SEMESTER-III

Core Course (CC-11) Gamete and Developmental Biology

Topic: Genetic Basis of Limb Development in Tetrapod

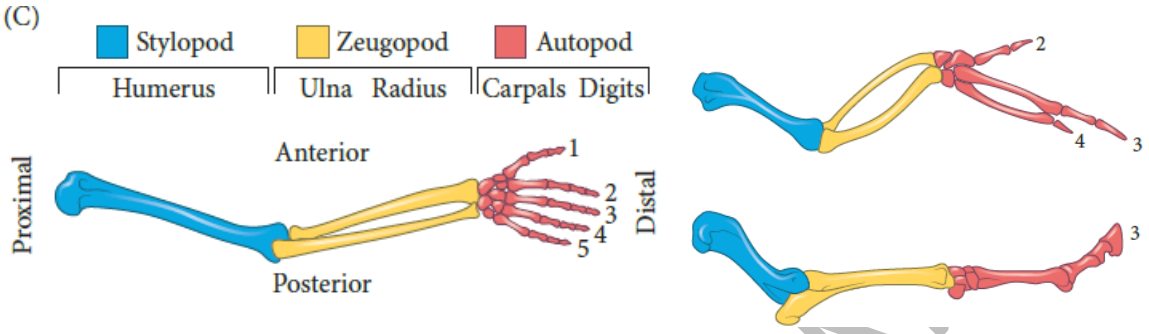
E-content for Post-Graduate students

Developed By:

Parimal K. Khan
Professor
Department of Zoology
Patna university, Patna

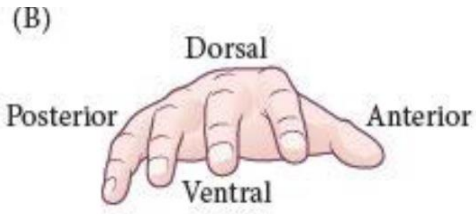
Introduction:

As the name denotes, tetrapods are four-limbed vertebrates (amphibians, reptiles, birds, and mammals). The bones of tetrapod limb consist of a **proximal stylopod (humerus/femur)** near body wall, a **zeugopod (radius-ulna/ tibia-fibula)** in the middle region, and a **distal autopod (carpals-fingers/tarsals-toes).** Fingers and toes can be referred to as phalanges or, more generally, digits.



The positional information needed to develop a limb. That has to function in a three-dimensional coordinate system:

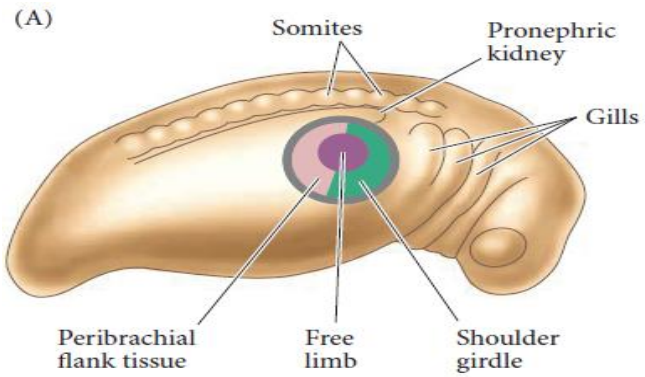
1. The first dimension is the proximal-distal axis ("close-far"; that is, shoulder-to-finger or hip-to-toe). The bones of the limb are formed by endochondral ossification. They are initially cartilaginous, but eventually most of the cartilage is replaced by bone.
2. The second dimension is the anterior-posterior axis (thumb-to-pinkie). Our little fingers or toes mark the posterior end, and our thumbs or big toes are at the anterior end.
3. Finally, limbs have a dorsal-ventral axis: our palms (ventral) are readily distinguishable from our knuckles (dorsal).



The Limb Bud:

The first visible sign of limb development is the formation of bilateral bulges called limb buds at the presumptive forelimb and hindlimb locations. Limb buds are derived from posterior lateral plate mesoderm. Limb bud will be formed from limb field.

Limb Field = Peribranchial flank tissue + Shoulder girdle + Free limb + Ring of cells



Lateral plate mesenchyme will form *skeletal precursor cell*. Mesenchymal cells from the somites *muscle precursor cell*. The limb bud is further regionalized into three functionally distinct domains:

a) Progress zone (PZ):

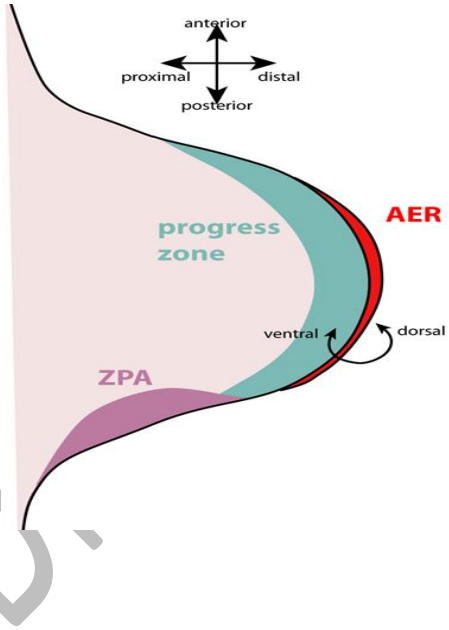
- Highly proliferative mesenchyme that fuels limb bud growth. Also known as *undifferentiated zone.*

b) Zone of polarizing activity (ZPA):

- Most posterior region of the progress zone.

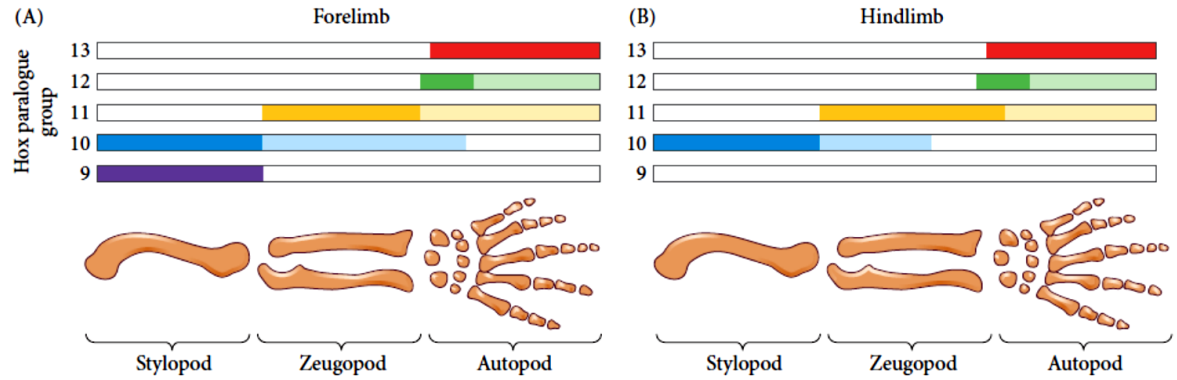
c) Apical ectodermal ridge (AER):

- A thickening of the ectoderm at the apex of the developing limb bud.



Hox Gene Specification of Limb Skeleton Identity:

Homeobox transcription factors, or Hox genes, play an essential role in specifying the mesenchymal cell to become stylopod, zeugopod, or autopod. The proximal to distal limb is specified by Hox genes. The development of limb bud in mice is specified by complex of *Hoxa* and *Hoxd* gene.

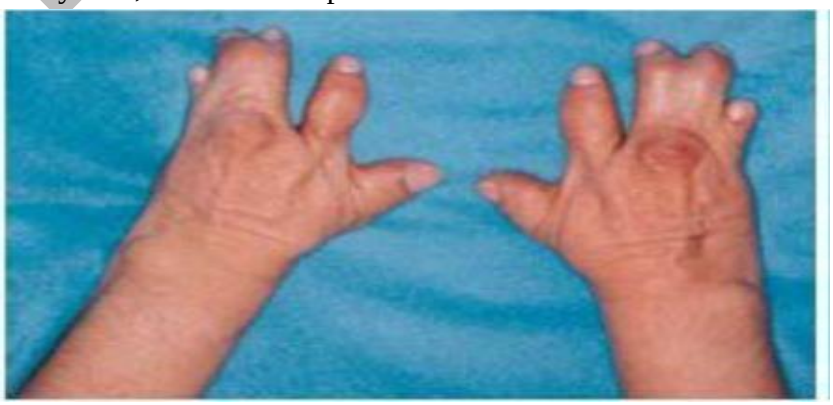


Abnormalities related to Hox genes:

a) Forelimb of a wild-type mouse (left) and of a double mutant mouse that lacks functional Hoxa11 and Hoxd11 genes (right). The ulna and radius are severely reduced or absent in the mutant

.

b) Human polysyndactyly ("many fingers joined together") syndrome results from a homozygous mutation at the HOXD13 loci. This syndrome includes malformations of the urogenital system, which also expresses HOXD13.



Morphogenetic rules for Limb Development:

- Certain basic "morphogenetic rules" for forming a limb appear to be the same in all tetrapods.

- Experimentally remove or transplant parts of the developing limb, or create limb-specific mutants, without interfering with the vital processes of the organism.
- Grafted pieces of reptile or mammalian limb buds can direct the formation of chick limbs, and regions taken from frog limb buds can direct the patterning of salamander limbs.

Specifying the limb fields:

The mesodermal cells that give rise to a vertebrate limb have been identified by-

- Removing certain groups of cells and observing that a limb does not develop in their absence **("lose it").**
- Transplanting groups of cells to a new location and observing that they form a limb in this new place **("move it").**
- Marking groups of cells with dyes or radioactive precursors and observing that their descendants partake in limb development **("find it").**

Induction of the early limb bud:

The limb bud formation is initiated by following series of processes. These processes are divided into four steps:

1. Making mesoderm permissive for limb formation
2. Specifying forelimb and hindlimb
3. Inducing epithelial-mesenchymal transitions
4. Establishing two positive feedback loops for limb bud formation.

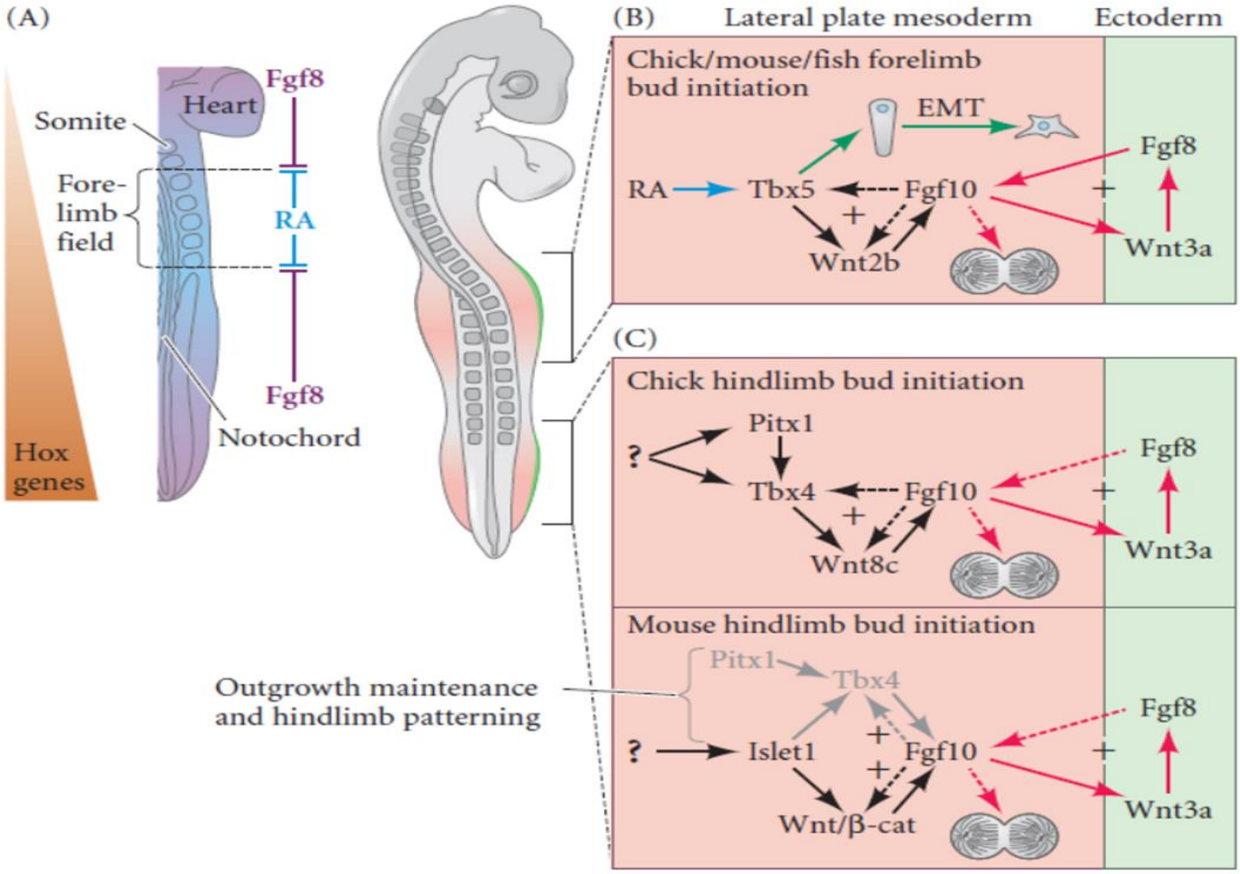
1. **Making mesoderm permissive for limb formation:**

   Fgf8 is expressed by the caudal (posterior) progenitor zone, which is located just posterior to the forelimb field. In contrast, RA is generated more anteriorly in somites and anterior presomitic mesoderm. RA is responsible for development of forelimb bud. whereas fgf8 inhibits the development of fore limb bud. So, Retinoic acid and fgf8 is antagonist to each other. RA made permissive the anterior presomitic region for the development of fore limb.

2. **Specifying forelimb and hindlimb:**

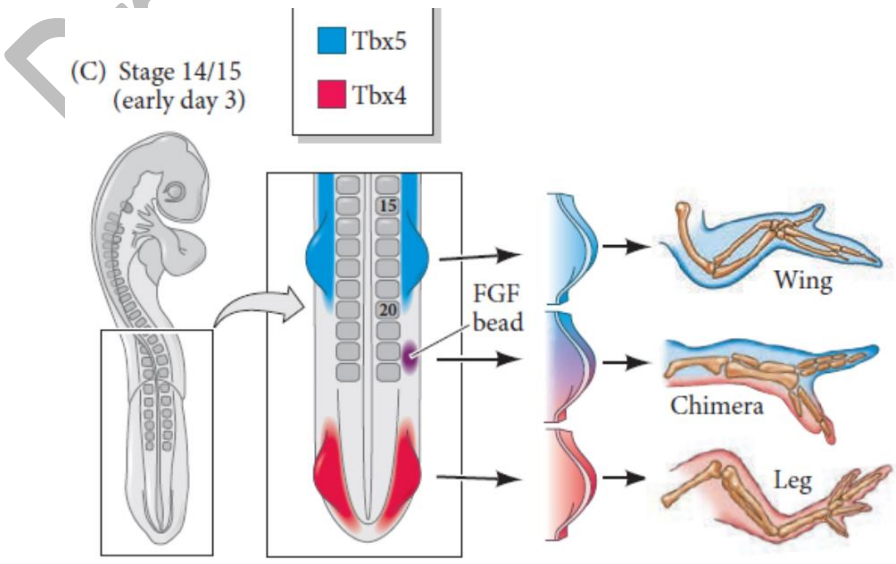
   The gene encoding the ***Tbx5*** transcription factor in mice is transcribed in the anterior lateral plate mesoderm and in the forelimbs. while the genes encoding the transcription factors ***Tbx4, Pitxl*** and ***Islet1*** are expressed in the posterior lateral plate mesoderm and in the hindlimbs*. Transcriptional regulation of Islet1 and of Pitx1 are independent of each other's function, as are their roles in hindlimb development.

*Tbx stands for T-box. The T (Brachyury) gene and its relatives have a sequence that encodes this specific DNA-binding domain. Humans heterozygous for the TBX5 gene have *Holt-Oram syndrome*, characterized by abnormalities of the heart and upper limbs (Basson et al. 1996; Li et al. 1996).



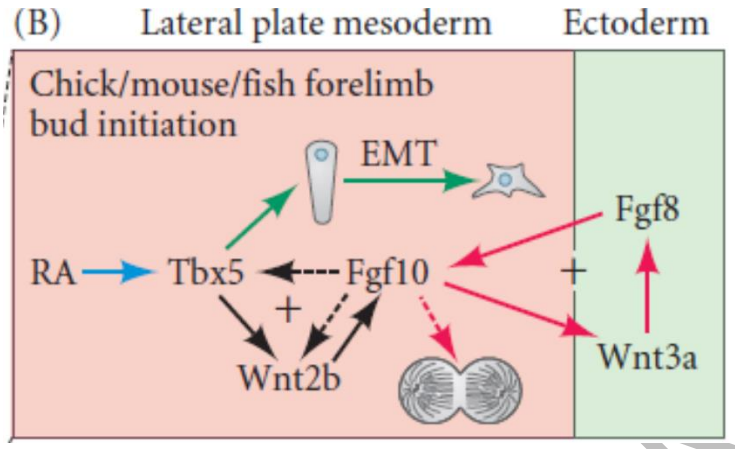
Case Study:

- If Fgf bead is placed in between forelimb and hindlimb than chimera of wing and limb is formed.



3. **Inducing epithelial-mesenchymal transitions:**

Prior to limb bud formation, the lateral plate mesoderm of the somatopleure displays characteristics of a pseudostratified epithelium with apical-basal polarity. Tbx5 cause transition of pseudostratified epithelium to mesenchyme.



Generating the Proximal-Distal Axis of the Limb:

The apical ectodermal ridge:

The apical ectodermal ridge is a multipurpose signaling center that will influence patterning along all axes of limb development.

The diverse roles of the AER include:

1. Maintaining the mesenchyme that enables the linear (proximal-distal, or shoulder-finger) growth of the limb.

2. Maintaining the expression of those molecules that generate the anterior-posterior (thumb-pinkie) axis; and

3. Interacting with the proteins specifying the anterior-posterior and dorsal-ventral (knuckle-palm) axis.

Manipulation of the apical ectodermal ridge (AER):

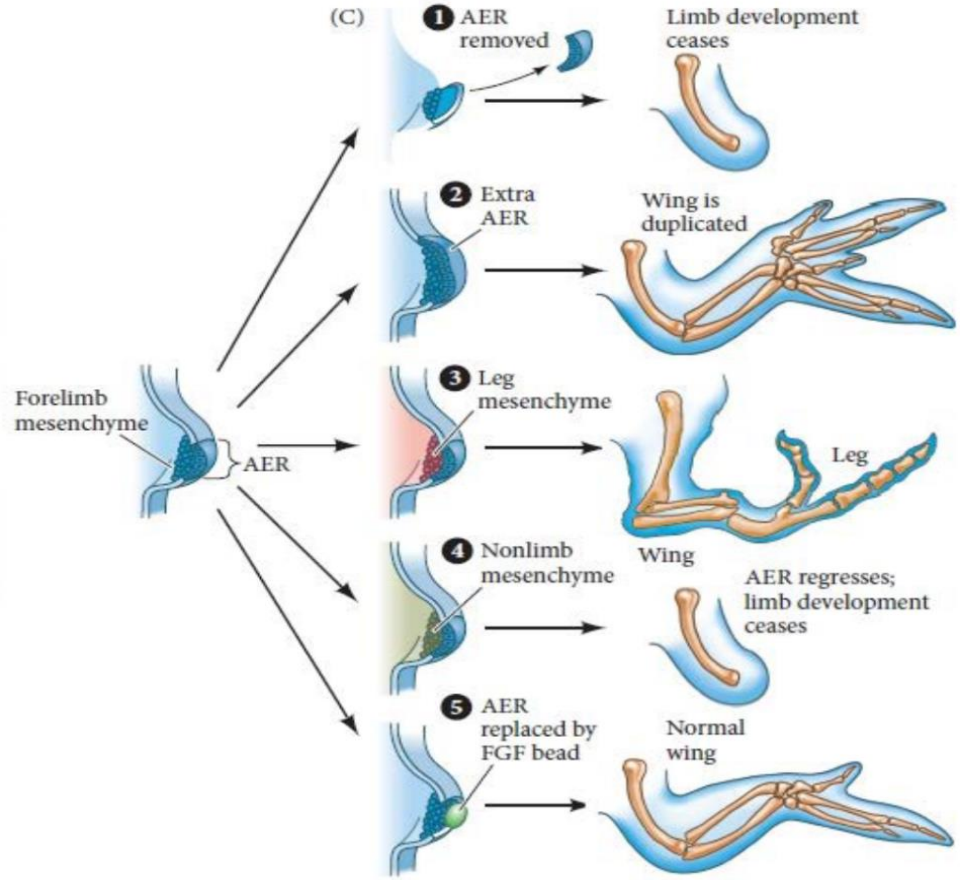
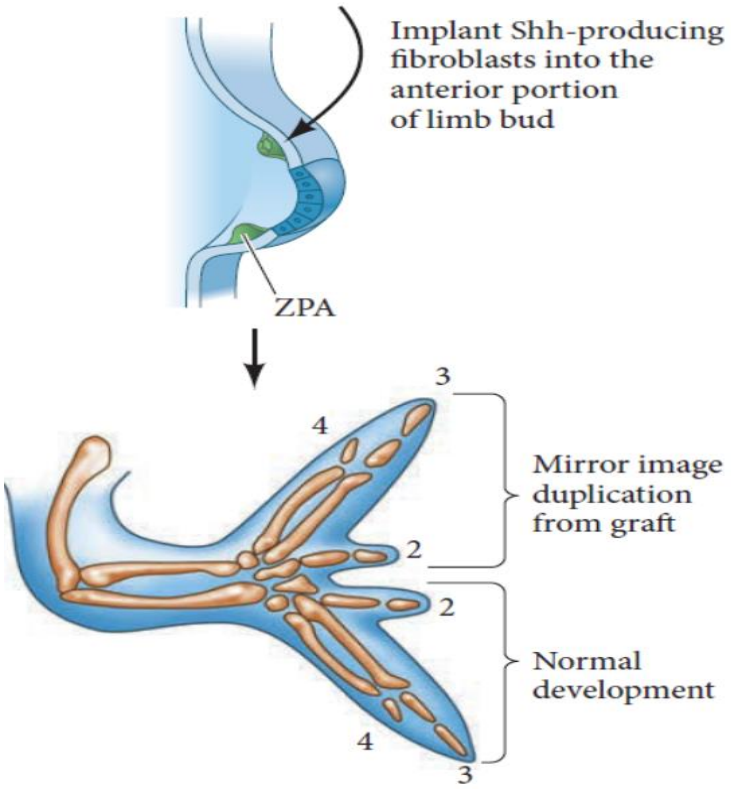


FIGURE: Manipulation of the apical ectodermal ridge (AER). (A) In the normal 3-day chick embryo, Fgf8 (dark purple) is expressed in the AER of both forelimb and hindlimb buds. (B) Expression of Fgf8 RNA in the AER, the source of mitotic signals to the underlying mesoderm. (C) Summary of experiments demonstrating the effect of the AER on the underlying mesenchyme. (C after N. K. Wessells. 1973. Tissue Interactions in Development: An Addison-Wesley Module in Biology, no 9. Addison-Wesley Longman: Boston.)

Specification of the Anterior-Posterior Axis

The zone of polarizing activity:

Several experiments suggest that the anterior-posterior axis is specified by a small block of mesodermal tissue near the posterior junction of the young limb bud and the body wall called the zone of polarizing activity (ZPA). In 1993, Riddle and colleagues showed by in situ hybridization that sonic hedgehog (shh), a vertebrate homologue of the Drosophila hedgehog gene, was expressed specifically in that region of the limb bud known to be the ZPA.



Generating Dorsal-Ventral Axis:

The dorsal-ventral patterning in the limb bud is done by *Wnt* and BMP signalling. *Wnt7a* induces dorsal cell fates of the limb bud through *Lmx1b*, whereas BMP signalling functions through Engrailed-1 (*En1*) to regulate ventral limb patterning.

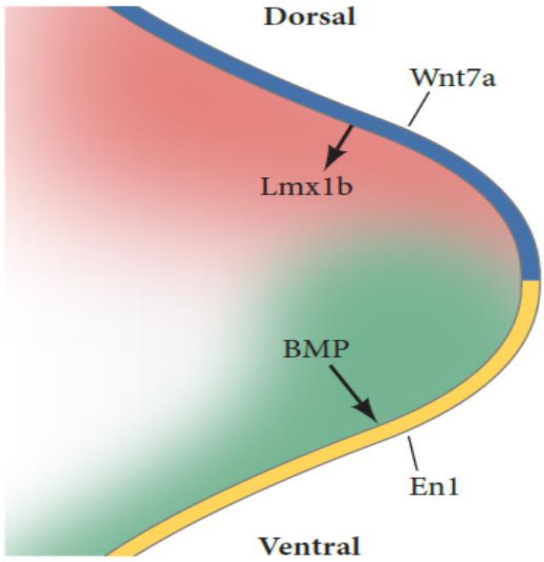


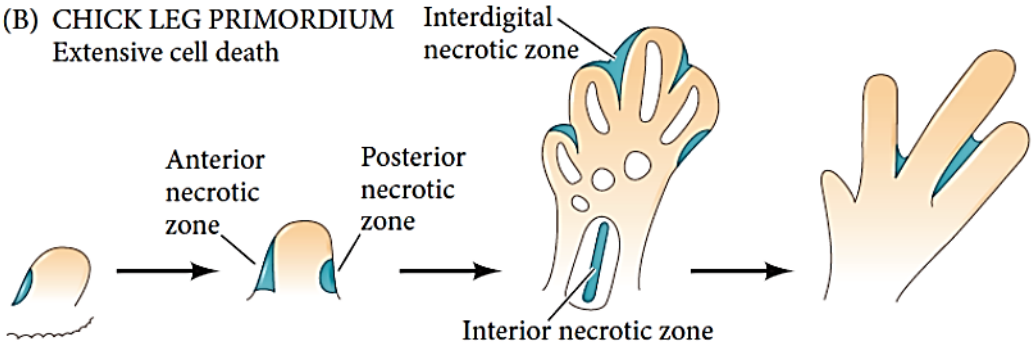
Fig: Model of Dorsal-Ventral patterning.

Cell Death and the Formation of Digits and Joints:

Cell death plays a role in sculpting the tetrapod limb. Indeed, cell death is essential if our joints are to form and if our fingers are to become separate. The death (or lack of death) of specific cells in the vertebrate limb is genetically programmed and has been selected for over the course of evolution.

Regions where cell death takes place:

Interdigital necrotic zone: Create the gap between digits and separate them.
Interior necrotic zone: The ulna and radius are separated from each other.
Anterior necrotic zone: } Further shape the end of the limb
Posterior necrotic zone: }



- The signal for apoptosis in the autopod is probably provided by the BMP proteins. BMP2, BMP4, and BMP7 are each expressed in the interdigital mesenchyme.

Formation of Digits:

Sonic hedgehog is the major derive for digit formation. It seems that specification of the digits is primarily dependent on the amount of time the Shh gene is expressed and only a little bit on the concentration of Shh protein that other cells receive.

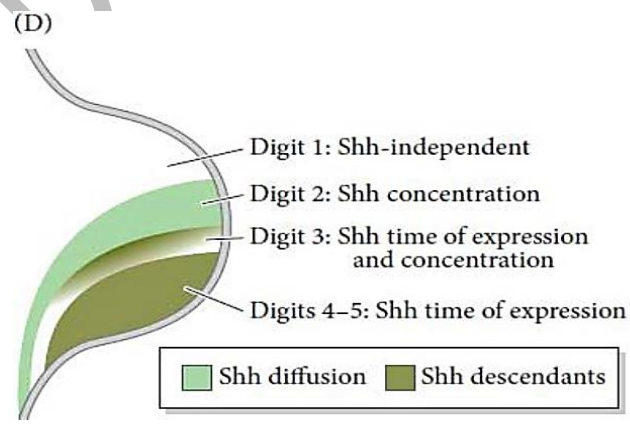


Figure: Schematic by which digits 4 and 5 are specified by the amount of time they were exposed to Shh in an autocrine fashion; digit 3 is specified by the amount of time the cells were exposed to Shh in both an autocrine and a paracrine fashion. Digit 2 is specified by the concentration of Shh its cells received by paracrine diffusion, and digit 1 is specified independently of Shh. (After B. D. Harfe et al. 2004. Cell 118: 517–528.)