● **PERSPECTIVE**

To myelinate or not to myelinate: fine tuning cAMP signaling in Schwann cells to balance cell proliferation and differentiation

cAMP signaling and the control of Schwann cell fate: The ubiquitous second messenger cyclic adenosine monophosphate (cAMP) controls a variety of cellular responses in a cell type-specific and stimulus-dependent manner through an elaborate network of signaling intermediaries that connect stimulation of cell membrane receptors (typically G protein-coupled receptors, GPCRs) to transcription factor activation. Schwann cells (SCs) are highly responsive to cAMP throughout their lifespan, as extensive research has shown that SC survival, lineage specification, proliferation and differentiation into myelin-forming cells require cAMP signaling.

The first evidence concerning the relevance of cAMP to SC function was documented in the 1970s with the discovery that mitotic cell division of isolated SCs was enhanced by cAMP-stimulating agents. Further mechanistic studies indicated that cAMP acts together with growth factors such as neuregulin to synergistically increase the rate of S-phase entry. In addition, cAMP has been known since the 1980s to directly drive the expression of proteins and lipids specific to the myelin sheath, including protein zero, periaxin, myelin associated glycoprotein (MAG) and galactocerebroside (Jessen et al., 1991). Yet, it was not until recent years that the molecular basis of cAMP-mediated signal transduction in SCs began to be understood. As described below, emerging data from independent *in vitro* and *in vivo* approaches have highlighted the identity of some key molecular players operating both upstream and downstream of cAMP biosynthesis that act in conjunction with other signals to differentially control SC proliferation and differentiation.

It is understood that myelination in SCs is an inducible process sensitive to extracellular signals. Whereas oligodendrocytes autonomously turn on the expression of myelin-related genes upon or even when deprived of axon contact, SCs tend to remain indefinitely undifferentiated despite maintaining extensive contact with axons. Examples provided by *in vitro* myelination studies and models of nerve regeneration *in vivo* have shown that some SCs may effectively extend their processes along those of axons and form a basal lamina, a pre-requisite for myelination, yet still do not proceed to form a myelin sheath. If axon contact is not sufficient for myelination, what are the factors limiting the process? In a recent study, we argued that one such factor is cAMP, as activation of cAMP signal transduction in SCs is sufficient to bundle and synchronize the differentiating responses of axon-associated SCs in such a way as to accelerate and greatly enhance myelin formation *in vitro* (Bacallao and Monje, 2015). By promoting the transition from an immature (proliferative) to a differentiated (growth arrested) state, cAMP acts in concert with, but still independently of, other axonal signals such as neuregulin to initiate myelin membrane wrapping. Indeed, cAMP seems to function as an on/off control switch for myelination, as the simple removal of the cAMP stimulus is sufficient to readily suppress the expression of myelin-associated genes and shift the SC's phenotype back to an immature proliferative state that resembles the one derived through dedifferentiation in response to nerve injury (Monje et al., 2010).

Though at first glance it may seem contradictory to assert that a single second messenger could positively control proliferation and differentiation, a specific cellular outcome is achieved *via* the use of distinct and independent signaling mechanisms (**Figure 1A**). Whereas the synergistic effect of cAMP on SC proliferation is achieved through gating or cross-talk with signals emanating from ligand-activated receptor tyrosine kinases such as neuregulin-activated ErbB/HER receptors (Monje et al., 2008), the effect of cAMP on differentiation is direct and seems not to require the concurrent activation of receptor tyrosine kinase pathways. The use of separate transduction elements also contributes to the specificity of outcome. As such, SC proliferation rather than differentiation relies on the activation of the transmembrane adenylyl cyclase (tmAC)-dependent, protein kinase A (PKA)-dependent pathway. SC myelination, by contrast, seems to be controlled by non-canonical

cAMP signaling, as this process is mediated by effectors and upstream activators that have been relatively understudied in comparison to the classical tmAC-PKA pathway. Novel transduction elements reported to control myelination include: (1) the exchange protein activated by cAMP (EPAC), which is a guanine nucleotide exchange factor for the small GTP-binding protein Rap1 and transduces cAMP signals through direct binding to cAMP (Bacallao and Monje, 2013); (2) the soluble adenylyl cyclase (sAC), which is an ubiquitous forskolin- and GPCR-insensitive adenylyl cyclase subtype that generates cAMP in various cell compartments (Bacallao and Monje, 2015); and (3) the adhesion receptor *Gpr126*, which is a highly conserved orphan GPCR that signals *via* G protein activation and cAMP to control myelination *in vivo* (Mogha et al., 2013). These signal transduction molecules represent attractive targets to control the state of differentiation that is conducive to myelination independently of the control of proliferation.

Manipulating and optimizing cAMP signaling in SCs for therapeutic applications: Our improved understanding of cAMP regulation of SC fate, along with the well-recognized role of cAMP in promoting axon growth in different types of neurons (Spencer and Filbin, 2004), can be exploited to delineate novel approaches to improve the outcome of SC-mediated nerve repair. The basic argument discussed herein postulates that balancing proliferation and differentiation through differential targeting of the cAMP signaling system may have an impact on the extent to which endogenous or transplanted SCs promote peripheral and central axon regeneration and myelination, thus contributing to functional repair.

SCs have been grafted in the injured or dysmyelinated CNS and PNS for decades on the assumption that they can foster axon growth and subsequently form a myelin sheath to insulate regenerated and/ or spared axons. Because the benefits of SC transplantation can be improved significantly if additional treatments are provided, attempts have been made to combine SC transplants with modulators of intracellular cAMP levels to augment nervous tissue repair (Fortun et al., 2009). One advantage of targeting the cAMP signaling system is that a single therapeutic approach can potentially improve various aspects linked to functional repair. Another advantage is that many of the molecular players within this system lend themselves suitable to pharmacological intervention; in addition, extensive information is available on their mechanism of action at the cellular and molecular levels. Considering the sophistication of cAMP networks, the potential for crosstalk, and the multiple cellular targets that are expected to react to cAMP stimulation, one may reason that any given cAMP therapy should be tailored to a desired cellular outcome. Most studies performed so far have relied on the use of broad-spectrum cAMP-stimulating agents administered either locally or systemically [see (Knott et al., 2014) for a recent review]. Though useful for proof of principle and feasibility assessment, this type of traditional approach may limit our understanding of the mechanism of action by which a given treatment promotes repair. An example is provided by a SC transplantation study in the contused spinal cord which showed a dramatic increase in axon growth and myelination within the SC transplants upon co-administration of dibutyryl-cAMP (a non-hydrolyzable cAMP analog) and rolipram (a phosphodiesterase, PDE, IV inhibitor); yet, whether the effect of cAMP was mediated by the SCs, the neurons or both could not be defined simply on the basis of the results obtained (Pearse et al., 2004).

The implementation of a cAMP-based strategy designed to modulate the rate and/or extent of myelin formation by SCs, alone or while concurrently preventing myelin loss, seem in principle rather straightforward based on our current knowledge on how the initiation and maintenance of myelination is controlled by cAMP. Yet, a strategy for SC-mediated nerve repair is more challenging, as treatment should balance at least two independent events: (1) promotion of axonal growth, which can be achieved by targeting cAMP-dependent pathways within the SCs and/or the neurons; and (2) promotion of myelination, which can be achieved by targeting pathways within the SCs. Novel research in the SC field has suggested that axon regeneration and SC differentiation are highly interdependent events (Jessen and Mirsky, 2008). Whereas the initiation and maintenance of an immature SC phenotype may foster axon growth, a premature or exacerbated differentiation of the SC may determine a poor or suboptimal regenerative response. The axon growth-promoting benefits of the SCs themselves are expected to be reduced upon their differentiation into myelin-forming cells. Not only do SCs cease to proliferate, migrate and secrete neurotrophic factors
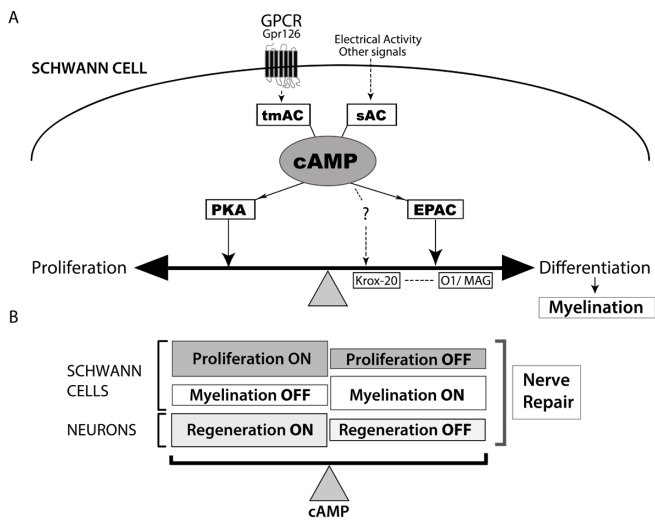
Figure 1 Balancing Schwann cell (SC) fate *via* cyclic adenosine monophosphate (cAMP).
A mechanistic model for the differential control of SC proliferation and differentiation by cAMP signals based on available data (A) and a suggested general strategy for otimizing cAMP-mediated, SC-dependent regeneration and myelination (B). Krox-20, a cAMP-dependent transcription factor that is a master regulator of myelination; O1: The myelin lipid galactocerebroside; EPAC: exchange protein activated by cAMP; GPCR: G protein-coupled receptor; PKA: protein kinase A; sAC: soluble adenylyl cyclase; tmAC: transmembrane adenylyl cyclase.

as they undergo differentiation, but the expression of myelin-specific proteins such as MAG on their surface may elicit a stop signal for axonal growth, a phenomenon which is particularly relevant in the context CNS regeneration.

The present line of reasoning implies that several independent parameters should be considered when optimizing cAMP therapies for SC-mediated repair and myelination. These parameters include: (1) the properties and specificity of the cAMP-inducing treatment on downstream effectors; (2) the possibility of positive or negative cross-talk of cAMP signaling with other pathways; (3) the timing of administration and the duration of the cAMP stimulus; (4) the expected cell type-specific outcome of cAMP elevation in SCs and neurons; and (5) the effect of environmental or context-specific factors.

Multiple tools currently available offer an exceptional opportunity to fine-tune cAMP signaling into a desired cellular outcome. Selective targeting and specificity of signaling is plausible if we understand that cAMP does not act as a unitary signaling pathway but orchestrates many differentially regulated pathways that are built around a common second messenger. First generation cAMP-modulating agents, which offered low or little power for target discrimination, can nowadays be replaced by the wide range of chemical agents (activators and inhibitors) with potential to distinguish among distinct cAMP-specific PDEs, adenylyl cyclase subtypes and downstream cAMP effectors. Novel pathway-specific, cell permeable cAMP derivatives offer the possibility to potently and selectively manipulate PKA and EPAC activation within living cells (Holz et al., 2008). We and others have used some of these analogs to more selectively control the rate of proliferation (*via* PKA) and differentiation (*via* EPAC) of SCs *in vitro*. Isoform-specific EPAC antagonists have also become available, which brings the unique potential to block EPAC signaling while maintaining PKA-initiated pathways. Differential targeting of tmAC and sAC activities can also provide a feasible route for selective pathway modulation based on their clearly different modes of activation and inhibition. Non-pharmacological treatments such as electrical stimulation, which is known to stimulate sAC, may contribute to modulating the potency and pathway specificity through cAMP in selected cell populations.

In optimizing the timing and duration of treatment, one should consider that SC differentiation may counterbalance axon growth. Thus, cAMP therapies aimed to increase myelination may be better implemented independently of those aimed to increase axon regeneration or alternatively, during the later stages of the regeneration process.

Additive or synergistic effects on SC-mediated axon regeneration may be achieved if treatments aimed at enhancing SC proliferation (by targeting SCs) are coupled to those aimed at enhancing axon growth (by targeting the neurons) as long as these are provided while concurrently halting or delaying SC differentiation (**Figure 1B**). A faster or more efficient myelination may be derived from the synchronization of the differentiating responses expected to result from cAMP elevation in SCs, if a similar phenomenon is observed during nerve development or repair *in vivo*. Despite no evidence so far indicates that the environment *per se* would preclude cAMP-induced SC proliferation and/or differentiation, the scenarios may differ considerably in light of the expected effects of cAMP on axon regeneration in PNS and CNS neurons.

To conclude, our significantly expanded understanding of cAMP signal transduction in SCs offers a unique opportunity for new therapeutic developments for SC-mediated nervous tissue repair. A re-interpretation of already available data in the context of new discoveries in signal transduction research is also needed, as the field continues to evolve swiftly. Remaining challenges include achieving complete elucidation of the non-canonical cAMP pathway that underlies myelination as well as a more in-depth understanding of the receptor-ligand interactions that differentially mediate the cAMP-dependent control of SC proliferation and myelination *in vivo*. In light of the revitalized concept that SCs myelinate (or not) as determined at least in part by cAMP, there is, in my opinion, extensive room for innovation in addressing the treatment of nerve system injuries and myelin diseases through cAMP-based therapies.

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