Biology 4361  
Developmental Biology Lab  
November 29, 2007

Pattern Formation during Chick Limb Development

Limb buds are induced by lateral plate and somatic mesoderm in the flank regions. The position of this induction may be controlled by Hox gene expression at the appropriate level. Induction of limb buds is controlled by Fgf10 (a paracrine factor), which is induced throughout the lateral plate mesoderm, but becomes restricted to the regions of the lateral plate mesoderm where the limbs will form by Wnt proteins, which stabilize Fgf10 expression in the limb-forming areas.

Limbs develop with certain patterns. Consider your hand; it has proximal-distal (wrist to fingertip), anterior-posterior (thumb-little finger), and dorsal-ventral (knuckle-palm) axes. All limbs and limb segments have similar patterns, which are established during early development by different molecular signaling pathways. Proximal-distal development is controlled by FGFs, anterior-posterior development is controlled by Sonic hedgehog (Shh), and dorsal-ventral by Wnts. Expression of these genes, combined with Hox genes expressed sequentially from posterior to anterior, results in the final pattern of the limb, which is laid down first as cartilage, and is eventually converted to bone.

Several models have been proposed to explain development along the limb axes. Anterior-posterior patterning can be explained by the interaction of cells (and gene expression) from various regions of cells within the developing limb. The apical ectodermal ridge (AER) is one of the first tissues specified by FGF signaling, and is required throughout limb development. Damage or excision of the AER results in a truncated limb, with characteristics dependent on the time of lesion.

Proximal to the AER is an area of mesodermal tissue called the progress zone (PZ), which provides the mitotic cells that will form the bulk of the limb tissue. Removal of PZ cells or replacement with non-limb mesenchyme results in a lack of limb formation, indicating that this mesoderm is specified to form limb tissues.

Anterior-posterior specification appears to be dependent on the presence of cells in the posterior region of the limb bud, called the zone of polarizing activity (ZPA). Removal or disruption of this region results in dysmorphogenesis (malformation) of the limb, and transplantation of ZPA tissue to another portion the developing limb bud can produce a secondary limb. By assessing the structure of these secondary limbs, it is evident that the ZPA defines the posterior (e.g. little finger) axis of the limb, and the anterior axis is defined as the region spreading away from it. Thus, the secondary limbs are often “mirror images” of the original limb.

The polarizing activity of the ZPA can be replicated by Sonic hedgehog (Shh); forcing expression of Shh in the anterior limb bud will also produce a mirror image secondary limb. In many embryos, duplicated limbs can also be produced by retinoic acid (RA),
Although it is not known if this is due to ectopic stimulation of Shh expression, or whether Hox genes are the primary targets. It may be significant that natural occurrences of limb duplication sometimes show similar patterns. For instance, recently wild frogs have appeared with missing, malformed, or duplicated legs, and one possible suggested cause was exposure to pesticides that act as retinoic acid mimics. Experiments such as the one you will perform today have been used to explore these questions.

The chick embryo serves as an excellent experimental model for limb development, since embryos are easily accessible and observable during the early developmental stages. We will test the ability of retinoic acid to stimulate limb duplication in early embryos. Small porous beads (chromatography media) will be soaked in RA, and placed within of the anterior region of developing limb buds. Control experiments will be done using non-exposed beads. After several days of development, the developing limbs will be processed and stained for cartilage, which will indicate the pattern of the wing.