S100A1 Regulates Neurite Organization, Tubulin Levels, and Proliferation in PC12 Cells*

(Received for publication, October 14, 1997, and in revised form, December 9, 1997)

Danna B. Zimmer‡, Emily H. Cornwall, Philip D. Reynolds, and Christopher M. Donald

From the Department of Pharmacology, School of Medicine, University of South Alabama, Mobile, Alabama 36688

As a first step in determining what cellular processes are regulated by the calcium-modulated protein S100A1 isoform in neurons, the effects of ablated S100A1 expression on neurite organization and microtubule/tubulin levels in PC12 cells were examined. A mammalian expression vector containing the rat S100A1 cDNA in the antisense orientation with respect to a cytomegalovirus promoter was constructed and transfected into PC12 cells. Indirect immunofluorescence microscopy confirmed decreased S100A1 protein levels in all three stable transfectants (pAntisense clones) that expressed exogenous S100A1 antisense mRNA. In response to nerve growth factor, pAntisense clones extended significantly more neurites than control cells (4.01 ± 0.16 neurites/cell). This increase in neurite number was accompanied by an increase in total α-tubulin levels in untreated (4.0 ± 0.6 versus 1.76 ± 0.4 ng of α-tubulin/mg of total protein) and nerve growth factor-treated pAntisense clones (4.15 ± 0.4 versus 2.04 ± 0.5 ng of α-tubulin/mg of total protein) when compared with control cells. At high cell densities, pAntisense clones exhibited a significant decrease in anchorage-dependent growth. In soft agar, pAntisense clones formed significantly more colonies (153 ± 8%) than control cells (116 ± 5%). However, the pAntisense soft agar colonies were significantly smaller than those observed in control cells (40.6 ± 3.0 versus 59.5 ± 1.2 μm). These data suggest that cell density inhibits both anchorage-independent and -dependent growth of pAntisense clones. In summary, ablation of S100A1 expression in PC12 cells results in increased tubulin levels, altered neurite organization, and decreased cell growth. Thus, S100A1 may directly link the cytoskeleton and calcium signal transduction pathways to cell proliferation.

The S100 protein family is a group of calcium-binding proteins that exhibit a high degree of conservation in amino acid sequence, secondary structure, and genomic organization (1–3). To date, there are 18 members of the S100 family, 13 of which are clustered in region q21 of human chromosome 1 (see Refs. 4 and 5). A new nomenclature that reflects the genomic organization of these proteins has recently been adopted, and the S100A1 antisense mRNA. In response to nerve growth factor, pAntisense clones extended significantly more neurites than control cells (4.01 ± 0.16 neurites/cell). This increase in neurite number was accompanied by an increase in total α-tubulin levels in untreated (4.0 ± 0.6 versus 1.76 ± 0.4 ng of α-tubulin/mg of total protein) and nerve growth factor-treated pAntisense clones (4.15 ± 0.4 versus 2.04 ± 0.5 ng of α-tubulin/mg of total protein) when compared with control cells. At high cell densities, pAntisense clones exhibited a significant decrease in anchorage-dependent growth. In soft agar, pAntisense clones formed significantly more colonies (153 ± 8%) than control cells (116 ± 5%). However, the pAntisense soft agar colonies were significantly smaller than those observed in control cells (40.6 ± 3.0 versus 59.5 ± 1.2 μm). These data suggest that cell density inhibits both anchorage-independent and -dependent growth of pAntisense clones. In summary, ablation of S100A1 expression in PC12 cells results in increased tubulin levels, altered neurite organization, and decreased cell growth. Thus, S100A1 may directly link the cytoskeleton and calcium signal transduction pathways to cell proliferation.

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

‡ To whom correspondence should be addressed: MSB 3130, Dept. of Pharmacology, University of South Alabama, Mobile, AL 36688. Tel.: 334-460-7056; Fax: 334-460-6798; E-mail: dzimmer@jaguar1.usouthal.edu.

‡‡ This work was supported by Grant NS 30660 from the National Institutes of Health, Grant BIO-920038 from the National Science Foundation, and a grant from the Pine Family Foundation, Inc. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: MSB 3130, Dept. of Pharmacology, University of South Alabama, Mobile, AL 36688. Tel.: 334-460-7056; Fax: 334-460-6798; E-mail: dzimmer@jaguar1.usouthal.edu.

The abbreviations used are: NGF, nerve growth factor; PCR, polymerase chain reaction; RT, reverse transcriptase; bp, base pair(s); ANOVA, analysis of variance; EB, Extraction Buffer; PIPES, 1,4-piperazinediethanesulfonic acid.

* This work was supported by Grant NS 30660 from the National Institutes of Health, Grant BIO-920038 from the National Science Foundation, and a grant from the Pine Family Foundation, Inc. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: MSB 3130, Dept. of Pharmacology, University of South Alabama, Mobile, AL 36688. Tel.: 334-460-7056; Fax: 334-460-6798; E-mail: dzimmer@jaguar1.usouthal.edu.

This paper is available online at http://www.jbc.org
at resting intracellular calcium levels. Furthermore, these studies directly implicate S100 proteins in the regulation of cell growth and cytoskeletal organization.

Previous studies have indirectly implicated another member of the S100 family, S100A1, in regulating cytoskeletal organization. Like S100A6, S100A1 expression is up-regulated in NGF-treated PC12 cells that are extending neurites (11). In addition, a number of S100A1 target proteins are essential structural elements of the cytoskeleton including tubulin, \( \tau \) protein, intermediate filaments, caldesmon, and myosin (see Ref. 1). Furthermore, several S100A1 target proteins are associated with the cytoskeleton including aldolase, glyceraldehyde-3-phosphate dehydrogenase, and phosphoglucomutase (see Refs. 1 and 11). The expression of both S100A1 and the microtubule-associated \( \tau \) protein in neuronal cells further suggests that S100A1 regulates microtubule assembly in neuronal cells.

Rat pheochromocytoma cells (PC12 cells) are an extensively used model system for studying neuronal cell differentiation and signal transduction. Furthermore, antisense/sense approaches have been used in PC12 cells to examine the in vivo functions of a wide variety of neuronal proteins including \( \tau \) (12), GAP-43 or neurabin (13–16), the \( \alpha \) subunit of \( G_\alpha \) (17), calmodulin-dependent protein kinase II (18), annexin II (19), and the plasma membrane calcium ATPase pump (20). To determine if S100A1 regulates cytoskeletal organization in neuronal cells, we have examined the effects of ablated S100A1 expression on \( \alpha \)-tubulin levels in PC12 cells as well as the number and length of neurites extended by PC12 cells in response to NGF. We observed increases in \( \alpha \)-tubulin levels in PC12 cells that do not express S100A1. Furthermore, PC12 cells that do not express S100A1 extended more neurites per cell in response to NGF. These cytoskeletal changes were accompanied by reductions in anchorage-dependent and anchorage-independent growth that were density-related. The fact that three members of the S100 protein family, S100B, S100A6, and now S100A1, modulate growth and cytoskeletal organization suggest that these proteins link the cytoskeleton and calcium signal transduction pathways to cell growth.

**EXPERIMENTAL PROCEDURES**

**Construction of the pAntisense Expression Vector—**To construct the pAntisense expression vector, a plasmid containing the rat S100A1 cDNA (21) was PCR-amplified using M13 reverse primer (CLONTECH, Palo Alto, CA) and a gene-specific primer containing an engineered XbaI restriction enzyme site (5'-GGTCTAGAGACCTCATCAAGT-3'). After digestion with XbaI and HindIII and gel purification, the fragment was ligated into gel-purified XbaI-HindIII-digested pCMV expression vector (22). The resulting DNA construct (Fig. 1A) was transformed into Escherichia coli, and ampicillin-resistant colonies were screened by restriction mapping and PCR analysis. DNA sequencing analysis (23) of the 5' region of the S100A1 cDNA coding sequence (5'-GGTCTAGAGACCTCATCAAGT-3') was performed to verify the orientation of the insert DNA.

**Cell Culture and Transfection—**PC12 cells were the generous gift of Dr. Jonathan Seammell (University of South Alabama, Mobile, AL) and grown as described previously (11). PC12 cells (1.7 \( \times \) 10\(^6\) cells in 0.8 ml) were transfected by electroporation at 400 V using a Bio-Rad Gene Pulser\(^\text{TM} \) apparatus (Bio-Rad), equipped with a 900-microfarad capacitor and 0.4-cm gap electrodes. A plasmid containing the neomycin resistance gene under the control of an SV40 promoter (Invitrogen, San Diego, CA) was cotransfected with the S100A1 antisense plasmid or plasmid containing no insert DNA. Cells were plated in 100-mm dishes, and ampicillin-resistant colonies were scored for number of neurites and neurite length on study prints (final magnification, \( \times 64 \)). Individual processes were measured in millimeters converted to micrometers during analysis, with processes \( \geq 2 \mu \)m or greater in length scored as neurites. Between 9 and 20 different fields consisting of 120–200 cells were scored for each clone. Each experiment was repeated using cells plated on different days. The data were expressed as the mean \( \pm \) standard error of the mean. An ANOVA (GraphPad, San Diego, CA) was used to determine the statistical significance of measured differences.

**Anchorage-Dependent Growth Analysis—**Anchorage-dependent growth was assessed by plating approximately 95,000 cells in triplicate 60-mm dishes and determining the cell number using a hemacytometer at 0, 24, 48, 72, and 192 h after plating. An ANOVA (GraphPad) was used to determine the statistical significance of measured differences.

**Measurement of Neurites—**Duplicate 35-mm plates were seeded at low density, and 48 h later two plates were fed with medium only and two plates with medium containing 10 ng/ml NGF (Collaborative Research, Inc., Waltham, MA). Forty-eight hours later, five random fields were photographed from each plate using a Leitz microscope equipped with phase optics and a \( \times 20 \) objective. Individual/discernible cells were scored for number of neurites and neurite length on study prints (final magnification, \( \times 64 \)). Individual processes were measured in millimeters converted to micrometers during analysis, with processes \( \geq 2 \mu \)m or greater in length scored as neurites. Between 9 and 20 different fields consisting of 120–200 cells were scored for each clone. Each experiment was repeated using cells plated on different days. The data were expressed as the mean \( \pm \) standard error of the mean. An ANOVA (GraphPad) was used to determine the statistical significance of measured differences.

**Quantitation of \( \alpha \)-Tubulin Levels—**Duplicate 100-mm plates were fed with media only or media containing 10 ng/ml NGF (Collaborative Research, Inc.). Forty-eight hours later, the plates were rinsed three times in phosphate-buffered saline and cell extracts prepared as described previously (24). After rinsing in Extraction Buffer (EB) (0.1 MPIPES, 0.1 mM MgSO\(_4\), 2 mM EGTA, 0.1 mM EDTA, pH 7.0) plates were
S100A1 Function in PC12 Cells

RESULTS

Isolation of pAntisense Clones—In generating the clones used in this study, approximately 200 stable transfecants were isolated. Of the 17 stable transfecants screened for expression of the exogenous pAntisense S100A1 mRNA, three clones (A1, A2, and A3) exhibited the predicted 265-bp PCR product (Fig. 1B). Our inability to detect a PCR product in the remaining 14 clones was not due to technical problems with the RT or PCR reactions, as all clones exhibited a 500-bp fragment. The clones A1, A2, and A3 exhibited the predicted 265-bp PCR fragment. The actin lane contains a PCR reaction using purified plasmid DNA as template rather than an RT reaction and contains the expected 265-bp PCR fragment. The actin lane contains a PCR reaction using β-actin oligonucleotide primers and contains the expected 500-bp fragment.

and A3 exhibited a S100A1 staining intensity indistinguishable from that of secondary antibody only and significantly less than that observed in parental PC12 cells or cells transfected with vector containing no insert DNA (Fig. 2). Altogether, these results demonstrate that the A1, A2, and A3 clones (pAntisense clones) have S100A1 protein levels that are significantly less than parental PC12 cells and are an appropriate model system for studying S100A1-regulated processes.

NGF Responsiveness of pAntisense Clones—Previous studies demonstrating that S100 proteins regulate microtubule assembly in vitro (see Ref. 1) and increased S100A1 levels in PC12 cells treated with NGF (11) suggest that S100A1 regulates microtubule polymerization/depolymerization. To test this hypothesis, phase contrast microscopy and morphometric techniques were used to examine the NGF-induced neurite extension in pAntisense clones. To maximize the length of neurites extended, the effects of NGF were assayed at low confluence. As shown in Fig. 3, the morphology of the three pAntisense clones was identical to that of control cells in the absence and presence of NGF. Furthermore, there was no difference in the

Fig. 1. Detection of the pAntisense S100A1 mammalian expression vector in potential clones. A, the mammalian expression vector used to ablare S100A1 expression. The shaded rectangle denotes the rat S100A1 cDNA sequence. The cytomegalovirus promoter is designated CMV and the human growth hormone termination signals hGH. The SpeI, HindIII, and XbaI restriction sites are indicated by the vertical arrowheads. The horizontal arrowheads indicate the position of the oligonucleotide primers used in RT-PCR analysis, and the connecting line the expected RT-PCR product. The arrow indicates the extent and direction of DNA sequence analysis. B, RT reactions of RNA isolated from neomycin-resistant clones, which were subjected to PCR using the oligonucleotide primers shown in A and size-fractionated on 1.5% agarose gels. The line denotes the 250-bp PCR product observed in the potential antisense clones A1, A2, and A3. The plasmid lane is a PCR reaction using purified plasmid DNA as template rather than an RT reaction and contains the expected 265-bp PCR fragment. The actin lane contains a PCR reaction using β-actin oligonucleotide primers and contains the expected 500-bp fragment.

Fig. 2. S100A1 levels and subcellular distribution in pAntisense clones. Indirect immunofluorescence micrographs of parental PC12 cells (A and B) and a pAntisense clone (C). The cells in A and C were incubated in a monoclonal S100A1 primary antibody; cells in B were incubated in secondary antibody only. Bar, 1 μm.

Downloaded from http://www.jbc.org/ by guest on July 24, 2018
NGF dose-response curve for the pAntisense clones and control cells (data not shown). These results demonstrate that reduced S100A1 levels do not inhibit or promote NGF-induced neurite extension in PC12 cells.

To determine if S100A1 regulates neurite organization, the number of neurites per cell and the average neurite length were determined in control and pAntisense clones. Although many investigators score only those extensions that are equal to or double the cell body width, we scored any extensions 2 μm or longer as neurites to ensure that subtle differences would be detected. In addition, cells were treated with saturating levels (10 ng/ml) of NGF. As shown in Fig. 4, the three pAntisense clones extended 4.01 ± 0.11 neurites/cell while control cells extended 2.93 ± 0.11 neurites/cell. However, the average neurite length in the pAntisense clones and control cells were not significantly different (23.17 ± 1.18 versus 27.53 ± 1.51 μm). In addition, the average neurite length that we observed in NGF-treated cells was almost identical to the previously reported average neurite lengths of 23.2 μm (27) and approximately 20–30 μm (12) for NGF-treated PC12 cells. Altogether, these data demonstrate that decreased S100A1 levels are associated with an increase in the number, but not the length, of neurites extended in response to NGF. These results suggest that S100A1 regulates NGF-induced neurite organization in PC12 cells.

Tubulin Levels in PC12 Cells—The increased number of neurites in NGF-treated pAntisense clones suggests that S100A1 may regulate the level of polymerized/unpolymerized tubulin in PC12 cells. To test this hypothesis, an immunodot assay was used to quantitate α-tubulin levels in PC12 cell total homogenates as well as in fractions containing polymerized or unpolymerized tubulin. Western blot analysis of PC12 cell homogenates confirmed that the commercial antibody used for the immunodot experiments recognized a single protein species (data not shown). As shown in Fig. 5A, pAntisense clones contained significantly more α-tubulin protein (4.0 ± 0.6 versus 1.76 ± 0.4 ng/mg of total protein) than control cells. Furthermore, NGF treatment did not significantly alter the α-tubulin levels in control or pAntisense clones. It should be noted that the total α-tubulin levels in this study are approximately 2-fold higher than those previously reported for PC12 cells (24), and this may account for the small processes that we observe in cells which are not treated with NGF, as well as the fact that we achieve maximal neurite extension in our cells after 48 h whereas other investigators routinely use 7 days. In summary, ablation of S100A1 expression in PC12 cells results in an NGF-independent increase in α-tubulin levels.

Although the analysis of total α-tubulin protein levels did reveal an important difference between control cells and pAntisense cells, it did not allow us to determine if the increased number of neurites in NGF-treated pAntisense clones was accompanied by an increase in polymerized tubulin. To test this hypothesis, the α-tubulin content in polymerized and nonpolymerized microtubule fractions was determined using an immuno-
FIG. 5. α-Tubulin levels in pAntisense clones. The total α-tubulin levels (A) as well as the fraction of polymerized and unpolymerized α-tubulin (B) were determined as described under “Experimental Procedures.” In A, total α-tubulin levels in untreated (white bars) and NGF-treated (gray bars) were expressed as the mean nanograms of α-tubulin/mg of total protein ± the standard error of the mean of two independent experiments (pAntisense, n = 8; pControl, n = 11). The asterisks denote p values < 0.05. Both untreated and NGF-treated pAntisense clones had significantly more α-tubulin than control cells. In B, the unpolymerized (speckled bars) and polymerized (hatched bars) α-tubulin levels are expressed as the mean nanograms/mg of total protein ± the standard error of the mean of two independent experiments (pAntisense, n = 8–10; pControl, n = 8–13). The + and – below the x axis denote NGF-treated and untreated cells, respectively. The asterisks denote p < 0.05. In the absence of NGF, pAntisense clones exhibited a significant increase in unpolymerized tubulin when compared with control cells. In the presence of NGF, pAntisense clones contained significantly more polymerized and unpolymerized α-tubulin than NGF-treated control cells.

Next, the effects of NGF on soluble and polymerized α-tubulin were examined. As shown in Fig. 5B, untreated control cells had significantly more polymerized α-tubulin than unpolymerized α-tubulin (1.14 ± 0.15 versus 2.10 ± 0.42 ng/mg of total protein). In untreated pAntisense clones, the unpolymerized α-tubulin levels were significantly higher than control cells (2.09 ± 0.39 versus 1.14 ± 0.15 ng/mg of total protein) while polymerized α-tubulin levels were indistinguishable. These results suggest that the increased α-tubulin levels observed in response to ablamed S100A1 expression are due to increases in soluble and not polymerized tubulin.

Next, the effects of NGF on soluble and polymerized α-tubulin were examined. As shown in Fig. 5B, NGF treatment of control cells resulted in a slight decrease in polymerized α-tubulin levels that was not statistically significant and no change in soluble α-tubulin levels. These data suggest that neurite extension in the PC12 cells used in this study occurs via a reorganization of existing microtubules without an increase in α-tubulin levels or the level of polymerized α-tubulin. This is in contrast to previous studies, which have demonstrated increases in α-tubulin levels in PC12 cells in response to NGF (24). The cells used in previous studies required up to 7 days of treatment to extend neurites, whereas the cells used in this study extend neurites within 48 h. One explanation for the significant reduction in time for neurite extension in our cells may be the fact that these cells already express sufficient levels of α-tubulin for neurite extension. pAntisense clones exhibited slight increases in soluble and polymerized α-tubulin levels, in response to NGF, that were not statistically significant. However, the level of soluble α-tubulin in NGF-treated pAntisense clones was significantly higher than those in NGF-treated control cells (2.93 ± 0.36 versus 1.18 ± 0.18 ng/mg of total protein), as was the level of polymerized α-tubulin (2.52 ± 0.50 versus 1.34 ± 0.22 ng/mg of total protein). The increased polymerized/unpolymerized α-tubulin levels observed in pAntisense clones is consistent with the increased number of neurites extended by pAntisense clones in response to NGF. However, because untreated and NGF-treated pAntisense clones have almost identical polymerized and unpolymerized α-tubulin levels, the increased number of neurites in NGF-treated pAntisense clones is most likely a result of the increased α-tubulin levels present before NGF treatment and not S100A1 effects on the NGF signaling pathway.

Growth Properties of pAntisense Clones—Because changes in the cytoskeleton are often associated with altered growth properties and members of the S100 family have been shown to regulate cell growth, the anchorage-dependent and anchorage-independent growth properties of the pAntisense clones were assayed. Although the growth properties of pAntisense clones at low densities were not different from control cells, at high densities pAntisense clones exhibited a significant decrease in anchorage-dependent growth (Fig. 6). Anchorage-independent growth was also altered in the pAntisense clones (Fig. 7). When all colonies larger than a single cell were scored, the three pAntisense clones formed significantly more colonies (153 ± 8%) in soft agar than control cells (116 ± 5%). Because cells were initially plated at equivalent numbers, this increase in colony number cannot be attributed to an increase in the number of cells plated. The increase in colony size cannot be attributed to experimental variability either, as the colony number in each experiment was normalized to the number of colonies formed by the parental PC12 cells which was set at 100%. These results demonstrate increased anchorage-independent growth in PC12 cells that do not express S100A1. Significant differences in colony size were also detected with the pAntisense clones forming colonies that were significantly smaller (40.6 ± 0.03 μm) than cells transfected with vector only and the parental PC12 cell line (59.5 ± 1.2 μm). Because control and
S100A1 Function in PC12 Cells

**DISCUSSION**

This study reports the first information regarding the function of the calcium-binding protein S100A1 in intact cells. Decreased S100A1 levels in PC12 cells resulted in an increase in tubulin levels and the number of neurites extended in response to NGF. In theory, cells that are assembling more processes would have higher levels of structural components such as microtubules. In fact, PC12 cells that do not express S100A1 did have increased levels of α-tubulin. We also report that decreased S100A1 levels in PC12 cells alter cell growth in a density-dependent manner. Although the reductions in S100A1 initially increase anchorage-independent growth, as cells become more dense, the net result in both anchorage-independent and anchorage-dependent growth is a reduction in cell number. These results suggest that S100A1 expression promotes cell growth and eliminates some aspect of contact-inhibited cell growth. One model that accounts for our results would be that S100A1 directly modulates tubulin/microtubule levels in PC12 cells and that the effects on cell growth and calcium homeostasis are indirect effects, which occur as a consequence of the changes in tubulin/microtubules. This model is consistent with previous studies, which have identified tubulin and tubulin-associated proteins as S100A1 target proteins (see Ref. 1). However, S100A1 has been shown to regulate the activity of a number of growth regulatory proteins in vitro, including the tumor suppressor p53 (28). Thus, it is possible that S100A1 interacts with multiple target proteins and directly regulates both tubulin/microtubule levels and cell growth. Additional experiments will be needed to determine if S100A1 directly or indirectly regulates various cellular processes.

The results that we observed in PC12 cells are in accordance with previous in vitro studies on S100 target proteins. First, previous studies have reported that S100A1 proteins interact with numerous in vitro target proteins, suggesting that S100A1 will regulate multiple diverse processes in cells and that S100A1 regulation of these processes can be modulated at multiple sites. Consistent with these studies are our observations that S100A1 regulates multiple cellular processes in PC12 cells and that the effects of S100A1 on these processes can change under various conditions such as cell density. Second, the fact that S100A1 regulates most in vitro target proteins in a stoichiometric rather than catalytic fashion suggests that the effects of S100A1 on cellular process will be modulatory and not all-or-none. As predicted, in PC12 cells we observed modulatory rather than all-or-none effects. Third, the fact that some S100A1 target proteins are regulated in a calcium-independent manner suggests that altered S100A1 expression would have significant effects on cell phenotype even in the absence of agents that raise intracellular calcium levels. This view is supported not only by our observations with S100A1, but also by studies on S100B (7), S100A4 (8), and S100A6 (10). Because we have observed a small decrease in resting intracellular calcium levels in pAntisense cells, we cannot completely rule out the possibility that the effects which we observe are calcium-dependent. Nonetheless, we can say that these processes are regulated by S100A1 in absence of the large changes in intracellular calcium usually associated with activation of the calcium signaling cascade. Additional studies will be needed before the target proteins involved in and calcium dependence of S100A1 regulation of tubulin/microtubule levels, neurite organization, and cell growth in PC12 cells can be ascertained.

The results presented in this study also provide new insights into the relationship between S100 family members. First, all of the S100 proteins that have been studied in intact cells have been documented to regulate the cytoskeleton and cell growth (7–10). These observations suggest that S100 family members have redundant functions. However, when one examines an individual cell type, each family member appears to regulate different aspects of these processes. For example, in PC12 cells, S100A6 initiates neurite extension in response to NGF (10) whereas S100A1 determines how many neurites will be extended. Furthermore, S100A6 does not alter cell growth whereas S100A1 does. Additional studies on other S100 family members will be required before the universality of nonredundant function for S100 family members can be established.

In summary, this study provides direct evidence that S100A1 regulates tubulin/microtubule levels, neurite organization, and cell growth in PC12 cells. In fact, S100A1 may be a molecule that directly links the cytoskeleton and cell growth. The observations that increases in microtubule stability can reverse neuronal cell death associated with Alzheimer’s disease (29) as well as neurotoxic agents such as acrylamide and carbon disulfide (30), glutamate (31), and N-methyl-4-phenyl-1,2,3,6-tetrahydropropyridine (32) suggest that S100A1 may be a suitable pharmacological target for treating neurological disorders. S100A1 antagonists may also be useful agents for the treatment of cancers such as renal carcinoma because these cancers express high levels of S100A1 (see Ref. 1), and this study demonstrates that decreased S100A1 expression/function is associated with decreased growth rates.

**Acknowledgments**—We thank Alexander Landar for technical assistance and J. Chessher for assistance in preparing the figures.

**REFERENCES**

1. Zimmer, D. B., Cornwall, E. H., Landar, A., and Song, W. (1995) Brain Res. Bull. 37, 417–429
2. Zimmer, D. B., Chessher, J. C., and Song, W. (1996) Biochim. Biophys. Acta 1313, 229–238
3. Schafer, B. W., and Heizmann, C. W. (1996) Trends Biochem. Sci. 21, 134–140
4. Wicki, R., Schafer, B. W., Erne, P., and Heizmann, C. W. (1996) Biochem. Biophys. Res. Commun. 227, 594–599
5. Wicki, R., Marenholz, I., Mischke, D., Schafer, B. W., and Heizmann, C. W.

2 D. B. Zimmer, E. H. Cornwall, P. D. Reynolds, and C. M. Donald, unpublished observation.
(1996) Cell Calcium 20, 459–464
6. Schafer, B. W., Wicki, R., Engelkamp, D., Mattei, M. G., and Heizmann, C. W. (1995) Genomics 25, 638–643
7. Selinfeld, R. H., Barger, S. W., Welsh, M. J., and Van Eldik, L. J. (1990) J. Cell Biol. 111, 2021–2028
8. Lakshmi, M. S., Parker, C., and Sherbet, G. V. (1993) Anticancer Res. 13, 269–304
9. Takenaga, K., Nakamura, Y., and Sakiyama, S. (1997) Oncogene 14, 331–337
10. Masaikowski, P., and Shooter, E. M. (1996) J. Neurosci. Res. 27, 264–269
11. Zimmer, D. B., and Landar, A. (1995) J. Neurochem. 64, 2727–2736
12. Esmaili-Asad, B., McCarty, J. H., and Feinstein, S. C. (1994) J. Cell Sci. 107, 869–879
13. Griakoff, V. K., Hammang, J. P., and Baetge, E. E. (1995) Mol. Brain Res. 29, 29–36
14. Meiri, K. F., Hammang, J. P., Dent, E. W., and Baetge, E. E. (1996) J. Neurobiol. 29, 213–232
15. Ivins, K. J., Neve, R. L., Feller, D. J., Fidel, S. A., and Neve, R. L. (1993) J. Neurochem. 60, 626–633
16. Niehlander, H. B., French, P., Oestreicher, A. B., Gispen, W. H., and Schotman, P. (1993) Neurosci. Lett. 162, 46–50
17. Xie, R., Li, L., Goshima, Y., and Strittmatter, S. M. (1995) Dev. Brain Res. 87, 77–86
18. Tashima, K., Yamamoto, H., Setoyama, C., Ono, T., and Miyamoto, E. (1996) J. Neurochem. 66, 57–64
19. Graham, M. E., Gerke, V., and Burgoyne, R. D. (1997) Mol. Biol. Cell 8, 431–442
20. Brandt, P. C., Sisken, J. D., Neve, R. L., and Vanaman, T. C. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 13843–13848
21. Zimmer, D. B., Song, W., and Zimmer, W. E. (1991) Brain Res. Bull. 27, 157–162
22. Anderson, S., Davis, D. L., Dahlback, H., Jornvall, H., and Russell, D. W. (1989) J. Biol. Chem. 264, 8222–8229
23. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
24. Strittmatter, S. M. (1995) J. Biol. Chem. 270, 11627–11631
25. Burke, W. J., Raghu, G., and Strong, R. (1994) Life Sci. 16, 313–319
26. Gupta, R. P., and Abou-Donia, M. B. (1997) Mol. Chem. Neuropathol. 30, 223–237
27. Bonfoco, E., Leist, M., Zhivotovsky, B., Orenius, S., Lipton, S. A., and Nicotera, P. (1996) J. Neurochem. 67, 2484–2493
28. Cappellletti, G., Incani, C., and Masi, R. (1995) Cell Biol. Int. 19, 687–693
