

Down-regulation of the POU transcription factor SCIP is an early event in oligodendrocyte differentiation in vitro

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Summary

The POU-domain transcription factor SCIP (also known as Tst-1) has been implicated in the development of Schwann cells, the myelinating cells of the peripheral nervous system (PNS). We have investigated the possibility that SCIP also might play a role in the development of oligodendrocytes, the myelinating cells of the central nervous system (CNS). We purified oligodendrocyte precursors (O-2A progenitors) by immunoselection and cultured them in the presence of platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF), which together keep O-2A progenitors proliferating and prevent oligodendrocyte differentiation. Under these culture conditions, O-2A progenitors expressed high levels of SCIP mRNA and protein, and did not express myelin-specific genes. When oligodendrocyte differentiation was initiated by withdrawing the growth factors, SCIP mRNA was rapidly down-regulated, followed by a decline in SCIP protein and the sequential activation of myelin-specific genes. **Rapid down-regulation of SCIP mRNA required con-**

tinued protein synthesis. In O-2A progenitors that were cultured in the presence of PDGF alone, SCIP expression declined to an intermediate level, and low levels of the myelin gene products were induced. Thus, the level of SCIP expression in O-2A progenitors is inversely related to the level of myelin gene expression, suggesting that SCIP may be involved in the developmental switch from proliferation to differentiation in the oligodendrocyte lineage. When O-2A progenitors are cultured in the presence of 10% fetal calf serum, they differentiate into type-2 astrocytes rather than oligodendrocytes. SCIP mRNA was also down-regulated in type-2 astrocytes, which do not express myelin genes, so down-regulation of SCIP seems to be more closely linked to the cessation of cell proliferation per se than the expression of a particular differentiated phenotype.

Key words: oligodendrocytes, CNS, development, POU transcription factors.

Introduction

Oligodendrocytes, the myelinating cells of the central nervous system (CNS), arise during development from glial progenitor cells known as O-2A progenitors. O-2A progenitors have been identified in several regions of the developing CNS including the optic nerve, cerebral cortex and cerebellum (see Richardson et al., 1990, for review). In vitro, O-2A progenitors are bipotential, giving rise to oligodendrocytes when cultured in defined medium containing 0.5% fetal calf serum (FCS), or type-2 astrocytes when cultured in the presence of 10% FCS (Raff et al., 1983). Type-2 astrocytes have been characterized in vitro (Lillien and Raff, 1990; Lillien et al., 1990), although it is not yet known whether they exist in vivo (Richardson et al., 1990).

Oligodendrocytes arise on a strict time schedule during development. In the rat optic nerve, for example, oligodendrocytes first appear on the day of birth and continue to be generated from proliferating O-2A progenitors for several weeks after birth (Miller et al., 1985). However, when O-2A progenitors from embryonic optic nerves are cultured in defined medium, most of them stop dividing prematurely and differentiate rapidly into oligodendrocytes (Raff et al., 1983). The correct timing of oligodendrocyte differentiation can be restored in vitro by culturing embryonic O-2A progenitors in medium containing platelet-derived growth factor (PDGF); then, O-2A progenitors continue to divide for some time, and oligodendrocytes are produced over a period of several weeks in vitro, just as in vivo (Raff et al., 1985; Noble et al., 1988; Richardson et al., 1988; Raff et al., 1988). PDGF is widely expressed

in the developing CNS (Richardson et al., 1988; Pringle et al., 1989; Yeh et al., 1991; Sasahara et al., 1991), and is likely to play a role in controlling O-2A progenitor proliferation and the timing of oligodendrocyte differentiation *in vivo*. Acidic and basic fibroblast growth factors (aFGF and bFGF) are also mitogenic for O-2A lineage cells in culture (Saneto and deVellis, 1985; Eccleston and Silberberg, 1985; McKinnon et al., 1990; Böglér et al., 1990). These and other members of the FGF family are present in the developing CNS (Gospodarowicz et al., 1984; Grothe et al., 1991), but it is not yet clear whether oligodendrocyte differentiation occurs normally in the presence of these factors (McKinnon et al., 1990; Böglér et al., 1990). We are trying to define the molecular events that initiate oligodendrocyte differentiation, and to understand how the timing of these events is influenced by polypeptide growth factors including PDGF and FGF.

POU-domain transcription factors have been implicated in the control of cell proliferation and differentiation in several cell lineages (Schöler, 1991). For example, there is evidence that the POU-domain transcription factor SCIP, also known as Tst-1 (He et al., 1989), is upregulated in rat Schwann cells, the myelinating cells of the peripheral nervous system (PNS), when they are stimulated to divide *in vitro* (Monuki et al., 1989). SCIP is also up-regulated *in vivo* in transected sciatic nerves, when Schwann cells are known to dedifferentiate and divide (Monuki et al., 1990). In addition, SCIP represses transcription from the myelin protein zero (P_0) and myelin basic protein (MBP) promoters when assayed by cotransfection in cultured Schwann cells (Monuki et al., 1990). On these grounds, it has been proposed that SCIP may regulate Schwann cell proliferation and myelin gene expression during peripheral nerve development and regeneration (Monuki et al., 1990).

Oligodendrocytes express some of the same myelin genes as Schwann cells (Hudson, 1990), although they belong to a different cell lineage. SCIP mRNA can be detected in the developing optic nerve (Monuki et al., 1989), which contains glial cells but no neurons, suggesting that SCIP is expressed by glial cells in the CNS. We therefore investigated the possibility that SCIP might be involved in the control of proliferation and/or myelin-specific gene expression in the oligodendrocyte lineage. We purified O-2A progenitors from perinatal rat optic nerves or from cultures established from newborn rat cerebral hemispheres, and cultured them in conditions that either maintained them in a state of continuous proliferation or allowed them to differentiate synchronously into oligodendrocytes. We used northern and western blot analyses to examine the expression of the SCIP gene and the myelin-specific genes 2',3'-cyclic nucleotide 3-phosphodiesterase (CNP) and MBP. We found that SCIP is expressed at a high level in proliferating O-2A progenitors but is down-regulated rapidly when the cells cease dividing and differentiate into oligodendrocytes, suggesting that SCIP may play a role in the transition from proliferation to differentiation in the oligodendrocyte lineage. SCIP was also down-regulated when O-2A progenitors were induced to differentiate into type-2 astrocytes, which do not express myelin genes. Therefore, SCIP expression correlates more closely with

O-2A progenitor cell proliferation *per se* than with the expression of a particular differentiated phenotype.

Materials and methods

Purification of O-2A progenitors from rat cerebral hemispheres

Neonatal Sprague-Dawley rat cerebral hemispheres were mechanically dissociated and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS, GIBCO) as described previously (Behar et al., 1988). After one week in culture, the cells were trypsinized and O-2A progenitors immunoselected by a modification of the method described by Barres et al. (1992), as follows. Petri dishes were coated with 5 µg/ml goat anti-mouse IgG (Sigma) or goat anti-mouse IgM (Sigma) in 50 mM Tris, pH 9, at 4°C for 4 to 16 hours, and washed with PBS. Anti-RAN-2 (Bartlett et al., 1981) or anti-galactocerebroside (GC) (Raff et al., 1978) cell supernatants (1:10 dilution) were added to the IgG dishes, and antibody A2B5 (Eisenbarth et al., 1979) ascites fluid (1:3000) to the IgM dishes, in Hepes-buffered minimal essential medium (MEMH) containing 0.2% bovine serum albumin (BSA), for at least one hour. The dishes were washed with MEMH immediately before use. The cell cultures were dissociated with trypsin (0.025%) in Ca^{2+} - Mg^{2+} -free DMEM, washed several times with Ca^{2+} - Mg^{2+} -free DMEM containing soybean trypsin inhibitor (0.2 mg/ml, Sigma) and deoxyribonuclease I (DNAaseI, 0.004%, Sigma), washed once in MEMH containing trypsin inhibitor and DNAaseI, and then resuspended in MEMH containing 0.5% FCS, DNAaseI and 5 µg/ml insulin (Sigma). The cells were then sequentially added to Petri dishes coated with anti-RAN-2 (to remove astrocytes and macrophages), anti-GC (to remove oligodendrocytes) and A2B5 (to select positively O-2A progenitors) for approximately 30 minutes on each set of dishes. The adherent cells were removed from the final A2B5 dishes with trypsin (0.025% in Ca^{2+} - Mg^{2+} -free DMEM), washed and resuspended in DMEM containing 10% FCS. The cells were added to 24-well tissue culture dishes (approximately 10^5 cells/well) containing poly-D-lysine (PDL)-coated coverslips, or PDL-coated 60 mm tissue culture dishes, in our modification (Richardson et al., 1988) of Bottenstein and Sato's defined medium (B-S) containing 16 µg/ml putrescine and 5 µg/ml insulin, so that the FCS was diluted to a final concentration of 0.5%. Growth factors (Peprotech recombinant human PDGF-AA and bFGF) were each added at a concentration of 10 ng/ml.

Purification of O-2A progenitors from rat optic nerve

O-2A progenitors from 8-day-old rat optic nerves were purified by immunoselection from suspensions of dissociated cells by the method of Barres et al. (1992). The cells were trypsinized from the final A2B5 dish as above, washed with trypsin inhibitor, and grown in serum-free B-S medium supplemented with PDGF-AA and bFGF (10 ng/ml each) and 20% cortical astrocyte-conditioned medium prepared as described previously (Richardson et al., 1988), until there were enough cells for northern analysis (about 2 months). At this time, the cell population consisted of 99% A2B5⁺ cells, of which 10% also expressed GC. There were no GFAP⁺ cells in these cultures.

Preparation of RNA and probes

Total cellular RNA was prepared using the guanidinium-thiocyanate method (Chomczynski and Sacchi, 1987). RNA samples (5-10 µg) were electrophoresed on 0.8%-1% agarose/2.2 M formaldehyde gels, transferred to Zetaprobe (Biorad) membranes, and hybridized sequentially with random primed, ³²P-labelled

DNA probes for SCIP, CNP, MBP and pyruvate kinase (PK). The SCIP probe was a 1.1 kb *Xho*I cDNA fragment corresponding to the 3' end of the coding region and the untranslated region (Monuki et al., 1989). The CNP probe was a 370bp *Eco*RI-*Hind*III fragment of plasmid pCNP7, corresponding to the 5'-untranslated end of the mRNA (Bernier et al., 1987) (kindly supplied by A. McMorris). The MBP probe was a 4 kb *Bam*HI fragment of the MPB cDNA in plasmid pCJ12 (Jordan et al., 1990) (kindly provided by R. Mirsky). We probed for PK mRNA as a control for gel loadings, as described previously (Richardson et al., 1988).

Preparation of nuclear extracts for western analysis

Cells were washed with ice-cold PBS, scraped into 1 ml of cold PBS and pelleted in a microcentrifuge at 4°C. The pellet was resuspended in 400 µl of cold [10 mM Hepes pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol (DTT)] containing the protease inhibitors [0.5 mM phenylmethylsulfonyl fluoride, 2 µg/ml leupeptin, 1 µg/ml pepstatin A, 1 µg/ml aprotinin (all from Sigma)]. Nonidet-P40 was then added to a final concentration of 0.5%, and the nuclear pellet collected. The pellet was extracted in 25 µl of cold [20 mM Hepes, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT and protease inhibitors] for 30 minutes, centrifuged, and the supernatant collected (Schreiber et al., 1989). Aliquots containing equal amounts of protein (12 µg) were run on a 12% SDS reducing polyacrylamide gel and electroblotted onto nitrocellulose membrane (Schleicher and Schuell). The blot was blocked in 5% non-fat dry milk and 0.5% Tween-20 in PBS (blocking buffer), incubated with anti-SCIP antibody in blocking buffer overnight, washed with 0.5% Tween-20 in PBS, incubated with swine anti-rabbit IgG conjugated to horseradish peroxidase (Dako) in blocking buffer, then developed using Enhanced Chemiluminescence (Amersham). The anti-SCIP antibody was raised in rabbits against a TrpE fusion protein containing SCIP amino acids 145-408 (which includes the entire POU domain), and affinity purified on a -galactosidase-SCIP fusion protein column (Kuhn et al., 1991).

Results

Characterization of purified O-2A progenitor cells

We purified O-2A progenitors from one-week-old cultures of dissociated neonatal rat cerebral hemispheres and cultured them in the presence of both PDGF and bFGF. This combination of growth factors has been shown to keep O-2A progenitors proliferating, in the absence of oligodendrocyte differentiation, in mixed cultures of optic nerve cells (Bögler et al., 1990). Our purified O-2A progenitors from brain were also prevented from differentiating in the presence of PDGF and bFGF, judging by the low proportion of cells expressing galactocerebroside (GC), a myelin glycolipid that is a specific cell-surface marker for oligodendrocytes (Raff et al., 1978). When PDGF and bFGF were withdrawn from the cultures, most O-2A progenitors stopped dividing and differentiated rapidly into oligodendrocytes, as indicated by a reduction in the proportion of cells incorporating bromo-deoxyuridine (BrdU) and an increase in the proportion of cells expressing GC (Fig. 1). In this way, we were able to generate homogeneous populations of O-2A progenitors that were either dividing or differentiating into oligodendrocytes, for biochemical studies of the differentiation process.

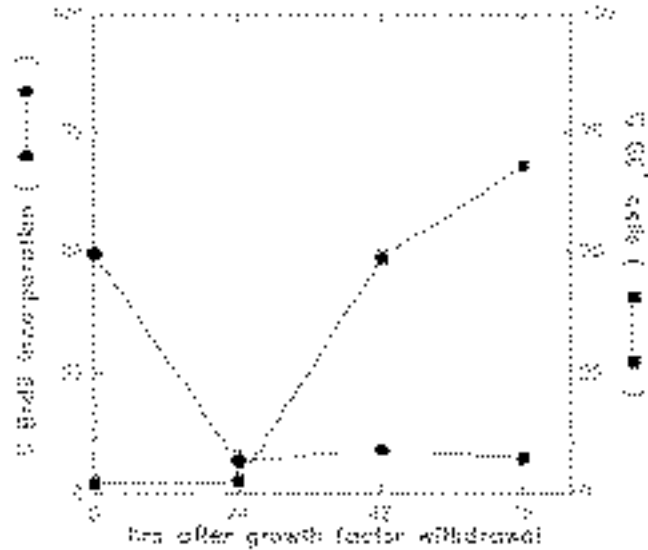


Fig. 1. O-2A progenitors cultured with both PDGF-AA and bFGF stop dividing and differentiate rapidly into oligodendrocytes following withdrawal of growth factors. O-2A progenitors were purified and cultured as described in Materials and methods. PDGF-AA and bFGF were added at 10 ng/ml each, and the cells were grown for 3 days, adding bFGF every 24 hours and PDGF-AA after 48 hours. The cultures were then washed three times with DMEM and incubated for 1, 2 or 3 days in B-S medium with 0.5% FCS. Bromodeoxyuridine (BrdU, 40 µM) was added for the final 8 hours of the culture period. At each time point, duplicate coverslips were fixed and immunolabelled with anti-galactocerebroside (GC), anti-gial fibrillary acidic protein (GFAP), a marker for astrocytes, or A2B5, followed by anti-BrdU, as described previously (Richardson et al., 1988; Pringle et al., 1989). They were then examined in the fluorescence microscope, and at least 200 cells scored on each coverslip. The error bars represent the range of the duplicates. The cell population in the presence of PDGF-AA and bFGF consisted of at least 98% A2B5⁺ cells, of which 1-2% also expressed GC, and about 3% were GFAP-positive cells. Following withdrawal of PDGF and bFGF, the cells stopped incorporating BrdU and the proportion of GC⁺ oligodendrocytes increased markedly, while the number of GFAP⁺ astrocytes did not change.

Expression of SCIP and myelin-specific gene products in purified O-2A progenitors

We examined the expression of SCIP mRNA in O-2A progenitors that were proliferating, or at various stages after oligodendrocyte differentiation had been initiated by withdrawal of PDGF-AA and bFGF. Northern analysis showed that proliferating O-2A progenitors expressed SCIP mRNA abundantly (Fig. 2A). Following growth factor withdrawal, SCIP mRNA was rapidly down-regulated, dropping to basal levels within 6 hours (Fig. 2A).

Down-regulation of SCIP mRNA was followed by a decline in the amount of SCIP protein detected in nuclear extracts from O-2A progenitors on western blots. SCIP was down-regulated to a basal level within 24 hours after growth factor withdrawal (Fig. 3B), but did not disappear completely. The major protein recognized in O-2A progenitors by our anti-SCIP antibody had the same apparent relative mass ($\sim 50 \times 10^3$) as that seen in rat Schwann cells (Kuhn

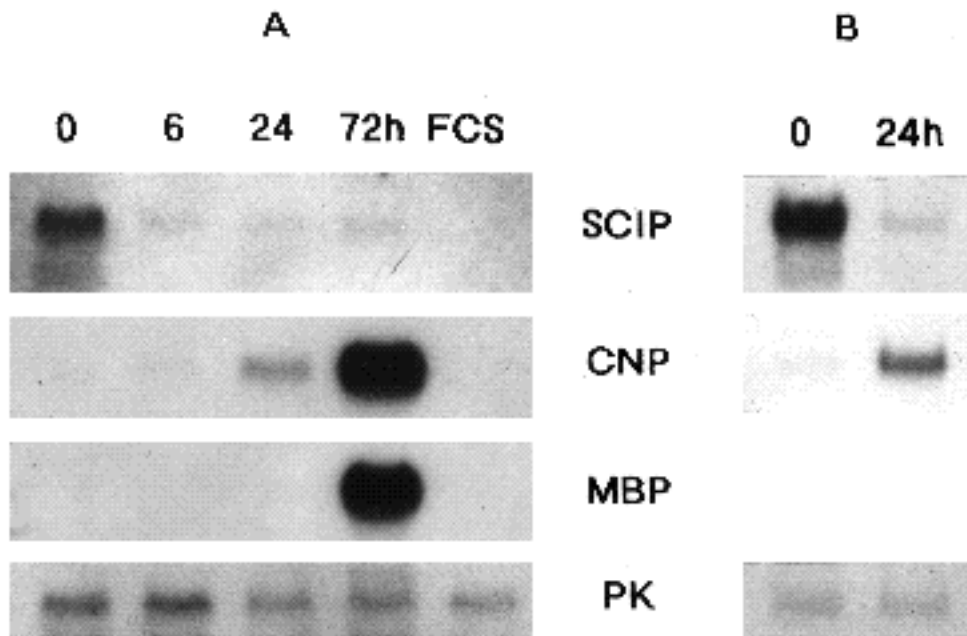


Fig. 2. Time course of expression of SCIP, CNP and MBP mRNAs in cultured O-2A lineage cells from rat cerebral hemispheres or optic nerves following the initiation of oligodendrocyte differentiation by growth factor withdrawal. (A) O-2A progenitors were prepared from cerebral hemispheres as described in Materials and methods, and plated in 60 mm tissue culture dishes (approximately 2×10^6 cells/dish). After 5 days in culture in the presence of both PDGF-AA and bFGF, the cells were washed and the medium replaced with B-S medium with 0.5% FCS. At the indicated times after growth factor withdrawal, total cellular RNA was isolated and analyzed on a northern blot. For the lane labelled "FCS", cells grown in the presence of PDGF-AA and bFGF were washed and the medium was replaced with B-S medium containing 10% FCS for 48 hours, to induce type-2 astrocyte differentiation, before RNA preparation. We probed for pyruvate kinase (PK) mRNA as a control for gel loadings. (B) Optic nerve O-2A progenitors were purified as described in Materials and methods, and RNA was isolated before and after oligodendrocyte differentiation had been initiated by growth factor withdrawal for 24 hours.

et al., 1991; Fig. 3A). However, O-2A progenitors contained smaller amounts of an additional immunoreactive protein not present in Schwann cells (Fig. 3A). Using the polymerase chain reaction, we have identified a novel SCIP-related POU protein-coding sequence in O-2A progenitor cultures (G. Stevens, E. Collarini and W. Richardson, unpublished). Since our anti-SCIP antibody was raised against a SCIP fusion protein that included the conserved POU motif (Kuhn et al., 1991), it may cross-react with this new POU protein.

We also examined the expression of two markers of oligodendrocyte differentiation, CNP and MBP. mRNA encoding CNP appeared in the O-2A progenitor cultures within 24 hours after growth factor withdrawal, and was strongly up-regulated during the next 48 hours (Fig. 2A). MBP expression was activated between 24 hours and 72 hours after growth factor withdrawal (Fig. 2A). These data concur with other observations on the sequential order of myelin gene expression *in vivo* and *in vitro* (see Vogel and Thompson, 1988, for review). SCIP mRNA was also down-regulated, and CNP mRNA up-regulated, in cultures of optic nerve O-2A progenitors that were grown in the presence of PDGF and bFGF, and then transferred to growth-factor-free medium (Fig. 2B).

O-2A progenitors are bipotential *in vitro*, giving rise to type-2 astrocytes rather than oligodendrocytes when they are cultured in defined medium containing 10% FCS (Raff et al., 1983). SCIP mRNA was down-regulated strongly in

our O-2A cultures when type-2 astrocyte differentiation was induced in this way (Fig. 2A). Type-2 astrocytes express a set of characteristic markers including the glial fibrillary acidic protein (GFAP), but not the myelin gene products (Fig. 2A). Thus, down-regulation of SCIP in O-2A lineage cells does not necessarily result in myelin-specific gene expression. Cultured cortical (type-1-like) astrocytes, which belong to a different cell lineage, do not express SCIP mRNA (Monuki et al., 1989) or protein (data not shown).

Down-regulation of SCIP mRNA in O-2A progenitors requires protein synthesis

We asked whether continuous protein synthesis is required for down-regulation of SCIP mRNA by adding cycloheximide to O-2A progenitor cultures at the time of growth factor withdrawal. In the presence of cycloheximide, SCIP mRNA levels remained elevated for at least 9 hours (Fig. 4), suggesting that down-regulation of SCIP requires continued protein synthesis. While this may indicate that synthesis of a new protein(s) is required, it is also possible that cycloheximide stabilizes SCIP mRNA by preventing its release from ribosomes (reviewed in Brawerman, 1989).

The effects of individual growth factors on SCIP expression

When O-2A progenitor cultures are switched from medium containing PDGF and bFGF to medium lacking growth factors, most of the cells differentiate within a few days into oligodendrocytes. While this is a convenient regimen for

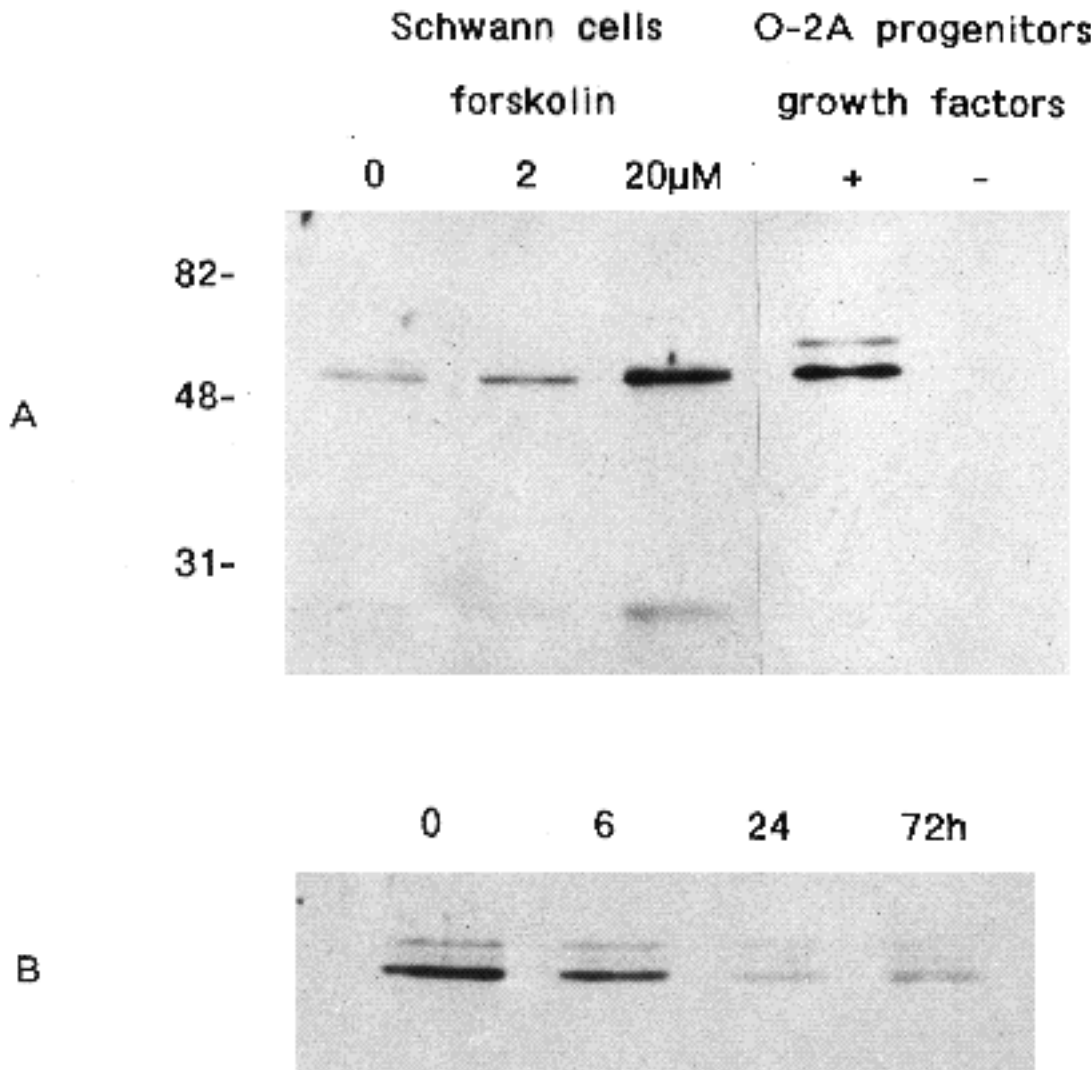


Fig. 3. Western blots of SCIP protein in nuclear extracts from cultured Schwann cells and O-2A lineage cells. (A) Schwann cells (kindly provided by M. Khan) were immunoselected from dissociated rat sciatic nerves using antibody O4, and cultured for 16 hours on the O4-coated dishes in DMEM containing 10% FCS in the presence or absence of forskolin, which has been shown to induce SCIP expression (Monuki et al., 1989). O-2A progenitors were cultured in the presence of both PDGF and bFGF for several days, then washed and transferred to B-S medium with 0.5% FCS and grown for a further 72 hours in the presence or absence of PDGF-AA and bFGF. Nuclear extracts of Schwann cells and O-2A progenitors were prepared and SCIP protein visualized on a western blot. (B) Time-course of SCIP down-regulation in O-2A progenitors following initiation of oligodendrocyte differentiation. O-2A progenitors were cultured in the presence of both PDGF-AA and bFGF for several days, then washed and transferred to B-S medium containing 0.5% FCS. At the indicated times after growth factor withdrawal, nuclear extracts were prepared and SCIP was visualized on a western blot.

analyzing homogeneous populations of proliferating or differentiating cells *in vitro*, it does not accurately reflect what happens *in vivo*. In the developing rat optic nerve, for example, oligodendrocytes are produced during a period of several weeks after birth; during this time proliferating and differentiating O-2A progenitors coexist in the nerve (Miller et al., 1985). This *in vivo* schedule of oligodendrocyte differentiation can be recreated *in vitro* by culturing O-2A progenitors continuously in PDGF alone rather than in PDGF and bFGF (Raff et al., 1988). PDGF mRNA and protein are present in the developing brain and optic nerve (Richardson et al., 1988; Pringle et al., 1989; Yeh et al., 1991; Sasahara et al., 1991), so it seems likely that

PDGF may play a key role in controlling the rate of oligodendrocyte production *in vivo*. Therefore, to relate our *in vitro* studies of SCIP expression to the mechanism of oligodendrocyte development *in vivo*, we needed to establish how PDGF (and FGF) individually regulate SCIP and myelin gene expression in O-2A progenitors.

We purified O-2A progenitors from cultures of dissociated rat cerebral hemispheres and examined the level of SCIP mRNA in cells either immediately after isolation, or after exposure to PDGF and bFGF separately or in combination. SCIP mRNA is present in O-2A progenitors before exposure to PDGF and bFGF, indicating that the combination of PDGF and bFGF does not artefactually elevate the

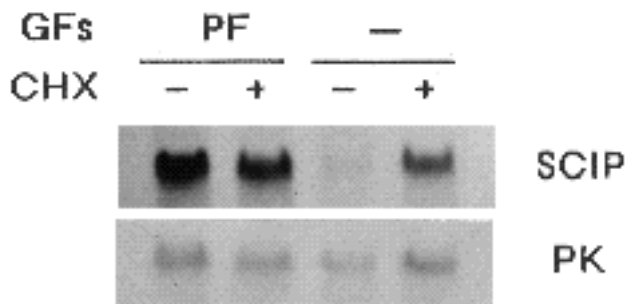


Fig. 4. The effect of cycloheximide on SCIP mRNA down-regulation in O-2A progenitors. O-2A progenitors were cultured in both PDGF-AA and bFGF. After 3 days the cells were washed three times and the medium replaced with B-S containing 0.5% FCS, with PDGF and bFGF together (PF) or without growth factors (-), with or without cycloheximide (CHX, 10 µg/ml) for 9 hours. RNA was isolated and analyzed on a northern blot.

level of SCIP mRNA (Fig. 5). This conclusion is not affected by the apparently uneven loading of this gel (judging by levels of PK mRNA; see legend to Fig. 5). Immediately after immunoselection, the O-2A progenitors contained a low level of CNP mRNA, suggesting that some of the cells in the starting population may already have started to differentiate into oligodendrocytes (Fig. 5). When O-2A progenitors were grown for 16 hours in PDGF-AA and bFGF together, CNP mRNA was no longer detected (Fig. 5), suggesting that this combination of growth factors may induce oligodendrocytes to de-differentiate in culture. In contrast, cells cultured in PDGF-AA alone (Figs 5 and 6A) or bFGF alone (Fig. 6B) expressed both SCIP and CNP mRNAs.

O-2A progenitors maintained in a proliferating state in

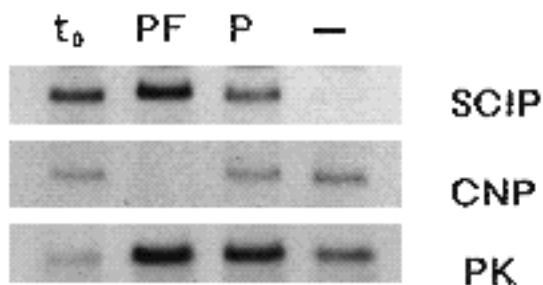


Fig. 5. Regulation of SCIP and CNP mRNA expression in O-2A progenitors before and after exposure to growth factors. Total cellular RNA was isolated from purified O-2A progenitors immediately after immunoselection (t₀), or after culturing for 16 hours in the presence of PDGF-AA and bFGF together (PF), PDGF-AA alone (P) or with no growth factors (-). SCIP, CNP and PK mRNAs were then visualized on a northern blot. Methylene blue staining of the blot (not shown) demonstrated that the underloading of lane 1 was not as marked as suggested by PK mRNA levels. It may be that a metabolic enzyme such as PK is not the best control for loadings in this particular case, since this figure compares RNA from cells immediately after purification, which have been at room temperature for several hours (lane 1), with cells that have been cultured at 37°C for 16 hours (lanes 2-4).

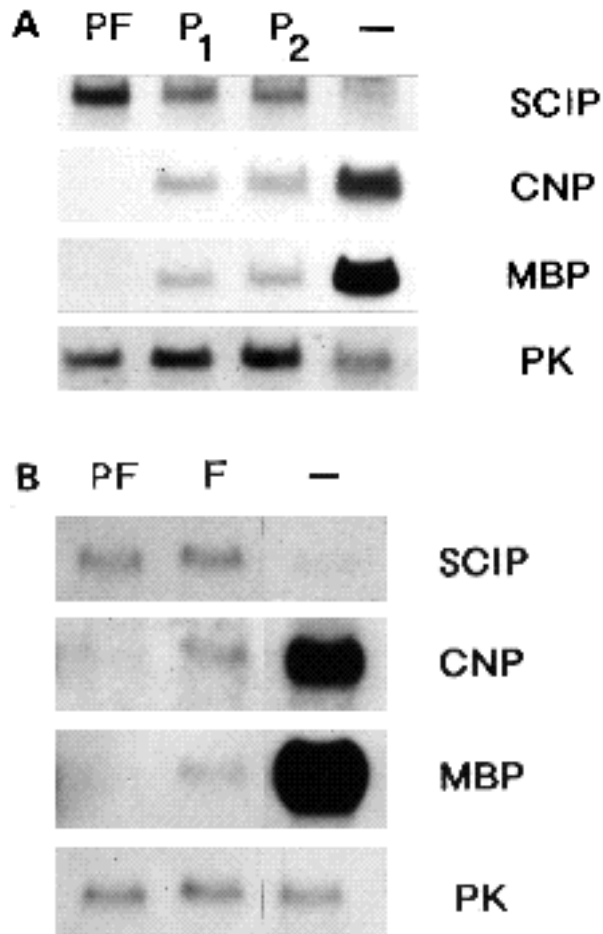


Fig. 6. Regulation of SCIP, CNP and MBP mRNA expression in O-2A progenitors by PDGF-AA and bFGF. (A) Cells were cultured for 3 days in the presence of PDGF-AA and bFGF together (PF), PDGF-AA alone, added every 24 hours (P₁) or 48 hours (P₂), or without growth factors (-). SCIP, CNP, MBP and PK mRNAs were then visualized on a northern blot. (B) Cells were cultured for 3 days in the presence of PDGF-AA and bFGF together (PF), bFGF alone (F), or without growth factors (-). SCIP, CNP, MBP and PK mRNAs were then visualized on a northern blot.

the presence of PDGF-AA and bFGF together expressed high levels of SCIP and no myelin-specific gene products, whereas cells cultured in the absence of growth factors expressed high levels of myelin-specific gene products and little or no SCIP. After three days in PDGF-AA alone, the O-2A progenitor cultures expressed intermediate levels of both SCIP and the myelin-specific gene products (Fig. 6). The level of SCIP expression in the O-2A lineage therefore appears to correlate inversely with the onset of oligodendrocyte differentiation. These findings are in keeping with previous evidence that PDGF is a mitogen for O-2A progenitors and delays, but does not prevent, oligodendrocyte differentiation in vitro (Raff et al., 1988), and are consistent with the idea that SCIP may be causally involved in the transition from proliferation to differentiation in the O-2A lineage.

Discussion

What is the role of SCIP in O-2A progenitors?

SCIP is expressed by proliferating Schwann cells in vitro and is expressed transiently in the sciatic nerve following transection (Monuki et al., 1990), a procedure that is known to induce Schwann cells to de-differentiate and proliferate in vivo. Oct-6, the mouse homologue of SCIP, is expressed in proliferating embryonic stem cells (Suzuki et al., 1990), together with the related POU factor Oct-3 (Okamoto et al., 1990); both are down-regulated when the cells are induced to stop dividing and differentiate in vitro with retinoic acid. Our experiments show that SCIP is down-regulated when O-2A progenitors stop dividing and differentiate in vitro. Therefore, the evidence suggests that down-regulation of SCIP and other POU domain transcription factors is coupled to the transition from proliferation to differentiation in several different cell lineages.

There is evidence that SCIP is directly involved in inhibiting transcription from myelin gene promoters in Schwann cells (Monuki et al., 1990; He et al., 1991), and the results of the experiments presented here are consistent with an analogous role for SCIP in O-2A progenitors. However, since SCIP is down-regulated when O-2A progenitors differentiate into either oligodendrocytes or type-2 astrocytes, which do not express the myelin genes, SCIP appears to be more closely linked to O-2A progenitor proliferation per se than to the repression of a particular set of differentiation genes. There is direct evidence that POU transcription factors are involved in controlling cell division: Oct-1 and Oct-2 can stimulate DNA replication in vitro (Verrijzer et al., 1990), and Pit-1/GHF1 antisense oligonucleotides inhibit proliferation of pituitary cell lines (Castro et al., 1991). It is possible that SCIP regulates both O-2A progenitor proliferation and myelin gene expression, but SCIP is probably only one component of a complex regulatory network that perhaps involves other POU domain transcription factors.

How do PDGF and bFGF individually regulate SCIP expression in O-2A progenitors?

We cultured O-2A progenitors in the presence of both PDGF and bFGF, to establish a homogeneous population of proliferating O-2A progenitors that could be induced to differentiate synchronously into oligodendrocytes by removing the growth factors. However, this experimental paradigm does not necessarily mimic the way oligodendrocytes are produced in vivo. Oligodendrocytes do not form all at once in vivo, but are continuously generated for several weeks after birth (Miller et al., 1985), during which time proliferating and differentiating O-2A progenitors coexist side-by-side. This in vivo pattern of proliferation and differentiation can be recreated in vitro if O-2A progenitors are cultured in the presence of PDGF without FGF (Raff et al., 1988). Under these culture conditions, we found that SCIP mRNA declined in comparison to levels in cells cultured in the presence of both PDGF and bFGF, and expression of CNP and MBP was activated, but to a lesser degree than in cells cultured without growth factors. Thus, under different conditions of culture (PDGF and bFGF,

PDGF alone, or no growth factors), expression of SCIP and the myelin-specific genes are inversely correlated, raising the possibility that there is a direct link between SCIP expression and the repression of oligodendrocyte differentiation. PDGF might cause oligodendrocytes to be generated at a controlled rate during development by regulating the rate of decline of SCIP in O-2A progenitors.

bFGF is also a mitogen for O-2A lineage cells, although it is not known if oligodendrocyte differentiation proceeds normally in the presence of bFGF alone (Saneto and deVellis, 1985; Eccleston and Silberberg, 1985; McKinnon et al., 1990; Böglér et al., 1990). Culturing O-2A progenitors in bFGF alone for 3 days allowed continued expression of SCIP, but also some expression of CNP and MBP (Fig. 6B). Thus, it appears that neither bFGF nor PDGF alone inhibit oligodendrocyte differentiation as effectively as do PDGF and bFGF together.

In conclusion, we have found that, under different conditions of culture, the level of SCIP expression in O-2A progenitors correlates with the proliferative state of the cells. This suggests a close link between SCIP activity and stimulation of proliferation and/or inhibition of differentiation. Direct demonstration of a causative role for SCIP in the switch from proliferation to differentiation in the O-2A lineage requires experiments aimed at interfering with SCIP expression or activity in O-2A progenitors.

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