

Lecture - Limb Development

From Embryology

Introduction

This lecture is an introduction to the events in limb development.

The limb has long been used as a model of how **developmental patterning** occurs by manipulation of the limb in animal models. This lecture will therefore also introduce some concepts and experiments that have identified patterning mechanisms within the limb.

The previous lecture covered the basics of bone, muscle and cartilage development, that can be applied to the same elements within the limb.

Cells of the ectoderm, cells derived from the dermatome and the hypaxial portion of the myotome mix with somatic component of the lateral plate mesoderm to give rise to the fore and hind limbs.

The appendicular skeleton consists of: Shoulder girdle, Upper limb (arm, hand), Pelvic girdle, Lower limb (leg, foot).

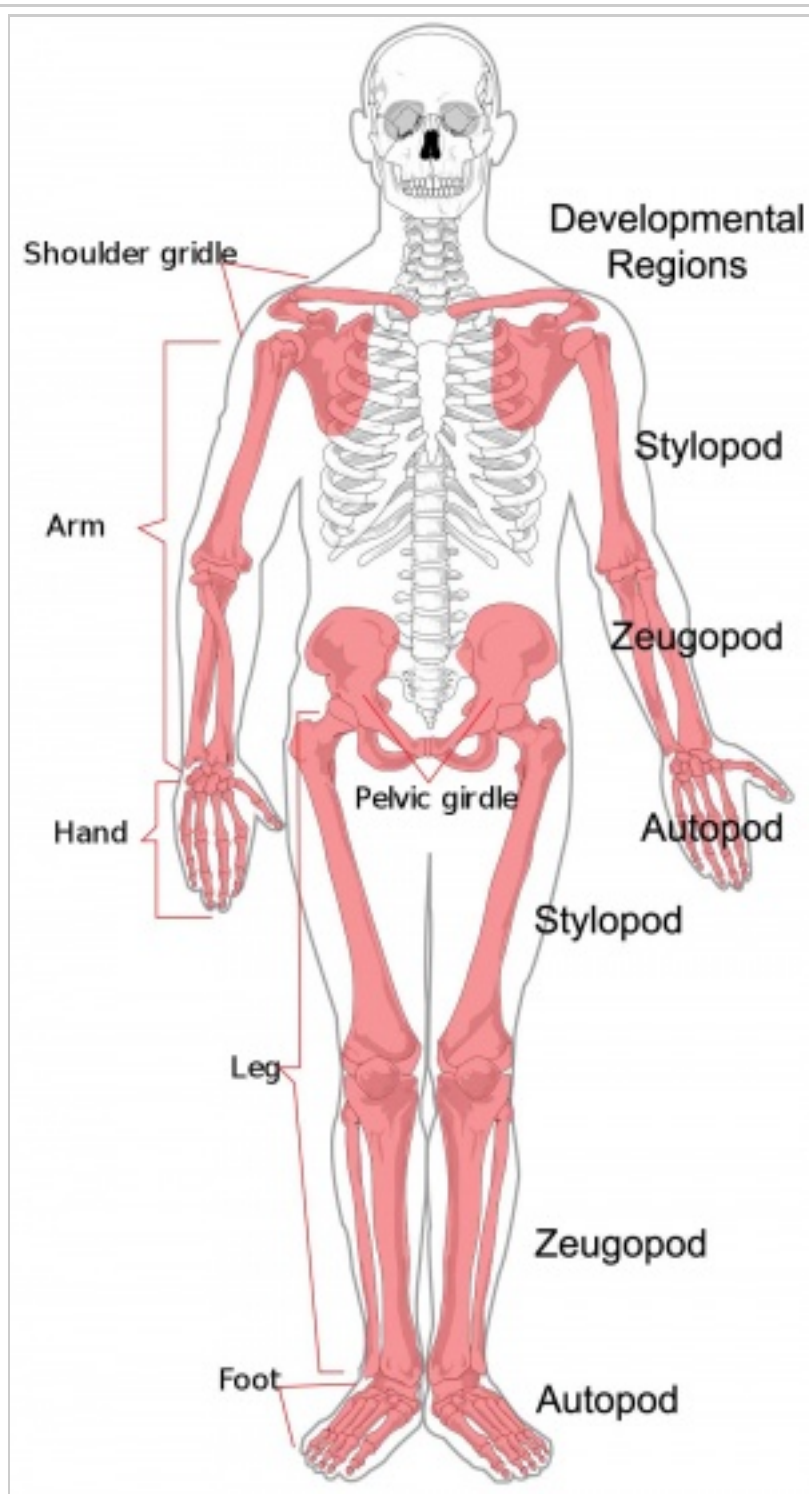
Carnegie stage 1-23

Lecture Objectives

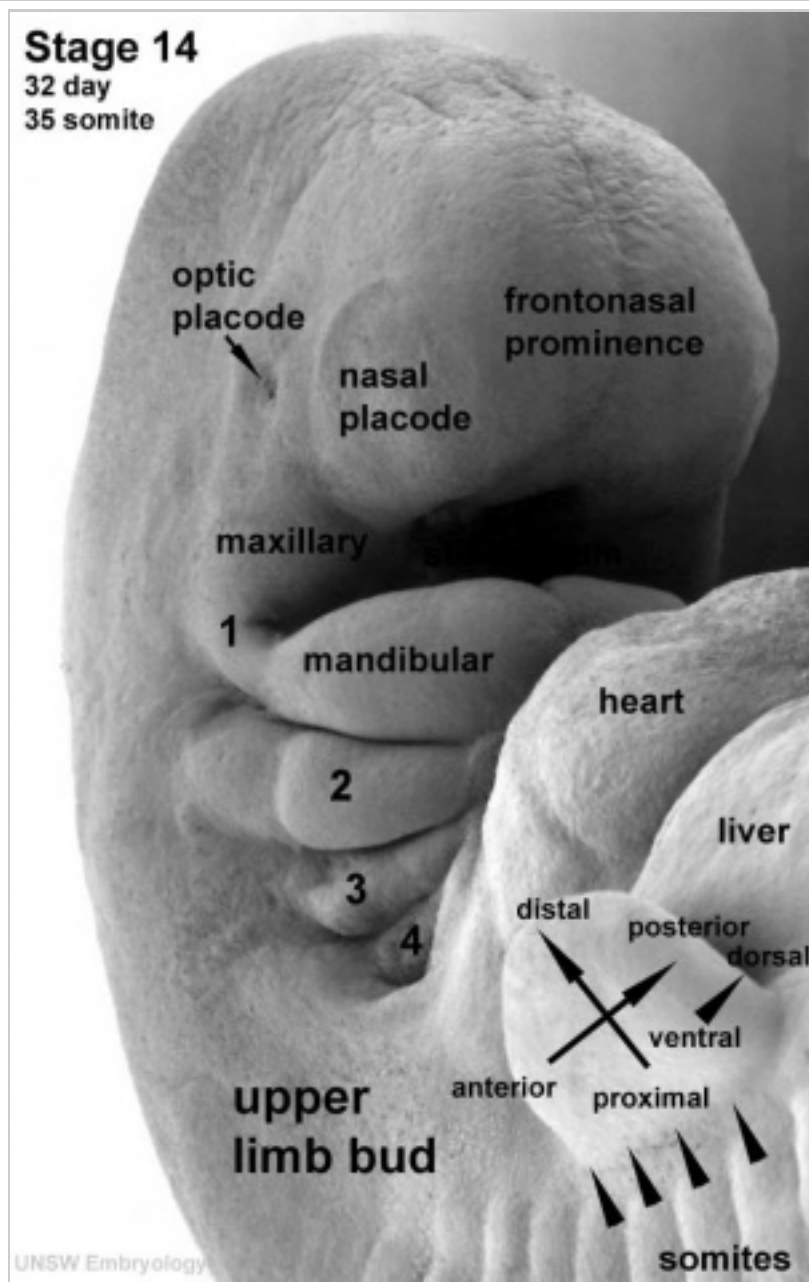
- Review of the subdivisions of mesoderm development.
- Differentiation of somites
- Limb patterning (axes)
- Cartilage formation
- Bone formation
- Development of skeletal muscle

Lecture Resources




Movies[Expand]



Appendicular skeleton



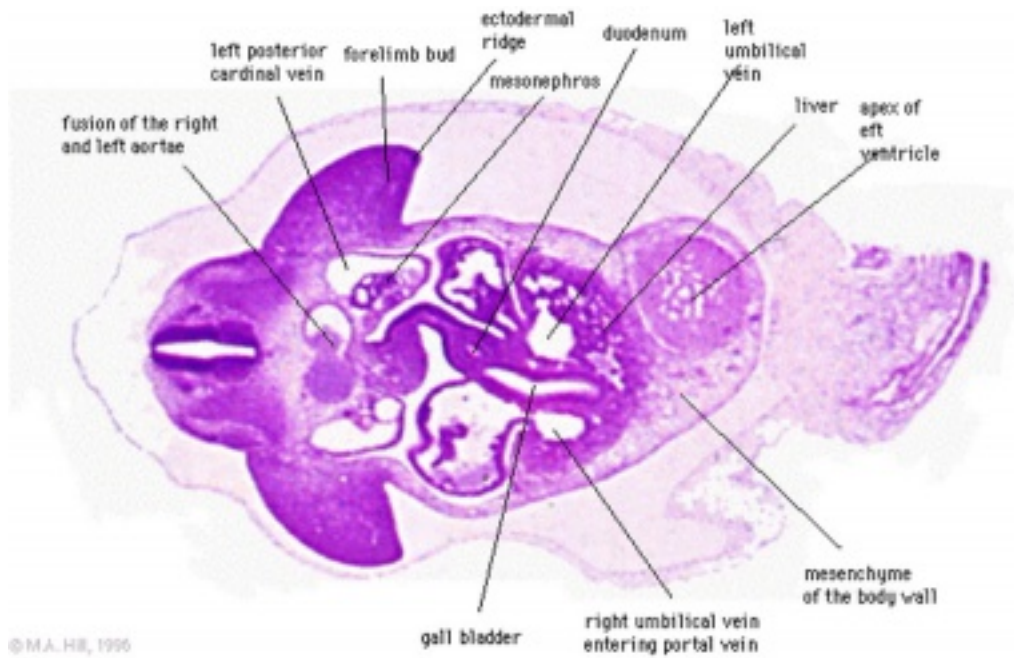
Human Embryo stage 14 SEM

| References | [Collapse] |
|---|--|
|  <p>Hill, M.A. (2015). <i>UNSW Embryology</i> (15th ed.) Retrieved October 6, 2015, from https://embryology.med.unsw.edu.au</p> | <ul style="list-style-type: none"> ■ Limb Development ■ Limb Abnormalities ■ Archive - 2014 (https://embryology.med.unsw.edu.au/embryology/index.php?title=Lecture_-_Limb_Development&oldid=146702) 2014 PDF ■ ANAT2341 Lab 9 <p>Factor Links: hCG BMP Sonic hedgehog Homeobox FGF Nanog Notch Fox Pax Retinoic acid Six Slit2/Robo1 Sox Tbx TGF-beta VEGF Wnt Hippo Category:Molecular</p> <ul style="list-style-type: none"> ■ Developmental Dynamics - Special Issue: Special Issue on Limb Development (http://onlinelibrary.wiley.com/doi/10.1002/dvdy.v240.5/issuetoc) May 2011 Volume 240, Issue 5 |
|  <p>Moore, K.L., Persaud, T.V.N. & Torchia, M.G. (2011). <i>The developing human: clinically oriented embryology</i> (9th ed.). Philadelphia: Saunders.</p> | <p>The following chapter links only work with a UNSW connection.</p> <ul style="list-style-type: none"> ■ Development of Limbs (http://www.unsw.eblib.com.wwwproxy0.library.unsw.edu.au/patron/Read.aspx?p=1430154&pg=393) |
|  <p>Schoenwolf, G.C., Bleyl, S.B., Brauer, P.R. & Francis-West, P.H. (2009). <i>Larsen's human embryology</i> (4th ed.). New York; Edinburgh: Churchill Livingstone.</p> | <p>The following chapter links only work with a UNSW Library connection (http://er.library.unsw.edu.au/er/cgi-bin/eraccess.cgi?url=http://www.unsw.eblib.com.wwwproxy0.library.unsw.edu.au/patron/FullRecord.aspx?p=2074524).</p> <ul style="list-style-type: none"> ■ Chapter 18 - Development of the Limbs |

ECHO360 Recording[Expand]

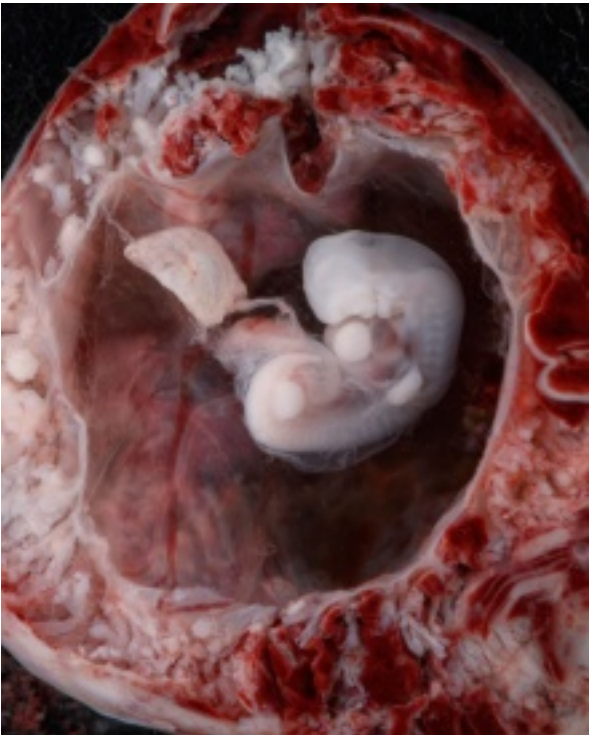
Limb Buds

- Limbs are initially undifferentiated mesenchyme (mesoderm) with an epithelial (ectoderm) covering. Uniform paddle shaped structures that grow outwards gradually.
- One the first noticeable changes is the development of a large blood vessel (marginal vein) which runs just underneath a thickening of the ectoderm at the tip of the limb bud called the Apical Ectodermal Ridge (AER).
- Positioning of the limbs is distant from final location



Stage 13

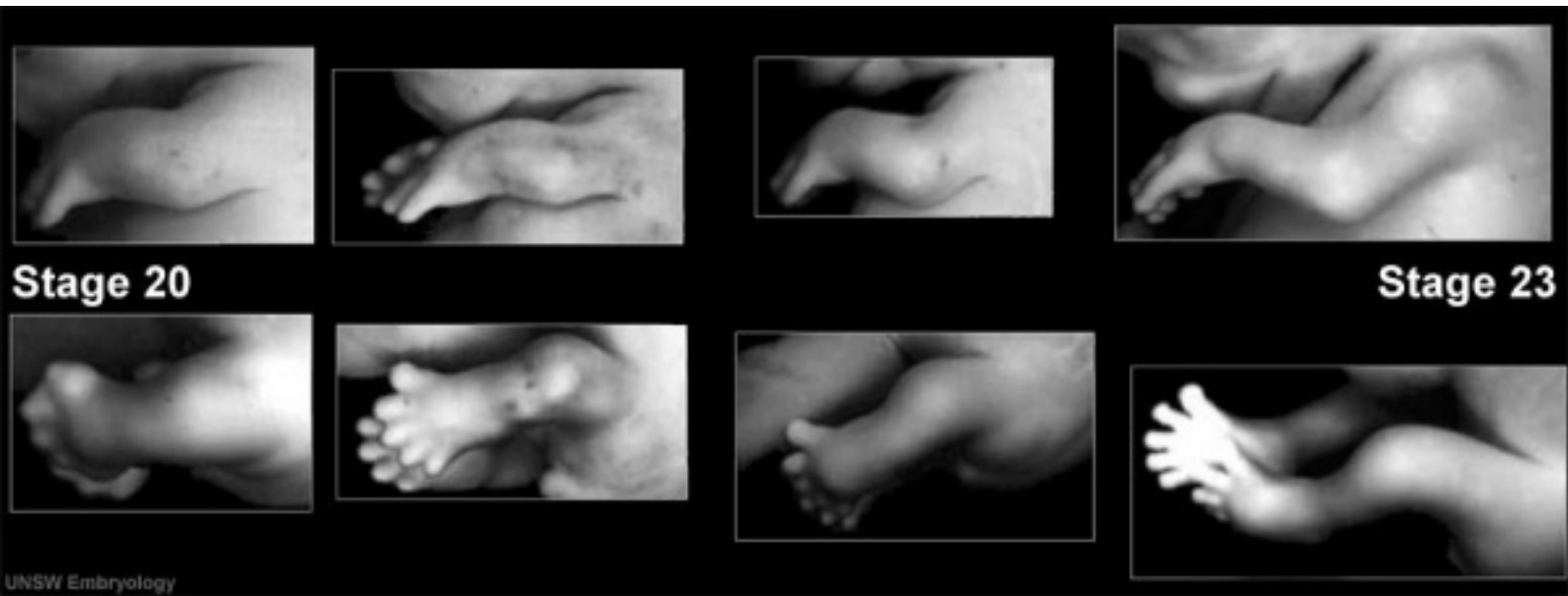
Stage 14 - Lateral View



Mobile
(http://embryology.med.unsw.edu.au/embryology/Slides/Embryo_Stages/Stage14/bf18/leaflet.html) | **Desktop**
(http://embryology.med.unsw.edu.au/embryology/Slides/Embryo_Stages/Stage14/bf18/stage14bf18.html) | **Original**

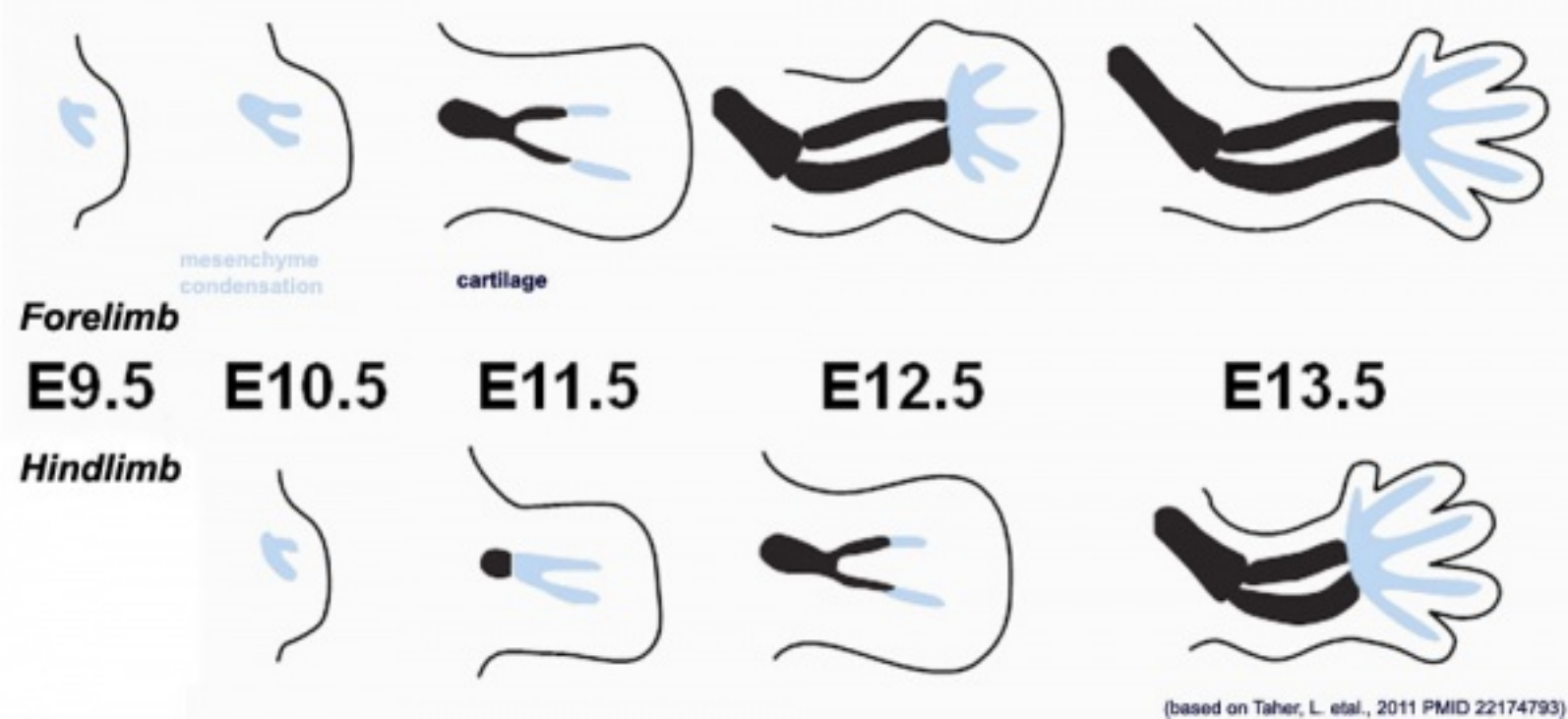
Stage 14 | Embryo Slides

Upper and Lower Limb



Limb development occurs at different times for forelimbs and hindlimbs. In the mid-4th week, human upper limb buds first form and lower limbs about 2 days later. The limbs form at vertebra segmental levels C5-C8 (upper limbs) L3-L5 (lower limbs).

Mouse Limb Development



Limb Axis Formation

Four Concepts - much of the work has been carried out using the chicken and more recently the mouse model of development.

1. Limb Initiation
2. Proximodistal Axis
3. Dorsoventral Axis
4. Anteroposterior Axis

Limb Initiation

- Fibroblast growth factor (FGF) coated beads can induce additional limb
- FGF10 is expressed in lateral plate mesoderm prior to bud formation induces expression of FGF8 in the overlying ectoderm. FGF8 induces continued growth in the underlying mesoderm - thus a positive feedback loop
- Anterior boundary of Hoxc6 expression coincides with the position of forelimb development

Autoregulatory loop of induction between FGF10 and FGF8 (<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=dbio&part=A3941&rendertype=figure&id=A3953>)

Site of FGF10 expression in the chick embryo (<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=dbio&part=A1038&rendertype=figure&id=A1041>)

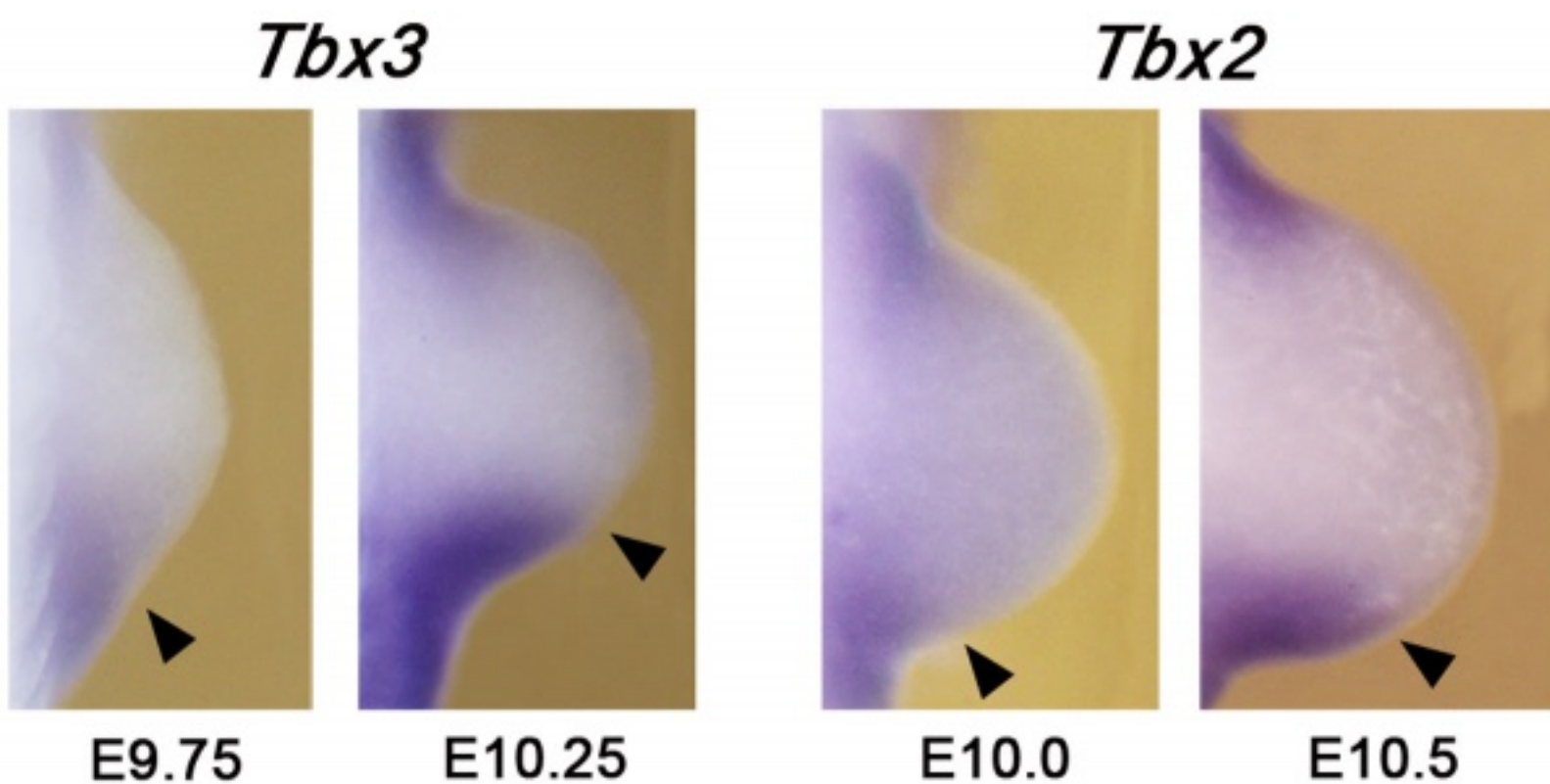
Positioning of the limb on the rostrocaudal (anteroposterior) axis is determined by the expression of Hox genes (<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=mboc4&part=A3871&rendertype=figure&id=A3881>)

Examples of Hox gene expression boundaries in the mouse Hoxb2 and Hoxb4 (<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=mboc4&part=A3871&rendertype=figure&id=A3882>)

Limb Identity

Forelimb and hindlimb (mouse) identity appears to be regulated by T-box (Tbx) genes, which are a family of transcription factors.

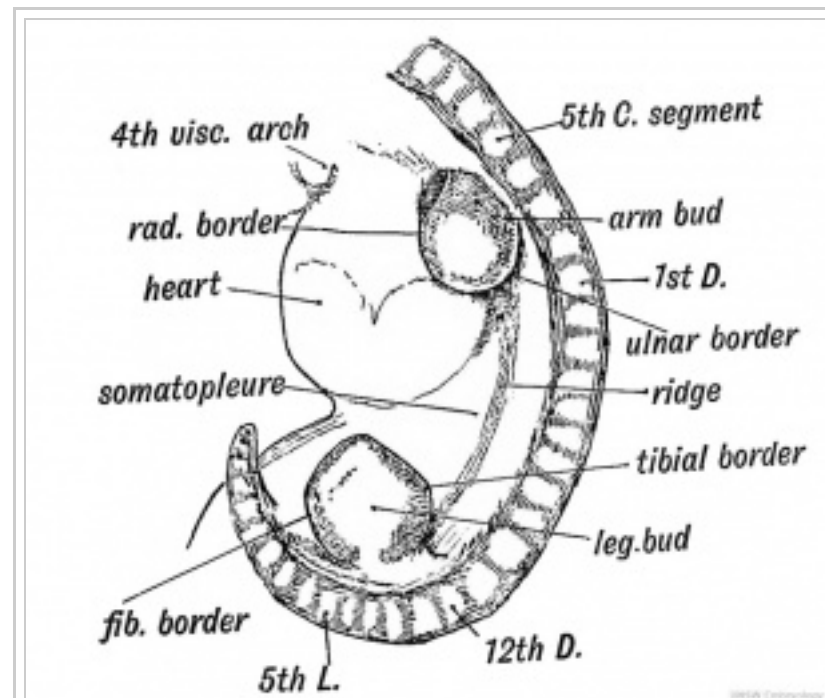
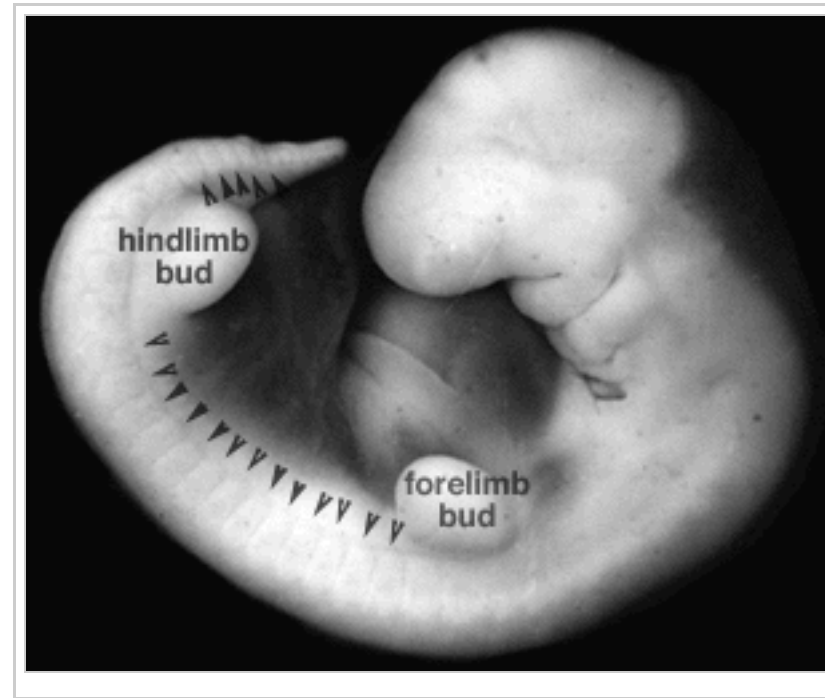
- hindlimb Tbx4 is expressed.
- forelimb Tbx5 is expressed.
- Tbx2 and Tbx3 are expressed in both limbs.



Tbx3 and Tbx2 expression in E9.75 to 10.5 wild-type mouse embryonic forelimb.^[1]

Related Research - PMID: 12490567 (<http://www.ncbi.nlm.nih.gov/pubmed/12490567?dopt=Abstract>) | Development 2003 Figures (<http://dev.biologists.org/cgi/content/figonly/130/3/623>) | Scanning electron micrographs of E9 Limb bud wild-type and Tbx5del/del (<http://dev.biologists.org/cgi/content/full/130/3/623/FIG1>) A model for early stages of limb bud growth (<http://dev.biologists.org/cgi/content/full/130/3/623/FIG7>) | PMID: 12736217 (<http://www.ncbi.nlm.nih.gov/pubmed/12736217?dopt=Abstract>) | Development 2003 Figures (<http://dev.biologists.org/cgi/content/figonly/130/12/2741>)

Tbx4 expression can turn an experimentally induced forelimb into a hindlimb (<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=dbio&part=A3928&rendertype=figure&id=A3936>)



Limbs 28 days

Axes and Morphogens

- **Anteroposterior** - (Rostrocaudal, Craniocaudal, Cephalocaudal) from the head end to opposite end of body or tail.
- **Dorsoventral** - from the spinal column (back) to belly (front).
- **Proximodistal** - from the tip of an appendage (distal) to where it joins the body (proximal).

Model of patterning signals in the vertebrate limb (<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=mboc4&part=A3885&rendertype=figure&id=A3902>)

Diffusible morphogens create a concentration gradient accross an embryonic field (<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=mboc4&part=A3792&rendertype=figure&id=A3812>)

Proximodistal Axis

- Apical Ectodermal Ridge (AER) initially formed at the site of FGF10 induction
- then AER secretes FGF8 and FGF4 slightly later
- FGFs stimulate proliferation and outgrowth in the underlying mesenchyme

limb development at embryo images online (http://www.med.unc.edu/embryo_images/unit-mslimb/mslimb_https/mslimb017.htm)

Morphogen production from the AER - The Fibroblast Growth Factors (FGFs)

- 22 FGF genes identified in humans
- bind membrane tyrosine kinase receptors
- Patterning switch with many different roles in different tissues

FGF receptors

- comprise a family of at least 4 related but individually distinct tyrosine kinase receptors (FGFR1- 4) similar protein structure
- 3 immunoglobulin-like domains in extracellular region
- single membrane spanning segment
- cytoplasmic tyrosine kinase domain

FGF receptors are paired proteins on the cell surface with an internal tyrosine kinase domain (<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=dbio&part=A1038&rendertype=figure&id=A1041>)

Dorsoventral Axis

- Important for patterning muscles - ventral muscles - flexors; Dorsal muscles - extensors
- Early grafting experiments showed that the D/V signalling centre resided in the dorsal ectoderm
- Wnt7a is a diffusible morphogen that is secreted by dorsal ectoderm cells
- Wnt7a induces the expression of the homeobox gene Lmx1 in the underlying mesoderm adjacent to the dorsal surface
- The homeobox gene Engrailed (En1) is expressed in the opposite ventral ectoderm

Consequence of Wnt7a deficiency in the mouse forelimb (<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=dbio&part=A3968&rendertype=figure&id=A3969>)

Morphogen production from the dorsal ectoderm - Wnt7a

- name was derived from 'wingless' and 'int'
- Wnt gene first defined as a protooncogene, int1
- Humans have 19 Wnt genes
- Wnt7a gene is at 3p25 encoding a 349aa secreted glycoprotein
- patterning switch with different roles in different tissues
- One WNT receptor is called Frizzled (FZD) - named after a drosophila phenotype
- Frizzled gene family encodes a G protein-coupled receptor with 7 transmembrane domains

Anteroposterior Axis

- Zone of polarizing activity (ZPA)
- a mesenchymal posterior region of limb
- secretes sonic hedgehog (SHH)

ZPA secretes SHH and determines the anteroposterior axis of the limb bud (<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=mboc4&part=A3792&rendertype=figure&id=A3815>)

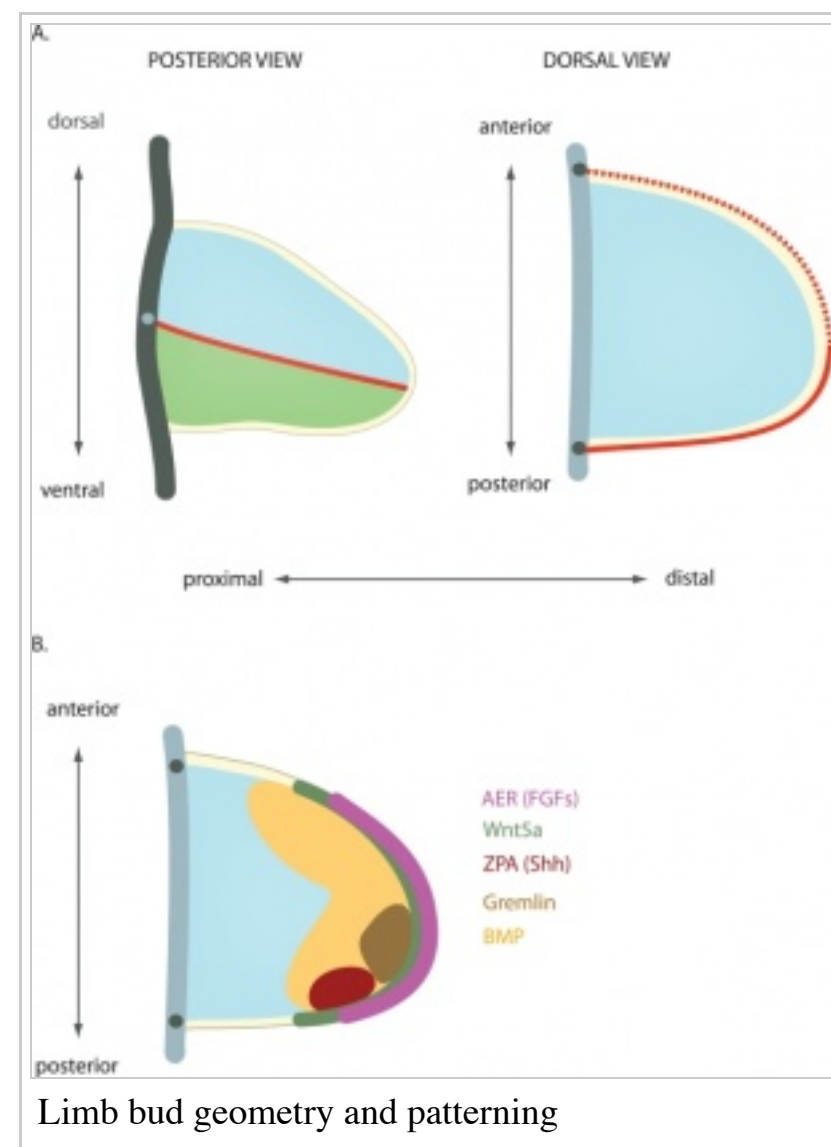
Morphogen production from the ZPA - Sonic Hedgehog (SHH)

- Sonic hedgehog (SHH) is a diffusible morphogen secreted from cells, the protein product of the SHH gene
- The protein is processed by cleavage of the preprotein and addition of a palmitate molecule to the amino terminus and cholesterol to the carboxy terminus
- The SHH receptor is a cell surface protein called Patched which interacts with another cell surface protein Smoothened.
- Binding of SHH to Patched blocks its inhibitory effect on Smoothened and allows it to initiate an intracellular signaling cascade

The Time Axis - Dynamic development and temporal gene expression

- Different Hox genes are expressed at different times in the developing limb bud and pattern the fine structure of the limb.
- Structures are determined in a proximal>distal direction with time, i.e. proximal structures such as the humerus bone are laid down first.

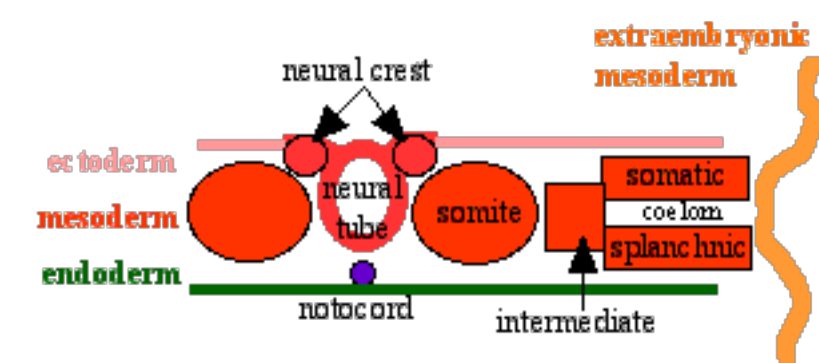
Hox genes and dynamic patterning of the limb (<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=dbio&part=A3941&rendertype=figure&id=A3956>)



Cellular origins of the limb

Limb cartilage and bone

- Derived from local proliferating mesenchyme derived from the somatic lateral plate mesoderm (somatopleure)
- BMP2 and BMP4 play crucial roles in the development of cartilage - sufficient BMP must be present to achieve chondrogenesis. However, the main role is in later bone formation. Loss of BMP2 and 4 leads to a severe impairment of osteogenesis



- Differentiation of somitic mesoderm in the chick embryo

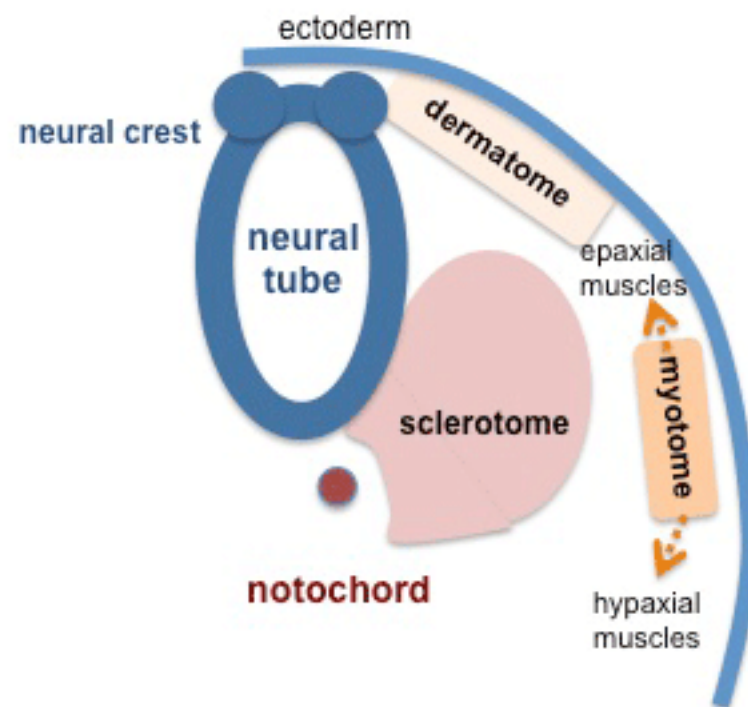
(<http://www.ncbi.nlm.nih.gov/books/NBK10085/figure/A3468/?report=objectonly>)

Limb muscle and dermis

- Skeletal muscle derived from somites at the level of the limb buds (C3-C5; L3-L5),
 - the hypaxial part of the myotome
- Pax3 positive migratory myoblasts invade the limb bud
- Similarly, dermal cells also invade derived from the dermomyotome
- Both maintain the identity of the somite from which they were derived so that innervation corresponds to the same spinal nerve root.
- Note that dermatomes are rotated due to embryonic limb rotations

Origin of limb muscle cells - Migrations traced by grafting cells from a quail embryo into a chick embryo

- two species very similar in development
- quail cells recognizable by distinctive nucleoli
- Quail somite cells substituted for somite cells of 2 day chick embryo
- wing of chick sectioned a week later
- found muscle cells in chick wing derive from transplanted quail somites



Shh expression in ZPA mouse forelimb (E11.5)^[2]

Dorsal/Ventral Muscle Mass - sometimes referred to as the anterior and posterior muscle compartments.

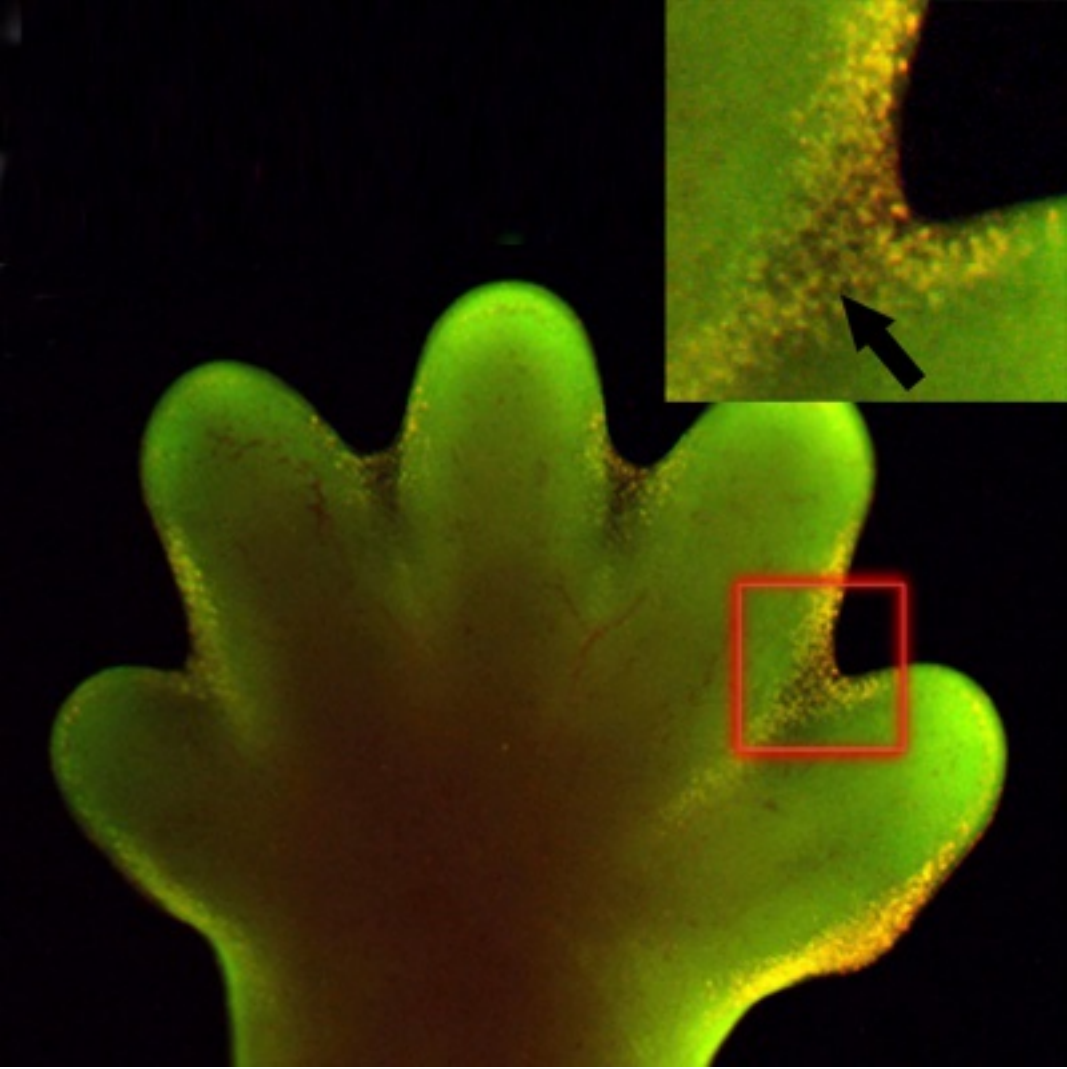
Limb Muscle - Differentiation of Skeletal muscle is the same as in the myotome blocks but involves an extra migratory step

Muscle Development

- Muscle precursor cells migrate to the muscle location
- Form beds of proliferating myoblasts
- Myoblasts fuse together to form a syncytial structure called a myotube
- Myotubes begin to express contractile proteins, form sarcomeres
- mature into myofibers with tendon connections at each end, motor and sensory innervation.

Hand and Footplates

- 5th week- hand and footplates appear at the ends of limb buds and ridges form digital rays
- Cells between the digital rays are removed by programmed cell death (apoptosis)
- 3-5 day difference between hand and foot development



Interdigital apoptosis in the mouse hindlimb.^[3]

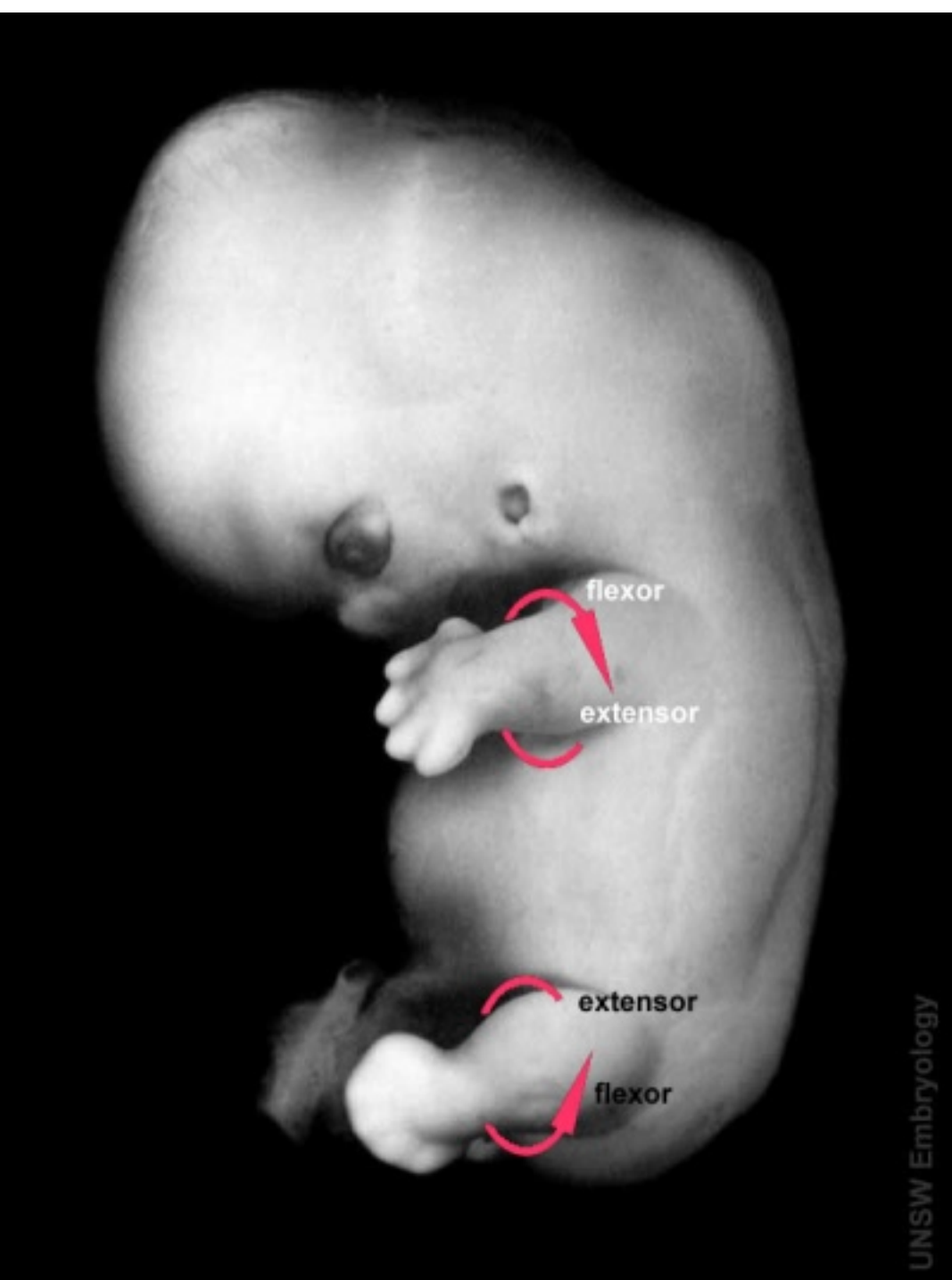
Links: hand growth (http://www.med.unc.edu/embryo_images/unit-mslimb/mslimb_htms/mslimb024.htm)

Apoptosis

Cell Biology - Apoptosis Lecture (http://php.med.unsw.edu.au/cellbiology/index.php?title=2009_Lecture_18)

Fluorescent staining of cells undergoing apoptosis in the limb (<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=mboc4&part=A3245&rendertype=figure&id=A3246>)

Limb Rotation

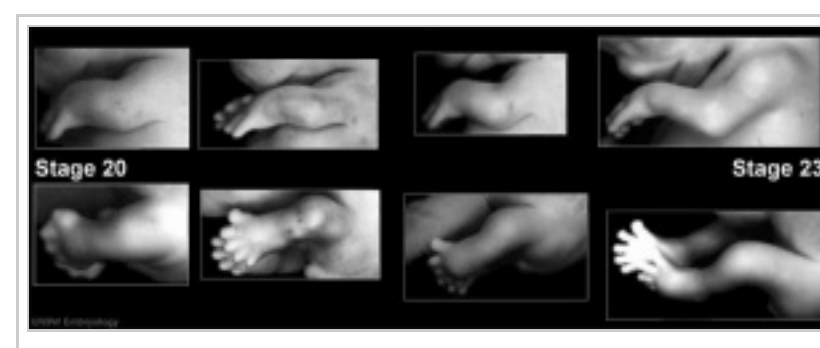
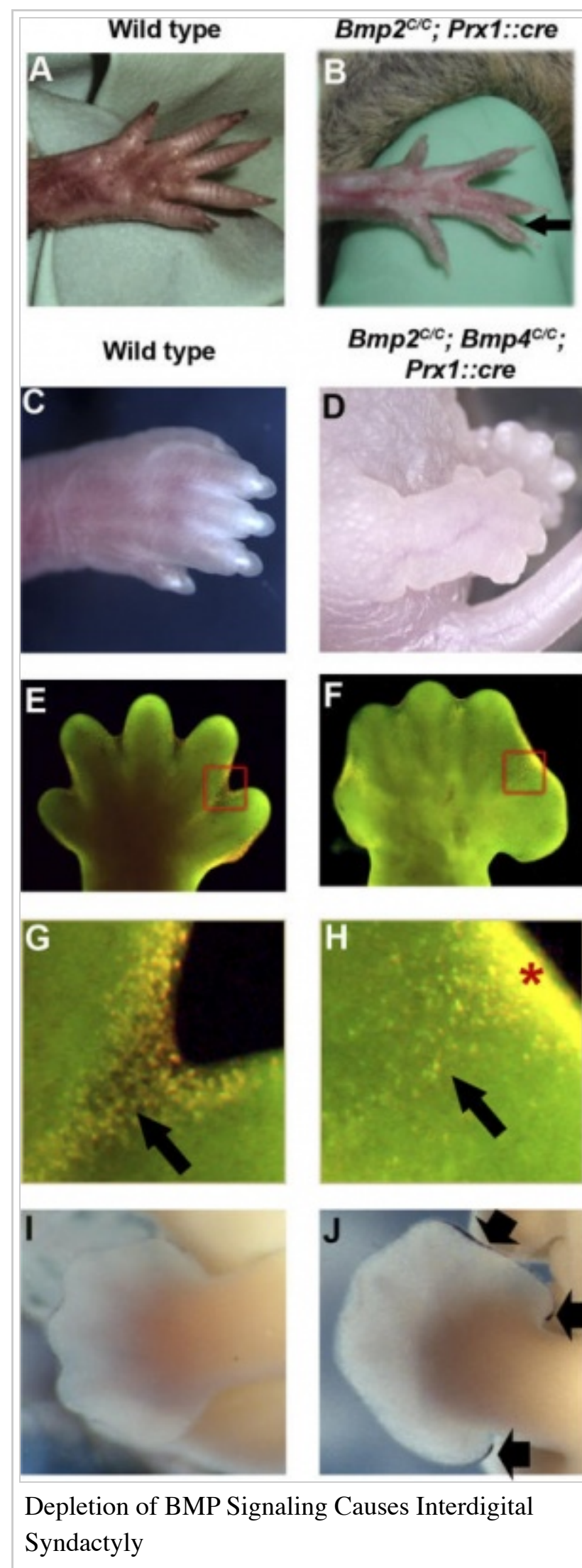


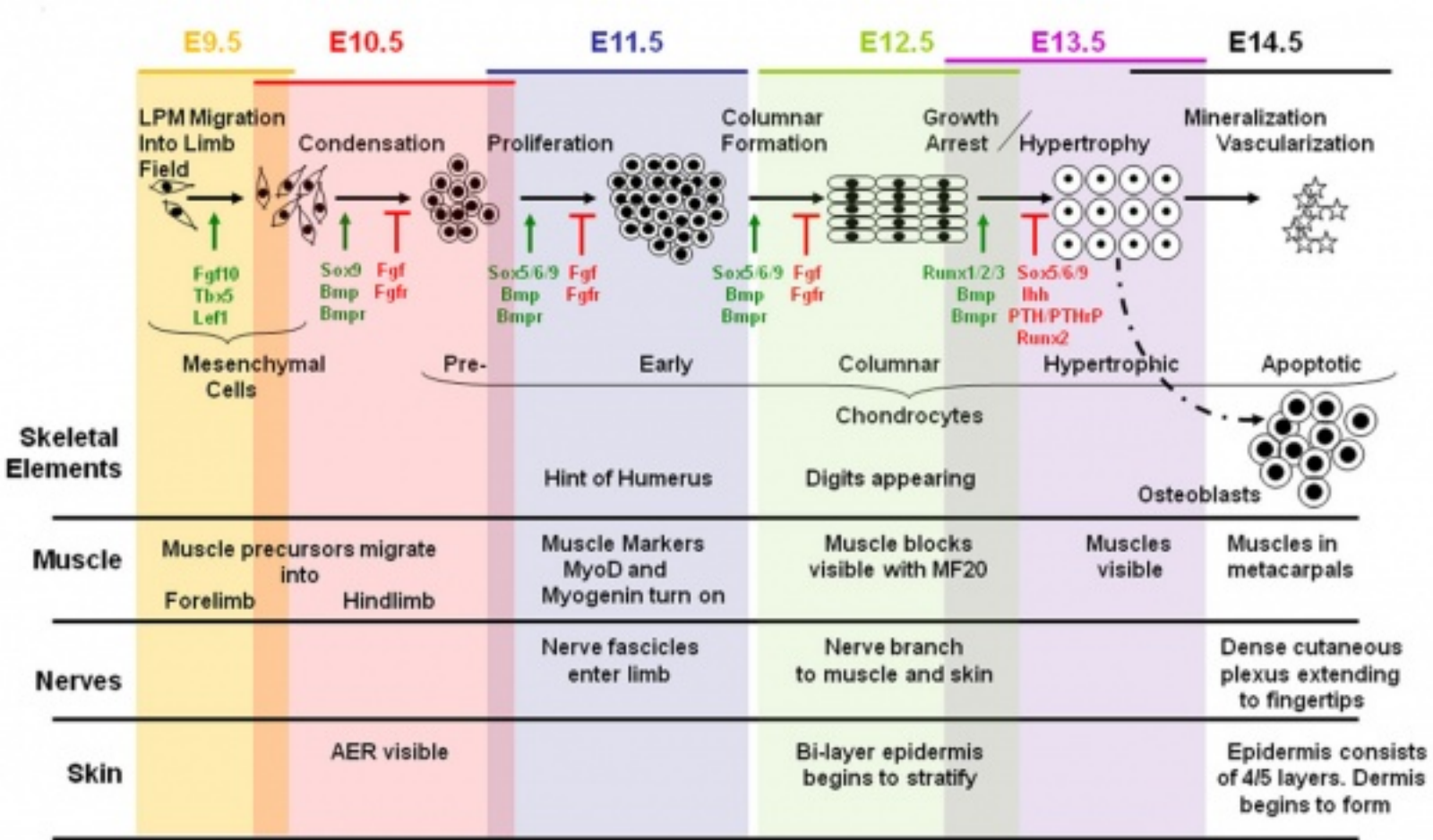
- 8th week limbs rotate in different directions (Humans Stage 20-23)
- thumb and toe rostral
- knee and elbow face outward
- **upper limb rotates dorsally**
- **lower limb rotates ventrally**

Limb Innervation

- spinal cord segmental nerves form a plexus adjacent to each limb
- Brachial (upper) lumbar (lower)
- Plexus forms as nerves invade the limb bud mesenchyme
- Fetal period - touch pads become visible on hands and feet

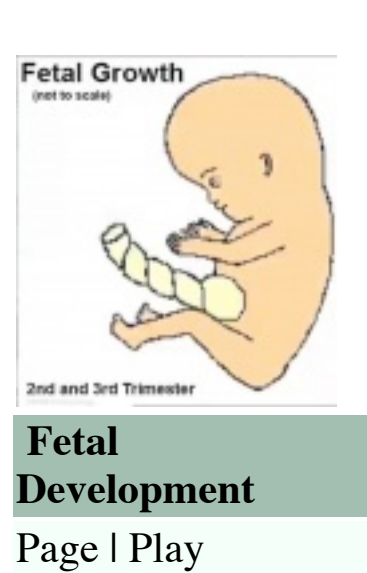
brachial plexus origin (http://www.med.unc.edu/embryo_images/unit-mslimb/mslimb_htms/mslimb019.htm)





Mouse Limb Timeline Leila Taher, Nicole M Collette, Deepa Murugesh, Evan Maxwell, Ivan Ovcharenko, Gabriela G Loots **Global gene expression analysis of murine limb development.** PLoS ONE: 2011, 6(12);e28358 PubMed 22174793

PMC3235105 (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3235105>) | PLoS One. (<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0028358>)



Embryonic period - the external appearance of both the upper and lower limb has been formed.

Fetal period - the limbs continue to grow significantly in length (elongate).

Play the associated animation to observe the relative change in limb dimensions.

Limb Abnormalities

Limb Abnormalities

Congenital Hip Dislocation

- Instability of the femoral head in the acetabulum - ligaments may stretch: 1:60 at birth
- congenital instability of hip, later dislocates by muscle pulls or gravity
- familial predisposition female predominance
- Growth of femoral head, acetabulum and innominate bone are delayed until the femoral head fits firmly into the acetabulum

Maternal

- thalidomide Phocomelia
- short ill-formed upper or lower limbs
- hyperthermia

Links: Thalidomide

Genetic

- Trisomy 21 - Downs syndrome
- Human Gene Mutations - mutation of any of the patterning genes will result in limb abnormalities

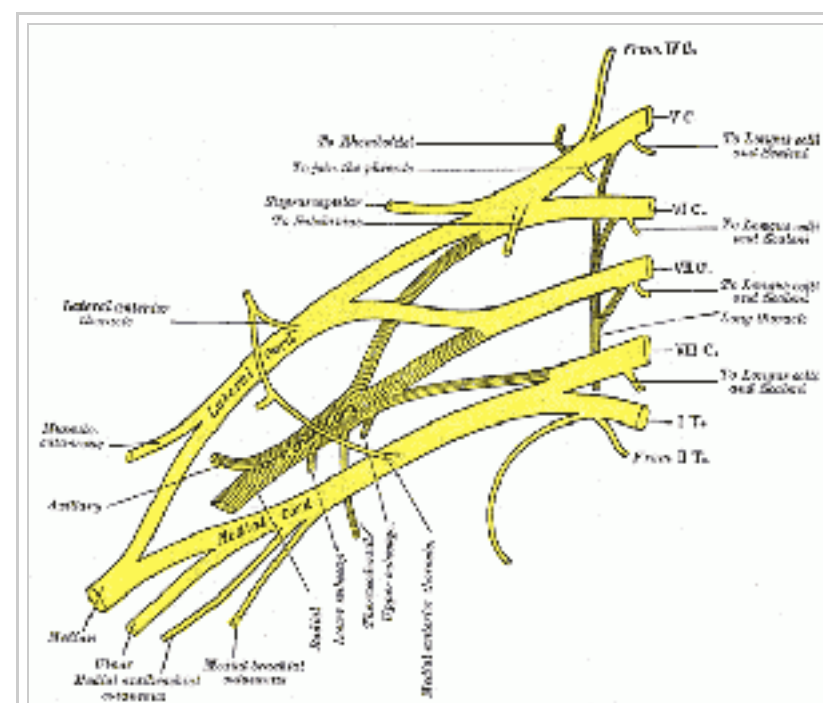
Type II syndactyly- HoxD13

Syndactyly

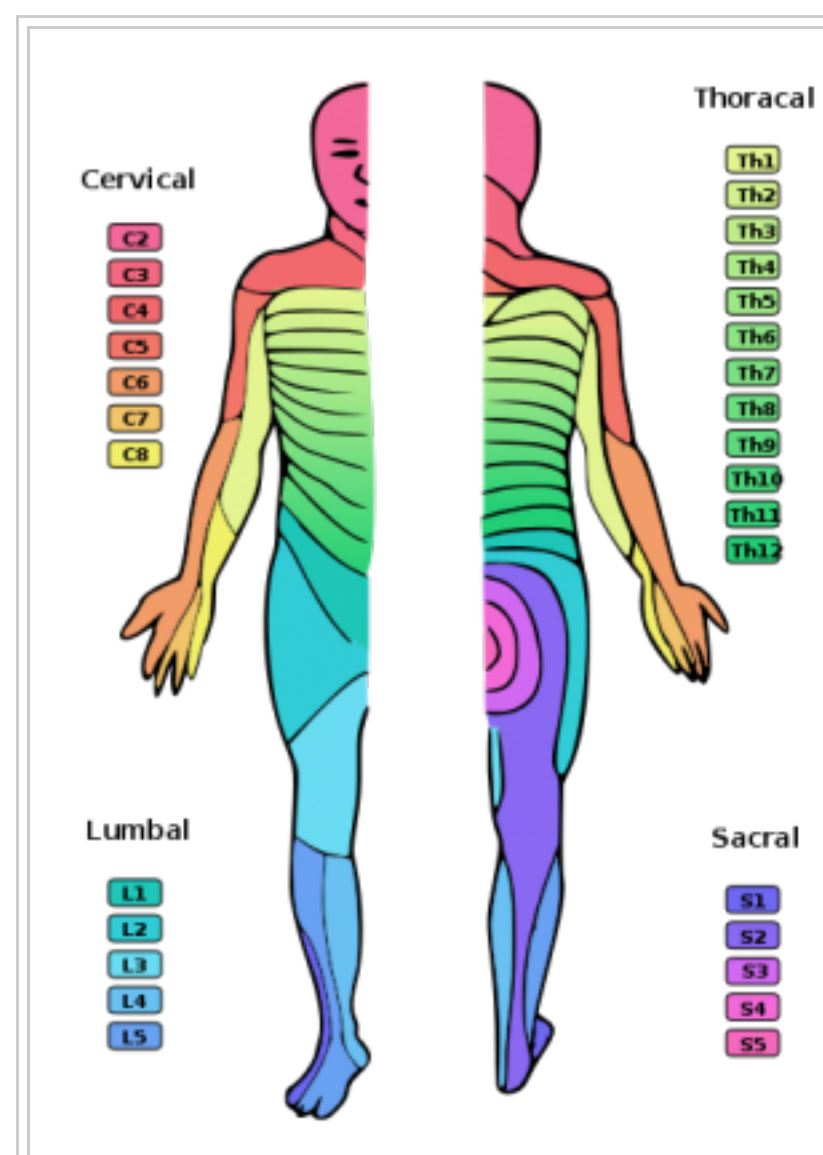
Fusion of fingers or toes (Greek, *syn* = together, *dactyly* = digit) which may be single or multiple and may affect: skin only, skin and soft tissues or skin, soft tissues and bone. The condition is unimportant in toes but disabling in fingers and requires operative separation and is frequently inherited as an autosomal dominant. The presence of this additional "webbing" reflects preservation of the developmental tissues that in normal development are removed by programmed cell death (apoptosis).

Talipes Equinovarus

(Latin, *talipes* = ankle bone, *pes* = foot, *equinus* = horse) Abnormality of the lower limb which begins in the embryonic period (first trimester of pregnancy) resulting in the foot is then turned inward and downward at birth, described as "club foot". Occurs in approximately 1 in 1,000 births, postnatally it affects how children walk on their toes with the foot pointed downward like a horse.



brachial plexus



Adult Dermatomes



Congenital Hip Dislocation



Muscle Development

Duchenne Muscular Dystrophy

- X-linked dystrophy
- large gene encoding cytoskeletal protein- Dystrophin
- progressive wasting of muscle, die late teens

Becker Muscular Dystrophy

- milder form, adult onset

References

1. ↑ Antonella Galli, Dimitri Robay, Marco Osterwalder, Xiaozhong Bao, Jean-Denis Bénazet, Muhammad Tariq, Renato Paro, Susan Mackem, Rolf Zeller **Distinct roles of Hand2 in initiating polarity and posterior Shh expression during the onset of mouse limb bud development.** PLoS Genet.: 2010, 6(4);e1000901 PubMed 20386744 | PMC2851570 (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2851570>) | PLoS Genet. (<http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1000901>)
2. ↑ Amitabha Bandyopadhyay, Kunikazu Tsuji, Karen Cox, Brian D Harfe, Vicki Rosen, Clifford J Tabin **Genetic analysis of the roles of BMP2, BMP4, and BMP7 in limb patterning and skeletogenesis.** PLoS Genet.: 2006, 2(12);e216 PubMed 17194222 | PMC1713256 (<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1713256>) | PLoS Genet. (<http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.0020216>)
3. ↑ Amitabha Bandyopadhyay, Kunikazu Tsuji, Karen Cox, Brian D Harfe, Vicki Rosen, Clifford J Tabin **Genetic analysis of the roles of BMP2, BMP4, and BMP7 in limb patterning and skeletogenesis.** PLoS Genet.: 2006, 2(12);e216 PubMed 17194222 | PMC1713256 (<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1713256>)

Online Textbooks

- **Developmental Biology** by Gilbert, Scott F. Sunderland (MA): Sinauer Associates, Inc.; c2000 Formation of the Limb Bud (<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?&rid=dbio.section.3928>) | Generating the Proximal-Distal Axis of the Limb (<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?&rid=dbio.section.3941>)
- **Molecular Biology of the Cell** Alberts, Bruce; Johnson, Alexander; Lewis, Julian; Raff, Martin; Roberts, Keith; Walter, Peter New York and London: Garland Science; c2002 Figure 21-13. Sonic hedgehog as a morphogen in chick limb development (http://www.ncbi.nlm.nih.gov/books/bv.fcgi?highlight=limb_development&rid=mboc4.figgrp.3815)

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- **Bookshelf** limb development (http://www.ncbi.nlm.nih.gov/sites/entrez?db=Books&cmd=search&term=limb_development)
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Images

Stage13



Stage14

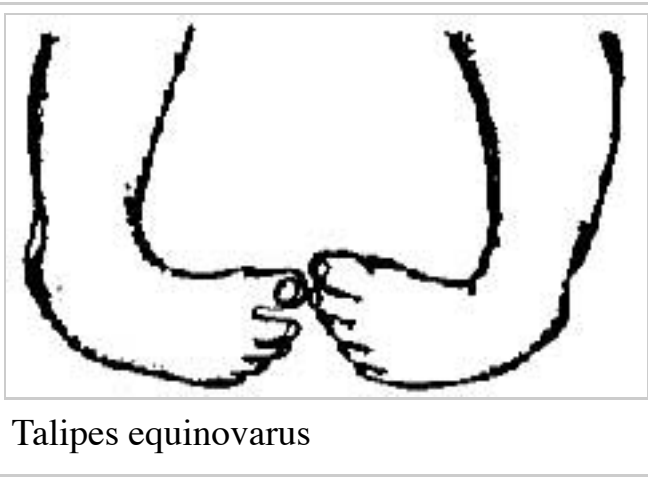
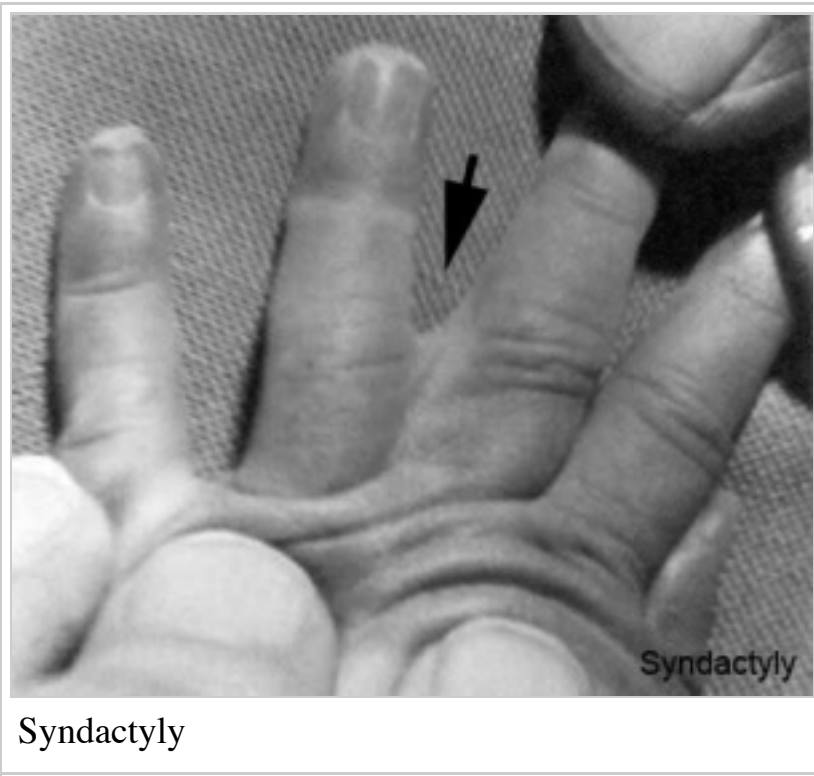




Fig. 251. Showing the Origin of the Ligamentum Teres and Reflected Bundle of the Capsular Ligament.

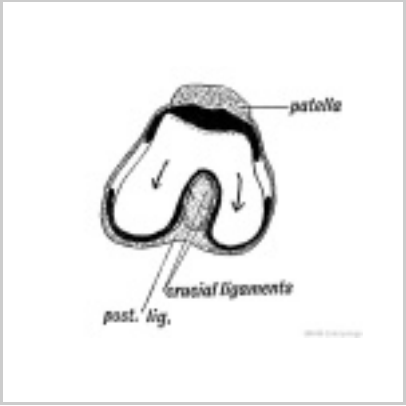


Fig. 252. Showing the Origin of the Crucial Ligaments of the Knee.

External Links

***External Links Notice** - The dynamic nature of the internet may mean that some of these listed links may no longer function. If the link no longer works search the web with the link text or name.*

- Embryo Images Limb Unit (https://syllabus.med.unc.edu/courseware/embryo_images/unit-mslimb/mslimb_htms/mslimb014.htm)
- International J. Dev. Biology Vol 46 Special Issue- Limb Development 2002 (<http://www.ijdb.ehu.es/0207contents.htm>)
- Research Labs - Rolf Zeller University of Basel Medical School (<http://pages.unibas.ch/anatomie/zeller/seiten/seite1.html>)

2015 Course: **Week 2** Lecture 1 Lecture 2 Lab 1 | **Week 3** Lecture 3 Lecture 4 Lab 2 | **Week 4** Lecture 5 Lecture 6 Lab 3 | **Week 5** Lecture 7 Lecture 8 Lab 4 | **Week 6** Lecture 9 Lecture 10 Lab 5 | **Week 7** Lecture 11 Lecture 12 Lab 6 | **Week 8** Lecture 13 Lecture 14 Lab 7 | **Week 9** Lecture 15 Lecture 16 Lab 8 | **Week 10** Lecture 17 **Lecture 18** Lab 9 | **Week 11** Lecture 19 Lecture 20 Lab 10 | **Week 12** Lecture 21 Lecture 22 Lab 11 | **Week 13** Lecture 23 Lecture 24 Lab 12 | **Projects:** Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Students | Student Designed Quiz Questions | Moodle page (<http://moodle.telt.unsw.edu.au/course/view.php?id=15814>)

Glossary Links

A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z | Numbers | Symbols

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