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Motoneuronal development in the embryonic zebrafish

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Summary

To learn how neurons find their appropriate targets, we have studied two populations of motoneurons in the embryonic zebrafish: *primary motoneurons*, individually identified cells whose growth cones pioneer the first nerve pathways in the muscle, and *secondary motoneurons*, cells which develop later and whose growth cones apparently extend along the axons of the primary motoneurons. Transplantation studies of single, identified primary motoneurons suggest that commitment of these cells to innervate their cell-specific muscle territories may be a multistep process in which they are first committed to be motoneurons and are later committed to extend axons along specific pathways. Ablation studies suggest that interactions among the primary motoneurons are unlikely to be necessary for proper pathfinding or commitment. However, interactions with the primary motoneurons may be important for proper development of the secondary motoneurons.

Key words: *Brachydanio rerio*, axonal guidance, cell commitment, pathfinding.

Introduction

Proper nervous system function requires that neurons make synaptic contacts with only the appropriate target cells. How this is accomplished is a central question in developmental neuroscience. At least three steps are likely to be crucial in the process of proper synapse formation. First, neurons must be committed to be a particular type, second they must select a pathway that will lead toward a region of the body containing an appropriate target, and finally, they must be able to form synapses with the correct type and number of cells (reviewed in Westerfield and Eisen, 1988). Many types of neurons develop cell-type specific morphologies in culture even when they are plated before axogenesis (see Banker and Waxman, 1988), suggesting that they may also be committed to select specific pathways or innervate particular targets. This idea is reinforced by studies in which changes in the expression of specific genes can switch the pathway selected by an identified neuron (Doe *et al.* 1988*a*,*b*). *En route* to their targets, navigating growth cones appear to interact with cells that influence the pathways they select (Lance-Jones and Landmesser, 1981; Goodman *et al.* 1982; Westerfield and Eisen, 1988). Early-developing pioneer neurons whose growth cones establish the first axonal pathways have been shown to interact with non-neuronal cells (Singer *et al.* 1979; Ho *et al.* 1983; Bastiani and Goodman, 1986; Kuwada, 1986) or undifferentiated neurons (Bate, 1976; Bentley and Caudy, 1983*a*,*b*) whose absence can prevent or alter the formation of specific axonal pathways (Silver *et al.* 1982; Ball *et al.* 1985; Bastiani and Goodman, 1986; Jellies and Kristan, 1988). The growth cones of later-developing neurons navigate through territories in which numerous axonal tracts may already be present. Apparently, these growth cones select specific axonal tracts on which to navigate; these tracts may be required for accurate pathway navigation (Raper *et al.* 1984; Landmesser and Honig, 1986). Interactions between navigating growth cones and cells in their environments are likely to be mediated by appropriate temporal and spatial expression patterns of specific molecules, for example extracellular matrix (ECM) components (Sanes, 1984; Tomaselli *et al.* 1986), cell-surface glycoproteins (Bastiani *et al.* 1987; Dodd *et al.* 1988), and diffusible factors (Letourneau, 1978; Lumsden and Davies, 1986; Tessier-Lavigne *et al.* 1988).

To learn how neurons become committed to innervate particular targets and to carry out proper pathfinding, my colleagues and I have been studying the development of motoneurons that innervate the axial muscles of the zebrafish. This system is well-suited for studies of neuronal differentiation, since the early embryo has relatively few neurons, many of which can be recognized as unique individuals; in addition, the optical clarity and rapid development of the embryo allow these cells to be observed at all stages *in situ*, in the living embryo. Zebrafish axial muscles are segmentally arranged; each muscle segment is innervated by motoneurons whose somata are located in the ipsilateral spinal hemisegment. Most muscles of adult vertebrates are innervated by a single motor axon at a single endplate (see Jansen and Fladby, 1990 for a review). In contrast, adult zebrafish axial muscles receive polyneuronal, multiterminal innervation (Westerfield *et al.* 1986). Each muscle fiber is innervated by motoneurons from two separate populations, a single, identified *primary motoneuron* that innervates all of the muscle fibers within a specific muscle territory and by from 1 to 3 *secondary motoneurons* that innervate subsets of muscle fibers. The primary motoneurons can be distinguished by their earlier birthdays, larger sizes, smaller numbers, and earlier stage of axonal outgrowth from the later developing secondary motoneurons (Kimmel and Westerfield, 1990). In addition, there are genetic differences between primary and secondary motoneurons (Grunwald *et al.* 1988), and the two types of neurons subserve different functions during swimming behavior (Liu and Westerfield, 1990).

This paper will review some of the recent work describing motoneuronal development in the zebrafish embryo (see also Kimmel *et al.* this volume). Our studies suggest that zebrafish primary motoneurons are committed to become a specific cell type before they are committed to innervate particular targets. Later, but before they extend growth cones, these cells become committed to develop specific axonal trajectories. Thus, each growth cone arrives directly in the muscle region appropriate for its adult function. Commitment is unlikely to involve interactions among the primary motoneurons, but interactions with primary motoneurons may be very important for the proper development of secondary motoneurons.

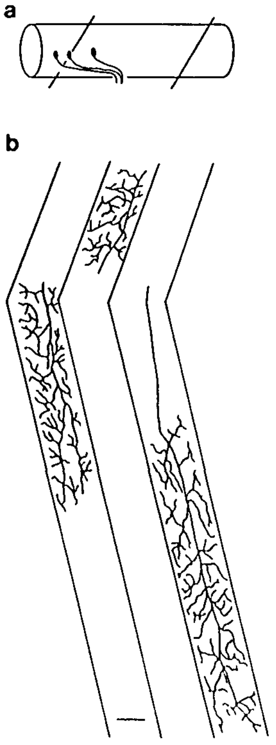

Fig. 1. Individual primary motoneurons can be identified in adult zebrafish by the positions of their somata in the spinal cord and the regions of muscle they innervate. (a) Schematic drawing of a portion of the spinal cord; rostral is to the left and dorsal is to the top in this and all subsequent figures. The diagonal lines represent segmental boundaries. Within each spinal hemisegment, RoP has the most rostral soma position, CaP has the most caudal soma position, and MiP has a soma position between RoP and CaP. (b) Drawing of three muscle segments of an adult zebrafish illustrating the arbors of the primary motoneurons. RoP is to the left, MiP is in the middle, and CaP is to the right. After Westerfield *et al.* 1986. Scale bar represents 225 μm for (a) and 500 μm for (b).

Development of the zebrafish neuromuscular system

Organization of motoneuronal somata and axonal trajectories

Primary motoneurons

In larval and adult zebrafish, every muscle segment is innervated by 3 primary motoneurons, each of which can be individually identified by the position of its soma in the spinal cord and the region of the muscle segment it innervates (Westerfield *et al.* 1986). CaP, the Caudal Primary, has the most caudally located soma in each spinal hemisegment and innervates the ventral region of the muscle segment. RoP, the Rostral Primary, has the most rostrally located soma and innervates the middle region of the muscle segment. MiP, the Middle Primary, has a soma between CaP and RoP and innervates the dorsal region of the muscle segment (Fig. 1).

Secondary motoneurons

In contrast to the primary motoneurons, we currently know much less about the secondary motoneurons. Some secondary motoneurons become postmitotic around the end of the first day of development, and new secondary motoneurons continue to be added at least through the second day of development (Myers *et al.* 1986). The secondary motoneurons with the earliest birthdays appear to develop in a pattern that is similar to the pattern of the primary motoneurons; cells with somata near CaP innervate ventral muscle and cells with somata near MiP innervate dorsal muscle (S. H. Pike, E. Melancon, and J. S. Eisen, unpublished data). The territories innervated by secondary motoneurons in larval and adult fish are smaller than those innervated by the primary motoneurons (Myers, 1985; Westerfield *et al.* 1986).

Pathfinding by motoneuronal growth cones

Primary motoneurons

In addition to having a segmentally repeating, stereotyped organization, axonal outgrowth by the primary motoneurons occurs in a stereotyped sequence in which CaP precedes MiP and MiP precedes RoP (Fig. 2a; Eisen *et al.* 1986; Myers *et al.* 1986). The CaP growth cone pioneers the first nerve pathway into the periphery in each myotome. The CaP growth cone extends directly ventrally from the spinal cord along the medial surface of the myotome, until it reaches the region of the nascent horizontal septum, which will later separate the dorsal and ventral muscle of the myotome, where it pauses for about an hour. After pausing, the CaP growth cone resumes extension along the medial surface of the ventral myotome. The MiP and RoP growth cones initially extend caudally within the spinal cord. When they encounter CaP, they reorient and follow the CaP axon to the nascent horizontal septum. Thus, the pathway between the spinal cord and horizontal septum is a common one, followed by the growth cones of all of the primary motoneurons. The MiP and RoP growth cones also pause in the region of the horizontal septum. However, after pausing, they take different pathways; MiP sprouts a collateral growth cone that extends along the medial surface of the dorsal myotome and withdraws its ventral growth cone, and RoP extends its growth cone laterally,

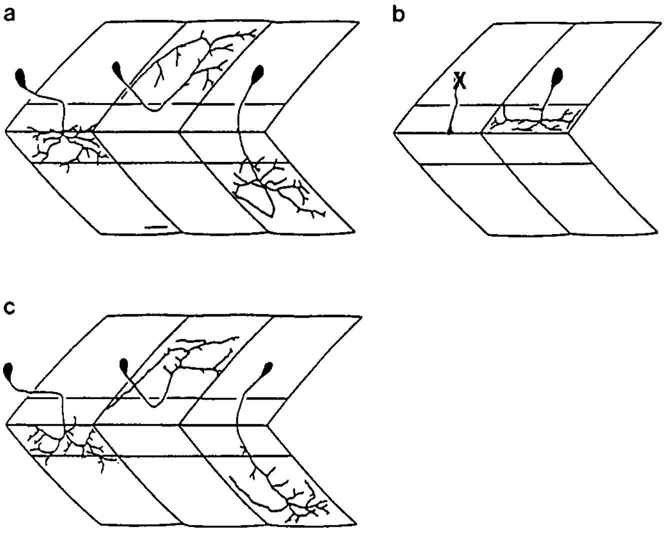

Fig. 2. Pathfinding is normal in the absence of interactions among primary motoneurons. (a) Drawing of three segments from three different embryos at the end of the third day of development illustrating the soma positions and arbors of each of the primary motoneurons. RoP is shown on the left, MiP is shown in the middle, and CaP is shown on the right. The three horizontal lines within the segments represent the ventral edge of the spinal cord (top), the horizontal septum (middle), and the ventral edge of the notochord (bottom). After Pike and Eisen (1990). (b) Drawing of two segments from two different embryos showing two VaPs. The VaP on the left died, represented by the X on the soma, during the second day of development; about 85 % of VaPs typically die at this time (see Eisen *et al.* 1990). The VaP on the right is at the fourth day of development; this surviving cell arborized in the region between the MiP and RoP arbors. (c) A drawing of three segments from three different embryos at the end of the third day of development. In each of the segments that are illustrated, one or more primary motoneurons were ablated by laser-irradiation prior to axogenesis. In the segment on the left, CaP, VaP, and MiP were ablated; the remaining RoP underwent normal pathfinding. In the segment in the middle, CaP was ablated; the remaining MiP underwent normal pathfinding. In the segment on the right, MiP and RoP were ablated; the remaining CaP underwent normal pathfinding. After Pike and Eisen (1990). Scale bar represents 20 μm.

through the horizontal septum. Thus, the region of the horizontal septum defines a 'choice point', from which the axons of the 3 primary motoneurons diverge along individual, cell-specific pathways.

In addition to CaP, MiP and RoP, about half the spinal hemisegments in the trunk of embryonic zebrafish contain a fourth primary motoneuron, named VaP, for Variable Primary, because it is variably present (Fig. 2b; Eisen *et al.* 1990). In spinal hemisegments in which VaP is present, its soma is adjacent to the CaP soma, and early in development the 2 cells cannot be distinguished. However, the VaP growth cone extends only to the horizontal septum choice point. VaPs typically die without extending their growth cones further or forming any axonal branches. A few VaPs survive longer and innervate muscle fibers between the regions innervated by MiP and RoP. Although these cells persist into larval development, they have not been followed in subsequent stages, so it is not known whether they are present in adults.

The precise temporal order of outgrowth by the primary motoneurons raises the possibility that the sequence in which they grow might determine the muscle regions they innervate. Thus, the first primary motoneuron to extend a growth cone would select the ventral pathway and innervate the ventral muscle territory, the second would select the dorsal pathway and innervate the dorsal muscle territory, and the third would select the lateral pathway and innervate the middle muscle territory. This hypothesis makes a specific prediction: the first primary motoneuron to extend an axon will select the CaP pathway, the second will select the MiP pathway, and the third will select the RoP pathway. Thus, in the absence of CaP, the MiP growth cone should extend into CaP territory, rather than into MiP territory, and in the absence of both CaP and MiP, the RoP growth cone should extend into CaP territory.

To test this idea, we ablated primary motoneurons by focusing a laser microbeam onto their somata prior to axogenesis (Eisen *et al.* 1989; Pike and Eisen, 1990; Eisen *et al.* 1990). We found that, even when we ablated all but one primary motoneuron in a spinal hemisegment, the remaining cell always extended its axon along the cell-specific pathway it would have chosen if the other primary motoneurons were still present (Fig. 2c). Thus, each primary motoneuron appears to have an independent ability to pioneer both the common part of the pathway and its cell-specific pathway, suggesting that interactions among the primary motoneurons based on their sequence of outgrowth are unlikely to be important in pathway choice. These observations raise the possibility that pathway choice is governed by other factors.

One alternative possibility is that even before axogenesis, the primary motoneurons may be committed to extend growth cones along specific pathways or to innervate particular muscle regions. Commitment might involve several different aspects of neuronal differentiation, for example, expression of a set of receptors that recognizes particular extrinsic cues that would provide guidance information to the navigating growth cones, or activation of an intrinsic program that causes the cell to develop a specific morphology, as has been described for regenerating neurons of the leech (Acklin and Nicholls, 1990). The common test for commitment is to transplant a cell to a new location and observe whether it behaves in a manner appropriate for that *new* location, in which case it was uncommitted when it was moved, or in a manner appropriate for its *original* location, in which case it was committed when it was moved.

To learn whether primary motoneurons are committed to extend axons along specific pathways before axogenesis, we transplanted primary motoneurons to new spinal cord locations just prior to the time at which

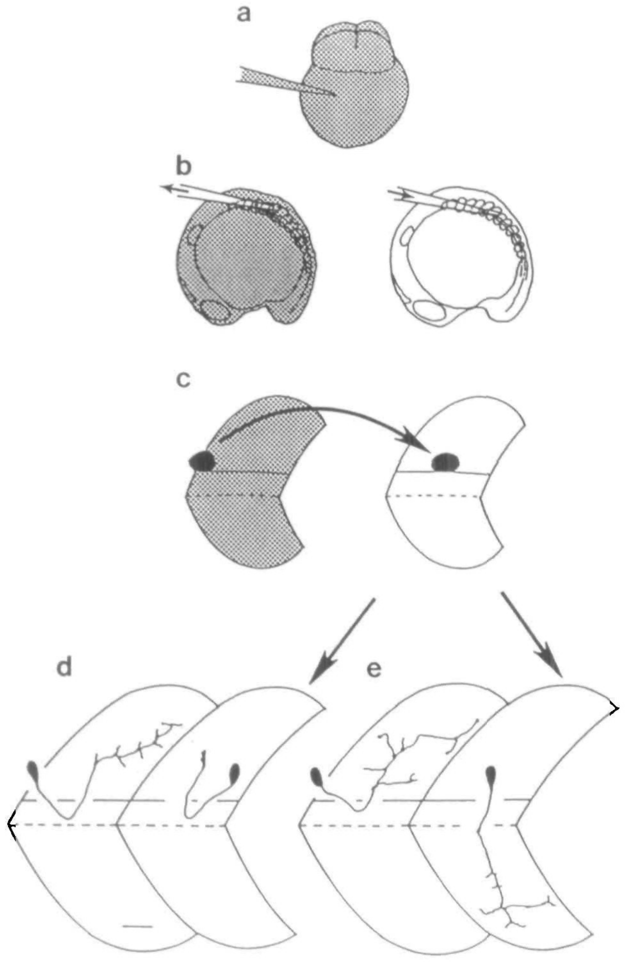

Fig. 3. Primary motoneurons are committed to develop specific axonal trajectories before axogenesis. (a) Donor embryos were labeled with rhodamine-dextran at the 1–16 cell stage by injection into the yolk (Eisen, 1991); the dye dispersed into all of the cells *via* cytoplasmic bridges. (b) Labeled donor (left) and unlabeled host (right) embryos were allowed to develop until primary motoneurons could be recognized. One or two identified primary motoneurons were removed from the donor by aspiration with a micropipette, the micropipette removed from the donor, transferred to the host, and the motoneurons expelled with gentle pressure. (c) An example of a cell transplanted from the MiP position of a labeled donor (left) to the CaP position of an unlabeled host (right). The solid horizontal line represents the ventral edge of the spinal cord and the broken horizontal line represents the horizontal septum. (d) There are two outcomes of transplanting a cell from the MiP position to the CaP position shortly before axogenesis (about 1 h). As shown on the left, some cells do not remain where they are placed. This cell moved back to the MiP position and developed a normal MiP morphology, thus it was not possible to evaluate whether it was committed. As shown on the right, some cells remain where they are placed. This cell developed a MiP axonal trajectory, although its soma was not in the MiP position. By the definition in the text, this cell was committed before it was transplanted. (e) There are two outcomes of transplanting a cell from the MiP position to the CaP position earlier (2–3 h before axogenesis). As shown on the left, some cells do not remain where they are placed. This cell moved back to the MiP position and developed a normal MiP morphology; again, it was not possible to evaluate whether this cell was committed. As shown on the right, some cells remain where they are placed. This cell developed a CaP axonal trajectory; by the definition in the text, this cell was uncommitted at the time it was transplanted. Transplanted cells were redrawn from the face of the video monitor. Scale bar represents 300 μm in (a,b) and 14 μm in (c–e).

they would elaborate growth cones (Eisen, 1991). A cell was considered to be committed if its axonal trajectory was appropriate for the cell's *original* position, and independent of the cell's new position; a cell was considered to be uncommitted if its axonal trajectory was appropriate for the cell's *new* position. We found that all of the transplanted cells developed axonal trajectories that were appropriate for their *original* soma positions (Fig. 3). By the definition of commitment set forth above, these cells could be considered to be committed. However, these cells behaved in two different ways, only one of which was compatible with the definition of commitment. Many of the cells in this experiment did something quite surprising. After they were transplanted, their somata moved from the new positions in which they were placed back to their original positions. Since the somata returned to their original positions, the axonal trajectories of these cells were appropriate for both their *new* (final) soma positions and their *original* soma positions. Thus, these axonal trajectories were not independent of soma position and we could not evaluate whether the cells were committed. The second result was that some of the transplanted cells remained in their new positions. These cells developed novel morphologies in which their axonal trajectories were inappropriate for their new soma positions but were appropriate for their original soma positions. Since the axonal trajectories of these cells were independent of their soma positions, by our definition these cells were committed before they were transplanted.

What features might be important in commitment? To begin to address this question, we transplanted primary motoneurons to new locations at earlier developmental stages (Fig. 3; Eisen, 1991). Again, many of these cells failed to remain in their new positions and returned to their original positions, making it difficult to evaluate whether they were committed. However, the ones that remained where they were placed developed axonal trajectories appropriate for their *new* soma positions, suggesting that these cells were uncommitted at the time they were transplanted. These results suggest that the soma position somehow influences the cell's choice of axonal trajectory. One possible mechanism is that primary motoneurons in particular spinal cord locations may express receptors that recognize cues that are localized on different cell-specific pathways. Preliminary results support this idea, since motoneurons transplanted from the CaP position in the spinal cord to the horizontal septum choice point characteristically extend axons along the normal CaP pathway, but not on the MiP or RoP pathways (Gatchalian and Eisen, 1990).

Secondary motoneurons

The development of at least some secondary motoneurons appears to recapitulate the development of the primary motoneurons. Secondary motoneurons whose somata are in the vicinity of the CaP soma are probably the first to extend axons out of the spinal cord. The growth cones of these cells appear to fasciculate with the axons of the primary motoneurons, and to follow them to the horizontal septum choice point. These growth cones then continue ventrally, following the CaP axon, and innervate muscle fibers in the ventral region of the myotome, although we do not yet know their final destinations. Secondary motoneurons with somata in the vicinity of the MiP soma also extend growth cones to the horizontal septum choice point. Later, these cells sprout dorsal collaterals that extend along the MiP axon into dorsal muscle. The spatiotemporal relationship between the axons of the primary motoneurons and the growth cones of the secondary motoneurons suggests that the growth cones of the secondary motoneurons might require the axons of the primary motoneurons for proper pathfinding.

To test this idea, we ablated CaP and followed the development of secondary motoneurons whose growth cones would normally follow the CaP axon (Pike *et al.* 1989). We found that their growth cones typically were delayed in the region of the horizontal septum choice point or slightly ventral to it, and they often formed a profusion of ectopic branches in this area. Although these results suggest that the CaP axon may be required for proper extension by the secondary motoneuron growth cones, we found that later at least some of the growth cones resumed extension and in many cases a normal looking ventral nerve was eventually formed. Thus, although the CaP axon appears to be important in facilitating outgrowth by the secondary motoneuron growth cones, it does not appear to be necessary for proper pathfinding. These results show that at least some of the secondary motoneuron growth cones can pioneer the nerve pathways, and they raise the possibility that these growth cones may be able to use the same cues normally followed by the CaP growth cone.

Conclusions

Our results suggest that normal development by motoneurons in the embryonic zebrafish involves a number of different steps. All of the surviving transplanted primary motoneurons developed motoneuronal morphologies and not, for example, interneuronal morphologies, although there are primary interneurons with similar soma positions (see Kuwada *et al.* 1990). Thus, these cells appear to be committed to become primary motoneurons before they are committed to innervate specific muscle territories. Later, each cell becomes committed to develop a specific axonal trajectory; this commitment appears to depend on the location of the cell in the spinal cord. These results suggest that there may be positional cues that somehow direct primary motoneurons to extend their growth cones along specific pathways. Thus, all of the primary motoneurons may initially be equivalent, or at least have an equivalent ability to respond to localized positional cues. The time course of acquisition of primary motoneuronal identity appears similar to that of some neurons in the grasshopper (Kuwada and Goodman, 1985). Both sets of cells appear to acquire their individual identities after they become postmitotic, but before axogenesis. Because the somata of at least some of the secondary motoneurons appear to have an arrangement that is similar to that of the primary motoneurons, they may be influenced by the same sorts of positional cues. This hypothesis could be tested by transplantation experiments, similar to those performed with the primary motoneurons.

Currently, we have little information about the nature of the putative positional cues that may be influencing the differentiation of the primary motoneurons. Since pathfinding is normal after ablation of neighboring primary motoneurons, it seems likely that the primary motoneurons themselves are not the sole providers of positional information. In addition, primary motoneurons appear to develop normally in the absence of neural activity (Westerfield *et al.* 1990; Liu and Westerfield, 1990), suggesting that acquisition and maintenance of identity is independent of retrograde signals transported from the muscle target to the soma *via* the growth cone. Analysis of the *spt-1(b104)* mutation (Eisen and Pike, 1991; see Kimmel *et al.* this volume) suggests that segmented mesoderm may be important in patterning the primary motoneurons, although its precise role remains unclear.

The pathway traversed by the growth cones of the developing motoneurons has three distinct regions, where the growth cones exhibit different types of behaviors. The proximal portion of the pathway is followed by the growth cones of all primary and secondary motoneurons, thus, the cues along this pathway region may be of a 'general' nature (Lewis *et al.* 1983). The second region of the pathway is the horizontal septum choice point. The growth cones of the primary motoneurons typically pause when they reach this location. It is not clear whether the growth cones of secondary motoneurons also pause at this position during normal development, however, they are delayed in this region for some time when the primary motoneurons are ablated. Thus, the choice point region seems to be a location where the growth cones wait for some event to occur. This event might be expression of a particular pathway cue, or expression by the motoneuron of a particular receptor. Pausing of the motoneuronal growth cones at the choice point is reminiscent of the extension of growth cones in the mammalian CNS (Dodd and Jessell, 1988) and grasshopper CNS and limbs (Raper *et al.* 1983; Caudy and Bentley, 1986*b*) where *en route* to their final destinations, growth cones appear to be guided by a series of intermediate targets. In the case of the mammalian CNS, it may be that contact with the intermediate target influences the expression of surface molecules by the navigating growth cone (Dodd *et al.* 1988). The third region of the pathway is the cell-specific portion. We postulate that each cell-specific pathway may be demarcated by a different set of cues. Preliminary experiments in which we have transplanted CaP to the choice point region as a bioassay to look for differences between the pathways suggest that this view may be correct (Gatchalian and Eisen, 1990). Thus, the final basis of cell-specific pathway selection may be the ability of individual primary motoneurons to discriminate between pathways that themselves express different sets of cues.

We still know little about the specifics of pathfinding by the secondary motoneurons. Our ablation studies suggest that the primary motoneurons are important, although not essential, for proper pathfinding by the secondary motoneurons. These observations raise the possiblity that the growth cones of the secondary motoneurons may be able to recognize and follow at least two sets of cues, one on the axons of the primary motoneurons, and one on the substratum; the latter set may be the same as that followed by the growth cones of the primary motoneurons. In the future it will be interesting to learn whether there are two different sets of cues, and how they might interact during normal pathfinding by the secondary motoneurons.

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References

Acklin, S. E. and Nicholls, J. G. (1990). Intrinsic and extrinsic factors influencing properties and growth patterns of identified leech neurons in culture. *J. Neurosci.* **10**, 1082–1090.

Ball, E. E., Ho, R. K. and Goodman, C. S. (1985). Development of neuromuscular specificity in the grasshopper embryo: Guidance of motoneuron growth cones by muscle pioneers. *J. Neurosci.* **5**, 1808–1819.

Banker, G. A. and Waxman, A. B. (1988). Hippocampal neurons generate natural shapes in cell culture. In *Intrinsic Determinants of Neuronal Form and Function* (eds. R. J. Lasek and M. M. Black). pp. 61–82. New York: Alan R. Liss, Inc.

Bastiani, M. J. and Goodman, C. S. (1986). Guidance of neuronal growth cones in the grasshopper embryo. III. Recognition of specific glial pathways. *J. Neurosci.* **6**, 3542–3551.

Bastiani, M. J., Harrelson, A. L., Snow, P. M. and Goodman, C. S. (1987). Expression of fasciclin I and II glycoproteins on subsets of axon pathways during neuronal development in the grasshopper. *Cell* **48**, 745–755.

Bate, C. M. (1976). Pioneer neurons in an insect embryo. *Nature* **260**, 54–56.

Bentley, D. and Caudy, M. (1983*a*). Pioneer axons lose directed growth after selective killing of guidepost cells. *Nature* **304**, 62–65.

Bentley, D. and Caudy, M. (1983*b*). Navigational substrates for peripheral pioneer growth cones: limb-axis polarity cues, limb-segment boundaries, and guidepost neurons. *Cold Springs Harb. Symp. quant. Biol.* **48**, 573–585.

Caudy, M. and Bentley, D. (1986). Pioneer growth cone steering along a series of neuronal and non-neuronal cues of different affinities. *J. Neurosci.* **6**, 1781–1795.

Dodd, J. and Jessell, T. M. (1988). Axon guidance and the patterning of neuronal projections in vertebrates. *Science* **242**, 692–699.

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

Doe, C. Q., Hiromi, Y., Gehring, W. J. and Goodman, C. S. (1988*a*). Expression and function of the segmentation gene *fushi tarazu* during *Drosophila* neurogenesis. *Science* **239**, 170–175.

Doe, C. Q., Smouse, D. and Goodman, C. S. (1988*b*). Control of neuronal fate by the *Drosophila* segmentation gene *even-skipped*. *Nature* **333**, 376–378.

Eisen, J. S. (1991). Determination of primary motoneuron identity in developing zebrafish embryos. *Science* **252**, 569–572.

Eisen, J. S., Myers, P. Z. and Westerfield, M. (1986). Pathway selection by growth cones of identified motoneurons in live zebrafish embryos. *Nature* **320**, 269–271.

Eisen, J. S. and Pike, S. H. (1991). The *spt-1* mutation alters the segmental arrangement and axonal development of identified neurons in the spinal cord of the embryonic zebrafish. *Neuron* **6**, 767–776.

Eisen, J. S., Pike, S. H. and Debu, B. (1989). The growth cones of identified motoneurons in embryonic zebrafish select appropriate pathways in the absence of specific cellular interactions. *Neuron* **2**, 1097–1104.

Eisen, J. S., Pike, S. H. and Romancier, B. (1990). An identified motoneuron with variable fates in embryonic zebrafish. *J. Neurosci.* **10**, 34–43.

Gatchalian, C. L. and Eisen, J. S. (1990). Growth cones of ectopic motoneurons select normal pathways in embryonic zebrafish. *Soc. Neurosci. Abstr.* **16**, 624.

Goodman, C. S., Raper, J. A., Ho, R. K. and Chang, S. (1982). Pathfinding by neuronal growth cones in grasshopper embryos. In *Developmental Order: Its Origin and Regulation* (ed. S. Subtelny and P. B. Green), pp. 275–316, New York: Alan R. Liss, Inc.

Grunwald, D. J., Kimmel, C. B., Westerfield, M., Walker, C. and Streisinger, G. (1988). A neural degeneration mutant that spares primary neurons in the zebrafish. *Devl Biol.* **126**, 115–128.

Ho, R. K., Ball, E. E. and Goodman, C. S. (1983). Muscle pioneers: large mesodermal cells that erect a scaffold for developing muscles and motoneurons in grasshopper embryos. *Nature* **301**, 66–69.

Jansen, J. K. S. and Fladby, T. (1990). The perinatal reorganization of the innervation of skeletal muscle in mammals. *Prog. Neurobiol.* **34**, 39–90.

Jellies, J. and Kristan, Jr, W. B. (1988). Embryonic assembly of a complex muscle is directed by a single identified cell in the medicinal leech. *J. Neurosci.* **8**, 3317–3326.

Kimmel, C. B. and Westerfield, M. (1990). Primary neurons of the zebrafish. In *Signal and Sense* (eds. W. E. Gall and M. W. Cowan), pp. 561–588, New York: Wiley-Liss.

Kuwada, J. Y. (1986). Cell recognition by neuronal growth cones in a simple vertebrate embryo. *Science* **233**, 740–746.

Kuwada, J. Y., Bernhardt, R. R. and Chitnis, A. B. (1990). Pathfinding by identified growth cones in the spinal cord of zebrafish embryos. *J. Neurosci.* **10**, 1299–1308.

Kuwada, J. Y. and Goodman, C. S. (1985). Neuronal determination during embryonic development of the grasshopper nervous system. *Devl Biol.* **110**, 114–126.

Lance-Jones, C. and Landmesser, L. (1981). Pathway selection by chick lumbosacral motoneurons during normal development. *Proc. R. Soc. London* B **214**, 1–18.

Landmesser, L. and Honig, M. G. (1986). Altered sensory projections in the chick hind limb following the early removal of motoneurons. *Devl Biol.* **118**, 511–531.

LETOURNEAU, P. C. (1978). Chemotactic response of nerve fiber elongation to nerve growth factor. *Devl Biol.* **66**, 183–196.

LEWIS, J., AL-GHAITH, L., SWANSON, G. AND KHAN, A. (1983). The control of axon outgrowth in the developing chick wing. In *Limb Development and Regeneration* (ed. J. F. Fallon and A. I. Caplan), pp. 195–205, New York: Alan R Liss, Inc.

LIU, D. W. AND WESTERFIELD, M. (1990). The formation of terminal fields in the absence of competitive interactions among primary motoneurons in the zebrafish. *J. Neurosci.* **10**, 3947–3959.

LUMSDEN, A. G. S. AND DAVIES, A. M. (1986). Chemotropic effect of specific target epithelium in the developing mammalian nervous system. *Nature* **323**, 538–539.

MYERS, P. Z. (1985). Spinal motoneurons of the larval zebrafish. *J. comp. Neurol.* **236**, 555–561.

MYERS, P. Z., EISEN, J. S. AND WESTERFIELD, M. (1986). Development and axonal outgrowth of identified motoneurons in the zebrafish. *J. Neurosci.* **6**, 2278–2289.

PIKE, S. H., BRANDENBURG, E. F. AND EISEN, J. S. (1989). Extension of motoneuronal growth cones is disrupted by the absence of pre-existing axonal pathways. *Soc. Neurosci. Abstr.* **15**, 1262.

PIKE, S. H. AND EISEN, J. S. (1990). Interactions between identified motoneurons in embryonic zebrafish are not required for normal motoneuron development. *J. Neurosci.* **10**, 44–49.

RAPER, J. A., BASTIANI, M. J. AND GOODMAN, C. S. (1983). Pathfinding by neuronal growth cones in grasshopper embryos. I. Divergent choices made by the growth cones of sibling neurons. *J. Neurosci.* **3**, 20–30.

RAPER, J. A., BASTIANI, M. J. AND GOODMAN, C. S. (1984). Pathfinding by neuronal growth cones in grasshopper embryos. IV. The effects of ablating the A and P axons upon the behavior of the G growth cone. *J. Neurosci.* **4**, 2329–2345.

SANES, J. (1984). Roles of extracellular matrix in neural development. *A. Rev. Physiol.* **45**, 581–600.

SILVER, J., LORENZ, S. E., WAHLSTEN, D. AND COUGHLIN, J. (1982). Axonal guidance during development of the great cerebral commissures: descriptive and experimental studies, in vivo, on the role of preformed glial pathways. *J. comp. Neurol.* **210**, 10–29.

SINGER, M., NORDLANDER, R. H. AND EGAR, M. (1979). Axonal guidance during embryogenesis and regeneration in the spinal cord of the newt: the blueprint hypothesis of neuronal pathway patterning. *J. comp. Neurol.* **185**, 1–22.

TESSIER-LAVIGNE, M., PLACZEK, M., LUMSDEN, A. G. S., DODD, J. AND JESSELL, T. M. (1988). Chemotropic guidance of developing axons in the mammalian central nervous system. *Nature* **336**, 775–778.

TOMASELLI, K. J., REICHARDT, L. F. AND BIXBY, J. L. (1986). Distinct molecular interactions mediate neuronal process outgrowth on non-neuronal cell surfaces and extracellular matrices. *J. Cell Biol.* **103**, 2659–2672.

WESTERFIELD, M. AND EISEN, J. S. (1988). Common mechanisms of growth cone guidance during axonal pathfinding. In *From Message to Mind: Directions in Developmental Neurobiology* (eds. S. S. Easter, K. F. Barald, and B. M. Carlson), pp. 110–120, Sunderland MA: Sinauer Assoc.

WESTERFIELD, M., LIU, D. W., KIMMEL, C. B. AND WALKER, C. (1990). Pathfinding and synapse formation in a zebrafish mutant lacking functional acetylcholine receptors. *Neuron* **4**, 867–874.

WESTERFIELD, M., MCMURRAY, J. AND EISEN, J. S. (1986). Identified motoneurons and their innervation of axial muscles in the zebrafish. *J. Neurosci.* **6**, 2267–2277.