Commentary & View

No Sidesteps on a beaten track

Motor axons follow a labeled substrate pathway

Hermann Aberle

Westfälische Wilhelms-Universität Münster; Institut für Neurobiologie; Münster, Germany

Key words: Drosophila, motor axon guidance, adhesion, sidestep, beaten path, labeled substrate pathway

The establishment of synaptic connections between motor neurons and muscle fibers is essential for controlled body movements in any higher organism. The wiring of the neuromuscular system in Drosophila serves as a model system for the identification of key regulatory proteins that control axon guidance and target recognition. Sidestep (Side) is a transmembrane protein of the immunoglobulin superfamily and plays a pivotal role in the coordination of motor axonal guidance decisions, as it functions as a target-derived attractant. Side, however, is expressed in a highly dynamic pattern during embryogenesis, making it difficult to deduce its precise function. We have recently shown that the expression of Side strongly correlates with the actual position of motor axonal growth cones. Motor axons seem to recognize and follow Side-positive surfaces until they reach their target fields.

The motor neuronal protein Beaten path Ia (Beat) is required to detect Side. In beat mutant embryos, motor axons are no longer attracted to Side-expressing tissues. In addition, Beat and Side interact biochemically, forming heterophilic adhesion complexes in vitro. Here, I discuss the model that preferential adhesion of Beat-expressing growth cones to Side-labeled substrates could be a powerful mechanism to guide motor axons.

During the development of Drosophila embryos, motor axons navigate along stereotypic routes to reach their target regions. Five major nerve branches innervate spatially restricted groups of muscles. For example, the intersegmental nerve (ISN) innervates a dorsal muscle group, whereas the segmental nerve (SNa) innervates a lateral muscle group. Forward genetic screens have identified a great variety of cell surface regulators of axonal pathfinding, including the netrins, semaphorins, receptor tyrosine phosphatases, and several members of the immunoglobulin superfamily (IgSF). However, it is still unclear how these molecules cooperate, both spatially and temporally, to steer growth cones from the central nervous system (CNS) to the peripheral muscle groups. The function of these different proteins might be best summarized by the “relative balance model,” according to which growth cones assess guidance information based on the combinatorial and simultaneous input of several attractive and repulsive cues. The fact that the majority of muscles is still innervated in mutants of all these guidance molecules further argues that several guidance systems contribute to the precise wiring of body wall muscles.

Most of our knowledge about the highly dynamic pathfinding process in Drosophila is derived from fixed and immunohistochemically stained embryos. Only recently, a GFP exon trap insertion into fasciclin II, a conserved gene encoding a motor axonal marker protein, made it possible to label motor axons during the period of axonal outgrowth. Time-lapse movies recorded through the translucent epidermis of living embryos show that the ISN forms a coherent axon bundle that migrates continuously through the lateral body wall. Unexpectedly, the growth cone of the ISN sprouts relatively few filopodia and does not seem to intensively search for guidance cues, implying that it might actually migrate along a preformed pathway. Of the known axon guidance regulators, Sidestep (Side) represents a promising candidate molecule that could be a determinant of such a pathway, as it has been shown to function as a target-derived attractant for motor axons. Side is expressed in a complex spatiotemporal pattern during embryonic development, but its expression in sensory neurons and muscles coincides with the arrival of motor axons at these intermediate and final targets, respectively. Since Side is a transmembrane protein, it most likely functions as a contact attractant, suggesting that its expression pattern provides pathway information. Simultaneous visualization of both motor axons and Side-expressing cells reveals an intriguing spatiotemporal relationship between these cell types. The growth cones are strongly associated with Side-expressing cell surfaces at any developmental stage. During the period of axonal outgrowth, motor axons migrate along Side-expressing cells towards the exit junction of the CNS. In the periphery, motor axons follow Side-positive, afferent sensory axons. Importantly, Side expression in the CNS is turned off once motor axons reach the sensory tracks. Based on antibody stainings the expression of Side appears to be generally downregulated after contact with motor axons, a regulatory process I refer to here as “postcontact...
elimination.” Consequently, when motor axons reach the end of the sensory pathways, Side is no longer detectable in sensory axons. It is then upregulated in muscle fibers. Side-positive muscle fibers likely attract the growth cones and cause them to leave the sensory tracks. In this respect, it is interesting to note that motor axons have been found to end up in clusters of sensory neurons when deprived of their target muscles. These findings provided the first hint that motor axons might use Side-expressing cell surfaces as migratory tracks. The fact that the tight association of motor axons with sensory axons was partially lost in side mutants supported these findings. Conversely, when Side is ectopically expressed on all tracheal branches of side mutant embryos—the only source of Side in these embryos—most, if not all, motor axons divert from their destined routes and project along tracheae. This occurs regardless of the presence of other endogenous guidance cues, supporting the idea that Side is an instructive cue that determines the growth direction of motor axons.

How do motor axons recognize Side? Aberrations in the motor neuronal protein Beat (Beat) cause an almost identical embryonic axon guidance phenotype as mutations in side. In addition, these phenotypic similarities persist until late larval stages. While alterations in the embryonic projection pattern can be transient, persistent guidance errors lead to permanent innervation defects in larvae. The neuromuscular innervation pattern of third instar larvae therefore preserves embryonic guidance errors at a high spatial resolution, as misprojecting nerves either fail to innervate their target muscles or innervate them at aberrant positions. Similar embryonic guidance defects lead therefore to comparable larval innervation errors. Most importantly, the frequency of missing or mislocalized neuromuscular junctions is not increased in beat side double mutants, indicating that both genes function in a common process. In addition, in the absence of Beat, motor axons are no longer attracted to Side expressing substrates, suggesting that Beat functions as a receptor or part of a receptor complex that is required to detect Side. This conclusion differs from the initial interpretation of the function of Beat. Beat was originally described as a secreted protein that regulates defasciculation at motor axon choice points by weakening homotypic axon-axon interactions. However, biochemical and genetic evidence support a cell-autonomous function of Beat. One possibility is that Beat is sequestered on extracellular surfaces of motor axons by a so far unknown co-receptor or by another member of the extensive Beat family. Interestingly, in transfected cells, protein complexes containing Beat and Side can be co-immunoprecipitated, suggesting that they interact either directly or indirectly. In addition, mixtures of Beat- and Side-expressing S2 cells form large, heterophilic cell aggregates, substantiating the idea that adhesive Beat-Side interactions might induce the formation of these cell clusters.

The implication of these results for the wiring of the neuromuscular system is that the recognition of Side-expressing cells by Beat-expressing growth cones induces the formation of transient adhesion complexes, comparable somehow to leucocytes rolling on endothelia. Assuming that the interactions between Beat and Side have a higher affinity than the homotypic axon-axon interactions within motor nerves, the affinity differences could favor attraction to the substrate. In a working model, I suggest that the principle of preferential adhesion to a contact attractant could be used more widely to regulate qualitatively different guidance decisions (Fig. 1). Searching for and detection of the attractive label (Side) could be achieved by randomly sprouting receptor-expressing (Beat) filopodia at the tip of the growth cone (Fig. 1A). Filopodia that adhere to the substrate are probably maintained for a certain time, whereas non-adhering filopodia are withdrawn. The transient adhesion likely attracts further filopodia, leading to growth cone turning and pursuit of the labeled path. In the absence of the label, e.g., when the path is interrupted or less well defined, the growth cone presumably sends out more and longer filopodia to scan
the environment for meaningful guidance information (Fig. 1B, stage B). According to the model, the attractive label is removed or inactivated shortly after the interaction with the growth cone, which eventually erases the path. Such a "postcontact elimination" mechanism would ensure that the highest levels of the attractant are located ahead of the growth cone, creating polarity cues that promote unidirectional growth.

The migration of a follower axon along an established fascicle might be interrupted when its growth cone detects high affinity binding sites on a substrate nearby (Fig. 1B, stages C and D). Under this scenario, the growth cone preferentially adheres to the attractive substrate label, which might trigger defasciculation. The increased affinity to the substrate likely overturns the homotypic axon-axon interactions within the motor nerve. Thus, local attraction to a nearby substrate could cause an anti-adhesive effect between less tightly adhering motor axons, a paradigm that is highly reminiscent to the originally proposed, defasciculation-promoting function of Beat at choice points. The ability to discriminate between different adhesion affinities by the selective recognition of an attractive label could be sufficient for growth cones to distinguish between permissive and restricted pathways. The relatively simple mechanism of preferential adhesion to a prelocalized attractant seems therefore to be able to regulate diverse guidance decisions such as migration along a path, growth cone turning, and defasciculation. Which of these pathfinding routines is executed depends on the position of the growth cone within the nerve bundle and the local expression of the attractant at a given developmental stage. In other words, the behavior of motor axons might be somehow comparable to a tracker dog following a scent trail. Such a tracking model might not be restricted to the neuromuscular system in Drosophila but might also apply to the formation of neural circuits in higher organisms. In vertebrates, thalamocortical fibers have been shown to depend on the spatiotemporal arrangement of guidance cues for their navigation from the thalamus to their cortical target regions.

Future experiments will help to clarify whether the tracking model suggested above is applicable to a wider range of pathfinding processes. With regard to the development of motor nerves in Drosophila, an important outstanding question concerns the motor axon-triggered elimination of Side. While it seems more likely that Side is downregulated by a posttranslational mechanism the dynamic regulation of its transcription could contribute to the short half-life on the cell surface. To understand the spatiotemporal expression pattern of Side is hence crucial, as it seems to provide a scaffold upon which the axonal projections develop. How can diverse cell types express Side in such a precisely choreographed pattern? Another open question relates to the finding that Beat is required to detect Side. This provokes experiments that examine whether Beat and Side interact directly or function in larger complexes. Which signaling cascades are activated upon binding? And how is the signal transduced into the growth cone to affect guidance decisions? It is tempting to speculate that Beat is somehow linked to the underlying cytoskeleton. Adhesive interactions could then be converted into mechanical forces, which could pull or turn growth cones.

In summary, Beat and Side function in an axon guidance mechanism that guides Drosophila motor axons from the ventral nerve cord to their peripheral target fields. It is likely that other guidance molecules contribute to the pathfinding process, for example by playing regulatory roles or functioning in selective target recognition events. Based on the severity of the mutant phenotypes, however, the tracking of a labeled substrate pathway seems to be one of the major mechanisms in Drosophila. Since Beat and Side are members of two related protein families that are highly conserved in other insects, similar functional principles may apply for the wiring of other circuits in the fly and in related species.

Acknowledgements

I am grateful to Christian Klambt, Marion Silies, Ann-Christin Bauke and Sven Bogdan for helpful comments on the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (DFG Ab116/4-1).

References

1. Mitchell KJ, Doyle JL, Serafini T, Kennedy TE, Tessier-Lavigne M, Goodman CS, Dickson BJ. Genetic analysis of Ntrin genes in Drosophila: Ntrins guide CNS commissural axons and peripheral motor axons. Neuron 1996; 17:203-15.
2. Kolodkin AL, Marthes DJ, Goodman CS. The semaphorin genes encode a family of transmembrane and secreted growth cone guidance molecules. Cell 1993; 75:1389-99.
3. Krueger NX, Van Vactor D, Wan HJ, Gelbart WM, Goodman CS, Saito H. The transmembrane tyrosine phosphatase Df(Sc) controls motor axon guidance in Drosophila. Cell 1996; 84:611-22.
4. Desai CJ, Gindhart JG Jr, Goldstein LS, Zinn K. Receptor tyrosine phosphatases are required for motor axon guidance in the Drosophila embryo. Cell 1996; 84:599-609.
5. Araiho SJ, Tear G. Axon guidance mechanisms and molecules: lessons from invertebrates. Nat Rev Neurosci 2003; 4:910-22.
6. Winkelm M, Mitchell KJ, Goodman CS. Genetic analysis of the mechanisms controlling target selection: complementary and combinatorial functions of netrins, semaphorins and IgCAMs. Cell 1993; 93:581-91.
7. Rasse TM, Fouquet W, Schmid A, Kirtel RJ, Mertel S, Sigrist CB, et al. Glutamate receptor dynamics organizing synapse formation in vivo. Nat Neurosci 2005; 8:898-905.
8. Stork T, Engelen D, Krudewig A, Silies M, Baillot RJ, Klambt C. Organization and function of the blood-brain barrier in Drosophila. J Neurosci 2008; 28:587-97.
9. Siebert M, Banovic D, Goellner B, Aberle H. Drosophila motor axons recognize and follow a Sidestep-labeled substrate pathway to reach their target fields. Genes Dev 2009; 23:1052-62.
10. Sink H, Rehm EJ, Richstone L, Bulls YM, Goodman CS. Sidestep encodes a target-derived attractant essential for motor axon guidance in Drosophila. Cell 2001; 105:57-67.
11. Langdraf M, Baylies M, Bate M. Muscle founder cells regulate defasciculation and targeting of motor axons in the Drosophila embryo. Curr Biol 1999; 9:589-92.
12. Fambrough D, Goodman CS. The Drosophila beaten path gene encodes a novel secreted protein that regulates defasciculation at motor axon choice points. Cell 1999; 87:1049-58.
13. de Jong J, Cavillo JA, Riss CD, Dvorak HA, Sink H. Target recognition and synaptogenesis by motor axons: responses to the sidestep protein. J Neurosci 2005; 23:397-410.
14. Meyer F, Aberle H. At the next stop sign turn right: the metalloproteinase Tolloid-related 1 controls defasciculation of motor axons in Drosophila. Development 2006; 133:4035-44.
15. Pipes GC, Lin Q, Riley SE, Goodman CS. The Beat generation: a multigene family encoding Igf3 proteins related to the Beat axon guidance molecule in Drosophila. Development 2001; 128:4945-52.
16. Lopez-Bendito G, Cautinat A, Sanchez JA, Biele F, Flames N, Garratt AN, et al. Tangential neuronal migration controls axon guidance: a role for neuroreglin-1 in thalamocortical axon navigation. Cell 2006; 125:127-42.
17. Zinn K. Choosing the road less traveled by: a ligand-receptor system that controls target recognition by Drosophila motor axons. Genes Dev 2009; 23:1042-5.