B-Myb Expression in Vascular Smooth Muscle Cells Occurs in a Cell Cycle-dependent Fashion and Down-regulates Promoter Activity of Type I Collagen Genes*

(Received for publication, August 14, 1995, and in revised form, November 3, 1995)

Darius J. Marhamati and Gail E. Sonenshein†

From the Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts 02118

The members of the Myb family of transcription factors are defined by homology in the DNA-binding domain; all bind the Myb-binding site (MBS) sequence (YG(A/G)(A/C/G)GT(G/A)). Here we report that cultured bovine vascular smooth muscle cells (SMCs) express B-Myb. Levels of B-Myb RNA found in exponential growth were reduced dramatically in serum-deprived quiescent SMCs; B-Myb mRNA levels increased in the cell cycle during the late G1 to S phase transition following re-stimulation with serum, epidermal growth factor, or phorbol ester plus insulin-like growth factor-1. Changes in the rate of B-Myb gene transcription could account for part of the observed increase following serum addition. Treatment of SMC cultures with actinomycin D indicated that a 4-h half-life for B-Myb mRNA during the S phase of the cell cycle. Cotransfection of either a bovine or human B-Myb expression vector down-regulated the activity of a multimerized MBS element-driven reporter construct in SMCs. Putative MBS elements were detected upstream of the promoters of the two chains of type I collagen, which we have found to be expressed inversely with growth state of the SMC (Kindy, M. S., Chang, C.-J., and Sonenshein, G. E. (1988) J. Biol. Chem. 263, 11426–11430). In cotransfection experiments, B-Myb expression down-regulated the promoter activity of α(I) and α(II) collagen constructs an average of 92 and 82%, respectively. Thus, B-Myb represents a potential link in the observed inverse relationship between collagen gene expression and growth of vascular SMCs.

SMCs are the major cellular constituents of the medial layer of an artery, being responsible for maintaining vascular tone in the adult blood vessel (Ross, 1993). During the formation of a developing artery, SMCs display a synthetic phenotype; an initial highly proliferative phase is followed by synthesis of extracellular matrix components such as collagen, elastin, and proteoglycans (Hughes, 1942; Wu et al., 1992). This matrix provides a structural framework for the artery and also presumably allows for cell layering. Once the artery has been fully formed, SMCs differentiate into a contractile phenotype, in which they normally remain (Chamley-Campbell et al., 1979). As a normal response to injury and in certain disease states, however, SMCs migrate to the intimal layer, where modest rounds of proliferation are followed by production and deposition of matrix components over extended periods of time (Poole

*This work was supported by National Institutes of Health Grant HL13262 (to G.E.S.) and Training Grant HL07429 (to D.J.M.). 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: Dept. of Biochemistry, Boston University School of Medicine, 80 East Concord St., Boston, MA 02118. Tel.: 617-638-4120; Fax: 617-638-5339.

The myb oncogene was first identified as the transforming gene of two retroviruses, avian myeloblastosis virus and E26, both of which cause myeloblastic leukemia in birds (Moscovici, 1975). In nontransformed cells, high levels of c-myb mRNA are observed only in immature hematopoietic cells (Gonda and Metcalf, 1984), while lower myb levels have been detected in embryonic neural tissue as well as neuroblastoma cells and in chick embryo fibroblasts (Thiele et al., 1988; Thompson et al., 1986). We found that cultured bovine vascular smooth muscle cells (SMCs) also express low levels of c-myb mRNA (Reilly et al., 1988; Brown et al., 1992). Two c-myb related genes have been isolated based on their high homology in the DNA-binding domains (Nomura et al., 1988; Lam et al., 1992). These genes, termed A- and B-myb, have only recently begun to be characterized.

B-Myb expression has been detected in many tissues (Nomura et al., 1988; Golay et al., 1991; Arsura et al., 1992, 1994). Cell synchronization studies have demonstrated that in 3T3 fibroblasts and hematopoietic cells, B-Myb displays a late G1-specific gene expression pattern, similar to that of c-myb (Lam et al., 1992; Golay et al., 1991; Reiss et al., 1991). Recent work has indicated that B-Myb protein is capable of binding to the consensus Myb-binding site (MBS) (YGRC(A/C/G)GT(T/G)(A)) (Howe and Watson, 1991), although R is preferably T/C for c-Myb and A/C for B-Myb. Furthermore, B-Myb has also been reported to recognize a second specific consensus sequence (CUNTTTCT) as well (Mizuguchi et al., 1990). The transactivation properties of B-Myb are controversial, as one group reported it to function as a positive regulator (Mizuguchi et al., 1990), while several others had found it to be a transcriptional inhibitor of c-myb-mediated transactivation (Foss et al., 1992; Watson et al., 1993). These apparently contradictory findings may be due to the fact that transfected B-Myb behaves differently in different cell lines (Tashiro et al., 1995). B-Myb inhibited c-myb-induced transactivation in 3T3 fibroblasts, whereas activation was observed upon transfection into HeLa cells. Although no mechanism has been established for this effect, Tashiro et al. (1995) proposed that cell-specific expression of binding partner proteins allows for differential formation of a functional dimer. In addition, B-Myb has also been found to transactivate the DNA polymerase α gene promoter independent of any identified MBS element (Venturelli et al., 1990; Watson et al., 1993).

The abbreviations used are: SMCs, smooth muscle cells; MBS, Myb-binding site; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; EGF, epidermal growth factor; TPA, 12-O-tetradecanoate; IGF-1, insulin-like growth factor-1; kb, kilobase(s); bp, base pair(s); CAT, chloramphenicol acetyltransferase; TK, thymidine kinase.
et al., 1971; Schwartz et al., 1985; Gordon et al., 1990; Ross, 1993; Strauss et al., 1994). These synthetic responses of SMCs, in association with deposition of lipids and minerals, can result in formation of an atherosclerotic plaque.

SMCs grown in culture maintain a dedifferentiated synthetic phenotype. At low cell density, they proliferate rapidly, but produce little connective tissue matrix (Beldekas et al., 1982; Stepp et al., 1986). As we and others have shown, production of connective tissue proteins, such as collagen types I, III, and IV, by SMCs increases dramatically as they approach confluence, when their growth slows and cells begin to form multilayers (Beldekas et al., 1982; Laiu and Chan, 1989; Ang et al., 1990; Brown et al., 1991). Since vascular SMCs express the c-myb oncogene, here we characterized expression of B-myb. Aortic SMCs were found to express B-myb in a cell cycle-dependent fashion: quiescent cells contained low levels of B-myb RNA, with increasing levels seen during the late G1 to S phase transition. Cotransfection of B-myb expression vectors in SMC cultures inhibited the activity of a multimerized MBS-driven promoter linked to the chloramphenicol acetyltransferase gene in dAX-TK-CAT (Ibanez and Lipsick, 1990). The vector dAX-TK-CAT was in turn constructed from pBLCAT2 by deletion of the AaII polylinker (XhoI) and restriction from the pUC18 backbone. A lower level of myb-induced transcription activity apparently caused by cryptic MBS elements (Ibanez and Lipsick, 1990). The vector p1.6Bgl-CAT contains bp –1114 to +513 of the murine c-myb gene linked to the CAT reporter construct as described previously (Duyao et al., 1992); pHMyb-CAT contains 1 kb of sequence upstream of the start site of transcription of the human myb promoter and MBS cloned into the pSV2-CAT vector (kindly provided by T. Bender, University of Virginia School of Medicine, Charlottesville, VA). The pMS-3.5/CAT construct contains bp –3500 to +58 of the human a2(I) promoter upstream of the CAT reporter gene (Boast et al., 1990). The plasmid pOB3.6 contains 3.5 kb of the rat a2(I) collagen promoter plus the first exon and first intron linked to the CAT reporter (Bedalov et al., 1994). The plasmid ColCAT3.6 is composed of a 3.6-kb fragment containing 3.5 kb of sequence upstream of the start site of transcription and 115 bp of the first exon of the rat a2(I) collagen gene linked to the CAT reporter (Lichtler et al., 1989).

Transfections and Reporter Gene Assays—Cells were plated at a density of 5 × 10^4 cells/100-mm^2 dish 24 h before transfection. The medium was changed 2–4 h before transfection. DNA (50 μg) was transfected by the modified CaPO4 transfection procedure of Chen and Okayama (1987). Cells were harvested 48–72 h after transfection, and lysates were prepared as described previously (Lawrence et al., 1994a). Protein concentrations of the lysates were determined using the Bradford assay as directed by the manufacturer (Bio-Rad). Equal amounts of total protein were incubated with 2.5 μCi of [3H]acetyl coenzyme A (DuPont NEN; 200 mCi/mmol), 50 μM acetyl coenzyme A, and 1.6 μM chloramphenicol for 4–8 h, and the acetylated bands were extracted with ethyl acetate and assayed by liquid scintillation counting (Lawrence et al., 1994a).

RNA Isolation and Hybridization Analysis—Total cellular RNA was isolated according to the method of Chirgwin et al. (1979) or with Tri-Reagent (Molecular Research Center, Inc.). Equal quantities of RNA (15–25 μg) were denatured and separated by electrophoresis on 1% agarose-formaldehyde gels. Separated RNA was transferred onto a GeneScreen Plus nylon membrane (DuPont NEN). RNA was cross-linked to the membrane by UV irradiation (Stratagene) at 0.12 J/cm^2 for 30 s. For RNA stability studies, cells were treated with 5 μg/ml actinomycin D (Boehringer Mannheim). Probes were prepared as described previously by Feinberg and Vogelstein (1982); hybridization reactions contained 1–2 × 10^6 cpm of 32P-labeled DNA/ml of buffer. Unhybridized probe was removed by washing blots at 68 °C with 2 × SSC (1 × SSC is 0.15 M NaCl, 15 mM sodium citrate), 0.1% SDS for 30 min, followed by 15–30 min washes with 1 and 0.3 × SSC, as needed. Autoradiography by scanning densitometry was performed using a Molecular Dynamics 300A computing densitometer.

Transcription Analysis—Nuclei were isolated from SMCs, and run-off analysis was performed by a modification of the method of Greenberg and Ziff (1984). Briefly, 1 × 10^7 nuclei were incubated in the presence of 250 μCi of [32P]UTP (DuPont NEN; 3200 Ci/mmol) for 30 min at 37 °C. Labeled RNA was isolated, and equal amounts of radiolabeled RNA (4.5 × 10^6 cpm/ml of hybridization buffer) were hybridized to transfected DNA (10 μg/sample) immobilized onto GeneScreen Plus by slot blotting followed by UV irradiation; after hybridization, blots were washed as described above.

RESULTS Expression of the B-myb mRNA in Aortic SMC Cultures—Expression of B-myb in hematopoietic cells and 3T3 fibroblasts has been shown to be cell cycle-regulated, with increasing levels detected during the G1 to S phase transition (Golay et al., 1991; Lam et al., 1992). To determine whether aortic SMCs express B-myb, Northern blot analysis was performed using RNA isolated from bovine aortic SMCs synchronized using the serum deprivation-stimulation protocol described previously (Kindy and Sonenshein, 1986; see "Materials and Methods"). Serum-deprived quiescent cells begin to enter S phase ~12 h after serum stimulation, and DNA synthesis peaks between 16 and 20 h (Kindy and Sonenshein, 1986). RNA was isolated from SMCs in exponential growth, in quiescence, and at various
B-Myb Expression Down-regulates Collagen Gene Transcription

Fig. 1. Cell cycle expression of B-myb RNA in bovine vascular smooth muscle cells. Bovine aortic SMC cultures were rendered quiescent by serum deprivation (DMEM plus 0.5% FBS) for 72 h. Serum was then added back (DMEM plus 10% FBS) to allow synchronous entry into S phase. Total RNA, isolated at the indicated time points and from cells in exponential growth and quiescence, was subjected to Northern blot analysis. A, autoradiogram of a blot probed with the human λ-B-myb cDNA clone; B, ethidium bromide-stained gel confirming RNA quality and equal loading. Lane E, exponential growth; lane Q, quiescence; lanes 2, 8, 14, 18, and 24, numbers indicate the hours after serum addition.

Fig. 2. Nuclear run-off analysis of cell cycle changes in the rate of transcription of the B-myb gene. SMC cultures were made quiescent and serum-stimulated as described in the legend to Fig. 1. Nuclei were isolated at 0.5, 12, and 18 h after serum addition and subjected to nuclear run-off analysis. The resulting radiolabeled RNAs were hybridized to the following cDNA probes (10 μg) immobilized on nylon membranes: bovine B-myb (pB14), ornithine decarboxylase (ODC), histone H3.2 (His H3.2), α1(I) collagen, α1(V) collagen, and pUC19 plasmid DNA.

after 4 h of actinomycin D treatment (Fig. 3). In contrast, levels of the 5 phase-expressed histone H3.2 mRNA, which were significant at 18 h, decayed substantially during this treatment, consistent with results obtained previously (Brown et al., 1992). Therefore, B-myb mRNA appears relatively stable at the time points where substantial steady-state levels are present.

Activation of B-myb by EGF and TPA plus IGF-1—We next investigated the ability of specific growth factors to induce expression of B-myb RNA. Subconfluent SMC cultures were made quiescent via serum deprivation and were then stimulated with addition of 20 ng/ml EGF. This growth factor is a weak mitogen for subconfluent SMCs, as evidenced by the appearance of histone H3.2 mRNA, which was detectable by 24 h (Fig. 4). EGF treatment resulted in a low level of induction of B-myb mRNA that began to be detectable at 12 h and was clearly seen at 24 h (Fig. 4). Thus, expression of B-myb RNA was inducible by EGF and appeared to precede entry into S phase, consistent with the G$_S$/S-phase specific nature of the B-myb expression observed above.

Phorbol ester treatment of quiescent cells has been found to induce genes mediating competence, such as c-fos and c-myc, and entry into the G$_1$ phase of the cell cycle (Greenberg and Ziff, 1984; Kelly et al., 1983). Further transit from G$_1$ into S phase requires stimulation with a progression factor, such as IGF-1 (Leef et al., 1982). To examine the effects of these agents on SMCs, serum-deprived quiescent cell cultures were stimulated with 100 nM TPA in the absence or presence of 35 ng/ml IGF-1. RNA was isolated from cells in quiescence (0 h) or 10, 16, and 24 h after stimulation. B-myb RNA levels were low in quiescence (Fig. 5), as observed above (Fig. 1). No significant increase in B-myb expression was seen with TPA treatment alone. In contrast, B-myb RNA levels increased in the cells treated with both TPA and IGF-1. Thus, treatment with TPA made SMCs competent to respond to the progression factor IGF-1, leading to increased expression of B-myb.

Activity of B-myb as a Transcriptional Regulator—Previously, we had shown that the reporter plasmid KHK-CAT-dAX, derived by insertion of nine copies of the MBS directly in front of the TK promoter linked to the CAT gene in the plasmid dAX-TK-CAT, was transcriptionally active in SMCs (Brown et al., 1992). To begin to assess the specific functional role of B-myb in transactivational control in the vascular SMC, a 3.4-kb bovine pB14 B-myb cDNA clone, in the pBK-CMV plasmid expression vector, was isolated from an aortic SMC cDNA library and used in cotransfection experiments. SMC cultures, at ~50% confluence, were cotransfected with the KHK-CAT-
dAX reporter construct and increasing amounts of pB14 expression vector. After 3 days, extracts were prepared, and equal amounts of proteins were analyzed. B-myb expression resulted in dose-dependent down-regulation of KHK-CAT-dAX activity, with a maximal decrease of 2.5-fold or 60% (Fig. 6). An average of three separate experiments yielded a drop in activity of 60.3 ± 3.7%. In contrast, pB14 had no effect on the activity of parental dAX-TK-CAT. Cotransfection with 12 μg of pB14 only reduced dAX-TK-CAT activity to 92.0 ± 4.0% of control in two separate experiments (data not shown). Cotransfection of 5 μg of a human pCEP-B-myb expression vector similarly resulted in a specific down-regulation of the activity of KHK-CAT-dAX (65%; data not shown). Thus, expression of B-Myb leads to repression of the transcriptional activity of an MBS element-driven heterologous promoter in SMCs.

Regulation of Collagen Promoters by B-myb—Previously, we had noted that type I collagen mRNA levels varied inversely with the growth state of the vascular SMC (Stepp et al., 1986; Kindy et al., 1988). Levels of type I collagen mRNA were low in actively proliferating SMCs and increased in quiescence due to either serum deprivation or growth to confluence. Since bovine B-myb appeared to be a negative regulator of transcription, we analyzed the promoters of the type I collagen genes and identified several putative MBS elements in both the α1 and α2 chains of type I collagen (see “Discussion”). Thus, cotransfection experiments were performed to test the effects of B-myb expression on collagen promoter activity. Increasing concentrations of pB14 were cotransfected with the α2(1) collagen promoter pMS-3.5/CAT construct, which contains 3.5 kb of sequence upstream of the start site of transcription and 58 bp of exon 1 driving the CAT reporter gene (Boast et al., 1990). The activity of the pMS-3.5/CAT vector was down-regulated 3.8-fold (72%) upon cotransfection with 10 μg of bovine B-myb vector.
assessed the effects of B-Myb expression on the activity of the promoter activity in 3T3 fibroblasts. Similarly, the activity of the promoters of the genes encoding both chains of type I collagen.

The expression of the α1 and α2 genes of type I collagen is often coordinately regulated (Stepp et al., 1986). Therefore, we assessed the effects of B-Myb expression on the activity of the α1(II) promoter. pOB3.6, which contains 3.6 kb of the α1(II) collagen promoter plus all of exon 1 and intron 1 upstream of the CAT reporter gene, was cotransfected with the bovine B-Myb expression vector pB14. The activity of pOB3.6 was downregulated 12.4-fold in a dose-dependent manner, by coexpression of B-Myb (Fig. 8). Cotransfection with 10 μg of bovine pB14 reduced pOB3.6 activity an average of 92 ± 2.5% in three experiments, and that with 10 μg of human pCEP-B-Myb expression vector reduced it 79% (data not shown). The plasmid ColCAT3.6, which contains 3.5 kb of the α1(II) collagen promoter plus 115 bp of exon 1 upstream of the CAT reporter gene, displayed fairly low levels of CAT activity in SMCs (data not shown). This activity was similarly down-regulated by the presence of either human or bovine B-Myb, but to a somewhat lesser extent, 57 ± 2.4% (data not shown). Therefore, B-Myb is a specific regulator of transcription that is able to down-regulate the activity of the promoters of the genes encoding both chains of type I collagen.

To determine whether B-Myb acts nonspecifically as a negative regulator of transcription in SMCs, cotransfection analysis was performed with the c-myc and c-myc promoters, both of which contain MBSs that have been shown to be regulated by c-Myb (Evans et al., 1990; Nakagoshi et al., 1992; Nicolaides et al., 1991). Cotransfection of 5 μg of pB14 with the c-myc promoter plasmid p1.6Bgl-CAT resulted in only an 15% reduction in its activity. These results agree with those of Watson et al. (1993), who found that B-Myb had no effect on c-myc promoter activity in 3T3 fibroblasts. Similarly, the activity of the c-myc promoter plasmid pHNmyb-CAT was down-regulated only 12% upon cotransfection with B-Myb. Thus, B-Myb expression did not appear to significantly affect the promoter activity of these two oncogenes, suggesting that the inhibition of collagen promoter activity described above is specific.

**DISCUSSION**

Proliferating primary bovine aortic SMCs were found to express B-Myb, a member of the myb gene family. B-Myb expression in SMCs occurred in a cell cycle-dependent fashion and displayed negative regulatory activity with respect to an MBS element-driven construct and the α1(II) and α2(II) collagen promoters. Quiescent SMCs expressed very little B-Myb mRNA, and levels increased as cells entered late G1 and peaked in S phase following stimulation with serum, EGF, or a combination of treatment with TPA and 1GF-1. Previous work in several laboratories, including our own, demonstrated an inverse rela-
B-Myb Expression Down-regulates Collagen Gene Transcription

The regulation of collagen gene transcription in SMCs is under investigation. The selective down-regulation of the activity of the promoters for the two chains of type I collagen upon B-Myb expression in transient cotransfection analysis in primary cultured SMCs may occur by either a direct or an indirect mechanism. DNA analysis of the rat and human collagen COL1A1 and A2 genes revealed the presence of several putative Myb-binding sites and B-Myb-specific sequence elements. The presence of these putative sites raises the possibility of a direct effect of B-Myb on collagen genes. The limited size of these sequences, however, necessitates more specific mapping analysis. It should also be noted that indirect mechanisms have been observed, e.g., with the DNA polymerase α promoter (Venturelli et al., 1990; Watson et al., 1993); these could similarly be involved with the down-regulation of transcription of collagen genes via expression of B-Myb and would be of equal functional significance for matrix formation by the SMC. We have recently found that co-microinjection of B-Myb with c-myc expression vectors into quiescent SMCs failed to induce entry into S phase, suggesting that the observed inhibition of collagen gene transcription is not simply due to a change in the proliferative state of the cell.

The 6–8-fold increase in B-Myb mRNA seen in the cell cycle can be partly accounted for by the 1.6–2-fold increase in the overall rate of transcription of the gene. In 3T3 fibroblasts, the mechanism of the cell cycle increase in B-Myb RNA levels was determined to be due to an increase in the rate of transcription of the gene. Deletion of an E2F site abrogated cell cycle regulation of B-Myb transcription (Lam and Watson, 1993). Gel shift analysis revealed that quiescent cells showed E2F binding that was supershifted only with antibodies to E2F and p107, while in S phase, this complex contained cyclin A as well. It is possible that other mechanisms play a significant role in B-Myb expression in SMCs. Alternative levels of control include either a change in the rate of elongation of RNA chains during synthesis or of RNA processing or altered stability. For example, c-myc mRNA levels in hematopoietic cells are controlled mainly by the rate of elongation of transcription (Bender et al., 1987), while in chick embryo fibroblasts, mRNA stability is the main level of regulation (Thompson et al., 1986). Interestingly, the increase in c-myc mRNA levels in SMCs during the late G1 to S phase transition could not be accounted for either by an enhanced rate of gene transcription or by a change in the stability of the c-myc RNA (Brown et al., 1992), suggesting additional levels of control.

The observation that there is an inverse relationship between matrix deposition and cellular proliferation is a long standing one that has been substantiated in many different systems. For example, viral transformation of fibroblasts enhanced the proliferative capacity of these cells while decreasing their level of synthesis of type I collagen (Adams et al., 1982). Overexpression of the ras oncogene in Rat1 fibroblasts had a similar effect on type I collagen gene expression by these cells (Slack et al., 1992). In density-arrested nondividing human fetal lung fibroblasts, type I and III collagen mRNA levels were significantly higher than those in logarithmically growing cells (Miskulin et al., 1986). Thus, it appears that when genes necessary for growth, such as oncogenes, are expressed, other genes that are inconsistent with or unnecessary for growth are turned off. The fact that B-Myb is expressed broadly in many different cell types presents the possibility that the signal transduction pathway that mediates activation of this gene may be involved in the inhibition of collagen gene expression in cells derived from many different tissues.

SMCs are responsible for synthesizing the extracellular matrix components in the medial layer of a normal artery, including collagen, elastin, fibronectin, and proteoglycans, as well as the enzymes involved in matrix protein deposition, such as lysyl oxidase (reviewed by Ross (1993)). During arterial development in the chick, an initial SMC synthetic phase is followed by deposition of matrix proteins and additional cell layering (Hughes, 1942; Wu et al., 1992). The most abundant collagen species produced by SMCs is type I collagen, with lesser but still significant amounts of collagen types III, V, and VI. In atherosclerosis, SMCs migrate from the medial layer to the intima, where some initial rounds of proliferation are followed by extensive synthesis and deposition of matrix proteins (Poole et al., 1971; Gordon et al., 1990; Ross, 1993; Strauss et al., 1994). The majority of the mass of a fibrous plaque is composed of the collagen proteins deposited by the SMC. The subsequent occlusion of the lumen of the artery and the clinical sequelae that follow are a primary cause of morbidity and mortality in the Western world. Coordinate regulation of many collagen species, including types I, III, and V, has been noted in SMC cultures under a variety of conditions that affect growth state (Jones et al., 1979; Stepp et al., 1986; Liau and Chan, 1989; Ang et al., 1990; Brown et al., 1991; Lawrence et al., 1994b). In addition, other genes necessary for matrix deposition, such as lysyl oxidase, have similar inverse expression patterns in relation to growth (Kenyon et al., 1991). Thus, the possibility that B-Myb plays a more general role in regulation of matrix gene expression in SMCs is under investigation.

Acknowledgments—We thank Drs. Joe Lipsick, Francesco Ramirez, Barbara Smith, David Rowe, Martino Introna, and T. Bender for gen-

References:

1. D. Marhamati, R. Bellas, M. Arsura, and G. E. Sonenshein, manuscript in preparation.

2. D. Marhamati, R. Bellas, M. Arsura, and G. E. Sonenshein, manuscript in preparation.

Downloaded from http://www.jbc.org/ on July 24, 2018
erously providing clone DNA reporter and expression constructs. The helpful comments and suggestions of Dr. Marcello Arsura are gratefully acknowledged. We also thank Dr. Judith Foster for use of the scanning densitometer.

REFERENCES

Adams, S. L., Boettiger, D., Focht, R., Holtzer, H., and Pacid, M. (1982) Cell 30, 373–384
Alterman, R., Ganguly, S., Schulze, D., Marzloff, W., Schildkraut, C., and Skoultchi, A. (1984) Mol. Cell. Biol. 4, 123–132
Ang, A. H., Tachas, G., Campbell, J. H., Bateman, J., and Campbell, G. R. (1990) Biochem. J. 265, 461–469
Arsura, M., Introna, A., Passerini, F., Mantovani, A., and Golay, J. (1992) Blood 79, 2708–2716
Arsura, M., Luchetti, M., Erba, E., Golay, J., Rambaldi, A., and Introna, M. (1994) Blood 83, 1778–1790
Bedalov, A., Breault, D., Sokolov, B., Lichtler, A. Bedalov, I., Clark, S., Mack, K., Khillian, J., Woody, C., Kream, B., and Rowe, D. (1994) J. Biol. Chem. 269, 4903–4909
Bédélolas, J., Gerstenfeld, L., Sonenschein, G. E., and Franzblau, C. (1982) J. Biol. Chem. 257, 12252–12256
Bender, T., Thompson, C. B., and Kuehl, W. M. (1987) Anal. Biochem. 166, 88–96
Bender, T., Thompson, C. B., and Kuehl, W. M. (1987) Science 236, 1473–1476
Bost, S., Su, S.-W., Ramirez, F., Sanchez, M., and Avvedimento, E. (1990) J. Biol. Chem. 265, 13331–13336
Brown, K. E., Lawrence, R., and Sonenshein, G. E. (1991) J. Biol. Chem. 266, 23268–23273
Brown, K. E., Kindy, M., and Sonenshein, G. E. (1992) J. Biol. Chem. 267, 4625–4630
Chanley-Campbell, J., Campbell, G. R., and Ross, R. (1979) Physiol. Rev. 59, 1–61
Chang, C. J., and Sonenshein, G. E. (1991) Matrix 11, 242–251
Chen, C., and Okayama, H. (1987) Mol. Cell. Biol. 7, 2745–2752
Chirgwin, J. M., Przybyla, A., MacDonald, R. J., and Rutter, W. J. (1979) Biochemistry 18, 5294–5299
Duyao, M., Kessler, D. J., Spicer, D. B., Bartholomew, C., Cleveland, J. L., Siekieritz, M., and Sonenshein, G. E. (1992) J. Biol. Chem. 267, 16288–16291
Evans, J., Moore, T., Kuehl, W. M., Bender, T., and Ting, J. (1990) Mol. Cell. Biol. 10, 5474–5752
Feinberg, A., and Vogelstein, B. (1983) Anal. Biochem. 132, 6–13
Foos, G., Grimm, S., and Klempnauer, K. H. (1992) EMBO J. 11, 4619–4629
Golay, J., Cherqui, A., Arsura, M., Castellano, M., Rizzo, V., and Introna, M. (1991) Blood 77, 149–158
Gonda, T. J., and Metcalf, D. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 540–544
Greenberg, M. E., and Ziff, E. B. (1984) Cell 33, 353–357
Hughes, A. F. W. (1942) Proc. Natl. Acad. Sci. U.S.A. 28, 12252–12256
Ishizake, R. (1988) J. Biol. Chem. 263, 14800–14806
Ishii, S. (1990) J. Biol. Chem. 265, 9280–9284
Kenyon, K., Cohan, B., Stiles, C., and Leder, P. (1983) Cell 35, 603–610
Kenyon, K., Conteste, S., Trackman, P. C., Tang, J., Kagan, H. M., and Friedman, R. (1991) Science 253, 802
Kindy, M. S., and Sonenshein, G. E. (1986) J. Biol. Chem. 261, 12865–12868
Kindy, M. S., Chang, C.-J., and Sonenshein, G. E. (1988) J. Biol. Chem. 263, 11426–11430
Law, E. W.-F., and Watson, R. (1993) EMBO J. 12, 2705–2713
Lehrach, H., Frischjauch, A. M., Hanahan, D., Wozney, J. F., Fuller, F., and Boedtker, H. (1979) Biochemistry 18, 3146–3152
Leof, E. B., Wharton, W., van Wyk, J., and Pledger, J. (1982) Exp. Cell Res. 141, 107–115
Liau, G., and Chan, L. M. (1989) J. Biol. Chem. 264, 10315–10320
Moscovici, C. (1975) Curr. Top. Microbiol. Immunol. 71, 79–101
Nomura, N., Takahashi, M., Matsu, M., Ishii, S., Date, T., Sasamoto, S., and Ishizaka, R. (1988) Nucleic Acids Res. 16, 11075–11083
Poll, E. C., Cromwell, S. B., and Benditt, E. P. (1971) J. Clin. Pathol. 24, 391–413
Relly, C. F., Kindy, M. S., Brown, K. E., Rosenberg, R. D., and Sonenshein, G. E. (1989) J. Biol. Chem. 264, 6990–6995
Reiss, K., Travali, S., Calabretta, B., and Baserga, R. (1991) J. Cell. Physiol. 148, 338–348
Rich, R. (1993) Science 262, 801–809
Schwartz, S. M., Reidy, M., and Clowes, A. (1985) Ann. N. Y. Acad. Sci. 454, 292–304
Stepp, M. A., Kindy, M. S., Franzblau, C., and Sonenshein, G. E. (1986) J. Biol. Chem. 261, 6542–6547
Thiele, C. J., Cohen, P. S., and Israel, M. A. (1988) Mol. Cell. Biol. 11, 4714–4723
Venturelli, D., Travali, S., and Calabretta, B. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 5963–5969
Venturelli, D., Travali, S., and Calabretta, B. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 5963–5969
Wu, Y., Rich, C., Lincecum, J., Trackman, P. C., Kagan, H. M., and Foster, J. A. (1992) J. Biol. Chem. 267, 24199–24206
B-Myb Expression in Vascular Smooth Muscle Cells Occurs in a Cell Cycle-dependent Fashion and Down-regulates Promoter Activity of Type I Collagen Genes
Darius J. Marhamati and Gail E. Sonenshein

Access the most updated version of this article at http://www.jbc.org/content/271/7/3359

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 61 references, 35 of which can be accessed free at http://www.jbc.org/content/271/7/3359.full.html#ref-list-1