Developmental Biology 361 (2012) 232–244

Contents lists available at SciVerse ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Time-lapse imaging reveals stereotypical patterns of *Drosophila* midline glial migration

Scott R. Wheeler, Joseph C. Pearson, Stephen T. Crews *

Department of Biochemistry and Biophysics, Program in Molecular Biology and Biotechnology, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3280, USA

ARTICLE INFO

Article history:
Received for publication 11 June 2011
Revised 16 September 2011
Accepted 8 October 2011
Available online 26 October 2011

Keywords:
Apoptosis
Central nervous system
Drosophila
Ensheathment
Midline glia
Migration

ABSTRACT

The *Drosophila* CNS midline glia (MG) are multifunctional cells that ensheath and provide trophic support to commissural axons, and direct embryonic development by employing a variety of signaling molecules. These glia consist of two functionally distinct populations: the anterior MG (AMG) and posterior MG (PMG). Only the AMG ensheath axon commissures, whereas the function of the non-ensheathing PMG is unknown. The *Drosophila* MG have proven to be an excellent system for studying glial proliferation, cell fate, apoptosis, and axon–glial interactions. However, insight into how AMG migrate and acquire their specific positions within the axon–glial scaffold has been lacking. In this paper, we use time-lapse imaging, single-cell analysis, and embryo staining to comprehensively describe the proliferation, migration, and apoptosis of the *Drosophila* MG. We identified 3 groups of MG that differed in the trajectories of their initial inward migration: AMG that migrate inward and to the anterior before undergoing apoptosis, AMG that migrate inward and to the posterior to ensheath commissural axons, and PMG that migrate inward and to the anterior to contact the commissural axons before undergoing apoptosis. In a second phase of their migration, the surviving AMG stereotypically migrated posteriorly to specific positions surrounding the commissures, and their final position was correlated with their location prior to migration. Most noteworthy are AMG that migrated between the commissures from a ventral to a dorsal position. Single-cell analysis indicated that individual AMG possessed wide-ranging and elaborate membrane extensions that partially ensheathed both commissures. These results provide a strong foundation for future genetic experiments to identify mutants affecting MG development, particularly in guidance cues that may direct migration. *Drosophila* MG are homologous in structure and function to the glial-like cells that populate the vertebrate CNS floorplate, and study of *Drosophila* MG will provide useful insights into floorplate development and function.

Introduction

In both vertebrates and invertebrates, there exist diverse glial cell types that carry-out a variety of important nervous system functions. One central issue in developmental neuroscience is understanding how each glial cell type divides, migrates, undergoes apoptosis, recognizes and ensheaths axons, and acquires its distinctive functional morphology. In this manner, it can be determined which genetic pathways are shared among different glia, and which are distinct. This will be important for understanding how glia function and evolve, and, in the case of humans, provide the foundation for potential therapies for glial-based diseases.

The *Drosophila* midline glia (MG) are a distinct glial cell type that ensheath the two axon commissures, anterior commissure (AC) and posterior commissure (PC), that cross the midline of the CNS (Crews, 2009). There are two MG subtypes, anterior MG (AMG) and posterior MG (PMG) that have distinct gene expression patterns and interactions with the commissures (Watson et al., 2011; Wheeler et al., 2009). MG are present throughout embryonic, larval, and pupal development, but are absent from the adult CNS, suggesting that their roles are developmental (Awad and Truman, 1997). One potential function of MG is to provide trophic support to crossing axons, and the recent identification of midline-expressed neurotrophins supports this concept (Zhu et al., 2008). The MG also comprise a multifunctional embryonic signaling center controlling aspects of axon guidance, epidermal formation, muscle cell migration, and formation of the mesodermal dorsal median cells (Crews, 2003). When embryonic MG are ablated, the axon commissures become severely disorganized (Bergmann et al., 2002). This emphasizes the importance of *Drosophila* MG.

Insect MG are highly related to the glial-like cells that lie at the ventral midline or floorplate of the developing vertebrate nerve cord and brain. The floorplate cells also ensheath and guide commissural axons (Campbell and Peterson, 1993; Garbe and Bashaw, 2004; Yoshioka and Tanaka, 1989) and act as a critical signaling center that patterns the spinal cord by secreting Sonic hedgehog (Dessaud et al.,

* Corresponding author. Fax: +1 919 962 8472.
E-mail address: steve_crews@unc.edu (S.T. Crews).

0012-1606/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2011.10.024

2008). Given the important roles of the floorplate, it is likely that floorplate-related dysfunction may lead to neurological and mental health disorders. Despite considerable knowledge regarding the signaling role of the floorplate in axon guidance and pattern formation, relatively little is known regarding the development of floorplate cells or how they ensheath commissural axons. In this regard, the *Drosophila* MG provide an excellent, generalizable system for the study of floorplate glia.

Previous studies on MG development have provided useful information regarding apoptosis, migration, and ensheathment (Bergmann et al., 2002; Crews, 2009). However, these studies were limited by visualizing MG in fixed embryos. In most cases, AMG could not clearly be distinguished from PMG, nor could accurate patterns of migration be discerned. In order to more fully understand MG development, we have utilized three complementary approaches for studying MG development. The first involved staining embryos with markers that provided the ability to distinguish AMG from PMG and to detect MG undergoing division and apoptosis. The second approach utilized live imaging of MG to track their movement throughout development. The third approach involved imaging individual MG in stained embryos to assess the extent of membrane elaboration and commissural axon ensheathment. Using these approaches, we have assembled a comprehensive view of MG development that will provide a strong foundation for future genetic studies.

This view incorporates a description of the migration, proliferation, and apoptosis of MG during embryonic development. We show that after their formation, both AMG and PMG migrate inward over midline neurons toward the nascent axon commissure. AMG then initiate a second posterior migration in which they move along stereotyped paths, ultimately residing in positions where they elaborate complex processes that surround and intercalate into the axon commissures. Analyses of individual AMG migration paths from time-lapse imaging indicate a correlation between AMG positions at the start of the posterior migrations with their choice of migration path and destination. Taken together, these data suggest the existence of guidance cues to direct AMG along different migration paths and the possibility that the interpretation of those cues by AMG is dictated by position. Near the end of the inward migration, a subset of AMG divides and produces, on average, 2 additional AMG per segment. Paradoxically, these and other AMG almost immediately undergo apoptosis. Apoptosis continues sporadically in both AMG and PMG until late in embryogenesis, leaving approximately 3 AMG and no PMG. The work described in this paper will be particularly useful for future studies in identifying genes that govern how glial number is regulated, how glia respond to guidance cues and migrate to specific locations around axons, and how they interact with those axons.

Materials and methods

Drosophila strains

Drosophila strains used included *380-slit-Gal4* (Wharton and Crews, 1993), *sim-Gal4 UAS-tau-GFP* (Wheeler et al., 2006), *UAS-GFP-actin* (Verkhusha et al., 1999), *UAS-mCD8-GFP* (Lee and Luo, 1999), *UAS-moesin*-GFP (Bloor and Kiehart, 2001), *UAS-tau-GFP* (Brand, 1995), and *P[12xSu(H)bs-lacZ]* (Go et al., 1998), and *P{nos-phiC31\int.NLS}*; *P{CaryP}attP2* (Groth et al., 2004).

Generation of MG-expressed Gal4 lines

Two novel MG-expressed *Gal4* transgenic lines were generated and used for either live imaging (*glec-O1.5-Gal4*) or single-cell analysis (*argos-G1.1-Gal4*). The *glec-O1.5* fragment resides in the 3′ flanking sequence, and was generated by PCR amplification of genomic DNA using the forward primer 5′-TCTCCCGGAAACGAAGGAGTTCCTG-3′ and reverse primer 5′-CATAATCGTTGTCCTGTGATCCTACGTTTG-3′ (Chr 3R: 17672381–17673859; Assembly: BDGP R5\dm3). Note that *glec-O1.5* spans a genomic region containing both *glec* MG enhancers described in Fulkerson and Estes (2010). The *argos-G1.1* fragment resides in intron 1, and was generated by PCR-amplification of genomic DNA using the forward primer 5′-GTGCACACGCACACTCAGACTCGCAC and reverse primer 5′-CTACTCTTCCAGCTTCTTCGCCAGCACAG-3′ (Chr 3L: 16466506–16467651). To clone the *sli-380* fragment (Wharton and Crews, 1993), forward primer 5′-ATTTAAGTTGCTTGCCATGCTGGAG-3′ and reverse primer 5′-GTGAGTGACATTCCATGGGGAGC-3′ were used to PCR amplify from genomic DNA (Chr 2R: 11775792–11776179). Fragments were cloned into either pCR8 or pENTR (Invitrogen), Gateway-cloned into pBPGw-UCP, and transformed into *P{nos-phiC31\int.NLS}*; *P{CaryP}attP2 Drosophila* embryos containing the PhiC31 destination site *attP2* (68A1-B2) (Groth et al., 2004) and expressing posteriorly-localized PhiC31 integrase.

Construction of the pBPGw-UCP PhiC31 transformation vector

The pBPGw-UCP plasmid is a *Drosophila* PhiC31-based *Gal4* transformation vector in which enhancer-containing fragments are cloned adjacent to a universal core promoter (UCP) followed by *Gal4*. The UCP sequence was kindly provided by Jim Kadonaga. To generate the UCP insert, fragment four oligonucleotides were designed and annealed, generating the double-stranded sequence: 5′-*CCGG*CAGCGGTATAAAAGGGCGCGGGGTGGCTGAGAGCATCAGTTGTGAATGAATGTTCGAGCCGAGCAGACGTGCCGCT*GTAC*-3′; italicized nucleotides were single-stranded overhangs to match FseI and KpnI restriction site overhangs, respectively; transcription start +1 nucleotide is underlined. The Gateway-compatible PhiC31 transformation *Gal4* reporter vector pBPGw (Pfeiffer et al., 2008) was cut with FseI and KpnI, and ligated to the annealed UCP fragment. The resulting pBPGw-UCP is missing 82 bases of promoter-flanking sequences compared to pBPGUw (Pfeiffer et al., 2008) due to the restriction sites used, but is otherwise functionally equivalent.

In situ hybridization, immunostaining, and microscopy

In situ hybridization and immunostaining were carried out as previously described (Kearney et al., 2004; Wheeler et al., 2006). Tyramide Signal Amplification (TSA) (Perkin Elmer) was used for indicated antibodies. Primary antibodies: mouse anti-β-galactosidase (1:500, Promega), mouse MAb BP102 (1:33, Developmental Studies Hybridoma Bank [DSHB]), rabbit anti-activated Caspase-3 (1:25 with TSA amplification, Abcam, ab13847), rabbit anti-GFP (1:500, Abcam, ab290), and rabbit anti-phospho-Histone H3 (1:250, Millipore, 06-570), guinea pig anti-Sim (1:200 with TSA) (Ward et al., 1998), and guinea pig anti-Wrapper (1:200) (Wheeler et al., 2009). The *wrapper* digoxygenin-labeled antisense RNA probe used for in situ hybridization was generated from cDNA clone GH03113 from the *Drosophila* Gene Collection (Open Biosystems). Midline cells were visualized in abdominal segments A1–A6 using Zeiss LSM Pascal and LSM-710 confocal microscopes.

Time-lapse imaging of MG

Imaging of MG migration was carried-out using the following transgenic strains: (1) *glec-O1.5-Gal4 UAS-mCD8-GFP*, (2), *glec-O1.5-Gal4 UAS-GFP-actin*, (3) *glec-O1.5-Gal4 UAS-moesin-GFP*, and (4) *380-slit-Gal4 UAS-mCD8-GFP*. Embryos were collected for 3 h at room temperature and aged for 14 h at 18 °C. They were then dechorionated for 2 min in 50% bleach, mounted on glass coverslips, and covered with halocarbon oil 700 (Sigma, H8898). Coverslips were placed oil-side down on PetriPERM 50 mm hydrophobic cell culture dishes (Sarstedt, 96077305) that had an oxygen-permeable membrane. GFP fluorescent images were captured using a Zeiss LSM-710 confocal microscope with a 40X oil-immersion objective. Embryos