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Molecular Correlates of Neuronal Specificity in the Developing Insect Nervous System

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Abstract

The development of the nervous system in insects, as in most other higher animals, is characterized by the high degree of precision and specificity with which synaptic connectivity is established. Multiple molecular mechanisms are involved in this process. In insects a number of experimental methods and model systems can be used to analyze these mechanisms, and the modular organization of the insect nervous system facilitates this analysis considerably. Well characterized molecular elements involved in axogenesis are the cell–cell adhesion molecules that underlie selective fasciculation. These are cell-surface molecules that are expressed in a regional and dynamic manner on developing axon fascicles. Secreted molecules also appear to be involved in directing axonal navigation. Nonneuronal cells, such as glia, provide cellular and noncellular substrates that are important pathway cues for neuronal outgrowth. Once outgrowing processes reach their general target regions they make synapses with the appropriate postsynaptic cells. The molecular mechanisms that allow growth cones to recognize their correct target cells are essential for neuronal specificity and are being analyzed in neuromuscular and brain interneuron systems of insects. Candidate synaptic recognition molecules with remarkable and highly restricted expression patterns in the developing nervous system have recently been discovered.

Index Entries: axogenesis; synaptogenesis; glia; adhesion molecules; recognition molecules; TERM-1; fasciclins; neuromuscular synapse; *Drosophila*; grasshopper; homology.

Introduction

A central question in neurobiology concerns the molecular mechanisms that underlie the formation of precise neuronal connectivity during embryonic development. Investigations on axonal pathfinding, neuronal recognition, and synapse formation in both invertebrates and vertebrates indicate that a multiplicity of molecular signals is involved in specifying circuit formation. These signals include cell surface molecules on neurites and glia, extracellular matrix molecules, and factors that are secreted by growth cones and by developing synaptic terminals (for reviews *see* Dodd and Jessel, 1988; Harrelson and Goodman, 1988; Jessel, 1988; McMahan and Wallace, 1989; Sanes, 1989; Grenningloh et al., 1990; Reichardt and Tomaselli, 1991; Patterson, 1992). Indeed, this very multiplicity of possible molecular mechanisms for establishing proper neuronal connectivity is rapidly becoming a conceptual and experimental limitation for understanding the way in which neuronal specificity is attained in the more complex developing brains of vertebrates. Moreover, in many of the higher vertebrate brains it has proved difficult to study the role of any putative recognition molecule in vivo. One approach to unravel the complex interactions among such molecular signals in the living embryonic nervous system has been to investigate the role of these molecules in the neuronal development of simpler model systems, notably of insects. The advantages of insects for molecular studies of neuronal specificity range from the relative accessibility of identified neurons for cell biological experiments, in preparations such as the grasshopper embryo, to the opportunity for molecular genetic manipulations and ablations in *Drosophila* (e.g., Thomas et al., 1984; Grenningloh et al., 1991).

From recent work on the molecular basis of neuronal development in insects it is becoming increasingly clear that a significant fraction of the information stored in the genome is used in the developmental assembly of the nervous system, and it is also becoming clear that little of it is used exclusively in the developmental assembly of the nervous system (Bellen et al., 1990; Datta et al., 1993). Given that the genetic information used to generate the nervous system is subjected to multiple usage, it is unlikely that relevant genes or gene products were optimized during evolution for a role exclusively in neuronal development. Since, therefore, the functional role of any single molecular type is likely to be subjected to inevitable compromise (Miklos, 1993), cellular recognition processes involving combinations of several different molecular types (some of which may appear to be functionally redundant) are to be expected to operate during the establishment of precise neuronal connectivity. This has already been amply documented for the molecular control elements that are responsible for the specification of neuronal precursor cells and the generation of different neuronal cell types from these precursors (for reviews *see* Ghysen and Dambly-Chaudiere, 1989; Campos-Ortega and Knust, 1990; Jan and Jan, 1990; Cabrera, 1992; Goodman and Doe, 1994). It is also a feature of the molecular mechanisms involved in axogenesis and synaptogenesis that are reviewed here.

Species, Systems, and the Size of the Problem

Neuronal development has been widely studied in a number of insect species. Among the large insects with accessible embryonic nervous systems are the grasshoppers *Schistocerca gregaria* and *Locusta migratoria*, the cricket *Acheta domesticus*, and the cockroach *Periplaneta americana* (Bentley and Keshishian, 1982; Murphey, 1986; Bastiani et al., 1987; Wang and Denburg, 1992). For classical genetic and molecular genetic analyses of neuronal development, the fly *Drosophila melanogaster* is unrivaled (Rubin, 1988; Harrelson and Goodman, 1988). The conceptual and experimental merger of work on the large insects, with their highly accessible identified embryonic neurons, with work on *Drosophila*, with its powerful genetics and molecular biology (Thomas et al., 1984; Goodman et al., 1984), has lead to a spectacular advance in terms of experimental potential. This has resulted in a combined cellular, genetic, and molecular approach to many basic

questions involved in the construction of a functioning nervous system (Patel et al., 1987; Zinn et al., 1988; Grenningloh et al., 1991). Thus, the various experimental approaches currently available for analyzing the molecular diversity involved in axogenesis and synaptogenesis in insects now include monoclonal antibody and cloning-by-homology methodologies, genetic screens and mutant analysis, enhancer-trap approaches, and others (e.g., Bastiani et al., 1987; Grenningloh et al., 1990; Seeger et al., 1993; Van Vactor et al., 1993; Bellen et al., 1990; Brand and Perrimon, 1993).

Virtually all parts of the insect nervous system have specific advantages for developmental studies. In many insects most of the sensory afferents as well as many of the central interneurons and motoneurons are repeatedly distinguishable as individuals based on their morphology and synaptic connectivity. This property of individual identifiability of neurons and their neuronal connectivity has made the insect nervous system an important model system for many fundamental cellular neurobiological analyses of structure and function (e.g., Rowell, 1971; Burrows, 1975; Robertson and Pearson, 1983; Reichert et al., 1985), and it is this same property that also recommends the various components of the insect nervous system for a molecular analysis of neuronal specificity during development.

In the sensory periphery, each sensory receptor is located directly adjacent to the sense organ it innervates, and many sense organs occupy reproducible positions in the body so that they can be uniquely recognized (Bate, 1978). In this respect, each sense organ can be considered as unique, and the pathway that each sensory neuron follows into the central nervous system can be analyzed (Ghysen, 1978). In insect sensory systems, the determinants of guided axonal growth have been primarily studied in appendages such as the legs, wings, mouthparts, antennae, and cerci (Bate, 1976; Edwards and Chen, 1979; Ho and Goodman, 1982; Bentley and Keshishian, 1982; Shankland and Bentley, 1983; Berlot and Goodman, 1984; Murphey, 1986; Chiba et al., 1988; Meier and Reichert, 1991). The development of axonal projections from the retina into the optic lobes as well as that of the body wall sensory organs have also received considerable attention (Steller et al., 1987; Campos et al., 1992; Ghysen et al., 1986; Meier and Reichert, 1990; Meier et al., 1991).

The motoneuronal system of insects has been well characterized because of its accessibility, and many of the motoneurons in the segmental ganglia have been individually identified on the basis of their anatomical structure and peripheral projection patterns (Tyrer and Altman, 1974; Hedwig and Pearson, 1984; Siegler and Pousman, 1990a,b). During development, these motoneurons extend their growth cones toward their target muscles and form neuromuscular synapses with these targets in a highly specific fashion. For example, in the *Drosophila* embryo, where the body wall musculature is arranged in a stereotyped array of 30 individual fibers in each abdominal hemisegment, most of these muscle fibers are innervated in a stereotyped manner by one or only a few of approx 35 identifiable segmental motoneurons (Johansen et al., 1989; Sink and Whitington, 1991a,b; Halpern et al., 1991).

In the central nervous system of insects, most of the work on neuronal development has focused on the segmental ganglia. This is because in these ganglia single neurons can be easily identified as individuals and the trajectory of single axons can be traced from the onset of axogenesis throughout the period of synapse formation with identified postsynaptic target cells. Each segmental ganglion consists of 500–3000 neurons, most of which have bilaterally symmetrical homologs. Many of these neurons can be identified on the basis of their lineage, their biochemistry, the position of their cell bodies, the projection patterns of their axons, and their synaptic connections (Goodman et al., 1979; Goodman et al., 1981, 1984; Taghert and Goodman, 1984; Bastiani et al., 1986). In many embryonic preparations, the developing neurons and their processes can be penetrated with microelectrodes and labeled with intracellular dye, that it is possible to follow the differentiation of identified neurons from their neuroblast of origin to their mature neuronal state (Goodman and Spitzer, 1979; Taghert et al., 1982; Kuwada and Goodman, 1985).

In contrast to the wealth of knowledge about the development of the segmental ganglia, very little is known about the embryonic development of the insect brain. However, recently work has been initiated on neuronal development in the early embryonic brain of the grasshopper and *Drosophila* (Zacharias et al., 1993; Meier et al., 1993; Boyan et al., 1993 a,b; Therianos et al., 1993). These studies indicate that many features of the complex mature insect brain derive through a surprisingly simple and stereotyped set of neuronal interactions that occur early in embryogenesis. Moreover, this work shows that many of the concepts and methods that have been used to study axogenesis and synaptogenesis in the simpler segmental ganglia are also, in principle, applicable to the more complex insect brain.

In the vertebrate central nervous system a cellular and molecular analysis of the mechanisms responsible for the formation of correct synaptic connections of individual neurons is complicated because billions of individual nerve cells are involved. In contrast, the average large insect has 100,000–1,000,000 neurons in its optic lobes and 50,000–100,000 neurons in the rest of its brain. There is considerable variability from species to species. The brain of the cockroach *Periplaneta americana* consists of 1,200,000 neurons (Farrel and Kuhlenbeck, 1964). *Drosophila* has only approx 200,000 neurons in its brain (as compared to approx 15,000 transcription units in its genome). Parasitic wasps of the families *Mymaridae* and *Trichogrammatidae* have head diameters in the order of only 30 μm (Edwards, 1977) and, thus, have entire brains that are only the size of a few vertebrate neurons; their brains probably consist of only several thousand neurons. In most cases, though, the total number of neurons in insect nervous systems is still very large, and analyzing the molecules responsible for correctly interconnecting them might appear to be a daunting task. However, the number of neurons that need to be interconnected in *different* neuronal circuits in the developing insect nervous system is considerably smaller. This is because there is an enormous degree of modularized and repetitive neuronal circuitry in the insect nervous system. It is important to realize that this repetitive modularization of a relatively small number of neuronal types greatly reduces the size of the developmental problem involved in constructing the circuits of the insect brain.

This is especially obvious in the optic lobes. The number of ommatidia in different insect eyes can range from tens to tens of thousands (Rensch, 1959) and underlying each ommatidium is a concomitant investment in neural circuitry. For example, the brain of the fly *Musca* consists of approx 340,000 neurons (Strausfeld, 1976). Of these, 75% are found in the optic lobe and a total of about 270,000 neurons can be related to the visual neuropils. However, this enormous number of neurons probably reduces to approx 150 cell types that are repeatedly interconnected in a stereotyped and modular circuitry for each ommatidium and neuro-ommatidium. Similar considerations hold for the most of the other interneurons in the brain. Of the remaining 70,000 neurons in the fly brain, 90% are found in the sensory parts of the brain and of these 42,000 are Kenyon cells, that may even constitute a single-cell type that is synaptically incorporated into stereotyped and modular circuitry. Thus, increasing the number of neurons in the larger sensory systems of some insects may refine systems sophistication and behavior, but it does not necessarily produce novel circuitry (Shaw, 1989), and is unlikely to require molecularly intensive *de novo* investment during the development of precise synaptic connectivity.

Modular circuitry is also characteristic of the segmental nervous system of insects, where it is based at least in part on serial homology (Bate et al., 1981; Robertson et al., 1982; Wilson et al., 1982; Pearson et al., 1985). The development of homologous nervous structures in insects has been studied in some detail. For example, in the peripheral nervous system of the grasshopper, serially repeated pathfinding mechanisms and serially repeated molecular guidance cues in all of the body segments are involved in guiding the axons of homologous sensory neurons and into their target regions (Meier and Reichert, 1990, 1991; Meier et al., 1991). Furthermore, comparative developmental studies show that aspects of the underlying basic neurodevelopmental pro-

gram are evolutionarily highly conserved. A comparison of the results obtained in the grasshopper *Schistocerca* and in the fly *Drosophila* shows that virtually the same pattern of peripheral sensory innervation is formed in both species. This indicates that the construction of the peripheral nervous system in extremely divergent modern insects relies on highly conserved molecular mechanisms that evolved in ancestral insects over 300,000,000 yr ago.

The implications of a modular organization in most parts of the insect nervous system for molecular mechanisms of axogenesis and synaptogenesis are profound. Although the central nervous system may contain hundreds of thousands of individual neurons, the different types of neurons in different circuit modules is probably only on the order of a few thousand. This is significantly less than the total number of transcription units in the insect genome. Thus, at least in principle, several different and unique gene products could be utilized to determine the specificity of synaptic interconnections for each neuronal cell type during the development of the insect brain.

Axogenesis: Molecular Mechanisms of Pathway Recognition

A first important step in the generation of neuronal specificity is the selection of the correct pathway for neurite outgrowth and navigation. The growing tips of neurons, the growth cones, are crucial elements in this process of pathway recognition. Neuronal growth cones can extend long distances along stereotyped pathways to find their targets. In this process each growth cone makes a series of specific navigational choices and in this way generates the distinctive morphology of each individual neuron (Raper et al., 1983a,b; Bastiani et al., 1986). In the developing peripheral and central nervous system of insects, early outgrowing growth cones pioneer a simple scaffold of axon pathways (Bate, 1976; Bentley and Keshishian, 1982; Goodman et al., 1984). The earliest pioneering neuronal growth cones use a variety of cell surface, extracellular matrix, and diffusible molecular signals for axonal guidance (Caudy and Bentley, 1986; Condic and Bentley, 1989; Kolodkin et al., 1992). In addition to these signals, later developing growth cones can also take advantage of the other axon bundles that are already in place in their near vicinity, and carry out directed pathfinding by distinguishing one axon fascicle from another. This phenomenon of selective fasciculation, first demonstrated by Wiggelsworth (1953) in the sensory periphery, has now been documented in all parts of the central and peripheral nervous system.

Selective fasciculation of growth cones and axons is correlated with the regional and dynamic expression of specific cell surface adhesion molecules on the interacting cellular structures. Several candidate molecules that are involved in selective fasciculation have been studied in the segmental nervous system of grasshopper and *Drosophila*. Among these are fasciclin I, fasciclin II, fasciclin III, fasciclin IV, and neuroglian (Patel et al., 1987; Zinn et al., 1988; Harrelson and Goodman, 1988; Snow et al., 1989; Bieber et al., 1989; Grenningloh et al., 1990; Kolodkin et al., 1992). Neuroglian, fasciclin II, and fasciclin III are members of the immunoglobulin superfamily (Fig. 1). Many of these molecules can function as homophilic cell adhesion molecules in vitro and a role for fasciclin II as a neuronal recognition molecule in vivo has been demonstrated both by antibody–block and by mutant analysis (Harrelson and Goodman, 1988; Grenningloh et al., 1991). All of these molecules are expressed on restricted subsets of axons in the developing nervous system and can, thus, in principle, be the molecular substrates predicted by the labeled pathways hypothesis for directed axonal outgrowth (Raper et al., 1984; Goodman et al., 1984). All of these molecules are also expressed outside of the developing nervous system (Bastiani et al., 1987; Bieber et al., 1989; Hortsch et al., 1990; Grenningloh et al., 1991; McAllister et al., 1992).

The role of cell adhesion molecules like the fasciclins in axogenesis has been studied most comprehensively in the segmental nervous system. However, evidence is accumulating that

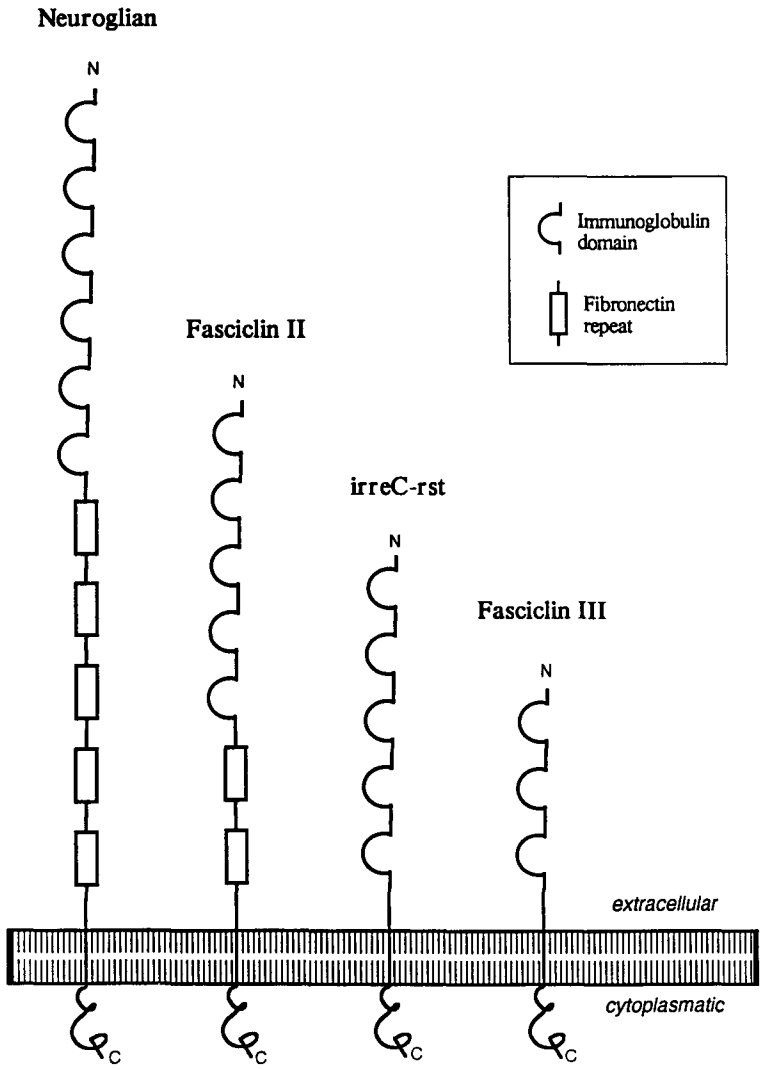

Fig. 1. A schematic diagram of some of the neural cell surface molecules in insects that are members of the immunoglobulin superfamily. Most of these function as cell surface adhesion molecules. The molecular domain structure is indicated.

some of these molecules are also important in the formation of axon tracts in the brain (Zacharias et al., 1993; Boyan et al., 1993a,b). In the embryonic brain of the grasshopper a small subset of neurons initially establishes a primary axon scaffold that prefigures the correct bilateral connections between the brain hemispheres and links the brain with the remainder of the segmental nervous system. The axons in this primary scaffold express fasciclin I in a regional and dynamic manner. Fasciclin I expression is also seen during axogenesis of the retinula cells of the eye and of the ocellar interneurons. Moreover, similar patterns of fasciclin I expression are seen during early embryonic brain axogenesis in *Drosophila* (Therianos et al., 1993). During the development of the optic lobes in *Drosophila* a further member of the immunoglobulin superfamily, the *irreC-rst* gene product, appears to play an important role in axon guidance (Boschert et al., 1990; Ramos et al., 1993).

A different type of molecular mechanism for axonal guidance involves the attraction or repul-

sion of growth cones. In contrast to the situation in vertebrate nervous systems, surprisingly little is known about molecules that might subserve these functions in insects. Contact-mediated attractive and repulsive cues for growth cones may be important at the midline of the developing insect central nervous system where specific cells play an important role in the formation of commissural tracts (Thomas et al., 1988; Crews et al., 1988; Klämbt et al., 1991). Mutant analysis in *Drosophila* indicates that contact-mediated cues on these cells may attract those growth cones that normally cross the midline and repel those growth cones that normally remain on their side of origin (Seeger et al., 1993). The molecules involved in this attraction/repulsion interaction are not yet known. Several of the membrane-associated cell adhesion molecules described above come in multiple forms, some of which could be released from the membrane (Grenningloh et al., 1990). Once released they could interact with membrane-bound forms of the same molecular type on navigating axons.

TERM-1, an unique neuron-specific glycoprotein that was discovered in the grasshopper embryo, is concentrated at and secreted from the growth cones of two pairs of identified brain interneurons into the surrounding extracellular matrix (Meier et al., 1993). It is not expressed by any other neurons in the entire nervous system. Its spatio-temporal expression pattern suggests that TERM-1 is involved in axonal navigation (Fig. 2). Secreted TERM-1 in the extracellular matrix surrounding the labeled neurons might be used by the growth cones of other axons as a temporally dynamic guidance cue for directed outgrowth or selective fasciculation. Alternatively, since TERM-1 does not appear to be tightly bound and may diffuse away from the labeled axons, it could function as a cell-specific chemoattractant for other neuronal processes, and might also interact biochemically with the extracellular matrix so as to alter the growth properties of the labeled axons through the extracellular matrix. Interestingly, during axonal navigation the pair of growth cones that secrete TERM-1 grow in tight apposition along an identified descending axon bundle in the connective that expresses fasciclin II. Nonneuronal expression of TERM-1 is seen in the compound eye (Gasser and Reichert, 1993). TERM-1 has recently been characterized in the *Drosophila* brain, where it is also expressed by an extremely small subset of interneurons. Other secreted molecules with this remarkable type of highly restricted spatio-temporal expression pattern during axogenesis have not yet been described.

The Role of Nonneuronal Cells in Neuronal Guidance

Growing axons and dendrites encounter a wide variety of cellular and noncellular substrates during development that are not neuronal but nevertheless provide important pathway cues for neurons. Glia cells (as well as other special midline cells) are important for the navigation of growth cones that pioneer initial axon pathways. This has been studied in detail in the segmental nervous system. Early in embryogenesis a glial scaffold marks the presumptive location of major axonal pathways before the onset of axonal outgrowth. The axons that pioneer the connectives, commissures, and nerve roots make extensive contact with these glial cells (Bastiani and Goodman, 1986; Jacobs and Goodman, 1989; Klämbt et al., 1991; Klämbt and Goodman, 1991). Comparable guidance phenomena by glial and midline cells are also observed in embryonic brain development (Boyan et al., 1993b). Glial pathways and compartment borders are established before axogenesis begins and are used by the pioneering brain interneurons in order to construct a primary axon scaffold. Moreover, brain glia appear to prefigure some of the peripheral brain nerves, such as the ocellar nerve.

Several cell surface molecules that are expressed by developing glial cells in insects have been identified (Meyer et al., 1987, 1988; Bieber et al., 1989; Carpenter and Bastiani, 1991). Moreover, both enhancer trap lines and mutant analysis indicate that the molecular and functional heterogeneity of the embryonic glia that are

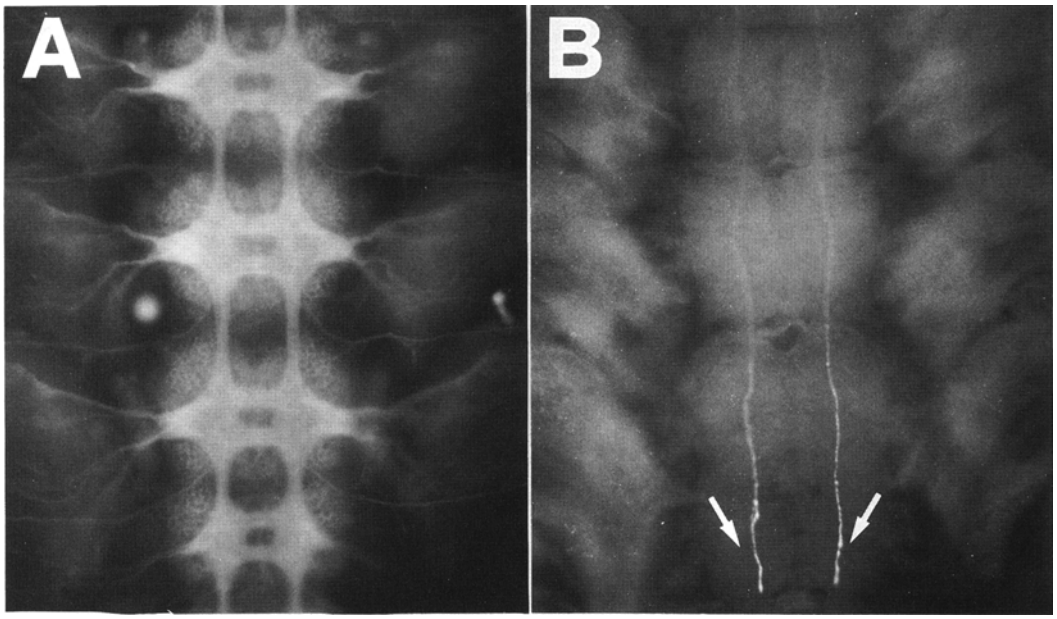

Fig. 2. Highly restricted spatio-temporal expression pattern of the TERM-1 glycoprotein. Chain of ventral ganglia in a 50% grasshopper embryo. (**A**) Immunostaining with an anti-HRP antibody reveals all of the neuronal processes and cell bodies in the developing ventral ganglia. Note the dense, ladderlike staining caused by the many axons in the commissures and connectives. (**B**) Immunostaining with an anti-TERM-1 antibody. Only the descending axons of two pairs of brain interneurons are labeled, and TERM-1 expression is strongest near the growth cones of these axons (arrows). **A** and **B** are the same set of ganglia in the same preparation.

involved in neuronal guidance is surprisingly extensive (Klämbt and Goodman, 1991; Klämbt et al., 1991).

In the peripheral nervous system pioneering growth cones can use epithelial cells, mesodermal cells, and extracellular matrix components secreted from nonneuronal cells as guidance cues. This has been especially well analyzed for sensory cells in the developing insect limb buds and wings (Condic and Bentley, 1989; O'Connor et al., 1990; Blair et al., 1987; Kolodkin et al., 1992; Wang and Denburg, 1992). Pathfinding by peripherally growing motoneurons involves transient interactions with glial cells (Bastiani and Goodman, 1986; Jacobs and Goodman, 1989; Klämbt and Goodman, 1991) as well as with trachea, muscle cells, and special mesodermal cells. Interestingly, many of these nonneuronal cells express fasciclin II transiently as the growth cones of the motoneurons approach and interact with them (Van Vactor et al., 1993).

Synaptogenesis: Molecular Mechanisms of Target Recognition

Once growth cones have navigated to their general target regions, they must recognize, contact, and make synapses with the appropriate postsynaptic partner cells. This ability of growth cones to recognize their correct target cells during development is central for the establishment of neuronal specificity. The precise choices made by embryonic insect neurons during synaptogenesis suggest that complex forms of target recognition are occurring. Yet in many cases, growth cones can both find and recognize their correct targets in the absence of neuronal activity (for review *see* Goodman and Shatz, 1993). What are the molecular signals that are involved in this activity-independent target recognition?

The biochemical and cell biological analysis of many neuronal cell–cell recognition phenomena in invertebrates and in vertebrates has lead to a marked resurgence of interest in the general concept of chemoaffinity (Sperry, 1963). According to this concept, individual developing neurons are thought to acquire and retain unique cell-specific molecular signals that promote both accurate axon outgrowth and the formation of correct synaptic connections. However, despite the prevailing influence of the chemoaffinity theory, neuron-specific recognition molecules that selectively label the growth cones and the developing synapses of individual neurons in the central nervous system have, until recently, not been described. The cell adhesion molecules that are implicated in pathway recognition during axogenesis can be expected to participate to some degree in target recognition. However, many of the biochemical methods used to analyze the molecules involved in axogenesis are biased toward selecting relatively abundant molecules, and may miss molecules involved in the formation of precise synaptic connectivity that are present only on a small subset of cells and possibly only for a short period during development. Fortunately, with the use of mutant analysis, enhancer trap lines, and monoclonal antibodies, even molecules of very low abundance in the developing nervous system, such as those that are expressed by only a few neurons, can be detected. Using these methods, candidate synaptic recognition molecules in insects have recently been discovered in the embryonic neuromuscular system and in the descending interneuron system of the brain.

Significant progress has been made in understanding cellular mechanisms involved in the development of specificity in neuromuscular synapses (Ball et al., 1985; Johansen et al., 1989; Sink and Whitington, 1991a; Broadie and Bate, 1993). As is the case for neurons in the central nervous system, motoneurons extend growth cones toward and innervate their correct target cells in a highly specific manner. This target specificity becomes especially manifest in experiments in which individual muscle cells are either duplicated or deleted and the ability of the innervating motoneurons to recognize appropriate targets is monitored (Ball et al., 1985; Cash et al., 1992; Sink and Whitington, 1991b; Chiba et al., 1993). During motoneuron pathfinding and neuromuscular innervation in *Drosophila* some motoneurons and muscles are known to express cell surface molecules that are members of the immunoglobulin superfamily, such as fasciclin II and fasciclin III. For example, fasciclin III as well as a novel adhesive cell surface protein, connectin, are expressed on subsets of motoneuron axons as well as the target muscles they innervate (Nose et al., 1992; Halpern et al., 1991). Both of these molecules are expressed by both synaptic partners as connections form and are downregulated once the neuromuscular synapses are established, suggesting an involvement in synaptic recognition. Mutant analysis shows that the loss of any one of these cell adhesion proteins does not, however, lead to a loss of synaptic connectivity. This is an indication that there are parallel molecular recognition mechanisms in operation that are sufficient to allow correct synaptic connections to form even if one species of cell adhesion molecule is defective (functional redundancy).

An important step toward the unraveling of the molecular signals involved in generating neuromuscular specificity is the recent (and ongoing) isolation of different classes of mutants that are defective in correct motoneuron–muscle target recognition in *Drosophila* (Van Vactor et al., 1993). Currently, approx 10 genes on the second chromosome have been identified that, when mutated, result in phenotypes that suggest that these genes control specific aspects of motoneuron pathfinding or target recognition. The different classes of mutant phenotypes observed suggest that neuromuscular specificity is controlled by a hierarchy of molecular mechanisms that culminate in the recognition of the correct target muscle by the growth cone of each motoneuron. Two of the genes isolated to date, *clueless* and *walkabout*, appear to be involved in specific muscle target recognition.

In the central nervous system, the mutant analysis approach has also led to the identification of a gene, *passover*, that is apparently impor-

tant for the connectivity of a pair of identified descending giant fibers in *Drosophila*. This gene appears to be expressed only in the giant fibers and their putative thoracic targets (Krishnan et al., 1993). Although the *passover* gene product could be involved in mediating homophilic adhesion between descending neurons and their targets, the gene product is not structurally related to any of the other axonal cell adhesion molecules that have been identified in the developing insect nervous system (Grenningloh et al., 1990; Goodman and Doe, 1994). Its highly restricted expression pattern also differentiates the *passover* gene product from the cell adhesion molecules that are expressed on relatively large subsets of axon bundles. A second candidate synaptic recognition molecule that also has a highly restricted expression pattern is the TERM-1 glycoprotein. TERM-1, which is expressed by two pairs of identified descending interneurons in the grasshopper brain (Meier et al., 1993), is not only present on the growth cones of the these interneurons during axogenesis. During synaptogenesis, as the terminal arbors of the two interneuron pairs are formed, TERM-1 also accumulates at these endings and becomes concentrated at discrete sites on the fine branches of the arborizations. The accumulation of TERM-1 in the cleft that separates the labeled terminal endings from other neighboring small diameter terminal profiles could indicate a role in the formation of adhesive contacts or in the organization of subcellular synaptic specializations. Alternatively, TERM-1 might be involved in the synaptic or secretory functions of the labeled interneurons. In either case, the highly restricted expression pattern of TERM-1 in only two pairs of interneurons in the central nervous system implies that individual developing neurons can acquire and retain unique molecular labels that may be important for neuron-specific pathway and target recognition.

Summary

Recent molecular studies on the development of neuronal specificity in insects are providing insight into the mechanisms, and in some cases even into the genes, that operate during axogenesis and synaptogenesis. It is becoming clear that the directed outgrowth of the neuronal processes carried out by growth cones depends on a variety of molecular cues located on cell surfaces, in the extracellular matrix, or secreted by distant target cells. It is also becoming clear that neuronal specificity is not established in one step. Pathway recognition precedes target recognition and in many cases the growth cones use intermediate neuronal or nonneuronal targets as substrates for correct navigation. Some of the cell adhesion molecules involved in neuronal development in insects have been identified and their genes cloned. Many of these genes and gene products have vertebrate homologs that are also involved in nervous system development. In several cases, novel molecular types that are only expressed by small subsets of neurons or even by unique individual cells are being discovered in the developing insect nervous system. It seems likely that these neuron-specific molecules will play a significant role in pathway and target recognition. The identification of further neuron-specific molecules of this type as well as the elucidation of their functional role in establishing neuronal specificity will be important goals for future research.

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