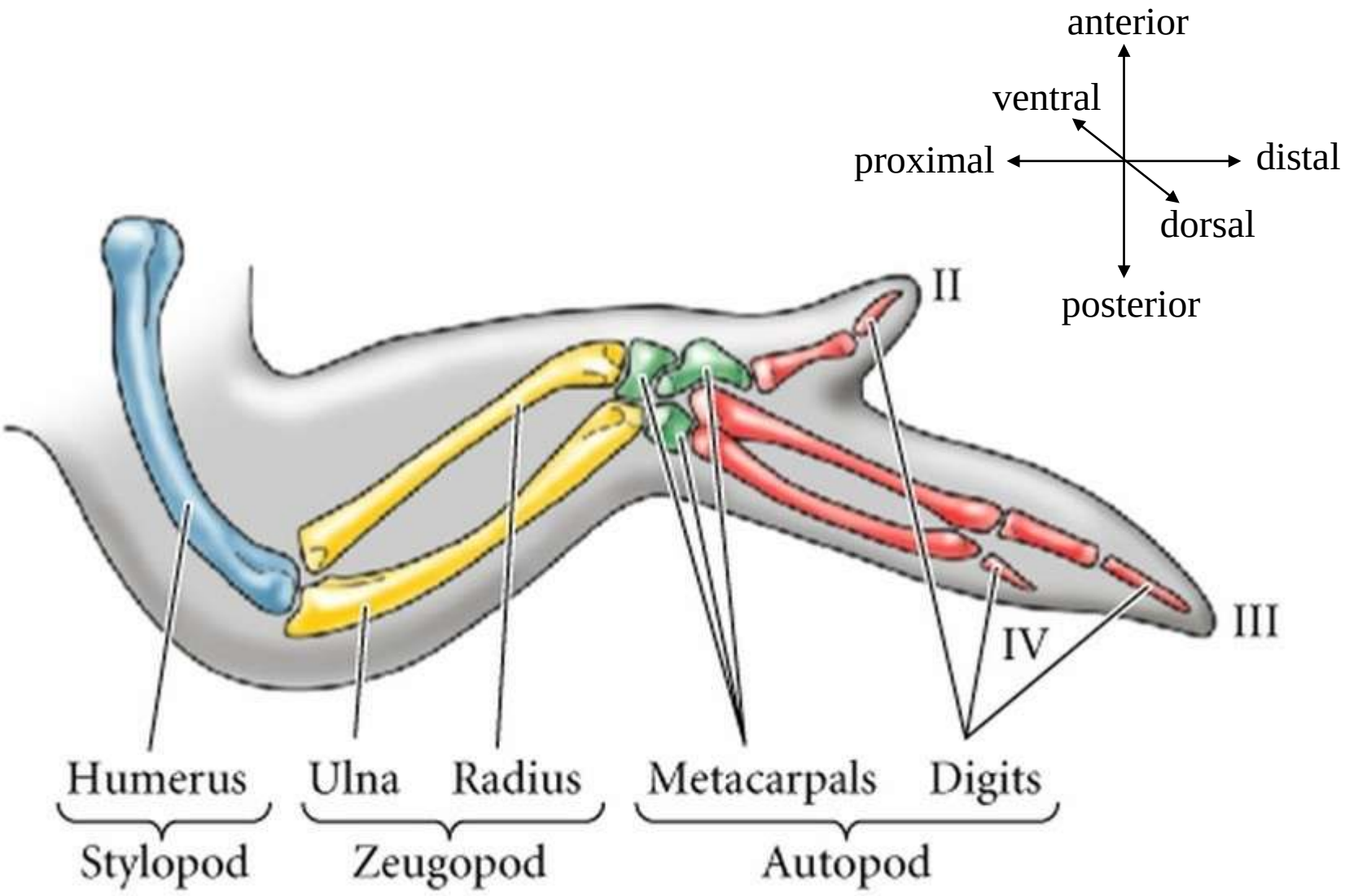
• DEVELOPMENT OF TETRAPOD LIMB

'PATTERN FORMATION

- process by which embryonic cells form ordered spatial arrangement of differentiated tissues.

- The developing limb has long been a pioneering model for understanding pattern formation: the process in which the spatial organization of differentiated cells and tissues is generated in the embryo.
- Pattern formation can be considered as a two-step process; first cells are informed of their position and, thus, acquire a positional value (specification); cells then remember and interpret this value to form the appropriate structures (differentiation) (Wolpert, 1969).

TETRAPOD LIMB



Development of the tetrapod limb has to function in a three-dimensional coordinated system

- Proximal-distal axis------ running in the human arm from shoulder to digits; Fibroblast Growth Factor family protein and Hox Genes

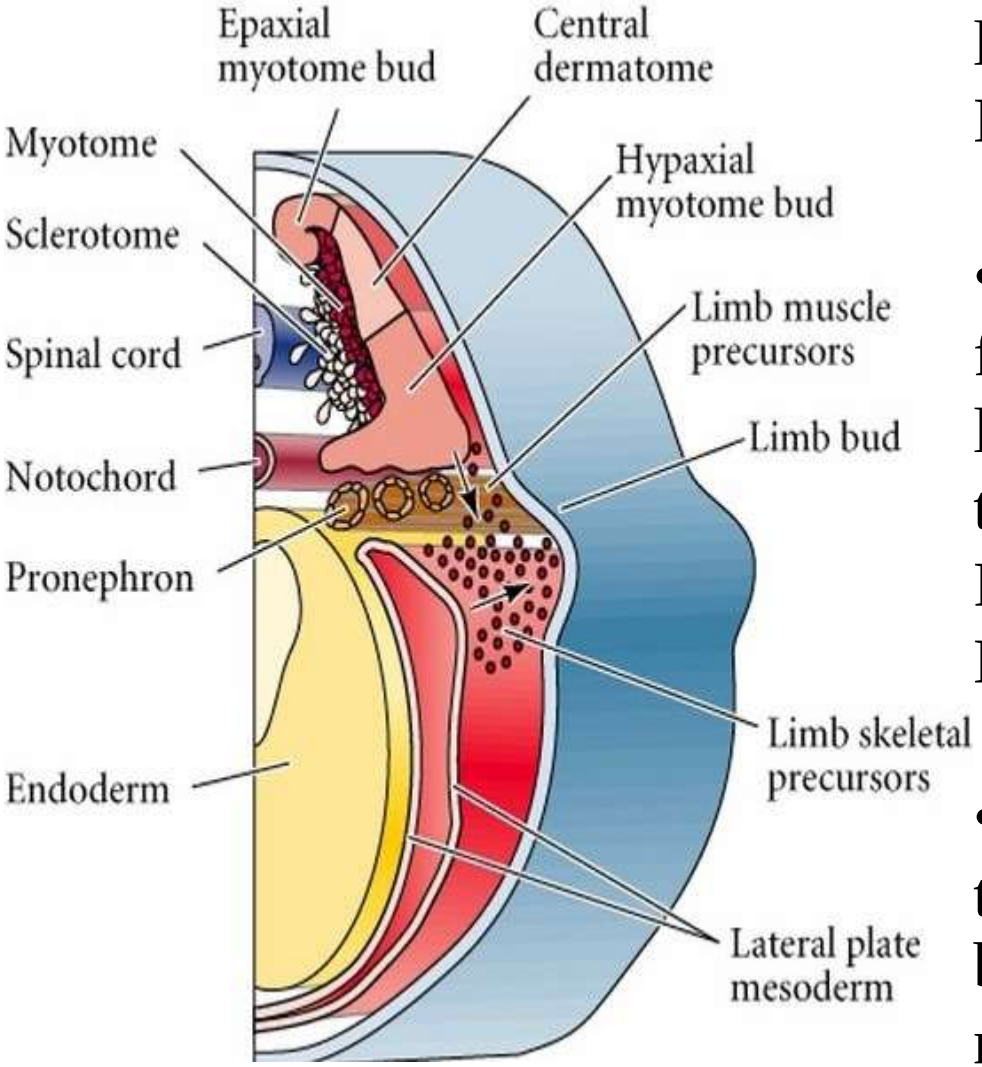
- Anterior-posterior axis---- from thumb to the little finger; Sonic hedgehog protein

- Dorsal-ventral axis----- from the back of the hand to the palm ,Wnt7a protein

DEVELOPMENT OF THE TETRAPOD LIMB

1. Formation of the Limb Bud
2. Generating the Proximal- Distal Axis of the Limb
3. Specification of Anterior- Posterior Limb Axis
4. Generation of the Dorsal- Ventral Axis
5. Cell Death and the Formation of Digits and Joints

INDUCTION OF EARLY LIMB BUD: FIBROBLAST GROWTH FACTORS

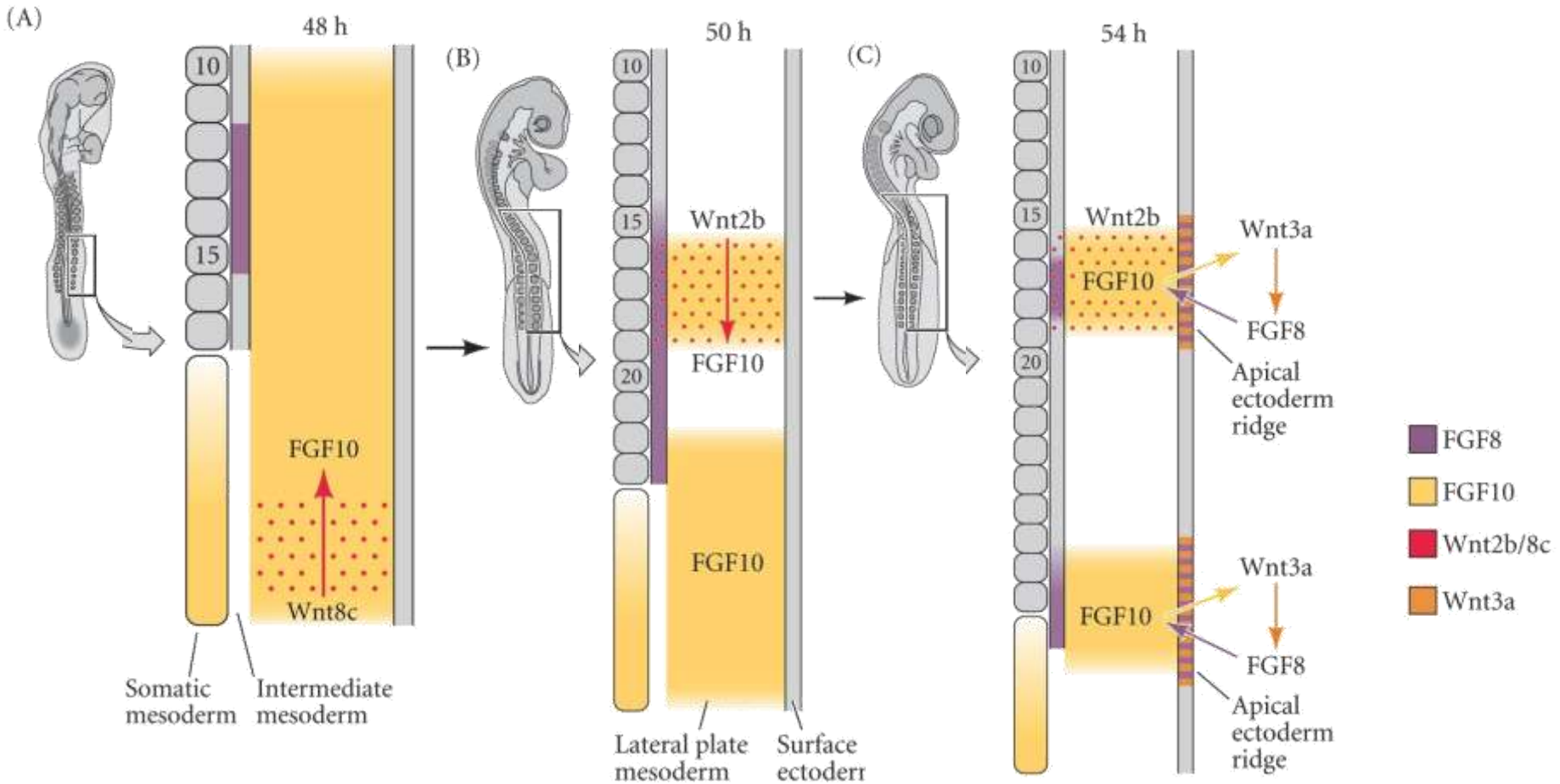


- Limb muscle precursors + limb skeletal precursors----- LIMB BUD

- Signal for limb bud formation comes from the lateral plate mesoderm cells that later will become PROSPECTIVE LIMB MESENCHYME CELLS

- FGF10 = capable of initiating the limb –forming interactions between ectoderm and mesoderm.

Establishment of Limb Fields



LIMB BUD FORMATION

Limb Field Specification

¬ regulated by Hox Genes and Retinoic Acid

♣Limb field position is determined with respect to the level of Hox gene expression along the anterior –posterior axis

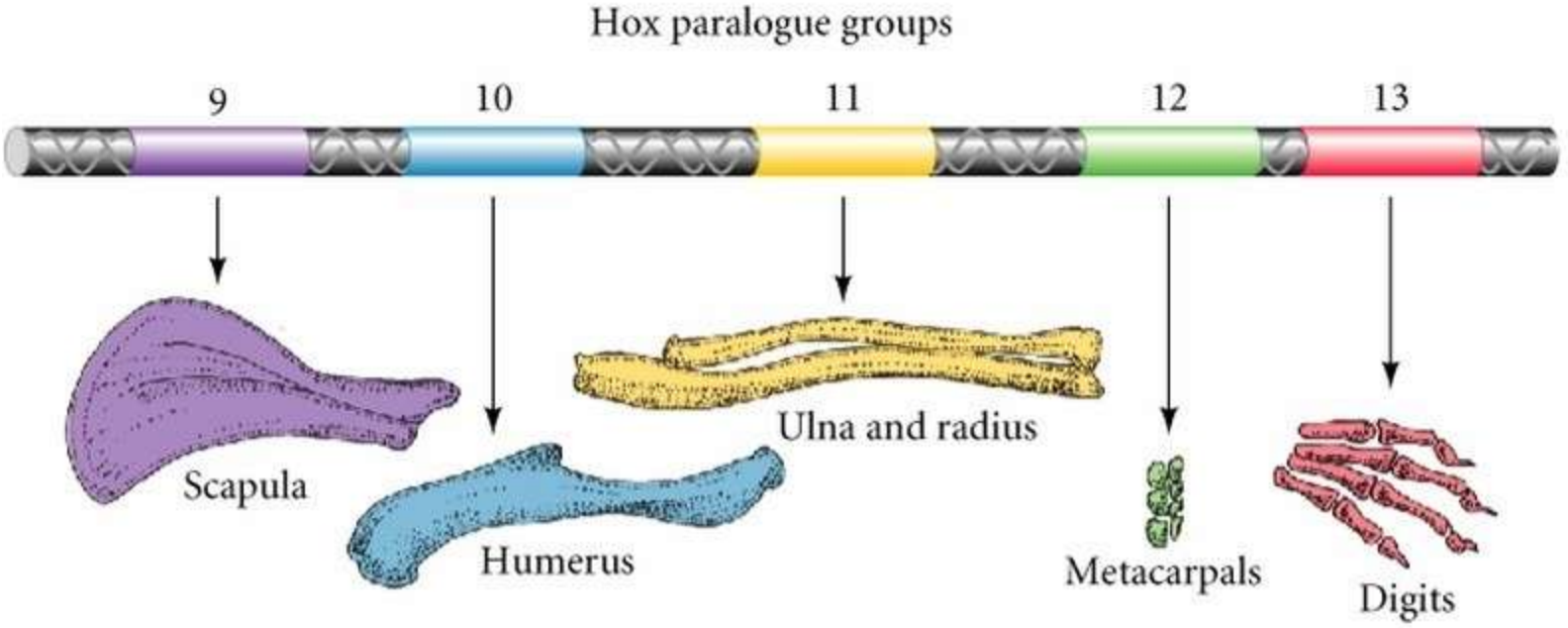
♣Retinoic acid is also critical in limb bud outgrowth.

Positioning of the Limb Field

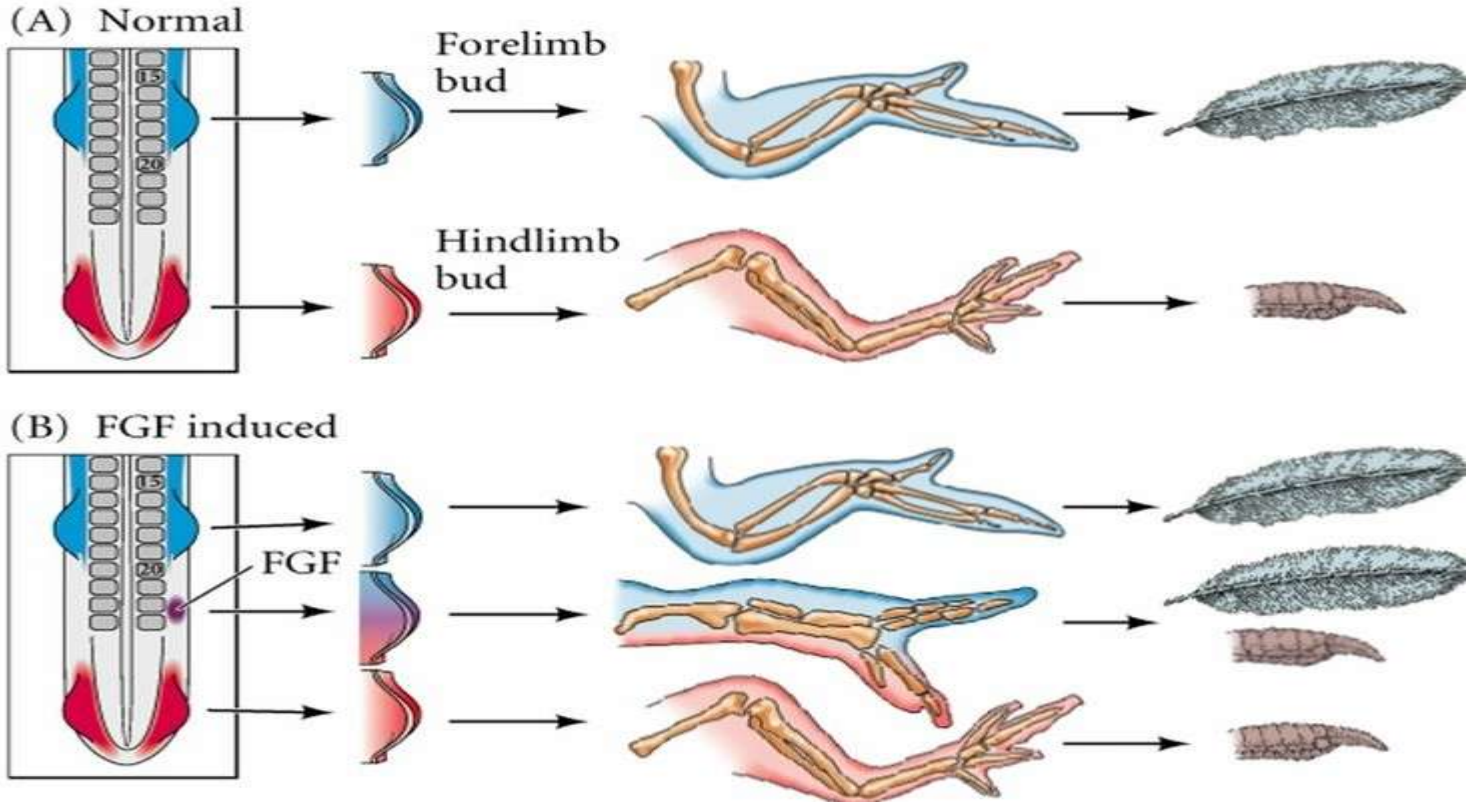
- Forelimb position lies near anterior expression boundary of hox b8, b9, c6, c9, & d9
- FGF8 expression in intermediate meso + hox genes induces FGF10 in the lateral plate meso
- Wnts in the lateral plate maintain FGF expression in regions of hoxd9 expression
- Between the fore & hindlimb buds, the developing kidney (mesonephros) inhibits FGF expression
- Exogenous FGF can induce supernumerary limbs

Hox Code of the Limb

Proximal ⟶ Distal



Hoxa & d = forelimb; Hoxc & d = hindlimb

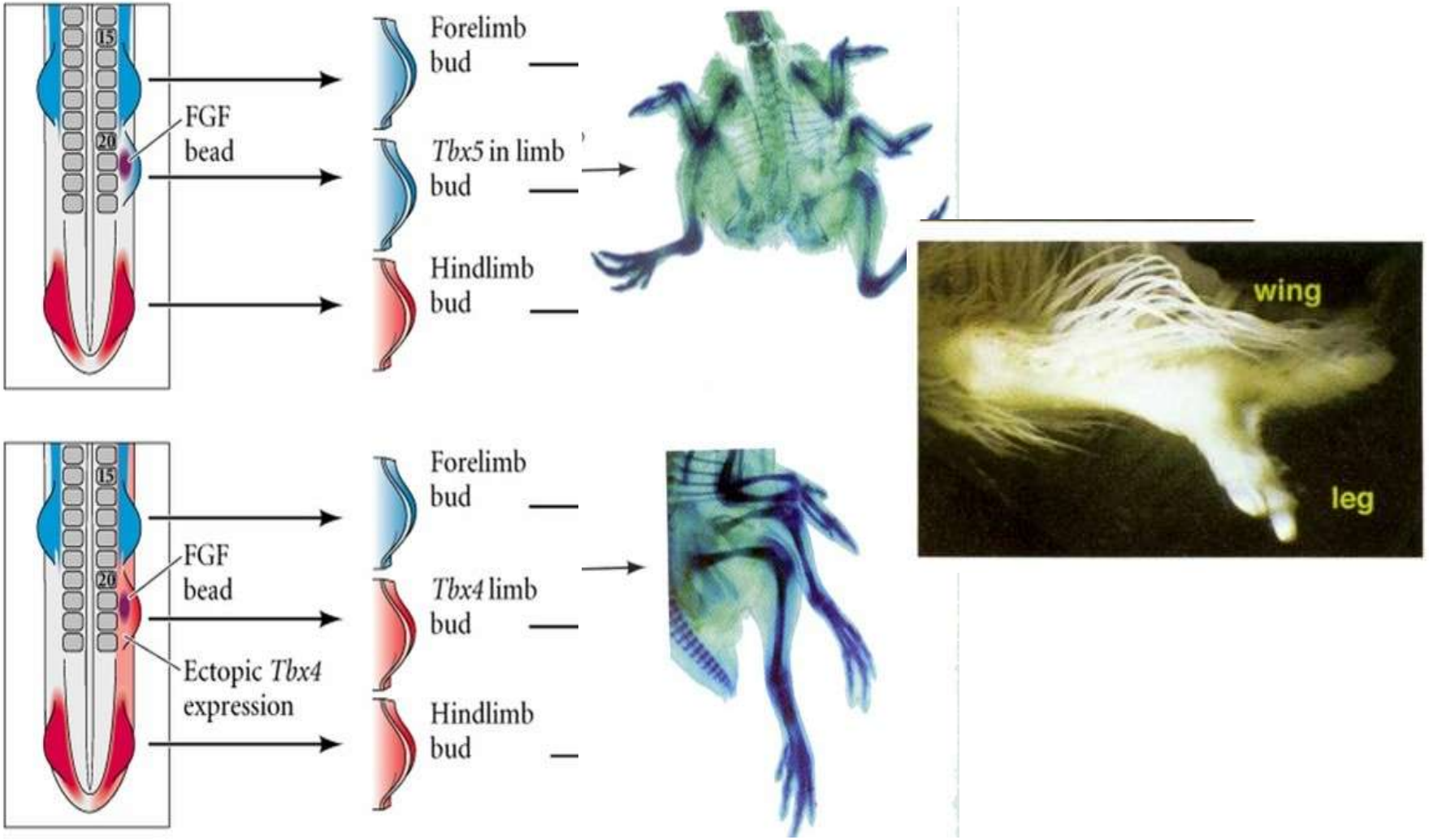


supernumerary limbs from implanting FGF10 beads

SPECIFICATION OF FORELIMB OR HINDLIMB: TBX4 AND TBX5

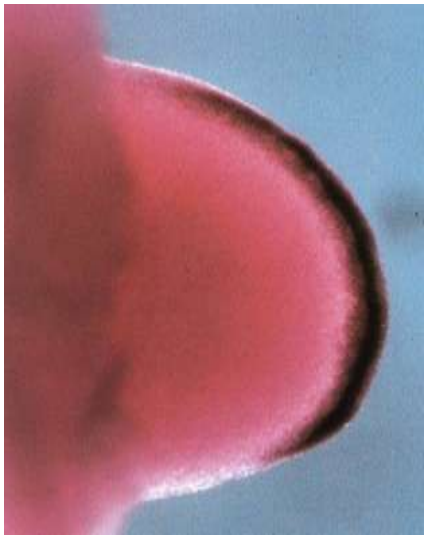
The gene encoding the Tbx5 transcription factor is transcribed in forelimbs of embryo while the gene encoding the Tbx4 transcription factor is expressed in hindlims.

TBX4 AND TBX5 GAIN OF FUNCTION STUDY



FIBROBLAST GROWTHFACTOR & HOX GENES

- AER (Apical Ectodermal Ridge)--- major signaling center for the developing limb

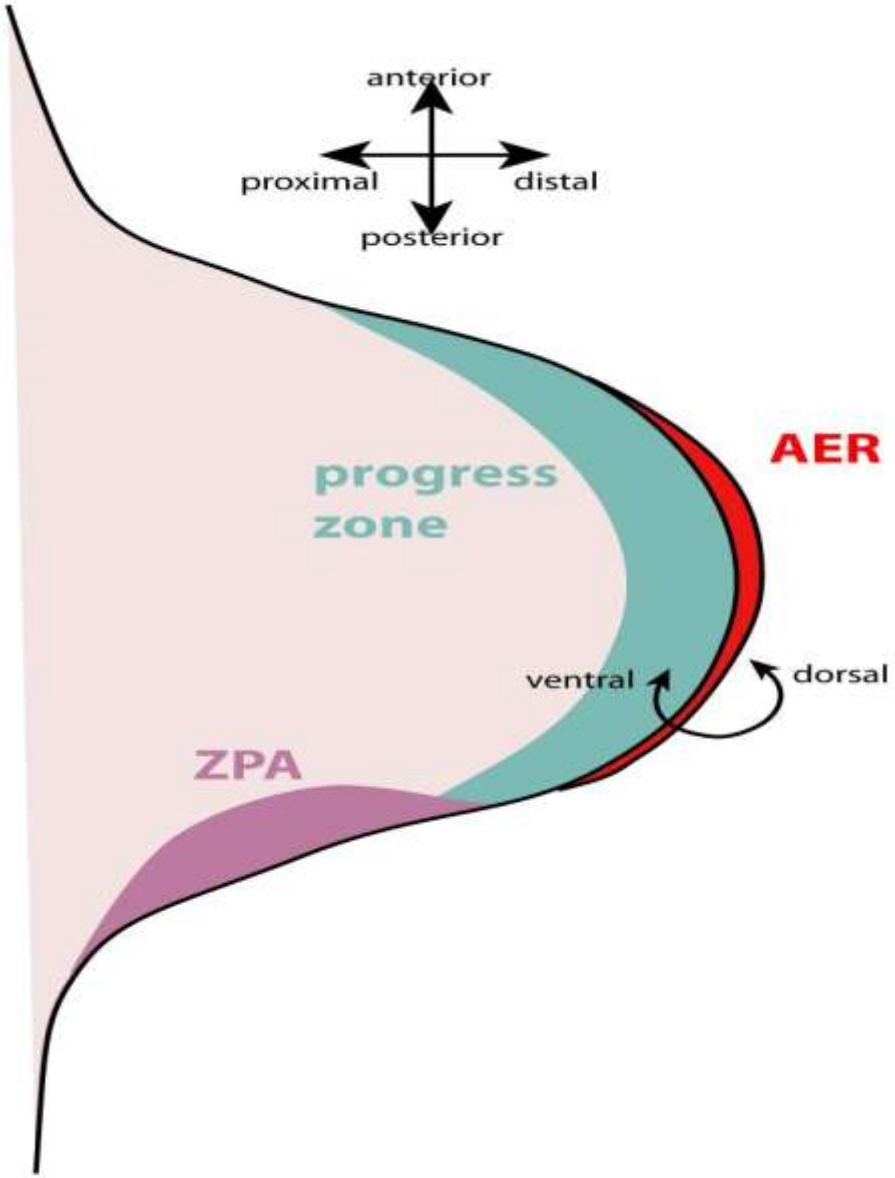


- maintaining the mesenchyme beneath it in a plastic, proliferating phase that enables the proximal-distal growth of the limb

- Maintain the expression of molecules that generate anterior-posterior axis

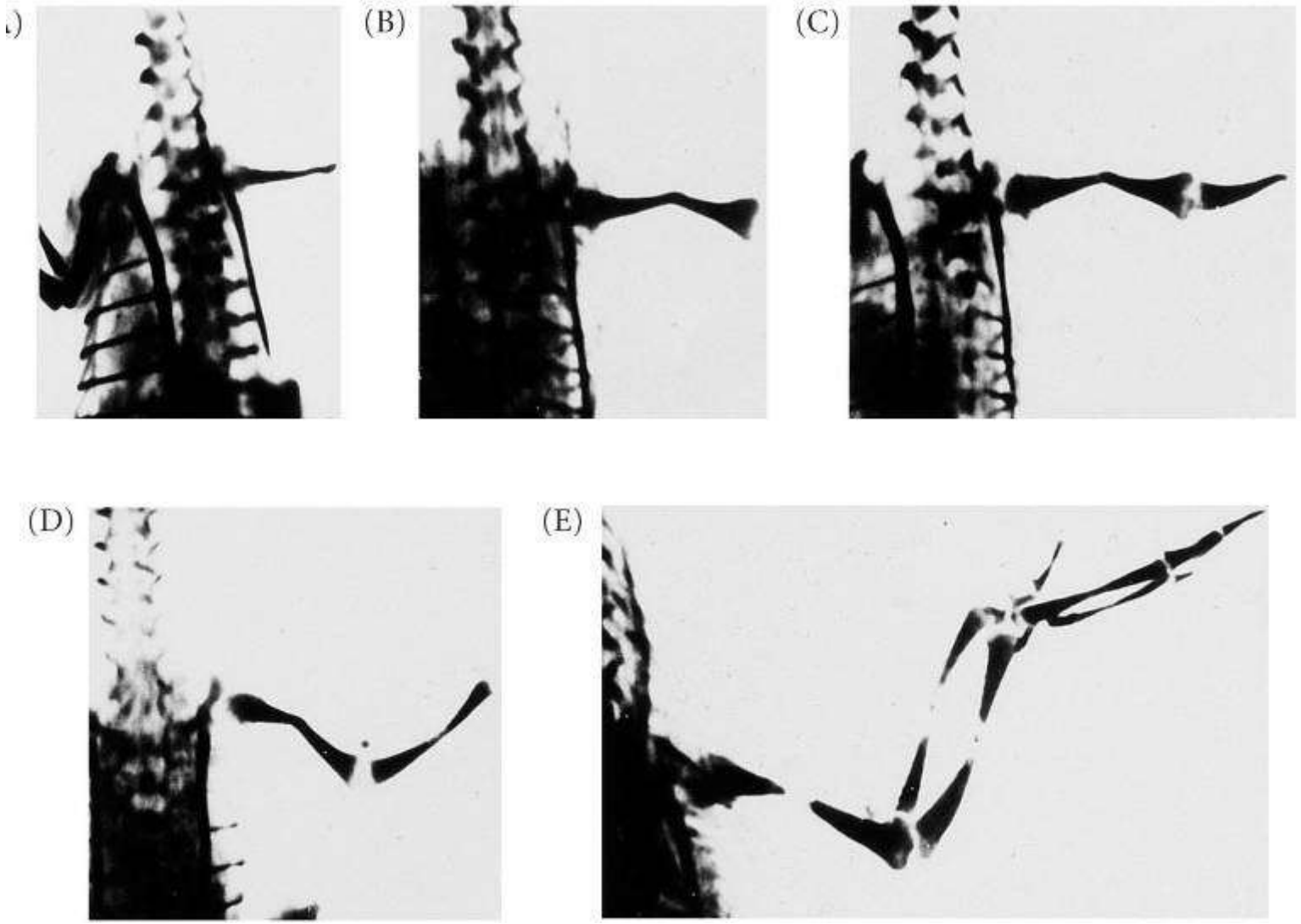
- Interacting with the proteins specifying the anterior-posterior and dorsal-ventral axes so that each cell is given instructions on how to differentiate.

THE PROGRESS ZONE (PZ)



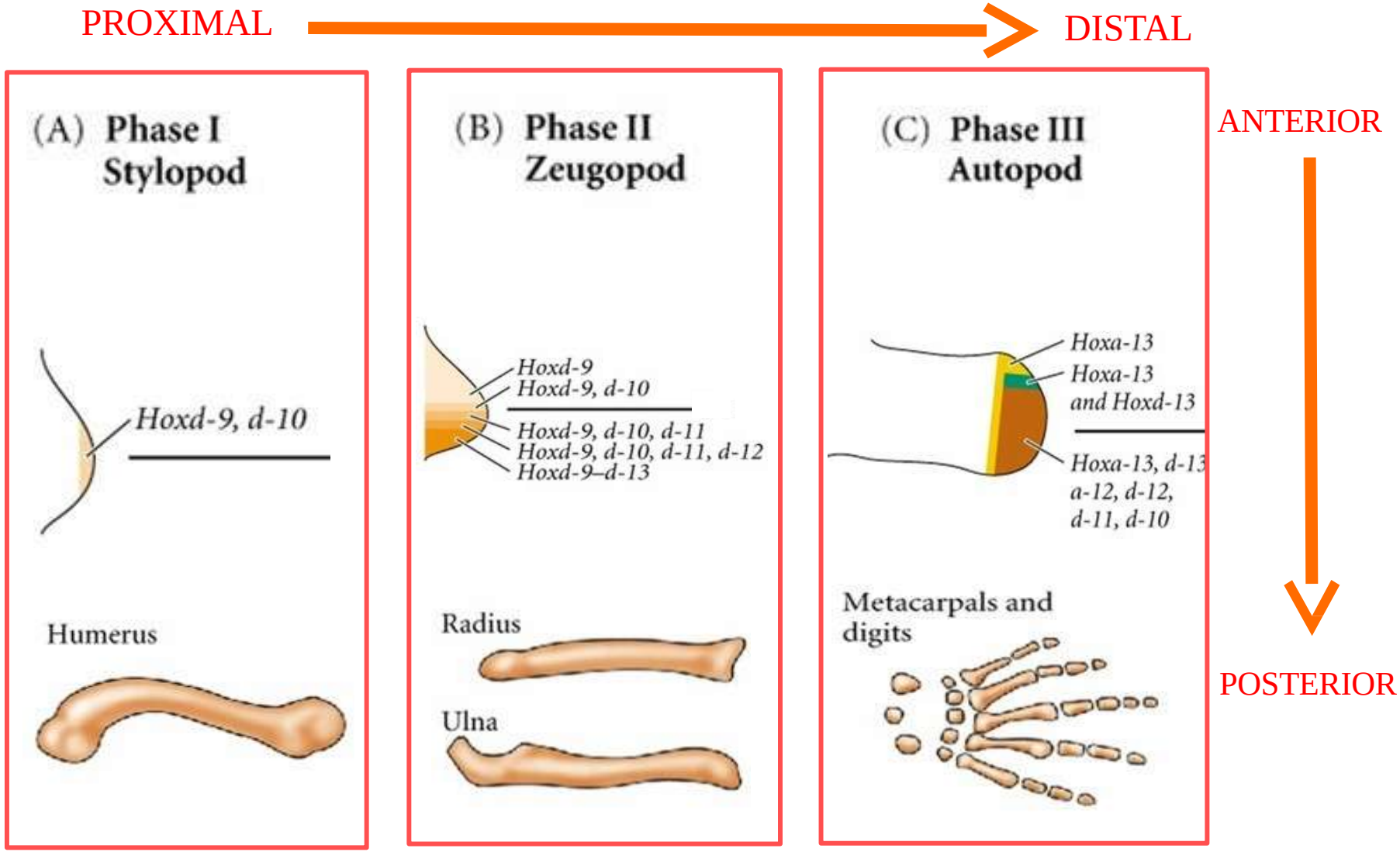
Molecules that are secreted from AER (FGF8) keep the PZ mesenchyme cells dividing.

OUTGROWTH

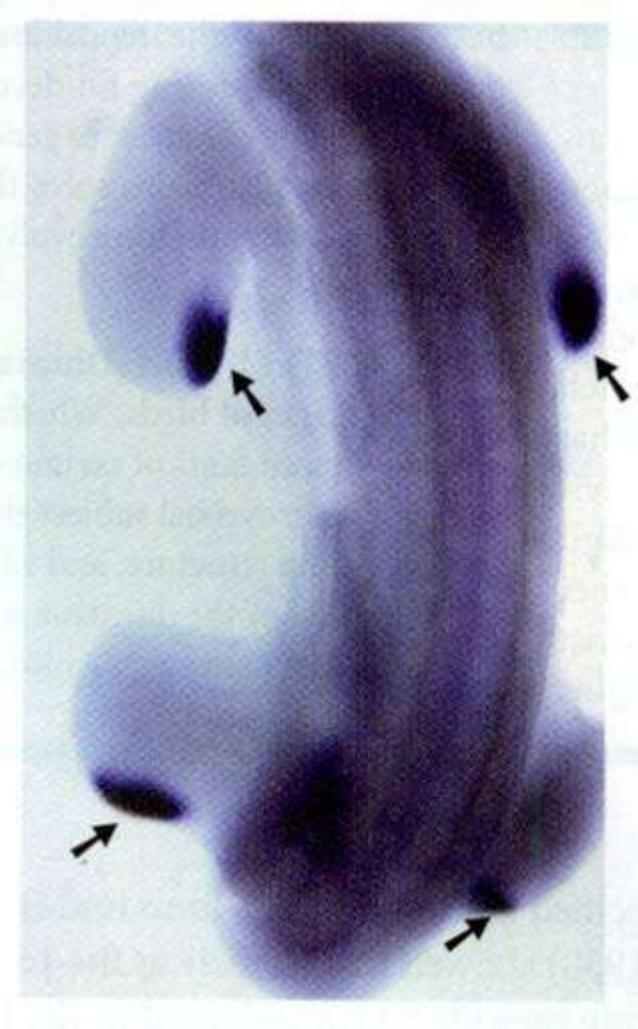


Removal of AER at various times prematurely truncates limb

SPECIFICATION OF THE PROXIMAL- DISTAL AXIS

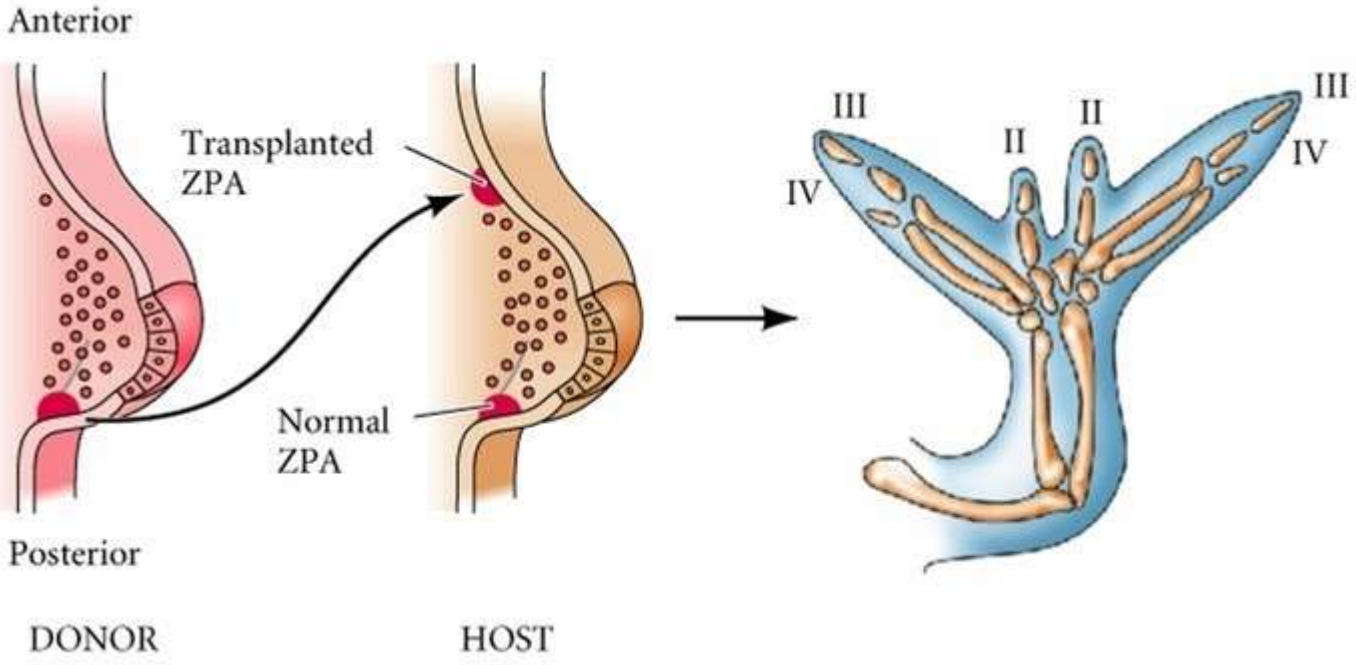


POSTERIOR LIMB AXIS: SONIC HEDGEHOG PROTEIN



Zone of Polarizing Activity (ZPA) --- block of mesodermal tissue near the posterior junction of the young limb bud.

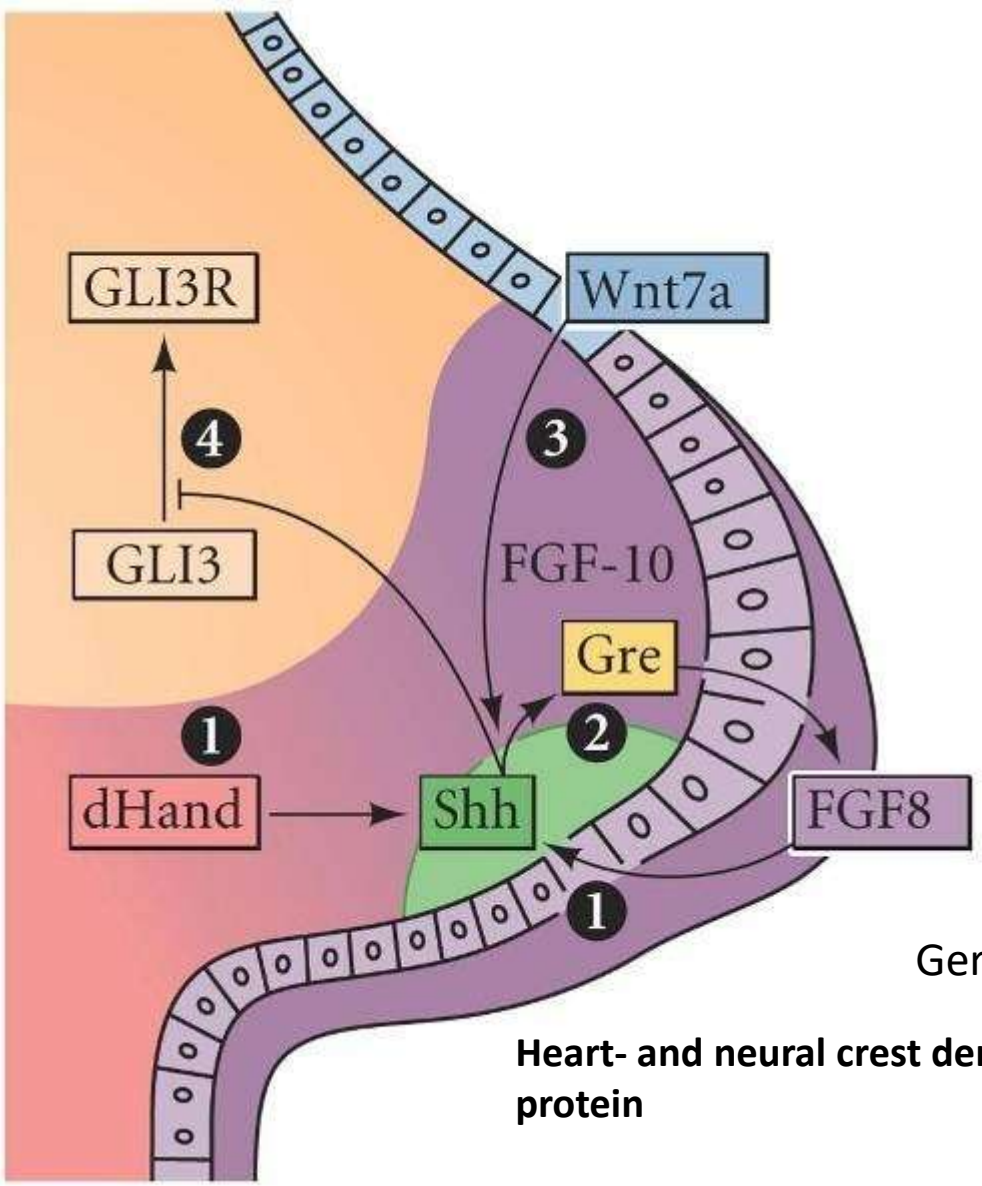
ZPA



SHH (Sonic hedgehog) works by initiating and sustaining a cascade of other proteins, such as BMP2 and BMP7. A gradient of BMPs expressed from ZPA specify the digits.

However, transplantation of ZPA to the anterior portion of limb bud leads to the formation of mirror-image digit pattern.

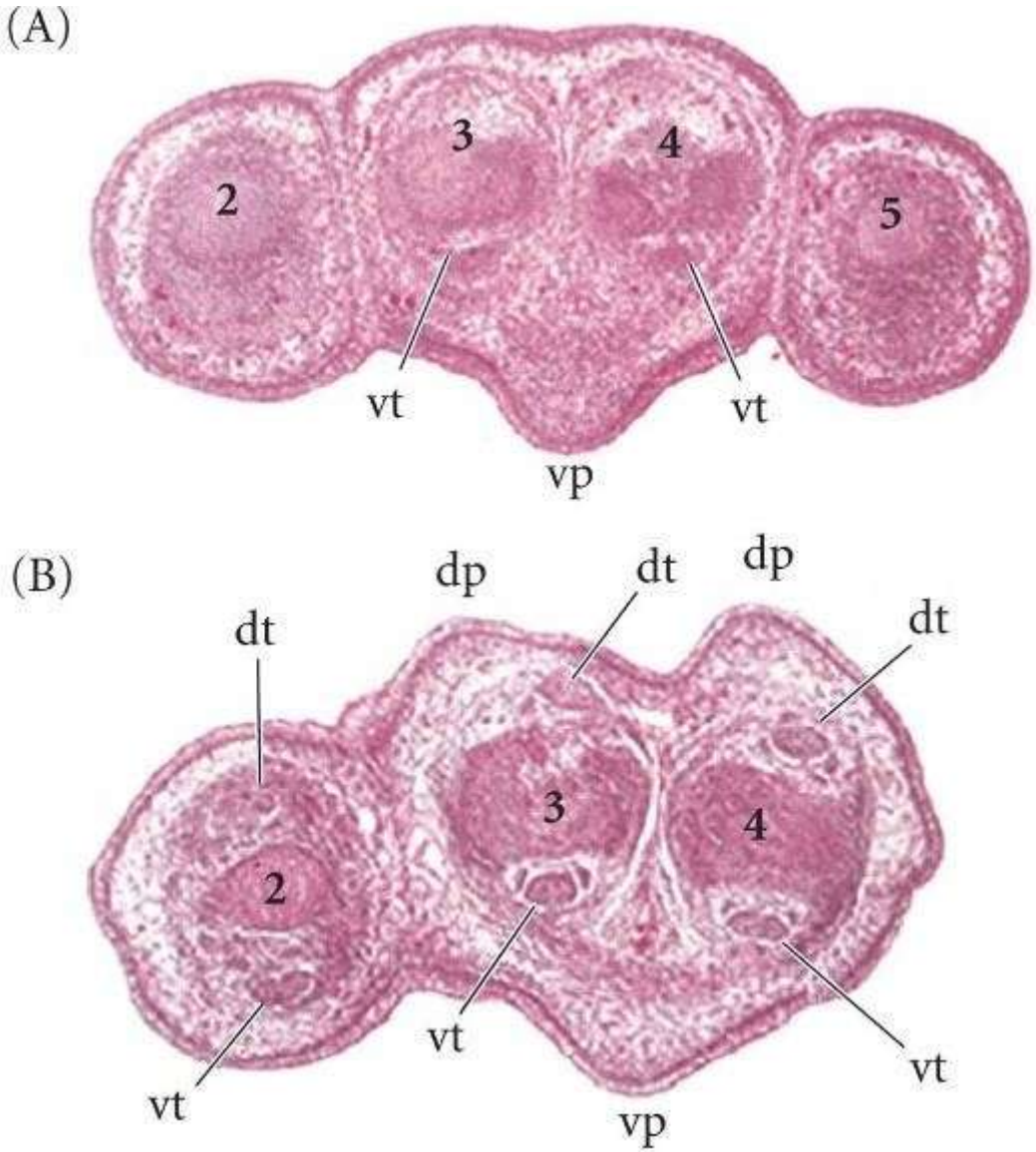
Molecular Interactions in Limb Bud



Germline

Heart- and neural crest derivatives-expressed protein

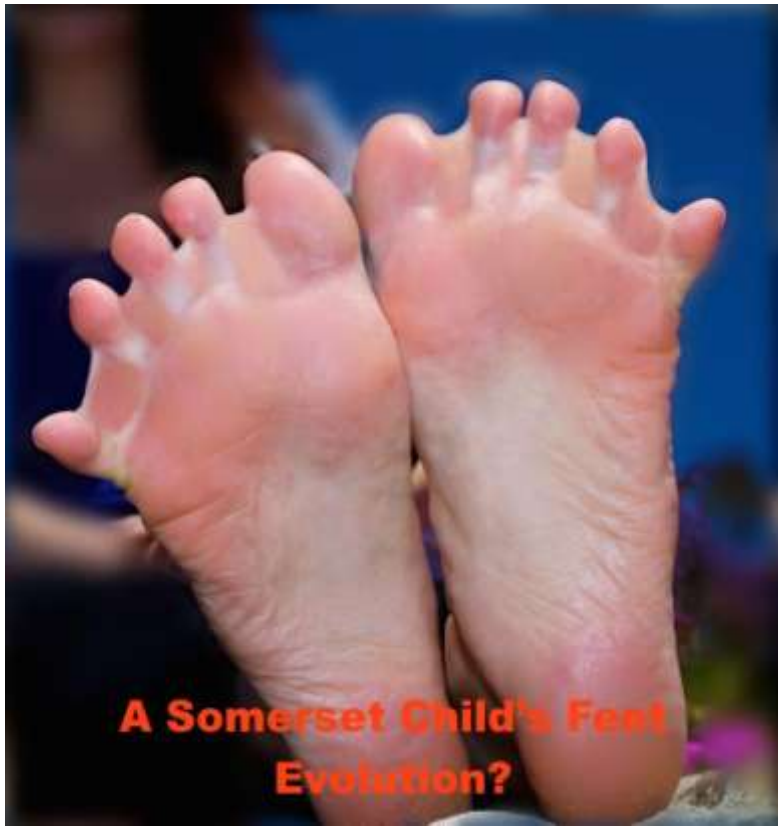
GENERATION OF THE DORSAL- VENTRAL AXIS: WNT7A



- *Wnt7a* gene is expressed only in dorsal portion of limb bud.

- It induces activation of the *Lmx1* gene in dorsal mesenchyme and this gene encodes a transcription factor that appears to be essential for specifying dorsal fates in the limb.

CELL DEATH AND THE FORMATION OF DIGITS AND JOINTS



•Signal for apoptosis in the autopod region is provided by the Bone Morphogenetic Proteins.

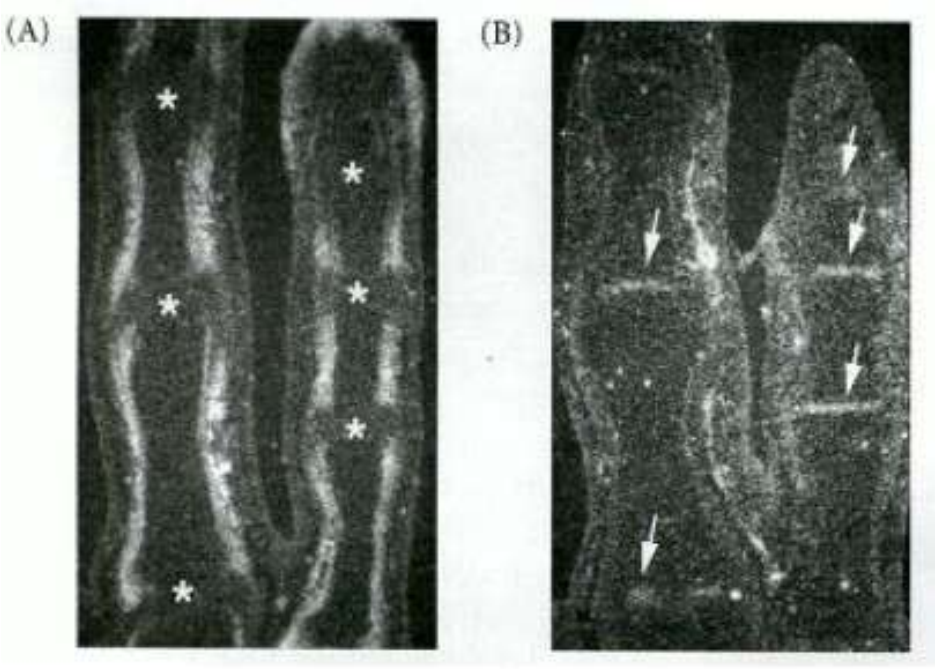
• BMP2, BMP4 and BMP7 are each expressed in the interdigital mesenchym e

•Nogging proteins supressed BMPs

•Noggin proteins is synthesized in developing digits.and perichondrial cells surrounding them.

•However Blocking BMP signaling between digits prevents interdigital apoptosis.

FORMING JOINTS



•Bone Morphogenetic Proteins are responsible in forming joints.

•BMP7 is synthesized in the perichondrial cells sorrounding the condensing chondrocytes and promotes cartilage formation.

•Other BMPs, BPM2 and GDF5, are expressed at the regions between the bones, where joints will form.

SUMMARY: THE DEVELOPMENT OF TETRAPOD LIMB

- The places where limbs emerge from the body axis depend upon Hox gene expression.

The specification of the limb field into a hindlimb or forelimb bud is determined by *Tbx4* and *Tbx5* expression.

The proximal-distal axis of the developing limb is determined by the induction of the ectoderm at the dorsal-ventral boundary to form the apical ectodermal ridge (AER). This induction is caused by an FGF10. The AER secretes FGF8, which keeps the underlying mesenchyme proliferative and undifferentiated. This mesenchyme is called progress zone

SUMMARY: THE DEVELOPMENT OF TETRAPOD LIMB

- As the limb grow outward, the stylopod forms first, then the zeugopod, and the autopod is fomed last. Each of these phases involves the expression of Hox genes, and the formation of the autopod involves a reversal of Hox gene expression that distinguishes fish fins from tetrapod limbs

- The anterior-posterior axis is defined by the expression of Sonic hedgehog in the posterior mesoderm of the limb bud. This region is called the zone of polarizing activity (ZPA). If the ZPA or Sonic hedgehog-secreting cells or beads are placed in the anterior margin, they establish a second, mirror-image pattern of Hox gene expression and a corresponding mirror-image duplication of the digits

SUMMARY: THE DEVELOPMENT OF TETRAPOD LIMB

- The ZPA is established by the interaction of FGF8 from the AER and mesenchyme made competent to express Sonic hedgehog by its expression of particular Hox genes. Sonic hedgehog acts, probably in an indirect manner, to change the expression of Hox genes in the limb bud

SUMMARY: THE DEVELOPMENT OF TETRAPOD LIMB

- ′The dorsal-ventral axis is formed, in part, by the expression of Wnt7a in the dorsal portion of the limb ectoderm. Wnt7a also maintains the expression of Sonic hedgehog in the ZPA and FGF4 in the posterior AER. FGF4 and Sonic hedgehog reciprocally maintain each other's expression.
- ′Cell death in the limb is necessary for the formation of digits and joints. It is mediated by BMPs. The effects of BMPs can be regulated by the Noggin protein and the BMPs can be involved both in inducing apoptosis and in differentiating the mesenchymal cells into cartilage