Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 5359–5363, June 1994
Developmental Biology

Astrocytosis and axonal proliferation in the hippocampus of *S100b* transgenic mice

(neurotrophin/neurodevelopment/Down syndrome/chromosome 21)

ROGER H. REEVES*†, JIBIN YAO*, MICHAEL R. CROWLEY*‡, SUZANNE BUCK§, XIAOFENG ZHANG§, PAUL YAROWSKY¶, JOHN D. GEARHART*‖, AND DANA C. HILT§

Departments of *Physiology and of ‖Obstetrics and Gynecology, Johns Hopkins University School of Medicine, Baltimore, MD 21205; and Departments of §Neurology and of ¶Pharmacology, University of Maryland Medical School, Baltimore, MD 21201

Communicated by John W. Littlefield, February 4, 1994

ABSTRACT **S100β is a calcium-binding protein that is expressed at high levels in brain primarily by astrocytes. Addition of the disulfide-bonded dimeric form of S100β to primary neuronal and glial cultures and established cell lines induces axonal extension and alterations in astrocyte proliferation and phenotype, but evidence that S100β exerts the same effects *in vivo* has not been presented. An 8.9-kb murine *S100b* genomic clone was used to produce two lines of transgenic mice in which S100β RNA is increased in a dose-related manner to 2-fold and 7-fold above normal. These lines show concomitant increased S100β protein throughout the brain. Expression in both lines is cell type- and tissue-appropriate, and expression levels are correlated with the transgene copy number, demonstrating that sequences necessary for normal regulation of the gene are included within the cloned segment. In the hippocampus of adult transgenic mice, Western blotting detects elevated levels of glial fibrillary acidic protein and several markers of axonal sprouting, including neurofilament L, phosphorylated epitopes of neurofilament H and M, and β-tubulin. Immunocytochemistry demonstrates alterations in astrocyte morphology and axonal sprouting, especially in the dentate gyrus. Thus, both astrocytosis and neurite proliferation occur in transgenic mice expressing elevated levels of S100β. These transgenic mice provide a useful model for studies of the role of S100β in glial–neuronal interactions in normal development and function of the brain and for analyzing the significance of elevated levels of S100β in Down syndrome and Alzheimer disease.**

S100β protein is the prototype for a family of small acidic calcium-binding proteins (1). While various members of the S100 protein family are found in many tissues, the *S100b* gene is expressed primarily in the nervous system by astrocytes and Schwann cells, and the protein is found in the extracellular fluid of the brain (2). *In vitro* studies have shown that added S100β increases neurite extension of embryonic chicken and fetal rat dorsal root ganglion neurons (3), embryonic chicken cortical neurons (4, 5), mesencephalic serotonergic neurons (6, 7), and neuro-2A cells (8). S100β also promotes prolonged survival of chick cortical neurons *in vitro* (5), and *in vivo* administration promotes the survival of chick motor neurons (9). In addition to its paracrine effects on neurons, S100β stimulates glial fibrillary acidic protein (GFAP) expression, proliferation, and morphologic alteration of C6 glioma cells and primary rat astrocytes in culture (10, 11). The varied roles of S100β suggest that it may be an important mediator of glial–neuronal interactions in the normal brain.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

The human *S100B* gene is located near the distal end of chromosome 21 and thus is present in three copies in individuals with Down syndrome (DS) (12). Increased levels of S100β protein are found in the brains of individuals with DS, as predicted for a gene at dosage imbalance, and in individuals with Alzheimer disease (AD) (13, 14). These two conditions have been linked previously because individuals with DS invariably develop, at an early age, neuropathology indistinguishable from that seen in AD. Increased S100β in AD brains may reflect the astrocytosis that is a feature of this disease. Alternatively, overexpression could play a pathogenic role in the development of astrocytosis and abnormal neurite proliferation seen in DS and AD (15, 16).

Transgenic mice were described that expressed the human *S100B* gene in a largely tissue-appropriate manner at levels 10–100 fold above normal (17). No pathological changes were reported to occur in the brains of these mice. In the present study, two lines of transgenic mice were constructed that express the normal murine *S100b* gene in a cell- and tissue-appropriate manner. Analysis of these lines demonstrates astrocytosis and axonal proliferation in levels proportional to transgene copy number, demonstrating that S100β is a growth factor in the brain and that several growth factor activities previously reported *in vitro* occur *in vivo*.

MATERIALS AND METHODS

Transgenic Mice. An 8.9-kb genomic *Eco*RI fragment containing the entire *S100b* gene (18) was isolated from plasmid sequences, suspended at 300 copies per picoliter in 10 mM Tris/0.1 mM EDTA, pH 7.2, and microinjected (2 pl) into (B6A × CD-1)F_1 pronuclear-stage embryos. DNA from putative transgenic mice was analyzed (19) by *Bam*HI digestion and blot hybridization with a 0.95-kb *Xba* I–*Eco*RI fragment containing 3′ untranslated sequences of *S100b* (Fig. 1). Homozygous line 3 and heterozygous line 5 mice were used in this study.

Northern and Western Blot Analysis. RNA for quantitative blot analysis was prepared from tissues of young adult animals (20). Hybridization analysis and quantification of autoradiographic bands were accomplished with an LKB Ultroscan XL laser densitometer (19). For Western blotting, 10 μg of total brain homogenate protein was electrophoresed in an SDS/15% polyacrylamide gel; proteins were transferred to nitrocellulose membrane, fixed, and blocked; visualization

Abbreviations: AD, Alzheimer disease; DS, Down syndrome; GFAP, glial fibrillary acidic protein; NF, neurofilament.

†To whom correspondence should be addressed at: Physiology 202, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205.
‡Present address: Roswell Park Memorial Institute, Buffalo, NY 14263.

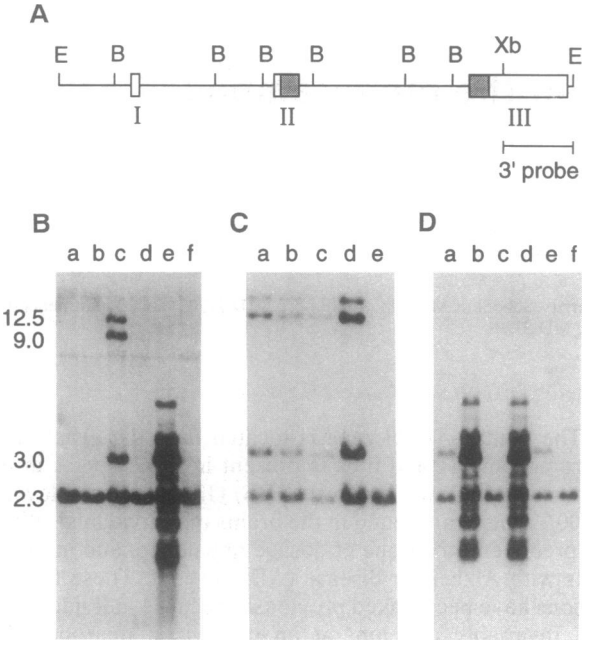

FIG. 1. Characterization of transgenic mice. (*A*) Map of the mouse genomic *S100b* clone injected into pronuclear-stage embryos. Boxed regions represent exons I–III. Shaded parts of exons II and III correspond to protein-coding regions of the 1.6-kb message. The 0.95-kb *Xba* I–*Eco*RI 3′ probe fragment is indicated. E, *Eco*RI; X, *Xba* I; B, *Bam*HI. (*B*) Genomic DNA was digested with *Bam*HI and hybridized with the 3′ *S100b* probe to identify transgenic mouse strains and determine transgene copy number and structure. Nontransgenic mice contain only the endogenous 2.3-kb fragment (lanes a, b, d, and f), whereas founders of line 3 (lane c) and line 5 (lane e) contain additional bands. (*C*) F_2 progeny of line 3 transgenic mice demonstrated different relative intensities of 2.3-kb endogenous and 3.0-kb transgene concatamer bands, permitting identification of animals heterozygous (lane b) and homozygous (lanes a and d) for the *S100b* transgene. (*D*) Line 5 transgenic mice used in this study presented a complicated transgene pattern including about six head-to-tail concatamers at one site (lanes b and d). The founder of this line also contained a second, independently segregating insertion of a single transgene copy (lanes a and e).

of proteins with specific antibodies was accomplished with the ECL system (Amersham).

Antibody monospecific for S100β was prepared in rabbits against gel-purified bovine S100β. The resulting antisera had a titer of 256,000 against S100β and no crossreactivity was detected with S100α at high titer by ELISA. Additional antibodies included Z334, anti-GFAP (Dako); SMI 35, which recognizes phosphorylated epitopes of neurofilament (NF)-H and -M chains (Sternberger Monoclonals, Baltimore); neurofilament-68, directed against NF-L (Chemicon); and TUJ-1, recognizing β-tubulin (kindly provided by A. Frankfurter, University of Virginia).

Immunocytochemistry. Adult animals were anesthetized and subjected to transcardial perfusion by flushing with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS (pH 7.2). Brains were removed to paraformaldehyde at 4°C and 25-μm sections were cut on a Vibratome. Floating sections were treated in 0.3% H_2O_2, washed in 0.1 M Tris buffer (pH 7.6), blocked in 10% normal goat serum containing 0.5% bovine serum albumin, and incubated overnight in primary antibody with 0.2% Triton X-100 in Tris buffer. For S100β, primary antibody was used at 1:3000, and sections were washed and incubated with fluorescein-conjugated goat anti-rabbit IgG F(ab′)$_2$ (Tago). For enzyme-coupled staining, the SMI 35 antibody was used at 1:2000 and Z334 at 1:4000. The secondary antibody was a biotinylated horse anti-mouse IgG (Vector Elite, Vector Laboratories) for mouse monoclonal antibodies (NF) or protein A for rabbit antibodies (GFAP) followed by reaction with ABC reagent in 0.1% Triton X-100 in 0.5 M Tris buffer (pH 7.6) for 1 hr. Reaction for peroxidase was carried out with 0.05% diaminobenzidine and the glucose/ammonium chloride/glucose oxidase method (21). Control slides reacted with preimmune serum from the appropriate species were always included as a control.

RESULTS

Production of Transgenic Mice. An 8.9-kb *Eco*RI fragment containing the entire *S100b* gene (18) was injected into 80 pronuclear-stage embryos. Three transgenic animals were identified among the 36 pups born, and 2 were analyzed further (Fig. 1). The founder of line 3 was backcrossed to C57BL/6J animals and the heterozygous progeny were mated to produce animals homozygous for the transgene. Progeny of the line 5 founder demonstrated the presence of two independently segregating transgene insertion sites. Some progeny contained a single extra copy of *S100b* (Fig. 1*D*) and were not analyzed further. Others contained multiple copies of the transgene. These high-copy-number animals were crossed onto C57BL/6J to produce line 5.

Transgene Conformation and Copy Number. *Bam*HI digestion and analysis with a probe representing the 3′ end of the genomic clone used to make the transgenic mice identified a 3.0-kb band in both lines 3 and 5 (Fig. 1*B*). This result is predicted if the transgenes are present as head-to-tail concatamers, which are common in transgenic mice. Four additional enzyme digests were consistent with the presence of concatamers of the original clone (data not shown).

Approximately one-quarter of line 3 mice produced from matings between F_1 individuals heterozygous for the transgene were nontransgenic, half demonstrated stronger hybridization to the 2.3-kb endogenous band than to the 3.0-kb transgene-specific band, and the remaining animals showed equal hybridization intensity (Fig. 1*C*). This is the expected 1:2:1 ratio if the intensely hybridizing DNA samples are from animals homozygous for the transgene. Ten putative homozygotes identified in this manner were outcrossed to nontransgenic mice to produce an average of 11 progeny (from 5 to 29 for each). Every offspring was transgenic, demonstrating that the parents selected by hybridization intensity were correctly identified as homozygous for the transgene.

There was no obvious difference in hybridization intensity between progeny of matings between line 5 heterozygous animals. Twelve of these animals were outcrossed to nontransgenic mice. In every case, these matings produced some nontransgenic offspring, demonstrating that the transgenic parent was always heterozygous and indicating that mice homozygous for this transgene are not viable. This would occur if the transgene insertion in the line 5 animals disrupted a sequence one copy of which is required for normal development. Half (42 of 86) of the line 5 outcross progeny were transgenic, demonstrating that this transgene insertion does not affect viability of heterozygous individuals.

The relative hybridization intensity of the 3.0-kb concatamer band was approximately half that of the 2.3-kb endogenous *Bam*HI fragment in line 3 mice that were heterozygous for the transgene and was of equal intensity in homozygotes (confirmed by scanning densitometry). This indicates that 1 copy of a head-to-tail concatamer (2 copies of the gene) is present per haploid genome. Estimation of copy number in line 5 by this method was less precise, but this line appeared to contain about 6 copies of the concatamer (12 copies of the gene). The presence of two additional flanking-sequence bands in line 3 animals instead of the single predicted band, as well as the complex hybridization pattern observed in line

5 animals, suggests that some of the transgene sequences are rearranged.

S100β RNA and Protein Are Expressed in a Tissue-Appropriate Manner at Levels Corresponding to Transgene Copy Number. S100β mRNA was expressed at high levels in brain, but no signal was detected in liver, spleen, muscle, testis, or adipose tissue. A faint signal was occasionally visualized in heart, lung, or kidney RNA of line 5 transgenic mice (data not shown). Northern blotting of RNA from various brain regions demonstrated differential levels of S100β expression (Fig. 2). The highest levels were observed in cerebellum and the lowest in cerebral cortex, with intermediate levels apparent in midbrain and brainstem. While relative S100β expression levels were conserved, absolute levels increased in all regions of the brains of transgenic mice. To estimate the overall increase in expression, RNAs from whole brains of 10- to 12-week-old line 3, line 5, and control mice were analyzed by quantitative Northern blotting (19). Four control and 12 line 3 transgenic mice were analyzed on duplicate gels and each was probed sequentially for S100β and β-actin RNA. The ratio of S100β/actin hybridization was determined for each individual, then the average transgenic value was divided by the average ratio for the controls to determine the relative increase in S100β expression. The same procedure was followed with RNA from 11 line 5 transgenic mice. In whole brain, S100β RNA levels were increased 2.2-fold in line 3 animals and 6.6-fold in line 5 animals (data not shown). Western blotting demonstrated that relative levels of S100β protein were directly correlated with the pattern of S100β RNA levels; that is, line 3 expressed more protein than controls and line 5 produced more than line 3 (Fig. 2*C*).

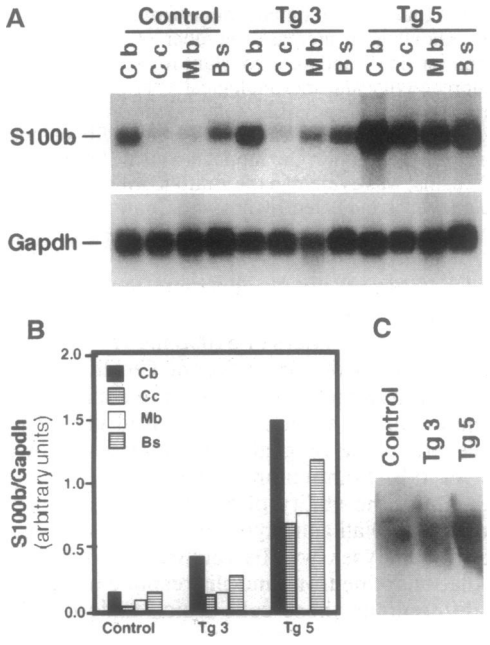

FIG. 2. Transgenic mice express elevated levels of S100β RNA and protein in a dose-dependent manner. (*A*) Ten micrograms of total RNA from four brain regions of control, line 3 (Tg3), or line 5 (Tg5) animals was analyzed by Northern blotting using sequentially probes for *S100b* (*Upper*) and *Gapdh* (*Lower*). In all, three or more individuals of each group were analyzed with consistent results. Cb, cerebellum; Cc, cerebral cortex; Mb, midbrain; Bs, brainstem. (*B*) Relative expression levels of S100β in control and transgenic mice are presented graphically for the same four brain regions. Hybridization intensity was determined by densitometry, and the average S100β/Gapdh ratio was determined for each sample. (*C*) Western blotting with antibodies specific for S100β shows protein levels commensurate with RNA levels in total brain extracts of control, line 3 (Tg3), and line 5 (Tg5) mice.

S100β Is Expressed in a Cell-Appropriate Manner in Transgenic Mice. Immunocytochemistry using the antibody to S100β detected expression in only the appropriate cells in the nervous system. In the hippocampus, S100β immunoreactivity was restricted to stellate astrocytes, whereas CA1 neurons and dentate granule neurons did not react with the antibody (Fig. 3 *A–C*). Increased expression was observed throughout the neuropil, probably within sectioned astrocyte processes. Elevated expression was observed in several additional populations of glia normally expressing S100β throughout the brain, but within the limits of immunocytochemistry, no neuronal expression of S100β was observed in the cerebral cortex, hippocampus, or cerebellum. Low expression was observed in neurons of the vestibular nucleus of control and transgenic mice, as reported previously (17).

Increased GFAP Immunoreactivity in the Hippocampus. GFAP immunoreactivity in astrocyte processes was increased throughout the hippocampus of both transgenic lines and was especially pronounced in the dentate gyrus (Fig. 3 *D–F*). An increased number of radial fibers were seen throughout the granule cell layer. This increase in GFAP expression was confirmed by Western blotting (Fig. 4). The number of astrocytes was not increased in any of several regions of the dentate gyrus, indicating that increased reactivity in hippocampal glia was due to increased expression of GFAP in each cell rather than expression by additional cells (Table 1). This astrocytosis showed a graded response correlated with S100β expression levels—that is, line 5 was greater than line 3 which was greater than control—and indicates that S100β is involved in the astrogliotic response *in vivo*.

Axonal Sprouting in the Hippocampus. Increased immunoreactivity in hippocampus was detected with antibodies directed against axonal NF proteins including epitopes of NF-H and -M chains, NF-L, and β-tubulin. NF immunoreactivity was elevated in the stratum oriens, stratum radiatum, and the stratum lacunosum moleculare in CA1 of transgenic mice (Fig. 3 *G–I*) and in the perforant pathway. These increases were confirmed by Western blotting and demonstrated the same graded response as S100β and GFAP expression in lines 5, 3, and control mice (Fig. 4). Neuronal cell bodies were counted in the granule cell layer of line 5 animals and were not significantly increased over control numbers (Table 1). As with increased GFAP expression in glia, increased levels of these axonal markers appeared to be due to elevated expression by individual cells. The results indicate that S100β acts as a neurite-promoting agent and confirms *in vivo* the previously reported ability of S100β to induce neurite proliferation *in vitro* (3, 6, 8, 22).

DISCUSSION

Two lines of transgenic mice have been produced that overexpress the murine *S100b* gene in a cell- and tissue-appropriate manner at levels correlated with transgene copy number. Hippocampal neurons in transgenic mice do not react with S100β-specific antisera, whereas the immunoreactivity of glial cells to S100β- and GFAP-specific antisera is enhanced. S100β RNA levels are elevated throughout the brains of transgenic mice compared with corresponding regions of control mice, but the relative levels of expression in the various brain regions are maintained. Preliminary results indicate that the transgene is expressed at the appropriate developmental stages, as well (J.Y. and R.H.R., unpublished observations). Thus, the signals necessary for appropriate regulation of *S100b* reside within the 8.9-kb segment utilized to construct these transgenic mice. This clone contains only 1.7 kb 5′ to the transcription start site and 14 bp 3′ to the

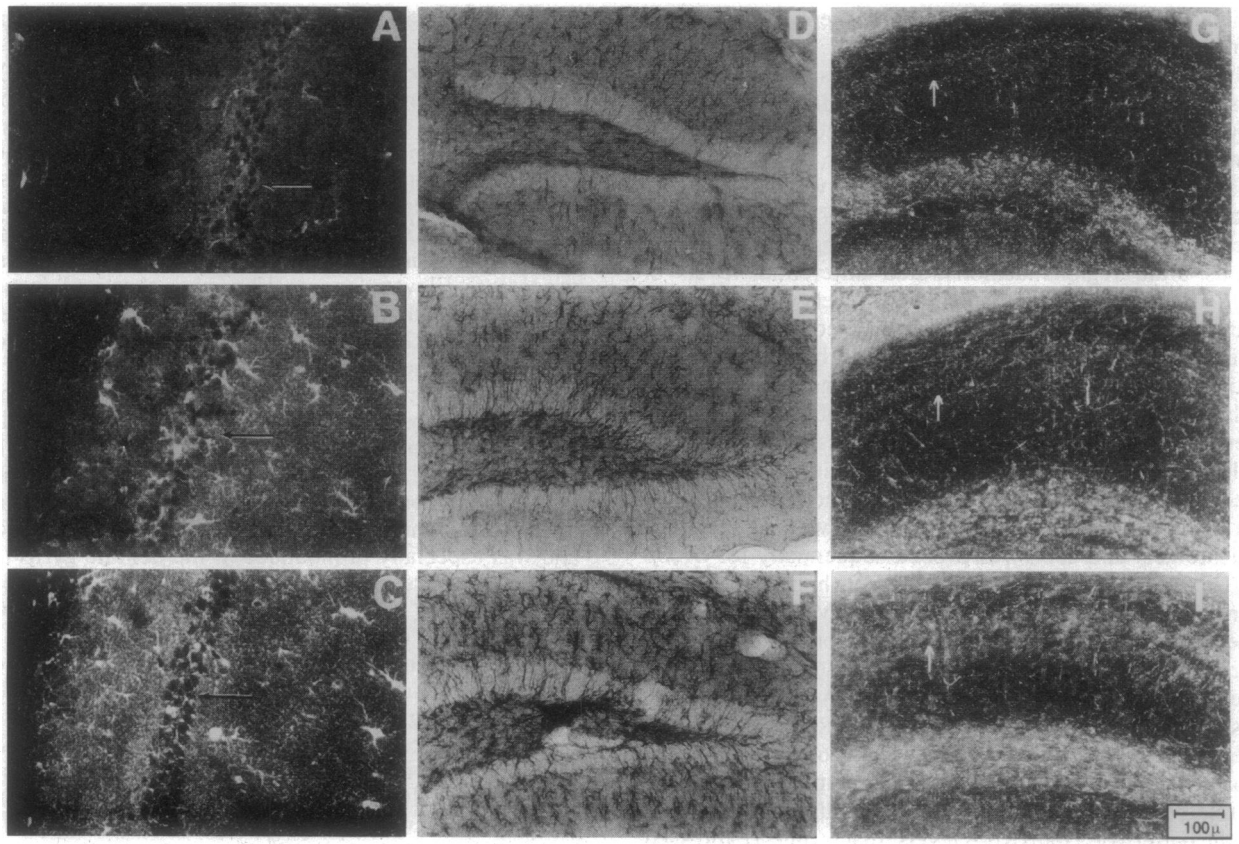

FIG. 3. Transgenic mice demonstrate astrocytosis and axonal proliferation. (*A–C*) Increased immunoreactivity for S100β in a cell type-appropriate manner was correlated with transgene dosage as illustrated in the CA1 area of the hippocampus from control (*A*), line 3 (*B*), and line 5 (*C*) mice. Astrocytes in transgenic mice were altered morphologically, showing an increased number of processes, while CA1 pyramidal neurons (arrows) remained unstained. Exposure metering was determined for line 5 and kept constant for sections from line 3 and control animals. (Immunofluorescence detection.) (*D–F*) Increased GFAP immunoreactivity and morphological alteration of astrocytes are evident in line 3 (*E*) and line 5 (*F*) transgenic mice and are correlated with transgene dosage. Astrocytosis was evident throughout the dentate gyrus, including the hilus, molecular layer, and stratum radiatum, and in the CA3 pyramidal layer. Control is shown for comparison (*D*). (Brightfield photomicrographs of peroxidase-stained sagittal sections.) (*G–I*) Axonal proliferation in the hippocampus of line 3 (*H*) and line 5 (*I*) transgenic mice, shown by increased reactivity with an antibody to NF-M and -H chains, was evident in the stratum oriens, stratum radiatum, and stratum lacunosum moleculare in CA1 of transgenic mice. The CA1 pyramidal layer is indicated by arrows. Control is also shown (*G*). (Darkfield photomicrographs of peroxidase-stained sections.) (Bar = 100 μm.)

poly(A)-addition site, delimiting the location of the locus control region for this gene (18). The inclusion of the entire gene and its regulatory elements assures that phenotypic manifestations in these transgenic animals are due to altered

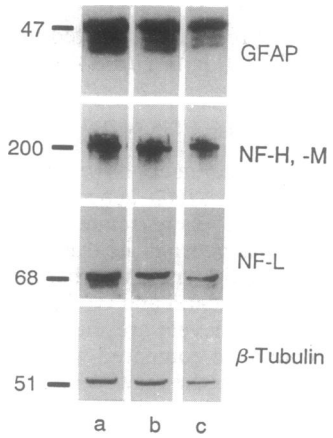

FIG. 4. Western blots demonstrate elevated levels of GFAP; NF-H, -M, and -L; and β-tubulin in hippocampus commensurate with S100β expression levels in line 5 (lane a), line 3 (lane b), and control (lane c) mice. Molecular masses (kDa) of reactive bands are indicated at left.

levels of S100β and not to abnormal location or timing of transgene expression.

Disulfide-bonded dimers of S100β induce proliferation and altered morphology of astrocytes and C6 glioma cells *in vitro* (11). *S100b* transgenic mice demonstrated astrocytosis in the hippocampus as evidenced by increased GFAP levels and extensive branching of glial cell processes. However, the number of GFAP-immunoreactive glial cells was not increased. Thus, the ability of S100β to induce proliferation does not apply to all astrocyte populations *in vivo*. Detailed quantitative analyses will be required to determine which subpopulations of neurons and glia respond to elevated S100β and to differentiate those cells which undergo a mitogenic response from those that undergo specific morphological changes. Overall, the induction of morphological changes by elevated levels of S100β *in vivo* is consistent with alterations observed *in vitro* and clearly validates the use of culture systems in elucidating the roles of this growth factor.

Addition of S100β dimers to various primary and established cells in culture also induces neurite extension. This *in vitro* activity of S100β was recapitulated *in vivo* in the hippocampus of *S100b* transgenic mice. While the number of neuronal cell bodies in hippocampus remained unchanged, immunocytochemistry and Western blotting demonstrated elevated expression of at least two types of NF proteins as well as β-tubulin. Increased immunoreactivity to neuronal markers was observed throughout the hippocampus, suggest-

Table 1. Numbers of hippocampal astrocytes and neurons are unchanged in transgenic mice

	Control	Line 5
GFAP$^+$ cells	No. of cells per mm^2	
CA1		
Stratum radiatum	583 ± 186	508 ± 118
Stratum oriens	600 ± 80	515 ± 134
Dentate gyrus		
Hilus	840 ± 144	590 ± 167
Molecular layer	520 ± 120	475 ± 80
Neurons	No. of cells	
Dentate gyrus		
Suprapyramidal blade	9,245 ± 524	9,957 ± 471
Infrapyramidal blade	8,179 ± 854	9,601 ± 540
Crest	11,912 ± 568	13,335 ± 888

Each measurement is the mean value from three animals plus or minus standard deviation. Dimensions of the hippocampus in matched sections from control and transgenic mice were identical. For GFAP, all immunoreactive cells in each of five 10-μm × 10-μm squares on six coronal sections of the dorsal hippocampus were counted in the indicated regions of each mouse. For neurons, three coronal sections of the dorsal hippocampus from each animal were stained (cresyl violet) and all cells in the indicated regions were counted. No significant difference was detected between control and line 5 by analysis of variance.

ing that S100β induces axonal proliferation from multiple populations of neurons with different neurotransmitter phenotypes *in vivo*.

Friend *et al.* (17) described transgenic mice expressing 10- to 100-fold elevated levels of the human *S100B* gene but did not report astrocytosis or axonal proliferation in these animals. Both were apparent in the present studies even when the murine *S100b* gene was expressed at only twice the normal level (line 3) and were further elevated as a consequence of 7-fold elevated expression (line 5). Human and mouse S100β differ by only one amino acid residue (18). Many *in vitro* studies have used bovine S100β (which differs from both human and mouse at three amino acid residues) to demonstrate trophic effects on primary and established cell lines from chicken, mouse, and rat. Thus, it is surprising that elevated expression of human *S100B* in mouse brain did not induce changes similar to those observed in the present study.

Because the human *S100B* gene is found on chromosome 21 and is expressed primarily in the nervous system, it has been suggested that dosage imbalance of this gene is a major contributor to the abnormalities of brain development and function that occur invariably in DS individuals (12). Overall, DS brains are markedly reduced in size and are hypocellular with the cerebellum consistently smaller than normal (23). In contrast, the brains of *S100b* transgenic mice are of normal size and show no gross external pathological features. It seems likely that the complex pathophysiology of the DS brain reflects interactions among many of the hundreds of genes at dosage imbalance in the syndrome. This study suggests that increased S100β contributes to the astrogliotic response and neurite proliferation seen with the early development of AD pathology in DS individuals. These transgenic mice provide a useful model system for further studies concerning the role of S100β in normal development and pathological processes in the brain.

We thank Brenda Klaunberg for excellent technical assistance and Drs. Cheryl Kitt, Douglas Kligman, Bruce Kreuger, and Phyllis Wise for helpful discussions. This work was supported by Public Health Service Grant HD24605 (R.H.R. and J.D.G.) and by grants from the National Institutes of Health and the Department of Veterans Affairs (D.C.H.), and from the National Science Foundation (P.Y.).

1. Kligman, D. & Hilt, D. C. (1988) *Trends Biochem. Sci.* **13**, 437–443.
2. Shashoua, V. E., Hesse, G. W. & Moore, B. W. (1984) *J. Neurochem.* **42**, 1536–1541.
3. Van Eldik, L. J., Christie-Pope, B., Bolin, L. M., Shooter, E. M. & Whetsall, W. O. (1991) *Brain Res.* **542**, 280–285.
4. Kligman, D. & Marshak, D. R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7136–7139.
5. Winningham-Major, F., Staecker, J. L., Barger, S. W., Coats, S. & Van Eldik, L. J. (1989) *J. Cell Biol.* **109**, 3063–3071.
6. Azmitia, E. C., Dolan, K. & Whitaker-Azmitia, P. M. (1990) *Brain Res.* **516**, 354–356.
7. Liu, J. & Lauder, J. M. (1992) *J. Neurosci. Res.* **33**, 248–256.
8. Kligman, D. & Hsieh, L.-S. (1987) *Dev. Brain Res.* **33**, 296–300.
9. Battacharyya, A., Oppenheim, R. W., Prevette, D., Moore, B. W., Brackenbury, R. & Ratner, N. (1992) *J. Neurobiol.* **23**, 451–456.
10. Dunn, R., Landry, C., O'Hanlon, D., Dunn, J., Allore, R., Brown, I. & Marks, A. (1987) *J. Biol. Chem.* **262**, 3562–3566.
11. Selinfreund, R. H., Barger, S. W., Pledger, W. L. & Van Eldik, L. J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3554–3558.
12. Allore, R., O'Hanlon, D., Price, R., Neilson, K., Willard, H. F., Cox, D. R., Mark, A. & Dunn, R. J. (1988) *Science* **239**, 1311–1313.
13. Griffin, W. S. T., Stanley, L. C., Ling, C., White, L., MacLeod, V., Perrot, L. J., White, C. L. & Araoz, C. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7611–7615.
14. Kato, K., Kurobe, N., Suzuki, F., Morishita, R., Asano, T., Satao, T. & Inagaki, T. (1991) *J. Mol. Neurosci.* **3**, 95–99.
15. Geddes, J. W., Anderson, K. J. & Cotman, C. W. (1986) *Exp. Neurol.* **94**, 767–776.
16. Jorgensen, O. S., Brooksbank, B. W. L. & Balazs, R. (1990) *J. Neurol. Sci.* **98**, 63–79.
17. Friend, W. C., Clapoff, S., Landry, C., Becker, L. E., O'Hanlon, D., Allore, R. J., Brown, I. R., Marks, A., Roder, J. & Dunn, R. J. (1992) *J. Neurosci.* **12**, 4337–4346.
18. Jiang, H., Shah, S. & Hilt, D. (1993) *J. Biol. Chem.* **268**, 20502–20511.
19. O'Hara, B. F., Fisher, S., Gearhart, J. D., Oster-Granite, M. L. & Reeves, R. H. (1989) *Dev. Brain Res.* **49**, 300–304.
20. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
21. Sloviter, R. S. (1989) *J. Comp. Neurol.* **280**, 183–196.
22. Kligman, D. (1982) *Brain Res.* **250**, 93–100.
23. Crome, R., Cowie, V. & Slater, E. (1966) *J. Ment. Defic. Res.* **10**, 69–72.