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# Neuronal and glial expression of the adhesion molecule TAG-1 is regulated after peripheral nerve lesion or central neurodegeneration of adult nervous system

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## Abstract

Expression of the cell adhesion molecule TAG-1 is down-regulated in adult brain, with the exception of certain areas exhibiting structural plasticity. Here, we present evidence that TAG-1 expression persists also in adult rat spinal cord and dorsal root ganglia (DRG), and can be up-regulated after injury. On Western blots of adult tissue, TAG-1 is detected as a 135-kDa band, with an additional specific 90-kDa band, not present in developing tissue. TAG-1 expression is found both in DRG neurons and in Schwann cells, particularly those associated with the peripherally projecting DRG processes. Quantitative *in situ* hybridization revealed that TAG-1 expression is significantly higher in small neurons that give rise to unmyelinated fibers, than in large DRG neurons. The regulation of TAG-1 was then examined in two different lesion paradigms. After a sciatic nerve lesion, TAG-1 expression is not up-regulated in DRG neurons, but decreases with time. At the lesion site, reactive Schwann cells up-regulate TAG-1, as demonstrated by both immunohistochemistry and *in situ* hybridization. In a second paradigm, we injected kainic acid into the spinal cord that kills neurons but spares glia and axons. TAG-1 is up-regulated in the spinal neuron-depleted area as well as in the corresponding dorsal and ventral roots, associated with both target-deprived afferent fibers and with the non-neuronal cells that invade the lesion site. These results demonstrate a local up-regulation of TAG-1 in the adult that is induced in response to injury, suggesting its involvement in axonal re-modelling, neuron–glia interactions, and glial cell migration.

## Introduction

Ig-superfamily cell adhesion molecules control cell migration, neurite outgrowth, fasciculation, and synaptogenesis through homophilic or heterophilic binding (Martini, 1994; Ide, 1996; Fu & Gordon, 1997; Karagogeos, 2003). Their expression is highly regulated during development in relation to axonal growth and guidance (Walsh & Doherty, 1997; Revest *et al*., 1999; Hortsch, 2000). These proteins are also maintained or re-expressed in the adult where they are considered to be important for neuronal plasticity in response to certain physiological stimuli or to a lesion (reviewed in Hoffman, 1998), mediating for example, peripheral axon regeneration (Martini, 1994; Ide, 1996; Fu & Gordon, 1997).

The Ig superfamily member TAG-1 exists in a GPI-anchored and a secreted form. It was first described as only transiently expressed during development (Yamamoto *et al*., 1986; Dodd *et al*., 1988; Furley *et al*., 1990), where it is involved in axon guidance (Stoeckli & Landmesser, 1995; Stoeckli *et al*., 1997), and cell migration (Denaxa *et al*., 2001; Kyriakopoulou *et al*., 2002). However, low levels of TAG-1 could also be detected in certain neurons of adult rodent brain, e.g. in olfactory bulb, hippocampus, and cerebellar granule cells (Yoshihara *et al*., 1995; Wolfer *et al*., 1998). In adult fish, TAG-1 expression is maintained in a restricted subpopulation of neurons, the nasal retinal ganglion cells (RGCs), in which an up-regulation of TAG-1 is observed after axotomy (Lang *et al*., 2001). TAG-1 activity appears to be mediated through both homophilic and heterophilic mechanisms, the latter of which can involve various binding partners, including several members of the L1 family (Buchstaller *et al*., 1996; Rader *et al*., 1996; Malhotra *et al*., 1998), NCAM, and extracellular matrix molecules (Milev *et al*., 1996).

A novel function of TAG-1 has emerged from the recent demonstration of its involvement in the molecular organization of juxtaparanodal regions of myelinated fibers. TAG-1 associates with Caspr2, a member of the neurexin IV-caspr-paranodin (NCP) protein family and the *shaker*-type $K^+$ channels (Poliak *et al*., 2003; Traka *et al*., 2003), and is required for their clustering in the juxtaparanodes. Accordingly, TAG-1 was shown to be expressed in both Schwann cells and oligodendrocytes (Traka *et al*., 2002).

Taken together, these studies suggest an involvement of TAG-1 in the formation of neuronal networks, the proper domain organization of myelinated fibers, and in the plasticity of the adult nervous system.

The central nervous system (CNS) in adult mammals exhibits only a limited capacity for axonal regeneration after mechanical injury. In contrast, axonal regeneration in the peripheral nervous system (PNS)

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is facilitated both by a permissive environment and by continuous expression and/or up-regulation of axon outgrowth-related genes. The present study focuses on TAG-1 expression in adult rat DRGs and spinal cord, and in dorsal and ventral roots. TAG-1 expression by DRG neurons has been shown previously to begin early in development, and to be maintained up to one week after birth (Dodd *et al*., 1988; Karagogeos *et al*., 1991). We show here that in addition, TAG-1 is highly expressed not only in Schwann cells but also in adult DRG neurons, and that this expression varies according to the type of DRG neuron. To address the potential function of TAG-1 in the adult, we also investigated changes in TAG-1 expression and distribution in two different lesion paradigms that induce axonal plasticity; axonal regeneration after peripheral nerve lesion (Nothias *et al*., 1993), and axonal re-modelling after target deprivation by excitotoxic central neurodegeneration in the spinal cord (Nothias *et al*., 1991).

## Materials and methods

### Animals and surgery

Adult female (200–250 g; $n = 35$), and postnatal day 15 (P15) Wistar rats were used in this study. Experimental procedures (authorization no. 91–78 for FN) were in accordance with EU Committees directives (86/609/EEC). Animals were anaesthetized by i.p. injection of a solution containing xylazine (5 mg/kg; Rompun 2%, Bayer, France) and ketamine (100 mg/kg; Imalgène 500, Merieux France).

#### *Peripheral nerve lesion*

Right sciatic nerves were cut at midthigh levels, and re-apposed by a single 9–0 suture through the epineurium to allow nerve regeneration. The left side (unoperated) nerve and DRG were used as normal controls, in addition to intact animals.

After survival times of 3–60 days, animals were deeply anaesthetized with pentobarbital then decapitated. Fresh DRGs, sciatic nerves and spinal cord were dissected for *in situ* hybridization and Western blots, frozen and kept at −80 °C. For immunohistochemical studies, animals were deeply anaesthesized with pentobarbital, and then perfused transcardially with NaCl (0.9% at 37 °C, supplemented with heparin) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. The lumbar spinal cord and L4 and L5 DRGs with the central and peripheral roots of both right (experimental) and left (control) sides were removed and cryoprotected in 30% sucrose in 0.1 M PB containing NaCl (0.9%; PBS). In some cases, to visualize individual Schwann cells, teased fibers were prepared from dissected fresh sciatic nerves, then fixed for 30 min in 4% paraformaldehyde in 0.1 M PB, followed by several rinses prior to immunostaining (see below).

#### *Kainic acid lesion*

A spinal neurodegenerative lesion was made according to our previously developed experimental model (Nothias & Peschanski, 1990). On adult rats ($n = 8$), the spine was freed from skin and muscles at the level of vertebrae T12–L2 and the animal was secured in a stereotaxic apparatus with spinal clamps. A laminectomy of the T13 vertebrae exposed the lumbar enlargement of the spinal cord. With a dentistry needle mounted on a microdrive, a 0.2 μL kainic acid solution (KA; 0.5% in buffered saline) was pressure-injected 1.5–2 mm below the surface and 0.7 mm from the midline. Three injections were made 3 mm apart to produce a large area of neuronal depletion. Care was taken not to damage the blood vessels running along the spinal cord. The wound was washed with saline, and muscles and skin sutured. Operated animals received daily doses of antibiotics (Bristopen, 200 mg/kg i.m.), and were killed after survival times of 8–20 days postlesion.

### Adult Schwann cell culture

The protocol used for purification and culture of adult Schwann cells is described in Manent *et al*. (2003). Briefly, sciatic and trigeminal nerves were removed from adult rats, cultured for one week to allow Wallerian degeneration to take place. After enzymatic and mechanical dissociation of nerves, Schwann cells were separated from contaminating fibroblasts by indirect magnetic cell sorting, using P75NTR (Euromedex, Souffelweyersheim, France) as the antigen. Purified adult Schwann cells were then plated on poly lysine/laminin (Sigma/Invitrogen, France)-coated coverslips in N2-HRG medium (R & D system, Lille, France), and kept at 37 °C, 7.5% $CO_2$. For immunochemistry, cells were fixed with 4% PFA in 7.5% sucrose and treated in the same way as tissue sections.

### Western blotting

After removal of attached nerves, spinal cords and L4 and L5 DRGs were homogenized at 4 °C in 5 mM Tris-HCl pH 7.2, 1% NP-40, 1 mM PMSF, 5 mM EGTA, 2 mg/mL leupeptin and 2 μg/mL aprotinin. Samples were centrifuged for 20 min at 10 000 × g at 4 °C, and the protein concentration in the supernatant determined with the Biorad Bradford kit. Samples were subjected to SDS-PAGE and Western blotting as described before (Dodd *et al*., 1988; Karagogeos *et al*., 1991), except that the ECL method (Amersham, Freiburg, Germany) was used for detection. Western blots were reacted with a polyclonal TAG-1 antibody (Dodd *et al*., 1988; Traka *et al*., 2002, 2003), and a monoclonal actin antibody (Amersham, Freiburg, Germany).

### Protein quantification

To normalize total protein levels in each sample, actin concentrations were used as internal control. Optical densities of TAG-1 and actin bands were evaluated using the Gel Plotting Macros of the NIH image software. TAG-1 protein levels from axotomized DRGs were first normalized with actin and then they were compared to the ones in the DRGs from the corresponding intact side (defined as 100%), and mean values for lumbar DRG samples from at least five animals for each survival period were examined. Statistical analysis was performed using a one-way ANOVA test (Microcal Origin).

### Immunohistochemistry

TAG-1 immunostaining was performed using the 1C12 monoclonal antibody (IgG2a; Dodd *et al*., 1988). Sciatic nerve segments and DRGs were cryoprotected, embedded in OCT, cut into 16-μm sections on a cryostat, and mounted on Superfrost Plus slides (Gassalem, Limeil-Brevannes, France). Floating sections of spinal cord (40 μm) were also performed and kept in PBS at 4 °C.

Sections were incubated with primary antibodies in 5% goat serum or 2.5% non-fat dry milk in 0.1 M PBS overnight at room temperature, followed by incubation with appropriate peroxidase-labelled or fluorochrome-labelled secondary antibodies (Cappel/ICN, France). Several combinations of double immunofluorescence were performed by using the following antibodies: S-100 (DAKO, Trappes, France),

Krox-20 (Eurogenetec, Belgium), L1 (provided by F. Rathjen), IB4 (Sigma, France), NG2 (Euromedex, Souffelweyersheim, France), GFAP (DAKO, France), MAP1B (Fischer & Romano-Clarke, 1990) polyclonal antibodies, and ED1 (Serotec, Cergy St Christophe, France) mouse monoclonal antibodies, combined in some cases with Dapi nuclear costaining. Spinal cord sections adjacent to immunostained sections were Nissl-stained to evaluate the extension of the lesion. Sections were analysed on a standard light-microscope (Leica DMRB), and confocal (Leica TCS) microscope.

### In situ *hybridization*

The full-length (3.5 kb) rat TAG-1 cDNA was subcloned into pBluescript SK(+) vector (Furley *et al*., 1990), linearized, and transcribed using either T3 (for antisense riboprobe) or T7 (for sense riboprobe) RNA-polymerases according to the manufacturer's specification (Stratagene, France), in the presence of 2.5 μM $^{35}$S-UTP (1000 Ci/mmol, Amersham, Orsay, France).

DRG and spinal cord sections (16-μm thick) from fresh frozen tissues were prepared for *in situ* hybridization histochemistry, as previously described (Nothias *et al*., 1993). Briefly, mounted sections were fixed in 4% paraformaldehyde, rinsed in 2× standard saline citrate (SSC) buffer, acetylated, treated with Tris-glycine, rinsed in 2× SSC, dehydrated, and finally hybridized overnight at 55 °C in a humid chamber with $10^6$ c.p.m. of the $^{35}$S-TAG-1 mRNA probe. Post-hybridization procedures included washes in 50% formamide/2× SSC at 52 °C, treatment with RNase A, and rinses in 2× SSC buffer. Autoradiography was carried out using NTB-2 emulsion (Kodak, Integra Bioscience, Cergy-le-haut, France). Sections were counter-stained with haematoxylin/eosin, and examined using bright- and dark-field microscopy.

For non-radioactive *in situ* hybridization, a digoxigenin (dig)-UTP labelled RNA probe was synthesized according to the manufacturer's protocol (Boehringer–Mannheim; France). Sections were processed as described above, except that a digoxigenin antibody coupled to alkaline phosphatase was used for immunodetection (for more details see Stettler *et al*., 1995). Sections were examined using bright-field microscopy.

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### *Quantification of TAG-1 mRNA levels from* in situ *hybridization data*

Quantification of *in situ* hybridization signals on DRG neurons was performed as described previously (Smolen & Beaston-Wimer, 1990; Soares *et al*., 2002). Images of adult rat DRGs 15 days after sciatic nerve transection, hybridized with $^{35}$S-UTP TAG-1 riboprobe, were acquired under bright-field illumination at high magnification (×40), using a digital camera (CoolSnap, Princeton Instruments, Evry, France) coupled to an appropriate analysis system (Metamorph software, Princeton Instruments). At 150 μm intervals, 16-μm sections were taken through DRGs both ipsilateral (experimental), and contralateral (control) to the sciatic nerve lesion site; L4 and L5 DRGs were pooled. Cells were identified, their perimeter measured, and the labelling intensity per individual cell was standardized as number of reduced silver grains per 100 μm$^2$ cell surface. Cells were classified as small (< 600 μm), medium (between 600 and 1000 μm) and large (> 1000 μm). Finally, mean values for labelling intensities in relation to the different cell sizes were compared for control (left) and experimental (right) DRGs using an ANOVA test (Statview software).

## Results

### *TAG-1 expression in intact adult DRGs and spinal cord*

TAG-1 expression in the CNS and in DRG neurons during embryonic development has been described in detail (Yamamoto *et al*., 1986; Dodd *et al*., 1988; Karagogeos *et al*., 1991; Wolfer *et al*., 1994; Denaxa *et al*., 2003). While several studies reported an expression of TAG-1 mRNA in adult brain and spinal cord (Furley *et al*., 1990; Yoshihara *et al*., 1995; Wolfer *et al*., 1998), the specific distribution profile of TAG-1 protein expression in the adult nervous system was not described. As a first step, we performed a Western blot analysis on protein extracts isolated from adult spinal cord, and from P15 and adult DRGs (Fig. 1A). In both tissues, the main TAG-1 protein band of 135 kDa, described for developing nervous system, was found to be expressed. When the protein levels loaded are normalized with reference to actin (Fig. 1B), TAG-1 expression is seen to be at lower levels in adult DRG neurons as compared to P15 DRGs. In both adult DRGs and spinal cord, an additional band of 90 kDa is consistently detected. This band is specific as it was not detected on Western blots of CNS protein extract isolated from adult TAG-1 knockout mice (Traka *et al*., 2003). This could be a product of an adult-specific alternative splicing or proteolytic event. Another band of 110 kDa that is sometimes seen on immunoblots of adult spinal cord material, is probably non-specific (Dodd *et al*., 1988).

TAG-1 expression in the adult was further examined by immunohistochemistry and *in situ* hybridization performed on DRG sections. Strong TAG-1 immunostaining is observed in adult DRGs, with the highest labelling intensity located around cell bodies of DRG neurons (Fig. 2A and C) and along their peripherally projecting axons (Fig. 2A and D). In contrast, TAG-1 staining in the centrally projecting dorsal roots was rather weak (Fig. 2A and B). Likewise, TAG-1 is almost undetectable on the central DRG projections within the spinal cord (Fig. 6A). The difference in TAG-1 staining in peripherally vs. centrally projecting DRG axons is seen where they emerge from the cell body (Fig. 2C). It should be noted that TAG-1 staining appears as strong in the ventral roots as in the more distally located sciatic nerve (not shown).

Expression of TAG-1 by Schwann cells *in vivo* has recently been demonstrated using *in situ* hybridization on sciatic nerve sections

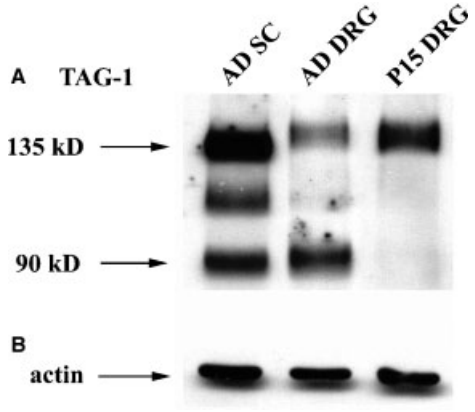


FIG. 1. TAG-1 protein continues to be expressed in the adult. (A) Western blot analysis shows that TAG-1 protein is expressed as a 135-kDa band in adult spinal cord (AD SC), in adult DRG neurons (AD DRG), and in P15 DRG. In addition, a lower molecular weight band (90 kDa) is detected in adult tissue, but it is absent from P15 DRG. (B) Actin levels were used to monitor protein quantities loaded in each lane. Sixty micrograms of total protein was loaded in each lane.

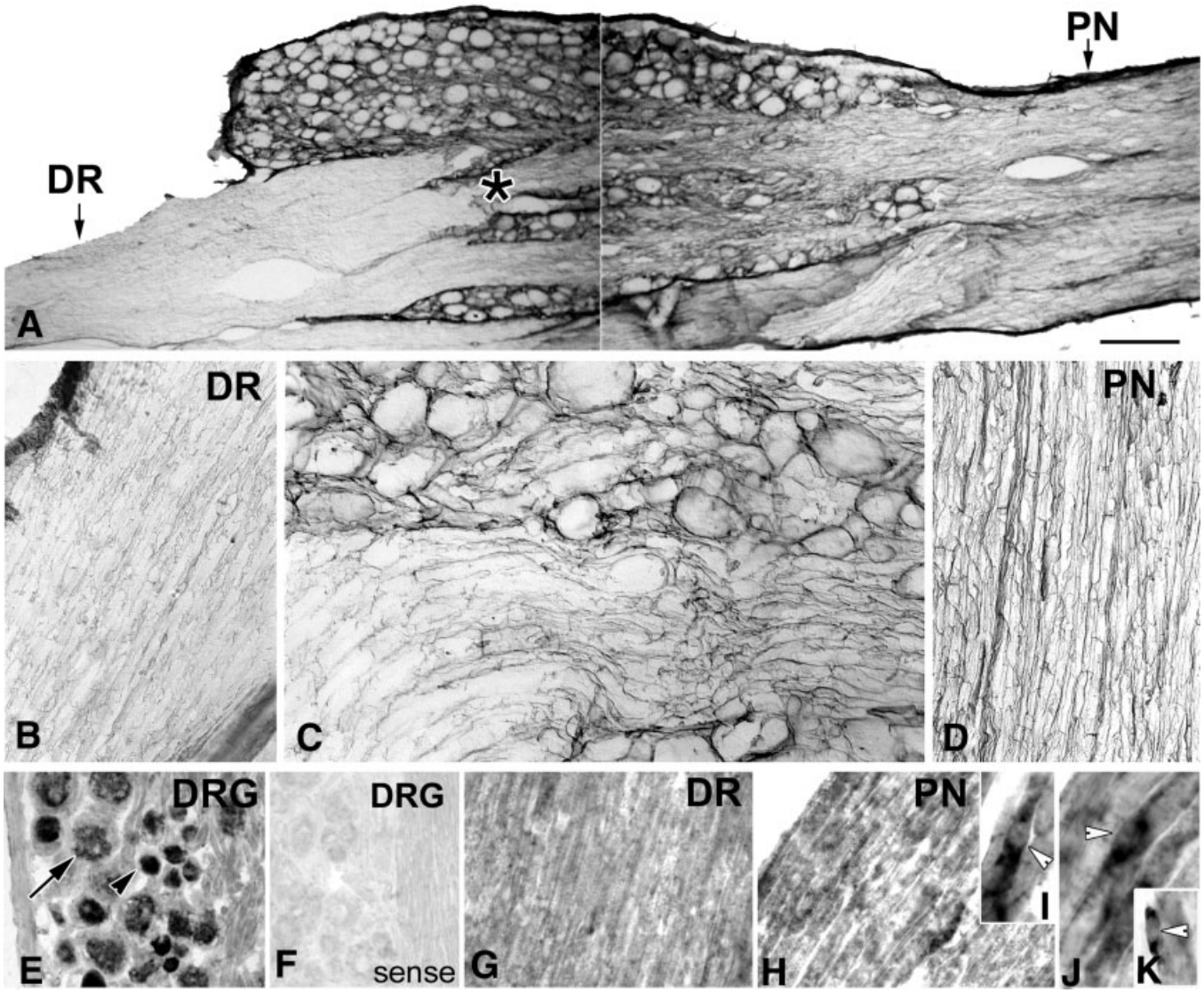


FIG. 2. TAG-1 expression in intact adult DRGs. (A–D) TAG-1 immunohistochemistry on intact adult DRG section at low (A), and high (B–D) magnification. Strong staining is observed around cell bodies of DRG neurons (A and C) and along their axons projecting into the peripheral nerve (PN; to the right in A and C, higher magnification in D. The dorsal root (DR), containing centrally projecting DRG axons, is weakly stained (to the left in A and C high magnification in B. (C) Higher magnification of the middle part of the DRG (A, star). (E–K) *In situ* hybridization for TAG-1 mRNA, using digoxigenin-labelled antisense (E and G–K), and sense (F) RNA probes. TAG-1 mRNA is detected in DRG neurons (E), and also in cells along their axons in the dorsal root (DR; G), and in the sciatic nerve (PN; H, and high magnification in I–K). Note that the hybridization signal appears on DRG section in (E) higher in large size (arrow) than in small size (arrowhead) neurons, and also higher along the peripheral vs. the central projections. Scale bar in A corresponds to all photographs as follow: 180 μm (A); 90 μm (B, D, G and H); 45 μm (C); 16 μm (E and F) and 30 μm (I–K).

(Traka *et al*., 2002). Therefore, it seemed likely that the TAG-1 immunostaining seen on DRG sections reflected both neuronal and glial expression. Indeed, our *in situ* hybridization data show that TAG-1 mRNA is present in both DRG neurons (Figs 2E and 4A), and cells associated with DRG axons in the peripheral nerve (Fig. 2H–K), which exhibit the Schwann cell morphology (Fig. 2I–K). Interestingly, the TAG-1 hybridization signal was significantly less intense in cells localized in the dorsal roots (Fig. 2H vs. G), as described above by immunohistochemistry for the protein. In the ventral roots, the hybridization signal appeared as high as in the peripheral nerve.

We then wanted to confirm that the TAG-1 immunoreactivity detected in the fibre tracts was indeed due to the presence of the protein in both DRG axons and in Schwann cells. First, we performed a double immunostaining for TAG-1 and the glial cell marker S-100, which showed individual TAG-1-positive cells in the nerve to be of glial origin (Fig. 3A–C). Second, it had been reported by Traka *et al*. (2002) that TAG-1 does not colocalize with MAG (myelin-associated glycoprotein), although it is present at the juxtaparanodal region of myelinated fibers in adult PNS. However, this observation does not exclude the possibility of TAG-1 to be expressed by myelinating Schwann cells. We therefore performed double-labelling experiments for TAG-1 and the krox-20 transcription factor, known be specifically expressed by myelinating Schwann cells (Topilko *et al*., 1994). This immunostaining showed that both TAG-1 and krox-20 proteins are colocalized in individual cells of the sciatic nerve (Fig. 3D–I). To confirm expression of TAG-1 by myelinating Schwann cells in a peripheral nerve (such as the sciatic nerve), krox-20 may in fact be better suited than other currently used Schwann cell markers, such as

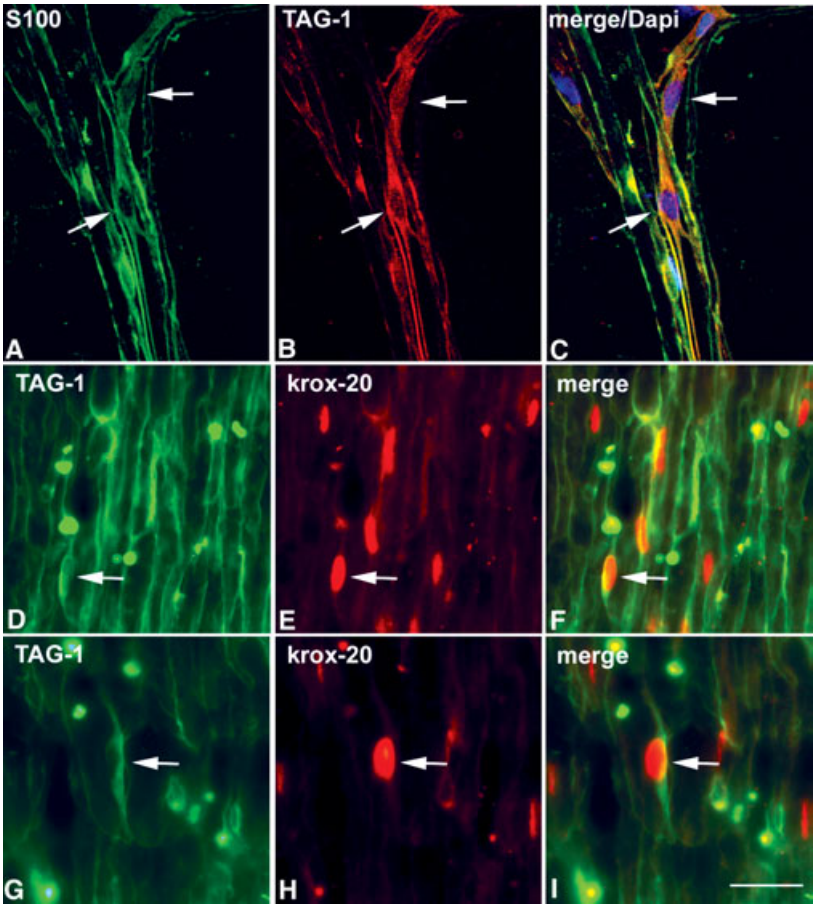


FIG. 3. TAG-1 immunostaining in adult sciatic nerve. TAG-1 (B, D and G) is present in Schwann cells, identified by double-immunofluorescence staining for S-100 (A; confocal images of Schwann cells along teased fibers), and krox-20 (E and H). Schwann cell bodies are also visualized by nuclear staining with DAPI in merge figure (C) of A and B. F and I are, respectively, the merge images of D and E, and G and H. Arrows mark reference point cell bodies seen on all images within the same row. Scale bar, 30 μm.

S-100 and L1. Indeed, although in the peripheral nervous system, S-100 and L1 are mainly detected in glial cells, both proteins are also expressed by adult DRG neurons and are present in their axons. From our own analysis (not shown) and as previously published, S-100 is expressed by large DRG neurons, and L1 by small DRG neurons, indicating that these two proteins are localized in thick myelinated and thin unmyelinated sciatic nerve axons, respectively (Vega *et al*., 1991; Haney *et al*., 1999; Akopians *et al*., 2003).

TAG-1 can act through a homophilic, as well as a heterophilic binding mechanism. Following the demonstration of its presence at juxtaparanodal regions of myelinated fibers, we asked whether TAG-1 was preferentially expressed by those DRG neurons whose axons are myelinated. DRGs are composed of heterogeneous neuronal populations that differ in function, neurotransmitter expression, and size; small cells give rise to unmyelinated, medium size to thinly myelinated, and large cells to myelinated fibers (for more details see Nothias *et al*., 1993). To compare expression levels of TAG-1 mRNA in these different DRG cell groups, we used an *in situ* hybridization protocol with radiolabelled probes. Quantification (Nothias *et al*., 1993) of the $^{35}$S-riboprobe hybridization signal shows that TAG-1 mRNA is indeed differentially expressed among adult DRG neurons. Surprisingly, the smallest neurons are the most intensely stained (relative number of reduced silver grains; Fig. 4A and B). In comparison to the small cells, the TAG-1 mRNA hybridization signal is 50% less intense in large cells, 25% in medium size cells (Fig. 4B).

Thus, although TAG-1 is expressed by all DRG neurons, the highest expression levels are found in small DRG neurons that give rise to unmyelinated fibers.

### *TAG-1 expression after a peripheral lesion*

As TAG-1 plays a role during development in axonal outgrowth and pathfinding, we examined its potential function in axonal plasticity in the adult. Thus, we analysed the regulation of its expression during sciatic nerve lesion-induced axonal regeneration in adult rats. In this well-characterized experimental model, the right (experimental) sciatic nerve is axotomized, inducing, after several days, the formation of regenerative growth cones by the lesioned axons. If proximal and distal portions of the nerve are re-apposed, the axotomized neurons can regenerate their axons and re-establish functional connections, a process which involves up-regulation of developmental growth promoting proteins such as L1 and NCAM (Martini & Schachner, 1988). Using *in situ* hybridization with a radioactive riboprobe, we examined the TAG-1 mRNA expression in regenerating DRG neurons at three and 15 days postlesion, i.e. during the period of active regeneration. At 3 days (data not shown), there was no significant change in TAG-1 mRNA expression levels, but a decrease in all cell populations was detected at 15 days (Fig. 4A and B). Although not highly significant at this postlesion stage, this decrease, of

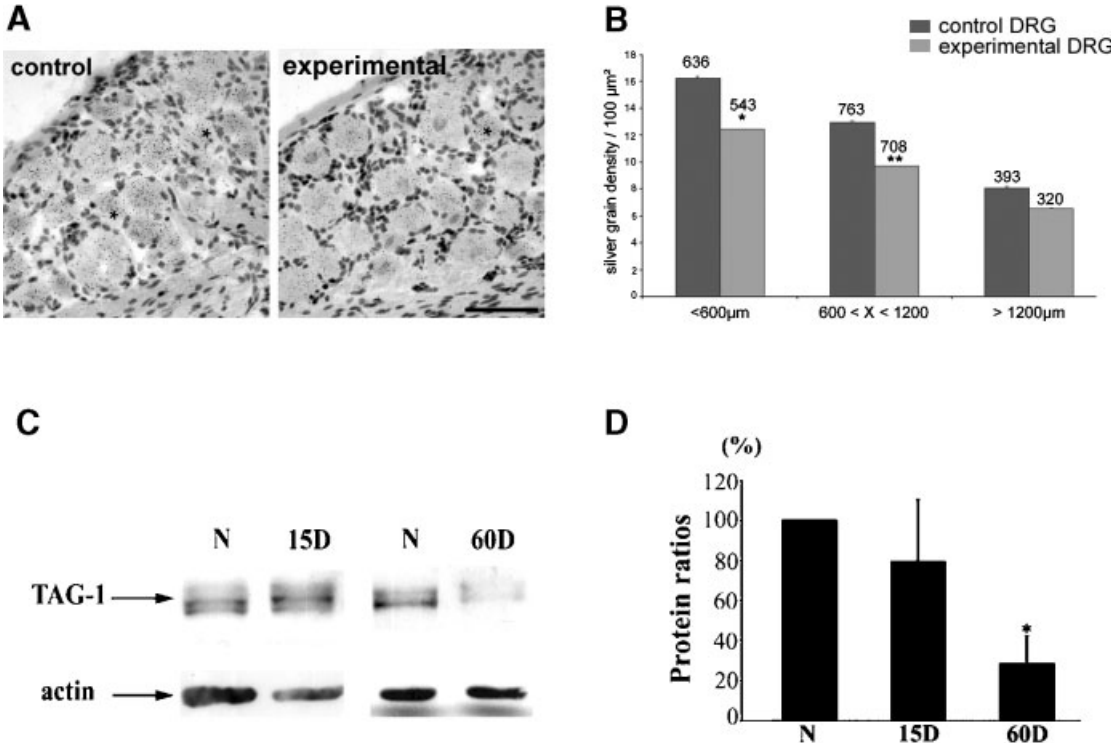


FIG. 4. Regulation of TAG-1 after a sciatic nerve lesion. (A) TAG-1 mRNA expression in the L5 DRG 15 days postlesion (left, control; right, experimental). Sections were radiolabelled with a $^{35}$S-labelled TAG-1 riboprobe, and counterstained with haematoxylin/eosin. Small neurons (asterisks) are the most intensely stained, as confirmed in B. Scale bar, 75 µm (B). Relative levels of TAG-1 mRNA hybridization signals in L4 and L5 DRGs (pooled) in small, medium and large neurons. At 15 days postlesion, the hybridization signal decreases by 20–24% in all three cell populations (**$P < 0.01$, ANOVA test. The number of cells evaluated is shown at the top of each column). (C and D) TAG-1 protein expression in DRGs at 15 days (15D) and 60 days (60D) after sciatic nerve lesion. N represents the intact/control DRG. (C) Representative Western blots; at 60 days postlesion, TAG-1 is expressed by regenerating DRGs at significantly lower levels than by contralateral control side DRGs (N). (D) Quantitative analysis; TAG-1 levels of regenerating DRGs 15 and 60 days postlesion, normalized to levels of the contralateral (N) DRG. Bars represent SEM. The decrease in TAG-1 expression in regenerating DRGs after 60 days is statistically significant (*$P < 0.05$, one-way ANOVA test).

approximately 20–24%, occurred in DRG neurons of all sizes. The small size neurons still displayed the highest signal (Fig. 4B).

Thus, unlike L1 and NCAM, TAG-1 appears down-regulated in regenerating adult DRG neurons, during the first 15 days after axotomy.

Using the same experimental paradigm, we then analysed the expression levels of TAG-1 protein on Western blots prepared from lysates of regenerating L4 and L5 DRG neurons at 15 and 60 days after sciatic nerve cut and re-apposition. Figure 4C shows an example of a Western blot from L5 DRGs, used to determine the staining intensities of the 135 kDa TAG-1 band under the different conditions. Statistical analysis was performed on data pooled from both L4 and L5 DRGs (Fig. 4D). No statistically significant change in TAG-1 protein expression was observed after 15 days. However, in regenerating DRGs 60 days postlesion, TAG-1 protein levels appeared significantly reduced (Fig. 4C and D), corroborating our *in situ* hybridization data.

The distribution of TAG-1 protein in DRGs at 15 days postlesion did not show any apparent changes in comparison to controls (not shown). In contrast, at the lesion site undergoing active regeneration (Fig. 5B), TAG-1 immunostaining was highly increased compared to the intact contralateral nerve, or to the proximal nerve region (close to the DRGs; Fig. 5A). Double labelling for S-100 and TAG-1 indicated expression of TAG-1 also in reactive Schwann cells at the lesion site (Fig. 5D–G). As in this area it was difficult to distinguish individual cells because of the high cell density and the presence of fibre debris, the expression of TAG-1 at the lesion site was further confirmed by *in situ* hybridization (Fig. 5C). In addition, TAG-1 is also expressed in reactive Schwann cells purified from cultured adult nerve segments undergoing Wallerian degeneration *in vitro* (Fig. 5H–M). In these cells, TAG-1 is indeed colocalized with S-100 (Fig. 5K–M), and with the microtubule-associated protein 1B (MAP1B; Fig. 5H–J), known to be re-induced in reactive Schwann cells *in vivo* after sciatic nerve section (Ma *et al.*, 1999).

In summary, axotomy of DRG neurons at the peripheral nerve is followed by a down-regulation of TAG-1 in injured neurons, while the same protein is increased in reactive Schwann cells at the lesion site.

### *TAG-1 expression and distribution is associated to spinal cord neurodegeneration*

Regulation of TAG-1 expression in adult CNS was further examined in another experimental model of lesion-induced plasticity, excitotoxin-provoked spinal cord neurodegeneration. This model allowed us to analyse potential changes in the distribution of TAG-1 in the area of the central projections of DRG neurons after elimination of their targets. Through injection of an excitotoxin such as kainic acid *in situ*, it is possible to obtain a spinal cord neurodegeneration without, except along the needle trajectory, creating additional mechanical lesions like those seen after spinal cord trauma (Nothias & Peschanski, 1990).

In intact adult spinal cord, TAG-1 immunostaining is rather weak in comparison to adult DRGs, although on Western blots the amount of TAG-1 protein seems rather high (Fig. 1). This is likely due to the fact that not all neuronal and glial cells in spinal cord express TAG-1. Indeed, as reported previously (Traka *et al.*, 2002), TAG-1 expression

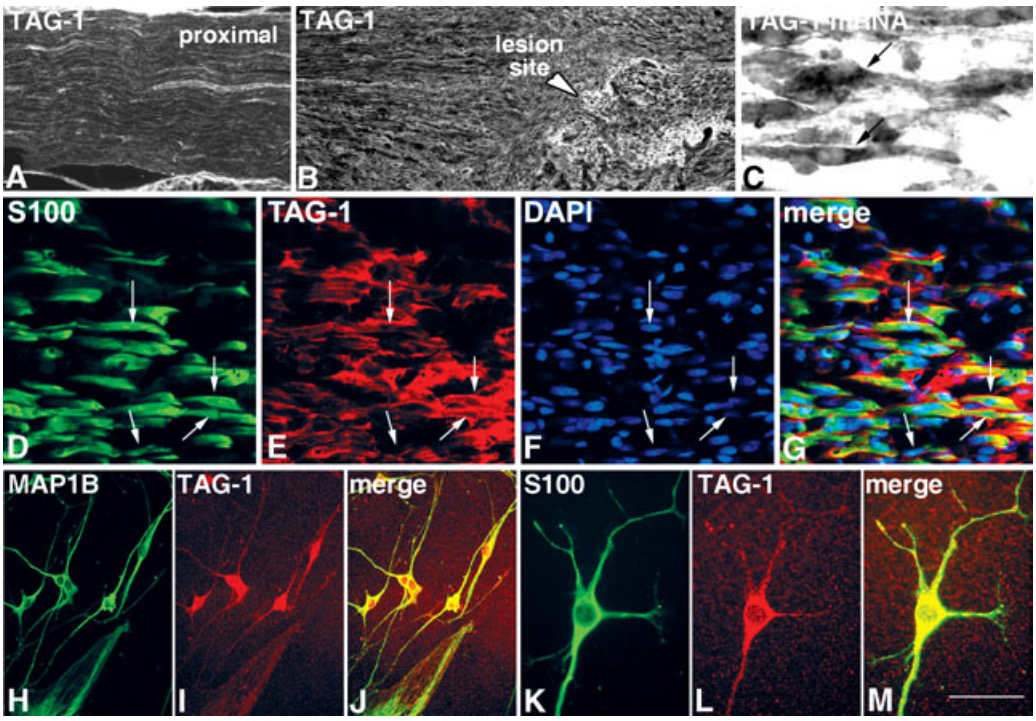


FIG. 5. TAG-1 immunohistochemistry in transected adult sciatic nerve (15 days postlesion). (A) Black and white photomicrographs showing low TAG-1 staining near the DRG (proximal) (B) but strongly increased at the lesion site (arrowhead). (C) *In situ* hybridization for TAG-1 mRNA with digoxigenin-labelled RNA antisense probe at the lesion site, showing hybridization signal within individual cells (arrows) that exhibit Schwann cell morphology. (D–G) Double immunofluorescence of S-100 (D) and TAG-1 (E), and Dapi nuclear staining (F) at sciatic nerve lesion-site, showing colocalization in individual cells (arrows). G corresponds to the merge image of D–F. (H–M) TAG-1 (I and L) double immunofluorescence on purified Schwann cells cultured from adult nerves (H) with MAP1B (K) with S-100. J and M are, respectively, the merge images of H and I, and K and L. Scale bar, 185 μm (A and B); 20 μm (C); 23 μm (D–G); 60 μm (H–J) and 12 μm (K–M).

in the adult spinal cord is detected only in motoneurons in the ventral horn (see also Fig. 6A, F and G both for protein and mRNA, respectively), and in oligodendrocytes in the white matter (*in situ* hybridization Fig. 6F). A faint immunostaining is also detected around the dorsal and ventral root entry points into the spinal cord.

Injection of kainic acid into the spinal cord provoked a degeneration of all neurons in the diffusion area of the excitotoxin in the grey matter (Fig. 6H; see also Nothias & Peschanski, 1990). With time, the lesion site underwent shrinkage, as it was occupied only by afferent fibers and non-neuronal cells. In some cases, the dorsal horn was spared by the neurodegeneration and did not show shrinkage (see Fig. 6), as in accordance with our previous report (Nothias & Peschanski, 1990), the neurons in this area are more resistant to kainic acid excitotoxicity than motoneurons.

Neuronal loss after kainic acid injection is followed by an increase in TAG-1 immunostaining that, in the spinal cord, is restricted to the neuron-depleted area (Fig. 6B). At higher magnification, this immunostaining is seen associated with fibers (Fig. 6C and D), and small cells (Fig. 6E). In addition, TAG-1 levels appeared to be increased in the dorsal and ventral roots (Fig. 6B). These results are corroborated by *in situ* hybridization data, showing TAG-1 mRNA signals in numerous small cells located both in the neuron-depleted spinal cord, and in the dorsal and ventral roots (Fig. 6I–K).

To identify the phenotype of the non-neuronal TAG-1 positive cells within the neurotoxic lesion, we performed a series of double-staining experiments. A likely candidate cell type to express TAG-1 was Schwann cells, as we had previously observed that these peripheral glial cells migrated into the central nervous system in case of a spinal cord neurodegeneration (Nothias & Peschanski, 1990). Indeed, Fig. 7G–I shows for certain cells a colocalization of TAG-1 with the Schwann cell marker S-100. However, the overall number of TAG-1 mRNA expressing cells appeared higher than what would have been expected from our analysis on Schwann cell invasion into the excitotoxic spinal cord lesion, suggesting that other non-neuronal cells might also express TAG-1. A second candidate cell type was activated macrophages/microglia, known to massively invade the neuron-depleted area. Using the ED1 antibody as a marker for these cells, we observed strong immunostaining associated to numerous cells located in the lesion site, and interestingly, also in both dorsal and ventral roots (Fig. 7J and K). As both TAG-1 and ED1 antibodies used in the present study were developed in the same species, the isolectin-directed IB4 antibody, another marker for microglia/macrophages, was used to confirm the presence of TAG-1 in this cell population (Fig. 7L–N). Furthermore, a subpopulation of activated macrophages/microglia within the excitotoxic lesion site has also been shown to transiently express the NG2 proteoglycan, normally known as oligodendroglial progenitor marker (reviewed in Dawson *et al*., 2000). These cells that coexpress ED1 and NG2 antigens invade the lesion by 2 weeks (Bu *et al*., 2001). Double-immunofluorescence staining revealed a high colocalization of TAG-1 with NG2 within cells located in the neuron-depleted area that displayed large rounded cell bodies and short processes (Fig. 7O–Q). NG2-positive cells outside the lesion, however, exhibiting the typical features of oligodendrocyte progenitors, were apparently not positive for TAG-1 (not shown). The same was true for reactive astrocytes, around the lesion site, as no colocalization of GFAP and TAG-1 was observed (not shown).

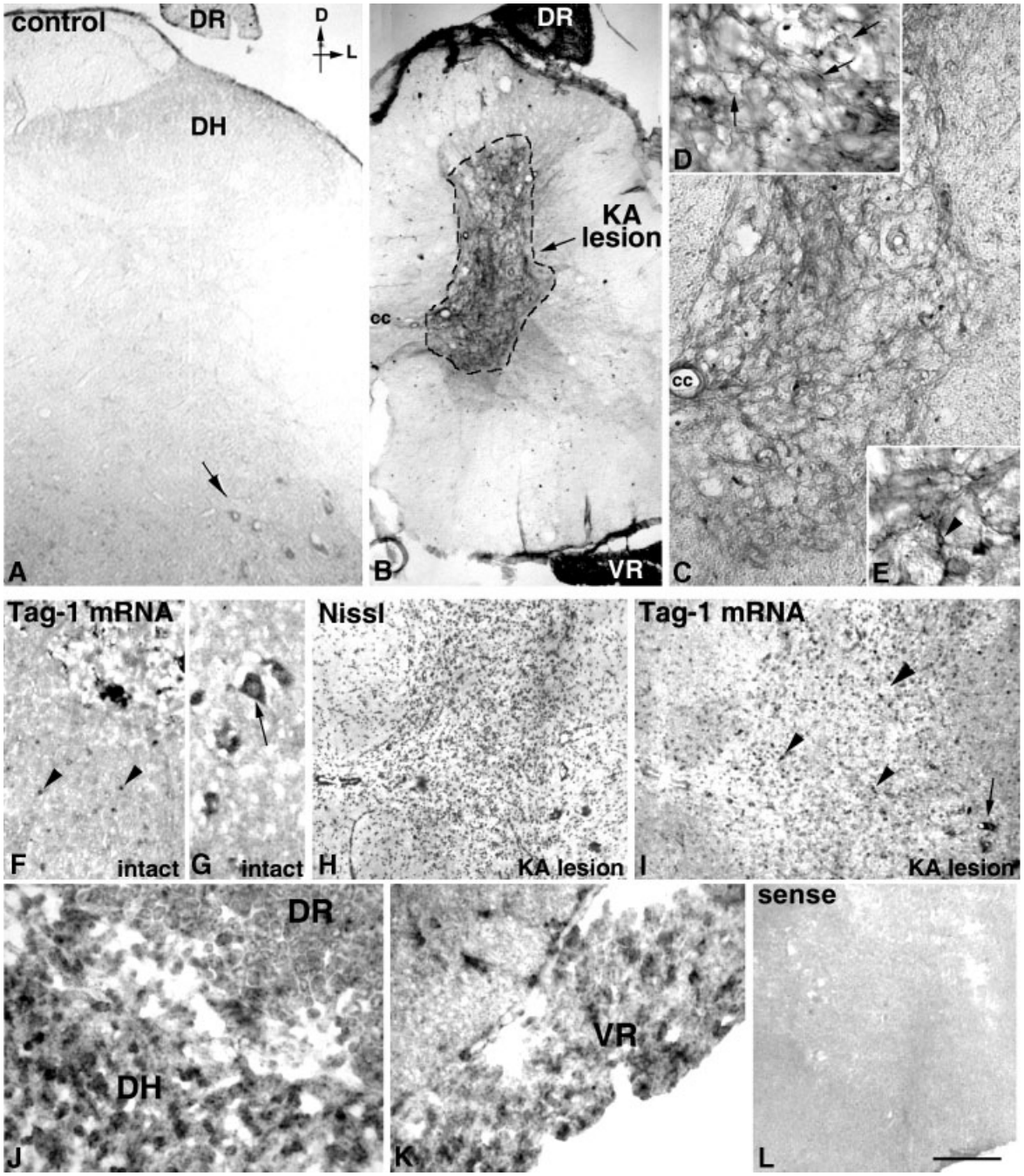


FIG. 6. TAG-1 expression in the spinal cord after a neurodegenerative lesion, 14 days after kainic acid (KA) injection into spinal cord. (A) In intact spinal cord, motoneurons are immunoreactive for TAG-1 (arrow). (B–E). In the neuron-depleted lesioned spinal cord TAG-1 immunostaining is induced both on fibers (C and D, arrows in D) and cells (E, arrowhead). Strong increases are also observed in B, in both dorsal (DR) and ventral (VR) roots. cc, central canal; DH, dorsal horn; D, dorsal; L, lateral. (H) Nissl staining on a section adjacent to I, the kainic acid lesion is characterized by neuronal loss (absence of large cell bodies), and large numbers of densely packed non-neuronal cells. (F, G and I–K) *In situ* hybridization with a digoxigenin-labelled TAG-1 antisense riboprobe. (F) In intact ventral horn, a hybridization signal is observed over non-neuronal cells (arrowheads) in white matter, and G over motoneurons (arrow). (I) After kainic acid injection, a specific hybridization signal appears on a subpopulation of glial cells (arrowheads) in the neuron-depleted area. Surviving motoneurons outside the lesion are also stained (arrow in I). A specific hybridization signal is also detected in numerous cells in J dorsal horn (DH) and dorsal root (DR), and K, ventral root (VR). L shows an intact spinal cord section at the level of the ventral horn, hybridized with a TAG-1 sense riboprobe for control. Scale bar, 110 μm (A); 170 μm (B); 100 μm (C); 80 μm (D and E); 45 μm (F and G); 60 μm (H, I and L) and 50 μm (J and K).

Interestingly, kainic acid-induced spinal cord neurodegeneration also affected the expression of TAG-1 within the dorsal roots. Thus, the labelling intensity ipsilaterally to the lesion site was increased in comparison to DRGs isolated from an intact animal (Fig. 2). Centrally projecting fibers within the dorsal roots now exhibited a staining intensity similar to that of the peripheral projections. As shown in Fig. 7D–F, MAP1B-immunostained DRG fibers at the dorsal root entry point into lesioned spinal cord were also TAG-1 positive, while in dorsal roots from intact animals no colocalization was noted (Fig. 7A–C).

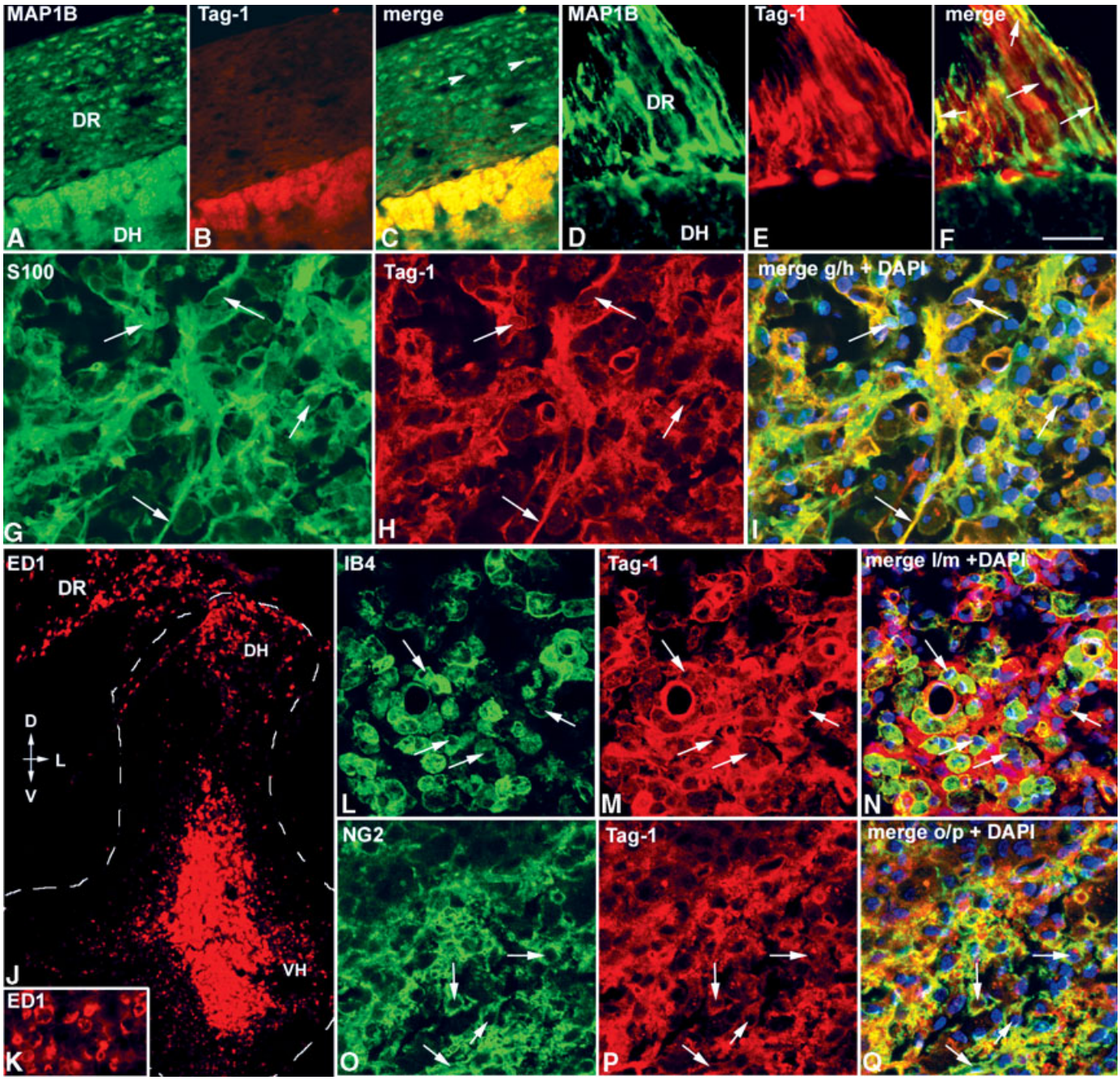


FIG. 7. TAG-1 double immunostaining in the spinal cord 14 days after kainic acid (KA) injection. (A–F) Colocalization of TAG-1 with MAP1B in the dorsal roots at their entry into the spinal cord; (A–C) intact animal, no TAG-1 (B) immunostaining is detectable within MAP1B (A) positive fibers (arrowheads), while in the experimental animal (D–F), a colocalization of the two markers is noted (arrows); C and F are, respectively, the merge images of A and B, and D and E. (G–I) Confocal images of S-100 (G) and TAG-1 (H) double immunofluorescence showing high colocalization of the two markers (arrows); I is the merge image of G and H, with nuclear DAPI staining. (J and K) Simple immunostaining for ED1 showing numerous microglia/macrophages that have invaded the lesioned spinal cord (K is a higher magnification of J). (I–N and O–Q) Confocal images of double immunostaining of TAG-1 (M and P) with IB4 (L) and NG2 (O), respectively, showing TAG-1 colocalization with both markers within the same cells (arrows). Dapi nuclear staining (blue colour) is added in merge photos N of L and M, and Q of O and P. Scale bar, 60 μm (A–F); 30 μm (G–I and L–Q); 140 μm (J) and 35 μm (K). D, dorsal; V, ventral; L; lateral; DR, dorsal root; DH, dorsal horn; VH, ventral horn.

## Discussion

### *TAG-1 expression in adult PNS and CNS*

While generally down-regulated in the adult nervous system, TAG-1 mRNA expression is maintained in certain areas of adult mammalian brain and spinal cord (Furley *et al*., 1990; Yoshihara *et al*., 1995), in particular those displaying cellular and structural plasticity (Wolfer *et al*., 1998). TAG-1 protein has also been detected in adult rat retinal ganglion cells (Jung *et al*., 1997), but the specific expression and distribution pattern of TAG-1 protein elsewhere in adult nervous system has not yet been described. The present study was intended to provide a detailed analysis of TAG-1 protein and mRNA expression in

adult rat DRGs and spinal cord, both in intact animals, and after mechanical or neurodegenerative lesions. We show that in both tissues, TAG-1 continues to be expressed as a 135-kDa protein, thus exhibiting the same apparent molecular weight as during development. An additional specific TAG-1 related band of 90 kDa is found exclusively in adult tissue, although no obvious splicing sites were detected in TAG-1 mRNA in the developing nervous system (Furley *et al*., 1990). This band is likely to be a proteolytic product of the 135-kDa protein, or a product of an adult-specific alternative splicing event, as it is absent in protein extracts from TAG-1 knockout brain (Traka *et al*., 2003).

Our immunohistochemistry data show the presence of TAG-1 protein on adult DRG neurons and along their centrally and peripherally projecting axons. Immunostaining is also present in spinal cord, notably in the ventral horn, associated with motoneurons and their axons in the ventral root. *In situ* hybridization confirmed TAG-1 expression by motoneurons, and also by oligodendrocytes (Traka *et al*., 2002; present study) but not by astrocytes, as TAG-1 was not colocalized with GFAP.

TAG-1 mRNA expression is detected in all adult DRG neurons. This has also been recently shown in adult mice, by using a tau-*lac*Z marker construct under the control of the TAG-1 promotor (Poliak *et al*., 2003). In addition, we show here that TAG-1 expression differs according to the size of DRG neurons, i.e. mRNA levels in small neurons are two-fold higher than in large neurons.

Expression of TAG-1 by large DRG neurons, giving rise to myelinated axons, corroborates recent reports on its expression in myelinated fibers where it associates with Caspr2. This interaction seems crucial for axon–glia interactions in that it is implicated in the molecular organization of juxtaparanodal regions of myelinated fibers (Poliak *et al*., 2003; Traka *et al*., 2003). These studies suggested a similar model in which neuronal TAG-1 would *cis*-interact with Caspr2, and *trans*-interact with glial TAG-1 (homophilic binding). The function of TAG-1 in small DRG neurons, whose axons are unmyelinated, remains to be elucidated. It is interesting to note that expression of L1, another Ig superfamily protein that binds to TAG-1 via a heterophilic interaction, is also maintained in adult peripheral nervous system, in particular in Schwann cells and small DRG neurons (Haney *et al*., 1999). In addition, L1 expressed by axons rather than Schwann cell is essential for adhesion, ensheathing, and viability of unmyelinated sensory fibers (Haney *et al*., 1999). Thus, in view of the colocalization of L1 and TAG-1 in peripheral nerves (Traka *et al*., 2002; our observation, not shown), we suggest that TAG-1 is also implicated in Schwann cell interactions with unmyelinated axons, probably through heterophilic biding with L1.

A particularly interesting result of our study concerns the presence of high levels of TAG-1 mRNA and protein in Schwann cells associated with peripheral DRG projections, while labelling in dorsal roots is much less intense. Because TAG-1 is coexpressed with krox-20 in myelinating Schwann cells, it may be related to the myelinating potential of adult Schwann cells. In addition, the percentage of myelinated axons in dorsal roots is much lower than in peripheral nerve or in ventral roots. Our observations on a differential expression of TAG-1 between dorsal root and peripheral nerve are an indication for a structural and molecular difference between these two DRG-derived nerves. This is in accordance with previous studies of Suh *et al*. (1984) and Lee e*t al*. (1986) that showed that peripherally projecting DRG neurites are different from the centrally projecting with respect to their axonal diameter, myelin thickness, and conduction velocity.

### *TAG-1 expression is down-regulated in DRG neurons during active peripheral nerve regeneration but not in Schwann cells*

During the first days following a peripheral nerve lesion, TAG-1 is obviously not up-regulated in DRG neurons undergoing regeneration. This finding does not exclude the importance of this protein for regeneration, as the persistence of TAG-1 expression might be sufficient to support axonal regrowth, as it has been proposed for L1 (Zhang *et al*., 2000). In addition, there is also a released form of this glycoprotein, which could account for the local increase in both protein and mRNA labelling for TAG-1 in association with reactive Schwann cells at the lesion site. Regeneration-induced regulation of TAG-1 expression has been studied in another experimental model, axotomized rat optic nerve (Jung *et al*., 1997). In that study, axotomized retinal ganglion cells (RGCs) showed a down-regulation of TAG-1 that was not compensated by the positive influence of a peripheral nerve graft on axonal regeneration. However, expression of other growth-associated proteins, such as GAP-43 and L1, was maintained or increased after optic nerve injury (Jung *et al*., 1997), as in peripheral nerve (Tetzlaff *et al*., 1989). After peripheral nerve injury, functional recovery is not always complete for axotomized DRGs either, because their axons are often misrouted within the endoneurium of the distal nerve stump. As a result, continuous axonal sprouting occurs in the target area, in an attempt to regenerate DRG neurons to re-establish proper peripheral connections (Fu & Gordon, 1997). The observed down-regulation of TAG-1 expression in DRGs at later stages of regeneration may thus be due to a lack of specific signals related to the absence of proper target connections.

### *TAG-1 up-regulation in a neurodegenerative lesion*

Death of spinal cord neurons, induced by the neurodegenerative lesion paradigm used in our study, results in an increase in TAG-1 protein and mRNA levels in both dorsal roots and spinal cord. The cells expressing TAG-1 mRNA within the lesioned area are non-neuronal (see Discussion in Nothias & Peschanski, 1990). The neurodegenerative lesion site is invaded by macrophages, microglia, reactive astrocytes, and in addition, by Schwann cells from the periphery, both in brain (Dusart *et al*., 1989) and spinal cord (Nothias & Peschanski, 1990). Axons spared by the lesion undergo a rapid demyelination and re-myelination process (over one month postlesion; Dusart *et al*., 1992), in which both oligodendrocytes and Schwann cells participate by ensheathing and myelinating available CNS axons. The invasion by peripheral Schwann cells probably originates at the perivascular space around large blood vessels after disruption of the glia limitans, and subsequently follows smaller capillaries (Dusart *et al*., 1992). In spinal cord with a disrupted glia limitans, Schwann cells, as revealed here by TAG-1/S-100 double staining, may also arrive from the dorsal and ventral roots, corresponding to regions that after KA injection exhibit high amounts of both TAG-1 protein and mRNA. However, cells expressing TAG-1 mRNA are not exclusively Schwann cells. As we demonstrate here, TAG-1 protein is also localized on reactive macrophages/microglia, cells that invade the excitotoxic lesion during the first two weeks and which are transiently expressing the NG2 proteoglycan (Bu *et al*., 2001). Oligodendrocytes may undergo apoptosis and are not detected in the lesion (Jamin *et al*., 2001). The surviving oligodendrocytes are found around the lesion site, together with reactive astrocytes (Jamin *et al*., 2001), on which TAG-1 immunostaining was undetectable (present study).

In addition to this non-neuronal cell response, the axon terminals of target-deprived afferents undergo morphological modifications (Peschanski & Besson, 1987). Some may form regenerative growth

cones that, after transplantation of fetal neurons, are able to connect to new postsynaptic targets (Peschanski & Isacson, 1988; Nothias & Peschanski, 1990). This axonal re-organization is different from that observed after traumatic CNS lesions, where regeneration is inhibited by the molecular environment and glial scar formation. In contrast, a release of trophic factors has been demonstrated in the excitotoxic lesion (Manthorpe *et al*., 1983; Mahy *et al*., 1996; Widenfalk *et al*., 2001). Schwann cells are a known source of trophic factors promoting survival and growth of both peripheral and central axons. In addition, ensheathing of central axons by Schwann cells may account for their survival and terminal re-modelling, including formation of regenerative growth cones (reviewed in Ide, 1996; Terenghi, 1999), in which expression of some developmental cytoskeletal protein such as MAP1B is re-capitulated (Soares *et al*., 1998). As we show here, TAG-1 is another developmentally regulated protein being induced in these fibers.

In summary, our results demonstrate a local expression of TAG-1 in non-neuronal cells, following both mechanical and neurotoxic lesions. Furthermore, neuronal expression is also noted, particularly in axons that project into the peripheral or central lesions. Thus, the observed local increase in TAG-1 protein can be attributed to its increased expression by neurons and glia. TAG-1 protein is an Ig superfamily member that exists in a GPI-anchored and/or a secreted form (see Introduction). Therefore, at lesion sites, TAG-1 is associated with membranes of cells and axons, and may also be released to diffuse locally. Concerning its expression by isolated cells within the lesions we were able to identify at least two TAG-1 positive non-neuronal cell types, Schwann cells and macrophages/microglia.

Taken together, it seems likely that TAG-1 will play a role during structural re-organization after injury that is similar to its role during development. We suggest that TAG-1 is implicated in axon–glia interactions, axonal re-modelling, glial cell migration into the lesioned area, and in the demyelination/re-myelination process.

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## Abbreviations

CNS, central nervous system; DRG, dorsal root ganglia; KA, kainic acid solution; PNS, peripheral nervous system.

## References

Akopians, A., Runyan, S.A. & Phelps, P.E. (2003) Expression of L1 decreases during postnatal development of rat spinal cord. *J. Comp. Neurol.*, **467**, 375–388.

Bu, J., Akhtar, N. & Nishiyama, A. (2001) Transient expression of the NG2 proteoglycan by a subpopulation of activated macrophages in an excitotoxic hippocampal lesion. *Glia*, **34**, 296–310.

Buchstaller, A., Kunz, S., Berger, P., Kunz, B., Ziegler, U., Rader, C. & Sonderegger, P. (1996) Cell adhesion molecules NgCAM and axonin-1 form heterodimers in the neuronal membrane and cooperate in neurite outgrowth promotion. *J. Cell Biol.*, **135**, 1593–1607.

Dawson, M.R.L., Levine, J.M. & Reynolds, R. (2000) NG2-Expressing cells in the central nervous system: are they oligodendroglial progenitor? *J. Neurosci. Res.*, **61**, 471–479.

Denaxa, M., Chan, C.H., Schachner, M., Parnavelas, J.G. & Karagogeos, D. (2001) The adhesion molecule TAG-1 mediates the migration of cortical interneurons from the ganglionic eminence along the corticofugal fiber system. *Development*, **128**, 4635–4644.

Denaxa, M., Pavlou, O., Tsiotra, P., Papadopoulos, G.C., Liapaki, K., Theodorakis, K., Papadaki, C., Karagogeos, D. & Papamatheakis, J. (2003) The upstream regulatory region of the gene for the human homologue of the adhesion molecule TAG-1 contains elements driving neural specific expression *in vivo*. *Brain Res. Mol Brain Res.*, **118**, 91–101.

Dodd, J., Morton, S.B., Karagogeos, D., Yamamoto, M. & Jessell, T.M. (1988) Spatial regulation of axonal glycoprotein expression on subsets of embryonic spinal neurons. *Neuron*, **1**, 105–116.

Dusart, I., Isacson, O., Nothias, F., Gumpel, M. & Peschanski, M. (1989) Presence of Schwann cells in neurodegenerative lesions of the central nervous system. *Neurosci. Lett.*, **105**, 246–250.

Dusart, I., Marty, S. & Peschanski, M. (1992) Demyelination, and remyelination by Schwann cells and oligodendrocytes after kainate-induced neuronal depletion in the central nervous system. *Neuroscience*, **51**, 137–148.

Fischer, I. & Romano-Clarke, G. (1990) Changes in microtubule-associated protein MAP1B phosphorylation during rat brain development. *J. Neurochem.*, **55**, 328–333.

Fu, S.Y. & Gordon, T. (1997) The cellular and molecular basis of peripheral nerve regeneration. *Mol. Neurobiol.*, **14**, 67–116.

Furley, A.J., Morton, S.B., Manalo, D., Karagogeos, D., Dodd, J. & Jessell, T.M. (1990) The axonal glycoprotein TAG-1 is an immunoglobulin superfamily member with neurite outgrowth-promoting activity. *Cell*, **61**, 157–170.

Haney, C.A., Sahenk, Z., Li, C., Lemmon, V.P., Roder, J. & Trapp, B.D. (1999) Heterophilic binding of L1 on unmyelinated sensory axons mediates Schwann cell adhesion and is required for axonal survival. *J. Cell Biol.*, **146**, 1173–1184.

Hoffman, K.B. (1998) The relationship between adhesion molecules and neuronal plasticity. *Cell Mol. Neurobiol.*, **18**, 461–475.

Hortsch, M. (2000) Structural and functional evolution of the L1 family: are four adhesion molecules better than one? *Mol. Cell Neurosci.*, **15**, 1–10.

Ide, C. (1996) Peripheral nerve regeneration. *Neurosci. Res.*, **25**, 101–121.

Jamin, N., Junier, M.P., Grannec, G. & Cadusseau, J. (2001) Two temporal stages of oligodendroglial response to excitotoxic lesion in the gray matter of the adult rat brain. *Exp. Neurol.*, **172**, 17–28.

Jung, M., Petrausch, B. & Stuermer, C.A. (1997) Axon-regenerating retinal ganglion cells in adult rats synthesize the cell adhesion molecule L1 but not TAG-1 or SC-1. *Mol. Cell Neurosci.*, **9**, 116–131.

Karagogeos, D. (2003) Neural GPI-anchored cell adhesion molecules. *Front Biosci.*, **8**, s1304–s1320.

Karagogeos, D., Morton, S.B., Casano, F., Dodd, J. & Jessell, T.M. (1991) Developmental expression of the axonal glycoprotein TAG-1: differential regulation by central and peripheral neurons *in vitro*. *Development*, **112**, 51–67.

Kyriakopoulou, K., de Diego, I., Wassef, M. & Karagogeos, D. (2002) A combination of chain and neurophilic migration involving the adhesion molecule TAG-1 in the caudal medulla. *Development*, **129**, 287–296.

Lang, D.M., Warren, J.T. Jr, Klisa, C. & Stuermer, C.A. (2001) Topographic restriction of TAG-1 expression in the developing retinotectal pathway and target dependent reexpression during axon regeneration. *Mol. Cell Neurosci.*, **17**, 398–414.

Lee, K.H., Chung, K., Chung, J.M. & Coggeshall, R.E. (1986) Correlation of cell body size, axon size, and signal conduction velocity for individually labelled dorsal root ganglion cells in the cat. *J. Comp. Neurol.*, **243**, 335–346.

Ma, D., Chow, S., Obrocka, M., Connors, T. & Fischer, I. (1999) Induction of microtubule-associated protein 1B expression in Schwann cells during nerve regeneration. *Brain Res.*, **823**, 141–153.

Mahy, N., Diaz, R., Andres, N. & Fuxe, K. (1996) Ibotenic acid in the medial septum increased brain-derived neurotrophic factor mRNA levels in the dorsal rat hippocampal formation. *Neurosci. Lett.*, **213**, 169–172.

Malhotra, J.D., Tsiotra, P., Karagogeos, D. & Hortsch, M. (1998) *Cis*-activation of L1-mediated ankyrin recruitment by TAG-1 homophilic cell adhesion. *J. Biol. Chem.*, **273**, 33354–33359.

Manent, J., Oguievetskaia, K., Bayer, J., Ratner, N. & Giovannini, M. (2003) Magnetic cell sorting for enriching Schwann cells from adult mouse peripheral nerves. *J. Neurosci. Meth.*, **123**, 167–173.

Manthorpe, M., Nieto-Sampedro, M., Skaper, S.D., Lewis, E.R., Barbin, G., Longo, F.M., Cotman, C.W. & Varon, S. (1983) Neuronotrophic activity in brain wounds of developing rat. Correlation with implant survival in the wound cavity. *Brain Res.*, **267**, 47–56.

Martini, R. (1994) Expression and functional roles of neural cell surface molecules and extracellular matrix components during development and regeneration of peripheral nerves. *J. Neurocytol.*, **23**, 1–28.

Martini, R. & Schachner, M. (1988) Immunoelectron microscopic localization of neural cell adhesion molecules (L1, N-CAM, and myelin-associated glycoprotein) in regenerating adult mouse sciatic nerve. *J. Cell Biol.*, **106**, 1735–1746.

Milev, P., Maurel, P., Haring, M., Margolis, R.K. & Margolis, R.U. (1996) TAG-1/axonin-1 is a high-affinity ligand of neurocan, phosphacan/protein-tyrosine phosphatase-zeta/beta, and N-CAM. *J. Biol. Chem.*, **271**, 15716–15723.

Nothias, F., Cadusseau, J., Dusart, I. & Peschanski, M. (1991) Fetal neural transplants into an area of neurodegeneration in the spinal cord of adult rat. *Restor. Neurol. Neurosci.*, **2**, 283–288.

Nothias, F. & Peschanski, M. (1990) Homotypic fetal transplants into an experimental model of spinal cord neurodegeneration. *J. Comp. Neurol.*, **301**, 520–534.

Nothias, F., Tessler, A. & Murray, M. (1993) Restoration of substance P and calcitonin gene-related peptide in dorsal root ganglia and dorsal horn after neonatal sciatic nerve lesion. *J. Comp. Neurol.*, **334**, 370–384.

Peschanski, M. & Besson, J.M. (1987) Structural alteration and possible growth of afferents after kainate lesion in the adult rat thalamus. *J. Comp. Neurol.*, **258**, 185–203.

Peschanski, M. & Isacson, O. (1988) Fetal homotypic transplant in the excitotoxically neuron-depleted thalamus: light microscopy. *J. Comp. Neurol.*, **274**, 449–463.

Poliak, S., Salomon, D., Elhanany, H., Sabanay, H., Kiernan, B., Pevny, L., Stewart, C.L., Xu, X., Chiu, S.Y., Shrager, P., Furley, A.J. & Peles, E. (2003) Juxtaparanodal clustering of Shaker-like $K^+$ channels in myelinated axons depends on Caspr2 and TAG-1. *J. Cell Biol.*, **162**, 1149–1160.

Rader, C., Kunz, B., Lierheimer, R., Giger, R.J., Berger, P., Tittmann, P., Gross, H. & Sonderegger, P. (1996) Implications for the domain arrangement of axonin-1 derived from the mapping of its NgCAM binding site. *EMBO J.*, **15**, 2056–2068.

Revest, J.M., Faivre-Sarrailh, C., Schachner, M. & Rougon, G. (1999) Bidirectional signaling between neurons and glial cells via the F3 neuronal adhesion molecule. *Adv. Exp. Med. Biol.*, **468**, 309–318.

Smolen, A. & Beaston-Wimer, P. (1990) Quantitative analysis of *in situ* hybridization using image analysis. In Chesselet, M.-F. (Ed), *In Situ Hybridization Histochemistry*, CRC Press, Boca Raton, FL, pp. 175–188.

Soares, S., Fischer, I., Ravaille-Veron, M., Vincent, J.D. & Nothias, F. (1998) Induction of MAP1B phosphorylation in target-deprived afferent fibers after kainic acid lesion in the adult rat. *J. Comp. Neurol.*, **396**, 193–210.

Soares, S., von Boxberg, Y., Lombard, M.C., Ravaille-Veron, M., Fischer, I., Eyer, J. & Nothias, F. (2002) Phosphorylated MAP1B is induced in central sprouting of primary afferents in response to peripheral injury but not in response to rhizotomy. *Eur. J. Neurosci.*, **16**, 593–606.

Stettler, O., Nothias, F., Tavitian, B. & Vernier, P. (1995) Double *in situ* hybridization reveals overlapping neuronal populations expressing the low molecular weight GTPases Rab3a and Rab3b in rat brain. *Eur. J. Neurosci.*, **7**, 702–713.

Stoeckli, E.T. & Landmesser, L.T. (1995) Axonin-1, Nr-CAM, and Ng-CAM play different roles in the *in vivo* guidance of chick commissural neurons. *Neuron*, **14**, 1165–1179.

Stoeckli, E.T., Sonderegger, P., Pollerberg, G.E. & Landmesser, L.T. (1997) Interference with axonin-1 and NrCAM interactions unmasks a floor-plate activity inhibitory for commissural axons. *Neuron*, **18**, 209–221.

Suh, Y.S., Chung, K. & Coggeshall, R.E. (1984) A study of axonal diameters and areas in lumbosacral roots and nerves in the rat. *J. Comp. Neurol.*, **222**, 473–481.

Terenghi, G. (1999) Peripheral nerve regeneration and neurotrophic factors. *J. Anat.*, **194**, 1–14.

Tetzlaff, W., Zwiers, H., Lederis, K., Cassar, L. & Bisby, M.A. (1989) Axonal transport and localization of B-50/GAP-43-like immunoreactivity in regenerating sciatic and facial nerves of the rat. *J. Neurosci.*, **9**, 1303–1313.

Topilko, P., Schneider-Maunoury, S., Levi, G., Baron-Van Evercooren, A., Chennoufi, A.B., Seitanidou, T., Babinet, C. & Charnay, P. (1994) Krox-20 controls myelination in the peripheral nervous system. *Nature*, **371**, 796–799.

Traka, M., Dupree, J.L., Popko, B. & Karagogeos, D. (2002) The neuronal adhesion protein TAG-1 is expressed by Schwann cells and oligodendrocytes and is localized to the juxtaparanodal region of myelinated fibers. *J. Neurosci.*, **22**, 3016–3024.

Traka, M., Goutebroze, L., Denisenko, N., Bessa, M., Nifli, A., Havaki, S., Iwakura, Y., Fukamauchi, F., Watanabe, K., Soliven, B., Girault, J.A. & Karagogeos, D. (2003) Association of TAG-1 with Caspr2 is essential for the molecular organization of juxtaparanodal regions of myelinated fibers. *J. Cell Biol.*, **162**, 1161–1172.

Vega, J.A., del Valle-Soto, M.E., Calzada, B. & Alvarez-Mendez, J.C. (1991) Immunohistochemical localization of S-100 protein subunits (alpha and beta) in dorsal root ganglia of the rat. *Cell Mol. Biol.*, **37**, 173–181.

Walsh, F.S. & Doherty, P. (1997) Neural cell adhesion molecules of the immunoglobulin superfamily: role in axon growth and guidance. *Ann. Rev. Cell Dev. Biol.*, **13**, 425–456.

Widenfalk, J., Lundstromer, K., Jubran, M., Brene, S. & Olson, L. (2001) Neurotrophic factors and receptors in the immature and adult spinal cord after mechanical injury or kainic acid. *J. Neurosci.*, **21**, 3457–3475.

Wolfer, D.P., Giger, R.J., Stagliar, M., Sonderegger, P. & Lipp, H.P. (1998) Expression of the axon growth-related neural adhesion molecule TAG-1/axonin-1 in the adult mouse brain. *Anat. Embryol. (Berl)*, **197**, 177–185.

Wolfer, D.P., Henehan-Beatty, A., Stoeckli, E.T., Sonderegger, P. & Lipp, H.P. (1994) Distribution of TAG-1/axonin-1 in fibre tracts and migratory streams of the developing mouse nervous system. *J. Comp. Neurol.*, **345**, 1–32.

Yamamoto, M., Boyer, A.M., Crandall, J.E., Edwards, M. & Tanaka, H. (1986) Distribution of stage-specific neurite-associated proteins in the developing murine nervous system recognized by a monoclonal antibody. *J. Neurosci.*, **6**, 3576–3594.

Yoshihara, Y., Kawasaki, M., Tamada, A., Nagata, S., Kagamiyama. H. & Mori, K. (1995) Overlapping and differential expression of BIG-2, BIG-1, TAG-1, and F3: four members of an axon-associated cell adhesion molecule subgroup of the immunoglobulin superfamily. *J. Neurobiol.*, **28**, 51–69.

Zhang, Y., Roslan, R., Lang, D., Schachner, M., Lieberman, A.R. & Anderson, P.N. (2000) Expression of CHL1 and L1 by neurons and glia following sciatic nerve and dorsal root injury. *Mol. Cell Neurosci.*, **16**, 71–86.