Limb Development

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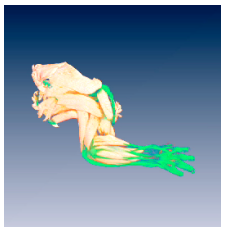
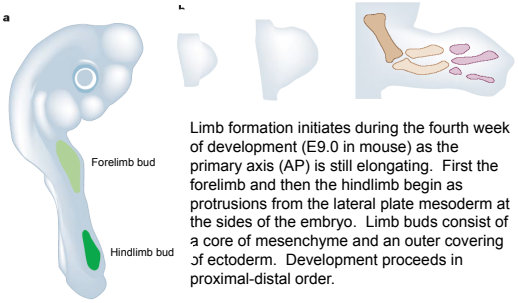
What we will cover...



What signals control initiation of limb bud formation ?

What signals transform the *embryonic limb bud* into a *mature limb* comprised of a precisely interconnected array of many different tissues?

EARLY LIMB PATTERNING:

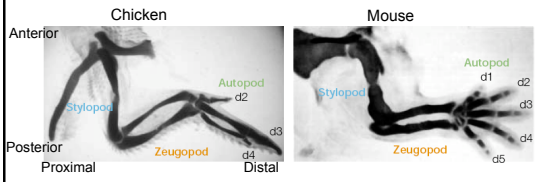


Limb formation initiates during the fourth week of development (E9.0 in mouse) as the primary axis (AP) is still elongating. First the forelimb and then the hindlimb begin as protrusions from the lateral plate mesoderm at the sides of the embryo. Limb buds consist of a core of mesenchyme and an outer covering of ectoderm. Development proceeds in proximal-distal order.

1 in 200 live human births display limb defects.

Limb skeletal elements:

Dorsal: top of hand/paw
Ventral: palm/sole

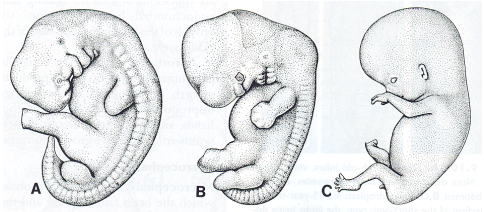


Stylopod: The proximal element of a limb.
The **humerus** in the forelimb; **femur** in the hindlimb

Zeugopod: The intermediate element of a limb.
The **radius** and **ulna** in the forelimb; **tibia** and **fibula** in the hindlimb

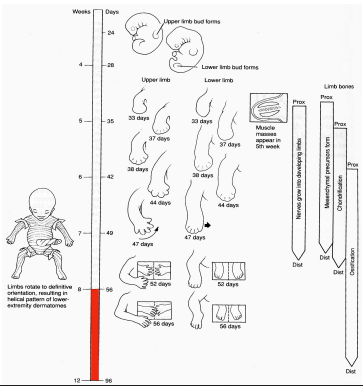
Autopod: The distal elements of a limb.
The **wrist** and the **fingers** in the forelimb; **ankle** and **toes** in the hindlimb

Human Limb Development

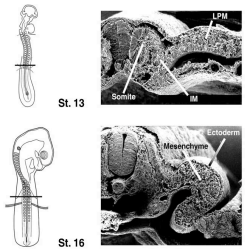


5 weeks 6 weeks 8 weeks

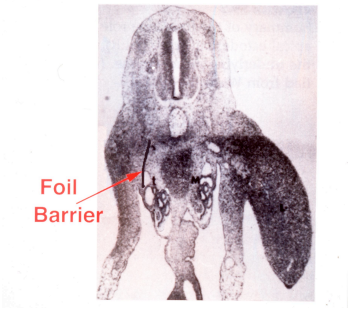
Proximal to distal progression of limb development



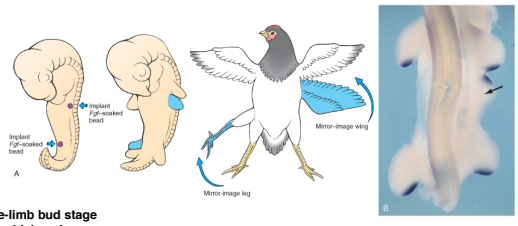
How is limb initiation controlled?



Barrier between the intermediate and lateral plate mesoderm prevents limb bud formation



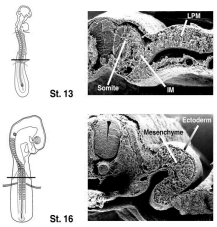
Secreted signaling molecules, fibroblast growth factors (FGFs) can induce ectopic limb formation in the flank of the chick embryo



Pre-limb bud stage chick embryo

-a single molecule (FGF) is capable of inducing cascade leading to limb formation
-cells in the flank are competent to respond to this secreted signal

Limb initiation



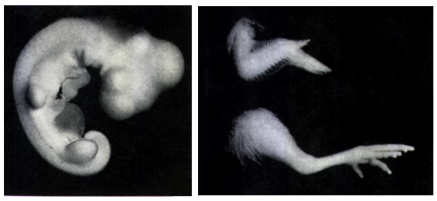
lpm: lateral plate mesoderm

Axial cue Wnt? → **LPM** Fgf10 → **Ectoderm** Wnt3a, Fgf8

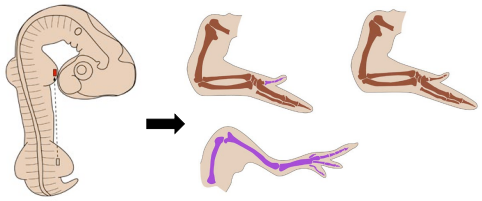
Transfers information from the body to the limb axis

Limb-type specification

Morphologically uniform limb buds develop to form morphologically distinct limb elements



Cell identity and plasticity in the developing limb bud



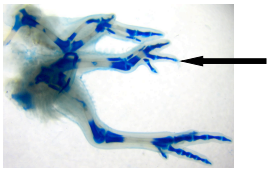
Following heterotopic transplant, cells retain forelimb vs hindlimb identity

Saunders et al., 1957, 1959

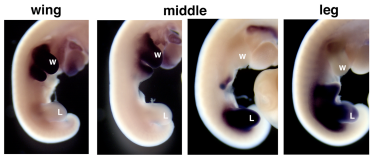
Limb-type specification: candidate genes

chick st.29

A source of FGF applied to the interlimb flank induces ectopic limb formation in the chick embryo: Tbx expression correlates with morphology



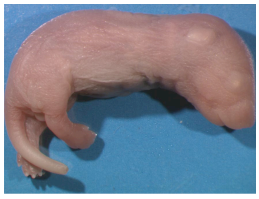
wing middle leg



Tbx5 Tbx4 Pitx1

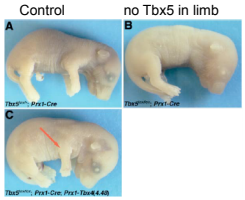
Conditional knock-out of Tbx5 in the limbs leads to the absence of the forelimbs

Tbx5$^{lox/lox}$;Prx1cre



Replacement of Tbx5 with Tbx4 in the limb rescues limb outgrowth. The limb remains a forelimb.

Control no Tbx5 in limb



Tbx5 and *Tbx4* Are Not Sufficient to Determine Limb-Specific Morphologies but Have Common Roles in Initiating Limb Outgrowth

Carolina Minguillon, Jo Del Buono, and Malcolm P. Logan*

Developmental Cell, Vol. 8, 75–84, January, 2005,

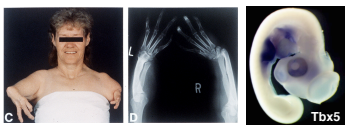
Tbx4 for Tbx5: Rescue

Correlation is not causality: causality must be determined experimentally

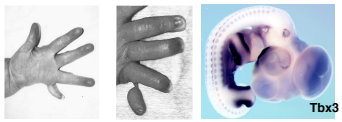
Limb abnormalities are associated with many human congenital syndromes

second most-common congenital abnormality in human live births
also common abnormality following environmental insult (eg Thalidomide)

Mutations in Human *TBX5* are associated with Holt-Oram Syndrome (HOS; OMIM 142900)

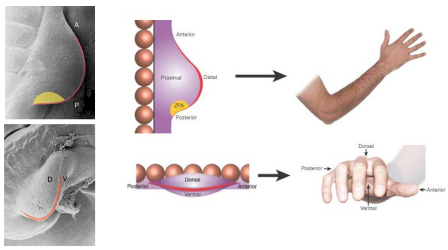


Mutations in human *TBX3* are associated with Ulnar-Mammary Syndrome (UMS; OMIM 181450)



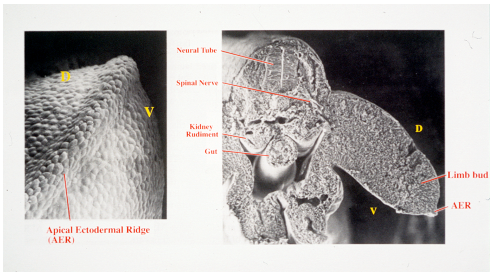
Three signaling centers pattern the three primary limb axes

Apical ectodermal ridge (AER) - proximal-distalZone of polarizing activity (Z

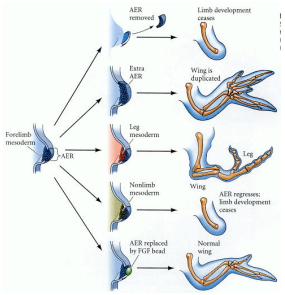


Apical ectodermal ridge (AER) - proximal-distal axis

Signals from the AER maintain limb outgrowth



AER manipulations give insight into AER function



required

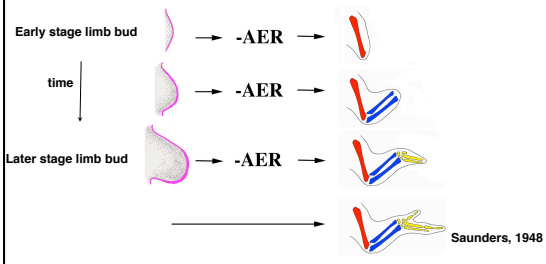
promotes outgrowth

not instructive

not instructive

FGF source

Conclusions from 'classical' embryological studies
- the AER is required for normal proximal-distal pattern

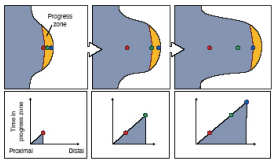


Saunders, 1948

- required for outgrowth, full P-D pattern
- elements laid down in proximal to distal progression

The progress zone (PZ) model

Summerbell et al. Nature 1973

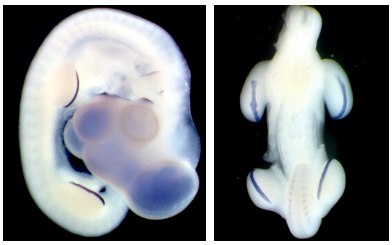


The length of time a cell spends in the PZ may determine proximal-distal identity

What factors are regulating outgrowth?

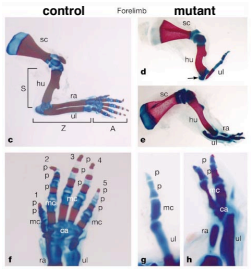
Evidence supporting a role for FGFs in proximal-distal patterning
- **Fgf8 is expressed in the apical ectodermal ridge (AER)**

i.e. present at right time, in right place



Whole mount RNA *in situ* hybridization - chick (st23)

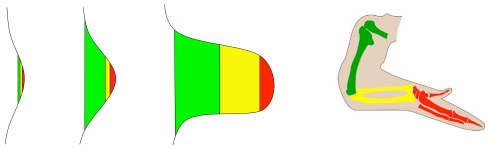
Fgf8/Fgf4 double knock-out mouse: a genetic equivalent to AER removal in the chick



Sun et al., Nature 2002

Limb outgrowth disrupted but distal structures do form. This would not be predicted by the progress zone model

Alternative model: early allocation followed by expansion

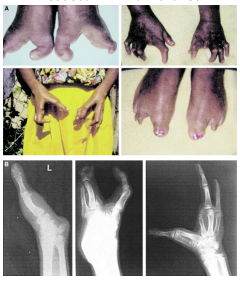


progenitor pools are specified early during limb outgrowth

A signal (FGF) from the AER progressively expands these preexisting populations

AER defects give rise to proximodistal outgrowth phenotypes

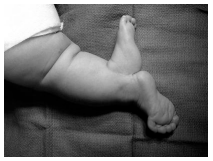
Split-hand/split-foot malformation (SHFM) caused by p63 mutation: reduced AER maintenance



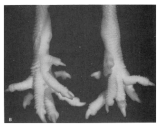
Am J Hum Genet. 2000 July; 67(1): 59–66. Published online 2000 June 5.

Pediatric Radiology 2003 10.1007/s00247-003-1017-3

Diplopodia: ectopic AER?



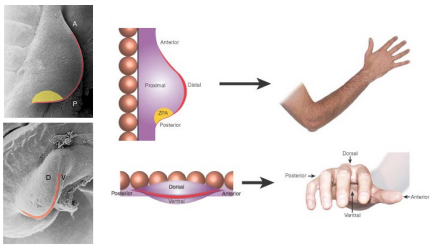
Eudiplopodia: ectopic AER (chicken)



J. Exp. Zool., 176: 219-236.

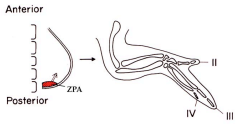
The axes of the limb

Zone of polarizing activity (ZPA) - anterior-posterior

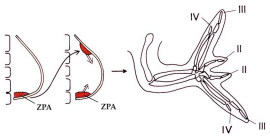


A region of cells in the posterior limb bud, the zone of polarizing activity (ZPA) is important for patterning the anterior-posterior axis of the limb

Again, an important signaling center in the limb was initially identified in 'classical' embryological experiments

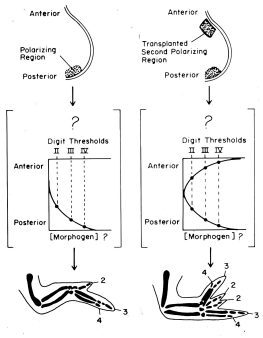


Ectopic digits are not derived from the ZPA graft itself. They are induced in the host tissue. This is a non cell-autonomous phenotype.



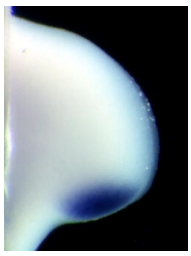
Saunders and Gasseling 1968

Morphogen model: cell identity via threshold responses to a gradient of signaling molecule



circa 1969

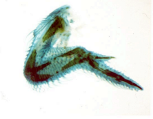
Sonic Hedgehog (Shh) is expressed in the ZPA



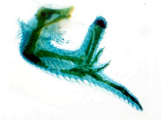
Direct evidence for a role of Shh in anterior-posterior patterning

Sonic hedgehog causes ZPA-like duplications

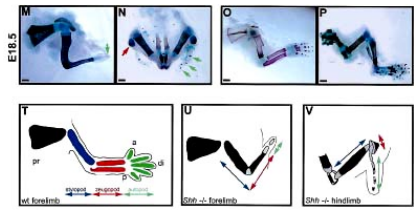
Wild Type



Sonic Protein Implant

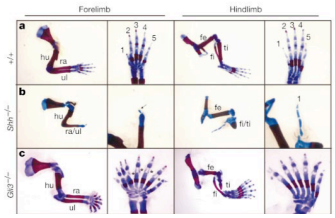


The complementary approach:
Deletion or 'knock-out' of the Shh gene disrupts anterior-posterior patterning of the limb



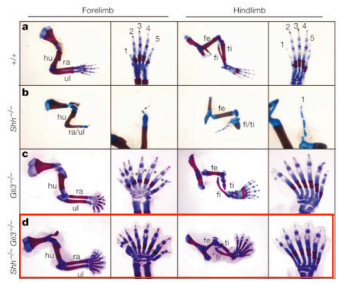
Chiang et al., Nature 1996
Kraus et al., Mech Dev. 2001

Biochemistry: Gli3 transcription factor mediates Shh function



Gli3 loss-of-function results in polydactyly.

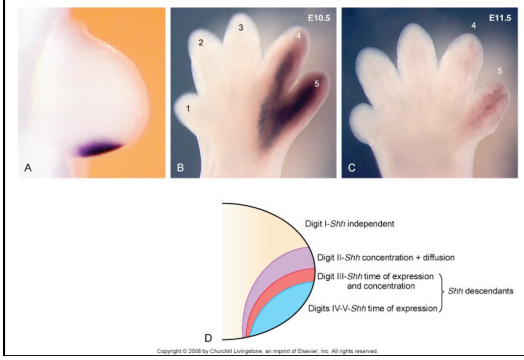
Surprisingly, *Shh/Gli3* double mutants look identical to *Gli3* nulls



Shh modulates inherent polydactylous limb 'ground state.'

Shh inhibits an inhibitor of digit formation and imposes polarity

Regulation of AP identity by Shh involves complex integration of concentration and time dependent signaling



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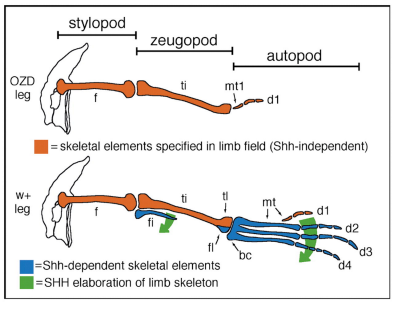
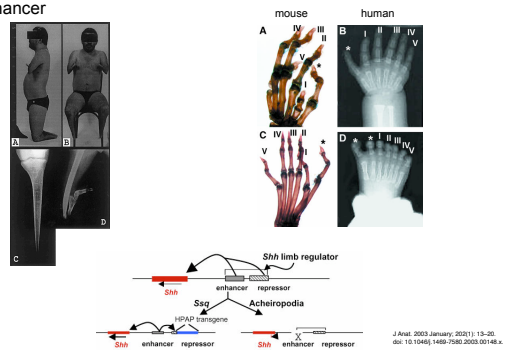
SHH function in generating the amniote limb skeleton



Ros, M. A. et al. Development 2003;130:527-537

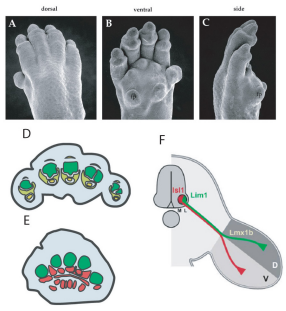
Acheiropodia (OMIM 200500): deletion of the SHH limb enhancer

preaxial polydactyly (PPD2; OMIM 174500): ectopic anterior SHH activity

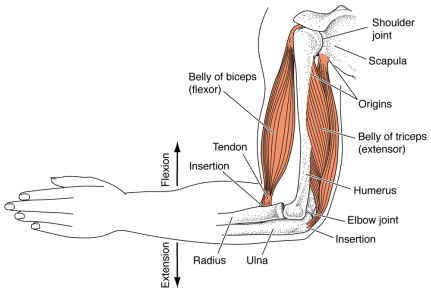


The Dorsal-Ventral Axis

Dorsal-ventral limb asymmetry

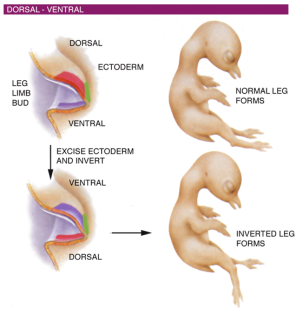


Dorsal-ventral asymmetry is required for coordinated limb movement

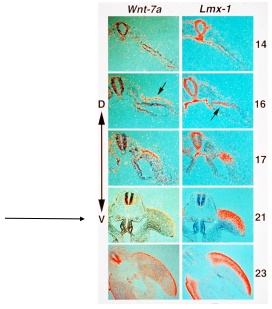


Signals from the dorsal ectoderm play an important role in patterning the dorsal-ventral axis

Initial observations in 'classical' embryological experiments

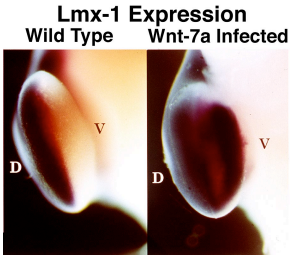


The Wnt7a signal and Lmx1 transcription factor: candidates for DV pattern regulation

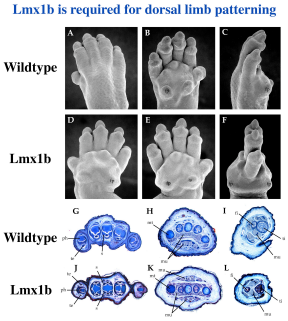


Chick limb buds

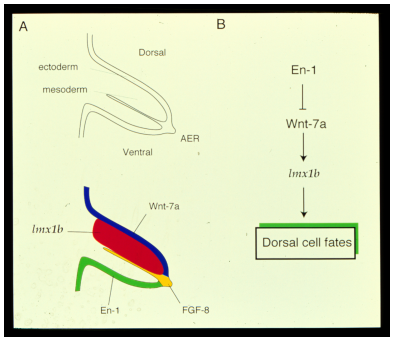
Misexpression of Wnt7a in the ventral ectoderm induces Lmx-1 in the ventral mesenchyme



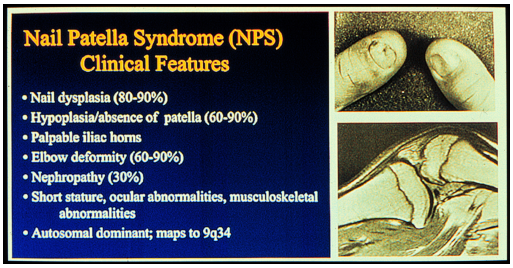
Genetic deletion of Lmx1b in the mouse leads to a loss of dorsal limb pattern



A cascade of factors in the ectoderm controls dorsal-ventral polarity in the mesoderm

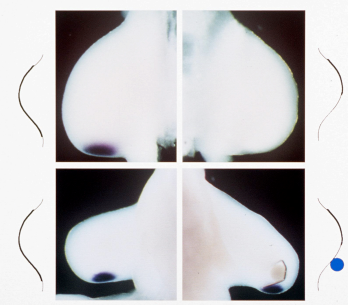


Human LMX1B mutation: Nail Patella Syndrome

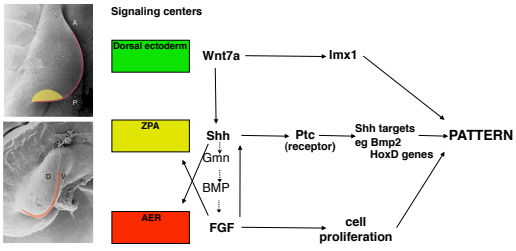


NPS; OMIM 161200

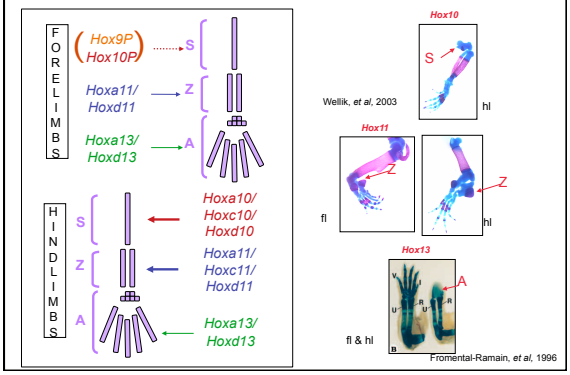
Signaling center crosstalk:
Signals from the AER (FGFs) maintain *Shh* expression



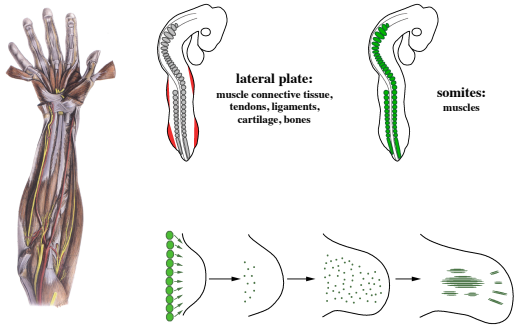
Limb patterning occurs through the coordination of signals from three signaling centers



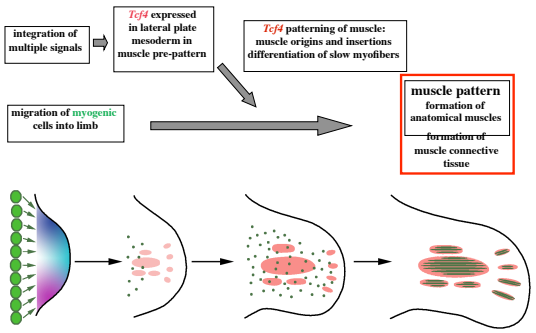
Patterning of the limb elements: *Hox9* through *Hox13* paralogous groups are responsible for establishing morphological pattern



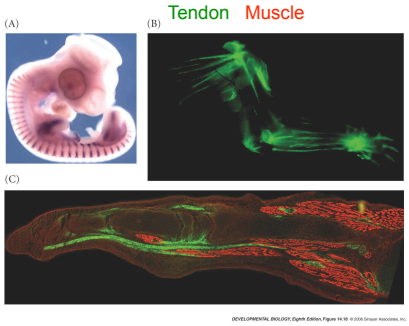
Development of the Limb Musculoskeleton



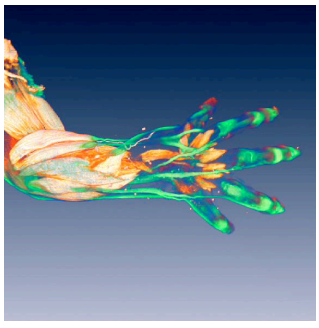
Model of Muscle Patterning in the Limb



Tendons develop in situ, attach to muscle as it develops



Integration of pattern and differentiation results in limb muscle-tendon connectivity



Summary

- Initiation: localized FGF activity
- Outgrowth: FGFs produced by the AER
- Anterior-posterior patterning: ZPA/Sonic hedgehog
- Dorsal-ventral patterning: engrailed-wnt7a-lmx1b
- Integration of signaling centers
- Tbx genes are required for limb outgrowth
- Hox genes are essential regulators of limb development and control segmental development
- Myogenic precursors migrate into the limb, and follow prepattern laid down by connective tissue fibroblasts. Tendons develop in situ