**Molecular Cloning, Sequencing, Tissue and Developmental Expression of Mouse Cartilage Oligomeric Matrix Protein (COMP)**

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Introduction: Cartilage oligomeric matrix protein (COMP) is a member of the recently characterized thrombospondin protein family and is an abundant noncollagenous extracellular matrix protein in cartilage. The importance of COMP for skeletal development and growth has been recently illustrated by identification of COMP gene mutations in two types of inherited chondrodysplasias and osteoarthritic phenotypes: pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED).

Although COMP was once believed to be a cartilage-specific molecule, studies showing that it is localized in both bovine and equine tendon as well as synovium indicate that it is more widely distributed. To understand more about the function and the integrated role of COMP molecule, we cloned and sequenced the full length of mouse COMP (mCOMP) cDNA, investigated the tissue distribution of COMP in adult mouse tissues and temporal and spatial expression of COMP at various stages of embryonic development in mouse.

Methods: *DNA cloning:* mCOMP gene fragment was first amplified by RT-PCR with total RNA from a 19-day-old mouse embryo using degenerative primers based on sequence alignments among human, rat and bovine COMP. Cloning the full length of murine sequence was completed by both 5' and 3' primers based on sequence alignments among human, rat and bovine COMP. The sequence of overlapping mCOMP clones was assembled into a 2438-nucleotide composite sequence with an open reading frame spanning from 15 to 2282 bp. The deduced amino acid sequence contains 755 residues with 98%, 94% and 91% overall sequence identity to rat, bovine and human COMP respectively.

To investigate the tissue distribution of COMP mRNA, the COMP messenger was RT-PCR-amplified with two sets of primers covering two different regions of COMP sequence. Our results showed that mCOMP is not only expressed in cartilage and tendon, but also in bone, eye, trachea, skeletal muscle, heart, and placenta. No expression was found in testes, ovary, stomach, thymus, spleen, intestine, pancreas, uterus, heart, liver, adrenal, kidney, thyroid and skin (data not shown), indicating that the expression of COMP is tissue-specific.

Immunostaining results using the pAb to bovine COMP on days 10, 13, 16 and 19 mouse embryos demonstrated that COMP was specifically expressed in the developing cartilage. The day 10 embryo was positive for COMP staining in predifferentiated mesenchymal cells alongside the neural tube; these cells likely represents sclerotome cells. The day 13 embryo demonstrated positive staining for COMP in the hypertrophic chondrocytes of prearticular condensations, such as the prearticular primordium of shaft of rib, vertebral bodies, limb buds, somites, and developing skeletal muscles. The positive COMP staining in these areas continued as embryo developed to 16 days of gestation (data not shown). At 19 days of gestation, endochondral ossification within cartilage primordium became prominent; COMP localized to the pericellular region and territorial extracellular matrix of hypertrophic chondrocytes and, to a less degree, the proliferating chondrocytes in the growth zone of vertebral bodies. COMP staining was absent in the resting zone adjacent to the vertebral disc. Immunostaining performed on the day 19 mouse embryo limbs, localized COMP to all cartilaginous regions, perichondrium and cortical bone peristeme (Fig 1). No staining was found in the resting zone of the growth plate. Diminished positive staining for COMP was noted in the calcified matrix in the diaphysis of the long bones, where ossification had occurred. The superficial layer of cartilage at the articular surface stained positive for COMP whereas the more central areas of the epiphysial cartilage were negative.

Discussion: mCOMP cloned and sequenced revealed a high degree of amino acid sequence identity and possessed all the molecular characteristic features of COMP from either rats or humans. COMP mutations characterized in both pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED) patients were noted for their heterozygosity by virtue of either a deletion, addition, or substitution in the amino acid sequence of the most conserved ype III repeating domain. Interestingly, all the affected amino acids were conserved among different species cloned so far, strongly suggesting that the mutated amino acids of COMP found in PSACH and MED play an important role in COMP structure and therefore in its function as well.

The expression of COMP during the early stages of skeletal development appears to be tightly regulated in the mouse embryo. Interestingly, prominent staining was not only observed in the perichondrium but also in periosteum in the long bone of a limb. The marked expression of COMP in areas of calcification simultaneously to the development of a diaphyseal bone collar and subsequent calcification of the cartilaginous matrix is different from that of type II collagen gene expression. These results suggested that COMP might have a specific role in proliferation and differentiatiation of chondrocytes, especially during endochondral bone formation. The detection of COMP expression in the early stages of both endochondral ossification and chondrogenesis may therefore help explain how a mutated form can result in disrupted longitudinal growth and early-onset osteoarthritis.

In addition to its expression in cartilaginous tissues, COMP was also expressed in cells of noncartilaginous origin in both mouse embryo (in developing skeletal muscle) and adult mouse tissues. Thrombospondin-4, originally cloned from heart and also identified from tendon (where COMP is also expressed), may have redundant functions, which might explain the absence of deformities noted in the organs of PSACH patients.

The unique expression pattern of COMP during embryogenesis indicates a different regulatory mechanism for COMP gene expression. Analysis of potential regulatory elements for the tissue- and site-restricted expression pattern of COMP protein in the future is needed to further enhance our understanding of COMP expression and function in physiological and pathological conditions.

**Fig. 1. Immunolocalization of COMP in day-19 mouse limb. (Left) Sagittal section of mouse arm (original magnification 2x). Arrows indicate the positive staining in the perichondrium (PC), periosteum (PO) and the superficial layer of articular surface (AS) of humerus. (Right) Higher-magnification (10x) view of C showing COMP staining patterns in the resting zone (R), proliferative zone (P), hypertrophic zone (H) and areas of calcification (C) zone. Reference: 1. Di Cesare PE et al. J. Orthop. Res. 14:946-55.1996. 2. Maddox BK et al. J. Biol.Chem.. 272:30993-7.1997.**

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