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Hypomyelinating Peripheral Neuropathies and Schwannomas in Transgenic Mice Expressing SV40 T-Antigen

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We have prepared transgenic mice carrying a temperature-sensitive mutant of the SV40 oncogene (tsA-1609) under the control of 5′ flanking sequences from the Schwann cell–specific P_0 gene. Four of six founder mice showed moderate to severe hypomyelination in peripheral nerves of tail biopsies, with only rare myelinated fibers. Offspring were obtained from three of these founders. Northern blot and immunohistochemical analyses showed that expression of T-antigen was restricted to the PNS. Mice expressing the highest levels of T-antigen exhibited the most severe hypomyelination. Mice expressing lower levels developed transient mild hypomyelination, but after long latencies developed sporadic schwannomas. An immortalized cell line exhibiting properties of Schwann cells at an arrested stage of differentiation, termed "SCT-1," was derived from one of these tumors.

[Key words: transgene, mouse, P_0, Schwann cell, cell line, SV40]

Papovaviruses comprise a group of circular DNA viruses whose early region genes are highly oncogenic in rodents. Transgenic studies in the mouse, in which expression of the SV40 T-antigen was controlled by various cell-specific promoters, have shown that this oncoprotein can transform a wide variety of cell types (Adams and Cory, 1991). Yet papovavirus infections cause demyelinating syndromes in their natural primate hosts (Zu Rhein, and Chou, 1965; Gribble et al., 1975), and when either the JC or SV40 viral T-antigens are expressed in myelinating glia of transgenic mice, hypomyelination rather than transformation results (Messing et al., 1985; Small et al., 1986; Jensen et al., 1993).

Several mechanisms exist by which T-antigens might cause dysmyelination. In the case of transgenic mice carrying the JC virus early region, in which T-antigen expression occurs in oligodendrocytes, dysmyelination was thought to occur because of arrested differentiation. Oligodendrocyte-specific genes such as myelin basic protein and proteolipid protein were transcribed into mRNA, but inadequate levels of protein were made (Trapp et al., 1988). In cultured Schwann cells, expression of SV40 T-antigen inhibits the P_0 promoter through a complex with the transcriptional factor c-jun (G. Tennekoon, personal communication). Nevertheless, these T-antigens are highly oncogenic in nearly all other cell types, including lens epithelium, which does not spontaneously give rise to neoplasms (Mahon et al., 1987). We therefore wondered whether myelinating glia (Schwann cells and oligodendrocytes) are resistant to the transforming effects of papovaviral T-antigens, despite the common involvement of these glial cells in spontaneous tumors of the nervous system.

We began a series of studies aimed at testing the susceptibility of Schwann cells to transformation *in vivo*. We generated transgenic mice in which the SV40 early region was placed under the control of the Schwann cell–specific, P_0 promoter. The P_0 protein is the major structural protein of peripheral myelin, and previous studies identified regulatory elements within the proximal 5′ flanking DNA that were sufficient to direct appropriate cell-specific expression of heterologous genes in cell culture and in transgenic mice (Lemke et al., 1988; Messing et al., 1992).

The developing Schwann cells of transgenic mice that express this construct appear to be trapped in the proliferative phase that precedes their full differentiation. The peripheral nerves of these mice contain elevated numbers of Schwann cells that are unable to form myelin. Mice expressing the highest levels of T-antigen exhibit the most severe hypomyelination and persistent Schwann cell hyperplasia. Mice expressing lower levels progress through a transient period of Schwann cell hyperplasia and mild hypomyelination (corresponding to the peak period of P_0 expression), but after long latencies develop sporadic schwannomas. An immortalized cell line exhibiting properties of Schwann cells at an arrested stage of differentiation was derived from one of these tumors.

Materials and Methods

Production of transgenic mice. To produce the P_0–T-antigen construct, the rat P_0 promoter (a HindIII-ApaI fragment; see Lemke et al., 1988) was fused to the StuI-BamHI fragment containing temperature-sensitive SV40 T-antigen (mutant tsA-1609) that was obtained from Dr. M.

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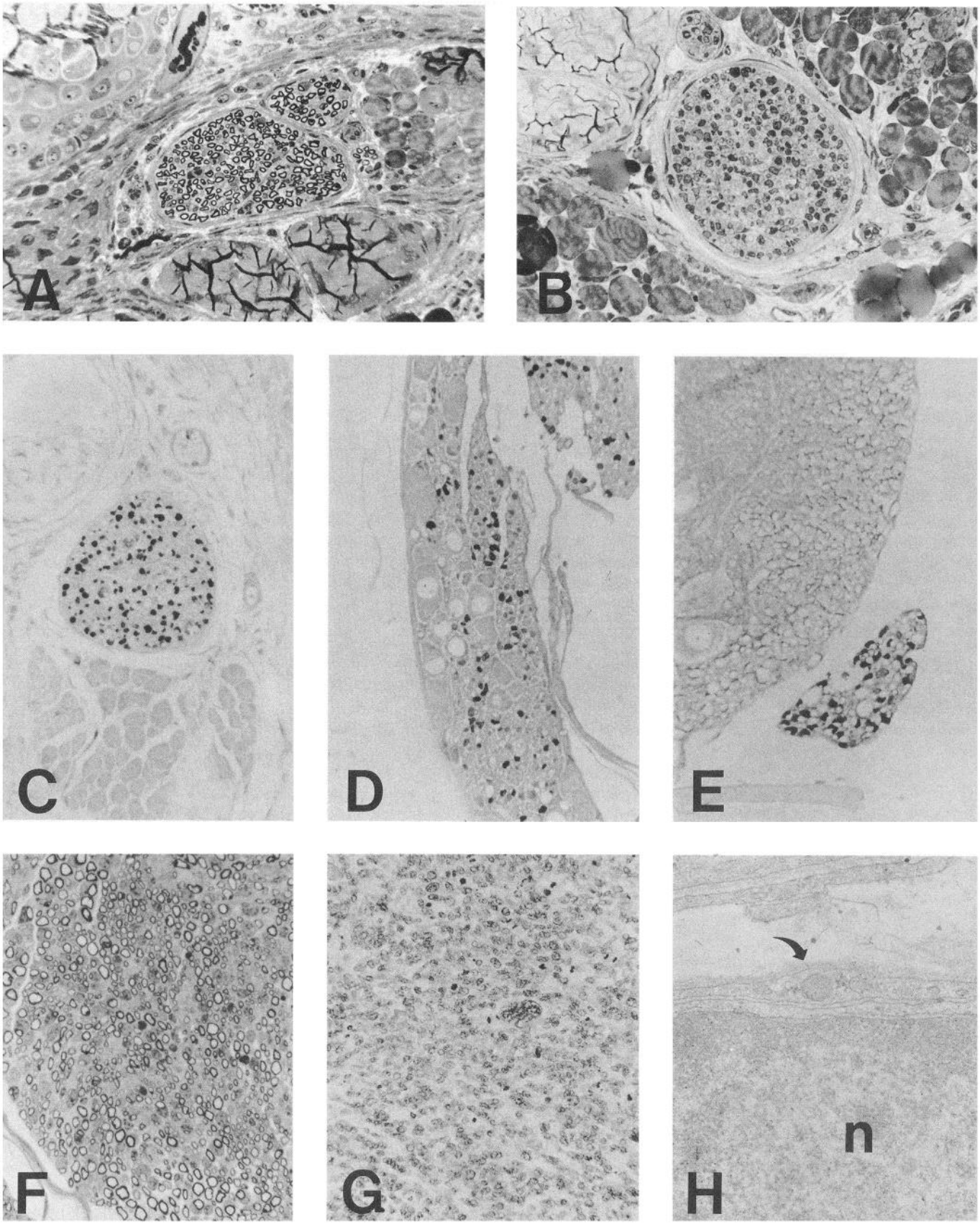

Figure 1. Morphology and immunohistochemistry of peripheral nerves and tumors. *A, B,* and *F*, Toluidine blue–stained 1 μm Epon sections. *C–E*, Immunoperoxidase stain of T-antigen in 1 μm Epon sections. *A*, Transverse section of nerve fascicle in tail biopsy taken at weaning from control mouse. *B*, Transverse section of nerve fascicle in tail biopsy taken at weaning from 1866-1 founder transgenic mouse, showing marked hypomyelination and increased number of endoneurial nuclei. *C*, Transverse section of tail nerve fascicle from first generation offspring of 1866-1 founder

Tevethia (Tevethia and Ripper, 1977). A 3.7 kilobase (kb) FspI-EcoRI fragment was isolated for microinjection. This fragment includes about 120 base pairs (bp) of pUC plasmid vector, 1.1 kb of P_0 promoter and 2.5 kb of SV40 T-antigen sequence. Transgenic mice were produced according to standard techniques (Brinster et al., 1985), using fertilized eggs obtained from the mating of F1 hybrid C57BL/6J × SJL mice. Breeding lines of animals were maintained by backcrosses to B6SJLF$_1$ mice. The two stable lineages described in this report have been assigned the following genetic designations: line 1868-1, Tg(*Mpz*,SV40E)Bri135; and line 1868-2, Tg(*Mpz*,SV40E)Bri136.

Nerve and tumor morphology. For morphologic and immunohistochemical evaluation of peripheral nerves in tail biopsies or sciatic nerve, tissues were immersion fixed with 3.6% glutaraldehyde, 0.1 M Na phosphate buffer, pH 7.4, followed by postfixation in 1% osmium tetroxide, dehydration, and embedding in Epon. Thick (1 μm) sections were stained with alkaline toluidine blue. Routine histopathology was performed on tissues that were immersion fixed in neutral buffered formalin, paraffin embedded, and stained with hematoxylin-eosin.

T-antigen staining in plastic sections. Immunohistochemical detection of T-antigen in nerve was performed by postembedding staining of thick (1 μm) sections from tail biopsies (immersion fixed) or other neural tissues (perfused) processed without osmium postfixation, using a rabbit polyclonal anti-T antiserum as previously described (Behringer et al., 1988).

Establishment and cloning of SCT-1 cell line. A schwannoma from the sciatic nerve of a 7-month-old mouse of the 1868-2 line was mechanically and enzymatically dissociated (0.125% trypsin, 0.02% collagenase in Hank's balanced salt solution for 30 min at 37°C), washed in Dulbecco's Minimal Essential Medium with 10% fetal calf serum, and seeded into 25 cm^2 plates for growth at 33°C in a 5% CO_2 atmosphere. The SCT-1 clonal cell line was isolated by limiting dilution in 96-well plates. The SCT-1 cells were routinely maintained in DMEM supplemented with 10% fetal calf serum at the permissive temperature for T-antigen (33°C). For temperature shift experiments, SCT-1 cells were cultured for 5 d at the nonpermissive temperature (39°C) prior to analysis or forskolin treatment. P_0 gene expression was activated in these cells at either temperature by culture in 4 μM forskolin for 3 additional days (Lemke and Chao, 1988).

Immunofluorescence on schwannoma cells. The transgenic tumor-derived Schwann cells were enzymatically dissociated and plated on poly-L-lysine–treated glass coverslips and grown at 33°C for 1–3 d. P_0 and T-antigen were detected using immunofluorescence. The cells were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) for 20 min, washed twice with PB, and permeabilized with 100% ethanol for 2 min. The cells were then washed, blocked with 3% normal goat serum (NGS) in PB for 1 hr, and then incubated for 2 hr at 22°C with the pooled primary antibodies diluted in 1% NGS-PB: anti-P_0 (final dilution 1:500) (Trapp et al., 1981) and anti-T-antigen (PAb-419, culture supernatant) (Harlow et al., 1981). The cells were then washed twice with PB and then incubated for 1 hr at 22°C with pooled labeled secondary antibodies: goat anti-rabbit-fluorescein and goat anti-mouse-rhodamine. Controls included transgenic Schwann cells treated as above but without either of the primary antibodies, or nontransgenic and non-Schwann cell lines that express neither P_0 nor T-antigen proteins.

Northern blot analysis. Tissues from transgenic and nontransgenic littermates, or washed SCT-1 cells, were frozen in liquid nitrogen and stored at −70°C until further processing. Total RNA was isolated by the guanidine/water-saturated phenol procedure of Chomczynski and Sacchi (1987), and analyzed by Northern blot as described previously (Weinmaster and Lemke, 1990). To control for the relative amount and quality of RNA in each lane, 18S and 28S ribosomal RNAs were visualized by staining transferred blots with 0.4% methylene blue prior to hybridization, as described previously (Weinmaster and Lemke, 1990), or were probed for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The following cDNAs were utilized as probes: P5, a 1.7 kb fragment of human neurofibromin (Wallace et al., 1990), a 0.7 kb BamH1 fragment of rat nerve growth factor receptor (Radeke et al., 1987), a 330 bp PstI/PvuII fragment of large T-antigen (Gruda and Alwine, 1993), a full-length cDNA of rat P_0 (Lemke and Axel, 1985), a 330 bp PvuII fragment of histone H3 (pFF 435C) (Plumb et al., 1983), and a full-length cDNA of rat GAPDH (Fort et al., 1985).

Results and Discussion

The transgene construct was made by attaching 1.1 kb of 5′ flanking DNA from the rat P_0 gene to the early region of SV40 virus. This region of the P_0 gene was previously shown to be effective in directing expression of heterologous genes to myelinating Schwann cells in transgenic mice (Messing et al., 1992). Since one of the long-term goals of the study was to develop immortalized cell lines, we used a temperature-sensitive mutant of SV40, tsA-1609, which contains a point mutation that results in conversion of an arginine to lysine at amino acid 357 in the protein (Tevethia and Ripper, 1977). This mutation occurs in the p53-binding domain of the T-antigen, and, *in vitro*, inactivates the T-antigen at the restrictive temperatures of 39–41°C (Rio et al., 1985). Because of previous reports that mouse body temperature might be restrictive for the mutant T-antigen (Lendahl and McKay, 1990), we first prepared transgenic mice carrying the tsA-1609 mutant early region under the control of the SV40 enhancer and promoter. Six of seven founder mice developed tumors in the choroid plexus, the expected site of SV40 enhancer/promoter activity (Brinster et al., 1984; Palmiter et al., 1985), showing that the tsA-1609 is transformation-competent at mouse body temperature (data not shown).

Six founder transgenic mice were born carrying the SV40 sequences, of which three (1866-1, 1868-1, 1865-8) developed an obvious neurological phenotype at 2 weeks of age. This phenotype was characterized by weakness of limbs and tremors. The neurologic deficits progressed to paralysis and death by 1.5–3 months. We obtained offspring from two of these three founders by ovarian transplantation and *in vitro* fertilization when the founders could not mate normally. A fourth founder (1868-2) that was less affected was naturally mated. Stable lines were obtained from the 1868-1 and 1868-2 founders, and in these lines the phenotypes have remained constant through more than nine generations. Although the clinical signs predominantly reflect PNS dysfunction, seizures are occasionally seen in mice from the 1868-1 line when they are stressed (i.e., during tail sampling).

Peripheral nerve morphology was initially assessed in transverse sections of the tail biopsies collected at weaning for DNA analysis. These sections were taken approximately 1 cm from

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sampled at weaning, stained for T-antigen. T-antigen–immunoreactive nuclei are confined to the Schwann cells of the nerve. *D*, Section of sensory dorsal root ganglion from first generation offspring of 1868-1 founder, killed at 6 weeks of age and stained for T-antigen. T-antigen–immunoreactive nuclei are confined to the axon-containing areas of the section, whereas the satellite Schwann cells surrounding neuronal cell bodies are negative. *E*, Section of ventrolateral spinal cord and ventral root from same mouse as in *D* (1868-1 line), stained for T-antigen. T-antigen–immunoreactive nuclei are confined to the Schwann cell–containing ventral root, whereas the oligodendrocytes of the spinal white matter are negative. *F*, Transverse section of sciatic nerve from mouse of the 1868-2 line sampled at 3 weeks of age, showing the mild hypomyelination and increased number of endoneurial nuclei typical of this line at weaning (compare with *A*). *G*, Histopathology of peripheral nerve tumor from transgenic mouse of the 1868-2 line, sampled at 7 months of age. The tumor consists of densely packed cells with irregularly shaped and occasional giant nuclei, embedded in a poorly defined background matrix. Hematoxylin-eosin–stained paraffin section. *H*, Ultrastructural appearance of peripheral nerve tumor from transgenic mouse of the 1868-2 line, sampled at 7 months of age. The neoplastic cells (*n* = tumor cell nucleus) produce numerous thin processes and have an obvious basal lamina (*arrow*), indicative of a Schwann cell origin. Magnification: *A–G*, 280×; *H*, 14,400×.

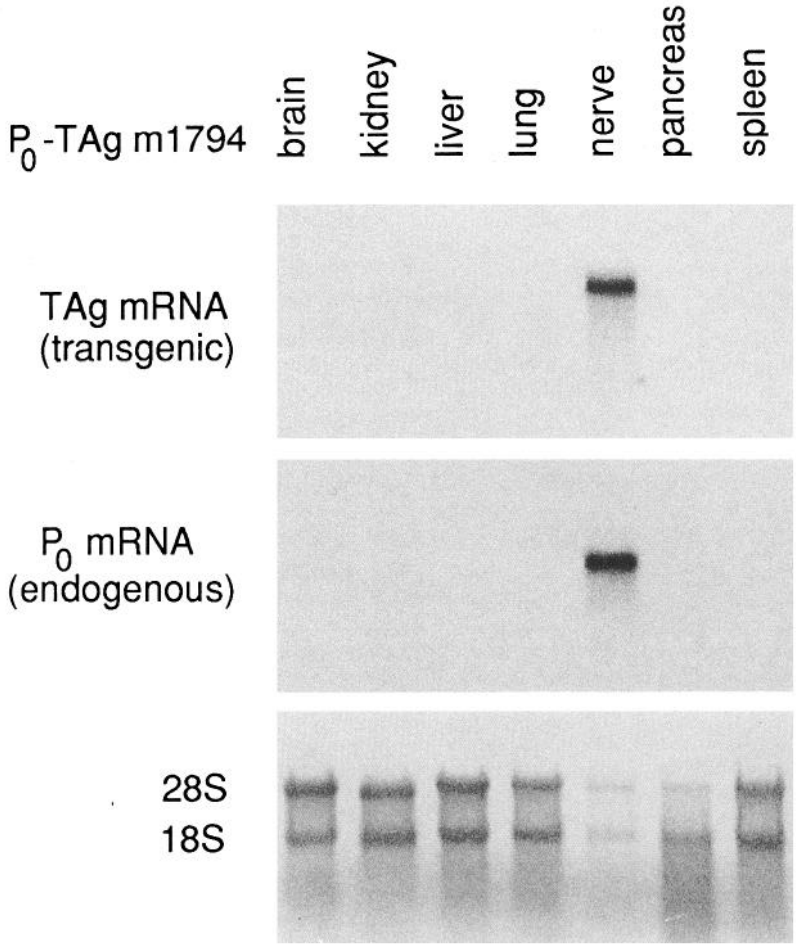

Figure 2. Northern blot analysis of transgene expression in several tissues. Peripheral nerve–specific expression of the P_0–T-antigen transgene. Total RNA was isolated from a variety of tissues of a mouse from the transgenic P_0–T-antigen line 1868-1 (mouse 1794), and analyzed by Northern blot for expression of T-antigen (*TAg*) mRNA and endogenous P_0 mRNA. The same blot was hybridized first with a radiolabeled TAg probe and then with a P_0 probe. The control panel at the *bottom* indicates the relative amount of RNA in each lane, as visualized by methylene blue staining of the blot prior to hybridization. Note that (due to the very small amount of tissue that can be recovered) the amount of RNA present in the peripheral nerve lane is markedly less than that present in all other lanes. Transcripts corresponding to the TAg transgene and the endogenous P_0 gene are seen only in peripheral nerve, whereas all other tissues tested, including brain, are negative.

the tip of the tail, a site that provides a consistent and reliable sampling of the four major nerve fascicles in the tail. In control mice, these nerve fascicles contain a mixed population of myelinated and unmyelinated fibers (Fig. 1*A*). However, tail nerves of founder 1866-1 were markedly depleted of myelin and had increased numbers of endoneurial nuclei (Fig. 1*B*). Expression of SV40 T-antigen was evaluated by immunohistochemical analysis of tail biopsies from an offspring of the 1866-1 founder, and immunoreactive nuclei were confined to the fascicle of nerve, with no staining in surrounding tissues (Fig. 1*C*). A more extensive survey of the PNS in offspring of the 1868-1 line showed T-antigen–positive cells in the myelinated areas of dorsal root ganglia, while the satellite cells surrounding the neuronal cell bodies were negative (Fig. 1*D*). In addition, sections of spinal cord with associated roots showed T-antigen expression confined to the PNS, and absent in the CNS (Fig. 1*E*). The specificity of the P_0 promoter for expression of transgenes in myelinating Schwann cells was further verified by ultrastructural identification of immunolabeled cells in serial thin sections (data not shown), consistent with the previously demonstrated efficacy of the P_0 promoter in other transgenic experiments (Messing et al., 1992). Similar results were obtained in offspring from the other two lines of mice.

To confirm our initial impression of tissue-specificity gained from the tail biopsies, we isolated total RNA from several tissues of a 3-month-old mouse from the 1868-1 line and analyzed these RNAs for T-antigen expression by Northern blot. We probed this blot first for expression of T-antigen mRNA transcribed from the transgene, and subsequently for P_0 mRNA transcribed from the endogenous P_0 gene. As shown in Figure 2, T-antigen transcripts were seen only in samples of peripheral (sciatic) nerve, the only tissue in which the endogenous P_0 transcript was also present. In addition, complete necropsies of 3–10 mice from each of the lines showed no significant gross or histologic abnormalities in any tissue other than nerve, except muscle atrophy caused by the neuropathy.

In addition to hypomyelination, focal space-occupying masses were sometimes seen arising within nerves. However, the appearance of tumors seemed to be inversely correlated with severity of the demyelinating neuropathy and the level of T-antigen expression (Table 1). For instance, in the most severely affected line (1866-1) only hypomyelination was seen, whereas in a slightly less affected line (1868-1) occasional microscopic foci of tumors were seen. Mice of the 1868-2 line have only a subtle gait abnormality when young (allowing their hind paws to drag slightly when walking), but they appear to recover. These mice have lower levels of T-antigen expression and a much milder neuropathy that peaks at weaning (Fig. 1*F*). However, by 6–12 months a small percentage of 1868-2 mice develop macroscopic tumors (to 2 cm in diameter) in peripheral nerves that consist of pleomorphic populations of spindle-shaped cells (Fig. 1*G*). Ultrastructural examination of a nerve tumor showed a distinct basal lamina around the tumor cells, suggesting a Schwann cell origin (Fig. 1*H*). In addition, Northern blot anal-

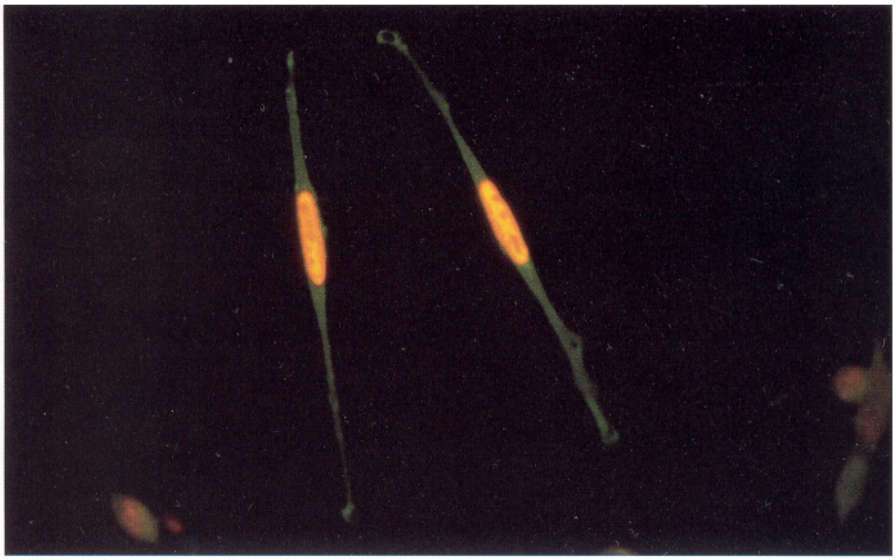

Figure 3. Double immunofluorescence detection of T-antigen and P_0 in schwannoma cells cultured from a peripheral nerve tumor (m1952) arising in a 7-month-old transgenic mouse from the 1868-2 transgenic line. The Schwann-like spindle cells contain both nuclear T-antigen (*red*) and cytoplasmic P_0 (*green*), whereas other cells in the culture are negative for both antigens. Note that the double exposure of the same frame of film using first rhodamine and then fluorescein filters changes the color balance from the deeper red and green that would be seen with either set of filters alone. Magnification, 440×.

ysis of RNA isolated from nerve tumors showed expression of P_0 mRNA (see below, Fig. 4*A*). These results demonstrate that Schwann cells can be transformed by SV40 T-antigen, but that tumorigenesis requires a significant amount of time to elapse after the onset of T-antigen expression. The more severely affected lines of mice may remain tumor free because their lifespan is too short to allow accumulation of sufficient secondary events to complete the process of transformation.

We attempted to utilize these transgenic mice to establish immortalized cell lines of Schwann cell origin. We prepared dissociated cell cultures from hypomyelinated sciatic nerves of mice from the 1868-1 line (high T-antigen expression), and from a schwannoma of the 1868-2 line (low T-antigen expression). Consistent with the *in vivo* tumorigenesis results described above, only the 1868-2 cells continued to grow. Schwann-like cells from the 1868-2–derived cultures expressed readily detectable levels of both T-antigen and P_0 proteins (Fig. 3). The primary cultures were subsequently cloned by limiting dilution, giving rise to a clonal cell line termed "SCT-1" (for Schwann cell tumor 1). Because the oncogene used was a temperature-sensitive mutant, we studied transcriptional regulation under both permissive (33°C) and restrictive (39.5°C) conditions. The expression of P_0 mRNA rises at the restrictive temperature (Fig. 4*A,B*), and is forskolin-inducible at both temperatures (Fig. 4*B*). However, proliferation continues at approximately the same rate at either temperature (note levels of histone H3 transcripts in Fig. 4*A*). In addition, other measures of growth (tritiated thymidine incorporation, BrDU uptake, cyclin B mRNA) also showed no effect of the temperature shift on proliferation (data not shown). The SCT-1 cell line also expresses high levels of other genes produced by premyelinating cells in the PNS, including the NGF receptor (NGFR) and neurofibromin (NF1) transcripts (Fig. 4*A*). Expression of mRNA for these four genes is also seen in samples of the primary schwannoma from which the cell line is derived (m1952, Fig. 4*A*). Interestingly, another tumor analyzed from a different mouse of the same line showed much higher levels of P_0 mRNA but lower levels of T-antigen mRNA and the other gene products (m1970, Fig. 4*A*).

In summary, Schwann cells are susceptible to transformation *in vivo* by the SV40 T-antigen, but tumors form only after a long latency. The hypomyelination that is a prominent feature in some lines of mice may be analogous to the preneoplastic hyperproliferative states observed in other models of T-antigen–induced tumorigenesis (Hanahan, 1985; Ornitz et al., 1987). As in these other models, one or more secondary events are presumably necessary for complete transformation. Previous cell culture experiments with rat Schwann cells implicated cooperative interactions with cellular proto-oncogenes such as *ras* as necessary for transformation of Schwann cells by large T-antigen (Ridley et al., 1988). In our transgenic model, it is interesting that continued expression of neurofibromin, a putative tumor-suppressor gene in which deletions are associated with spontaneous schwannomas in humans, does not counteract the transforming effects of T-antigen. Alternatively, the hypomyelination in these transgenic mice may result from direct inhibition of expression of the endogenous P_0 gene by the SV40 T-antigen (Tennekoon, personal communication), since the P_0 protein is known to be essential for peripheral myelination (Giese et al., 1992). By whatever mechanism, high level expression of T-antigen appears to be incompatible with full Schwann cell differentiation.

The SCT-1 cell line exhibits properties of early Schwann cells that are just beginning to activate the program of myelin gene expression, expressing both the NGFR and P_0. Other genes that are part of this same program, such as myelin basic protein (MBP), are also expressed (L. Wrabetz et al., unpublished observations). SV40 or its T-antigens have been previously used to derive Schwann cell lines with varying degrees of differentiation, but none of these expressed both P_0 and MBP (Chen et al., 1987; Tennekoon et al., 1987; Ridley et al., 1988; Peden et al., 1989; Watabe et al., 1990). In the case of SCT-1 cells, it is possible that expression of T-antigen prevents further differentiation, even at the restrictive temperature (39°C). Under these

Table 1. Phenotypes of P_0-T-antigen transgenic mice

Line	Life-span	T-antigen	Neuropathy	Tumors
1866-1	1 month	+++	+++	–
1868-1	2–4 months	+++	+++	Micro
1868-2	8–18 months	+	+	Macro

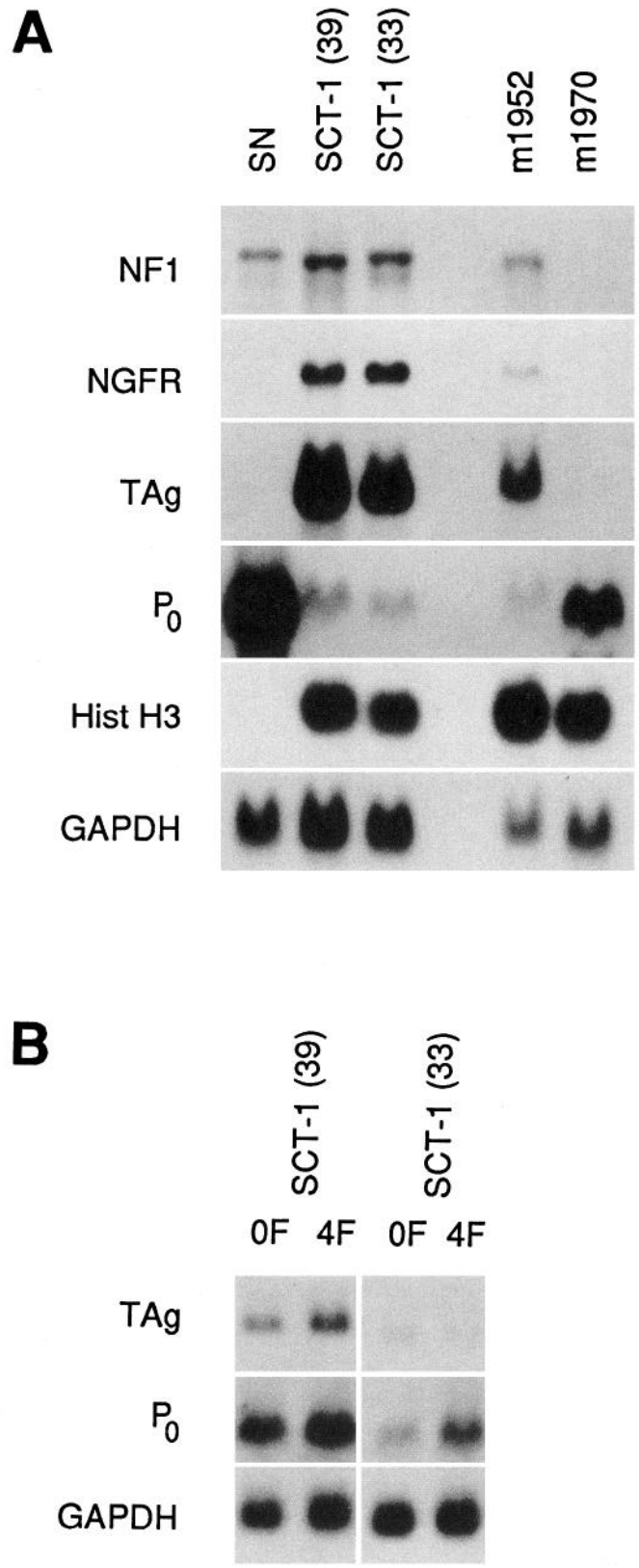

Figure 4. Northern blot analysis for Schwann cell–specific and T-antigen gene expression in peripheral nerve tumors from transgenic mice and SCT-1 cells. *A*, A Northern blot of total RNA (10 μg per lane) prepared from two peripheral nerve tumors (*m1952* and *m1970*); SCT-1 cells cultured at either the permissive (33°C) or nonpermissive (39°C) temperatures for the mutant T-antigen; and adult rat sciatic nerve (*SN*). Both SCT-1 cells, and the m1952 tumor from which they are derived express several Schwann cell–enriched genes including P_0, neurofibromin (*NF1*), and NGF receptor (*NGFR*), in addition to T-antigen (*TAg*). Reprobing the blot for histone H3 (*Hist H3*) mRNA demonstrates that the rate of proliferation is similar in both tumor samples and in SCT-1 cells at both the permissive and nonpermissive temperatures. Histone H3 mRNA is selectively synthesized during the S-phase of the cell cycle, and serves as a convenient marker of cell proliferation (Plumb et al., 1983). Reprobing the blot for GAPDH mRNA demonstrates equal amounts of RNA in each lane. The films were exposed for 6 d, 4 d, 4 d, 18 hr, 2 hr, and 18 hr for NF1, NGFR, TAg, P_0, Hist H3, and GAPDH, respectively. *B*, A Northern blot of total RNA (10 μg per lane) prepared from SCT-1 cells cultured in the presence or absence of forskolin at both 39°C and 33°C. The expression of P_0 and T-antigen (*TAg*) mRNAs is increased at the restrictive temperature (39°C). Further, the expression of both P_0 and TAg mRNAs is induced by 4 μM forskolin at the nonpermissive temperature (39°C). Reprobing the blot for GAPDH demonstrates equal loading of total RNA for all of the lanes. Under these temperature conditions, expression of histone H3 does not change (see Fig. 4*A*). *0F*, cultured in the absence of forskolin; *4F*, cultured in the presence of 4 μM forskolin. The films were exposed for 24 hr, 18 hr, and 24 hr for TAg, P_0, and GAPDH, respectively.

conditions, the NGFR continues to be expressed and the cells actively proliferate, although both P_0 and T-antigen levels rise. We believe that these unusual temperature effects reflect the consequence of having the oncogene under the control of a promoter that is itself regulated by differentiation. That is, T-antigen function is compromised at the restrictive temperature, but as the cells attempt to differentiate, and thereby increase expression of the endogenous P_0, the transgene is similarly upregulated. Partial function of the oncogene at the restrictive temperature could be compensated for by increased levels of expression. The result would be an equilibrium of T-antigen self-regulation through the P_0 promoter, selecting for continued expression of P_0 but suppressing full differentiation (note continued expression of NGFR and NF1 transcripts). These experiments may indicate a general limitation of using differentiation-specific promoters to direct the expression of temperature-sensitive oncogenes for the purpose of deriving differentiated cell lines.

Alternatively, the lack of dramatic changes in the differentiated state of the SCT-1 cells at the restrictive temperature for T-antigen may reflect the dominating effect of putative secondary mutations in cooperating oncogenes. In this case, temperature shifts would still affect T-antigen function and compromise its ability to inhibit the P_0 promoter, but other markers of Schwann cell differentiation would not change significantly (such as expression of NGFR and NF1).

Finally, the phenotype of the severely affected 1866-1 and 1868-1 lines is considerably worse than transgenic mice with comparable levels of hypomyelination induced by expression of diphtheria toxin under the control of the P_0 promoter. However, the spontaneous murine mutant *Trembler,* in which peripheral hypomyelination is associated with a point mutation in a myelin gene PMP-22 (Suter et al., 1992), and the P_0-deficient mice created by gene targeting (Giese et al., 1992), both have much longer life-spans than any of the P_0-transgenic lines (diphtheria toxin or T-antigen). It is clear that these four models of peripheral neuropathy represent different alterations in Schwann cell functions apart from simply hypomyelination. Comparative studies of these neuropathies may reveal additional mechanisms by which Schwann cells regulate the physiological properties of axons independent from ensheathment with myelin.

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