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Oligodendrocyte progenitor cells: the ever mitotic cells of the CNS

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1. ABSTRACT

Oligodendrocyte Progenitor Cells (OPCs) first appear at mid embryogenic stages during development of the mammalian CNS and a mitotically active population of them remains present even into late adulthood. During the life-time of the organism they initially proliferate and migrate in order to populate the whole nervous tissue, then they massively generate oligodendrocytesand finally they switch to a less mitotically active phase generating new oligodendrocytes at a slow rate in the adult brain; importantly, they can regenerate acutely or chronically destroyed myelin. All the above depend on the capacity of OPCs to regulate their cell cycle within different contexts. In this review we describe the development of OPCs, their differential mitotic behavior in various conditions (embryo, disease, ageing), we discuss what is known about the mechanisms that control their cell cycle and wehighlightfew interesting and still open questions.

2. INTRODUCTION

The generation of Oligodendrocytes (OLs) from Oligodendrocyte Progenitor Cells (OPCs) constitutes a crucial step during the development of the Central Nervous System (CNS). Every OL extends multiple processes, each one of which wraps around a neuronal axon in order to form a membranous multilayered sheath called myelin, thus ensuring the integrity and trophic support of the axon, as well as the successful propagationof neuronal activity via saltatory conduction. OPCs are derived from different areas of the ventral and dorsal embryonic neuro-epithelium (1), they are massively generated after mid-gestation in the mouse embryo and subsequently populate the whole CNS through extensive proliferation and migration. Myelination of axons largely occurs in the early postnatal stages and with its completion the majority of perinatal OPCs have exited the cell cycle in order to terminally differentiate into mature OLs. However, a subpopulation of OPCs survives for the life span of the organism (both in humans and rodents) as adult OPCs scattered throughout the grey and white matter where it represents approximately 5% of all cells (2) (Figure 1). Recent experimental work in the mouse has revealed that adult OPCs probably sustain myelin homeostasis by continuing to proliferate and to generate new OLs in the adult brain (3-5); albeit exhibiting a gradually slower cell cycle and with a fraction of them eventually entering senescence (6). Importantly, in response to demyelination (as in the context of multiple sclerosis) adult OPCs can drive a robust endogenous repair process called **remyelination** during which denuded axons are ensheathed by newly-formed OLs. This is achieved through the proliferation and differentiation of local OPCs and the recruitment of more distant progenitors that rapidly migrate to the area. Failure of remyelination can lead to impaired integrity and function of the axon and subsequent neuropathologies (reviewed in Franklin &ffrench-Constant)(7). In addition to their role in repair, adult OPCs –for example in the grey mater- might also contribute in the homeostasis of neurons through a range of functions, such as metabolic support (8, 9).

As in other types of stem and progenitor cells, cell cycle regulation is crucial for OPCs to achieve their developmental role, as well as to maintain their population in the adult and aging CNS so as to contribute to homeostasis and to respond efficiently in cases of degeneration. In this review we will discuss the cell cycle characteristics of OPCs throughout development, ageing and disease and we will summarize the key controlling mechanisms. Most of the information we will refer to is derived from experimental work in mice and rats; however,

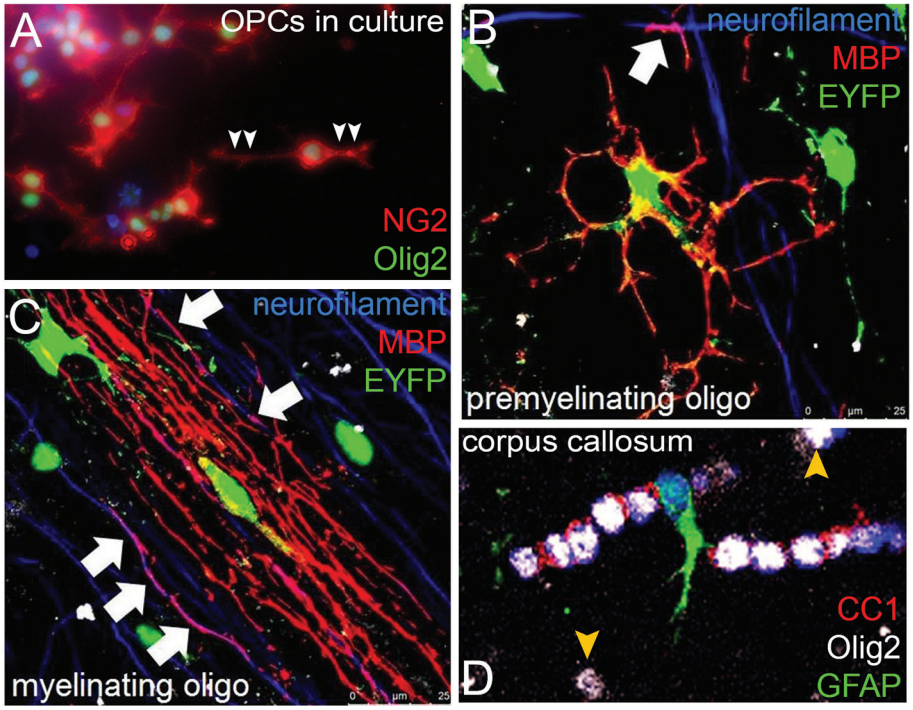

Figure 1. OPCs and Oligodendrocytes in vitro and in vivo.(A) Microphotograph of OPCs isolated from adult rat brain and kept in growth medium after immunostaining for the proteoglycan NG2 (in red) that is a characteristic marker of OPCs and for the key oligodendroglial lineage transcription factor Olig2 (in green). Arrowheads point to the characteristic bipolar morphology of an OPC in culture. (B) Microphotograph of a premyelinatingoligodendrocyte, as identified by the expression of Myelin Based Protein (in red). The arrow indicates one process that is in contact with a neuronal axon (axons are marked by neurofilament staining, in blue). Expression of EYFP (in green, both in B and C) indicates that this cell is derived from adult neural stem cells located in the subependymal zone stem cell niche (OPCs generated by stem cells isolated from hGFAP-Cre^{ERT2} x Rosa26-EYFP mice were co-cultured with dorsal root ganglion cells). (C) Microphotograph of a myelinatingoligodendrocyte, as identified by the expression of Myelin Based Protein (in red) and the formation of characteristic myelin sheaths surrounding neuronal axons (characteristic examples are indicated by arrows, axons are marked by neurofilament staining, in blue). (D) Microphotograph of chains of oligodendrocytes, as identified by expression of both Olig2 (in white) and CC1 (a marker of mature oligodendrocytes, in red). An astrocyte (marked by expression of GFAP, in green) is intercalated, whilst the two cells indicated by the yellow arrowhead express only Olig2 and are probably OPCs (with no expression of CC1).

reference to the human CNS will be made whenever possible. It should also be noted that here we pool together published information that has been generated by experiments in which cells expressing a subset from a range of OPC-specific markers (such as A2B5, NG2, Nkx2.2., Olig2, O4 and Sox10) are investigated each time, even though there is evidence that differences might exist amongst different OPC subtypes(10).

3. OPCS REMAIN MITOTICALLY ACTIVE THROUGHOUT THE LIFESPAN OF THE ORGANISM

3.1. Development

During embryogenesis of the mouse the first OPCs emerge at around embryonic day 12 (E12) at the ventral neuroepithelium of the spinal cord (pMN domain), generated from the same progenitors that had previously generated motor neurons. Few days later, at E15 a second wave of OPCs emerges from the dorsal neuroepithelium of the spinal cord (11-13), while a third less well-characterized wave of OPC generation occurs around birth (14). In the forebrain, the bulk of OPCs emerge after the peak of neurogenesis, again in three waves: the first is driven by neuroepithelial cells of a transient proliferative zone called medial ganglionic eminence at E12, the second from progenitors of the lateral ganglionic eminence at around E15 and the third perinatally from progenitors of dorsal location (1). In the human brain OPCs are already detectable at 5 gestational weeks and it is suggested that a subpopulation is generated from dorsal proliferative zones (15). Interestingly, the switch in lineage commitment of forebrain neural progenitors from neurogenesis to oligodendrogenesis is accompanied by a significant change in their mode of proliferation, with an increase in the number of symmetric self-renewing divisions. This phenomenon has been observed in single-cell cultures *in vitro*; thus must be an inherent property of these progenitors (Figure 2A-C) (16).

Embryonic OPCs migrate long distances whilst continuing to proliferate and eventually populate the whole CNS, although this process is not homogeneous. For example, in the spinal cord OPCs of ventral origin

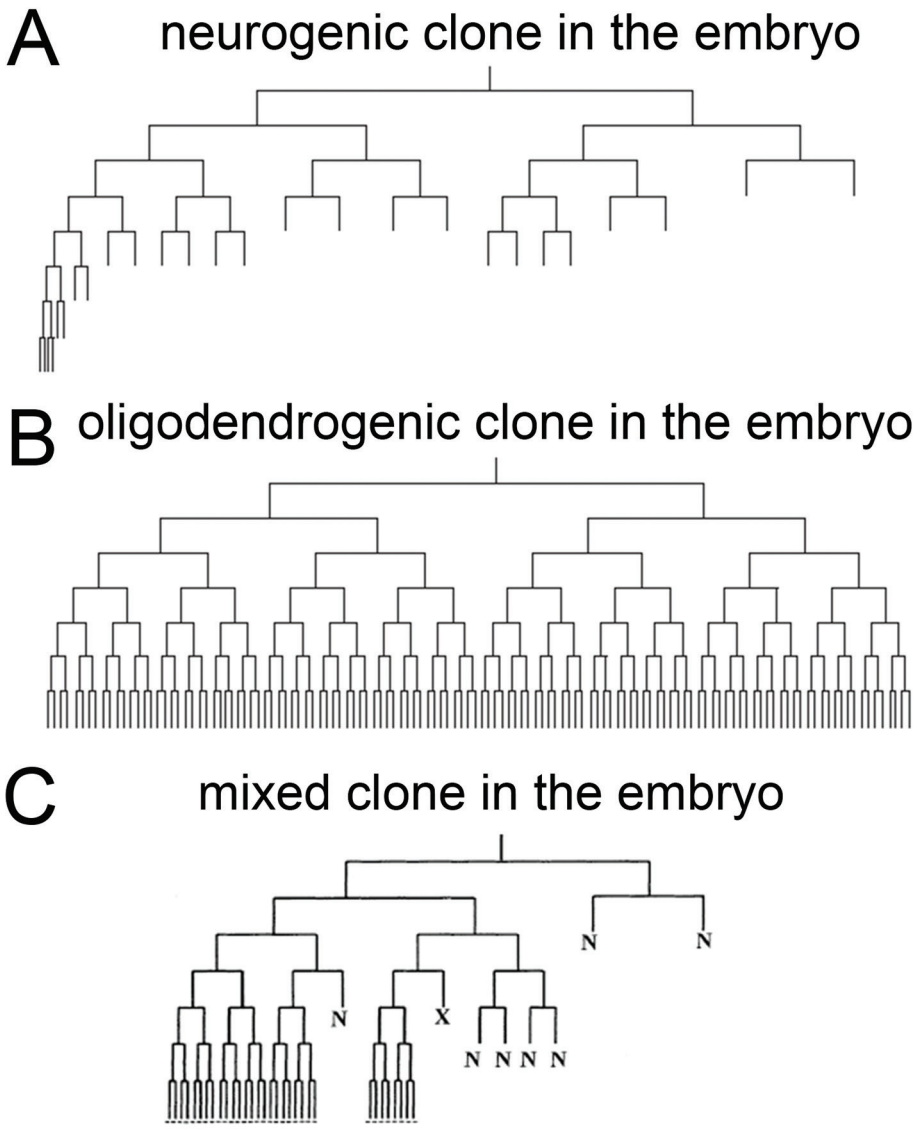

Figure 2. Patterns of proliferation and differentiation of single neural progenitors and OPCs. (A) Proliferation and differentiation tree of a clone generated in vitro by a single embryonic cortical neural progenitor. Note that all differentiated cells that were generated were neurons. (B)Proliferation and differentiation tree of a clone generated in vitro by a single embryonic cortical neural progenitor that gave rise exclusively to oligodenrocytes. (C) Proliferation and differentiation tree of a clone generated in vitro by a single embryonic cortical neural progenitor that gave rise in both neurons (marked by "N") and oligodendrocytes (marked by "-"). Note that the generation of oligos coincided with additional symmetrical divisions of the progenitors. (D) Proliferation and differentiation tree of clones generated in vivo by single adult cortical OPCs using time lapse imaging. Note that the main pattern within the 40-day chase period was that of proliferation without differentiation events. (E) Proliferation and differentiation tree of clones generated in vitro by single adult NSCs isolated from the SEZ using time lapse imaging. Note that -bearing in mind the in vitro/in vivo differences- NSC-derived cells seem to have significantly shorter cell cycle. ((A,C) Reproduced with permission from (19); (B) reproduced with permission from (16))

are more mobile than those of dorsal origin (13, 17). As gestation progresses the length of the cell cycle of OPCs gradually increases from 6h at E13, to 15h at E15 only to reach 22h at E17(18). This, in combination with the initiation of differentiation and the increase in levels of apoptotic cell death, leads to a plateau in the number of OPCs within the early postnatal CNS, as revealed by pulses of BrdU labeling (18). Two main hypotheses were proposed in order to explain the mechanism by which this deceleration of the cell cycle is controlled: i) that it is an inherent property of OPCs, in concert with results that revealed that the switch from neurogenesis to oligodendrogenesis is maintained in single-cell cultures (19), or ii) that it is controlled through the concerted effects of mitogenic and anti-mitogenic extracellular stimuli. In order to address this question transgenic mouse in which expression of platelet-derived growth factor (PDGF) was either decreased

or increased were investigated. PDGF acts on OPCs via activation of PDGFRα signaling and enhances their migration, proliferation and survival (20-22). In mice deficient for PDGF the population of OPCs was found to be significantly reduced, leading to severe hypomyelination (18, 23). In contrast, over-expression of PDGF resulted in increased numbers of OPCs in the spinal cord, caused by a shortening of the cell cycle during early developmental stages (E13-E15). Notably though, at E17 cell cycle duration of OPCs was similar when comparing transgenic and wild-type animals (18). These results strongly suggest that cell cycle length in OPCs is regulated by mitogens and that its deceleration is caused by the limited access to PDGF, although other factors (increased levels of anti-mitotic molecules and inherent properties) must also participate in the regulation. Interestingly, the overpopulation of the spinal cord with OPCs did not translate into excess numbers of OLs due to increased levels of apoptosis in premyelinating OLs. Therefore, although cell cycle exit is directly coupled with initiation of differentiation in embryonic and perinatal OPCs, an increase in the proliferative capacity of the overall OPC pool in the normal brain is not linearly linked with increased myelination activity. In contrast, in cases of perinatal hypoxia (for example in premature births), in which large-scale apoptosis occurs in OPCs leading to delayed expansion of their population and to hypomyelination (24, 25), the induction of proliferation in OPCs can restore the pathology (24).

Many other growth factors, morphogens and intracellular pathways have been identified to control proliferation of OPCs and this is normally coupled with inhibition of differentiation (26) (Table 1). Notably, the same growth molecule can induce proliferation of OPCs in one developmental stage and stimulate differentiation in another, with PDGF and neuregulin being two such examples (18, 27). In the case of neuregulin, this switch is promoted by the regulated alteration in the expression of integrins in OPCs and is manifested as a change in the neuregulin-induced intracellular signaling cascade from the phosphatidylinositol-3-OH kinase (PI(3)K) pathway to that of the mitogen-activated kinase (MAPK)(27). Nevertheless, in *Erk2-/-* conditional mice (ERK2 is a prototypic member of the MAPK family), in which ERK2 was knocked out specifically in neural progenitors, no defect in OPC proliferation was observed (28). The emergence of OPCs from their ancestral neural progenitors is largely dependent on fibroblast growth factor (FGF) signaling (29), a factor that controls their proliferation also in later stages (30). Similarly, insulin-like growth factor-I (IGF-I) is important both in the initial specification/expansion stages of OPC development and during their main proliferative phase (31, 32). Another intriguing process that controls OPC proliferation is neuron-derived signaling. Although this appears to be a major regulating factor, since myelination involves the coupling of OPCs and neurons, not much is still known after the seminal work of Barres and Raff (33) (and see Table 1). In a recent study, *in vivo* optogenetic stimulation of deep layer cortical neurons resulted in significant local increases in OPC proliferation, as well as to increased differentiation (34). Therefore, although this suggests the existence of a direct link between electrical activity and OPC division and complements earlier reports of synaptic activity on OPCs (35), it is still impossible to rule out a primary effect on differentiation subsequently leading to the proliferation of OPCs in order to maintain their numbers (43). Nevertheless, the significance of the neuron-OPC communication in the control of OPC proliferation seems to be an evolutionary conserved mechanism that in Drosophila is mediated by the DNA-binding protein Prospero that is important in the control of asymmetric divisions of neuroblasts (36).

OPEN QUESTIONS: The expression of molecules such as NG2 (37) and integrins (27) has been shown to be important in the regulation of OPC proliferation. What other molecules with auto- and paracrine function are expressed in OPCs? Are they necessary only for the fine tuning of OPC mitotic behavior?

3.2. Adult brain homeostasis and pathology

After the completion of myelination at the early postnatal period (only in few areas -such as the corpus callosum- myelination continues in adulthood in rodents) (38) a fraction of perinatal OPCs retain their progenitor properties and survive in the brain parenchyma as adult OPCs (39-41). Recent work using BrdU or EdU pulse-chasing protocols has revealed that all adult OPCs remain mitotic with cell cycle lengths significantly longer than those of perinatal OPCs (3, 42). Furthermore, both the length of the cell cycle and the mode of division (whether eventually resulting in new OPCs or in OLs) are area-dependent. In juvenile mice the cell cycle varies from ≈3 days in the corpus callosum to ≈20 days in the cortex and it slows down further with ageing reaching ≈10 days in the corpus callosum and ≈35 days in the cortex (3, 43). Hence, OPCs are considered to be a relatively quiescent progenitor population in rodents (44) and the same seems to apply for the human brain, in which almost all cycling cells are OPCs proceeding through a long G1 phase (45). Recent experimental work in which cortical OPCs were imaged *in situ* revealed that they remain in a dynamic and motile state, constantly extending and withdrawing processes that allow them to sense their microenvironment (46). The disappearance of one OPC either due to cell death or due to differentiation induces a neighboring cortical OPC to undergo cell division in order to maintain cell density. These cell mitoses are only rarely directly coupled with the initiation of differentiation as the majority of them leads to two OPCs that can differentiate within a range of time scales (4, 43, 46, 47). In that way the pool of OPC remains capable to react in future challenges, although it still remains elusive if injury or myelination-derived stimuli

Table 1. Summary of the effects of different factors in the proliferation/differentiation of OPCs

Factor	Effect on OPC proliferation and/or differentiation	Reference
PDGF	Enhances and is necessary for OPC proliferation *in vitro* and *in vivo*. Blocks cell cycle exit and preservees OPCs in an immature progenitor state.	(18, 20, 23, 93, 107)
FGF-2	Increases the expression of PDGFR-A, thereby indirectly enhancing sensitivity to PDGF signalling	(30, 108, 109)
IGF-1	Increases proliferation of OPCs *in vitro* through regulation of G2/M progression *in vitro*	(31, 32, 56, 85, 86, 110)
Insulin	Higher concentrations, i.e., in cell culture medium lead to an activation of IGF1-receptor, promotes survival of OPCs *in vitro*	(111)
CNTF	Can act synergistically with PDGF-AA. Increases survival of OPCs *in vitro*	(111)
BDNF	Enhances OPC proliferation *in vitro* synergistically with NT-3, increased OPC proliferation in a spinal cord contusion model in rats	(112)
NT-3	Enhances OPC proliferation *in vitro*, acts synergistically with PDGF-AA, block of NT-3 signalling with anti-NT-3 antibodies reduced the number of proliferating OPCs in the optic nerve during development in rats, promotes proliferation of OPCs in a contused spinal cord model in adult rats	(111-113)
Il-6	Enhances OPC survival	(111)
LIF	Enhances OPC survival	(111, 114)
GGF2/neuregulin	Enhances OPC proliferation *in vitro*. Enhances OPC proliferation in EAE models	(115, 116)
TGF-beta	Promotes OPC differentiation, ihibits progenitor proliferation	(117)
cAMP	Promotes OPC differentiation	(88, 118-120)
Wnt3/3a	Promotes specifically the proliferation of oligodendrogneic adult NSCs	
Beta-adrenergic receptor agonists	Promotes OPC cell cycle exit in G1 and blocks S phase entry *in vitro*	(87)
Glutamate	Promotes OPC cell cycle exit *in vitro* and in cerebellar slice cultures	(87)
Thyroid hormones (T3 and T4)	Important for OPC differentiation *in vitro* and *in vivo*, promotes cell cycle exit and blocks OPC proliferation *in vitro*	(121-124)
Electrical activity	Enhances OPC proliferation *in vivo*	(33, 34)
M1 polarised macrophages	Conditioned medium from M1 polarised macrophages enhanced proliferation of OPCs *in vitro*	(125)
M2 polarised macrophages	Conditioned medium from M2 polarised macrophages enhanced proliferation and differentiation of OPCs *in vitro*	(125)

BDNF: Brain-Derived Neurotrophic Factor; cAMP: cyclic Adenosine MonoPhosphate; CNTF: CiliaryNeuroTrophic Factor; FGF-2: Fibroblast Growth Factor 2; GGF2: GliaGrowth Factor 2; IGF-1: Insulin-like Growth Factor 1; Il-6: Interleukin-6; LIF: Leukemia Inhibitory Factor; NT-3: Neurotrophin-3; PDGF: Platelet-Derived Growth Fcator; TGF-beta: Transforming Growth Factor beta

might also affect directly the proliferative behavior of OPC. The existence of wide differentiation time-frames for adult OPCs observed by Hughes *et al*. (43) is in concert with the conclusions of another recent report (48) in which the distribution of myelin in different layers of cortical pyramidal neurons was meticulously explored and that revealed that although OPCs are in similar abundance throughout all layers significant differences in the levels of myelin are maintained. Notably, morphological studies of proliferating OPCs in adult brain slices and *in vivo* on one side confirmed the existence of symmetric divisions of adult OPCs and showed that these cell retain their structural and even electrophysiological properties during the cell cycle (49) and on the other side revealed a significant proportion of divisions with very early signs of asymmetry between sister cells (50).

In contrast, in white matter tracts, such as the corpus callosum, the cell generation pattern seems to be substantially different, as within a 65-day chase experiment the majority of OPCs were found to generate myelinating oligodendrocytes and not to self-renew (4, 47). These area-specific differences in the mitotic behavior of adult OPCs could again be intrinsic or extrinsic and the investigation of this question is still pending, one reason being the lack of protocols allowing the isolation of sufficient numbers of adult OPCs. Recent experimental work revealed that areas such as the corpus callosum

retain a higher level of plasticity in teenagers with myelination patterns being altered in response to external stimuli such as learning or abuse (51-53). However, it is far from clear whether this plasticity depends on the proliferative capacity of OPCs, or on changes in rates of differentiation and of OL survival. The performance of heterotopic transplantation experiments, in which grey and white matter OPCs were grafted into white and grey matter, respectively, confirmed the existence of intrinsic properties that underline the higher capacity of white matter OPCs to generate myelin; albeit, this capacity was not linked to significant differences in their proliferation or survival abilities (54). In the rodent brain, specific areas of the corpus callosum have also been shown to be populated by OPCs of a totally different origin. Those generated by oligodendrogenic adult Neural Stem Cells (NSCs) that are located in the stem cell niche found within the Subependymal Zone (SEZ) of the lateral walls of the lateral ventricles (55). When isolated and cultured *in vitro*, these SEZ-derived OPCs exhibit a very active proliferative behaviour, with cell cycle duration much shorter that those described for the parenchymal OPCs *in vivo*. Similarly to embryonic OPCs, growth factors such as IGF-I and EGF enhance mitotic activity of adult OPCs (56), whilst exercise was shown to result in further slow-down of their cell cycle in contrast to its effects in adult neurogenic progenitors (43). An interesting, but still largely unexplored, mechanism of control of OPC behavior seems to be the release of neurotransmitters (mainly of glutamate) from neurons. OPCs have been found to express NMDA receptors but it still remains to be elucidated whether and how glutamate signaling affects proliferation or differentiation (57, 58).

Although the mammalian CNS is often referred to as a non-plastic tissue with minimal capacity for regeneration, experimental and clinical work over the last decades has clearly shown that this does not apply to the myelin component. Both in cases of acute demyelination, such as after traumatic brain injury, but also in chronic demyelinating diseases, such as multiple sclerosis, there is strong evidence that substantial or even full repair of myelin (remyelination) occurs (59-62). In experimental models of focal demyelination (induced by cortical injury or the intra-CNS administration of toxins such as lysolecithin and ethidium bromide that damage OLs and OPCs) it has been shown that OPCs local to the lesion exit quiescence by reducing the G1 phase time-length (43) and become highly mitotic, often reaching cell densities higher than normal (63-65). Mitotic activation of OPCs is also observed in the periphery of the lesion (63, 66) and experiments in which OPCs are depleted locally using X-ray radiation have shown the repopulation of the lesioned areas by OPCs recruited from non-depleted areas (67,68). Similar mitotic activation of OPCs has been described in experimental models of multiple sclerosis (such as in Experimental Autoimmune Encephalomyelitis) (69). Defects in the control of proliferation or of the mode of division (a bias towards symmetric expansive divisions, Figure 2D)(70) in adult OPCs have been suggested to result in the formation of human low-grade and aggressive gliomas(71-73) and in highly invasive rodent gliomas (74). Importantly, inhibition of the EGFR signaling pathway prevented OPC-driven, but not adult neural stem cell-driven, tumorigenesis suggesting new possible therapeutic strategies (72).

The ensheathing of neurons by specialized glial cells is important for their function, as suggested by the fact that this type of cell-cell interaction has emerged at least three times –independently- during evolution (75) and myelination of nerve fibers is considered to be a key factor in the evolution of mammalian and primate cognitive abilities (76,77). As a consequence it has been hypothesized that the cognitive decline observed during ageing and especially in the context of pathologies such as late-onset alzheimer's disease might be caused by a failure to maintain myelin homeostasis (77-79). To what extend this involves defects in the mitotic capacity of OPCs thatcould lead to the occurrence of OPC-poor areas in the brain remains contradictory. According to a recent study, a fraction of OPCs enters senescence in the ageing brain (6) and decreased proliferation of OPCs in response to demyelination has been observed in the aged mouse brain (80). On the other hand the self-renewing potential of OPCs is not exhausted upon serial demyelinating insults (81), and even high losses of OPCs can be efficiently compensated by massive migration of progenitors from unaffected areas (82). Rather than caused by a defect in proliferation of OPCs, perturbations in their differentiation capacity are more likely responsible for the age-related decline in remyelination(47, 81, 83, 84). Importantly, the mitotic capacity of OPCs in the aged rodent brain can be rejuvenated upon exposure to young milieu, as has been demonstrated in parabiosis experiments(80).

OPEN QUESTIONS

1. What defines when and how many perinatal OPCs will switch to the adult OPC fate (with slower cell cycle, different mode of division)?
2. Is the proliferative behavior of OPCs a major cause for the failure of remyelination in diseases such as multiple sclerosis?
3. Is OPC exhaustion a major cause for cognitive decline in the ageing brain and in degenerative diseases?
4. Do adult OPCs retain an element of plasticity as to their mitotic behavior that might underlinethe plasticityin myelination observed during learning?

4. MECHANISMS OF CONTROL OF MITOSIS IN OPCS

In mammals progression through G1 and entry into S phase of the cell cycle is mainly controlled

by three different protein families and their activity. The key process involves the sequential phosphorylation of the Retinoblastoma protein (Rb) by two cyclin/cyclin-dependent kinase (CDK) complexes. In the middle of the G1 phase cyclinDs can form a protein complex with CDK4/6 that phosphorylates Rb which is then targeted for additional phosphorylation by the cyclinE-CDK2 complex. The phosphorylated RB inhibits the sequestration of E2F transcription factors and leads to the expression of S phase specific genes that promote the G1/S transition. On the other side this transition can be blocked by the activity of cell cycle inhibitory proteins –the most important belonging to theINK4 and the Kip/Cip families- that often function as tumor suppressors. In OPCs FGF-2 and IFG-I dependent stimulation of proliferation is mediated through different intracellular pathways (GSK3β and ERK1/2) that converge in the activity of cyclin D1 (85, 86) and neurotransmitters have also been shown to affect directly cell cycle inhibitors(87, 88).

Regarding OPCs, based on single progenitor cultures Temple and Raff proposed the intrinsic clock hypothesis, according to which each OPC can divide 8 times before initiating differentiation (89). This was subsequently refined as it was revealed that the clock was measuring time rather than number of divisions(90) with PDGF being essential for its operation (21). Notably though, the degree to which the operation of such a clock mechanism controls the behavior of OPCs *in vivo* remains controversial. From one hand there is evidence for a close association between OPC birth-date and the timing of their differentiation. Dorsally-derived OPCs in the spinal cord are born later than their ventrally-derived counterparts and they start generating oligodendrocytes with a similar delay and irrespective of whether ventral OPCs (and the oligodendrocytes they generate) are genetically ablated (91,92). On the other hand we now know that adult OPCs remain ubiquitously in the cell cycle (3, 93), that they proliferate in response to demyelination, that their numbers are not exhausted even after repeated degenerative insults (81) and that they can be rejuvenated (80). A central hypothesis formed to explain how the clock model works was that cell cycle inhibitory factors gradually accumulate, leading -once over a threshold- into mitosis inhibition. However, long-term culture experiments of perinatal OPCs revealed that the accumulation $p21^{cip/kip}$, $p27^{cip/kip}$ and $p57^{cip/kip}$cyclin kinase inhibitors and of p53 was counterbalanced by gradual increases in the expression of CDKs 2, 4 and of cyclins D1, D3 and E(93). Indeed, the operation of such compensatory mechanisms, either at a cellular or molecular level, safeguards normal developmental and myelination programs. Mice deficient for $p27^{cip/Kip}$ did not show a major defect in myelination and were only characterized by an increased number of progenitor cells that still differentiated appropriately *in vivo*(94). Furthermore, in *cdk2*knockout mice myelination was completely unaffected, possibly due to over expression of CDK4 (24). Notably, expression of CDK4 is downregulated after postnatal day 15 in the mouse brain and proliferation of adult OPCs in response to demyelination is decreased in*cdk2*-/- mice (95). In the adult rodent brain OPCs are also generated from neural stem cells located within the subependymal zone (SEZ) cytogenic niche (96-99) and the proliferation of SEZ-derived OPCs is also impaired in *cdk2*-/- mice(95); thus one of the features distinguishing embryonic/perinatal and adult OPCs is the dependency of the latter to CDK2 due to the absence of compensatory mechanisms. Embryonic/perinatal fast-cycling and adult slowly-cycling OPCs also differ to their dependency on cyclinD1, with only the adult ones being significantly affected in *cdkD1*-/- mice (100,101). As discussed above, during development it is necessary to couple the exit of OPCs from the cell cycle with the initiation of differentiation and the engagement with neuronal axons in order to generate myelinating OLs at the correct place and time. This requires the operation of multiple layers of fine regulation and the study of several knockout mice, in which expression of various transcription factors (such as Olig2, Nkx2.2., Sox5 and 6, Id2 and 4) is perturbed, has shown that the usual effect is not a total block of self-renewal or a total block of exit from the cell cycle (indicating exhaustion or expansion of OPCs), as a clock mechanism would dictate, but rather defects in the fine tuning resulting in delayed or incomplete formation of myelin (102-106). This coupling apparently become less important during adult brain homeostasis, when differentiation is not tightly linked to cell cycle exit, and this might be why there are fewer compensatory mechanisms in adult OPCs (rendering them more vulnerable to cdk2 knocking out) and why remyelination can often be inadequate.

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