Introduction
COMP is an extracellular matrix (ECM) protein of cartilage, synovium and tendon. The proteins of the ECM must perform the difficult task of interacting in such a manner as to create both a stable environment for support and one conducive to growth and development. Various novel mutations in the type III molecular domain repeats of COMP (the calcium binding domain) have been identified in conjunction with two types of human dwarfism, pseudoachondrodysplasia (PSACH) and multiple epiphyseal dysplasia (MED). These autosomal dominant dwarfsims are characterized by stunted long-bone development in conjunction with early-onset osteoarthritis. It has yet to be determined how these mutations result in a growth disturbance. The purpose of this study was to examine the tissue distribution and the cell source of COMP in the normal developing human fetal and in adult bone by both immunostaining and in situ hybridization.

Methods
A 21-week-old human fetal foot and adult subchondral bone (obtained from knee replacement surgery) were subjected to immunostaining. Sections were probed with either a monoclonal or polyclonal antibody to human COMP; bound antibody detected with a biotinylated goat antiprimary antibody followed by alkaline phosphatase-conjugated streptavidin, developed with Vector Red I and counterstained with Mayer's hematoxylin. Immunostaining performed on embryonic specimens was performed on sections using either an antisense or sense (negative control) digoxigenin-labeled riboprobes (human COMP base pairs 86-281; exons 1-4, amino acids 1-65). Bound probe was detected by an alkaline-phosphatase-linked sheep antidigoxigenin antibody. Nitroblue tetrazolium was added with 5-bromo-4-chloro-3-indolylphosphate as a substrate, and counterstaining was performed with green PCF. The cells expressing COMP messenger exhibited a dark blue-black reaction by light microscopy.

Results
Various stages of endochondral bone development were seen on the embryonic histological specimens. Sections were subjected to in situ hybridization [with antisense (positive) and sense (negative control) riboprobes to human COMP] and immunostaining. The cartilage anlage of the distal phalanx showed little to no staining for COMP and was negative on in situ hybridization. The mature phalanx had the presence of a bone collar about the cartilaginous anlage; it stained positive for COMP and the cells were positive by in situ hybridization. The proximal phalanx demonstrated endochondral bone formation; intense staining by in situ hybridization was seen in the endosteum of the medullary canal (Fig. 1) and in the osteoblasts lining the newly formed osteoid at the base of the growth plate. Immunostaining confirmed the deposition of COMP within the newly formed metaphyseal bone and at the base of the growth plate. No staining was seen in the control sections probed with a sense probe constructed from the analogous region of cDNA. Immunostaining performed on adult subchondral bone showed positive intracellular staining for COMP of the osteoblasts lining the trabecular bone; osteocytes were negative.

Conclusions
COMP is implicated as an important component of both endochondral ossification and osteogenesis. The presence of COMP within the osteoblasts of developing bone and in adult tissues suggests a more complex role than that of simply a supporting glycoprotein within the host of noncollagenous ECM proteins that comprise cartilage. These results indicate that COMP is present from the initial stages of osteogenesis, as COMP is localized to the bone collar and at the newly formed bone at the growth plate. Synthesis by osteoblasts appears to last into adulthood, as adult osteoblasts still stained positive for COMP. The altered structure of COMP by the mutations seen in PSACH and MED may have direct effects on osteoblasts or long-bone development leading to the pathogenesis of these genetic disorders.


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