

## Signal Transduction & Hox Genes during Limb Development: On-Line Self-Study

### Retinoic Acid: A Developmental Morphogen

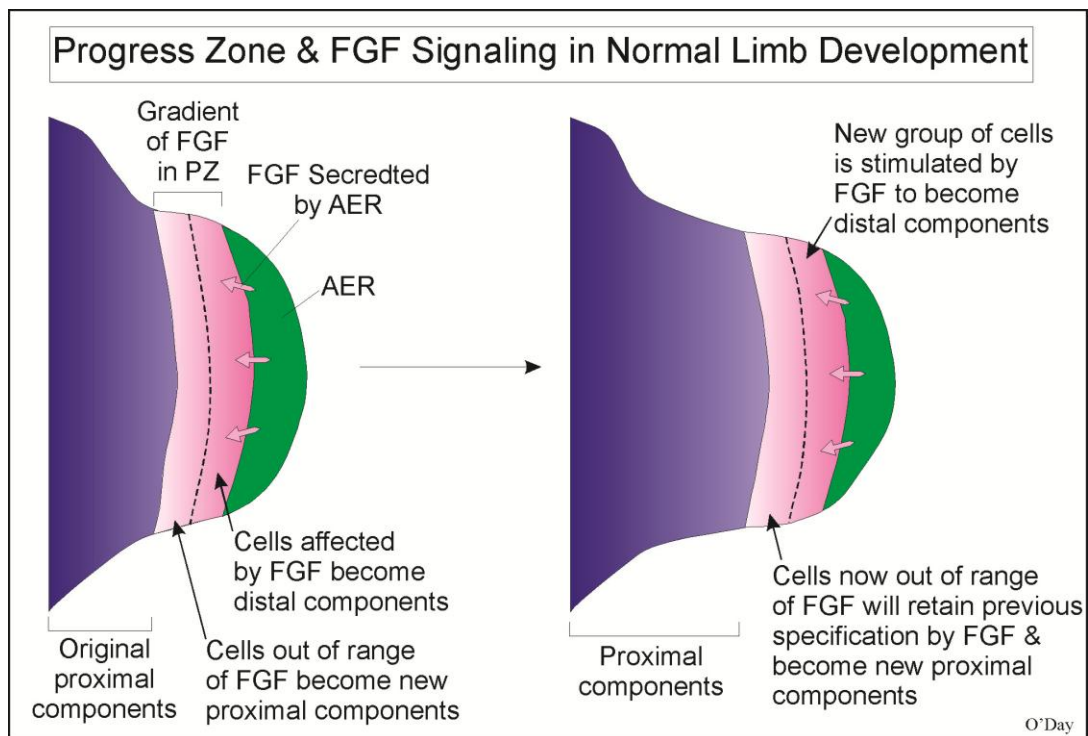
Retinoic acid (RA) is the active form of Vitamin A and has many developmental functions. Excess RA and vitamin A deficiency each affect left-right symmetry (for example, for normal formation of the mammalian heart) through alterations in gene expression.

### Arguments for retinoic acid as a morphogen in limb development include:

- RA: Exists in limb, Receptors present
- The ZPA likely controls the A/P polarity of the limb through the secretion of sonic hedgehog

### Signal Transduction & Limb Formation

During limb development the limb bud grows away from the body in a proximo- (close) distal (away from) fashion. Developmentally, it is important to realize that as the limb bud lengthens and limb components are specified and start to differentiate, what were once distal regions become proximal as new distal regions form. Thus in early embryogenesis, the humerus as it forms is initially the most distal component but once the radius and ulna and subsequent components form, it becomes proximal to them. This continues until the limb is fully developed and the final relationship of limb components is defined.



By the progress-zone model of limb development, the AER secretes FGF that influences the closest cells (those in the progress zone) to develop into distal structures. FGF is a distalizing factor in limb development. Those cells that are not within range of the AERs influence remain proximal in nature. As the AER extends out due to

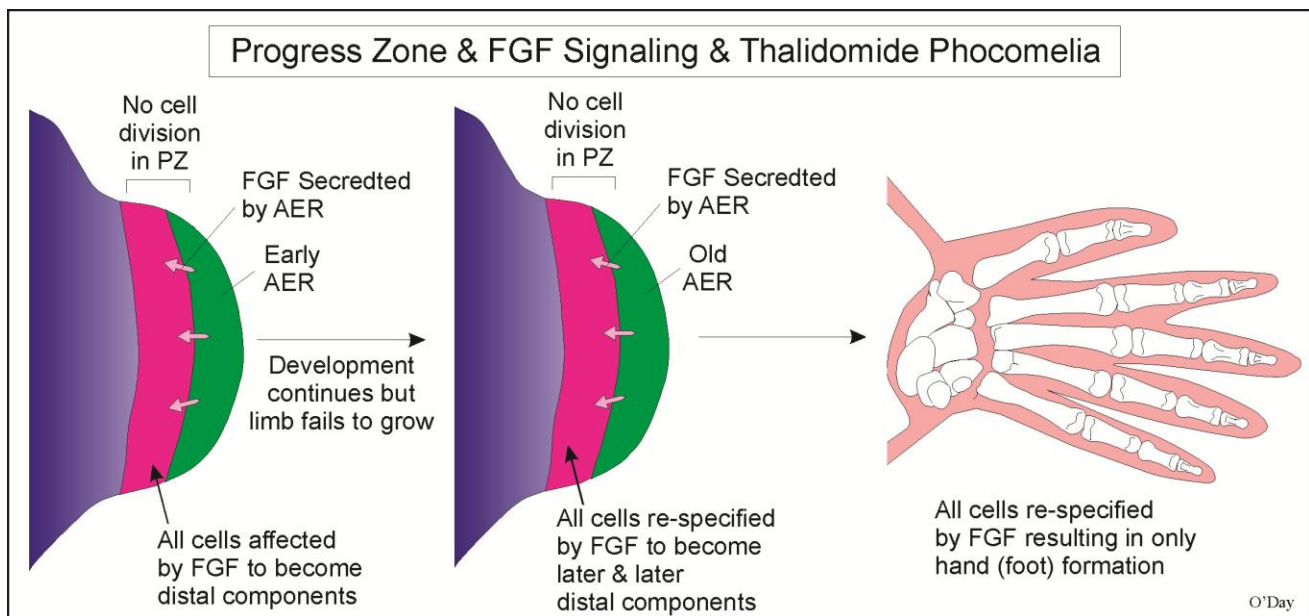
## Limb Development: Hox Genes

the continued division of cells in the progress zone, it continues to affect the closest cells by causing them to be specified as distal structure cells. Those that fall out of the range of influence of the AER are no longer influenced by the effects of FGF and retain their previously defined status (i.e., are now proximal components not influenced by the distalizing effect of FGF).

### Understanding Thalidomide and Limb Development

In the lecture on Critical Periods in Development we mentioned the effect of the teratogen thalidomide as an inducer of congenital limb malformations. The mode of action of thalidomide in limb development is still a controversial issue. However, it is generally believed that exposure to thalidomide leads to an inhibition of the growth of the limb bud mesenchyme. The end product is a limb with distal structures (hand/fingers) attached directly to the shoulders (i.e., the long bones are lost) resembling the flippers of a seal.

The problem is fitting the effects of thalidomide into current models of limb development. By the progress-zone model one interpretation is that the lack of growth of the limb mesenchyme means that all of those cells are continually influenced by FGF released by the AER. Thus these cells never fall out of the sphere of influence of the AER to become proximal cells. Instead they only receive the distalizing signals ultimately resulting in their differentiation only into distal components. This is shown in the following diagram.

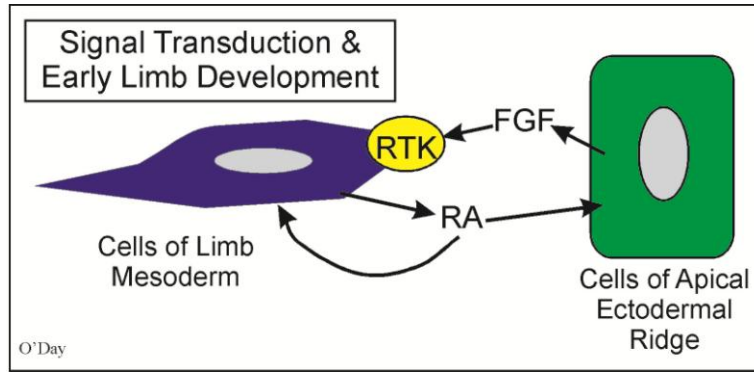


Clearly there is a range of limb shortening (phocomelia) syndromes reflected in thalidomide syndromes but this model begins to elucidate the possible underlying mechanisms and may lead to a better understanding of normal and defective limb development.

### Fibroblast Growth Factor (FGF) and Limb Formation

- FGF is linked to the initiation of bud formation, maintaining bud outgrowth, and the induction of a regeneration
- FGFs are secreted primarily by AER
- Tyrosine kinase FGF receptor is expressed on the surface mesenchyme cells

# Limb Development: Hox Genes



## Events of Signal Transduction & Limb Formation

- FGF Released by AER binds to FGF Receptor (a receptor tyrosine kinase or RTK) & activates It
- RTK then phosphorylates critical proteins
- This causes the mesenchyme cells to release retinoic acid (RA)
- RA induces Hox Gene Expression in target cells

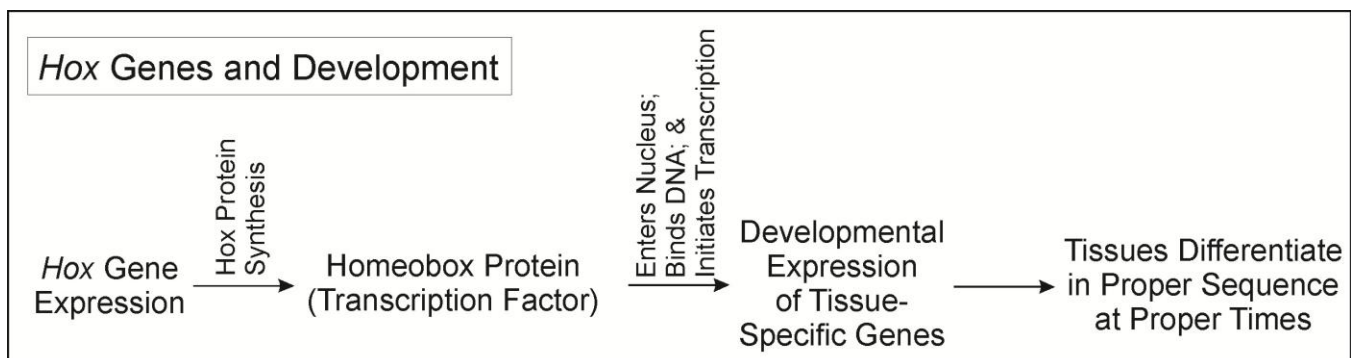
## Homeobox (Hox) Genes: Master Developmental Genes

- 1<sup>st</sup> identified in *Drosophila* (homeotic genes)--regulate development of body segments--bizarre development when mutated
- Homologous genes are present from Arthropods to Vertebrates (homeobox genes)
- Highly conserved genes
- Crucial to development
- Functions still not well understood

## Human HOX Genes

- 39 Hox genes (i.e., they must be important) distributed in 4 linkage groups
- Contain homeobox domain (highly conserved)
- This domain gives them their name
- Homeobox domain encodes a small protein called homeodomain protein segment
- This protein binds DNA & regulates gene activity (i.e., transcription factor)
- Genes are expressed in sequences that correlate with development of specific regions

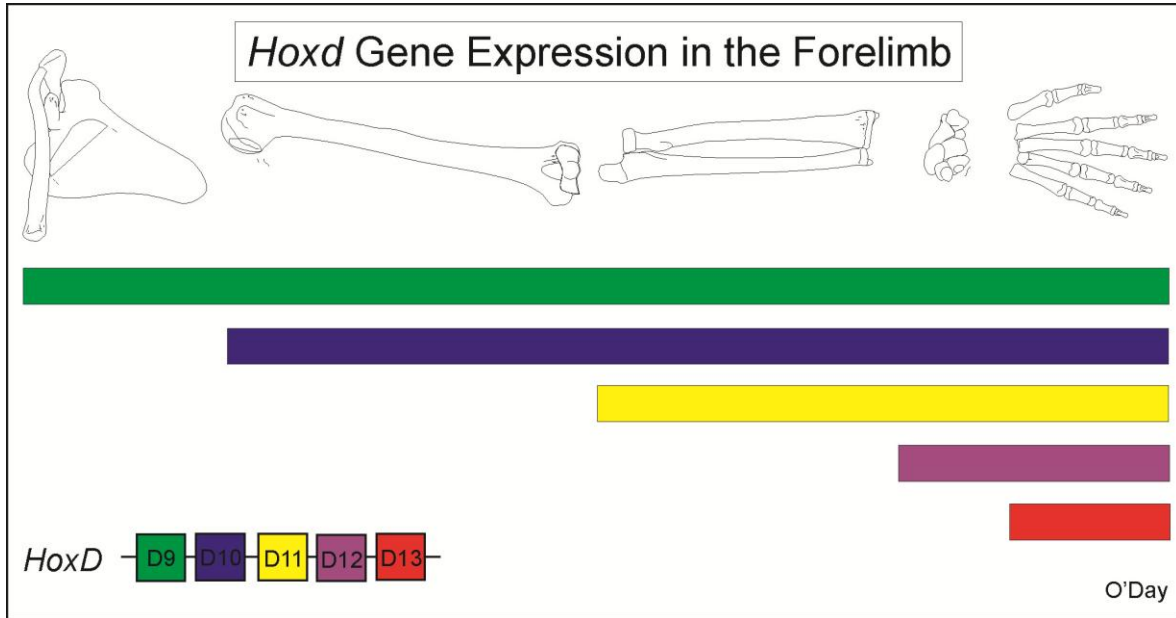
## Homeobox Regulation of Gene Expression



# Limb Development: Hox Genes

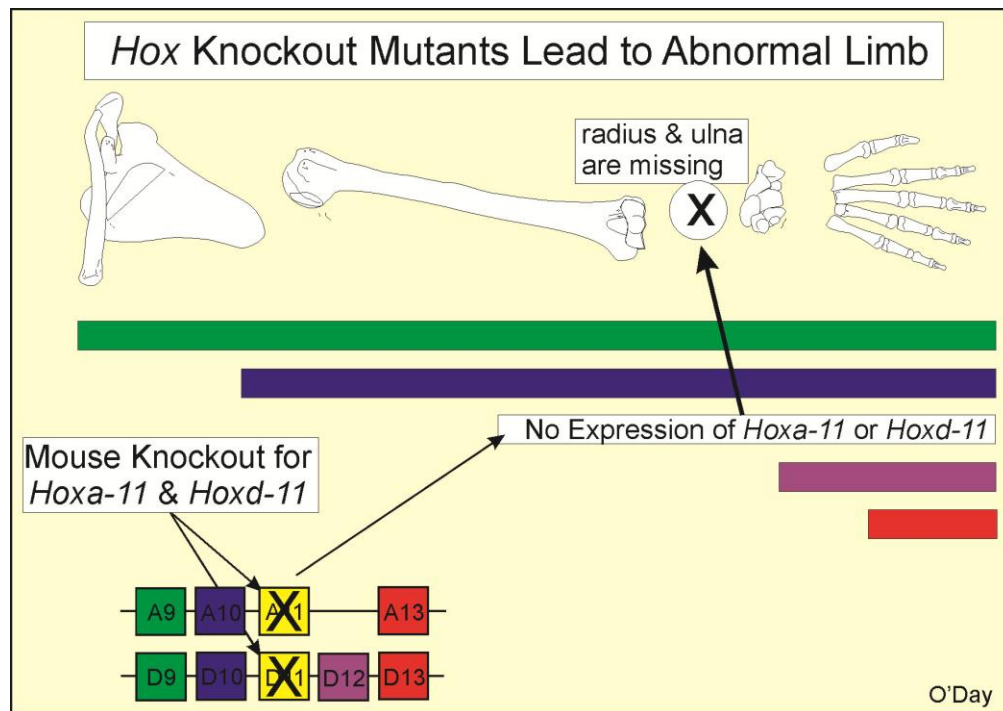
## Hox Gene Expression in the Mammalian Limb

The HoxD gene complex is expressed in a specific pattern in the developing mouse forelimb. The pattern of gene expression correlates with the linear arrangement of the genes in the genome. The following figure shows a summary of the expression of the Hox genes relative to the final developed limb.



## Hox Gene Knockouts in Mice Lead to Limb Defects

The patterns of Hox gene expression mediate specific events in limb development. The following figure shows how knockouts of *Hoxa11* or *Hoxd11* lead to the loss of the radius and ulna from the mouse forelimb.



## HOX GENES: Mutation in Michigan Family

- Defects: thumbs and toes
- Women: uterine abnormalities & sterility
- Examine DNA: Mutation present in HOXA13
- Thus, HOXA13 gene important for development

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