The Upstream Regulatory Regions of the Hepatocyte Growth Factor Gene Promoter Are Essential for Its Expression in Transgenic Mice*

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Hepatocyte growth factor (HGF) is a unique secreted regulator molecule with multiple biological activities. It is a strong mitogen for various epithelial cells such as hepatocytes as well as endothelial cells and melanocytes (1–6) and has been shown to have motogenic, morphogenic, and antitumor activities (7–11). It exerts these diverse activities through its specific transmembrane tyrosine kinase receptor c-Met, also called the HGF receptor (12–14). HGF gene expression is mainly confined to the mesenchymal/stromal cells of a variety of tissues under normal and pathophysiological conditions (15, 16). The HGF receptor, on the other hand, is expressed predominantly in epithelial cells (17, 18). This ligand/receptor system is thought to play a significant role in mediating stromal-epithelial interactions (5, 6).

Studies have shown that the HGF gene is expressed in developing embryos (19), whereas gene knockout studies have revealed that HGF is essential for normal development of liver, placenta, and muscle (20, 21). HGF gene expression has been shown to be modulated in vivo by estrogen, dexamethasone, transforming growth factor-β, tumor necrosis factor-α, and other cytokines (29–31), and it increases dramatically in vivo in remnant tissues and distal organs following tissue loss such as partial hepatectomy, unilateral nephrectomy, or acute lung injury (22, 23, 26, 32). Thus, it follows that HGF and the HGF receptor are implicated as an important paracrine system involved in embryogenesis, organ regeneration, wound repair, and cancer (19–28). Unveiling the molecular mechanisms that govern HGF and HGF receptor expression is crucial to understanding the biology and pathobiology of each of these processes.

In an effort to elucidate the molecular mechanisms responsible for the complex regulation of HGF gene expression, we have previously reported the cloning and partial characterization of the 5′-flanking region of the mouse HGF gene (33). Transient transfection studies using in vitro models have identified several regulatory elements that may be involved in the cell type-specific and inducible expression of the HGF gene (29, 34–38). Promoter analysis using in vitro transfection assays, however, suffers from many shortcomings and is limited to few cell types and experiments. Thus, to expand our in vitro findings as well as to further characterize other mechanisms involved in regulation of HGF expression in vivo, we developed transgenic mice harboring chimeric genes containing various lengths of the mouse HGF promoter region fused to the chloramphenicol acetyltransferase (CAT) reporter gene. Our data show that the upstream regulatory regions of the HGF promoter are essential for efficient promoter activity and contain the necessary DNA elements to dictate expression in embryonic and adult tissues and to confer inducibility in response to cues such as tissue loss, which triggers cell growth and regeneration. These transgenic mice provide a good model system for studying the transcriptional regulation of HGF gene expression in normal development and in pathological conditions such as cancer in which HGF gene expression is unregulated.

EXPERIMENTAL PROCEDURES

Generation and Identification of Transgenic Mice—The cloning of the mouse HGF promoter region into pCAT basic vector designated 2.7HGF-CAT (-2674 to +29 bp), 0.7HGF-CAT (-699 to +29 bp), and 0.1HGF-CAT (-70 to +29 bp) has been described in our previous study (33). The transgene constructs used for microinjection were derived from these plasmids by digesting them with HindIII and HpaI to produce fragments of various lengths. These constructs were microinjected into fertilized mouse ova, and the resulting embryos were implanted into pseudopregnant host mothers. The progeny were screened by polymerase chain reaction to identify transgenic mice. The transgenic mice were genotyped by Southern blotting using a 200-bp probe made by PCR using primers specific for mouse HGF and CAT genes. The transgenic mice were then mated to generate the homozygous transgenic mice. The transgenic mice were then mated to generate the homozygous transgenic mice.
release the corresponding fragment, which consisted of the HGF promoter, CAT gene, and the late SV40 poly(A) site as depicted in Fig. 1. These linearized DNA fragments were separated by agarose gel electrophoresis and purified by glassmill (BIO 101, Inc., Vista, CA). Transgenic mice were generated according to established methods (39) by the transgenic mouse facility at our institution using the mouse strain B6D2 (C57B6 x DBA2). Potential founders were screened for transgene integration by PCR analysis of blood DNA using CAT-specific primers. Whole blood (50 μl) was obtained from 3-week-old mice by retro-orbital bleeding, and the DNA was prepared according to Innis et al. (40). Oligonucleotide primers designed from the CAT gene sequence (sense, 5′-CACGTTGATATATCCACTCCAGCATCATCATCAT-3′; and antisense, 5′-GCCACTCATCCGATGAAATAGGGCAATAATCCCAAGGAA-3′) were used to generate a 620-bp PCR product from the transgene. PCR was carried out using Taq DNA polymerase (Boehringer Mannheim), 1 μg of blood DNA, and 15 pmol of primers according to the manufacturer’s instructions.

Southern blot hybridization of tail DNA was also performed to confirm the PCR results. Tails were clipped at postnatal day 21, and the genomic DNA was extracted according to Hogan et al. (39). Tail DNA (20 μg) was digested with EcoRI restriction endonuclease, fractionated on 0.8% agarose gel, transfected to GeneScreen Plus membranes (NEN Life Science Products), and hybridized with a 32P-labeled 0.6-kb CAT DNA fragment. Hybridization conditions have been described (41).

**CAT Assay**—To determine the expression of the HGF promoter-CAT reporter constructs in various tissues, CAT activity was analyzed as follows. Tissues were dissected and homogenized in 0.25 M Tris-HCl (pH 7.5) using a Dounce homogenizer. For cell suspensions, cells were pelleted, and their membranes were disrupted using three freeze-thaw cycles. The homogenates were incubated at 65 °C for 5 min and then centrifuged at 15,000 × g for 10 min at 4 °C. The protein concentration was determined using a protein assay kit (Bio-Rad). CAT activity was determined by incubating 20–100 μg of total protein with [3H]chloramphenicol (Amersham Pharmacia Biotech) as a substrate for 24 h as described (42). The acetylated products were separated by thin-layer chromatography (Eastman Kodak Co.) and visualized by autoradiography. densitometric analysis of autoradiographs was performed using the BioImage analytical scanning densitometer (Millipore/BioImage, Bedford, MA) in conjunction with the Whole Band Analysis software package. CAT activity (percent conversion of CAT substrate to acetylated products) was normalized by comparison with protein concentration.

**RNA Isolation and Analysis**—Various tissues were dissected from both wild-type and HGF-CAT transgenic mice anesthetized with methoxyflurane (Pittman-Moore, Mundelein, IL). Total RNA was then isolated using RNAzol B solution (Cinna/Biotex, Friendswood, TX) according to the manufacturer’s instructions. RNA concentration was determined by measuring the optical density at 260 nm. Northern blots were prepared by separating 20 μg of total RNA on formaldehyde-gel electrophoresis. These electrophoretic products were separated by thin-layer chromatography (Eastman Kodak Co.) and visualized by autoradiography. Densitometric analysis of autoradiographs was performed using the BioImage analytical scanning densitometer (Millipore/BioImage, Bedford, MA) in conjunction with the Whole Band Analysis software package. CAT activity (percent conversion of CAT substrate to acetylated products) was normalized by comparison with protein concentration.

**DNA Isolation**—Lung and liver tissues were dissected from control and 70% partially hepatectomized mice; rinsed in ice-cold phosphate-buffered saline; and homogenized in 2 ml of 0.2% Nonidet P-40, 60 mM KCl, 15 mM NaCl, 0.05 mM CaCl2, 3 mM MgCl2, 0.5 mM dithiothreitol, 250 mM sucrose, and 15 mM Tris-Cl (pH 7.4) by 20 strokes in a Dounce homogenizer. Aliquots of 400 μl were then treated with proteinase K, followed by phenol/chloroform extraction and RNA precipitation with ethanol.

**DNase I-hypersensitive Site Analysis**—Lung and liver tissues were dissected from control and 70% partially hepatectomized mice; rinsed in ice-cold phosphate-buffered saline; and homogenized in 2 ml of 0.2% Nonidet P-40, 60 mM KCl, 15 mM NaCl, 0.05 mM CaCl2, 3 mM MgCl2, 0.5 mM dithiothreitol, 250 mM sucrose, and 15 mM Tris-Cl (pH 7.4) by 20 strokes in a Dounce homogenizer. Aliquots of 400 μl were then treated with proteinase K, followed by phenol/chloroform extraction and RNA precipitation with ethanol.
with DNase I (0–30 units; Boehringer Mannheim) for 1 min at room temperature. The reaction was terminated by the addition of EDTA and SDS to final concentrations of 12.5 mM and 0.5%, respectively. DNA was isolated by phenol extraction following a 30-min RNAse A digestion. DNA was then digested with HindIII/EcoRI, run on 1.5% agarose gel, transferred to GeneScreen Plus membranes, hybridized with a DNA fragment that corresponded to the 5′-flanking region of the mouse HGF promoter, and autoradiographed.

RESULTS

Development of the Chimeric HGF Promoter-CAT Transgenic Mice—We generated transgenic mouse lines that harbor various lengths of the mouse HGF promoter region (5′-deletions) fused to the CAT reporter gene. The constructs designated 2.7HGF-CAT (−2674 to +29 bp), 0.7HGF-CAT (−699 to +29 bp), and 0.1HGF-CAT (−70 to +29 bp) (Fig. 1) were used since our previous in vitro transient transfection studies using these constructs revealed that they are transcriptionally active and may contain important regulatory elements (33, 34, 36). Transgenic offspring were screened for germ line integration of the transgene by both PCR amplification of peripheral blood cell DNA and Southern blot hybridization of tail DNA (data not shown). Four lines with germ line integration of the 2.7HGF-CAT transgene construct were propagated. Of these, two lines contained ~10 copies of the transgene, whereas the other two had five copies. Three transgenic founders harboring the 0.7HGF-CAT construct were also established. Estimates of transgene copy numbers indicated that all founders possessing the 0.7HGF-CAT construct contained approximately five copies; however, one founder did not yield germ line transmission. Germ line transmission of the 0.1HGF-CAT construct was propagated in four transgenic lines, two of which had approximately five copies and two of which harbored 10–15 copies of the transgene (data not shown).

Tissue Distribution of HGF-CAT Transgene Expression—To determine whether the HGF promoter fragments described above were transcriptionally active in mice, extracts from various tissues of HGF-CAT transgenic animals (8–10 weeks of age) were isolated and assayed for reporter gene expression by CAT assay and by Northern blot analysis to determine CAT mRNA levels. Tissues from nontransgenic mice were also harvested and analyzed for expression of the endogenous HGF gene by RT-PCR and Northern blot analysis. In the 2.7HGF-CAT mice, we observed a widespread tissue distribution of the reporter gene expression, which mimicked that of the endogenous HGF gene, with nearly all tissues examined expressing a detectable level of CAT activity (Fig. 2 and Table I). A similar expression pattern was observed with the 0.7HGF-CAT transgenic lines, although the expression in the 2.7HGF-CAT transgenic lines was much more robust than that observed with the 0.7HGF-CAT animals, particularly in the kidney, stomach, and intestine (Table I). Interestingly, the 0.1 HGF-CAT transgenic mice did not express the transgene in any tissues assayed from the four independent founder lines (Fig. 3 and Table I). Akin to the expression of the endogenous HGF gene, the highest expression of the transgene was reproducibly noted in the lung, skin, and spleen, especially in the 2.7HGF-CAT mice (Figs. 2 and 3 and Table I).

To substantiate these findings, we analyzed the expression pattern of the endogenous mouse HGF gene in wild-type littersmates by RT-PCR and Northern blot analysis (Table I). Tissues from nontransgenic littersmates were also screened for CAT activity, and none was detected in any of their extracts, as expected (Fig. 3).

Cellular Compartments of HGF-CAT Expression—It is well known that HGF gene expression is mainly confined to mesenchymally derived cells of various tissues. For example, in the liver, the HGF gene is expressed in non-parenchymal cells such as Ito cells, Kupffer cells, and endothelial cells, but not in parenchymal hepatocytes. To define the cellular localization of transgene expression, specific cell types were isolated from HGF-CAT transgenic mice and assayed for CAT expression. Similar to endogenous HGF gene transcription, transgene expression (2.7HGF-CAT and 0.7HGF-CAT constructs) was directed to the non-parenchymal liver cell fraction, but not to the hepatocyte fraction of the liver (Table II).

Previous studies have shown that peripheral blood leukocytes, human promyelocytic leukemia cells (HL-60 cell line),

![Fig. 2. Tissue distribution of 2.7HGF-CAT construct expression in transgenic mice. Shown is a graphic representation of several CAT assay experiments using 50 μg of protein extracted from various tissues of 10-week-old 2.7HGF-CAT transgenic mice. The results are presented as mean CAT activity (percent conversion) ± S.D. from at least four independent experiments. Three out of four independent 2.7HGF-CAT lines expressed the transgene in various tissues.](http://www.jbc.org/)

### Table I

| Tissue | Lung | Skin | Spl | Thy | Utr | Ovr | Tes | Mus | Kid | Stm | Int | Pan | Liv | Hrt | Bra |
|--------|------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| **HGF mRNA**<sup>a</sup> | ++ | ++ | ++ | + | + | + | + | + | + | + | + | + | + | + | + |
| **2.7HGF-CAT (4)**<sup>b</sup> | ++ | ++ | ++ | + | + | + | + | + | + | + | + | + | + | + | + |
| **0.7HGF-CAT (2)** | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **0.1HGF-CAT (4)**<sup>c</sup> | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

<sup>a</sup> Spl, spleen; Thy, thymus; Utr, uterus; Ovr, ovary; Tes, testis; Mus, muscle; Kid, kidney; Stm, stomach; Int, intestine; Pan, pancreas; Liv, liver; Hrt, heart; Bra, brain.

<sup>b</sup> The expression of HGF mRNA in normal wild-type mouse tissues were determined by RT-PCR and/or Northern blot analysis.

<sup>c</sup> Transgene expression was determined by CAT assays and confirmed by RT-PCR and/or Northern blot analysis.

<sup>d</sup> The numbers in parentheses indicate the number of independent transgenic lines for each construct. The highest level of 6.0-kb HGF mRNA and transgene expression was detected in the lung, skin, and spleen and is denoted by +++. + indicates that HGF or CAT expression was barely detectable by Northern blot but readily detected by RT-PCR. − indicates no detectable expression.
and human promonocytic leukemia cells (THP-1 cell line) express the HGF gene and that the expression of HGF in these cells is inducible by 12-O-tetradecanoylphorbol-13-acetate and cytokines such as tumor necrosis factor-α (45, 46). High levels of HGF mRNA and CAT mRNA were detected in both the monocyte- and polymorphonuclear cell-enriched fractions of the spleens of 2.7HGF-CAT and 0.7HGF-CAT transgenic mice (Table II). The spleen mononuclear cell fraction exhibited a significantly higher level of expression of the HGF-CAT transgenes than the polymorphonuclear cell-enriched fraction. Cells isolated by peritoneal lavage and lung lavage also had detectable levels of both endogenous HGF mRNA and CAT mRNA in both 2.7HGF-CAT and 0.7HGF-CAT transgenic mice (Table II).

Since it is well known that skin fibroblasts express the endogenous HGF gene, they were isolated from the 2.7HGF-CAT transgenic mice, cultured, and assayed for CAT activity. CAT assays revealed that these fibroblasts clearly express detectable levels of CAT activity (Table II). In addition, these cultures maintained transgene expression when they were propagated and subcultured up to four passages over the period of 1 month, suggesting that these cells may provide a unique system for studying the transcripational regulation of the mouse HGF gene under conditions of stable transfection.

**Developmentally Dependent Expression of the HGF Promoter—**HGF mRNA and protein are expressed during embryogenesis (17–19), and the proper development of organs such as the liver and placenta is dependent on HGF (20, 21). To examine whether the 2.7-kb fragment of the HGF promoter region is sufficient to dictate HGF promoter activity during embryogenesis, CAT activity was measured in tissues isolated from 2.7HGF-CAT transgenic fetuses at different stages of development.

**The Upstream Region of the HGF Promoter Is Necessary for Its Maximal Inducibility in the Lung and Spleen after Partial Hepatectomy—**It has been well documented by several independent studies that HGF expression is induced in the spleen and lung following partial hepatectomy (22–24). Based on these findings, it has been suggested that HGF also functions in an endocrine manner to facilitate organ regeneration (1–5). Interestingly, in our HGF-CAT transgenic partial hepatectomy models, we noted a significant activation (<3-fold) of the HGF-CAT transgene in the lungs and spleens of the 2.7HGF-CAT transgenic mice and the expression pattern of CAT transgene levels in three genic mice. Shown are the results from a representative CAT assay cells collected by bronchoalveolar lavage; SKF, skin fibroblasts. NPC, hepatocytes and non-parenchymal cells, respectively, isolated from collagenase-perfused livers; PEL, cells isolated by peritoneal lavage; LUL, cytokines such as tumor necrosis factor-α cells is inducible by 12-press the HGF gene and that the expression of HGF in these other tissues.

**Fig. 3. Lack of transgene expression in the 0.1HGF-CAT transgenic mice.** Shown are the results from a representative CAT assay demonstrating the expression pattern of CAT transgene levels in three separate HGF-CAT transgene constructs (i.e. 2.7, 0.7, and 0.1 kb). Protein extract (50 μg) from various tissues of 0.1HGF-CAT, 0.7HGF-CAT, and 2.7HGF-CAT transgenic mice and nontransgenic (NTG) mice was analyzed for CAT expression (see Table I). Representative results of CAT assays are shown for skin, spleen, and lung since these tissues reproducibly exhibited the highest CAT expression as compared with the other tissues.

**Table II**

|                  | MNC    | PMN    | HEP    | NPC    | PEL    | LUL    | SKF     |
|------------------|--------|--------|--------|--------|--------|--------|---------|
| HGF mRNA<sup>a</sup> | ++     | +      | –      | –      | ++     | +      | +       |
| 2.7HGF-CAT* (4)<sup>b</sup> | ++     | +      | –      | –      | ++     | +      | +       |
| 0.7HGF-CAT (2)    | +      | +      | –      | –      | +      | +      | +       |
| 0.1HGF-CAT (4)    | –      | –      | –      | –      | +      | +      | +       |

<sup>a</sup> MNC and PMN, monocytes/lymphocytes and polymorphonuclear cells, respectively, isolated by Ficoll density gradient from spleen; HEP and NPC, hepatocytes and non-parenchymal cells, respectively, isolated from collagenase-perfused livers; PEL, cells isolated by peritoneal lavage; LUL, cells collected by bronchoalveolar lavage; SKF, skin fibroblasts.

<sup>b</sup> The expression of HGF mRNA in normal wild-type mouse cells was determined by RT-PCR and/or Northern blot analysis.

<sup>c</sup> Transgene expression was determined by CAT assays and confirmed by RT-PCR and/or Northern blot analysis.

<sup>d</sup> The numbers in parentheses indicate the number of independent transgenic lines for each construct. Among the cells examined the highest level of 6.0-kb HGF mRNA and transgene expression was detected in the macrophages and lymphocytes isolated from the spleen and the non-parenchymal liver cells and is denoted by ++. + indicates that HGF or CAT expression was barely detectable by Northern blot but readily detected by RT-PCR. – indicates no detected HGF or CAT expression.
genic mice, but only a modest, yet significant increase (1.5-fold) in these organs from the 0.7HGF-CAT animals (Fig. 6).

We also examined HGF promoter activity in another injury response model. We analyzed cells isolated from the peritonea of wild-type and transgenic mice by lavage following the simple abdominal incision and closing used in the partial hepatectomy procedure. The endogenous HGF mRNA was virtually undetectable in cells obtained from peritoneal lavage of unoperated wild-type mice. However, cells obtained from peritoneal lavage of wounded wild-type mice (i.e. those receiving the incision) exhibited significant levels of HGF mRNA expression at 24 h post-operation as determined by RT-PCR (Fig. 7A). Similarly, peritoneal cells from the 2.7HGF-CAT transgenic mice showed markedly induced expression of the CAT transgene in this injury model (Fig. 7B). When the lavaged cells, which consisted mostly of macrophages and occasional polymorphonuclear leukocytes, were separated into adherent and nonadherent cell populations by placing them in culture, CAT expression was mainly detected in the adherent cell population, of which macrophages are the majority (Fig. 7B). Despite the fact that transgene expression was very low (yet detectable) in the peritoneal cells from the 0.7HGF-CAT transgenic lines, absolutely no induction was noted following incision and closure (data not shown).

Our in vivo data clearly demonstrated that the 2.7-kb promoter region is sufficient to confer high and inducible expression to tissues such as lung and liver and that this promoter region behaves in a manner similar to that seen with the endogenous HGF gene promoter. Because of these findings, we performed DNase I-hypersensitive site assays using freshly isolated nuclei from lungs and livers of wild-type mice to define the region(s) in this DNA segment that may bind or be accessible to transcription factors in vivo. The results indicated that at least five hypersensitive sites (HSS) are scattered throughout the 2.7-kb HGF promoter region, which roughly map to positions at -2.2, -1.5, -1.2, -0.7, and -0.3 kb from the transcription start site. These HSS were more prominent in the DNA prepared from the lungs of the operated animals (PHX) as compared with the unoperated controls (Fig. 8). Similar HSS in the 2.7-kb promoter region were also present in the DNA prepared from liver nuclei (data not shown). These data are indicative of protein-DNA interactions and transcriptional activity in the endogenous HGF promoter in vivo.

Since the 0.7HGF-CAT transgenic construct responded to PHX in the liver, lung, and spleen (Figs. 5B and 6) and two injury-induced HSS were detected in this region of the promoter, we surmised that some injury-inducible response elements are localized to this 0.7-kb region. Interestingly, our previous in vitro functional analysis of the 2.7-kb promoter using various 5' and internal deletion constructs localized regulatory element(s) to the -538 to -274 bp region, which

![Fig. 4. Expression of the HGF-CAT promoter construct in tissues of transgenic mice at various stages of development.](http://www.jbc.org/)  

![Fig. 5. Induction of the HGF-CAT transgene in the liver during liver regeneration stimulated by 70% partial hepatectomy.](http://www.jbc.org/)
exerted a strong enhancing effect on the activity of the HGF promoter (33). Thus, we performed gel mobility band shift assays using this fragment of the 0.7-kb HGF promoter as a probe and liver nuclear protein extracts prepared from normal and partially hepatectomized wild-type mice. The probe that corresponded to the promoter region from −538 to −274 base pairs formed a major specific complex with liver nuclear extracts, which increased in intensity at 6 h post-hepatectomy (Fig. 9A). The same nuclear extracts were subjected to gel shift assays using a radiolabeled Sp1 site as a probe to control for integrity of the nuclear protein extracts (Fig. 9B). The complexes formed by the Sp1 probe, which are not induced by PHX, are composed of Sp1 and Sp3 proteins, as we described previously (37). Computer analysis of this −538 to −274 bp DNA segment identified potential AP-1, cAMP, nuclear factor-IL-6, and IL-6 response elements. However, except for the slight competition observed with the nuclear factor-IL-6 site (C/EBP site) and the IL-6 response element (Fig. 9C), none of the binding sites competed strongly with the labeled probe for formation of the major binding complex when they were used as competitors in gel mobility band shift assays.

DISCUSSION

HGF is a pleiotropic growth factor that regulates growth and regeneration of various tissues under normal conditions as well as during neoplastic growth. Its gene expression is restricted to mesenchymally derived cells in a multitude of tissues and is induced in response to tissue loss to facilitate regeneration. HGF gene expression is influenced by some cytokines and induced in response to tissue loss to facilitate regeneration. The expression is developmentally regulated and is induced in response to injury similar to the endogenous gene.

Previous transient transfection investigations from independent laboratories showed that the 0.1-kb basal promoter region (−70 to +29 bp) was necessary and sufficient to efficiently drive the CAT reporter gene in vitro (33, 35). The present study on transgenic mice revealed that the 0.1-kb promoter region is not sufficient to dictate efficient expression in vivo (Table 1). Thus, it is evident that other distal elements, in addition to those present within the first 0.1 kb, are required to enforce HGF expression in vivo. The functional differences observed between transient transfected cells and transgenic animals may reflect a difference in the chromatin structure of the transferred genes. Indeed, in many cases, genes are regulated correctly in transgenic mice, but inappropriately after transfection into cells (47–49). Furthermore, while transfection studies in cell culture models may help define regulatory elements in proximal promoter regions, they may not be able to identify the importance of distal enhancers or locus control regions whose functions are critically dependent on higher order chromatin structure. Such structures help to approximate upstream sequences with their cognate basal promoters to mediate proper and maximal gene activation (50–52). For example, the multiple Sp1 sites identified in the 2.7-kb 5′-flanking region of the HGF promoter may not only function as enhancers (37), but may also facilitate loop formation. This higher order structure may in turn provide additional transcription factors such as C/EBP, the estrogen receptor, and the chicken ovalbumin upstream promoter transcription factor access to their binding sites in the HGF promoter for fine-tuned regulation (36, 38).

In vitro studies of the HGF promoter revealed that the HGF promoter constructs including 2.7HGF-CAT, 0.7HGF-CAT, and 0.1HGF-CAT are active not only in stromal cells such as...
NIH3T3 fibroblasts, but also in epithelial cells such as RL95-2 endometrial carcinoma cells and HepG2 hepatocellular carcinoma cells, which do not express the HGF gene (33, 36). Our current data show that the promoter indeed behaves like the endogenous HGF gene in vivo and that the HGF promoter constructs (2.7 and 0.7 kb) are not expressed in epithelial cells such as hepatocytes (Table II). These results again imply that in vitro data obtained by transient transfection are often misleading in some cases. The data also suggest that the HGF gene promoter in epithelial tissues may be inactivated during tissue development by some mechanisms such as DNA methylation and nucleosome phasing.

In response to certain types of injury and cues that trigger tissue regeneration, many genes are transcriptionally induced or repressed. The HGF gene is induced in the liver as well as in more distal sites such as the lung in response to loss of liver mass induced by PHX (22–24). We have shown that transgene expression driven by the 2.7- and 0.7-kb HGF promoter fragments is also up-regulated in these tissues following 70% hepatectomy (Figs. 5 and 6). CAT activity in the liver increased by 12 h post-hepatectomy, peaked at 24 h, and remained elevated through several days, during which tissue regeneration occurred. Northern blot analysis showed that CAT mRNA levels increased at 6–12 h post-PHX in a manner identical to that seen with endogenous HGF mRNA expression (data not shown). The differential in time between detected CAT activity and mRNA expression is consistent with the lag time for protein synthesis and accumulation.

Our present study indicated that additional response elements lying between −2.7 and −0.7 kb in the HGF promoter enhance the induction of the HGF promoter in a tissue- and cell-specific manner. We based this hypothesis on the following observations. Substantial induction of the 2.7-kb HGF-CAT construct was noted in the lung and spleen as well as the liver after PHX (Figs. 5 and 6). In contrast, the expression of the 0.7-kb HGF-CAT construct was only modestly up-regulated in the liver, lung, and spleen in response to PHX. Analysis of another injury response model revealed that a simple abdominal incision or hepatectomy (which requires such an incision) resulted in a dramatic activation of HGF expression in lavaged peritoneal cells by 24–48 h post-operation (Fig. 7B). Surprisingly, only the 2.7-kb HGF-CAT transgenic construct, but not the 0.7-kb HGF-CAT transgenic construct, was induced in this model. This again implies that an additional regulatory element(s) between −2.7 and −0.7 kb of the mouse HGF promoter, by itself or in concert with other element(s), is responsible for the induction of HGF promoter activity. This claim is supported by the presence of multiple DNase I-hypersensitive sites within the 2.7-kb HGF promoter fragment (Fig. 8), which map to −2.2, −1.5, −1.2, −0.7, and −0.3 kb of the HGF 5′-flanking promoter region.
We have shown that at $-872$ to $-860$ bp in the HGF promoter, an estrogen response element (5'GGTCAGAAAGACC-3') is present. We demonstrated that the chicken ovalbumin upstream promoter transcription factor, a nuclear orphan receptor belonging to the steroid/thyroid hormone receptor superfamily, through binding to this site, effectively silenced the basal transcriptional activity of the HGF promoter (36). The estrogen receptor, on the other hand, relieved the repressive action of the chicken ovalbumin upstream promoter transcription factor, resulting in the induction of the HGF promoter. Injection of estradiol stimulated HGF promoter activity in tissues such as mammary gland and ovary of mice harboring the 2.7-kb region, but not the 0.7-kb region, of the mouse HGF promoter (36). Furthermore, our laboratory recently reported that members of the C/EBP family (especially C/EBPb) of transcription factors bound to a region in the basal HGF promoter (at $+1$ bp) and were responsible for the inducibility of the promoter by cytokines such as IL-6 and tumor necrosis factor-α in NIH3T3 cells in vitro (38). The activity of binding of C/EBPβ and C/EBPδ to this region of the HGF promoter was strongly induced in the liver after partial hepatectomy (38). The fact that the 0.7-kb construct, but not the 0.1-kb construct, was active in vivo and was induced in the liver after PHX suggests that other sites, novel or known, within the 0.7-kb region are responsible for facilitating induction of the HGF gene in this scenario. Of course, regions upstream of 0.7 kb also contributed for maximum inducibility, especially in the lung, spleen, and peritoneal macrophages. In summary, our present results demonstrate that HGF promoter-CAT transgenic mice provide a unique system by which we can analyze the true in vivo transcriptional regulation of the HGF promoter under various normal and pathological conditions and further characterize the important regulatory cis-acting elements and their cognate transcription factors involved in these processes.

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