

Developing limb region is an example of a "morphogenetic field"

Properties of a "morphogenetic field" (or in an embryo, an "embryonic field")

Any part of field is competent to form complete structure.

Partial field removal results in 'regulation' of remaining tissue.

Splitting field into non-communicating parts can result in structure duplication.

Field region larger than portion normally generating structure.

Removal of region normally forming structure reveals nearby tissue within field competent to form structure.

Figure 16.3 *The Regulative Ability of the Limb Field as Demonstrated by an Experiment of Nature*



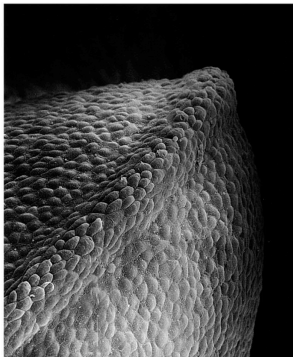
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Francesco Lentini, the 'handsome three-legged man' of P.T. Barnum's 'Freak Show'



Possible victim of a 'split' limb field during embryogenesis?

Figure 16.9 *An Early Chick Forelimb Bud*



SEM showing the Apical Ectodermal Ridge (AER)

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Limb bud development involves **reciprocal interaction** between overlying ectoderm (AER) and mesoderm (PZ).

Secreted signaling protein: Fibroblast Growth Factor (FGF)

1. FGF10 from limb mesenchyme induces formation of AER; continued secretion maintains its presence.
2. AER maintains PZ mesoderm via FGF8 secretion.

AER = apical ectodermal ridge

PZ = progress zone (mesoderm)

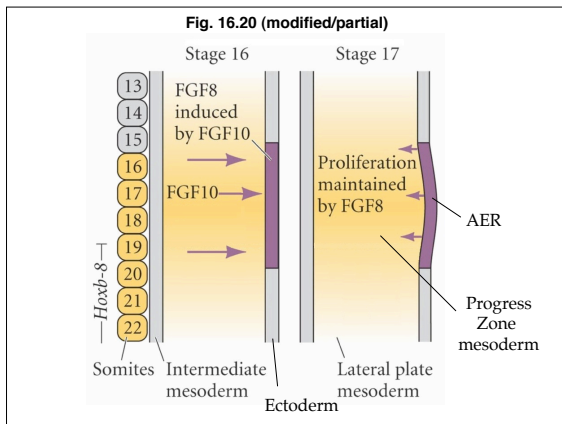
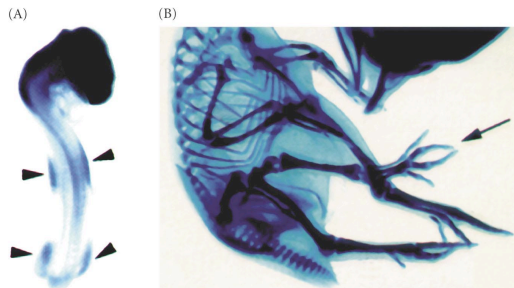


Figure 16.6 FGF10 Expression and Action in the Developing Chick Limb

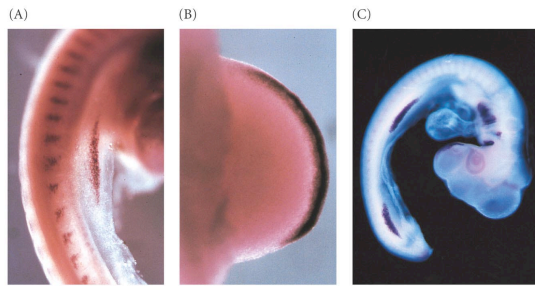


FGF10 is found at sites of limb bud formation

Ectopic FGF10 (implanted bead) can cause formation of ectopic limb (arrow)

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Figure 16.11 FGF8 in the Apical Ectodermal Ridge



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Fig. 16.10(modified) Effect of AER On Underlying Mesenchyme

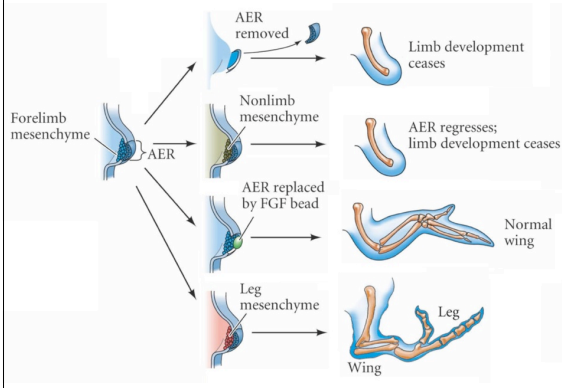
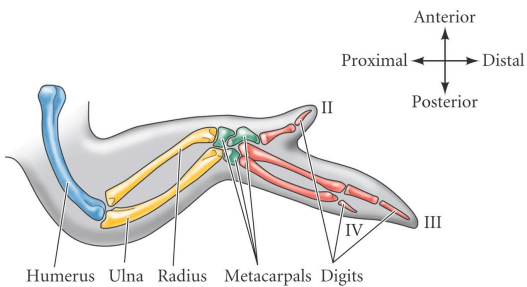
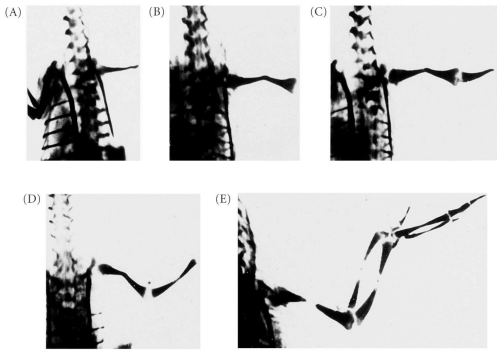


Figure 16.1 Skeletal Pattern of the Chick Wing



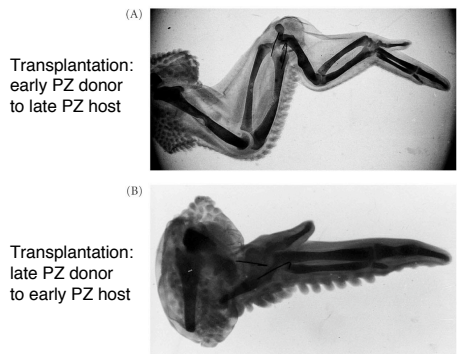
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Figure 16.12 Dorsal Views of Chick Skeletal Patterns after Removal of the AER from the Right Wing Bud of Chick Embryos



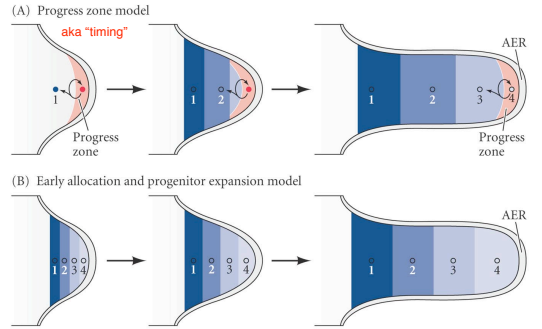
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Figure 16.13 Control of Proximal-Distal Specification by the Progress Zone Mesenchyme



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Figure 16.14 Two Models for the Mesodermal Specification of the Proximal-Distal Axis of the Limb



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Specification of Anterior-Posterior Axis in Limb

Zone of Polarizing Activity (ZPA) in the posterior limb bud discovered by transplantation experiments.

Properties of the ZPA are consistent with its release of a 'morphogen' that creates a 'gradient of positional information' (this is like *bicoid* protein in fly embryo).

Normal Chick Forelimb Development

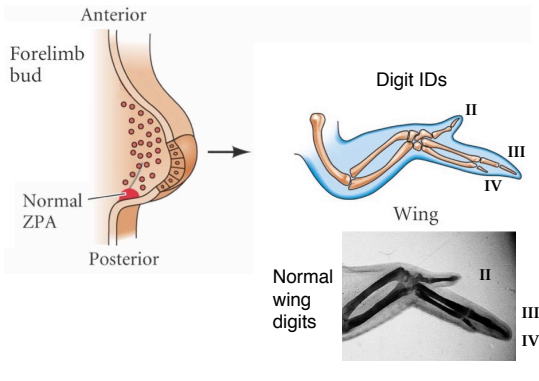
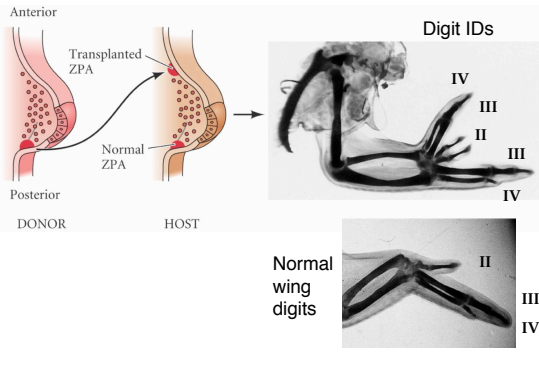
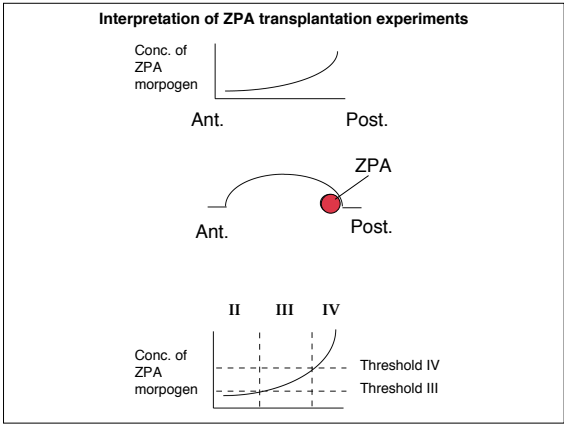
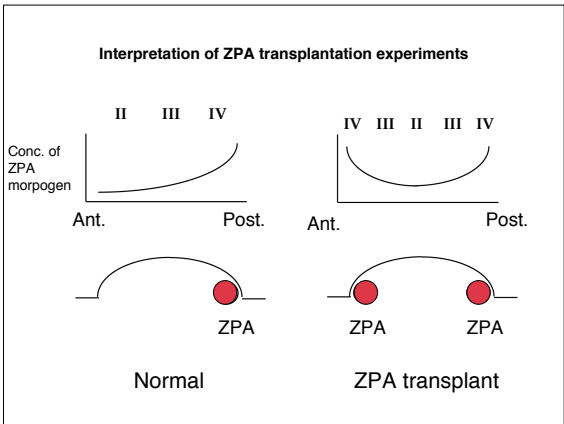


Figure 16.17 ZPA Grafted to Anterior Limb Bud Mesoderm







What is the ZPA morphogen?

ZPA morphogen was once thought to be **retinoic acid (RA)**.

RA was present in posterior of early limb bud.

RA-soaked bead transplants could mimic ZPA transplant.

ZPA morphogen is now known to be **Sonic Hedgehog** protein.

Where did we go wrong / how were we misled?

RA can artificially induce secretion of Shh.

Criteria for an "inducer" substance

Qualitative

- Substance is present in correct location.
- Substance is present throughout period of inducer activity.
- Addition or deletion of substance has predicted effect.

Quantitative

Substance is present at concentrations consistent with addition (transplantation) or deletion (removal) experiments.

Figure 16.18 Sonic Hedgehog Protein Is Expressed in the ZPA

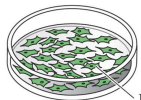


Sonic Hedgehog present throughout period of ZPA morphogen activity & in concentrations consistent with experimental results.

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Figure 16.19(1) Assay for Polarizing Activity of Sonic Hedgehog

Transfect *shh*-expressing virus and allow viral spread

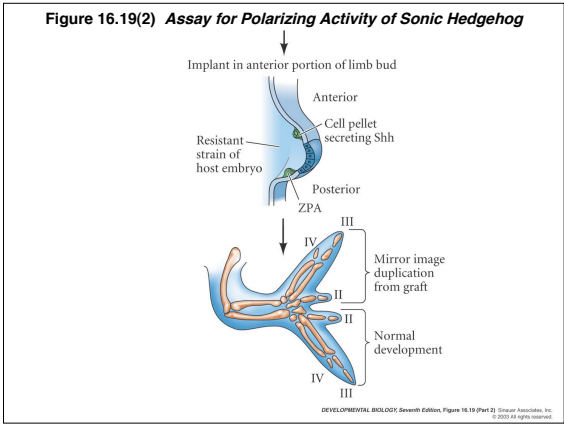


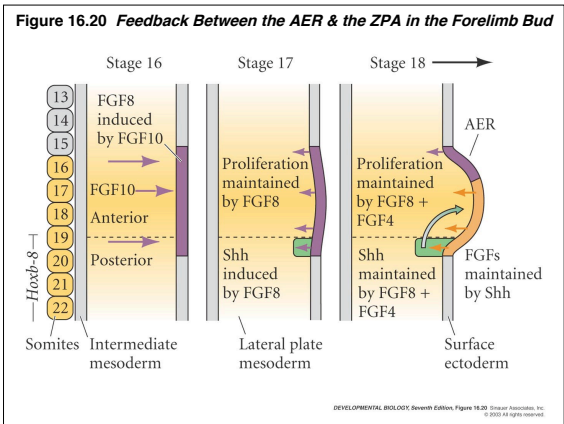
Infectable strain of chick embryo fibroblast cells

Centrifuge cells



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How does Shh pattern the limb bud?

Shh gradient leads to activation of Hox genes in the limb bud.

'Posterior' group Hox genes are activated progressively in an anterior-posterior / proximal-distal pattern.

HoxD-9 through -13 are especially important; also comparable HoxA genes.

Deletion of posterior Hox genes can cause loss of proximal-distal structures.

Figure 16.15(2) Deletion of Limb Bone Elements by the Deletion of Paralogous Hox Genes

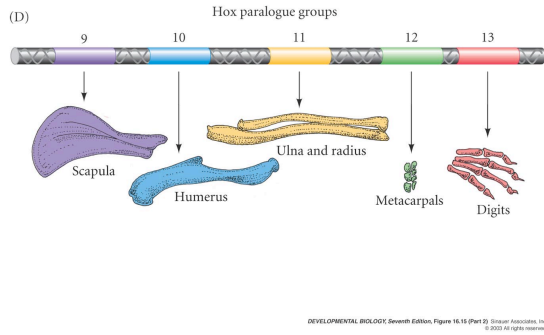
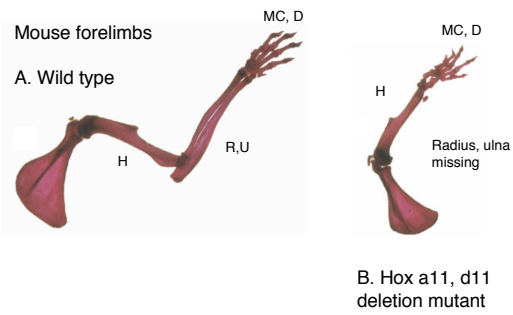


Figure 16.15(A,B) Deletion of Limb Bone Elements by the Deletion of Paralogous Hox Genes



Synpolydactyly (SPD) caused by dominant HoxD13 mutation



Poly-Alanine tracts are common in transcription factors, especially homeodomain tfs, have some unclear role in regulating transcription.

Normal HoxD13 gene **poly-Alanine tract** encodes 15 Alanines.

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>Homeobox D13 [Homo sapiens]
MSRAGSWDMDGLRADGGGAGGAPASSSSSSVAAAAASGQCRGFL
SAPVFAGTHSGRAAAAAAAAAAAAASGFAYPGTSERTGSSSS
SSSSAVVAARPEAPPAKECPAPTPAAAAAPPAPALGYGYHFG
NGYYS CRM SHGVLQONALKSSPHASLGGFPVEKYMDVSGGLASS
SVPANEVPARAKEVSYFYQGYTSPYQHVPGYIDMVSTFGSGEPRH
EAYISMEGYQSWTLANGWNSQVYCTKDQPQGS SHFWKSSFPGDVA
LNQPDMCVYRGRKKRVPYTKLQLKELENEYAINKFINKDKRRR
ISAATNLSERQVTIWFQNRVRVKKKIVSKLKDTVS
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SPD-causing HoxD13 mutations are increased lengths of poly-Alanine tracts.

SPD-causing HoxD13 mutations are increased lengths of poly-Alanine tracts.

Normal HoxD13 gene encodes 15 Alanines, mutant versions have 7-14 more Alanines.

Frequency and severity of effect is correlated with the length of the additional poly-Alanine tract.

