Session V: Regulation and Expression of Recombinant Matrix Proteins

DNA Binding Specificity of CBF.

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A transcriptional regulatory element containing the CCAAT pentanucleotide is present in the promoters of many eucaryotic genes including the two type I collagen genes. The CCAAT binding factor CBF (also named CP1 and NF-Y) consists of three subunits, CBF-A, CBF-B, and CBF-C, all of which are needed in DNA binding and are present in the DNA-CBF complex. Previous experiments showing that CBF was unable to bind to some segments containing a CCAAT motif, had suggested that the CCAAT pentanucleotide is critical but not sufficient for CBF binding. Here we utilized a PCR-mediated random site selection method to investigate the DNA-binding specificity of CBF in an unbiased manner. A consensus sequence 5' T/C A/G A/G C C A A T C A 3' was derived, indicating that the sequences flanking the CCAAT motif display specificity for DNA binding of CBF. The CCAAT containing sequences in the promoters can be divided into high, moderate and low affinities to CBF according to their sequences around the CCAAT motif. The consensus site of CBF generated from our results of the selection data is similar to the CCAAT motif defined by comparative sequence analysis of 175 appropriately positioned CCAAT elements in unrelated RNA polymerase II promoter regions; this suggests that CBF is the protein that binds most CCAAT motifs in promoters. A mutant that can not bind CBF was constructed by changing the nucleotides around the CCAAT motif to a nonconsensus sequence. In vitro transcription assay showed that a promoter containing this mutant CBF binding site can not be activated by recombinant CBF subunits in contrast to a promoter with a wildtype CBF binding site. Our studies suggest that the sequences around the CCAAT motif are important for both CBF binding and transcription activity.

An 18-bp Sequence in the Mouse Proα1(II) Collagen Gene is Sufficient for Cartilage Expression and Binds Nuclear Proteins that are Selectively Expressed in Chondrocytes

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The molecular mechanisms which control the differentiation of mesenchymal cells into chondrocytes are still poorly understood. We have used the gene for a chondrocyte marker, the proα1(II) collagen gene (Col2a1) to delineate a minimal sequence needed for cartilage expression and identify chondrocyte-specific proteins binding to this element. We previously localized a 156-bp cartilage-specific enhancer in the mouse Col2a1 intron 1. We show here that four copies of a 48-bp subsegment strongly enhanced promoter activity in transiently transfected rat chondrosarcoma (RCS) cells and mouse primary chondrocytes but not in 10T1/2 fibroblasts. They also directed cartilage-specific expression in transgenic mice. These 48 bp include two 11-bp inverted repeats with only one mismatch. Multiple copies of an 18-bp element containing the 3' repeat strongly increased promoter activity in RCS cells and chondrocytes but not in fibroblasts. Transgenic mice harboring a 12-copy 18-mer expressed luciferase in ribs, vertebrae, and isolated chondrocytes but not in non-cartilaginous tissues except skin and brain. In gel retardation assays, an RCS cell-specific protein and another related protein expressed only in RCS cells and chondrocytes bound to a 10-bp sequence within the 18-mer. Mutations in the 10-bp sequence abolished activity of the multimerized 18-bp enhancer, and deletion of these 10 bp abolished enhancer activity of larger intron-1 segments. These 10 bp contain a low-affinity binding site for POU domain proteins and competition experiments with a high-affinity POU domain binding site strongly suggested that the chondrocyte-specific proteins belong to this family. Together, our results indicate that an 18-bp sequence in the Col2a1 intron 1 controls cartilage expression and suggest that RCS cells and chondrocytes contain specific POU domain proteins involved in enhancer activity.