0023-6837/01/8109-1299$03.00/0
Laboratory Investigation
Vol. 81, No. 9, p. 1299, 2001
Printed in U.S.A.
Copyright © 2001 by The United States and Canadian Academy of Pathology, Inc.

# Expression of Presumed Specific Early and Late Factors Associated with Liver Regeneration in Different Rat Surgical Models

Stéphanie Laurent, Masayuki Otsuka, Christine De Saeger, Dominique Maiter, Luc Lambotte, and Yves Horsmans

*Gastroenterology (SL, CD, YH), Diabetology (DM), and Experimental Surgery (MO, LL) Laboratories, Université Catholique de Louvain, Brussels, Belgium*

**SUMMARY:** Experiments performed on the portal branch ligation (PBL) model indicate that early changes observed after surgery are not related to the regenerative process because they also occur in atrophying lobes. To further confirm the lack of specificity of the early events and to exclude the influence of circulatory factors released by proliferating lobes on their occurrence, we investigated this response after sham operation (SO) and portacaval shunt (PCS), a model characterized by liver atrophy. We also attempted to determine expression of later events associated specifically with regeneration, ie, expression of p53 or c-Ha-*ras*, or inhibition of proliferation, ie, interleukin-1$\beta$ (IL-1$\beta$) and transforming growth factor-$\beta$1 (TGF-$\beta$1) after partial (PH) and temporary partial (TPH) hepatectomy, SO and PCS. Nuclear factor-$\kappa$B (NF-$\kappa$B) and signal transducer and activator of transcription 3 (STAT3) DNA binding were assessed by electrophoretic mobility shift assay (EMSA), interleukin-6 (IL-6) mRNA by reverse transcription-polymerase chain reaction (RT-PCR), c-*myc* and c-*jun* mRNAs by Northern blot analysis at 0.5 and 2 hours, p53 and c-Ha-*ras* mRNAs by Northern blot analysis at 8 and 24 hours, and IL-1$\beta$ and TGF-$\beta$1 by RT-PCR at 24 hours. The early response including an increase of NF-$\kappa$B, STAT3, IL-6, and immediate-early genes expression was present after PH, PCS, and SO. In SO, slight differences were observed in comparison with PH: no NF-$\kappa$B p65/p50 DNA binding was observed, only three of six SO rats were positive for IL-6, and immediate-early genes induction showed differences in the intensity of the response. At later times, p53 mRNA increased at 8 hours after PH and TPH, c-Ha-*ras* mRNA at 24 hours after PH, and IL-1$\beta$ mRNA at 24 hours after PCS. Early events are not specifically associated with the reduction of liver mass or with the regenerative process, are not predictive of future cell fate, and are most likely related to surgical stress. p53 and c-Ha-*ras* induction is closely associated with cell cycle progression whereas IL-1$\beta$, but not TGF-$\beta$1, appears to be one of the negative growth regulators that might play an important role in atrophy. (*Lab Invest 2001, 81:1299–1307*).

Liver regeneration is a complex regulated process divided into several phases resulting in the restoration of the original and functional liver mass. In rats, the proliferative response is preceded by a stage called "priming" in which quiescent hepatocytes acquire proliferative competences (Fausto and Mead, 1989; Fausto, 2000; Webber et al, 1994). This period corresponds, in the current 2/3 partial hepatectomy model (PH), to the first hours after surgery during which tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and interleukin-6 (IL-6) expression (Diehl and Rai, 1996a, 1996b; Fausto et al, 1995; Michalopoulos and DeFrances, 1997; Yamada et al, 1997), as well as DNA binding of the transcription factors, nuclear factor-$\kappa$B (NF-$\kappa$B) and signal transducer and activator of transcription 3 (STAT3) (Cressman et al 1994, 1995; FitzGerald et al, 1995; Taub, 1996; Trautwein et al, 1996), and c-*myc*, c-*fos*, and c-*jun* transcripts are increased (Alcorn et al, 1990; Thompson et al, 1986; Webber et al, 1994). Although studies in knockout animals seem to emphasize the role of these factors in triggering liver regeneration, hepatocyte proliferation has been obtained in several models independently of this cascade (Cressman et al, 1996; Menegazzi et al, 1997; Sakamoto et al, 1999; Yamada et al, 1997, 1998). Moreover, our previous observations from experiments performed on the temporary partial hepatectomy (TPH) and the portal vein ligation (PBL) models (Lambotte et al, 1997; Stärkel et al, 1999) indicate that early changes are not specifically related to the reduction of liver mass and the subsequent regenerative process. The increases in NF-$\kappa$B, STAT3, IL-6, and immediate-early genes expression in the atrophying lobes of the PBL model suggests that these events are not predictive of the future proliferative response and are most likely due to the surgical stress (Stärkel et al, 1999). However, in the PBL model, it was not excluded that the response observed in the atrophying lobes could be induced by changes occurring in the regenerating ones. We, therefore, investigated the response after a termino-lateral portacaval shunt (PCS), a model in which the entire liver, deprived of

Received June 13, 2001.
*This work was supported by a grant of Glaxo-Wellcome, Belgium, a grant of Commission du Patrimoine UCL, Belgium, and a grant (3-4598) of Fonds de la Recherche Scientifique Medicale (FRSM), Belgium.*
*Address reprint requests to: Dr. Yves Horsmans, Department of Gastroenterology, St. Luc University Hospital, Av. Hippocrate 10, 1200 Brussels, Belgium. E-mail: Horsmans@gaen.ucl.ac.be*

portal blood flow, begins to atrophy (Fisher et al, 1961, 1979). Because the response observed in this model was similar to that after PH, we investigated the occurrence of the early response after sham operation (SO) (laparotomy and liver lobes handling). The results clearly confirmed that the initial response is not related to the reduction of the liver mass, but more likely to surgical stress.

The divergences observed between proliferating and atrophying lobes after PBL (Uemura et al, 2000) and the absence of specificity in activation of early factors prompted us to evaluate the expression of p53, c-Ha-*ras*, interleukin-1$\beta$ (IL-1$\beta$), and transforming growth factor-$\beta$1 (TGF-$\beta$1) at later time points in four models characterized by different outcomes after a similar initial response: the classical 2/3 PH, TPH (PH followed by a reconnection of ligated lobes at 3 hours), SO, and PCS.

## Results

### NF-κB and STAT3 Activation

By electrophoretic mobility shift assay (EMSA), no NF-κB p65/p50 complex (Fig. 1A, Lane 3) or STAT3 (Fig. 2A, Lane 1) DNA binding was observed in liver nuclear extracts from controls. The p65/p50 complex was observed 0.5 hours after PH (Fig. 1A, Lane 2) but was not found at 2 hours (not shown). STAT3 was detected at 2 hours after PH (not shown).

In SO animals, only dimers containing the p50 sub-unit but not the p65/p50 transcriptionally active heterodimer were expressed (Fig. 1A, Lanes 4 to 5). A positive STAT3 DNA binding was shown at 2 hours (Fig. 2A, Lane 3).

Following PCS, a strong increase in p65/p50 NF-κB binding activity was observed at 0.5 hours, whereas it was not found anymore at 2 hours (Fig. 1A, Lanes 6 to 7). The lower complex was partially supershifted by p50 (Fig. 1, Lane 9) and the upper complex was supershifted by p65 antibody (Fig. 1A, Lane 10). STAT3 was only detected at high levels at 2 hours (Fig. 2A, Lane 5).

By immunoblotting, a faint p65 NF-κB (Fig. 1B) and STAT3 (Fig. 2B) immunoreactive band was noticed in nuclear extract from controls. After PH, a significant increase was observed at 0.5 hours for NF-κB ($p < 0.01$) and at 2 hours for STAT3 ($p < 0.01$).

After SO, there was no significant change of p65 NF-κB level at 0.5 and 2 hours. In contrast, a significant increase in STAT3 was observed at 2 hours ($p < 0.01$). After PCS, a significant elevation above baseline levels in NF-κB p65 protein was present at 0.5 hours ($p < 0.05$), whereas a nuclear abundance of STAT3 was increased at 2 hours ($p < 0.01$).

### Expression of IL-6 mRNA

IL-6 mRNA expression was not found in the liver of control animals. After SO and PCS, IL-6 mRNA ($p < 0.05$) was significantly induced at 2 hours, but not at 0.5 hours (Fig. 3), whereas it was significantly increased at both times after PH ($p < 0.001$, not shown).

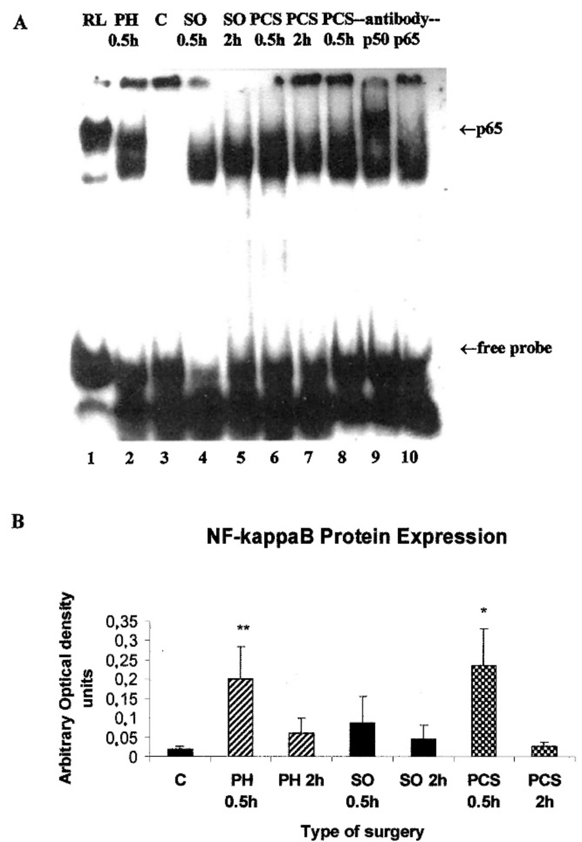


**Figure 1.**

A, Identification of nuclear factor (NF)-κB binding activity by electrophoretic mobility shift assays (EMSA). Nuclear extracts were prepared from partial hepatectomy (PH) (0.5 hours, Lane 2), control (Lane 3), sham (0.5 and 2 hours, Lanes 4 and 5), and portacaval shunt (PCS, 0.5 and 2 hours, Lanes 6 and7). Ten micrograms of nuclear proteins were incubated with a $^{32}$P-labeled oligonucleotide containing the NF-κB binding site followed by electrophoresis through a 5% polyacrylamide gel. A rabbit reticulocyte lysate expressing selectively p65 NF-κB was used as positive control (Lane 1). Supershift experiments with NF-κB p50- and p65-specific antibodies confirmed the specificity of the complexes detected after sham operation (SO) and PCS (Lanes 8–10), which do contain p50 and p65 subunits of the transcription factor. B, Quantification of NF-κB protein by densitometric analysis of Western blots. Mean values ± SEM obtained from individual densitometric measures are represented ($n = 3$). Weak expression of the factor was noticed in control animals. NF-κB p65 protein was significantly induced at 0.5 hours after PH and PCS. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

However, among SO rats, a strong variability in IL-6 expression was observed: only three of six rats were positive for IL-6 mRNA.

### Expression of c-myc and c-jun mRNA

c-*myc* and c-*jun* transcripts were detected in livers of controls (Fig. 4). After PH, an increase ($p < 0.001$) in c-*myc* mRNA was observed at 0.5 hours (7-fold) and 2 hours (5-fold), and c-*jun* expression was also increased 4-fold at 0.5 hours ($p < 0.001$) and 2-fold at 2 hours ($p < 0.05$) (not shown).

After SO, c-*myc* expression increased 3-fold at 0.5 hours ($p < 0.01$) and 5-fold at 2 hours ($p < 0.001$). c-*jun* mRNA showed 1.5-fold and 4-fold increase at 0.5 and 2 hours ($p < 0.01$ and $p < 0.001$, respectively). After PCS, c-*myc* expression showed a statistically

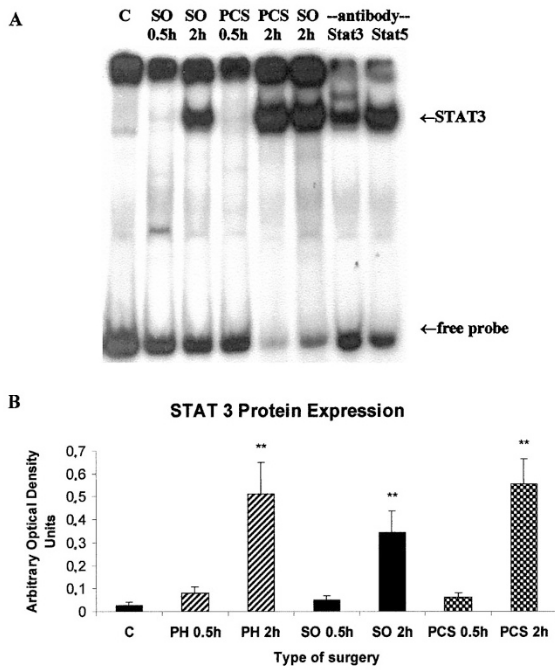


**Figure 2.**

A, Identification of signal transducer and activator of transcription 3 (STAT3) binding activity by EMSA. Ten micrograms of nuclear proteins from control (Lane 1), SO (0.5 and 2 hours, Lanes 2 and 3), and PCS (0.5 and 2 hours, Lanes 4 and 5) were incubated with a $^{32}$P-labeled oligonucleotide containing the STAT3 binding site followed by electrophoresis through a 5% polyacrylamide gel. Supershift experiments with STAT3- and STAT5-specific antibody confirmed that the complexes detected after SO (Lanes 6–8) and PCS did contain STAT3, but not STAT5. B, Quantification of STAT3 protein by densitometric analysis of Western blots. Mean values ± SEM obtained from individual densitometric measures are represented ($n = 3$). Weak expression of this factor was noticed in control animals. STAT3 protein was significantly induced 2 hours after PH, SO, and PCS. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

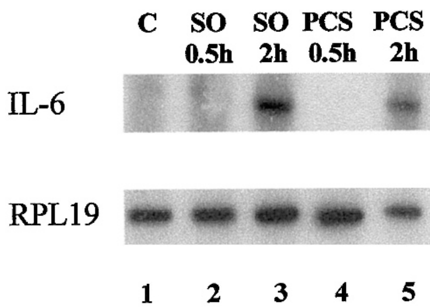


**Figure 3.**

Expression of interleukin-6 (IL-6) mRNA. Representation of reverse transcription-polymerase chain reaction (RT-PCR) of IL-6 and ribosomal protein L19 (RPL-19) mRNAs in controls (Lane 1), 0.5 hours and 2 hours after SO (Lanes 2 and 3), and 0.5 hours and 2 hours after PCS (Lanes 4 and 5). Total liver mRNA was subjected to RT-PCR as described in "Materials and Methods." The results were adjusted for their respective RPL-19 levels and then compared to a standard dilution curve obtained by amplification of liver mRNA from lipopolysaccharide (LPS)-treated rats in the same PCR procedure. IL-6 expression was significantly increased at 2 hours after SO and PCS ($p < 0.05$).

significant increase (3-fold) at 0.5 hours and at 2 hours ($p < 0.01$), whereas a rapid and highly significant increase in c-*jun* transcript at 0.5 (20-fold) and 2 hours (15-fold) was found ($p < 0.001$).

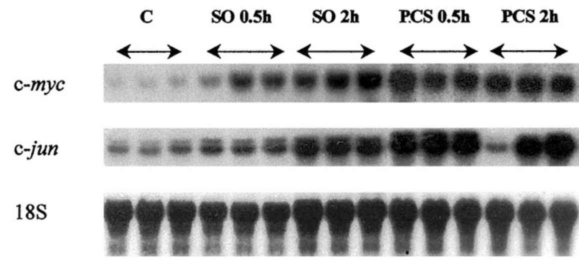


**Figure 4.**

Expression of c-*myc* and c-*jun* mRNAs. Representation of a Northern blot analysis of c-*myc* and c-*jun* after SO and PCS. Rats were killed 0.5 and 2 hours after surgery. Samples (20 $\mu$g of total liver mRNA) were separated by electrophoresis on agarose gel and hybridized with their respective $\alpha^{32}$P-labeled cDNAs and blotted. 18S mRNA hybridization was used as a control for loading. c-*myc* and c-*jun* were significantly increased at all investigated time points.

### *Expression of p53 and c-Ha-*ras *mRNA*

p53 (Fig. 5A) and c-Ha-*ras* (Fig. 5B) transcripts were detected in livers of controls. p53, but not c-Ha-*ras*, mRNA levels were significantly up-regulated 8 hours after PH (2.5-fold) and TPH (2-fold)($p < 0.001$). At 24 hours, p53 returned to baseline levels in both groups. In contrast to p53, c-Ha-*ras* mRNA was only significantly increased at 24 hours after PH (2-fold) ($p < 0.001$). Following SO and PCS, no significant change in p53 and c-Ha-*ras* mRNA was observed at 8 hours. p53 and c-Ha-*ras* showed a significant decrease 24 hours after SO and TPH, respectively ($p < 0.05$).

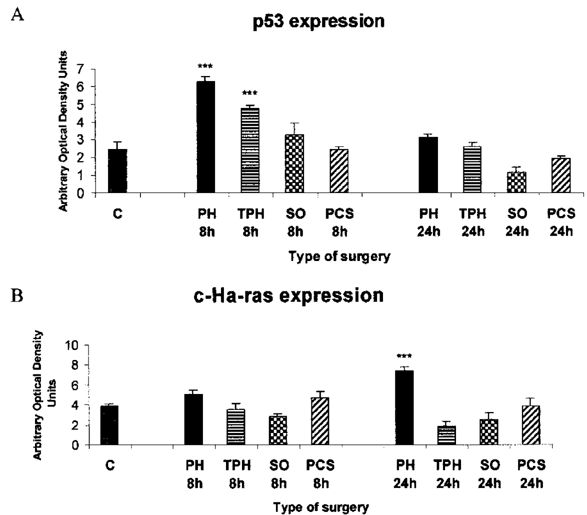


**Figure 5.**

Quantification of p53 and c-Ha-*ras* mRNAs by densitometric analysis of Northern blots. Mean values ± SEM obtained from individual densitometric measures are represented ($n = 3$). A, Quantification of p53 mRNA by densitometric analysis of the blots. After the adjustment for the respective 18S ribosomal signals, p53 was significantly up-regulated 8 hours after PH and temporary partial hepatectomy (TPH). B, Quantification of c-Ha-*ras* mRNA by densitometric analysis of the blots. After the adjustment for the respective 18S ribosomal signals, c-Ha-*ras* was only significantly up-regulated 24 hours after PH. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

### *Expression of IL-1β mRNA*

No significant change was seen at 8 hours (not shown) and 24 hours after PH, TPH, and SO. IL-1β mRNA was elevated 24 hours after PCS ($p < 0.01$) (Fig. 6).

### *Expression of TGF-β1 mRNA*

No change in liver TGF-β1 mRNA levels was observed at 24 hours after PH, TPH, SO, and PCS (Fig. 6).

## Discussion

Induction of transcription factors (NF-κB and STAT3), immediate-early proto-oncogenes (c-*fos*, c-*myc*, c-*jun*), and proinflammatory cytokines (TNF-α, IL-6) constitutes some of the earliest responses to a PH (Fausto and Mead, 1989; Fausto et al, 1995). It is generally accepted that these early modifications play an important role in the process that allows the hepatocyte to enter the cell cycle as a consequence of the reduction of liver mass. As pointed out years ago by Fausto and Mead (1989), the full responses "unfold automatically" after PH, and additional methods are therefore useful in clarifying the origin and importance of the early events. One of the first experiments was to show that the dietary manipulations could induce a similar, but probably incomplete, response and accelerate the in vitro proliferation of primed hepatocytes after the administration of growth factors (Mead et al, 1990). The importance of early events was also suggested as early as 1970 (Moolten et al, 1970): a sham operation, performed before a PH, produced an acceleration of the proliferative response after PH. No measurement of the early factors was performed in this and a subsequent paper on the topic by Sakamoto et al (1977). More recently, studies focusing on the early factors implicated in liver regeneration have emphasized their essential role in the process that allows the hepatocyte to enter the cell cycle (Cressman et al, 1996; Yamada et al, 1997). Nevertheless, our previous studies in other surgical models, such as TPH and PBL, have rather suggested that these early events are more likely related to accompanying surgical stress and do not provide specific signals for cell cycle progression (Lambotte et al, 1997; Stärkel et al, 1999). Moreover, they do not seem to be required in some models of liver hypertrophy (Ledda-Columbano et al, 1998; Menegazzi et al, 1997). Similarly, recent studies using knockout mice for receptor gp130 also suggest that liver regeneration after PH was not affected by an inhibition of STAT3 activation (Wustefeld et al, 2000).

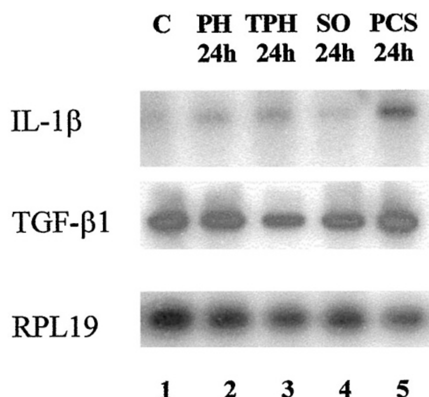


**Figure 6.**
Expression of interleukin-1β (IL-1β) and transforming growth factor-β1 (TGF-β1) mRNAs. Representation of RT-PCR of IL-1β, TGF-β1, and RPL-19 mRNAs in controls (Lane 1), 0.5 hours and 2 hours after SO (Lanes 2 and 3), and 0.5 hours and 2 hours after PCS (Lanes 4 and 5). Total liver mRNA was subjected to RT-PCR as described in "Materials and Methods." The results were adjusted for their respective RPL-19 levels and then compared with a standard dilution curve obtained by amplification of liver mRNA from LPS-treated rats in the same PCR procedure. IL-1β expression was significantly increased at 24 hours after PCS ($p < 0.01$), whereas there was no change in TGF-β1 expression at all investigated time points.

In the present study, the origin and specificity of the early cellular response was further assessed after SO, a model devoid of cell mass reduction and proliferation, and after PCS, which leads to a marked liver atrophy (Fisher et al, 1979). Activation of early factors occurred in both models, confirming that they are not specifically associated with a reduction of liver mass and with subsequent liver regeneration. After PCS, the early events, including the rapid activation of NF-κB and STAT3 and the expression of IL-6 and immediate-early genes, were similar to those observed by us and many others after PH (Alcorn et al, 1990; Cressman et al, 1994, 1995; FitzGerald et al 1995; Thompson et al, 1986; Trautwein et al, 1996). Because this response occurs in the absence of proliferating lobes, we can reasonably exclude an influence of the latter on the response obtained in the atrophying lobes of the PBL model. The absence of portal blood flow in PSC model allows us to refute the role of portal circulatory factors as potential inducers of early events observed after PH.

After SO, STAT3 was activated in all livers, but IL-6 was increased only in three of six rats. The variability of the response is consistent with the role of surgical stress in the activation of IL-6 in the liver, which has also been demonstrated as a consequence of other forms of stress as, eg, immobilization stress in mice (Kitamura et al, 1997). These results further suggest that factors other than IL-6 are responsible for STAT3 activation in such conditions. One of them could possibly be the hepatocyte growth factor as demonstrated in hepatocyte cell cultures (Schaper et al, 1997). DNA binding of a transcriptionally active p65/p50 NF-κB complex was not found after SO. However, a faster migrating complex was observed, which could represent another heterodimer such as p50/p35, previously described after PH. This might result from the turnover of nuclear p65 via a degradative mechanism that converts it to p35, preserving the κB binding activity (Cressman et al, 1994).

If the initial response can be induced by a great variety of stimuli ranging from PH to dietary manipulations (Fausto and Mead, 1989), the course of events diverges at some time point. The divergence may exist from the very beginning, involving mechanisms that remain presently undetected. To support this hypothesis, it can be pointed out that the patterns of activation of c-*myc* and c-*jun* were different after PH, SO,

and PCS. This suggests that the early response might be influenced either by the severity or by the nature of the surgical stress. The differences in the peak levels of transcription factors and immediate-early genes after SO and PCS, but also between anterior and posterior lobes of the PBL model (Stärkel et al, 1999), could be of some importance, because overcoming a speculative threshold level might be required to induce the complete sequence of events leading to hepatocyte proliferation. The methods used were semiquantitative and therefore did not allow us to exclude this possibility. The absence of proliferation observed when the liver mass is reconstituted 3 hours after PH demonstrates that these early changes are not sufficient to promote a regenerative response (Lambotte et al, 1997)

The initial response seems to be common for divergent outcomes, and the recruitment of early factors may be part of a general response to liver stress resulting in protective and anti-apoptotic measures, as suggested by recent works (Iimuro et al, 1998; Leu et al, 2001; Plumpe et al, 2000). The separation of downstream signaling could occur later, and the precise moment at which the evolution toward proliferation, atrophy, or maintenance of the liver mass starts to diverge after a similar, if not identical, early response remains unknown. We, therefore, investigated the expression of elements implicated either in the control of the progression through the cell cycle, such as p53 and c-Ha-*ras* (Fausto and Mead, 1989; Kren et al, 1996; Thompson et al, 1986), or in the inhibition of hepatocyte proliferation, such as IL-1$\beta$ (Boulton et al, 1997; Nakamura et al, 1988) and TGF-$\beta$1 (Nakamura et al, 1985; Russel et al, 1988), at later time points after PH, TPH, SO, and PCS.

The TPH model corresponds to a functional PH during the first 3 hours, followed by a reconnection of the devascularized lobes at 3 hours and an absence of DNA synthesis at 24 hours (Lambotte et al, 1997). In the present work, we have shown that expression of p53 mRNA was increased at 8 hours after TPH, as it was after PH in our study and in previous studies (Thompson et al, 1986; Kren et al, 1996). In contrast, c-Ha-*ras* expression was elevated at 24 hours after PH only, in direct correlation to the peak of DNA synthesis, confirming reported findings (Goyette et al, 1983; Thompson et al, 1986). Activation of c-Ha-*ras* seems clearly associated with S phase progression, and this hypothesis is reinforced by the findings that the blocking of *ras* activity by anti-*ras* antibodies results in G1 cycle cell arrest (Dobrowolski et al, 1994). After SO and PCS, no significant increase in p53 and c-Ha-*ras* mRNAs was observed at 8 and 24 hours. The results suggest that p53 in TPH may drive hepatocytes to progress toward the late G1 phase of the cell cycle, whereas they do not progress beyond the early G1 phase in the SO and PCS models. Indeed, it has been suggested that p53 is a key element of the transition from G1 to S phases and may act as an inhibitor of the "mitogen restriction point" (Bellamy et al, 1997). The restriction point may thus vary depending upon the initial stimulus. The absence of progression through the cell cycle in TPH, SO, and PCS could be due to a lack of activation of stimulating factors, a predominance of inhibitory factors, or an imbalance between the two. We have investigated two potential inhibitors, IL-1$\beta$ and TGF-$\beta$1, implicated in vitro and in vivo in the inhibition of hepatocyte proliferation (Boulton et al, 1997, 1998; Nakamura et al, 1985, 1988; Russel et al, 1988). After PH, a transient and rapid increase in hepatic expression of IL-1$\beta$ mRNA has been shown (Boulton et al, 1997; Rai et al, 1997), as well as an increase in TGF-$\beta$ mRNA, starting at 4 hours after surgery and remaining elevated until 96 hours, with a progressive increase of the TGF-$\beta$1 isoform until 48 hours (Bissell et al, 1995, Braun et al, 1988). These two cytokines may act as inhibitors of the progression through the cell cycle and, in this way, may play a significant role in the control of liver regeneration. It was therefore intriguing to determine whether IL-1$\beta$ and TGF-$\beta$1 would be particularly expressed in circumstances where a surgical stimulus does not lead to subsequent proliferation after an initial response. IL-1$\beta$ remained at low control values 24 hours after PH, but also after TPH and SO. It was, however, increased after PCS, as it was in the ligated lobes after PBL in our study (Stärkel et al, 2001) and in other studies (Uemura et al, 2000). It has been demonstrated that administration of exogenous recombinant IL-1$\beta$ in vivo diminishes the replicating surge of hepatocyte DNA synthesis after PH (Boulton et al, 1997), whereas blocking its effect by administration of IL-1$\beta$ receptor antagonists during the first 24 hours after PH enhances hepatocyte proliferation in rats (Boermeester et al, 1995). This cytokine might exert a negative growth modulator effect preventing uncontrolled hepatocyte proliferation (Higashitsuji et al, 1995). However, absence of IL-1$\beta$ expression after TPH and SO does not favor a role for this cytokine as a major negative regulator of hepatocyte proliferation during the first 24 hours. Nevertheless, marked expression of IL-1$\beta$ at 24 hours was found in atrophying livers after PBL and PCS. It is conceivable that IL-1$\beta$ may act as a negative modulator of cell cycle events, required for inducing and/or maintaining the commitment of the hepatocyte toward atrophy. Our results do not support the hypothesis of an inhibitory role of TGF-$\beta$ on hepatocyte proliferation, because no change in TGF-$\beta$ mRNA was noticed at 24 hours in contrast to the results of Bissell et al (1995). It is, however, possible that TGF-$\beta$ exerts an inhibitory effect at later times. Indeed, a peak of TGF-$\beta$ expression following PH has been reported after the major wave of DNA synthesis (Braun et al, 1988), but later times have not been evaluated in our studies. It is also possible that the sensitivity of hepatocytes to the TGF-$\beta$ inhibitory effect is dependent on the expression of its receptors during regeneration or atrophy as described after PH (Chari et al, 1995).

In conclusion, in agreement with the data previously obtained from the PBL model, we demonstrate, using SO and PCS models, that activation of early factors such as NF-$\kappa$B, STAT3, and IL-6 is not specifically associated with the reduction of liver mass or with the

regenerative process. All these changes are more likely related to nonspecific surgical stress and, as such, are not predictive of future cell evolution, which is determined at later times.

In this regard, investigating four models (PH, TPH, SO, and PCS), a marked difference in p53 expression seemed to be correlated with hepatocyte progression through the cell cycle, whereas c-Ha-*ras* was associated with the subsequent evolution toward regeneration. By contrast, prolonged IL-1$\beta$ expression appears to be linked to the absence of portal blood flow in PBL and PCS. This cytokine may exert a major negative growth effect in these models, and it possibly plays a role in the mechanisms leading to and/or maintaining atrophy. TGF-$\beta$, whose expression remained unchanged, does not seem to be important at least at 24 hours after surgery. As there were great similarities between the initial responses, despite very different outcomes, a divergent response appears after a few hours concerning factors that are related either to the progression in the cell cycle or to the inhibition of this progression. These elements may be activated by common initiating mechanisms, or they may simply require the changes induced to get expressed.

## Materials and Methods

### *Animals*

Male Wistar rats (210–275 g body weight) were obtained from the Rat Breeding Facilities of the Catholic University of Louvain Medical School, Brussels, Belgium. All animals were kept in a temperature- and humidity-controlled environment in a 12-hour light-dark cycle. At all times, they were allowed free access to water and standard food pellet diet (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orges, France). The animals were handled according to the guidelines established by the Catholic University of Louvain.

### *Surgical Procedures and Experimental Design*

All operations were carried out under light ether anesthesia at room air between 9:00 am and 12:00 noon with use of a clean, but not sterile, technique. Hepatectomy consisted in mid-ventral laparotomy and resection of the left lateral and median lobes (70% of the liver), according to Higgins and Anderson (1931). Temporary partial hepatectomy (TPH), described by Lambotte et al (1997), consisted in mid-ventral laparotomy and devascularization with a clamp of the median and left lateral lobes, secondarily immersed in 0.9% NaCl solution at 8° C. After 3 hours, the clamp was removed and the anterior lobes (AL) were replaced in the abdomen. SO rats underwent mid-ventral laparotomy followed by dissection of the relevant ligaments without ligature. PCS consisted in dissection of the portal vein and inferior vena cava from the entrance of the right renal vein to its disappearance beneath the liver. The portal vein was clamped for 10 to 15 minutes and end-to-side anastomosis was performed (Lee and Fisher, 1961). The operative field was irrigated with warm saline solution during the intervention. In the control group (0 hours), the livers were removed without any prior surgical aggression. Postoperatively, the animals had free access to water and food.

The rats were killed under ether anesthesia by exsanguination after puncture of the abdominal aorta and transection of the inferior vena cava in the thoracic cavity. They were killed at 8 hours and 24 hours after PH and TPH, at 0.5 hours and 2 hours after SO, and at 0.5 hours, 2 hours, 8 hours, and 24 hours after PCS. The livers were removed and the lobes were rapidly weighed, snap-frozen in liquid nitrogen, and stored at −80° C. A minimum of three rats was killed in all groups at each of the indicated time points.

### *Preparation of Nuclear Extracts and Total RNA Isolation*

Nuclear extracts were prepared as described by Hattori et al (1990), with slight modifications. All steps were performed at 4° C. All buffers were supplemented with protease and phosphatase inhibitors as follows: 2 $\mu$g/ml each of antipain, aprotinin, bestatin, and leupeptin; 0.5 mM NaF; and 1 mM $Na_3VO_4$ (all protease and phosphatase inhibitors were purchased from Sigma Chemical Company, Bornem, Belgium). The nuclear extracts were resuspended in the nuclear extract dialysis buffer (NED) (25 mM Hepes, 40 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol [DTT], 0.5 mM phenylmethylsulfonyl fluoride [PMSF], 0.5 mM NaF, 1 mM $Na_3VO_4$) and dialyzed for 4 hours against 250 ml NED with one change of dialysis solution. After dialysis, the extracts were centrifuged at 14,000 rpm for 5 minutes at 4° C in a microcentrifuge (Sorvall RMC 14; DuPont, Newtown, Connecticut) to remove insoluble material, frozen in aliquots on liquid nitrogen, and stored at −80° C until use.

Protein content was determined using a bicinchoninic acid (BCA) protein assay with serum albumin as a standard (Pierce Chemical, Rockford, Illinois). Total RNA was prepared from frozen liver tissue using the guanidine thiocyanate and cesium chloride method (Chirgwin et al, 1977).

### *Electrophretic Mobility Shift Assays*

Five to fifteen micrograms of nuclear proteins were preincubated for 30 minutes at room temperature with 2 $\mu$g poly(dI-dC) in the following binding buffers: NF-$\kappa$B (20 mM Hepes, 60 mM KCl, 5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM PMSF, 0.5 mM DTT, glycerol 10%, Nonidet P40 1%); STAT3 (10 mM Hepes, 50 mM NaCl, 1 mM EDTA, glycerol 10%). Double stranded oligonucleotides were $^{32}$P end-labeled with $\gamma$-$^{32}$P ATP and added to the extracts ($10^5$ cpm). The mixtures were further incubated for 30 minutes at room temperature and then electrophoresed (200V, 2 hours) on a 5% polyacrylamide gel (29:1 cross-linking) in a $\times$1 TAE buffer (6.7 mM Tris-HCl, 1 mM EDTA, 3.3 mM NaAc). For antibody supershift assays, 4 $\mu$l of the antibody (1 $\mu$g/$\mu$l) was added to the respective samples after 30 minutes of incubation with the labeled probe to confirm the identity of NF-$\kappa$B and STAT3. The samples were incubated at room tempera-

ture for an additional 30 minutes before electrophoresis. The anti-p50 and p65-specific polyclonal antibodies for the NF-κB components, as well as the STAT3, were purchased from Santa Cruz Biotechnology (Santa Cruz, California). The following probes were used: preannealed chromatography-purified double-stranded oligonucleotides from the class I major histocompatibility complex enhancer element H2-κB; TCGAGGGCTGGGGATTCCCCATCTC (NF-κB) and from the serum-inducible factor binding element in the c-fos promoter; GATCCTCCAGCATTTCCCGTAAATCCTCCAG (STAT3). A rabbit reticulocyte (Promega Benelux, Leiden, Netherlands) and an EGF-stimulated A431 cell nuclear extract (Santa Cruz Biotechnology) were used as standards for NF-κB and STAT3, respectively. Gels were dried and exposed to a Kodak Biomax MS film (NEN Life Science Products, Inc., Boston, Massachusetts) for 16 to 24 hours.

### *RT-PCR of IL-6, IL-1β, TGFβ1 m-RNAs*

Total liver RNA (40 μg) was treated with 10 U RNasin (Promega) and 1 U DNase (Promega) during 15 minutes at 37° C in an incubation buffer (Tris-HCl 10 mM, NaCl 50 mM, $MgCl_2$ 25 mM) to avoid any DNA contamination during PCR. The absence of genomic DNA contamination was assured by submitting samples, where reverse transcriptase had been omitted from the reaction, to PCR amplification. After incubation, the purified RNA was extracted using phenol-chloroform-isoamyl alcohol (25:24:1) (Sigma) and chloroform (Sigma) and then precipitated by addition of sodium acetate (3 M) and ethanol 100%. Five micrograms of purified RNA were preincubated with random hexamer and water for 10 minutes at 70° C. Four hundred units of moloney murine leukemia virus (M-MLV) reverse transcriptase (Gibco BRL, Merelbeke, Belgium) were added together with DTT and deoxynucleoside triphosphate (dNTP; Pharmacia LKB, Uppsala, Sweden), and the reaction was continued for further 60 minutes at 37° C. The reverse transcription products were amplified in a two-step PCR using *Taq* DNA polymerase (Boehringer Mannheim, Brussels, Belgium) and specific IL-6, IL-1β, and TGFβ1 primers. In our models, we have assessed TGF-β1 expression, which is the most abundant isoform in liver (De Bleser et al, 1997). Ribosomal protein L19 (RPL19) cDNA was co-amplified as standard. A PCR of 30 cycles (94° C for 1 minute; 60° C for 1 minute, 30 seconds; 72° C for 2 minutes) was performed using the IL-6 primers, a PCR of 24 cycles (94° C for 1 minute; 56° C for 1 minute, 30 seconds; 72° C for 2 minutes) was performed using the RPL19 primers, a PCR of 24 cycles (94° C for 1 minute; 60° C for 1 minute, 30 seconds, 72° C for 2 minutes) was performed using the IL-1β primers, and a PCR of 28 cycles (94° C for 1 minute; 60° C for 1 minute, 30 seconds; 72° C for 2 minutes) was performed using the TGF-β1 primers. The following primers were used: IL-6 sense CTTCCAGCCAGTTGCCTTCT; antisense AGCCAGAGTCATTCAGAGCA, RPL19 sense AGTATGCTTAGGCTACAGAA; antisense GCAGGTCTAAGACCAAGGAA, IL-1β sense GTGGCAGCTACCTATGTCTT; antisense GAGAGGTGCTGATGTACCAG, TGF-β1 sense GGACTCTCCACCTGCAAGAC; antisense CAGAGCTGCGCCTGCAGAG.

In parallel, RNA samples obtained from lipopolysaccharide-treated rats (positive control for IL-6, IL-1β, TGF-β1, and RPL19) were serially diluted and coamplified in the same PCR procedure. Standard dilution curves for IL-6, IL-1β, TGF-β1, and RPL19 were thus obtained. [$\alpha$-$^{32}$P]dCTP was included for detection of the PCR products that were separated by electrophoresis through an agarose gel (1.5%) and dried and exposed to a Kodak Biomax MS film (NEN Life Science Products, Inc.) for 1 hour.

The bands were subjected to densitometric analysis (GelDoc 2000 Scan; Bio-Rad, Nazareth, Belgium), and semiquantitative analysis of signal strength was obtained by comparing the measured values to their standard dilution curves. IL-6, IL-1β, and TGF-β1 values were finally adjusted for the respective RPL19 values.

### *Northern Blotting*

Twenty micrograms of RNA were resolved on 1% agarose gels containing 1% formaldehyde and transferred to Hybond-N membranes (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, England). cDNA probes were $^{32}$P-labeled by the method of "random priming" using a Megaprime DNA labeling System (Amersham). Oligonucleotide probes were $^{32}$P end-labeled using a $T_4$ polynucleotide kinase (Amersham). After ultraviolet fixation, the filters were hybridized with the $^{32}$P-labeled oligonucleotide or cDNA probes. The blots were exposed to a Hyperfilm MP (Amersham) for 4 hours to 7 days. Densitometry using an Ultroscan XL device (Pharmacia) was performed. The results are expressed in arbitrary densitometric units adjusted for the respective 28S ribosomal signal. The c-*jun* cDNA was kindly provided by D. Morello (Institut Pasteur, Paris, France). The c-*myc* cDNA was purchased from Oncor (Gaithersburg, Maryland). The p53 cDNAs were kindly provided by T. Soussi (INSERM U301, Paris, France) (Soussi et al, 1988). The c-Ha-*ras* cDNAs were purchased from Oncor. The 28S rRNA oligonucleotide was obtained from Oncogene Sciences (Uniondale, New York).

### *Western Blotting*

Nuclear protein (30 μg [STAT3] to 40 μg [NF-κB]) was resolved on an SDS-PAGE gel and transferred to a HYBOND-P membrane (optimized for protein transfer) (Amersham). The blotted membrane was blocked (1 hour, room temperature) in Tris buffered saline (TBS) containing 5% of membrane blocking reagent (Amersham). All of the following incubations were carried out at room temperature in TBS containing 1% of membrane blocking reagent. The incubation steps were followed by five washing steps of 3 minutes with TBS containing 0.1% Tween 20. The following antibodies were used: a STAT3 rabbit polyclonal primary antibody (Santa Cruz Biotechnology; 1:3000; 1 hour), a NF-κB p65 rabbit polyclonal primary antibody (sc-372-G, Santa Cruz Biotechnology; 1:2000; 1 hour), a secondary bio-

tinylated antibody (donkey anti-rabbit IgG, Amersham; 1:30000; 1 hour). The antigen-antibody reaction was visualized using the Amersham chemiluminescent detection system followed by exposure of the membranes to a Kodak X-Omat Blue XB film (NEN Life Science Products, Inc.) for 2 minutes. Equal protein load on membranes and complete transfer were checked by staining gels and membranes with Coomassie blue. Liver cytoplasmic extracts, obtained 45 minutes and 75 minutes after PH, were used as positive controls for NF-κB and STAT3, respectively. All blots were produced twice and the amount of immunoreactive nuclear protein on each membrane was quantified using a GelDoc 2000 Scan (Bio-Rad). The volume values were adjusted for total nuclear protein load. The final result of each sample was defined as the mean of two immunoblots performed on an identical nuclear extract sample.

### *Statistical Analysis*

Results are expressed as means ± SEM in comparison with control values. The statistical differences between the groups were tested using the one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls multiple comparison tests. Statistical significance was admitted for a $p$ value $<0.05$.

## Acknowledgements

We thank Valérie Lebrun and Pascale Lause for expert technical assistance and Alain Saliez for carrying out the surgical procedures.

## References

Alcorn JA, Feitelberg SP, Brenner DA (1990). Transient induction of c-jun during hepatic regeneration. Hepatology 11:909–915.

Bellamy CO, Clarke AR, Wyllie AH, and Harrison DJ (1997). p53 deficiency in liver reduces local control of survival and proliferation, but does not affect apoptosis after DNA damage. FASEB J 11:591–599.

Bissell DM, Wang SS, Jarnagin WR, and Roll FJ (1995). Cell-specific expression of transforming growth factor β in rat liver. Evidence for autocrine regulation of hepatocyte proliferation. J Clin Invest 96:447–455.

Boermeester MA, Straatsburg IH, Houdijk AP, Meyer C, Frederiks WM, Wesdorp RI, van Noorden CJ, and van Leeuwen PA (1995). Endotoxin and interleukin-1 related hepatic inflammatory response promotes liver failure after partial hepatectomy. Hepatology 22:1499–1506.

Boulton R, Alison MR, Golding M, and Hodgson HJ (1998). Augmentation of the early phase of liver regeneration after 70% partial hepatectomy in rat following selective kupffer cell depletion. J Hepatol 29:271–280.

Boulton R, Woodman A, Calnan D, Selden C, Tam F, and Hodgson H (1997). Nonparenchymal cells from regenerating rat liver generate interleukin-1alpha and -1beta: A mechanism of negative regulation of hepatocyte proliferation. Hepatology 26:49–58.

Braun L, Mead JE, Panzica M, Mikumo R, Bell GI, and Fausto N (1988). Transforming growth factor β mRNA increases during liver regeneration: A possible paracrine mechanism of growth regulation. Proc Natl Acad Sci USA 85:1539–1543.

Chari RS, Price DT, Sue SR, Meyers WC, and Jirtle RL (1995). Down-regulation of transforming growth factor beta receptor type I, II, and III during liver regeneration. Am J Surg 169: 126–132.

Chirgwin JM, Przybla AE, MacDonald RJ, and Rutter WJ (1977). Isolation of biologically active ribonucleic acid from sources enriched in ribonucleases. Biochemistry 18:5294–5299.

Cressman DE, Diamond RH, and Taub R (1995). Rapid activation of STAT3 transcription complex in liver regeneration. Hepatology 21:1443–1449.

Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, and Taub R (1996). Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. Science 274:1379–1383.

Cressman DE, Greenbaum LE, Haber BA, and Taub R (1994). Rapid activation of post-hepatectomy factor/Nuclear factor kB in hepatocytes, a primary response in the regenerating liver. J Biol Chem 269:30429–30435.

De Bleser PJ, Niki T, Rogiers V, and Geerts A (1997). Transforming growth factor-β gene expression in normal and fibrotic rat liver. J Hepatol 26:886–893.

Diehl AM and Rai R (1996a). Regulation of liver regeneration by pro-inflammatory cytokines. FASEB J 11:466–470.

Diehl AM and Rai R (1996b). Regulation of signal transduction during liver regeneration. FASEB J 10:215–227.

Dobrowolski S, Harter M, and Stacey DW (1994). Cellular ras activity is required for passage through multiple points of G0/G1 phase in BALB/c 3T3 cells. Mol Cell Biol 14:5441–5449.

Fausto N (2000). Liver regeneration. J Hepatol 32:19–31.

Fausto N and Mead JE (1989). Biology of disease. Regulation of liver growth: Protooncogenes and transforming growth factors. Lab Invest 60:4–13.

Fausto N, Laird AD, and Webber EM (1995). Role of growth factors and cytokines in hepatic regeneration. FASEB J 9:1527–1536.

Fisher B, Lee SH, and Fisher ER (1961). Liver regeneration following portacaval shunt. Surgery 52:88–102.

Fisher B, Gebhardt MC, Saffer EA, and Fisher ER (1979). Effect or corynebacterium parvum in prevention and reversal of atrophy of the liver following portacaval shunt. Surg Gynecol Obstet 149:2–5.

FitzGerald MJ, Webber EM, Donovan JR, and Fausto N (1995). Rapid DNA binding by nuclear factor κB in hepatocytes at the start of liver regeneration. Cell Growth Differ 6:417–427.

Goyette M, Petropoulos CJ, Shank PR, and Fausto N (1983). Expression of cellular oncogene during liver regeneration. Science 219:510–512.

Hattori M, Tugores A, Veloz L, Karin M, and Brenner DA (1990). A simplified method for the preparation of transcriptionally active liver nuclear extracts. DNA Cell Biol 9:777–781.

Higgins GM and Anderson RM (1931). Restoration of the liver of the white rat following partial surgical removal. Arch Pathol 12:186–202.

Higashitsuji H, Arii S, Furutani M, Mise M, Monden K, Fujita SI, Ishiguro S, Kitao T, Nakamura T, Nakayama H, Fujita J,

and Imamura M (1995). Expression of cytokine genes during liver regeneration after partial hepatectomy in rats. J Surg Res 58:267–272.

Iimuro Y, Nishiura T, Hellerbrand C, Behrns KE, Schoonhoven R, Grisham JW, and Brenner DA (1998). Nf-κB prevents apoptosis and liver dysfunction during liver regeneration. J Clin Invest 101:802–811.

Kitamura H, Konno A, Morimatsu M, Jung BD, Kimura K, and Saito M (1997). Immobilization stress increases hepatic IL-6 expression in mice. Biochem Biophys Res Commun 238: 707–711.

Kren BT, Trembley JH, and Steer CJ (1996). Alterations in mRNA stability during liver regeneration. Am J Physiol 270: G763–G777.

Lambotte L, Saliez A, Triest S, Tagliaferri EM, Barker AP, and Baranski AG (1997). Control of rate and extent of the proliferative response after partial hepatectomy. Am J Physiol 273:G905–G912.

Ledda-Columbano GM, Curto M, Piga R, Zedda AI, Menegazzi M, Sartori C, Shinozuka H, Bluethmann H, Poli V, Ciliberto G, and Columbano A (1998). In vivo hepatocyte proliferation is inductible through a TNF and IL-6-independent pathway. Oncogene 17:1039–1044.

Lee SH and Fisher B (1961). Portacaval shunt in the rat. Surgery 50:668–673.

Leu JI, Crissey MA, Leu JP, Ciliberto G, and Taub R (2001). Interleukin-6-induced STAT3 and AP-1 amplify hepatocyte nuclear factor 1-mediated transactivation of hepatic genes, an adaptative response to liver injury. Mol Cell Biol 21:414–424.

Mead JE, Braun L, Martin DA, and Fausto N (1990). Induction of replicative competence ("priming") in normal liver. Cancer Res 50:7023–7030.

Menegazzi M, Carcereri-De Prati A, Suzuki H, Shinozuka H, Pibiri M, Piga R, Columbano A, and Ledda-Columbano GM (1997). Liver cell proliferation induced by nafenopin and cyproterone acetate is not associated with increases in activation of transcription factors NF-κB and AP-1 or with expression of tumor necrosis factor $\alpha$. Hepatology 25:585–592.

Michalopoulos GK and DeFrances MC (1997). Liver regeneration. Science 276:60–66.

Moolten FL, Oakman NL, and Bucher NLR (1970). Accelerated response of hepatic DNA synthesis to partial hepatectomy in rats pretreated with growth hormone or surgical stress. Cancer Res 30:2353–2357.

Nakamura T, Arakaki R, and Ischihara A (1988). Interleukin-1$\beta$ is a potent growth inhibitor of adult rat hepatocytes in primary culture. Exp Cell Res 179:488–497.

Nakamura T, Tomita Y, Hirai R, Yamaoka K, Kaji K, and Ichihara A (1985). Inhibitory effect of transforming growth factor-$\beta$ on DNA synthesis of adult rat hepatocytes in primary culture. Biochem Biophys Res Commun 133:1042–1050.

Plumpe J, Malek NP, Bock C, Rakemann T, Manns, and Trautwein C (2000). NF-kappaB determines between apoptosis and proliferation in hepatocytes during liver regeneration. Am J Physiol Gastrointest Liver Physiol 278:G173–G183.

Rai RM, Loffreda S, Karp CL, Yang SQ, Lin HZ, and Diehl AM (1997). Kupffer cell depletion abolishes induction of interleukin-10 and permits sustained overexpression of tumor necrosis factor alpha messenger RNA in the regenerating rat liver. Hepatology 25:889–895.

Russel WE, Coffey RJ, Ouellette AJ, and Moses HL (1988). Type $\beta$ transforming growth factor reversibly inhibits the early proliferative response to partial hepatectomy in the rat. Proc Natl Acad Sci USA 85:5126–5130.

Sakamoto T, Liu Z, Murase N, Ezure T, Yokomuro S, Poli V, and Demetris AJ (1999). Mitosis and apoptosis in the liver of interleukin-6-deficient mice after partial hepatectomy. Hepatology 29:403–411.

Sakamoto Y, Jehn D, Nicolson MO, Ohashi E, and Hays DM (1977). The acceleration of hepatic regeneration by prior laparotomy. J Surg Res 23:306–310.

Schaper F, Siewert E, Gomez-Lechon MJ, Gatsios P, Sachs M, Birchmeier W, Heinrich PC, and Castell J (1997). Hepatocyte growth factor/scatter factor (HGF/SF) signals via the STAT3/APRF transcription factor in human hepatoma cells and hepatocytes. FEBS Lett 405:99–103.

Soussi T, Caron de Fromentel C, Breugnot C, and May E (1988). Nucleotide sequence of cDNA encoding the rat p53 nuclear oncoprotein. Nucleic Acids Res 16:11384.

Stärkel P, Horsmans Y, Sempoux C, De Saeger C, Wary J, Lause P, Maiter D, and Lambotte L (1999). After portal branch ligation in rat, nuclear factor κB, interleukin-6, signal transducers and activators of transcription 3, c-fos, c-myc, and c-jun are similary induced in the ligated and non-ligated lobes. Hepatology 29:1463–1470

Stärkel P, Lambotte L, Sempoux C, De Saeger C, Saliez A, Maiter D, and Horsmans Y (2001). After portal branch ligation in the rat, cellular proliferation is associated with selective induction of c-Ha-ras, p53, cyclin E and Cdk2. Gut 49:119–130.

Taub R (1996). Transcriptional control of liver regeneration. FASEB J 10:413–427.

Thompson NL, Mead JE, Braun L, Goyette M, Shank PR, and Fausto N (1986). Sequential protooncogene expression during rat liver regeneration. Cancer Res 46:3111–3117.

Trautwein C, Rakemann T, Niehof M, Rose-John S, and Manns MP (1996). Acute phase response factor, increased binding, and target gene transcription during liver regeneration. Gastroenterology 110:1854–1862.

Uemura T, Miyazaki M, Hirai R, Matsumoto H, Ota T, Ohashi R, Shimizu N, Tsuji T, Inoue Y, and Namba M (2000). Different expression of positive and negative regulators of hepatocyte growth in growing and shrinking hepatic lobes after portal vein branch ligation in rats. Int J Mol Med 5:173–179.

Webber EM, Godowski PJ, and Fausto N (1994). In vivo response of hepatocytes to growth factors requires an initial priming stimulus. Hepatology 14:489–497.

Wustefeld T, Graw A, Betz U, Manns MP, Mueller W, and Trautwein C (2000). Inducible GP130 deletion in the liver blocks Stat3 activation and has no effect on DNA synthesis after hepatectomy. Hepatology 32:326A.

Yamada Y, Kirillova I, Peschon JJ, and Fausto N (1997). Initiation of liver growth by tumor necrosis factor: Deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. Proc Natl Acad Sci USA 94:1441–1446.

Yamada Y, Webber EM, Kirillova I, Peschon JJ, and Fausto N (1998). Analysis of liver regeneration in mice lacking type 1 or type 2 tumor necrosis factor receptor: Requirement for type 1 but not type 2 receptor. Hepatology 28:959–970.