Molecular genetics of neuronal development in the *Drosophila* embryo

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ABSTRACT

Making a functional nervous system involves the production of specific types of neurons in characteristic locations and their ability to find and synapse with appropriate target cells. By capitalizing on the advanced genetics and molecular biology of *Drosophila*, a rapidly growing number of genes have been identified that control these events. Studies of the expression and function of these genes in single, uniquely identified cells is possible because of the relative simplicity of the *Drosophila* embryonic nervous system. A class of neurogenic genes, including *N*, *Dl*, and *E(spl)*, controls the emergence of the entire neuronal precursor population, whereas some of the segmentation genes, such as *ftz* and *eve*, control the fates of individual neurons. Later in development, genes encoding cell-surface molecules, called fasciclins, may be involved in the ability of growing neurons to recognize and elongate axons along specific pathways to reach their synaptic targets.—THOMAS, J. B.; CREWS, S. T. Molecular genetics of neuronal development in the *Drosophila* embryo. *FASEB J.* 4: 2476–2482; 1990.

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HOW THE LARGE NUMBERS OF distinct cell types are generated and subsequently assembled during development to produce a functioning nervous system is largely unknown, and remains one of the most important questions in developmental neurobiology. Understanding how even a relatively simple nervous system such as that of an insect develops is a daunting task. For instance, the adult central nervous system (CNS)[1] of the fruitfly *Drosophila* contains more than 100,000 nerve cells that are, for the most part, uniquely specified and precisely interconnected. During the past several years, many researchers have turned to *Drosophila* for a molecular genetic approach to uncover the mechanisms underlying these developmental processes. In so doing, a number of different aspects of neural development in the fly have been studied. In this review, we will concentrate on one of the better studied systems, the embryonic nervous system.

A major advantage of studying neuronal development in the embryo is that the nervous system at this early stage is simple enough to allow detailed studies at the cellular level. Instead of thousands of neurons with highly interwoven processes, the embryonic nervous system consists of only dozens of neurons in each segment, laying down an initial framework of axons on which the rest of the nervous system will subsequently be built. In some cases, the lineages of these early neurons have been traced to their specific precursor cells, allowing one to follow the development of a neuron from its birth to maturity. This ability to easily study the behavior of identified cells from animal to animal (or, in the case of *Drosophila*, from mutant to mutant) is one of the strengths of using insects to study the development of the nervous system. Although there are many genes in *Drosophila* that appear to be involved in neuronal development, we will confine ourselves for the most part to genes whose roles have been studied at the level of identified single cells.

STRATEGIES FOR A MOLECULAR ANALYSIS

Two basic strategies have been used for uncovering molecular mechanisms underlying *Drosophila* neuronal development. Using the first strategy, one searches for mutations that alter a particular developmental event of interest. Such mutations identify genes involved in these events, and may also suggest functions for their gene products. By subsequently cloning such a gene, one can begin to study the function of its gene product at a molecular level. Although it is safe to say that once a gene in *Drosophila* has been identified by mutation it can be cloned, the process can sometimes become laborious, requiring an extensive genetic analysis of the region and lengthy molecular walks along the chromo-

[1]Abbreviations: AS-C, *achaete-scute* complex; ch, chordotonal; CNS, central nervous system; *Dl*, *Delta;* es, external sensory; *E(spl)*, *Enhancer of split; eve, even-skipped; ftz, fushi tarazu;* GMC, gaglion mother cell; ISN, intersegmental nerve; MNB, median neuroblast; N, Notch; NB, neuroblast; PNS, peripheral nervous system; *sim*, *single-minded;* SN, segmental nerve.

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some to reach the mutated gene. The second strategy is essentially the reverse of the first. If one has identified a protein (for example, with a monoclonal antibody) that is expressed in a temporal and spatial pattern suggesting a role in neuronal development, one can clone the gene encoding the protein, physically map the gene's chromosomal location, and make mutations to determine the effects of removing the gene and its product.

A powerful new technique has recently been developed that combines advantages of each of the two strategies described above. This technique, called enhancer trap mutagenesis (1-3), relies on the ability of transposable genetic elements, P elements, to mobilize at high frequency within the *Drosophila* genome. By mobilizing a single in vitro modified P element containing the bacterial *lacZ* reporter gene (which encodes the enzyme β-galactosidase) driven by a weak neutral promoter, a wide range of tissue-specific β-galactosidase expression is seen, depending on the site of insertion. In many cases it has been shown that the pattern of expression associated with a particular insertional event is due to the influence of regulatory elements of a gene near the site of insertion. Therefore, such P element constructs can act as reporters of a gene into which they have inserted (or one nearby) by expressing β-galactosidase in a manner reflecting the gene's normal expression pattern. A remarkable finding has been that approximately 30% of all insertions cause patterns of β-galactosidase expression within the nervous system, either exclusively or in combinations with other tissues (2). This high percentage may be a reflection of both the complexity of the nervous system and the number of genes involved in its development.

Because P elements also have the ability to cause insertional mutations, genes involved in neuronal development can be identified both by their β-galactosidase expression patterns and the phenotypes caused by insertion of the P element. For those insertions with interesting patterns of expression that do not result in an observable phenotype, there are routine genetic techniques for creating deletional mutations within the genomic region surrounding the P element insert. These deletions can be used to assess the phenotypic effects of the removal of the gene of interest and to isolate additional mutations.

Many of these in vitro modified P elements have been engineered to contain, in addition to the *lacZ* coding sequence, a bacterial origin of replication and an antibiotic resistance gene. These sequences allow for direct plasmid rescue cloning of the *Drosophila* genomic sequences flanking the insertional point by simply cutting the DNA from an individual containing the insert, religating to form the plasmid, and finally transforming bacteria. By selecting for antibiotic resistance (conferred by the drug resistance gene within the P element), surviving bacterial colonies will contain the P element sequences plus some *Drosophila* DNA flanking the point of insertion. This DNA can then be used as a probe to isolate the intact gene from wild-type libraries.

CELLULAR EVENTS OF EMBRYONIC NEURONAL DEVELOPMENT

To lay the foundation for molecular analyses, detailed cellular studies of embryonic neuronal development have been carried out in a number of laboratories. These studies have focused on both the CNS, which contains the bulk of neurons, and the peripheral nervous system (PNS), which contains a smaller number of sensory neurons. In *Drosophila,* as in other animals, neuronal development can be crudely divided into two phases. The first phase, termed neurogenesis, comprises the emergence of neuronal precursor cells and production of neurons. During this period, the precursors, and most likely the variety of neurons they generate, acquire their unique developmental fates. During the second phase, the neurons elongate axons that must find and eventually synapse with their appropriate target cells.

Many of the details of these cellular events in *Drosophila* have been elucidated with methods developed over the years for analysis in other animals, such as dye-fills of individual neurons with microelectrodes (4), serial light and electron microscope reconstructions (5), and monoclonal antibodies that recognize restricted subsets of neurons (6). In addition, many of the enhancer trap lines, which express β-galactosidase in subsets of cells within the nervous system, have proved to be excellent markers for specific neural cells and their lineages (7, 8). Using these techniques, many neurons and their precursors can be routinely identified from individual to individual based on their characteristic cell body position and distinct axonal morphology.

In the *Drosophila* embryo, as in all insects, neurons within the CNS are generated by a segmentally repeated set of neuronal precursor cells derived from the ventral region of the ectoderm called the neurogenic region (9). In each bilateral hemisegment approximately 100 ectodermal cells are found within the neurogenic region. Beginning just after gastrulation, approximately one in four of these ectodermal cells begins to enlarge and delaminate inward from the ventral surface to become the major precursors of the nervous system, the neuroblasts (NBs). The remaining ectodermal cells become precursor cells that give rise to the epidermis. Emerging from the ectoderm within each hemisegment of the *Drosophila* ventral nerve cord are approximately 25 NBs, a glioblast that generates a set of glial cells within the CNS, plus a single unpaired median neuroblast (MNB) at the midline (10, 11). As diagramed in **Fig. 1**, the NBs act as stem cells, dividing asymmetrically to produce families of ganglion mother cells (GMCs), each of which in turn divides symmetrically to give rise to two daughter neurons. As in other insects, individual neuroblasts can be uniquely identified by virtue of their positions within the neuroepithelium and by the characteristic families of neurons they produce.

The earliest events of neuronal development, includ-

ing the formation of certain NB lineages, the production of the first identified neurons, and the pathway choices made by their growth cones, are highly conserved among the insects. Thus, studies of other species can be used as a guide in deciphering developmental events in *Drosophila*. The paradigm for cellular analyses of *Drosophila* neurogenesis is a series of elegant cell ablations studies carried out in the larger grasshopper embryo (e.g., ref 12). These studies have shown that within a region in which a particular NB will emerge, each of the surrounding ectodermal cells is competent to become that NB. For example, if an NB is ablated soon after it begins to emerge, one of the nearby ectodermal cells will take its place. It appears, therefore, that some type of inhibitory signal delivered by the NB itself prevents the remaining ectodermal cells within its vicinity from also becoming NBs. Furthermore, as two adjacent NBs will each proceed to generate a distinct family of neuronal progeny, there must be additional mechanisms operating that are capable of imparting a unique identity to each NB.

The PNS is considerably simpler than the CNS. It is composed of a number of sensory organs, found in characteristic locations along the body wall, which are innervated by neurons that send axons into the CNS (13). Each hemisegment contains about 40 sensory organs. Each sensory organ is composed of an organ-specific number of different cell types, including support cells and neurons. Five different types of sensory neurons can be distinguished based on their morphology and the type of organ innervated, including two particularly well-studied sensory organs, the external sensory (es) and the chordotonal (ch). Studies of the development of the PNS in *Drosophila* as well as in a number of other insects have shown that the cells within an individual sensory organ appear to be derived from a characteristic set of cell divisions from a single precursor cell (14). Clusters of proneural ectodermal cells form at sites of future sensory organs. However, only one cell within each cluster develops into a sensory organ precursor cell, and as in the CNS during NB formation, this cell inhibits the surrounding cells from also becoming precursor cells. As shown in **Fig. 2** for an external sensory (es) organ, the sensory organ precursor divides, giving rise to two cells, each of which divides once more to generate the four cells of the sensory organ. These cells then differentiate into the characteristic cell types of a particular sensory organ.

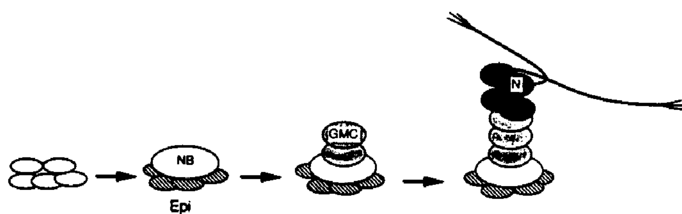

Figure 1. The basic features of *Drosophila* neuronal development illustrated by the emergence and differentiation of a single neuroblast (NB) and its lineage. One of four to five ectodermal cells enlarges and delaminates to become an NB; the remaining ectodermal cells become epidermal precursors (Epi). The NB divides asymmetrically, generating a chain of ganglion mother cells (GMC), each of which divides symmetrically to give rise to two daughter neurons (N). Shortly after birth, the neurons begin elongating axons along specific routes to their synaptic targets.

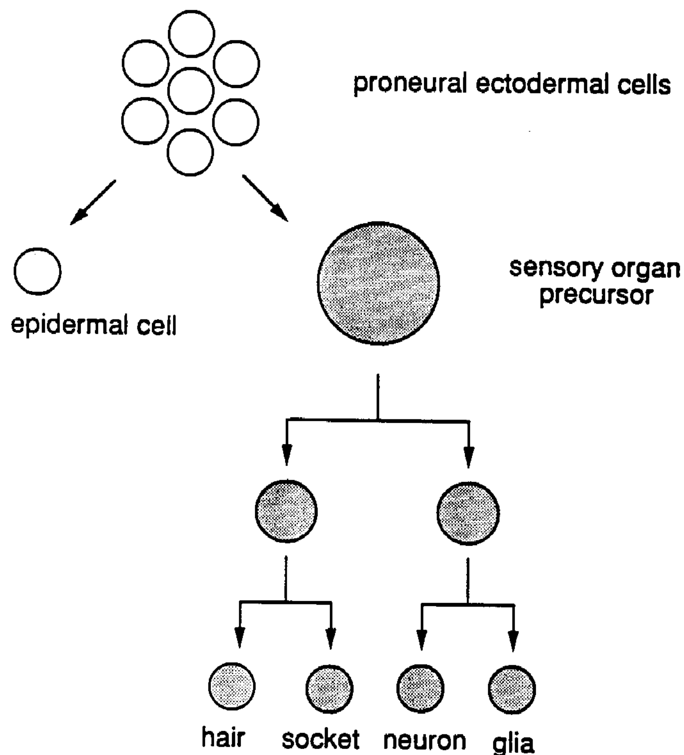

Figure 2. Lineage of an es sensory organ. A proneural cell cluster consists of a small group of ectodermal cells, one of which will form a sensory organ precursor cell, and the others epidermal cells. Once formed, the sensory organ precursor divides into two cells. One of these cells undergoes an additional division to form a hair cell and a socket cell. The other precursor gives rise to a neuron and a glial cell.

Shortly after birth, neurons within the CNS and PNS begin to differentiate, elongating axons along specific routes that will ultimately lead them to their appropriate target cells. Axons are not extended randomly. Instead, there is a stereotyped temporal and spatial progression of outgrowth and recognition events between subsets of neurons, resulting in a highly ordered framework of discrete axon bundles, as shown in **Fig. 3**. These axon bundles are organized into two bilaterally symmetrical connectives running the length of the embryo, two commissural tracts in each segment connecting one side to the other, and two nerves, the segmental and intersegmental nerves (SN and ISN), in each segment exiting the CNS to the periphery. Figure 3 shows some of the earliest differentiating neurons, named aCC, RP1, and RP2, which show affinity for one another to form the ISN. Such patterns of axon outgrowth suggest that the growing tips of the axons, the growth cones, can differentially recognize the cell surfaces of other axons and glial cells, and that these cell recognition events mediate the specific pathway choices made by neurons (6). Actual functioning of the nervous system begins later in development with the es-

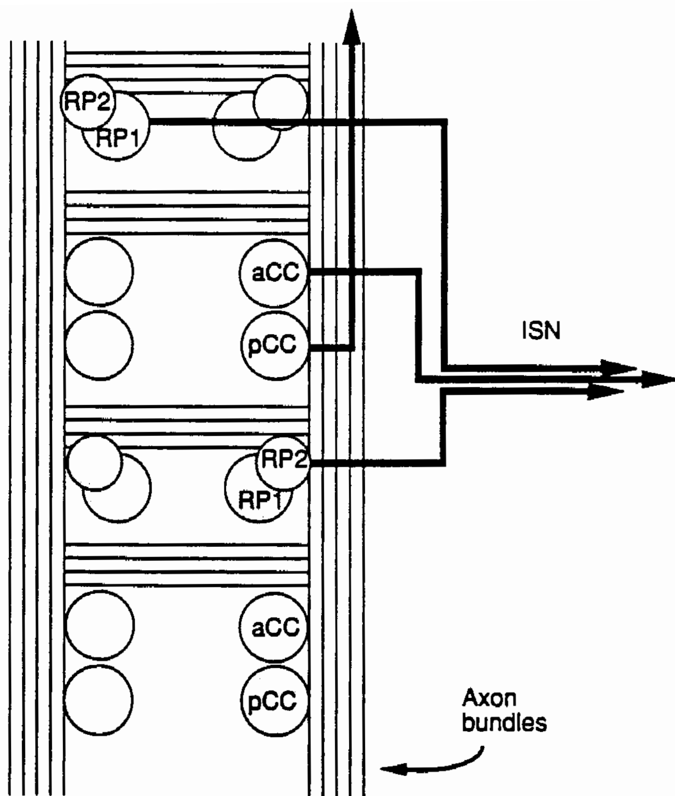

Figure 3. Early differentiating neurons form an initial scaffold consisting of discrete axon bundles. The axons of the aCC, RP1, and RP2 neurons show affinity for one another and form one of these bundles, the intersegmental nerve (ISN), which exits the CNS to the periphery. The axon of the pCC (the sibling of aCC) extends anteriorly, forming one of the first longitudinal axon bundles within the connective by fasciculating with other identified neurons (not shown).

tablishment of synaptic connections and the acquisition of electrical excitability, both of which represent later steps in the differentiation process.

GENES INVOLVED IN NEUROGENESIS

The neurogenic genes control the decision between the NB and epidermal precursor cell fate. This class of genes, which includes *Notch (N), Delta (Dl), Enhancer of split (E(spl)) mastermind, big brain, and neuralized,* was initially identified by the similar phenotype each displays when mutated (15). Mutations in any one of these genes cause an overproduction of NBs within the neurogenic region at the expense of epidermal precursors. In addition, genetic interactions between certain members of the neurogenic group have been demonstrated, particularly between *(E(spl))* and both *N* and *Dl,* which suggests that interactions between their gene products are important for NB production. Consistent with the model of cell-cell communication and inhibition implied by cellular studies, the *N* and *Dl* genes encode membrane-spanning proteins with extracellular domains containing regions of striking similarity to mammalian epidermal growth factor (16, 17). The *N* gene product appears to be a more ubiquitous enabler of cell interaction in a variety of other tissues such as the fly retina, where it is required for proper development of a number of different cell types (18). A transcript from the *E(spl)* locus encodes a putative membrane-associated protein with similarity to G proteins (19), which act as mediators of signal transduction. Three additional products of the *E(spl)* locus have sequence homology to known transcription factors and thus are presumably involved in controlling the expression of other genes (20). Exactly how these and the other neurogenic genes combine to generate the NBs is not yet know, but it is tempting to speculate that within this class might be found the repertoire of gene products needed for cell signaling events and the transduction and implementation of the inhibitory signal.

In addition to producing the correct number and spacing of NBs, there must be some mechanism to ensure that each NB is correctly specified to produce its stereotyped set of GMCs and subsequent neuronal progeny. As ablated GMCs are not replaced, the identity of a GMC appears to depend strictly on position of birth within its particular NB lineage (12). In contrast, the two postmitotic daughter neurons from a single GMC division require early cell interactions to acquire their unique fates (6). Therefore, neuronal identity is specified by a combination of position within an NB lineage and cell interactions soon after birth. Of the ~250 progeny neurons per hemisegment generated during embryonic development, most if not all are uniquely determined for their specific functions. The extent of this identity ranges from a neuron's distinct morphology to its choice of synaptic targets and use of particular transmitters and receptors. It is apparent that the specification of the insect nervous system requires an equally extensive array of genetic control elements.

An interesting finding over the past few years has been that some members of the *Drosophila* segmentation genes, in addition to being expressed early in development within the ectoderm to establish the pattern of segmentation, are also expressed during neurogenesis in restricted subsets of NBs, GMCs, and neurons. Given the regulatory role that these genes play in specification of the segmental body plan, it seemed plausible that they might serve an additional role in specification of NBs or their lineal progeny. However, this relationship may prove to be reversed. Based on a recent study of the expression of the *engrailed* segmentation gene within the nervous system of arthropods and chordates, it has been proposed that the segmentation genes may have evolved initially to function during neurogenesis and were only later adopted by insects and other arthropods to control the segmentation process (21).

A major obstacle to studying the effects on neuronal development of mutations in certain classes of segmentation genes is that the early segmentation defects caused by such mutations interfere with any attempt to analyze the nervous system. This problem was overcome for two of the segmentation genes, *fushi tarazu (ftz)* and *even-skipped (eve).* The use of a temperature-sensitive allele of *eve* allowed early development to proceed normally with subsequent elimination of *eve* function during neuronal development by a shift in temperature (22). For *ftz,* transgenic individuals carrying an in vitro modified gene in-

capable of CNS expression allowed removal of *ftz* function within the nervous system only (11). Both *ftz* and *eve* are expressed in restricted subsets of GMCs and neurons. The neuronal subsets are distinct but overlapping, and fortunately include several cells that have been particularly well studied: the aCC and pCC (sibling neurons) and the RP2 neuron. In the absence of *eve* function, the pCC pathway choice is unaltered, but both the aCC and RP2 axons choose inappropriate pathways and thus exhibit abnormal axon morphologies. The abnormal choices are not random; instead both the aCC and RP2 each choose a preferred aberrant pathway. This suggests a model in which *eve* functions to regulate the expression of downstream genes controlling pathway choice, and that in the absence of *eve*, transformations of cell fate take place. Removal of *ftz* function causes the absence of *eve* expression in RP2 (resulting in abnormalities in RP2 morphology identical to those seen in *eve* mutants), but has no effect on either *eve* expression or morphology of aCC. This implies, as has been proposed for segmentation gene function in the early embryo (23), the existence of multiple regulatory controls interacting with one another to generate the diversity of unique fates within the developing nervous system.

This regulatory control will most likely consist of additional genes acting at different points in the developmental pathway to specify NBs, GMCs, and their progeny neurons. Although less well studied phenotypically for their effects on embryonic CNS development, genes of the *achaete-scute* gene complex (AS-C) are good candidates. These genes are express within the developing neuroepithelium at the time of NB segregation, and preliminary examination of mutations within the complex shows that a variety of identified neurons appear to be either missing or altered (24). Genes of the AS-C encode proteins with similarity to transcription factors of the helix-loop-helix family (25). Because this family of factors appears to bind DNA as heterodimers to activate transcription of other genes, it is possible that a fair amount of diversity may be generated by the formation of heterodimers between products of the AS-C and with other helix-loop-helix proteins (26). Another candidate gene, called *single-minded (sim)*, controls the emergence of a restricted subset of neuronal and glial precursor cells at the midline, including the MNB (27, 28). The *sim* gene encodes a nuclear protein expressed exclusively within the group of midline cells that fail to emerge in the mutant. Although the phenotype and the pattern of *sim* expression suggest that it controls downstream genes involved in the differentiation of these precursors, its gene product shows no homology to any known transcription factor, and thus may define a novel class of regulatory protein within the nervous system. Finally, new genes controlling cell fate will surely be identified from the enhancer trap lines by virtue of their expression patterns and mutant phenotypes. These lines will also yield a variety of markers for different lineages, which can then be used in studies of the phenotypic effects of mutations.

GENES INVOLVED IN PNS DEVELOPMENT

Recent work has identified a number of genes that play specific roles in the steps of sensory organ formation, from the emergence of the precursor cells to the identity of the sensory organ itself. Early in PNS development, the *daughterless (da)* and AS-C genes participate in the formation of proneural cell clusters. Mutations in *da* cause a complete absence of all sensory cells (29), whereas deletions of the entire AS-C result in a loss of all sensory cells except the chordotonal (ch) organs (30). Further genetic analysis has revealed that the AS-C is composed of multiple genes, each of which controls the formation of a subset of sensory organs. The *da* gene, like the AS-C genes, encodes a protein with similarity to the helix-loop-helix family of transcription factors (31), and several lines of evidence suggest that the *da* and AS-C gene products interact together to regulate a set of genes that result in proneural cell cluster formation (26).

From each cluster of proneural cells, a sensory organ precursor emerges and inhibits the adjacent cells from also becoming sensory organ precursors; these surrounding cells will generally differentiate into epidermal cells. Members of the neurogenic group of genes, such as *N* and *Dl*, previously shown to play a role in CNS development, are involved in this lateral inhibition. In a manner similar to that in the CNS, mutations in these genes result in an increased number of sensory cells at the expense of the epidermis (32).

Once a sensory organ precursor forms, it proceeds through a fixed series of divisions to produce the four cells of the sensory organ. The *numb* gene, which encodes a putative transcription factor of the zinc finger class, appears to control proper sensory cell lineage (33). Mutations in this gene alter the lineages of sensory organ precursors such that the socket and hair cells are duplicated and the neurons and glia are absent. In contrast to *numb*, which affects all sensory organs, the *cut* gene is required for the realization of a particular sensory organ identity (34). The es and ch organs both contain four cells, but the morphology and function of these cells differ between the two types of organ. Mutations of *cut* result in transformation of es organs into ch organs. Since the *cut* gene product is a homeobox-containing protein and is expressed by all es cells but not by any ch cells, it is likely to control the expression of a set of genes involved in the differentiation of the es organ.

GENES INVOLVED IN NEURONAL PATHFINDING

Studies of both vertebrates and invertebrates over the past several years have shown that the growing tips of neurons, the growth cones, are able to recognize and grow along specific glial and neuronal cell surfaces (e.g., 35, 36). Ablation studies in the grasshopper have provided insight into possible mechanisms. In one series of ablations, the behavior of the aCC neuron was as-

sayed in the absence of its normal pathway substrate, the U neurons (35). The aCC growth cone normally contacts a number of different cell surfaces but always grows along only one, that of the earlier differentiating U neurons. In contrast, the aCC's sibling, the pCC, ignores the U neurons and grows along the surface of an entirely different neuron. If the U neurons are ablated, the aCC fails to follow its usual path and does not reproducibly choose any particular alternative path. These results suggest a model in which cell-surface pathways express some system of molecules on their cell surfaces that renders them sufficiently distinct from one another such that the growth cones of developing neurons can distinguish their appropriate route (6). The most likely possibility is that these molecules will be involved in some form of specific adhesive interaction between the growth cone of a developing neuron and its appropriate cell-surface substrate.

To find genes encoding such molecules, one could search for mutations in which all neurons are present, their cell bodies residing in their normal locations, the general processes of axon elongation intact, but the normal recognition and axon pathway choice of subsets of neurons are altered. The *eve* mutation and its effect on the aCC and RP2 neurons, as described above, could fall into this category. But because *eve* is a nuclear transciptional regulatory protein, it probably functions to regulate the expression of cell-surface molecules that mediate the growth cone choices and thus the ultimate morphology of these neurons. Other than a few examples of mutations in such regulatory genes that likely cause changes in cell fate, no convincing example of such a mutation has yet been described, although it is fair to say that a systematic search for such mutations has not been undertaken.

A number of cell-surface molecules, however, show patterns of expression on subsets of neurons and glia that suggest a role in neuronal recognition events underlying pathfinding. Three such molecules, named fasciclin I and III and neuroglian, have been identified with monoclonal antibodies raised against grasshopper or *Drosophila* embryonic nervous systems; the genes encoding them were subsequently cloned (37–39). Each of the fasciclins is expressed on the surface of a restricted subset of neurons whose axons show affinities for one another during pathfinding to form axon bundles or fascicles (thus the term fasciclins). They therefore represent good candidates for molecules involved in the recognition and/or adhesion events mediating specific neuronal pathway choices. Fasciclin I was originally identified in the grasshopper, but its homolog in *Drosophila* is highly conserved. It is attached to the cell surface by a phosphatidylinositol linkage and has no similarity to any known proteins; it is therefore difficult to predict its function based solely on sequence. Fasciclin III is an intergral membrane protein and also has a novel sequence. Functional studies have shown that fasciclin III acts as a homophilic adhesion molecule when introduced into tissue culture cells (40).

Neuroglian, as its name implies, is expressed on the surface of both neurons and glial cells. Highest levels of neuroglian are found on the axons and associated glial cells of the peripheral nerves, the ISN and SN. Neuroglian is a member of the immunoglobulin superfamily and shows highest similarity to the vertebrate neural adhesion molecule L1, suggesting that it, like fasciclin III, functions as an adhension molecule during development of the nervous system (39). Surprisingly, loss of function mutations in the *neuroglian* gene cause no gross abnormalities within the developing nervous system. This result has led to the proposal that, as has been found in in vitro neurite outgrowth experiments of vertebrate neurons (41), there may be sufficient mechanistic redundancy that multiple adhension/recognition molecules must be eliminated to have a dramatic effect on neuronal development. In this scenario of redundancy, the abnormal morphologies of the RP2 and aCC neurons in *eve* mutants would result from the absence of regulated expression of a coordinated set of cell-surface molecules needed for proper pathway recognition and selection. One might also expect to obtain a result similar to that of the *neuroglian* gene when individual fasciclins are inactivated by mutations in their genes. However, inasmuch as the expression of the two fasciclins and neuroglian overlap in a small subset of identified neurons, double or even triple mutants can be tested for effects on neuronal development.

CONCLUSIONS AND FUTURE PROSPECTS

One of the strengths insects offer for studying nervous system development is that neurons and their precursors can be uniquely identified and their differentiation readily charted. This feature, coupled with its advanced genetics and molecular biology, has made *Drosophila* an attractive system to uncover molecular mechanisms underlying neuronal development. Although in the early stages of analysis, several classes of genes have been identified that control various aspects of neuronal development. The new technique of enhancer trap mutagenesis should significantly enlarge this number of genes. The challenge will be to determine how these genes act, and in many cases interact with one another, to generate a functioning nervous system. As many developmentally important genes in *Drosophila* have been shown to have homologs in vertebrates, it can be expected that results of these studies will lead to the discovery of developmental strategies used by other nervous systems, including those of higher vertebrates. FJ

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