Revisiting remyelination: towards a consensus on the regeneration of CNS myelin

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Abstract

The biology of CNS remyelination has attracted considerable interest in recent years because of its translational potential to yield regenerative therapies for the treatment of chronic and progressive demyelinating diseases such as multiple sclerosis (MS). Critical to devising myelin regenerative therapies is a detailed understanding of how remyelination occurs. The accepted dogma, based on animal studies, has been that the myelin sheaths of remyelination are made by oligodendrocytes newly generated from adult oligodendrocyte progenitor cells in a classical regenerative process of progenitor migration, proliferation and differentiation. However, recent human and a growing number of animal studies have revealed a second mode of remyelination in which mature oligodendrocytes surviving within an area of demyelination are able to regenerate new myelin sheaths. This discovery, while opening up new opportunities for therapeutic remyelination, has also raised the question of whether there are fundamental differences in myelin regeneration between humans and some of the species in which experimental remyelination studies are conducted. Here we review how this second mode of remyelination can be integrated into a wider and revised framework for understanding remyelination in which apparent species differences can be reconciled but that also raises important questions for future research.

1. Introduction

The poor regenerative capacity of the mammalian central nervous system (CNS) has long been one of the central tenets of neurobiology. However, in 1961 a paper was published by the laboratory of Mary and Dick Bunge, in a relatively obscure journal, describing the then unexpected regenerative process of remyelination [1]. Using the somewhat outre model of CNS barbotage, where cerebrospinal fluid is withdrawn and then injected at force onto the surface of the cat spinal cord, combined with the then emergent technique of electron microscopy, it was shown that myelin sheaths first disappear from axons that remain intact (demyelination) and that new myelin sheaths are subsequently restored to the axons (remyelination), albeit often thinner than the original sheaths [2].
This phenomenon was explored in more detail by, amongst others, Bill Blakemore and Sam Ludwin, using toxins such as cuprizone in rodents and it was confirmed that the loss of myelin was followed by the subsequent recovery of thinner myelin sheaths [3]. Examination of post-mortem tissue by electron microscopy revealed thin myelin sheaths in the demyelinating disease multiple sclerosis (MS) [4], suggesting, but not proving, that this regenerative process could also occur in human neurological disease. This prompted a reassessment of MS shadow plaques (areas of pale myelin staining) as being areas of remyelination and not, as was previously proposed, areas of ongoing or partial demyelination. However, despite it being recognised as being of enormous therapeutic potential, the study of remyelination biology and its clinical application remained at the margins of mainstream neuroscience. It is only in recent years that there has been an explosion of interest in this relatively achievable regenerative medicine strategy, based on small molecules targeting endogenous regeneration [5,6].

2. The origins of new myelin

Given evidence that remyelination can take place, the question arose as to where the new myelin sheaths made during remyelination might be coming from? Two possible sources of new myelin in principle exist, myelin sheaths produced by newly generated oligodendrocytes, and those made by oligodendrocytes that survived demyelination. If demyelination occurs concomitant with significant oligodendrocyte loss, then those cells that survive demyelination are unlikely to represent a major source of remyelination, given that their ability to migrate would be limited by their own myelin sheaths and their inability to proliferate as postmitotic cells [6]. If, however, demyelination occurs and cells that lose their myelin sheaths can survive, then those cells, could, in principle, contribute significantly to remyelination. As will be discussed in this review, and given that regenerative processes in other tissues generally involve, at some stage, cell migration and proliferation, the notion that new oligodendrocytes might derive from a CNS resident stem or progenitor cell gained momentum, with the case for remyelination by cells that survive demyelination only recently garnering attention. The idea that new myelin might be made by newly generated cells was substantiated first by the discovery of adult neural progenitors in the vertebrate brain [7], and second, by the description of the glial (O-2A) progenitor as a source of oligodendrocytes during development by Raff and colleagues during the 1980s [8]. The discovery of O-2A cells, or oligodendrocyte progenitor cells (OPCs) as they are now more commonly called, in the adult rodent and human CNS made them obvious candidates for new oligodendrocyte generation during remyelination [9]. Several lines of evidence supported this view: first, proliferating cells, labelled either by injecting a LacZ-expressing retrovirus into normal adult white matter or with tritiated thymidine, and likely to be OPCs, gave rise to labelled remyelinating oligodendrocytes [10,11], second, transplanted adult OPCs can remyelinate areas of demyelination [12,13]. However, it was not until the advent of genetic fate mapping approaches that it could be unequivocally shown that adult OPCs give rise to remyelinating oligodendrocytes (and remyelinating Schwann cells that can remyelinate areas of CNS demyelination from which astrocytes are absent) [14-16]. This evidence confirmed the OPC as a target for remyelination biology as well as a target for approaches to therapeutically enhance remyelination in chronic demyelinating disease such as MS [6], where remyelination efficiency declines with age [17].
While the role of the OPCs in remyelination was ascendant, the case for differentiated surviving oligodendrocytes contributing to myelin regeneration weakened. Employing a similar fate-mapping approach used to demonstrate that OPCs can give rise to new remyelinating oligodendrocytes it was shown that intact mature oligodendrocytes do not generate new cells capable of contributing to remyelination [18], unlike myelinating Schwann cells in the peripheral nervous system, which can dedifferentiate and generate new cells that contribute to regeneration. It was also argued that mature oligodendrocyte cell bodies surviving within an area of demyelination, shorn of some or all of their processes, could not regenerate new myelin sheaths. This latter claim derived largely from a study in which a combination of antibodies and complement were used to induce demyelination while preserving oligodendrocyte cell bodies [19]. In order to prevent OPCs entering the lesion and remyelinating the demyelinated axons, the tissue containing the lesion was exposed to high level of X-irradiation. Since no remyelination occurred, it was inferred that the surviving oligodendrocytes were unable to generate new myelin sheaths. However, such were the levels of X-irradiation to which the surviving oligodendrocyte cell bodies were exposed, it remained likely that the normal functional repertoire of these cells was severely compromised. Indeed, recent studies have reinvigorated the idea that mature oligodendrocytes might represent a major source of myelin regeneration, particularly in the adult human brain.

3. Oligodendrocyte and myelin dynamics in humans

The generation of new myelin in the context of the regenerative sequela of demyelination has now been recognised for several decades, but more recently it has emerged that the generation of new oligodendrocytes and new myelin can also occur in the healthy adult CNS as part of neural circuit function associated with adaptation and learning. This concept is often referred to as adaptive myelination or myelin plasticity, and appears to be a fundamental feature of the adaptable vertebrate nervous system, evidenced from fish to man [20]. However, while both adaptive myelination and remyelination can be studied using the whole panoply of available tools and techniques in various vertebrate models, studying these processes in humans is especially challenging. Nonetheless, imaging studies have demonstrated increasing white matter volume in circuits employed by individuals who practice a task, both over short time frames as when taking a course in juggling and over long time periods as developing into a skilled musician [21-23]. Given that white matter tracts are principally comprised of myelinated axons, this suggests that adaptive myelination also occurs in humans. Dynamic regulation of myelination throughout adult human life is further supported by the fact that many MS patients have periods of remissions during which previous symptoms may dissipate, possibly reflecting remyelination, evidenced by PET imaging [24]. However, the cellular basis of adaptive myelination and remyelination in the human CNS remain to be determined.

Whereas proliferating OPCs and new oligodendrocytes are abundant in the adult rodent CNS and play key roles in both adaptive myelination and remyelination [14,25-27], it has been challenging to assess oligodendrocyte generation and myelination in humans since many of the methods used in experimental animals are not readily applicable in people. However, key
insights have recently emerged with the use of new methodology. The integration into cellular DNA of $^{14}$C, derived from nuclear bomb tests during the Cold War, can be used to birth date and establish cell generation dynamics of FACS-isolated cells or cell nuclei from the post-mortem human brain [28]. This has revealed a very low generation/exchange rate of oligodendrocytes in human white matter after the initial complement of oligodendrocytes is established in childhood, with only 1 in 300 (0.3%) oligodendrocytes being exchanged in a year [29]. This low degree of oligodendrocyte generation in the adult human brain was corroborated in cancer patients that received IdU as a radiosensitizer, where only very sparse oligodendrocytes with the nucleotide were found. However, whereas new oligodendrocyte generation and turnover is very low, carbon dating of myelin itself revealed that it is contemporary and thus continuously made through adult life [29]. This indicates that the active growth and maintenance of myelin in white matter tracts in humans is driven by mature oligodendrocytes. Given this conclusion, it is difficult to see how the low level of oligodendrocyte generation could underlie the rapid changes in white matter/ myelin volume seen in healthy humans [23]. Therefore, it may be that myelin plasticity is principally mediated by remodelling of pre-existing oligodendrocytes in human white matter.

The limited generation of oligodendrocytes in the adult white matter posed the question as to whether it is increased in MS in response to demyelination, as occurs in animal models [14,15]. Despite expectations that it might, carbon dating of oligodendrocytes in shadow plaques, thought to reflect areas of remyelination in MS, did not provide evidence for generation of new oligodendrocytes in these lesions. On the contrary, even the normally low generation rate of oligodendrocytes observed in the healthy nervous system appeared to have ground to a halt in shadow plaques around the time of disease diagnosis [30]. The evidence that areas of putative remyelination principally contained old oligodendrocytes suggests that it is pre-existing oligodendrocytes, rather than newborn cells that contribute to remyelination in MS. Despite the fact that shadow plaques in MS have a similar appearance to remyelinated lesions in experimental models, it is not possible to conclude that remyelination has taken place in these lesions in MS, and there may be other causes for this histology [31], including partial loss of myelin, as was first thought to characterise these lesions. Nonetheless, recent single cell RNA sequencing of the oligodendrocyte lineage in humans and mice has provided additional support to the premise that mature oligodendrocytes contribute to remyelination in humans [32,33]. These RNA-seq studies delineated the molecular characteristics of the progression of cells from OPCs through distinct intermediate states of oligodendrocyte maturation, to myelination in both healthy and diseased mice and humans. In an EAE model of demyelination in mice, OPCs were shown to proliferate and differentiate as expected, corroborating and providing a transcriptional map of their response to demyelination in the mouse. In addition, OPCs in EAE exhibit states not seen in healthy animals, with reduced expression of genes typical for the progenitor state and higher expression of differentiation-associated genes, including, unexpectedly, those associated with immune functions [32], as found also by others [34] [35]. These transcriptomic features were also documented in MS, highlighting complexity in the response of the oligodendrocyte lineage disease. The comparison of mouse and human transcriptomic signatures also indicated some differences in the response of the oligodendrocyte lineage to EAE and MS, with OPCs and intermediate cell states being
significantly reduced in number in MS (unlike in EAE), and with an increase in the number of actively myelinating mature oligodendrocytes in MS relative to healthy controls. Although RNA-seq cannot speak to the specific origin or remyelination proficiency of oligodendrocytes in MS, these observations, together with the lack of new oligodendrocytes in shadow plaques, support the conclusion that remyelination in MS may be mediated by old rather than new oligodendrocytes.

The suggestion that remyelination in MS may derive from old oligodendrocytes represented a paradigm shift in our understanding of remyelination biology and consideration of options to treat disease. However, MS is a heterogeneous disease, with great inter-individual variability, including in remyelination. Indeed, the ability to generate new oligodendrocytes in MS also appears to vary between individuals. For example, in the 14C-based cellular birth-dating study, although the majority of people with MS exhibited little evidence of new oligodendrocyte generation, about a quarter showed a more than threefold increase in the generation of new oligodendrocytes in normal appearing white matter, demonstrating the potential to greatly increase oligodendrocyte generation in the adult human MS brain [30], as would have been expected based on studies of animal models. These observations do not provide direct insight into the specific origin or functions of these newly-generated oligodendrocytes in MS patients, but the cells likely derive from OPCs and may participate in remyelination. It is possible for example that such cells contribute to remyelination in areas that do not leave a histological mark associated with remyelination at all. Indeed, it has recently been documented that areas of remyelination of experimentally-induced demyelination in animal models can be essentially indistinguishable from the surrounding normal white matter given sufficient time for full tissue resolution [31]. To better understand pathology and regeneration in MS, future studies will need to integrate new molecular and cellular approaches with imaging-based measures to determine the relationship between cell states, tissue appearance and the dynamics of prospective demyelination and remyelination during disease.

The recent molecular profiling and cellular based investigations of oligodendrocytes in health and disease in humans have uncovered previously unappreciated complexity and indicated that mature oligodendrocytes might significant contribute to remyelination. This begs the question as to whether there are major differences in oligodendrocyte biology between species, or whether the apparent differences in the relative importance of OPC-driven versus mature oligodendrocyte driven remyelination can be explained by the different contexts, timelines and methods of analyses of model systems and humans. This is an issue that we consider of great importance to resolve.

4. Reconciling apparent differences between animal models and humans

A cursory review of the current literature could lead to the conclusion that remyelination has been subject to an evolutionary divergence that has led to profoundly different mechanisms in humans versus animal models. This is not our view. Although undoubtedly inter-species differences occur in the gene expression profiles of the various stages of the oligodendrocyte lineage and possibly context-specific functions, we are not aware of fundamental differences
in the mechanisms by which oligodendrocytes are formed from their progenitors throughout vertebrates. No major differences have emerged in the key regulatory machineries that drive oligodendrocyte differentiation. Furthermore, with respect to the key difference in myelin regeneration re-ignited by the human-based investigations, recent studies have indicated that mature oligodendrocytes can also contribute to remyelination in animal models. Firstly, based on the premise that the appearance of thin myelin is indicative of sheaths made during remyelination, Duncan et al. showed that following demyelination in a cat model, single oligodendrocytes had both thick myelin sheaths and thin myelin sheaths, taken as evidence of the former being an old sheath that survived demyelination and the latter one made during remyelination [36]. Although it is emerging that thicker myelin sheaths can result from remyelination, given enough time, two very recent live imaging studies (one in mice, one in zebrafish) have provided further and direct evidence of new myelin sheath generation by oligodendrocytes that survive demyelination.

Advances in microscopy and the development of reporter tools have revolutionised the analyses of cell behaviours in the vertebrate nervous system. In a recent study, Bacmeister et al. used multiphoton-imaging of transgenic mice in which oligodendrocyte lineage cells expressed a fluorescent reporter to track the response of cells to demyelination of superficial layers of the cerebral cortex of mice in response to the dietary toxin cuprizone [37]. In addition to characterising the nature of remyelination by newly generated oligodendrocytes, the authors also observed that oligodendrocytes that survived losing some of their myelin sheaths occasionally formed a new one. Remarkably though, training in a specific skilled reaching task increased the number of the demyelinated oligodendrocytes that contributed to remyelination. In a separate study using zebrafish, Neely et al. found, similarly, that newly generated oligodendrocytes exhibited robust remyelination potential, while those that survived demyelination were much less efficient [38]. Together these recent studies indicate that the generation of new sheaths by oligodendrocytes that survive demyelination is both demonstrably possible and conserved from zebrafish through mammals, in line with the predictions of recent studies in humans.

Therefore, what has emerged are (at least) two distinct mechanisms of new myelin formation following demyelination - one involving the generation of new oligodendrocytes, and one with new myelin formed by existing oligodendrocytes (Figure 1).

Many key questions remain, including how the relative balance of the two modes of remyelination is determined, and whether one or other might represent more promising targets to promote remyelination for the treatment of disease?

4.1 Might current classification of MS lesions skew observations of modes of remyelination?
A potential explanation for the apparent differences in the modes of remyelination observed in models and humans may be due to the ease with which one can tell where demyelination is induced (and thus remyelination occurs) in animal models compared to the difficulties of doing
so in disease. In animal models the timing and area of demyelination are controlled and so the subsequent time-course of regeneration can be carefully mapped. In contrast, a significant caveat in the pathology-based analyses of MS tissue is that they are typically end-stage analyses, as biopsies are rare, and are snapshots from which one cannot be certain what happened before or what might have happened afterwards. Detailed histological studies, in depth discussion of which are beyond the scope of this piece, indicate the complexity of cellular pathology in MS. For example, differentiated oligodendrocytes are often abundant at the edges of demyelinated lesions, where they are positioned to, but fail to contribute to sufficient remyelination, either due to a limited regenerative potential (e.g. perhaps cells that survived myelin loss), and or environmental factors that limit remyelination (by newly made or surviving cells). In contrast, the number of differentiated oligodendrocytes in lesion cores is often greatly reduced, and may remain refractory to remyelination without significant intervention [39]. As noted above, shadow plaques may represent regions of partial damage, or areas of remyelination that only surviving cells exhibit, or may not have even undergone remyelination [31]. Similarly, some remyelination could occur in areas with no histological mark upon end-stage analyses. Therefore, it is possible that lesions examined at the end-stage of disease primarily represent lesions that are refractory to remyelination and that fully resolved areas of remyelination, perhaps mediated by the generation of new oligodendrocytes, have remained undetected to date.

4.2 Might distinctions between grey and white matter or distinct neuronal subtypes exhibit distinct modes of remyelination?

Studies in model organisms have documented distinctions in the fate and potential of OPCs and oligodendrocytes to contribute to myelination of the grey and white matter. For example, OPCs appear to be able to directly differentiate into oligodendrocytes without proliferation in the grey matter [40], whereas in white matter (and in vitro) proliferation is required for differentiation [41]. OPCs can also receive very different modes of synaptic input (e.g. plentiful GABAergic input in the grey matter, but prominently glutamatergic in the white matter), and may even have distinct functions in the grey matter such as locally regulating synapse function [42]. Furthermore, cell transplantation studies have indicated that the grey matter is less permissive for oligodendrocyte differentiation than the white matter, and that white matter-derived oligodendrocytes are more likely to differentiate than grey matter derived cells, irrespective of their environment [43]. Also, OPCs derived from the brain and spinal cord generate oligodendrocytes with profiles of myelination (sheath number and length) indicative of their donor environment, even when cultured with plastic synthetic microfibers, indicating that regional differences may also exist in the myelinating potential of single mature oligodendrocytes [44]. Cellular 14C-based birth-dating in humans has indicated that the final number of oligodendrocytes is reached much later in grey matter, well into adulthood depending on brain area, with the generation rate of oligodendrocytes being almost 8-fold higher than in white matter [29]. This higher generation rate of oligodendrocytes in the grey matter, which may relate to the protracted myelination of cortical axons [26,45,46], suggests that grey matter remyelination in humans might also be largely driven by generation of new oligodendrocyte, as predominates throughout most animal models. Although remyelination of the human grey matter is thought to be more efficient than that of the white matter, comparison
of the mechanisms of remyelination in the human grey and white matter remains to be undertaken.

In addition to potentially gross differences in modes of remyelination being evident due to how lesions are analysed in humans and models and possible distinctions between grey matter and white matter-located OPCs and oligodendrocytes in remyelination, there are likely also more local factors that could greatly influence remyelination. Another recent live imaging-based study of cortical remyelination in rodents showed, for example that the rate of new oligodendrocyte generation was reduced in deeper cortical layers, coinciding with increased gliosis [47]. This study also showed that newly generated oligodendrocytes in superficial layers of the cortex often made new myelin sheaths on new axons (or stretches of axon) not previously myelinated, indicating that the local environment may also influence the pattern of myelination versus remyelination, and therefore also potentially regulating circuit function. It is possible, that there are redundancies in the requirement for myelination (and in turn remyelination) of some circuits compared to others. For example, developmental myelination has previously been shown to vary significantly between axons belonging to neurons located in distinct cortical layers, with neurons in superficial cortical layers having axons with intermittent myelination, compared to those in deeper layers being essentially completely myelinated along their length [45]. This might mean that remyelination of intermittently myelinated axons does not have to be as stringent to ensure the maintenance of their health and function compared to more completely myelinated axons. Whereas the recent analyses of newly generated oligodendrocytes in superficial cortical layers showed that oligodendrocytes can exhibit de novo myelination concomitant with remyelination, other studies in both the mammalian cortex and zebrafish spinal cord indicate that very precise restoration of original patterns of myelin can be restored by remyelination, following the ablation of single oligodendrocytes lead to [48,49]. Further evidence of neuron-driven local regulation of myelination in vivo, comes from analyses of the zebrafish spinal cord in which distinct modes of myelination (activity regulated or not) are exhibited by different neuronal subtypes with axons that can in principle be myelinated by the same oligodendrocyte [50]. As per the comparison of grey matter and white matter remyelination, investigation of the mechanisms underpinning remyelination of axons of different sizes, functional characteristics, or original patterns of myelination remain largely unexplored and represent important areas of future investigation.

4.3 Could fundamental differences in human OPCs skew modes of remyelination?

Despite the likelihood that spatial, temporal and functional characteristics of areas of the CNS and their local microenvironments will have a bearing on remyelination, it remains the case that OPCs are present throughout the grey and white matter in the healthy nervous system in roughly consistent densities irrespective of brain area, and that OPCs respond to demyelination in model organisms. This begs the question as to whether human OPCs simply cannot generate remyelinating oligodendrocytes as well those of model systems. Pioneering inter-species transplantation studies suggest, however, that they can. First it was shown that human glial progenitor cells have the capacity to myelinate essentially the entire brain of mutant mice that cannot make their own myelin sheaths, due to a mutation in a major myelin protein. The transplanted glial progenitors even outcompete the endogenous glial cell populations, meaning
that chimeric mice acquire principally human-derived OPCs [51]. More recently, such mice with now-resident human OPCs and human myelin were experimentally demyelinated using cuprizone. It was found that the human OPCs were capable of responding to demyelination and of generating new oligodendrocytes that exhibited *bona fide* remyelination [52]. This study indicates that human OPCs and the oligodendrocytes that they generate are not fundamentally different from those of rodents and are potent myelin regenerators.

So, why might there be a skew against remyelination by newly generated oligodendrocytes in humans? One possibility is that the demyelinated lesion environment in human disease may be more hostile to OPC migration and/or proliferation than in animal models, meaning that in certain areas remyelination by mature oligodendrocytes is the only option. Indeed, nearly 40% of lesions in MS are thought to have deficiencies in OPCs [53]. Furthermore, exogenous expression of a chemorepellent abundant in lesions that lack OPCs can impair otherwise successful remyelination in mice [54]. Furthermore, the difference in scale between lesions typically assessed in animal models and those of humans may provide constraints that are more difficult to overcome. Therefore, it may be that it is the lesion environment that principally impairs remyelination by OPCs, and that, context-aside, mechanisms of remyelination may otherwise be largely conserved across species. One major regulator of the tissue microenvironment that contributes to remyelination success or failure is the process of ageing. Indeed, because longevity is not greatly reduced in MS, pathological analyses of disease are typically made of tissue that has experienced significant effects of ageing, which may contribute the observation of specific modes of remyelination.

### 4.4 Does ageing skew modes of remyelination?

The apparently vast differences in the extent of oligodendrocyte generation between adult rodent and human brain may in part be related to the ages of the species that are compared. In most studies of adult rodents, animals a few months of age are typically used, whereas in humans the onset of clinical pathology in MS typically occurs in the third decade of life. Therefore, one possibility is that the progenitor-mediated remyelination that occurs in young animals is essentially a continuation of the developmental process of myelination and that this has long-since passed in many brain areas in humans by the time disease symptoms emerge. Indeed with ageing the efficiency of progenitor-driven remyelination also declines in rodents [17,55] and this may be naturally superseded by mature oligodendrocyte-mediated remyelination. In this regard, it is interesting that progenitor-based remyelination is predominantly a feature of short-lived species such as rodents and fish, and that oligodendrocyte-mediated remyelination may become more prominent in cats, non-human primates and humans. In humans, progenitor-mediated remyelination may only occur in young adults, and/or only in specific areas (grey vs white matter), and the now-well established effects of ageing on OPCs may render progenitor cells incapable of effectively contributing to remyelination much earlier than was previously thought. Nonetheless, OPCs clearly persist throughout the human lifespan, and, based on rodent studies that aged OPCs can be rejuvenated to undergo enhanced remyelination [17,56,57]. Therefore, the OPC and the ability to generate new oligodendrocytes undoubtedly remain legitimate targets for therapeutic remyelination.
5. New oligodendrocytes exhibit better remyelination than oligodendrocytes that survive demyelination

Two recent live imaging-based studies have allowed a direct comparison of the relative remyelinating potential of oligodendrocytes that survived demyelination with those newly generated following demyelination. In one study that employed zebrafish as a model, Neely et al generated a transgenic line in which almost complete myelin sheath loss could be induced whilst leading to relatively limited oligodendrocyte death [38]. By exploiting the advantages of zebrafish for in vivo live imaging, the authors also followed single oligodendrocytes over time, both newly generated cells and those that survived demyelination. They showed that newly generated oligodendrocytes made large numbers of myelin sheaths correctly targeted to axons. In contrast, oligodendrocytes that survived losing their myelin sheaths made very few new sheaths (only two per cell compared to 15 prior to demyelination). Secondly, a majority of surviving cells mistargeted newly-made myelin, placing it on neuronal cell bodies instead of axons, suggesting that the ability of mature oligodendrocytes to sense correct targets is impaired following demyelination (Figure 1). Interestingly the mistargeting of myelin to cell bodies was also observed in areas of prospective remyelination in the grey matter of MS, suggesting that myelin mistargeting may be a conserved feature of remyelination by surviving cells. Despite an apparently limited ability to form and correctly target new sheaths, surviving oligodendrocytes were able to support the elongation of myelin sheaths along axons. In parallel, live imaging of oligodendrocytes in the rodent cortex over time following treatment with the demyelinating toxin cuprizone provided broadly similar findings. Work by both Bacmeister et al., and Orthmann-Murphy et al., showed that newly generated oligodendrocytes were produced (as expected) following demyelination and were capable of generating large numbers of myelin sheaths [37,47]. Although cuprizone treatment ultimately leads to oligodendrocyte cell death following demyelination, Bacmeister et al., were able to follow the fate of oligodendrocytes that survived demyelination, and found that they were also capable of generating new sheaths. These oligodendrocytes made very few new sheaths, mirroring observations made in zebrafish, but very interestingly, could do so over a protracted period of time, suggesting that they might be manipulable. Indeed, Bacmeister et al., made the remarkable finding that both the proportion of oligodendrocytes that contributed to remyelination and the amount of myelin that those cells made could be enhanced by training animals following demyelination in region and circuit specific tasks [37], validating the principle that surviving oligodendrocytes also represent therapeutic targets for the enhancement of remyelination. Future analyses of remyelination by surviving oligodendrocytes in different models and contexts of different brain areas, times, and in yet further environmental contexts will further explore the potential of these cells to contribute to remyelination. It is also clear that deeper analyses of individual oligodendrocytes in MS will also be required to determine how their myelination characteristics relates to their molecular profiles, age and environment.

6. Conclusions
Many questions remain as to the potential for remyelination by new and surviving oligodendrocytes. In human disease, the extent of remyelination by these respective cell types remains to be more fully elucidated. Deep molecular profiling and comparison of newly generated and surviving oligodendrocytes in disease and animal models will also help identify further strategies to promote their ability to contribute to remyelination. Similarly, phenotypic screens in models in which surviving cells are experimentally tractable may identify drug candidates to promote remyelination, as have been successful in finding compounds that promote new oligodendrocyte production. In addition, ongoing studies focused on the cell biology of remyelination in diverse species and models that reveal the myriad cell-cell interactions and molecular pathways that promote and prevent remyelination will aid both our understanding of this fundamental feature of regeneration of the CNS and provide insights to help treat human disease.

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Figure 1. Two modes of remyelination by oligodendrocytes

**Top.** In the healthy CNS, oligodendrocyte progenitor cells (OPCs) generate the oligodendrocytes that myelinate axons (sky blue).

**Middle.** Upon demyelination, some oligodendrocytes survive losing some or all of their myelin sheaths whereas others die. This can lead to activation of OPCs (dark blue).

**Bottom.** During remyelination, OPCs can generate new myelinating-oligodendrocytes (purple). In addition oligodendrocytes that survive demyelination can contribute to remyelination, but to a limited extent (green cells left and right), and sometimes mistargeting myelin to cell bodies (green cell left).
Revisiting remyelination: towards a consensus on the regeneration of CNS myelin

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Abstract

The biology of CNS remyelination has attracted considerable interest in recent years because of its translational potential to yield regenerative therapies for the treatment of chronic and progressive demyelinating diseases such as multiple sclerosis (MS). Critical to devising myelin regenerative therapies is a detailed understanding of how remyelination occurs. The accepted dogma, based on animal studies, has been that the myelin sheaths of remyelination are made by oligodendrocytes newly generated from adult oligodendrocyte progenitor cells in a classical regenerative process of progenitor migration, proliferation and differentiation. However, recent human and a growing number of animal studies have revealed a second mode of remyelination in which mature oligodendrocytes surviving within an area of demyelination are able to regenerate new myelin sheaths. This discovery, while opening up new opportunities for therapeutic remyelination, has also raised the question of whether there are fundamental differences in myelin regeneration between humans and some of the species in which experimental remyelination studies are conducted. Here we review how this second mode of remyelination can be integrated into a wider and revised framework for understanding remyelination in which apparent species differences can be reconciled but that also raises important questions for future research.

1. Introduction

The poor regenerative capacity of the mammalian central nervous system (CNS) has long been one of the central tenets of neurobiology. However, in 1961 a paper was published by the laboratory of Mary and Dick Bunge, in a relatively obscure journal, describing the then unexpected regenerative process of remyelination [1]. Using the somewhat outré model of CNS barbotage, where cerebrospinal fluid is withdrawn and then injected at force onto the surface of the cat spinal cord, combined with the then emergent technique of electron microscopy, it was shown that myelin sheaths first disappear from axons that remain intact (demyelination) and that new myelin sheaths are subsequently restored to the axons (remyelination), albeit often thinner than the original sheaths [2].
This phenomenon was explored in more detail by, amongst others, Bill Blakemore and Sam Ludwin, using toxins such as cuprizone in rodents and it was confirmed that the loss of myelin was followed by the subsequent recovery of thinner myelin sheaths [3]. Examination of post-mortem tissue by electron microscopy revealed thin myelin sheaths in the demyelinating disease multiple sclerosis (MS) [4], suggesting, but not proving, that this regenerative process could also occur in human neurological disease. This prompted a reassessment of MS shadow plaques (areas of pale myelin staining) as being areas of remyelination and not, as was previously proposed, areas of ongoing or partial demyelination. However, despite it being recognised as being of enormous therapeutic potential, the study of remyelination biology and its clinical application remained at the margins of mainstream neuroscience. It is only in recent years that there has been an explosion of interest in this relatively achievable regenerative medicine strategy, based on small molecules targeting endogenous regeneration [5,6].

2. The origins of new myelin

Given evidence that remyelination can take place, the question arose as to where the new myelin sheaths made during remyelination might be coming from? Two possible sources of new myelin in principle exist, myelin sheaths produced by newly generated oligodendrocytes, and those made by oligodendrocytes that survived demyelination. If demyelination occurs concomitant with significant oligodendrocyte loss, then those cells that survive demyelination are unlikely to represent a major source of remyelination, given that their ability to migrate would be limited by their own myelin sheaths and their inability to proliferate as postmitotic cells [6]. If, however, demyelination occurs and cells that lose their myelin sheaths can survive, then those cells, could, in principle, contribute significantly to remyelination. As will be discussed in this review, and given that regenerative processes in other tissues generally involve, at some stage, cell migration and proliferation, the notion that new oligodendrocytes might derive from a CNS resident stem or progenitor cell gained momentum, with the case for remyelination by cells that survive demyelination only recently garnering attention. The idea that new myelin might be made by newly generated cells was substantiated first by the discovery of adult neural progenitors in the vertebrate brain [7], and second, by the description of the glial (O-2A) progenitor as a source of oligodendrocytes during development by Raff and colleagues during the 1980s [8]. The discovery of O-2A cells, or oligodendrocyte progenitor cells (OPCs) as they are now more commonly called, in the adult rodent and human CNS made them obvious candidates for new oligodendrocyte generation during remyelination [9]. Several lines of evidence supported this view: first, proliferating cells, labelled either by injecting a LacZ-expressing retrovirus into normal adult white matter or with tritiated thymidine, and likely to be OPCs, gave rise to labelled remyelinating oligodendrocytes [10,11], second, transplanted adult OPCs can remyelinate areas of demyelination [12,13]. However, it was not until the advent of genetic fate mapping approaches that it could be unequivocally shown that adult OPCs give rise to remyelinating oligodendrocytes (and remyelinating Schwann cells that can remyelinate areas of CNS demyelination from which astrocytes are absent) [14-16]. This evidence confirmed the OPC as a target for remyelination biology as well as a target for
approaches to therapeutically enhance remyelination in chronic demyelinating disease such as MS [6], where remyelination efficiency declines with age [17].

While the role of the OPCs in remyelination was ascendant, the case for differentiated surviving oligodendrocytes contributing to myelin regeneration weakened. Employing a similar fate-mapping approach used to demonstrate that OPCs can give rise to new remyelinating oligodendrocytes it was shown that intact mature oligodendrocytes do not generate new cells capable of contributing to remyelination [18], unlike myelinating Schwann cells in the peripheral nervous system, which can dedifferentiate and generate new cells that contribute to regeneration. It was also argued that mature oligodendrocyte cell bodies surviving within an area of demyelination, shorn of some or all of their processes, could not regenerate new myelin sheaths. This latter claim derived largely from a study in which a combination of antibodies and complement were used to induce demyelination while preserving oligodendrocyte cell bodies [19]. In order to prevent OPCs entering the lesion and remyelinating the demyelinated axons, the tissue containing the lesion was exposed to high level of X-irradiation. Since no remyelination occurred, it was inferred that the surviving oligodendrocytes were unable to generate new myelin sheaths. However, such were the levels of X-irradiation to which the surviving oligodendrocyte cell bodies were exposed, it remained likely that the normal functional repertoire of these cells was severely compromised. Indeed, recent studies have reinvigorated the idea that mature oligodendrocytes might represent a major source of myelin regeneration, particularly in the adult human brain.

3. Oligodendrocyte and myelin dynamics in humans

The generation of new myelin in the context of the regenerative sequela of demyelination has now been recognised for several decades, but more recently it has emerged that the generation of new oligodendrocytes and new myelin can also occur in the healthy adult CNS as part of neural circuit function associated with adaptation and learning. This concept is often referred to as adaptive myelination or myelin plasticity, and appears to be a fundamental feature of the adaptable vertebrate nervous system, evidenced from fish to man [20]. However, while both adaptive myelination and remyelination can be studied using the whole panoply of available tools and techniques in various vertebrate models, studying these processes in humans is especially challenging. Nonetheless, imaging studies have demonstrated increasing white matter volume in circuits employed by individuals who practice a task, both over short time frames as when taking a course in juggling and over long time periods as developing into a skilled musician [21-23]. Given that white matter tracts are principally comprised of myelinated axons, this suggests that adaptive myelination also occurs in humans. Dynamic regulation of myelination throughout adult human life is further supported by the fact that many MS patients have periods of remissions during which previous symptoms may dissipate, possibly reflecting remyelination, evidenced by PET imaging [24]. However, the cellular basis of adaptive myelination and remyelination in the human CNS remain to be determined.

Whereas proliferating OPCs and new oligodendrocytes are abundant in the adult rodent CNS and play key roles in both adaptive myelination and remyelination [14,25-27], it has been
challenging to assess oligodendrocyte generation and myelination in humans since many of the methods used in experimental animals are not readily applicable in people. However, key insights have recently emerged with the use of new methodology. The integration into cellular DNA of $^{14}$C, derived from nuclear bomb tests during the Cold War, can be used to birth date and establish cell generation dynamics of FACS-isolated cells or cell nuclei from the post-mortem human brain [28]. This has revealed a very low generation/exchange rate of oligodendrocytes in human white matter after the initial complement of oligodendrocytes is established in childhood, with only 1 in 300 (0.3%) oligodendrocytes being exchanged in a year [29]. This low degree of oligodendrocyte generation in the adult human brain was corroborated in cancer patients that received IdU as a radiosensitizer, where only very sparse oligodendrocytes with the nucleotide were found. However, whereas new oligodendrocyte generation and turnover is very low, carbon dating of myelin itself revealed that it is contemporary and thus continuously made through adult life [29]. This indicates that the active growth and maintenance of myelin in white matter tracts in humans is driven by mature oligodendrocytes. Given this conclusion, it is difficult to see how the low level of oligodendrocyte generation could underlie the rapid changes in white matter/myelin volume seen in healthy humans [23]. Therefore, it may be that myelin plasticity is principally mediated by remodelling of pre-existing oligodendrocytes in human white matter.

The limited generation of oligodendrocytes in the adult white matter posed the question as to whether it is increased in MS in response to demyelination, as occurs in animal models [14,15]. Despite expectations that it might, carbon dating of oligodendrocytes in shadow plaques, thought to reflect areas of remyelination in MS, did not provide evidence for generation of new oligodendrocytes in these lesions. On the contrary, even the normally low generation rate of oligodendrocytes observed in the healthy nervous system appeared to have ground to a halt in shadow plaques around the time of disease diagnosis [30]. The evidence that areas of putative remyelination principally contained old oligodendrocytes suggests that it is pre-existing oligodendrocytes, rather than newborn cells that contribute to remyelination in MS. Despite the fact that shadow plaques in MS have a similar appearance to remyelinated lesions in experimental models, it is not possible to conclude that remyelination has taken place in these lesions in MS, and there may be other causes for this histology [31], including partial loss of myelin, as was first thought to characterise these lesions. Nonetheless, recent single cell RNA sequencing of the oligodendrocyte lineage in humans and mice has provided additional support to the premise that mature oligodendrocytes contribute to remyelination in humans [32,33]. These RNA-seq studies delineated the molecular characteristics of the progression of cells from OPCs through distinct intermediate states of oligodendrocyte maturation, to myelination in both healthy and diseased mice and humans. In an EAE model of demyelination in mice, OPCs were shown to proliferate and differentiate as expected, corroborating and providing a transcriptional map of their response to demyelination in the mouse. In addition, OPCs in EAE exhibited states not seen in healthy animals, with reduced expression of genes typical for the progenitor state and higher expression of differentiation-associated genes, including, unexpectedly, those associated with immune functions [32], as found also by others [34] [35]. These transcriptomic features were also documented in MS, highlighting complexity in the
response of the oligodendrocyte lineage disease. The comparison of mouse and human transcriptomic signatures also indicated some differences in the response of the oligodendrocyte lineage to EAE and MS, with OPCs and intermediate cell states being significantly reduced in number in MS (unlike in EAE), and with an increase in the number of actively myelinating mature oligodendrocytes in MS relative to healthy controls. Although RNA-seq cannot speak to the specific origin or remyelination proficiency of oligodendrocytes in MS, these observations, together with the lack of new oligodendrocytes in shadow plaques, support the conclusion that remyelination in MS may be mediated by old rather than new oligodendrocytes.

The suggestion that remyelination in MS may derive from old oligodendrocytes represented a paradigm shift in our understanding of remyelination biology and consideration of options to treat disease. However, MS is a heterogeneous disease, with great inter-individual variability, including in remyelination. Indeed, the ability to generate new oligodendrocytes in MS also appears to vary between individuals. For example, in the 14C-based cellular birth-dating study, although the majority of people with MS exhibited little evidence of new oligodendrocyte generation, about a quarter showed a more than threefold increase in the generation of new oligodendrocytes in normal appearing white matter, demonstrating the potential to greatly increase oligodendrocyte generation in the adult human MS brain [30], as would have been expected based on studies of animal models. These observations do not provide direct insight into the specific origin or functions of these newly-generated oligodendrocytes in MS patients, but the cells likely derive from OPCs and may participate in remyelination. It is possible for example that such cells contribute to remyelination in areas that do not leave a histological mark associated with remyelination at all. Indeed, it has recently been documented that areas of remyelination of experimentally-induced demyelination in animal models can be essentially indistinguishable from the surrounding normal white matter given sufficient time for full tissue resolution [31]. To better understand pathology and regeneration in MS, future studies will need to integrate new molecular and cellular approaches with imaging-based measures to determine the relationship between cell states, tissue appearance and the dynamics of prospective demyelination and remyelination during disease.

The recent molecular profiling and cellular based investigations of oligodendrocytes in health and disease in humans have uncovered previously unappreciated complexity and indicated that mature oligodendrocytes might significant contribute to remyelination. This begs the question as to whether there are major differences in oligodendrocyte biology between species, or whether the apparent differences in the relative importance of OPC-driven versus mature oligodendrocyte driven remyelination can be explained by the different contexts, timelines and methods of analyses of model systems and humans. This is an issue that we consider of great importance to resolve.

4. Reconciling apparent differences between animal models and humans

A cursory review of the current literature could lead to the conclusion that remyelination has been subject to an evolutionary divergence that has led to profoundly different mechanisms in
humans versus animal models. This is not our view. Although undoubtedly inter-species differences occur in the gene expression profiles of the various stages of the oligodendrocyte lineage and possibly context-specific functions, we are not aware of fundamental differences in the mechanisms by which oligodendrocytes are formed from their progenitors throughout vertebrates. No major differences have emerged in the key regulatory machineries that drive oligodendrocyte differentiation. Furthermore, with respect to the key difference in myelin regeneration re-ignited by the human-based investigations, recent studies have indicated that mature oligodendrocytes can also contribute to remyelination in animal models. Firstly, based on the premise that the appearance of thin myelin is indicative of sheaths made during remyelination, Duncan et al. showed that following demyelination in a cat model, single oligodendrocytes had both thick myelin sheaths and thin myelin sheaths, taken as evidence of the former being an old sheath that survived demyelination and the latter one made during remyelination [36]. Although it is emerging that thicker myelin sheaths can result from remyelination, given enough time, two very recent live imaging studies (one in mice, one in zebrafish) have provided further and direct evidence of new myelin sheath generation by oligodendrocytes that survive demyelination.

Advances in microscopy and the development of reporter tools have revolutionised the analyses of cell behaviours in the vertebrate nervous system. In a recent study, Bacmeister et al. used multiphoton-imaging of transgenic mice in which oligodendrocyte lineage cells expressed a fluorescent reporter to track the response of cells to demyelination of superficial layers of the cerebral cortex of mice in response to the dietary toxin cuprizone [37]. In addition to characterising the nature of remyelination by newly generated oligodendrocytes, the authors also observed that oligodendrocytes that survived losing some of their myelin sheaths occasionally formed a new one. Remarkably though, training in a specific skilled reaching task increased the number of the demyelinated oligodendrocytes that contributed to remyelination. In a separate study using zebrafish, Neely et al. found, similarly, that newly generated oligodendrocytes exhibited robust remyelination potential, while those that survived demyelination were much less efficient [38]. Together these recent studies indicate that the generation of new sheaths by oligodendrocytes that survive demyelination is both demonstrably possible and conserved from zebrafish through mammals, in line with the predictions of recent studies in humans.

Therefore, what has emerged are (at least) two distinct mechanisms of new myelin formation following demyelination - one involving the generation of new oligodendrocytes, and one with new myelin formed by existing oligodendrocytes (Figure 1).

Many key questions remain, including how the relative balance of the two modes of remyelination is determined, and whether one or other might represent more promising targets to promote remyelination for the treatment of disease?
4.1 Might current classification of MS lesions skew observations of modes of remyelination?

A potential explanation for the apparent differences in the modes of remyelination observed in models and humans may be due to the ease with which one can tell where demyelination is induced (and thus remyelination occurs) in animal models compared to the difficulties of doing so in disease. In animal models the timing and area of demyelination are controlled and so the subsequent time-course of regeneration can be carefully mapped. In contrast, a significant caveat in the pathology-based analyses of MS tissue is that they are typically end-stage analyses, as biopsies are rare, and are snapshots from which one cannot be certain what happened before or what might have happened afterwards. Detailed histological studies, in depth discussion of which are beyond the scope of this piece, indicate the complexity of cellular pathology in MS. For example, differentiated oligodendrocytes are often abundant at the edges of demyelinated lesions, where they are positioned to, but fail to contribute to sufficient remyelination, either due to a limited regenerative potential (e.g. perhaps cells that survived myelin loss), and or environmental factors that limit remyelination (by newly made or surviving cells). In contrast, the number of differentiated oligodendrocytes in lesion cores is often greatly reduced, and may remain refractory to remyelination without significant intervention [39]. As noted above, shadow plaques may represent regions of partial damage, or areas of remyelination that only surviving cells exhibit, or may not have even undergone remyelination [31]. Similarly, some remyelination could occur in areas with no histological mark upon end-stage analyses. Therefore, it is possible that lesions examined at the end-stage of disease primarily represent lesions that are refractory to remyelination and that fully resolved areas of remyelination, perhaps mediated by the generation of new oligodendrocytes, have remained undetected to date.

4.2 Might distinctions between grey and white matter or distinct neuronal subtypes exhibit distinct modes of remyelination?

Studies in model organisms have documented distinctions in the fate and potential of OPCs and oligodendrocytes to contribute to myelination of the grey and white matter. For example, OPCs appear to be able to directly differentiate into oligodendrocytes without proliferation in the grey matter [40], whereas in white matter (and in vitro) proliferation is required for differentiation [41]. OPCs can also receive very different modes of synaptic input (e.g. plentiful GABAergic input in the grey matter, but prominently glutamatergic in the white matter), and may even have distinct functions in the grey matter such as locally regulating synapse function [42]. Furthermore, cell transplantation studies have indicated that the grey matter is less permissive for oligodendrocyte differentiation than the white matter, and that white matter-derived oligodendrocytes are more likely to differentiate than grey matter derived cells, irrespective of their environment [43]. Also, OPCs derived from the brain and spinal cord generate oligodendrocytes with profiles of myelination (sheath number and length) indicative of their donor environment, even when cultured with plastic synthetic microfibers, indicating that regional differences may also exist in the myelinating potential of single mature oligodendrocytes [44]. Cellular 14C-based birth-dating in humans has indicated that the final number of oligodendrocytes is reached much later in grey matter, well into adulthood depending on brain area, with the generation rate of oligodendrocytes being almost 8-fold
higher than in white matter [29]. This higher generation rate of oligodendrocytes in the grey matter, which may relate to the protracted myelination of cortical axons [26,45,46], suggests that grey matter remyelination in humans might also be largely driven by generation of new oligodendrocyte, as predominates throughout most animal models. Although remyelination of the human grey matter is thought to be more efficient than that of the white matter, comparison of the mechanisms of remyelination in the human grey and white matter remains to be undertaken.

In addition to potentially gross differences in modes of remyelination being evident due to how lesions are analysed in humans and models and possible distinctions between grey matter and white matter-located OPCs and oligodendrocytes in remyelination, there are likely also more local factors that could greatly influence remyelination. Another recent live imaging-based study of cortical remyelination in rodents showed, for example that the rate of new oligodendrocyte generation was reduced in deeper cortical layers, coinciding with increased gliosis [47]. This study also showed that newly generated oligodendrocytes in superficial layers of the cortex often made new myelin sheaths on new axons (or stretches of axon) not previously myelinated, indicating that the local environment may also influence the pattern of myelination versus remyelination, and therefore also potentially regulating circuit function. It is possible, that there are redundancies in the requirement for myelination (and in turn remyelination) of some circuits compared to others. For example, developmental myelination has previously been shown to vary significantly between axons belonging to neurons located in distinct cortical layers, with neurons in superficial cortical layers having axons with intermittent myelination, compared to those in deeper layers being essentially completely myelinated along their length [45]. This might mean that remyelination of intermittently myelinated axons does not have to be as stringent to ensure the maintenance of their health and function compared to more completely myelinated axons. Whereas the recent analyses of newly generated oligodendrocytes in superficial cortical layers showed that oligodendrocytes can exhibit de novo myelination concomitant with remyelination, other studies in both the mammalian cortex and zebrafish spinal cord indicate that very precise restoration of original patterns of myelin can be restored by remyelination, following the ablation of single oligodendrocytes lead to [48,49]. Further evidence of neuron-driven local regulation of myelination in vivo, comes from analyses of the zebrafish spinal cord in which distinct modes of myelination (activity regulated or not) are exhibited by different neuronal subtypes with axons that can in principle be myelinated by the same oligodendrocyte [50]. As per the comparison of grey matter and white matter remyelination, investigation of the mechanisms underpinning remyelination of axons of different sizes, functional characteristics, or original patterns of myelination remain largely unexplored and represent important areas of future investigation.

4.3 Could fundamental differences in human OPCs skew modes of remyelination?

Despite the likelihood that spatial, temporal and functional characteristics of areas of the CNS and their local microenvironments will have a bearing on remyelination, it remains the case that OPCs are present throughout the grey and white matter in the healthy nervous system in roughly consistent densities irrespective of brain area, and that OPCs respond to demyelination
in model organisms. This begs the question as to whether human OPCs simply cannot generate remyelinating oligodendrocytes as well as those of model systems. Pioneering inter-species transplantation studies suggest, however, that they can. First it was shown that human glial progenitor cells have the capacity to myelinate essentially the entire brain of mutant mice that cannot make their own myelin sheaths, due to a mutation in a major myelin protein. The transplanted glial progenitors even outcompete the endogenous glial cell populations, meaning that chimeric mice acquire principally human-derived OPCs [51]. More recently, such mice with now-resident human OPCs and human myelin were experimentally demyelinated using cuprizone. It was found that the human OPCs were capable of responding to demyelination and of generating new oligodendrocytes that exhibited *bona fide* remyelination [52]. This study indicates that human OPCs and the oligodendrocytes that they generate are not fundamentally different from those of rodents and are potent myelin regenerators.

So, why might there be a skew against remyelination by newly generated oligodendrocytes in humans? One possibility is that the demyelinated lesion environment in human disease may be more hostile to OPC migration and or proliferation than in animal models, meaning that in certain areas remyelination by mature oligodendrocytes is the only option. Indeed, nearly 40% of lesions in MS are thought to have deficiencies in OPCs [53]. Furthermore, exogenous expression of a chemorepellent abundant in lesions that lack OPCs can impair otherwise successful remyelination in mice [54]. Furthermore, the difference in scale between lesions typically assessed in animal models and those of humans may provide constraints that are more difficult to overcome. Therefore, it may be that it is the lesion environment that principally impairs remyelination by OPCs, and that, context-aside, mechanisms of remyelination may otherwise be largely conserved across species. One major regulator of the tissue microenvironment that contributes to remyelination success or failure is the process of ageing. Indeed, because longevity is not greatly reduced in MS, pathological analyses of disease are typically made of tissue that has experienced significant effects of ageing, which may contribute the observation of specific modes of remyelination.

**4.4 Does ageing skew modes of remyelination?**

The apparently vast differences in the extent of oligodendrocyte generation between adult rodent and human brain may in part be related to the ages of the species that are compared. In most studies of adult rodents, animals a few months of age are typically used, whereas in humans the onset of clinical pathology in MS typically occurs in the third decade of life. Therefore, one possibility is that the progenitor-mediated remyelination that occurs in young animals is essentially a continuation of the developmental process of myelination and that this has long-since passed in many brain areas in humans by the time disease symptoms emerge. Indeed with ageing the efficiency of progenitor-driven remyelination also declines in rodents [17,55] and this may be naturally superseded by mature oligodendrocyte-mediated remyelination. In this regard, it is interesting that progenitor-based remyelination is predominantly a feature of short-lived species such as rodents and fish, and that oligodendrocyte-mediated remyelination may become more prominent in cats, non-human primates and humans. In humans, progenitor-mediated remyelination may only occur in young
adults, and/or only in specific areas (grey vs white matter), and the now-well established effects of ageing on OPCs may render progenitor cells incapable of effectively contributing to remyelination much earlier than was previously thought. Nonetheless, OPCs clearly persist throughout the human lifespan, and, based on rodent studies that aged OPCs can be rejuvenated to undergo enhanced remyelination [17,56,57]. Therefore, the OPC and the ability to generate new oligodendrocytes undoubtedly remain legitimate targets for therapeutic remyelination.

5. New oligodendrocytes exhibit better remyelination than oligodendrocytes that survive demyelination

Two recent live imaging-based studies have allowed a direct comparison of the relative remyelinating potential of oligodendrocytes that survived demyelination with those newly generated following demyelination. In one study that employed zebrafish as a model, Neely et al generated a transgenic line in which almost complete myelin sheath loss could be induced whilst leading to relatively limited oligodendrocyte death [38]. By exploiting the advantages of zebrafish for in vivo live imaging, the authors also followed single oligodendrocytes over time, both newly generated cells and those that survived demyelination. They showed that newly generated oligodendrocytes made large numbers of myelin sheaths correctly targeted to axons. In contrast, oligodendrocytes that survived losing their myelin sheaths made very few new sheaths (only two per cell compared to 15 prior to demyelination). Secondly, a majority of surviving cells mistargeted newly-made myelin, placing it on neuronal cell bodies instead of axons, suggesting that the ability of mature oligodendrocytes to sense correct targets is impaired following demyelination (Figure 1). Interestingly the mistargeting of myelin to cell bodies was also observed in areas of prospective remyelination in the grey matter of MS, suggesting that myelin mistargeting may be a conserved feature of remyelination by surviving cells. Despite an apparently limited ability to form and correctly target new sheaths, surviving oligodendrocytes were able to support the elongation of myelin sheaths along axons. In parallel, live imaging of oligodendrocytes in the rodent cortex over time following treatment with the demyelinating toxin cuprizone provided broadly similar findings. Work by both Bacmeister et al., and Orthmann-Murphy et al., showed that newly generated oligodendrocytes were produced (as expected) following demyelination and were capable of generating large numbers of myelin sheaths [37,47]. Although cuprizone treatment ultimately leads to oligodendrocyte cell death following demyelination, Bacmeister et al., were able to follow the fate of oligodendrocytes that survived demyelination, and found that they were also capable of generating new sheaths. These oligodendrocytes made very few new sheaths, mirroring observations made in zebrafish, but very interestingly, could do so over a protracted period of time, suggesting that they might be manipulable. Indeed, Bacmeister et al., made the remarkable finding that both the proportion of oligodendrocytes that contributed to remyelination and the amount of myelin that those cells made could be enhanced by training animals following demyelination in region and circuit specific tasks [37], validating the principle that surviving oligodendrocytes also represent therapeutic targets for the enhancement of remyelination. Future analyses of remyelination by surviving oligodendrocytes in different models and contexts of different brain areas, times, and in yet
further environmental contexts will further explore the potential of these cells to contribute to remyelination. It is also clear that deeper analyses of individual oligodendrocytes in MS will also be required to determine how their myelination characteristics relates to their molecular profiles, age and environment.

6. Conclusions

Many questions remain as to the potential for remyelination by new and surviving oligodendrocytes. In human disease, the extent of remyelination by these respective cell types remains to be more fully elucidated. Deep molecular profiling and comparison of newly generated and surviving oligodendrocytes in disease and animal models will also help identify further strategies to promote their ability to contribute to remyelination. Similarly, phenotypic screens in models in which surviving cells are experimentally tractable may identify drug candidates to promote remyelination, as have been successful in finding compounds that promote new oligodendrocyte production. In addition, ongoing studies focussed on the cell biology of remyelination in diverse species and models that reveal the myriad cell-cell interactions and molecular pathways that promote and prevent remyelination will aid both our understanding of this fundamental feature of regeneration of the CNS and provide insights to help treat human disease.

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Figure 1. Two modes of remyelination by oligodendrocytes

**Top.** In the healthy CNS, oligodendrocyte progenitor cells (OPCs) generate the oligodendrocytes that myelinate axons (sky blue).

**Middle.** Upon demyelination, some oligodendrocytes survive losing some or all of their myelin sheaths whereas others die. This can lead to activation of OPCs (dark blue).

**Bottom.** During remyelination, OPCs can generate new myelinating-oligodendrocytes (purple). In addition oligodendrocytes that survive demyelination can contribute to remyelination, but to a limited extent (green cells left and right), and sometimes mistargeting myelin to cell bodies (green cell left).
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