strong indication that there is only one rat 3.2.3 gene and that this gene may consist of at least seven exons. Further, genomic DNA analysis from either $3.2.3^+$ or $3.2.3^-$ rat cell lines showed the same pattern of fragments (*18*), demonstrating that the gene does not undergo rearrangement in order to be expressed.

All these data indicate that the gene encoding the 3.2.3 antigen is not related to the genes encoding other NK cell–associated transduction molecules, including CD2, CD16, and CD45. The membrane orientation and the similarities of the deduced protein sequence to the sequences of the receptor molecules shown in Fig. 3 qualify our NK cell protein as a member of the C-type animal lectin family (*19*). This similarity to the C-type lectins implicates a calcium-dependent ligand binding process and also suggests that the ligand is a carbohydrate because many of these lectins specifically bind oligosaccharides with terminal galactose, *N*-acetylglucosamine, or mannose. However, the possibility that the NK cell protein could bind specific peptides is not excluded, because such binding has been demonstrated in the case of the low-affinity IgE receptor (FcεR) (*20*). Finally, the lectin-like domain of the NK cell protein has twice as many lysine residues as the majority of the C-type lectins. This suggests that the receptor ligand may be more negatively charged than the ligands of the other C-type lectins.

One could speculate that the NK protein also shares functional characteristics with the most similar C-type lectins. As an example, the low-affinity IgE receptor has been demonstrated to be involved in B cell activation (*20*). The participation of our protein in activation of NK cells is suggested by its structural characteristics and is also implied by the effects of the 3.2.3 antibody on NK function.

We conclude that our protein (NKR-P1) functions as a receptor expressed selectively on NK cells, which shares similarities with molecules whose surface expression is linked to cell activation programs.

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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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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*).

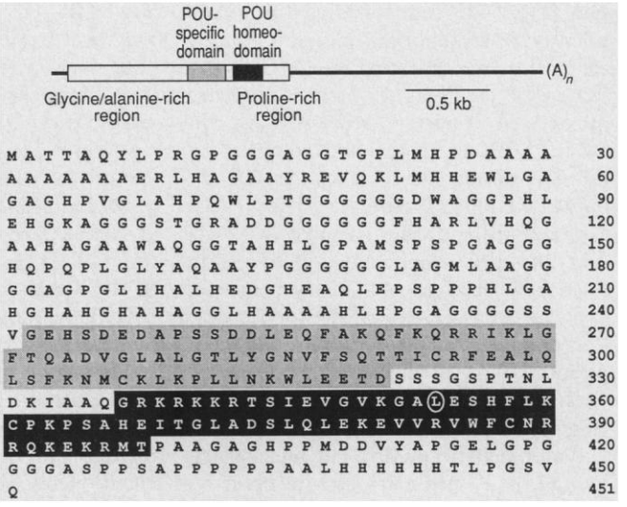

Fig. 1. Schematic diagram of the SCIP mRNA and deduced amino acid sequence of the SCIP protein (*16*) derived from the nucleotide sequence of six overlapping cDNA clones. Double-stranded dideoxy sequencing (USB) was performed with the use of nested Erase-a-base deletions (Promega). The SCIP POU-specific 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.

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, expressed 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 (P_0) 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-

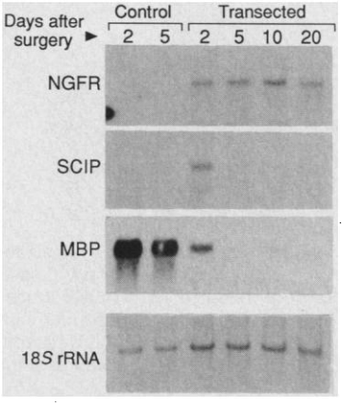

Fig. 2. Northern (RNA) analysis of NGFR, SCIP, and MBP mRNA expression after rat sciatic nerve transection. Transections were performed as described previously (*6*). After surgery, the proximal nerve stump and attached L4 and L5 roots were removed to prevent regeneration of axons into the distal segment. Unoperated contralateral sciatic nerves were used for control time points. Methylene blue–stained 18*S* 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 ^{32}P-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 P_0 promoter (*8*) and human NGFR (*21*) promoter. These effects appeared to be specific, since less than twofold 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 P_0 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 P_0 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 P_0 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 P_0 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 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.

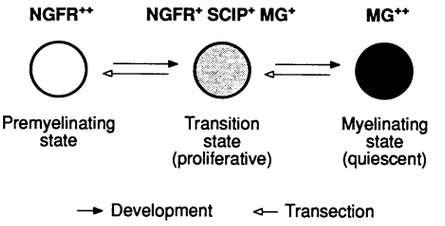

Fig. 4. Summary of the phenotypic progression of Schwann cell populations during normal development and in response to nerve transection. See text for details. MG, myelin genes.

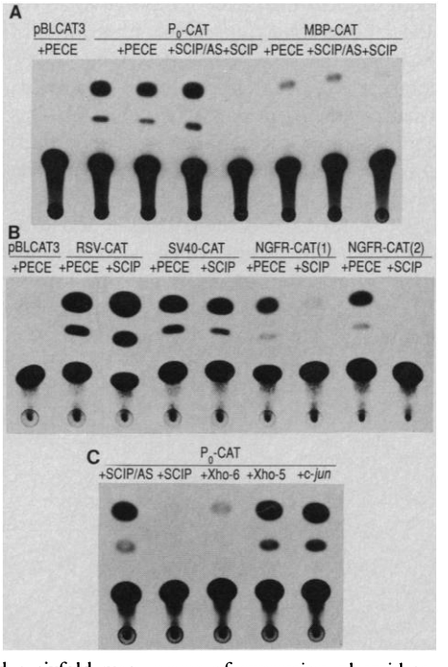

Fig. 3. Transient cotransfection analysis of SCIP in cultured Schwann cells. (**A**) SCIP represses major myelin gene promoters. PECE, a eukaryotic expression vector (*29*) that utilizes the SV40 early promoter; SCIP/AS, a SCIP cDNA fragment (nucleotides 1 to 1747, containing the full coding sequence) 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*); P_0-CAT, a 1.1-kb rat P_0 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 frameshift 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-AΔ5′ (*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 μM 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.

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Cloning and Expression of a Rat Brain GABA Transporter

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A complementary DNA clone (designated GAT-1) encoding a transporter for the neurotransmitter γ-aminobutyric acid (GABA) has been isolated from rat brain, and its functional properties have been examined in *Xenopus* oocytes. Oocytes injected with GAT-1 synthetic messenger RNA accumulated [^{3}H]GABA to levels above control values. The transporter encoded by GAT-1 has a high affinity for GABA, is sodium- and chloride-dependent, and is pharmacologically similar to neuronal GABA transporters. The GAT-1 protein shares antigenic determinants with a native rat brain GABA transporter. The nucleotide sequence of GAT-1 predicts a protein of 599 amino acids with a molecular weight of 67 kilodaltons. Hydropathy analysis of the deduced protein suggests multiple transmembrane regions, a feature shared by several cloned transporters; however, database searches indicate that GAT-1 is not homologous to any previously identified proteins. Therefore, GAT-1 appears to be a member of a previously uncharacterized family of transport molecules.

THE PLASMA MEMBRANES OF NEURONS and glia, and the synaptic vesicle membranes of neurons, have ion-dependent transporters capable of bidirectional neurotransmitter transport. The plasma membrane transporters probably terminate synaptic activity, although other functions have been proposed. Neurotransmitter transporters have been extensively studied pharmacologically, but the molecular features of these proteins are largely unknown.

The amino acid GABA is the predominant inhibitory neurotransmitter in the mammalian brain. Plasma membrane transporters for GABA are widely distributed in the central and peripheral nervous systems (*1, 2*). Pharmacological and kinetic studies suggest the presence of a variety of GABA transporter subtypes (*1–3*). Thus, GABA transporters may make up a family of proteins possessing considerable molecular diversity. The purification of a rat brain GABA transporter protein (*4*) provided a reagent for the molecular cloning of a GABA transporter cDNA. We report the isolation and sequence of such a cDNA clone and its functional characterization in *Xenopus* oocytes.

Rat brain GABA transporter protein, purified as described (*4*), was subjected to cyanogen bromide degradation, and several of the resulting fragments were sequenced (*5*). The sequence of the longest peptide (QPSEDIVRPENG) (*6*) was used to design oligonucleotide probes. Because sucrose density gradient RNA fractionation had shown that GABA transporter mRNA was in the 4- to 5-kb size range (*7*), a λ-ZAPII rat brain cDNA library containing inserts of 4 kb and greater (*8*) was screened with conventional plaque hybridization techniques. Two plaques were positive through successive platings. The mRNA was synthesized in vitro from each of these clones and was tested for its ability to express functional GABA transporters in *Xenopus* oocytes. One clone, which tested positive in the oocyte assay, was selected for detailed characterization and designated GAT-1 (GABA transporter 1).

Oocytes injected with GAT-1 RNA accumulated 50 to 100 times as much [^{3}H]GABA as water-injected or uninjected control oocytes (*9*) (Fig. 1A). In contrast, oocytes injected with 50 ng of rat brain polyadenylated RNA accumulated only 10 to 15 times as much [^{3}H]GABA as controls. Kinetic studies showed that GABA uptake by oocytes injected with GAT-1 mRNA is saturable (Fig. 1B), indicating the expression of a carrier-mediated uptake system. Eadie-Hofstee plots of three experiments revealed a Michaelis constant (K_m) of 3.1 to 10.6 μM (mean, 7.0 μM), which is

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