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REVIEW ARTICLE

Emerging concepts in oligodendrocyte and myelin formation, inputs from the zebrafish model

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Abstract

Oligodendrocytes (OLs) are the myelinating cells of the central nervous system (CNS), which are derived from OL precursor cells. Myelin insulates axons allowing the saltatory conduction of action potentials and also provides trophic and metabolic supports to axons. Interestingly, oligodendroglial cells have the capacity to sense neuronal activity, which regulates myelin sheath formation via the vesicular release of neurotransmitters. Neuronal activity-dependent regulation of myelination is mediated by specialized interaction between axons and oligodendroglia, involving both synaptic and extra-synaptic modes of communications. The zebrafish has provided key advantages for the study of the myelination process in the CNS. External development and transparent larval stages of this vertebrate specie combined with the existence of several transgenic reporter lines provided key advances in oligodendroglial cell biology, axo-glial interactions and CNS myelination. In this publication, we reviewed and discussed the most recent knowledge on OL development and myelin formation, with a focus on mechanisms regulating these fundamental biological processes in the zebrafish. Especially, we highlighted the critical function of axons and oligodendroglia modes of communications and calcium signaling in myelin sheath formation and growth. Finally, we reviewed the relevance of these knowledge's in demyelinating diseases and drug discovery of pharmacological compounds favoring myelin regeneration.

KEYWORDS
axo-glial communications, demyelination, myelination, oligodendroglial cell lineage, zebrafish

1 | INTRODUCTION

In the central nervous system (CNS), myelin is a lipid-rich substance produced by oligodendrocytes (OLs) that wrap and compact cytoplasmic membrane extensions around axons. Myelination plays major roles in vertebrates: (1) it protects and insulates axons, allowing saltatory conduction of action potentials (AP), (2) it provides trophic and metabolic support to axons (reviewed in Nave, 2010; Saab & Nave, 2017; Stadelmann et al., 2019), and (3) it allows higher cognitive and intellectual functions.

OLs originate from multipotent neural stem cells, which are specified into OL precursor cells (OPCs), migrate through the CNS, proliferate and finally differentiate into mature myelinating OLs. Oligodendroglial development and myelination are highly regulated by numerous intrinsic and extrinsic signals (reviewed in Rowitch & Kriegstein, 2010; Mitew et al., 2014; Emery & Lu, 2015;

Takebayashi & Ikenaka, 2015). Among extrinsic signals, neuronal activity has emerged in the last decade as a major regulator of the myelination process (reviewed in de Faria et al., 2019; Thornton & Hughes, 2020).

The first evidence that myelination is regulated by neuronal activity was observed in the rodent developing visual system either with dark-rearing mice (Gyllensten & Malmfors, 1963), transected optic nerve (Barres & Raff, 1993) and tetrodotoxin (TTX) treated rats (Barres & Raff, 1993; Demerens et al., 1996). Since then, different *in vitro* and *in vivo* models and protocols have been used and developed to study how neuronal activity affects oligodendrogenesis and myelogenesis. These studies mainly used drugs or toxins, like TTX, clostridial botulinum toxin A (BoNT/A) or α-scorpion toxin (α-ScTx) to modulate neuronal activity (Barres & Raff, 1993; Demerens et al., 1996; Etxeberria et al., 2016; Gautier et al., 2015; Lundgaard et al., 2013; Stevens et al., 2002; Wake et al., 2011). These data showed that electrical activity regulates the myelination process, but not its initiation step. Myelination process is regulated by synaptic glutamate vesicular release along axons by acting on local synthesis of *mbp* mRNA (Wake et al., 2015). Opto- and chemo-genetic stimulations of cortical neurons were also developed and showed an increase in OL number and differentiation, and consequently of myelin synthesis (Gibson et al., 2014; Mitew et al., 2018). Finally, social isolation (Liu et al., 2012; Makinodan et al., 2012), environmental deprivation (Narducci et al., 2018), sensory experience (Hughes et al., 2018) and motor learning (e.g., running on an irregular wheel or reaching a pellet) (McKenzie et al., 2014; Sampaio-Baptista et al., 2013; Xiao et al., 2016) showed an impact on oligodendroglial lineage progression and myelination in rodents. For instance, social isolation has a negative impact on oligodendrogenesis and myelination, with a decrease number of myelinated axons and reduced myelin thickness. This has a long term behavioral and cognitive impacts in adulthood (Liu et al., 2012). Human studies also showed that learning new skills, such as juggling and playing piano, increases white matter volume (Hofstetter et al., 2013; Lakhani et al., 2016).

All these mentioned studies mainly used mouse or rat as experimental models, but the zebrafish has recently emerged as a suitable model system for the studies of OL development and myelination. Here, we reviewed OL development and myelination, with a special focus on activity-dependent regulation of myelination and axo-glial communications in the zebrafish. Finally, we reviewed how the zebrafish is used to study myelin diseases.

2 | THE ZEBRAFISH MODEL

Also known as *Danio rerio*, the zebrafish is a vertebrate originating from South Asia. The zebrafish is widely used as a model system in neurosciences as it provides several advantages. It can easily breed (around 200 eggs per week and per couple) and its external development simplifies the manipulation by an experimenter. Embryogenesis is completed within 3 days post fertilization (dpf), and by 5 dpf, larva can freely swim and start to hunt and feed themselves. Thanks to its transparent larval development, the zebrafish is a model of choice for the application of various optical techniques. The development of optogenetic tools (Del Bene & Wyart, 2012) like fluorescent sensors or light-gated channels are easier in the zebrafish than in rodent, as *in vivo* implantation of optical fibers is not needed. Live imaging studies of biological processes is simplified especially with the development of several transgenic reporter lines for neurons and glia. The generation of transgenic zebrafish lines was greatly improved by the discovery of the *Tol2* transposase element, derived from the medaka fish (Kawakami et al., 2000; Kawakami & Shima, 1999). The *Tol2* transposition system allows to randomly integrate an exogenous DNA sequence in germinal cells with a 50%–70% rate of genomic integration in zebrafish (Kawakami, 2007; Kawakami et al., 2004; Urasaki et al., 2006). Other transgenic tools like morpholino-mediated knockdown or CRISPR-mediated knock-out were also developed to mediate loss-of-function of specific genes (Albadri et al., 2017; De Santis et al., 2016). In research laboratories, zebrafish housing and husbandry are classically maintained under 14/10 h of dark/light cycle, with constant water temperature at 28.5°C. Zebrafish larva are fully developed and have reach maturity by 2–3 months.

3 | OLIGODENDROGENESIS

3.1 | Oligodendrocyte development

OLs are resident cells of the CNS where they play a major role in the formation of myelin around axons. Mature myelinating OLs are generated from OPCs that are specified from CNS multipotent neuroepithelial stem cells (NSCs). These cells, which are located along the spinal cord (SC) central canal and forebrain ventricles, possess a radial polarity and have the capacity to differentiate into neurons or highly proliferative intermediate progenitors by asymmetric cell divisions (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004, 2008). OPCs will then arise from these intermediate progenitors.

3.1.1 | OPC specification

In rodents, two major waves of OPC production have been described in the SC. At the embryonic day (E) 12.5, OPCs are generated in the pMN domain of the neural tube, which also give rise to motor neurons (Wu et al., 2006). Then, a second wave occurs in the dorsal dP6 domain around E14.5 (Cai et al., 2005; Fogarty et al., 2005; Noll & Miller, 1993). In the telencephalon, three successive waves of OPC specification have been reported (Kessaris et al., 2006). The first wave of oligodendrogenesis starts in the medial ganglionic eminence (MGE) and in the pre-optic area at E12.5 (Spassky et al., 2001), followed by a second wave in lateral ganglionic eminence (LGE) at E14.5. Then, in late embryonic development and in early post-natal life, the third wave of OPCs is generated from the dorsal part of the telencephalic ventricular zone (Vallstedt et al., 2005). Interestingly, the first and second waves of OPCs generated in the forebrain are completely

eliminated and replaced by OPCs of the third wave (Kessaris et al., 2006). During the postnatal period and following their production within the subventricular zone (SVZ), OPCs migrate to distant zones to colonize white and gray matter, following attractive and repulsive signals (Simpson & Armstrong, 1999; Tsai et al., 2002, 2003).

Gradients of morphogens along the dorsal-ventral axis of the neural tube act as spatial inducer or repressor of specific transcription factors such as Olig2, Nkx2.2/2.6 or Sox8/9/10. After their determination and migration, OPCs differentiate into OLs. The generation of immature pre-myelinating OLs is favored by several intrinsic and extrinsic factors including growth factors such as IGF-1 (insulin growth factor) or the T3 thyroid hormone, and previously mentioned transcription factors expression (reviewed in Baumann & Pham-Dinh, 2001; Miron et al., 2011; Mitew et al., 2014).

While rodent oligodendrogenesis has been extensively described, studies of OL development emerged much later in the zebrafish model, notably with the development of several transgenic reporter lines, allowing *in vivo* fluorescence imaging of oligodendroglia and myelin. The first studies on oligodendrogenesis in zebrafish used the transgenic Tg(Olig2:GFP) line (Buckley, Marguerie, Alderton et al., 2010; Park et al., 2002, 2007; Shin et al., 2003). It was showed that Olig2+ multipotent proliferative cells arise from the ventral pMN domain (Park et al., 2002), migrate to the dorsal SC (Shin et al., 2003) where they divide asymmetrically at 36 hours post fertilization (hpf) to produce only OPCs (Buckley, Marguerie, Alderton et al., 2010; Park et al., 2002, 2007). Sox10 + Olig2+ cells were observed from 2 dpf in the ventral SC, implying that the Olig2-precursors effectively differentiate into oligodendroglial cells (Buckley, Marguerie, Alderton et al., 2010), which will finally start to ensheath axons around 3 dpf. As in rodent, the pMN domain of the SC gives birth first to motor neurons and then to oligodendroglial cells, following the similar mechanisms described in rodents (Orentas et al., 1999; Spassky et al., 2001; Tekki-Kessaris et al., 2001). For instance, the specification of NSCs into OPCs in the ventral neural tube is also dependent on sonic hedgehog (SHH) signaling (Chung, et al., 2013; Doll et al., 2021; Park et al., 2004; Ravanelli et al., 2018; Schebesta & Serluca, 2009).

The second wave of OPCs derived from the dorsal SC requires fibroblast growth factor (FGF) (Cai et al., 2005; Chandran et al., 2003). FGF is involved in the specification and proliferation of OPCs (Esain et al., 2010) in the forebrain in combination with an inverse gradient of WNT (Azim & Butt, 2011; Tawk et al., 2011) and bone morphogenetic proteins (BMPs), which are known to promote astrocyte specification (Agius et al., 2004; Shimizu et al., 2005) and to inhibit OL differentiation and myelination (Fancy et al., 2009; Feigenson et al., 2009; Ye et al., 2009). Other signaling cascades are known to be conserved between zebrafish and rodents such as Notch signaling influencing the switch from motor neurons to OPC specification in the pMN domain (Appel et al., 2001; Kim et al., 2008; Park et al., 2005; Ravanelli et al., 2018; Schebesta & Serluca, 2009) and the ErbB/EGF signaling pathway that promotes OL development and myelination (Lyons et al., 2005; Pruvot et al., 2014).

More recently, single-cell RNA sequencing studies of pMN progenitor cells on 24, 36 and 48 hpf Tg(Olig2:eGFP) larval SC allowed to identify less than 30 cell clusters among them Olig2+ cells but also an Olig2- population of post-mitotic neuroblasts that do not express Olig2 transcripts once differentiated. At 36 hpf, few Sox10+ cells were sorted by fluorescence-activated cell sorting, indicating that at this developmental time, OPCs were not yet specified. Moreover, a population of Sox19a+ Olig2+ Nkx2.2a+ Olig1+^{low} cells were also isolated, suggesting the presence of a pre-OPC population within this domain. Altogether, the pMN domain contains two distinct progenitor populations: a pre-OPC one and a pre-neuronal progenitor population. OPC specification occurs in parallel with a decrease in expression of GS Homeobox 2 (Gsx2) gene, which is involved in neuronal fate during development (Scott et al., 2021) (Figure 1).

3.1.2 | OPC migration

Using another transgenic line, the Tg(Nkx2.2a:GFP), the detailed migration of OPCs was studied in the developing zebrafish SC (Kirby et al., 2006). OPCs originating from the pMN domain migrate along axons of the SC, and more preferentially along the dorsal longitudinal fascicle (DLF). They sometimes migrate more dorsally but always come back along this fascicle. Migrating OPCs have been described as highly branched and dynamic cells with filopodium-like processes, sensing the surrounding environment. Most of these processes were oriented toward the direction of migration. Moreover, when an OPC contacts another neighboring OPC, it retracts its processes and changes its migratory trajectory. These migratory movements and extension-retraction cycles last several hours and stop at the onset of myelination around 60–72 hpf (Kirby et al., 2006).

OPC migration is regulated by several extracellular and intracellular components, such as growth factors, chemokines, and actin cytoskeleton remodeling. Recently, the Gria4a (GluR4a) AMPA receptor subunit has been also implicated in OPC migration in the zebrafish SC, as CRISPR-mediated loss-of-function of *Gria4a* leads to fewer OPCs distributed along DLF and to a decrease in OPC migration speed at 3 dpf. These migration defects came from a cell-autonomous mechanism as grafting of mutated cells in wild type zebrafish lead to the same phenotype. Moreover, Gria4a$^{-/-}$ mutated animals possess shorter internodes suggesting a dual role of the GluR4a subunit in OPC migration and myelination process (Piller et al., 2021). Neurons are also involved in the regulation of OPC migration via the activation of adenosine receptor A2A and further in activity-dependent control of myelination. Inhibition of A2A receptors with chemical antagonists results in decrease neuronal activity and ectopic OPC migration to the PNS and specific loss-of-function of both A2A isoforms a and b suggests that the isoform b regulates OPC migration in the CNS (Fontenas et al., 2019).

3.2 | OPC diversity

Several *in vivo* and *in vitro* studies revealed heterogeneity of OPCs in the rodent CNS (Dimou & Simons, 2017; Foerster et al., 2019; Soreq

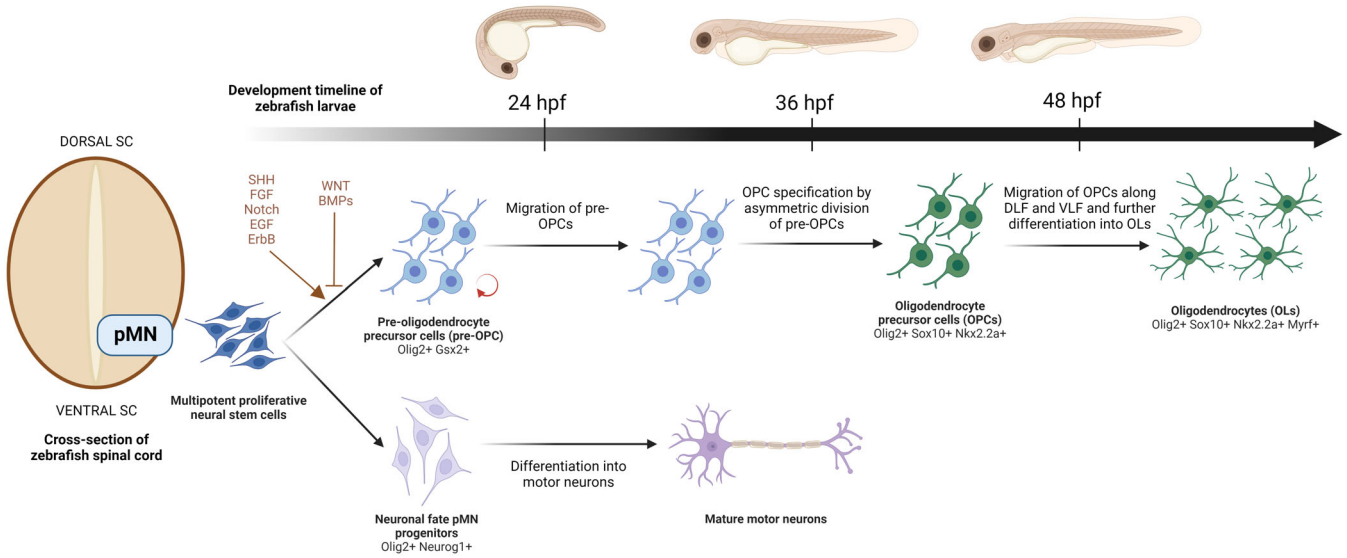

FIGURE 1 OPC specification and differentiation in the zebrafish. Oligodendroglial cells arise from multipotent neural stem cells localized in the pMN domain of the zebrafish SC. At 24 hpf, those neural cells first give rise to neuronal progenitors expressing Olig2 and Neurog1. Then at 36 hpf, pre-OPCs are specified from PMN progenitor cells and migrate along SC. Finally, following a ventral-dorsal SHH gradient, pre-OPCs divide asymmetrically to give OPCs and more precursor cells. As soon as 48 hpf, OPCs will differentiate into Olig2+ Sox10+ Nkx2.2a + Myrf+ OLs. OLs, oligodendrocytes; OPCs, OL precursor cells; SC, spinal cord; SHH, sonic hedgehog

et al., 2017; Zhang et al., 2014). This diversity occurs with age but also between CNS regions, and thus in term of ions channels and receptors expression (Spitzer et al., 2019) or myelination capacities (Viganò et al., 2013). RNA-sequencing experiments showed different transcriptional profiles between young and aged mice (Marques et al., 2016, 2018; Moyon et al., 2015).

As in the rodent CNS, OPCs are also a heterogeneous cell population in the zebrafish both in term of gene expression profile and ability to differentiate and myelinate. For instance, at least two populations of OPCs have been described in the zebrafish SC. A first one mainly located in the lateral SC, near axons and dendrites, and a second located in the central SC in close proximity with neuronal cell bodies (Marisca et al., 2020). 3D imaging of oligodendroglial cell morphology revealed that both populations extend their processes within the lateral SC, even those with their soma located in the vicinity of neurons. Moreover, this OPC population possesses a much more elaborate process network than OPCs in the lateral SC. Transcriptomic analysis showed that both populations have a similar cell division capacity, but OPCs with their soma located within the central SC does not differentiate or myelinate permissive axons even at late developmental stages. These intriguing data raise the question of how these two subpopulations are linked. Time-lapse videomicroscopy showed that OPCs of the neuron-rich central area divide and one of the two daughter cells migrates to the lateral SC where it differentiates into myelinating OL. It was also showed that both OPC populations exhibit spontaneous and evoked calcium waves. These calcium waves, which are more frequent and of higher amplitudes in OPCs located in central SC, are enhanced by neuronal activity and precedes OPC proliferation and further differentiation within the lateral SC. Moreover, OPCs of the lateral SC mainly express genes involved in oligodendroglial differentiation and proliferation, compared to the other population that mainly expresses neurotransmitter (NT) receptors and transsynaptic adhesion proteins. Overall, these data suggest that myelinating OLs of the lateral SC come from proliferating OPCs of the neuron-rich SC and that axon-OPC synapses in this SC area are not implicated in the regulation of differentiation and myelination processes (Marisca et al., 2020).

4 | MYELIN SHEATHS FORMATION AND REGULATION

4.1 | Myelin composition and structure

Most zebrafish genes share homology with human genes (Barbazuk et al., 2000; Postlethwait et al., 2000). Regarding myelin genes, a homology of 70%–90% exists between zebrafish and human (Howe et al., 2013). The major myelin proteins such as myelin basic protein (MBP), myelin protein zero (MPZ/P0) (Brösamle & Halpern, 2002; Schweitzer et al., 2003) and proteolipid protein (PLP/DM20) (Brösamle & Halpern, 2002; Schweitzer et al., 2006) are also present in *D. rerio*. The expression of genes encoding for myelin proteins starts as soon as 2 dpf and precedes the formation of compact myelin, suggesting similar functions of these genes between zebrafish and mammals (Brösamle & Halpern, 2002). Moreover, because of the tetrapods divergence during evolution, most of the zebrafish genes have been duplicated in several copies. For instance, *plp* and its splice variant *dm20* are present in three pairs of orthologues (Schweitzer et al., 2006).

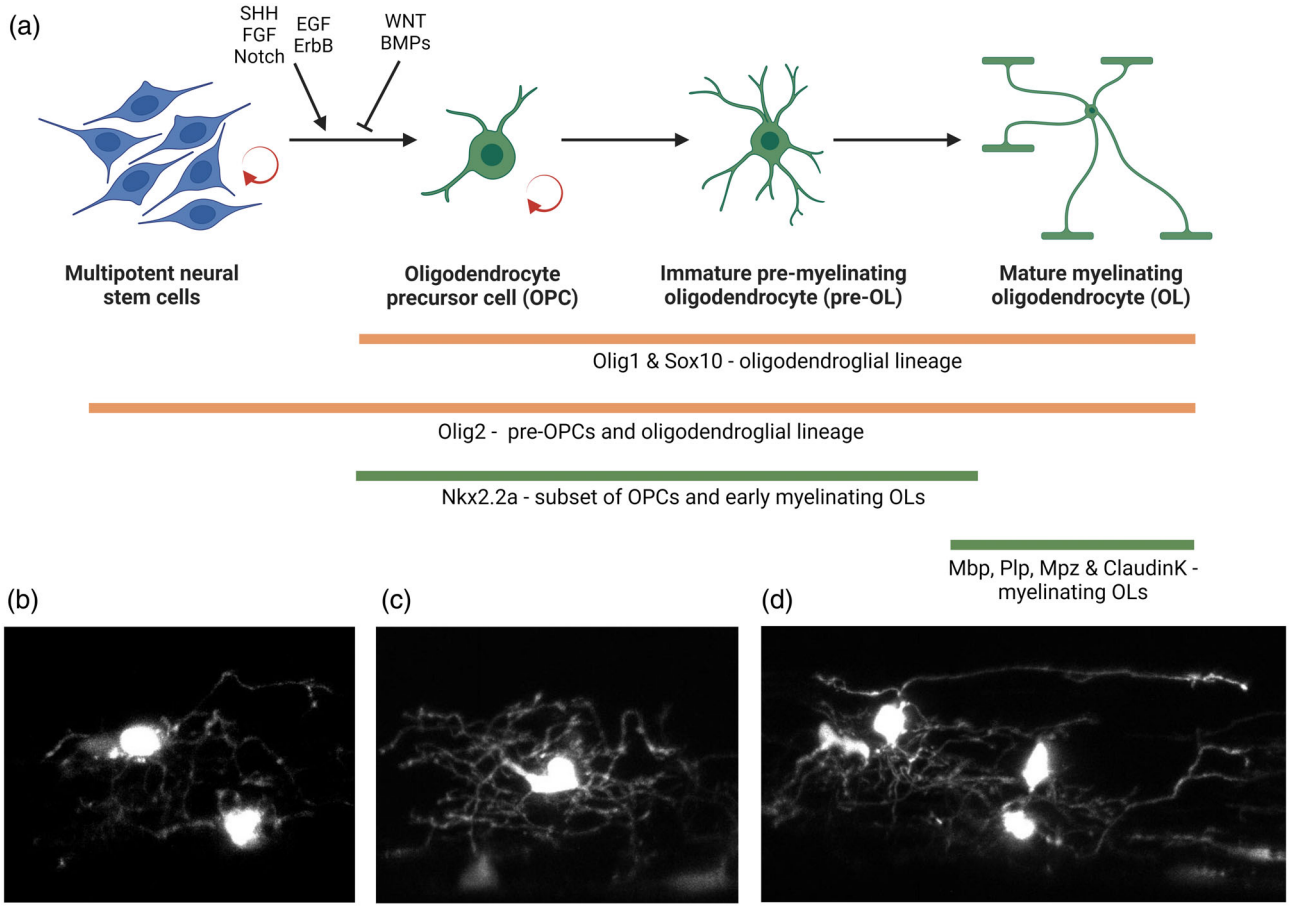

FIGURE 2 Regulation of OPC differentiation and associated oligodendroglial markers. (a) OPC specification is favored by SHH, Notch, EGF, FGF, and ErbB signaling pathways. On the contrast, OPC specification is inhibited by WNT and BMPs signaling. Zebrafish oligodendroglial cells express Olig2, Olig1 and Sox10 transcription factors. Nkx2.2a is expressed in early OPCs and immature OLs, while PLP, MBP, MPZ, and Claudin K are markers of more mature myelinating OLs. Following their migration from pMN domain, OPCs (b) can differentiate into pre-myelinating ramified OLs (c) and then into mature myelinating OLs (d) with myelin sheaths. Oligodendroglial cell morphologies are easily distinguishable using transgenic animal like here with Tg(Olig1:KalTa4, UAS:mRFP) 5 dpf larva. OLs, oligodendrocytes; OPCs, OL precursor cells; SHH, sonic hedgehog

The major difference of the zebrafish myelin as compared to mammals is the expression of MPZ/P0 in the CNS (Bai et al., 2011). Indeed, in rodents and humans, MPZ/P0 protein is only expressed within the peripheral nervous system (PNS) and MBP within both nervous systems. MPZ/P0 is considered as the major CNS myelin protein in the zebrafish (Howe et al., 2013; Jeserich et al., 1997, 2008). New myelin proteins were also described in this model, like Zwilling-A and -B (Schaefer & Brösamle, 2009) or claudin K (claudin31) (Münzel et al., 2012) (Figure 2). Very recent proteomic study reported that only 38% of myelin proteins were known nowadays with MBP, MPZ, and PLP constituting respectively 8%, 12%, and 3% of total myelin proteins (Siems et al., 2021).

4.2 | Myelination in the zebrafish

As early as 2 dpf, RNA in situ hybridization showed *dm20*, *mpz/p0*, and *mbp* transcripts expression in the ventral medial hindbrain (Brösamle & Halpern, 2002). At 3 dpf, expression of *mbp* mRNA and protein were confirmed by qPCR and immunohistochemistry, respectively, near Mauthner cells and ventral SC axons (Buckley, Marguerie, Alderton, et al., 2010). At 4 dpf, positive cells for DM20, P0, and MBP increased in number and expanded into the ventral hindbrain bundle, but also in the midbrain and the SC. At the same time point, electron microscopy analysis showed large-diameter axons of the lateral line surrounded by OLs, and at 7 dpf, only large axons of the lateral line and of the optic and peripheral nerves were surrounded by compact myelin, especially the large Mauthner axons (Brösamle & Halpern, 2002). However, zebrafish myelin is less compact than in mammals, even at 15 dpf (Brösamle & Halpern, 2002; Buckley, Marguerie, Alderton, et al., 2010; Kazakova et al., 2006; Voas et al., 2007).

The first axons to be myelinated in the zebrafish are the large Mauthner axons, located within the ventral longitudinal fasciculus (VLF). Myelination starts around 60 hpf on the proximal anterior part, around somite 3, and then move posteriorly (Almeida et al., 2011)

(Figure 3a,b). Using the Tg(Nkx2.2a:meGFP) line, an anterior–posterior gradient of OL maturation was observed with mature myelinating OLs located more anteriorly than immature pre-myelinating OLs (Czopka et al., 2013). Myelination occurs along a ventral-dorsal gradient, with first the myelination of ventral Mauthner axons followed by those of DLF axons (Almeida et al., 2011) (Figure 3b).

Between 4 and 8 dpf, a significant increase of myelin internode length occurs (Figure 3a). This increase is due to two distinct mechanisms: (1) the longitudinal growth of internodes and (2) the production of new myelin sheaths. Similarly to the data reported in mice (Watkins et al., 2008), a short time window is necessary for a single OL to wrap axons in the zebrafish. In fact, each individual OL only has 5 h to

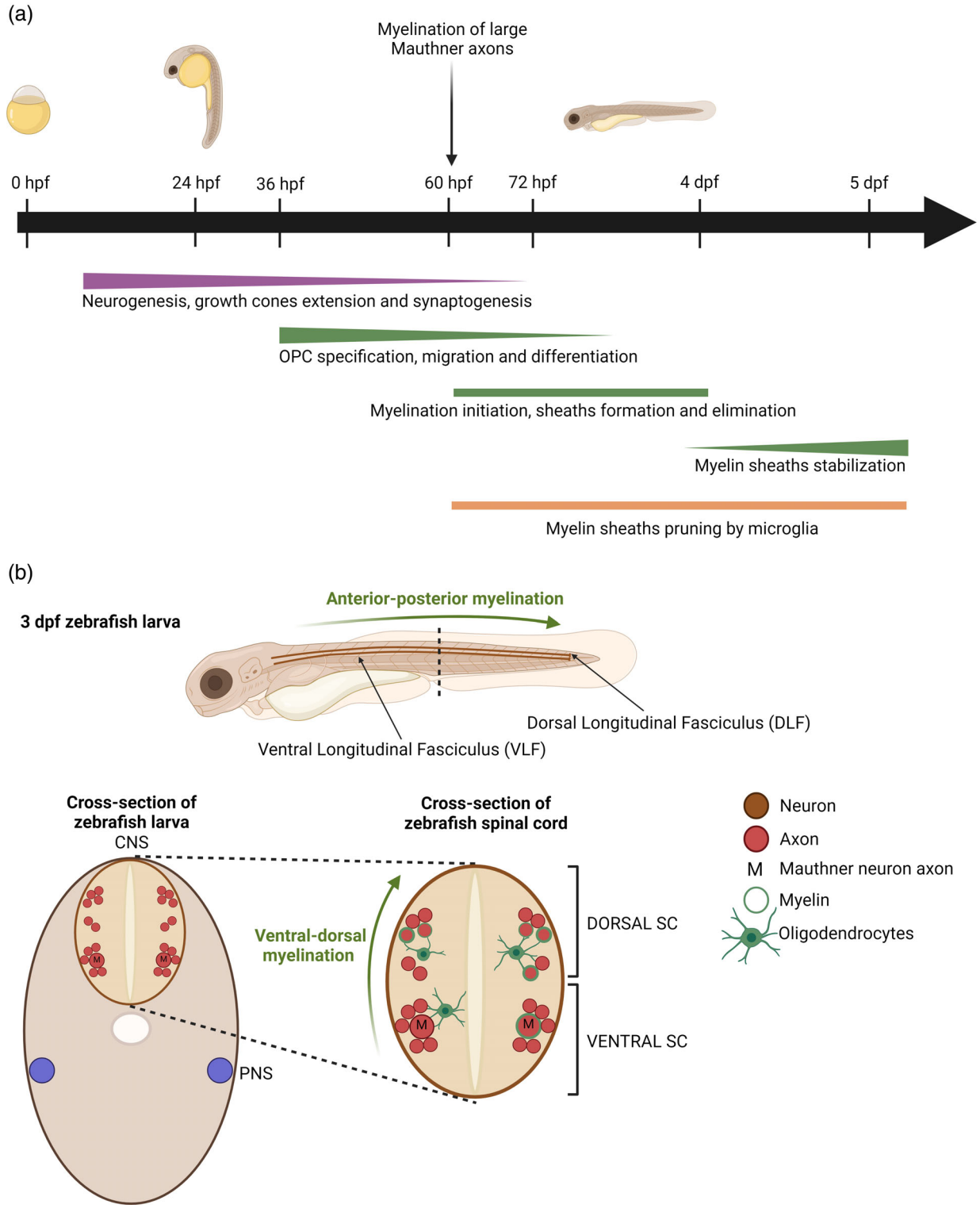

FIGURE 3 Legend on next page.

initiate and form myelin around an axon, with in average 5 to 20 internodes per OL. After this 5 h time window, new sheaths are not produced but internodes continue to elongate (Czopka et al., 2013) during 3 days until they reach their final lengths (Auer et al., 2018). Myelin sheath growth is highly variable, with phases of retraction, elongation, and stabilization (Czopka et al., 2013; Liu et al., 2013) (Figure 3a). The growth rate of myelin internode varies between minus 6 to plus 41 μm/day (Auer et al., 2018). These studies suggest that axon-OPCs communications, OPCs migration, as well as sheath retractions, are highly regulated events that control which axons will be fully myelinated.

Three different mechanisms could explain sheaths retraction. First, the constitutive activation of Fyn kinase in myelinating OLs leads to the production of 33% more myelin internodes, with 68% more myelin covering axons in animals aged of 7 dpf. Moreover, the number of OLs do not seem to be affected by Fyn overexpression. On the other hand, the use of Fyn morpholino led to a decrease in myelin production by individual OLs (Czopka et al., 2013). This work together with a previous study highlighted the critical role of Fyn kinase in the local translation of *mbp* mRNA (Wake et al., 2011). Neuregulin1 (NRG1) – ErbB2/ErbB3 signaling also regulates myelin sheath formation, as in NRG1 and ErbB morpholino-mediated knockdown animals less myelin segments were retracted, so more sheaths were stabilized (Liu et al., 2013). Finally, microglial cells have the capacity to remodel myelin pattern by engulfing myelin internodes. Myelin sheaths that were pruned by mpeg+ microglial cells were longer than sheaths that just retracted. Microglial engulfment appeared to count for 70% of total myelin pruning (Hughes & Appel, 2020) (Figure 3a).

Besides intrinsic and secreted extrinsic signals regulating myelination (Emery & Lu, 2015; Mitew et al., 2014; Rowitch & Kriegstein, 2010; Takebayashi & Ikenaka, 2015), axonal diameter is also a critical parameter in this process. *In vitro* experiments, mainly using rodents cells, showed that OLs can wrap engineered polymer nanofibers, especially those with a diameter superior to 0.4 μm (Lee et al., 2012) and even inert paraformaldehyde-fixed axons (Rosenberg et al., 2008). In both cases, OLs have the capacity to produce myelin sheaths of the same length. Moreover, it appears that axon diameter impacts sheath length as large diameter axons possess longer sheaths than small diameter ones (Bechler et al., 2015; Murray & Blakemore, 1980). However, it is still unclear how the myelination pattern of variable internode lengths along an axon occurs over time. In the zebrafish, internode length variability seems to be established within the 3 days during the myelination process. After this time window, myelin internodes have a length ranging from 6 to 87 μm at 7 dpf, period coinciding to the end of the active myelination period. After this developmental period, differences between ventral and dorsal SC axons were not observed (Auer et al., 2018). Moreover, no correlation between axon caliber and sheath length have been noted (Auer et al., 2018). The number of neighboring neurons also regulates the myelination potential of individual OLs. In fact, when the number of Mauthner axons is increased either by injection of Notch1a morpholino (Liu et al., 2003) or of Hoxb1 mRNA (Hale et al., 2004), the number of myelinating OLs do not change, but they rather adapt their behavior to produce more myelin to wrap additional axons. Also, OLs located dorsally can also extend their processes to myelinate supernumerary ventral Mauthner neurons (Almeida et al., 2011).

It is important to highlight that even after the 3 to 7 dpf period of massive oligodendrogenesis and myelination, OPCs can still differentiate into mature myelinating OLs up to at least 28 dpf (Marisca et al., 2020). OPCs of the central SC differentiate in a 3 days period following cell division, and OPCs migrate to the lateral SC at least 24 h after mitosis.

4.3 | Neuronal activity-dependent myelination in the zebrafish

Several studies performed in rodents showed that neuronal activity regulates myelination (Demerens et al., 1996; Gibson et al., 2014; Kukley et al., 2007; Lundgaard et al., 2013; Mitew et al., 2018; Stevens et al., 2002; Wake et al., 2011; Ziskin et al., 2007). AP firing favors myelination, and oppositely, a decrease in AP propagation leads to a decrease in the number of myelinated axons in dorsal root ganglion neurons and OPC co-cultures. These results were corroborated by *in vivo* experiments in mouse optic nerve and rat cerebellum (Barres & Raff, 1993; Demerens et al., 1996; Etxeberria et al., 2016; Gautier et al., 2015). Interestingly, a blockade of electrical activity has no impact on myelination initiation, thus suggesting a possible effect of electrical activity on myelination progression (Demerens et al., 1996). Several years after these pioneer studies, activity-dependent glutamate vesicular release along active axons and downstream signaling pathways in oligodendroglial cells emerged as key regulator of the myelination process. Initial myelination events of such as MBP production, are controlled by synaptic glutamate vesicular release by active neurons and activation of NMDA or metabotropic receptors on OPCs/OLs. It has also been shown that different

FIGURE 3 Neurogenesis, oligodendrogenesis and myelination in the zebrafish spinal cord. (a) Timeline of neurogenesis and oligodendrogenesis. Soon after the neurogenesis, OPCs specification from pMN precursors starts around 36 hpf. Rapidly, OPCs migrate along spinal cord axons by extension of their processes to sense their environment. Finally, OPCs differentiate into mature myelinating OLs, which will form myelin sheaths in a 5 h time-window. Sheaths elimination can still occur between 3 and 4 dpf, and internodes then remain mainly stable. (b) the zebrafish spinal cord (SC) is composed of two parallel tracts: The dorsal longitudinal fasciculus (DLF) and the ventral one (VLF). Large diameter Mauthner axons are located within the VLF and are the first axons to be myelinated at 60 hpf following an anterior–posterior myelination gradient. Soon after, CoPA axons located in the dorsal SC are myelinated following a ventral-dorsal myelination gradient. Two populations of OPCs are present in the SC: A proliferative population in the neuron-rich SC, and a differentiated one in the lateral SC. OPCs, OL precursor cells

processes from a single OL act independently from each other to myelinate active axons via local translation of MBP protein that is controlled by activity-dependent vesicular release (Wake et al., 2011, 2015). Moreover, the vesicular release of glutamate induced by neuronal activity effectively modulates myelin formation by providing spatial information for myelin ensheathment, as shown with the vGlut2 knock-out mouse line (Etxeberria et al., 2016). In fact, when vGlut2 is deleted on sensory neurons, an increased number of paranodes and a decreased internodes length were observed as compared to controls, but without any changes of myelin thickness. Furthermore, optogenetic or pharmacogenetic stimulations of the premotor cortex promote oligodendroglial development and myelination in an activity-dependent manner (Gibson et al., 2014; Mitew et al., 2018). These findings were also corroborated by classical electrophysiological stimulations of neurons in the rat SC (Li et al., 2010).

In zebrafish, toxins like TTX were also used and showed similar results. First, TTX applications on 2 dpf larva showed that electrical activity affects the axonal choice by individual OLs, without affecting oligodendroglial cell lineage differentiation or myelination initiation. Similar results were also obtained when the inward rectifier potassium channel Kir2.1 was selectively expressed on phox2b + neurons (Hines et al., 2015). Interestingly, when neuronal activity is modulated by Veratridine, a well-known sodium channel modulator or by photon stimulation, no increase in the myelination process of phox2b + axons was noted, suggesting that neuronal activity is not sufficient to favor myelin elongation (Hines et al., 2015). Nonetheless, larva treated with pentylenetetrazol, a GABA receptor antagonist also known to increase neuronal activity, showed a significant increase in the number of oligodendroglial cells and of myelin internodes in the SC (Mensch et al., 2015), suggesting that NT synaptic vesicular release promotes myelin sheath elongation (Hines et al., 2015; Mensch et al., 2015).

Neuronal activity is efficiently relayed into oligodendroglial cells by their expression of receptors for neuronal NTs and neurotrophic factors (Bergles et al., 2000; De Biase et al., 2010; Marisca et al., 2020; Marques et al., 2016; Zhang et al., 2014). For instance, OPCs express a repertoire of NT receptors such as GABA, NMDA and AMPA receptors, as well as ion channels. NT synaptic vesicular release mediated by VAMP proteins is also necessary to promote myelin internodes elongation in zebrafish larva. Indeed, specific expression of TeNT, a specific inhibitor of synaptic vesicle exocytosis, in phox2b + neurons leads to a drastic decrease of the number of myelinating axons and myelin internodes were also shorter (Hines et al., 2015). However, the major question that remain not fully understood yet is how does synaptic vesicle release regulate myelin sheath elongation. By crossing the transgenic lines Tg(phox2b:Gal4) with Tg(UAS:synaptophysin-GFP), synaptic vesicles were observed in stationary state along axons at ensheathment sites and in motile state along unmyelinated segments (Hines et al., 2015). An elegant study using the synaptophysin marker (Wiedenmann & Franke, 1985) fused with a pH-sensor (SypHy) (Zhu et al., 2009) showed that sites of vesicle exocytosis occurred at the growing extremity of collateral branches but also at axon-OL contact sites, in ventral reticulospinal neurons. Moreover, SypHy fluorescent sites co-localized with the vesicular glutamate transporter vGlut1 and with Neurofascin. This suggests that glutamate vesicle exocytosis occurs at hemi-nodal region, where nascent myelin sheaths elongate, and that glutamate vesicular release promote sheaths elongation. Expression of BoNT completely abrogates SypHy activity and reduces sheaths stability and growth (Almeida et al., 2021).

Finally, what is the role of vesicles release and neuronal activity in sheaths retraction? To answer this question, TeNT toxin was expressed in zebrafish neurons, and a decrease in oligodendroglial cells number was observed but myelin sheaths initiation was not affected, confirming that neuronal activity is not required for the initiation of ensheathment by individual OLs. Nevertheless, 75% of myelin sheaths disappeared 90 minutes after their initiation. Sheaths that more frequently retract were shorter (length inferior at 10 μm) with a slow ensheathment and wrapping processes. This suggests that maintenance of newly formed internodes is dependent on axonal activity, just as the elongation phase (Hines et al., 2015).

4.4 | Calcium regulates the myelination process

Calcium is a very well-known second messenger implicated in many intracellular signaling pathways downstream vesicular release of glutamate or GABA (Gallo et al., 1996; Hamilton et al., 2017; Lundgaard et al., 2013; Sun et al., 2016). Several studies, in rodents and zebrafish, showed localized calcium events in OPCs (Sun et al., 2016), myelinating OLs (Wake et al., 2011) and myelin sheaths (Baraban et al., 2018; Krasnow et al., 2018; Micu et al., 2016). In the zebrafish SC, calcium events occur in cell processes but also in the soma of OPCs, pre-OLs and myelinating OLs (Baraban et al., 2018; Krasnow et al., 2018). These calcium transients last longer than 3.5 s (Baraban et al., 2018), but appear to have a short duration in cell processes as compared to the soma (Krasnow et al., 2018). Half of those calcium events appear to regulate myelin sheaths formation, as when neuronal activity is inhibited by TTX, a 2-fold decrease in the number of internodes was noted. On the other hand, an increase in neuronal activity leads to internodes elongation. Furthermore, sheaths elongation tends to occur in a time window of 5 h after calcium transients, highlighting a role of these transients in sheaths elongation (Krasnow et al., 2018). Ca^{2+} waves in myelin sheaths exhibited a significant diversity in term of frequency, amplitude, and duration. High-amplitude long duration calcium waves traveling from myelin internodes to cell processes are responsible for sheaths retraction. Those types of calcium signatures precede the activation of the calpain enzyme, a well-known protease involved in dendrites elimination in Drosophila development. Using both a chemical and a genetic approaches, it was showed that calpain inhibition leads to less sheath retraction and thus to an increase number of myelin segments, suggesting a role of this calcium-dependent protease in the myelin sheath formation (Baraban et al., 2018). In rodents, longitudinal myelin sheaths remodeling was observed mainly at postnatal (P) days P13-P15 following large amplitude of Ca^{2+} waves. Here, myelin elongation happens thanks to calcium release by mitochondria located within the non-compacted inner cytoplasmic

tongue and at paranodal loops in P15 but also in adult mice (Battefeld et al., 2019). Cyclosporin A/rotenone-mediated inhibition of the mitochondrial permeability transition pore (mPTP) completely abolished calcium transients in myelin internodes during active myelination step suggesting a role for mPTP in myelin remodeling. Nevertheless, unlike Krasnow and colleagues, they did not observe a correlation between neuronal activity and calcium transients (Battefeld et al., 2019; Krasnow et al., 2018).

In rodents, voltage-gated calcium ($VGCa^{2+}$) channels are known to be involved with AMPA receptors in OPCs development (Gudz et al., 2006; Harlow et al., 2015; Paez et al., 2010). In the zebrafish, activation of $VGCa^{2+}$ with its agonist (±)-Bay K8644 rescues the defective migration and myelination phenotypes in Gria4a larva mutants suggesting that both processes are regulated by Ca^{2+} signaling downstream to AMPA receptor activation and further $VGCa^{2+}$ stimulation and extracellular calcium influx (Piller et al., 2021). A very recent study showed that $VGCa^{2+}$ antagonist cadmium decreases by half the frequency of intrinsic Ca^{2+} in OPC micro-domain, confirming that those voltage-gated calcium channels and not glutamatergic NMDA or AMPA receptors play a role in the generation of intrinsic calcium waves in OPCs. Moreover, by injecting TTX in 3 dpf larvae ventricles, they showed that 25% to 33% of OPCs intrinsic Ca^{2+} activity are mediated by neuronal activity and further synaptic vesicle release. At 5 dpf, this role was not anymore observed suggesting a limited role of neuronal activity in the generation of calcium signaling (Li et al., 2022).

4.5 | The zebrafish as a suitable model to clarify the debate of synaptic versus extra-synaptic axo-glial communications?

Finally, how do neurons and oligodendroglial cells interact and communicate with each other? How do oligodendroglial cells receive axonal signals?

Neuron-OPC synapses were first described in 2000 (Bergles et al., 2000). Whole-cell patch-clamp experiments on Sprague–Dawley rat hippocampus slices showed small Na^{+} currents and delayed rectifier K^{+} currents into OPCs in response to stimulated afferent axons. These inward currents are mediated by Ca^{2+}-permeable AMPA glutamatergic receptors. These electrophysiological data allowed to conclude that these currents are synaptic and that numerous axon-OPC synapses are formed during development. Electron microscopy analysis support the existence of axon-OPC synapses, with OPC processes directly in contact to presynaptic-like domains along axons, where an accumulation of vesicles is observed. However, OPC post-synaptic membranes were described thinner than classical neuronal post-synaptic membranes. It is noteworthy that OPCs do not fire AP (Bergles et al., 2000; Clarke et al., 2012). In addition, newborn OPCs kept their synapses with axons during their cell division: half of the initial number of axon-OPC synapses are found for each daughter OPCs (Kukley et al., 2007). No changes in synaptic current (same amplitude, kinetic and frequency) were noticed between parental OPCs and daughter cells.

We previously mentioned the heterogeneity of OPCs and neuronal populations in the CNS, and that OPCs have the capacity to ensheath various axonal populations. A recent *in vivo* mouse study using a modified virus ligand to trace monosynaptic retrograde axo-oligodendroglia communication revealed that PDGFR + OPCs receive brain-wide synaptic inputs. As examples, OPCs located within the premotor cortex receive synaptic inputs from premotor cortical and thalamic neurons, or OPCs of the corpus callosum receive inputs from various brain regions like medial prefrontal, primary motor and somatosensory cortices, and even thalamic inputs (Mount et al., 2019). Moreover, previous studies highlight that these axon-OPC synapses can be either glutamatergic (De Biase et al., 2010; Wake et al., 2011; Ziskin et al., 2007) or GABAergic (Benamer et al., 2020; Kukley et al., 2007; Lin & Bergles, 2004; Orduz et al., 2015). An elegant hypothesis is that prior to myelin formation, OPC processes explore their environment and neighboring axons in a similar chemo-affinity manner as dendritic filopodia (Almeida & Lyons, 2014). It involves match-maker and organizer adhesion molecules, which promote respectively the local specific interaction between an axon and an OPC process and stabilize the pairing between both cell types. Morphological criteria of axon-OPC synapses are the same than classical neuronal synapses (Bergles et al., 2000; Kukley et al., 2007; Maldonado et al., 2011), with a pre-synaptic accumulation of NT vesicles apposed to OPC processes and a post-synaptic density rich area, characterized by electron-dense materials. In zebrafish, ectopic PSD-95-GFP or gephyrin-GFP expression, two well-known glutamatergic and GABAergic post-synaptic neuronal markers respectively, was observed in OPCs. These proteins form highly dynamic structures, with a half-life shorter than neuronal post-synaptic structures and they apposed to pre-synaptic structures on the axonal side (Li et al., 2022).

Nonetheless, even if the existence of axon-OPC synapses is now well accepted, these synapses are not a permanent structure and are restricted to the OPC stage. Indeed, De Biase and colleagues showed that NG2+ mice OPCs have the capacity to form synapses with axons, which are lost upon their differentiation into OLs (De Biase et al., 2010). The restriction of synaptic connectivity to the OPC stage was further corroborated by electrophysiological recordings of inward currents in OPCs but not in differentiated OLs (Bergles et al., 2000). However, myelinating OLs express functional glutamatergic AMPA and NMDA receptors, which are activated by activity-dependent vesicular release of glutamate, arguing for the existence of "axo-myelinic synapses" (Micu et al., 2016). Additionally, RNA-seq data on rodent brains showed that OLs express synaptogenic adhesion molecules (Zhang et al., 2014). Recently, Hugues and Appel confirmed that some trans-synaptic adhesion molecules are expressed on oligodendroglia and showed that Cadm1b (also known as SynCAM1) is expressed by zebrafish OLs and is located underneath myelin sheaths and coincided to sites of synaptic vesicles accumulation along axons. Furthermore, SynCAM1 loss-of-function influences the number and length of myelin internodes produced by OLs. Similar results were also obtained when this adhesion molecule cannot interact with its axonal partners. Overall, these data indicate that pre-synaptic adhesion

molecules on the axonal side and post-synaptic proteins on oligodendroglial membrane and myelin are effectively involved in axon-OL interactions regulating myelination (Hughes & Appel, 2019). However, it remains unclear whether these axon-oligodendroglial domains are functional classical synapses, and what are their functional roles in myelination.

5 | THE ZEBRAFISH MODEL TO STUDY DEMYELINATING DISEASES

Myelin damage or loss is implicated in numerous neurodegenerative diseases such as leukodystrophies (Wolf et al., 2021) or multiple sclerosis (Thompson et al., 2018), but also in neuropsychiatric diseases including autism (Graciarena et al., 2019) and schizophrenia (Maas et al., 2017, 2020). To study demyelination and remyelination processes, rodents are commonly used. However, rodents are complex organisms and the number of axons, as well as the complexity of the cellular environment and interaction, do not allow to follow the demyelination and remyelination processes of a single axon easily. The zebrafish offers several advantages to study demyelination and remyelination as axonal population and myelination are well defined at least in the SC.

5.1 | Laser ablation

Different methods were developed to perform demyelination or OLs ablation in the zebrafish. The less complicated appears to be the laser ablation of fluorescent cells (Figure 4a). Kirby and colleagues specifically targeted eGFP+ oligodendroglial cells of 4 dpf Tg(Olig2:eGFP) larva and killed these cells with a 440 nanometer (nm) laser short duration pulses. Following OLs dorsal SC injury, neighboring OPCs rapidly migrated as soon as 3 h post lesion, leading to the replacement of half of the ablated cells the day after the lesion and of two-third within 4 days. This technic appears very specific as no neural cells died in the process and as myelination is still possible 12 h after the laser microsurgery (Kirby et al., 2006).

5.2 | Chemical ablation

In zebrafish, only lysophosphatidylcholine (LPC) is used as a membrane-dissolving agent to induce focal lesions. First protocol developed generated demyelination by inserting 1% lysolecithin into small gelatin pieces placed in close proximity with the optic nerve, just behind the eyeball (Münzel et al., 2014) (Figure 4b). Other recent protocols directly injected LPC within the zebrafish dorsal SC using glass microcapillaries and microinjector (Cunha et al., 2020; Morris & Kucenas, 2021) (Figure 4b). In the injection site, a decrease in the proportion in Sox10+ oligodendroglial cells is quantified at 8 h post-injection (hpi) following by an increase of these cells number at 20 hpi, suggesting OPCs proliferation at the demyelination site, but also a migration of most anterior OPCs to the lesion (Morris & Kucenas, 2021). This proliferation and migration processes were also observed in lesioned rodents (Nait-Oumesmar et al., 1999). In these demyelinated zebrafish, remyelination happened within 4 weeks but internodes appeared shorter (Münzel et al., 2014). One of the major advantages of this method is that neurons do not appear affected by LPC injection. However, microglial cells and macrophages tend to respond, migrate to the injection site between 2 and 6 hpi and to proliferate (Cunha et al., 2020; Morris & Kucenas, 2021).

5.3 | Chemical-genetic cell ablation

Another well-developed demyelinated method in the zebrafish is the used of the bacterial nitroreductase (NTR) to convert the non-toxic Metronidazole (Mtz) pro-drug into a cytotoxic chemical agent (Figure 4c). This technic allows a spatial and temporal cell ablation when the *nfsB* gene (encoding for the NTR) is expressed under the control of a cell specific promoter (Karttunen & Lyons, 2019). This method was first developed to ablate cardiomyocytes, pancreatic beta cells and hepatocytes (Curado et al., 2007). Different transgenic zebrafish lines to specifically ablate OLs were also developed such as Tg(Mbp:Gal4; UAS:nfsB-mcherry), Tg(Sox10:Gal4; UAS:nfsB-mcherry) (Chung et al., 2013) or Tg(Mbp:nfsB-eGFP) (Fang et al., 2015). Treatment of these animals showed that 2 days of 5–10 mM Mtz suppress more than two-third of MBP+ cells (Karttunen et al., 2017), with a demyelination observe as soon as 12 h post-treatment (Chung, et al., 2013) accompanied by behavioral changes and a reduced movement distance, time and velocity (Fang et al., 2015). The zebrafish innate immune system was also activated with an increase in microglial cells and macrophages, engulfing myelin debris between 3 and 4 days post-treatment, as shown by mpeg+ cells positive for mCherry protein in Tg(mbp:mCherry-NTR) transgenic line (Karttunen et al., 2017). One of the major advantages of this chemical-genetic induced cell ablation, is that the effect of Mtz is reversible when the drug treatment is stopped, with full myelin and locomotor recovery 2 weeks after the drug removal (Chung, et al., 2013; Fang et al., 2015; Karttunen et al., 2017).

As the cell ablation can be temporally and spatially controlled, we can easily envision the combination of this demyelination method with high-throughput screening of chemical compounds promoting remyelination *in vivo* (Buckley, Marguerie, Roach, et al., 2010; Peterson et al., 2000).

5.4 | Photosensitive cell ablation

Photosensitizer can also be used to delete specific cells. Under a specific light illumination, the KillerRed photosensitizer generates reactive oxygen species (Bulina et al., 2006) and so permit a precise and rapid neuronal (Teh et al., 2010) or oligodendroglial cell (Auer et al., 2018) deletion, depending on the specific promoter used

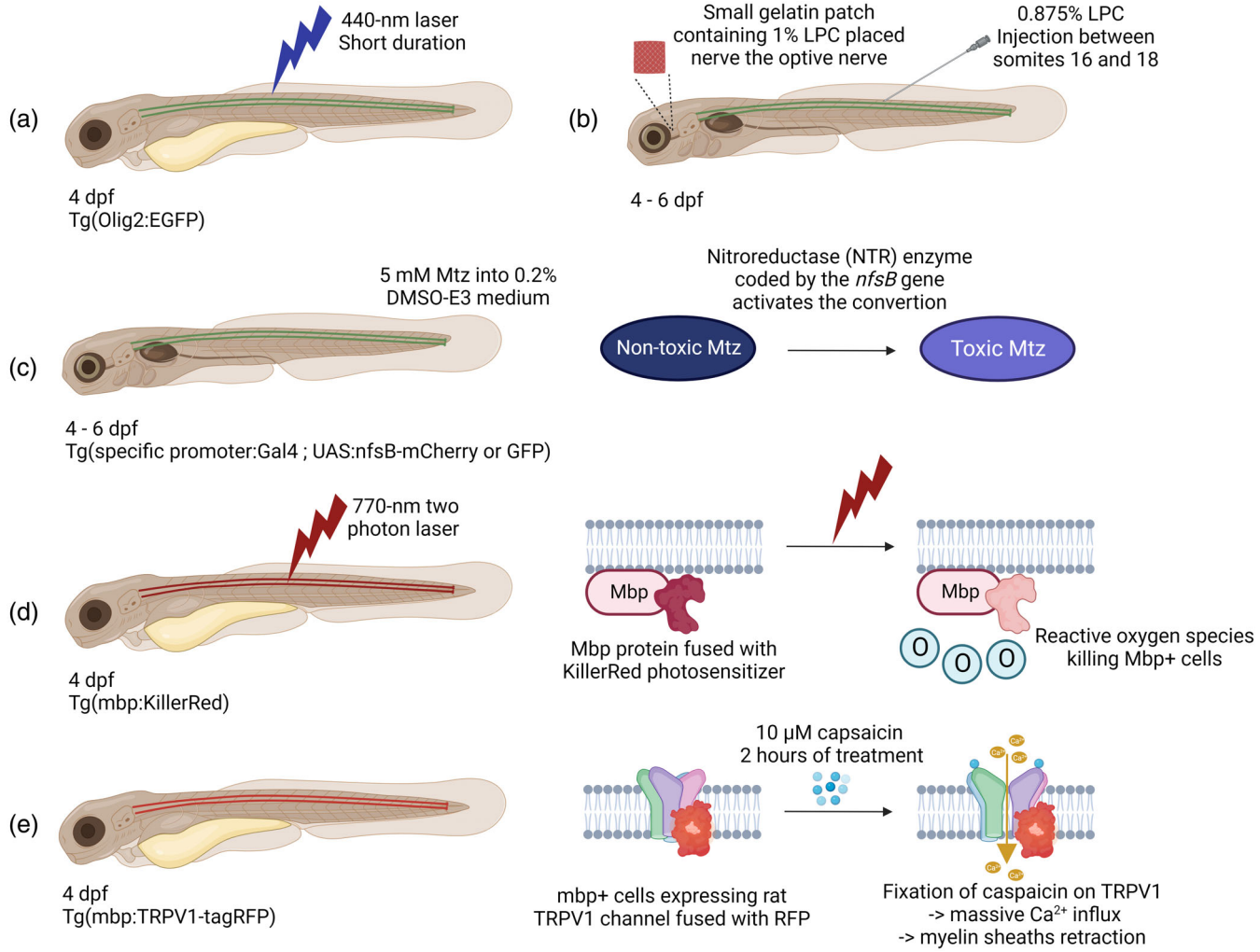

FIGURE 4 Methods of demyelination in the zebrafish. Laser (a) and LPC-induced chemical (b) ablations are the easiest technics implemented in the zebrafish to delete oligodendroglial cells. Genetic approaches include the expression of the nitroreductase (NTR) to activate the pro-drug metronidazole (Mtz) (c) or of the KillerRed photosensitizer to generate reactive oxygen species (d) in specific cell populations. The rat transient receptor potential V1 (TRPV1) cations channel can also be specifically expressed in oligodendroglial cells in order to induce a massive influx of calcium thanks to a short treatment by the capsaicin, leading to myelin sheaths retraction.

(Figure 4d). In their last paper, Auer et al. used a 770-nm two photon laser light to ablate Mbp + cells in transgenic Tg(Mbp:KillerRed) animals which already possess stable myelin segments. Within 1–2 h post light application, OLs soma disintegrated and then myelin sheaths disappeared. Myelin injury necessarily leads to remyelination either by formation of new myelin segments in the demyelinated axons, or by extension of neighboring sheaths. The second process was observed from 1 day post-ablation (dpa) while the formation of new internodes happened between 2 and 4 dpa. Moreover, at 8 dpa, half of the eliminated sheaths were already replaced by new sheaths possessing the exact same length and located at the exact same place than the original sheaths, suggesting the existence of spatial permissive axonal signals controlling myelination (Auer et al., 2018). With this method, surrounding neurons and axons were not affected just like in the others methods described above (Chung, et al., 2013; Cunha et al., 2020; Fang et al., 2015; Karttunen et al., 2017; Karttunen & Lyons, 2019; Morris & Kucenas, 2021; Münzel et al., 2014).

5.5 | High amplitude calcium influx

Finally, a last method developed in zebrafish to perform demyelination is the use of massive cations influx specifically within mature myelinating OLs (Figure 4e). This recent method is based on the fact that: (1) high amplitude long duration calcium transients within myelinating OLs lead to sheaths retraction as aforementioned (Baraban et al., 2018), (2) cations influx mimic myelin pathology (Hamilton et al., 2016), and (3) TRPV1 channels in zebrafish are not capsaicin sensitive (Chen et al., 2016). Thereby, Neely and colleagues developed a transgenic Tg(mbp:TRPV1-tagRFP) line in which the capsaicin-inducible transient receptor potential V1 (TRPV1) ion channel is fused

with a red fluorescent reporter and specifically expressed in Mbp + OLs (Neely et al., 2022). With this method, following a 10 μM capsaicin treatment for 2 h, demyelination was observed as soon at 1 day post-treatment with 95% of dorsal axons which were demyelinated. No changes were observed in axon number meaning that capsaicin treatment and TRPV1 expression did not affect neuronal survival. Moreover, this method permits a good oligodendroglial survival with only 1/3 of myelinating OLs that completely disappear at 3 h post-treatment. By 5 days post-treatment, the number of OLs was the same in treated animals versus control ones. An interesting observation that they made is that both surviving and newly formed myelinating OLs have the capacity to remyelinate axons but surviving OLs possess low remyelination capacities and tend to make many miss-targeting sheaths near neuronal cell body. This miss-targeting process was made by 60% of surviving OLs and was also observed within human post-mortem multiple sclerosis tissues. In order to increase surviving OLs differentiation and myelination capacity, demyelinated animals were treated with a known rho kinase inhibitor (Y27632) (Harboe et al., 2018; Melendez-Vasquez et al., 2004; Wang et al., 2008) but no changes in cells number or miss-targeting events were displayed suggesting that surviving OLs possess a poor remyelination capacity. On the other hand, newly formed OLs generate more myelin internodes per cells and per axons, but sheaths were generally shorter in length (Neely et al., 2022).

5.6 | High-throughput screening using zebrafish

The zebrafish was first used for high-throughput screening in 2000, based on phenotypic effects of drug treatments (Peterson et al., 2000). Then, Buckley and colleagues tested several approved drugs on Tg(Olig2:eGFP) in order to found new compounds which could be repositioned as treatments for demyelinating diseases. Larva of 30 hpf were treated for 2 days during the OPC specification period, then Olig2-positive cells were counted and, if the number of oligodendroglial cells was affected, *mbp* mRNA expression was quantified by qPCR and effects on neurons were observed by immunohistochemistry. With this method, 15/1120 compounds increased the number of Olig2+ cells that migrated from the pMN domain to the dorsal SC (Buckley, Marguerie, Roach, et al., 2010). However, thanks to further investigations, it was observed that one compound promoted differentiation of pMN-derived precursors into motor neurons instead of oligodendroglial cells, highlighting the importance of neurons-OLs co-labeling to correctly distinguish Olig2+ cells. Recently, David A Lyons' team at the University of Edinburgh developed an automated image acquisition and analysis pipeline in order to discover new pro-myelinating compounds, but also to reduce manual counting bias. Using this high resolution screening method, the team discovered and validated four compounds which increase OLs differentiation (Early et al., 2018). However, one disadvantage of the zebrafish larva for drug screening is, that even with a good permeability during embryogenesis and organogenesis, some molecules cannot penetrate inside the organism and so no effects can be studied.

6 | CONCLUSION AND PERSPECTIVES

In the last decade, the zebrafish model has greatly contributed to our understanding of the mechanisms regulating OL development and myelination. Due its external development and to the existence of several transgenic reporter lines for both oligodendroglia and axons, this model allowed to unravel the fundamental roles of axo-glial interactions in myelin formation and growth, using *in vivo* time lapse imaging. This model is also a powerful tool for assessing the impacts of gene mutations and/or axonal activity on myelination, using state-of-art optogenetic, chemogenetic and live imaging approaches in a relatively flexible manner. It also provides a unique model system to monitor myelin dynamics in health and disease. Importantly, many of the mechanisms driving OPC specification, differentiation and myelination are conserved from zebrafish to humans. In zebrafish and rodent, CNS myelination occurs following an anterior–posterior and ventral-dorsal gradients (Almeida et al., 2011; Czopka et al., 2013) (Figure 3b) and the myelin ultrastructure is fundamentally comparable from fish to humans (Brösamle & Halpern, 2002; Howe et al., 2013; Schweitzer et al., 2003, 2006). Neuronal activity and evoked synaptic vesicle release were showed to promote myelination process especially via le local translation of myelin proteins (Wake et al., 2015) but also to increase sheaths elongation and stabilization (Hines et al., 2015). Intrinsic Ca^{2+} activity mediated by neuronal activity is one of the major regulator of myelination (Baraban et al., 2018; Krasnow et al., 2018; Micu et al., 2016; Wake et al., 2011), firstly via calpain (Baraban et al., 2018), but also mitochondria (Battefeld et al., 2019) and VGCa^{2+} (Gudz et al., 2006; Harlow et al., 2015; Li et al., 2022; Paez et al., 2010; Piller et al., 2021). This model will certainly provide a powerful study system to decipher more accurately the contributions of synaptic versus extra-synaptic modes of interactions between axons and oligodendroglia in shaping myelination and how these axo-glial interactions impact the fine-tuning of neuronal circuits. Another major question in the field is how do these axo-glial interactions regulate neuronal circuit formation and functions, and finally their impacts on behavior? The zebrafish could also provide key insights into novel functions of OPCs that go beyond their classic role in OL formation.

Finally, the zebrafish model could also contribute to better knowledge of the cellular and molecular mechanisms regulating demyelination and myelin regeneration following injury. Although, this model has not been extensively used yet in remyelination studies, several demyelination methods have been developed in the zebrafish (Auer et al., 2018; Chung, et al., 2013; Cunha et al., 2020; Fang et al., 2015; Karttunen et al., 2017; Karttunen & Lyons, 2019; Morris & Kucenas, 2021; Münzel et al., 2014; Neely et al., 2022) (Figure 4). These demyelination methods allowed large scale screening of compounds favoring OL development and myelination in the zebrafish.

AUTHOR CONTRIBUTIONS

Mary-Amélie Masson and Brahim Nait-Oumesmar wrote the manuscript and Mary-Amélie Masson designed the figures. Both authors made substantial contributions to the discussion of content, reviewed, and edited the manuscript.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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