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Acute-phase reaction induces a specific complex between hepatic nuclear proteins and the interleukin 6 response element of the rat α_2-macroglobulin gene

(cytokines/inflammation/acute-phase genes/liver gene regulation)

MASAHIRA HATTORI, LAWRENCE J. ABRAHAM*, WOLFGANG NORTHEMANN, AND GEORG H. FEY†

Department of Immunology, The Research Institute of Scripps Clinic, La Jolla, CA 92037

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ABSTRACT **Interleukin 6 (IL-6) was established as a transcriptional inducer of the rat α_2-macroglobulin gene, a prototype liver acute-phase gene. Maximum induction occurred when the 5′ flanking sequences of this gene (position −209 to −43) directed expression from the gene's own TATA box and transcription start site. Removal of the hexanucleotide CTGGGA (position −164 to −159) abolished 60–70% of the hormonal induction in FAO1 rat hepatoma cells. This hexanucleotide was defined as the IL-6 response element (IL-6-RE). The IL-6-RE is well conserved in the cytokine-responsive regions of other acute-phase genes and serves as a binding site for nuclear proteins. A characteristic DNA–protein complex (complex I) was formed with nuclear proteins from normal rat livers. A different, hormone-inducible complex (complex II) was assembled specifically with nuclear proteins from acute-phase rat livers or from IL-6-treated human Hep 3B hepatoma cells. Complex II was competitively inhibited by oligonucleotides representing the conserved IL-6-RE sequence from other acute-phase genes. Thus, the proteins building complex II likely participate in a general signal transduction mechanism mediating the transcriptional activation by IL-6 of several acute-phase genes.**

Interleukin 6 (IL-6) coordinates several important reactions in inflammation and the host defense against tissue damage and infections (1). It induces the synthesis of protective plasma proteins (acute-phase proteins) in the liver. It also modulates the immune response and regulates body temperature and fever (2–5). We have chosen the rat α_2-macroglobulin (α_2M) gene to study gene activation by IL-6 because this gene is dramatically induced by IL-6. α_2M contributes to the host defense as a broad-range proteinase inhibitor and as a carrier of various hormones (6–13). During an acute inflammatory reaction, α_2M plasma concentrations rise several 100-fold within 48 hr, following a corresponding elevation of hepatic α_2M mRNA levels (6, 7, 14). Transcription rates of the gene were reportedly increased up to 9-fold (6, 7, 15, 16). In living rats and most hepatic cell culture systems, both IL-6 and glucocorticoids are required for the full induction of the α_2M gene (3, 17, 18). Here we have evaluated the respective contributions of IL-6 and glucocorticoids to the transcriptional induction of the α_2M gene by using a hepatoma cell line stably transfected with an α_2M gene promoter region construct. In this assay system, IL-6 alone induced transcription of the α_2M gene, and glucocorticoids enhanced its effect. Using mutagenesis and transfection into hepatoma cells, we have mapped an IL-6-responsive cis-acting transcriptional control element in the 5′ flanking region of the α_2M gene. This element serves as a binding site for specific nuclear proteins, and we have observed a hormone-induced characteristic protein–DNA complex between this element and nuclear proteins from acute-phase rat livers and IL-6-treated cultured hepatoma cells.

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MATERIALS AND METHODS

Cell Cultures, DNA Transfections, and Luciferase Assays. The hepatoma lines FAO1 and Hep 3B were cultured as described (19, 20). Recombinant human IL-6 [specific activity, 5×10^6 IL-6/B cell-stimulating factor (BSF-2) units/mg; IL-6/BSF-2 units are a measure of the BSF-2 activity of IL-6] was from T. Hirano and T. Kishimoto of Osaka University and T. Miyake of Tosoh, Tokyo, Japan. FAO1 cells were transfected by the calcium phosphate method (21) with 18 μg of test plasmid DNA carrying a luciferase reporter gene (22) and 2 μg of the plasmid pRSVCAT (23) to monitor the efficiency of transfection. The cells were incubated for 14 hr with the DNA/calcium phosphate precipitate, shocked for 2 min with 20% glycerol (21), cultured in fresh medium for 12 hr, and exposed to 0.1 μM dexamethasone (Dex) for another 14 hr. Finally, the medium was replaced by fresh medium containing either 0.1 μM Dex alone or 0.1 μM Dex and 200 IL-6/BSF-2 units/ml. After treatment for 5 hr, cellular extracts were prepared and assayed for luciferase and chloramphenicol acetyl transfersase activities as reported (22, 24).

Plasmid Construction. Plasmid pRα_2MG/HB1.2 was constructed by insertion of the *Hin*dIII–*Bgl* II fragment (position −1151 to +17) of the rat α_2M gene (8, 25) into the *Hin*dIII site of the luciferase reporter vector pSVOAΔ5′ (22) and plasmid pRα_2MG/HH1.2 by insertion of the *Hin*dIII–*Hin*dIII fragment (position −1151 to +54) of the α_2M gene into the same site. The *Hin*dIII site at +54 was generated by digestion with BAL-31 exonuclease from the *Eco*RV site in the first intron of the α_2M gene, followed by ligation with a *Hin*dIII linker. For mutagenesis, exonuclease III-treated partially single-stranded plasmid DNA was hybridized with a complementary synthetic oligonucleotide (40 nucleotides) containing the desired mutation and was repaired with Klenow DNA polymerase and T4 DNA ligase.

Generation of the Stable Cell Line FAO/HB3. FAO1 cells were cotransfected with plasmids pRα_2MG/HB1.2 and pRSVNeo in a 50:1 mass ratio. Neomycin-resistant colonies were selected by culture for 3 weeks in medium (19, 20) containing 400 μg of G418 (neomycin, GIBCO) per ml. Nine of 12 isolates, including FAO/HB3, showed a luciferase response to IL-6.

Abbreviations: α_2M, α_2-macroglobulin; IL-6, interleukin 6; IL-6-RE, interleukin 6 response element; BSF-2, B cell-stimulating factor 2; IL-6/BSF-2, the BSF-2 activity of IL-6; SAA, serum amyloid A; α_1AGP, α_1 acid glycoprotein; Dex, dexamethasone.

*Present address: Department of Clinical Immunology, Royal Perth Hospital, Perth, Western Australia, Australia.
†To whom reprint requests should be addressed.

Nuclear Protein Extracts. Nuclear extracts from cultured cells were prepared as described (26). Rat liver nuclei were isolated by homogenization of liver tissue in 0.3 M sucrose/10 mM Hepes, pH 7.6/0.75 mM spermidine/0.15 mM spermine/1 mM dithiothreitol/0.1 mM EDTA/0.1 mM EGTA/10 mM KCl/0.5 mM phenylmethylsulfonyl fluoride. Nuclei were purified by centrifugation through a cushion of 2.2 M sucrose in the same buffer. Nuclear proteins were then isolated as described (26).

Gel Mobility Shift, DNase I Footprinting, and Methylation Interference. Gel mobility shift experiments were performed as described (27, 28). Binding reactions were carried out in a final volume of 20 μl for 20 min at room temperature in 10 mM Hepes (pH 7.6) containing 50 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 6 mM $MgCl_2$, 1.2 mM $CaCl_2$, 10% (vol/vol) glycerol, and 10 μg of poly(dI·dC). DNase I cleavage inhibition patterns (footprinting) were obtained as described (29). Methylation interference experiments were performed by the procedure of Sen and Baltimore (30).

RESULTS

IL-6 Is a Strong Transcriptional Inducer of the α_2M Gene. A survey of six different rat hepatoma cell lines showed that the line FAO1 responded most faithfully to IL-6 and glucocorticoids, resembling closely the hormonal response in living rats (data not shown). Therefore, the FAO1 line was chosen to generate transformed cell lines that carry α_2M gene promoter region constructs directing the expression of luciferase stably associated with the host cell genome. Treatment of one of these lines, FAO/HB3, with glucocorticoids alone caused only a 2-fold increase in transcription of the α_2M gene promoter, while treatment with IL-6 alone caused a 10- to 20-fold induction (Fig. 1*A*). Treatment with a combination of both hormones produced up to a 40-fold increase. Similar results were obtained with a second, independently established line, FAO/XB218, that carries a luciferase construct with 2180 base pairs (bp) from the 5′ flanking region of the α_2M gene (data not shown). The identical response of both lines indicates that the response is characteristic of the constructs rather than their chromosomal integration sites. Thus, IL-6 alone is a strong transcriptional inducer of the α_2M gene promoter region in hepatoma cells, and glucocorticoids enhance its effect. The maximal response to IL-6 alone was reached in both cell lines 4–6 hr after addition of the hormone. Combined treatment with IL-6 and glucocorticoids led to a secondary response after 24 hr (Fig. 1*A*). Thus, major cis-acting IL-6-responsive DNA control elements are contained in the 5′ flanking region (position −1151 to +17) of the α_2M gene.

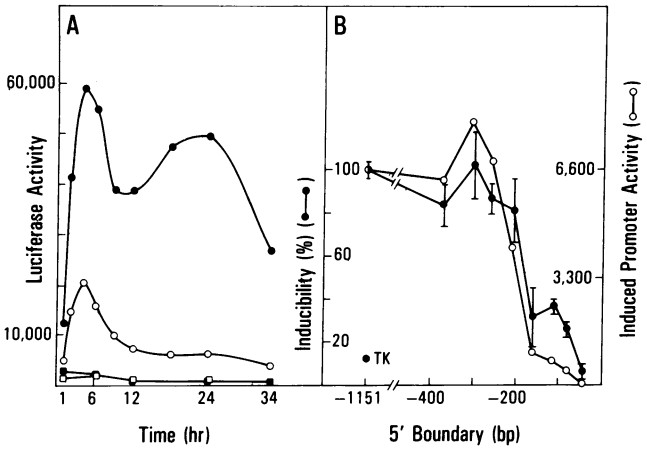
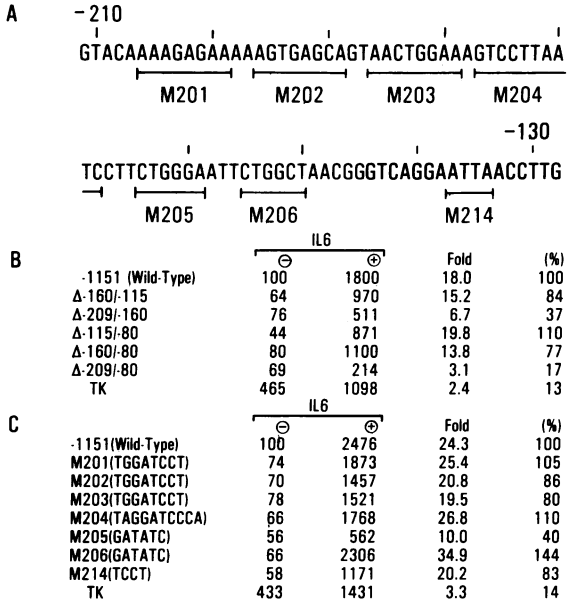


FIG. 1. Stable (*A*) and transient (*B*) expression of α_2M gene promoter constructs. (*A*) The rat hepatoma line FAO/HB3 carries stably integrated copies of the α_2M gene promoter construct pRα_2MG/HB1.2. The cells were either mock-treated (□) or were treated with 0.1 μM Dex (■), with 200 IL-6/BSF-2 units/ml of recombinant human IL-6 (○), or with IL-6 plus Dex at the same concentrations (●). (*B*) FAO cells were transfected with a series of 5′ deletion constructs of the α_2M gene promoter region. ○, Induced promoter activity; luciferase activities obtained from cells treated with 200 IL-6/BSF-2 units/ml and 0.1 μM Dex; ●, specific inducibility: luciferase activity obtained with IL-6/Dex, divided by the activity of the same construct after treatment with Dex alone, and normalized to the value obtained with the longest construct (−1151 bp); TK, inducibility of a control construct carrying the enhancerless herpesvirus thymidine kinase (TK) promoter coupled to the luciferase reporter. Specific inducibilities are mean values over at least three independent experiments. The length of error bars corresponds to 1 SD from the mean to either side.

Mapping of an IL-6-Response Element (IL-6-RE) in the α_2M Gene. To map IL-6-responsive cis-acting DNA control elements, a series of 5′ deletion constructs were prepared in the plasmid pRα_2MG/HH1.2 and transfected into FAO1 cells. The transfected cells were treated either with glucocorticoids alone or with glucocorticoids and IL-6, and transient expression was monitored. The promoter activities (luciferase activities) and the specific inducibilities by IL-6 of each construct are given in Fig. 1*B*. Constructs carrying deletions of 5′ sequences from position −1151 to −210 retained full inducibility. About 60–70% of the inducibility was lost when sequences between position −209 and −160 were deleted, and the remainder upon deletion of the sequences from −115 to −43 (Fig. 1*B*). The stepwise loss of inducibility indicated that several subregions within the area −209 to −43 contribute to induction by IL-6, but the single most critically required element resided between positions −209 and −160.



B

	IL6 ⊖	IL6 ⊕	Fold	(%)
-1151 (Wild-Type)	100	1800	18.0	100
Δ-160/-115	64	970	15.2	84
Δ-209/-160	76	511	6.7	37
Δ-115/-80	44	871	19.8	110
Δ-160/-80	80	1100	13.8	77
Δ-209/-80	69	214	3.1	17
TK	465	1098	2.4	13

C

	IL6 ⊖	IL6 ⊕	Fold	(%)
-1151(Wild-Type)	100	2476	24.3	100
M201(TGGATCCT)	74	1873	25.4	105
M202(TGGATCCT)	70	1457	20.8	86
M203(TGGATCCT)	78	1521	19.5	80
M204(TAGGATCCCA)	66	1768	26.8	110
M205(GATATC)	56	562	10.0	40
M206(GATATC)	66	2306	34.9	144
M214(TCCT)	58	1171	20.2	83
TK	433	1431	3.3	14

FIG. 2. Mapping of the IL-6-RE. (*A*) The sequences from the α_2M gene 5′ flanking region replaced in mutants M201 to M214 by nonrelated sequences of similar base composition are indicated by bars under the wild-type sequence. (*B*) Internal deletion constructs were prepared from pRα_2MG/HH1.2 lacking the internal fragments indicated by Δ. ⊖, Relative luciferase activity after treatment with 0.1 μM Dex alone; ⊕, relative activity with 0.1 μM Dex and 200 IL-6/BSF-2 units/ml of recombinant human IL-6. Values are normalized to the wild-type construct taken as 100%. Specific inducibilities by IL-6 (column "Fold") were calculated as the ratios of the values from the two preceding columns. Relative specific inducibilities (column "%") were normalized to the wild-type construct taken as 100%. TK, inducibility of the thymidine kinase (TK) promoter construct. (*C*) Inducibilities obtained with the linker scan mutants specified in *A*.

Transfection experiments with a series of internal deletions confirmed this result (Fig. 2*B*). An internal deletion of the base pairs between −209 and −160 (Δ −209/−160) reduced the inducibility to 37% of the value obtained with the wild-type construct pRα_2/HH1.2. Linker scanning mutants were generated for more precise mapping of the critical elements (Fig. 2 *A* and *C*). Mutant m205, replacing the sequence from −164 to −159, had the strongest effect and an inducibility reduced to 40% of the wild-type value (Fig. 2*C*). The transcriptional start site of the transgene was mapped in cells transfected with the wild-type construct pRα_2MG/HH1.2 and was found to be the same as in the endogenous α_2M gene (data not shown; ref. 8). From these data we concluded that the hexanucleotide CTGGGA (from position −164 to −159) mediated the single largest contribution to IL-6 responsiveness and that additional sequences in the promoter proximal region of the gene were responsible for the balance of the induction. The hexanucleotide is referred to as the core of the IL-6-RE.

IL-6-RE Core Is a Binding Site for Nuclear Proteins. Protein extracts from purified rat liver nuclei were used for DNase I protection (footprinting) experiments (Fig. 3). Two regions, I (−190 to −173) and II (−169 to −152), on the coding strand and the corresponding regions I (−193 to −176) and II (−172 to −155) on the noncoding strand were protected. Region I was protected less efficiently than region II but was observed reproducibly. Regions I and II were separated by DNAse I hypersensitive sites (−171 and −170 of the coding strand and −173 and −174 of the noncoding strand), as indicated by arrows in Fig. 3. The precise area covered by proteins cannot be determined by footprinting alone, and the coordinates given here delineate only the maximum boundaries of each region. Nuclear extracts from acute-phase rat livers conferred a stronger protection than did comparable amounts of nuclear proteins from normal rat liver, but the extent of the protected sequences was indistinguishable. Region II was centered around the IL-6-RE core sequence CTGGGA. When a mutated DNA fragment was used in which the sequence CTGGGA was converted to GATATC (mutant M205, Fig. 2 *A* and *C*), then the protected region II disappeared, while protection of region I remained. This was observed with extracts from both normal and acute-phase rat livers (data not shown). Thus, nuclear proteins protecting region II specifically recognize the IL-6-RE core or part of it. Such proteins are present in normal and in acute-phase liver cell nuclei. The region covered by footprint I contains the sequence CTGGAAA, identical with the core sequence CTGGGAA in six of seven nucleotides. This sequence is referred to as the IL-6-RE core homology.

Specific DNA protein complexes were also observed in gel mobility shift experiments (Fig. 4). An 82-base-pair (bp) DNA fragment containing both the IL-6-RE core and the region of footprint I (−209 to −130) was used as a target for complex formation. A specific complex (complex I, Fig. 4, track 1) was formed with nuclear extracts from normal rat livers and a different complex with greater electrophoretic mobility (complex II, Fig. 4, track 2) with nuclear extracts from 6-hr acute-phase rat livers. When using nuclear extracts from untreated or IL-6-treated FAO1 cells, only complex II was observed (Fig. 4, tracks 3–5). With extracts from untreated Hep 3B cells, no complex was seen (Fig. 4, track 6), and with extracts from IL-6-treated Hep 3B cells, only complex II was observed (Fig. 4, track 7). Thus, a clear qualitative difference in the pattern of protein–DNA complexes was observed with extracts from normal and acute-phase livers and from untreated and IL-6-treated human hepatoma cells. Proteins capable of forming complex II appeared to be induced by the acute-phase reaction *in vivo* and by treatment of cultured hepatic cells with IL-6. Gel shift competition experiments demonstrated that formation of complexes I and II depended critically on the hexanucleotide core of the IL-6-RE. A large molar excess of a 30-bp IL-6-RE core oligonucleotide partially prevented formation of complex I and completely inhibited complex II (Fig. 5, tracks 1 and 2). A comparable excess of a 30-bp oligonucleotide with a mutated IL-6-RE hexanucleotide core did not prevent these complexes (Fig. 5, track 3). Thus, proteins involved in both complexes I and II must recognize either the hexanucleotide core of the IL-6-RE or part of it.

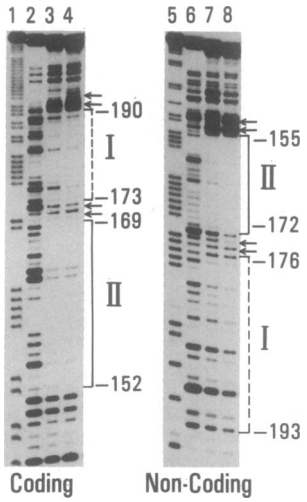


FIG. 3. DNAse I footprint analysis. Nuclear extracts were prepared from normal adult rat livers (T_0 extracts) and from rats 6 hr after induction of an acute-phase response by intraperitoneal injection of complete Freund's adjuvant (T_6 extracts). The 82-bp fragment (position −209 to −132) was used as a probe. Tracks: 1 and 5, Maxam and Gilbert G+A sequence of the coding and noncoding strands, respectively; 2 and 6, no addition of nuclear extracts; 3 and 7, protection with T_0 extracts; 4 and 8, protection with T_6 extracts. Arrows indicate DNAse I hypersensitive sites.

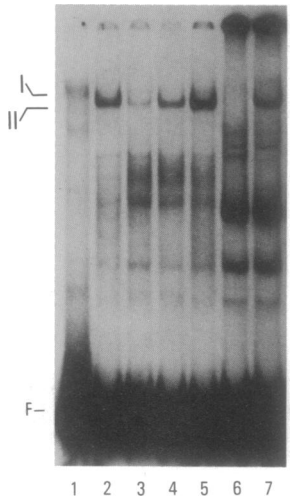


FIG. 4. Gel mobility shift assays. An end-labeled double-stranded 82-bp DNA fragment covering both footprint regions I and II (position −209 to −132) was used as the probe. Ten micrograms of nuclear extracts from rat livers and 20 μg of nuclear extracts from hepatoma cells were incubated with 0.7 ng of the 82-bp fragment. Tracks: 1 and 2, normal (T_0) and 6-hr-treated (T_6) rat liver extracts; 3–5, nuclear extracts from FAO1 cells untreated (track 3), treated with 0.1 μM Dex (track 4), or treated with 0.1 μM Dex and 200 IL-6/BSF-2 units/ml (track 5); 6 and 7, extracts from Hep 3B cells untreated (track 6) or treated with 200 units/ml of recombinant human IL-6 for 6 hr (track 7). Complexes I and II and the free (F) DNA fragment are marked.

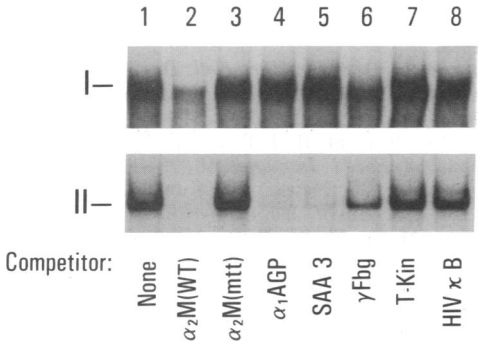


FIG. 5. Gel mobility shift competition analysis. (*Upper*) Complex I with normal (T_0) rat liver extracts and the 82-bp fragment. (*Lower*) Complex II with 6-hr-treated (T_6) acute-phase rat liver extracts and the 82-bp fragment. Molar excess of nonlabeled 30-bp competitor oligonucleotides was 120-fold. Tracks: 1 , no competitor; 2, 30-bp wild-type (WT) α_2M oligonucleotide GATCCTTAATCCTTCTGGGAATTCTGGCTA as competitor; 3, α_2M mutant (mtt) M205 oligonucleotide GATCCTTAATCCTTGATATCATTCTGGCTA; 4, rat α_1AGP oligonucleotide GATCGGCTGGGCTTCTGGGAAAACTCAAG (33); 5, murine SAA3 oligonucleotide GATCTCACACAATTTCTGGAAATGCCTAGATG (35); 6, rat γ-fibrinogen (Fbg) oligonucleotide GATCGTGTGCAAAATCTGGGAATCCCTCGG (34); 7, rat T_1 and T_2 kininogen (Kin) oligonucleotide GATCAATGCCCCAGCTGGGTACCTGCTTGG (32); 8, oligonucleotide from the κB site of the HIV enhancer GATCGGACTTTCCGCTGGGGACTTTCCAGG (36) (conserved sequences are underlined).

Same Proteins Interact with the Conserved IL-6-RE Sequence in Different Acute-Phase Genes. The IL-6-RE core is conserved in the cytokine-responsive regions of several other major rodent acute-phase genes, including the rat α_1 acid glycoprotein (α_1AGP) gene, the γ-fibrinogen gene, the T_1 and T_2 kininogen genes, and the murine serum amyloid A3 (SAA3) gene (31–35). To investigate whether common nuclear factors bind at the conserved sequences, gel shift competition experiments were performed with synthetic oligonucleotides representing the conserved IL-6-RE regions from the other acute-phase genes (Fig. 5). The oligonucleotides representing the IL-6-RE from the rat α_2M and γ fibrinogen genes were partially efficient as competitors of complex I (Fig. 5, track 6). Formation of complex II was competitively inhibited by the rat α_2M and α_1AGP oligonucleotides and the murine SAA3 oligonucleotide (Fig. 5, tracks 2, 4, and 5) and partially inhibited by the γ-fibrinogen oligonucleotide (Fig. 5, track 6). The data further indicate that complexes I and II have different, characteristic spectra of inhibition and possibly have different molecular composition. The disappearance of complex I and the appearance of complex II in acute-phase liver extracts and after treatment of hepatoma cells with IL-6 precedes most closely the hormonal induction of the α_2M gene. Therefore, the hormone-induced complex II is a candidate for a complex involved in the IL-6-induced transcription of the α_2M gene. The competition experiments suggest that common nuclear factors interacting with the conserved IL-6-RE sequences may be involved in the IL-6-induced transcription of several of these acute-phase genes.

The IL-6-RE core region of the rat α_2M gene matches a sequence overlapping the κB site of the human immunodeficiency virus enhancer (HIV; ref. 36) in 8 of 11 nucleotides. A synthetic 30-bp oligonucleotide representing the κB region of the HIV enhancer did not inhibit complexes I and II (Fig. 5, track 8). Thus, the proteins involved in formation of complexes I and II do not bind efficiently at the HIV κB site. Furthermore, complexes I and II were not detected with nuclear extracts from either normal HeLa cells or HeLa cells treated with phorbol 12-myristate 13-acetate, an agent known to induce the transcription factor NF-κB that binds at κB sites (37). These and other studies (data not shown) suggest that the proteins involved in complexes I and II are probably distinct from NF-κB.

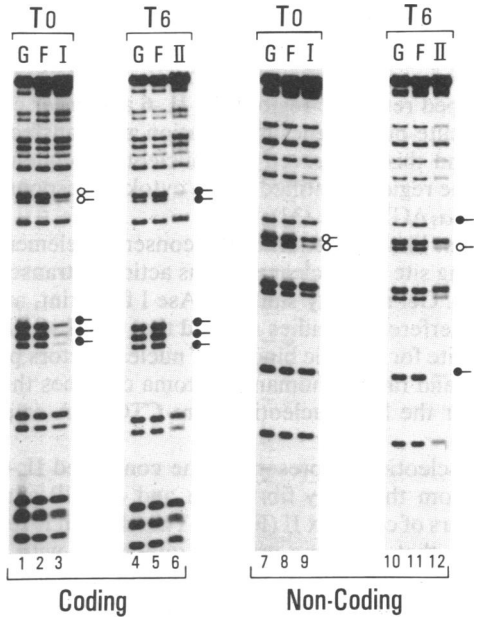


FIG. 6. Methylation interference analysis. The 82-bp fragment was methylated at G residues and allowed to react with normal (T_0) and acute-phase (T_6) rat liver nuclear extracts. Complexes I and II and the free probe (F; tracks 2, 5, 8, and 11) were separated by acrylamide gel electrophoresis and excised from the gel. Tracks G (tracks 1, 4, 7, 10) show the Maxam and Gilbert G reaction of the unreacted probe fragment. Methylation of nucleotides marked by ●— strongly interfered with complex formation; ○— shows residues of weak interference.

IL-6-RE Core Is Directly Involved in the Formation of Complex II. Direct contact between the IL-6-RE core sequence CTGGGA and nuclear proteins forming complex II was shown by methylation interference experiments (Fig. 6). Methylation of the three guanosyl residues contained in the core sequence prevented formation of complex I with extracts from normal rat liver nuclei and of complex II with extracts from acute-phase liver nuclei (Fig. 6, tracks 3 and 6). Methylation of these three residues affected complex formation more severely with acute-phase extracts than with normal liver extracts. Additional nucleotides involved in DNA–protein contacts on the noncoding DNA strand were revealed by using acute-phase extracts that were not detected with normal rat liver extracts (Fig. 6, tracks 9 and 12). Thus, the patterns of DNA–protein contacts were different for complexes I and II, but the IL-6-RE core and the core-homology sequences were directly involved in the formation of both complexes.

DISCUSSION

The IL-6-RE core is the IL-6 responsive cis-element that could be identified with the greatest precision by mutation and transfection. However, it is not the only sequence element involved in the IL-6 response. Using a 5′ flanking fragment from −209 to +54, we obtained about a 20-fold induction, while a fragment from −209 to −33 linked to the simian virus 40 TATA box region and multiple copies of the fragment −209 to −130 linked to the enhancerless herpesvirus thymidine kinase promoter generated an increase of only 2- to 3-fold. Thus, other sequences located between −33 and +54 are probably also important for the full hormonal effect.

The hexanucleotide CTGGGA was previously identified as a sequence conserved among several acute-phase genes on the basis of sequence comparison (25, 31). Other authors have mapped response regions for IL-6 and other cytokines in other acute-phase genes by mutation and transfection (33, 38–41), and this conserved hexanucleotide sequence is located in the region identified as the cytokine response region of the rat α_1AGP gene (33).

The most likely function of the conserved element is that of a binding site for nuclear proteins acting as transcriptional regulators. Gel mobility shift, DNAse I footprint, and methylation interference studies showed that the IL-6-RE core is indeed a site for specific binding of nuclear factors present in rat livers and rat and human hepatoma cell lines that recognize either the hexanucleotide core CTGGGA or part of it (Fig. 3–6).

Oligonucleotides representing the conserved IL-6-RE sequence from the rat γ fibrinogen and α_1AGP genes were competitors of complex II (Fig. 5). Therefore, it is reasonable to assume that the same proteins interacting with this core sequence *in vitro* will also interact with it *in vivo* and are involved in the regulation of these other acute phase genes by IL-6. However, a role of these DNA binding proteins in transcriptional regulation has not yet been established.

The composition of complexes I and II is unknown. These complexes were formed with an 82-bp DNA fragment covering both regions I and II defined by DNase I footprint analysis (Fig. 3). Therefore, complexes I and II conceivably contain several proteins bound to DNA, including some that bind at footprint region I. The DNA segment covered by footprint region I contains the sequence CTGGAAA, referred to as the IL-6-RE core homology. The same protein binding at the IL-6-RE core could conceivably also bind at this second site located 20 nucleotides upstream. The fact that both complexes I and II can be inhibited with the 30-bp oligonucleotide covering the IL-6-RE core is consistent with this interpretation. However, in the transfection experiments (Fig. 2*C*), the mutant m203 in the core homology sequence had only a weak effect on the inducibility by IL-6. Thus, the IL-6-RE core provides the dominant contribution to the overall induction by IL-6.

Recently, IL-6-REs were identified in the human C-reactive protein, haptoglobin, and hemopexin genes (39–41). However, there is little sequence homology between these elements and the IL-6-RE core of the rat α_2M gene as identified here. Thus, the IL-6 signal may activate different types of cis-acting response elements and possibly different factors binding at these elements.

We anticipate that the IL-6-RE and some of the proteins involved in complexes I and II described here may be part of at least one general IL-6 signal transduction mechanism, operating not only in hepatic cells but possibly also in other target cells of IL-6.

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1. Sehgal, P. B., Grieninger, G. & Tosato, G., eds. (1989) *Ann. N.Y. Acad. Sci.* **557.**
2. Fey, G. & Gauldie, J. (1990) in *Progress in Liver Disease*, eds. Popper, H. & Schaffner, F. (Saunders, Philadelphia), 9th Ed., in press.
3. Baumann, H. (1989) *In Vitro Cell. Dev. Biol.* **25,** 115–126.
4. Kishimoto, T. & Hirano, T. (1988) *Annu. Rev. Imunol.* **6,** 485–512.
5. Helle, M., Brakenhoff, J. P. J., de Groot, E. R. & Aarden, L. A. (1988) *Eur. J. Immunol.* **18,** 957–959.
6. Northemann, W., Heisig, M., Kunz, D. & Heinrich, P. C. (1985) *J. Biol. Chem.* **260,** 6200–6205.
7. Gehring, M. R., Shiels, B. R., Northemann, W., de Bruijn, M. H. L., Kan, C.-C., Chain, A. C., Noonan, D. J. & Fey, G. H. (1987) *J. Biol Chem.* **262,** 446–454.
8. Northemann, W., Shiels, B. R., Braciak, T. A., Hanson, R. W., Heinrich, P. C. & Fey, G. H. (1988) *Biochemistry* **27,** 9194–9203.
9. Sottrup-Jensen, L. (1987) in *The Plasma Proteins*, ed. Putnam, F. W. (Academic, Orlando, FL), Vol. 5, pp. 191–291.
10. Huang, J. S., Huang, S. S. & Deuel, T. F. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 342–346.
11. Borth, W. & Luger, T. A. (1989) *J. Biol. Chem.* **264,** 5818–5825.
12. Dennis, P. A., Saksela, O., Harpel, P. & Rifkin, D. B. (1989) *J. Biol. Chem.* **264,** 7210–7216.
13. Matsuda, T., Hirano, T., Hagasawa, S. & Kishimoto, T. (1989) *J. Immunol.* **142,** 148–153.
14. Hayashida, K., Okubo, H., Noguchi, M., Yoshida, H., Kanagawa, K., Matsuo, H. & Sakaki, Y. (1985) *J. Biol Chem.* **260,** 14224–14229.
15. Birch, H. & Schreiber, G. (1986) *J. Biol. Chem.* **261,** 8077–8080.
16. Northemann, W., Shiels, B. R. & Braciak, T. A. & Fey, G. H. (1989) *Biochemistry* **28,** 84–95.
17. Bauer, J., Tran-Thi, T. A. & Northoff, H. (1986) *Eur. J. Cell Biol.* **40,** 86–93.
18. Baumann, H., Prowse, K. R., Marinkovic, S., Won, K. A. & Jahreis, G. P. (1989) *Ann. N.Y. Acad. Sci.* **557,** 280–296.
19. Deschatrette, J. & Weiss, M. C. (1974) *Biochimie* **56,** 1603–1611.
20. Knowles, B. B., Howe, C. C. & Aden, D. P. (1980) *Science* **209,** 497–499.
21. Ott, M. O., Sperling, L., Herbomel, P., Yaniv, M. & Weiss, M. C. (1984) *EMBO J.* **3,** 2505–2510.
22. DeWet, J. R., Wood, K. V., DeLuca, M., Helinski, D. R. & Subramani, S. (1987) *Mol. Cell. Biol.* **7,** 725–737.
23. Fordis, C. M. & Howard, B. H. (1987) *Methods Enzymol.* **151,** 382–397.
24. Sleigh, M. (1986) *Anal. Biochem.* **156,** 251–254.
25. Tsuchiya, Y., Hattori, M., Hayashida, K., Ishibashi, H., Okubo, H. & Sakaki, Y. (1987) *Gene* **57,** 73–80.
26. Shapiro, D. J., Sharp, P. A., Wahli, W. W. & Keller, M. J. (1988) *DNA* **7,** 47–55.
27. Fried, M. & Crothers, D. M. (1981) *Nucleic Acids Res.* **9,** 6505–6525.
28. Garner, M. M. & Revzin, A. (1981) *Nucleic Acids Res.* **9,** 3047–3060.
29. Galas, D. & Schmitz, A. (1978) *Nucleic Acids Res.* **5,** 3157–3170.
30. Sen, R. & Baltimore, D. (1986) *Cell* **46,** 705–716.
31. Fey, G. H., Hattori, M., Northemann, W., Abraham, L. J., Baumann, M., Braciak, T. A., Fletcher, R. G., Gauldie, J., Lee, F. & Reymond, M. (1989) *Ann. N.Y. Acad. Sci.* **557,** 317–331.
32. Kageyama, R., Kitamura, N., Ohkubo, H. & Nakanishi, S. (1987) *J. Biol. Chem.* **262,** 2345–2351.
33. Prowse, K. R. & Baumann, H. (1988) *Mol. Cell. Biol.* **8,** 42–51.
34. Fowlkes, D. M., Mullis, N. T., Comeau, C. M. & Crabtree, G. R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2313–2316.
35. Lowell, C. A., Potter, D. A., Stearman, R. S. & Morrow, J. F. (1986) *J. Biol. Chem.* **261,** 8442–8452.
36. Nabel, G. & Baltimore, D. (1987) *Nature (London)* **326,** 711–713.
37. Lenardo, M. J. & Baltimore, D. (1989) *Cell* **58,** 227–229.
38. Kunz, D., Zimmermann, R., Heisig, M. & Heinrich, P. C. (1989) *Nucleic Acids Res.* **17,** 1121–1138.
39. Oliviero, S. & Cortese, R. (1989) *EMBO J.* **8,** 1145–1151.
40. Arcone, R., Gualandi, G. & Ciliberto, G. (1988) *Nucleic Acids Res.* **16,** 3195–3207.
41. Poli, V. & Cortese, R. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8202–8206.