Zebrafish myelination: a transparent model for remyelination?

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INTRODUCTION

Multiple sclerosis (MS) is one of the most common diseases of the human central nervous system (CNS), affecting over 1.1 million people worldwide (Zamvil and Steinman, 2003). There are bimodal peaks in disease prevalence; the highest number of cases occurs in young adults and middle-aged people, but MS can occur at any age. It is an inflammatory autoimmune disease that is initiated by a combination of genetic susceptibility and environmental triggers that cause myelin sheath breakdown through recurrent immune attacks on the CNS (Compston and Coles, 2002). This myelin breakdown is probably one of the reasons why axons eventually degenerate, causing most of the disability associated with the progressive stages of MS. There are two broad ways in which MS could be therapeutically targeted. First, the inflammatory immune response could be suppressed. This is the basis of current MS therapies. The second approach is to attempt to halt disease progression by developing therapies aimed at maintaining axonal survival, for example, by promoting the process of remyelination (Franklin and ffrench-Constant, 2008). Zebrafish larvae could provide a high-throughput in vivo vertebrate model for testing remyelination therapies. The second approach is to attempt to halt disease progression by developing therapies aimed at maintaining axonal survival, for example, by promoting the process of remyelination. Zebrafish larvae could provide a high-throughput in vivo vertebrate model for testing potential remyelination therapies because of their small size, external development, transparency and homology with mammalian myelin biology.

PROMOTING REMYELINATION IS AN IMPORTANT OBJECTIVE FOR FUTURE MS THERAPY

Remyelination occurs naturally in the early stages of MS but often fails during the later progressive phases. Therefore, in order to combat MS effectively, there is a need to devise therapies that can promote the regeneration of myelin sheaths around demyelinated axons in the CNS (remyelination; see Box 1) (Dubois-Dalcq et al., 2005; Dubois-Dalcq et al., 2008; Franklin and ffrench-Constant, 2008; Irvine and Blakemore, 2008).

Several anti-inflammatory and immunomodulatory therapies are currently in routine clinical use. However, these are only partly effective and may not have a significant effect on overall disease progression (DeAngelis and Lublin, 2008b). New therapies are being developed, including novel immunomodulatory agents that are aimed at inhibiting specific aspects of the immune system, thereby reducing the autoimmune attack on oligodendrocytes and myelin. For example, alemtuzumab is an antibody that targets lymphocytes and monocytes and has generated promising results in clinical trials (Coles et al., 2008). These new therapies are reviewed elsewhere (DeAngelis and Lublin, 2008a; DeAngelis and Lublin, 2008b; Kieseier et al., 2007).

Although these therapies might help to reduce initial inflammation and/or the autoimmune attack on myelin, and therefore reduce the number of relapses in early stages of MS, none specifically target the process of remyelination. In fact, although current MS therapies try to reduce the inflammatory response, aspects of this response such as macrophage-mediated removal of myelin debris are important in creating an environment that supports OPC differentiation, a process which is necessary for remyelination (Foote and Blakemore, 2005; Kotter et al., 2006; Setzu et al., 2006).

REMYELINATION THERAPIES – WHERE TO START?

The regenerative process of remyelination occurs in a similar way to the developmental process of myelination, proceeding through the processes of OPC activation, proliferation, recruitment and differentiation (Box 1) (Franklin and ffrench-Constant, 2008; Miller and Mi, 2007). Therefore, remyelination in MS may fail either because no OPCs are present in the lesion, or because the OPCs...
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models, must concentrate on manipulating the processes of OPC differentiation, rather than that of recruitment, might be predominantly responsible for inhibiting remyelination. When the OPCs have fully differentiated into myelinating oligodendrocytes, these processes compact to form myelin sheaths around the axons. Although the processes of developmental myelination and regenerative remyelination are very similar, differences do occur. One important distinction is that remyelination occurs in a pathological environment, whereas myelination occurs in a normal environment (Franklin, 2002b). However, spontaneous remyelination in response to injury has been demonstrated and shown to correlate with functional recovery of axons and axonal preservation (Irving and Blakemore, 2008; Kornel et al., 2000; Liebetanz and Merkler, 2006). This suggests that the function of the remyelination sheaths is essentially similar to that of the myelin sheaths of myelination.

Box 1. Myelin, myelination and remyelination

Myelin is an insulating membranous sheath, made up of around 70% lipid and 30% protein. It is produced by myelinating oligodendrocyte cells in the CNS and by Schwann cells in the peripheral nervous system (PNS). Myelin surrounds nerve axons, allowing saltatory nerve conduction and maintenance of the axon at a long distance from the cell body (Griffiths et al., 1998; Lappe-Siefke et al., 2003; Nave and Trapp, 2008). In the CNS, myelinating oligodendrocytes develop from a population of cells called oligodendrocyte precursor cells (OPCs). These cells are sometimes referred to as oligodendrocyte progenitor cells. During development, OPCs are normally restricted to an oligodendrocyte fate; however, recent evidence suggests that adult OPCs are multipotent, especially following injury. Consequently, it has been suggested that the adult OPC should be regarded as an ‘adult neural precursor cell’ (reviewed by Zawadzka and Franklin, 2007; Zhao et al., 2008). For the sake of simplicity, we will retain the term OPC in this review. When OPCs are activated, they proliferate and are recruited to unmyelinated (development) or demyelinated (regeneration) axons. As the OPCs differentiate, they extend multiple processes, each wrapping an axon. When the OPCs have fully differentiated into myelinating oligodendrocytes, these processes compact to form myelin sheaths around the axons.

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present are unable to differentiate into myelin-producing oligodendrocytes. By studying circumstances where remyelination is impaired, such as in older animals, it appears that both OPC recruitment and differentiation are important in maintaining remyelination efficiency. OPC colonisation of demyelinated lesions (recruitment) was delayed in older rats following ethidium bromide (EB)-induced demyelination, as was the time interval between equivalent OPC marker expression and differentiated oligodendrocyte marker expression (differentiation) (Sim et al., 2002). However, when the process of OPC recruitment into demyelinated focal lesions was artificially increased by overexpressing platelet-derived growth factor A (PDGF-A) in transgenic mice, there was no change in remyelination efficiency (Woodruff et al., 2004). A recent study also illustrated that in older mice brains, recruitment of histone deacetylase 1 (HDAC1) and, therefore, the ability to downregulate oligodendrocyte differentiation inhibitors is reduced, causing remyelination impairment. In further support of this result, defective remyelination and oligodendrocyte differentiation were induced in vivo and in vitro, respectively, by the application of HDAC inhibitors (Shen et al., 2008). These studies suggest that, although both processes become less efficient in older animals, the failure of OPC differentiation, rather than that of recruitment, might be predominantly responsible for inhibiting remyelination.

This conclusion mirrors clinical findings, where it is common to see MS plaques containing OPCs with no evidence of remyelination (Chang et al., 2000; Chang et al., 2002; Kuhlmann et al., 2008; Wolswijk, 2002). Therefore, potential endogenous remyelination therapies, and consequently remyelination screening models, must concentrate on manipulating the processes of OPC recruitment and, in particular, OPC differentiation into myelinating oligodendrocytes (Dubois-Dalcq et al., 2005; Franklin and ffrench-Constant, 2008).

Transplantation of myelinating cells is one approach for therapeutic remyelination. There are many studies showing repair of focal lesions by direct cell transplantation (Franklin, 2002a). Recent studies have also shown amelioration of experimental autoimmune encephalomyelitis (EAE) using systemic cell transplantation (Einstein et al., 2006; Pluchino et al., 2003). However, the findings from the EAE studies are thought to be the result of neural stem cell immunosuppression of T cells, rather than an increased capacity for remyelination (Einstein et al., 2007; Pluchino et al., 2005). Tissue incompatibility and cell delivery problems also make cell transplantation a challenging option. Therefore, a more attractive approach for promoting remyelination is through pharmacological promotion of endogenous remyelination.

The signalling systems that are responsible for remyelination are complex (for a review, see Franklin and ffrench-Constant, 2008). This makes elucidation of tractable remyelination targets difficult, especially when extrapolating from in vitro to in vivo models. The most widely used in vivo model of MS is EAE, in which the processes of demyelination and remyelination occur simultaneously. As the inflammatory process of EAE is damaging to oligodendrocytes, this lack of temporal separation makes it challenging to distinguish between an effect that is enhancing remyelination and one that is ameliorating the inflammatory response to EAE and, therefore, enabling the normal process of remyelination to occur. Some types of EAE cause inflammatory disease without extensive demyelination and so do not provide easily interpretable models of the neurobiological aspects of MS. The benefits and drawbacks of using EAE models to recapitulate MS are reviewed elsewhere (Altmann and Boyton, 2004; Dubois-Dalcq et al., 2005; Friese et al., 2006; Gold et al., 2006).

More reductionist in vivo rodent models based on the use of toxins such as EB and lysolethicin allow a clear temporal separation between the myelin insult and its subsequent regeneration. However, a higher throughput system would enable rapid drug screening for potential remyelination enhancement. Ideally, a logical hierarchy of models could be built, starting with a high-throughput model to identify potential therapeutic agents. In vitro cell culture models are not appropriate for studying myelination unless axons are present. Recently, in vitro myelinating co-cultures of dorsal root ganglion (DRG) neurons and oligodendrocytes have been used to investigate processes such as the effect of growth factors on myelination (Chan et al., 2004; Wang et al., 2007c). This system allows for specific investigation of myelination by oligodendrocytes in the absence of any other cell types such as astrocytes. However, myelinating co-culture systems are time-consuming to set up, with each experiment taking several weeks, and are therefore not amenable to high-throughput screening projects. It is also technically challenging to carry out manipulations such as gene knockdown in vitro. Zebrafish can potentially fulfil the requirement for a high-throughput myelination model, combining the speed of an in vitro model with the context of an in vivo vertebrate system (Fig. 1, discussed later), and are very easy to genetically manipulate. Zebrafish myelination can be assessed after only a few days postfertilisation. However, owing to the many cell types present in a whole animal, interpretation of results might

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Fig. 1. Zebrafish myelination: high-throughput drug screening.
(A) Anatomy of a transparent 3 d.p.f. zebrafish larva. (B) Several zebrafish larvae were placed in each well of a 96-well plate, with different drugs in each well. (C) Live sagittal image of a 3 d.p.f. olig2:EGFP zebrafish larva spinal cord. Dorsally migrated cells were counted in response to different drugs. Green dots in the middle of the magnified region indicate some of these cells. Examples of fainter, elongated migrating cells can be seen below the green star. The green arrow indicates a cell on the border of the pnMN. (D) An automated system was used to count olig2-positive cells. Larvae (the arrow indicates one larva) were aspirated in methylicellulose into a capillary tube. The capillary was inserted through a water chamber (blue square) to reduce refraction of light, then anchored onto a movable stage (arrowhead) and remotely positioned until the larvae were orientated sagittally above an inverted microscope (outer circle). A z-stack of images was taken and combined into one collapsed image. The number of dorsally migrated olig2-positive cells was automatically counted. (E) Mbp immunohistochemistry of a transverse hindbrain section from a 5 d.p.f. zebrafish larva. Arrows indicate the medial longitudinal fascicle and arrowheads indicate the ventral commissure. (F) Electron micrograph of a transverse spinal cord section from a 10 d.p.f. zebrafish larva. Arrows indicate the large Mauthner axons. These are surrounded ventrally by axons with a larger diameter.

Zebrafish models: applications for understanding myelination

Zebrafish provide an ideal in vivo model for high-throughput experiments. Their larvae are very small (only a few millimetres long) and transparent (Fig. 1A). Embryogenesis occurs ex vivo and is complete by 3 days post fertilisation (d.p.f.) (Berger and Currie, 2007; Kimmel, 1989; Kimmel et al., 1995). This allows for easy phenotypic assessment. Each pair of fish produces approximately 100 to 300 embryos per week and maintenance costs are 100-fold lower than for mice (Goldsmith and Solari, 2003; Kari et al., 2007).

Zebrafish and mammalian myelin: a comparison

The molecular and cellular organisation of zebrafish is remarkably similar to that of humans, and homologues for most human genes can be found and studied in zebrafish (Barbazuk et al., 2000; Postlethwait et al., 2000). However, owing to genome duplication after the divergence of the tetrapods, there are often several zebrafish genes for each mammalian gene, as in the case for the genes encoding one of the major myelin-associated proteins, proteolipid protein (Plp), and its splice variant Dm20 (Schweitzer et al., 2006). The genetic sequences of the major myelin proteins are also diverse among different species of fish, whereas they are often highly conserved among mammals (Geltner et al., 1998). In addition, there are physiological differences between zebrafish and mammals (reviewed by Lieschke and Currie, 2007).

The major biochemical difference between zebrafish and mammalian myelin is the presence of protein zero (P0), a major CNS myelin protein in zebrafish, rather than PLP in mammals (Table 1) (Jeserich et al., 2008; Waehneldt et al., 1986). This suggests an evolutionary neuroprotective change in myelin chemistry between aquatic and terrestrial vertebrates, which is supported by the degenerative phenotype observed in transgenic mice that express P0 instead of Plp in their CNS (Yin et al., 2006). Although there is sequence conservation between zebrafish and mammalian P0 (Schweitzer et al., 2003), the zebrafish p0 gene shows greater promoter region sequence conservation with the mammalian Plp gene rather than mammalian P0 (Jeserich et al., 2008; Jeserich et al., 1997). In keeping with this, a zebrafish transgenic line, which expresses enhanced green fluorescent protein (EGFP) under the regulation of a mouse Plp promoter, exhibits strong EGFP expression in oligodendrocytes and their precursors, although it is not clear whether this promoter regulates zebrafish dm20 or p0 genes (Yoshida and Macklin, 2005). This suggests that there is not a clear biochemical distinction between oligodendrocytes and Schwann cells in the zebrafish (Jeserich et al., 2008).

Nevertheless, the structural properties and cell lineage relationship of oligodendrocytes is highly comparable between zebrafish and mammals (Jeserich et al., 2008; Jeserich and Stratmann, 1992; Jeserich and Waehneldt, 1986; Sivron et al., 1990). Also, orthologous genes for all of the major mammalian myelin-associated genes have been found in zebrafish (dm20, mbp and p0) (Brosamle and Halpern, 2002). Although there is some variation between zebrafish and mammals in the expression pattern and sequence of these genes, there is enough conservation of predicted protein properties to suggest that the zebrafish orthologues function in a comparable way to the mammalian proteins (see Table 1). Further, the coexpression of all three major myelin-associated...
Feasible genetic manipulation and analysis

The zebrafish genome sequence is nearing completion and most genetic manipulation techniques can be carried out efficiently in zebrafish owing to the high number of offspring that can be produced, the transparency of larval stages and their fast, external embryonic development. Although some individual genetic manipulation techniques may not be more rapid than in other organisms, zebrafish are superior in efficiency and ease of technique.

External embryonic development enables straightforward microinjection technique. This allows transgenic lines to be generated through the injection of large insert clones such as P1 artificial chromosomes (PACs) or bacterial artificial chromosomes (BACs) (Lee et al., 2001; Shin et al., 2003). The recent development of the Tol2 and Sleeping Beauty (SB) transposon systems allows another method of transgenic generation and produces much higher germline transmission efficiencies (Kawakami, 2005). The development of the GAL4-UAS system in zebrafish is especially versatile as it allows gene expression to be targeted both spatially and temporally (Scheer and Campos-Ortega, 1999; Scheer et al., 2001). Homologous recombination of DNA into embryonic stem cells and the subsequent transplantation of these cells into an embryo has allowed targeted gene deactivation in mice and chicks. So far, it has not been possible to knockout a specific zebrafish gene in this way because zebrafish embryonic stem cell cultures were not able to contribute to germ cell lineage. However, recent attempts at generating gene knockout zebrafish are promising (Ma et al., 2001; Wang et al., 2007b) and the development of these techniques will make the zebrafish even more tractable as an experimental model. In the meantime, insertional mutagenesis in zebrafish is possible through transposon or retroviral systems (Ellingsen et al., 2005; Sivasubbu et al., 2007; Wang et al., 2007a), and target-induced local lesions in genomes (TILLING) (McCallum et al., 2000) have allowed the production of gene-specific mutations (Wienholds et al., 2002). Zebrafish embryos also allow fast investigation of in vivo gene function at early embryonic stages through RNA overexpression and morpholinoc gene knockdown experiments, because transgenic or mutant generation is not necessary, and gene alteration occurs within a few hours of microinjection (Nasevicius and Ekker, 2000). For example, in a morpholinoc study, hdac1 was shown to be essential in allowing the expression of oligodendrocyte-lineage-specific genes, such as olig2 and sox10, whilst suppressing the expression of neural progenitor determinants such as nks2.2 and her6 (Cunliffe and Casaccia-Bonnefil, 2006).

High mutagenesis rates can be achieved in zebrafish because of their resistance to N-ethyl-N-nitrosourea (ENU) toxicity and consequent survival of mutated embryos. The first use of this method of mutagenesis in zebrafish was in genome-wide forward genetic screens (Driever et al., 1996; Haffter et al., 1996; Lieschke and Currie, 2007). For example, screening for defects in myelin basic protein (Mbp) expression demonstrated that N-ethylmaleimide sensitive factor (Nsf), which is essential for membrane fusion (Wilson et al., 1989), also plays a role in myelination and the organisation of the nodes of Ranvier (Woods et al., 2006). Similar
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forward genetic screens have identified several other myelin-related genes (Kazakova et al., 2006; Pogoda et al., 2006).

Imaging techniques
Labelling and imaging techniques can be carried out in whole zebrafish larvae owing to their small size. For example, whole-mount immunohistochemistry (IHC) staining of zebrafish axons with myelin, axonal and nodal markers was used to determine sodium channel disruption in response to mutations (Voas et al., 2007; Woods et al., 2006). Such clear staining of nodes could also be used as a way of defining whether an axon is myelinated or not, as nodes are only formed in myelinated axons. In vivo retrograde fluorescent labelling of individual neurons is another way in which neurons can be visualised in whole larvae. This can be performed just 5–10 hours after injection of a fluorescent dye, such as dextran, into the spinal cord (Gahtan and O’Malley, 2001; Gahtan and O’Malley, 2003; Hale et al., 2001). However, the damage caused to the axons through this procedure may confound results (Gahtan and O’Malley, 2001). The ability to trace the lineage, and fate, of individual cells through fluorescent labelling in vivo is possible in zebrafish larvae. For example, the establishment of normal sodium channel clusters in α-spectrin mutant zebrafish larvae, which would otherwise have abnormal clustering, was achieved by transferring dextran-labelled wild-type cells into the mutants (Voas et al., 2007). This technology could have potential applications in many fields, such as cancer research (Lee et al., 2005).

One of the most dramatic imaging methods used in zebrafish is fluorescence transgenesis (Gong et al., 2001; Udvardia and Linney, 2003). There are several transgenic zebrafish lines that are directly relevant for studying myelin biology, including the plp:EGFP and the olig2:EGFP lines, both of which express EGFP in cells belonging to the oligodendrocyte lineage. The olig2:EGFP transgenic line has been used as an oligodendrocyte and neuronal marker to investigate several myelination-related pathways; for example, the control of cell cycle exit, primary neurogenesis and maintenance of precursor populations by Delta-Notch was shown to occur through the regulation of cyclin-dependent kinase inhibitor 1C (cdkn1c) expression, which is itself necessary for oligodendrocyte specification (Park et al., 2005). These experiments used Notch mutants, inducible constitutive expressers of Notch and cdkn1c morpholinos in combination with IHC co-labelling and in situ studies. Clonal analysis of olig2:EGFP motor neuron precursor domain (pMN) cells has also been used to show that, rather than being binary, olig2 precursors can produce several types of interneuron as well as motor neurons and oligodendrocytes. The fate of these olig2 precursors is under the control of Hedgehog signalling (Park et al., 2004).

More relevant to remyelination, olig2:EGFP larvae were used with another transgenic line that labels a subset of OPCs, nks2.2a:EGFP, to illustrate the density-dependent regulation of oligodendrocyte migration and division in response to the laser ablation of OPCs. The extension and retraction of filopodium-like processes was imaged through real-time in vivo movies (Kirby et al., 2006). The same model could be used to determine the time it takes for OPCs to repopulate a lesioned area in response to mutation or drug treatment, therefore, providing a measurement of OPC recruitment during remyelination. However, laser ablation might cause non-specific damage to the tissue surrounding the OPCs.

REMYELINATION SCREENING USING ZEBRAFISH MODELS
The examples discussed above illustrate the tractability of zebrafish models in the elucidation of a wide range of oligodendrocyte- and myelination-related pathways. If such flexibility in an animal system can be harnessed for screening purposes, it has the potential for not only screening the effects of drugs, but also clarifying the great quantities of genomic and proteomic information generated by recent microarray studies (Arnett et al., 2003; Han et al., 2008; Lock et al., 2002). The key to a successful screen for remyelination is to use an appropriate model at each screening stage. The use of larval (rather than adult) stages of the zebrafish allows a rapid and relatively low-cost method of in vivo vertebrate analysis. Many successful high-throughput zebrafish larvae screens have been established, both for forward genetic screens and for identifying and optimising lead drugs (for reviews, see Berger and Currie, 2007; Kari et al., 2007; Lieschi and Currie, 2007; Rubinstein, 2003).

Using a model of myelination rather than remyelination allows for much faster screening because it does not require myelin injury and can be assessed at a single time point. For example, Fig. 1 illustrates the methods we used to screen drug libraries for their potential enhancement of myelination. olig2:EGFP transgenic larvae were placed into 96-well plates and treated with different drugs during oligodendrocyte specification (from 1 d.p.f. to 3 d.p.f.) (Fig. 1B). The drugs used were either part of reprofiled drug libraries or identified in the literature as being relevant to myelination. Oligodendrocyte recruitment was assessed in response to each drug by counting the number of olig2-positive cells that had dorsally migrated from the pMN in the spinal cord (oligodendrocyte-lineage-specific) (Fig. 1C). The olig2:EGFP transgenic line had previously been used to find a zebrafish gene which increased the number of olig2-
positive cells (Bruce Appel, personal communication). Therefore, we attempted to find a drug that replicated this result. An automated counting system was developed that allowed us to screen approximately 80 drugs per week (Fig. 1D). Drugs that produced a concentration-dependent change in olig2-positive cell number were tested for their effects on OPC differentiation by measuring relative levels of larval mbp mRNA using real-time PCR. Drugs that altered recruitment and/or differentiation of OPCs are currently being validated through IHC co-labeling with neuron and oligodendrocyte markers to ensure that their effect is specific to the oligodendrocyte lineage. IHC is also being used in combination with electron microscopy to assess whether there is a visual effect of these drugs on myelin (Fig. 1E,F) (Buckley et al., 2007). Any drugs that specifically increase the number of oligodendrocyte lineage cells, without depleting other cell types such as neurons, or that cause an increase in OPC differentiation and/or zebrafish myelination could then be transferred to an appropriate mammalian remyelination model, such as those described previously (Ibanez et al., 2004; Penderis et al., 2003) (Fig. 2).

There are some important critiques for this screening model. First, there was quite a high variation between larvae in the numbers of dorsally migrated olig2-positive cells. Second, it was often difficult to identify which olig2-positive cells to count. Some cells were on the border of the pMN and were, therefore, hard to distinguish from non-oligodendrocyte lineage cells (Fig. 1C). As a result, it was challenging to automate the counting of these cells. Finally, using microarray technology rather than real-time PCR would increase the throughput of the differentiation screen, allowing OPC differentiation to be assessed in parallel with OPC recruitment. This would prevent any compounds that exclusively altered differentiation and not recruitment from being missed.

There are also some other caveats of the zebrafish myelination model in general that require consideration. First, high-throughput screening of drugs is usually accomplished by adding them to the medium in which the larvae swim. Although zebrafish embryos are permeable to small molecules and drugs during organogenesis (Kari et al., 2007), some potentially therapeutic drugs might be missed owing to a lack of penetration. Second, zebrafish larvae are used in the techniques described here, rather than adults. In other species there is a decrease in remyelination efficiency in older animals, which is associated with a decrease in the processes of recruitment and differentiation of OPCs, as discussed earlier (Sim et al., 2002; Woodruff et al., 2004) (for a review about the effects of ageing on remyelination, see Rist and Franklin, 2008). Finally, although using a model of the developmental process of myelination rather than the regenerative process of remyelination allows for much faster screening, there are differences between myelination and remyelination (see Box 1).

It is possible that a zebrafish model of remyelination could be developed through the use of laser ablation or expression of the recently developed cyan fluorescent protein and nitroreductase (NTR) (Curado et al., 2007) in oligodendrocytes. As more zebrafish-specific markers and fluorescent lines are created, real-time visualisation of remyelination is a realistic goal. Also, the development of a transparent adult zebrafish line (White et al., 2008) means that it might soon be possible to visualise remyelination in vivo in adult fish. However, zebrafish have a high regenerative capacity when compared with mammals. For example, their hearts are able to regenerate completely without scarring, a phenomenon that has lead to the discovery that fibroblast growth factor (Fgf) and platelet-derived growth factor (Pdgf) signalling pathways positively influence cardiac regeneration (Lepilina et al., 2006; Lien et al., 2006; Poss, 2007). Spinal cord neurons are also able to regenerate after spinal cord transection (Becker et al., 1997). Similarly, OPC recruitment in response to injury in zebrafish larvae occurs rapidly following a lesion (Kirby et al., 2006). Although an in vivo real-time remyelination model is an exciting concept, it might be challenging to quantifiably improve this already efficient process. If such a visual and temporal technique is used as a screen for pro-myelination mutations or treatments, a lot of thought must be put into designing a realistic output.

CONCLUSION

Zebrafish provide a highly versatile model, both genetically and experimentally, and their myelination biology is homologous to the mammalian system. Therefore, they are very useful models for exploring the process of developmental myelination. High-throughput drug screens have been carried out looking for enhancers of OPC recruitment and differentiation during myelination; the drugs obtained from these screens can be tested in mammals for their potential effects on remyelination. Thus, zebrafish provide a realistic and resource-effective starting point towards developing a therapy that may eventually target CNS remyelination in MS. As the techniques for transgenic manipulation progress, zebrafish have the potential to provide the first real-time in vivo imaging models of myelination and remyelination. However, care must be taken when designing realistic experimental outputs for zebrafish remyelination models. The real power of zebrafish may be in modelling the developmental, rather than the regenerative, process.

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COMPETING INTERESTS

The authors declare no competing financial interests.

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