Developmental Biology 414 (2016) 170–180

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Borderless regulates glial extension and axon ensheathment

Scott Cameron [a,b,1], Yixu Chen [a,1], Yong Rao [a,b,c,*]

[a] McGill Centre for Research in Neuroscience, Department of Neurology and Neurosurgery, McGill University Health Centre, 1650 Cedar Avenue, Montreal, Quebec, Canada H3G 1A4
[b] Integrated Program in Neuroscience, McGill University Health Centre, 1650 Cedar Avenue, Montreal, Quebec, Canada H3G 1A4
[c] Department of Medicine, McGill University Health Centre, 1650 Cedar Avenue, Montreal, Quebec, Canada H3G 1A4

ARTICLE INFO

Article history:
Received 16 April 2015
Received in revised form
15 March 2016
Accepted 26 April 2016
Available online 27 April 2016

Keywords:
Bdl
Glia
Drosophila
Eye disc
Axon ensheathment

ABSTRACT

Ensheathment of axons by glial processes is essential for normal brain function. While considerable progress has been made to define molecular and cellular mechanisms underlying the maintenance of axon ensheathment, less is known about molecular details of early events for the wrapping of axons by glial processes in the developing nervous system. In this study, we investigate the role of the transmembrane protein Borderless (Bdl) in the developing *Drosophila* visual system. Bdl belongs to the immunoglobulin (Ig) superfamily, and its in vivo function is unknown. We show that Bdl is expressed in wrapping glia (WG) in the developing eye disc. Cell-type-specific transgene rescue and knockdown indicate that Bdl is specifically required in WG for the extension of glial processes along photoreceptor axons in the optic lobe, and axon ensheathment. Our results identify Bdl as a novel glia-specific cell-surface recognition molecule in regulating glial extension and axon ensheathment.

1. Introduction

Axon ensheathment plays an essential role in insulating axons for propagating action potentials (Sherman and Brophy, 2005). In vertebrates, axons in the central and peripheral nervous systems are wrapped and insulated by oligodendrocytes and Schwann cells, respectively (Sherman and Brophy, 2005). Accumulated studies have identified a growing number of proteins that are important for axon-glia communications and the maintenance of axon ensheathment. However, the molecular mechanisms underlying initial axon-glia recognition for establishing axon ensheathment during development are not yet completely understood.

Drosophila has proven to be a valuable model for understanding glia development and axon ensheathment (Silies and Klambt, 2011). For instance, Gliotactin and Neurexin IV are identified as key regulators of glia-glia interaction in regulating the formation of septate junction and blood-nerve barrier in *Drosophila* (Auld et al., 1995; Baumgartner et al., 1996). A neuronal isoform of Neurexin IV has also been shown to bind to Wrapper on the surface of midline glia in mediating the ensheathment of commissural axons (Noordermeer et al., 1998; Stork et al., 2009).

In this study, we investigate the role of the cell adhesion molecule Borderless (Bdl) in regulating the wrapping of photoreceptor (R cell) axons in the developing *Drosophila* visual system. The formation of the adult visual system begins at the third-instar larval stage. At this stage, differentiating R cells in the eye-imaginal disc project axons through the optic stalk into the developing optic lobe. R-cell axons are ensheathed by WG that derive from perineurial glia (PG). At the mid-third instar larval stage, PG migrate from the optic stalk into the eye disc, and then differentiate into WG in response to neuron-derived signals (Silies and Klambt, 2011; Silies et al., 2007). Glial proliferation and migration into the eye disc require FGF8-like ligand Pyramus (Franzdottir et al., 2009), Gilgamesh and Hedgehog (Hummel et al., 2002), whereas the differentiation of PG into WG and the initiation of axonal wrapping require neuron-derived FGF8-like ligand Thisbe (Franzdottir et al., 2009). The adhesion system that mediates axon-glia recognition for axon ensheathment, however, remains unknown.

Understanding the mechanisms controlling the initial events of WG extension and axon ensheathment requires the identification of key cell-surface recognition molecules that are expressed on axons and glia. Here, we show that Bdl is specifically expressed in WG at the third-instar larval stage when WG interacts with R-cell axons in initiating axon ensheathment. Bdl is homologous to several members of the Ig superfamily, such as IgSF9/Dasm1/Tutl, Frazzled/DCC/UNC-40, Robo, and Dscam2 (Cameron et al., 2013; Ferguson et al., 2009). In our previous study (Cameron et al., 2013), we show that Bdl is expressed on R7 axons at pupal stage, and

* Correspondence to: Centre for Research in Neuroscience, McGill University Health Centre, Room L7-136, 1650 Cedar Avenue, Montreal, Quebec, Canada H3G 1A4.
E-mail address: yong.rao@mcgill.ca (Y. Rao).
[1] These authors contribute equally and are listed alphabetically.

http://dx.doi.org/10.1016/j.ydbio.2016.04.020
0012-1606/

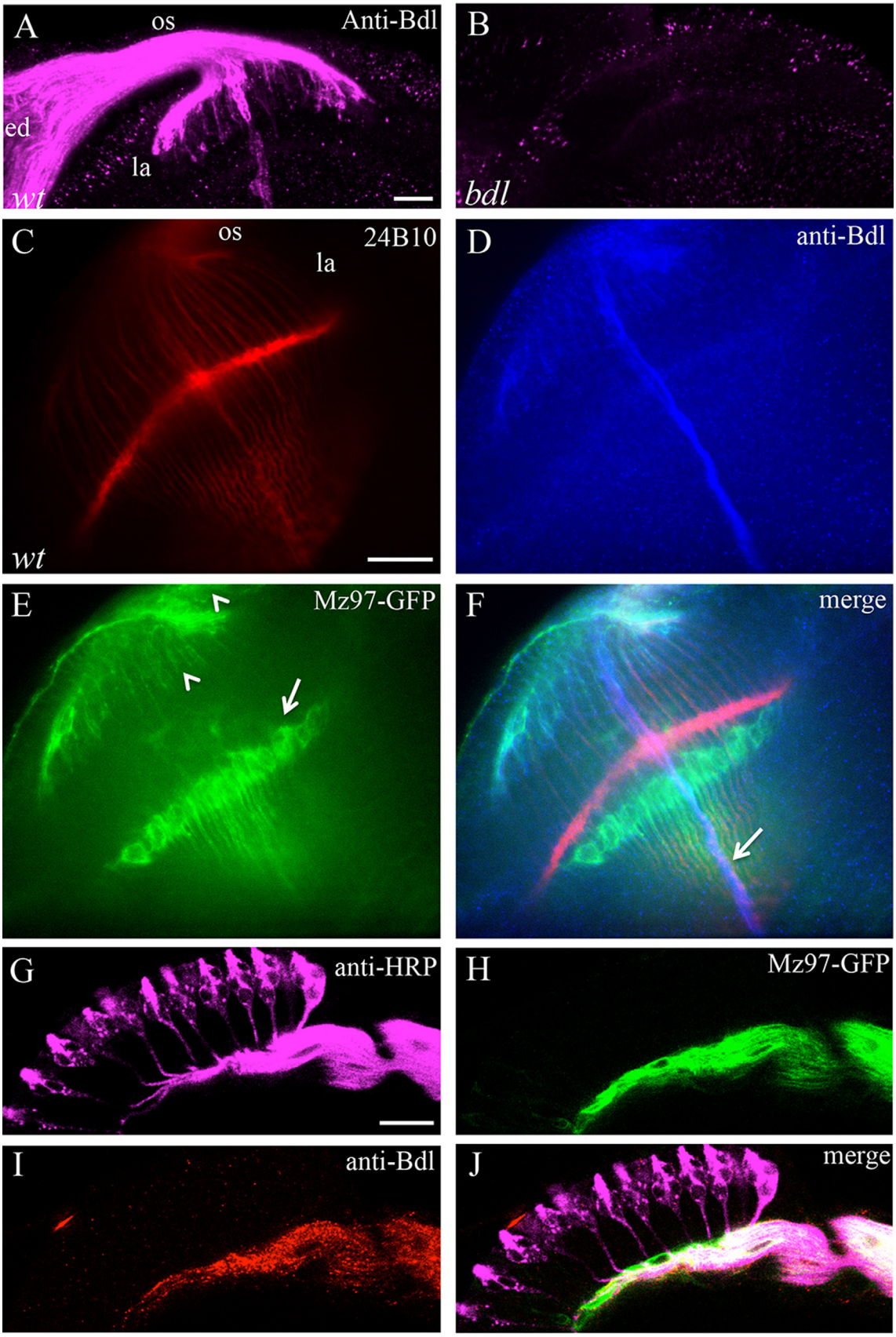

Fig. 1. Bdl is expressed in wrapping glia in the visual system at third-instar larval stage. (A and B) Third-instar eye-brain complexes were stained with anti-Bdl antibody. (A) In wild type, anti-Bdl staining visualized cellular processes from the eye disc (ed) through the optic stalk (os) into the developing lamina (la). (B) In homozygous bdl^{EX2} mutants, anti-Bdl staining was absent. (C–F) Wild-type third-instar eye-brain complexes were triple-stained with R-cell-specific monoclonal antibody MAb 24B10 (red), anti-Bdl (blue) and anti-GFP (green). (C) R-cell axons were visualized with MAb 24B10 staining. (D) Anti-Bdl staining. (E) WG processes (arrowheads) in the optic stalk and the proximal region of lamina were visualized with mCD8-GFP under control of the WG-specific driver Mz97-GAL4. Mz97-GFP also visualizes other glial processes (arrow) in the distal region of lamina. (F) The patterns of Mz97-GFP and anti-Bdl staining are superimposable in the optic stalk and the proximal region of lamina. Note that Bdl is also expressed in glial processes that wrap larval photoreceptor axons of Bolwig organ (arrow), the visual organ of the larva. (G–J) Longitudinal optic sections of wild-type third-instar eye discs triple-stained with Mz97-GFP (green), anti-Bdl (red) and anti-HRP (magenta). Apical is up, and posterior is to the right. (G) R-cell bodies and axons were visualized with anti-HRP staining. (H) WG processes at subretinal and optic stalk were stained with Mz97-GFP. (I) Anti-Bdl staining. (J) The patterns of Mz97-GFP and anti-Bdl staining are superimposable. Mz97-GFP denotes UAS-mCD8-GFP driven by Mz97-GAL4. Scale bar: 10 μm.