

Expression and Activity of the POU Transcription Factor SCIP

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POU proteins have been shown to transcriptionally activate cell-specific genes and to participate in the determination of cell fate. It is therefore thought that these proteins function in development through the stable activation of genes that define specific developmental pathways. Evidence is provided here for an alternative mode of action. The primary structure of SCIP, a POU protein expressed by developing Schwann cells of the peripheral nervous system, was deduced and SCIP activity was studied. Both in normal development and in response to nerve transection, SCIP expression was transiently activated only during the period of rapid cell division that separates the premyelinating and myelinating phases of Schwann cell differentiation. In cotransfection assays, SCIP acted as a transcriptional repressor of myelin-specific genes.

THE DEVELOPMENT OF MYELIN-forming Schwann cells, the principal glial cells of the vertebrate peripheral nervous system, can be divided into premyelinating and myelinating phases (1). These phases are defined by the reciprocal expression of marker genes (2, 3) and are separated

by a transition period during which Schwann cells rapidly divide (4). Both the initial progression of Schwann cells to the myelinating phase, as well as the subsequent maintenance of this phenotype, depend on a contact-mediated interaction with axons (5, 6). In cultured Schwann cells, early features of this interaction, including the triggering of cell division and the partial induction of myelin-specific genes, can be induced by agents that elevate intracellular concentrations of adenosine 3',5'-monophosphate (cAMP) (7, 8).

We asked whether a POU protein might be involved in mediating Schwann cell differentiation. We used degenerate oligonucleotides corresponding to the homeobox

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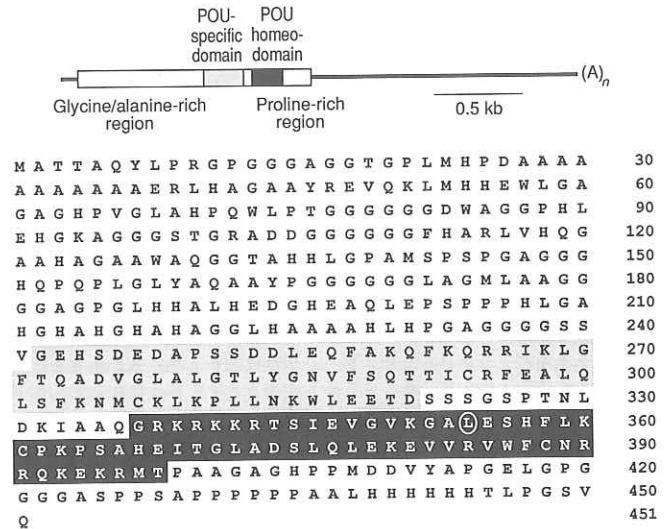
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and "POU-specific" domains of previously described POU proteins (9-12) to screen a Schwann cell cDNA library for clones encoding POU mRNAs expressed by myelin-forming cells (13). One set of overlapping cDNA clones with these properties was isolated. We have reported that in the neonatal rat these clones hybridize to a 3.1-kb mRNA expressed in developing brain and peripheral nerve, but not in most nonneural tissues (13). In cultured Schwann cells, expression of this mRNA is strongly induced by elevation of intracellular cAMP. Induction is not dependent on new protein synthesis, is stable as long as cAMP levels remain elevated, and precedes cAMP induction of the major myelin genes by ~12 hours (13). We have therefore named the protein SCIP ("skip," for suppressed, cAMP-inducible POU) (13).

The primary structure of SCIP (Fig. 1) was deduced from a composite cDNA sequence of 2977 nucleotides (14), excluding the polyadenylated tail, a length that correlated well with the estimated size of the SCIP mRNA. Translation initiated at the first in-frame ATG predicted a protein of 451 amino acids. The POU domain, composed of the POU-specific domain and the POU homeobox together with an intervening linker region (9), occupied ~35% of this protein and terminated 53 residues from the carboxyl terminus. This region exhibited strong amino acid sequence similarity to the equivalent regions of Pit-1, Oct-1, and Oct-2, and to the partially characterized domains of more recently identified POU proteins (13, 15). Among these is Tst-1, a fragment of the SCIP POU domain cloned from rat testis cDNA (15). Upstream of the POU domain, the SCIP protein contained tracts of reiterated single (A, alanine, or G, glycine) (16) and alternating (for example, AHGHAHGHAH) amino acids; alanine and glycine accounted for 48% of the residues in this region. Outside of the POU domain, the SCIP coding region was unusually GC-rich (78% G + C). Proline was the most frequently represented amino acid downstream of the POU domain (14 out of 53 residues). This may be of functional significance, given that a carboxyl terminal proline-rich region functions as a transcriptional regulatory domain in the CCAAT box-binding factor CTF (17). The deduced SCIP sequence did not otherwise exhibit significant similarity to the primary structures of previously characterized transcription factors.

In response to nerve injury, Schwann cells are able to reversibly transit between myelinating and premyelinating states (6, 18). These states are characterized by reciprocal expression of the major myelin genes, ex-

Fig. 1. Schematic diagram of the SCIP mRNA and deduced amino acid sequence of the SCIP protein (16) derived from the nucleotide sequence of six overlapping cDNA clones. Double-stranded dideoxy sequencing (USB) was performed with the use of nested Erase-a-base deletions (Promega). The SCIP POU-specific domain (black characters on gray) and POU homeodomain (white characters on black) are delineated as previously defined (9, 15). The circled leucine residue at position 354 marks the location of the Xho I site used for construction of the Xho-5 and Xho-6 transfection mutants described in Fig. 3.



pressed by myelinating cells (2), and the nerve growth factor receptor (NGFR) gene, expressed by premyelinating cells (3). The potential role of SCIP in the developmental plasticity of Schwann cells was investigated with the use of a peripheral nerve transection model in which the already myelinated sciatic nerves of 35-day-old rats were permanently severed (6). This manipulation results in axonal degeneration and Schwann cell dedifferentiation distal to the site of transection. We examined Schwann cell gene expression in this distal region at 2, 5, 10, and 20 days after transection. Expression of SCIP mRNA was barely detectable in the intact nerve, but was transiently up-regulated after permanent transection (Fig. 2). This expression profile differed markedly from those of genes specifically expressed by premyelinating and myelinating Schwann cells. The myelin basic protein (MBP) and protein zero (P₀) genes, which encode abundant structural proteins unique to myelin (2), were stably repressed after permanent transection, as has been demonstrated (6). In contrast, the reciprocally regulated

NGFR gene was stably induced. Transient activation of the SCIP gene correlated well with the onset, duration, and relative magnitude of a burst of Schwann cell division previously demonstrated to closely follow sciatic nerve transection (19). The transection-induced transformation of Schwann cell phenotype demonstrated in Fig. 2, including the transient activation of SCIP expression and the transient stimulation of proliferation, reverses the progression of Schwann cell gene expression and cell division that occurs in normal peripheral nerve development (3, 13).

POU proteins have generally been analyzed in the context of transcriptional activation (10, 11); three of these proteins are known to be key activators of cell-specific genes (11). In order to investigate the regulatory properties of SCIP, cultured Schwann cells were transfected with an SV40-based SCIP expression plasmid together with plasmids in which transcription of the chloramphenicol acetyltransferase (CAT) reporter gene (20) was driven by different eukaryotic promoters. Contrary to our initial expecta-

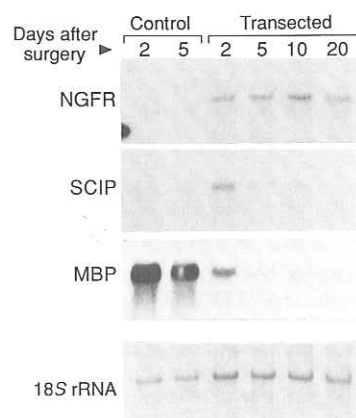


Fig. 2. Northern (RNA) analysis of NGFR, SCIP, and MBP mRNA expression after rat sciatic nerve transection. Transections were performed as described previously (6). After surgery, the proximal nerve stump and attached L4 and L5 roots were removed to prevent regeneration of axons into the distal segment. Unoperated contralateral sciatic nerves were used for control time points. Methylene blue-stained 18S ribosomal RNA (rRNA) is shown in the lowest panel to indicate relative amounts of total RNA per lane (3 µg for control points; 4 µg for transection points). The same Northern blot was used in successive hybridizations with ³²P-labeled NGFR, SCIP, and MBP cDNA probes.

tion (13), SCIP functioned as a transcriptional repressor of the major myelin promoters in these experiments (Fig. 3A). Cotransfection of the SCIP expression construct resulted in moderate repression of the mouse MBP promoter, and complete repression of the rat P₀ promoter (8) and human NGFR (21) promoter. These effects appeared to be specific, since less than two-fold differences in promoter activity (in the presence and absence of SCIP) were seen for CAT reporters driven by the mouse *c-jun* (22) promoter (not shown), the SV40 early promoter (20), or the Rous sarcoma virus (RSV) long terminal repeat (LTR) (20) (Fig. 3B).

We observed a modest but reproducible increase in P₀ promoter activity upon cotransfection with SCIP antisense expression constructs, which is consistent with the hypothesis that the low level of endogenous SCIP mRNA present in transfected Schwann cells (see legend to Fig. 3) was sufficient to partially repress the P₀ promoter. This finding is also consistent with previous demonstrations that cAMP-treated (SCIP⁺) cultured Schwann cells express (i) less major myelin mRNA than do actively myelinating Schwann cells in vivo (7), which are SCIP⁻ (Fig. 2); and (ii) less NGFR mRNA than do Schwann cells cultured in the absence of cAMP (7), which are

also SCIP⁻ (13). Taken together, these data suggest that SCIP antagonizes rather than potentiates expression of myelin-specific genes. It is likely that this antagonism was more obvious with the transfected promoters in the transient cotransfection experiments than with the endogenous genes expressed by cAMP-treated cultured cells (that is, complete repression was observed) as a result of abnormally elevated expression of SCIP protein.

SCIP repression of the P₀ promoter required an intact DNA binding domain. Introduction of a frame-shift mutation at leucine-354 in the predicted first α helix of the SCIP homeobox resulted in complete loss of repressor activity (Fig. 3C). A less drastic mutation involving the in-frame insertion of an additional valine at this same position resulted in a more modest reduction in repressor activity. Coexpression of the unrelated DNA binding protein *c-jun* (22) had no significant effect on transcription driven by the P₀ promoter.

The phenotypic progression of myelinating Schwann cell populations during normal development (3, 13, 23) and as reversed upon disruption of axonal contact (6, 24) is summarized in Fig. 4. The transition state population intermediate between premyelinating and myelinating Schwann cells is characterized by (i) elevated expression of

Fig. 3. Transient cotransfection analysis of SCIP in cultured Schwann cells. **(A)** SCIP represses major myelin gene promoters. PECE, a eukaryotic expression vector (29) that utilizes the SV40 early promoter; SCIP/AS, a SCIP cDNA fragment (nucleotides 1 to 1747, containing the full coding sequence) subcloned in the antisense orientation in PECE; SCIP, the same SCIP cDNA fragment in the sense orientation in PECE; pBLCAT3, a promoterless CAT reporter plasmid (30); P₀-CAT, a 1.1-kb rat P₀ promoter fragment (8) in pBLCAT3; MBP-CAT, a 1.4-kb mouse MBP promoter fragment in pBLCAT3. **(B)** SCIP represses the NGFR promoter, but not the RSV or SV40 promoters. RSV-CAT, the Rous sarcoma virus LTR in pSV0-CAT (20); SV40-CAT, the pSV2CAT construct containing the SV40 early promoter (20); NGFR-CAT(1), a 3-kb human NGFR promoter-CAT construct (21); NGFR-CAT(2), a 300-bp human NGFR promoter-CAT construct (21). **(C)** An intact SCIP homeodomain is required for repression. Xho-6, an insertion mutant of the SCIP sense construct in which an extra valine residue is inserted between leucine-354 and glutamate-355; Xho-5, a frame-shift mutant at the same position created by scission, fill-in, and ligation at the Xho I site; *c-jun*, the pSVc-*jun* expression construct (22). Transfections into cultured Schwann cells were performed by calcium phosphate precipitation and a sixfold mass excess of expression plasmid to reporter plasmid, as described previously (8). The luciferase plasmid pSV2.L- $\Delta\Delta 5'$ (31) was cotransfected in each experiment to normalize for transfection efficiency. All transfections were performed with cells cultured in the presence of 1 to 4 μ M forskolin, which induces a low level of endogenous SCIP mRNA (13). CAT assays were performed as described (8, 20), and quantitation of CAT production (measured as percent conversion of substrate to products) was performed by scintillation counting of modified and unmodified forms of chloramphenicol.

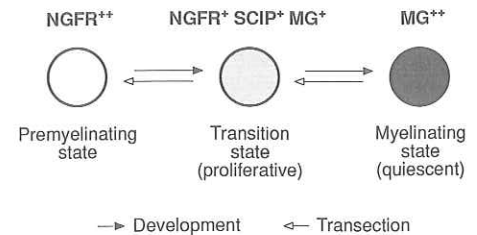
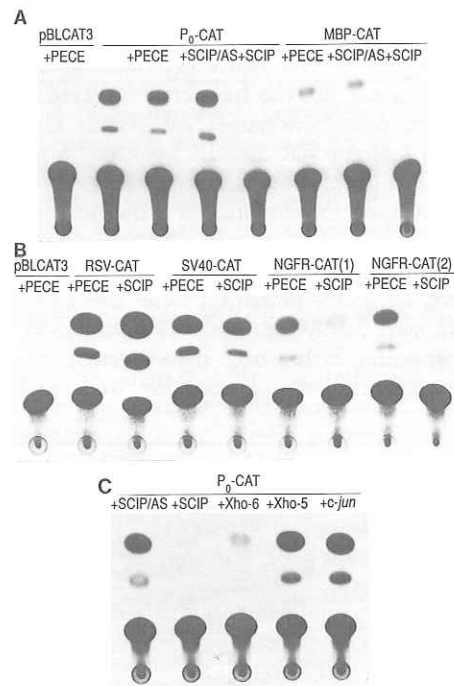


Fig. 4. Summary of the phenotypic progression of Schwann cell populations during normal development and in response to nerve transection. See text for details. MG, myelin genes.

SCIP mRNA; (ii) coexpression of NGFR and major myelin mRNAs at submaximal levels; and (iii) rapid Schwann cell proliferation. We have recognized a close association between SCIP expression and Schwann cell proliferation in three independent contexts; transient expression of the SCIP gene is correlated with transient Schwann cell division both in normal development (4, 13) and in response to transection (Fig. 2) (19), and continuous expression of this gene in cultured Schwann cells (in response to sustained elevation of intracellular cAMP) is associated with continuous proliferation (13, 25). This association may be of functional relevance, since the POU transcription factors Oct-1 and Oct-2 have been shown to stimulate DNA replication in vitro (26).

The developmental expression and repressor activity we have observed for SCIP may reflect a general role played by POU transcription factors in early vertebrate development. A recently described POU protein, closely related to SCIP in its DNA binding domain and variously designated Oct-3 (27), Oct-4, or NF-A3 (28), is expressed by the embryonic stem cell line P19 only when this line is cultured under conditions that permit cell proliferation but prevent differentiation. When P19 cells are induced to differentiate by retinoic acid, expression of Oct-3 is extinguished (27). In addition, nearly all of the POU proteins analyzed to date are expressed at relatively high amounts in regions of the early embryo, such as the ventricular zone of the neural tube, that are populated by rapidly proliferating progenitor cells (15). As development proceeds, this early expression is extinguished, and replaced in the adult by a pattern in which individual POU domain genes are stably expressed in restricted sets of fully differentiated cells (15). Together with our data, these observations suggest that in addition to their role as stably expressed transcriptional regulators in terminally differentiated cells, POU proteins may first function as transiently expressed regulators in proliferating progenitors.

REFERENCES AND NOTES

1. H. deF. Webster and J. T. Favilla, in *Peripheral Neuropathy*, P. J. Dyck et al., Eds. (Saunders, Philadelphia, 1984), pp. 329-359.
2. G. Lemke, *Neuron* **1**, 535 (1988).
3. Q. Yan and E. M. Johnson, Jr., *J. Neurosci.* **8**, 3481 (1988); K. R. Jessen, L. Morgan, H. J. S. Stewart, R. Mirsky, *Development (Cambridge)* **109**, 91 (1990).
4. M. J. Brown and A. K. Asbury, *Exp. Neurol.* **74**, 170 (1981).
5. A. J. Aguayo et al., *Nature* **268**, 753 (1977).
6. B. D. Trapp, P. Hauer, G. Lemke, *J. Neurosci.* **8**, 3515 (1988).
7. G. Lemke and M. Chao, *Development (Cambridge)* **102**, 499 (1988).
8. G. Lemke, E. Lamar, J. Patterson, *Neuron* **1**, 73 (1988).
9. W. Herr et al., *Genes Dev.* **2**, 1513 (1988).
10. R. A. Sturm, G. Das, W. Herr, *ibid.*, p. 1582; M. Tanaka and W. Herr, *Cell* **60**, 375 (1990).
11. H. A. Ingraham et al., *Cell* **55**, 519 (1988); M. Bodner et al., *ibid.*, p. 505; M. M. Müller, S. Ruppert, W. Schaffner, P. Matthias, *Nature* **336**, 544 (1988); C. Scheidereit et al., *ibid.*, p. 551.
12. M. Finney et al., *Cell* **55**, 757 (1988).
13. E. S. Monuki et al., *Neuron* **3**, 783 (1989).
14. The sequence of SCIP has been deposited with GenBank, accession number M35205.
15. X. He et al., *Nature* **340**, 35 (1989). In neonatal rats, the testis is the only nonneural tissue in which SCIP expression has been detected.
16. The single-letter abbreviations for the amino acids are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
17. N. Mermod, E. A. O'Neill, T. J. Kelly, R. Tjian, *Cell* **58**, 741 (1989).
18. G. M. Bray, M. Rasminsky, A. J. Aguayo, *Annu. Rev. Neurosci.* **4**, 127 (1981).
19. W. G. Bradley and A. K. Asbury, *Exp. Neurol.* **26**, 275 (1970); M. Abercrombie and J. E. Santler, *J. Cell. Comp. Physiol.* **50**, 429 (1957).
20. C. M. Gorman et al., *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6777 (1982).
21. A. Sehgal, N. Patil, M. Chao, *Mol. Cell. Biol.* **8**, 3160 (1988).
22. W. W. Lamph et al., *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4320 (1990).
23. G. Lemke and R. Axel, *Cell* **40**, 501 (1985).
24. R. Heumann, S. Korsching, C. Bandtlow, H. Thoenen, *J. Cell Biol.* **104**, 1623 (1987).
25. M. C. Raff, E. Abney, J. P. Brookes, A. Hornby-Smith, *Cell* **15**, 813 (1978).
26. C. P. Verijzer, A. J. Kal, P. C. Van der Vliet, *EMBO J.* **9**, 1883 (1990).
27. K. Okamoto et al., *Cell* **60**, 461 (1990); M. H. Rosner et al., *Nature* **345**, 686 (1990).
28. H. R. Schöler et al., *Nature* **344**, 435 (1990); M. J. Lenardo et al., *Science* **243**, 544 (1989).
29. L. Ellis et al., *Cell* **45**, 721 (1986).
30. B. Luckow and G. Schutz, *Nucleic Acids Res.* **15**, 5490 (1987).
31. J. R. deWet et al., *Mol. Cell. Biol.* **7**, 725 (1987).
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