Transforming Growth Factor (TGF)-β Stimulates Hepatic *jun*-B and *fos*-B Proto-oncogenes and Decreases Albumin mRNA

R. DANIEL BEAUCHAMP, M.D., HONG-MIAO SHENG, M.D., JIN ISHIZUKA, M.D., COURTNEY M. TOWNSEND, JR., M.D., and JAMES C. THOMPSON, M.D.

From the Department of Surgery, The University of Texas Medical Branch, Galveston, Texas

Transforming growth factor-β (TGF-β) modulates some components of the acute phase response in hepatic cells. The mechanisms for these actions of TGF-β are largely unknown. The authors recently found that the decrease in albumin mRNA after TGF-β1 treatment required *de novo* RNA and protein synthesis, suggesting that TGF-β acts through induction of another gene. The purpose of the current study was to determine whether TGF-β1 could regulate the expression of both the *jun* and *fos* genes that encode transcriptional regulatory proteins that constitute the AP-1 complex, and to determine whether expression of these genes may be coordinated with the decrease in albumin mRNA. Northern blot hybridization was used to determine levels of specific mRNAs. Transforming growth factor-β1 increased the levels of both *jun*-B and *fos*-B mRNA by 60 minutes after treatment of mouse hepatoma (BWTG3) cells. When TGF-β1 was removed from the media after 4 hours, there was a sustained effect of increased *jun*-B and decreased albumin mRNA (>48 hours), and the subsequent decrease in *jun*-B levels coincided with the increase in albumin mRNA. The tumor-promoting phorbol ester (phorbol 12-myristate 13-acetate [PMA]), known to induce *jun* and *fos* gene expression, caused increases in *jun*-B and *fos*-B that preceded the decrease in albumin mRNA levels at 24 hours. These observations are consistent with our hypothesis that *jun*-B and *fos*-B induction may participate in downregulation of albumin synthesis as well as other hepatic responses to TGF-β.

THE TRANSFORMING GROWTH factor-β (TGF-β) family of molecules comprise the protein products of five separate genes (there are three mammalian isoforms) that share a high degree of homology and all act through the same cell surface receptors.[1-3] In this report, the term TGF-β refers to the TGF-β family (TGF-β1, 2, or 3); otherwise, specific isoforms are designated by number (*e.g.*, TGF-β1). The TGF-βs are secretory proteins with numerous biologic activities that include inhibition of cell proliferation, regulation of cell adhesion and extracellular matrix deposition, immunosuppression, fibroblast and monocyte chemotaxis, and regulation of cell differentiation. Transforming growth factor-β probably has important roles in the liver. Transforming growth factor-β is expressed in hepatic megakaryocytes during development and in the fetal mouse liver capsule.[4] Levels of TGF-β expression in adult liver are very low; however, TGF-β1 expression in hepatic nonparenchymal cells is induced during hepatic regeneration after partial hepatectomy[5] and in cirrhosis.[6,7] Transforming growth factor-β inhibits the growth of cultured nontransformed hepatocytes[8]; either TGF-β1 or TGF-β2 can inhibit the first wave of DNA synthesis in the regenerating rat liver, but does not prevent or delay the recovery of hepatic mass or DNA content, which are normal 8 days after 70% hepatectomy.[9] Furthermore, the liver is the major site for metabolism of activated TGF-β1.[10,11] Most intravenously injected TGF-β1 is rapidly (half-life [$t_{1/2}$] = 2.2 minutes) cleared from the blood by the liver, where it is metabolized and the metabolites are excreted in the bile.[10] Uptake of active TGF-β1 in the liver is by hepatocytes, and not by nonparenchymal cells.[11]

In addition to effects on liver cell growth, TGF-β may have a role in the hepatic acute phase response.[12-15] After a systemic injury such as acute inflammation, bacterial infection, major surgery, metal toxicity, burn, or the development of certain forms of cancer, the liver responds with a striking increase in the synthesis of a subset of serum proteins that are called the positive acute phase response proteins.[16] There is another class of negative acute phase response proteins (including albumin) whose

Presented at the 112th Annual Meeting of the American Surgical Association, April 6-8, 1992, Palm Desert, California.

Supported by grants from the National Institutes of Health (CA01309, DK35608, DK15241), the Shriners Burns Institute (15860), and the National Cancer Society (PDT-220).

Address reprint requests to R. Daniel Beauchamp, M.D., Department of Surgery, The University of Texas Medical Branch, Galveston, TX 77555.

Accepted for publication April 10, 1992.

concentrations decrease after injury. Transforming growth factor-β decreases albumin mRNA levels and the secretion of albumin protein[12–14] and increases the secretion of some of the positive acute phase reactants (α1-protease inhibitor and α1-antichymotrypsin) from human hepatoma cells.[13] Transforming growth factor-β also inhibits albumin synthesis in primary cultures of normal human hepatocytes.[14] The mechanisms for these actions of TGF-β in liver cells have not been determined. We have reported previously that TGF-β1 downregulates albumin and α-fetoprotein (AFP) mRNA in the mouse hepatoma cell line (BWTG3) by a post-transcriptional mechanism that requires *de novo* RNA and protein synthesis, and probably involves destabilization of the mRNA for these two genes.[17] Our previous studies have suggested that the downregulation of albumin mRNA levels by TGF-β requires the expression of another, as yet unidentified, gene or set of genes.

A group of transcriptional regulatory proteins (the AP-1 complex) appears to be involved in mediating some of the effects of TGF-β on gene transcription.[18,19] AP-1 is a complex of nuclear proteins that was originally defined by the interaction of the proteins with DNA sequences in the transcriptional-regulatory regions of genes whose transcription could be induced by phorbol ester (12-0 α-tetradecanoylphorbol-13-acetate [TPA or PMA]).[20,21] Genes that are induced by PMA have a consensus DNA sequence [TGA(C/G)TCA] in the regulatory region that can bind to the AP-1 protein complex, and this interaction regulates transcription. The AP-1 complex can be composed of either homodimers of JUN protein or heterodimers of JUN and FOS proteins.[22,23] The c-*jun* proto-oncogene is the cellular homologue of the transforming v-*jun* gene, first isolated from a retrovirus that causes tumors in chickens.[24] Three separate but homologous members of the *jun* proto-oncogene family have been identified and are called c-*jun*, *jun*-B, and *jun*-D. In differentiated myocytes, the rapid transcriptional induction of *jun*-B by TGF-β is independent of protein synthesis and is among the earliest responses to TGF-β.[25] The other component of the AP-1 complex is the FOS protein. The *fos* family includes c-*fos*,[26] and more recently identified genes encoding homologous proteins: *fos*-B,[27] *fra*-1,[28] and *fra*-2.[29] FOS:JUN protein heterodimers form more stable complexes with the AP-1 site than JUN:JUN homodimers. Any JUN protein can interact with any FOS protein.[30] The purpose of this study was to determine whether TGF-β can induce expression of *jun* and *fos* genes, and to determine whether expression of these genes is coordinated with the decrease in albumin mRNA.

Materials and Methods

Cell Cultures

The BWTG3 cell line, derived from a mouse hepatoma (BW7756),[31] was cultured in Dulbecco's Modified Eagles Medium supplemented with 10% fetal bovine serum, 100 units of penicillin, and 0.1 mg streptomycin/mL. Cultures were maintained in log growth phase at all times, and all experiments were performed on cells within 6 weeks of culture from liquid nitrogen stocks. Lyophilized, platelet-derived, purified porcine TGF-β1 was obtained from R & D Systems, Inc. (Minneapolis, MN) and was hydrated with 4 mmol/L HCl and 0.1% bovine serum albumin. Phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma (St. Louis, MO) and dissolved in Dulbecco's Modified Eagles Medium containing 10% dimethylsulfoxide (vehicle). Preliminary experiments determined that the vehicle (added at 3 μL per milliliter medium) had no effect on the BWTG3 cells; therefore, controls received no additions in the present experiments. The protein kinase inhibitors 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H7) and N-(2-guanidine ethyl)-5-isoquinolinesulfonadmide hydrochloride (HA1004) were purchased from Seikagaku America, Inc. (St. Petersburg, FL). H7 and HA1004 were dissolved in distilled water at stock concentration of 10 mmol/L.

Isolation and Analysis of RNA

Total RNA from BWTG3 cells was isolated by the methods of Chirgwin et al.[32] The cells were washed twice in cold phosphate-buffered saline solution, then were homogenized in 7.5 mol/L guanidine hydrochloride, 1% 2-mercaptoethanol, and 25 mmol/L sodium citrate followed by ultracentrifugation through a 5.7 mol/L CsCl cushion. Northern blot analysis was done as described.[33] Filters were probed with [^{32}P]-labeled cDNA in hybridization buffer containing 50% formamide, 4 × SSC, 0.2 mg/mL sheared salmon sperm DNA, 4 × Denhardt's solution, 0.1% sodium dodecyl sulfate, and 50 mmol/L $NaPO_4$, pH 6.5. After hybridization, filters were washed three times for 15 minutes at 22 C in 2 × SSC, 0.1% sodium dodecyl sulfate, and then three times for 45 minutes at 60 C in 0.1 × SSC, 0.1% sodium dodecyl sulfate. The mouse *jun*-B and c-*fos* cDNA clones were obtained from the American Type Culture Collection (Rockville, MD). The *fos*-B cDNA was provided by Rodrigo Bravo.[27] The 18S ribosomal RNA signal was used as an internal control to ensure integrity of RNA and equality of loading among lanes.

Results

TGF-β1 Increases jun *and* fos-*B mRNA Levels*

In previous studies,[17] we found that TGF-β1 decreased albumin mRNA to 60% of control by 12 hours, 23% by 24 hours, and to less than 10% of control by 48 hours after treatment. Further studies suggested that TGF-β led to the transcription and translation of another (perhaps more than one) gene whose product causes degradation of albumin mRNA. AP-1–like sites are necessary for

transcriptional induction by TGF-β of at least two genes (plasminogen activator inhibitor[19] and TGF-β1 itself[18]); therefore, we first examined whether TGF-β could induce the genes encoding the AP-1 components. After TGF-β1 treatment of BWTG3 cells after 24 hours in serum-free conditions, *jun*-B mRNA abundance was examined (Figs. 1A & B). Transforming growth factor-β caused a rapid increase (30 minutes) in the level of *jun*-B mRNA (2.0 kb) that was sevenfold greater than control by 60 minutes. Subsequent experiments showed that c-*jun* mRNA was also increased, but to a lesser degree (data not shown). Transforming growth factor-β1 treatment also resulted in a 6.7-fold increase in the *fos*-B mRNA (~4.0 kb) at 60 minutes (Fig. 2). No c-*fos* mRNA was detectable even with prolonged (1 week) exposure of the autoradiograph (Fig. 2A). Increases in *jun*-B (4.75-fold) and *fos*-B (2.1-fold) mRNAs were still detectable at 24 hours after a single addition of TGF-β1 (120 pmol/L), whereas albumin mRNA levels were decreased to 28 ± 3.8% of control values (Fig. 3). There was no change in 18S ribosomal RNA abundance after TGF-β1 treatment.

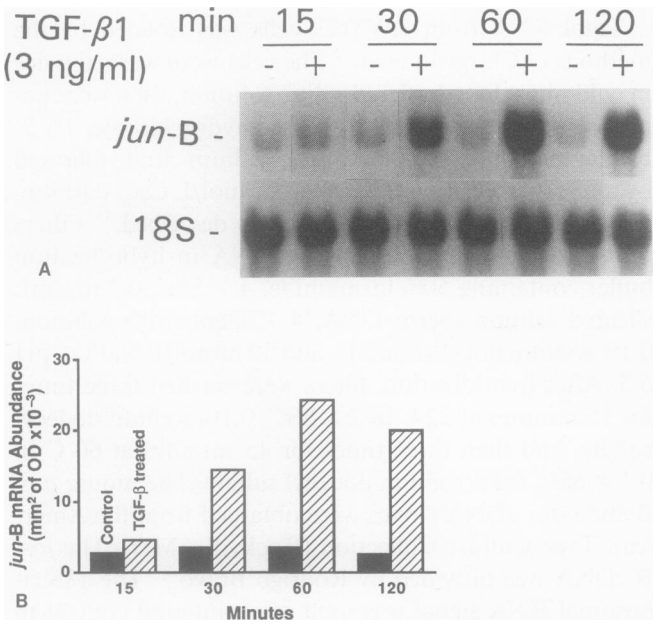


FIG. 1. TGF-β1 induction of *jun*-B and *fos*-B mRNA. BWTG3 cells at 70–80% confluence in 100-mm tissue culture plates in DMEM supplemented with 10% FBS were washed and the medium was replaced with serum-free DMEM. After 24 hours the cells were treated with TGF-β1 (120 pmol/L) as indicated by the (+) columns. Control cells (−) received no additions. Cells were harvested at the indicated times after treatment and total cellular RNA was extracted, fractionated, and immobilized on nitrocellulose filters (20 μg/lane). The filters were hybridized with ^{32}P-labeled cDNA probes for *jun*-B. After autoradiography, probe was removed and the filters were then hybridized with ^{32}P-labeled 18S ribosomal DNA to determine relative equality of sample loading in this and subsequent experiments. After hybridization and posthybridization washes, the filters were exposed to x-ray film at −70 C with intensifying screens. Exposure of filters hybridized with *jun*-B was for 24 hours. The graph below (B) shows the results from densitometry of the autoradiogram shown above (A).

Reversibility After Short-term TGF-β1 Treatment

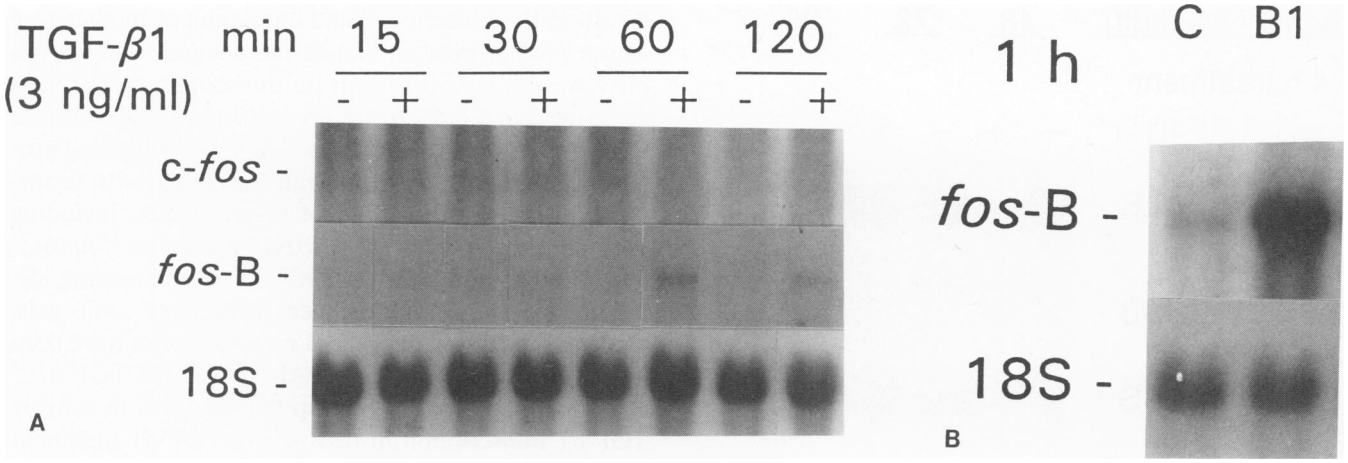
To determine whether a relatively brief exposure to TGF-β1 had a prolonged effect on albumin and *jun*-B mRNA levels in BWTG3 cells, the cells were treated with TGF-β1 for 4 hours, washed, and refed with medium without TGF-β1 and harvested at 48, 72, or 96 hours (Fig. 4). Controls ([−] lanes) were treated identically, except they received no TGF-β. The brief exposure to TGF-β1 resulted in prolonged depression of the albumin mRNA compared with controls (28% at 48 hours). There was a corresponding prolonged increase in the levels of *jun*-B mRNA (2.4-fold greater than control at 48 hours). The decrease toward normal levels of *jun*-B mRNA appeared to temporally coincide with the increase in albumin mRNA toward control levels. The higher albumin mRNA levels in the confluent cultures (>48 hours) is characteristic of these cells in the stationary phase of growth (John Papaconstantinou, personal communication).

Effect of PMA Treatment on jun-*B,* fos-*B, and Albumin mRNA*

Because the AP-1 complex was originally defined by interaction with phorbol ester-responsive enhancer elements, a decrease in albumin mRNA in response to phorbol ester would be consistent with the hypothesis that this transcriptional complex is involved in the TGF-β1 effect. Phorbol 12-myristate 13-acetate (162 nmol/L) caused a decrease in albumin mRNA levels by 24 hours after treatment (Fig. 5). Densitometric analysis of two separate experiments showed albumin mRNA to be 24% and 38% of control 24 hours after PMA treatment. There was no change in albumin mRNA 3 and 6 hours after PMA treatment. Phorbol 12-myristate 13-acetate (162 nmol/L) treatment also resulted in increased levels of *jun*-B and *fos*-B mRNA at 3, 6, and 24 hours, and these increases preceded and coincided with the decrease in albumin mRNA at 24 hours (Fig. 5).

Effect of Protein Kinase Inhibitor

Ohtsuki and Massague[34] recently reported that the protein kinase inhibitor H7 was able to block the rapid induction of both *jun*-B and plasminogen activator inhibitor in the mink lung epithelial cell line, Mv1Lu, in response to TGF-β1. We used H7 in an attempt to determine whether blocking induction of *jun*-B could prevent the observed decrease in albumin. H7 inhibits protein kinase C, protein kinase A, protein kinase G, and myosin light-chain kinase (Ki, 6.0 μmol/L, 3.0 μmol/L, 5.8 μmol/L and 97 μmol/L, respectively).[35] Another related compound, HA1004, also inhibits protein kinases but has less potency against protein kinase C (Ki, 40 μmol/L) and myosin light-chain kinase (Ki, 150 μmol/L) than it does against protein kinase A or protein kinase G (Ki, 2.3 μmol/



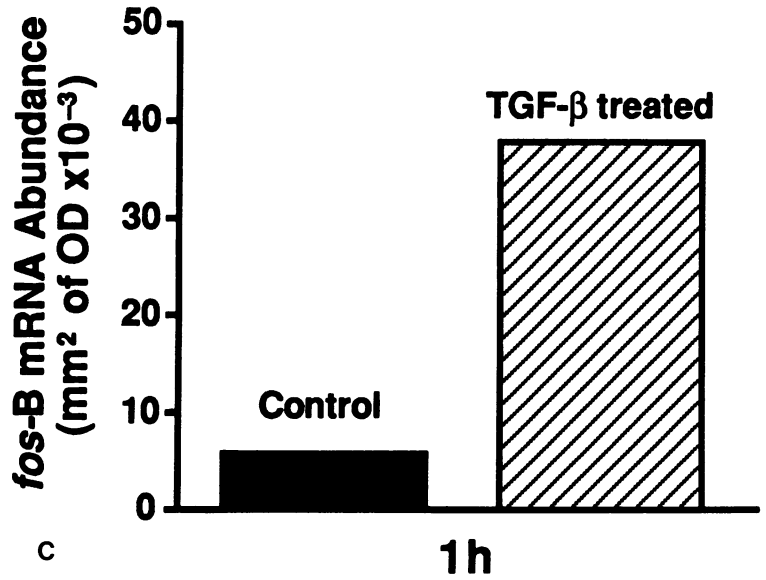


FIG. 2. TGF-β induction of *fos*-B. (A) The blot shown in Figure 1 was stripped of probe and rehybridized with radiolabeled *fos*-B cDNA and exposed to x-ray film for 24 hours. The filter hybridized with radiolabeled c-*fos* cDNA was exposed to x-ray film for 7 days. (B) In a separate experiment, serum-deprived BWTG3 cells were treated with TGF-β1 (120 pmol/L) for 1 hour, then harvested. The Northern blot was hybridized with ^{32}P-labeled *fos*-B cDNA. Radiographic exposure was for 3 days. (C) The bar graph shows the densitometric results.

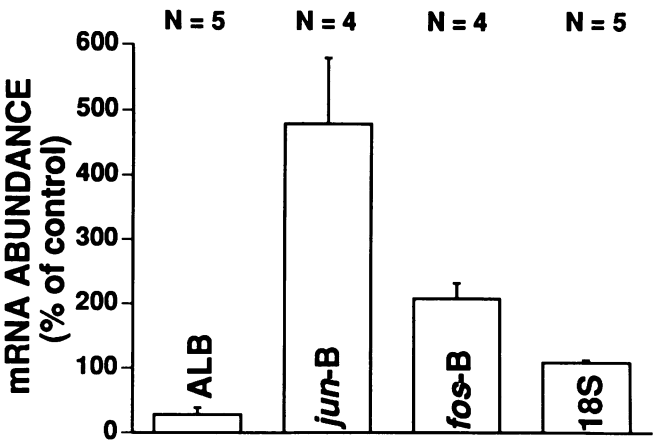


FIG. 3. Treatment with TGF-β has prolonged effects on gene expression. RNA from BWTG3 cells was analyzed after 24 hours of treatment with TGF-β1 (120 pmol/L). Controls received no additions. The RNA filters were hybridized with the indicated ^{32}P-labeled probes, followed by autoradiography. The combined densitometric results from repeated experiments is shown by the bar graph.

L and 1.3 μmol/L, respectively).[35] In our study, H7 (30 μmol/L) partially inhibited the induction of *jun*-B by TGF-β1 at 1 hour (Fig. 6A), but by 24 hours (Fig. 6B) the level of *jun*-B mRNA was increased. Albumin mRNA levels at 24 hours were decreased by TGF-β treatment after a single addition of H7. At 30 μmol/L, H7 alone had no effect on albumin mRNA levels, but at higher concentrations (60 μmol/L), H7 alone decreased albumin mRNA at 24 hours. At the 60-μmol/L concentration, H7 completely blocked the increase in *jun*-B observed at 1 hour after TGF-β1 treatment, but not at 24 hours (data not shown). HA1004 had no apparent effect on levels of *jun*-B or albumin mRNA.

Discussion

In this study, we found that TGF-β1 caused rapid and sustained increases in *jun*-B and *fos*-B expression in mouse hepatoma cells. Induction of *fos*-B by TGF-β has not been

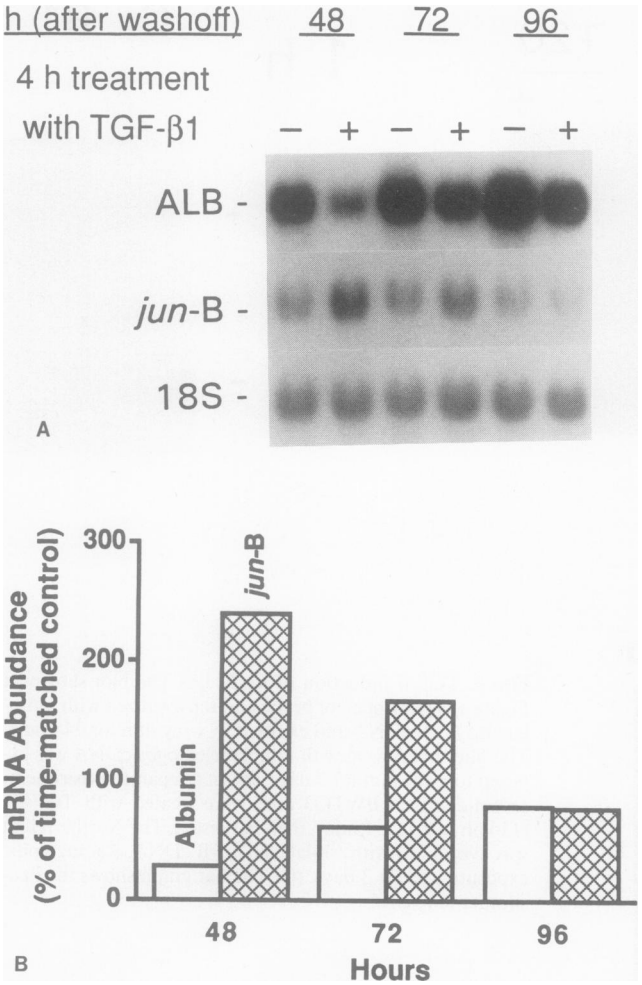


FIG. 4. Reversibility of the TGF-β effect. BWTG3 cells were treated with TGF-β where indicated (+) for 4 hours, then all plates were washed three times with fresh TGF-β-free medium and were refed medium without TGF-β1 (120 pmol/L). The cells were harvested at the indicated intervals and RNA was extracted for hybridization analysis. Each time point has a control that was washed and processed identically but received no TGF-β. The bar graph (B) represents the densitometric analysis of the autoradiograms shown above (A).

reported previously. Although JUN proteins can potentially regulate gene expression through homodimer formation, the JUN and FOS heterodimer form the more stable complex.[30] Induction of these genes after TGF-β1 treatment precedes the downregulation of albumin mRNA and recovery of albumin mRNA levels coincides with the decrease in *jun*-B mRNA. We also found that phorbol ester, another inducer of AP-1, also caused a decrease in albumin mRNA that was preceded by increases in *jun*-B and *fos*-B expression, thus supporting the hypothesis that induction of AP-1 may be involved in the albumin mRNA decrease.

Our previous studies[17] have shown that TGF-β1 caused a greater than 10-fold decrease in albumin mRNA by 48 hours after treatment and that *de novo* RNA and protein synthesis are required. These results suggested that TGF-β induces the transcription and translation of another gene whose protein product causes the decrease in albumin mRNA. For TGF-β to activate transcription of another gene, the signal must be transduced from the cell surface TGF-β receptor to the nucleus, where DNA binding proteins regulate transcription. Transforming growth factor-β can induce transcription of several genes, including α2(I)collagen,[36] plasminogen activator inhibitor,[19] *jun*-B,[25] fibronectin,[37] and TGF-β1 itself.[18,38] The *cis*-acting elements (specific DNA sequences within the same gene complex) necessary for TGF-β responsiveness have been identified as AP-1 sites for PAI-1[19] and for TGF-β1.[18] JUN and FOS proteins bind to the AP-1 site to activate TGF-β1 transcription in response to TGF-β1 treatment (autoactivation).[18] Because increased expression of *jun*-B and *fos*-B is among the earliest responses to TGF-β treatment and the response is sustained, it is likely that these transcriptional regulatory factors play important roles in regulating the expression of other genes after TGF-β treatment.

The present results showing that the protein kinase inhibitor, H7 (30 μmol/L), could inhibit TGF-β induction of *jun*-B at 1 hour in the mouse hepatoma cells are consistent with the observations of Ohtsuki and Massague[34]

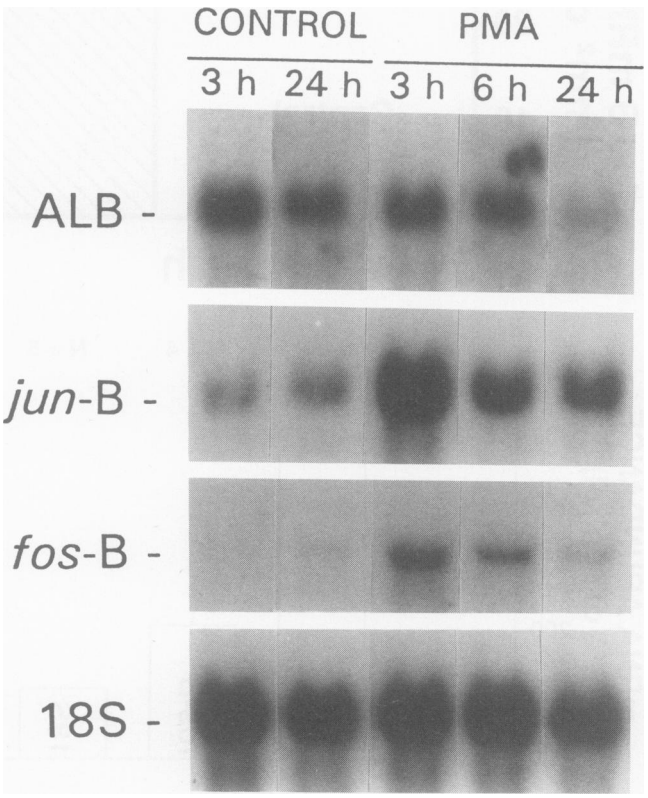


FIG. 5. Phorbol ester (PMA) effects on *jun*-B, *fos*-B, and albumin mRNA levels. BWTG3 cells growing in DMEM supplemented with 10% FBS were treated with either PMA or vehicle (control) and were harvested at the indicated times. RNA was subjected to hybridization analysis with the indicated probes.

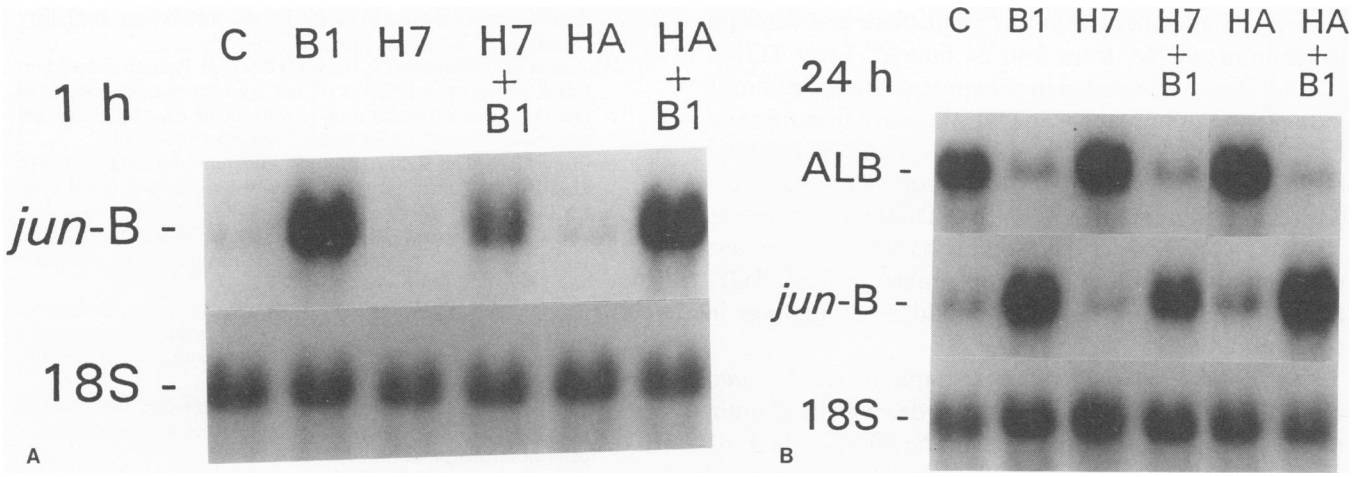


FIG. 6. Effect of protein kinase inhibitors. BWTG3 cells were deprived of FBS for 24 hours before the addition of TGF-β1 [120 pmol/L (lane B1)], H7 [30 μmol/L (lane H7)], HA1004 [30 μmol/L (lane HA)], or the combinations as indicated. The control received no additions. RNA was analyzed from cells harvested after (A) 1 hour or (B) 24 hours of treatment.

in mink lung epithelial cells. This effect was not examined after 90 minutes in the mink lung epithelial cells. We employed H7 to prevent the increase in *jun*-B caused by TGF-β to determine whether this would prevent the subsequent decrease in albumin mRNA. In the current study, this early inhibition of *jun*-B occurred, but the subsequent increase in *jun*-B at 24 hours was not prevented; nor was there any abrogation of the effect of TGF-β on albumin mRNA. This indicates that the inhibitory action of H7 is transient, compared with the long-lasting effects of TGF-β. Because complete recovery of *jun*-B induction has occurred by 24 hours, it is not possible to determine whether abrogation of *jun*-B expression prevents the decrease in albumin mRNA from this experiment. The type II TGF-β receptor was recently cloned and found to be a serine/threonine kinase protein.[39] Thus, the short-term actions of H7 on *jun*-B are likely due to the inhibition of serine/threonine kinase activity stimulated by TGF-β.

Given the temporal relationship between TGF-β1– or PMA-induced expression of *jun*-B and *fos*-B and the decrease in albumin mRNA, we suspect that these events may be causally related. To more directly test this hypothesis, it will be necessary to show that increased expression of both *jun*-B and *fos*-B are sufficient to cause the decrease in albumin mRNA levels in the absence of TGF-β or PMA treatment. In addition, if TGF-β induction of either *jun*-B or *fos*-B expression can be selectively blocked (*e.g.*, with antisense), and if the decrease in albumin mRNA was prevented, this would provide strong evidence that AP-1 is necessary in this role. These types of studies are underway in our laboratory.

The biologic relevance of the liver as an important target for TGF-β action can be inferred when considered in light of previous observations. The acute phase response of the liver to distant injury is a homeostatic process[40] and represents an early defense mechanism. In addition to the release of several cytokines (interleukin-6 [IL-6], IL-1, tumor necrosis factor, and interferon-gamma) that are known to regulate synthesis of acute phase proteins,[16] the same injuries are typically where one would expect TGF-β to be produced, released, and activated.[1-3] The most concentrated source of TGF-β in the body is in the α-granules of platelets,[41] and it is released at sites of tissue injury. Transforming growth factor-β is also released by other cells such as activated macrophages,[42] lymphocytes,[43] and fibroblasts[44] that are abundant in tissue that is injured and undergoing repair. Release of TGF-β is increased from circulating monocytes of severely injured patients.[45] Thus, tissue injury can lead to increased, circulating TGF-β that may alter the functions of hepatocytes and other cell types.

The effect of TGF-β1 on the production of liver-specific proteins is consistent with the regulation of differentiated function by TGF-β1 in other systems.[1-3] During the acute phase response, there is significant reprogramming of hepatocytes such that increased production of positive acute phase reactants (such as c-reactive protein, antichymotrypsin, haptoglobin, hemopexin, complement factor β, α1 acid glycoprotein) coincides with decreased production of negative acute phase reactants (albumin and apolipoprotein A1).[12,13,15,16] Teleologically, there would be an advantage to post-transcriptional regulation of the stability of ordinarily long-lived and abundant mRNAs such as albumin so that more substrate and translational components are available for synthesis of the necessary positive acute phase proteins.

In addition to the inflammatory response, increased TGF-β and decreased albumin synthesis appear to be coordinated in hepatic regeneration and in hepatic fibrosis. Transforming growth factor-β mRNA and protein are increased in regenerating rat liver after partial hepatectomy.[5,46] Levels of TGF-β1, 2, and 3 mRNAs are increased

by 4 hours after hepatectomy,[46] and there is a decrease in albumin mRNA from 8 to 24 hours.[47] Liver TGF-β mRNA levels are elevated in experimental hepatic fibrosis in rats,[6] and in humans with chronic active hepatitis and hepatic fibrosis.[7] The increase in TGF-β in experimental rat hepatic fibrosis also temporally coincides with a decrease in albumin mRNA levels.[6] These are further *in vivo* examples in which TGF-β may be acting to suppress the synthesis of albumin. We are examining whether TGF-β administration *in vivo* can reproduce our findings in the cultured cells.

In summary, TGF-β1 induces a rapid increase in *jun*-B and *fos*-B mRNA and a subsequent decrease in albumin mRNA in mouse hepatoma cells. The effect of TGF-β1 on these mRNAs is prolonged, but reversible on removal of the TGF-β (after 48 hours). Induction of *jun*-B and *fos*-B expression by phorbol ester is also followed by the subsequent decrease in albumin mRNA levels. These observations are consistent with the hypothesis that *jun*-B and *fos*-B induction may participate in the downregulation of albumin synthesis by regulation of another, as yet unidentified, gene.

Acknowledgments

The authors thank Wilfred Saito and Michael J. Ibarra for their technical support on this project. They thank E. Aubrey Thompson for providing the 18S ribosomal DNA clone, John Papaconstantinou for providing the BWTG3 cell line, and Achilles Dugaiczyk the mouse albumin cDNA.

References

1. Moses HL, Barnard JA, Bascom CC, et al. Regulation of fibroblastic and epithelial cell function by transforming growth factors. *In* Etievant C, Cros J, Rustum YM, eds. New Concepts in Cancer: Metastasis, Oncogenes, and Growth Factors. London: MacMillan Press Ltd, 1990; pp 218–230.
2. Sporn MB, Roberts AB. TGF-β: problems and prospects. Cell Regulation 1990; 1:875–882.
3. Barnard JA, Lyons RM, Moses HL. The cell biology of transforming growth factor beta. Biochim Biophys Acta 1990; 1032:79–87.
4. Pelton RW, Dickinson ME, Moses HL, Hogan BLM. *In situ* hybridization analysis of TGFβ3 RNA expression during mouse development: comparative studies with TGFβ1 and β2. Development 1990; 110:609–620.
5. Braun L, Mead JE, Panzica M, et al. Transforming growth factor β mRNA increases during liver regeneration: a possible paracrine mechanism of growth regulation. Proc Natl Acad Sci USA 1988; 85:1539–1543.
6. Czaja MJ, Weiner FR, Flanders KC, et al. In vitro and in vivo association of transforming growth factor-β1 with hepatic fibrosis. J Cell Biol 1989; 108:2477–2482.
7. Castilla A, Prieto J, Fausto N. Transforming growth factors β1 and α in chronic liver disease. Effects of interferon alfa therapy. N Engl J Med 1991; 324:933–939
8. Nakamura T, Tomita Y, Hirai R, et al. Inhibitory effect of transforming growth factor-β on DNA synthesis of adult rat hepatocytes in primary culture. Biochem Biophys Res Commun 1985; 133:1042–1050.
9. Russell WE, Coffey RJ Jr, Ouellette AJ, Moses HL. Type β transforming growth factor reversibly inhibits the early proliferative response to partial hepatectomy in the rat. Proc Natl Acad Sci USA 1988; 85:5126–5130.
10. Coffey RJ Jr, Kost LJ, Lyons RM, et al. Hepatic processing of transforming growth factor β in the rat: uptake, metabolism, and biliary excretion. J Clin Invest 1987; 80:750–757.
11. Wakefield LM, Winokur TS, Hollands RS, et al. Recombinant latent transforming growth factor β1 has a longer plasma half-life in rats than active transforming growth factor β1, and a different tissue distribution. J Clin Invest 1990; 86:1976–1984.
12. Morrone G, Cortese R, Sorrentino V. Posttranscriptional control of negative acute phase genes by transforming growth factor beta. EMBO J 1989; 8:3767–3771.
13. Mackiewicz A, Ganapathi MK, Schultz D, et al. Transforming growth factor β1 regulates production of acute-phase proteins. Proc Natl Acad Sci USA 1990; 87:1491–1495.
14. Busso N, Chesne C, Delers F, et al. Transforming growth-factor-β (TGF-β) inhibits albumin synthesis in normal human hepatocytes and in hepatoma HepG2 cells. Biochem Biophys Res Commun 1990; 171:647–654.
15. Mackiewicz A, Kushner I. Transforming growth factor β 1 influences glycosylation of alpha 1-protease inhibitor in human hepatoma cell lines. Inflammation 1990; 14:485–497.
16. Kushner I, Ganapathi M, Schultz D. The acute phase response is mediated by heterogeneous mechanisms. Ann NY Acad Sci 1989; 557:19–30.
17. Beauchamp RD, Alam T, Sheng H-M, et al. Regulation of alpha fetoprotein and albumin mRNA levels by TGFβ1. Surg Forum 1991; 42:150–152.
18. Kim S-J, Angel P, Lafyatis R, et al. Autoinduction of transforming growth factor β1 is mediated by the AP-1 complex. Mol Cell Biol 1990; 10:1492–1497.
19. Westerhausen DR Jr, Hopkins WE, Billadello JJ. Multiple transforming growth factor-β-inducible elements regulate expression of the plasminogen activator inhibitor type-1 gene in HepG2 cells. J Biol Chem 1991; 266:1092–1100.
20. Angel P, Imagawa M, Chiu R, et al. Phorbol ester-inducible genes contain a common *cis* element recognized by a TPA-modulated trans-acting factor. Cell 1987; 49:729–739.
21. Lee W, Mitchell P, Tjian R. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. Cell 1987; 49: 741–752.
22. Chiu R, Boyle WJ, Meek J, et al. The c-Fos protein interacts with c-Jun/AP-1 to stimulate transcription of AP-1 responsive genes. Cell 1988; 54:541–552.
23. Schuermann M, Neuberg M, Hunter JB, et al. The leucine repeat motif in Fos protein mediates complex formation with Jun/AP-1 and is required for transformation. Cell 1989; 56:507–516.
24. Vogt PK, Bos TJ, Doolittle RF. Homology between the DNA-binding domain of the GCN4 regulatory protein of yeast and the carboxyl-terminal region of a protein coded for by the oncogene *jun*. Proc Natl Acad Sci USA 1987; 84:3316–3319.
25. Li L, Hu J-S, Olson EN. Different members of the *jun* proto-oncogene family exhibit distinct patterns of expression in response to Type β transforming growth factor. J Biol Chem 1990; 265:1556–1562.
26. Greenberg ME, Ziff EB. Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. Nature (London) 1984; 311: 433–438.
27. Zerial M, Toschi L, Ryseck R-P, et al. The product of a novel growth factor activated gene, *fos*-B, interacts with JUN proteins enhancing their DNA binding activity. EMBO J 1989; 8:805–813.
28. Cohen D, Curran T. *fra*-1: a serum-inducible, cellular immediate-early gene that encodes a *fos*-related antigen. Mol Cell Biol 1988; 8:2063–2069.
29. Nishina H, Sato H, Suzuki T, et al. Isolation and characterization of *fra*-2, an additional member of the fos family. Proc Natl Acad Sci USA 1990; 87:3619–3623.
30. Ryseck R-P, Bravo R. c-JUN, JUN B, and JUN D differ in their binding affinities to AP-1 and CRE consensus sequences: effect of FOS proteins. Oncogene 1991; 6:533–542.
31. Szpirer C, Szpirer J. A mouse hepatoma cell line which secretes several serum proteins including albumin and α-foetoprotein. Differentiation 1975; 4:85–91.
32. Chirgwin JM, Przybyla AI, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 1979; 18:5294–5299.

33. Beauchamp RD, Barnard JA, McCutchen CM, et al. Localization of TGFα and its receptor in gastric mucosal cells: implications for a regulatory role in acid secretion and mucosal renewal. J Clin Invest 1989; 84:1017–1023.
34. Ohtsuki M, Massague J. Evidence for the involvement of protein kinase activity in transforming growth factor-β signal transduction. Mol Cell Biol 1992; 12:261–265.
35. Hidaka H, Inagaki M, Kawamoto S, Sasaki Y. Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase and protein kinase C. Biochemistry 1984; 23:5036–5041.
36. Rossi P, Karsenty G, Roberts AB, et al. A nuclear factor 1 binding site mediates the transcriptional activation of a type I collagen promoter by transforming growth factor-β. Cell 1988; 42:405–414.
37. Blatti SP, Foster DN, Ranganathan G, et al. Induction of fibronectin gene transcription and mRNA is a primary response to growth-factor stimulation of AKR-2B cells. Proc Natl Acad Sci USA 1988; 85:1119–1123.
38. Kim S-J, Jeang K-T, Glick AB, et al. Promoter sequences of the human transforming growth factor-β1 gene responsive to transforming growth factor-β1 autoinduction. J Biol Chem 1989; 264: 7041–7045.
39. Lin HY, Wang X-F, Ng-Eaton E, et al. Expression cloning of the TGF-β type II receptor, a functional transmembrane serine/threonine kinase. Cell 1992; 68:775–785.
40. Fey GH, Fuller GM. Regulation of acute phase gene expression by inflammatory mediators. Mol Biol Med 1987; 4:323–338.
41. Assoian RK, Sporn MB. Type β transforming growth factor in human platelets: release during platelet degranulation and action on vascular smooth muscle cells. J Cell Biol 1986; 102:1217–1223.
42. Rappolee DA, Mark D, Banda MJ, Werb Z. Wound macrophages express TGF-α and other growth factors *in vivo:* analysis by mRNA phenotyping. Science 1988; 241:708–712.
43. Kehrl JH, Wakefield LM, Roberts AB, et al. Production of transforming growth factor β by human T lymphocytes and its potential role in the regulation of T cell growth. J Exp Med 1986; 163:1037–1050.
44. Bascom CC, Wolfshohl JR, Coffey RJ Jr, et al. Complex regulation of transforming growth factor β1, β2, and β3 mRNA expression in mouse fibroblasts and keratinocytes by transforming growth factors β1 and β2. Mol Cell Biol 1989; 9:5508–5515.
45. Miller-Graziano CL, Szabo G, Griffey K, et al. Role of elevated monocyte transforming growth factor β (TGFβ) production in posttrauma immunosuppression. J Clin Immunol 1991; 11:95–102.
46. Jakowlew SB, Mead JE, Danielpour D, et al. Transforming growth factor-β (TGF-β) isoforms in rat liver regeneration: messenger RNA expression and activation of latent TGF-β. Cell Regul 1991; 2:535–548.
47. Ito Y, Hayashi H, Taira M, et al. Depression of liver-specific gene expression in regenerating rat liver: a putative cause for liver dysfunction after hepatectomy. J Surg Res 1991; 51:143–147.

Discussion

DR. STEPHEN F. LOWRY (New York, New York): I congratulate the authors for leading us into new frontiers with respect to regulation of the acute phase response.

I would address two issues. One is the possibility that other potential mediators may be responsible for proto-oncogene expression and the possibility that they may be involved in the regulation of the acute phase response. I wonder if the investigators have looked at some of these alternative explanations, in that transforming growth factor beta (TGF-β) or members of the TGF-β family can cause some rather dramatic alterations in production of other mediators such as cytokines, such as tumor necrosis factor, and perhaps more importantly in this particular model, interleukin-6.

I would applaud their efforts looking at transcriptional regulatory proteins. This is clearly an important interface between second messenger systems and DNA regulatory pathways. I wonder if they can give us some insights as to how they are going to look at this mechanistically. Clearly what we have today are some associative responses. There are probably some novel techniques with respect to transfection as well as anti-sense RNA probing techniques that may give us some precision as to cause/effect relationships.

DR. DANA K. ANDERSEN (Chicago, Illinois): I think there may be one or two people who are still struggling with what may be a linguistics problem because we are obviously having to learn a new language as we think about some of these issues.

Dr. Beauchamp has helped me to understand some of these issues in a little simpler model, which I would like to share with you.

I sort of think of the liver as a giant synthetic machine or factory that has the capacity to generate positive acute phase reactants, which you might think of as military vehicles, as well as negative acute phase response proteins, which you might think of as luxury automobiles. The liver clearly has the capacity to do both, but something has to tell the liver whether to make military vehicles or luxury automobiles.

And using that very crude analogy, this study shows that there is a protein called transforming growth factor beta (TGF-β), which is the trigger that shifts the synthetic machinery to military vehicles and away from negative acute phase response proteins, one of which happens to be albumin. That is why it is significant that albumin synthesis drops in response to TGF-β. It is the clue that the synthetic machinery is shifting toward a more defensive or reactive form and away from the baseline or the affluent side of liver protein synthesis. The data would suggest that TGF-β is in turn activating the *fos*-genes and the *jun*-genes and these in turn serve as intermediate triggers that shift the synthetic machinery toward the positive acute phase response proteins.

Dr. Beauchamp suggests that there is yet another gene involved in this, and my question would be, why not stop here? Why are not the *fos*-genes and the *jun*-genes in fact the gene X that he employs in his hypothesis? What are the data that suggest that those genes are not in fact the regulatory genes that are shutting off the synthesis of albumin?

Second, I would like to know what steps he needs to follow to prove this causal relationship.

And finally, I would like to hear his thoughts about the biologic significance of this, such as issues of the growth of the organ, the "regeneration" of the liver, and the development of tumors.

DR. R. D. BEAUCHAMP (Closing discussion): I would like to thank Dr. Andersen and Dr. Lowry for their nice comments and their questions. I think the question of biologic significance is relevant, and when we talk about the biologic significance, we can probably answer a number of the questions that were raised.

Whenever the liver has to reprogram itself to respond to systemic injury, it has to shift from producing albumin, which constitutes approximately 25% of the hepatic protein product and has to provide these homeostatic proteins (the acute phase proteins) that are necessary in the acute phase response. I think that, in part, gives you the answer to the biologic significance of our observations.

Another aspect of the biologic significance would be when we see the liver regenerating after resection or we see regenerating nodules in sclerotic liver. In those two circumstances, there is a decrease in albumin synthesis. Also in those two circumstances, increase in transforming growth factor beta (TGF-β) expression has been observed. We might speculate that the liver must decrease albumin production to provide substrate for cell replication and for the acute phase response.

As far as Dr. Andersen's question about why the *jun* and *fos* genes are not gene X: from our understanding of the *jun* and *fos* proteins, they act to regulate transcription of selected genes in the nucleus. They directly interact with the DNA. From our previously reported studies, the effect of TGF-β appears to be post-transcriptional. It is not at the level of transcription. And what we have found is that TGF-β causes the degradation of the messenger RNA. The messenger RNA for albumin is