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Association of hepatic nuclear factor-4 in the apolipoprotein B promoter: a preliminary report

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Abstract

Previous studies have examined the arrangement of regulatory elements along the apolipoprotein B (apoB) promoter region (-3067 to +940) and a promoter fragment extending from nucleotides -150 to +124 has been demonstrated to be essential for transcriptional activation of the apoB gene in hepatic and intestinal cells. It has also been shown that transcriptional activation of apoB requires a synergistic interaction between hepatic nuclear factor-4 (HNF-4) and CCAAT/enhancer-binding protein α (C/EBPα) transcription factors. Here, we have examined the hypothesis that HNF-4 factor binding to DNA may induce a DNA helix bend, thus facilitating the communication with a C/EBPα factor located one helix turn from this HNF-4 factor in the apoB promoter. A gel electrophoretic mobility shift assay using wild type double-stranded oligonucleotides or modified wild type duplex oligonucleotides with 10 nucleotides inserted between HNF-4 and C/EBPα factor motifs showed similar retarded complexes, indicating that HNF-4 and C/EBPα factors interact independently of the distance between binding sites. However, when only one base, a thymidine, was inserted at the -71 position of the apoB promoter, the complex shift was completely abolished. In conclusion, these results regarding the study of the mechanisms involving the interaction between HNF-4 and C/EBPα factors in the apoB promoter suggest that the perfect 5'-CCCTTTGGA-3' motif is needed in order to facilitate the interaction between the two factors.

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• C/EBPα

Apolipoprotein B (apoB) is a constituent of several lipoproteins and acts as a ligand for the cellular recognition and catabolism of low density lipoprotein (LDL) by the LDL receptor (1,2). ApoB mRNA has been detected in the liver, intestine and placenta, indicating that apoB gene transcription is regulated in a tissue-specific manner (3,4). The regulatory elements of the human apoB gene are located in the proximal promoter region (5,6). Kardassis et al. (5) have demonstrated that four elements (named A, B, C and E) are recognized by nuclear factors. Three of these elements (A, B, and C) are spaced within the apoB promoter -36 to -118, and element E is located in the +35 to +53 region within the first exon of the apoB gene (5). Analysis of the interaction of nuclear

proteins with element A (5'-GCGCCCTTTG GACCTTTTGCAATCC-3') localized in the -79 to -63 apoB promoter showed the existence of multiple sequence-specific DNA-complexes. Two liver-enriched transcription factors, hepatic nuclear factor-4 (HNF-4), a transcription factor that belongs to the steroid-thyroid hormone receptor superfamily (7), and CCAAT/enhancer-binding protein α (C/EBPα), a transcription factor that belongs to the bZip family of proteins (8), bind to the -81 to -52 region of the apoB promoter (6); both factors are critical for gene expression in hepatocytes. Furthermore, both transcription factors have an overlapping binding site in the apoB promoter and act synergistically in order to activate transcription (6). In this study, we have examined the role of the HNF-4 factor in the interaction between C/EBPα and HNF-4 proteins within the -81 to -52 region of the apoB promoter. The transcription factors HNF-4 and C/EBPα used in gel mobility shift assays were purified from rat liver nuclei using DNA-specific affinity chromatography, as described by Kadonaga and Tijan (9). HNF-4 protein was purified from crude rat liver nuclear extract by chromatography, using three different supports (Q-Sepharose, Bio-Rex 70 and S-Sepharose). The columns were washed with KCl gradients and then fractions containing HNF-4 protein activity were pooled and the solution was brought to 100 mM KCl and applied to affinity columns (10). The specific affinity column was prepared with a double-stranded oligonucleotide (5'-AGG CGCCCTTTGGACCTT-3') corresponding to the -81 to -64 region from the apoB promoter coupled to a cyanogen bromide-activated Sepharose 4B column. Twenty-five mg of protein from S-Sepharose fractions was loaded onto a 10-ml specific affinity column equilibrated with NDB buffer (25 mM HEPES, pH 7.6, 5 mM $MgCl_2$, 0.1 mM EDTA, and 10% glycerol) containing 0.1 mM KCl. Active fractions were concentrated by Mono-Q column chromatography. Eluted HNF-4 protein was dialyzed in NDB solution containing 40 mM KCl, and stored at -70°C. C/EBPα protein was purified using heparin-agarose and DNA-cellulose columns, followed by heating for 6 min at 85°C and cooling immediately on ice. Fractions were centrifuged at 9,500 rpm for 15 min and a soluble material which contained the proteins was obtained. Twenty mg of protein was then passed through an affinity column. The affinity column for purification of C/

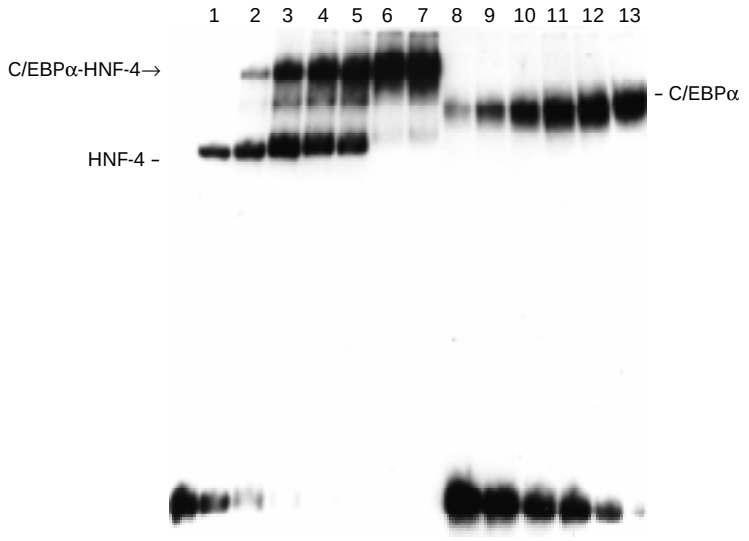

Figure 1 - Synergistic effect of C/EBPα and HNF-4 in the apoB promoter. Transcription factors HNF-4 and C/EBPα were purified by affinity chromatography. The transcription factors were incubated with ^{32}P-labeled double-stranded wild type oligonucleotides (5'-AGGCGCCCTTTGGACCTTTTGCAATCC TGG-3'). This sequence corresponds to the -81 to -52 region of the apoB promoter. Lane 1: 0.5 µg of HNF-4 protein. Lanes 2-7: increasing concentrations of C/EBPα factor (0.8, 3, 5 and 6.4 µg, respectively), and a constant concentration of HNF-4 protein (0.5 µg). Lanes 8-13: increasing amounts of C/EBPα factor (0.4, 0.8, 2, 4, 5 and 6.4 µg, respectively). The arrow shows the low mobility band derived from HNF-4 and C/EBPα specific complexes. High mobility bands correspond to unbound probes.

EBPα protein was prepared with double-stranded oligonucleotide (5'-TGCAATCCTG G-3') corresponding to the -62 to -51 region from the apoB promoter coupled to a cyanogen bromide-activated Sepharose 4B column. The active protein C/EBPα was eluted from the affinity column with 0.8 M NaCl and stored at -70°C.

Gel mobility shift assays were prepared with 0.2-6.4 µg of purified nuclear protein. Samples were incubated for 30 min with wild type (5'-AGGCGCCCTTTGGACCTTT TGCAATCCTGG-3') or modified oligonucleotides (insertion of 1 or 10 nucleotides) were ^{32}P labeled (sp. act. 5 x 10^8 cpm/µg) as described in Figure 2. The DNA binding reaction was carried out in a final volume of 20 µl solution containing 60 mM KCl, 20 mM HEPES, pH 7.9, 3% Ficoll, 0.5 mM dithiotreitol, and 1 mM $MgCl_2$ and incubated at room temperature for 30 min. Two µg poly-(dI-dC) (Pharmacia, Uppsala, Sweden) was added as a nonspecific competitor. Then, the samples were submitted to 4% PAGE in 1 x TAE (10 x TAE: 67 mM Tris, 33 mM sodium acetate, 10 mm EDTA, pH 7.9), as described by Novak and Bydlowski (11). After drying, gels were exposed to Fuji RX X-ray film for at least 4 h at -70°C. Bands were visualized by autoradiography.

When both transcription factors were used together, as shown in Figure 1 (increasing concentrations of C/EBPα with a constant amount of HNF-4), a ternary complex was formed (slower migrating shift complex). However, when C/EBPα or HNF-4 proteins were used alone, no ternary complex was observed (Figure 1, lanes 1 and 8-13, and Figure 2, lanes 1-4 and 9).

Our results confirm previous data about the synergistic interaction between HNF-4 and C/EBPα in the apoB promoter (6). Surprisingly, when one helical turn was created by the insertion of 10-base pairs (5'-AGGCG CCCTTTGGAACCTTTCGGTCCTTTT GCAATCCTGG-3') between the HNF-4 and C/EBPα motifs in the -81 to -52 region of the apoB promoter, a ternary complex was also formed despite the distance between these factors (Figure 2, lines 5-8). These data suggest that the HNF-4 factor may be acting by direct communication with distant C/EBPα. Lymphoid enhancer-binding factor 1 (LEF-1) has been reported to have a similar effect regarding bent DNA (12). The LEF-1 factor recognizes the 5'-CCTTTGAA-3' sequence and induces a sharp bend in the DNA helix, facilitating an interaction between proteins

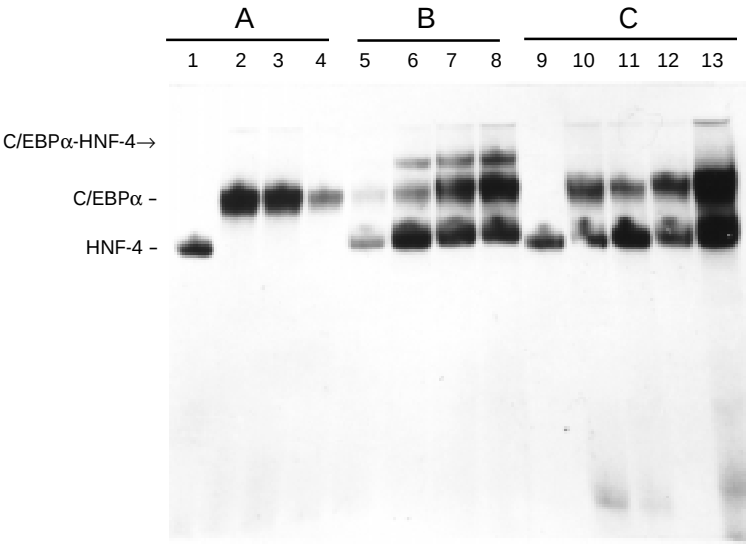

Figure 2 - Effect of the insertion of nucleotides into the -81 to -52 region of the apoB promoter. Gel electrophoresis mobility shift assays were performed with HNF-4 and C/EBPα purified by affinity chromatography and each of the following ^{32}P-labeled double-stranded oligonucleotides: *A*, Wild type probe (5'-AGGC GCCCTTTGGACCTTTTGCAATCCTGG-3') (lanes 1-4). Lane 1: 0.5 µg of HNF-4 and lanes 2-4: decreasing concentrations of C/EBPα (4, 2 and 0.8 µg, respectively). *B*, Probe modified by the insertion of ten nucleotides between the HNF-4 and C/EBPα motifs in the wild type sequence (5'-AGGCGCCCTTTGGAACCTTTCGGTCC TTTTGCAATCC-3') (lanes 5-8). Lane 5: 0.4 µg of C/EBPα and 0.2 µg of HNF-4. Lane 6: 0.8 µg of C/EBPα and 0.5 µg of HNF-4. Lane 7: 2 µg of C/EBPα and 0.5 µg of HNF-4. Lane 8: 4 µg of C/EBPα and 0.5 µg of HNF-4. *C*, Probe modified by the insertion of a thymidine nucleotide at the -71 position of the apoB promoter (5'-AGGCGCCCTTTTGGACCTTTTGCAATCCTGG-3') (lanes 9-13). Lane 9: 0.5 µg of HNF-4. Lane 10: 0.5 µg of HNF-4 and 1.5 µg of C/EBPα. Lane 11: 1 µg of HNF-4 and 0.8 µg of C/EBPα. Lane 12: 0.5 µg of HNF-4 and 2 µg of C/EBPα. Lane 13: 5 µg of C/EBPα and 2 µg of HNF-4. The arrow indicates the ternary complexes formed by interaction between HNF-4 and C/EBPα factors.

bound at the distant 5' and 3' enhancer (12). In the LEF-1 DNA-complex, the A-T-rich sequences favor minor groove compression, whereas G-C-rich sequences favor major groove compression. Moreover, Erie et al. (13), using scanning force microscopy to visualize the DNA molecule conformation, have described bent DNA within specific complexes (12). The HNF-4 factor recognizes the 5'-CCTTTGGA-3' sequence in the -81 to -61 region of the apoB promoter. On the other hand, when a thymidine nucleotide was added at the -71 position, a 5'-CCTTTT GGA-3' sequence was created and the ternary complex was abolished (Figure 2, lanes 10-13). This insertion probably destroyed the perfect 5'-CCTTTGGA-3' motif. These results support the hypothesis that HNF-4 has an action similar to that of LEF-1 factors, probably inducing DNA bending by a minor groove through 5'-TTTGGA-3' and favoring binding of the C/EBPα to the -81 to -52 apoB promoter region. Therefore, the absence of the perfect motif 5'-CCTTTGGA-3' obstructed the DNA bend by the minor groove and consequently the communication between the HNF-4 and C/EBPα factors.

In summary, these data suggest that HNF-4 protein may be related to the structural changes which confer on the C/EBPα and HNF-4 factors the ability to form ternary complexes, as shown for other proteins, including LEF-1 (13-15). These interactions have an important role in the control of transcriptional regulation of the human apoB gene, as observed in cotransfection experiments with HeLa cells, where plasmids expressing C/EBPα and HNF-4 factors have increased the transcription of the reporter gene by 50- to 60-fold (6).

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