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Research report

# SC1: a marker for astrocytes in the adult rodent brain is upregulated during reactive astrocytosis

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**Abstract**

Astrocytes are the most abundant cell type in the mammalian central nervous system (CNS), and are involved in many processes critical for normal CNS maintenance and function. We have used double-label immunocytochemistry and in situ analysis to show that the SPARC (secreted protein acidic and rich in cysteine)-related protein SC1, co-localizes with the astrocyte marker glial fibrillary acidic protein (GFAP) in the adult rodent brain. Thus, SC1 is an astrocyte marker that may be used to investigate astrocyte heterogeneity and analyze glial cell lineages during neural development. Consistent with the presence of SC1 and GFAP in astrocytes, both proteins were markedly upregulated following reactive astrocytosis induced by focal mechanical trauma. Therefore, SC1 may play an important role in reactive astrocytosis subsequent to a wide variety of neural trauma, including neurodegenerative diseases and acute neural damage.

*Keywords:* Astrocyte; Glia; SC1; SPARC; Reactive astrocytosis; Extracellular matrix

## 1. Introduction

Astrocytes are a class of glial cells involved in many aspects of the development and maintenance of the central nervous system (CNS) (reviewed in [9]). Previously, astrocytes were thought to be electrically passive, however several types of ion channels including glutamate receptors, have been found in astrocytes [2,28], indicating a more active role for these cells in electrical signalling. Moreover, several lines of evidence argue for a very close astrocyte/neuron interrelationship [40]; for example astrocytes have been shown to directly signal neurons via gap junctions [29]. The role played by astrocytes changes during development and maturation of the nervous system. In the developing brain immature astrocytes are permissive for axonal growth, while mature astrocytes inhibit growth [12,37]. This may reflect changes on the cell surface or extracellular matrix of the glial cells [15].

Additionally, astrocytes undergo reactive astrocytosis in response to various types of neurodegenerative disorders such as Alzheimers, and a wide range of other neural insults (reviewed in [1,8,14,30]). One hallmark of reactive astrocytosis is increased expression of glial fibrillary acidic protein (GFAP) [3,7]. The exact role of reactive astrocytosis is unknown, although the rapidity of the response and its conservation across-species suggest that it may be important for nervous system maintenance [8]. Clearly, astrocytes are a dynamic component of the mammalian CNS, and characterization and functional studies of astrocyte-associated molecules will provide key information regarding CNS form and function.

This report describes the presence of SC1 in astrocytes of the adult rodent brain. SC1 is a secreted glycoprotein, closely related to the extracellular matrix protein SPARC (secreted protein, acidic and rich in cysteine). SPARC is thought to be involved in processes that modulate cell-extracellular matrix (ECM) interactions (reviewed in [20]). SC1 was originally cloned as a candidate synaptic junction glycoprotein and thought to be associated with a variety of neuronal subtypes of the rat brain [17], however, SC1 was recently identified in the Bergmann glia of the cerebellum [27]. The function of SC1 is presently unknown, although the high degree of relatedness to SPARC suggests a functional similarity.

We have used in situ analysis and immunohistochemistry to show that SC1 is a component of astrocytes in all areas of the adult rat brain examined, and that this protein

Abbreviations: GFAP, glial fibrillary acidic protein; ECM, extracellular matrix; SPARC, sectreted protein acidic and rich in cysteine

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may play an important role in reactive astrocytosis. Since SC1 is secreted, it may be involved in astrocyte-mediated recognition or signaling events.

## 2. Materials and methods

### 2.1. In situ analysis

The polymerase chain reaction (PCR) was used to generate a 423bp fragment that was cloned into the Not I/Sal I polylinker site of pSPORT (BRL, Bethesda, MD) to create pSC1-423. The following primers were used; forward, 5'CTC **GTCGAC**CTGGAAGGGAC-CAAAAAGG and reverse, 5'CATAAGAAT **GCGGCCGC** CCAAAGAAGCGCGTTATGCAGT-GCTCCATGGG (restriction sites are underlined). Antisense transcript was generated by SP6 polymerase (Promega, Madison, WI) using Sal I linearized pSC1-423 template; the sense transcript was generated by T7 polymerase (Promega, Madison, WI) with Not I linearized pSC1-423. Run-off transcripts were generated with Promega reagents under conditions described by the manufacturer. In situ hybridization was performed on coronal cryostat sections (10 $\mu$m), as described [26]. Slides were emulsion coated with Kodak NBT-2 (IBI/Eastman Kodak, New Haven, CT) exposed for 14 days, developed and counter-stained with haematoxylin and eosin.

### 2.2. Antibody production

A 1100bp Sal I/Hind III fragment of the rat SC1 gene was cloned into pQE32 (Qiagen, Chatsworth, CA) to generate pQE-SC1. Expression in *E. coli* from this clone produced a 536aa polypeptide containing the unique amino terminal portion of SC1 (a region with no homology to SPARC). This recombinant protein contains a hexa-histidine tag, derived from the pQE32 vector, and was purified using Nickel-NTA resin (Qiagen, Chatsworth, CA) according to the manufacturers instructions. The eluted protein was dialysed against phosphate-buffered saline (PBS) and used for rabbit immunization and antibody blocking experiments. SC1 antisera from 2 rabbits were produced under contract from Pocono Rabbit Farm and Laboratories (Canadensis, PA): 100$\mu$g of SC1 fusion protein was injected subcutaneosly, the rabbits were boosted 56 days later, and then every month with 50 $\mu$g protein. Both animals produced similar titers of anti-SC1 as judged by Western analysis and immunohistochemistry. The antibody was purified as an IgG fraction using caprylic acid; sera were adjusted to pH 4.3, 0.075 vol. of caprylic acid (Sigma, St Louis, MO) added, then the precipitate was cleared and the soluble IgG fraction was precipitated with ammoniun sulfate. The IgG was then resuspended in PBS to the original starting volume of sera and dialyzed extensively against PBS/0.05% sodium azide.

### 2.3. Immunohistochemistry

Coronal cryostat sections (10 $\mu$m) were obtained from formaldehyde perfused rat tissue. Sections were washed in PBS (5 min), blocked in 1% BSA/0.5% Tween 20/PBS (30 min). Sections were then washed in PBS (10 min) and incubated for 1 h at room temperature with two primary antibodies in PBS (anti-SC1 and either anti-neurofilament or anti-GFAP antibodies). Caprylic acid purified rabbit anti-SC1 was used at a dilution of 1/100 in PBS. Monoclonal mouse anti-GFAP, clone #G-A-5 (Boehringer, Indianapolis, IN) and monoclonal mouse anti-NF 160 (neurofilament protein of 160kD molecular weight), clone #NN18 (Boehringer, Indianapolis, IN), were each used at 0.1 $\mu$g/section (40 $\mu$l total volume). Sections were washed twice with PBS (10 min), incubated with 5% goat serum (Jackson Immunologicals, West Grove, PA) (30 min), washed in PBS (5min) and then incubated with fluorescein-labeled goat anti-rabbit or rhodamine-labeled goat anti-mouse sera (30 min). Finally, sections were washed twice in PBS (15 min), mounted in PBS/glycerol and viewed under fluorescent illumination on a Zeiss axioplan microscope. Appropriate cut-off filters were used to prevent overlap in the fluorescein/rhodamine channels. All photographs were taken on Kodak EPH 1600ASA film, using automatic exposure controls of the Leitz camera with a + 1 exposure compensation for fluorescein label.

### 2.4. Focal mechanical trauma

All surgical procedures adhered to guidelines outlined by the RIMB ACC (Roche animal care committee). Utilizing sterile technique, 2 month old 129SV/J (Jackson Laboratories, Bar Harbor, ME) mice were deeply anesthetized with intraperitoneal injections of Ketaset (90 mg/ml) (Aveco Co., Fort Dodge, IO) and Rompun (10 mg/ml) (Miles Inc., Shawnee Mission, KS) and focal mechanical trauma was produced by penetration of one cerebral hemisphere with a 27 gauge needle. Tissue penetration extended to the hippocampus. No overt post-lesion neurological defects were observed in the mice. At 48 h post- lesion, the mice were again anesthetized, perfused with 4% formaldehyde, and the brain sectioned by cryostat (as described above). Horizontal sections of 10 $\mu$m thickness were used, to maximize the number of sections containing the focal injury.

## 3. Results

### 3.1. SC1 is present in astrocytes

In situ analysis showed a general distribution of SC1 mRNA in most areas of the adult rat brain examined. In

the cerebellum, mRNA was present in a number of regions including the Purkinje cell layer (which includes the Bergmann glial cells), that was previously shown to express SC1 [27]. SC1 mRNA was also identified in a wide number of other brain regions; in particular the meningeal layers showed abundant staining (Fig. 1).

Immunohistochemistry of cerebellar sections showed that the pattern of expression of SC1 and GFAP was quite similar with a distribution along the radial processes of the Bergmann glia (Fig. 2). The SC1 antiserum was shown to be specific by blocking with SC1 recombinant protein (Fig. 2a) and by Western analysis (data not shown). How-

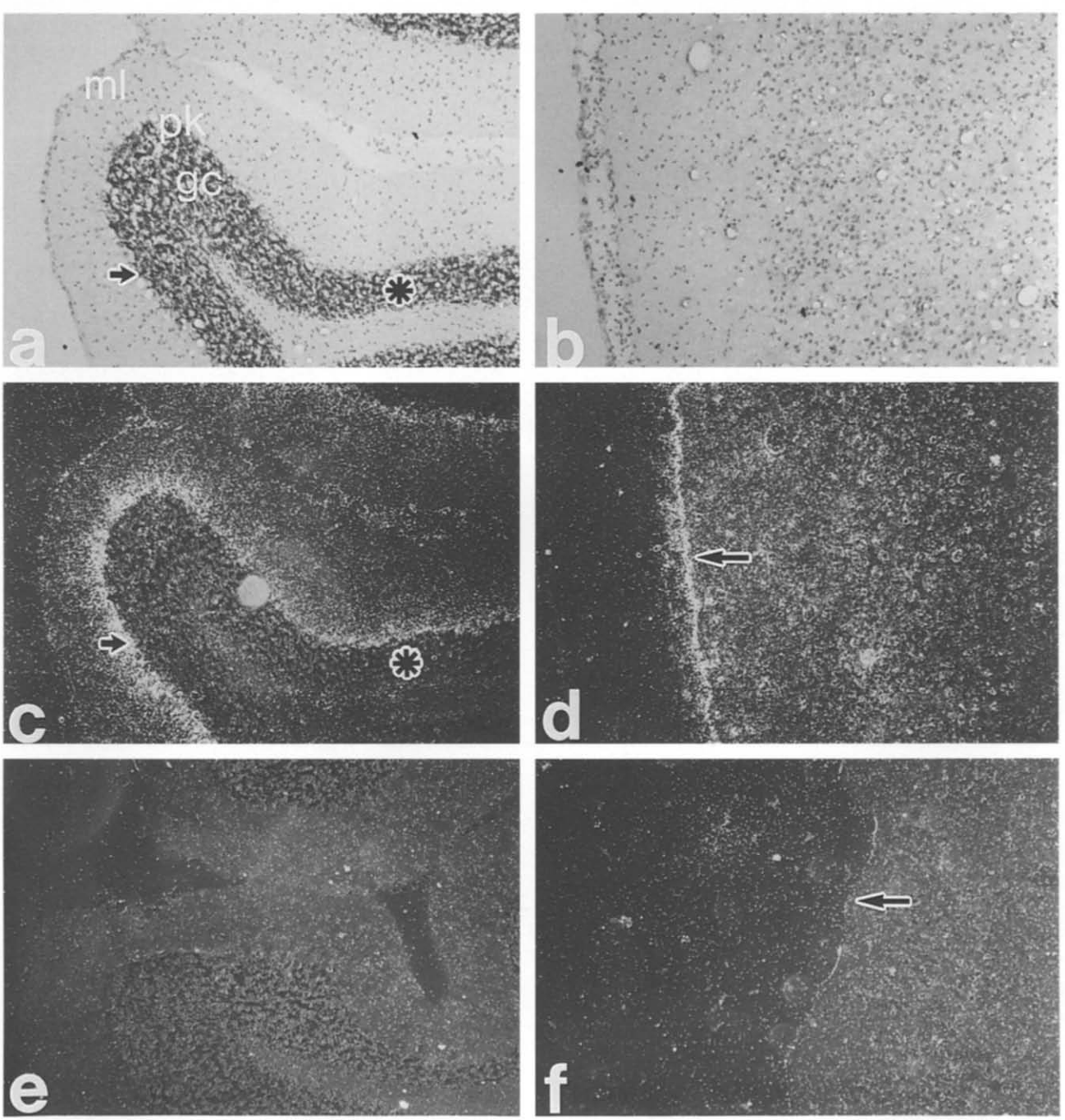


Fig. 1. In situ localization of SC1 message in the rat cerebellum and forebrain. Hybridization of [$^{35}$S]-labelled antisense SC1 RNA probe identifies defined regions of SC1 message in the rat forebrain. Plates a,c and plates b,d correspond to light and dark field images of the same section. Sense probe controls do not show a pattern of brain expression (e, f). The labelled regions in the cerebellum (a) are the molecular layer (ml), the Purkinje layer (pk) and the granule cell layer (gc). The asterisk used for alignment in a and c indicates the same position in the granule cell layer, and the arrows in c shows the intense staining in the Purkinje layer. Arrows in d and f indicate the high level of signal observed in the meningial layer with antisense (d), but not with sense transcript (f).

ever, the SC1 recombinant protein did not affect GFAP staining (Fig. 2b). Higher magnification analysis (×1000) of SC1 and GFAP immunofluorescence labelling (Fig. 2e–h) revealed a difference in distribution of the two proteins: SC1 showed a punctate pattern along the processes of the Bergmann glia (indicated by arrows in Fig. 2g), whereas GFAP was evenly distributed (Fig. 2h). The punctate pattern may indicate contact points for other molecules associated with SC1 (e.g. gap junction proteins, cytoskeletal elements) or areas of focal adhesion. The distribution of SC1 in astrocytes found in other regions of the brain (e.g. the striatum) also showed a punctate pattern although generally less pronounced than seen with the radial Bergmann glia. To exclude the possibility that SC1 is secreted by non-astroglial cells and subsequently becomes associated with astrocytes, SC1 message was shown to co-localize with GFAP antibody staining (results not shown). Additionally, mice transgenic for the SC1 promoter fused to a LacZ reporter gene, showed LacZ staining in the Bergmann glia (and other astrocytes) providing

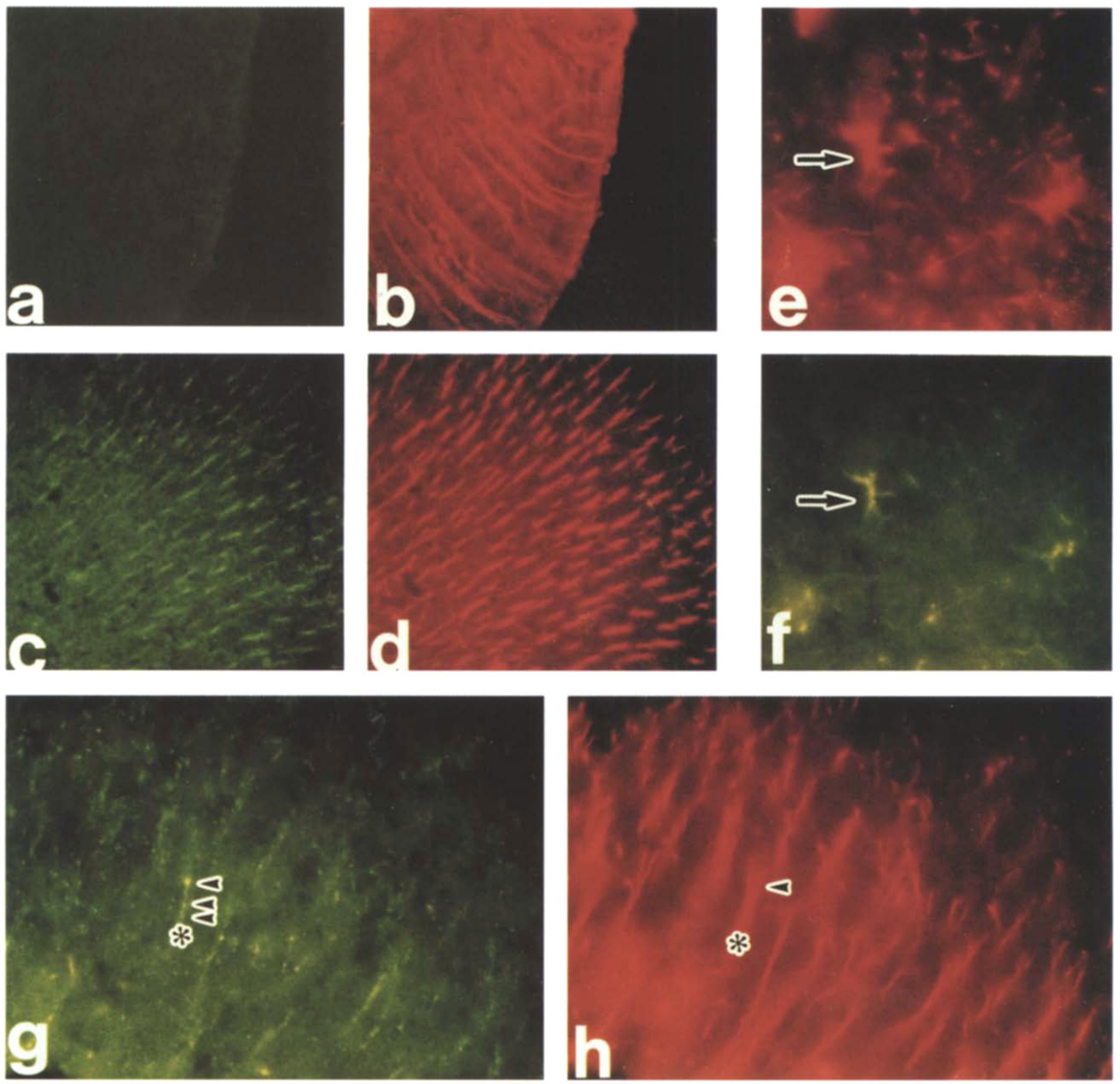


Fig. 2. SC1 in Bergman glia is distributed in a punctate manner along the radial processes. Double label immunocytochemistry was used to identify SC1 and GFAP-containing regions of the cerebellum. SC1 (c,f,g: fluorescein) and GFAP (b,d,e,h: rhodamine) colocalize in all regions examined: e.g. the Bergman glia (b,c,d,g,h), and the cerebellar white matter (e,f). At higher magnification (×1000) the distribution of SC1 associated with the Bergmann glia has a punctate appearance (g) while GFAP does not (h). The specificity of the SC1 antibody is demonstrated by a complete block of SC1 immunoreactivity when preincubated with recombinant SC1 protein (a), while GFAP staining on the same section was unaffected (b). Plates a,b,c,d are x400 and e,f,g,h are ×1000.

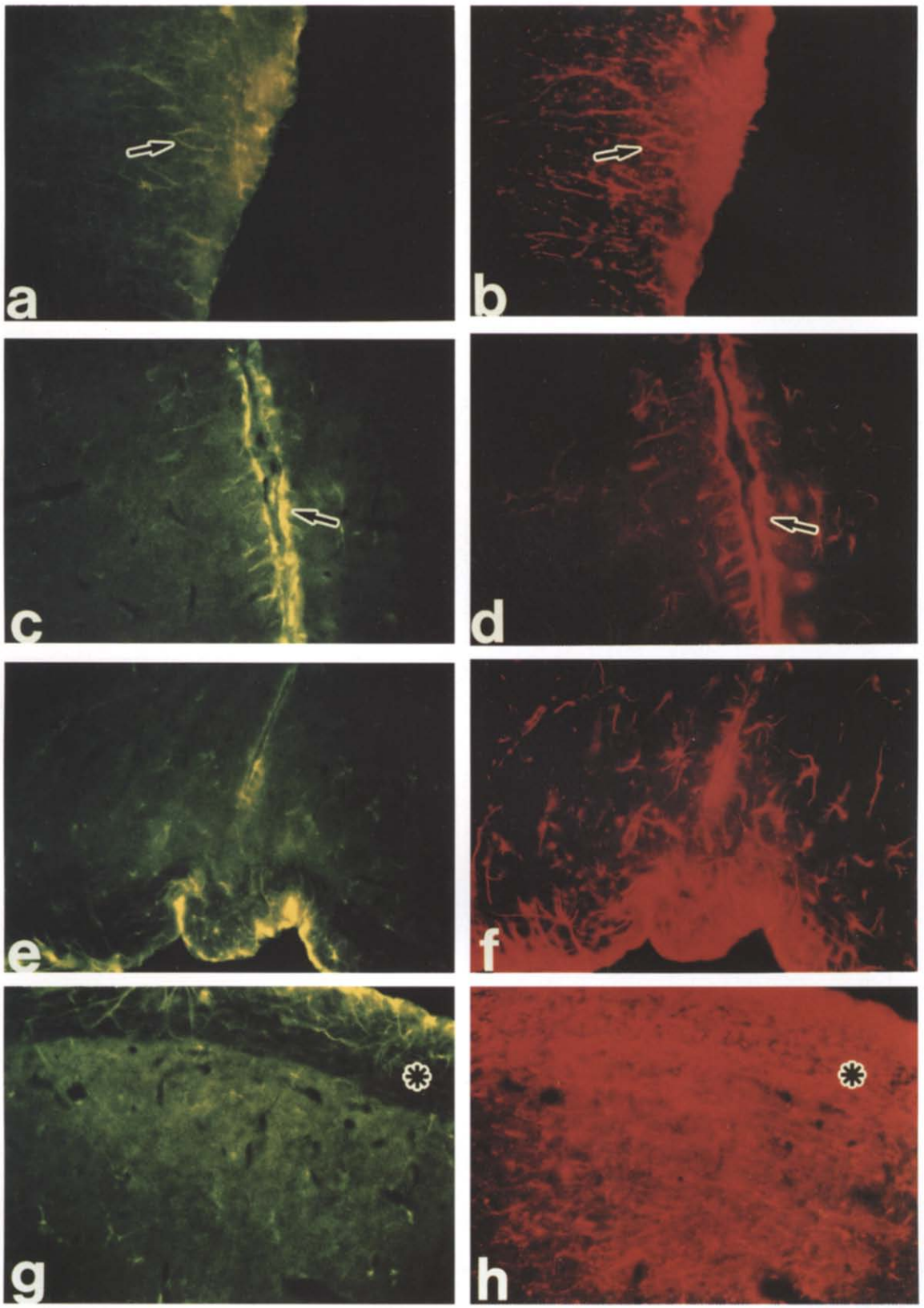


Fig. 3. SC1 co-localizes with GFAP in astrocytes at the glia limitans and meningial surfaces. SC1 (a,c,e,g: fluorescein) and GFAP (b,d,f: rhodamine) were present at the meningial surface (a,b) the third ventricle region (c,d) and the glia limitans and median eminence (e,f). Arrows are used to align the sections and to indicate regions of pronounced SC1 staining. Identification of neurons using anti-neurofilament antibodies (h) showed a different distribution to that of SC1 (g). The asterisk identifies the same region in (g) and (h). All photos are ×400.

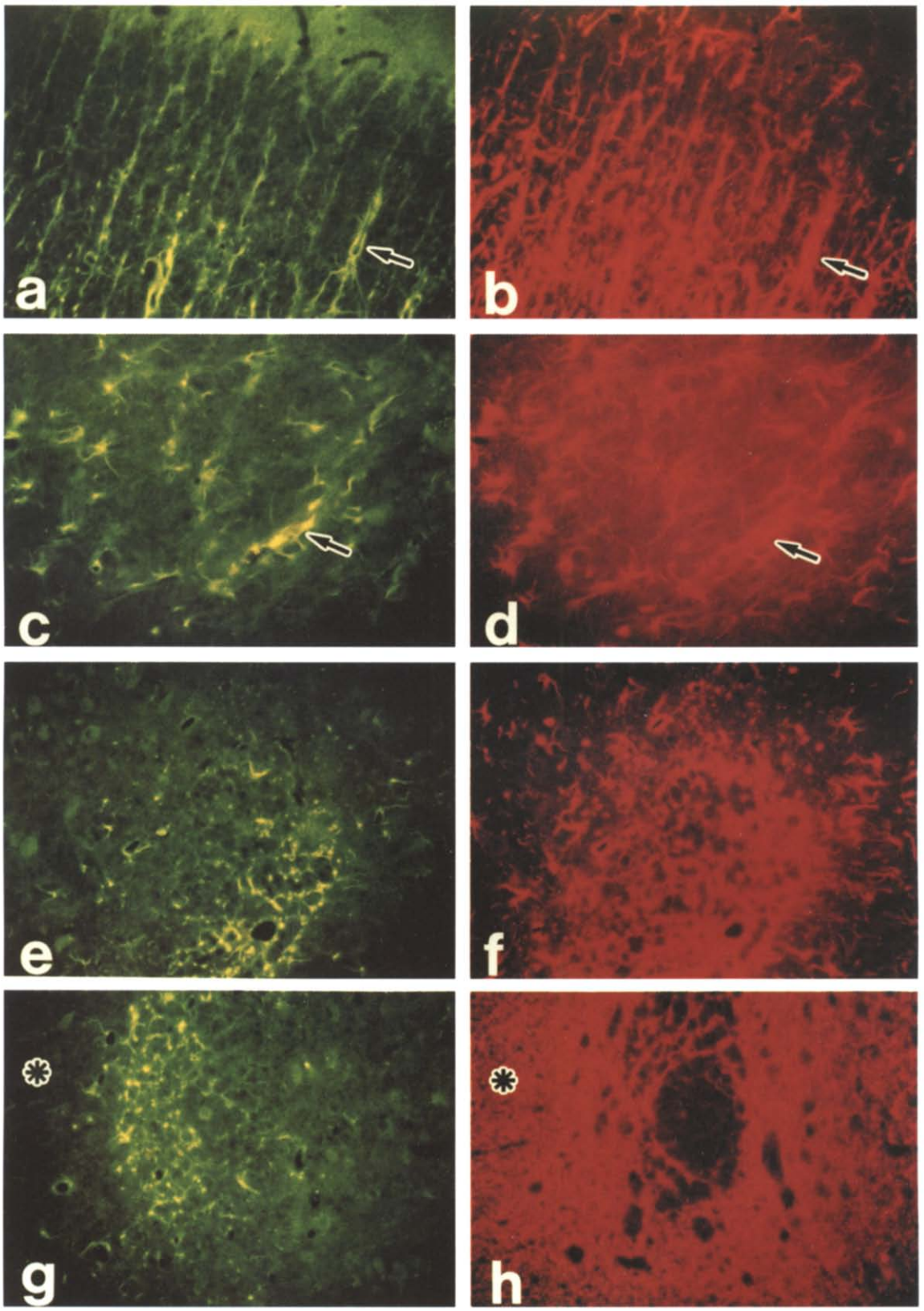


Fig. 4. SC1 is present in astrocytes of the striatum, neocortex and medulla. SC1 (a,c,e,g) showed identical distribution to GFAP (b,d,f) but not neurofilament (h) in the adult rat brain. The neocortex (a,b) and striatum (c,d) show localization of SC1 and GFAP to the astrocytic processes (indicated by arrows). In the medulla (e,g), SC1 is distributed similarly to GFAP (f), but differently from neurofilament (h); the asterisk indicates the same position in the double-labelled section. All photos are ×400.

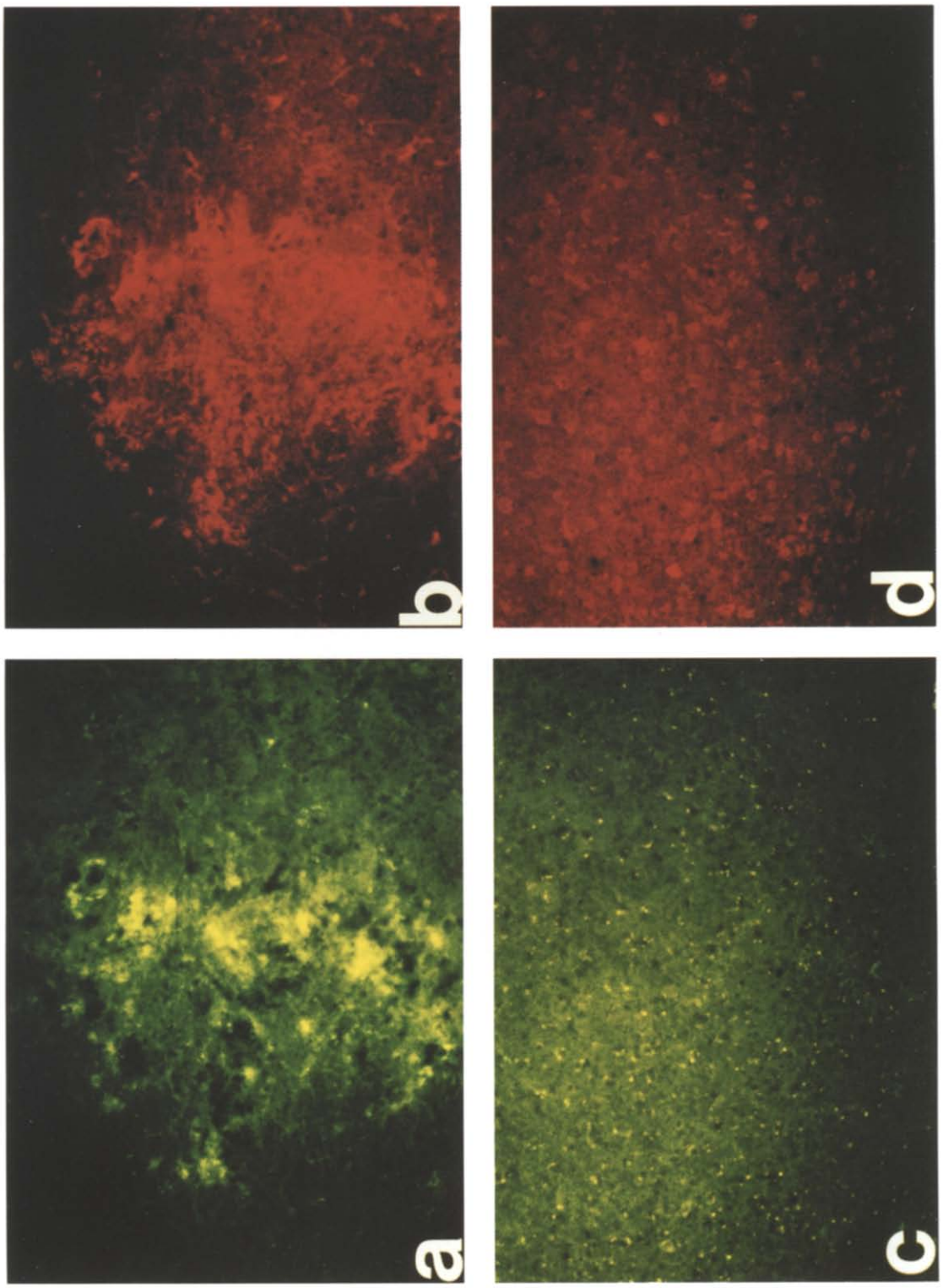


Fig. 5. SC1 and GFAP are upregulated following focal mechanical lesion to the mouse brain. Following a focal injury to one hemisphere of an adult mouse brain, sections including the injured right hemisphere and the non-injured left hemisphere (control) were stained for SC1 (a,c) and GFAP (b,d). As shown in a, SC1 levels increased following the focal injury compared to the same region in the contra-lateral control area (c). GFAP was likewise upregulated at the lesioned area, but not in a similar region of the control hemisphere (d). This section was 24 hours post-lesion, and magnification was ×200. Panels c and d exposure was twice that of a and b.

further support that astrocytes are the site of synthesis of SC1 (McKinnon et. al., manuscript in preparation).

Double label immunocytochemistry of other brain regions shows that SC1 and GFAP co-localize to the same cells (Fig. 3a–f, Fig. 4a–f), but that the 160kD neurofilament protein shows quite a different pattern (Fig. 3g,h and Fig. 4g,h). Figs. 3 and 4 show a gross survey of different brain areas. SC1 is particularly enriched along the pia mater (Fig. 3a,c,d), the third ventricle (Fig. 3c,d) and the median eminence (Fig. 3e,f) with all showing relatively high levels of SC1 protein. The neocortex (Fig. 4a,b) and striatum (Fig. 4c,d) also both show SC1 and GFAP to co-localize, with signal present in the intricate processes typical of astrocytes (Fig. 4a–d, indicated by arrows). In the olfactory bulb (Fig. 4e–h), increased SC1 protein is found in the region of the anterior commissure.

In all other areas of the adult rat brain examined, SC1 and GFAP showed an identical pattern of distribution. Double label immunofluorescence for neurofilament protein and SC1 showed distinct labelling patterns, indicating that SC1 is absent from neurons. Studies using GalC monoclonal antibodies to identify oligodendrocytes showed a staining pattern distinct from that obtained with anti-SC1 (results not shown). Additionally, other markers including anti-vimentin, enolase and calbindin all showed dissimilar staining patterns to that obtained with anti-SC1 (data not shown). From the above results it is clear that SC1 is a marker for most, if not all, astrocytes in the adult rat brain. While the distribution of SC1 and GFAP was very similar, we consistently observed different intensities of SC1 staining in particular astrocytes or groups of astrocytes, (see examples from the neocortex and striatal regions, indicated by arrows in Fig. 4a,c). This may be indicative of astrocyte heterogeneity, for example reflecting various stages of astrocyte maturation (discussed in [9,15].).

### *3.2. Focal mechanical trauma upregulates SC1*

A general response to a wide variety of neural trauma is reactive astrocytosis, characterized by astrocyte hypertrophy and up-regulated GFAP expression (reviewed in [8,14]). To investigate the involvement of SC1 in reactive astrocytosis, a single focal lesion from a needle stab was introduced into one hemisphere of the mouse brain. Consistent with their presence in astrocytes, SC1 and GFAP were both upregulated in the area immediately surrounding the lesion (Fig. 5a,b). Contralateral control regions showed no upregulation of either GFAP or SC1 (Fig. 5c and d). That anti-ratSC1 antibodies identify mouse SC1 is expected based on the relatedness between the two genes (McKinnon et al., unpublished). Additionally, Western analysis of mouse and rat brain extracts gave indistinguishable staining patterns that were completely blocked following preincubation with recombinant SC1. The levels of SC1 upregulation appear similar to that of GFAP, suggesting that SC1 may play an important role in reactive astrocytosis. It will be informative to investigate the effects of other types of neural trauma on the regulation of SC1, and compare this to the regulation of GFAP expression.

## 4. Discussion

A variety of markers, including extracellular matrix (ECM) and cell surface proteins, have been described for various neural (i.e. neuronal and glial) cell types. These markers have been essential for an understanding of the process of neural differentiation and neural cell lineage [5]. We have shown here that SC1 is a marker for astrocytes in adult rodent brain. The epitope profile of neural precursor cells, as defined by antibodies, undergoes a dynamic series of changes as these cells progress to terminal differentiation. GFAP appearance during glial differentiation occurs relatively late, as the glial lineage progenitor cells differentiate to type-1 astrocytes, or more controversially, type 2 astrocytes from the bipotential O-2A progenitor [5,22]; corresponding to GFAP expression at embryonic day 15–16 in the rat which is equivalent to E12–E14 in the mouse. However, SC1 expression occurs much earlier than GFAP expression; at around E9–E10 in the mouse (McKinnon et al, in preparation). This suggests that SC1 may be an early, glial-precursor marker that becomes restricted to astrocytes in the adult CNS. Although we have not observed SC1 staining independent of GFAP in the adult rat brain, there may be unique astrocyte subtypes that either express SC1 or GFAP. In this regard it would be interesting to examine SC1 in NG2 + astrocytes [21], in which GFAP expression was not detected.

While the function of SC1 is presently unclear, the high degree of relatedness to SPARC (and the gene QR1, present in quail retinal Müller cells [13]) suggests some functional similarity. This is especially intriguing as the areas of homology are associated with the known functional domains of SPARC. The enrichment of SC1 in the glia limitans or pia mater may reflect a role in astrocyte functions associated with the blood/brain barrier. This is consistent with the involvement of SPARC in endothelial functions [20] and a role in angiogenesis [34]. SPARC has been shown to have a number of diverse properties, with each relating to a defined region of the protein. These properties include binding of certain growth factors, the ability to alter progression through the cell cycle and a cellular anti-adhesiveness (reviewed in [20,34]). SPARC has been associated with tissue morphogenesis and remodeling, which probably relates to the ability of SPARC to modulate metalloproteinase activity in tissue culture lines [39]. The perturbation of SPARC levels during development in *X. laevis* causes embryonic abnormalities at and around neurulation, and in *C. elegans* altered SPARC expression produces embryonic defects and an uncoordinated (Unc) phenotype [31,36]. As some of these properties of SPARC reside in regions of homology shared

between SPARC and SC1, it is likely SC1 may have similar functions.

Growth factors (e.g. PDGF, FGF and TGF-$\beta$) are known to be important for glial cell proliferation, migration and maturation [16,25]. Certain ECM molecules, including SPARC are known to bind growth factors [18,20,33]. SPARC has been shown to modulate binding of PDGF to its receptor [20], and the protein domain responsible for this has been mapped to a region that is present in SC1. Therefore, SC1 may also be capable of modulating growth factor/receptor interactions. Growth factor levels (e.g. TGF-$\beta$ and IL-1) are known to be altered during reactive astrocytosis [6,10,11,23,24,32]. Thus, SC1 upregulation during reactive astrocytosis may be an important regulatory step involved in growth factor responses during neural injury.

SC1 is a secreted protein [27], but it is as yet unclear if SC1 is a bona fide ECM component. However, the similarity to SPARC suggests that this is the case. In support of this, ECM components such as tenascin, laminin, thrombospondin, proteoglycans and SPARC have been shown to occur in neural tissue and astrocytes in vitro, in vivo and at various developmental times [41] (reviewed in [4,19,35,38]). These ECM components are believed to be involved in a number of astrocyte-mediated events, including cell migration, response to neural injury and process extension, although assignment of exact neural function is uncertain [19,35,38]. In most cases, extracellular matrix gene expression occurs transiently during development (e.g. fibronectin and laminin), and is thought not to play a significant role in the function of the adult brain. SC1 is unusual in this regard as it is expressed during the early development and differentiation of the nervous system, and is also present at high levels in the adult nervous system. This property places SC1 in a unique category, suggesting that knowledge of SC1 function may have important implications for our understanding of the role of the neural ECM.

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