Mornet, D., Pantel, P., Audemard, E., Derancourt, J., & Kassab, R. (1985) *J. Mol. Biol. 183*, 479-489.
Mossakowska, N., & Strzelecka-Golaszewska, H. (1985) *Eur. J. Biochem. 153*, 373-381.
Muszbeck, L., Gladner, J. A., & Laki, K. (1975) *Arch. Biochem. Biophys. 167*, 99-103.
Offer, G., Moos, C., & Starr, R. (1973) *J. Mol. Biol. 74*, 653-676.
Peters, K., & Richards, F. M. (1977) *Annu. Rev. Biochem. 46*, 523-551.
Rouayrenc, J. F., Bertrand, R., Kassab, R., Walzthöny, D., Bähler, M., & Wallimann, T. (1985) *Eur. J. Biochem. 146*, 391-401.
Roustan, C., Boyer, M., Fattoum, A., Jeanneau, R., Benyamin, Y., Roger, M., & Pradel, L. A. (1982) *Eur. J. Biochem. 129*, 149-155.
Roustan, C., Benyamin, Y., Boyer, M., & Cavadore, J. C. (1986) *Biochem. J. 233*, 193-197.
Schwyter, D., & Reisler, E. (1988) *Biophys. J. 53*, 575a.
Sevensson, E. C., & Thomas, D. (1987) *Biophys. J. 50*, 999-1002.
Sutoh, K. (1982) *Biochemistry 21*, 3654-3661.
Sutoh, K. (1983) *Biochemistry 22*, 1579-1585.
Sutoh, K., & Mabuchi, I. (1986) *Biochemistry 25*, 6186-6192.
Taylor, K. A., & Amos, L. A. (1981) *J. Mol. Biol. 147*, 297-324.
Toyoshima, C., & Wakabayashi, T. (1985) *J. Biochem. (Tokyo) 97*, 219-243.
Toyoshima, Y. Y., Kron, S. J., Mc Nally, E. M., Niebling, K. R., Toyoshima, C., & Spudich, J. A. (1987) *Nature (London) 328*, 536-539.
Vibert, P., & Craig, R. (1982) *J. Mol. Biol. 157*, 299-319.
Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406-4412.
Weeds, A. G., & Taylor, R. A. (1975) *Nature (London) 257*, 54-56.

Expression and Stability of c-sis mRNA in Human Glioblastoma Cells†

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ABSTRACT: The production of platelet-derived growth factor like (PDGF-like) material by glioblastomas may be involved in the conversion of normal cells to tumor cells. In an investigation of this problem, we have examined some of the properties of the platelet-derived growth factor B-chain mRNA (c-sis mRNA) by a sensitive and quantitative RNA–RNA solution hybridization method. In 5 out of 8 human glioblastoma cell lines, c-sis mRNA was present, and in the line with the highest level, there were approximately 4–10 molecules per cell. The half-lives of the c-sis mRNA in two glioblastoma cell lines were 2.6 and 3.4 h, while in human umbilical vein endothelial (HUVE) and bladder carcinoma (T24) cells they were 1.6 and 2.5 h, respectively. Inhibiting protein synthesis produced no significant alteration of the c-sis mRNA half-lives in the glioblastoma or HUVE cells. The A-U-rich sequence at the 3′ end of the c-sis mRNA therefore does not appear to affect the mRNA stability in the presence of cycloheximide as it does in other transcripts. The similarity of the c-sis mRNA half-lives in normal and tumor cells suggests that regulation of stability of c-sis mRNA is not a major factor in tumorigenesis in the glioblastoma cell lines examined.

Mechanisms by which normal glial cells are converted to glioblastomas are still poorly understood. Some data are now available, however, suggesting the involvement of an autocrine growth loop (Sporn & Todaro, 1980) in glial cell tumorigenesis, whereby growth factor receptors are altered and/or growth factors themselves are abnormally produced. The epidermal growth factor (EGF) receptor gene, for instance, has been shown to be either amplified or structurally altered in a number of human glioblastomas (Libermann et al., 1985). Rat neuroglioblastomas induced by transplacental ethylnitrosourea (ENU) contain a point mutation in the transmembrane domain of the c-erb-B-2 (neu) transforming gene (Bargmann et al., 1986), the structure of which has considerable homology to the EGF receptor gene (Schechter et al., 1984). Long-term culture of neonatal rat brain cells similarly treated with ENU results in phenotypic transformation, the timing of which correlates with expression of the c-sis protooncogene (Lens et al., 1986), the gene encoding the B chain of platelet-derived growth factor (PDGF). These ENU-induced rat gliomas, as well as numerous cultured human glioblastomas, produce a PDGF-like mitogenic protein (Lens et al., 1986; Betsholtz et al., 1983; Nister et al., 1984; Pantazis et al., 1985), while normal human glial cells do not (Betsholtz et al., 1983). Because glial cells contain cell-surface receptors for PDGF (Heldin et al., 1981) and respond to exogenous PDGF by proliferating (Westermark & Wasteson, 1976; Besnard et al., 1987), the autocrine production of PDGF by glioblastomas may be a mechanism for constitutive stimulation of cellular growth. In support of this hypothesis, the growth rate in serum-free medium of various clonal glioblastoma cell lines correlates fairly well with their level of PDGF secretion (Nister et al., 1986). In addition, the in vivo growth of nude mouse tumors induced by v-sis-transformed fibroblasts correlates directly with their level of PDGF secretion (Huang et al., 1984). Although glioblastomas can express mRNA corresponding to both the A (Betscholtz et al., 1986a) and B (Pantazis et al., 1985; Betscholtz et al., 1986a; Eva et al., 1982) chain of PDGF, it is not clear whether glioblastoma-derived PDGF is composed of PDGF A chains, B chains, or both. The formation of glioblastomas in monkeys injected intracerebrally with simian sarcoma virus (SSV) (Swenberg, 1977), an acutely

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transforming retrovirus carrying the v-sis oncogene, suggests that overexpression of the sis-encoded PDGF B chain may be all that is necessary for glial cell transformation.

Expression of the sis/PDGF gene appears to be involved in the transformation of fibroblast cells since NIH 3T3 cells can be transformed not only by the viral form of the sis gene, v-sis (Robbins et al., 1982), but also by the normal c-sis gene (Chiu et al., 1984) driven by a strong promoter (Clarke et al., 1984; Gazit et al., 1984). An altered primary structure of the PDGF protein is therefore not needed for phenotypic transformation. Maintenance of the transformed phenotype also appears to be dependent on the presence of the sis/PDGF gene product, since either antibody to PDGF (Johnsson et al., 1985) or suramin (Betsholtz et al., 1986b), a drug known to bind free PDGF, can cause reversion of the transformed phenotype of SSV-infected fibroblasts. Whether this probable involvement of a sis-encoded PDGF-like protein in the transformation of fibroblasts is also applicable to the transformation of glial cells is not clear. The inability of PDGF antibody to alter the growth of a glioblastoma cell line (Heldin et al., 1985) could be due to many factors, one of which may be an intracellular action of the PDGF-like protein (Robbins et al., 1985).

A major focus of this laboratory is the question of whether PDGF-like growth factors are involved in the conversion of glial cells to glioblastomas. Initial experiments described here have been done to define some of the properties of the c-sis RNA expressed in some glioblastomas but not in normal human glial cells.

Experimental Procedures

Cell Culture. Human glioblastoma cell lines U87MG, U138MG, and U373MG were obtained from the American Type Culture Collection. Human glioblastoma cell lines A1207, A1235, A172, and A2781 and T24 bladder carcinoma cells were provided by Dr. Stuart Aaronson (NIH). Glioblastoma 05 was obtained from Dr. Sharad Deodhar (Cleveland Clinic). Human glioblastoma cell lines were grown at 37 °C in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% fetal calf serum (FCS) (GIBCO) and either glucose (3.4 g/L; glioblastomas A1207, A1235, A172, and A2781) or MEM nonessential amino acids (Glioblastomas 05, U87MG, U138MG, and U373MG). Normal human glial cells, provided by Dr. Steven D'Ambrosio (The Ohio State University) (Gibson-D'Ambrosio et al., 1983), were grown in a supplemented DMEM medium. Human umbilical vein endothelial (HUVE) cells were provided by Dr. P. Silverman (Case Western Reserve University) and were grown in medium 199 supplemented with *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (Hepes) (15 mM), endothelial cell growth factor (50–100 μg/mL; Collaborative Research), heparin (90 μg/mL), and 20% FCS.

Preparation of Total Cellular RNA. Total cellular RNA was prepared from cultured cells approaching 50–80% confluence by a modification of the CsCl ultracentrifugation method (Chirgwin et al., 1979). Cells were lysed with 4 M guanidinium thiocyanate (GTC) buffer [4 M GTC, 0.5% sodium *N*-laurylsarcosinate, 20 mM ethylenediaminetetraacetic acid (EDTA), 0.2 M 2-mercaptoethanol, and 50 mM 1,4-piperazinediethanosulfonic acid hydrochloride (Pipes-HCl), pH 7.0], the homogenate was layered over a 3.5-mL neutral cushion of 5.7 M CsCl/0.1 M EDTA in a 12-mL polyallomer centrifuge tube, and RNA was sedimented in a Beckman SW40 rotor at 25 000 rpm for 16 h at 22 °C. RNA pellets were suspended in 0.2 mL of water, extracted with chloroform, precipitated with ethanol, resuspended in 0.2 mL of water, and quantitated by the optical density at 260 nm. The RNA was then distributed into 5–15-μg aliquots, precipitated with ethanol, and stored as an alcohol slurry at −70 °C until use.

Plasmid Constructs. The antisense c-sis plasmid (C6) was made by subcloning a 960 base pair (bp) *Ban*II fragment of normal human c-sis DNA (Chiu et al., 1984) in the reverse orientation into the *Sma*I site of Genescribe plasmid pT7-2 (U.S. Biochemical) containing a specific T7 RNA polymerase promoter. This *Ban*II fragment encompasses exons 6 and 7 of c-sis genomic DNA and was derived from the λ--c-sis clone 8 (Chiu et al., 1984) provided by Dr. K. C. Robbins (NIH). The sense-strand sis plasmid (R22) was made by subcloning a 2.0-kilobase (kb) *Pst*I–*Bam*HI fragment of human c-sis cDNA clone pSM-1 (Clarke et al., 1984), provided by Dr. F. Wong-Staal (NIH), in the correct orientation into the analogous site of pT7-2. The antisense α-tubulin plasmid (G1) was made by subcloning a 135 bp *Sac*I–*Pst*I fragment of human α-tubulin cDNA clone Kα1 (Cowan et al., 1983), provided by Dr. D. Cleveland (The Johns Hopkins University), in the reverse orientation into the analogous site of Genescribe plasmid pT7-1 (U.S. Biochemical). For T7 RNA polymerase reactions, plasmid C6 was linearized with *Eco*RI, plasmid R22 was linearized with *Bam*HI, and plasmid G1 was linearized with *Rsa*I.

Synthesis of RNA Probes with T7 RNA Polymerase. Final reaction conditions for RNA synthesis by T7 RNA polymerase were 40 mM tris(hydroxymethyl)aminomethane (Tris) (pH 8.0), 5 mM dithiothreitol (DTT), 20 mM $MgCl_2$, 1 mM each of ATP, CTP, and GTP, 0.5 mg/mL bovine serum albumin, 1000 units/mL RNasin ribonuclease inhibitor (Promega Biotec), 20 μM UTP (labeled plus unlabeled), 20 μg/mL linearized template DNA, and 1000 units/mL T7 RNA polymerase. The final reaction volume was 10 μL. c-sis probes were synthesized with 130 pmol of [α-^{32}P]UTP (110 μCi, New England Nuclear), α-tubulin probes with 40 pmol of [α-^{32}P]UTP (30 μCi), and ^{3}H-labeled sense-strand sis RNA with 290 pmol of [^{3}H]UTP (10 μCi, ICN) and 210 pmol of unlabeled UTP (50 μM UTP final concentration). The reactions were incubated at 37 °C for 25 min. Template DNA was removed by incubation for 10 min at 37 °C with DNase I (10 μg/mL) purified free of ribonuclease (Tullis & Rubin, 1980). Following phenol/chloroform extraction and ethanol precipitation, the labeled RNA was resuspended in water to a final concentration of 1.0 μg/mL. Under these reaction conditions, approximately 50% of labeled UTP was incorporated into trichloroacetic acid (TCA)-precipitable RNA such that quantitative yields of ^{3}H- and ^{32}P-labeled sis transcripts could be determined. This labeled RNA appeared predominantly full length in denaturing agarose or acrylamide gels.

RNA–RNA Solution Hybridization Analysis. Ethanol-precipitated aliquots of total cellular RNA (5–15 μg) were suspended in 30 μL of hybridization buffer consisting of 80% deionized formamide, 40 mM Pipes-HCl (pH 6.4), 0.4 M NaCl, 1 mM EDTA, and 1 ng of ^{32}P-labeled RNA probe. To determine the sensitivity of the assay, a variable amount of ^{3}H-labeled sense-strand sis RNA (0.15–30 pg) was added to 10 μg of yeast tRNA and dissolved in the same buffer. The hybridization reactions were heated to 85 °C for 10 min and then annealed at 52 °C for at least 12 h. Hydrolysis of unprotected single-stranded regions of RNA was initiated by adding RNase A (to 50 μg/mL) and RNase T1 (to 2 μg/mL) in 300 μL of 10 mM Tris-HCl (pH 7.5), 5 mM EDTA, and 0.3 M NaCl. The reactions were incubated at room temperature for 30 min and at 30 °C for 30 min and were then terminated by the addition of 50 μg of proteinase K and 10 μL of 20% sodium dodecyl sulfate (SDS) and incubated for

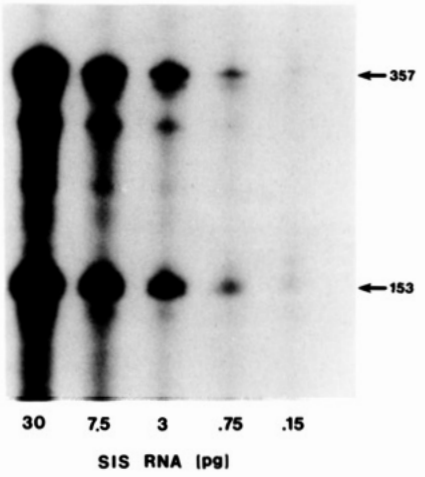

FIGURE 1: Quantitative RNA–RNA solution hybridization analysis of c-sis transcripts. ^{3}H-Labeled sense-strand sis RNA (0.15–30 pg) was hybridized in solution to a ^{32}P-labeled antisense c-sis RNA probe and analyzed as described under Experimental Procedures. The gel was exposed to film for 10 h.

15 min at 37 °C. Following phenol/chloroform extraction and two sequential ethanol precipitations with 10 μg of tRNA as carrier, the final RNA pellets were suspended in gel loading buffer consisting of 0.089 M Tris–borate, pH 7.5, 2 mM EDTA (TBE), 90% formamide, 0.02% bromophenol blue, and 0.02% xylene cyanol. The tubes were heated at 85 °C for 15 min and loaded into the wells of gels made with 5% acrylamide and 8 M urea in TBE. Following electrophoresis, the gels were applied to Kodak XAR-5 film for 0.5–3 days, and the resulting autoradiographic signals were quantitated by scanning densitometry (Transidyne General). Alternatively, the regions of the gel producing major autoradiographic signals were excised, and the radioactivity was determined by a liquid scintillation spectrometer (Pentz et al., 1986).

RESULTS

Detection of c-sis RNA by an RNA–RNA Solution Hybridization Assay. The quantitative nature of the RNA–RNA solution hybridization assay for the detection of c-sis mRNA was determined by annealing a ^{32}P-labeled antisense c-sis RNA probe with variable amounts of ^{3}H-labeled sense-strand c-sis RNA (Figure 1). This sense-strand sis RNA was synthesized in vitro from a 2.0-kb sis cDNA template and is thus a truncated version of the intact 3.4-kb c-sis mRNA (Rao et al., 1986). The ^{3}H labeling allowed accurate quantitation of the sense-strand message. The sense-strand sis RNA protected the two expected ^{32}P-labeled RNA fragments of 357 bases (exon 7) and 153 bases (exon 6) in a concentration-dependent manner. The detection of only 0.15 pg of c-sis RNA shows that this assay is quite sensitive. In this case, the film was exposed for just 10 h. The lighter minor bands in this figure are probably size-specific degradation products of the two predicted RNA fragments. These minor bands persisted to some degree in all experiments.

The signal intensity of the 357-base-protected fragment was quantitated by both scanning densitometry of the autoradiogram and liquid scintillation counting of the excised, solubilized gel slices. The results shown in Table I confirm that both methods yield c-sis signal intensity ratios that compare favorably to the amounts of ^{3}H-labeled sense-strand RNA initially added. The signals observed with the 153-base-protected fragment yielded similar results (data not shown).

c-sis RNA Is Expressed in Some Human Glioblastoma Cell Lines but Not in Normal Glial Cells. Total cellular RNA was isolated from eight different human glioblastoma cell lines, from normal human glial cells, from human fibroblast cells, from human umbilical vein endothelial (HUVE) cells, from HeLa cells, and from T24 bladder carcinoma cells. RNA aliquots were annealed in solution, first to the antisense c-sis RNA probe and later to the antisense α-tubulin RNA probe. The predicted c-sis-protected fragments were observed in five out of eight human glioblastoma cell lines, in HUVE, HeLa, and T24 cells, but not in untransformed glial of fibroblast cells (Table II). The presence of a 136-base α-tubulin RNA fragment provided a control for the amount of RNA added to each lane. The results are presented as a ratio of sis to tubulin RNA as measured by densitometry and with the highest ratio arbitrarily assigned a value of 100. On the less sensitive Northern blot analysis, a 4.0-kb c-sis mRNA has been detected in glioblastoma cell lines A1207, A172, and A2781, but not in glioblastomas U373MG or A1235 (data not shown). In A2781, a smaller RNA band was also observed which accounted for approximately 30% of the total sis mRNA. These bands were distinct which suggested no or minimal degradation of the RNA.

Half-Life of c-sis RNA in Human Cells. To determine the intracellular stability of c-sis RNA, cells expressing this transcript were treated for variable times with the transcription inhibitor actinomycin D. Efficient inhibition of cellular transcription was confirmed by demonstrating a greater than 95% reduction in the incorporation of [^{3}H]uridine into TCA-precipitable RNA in drug-treated (10 μg/mL) versus control cultures of glioblastoma A2781 cells. During the incubation, the morphology of the cells was unaltered, and cells were not released from the plates. Equal amounts of total cellular RNA from each actinomycin D exposure time were assayed for sis expression by RNA–RNA solution hybridization. sis mRNA half-life values were then calculated from the slope of the

Table I: Quantitation of the RNA–RNA Solution Hybridization Assay[a]

sense-strand c-sis RNA (pg)	357 band rel density	357 band total cpm	357 band rel cpm
30	ND[b]	3700	30
7.5	7.5	940	7.5
3.0	2.9	340	2.7
0.75	0.54	99	0.8
0.15	0.14	0	0

[a] The amount of the 357-base-protected fragment was measured by scanning densitometry of the autoradiogram (Figure 1) or by liquid scintillation counting. For each method, the band from the 7.5-pg lane was assigned a relative value of 7.5. [b] Not determined.

Table II: Steady-State Levels of c-sis mRNA in Human Cells[a]

cells	sis:tubulin RNA ratio		
	expt 1	expt 2	expt 3
glioblastoma A2781	100	100	100
glioblastoma A172	45	46	
glioblastoma A1207	38	44	
glioblastoma U373MG	7	12	
glioblastoma U87MG	0	0	
glioblastoma A1235			1.6
glioblastoma U138MG			0
glioblastoma 05			0
T24 bladder carcinoma			290
HUVE			220
HeLa			4
normal glial			0
fibroblast			0

[a] The results are expressed as the ratio of c-sis to tubulin RNA with the maximum c-sis glioblastoma level set at 100 for each of three separate experiments.

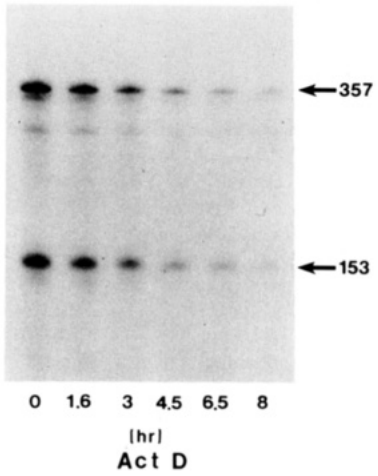

FIGURE 2: Half-life of c-sis mRNA in glioblastoma A2781 cells. Cells were treated with actinomycin D (5 μg/mL) for variable times. Equal amounts of RNA were analyzed. Quantitation of the data is shown in Figure 3.

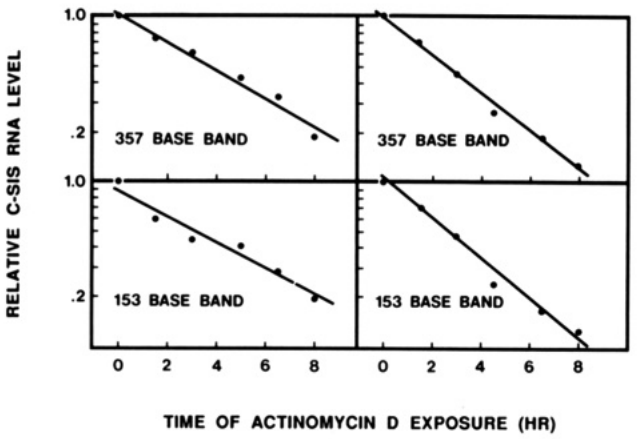

FIGURE 3: Half-life of c-sis mRNA in glioblastoma A172 (left) and A2781 (right) cells. The data were obtained from the experiment in Figure 2 (glioblastoma A2781) or from a similar experiment with glioblastoma A172 cells. The densitometric data on the abscissa are on a logarithmic scale and are not normalized to tubulin RNA. The c-sis mRNA decays in this experiment with half-lives of 3.5 h (357-base fragment) and 3.8 h (153-base fragment) for glioblastoma A172 cells and 2.6 h (357-base fragment) and 2.5 h (153-base fragment) for glioblastoma A2781 cells.

best-fit line in a plot of the log of sis mRNA signal intensity vs the time of transcription inhibition. Levels of mRNA for these half-life experiments were not normalized to tubulin expression. Representative half-life experiments using glioblastoma A172 and A2781 cells are shown in Figures 2 and 3, where the 357- and 153-base-protected fragments decay logarithmically. The comparable decay rate of each of the two protected RNA fragments served as an internal control. The results of similar actinomycin D half-life experiments using these and other cell lines are summarized in Table III. These experiments revealed a c-sis mRNA half-life value of 3.4 h in glioblastoma A172 cells, 2.6 h in glioblastoma A2781 cells, 2.5 h in T24 bladder carcinoma cells, and 1.6 h in normal HUVE cells. To demonstrate that the decline in c-sis mRNA was not the result of a generalized drug-induced degradation of all cellular transcripts, levels of α-tubulin RNA were also examined. The half-life for α-tubulin mRNA in the glioblastoma A172 cells was approximately 36 h and in the glioblastoma A2781 cells was approximately 23 h.

Protein Synthesis Inhibition Changes neither the Concentration nor the Intracellular Stability of c-sis RNA. Inhibition of cellular protein synthesis has been shown to increase both

Table III: Half-Life of c-sis mRNA in Human Cells[a]

	A172	A2781	T24	HUVE
no. of expt	2	2	2	2
$t_{1/2}$, 357 b	3.4 ± 0.2	2.6 ± 0.02	2.5 ± 0.1	1.6 ± 0.03
$t_{1/2}$, 153 b	3.4 ± 0.4	2.6 ± 0.03	2.5 ± 0.2	1.6 ± 0.1
		Plus CHX		
no. of expt	4	2		1
$t_{1/2}$, 357 b	3.4 ± 0.2	3.1 ± 0.1		1.6
% change	0	+20		0
		Plus Pur		
no. of expt		3		
$t_{1/2}$, 357 b		3.5 ± 0.4		
% change		+35		

[a] Cells were treated with actinomycin D (5–10 μg/mL) for variable times before RNA isolation. Half-lives ($t_{1/2}$) recorded in hours were determined from the best-fit straight lines and are the average of the values from the indicated number of experiments ± 1 standard deviation. Each experiment consisted of 4–6 times points, and the correlation coefficients were always greater than 0.90 Cycloheximide (10 μg/mL) was added 35–50 min and puromycin (0.4 mM) 4 min before actinomycin D. Abbreviations: CHX, cycloheximide; Pur, puromycin; T24, bladder carcinoma; HUVE cells, human umbilical vein endothelial cells; b, base or nucleotide residue.

the concentration and the intracellular stability of several RNAs including the protooncogenes c-fos (Rahmsdorf et al., 1987), c-myc (Dani et al., 1984; Thompson et al., 1986), and c-myb (Thompson et al., 1986). Although c-sis mRNA is not as labile as the mRNA of these other protooncogenes, its expression may be regulated by similar posttranscriptional mechanisms. To examine this possibility, protein synthesis inhibitors were added to various human cells, and their effect on both the concentration and stability of c-sis RNA was determined. For example, when 10 μg/mL cycloheximide was added to glioblastoma A172 cells for 2.8 or 6.7 h, levels of both c-sis and α-tubulin RNA remained unchanged compared to untreated cells (data not shown). To ensure that cycloheximide actually inhibited cellular protein synthesis, the incorporation of [^{35}S]methionine into TCA-precipitable protein was found to be reduced by an average of 80% in two experiments with cycloheximide-treated, compared to control, glioblastoma A172 cells.

The half-life of c-sis RNA was also determined in actinomycin D treated cells which had been pretreated with either cycloheximide or puromycin (Table III). In the absence of protein synthesis, the half-life for the 357-base-protected fragment of c-sis RNA in glioblastoma A172 cells was unchanged from the control value of 3.4 h, while in glioblastoma A2781 cells it was increased by 20–35%. In HUVE cells, the c-sis RNA half-life was 1.6 h with or without cycloheximide. Thus, inhibition of cellular protein synthesis has little if any effect on either the concentration or the intracellular stability of c-sis RNA in glioblastoma or normal endothelial cells.

Discussion

The RNA–RNA solution hybridization procedure has proven to be a quantitative and sensitive method for the detection of the relatively rare c-sis RNA species in human cells. The estimated number of c-sis mRNA molecules in each glioblastoma A2781 cell, the cell line in our series with the highest expression level, varied from approximately 4 to 10 copies per cell in 5 different experiments. These estimates required a quantitative comparison of the intensity of the c-sis mRNA signal in a known amount of total cellular RNA to that of a known amount of ^{3}H-labeled sense-strand sis mRNA. The number of cells represented in each lane of a gel was estimated from the number of cells isolated and the yield of total RNA from those cells. The level of c-sis mRNA observed

in T24 bladder carcinoma cells was approximately 3 times that in the glioblastoma A2781 cells as normalized to tubulin (Table II). Assuming comparable levels of tubulin mRNA in the glioblastoma and T24 cells, our estimate of the c-sis mRNA copy number in T24 cells is similar to another estimate of 10–20 copies per T24 cell [Weich et al. (1987) compared with Graves et al. (1984)]. The presence of RNA for the PDGF B chain in five of eight glioblastoma cell lines compared to its absence in normal glial cells suggests that constitutive synthesis of the B chain of this growth factor may contribute to either the formation or the maintenance of glioblastomas. In contrast, the absence of c-sis transcripts in other glioblastomas argues that expression of PDGF B-chain mRNA is not essential for glial cell transformation. Expression of PDGF-B may, however, by important in providing transformed cells a selective growth advantage during in vivo tumor progression. Levels of PDGF A-chain mRNA in these cells are currently under investigation.

One criticism of the methodology employed for measuring c-sis mRNA is that it measures only sis transcripts which include exons 6 and 7. If there were alternative c-sis mRNA splicing, a transcript lacking these exons would be missed by this assay. Although c-sis mRNA species smaller in size than the full-length spliced transcript have been identified in some human cell types (Barrett et al., 1984; Eva et al., 1982; Graves et al., 1984; Collins et al., 1985), there is no evidence provided by others that any human glioblastoma contains a c-sis mRNA of any size other than full-length. The smaller c-sis mRNA identified in these other cell types may in fact represent not the PDGF B chain but the highly homologous PDGF A chain, having known mRNAs of length 2.8, 2.3, and 1.9 kb (Betsholtz et al., 1986a). Northern blots of RNA from glioblastoma cell lines A172 (Eva et al., 1982; Pantazis et al., 1985; Igarashi et al., 1987; Tong et al., 1987) and A2781 (Igarashi et al., 1987; Gazit et al., 1984) all detect only a single visible 4.2–4.4-kb c-sis mRNA band. Only a single 4.4-kb RNA band has been detected in A172 cells using DNA probes from each of six different c-sis exons, including exons 6 and 7 (Pantazis et al., 1985). Alternatively spliced c-sis mRNAs are therefore absent in the A172 glioblastoma cells and present as a minor subpopulation in the A2781 cells. Finally, there is no evidence that smaller c-sis transcripts involve alternative splicing involving exons 6 and 7.

This is the first report of message stability of the PDGF B chain in normal and transformed cells. The shortest c-sis mRNA half-life observed with the HUVE cells was 1.6 h. In the T24 bladder carcinoma cells, it was 2.5 h while in the glioblastoma cell lines A2781 and A172 it was 2.6 and 3.4 h, respectively. Intracellular stability of mRNA in eucaryotic cells is quite variable with half-lives ranging from around 500 h (Brock & Shapiro, 1983) to less than 10 min (Rahmsdorf et al., 1987). The best studied examples of labile protooncogene mRNAs are c-fos, c-myc, and c-myb with half-lives in human cells of around 9 min (Rahmsdorf et al., 1987), 15 min (Dani et al., 1984), and 31 min (Thompson et al., 1986), respectively. Serum stimulation of quiescent fibroblasts results in the rapid but transient expression of c-fos, c-myc, and c-myb mRNA, implying a temporary role for these nuclear proteins in the cell's proliferative response. While the serum-stimulated induction of c-fos mRNA is the result of an increase in its transcription rate (Greenberg & Ziff, 1984), the induction of both c-myc (Thompson et al., 1986) and c-myb (Thompson et al., 1986) mRNA is primarily the result of increased mRNA stability. Posttranscriptional control of c-myc is also seen in interferon-inhibited Daudi lymphoblastoid cells (Dani et al., 1985), in differentiating F9 embryonal carcinoma cells (Dean et al., 1986; Dony et al., 1985), and in mouse plasmacytomas without an intact first exon (Piechaczyk et al., 1985). Additional evidence for posttranscriptional regulation is the observation that cycloheximide increases both the cytoplasmic concentation and the half-lives of c-fos (Rahmsdorf et al., 1987), c-myc (Dani et al., 1984), and c-myb (Thompson et al., 1986) mRNAs. Although these data imply the presence of a labile mRNA-specific ribonuclease of related protein, the existence of this protein has yet to be confirmed.

A major question approached by this study was whether there was any evidence for posttranscriptional regulation of c-sis RNA in glioblastoma cells. In comparison with normal endothelial cells, the c-sis mRNA half-lives in the two glioblastoma cell lines were 1.6 and 2.1 times longer. These differences are minor when compared to the examples cited above. Since normal glial cells in culture do not produce detectable c-sis RNA (Table II), we have no appropriate cell-specific control. It is possible that the observed decay rate of c-sis RNA could be an artifact induced by actinomycin D treatment, but this seems unlikely since the decay of c-sis RNA after induction with phorbol ester or TGF-β occurs at a rate very similar to the half-lives noted here (unpublished observations). In glioblastoma cells, α-tubulin RNA is a good control since in two cell lines its mRNA half-life was 23 and 36 h, in agreement with some previous reports that it is a relatively stable transcript with a half-life greater than 6 h (Rice & Roberts, 1983; Howe et al., 1984). Is is of interest that the H-ras oncogene, which on transfection can increase production of growth factors (Zhan & Goldfarb, 1986) and c-sis mRNA (Owen & Ostrowski, 1987) by a cell, does not appear to affect the c-sis mRNA in the T24 bladder carcinoma cells by an alteration of the half-life.

Although c-sis mRNA is significantly more stable than that of the nuclear protooncogenes myc, myb, and fos, it is less stable than the majority of the mRNA species of the cell, since the average mammalian poly(A) mRNA decays with a half-life greater than 10 h (Greenberg, 1972; Murphy & Attardi, 1973). The inability of protein synthesis inhibitors to alter either the concentration or the intracellular stability of c-sis RNA represents additional evidence that the mechanisms controlling c-sis gene expression are different than those controlling expression of fos, myc, or myb.

Various sequences at both the 5′ and 3′ end of mRNAs have been identified which affect the half-lives of messages (Brawerman, 1987). A 51-residue A-U-rich sequence was found at the 3′ end of a human lymphokine gene which, when added to the 3′ end of a β-globin gene, converted the mRNA from a structure stable over a 2-h period to one with a half-life of less than 30 min (Shaw & Kamen, 1986). By addition of cycloheximide, the level of β-globin mRNA with the normal 3′-terminal sequence increased 2-fold while that with the A-U sequence substitution increased 25-fold. The 3′-terminal A-U sequence may therefore be a specific cleavage site for a labile cellular ribonuclease or enzyme complex. The protooncogenes c-fos, c-myc, and c-myb, each of which contains a consensus A-U-rich sequence near its 3′ terminus (Shaw & Kamen, 1986), encode labile transcripts that are also significantly stabilized in the presence of cycloheximide (Rahmsdorf et al., 1987; Dani et al., 1984; Thompson et al., 1986). Although this A-U-rich sequence is also present near the 3′ terminus of the c-sis gene (Shaw & Kamen, 1986), the c-sis mRNA is much more stable and its half-life does not significantly change when cycloheximide is added. The expression of c-sis RNA (in these glioblastoma and HUVE cells) therefore ap-

pears to be controlled by stable rather than labile protein elements. In addition, these data suggest that the presence of a 3′-terminal A-U-rich sequence in the sis mRNA is not, by itself, sufficient to result in the formation of a labile transcript.

REFERENCES

Bargmann, C. I., Hung, M.-C., & Weinberg, R. A. (1986) *Cell (Cambridge, Mass.) 45*, 649–657.

Barrett, T. B., Gajdusek, C. M., Schwartz, S. M., McDougall, J. K., & Benditt, E. P. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 6772–6774.

Besnard, F., Perraud, F., Sensenbrenner, M., & Labourdette, G. (1987) *Neurosci. Lett. 73*, 287–292.

Betsholtz, C., Heldin, C.-H., Nister, M., Ek, B., Wasteson, A., & Westermark, B. (1983) *Biochem. Biophys. Res. Commun. 117*, 176–182.

Betsholtz, C., Johnsson, A., Heldin, C.-H., Westermark, B., Lind, P., Urdea, M. S., Eddy, R., Shows, T. B., Philpott, K., Mellor, A. L., Knott, T. J., & Scott, J. (1986a) *Nature (London) 320*, 695–699.

Betsholtz, C., Johnsson, A., Heldi, C.-H., & Westermark, B. (1986b) *Proc. Natl. Acad. Sci. U.S.A. 83*, 6440–6444.

Brawerman, G. (1987) *Cell (Cambridge, Mass.) 48*, 5–6.

Brock, M. L., & Shapiro, D. J. (1983) *Cell (Cambridge, Mass.) 34*, 207–214.

Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., & Rutter, W. J. (1979) *Biochemistry 18*, 5294–5299.

Chiu, I. M., Reddy, E. P., Givol, D., Robbins, K. C., Tronick, S. R., & Aaronson, S. A. (1984) *Cell (Cambridge, Mass.) 37*, 123–129.

Clarke, M. F., Westin, E., Schmidt, D., Josephs, S. F., Ratner, L., Wong-Staal, F., Gallo, R. C., & Reitz, M. S. (1984) *Nature (London) 308*, 464–467.

Collins, T., Ginsburg, D., Bass, J. M., Orkin, S. H., & Pober, J. S. (1985) *Nature (London) 316*, 748–750.

Cowan, N. J., Dobner, P. R., Fuchs, E. V., & Cleveland, D. W. (1983) *Mol. Cell. Biol. 3*, 1738–1745.

Dani, Ch., Blanchard, J. M., Piechaczyk, M., Sabouty, S. E., Marty, L., & Jeanteur, P. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 7046–7050.

Dani, Ch., Mechti, N., Piechaczyk, M., Lebleu, B., Jeanteur, P., & Blanchard, J. M. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 4896–4899.

Dean, M., Levine, R. A., & Campisi, J. (1986) *Mol. Cell. Biol. 6*, 518–524.

Dony, C., Kessel, M., & Gruss, P. (1985) *Nature (London) 317*, 636–639.

Eva, A., Robbins, K. C., Andersen, P. R., Srinivasan, A., Tronick, S. R., Reddy, E. P., Ellmore, N. W., Galen, A. T., Lautenberger, J. A., Papas, T. S., Westin, E. H., Wong-Staal, F., Gallo, R. C., & Aaronson, S. A. (1982) *Nature (London) 295*, 116–119.

Gazit, A., Igarashi, H., Chiu, I. M., Srinivasan, A., Yanin, A., Tronick, S., Robbins, K. C., & Aaronson, S. A. (1984) *Cell (Cambridge, Mass.) 39*, 89–97.

Gibson-D'Ambrosio, R. E., Leong, Y., & D'Ambrosio, S. M. (1983) *Cancer Res. 43*, 5846–5850.

Graves, D. T., Owen, A. J., Barth, R. K., Tempst, P., Winoto, A., Fors, L., & Hood, L. E. (1984) *Science (Washington, D.C.) 226*, 972–974.

Greenberg, J. R. (1972) *Nature (London) 240*, 102–104.

Greenberg, M. E., & Ziff, E. B. (1984) *Nature (London) 311*, 433–438.

Heldin, C.-H., Westermark, B., & Wasteson, A. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 3664–3668.

Heldin, C.-H., Betsholtz, C., Johnsson, A., Nister, M., Ek, B., Ronnstrand, L., Wasteson, A., & Westermark, B. (1985) *J. Cell Sci. Suppl. 3*, 65–76.

Howe, C. C., Lugg, D. K., & Overton, G. C. (1984) *Mol. Cell. Biol. 4*, 2428–2436.

Huang, J. S., Huang, S. S., & Deuel, T. F. (1984) *Cell (Cambridge, Mass.) 39*, 79–87.

Igarashi, H., Rao, C. D., Siroff, M., Leal, F., Robbins, K. C., & Aaronson, S. A. (1987) *Oncogene 1*, 79–85.

Johnsson, A., Betsholtz, C., Heldin, C.-H., & Westermark, B. (1985) *Nature (London) 317*, 438–440.

Lens, P. F., Altena, B., & Nusse, R. (1986) *Mol. Cell. Biol. 6*, 3537–3540.

Libermann, T. A., Nusbaum, H. R., Razon, N., Kris, R., Lax, I., Soreq, H., Whittle, N., Waterfield, M. D., Ullrich, A., & Schlessinger, J. (1985) *Nature (London) 313*, 144–147.

Murphy, W., & Attardi, G. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 115–119.

Nister, M., Heldin, C.-H., Wasteson, A., & Westermark, B. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 926–930.

Nister, M., Heldin, C.-H., & Westermark, B. (1986) *Cancer Res. 46*, 332–340.

Owen, R. D., & Ostrowski, M. C. (1987) *Mol. Cell. Biol. 7*, 2512–2520.

Pantazis, P., Pelicci, P. G., Dalla-Favera, R., & Antoniades, H. N. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 2404–2408.

Pentz, M., Vatev, R., & Goldthwait, D. A. (1986) *Nucleic Acids Res. 14*, 5513–5529.

Piechaczyk, M., Yang, J.-Q., Blanchard, J. M., Jeanteur, P., & Marcu, K. (1985) *Cell (Cambridge, Mass.) 42*, 589–597.

Rahmsdorf, H. J., Schonthal, A., Angel, P., Litfin, M., Ruther, U., & Herrlich, P. (1987) *Nucleic Acids 15*, 1643–1659.

Rao, C. D., Igarashi, H., Chiu, I.-M., Robbins, K. C., & Aaronson, S. A. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 2392–2396.

Rice, A. P., & Roberts, B. E. (1983) *J. Virol. 47*, 529–539.

Robbins, K. C., Devare, S. G., Reddy, E. P., & Aaronson, S. A. (1982) *Science (Washington, D.C.) 218*, 1131–1133.

Robbins, K. C., Leal, F., Pierce, J. H., & Aaronson, S. A. (1985) *EMBO J. 4*, 1783–1792.

Schechter, A. L., Stern, D. F., Vaidyanathan, L., Decker, S. J., Drebin, J. A., Green, M. I., & Weinberg, R. A. (1984) *Nature (London) 312*, 513–516.

Shaw, G., & Kamen, R. (1986) *Cell (Cambridge, Mass.) 46*, 659–667.

Sporn, M. B., & Todaro, G. J. (1980) *N. Engl. J. Med. 303*, 878–880.

Swenberg, J. A. (1977) *Natl. Cancer Inst. Monogr. No. 46*, 3–10.

Thompson, C. B., Challoner, P. B., Neiman, P. E. & Groudine, M. (1986) *Nature (London) 319*, 374–380.

Tong, B. D., Auer, D. E., Jaye, M., Kaplow, J. M., Ricca, G., McConathy, E., Drohan, W., & Deuel, T. F. (1987) *Nature (London) 328*, 619–621.

Tullis, R. H., & Rubin, H. (1980) *Anal. Biochem. 107*, 260–264.

Weich, H. A., Herbst, D., Schairer, H. U., & Hoppe, J. (1987) *FEBS Lett. 213*, 89–94.

Westermark, B., & Wasteson, A. (1976) *Exp. Cell Res. 98*, 170–174.

Zhan, X., & Goldfarb, M. (1986) *Mol. Cell. Biol. 6*, 3541–3544.