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Biochemistry

Interleukin 6 induces a liver-specific nuclear protein that binds to the promoter of acute-phase genes

(acute-phase response/interleukin 6 responsive elements/hepatoma cells/hemopexin)

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ABSTRACT **Interleukin 6 (IL-6) is responsible for a variety of biological effects related to the activation of defenses against infection or inflammation, including the immune response and the acute-phase reaction. Its mechanism of action is unknown. It has recently been shown to induce transcription of several genes encoding acute-phase proteins. Here we describe the identification of an IL-6 responsive element (IL-6RE) present in the promoter of the human hemopexin gene. This element is necessary and sufficient for the IL-6-dependent activation of transcription. The IL-6 effect does not require *de novo* protein synthesis. A liver-specific nuclear protein (IL-6DBP) binds to the hemopexin IL-6RE as well as to similar sequences on the promoter of other acute-phase genes. IL-6DBP DNA binding activity is induced by IL-6 via a posttranslational mechanism.**

Interleukin 6 (IL-6) is a cytokine synthesized and secreted by several cell types (1). It has a wide range of biological activities related to differentiation of the immune system and to the immune response, including stimulation of B-cell differentiation and IgG secretion, stimulation of growth of hybridoma and plasmacytoma cells, activation of T cells, and costimulation of thymocyte proliferation (for a review, see ref. 2). IL-6 is also a potent inducer of the acute-phase reaction (1), a response of the organism to several pathological conditions, which is believed to constitute a rapid and general defense mechanism against injury prior to the onset of the immune response. IL-6 binds to a specific receptor present on the surface of most cell types (3). How the signal is transduced and whether there is a unique pathway mediating the various biological activities of IL-6 is at present unknown. An opportunity for a straightforward experimental strategy to identify one of the molecular targets of IL-6 is offered by analysis of the mechanisms involved in activation of several genes during the acute-phase phenomenon.

The acute-phase reaction is characterized by a dramatic change, triggered by infection or inflammation, in the concentration of several plasma proteins. In a variety of experimental systems, it has been shown that the increased protein synthesis occurring in the liver during the acute-phase reaction is mainly a consequence of an increased rate of transcription of the positive acute-phase genes (4–7). It has been established that transcription of transfected acute-phase genes in the human hepatoma cell line Hep3B can be induced by IL-6 (7, 8).

The gene encoding human hemopexin (*HPX*), a positive acute-phase reactant, has been isolated (9). It is expressed specifically in liver cells and it is activated during the acute-phase reaction. In this paper, we describe the characterization of an IL-6-responsive element (IL-6RE) present on the *HPX* promoter, which is necessary for IL-6 responsiveness and is sufficient to confer inducibility to a heterologous promoter.

MATERIALS AND METHODS

Cell Cultures, DNA Transfections, and Chloramphenicol Acetyltransferase (CAT) Assays. The human hepatoma cell line Hep3B (10) and HeLa cells were cultured as monolayers in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Human recombinant IL-6 was obtained from a medium of HeLa cells infected with a recombinant vaccinia virus carrying the human IL-6 coding region. Its activity was 2×10^5 units/ml. Induced cells were treated with the recombinant IL-6 at a concentration of 500 units/ml. DNA transfections and IL-6 induction were performed as described (7). The plasmids Rous sarcoma virus luciferase (11) and pSV-β-globin (12) were used as internal markers for the CAT assay and for S1 nuclease mapping, respectively. Cells were harvested 48 hr after transfection for measuring both CAT and luciferase activity and 20 hr after transfection for RNA extraction. CAT assays were done according to Gorman *et al.* (13). The CAT activity (ratio of the acetylated form to the total) was normalized by comparison with the luciferase activity of the corresponding sample measured as described by de Wet *et al.* (11).

S1 Nuclease Mapping Assay. S1 nuclease mapping was performed as described by Ciliberto *et al.* (14). The uniformly labeled probes were obtained as described (6). The simian virus 40 (SV40) probe is 484 nucleotides long and originates a protected band of 309 bases. The β-globin probe is 695 nucleotides long and gives origin to a protected band of 201 bases.

Northern Blot Analysis and CHX Treatment. Cells were treated for 12 hr with IL-6 alone or in the presence of CHX at a concentration of 10 μg/ml, sufficient to inhibit >95% of protein synthesis. Total cellular RNA was prepared according to Chirgwin *et al.* (15), fractionated by electrophoresis (10 μg per slot), and hybridized as described (7) with a ^{32}P-labeled HPX cDNA (16).

Plasmid Constructions and Site-Directed Mutagenesis. 5′-Deletion (5′Δ) and 3′-deletion (3′Δ) constructs were derived from BAL-31 deletions of a clone spanning between nucleotide −500 and nucleotide +22 of the *HPX* promoter region. Plasmids pUMS, 5′Δ-130/UMS, and 5′Δ-106/UMS were generated from the plasmid pUMS ΔE_2 (17), containing a fragment of the rat albumin promoter inserted between the CAT gene and a sequence named UMS (upstream mouse sequence) originating from the upstream region of the mouse c-*mos* gene, which has been shown to act as a potent transcription terminator in eukaryotic cells (18). The albumin sequences were eliminated to generate the promoterless

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Abbreviations: IL-6, interleukin 6; IL-6RE, IL-6 responsive element; IL-6DBP, IL-6-dependent DNA binding protein; HPX, hemopexin; CHX, cycloheximide; SV40, simian virus 40; CAT, chloramphenicol acetyltransferase.

plasmid pUMS and were substituted with the *HPX* sequences from nucleotide −130 or −106 to nucleotide +22 to generate the plasmids 5′Δ-130/UMS and 5′Δ-106/UMS, respectively. The oligonucleotide-directed mutagenesis of the clone 5′Δ-130/UMS, generating the plasmid M-130/UMS, was carried out by the oligonucleotide-directed procedure described by Kunkel (19). The 6 base pairs (bp) between positions −113 and −108 were replaced by the sequence GGATCC. 3′-Deletions were fused to a truncated SV40 promoter in the vector pUC19CAT (20). To generate the plasmid pOL-HpxA, the double-stranded oligonucleotide OL-HpxA (see below) was polymerized by ligation, gel-purified, and inserted in the *Bam*HI site of the vector pUC19CAT. The resultant plasmid contains three copies of the OL-HpxA oligonucleotide—the two proximal ones in the 5′–3′ orientation and the third one in the inverted orientation.

Cell Protein Extracts and Binding Assays. Protein extracts from cultured cell nuclei were prepared according to Shapiro *et al.* (21). Whole cell extracts were prepared according to Zimarino and Wu (22). The wild-type probe for the gel-retardation assays was a −178/−86 fragment from the *HPX* promoter. The mutant probe was a −130/−93 fragment obtained from the mutant plasmid M-130. The gel-retardation assays were performed as described (23). For each assay, 4 μg of nuclear extract was used. Three micrograms of poly(dI-dC) and 50 ng of sonicated salmon sperm DNA were added as competitors. For the competition assays, a 400-fold molar excess of unlabeled double-stranded oligonucleotides was used. Footprinting in the gel matrix was performed according to Kuwabara and Sigman (24).

Oligonucleotides. The following oligonucleotides were used: OL-HpxA, GATCCTATTTGCAGTGATGTAATCAGCG; OL-HpA, GATCCAAGTGTGAAGCAAGAGCG; OL-HPC, GATCCAGGAATTACGAAATGGAGGAG; OL-CRPα, GGGCATAGTGGCGCAAACTCCCTTACTG; OL-Lynk12, GGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGAC.

RESULTS

Induction of the HPX mRNA by IL-6 Does Not Require *de Novo* Protein Synthesis. In previous studies, we and others have shown that the human hepatoma cell line Hep3B is a good system for the investigation of the acute-phase reaction (4, 6–8). Picomolar amounts of IL-6 induce the accumulation of the mRNA encoding several acute-phase proteins. Untreated Hep3B cells contain a basal level of HPX mRNA, which can be induced at least 6-fold by IL-6 (Fig. 1). The kinetics of induction (data not shown) indicates that HPX mRNA reaches almost maximum levels after 12 hr of treatment with IL-6, and after 24 hr the induction reaches a plateau. Equivalent induction is also observed in the presence of CHX (Fig. 1), thus indicating that *de novo* protein synthesis is not required for HPX mRNA induction.

Definition of an IL-6RE on the *HPX* Promoter. The *HPX* gene has been cloned and its promoter region was identified and sequenced (9). To study the mechanism of induction by IL-6, transcription of HPX–CAT fusions transfected into the human hepatoma cell line Hep3B either in the presence or in the absence of IL-6 was examined. We have shown that a segment of DNA containing residues −130 to −86 relative to the transcriptional start site is sufficient to direct transcription in Hep3B cells but is inactive in HeLa cells (V.P., L. Silengo, F. Altruda, and R. Cortese, unpublished data). Here we show that transcription from a plasmid containing the *HPX* promoter from nucleotide −130 to nucleotide +22 is induced 6-fold by treatment of Hep3B cells with IL-6, while a further 5′ deletion up to nucleotide −106 is no longer inducible (Table 1). Similar induction is observed when a plasmid carrying the *HPX* promoter from nucleotide −500 to nucleotide −86 fused to the SV40 early promoter (21-bp repeats plus TATA box) is used as a template. Again, a further 3′ deletion up to nucleotide −130 abolishes inducibility. It is worthwhile to note that induction is never obtained in HeLa cells. The results of S1 nuclease mapping analysis confirm that in each case the correct start of transcription is used (data not shown). Therefore, the same minimal promoter element, between positions −130 and −86, directs hepatoma-specific transcription and is responsible for the induction by IL-6. We conclude that cis-acting element(s) responsive to IL-6 (IL-6RE) must be present within this promoter fragment.

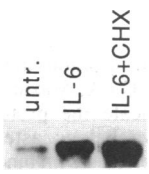

FIG. 1. Induction of HPX mRNA by IL-6. Hep3B cells were treated for 12 hr with control medium (untr.), with human recombinant IL-6 alone, or with IL-6 in the presence of CHX.

Identification of Nuclear Proteins Binding to the IL-6RE. Having identified the region of the *HPX* promoter containing the transcriptional signals involved in inducibility, we searched for the corresponding trans-acting factors by analyzing the interaction between the *HPX* promoter and nuclear proteins extracted from Hep3B cells, with or without prior treatment with IL-6.

A preliminary analysis by footprinting revealed several regions protected from DNase I digestion, including a footprint within the minimal element defined above, but no difference between extracts from treated and untreated cells was observed (data not shown).

The analysis of DNA–protein complexes by gel retardation was more informative. The minimal element of the *HPX* promoter forms several complexes with nuclear proteins, which can be separated by gel electrophoresis (Fig. 2*A*). Complexes 1, 2, 6, and 6′ are due to apparently ubiquitous proteins present in treated and untreated Hep3B (Fig. 2*A*) and HeLa cells (data not shown). It can be noted that complex 6 appears more abundant when extracts from IL-6-treated Hep3B cells are used. However, the amounts of complex 6 were highly variable from experiment to experiment; for this reason, although we cannot rule out the possibility that the protein giving origin to this complex plays some role in induction by IL-6, we decided not to investigate it any further. In contrast, complex 4 is reproducibly observed exclusively with extracts from IL-6-treated Hep3B cells. In some experiments, complex 4 separates into at least two closely spaced bands (4a and 4b). The protein(s) responsible for the formation of complex 4 has been named IL-6DBP. The induction of IL-6DBP activity is rapid: it is already detectable after 1 hr of treatment with IL-6; maximal levels are achieved within 3 hr (Fig. 2*B*).

Induction of IL6-DBP Is Hepatoma Specific. IL-6 is a multifunctional cytokine, triggering different responses in

Table 1. Induction ratios obtained with the HPX–CAT fusions in Hep3B cells

Template	Induction ratio
pUMS	1
5′Δ-130/UMS	6
5′Δ-106/UMS	1
M-130/UMS	1.3
pUC19CAT	1
3′Δ-86	7.2
3′Δ-130	1.2

For the 5′Δ as well as the 3′Δ constructs, the value of 1 corresponds to the induction ratio obtained with the vector pUMS or pUC19CAT, respectively.

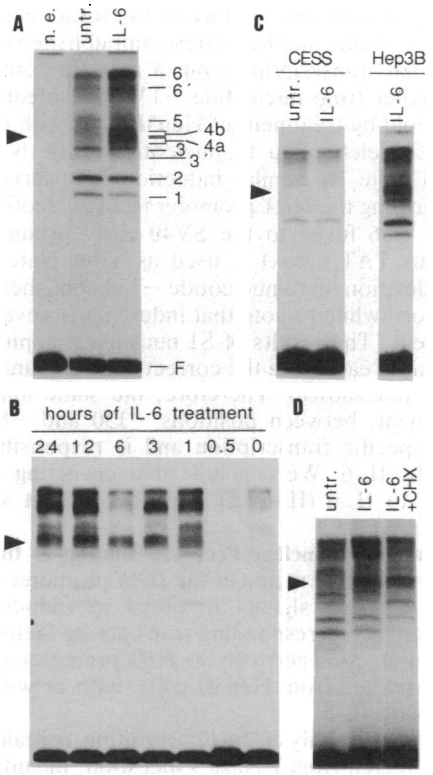

FIG. 2. Gel-retardation assays. (*A*) Appearance of complex 4 on induction. Nuclear extracts from untreated (untr.) or from IL-6-treated Hep3B cells were used. Numbers indicate the different complexes obtained. n. e., No extract; F, free DNA. (*B*) Kinetics of induction of complex 4. Whole cell extracts from Hep3B cells either untreated (0 hr) or treated with IL-6 (0.5–24 hr) were used. (*C*) IL-6DBP activity is not detectable in extracts from CESS cells. Whole cell extracts from untreated (untr.) or from IL-6-treated CESS cells and nuclear extracts from IL-6-treated Hep3B cells were used. (*D*) IL-6DBP activity is induced by IL-6 in the presence of CHX. Whole cell extracts from untreated, from IL-6-treated, or from IL-6-treated cells in the presence of CHX were used. Arrowheads indicate the position of complex 4.

different cell types. It is therefore interesting to establish whether induction of IL-6DBP is a general phenomenon, perhaps mediating all the transcriptional effects of IL-6 in various cell types, or whether it is induced only in hepatoma cell lines. The lack of IL-6DBP in IL-6-treated HeLa cells (data not shown) could simply be a consequence of the lack of receptors for IL-6 in this cell line. We therefore analyzed nuclear extracts from treated and untreated CESS cells, a lymphoblastoid cell line whose response to IL-6 is well characterized and consists of an increase in the secretion of IgG (25). The result shown in Fig. 2*C* clearly indicates that the ubiquitous proteins, giving rise to the complexes detected both in HeLa and in Hep3B cells, are also detectable in CESS nuclear extracts, but there is no trace of IL-6DBP activity. We can conclude that induction of IL-6DBP is not universally associated with treatment with IL-6, but it appears to be a hepatoma-specific phenomenon.

Activation of IL-6DBP Does Not Require *de Novo* Protein Synthesis. As described above, induction of the *HPX* mRNA by IL-6 is independent of *de novo* protein synthesis (Fig. 1). A further correlation between the induction of HPX mRNA and the appearance of IL-6DBP activity is that induction of IL-6DBP does not require *de novo* protein synthesis (Fig. 2*D*). Therefore, IL-6DBP acquires its binding activity through the IL-6-dependent activation of a preexisting inactive molecule (pre-IL-6DBP). This situation is reminiscent of the inhibition of DNA binding activity of NFkB by a cytoplasmic protein (IkB), which prevents NFkB nuclear transport by binding to it (26). In this case, however, it is unlikely that inhibitory protein–protein interactions play any role in the inhibition or activation of IL-6DBP. The protein giving origin to complex 4 is very stable, is resistant to heat treatment at 95°C, and can be efficiently renatured after denaturation with guanidine hydrochloride (data not shown). Neither of these treatments leads to the induction of IL-6DBP in extracts derived from uninduced cells. Furthermore, mixing either nuclear or whole cell extracts from induced and uninduced cells in different ratios does not affect IL-6DBP activity (data not shown). Even though these are only negative results, they indicate as more likely that IL-6DBP activity is generated by a covalent posttranslational modification of a preexisting inactive protein.

The Integrity of the IL-6DBP Binding Site Is Essential for Transcription. The complex pattern of DNA–protein interactions shown in Fig. 3 was further investigated by direct *in situ* footprinting of the various DNA–protein complexes with *o*-phenanthroline (24). Surprisingly, exactly the same DNA sequence, from nucleotides −120 and −104, was protected in each case (Fig. 3). The corresponding sequence was named the HPXA site. Identical results with each DNA–protein complex were also obtained with methylation interference experiments. These data might explain why we failed to observe differences in the pattern of DNase I footprinting between extracts from untreated or IL-6-treated Hep3B cells. The same DNA sequence (the HPXA site) is recognized by several proteins present in extracts from both untreated and IL-6-treated cells. The appearance of IL-6DBP, also interacting with the HPXA site, could only be detected by gel retardation, because it generates a complex of distinct electrophoretic mobility.

At present, we have not established the functional significance of the interaction between the various proteins and the A site on the *HPX* promoter. However, this site is both essential for IL-6 inducibility of the *HPX* promoter (Table 1) and for binding to each of the proteins responsible for the various complexes (Fig. 4*A*), since a 6-bp substitution in this sequence abolishes both phenomena. These results indicate that the HPXA site is an IL-6RE.

The HPX IL-6RE Is Sufficient to Confer IL-6 Inducibility on the SV40 Promoter. The data described above indicate that the IL-6RE present in the *HPX* promoter is essential for transcription. On this basis, it is not possible to distinguish between a general effect on transcription and a specific effect on transcriptional inducibility. To address this point, an

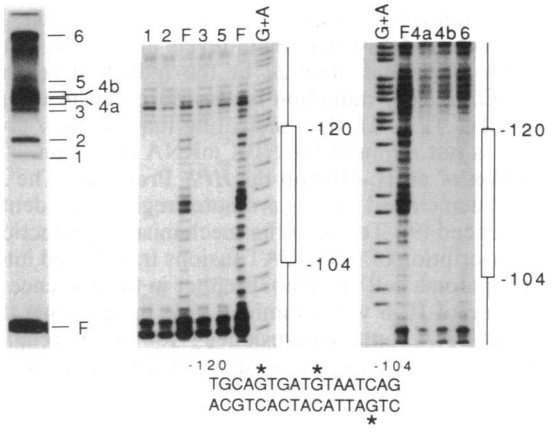

FIG. 3. *In situ* footprinting experiment. Lane designations indicate the different complexes analyzed. F, free DNA; G+A, Maxam and Gilbert sequencing reactions. Open boxes indicate the protected region, whose sequence (HPXA site) is shown below; asterisks indicate the G nucleotides whose methylation interferes with binding.

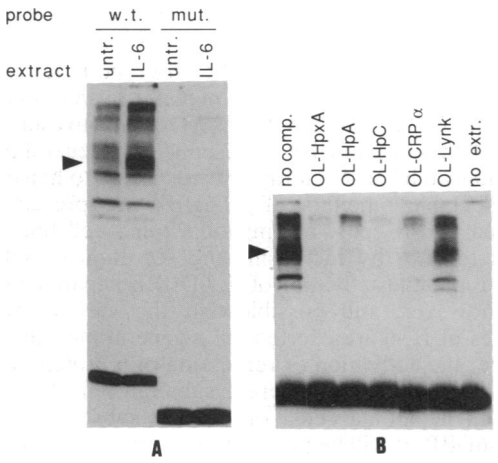

FIG. 4. Gel-retardation assays. (*A*) Mutation of the HPXA site abolishes binding. Nuclear extracts from untreated (untr.) or from IL-6-treated Hep3B cells were used either with the wild-type (w.t.) probe or with the mutant (mut.) probe. (*B*) Competition experiments. Nuclear extract from IL-6-treated Hep3B cells was used. Either no competitor (no comp.) or a 400-fold molar excess of different unlabeled double-stranded oligonucleotides was used: OL-HpxA, carrying the HPXA site; OL-HpA and OL-HpC, spanning the haptoglobin distal and proximal IL-6REs, respectively; OL-CRPα, carrying the C-reactive protein IL-6RE; OL-Lynk, a nonspecific competitor; no extr., no extract. Arrowheads indicate the position of complex 4.

enhancer–substitution experiment was performed. Transcription directed by the SV40 promoter and enhancer segment (Fig. 5 *Left*) is insensitive to IL-6, indicating that there are no IL-6REs in this gene. Furthermore, removal of the 72-bp enhancer, generating the enhancerless vector pUC19-CAT, leads to a strong decrease in transcription, both in the presence and in the absence of IL-6. It was then possible to substitute the SV40 enhancer with three copies of the HPX IL-6RE, generating the plasmid pOL-HpxA. This plasmid is poorly transcribed when transfected into untreated Hep3B cells, but its transcription is induced severalfold by IL-6 (Fig. 5 *Left*). Therefore, the HPX IL-6RE is sufficient to confer IL-6 inducibility on a promoter otherwise not responsive.

Induction of Transcription of IL-6RE–SV40 Fusions Does Not Require *de Novo* Protein Synthesis. Induction by IL-6 of both the HPX mRNA and the DNA binding activity of IL-6DBP is independent of *de novo* protein synthesis. A further correlation between these phenomena is provided by the observation that the transcriptional activity of the plasmid pOL-HpxA transfected into Hep3B cells is stimulated by IL-6 in the presence of CHX (Fig. 5 *Right*). For this experiment, it was necessary to measure transcription by S1 nuclease mapping because synthesis of CAT was prevented by blocking protein synthesis. Induction of transcription in the presence of IL-6 and CHX is lower than in the presence of IL-6 alone. At present, we ignore the reasons for this difference, and we cannot rule out a nonspecific toxic effect of CHX on transcription. On the other hand, it is also possible that the overall effect of IL-6 on HPX transcription is complex, involving CHX-sensitive as well as CHX-insensitive processes.

The IL-6DBP Binding Site Is Present on Other Acute-Phase Genes. In an independent study on the human haptoglobin gene, we identified two elements that are essential for activation of that promoter by IL-6 (8). Likewise, in their study of the human C-reactive protein promoter, Ciliberto and co-workers identified two different IL-6REs, α and β, located in the proximity of the TATA box (B. Majello, R. Arcone, C. Toniatti, and G. Ciliberto, personal communication). The two haptoglobin IL-6REs, the C-reactive protein α IL-6RE, and the HPX IL-6RE bind to the same proteins, as shown by the results of cross-competition experiments with oligonucleotides containing these sequences (Fig. 4*B*). We therefore propose that a general mechanism is responsible for the induction of transcription of acute-phase genes by IL-6, based on the interaction of an IL-6DBP(s) with the promoter of IL-6-responsive genes.

DISCUSSION

Treatment with IL-6, a potent inducer of several acute-phase genes, increases by 6-fold the HPX mRNA levels in the human hepatoma cell line Hep3B, and this induction does not require *de novo* protein synthesis. We show that the *HPX* promoter region is able to confer the same degree of induction to the transcription of the reporter gene. Therefore, IL-6 acts through a transcriptional activation of the *HPX* gene. It was possible to establish that the information required for the IL-6 response is contained within a short stretch of DNA (the HPXA site) between nucleotide −120 and nucleotide −104. Mutation in this site abolishes induction, and its fusion with a heterologous promoter results in acquisition of the ability to respond to IL-6.

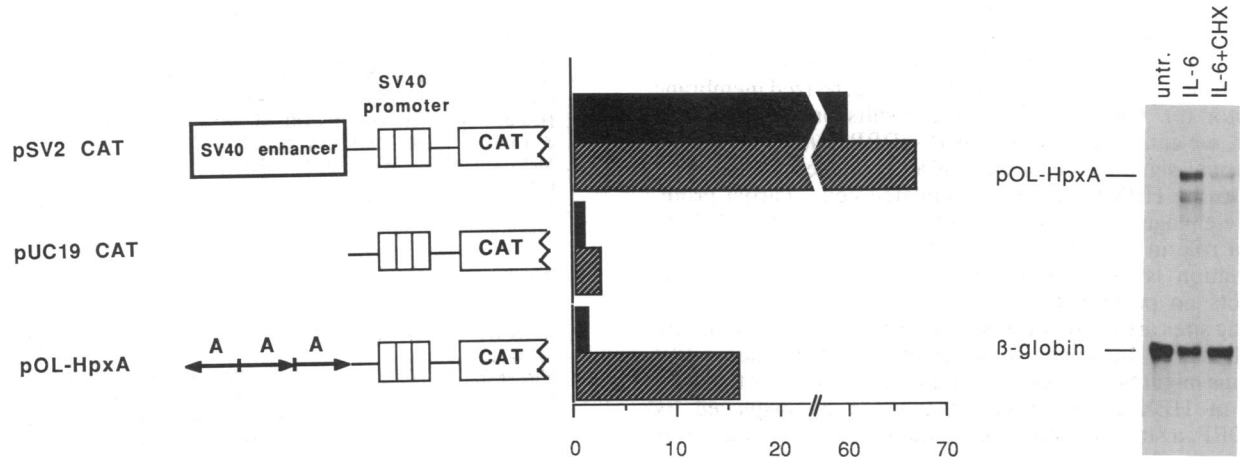

FIG. 5. Expression of IL-6RE–SV40 fusion on transfection into Hep3B cells. (*Left*) Histograms indicate the levels of normalized CAT activity on transfection of the depicted constructs into untreated (solid bars) or IL-6-treated (hatched bars) Hep3B cells. (*Right*) S1 nuclease mapping analysis. Total cellular RNA from Hep3B cells transfected with the pOL-HpxA plasmid was used. Cells were treated for 12 hr with control medium (untr.), with IL-6, or with IL-6 in the presence of CHX. Positions of the pOL-HpxA and β-globin protected bands are indicated.

The HPXA site is involved in complex interactions with different nuclear proteins, yielding identical footprint and methylation interference patterns. The different DNA–protein complexes can only be distinguished by their different electrophoretic mobilities. The existence of multiple proteins interacting with the same DNA sequence is not an uncommon phenomenon. Similar or identical sequences are recognized by the ubiquitous and lymphoid-specific octamer binding proteins (OTF-1 and OTF-2) as well as by several homeobox-containing DNA binding proteins (ref. 27 and refs. therein). Modulation of transcription by α and β interferons also apparently involves at least three different proteins, all recognizing the same sequence (28).

At present, it is not clear whether the different DNA binding activities detected are due to different modifications of the same protein or to different proteins sharing the same binding specificity. The functional importance of each of these interactions remains to be established. However, the properties of the protein(s) giving origin to complex 4 (IL-6DBP) are very interesting and suggestive. Unlike all the other complexes, complex 4 is only observed when extracts from IL-6-treated Hep3B cells are used. The kinetics of activation of the IL-6DBP activity is compatible with it being a prerequisite for the transcriptional activation of the *HPX* promoter. There is also a strong correlation between the fact that IL-6 induction of HPX mRNA does not require *de novo* protein synthesis and the observation that the appearance of IL-6DBP activity is not prevented by treatment with CHX. Furthermore, IL-6-dependent induction of transcription of the SV40 promoter is solely dependent on the fusion to multimers of HPX IL-6RE and is observed in the presence of CHX (Fig. 5 *Right*).

IL-6DBP also binds to sequences characterized as IL-6REs on the promoter of haptoglobin and C-reactive protein genes. This finding adds to the idea that IL-6DBP plays a crucial role in IL-6-dependent activation of transcription of several acute-phase genes.

A direct demonstration of the role of IL-6DBP must await the development of a functional assay. We believe, however, that the circumstantial evidence presented in this paper is very strong, and we propose that the final step of IL-6-dependent induction of HPX, haptoglobin, C-reactive protein, and possibly of other acute-phase genes is the interaction between IL-6DBP and IL-6REs. The activation of IL-6DBP does not require *de novo* protein synthesis, and it is likely to involve covalent modifications of a preexisting inactive molecule (pre-IL-6DBP) rather than changes in protein–protein interactions, as it has been demonstrated in the case of NFkB (26), or in the interaction of OTF-1 with VP16 (29). The activation of IL-6DBP might thus represent the penultimate step of the series of reactions started by the interaction of IL-6 with the recently characterized membrane receptor (3). On the basis of the results presented in this paper, we can suggest a role for IL-6DBP, but we ignore the functional significance, if any, of all the other proteins that bind to the HPXA site in unstimulated cells. Partial proteolitic cleavage of a single DNA binding protein is unlikely to play a role in this phenomenon, because the pattern of gel retardation is very reproducible. Moreover, in the same extracts no protein degradation is noticed when different binding sites are used as probes. Alternative explanations are possible. For instance, some or all the IL-6-independent proteins might be weaker activators, responsible for the basal level of HPX transcription, which can be displaced by IL-6DBP, a stronger activator. A variant of this hypothesis is that some of them are antagonists of IL-6DBP. The latter would imply that the level of transcription of the *HPX* promoter is a consequence of the relative intranuclear concentration of activators (IL-6DBP) and repressors (some or all the other proteins). In light of this possibility, IL-6 would have the role of increasing the ratio of activators over repressors.

Different effects of IL-6 can be observed in different cell targets, and the pathways of signal transmission are at present poorly understood. It was interesting to establish that the activation of IL-6DBP represents not a general phenomenon induced by IL-6 but rather is restricted to hepatic cells. No detectable amounts of IL-6DBP are present in IL-6-treated CESS cells, a lymphoblastoma cell line that responds to IL-6 by increasing the secretion of IgG. This indicates that the activation of IL-6DBP is hepatoma specific. It is, however, still possible that the various biological activities of IL-6 are exerted via a general mechanism—for instance, the activation or repression of a protein modification system involving different substrates in different cell types. In hepatoma cells, one of the substrates could be pre-IL-6DBP. It will be possible to test this hypothesis when the molecular basis for the activation of IL-6DBP has been clarified.

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