**Oncogene (1998) 17**, 1039–1044
© 1998 Stockton Press All rights reserved 0950–9232/98 $12.00
http://www.stockton-press.co.uk/onc

# *In vivo* hepatocyte proliferation is inducible through a TNF and IL-6-independent pathway

GM Ledda-Columbano[1], M Curto[1], R Piga[1], AI Zedda[1], M Menegazzi[3], C Sartori[2], H Shinozuka[4], H Bluethmann[5], V Poli[6], G Ciliberto[6] and A Columbano[1]

[1]*Dipartimento di Tossicologia, Sezione di Oncologia e Patologia Molecolare, University of Cagliari, Cagliari;* [2]*Dipartimento Di Biologia Cellulare e dello Sviluppo, University of Rome, Italy;* [3]*Istituto di Scienze Biochimiche, University of Verona, Italy;* [4]*Department of Pathology, University of Pittsburgh, USA;* [5]*Hoffman LaRoche, Basel, Switzerland;* [6]*IRBM, Rome, Italy*

**Recent studies in mice harboring a targeted disruption of genes encoding TNF receptor 1 (TNFR-1) or Interleukin 6 (IL-6) suggested a critical role for TNF and IL-6 in initiation of liver regeneration after 2/3 partial hepatectomy. However, hepatocyte proliferation can also occur following treatment with agents that do not induce tissue loss (primary mitogens). To determine whether the above cytokines could also be involved in mitogen-induced liver cell proliferation, we studied the hepatocyte proliferative response after treatment with primary mitogens in mice knock-out for TNFR-1 or IL-6. Our results showed no difference in the proliferative response of the liver between the wild type and the knock-out mice following treatment with the mitogens 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP), or the peroxisome proliferator, ciprofibrate, suggesting that TNF or IL-6 may not play a major role in this type of proliferation. Gel shift assay indicated that TCPOBOP-induced hepatocyte proliferation is not associated with activation of STAT3 transcription factor, a major target of IL-6 and other growth factors/cytokines. Our results thus indicate that hepatocyte proliferation can be induced by at least two different pathways; compensatory regeneration being TNF and IL-6-dependent, and mitogen-induced direct hyperplasia which does not require TNF or IL-6.**

**Keywords:** cell proliferation; TNF; IL-6; TCPOBOP; ciprofibrate; liver

## Introduction

The liver has the unique capacity to regenerate after removal of part of its cellular mass. During the last decade, much new information has become available on the events that may initiate liver regeneration. Several converging lines of evidence from recent works have proposed that Tumor necrosis factor-α (TNF-α) and Interleukin-6 (IL-6) are important components of the early signaling pathways leading to regeneration (Akerman *et al*., 1992; Yamada *et al*., 1997; Cressman *et al*., 1996). Using mice harboring a targeted disruption of the gene encoding TNFR-1 (Rothe *et al*., 1993) and IL-6 (Poli *et al*., 1994), it was shown that liver regeneration after 2/3 partial hepatectomy (PH) is severely impaired in both types of gene knock-out mice (Yamada *et al*., 1997; Cressman *et al*., 1996). These results were interpreted to suggest that TNF, signaling through the TNFR-1, can initiate liver regeneration and acts by activating an IL-6-dependent pathway that involves STAT3 transcription factor. Hepatocytes, however can be stimulated to proliferate by treatment with a variety of agents such as triiodothyronine, retinoic acid, peroxisome proliferators, lead nitrate and barbiturates that do not cause tissue loss/injury, but rather an excess of cells (Columbano and Shinozuka, 1996). Unlike compensatory regeneration observed after 2/3 PH or chemically-induced cell necrosis, direct hyperplasia induced by primary mitogens is neither accompanied by increased hepatic mRNA levels of growth factor genes such as TGF-α and HGF (Ohmura *et al*., 1996), nor by increased levels of circulating HGF (Kubo *et al*., 1996). These findings suggest that the growth factors critical for liver regeneration may not be involved in direct hyperplasia. Furthermore, mitogens such as ethylene dibromide and lead nitrate induce a rapid increase in TNF-α mRNA hepatic levels as well as an activation of the transcription factor NF-κB, one of the main targets of TNF-α. However, such changes were not observed following treatment with mitogens acting through receptors of the superfamily of hormone nuclear receptors (Menegazzi *et al*., 1997). All together, these findings suggest that TNF-α dependent and TNF-α independent, i.e. nuclear receptor-mediated, pathways leading to hepatocyte proliferation exist.

To directly test the role of TNF-α and IL-6-dependent signaling in the initiation of direct hyperplasia induced by primary mitogens of different nature, we analysed the growth response of hepatocytes in knock out mice lacking either TNFR-1 or IL-6. We report that in both cases mice respond as efficiently as their wild type counterparts to proliferative stimuli induced by two primary mitogens, TCPOBOP (an halogenated hydrocarbon) (Manenti *et al*., 1987) and ciprofibrate (CYP, a peroxisome proliferator) (Reddy *et al*., 1986). Proliferation by these agents occurred without activation of STAT3, a major target of IL-6 and other cytokines/growth factors. These results demonstrate that although TNF-α and IL-6 appear to play a critical role in triggering liver regeneration, they are not necessarily required for initiation of hepatocyte proliferation. These results support the hypothesis that

Correspondence: GM Ledda-Columbano
Received 12 January 1998; revised 31 March 1998; accepted 31 March 1998

cell proliferation caused by certain primary mitogens may be triggered by signaling pathways different from those responsible for the transition of hepatocytes from G0-to G1 after PH or cell necrosis.

## Results and discussion

### *TNFR-1 knock-out mice*

We previously reported that TCPOBOP-induced hepatocyte proliferation, unlike compensatory regeneration, is not associated with increased hepatic mRNA levels of c-*fos*, c-*jun*, LRF-1 and other immediate early genes (Columbano *et al*., 1997). Together with the observation that 24 h after TCPOBOP there is a higher number of hepatocytes entering S phase than that observed after PH, these findings suggest that the signaling pathways leading to S phase entry after TCPOBOP are different. To directly test the role of TNF-α in TCPOBOP-induced liver cell proliferation, mice lacking the TNFR-1 receptor, and their normal counterparts, were treated with a single dose of TCPOBOP. BrdU (0.5 mg/ml) in drinking water was given 2 h after treatment to determine the labeling index of hepatocytes. Animals were sacrificed after 3 days. Separate experiments showed that administration of BrdU in drinking water for 4 days resulted in a labeling index higher than 95% in CD-1 mice subjected to 2/3 PH, thus demonstrating a clear effectiveness of this protocol (Figure 1). Treatment with TCPOBOP caused no signs of liver cell damage, although an increased fat accumulation in hepatocytes from both wild and mutant mice was observed following mitogen administration. Labeling index of hepatocytes of TCPOBOP-treated mice (Figure 2), showed an almost similar response in both wild type as well as mutant type (15% and 20% of BrdU-positive hepatocytes, respectively) indicating that lack of TNFR-1 does not affect the proliferative response of hepatocytes to this mitogen. In addition, TCPOBOP did not induce any change in hepatic TNF-α mRNA levels (data not shown). These findings suggest that TCPOBOP triggers hepatocyte proliferation through a TNF-independent pathway.

### *IL-6 knock out mice*

IL-6 is a major regulator of the hepatic acute phase response *in vivo* and a STAT3 inducer (Trautwein *et al*., 1994; Ruff-Jamison *et al*., 1993). Its transcription is stimulated by TNF through NF-κB activation (Kishimoto *et al*., 1992). Since it was previously reported that IL-6 deficiency impairs liver regeneration for at least 4–7 days (Cressman *et al*., 1996), we have determined whether the absence of IL-6 could also inhibit the proliferative response of the liver to TCPOBOP. Results indicate that the extent of proliferation induced by TCPOBOP 4 days after treatment was similar in both mutant and wild type mice (50% and 43%, respectively *versus* 1% of oil-treated mice) (Figure 3 and 4a). The increased labeling index was associated to an increase in total hepatic DNA content (Figure 4b). Therefore, lack of IL-6 does not affect the proliferative response of hepatocytes to this liver mitogen.

### *STAT3 activation following 2/3 PH or TCPOBOP*

Activation of the transcription factor STAT3 has been suggested to play a critical role in regulating a cascade

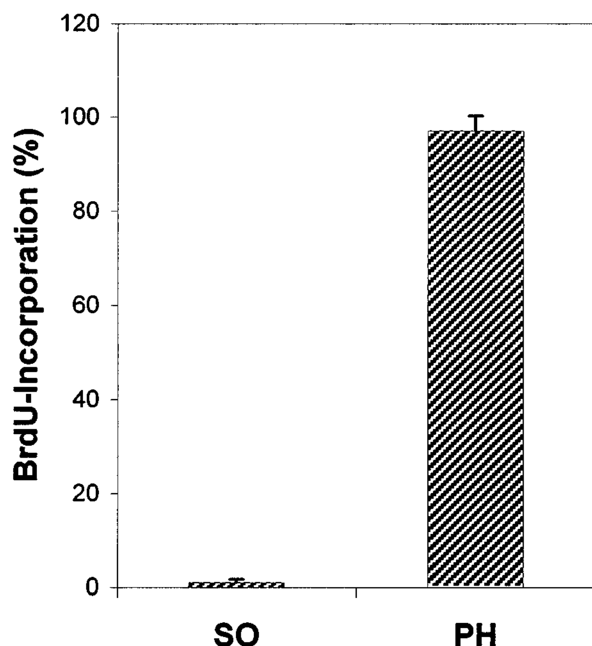


**Figure 1** Labeling index of CD-1 mice 4 days after PH. Labeling index expressed as number of BrdU-positive hepatocyte nuclei/100 nuclei. BrdU (0.5 mg/ml) was dissolved in drinking water and given *ad libitum* for 4 days. At least 5000 hepatocyte nuclei per liver were scored in mice subjected to partial hepatectomy (PH) or sham operated (SO)

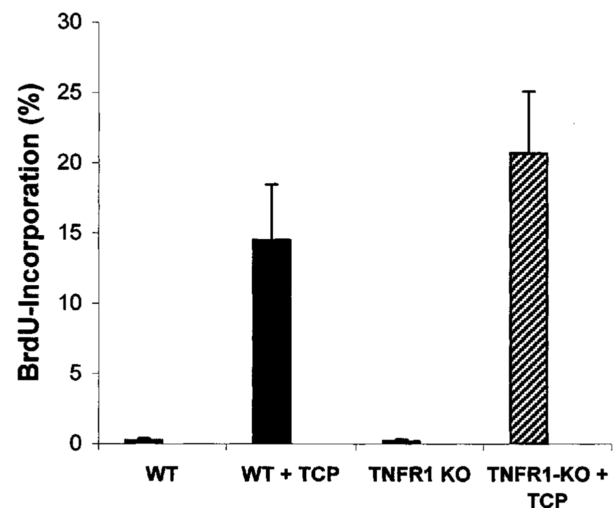


**Figure 2** Labeling index of TNFR-1 mouse hepatocytes following TCPOBOP. TNFR1° (KO) and wild type (WT) mice treated with a single dose of TCPOBOP (TCP, 3 mg/Kg, i.g.) or oil were sacrificed 3 days later. Two hours after treatment, all mice were given BrdU (0.5 mg/ml in drinking water) until sacrificed. At least 5000 hepatocyte nuclei per liver were scored. Labeling index was expressed as number of BrdU-positive hepatocyte nuclei/100 nuclei. Results are expressed as means±s.e. of 5–6 mice per group

of events leading to liver regeneration. In addition to IL-6, other molecules including Epidermal growth factor (EGF), may activate STAT3 (Ruff-Jamison *et al*., 1994). To determine whether STAT3 activation could still occur in TCPOBOP-induced hepatocyte proliferation via alternative mechanisms, gel shift assay was performed in both wild type and IL-6$^{-/-}$ mice. Livers from IL-6$^{+/+}$ mice subjected to 2/3 PH were also analysed as controls. As expected from previous studies, STAT3 activation is strongly induced 2 and 4 h after 2/3 PH (Figure 5a) (Cressman *et al*., 1995). On the contrary, no changes in STAT3 activation were observed after TCPOBOP treatment, neither in IL-6$^{-/-}$ or in IL-6$^{+/+}$ mice (Figure 5a). From these results it can be concluded that STAT3 activation is not required for TCPOBOP-induced direct hyperplasia of the mouse liver. Similarly another transcription factor, AP-1, was activated in mouse liver following PH, but not in the liver of TCPOBOP-treated mice (Figure 5b).

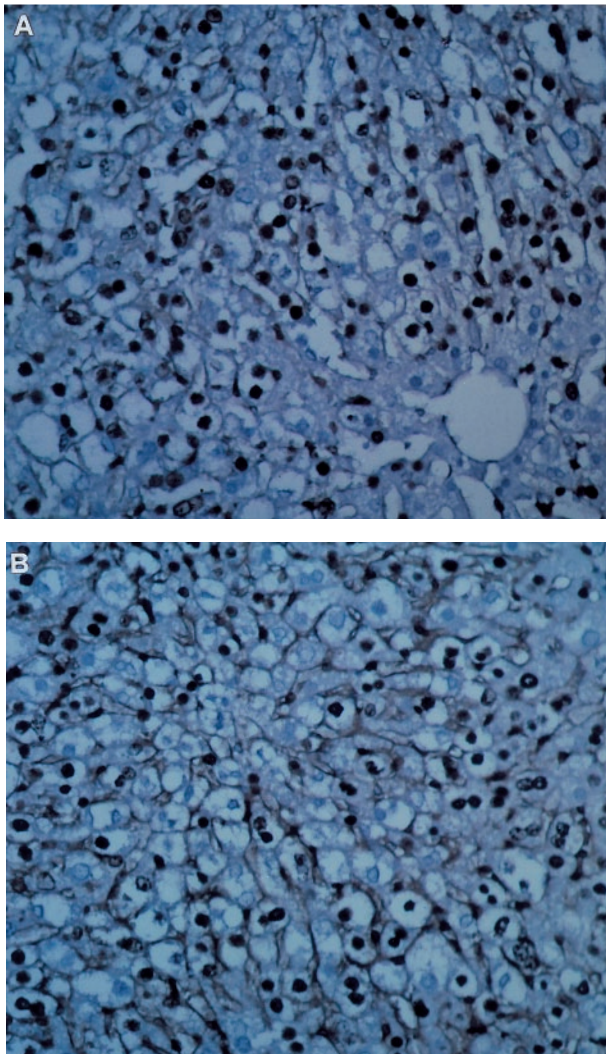


**Figure 3** Representative microphotography which illustrates: (**a**), the presence of several BrdU-positive hepatocytes 4 days after TCPOBOP treatment in IL-6$^{+/+}$, and (**b**), IL-6$^{-/-}$ mice. (X200, sections counterstained with hematoxylin)

### *Liver cell proliferation by ciprofibrate in IL-6*$^{-/-}$ mice

To determine whether the existence of TNF and IL-6 independent pathways in mouse liver cell proliferation is unique to TCPOBOP or a more general phenomenon, further experiments were conducted using the mitogen ciprofibrate, a peroxisome proliferator (Reddy *et al*., 1986). As shown in Figure 6, the labeling index of the IL-6$^{-/-}$ mice hepatocytes was slightly higher than that of IL-6$^{+/+}$ mice, though the differences were not statistically significant because of individual variability. The results indicate that liver cell proliferation induced by this member of PP, in addition to TCPOBOP, occurs in the absence of IL-6 involvement.

### *STAT3 activation in rat liver following 2/3 PH or Nafenopin*

Nafenopin, like ciprofibrate, is a peroxisome proliferator and a strong rat liver mitogen (Levine *et al*., 1977). Previously, we have shown that a single dose of this agent does not induce activation of NF-$\kappa$B or AP-1 (Menegazzi *et al*., 1997). To determine whether hepatocyte proliferation induced by mitogens could occur in the absence of STAT3 activation also in rat, gel shift analysis was performed in animals given a single dose of nafenopin or subjected to 2/3 PH. While

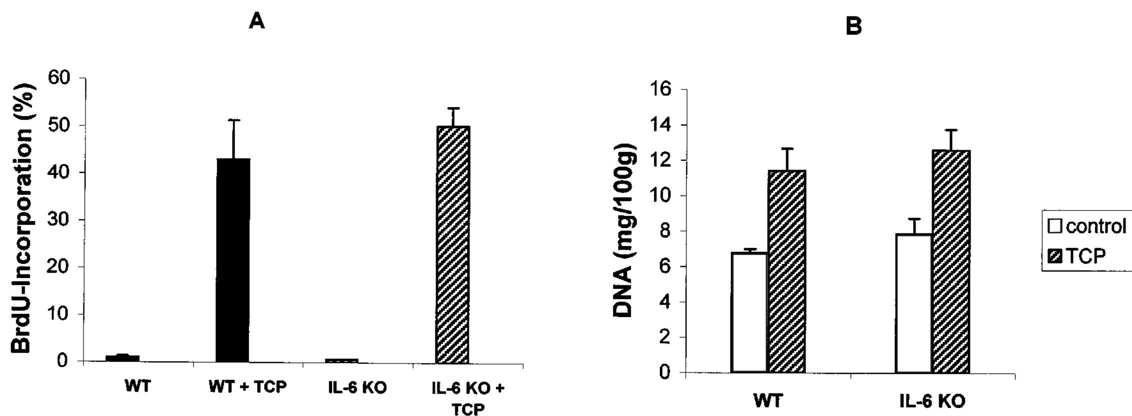


**Figure 4** Labeling index of IL6$^{-/-}$ and IL-6$^{+/+}$ mouse hepatocytes and DNA content following TCPOBOP treatment. (**a**) IL-6$^{-/-}$ (KO) and wild type (WT) mice were treated with a single dose of TCPOBOP (TCP, 3 mg/Kg, i.g.), or oil, and sacrificed 4 days later. Two hours after treatment, all mice were given BrdU (0.5 mg/ml in drinking water) until sacrifice. At least 5000 hepatocyte nuclei per liver were scored. Labeling index was expressed as number of BrdU-positive hepatocyte nuclei/100 nuclei. Results are expressed as means$\pm$s.e. of 5–6 mice per group; (**b**) Total hepatic DNA content in TCPOBOP and oil treated mice

a rapid activation of STAT3 occurs after PH, no STAT3 activation could be observed at different times after Nafenopin treatment (Figure 7a). In agreement with our previous results (Menegazzi *et al*., 1997), Nafenopin-induced liver hyperplasia proceeds in the absence of activation of the transcription factor AP-1 (Figure 7b).

## Conclusions

We have shown that two different primary mitogens, TCPOBOP and ciprofibrate induce liver cell proliferation in mice independent of TNFR-1 or IL-6. We have also shown that while the transcription factor STAT3, a main target of IL-6 and other cytokines and growth factors, is strongly activated after 2/3 PH in mouse and rat liver, there is no such an activation in mouse liver following a single administration of TCPOBOP nor in rat liver after administration of the peroxisome proliferator nafenopin. Together with previous findings indicating that certain rat or mouse hepatomitogens neither induce activation of NF-$\kappa$B, nor stimulate hepatic transcription of immediate early genes (c-*fos*, c-*jun*, LRF-1, egr-1 and c-*myc*) (Coni *et al*., 1993; Ohmura *et al*., 1996; Columbano *et al*., 1997) our HGF or TGF-$\alpha$ (Ohmura *et al*., 1996), our results suggest that liver cell proliferation in the absence of cell loss/death may occur through different signal transduction pathways.

It was previously suggested (Columbano and Shinozuka, 1996) that PPs such as ciprofibrate, exert their mitogenic effect through activation of PPAR$\alpha$, a member of steroid hormone nuclear receptor superfamily (Bardot *et al*., 1993). The critical role of PPAR$\alpha$ in hepatocyte proliferation induced by PP is supported by recent work on mice knock-out for this receptor (Peters *et al*., 1997). On the other hand, no satisfactory explanation has yet been proposed for the mechanisms underlying the mitogenic effect of TCPOBOP. TCPOBOP is a Phenobarbital-like agent in respect to its ability to induce proliferation of smooth endoplasmic reticulum and P450 isozymes (Beebe *et al*., 1996; Honkakoski *et al*., 1992). Phenobarbital is known to induce an Ah-receptor-like protein (Benedetti *et al*., 1994) that also binds TCDD. In several respects, the TCDD receptor is analogous to the peroxisome-proliferator activated receptor: both receptors have no known endogenous ligands clearly identified, activate transcription of cytochrome P450 isoenzymes and bind DNA response elements that can potentially interfere with estrogen receptor transcriptional activity (Poellinger *et al*., 1992; White and Gasiewicz, 1993; Keller *et al*., 1995). Whether the mitogenic activity of TCPOBOP is mediated through a receptor analogous to members of the nuclear receptor superfamily is not known. However, in view of the association between the

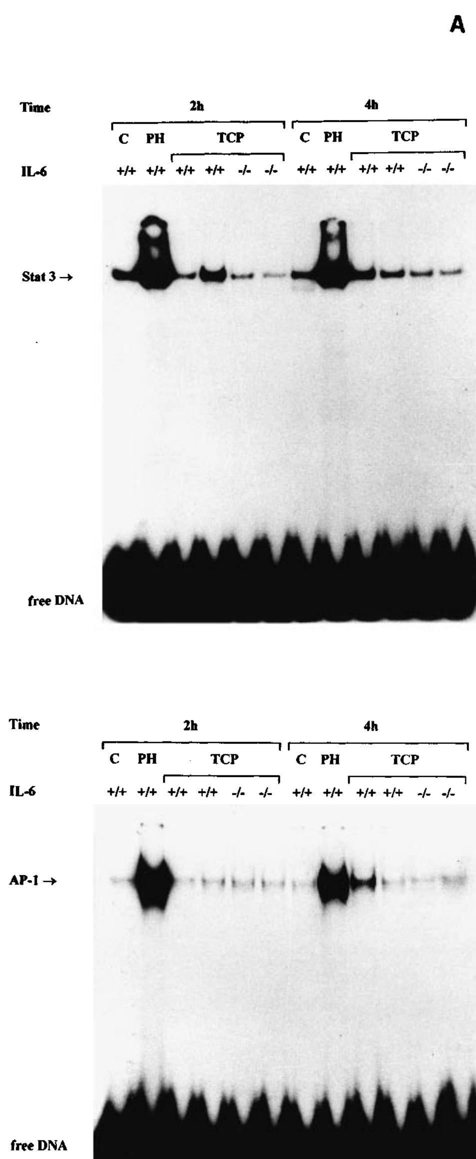


**Figure 5** DNA binding activity of STAT3 (**a**) and AP-1 (**b**) transcription factors in the liver of PH and TCPOBOP-treated mice. Nuclear extract from liver of mice killed at 2 and 4 h after PH or TCPOBOP administration were incubated with $^{32}$P-labeled double stranded oligonucleotide containing the consensus sequence for STAT3 or AP-1 binding site

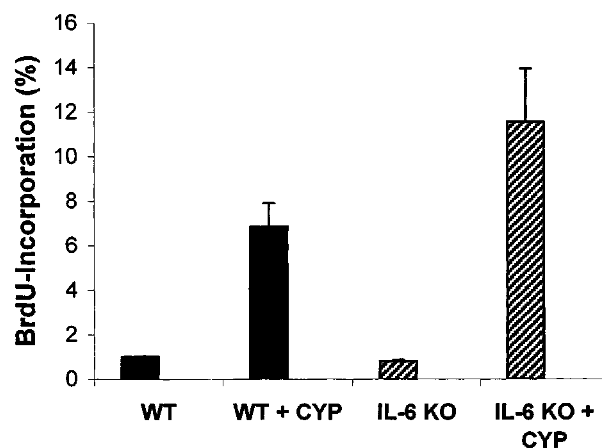


**Figure 6** Labeling index of IL6$^{-/-}$ and IL-6$^{+/+}$ mouse hepatocytes following ciprofibrate. IL-6$^{-/-}$ (KO) and wild type (WT) mice were fed a ciprofibrate-supplemented diet (CYP, 0.025%) or basal diet and killed 4 days afterwards. Two h after treatment, all mice were given BrdU (0.5 mg/ml in drinking water) for 4 days. At least 5000 hepatocyte nuclei per liver were scored. Labeling index was expressed as number of BrdU-positive hepatocyte nuclei/100 nuclei. Results are expressed as means$\pm$s.e. of 8–10 mice per group

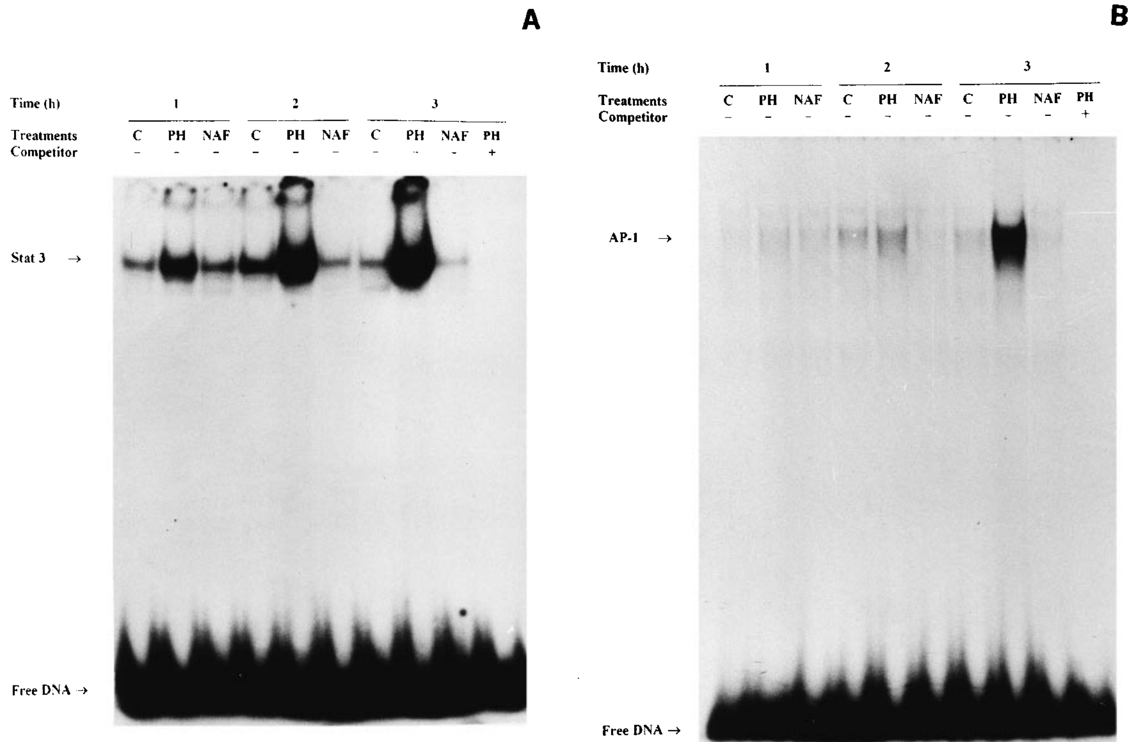


**Figure 7** DNA binding activity of STAT3 (**a**) and AP-1 (**b**) transcription factors in the liver of PH or NAF-treated rats. Nuclear extracts from liver of rats killed at 1, 2 and 3 h after 2/3 PH or NAF administration (200 mg/kg, in oil) were processed as described in Figure 5. The specificity of the bands was demonstrated by competing with 200-fold excess of SIE/m67 or AP-1 unlabeled oligonucleotides

mitogenic and carcinogenic effect of TCPOBOP in mouse liver, in depth studies might help to clarify the molecular basis of its action and, in particular, the possible existence of specific receptor(s) responsible for both mitogenic and tumorigenic effect of this chemical.

## Materials and methods

### *Animals*

TNFR-1 knock-out male mice ($p55^{-/-}$) of the B6 × 129 strain (Rothe *et al*., 1993) and IL-$6^{-/-}$ female mice generated in 129 and backcrossed to C57/BL6 (Poli *et al*., 1994) were used in these experiments. The animals were fed a laboratory chow diet provided by Ditta Piccioni (Brescia, Italy) and had free access to food and water. All animals were acclimated 1 week before treatment, and the National Research Council criteria for the care and use of laboratory animals in research were followed. All experiments were performed in a temperature-controlled room with alternating 12 h dark/light cycles. TCPOBOP (3 mg/Kg) was administered intragastrically in oil. Ciprofibrate (CYP) was fed in the diet at a concentration of 0.025%. Ciprofibrate was a kind gift from Sanofi Winthrop (Guilford, UK). Nafenopin (NAF, 200 mg/Kg, in oil), was administered intragastrically to male Wistar rats (Charles River, Milano, Italy) and were sacrificed at 1, 2 and 3 h thereafter. Two/third PH was performed according to Higgins and Anderson (1931). Most treatments were performed between 9 and 12 am. For determination of hepatocyte proliferation, bromodeoxyuridine (BrdU) was given in drinking water at a final concentration of 0.5 mg/ml all throughout the experiment. Immediately after sacrifice liver sections were fixed in 10% buffered formalin and processed for H&E staining or for immunohistochemistry. Remaining liver was frozen in liquid nitrogen and stored at −80°C for further studies.

### *Electrophoretic mobility shift assay (EMSA)*

Nuclear extracts were prepared from 200 mg of liver tissue according to Schreiber *et al*. (1989) in the presence of 10 μg/ml leupeptin, 5 μg/ml antipain and pepstatin, and 1 mM PMSF (Sigma Chemical Co.). Protein concentration in the nuclear extracts was determined using the method of Bradford (1976). Eight micrograms of nuclear extracts were incubated at room temperature for 30 min with (2 – $5 \times 10^4$) of a $^{32}$P-labeled double stranded oligonucleotide containing the sis-inducible factor binding recognition element (SIE/m67) from the c-*fos* promoter (5′-gtcga-CATTTCCCGTAAATCg-3′) (Wagner *et al*., 1990) or the AP-1 DNA binding site (5′-CTAGTGATGAGTCAGCCG-GATC-3′), in a 15 μl reaction mixture containing 20 mM HEPES, pH 7.9, 50 mM KCl, 10% glycerol, 0.5 mM DTT, 0.1 mM EDTA, 2 μg poly(dI-dC), 1 μg salmon sperm DNA. Products were fractionated on a non-denaturing 5% polyacrylamide gel. In competition assays, 200-fold excess of competitor oligonucleotide was added 15 min before addition of the labeled probe. The intensity of the retarded bands was measured on a Phosphor Imager (Molecular Dynamics, Sunnyvale, CA, USA).

### *DNA hepatic content*

After sacrifice the livers were frozen at −80°C. Total hepatic DNA was quantitatively assayed from frozen tissue by Burton's diphenylamine method. (Burton, 1956).

*BrdU-Incorporation*

Formalin-fixed sections were embedded in paraffin and deparaffined sections processed for immunohistochemistry. BrdU incorporation into nuclei was determined immunohistochemically by the avidin-biotin-peroxidase complex (ABC) method using a mouse anti-BrdU monoclonal antibody (Becton Dickinson Immunocytometry Systems, San Josè, CA) and Vectastain ABC kit (PK-4002, Vector Laboratories Inc., Burlingame, CA). In brief, tissue sections fixed in 10% formalin were embedded in paraffin, deparaffinized, exposed to 0.3% hydrogen peroxide in ethanol for 10 min to block endogenous peroxidase, treated with 2N HCl, incubated with trypsin 0.1% for 20 min and then with normal horse serum for 20 min at room temperature. Sections were then incubated for 2 h with an anti-BrdU monoclonal antibody, followed by biotinylated horse anti-mouse IgG and avidin-biotin peroxidase complex. Sites of peroxidase binding were detected with diaminobenzidine (Graham and Karnovski, 1966), and the sections were counterstained with hematoxylin. A segment of duodenum, an organ with a high rate of cell proliferation, was included from each mouse to confirm delivery of the DNA precursor. At least 5000 hepatocyte nuclei per mouse were scored.

**Acknowledgements**
Supported by Associazione Italiana Ricerca sul Cancro (AIRC) and MURST (40% and 60%), Italy.

## References

Akerman P, Cote P, Yang S-Q, McClain C, Nelson S, Bagby GJ and Diehl AM. (1992). *Am. J. Physiol.,* **263,** G579 – G585.
Bardot O, Aldridge TC, Latruffe ND and Green S. (1993). *Biochem. Biophys. Res. Commun.,* **192,** 37 – 45.
Beebe LE, Roberts ES, Fornwald LW, Hollenberg PF and Alworth WL. (1996). *Biochem. Pharmacol.,* **52,** 1507 – 1513.
Benedetti I, MacCormack LS and Bunce NJ. (1994). *Arch. Biochem. Biophys.,* **309,** 1 – 9.
Bradford M. (1976). *Anal. Biochem.,* **72,** 248 – 251.
Burton K. (1956). *Biochem. J.,* **62,** 315 – 323.
Columbano A and Shinozuka H. (1996). *FASEB J.,* **10,** 1118 – 1128.
Columbano A, Ledda-Columbano GM, Pibiri M, Piga R, Shinozuka H, De Luca V, Cerignoli F and Tripodi M. (1997). *Oncogene,* **14,** 857 – 863.
Coni P, Simbula G, Carcereri De Prati A, Menegazzi M, Suzuki H, Sarma DSR, Ledda-Columbano GM and Columbano A. (1993). *Hepatology,* **17,** 1109 – 1116.
Cressman DE, Diamond RH and Taub R. (1995). *Hepatology,* **21,** 1443 – 1449.
Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V and Taub R. (1996). *Science,* **274,** 1379 – 1383.
Graham RC and Karnovsky M. (1966). *J. Histochem. Cytochem.,* **14,** 291 – 302.
Higgins GM and Anderson RM. (1931). *Arch. Pathol.,* **12,** 186 – 202.
Honkakoski P, Auriola S and Lang MA. (1992). *Biochem. Pharmacol.,* **43,** 2121 – 2128.
Keller H, Givel F, Perroud M and Wahli W. (1995). *Mol. Endocrinol.,* **9,** 794 – 804.
Kishimoto T, Akira S and Taga T. (1992). *Science,* **258,** 593 – 597.
Kubo Y, Yasunaga M, Masuhara M, Terai S, Nakamura T and Okita T. (1996). *Hepatology,* **23,** 104 – 114.
Levine WG, Ord MG and Stocken LA. (1977). *Biochem. Pharmacol.,* **26,** 939 – 942.
Manenti G, Dragani T and Della Porta G. (1987). *Chem-Biol. Interact.,* **64,** 83 – 92.
Menegazzi M, Carcereri De Prati A, Suzuki H, Shinozuka H, Pibiri M, Piga R, Columbano A and Ledda-Columbano GM. (1997). *Hepatology,* **25,** 585 – 592.
Ohmura T, Ledda-Columbano GM, Piga R, Columbano A, Glemba J, Katyal SL, Locker J and Shinozuka H. (1996). *Am. J. Pathol.,* **148,** 815 – 824.
Peters JM, Catley RC and Gonzalez FJ. (1997). *Carcinogenesis,* **18,** 2029 – 2033.
Poellinger LM, Gottlicher M and Gustafson JA. (1992). *Trends. Pharmacol. Sci.,* **13,** 241 – 245.
Poli V, Balena R, Fattori E, Markatos A, Yamamoto M, Tanaka H and Ciliberto G. (1994). *EMBO J,* **13,** 1189 – 1196.
Reddy JK, Goel SK, Nemali MR, Carrino JJ, Lafler TG, Reddy MK, Sperback SJ, Osumi T, Hashimoto T, Lalwani ND and Rao MS. (1986). *Proc. Natl. Acad. Sci. USA,* **83,** 1747 – 1751.
Rothe J, Lesslauer W, Lotscher H, Lang Y, Koebel P, Kontgen F, Althage A, Zinkernagel R, Steinmetz M and Bluethmann H. (1993). *Nature,* **364,** 798 – 802.
Ruff-Jamison S, Chen K and Cohen S. (1993). *Science,* **261,** 1733 – 1736.
Ruff-Jamison S, Zhong Z, Wen Z, Chen K, Darnell JE and Cohen S. (1994). *J. Biol. Chem.,* **269,** 21933 – 21935.
Schreiber E, Matthias P, Muller MM and Schaffner W. (1989). *Nucleic Acid Res.,* **17,** 6419A.
Trautwein C, Boker K and Manns MP. (1994). *Gut,* **35,** 1163 – 1166.
Wagner BJ, Hayes TE, Hoban CJ and Cochran BH. (1990). *EMBO J.,* **9,** 4477 – 4484.
White TEK and Gasiewicz TA. (1993). *Biochem. Biophys. Res. Commun.,* **183,** 956 – 962.
Yamada Y, Kirillova I, Peschon JJ and Fausto N. (1997). *Proc. Natl. Acad. Sci. USA,* **94,** 1441 – 1446.