The human coagulation protease factor VII plays a pivotal role in the initiation of the coagulation cascade by both the extrinsic and the intrinsic pathway. Although the gene, encoding factor VII, is expressed predominantly in the liver, the mechanisms underlying this tissue-specific expression have not been elucidated. In this study, we have analyzed the contribution of 5 kilobases upstream of the ATG translational initiation codon upon hepatic factor VII gene transcription. Transient transfection assays of a set of nested deletions in both liver and non-liver cell lines, HepG2 and HeLa respectively, indicate that several regions are involved in liver-specific expression. A slight negative effect on factor VII promoter activity in HepG2 cells is mediated by sequences upstream of position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNase I protection experiments reveal six footprints, FVVII1 through FVVII6, within the proximal 714 base pairs but a minimal promoter of 165 base pairs containing only FVVII3–6 is sufficient to confer liver-specific expression in HepG2 cells. Interestingly, FVVII6, at position –22988, binds specifically to footprint FVVII4 at position –1212. DNA sequences reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U14580. † To whom correspondence should be addressed. Tel.: +41-61-69-66-412; Fax: +41-61-69-66-323; E-mail: erdmanf@imi.ch.
† The abbreviations used are: kb, kilobase(s); bp, base pair(s); apo, apolipoprotein; EMSA, electrophoretic mobility shift assay; FVVIIc, factor VII procoagulant activity; FVVIIag, factor VII antigen; IL1-β, interleukin 1-β; IL6, interleukin 6; wt, wild-type; PCR, polymerase chain reaction; CMV, cytomegalovirus.

The human factor VII gene is a single copy gene on chromosome 13q34, 3 kb upstream of the coagulation factor X gene (2, 3), which consists of eight exons spread over 12 kb of genomic DNA, which produces a 2.4-kb mRNA encoding a mature protein of 254 amino acids (4).

A positive correlation between high plasma factor VII antigen levels (FVVIIag), procoagulant activity (FVVIIc), and an increased risk of coronary heart disease has been demonstrated (5). Although both increased FVVIIag and FVVIIc levels are associated with other risk parameters such as higher age, use of oral contraceptives, and plasma triglyceride and cholesterol (see Ref. 6 and references therein), the picture is complicated as very low density lipoprotein and low density lipoprotein particles and FVVIIag levels (8–10). Intriguingly, males heterozygous for an Arg353 to Gln353 polymorphism found in about 20% of the caucasian population have FVVIIag concentrations reduced by 20–25%, which presumably reduces the risk of coronary heart disease and thrombosis by lowering the proportion of factor VII molecules being in the activated state (11, 12).

From these prospective studies it is possible that a drug that interferes with factor VII expression levels could be an anti-thrombotic, and the first step toward such a drug would be to understand the regulatory mechanisms of factor VII expression. As only 522 nucleotides of the factor VII 5’-flanking region have been published (4), we have cloned and sequenced a further 4291 bp and functionally characterized this sequence in reporter gene assays. We have identified cis-acting regulatory sequences in the promoter by DNase I footprinting analysis, and we show that HNF-4 is a positive regulator of factor VII expression.

**MATERIALS AND METHODS**

Isolation and Sequencing of a Recombinant Cosmid Clone—Sequence from the promoter previously published extends only 522 bp upstream of the ATG (4). A 654-bp fragment was amplified from human genomic DNA (Clontech) by PCR and sequenced. This showed some differences with the published sequence which included a 10-bp insertion (CCTATATCCT) located at position –324 with respect to the ATG that has been previously described as a polymorphism by Marchetti and co-workers (13). To obtain additional 5’-flanking sequences, six aliquots of 1 × 106 clones each of a human placenta genomic DNA cosmid library (Clontech) were screened by PCR using the same primers. One positive aliquot was subjected to screening by colony hybridization using the PCR fragment as probe, and one positive cosmid clone was obtained. Based on restriction digests and Southern blotting the clone was estimated to contain at least 5 kb of 5’-flanking sequences. A 3.5-kb EcoRI fragment and a 0.8-kb SmaI fragment overlapping each other in the already known sequence were subcloned into vector pBluescript® (Stratagene). To recombine both fragments, the 3.5-kb EcoRI fragment was cloned into the EcoRI sites in the vector containing the 0.8-kb SmaI fragment (Fig. 1). Additional 5’-flanking sequences were subcloned as a 2.5-kb Apal fragment that overlaps the EcoRI fragment. Enzymatic sequencing of both strands was performed by both ExoIII/S1-generated nested deletion templates (Erase a base kit, Promega) or primer walking techniques.
Construction of Plasmids—The luciferase reporter gene plasmids of the pGL2 series were obtained from Promega, and reporter plasmids were constructed in several steps. First the factor VII open reading frame was deleted by subcloning the proximal sequence as a 280-bp EcoRI/HindIII fragment into EcoRI/Smal of pBlKKS. In the next step the 3.5-kb EcoRI fragment was inserted in order to obtain a long consecutive promoter fragment.

Starting from this construct a 1.4-kb KpnI/BgII promoter fragment and exon 1/5I deletion derivatives were ligated into the KpnI/BamHI sites of the promoterless luciferase vector pGL2-basic (pUC-1.6/-34, pUC-1.2/-34, pGL-474/-34, see Fig. 3).

Larger promoter constructs were obtained by inserting the original KpnI promoter fragment as an exon 1/5I deletion derivative into a single KpnI site in plasmid pUC-1.6/-34 giving pUC-3.9/-34 and pUC-2.5/-34 (Fig. 3). The plasmids pUC-981/-34 and pUC-712/-34 are Apal and Smal deletion derivatives of pUC-1.2/-34, respectively.

To generate the plasmids pUC-237/-34 and pUC-165/-34, the PstI or NsiI sites in the progresor plasmid pGL-474/-34 were treated with T4 DNA-polymerase and fused to Smal. Construct pUC-2.5/-40 was generated by blunt end fusion of a PCR product amplified from promoter and luciferase parts of pUC-712/-131 and replacing the promoter-luciferase EcoRI fragment in pUC-2.5/-34. Plasmid pUC-712/-131 itself originates from inserting the Smal digested Smal fragment into the filled-in HindIII site of pGL2-basic. All PCR fragments were sequenced to rule out amplification artifacts.

The plasmids pUC-747/-34Leyden and pUC-165/-34Leyden were generated by replacing the StyI-NcoI fragments containing the putative HNF-4 site by a double-stranded oligonucleotide that contained the Leyden-specific point mutation (Table I). The mutation was also introduced into plasmid pUC-1.6/-34 by replacing the EcoRI fragment that contained the HNF-4-binding site by the corresponding fragment from pUC-747/-34Leyden. All plasmid derivatives containing the SV40 enhancer were generated by replacing the BamHI-HindIII vector fragment, which contains luciferase gene following by the SV40 T antigen enhancer/SA polymerase

Cell Culture and Transfections—Human hepatoma cells HepG2 (14) and HeLa cells were obtained from the American Type Culture Collection. COS-7 cells and L132 cells were taken from our in-house collection. HepG2 cells were cultured in Dulbecco's modified Eagle's medium without F12 nutrient mix 1:1 (Life Technologies Inc.) supplemented with 10% fetal calf serum (Boehringer Mannheim). HeLa cells, L132 cells, and COS-7 cells were grown in minimal essential medium with 5% fetal calf serum. Both media were supplemented with gentamicin and cells were grown in a 10% CO2 atmosphere.

COS-7 cells were transfected with Lipofectin Reagent (Life Technologies Inc.) following the manufacturer's protocol. Transient transfections into HepG2 and HeLa cells were performed by the calcium phosphate coprecipitation technique as described by Ausubel et al. (15). Transfection experiments with transfected HepG2 cells were started 24 h after glycerol shock. Cells were harvested after incubation for 0.5, 1, 3, 6, 12, and 24 h with either one, or combinations, of the following substances: 100 units/ml IL-6, 100 units/ml IL-1β, 1 µM dexamethasone, 100 µM dexamethasone, 13-acetate, 10 µM forskolin, 0.4 M sodium carbonate, and grown in fresh medium for 48 h. In HeLa cell transfections the glycerol shock was omitted. Cells were harvested using 150 µl of reporter lysis buffer (Promega) according to the manufacturer’s recommendations.

Induction experiments with transfected HepG2 cells were started 24 h after glycerol shock. Cells were harvested after incubation for 0.5, 1, 3, 6, 12, and 24 h with either one, or combinations, of the following substances: 100 units/ml IL-6, 100 units/ml IL-1β, 1 µM dexamethasone, 100 µM forskolin, 13-acetate, 10 µM forskolin.

Luciferase and β-galactosidase Assays—Luciferase assays were carried out in microtiter plates using the Luciferase assay system (Promega) as described in the manufacturer’s protocol. β-Galactosidase assays were performed in microtiter plates as follows: 100 µl of prewarmed assay buffer (40 mM Tris-Cl, pH 7.5, 2 mM MgCl2, 0.5% Nonidet P-40, 0.5% Triton X-100) were added to 20 µl of cell extract and incubated at 37°C for 15 min. The reaction was stopped by adding 75 µl of 1 M sodium carbonate and the A500 determined. Luciferase data were normalized with respect to β-galactosidase values in order to correct for differences in transfection efficiency.

RNA Isolation and Northern Blotting—Single step RNA isolation from cultured cells and Northern blotting were done as described in Ref. 15. Radioactive hybridization was performed with [32P]dCTP-labeled DNA fragments in 5 × SSPE, 10 × Denhardt's, 100 µg/ml single-stranded DNA, 50% formamide, and 2% SDS at 42°C. After 16 h of incubation, the filters were washed with 2 × SSC, 0.5% SDS for 5 min at room temperature and 2 times 30 min at 42°C. For non-radioactive hybridization DIG-labeled RNA probes were used according to the manufacturer’s recommendations (Boehringer Mannheim).

DNase I Footprint Assays—Nuclear extracts from HepG2, HeLa, L132, and COS-7 cells were prepared by the method of Dignam et al. (16) with minor modifications as described by Ausubel et al. (15).

DNase I footprints were performed in a total volume of 50 µl containing 20 mM HEPES, pH 7.5, 30 mM KCl, 4 mM MgCl2, 0.5 mM EDTA, 0.5 mM dithiothreitol, 4% glycerol, and 1.5 µg of poly(dI-dC)poly(dI-dC). Nuclear extracts containing 10–60 µg of total protein were incubated for 15 min at room temperature. One to two ng of end-labeled fragments (1–2 × 105 counts/min) were added and incubated for another 15 min at room temperature. Limited digestion was achieved by adding 5 µl of Ca2+/Mg2+ solution (final concentration 1 mM MgCl2, 0.5 mM CaCl2) and 0.33–1 units of freshly diluted DNase I for 1 min at room temperature. The reaction was stopped by 140 µl of DNase I stop buffer (192 mM sodium acetate, 32 mM EDTA, 0.14% SDS, and 64 µg/ml yeast RNA). The DNA was treated with phenol, ethanol precipitated, and analyzed on 6% polyacrylamide, 7 M urea sequencing gels.

Electrophoretic Mobility Shift Assays—Duplex oligonucleotides were labeled at both ends by filling in with Klenow fragment of DNA-polymerase I. Ten to 30 µg of crude nuclear extracts were preincubated in presence of 2 µg of poly(dI-dC)poly(dI-dC) in a total volume of 25 µl of DNase I footprint buffer at room temperature. After 10 min, 0.25 ng of labeled oligonucleotide (3 × 105 counts/min) were added, and incubation was continued for an additional 10 min. Protein-DNA complexes were fractionated on 5% polyacrylamide gels (29:1, 0.5 × Tris-borate, 5% glycerol) with 0.4 × TBE running buffer at 4°C.

Antibody Super Shift Assays—The electrophoretic mobility shift assays were done with HNF-4 antisem kindly provided by Dr. Francis Sladek (University of California, Riverside). One µl of undiluted antisem was added to 10 µg of crude nuclear extract preincubated with labeled oligonucleotide.

RESULTS

5' Flanking Sequence of the Human Factor VII Gene—Based on the limited published sequence (4) we cloned a PCR fragment of 523 bp immediately upstream of the factor VII translational initiation codon from human genomic DNA (Clontech). This clone identified one positive clone cosSVII on screening a human placenta cosmid library. The cosmid DNA, which was shown to be colinear with genomic DNA by Southern blotting using the PCR fragment as a probe, contains at least 5 kb of the 5' flanking sequence which was sequenced on both strands. Compared to the published sequence additional nucleotides were found, an additional C at –140 and an additional G at position –460 (Fig. 1), which were probably not detected previously due to band compressions typically found in GC-rich sequences.

The 5'-flanking sequence has neither a typical TATA nor CAAT box as found in the promoters of the other coagulation proteins: factor IX (17), X (3, 18), XII (19), and prothrombin (20, 21).

A homology search of the 4813-bp 5'-flanking sequence against the EMBL databank identified three Alu repeats at position –4742 to –4440, –2739 to –2518, and –942 to –642. Sequence alignment of the factor VII and factor X promoters revealed a similarity of 86% for a small 37-bp element located in factor VII promoter at position –2340 to –2304 and in the factor X promoter in the same orientation at position –520 to –485. The functional relevance, if any, of this element is unclear as deletion of these sequences in the factor X or factor VII promoters did not alter reporter gene expression significantly (see Fig. 3 and Refs. 3, 18).

Tissue Distribution of Factor VII Transcription—In order to determine the tissue distribution of factor VII expression, we...
Transcriptional Control of the Factor VII Gene in HepG2 Cells—To investigate the transcriptional regulation of factor VII, a promoterless luciferase reporter gene in the vector pGL2-basic was fused to a variety of promoter fragments at position -34 as a HindIII/Smal fusion which is 3' of the transcriptional start site (Fig. 3). The constructs were all transfected into human hepatoma HepG2 cells, known to express factor VII (22), as well as HeLa and COS-7 cells, and transient promoter activity was monitored by measuring luciferase activity in cell extracts. Differences in transfection efficiencies were corrected for by cotransfection of pCMVβ that carries the β-galactosidase reporter gene under the control of the CMV promoter. As negative control the promoterless plasmid pGL2-basic was used while as a positive control plasmid the luciferase gene was transcribed from the SV40 early promoter-enhancer in pGL2-control which gave rise to very high luciferase values in all three cell lines tested. In HepG2 cells, expression from the factor VII promoter in plasmid pLUC-3.9/-34 was significantly higher than the negative control pGL2-basic, but, even in hepatocytes, the factor VII promoter is remarkably weak as compared to the SV40 promoter, which gave about 50-fold higher luciferase values.

Deletion of sequences from -4813 down to position -1601 did not significantly alter luciferase expression, but a longer deletion to position -1212 doubled luciferase expression (Fig. 3). Further truncation of the promoter down to position -165 retained the high promoter activity of pLUC-981/-34, but a longer deletion to position -46 drastically reduced promoter activity down to values seen with the promoterless control vector. These results suggested that the first 165 bp upstream from the translational initiation codon are sufficient to confer expression in hepatocytes. Activity was restored to 73% on plasmid pLUC-474d3/-34 by sequences from 461 to 355, although the presence or absence of this fragment on the longer constructs pLUC-474/-34, pLUC-237/-34, and pLUC-165/-34 had no impact on expression.

Hepatocyte Specificity of Factor VII Expression—Direct comparison of expression data from HepG2 and HeLa cells was difficult because transfection efficiencies differed by a factor greater than 20, as judged from β-galactosidase values, but expression from the factor VII promoter was detected in both cell lines. Virtually no promoter activity was detectable in COS-7 cells as none of the factor VII promoter constructs expressed luciferase better than the negative control pGL2-basic (data not shown). In HeLa cells sequential truncation of the promoter led to a steady reduction in activity down to about 50% of the original values in pLUC-165/-34 and to 10% in plasmid pLUC-46/-34 (Fig. 3) which does not identify any specific promoter in the 5'-flanking sequence. Constructs containing the heterologous SV40 enhancer were more informative as only the constructs containing sequences upstream of position -1212 can be activated by a factor of three to four by the SV40 enhancer, which suggests that the enhancer interacts with some promoter-like structures located upstream from position -1212 (Fig. 3).

Identification of Regulatory Sequences in the Factor VII Promoter—A homology search for putative transcription factor-binding sites, using the Findpatterns algorithm of the CCG software package on the data base Transcription Factor Sites Release 6.5 (23, 24), revealed four putative Sp1 binding sites at positions -2295, -1862, -1847, and -1785 and consensus sequences for AP-1 binding at position -2488, -1006, and -750 although no inducibility of factor VII expression by phorbol esters was observed (data not shown). A putative C/EBP-β/NF-IL6-binding site at position -690 to -682 seems not to be

FIG. 1. Partial nucleotide sequence of the human factor VII 5'-flanking region. 613 nucleotides out of 4813 nucleotides cloned and sequenced are shown. The most 5'-nucleotide at position -523 previously published is indicated by an asterisk. Additional nucleotides not present in the published sequence are shown in bold. Restriction sites important for cloning purposes are shown above the sequence. The amino acid sequence of the first exon is shown.

![Diagram A](image1)

![Diagram B](image2)

FIG. 2. Tissue distribution of factor VII transcripts determined by a multiple tissue Northern blot. The multiple tissue Northern blot purchased from Clontech was hybridized with a randomly labeled factor VII cDNA fragment corresponding to codons -60 to +152 (panel A) and with a β-actin-specific probe (panel B), respectively. Lane 1, heart; 2, brain; 3, placenta; 4, lung; 5, liver; 6, skeletal muscle; 7, kidney; 8, pancreas. A strong signal corresponding to a transcript of the correct size is visible in liver (lane 5).
relevant as adding IL6, IL1-β, and combinations of IL6 with IL1-β or dexamethasone did not effect transient transfection assays nor RNA levels measured by Northern blots. This is consistent with a report by Hassan et al. (25) that IL6 does not affect factor VII mRNA levels in Hep3B cells. The find patterns search for cis-acting regulatory sequences did not identify any further putative binding sites for liver-enriched transcription factors, but when repeated with more degenerated consensus sequences a potential binding site for the liver-enriched transcription factor HNF-4 at position -2767 to -256 was seen. 

HNF-4 Binds to the Factor VII Promoter—The transcription factor HNF-4 (26) has been shown to be involved in liver-specific expression of coagulation factors IX (17, 27) and X (3, 18). So, to investigate whether the presence of the putative HNF-4-binding site is of any significance for factor VII transcriptional regulation, electrophoretic mobility shift assays (EMSA) were performed with double-stranded 32P-labeled oligonucleotides that correspond to wt factor VII sequence from -2777 to -243 and a mutated oligonucleotide carrying a T → A transversion (Table I). EMSA analysis with nuclear extracts from HepG2 cells revealed a concentration-dependent shift of protein-DNA complexes as compared to free probe (Fig. 4A, lanes 1–4, C, and F). This shift could be specifically competed for by preincubation with unlabeled wt oligonucleotide (lanes 5 and 6), but not by the T → A transversion mutant oligonucleotide (lane 7) which when present in the factor IX promoter causes the Leyden phenotype of factor IX deficiency (27). The affinity of a DNA-binding protein to this mutated oligonucleotide was strongly reduced in our experiments (lane 9) and could be competed for much more efficiently by the wt sequence as compared to the mutated oligonucleotide itself (lanes 10 and 11) demonstrating that protein binding is highly specific and dependent on the sequence of the binding site. 

To prove unambiguously that the protein binding is indeed HNF-4, we repeated the EMSA assays in the presence or absence of an antiserum raised against HNF-4 (26). The slower migrating complex obtained with HepG2 nuclear extracts (Fig. 4B, lane 2, C) was supershifted upon addition of the HNF-4 antiserum (lanes 3 and 4, S). In contrast, the slower migrating complexes obtained with crude nuclear extracts from HeLa, L132, and COS-7 cells (lanes 5, 7, and 9) that could be competed for by cold oligonucleotide (data not shown) were not recognized by the anti HNF-4 antiserum (lanes 6, 8, and 10). 

To further investigate the functional role of the HNF-4 site in HepG2 cell experiments, we replaced the Sty/Ncol fragment containing the wt-binding site in plasmid pLUC-165/-34 and pLUC-474/-34 by an oligonucleotide that contains the Leyden-specific point mutation (Table I). Out of the longer resulting plasmid pLUC-474/-34 "Leyden," we subcloned the mutated putative HNF-4-binding site into pLUC-1.6/-34 and assayed all three constructs by transient transfection assays. This point

| Relative luciferase activity % | HepG2 | HeLa |
|-----------------------------|-------|------|
|                            | + SV40 enhancer | + SV40 enhancer |
| pLUC-1.6/-34 | 88 ± 5 | ND | ND | ND |
| pLUC-3.3/-34 | 88 ± 5 | ND | ND | ND |
| pLUC-2.5/-34 | 100 | 620 ± 109 | 100 | 277 ± 55 |
| pLUC-1.6/-34 | 100 | 377 ± 35 | 70 ± 19 | 283 ± 240 |
| pLUC-1.2/-34 | 106 ± 28 | 765 ± 134 | 88 ± 22 | 172 ± 39 |
| pLUC-0.8/-34 | 223 ± 107 | 833 ± 16 | 238 ± 192 | 205 ± 42 |
| pLUC-712/-34 | 167 ± 24 | ND | ND | ND |
| pLUC-474/-34 | 179 ± 49 | ND | ND | ND |
| pLUC-474d1/-34 | 145 ± 52 | 653 ± 29 | 73 ± 23 | 88 ± 4 |
| pLUC-474d2/-34 | 152 ± 33 | 828 ± 38 | 61 ± 20 | 76 ± 6 |
| pLUC-474d3/-34 | 155 ± 30 | 839 ± 55 | 42 ± 14 | 60 ± 10 |
| pLUC-465/-34 | 8 ± 2.4 | 40 ± 4.8 | 10 ± 3 | 35 ± 7 |

**Fig. 3.** Factor VII promoter deletion analysis by luciferase reporter gene assay. Left, schematic drawing of the factor VII promoter sequences used in transient transfection experiments. The 5′-most nucleotide of each factor VII promoter derivative either generated by restriction enzyme digests or exonucleaseIII/S1 treatment is indicated by its position number with reference to the translational initiation codon. Factor VII promoter sequences are shown as a single line. Double lines represent vector sequences. Plasmids named by the extension "Leyden" differ from their progenitor plasmids by a single base exchange in the putative HNF-4-binding site as indicated by an A. Internal deletions in plasmids pLUC-474d1 through d3 are indicated by gray boxes and position numbers. Right, relative luciferase expression in percent of pLUC-3.3/-34 expression as means ± standard deviation of four to eight independent transfections with at least two DNA preparations in HepG2 and HeLa cells, respectively. ND, not determined.
Table I
Sequences of double stranded oligonucleotides used in EMSA assays

| A.                     | B.                     | C.                     |
|------------------------|------------------------|------------------------|
| TGGAGCCGAGAAGCTTTGCCCCGTCAG | CTCCGGCTCTTGGAAACGGGCACTGAGGTAC | AGGCTGGAGCTTTCATCCAGGTCAG |
| −77                    | −47                    | +12                    |

For EMSA assays the 5′-protruding ends were blunted and labeled with Klenow polymerase. A, wild-type sequence comprising the HNF-4 binding site, B, analogous to the "Leyden" phenotype of factor IX deficiency the thymidine at position −59 was replaced by adenine (bold letter). C, oligonucleotide comprising the binding site for the as yet unidentified factor causing footprint FPVII6. The translational initiation codon is underlined.

mutation reduced the promoter activity to about 80−50% of the particular wt plasmid in HepG2 cells (Fig. 3) which confirms the functional importance of the HNF-4-binding site for liver-specific expression.

The Factor VII Promoter Is Regulated by Additional Cis-acting Regulatory Sequences—To identify further cis-acting regulatory sequences in the promoter, DNase I protection assays were performed. Besides the footprint FPVII4 originating from HNF-4 on the antisense strand at position −50 to −76, five more regions protected against DNase I digestion were detected (Fig. 5). All footprints were confirmed on the sense strand, on which HNF-4 protects position −49 to −71 (data not shown), but none of additional sites were similar to known cis-acting regulatory sequences. The strong footprint FPVII6, at position −13 to +9, interestingly covers the ATG translational initiation codon, and we have tested the specificity of this footprint in EMSA experiments using two complementary oligonucleotides spanning nucleotides −20 to +12 on the sense strand and nucleotides +14 to −18 on the antisense strand (Table I). A protein-DNA complex produced on incubation with HepG2 nuclear extracts (Fig. 6, lanes 1–3, C) was shown to be specific as unlabeled double-stranded oligonucleotide competed successfully (lane 4), but the HNF-4 oligonucleotide did not (lane 5). In COS-7 cell nuclear extracts a distinct but different binding activity was present (lane 6), leading to a complex with different migration pattern.

None of the promoter-reporter fusion discussed above contained this cis-acting site. To test the functional importance of this site, we constructed the new plasmid pLUC-2.5/+40, where the translational initiation codon of the luciferase gene was fused to position +40 in the factor VII coding region. In transient transfection experiments, luciferase expression from construct pLUC-2.5/+40 was 1.9-fold higher than from plasmid pLUC-2.5/+34 (Fig. 7). Furthermore, when the first factor VII exon and part of the first intron up to position +131 were included in plasmid pLUC-712/+131 reporter gene expression was about 2.7 times higher than from plasmid pLUC-712/-34. These experiments suggest that sequences downstream of the putative HNF-4-binding site and the first exon are also involved in expression of factor VII.

Discussion
During the past few years, a number of studies on promoters of coagulation proteases like prothrombin (20, 21, 28), factor IX (27, 29–39), and factor X (3, 18) have been published. The regulation of factor IX gene expression is of particular interest, since the Leyden phenotype of the severe bleeding disorder hemophilia B is caused by several point mutations within the promoter region, of which a number map in the HNF-4-binding site, reducing the affinity of HNF-4 for the promoter. Both the factor IX and the factor X promoter are positively regulated by the liver-specific transcription factor HNF-4.

In this work we show that measurable amounts of factor VII transcripts are present only in liver suggesting that regulation takes place at the transcriptional rather than at the translational level (Fig. 2), and we have defined promoter elements involved in liver-specific expression of the coagulation factor VII present in the 4813 bp of factor VII 5′-flanking sequence.

Factor VII expression is tissue-specific—Factor VII promoter transient transfections showed cell line specificity of luciferase expression to human hepatocyte cell line HepG2 (Fig. 2). In contrast, the factor VII promoter is not active at all when compared with the promoterless vector pGL2-basic in non-human COS-7 cells. Although HeLa cells did express luciferase from the factor VII promoter, it seems likely that in HeLa cells the slight decrease of expression upon successive truncations is the effect of a rather unspecific fortuitous transcription initiation in HeLa cells, which is further substantiated by the observation that the heterologous SV40 enhancer only increases expression in HeLa cells when present with 1.6 kb of promoter whereas it increases expression in HepG2 cells with a promoter fragment of only 165 bp (Fig. 3). Thus it is unlikely that identical sequences act as promoters in HepG2 and HeLa cells.

A number of studies show that triglyceride and cholesterol levels in plasma are positively correlated with the risk for coronary heart diseases and thrombosis (5) and the plasma levels of lipoproteins and activity of vitamin K-dependent coagulation proteases are positively correlated with lipid concentrations in plasma (8–10). The apolipoprotein genes AII, CIII, and AI form a gene cluster, and, since coregulation has been shown, it has been hypothesized that apoCIII, AII, AIV, and E have evolved from a common ancestor (40, 41). Interestingly, the genes encoding factor VII and factor X are also clustered and separated by only 2823 nucleotides (3). The significant similarity of vitamin K-dependent blood coagulation proteins suggests that the genes could have also evolved from a common ancestral gene and that they might be regulated by a common mechanism. The close correlation of FVIIa level and activity of factor IX, factor X, and prothrombin with the lipoprotein metabolism tempted us to compare the factor VII promoter and promoters of apolipoprotein genes and coagulation proteases factor IX and X with respect to common regulatory mechanisms.

Factor VII expression is regulated by several factors—Functional studies of promoter deletions in the HepG2 cell line showed that deletion of sequences from −1601 down to −1212 increases expression about 2-fold which suggests a negative element is present upstream of −1212 (Fig. 3). A 25-bp sequence element at position −1230 to −1214 that has a similarity of 76% to position −122 to −98 of the apoAIV promoter element AIVC is a good candidate for a binding site of such a negatively acting factor. The element AIVC was defined by a footprint covering position −148 to −92 in the apoAIV promoter, and proteins binding to the 5′-half of the motif at
In our experiments the proximal 165 bp upstream from the ATG translational initiation codon were sufficient for liver-specific expression (Fig. 3), and DNase I protection assays on the proximal 714-bp promoter fragment revealed a set of six footprints of which four map in the first 145 nucleotides (Fig. 5). Removal of the protein-binding sites VII1 and 2 at a distance from 380 to 450 from the translational initiation codon had no effect on the transcriptional activity (compare pLUC-474/-34topLUC-237/-34). However, the presence of these binding sites might account for the higher promoter activity in plasmid pLUC-474d3/-34 as compared to pLUC-46/-34 (Fig. 3).

Including sequences up to position +40 in the promoter-luciferase construct pLUC-2.5/+40 raised expression levels to about two times the values obtained with the plasmid pLUC-2.5/-34 (Fig. 7). The effect is cell line specific as almost no expression from this plasmid is detectable in HeLa cells. As demonstrated by the strong footprint FPVII16 in DNase I protection experiments (Fig. 5) and by EMSA experiments (Fig. 6), an as yet unidentified regulatory protein binds specifically to this sequence which covers the ATG translational initiation codon.

Inclusion of the entire factor VII exon I and the 5′-terminal part of the first intron in plasmid pLUC-712/-34 has a similar effect; plasmid pLUC-712/-34 and pLUC-712/+131 differ

---

**FIG. 4. EMSA of the HNF-4-binding site with crude nuclear extracts.** A, a double-stranded end-labeled oligonucleotide, with nucleotides -77 to -43 containing the HNF-4-binding site was incubated with crude nuclear extracts from HepG2 cells. Lane 1, free oligonucleotide (F) without added nuclear extract; lanes 2–4, incubated with 10, 30, and 60 µg of HepG2 nuclear extract, respectively (C indicates the protein-DNA complex); lanes 5 and 6: incubation with 30 µg of extract and competition by 20- and 400-fold molar excess of unlabeled wt oligonucleotide; lane 7, competition by 400-fold molar excess of unlabeled Leyden-specific oligonucleotide; lane 8, free Leyden-specific oligonucleotide; lane 9, with 30 µg of HepG2 nuclear extract; lanes 10 and 11, competition by 400-fold molar excess of mutated and wt oligonucleotide, respectively. B, the free HNF-4 oligonucleotide (lane 1, F) was retarded by incubation with 10 µg of HepG2 nuclear extracts (lane 2, C) and supershifted by 1 and 2 µl of undiluted HNF-4 antiserum, respectively (lanes 3 and 4, S); lanes 5, 7, and 9, the same oligonucleotide with HeLa, L132, and COS-7 cell nuclear extracts, respectively; lanes 6, 8, and 10, after incubation with HNF-4 antiserum. A supershift of the protein-DNA complex did not occur.

**FIG. 5. DNase I protection assay of the factor VII promoter antisense strand.** Prior to DNase I digestion the labeled fragment (U lane) was incubated either with bovine serum albumin (B lane) or with 10, 20, 30, 40, and 60 µg of HepG2 nuclear extract, respectively. G/A denotes G- and A-specific Maxam-Gilbert sequencing reaction. Footprints FPVII3–6 protected from DNase I digestion are indicated by squares. The antisense strand (shown here) of promoter fragment -314 to -129 was labeled by filling in the EcoRI site with [α-32P]dATP. Incorporation of [α-32P]dCTP into the AvoI end of this fragment labeled the sense strand which gave similar results. Footprints FPVII11 and 2 were identified on antisense and sense strands of promoter sequences from position -714 to -315 (data not shown).
2.7-fold (Fig. 7). This effect is slightly more pronounced than in the plasmid pair pLUC-2.5/-34 and pLUC-2.5/-40 which argues for an additional regulatory function located within exon I or the 5'-end of intron I. This is similar to transcriptional regulation of von Willebrand factor which has a positive regulatory region located within the first exon (42) and in apoB where HNF-1 and C/EBP bind to an enhancer in the second intron (43).

C/EBP-α and related factors that recognize the same binding sites as C/EBP-β play an important role in expression of apolipoproteins A1 (44–46), AII (47, 48), B (49–51), and coagulation factor IX (32, 33, 38), and we anticipated a similar function of C/EBP-α at position −690 to −682 at the putative C/EBP-β/NF-IL6-binding site in the factor VII expression. However, the DNase I protection assay performed on the factor VII promoter did not show a footprint over the putative C/EBP-binding site (see below), and in transient transfections deletion of this binding site in plasmid pLUC-474/-34 does not alter expression when compared to pLUC-712/-34 (Fig. 3) and probably does not mediate acute phase response of factor VII expression by C/EBP-β as examined by induction and cotransfection experiments (data not shown). One explanation for the lack of this interaction could be the very low C/EBP concentration in HepG2 cells, which also accounts for the weak factor IX expression in HepG2 cells (32).

Transcription Factor HNF-4 Binds to the Factor VII Promoter—One of the footprints which we detected corresponds to the liver-enriched transcription factor HNF-4. HNF-4 is an orphan member of the steroid hormone receptor superfamily which has been highly conserved throughout evolution, suggesting that it might respond to an as yet unidentified ligand. Initially, it was characterized as a factor that activates apolipoprotein CIII and transthyretin (TTR) gene expression (26, 52) and it also positively regulates numerous genes (for a review, see Ref. 52). HNF-4 exists in two different isoforms as a result of differential splicing from which the largest is by far the most common in HepG2 cells (53). Despite the presence of binding activity specific for the HNF-4 site in all cells investigated, HNF-4 is mainly restricted to hepatocytes. With high levels in liver and moderate levels in kidney and intestine, the expression pattern of HNF-4 reflects the tissue distribution of genes regulated by HNF-4 (54). HNF-4 has been demonstrated to confer liver-specific expression on coagulation proteases factor IX (30, 33, 34) and factor X (3, 18). A number of point mutations in the factor IX promoter between position −221 and +13 cause the Leyden phenotype of the severe bleeding disorder hemophilia B (Table I; (26, 31, and references therein), and the mutations at position −221 and −20 disrupt the HNF-4-binding site. As shown by our DNase I protection experiments and EMSA assays, HNF-4 specifically binds to position −71 to −49 on the factor VII promoter (Figs. 4 and 5).

In order to prove the functional importance of the HNF-4-binding site for factor VII expression, we introduced the Leyden-specific +20 T to A mutation (ACTTTG → ACTTAG; Tables I and II) into the HNF-4-binding site of the factor VII promoter which lowered promoter activity in HepG2 cells by 20–50% (Fig. 3) and reduced the binding of HNF-4 in crude nuclear extracts by 50% (Fig. 5). The HNF-4 oligonucleotide fails to compete for binding (lane 5). In COS-7 nuclear extracts a distinct but different binding activity is present (lane 6).

Relative luciferase activity %

HepG2 | HeLa
---|---
95 ± 8 | 70 ± 19
186 ± 33 | 35 ± 0.5
179 ± 49 | ND
480 ± 37 | 88 ± 3.6
In the factor IX HNF-4-binding site, the point mutations causing the Leyden phenotype of hemophilia B are underlined.

| Sequence | Gene | Element |
|----------|------|---------|
| CAGGAGACTTTCGCCCAGT | Factor VII | VII4 |
| CAGGAGACTTACTCGCCCTG | Factor VII “Leyden” | This study |
| GCTGGAATTCATGACAAT | Factor IX | Site 3 |
| GCGGAGATCTTGGCCAGG | Factor X | FXI |
| AGGGTCCTTGGCCACAG | ApoCIII | CIIIB |
| AGGGCGCTTTGAGACT | ApoB | BAI |
| CTTCACATGGCTGGCTT | ApoAI | AlII |
| ACTGAACCTTGGCCTCCT | ApoAI | AlI (A) |
| ACTGAACCTTGGCCACT | ApoAI | C |
| GACCTGACTTGGGACTT | HNF-1 | TRH |
| TGACXXCTGCC | Consensus | 52 |

RESULTS

1. Furie, B. and Furie, B. C. (1988) Cell 53, 505–518

2. Hagen, F. S., Gray, C. L., O’Hara, P., Grant, F. J., Saari, G. C., Woodbury, R. G., Hart, C. E., Insel, M., Kisiel, W., Kurachi, K., and Davie, E. W. (1985) Proc. Natl. Acad. Sci. U. S. A. 83, 2422–2426.

3. Miao, C. H., Leytus, S. P., Chung, D. W., and Davie, E. W. (1992) J. Biol. Chem. 267, 7395–7401.

4. O’Hea, P. J., Grant, F. J., Haldeman, B. A., Gray, C. L., Insel, M. Y., Hagen, F. S., and Murray, M. J. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 1518–1526.

5. Meade, T. W., Melloas S., Bologzo, M., Miller, G. J., Chakrabarti, R. R., North, W. R. S., Haines, A. P., Stirling, Y., Imeson, J. D., and Thompson, S. G. (1986) Lancet 2, 533–537.

6. Howard, P. R., Bovill, E. G., Pike, J., Church, W. R., and Tracy, R. P. (1994) Thromb. Haemostasis 72, 67–72.

7. Negri, M., Ariglino, P. L., Talagini, G., Carlini, S., Mannato, F., and Bonadonna, G. (1993) Timer Reproduction 99, 55–61.

8. March, G., Hamsten, A., Karp, J., Bavenheim, P., Blombak, M., and Silber, A. (1994) Thromb. Haemostasis 71, 707–712.

9. Hoffmann, C. J., Miller, R. H., and Hultin, M. B. (1992) Arteriosclerosis. Thromb. 12, 267–270.

10. Hoffmann, C. J., Lawson, W. E., Miller, R. H., and Hultin, M. B. (1994) Arteriosclerosis. Thromb. 14, 1737–1740.

11. Humphries, S. E., Lane, A., Green, F. R., Cooper, J., and Miller, G. J. J. (1993) Arteriosclerosis. Thromb. 13, 193–198.

12. Silveira, A., Green, F., Karpe, F., Blombak, M., Humphries, S., and Hamsten, A. (1994) Thromb. Haemostasis 72, 734–739.

13. Marchetti, G., Patracchini, P., Papacchini, M., Ferrati, M., and Bernardi, F. (1993) Hum. Genet. 90, 575–576.

14. Knowles, B. B., Howe, C. W., and Aden, D. P. (1980) Science 209, 497–499.

15. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994) Current Protocols in Molecular Biology, Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., New York.

16. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1475–1489.

17. Kurachi, S., Furukawa, M., Safer, J. P., Wu, C.-T., Wilson, E. J., French, F. S., and Kurachi, K. (1994) Biochemistry 33, 1580–1591.

18. Hudson, H. L., Hung, H. L., Stanford-Oakley, S. A., and High, K. A. (1992) J. Biol. Chem. 267, 15440–15446.

19. Cifarello, F., Misti, S., Falcicci, A., Porta, C., Santoni, A. (1993) Biochim. Biophys. Acta 1172, 197–199.

20. Banserott, J. D., Schaefer, L. A., and Frieren Degen, S. J. (1990) Genet. Am. 95, 252–260.

21. Gehm, B. C., Ting, V., Tufaro, F., and Magdillarv, R. T. A. (1991) J. Biol. Chem. 266, 18927–18933.

22. Fair, D. S., and Marlan, S. A. (1986) Blood 67, 64–70.

23. Ghosh, D. (1993) Nucleic Acids Res. 5137–5146.

24. Hasson, J. H., Cherucci, C., Peschle, C., and Sorrentino, V. (1992) Thromb. Haemostasis 67, 478–483.

25. Sladek, F., Zheng, W., Lau, E., and Darnell, J. E. (1990) Genes & Dev. 4, 2363–2368.

26. Picketts, D. J., D’Souza, C., Bridge, P. J., and Lilllicrap, D. P. (1992) Genomics 15, 161–163.

27. Picketts, D. J., Mcdowell, S. A., and Frieren Degen, S. J. (1992) Biochemistry 31, 12469–12476.

28. Briet, E., Bertina, R. M., Van Tilburg, N. H., and Veltkamp, J. J. (1982) New J. Med. 306, 778–781.

29. Crossley, M., Ludwig, M., Stowell, K. M., deVos, P., Olek, K., and Brownlee, S. G. (1986) Science 2353–2365.

30. Sladek, F. M., Zhong, W., Lai, E., and Darnell, J. E. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4421–4425.

31. Hassan, J. H., Chelucci, C., Peschle, C., and Sorrentino, V. (1992) Thromb. Haemostasis 21, 67, 734–739.

32. Picketts, D. J., Lillicrap, D. P., and Mueller, C. R. (1993) Thromb. Haemostasis 71, 707–712.

33. Picketts, D. J., Mueller, C. R., and Lillicrap, D. (1994) Blood 84, 2929–3000.

34. Reijnen, M. J., Peerlink, K., Maasdam, D., Bertina, R. M., and Reitsma, P. H.
(1993) Blood 82, 151–158
35. Reitsma, P. H., Bertina, R. M., Ploos Van Amstel, J. K., Riemans, A., and Briet, E. (1988) Blood 72, 1074–1076
36. Reitsma, P. H., Mandalaki, T., Kasper, C. K., Bertina, R. M., and Briet, E. (1989) Blood 73, 743–746
37. Royle, G., Van de Water, N. S., Berry, E., Ockelford, P. A., and Browett, P. J. (1991) Br. J. Haematol. 77, 191–194
38. Crossley, M., and Brownlee, G. G. (1990) Nature 345, 444–446
39. Anson, D. S., Choo, K. H., Rees, D. J., Kasper, C. K., Bertina, R. M., and Briet, E. (1984) J. Biol. Chem. 259, 10531–10536
40. Luo, C. C., Li, W. H., Moore, M. N., and Chan, L. (1986) J. Biol. Chem. 261, 325–340
41. Ktistaki, E., Lacorte, J.-M., Kastrakili, N., Zannis, V. I., and Talianidis, I. (1994) Nucleic Acids Res. 22, 4689–4696
42. Jahroudi, N., and Lynch, D. C. (1994) Mol. Cell. Biol. 14, 999–1008
43. Brooks, A. R., and Levy-Wilson, B. (1992) J. Biol. Chem. 267, 1134–1148
44. Ge, R., Rhee, M., Malik, S., and Karathanasis, S. K. (1994) J. Biol. Chem. 269, 13189–13192
45. Papazafiri, P., Ogami, K., Ramji, D. P., Nicosia, A., Monaci, P., Cladaras, C., and Zannis, V. I. (1994) J. Biol. Chem. 269, 5790–5797
46. Widom, B. L., Ladias, J. A., Koudou, S., and Karathanasis, S. K. (1991) J. Biol. Chem. 266, 677–687
47. Chambaz, J., Cardot, P., Pastier, D., Zannis, V. I., and Cladaras, C. (1991) J. Biol. Chem. 266, 11676–11685
48. Cardot, P., Chambaz, J., Cladaras, C., and Zannis, V. I. (1991) J. Biol. Chem. 266, 24460–24470
49. Kardassis, D., Hadzopoulou-Cladaras, M., Ramji, D. P., Cortese, R., Zannis, V. I., and Cladaras, C. (1990) Mol. Cell. Biol. 10, 2653–2659
50. Kardassis, D., Zannis, V. I., and Cladaras, C. (1992) J. Biol. Chem. 267, 2622–2632
51. Metzger, S., Halaas, J. L., Breslow, J. L., and Sladek, F. M. (1993) J. Biol. Chem. 268, 16831–16838
52. Sladek, F. M. (1993) Receptor 3, 223–232
53. Chartier, F. L., Bossu, J.-P., Lauvet, V., Fruchtart, J.-Ch., and Laine, B. (1994) Gene (Amst.) 147, 269–272
54. Xanthopoulos, K. G., Prezioso, V. R., Chen, W. S., Sladek, F. M., Cortese, R., and Darnell, J. E., Jr. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3807–3811
55. Chan, J., Nakabayashi, H., and Wong, N. C. W. (1993) Nucleic Acids Res. 21, 1205–1211
56. Ladias, J. A. A., Hadzopoulou-Cladaras, M., Kardassis, D., Cardot, P., Cheng, J., Zannis, V. I., and Cladaras, C. (1992) J. Biol. Chem. 267, 15849–15860
57. Mietus-Snyder, M., Sladek, F. M., Ginsburg, G. S., Kuo, C. F., Ladias, J. A. A., Darnell, J. E., Jr., and Karathanasis, S. K. (1992) Mol. Cell. Biol. 12, 1708–1718
58. Ochoa, A., Bovard-Houppermans, S., and Zakin, M. M. (1993) Biochim. Biophys. Acta 1210, 41–47
59. Courtois, G., Baumhueter, S., and Crabtree, G. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7937–7941
60. Kuo, C. J., Conley, P. B., Chen, L., Sladek, F. M., Darnell, J. E., Jr., and Crabtree, G. R. (1992) Nature 355, 457–461
61. Tian, J.-M., and Schibler, U. (1991) Genes & Dev. 5, 2225–2234
62. Kritis, A. A., Ktistaki, E., Barda, D., Zannis, V. I., and Talianidis, I. (1993) Nucleic Acids Res. 21, 588–589
