

**CONTROL OF SKELETOGENESIS AND PROGRAMMED CELL DEATH IN THE DEVELOPING AVIAN LIMB BUD BY GROWTH FACTORS.**Yolanda GAÑAN<sup>1</sup>, Domingo MACIAS<sup>1</sup>, Maria Angeles ROS<sup>2</sup>, Ramon MERINO<sup>2</sup>, and Juan M HURLE<sup>2</sup>

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At the latest stages of limb morphogenesis the undifferentiate growing mesoderm of the autopod is segregated into digit forming regions and interdigital mesoderm destined to cell death. During this period a variety of growth factors are expressed in precise temporal and spatial patterns in the autopodal tissues. Members of the family of the fibroblast growth factors (FGF) including FGF-2, FGF-4 and FGF-8 are expressed in the apical ectodermal ridge (AER) riming the distal margin of the autopod. Transforming growth factors  $\beta$  (TGF- $\beta$ ) are expressed in the condensing mesenchyme of the digital forming regions. Bone morphogenetic proteins (BMPs), including BMP-2, BMP-4 and OP-1 (BMP-7) are expressed in the interdigital regions. In the present study we have analyzed the possible role of these growth factors in the commitment of the autopodial mesoderm for chondrogenesis or for programmed cell death. The experimental design consisted of in the local administration of those growth factors in different regions of the developing autopod using heparin-acrylic beads (Sigma) or Affi-gel blue beads (Bio-Rad) as carriers. Human recombinant BMP-2, -4 and -7, TGF- $\beta$ 1 and  $\beta$ 2, and FGF-2 and -4 were employed. The experiments were performed in chick and duck embryos at the stages preceding the occurrence of interdigital cell death.

Our results in the chick embryo show that implantation of TGF $\beta$ -beads into the interdigital spaces inhibits cell death leading to the formation of ectopic interdigital extra-digits. The opposite occurred following implantation of BMP-beads. When BMP-beads were implanted in the interdigital spaces cell death was accelerated. When BMP-beads were implanted at the tip of the digit forming regions, the digits bifurcated and an extra interdigital region of cell death by apoptosis was formed. Implantation of FGF-beads caused a transitory inhibition of both interdigital chondrogenesis and cell death. These results indicate that the distal undifferentiate mesodermal cells located under the AER are maintained alive and proliferating by the influence of FGFs. When the cells became displaced from the influence of the AER they differentiate into cartilage or entered programmed cell death depending on whether they became influenced by the domains of expression of TGF $\beta$ s or BMPs. Similar experiments performed in the duck leg which is characterized by a reduced extension of interdigital cell death and persistence of interdigital webbs indicate that the apoptotic response of the undifferentiated mesoderm to BMPs is critically dependent on the previous influence of FGFs. The molecular basis for this process was analyzed studying changes in the pattern of expression of genes related with cell death and cartilage differentiation.