Neuron
Article

Intra-axonal Patterning: Intrinsic Compartmentalization of the Axonal Membrane in *Drosophila* Neurons

Takeo Katsuki,[1,2] Deepak Ailani,[1,2] Masaki Hiramoto,[1,3] and Yasushi Hiromi[1,2,*]
[1]Department of Developmental Genetics, National Institute of Genetics, 1111 Yata, Mishima, Shizuoka 411-8540, Japan
[2]Department of Genetics, SOKENDAI, 1111 Yata, Mishima, Shizuoka 411-8540, Japan
[3]Present address: ICND 216, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA
*Correspondence: yhiromi@lab.nig.ac.jp
DOI 10.1016/j.neuron.2009.08.019

SUMMARY

In the developing nervous system, distribution of membrane molecules, particularly axon guidance receptors, is often restricted to specific segments of axons. Such localization of membrane molecules can be important for the formation and function of neural networks; however, how this patterning within axons is achieved remains elusive. Here we show that *Drosophila* neurons in culture establish intra-axonal patterns in a cell-autonomous manner; several membrane molecules localize to either proximal or distal axon segments without cell-cell contacts. This distinct patterning of membrane proteins is not explained by a simple temporal control of expression, and likely involves spatially controlled vesicular targeting or retrieval. Mobility of transmembrane molecules is restricted at the boundary of intra-axonal segments, indicating that the axonal membrane is compartmentalized by a barrier mechanism. We propose that this intra-axonal compartmentalization is an intrinsic property of *Drosophila* neurons that provides a basis for the structural and functional development of the nervous system.

INTRODUCTION

The process by which different parts of the body acquire distinct properties such as morphology, function, and molecular distribution is generally called patterning. Likewise, patterning events that take place within a single cell may be called intracellular patterning. In the nervous system of vertebrates and invertebrates, different regions of axons are often characterized by differential expression of membrane molecules (Bastiani et al., 1987; Brittis et al., 2002; Callahan et al., 1995; Dodd et al., 1988; Kidd et al., 1998; Patel et al., 1987; Rajagopalan et al., 2000a, 2000b; Simpson et al., 2000a, 2000b), suggesting that axons are "patterned" into intra-axonal segments. For example, in the ventral nerve cord of *Drosophila* embryos, axon guidance receptor Roundabout (ROBO), and other members of this family, ROBO2 and ROBO3, accumulate on longitudinal axon tracts, but are excluded from commissures (Kidd et al., 1998; Rajagopalan et al., 2000a, 2000b; Simpson et al., 2000a, 2000b) (Figures 1A and 1C). This specific distribution pattern is due to the localization of ROBO proteins to distal axon segments of individual commissural neurons (Kidd et al., 1998; Simpson et al., 2000a) (Figure 1D). The distribution of another guidance receptor, Derailed (DRL), which, by contrast, is enriched on the anterior commissures, provides a second example of this regulated distribution (Bonkowsky et al., 1999; Callahan et al., 1995) (Figures 1B–1D). In the spinal cord of mouse embryos, commissural axons express elevated levels of Robo1 and Robo2 proteins on the axon segments that have crossed the floor plate, while an isoform of Rig-1/Robo3 is mostly present on the precrossing axon segment (Chen et al., 2008; Sabatier et al., 2004). Thus, the spatial regulation of axon guidance receptors within an axon appears to be conserved across species.

Despite the generality of the intra-axonal localization of membrane molecules, little is known about how such elaborate patterns emerge. These patterns may result from the influence of extrinsic cues, an intrinsic ability of cells, or both. It is also elusive how polarized distribution of these membrane proteins along axons can be established and maintained without diffusing into a uniform distribution. Here, using a low-density primary cell culture prepared from *Drosophila* embryos, we show that neurons possess an ability to generate intra-axonal patterns of membrane molecules cell-autonomously. ROBO3 and ROBO2 are localized to distal axon segments, while DRL is localized to proximal axon segments in a complementary manner. These localization patterns are not explained by a simple temporal control of receptor expression, and likely involve spatially controlled vesicular targeting or retrieval pathways. The temperature-sensitive *dynamin* mutant reveals that DRL requires Dynamin-dependent endocytosis for its localization to proximal axon segments, whereas ROBO3 localization is relatively insensitive to blocking Dynamin function. We also show that the exchange of membrane proteins between the distal and proximal axon segments is restricted at a medial point of the axon, which may maintain the compartmentalized distribution of membrane proteins along axons.

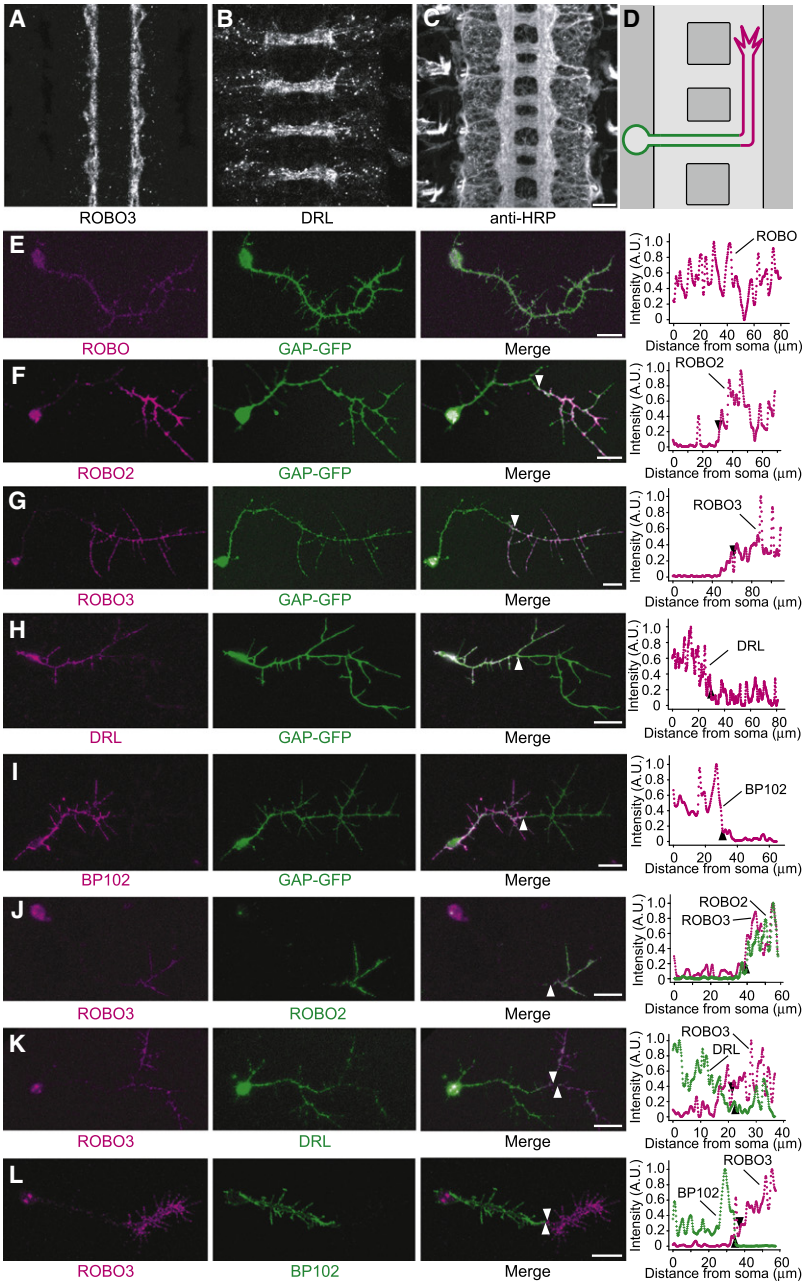

Figure 1. Intra-axonal Localization of Axon Guidance Receptors in Cultured *Drosophila* Neurons

(A–D) Distribution of ROBO3 (A) and DRL (B) axon guidance receptors in the ventral nerve cord of stage 16 *Drosophila* embryos. The anti-HRP antibody stains all neuronal surfaces (C). (D) Trajectory of a commissural neuron illustrating the localization of ROBO3 (magenta) and DRL (green) within a single axon.

(E–I) Cultured neurons in isolation labeled for ROBO (E), ROBO2 (F), ROBO3 (G), DRL (H), and BP102 (I) (magenta). GAP-GFP (green) labels the entire plasma membrane, and was used as a membrane marker for the quantitative analysis of localization (see Experimental Procedures).

(J) Overlapping distribution patterns of ROBO3 (magenta) versus ROBO2 (green).

(K and L) Complementary distribution patterns of ROBO3 (magenta) versus DRL (green) (K) or BP102 (green) (L).

Throughout figures, arrowheads pointing downward and upward indicate the boundaries of distal and proximal localization, respectively. Right-most panels, fluorescence intensity profiles along axons. Scale bars, 10 μm.

RESULTS

Intra-axonal Localization of Axon Guidance Receptors Can Be Generated Cell Autonomously

We sought to examine whether *Drosophila* neurons possess any intrinsic ability to generate intra-axonal distribution patterns of membrane molecules, and if so, by what mechanism. To explore the cell-autonomous properties of *Drosophila* neurons, we used a low-density primary cell culture system, in which neurons extend their axons in the absence of cell-cell contacts. We tested whether or not neurons placed in culture exhibit a localized distribution of ROBO receptors. Immunostaining revealed that ROBO is distributed uniformly along axons, suggesting that ROBO requires extrinsic signals for its distal localization pattern (Figure 1E, Table 1). By contrast, ROBO2 and ROBO3 were localized to distal axon segments in many neurons, with a discrete boundary at a medial point in the axonal process (Figures 1F and 1G, Table 1). Likewise, DRL protein and the antigen of a monoclonal antibody BP102 (Seeger et al., 1993) were also localized cell-autonomously to the proximal region of axons (Figures 1H and 1I, Table 1). Thus, we conclude that *Drosophila* neurons possess an ability to create intra-axonal localization patterns via a cell-intrinsic mechanism (or mechanisms).

Intra-axonal Localization of Guidance Receptors Shares a Common Boundary

To unveil the mechanism for this intrinsic intra-axonal patterning, we first asked how many distinct localization patterns can be formed in a single axon. We reasoned that if each localized molecule is based on a distinct localization mechanism, there could be as many distinct localization patterns and boundaries as the number of different molecules. Alternatively, if multiple molecules employ the same mechanism or related mechanisms, their localization patterns could share a common boundary. To test this, we visualized the distribution of multiple receptors simultaneously using double immunostaining. When ROBO2 and ROBO3 were detected by double staining, their distal localization pattern overlapped and shared a common boundary (Figure 1J). Moreover, the distal pattern of ROBO3 and the proximal pattern of DRL (or BP102) were complementary and their boundaries largely coincided (Figures 1K and 1L). These results suggest that intra-axonal patterning may involve a mechanism that generates or maintains multiple molecules.