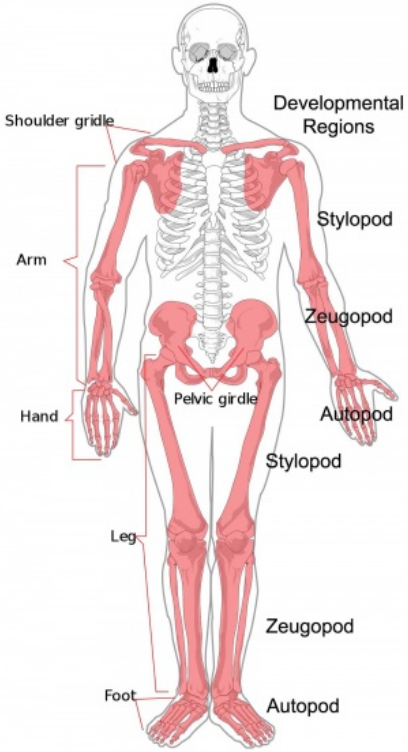
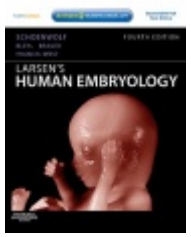
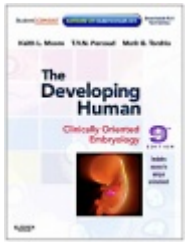
Limb Development





Resources:
http://php.med.unsw.edu.au/embryology/
Larsen's Human Embryology
The Developing Human: Clinically Oriented Embryology



Dr Annemiek Beverdam – School of Medical Sciences, UNSW
Wallace Wurth Building Room 234 – A.Beverdam@unsw.edu.au

Lecture overview
Limb Development

Limb Anatomy

Embryonic Tissues Contributing to Limb Development

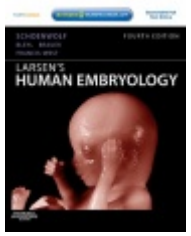
Early Limb Development and Limb Axes

Initiation

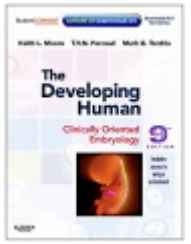
Outgrowth and Patterning

Limb Tissue Differentiation and Ossification

Limb rotation

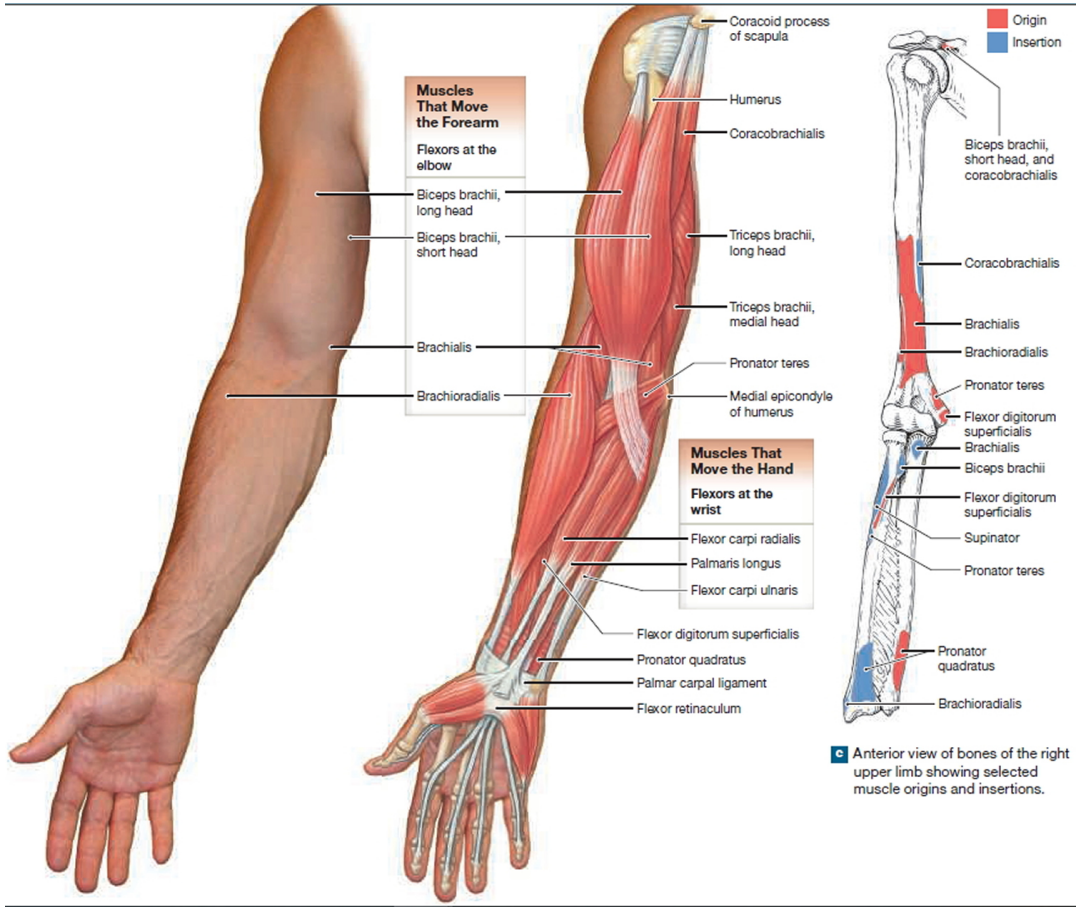


Resources:
http://php.med.unsw.edu.au/embryology/
Larsen's Human Embryology
The Developing Human: Clinically Oriented Embryology



Dr Annemiek Beverdam – School of Medical Sciences, UNSW
Wallace Wurth Building Room 234 – A.Beverdam@unsw.edu.au

Limb Anatomy



Defined asymmetric anatomy

Skin
Muscles
Bones
Vasculature
Nerves

End product gastrulation:

Trilaminar embryo

Ectoderm *(Neural crest)*
brain, spinal cord, eyes, *peripheral nervous system*
epidermis of skin and associated structures,
melanocytes, cranial connective tissues (dermis)

Mesoderm
musculo-skeletal system, limbs,
connective tissue of skin, organs and cranium,
urogenital system, heart, blood cells

Endoderm
epithelial linings of gastrointestinal, liver, pancreas,
thyroid and respiratory tracts

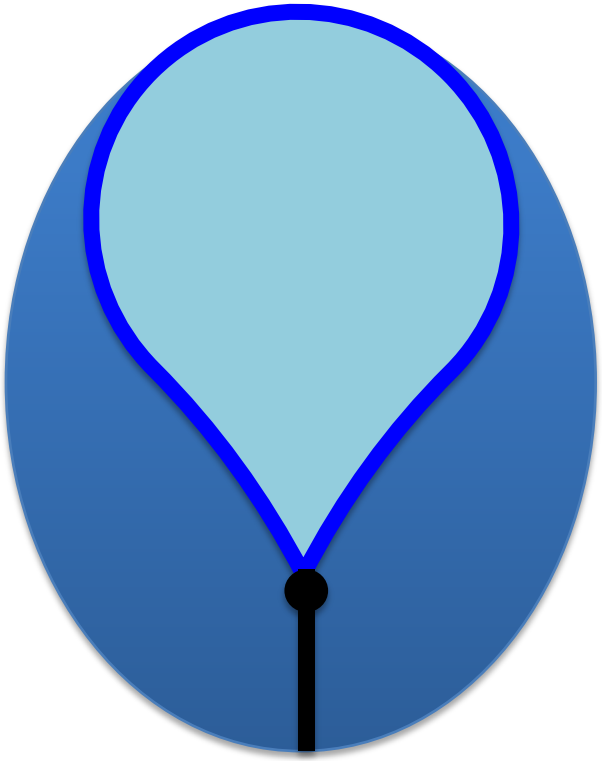
Embryonic tissues contributing to limb development

Lateral ectoderm
Lateral Plate Mesoderm
Paraxial Mesoderm

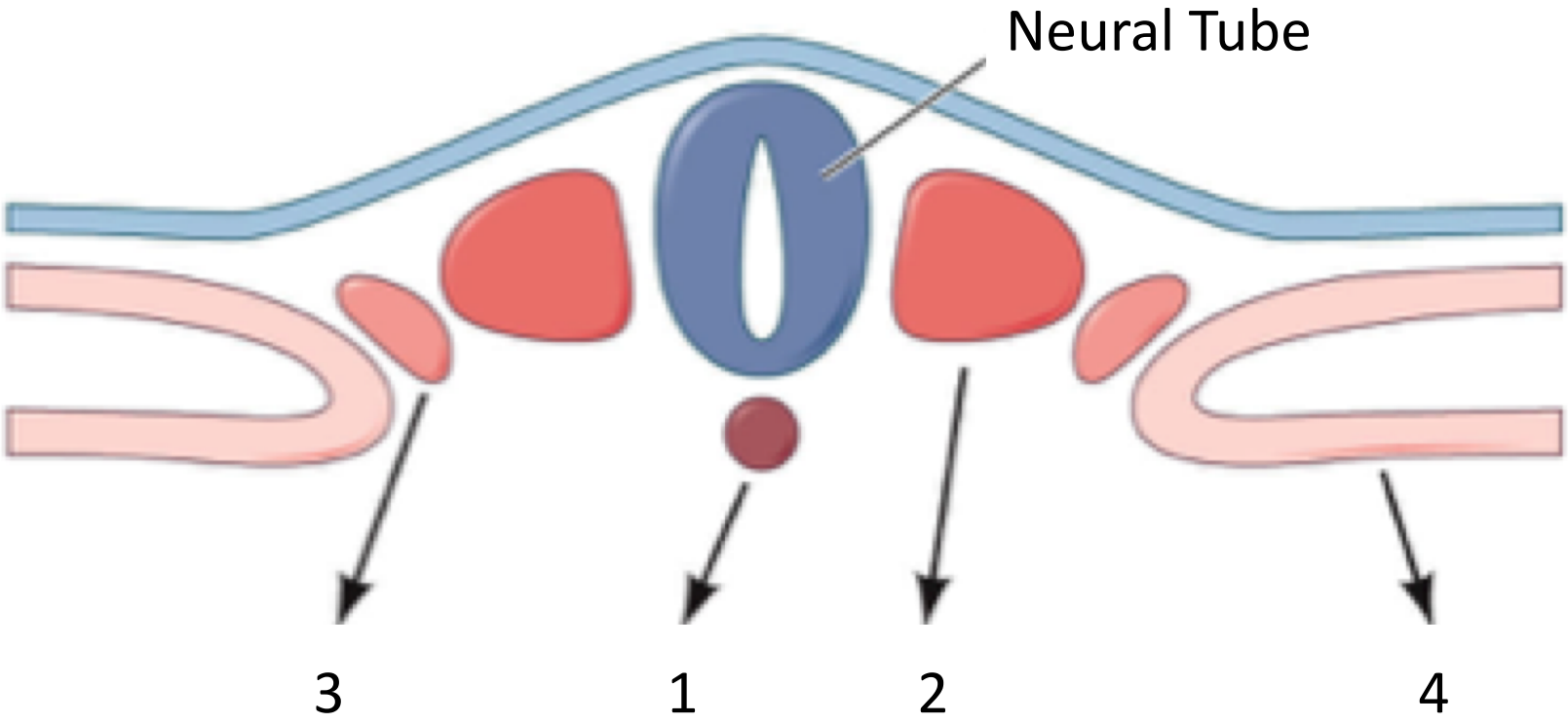
Week 4 embryo

Embryonic tissues contributing to limb development

Lateral Ectoderm contributes to the epidermis covering the limbs



Embryonic tissues contributing to limb development



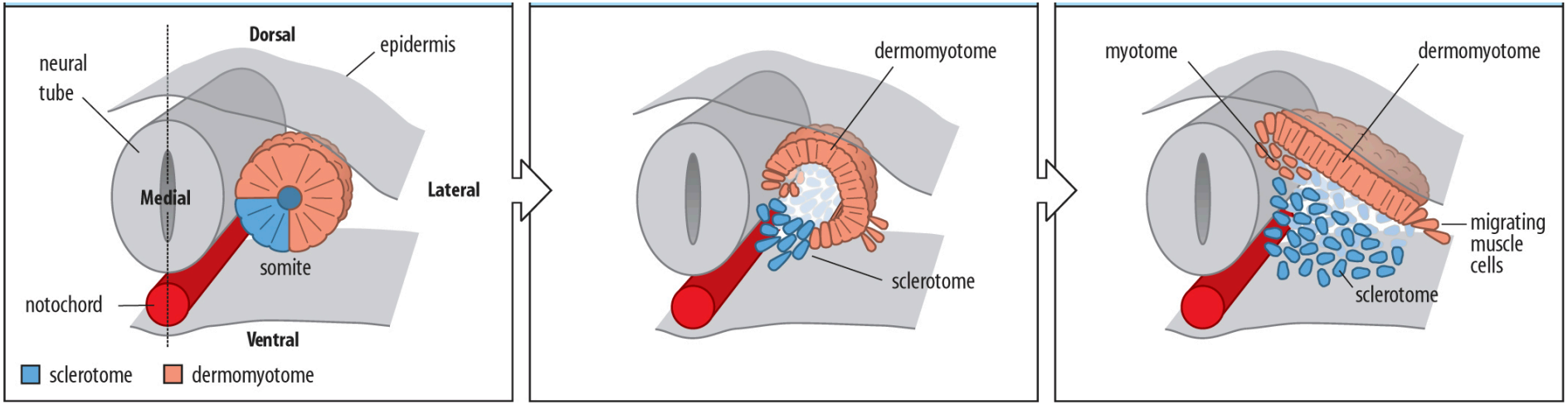
1: notochord

2: paraxial mesoderm: contributes limb musculature

3: intermediate mesoderm

4: lateral plate mesoderm: contributes to limb dermis, connective tissues, skeleton, vasculature

Embryonic tissues contributing to limb development



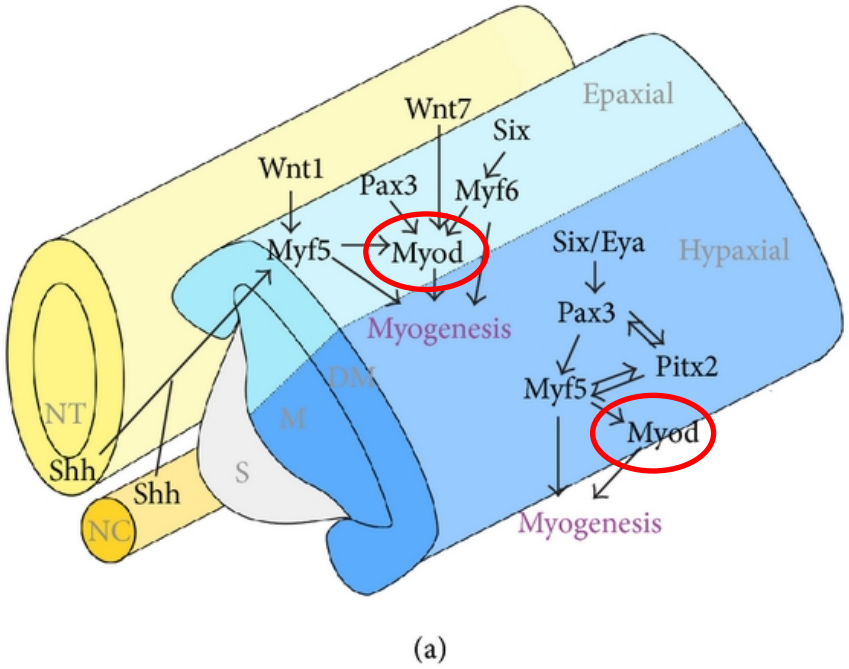
Somites develop into:

- Sclerotome: mesenchymal cells (vertebral body and intervertebral disk)
- Dermomyotome: columnar epithelium

Dermomyotome develops into:

- Dermatome: dermis of the trunk and limbs
- **Myotome:** trunk and **limb musculature**

Embryonic tissues contributing to limb development

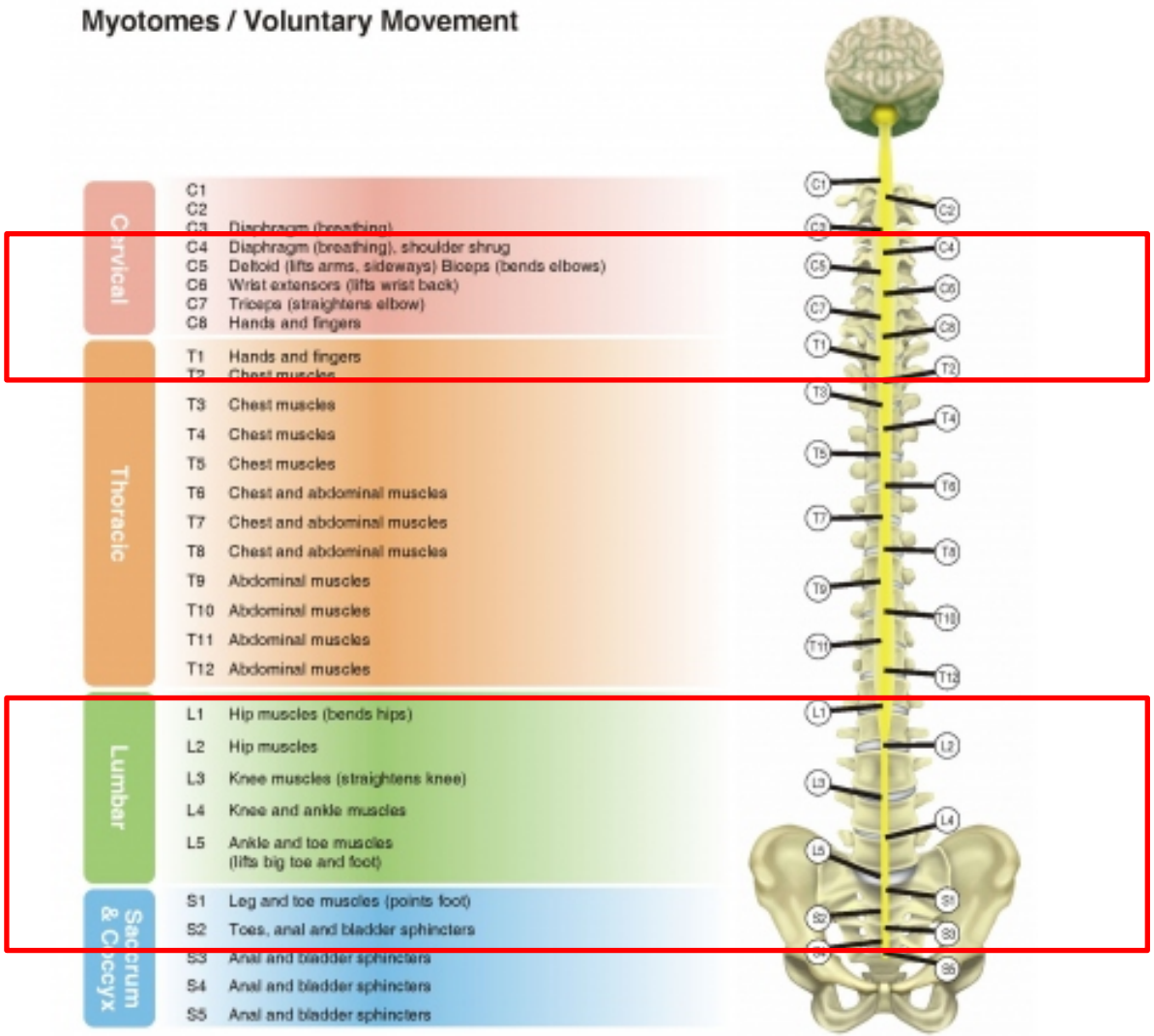


Epaxial myotome: epimere: erector spinae (muscles of the deep back)

Hypaxial myotome: hypomere: 3 primary muscle layers (body wall, **limbs**)

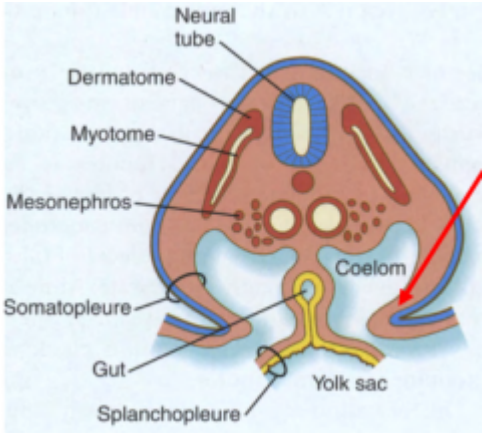
MyoD initiates myogenesis

Embryonic tissues contributing to limb development

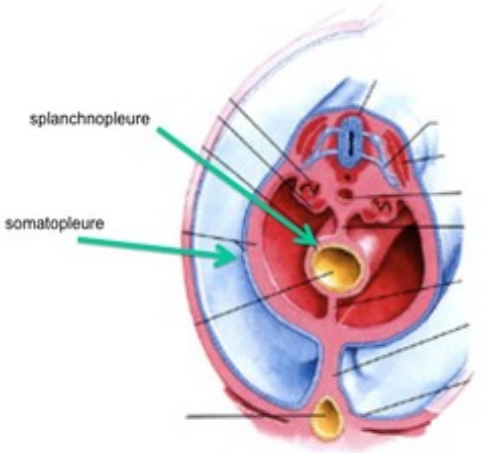


Embryonic tissues contributing to limb development

Somatic/parietal mesoderm: **somatopleure**
- Closest to ectoderm
- Gives rise to:
  - Connective tissue and lining of the body wall
  - Bones, ligaments and dermis of the limbs



Splanchnic/visceral mesoderm: **splanchnopleure**
- Closest to endoderm
- Gives rise to:
  - Cardiac mesoderm (prechordal splanchnic mesoderm)
  - Blood vessels
  - Smooth muscles of the gut



Lecture overview
Limb Development

Limb Anatomy

Embryonic Tissues Contributing to Limb Development

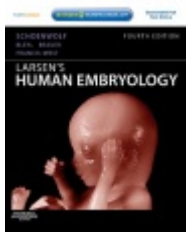
Early Limb Development and Limb Axes

Initiation

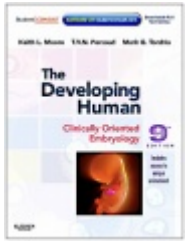
Outgrowth and Patterning

Limb Tissue Differentiation and Ossification

Limb rotation



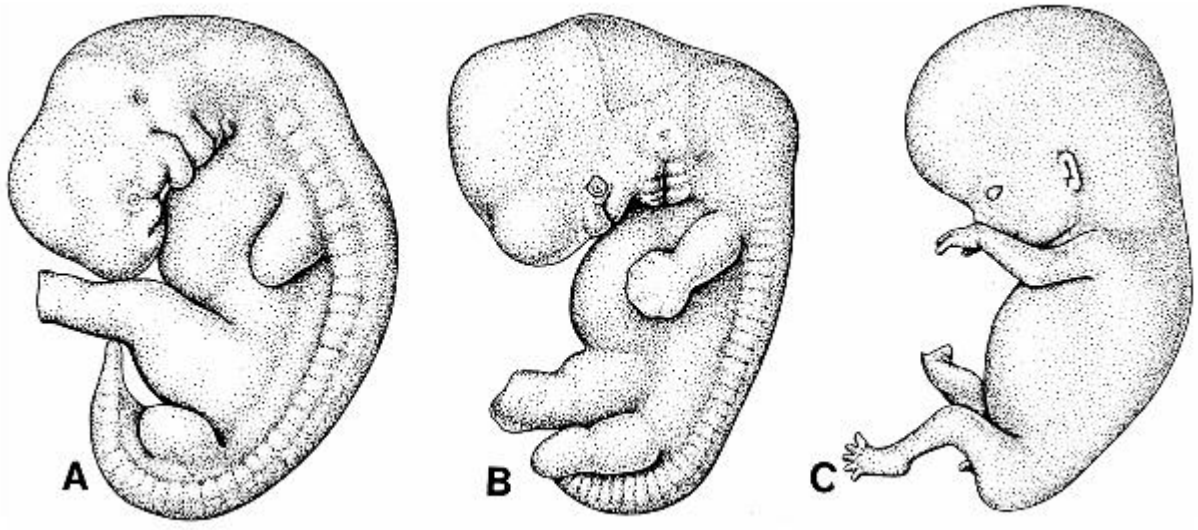
Resources:
http://php.med.unsw.edu.au/embryology/
Larsen's Human Embryology
The Developing Human: Clinically Oriented Embryology



Dr Annemiek Beverdam – School of Medical Sciences, UNSW
Wallace Wurth Building Room 234 – A.Beverdam@unsw.edu.au

Early Limb Development

From week 4
Forelimb development ahead of hindlimb development
Proliferation of lateral plate mesoderm
Limb bud: outer ectodermal cap, inner mesodermal core
Apical Ectodermal Ridge



1: Initiation
2: Outgrowth and Patterning
3: Differentiation and ossification

Early Limb Development

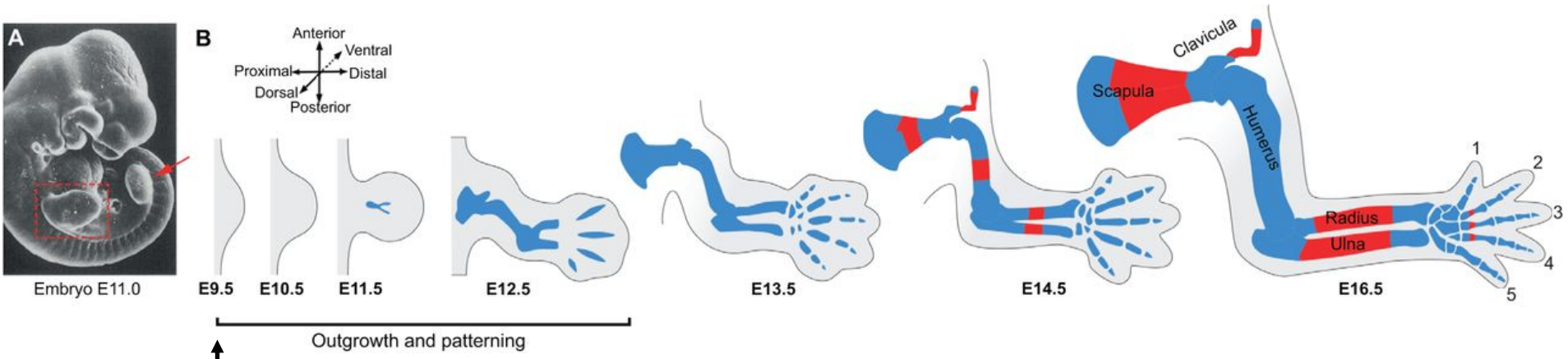
From week 4

Fore limb development ahead of hind limb development

Proliferation of lateral plate mesoderm

Limb bud: outer ectodermally-derived epithelial cap, inner mesodermally-derived core

Apical Ectodermal Ridge



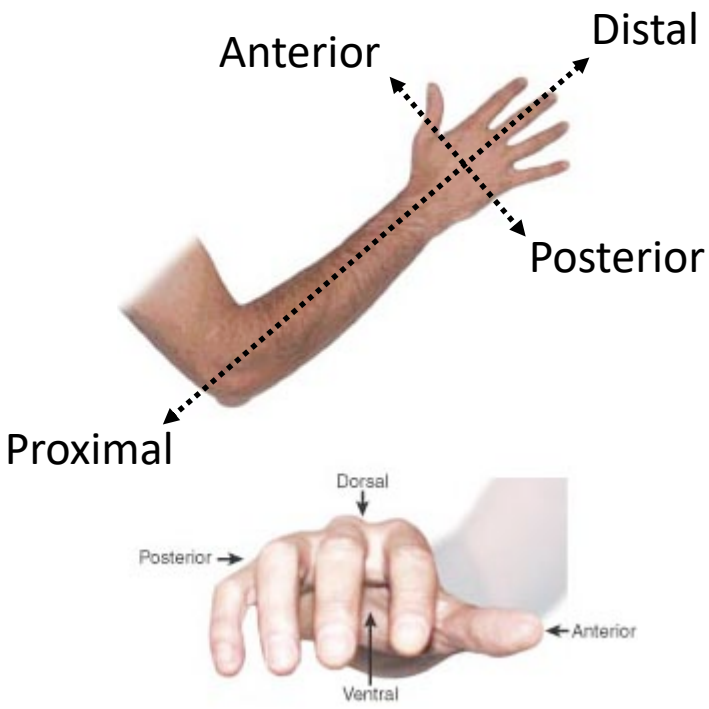
2: Outgrowth and patterning

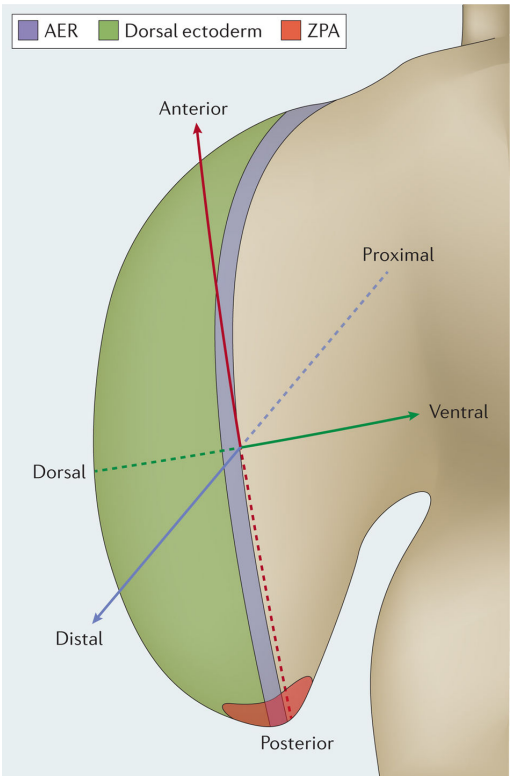
3: Differentiation, Ossification

1: Initiation

Limb Development
Limb axes

Proximodistal axis
Anteroposterior axis
Dorsoventral axis





Lecture overview
Limb Development

Limb Anatomy

Embryonic Tissues Contributing to Limb Development

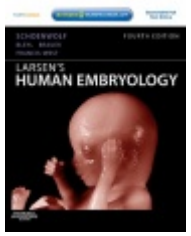
Early Limb Development and Limb Axes

Initiation

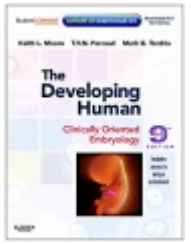
Outgrowth and Patterning

Limb Tissue Differentiation and Ossification

Limb rotation

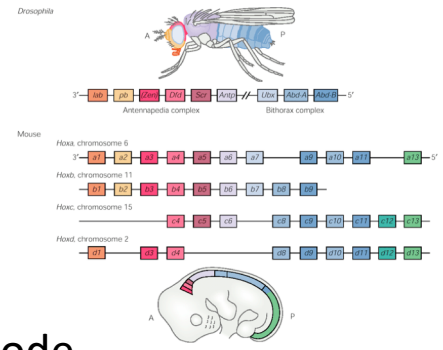


Resources:
http://php.med.unsw.edu.au/embryology/
Larsen's Human Embryology
The Developing Human: Clinically Oriented Embryology



Dr Annemiek Beverdam – School of Medical Sciences, UNSW
Wallace Wurth Building Room 234 – A.Beverdam@unsw.edu.au

Limb Development
Initiation



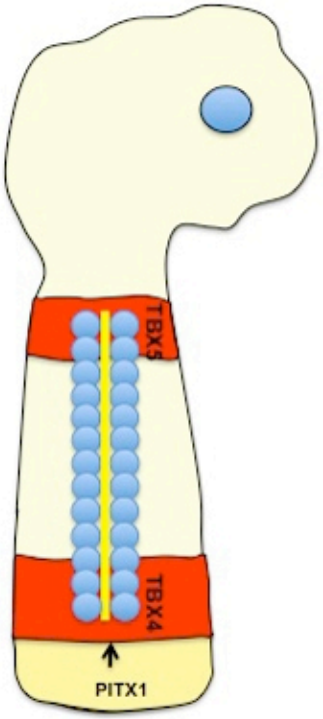
Lateral Plate Mesoderm

Anterior and posterior limb fields defined by Hox code

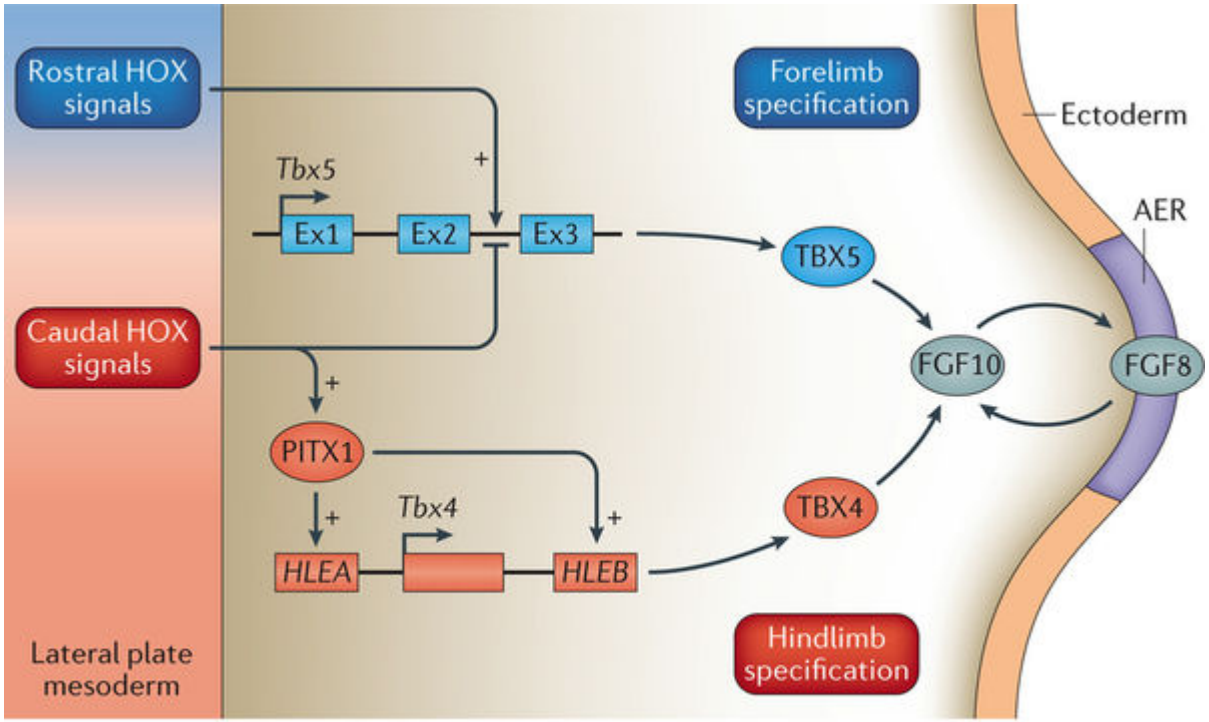
Hox 6/10 codes -> Retinoic Acid -> Tbx4/5

Tbx4/5 -> FGF10 ⇔ FGF8

A



Chick wing St. 14
Mouse limb E. 8.0



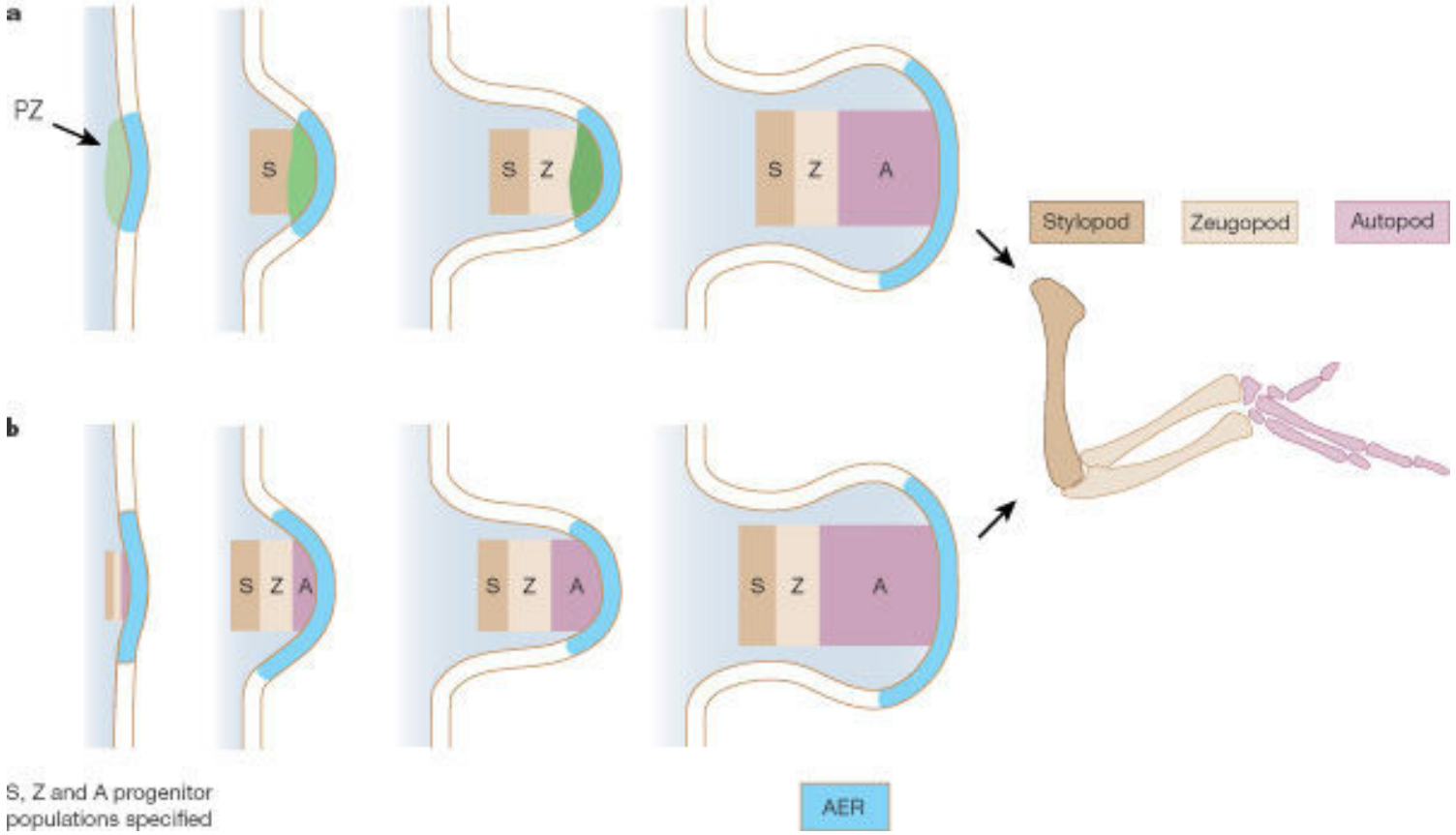
Nature Reviews | Genetics

Limb Development
Proximo-distal outgrowth

FGF10 (mesenchyme) ⇔ FGF8 (AER)
Stylopod, zeugopod, autopod

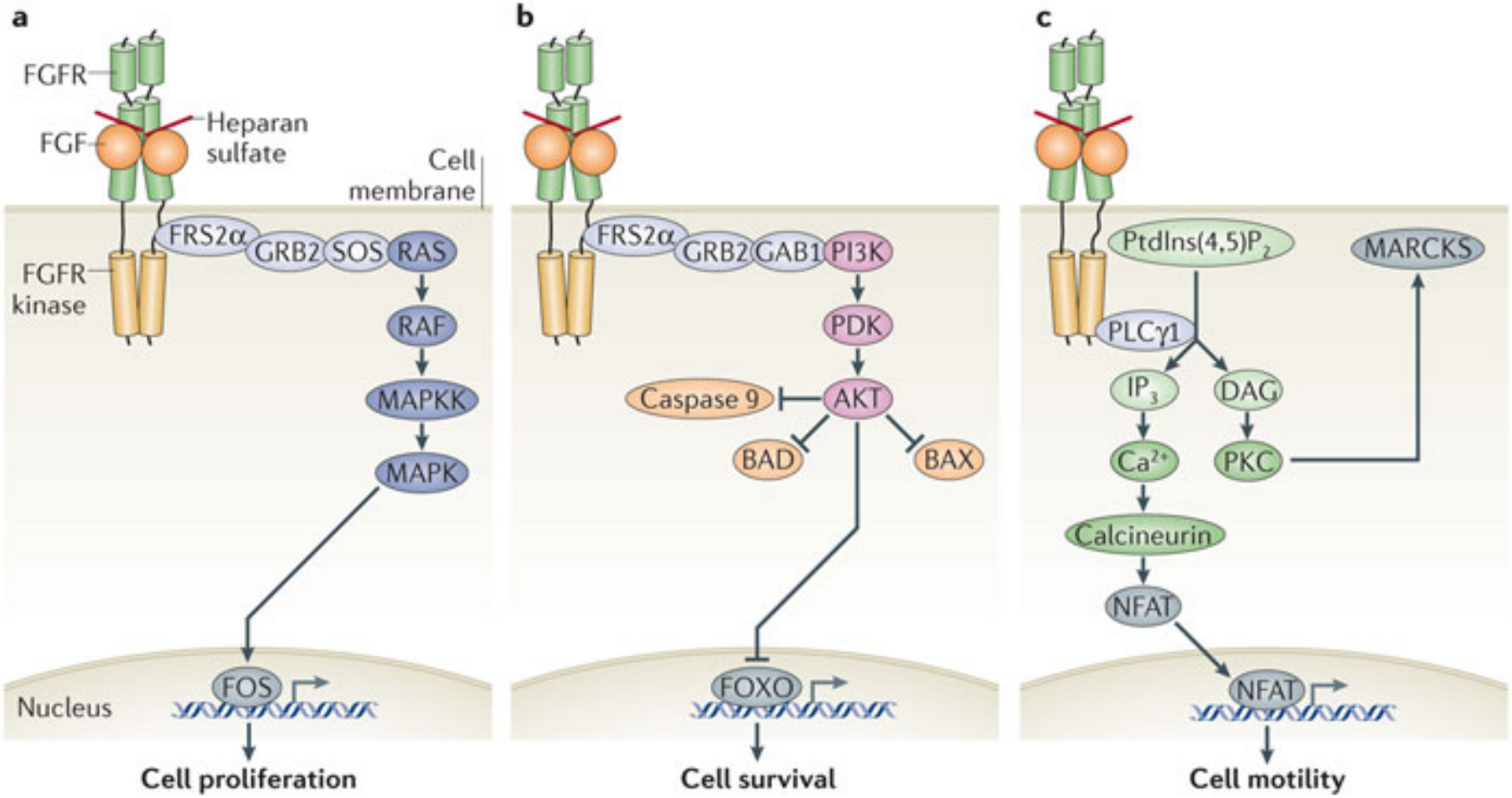
Progress Zone Model

Early Specification Model



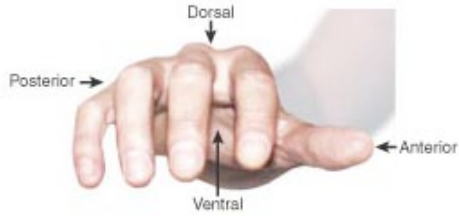
Limb Development
Proximo-distal outgrowth

FGF signalling

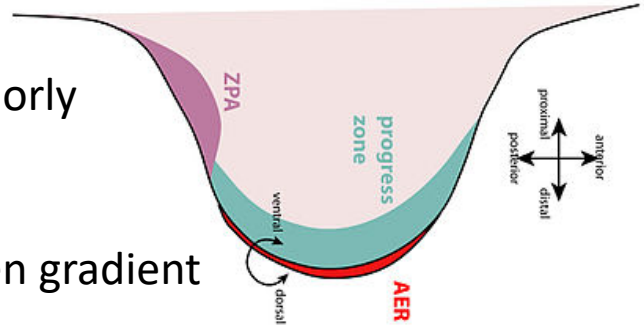


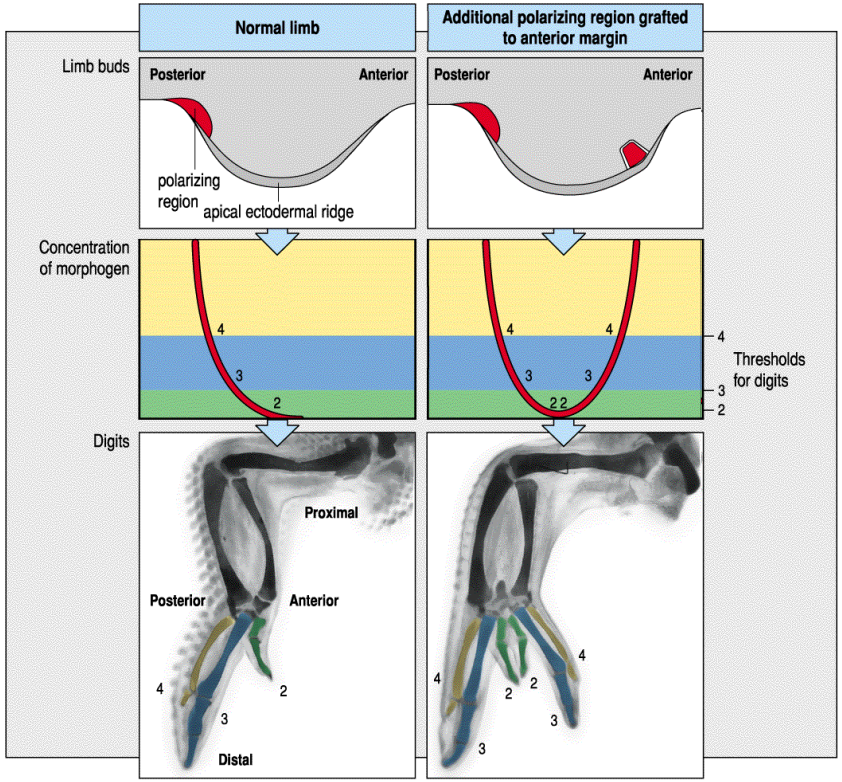
Nature Reviews | Molecular Cell Biology

Limb Development
Antero-posterior patterning

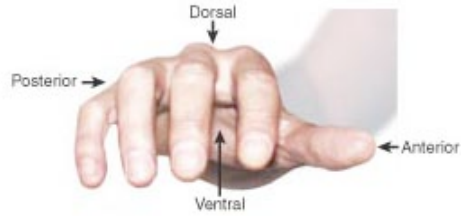


Thumb anteriorly, pinky posteriorly
ZPA: Zone of polarizing activity
ZPA produces morphogen
Posterior to anterior morphogen gradient

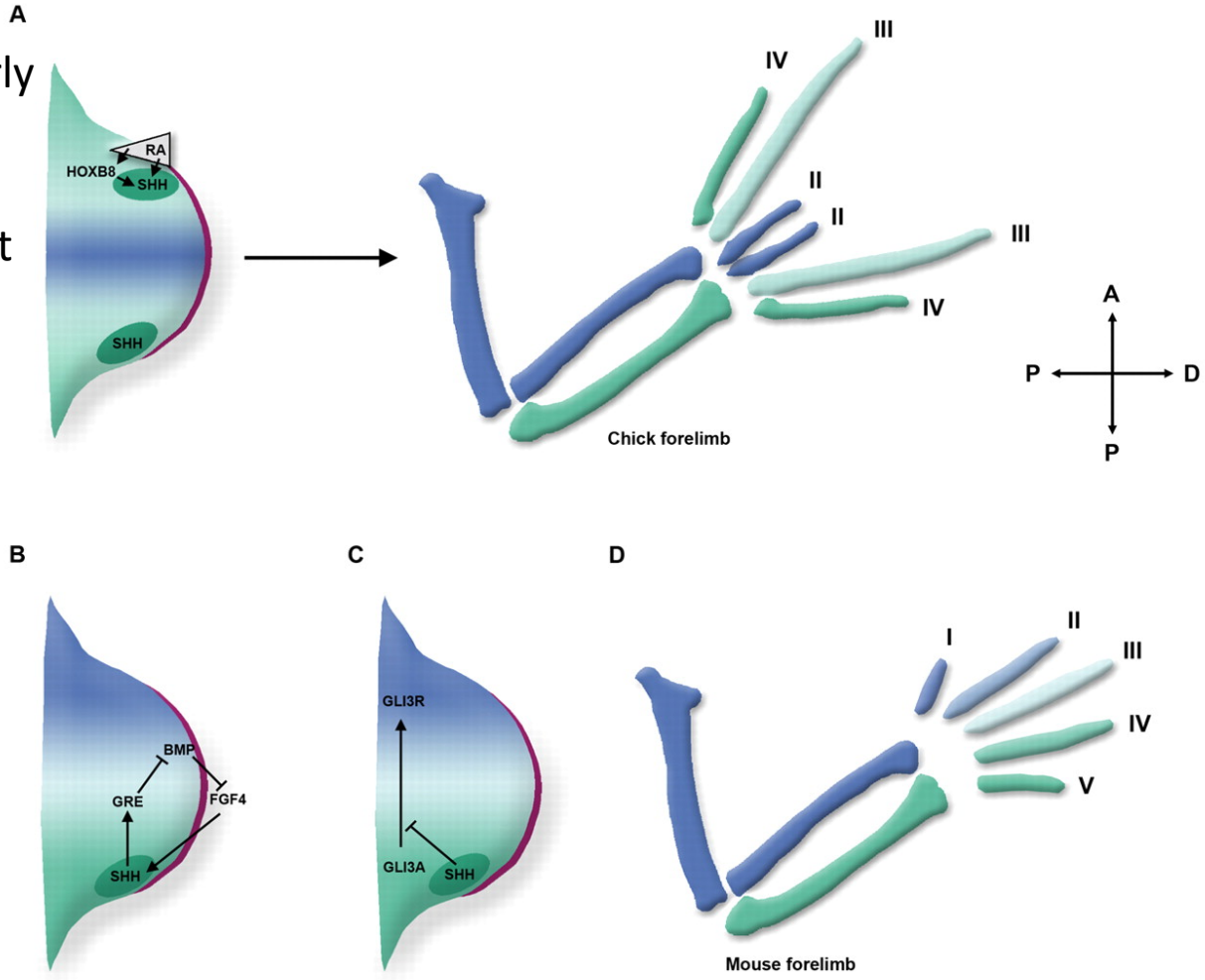




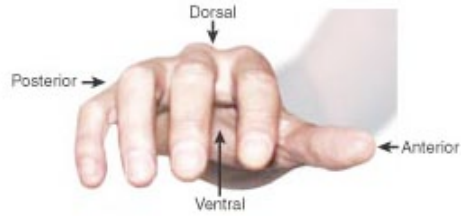
Limb Development
Antero-posterior patterning



Thumb anteriorly, pinky posteriorly
ZPA: Zone of polarizing activity
ZPA produces SHH
Posterior to anterior SHH gradient

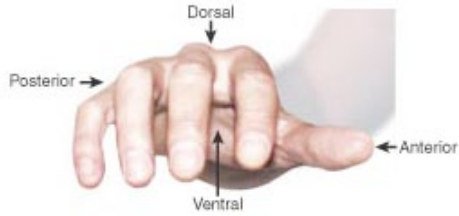


Limb Development
Antero-posterior patterning

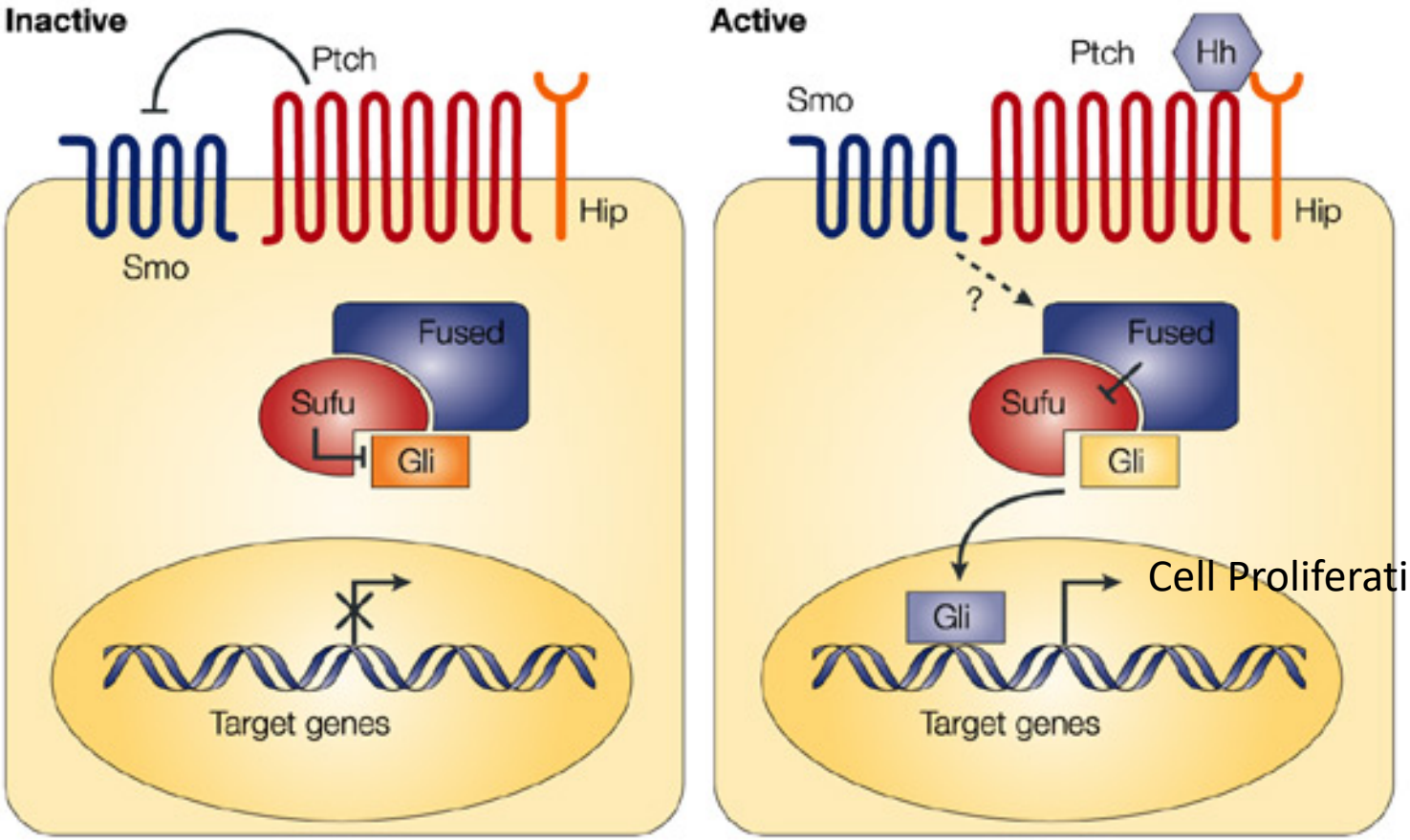


Polydactyly is a caused by an incorrect establishment of the anteroposterior axis

Limb Development
Antero-posterior patterning

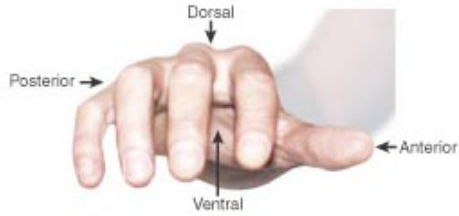


SHH signalling



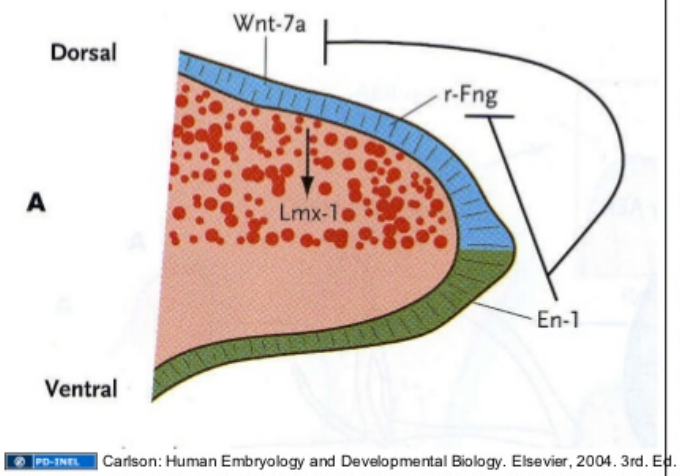
Cell Proliferation

Limb Development
Dorso-ventral patterning

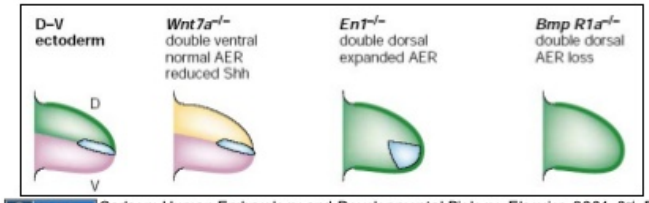


Muscles, tendons dorsally; palms, soles ventrally
Wnt7a (dorsal epithelium) -> Lmx1 (dorsal mesenchyme)
En1 (ventral epithelium) -| Wnt7a

Molecular control of dorsoventral (DV) patterning:



PD-INEL Carlson: Human Embryology and Developmental Biology. Elsevier, 2004. 3rd. Ed.



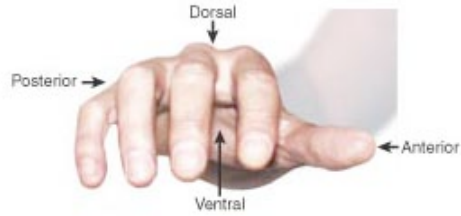
PD-INEL Carlson: Human Embryology and Developmental Biology. Elsevier, 2004. 3rd. Ed.

Early mesenchymal signals establish *Wnt7a* expression in the dorsal ectoderm and *Lmx1* expression in the dorsal mesenchyme.
- Loss of expression results in bi-ventral limbs.

Expression of *Wnt7a* is restricted to the dorsal ectoderm because it is repressed in the ventral ectoderm by *En1*. *En1* expression is induced by BMP signaling.
-Loss of expression results in bi-dorsal limbs.

-Important Note: Disruption of DV patterning does NOT affect specification of the skeletal elements.

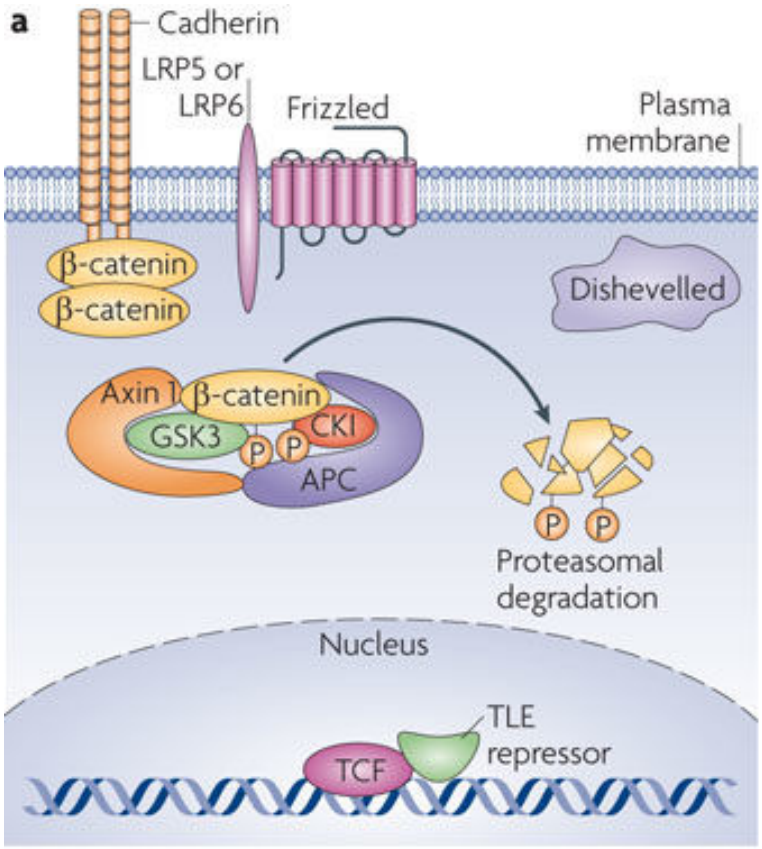
Limb Development
Dorso-ventral patterning

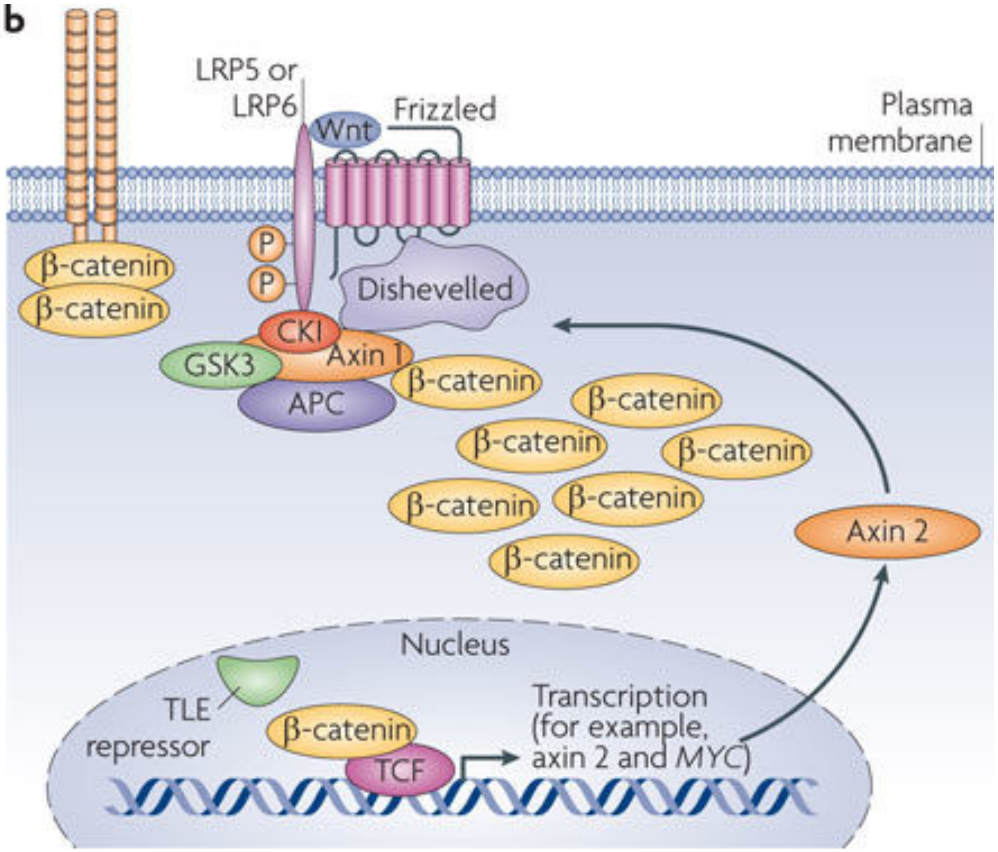


WNT signalling

Inactive

Active



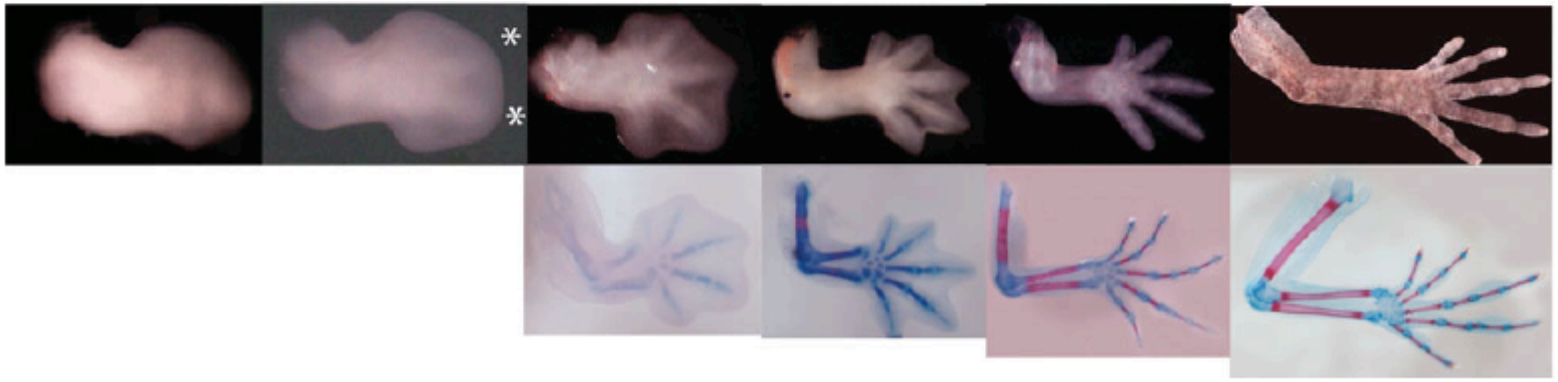


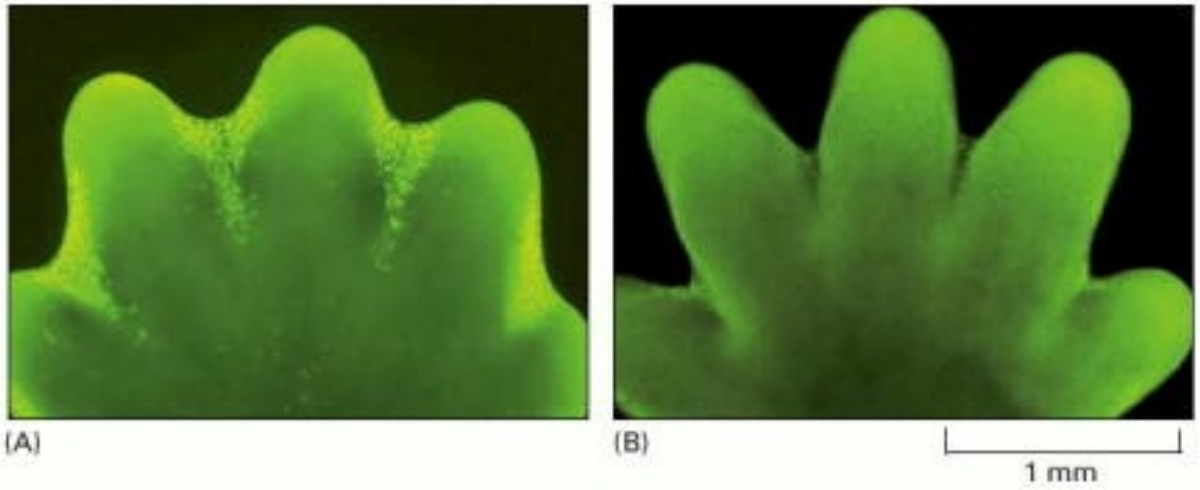
β-catenin acts as adherens junction molecule at the membrane or it is degraded

β-catenin acts as transcriptional activator in the nucleus

Limb Development
Autopod development

Interdigital apoptosis





Limb Development
Syndactyly: persistent webbing between the digits

Lecture overview
Limb Development

Limb Anatomy

Embryonic Tissues Contributing to Limb Development

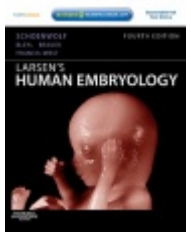
Early Limb Development and Limb Axes

Initiation

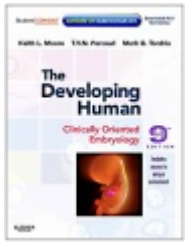
Outgrowth and Patterning

Limb Tissue Differentiation and Ossification

Limb rotation



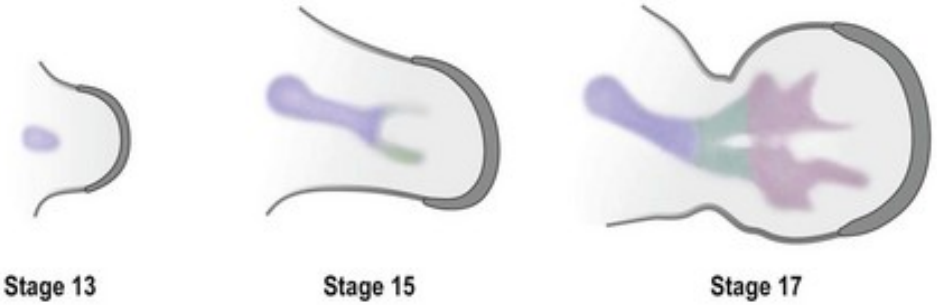
Resources:
http://php.med.unsw.edu.au/embryology/
Larsen's Human Embryology
The Developing Human: Clinically Oriented Embryology

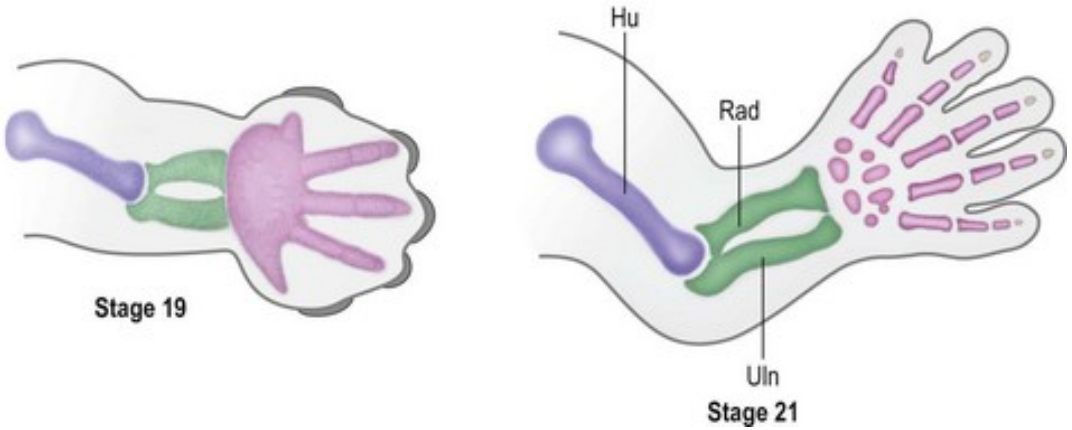


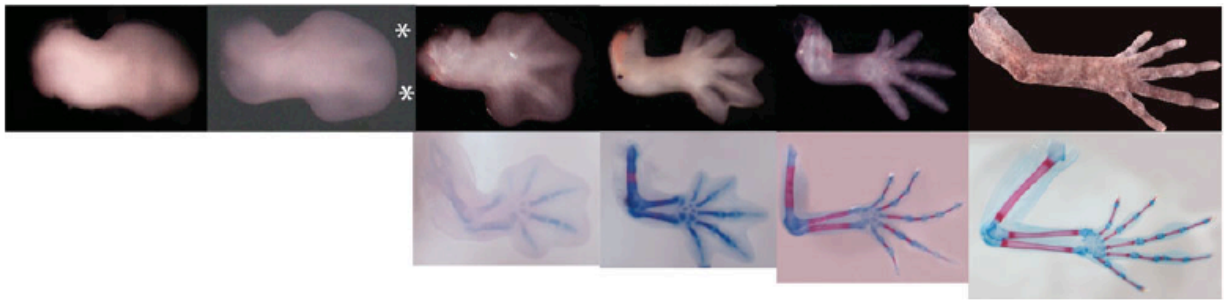
Dr Annemiek Beverdam – School of Medical Sciences, UNSW
Wallace Wurth Building Room 234 – A.Beverdam@unsw.edu.au

Limb Development
Limb Skeleton

Endochondral ossification of Lateral Plate Mesoderm

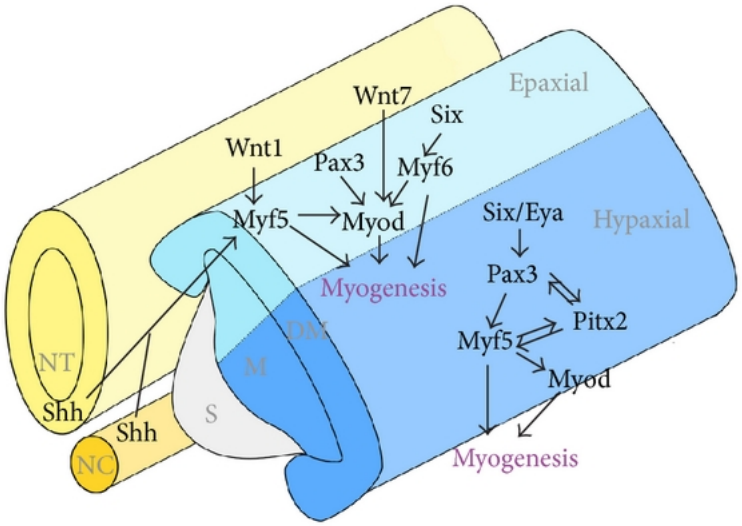




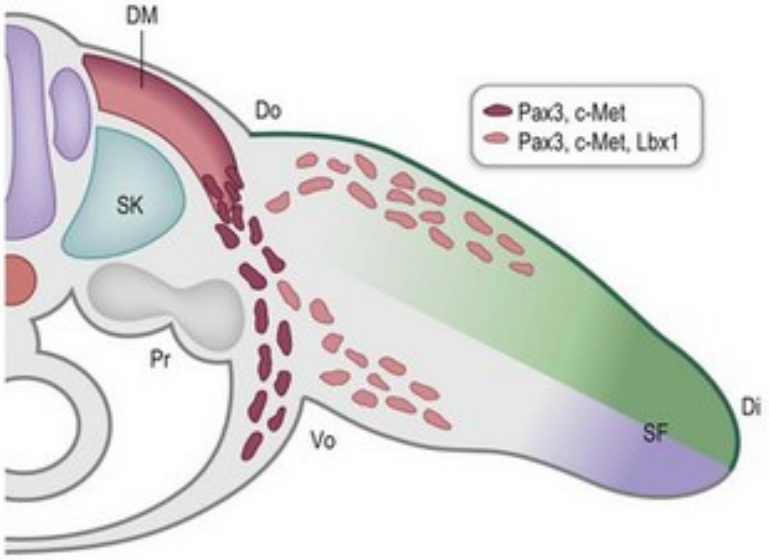


Limb Development
Limb Musculature

Myoblasts from hypaxial myotome migrate into developing limb buds

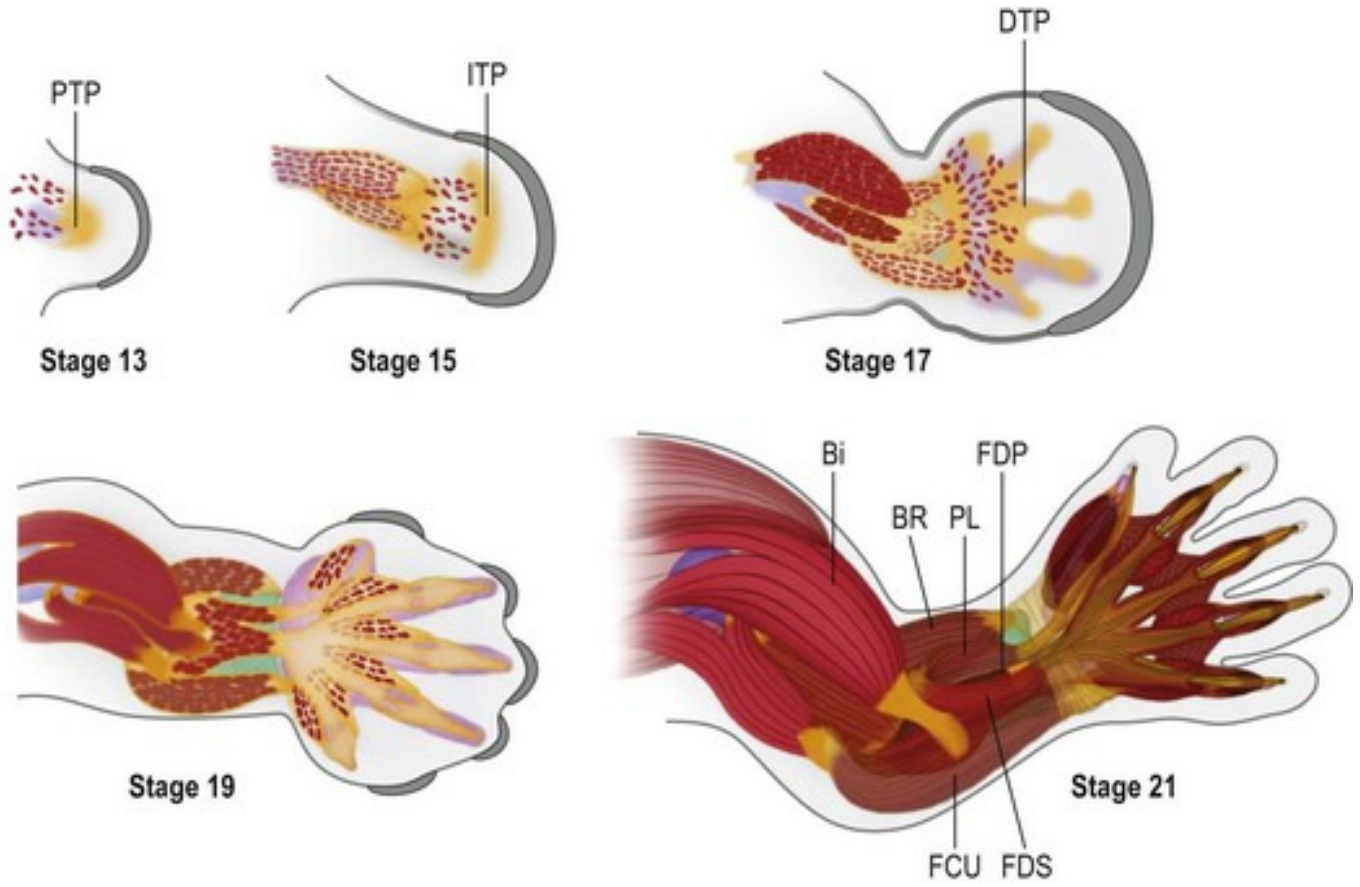


(a)



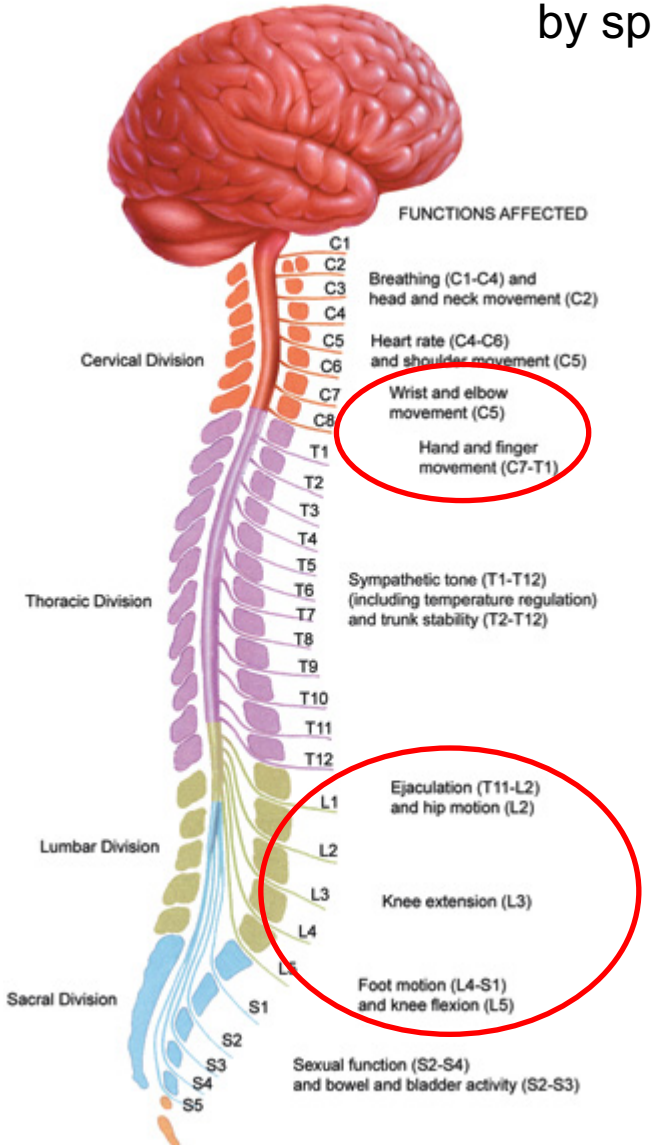
Limb Development
Limb Musculature

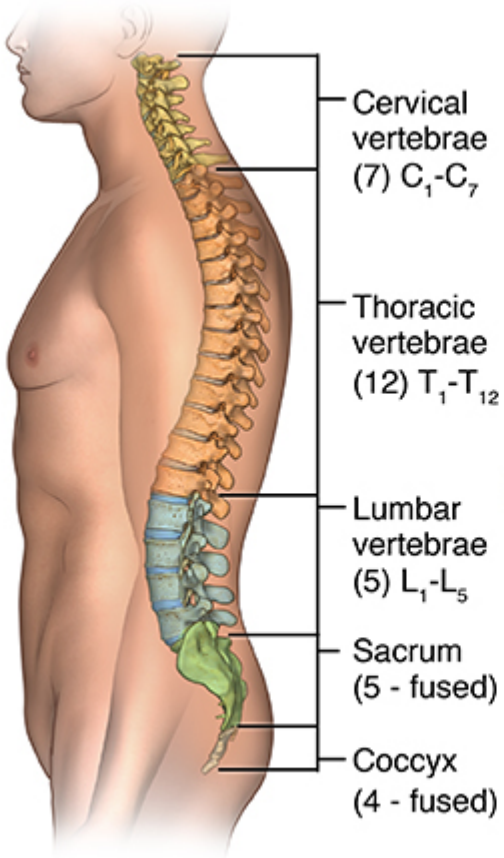
Hypaxial myotome

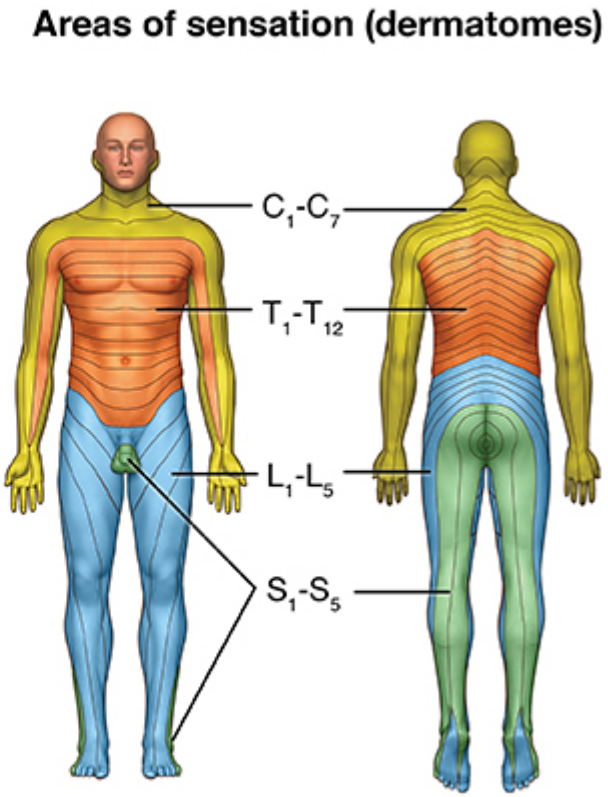


Limb Development
Limb Innervation

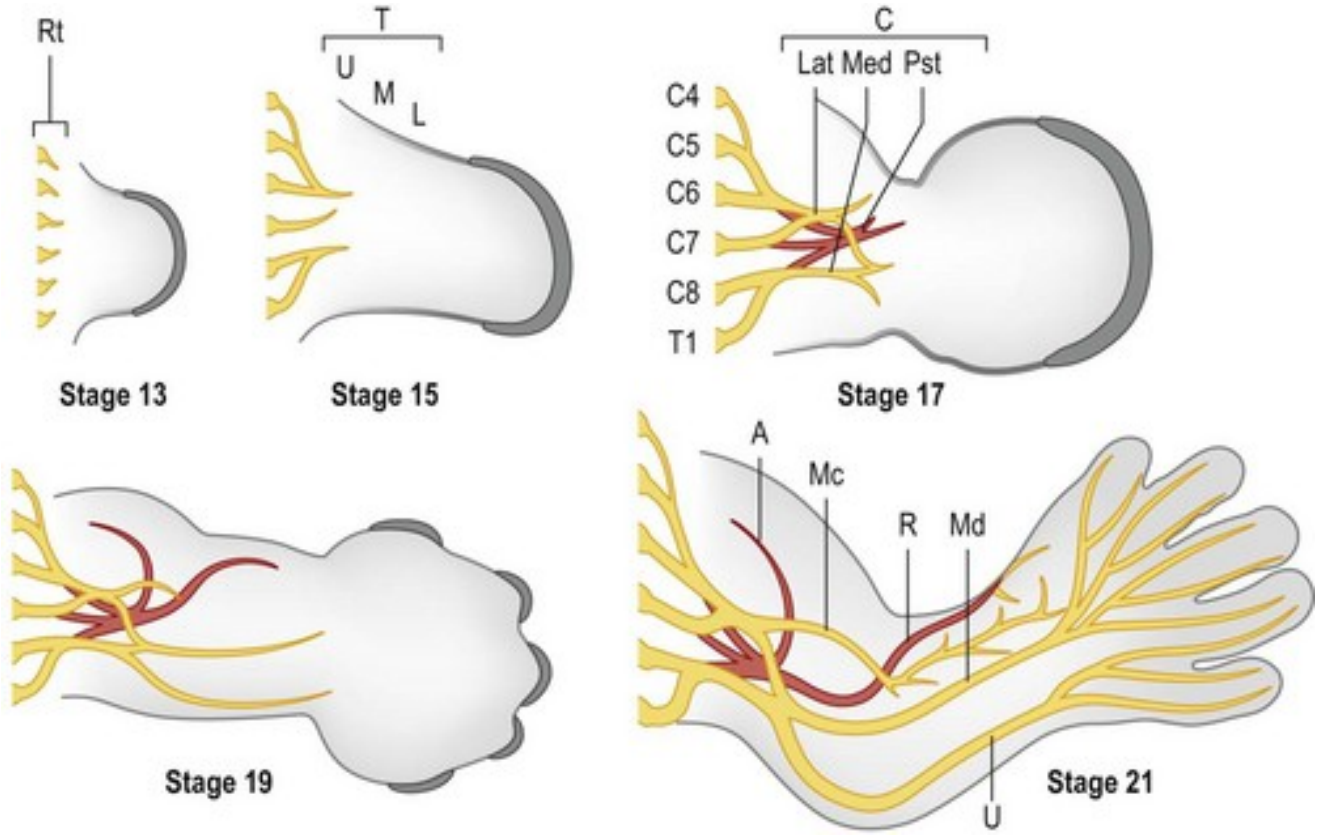
by spinal nerves C5-T1 and T11-L5
Hox 6 and 10 codes







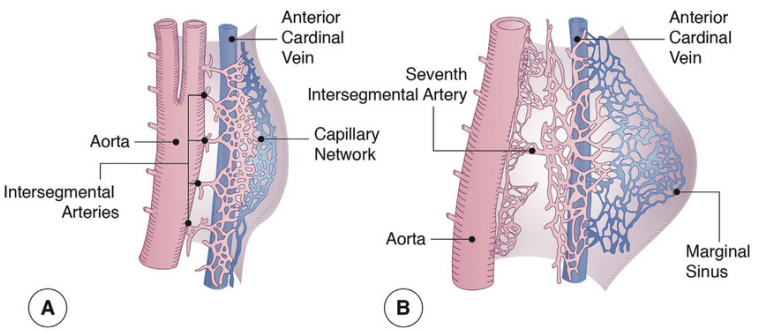
Limb Development
Limb Innervation

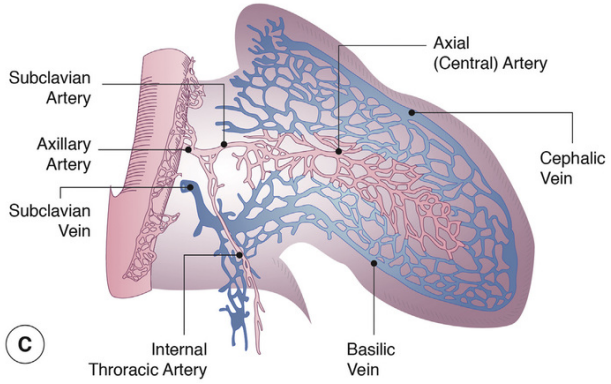


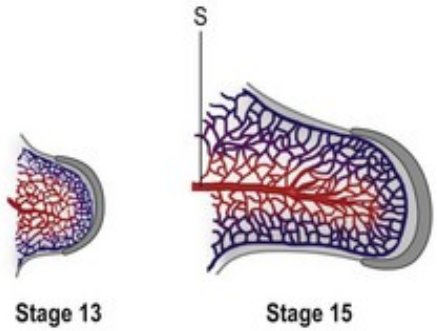
Hox6/10 code

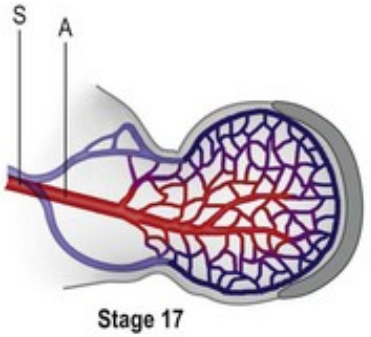
Limb Development
Limb Vasculature

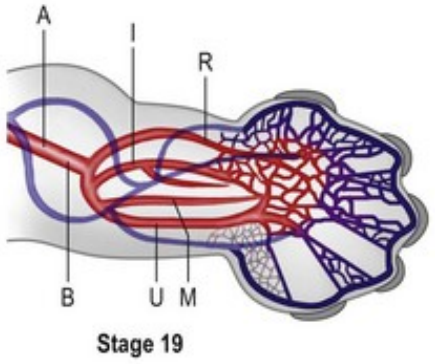
Angiogenesis from dorsal aorta
Vasculogenesis within Limb bud mesenchyme

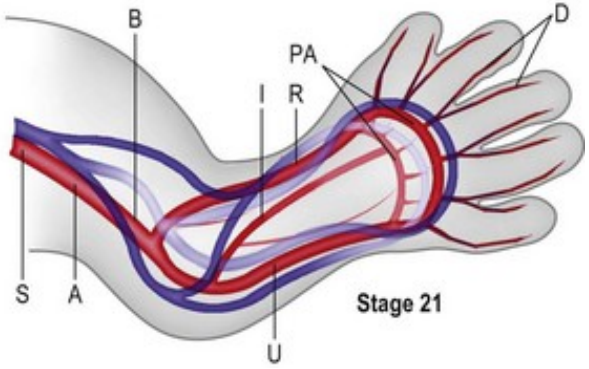








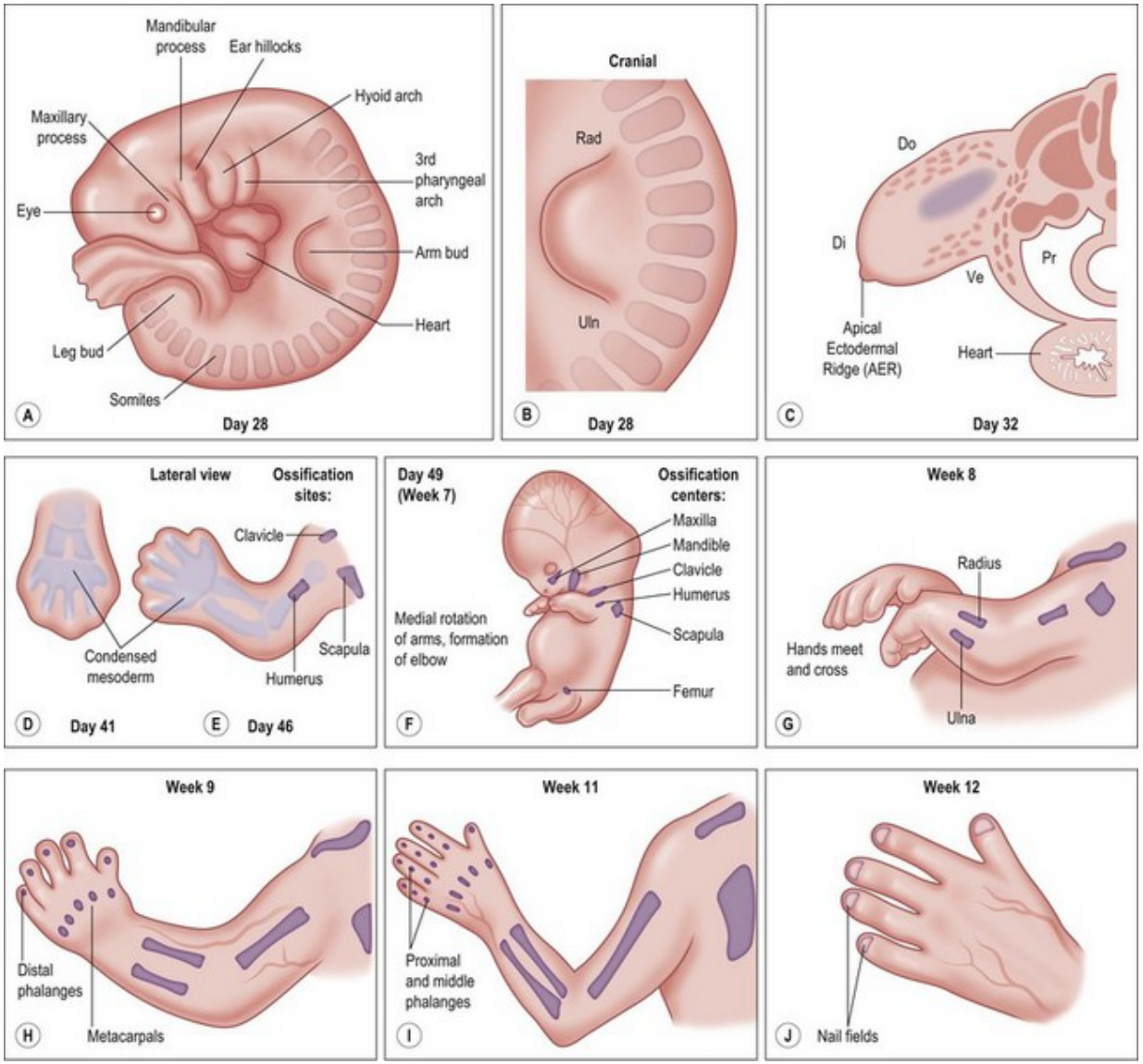




Limb Development
Limb rotation

8th week limbs rotate in different directions
thumb and toe rostral
knee and elbow face outward
upper limb rotates dorsally
lower limb rotates ventrally

Limb Development Summary



Lecture overview
Limb Development

Limb Anatomy

Embryonic Tissues Contributing to Limb Development

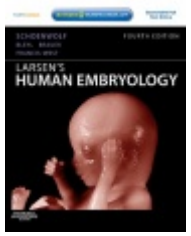
Early Limb Development and Limb Axes

Initiation

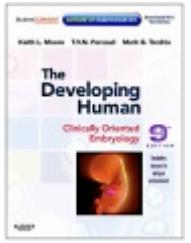
Outgrowth and Patterning

Limb Tissue Differentiation and Ossification

Limb rotation



Resources:
http://php.med.unsw.edu.au/embryology/
Larsen's Human Embryology
The Developing Human: Clinically Oriented Embryology



Dr Annemiek Beverdam – School of Medical Sciences, UNSW
Wallace Wurth Building Room 234 – A.Beverdam@unsw.edu.au