Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 1539–1543, March 1988
Cell Biology

Transforming growth factor β mRNA increases during liver regeneration: A possible paracrine mechanism of growth regulation

(cell proliferation/hepatocyte/nonparenchymal cell/rat)

LUNDY BRAUN*, JANET E. MEAD*, MARILYN PANZICA*, RYOKO MIKUMO*, GRAEME I. BELL†, AND NELSON FAUSTO*‡

*Department of Pathology and Laboratory Medicine, Division of Biology and Medicine, Brown University, Providence, RI 02912; and †The Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637

Communicated by Philip Siekevitz, November 2, 1987 (received for review September 15, 1987)

ABSTRACT **Transforming growth factor β (TGF-β) is a growth factor with multiple biological properties including stimulation and inhibition of cell proliferation. To determine whether TGF-β is involved in hepatocyte growth responses *in vivo*, we measured the levels of TGF-β mRNA in normal liver and during liver regeneration after partial hepatectomy in rats. TGF-β mRNA increases in the regenerating liver and reaches a peak (about 8 times higher than basal levels) after the major wave of hepatocyte cell division and mitosis have taken place and after the peak expression of the *ras* protooncogenes. Although hepatocytes from normal and regenerating liver respond to TGF-β, they do not synthesize TGF-β mRNA. Instead, the message is present in liver nonparenchymal cells and is particularly abundant in cell fractions enriched for endothelial cells. TGF-β inhibits epidermal growth factor-induced DNA synthesis *in vitro* in hepatocytes from normal or regenerating liver, although the dose–response curves vary according to the culture medium used. We conclude that TGF-β may function as the effector of an inhibitory paracrine loop that is activated during liver regeneration, perhaps to prevent uncontrolled hepatocyte proliferation.**

The regenerating rat liver is an ideal model system in which to study the mechanisms that control cell proliferation. Under normal physiological conditions, adult rat hepatocytes rarely divide. However, in response to tissue injury or surgical removal of portions of the liver (partial hepatectomy), mature hepatocytes undergo a partially synchronous wave of DNA replication followed by cell division. After partial hepatectomy in rats, the major wave of DNA synthesis starts ≈14 hr after the operation and reaches a peak at 24 hr. The mass of the liver remnant doubles in the first 36 hr of the growth process, and within a week the normal liver mass is fully restored and the quiescent state reestablished (1, 2). The factors that control this precisely regulated growth process are not known but are often assumed to involve the turning on and off of a positive stimulus for cell proliferation. More likely, however, is the possibility that this tight regulation requires an interplay between growth-stimulatory factors, operative in the early prereplicative stage of the regenerative process, and inhibitory factors, which may be important in later stages as cell division ceases. We have suggested that when quiescent hepatocytes enter the cell cycle, progression to DNA synthesis is controlled by events specifically occurring in the liver—that is, by autocrine or paracrine secretion of growth-stimulatory and -inhibitory factors by hepatic cells (3, 4).

It has been shown by several laboratories that transforming growth factor β (TGF-β) is a potent inhibitor of hepatocyte proliferation *in vitro* (5–7). Under serum-free conditions, as little as 0.1 ng of TGF-β per ml (4 pM) causes 85–90% inhibition of epidermal growth factor (EGF)-induced DNA synthesis of hepatocytes in primary culture. TGF-β is a 25-kDa, dimeric peptide growth factor originally described as a transforming growth factor by virtue of its ability to reversibly induce phenotypic transformation of non-neoplastic rat fibroblasts (8). It is now well established that the biological effects of this molecule are multiple; that is, depending on the cell type (epithelial or mesenchymal) and the presence of other growth factors, TGF-β can stimulate or inhibit cell proliferation and in some systems function as an important agent of cellular differentiation (9–13). In addition, TGF-β appears to control other metabolic functions of some cells, such as the stimulation of collagen and fibronectin synthesis (14). These observations, as well as the fact that the amino acid sequence of TGF-β is highly conserved, have led to the suggestion that this molecule has a physiologically important function in the regulation of normal cell growth (15) and might be a negative regulator of hepatocyte proliferation *in vivo*.

We have examined the levels of TGF-β mRNA in normal and regenerating liver, its presence among different liver cell types, and the sensitivity of hepatocytes isolated from intact and regenerating liver to DNA synthesis inhibition by TGF-β *in vitro*. We conclude that TGF-β may function as the effector of an inhibitory paracrine mechanism that is activated during liver regeneration, perhaps to prevent uncontrolled hepatocyte growth.

MATERIALS AND METHODS

Animals. Male albino rats (CD strain, Charles River Breeding Laboratories; 140–180 g) were used for all experiments, including partial hepatectomy and cell-isolation procedures. Partial hepatectomies consisted of the removal of 70% of the liver as described by Higgins and Anderson (16) and were performed on rats under ether/oxygen anesthesia. For sham operations, rats were anesthetized, an abdominal incision was made, and the liver was manipulated but not removed. All rats were maintained in temperature-controlled rooms under 12-hr dark/light cycles to synchronize feeding and were killed between 0900 and 1100 to minimize diurnal variation in metabolic activity.

Cell-Separation Procedures. Hepatocytes and nonparenchymal cells were isolated by a two-step collagenase procedure (17, 18). In brief, the liver was perfused via the portal vein with Ca^{2+}- and Mg^{2+}-free Hanks' balanced salts solution for 10 min at a flow of 30–40 ml/min followed by perfusion with 0.05% collagenase in Ca^{2+}-containing me-

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

Abbreviations: EGF, epidermal growth factor; Gc, group-specific component; IGF-II, insulin-like growth factor II; TGF-β, transforming growth factor β.

‡To whom reprint requests should be addressed.

dium (Hepes buffer) for 10 min (19). The liver capsule was removed, and the cells were shaken loose from the tissue and filtered. Hepatocytes were collected by repeated centrifugation of the cell suspension at 50 × *g* for 2.5 min. For the isolation of nonparenchymal cells, the undissociated tissue remaining after collagenase digestion and mechanical dispersion was further digested with 0.1% collagenase/0.1% Pronase/0.004% DNase for 20 min at 37°C as described (17, 18). The cell suspension was diluted with cold minimal essential medium (MEM) containing 10% calf serum to inactivate the enzymes, filtered through a 45-μm nylon mesh, and centrifuged at 300 × *g* for 10 min. The digestion procedure was done three times and the cell pellets resulting from each digestion were combined. Hepatocyte suspensions are virtually free of nonparenchymal cell contamination; the nonparenchymal cell fraction contains a maximum of 1–2% hepatocytes (17, 18).

For the purification of Kupffer and endothelial cells, the nonparenchymal cell fraction was further separated by centrifugal elutriation as described by Bodenheimer *et al.* (20) and Knook and Sleyster (21). Kupffer cells (isolated by H. C. Bodenheimer) had a viability >95%; in the viable cells, endogenous peroxidase and phagocytic activity were >85%. Endothelial cell viability was >90%.

Hepatocyte Cultures. Cells (2×10^5 per dish) were plated on 35-mm dishes coated with rat tail collagen in MEM containing 5% fetal bovine serum, 1 mM pyruvate, 0.2 mM aspartate, 1 mM proline, 0.2 mM serine, 2 mM glutamine, and 0.5 μg of hydrocortisone and 1 μg of insulin per ml. After 3 hr, fresh serum-free medium containing EGF (20 ng/ml) and appropriate concentrations of TGF-β (human platelet-derived TGF-β kindly supplied by M. B. Sporn, Laboratory of Chemoprevention, National Cancer Institute, National Institutes of Health, Bethesda, MD) was added and replaced every 24 hr. For DNA-synthesis determination, the cells were labeled with [^{3}H]thymidine for 24 hr.

In some experiments hepatocytes were plated in Williams E medium containing 5% fetal bovine serum and then incubated in serum-free Williams E medium containing 0.65 μg of insulin, 1.8 μg of hydrocortisone, and 20 ng of EGF per ml and appropriate concentrations of TGF-β.

RNA Extraction and Hybridization. For isolation of total RNA from purified cell fractions, the cell pellets were homogenized in 7.5 M guanidine thiocyanate and layered on a 3.5-ml cushion of 5.7 M CsCl in 25 mM sodium acetate (pH 5) by the method of Chirgwin *et al.* (22), essentially as described (23). Centrifugation was for 20 hr at 28,000 rpm in a Beckman SW40 Ti rotor. The RNAs were dissolved in water and precipitated in ethanol. Poly(A)$^+$ RNA was prepared from livers of intact, sham-operated, and partially hepatectomized rats as previously described (24), with the exception that oligo(dT)-cellulose was substituted for poly(U)-Sepharose. RNA samples (5 μg for poly(A)$^+$ RNA; 15–20 μg for total RNA from cell fractions) were fractionated by electrophoresis in 6.2% formaldehyde/1% agarose gels and transferred to nitrocellulose filters in 20× SSC (1× SSC is 0.15 M NaCl/0.015 M trisodium citrate). The blots were then hybridized with a ^{32}P-labeled 1.1-kilobase (kb) *Bgl* I fragment of a human TGF-β cDNA (isolated by G.I.B.) or a 1.5-kb *Eco*RI fragment of a mouse insulin-like growth factor II (IGF-II) cDNA [pMIGF-II, clone 3, isolated by Stempien *et al.* (25)] at 42°C for 72 hr. After washing at 50°C, filters were exposed to Kodak XAR-2 film at −70°C with intensifying screens (23, 24). Quantitation of autoradiographs was done by scanning densitometry with a Gilford spectrophotometer.

RESULTS

TGF-β mRNA During Liver Regeneration. We examined the levels of TGF-β mRNA in livers of normal, sham-operated, and partially hepatectomized rats at various times after the operations. Hybridization of liver poly(A)$^+$ RNA with the TGF-β cDNA probe detected a major 2.5-kb RNA that is the coding transcript for the 391 amino acid TGF-β precursor (Fig. 1). Normal liver contained very low levels of this mRNA. By 4 hr after partial hepatectomy, levels of the 2.5-kb TGF-β transcript increased ≈3-fold (as determined by scanning densitometry) and remained unchanged until about 24 hr after the operation. Between 24 and 72 hr, a steady increase in TGF-β mRNA occurred, with the peak of expression (≈8-fold above normal) at 72 hr. By 96 hr after partial hepatectomy, a time when the regenerative response is not yet complete but the major waves of hepatocyte DNA synthesis and mitosis have passed (1, 2), the levels of the message had declined significantly but were still higher than normal. That the changes in TGF-β mRNA detected in regenerating liver were not a consequence of simple surgical stress and anesthesia was indicated by the lack of increase in the message in livers of rats at various times after sham operation (Fig. 1). TGF-β mRNA was also not increased in livers of sham-operated or partially hepatectomized rats in the first 2 hr after surgery (data not shown).

TGF-β mRNA in Hepatocytes and Nonparenchymal Cells. Although hepatocytes constitute >90% of the hepatic mass, they represent only 60–65% of the total cell population in the liver. If the accumulation of TGF-β mRNA detected during liver regeneration takes place in hepatocytes, one could postulate that TGF-β functions as an autocrine regulator of hepatocyte replication. To test this hypothesis, we purified hepatocytes and nonparenchymal cells from normal and regenerating liver and looked for the presence of TGF-β mRNA in these cell populations. TGF-β mRNA was found in nonparenchymal cells (at both 24 and 48 hr after partial hepatectomy) but not in hepatocytes (Fig. 2*a*). Even after a long autoradiographic exposure we did not detect the 2.5-kb message in hepatocytes from either regenerating (Fig. 2*a*) or normal liver (Fig. 3 and ref. 4). Hybridization of total, but not poly(A)$^+$ liver RNA with the TGF-β cDNA probe revealed an additional RNA of ≈1.5 kb, which appeared to vary in abundance in parallel with the major band (Fig. 2*a*). The significance of the minor band is not understood, but it may represent the transcript from another TGF-β gene or a partial degradation product (13).

To determine whether the lack of TGF-β mRNA in purified hepatocytes might have been due to cell injury caused by the isolation procedure, we hybridized filters containing RNA from hepatocytes and nonparenchymal cells with a cDNA probe for the group-specific component (Gc). The Gc gene codes for the major vitamin D-binding protein produced in the liver by hepatocytes (26). We found (Fig. 2*b*) that the Gc message was present in hepatocytes but

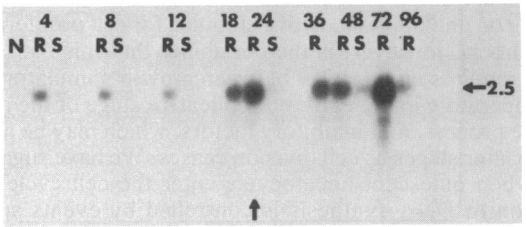

FIG. 1. TGF-β mRNA expression in regenerating rat liver. Poly(A)$^+$ RNA was prepared from rat livers at various times (4–96 hr) after partial (two-thirds) hepatectomy (R), after sham operation (S), or from intact rats (N). Five micrograms of each RNA was electrophoresed in an agarose gel, transferred to a nitrocellulose filter, and hybridized with a ^{32}P-labeled 1.2-kb *Bgl* I fragment of TGF-β cDNA. This probe detects the major 2.5-kb TGF-β transcript. Arrow at bottom denotes the peak of hepatocyte DNA synthesis.

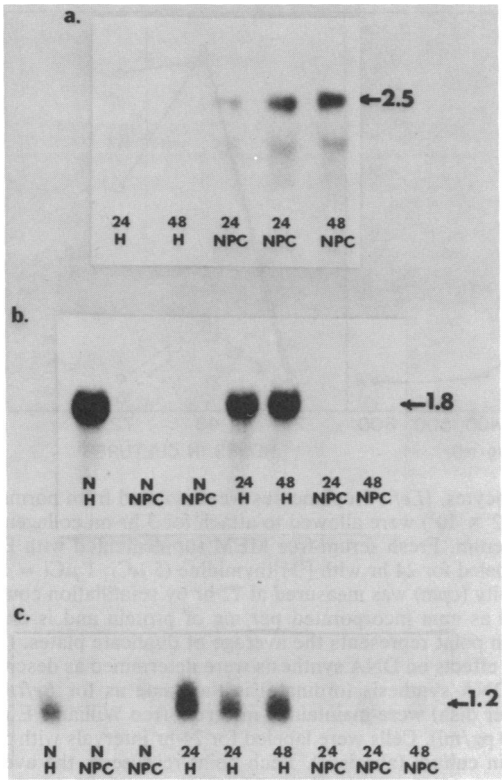

FIG. 2. TGF-β mRNA expression in different liver cell populations. Hepatocytes (H) and nonparenchymal cells (NPC) were obtained from normal (N) or regenerating liver 24 and 48 hr after partial hepatectomy. Total cell RNA was extracted from each cell fraction and hybridized with ^{32}P-labeled TGF-β cDNA (*a*), Gc (vitamin D-binding protein) cDNA (*b*), and BS-9 probe for c-Ha-*ras* (*c*). Arrows indicate the size of the major transcript for TGF-β (2.5 kb), Gc (1.8 kb), and c-Ha-*ras* (1.2 kb).

not in nonparenchymal cells from normal and regenerating liver, indicating that hepatocyte RNA was not degraded.

We previously showed that various protooncogenes are expressed in a sequential and regulated manner during liver regeneration (3, 4). In particular, the expression of *ras* genes during this process is elevated at the time of the major wave of hepatocyte DNA synthesis and mitosis (27, 28). To verify whether the hepatocytes purified from partially hepatectomized rats contain *ras* gene transcripts, we hybridized RNA from hepatocytes and nonparenchymal cells with the BS-9 probe for Ha-*ras*. The 1.2-kb c-Ha-*ras* transcript was clearly

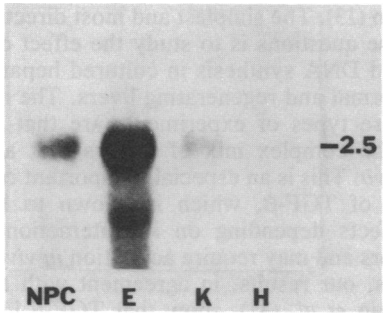

FIG. 3. TGF-β mRNA expression in nonparenchymal cell types. Normal liver nonparenchymal cells (NPC) were subfractionated into Kupffer cells (K) and endothelial cells (E). Sixteen micrograms of total RNA from each of these fractions, as well as RNA from hepatocytes (H), was hybridized to a TGF-β cDNA probe. The position of the 2.5-kb TGF-β transcript is indicated at right.

detected in hepatocytes but not in nonparenchymal cells (Fig. 2*c*). Since nonparenchymal cells also replicate in the regenerating liver (a few days later than hepatocytes), it is likely that protooncogene mRNAs would also be found in nonparenchymal cell types purified at later stages of liver regeneration. In any event, the data shown in Fig. 2 indicate that the differential expression of TGF-β mRNA in liver cell populations is not due to injury or artifacts in the isolation of hepatocytes. These findings argue against TGF-β being an autocrine regulator of hepatocyte proliferation and suggest that hepatocytes may respond to TGF-β produced by other liver cells.

TGF-β mRNA in Nonparenchymal Cell Types. Nonparenchymal cell fractions contain a mixed population that includes bile-duct epithelial cells, Kupffer cells, and endothelial cells. To determine whether TGF-β mRNA might be localized in a specific cell type, we separated nonparenchymal cells isolated from normal liver into endothelial and Kupffer cells. There was a major enrichment of TGF-β mRNA in the endothelial cell fraction, with much lower levels of the message detected in Kupffer cells (Fig. 3). TGF-β mRNA was not found in significant amounts in bile-duct epithelial cells purified by centrifugal elutriation from livers of bile-duct-ligated rats (data not shown). However, the message is present in great abundance in nonparenchymal liver epithelial cells (oval cells) that proliferate during hepatocarcinogenesis (L.B. and N.F., unpublished data).

IGF-II mRNA During Liver Development and Regeneration. To assess whether mRNAs for other growth factors would show a similar pattern of change during liver regeneration as that of TGF-β mRNA, we hybridized normal and regenerating liver poly(A)$^+$ RNAs with a cDNA probe for IGF-II. We selected this growth factor because it is considered to be a mitogenic factor during fetal growth (29). Thus, it was of interest to determine whether or not transcripts from this growth factor gene would increase when liver growth was induced in adult rats by partial hepatectomy. In agreement with data from other laboratories (29, 30), levels of IGF-II mRNAs were high in fetal livers but decreased drastically after birth. However, no changes in IGF-II mRNAs were detected during liver regeneration (data not shown).

Sensitivity of Hepatocytes from Normal and Regenerating Liver to TGF-β Effects on DNA Synthesis *in Vitro*. TGF-β is a potent inhibitor of EGF-induced DNA synthesis in normal hepatocytes in culture (5–7). It is, however, not yet clear if hepatocytes are normally maintained in a quiescent state by TGF-β but become insensitive to the inhibitory effects of the factor when stimulated to proliferate during liver regeneration. Strain *et al.* (31) have shown that DNA synthesis in cultured hepatocytes obtained from 18-hr regenerating livers is strongly inhibited by TGF-β. A preliminary report (32) suggests that hepatocytes lose TGF-β receptors between 3 and 5 hr after partial hepatectomy. Although *in vitro* experiments cannot completely mimic the situation *in vivo*, we determined whether hepatocytes from normal and regenerating livers differ in their sensitivity to TGF-β inhibition of DNA synthesis. Hepatocytes isolated from normal and partially hepatectomized rats were maintained in either modified MEM or Williams E medium in the absence of serum. EGF and TGF-β (when appropriate) were added together at the start of the cultures and at every medium change, done at 24-hr intervals. In normal hepatocytes and hepatocytes isolated from 3-hr, 5-hr and 12-hr regenerating liver cultured in MEM, very low concentrations of TGF-β inhibited EGF-induced DNA synthesis measured between 48 and 72 hr in culture (Fig. 4 *Left*). The dose–response curves for hepatocytes from normal and regenerating livers are very similar; TGF-β at 80 pg/ml inhibited 70–80% of EGF-induced DNA synthesis. TGF-β also inhibited EGF-

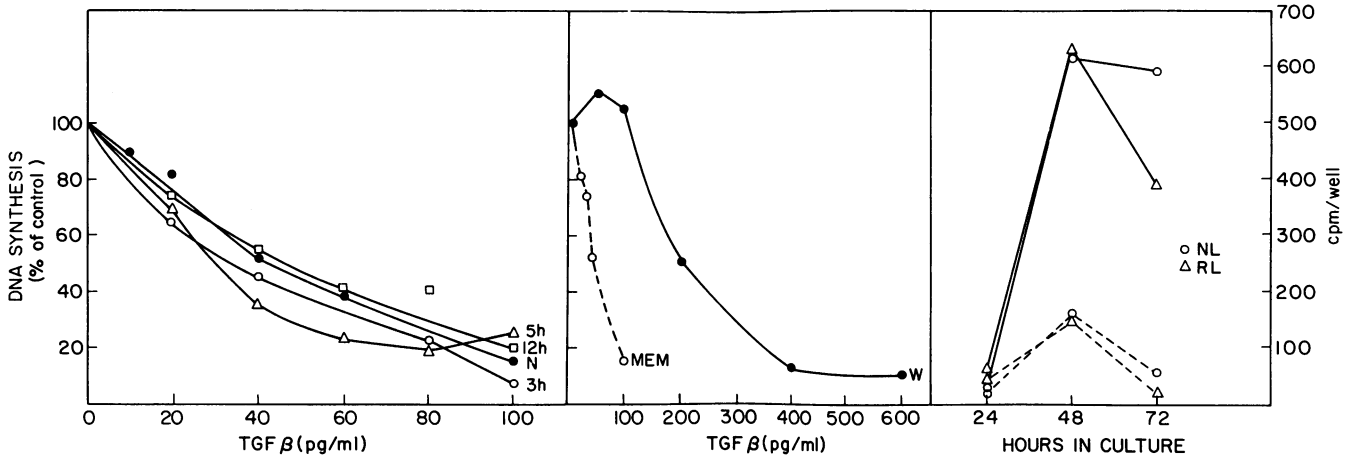

FIG. 4. Effects of TGF-β on EGF-induced DNA synthesis of cultured hepatocytes. (*Left*) Hepatocytes were isolated from normal livers (N) and from regenerating livers 3, 5, and 12 hr after partial hepatectomy. Cells (2×10^5) were allowed to attach for 3 hr on collagen-coated 35-mm dishes in minimal essential medium (MEM) containing 5% fetal bovine serum. Fresh serum-free MEM supplemented with EGF (20 ng/ml) and TGF-β (0–100 pg/ml; abscissa) was added every 24 hr. Cells were labeled for 24 hr with [^{3}H]thymidine (5 μCi; 1 μCi = 37 kBq) between 48 and 72 hr in culture (peak of DNA synthesis). Incorporated radioactivity (cpm) was measured at 72 hr by scintillation counting of trichloroacetic acid precipitates of cell extracts. DNA synthesis was calculated as cpm incorporated per mg of protein and is expressed (ordinate) as a percentage of DNA synthesis in cells not exposed to TGF-β. Each point represents the average of duplicate plates. (*Middle*) Normal hepatocytes were maintained in MEM or Williams E medium (W). TGF-β effects on DNA synthesis were determined as described for *Left*. Note that TGF-β concentrations are 0–600 pg/ml (abscissa). Scale for DNA synthesis (ordinate) is the same as for *Left*. (*Right*) Hepatocytes from normal (NL) and 5-hr regenerating (RL) liver (2×10^5 cells per dish) were maintained in serum-free Williams E medium containing EGF (20 ng/ml) with (broken lines) or without (solid lines) TGF-β (600 pg/ml). Cells were labeled for 24-hr intervals with 5 μCi of [^{3}H]thymidine and incorporation into DNA was measured at 24, 48, and 72 hr in culture (abscissa). Each point represents the average of duplicate plates. DNA synthesis is expressed as cpm/well (ordinate).

induced DNA synthesis in hepatocytes from normal and regenerating livers maintained in Williams E medium. However, in this medium, the concentration of TGF-β required to achieve 50% reduction in DNA synthesis was ≈200 pg/ml, whereas the corresponding concentration was ≈40 pg/ml in MEM (Fig. 4 *Middle*). In either of these media and regardless of concentration, TGF-β did not inhibit EGF-induced DNA synthesis during the first 24 hr in culture (Fig. 4 *Right*) despite the fact that both growth factors were present from the start of the cultures. These results are in complete agreement with those reported by Strain *et al.* (31). TGF-β is inhibitory at 48 or 72 hr in culture, when EGF-induced DNA synthesis reaches its maximal values (Fig. 4 *Right*).

DISCUSSION

In this paper we show that TGF-β mRNA increases after partial hepatectomy and that the message is present in nonparenchymal cells but not in hepatocytes. The message is particularly abundant in cell fractions enriched for endothelial cells. The increase in TGF-β mRNA during liver regeneration occurs after the major wave of hepatocyte DNA synthesis and mitosis have taken place and after maximal expression of the *ras* protooncogenes. In contrast, levels of mRNAs for IGF-II, a growth factor for fetal liver, are not changed in regenerating rat livers.

The kinetics of the increase in TGF-β mRNA in regenerating liver, the localization of the message in nonparenchymal cells, and the sensitivity of hepatocyte DNA synthesis *in vitro* to TGF-β are consistent with the view that TGF-β may function as a regulator of hepatocyte proliferation *in vivo*. Hepatocytes (which do not synthesize TGF-β but possess functional receptors for the growth factor) may respond to TGF-β produced in the liver by nonparenchymal cells through a paracrine regulatory mechanism that is activated during liver regeneration. This seemingly paradoxical sequence of events in which a growth inhibitor increases at the time of hepatocyte replication may, in fact, be an important mechanism that prevents uncontrolled growth during liver regeneration. Similarly, work with other systems suggests that the induction of inhibitory factors by growth stimuli might be a general means of regulation in non-neoplastic growth processes. Kehrl *et al.* (33) reported that TGF-β mRNA increases in T lymphocytes stimulated to proliferate and have suggested that TGF-β may prevent unregulated clonal expansion of stimulated lymphocytes. Another interesting example of the induction of genes coding for inhibitory substances during cell growth is the expression of the β-interferon gene in 3T3 fibroblasts stimulated by platelet-derived growth factor (34). The interplay between positive and negative stimuli may be similar in hepatocyte, lymphocyte, and fibroblast growth, but it appears that growth inhibition in hepatocytes takes place through a paracrine rather than autocrine mechanism.

Hepatocytes could escape the inhibitory effects of TGF-β by modulating their sensitivity to the growth factor. This could take place at the receptor level (32), by postreceptor mechanisms, or by alterations in the capacity of the hepatocyte to activate TGF-β if it were presented to the cell in an inactive form (13). The simplest and most direct approach to analyze these questions is to study the effect of TGF-β on EGF-induced DNA synthesis in cultured hepatocytes purified from normal and regenerating livers. The major limitations of these types of experiments are that they cannot reproduce the complex mix of factors that act on hepatocytes *in vivo*. This is an especially important consideration in the case of TGF-β, which is known to have widely different effects depending on its interaction with other growth factors and may require activation *in vivo* (9–13, 15). Nevertheless, our results, in agreement with the observations of Strain *et al.* (31), show that TGF-β inhibits DNA synthesis in both normal and regenerating liver. Under our experimental conditions (replenishing the serum-free, growth factor-containing medium every 24 hr and assessing DNA synthesis for each 24-hr period), TGF-β was always inhibitory to EGF-induced DNA synthesis measured at 48 or 72 hr in culture. The inhibition was observed for cells maintained in either MEM or Williams E medium, although 5 times more

TGF-β was required in Williams E medium to obtain the same amount of inhibition as observed in MEM. The difference in sensitivity to TGF-β inhibition in these two media is not understood but does not appear to be due to the high content of hydrocortisone added to the Williams E medium (unpublished data). The results from these experiments indicate that at least *in vitro*, hepatocytes from normal and regenerating liver are sensitive to the inhibitory effects of TGF-β. However, since the range of the sensitivity varies with the growth media and experimental conditions, we cannot exclude the possibility that during liver regeneration *in vivo*, hepatocytes may modulate their sensitivity to TGF-β. Recent data show, however, that in a variety of cell types, the primary control mechanism for TGF-β action is likely to be the activation of the latent form of the growth factor rather than the binding of TGF-β to its receptors (35).

Although we cannot rule out the possibility that the increased expression of TGF-β during liver regeneration is solely associated with nonparenchymal cell metabolism, it is clear that local interactions between different cell types play a role in the control of cell proliferation within some tissues (13). Particularly pertinent to our findings is the recent work with human fetal liver, in which the mRNAs for IGF-I and IGF-II were localized in liver sinusoidal cells, whereas their protein products were detected in hepatocytes (36, 37).

An interesting question is whether the quiescent state of normal hepatocytes is a consequence of the continuous inhibitory effects of TGF-β. Data obtained so far argue against this view: (*i*) hepatocytes do not proliferate spontaneously in culture in TGF-β-free medium, and neither do they synthesize the factor; (*ii*) EGF-induced DNA synthesis in hepatocytes is not inhibited by TGF-β during the first 24 hr in culture, even at high TGF-β concentrations (6, 31); (*iii*) normal liver contains negligible amounts of TGF-β mRNA; (*iv*) although we do not know the levels of hepatic TGF-β during liver regeneration, the timing of the change in TGF-β mRNA suggests that the growth factor is maximally increased in the liver only after the major wave of cell replication has passed. Alternatively, it is conceivable that with the increased synthesis of TGF-β mRNA after partial hepatectomy, sufficient amounts of TGF-β accumulate to prevent subsequent rounds of hepatocyte replication. In this regard, it would be of interest to determine whether the administration of TGF-β inhibitors after partial hepatectomy would lead to uncontrolled liver growth or alter the kinetics of the regenerative response. On the other hand, despite these arguments, we cannot exclude the possibility that the quiescent state of normal hepatocytes is maintained by low levels of TGF-β.

We thank Dr. Henry C. Bodenheimer for providing isolated cells, Dr. Barbara Bowman for her gift of the Gc probe, Dr. Michael B. Sporn for the gift of purified TGF-β, and Mrs. Anna-Louise Baxter for her help in preparing the manuscript. This work was supported by National Cancer Institute Grants CA23226 and CA35249 (to N.F.) and Postdoctoral Fellowship CA07763 (to L.B.).

1. Bucher, N. L. R. & Malt, R. A. (1971) *Regeneration of Liver and Kidney* (Little, Brown, Boston).
2. Grisham, J. W. (1962) *Cancer Res.* **22,** 842–849.
3. Thompson, N. L., Mead, J. E., Braun, L., Goyette, M., Shank, P. R. & Fausto, N. (1986) *Cancer Res.* **46,** 3111–3117.
4. Fausto, N., Mead, J. E., Braun, L., Thompson, N. L., Panzica, M., Goyette, M., Bell, G. I. & Shank, P. R. (1987) *Symp. Fundam. Cancer Res.* **39,** 69–86.
5. Nakamura, T., Tomita, Y., Hirai, R., Yamaoka, K., Kaji, K. & Ichihara, A. (1985) *Biochem. Biophys. Res. Commun.* **133,** 1042–1060.
6. Carr, B. I., Hayashi, I., Branum, E. L. & Moses, H. L. (1986) *Cancer Res.* **46,** 2330–2334.
7. McMahon, J. B., Richards, W. L., DelCampo, A. A., Song, M.-K. & Thorgeirsson, S. S. (1986) *Cancer Res.* **46,** 4665–4671.
8. Roberts, A. B., Anzano, M. A., Lamb, L. C., Smith, J. M. & Sporn, M. B. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 5339–5343.
9. Moses, H. L., Tucker, R. F., Leof, E. B., Coffey, R. J., Jr., Halper, J. & Shipley, G. D. (1985) in *Cancer Cells: Growth Factors and Transformation*, eds. Feramisco, J., Ozanne, B. & Stiles, C. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), Vol. 3, pp. 65–78.
10. Roberts, A. B., Anzano, M. A., Wakefield, L. M., Roche, N., Stern, D. F. & Sporn, M. B. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 119–123.
11. Goustin, A. S., Leof, E. B., Shipley, G. D. & Moses, H. L. (1986) *Cancer Res.* **46,** 1015–1029.
12. Masui, T., Wakefield, L. M., Lechner, J. F., LaVeck, M. A., Sporn, M. B. & Harris, C. C. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8206–8210.
13. Sporn, M. B., Roberts, A. B., Wakefield, L. M. & de Crombrugge, B. (1987) *J. Cell Biol.* **105,** 1039–1045.
14. Ignotz, R. A., Endo, T. & Massague, J. (1987) *J. Biol. Chem.* **262,** 6443–6446.
15. Roberts, A. B. & Sporn, M. B. (1985) *Cancer Surveys* **4,** 633–705.
16. Higgins, G. M. & Anderson, R. M. (1931) *Arch. Pathol.* **12,** 136–202.
17. Yaswen, P., Hayner, N. T. & Fausto, N. (1984) *Cancer Res.* **44,** 324–331.
18. Fausto, N., Thompson, N. L. & Braun, L. (1987) in *Cell Separation: Methods and Selected Applications*, eds. Pretlow, T. G., II, & Pretlow, T. P. (Academic, Orlando, FL), Vol. 4, pp. 45–77.
19. Seglen, P. O. (1976) *Methods Cell Biol.* **13,** 30–78.
20. Bodenheimer, H. C., Charland, C. & McCrow, L. T. (1986) in *Cells of the Hepatic Sinusoid*, eds. Kim, A., Knook, D. L. & Wisse, E. (Kupffer Cell Foundation, Rijswijk, The Netherlands), Vol. 1, pp. 141–142.
21. Knook, D. L. & Sleyster, E. Ch. (1976) *Exp. Cell Res.* **99,** 444–449.
22. Chirgwin, J. M., Przybyla, A. E. & Rutter, R. J. (1979) *Biochemistry* **18,** 5294–5299.
23. Yaswen, P., Goyette, M., Shank, P. R. & Fausto, N. (1985) *Mol. Cell. Biol.* **5,** 780–786.
24. Petropoulos, C. J., Yaswen, P., Panzica, M. & Fausto, N. (1985) *Cancer Res.* **45,** 5762–5768.
25. Stempien, M. M., Fong, N. M., Rall, L. B. & Bell, G. I. (1986) *DNA* **5,** 357–361.
26. Yang, F., Brune, J. L., Naylor, S. L., Cupples, R. L., Naberhaus, K. H. & Bowman, B. H. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7994–7998.
27. Goyette, M., Petropoulos, C. J., Shank, P. R. & Fausto, N. (1983) *Science* **219,** 510–512.
28. Goyette, M., Petropoulos, C. J., Shank, P. R. & Fausto, N. (1984) *Mol. Cell Biol.* **4,** 1493–1498.
29. Graham, D. E., Rechler, M. M., Brown, A. L., Frunzio, R., Romanus, J. A., Bruni, C. B., Whitfield, H. J., Nissley, S. P., Seelig, S. & Berry, S. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4519–4523.
30. Soares, M. B., Ishii, D. N. & Efstratiadis, A. (1985) *Nucleic Acids Res.* **13,** 1119–1134.
31. Strain, A. J., Frazer, A., Hill, D. J. & Milner, R. D. G. (1987) *Biochem. Biophys. Res. Commun.* **145,** 436–442.
32. Carr, B. I., Thall, A., Whitsen, R. H. & Itakura, K. (1986) *J. Cell Biol.* **103,** 443 (abstr.).
33. Kehrl, J. H., Wakefield, L. M., Roberts, A. B., Jakowlew, S. B., Alvarez-Mon, M., Derynck, R., Sporn, M. B. & Fauci, A. S. (1986) *J. Exp. Med.* **163,** 1037–1050.
34. Zullo, N. J., Cochran, B. H., Huang, A. S. & Stiles, C. D. (1985) *Cell* **43,** 793–800.
35. Wakefield, L. M., Smith, D. M., Masui, T., Harris, C. C. & Sporn, M. B. (1987) *J. Cell Biol.* **105,** 965–975.
36. Han, V. K. M., D'Ercole, J. & Lund, P. K. (1987) *Science* **236,** 193–196.
37. Han, V. K. M., Hill, D. J., Strain, A. J., Towle, A. C., Lauder, J. M., Underwood, L. E. & D'Ercole, J. (1987) *Pediatr. Res.* **22,** 234–249.