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REVIEW

Comparing peripheral glial cell differentiation in *Drosophila* and vertebrates

Floriano Rodrigues · Imke Schmidt · Christian Klämbt

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Abstract In all complex organisms, the peripheral nerves ensure the portage of information from the periphery to central computing and back again. Axons are in part amazingly long and are accompanied by several different glial cell types. These peripheral glial cells ensure electrical conductance, most likely nuture the long axon, and establish and maintain a barrier towards extracellular body fluids. Recent work has revealed a surprisingly similar organization of peripheral nerves of vertebrates and *Drosophila*. Thus, the genetic dissection of glial differentiation in *Drosophila* may also advance our understanding of basic principles underlying the development of peripheral nerves in vertebrates.

Keywords Peripheral glia · Schwann cell · Wrapping glia · *Drosophila* · Vertebrates · Septate junctions · Myelin

Introduction

Most animals rely on their ability to sense environmental signals, to compute them and to finally trigger the appropriate responses. For this task, the central and the peripheral nervous systems (CNS, PNS) with their highly sophisticated cellular adaptations have evolved. The computing is hardwired in the numerous neuronal connections and establishes an astonishing complex and interwoven lattice. As complex as the formation of such intricate networks can be, the greater challenge may lie in the fact that the neuronal ensemble has to be functional for a very long time. Neuronal signals have to be faithfully transmitted over long distances and neurons have to reproducibly elicit the required responses in their target cells over many years. Thus, the need for a tight electrical insulation and metabolic support of the nervous system is directly evident. This task is executed by a set of glial cells that ensures the comfortable life of neurons. To dissect the functional characteristics of glial cells, the PNS can be used in a simple reductionist approach, since it is mostly comprised of axons and glial cells.

The organization of peripheral nerves is relatively simple and is not complicated by synaptic connections. Thus, glial cells have to fulfill only a limited set of functional requirements. They provide trophic support to the peripheral axons [1]. In addition, glial cells insulate the different axons to allow fast electrical conductance and to participate in the establishment of the blood–brain barrier to ensure constant reaction conditions during signal propagation. In the following, we compare how glial cells differentiate in the PNS of vertebrates and invertebrates to cover these different functional needs.

Peripheral nerves in vertebrates

During development of the vertebrate PNS, neural crest cells detach from the dorsal neural tube and give rise to neurons and glial cells of almost the entire PNS, as well as to many endocrine cells and to other mesenchymal cells [2–4]. The glial cell complement within the peripheral nerves is comprised of myelinating and non-myelinating Schwann cells also called Remak fibers [5]. Schwann cells always ensheath a single axon whereas Remak fibers sort more than one axon into so-called Remak pockets. These cell types, together with the motor axons and the sensory

F. Rodrigues · I. Schmidt · C. Klämbt (✉)
Institut für Neurobiologie, Badestr. 9, 48149 Münster, Germany
e-mail: klaembt@uni-muenster.de

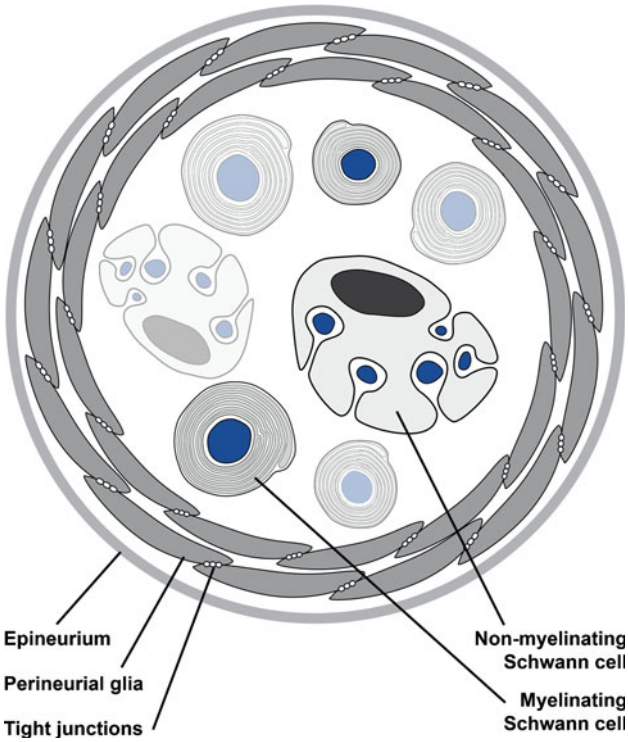

Fig. 1 Schematic view of a cross-section through a vertebrate peripheral nerve. The tight junction forming perineurial glial cells are surrounded by an epineurium. The endoneurium hosts myelinating Schwann cells, which ensheath large caliber axons and and non-myelinating Schwann cells which engulf axons of a small caliber size

axons, pericytes, endothelial and some endoneurial fibroblastic cells within the nerve, are engulfed by perineurial glial cells, which form a tight barrier between nerve and tissue fluids (Fig. 1) [6–8].

Schwann cells

Neural crest cells first differentiate into so-called Schwann cell precursors (SCPs) [9]. SCPs migrate along peripheral axon projections to reach their final position and do not seem to be important for correct axon targeting [10]. Later on, SCPs give rise to immature Schwann cells, which persist until the time of birth. Some marker genes for the different developmental stages are known (e.g., Cadherin19 as exclusive marker for SCPs; [11, 12]). Immature Schwann cells either develop into myelinating glia or non-myelinating Remak fibers. This differentiation correlates with the diameter of axons and is regulated by activation of the ErbB receptor on glial cell membranes by expression of axonal Neuregulin1 (NRG1). This not only triggers the differentiation of immature Schwann cells into either myelinating Schwann cells or Remak fibers but also determines the thickness of myelin sheath [13–15].

Perineurial cells

The mature peripheral nerves are engulfed by a layer of perineurial glial cells that will form a functional barrier providing a constant milieu for the centrally located axons and glial cells (Fig. 1). The perineurial glia forms a multilayered sheath. This cell layer is characterized by intensive interdigitated cell–cell contacts with extensive tight junctions, which establish an efficient barrier preventing paracellular transport of solutes. In vertebrates, the barrier function is established only 10–20 days after birth, which correlates with the detection of tight junction at the electron microscopic level [6–8, 16]. The origin of the perineurium has long been a matter of debate, but recent lineage tracing and live imaging technologies have convincingly demonstrated in zebrafish embryos that the perineurium is mostly, if not exclusively, derived from the CNS [17]. Thus, the peripheral nerves of vertebrates are comprised of neural tube and neural crest derivatives, and the interplay of the two cell types appears important for normal development.

Peripheral nerves in *Drosophila*

The organization of the *Drosophila* peripheral nerves is surprisingly similar to the one in vertebrates and can be used as a model for peripheral nerves (Fig. 2). The cellular complements of the segmentally arranged peripheral nerves are well known and all lineages have been described [18, 19]. In every abdominal segment, 30 motor neurons are born that send their axons to the lateral musculature [20, 21]. In the lateral body wall of the *Drosophila* larva, 42 sensory neurons are generated in each abdominal hemisegment [22, 23]. They all project their axons towards the ventral nerve cord and fasciculate with the motor axon tracts [22]. The axons are engulfed by several layers of glial cells [24]. After completion of embryogenesis, only 12 glial cells populate every segmental nerve. Seven of these glial cells originate from neuroblasts located in the ventral nerve cord and thus have to migrate along motor axons towards their final destinations. The remaining five glial cells are born in the periphery [18].

The outermost layer of the peripheral nerve is covered by a thick extracellular matrix deposited by macrophages circulating in the hemolymph (Fig. 2) [24, 25]. Below this matrix are the perineurial cells that, unlike the perineurium of the vertebrate nerve, do not appear to form special junctional cell–cell contact structures [24]. Instead, these are found between the subperineurial cells that are highly interdigitated with pronounced septate junctions. Within the nerve are the wrapping glial cells, which towards the

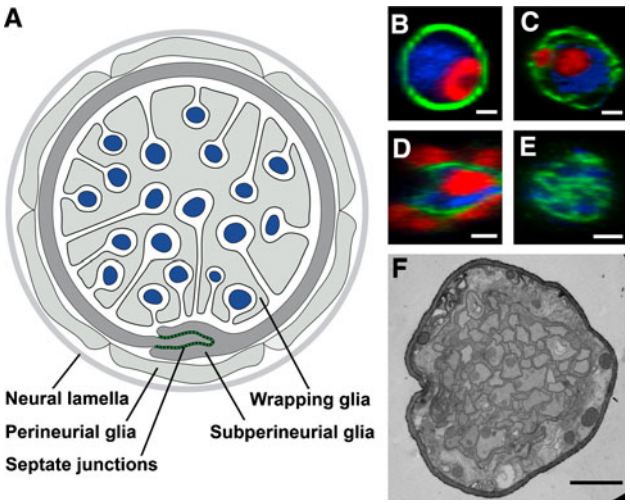

Fig. 2 *Drosophila* peripheral nerve. **a** Schematic drawing of a cross-section of a third instar larval peripheral nerve. Perineurial glial cells are covered by an extracellular matrix called neural lamella. **b–e** Orthogonal cross-sections of segmental nerves. GFP-expression is shown in *green*, glial nuclei express Repo (*red*), axonal membranes are labeled by HRP staining (*blue*). **b** The neural lamella is marked by the GFP-gene trap insertion into the *viking* gene, which encodes *Drosophila* CollagenIV. **c** The perineurial glial cells are labeled in *c527*Gal4; UAS*CD8GFP* flies. **d** The septate junction forming subperineurial glial cells express the *moody*Gal4 driver (genotype: *moody*Gal4; UAS*CD8GFP*). **e** Wrapping glial cells are marked by expression of the *nervana2*Gal4 driver (genotype: *nrv2*Gal4; UAS*S65TGFP*). **f** Electron micrograph of a cross-section through a larval peripheral nerve at the third instar stage. *Scale bars* 2 μm

end of larval development have engulfed every single axon. Markers to follow the different glial cells in wild-type and mutant backgrounds are available as the result of extensive enhancer trap and exon trap experiments (Fig. 2).

Wrapping glia

The innermost glial cells of the peripheral nerve are the wrapping glial cells. During embryonic development, three to four cells are found along the nerve, but the cells have not yet initiated their differentiation [24]. Towards the end of larval development, these glial cells enwrap every single peripheral axon in a way very similar to Remak cells. Within the peripheral nerves, the wrapping glia can be specifically identified using the *nervana2*Gal4 driver. *nervana2*Gal4 was generated by using a 7-kb promoter fragment from the *nervana2* (*nrv2*) gene, which encodes one of the 3 β-subunits of the P-type Na/K-ATPase [26]. It was originally reported as a neuronal marker but was later noted as being expressed in a large subset of glial cells [26–30]. In our hands, this Gal4 driver line and two additional GFP exon trap insertions, that faithfully mimic the endogenous *nrv2* expression pattern (*line173* [24] and *ZCL2903* [31]), show a surprisingly strong expression in the wrapping glia of the peripheral nerves, in addition to a weak expression in the subperineurial glia that build the blood brain barrier. No expression can be detected in the glia of the eye imaginal disc.

The wrapping glia tightly associates with axonal membranes. Previously, septate junctions between axons and non-neuronal cells were described at the nerve endings, close to the neuromuscular junctions [32]. However, we have failed to detect such junctional structures along the peripheral nerves.

Subperineurial glia

The subperineurial glial cells are the main constituents of the *Drosophila* hemolymph brain barrier, which corresponds to the blood–brain barrier (BBB) [24, 33–35]. They form a single cell wide, squamous epithelia-like structure with highly interdigitated cell–cell contacts. At the end of embryogenesis, extensive septate junctions are generated along these interdigitated cell contact sites, which morphologically resemble the paranodal septate-like junctions. The subperineurial glial cells do not divide; however, endoreplication is likely since the nuclei of the subperineurial glial cells are very large. These cells can be labeled throughout development using the markers *moody*Gal4 and *gliotactin*Gal4 [33, 36–38]. The *moody*Gal4 driver has been generated using 2.4-kb large promoter fragment of the *moody* gene and has been shown to rescue the mutant phenotype. *moody* encodes a G-protein-coupled receptor (GPCR) and transmits a still unknown signal regulating the formation of septate junctions (see below). In *moody* mutants, the length of the septate junctions is reduced, which might cause the leaky BBB phenotype. However, this may not necessarily be the direct cause of the leakage of the BBB, since mutant *yurt* animals also lack a functional barrier but show morphologically intact septate junctions [33, 36, 39–41].

One additional signaling pathway that has been recently identified to control the differentiation of the subperineurial glial cells in *Drosophila* may be defined by the PAK-like serine-threonine kinase Fray. Fray is required for the establishment or the maintenance of the axonal ensheathment by wrapping glia [42]. In *fray* mutants, peripheral nerves develop normally during embryonic development but exhibit severe swellings during larval stages [42]. The mammalian homolog of *fray* (PASK) directly phosphorylates the Na–K-2Cl co-transporter, and thereby activates solute transport, suggesting that the Fray kinase regulates ionic homeostasis [43].

Rescue experiments indicate that *fray* acts in the subperineurial glia and point to the important role of these cells in defining ion homeostasis in the nerve preventing severe nerve swellings. In addition, *fray* may control

axonal ensheathment by the wrapping glia in a non-cell-autonomous manner; however, electron microscopic data are still missing [42]. The signaling cascade involved is presently unclear.

The subperineurial glial cells may also influence the development of the perineurial glial cells. Upon activation of the Ras effector phosphatidylinositol 3-kinase (PI3 K) and its downstream kinase Akt in the subperineurial glia (*gliotactin*Gal4), the perineurial glial cells enlarge. This process depends on the FOXO transcription factor, suggesting that Ras-PI3 K-Akt signaling pathway in the subperineurial glia promotes growth of the perineurial glia [44].

Perineurial glial cells

The perineurial glial cells have the ability to divide, but currently no function is assigned to this cell type either in the PNS or in the CNS [24, 45]. Unlike the other peripheral glial cells, the perineurial glia does not contact neurons [24, 45]. Possibly, the perineurial glia exerts an accessory function during BBB formation [24] or these cells might serve as a reserve pool for structural plasticity. The best marker to label the perineurial glial cells is the Gal4 enhancer trap insertion *NP6293* [45], which carries an P[Gal4] insertion within the *basigin* locus. Basigin encodes an Ig-domain adhesion protein that, interestingly, is involved in the neuron–glia interaction in the optic ganglia of *Drosophila* [46]. The perineurial glial cells abut the extracellular matrix and Basigin has been reported to interact with Integrin signaling [47].

Molecular control of axonal wrapping

In vertebrates, SCPs can either differentiate into myelinating or non-myelinating glial cells. This depends on the activity level of two members of the EGF-receptor tyrosine kinase family, ErbB3 and ErbB4. ErbB signaling results in the activation of two downstream signaling cascades: the PI3 K pathway and the Ras/MAPK pathway [48]. The initiation of myelination appears to be at least in part controlled by the PI3 K pathway and is not influenced by the Ras/MAPK pathway [49]. In mice, the activating ligand of the ErbB receptors is Neuregulin1 (NRG1). Genetic analysis demonstrated that the level of axonal NRG1 determines the extent of myelination. Reduced levels of NRG1 result in hypomyelination, whereas increased NRG1 expression leads to hypermyelination. Interestingly, the NRG1-ErbB pathway does not exclusively regulate myelination but also affects the sorting of axons into Remak fiber pockets [13]. Moreover, together with Sox2, Pax3 and Laminin, NRG1 induces cell proliferation of SCPs and can act as a survival factor for SCPs [11]. Recently, direct forward genetic screens in zebrafish are revealing further molecules underlying glial differentiation [50, 51].

In *Drosophila*, axonal wrapping is regulated similarly to vertebrates. Axonal wrapping has been well studied using the example of the developing compound eye, where FGF-receptor signaling regulates proliferation, migration and differentiation of glial cells [52, 53]. Most of the *Drosophila* compound eye is formed from the imaginal disc epithelia [54]. The only exception is seen in the retinal glial cells, which are derived from progenitor cells located in the larval CNS. These progenitor cells proliferate and migrate onto the eye imaginal disc as neuronal differentiation proceeds in this epithelium [52, 53, 55–59]. Glial cell proliferation and migration is regulated by a sustained activation of the FGF-receptor Heartless. Heartless, which is expressed specifically in the retinal glial cells, becomes activated by the FGF8-like ligand Pyramus. Interestingly, Pyramus is also expressed in the glia and thus the FGF-receptor is activated by paracrine or possibly autocrine mechanisms. Heartless activity is then transmitted to the nucleus via the function of Rap1, a small GTPase of the Ras family [52]. Once the glial cells have reached the forming photoreceptor neurons, they contact the nascent photoreceptor axons. This triggers the differentiation of glial cells into a wrapping glial cell type in a Heartless-dependent manner. However, now Heartless becomes activated via the neuronally expressed FGF8-like molecule Thisbe [52]. In conclusion, the sequential activation of a single FGF-receptor in glial cells first triggers proliferation and then stimulates differentiation.

How is this differential response to a seemingly very same signal, namely the activation of the Heartless receptor, controlled during the life of a glial cell? The first clue towards an understanding of this question stems from the observation that prior to glial differentiation, photoreceptor axons and glial cells contact each other for the first time. Concomitantly, the expression of Sprouty, a negative regulator of receptor tyrosine kinase signaling, is activated [52]. Thus, neuron–glia interaction appears to attenuate the intensity of FGF-receptor activity and sets the stage for a differential response to FGF-receptor signaling. The dampening of the FGF-receptor activity in differentiating glial cells may be of more general relevance, since we recently identified a novel negative regulator of *heartless* signaling expressed in glial cells in response to axonal contact (F. Sieglitz and C.K., unpublished). The combined action of these negative regulators ensures reduced levels of FGF-receptor activity and results in the inactivation of Rap1. Subsequently, target genes required for glial differentiation are then activated. Interestingly, the level of FGF-receptor activity is still decisive in setting the amount of glial wrapping of axons and more FGF-receptor activity can induce more wrapping [52].

In general, however, a typical invertebrate wrapping glial cell will only wrap around any axon once. Structures such as myelin are thought to be an invention of the vertebrate lineage. Nevertheless, myelination and even the formation of nodes have been described for annelids, malacostracan crustaceans and copepods [60–68]. In most cases, motor axons involved in escape responses are wrapped multiple times, but for some shrimps even sensory axons are additionally myelinated [65]. The tight wrapping of axonal segments induces the question how the propagation of the action potential is regulated in the invertebrate system. For some invertebrates like *Aplysia* voltage-gated ion channels are reported to be unevenly distributed and show clustering along the axon [69]. In cultured neurons from *Manduca* pupae, high expression of a voltage-gated sodium channel is noted in the axon close to the cell soma with more even expression along the remaining axonal segment [70]. In the larval nervous system of *Drosophila*, a similar distribution of the voltage-dependent Na^+ channel Para has been observed (I.S. and C.K., unpublished).

In conclusion, although in the *Drosophila* nervous system myelin-like wrapping of axons is not used to facilitate faster electrical conductance, the developmental program underlying the formation of such multiple membrane wraps may be be evolutionary ancient. Receptor tyrosine kinase activity regulates the extension of glial membranes around axons in flies and mammals. Possibly, the single ensheathment is primarily needed to provide enough trophic support for the different axons [1].

The blood–brain barrier and septate junctions

In the vertebrate system, the peripheral nerves are engulfed by the perineurium, and in invertebrates, such as *Drosophila*, the peripheral nerves are surrounded by a layer of perineurial and subperineurial glia (Figs. 1 and 2). Not much is known on the definition of these particular lineages but it is without doubt that these cells fulfill important tasks in setting the blood–brain barrier by establishing tight junction (vertebrates) or septate junctions (invertebrates).

Formation of septate-like junctions in vertebrates

In the vertebrate peripheral nervous system, saltatory conduction requires gaps between the myelinating cells called the nodes of Ranvier. The action potential is evoked in this small area which represents 0.1–0.3% of the surface of the entire axon. The concentration of voltage-gated ion channels restricts the ion flux needed for generation of action potential to a very small area. The tight association of the myelin sheath to the axon at paranodal regions flanking the node is critical for proper saltatory conductance and is characterized by a series of septate-like junctions [71–74]. The septate-like junctions function similarly to tight junctions and restrict paracellular transport of small molecules and ions. Septate-like axo-glial junctions, however, do not prevent the diffusion of lanthanum into the periaxonal space and hence do not provide a "tight" seal as do septate junctions found in the invertebrate nervous system [75, 76]. Nevertheless, many of the molecules that are associated with septate-like junctions have been highly conserved during evolution (Table 1 [77–79]).

Axonal Caspr and Contactin form a tight cis-complex required for the expression of Caspr at the cell surface and for the localization of Contactin at the paranode [80–87]. This axonal complex binds to glial Neurofascin155 although the precise means of interaction remain unclear and may involve other proteins or protein complexes [85, 87, 88]. Animals with loss of function mutations affecting septate-like junction components fail to form the paranodal junctions [89–91]. Additionally, the nerve conduction is significantly slower and intracellular organelles accumulate suggesting that normal axonal transport depends on the integrity of the paranodal junctions [79, 92]. Interestingly, loss of several other proteins like Netrin-1 or Deleted in Colorectal Cancer (DCC), CD9, ceramide galactosyl transferase and cerebroside sulfotransferase results in a similar destabilization of the paranodal complex, which indicates a rather complicated establishment of septate-like junctions in vertebrates [93–96].

Drosophila septate junctions

Invertebrates often show septate junctions that morphologically resemble those found in the paranodal regions at the nodes of Ranvier [34, 72]. Within the nervous system, they are prominently formed between subperineurial glial cells. A hallmark of all septate junctions is the NeurexinIV protein, which is the fly homolog of Caspr [97, 98]. In addition, Dcontactin and the Neurofascin155 homolog Neuroglian are localized to septate junctions. At least 15 other components localizing to the septate junctions are known (Table 1). In most cases, loss of any septate junction component results in the disassembly of morphologically discernable septate junctions [40, 99]. Based on the large number of proteins that are required to build functional septate junctions, it is hard to imagine how they assemble the elaborate ladder-like structures that are recognized in the TEM. Questions concerning the stability of junctions and their remodeling during epithelia morphogenesis are currently unclear and need further research. Likewise, it is not known how a possible paracellular

Table 1 Molecular composition of different cellular junctions

Septate junction	Septate-like junction	Tight junction	References
NeurexinIV	Caspr		[98, 132]
Gliotactin	Neuroligin		[38, 133]
Neuroglian	NeurofascinNF15		[134]
Megatrachea		Claudin	[135, 136]
Sinuous		Claudin	[136, 137]
Kune-kune		Claudin	[136, 138]
Nervana2			[100, 101]
Fasciclin II			[139]
Fasciclin III			[140]
Boudin			[141]
Coracle	4.1 Protein		[140]
Dcontactin	Contactin3		[83, 84]
Lachesin			[142]
Varicose			[143]
Yurt			[39, 40]
Disc Large		Dlg1	[144, 145]
Scribble		Scribble	[146, 147]
Lethal giant larva		Hugl-1	[148]
		Occludin	[149]
		Tricellulin	[150]
		JAMs (A-C)	[151]
		JAM4	[152]
		CAR	[153]
		ESAM	[154]
		MarvelD3	[155]
Polychaetoid		ZO-1, ZO-2, ZO-3	[156, 157]
		MAGI-1, MAGI-3	[158]
		MUPP1	[159]
		PATJ	[160]
		Cingulin	[161]
		JACOP/cingulin-like protein 1	[162]
aPKC-baz-Par6 complex		aPKC-Par3-Par6 complex	[163]
		ZONAB	[164]

transport of small molecules can be controled by septate junctions. Possibly, the difference between flies, where septate junctions define the paracellular seal, and the septate-like junctions of paranodes in vertebrates, which do not provide such a tight sealing function, resides in the integration of Claudin-like proteins in the *Drosophila* septate junctions [24]. One interesting component of the septate junctions in *Drosophila* is the Nervana2 (Nrv2) protein. Interestingly, Nrv2 was reported to be a component of septate junctions found in epithelial cells and *nrv2* mutants show leaky trachea and a defective blood–brain barrier (BBB) [24, 100–102]. The function of Nrv2 in cell adhesion seems to be pump-independent, as it is already described for Na/K-ATPases in the vertebrate system [100]. Interestingly, Nrv2 is strongly expressed in the wrapping glial cells, which do not appear to form septate junctions [24].

A clear hint that septate junctions control paracellular transport stems from the analysis of the gene *moody*. Mutations in the gene *moody* have been identified in a forward genetic screen for *Drosophila* mutants with altered cocaine sensitivity [36]. *Moody* mutants have abnormal subperineurial cells, which exhibit septate junctions of reduced length [33]. In consequence, the BBB is leaky and fluorescently labeled dextrane penetrates into the nerve cord [33]. Within the nervous system, only the subperineurial glial cells continuously express the GPCR Moody. Interestingly, transient RNAi expression leads to a temporary and reversible breakdown of the BBB [33]. Thus, the GPCR Moody is required for both the establishment

Table 2 *Drosophila* homologs of the vertebrate mylein proteom

Vertebrate ID	Vertebrates symbol	*Drosophila* CG	*Drosophila* symbol
P35762/P40240/Q922J6/P40237	Cd81/Cd9/Tspan2/Cd82	CG6120	Tsp96F
Q8VDN2/Q6PIE5/Q9WV27	Atp1a1/2/4	CG5670	Atp α*
P56371/Q91ZR1	Rab4a/4b	CG4921	Rab4
P62835/Q99JI6	Rap1a/b	CG1956	R
P07901/P11499	Hsp90aa1/ab1	CG1242	Hsp83*
Q7TQD2/Q9CRB6	Tppp/Tppp3	CG4893	CG4893
P16388/P63141/P16390	Kcna1/2/3	CG12348	Sh
B2RSH2/P08752/Q9DC51	Gnai1/2/3	CG10060	G-i 65A
O35526/P61264	Stx1a/b	CG31136	Syx1A
P26040/P26041/P26043	Ezr/Msn/Rdx	CG10701	Moe
P63321/Q9JIW9	Rala/b	CG2849	Rala
P21278/P21279	Gna11/q	CG17759	G 49B
P50396/Q61598	Gdi1/2	CG4422	Gdi*
Q9CQV8/P63101	Ywhab/z	CG17870	14-3-3zeta*
P35803/P60202	Gpm6b/Plp1	CG7540	M6*
P63011/P62823	Rab3a/c	CG7576	Rab3
Q504M8/Q9JKM7	Rab26/37	CG34410	Rab26
Q7TMM9/P68372/Q9D6F9/P99024	Tubb2a/2c/4/5	CG9359	βTub85D
P62874/P62880/P29387	Gnb1/2/4	CG10545	G13F
P06151/P16125/P00342	Ldha/b/c	CG10160	ImpL3
P17182/P17183/P21550	Eno1/2/3	CG17654	Eno
P68369/P05213/P68373/P05214	Tuba1a/1b/1c/3a	CG1913; CG2512	αTub84B; αTub84D
P15532/Q01768	Nme1/2	CG2210	Awd
P09411/P09041	Pgk1/2	CG3127	Pgk
P62821/Q9D1G1	Rab1/1b	CG3320	Rab1
O88935/Q64332	Syn1/2	CG3985	Syn
Q8CHH9/Q8C1B7	Sep8/11	CG4173; CG2916	Sep2; Sep5
P45591/Q9R0P5	Cfl2/Dstn	CG4254; CG6873	Tsr; CG6873
P05064/P05063	Aldoa/c	CG6058	Ald
Q9WVK4/Q9QXY6/Q9EQP2	Ehd1/3/4	CG6148	Past1
Q9DB05/P28663	Napa/b	CG6625	Snap
Q91X97/P62748	Ncald/Hpcal1	CG7641	Nca
P10126/P62631	Eef1a1/2	CG8280; CG1873	Ef1α48D; Ef1α100E
P12960	Cntn1	CG1084	Cont
Q8R464	Cadm4	CG10095; CG12591	dpr15; dpr16
P13595	Ncam1	CG3665	Fas2*
Q63912	Omg	CG42709	CG42709
Q8VDQ8	Sirt2	CG5085	Sirt2
P62259	Ywhae	CG31196	14-3-3ε
P60710	Actb	CG12051	Act42A
P40124	Cap1	CG33979	Capt
P84078	Arf1	CG8385	Arf79F
P84084	Arf5	CG11027	Arf102F*
P62331	Arf6	CG8156	Arf51F
O08539	Bin1	CG8604	Amph
Q8K298	Anln	CG2092	Scra
P16460	Ass1	CG1315	CG1315

Table 2 continued

Vertebrate ID	Vertebrates symbol	*Drosophila* CG	*Drosophila* symbol
Q0VF55	Atp2b3	CG42314	PMCA
P62204	Calm3	CG8472	Cam*
P35564	Canx	CG9906; CG11958; CG1924	CG9906; Cnx99A; CG1924
Q923T9	Camk2 g	CG18069	CaMKII
P00920	Car2	CG7820	CAH1
P61164	Actr1a	CG6174	Arp87C
O54991	Cntnap1	CG6827	Nrx-IV
Q9WUM4	Coro1c	CG9446	Coro
Q04447	Ckb	CG32031; CG5144	Argk*, **; CG5144
P23927	Cryab	CG4533	l(2)efl
O08553	Dpysl2	CG1411	CRMP
Q99KJ8	Dctn2	CG8269	Dmn
Q9JHU4	Dync1h1	CG7507	Dhc64C
O70251	Eef1b2	CG6341	Ef1β
P58252	Eef2	CG2238	Ef2b
P19096	Fasn	CG3524; CG3523	v(2)k05816; CG3523
O08917	Flot1	CG8200	Flo*
P62881	Gnb5	CG10763	Gβ5
Q9DAS9	Gng12	CG8261	Gγ1
P16858	Gapdh	CG12055; CG8893	Gapdh1; Gapdh2*
P13020	Gsn	CG1106	Gel
P06745	Gpi1	CG8251	Pgi
P05201	Got1	CG8430	Got1*
P56564	Slc1a3	CG3747	Eaat1**
P15105	Glul	CG1743	Gs2*,**
P62827	Ran	CG1404	ran
Q61316	Hspa4	CG6603	Hsc70Cb
P20029	Hspa5	CG4147	Hsc70-3
P63017	Hspa8	CG4264	Hsc70-4
P62482	Kcnab2	CG32688	Hk
Q9D1G5	Lrrc57	CG3040	CG3040
Q8BLK3	Lsamp	CG12369; CG2198	Lac*,**; Ama*
P14152	Mdh1	CG5362	CG5362
P14873	Mtap1b	CG34387	futsch
P63085	Mapk1	CG12559	rl
Q5SYD0	Myo1d	CG7438	Myo31DF
P14094	Atp1b1	CG9261; CG9258; CG8663	nrv2*,**; nrv1; nrv3
P55012	Slc12a2	CG4357	Ncc69
P46460	Nsf	CG33101; CG1618	Nsf2; comt
Q99K10	Nlgn1	CG13772	neuroligin
Q62433	Ndrg1	CG15669	MESK2
Q99LX0	Park7	CG1349; CG6646	dj-1β; DJ-1α
P99029	Prdx5	CG7217	Prx5
P53810	Pitpna	CG5269	Vib
P47857	Pfkm	CG4001	Pfk
Q61753	Phgdh	CG6287	CG6287

Table 2 continued

Vertebrate ID	Vertebrates symbol	*Drosophila* CG	*Drosophila* symbol
Q9DBJ1	Pgam1	CG1721; CG17645	Pglym78; Pglym87
Q9Z1B3	Plcb1	CG4574	Plc21C
Q99K85	Psat1	CG11899	CG11899
P67778	Phb	CG10691	l(2)37Cc
O35129	Phb2	CG15081	l(2)03709*
P27773	Pdia3	CG8983	ERp60*
P63318	Prkcc	CG6622	Pkc53E
P52480	Pkm2	CG7070	PyK
Q8BVI4	Qdpr	CG4665	Dhpr
P53994	Rab2a	CG3269	Rab2
P51150	Rab7	CG5915	Rab7*
P61028	Rab8b	CG8287	Rab8
P61027	Rab10	CG17060	Rab10
Q91V41	Rab14	CG4212	Rab14
P35293	Rab18	CG3129	Rab-RP4*
Q923S9	Rab30	CG9100	Rab30
Q8BHC1	Rab39b	CG12156	Rab39
Q8CG50	Rab43	CG7062	Rab-RP3
P60764	Rac3	CG2248; CG8556	Rac1; Rac2**
Q80ZJ1	Rap2a	CG3204	Rap2 l
Q99PT1	Arhgdia	CG7823	RhoGDI
Q9QUI0	Rhoa	CG8416	Rho1
P42208	Sep1	CG1403	Sep1
Q91V61	Sfxn3	CG11739	CG11739
Q9CWZ7	Napg	CG3988; CG6208	γSnap; CG6208
Q62261	Spnb2	CG5870	β-Spec*
Q60864	Stip1	CG2720	Hop
P08228	Sod1	CG11793	Sod*
P60879	Snap25	CG9474; CG40452	Snap24; Snap25
P46096	Syt1	CG3139	Syt1
Q61644	Pacsin1	CG33094	Synd
P11983	Tcp1	CG5374	T-cp1
P80314	Cct2	CG7033	CG7033*
P80315	Cct4	CG5525	CG5525
P80316	Cct5	CG8439	Cct5
P80318	Cct3	CG8977	Cctγ
Q9R1Q8	Tagln3	CG14996; CG5023	Chd64; CG5023
Q01853	Vcp	CG2331	TER94
P40142	Tkt	CG8036; CG5103	CG8036; CG5103
P17751	Tpi1	CG2171	Tpi
P62991	Ub	CG5271; CG2960; CG11624	RpS27A; RpL40; Ubi-p63E
Q02053	Uba1	CG1782	Uba1*
Q9R0P9	Uchl1	CG4265	Uch
P50516	Atp6v1a	CG3762; CG12403	Vha68-2; Vha68-1
P62814	Atp6v1b2	CG17369	Vha55
Q9Z1G3	Atp6v1c1	CG8048	Vha44

Table 2 continued

Vertebrate ID	Vertebrates symbol	*Drosophila* CG	*Drosophila* symbol
P50518	Atp6v1e1	CG1088	Vha26
O88342	Wdr1	CG10724	Flr

The left hand column lists the myelin proteom according to Jahn et al. (2009). Only proteins with *Drosophila* homologs are shown. *Drosophila* proteins marked with a * were already identified as being expressed in glial cells by Altenhein et al. [127] and proteins marked with ** were identified by Freeman et al. [126]

and the maintenance of the BBB. Possibly, Moody regulates the actin cytoskeleton, which has been demonstrated to have a pivotal function in the peripheral glia [103], to position the different components of the septate junctions.

Surprisingly, however, flies lacking all *moody* function are viable and can be easily kept as homozygous stock. GPCRs transmit signals through trimeric G-proteins, inducing a guanine nucleotide exchange on their Gα-subunits [104]. A role for G-protein signaling in *Drosophila* BBB formation was first suggested by the identification of the gene *loco*, which encodes an RGS-type protein expressed in a larger subset of glial cells [105]. Although *loco* mutants have been studied for a long time, the precise integration of Loco function into glial G-protein signaling has not yet been elucidated.

Control of septate junction formation via mRNA splicing

The septate junctions form relatively late at the very end of embryonic development [34]. Genetic screens have identified two mRNA processing factors as being essential for septate junction formation. *crooked neck* (*crn*) was shown to be required for the correct splicing of the *neurexinIV* pre-mRNA [106]. The gene *how* encodes three different mRNA binding proteins [HOW(S), HOW(M) and HOW(L)] which are linked to mRNA stability and splicing. Crn is an essential and evolutionary well-conserved general splicing factor, which binds to HOW(S) in the cytoplasm [106–108]. The HOW proteins are members of the STAR family (Signal Transduction and Activation of RNA) of RNA-binding proteins [109]. The STAR proteins are evolutionary well conserved and Quaking and Sam68 are well-known homologs in mammals [110, 111]. The Crn/HOW(S) complex must then be shuttled into the nucleus to modulate the splicing of *neurexinIV* and *nervana2*, which are both essential septate junction components [98, 101, 102, 106]. The *neurexinIV* gene generates two different isoforms by mutually exclusive and "tissue-specific" usage of exons 3 and 4. The protein encoded by the $neurexinIV^{Exon3}$ mRNA is enriched in septate junction-forming tissues and interacts with Dcontactin and Neuroglian, whereas the protein encoded by the $neurexinIV^{Exon4}$ mRNA is expressed by axons where it binds to the Ig domain protein Wrapper, which is expressed on the midline glia. This binding does not result in the formation of septate junctions [112–114].

What could be the benefit of a splicing-related mechanism in controlling septate junction formation? Possibly, in an initial phase, both NeurexinIV isoforms are generated and only upon a differentiation-dependent signal, e.g., when septate junctions reach certain maturity, is the nuclear import of the Crn/HOW(S) complex initiated and expression of the $NeurexinIV^{Exon3}$ isoform dominates. The exact molecular mechanism underlying this tissue-specific splicing is still unknown. Members of the STAR protein family, such as HOW, are often expressed tissue-specifically and have the ability to undergo post-transcriptional modification such as phosphorylation [115–118]. In humans, for example, a signal-dependent regulation of splicing can be regulated by phosphorylation of Sam68, which modulates the inclusion of exon-v5 of the CD44 mRNA [115]. A similar mechanism may operate for HOW function.

Conclusions and outlook

Here, we have shown that the overall organization of peripheral nerves of vertebrates is akin to the one of peripheral nerves in *Drosophila*. Both on a morphological and on a molecular level, astonishing homologies have been revealed in the past years. Wrapping of axons is regulated in very similar ways in *Drosophila* and mammals—despite the fact that in our nervous system myelin predominates. The apparent conservation of basic cellular mechanisms will very likely promote the analysis of genes and proteins first identified in mammalian myelin. The myelin proteome has been determined and long lists of proteins exist, which now need to be tested for their biological relevance [119–121]. A large fraction of the identified proteins have clear homologs in *Drosophila* and their relevance for glial differentiation is now being assayed using cell-type-specific RNA interference technologies (Table 2; [122–125]). In fact, for some of the fly homologs of the 294 proteins of the myelin proteome, glial expression has been noted [126, 127]. The regulation of

septate junction formation by splicing and possibly post-translational control of the nuclear access of splicing factors appears also evolutionary conserved. The HOW homolog Quaking is needed for proper development of myelinated nerves and has also been linked to the control of splicing [128–131]. This suggests that elucidation of the mechanism underlying the action of the Crn/HOW complex will shed some light on the biology of myelination.

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