**Introduction:**
Chondrocyte growth and differentiation is a highly complex and regulated process. Previous studies showed that Wnt factors control a number of processes during limb development ranging from outgrowth initiation and patterning to differentiation control in a wide number of tissues. Several Wnt proteins are expressed during skeletal formation. It has been reported that Wnt3a, Wnt5a, Wnt7a are expressed in the early limb bud, and regulate skeletal patterning along the proximo-distal and dorso-ventral axes. Wnt14 is involved in joint formation in vivo. Wnt4 expression is also found in the joint region and has been shown to accelerate chondrocyte hypertrophy. In contrast, Wnt5 is expressed in the perichondrium where it delays cartilage maturation. In this study, we investigated the expression patterns of Wnt proteins during chondrocyte differentiation, and examined the role of Wnt/β-Catenin signaling in regulating chondrocyte differentiation in response to PTHrP and TGF-β, two well known factors that regulate chondrocyte maturation.

**Materials and Methods:**
Upper sternal chondrocytes (USC) and lower sternal chondrocytes (LSC) were isolated from 14 day old chick embryos and culture for 8 days in DMEM supplemented with 10% fetal bovine serum. Media were changed every other day. Cells were treated with PTHrP (10^{-7}M) or TGF-β (3ng/ml) for 48 hours before RNA extraction.

In some experiments, cells were co-transfected with the full length chicken type X collagen promoter driving the luciferase reporter (ABC-640), and wild type or mutant β-Catenin expression vectors. RCAS / β-Catenin viruses were also used to infect USC for 2 days prior to total RNA isolation at day 6. Quantitative Real time RT-PCR was performed using 1ul of reverse transcribed RNA in the SyberGreen reagent (ABI) according to the manufacturer instructions. Specific primers were made for chicken collagen type X and Wnts 4, 5, 7, 8, and 14 with specificity confirmed by sequence analysis, and expression normalized to GAPDH. For luciferase assays, Firefly and Renilla luciferase activities were determined using the dual-luciferase reporter kit (Promega).

**Results:**
We assessed the expression patterns of the Wnts during differentiation of upper and lower sternal chondrocytes. In USC Wnts 4, 5, 7, 8, and 14 are all up-regulated throughout cell differentiation as evidenced by collagen type X expression between two and eight days of culture. Wnt 8 is however predominantly expressed in comparison to the other Wnt transcripts. In LSC all Wnts are also upregulated during chondrocyte differentiation. However, Wnt4, Wnt8 and Wnt14 are predominantly expressed and dramatically up-regulated between 2 and 4 days in culture (Fig.1).

Our results indicate a differential regulation of Wnt transcripts throughout chondrocyte maturation that suggests a stage-specific role of these factors in mediating chondrocyte differentiation. We further assessed the effect of PTHrP and TGF-β on Wnt mRNA levels in USC (Figure 2.).

**Discussion:**
Our work demonstrates for the first time that multiple Wnts are highly expressed and regulated during chondrocyte differentiation. In both upper sternal chondrocytes, Wnt 8, a factor not previously studied in cartilage, is increased more than type X collagen levels. Wnt 4 and Wnt 14 were also predominantly expressed, suggesting that these factors may act in an autocrine fashion during maturation. Consistent with a role in differentiation was the finding that TGF-β suppresses Wnt expression. Moreover, TGF-β inhibited the ability of β-Catenin to induce maturation. Since β-Catenin is the major downstream signaling molecule of the Wnts, it appears that the Wnt pathway may be a molecular target for TGF-β effects on maturation at two levels: 1) by suppression of Wnt expression; and 2) by inhibition of activated β-catenin effects. Further experiments will determine the interaction between TGF-β and Wnt signaling in cartilage.