Oct-1 and CCAAT/Enhancer-binding Protein (C/EBP) Bind to Overlapping Elements within the Interleukin-8 Promoter

THE ROLE OF Oct-1 AS A TRANSCRIPTIONAL REPRESSOR

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Interleukin-8 (IL-8), a potent neutrophil chemoattractant, can be expressed at high levels by many different cell types after immune stimulation. In contrast, expression of IL-8 in these same cells is virtually absent in the unstimulated state, demonstrating the tight regulation of the IL-8 gene. Although much is known about how this gene is transcriptionally activated after immune stimulation, little is known about the regulation of the IL-8 promoter in the absence of immune activation. In this study we examine how the IL-8 promoter is transcriptionally regulated in the uninduced state and how these mechanisms are altered in response to immune stimulation by IL-1β. Electrophoretic mobility shift assay and transfection studies show that the IL-8 promoter is transcriptionally regulated by both positive and negative elements. Although the nuclear factor-κB (NFκB) element regulates only inducible activity of the IL-8 promoter in response to stimulation with IL-1β, the AP-1 and CCAAT/Enhancer-binding Protein (C/EBP) elements influence both basal and inducible activities. In contrast to these three positive regulatory elements, the binding of the ubiquitously expressed POU-homeodomain transcription factor, Oct-1, strongly represses transcriptional activity of the IL-8 promoter by binding independently to an element overlapping that of C/EBP.

regulated in a cell line-specific fashion requiring a NFκB element plus either an AP-1 or a C/EBP element (Fig. 1). For example, the AP-1 element along with the NFκB site are sufficient for the full expression of this promoter in gastric cell lines (4), whereas in a fibrosarcoma cell line, only the C/EBP and NFκB sites are required (5). Recently, several investigators have shown that NFκB/Rel transcription factors can interact with those of the C/EBP family through a rel domain-bZIP interaction to alter the transcriptional activation of the IL-8 promoter (6–8). In contrast to transcriptional induction, there are no previous reports which describe how the IL-8 gene is regulated in the absence of inflammatory stimulation, a state which we will refer to as basal transcriptional activity. In this study we investigate how the IL-8 promoter is transcriptionally regulated in two epithelial cell lines, Caco-2 and HepG2, in the unstimulated state and in response to a physiologically relevant mediator of inflammation, IL-1β. These two cell lines constitutively express certain bZIP transcription factors, which play a role in the transcriptional activation of the IL-8 promoter. The AP-1 and C/EBP elements bind proteins that significantly influence not only induced transcription activity of the IL-8 promoter but also activate this promoter in the absence of immune stimulation. Despite this, these two cell lines have no detectable expression of IL-8 mRNA by Northern blot analysis in the absence of an inflammatory stimulus (9). We provide the first evidence that the ubiquitously expressed transcription factor, Oct-1, binds to a motif overlapping that of the C/EBP element and acts as a potent transcriptional repressor, which may help to prevent expression of the IL-8 promoter in the uninduced state.

EXPERIMENTAL PROCEDURES

Cell Culture Line and Conditions—Caco-2 and HepG2 cells (from ATCC), used for both nuclear protein isolation and in the transfection experiments described below, were plated at a density of 4 X 10⁴ cells/cm² in 10-cm dishes containing Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum and penicillin/streptomycin as described previously (10, 11).

Nuclear Protein Isolation and Electrophoretic Mobility Shift Assay (EMSA)—Caco-2 cells, day 5 post plating, were stimulated with complete medium containing 5 ng/ml IL-1β for 45 min. Nuclear proteins were isolated from unstimulated and IL-1β-stimulated Caco-2 cells using a modification (12) of the method of Dignam et al. (13). The protein concentrations of the extracts were determined (14), and aliquots were stored at −80 °C. Complimentary oligonucleotides with overlapping ends were synthesized, annealed, and labeled with ³²P using Klenow enzyme as described previously (11). Binding reactions, each containing 10 µg of nuclear extract, were performed as described previously (11) and subsequently separated on a 4% polyacrylamide gel. Competition and antibody supershift experiments were performed in a fashion described previously (15). Supershift assays used 1 µl of each antibody for the three C/EBP isoforms α, β, and δ (Santa Cruz Biotechnology, Santa Cruz, CA).

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Fig. 1. Sequence analysis of the IL-8 promoter and description of oligonucleotides used in EMSA experiments. The IL-8 promoter region contains three cis-acting elements that have been shown to be functional in cell lines, AP-1, C/EBP (NF-IL6), and NF-κB. The IL-8 promoter sequence encompassing the C/EBP and NF-κB elements are shown with these two motifs identified by the boxes. The consensus sequences for these two elements are located above the IL-8 promoter sequence. The C/EBP and NF-κB motifs are adjacent to each other, while a binding motif for octamer transcription factors is found on the complementary strand directly overlapping that of the C/EBP element. The sequences of the sense strand, in the 5’ to 3’ orientation, of various oligonucleotides used for EMSA have been aligned to the genomic sequence for comparison and are listed below. Site-directed mutations are underlined. Several base extensions, which were added to certain oligonucleotides to permit Klenow labeling, are shown in lower case letters.

Herr, Cold Spring Harbor Laboratory, and c-fos (Upstate Biotechnology, Lake Placid, NY).

The sense strand sequences of the various oligonucleotides used for EMSA (described under “Results”) are listed in Fig. 1. In particular, the C/EBP.cons oligonucleotide contains a previously described consensus sequence for C/EBP (16). The Oct.cons oligonucleotide contains a octamer consensus sequence which was commercially available (Santa Cruz Biotechnology) and was labeled using T4 kinase. Two additional oligonucleotides, not listed in Fig. 1, include AP-1 (a 1-AP-1 element of the IL-8 promoter, designated in boldface type) and mAP-1 (a 2-bp mutation, underlined, of the IL-8 AP-1 element): AP-1, 5’-gtgagatctGAAGTGTAAG-3’ and mAP-1, 5’-gtgagatctGAAGTGTAAGccc-3’. The consensus sequences for these elements are in bold face type with the corresponding sites underlined.

Construction of Luciferase Reporter Plasmids—The IL-8 promoter region (bp 135 to +46) was amplified by polymerase chain reaction using human liver genomic DNA as a template with the primers IL-8(135)5’-GTGAGATCTGAAGTGTAAGG-3’ (Promega, Madison, WI) yielding the reporter construct (wt)LUC. The fidelity of each polymerase chain reaction product was verified by dideoxy chain-termination sequencing using Sequenase 2.0 (U.S. Biochemical Corp.). Each reporter plasmid was amplified in DH5α Escherichia coli and purified by alkaline lysis followed by two successive bandings of plasmid DNA in cesium chloride gradients (18).

Cell Transfections; Luciferase and β-Galactosidase Assays—Caco-2 or HepG2 cells were plated at a density of 4 × 10⁴ cells/cm² in 10-cm dishes containing complete medium. After 24 h, 7 μg of reporter plasmid were co-transfected with 1 μg of SV40 β-galactosidase (transfection control) using the calcium phosphate precipitation method of Chen and Okayama (19). To determine the effect of IL-1β on reporter gene expression, products into pGL2-Basic yielded the reporter constructs (mAP-1)LUC and (mAP-1)LUC. The binding elements are in boldface type with the point mutation underlined: −135IL8(mAP-1), 5’-GTGAGATCTGAAGTGTAAGGTGGAATTCCCTCTGAGGGATGGGCCATCAGCTA-3’; −135IL8(mC/EBP), 5’-GTGAGATCTGAAGTGTGATGACTCAGG-3’; and (mC/EBP)LUC. Similarly, the 5-bp mutation of the C/EBP element used in the IL-8(C/EBP.cons)LUC EMSA oligonucleotide was incorporated into the IL-8 promoter in a similar fashion to yield the reporter construct, (Oct.cons)LUC. The fidelity of each polymerase chain reaction product was verified by dideoxy chain-termination sequencing using Sequenase 2.0 (U.S. Biochemical Corp.). Each reporter plasmid was amplified in DH5α Escherichia coli and purified by alkaline lysis followed by two successive bandings of plasmid DNA in cesium chloride gradients (18).
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FIG. 2. Functional effect of site-directed mutations on IL-8 promoter activity in Caco-2 cells. Transfection of the wild-type IL-8 promoter construct, (wt)LUC, as well as those containing site-directed mutations of the NFκB ((mNFκB)LUC), C/EBP ((mC/EBP)LUC), or AP-1 (mAP-1)LUC) binding elements into Caco-2 cells; ■, control cells; □, IL-1β stimulation (5 ng/ml) for 6 h. The results in the graph are reported as the mean ± S.D. of three replicates normalized for transfection efficiency using β-galactosidase.

![Graph showing functional effect of site-directed mutations on IL-8 promoter activity in Caco-2 cells.](image)

| % of Wild-type | Fold Induction (Stim/Basal) |
|---------------|---------------------------|
| Basal         | Stimulated                |
| (wt)LUC       | 100%                      | 100% | 11.4X |
| (mNFκB)LUC    | 99%                       | 1%   | 2X   |
| (mC/EBP)LUC   | 160%                      | 11%  | 7.5X |
| (mAP-1)LUC    | 30%                       | 26%  | 98X  |

RESULTS

Binding of Nuclear Proteins to the IL-8 NFκB Element Are Necessary but Not Sufficient for Full Transcriptional Activity of the IL-8 Promoter in Response to IL-1β—In order to determine the functional effect of previously characterized DNA binding elements in the IL-8 promoter in the colon cancer cell line, Caco-2, transcriptional activation of the wild-type IL-8 promoter ((wt)LUC) or the promoter with point mutations in the NFκB ((mNFκB)LUC), C/EBP ((mC/EBP)LUC), or AP-1 (mAP-1)LUC) binding elements was determined by transient transfection into Caco-2 cells as described under “Experimental Procedures” (Fig. 2). After stimulation with IL-1β, the activity of this promoter is increased by 114-fold. Although a 3-bp mutation in the NFκB element of the IL-8 promoter does not affect the basal activity of this promoter, it does eliminate induction of promoter activity by IL-1β. A 4-bp mutation of the C/EBP binding element increases basal activity by 60% and decreases, but does not eliminate, the induction of the IL-8 promoter in response to IL-1β. In contrast, mutation of the AP-1 binding element decreases the basal activity of the IL-8 promoter by 3–4-fold, but does not affect the inducible response (98-fold) of the promoter to IL-1β. These results demonstrate that, although protein binding to the NFκB element of the IL-8 promoter is critical for transcriptional induction in response to IL-1β, the AP-1 and C/EBP binding elements are required for the full expression of the IL-8 promoter.

The Proto-oncogene Product fos Is Part of the Protein Complex That Binds Constitutively to the AP-1 Element of the IL-8 Promoter—EMSA using a double-stranded oligonucleotide spanning the AP-1 element of the IL-8 promoter shows that nuclear proteins from Caco-2 cells bind constitutively to this motif (Fig. 3). Effective competition using the unlabeled wild-type AP-1 oligonucleotide demonstrates the specific nature of this DNA-protein interaction. In contrast, ineffective competition using the mutant oligonucleotide, mAP-1, demonstrates that a 2-bp mutation of the AP-1 element significantly reduces protein binding to this element. Supershift of this band with the addition of fos antiserum shows that the proto-oncogene product, fos, is a component of the protein complex that binds to the AP-1 element of the IL-8 promoter. The specific nature of this supershift is demonstrated by effective competition with the addition of excess unlabeled wild-type AP-1 oligonucleotide.

Transcription Factors of Both the Octamer and C/EBP Families Bind Independently to the C/EBP Element of the IL-8 Promoter—The C/EBP binding motif of the IL-8 promoter, located between bp −82 and −94, also contains an overlapping octamer motif located on the complementary strand in the opposite orientation (Fig. 1). Pictured in Fig. 4 is an EMSA of Caco-2 nuclear extracts using a double-stranded oligonucleotide as a probe, named C/EBP, that spans this region. This sequence binds nuclear transcription factors of both the octamer (slower mobility complex) and C/EBP families in both unstimulated Caco-2 cells and those stimulated with IL-1β, lanes 2 and 3, respectively. Near complete competition of both the bands by addition of 100-fold excess unlabeled C/EBP oligonucleotide in lane 4 demonstrates the specific nature of the binding. Competition experiments using double-stranded oligonucleotides encoding consensus sequences for C/EBP (C/EBP.cons) and octamer (Oct.cons) in lanes 5 and 6, respectively, show that these two transcription factors can bind to the C/EBP probe independently. As expected, competition with both of these consensus oligonucleotides in lane 7 eliminates all specific binding. Identical results were observed in EMSAs performed using nuclear extracts isolated from both HepG2 cells and Hela cells (data not shown). Supershift experiments using antiserum specific for the nuclear transcription factor Oct-1 in lane 8 (20) demonstrate that the slower mobility complex is due to binding of Oct-1. Competition with the octamer consensus oligonucleotide in lane 9 shows the specific nature of

![Graph showing results of functional effect of site-directed mutations on IL-8 promoter activity in Caco-2 cells.](image)
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The supershifted band. In a similar fashion, supershift experiments using antisera specific for the C/EBP isoforms α, β, and δ (lanes 10, 11, and 12, respectively) show that all three isoforms are present in Caco-2 cells and can bind to C/EBP probe.

Adjacent C/EBP and NFκB Elements in the IL-8 Promoter Allow Interactions of NFκB Proteins with that of C/EBP and Octamer—Physical interaction between NFκB and C/EBP transcription factors through the rel and bZIP domains of these proteins lead to synergistic activation of the IL-8 promoter (6–8). In order to demonstrate this interaction in Caco-2 cells and to determine if Oct-1 binding occurs within the context of this interaction, EMSAs of nuclear extracts isolated from both control (C) and IL-1β-stimulated (S) Caco-2 cells were performed using a double-stranded oligonucleotide probe, named IL-8wt, which spans both the C/EBP and NFκB elements of the IL-8 promoter (Fig. 5, lanes 3 through 13). EMSA using the NFκB probe in lanes 1 and 2 demonstrate the relative mobility of proteins which bind to the NFκB element and confirm their induction of binding by IL-1β. The specific binding patterns in both control and IL-1β-stimulated extracts using the IL-8wt probe, in lanes 3 and 4, respectively, is demonstrated by effective competition using the IL-8wt oligonucleotide in lanes 5 and 6. Oct-1 and C/EBP bind independently to the IL-8wt probe in unstimulated Caco-2 cells (lane 9). This is confirmed by effective competition with the C/EBP and Oct heterodimeric complexes formed using the Oct-1 oligonucleotide in lanes 9 and 11, respectively. Upon stimulation with IL-1β, NFκB and C/EBP form a heterodimeric complex of slower mobility (lane 4). This is confirmed by the loss of this band upon competition with either the NFκB or Oct oligonucleotide (lane 8) or the C/EBP oligonucleotide (lane 10). Oct-1 binding appears to be diminished upon cell stimulation in lane 4 as confirmed by the similarity in binding patterns when the Oct-1 oligonucleotide is used as a competitor in stimulated cell extracts in lane 12. Lane 13 represents the IL-8wt probe alone. In summary, these results demonstrate that both C/EBP and Oct-1 bind to the promoter in control cells. Upon stimulation with IL-1β, Oct-1 binding may be reduced and the induction of NFκB binding leads to the formation of a heteromeric complex of C/EBP and NFκB.

Separation of Oct-1 and C/EBP Binding by Site-directed Mutagenesis of the IL-8 C/EBP Element—The EMSA using the IL-8wt oligonucleotide in Fig. 5 shows the complex nature of the DNA-protein interactions at this motif involving proteins of the NFκB/rel, C/EBP, and Oct-1 families. The consensus sequence for either C/EBP or octamer (Fig. 1) were used to create site-directed mutations of this oligonucleotide to separate C/EBP from Oct-1 binding. The effect of these mutations on DNA-protein interactions using Caco-2 nuclear proteins were determined by EMSA. Fig. 6A shows that the oligonucleotide IL-8(C/EBP.cons) which contains a 5-bp mutation, enhances C/EBP binding but eliminates Oct-1 binding. Competition with the C/EBP.cons oligonucleotide eliminates all specific binding in extracts from unstimulated cells (lane 7) and permits binding of NFκB/rel factors in the stimulated state (lane 8). In contrast, the DNA-protein interactions are not altered by competition with the Oct.cons oligonucleotide in extracts isolated form either control or IL-1β-stimulated extracts (compare lanes 1 and 2 to lanes 9 and 10). This oligonucleotide still permits the formation of the slower mobility heteromeric C/EBP-NFκB complex upon IL-1β stimulation (lane 2). This complex is lost upon competition with oligonucleotides specific for either NFκB (lane 6) or C/EBP (lane 8) but not Oct-1 (lane 10). Although this mutation clearly increases binding of nuclear proteins specific for a C/EBP element, it is not known if the proportion of the various C/EBP isoforms which bind has been altered compared to those which bind to the wild-type C/EBP element (Fig. 4). The oligonucleotide IL-8(Oct.cons) contains a 3-bp mutation which enhances Oct-1 binding and eliminates C/EBP binding (Fig. 6B). Competition with an oligonucleotide specific for octamer eliminates the predominant band (lanes 11 and 12) in contrast to competition with C/EBP.cons which does not alter the DNA-protein interactions (compare lanes 3 and 4 to lanes 9 and 10). Although proteins can still bind to the NFκB element in this oligonucleotide (lane 12), there is no heteromeric complex formation between these proteins and Oct-1. Pictured in
Fig. 6. EMSAs confirm that site-directed mutations of the IL-8wt oligonucleotide separate C/EBP from Oct-1 binding to the C/EBP element in the IL-8 promoter. Competition experiments were performed by preincubation of the reaction for 20 min at room temperature using 100-fold molar excess of the unlabeled oligonucleotides shown. A, an oligonucleotide with 5-bp mutation named IL-8(C/EBP.cons) enhances protein binding specific for the C/EBP element in the absence of Oct-1 binding in both control (C) and IL-1β-stimulated (S) nuclear extracts. B, an oligonucleotide with a 3-bp mutation named IL-8(Oct.cons) enhances protein binding specific for the Oct-1 in the absence of C/EBP binding in both control (C) and IL-1β-stimulated (S) nuclear extracts. C, EMSA using an oligonucleotide named IL-8(mC/EBP) which contains a 4-bp mutation of the IL-8wt oligonucleotide, eliminates binding of both C/EBP and Oct-1 but preserves binding to the NFκB element.

Oct-1 Is a Potent Repressor of Both Basal and Activated Transcription of the IL-8 Promoter in Caco-2 and HepG2 Cells—In order to determine the effect of Oct-1 and C/EBP binding on both basal and stimulated activity of the IL-8 promoter, two site-directed mutants of the wild-type IL-8 promoter, (C/EBP.cons)LUC and (Oct.cons)LUC, were transfected into both Caco-2 and HepG2 cells. Enhanced binding of C/EBP in the absence of Oct-1 (the C/EBP.cons mutant) increases the unstimulated transcriptional activity in both Caco-2 and HepG2 cells by severalfold (Fig. 7, A and C, respectively) compared to the wild-type IL-8 promoter. In contrast, enhanced binding of Oct-1 in the absence of C/EBP (the Oct.cons mutant) virtually abolishes the basal activity of this promoter in both these cell lines. Fig. 7C also shows that elimination of both C/EBP and Oct-1 binding with the (mC/EBP)LUC mutant actually enhances the basal activity of the IL-8 promoter in HepG2 cells compared to the wild-type; a finding also observed in Caco-2 cells (Fig. 2). These results demonstrate that C/EBP is an activator of basal transcription in contrast to Oct-1 which acts as a repressor. Fig. 7, B and D, shows that, upon stimulation of Caco-2 and HepG2 cells, respectively, with IL-1β, transcriptional activation of the IL-8 promoter is preserved with enhanced C/EBP binding(C/EBP.cons) but is virtually eliminated upon binding of Oct-1 (Oct.cons).

DISCUSSION

Transcriptional regulation of the IL-8 promoter involves the interaction of transcription factors which are activated by immune stimulation, such as those of the NFκB/rel family, and constitutively expressed proteins of the bZIP family, such as C/EBP and AP-1 (3). The transcriptional regulation of this promoter in the basal state and after induction by the proinflammatory cytokine, IL-1β, was studied in two epithelial cell lines. As anticipated, inducible activation of NFκB transcription factors by IL-1β is critical for the transcriptional induction of the IL-8 promoter but plays no role in uninduced transcrip-
Full transcriptional activation of this promoter, however, requires the binding of nuclear transcription factors to both the C/EBP and AP-1 elements (Fig. 2). Therefore, all three previously described cis-acting elements in the IL-8 promoter, AP-1, C/EBP, and NFκB, act as positive elements in the activation of the IL-8 promoter in Caco-2 cells. This is in contrast to previously published results obtained from other cell lines where the NFκB element along with either the C/EBP or AP-1 element are sufficient for full activation of this promoter (4, 5). It is likely that different sets of transcription factors may be involved in the transcriptional regulation of the IL-8 promoter in a cell type-specific manner.

In contrast to the inducible binding of NFκB, EMSAs of Caco-2 nuclear proteins show that transcription factors such as fos bind constitutively to the AP-1 element of the IL-8 promoter (Fig. 3). It is not surprising, therefore, that the transfection of AP-1 mutants of the IL-8 promoter into Caco-2 cells show that this site has no effect on the inducibility of this promoter but is rather involved in the basal level of transcription (Fig. 2). It is interesting to note, however, that the induced transcriptional activity of this promoter is reduced in proportion to the reduction in basal activity when the AP-1 element is mutated. This suggests that the potential for transcriptional induction by IL-1β is influenced by the basal activity of this promoter.

In similar fashion, proteins that bind to the C/EBP element of the IL-8 promoter play a role in both the basal and induced transcriptional activity of this promoter. Several investigators have shown that physical interactions of C/EBP transcription factors with those of the NFκB/rel family lead to synergistic transactivation of the IL-8 promoter through the adjacent C/EBP and NFκB DNA binding elements (6–8). EMSAs confirm that stimulation of Caco-2 cells lead to the formation of a heteromeric complex of NFκB/rel- and C/EBP-binding proteins (Fig. 5). EMSAs using the C/EBP element show that three isoforms of C/EBP, α, β, and δ, are able to bind to the IL-8 promoter in Caco-2 cells (Fig. 4). Although we did not characterize the specific C/EBP isoforms, which interact with the NFκB/rel proteins in Caco-2 cells, Stein et al. (21) have shown that all three isoforms can physically interact with p65 to enhance gene expression of a reporter gene regulated by the SRE of the human c-fos promoter.

In contrast to the role of C/EBP in the induction of IL-8 promoter activity, the role that this element plays in the regulation of this promoter in the absence of immune stimulation has not been previously studied. EMSAs of the C/EBP element clearly demonstrate that not only C/EBP but also Oct-1 can bind to this motif. The binding of Oct-1 to the IL-8 promoter has not been previously reported. Oct-1 belongs to the POU-homeodomain family of transcription factors which bind to the 8-bp octamer sequence ATGCAAAT (22). The IL-8 C/EBP element diverges from this consensus sequence by 1 bp (Fig. 1). It is well known, however, that Oct-1 can bind promiscuously to a divergent array of DNA binding elements (23). The overlapping nature of the C/EBP and Oct-1 binding sequences in the IL-8 promoter is consistent with the observation that these two proteins bind independently to this element (Fig. 4). Oct-1 is ubiquitously expressed and plays a critical role in basic cellular functions. It has been shown to both activate and
C/EBP and Oct-1 to overlapping elements permits three states of promoter activity in the absence of IL-1

Emerging evidence suggests that transcriptional activation via Oct-1 is dependent upon interactions with additional proteins such as Pit-1 (25), OAP (26), or VP-16 (27). In contrast, Oct-1 has also been shown to act as a transcriptional repressor for a number of regulatory regions such as the LCR enhancer of HPV 16 (28), the SV40 enhancer (29), and the HPV 18 enhancer (30). Each one of these regulatory regions contains an Oct-1 element, which overlaps that of a specific transcriptional activator (PEF-1, Sp1, and KRF-1, respectively). Transcriptional activation by each of these regulatory regions is repressed by displacement of the transcriptional activator by the binding of Oct-1. Therefore, the overall level of transcriptional activity is determined by balance between the binding of Oct-1 and the particular transcriptional activator (28). In order to ascertain the functional significance of Oct-1 binding in the context of the IL-8 promoter, we compared the transcriptional activity of three site-directed mutants of this promoter: 1) IL-8(C/EBP.cons), a 5-bp mutation which enhances C/EBP binding but eliminates the binding of Oct-1; 2) IL-8(Oct.cons), a 3-bp mutation which enhances Oct-1 binding but eliminates the binding of C/EBP; and 3) IL-8(mC/EBP), a 4-bp mutation which eliminates binding of both Oct-1 and C/EBP (Fig. 6, A–C).

In a fashion analogous to the regulatory elements of the HPV and SV40 enhancers, EMSA and site-directed mutagenesis of the C/EBP element of the IL-8 promoter show that Oct-1 acts as a potent transcriptional repressor of both the basal and induced IL-8 promoter activity in Caco-2 and HepG2 cells (Fig. 7). It is likely that the loss of transcriptional activity observed with the IL-8(Oct.cons) mutation is due to a combination of the loss of C/EBP binding and the gain of Oct-1 binding. However, the fact that a mutation which eliminates binding of both C/EBP and Oct-1 to this element actually increases basal activity while retaining a significant degree of transcriptional inducibility (Figs. 2 and 7) supports the role of Oct-1 as a transcriptional repressor. Furthermore, the IL-8(C/EBP.cons) mutation demonstrates that C/EBP binding can act to increase transcriptional activity of this promoter in unstimulated cells (Fig. 7, A and C). Indeed, although C/EBP binding to the IL-8 promoter in the unstimulated state is relatively weak (Fig. 4), other investigators have shown that this promoter can be transactivated by several fold upon co-transfection of an expression vector for NF-IL6 (C/EBP β) (7, 8). Therefore, in light of the EMSAs, which show that C/EBP binding is present in the uninduced state (Figs. 4 and 5), transcriptional repression by Oct-1 would be necessary to maintain the low level of basal activity of the wild-type IL-8 promoter in the absence of immune stimulation.

The results of this study would support the following model regarding regulation of the transcriptional activity of the IL-8 promoter in the uninduced state (Fig. 8). Basal activity is determined by constitutive binding of transcription factors to the AP-1 element and the proportional binding of C/EBP, a transcriptional activator, and Oct-1, a transcriptional repressor, to overlapping motifs located at the C/EBP element of this promoter. The site-directed mutants used in this study provide evidence that protein binding to the C/EBP element, which can be divided into three different states, has a significant influence on the basal activity of this promoter. Exclusive binding of Oct-1 with the (Oct.cons)LUC reporter leads to low basal activity while exclusive binding of C/EBP with the (C/EBP.cons)LUC reporter results in high basal activity. Intermediate basal activity is observed with the (mC/EBP)LUC reporter where protein binding to this element is absent. The observed basal activity of the wild-type IL-8 promoter is, therefore, a composite of these three promoter states.

Although it is not clear by EMSA whether or not Oct-1 binding is lost when Caco-2 cells are stimulated by IL-1β (Fig. 5), the loss of any detectable transcriptional activation when Oct-1 binding is present (Fig. 7, B and D) in contrast to the 100-fold transcriptional induction noted with the wild-type promoter (Fig. 2) strongly suggests that Oct-1 binding is replaced by that of NFκB and C/EBP upon IL-1β stimulation (Fig. 8). The lack of transcriptional activity with the (Oct.cons)LUC reporter after stimulation with IL-1β is likely an artificial state due to the mutation of the C/EBP element which disrupts the cooperative binding of C/EBP and NFκB upon cell stimulation. The physiologic role of Oct-1 binding may, therefore, not be to inhibit transcriptional induction to an inflammatory stimulus, but rather to inhibit basal activity perhaps in cell types which constitutively express high levels bZIP proteins which are capable of binding to the C/EBP and/or AP-1 elements of the IL-8 promoter. IL-8 is an initiator of the acute inflammatory response and can be induced in many different cell types (31). Oct-1, a transcription factor that is ubiquitously expressed (22), would therefore be well suited as a transcriptional repressor which inhibits expression of this potent chemoattractive substance in the absence of immune stimulation.

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Fig. 8. A model for interaction between the positive and negative regulatory elements of the IL-8 promoter. Independent binding of C/EBP and Oct-1 to overlapping elements permits three states of promoter activity in the absence of IL-1β stimulation. Evidence for the level of activity is provided by the three site-directed mutants of the IL-8 promoter, (C/EBP.cons)LUC, (mC/EBP)LUC, and (Oct.cons)LUC. The observed basal transcriptional activity of the wild-type IL-8 promoter is a composite of these three states. Binding to the NFκB element transactivates this promoter after stimulation with IL-1β.
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