Glial interactions with neurons during *Drosophila* embryogenesis

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**Summary**

A monoclonal antibody (Mab5B12) demonstrating specificity for glial cells within the central and peripheral nervous systems of *Drosophila* has been used in combination with neural-specific antibodies to study the early organization of the *Drosophila* embryo. The embryonic central nervous system of *Drosophila* contains cells within the ventral midline that are recognized by monoclonal antibody 5B12. These cells are not recognized by either a polyclonal antiserum to horse radish peroxidase, which recognizes several antigens on the surface of *Drosophila* neurons, or Mab22C10, which recognizes an antigen specific to the peripheral nervous system. Mab5B12-positive cells lie dorsal both to the developing anterior and posterior commissures in each thoracic and abdominal segment and to the supraoesophageal commissure. They ensheath these commissures in later stage embryos. Other Mab5B12-positive cells lie dorsolateral to the CNS and send processes laterally to the lateral sensilla during axonogenesis in the PNS. These cells surround the axons of the intersegmental and segmental nerves. Other cells that line the advancing ectoderm during dorsal closure and surround the anal pads also express the Mab5B12 antigen. Neuronal cell cultures derived from *Drosophila* gastrulae contain cells expressing the Mab5B12 antigen. These cells can be found separate or in close association with neuronal clusters and their axons.

**Key words:** *Drosophila*, glia, cell culture, axon guidance.

**Introduction**

A number of important events occurring during development of the nervous system have been shown to require non-neuronal cells for specific functions and support. For example, determination of neuroblasts during neurogenesis in the grasshopper, migration of neurons from their germinal zone towards their final locations, and pathfinding by neuronal growth cones in vertebrates all depend upon non-neuronal cells for instructive cues or substrate support (Doe & Goodman, 1985a; Silver & Rutishauser, 1984; Rakic, 1971). Although glial cells have been studied extensively in vertebrate systems, many invertebrate nervous systems also possess extensive neuroglial populations, which arise during critical periods of neurogenesis and persist throughout development (Radojcic & Pentreath, 1979). Complex neuron–glia interactions during development and in adulthood have been described in a number of insect species (Jacobs & Goodman, 1989; Carr & Taghert, 1988; Meyer *et al.*, 1987; Bastiani & Goodman, 1986; Hoyle, 1986; Swales & Lane, 1985; Smith & Treherne, 1963; Wigglesworth, 1959; Poulson, 1950).

*Drosophila* offers an especially useful model system for the study of insect neuroglia due to its long history as an organism for genetic studies, its suitability for molecular studies, and the wealth of information concerning the principle events in nervous system development. The *Drosophila* embryonic central nervous system (CNS) begins to differentiate between 4 and 5 h after fertilization with the commitment of ectodermal cells to the neuroblast lineage (Hartenstein *et al.*, 1987; Hartenstein & Campos-Ortega, 1984). Neuroblasts enlarge and move internally where they proceed through a series of asymmetric divisions to produce ganglion mother cells. Each ganglion mother cell then divides symmetrically into a pair of neurons. One neuroblast is capable of producing 5–20 neurons between 7 and 13 h after fertilization (Hartenstein *et al.*, 1987; Furst & Mahowald, 1985) all of which are recognized by antisera raised against horseradish peroxidase (anti-HRP) (Jan & Jan, 1982). Axonogenesis begins between 8 and 9 h after fertilization and proceeds throughout embryonic life (Thomas *et al.*, 1984). Early axonal growth cones cross the ventral midline to the contralateral hemi-segmental ganglion and pioneer the anterior and posterior commissures of the embryonic CNS.

In 1950, Poulson suggested that some cells of the ventral median cord in the embryonic CNS of *Drosophila* were glia. Citing earlier studies, he raised the possibility that these cells play a role in the formation of the central neuropil and peripheral nerve projections. More recently, a defined set of cells within the ventral midline of *Drosophila* have been identified as midline glial cells based on morphological criteria and lineage analysis (Jacobs & Goodman, 1989). These cells,
through mutant analysis, have been ascribed a role in formation of the anterior and posterior segmental commissures (Rothberg et al. 1988; Thomas et al. 1988; Doe et al. 1988; Smouse et al. 1988).

The present study focuses on cells in the CNS and peripheral nervous system that are recognized by a specific monoclonal antibody, some of which possess characteristics consistent with their classification as neuroglia. The monoclonal antibody that we have used, Mab5B12, was raised against adult Drosophila antigens by Dr S. Benzer and colleagues. To further characterize these cell populations, we examined the developmental profile of the Mab5B12 immunoreactive (Mab5B12-IR) cells in vivo and in vitro in relation to cells recognized by anti-HRP and Mab22C10, the latter being specific for components of the peripheral nervous system. We have found that a prominent subpopulation of Mab5B12-IR non-neuronal cells are intimately associated with neurons and their processes during neurogenesis and axonogenesis in vivo and in vitro. Evidence presented here suggests that glial cells may play an important role in the formation of central neuropil and peripheral axon tracts during Drosophila embryogenesis.

Materials and methods

Egg collection and fixation

Embryos from population cages of stock Drosophila melanogaster (Oregon R-P2) were collected on yeast-coated molasses plates, washed in 0-2% Triton X-100 and dechorionated in 50% commercial bleach (active ingredient is 5% sodium hypochlorite). The vitelline membranes were removed and the eggs fixed according to the method of Mitchison and Sedat (1983), rinsed in three changes of PBS, pH7-4 and incubated overnight in PBS, 5% BSA, 0-1% Triton X-100, pH7-4 at 4°C. All timed egg collections were staged according to Campos-Ortega and Hartenstein (1985).

Cell culture

Cells from manually dissociated whole embryos and from centrifugal elutriated fractions were obtained and grown as previously described (Furst & Mahowald, 1985) 18–24h cultures were washed in PBS and fixed in 4% paraformaldehyde for 30 min at room temperature. Cultures were then incubated in PBS, 5% BSA, 0-1% Triton X-100 for 2h at room temperature before incubation with primary antibodies.

Immunohistochemistry

Mab22C10 and Mab5B12 were generously supplied by Dr Seymour Benzer and Dr John Pollack. Polyclonal antisera to HRP was obtained from Organon Technika and further purified by affinity chromatography with HRP bound to agarose beads. Fixed whole embryos and cultures were incubated overnight at 4°C in monoclonal and polyclonal antibodies diluted in incubation media. Tissue was washed in PBS for 12–24h with a total of 3 changes and incubated overnight at 4°C in dilute secondary antibody conjugated to either FITC, RITC or HRP. HRP-conjugated secondary antibodies were reacted in 10mg DAB in 10mM-Tris buffer, pH7-2 with 0-002% H2O2. Reacted tissue was then dehydrated in graded ethanol, cleared in xylene, and mounted in Permount. Fluorescently labeled tissue was mounted in glycerol: ethanol (1:1) and viewed under epifluorescence on a Leitz microscope fitted with a 100W mercury lamp. HRP-labeled tissue was viewed under bright-field and interference-contrast optics.

Embryos for transmission electron microscopy were processed in the same manner except without the 0-1% Triton X-100 in the incubation media. After antibody incubation, embryos were reacted in the DAB solution, washed 2h in PBS with 3 changes, fixed in 1-2% OSO4, stained en bloc with uranyl acetate, and embedded in epon. Thin sections (50–90nm) were collected on either 200 mesh or Formvar-coated slot grids. Some sections were stained with lead citrate and all were viewed on a Jeol 100CX at 80kV.

Results

Mab5B12-IR cell distribution in the embryo

Four to six cells which express the Mab5B12 antigen lie along the pathway traversed by pioneer axons crossing the ventral midline to form the anterior and posterior commissures evident in later embryos (Fig. 1A). These cells are not recognized by either anti-HRP or Mab22C10, although other cells within the ventral midline are recognized by both (Fig. 1B). The Mab5B12-IR cells are first detected between 9 and 10h of development (late stage 12 to stage 13) (Fig. 1C) and persist throughout embryonic life. In later embryonic stages, Mab5B12-IR cells elongate and possess nuclei polarized either dorsally or ventrally in relation to the commissural axons. They project short cellular processes which surround the anterior and posterior segmental commissures (Figs 1D,E; 2A,D). These processes envelop the entire individual commissure in a dorsal/ventral orientation and spread slightly laterally into each hemi-segmental ganglion.

Mab5B12-IR cells are also present peripherally in association with cells of the PNS. Axons entering and leaving the CNS via the intersegmental nerve (ISN) and the segmental nerve (SN) encounter Mab5B12-IR cells which are positioned dorsolateral to the CNS in each thoracic and abdominal hemi-segment (Figs 1A,E; 2B,C; 7). These cells are first detectable with Mab5B12 at about 9h (late stage 12) (Fig. 1C) or during germ band shortening and continue to stain throughout later embryonic stages. Mab5B12-IR cells in the thoracic and abdominal segments send processes ventrolaterally to the lateral wall of the embryo where they turn dorsally and extend past the lateral sensilla of the PNS (Figs 1E; 2B; 7). These cells and the peripheral axon projections in abdominal segments 8 and 9 are in close apposition adjacent to the CNS where they extend to the sensory receptors of the telson. The SN is accompanied by an additional Mab5B12-IR cell, which extends processes to the ventral sensilla of the PNS (Figs 1A; 2B; 7). Peripheral Mab5B12-IR cells extend processes during the period of initial pathway formation of the ISN and SN. Dorsal to the lateral sensilla is an additional Mab5B12-IR cell, which may be analogous to the bipolar dendrite (Bodmer & Jan, 1987) or the lateral line (Canal & Ferrus, 1986). This cell extends processes in anterior and posterior directions (Figs 2B; 7).
Glial development in Drosophila

Fig. 1. Mab5B12-IR cell distribution in the Drosophila embryo. (A) Ventral view of a 15 h (late stage 16) embryo incubated in Mab5B12 followed by an FITC-conjugated secondary antibody. Anterior is to the top of the page. Cells within the ventral midline of the CNS (arrows) are visible occupying positions associated with the anterior and posterior segmental commissures. The perineurium surrounding the CNS is also recognized by Mab5B12. Cells lateral to the CNS in each hemisegment (arrowhead) send processes dorsolaterally into the periphery. Bar = 50 μm. (B) Same view of a 15 h (stage 16) embryo incubated in Mab22C10. All peripheral and central components of the PNS are recognized by this antibody. The ISN and SN occupy positions coincident with cells recognized by Mab5B12 in A. (C) 10 h (late stage 12) embryo stained with Mab5B12. Cells within the ventral midline (arrows) are visible, as well as cells lateral to the CNS (arrowhead). (D) Interference contrast image of approximately 1 μm plastic cross-section of a 15 h (stage 16) embryos stained with Mab5B12. Ventral is toward the bottom of the page. Mab5B12-IR cell is present within the midline of the CNS between the positions of the anterior and posterior commissures. Bar = 5 μm. (E) Mab5B12-IR cell is present where the segmental commissures cross the midline (arrow). Also present are cells positioned dorsolateral to the CNS which send Mab5B12-IR processes to the lateral wall of the embryo (arrowhead). lc, longitudinal connective; vnc, ventral nerve cord.

Peripheral Mab5B12-IR cells and their relationship to the PNS are diagrammed in Fig. 3. At least 6 Mab5B12-IR cells/hemisegment in each abdominal segment, including the bipolar dendrite, can be discerned in close apposition to the PNS. Other Mab5B12-IR cells, present in cephalic segments, are closely associated with axons of the antenno-maxillary complex, the labral nerve, and other cephalic axon projections (Fig. 1A,B).

Ultrastructural analysis reveals that the Mab5B12
Further distribution of Mab5B12-IR cells. (A) Lateral view of a 15 h (stage 16) embryo incubated in Mab5B12. Image is focused on the cells in the ventral midline showing envelopment of each segmental commissure (arrows). Anterior is toward the top of the page and ventral is to the left. Bar = 50 μm. (B) More lateral view of (A). Cell processes, which begin dorsolateral to the CNS, extend processes along the pathway transversed by PNS axon projections (arrowheads). Arrows indicate bipolar cells or lateral line, which extend anterior and posterior in each abdominal segment. Note anal pads at posterior extreme (asterisk). (C) Slightly older embryo as in B incubated in Mab22C10 to delineate the PNS. Note absence of anal pad stain. (D) Ultrastructural localization of the Mab5B12 antigen. Cross-section through a ventral midline commissure. Cells possessing Mab5B12 antigen on their surfaces envelop the entire commissure, as well as, individual axons (arrows) coursing through the commissure. Bar = 1 μm. (E) Cross-section of the ISN demonstrating four profiles of Mab5B12-IR cells surrounding the axons of the PNS (asterisk). Antigen is localized between these cells and around axons. Bar = 0.5 μm. cl, clypeolabrum; hg, hindgut; ls, lateral sensilla; ps, posterior spiracle; vnc, ventral nerve cord; vs, ventral sensilla.
Glial development in Drosophila

Fig. 3. Diagram of peripheral Mab5B12-IR cells association with the PNS in a late embryo. (A) Mab5B12-IR cells in an abdominal segment. Nuclei of the Mab5B12-IR cells can be identified in the embryo by a visible decrease in the intensity of the stain. At least six cells can be identified. Anterior is to the left of the page. (B) PNS as demonstrated by Mab22C10. Peripheral neurons can be subdivided into ventral, lateral, and dorsal sensilla. (C) A and B in close association as in the embryo.

antigen is localized to the surface of cells in the ventral midline and the peripheral projections (Fig. 2D,E). Antigen is present between cells that ensheathe the anterior and posterior commissures and around some of the cortical axon bundles that pass through these fiber tracts. Peripherally, Mab5B12 antigen is present on the surfaces of cell processes that surround the ISN and SN. Sections of the ISN reveal distinct cellular processes surrounding the peripheral axons that express the Mab5B12 antigen (Fig. 2E). Each process possesses Mab5B12-IR at its interface with the other cellular processes and axons.

A separate population of Mab5B12-IR cells, which line the edge of the dorsal epidermal primordium, become detectable before completion of germ band shortening (late stage 12) (Fig. 4A). These cells advance dorsally over the amnioserosa and meet at the midline during dorsal closure (Fig. 4B,C). The Mab5B12-IR cells appear to be incorporated into structures of the dorsal vessel along the entire dorsal midline of the abdomen. Anteriorly, the Mab5B12-IR cells end directly beneath the supraoesophageal commissure (Fig. 4D,E) and appear to contribute to the formation of the dorsal vessel and structures of the ring gland. Posteriorly, Mab5B12 recognizes epidermal cells surrounding the anal pads (Fig. 2B).

The perineurium, which encloses the entire CNS, is also recognized by Mab5B12 (Fig. 1A). Although not apparent at the initial appearance of Mab5B12-IR in the ventral midline, the Mab5B12-IR of the perineurium increases during later embryonic stages as the CNS condenses.

Mab5B12-IR cells in culture

Subsets of cells within unfractionated and neuroblast-enriched cultures express the Mab5B12 antigen (Figs 5, 6, 8). Some Mab5B12-IR cells exist in tight clusters with one or more broad processes possessing growth-cone-like structures extending away from the cell bodies (Fig. 5A,B). Others appear as individual cells with long thin processes resembling neurites (Fig. 5C,D). Axons emanating from nearby neuronal clusters are frequently found in association with some of the Mab5B12-IR cells (Figs 5C,D; 6A,B). These interactions may occur for a
Fig. 4. Mab5B12-IR cells are present at the leading edge of the advancing ectoderm during dorsal closure. (A) Dorsal view of a 9h (late stage 12) embryo incubated in Mab5B12. Dorsal closure has begun. Anterior is toward the left of the page. Ectoderm has begun to advance dorsally from either side of the embryo (arrows in A and B). (B) Dorsolateral view of a slightly older embryo than in A. Lateral edges of the ectoderm have advanced dorsally. (C) Higher magnification than in A and B of the dorsal midline of the thoracic and first abdominal segments of a 13 h (stage 15) embryo after dorsal closure. Advancing ectoderm from either side of the embryo have met to complete closure. (D) Cross-section through a 15h (stage 16) embryo at the level of the supraoesophageal commissure (arrows). Mab5B12-IR cells lie dorsal to the commissure. (E) Sagittal section at level of dorsal midline. Anterior is toward the left of the page. Asterisk denotes longitudinal connectives in the brain lobe. as, amnioserosa; cc, corpus cardiacum; dr, dorsal ridge; dv, dorsal vessel; fs, frontal sac; ps, posterior spiracles; spg, supraoesophageal ganglion.
short distance, with the axons contacting the Mab5B12-IR cell then extending elsewhere, or axons may contact the Mab5B12-IR cell and course along its length. Additional Mab5B12-IR cells in culture appear fibroblast-like (Fig. 5E,F), similar to the cells in the intact embryo that are lateral to the CNS and extend processes peripherally.

Some Mab5B12-IR cells are closely associated with

Fig. 5. Mab5B12-IR cells are present in tissue cultures of Drosophila gastrula-staged embryos. (A) Interference contrast image of 24 h whole-cell culture incubated in Mab5B12. Cluster of Mab5B12-IR cells with large processes possessing growth cone-like structures (arrows). Bar = 5 μm. (B) Bright-field micrograph of A. (C) Single Mab5B12-IR cells with long process. Axons (arrows) from nearby neuronal clusters interact with Mab5B12-IR process. (D) Bright-field micrograph of C. (E) Neuronal cluster with multiple Mab5B12-IR cells. (F) Bright field micrograph of E.
Fig. 6. Neuronal cell clusters in culture possess Mab5B12-IR cells. (A) Two neuronal clusters, one of which possesses a Mab5B12-IR cell. Axons from the Mab5B12-negative cluster project toward and contact the Mab5B12-IR cell in another neuronal cluster (arrow). Bar = 5 μm. (B) Bright-field micrograph of A.

the neuronal clusters. In a neuroblast-enriched culture, many neuronal clusters possess one or more Mab5B12-IR cells (Fig. 6). These Mab5B12-IR cells are either spindle shaped with nuclei lying distal to the neuronal cluster or variably shaped and lying between the substrate and the cluster of neurons. Axons emanating from the neuronal clusters course along the surface of these Mab5B12-IR cells either in close contact along the axon’s entire length or with intermittent contact through short processes (Figs 5C,D; 6A,B). Fasciculated axons are also seen to course along the surface of the Mab5B12-IR cell.

A small number of cells in culture possess antigens to both Mab5B12 and anti-HRP (Fig. 8). These cells occur individually or in relatively small clusters of 3–4 cells and possess long, thin processes which are also recognized by both Mab5B12 and anti-HRP. Cells possessing both Mab5B12 and anti-HRP antigens are often associated with neuronal clusters.

Discussion

Mab5B12 recognizes several distinct sets of cells in the *Drosophila* embryo, some of which are closely associated with axon fascicles of the central and peripheral nervous system. Mab5B12-IR cells are identified as non-neuronal due to the absence of neural antigens recognized by anti-HRP and Mab22C10. A subset of these cells is detectable within the ventral midline between 9 and 10 h after fertilization, at a time and position corresponding to the formation of the anterior and posterior segmental commissures. Mab5B12-IR cells within the ventral midline have been identified elsewhere as midline ectodermal cells (MECs) by their expression of the *sim* and *slit* gene products, although the Mab5B12 antigen first appears at the start of axonogenesis while *sim* and *slit* are expressed hours earlier (Rothberg et al. 1988; Thomas et al. 1988). Also, unlike the *slit* gene product, the Mab5B12 antigen is not associated with the longitudinal connectives of the CNS. Other Mab5B12-IR cells appear in a position dorsolateral to the CNS in the thoracic and abdominal segments and extending processes to the lateral wall of the embryo. These cells become detectable with Mab5B12 during elongation of axons that pioneer the pathways of the PNS and may correspond to the segmental boundary cells (SBCs) of *Drosophila* identified elsewhere (Bastiani & Goodman, 1986). Peripheral axon fascicles in the cephalic segments are also accompanied by Mab5B12-IR cells. The Mab5B12 antigen is present on the surface of cells that ensheathe some axon tracts of the CNS commissures and most axons within the periphery. Antigen can be localized between closely adjacent glial surfaces, between axon and glial surfaces, and between adjacent axonal surfaces.

Mab5B12-IR cells in unfractionated and neuroblast-enriched cultures of dissociated gastrula-stage embryos are present in various morphologically distinct cell types. Individual and clusters of Mab5B12-IR cells are frequently seen to project long processes away from the cell bodies and possess growth-cone-like structures at the ends of these processes. A small percentage of these cells may also express anti-HRP antigens. Variably shaped Mab5B12-IR cells are observed in close association with neuronal clusters. Axons projecting from neurons within these and nearby clusters frequently travel along the length of the Mab5B12-IR cell and may extend from one Mab5B12-IR cell to another. Thus Mab5B12-IR cells in culture possess characteristics similar to those found in the intact embryo, in particular, an association with neuronal processes.

*Drosophila* has received attention in the investigation of axonal pathway formation through study of embryonic and postembryonic neural development (Blair et al. 1987; Blair & Palka, 1985a; Thomas et al. 1984, 1988). Other studies have relied upon individual neuronal identity to investigate cellular determination and pattern formation in neuromere development (Bodmer & Jan, 1987; Dambly-Chaudière & Ghysen, 1986; Ghysen et al. 1986). Perhaps the most comprehensive
Fig. 7. Mab5B12-IR cells are in close association with the PNS. Abdominal segments of an approximately 13 h (early stage 16) embryo stained with both Mab5B12 (FITC) and rabbit anti-HRP (RITC). Dorsal is toward the top of the figure. Mab5B12-IR cells are present along axon tracts of the PNS between the CNS and the lateral sensilla. bd, bipolar dendrite; ls, lateral sensilla; vs, ventral sensilla.
Fig. 8. Some cells in culture possess antigens to both Mab5B12 and anti-HRP. (A) Phase image of two clusters of cells and two unidentified cells. (B) Same field as in A showing a Mab5B12-IR cluster of cells (arrow) with a long process extending away from the cluster. (C) Same field as in A showing the same Mab5B12-IR cluster of cells in B also possesses antigens to anti-HRP (arrow). Also present is an anti-HRP-positive neuronal cluster (arrowheads in B and C).
investigations have focused on specific molecules present on the neuronal cell surface which may influence proper routing of CNS axons on pathways previously supplied through pioneer axons (Bastiani et al. 1987; Paterson et al. 1987). The present study has relied upon evidence obtained elsewhere of the growth cone guidance role of non-neuronal cells in the developing vertebrate and invertebrate nervous system. Evidence presented here supports the hypothesis that Mab5B12-IR glial cells execute this guidance role during axonogenesis because of their presence along the pathway transversed by some of the initial pioneer growth cones and by apparent selective affinities of some growth cones for Mab5B12-IR cells in culture. Midline glial cells, identified here as Mab5B12-IR cells, interact with the initial pioneer growth cones which cross the ventral midline to form the anterior and posterior segmental commissures (Jacobs & Goodman, 1987; Poulson, 1950). Whether pioneer axonal processes have a direct affinity for the Mab5B12-IR midline glia remains for further study. However, when presented with a choice between a Mab5B12-IR cell and other non-neuronal cell types in culture, some axons associate with the Mab5B12-IR cell surface suggesting some affinity of these axons for the Mab5B12-IR cell surface. As demonstrated by others, the non-neuronal cells destined to become Mab5B12-IR are present before axonogenesis and provide a substrate for pioneer growth cones crossing the ventral midline and for those extending into the periphery (Jacobs & Goodman, 1989). Whether growth cones contact with the glial cell surface in the embryo induces expression of the antigen or antigen is present before growth cone contact cannot be resolved here. However, the presence of Mab5B12-IR cells in culture without any apparent neurons in close apposition would suggest the latter. The specificity of pioneer growth cone guidance in Drosophila may require tight temporal constraints due to the small distances between directional decision points and the number of growth cones within a hemi-neuromere at 9-10 h of development. Resolution of such constraints may be beyond the methods used here.

Other Mab5B12-IR cells are present just dorsal to the pathway of the future supraoesophageal commissure. In older embryos, the large supraoesophageal commissure crossing the midline just ventral to these cells is reminiscent of the 'glial sling' described in the development of the vertebrate corpus callosum, which provides a substrate for growth cones crossing between cerebral hemispheres (Silver et al. 1982). Mab5B12-IR cells extend posteriorly from dorsal to the supraoesophageal commissure occupying the leading edge of the advancing ectoderm during dorsal closure. The presence of antigen on such varied cell types requires further study to determine whether this represents shared epitopes or a common function.

Wheeler (1983) and Nusbaum (1883) suggested that glial elements in the CNS of arthropods originate from components contained in the ventral midline, but disagreed over their ectodermal or mesodermal origin. More recently, others have suggested the presence of glial cells within the ventral midline of Drosophila having a 'mesectodermal' origin and a non-neuronal phenotype (Canal & Ferrus, 1986; Springer, 1967, 1968; Poulson, 1950). Thomas and colleagues (1988) suggest the derivation of ventral midline glia (MECs) from ectoderm and propose their role in formation of the anterior and posterior segmental commissures. In this study, the presence of Mab5B12-IR cells in close association with some neuronal clusters regardless of cell culture densities and the presence, although rare, of cells possessing both Mab5B12-IR and neuronal antigens may reflect a common lineage between a subset of neurons and a subset of Mab5B12-IR cells. Other possibilities including cellular migration or adhesion affinities at the time of plating cannot, as yet, be ruled out. Clearly the occurrence of individual or clusters of Mab5B12-IR cells in culture suggests the presence of glioblasts at the same time as neuroblasts. While it appears that some cells express both Mab5B12 and anti-HRP antigens, it is possible that this staining pattern represents neurons ensheathed by Mab5B12-IR cells. As demonstrated ultrastructurally in Fig. 2E, some neurons may express the Mab5B12 antigen. It is possible that similar events as suggested for the slit gene product (Rothenberg et al. 1988) also occur with the Mab5B12 antigen in that a secreted form of the antigen is associated with some axons.

This study presents evidence supporting the hypothesis that Mab5B12-IR cells within the ventral midline of Drosophila interact with axons emanating from the ventral segmental ganglia. Other Mab5B12-IR cells in the periphery interact with cells of the PNS and their afferent or efferent axons. Glial cell identity has been proposed to conform to specific criteria in their relationship with neurons (Radojcic & Pentreath, 1979). Mab5B12-IR cells comply with these criteria of ectodermal ('meso-ectodermal') origin and association with neurons and are identified here as glia. The presence of Mab5B12-IR cells within the nervous system of Drosophila at critical periods of axonogenesis and at critical positions within the pathways followed by elongating axons may be indicative of the participation of these cells in the guidance of pioneer growth cones towards their specific targets. The pioneer growth cones, which cross the ventral midline to initiate the anterior and posterior commissures, encounter and may utilize these Mab5B12-IR cells as a guidance substrate. Groups of Mab5B12-IR cells located lateral to the CNS may act as a guidance substrate for axons exiting or entering the CNS through peripheral axon tracts. Ensuing experiments have been designed to address the issues of the function and lineage of the Mab5B12-IR glial cells during development in the Drosophila embryo and to develop other probes for their investigation.
References


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