Neurosci Bull August 1, 2014, 30(4): 584–594. http://www.neurosci.cn
DOI: 10.1007/s12264-014-1448-2

·Review·

Glial cells in neuronal development: recent advances and insights from *Drosophila melanogaster*

Jiayao Ou[1], Yijing He[1], Xi Xiao[1], Tian-Ming Yu[1], Changyan Chen[2], Zongbao Gao[1], Margaret S. Ho[1]
[1]*Department of Anatomy and Neurobiology, School of Medicine,* [2]*School of Life Sciences, Tongji University, Shanghai 200092, China*
Corresponding author: Margaret S. Ho. E-mail: margaret_ho@tongji.edu.cn

Glia outnumber neurons and are the most abundant cell type in the nervous system. Whereas neurons are the major carriers, transducers, and processors of information, glial cells, once considered mainly to play a passive supporting role, are now recognized for their active contributions to almost every aspect of nervous system development. Recently, insights from the invertebrate organism *Drosophila melanogaster* have advanced our knowledge of glial cell biology. In particular, findings on neuron-glia interactions *via* intrinsic and extrinsic mechanisms have shed light on the importance of glia during different stages of neuronal development. Here, we summarize recent advances in understanding the functions of *Drosophila* glia, which resemble their mammalian counterparts in morphology and function, neural stem-cell conversion, synapse formation, and developmental axon pruning. These discoveries reinforce the idea that glia are substantial players in the developing nervous system and further advance the understanding of mechanisms leading to neurodegeneration.

Keywords: glia; neuronal development; Gcm; neurodegeneration; neural stem cell; synapse formation; axon pruning

Introduction

Nervous systems sense environmental inputs and cellular cues and their development, which mainly relies on the correct differentiation of two cell types, neurons and glia, is a vital process for animals to execute functions such as cognition, learning, and memory. At the cellular level, neurons develop from undifferentiated progenitor cells (neural stem cells) to differentiated cells with compartmentalized structures like axons and dendrites that mediate pathfinding, information processing, and synaptic connections. Almost every aspect of this developmental process and subsequent neuronal activity are under precise regulation by factors such as signaling components and the surrounding milieu. Interestingly, a major part of these regulatory mechanisms is mediated by glia, the partners of neurons. It is known that glia play essential roles by providing extrinsic signals to neurons and acting as part of the niche required for neuronal development and function.

Conventionally, glia have been considered to play a passive supporting role due to a lack of electrical excitability for transducing information like neurons. Nonetheless, compelling evidence has demonstrated that glia participate actively in mediating a number of neuronal events such as axon guidance, peripheral axon ensheathment, and formation of the blood-brain barrier to protect the central nervous system (CNS)[1-5]. On the other hand, a tripartite model that includes glia has recently been proposed to revise the classical view of synaptic structure[6-8]. In addition to the presynaptic and postsynaptic compartments, adjacent glia, particularly mammalian astrocytes, are now envisioned as one of the major components integrating synaptic function by releasing gliotransmitters, promoting synapse formation, and regulating synaptic plasticity[9]. Intriguingly, studies from the invertebrate model organism *Drosophila melanogaster* have offered abundant insights

into how *Drosophila* glia, resembling their mammalian counterparts, function to interact with neurons and regulate development. These recent advances have now implicated glia in other previously-unrecognized functions.

Several recent articles have provided excellent overviews of the origin and development of glia[10-14]. In this review, we explicitly summarize glial functions that have emerged as key mechanisms in the regulation of neuronal development in *Drosophila*. We describe the distinct classes of *Drosophila* glia, followed by a discussion of how they modulate neural stem-cell behavior, an extrinsic regulatory step during the early stage of neural fate decision. Next, we discuss how glia secrete different factors to affect the development and function of the neuromuscular junction (NMJ). Finally, we compare the glia-derived two-step secretion/engulfment mechanism in NMJ remodeling with axon pruning of mushroom body (MB) γ-neurons. Altogether, these recent discoveries point to a significant role for glia during neuronal development, and provide novel insights into mechanisms leading to a destabilized state of the nervous system, as in neurodegeneration.

Drosophila Glia

The genetically-tractable organism *Drosophila* has been an excellent animal model in advancing our understanding of glial biology. Distinct classes of glia are based on their morphology and function similar to their mammalian counterparts[10, 13, 15-17] (Table 1 and Fig. 1). Surface glia, the outermost layer of protection surrounding the larval and adult CNS, comprises two subtypes, the perineurial and subperineurial glia (SPG). These glial cells exclusively function as a blood-brain-barrier to prevent unwanted molecules over a certain size from entering the CNS[2,5,16]. The protection is mainly mediated by SPG, which form

Table 1. *Drosophila* glial cells are categorized into four groups according to their function and distribution[9,10,13,16]

Drosophila glia	Subtype	Distribution	Primary function Corresponding mammalian counterparts
CNS			
Surface glia	Perineurial glia (PG)	CNS surface (outer layer)	Blood-brain barrier (BBB)
	Subperineurial glia (SPG)	CNS surface (underneath PG)	Blood-brain barrier (BBB) Glia-glia pleated septate junctions (pSJs)
Neuropil glia	Ensheathing glia	Synaptic neuropil (outside)	Axon ensheathment Mammalian counterpart: Oligodendrocytes
	Astrocyte-like glia (Reticular glia)	Synaptic neuropil (infiltrated)	Axon ensheathment and pruning Engulfment activity Mammalian counterpart: Astrocytes
Cortical glia		In the cortex around neuronal cell bodies	Gas and nutrient exchange Mammalian counterpart: Astrocytes
PNS			
Peripheral glia	PG	Peripheral nerves (outer layer)	Axon ensheathment Mammalian counterpart: Schwann cells
	SPG	Peripheral nerves (underneath peripheral PG)	
	Wrapping glia	Inner layer contacting axon	

CNS: central nervous system; PNS, peripheral nervous system.

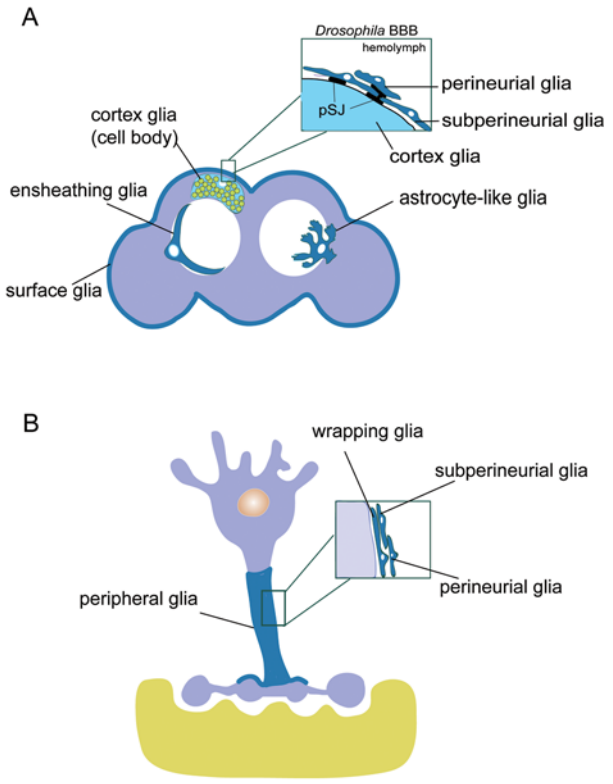

Fig. 1. *Drosophila* glia. A: In this schematic cross-section of *Drosophila* brain, four types of CNS glia are shown in blue: cortical glia (neuronal cell bodies in green), surface glia, and neuropil glia, which include ensheathing glia and astrocyte-like glia. The insert shows the subtypes of surface glia. Perineurial and subperineurial glia are shown in close association with the cortical glia. These glial cells function as a blood-brain-barrier to protect the CNS. Pleated septate junctions (pSJs) within subperineurial glia are in black. B: Schematic of *Drosophila* peripheral glia in the neuromuscular junction with a presynaptic axon (purple) and postsynaptic muscle (yellow). Three subtypes of peripheral glia are shown in blue: perineurial, subperineurial, and wrapping glia. These glia wrap around the axons of motor neurons up to the proximal synaptic bouton and regulate synapse formation and function.

pleated septate junctions among themselves. The components of pleated septate junctions are known to be homologs of proteins forming the paranodal junctions between axons and glia at the node of Ranvier in mammals[3,18]. In regards to size, SPGs have large and flatted cell bodies and are few in number, whereas perineurial glia have smaller cell bodies and higher numbers (Fig. 1A).

Cortical glia, also termed cell-body-associated glia, are structurally similar to mammalian astrocytes. Cortical glia wrap around the neuronal cell bodies at the outer surface of the brain, mediate gas exchange between cell bodies and the trachea, and provide trophic support. The third glial subtype is neuropil glia; these are closely associated with the neuropil regions containing bundles of axons and ensheath the synaptic neuropil like mammalian oligodendrocytes. Two types of neuropil glia are present in *Drosophila*: ensheathing glia, which surround the synaptic neuropil, and astrocyte-like glia that infiltrate into the inner region of the neuropil volume. Finally, CNS-derived peripheral glia are also subcategorized into three types[3-5,16]. The innermost type in contact with axons is termed wrapping glia; this is also considered to be a subtype of neuropil glia due to its association with nerves. Immediately above the wrapping glia is the peripheral perineurial glia and SPG. As in the CNS, these SPGs also form pleated septate junctions and provide insulation for axons (Fig. 1B).

It is noteworthy that microglia, the resident immune cells, engulf cell debris to protect the integrity of the nervous system. Unlike mammals, there is no corresponding microglial subtype in *Drosophila*. In terms of engulfing activity, at least two subtypes of *Drosophila* glia have been shown to execute this function[19, 20].

Glia Modulate Neural Stem-Cell Behavior

Drosophila neural stem cells, also termed neuroblasts (NBs), are plastic with an undifferentaited nature and serve as an excellent model to study stem-cell biology[21-23]. During the first wave of neurogenesis, embryonic NBs undergo asymmetric division to generate a smaller ganglion mother cell, which divides once more to produce differentiated neurons and/or glial cells, and another NB with self-renewal potential[21, 24]. These NBs generate most of the larval CNS neurons and enter a quiescence period for ~24 h at the end of embryogenesis[24-27]. How these NBs are reactivated during the larval stage remains largely unclear. However, once reactivated, they continue to divide and generate the neurons needed for the adult CNS. During larval neurogenesis, a different NB type, type II, produces a transient amplifying intermediate neural progenitor cell which undergoes extra rounds of division to

produce greater numbers of neurons than type I NBs[28-30]. These NBs, patterned by the distinct temporal and spatial expression of transcription factors, orchestrate the order and diversity of neural progeny in both the larval and adult CNS[22,23,28].

Glia participate in distinctive ways throughout this developmental process[31-37]. A fat body-glia-NB signaling relay has been demonstrated to regulate NB reactivation after quiescence[38, 39]. Within this relay, the insulin/insulin-like growth factor signaling pathway with the downstream effector PI3K/Akt, the central regulator of growth and metabolism, is activated in NBs by insulin-like peptides (dILPs) secreted by glia. These dILPs, in particular dILP2 and dILP6, bind to the single insulin/insulin-like growth factor receptor and are secreted upon the delivery of a nutrient signal from the fat body. This tripartite relay then allows the NBs to exit from quiescence and reactivate.

Typically, dILPs are secreted by insulin-producing cells in the larval brain to execute their function during cell growth and proliferation[40]. The discovery that glia are capable of secreting some of these peptides suggests an alternate route for converting the fat body signals into paracrine dILP function, hence diversifying their target list. This particular group of glia, surface glia, is adjacent to the NBs and associates with the surface to wrap around the CNS (note that Sousa-Nunes *et. al.* suggested that cortical glia are responsible for the secretion[39]). Surface glia are ideally positioned to transmit signals from the fat body to modulate NB reactivation. It is worth noting that glia also express additional factors such as the glycoprotein Anachronism (Ana)[41], dPerlecan[42-44], the RNA-binding protein FMRP implicated in Fragile X syndrome[45], and another type of secretory peptide, the activin-like peptides[46, 47], all of which have been reported to contribute to NB reactivation in different ways (Fig. 2A).

Later during development, after NB reactivation,

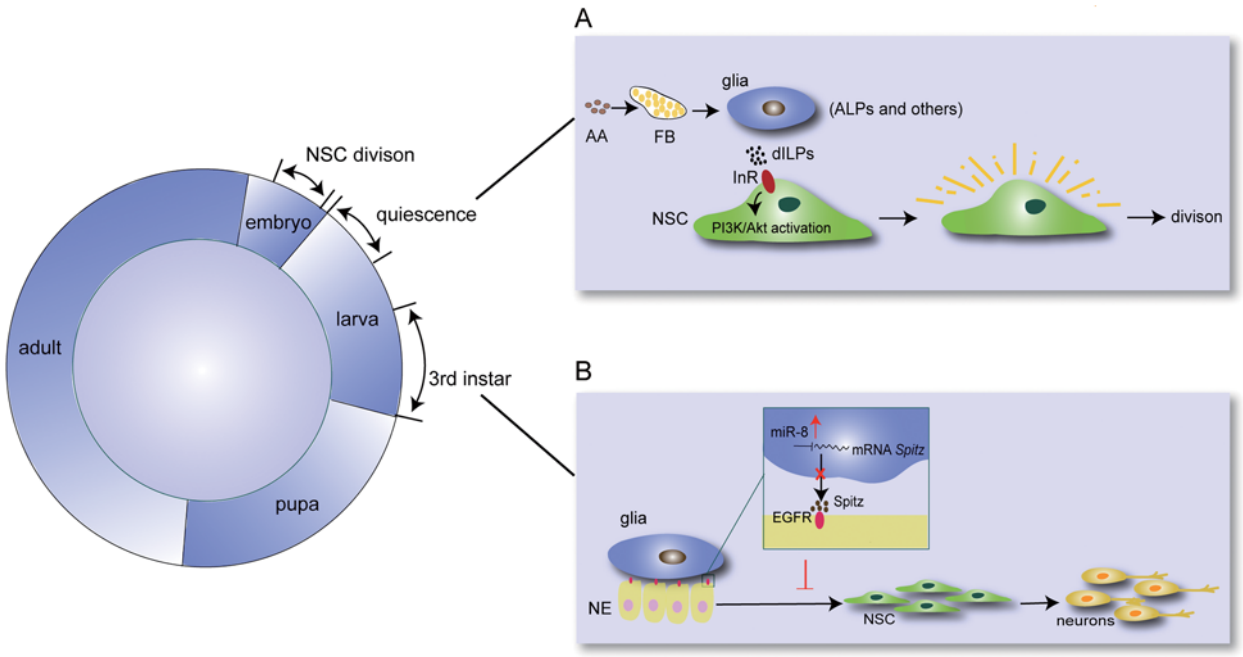

Fig. 2. ***Drosophila*** **glia modulate neural stem-cell behavior. The life cycle of** ***Drosophila*** **from embryo to adult is illustrated in the left panel. A: The three-step fat body-glia-NB relay. An amino-acid-triggered fat body signal is delivered to the surface glia, which are ideally positioned to release dILPs to activate the insulin receptor (InR) expressed in the NBs. This action in turn reactivates NBs from quiescence. Glia also secrete other factors such as activin-like peptides (ALPs) to modulate NB reactivation. B: In** ***Drosophila*** **optic lobe, neural stem cells (NSCs) are transformed from neuroepithelial (NE) cells and this transition is regulated by the optic-lobe-associated glia expressing the microRNA miR-8. miR-8 inhibits the translation of the epidermal growth factor receptor (EGFR) ligand** ***Spitz*****, abolishes its secretion by glia and interaction with EGF receptors on NE cells. This glial regulation suppresses the NE-to-NSC transition.**

glia regulate the transition from neuroepithelial (NE) to neural stem cells in the developing larval optic lobe. This transition, an event orchestrated in a manner similar to the epithelial-to-mesenchymal transition in mammals[48], is an ideal system to study an effect of the glial niche on stem-cell behavior. One of the recent studies using this system has revealed a specific glial subtype below the SPGs, the optic-lobe-associated glia, that express the microRNA miR-8, a homolog of mammalian miR-200. Glial-specific expression of miR-8 locally inhibits translation of the epidermal growth factor receptor ligand Spitz, affecting the ligand-receptor interaction on the NE cell membrane, and leading to the dysregulation of NE expansion and NB transition. In contrast, miR-8 positively regulates glial size, suggesting a dual effect on both glia and the neighboring NE cells[49] (Fig. 2B).

In summary, different populations of *Drosophila* glia function in diverse ways to regulate stem-cell behavior. Similar to mammals, a glial niche environment organized by glia and other cell types is required for NB conversion, reactivation after quiescence, and ultimately during the proliferative developmental phase (Fig. 2).

Glia-derived Factors during Synapse Formation and Function

The *Drosophila* NMJ is a widely-used model for studying synapse formation and activity. These synapses are glutamatergic, stereotypically positioned, and resemble mammalian central synapses in terms of the neurotransmitter used[50, 51]. Compelling evidence has shown that glia, closely associated with these synapses, modulate synaptic activity and synapse formation[52-56]. Among the three types of peripheral glia, perineurial glia and SPGs, but not wrapping glia, send processes into the NMJ[56]. These processes display a variety of morphological structures along the motoneuron axons to the point of nerve-muscle contact, and sometimes extend into the proximal synaptic bouton, yet never completely cover the NMJ[5, 53, 56, 57].

Recent advances have uncovered a critical role for peripheral glia during NMJ formation and function. Wingless (Wg)/Wnt, identified by chromatin immunoprecipitation analysis as a downstream target of the glial transcription factor Reversed polarity (Repo), is secreted by glia to mediate postsynaptic glutamate receptor clustering[55]. Unlike the Wg/Wnt released from motoneurons, which regulates both NMJ growth and postsynaptic glutamate receptor clustering in a manner dependent on dFrizzled2 (dFz2) receptors[58-61], glia-derived Wg/Wnt does not affect NMJ size, but regulates postsynaptic function as revealed by electrophysiological studies[55]. Furthermore, peripheral glia secrete another factor, the TGF-β ligand Maverick (Mav), that binds postsynaptically to a not-yet-identified receptor (likely the TGF-β type II receptor Punt) and turns on Gbb transcription *via* the cascade of Mad phosphorylation and Co-Smad Medea (Med) interaction. Gbb is the central effector of the retrograde signaling from muscle to presynaptic motoneuron and it does do by interacting with the bone morphogenetic protein (BMP) receptors Wishful thinking (Wit), Saxophone (Sax), and/or Thickvein (Tkv). Interaction with this receptor brings about Mad phosphorylation, hence regulating the expression of the Rac-activating gene *trio* and synaptic growth[52, 62, 63] (Fig. 3).

Bimodal Regulation of Synaptic Remodeling by Glia

In addition to synapse formation and function, remodeling events that occur during synaptogenesis to shape the synaptic contact are also regulated by glia. For instance, the tumor necrosis factor-alpha (TNF-α) factor Eiger expressed by peripheral SPGs mediates a glia-derived pro-degenerative signaling event that controls axonal and synaptic degeneration. Severe presynaptic degeneration of the NMJ, indicated by fragmentation of presynaptic membranes, occurs when the functions of cytoskeletal molecules like Spectrin or Ankyrin are disrupted[64-66]. Loss of Eiger significantly suppresses the presynaptic degeneration induced by the absence of Ankyrin, suggesting a role for Eiger in mediating the degeneration of these presynaptic materials[54]. Upon secretion from glia, Eiger interacts with the TNF receptor Wengen in neurons, triggering the downstream caspase Dronc-Dcp1 pathway that induces axonal and synaptic degeneration. In addition, mitochondria-based signaling mediated by DARK and Debc1 is proposed to work with the caspase pathway to augment the response to the glia-derived pro-degenerative signal[54].

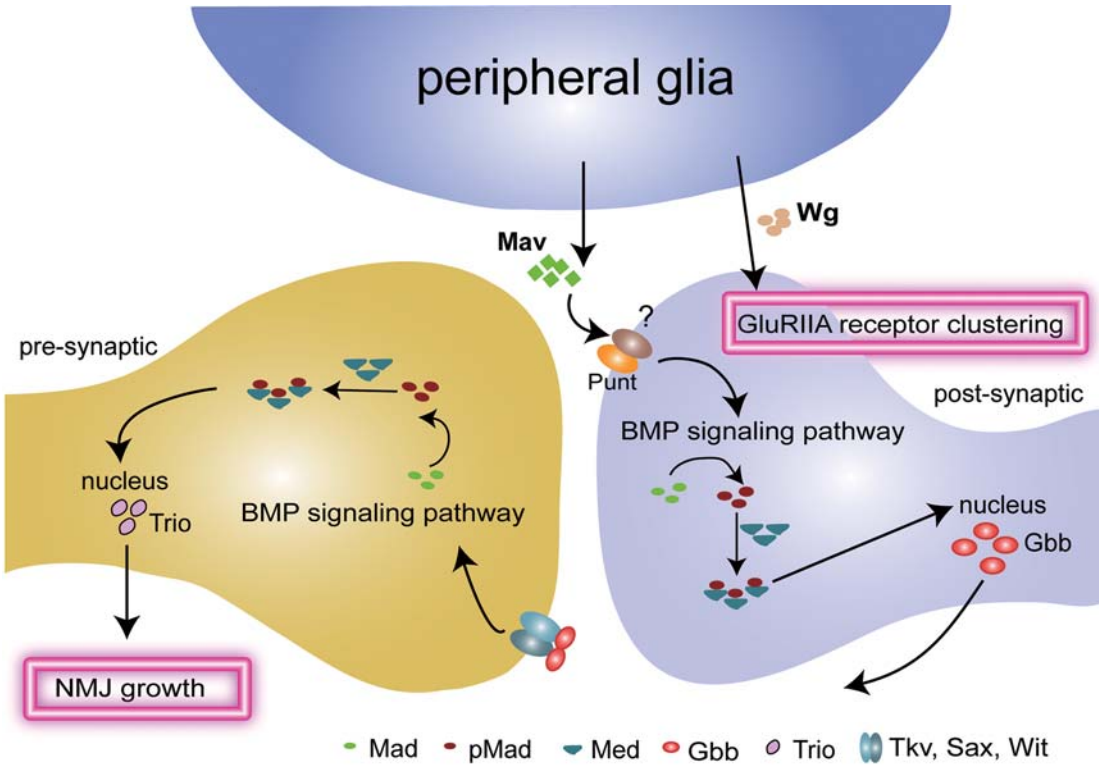

Fig. 3. Glia-derived factors regulate NMJ formation and function. In the *Drosophila* NMJ, adjacent peripheral glia secrete Wg to regulate postsynaptic function *via* glutamate receptor clustering. Glia also secrete another TGF-β ligand Mav, which acts postsynaptically to turn on BMP signaling *via* Mad phosphorylation and Mad-Med interaction. Upregulated BMP signaling tunes the transcription of Gbb, which is released from muscle to the presynaptic compartment to activate BMP signaling in motoneurons. This retrograde Gbb signaling controls Trio expression, which then regulates NMJ growth and size.

Reasonably, upon degeneration, these fragmented presynaptic membranes need to be removed to create an environment for the synapse to "remodel" under normal cellular dynamics. A recent study has shown that these extra presynaptic materials, including fragmented membranes (also termed presynaptic debris) and undifferentiated boutons (also termed ghost boutons) represented by the lack of active zones and postsynaptic proteins[53, 67], have been detected in motoneurons either naturally or upon light-stimulation of neurons expressing channelrhodopsin-2. In addition to the Eiger-dependent instructive signals provided by glia, these extra materials of presynaptic origin are removed *via* an engulfment process also mediated by adjacent peripheral glia. In particular, downregulating the expression of the engulfment receptor *draper* in glia results in an accumulation of presynaptic debris, but does not affect the presence of ghost boutons. It is worth noting that *draper* expression in muscle is also required for the engulfment process, but only for the disappearance of ghost boutons, indicating distinctive mechanisms by which glia and muscle control different presynaptic materials[53] (Fig. 4A).

Developmental Axon Pruning: Two-step Mechanism Mediated by Glia

A similar two-step glia-mediated mechanism has been ascribed to the axon pruning of MB γ-neurons. During metamorphosis, extensive remodeling of axons and dendrites occurs in order to accommodate the need for an adult neuronal circuitry. Notably, γ-neurons of the MB, the center for learning and memory in *Drosophila*, serves as an excellent model for understanding the mechanism underlying this dynamic process. Beginning in the late

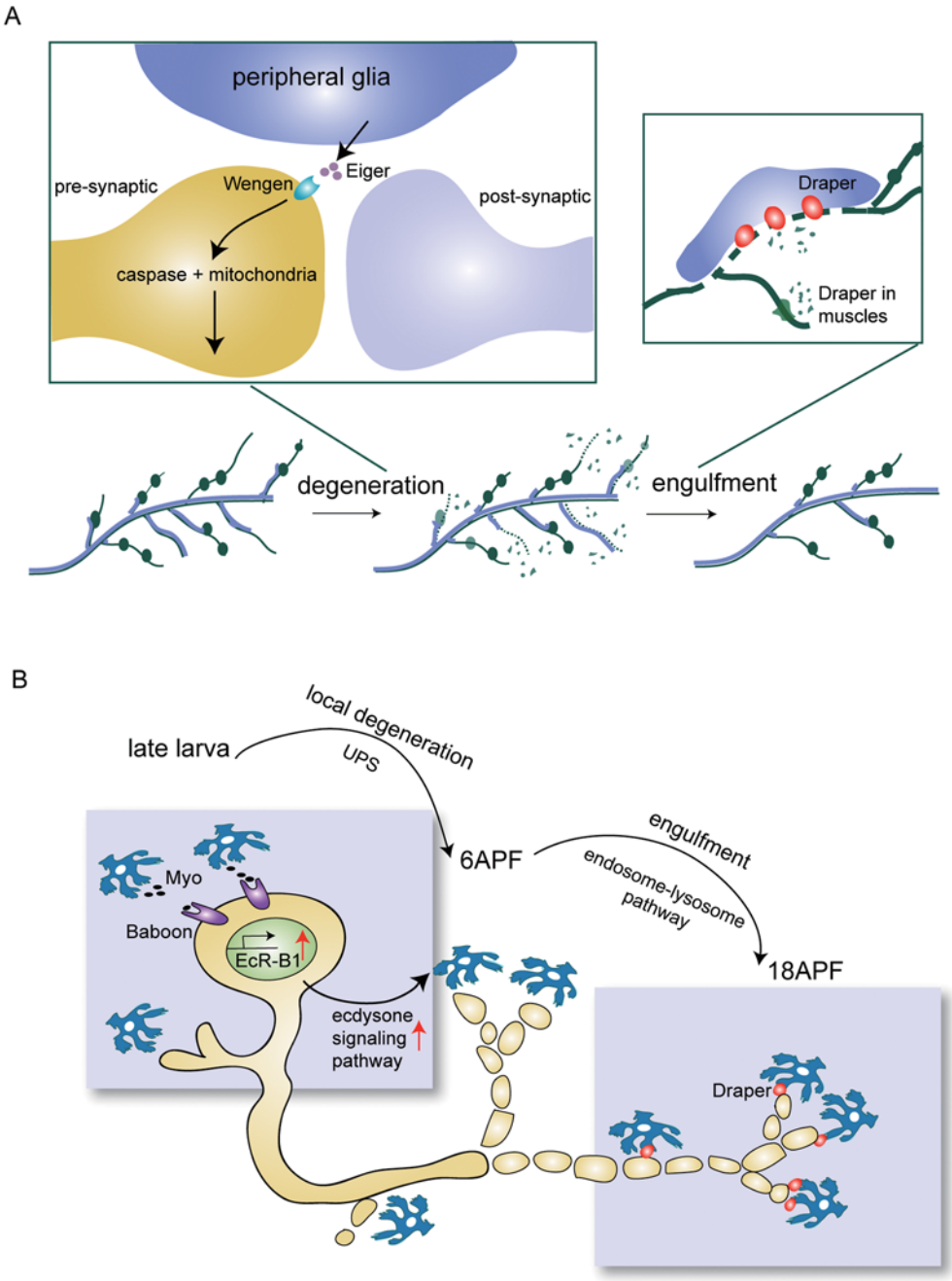

Fig. 4. Synaptic remodeling and axon pruning: two-step mechanism mediated by glia. A: In addition to releasing Wg and Mav, peripheral glia secrete the TNF ligand Eiger to interact presynaptically with the TNF receptor Wengen. This interaction activates the downstream caspase pathway (Dronc and Dcp1), which mediates axonal and synaptic degeneration in the NMJ. Two types of presynaptic degeneration materials, presynaptic debris and undifferentiated ghost boutons, are engulfed by glia and muscle respectively. Draper (red circles on the right), the engulfment receptor expressed in both glia and muscle, is responsible for engulfment activity in the NMJ. B: During axon pruning of MB γ-neurons, at an initial step, astrocytic glia (blue) secrete the TGF-β ligand Myo (black dots) to interact with the receptor Baboon (purple) on the neurons (upper left). This interaction activates TGF-β signaling in MB neurons, then upregulates ecdysone signaling by increasing the ecdysone receptor B1 (EcR-B1) levels. Upregulation of ecdysone signaling actively recruits astrocyte-like glia to infiltrate γ-neurons and initiate axon pruning. In the late pupa, glial cells engulf the degenerating axon materials *via* the activity of Draper (lower right). MB neurons are yellow and Draper is red.

larval stage, MB γ-neurons project with dendrites and axons that bifurcate into a dorsal and medial branch. At ~6 h after puparium formation, the axons and dendrites undergo a pruning process triggered by the metamorphic hormone ecdysone[68], so that local axon degeneration is induced and both the dorsal and medial branches are pruned, leaving only the axon peduncle. Later during the pupal stage, the medial branch re-extends and establishes the adult-specific axonal connection. It has been previously shown that, in addition to ecdysone signaling, the ubiquitin-proteasome system also plays a role in initiating the axon pruning of MB γ-neurons[68, 69].

Intriguingly, glia are involved in regulating the axon-pruning process by a consecutive two-step mechanism. Initially, upregulation of MB ecdysone receptor B1 (EcR-B1) expression is required to trigger pruning, and this upregulation is effected by the activation of TGF-β signaling in MB neurons. To achieve this, surrounding cortical and astrocyte-like glia secrete the TGF-β ligand myoglianin (Myo) that interacts with the type-I receptor Baboon in MB neurons to activate intrinsic TGF-β signaling[70]. Interestingly, the immunoglobulin superfamily molecule Plum has been shown to regulate TGF-β signaling at the receptor level and may participate in the glia-MB neuron interaction during developmental axon-pruning[71].

Upon the upregulation of ecdysone signaling, astrocyte-like glia infiltrate the MB and axon pruning is initiated. Further down the road, astrocyte-like glia also take on a scavenger-like role in cleaning up the degenerating axon fragments[72-75]. This glial degradation pathway, mediated by endosomes and lysosomes, is strictly required for axon pruning since inhibition of glial function in this case results in a delay in pruning and accumulation of degenerating materials. On the other hand, in the absence of ecdysone signaling from MB neurons, astrocyte-like glia do not infiltrate γ-neurons and engulfment activity is silenced. These results suggest that astrocyte-like glia take on an active role during pruning and that a bi-directional interaction between MB neurons and glia is required for the correct pruning process to occur[72].

Interestingly, similar to the NMJ, the glial engulfment receptor Draper is also required for the engulfment of axonal debris during MB γ-neuron pruning[73]. Although a notable amount of data has demonstrated that *draper* expression in glia is required for the engulfment of apoptotic neurons in embryos[76, 77], glial engulfment during γ-axon pruning differs from the engulfment mechanism for apoptotic cells. Expression of the caspase inhibitor *p35* in MB neurons does not lead to pruning defects[69], suggesting that apoptosis is not the major mechanism. On the other hand, γ-axon pruning is similar to the Wallerian degeneration of axon injury, which does not involve apoptosis, and the ubiquitin-proteasome system is one of the major mechanisms[78]. A Wallerian degeneration process has recently been well exemplified in *Drosophila* olfactory receptor neurons[79] and it has been shown that *draper* expression is similarly upregulated when axons undergo injury in this model[79-82]. Yet, unlike γ-axon pruning where astrocyte-like glia are the major subtype responsible[75, 83], ensheathing glia have been shown to engulf debris during axon injury of olfactory receptor neurons[84] (Fig. 4B).

Concluding Remarks

Understanding the mechanisms of how a nervous system develops from single progenitor cells to a functional unit integrating responses has always been the central area of interest in modern neuroscience. In-depth experimental analysis and pioneering work on model organisms like *Drosophila* have allowed researchers to draw conclusions about the important contributions of glia to the series of events leading to the maturation of neuronal circuitry. As both a long-term supporter and an active participant, glia modulate neural stem-cell behavior, secrete factors to regulate synapse formation, and are involved in redefining the nature of synaptic connections *via* degeneration and regrowth. Intriguingly, bi-directional communication between neurons and glia powerfully orchestrates developmental progression and serves as the basis for the mechanisms underlying neurodegenerative diseases. It is increasingly clear that glia, like neurons, are major mediators in regulating various aspects of neuronal development and function; their importance can no longer be neglected. Future work is required to further elucidate the glia-derived regulatory mechanisms, both intrinsic and extrinsic, in other developmental contexts and a fruitful outcome advancing our knowledge is envisioned.

ACKNOWLEDGEMENTS

We apologize to colleagues whose work could not be cited here

due to space restrictions. This work was supported by grants from the National Basic Research Program of China (973 Program 2010CB944900 and 2013CB945602), the National Natural Science Foundation of China (31270825 and 31171043), and Fundamental Research Funds for the Central Universities. We thank members of the Ho lab for discussion and comments.

Received date: 2014-04-04; Accepted date: 2014-05-22

REFERENCES

[1] Chotard C, Salecker I. Neurons and glia: team players in axon guidance. Trends Neurosci 2004, 27: 655–661.

[2] Bainton RJ, Tsai LT, Schwabe T, DeSalvo M, Gaul U, Heberlein U. moody encodes two GPCRs that regulate cocaine behaviors and blood-brain barrier permeability in *Drosophila*. Cell 2005, 123: 145–156.

[3] Banerjee S, Pillai AM, Paik R, Li J, Bhat MA. Axonal ensheathment and septate junction formation in the peripheral nervous system of *Drosophila*. J Neurosci 2006, 26: 3319–3329.

[4] Blauth K, Banerjee S, Bhat MA. Axonal ensheathment and intercellular barrier formation in *Drosophila*. Int Rev Cell Mol Biol 2010, 283: 93–128.

[5] Stork T, Engelen D, Krudewig A, Silies M, Bainton RJ, Klambt C. Organization and function of the blood-brain barrier in *Drosophila*. J Neurosci 2008, 28: 587–597.

[6] Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci 1999, 22: 208–215.

[7] Eroglu C, Barres BA. Regulation of synaptic connectivity by glia. Nature 2010, 468: 223–231.

[8] Danjo R, Kawasaki F, Ordway RW. A tripartite synapse model in Drosophila. PLoS One 2011, 6: e17131.

[9] Ransom HKaBR. Neuroglia. 3rd ed: Oxford University Press, 2013.

[10] Coutinho-Budd J, Freeman MR. Probing the enigma: unraveling glial cell biology in invertebrates. Curr Opin Neurobiol 2013, 23: 1073–1079.

[11] Barres BA. The mystery and magic of glia: a perspective on their roles in health and disease. Neuron 2008, 60: 430–440.

[12] Corty MM, Freeman MR. Cell biology in neuroscience: Architects in neural circuit design: glia control neuron numbers and connectivity. J Cell Biol 2013, 203: 395–405.

[13] Freeman MR, Doherty J. Glial cell biology in *Drosophila* and vertebrates. Trends Neurosci 2006, 29: 82–90.

[14] Freeman MR, Rowitch DH. Evolving concepts of gliogenesis: a look way back and ahead to the next 25 years. Neuron 2013, 80: 613–623.

[15] Edwards TN, Meinertzhagen IA. The functional organisation of glia in the adult brain of *Drosophila* and other insects. Prog Neurobiol 2010, 90: 471–497.

[16] Hartenstein V. Morphological diversity and development of glia in Drosophila. Glia 2011, 59: 1237–1252.

[17] Stork T, Bernardos R, Freeman MR. Analysis of glial cell development and function in *Drosophila*. Cold Spring Harb Protoc 2012, 2012: 1–17.

[18] Poliak S, Peles E. The local differentiation of myelinated axons at nodes of Ranvier. Nat Rev Neurosci 2003, 4: 968–980.

[19] Kurant E. Keeping the CNS clear: glial phagocytic functions in *Drosophila*. Glia 2011, 59: 1304–1311.

[20] Logan MA, Freeman MR. The scoop on the fly brain: glial engulfment functions in *Drosophila*. Neuron Glia Biol 2007, 3: 63–74.

[21] Homem CC, Knoblich JA. *Drosophila* neuroblasts: a model for stem cell biology. Development 2012, 139: 4297–4310.

[22] Li X, Chen Z, Desplan C. Temporal patterning of neural progenitors in *Drosophila*. Curr Top Dev Biol 2013, 105: 69–96.

[23] Maurange C. Temporal specification of neural stem cells: insights from *Drosophila* neuroblasts. Curr Top Dev Biol 2012, 98: 199–228.

[24] Hartenstein V, Rudloff E, Campos -Ortega J. The pattern of proliferation of the neuroblasts in the wild-type embryo of Drosophila melanogaster. Roux's archives of developmental biology 1987, 196: 473–485.

[25] Ito K, Hotta Y. Proliferation pattern of postembryonic neuroblasts in the brain of *Drosophila melanogaster*. Dev Biol 1992, 149: 134–148.

[26] Prokop A, Technau GM. The origin of postembryonic neuroblasts in the ventral nerve cord of *Drosophila melanogaster*. Development 1991, 111: 79–88.

[27] Truman JW, Bate M. Spatial and temporal patterns of neurogenesis in the central nervous system of *Drosophila melanogaster*. Dev Biol 1988, 125: 145–157.

[28] Bayraktar OA, Doe CQ. Combinatorial temporal patterning in progenitors expands neural diversity. Nature 2013, 498: 449–455.

[29] Viktorin G, Riebli N, Reichert H. A multipotent transit-amplifying neuroblast lineage in the central brain gives rise to optic lobe glial cells in *Drosophila*. Dev Biol 2013, 379: 182–194.

[30] Wang YC, Yang JS, Johnston R, Ren Q, Lee YJ, Luan H*, et al*. *Drosophila* intermediate neural progenitors produce lineage-dependent related series of diverse neurons. Development 2014, 141: 253–258.

[31] Hosoya T, Takizawa K, Nitta K, Hotta Y. Glial cells missing: a binary switch between neuronal and glial determination in *Drosophila*. Cell 1995, 82: 1025–1036.

[32] Jones BW, Fetter RD, Tear G, Goodman CS. Glial cells missing: a genetic switch that controls glial versus neuronal fate. Cell 1995, 82: 1013–1023.

[33] Vincent S, Vonesch JL, Giangrande A. Glide directs glial fate commitment and cell fate switch between neurones and glia. Development 1996, 122: 131–139.

[34] Viktorin G, Riebli N, Popkova A, Giangrande A, Reichert H. Multipotent neural stem cells generate glial cells of the central complex through transit amplifying intermediate progenitors in *Drosophila* brain development. Dev Biol 2011, 356: 553–565.

[35] Flici H, Erkosar B, Komonyi O, Karatas OF, Laneve P, Giangrande A. Gcm/Glide-dependent conversion into glia depends on neural stem cell age, but not on division, triggering a chromatin signature that is conserved in vertebrate glia. Development 2011, 138: 4167–4178.

[36] Hitoshi S, Ishino Y, Kumar A, Jasmine S, Tanaka KF, Kondo T*, et al.* Mammalian Gcm genes induce Hes5 expression by active DNA demethylation and induce neural stem cells. Nat Neurosci 2011, 14: 957–964.

[37] Mao H, Lv Z, Ho MS. Gcm proteins function in the developing nervous system. Dev Biol 2012, 370: 63–70.

[38] Chell JM, Brand AH. Nutrition-responsive glia control exit of neural stem cells from quiescence. Cell 2010, 143: 1161–1173.

[39] Sousa-Nunes R, Yee LL, Gould AP. Fat cells reactivate quiescent neuroblasts via TOR and glial insulin relays in *Drosophila*. Nature 2011, 471: 508–512.

[40] Goberdhan DC, Wilson C. The functions of insulin signaling: size isn't everything, even in *Drosophila*. Differentiation 2003, 71: 375–397.

[41] Ebens AJ, Garren H, Cheyette BN, Zipursky SL. The *Drosophila* anachronism locus: a glycoprotein secreted by glia inhibits neuroblast proliferation. Cell 1993, 74: 15–27.

[42] Friedrich MV, Schneider M, Timpl R, Baumgartner S. Perlecan domain V of Drosophila melanogaster. Sequence, recombinant analysis and tissue expression. Eur J Biochem 2000, 267: 3149–3159.

[43] Lindner JR, Hillman PR, Barrett AL, Jackson MC, Perry TL, Park Y*, et al.* The *Drosophila* Perlecan gene *trol* regulates multiple signaling pathways in different developmental contexts. BMC Dev Biol 2007, 7: 121.

[44] Voigt A, Pflanz R, Schafer U, Jackle H. Perlecan participates in proliferation activation of quiescent *Drosophila* neuroblasts. Dev Dyn 2002, 224: 403–412.

[45] Callan MA, Clements N, Ahrendt N, Zarnescu DC. Fragile X Protein is required for inhibition of insulin signaling and regulates glial-dependent neuroblast reactivation in the developing brain. Brain Res 2012, 1462: 151–161.

[46] Brummel T, Abdollah S, Haerry TE, Shimell MJ, Merriam J, Raftery L*, et al.* The *Drosophila* activin receptor baboon signals through dSmad2 and controls cell proliferation but not patterning during larval development. Genes Dev 1999, 13: 98–111.

[47] Zhu CC, Boone JQ, Jensen PA, Hanna S, Podemski L, Locke J*, et al. Drosophila* Activin- and the Activin-like product Dawdle function redundantly to regulate proliferation in the larval brain. Development 2008, 135: 513–521.

[48] Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY*, et al.* The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008, 133: 704–715.

[49] Morante J, Vallejo DM, Desplan C, Dominguez M. Conserved miR-8/miR-200 defines a glial niche that controls neuroepithelial expansion and neuroblast transition. Dev Cell 2013, 27: 174–187.

[50] Menon KP, Carrillo RA, Zinn K. Development and plasticity of the Drosophila larval neuromuscular junction. Wiley Interdiscip Rev Dev Biol 2013, 2: 647–670.

[51] Ruiz-Canada C, Budnik V. Introduction on the use of the Drosophila embryonic/larval neuromuscular junction as a model system to study synapse development and function, and a brief summary of pathfinding and target recognition. Int Rev Neurobiol 2006, 75: 1–31.

[52] Fuentes-Medel Y, Ashley J, Barria R, Maloney R, Freeman M, Budnik V. Integration of a retrograde signal during synapse formation by glia-secreted TGF-beta ligand. Curr Biol 2012, 22: 1831–1838.

[53] Fuentes-Medel Y, Logan MA, Ashley J, Ataman B, Budnik V, Freeman MR. Glia and muscle sculpt neuromuscular arbors by engulfing destabilized synaptic boutons and shed presynaptic debris. PLoS Biol 2009, 7: e1000184.

[54] Keller LC, Cheng L, Locke CJ, Muller M, Fetter RD, Davis GW. Glial-derived prodegenerative signaling in the *Drosophila* neuromuscular system. Neuron 2011, 72: 760–775.

[55] Kerr KS, Fuentes-Medel Y, Brewer C, Barria R, Ashley J, Abruzzi KC*, et al.* Glial wingless/wnt regulates glutamate receptor clustering and synaptic physiology at the *Drosophila* neuromuscular junction. J Neurosci 2014, 34: 2910–2920.

[56] Brink DL, Gilbert M, Xie X, Petley-Ragan L, Auld VJ. Glial processes at the *Drosophila* larval neuromuscular junction match synaptic growth. PLoS One 2012, 7: e37876.

[57] Brink D, Gilbert M, Auld V. Visualizing the live *Drosophila* glial-neuromuscular junction with fluorescent dyes. J Vis Exp 2009, pii: 1154.

[58] Ataman B, Ashley J, Gorczyca D, Gorczyca M, Mathew D, Wichmann C, *et al.* Nuclear trafficking of *Drosophila* Frizzled-2 during synapse development requires the PDZ protein dGRIP. Proc Natl Acad Sci U S A 2006, 103: 7841–7846.

[59] Mathew D, Ataman B, Chen J, Zhang Y, Cumberledge

S, Budnik V. Wingless signaling at synapses is through cleavage and nuclear import of receptor DFrizzled2. Science 2005, 310: 1344–1347.

[60] Miech C, Pauer HU, He X, Schwarz TL. Presynaptic local signaling by a canonical wingless pathway regulates development of the Drosophila neuromuscular junction. J Neurosci 2008, 28: 10875–10884.

[61] Packard M, Koo ES, Gorczyca M, Sharpe J, Cumberledge S, Budnik V. The *Drosophila* Wnt, wingless, provides an essential signal for pre- and postsynaptic differentiation. Cell 2002, 111: 319–330.

[62] Ball RW, Warren-Paquin M, Tsurudome K, Liao EH, Elazzouzi F, Cavanagh C*, et al.* Retrograde BMP signaling controls synaptic growth at the NMJ by regulating trio expression in motor neurons. Neuron 2010, 66: 536–549.

[63] Fuentes-Medel Y, Budnik V. Menage a Trio during BMP-mediated retrograde signaling at the NMJ. Neuron 2010, 66: 473–475.

[64] Pielage J, Cheng L, Fetter RD, Carlton PM, Sedat JW, Davis GW. A presynaptic giant ankyrin stabilizes the NMJ through regulation of presynaptic microtubules and transsynaptic cell adhesion. Neuron 2008, 58: 195–209.

[65] Pielage J, Fetter RD, Davis GW. Presynaptic spectrin is essential for synapse stabilization. Curr Biol 2005, 15: 918–928.

[66] Massaro CM, Pielage J, Davis GW. Molecular mechanisms that enhance synapse stability despite persistent disruption of the spectrin/ankyrin/microtubule cytoskeleton. J Cell Biol 2009, 187: 101–117.

[67] Ataman B, Ashley J, Gorczyca M, Ramachandran P, Fouquet W, Sigrist SJ*, et al.* Rapid activity-dependent modifications in synaptic structure and function require bidirectional Wnt signaling. Neuron 2008, 57: 705–718.

[68] Lee T, Marticke S, Sung C, Robinow S, Luo L. Cell-autonomous requirement of the USP/EcR-B ecdysone receptor for mushroom body neuronal remodeling in *Drosophila*. Neuron 2000, 28: 807–818.

[69] Watts RJ, Hoopfer ED, Luo L. Axon pruning during *Drosophila* metamorphosis: evidence for local degeneration and requirement of the ubiquitin-proteasome system. Neuron 2003, 38: 871–885.

[70] Awasaki T, Huang Y, O'Connor MB, Lee T. Glia instruct developmental neuronal remodeling through TGF-beta signaling. Nat Neurosci 2011, 14: 821–823.

[71] Yu XM, Gutman I, Mosca TJ, Iram T, Ozkan E, Garcia KC*, et al.* Plum, an immunoglobulin superfamily protein, regulates axon pruning by facilitating TGF-beta signaling. Neuron 2013, 78: 456–468.

[72] Awasaki T, Ito K. Engulfing action of glial cells is required for programmed axon pruning during *Drosophila* metamorphosis. Curr Biol 2004, 14: 668–677.

[73] Awasaki T, Tatsumi R, Takahashi K, Arai K, Nakanishi Y, Ueda R*, et al.* Essential role of the apoptotic cell engulfment genes draper and ced-6 in programmed axon pruning during *Drosophila* metamorphosis. Neuron 2006, 50: 855–867.

[74] Watts RJ, Schuldiner O, Perrino J, Larsen C, Luo L. Glia engulf degenerating axons during developmental axon pruning. Curr Biol 2004, 14: 678–684.

[75] Hakim Y, Yaniv SP, Schuldiner O. Astrocytes play a key role in *Drosophila* mushroom body axon pruning. PLoS One 2014, 9: e86178.

[76] Freeman MR, Delrow J, Kim J, Johnson E, Doe CQ. Unwrapping glial biology: Gcm target genes regulating glial development, diversification, and function. Neuron 2003, 38: 567–580.

[77] Kurant E, Axelrod S, Leaman D, Gaul U. Six-microns-under acts upstream of Draper in the glial phagocytosis of apoptotic neurons. Cell 2008, 133: 498–509.

[78] Hoopfer ED, McLaughlin T, Watts RJ, Schuldiner O, O'Leary DD, Luo L. Wlds protection distinguishes axon degeneration following injury from naturally occurring developmental pruning. Neuron 2006, 50: 883–895.

[79] MacDonald JM, Beach MG, Porpiglia E, Sheehan AE, Watts RJ, Freeman MR. The *Drosophila* cell corpse engulfment receptor Draper mediates glial clearance of severed axons. Neuron 2006, 50: 869–881.

[80] Logan MA, Hackett R, Doherty J, Sheehan A, Speese SD, Freeman MR. Negative regulation of glial engulfment activity by Draper terminates glial responses to axon injury. Nat Neurosci 2012, 15: 722–730.

[81] Ziegenfuss JS, Biswas R, Avery MA, Hong K, Sheehan AE, Yeung YG*, et al.* Draper-dependent glial phagocytic activity is mediated by Src and Syk family kinase signalling. Nature 2008, 453: 935–939.

[82] Ziegenfuss JS, Doherty J, Freeman MR. Distinct molecular pathways mediate glial activation and engulfment of axonal debris after axotomy. Nat Neurosci 2012, 15: 979–987.

[83] Tasdemir-Yilmaz OE, Freeman MR. Astrocytes engage unique molecular programs to engulf pruned neuronal debris from distinct subsets of neurons. Genes Dev 2014, 28: 20–33.

[84] Doherty J, Logan MA, Tasdemir OE, Freeman MR. Ensheathing glia function as phagocytes in the adult *Drosophila* brain. J Neurosci 2009, 29: 4768–4781.