CCAAT/Enhancer-binding Proteins $\beta$ and $\delta$ Mediate the Repression of Gene Transcription of Cartilage-derived Retinoic Acid-sensitive Protein Induced by Interleukin-1$\beta$*

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Cartilage-derived retinoic acid-sensitive protein (CD-RAP) is a secreted protein expressed by chondrocytes; the expression is repressed by interleukin 1$\beta$ (IL-1$\beta$). To investigate the transcriptional mechanism, by which CD-RAP expression is suppressed by IL-1$\beta$, deletion constructs of the mouse CD-RAP promoter were transfected into rat chondrocytes treated with or without IL-1$\beta$. The results revealed an IL-1$\beta$-responsive element located between $-2138$ and $-2068$ bp. As this element contains a CAAT/enhancer-binding protein (C/EBP) motif, the function of C/EBP$\beta$ and C/EBP$\delta$ was examined. IL-1$\beta$ stimulated the expression of C/EBP$\beta$ and $-\delta$, and the direct binding of C/EBP$\beta$ to the C/EBP motif was confirmed. The $-2251$-bp CD-RAP promoter activity was down-regulated by co-transfection with C/EBP expression vectors. Mutation of the C/EBP motif abolished the inhibitory response to IL-1$\beta$. Additionally, C/EBP expression vectors were found to down-regulate the construct containing the promoter and enhancer of the type II collagen gene. Finally, the enhancer factor, Sox9, was shown to bind adjacent to the C/EBP site competing with C/EBP binding. Taken together, these results suggest that C/EBP$\beta$ and $-\delta$ may play an important role in the IL-1$\beta$-induced repression of cartilage-specific proteins and that expression of matrix proteins will be influenced by the availability of positive and negative trans-acting factors.

Cartilage-derived retinoic acid-sensitive protein (CD-RAP)$^1$ is a small secreted protein expressed in cartilage throughout chondrogenesis and in mature chondrocytes (1). Its physiological expression is primarily restricted to cartilage with transient expression in mammary buds and salivary glands (2). Pathologically, CD-RAP and its human homologue, melanoma inhibitory activity (MIA), are also expressed in various tumor tissues, such as chondrosarcoma, melanoma, and breast cancer (3–5). CD-RAP was originally cloned as a protein expressed in cartilage and down-regulated in chondrocytes that had been de-differentiated by treatment with retinoic acid (6). The protein structure of CD-RAP/MIA showed that it contains an Src homology 3 domain, which is unique for an extracellular protein. CD-RAP/MIA can interact with the type III repeat of fibronectin suggesting a model whereby CD-RAP/MIA binds to fibronectin and interferes with integrin binding (7). A recent study of CD-RAP-deficient mice showed that the mice have normal expression and distribution of type II collagen, aggrecan, and type X collagen but have increased density, increased diameter, and irregular arrangement of collagen fibrils (8).

Thus, CD-RAP may function to organize highly ordered ultrastructural fiber architecture. CD-RAP is regulated by various cytokines or growth factors; insulin and insulin-like growth factor I stimulate and basic fibroblast growth factor and interleukin 1$\beta$ (IL-1$\beta$) suppress CD-RAP expression in bovine articular chondrocytes (9).

IL-1$\beta$ is one of the major cytokines that mediate inflammatory reactions. IL-1$\beta$ promotes the arachidonic acid cascade resulting in production of prostaglandin (10). In joint diseases, such as rheumatoid arthritis and potentially osteoarthritis, IL-1$\beta$ is thought to contribute to degradation of matrix proteins not only by the production of proteases such as matrix metalloproteinases but also by down-regulation of expression of the matrix proteins such as types II, IX, and XI collagen and aggrecan, resulting in the loss of the cartilage structure and prevention of repair (10–13). Because CD-RAP is thought to be required for formation of the highly ordered ultrastructural fiber architecture (8), down-regulation of CD-RAP gene may contribute further to loss of cartilage integrity.

To date, little is known about the mechanism of IL-1$\beta$-induced repression of matrix proteins. IL-1$\beta$ induces or activates many transcription factors, such as nuclear factor-κB (NF-κB) (14), Fos/Jun, early growth response-1 (15, 16), and CCAAT/enhancer-binding protein (C/EBP) families in chondrocytes (17). However, direct functions of these factors with respect to repression of matrix protein gene transcription have not been clearly determined. CD-RAP is a family of basic leucine-zipper transcription factors with six known family members of C/EBPs as follows: C/EBP$\alpha$, $\beta$, $\delta$, $\epsilon$, $\gamma$, and $\zeta$. Among these, C/EBP$\beta$ and $-\delta$ are known to be activated by IL-1$\beta$ and TNF-$\alpha$ at the mRNA level (18–22) and protein level (for $\beta$ by phosphorylation and intracellular translocation) (23–28). In response to these proinflammatory cytokines, C/EBP$\beta$ and/or $-\delta$ activate various genes related to inflammation, such as phospholipase A$_2$ and cyclooxygenase-2 and manganese superoxide dismutase (17, 20, 21, 29). C/EBP$\beta$ also regulates matrix proteins, such as
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pro-α1 and -α2 type I collagen, matrix Glα protein, and osteocalcin (22, 30–32). C/EBPβ has two major isoforms, liver-enriched activator protein (LAP) and liver-enriched inhibitory protein (LIP). LAP is generally considered to be an activator, whereas LIP, which lacks most of trans-activation domain of LAP, can act as a dominant-negative inhibitor (18, 33). Thus, C/EBPβ can directly activate or inhibit the target gene.

Here, we investigated the factors responsible for down-regulation of the CD-RAP gene by IL-1β, and we show that C/EBPβ plays a key role in this regulation. Furthermore, we show that C/EBPβ can down-regulate type II collagen gene transcription, thus suggesting that C/EBPβ might play an important role in down-regulation of various matrix proteins induced by IL-1β. We provide the first demonstration that C/EBPβ down-regulates the expression of a cartilage-specific matrix-associated gene and that it is a novel pathway mediating repressive effects of IL-1β.

EXPERIMENTAL PROCEDURES

Materials—The materials used in this work were purchased as follows: α-modified Eagle's minimum medium (αMEM), Dulbecco's modified Eagle's medium (DMEM), DMEM/F-12 medium, non-essential amino acids, Penicillin-Streptomycin, and FBS from Gibco-BRL (Gaithersburg, MD); fetal bovine serum from HyClone Laboratories, Inc., Logan, UT; penicillin/streptomycin solution, dexamethasone, and Nu-Clear Extraction Kit™ from Sigma; Human IL-1 α, β, or β-2 cells were cultured in DMEM with 10% FBS and 2% charcoal-treated horse serum. Isolate chondrocytes were plated at a density of 10^4/cm² and cultured in DMEM/F-12 containing 10% FBS for 8 days, and then harvested with Reporter Lysis Buffer™, and the lysate was analyzed for luciferase activity using Promega Luciferase Assay Reagent™. The β-galactosidase activities were also measured to normalize variations in transfection efficiency. Each transfection experiment was repeated at least twice. For the antibody interference experiments, the probe and incubated for 15 min at room temperature before adding the secondary antibodies were recognized by anti-rabbit IgG antibodies diluted to 1:2000 in PBS containing 1% dry milk. The anti-Sox9 antibodies was generous gift from Dr. de Crombrugghe (43). DNA protein complexes were resolved on a 5% polyacrylamide gel at 100 V for several hours. The gels were dried and autoradiographed.

Electrophoretic Mobility Shift Assay—Fragment A (between –2138 and –2037 bp relative to the mouse CD-RAP translation start site) was amplified by PCR. All oligonucleotides were synthesized by Invitrogen, and complementary oligonucleotide was annealed to make double-stranded oligonucleotide. The fragment A or various double-stranded oligonucleotides were end-labeled using T4 polynucleotide kinase and [γ-32P]ATP. Bandshifts were performed by incubating 4 μg of nuclear extracts in the mobility shift buffer (10 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 50 mM NaCl, 0.5 mM dithiothreitol, 1 mM MgCl₂, 4% glycerol, 2 μg of poly(dI-dC) or poly(dG-dC)) with the DNA probe at room temperature for 25 min. In vitro synthesized proteins were assayed as described before (35, 42). For the competition studies, the cold DNA probe (100-fold) was added to the binding reaction with the DNA probe and incubated for 15 min at room temperature before adding the DNA probe. For the antibody interference experiments, the nuclear extracts and 1 μl of antibody were preincubated in the buffer for 1 h at 4 °C. The anti-Sox9 antibodies was generous gift from Dr. de Crombrugghe (43). DNA protein complexes were resolved on a 5% polyacrylamide gel at 100 V for several hours. The gels were dried and autoradiographed.

Western Blot—Thirty μg of cytoplasmic protein or nuclear extracts were denatured in SDS sample buffer contains 0.1 M dithiothreitol at 100 °C for 5 min and separated on a 4–20% Express Gel™ in Tris/glycine/SDS buffer. The gels were then transferred to Hybond-C extra™ nitrocellulose membrane in Tris/glycine buffer, pH 8.3, containing 20% methanol. The membranes were saturated in 7% non-fat dry milk in PBS at room temperature for 1 h and hybridized with anti-C/EBP antibodies diluted to 1:2000 in PBS containing 1% dry milk. The hybridized antibodies were recognized by anti-rabbit IgG antibodies coupled to horseradish peroxidase, and the secondary antibodies were detected by autoradiography using SuperSignal™ peroxidase substrate.

Quantitative Real Time PCR—Total RNA was isolated from the primary human articular chondrocytes, and 2 μg of RNA was reverse-transcribed in the presence of avian myeloblastosis virus-reverse transcriptase (1.2 μl/μl), oligo(dT)15 (40 ng/μl), dNTP (1 mM each), and RNasin Ribonuclease Inhibitor (1.6 units/μl) in 10 μl reaction mixture containing 25 μl of SYBR™ Green PCR master mix, 1 μl of RT products, and 300 nM of primers using GeneAmp® 5700 Sequence Detection System (PerkinElmer Life Sciences). The primer sequences follows: 5′-ATAGGACCCGAAAGAAGCTACAG-3′ (antisense); 5′-GGGTGTCGGTGTTGAAGTC-3′ (sense); human C/EBPβ, 5′-ATCGGGACCTCGGGAAGCAGAAGAGG-3′ (sense) and 5′-TGGTCCGTCGTCGTCGGTTCG-3′ (antisense); human C/EBPα, 5′-TGGTCCGTCGTCGTCGGTTCG-3′ (sense) and 5′-TGGTCCGTCGTCGTCGGTTCG-3′ (antisense); human C/EBPβ, 5′-CGGTGAGCGGAAAGAAGCTACAG-3′ (sense) and 5′-TCCTCGCGAGCGGAAAGAAGCTACAG-3′ (antisense).
**RESULTS**

**IL-1β-responsive Element Is Located between −2138 and −2068 bp of the CD-RAP promoter**—We have shown previously that the −2251-bp CD-RAP promoter contains elements sufficient for cartilage-specific expression in transgenic mice (2) and that the expression of CD-RAP is down-regulated by IL-1β in primary chondrocyte cultures (9). To identify the IL-1β-responsive element within CD-RAP promoter, three 5′-deletion constructs were transiently transfected into RCS cells and incubated in the absence or presence of IL-1β (Fig. 1). Regarding basal activities of the constructs in untreated RCS cells, −2251-bp promoter was stronger than −2138 bp, and −2138 bp was stronger than −2068 bp, suggesting there are activating elements between −2251 to −2138 bp and between −2138 to −2068 bp. The expressions of the −2251- and −2138-bp constructs were down-regulated by the IL-1β treatment, whereas the response was lost in the −2068 bp, thus suggesting that the IL-1β-responsive element is located between −2138 and −2068 bp.

**IL-1β Stimulates the Expression of C/EBPβ and C/EBPδ in RCS Cells**—The expressions of C/EBPβ and -δ are known to be stimulated by IL-1β (18, 20). As computer analysis revealed there was a potential binding site for C/EBPβs between −2138 and −2068 bp, we examined whether IL-1β stimulates the expression of C/EBPβ or C/EBPδ in RCS cells (Fig. 2, A and B). The expression levels of C/EBPβ and C/EBPδ were very low in steady state but were stimulated by IL-1β in 4–24 h increasing up to 48 h. The expression of CD-RAP was repressed by 48 h. Western blots for C/EBPβ revealed that in the absence of IL-1β treatment, a very small amount of C/EBPβ protein exists in the cytoplasm but not in nuclei (Fig. 2C). The treatment of IL-1β stimulated the expression of both of the isoforms of C/EBPδ, LAP (36 kDa), and LIP (20 kDa) that were localized in the nuclei. Protein expression of C/EBPδ was also stimulated by the IL-1β treatment (Fig. 2C).

**C/EBPβ Binds to the IL-1β-responsive Element of the CD-RAP Promoter**—To determine whether C/EBPβ functions within the IL-1β-responsive element, EMSA was carried out using nuclear extract from the RCJ3.1C5.18 cells. These cells were chosen because C/EBPβ is expressed in steady state (Fig. 3A). Fragment A containing the sequence between −2138 and −2037 bp was used as a probe, and various cold oligonucleotides were used as competitors (Fig. 3B). Competitor 3, which has a potential binding site for C/EBPβs at −2085/−2077 bp, competed with the binding of nuclear proteins and the probe (Fig. 3C). As expected, the mutant oligonucleotide 3, in which C/EBP-binding site was mutated, did not compete with the binding, and supershift analysis confirmed that C/EBPβ bound to the sequence of native oligonucleotide 3 (Fig. 3D). The antibody for Ikars, a transcription factor that is also a potent binding protein for the sequence of oligonucleotide 3, did not affect the band shift. EMSA for the nuclear extract of RCS cells treated with 10 ng/ml of IL-1β bound to the sequence of oligonucleotide 3 as a probe to confirm that the IL-1β-induced C/EBPβ bound to the element (data not shown).

**C/EBPβ Functions as a Repressor for the CD-RAP Promoter Activities**—The C/EBP-binding site was mutated in the −2251-bp construct using mutant oligonucleotide 3. The promoter activity of the mutant −2251-bp construct was about 2-fold stronger than that of wild type −2251-bp construct after transient transfection into RCJ3.1C5.18 cells, suggesting that C/EBPβ is acting as a repressor (Fig. 4). To investigate the C/EBP function in detail, the expression vectors for C/EBPβ and C/EBPδ were co-transfected with wild type −2251-bp construct into RCS cells, in which endogenous C/EBP expression is very low (Fig. 5A). Because C/EBPβ is known to act in different ways depending on the isoforms, we used the following three different expression vectors: pCMV-C/EBP-full-length, pCMV-LAP, and pCMV-LIP. The promoter activity of the −2251-bp construct was down-regulated in a dose-dependent manner by
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Fig. 3. C/EBPβ binds the sequence between −2094 and −2069 bp of the CD-RAP promoter. A, Western blot of nuclear extracts (N.E.) from RCJ3.1C5.18 cells for C/EBPβ, C/EBPβ-LAP (38 and 36 kDa) and LIP (20 kDa) are expressed in steady state. B, A diagram showing DNA fragments used for EMSA. Fragment A from −2138 to −2037 bp and relative locations of oligonucleotides 1–4 are shown. The oligonucleotide 3 from −2094 to −2069 bp contains C/EBP motif (underlined). mu, mutant oligonucleotide 3 containing two base pair mutations (AA → CC) is underlined within the C/EBP motif. C, EMSA for RCJ3.1C5.18 nuclear extracts using fragment A as a probe and various cold competitors at 100-fold molar excess. The oligonucleotide 3 competes with the binding of nuclear proteins to the probe. D, EMSA and supershift analysis for RCJ3.1C5.18 nuclear extracts using oligonucleotide 3 as a probe. Mutant oligonucleotide 3 does not compete with the binding of nuclear proteins to the probe. Antibodies against C/EBPβ or Ikaros were preincubated with the nuclear extracts. Arrow shows supershift band of the complex of C/EBPβ, antibody (Ab), and probe.

Fig. 4. Mutation of the C/EBP-binding site up-regulates the promoter activity of the −2251-bp construct. Site-directed mutagenesis was performed within the C/EBP-binding site of the −2251-bp construct using the mutant oligonucleotide 3 sequence. The mutant and wild type −2251-bp constructs were transiently transfected into RCJ3.1C5.18 cells. The activity of promoterless pGL3b is set as 1. Each bar represents the mean ± S.D.

Fig. 5. The promoter activity of the −2251-bp construct is down-regulated by co-transfection with C/EBP expression vectors. A, the −2251-bp construct was co-transfected into RCS cells with various C/EBP expression vectors. Expression vectors for C/EBPβ-full-length (FL), C/EBPβ-LAP, C/EBP-LIP, or C/EBPβ were added to the transfection mixture as indicated. Total amounts of expression vectors were adjusted to 500 ng using the empty expression vector in each transfection. The activity of −2251-bp constructs co-transfected with 500 ng of the empty expression vector is set at 100. Each bar represents the mean ± S.D. B, Western blot of the lysate from the co-transfection assay for C/EBPβ confirming that the expression vectors form the protein products.

Mutation of C/EBP-binding Site Eliminates the Response to IL-1β Treatment—To determine whether the C/EBP-binding site on the CD-RAP promoter was required for the response to IL-1β, the mutant −2251-bp construct, in which the C/EBP-binding site was mutated, was transfected into RCS cells and incubated in the absence or presence of IL-1β for a further 48 h. As shown Fig. 6A, the mutant −2251-bp construct did not respond to IL-1β treatment. Furthermore, co-transfection of the pCMV-C/EBPβ-full-length vector did not result in repression of activity of the mutant −2251-bp construct. These results indicate that the C/EBP-binding site at −2085/−2077 bp of the CD-RAP promoter is required for the functional response to IL-1β and suggest that C/EBPs are key factors in this response.

Sox9 Binds to the Element Overlapping with the C/EBP Motif—Because the IL-1β-responsive element seems to contain an activating element as well (see Fig. 1), we hypothesized that C/EBP may interact with another activator protein conferring an indirect repressive effect. Because both of the flanking sequences of the C/EBP motif contain an HMG-like motif, the binding of Sox9 to this element was tested (Fig. 7). EMSA using
IL-1 mutated, was transfected into RCS cells and incubated with or without IL-1β (10 ng/ml). B, the mutant −2251-bp construct was co-transfected into RCS cells with empty or C/EBP-FL expression vector. The promoter activity of the construct without IL-1β and that co-transfected with empty vector were set at 100. Each bar represents the mean ± S.D.

RCS nuclear extract revealed the Sox9 binding to the sequence of oligonucleotide 3 (Fig. 7B). To see the interaction between Sox9 and C/EBPβ within this element, EMSA was carried out using Sox9 and C/EBP-LIP protein synthesized by in vitro translation. The binding of Sox9 protein to the sequence of oligonucleotide 3 was confirmed by supershift using anti-Sox9 antibody (Fig. 7C). To screen the binding site, each of two different competitors was preincubated with the binding reaction mix. The competitor b, which has the sequence TTCTAAAA that is close to the Sox protein motif (A/T)(A/T)CAA(A/T), competed with the Sox9 binding to the probe more strongly than the competitor a, suggesting Sox9 prefers the motif in b for its binding (Fig. 7C). When C/EBPβ and Sox9 proteins were incubated together with the probe, two major independent bands were observed to be the same size as Sox9 alone and C/EBP alone (Fig. 7D). The addition of antibodies for either Sox9 or C/EBPβ generated supershift of the related band and did not affect the other band (Fig. 7D). These results indicate that the supershifted complex did not contain the other protein, suggesting that Sox9 and C/EBPβ cannot bind to the probe in the same time probably because the binding sites partially overlap. Therefore, C/EBPβ may compete with Sox9 binding to this element thus eliminating the enhancer effect of Sox9.

Type II Collagen Promoter-Enhancer Construct Is Down-regulated by C/EBPs—Because the expression of type II collagen is also down-regulated by IL-1β and the data base analysis revealed that the first intron of type II collagen gene has multiple C/EBP motifs, we examined whether C/EBPs also regulate the promoter activity of the type II collagen gene. Co-transfection of the type II collagen promoter-enhancer construct, pGL2-COL2-577/+3426, into RCS cells with the expression vectors for C/EBPβ-LIP, C/EBPβ-LIP, or C/EBPβ also down-regulated the reporter gene activity (Fig. 8). Similar to the response of the CD-RAP promoter (see Fig. 5A), overexpression of C/EBPβ-LAP produced more potent inhibition than C/EBP-LIP or C/EBPβ. These results indicate that C/EBPβ could mediate, at least partially, the inhibitory effect of IL-1β on the type II collagen gene as well.

C/EBPβ and -δ Are Stimulated in Normal Chondrocytes from Human Articular Cartilage in Response to IL-1β Treatment—Our studies have been focused on two chondrocytic cell lines producing low (RCS3.1C5.18) and high (RCS) amounts of CD-RAP. In order to examine this response in normal chondrocytes, the levels of C/EBPβ and -δ, CD-RAP, and type II collagen mRNAs were investigated in IL-1β-treated chondrocytes isolated from human articular cartilage (Fig. 9). Cells were treated with 2 ng/ml IL-1β for up to 48 h. By using the real time quantitative RT-PCR method, the expression of C/EBPβ and -δ were shown to increase at 24 h up to 3- and 2-fold, respectively, and returned to basal levels by 48 h. Type II collagen mRNA significantly decreased to 8% within 24 h, whereas CD-RAP mRNA decreased relatively slowly to 56% compared with the controls within 48 h. Consistent with the results from cell lines, these results suggest that C/EBPβ and -δ can regulate the expression of CD-RAP and type II collagen in articular cartilage in the same manner.

DISCUSSION

The mechanism by which the proinflammatory cytokines IL-β and TNF-α stimulate inflammation and degradative enzymes as well as repress synthesis of structural matrix is
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The promoter activity of the type II collagen is also down-regulated by C/EBPβ and α. The 4.0-kb construct containing the promoter and intron enhancer of the human type II collagen gene was co-transfected into RCS cells with 500 ng of the empty vector C/EBPβ-LAP, C/EBPβ-LIP, or C/EBPβ expression vector. The activity of promoterless pGL3b is set at 1. Each bar represents the mean ± S.D.

FIG. 8. The promoter activity of the type II collagen is also down-regulated by C/EBPβ and α. The 4.0-kb construct containing the promoter and intron enhancer of the human type II collagen gene was co-transfected into RCS cells with 500 ng of the empty vector C/EBPβ-LAP, C/EBPβ-LIP, or C/EBPβ expression vector. The activity of promoterless pGL3b is set at 1. Each bar represents the mean ± S.D.

FIG. 9. IL-1β stimulates the expression of C/EBPα and α in normal chondrocytes from human articular cartilage. The relative expression levels of C/EBPα, δ, CD-RAP, and type II collagen were examined in normal chondrocytes from human articular cartilage treated with IL-1β (2 ng/ml) using quantitative real time RT-PCR method. Each point represents the mean ± S.D. of fold change compared with zero time.

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The mechanism of C/EBPβ activation in response to IL-1β is complex and still controversial. It is reported that C/EBPβ activates the expression of CD-RAP mRNA using the RNA polymerase II inhibitor, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (60 μM) in RCS cell and in bovine articular chondrocyte (9). CD-RAP mRNA was still present at 90% after 6 h of treatment and at 60 (RCS cell) to 40% (bovine chondrocyte) after 24 h of treatment.

C/EBPβ is an unusual transcription factor in that it can activate or repress gene transcription. For example, C/EBPβ activates the gene encoding the α1 chain of type I collagen (30), whereas it represses the gene encoding the α2 chain of type I collagen (22) and albumin genes (47). We found an HMG-like motif partially overlapping with the C/EBP motif that can bind to Sox9. As binding of one protein appears to preclude binding of the other, the balance between C/EBP and Sox proteins may regulate the transcription of CD-RAP. The interaction of C/EBPs with other transcription factors has been observed in other genes, although the mechanisms of binding and the results of binding are specific to the transcription factor and gene. For example, in the CD11c and rat cytochrome CYP2D5 genes, C/EBP acts synergistically with the transcription factor Sp1 to enhance gene expression through a mechanism whereby Sp1 facilitates the binding of C/EBP to a low affinity site in the DNA (48, 49). C/EBP also interacts with retinoblastoma protein and various other transcriptional activators or co-activators such as c-Myb, PU.1, and ATF-2 (41, 50). In the osteocalcin gene, C/EBPβ binds to DNA and, in turn, binds to the transcription factor Runx2/Cbfa1 to greatly enhance gene expression (32).

We have found that C/EBP also repressed the transcription of the type II collagen gene. There are multiple C/EBP motifs in the first intron of type II collagen gene. Interestingly, analysis revealed that there is a C/EBP motif within the 48-bp core enhancer element defined by Lefebvre et al. (42, 43) that regulates cartilage-specific expression of type II collagen gene; the C/EBP motif is next to the Sox9-binding motif in the element. A zinc finger transcription factor, αA-crystallin-binding protein 1 (CRYBP1), that is expressed in a reciprocal pattern compared with that of type II collagen is reported to bind to the sequence overlapping the C/EBP motif and compete with Sox9 binding thereby repressing gene expression of type II collagen (51).

C/EBP may participate in IL-1β-induced repression of type II collagen by taking place of the CRYBP1 binding and competing with Sox9 binding in a similar manner. It has been reported that Sox9 is down-regulated by IL-1β and TNF-α (52). In that report, the expression of Sox9 was decreased within a few hours via direct interaction of IL-1β-induced NF-κB with the Sox-9 promoter, whereas the decrease of type II collagen takes much longer. Because no further Sox9 is then expressed but the existing Sox9 remains bound to the enhancer of type II collagen gene, C/EBP might be involved in this regulation by displacing Sox9 from the element. Further studies will be performed to
transcriptional repression of chondrocyte extracellular matrix production in addition to the promotion of inflammatory cascade and catabolic processes in joint diseases, such as rheumatoid arthritis and osteoarthritis.

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