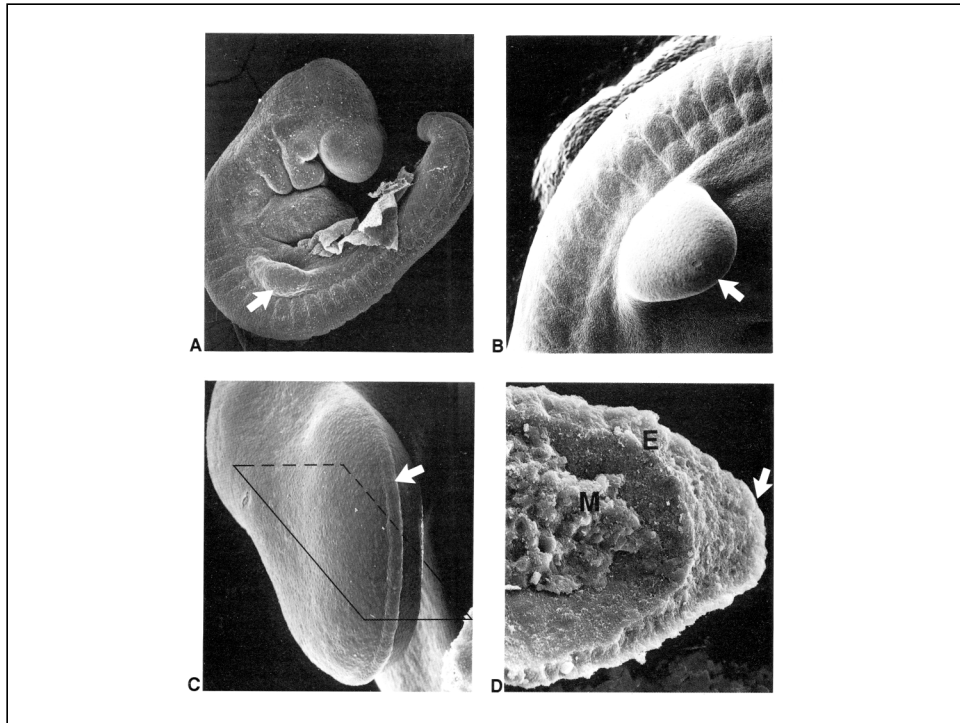
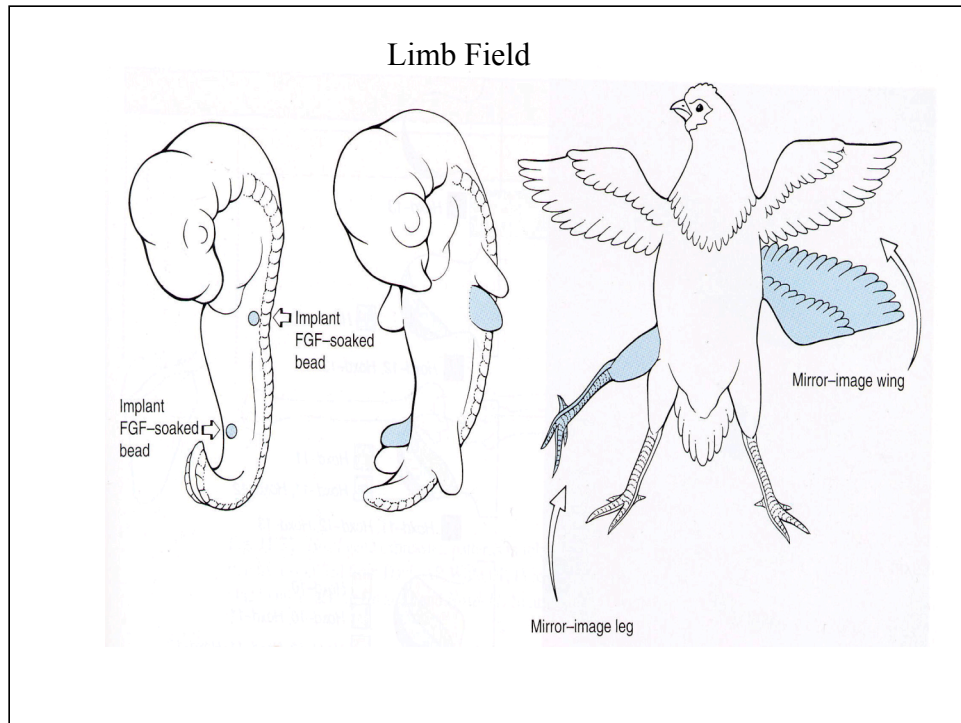


This slide shows all of the major events in limb development.



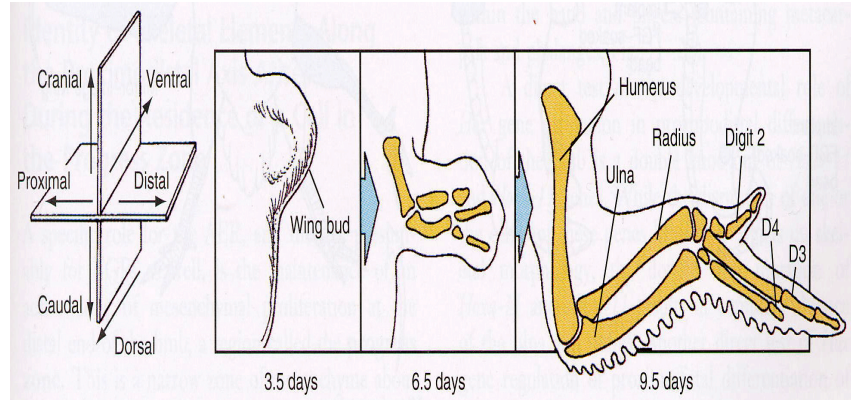
Limb begins as an elevation on the trunk body wall. The upper limb appears on day 24 at C5-C8 and the lower limb at day 28 at L3-L5. The bud consists of an ectodermal cover (E) and a mesodermal/mesenchymal (M) core. The white arrows indicate the apical epidermal ridge (AER) which is essential for limb elongation. Mesodermal core is composed of lateral plate mesoderm (skeleton) and somitic mesoderm (skeletal muscle). The PROGRESS ZONE is the mesoderm immediately under the AER and is where the proximal to distal patterning of limb occurs.



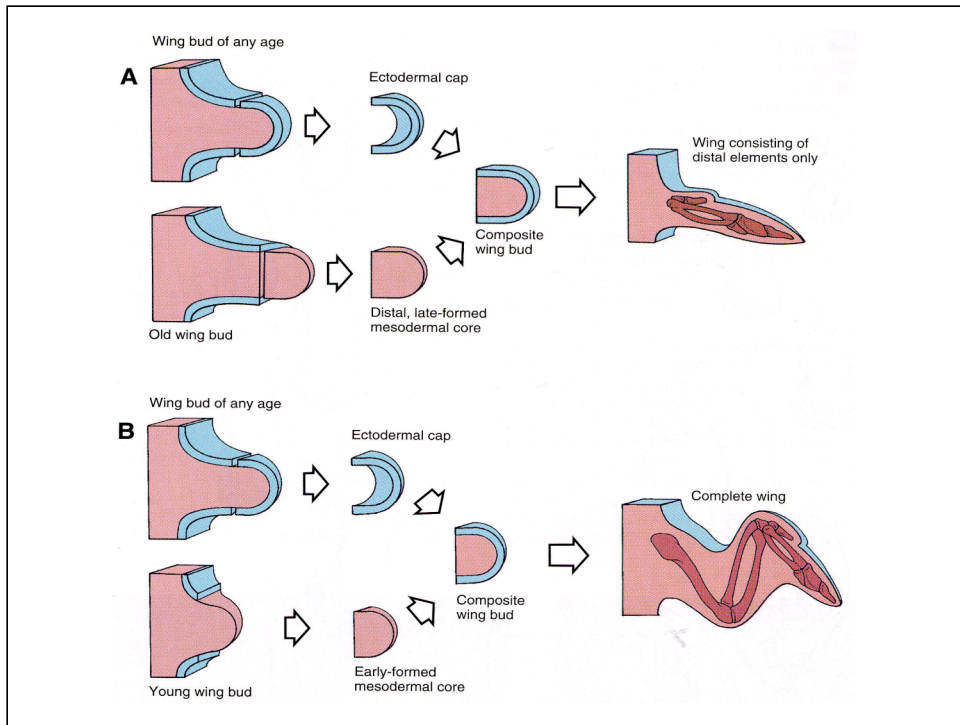
WHAT DETERMINES A LIMB FIELD. TRANSPLANTATION EXPERIMENTS. Mesoderm of limb field is committed prior to appearance of limb bud. Mesenchyme has its own identity

The same signal – FGF- induced the formation of either a wing or a leg. This signal is from the overlying ectoderm. This and other evidence indicates that identity of the limb (how it responds to signals) resides in the mesenchyme (lateral plate mesoderm) which gives rise to the skeleton.

Limb Elongation

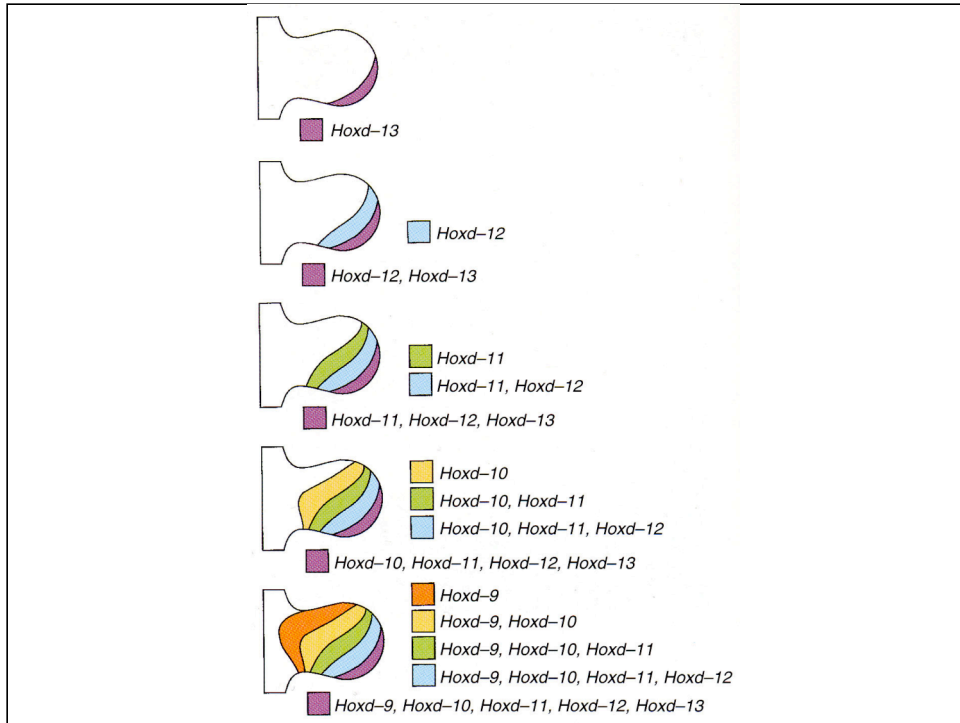


Initial elongation begins on day 33 and continues through the 7th week. Limb outgrowth is orderly and proceeds in a proximal to distal fashion. Limb must be patterned in the three axes indicated on the right side of the slide.

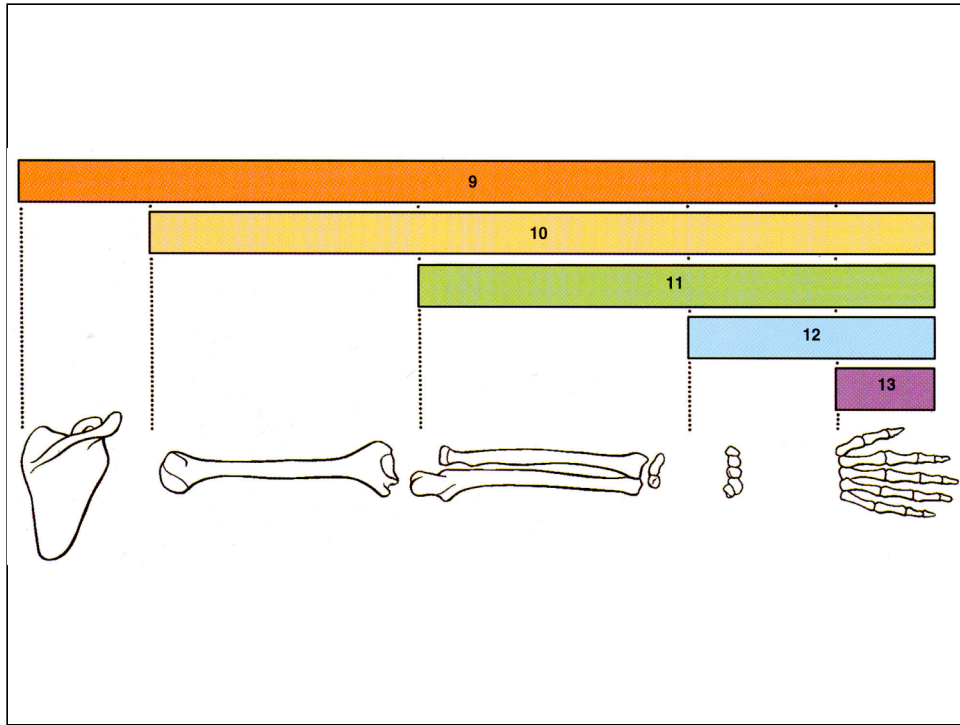


Outgrowth is dependent on the AER. In A remove AER at any age and limb will be truncated. The signal from the AER is constant as an AER of any age can restore orderly growth.

What would you predict would happen if you placed leg mesenchyme under the AER of the wing field?

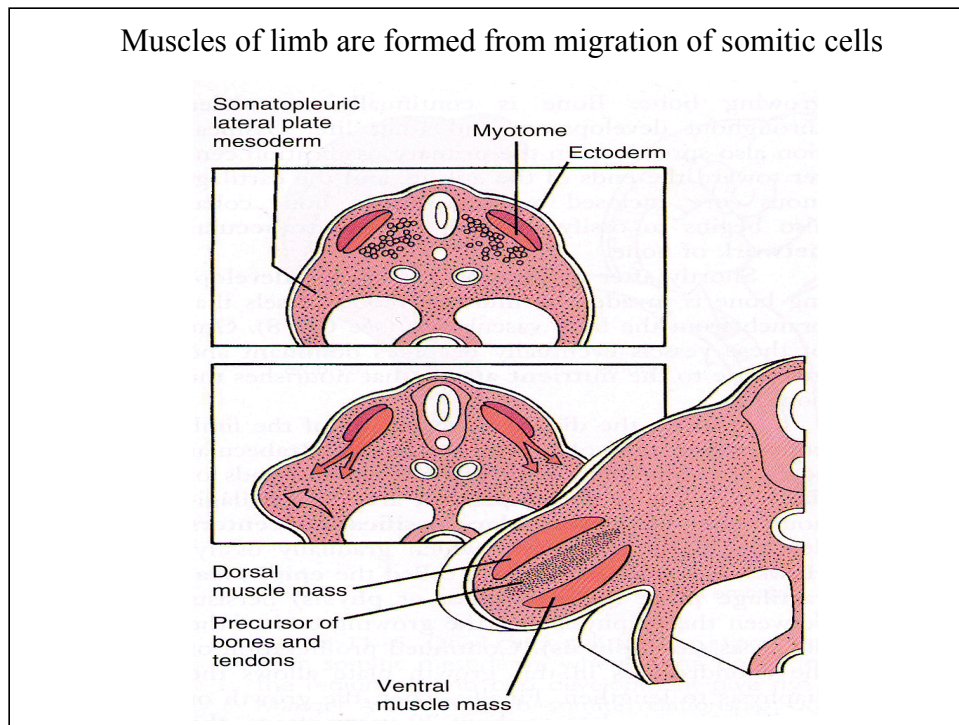


Expression of hox genes over time and limb elongation that underlies differentiation of specific tissues of limb.



Overlapping pattern of hox genes specifying skeletal elements.

Muscles of limb are formed from migration of somitic cells



Migration of somitic cells into presumptive limb begins in the 5th week and give rise to a dorsal and ventral muscle mass. In general the dorsal muscle mass gives rise to the flexors and supinators of the upper limb; extensors and supinators of the lower limb. The ventral muscle mass flexors and pronators in the upper limb and adductors in the lower. Not an absolute rule – see Table in next slide. At the core are the precursors first of the chondrocytes that will form the model and then the osteoblast/tendons.

**Table 11-1
Muscles Derived from the Ventral and Dorsal Muscle Masses of the
Limb Buds**

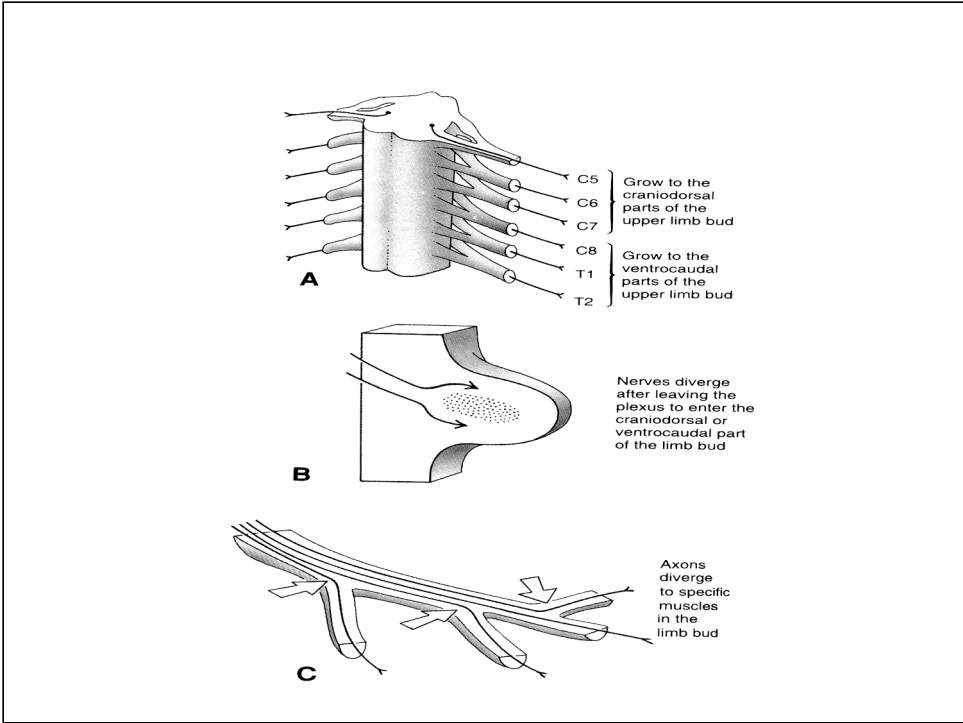
VENTRAL MUSCLE MASS	DORSAL MUSCLE MASS	
<i>Upper Limb</i>	<i>Upper Limb</i>	<i>Lower Limb</i>
Anterior compartment of the arm and forearm	Posterior compartment muscles of the arm and forearm	Anterior compartment muscles of the thigh and leg
All muscles on the palmar surface of hand	Deltoid	Tensor fasciae latae
<i>Lower Limb</i>	Lateral compartment muscles of the forearm and hand	Short head of the biceps femoris
Medial compartment muscles of the thigh	Latissimus dorsi	Lateral compartment muscles of the leg
Posterior compartment muscles of the thigh except for the short head of the biceps femoris	Rhomboids	Muscles of the dorsum of the foot
Posterior compartment muscles of the leg	Levator scapulae	Gluteus maximus, medius, and minimus
All muscles on the plantar surface of the foot	Serratus anterior	Piriformis
Obturator internus	Teres major and minor	Iliacus
Gemellus superior and inferior	Subscapularis	Psoas
Quadratus femoris	Supraspinatus (?)	
	Infraspinatus (?)	

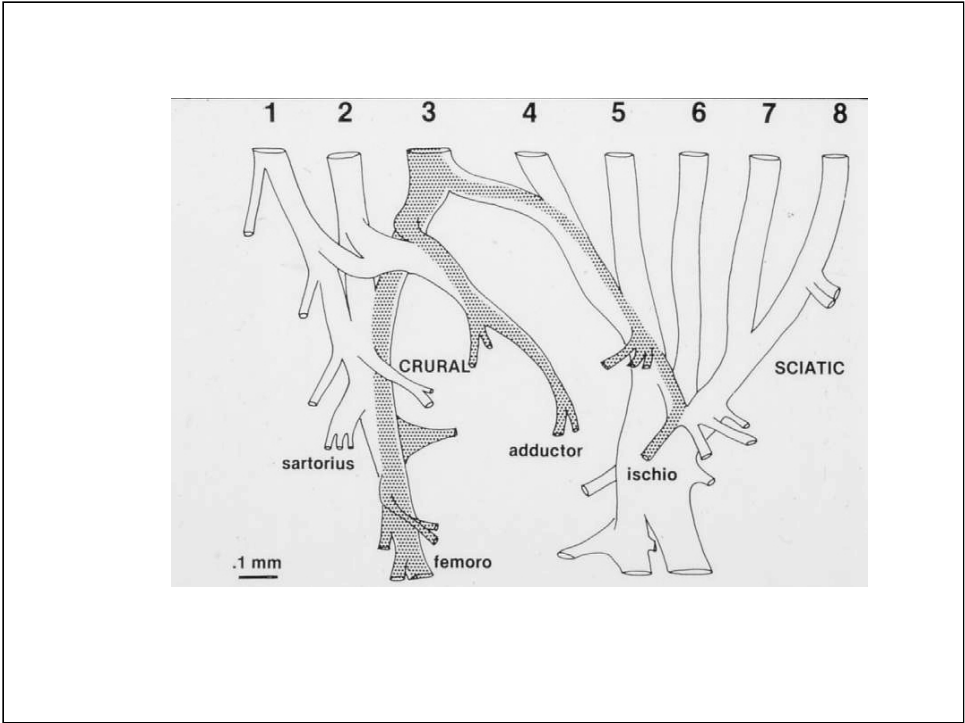
Data from Crafts RC. 1985. A Textbook of Human Anatomy. 3rd Ed. Churchill Livingstone, New York.

Axonal outgrowth to limb prior to muscle formation

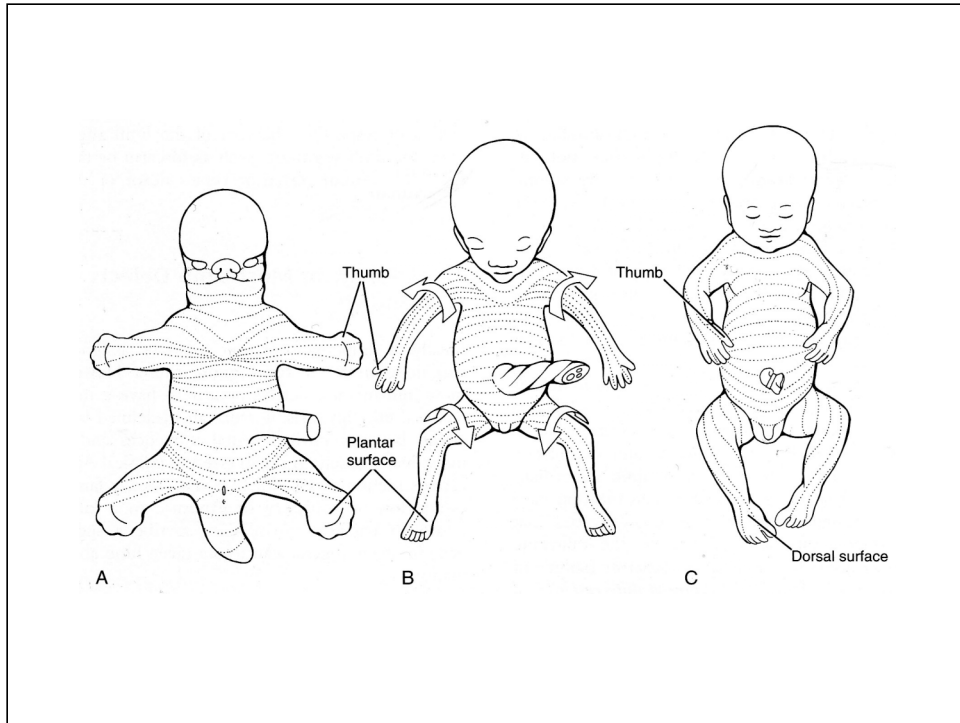


Axons grow into limb bud prior to condensation of the muscle masses

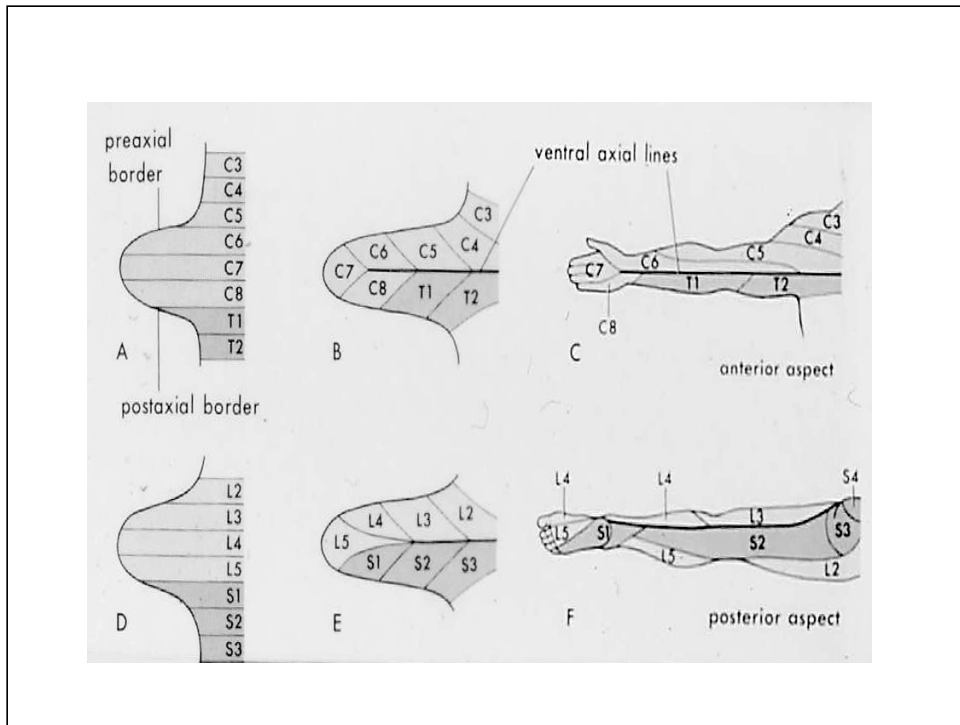




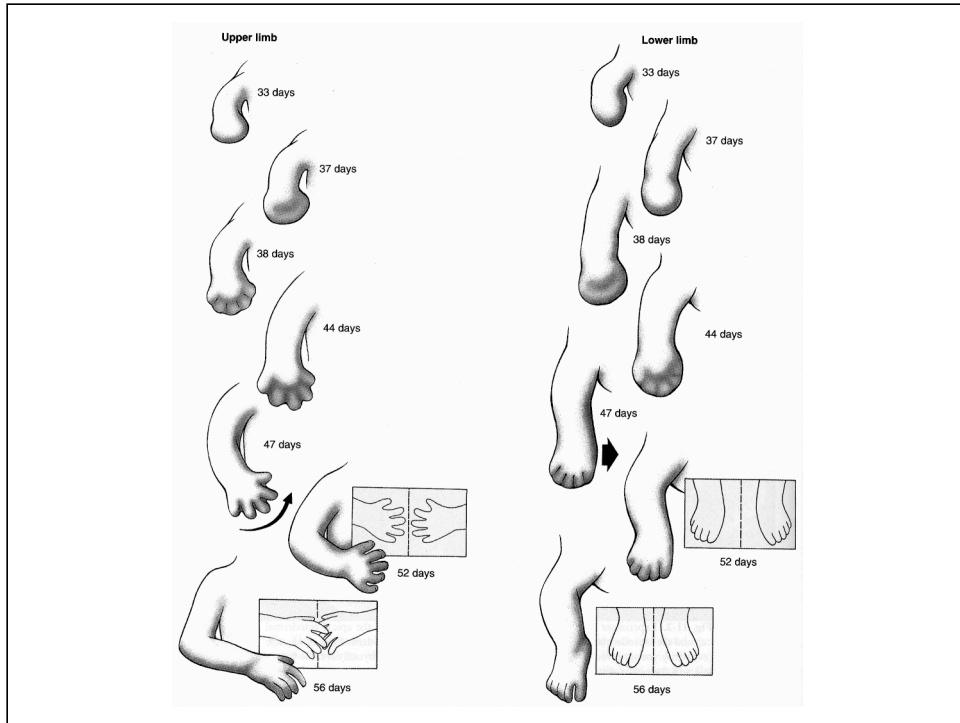
Tracing of specific pattern of innervation from spinal nerves.



Initial outgrowth of innervation to limbs is in the coronal plane (A; end of 6th week) which is “rearranged” following limb rotation (B&C). Both limbs rotate from initial coronal plane into a roughly parasagittal one (B) the upper limb rotates laterally though 90 degrees on the longitudinal axis. The lower limb rotates medially. Therefore segmental pattern of innervation becomes more or less spiral

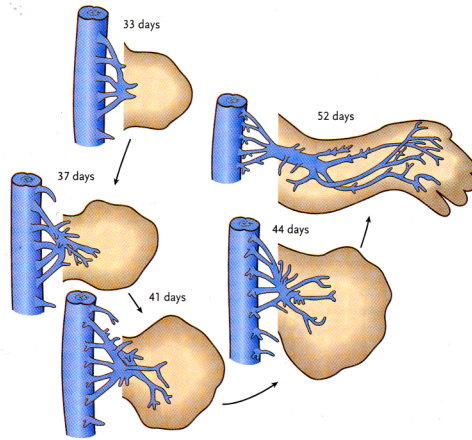


Consequences of rotation.



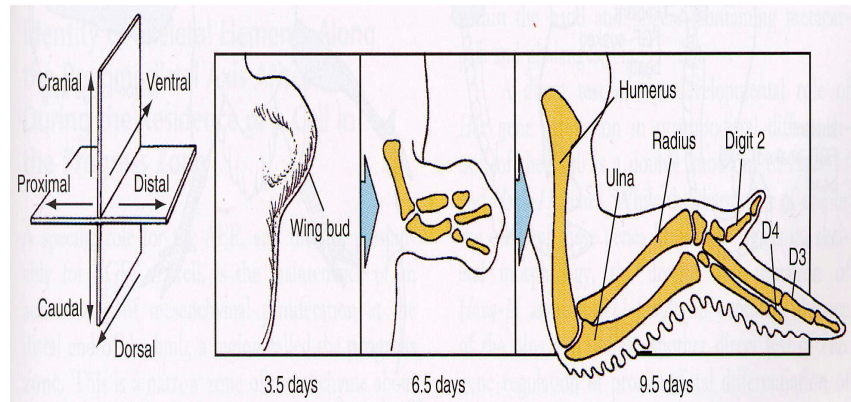
More detailed time course of limb rotation

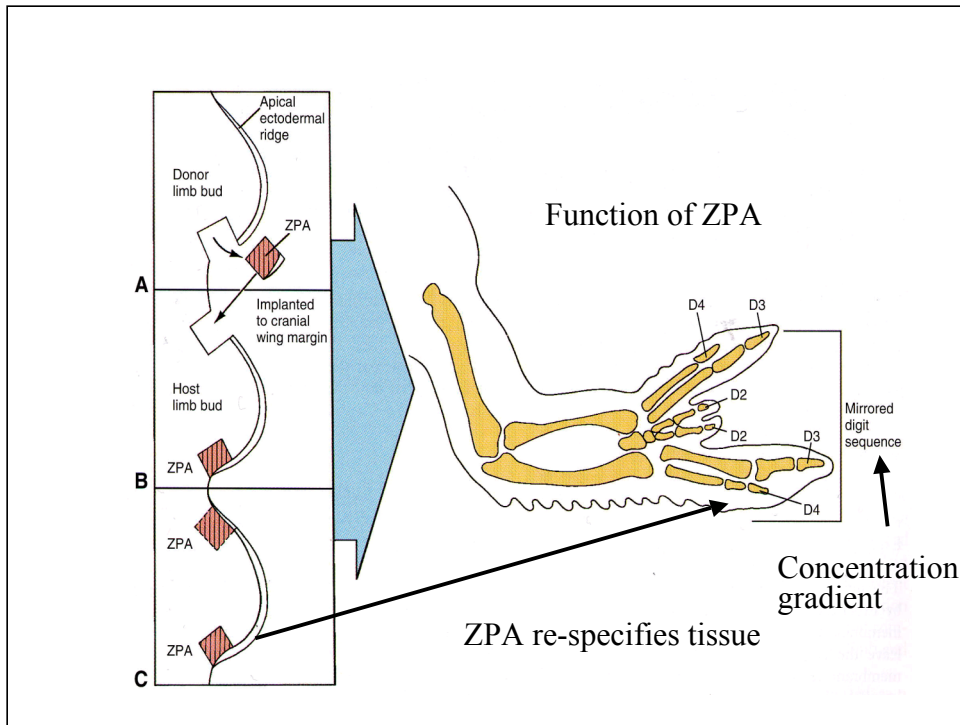
Blood vessel growth into limb.



Pattern of vasculature. Parallels nerves.

Patterning of digits



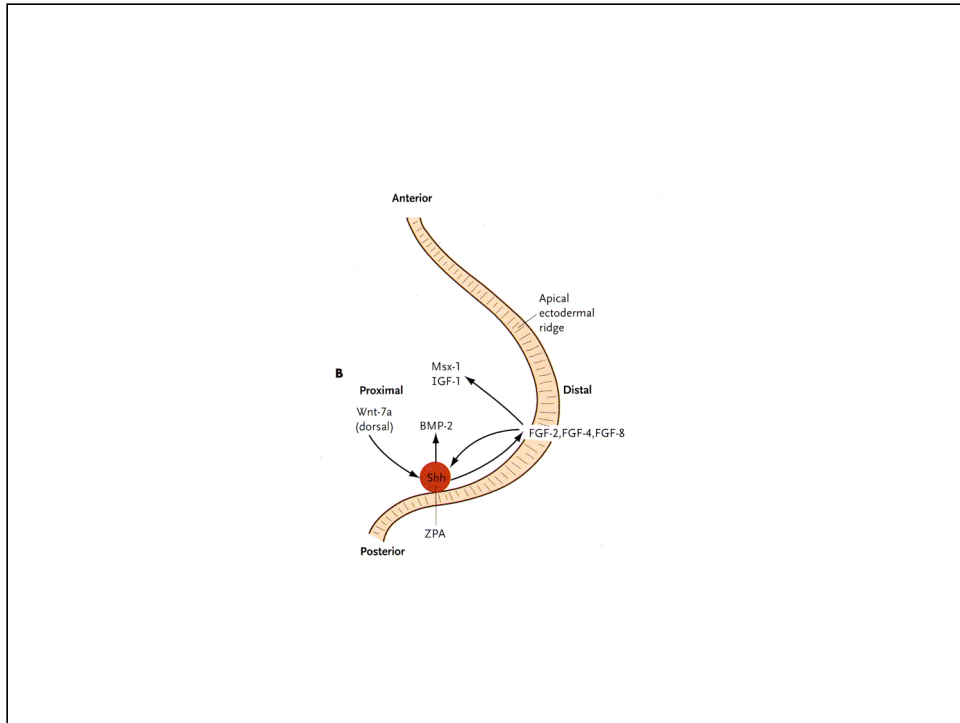


Mirror image suggests diffusible substance, morphogen, that establishes a concentration gradient.

Discuss moving the zpa nearer the original.

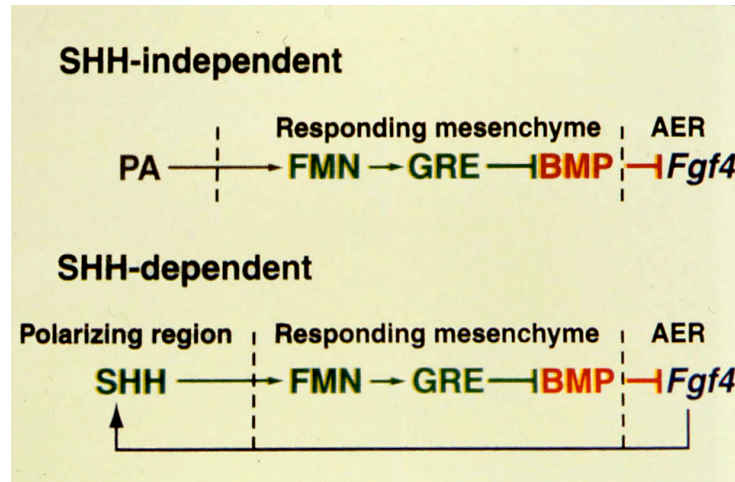
What are the known characteristics of the ZPA?

1. If ZPA is transplanted (at the correct time) to an ectopic site it will respecify tissue.
2. It acts to induce the correct A/P pattern via a concentration gradient.
3. Acts over a very narrow time frame.
4. The activity of the ZPA is universal among quadrapeds and is the same for the fore limb and hind limb.



Proper outgrowth of the limb bud requires a positive feedback loop between Sonic hedgehog (Shh) in the zone of polarizing activity (ZPA) and Fgfs in the overlying apical ectodermal ridge. FGF's of AER are necessary to maintained the zone of polarizing activity (ZPA). The ZPA in turn secretes sonic hedge hog (Shh) which maintains AER and patterns hand. Can mimic ZPA with cells transfected with Shh)

Shh dependent and independent signaling.



Limb-bud mesenchyme to AER signaling and establishment of the SHH/FGF4 feedback loop through FMN- and GRE-mediated BMP antagonism. Top, SHH-independent induction of the mesenchymal response cascade (*Fmn* and *Gre*) by an unknown posterior activity. Presumed early inhibition of BMP activity by *Gre* allows *Fgf4* activation in the AER independently of SHH signaling. Bottom, SHH-dependent maintenance and distal-anterior propagation of the SHH/

FGF-4 feedback loop. In contrast to the activation phase (top), SHH signaling by the polarizing region is essential to maintain and propagate *Fmn* and *Gre* expression in responding mesenchymal cells and *Fgf4* expression in the overlying AER. FGF4 signaling in turn maintains *Shh* expression by the polarizing region, resulting in establishment and maintenance of the SHH/FGF4 feedback loop.

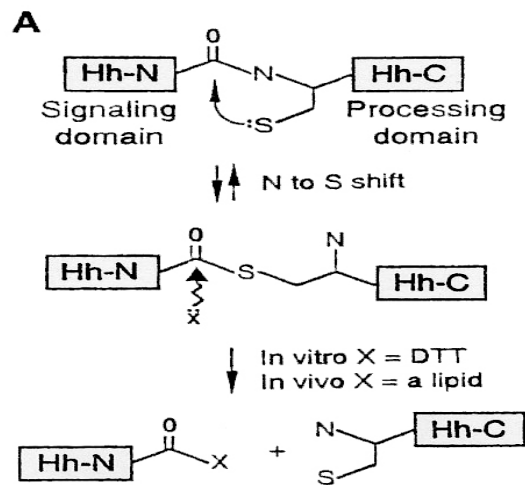
1. What is the polarizing signal?
 - (a) Sonic hedgehog (Shh) is the polarizing signal.
 - (b) Beads soaked in Shh can substitute for the ZPA.
 - (c) The notochord (which makes Shh) can substitute for the ZPA.

2. Concentration gradient.

As suggested by the transplantation experiments, the polarizing activity stimulates differentiation of the mesenchyme in a concentration dependent manner.

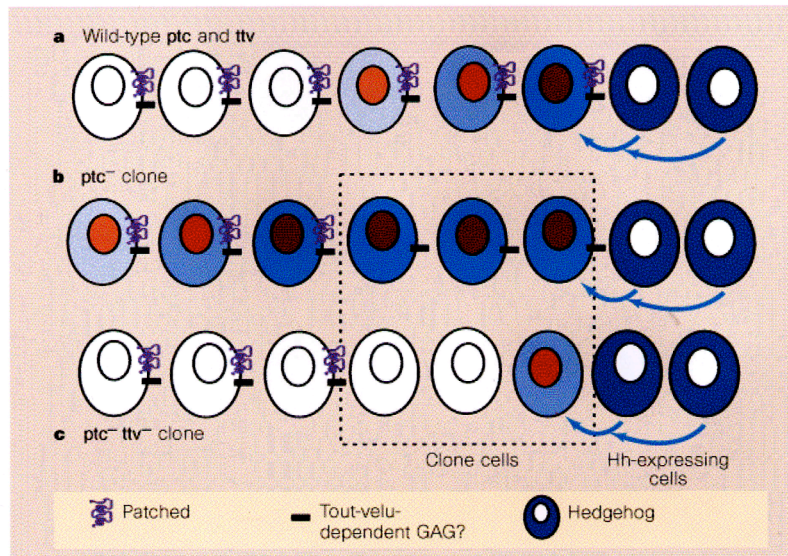
This has been verified by using fibroblasts that have been transfected with Shh. Depending on the number of cells used to substitute for the ZPA different patterns of digit formation occur.

How to limit diffusion of Shh.

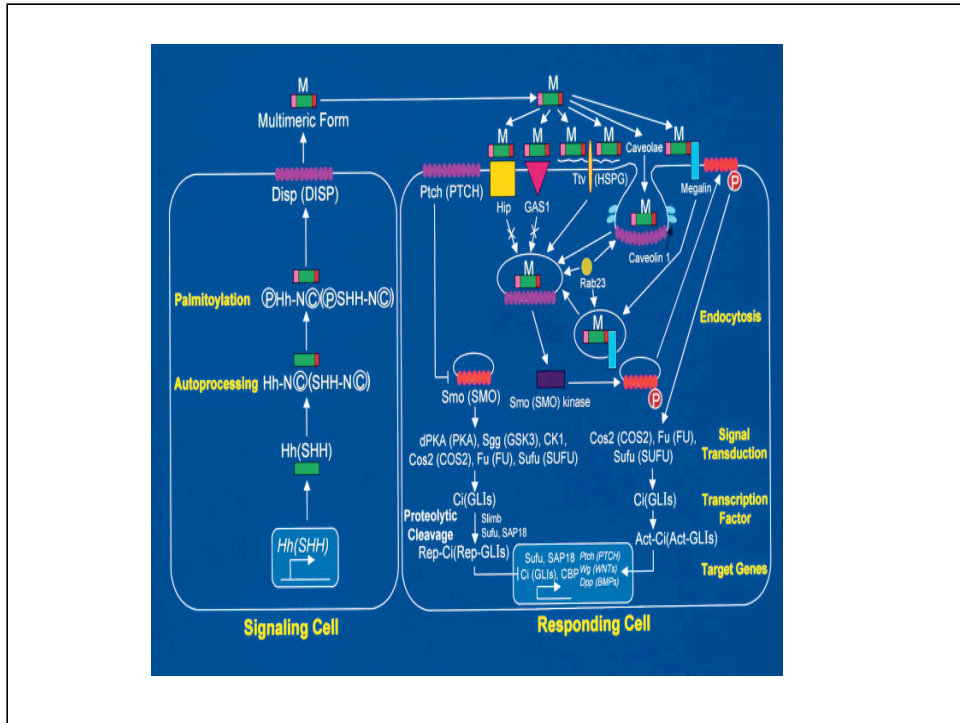


Diffusion of Shh is limited by a post-translational modification. Shh protein has two domains. The processing domain autonomously inserts a sulfate group as shown. The S/N domain is unstable and in vivo it is replaced by a lipid such as cholesterol so that the diffusion of Shh has to be in a lipophilic environment.

Sharpening the concentration gradient but getting to where you need to go.



Further sharpening of SHH gradient. The diffusion of Shh is limited by sequestration by Patched a transmembrane multiple span protein. A second protein, tout-velu – which interacts with glycosaminoglycans in the extracellular matrix to – promotes diffusion. a) HH travels X cell diameters, (b) no patch goes further (isn't internalized), c) no tv goes fewer cell diameters.



This pathway is also important and/or reactivated in neoplasms such as basal cell carcinoma.

What maintains the ZPA?

In vitro ZPA loses polarizing activity if cultured alone.

In vitro ZPA retains activity if cultured with AER.

If one ablates the AER completely, the ZPA is lost.

As in the leg/wing experiment FGF (4/8) can replace the AER.

FGF is made in the right place (over ZPA)

FGF diffusion is limited by binding to ECM.

In summary:

1. Two sources of mesoderm limb: lateral plate forming cartilage and bone; somite-derived cells forming muscle.
2. The trunk level of the lateral plate mesoderm determines whether it become forelimb or hindlimb.
3. The ectoderm is a signaling center regulating growth and it participates in patterning this outgrowth.
4. Because limb innervation arrives just as the muscle masses are forming, the segmental pattern of innervation will be altered as limb rotate.