Transcriptional regulation of the gene encoding cholesterol 7α-hydroxylase in the rat

ARTICLE *in* GENE · SEPTEMBER 1993

Impact Factor: 2.14 · DOI: 10.1016/0378-1119(93)90422-Y · Source: PubMed

Gene, 130 (1993) 217–223

GENE 07197

Transcriptional regulation of the gene encoding cholesterol 7α-hydroxylase in the rat

(Bile acid; taurocholate; promoter region; *cis*-acting element; transfection; posttranscriptional regulation)

Marco F.M. Hoekman[a], Jeanet M.J. Rientjes[a], Jaap Twisk[b], Rudi J. Planta[a], Hans M.G. Princen[b] and Willem H. Mager[a]

[a] *Department of Biochemistry and Molecular Biology, Vrije Universiteit, 1081 HV Amsterdam, Netherlands; and* [b] *Gaubius Laboratory IVVO-TNO, P.O. Box 430, 2300 AK Leiden, Netherlands. Tel. (31-71) 181470*

Received by H. van Ormondt: 11 December 1992; Revised/Accepted: 1 January/5 February 1993; Received at publishers: 14 April 1993

SUMMARY

The cytochrome P450 enzyme, cholesterol 7α-hydroxylase (CYP7A), catalyses the first and rate-limiting step in the conversion of cholesterol to bile acids. Expression of the *CYP7A* gene is under complex physiological control, encompassing amongst others a feedback down-regulation by bile acids. Using the *CYP7A* cDNA of the rat as a probe, we isolated a rat genomic clone containing the 5′ part of the gene, including approximately 3.6 kb of upstream sequences. Sequence analysis revealed the presence of several putative regulatory elements. Transient expression analyses of transfected primary hepatocytes demonstrated that the major transcription-activating region is located in the proximal 145 nucleotide (nt). Upon addition of taurocholate to the culture, a significant reduction of the transcriptional activity was observed, suggesting the presence of a bile acid-responsive element in the proximal region of the *CYP7A* promoter. In addition, evidence was obtained for the presence of a thyroxine-responsive site further upstream. After addition of taurocholate, steady-state *CYP7A* mRNA levels, as judged by Northern analysis of hepatocyte RNA, are eightfold reduced. On the other hand, the transcriptional activity of *CYP7A*, as shown both in CAT assays and run-on experiments, revealed only a threefold decrease. These experiments suggest that both transcriptional control and regulation of *CYP7A* mRNA stability play an important part in the feedback regulation of *CYP7A* activity in the rat.

INTRODUCTION

Cholesterol 7α-hydroxylase catalyses the first and rate-limiting step in the conversion of cholesterol to bile acids in vertebrate liver (Shefer et al., 1970). In fact, 7α-hydroxylation of cholesterol represents the major activity to remove cholesterol from the body and to maintain cholesterol homeostasis (Myant and Mitropoulos, 1977; Danielsson and Sjövall, 1975). The enzyme in question belongs to the extended family of cytochrome P450 isozymes and the corresponding gene is referred to as *CYP7A* (Gonzalez, 1989). The cholesterol 7α-hydroxylase activity depends on a variety of physiological signals. Glucocorticoids, thyroxine and insuline affect the enzyme activity (Princen et al., 1989; Ness et al., 1990; Subbiah and Yanker, 1984; Vlahcevic et al., 1991) and hormonal control most likely underlies the observed diurnal variations (Chiang et al., 1990; Noshiro et al., 1990). A second important regulatory effect is exerted by the end products of the pathway, viz. bile acids. Bile acids suppress the

Correspondence to: Dr. W.H. Mager, Department of Biochemistry and Molecular Biology, Vrije Universiteit, 1081 HV Amsterdam, Netherlands. Tel. (31-20) 5485652 or 5485606; Fax (31-20) 6461479; e-mail: mager@chem.vu.nl

Abbreviations: ACT, actin; bp, base pair(s); *BTE*, basic transcription element; CAT, chloramphenicol acetyltransferase; *cat*, gene encoding CAT; cDNA, DNA complementary to RNA; core*GRE*, core sequence of the glucocorticoid-responsive element; CYP7A, cholesterol 7α-hydroxylase; *CYP7A*, gene encoding CYP7A; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; *HGRE*, human glucocorticoid-responsive element; IV, international unit(s); kb, kilobase(s) or 1000 bp; nt, nucleotide(s); *LFB1*, liver factor B1; oligo, oligodeoxyribonucleotide; PCR, polymerase chain reaction; TCh, taurocholic acid.

CYP7A activity via the enterohepatic circulation (Björkhem, 1985; Carey and Cahalane, 1988). Accordingly, administration of the bile-acid-binding resin cholestyramine or biliary drainage results in stimulation of enzyme activity (Pandak et al., 1992).

Thanks to the recent availability of gene-specific DNA probes, studies could be started in order to elucidate at which level of *CYP7A* expression these regulatory events take place. Results obtained by *in vivo* analyses indicated a correlation between enzyme activity and mRNA levels, suggesting that regulation primarily occurs at the transcript level (Sundseth and Waxman, 1990). Comparison of the results of Northern hybridizations (steady-state mRNA amounts) and those obtained by run-on assays (*de novo* mRNA synthesis) led to the hypothesis that transcription of *CYP7A* may indeed be the major target for regulation (Li et al., 1990; Pandak et al., 1992). Recently, we were able to demonstrate that primary (rat and pig) hepatocytes in culture exhibit similar regulatory responses as observed *in vivo* (Twisk et al., 1993; Kwekkeboom et al., 1990). These findings prompted us to isolate and characterize the promoter of rat *CYP7A* and examine its transcription by transfection of primary hepatocytes.

RESULTS AND DISCUSSION

(a) Cloning of a rat liver *CYP7A* cDNA probe

Based on the published cDNA sequence coding for rat CYP7A (Noshiro et al., 1989), primers were selected to prepare a *CYP7A*-specific probe using the PCR technique. As a source of template we used total liver RNA isolated from cholestyramine-fed rats, which was previously shown to contain a high content of *CYP7A* mRNA (Chiang et al., 1990; Jelinek et al., 1990a). Two oligo primers, viz. 5′-AGCCGCCAAGTGACATCATC-CAGTGTTCGCTTCTTCC and 5′-ATGATGACTAT-TTCTTTGATTTGGGGAATTGCCGTG, which correspond to the termini of the coding region of the *CYP7A* mRNA and its cDNA, respectively, were synthesized. Polymerase chain reaction was performed according to the manufacturer's protocol (Superscript, Bethesda Research Laboratories). The major reaction product of 1.6 kb was isolated and cloned in pUC18. The identity of the *CYP7A* cDNA probe was confirmed by nt sequence analysis (result not shown) according to the chain termination technique (Sanger et al., 1977).

(b) Cloning and sequencing of the 5′-flanking region of rat *CYP7A*

With this cDNA fragment as a probe, from a λ-library of total rat genomic DNA (λEMBL3, kindly provided by Dr. W. Lamers; Academic Medical Centre, Amsterdam) several positive clones were isolated. A second screening was performed with the 5′-located PCR-primer to select clones that might contain the 5′ part of *CYP7A*. One of the resulting positive clones, designated R7α21, was further characterized by subcloning in pUC18 and restriction-site mapping. In Fig. 1 the physical map of the insert of this subclone pSSRλ3 is shown. This insert contains about 3.6 kb of 5′-flanking region, as well as the first intron and a part of the second intron. Sequence-specific oligonucleotides were used as primers in the sequence analysis of the flanking region. The primary structure of the 3.6-kb 5′ flanking region of rat *CYP7A* is presented in Fig. 2. The proximal 590 bp are identical to the sequence published previously (Jelinek et al., 1990b), except that we did not find an extra thymidine residue between nt positions −584 and −585.

A computer-aided search revealed the presence of several putative *cis*-acting elements in the upstream region of the gene (indicated in Fig. 2). It has been suggested that transcription of *CYP7A* is under positive control by cholesterol (Li et al., 1990; Jelinek et al., 1990a). Therefore, these authors assumed that the extended promoter of the gene may contain sterol-responsive sites, as has been found for other genes (Goldstein and Brown, 1990).

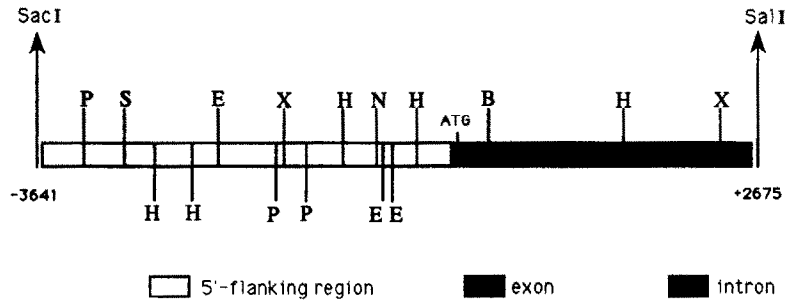

Fig. 1. Physical map of the 6.3-kb *SacI-SalI* insert of clone pSSRλ3. The organization of the gene is shown, drawn in scale. *Eco*RI (E), *Hin*dIII (H), *Pst*I (P), *Sma*I (S), *Xba*I (X), *Nco*I (N), *Bgl*II (B).

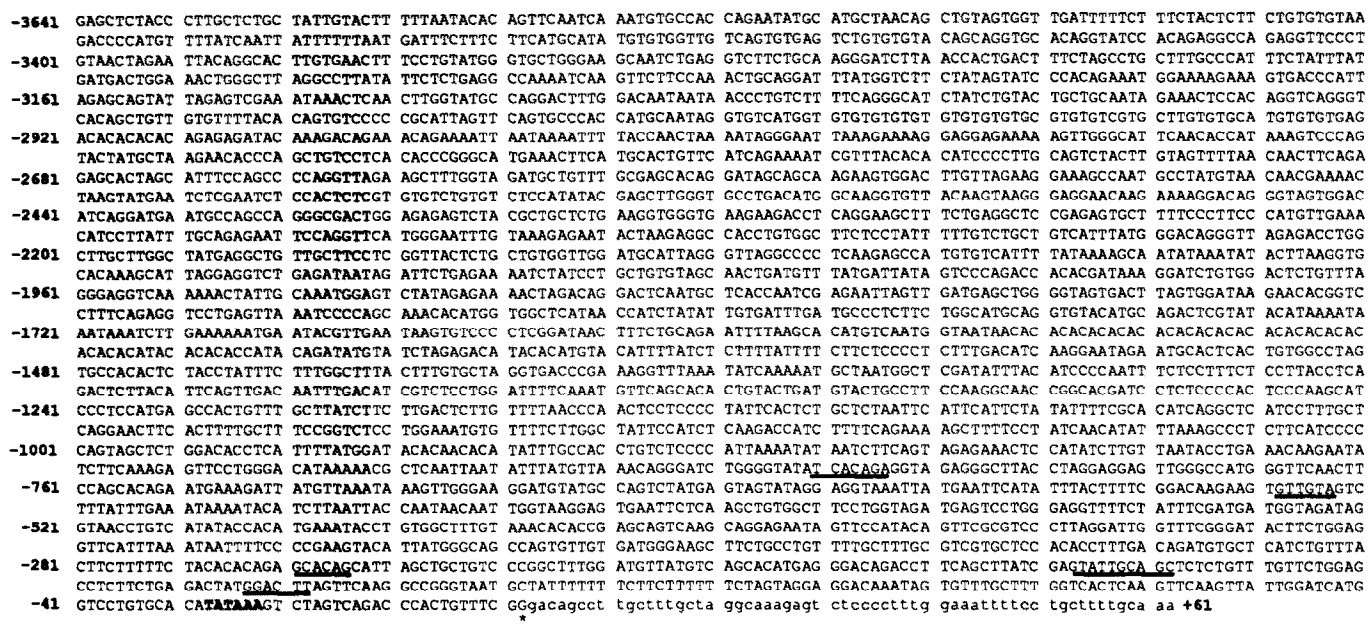

Fig. 2. Nucleotide sequence of the 5′-flanking region of rat *CYP7A*. The transcription start point (*tsp*), as determined previously (Jelinek et al., 1992), is indicated by the asterisk (+1). The TATA box is printed in boldface. The beginning of the leader region is shown by lower-case letters. Putative regulatory elements, which were identified on the basis of known *cis*-acting elements, are overlined: *LFB1* (5′-GTTATT), liver factor B1 (Frain et al., 1989); *BTE* (5′-AGTAGGAGG), basic transcription element (Yanagida et al., 1990); *HGRE5* (5′-TGTTCT), human glucocorticoid responsive element 5 (von der Ahe et al., 1985); *HGRE7* (5′-AGTCCT), human glucocorticoid responsive element 7 (Cato et al., 1984); core*GRE* (5′-AGGATGT), core sequence of the glucocorticoid responsive element (Langer and Ostrowski, 1988). The nt sequence data for the *CYP7A* promoter sequence have been assigned the GenBank accession No. Z18860.

However, analysis of the rat sequence, contrary to that of the recently published human gene promoter (Molowa et al., 1992; Cohen et al., 1992), did not reveal such a motif. In addition, *HNF3* (hepatic nuclear factor 3) sites, which were proposed to determine the liver-specific transcription of human *CYP7A*, are not present in the flanking region of rat *CYP7A* (see Fig. 2). On the other hand, elements homologous with the so-called human glucocorticoid-responsive elements (*HGRE*s), as well as sites designated as *BTE* and cognate to *LFB1*, are present in the *CYP7A* upstream region (see Fig. 2).

(c) Analysis of the *CYP7A* promoter activity

In order to identify the promoter elements that play a functional part in the regulation of transcription of *CYP7A*, different portions of the upstream DNA fragment were fused to the bacterial *cat* reporter gene (see Fig. 3). The resulting hybrid genes contain the transcription initiation site of *CYP7A* as determined previously (Jelinek et al., 1992). To construct −348R*cat*, we synthesized an oligo corresponding to nt −325 to −348 of the *CYP7A* upstream sequence containing a 5′ *Sal*I site and an oligo corresponding to the inverse complement of nt +23 to +2 containing a 5′ *Sph*I site. These oligos served as primers in a PCR experiment utilizing the 6.3-kb genomic fragment as a template. The resulting 371-bp product was cloned between the *Sph*I and *Sal*I sites of the Super*cat* vector, of which the *Hin*dIII site had been destroyed. Super*cat* is a derivative of pSV2*cat* (Gorman et al., 1985), containing the bacterial *cat* gene and the simian virus 40 (SV40) splice and polyadenylation signals on a *Hin*dIII-*Bam*HI fragment (nt 3370 to 5003 in pSV2*cat*) cloned between the *Hin*dIII and *Nde*I sites of pUC12. By the use of the *Sph*I site an 'upstream ATG' was introduced in the leader region of −348R*cat*, which has been demonstrated to lower the translation efficiency of the respective mRNAs (Alam et al., 1991). To avoid this effect the *Sph*I site was destroyed. Further 5′ deletion of the *CYP7A* promoter (leading to constructs −145R*cat*, −79R*cat*, and −49R*cat*, respectively) was performed by digestion with BAL31 starting from nt −348. The deletion endpoints were determined by nt sequencing. For the construction of plasmids −1571R*cat*, −2769R*cat* and −3641R*cat* an oligo was synthesized corresponding to nt −786 to −807. This oligo, together with the oligo corresponding to the inverse complement of nt +23 to +2 (containing the 5′ *Sph*I site) was used as a primer in a PCR-experiment in which clone pSSRλ3 served as a template. The product, a 830-bp DNA fragment, was cut with *Hin*dIII + *Nco*I and, in a triple ligation, fused to −348R*cat* (cut with *Hin*dIII + *Sac*I) and the *Nco*I-*Sac*I fragment of pSSRλ3, giving rise to −3641R*cat*. Subsequently, constructs −2769R*cat* and −1571R*cat* were made from −3641R*cat* by digestion

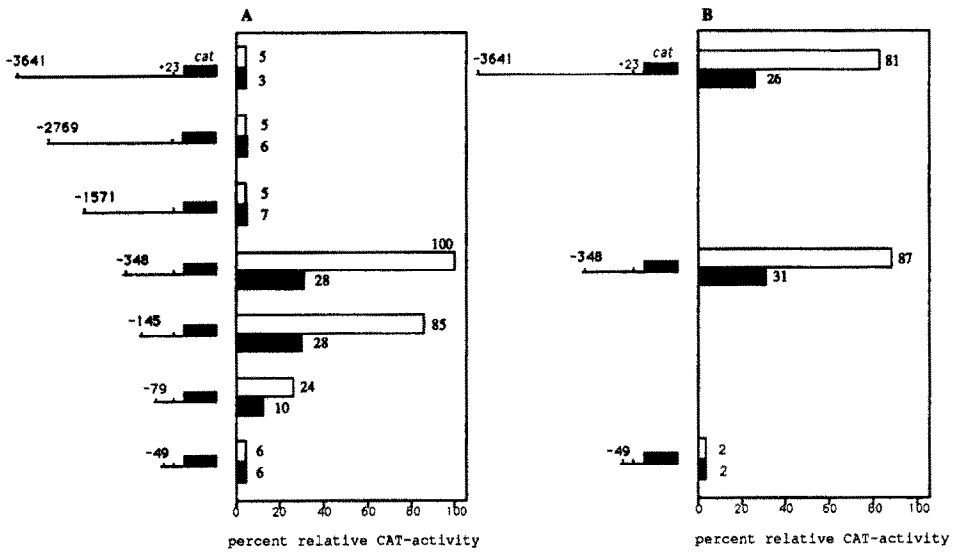

Fig. 3. Transient activity of the *CYP7A* promoter in primary rat hepatocytes. CAT activity was determined in primary cultures of rat liver cells transfected with the Ca·phosphate method. The used constructs contain various fragments of the 5'-flanking region of rat *CYP7A* fused to the *cat* reporter gene (black bar) at +23 of the *CYP7A* sequence. The lengths of *CYP7A* fragments (in nt) are indicated relative to the cap-site (+1). The *CYP7A* promoter activity of each reporter construct is expressed relative to that of the −348R*cat* construct (value set at 100%). Open bars represent CAT activity measured in hepatocytes without the addition of bile acids as opposed to hatched bars indicating CAT activity in hepatocytes cultured in the presence of 50 μM taurocholate. (**A**) Transfected cells were cultured in Williams E medium supplemented with 10% heat-inactivated FBS/2 mM L-glutamine/140 nM insuline/50 nM dexamethasone/100 IU penicillin and 100 μg streptomycin (per ml), in the presence or absence of 50 μM taurocholate. (**B**) Transfected cells were cultured in Williams E medium containing 1 μM thyroxine/100 nM dexamethasone/1.4 μM insuline/2 mM L-glutamine and 100 IU penicillin and 100 μg streptomycin (all per ml), in the presence or absence of 50 μM taurocholate. Data represent means of three independent experiments. **Methods: (a) Isolation and culture of rat hepatocytes:** Male Wistar rats weighing 250–300 g were maintained on standard chow and water ad libitum. Two days before isolation of hepatocytes, rats were fed a diet supplemented with 2% cholestyramine (Questran, Bristol Myers B.V., Weesp, The Netherlands). Rat liver cells were isolated by perfusion with 0.05% collagenase/0.005% trypsin inhibitor, as described previously (Princen et al., 1986). Viability, as determined by trypan blue exclusion, was higher than 90%. Cells were seeded on 60-mm diameter plastic tissue-culture dishes or 6-well cluster plates (Costar, Cambridge, MA, USA) at a density of 1.0×10^5 cells/cm^2 in Williams E medium supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine/140 nM insuline/50 nM dexamethasone/100 IU penicillin/100 μg streptomycin (per ml), (unless stated otherwise), and maintained at 37°C in a 5% CO_2/95% air atmosphere. After a 4-h attachment period, medium was refreshed with culture medium supplemented with hormones as described above. Cells were left to recover for another 18 h before use for further experiments. **(b) Transfection experiments and CAT assays:** At 22 h after their isolation, cells were subjected to transfection. Recombinant plasmids to be used for transfections were purified by centrifugation to equilibrium in CsCl-ethidium-bromide density gradients (Maniatis et al., 1982). In transient-expression assays, 3 μg of test plasmid and 1 μg of standard *lacZ* plasmid (Hall et al., 1983) were used for cotransfection as a Ca·phosphate precipitate (Pasco and Fagan, 1989). After 4 h the precipitate was removed, cells were treated with Williams E medium containing 15% glycerol for 1 min, and supplied with fresh culture medium with and without taurocholate. At 42 h after transfection, cells were harvested and cell extracts were prepared. Preparation of cell extracts and CAT assays were performed essentially as described by Gorman et al. (1985). Protein concentrations were determined with BCA protein-assay reagent (Pierce). The amounts of acetylated product as represented by signals on the autoradiograms were quantified with a Phosphorimager 400B (Molecular Dynamics). Data were corrected for protein concentration and transfection efficiency.

with *Sac*I + *Xba*I or *Sac*I + *Sma*I, respectively, isolation of the correct fragment, blunt-end formation with T4 DNA polymerase and religation with T4 DNA ligase. SV40*cat*, used as a control in transfection studies, contains the SV40 early promoter on a *Pvu*II-*Hin*dIII fragment of pSV2*cat* cloned into Super*cat* digested with *Sma*I + *Hin*dIII. The various deletion mutants were then tested as to their promoter activity in primary rat hepatocytes, cultured under conditions in which CYP7A and bile-acid-synthetic capacity were maintained (Princen and Meijer, 1990). Fig. 3A shows the results of the CAT assays. From these data it is apparent that the proximal part of the *CYP7A* promoter region, up to −145, confers most of the transcription-activating capacity of the *CYP7A* promoter, which appeared to be about 10% of the activity observed with the SV40*cat* used as a control (result not shown). Obviously, in this part of the promoter the major transcription-activating elements of *CYP7A* are located. We hypothesize that the *BTE* sequence present in this region (see Fig. 2) and previously shown to be involved in the transcription of other cytochrome P-450 genes (Yanagida et al., 1990), may have an important functional role in the transcription activation of *CYP7A* in the rat. A further deletion to −79 led to a reduced CAT activity, while the −49R*cat* construct showed only basic transcriptional activity, probably only due to the presence of the TATA box. Extending the promoter fragment up to −348 had only a slight effect on

CAT activity, whereas the −1571, −2769 and −3641 constructs only showed a surprisingly low level of transcription activity, comparable to the basic transcriptional activity found for construct −49R*cat*. These results suggest the presence of a negative regulatory element between nt −348 and −1571.

Recent results obtained by Hylemon et al. (1992) indicated that *CYP7A* transcriptional activity is enhanced, when primary hepatocytes are cultured in a serum-free medium containing thyroxine. Therefore, constructs −49R*cat*, −348R*cat* and −3641R*cat* were used for transfection of hepatocytes, cultured as described by Hylemon (Fig. 3B). As can be concluded from the presented data, constructs −49R*cat* and −348R*cat* displayed an activity similar to that in hepatocytes cultured without thyroxine. However, in contrast with the low level of CAT-activity in medium without thyroxine, the −3641R*cat* construct showed a relatively strong transcriptional activity, comparable to that of the −348R*cat* construct. These results suggest the presence of a thyroxine-responsive element in the *CYP7A* promoter, between nt −348 and −3641. Obviously, this element can serve to compensate the repressing action of the putative negative control site between nt −348 and −1571.

(d) Regulation of *CYP7A* expression

One of the most intriguing regulatory responses supposed to play a part in the expression of *CYP7A* is a feedback control exerted, directly (Kwekkeboom et al., 1990; Twisk et al., 1993) or indirectly (Akerlund et al., 1990), by bile acids. Previously obtained results indicated that taurocholate (as well as other conjugated and unconjugated bile acids) represses the CYP7A activity, both *in vivo* (Heuman et al., 1988) and in primary hepatocytes in culture (Kwekkeboom et al., 1990; Twisk et al., 1993). In agreement with these findings, this inhibitory effect also appeared to be manifest at the cellular level of *CYP7A* mRNA concentrations (see Fig. 4A and 4C). Northern analysis of RNA isolated from primary hepatocytes, cultured in the absence or presence of 50 μM taurocholate, demonstrates that the addition of taurocholate led to an eightfold decrease in the steady-state level of *CYP7A* mRNA. These results indicate that the regulation of *CYP7A* expression upon addition of taurocholate mainly takes place at the transcript level. In order to further study this regulatory response, the *cat*-constructs described above were used in transient-expression experiments in which primary hepatocytes were grown in a medium containing 50 μM taurocholate. The data presented in Fig. 3A show that the constructs −79R*cat*, −145R*cat* and −348R*cat* displayed a response upon the addition of bile acid to the medium. The CAT activity, reflecting the transcriptional activity of the proximal promoter fragments, appeared to be threefold decreased in taurocholate-exposed cells, whereas the control SV40*cat*-signal was not affected (result not shown). Since the −49R*cat* directed signal did not display a taurocholate response, the region between −79 and −49 of the *CYP7A* promoter apparently is essential for the bile acid response of *CYP7A* in the rat. Therefore this region is likely to contain a bile-acid-responsive element. The pertinent region of the *CYP7A* promoter contains some notable nucleotide motifs, for example, a direct repeat between nt −54 and −65 (i.e., TCAAGT). Experiments are in progress to identify the actual bile-acid-responsive site. The extent to which transcriptional control contributes to the negative regulation of *CYP7A* expression upon bile-acid treatment can not entirely explain the eightfold reduced *CYP7A* mRNA levels as shown by the Northern analyses (see Fig. 4A and 4C). Consistently, run-on assays using isolated nuclei from primary hepatocytes cultured in the presence or absence of taurocholate (see Fig. 4B and C) revealed a moderate (about twofold) feedback control.

The data taken together, therefore, led us to propose that both transcriptional and post-transcriptional regulation play an important role in the expression of rat *CYP7A*. Post-transcriptional control, most likely, affects the stability of the *CYP7A* mRNA. Indeed, the occurrence of putative mRNA-destabilizing motifs in the respective trailer region has been indicated previously (Noshiro et al., 1990). It remains to be solved whether these elements actually are involved in the regulatory response to bile acids.

(d) Conclusions

(*1*) A genomic clone containing approximately 3.6 kb of flanking region of rat *CYP7A*, was isolated and the primary structure of the flanking region was determined. A computer-aided search revealed the presence of several putative regulatory elements in this upstream region.

(*2*) Transient-expression experiments using cultured primary rat hepatocytes indicated that the major transcription-activating region is located within the proximal 145 nt of the *CYP7A* promoter. Furthermore, evidence was obtained for the presence of a bile-acid-responsive element between nt −49 and −79, and a possible thyroxine-responsive element between nt −348 and −3641.

(*3*) Combined data, obtained from Northern hybridizations, run-on transcription experiments and transient-expression studies in primary hepatocytes in culture, indicate that both transcriptional and posttranscriptional control play an important role in the feedback regulation of the *CYP7A* activity in the rat.

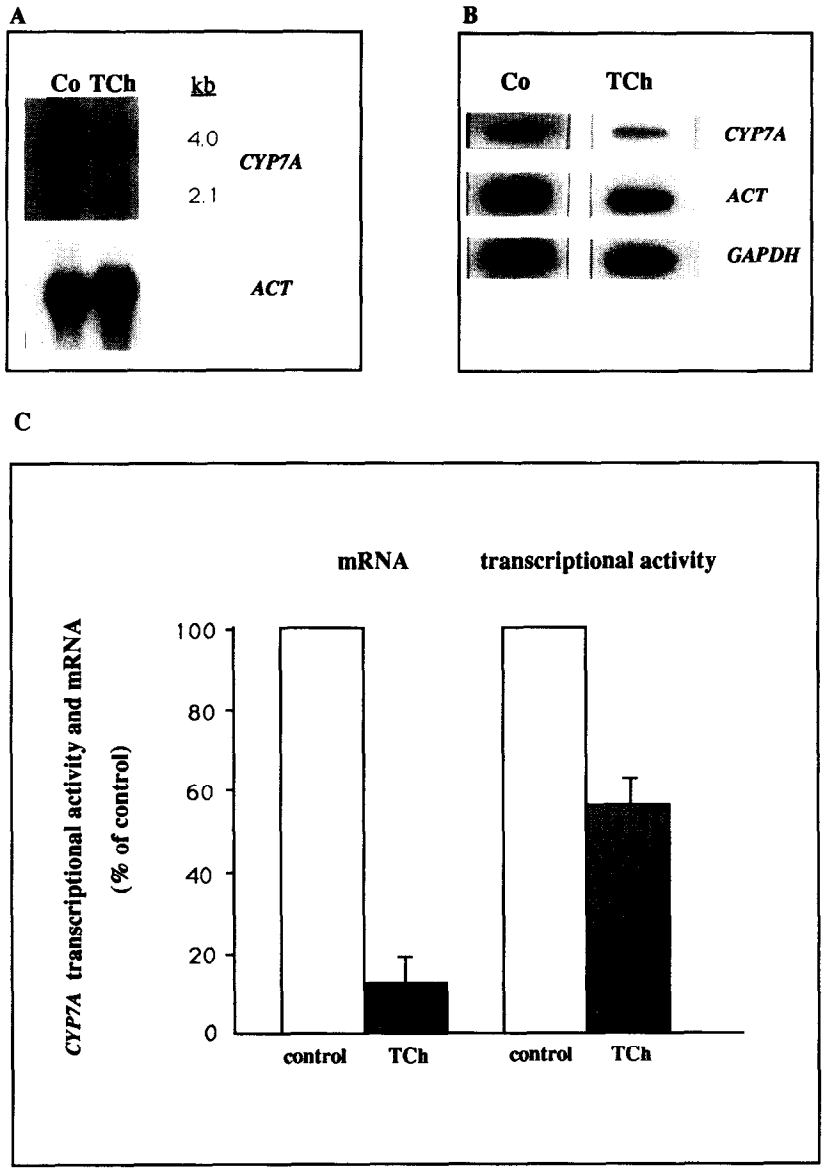

Fig. 4. *CYP7A* transcriptional activity and mRNA levels in hepatocytes cultured in the presence or absence of taurocholate. Cells were exposed to 50 μM taurocholate (TCh) for 24 h, between 18 and 42 h of culture, and were harvested simultaneously with untreated cells (control) for the isolation of nuclei and RNA. (**A**) Total RNA was isolated from cultured rat hepatocytes via the isolation procedure described by Chomczynski and Sacchi (1987). Northern blot analysis was performed with the 1.6-kb *CYP7A* cDNA and a 1.2-kb *PstI* fragment of hamster actin cDNA (*ACT*) as probes. *ACT* was used as a loading control; 10 μg of total RNA was applied in both lanes. (**B**) Nuclear run-on experiments were performed as described previously by Twisk et al. (1993). As target DNA for slot-blot hybridizations plasmid DNA containing cDNA sequences of rat *CYP7A*, hamster actin (*ACT*) and rat *GAPDH*, respectively, were used. (**C**) Transcriptional activity of *CYP7A* is presented relative to that of *actin* (*CYP7A* transcriptional activity relative to *GAPDH* showed the same results). The amount of mRNAs or labelled transcripts were assessed by densitometric scanning of the respective autoradiographs. Data are expressed as means (±S.D.) of duplicate incubations, using hepatocytes from 12 (mRNA) or 3 (transcriptional activity) rats. S.D., standard deviation.

ACKNOWLEDGEMENTS

We wish to thank Drs. Marcel Hoffmann for fruitful discussions and Elly de Wit and Eline Lehmann for technical assistance during part of the experiments. This work was supported by HGO-TNO, grant 900-523-138 to M.F.M. Hoekman and J.M.J. Rientjes, and the Netherlands Heart Found., grant 89.079 to J. Twisk.

REFERENCES

Alam, J., Yu, N., Irias, S., Cook, J.L. and Vig, E.: Reduced chloramphenicol acetyl transferase activity observed with vectors containing an upstream *SphI* recognition sequence. Biofeedback 10 (1991) 423–425.

Björkhem, I.: Mechanism of bile acid biosynthesis in mammalian liver. In: Danielsson, H. and Sjöval, J. (Eds.), Sterols and Bile Acids. Elsevier, New York, 1985, pp. 231–278.

Carey, M.C. and Cahalane, M.J.: Enterohepatic circulation. In: Arias,

I.M., Jakoby, W.B., Popper, H., Schachter, D., Shafritz, D.A. (Eds.), The Liver, Biology and Pathology. Raven Press, New York, 1988, pp. 573–616.

Cato, A.C.B., Geisse, S., Wenz, M., Westphal, H.M. and Beato, M.: The nucleotide sequences recognized by the glucocorticoid receptor in the rabbit uteroglobin gene region are located far upstream from the initiation of transcription. EMBO J. 3 (1984) 2771–2778.

Chiang, J.Y.L., Miller, W.F. and Lin, G.M.: Regulation of cholesterol 7α-hydroxylase in the liver. Purification of cholesterol 7α-hydroxylase and the immunochemical evidence for the induction of cholesterol 7α-hydroxylase by cholestyramine and circadian rhythm. J. Biol. Chem. 265 (1990) 7646–7650.

Chomczynski, P. and Sacchi, N.: Single-step method of RNA isolation by guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162 (1987) 156–159.

Cohen, J.C., Cali, J.J., Jelinek, D.F., Mehrabian, M., Sparkes, R.S., Lusis, A.J., Russell, D.W. and Hobbs, H.H.: Cloning of the human cholesterol 7α-hydroxylase gene (CYP7) and localization to chromosome 8q11-q12. Genomics 14 (1992) 153–161.

Danielsson, H., and Sjöval, J.: Bile acid metabolism. Annu. Rev. Biochem. 44 (1975) 233–253.

Frain, M., Swart, G., Monaci, P., Nicosia, A., Stämpfli, S., Frank, R. and Cortese, R.: The liver-specific transcription factor LF-B1 contains a highly diverged homeobox DNA binding domain. Cell 59 (1989) 145–157.

Goldstein, J.L. and Brown, M.S.: Regulation of the mevalonate pathway. Nature 343 (1990) 425–430.

Gonzalez, F.J.: The molecular biology of cytochrome P450s. Pharmacol. Rev. 40 (1989) 243–288.

Gorman, C.: High efficiency gene transfer into mammalian cells. In: Glover, D.M. (Ed.), DNA Cloning: A Practical Approach. IRL Press, Oxford, England, 1985, vol. II, pp. 143–190.

Hall, C.V., Jacob, P.E., Ringold, G.M. and Lee, F.J.: Expression and regulation of *Escherichia coli lacZ* gene fusions in mammalian cells. Mol. Appl. Gen. 2 (1983) 101–109.

Heuman, D.M., Vlahcevic, Z.R. and Bailey, M.L.: Regulation of bile acid synthesis. II, Effect of bile acid feeding on enzymes regulating hepatic cholesterol and bile acid synthesis in the rat. Hepatology 8 (1988) 892–897.

Hylemon, P.B., Gurley, E.C., Stravitz, R.T., Litz, J.S., Pandak, W.M., Chiang, J.Y.L. and Vlahcevic, Z.R.: Hormonal regulation of cholesterol 7α-hydroxylase mRNA levels and transcriptional activity in primary rat hepatocyte cultures. J. Biol. Chem. 267 (1992) 16866–16871.

Jelinek, D.F., Andersson, S., Slaughter, C.A. and Russell, D.W.: Cloning and regulation of cholesterol 7α-hydroxylase, the rate limiting enzyme in bile acid biosynthesis. J. Biol. Chem. 265 (1990a) 8190–8197.

Jelinek, D.F. and Russell, D.W.: Structure of the rat gene encoding cholesterol 7α-hydroxylase. Biochemistry 29 (1990b) 7781–7785.

Kwekkeboom, J., Princen, H.M.G., Van Voorthuizen, E.M. and Kempen, H.J.M.: Bile acids exert negative feedback control on bile acid synthesis in cultured pig hepatocytes by suppression of cholesterol 7α-hydroxylase activity. Hepatology 12 (1990) 1209–1215.

Langer, S.J. and Ostrowski, M.C.: Negative regulation of transcription in vitro by a glucocorticoid response element is mediated by a *trans*-acting factor. Mol. Cell. Biol. 8 (1988) 3872–3881.

Li, Y.C., Wang, D.P. and Chiang, J.Y.L.: Regulation of cholesterol 7α-hydroxylase in the liver. Cloning, sequencing and regulation of cholesterol 7α-hydroxylase mRNA. J. Biol. Chem. 265 (1990) 12012–12019.

Maniatis, T., Fritsch, E.F. and Sambrook, J.: Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982.

Molowa, D.T., Chen, W.S., Cimis, G.M. and Tan, C.P.: Transcriptional regulation of the human cholesterol 7α-hydroxylase gene. Biochemistry 31 (1992) 2539–2544.

Myant, N.B. and Mitropoulos, K.A.: Influence of thyroid state on cholesterol hydroxylation and absorption in the rat. J. Lipid Res. 18 (1977) 133–153.

Ness, G.C., Pendleton, L.C., Li, Y.C. and Chiang, J.Y.L.: Effect of thyroid hormone on hepatic cholesterol 7α-hydroxylase, LDL receptor, HMG-CoA-reductase, farnesyl pyrophosphate synthetase and apolipoprotein A-I mRNA levels in hypophysesectomized rats. Biochim. Biophys. Res. Commun. 172 (1990) 1150–1156.

Noshiro, M., Nishimoto, M., Morohashi, K. and Okuda, K.: Molecular cloning of cDNA for cholesterol 7α-hydroxylase from rat liver microsomes. FEBS Lett. 257 (1989) 97–100.

Noshiro, M., Nishimoto, M. and Okuda, K.: Rat liver cholesterol 7α-hydroxylase: pretranslational regulation for circadian rhythm. J. Biol. Chem. 256 (1990) 10036–10041.

Pandak, W.M., Vlahcevic, Z.R., Chiang, J.Y.L., Heuman, D.M. and Hylemon, P.B.: Bile acid synthesis, VI. Regulation of cholesterol 7α-hydroxylase by taurocholate and mevalonate. J. Lipid Res. 33 (1992) 659–668.

Pasco, D.S. and Fagan, J.B.: Efficient DNA-mediated gene transfer into primary cultures of adult rat hepatocytes. Lab. Methods 8 (1989) 535–541.

Princen, H.M.G., Huijsmans, C.M.G., Kuipers, F., Vonk, R.J. and Kempen, H.J.M.: Ketoconazole blocks bile acid synthesis in hepatocyte monolayer cultures and in vivo in rat by inhibiting cholesterol 7α-hydroxylase. J. Clin. Invest. 78 (1986) 1064–1071.

Princen, H.M.G., Meijer, P. and Hofstee, B.: Dexamethasone regulates bile acid synthesis in monolayer cultures of rat hepatocytes by induction of cholesterol 7α-hydroxylase. Biochem. J. 262 (1989) 341–348.

Princen, H.M.G. and Meijer, P.: Maintenance of bile acid synthesis and cholesterol 7α-hydroxylase activity in cultured rat hepatocytes. Biochem. J. 272 (1990) 273–275.

Sanger, F., Nicklen, S. and Coulson, A.R.: DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74 (1977) 5463–5469.

Shefer, S., Hauser, S., Bekersky, I. and Mosbach, E.H.: Biochemical site of regulation of bile acid synthesis in the rat. J. Lipid Res. 11 (1970) 404–411.

Subbiah, M.T.R. and Yanker, R.L.: Cholesterol 7α-hydroxylase of rat liver: an insulin sensitive enzyme. Biochem. Biophys. Res. Commun. 124 (1984) 896–902.

Sundseth, S. and Waxman, D.J.: Hepatic P-450 cholesterol 7α-hydroxylase. Regulation in vivo at the protein and mRNA level in response to mevalonate, diurnal rhythm and bile acid feedback. J. Biol. Chem. 265 (1990) 15090–15095.

Twisk, J., Lehmann, E.M. and Princen, H.M.G.: Differential feedback regulation of cholesterol 7α-hydroxylase mRNA and transcriptional activity by rat bile acids in primary monolayer cultures of rat hepatocytes. Biochem. J. (1993) In press.

Vlahcevic, Z.R., Heuman, D.M. and Hylemon, P.B.: Regulation of bile acids synthesis. Hepatology 13 (1991) 590–600.

von der Ahe, D., Janich, S., Scheidereit, C., Renkawitz, R., Schütz, G. and Beato, M.: Glucocorticoid and progesteron receptors bind to the same sites in two hormonally regulated promoters. Nature 313 (1985) 706–709.

Yanagida, A., Sogawa, K., Yasumoto, K.I. and Fujii-Kuriyama, Y.: A novel *cis*-acting DNA element required for a high level of inducible expression of the rat P-450c gene. Mol. Cell. Biol. 10 (1990) 1470–1475.