Transcriptional Regulation of the Hepatocyte Growth Factor Gene by the Nuclear Receptors Chicken Ovalbumin Upstream Promoter Transcription Factor and Estrogen Receptor*

(Received for publication, August 17, 1996, and in revised form, October 22, 1996)

Jie-Gen Jiang, Aaron Bell, Youhua Liu†,‡, and Reza Zarnegar§

From the Division of Cellular and Molecular Pathology, Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

Hepatocyte growth factor (HGF) is a multifunctional cytokine that controls the growth and differentiation of various tissues. Previously, we described the existence of a negative cis-acting regulatory element (s) within the −1 to −0.7-kilobase pair (kb) portion of the 5′-flanking region of the mouse HGF promoter. In the present study, we show that the repressor element is located at position −872 to −860 base pairs and comprises an imperfect estrogen-responsive element 5′-AGGTCAAGAGCCACCTGC-3′. We demonstrate that chicken ovalbumin upstream promoter transcription factor (COUP-TF), a nuclear orphan receptor belonging to the steroid/thyroid hormone receptor superfamily, through binding to this site effectively silences the transcriptional activity of the HGF promoter. We show that estrogen receptor, on the other hand, relieves the repressive action of COUP-TF, resulting in the induction of the HGF promoter. Using mice transgenic for either 2.7 or 0.7 kb of the HGF promoter region linked to the chloramphenicol acetyltransferase reporter gene, we found that injection of estradiol stimulates HGF promoter activity in tissues such as the mammary gland and ovary of mice harboring 2.7 but not 0.7 kb of the mouse HGF promoter region. Potential involvement of the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors in the regulation of HGF gene expression is also discussed.

Hepatocyte growth factor (HGF)† was originally identified as the major mitogenic component of serum and platelets for normal adult rat hepatocytes (1, 2). Further investigations revealed that HGF is a multifunctional cytokine with a broad range of target cells (3, 4). HGF elicits its biological effects through binding to and activating a specific transmembrane tyrosine kinase (4–6) known as Met (HGF receptor) (7). HGF gene expression is tightly regulated at the transcriptional level and is restricted to stromal cells such as fibroblasts, macrophages, leukocytes, and endothelial cells in various tissues. Numerous studies have shown that this growth factor is involved in the growth and regeneration of several organs (8–10). In response to tissue loss, such as in the case of partial hepatectomy or hepatotoxin injury, the level of HGF mRNA dramatically increases in the liver as well as in other organs such as the lung and spleen (9, 10). HGF mRNA also increases in the remaining kidney or lung after unilateral nephrectomy or pneumonectomy, respectively (9, 10). Recent investigations in transgenic mice homozygous for a null mutation in the HGF gene (knock-out mice) have proved that HGF is essential for the development of various tissues such as liver, muscle, and placenta (11, 12).

HGF expression is modulated by several cytokines and hormones. For example, it has been shown that in MRC-5 fibroblasts and HL-60 cells HGF expression is down-regulated by transforming growth factor β1 and dexamethasone or by coculture with epithelial cells (13–15). In contrast, phorbol esters, interleukin-1, or tumor necrosis factor α increase HGF mRNA level (16–19). Overexpression of HGF mRNA in various neoplastic tissues has also been reported. To understand the transcriptional regulation of HGF, the sequences of the human, rat, and mouse HGF gene promoters have been determined and the major transcriptional start sites have been mapped (20–23). Several potential regulatory sites including NF-IL6 (also known as C/EBPβ) were identified in the 5′-flanking region of the mouse HGF promoter by our laboratory (22) as well as by others (20–24). We reported the identification and partial characterization of a transcriptional repressor in the promoter of the mouse HGF gene (25). We also localized a negative regulatory region in the 5′-flanking region of the mouse HGF promoter residing at position −1 to −0.7 kb (22). In the current study, we show that COUP-TF binds to an imperfect estrogen response element (ERE) in this region and represses HGF promoter activity. We show that high expression of the estrogen receptor counteracts the suppressive action of COUP-TF by competing for the same binding site.

COUP-TF, which belongs to the orphan nuclear receptor family, was originally found through its interaction with a response element in the chicken ovalbumin gene promoter (26, 27) and has been shown to play both positive and negative roles in gene regulation upon binding to its cognate DNA regulatory element (28). The COUP-TF family (which consists of COUP-TF I and COUP-TF II) binds to the AGGTCA repeat sequence, which also is the DNA half-site for several other
nuclear receptors such as the estrogen receptor (ER), vitamin D3 receptor, thyroid hormone receptor, retinoic acid receptors (RAR and RXR), peroxisome proliferator-activated receptor, and hepatic nuclear factor 4 (HNF4) (28–31). In previous studies we reported that estrogen stimulates HGF mRNA expression in mouse ovaries (32). Our current results implicate COUP-TF as a potent repressor of HGF gene transcription and provide new insights into the molecular mechanisms that regulate HGF gene expression.

**Experimental Procedures**

**Plasmid Construction—**The 1.4 mouse HGF CAT (28) was digested with HindIII/IP/III. The 0.85 mouse HGF-CAT construct containing this region, two additional HGF-CAT chimeric constructs, 1.0 HGF-CAT, 0.85 HGF-CAT, 0.85 DHGF-CAT, and deletion (0.85DHGF-CAT) were generated by using the restriction enzyme AflII. The 0.85 mouse DHGF-CAT construct containing this region was inserted into the Blg/Ili site of the pCUT promotor (32).

**Cell Culture, DNA Transfection, and CAT Assay—**Human endometrial carcinoma RL95-2 cells, human hepatoma HepG2 cells, and mouse fibroblast NIH3T3 cells were obtained from American Type Culture Collection (Rockville, MD) and cultured as described previously (22). For transfection, the recipient cells were cultured in six-well plates for 24 h and then transfected with various mouse HGF promoter/CAT chimeric plasmids and heterologous plasmid constructs using the DNA calcium phosphate method according to the instructions of the CellPhct transfaction kit (Pharmacia Corp.) by filling in and blunt ending with Klenow enzyme (Life Technologies, Inc.).

**Preparation of Nuclear Extracts—**Human endometrial carcinoma RL95-2 cells, human hepatoma HepG2 cells, and mouse fibroblast NIH3T3 cells were originally obtained from ATCC. Cells were cultured under conditions described previously (22, 32). The nuclear extracts were prepared as already reported (25). Briefly, cells growing to about 90% confluence were washed with cold phosphate-buffered saline and dislodged with a rubber policeman into phosphate-buffered saline. Cells were pelleted by low speed centrifugation, resuspended in 5 volumes of Buffer A containing protease inhibitors (10 mM HEPES (pH 7.9), 1.5 mM MgCl2, 10 mM KCl, 0.2 mM phenylmethylsulfonyl fluoride, 0.15 mM spermidine, 0.5 mM spermine, 0.5% Nonidet P-40, and 0.5 mM dithiothreitol, plus 1 μg each of leupeptin and chymostatin per ml) and then placed on ice for 10 min. Immediately after lysis, the solution was transferred to a glass Dounce homogenizer and homogenized with 10 up and down strokes using a type B pestle. The nuclei were collected by centrifugation for 15 min at 3,300 × g at 4 °C, and the supernatant was then collected and dialyzed against 50 mM KCl-TGM (10 mM Tris-HCl (pH 7.6), 1% glycerol, 3 mM MgCl2, 3 mM EGTA buffer using a mini-dialysis system (Life Technologies, Inc.). The insoluble materials were removed by centrifugation, and small aliquots of protein extract were quickly frozen and stored at −80 °C after the protein concentration had been determined by protein assay (Bio-Rad).

Rat liver nonparenchymal cells were isolated by differential centrifugation of the collagenase-digested rat liver perfusate (provided by George Michalopoulos, Department of Pathology, University of Pittsburgh). Cells were washed twice with cold phosphate-buffered saline and then used to make nuclear extracts with the method described above.

**Oligonucleotides—**The following oligonucleotides were used in gel mobility shift assays: vitellogenin A2 ERE (VitA2ERE), 5′-GATCTGACGTCAGTACGCTA-3′ (33); and COUP, 5′-GATCTTTCCTGAGTGTGTA-3′ (30). The following sequences were derived from the mouse HGF gene promoter (22): HERE1, 5′-GATCAAGTTGCAAAAGACGACATT-3′; HERE2, 5′-GATCTGGCTCAACTACACGAA-3′; 5′-GATCATCGGACCTTACTACCGG-3′; IL-6ERE, 5′-GATCACAGCTGGAATTCCGTG-3′; GRE, 5′-GATCTTGAAGCGTGCTTAC-3′; CRE, 5′-GATCATCAAGCGTCTTTTTA-3′; and TIE, 5′-GATCAGGCGGATTTGGTCTGTC-3′.

**Gel Retardation Assays—**A DNA fragment corresponding to nucleotide position −964 to −812 of the 5′-flanking region of the mouse HGF gene was isolated from the chimeric 1.4 mouse HGF-CAT construct by restriction digestion with XhoI/AIII. The 154-bp fragment was purified and labeled with 32P-dATP (3,000 Ci/mmol, Amersham Corp.) by filling in and blunt ending with Klenow enzyme (Life Technologies, Inc.). The double-stranded oligonucleotides used in gel shift assays were also labeled with 32P by end labeling with T4 kinase. The labeled probes were then gel purified and used in gel mobility shift assays as described previously (25). Two micrograms of poly(dI-dC) (Pharmacia) were used as the nonspecific competitor in 10 μl of reaction mixture. When antibodies were used in supershift experiments, they (1 μl) were incubated with nuclear extracts for 15 min at room temperature before carrying out DNA binding shift assays. The binding reactions (20 μl total volume/reaction) were carried out at room temperature for 20 min before loading on 5% nondenaturing polyacrylamide (19:1 acrylamide/ bisacrylamide) gels. The concentration of nuclear protein extracts used in each reaction was 2 μg and that of the labeled probe was between 0.2 and 0.4 ng. For competition experiments, a 100-fold molar excess of unlabeled DNA fragments or oligonucleotides was included in the reaction mixture. Gels were run in 0.5 × TBE buffer (0.045 M Tris borate, 0.001 M EDTA) at a constant voltage of 190 V, dried, and autoradiographed using intensifying screens.

**Transgenic Mice—**Transgenic lines were generated by microinjection of a 15-kb construct, 1.4 mouse HGF-CAT, into C57BL/6.C57BL/6 mice were produced using various lengths (2.7, 0.7, and 0.1 kb) of the HGF promoter region fused to the CAT reporter gene were recently generated and characterized in our laboratory. Two different HGF-CAT transgenic mouse lines were used in the present study to evaluate HGF promoter activity in response to estradiol. The 2.7 HGF-CAT transgenic mouse lines (three different founders) harbor 2.7 kb of the HGF promoter that contains the HER1 element. The 0.7 HGF-CAT transgenic lines (two different founders) carry 0.7 kb of the HGF promoter region that lacks the HER1 element. Transgenic mice were maintained in animal facilities at the University of Pittsburgh Medical Center. All animal experiments were conducted in accordance with National Institutes of Health standards established by the “Guidelines for the Care and Use of Experimental Animals.” Mice were injected subcutaneously with 100 mg of 17β-estradiol (Sigma) per kg of body weight in a volume of 0.1 ml of buffered saline containing 0.2% ethanol. At 24 h after injection, groups of eight animals were killed by cervical dislocation, and the ovaries and other organs were dissected and immediately frozen for CAT assays.

**Results**

Identification of a c-Actin Repressor in the HGF Promoter—We previously reported the existence of a negative regulatory region in the 5′-flanking region of the mouse HGF gene located between nucleotides −964 and −699 bp (22). To further define this region, two additional HGF-CAT chimeric constructs containing a 5′ deletion (0.85 HGF-CAT) or an internal deletion (0.85 DHGF-CAT) were generated by using the restriction enzyme AluI (Fig. 1). Four different HGF-CAT chimeric constructs, 1.0 HGF-CAT, 0.85 HGF-CAT, 0.85 DHGF-CAT,

2 A. Bell, J.-G. Jiang, Y. Liu, G. K. Michalopoulos, and R. Zarnegar, manuscript in preparation.

3929
and 0.7 HGF-CAT, were subsequently tested for promoter activity by transfection into human endometrial carcinoma RL95-2 cells, human hepatoma HepG2 cells, and mouse fibroblast NIH3T3 cells. Promoter activity of each construct as determined by CAT assays revealed that the 0.7 and 0.85 HGF-CAT constructs had consistently stronger promoter activity than the 0.85 DHGF-CAT and the 1.0 HGF-CAT constructs (Fig. 1). These results suggest that the region between nucleotides −964 and −812 bp contains a negative cis-acting element(s) and that a negative trans-acting factor(s) may interact with this 154-bp DNA fragment to repress HGF gene transcription.

**Trans-Acting Factors Interact with the cis-Acting Negative Element in a Distinct Binding Region**—To examine whether trans-acting factors specifically bind to the 154-bp DNA fragment, we performed gel mobility band shift analysis using the end-labeled 154-bp fragment with nuclear protein extracts from NIH3T3 cells. Examination of the DNA-protein complexes by polyacrylamide gel electrophoresis under nondenaturing conditions revealed a prominent and specific band with a slow electrophoretic mobility. This complex was totally abrogated in the presence of 100-fold molar excess of the unlabeled "self" DNA probe. Several double-stranded synthetic oligonucleotide sequences including HERE1, GRE, CRE, TIE, AP1, IL-6RE, NF-IL6, and HERE2 were added to the reaction mixture to compete for binding as indicated. C, binding complex; F, free 32P-labeled DNA probe.

**Transcriptional Regulation of HGF Gene by COUP-TF and ER**

![Diagram](Image)

**Fig. 1. Localization of a cis-acting repressor element in the 5′-upstream flanking region of the mouse HGF promoter.** Mouse HGF-CAT constructs consisting of a series of 5′ deletions or an internal deletion were transiently transfected into NIH3T3, HepG2, and RL95-2 cells. Graphical representation of relative CAT activity after normalization against β-galactosidase is shown. The results presented here are the average from at least three independent experiments performed in duplicate. The transfected plasmids were from two independent preparations. Numbers indicate the insert sizes (kb) of the mouse HGF promoter region 5′ to the CAT reporter gene. DNA fragments were inserted in correct (5′ to 3′) orientation relative to the transcription initiation site, and the nucleotide boundaries were confirmed by sequencing.

**Fig. 2. Specific interaction of the 5′-flanking region of the mouse HGF gene with a negative regulatory activity in nuclear protein extracts.** The 154-bp DNA fragment that exerted negative regulatory activity was labeled with 32P and incubated with nuclear protein extract (NPE) from NIH3T3 cells. The binding reactions were performed at room temperature for 20 min in the presence of the nonspecific competitor poly(dI-dC). Several double-stranded synthetic oligonucleotide sequences including HERE1, GRE, CRE, TIE, AP1, IL-6RE, NF-IL6, and HERE2 were added to the reaction mixture to compete for binding as indicated. C, binding complex; F, free 32P-labeled DNA probe.

To assess the functionality of this element, synthetic HERE1 and HER2 (a mutated version of HERE1, 5′-GGTCAATCTA-ACC-3′) oligonucleotides were linked to the heterologous pCAT promoter reporter gene (Promega), which contains the SV40 promoter, and the promoter activity in three different cell lines using transient transfection and CAT assays was determined. The results are summarized in Fig. 3. HER1 efficiently repressed the SV40 promoter activity in HepG2, RL95-2, and NIH3T3 cells, whereas HER2 (which failed to specifically bind to the nuclear protein, Fig. 2, lane 11) did not have any function. These results indicate that HER1 negatively modulates HGF promoter activity and that this element functions not only in the context of the autologous promoter (Fig. 1) but also in the context of a heterologous promoter (Fig. 3).

**Identification of the Binding Protein(s) as COUP-TF**—To
**Transcriptional Regulation of HGF Gene by COUP-TF and ER**

**Fig. 3.** Synthetic mouse HERE1 sequence confers repression on a heterologous promoter. Shown on the left is the diagrammatic representation of the chimeric CAT constructs. Synthetic oligonucleotides corresponding to the mouse HERE1 and HERE2 were cloned into a position upstream of the SV40 promoter (SV). These constructs were transiently transfected into NIH3T3 cells followed by CAT assay as described under “Experimental Procedures.” Depicted on the right is a graphic representation of relative promoter activity as determined by CAT assays. Each bar represents the mean of three independent transfection experiments. The nucleotide sequences of wild type ERE (VitA2ERE), mouse HERE1, and mouse HERE2 are shown for comparison.

Identify the HERE1 binding protein(s), labeled HERE1 was used as a probe in gel mobility shift assays with the nuclear extracts from NIH3T3 and RL95-2 cells. In NIH3T3 nuclear extract, the specific complex was abolished by unlabeled HERE1, VitA2ERE (wild type ERE), and COUP binding sites (Fig. 4A, lanes 3–5) but not by HERE2 and GRE (Fig. 4A, lanes 6 and 7). We used COUP binding site because it is well known that COUP-TF binds to the AGGTCA motif regardless of its configuration (single half-site, direct as well as indirect repeats, and various spacings) (29). Although RL95-2 cell nuclear extract formed two different bands with the HERE1 probe, these bands were totally abolished by 100-fold molar excess of unlabeled HERE1, VitA2ERE, and COUP oligonucleotides (Fig. 4A, lanes 9–11) but not by HERE2 and GRE (Fig. 4A, lanes 12 and 13). It is known that RL95-2 and NIH3T3 cells express small amounts of ER, and that the major function of ER on HERE1 but had no effect on the HERE2-SV40 promoter activity (Fig. 5). Furthermore, overexpression of COUP-TF (pRS-COUP) in NIH3T3 cells counteracted the stimulatory function of ER on HERE1 but had no effect on the HERE2-SV40-CAT construct (Fig. 6B). These results demonstrate that the mouse HGF gene is transcriptionally suppressed by COUP-TF binding to the HERE1 element at nucleotide –872 bp in the 5'-flanking region of the mouse HGF gene. High expression of ER counteracts the suppressive function of COUP-TF by competing with ER for binding to HERE1.

**Stimulation of the Mouse HGF Promoter in Transgenic Mice by Estradiol**—We previously showed that injection of estradiol into mice dramatically stimulated HGF mRNA expression in mouse ovary (32). Our present data reveal that the molecular mechanism by which estradiol stimulates the HGF promoter is through the counteraction by ER of COUP-TF, which directly down-regulates the mouse HGF promoter. Only high levels of ER counteract the suppression caused by COUP-TF bound to HERE1. To further confirm that HERE1 is functional and responsible for the stimulation of HGF mRNA by estradiol in vivo, we analyzed transgenic mice that harbor 2.7 and 0.7 kb of the mouse HGF promoter linked to the CAT reporter gene. The activity of the 2.7 HGF-CAT promoter construct in transgenic mice that carries the HERE1 element was up-regulated in the mammary tissue and ovary after injection of estradiol (Fig. 7). On the other hand, the activity of the 0.7 HGF-CAT construct that does not contain the HERE1 element did not change upon treatment with estradiol (data not shown). This result strongly suggests that in vivo high expression of ER counteracts COUP-TF and stimulates HGF mRNA expression.

**DISCUSSION**

Our previous studies have shown that a negative regulatory element is located within –964 to –699 of the 5'-flanking region of the mouse HGF gene (22). In this region, an estrogen response element (HERE1) was identified that binds to ER and is responsible for the estradiol stimulation of the mouse HGF promoter in RL95-2 cells co-transfected with an ER expression vector (32). We now show that this same element binds with high affinity to the nuclear orphan receptor COUP-TF, which binds to the steroid/thyroid receptor superfamly, and silences the activity of the HGF promoter. We demonstrate that the estrogen receptor competes with COUP-TF for binding to this site, thereby relieving the repressive action of COUP-TF.
There are two imperfect EREs found in the mouse HGF promoter region that we have called HERE1 and HERE2 (22, 32). HERE1 is localized in the 5′-flanking region and HERE2 is in the first intron of the HGF gene. By using gel mobility band shift and supershift assays, we demonstrated that HERE1 is similar to the ERE found in the vitellogenin (vit) gene (37) and that it binds not only to ER but also to COUP-TF (Fig. 4) (30 and 32). Co-transfection of ER or COUP-TF expression vectors in NIH3T3 cells revealed that HERE1 confers the repressive function of COUP-TF to the heterologous SV40 promoter and that not only did ER overcome the suppression of COUP-TF but it also stimulated the SV40 promoter (Fig. 6). In the homologous mouse HGF promoter, ER only counteracted the negative effects of COUP-TF (Fig. 5).

The molecular mechanisms of COUP-TF-mediated transcriptional repression are partially understood. COUP-TF acts via several different mechanisms to inhibit target gene transcription including competition with other nuclear receptors for the occupancy of DNA binding sites, active repression of basal transcription, active repression of transactivator-dependent transcription, quenching of transactivator-dependent transcription, transrepression of activated transcription, and titration of the common coregulator RXR (31, 38). Our data shown here reveal that COUP-TF may down-regulate the mouse HGF promoter by active repression of basal transcription because HERE1 suppresses the basal SV40 promoter and that ER may activate HGF transcriptional activity by replacing COUP-TF and relieving the promoter from repression (Fig. 5). COUP-TF belongs to the orphan (thus far, no ligand has been found that can bind and directly regulate the activity of this factor) nuclear receptor family and resembles the thyroid hormone receptor/steroid hormone receptor superfamily (31, 36). The mode by which it represses its target gene involves the binding of COUP-TF to the AGGTCA motif arranged in various configurations (i.e. direct repeats (DR) or inverted repeats (IR) with different numbers of spacers ranging from 0 to 8 nucleotides) (31). Interestingly, the AGGTCA half-site depending on its configuration forms the response elements for other nuclear receptors such as the estrogen receptor (which binds to IR3), thyroid hormone receptor (which binds to IR0 or DR4), retinoid receptors (RAR, which binds to IR0 and DR2, and RXR, which binds to DR5 and DR11), vitamin D receptor (which binds to DR3), HNF4, and peroxisome proliferator-activated receptor (31). It is believed that COUP-TF directly competes with these receptors for binding to the AGGTCA sequence and thus inhibits their transactivating functions. Suppression by COUP-TF is also achieved in part through heterodimerization of COUP-TF with RXR (which is the essential transactivating partner for vitamin D1 receptor, thyroid hormone receptor, and RAR), thereby titrating RXR to a transcriptionally inactive complex.
(31, 38). It is tempting to speculate that in addition to ER other members of the nuclear receptor family mentioned above may bind to the COUP-TF binding site in the HGF promoter HEREl and thus activate HGF transcription in a tissue-specific manner. The COUP-TF site in the HGF promoter, however, is composed of an estrogen response element (IRE3) and apparently binds only to the estrogen receptor.

Previous observations in our laboratory showed that administration of 17β-estradiol to immature female mice caused a significant induction of HGF mRNA in the ovaries (32). The tissue- and cell type-specific expression of the mouse HGF gene by estrogen may be due to different amounts of hormone receptors in these tissues, as shown by other studies (37). Our present studies show that high expression of estrogen receptor in NIH3T3 cells is sufficient to counteract the suppression of COUP-TF on the mouse HGF promoter (Fig. 5). HGF promoter activity or lack thereof in transgenic mice also supports this hypothesis because estradiol stimulated the HGF promoter in mice carrying the 2.7 HGF-CAT construct that contains HEREl, particularly in tissues that have high expression of ER (Fig. 7). In this regard, it is important to understand how HGF gene expression is relieved from COUP-TF repression in other tissues in which ER may not be operational. The factors that are known to activate HGF expression include cytokines such as IL-1, IL-6, and tumor necrosis factor α and polypeptide growth factors such as epidermal growth factor. These agents do not exert their gene-regulatory function directly through the AGGTCA motif, the DNA binding site for COUP-TF. Although COUP-TF do not exert their gene-regulatory function directly through the AGGTCA motif, the DNA binding site for COUP-TF. Although the molecular mechanisms by which these agents induce their target genes are not fully understood, it is well established that they utilize members of the AP1, C/EBP, nuclear factor κB (NFκB), and signal transducer and activator of transcription (STAT) family of transcription factors. Indeed, in our previous report (22) we demonstrated that several NF-IL6 (C/EBPβ) response elements exist in the 5′-flanking region of the HGF promoter region and that IL-6 induces HGF promoter activity in a stromal (fibroblast) cell line stably transfected with an HGF-CAT chimeric construct. In our very recent investigations using gel shift, supershift, and CAT assays, we have found that functional C/EBP binding sites exist in the HGF promoter. We also found that co-transfection of expression vectors encoding C/EBPβ or C/EBPα with HGF-CAT vectors dramatically (more than 20-fold) stimulates the transcriptional activity of various HGF-CAT promoter constructs (even those constructs that do not contain COUP-TF binding sites, thus indicating that promoter activation is not due to interference with COUP-TF binding). These observations provide an explanation for how the HGF promoter is released from repression exerted by negative acting factors such as COUP-TF. The C/EBP family of transcription factors is known to consist of regulators of important cellular functions ranging from metabolism to cell growth to differentiation.

The physiological significance of the transcriptional regulation of the HGF gene by ER and COUP-TF also remains to be elucidated. Nevertheless, it has been shown that both HGF and COUP-TF play an important role during embryonic development. COUP-TFs are differentially expressed in a restricted manner during organogenesis (40). Both COUP-TF I and COUP-TF II genes are essential since COUP-TF I mutants die prenatally and COUP-TF II mutants die in utero (41). Additionally, HGF is believed to be one of the major mediators of stromal-epithelial interactions controlling growth and morphogenesis of various tissues such as breast (39). Studies on the

---

3 J.-G. Jiang and R. Zarnegar, manuscript in preparation.
embryological development of such tissues have revealed that steroid hormone-induced ductal morphogenesis and growth is accomplished via the interactions between the stromal and epithelial compartments of these tissues (34). Therefore, the functions of COUP-TF and ER during development may be mediated in part by their ability to regulate HGF gene expression. Our data shown here shed light on the significant interplay among transcription factors such as ER, COUP-TF, and C/EBP and polypeptide growth factors such as HGF.

Acknowledgments—We are grateful to Drs. S. Y. Tsai and M.-J. Tsai for generously providing us with the anti-COUP-TF antibody and the COUP-TF expression vector pRS-COUP. We also thank Dr. M. C. DeFrances for critical review of the manuscript.

REFERENCES

1. Zarnegar, R., and Michalopoulos, G. K. (1989) Cancer Res. 49, 3314–3320
2. Nakamura, T., Nishizawa, T., Hagiyu, M., Seki, T., Shimonishi, M., Sugimura, A., Tashiro, K., and Shimizu, S. (1989) Nature 342, 440–443
3. Zarnegar, R., and Michalopoulos, G. K. (1995) J. Cell Biol. 129, 1177–1180
4. Bottaro, D. P., Robin, J. S., Faletto, D. L., Chan, A. M.-L., Rmiek, T. E., Vande Woude, G. F., and Aaronson, S. A. (1991) Science 251, 802–804
5. Naldini, L., Vigna, E., Nasrinman, B., Gaudino, G., Zarnegar, R., Michalopoulos, G. K., and Comoglio, P. M. (1991) Oncogene 6, 501–504
6. Weidner, K. M., Sachs, M., and Birchmeier, W. (1993) J. Cell Biol. 121, 145–154
7. Park, M., Dean, M., Kaul, K., Braun, M. J., Gonda, M., and Vande Woude, G. F. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 6379–6383
8. Lindroos, P., Zarnegar, R., and Michalopoulos, G. K. (1991) Hepatology 13, 745–750
9. Matsumoto, K., Hashimoto, K., Yoshikawa, K., and Nakamura, T. (1991) Exp. Cell Res. 196, 114–120
10. Kono, S., Nagaike, M., Matsumoto, K., and Nakamura, T. (1992) Biochem. Biophys. Res. Commun. 79, 991–998
11. Schmidt, C., Bladt, F., Goecke, S., Brinkmann, V., Zichiesche, W., Sharpe, M., Gherardi, E., and Birchmeier, C. (1995) Nature 373, 699–702
12. Uehara, Y., Minowa, O., Mori, C., Shiota, K., Kuno, J., Noda, T., and Kitamura, N. (1995) Nature 373, 702–705
13. Matsumoto, K., Taima, H., Okazaki, H., and Nakamura, T. (1992) J. Biol. Chem. 267, 24917–24920
14. Nishino, T., Kaise, N., Sindo, Y., Nishino, N., Yasuda, S., and Masui, Y. (1991) Biochem. Biophys. Res. Commun. 188, 235–245
15. Seslar, S. P., Nakamura, T., and Byers, S. W. (1993) Cancer Res. 53, 1233–1238
16. Tamura, M., Arakaki, N., Tsuouchi, H., Takada, H., and Daikuhara, Y. (1993) J. Biol. Chem. 268, 8140–8145
17. Matsumoto, K., Taima, H., Hamaouchi, M., Kohn, S., Kinoshita, T., and Nakamura, T. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 3800–3804
18. Nishino, T., Kaise, N., Nishio, N., Nishida, T., Yasuda, S., and Masui, Y. (1991) Biochem. Biophys. Res. Commun. 181, 523–330
19. Gehda, E., Kataoka, H., Tsuouchi, H., Daikuhara, Y., and Yamamoto, I. (1992) FEBS Lett. 301, 107–110
20. Miyazawa, K., Kitamura, A., and Kitamura, N. (1991) Biochemistry 30, 9170–9176
21. Okajima, K., Miyazawa, K., and Kitamura, N. (1993) Eur. J. Biochem. 213, 113–119
22. Liu, Y., Michalopoulos, G. K., and Zarnegar, R. (1994) J. Biol. Chem. 269, 24917–24920
23. Aravamudan, B., Watabe, M., and Watabe, K. (1993) Biochem. Biophys. Res. Commun. 195, 346–353
24. Plaschke-Schlutter, A., Behrens, J., Gherardi, E., and Birchmeier, W. (1995) J. Biol. Chem. 270, 830–836
25. Liu, Y., Beedle, A., Liu, L., Bell, A., and Zarnegar, R. (1994) Mol. Cell. Biol. 14, 7046–7058
26. Sagami, I., Tsai, S. Y., Wang, L. H., Tsai, M. J., and O’Malley, B. W. (1986) Mol. Cell. Biol. 6, 4259–4267
27. Wang, L. H., Tsai, S. Y., Cook, R. G., Beattie, W. G., Tsai, M. J., and O’Malley, B. W. (1989) Nature 340, 163–166
28. Connelly, O. M., and O’Malley, B. W. (1994) in Molecular Biology Intelligence Unit: Mechanism of Steroid Hormone Regulation of Gene Transcription (Tsai, M. J., and O’Malley, B. W., eds) pp. 111–133, R. G. Landes Co., Austin, TX
29. Cooney, A. J., Tsai, S. Y., O’Malley, B. W., and Tsai, M. J. (1991) Mol. Cell. Biol. 12, 4153–4163
30. Liu, Y., Yang, N., and Teng, C. T. (1993) Mol. Cell. Biol. 13, 1836–1846
31. Mangelersdorf, D., and Evans, R. M. (1995) Cell 83, 841–850
32. Liu, Y., Lin, L., and Zarnegar, R. (1994) Mol. Cell. Endocrinol. 104, 173–181
33. Klein-Hitpass, L., Ryfel, G. U., Heitlinger, E., and Cato, A. C. B. (1988) Nucleic Acids Res. 16, 647–663
34. Beato, M. (1989) Cell 56, 335–344
35. Kadowaki, Y., Toyoshima, K., and Yamamoto, T. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4432–4436
36. Pereira, F. A., Qiu, Y., Tsai, M.-J., and Tsai, S. Y. (1995) J. Steroid Biochem. Mol. Biol. 53, 503–508
37. Meyer, M. E., Gronemeyer, H., Turcotte, B., Bocquel, J. T., Tasset, D., and Chambon, P. (1989) Cell 57, 433–442
38. Leng, X., Cooney, A. J., Tsai, S. Y., and Tsai, M.-J. (1996) Mol. Cell. Biol. 16, 2332–2340
39. Niranjan, B., Buluwela, L., Yant, J., Perusiching, N., Atherton, A., Phipard, D., Dale, T., Gustedt, B., and Kamalati, T. (1995) Development 121, 2697–2706
40. Qiu, Y., Cooney, A. J., Kuratani, S., DeMayo, F. J., Tsai, S. Y., and Tsai, M.-J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 4451–4455
41. Kastner, P., Mark, M., and Chambon, P. (1995) Cell 83, 859–869