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A role for fasciclin II in the guidance of neuronal migration

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SUMMARY

The insect cell adhesion receptor fasciclin II is expressed by specific subsets of neural and non-neural cells during embryogenesis and has been shown to control growth cone motility and axonal fasciculation. Here we demonstrate a role for fasciclin II in the guidance of migratory neurons. In the developing enteric nervous system of the moth *Manduca sexta*, an identified set of neurons (the EP cells) undergoes a stereotyped sequence of migration along the visceral muscle bands of the midgut prior to their differentiation. Probes specific for *Manduca* fasciclin II show that while the EP cells express fasciclin II throughout embryogenesis, their muscle band pathways express fasciclin II only during the migratory period. Manipulations of fasciclin II in embryonic culture using blocking antibodies, recombinant fasciclin II fragments, and enzymatic removal of glycosyl phosphatidylinositol-linked fasciclin II produced concentration-dependent reductions in the extent of EP cell migration. These results support a novel role for fasciclin II, indicating that this homophilic adhesion molecule is required for the promotion or guidance of neuronal migration.

Key words: Migration, Adhesion molecule, Guidance, Fasciclin II, *Manduca sexta*

INTRODUCTION

The directed migration of neurons or their precursors along specific pathways is essential to the formation of both the central and peripheral nervous system and can affect the expression of mature phenotypes by the post-migratory cells. While the phenomenon of neuronal migration was first characterized in vertebrates, it is now clear that this same process is also critical to the formation of invertebrate nervous systems, where many cells can be uniquely identified throughout development (Hedgecock et al., 1987; Klambt et al., 1991). A particularly dramatic example of directed migration has been documented within the developing enteric nervous system (ENS) of the moth, *Manduca sexta*. During the formation of the ENS, a population of approximately 300 post-mitotic cells (the EP cells) delaminates from a neurogenic placode to form a discrete packet of undifferentiated neurons at the foregut-midgut boundary (Copenhaver and Taghert, 1991). The completion of their development is delayed, however, until a new set of migratory pathways differentiates on the adjacent midgut, where a set of visceral muscle bands coalesce at eight specific locations around the midgut surface. Once these pathways have formed, subsets of EP cells then migrate rapidly onto each muscle band, traveling several hundred μm over the course of 7-10 hours (Copenhaver and Taghert, 1989b). Only after this migratory phase is complete do the neurons form mature synaptic contacts (Wright et al., 1998) and express transmitter phenotypes (Copenhaver and Taghert, 1989a), a developmental sequence that is regulated in part by the migratory process (Copenhaver et al., 1996).

Exploiting the relative simplicity and accessibility of the ENS in *Manduca*, we previously characterized the cellular mechanisms regulating neuronal migration within the developing embryo. Intracellular injections of individual EP cells in vivo have revealed a typical migratory profile: each neuron extends a leading process enriched with dynamic filopodial extensions that contact both the muscle bands and the non-supportive interband musculature as the neurons migrate (Horgan and Copenhaver, 1998). Manipulations of the developing ENS in embryonic culture have shown that contact with a muscle band pathway is both necessary and sufficient for EP cell migration, whereas muscles in the interband regions (which are eventually innervated by these neurons) are strongly inhibitory for migration (Copenhaver et al., 1996). These and other studies have indicated that molecular components associated with the muscle bands (but not the interband musculature) are essential for the support of neuronal migration in this system.

One candidate molecule that may function as a guidance cue for EP cell migration is the adhesion receptor fasciclin II. Originally identified by a monoclonal antibody screen of the grasshopper nervous system (Bastiani et al., 1987), cDNA clones encoding fasciclin II were subsequently isolated from both grasshopper and *Drosophila* (Grenningloh et al., 1991; Harrelson and Goodman, 1988; Snow et al., 1988). Fasciclin II is a member of the immunoglobulin (Ig)-related superfamily of cell adhesion receptors (Brummendorf and Rathjen, 1993) with structural similarity to the vertebrate receptor NCAM (Grenningloh et al., 1990). Like NCAM, fasciclin II is a membrane glycoprotein that has an extracellular domain

containing five Ig-like C2 domains and two fibronectin (FN)-type III domains (Brummendorf and Rathjen, 1993). Also like NCAM, fasciclin II is expressed in multiple isoforms, including one or more transmembrane forms and a membrane-associated form that is attached via a glycosyl phosphatidylinositol (GPI) linkage to the outer leaflet of the plasma membrane (Grenningloh et al., 1991; Lin and Goodman, 1994). Extensive studies on the function of fasciclin II in the developing insect nervous system have demonstrated that this molecule acts primarily as a homophilic cell adhesion receptor (Grenningloh et al., 1991) and participates in a number of different aspects of neuronal differentiation, including the regulation of axonal fasciculation (Lin et al., 1994; Lin and Goodman, 1994), synaptic stabilization and growth (Schuster et al., 1996), and the control of proneural gene expression and neurogenesis during metamorphosis (Garcia-Alonso et al., 1995; Whitlock, 1993). However, unlike NCAM, which has been implicated in the guidance of several different classes of migratory neurons (Cremer et al., 1994; Ono et al., 1994), a role for fasciclin II in the control of neuronal migration has not been explored.

In this paper, we report the cloning and characterization of fasciclin II from *Manduca* (hereafter designated MFas II), and we describe the developmental patterns of fasciclin II expression with respect to EP cell migration. We have also shown that during the migratory period, both the neurons and their muscle band pathways express MFas II, whereas the non-supportive interband musculature does not. Lastly, using an embryonic culture preparation, we have tested the functional role of MFas II with respect to the guidance of EP cells along their normal migratory pathways. Our results indicate that MFas II plays a key role in the migration of these identified neurons, serving to promote or guide their motile behavior in a tightly restricted manner within the developing ENS.

MATERIALS AND METHODS

Tissue preparation and immunoanalysis

Synchronized egg collections were obtained from a colony of *Manduca sexta* and maintained at 25°C, at which temperature embryogenesis is complete in 100 hours (1% of development = 1 hour). Embryo staging and whole-mount immunohistochemistry were performed as previously described (Copenhaver and Taghert, 1989a; Wright et al., 1998). For immunoblot analysis, tissues were collected on dry ice and rapidly homogenized in sample buffer (1% SDS, 10% glycerol, 50 mM Tris, pH 6.7) at 100°C. Approximately 100 μg of protein from each sample was separated on a 10% SDS-polyacrylamide gel under non-reducing conditions, transferred to nitrocellulose, and reacted with antisera (Horgan et al., 1994). For detecting MFas II, we used either the monoclonal antibody TN-1 at 1:20,000 dilution (gift of Dr Paul Taghert; Carr and Taghert, 1988) or a mouse polyclonal antiserum that was generated against gel-purified 91 kDa MFas II (at 1:2000; gift of Dr James Nardi). Both of these antibodies produced identical immunohistochemical staining patterns and recognized the same protein bands in immunoblots of embryonic and post-embryonic tissues. Also used in this study were antibodies against *Drosophila* fasciclin I, fasciclin II and semaphorin Ia (each at 1:5 and 1:20; gifts of Drs Corey Goodman and Alex Kolodkin), fasciclin III (at 1:20; gift of Dr Peter Snow); and *Manduca* neuroglian (at 1:2000; gift of Dr James Nardi). Concentrated IgG fractions of several of these antibodies were also used for the in vivo blocking experiments described below.

Microsequence analysis and cloning of MFas II

An affinity-purified fraction of MFas II (generously provided by Dr James Nardi; see Nardi, 1992) was analyzed at the HHMI Biopolymer Laboratory and W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University (New Haven). Primary and secondary sequences were obtained from two fragments of this protein and compared with known sequences in the Non-Redundant Protein Sequences database using the NCBI BLAST program (Altschul et al., 1990). Oligonucleotide primers based on this microsequence data were used in a PCR reaction to amplify a partial clone of MFas II from cDNA prepared from embryonic midgut mRNA. Random primed ^{32}P-labeled probes were prepared from this PCR product and used to screen a cDNA library (Uni-Zap^{tm} XR Custom Library from Stratagene; gift of Dr James Nardi) generated from mRNA derived from larval CNS. Approximately 6×10^5 plaques were screened, resulting in the isolation of 18 positive clones. Nucleic acid sequencing was performed on an automatic sequencer (Model 373 Stretch; ABI) and confirmed by manual sequencing.

Northern blot analysis and in situ hybridization histochemistry

For northern blots, ^{32}P-labeled probes were prepared from a 3 kb subclone of the extracellular domain of MFas II after restriction digest with either *Pst*I (sense) or *Apa*I (antisense) and used at final concentrations of 1×10^5 cpm/ml in hybridization buffer (after Horgan et al., 1995). Poly(A)$^+$-mRNA was purified from pupal wing using the Oligotek Direct mRNA kit (Qiagen), and 1 μg of each sample was separated on a 1% agarose gel. Samples were then transferred to nylon membranes (Zeta Probe; from Biorad) and hybridization with probe was performed in hybridization buffer at 65°C overnight. Blots were washed repeatedly and exposed to film. For whole-mount in situ hybridization histochemistry, both sense and antisense digoxygenin-labeled probes were also prepared from the same inserts described above using digoxigenin-11-UTP (Boehringer) after the methods of Patel and Goodman (1992). Unhydrolyzed probes (approx. 3,000 b) were found to yield a stronger signal in the EP cells than probes that had been hydrolyzed to approx. 400 b fragments. Embryos were fixed in 5% paraformaldehyde plus 0.8% Triton X-100 in PBS (pH 8.0) for 1 hour, rinsed and incubated with the probes (1:100 in hybridization buffer; Horgan et al., 1995) overnight at 58°C. Embryos were then washed, reacted with an alkaline phosphatase-conjugated anti-digoxygenin antibody (at 1:2,000; Boehringer), and bound antibody was visualized by reaction with the appropriate substrates (Horgan et al., 1995).

Preparation of GST fusion proteins containing MFas II fragments

A cDNA fragment of MFas II encoding Ig-domains III-V (amino acids 207-519; see Fig. 1) was amplified by PCR and subcloned into pGEX-KG (Pharmacia), which encodes glutathione S-transferase (GST) with an in-frame glycine-rich linker domain to facilitate thrombin cleavage (Guan and Dixon, 1991). The plasmid was then transformed into DH5α cells containing the pLysS plasmid. Subclones were screened for the expression of GST-MFas II by SDS-PAGE. Control plasmids containing GST alone were also expressed. Individual colonies were then grown overnight in 800 ml LB broth (containing 75 μg/ml ampicillin, 33 μg/ml chloramphenicol and 10 mM $MgSO_4$) at 37°C. The cultures were diluted 1:10 into 8 l of fresh LB broth and incubated for 3 hours at 37°C. IPTG was then added to a final concentration of 0.5 mM, and the cultures were incubated for an additional 2.5 hours at 30°C. Cells were harvested by centrifugation, resuspended in 50 ml chilled PBS, and lysed by passing them through a French press twice at 20,000 lb/in^2. Triton X-100 was added to a final concentration of 1% and the insoluble material was removed by centrifugation. 0.5 ml of a glutathione-agarose bead suspension (50% v/v) was added to the supernatant and incubated overnight at 4°C. The beads were washed 3 times in 50 ml

PBS and the fusion protein was eluted in 20 ml of reduced glutathione (25 mM). For some experiments, the eluted fusion protein was incubated with thrombin (2 U) for 2 hours in 10× thrombin cleavage buffer (Guan and Dixon, 1991), followed by removal of residual GST with glutathione agarose and removal of thrombin with streptavidin-agarose beads coupled to biotinylated anti-thrombin antibodies (Novagen). The fusion protein was then dialyzed in sterile defined saline (Horgan and Copenhaver, 1998) and concentrated in a stirred ultrafiltration cell (Amicon). Protein concentrations were determined using the BCA method (Pierce).

Embryonic culture

Staged embryos were isolated in a modified culture medium (Horgan et al., 1994), restrained in Sylgard-coated chambers, and a small incision was made in the dorsal body wall to expose the developing ENS. For experiments employing GST fusion proteins, embryos were dissected and incubated in sterile defined saline (Horgan and Copenhaver, 1998). Experimental and control solutions were then flushed repeatedly through the incision to ensure complete exposure of the EP cells and muscle bands to the experimental solutions. Embryos were then allowed to continue to develop at 28°C for 12-20 hours, then fixed in 4% paraformaldehyde and processed for whole-mount immunohistochemistry with anti-MFas II antibodies. For in vivo applications of blocking antibodies, IgG fractions were purified using HiTrap Protein G columns (Pharmacia). Phosphatidylinositol-specific phospholipase C (PI-PLC) and phospholipase B (PLB) were obtained from Boehringer. All other reagents were obtained from Sigma, unless otherwise specified. The distance of EP cell migration and axon outgrowth (measured from the foregut-midgut boundary on each of the four dorsal muscle bands) was then analyzed by photomicroscopy and camera lucida techniques. Data from experimental preparations were normalized to matched control groups and subjected to statistical analysis using a two-tailed Students *t*-test. Histograms indicate means ± s.e.m.

RESULTS

Multiple isoforms of fasciclin II are expressed in the developing ENS

In previous work, Carr and Taghert (1988) showed that the TN-1 antibody produced a staining pattern in the developing CNS of *Manduca* resembling the expression pattern of fasciclin II in grasshopper and *Drosophila* (Bastiani et al., 1987; Grenningloh et al., 1991). Subsequently, Nardi (1990) showed that TN-1 recognized two proteins with apparent molecular masses of 91 kDa and 94 kDa, and that partial amino acid sequences obtained from the 91 kDa protein shared some similarity with fasciclin II sequences from other species (Nardi, 1992). Because these sequences were insufficient for the complete characterization of the TN-1 epitope, we obtained two additional microsequences from tryptic fragments of the 91 kDa protein (Glu-Met-Gln-Glu-Arg-Glu-Ser-Arg-Val-Glu-Ile and Val-Phe-Ala-His-Ser-Gly-Glu-Phe-Iso-Asp-Lue-Tyr-Glu-Ile-Gln-Tyr-Cys-Phe-Val-Leu; see Materials and methods). These peptides showed 63% and 65% sequence identity with *Drosophila* fasciclin II, respectively, and were used to construct degenerate oligonucleotide primers for a PCR reaction against cDNA obtained from embryonic midgut mRNA. The resultant product was found to have strong sequence similarity with the coding regions for fasciclin II in other species (not shown).

Using probes derived from this PCR product, we isolated eight separate clones from a *Manduca* cDNA library that contained sequences encoding fasciclin II-like proteins. Two of these clones (with inserts of 4.0 and 5.5 kb, respectively) were found to contain full-length coding regions for proteins with significant sequence identity to *Drosophila* and grasshopper fasciclin II (Fig. 1). Each clone contains one long open reading frame encoding proteins with calculated masses of approximately 86.5 kDa and 92.5 kDa, respectively. The two clones share the same 5′ untranslated region and signal sequence and are 100% identical over the first 2547 nucleotides. As illustrated in Fig. 1, the proteins encoded by this region (amino acid residues 1-730) align well with the extracellular domains of fasciclin II from other species, containing five putative Ig-like C2 domains, two FN III domains, and seven potential sites for N-glycosylation (Fig. 1, asterisks). However, the 3′ ends of the two clones are divergent. The larger clone contains a predicted transmembrane domain (TM) and a short cytoplasmic tail with potential sites for N-myristoylation and phosphorylation by casein kinase II. The smaller clone encodes a protein with the characteristic features of a 3′ GPI attachment site (Udenfriend and Kodukula, 1995), including a triplet of small amino acids that comprise the putative GPI linkage site (boxed region) followed by a short linker region and a C-terminal hydrophobic domain (dotted region). The deduced transmembrane protein shares substantial sequence identity with transmembrane isoforms of fasciclin II in *Drosophila* (44%) and grasshopper (39%), and lower sequence similarity to mouse NCAM (26%). The putative GPI-linked protein shares 42% amino acid identity with the GPI-linked isoform of *Drosophila* fasciclin II. Based on these similarities, we conclude that these two clones encode transmembrane and GPI-linked forms of MFas II.

We next used a combination of northern blot and protein immunoblot analyses to verify that both isoforms of fasciclin II are expressed in *Manduca*. Riboprobes generated from a portion of the shared 5′ region of the two MFas II clones were hybridized with mRNA from developing adult wing (an abundant source of MFas II protein; Nardi, 1990). As shown in Fig. 2A, two strongly hybridizing transcripts of approximately 5 kb and 6.5 kb were consistently detected by these probes, as well as a less prevalent mRNA species at approximately 11 kb. These results indicate that the native mRNA species encoding MFas II contain additional untranslated sequences that are not present in our cDNA clones. Whether the 11 kb band represents an additional, as yet uncharacterized isoform of MFas II or is simply an unprocessed form of one of the smaller transcripts remains to be determined. Similarly, immunoblots using a polyclonal antiserum against MFas II (see Materials and methods) also revealed two strongly labeled bands (Fig. 2B) in protein extracts from embryonic midgut (g; containing the migratory EP cells) and from larval midgut muscle bands (b; containing the post-migratory EP cells). Both bands were also detected in immunoblots of developing adult wing (w), although only the lower band was consistently stained in extracts from larval central nervous system (cns). An identical pattern of immunopositive bands was recognized by TN-1 (not shown). Under the non-reducing conditions used in this study, the apparent molecular masses of the two proteins were about 90 and 95 kDa, consistent with the predicted sizes of the proteins encoded by our cDNA clones after glycosylation and similar to the pattern reported by Nardi (1992).

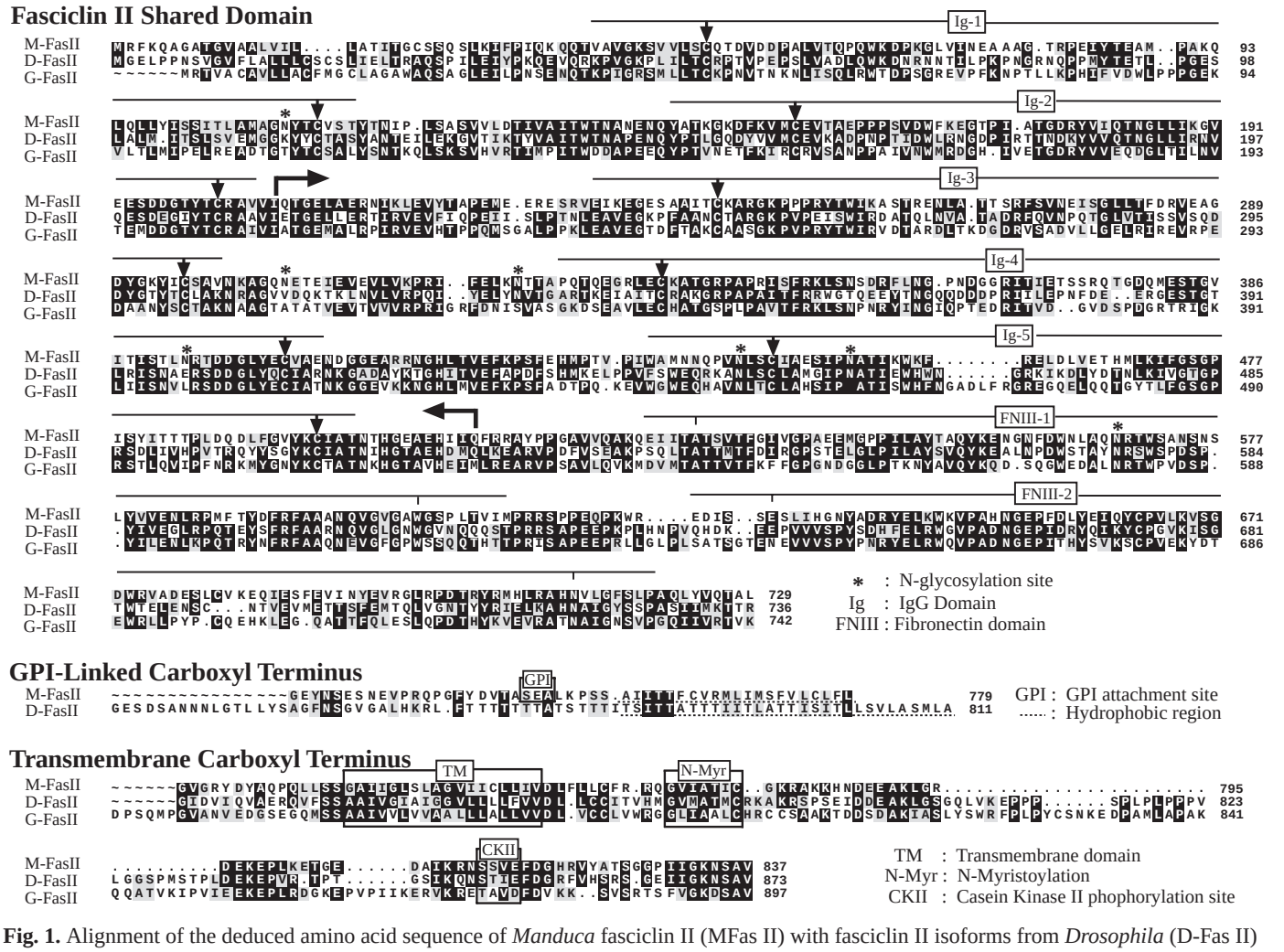

Fig. 1. Alignment of the deduced amino acid sequence of *Manduca* fasciclin II (MFas II) with fasciclin II isoforms from *Drosophila* (D-Fas II) and grasshopper (G-Fas II). Conserved amino acid residues are highlighted in black; similar residues are shaded in gray. The five immunoglobulin C2 domains (Ig-1 through Ig-5) and the two fibronectin type III domains (FNIII-1 and FNIII-2) are indicated by the labeled bars above the appropriate sequences. Potential sites for N-glycosylation are indicated by asterisks. The two isoforms of MFas II share a common extracellular domain (residues 1-730) but have divergent C-terminal extensions, as indicated. Characteristic features distinguishing the GPI-linked isoform of MFas II include a putative site for GPI attachment (boxed residues) separated from a C-terminal hydrophobic domain (dotted region) by a short linker region. The transmembrane isoform contains a predicted transmembrane domain (TM) of hydrophobic residues and potential sites for N-myristoylation (N-Myr) and casein kinase II phosphorylation (CKII) within the cytoplasmic domain. Right-angled arrows indicate sequences contained in the GST-MFas II fusion protein used in Figs 6-7.

To demonstrate that one isoform of MFas II is anchored to the external leaflet of the plasma membrane by a GPI-modification, we treated the developing ENS in culture with PI-PLC, an enzyme that has been shown to cleave GPI-linkages and release glypiated molecules into the surrounding medium (Udenfriend and Kodukula, 1995). Sets of embryonic guts containing the EP cells (at 60-65% of development; 10 guts per set) were incubated in culture medium in the presence or absence of PI-PLC for 2 hours. The tissue was then gently pelleted, and detergent-solubilized extracts of both the gut tissue (g) and the surrounding supernatant (sn) were assayed by immunoblot analysis to determine the distribution of the two MFas II isoforms (Fig. 2C). In control medium without PI-PLC, both isoforms remained associated with the embryonic gut tissue, with no apparent degradation of the proteins (compare with the control immunoblot in Fig. 2B). In contrast, incubation with PI-PLC caused virtually all of the approx. 90 kDa isoform protein to be released from the gut tissue into the surrounding medium. As an additional control, phospholipase B (PLB) was shown not to alter the distribution of either isoform. In summary, the results shown in Fig. 2 demonstrate that two isoforms of MFas II are associated with the developing ENS: a transmembrane isoform (approx. 95 kDa) and a GPI-linked isoform (approx. 90 kDa), as predicted by the deduced amino acid sequences of the clones described above.

Fasciclin II is expressed by both the migratory EP cells and their muscle band pathways

In previous work, antibodies against MFas II were found to label the EP cells at various stages of their differentiation (Copenhaver and Taghert, 1989b, 1991). Since fasciclin II has been shown to serve as a neuronal recognition molecule for

axon guidance in the developing insect CNS (Harrelson and Goodman, 1988; Lin and Goodman, 1994), we examined the developmental expression of MFas II with respect to EP cell migration. Staged embryos were immunostained either with TN-1 or with the polyclonal antiserum against MFas II, both of which recognize the two isoforms of MFas II (Fig. 2, and unpublished observations) and which produced identical staining patterns in the ENS (summarized below). A matched set of embryos were labeled by whole-mount in situ hybridization histochemistry, using a digoxygenin-labeled probe against the shared extracellular domain of the two MFas II clones.

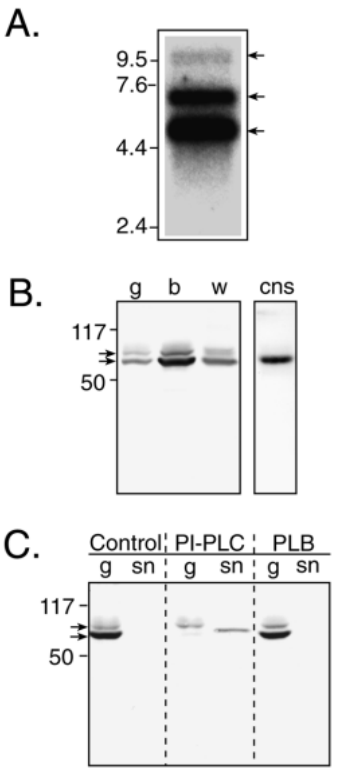

Fig. 2. Analysis of MFas II-specific mRNA transcripts and protein isoforms. (A) Northern blot of poly(A)$^+$-mRNA (purified from pupal wing) reacted with a ^{32}P-labeled probe that was made from a 3 kb subclone of the shared extracellular domain of MFas II. Two transcripts of approximately 5 and 6.5 kb were strongly labeled, while a third transcript (approximately 11 kb) was more faintly labeled. (B) Immunoblot of proteins extracted from embryonic gut (g), larval midgut muscle bands (b), developing adult wing (w) and larval nervous system (cns) that were labeled with a polyclonal MFas II antibody. Under non-reducing conditions, two prominent bands with apparent molecular masses of approximately 90 and 95 kDa were consistently seen in tissue extracts containing the EP cells (including embryonic guts and larval muscle bands), as well as in developing adult wing extracts. Only the smaller band was detected in the larval nervous system. (C) Immunoblots of proteins extracted from embryonic guts (g) or from the surrounding supernatant (sn) after 2 hours in culture and brief centrifugation (stained with polyclonal MFas II antiserum). In control medium, both MFas II-specific bands (approximately 90 and 95 kDa) remained associated with the gut tissue, whereas in medium containing PI-PLC, virtually all of the 90 kDa band was liberated into the surrounding medium. Incubation in PLB did not result in a change in distribution of either protein band. For each sample, gut tissue was collected from 10 embryos (60-65% of development) and pooled in culture.

Prior to the onset of EP cell migration (50% of development), both MFas II protein and MFas II-specific mRNAs were expressed throughout the packet of EP cells at the foregut-midgut boundary, as well as by cells within the developing esophageal nerve on the foregut (en; Fig. 3A,D). In contrast, no detectable expression of MFas II was observed within the adjacent muscle cell layers on the foregut and midgut, although a variety of other ectodermal and mesodermal cell types stained positively for MFas II at this time (not shown). During the subsequent period of EP cell migration (55-60% of development), all of the migratory neurons continued to express MFas II as they traveled onto the coalescing muscle bands of the midgut (Fig. 3B,E, arrows), including strong immunolabeling of the leading processes of the migratory neurons (visible in Fig. 3B). In addition, however, both MFas II protein and MFas II-specific mRNA could also now be seen within the muscle cells forming the band pathways (open arrows in Fig. 3B,E), but not within the adjacent interband musculature. By the end of the migratory period (65% of development), this dual pattern of expression had changed: while the post-migratory neurons continued to show strong levels of MFas II expression (Fig. 3C,F), including robust levels of MFas II immunoreactivity within their axonal processes (arrowheads), the muscle bands no longer contained detectable levels of either MFas II mRNA or protein.

This transient pattern of fasciclin II expression by the midgut muscle band pathways was unexpected and is better illustrated in Fig. 4. As previously noted, the muscle bands coalesce from subsets of the longitudinal muscle cells at eight specific locations around the midgut circumference, just prior to the onset of EP cell migration (Copenhaver and Taghert, 1989b). Shortly after the formation of the bands, MFas II expression could be detected along their entire length (Fig. 4A,D, open arrows), coincident with the migration of the EP cells onto the anterior ends of the bands at the foregut-midgut boundary (black arrows). Notably, positive staining within the visceral mesoderm was restricted to the component cells of the muscle bands, a distinction that was readily apparent in regions of the midgut posterior to the migratory neurons (Fig. 4B,E). As shown in Fig. 3, by 65% of development, the post-migratory EP cells had begun to extend axons along the muscle bands (Fig. 4C, arrowheads), a process that normally continues over the next 20% of development before the axons branch laterally to innervate the interband musculature (Copenhaver and Taghert, 1989a; Wright et al., 1998). However, by the time that migration was complete, MFas II expression could no longer be detected within the muscle band cells at any position along the midgut (Fig. 4C,F). Thus, both the EP cells and their muscle band pathways express MFas II during the migratory period but do so in developmentally distinct patterns: whereas the migratory neurons maintain strong levels of MFas II both before and after migration, the muscle bands express this cell adhesion receptor transiently, exhibiting detectable levels of MFas II only during the specific time when the neurons are migrating on them.

Fasciclin II is necessary for EP cell migration

As noted earlier, surgical manipulations of the developing ENS in vivo have shown that the muscle band pathways are both

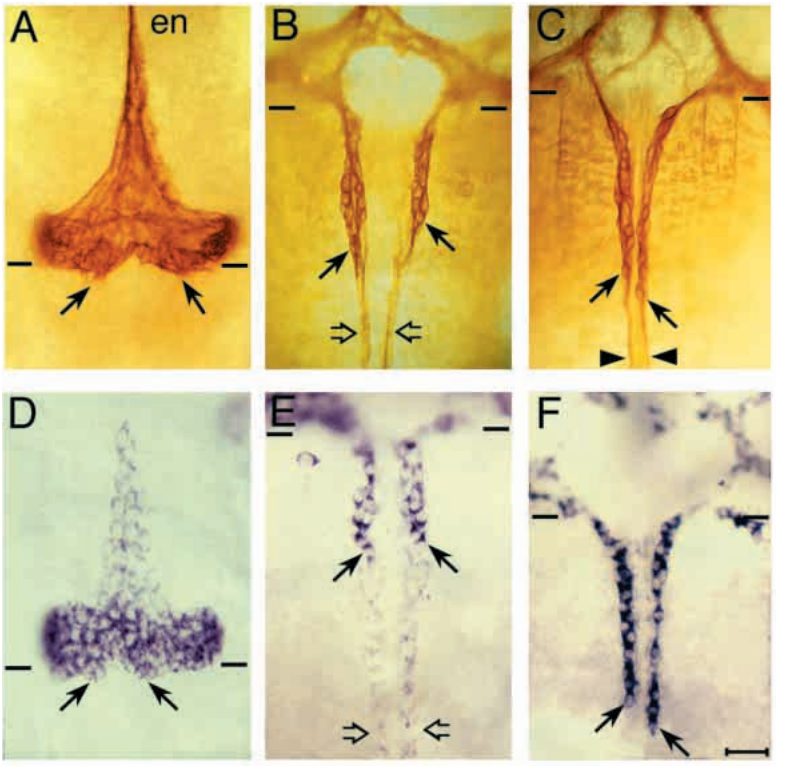

Fig. 3. Developmental expression of MFas II transcripts and protein in the developing ENS. (A-C) Whole-mount immunostaining with an anti-MFas II antibody (TN-1). (D-F) Whole-mount in situ hybridization histochemistry with digoxygenin-labeled probes against MFas II-specific mRNA. (A,D) At 50% of development, the premigratory EP cells form a tight packet at the foregut-midgut boundary and show strong levels of MFas II expression. Immunostaining can be seen in the short filopodial processes extended by some of the EP cells onto the adjacent midgut epithelium (A, black arrows). MFas II expression can also be seen within axon bundles and the presumptive glial cells forming the esophageal nerve (en) that joins the EP cells to the more anterior enteric ganglia (out of view). (B,E) At 58% of development, subsets of EP cells (black arrows) have begun to migrate out of the original packet and continue to express MFas II. In addition, the muscle cells forming the band pathways also contain detectable levels of MFas II protein and message (open arrows; only the mid-dorsal pair of muscle bands is shown), whereas the adjacent interband muscle cells do not (see also Fig. 4). (C,F) At 65% of development, the EP cells have completed their migration, but they continue to extend axonal processes posteriorly along the muscle bands. MFas II expression remains high in the post-migratory EP cells (black arrows), including robust immunostaining within the elongating bundles of axons (C, arrowheads). In contrast, the underlying muscle bands no longer exhibit detectable levels of either MFas II-related protein or mRNA. Similar immunostaining was also seen with polyclonal antisera against MFas II (see Fig. 4). Paired black hatchmarks indicate the foregut-midgut boundary. Bar, 25 μm.

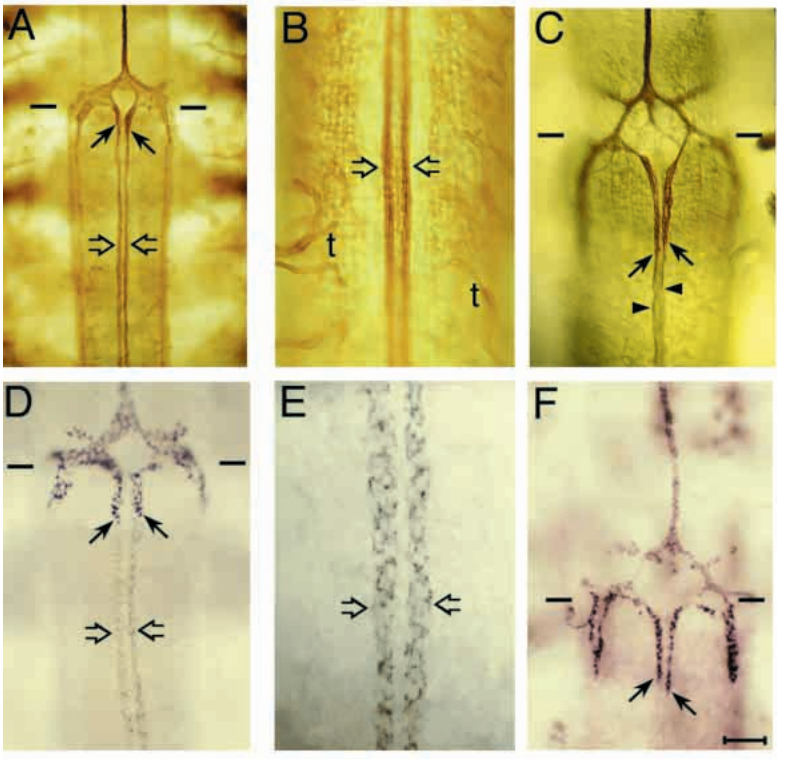

Fig. 4. The muscle band pathways transiently express MFas II during EP cell migration. (A-C) Whole-mount immunostaining of the developing ENS with a polyclonal antibody against MFas II. (D-F) Whole-mount in situ hybridization histochemistry of the ENS with digoxygenin-labeled probes against MFas II-specific mRNA. At 58% of development (A,D), the EP cells have commenced their migration onto the midgut muscle bands (black arrows). In addition, the eight longitudinal muscle bands of the midgut have also begun to express MFas II (open arrows indicate the mid-dorsal pair of bands; the dorsolateral pair of bands can also be seen near the margins of the midgut in A). The muscle bands extend the entire length of the midgut (>1 mm), whereas the EP cells will only migrate for approximately 200-250 μm before stopping (compare with Fig. 3). (B,E) Higher magnification view of the mid-dorsal muscle bands at 58% of development on the posterior midgut (distal to the position of the migratory EP cells). Positive expression of both MFas II-related protein and mRNA can clearly be detected within the muscle band cells (open arrows) but not in the surrounding interband musculature. Structures labeled ‘t’ are tracheolar branches growing onto the midgut. (C,F) At 65% of development, the post-migratory EP cells (black arrows) have begun to extend axonal processes along the muscle bands (arrowheads; see Fig. 3). By this stage, the muscle bands have ceased to exhibit detectable levels of MFas II. Similar immunostaining was seen with TN-1 (see Fig. 3). Paired black hatchmarks indicate the foregut-midgut boundary, where appropriate. Bar, 60 μm (A,C,D, F); 25 μm (B,E).

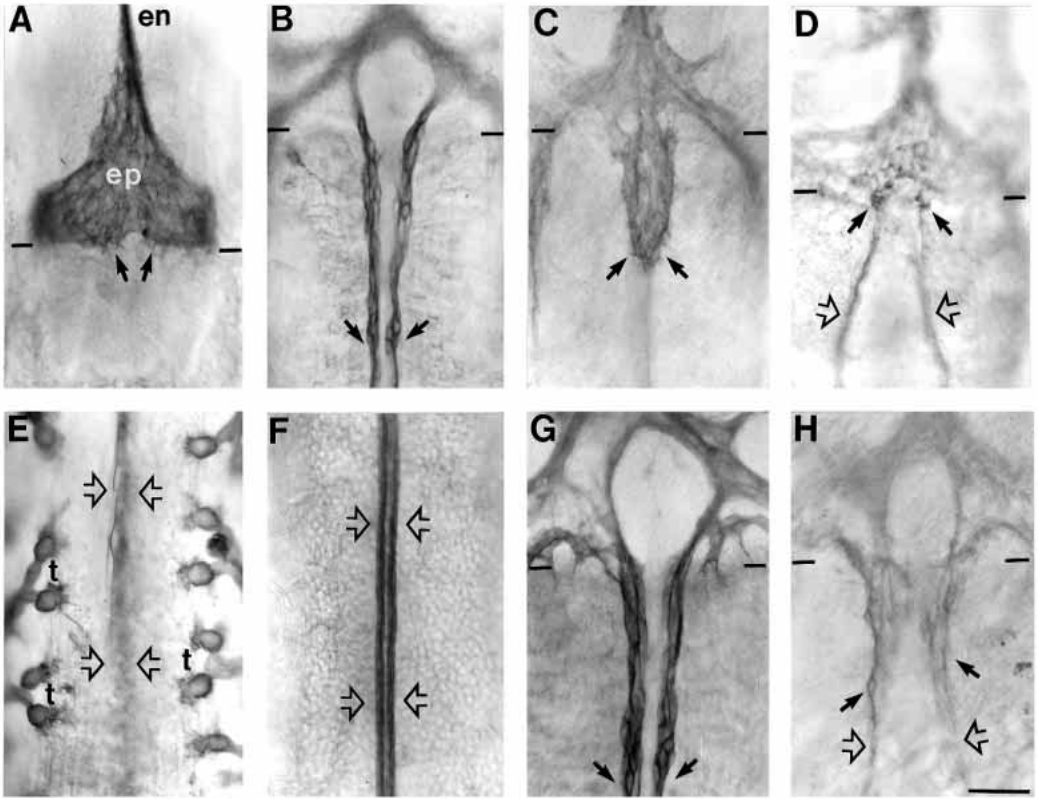

Fig. 5. Perturbations of MFas II in embryo culture inhibit EP cell migration. Preparations were fixed and immunostained with TN-1 unless otherwise noted. (A) Premigratory EP cells (ep) in an embryo placed in culture at approximately 53% of development and then fixed immediately to show the initial positions of these neurons (black arrows) at the onset of the culture experiments. MFas II expression was also present in the cells and processes of the adjacent esophageal nerve (en). (B) Control embryo that was placed in culture at 53% of development (A) and allowed to develop in normal medium for 12 hours before fixation; note that the migration of the EP cells (arrows) along the muscle bands proceeded normally. (C) Embryo that was cultured in medium containing 20 μg/ml anti-MFas II antibody (affinity-purified IgG). The extent of EP cell migration was reduced, and the neurons exhibited some aberrant clumping on the muscle bands (arrows). (D) Embryo that was cultured in the presence of 200 μg/ml anti-MFas II IgG. In this preparation, EP cell migration was completely inhibited, with the leading neurons (arrows) remaining at the foregut-midgut boundary. Formation of the mid-dorsal muscle bands (open arrows) was also partially disturbed, possibly due to incomplete gut closure. (E) Immunohistochemical staining of a 65% embryo with anti-fasciclin III antibodies labeled a stripe of epithelium underneath the mid-dorsal muscle bands (open arrows); the ingrowing tracheolar cells (t) were also positively stained. (F) Immunohistochemical staining of a 65% embryo with anti-neuroglian antibodies labeled the muscle bands (open arrows). (G) Embryo that was cultured in the presence of 220 μg/ml anti-neuroglian IgG; the migration of the EP cells and formation of the muscle bands appeared normal (compare with B). (H) Embryo that was treated in culture with PI-PLC (1 U for 60 minutes) at 50% of development and then allowed develop in normal medium for 12 hours. Migration of the EP cells (black arrows) was inhibited compared to control preparations, and the overall level of MFas II immunoreactivity was reduced. The coalescence of the muscle bands was also partially disrupted (open arrows), although they still appeared to support neuronal migration. Paired black hatchmarks indicate the foregut-midgut boundary, where appropriate. Bar, 25 μm.

necessary and sufficient for the normal migratory dispersal of the EP cells: manipulations that prevent contact between the EP cells and a muscle band preclude neuronal migration, while transplantation of the EP cells onto a muscle band will promote their migration (Copenhaver et al., 1996). Because fasciclin II has been shown to act as a homophilic adhesion molecule in other systems (Grenningloh et al., 1990; Lin and Goodman, 1994), the transient upregulation of MFas II on the muscle bands coincident with the onset of EP cell migration (Figs 3 and 4) suggested a role for MFas II in the guidance of the migratory neurons. To test this hypothesis, we first used antibodies against MFas II as a means of inhibiting fasciclin II-mediated interactions in vivo. Embryos were placed in culture at 50-52% of development (prior to migration onset; Fig. 5A) and minimally dissected to expose the ENS. The EP cells were then treated either with control medium or with medium containing affinity-purified antibodies against MFas II, and the preparations were allowed to develop for an additional 12-15 hours (through the completion of the migratory period). In contrast to control embryos, in which the EP cells migrated normally along their muscle band pathways (Fig. 5B), exposure to anti-MFas II antibodies caused a significant inhibition in neuronal migration (Fig. 5C,D). At relatively low antibody concentrations, the effects on migration were variable, resulting in abnormal clumping of EP cells that had partially migrated onto the muscle bands (Fig. 5C). At higher antibody concentrations, migration was significantly inhibited: whereas in control embryos, EP cells had migrated an average of 164 μm after 8 hours, in embryos treated with 200 μg/ml anti-MFas II IgG, the average distance of migration was only 65 μm, and in 20% of the preparations, virtually all of the EP cells remained clustered at the foregut-midgut boundary (Fig. 5D). The coalescence of the muscle band pathways also appeared to be perturbed, a finding that is discussed below. In addition, the overall level of MFas II staining was reduced in these preparations, possibly due to antibody-induced receptor internalization; however, we did not observe any evidence of necrosis or cell death within the EP cell packet or the muscle bands.

The effect on migration following treatment with either anti-MFas II antibodies or antibodies against a number of other cell adhesion receptors is also shown in Fig. 6A. As compared with

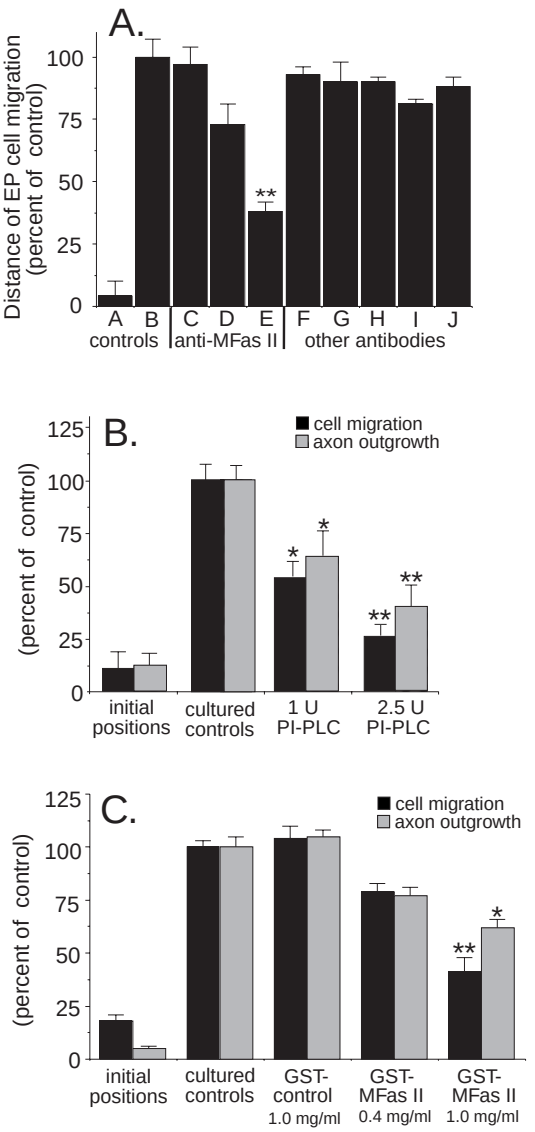

Fig. 6. Inhibition of MFas II-mediated interactions significantly inhibits migration. (A) The extent of EP cell migration in control embryos and in embryos incubated with antibodies against a variety of cell adhesion receptors. The distance of migration was measured from the foregut-midgut boundary for the leading neuron on each of the four dorsal muscle bands and normalized with respect to control embryos. Columns A and B show the extent of migration in cultured embryos at the onset and completion of the culture period. Columns C-E show the extent of migration in embryos treated with anti-MFas II IgG at 1 μg/ml, 20 μg/ml and 200 μg/ml, respectively. Treatment with 200 μg/ml anti-MFas II IgG caused a significant inhibition of migration (*P<0.001). In contrast, treatment with a variety of other antibodies produced no detectable effect on migration, including antibodies against fasciclin I (F), fasciclin III (G), semaphorin 1a (H) and neuroglian (I: 100 μg/ml IgG; J, 220 μg/ml IgG). (B) Both EP cell migration (black histograms) and axon outgrowth (gray histograms) were reduced by exposure to PI-PLC to remove GPI-MFas II in a concentration-dependent manner. (C) Application of GST-MFas II fusion proteins (encoding Ig domains III-V; see Fig. 1) to the EP cells at the onset of migration caused a dose-dependent inhibition of both migration and subsequent axon outgrowth. Equivalent concentrations of fusion protein containing GST sequence alone had no effect. *P<0.01; **P<0.001. n≥10 for each histogram (values are means ± s.e.m.).

cultured control embryos, antibodies against MFas II caused a concentration-dependent inhibition of EP cell migration along the midgut muscle bands. In contrast, antibodies against *Drosophila* fasciclin I, fasciclin III and semaphorin 1a (fasciclin IV) had no detectable effect on migration. While not all of these antibodies labeled components of the ENS in *Manduca*, anti-fasciclin III antibodies did stain the midgut epithelium underneath the muscle bands (Fig. 5E, open arrows), as well as the adjacent tracheolar cells growing onto the midgut surface. More significantly, affinity-purified antibodies against *Manduca* neuroglian, another member of the Ig-superfamily of cell adhesion receptors (Chen et al., 1997; Hortsch et al., 1990), showed strong immunolabeling of the midgut muscle bands (Fig. 5F) but had no effect on EP cell migration at equivalent concentrations (Figs 5G, 6A). All of these experiments were done in complement-free conditions, and the maximal IgG concentration (200 μg/ml) was substantially lower than antibody concentrations used to block related adhesion receptors in a number of previous studies (Bronner-Fraser et al., 1992; Burgoon et al., 1995; Chuong et al., 1987). Because the limited amount of available antibody precluded the use of Fab fragments, it is possible that some of the effects induced by anti-MFas II IgG were due to steric interference. Nevertheless, these results support the hypothesis that the coordinated expression of MFas II by the EP cells and their muscle band pathways promotes neuronal migration within the developing ENS.

As noted above, both the transmembrane and GPI-linked isoforms of MFas II appear to be expressed within the developing ENS (Fig. 2). In many systems, the enzyme PI-PLC has been shown to selectively remove GPI-linked forms of adhesion receptors from living cells without disrupting neuronal viability (e.g. Chang et al., 1992; Doherty et al., 1990; Karlstrom et al., 1993; Yoshihara et al., 1994). Accordingly, we used our embryonic culture preparation to expose the premigratory EP cells to PI-PLC for 30-60 minutes and then allowed the embryos to continue development in normal medium. As shown in Fig. 5H, treatment with PI-PLC caused a marked reduction in the extent of EP cell migration and also reduced the overall level of MFas II immunoreactivity. The effects of PI-PLC pre-treatment (before migration onset) were concentration-dependent (Fig. 6B), inhibiting both the migration and axon elongation of the EP cells. In contrast, phospholipase B had no significant effect on migration (not shown). Although PI-PLC treatments should remove other GPI-linked receptors besides MFas II, these treatments did not result in a general disruption of gut development. Rather, the enzymatic removal of GPI-linked MFas II at this stage of development appeared to affect selectively the progression of EP cell migration and outgrowth.

Previous work involving NCAM showed that synthetic peptides with sequences derived from the third Ig-domain of this receptor blocked homophilic binding (Rao et al., 1992), while recombinant protein fragments encoding the fourth Ig-domain blocked heterophilic interactions between NCAM and a related receptor, L1 (Luthl et al., 1994). As an independent means of testing whether MFas II-dependent interactions are required for EP cell migration, we applied GST-fusion proteins containing Ig-domains III-V of MFas II in culture. As shown in Fig. 6C, increasing concentrations of the fusion protein caused a dose-dependent inhibition of migration. At lower

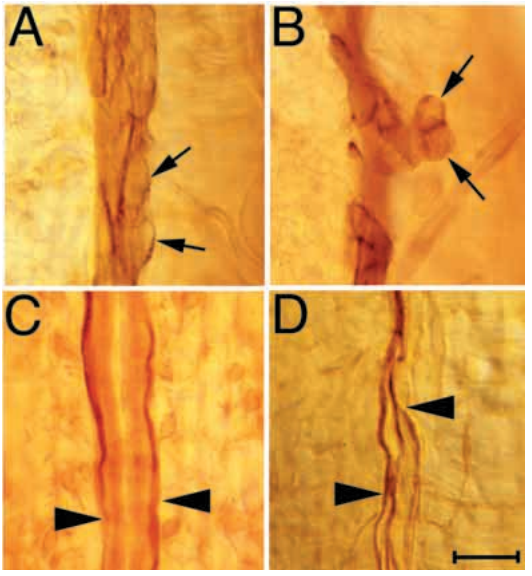

Fig. 7. Application of GST-MFas II fusion proteins reduce the adhesion of both the migratory EP cells and their axons from the midgut muscle bands. (A) Clusters of migrating EP cells (arrows) in cultured control embryo remain tightly apposed to their muscle band pathway. (B) EP cells treated with 1.0 mg/ml GST-MFas II show significant rounding and separation from the adjacent muscle band. (C) Axons and growth cones (arrowheads) of a cluster of EP cells in a control preparation remain tightly fasciculated and stay restricted to their muscle band pathway. (D) Leading growth cones of EP cells treated with GST-MFas II show a substantial increase in defasciculation and extensions off the muscle band. All preparations were immunostained with a polyclonal antibody against MFas II. Bar, 10 μm.

concentrations of GST-MFas II, this effect was variable, with some preparations exhibiting substantial reductions in migration while other embryos appeared relatively normal at the end of an experiment. At higher concentrations, however, the inhibitory effect of the fusion protein was consistent and robust, and many of the EP cells were found to have rounded partially off their migratory pathways (Fig. 7). No evidence of cell death or aberrant migration onto the interband musculature was detected in any of these cultures. The subsequent outgrowth of axons by the EP cells following migration was also reduced, although to a lesser degree (Fig. 6C). In addition, their leading growth cones (which normally remain tightly fasciculated on the muscle bands; Fig. 7C) exhibited a substantial increase in defasciculation and in some cases extended partially off their normal pathways (Fig. 7D). Preparations treated with equivalent concentrations of fusion proteins containing GST sequence alone developed normally (Fig. 6C). Together with the inhibitory effects of the blocking antibodies and PI-PLC treatments described above, these results indicate that fasciclin II plays an essential role as a guidance molecule for neuronal migration in the developing ENS.

DISCUSSION

Fasciclin II was originally characterized as a neuronal recognition molecule in the developing insect nervous system, based on its selective pattern of expression by specific subsets of growing axons within both the CNS and PNS (Bastiani et al., 1987; Harrelson and Goodman, 1988). A combination of molecular and genetic manipulations of fasciclin II subsequently demonstrated that it may serve a variety of functions, including the selective fasciculation of growing axons (Lin et al., 1994), synaptic stabilization and plasticity (Schuster et al., 1996; Thomas et al., 1997), and possibly the modulation of growth cone responses to other cell adhesion receptors (Fambrough and Goodman, 1996; Lin and Goodman, 1994). In the present study, we have shown that fasciclin II is also expressed by an identified class of migratory neurons (the EP cells) and their cellular pathways (the midgut muscle bands) during the normal period of migration. Moreover, we have shown that in vivo manipulations designed to perturb fasciclin II-mediated interactions consistently inhibited EP cell migration. These results demonstrate a new role for fasciclin II, indicating that this adhesion receptor participates in the control of multiple forms of neuronal motility.

The overall structure of fasciclin II (five Ig C2 domains plus two FN-III domains, expressed by multiple isoforms with divergent C-terminal sequences) suggests that it shares a common ancestral molecule with the vertebrate adhesion receptor NCAM (Grenningloh et al., 1990). Like fasciclin II, NCAM can be detected on migrating neurons and growing axons in many regions of the CNS and PNS (Goridis and Brunet, 1992), although its distribution is much more widespread than the restricted patterns of cell-specific expression described for fasciclin II (Harrelson and Goodman, 1988). Also like fasciclin II, the specific functions of NCAM appear to be more complex than originally proposed: genetic deletions of both of these receptors produced surprisingly subtle defects (Cremer et al., 1994; Grenningloh et al., 1991; Tomasiewicz et al., 1993), suggesting that their primary role may be to promote axonal adhesion rather than directional guidance per se (Lin and Goodman, 1994; Tang and Landmesser, 1993). However, one striking effect of deleting NCAM was a significant reduction in granule cell populations within the olfactory bulb, due to a failure in the migration of their precursors from the subventricular zone (Hu et al., 1996; Ono et al., 1994). Similarly, our results indicate that fasciclin II-dependent events are essential for the migration of the EP cells along their muscle pathways. Thus, just as the phenomenon of cell migration occurs in all developing nervous systems, the molecular mechanisms underlying this basic process may be evolutionarily conserved, as well.

The deduced amino acid sequences that we obtained for MFas II (Fig. 1) aligned well with sequences for fasciclin II from other species (Grenningloh et al., 1991; Snow et al., 1988), including the transmembrane isoforms of fasciclin II from both *Drosophila* and grasshopper and the GPI-linked isoform from *Drosophila* (curiously, no GPI-linked form has been identified in grasshopper). The proteins from all three species share similar degrees of sequence conservation (between 39-44% amino acid identity) and are similarly divergent from related vertebrate molecules (MFas II shares 26% identity with mouse NCAM). Unlike the transmembrane forms of fasciclin II described for grasshopper and fly, the cytoplasmic region of transmembrane MFas II does not contain a PEST domain (a motif that may confer instability to proteins

intended for rapid turnover; Rechsteiner, 1988). The existence of a transmembrane isoform of fasciclin II lacking a PEST domain in *Drosophila* has also been mentioned (Lin and Goodman, 1994). Whether a third, $PEST^+$ isoform of MFas II is expressed in *Manduca* remains to be determined. However, we did detect numerous ATTTA motifs in the 3′ untranslated regions of both isoforms of MFas II (eight in the GPI-linked form and ten in the transmembrane form), which have been shown to affect mRNA stability in other systems (Chen and Shyu, 1995). The transient expression of MFas II by the muscle band pathways during the migratory period may therefore be regulated in part by a rapid turnover of MFas II-specific mRNA within these cells.

How does fasciclin II regulate EP cell migration?

The manipulations that we performed in embryo culture to perturb fasciclin II-mediated interactions consistently inhibited EP cell migration: both the application of blocking antibodies against MFas II and removal of GPI-linked MFas II with PI-PLC caused significant reductions in the extent of migration without inducing obvious signs of damage to the EP cells. While both of these experimental approaches must be interpreted with some caution, a number of other antibodies (including anti-neuroglian, which also labels the muscle bands) and other enzymes (including phospholipase B) had no effect on ENS development. In addition, recombinant protein fragments containing immunoglobulin domains III-V of MFas II similarly interfered with migration, reducing the normal apposition of the migratory neurons with their muscle band pathways and causing a noticeable defasciculation of their growth cones. A similar region of NCAM has been implicated in both its homophilic and heterophilic adhesive interactions (Rao et al., 1992; Luthl et al., 1994), suggesting that the MFas II fragments used in this study should interfere with MFas II-mediated events in the developing ENS. Although we have not yet attempted to map the specific epitopes within the Ig-domains that affect EP cell development, these results support our conclusion that fasciclin II plays an essential role in promoting neuronal migration.

Still unresolved is the precise mechanism by which fasciclin II-mediated interactions affect neuronal motility. The simplest interpretation of our results is that the onset of MFas II expression within the newly formed muscle bands establishes a permissive substrate for the EP cells on the midgut, so that MFas II-mediated homophilic interactions between the neurons and the muscle bands lead to their migration along these pathways. This hypothesis is supported by previous work which showed that the muscle bands are both necessary and sufficient for neuronal migration, in that the EP cells will not migrate onto the midgut musculature unless they are in direct contact with one of the muscle bands (Copenhaver et al., 1996). Moreover, neither the application of anti-MFas II antibodies nor recombinant MFas II fragments resulted in a general dissociation of the premigratory packet of EP cells; rather, these treatments appeared to interfere specifically with the formation of homophilic interactions between the neurons and the newly formed muscle bands. However, our manipulations of MFas II in culture also perturbed the coalescence of the muscle bands themselves (Fig. 5D,H), suggesting that fasciclin II may play a role in the differentiation of the bands from the visceral mesoderm. Although we have observed in culture that the EP cells will migrate onto the muscle band cells even when the bands have not completely coalesced (unpublished data), it is possible that our manipulations of MFas II affected migration indirectly by preventing the normal differentiation of their migratory pathways. We are currently testing whether MFas II expressed ectopically or in the absence of other muscle band proteins can support EP cell migration in vitro.

Manipulations of fasciclin II by a variety of means have suggested that it may play a number of different roles within the developing nervous system. For example, application of blocking antibodies to cultured grasshopper embryos caused fasciclin II-expressing growth cones to stall and to elaborate filopodia in inappropriate directions (Harrelson and Goodman, 1988). Removal of fasciclin II from peripheral pioneer neurons using chromophore-assisted laser inactivation also inhibited axonogenesis, but only at a restricted stage of neuronal differentiation (Diamond et al., 1993). The results of these acute treatments were qualitatively similar to the dramatic effects that we observed with respect to neuronal migration, suggesting that fasciclin II plays an important role in the guidance of motile cells and processes.

In contrast, genetic deletions of fasciclin II have produced more subtle alterations in the developing nervous system, primarily due to errors in axonal fasciculation (Lin et al., 1994; Lin and Goodman, 1994). In like manner, we observed a marked increase in the defasciculation of EP cell growth cones following treatment with recombinant MFas II fragments (Fig. 7). It is therefore possible that the true role of fasciclin II in the developing ENS of *Manduca* is simply to maintain the apposition of the EP cells with the muscle bands, while other pathway-specific cues are needed to promote directional migratory behavior (cf. Winberg et al., 1998). As has been shown in other systems (Fishman and Hatten, 1993; Newgreen and Minichiello, 1995), it is likely that a combination of positive and negative guidance cues regulate the timing and directionality of migration in the ENS. However, acute inhibition of fasciclin II in culture (such as with blocking antibodies or enzymatic cleavage) may also reveal fasciclin II-dependent processes that are obscured in a genetic mutation, where other guidance cues with overlapping functions might be able to compensate for the lack of fasciclin II over time. Manipulations of MFas II expression within the EP cells and the muscle band pathways will be needed to determine which aspect of neuronal migration is specifically regulated by this adhesion receptor.

A related question concerns the mechanism by which EP cell migration is first initiated. As noted above (Fig. 3), all of the EP cells express MFas II prior to the onset of migration, at which time the neurons are tightly clustered at the foregut-midgut boundary. Although our data support a role for MFas II in guiding the EP cells along the muscle bands once these pathways have formed, an additional process must stimulate the preferential release of fasciclin II-dependent adhesive interactions within the premigratory cluster in order for migration to occur. By analogy, NCAM is also expressed by a variety of premigratory neurons in the vertebrate nervous system (e.g. Chuong et al., 1987; Rutishauser et al., 1988). While some cells may downregulate NCAM as they become motile (Akitaya and Bronner-Fraser, 1992; Hynes and Lander, 1992), the migration of granule cell precursors into the olfactory bulb coincides with the expression of a polysialated

NCAM (NCAM-180), an isoform of NCAM that has anti-adhesive properties and promotes neuronal plasticity and growth (Hu et al., 1996; Tang and Landmesser, 1993). Although fasciclin II has not been found to be polysialated, several different isoforms have been identified (Grenningloh et al., 1991; Lin and Goodman, 1994; this paper) that may play distinct roles in development. Alternatively, modulation of fasciclin II-fasciclin II interactions by other guidance cues associated with the muscle bands may facilitate the exodus of the EP cells from their original packet (Fambrough and Goodman, 1996). An analysis of isoform-specific expression patterns for MFas II should lend insight into the role that these molecules play with respect to EP cell migration.

As with other examples of neuronal migration, the EP cells remain migratory for only a limited time during development. However, following the migratory period, they continue to extend axonal processes along the same muscle band pathways on the midgut. Intriguingly, this behavioral transition from migration to axon outgrowth coincides with the disappearance of MFas II expression from the muscle bands, although MFas II levels within the axons of the EP cells remain high (Fig. 3C; and unpublished observations). It is possible that the termination of fasciclin II expression in the muscle bands precludes further migration by the neurons, while other guidance cues associated with the muscle bands (such as neuroglian; Fig. 5F) continue to support axonal outgrowth. The persistent expression of MFas II in the EP cell axons may simply serve to promote their fasciculation until they branch laterally to innervate the interband musculature (Wright et al., 1998). Alternatively, fasciclin II may also interact with other adhesion molecules expressed by the muscle bands once migration is complete. Heterophilic interactions of this type have been reported for a number of related adhesion receptors, including NgCAM, F_3/F_{11} and possibly NCAM (Brummendorf and Rathjen, 1993; Horstkorte et al., 1993), but have not yet been investigated with respect to fasciclin II. Similarly, several related molecules (including NCAM and L1) have been shown to associate with non-receptor tyrosine kinases, a relationship that is required for at least some aspects of their adhesive functions (Beggs et al., 1994; Ignelzi et al., 1994). In preliminary studies, we have shown that changes in tyrosine kinase activity accompany the transition of the EP cells from a premigratory to a migratory state (unpublished data), but a functional link between fasciclin II and a particular intracellular signaling pathway remains to be determined.

Besides contributing to the formation of the nervous system, the process of cell migration is essential to the formation of many other embryonic tissues, where similar molecular mechanisms may regulate the motility of markedly different cell types (reviewed in Caterina and Devreotes, 1991; Hynes and Lander, 1992). In this regard, it is noteworthy that MFas II is also expressed by subsets of cells within a number of different ectodermal and mesodermal tissues at specific times during embryogenesis (Carr and Taghert, 1989; Grenningloh et al., 1991; J. W. Wright and P. F. Copenhaver, unpublished observations). Moreover, high levels of MFas II expression have been specifically correlated with periods of cell migration and reorganization in both the embryonic tracheal system (Nardi, 1990) and the developing adult wing (Nardi, 1992). The results that we have obtained concerning the role that fasciclin II plays in controlling neuronal migration may therefore be relevant to more general aspects of morphogenesis and regulated cell movements within the developing embryo.

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