# A Novel Large Latent Complex of TGFb Is Produced by Hypertrophic Chondrocytes

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Chondrocytes produce transforming growth factor beta (TGFb) and store it in their matrix in a large latent complex (LLC) that includes latent TGFb binding protein (LTBP). Large quantities of activated TGFb are produced as a result of hypertrophy in chondrocytes and collagenase 3 (MMP-13) is involved in the activation of TGFb from its latent complexes. In this study, tibial hypertrophic chondrocytes were isolated from day 19 chicks, immunoprecipitated with antiserum to LTBP1 and MMP-13 and immunoblotted with antiserum to TGFb. Immunoblots contained bands of immunoreactivity corresponding to a novel 250 kDa LLC, the 60kDa bLAP N-terminus fragment of TGFb and the 25kDa homodimer of TGFb. Thus, the novel LLC for TGFb produced by hypertrophic chondrocytes includes MMP-13. An alternatively spliced, short form of LTBP 1 has been identified whose N-terminus plays a role in tissue transglutaminase-dependent TGFb activation mechanism. To determine the involvement of our novel LLC in more efficient activation of TGFb, we designed primers to differentiate the long form of LTBP from the short form. Reverse-transcription PCR of total RNA isolated from serum-free alginate cultures revealed that early hypertrophic chondrocytes produce both the long and short forms of LTBP, but the late hypertrophic chondrocytes only produce LTBP short. Therefore, as a chondrocyte nears terminal differentiation, LTBPshort is preferentially produced suggesting that chondrocytes shift to a more easily activated form of the LLC. Since the protease sensitive hinge region on LTBPshort is the target for LLC release by enzymes studied to date, it is plausible that association of MMP-13 to LTBPshort places the metalloproteinase in a position for more efficient activation of the chondrocyte-unique TGFb LLC. Through our own bioinformatics assessment based on previously published models of the TGFb LLC, we are suggesting that the hemopexin domain of MMP-13 interacts with EGF-CA domains of LBTPshort in such an orientation that the peptidase domain of MMP-13 lies in close proximity to the linker region of LBTPshort. This orientation is supported by existence of candidate amino acids in the linker region of LTBPshort, Gly-Ile bonds at positions 807/808 and 819/820, that are known to interact with the peptidase domain of MMP-13. The association of MMP-13 with LLC points to a novel storage and activation process for chondrocyte-produced TGFb that would allow for local and rapid control of release and activation of TGFb from matrix storage.