nambu *et al.*, 1991). This motif is characteristic of a large family of vertebrate and invertebrate proteins that act as DNA binding transcription factors (Murre *et al.*, 1989b; Benezra *et al.*, 1990). These proteins function as dimers; the two amphipathic alpha helices, separated by a variable loop, mediate dimerization, while the adjacent basic region functions in DNA binding (Murre *et al.*, 1989b; Davis *et al.*, 1990). The *sim* bHLH sequence is distinct from other bHLH proteins and *sim* is not a member of the known subfamilies of bHLH proteins. Nevertheless, the presence of a bHLH domain strongly suggests that *sim* acts by forming homodimers or heterodimers which bind DNA to directly regulate the transcription of target genes.

Additional sequence motifs further suggest that *sim* functions as a transcriptional activator. Following the bHLH domain is a large 270 amino acid region (Crews *et al.*, 1988) which is present in at least three other proteins, the *Drosophila period* (*per*) protein (Jackson *et al.*, 1986; Citri *et al.*, 1987), and two components of the human *aryl hydrocarbon receptor complex* (*AHRC*): the *aryl hydrocarbon nuclear translocator* (*arnt*) (Hoffman *et al.*, 1991) and the *aryl hydrocarbon receptor* (*ahr*) (Burbach *et al.*, 1992; Ema *et al.*, 1992). This region has been designated the PAS (Per-Arnt-Sim) domain and it consists of the PAS-A and PAS-B boxes, two 51 amino acid repeats separated by approximately 100 amino acids (Crews *et al.*, 1988; Nambu *et al.*, 1991). The function of the PAS domain is unknown, yet it is found only in putative regulatory proteins. The *per* gene encodes a protein which is localized mostly to cell nuclei (Siwicki *et al.*, 1988; Liu *et al.*, 1992). Although it does not contain a bHLH domain or other known DNA binding motif, *per* does autoregulate its own expression (Siwiki *et al.*, 1988; Hardin *et al.*, 1990). The multisubunit *AHRC* resides in the cytoplasm where it binds a small hydrophobic ligand such as dioxin. The receptor–ligand complex then translocates into the nucleus where it binds DNA and activates a group of genes involved in hydrocarbon metabolism, including cytochrome P450 (reviewed in Johnson, 1991). Like *sim*, both *arnt* and *ahr* also encode bHLH proteins and the relative positions of the bHLH and PAS domains in all three proteins are very similar, suggesting shared functions. The *sim*, *arnt* and *ahr* gene products thus define a novel subtype of bHLH proteins which may have distinctive functional properties due to conserved activities of the PAS domain. Finally, the C-terminal portion of the *sim* protein contains homopolymeric stretches of hydrophilic amino acids including glutamine and proline. These motifs act as transcriptional activation domains in other regulatory proteins (reviewed in Mitchell and Tijan, 1989).

The observation that *sim* contains a bHLH domain raises two important questions. Since bHLH proteins generally function *in vivo* as heterodimers what is the bHLH dimerization partner of *sim*? The second question concerns that nature of the DNA elements that the *sim*/bHLH dimer interacts with to regulate transcription. The identity of a bHLH partner for *sim* is unknown. However, the *sim* ectopic expression experiments do indicate that minimally, the bHLH partner(s) as well as other factors required for midline gene transcription are present throughout the neuroectoderm and cephalic regions (Nambu *et al.*, 1991). Molecular and genetic approaches will be used to identify and characterize such bHLH partners. In order to identify *sim* target DNA elements, germline transformation experiments have been utilized to identify small fragments of DNA from the *slit*, *Toll* and *sim* genes that are sufficient for midline gene transcription, and thus should contain *sim* binding elements (Nambu *et al.*, 1991; Kasai *et al.*, 1992; K. Wharton, Y. Kasai and S.T.C., unpublished). The identification of *sim* DNA binding sites through germline transformation and biochemical experiments will allow determination of the nature and number of genes directly regulated by *sim*.

In principal, *sim* may guide CNS midline development by activating a small set of secondary regulatory genes which in turn activate a cascade of other midline genes. Alternatively, *sim* may function continuously during midline development to regulate distinct genes at different times in development or in different subsets of cells by acting in combination with different transcription factors. Answers to these questions will shed considerable light on how master regulatory proteins control the development of particular cell lineages.

DIFFERENTIATION OF CNS MIDLINE NEURONS AND GLIA

As we have discussed, the *sim* gene plays a prominent role in the development of the entire CNS midline lineage. Genetic studies have indicated that a number of other genes are involved in formation of particular midline neurons and glia. Several such genes are described below.

The achaete–scute complex

The *achaete–scute complex* (*AS-C*) contains four genes (*achaete*, *asense*, *lethal-of-scute* and *scute*) that encode bHLH transcription factors (Villares and Cabrera, 1987; Gonzalez *et al.*, 1989). Genetic studies have shown that one or more of these genes are required for CNS neuroblast formation, including the median neuroblast (MNB) (Cabrera *et al.*, 1987; Jimenez and Campos-Ortega, 1990). The *lethal-of-scute* gene is expressed in the MNB (Cabrera, 1990; Martin-Bermudo *et al.*, 1991). The *AS-C* proteins can form DNA binding heterodimers with the bHLH protein *daughterless* (*da*), which is broadly expressed (Caudy *et al.*, 1988; Murre *et al.*, 1989a). The *AS-C* and *da* genes are required genetically for

the expression of genes involved in lateral neuroblast development, and they may play a similar role in formation of the MNB and its progeny.

The slit *gene*

The *slit* gene encodes a large secreted protein that contains multiple sequence motifs consistent with a role in intercellular signalling or cell adhesion (Rothberg *et al.*, 1988; Rothberg *et al.*, 1990; Rothberg and Artavanis-Tsakonas, 1992). These motifs include epidermal growth factor (EGF) repeats, leucine-rich repeats, and the Agrin–Laminin–Perlecan–Slit (ALPS) domain. The *slit* gene is expressed in all of the midline nerve cell precursors and then becomes restricted to the midline glia (Rothberg *et al.*, 1988). Null mutations of *slit* indicate that formation of the midline nerve cell precursors is normal, but during the time of differentiation and cell migration, the mutant cells fail to take their appropriate positions and are ultimately excluded from the CNS. This results in a collapsed CNS phenotype similar to that observed in *sim* mutants (Nambu *et al.*, 1990; Rothberg *et al.*, 1990). The function of *slit* is a mystery; it may promote adhesion of midline cells or be a factor required for their proper differentiation and/or survival.

The ocelliless *gene*

The *ocelliless* (*oc*) gene (previously called *orthodenticle*) encodes a homeobox protein and is likely a DNA binding transcriptional regulator (Finkelstein *et al.*, 1990). Initially it is expressed in all of the midline nerve cell precursors, but later becomes restricted to the midline neurons. Genetic studies indicate that loss-of-function mutations result in a partially collapsed CNS and an absence of midline neurons, including the VUMs and the MNB (Finkelstein *et al.*, 1990; Klämbt *et al.*, 1991).

The spitz *group*

The *spitz* group of genes includes *pointed* (*pnt*), *sichel* (*sic*), *sim*, *spitz* (*spi*), *Star* (*S*) and *veinlet* (*vn*) (Mayer and Nusslein-Volhard, 1988). Several members of this group, including *spi*, *S*, *vn*, as well as a *Drosophila* Epidermal growth factor receptor homolog (Egfr), all share similar mutant CNS defects, a narrowing of the axon scaffold (Mayer and Nusslein-Volhard, 1988; Zak *et al.*, 1990). Furthermore, these mutants each exhibit defects in midline glial formation as well as in ventral epidermis formation (Mayer and Nusslein-Volhard, 1988; Klämbt *et al.*, 1991; S. H. Kim and S.T.C., unpublished). The *spi* gene encodes a protein related to vertebrate transforming growth factor-α (TGF-α) (Rutledge *et al.*, 1992). TGF-α has been shown in vertebrates to be a ligand for Egfr, suggesting that *spi* may be a ligand for *Drosophila* Egfr (Todaro *et al.*, 1980). The *vn* gene encodes a transmembrane protein, and both *vn* and Egfr are expressed in the cells of CNS midline, including the midline glia (Bier *et al.*, 1990; Zak *et al.*, 1990). Although it is attractive to think that these genes function in a signalling pathway required for midline glia formation, it is unclear which cells are involved and exactly what step in the formation of midline glia is affected by the mutants. Further insight will be obtained from mosaic analyses and additional molecular studies of the expression of these genes and the biochemical activities of their products.

Enhancer trap genes

The genes described above have been studied primarily due to their genetic defects in CNS midline formation. Another large set of midline-expressed genes have been identified in enhancer trap screens. This technique utilizes *Drosophila* strains which each bear a P-element insertion located at a distinct site within the genome. The P-element contains the *E. coli* *lacZ* gene coupled to a weak promoter element and acts as an enhancer tester (O'Kane and Gehring, 1987; Bellen *et al.*, 1989; Bier *et al.*, 1989). Several large screens have identified a sizeable number of genes with prominent CNS midline expression (Klämbt *et al.*, 1991; Crews *et al.*, 1992). These include lines with early midline expression in all of the precursor cells and those that are expressed in subsets of cells at later times of development. The validity of this technique has been demonstrated by the identification of genes known to function in CNS midline development, including *slit* and *vn*. The ability to quickly clone the relevant gene and create mutations suggest this will be an important tool in understanding CNS midline development and function.

ROLE OF THE CNS MIDLINE INFORMATION OF THE VENTRAL EPIDERMIS AND AXON COMMISSURES

The midline cells comprise a distinct neuroanatomical structure whose characteristic development, morphology and patterns of gene expression suggest crucial roles in the developing CNS. There are two aspects of midline cell function which will be discussed: induction of cell fate in the ventral epidermis and formation of the commissural axon tracts.

Ventral epidermis formation

The ventral epidermis (VE) of *Drosophila* is defined as the ventral-most 50–60 epidermal cells per segment. Specific cytological and morphological markers exist for these cells, including trap lines and the ventral cuticular denticles. Using these markers the fate of the VE cells can be followed in mutant backgrounds. Initially, the *spitz* group of genes (*pnt*, *sic*, *sim*, *spi*, *S* and *vn*) was shown to have ventral cuticular defects (Mayer and Nusslein-Volhard, 1988). More recently it was demonstrated that VE cell formation and gene transcription was absent or severely reduced in these mutants as well as in Egfr and *oc* mutants (S. H. Kim and S.T.C., unpublished).

This is particularly instructive with respect to *sim* mutants, since it has been clearly shown that the *sim* gene is never expressed in the VE precursor cells (Thomas *et al.*, 1988; Crews *et al.*, 1988). Thus, the conclusion is that VE cell fate requires an inductive signal from the adjacent CNS midline cells (Fig. 5).

While mutations in *sim* and the *spitz* group genes show defects in both CNS midline and VE development, current information suggests that the roles of these genes in these processes are distinct. The model proposed is that *sim* function is required for a signal (probably *spi*/*TGF-α*) to be sent from the midline and received by E*gfr* in the adjacent ventral ectodermal cells (S. H. Kim and S.T.C., unpublished). This signal results in the activation of VE specific gene expression as well as inducing these cells to divide and differentiate.

Commissure formation

Individual embryonic nerve cord ganglia consist of 500 neurons which extend axons that associate together in an intricate array of fascicles, each containing 10–15 axons on average. These fascicles form the longitudinal connectives that lie on either side of the CNS midline and the two segmentally repeated commissures that cross the midline. The formation of the commissures has been examined in wild type and mutants with a defective CNS midline. Normally, the posterior commissure (pc) forms first and contacts the VUM cells and MP1s (Klämbt *et al.*, 1991). The anterior commissure (ac) forms next in close proximity to the pc. The MGM cells then migrate between the two commissures and separate them. Mutations in *oc* affect the formation of the VUM cell neurons and progeny of the median neuroblast; in these mutants no pc forms. Mutations in *spi*, *S* and *vn* both affect the formation and migration of the midline glia, but the VUMs appear normal. These mutations result in a fusion of the axon commissures. A model based on these experiments suggests that pc formation is dependent on a chemotactic signal or localized interaction with the midline neurons (VUMs and/or

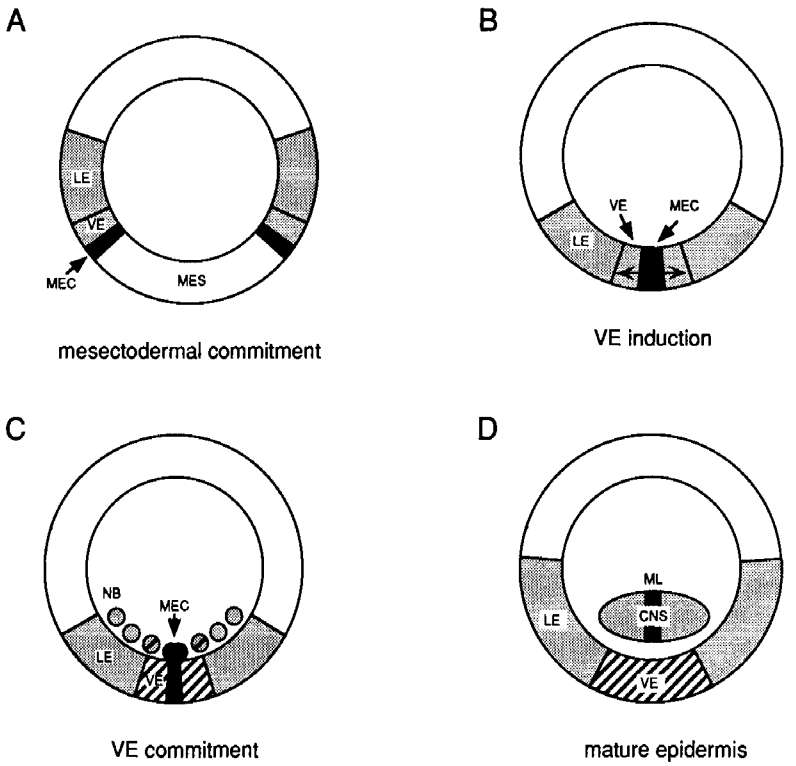

Fig. 5. Model of ventral epidermal (VE) cell commitment. This series of schematic embryonic cross-sections illustrates the formation of the VE. (A) The mesectodermal (MEC) lineage (filled), which lies adjacent to the mesodermal anlage (MES), becomes committed around the cellular blastoderm and gastrulation stages, coincident with the expression of the *sim* gene. The VE and lateral ectodermal (LE) precursors (stippled) may be equivalent at this time. (B) During germband extension, *sim* activates expression of genes that send an inductive signal (arrows) from the MEC to the adjacent VE precursors. The *spitz* class genes *pnt*, *spi*, *S* and *ve*, as well as E*gfr* and *oc*, are required for proper VE cell fate. (C) After the inductive signal, the VE precursors become committed to the VE lineage (dashed). At this time, neuroblasts (NB) delaminate from the neurogenic ectoderm and form a neuronal precursor cell layer (stippled and dashed to indicate common origin with LE and VE precursors). The MEC cells undergo nuclear migration inwards, but remain in close cytoplasmic proximity to the adjacent VE. (D) The VE cells spread out during dorsal closure and cover the ventral surface of the mature embryo. The NBs and MEC cells differentiate into a distinctive set of CNS midline cells (ML).

MP1s) and that separation of the commissures occurs via the migration of the MGM between the two commissures.

What molecules are involved in commissure formation? Two candidates are *fasciclin I* (*fas I*) and the gene encoding the *Drosophila* homolog of the *abelson* oncogene (*abl*). The first molecule, *fas I*, is a homophilic cell adhesion protein that has a novel four domain structure and a phosphatidylinositol linkage to the cell surface (Zinn *et al.*, 1988; Elkins *et al.*, 1990a; Horscht and Goodman, 1990). During commissure formation in *Drosophila* this protein is expressed on all commissural axons as well as the surface of the VUMs. Null mutations of *fas I* are viable and do not have a CNS phenotype. The second molecule, *abl*, is a non-receptor tyrosine kinase that is expressed on all CNS axons (Gertler *et al.*, 1989). *Abl* mutants also lack a strong CNS phenotype. However, *fas I*/*abl* double mutants show a range of CNS defects including a lack of both the ac and pc (Elkins *et al.*, 1990b). This suggests that *fas I* and *abl* mediate interactions between commissural axons and the midline as well as fasciculation of the commissural axons themselves.

Later in development, the expression of both *fas I* and *fasciclin III* (*fas III*) adhesion proteins is predominantly confined to segments of axons within the commissures and are largely absent from segments of the same axons outside the commissures (Patel *et al.*, 1987; McAllister *et al.*, 1992). This indicates that axonal expression of insect surface proteins is precisely regulated. It will be important to determine whether the CNS midline plays a causative role in this process.

THE VERTEBRATE CNS MIDLINE

Although far greater in cellular complexity, the development of the vertebrate spinal cord in many ways resembles insect CNS development. A specialized set of ectodermal cells, the floor plate, lie at the ventral midline of the neural tube and play an important role in commissure formation and establishing cell identity in the adjacent neuroectoderm (reviewed in Jessel *et al.*, 1989). The floor plate cells acquire their identity via an inductive signal from the underlying notochord (Placzeck *et al.*, 1990). The floor plate and the notochord then act to induce cell fate in the adjacent neuroectoderm (Yamada *et al.*, 1991). This formally resembles the role of the *Drosophila* CNS midline cells in inducing cell fate in the adjacent ventral neuroectoderm. Experimental work has also shown that the floor plate emits a diffusible molecule that attracts commissural growth cones (Tessier-Lavigne *et al.*, 1988). As the growth cones traverse the floor plate, new surface proteins likely to mediate axon guidance on the contralateral neuroectoderm are induced (Dodd *et al.*, 1988; Furley *et al.*, 1990). Thus, there are several features which link the CNS midline cells of vertebrates and insects.

Given this similarity it will be particularly interesting to see if genes involved in *Drosophila* CNS midline development and function are conserved in vertebrates. Genetic and molecular approaches are currently being pursued. In both the *cyclops* mutation of zebrafish and the mouse *Danforth short tail* mutant the floor plate cells fail to form, resulting in a fusion of the lateral sides of the brain and spinal cord (Hatta *et al.*, 1991; Bovalenta and Dodd, 1991). Of particular interest are a group of human birth defects that show midline abnormalities (reviewed in Munke, 1989). These related disorders, referred to as holoprosencephalies, result in fusion of both lateral brain hemispheres and facial structures. Genetic studies have indicated that there are at least five loci associated with holoprosencephaly.

CONCLUSION

The *Drosophila* CNS is separated by at least 600 million years of evolution from the human brain and, in comparison, is greatly simplified in structure. Yet it has become increasingly clear that both organisms utilize many of the same components and processes to direct nervous system formation. In the construction of symmetric neuroanatomical structures, it may be that there exist important and evolutionarily conserved properties provided by specialized midline placed cells. It is likely that further studies of the development and function of such cells as well as the genes that control these processes will provide significant insight into aspects of CNS formation, function and evolution.

Acknowledgements—Work in the author's laboratory was supported by grants from the NIH, Jonsson Cancer Center and the Lucille P. Markey Charitable Trust. S.T.C. is a Lucille P. Markey Scholar.

REFERENCES

Alberga A., Boulay J. L., Kempe E., Dennefeld C. and Haenlin M. (1991) The *snail* gene required for mesoderm formation in *Drosophila* is expressed dynamically in derivatives of all three germ layers. *Development* **111**, 983–992.

Anderson K. V. (1987) Dorsal–ventral embryonic pattern genes of *Drosophila*. *Trends in Genet.* **3**, 91–97.

Bellen H. J., O'Kane C. J., Wilson C., Grossniklaus U., Kurth-Pearson R. and Gehring W. (1989) P-element-mediated enhancer detection: a versatile method to study development in *Drosophila*. *Genes Dev.* **3**, 1288–1300.

Benezra R., Davis R. L., Lockshon D., Turner D. L. and Weintraub H. (1990) The protein *Id*: a negative regulator of helix–loop–helix DNA binding proteins. *Cell* **61**, 49–59.

Bier E., Jan L. Y. and Jan Y. N. (1990) *Rhomboid*, a gene required for dorsoventral axis establishment and peripheral nervous system development in *Drosophila melanogaster*. *Genes Dev.* **3**, 190–203.

Bier E., Vässin H., Shephard S., Lee K., McCall K., Barbel S., Ackerman L., Carretto R., Uemara T., Grell E. H., Jan L. Y. and Jan Y. N. (1989) Searching for pattern and mutations in the *Drosophila* genome with a P-*lacZ* vector. *Genes Dev.* **3**, 1273–1287.

Blackwell T. K. and Weintraub H. (1990) Differences and similarities in DNA-binding preferences of MyoD and E2A protein complexes revealed by binding site selection. *Science* **250,** 1104–1110.

Boulay J. L., Dennefeld C. and Alberga A. (1987) The *Drosophila* developmental gene *snail* encodes a protein with nucleic acid binding fingers. *Nature* **330,** 395–398.

Bovolenta P. and Dodd J. (1991) Perturbation of neuronal differentiation and axon guidance in the spinal cord of mouse embryos lacking a floor plate: analysis of Danforth's short-tail mutation. *Development* **113,** 625–639.

Burbach K. M., Poland A. and Bradfield C. A. (1992) Cloning of the AH-receptor cDNA reveals a novel ligand activated transcription factor. *Proc. natn. Acad. Sci. U.S.A.* **89,** 8185–8189.

Cabrera C. V. (1990) Lateral inhibition and cell fate during neurogenesis in *Drosophila*: the interactions between *scute*, *Notch*, *Delta*. *Development* **109,** 733–742.

Cabrera C. V., Martinez-Arias A. and Bate M. (1987) The expression of three members of the *achaete–scute* gene complex correlates with neuroblast segregation in *Drosophila*. *Cell* **50,** 425–433.

Campos-Ortega J. A. and Hartenstein V. (1985) *The Embryonic Development of Drosophila melanogaster*. Springer-Verlag, Berlin.

Caudy M., Vassin H., Brand M., Tuma R., Jan L. Y. and Jan Y. N. (1988) *Daughterless*, a gene essential for both neurogenesis and sex determination, has sequence similarities to *myc* and the *achaete–scute* complex. *Cell* **55,** 1061–1067.

Citri Y., Colot H. V., Jacquier A. C., Yu Q., Hall J. C., Baltimore D. and Rosbash M. (1987) A family of unusually spliced biologically active transcripts encoded by a *Drosophila* clock gene. *Nature* **326,** 42–47.

Crews S. T., Thomas J. B. and Goodman C. S. (1988) The *Drosophila single-minded* gene encodes a nuclear protein with sequence similarity to the *per* gene product. *Cell* **52,** 143–151.

Crews S., Franks R., Hu S., Matthews B. and Nambu J. (1992) The *Drosophila single-minded* gene and the molecular genetics of CNS midline development. *J. exp. Zoology* **261,** 234–244.

Davis R. L., Cheng P. F., Lassar A. B. and Weintraub H. (1990) The *MyoD* DNA binding domain contains a recognition code for muscle-specific gene activation. *Cell* **60,** 733–746.

Dodd J., Morton S. B., Karagogeos D., Yamamoto M. and Jessell T. M. (1988) Spatial regulation of axonal glycoprotein expression on subsets of embryonic spinal neurons. *Neuron* **1,** 105–116.

Elkins T., Hortsch M., Bieber A. J., Snow P. M. and Goodman C. S. (1990a) *Drosophila* fasciclin I is a novel homophilic adhesion molecule that along with fasciclin III can mediate cell sorting. *J. Cell Biol.* **110,** 1825–1832.

Elkins T., Zinn K., McAllister L., Hoffman F. M. and Goodman C. (1990b) Genetic analysis of a *Drosophila* neural cell adhesion molecule: interaction of fasciclin I and abelson tyrosine kinase mutations. *Cell* **60,** 565–575.

Ema M., Sogawa K., Watanabe N., Chujoh Y., Matsushita N., Gotoh O., Funae Y. and Fujii-Kuriyama Y. (1992) cDNA cloning and structure of mouse putative Ah receptor. *Biochem. Biophys. Res. Comm.* **184,** 246–253.

Ferguson E. L. and Anderson K. V. (1991) Dorsal–ventral pattern formation in the *Drosophila* embryo: the role of zygotically active genes. *Curr. Top. Dev. Biol.* **25,** 17–43.

Finkelstein R., Smouse D., Capaci T. M., Spradling A. C. and Perrimon N. (1990) The *orthodenticle* gene encodes a novel homeo domain protein involved in the development of the *Drosophila* nervous system and ocellar visual structures. *Genes Dev.* **4,** 1516–1527.

Foe V. E. (1989) Mitotic domains reveal early commitment of cells in *Drosophila* embryos. *Development* **101,** 1–22.

Furley A. J., Morton S. B., Manato D., Karagogeos D., Dodd J. and Jessell T. M. (1990) The axonal glycoprotein TAG-1 is an immunoglobulin superfamily member with neurite outgrowth-promoting activity. *Cell* **61,** 157–170.

Gertler F. B., Bennett R. L., Clark M. J. and Hoffman F. M. (1989) *Drosophila abl* tyrosine kinase in embryonic CNS axons: a role in axonogenesis is revealed through dosage-sensitive interactions with *disabled*. *Cell* **58,** 103–113.

Gonzalez F., Romani S., Cubas P., Modolell J. and Campuzano S. (1989) Molecular analysis of the asense gene, a member of the *achaete–scute* complex of *Drosophila melanogaster*, and its novel role in optic lobe development. *EMBO J.* **8,** 3553–3562.

Hardin P. E., Hall J. C. and Rosbash M. (1990) Feedback of the *Drosophila* period gene produce on circadian cycling of its messenger RNA levels. *Nature* **343,** 536–540.

Hatta K., Kimmel C. B., Ho R. K. and Walker C. (1991) The *cyclops* mutation blocks specification of the floor plate of the zebrafish central nervous system. *Nature* **350,** 339–341.

Hoffman E. C., Reyes H., Chu F-F., Sander F., Conley L. H., Brooks B. A. and Hankinson O. (1991) Cloning of a factor required for activity of the Ah (dioxin) receptor. *Science* **252,** 954–958.

Hortsch M. and Goodman C. S. (1990) *Drosophila* fasciclin I, a neural cell adhesion molecule, has a phosphatidylinositol lipid membrane anchor that is developmentally regulated. *J. biol. Chem.* **265,** 15104–15109.

Jackson R. R., Bargiello T. A., Yun S-H. and Young M. W. (1986) Product of *per* locus of *Drosophila* shares homology with proteoglycans. *Nature* **320,** 185–188.

Jacobs J. R. and Goodman C. S. (1989) Embryonic development of axon pathways in the *Drosophila* CNS. I. A glial scaffold appears before the first growth cones. *J. Neurosci.* **9,** 2402–2411.

Jessell T. M., Bovolenta P., Placzek M., Tessier-Lavigne M. and Dodd J. (1989) Polarity and patterning in the neural tube: the origin and function of the floor plate. In *Cellular Basis of Morphogenesis, Ciba Foundation Symposium*, Vol. 144, pp. 255–280. Wiley, Chichester.

Jimenez F. and Campos-Ortega J. A. (1990) Defective neuroblast commitment in mutants of the *achaete–scute* complex and adjacent genes of *D. melanogaster*. *Neuron* **5,** 81–89.

Johnson E. F. (1991) A partnership between the dioxin receptor and a basic helix–loop–helix protein. *Science* **252,** 924–925.

Kasai Y., Nambu J. R., Lieberman P. and Crews S. T. (1992) Dorsal–ventral patterning in *Drosophila*: DNA binding of snail protein to the single-minded gene. *Proc. natn. Acad. Sci. U.S.A.* **89,** 3414–3418.

Klämbt C., Jacobs J. R. and Goodman C. S. (1991) The midline of the *Drosophila* central nervous system: a model for the genetic analysis of cell fate, cell migration, and growth cone guidance. *Cell* **64,** 801–815.

Kosman D., Ip Y. T., Levine M. and Arora K. (1991) Establishment of the mesoderm–neuroectoderm boundary in the *Drosophila* embryo. *Science* **254,** 118–122.

Leptin M. (1991) *Twist* and *snail* as positive and negative regulators during *Drosophila* mesoderm development. *Genes Dev.* **5,** 1568–1576.

Lin X., Zwiebel L. J., Hinton D., Benzer S., Hall J. C. and Rosbash M. (1992) The *period* gene encodes a predominantly nuclear protein in adult *Drosophila*. *J. Neurosci.* **12,** 2735–2744.

Martin-Bermudo M. D., Martinez C., Rodriguez A. and Jimenez F. (1991) Distribution and function of the *lethal of scute* gene during early neurogenesis in *Drosophila*. *Development* **113,** 445–454.

Mayer U. and Nüsslein-Volhard C. (1988) A group of genes required for pattern formation in the ventral ectoderm of the *Drosophila* embryo. *Genes Dev.* **2,** 1496–1511.

McAllister L., Goodman C. S. and Zinn K. (1992) Dynamic expression of the cell adhesion fasciclin I during embryonic development in *Drosophila*. *Development* **115**, 267–276.

Mitchell P. M. and Tjian R. (1989) Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. *Science* **245**, 371–378.

Munke M. (1989) Clinical, cytogenetic, and molecular approaches to the genetic heterogeneity of holoprosencephaly. *Am. J. Med. Genet.* **34**, 237–245.

Murre C., Schonleber McCaw P., Vaessin H., Caudy M., Jan L. Y., Jan Y. N., Cabrera C. V., Buskin J. N., Hauschka S. D., Lassar A. B., Weintraub H. and Baltimore D. (1989a) Interactions between heterologous helix–loop–helix proteins generate complexes that bind specifically to a common DNA sequence. *Cell* **58**, 537–544.

Murre C., McCaw P. S. and Baltimore D. (1989b) A new DNA binding and dimerization motif in immunoglobulin enhancer binding, *daughterless*, *MyoD*, and *myc* protein. *Cell* **56**, 777–783.

Nambu J. R., Franks R. G., Hu S. and Crews S. T. (1990) The *single-minded* gene of *Drosophila* is required for the expression of genes important for the development of CNS midline cells. *Cell* **63**, 63–75.

Nambu J. R., Lewis J. L., Wharton K. A. and Crews S. T. (1991) The *Drosophila single-minded* gene encodes a helix–loop–helix protein which acts as a master regulator of CNS midline development. *Cell* **67**, 1157–1167.

O'Kane C. J. and Gehring W. J. (1987) Detection *in situ* of genomic regulatory elements in *Drosophila*. *Proc. natn. Acad. Sci. U.S.A.* **84**, 9123–9127.

Patel N. H., Snow P. M. and Goodman C. S. (1987) Characterization and cloning of fasciclin III: a glycoprotein expressed on a subset of neurons and axon pathways in *Drosophila*. *Cell* **48**, 975–988.

Placzek M., Tessier-Lavigne M., Yamada T., Jessell T. and Dodd J. (1990) Mesodermal control of neural cell identity: floor plate induction by the notochord. *Science* **250**, 985–988.

Rao Y., Vaessin H., Jan L. Y. and Jan Y. N. (1991) Neuroectoderm in *Drosophila* embryos is dependent on the mesoderm for positioning but not for formation. *Genes Dev.* **5**, 1577–1587.

Roth D., Stein D. and Nusslein-Volhard C. (1989) A gradient of nuclear localization of the dorsal protein determines dorso–ventral pattern in *Drosophila* embryo. *Cell* **59**, 1189–1202.

Rothberg J. M. and Artavanis-Tsakonas S. (1992) The modularity of the *slit* protein: characterization of a conserved carboxy-terminal sequence in secreted proteins and a motif implicated in extracellular protein interactions. *J. Molec. Biol.* (in press).

Rothberg J. M., Hartley D. A., Walther Z. and Artavanis-Tsakonas S. (1988) *slit*: an EGF-homologous locus of *D. melanogaster* involved in the development of the embryonic central nervous system. *Cell* **55**, 1047–1059.

Rothberg J. M., Jacobs J. R., Goodman C. S. and Artavanis-Tsakonas S. (1990) *slit*: an extracellular protein necessary for development of midline glia and commissural axon pathways contains both EGF and LRR domains. *Genes Dev.* **4**, 2167–2187.

Rushlow C. and Arora K. (1990) Dorsal ventral polarity and pattern formation in the *Drosophila* embryo. *Semin. Cell Biol.* **1**, 137–149.

Rushlow C. A., Han K., Manley J. L. and Levine M. (1989) The graded distribution of the dorsal morphogen is initiated by selective nuclear transport in *Drosophila*. *Cell* **59**, 1165–1177.

Rutledge B. J., Zhang K., Bier E., Jan Y. N. and Perrimon N. (1992) The *Drosophila spitz* gene encodes a putative EGF-like growth factor involved in dorsal–ventral axis formation and neurogenesis. *Genes Dev.* **6**, 1503–1517.

Siwicki K. K., Eastman C., Petersen G., Rosbash M. and Hall J. C. (1988) Antibodies to the *period* gene product of *Drosophila* reveal diverse tissue distribution and rhythmic changes in the visual system. *Neuron* **1**, 141–150.

Steward R. (1987) *Dorsal*, an embryonic polarity gene in *Drosophila*, is homologous to the vertebrate proto-oncogene, c-*rel*. *Science* **238**, 692–694.

Steward R. (1989) Relocalization of the *dorsal* protein from the cytoplasm to the nucleus correlates with its function. *Cell* **59**, 1179–1188.

Tessier-Lavigne M., Placzek M., Lumsden A. G. S., Dodd J. and Jessell T. M. (1988) Chemotropic guidance of developing axons in the mammalian central nervous system. *Nature* **336**, 775–778.

Thisse B., Stoetzel C., Gorostiza-Thisse C. and Perrin-Schmitt F. (1988) Sequence of the *twist* gene and nuclear localization of its protein in endomesodermal cells of early *Drosophila* embryos. *EMBO J.* **7**, 2175–2183.

Thomas J. B., Crews S. T. and Goodman C. S. (1988) Molecular genetics of the *single-minded* locus: a gene involved in the development of the *Drosophila* nervous system. *Cell* **52**, 133–141.

Todaro G. J., Fryling C. and De Larco J. E. (1980) Transforming growth factors produced by certain human tumor cells: polypeptides that interact with epidermal growth factor receptors. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5258–5262.

Villares R. and Cabrera C. V. (1987) The *achaete–scute* gene complex of *D. melanogaster*: conserved domains in a subset of genes required for neurogenesis and their homology to *myc*. *Cell* **50**, 415–424.

Yamada T., Placzek M., Tanaka H., Dodd J. and Jessell T. M. (1991) Control of cell pattern in the developing nervous system: polarizing activity of the floor plate and notochord. *Cell* **64**, 635–647.

Zak N. B., Wides R. J., Schejter E. D., Raz E. and Shilo B.-Z. (1990) Localization of the *DER/flb* protein in embryos: implications on the *faint little ball* lethal phenotype. *Development* **109**, 865–874.

Zinn K., McAllister L. and Goodman C. S. (1988) Sequence analysis and neuronal expression of fasciclin I in grasshopper and *Drosophila*. *Cell* **53**, 577–587.