INTRODUCTION: Longitudinal bone growth results from coordination of proliferation and hypertrophy of chondrocytes, calcification of the matrix, vascular invasion, and completion of endochondral bone formation in the growth plate. Although proliferative and hypertrophic chondrocytes are well characterized histomorphologically, an understanding of the factors regulating progression in the growth plate is not fully explained. Whether the pathways currently implicated in control of longitudinal growth are the primary ones responsible is unknown. Combination of laser microdissection and cDNA microarray can enable a comprehensive investigation of these complex interactions in different growth plate zones. Our hypothesis was that significant differential gene expression exists between proliferative (PZ) and hypertrophic (HZ) chondrocytes that would provide clues to the regulation of this transition at the transcriptional level.

METHODS: Laser Microdissection and Microarray Analysis: Three 6 week-old and three 7 week male SD rats were sacrificed and the PZ and HZ chondrocytes were harvested by laser microdissection (Fig 1). The RAE230A chip was used to evaluate genes by pooled RNA samples extracted from 3 animals/time point for the PZ and the HZ chondrocytes. Analyses of Changes in Gene Expression: To identify differentially expressed genes in the PZ and HZ, Affymetrix MAS and Gene Traffic 2.7 were used to make separate, age-matched comparisons and determine fold-changes between pooled PZ and HZ specimens using RMA method. All differentially expressed genes with fold change > 2 at both 6 and 7 weeks for the PZ and HZ from this list were identified as well.

RESULTS: Highly Expressed Genes in PZ and HZ: Of the 15,923 probe sets arrayed on the RAE 230A chips, the 30 genes showing the greatest absolute expression levels within the PZ and HZ were selected for further analysis. Fifteen genes from the top 30 genes expressed within the PZ were also present among the top 30 genes expressed within the HZ, so only 15 genes from each list were unique to the respective zone (Fig 2).

Fig 1. The growth plate section Before and after LMD in the PZ (A, B) and HZ (C, D).

Confirmation of Microarray Results with In Situ Hybridization and Real-Time RT-PCR: To confirm the results of microarray, fibromodulin (Fmod) and proline arginine-rich end leucine-rich repeat protein (Prelp) were chosen from the PZ and integrin binding sialoprotein (Ibsp) and matrix metalloproteinase (MMP-13) were chosen from the HZ (Fig 3). MMP-13 was also confirmed by Real-Time RT-PCR with the data (4.59 fold at 6 week, 59475.2 fold at 7 week in the HZ compared to the PZ).

Fig 3. Localization by in situ hybridization is shown in the PZ (Fmod, Prelp) and HZ (Ibsp, MMP-13) in 6 week growth plate. Bar = 100µm.

Function Categorization of Differentially Expressed Genes: Comparison of the relative distribution of genes differentially expressed by two fold or greater within the PZ compared to the HZ and vice-versa shows that a greater percentage of PZ differentially expressed genes were devoted to cell cycle (7.5% PZ vs. 0% HZ) and transcription (7.5% PZ vs. 1.9% HZ) functions. Conversely, a greater percentage of HZ differentially expressed genes were involved in structure (11.5% HZ vs. 5.0% PZ) and receptor/transporter function (28.8% HZ vs. 10.0% PZ).

Analysis of Established Growth Plate Pathways: TGF-β, PTHrP, BMP, and IGF pathways previously shown to be important in musculoskeletal tissues were utilized as a framework with which to display the differential expression levels of a number of genes. BMP and IGF pathways along with mediators and differential PZ and HZ expression are shown in Fig 4.