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DISSERTATION SUMMARY

The chondrogenic master transcription factor Sox9 binds to the regulatory region of matrilin-1 gene

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Sox9 is a high-mobility-group (HMG) DNA-binding domain transcription factor which plays an essential role in chondrogenesis and in transcriptional regulation of the type II collagen gene. In humans, *SOX9* haploinsufficiency results in campomelic dysplasia, a lethal skeletal malformation syndrome with XY sex reversal. Furthermore, in chimera studies *Sox9*$^{-/-}$ cells were unable to express chondrocyte-specific extracellular matrix genes. Matrilin-1 (also known as CMP-cartilage matrix protein), one of the major non-collagenous proteins in most cartilages, binds to aggrecan and type II collagen. It is a homotrimer of 54-kDa subunits assembled via a coiled-coil a-helix and stabilized by disulfide bridges. Matrilin-1 is expressed almost exclusively in chondrocytes, and may function as a bridging molecule between two major macromolecular networks of cartilage.

The understanding of the transcriptional regulation of the gene is still limited. To get further insight into the transcriptional regulation of the gene and to analyze protein-DNA interactions *in vivo*, we performed *in vivo* footprinting on the chicken matrilin-1 gene. Ligation-Mediated PCR technique (LMPCR) was introduced that allows the amplification of DNA fragments between positions –227 and +140 and detection of control elements. Chicken embryo chondrocytes (CEC) in comparison with chicken embryo fibroblasts (CEF), the non-expressing cell type, were subjected to *in vivo* analysis in which the total genomic DNA underwent a specific cleavage procedure carried out inside the cell. Sets of experiments revealed tissue-specific binding of transcription factors to the promoter and intronic control regions. For example, a cartilage-specific protection was observed at an inverted repeat carrying two putative Sox9-binding sites in the promoter upstream region of the matrilin-1 gene. To provide evidence that Sox9 is indeed involved in the transcriptional regulation of the matrilin-1 gene, we designed synthetic oligonucleotides (wild type and several mutant ones) for gel retardation assays. *In vitro* analysis using electrophoretic mobility shift and supershift assays confirmed the binding of Sox9 transcription factor to the identified control element. Furthermore, using *in vivo* footprinting, we could prove tissue-specific binding of NFI family transcription factors to the silencer elements SI and SII identified previously.

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