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Expression Specificity of GFAP Transgenes*

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Glial fibrillary acidic protein (GFAP) is an intermediate filament protein found predominantly in astrocytes. This specificity has recommended the GFAP gene promoter for targeting transgene expression to astrocytes. Although both we [Brenner et al. J. Neurosci. 14:1030–1037, (1994)] and others [Mucke et al. New Biol. 3:465–474, (1991)] have reported astrocyte specificity for GFAP promoters, we demonstrate here that these DNA sequences can also direct activity in neurons. The pattern of neuronal activity varied with both the nature of the expressed sequence and the transgene insertion site. Specifically, neuronal expression was very high for a protective protein/cathepsin A minigene, moderate for *lacZ* and undetectable for *GFP*. These findings, coupled with a survey of the literature, recommend that investigators using GFAP-driven transgenes verify specificity for each line studied, using a detection system whose sensitivity is sufficient to detect a compromising level of misexpression.

KEY WORDS: Astrocyte; GFAP; promoter; transgene.

INTRODUCTION

Glial fibrillary acidic protein (GFAP) was discovered by Eng et al. (1) in 1971 during an analysis of multiple sclerosis plaques. Subsequently, GFAP was found to be an intermediate filament protein expressed almost exclusively in astrocytes, leading to its adoption as an astrocytic marker for both clinical and basic studies (reviewed in (2, 3)). The cell specificity of GFAP expression in the CNS has recommended its promoter for directing transgene activity to astrocytes. Both Mucke's group, using a mouse GFAP promoter (4), and our group, using a human GFAP promoter (5), have presented results indicating this can be achieved, and over 150 papers have now been published involving GFAP driven transgenes (for a comparison of the expression properties of the mouse and human GFAP promoters see (6)). Applications have included *in vivo* functional tests of various gene products, use of astrocytes as factories for bioactive molecules, and creation of disease models. Adding to the attractiveness of the GFAP promoter is its high level of expression, with the human GFAP promoter capable of expressing a transgene at over 0.1% of total brain protein (7). However, we report here that both the human and mouse GFAP

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promoters may also direct expression in neurons, and a survey of the literature indicates that significant expression can occur outside of the CNS. Thus caution is required in the validation and interpretation of results obtained with GFAP transgenes.

EXPERIMENTAL PROCEDURES

Transgenic Animals. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham and St. Jude Children's Research Hospital. Previously described lines used in this study include tg6.4, a standard cytoplasmic *lacZ* (clac) driven by the human gfa2 promoter; tg7.2, a nuclear-targeted *lacZ* (nlac) driven by gfa2 (5) (Jackson Labs line Tg(X:GFAP,*lacZ*)Mes3); GFAP-S65T, *GFP* driven by gfa2 (8); and C-445, which contains clac embedded in the first exon of a genomic clone of the mouse GFAP gene (4). In this paper more descriptive names will be used for these lines as follows: tg6.4 = gfa2-clac; tg7.2 = gfa2-nlac1; GFAP-S65T = gfa2-*GFP*; C-445 = mGFAP-clac. Several additional lines were newly made for this study. These include two more gfa2-nlac lines, gfa2-nlac2 and gfa2-nlac3, made using the identical transgene and methodology as for gfa2-nlac1 (5); and three lines in which a human protective protein/cathepsin A (PPCA) minigene is driven by the gfa2 promoter. The gfa2-PPCA transgene was made by excising the 2.2 kb gfa2 promoter from pGfa2-clac with *Bam*HI and *Eco*RI and inserting it into the corresponding sites of plasmid pGEMEX-2 (Promega, Madison, WI). A 3.35 kb human PPCA minigene (9) was excised from plasmid pBKS by partial *Hin*dIII digestion and inserted into the *Hin*dIII site of plasmid pGEMEX-2 downstream of the gfa2 fragment. The 5.55 kb gfa2-PPCA transgene was obtained by complete digestion with *Sfi*I, followed by partial digestion with *Hin*d III. All lines were maintained on an FVB background except mGFAP-clac mice were either B57BL/6J or B57BL/6J × FVB hybrids (identical results were obtained for both).

Animal Perfusion and Tissue Preparation. All animals used were between 4–6 months of age, and 4–6 mice were investigated for each line. Animals were perfused transcardially with 4% paraformaldehyde in phosphate buffer (PB), pH 7.4, for light microscopy (LM), histochemistry (HC) and immunohistochemistry (IHC), or with 2.5% glutaraldehyde/0.5% paraformaldehyde in PB, pH 7.4, for electron microscopy (EM). For LM of the *lacZ* and *GFP* transgenic mice, the brains were removed and immersed in the same fixative for 1 h. The tissues were then washed with PB, immersed in a series of 10%, 20% and 30% sucrose in PB, embedded into tissue freezing medium (Triangle Biomedical Sciences, Durham, NC) and quickly frozen in methylbutane precooled in dry ice. Perfused brains analyzed for PPCA expression were embedded in paraffin (9). For EM the fresh fixed tissues were cut into 50 or 100 μm-thick vibratome sections, and stained either with 5-bromo-3-indolyl-β-D-galactopyranoside (Bluo-gal, Research Products International Corp., Mt. Prospect, IL) as described (5) or with the immuno-peroxidase method for GFP (see below). After staining and graded dehydration, sections were embedded in resin (Spurr's kit, Electron Microscopy Sciences, Hatfield, PA), and ultrathin sections cut.

Light Microscopy, Histochemistry and Immunohistochemistry. Cryosections about 15 μm thick were used for LM studies of the *lacZ* and *GFP* transgenics. HC for *lacZ* was performed using 5-bromo-4-chloro-β-D-galactoside (X-gal, Research Products International Corp.) as described previously (5). IHC employed the following primary antibodies: rabbit anti-cow GFAP (Dacopatts, Glostrup, Denmark; 1:5,000 dilution), rabbit anti-GFP (Chemicon, Temecula, CA; 1:100 dilution), mouse anti-β-galactosidase (β-gal, Promega, 1:100 dilution for standard immunofluorescence and 1:50,000 for the direct tyramide signal amplification (TSA) method), mouse anti-neuronal nuclei (NeuN, Chemicon, 1:1,000 dilution), mouse anti-calbindin-D-28k (CaBP, Sigma, St. Louis, MO; 1:2,500 dilution) and mouse anti-CNPase (Sigma; 1:1,000 dilution) (all the mouse antibodies are monoclonals). Secondary antibodies were used to produce signals by either reaction with 3,3′-diaminobenzidine tetrachloride (DAB, metal enhanced Substrate Kit, Pierce, Rockford, IL) or fluorescence. For the peroxidase method, secondary antibodies were peroxidase conjugated donkey anti-rabbit IgG or donkey anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA; 1:1,000 dilution for each). For immunofluorescence, secondary antibodies were Alexa FluorR488 goat anti-rabbit IgG, Alexa Fluor594 goat anti-mouse IgG, fluorescein goat anti-mouse IgG or tetramethylrhodamine goat anti-rabbit IgG (Molecular Probes, Eugene, OR; 1:100 dilution for each). In several instances, double label staining was performed when both primary antibodies were mouse monoclonals (GFAP/GFP, β-gal/NeuN, β-gal/CaBP). In each case, the first antigen was detected using the appropriate peroxidase conjugated secondary antibody described above in conjunction with the TSA method with a cyanine three reagent (10) (Perkin–Elmer Life Sciences Inc., Boston, MA), and the second antigen was detected by standard immunofluorescence. For analysis of PPCA expression, 7 μm paraffin sections were deparaffinated, subjected to antigen retrieval by microwave (9), immersed in blocking reagent (Roche, Indianapolis, IN) for 2 h, and then incubated overnight in the same blocking reagent containing rabbit anti-human PPCA antibody (9) and mouse anti-neuronal nuclei antibody (both at 1:500 dilution). Visualization with fluorescent secondary antibodies was as described above.

RESULTS

Neuronal Activity of Human GFAP-*lacZ* Transgenes

In initial investigations of the human GFAP promoter using transient transfection assays we found that a 2.2 kb fragment, gfa2, showed promise for astrocyte specific expression (11). The gfa2 fragment extends from bp −2163 to +47 relative to the RNA start site of +1, and has the ATG at +15 changed to TTG so that protein synthesis commences in the attached protein coding sequence. When this promoter was linked to either a standard cytoplasmic *lacZ* reporter gene (clac) or a nuclear targeted *lacZ* (nlac) to yield gfa2-clac and gfa2-nlac transgenes, expression appeared to be astrocyte specific in mice (5). However, in the course of continuing studies we have now found that gfa2 can also direct expression in neurons.

Neuronal activity was first noticed in the previously studied gfa2-nlac1 line (line 7.2 of (5)) while performing some supposedly control double label experiments. In these experiments reporter activity was detected by X-gal staining, and astrocytes and neurons identified by GFAP and NeuN IHC, respectively. While *lacZ* activity was found predominantly and most strongly in astrocytes, apparent neuronal expression was detected in the cerebral cortex, hippocampus and cerebellum (Fig. 1A–D). Since the gfa2-nlac1 line was known to express somewhat differently from other gfa2-*lacZ* mice (5), including expression in cerebellar Purkinje cells (12), we next examined the previously described gfa2-clac line (line 6.4 of (5)). Use of this line also allowed testing the possibility that the nuclear targeting signal may be responsible for the neuronal expression. Although it was significantly weaker and sparser than for gfa2-nlac1, significant neuronal activity was also detected for gfa2-clac, especially in the lateral septal nucleus, hippocampus (Fig. 1E and F) and cerebellum (data not shown). Apparent colocalization could be an artifact of closely apposed astrocytes and neurons. To examine this possibility, we analyzed expression by electron microscopy of Bluo-gal stained sections. *LacZ* expressing neurons, identified by Bluo-gal staining, were identified in both the gfa2-nlac1 and gfa2-clac lines by the presence of synapses (Fig. 2).

Although both the gfa2-nlac1 and gfa2-clac lines show neuronal expression, the patterns and intensities of these activities vary. We thus produced two additional lines, gfa2-nlac2 and gfa2-nlac3, to further characterize the specificity of expression. The astrocyte expression of both lines is similar to that of gfa2-nlac1, although the activity level of gfa2-nlac2 is only about half that of gfa2-nlac1. The neuronal expression of gfa2-nlac3 closely mirrors that of gfa2-nlac1 (data not shown), while that of gfa2-nlac2 is far less than the other three lines, with only weak expression being observed in a group of paranigral neurons (Fig. 3).

The distribution of neuronal activity for the gfa2-clac, gfa2-nlac1 and gfa2-nlac2 lines were compared in greater detail by quantitating the expression in multiple brain regions. No consistent pattern was found among these lines (Table I). For example, although the neuronal activity of gfa2-nlac2 is overall much less than gfa2-nlac1, it is much higher in the paranigral nucleus. On the other hand, only gfa2-nlac1 expresses in cerebellar Purkinje cells, whereas gfa2-clac is more active than the others in neurons in the cerebellar molecular layer. In contrast to the neuronal expression seen for these lines, no *lacZ* activity was found to colocalize with the oligodendrocyte marker CNPase (data not shown).

Neuronal Activity of a Mouse GFAP-*lacZ* Transgene

A mouse genomic GFAP clone had also been reported to drive *lacZ* expression almost exclusively in astrocytes (4). That transgene consists of a genomic fragment of the mouse GFAP gene extending from about 2,000 bp upstream of the RNA start site (similar to our human gfa2 promoter) to about 1,500 bp downstream of the end of the coding region, and having the *lacZ* gene embedded within the first exon. Accordingly, we re-examined cell specificity in these mice, which were kindly provided by Dr. Lennart Mucke at the University of California at San Francisco. In the single line still available for analysis, we also found that activity was not specific to astrocytes, but was present in many cerebellar granule neurons, a few neurons in the hippocampal CA3 region, pontine nuclei, and in epithelial cells of the lens and cornea of the eyes (Fig. 4). Electron microscopy confirmed neuronal expression of *lacZ* in cerebellar granule cells (data not shown).

Astrocyte-Specificity of Human GFAP-*GFP* Transgenes

In contrast to these findings of neuronal expression, multiple laboratories have published studies using our gfa2 promoter for transgenic studies, and most have reported astrocyte specificity of expression (these are reviewed in the Discussion). Striking among these have been the generation of gfa2-*GFP* (8) and gfa2-*EGFP* (13) mice. However, in these other studies, cell specificity was determined either by innate fluorescence (GFP) or fluorescent secondary antibodies. These detection methods are unlikely to have the same sensitivity as X-gal staining, which involves an enzymatic amplification. Indeed, when we used standard fluorescence IHC for *lacZ* in the gfa2-nlac1 line, no neuronal expression was detected (Fig. 5A). However, when the enzymatically amplified TSA method was used for fluorescence IHC, the neuronal *lacZ* expression was readily visualized (Fig. 5B and C). We thus reinvestigated the specificity of expression of the Zhuo et al. (8) gfa2-*GFP* mice using both innate

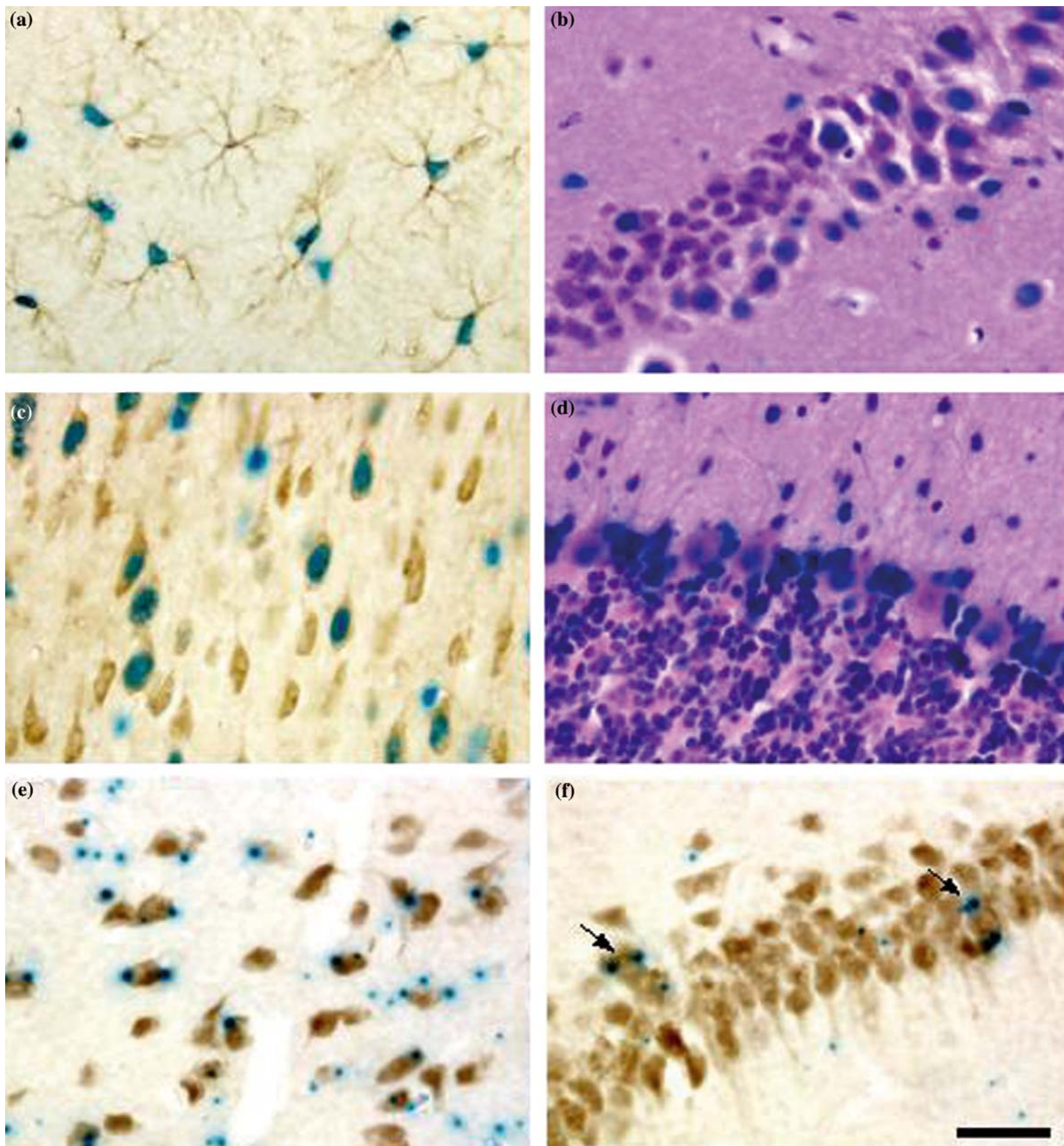

Fig. 1. Neuronal expression in the gfa2-nlac1 and gfa2-clac lines. The lacZ reporter was detected using X-gal staining (blue), and GFAP and NeuN by IHC using the peroxidase method (brown). A–D, gfa2-nlac1 line. (A) Control showing cerebral white matter stained for X-gal and GFAP. Note lacZ expression in astrocytes. (B) Hippocampus stained with X-gal and H&E. Many CA2 neurons are positive for X-gal. (C) Cerebral frontal cortex stained for X-gal and NeuN. Many neurons are positive for X-gal. (D) Cerebellar cortex stained with X-gal and H&E. Many Purkinje cells are positive for X-gal. (E,F), gfa2-clac line. (E) Cerebral lateral septal nucleus stained with X-gal and NeuN. X-gal-positive dots appear to be localized in the cellular membrane and cell bodies of neurons. (F) Hippocampus stained for X-gal and NeuN. A few neurons appear to express lacZ (arrows). Bar = 25 μm.

GFP fluorescence and TSA IHC. Even with this highly sensitive procedure, expression still appeared to be completely astrocyte specific (Fig. 6, Table I). Immuno-EM for GFP also failed to show any neuronal expression (data not shown).

Neuronal Activity of Human GFAP-PPCA Transgenes

As a further test of the properties of the gfa2 promoter we examined three transgenic lines in which this element drove expression of the lysosomal

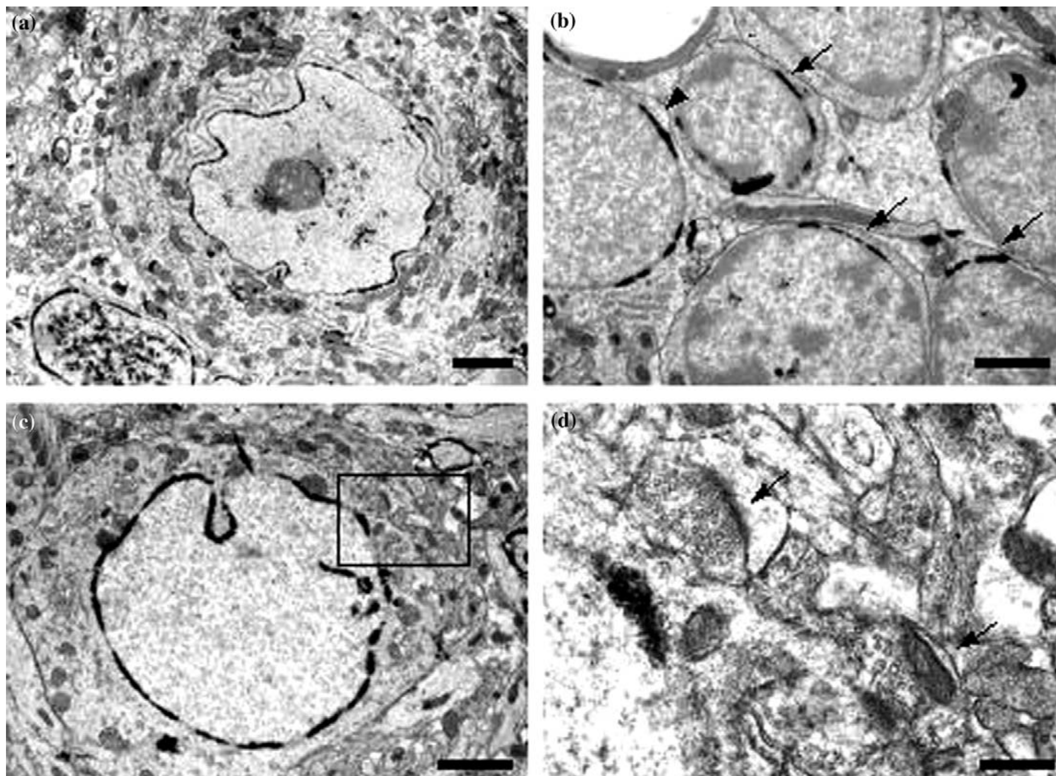

Fig. 2. Electron micrographs of Bluo-gal stained cerebellar cortex. (A) Cerebellar cortex of the gfa2-nlac1 line. A Bluo-gal positive Purkinje cell (upper right) and a Bluo-gal positive Bergmann glial cell (lower left) are seen. (B) Cerebellar cortex of the gfa2-clac line. A Bergmann glial cell (arrowhead), and three granule cells are positive for Bluo-gal staining (arrows). (C) Cerebral cortex of the gfa2-clac mouse. A Bluo-gal positive medium sized neuron is seen in the cerebral cortex. (D) A magnified field indicated in C shows two presynaptic terminals with pleomorphic vesicles in direct contact with the Bluo-gal stained cell (arrows). Bar = 3 μm in A, 1.5 μm in B, 2 μm in C and 0.4 μm in D.

enzyme, PPCA. One of these lines gave barely detectable activity in the CNS, but the other two were strongly active. Detection by both *in situ* hybridization (data not shown) and IHC (Fig. 7) revealed strong neuronal expression, comparable to that seen in astrocytes. In contrast to the results with the gfa2-*lacZ* transgenics, the gfa2-PPCA transgenics expressed in neurons in most brain regions, with the exception of cerebellar Purkinje cells.

DISCUSSION

Neuronal Activity Depends on Integration Site and Expressed Sequence

Our results show that both human and mouse GFAP-*lacZ* transgenes can express significantly in neurons. Detailed examination of the regional pattern of this expression reveals that it varies from line to line in both frequency and distribution (Table I), suggesting that the extent of neuronal activity is highly dependent on the transgene integration site. Results similar to these were previously reported for a mouse GFAP promoter from the *Mus spretus* strain (14). In that study, seven lines expressing an nlac reporter were all found to express in various populations of neurons. Double label IHC with a neuron specific marker was not performed, but neuronal activity was confirmed in several instances by electron microscopy. Similar to our mice, no two lines displayed the same neuronal expression pattern, but activity was most frequent in the cerebellar granule cell layer and the hippocampal region. The transgene copy number for these *Mus spretus* mice ranged from just 1 to 6, with no correlation between copy number and expression pattern. This rules out an alternative explanation for loss of tissue specificity—that a repressor plays a role in the regulation and is titrated out by a very high transgene copy number.

The sequence of the targeted gene appears to be another factor that can contribute to the extent

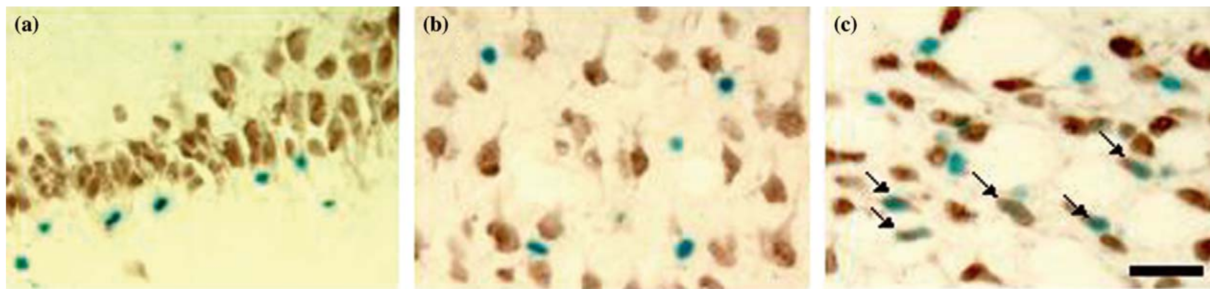

Fig. 3. LacZ expression in the gfa2-nlac2 line. Hippocampus (A), cerebral cortex (B) and paranigral nucleus of the brain stem (C) stained with X-gal and NeuN. No X-gal-positive neurons are seen in the hippocampus and cerebral cortex. Some neurons are positive for X-gal in the paranigral nucleus of the brain stem (arrows). Bar = 25 μm.

Table I. Distribution of Neuronal Expression in Four Different Transgenic Lines

	Transgenic lines			
Region	gfa2-clac	gfa2-nlac1	gfa2-nlac2	gfa2-GFP
Cerebral cortex	11/100 (EM)	**32/100**	0/100	0/100
Hippocampal CA1-2	3/100 (EM)	**30/50 (CA2)**	0/100 (EM)	0/100 (EM)
Lateral septal nucleus	**31/100**	**22/100**	0/100	0/100
Caudate-putamen	6/100	7/100	0/100	0/100
Thalamus	2/100	**24/100**	0/100	0/100
Superior colliculus	3/100	10/100	0/100	0/100
Nucleus of the trapezoid body	0/100	**35/60**	0/67	0/89
Spinal vestibular nucleus	0/100	6/100	0/100	0/100
Paranigral nucleus	ND	0/100	12/100	0/100
Cerebellar Purkinje cells	0/100 (EM)	**78/100 (EM)**	0/100 (EM)	0/100 (EM)
Cerebellar molecular layer	**15/25 (EM)**	7/36 (EM)	0/100 (EM)	0/100 (EM)
Olfactory bulb granule cells	3/100	8/100	0/100	0/100
Spinal gray matter	0/100	0/100	0/100	0/100

Data are the number of positive neurons/total number of neurons examined; bold : high level of neuronal expression (> 20% of neurons); ND: not done. *LacZ* and *GFP* transgenics were analyzed at the light microcopic level by double label staining for X-gal and NeuN or GFP IHC and NeuN, respectively; or by EM using Bluo-gal and morphology or GFP IHC and morphology, respectively (data obtained by EM are indicated).

of neuronal expression. Particularly strong neuronal activity was observed throughout the brain for two of three gfa2-PPCA transgenic lines. The pattern of activity was similar for the two lines, but differed from that of the gfa2-*lacZ* transgenics in both the intensity and ubiquity of neuronal activity. At the other end of the neuronal activity spectrum, two groups have reported complete astrocyte specificity for gfa2-*GFP* (8) and gfa2-*EGFP* transgenics (13), and we have here confirmed this result for the gfa2-*GFP* line.

Survey of the Literature for Astrocyte Specificity of GFAP Transgenes

To obtain a more complete view of the specificity of GFAP promoter expression, we performed a PubMed search using "GFAP and transgen*", and Citation Index searches for the original papers describing the GFAP promoters from mouse (4) and human (5). This search yielded 144 papers involving use of GFAP promoters, of which 45 included an examination of the cell specificity of expression within the CNS (Table II). All but nine of these publications reported activity only in astrocytes, and for six of these nine, the non-astrocytic expression was extremely rare or even uncertain (15,17,19,28,35,43). One of the remaining three papers that did observe significant non-astrocytic activity is that of Galou et al. (14) discussed above. A second is the report of Peel and Klein (26) that an adeno-associated virus carrying a gfa2-*GFP* transgene produced abundant neuronal expression when injected into rat spinal cord. This finding suggested that other promoters present in a viral vector might override the GFAP promoter specificity. However, this does not appear to be a general problem, as a high level of astrocyte tar-

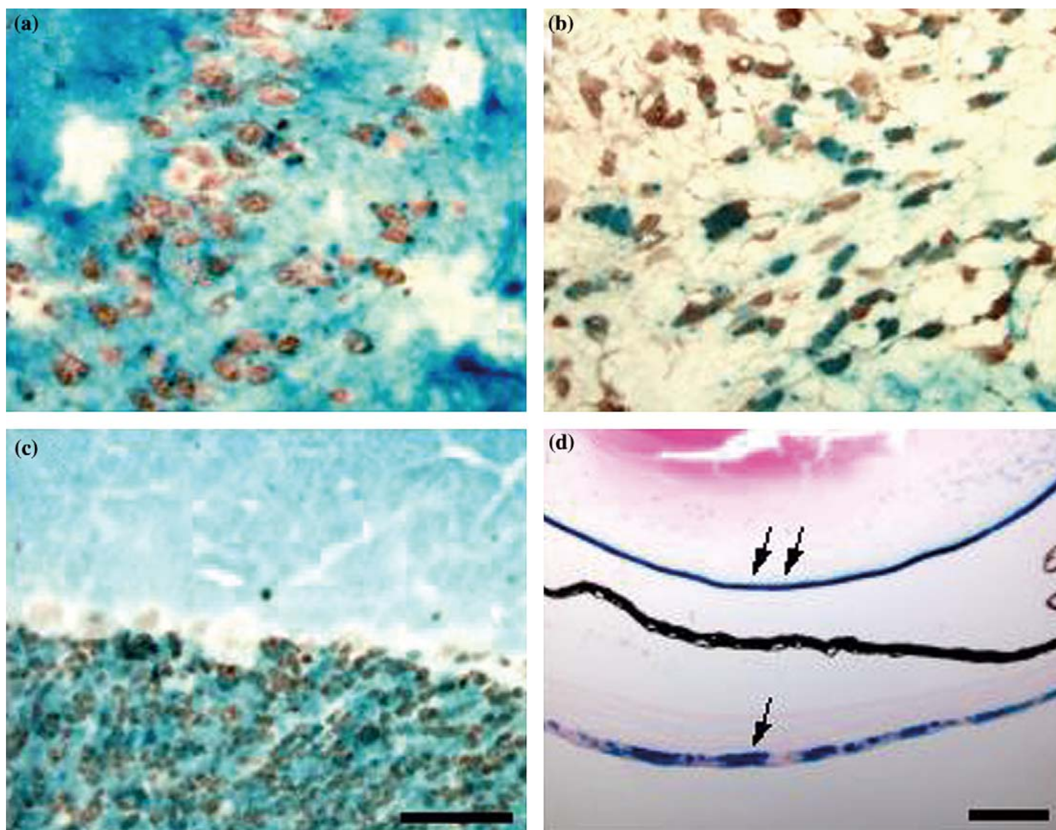

Fig. 4. LacZ expression in the mouse GFAP-lacZ line. (A) Hippocampal CA3 region stained with X-gal and NeuN. Some X-gal positive dots are seen in the peripheral region of NeuN positive neurons. (B) Pontine nucleus stained with X-gal and NeuN. Many neurons are positive for X-gal staining. (C) Cerebellar cortex stained with X-gal and NeuN. Many X-gal positive granule cells are seen in the granule cell layer. (D) Eye stained with X-gal and H&E. X-gal-positive material is seen in lens (double arrows) and cornea (arrow). Bar = 50 μm in A–C, and 250 μm in D.

geting has been reported for GFAP transgenes using adenovirus (51), herpes simplex virus (36) and lentivirus (28). The final report of failed specificity is from the study of Zhang et al. (53) in which 6-OHDA lesioned rats were treated by vascular delivery of a gfa2-TH transgene plasmid encapsulated in pegylated immunoliposomes. These authors reported that this transgene therapy resulted solely in activity in NeuN positive cells in the substantia nigra, with no expression in astrocytes. This surprising finding contrasts with a previous report from the same laboratory, in which astrocyte specific expression was observed when an encapsulated gfa2-clac plasmid was used (37). It is also contrary to observations of Segovia et al. (52), in which lipofection of a gfa2-TH plasmid into the striatum of 6-OHDA lesioned rats resulted in astrocyte specific expression. It remains an open question whether the particular combination of conditions used by Zhang et al. (53) produced a reversal of promoter specificity, or whether the TH monitored was of endogenous rather than transgenic origin.

None of the papers surveyed reported any expression in oligodendrocytes or microglia, though several observed activity in cells of unknown type. A potentially important methodological point is that most of the studies of cell specificity relied on either morphology or GFAP antibodies to identify cell types. Only nine studies used a neuronal marker, and nearly half (four) of these reported non-astrocytic expression, with two being in neurons (28,53) and two in undefined cells in the subfornical organ that stained for GFAP, MAP-2 and NeuN (15,43). In contrast, in the absence of a neuronal marker, only about 15% (five of 36) of the investigations reported neuronal activity (14,17,19,26,35). It is possible

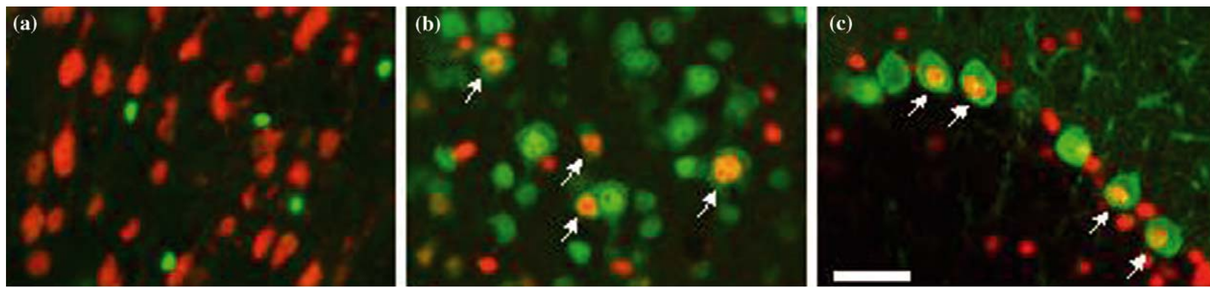

Fig. 5. Tyramide amplification method for detection of neuronal expression of gfa2-nlac1. (A) Cerebral cortex stained using standard immunofluoresence for β-gal (green) and the TSA method for NeuN (red). No neuronal expression of lacZ could be detected. (B) Cerebral cortex stained using the TSA method for β-gal (red) and standard IHC for NeuN (green) (note reversal of colors from A). Neuronal expression of lacZ was clearly detected (arrows). (C) Cerebellar cortex stained using the TSA method for β-gal (red) and standard IHC for CaBP (green). LacZ expression was detected in Purkinje cells (arrows). Bar = 25 μm.

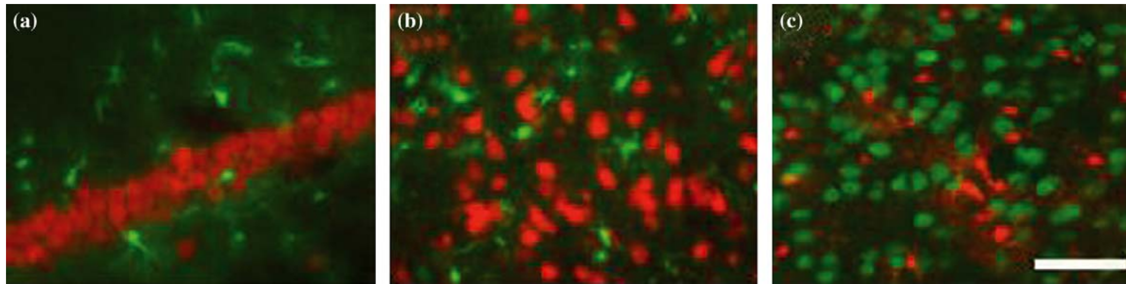

Fig. 6. GFP expression in the gfa2-GFP line. Hippocampus (A) and cerebral cortex (B) with standard double label immunofluorescent staining for GFP (green) and NeuN (red). No colocalization of GFP-positive cells and NeuN-positive neurons is observed. (C). Cerebral cortex stained by the tyramide method for GFP (red) and by standard immunofluorescence for NeuN (green) (note reversal of colors from A and B). No GFP signals could be detected in neuronal cell bodies. Bar = 50 μm.

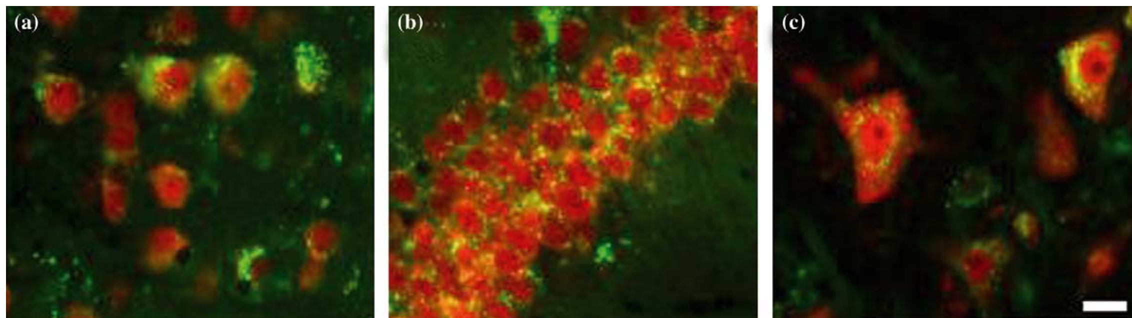

Fig. 7. Expression of a gfa2-PPCA line. Cerebral cortex (A), hippocampus (B) and medial vestibular nucleus of the brain stem (C) stained for PPCA (green) and NeuN (red). Many PPCA-positive dots are seen in the cytoplasm of the NeuN-positive neurons in all regions. Bar = 25 μm.

the observed incidence of neuronal expression of GFAP transgenes would be much higher were neuronal markers routinely used; on the other hand, it is also possible that they were used in the cited instances because morphology or absence of GFAP staining had raised suspicion of such activity.

Sensitivity of the Transgene Assay

Overall, our survey of studies of a wide range of GFAP-driven transgenes has yielded only a few instances in which expression clearly occurs in neurons. This suggests that the neuronal expression we have shown in Results, and that was previously

Table II. Survey of Findings for CNS Expression of GFAP Transgenes

Tg	Method	Cell Types				Comments	Ref
		as	nr	ol	Mg		
h-angiotensin (3); (genomic)	fIHC/fIHC#	y	*			*; fIHC# = GFAP and MAP-2	15
h-ApoE3 & h-ApoE4	fIHC/fIHCg	y	n	n	n		16
h-ApoE4* (1); genomic	fIHC/fIHCg	y	n	n	n	*	7
h-ApoE4 (2); genomic	ISH/mor	y	?#	n	n	could not rule out weak expression in cortical layer II and V neurons using ISH; not seen by	17
	pIHC/mor	y	n	n	n	pIHC	
h-CCL2; genomic	ISH/#	y#	n#	n#	n#	method for identifying astrocytes by ISH not stated	18
m-cre	ISH/pIHCg	y	?			some expression in undefined cell population in hippocampal C4 region	19
h-cre (1)	fIHC/fIHCg	y	n	n	n	*	20
	fIHC/mor	y	*	*	n		
h-cre-IRES-lacZ	X-gal/pIHC#	y	n	n		pIHC# = GFAP, adenomatous polyposis coli (oligodendrocytes) and MAP2	21
h-cre	fIHC1/fIHCg	y				*; fIHC1 = lacZ	22
sm-ecdysone-inducible VgRXR	fIHC1/fIHCg	y	n	n	n	*; fIHC1 = ErbB1	23
h-dominant negative ErbB4	fIHC/fIHCg	y	n	n	n		24
gfa2-GFP (2)	GFPf/mor	y	n	n	n	*	8
	GFPf/fIHCg	y	n	n	n		
h-GFP (1)	GFPf/mor	y	n	n	n	*; # = GFAP, NeuN, MAG, MAC-1; Ephys of over 200 GFPf cells was consistent with their	13
	GFPf/fIHC#	y	n	n	n	being astrocytes	
	pIHC/EM	y	n	n	n		
	Ephys#	y	n	n	n		
h-GFP	GFPf/fIHCg	y	n	n	n	examined along a needle track only	25
h-GFP; AAV	#	y	y			injection or recombinant AAV into adult rat spinal cord yielded primarily neuronal expression (detection methods not specified)	26
sm-GFP (1)	GFPf/fIHC#	y	n			*; yes = Müller cells; fIHC# = GFAP, CRALBP, glutamine synthetase	27
h-GFP; lentivirus	GFPf/fIHCgn	y	y#			70% of the GFP-positive cells stained for GFAP, and 0.6% for NeuN	28
m-gp120	ISH/pIHCg	y	n	n	n	*	29
sm-growth hormone (3)	pIHC/mor	y	n	n	n	*	30
h- hemagglutinin (1)	pIHC/mor	y	n	n	n		31
m-IL-12 (1)	ISH/pIHCg	y	n	n	n		32
m-clac (4)	X-gal/mor	y	n	n	n		4
	X-gal/pIHCg	y	n	n	n		
gfa2-clac (5)	X-gal/pIHCg (6)	y	n	n	n	only 1 line (gfa2-nlac1) expressed in retinal Müller cells (active in both normal and reactive	12
gfa2-nlac (5)	Bluo-gal/EM (3)	y	n	n	n	Müller cells); X-gal/pIHCg in cerebellum only; Bluo-gal/EM of optic nerve only	
sm-nlac (7)	X-gal/mor	y	y	n		*; pIHC#=GFAP & carbonic anhydrase	14
	X-gal/pIHC#	y		n			
	X-gal/EM		y				
m-clac (2) & sm-clac (2)	gal/pIHCg	y	n	n	n	*	33
m-lacZ	X-gal/pIHCg	y	n			examined eye only; no = resting & reactive Müller cells, cornea, choroid; yes = lens epithelium	34
h-lacZ; plasmid#	X-gal/pIHCg	y				naked DNA was injected into rat thalamus; most activity was in astrocytes, though some other	35
	X-gal/mor		#	#	#	unspecified cell types also stained with X-gal based on morphology	
h-lacZ; HSV*	X-gal/pIHC#	y	n	n		*; pIHC# = GFAP, S100, neurofilament, galactocerebroside	36
gfa2-clac; liposome*	fIHC/fIHCg	y				*	37
h-MHC class II transactivator (2)	fIHC/fIHCg	y	n	n	n		38
h-cMyc minigene*	tumors/fIHCgn	y	n	n	n	*; induced tumors stain for GFAP and not NeuN	39

Table II (Continued)

Tg	Method	Cell Types				Comments	Ref
		as	nr	ol	Mg		
sm-nitroreductase (1)	pIHC/pIHCg	y	n	n	n	*	40
m-prion protein	ISH/pIHCg	y	n#	n#	n#	cryoinjury was required to see the ISH signal, which prejudices to reactive astrocytes	41
h-Ras-IRES-lacZ	pIHCz/pIHCg	y#	n#	n#	n#	data presented are low power images of pIHCz/pIHCg in adjacent sections; state no	42
	tumors/pIHCg	y	n	n	n	expression in microglia (data and methods of identification not given)	
h-renin(2)*	fIHC/fIHC*	y	*			*; fIHC# = GFAP, MAP-2 and NeuN	43
h-SOD1 (2)	ISH/mor	y	n	n	n	analyzed spinal cord and brainstem	44
m-v-src	ISH/pIHC#	y	n	n	n	IHC# = GFAP & synaptophysin	45
	tumors/pIHCg	y	n	n	n		
h-SV40 T antigen	fIHC/fIHCg	y	n	n	n	*; pIHC# = GFAP, S100, synaptophysin, TuJ-1, PSA-NCAM	46
	tumors/pIHC#	y	n	n	n		
m-TGF-β1 (2)	fIHC/fIHC#	y			n*	*; fIHC# = GFAP & F4/80	47
h-TK	ISH/mor	y	n			examined cerebellum only; low power images	48
m-TK	fIHC/fIHCg	y	n	n	n	*	49
m-TK	fIHC/fIHCg	y	n	n	n	examined periventricular forebrain germinal zone of adult mice	50
h-tTA; AdV	GFPf/mor	y	n	n	n	*	51
h-TH; liposome	fIHC/fIHCg	y	n	n	n	plasmid in liposomes injected into the stiatum of 6-OHDA lesioned rats	52
h-TH; liposome	fIHC/fIHCgn	n	y			*	53

Entries were obtained by a PubMed search using "GFAP and transgen*", and Citation Index searches for the original papers describing the GFAP promoters from mouse (4) and human (5); they are arranged alphabetically by transgene, ignoring prefixes such as c, v and dominant negative; the "E" has been omitted from EGFP constructs. Notations used are as follows: # = see explanation in Comments column; * = see Table IV for additional information. Tg column: tg = transgene or transgenic; h- = human gfa2 promoter unless indicated otherwise (to facilitate identification, "gfa2" is specifically used for constructs analyzed in Results); m- = genomic mouse promoter as described by Mucke et al. (4); sm- = smaller mouse promoter consisting of the 5′-flanking regions (C-259 of Johnson et al. (33)) unless otherwise stated; tTA ; tetracycline sensitive transactivator; TK ; HSV thymidine kinase; TH = tyrosine hydroxylase. Expressed sequences were cDNAs unless noted as minigenes or genomic fragments. Number in parenthesis following the tg description or analysis method is the number of lines analyzed; no number is given if it is not stated in the paper, even though multiple lines may have been made. Analysis was in tg mice unless noted otherwise as a viral vector (AAV, adeno-associated virus; AdV, adenovirus; HSV, herpes simplex virus), plasmid (transfected with naked DNA) or liposome (plasmid transfected as a liposome). Method: ISH ; *in situ* hybridization; RPA ; riboprobe protection assay, X-gal = histochemical assay with X-gal; Bluo-gal ; histochemical assay with Bluo-gal; pIHC = peroxidase linked IHC; fIHC = fluorescent IHC; GFPf = innate GFP fluorescence; Ephys = electrophysiology; mor = morphology of stained cells, tumors = based on tissue location of tumors; IHC = Ab against the tg, IHCz = Ab against lacZ; IHCg = GFAP Ab; fIHCgn = separate use of GFAP Ab and NeuN Ab. Methods separated by a slash (/) = both applied to same cells (e.g., double label fIHC). Cell types: as = astrocytes; nr = neurons; ol = oligodendrocytes, mg = microglia; y = yes (activity present); n = no (no activity); ? = nature of expressing cells or presence of activity is uncertain.

Table III. Survey of Findings for Non-CNS Expression of GFAP Transgenes

Transgene	Method	Tissue: Ectopic: hr	lg	kd	sk	sp	th	GFAP+: lv	sc	ts	Comments	Ref
h-angiotensinogen antisense* (2); rat	northern	y						y			*; yes = adrenals (northern & ISH)	66
	ISH	y						y				
h-angiotensin (3) (genomic)	RPA	y	y	y	y	n		y*		y	*; no = ovary; yes = adrenal gland, aorta, salivary gland, diaphragm, white adipose tissue, brown adiopose tissue	15
h-angiotensinogen II (1)	RPA	n	n	n				n			no = stomach	67
h-cre (1)	ELISA	n	n	n		n		n			*; no = sciatic nerve & pancreas (ELISA)	20
	X-gal							y				
h-cre-IRES-lacZ	X-gal	n		n				n	n		no = intestine, other	21
h-EAAT2	western	n	n	n							no = other	68
sm-ecdysone-inducible VgRXR	western	n						n			*; no = ovary; yes = stomach (enteric glia?)	23
h-dominant negative ErbB4	western & RPA#							n			no = uterus, ovary, other; used RPA as well as western, but not stated for which tissues each method was used	24
h-dominant negative ErbB4	fIHC/fIHC#								y		no = myelinating sc; some lines expressed predominantly in astrocytes and others in non-myelinating sc; fIHC* = GFAP, p75 (non-myelinating sc), P0 (myelinating sc)	69
h-GFP (1)	GFPf/mor								y*		*	13
gfa2-GFP	GFPf/mor								y		*; yes = inner ear	62
sm-growth hormone (3)	RT-PCR	n	y	y	n	n		n		n	*	30
	western	n	n	n	n	n		n		n		
h- hemagglutinin (1)	northern	n					n	n			no = small bowel (northern); yes = small and	31
	RT-PCR	n				n	n				large intestines (RT-PCR), eteric glia (pIHC/mor)	
h- hemagglutinin	RT-PCR					y	y	y			Same mice as (31)	70
m-IFN-α (2)	RPA			n		n		n				71
sm-IFNγ#	RT-PCR	n	n	n	n	n		n			used 2.7 kb 5'flanking sequence for promoter; no = skin, stomach, other	72
m-IL-6 (3)	northern			n		n		n				73
m-IL-12 (1)	RPA	n	n	n	n	n	n	n				32
m-clac (4)	X-gal	n	n	n#		n#	n#	n	n	n#	n# = equivalent to non-transgenic controls; also for salivary gland, epidydimus (X-gal); no = salivary gland, pancreas (northern)	2
	northern		n	n		n	n	n				
gfa2-clac (5)	enz assay (4)	n	n	n		n		n			only 1 line (gfa2-nlac1) expressed in sc and enteric	12
gfa2-nlac (5)	X-gal (10)								y#		glia	
m-clac (2)	RPA	n	n	n		n	n	n	n	y	*; yes = lens epithelium, salivary gland; no = cer-	33
sm-clac (2)	X-gal or pIHC	n	n	n		n	n	n	n	y	ebellar Bergmann glia, pancreas (X-gal)	
h-lacZ; HSV*	X-gal/pIHC*										*; no = DRG	36
gfa2-clac; liposome*	X-gal	n	n			n		n			*	37

Table III. (Continued)

Transgene	Method	Tissue									Comments	Ref
		Ectopic						GFAP+				
		hr	lg	kd	sk	sp	th	lv	sc	ts		
gfa2-nlac1	X-gal/mor								y		*; yes = inner ear	62
h-MCP-1; genomic	northern	n	n	n	n	n		n	y			74
m-MHC class I antigen D^b minigene	RT-PCR			n		n	y#	n	n	n	no = pancreas; # = very low	75
h-MHC class II transactivator (2)	pIHC	n		n		n	n				no = lymph node	38
sm-nitroreductase (1)	northern	n	n	n				n		y	*; no = intestine, ovaries, uterus	40
	western	n	n	n				n		n		
m-prion protein	western	n	n	n	n	n	n	n				41
h-Ras-IRES-lacZ	northern	n	n	n	n	n		n				42
h-renin (2)*	RPA	y	y	y	y	y		y		y	*; yes = adrenal gland, aorta, salivary gland, diaphragm, white adipose tissue, brown adiopose tissue	43
m-v-src	RT-PCR		y			y	y			y	IHC# = GFAP & synaptophysin	45
	northern									y		
	ISH/pIHC#								y			
	tumors/pIHCg	n	n	n	n	n	n	n	y	n		
m-v-src	tumors	n	n	n	n	n	n	n	y	n	no = intestine; yes = fibroblasts, endoneurial cells, spinal bone marrow stromal cells, eteric glia	59
h-SV40 T antigen	tumors/fIHC	n	n	n	n	n	n	n	n	n	*	46
h-tTA (1); rat	RT-PCR	n	n	n			n	n			recreated gfa2 promoter by PCR	76
h-TK	RPA							n			TK known to inherently express in ts	48
	ISH									y#		
m-TK	RT-PCR	y	y	y		y		y	n		yes = intestine, trigeminal nerve, adrenal gland no = pancreas, esophagus, stomach	77
h-TH; liposome	TH enzyme assay	n	n	n				n			*	53

Abbreviations are the same as for Table II, with the following additions:
Tissue: GFAP+: reported to express endogenous GFAP; ectopic: not reported to express GFAP; hr: heart; lg: lung; kd: kidney; sk: skeletal muscle; sp: spleen; th: thymus; lv: liver; sc: Schwann cells; ts: testes; other: unspecified other non-CNS organs.

observed by Galou et al. (14), may be attributable to the *lacZ* reporter. However, an alternative explanation is that the level of neuronal expression is so low that its detection requires a highly sensitive assay. *LacZ* staining using X-gal or Bluo-gal has an inherent enzymatic amplification step. Indeed, although we could readily detect neuronal expression in the several gfa2-*lacZ* lines by using X-gal, we could not do so by standard fluorescence IHC (Fig. 5A) or peroxidase linked IHC (data not shown). Possibly, neuronal expression of other transgenes has been missed because of insufficiently sensitive assays. On the other hand, use of the TSA method did reveal neuronal staining in the gfa2-*lacZ* lines (Fig. 5B and C), yet with this procedure gfa2-*GFP* activity continued to appear astrocyte specific. It cannot be excluded that the TSA method is less sensitive for GFP than for β-galactosidase, but other activity differences between gfa2-*lacZ* and gfa2-*GFP* transgenes suggest that the attached gene (*lacZ* or *GFP*) plays a role. In particular, both the mouse and human GFAP-*lacZ* transgenes have been found to express poorly if at all in reactive Müller cells (5,34), although these cells can produce abundant amounts of endogenous GFAP (54). In contrast, both mouse and human GFAP-*GFP* constructs do express in these cells (8,13,27). Clearly this difference cannot also be attributed to GFP detection being less sensitive than X-gal staining! We thus conclude that the sequences attached to the promoter for expression can significantly affect the activity pattern. This is also a likely explanation for the particularly strong and widespread neuronal expression of the gfa2-PPCA transgenes. In addition to the influence that the transgene integration site might have on PPCA expression levels, the ectopic expression of the PPCA fragment is likely due to its being an intron-containing minigene; it is not unusual for enhancers to be present within introns; and if present, they could influence the transgene expression pattern.

Level of Expression in Astrocytes

The nature of the expressed sequence appears to influence not only the extent to which it is expressed in neurons, but also in astrocytes. Even with the highly sensitive X-gal assay, the *lacZ* reporter is usually detected in only a small fraction of astrocytes (4,5,14), whereas Bush et al. (55) report that 85% of GFAP positive astrocytes express mGFAP-TK in the hippocampus, and this increases to 92% following a stab injury. In the thalamus the percentage of GFAP positive cells expressing TK was as high as 98% following a stab injury, and similarly high levels were found in the cerebral cortex. High levels of expression were also observed for the gfa2-*EGFP* transgenic line, although the pattern of transgene activity did not always correlate with that for endogenous GFAP (13). For example, in several thalamic nuclei far more astrocytes expressed EGFP than GFAP, whereas the reverse was true for the superchiasm nuclei. For such presumably innocuous reporters as *lacZ*, *EGFP* and TK (the latter in the absence of drug), these differences may reflect differential susceptibility to transgene inactivation (56). On the other hand, if expression of the transgene results in a reactive response, the pattern of transgene activity could be altered by positive feedback on the GFAP promoter in a region-specific manner (reviewed in (6)). This might account for the mGFAP-gp120 transgene showing strong expression in the cerebral cortex, and relatively weak activity in cerebellum (29), which is the opposite of what is generally observed for GFAP-driven transgenes.

Non-CNS Expression of GFAP Transgenes

In addition to the potential for neuronal expression, GFAP-driven transgenes may also be active outside of the CNS. Endogenous GFAP expression has been reported for several non-CNS tissues, albeit at levels generally much lower than in astrocytes. These include non-myelinating Schwann cells (57), enteric glia (57), lens epithelium (34), fibroblasts (59), liver perisinusoidal stellate cells (60), Leydig cells in the testes (61), support cells in the inner ear (62), breast myoepithelial cells (63), respiratory tract chondrocytes (64) and lymphocytes (65). The literature search described above provides evidence for GFAP transgene activity in all but the last three of these tissues (Table III). However, as for neuronal expression discussed above, these activities have been highly variable and likely depend both on sequences present in the expressed sequence and the particular transgene integration site. For example, only 1 of 10 gfa2-*lacZ* transgenic lines examined expressed in non-myelinating Schwann cells (5), whereas both gfa2-*GFP* transgenic lines do so (8,13). A particularly interesting case is reported by Chen et al. (69), who found that a gfa2-driven dominantly negative ErbB receptor was expressed predominantly in astrocytes by some lines, and predominantly in Schwann cells by

Table IV. Specificity of GFAP Transgene Expression: Additional Comments

Ref	Additional comments
7	hGFAP promoter extended to −1992 rather than the −2163 used for gfa2. Lines also made for hGFAP-ApoE2 and hGFAP-ApoE3, but dbl fIHC reported only for ApoE4 in the hippocampus.
8	Autofluorescence of non-CNS/PNS tissues precluded their study. Activity found specific to astrocytes based on colocalization of GFPf/fIHC for GFAP in the cerebellum and GFPf/mor elsewhere in the brain. Activity found in reactive, but not resting retinal Müller cells. Preliminary results (not shown) suggest activity in Schwann cells.
13	Extremely strong activity; fluorescence could be seen through the skull under UV light. In the retina there was strong expression in Müller cells (presumptively reactive), but not in astrocytes. There was no correlation between the level of GFAP staining and GFP signal. Fluorescence in peripheral nerves was presumed due to labeling of non-myelinating Schwann cells.
14	Promoter spans −1913 to +92 of *Mus spretus* GFAP gene. Neuronal expression was weaker than in astrocytes, present in multiple brain regions, and highly variable among the lines. In the cerebellum, only one line expressed in Purkinje cells, whereas all 7 expressed in granule cells. EM of X-gal stained cells indicated neuronal expression in most cerebellar granule cells and sporatically in the hippocampus in both granule cells and interneurons.
15	Consistently high levels in liver, variably low to extremely low in the other non-CNS organs. In the brain, activity was astrocyte specific in all 26 regions examined except for an unusual set of cells that stained for both GFAP and MAP-2 in the subfornical organ.
20	Cre activity found specific to astrocytes based on cre/GFAP dbl fIHC in the cerebellum and cre fIHC/mor elsewhere in the brain. ELISA and X-gal were for β-gal following crosses to lacZ indicator lines; X-gal liver activity was in a subpopulation of cells (perhaps ductal cells). Expression in neural progenitor cells inferred from X-gal staining at e13.5 and widespread staining in adult neurons.
22	Gfa2-cre used to activate lacZ embedded in the endogenous connexin43 gene. In adult mice nearly all astrocytes display lacZ, indicating near universal expression of the tg in astrocytes or their progenitors. Strong activity also found in ependymal cells lining the ventricles, but not in endothelial cells or leptomeningeal cells. Activity detected by E12.5.
23	Tg is a Pon A inducible transcriptional activator. When crossed to a responsive mutant ErbB-1 transgenic and induced with Pon A, the ErbB-1 protein was detected specifically in astrocytes by dbl fIHC for ErbB-1/GFAP.
27	Promoter is a mouse GFAP fragment extending from −2560 to +61. Expression in resting and reactive Müller cells.
29	Pattern of expression differs from that seen for lacZ: have strong activity in all cerebral cortical regions and hippocampus, but little in midbrain, brainstem and cerebellum.
30	Promoter used is not clearly specified, but appears to be smGFAP; also not clear if tg is cDNA or genomic
33	Compared the genomic mouse GFAP promoter (mGFAP = C-445) with a 5′-flanking mouse promoter (smGFAP = C-259) similar to gfa2. One of two C-259 lines expressed weakly in testes, and the other weakly in salivary gland; neither of the two C-445 lines tested expressed in these two tissues.
36	Gfa2-lacZ recombinant HSV were injected into the cerebral hemisphere of mice. No expression was observed in the dorsal root ganglia following injection into the rear footpad, whereas a positive control was active.
37	Plasmid encapsulated in pegylated immunoliposomes was injected intravenously.
39	Created own 2.2 kb human promoter by PCR. Presumably identical to gfa2, but do not provide exact coordinates or state if introduced ATG → TTG at +15.
40	Used 2.7 kb mGFAP promoter extending from about −2600 to +92. Small transcripts but no protein detected in testes. By dbl pIHC, all tg expressing cells also express GFAP, and most but not all GFAP positive cells express the tg. Most activity in hippocampus and cerebellum. Find variable expression within different mice of the same line.
43	Tg includes a β-globin/IgG chimeric intron between the promoter and the cDNA. Non-CNS expression levels varied between the two lines investigated, but both were above 5% of the brain level in salivary gland, white adipose tissue and skeletal muscle. Astrocytic expression was investigated throughout the brain (26 regions) and found to be astrocyte specific except for the subfornical organ, where staining was found in an unusual set of cells that stained for GFAP, MAP-2 and NeuN, and questionable neuronal staining in the area postrema, parabrachial nucleus, mesencephalic trigeminal nucleus and cerebellum.
46	CNS specificity indicated by presence of tumors only in brain. All tumors judged to be of astrocytic origin based on staining for GFAP or S100 and not for synaptophysin, TuJ-1 or PSA-NCAM (pIHC). Dbl fIHC shows that most cells expressing the SV40 T antigen also express GFAP.
47	No expression stated for microglia, but figure presented appears to show weak staining. No investigation of neurons or oligodendrocytes. Tg expression detected in most GFAP+ astrocytes.
49	Examined expression in the subependymal zone by dbl fIHC for TK and GFAP using confocal microscopy and 3D reconstruction of entire cells. Found coexpression in all 62 cells examined.
51	Assayed gfa2-tTa tg activity by coinjection of a GFP indicator AdV into rat hippocampus. Expression was astrocyte specific based on GFPf and cell morphology; a positive control showed the indicator could express in neurons.
53	Plasmid encapsulated in pegylated immunoliposomes was injected intravenously into 6-OHDA lesioned rats. Dbl fIHC showed the TH to be present in striatal nerve terminals and NeuN positive cells in the substantia nigra, with no expression in GFAP positive astrocytes. (Note different result from previous report above (37) from the same lab using the gfa2-clac transgene.)
62	Endogenous GFAP expression was present in the organ of Corti in supporting cells of both inner and outer hair cell areas and in satellite and sc in the osseous spiral lamina and spiral ganglion (fIHC/mor). X-gal and GFPf signals were seen in these same regions, with some X-gal activity (but not GFAP) also present in the spiral limbus. In the vestibular organ both GFAP and X-gal staining were observed in the supporting cells of the sensory epithelia of the semicircular canal and utrical. X-gal but not GFAP staining was present in the transitional epithelium adjacent to the utricular macula (GFP was not examined in the vestibular organ).
66	Construct included rabbit β-globin intron 2 inserted within the antisense sequence. ISH indicated expression in heart, liver and adrenals was from endothelial cells.

Abbreviations are the same as for Tables II and III with the following addition: dbl = double label

others. These authors also noted that transgene activity was present in non-myelinating Schwann cells but absent from myelinating Schwann cells, consistent with what is observed for GFAP. Among the publications surveyed (Table III), six groups found Schwann cells to express GFAP transgenes and five reported negative results. Testes were another frequently analyzed tissue, with five groups finding expression and three obtaining negative results.

Investigations of other cell types believed to express endogenous GFAP have been quite limited. The mGFAP-TK transgene is presumed to be active in enteric glia, as these cells are ablated by treatment with ganciclovir (77,78); one of six gfa2-*lacZ* lines (gfa2-nlac1, A.M., unpublished observations) was also found to express in enteric glia, as well as a hGFAP-hemagglutin transgene (31). Rio et al. (62) found that both the gfa2-nlac1 and gfa2-*GFP* transgenic mice express in support cells and other epithelial cells of the inner ear, and Hainfellner et al. (59) deduced the expression of a mGFAP-*v-src* transgene in fibroblasts, Schwann cells and enteric glia based on the development of tumors in these tissues. Several groups have reported that the original mGFAP-*lacZ* line expresses in lens epithelium (33,34,79), a finding that we have also confirmed (Fig. 4). Curiously, no other transgene, including the gfa2-*lacZ* constructs (A.M and M.S., unpublished experiments), has been noted to express in this tissue. There has been no published report commenting on whether or not there was expression in liver stellate cells, but we have not seen such activity in any of six gfa2-*lacZ* lines examined (A.M., unpublished experiments). There also has been no report specifically stating the absence of transgene expression in any of these less commonly investigated tissues. However, many papers do make the general statement that there is no expression in non-CNS organs.

Table III also presents data indicating that GFAP transgenes may express in non-CNS tissues that do not stain for endogenous GFAP. Reports for the most common tissues surveyed, heart, lung, kidney, skeletal muscle, spleen and thymus, are indicated in individual columns; those for other tissues are given in the comments. Of the 33 papers reporting on at least one ectopic tissue, 10 found some level of activity, with half being with the human promoter and half with the mouse. The highly sensitive RT-PCR technique was used in five of these cases, suggesting that there is a low level of leakiness (in only three reports did RT-PCR not find expression outside the CNS). In the one instance where expression was checked both by RT-PCR and a less sensitive method (western blotting), no signal was found for tissues that were positive by RT-PCR (30).

Of the five reports of ectopic expression in which less sensitive detection methods were used, three involved transgenes which include introns which could carry enhancer elements. In one case, the expressed sequence was an intron-containing genomic fragment (15); in another, a chimeric β-globin/IgG intron was placed between the promoter and the cDNA (43); in the third, a β-globin intron was inserted into the expressed sequence in a position relatively close to the RNA start site (66). The final two cases involve expression in the epithelium of the cornea (79, and confirmed in Results), and activity in a small set of liver cells tentatively identified as ductal cells (20). In this latter case, X-gal staining was noted in the cells following a cross of a gfa2-*cre* transgenic with the R26R indicator line. Whether this represents *cre* transgene activity in the adult liver or in a transient progenitor cell population (see below) is not known.

These results show that GFAP transgenes can express outside of the CNS both ectopically and in cells believed to express endogenous GFAP (Table IV). While ectopic activity appears to be extremely weak, with no biological effects being noted, expression in GFAP producing non-CNS cells can have profound consequences. A striking example is the ganciclovir induced lethal fulminant ileitis in mGFAP-TK transgenics due to killing of enteric glia (77). Another is the severe loss of non-myelinated axons resulting from expression of a dominant negative ErbB4 transgene in Schwann cells (69). Tumors originating in fibroblasts and bone marrow stromal cells have been reported for mice carrying a mGFAP-*v-src* transgene (59); however, the formation of such non-CNS tumors appears to be unusual, as they have not been reported for expression of such oncogenes as *ras* (42), *myc* (39), SV40 T antigen (46) or even another mGFAP-*v-src* transgene (45).

Expression in Progenitor Cells

A final caveat in the use of GFAP transgenes is their expression in progenitor cells. Both human and mouse GFAP-*lacZ* transgenes express as early as e12.5 (6), well before astrocytes have differentiated. Thus embryonic effects need to be considered

in interpreting results for GFAP transgenics. A particularly striking example was the attempt to use GFAP-*cre* transgenes to achieve astrocyte specific gene knockouts. Instead, large numbers of neurons and oligodendrocytes also experienced gene deletions (19,20), presumably due to *cre* expression in embryonic radial glia progenitors which give rise to a major portion of CNS neurons and oligodendrocytes in addition to astrocytes (80,81). The expression of GFAP driven transgenes in adult progenitor cells could also be a factor for certain experimental paradigms (49,50,82).

CONCLUSIONS

In summary, a large number of informative studies have been published using GFAP promoters to drive transgenes in mice, and in the large majority the incidence of non-astrocytic expression has been minimal. However, use of these promoters does require careful consideration of the requirements of the particular experiment for the extent, specificity and timing of expression. In particular, our results and a survey of the literature indicate that both the insertion site and the attached sequences can significantly affect the expression pattern of GFAP transgenes.

Especially problematical is the placement of an intron near the RNA start site, perhaps because the intron may contain regulatory elements that would then be well-positioned to influence assembly of the transcription complex. Thus, it is incumbent upon investigators to verify specificity for each line studied, using an assay system whose sensitivity is sufficient to detect a compromising level of misexpression.

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