“Limb development”

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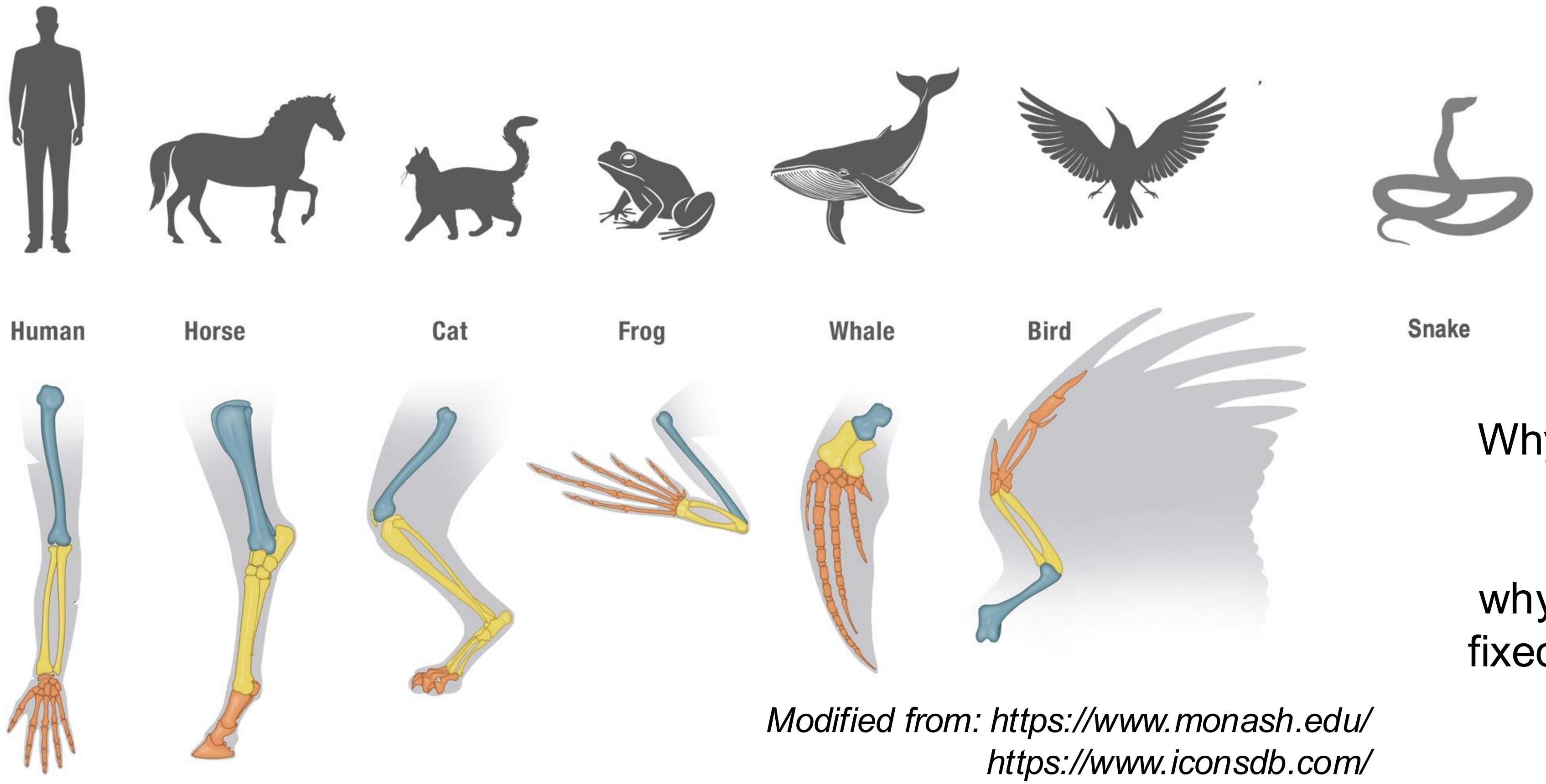
Symposium
New Frontiers in Molecular and Evolutionary Zoology

Sample lecture

1 December 2025

Limb diversity in vertebrates



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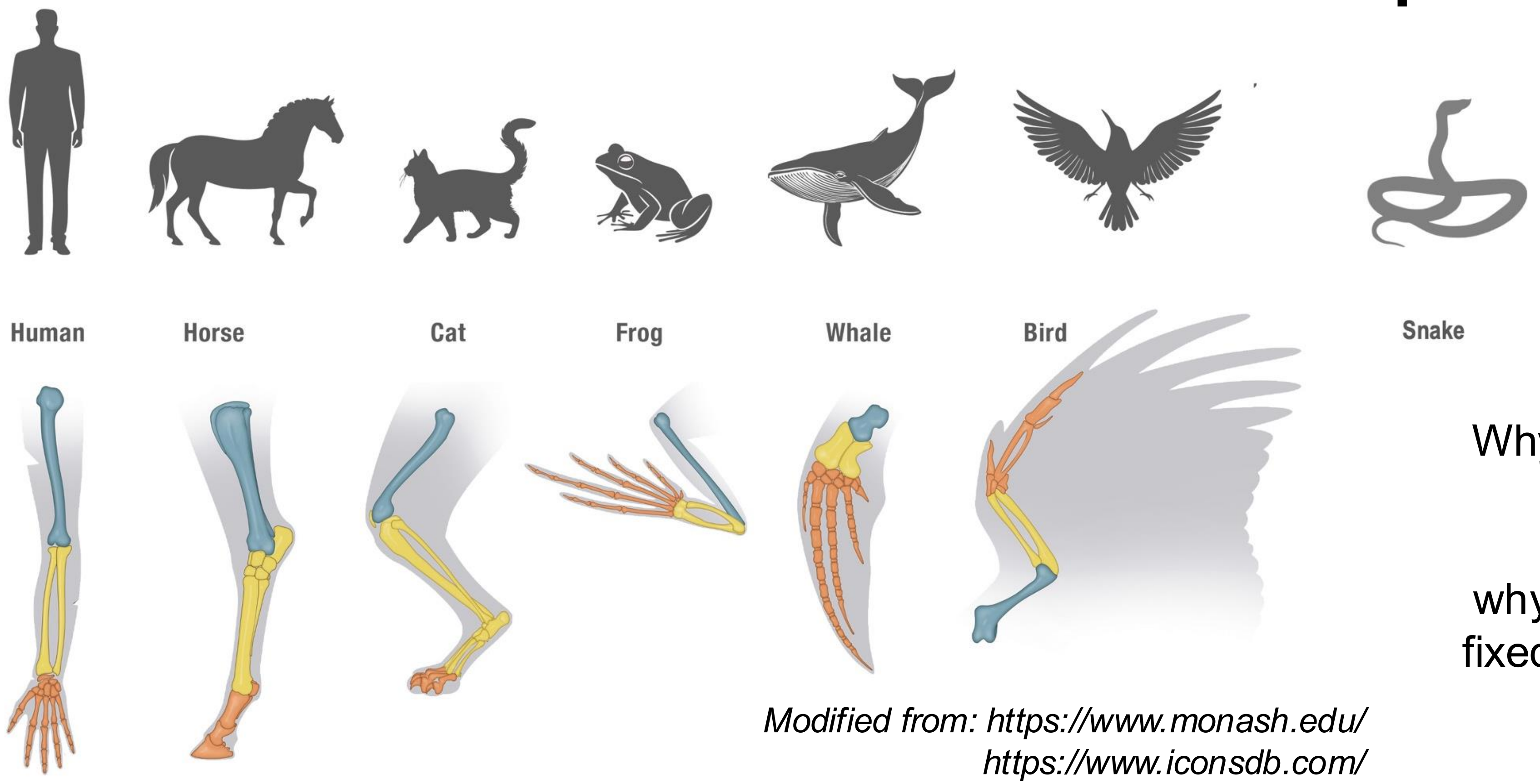
Why do tetrapods have exactly two pairs of limbs (or less) and why has this number remained fixed for over 380 million years?

- The vertebrate limbs vary a lot in shape and function
- The number of limbs is fixed

Why?
How?

Positional control of limb development



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Where?

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Why?
How?

Outline

Positional control of limb development

Local initiation of the limb bud

What factors determine the location of limb buds?

Inhibition of additional limb buds

What factors suppress additional limb buds' development?

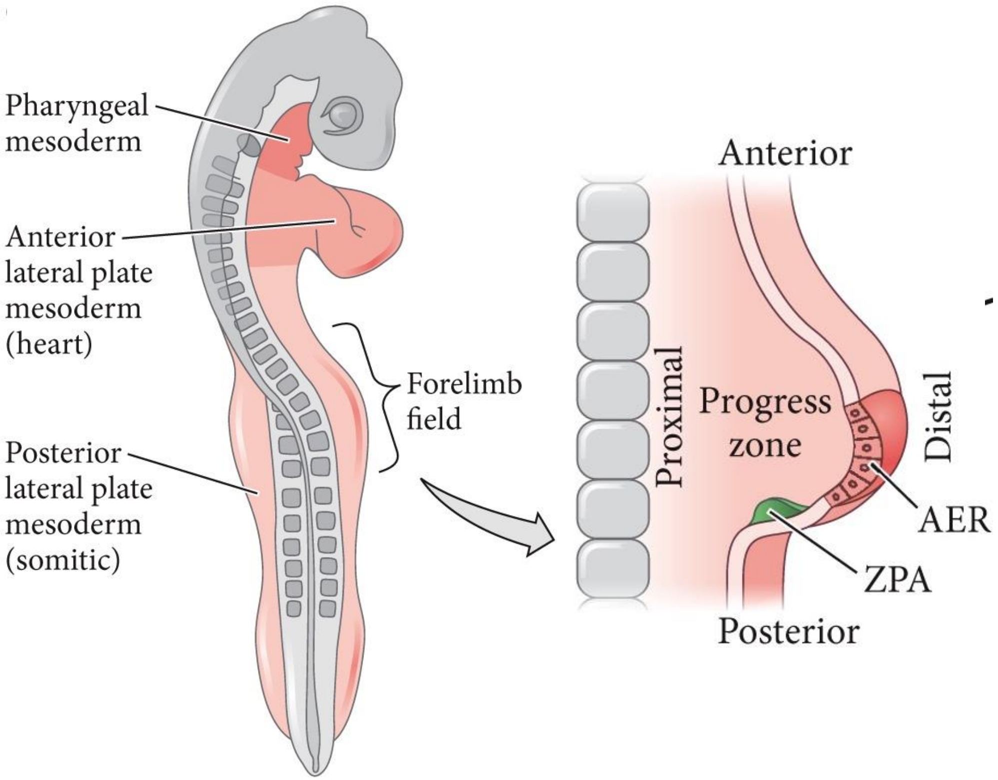
Evolution of paired appendages and developmental burden

What has constrained the evolution of the limb bud number?

Limb buds emerge from the lateral plate mesoderm

Where are limbs formed?

Tetrapod limbs develop from embryonic primordia – **the limb buds**



DEVELOPMENTAL BIOLOGY 11e, Figure 19.1 (Part 1)

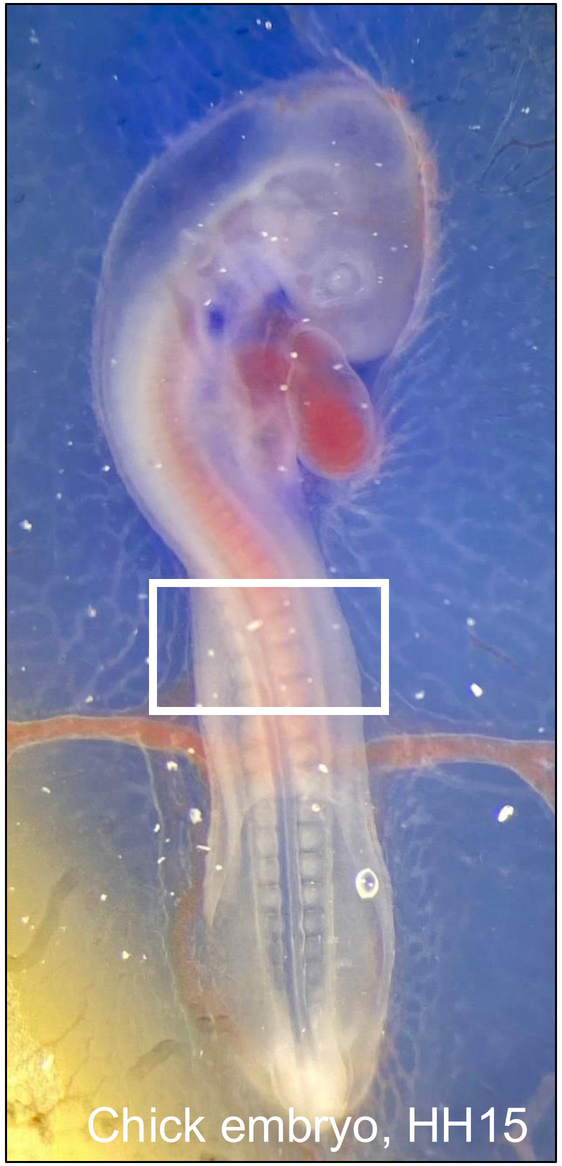


Image: A.Klimovich / CAU

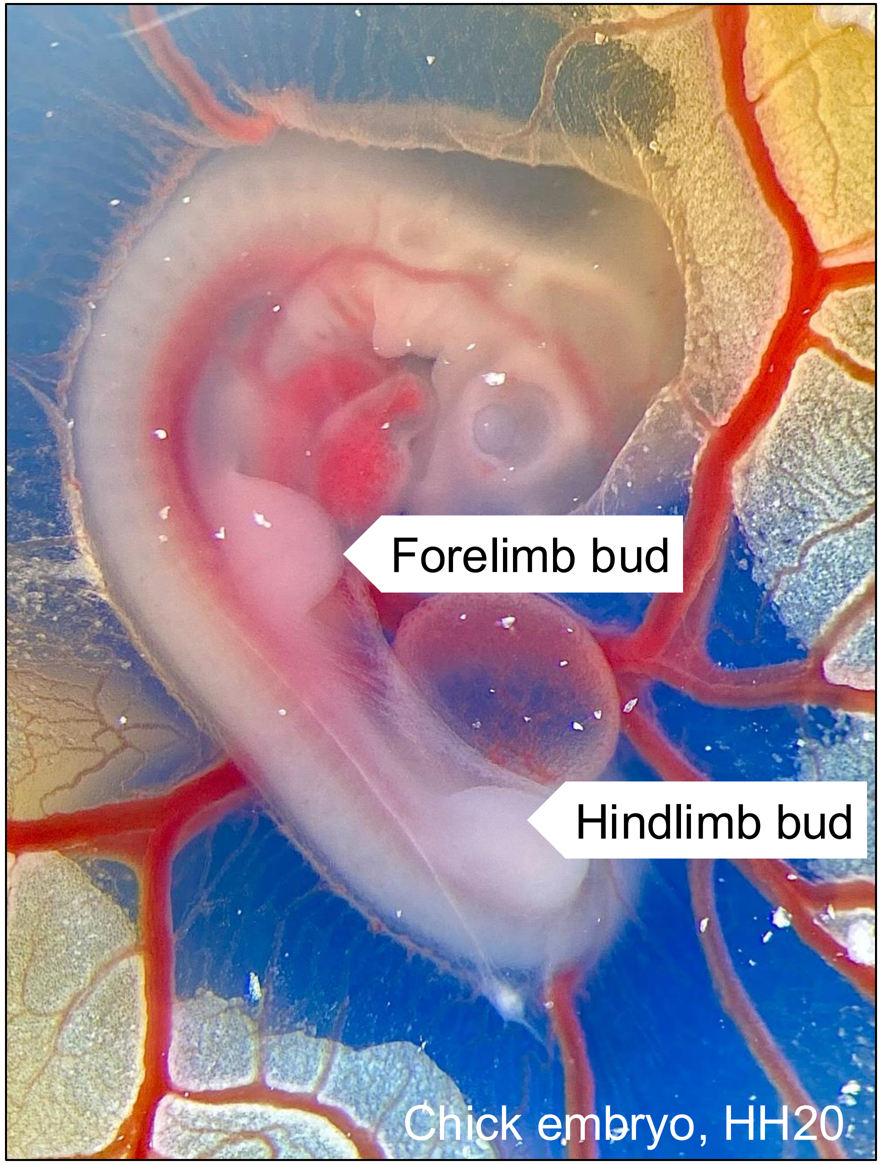


Image: A.Klimovich / CAU

Limb buds emerge from the lateral plate mesoderm

Where are limbs formed?

Tetrapod limbs develop from embryonic primordia – **the limb buds**

Two pairs of limb buds are specified in the **lateral plate mesoderm (LPM)**

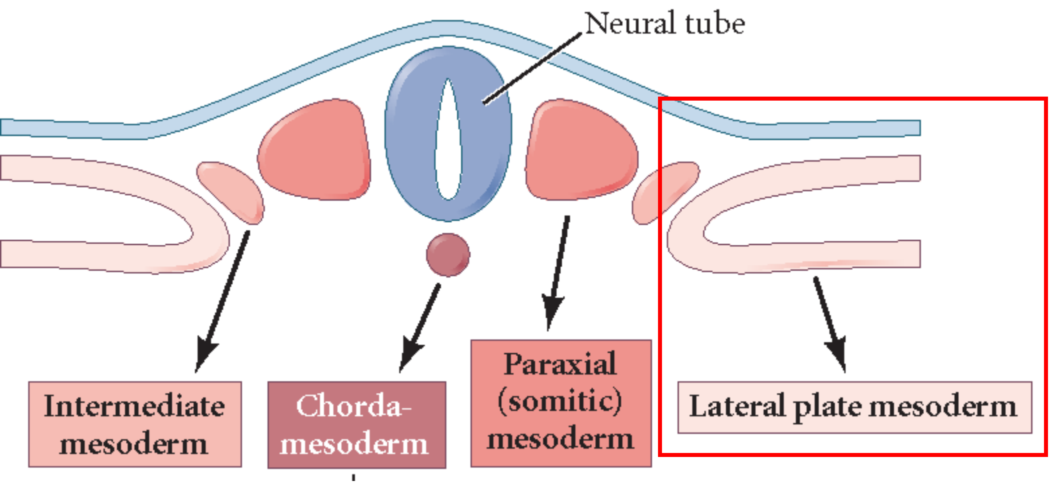
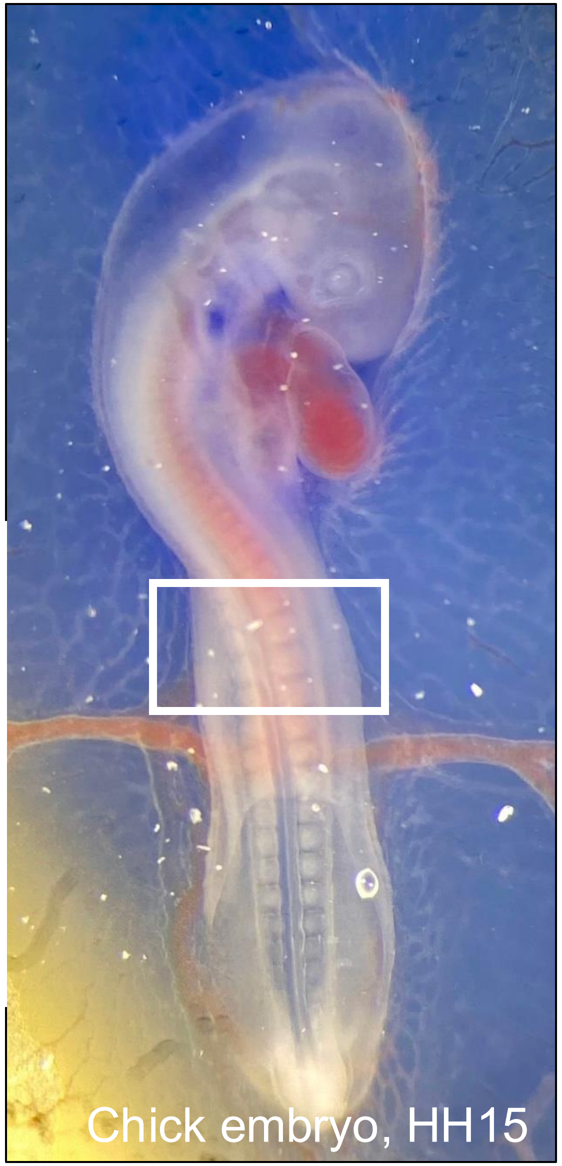


Image: Gilbert S.F. "Paraxial Mesoderm: The Somites and Their Derivatives." (2000).



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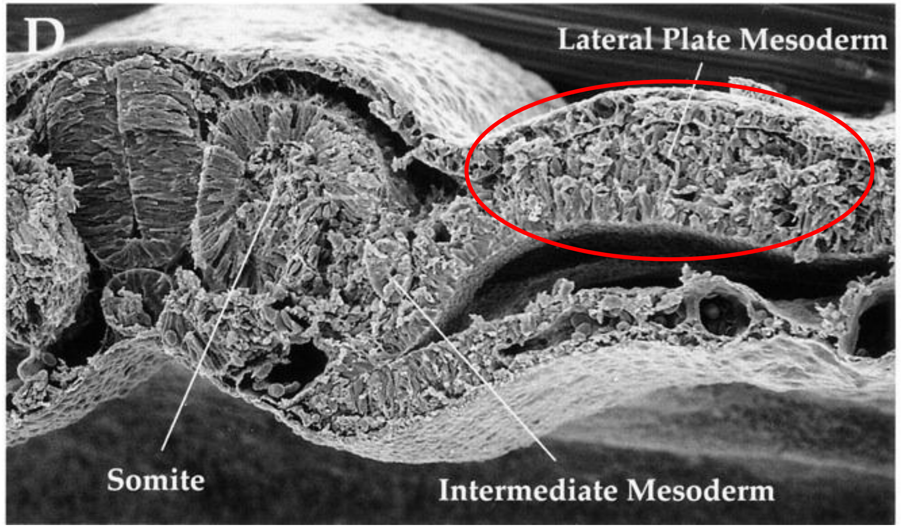


Scanning electron micrograph section of a 50 hr chick embryo. The limbs form from the lateral plate mesoderm, and a migratory contribution from adjacent somites. The intermediate mesoderm lies in between the lateral and somitic mesoderm. Courtesy of Gary C. Schoenwolf, University of Utah School of Medicine.

Image: Johnson RL & Tabin CJ, Cell 1997

What determines the position of the limb buds and gives the LPM the ability to form limbs?

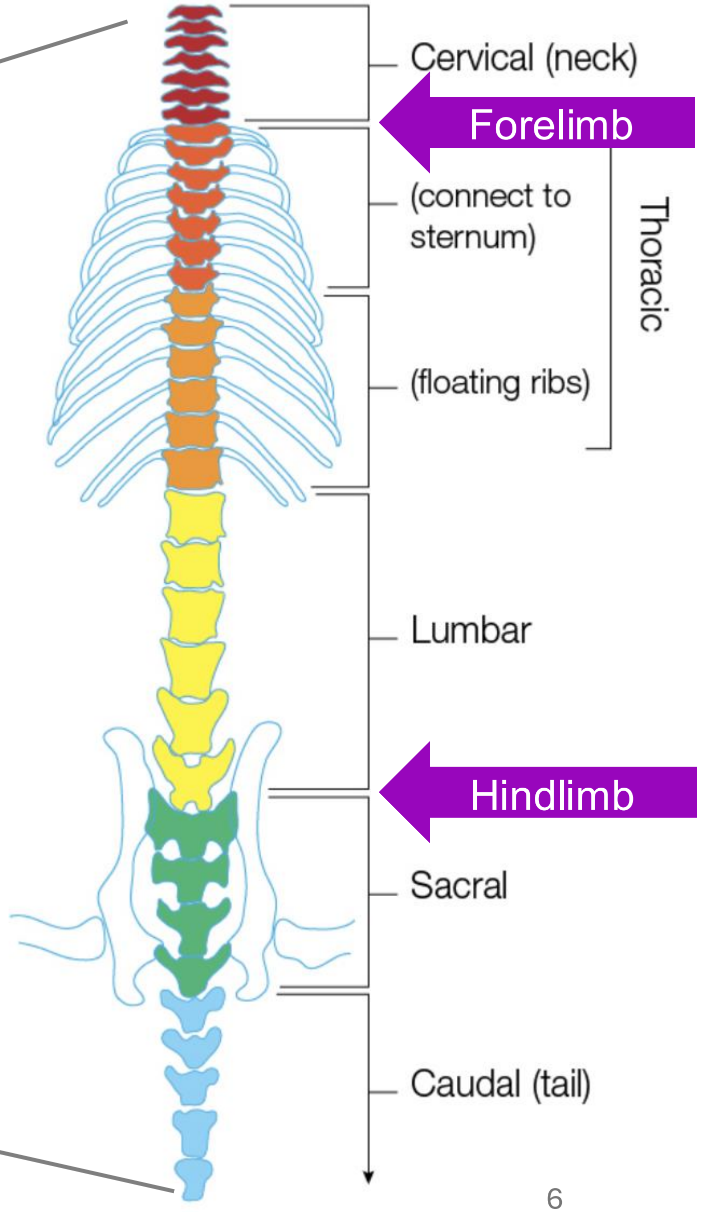
Hox-genes code marks the competent zones

Where are limbs formed?

Tetrapod limbs develop from embryonic primordia – **the limb buds**

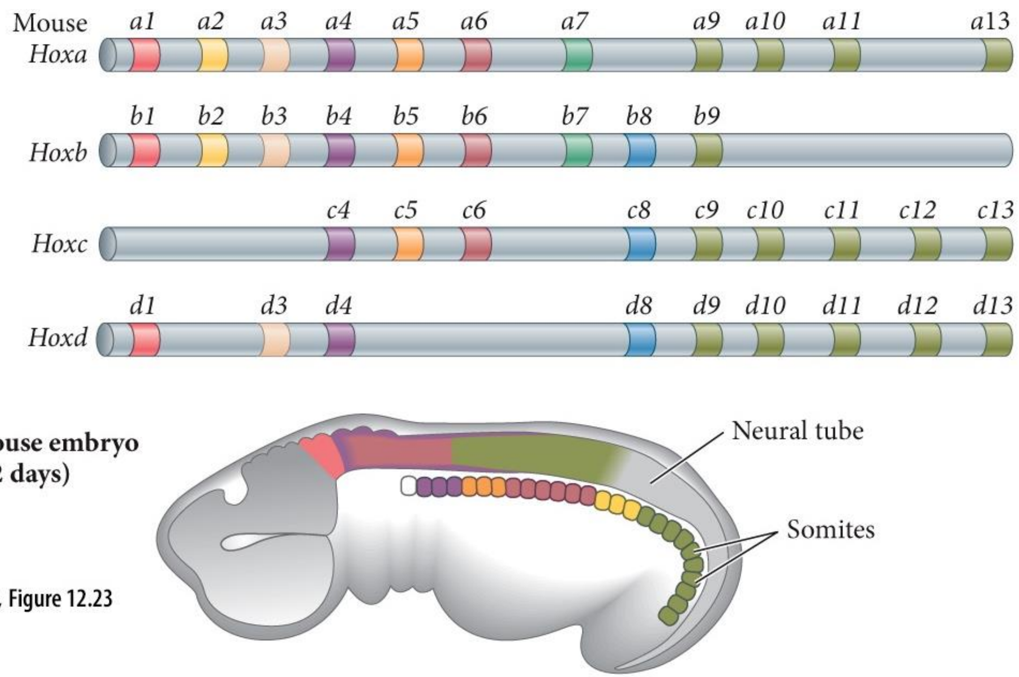
Two pairs of limb buds are specified in the **lateral plate mesoderm (LPM)**

The LPM is patterned along the anterior-posterior (rostro-caudal) axis by **expression of specific Hox genes**



Modified from: https://learn.genetics.utah.edu/content/basics/hoxgenes/

Hox-genes code marks the competent zones



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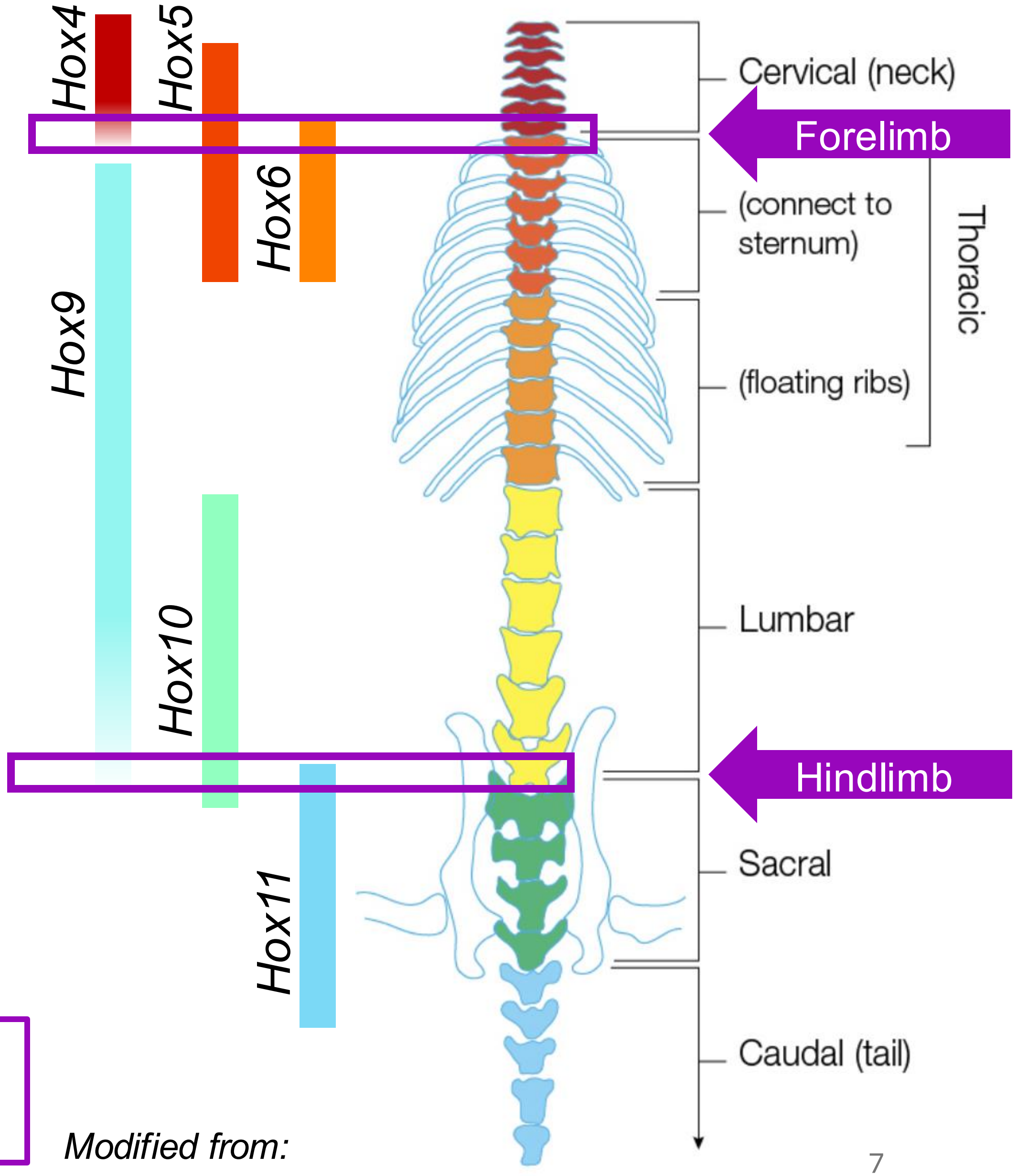


- Hox genes are expressed in a collinear manner *(low numbers – genes expressed earlier and anterior)*
- Hox genes pattern the vertebrate body, provide positional identity code and segment the trunk into functional regions

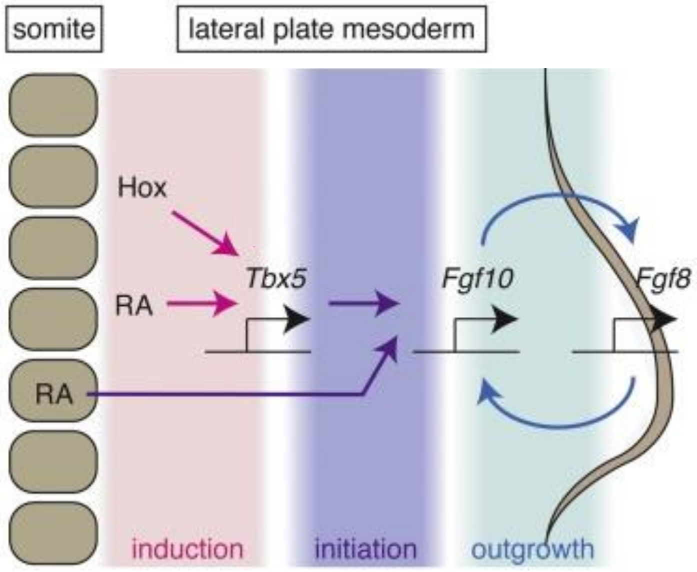
- Combinatorial Hox code confers the limb-forming potential to specific LPM domains

Modified from: https://learn.genetics.utah.edu/content/basics/hoxgenes/

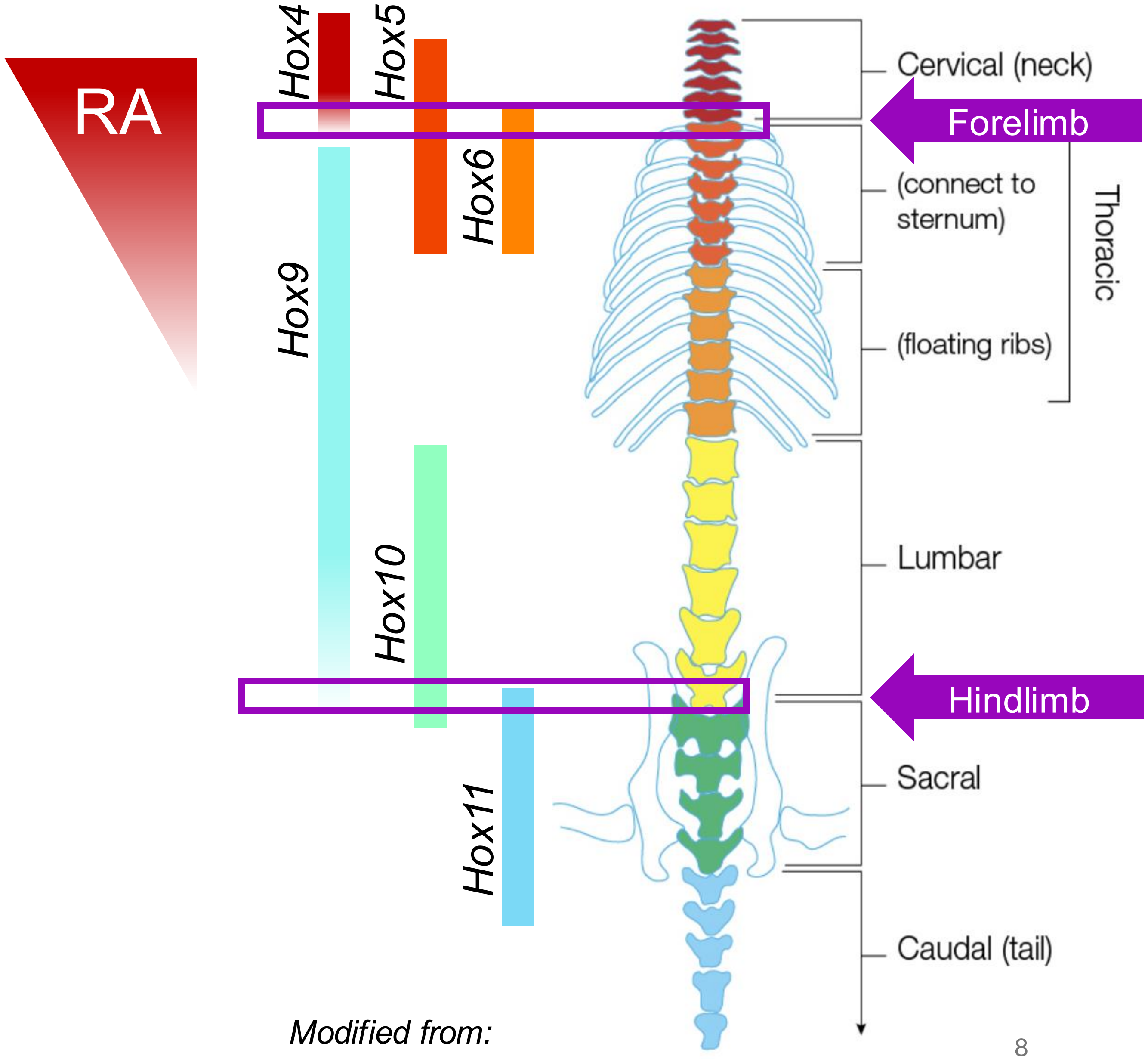
Retinoic acid (RA) restricts the competent zone

Local production of **retinoic acid (RA)** by RALDH enzyme in somites and local degradation of RA by CYP26 enzyme in flank mesoderm create **a gradient of RA activity**

High RA activity in the context of Hox4/5 and Hox6 activity **permits Tbx5 expression**



Modified from: Nishimoto et al., Cell Reps 2015



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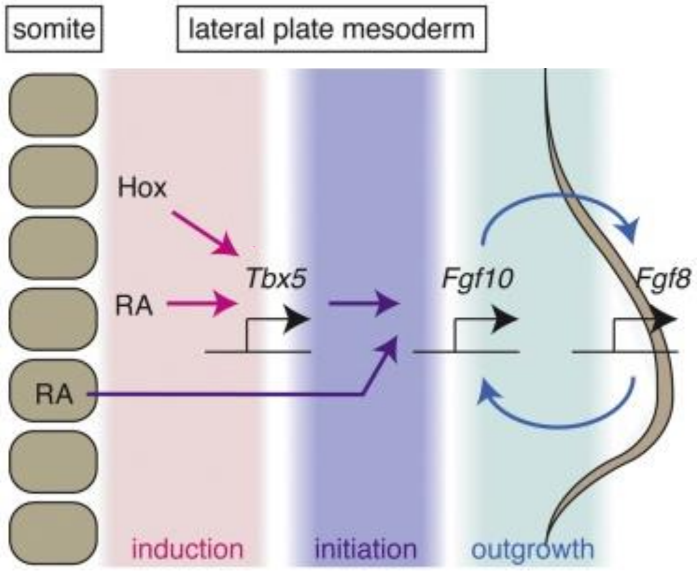

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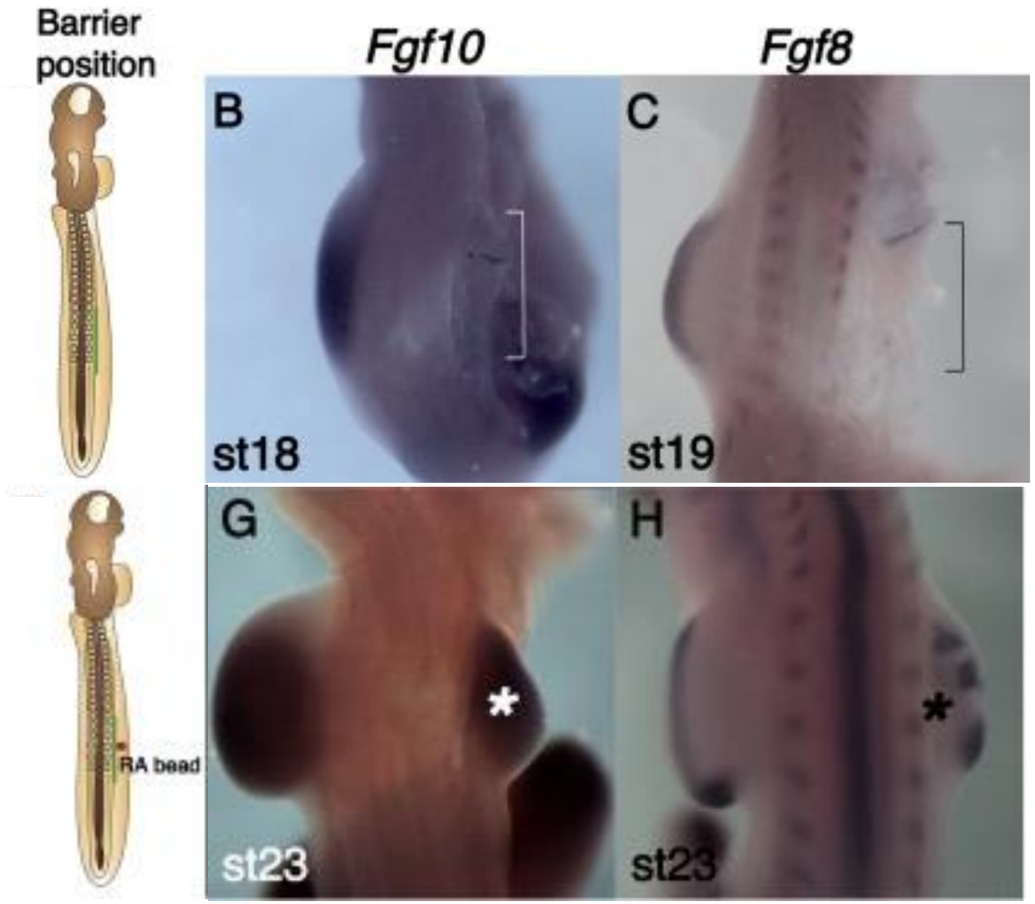


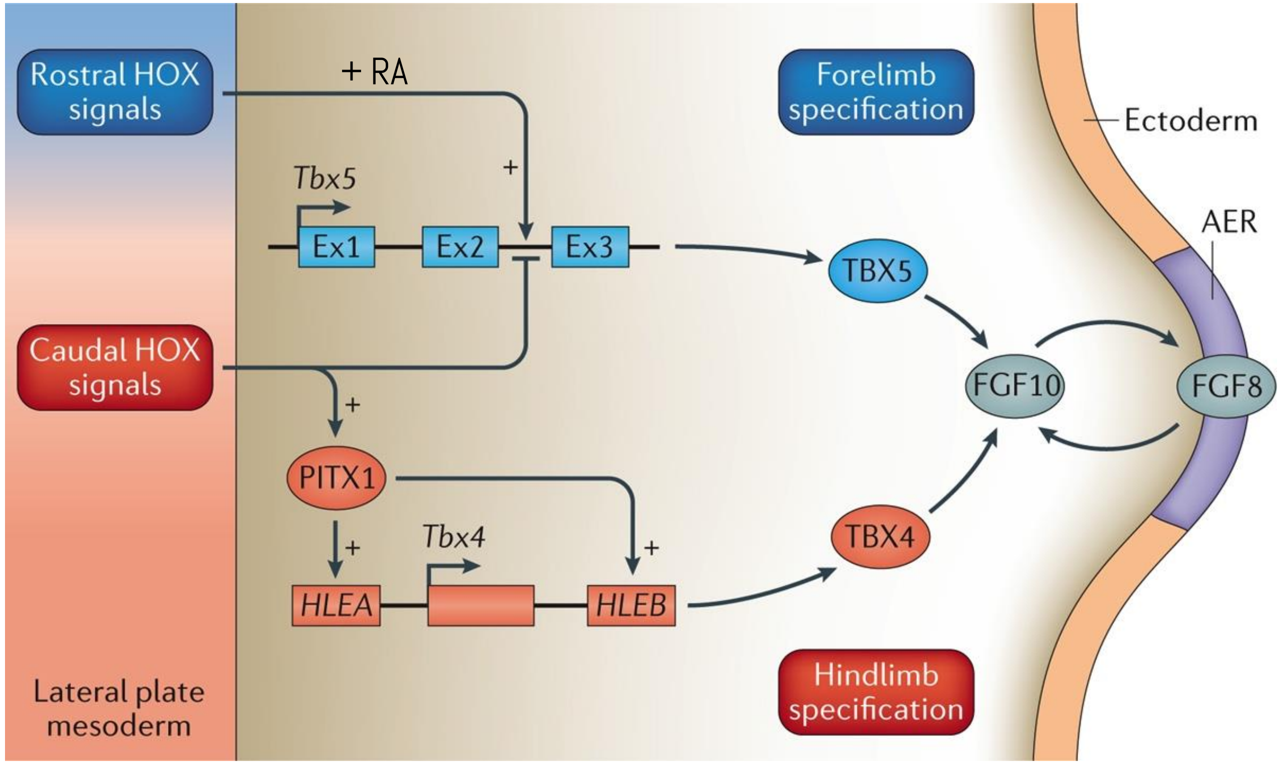
Experimental validation of the limb bud induction:
Insertion of an impermeable barrier between somites and LPM **eliminates FGF expression and limb bud outgrowth.**
Addition of a RA-soaked bead rescues the limb bud growth.

Modified from: Nishimoto et al., Cell Reps 2015

Summary: Induction of the limb buds

The **forelimb** program depends on the combinatorial action of **Hox4/5/6 genes** and **local RA activity**, which allow the expression of **Tbx5**.

The **hindlimb** induction program follows similar molecular logic: Specific Hox-genes code (**Hox9/10/11**) is complemented by activity of **Pitx** factor, which jointly allow **Tbx4** expression and hindlimb bud growth.



Modified from: Petit et al., Nature Review Genetics 2017

In the forelimb bud LPM, Tbx5 expression is induced by a combinatorial code of rostral Hox proteins that bind directly to its limb-specific enhancer in intron 2. In the caudal LPM, Pitx1 expression is induced by a combinatorial code of caudal Hox genes while Tbx5 is repressed. PITX1 directly binds to hindlimb-specific enhancer A and B (HLEA and HLEB) to drive the expression of Tbx4 in the prospective hindlimb bud. Both Tbx4 and Tbx5 trigger the expression of Fgf10 in the mesenchyme. After this stage, FGF10 interacts in a positive regulatory loop with FGF8 in the ectoderm, and this reciprocal regulation is crucial for limb bud outgrowth.

Why **no limb buds** are formed in the mid-trunk region, between the fore- and hindlimb buds?

Inhibition of the limb buds

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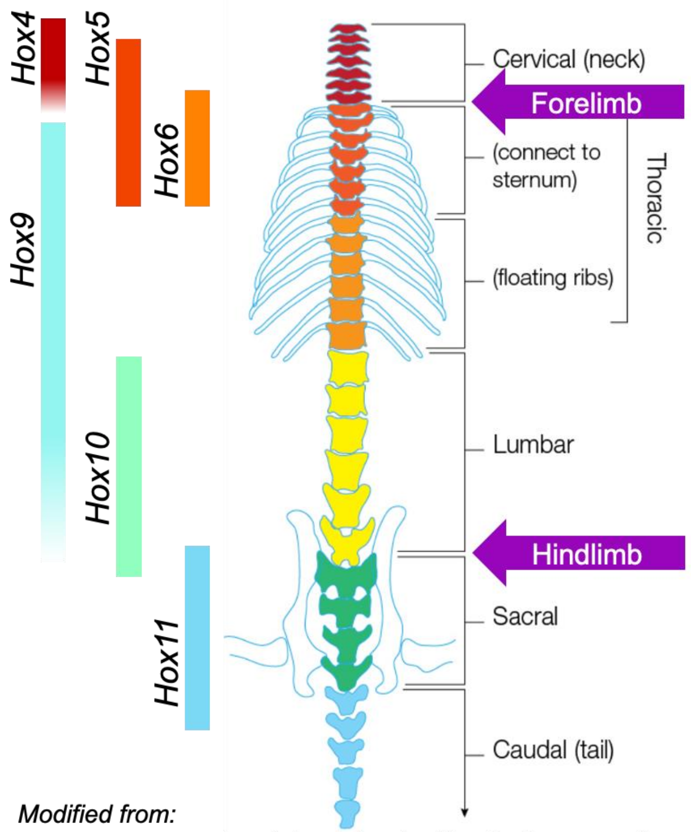

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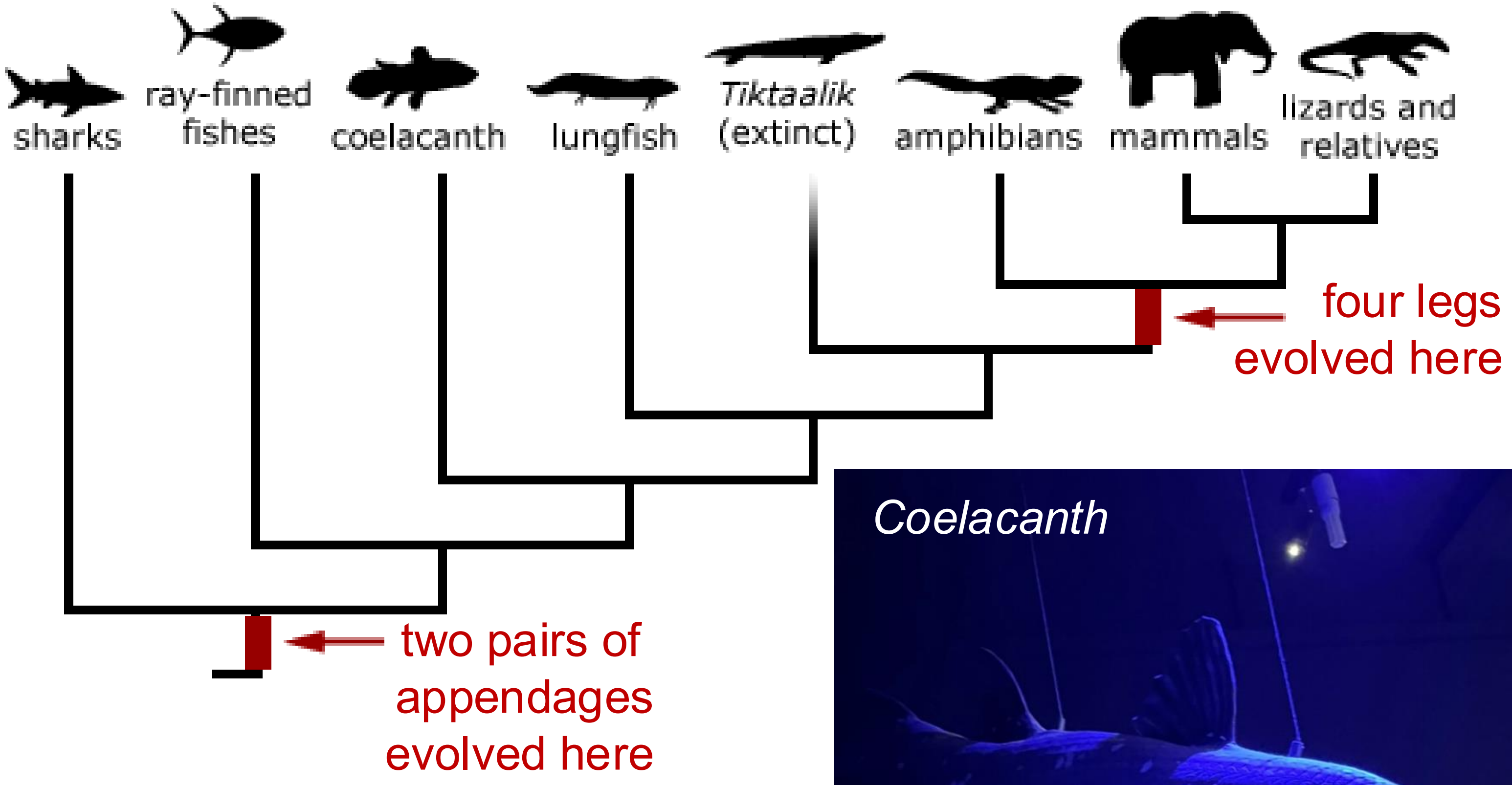
Image: A.Klimovich / CAU

Why **no limb buds** are formed in the mid-trunk region, between the fore- and hindlimb buds?

The trunk LPM expresses a Hox code (Hox6/7/8) that is inhibitory for limb initiation. Additionally, inductive signals like RA are missing.
Thus, expression of Tbx5/Tbx4 is genetically shut off, and an additional limb cannot form.

Developmental constraints in the evolution of paired appendages



Modified from: https://evolution.berkeley.edu/

All jawed vertebrates, including nearly all living fish, have exactly two pairs of paired appendages: pectoral fins (forelimb homolog) and pelvic fins (hindlimb homolog).

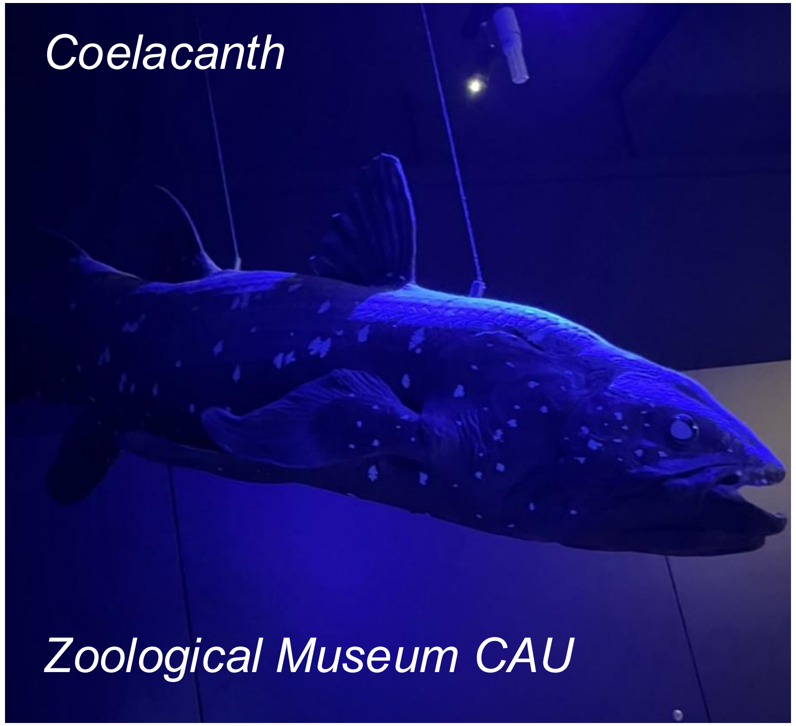


Image: zoologischesmuseumkiel @ Instagram

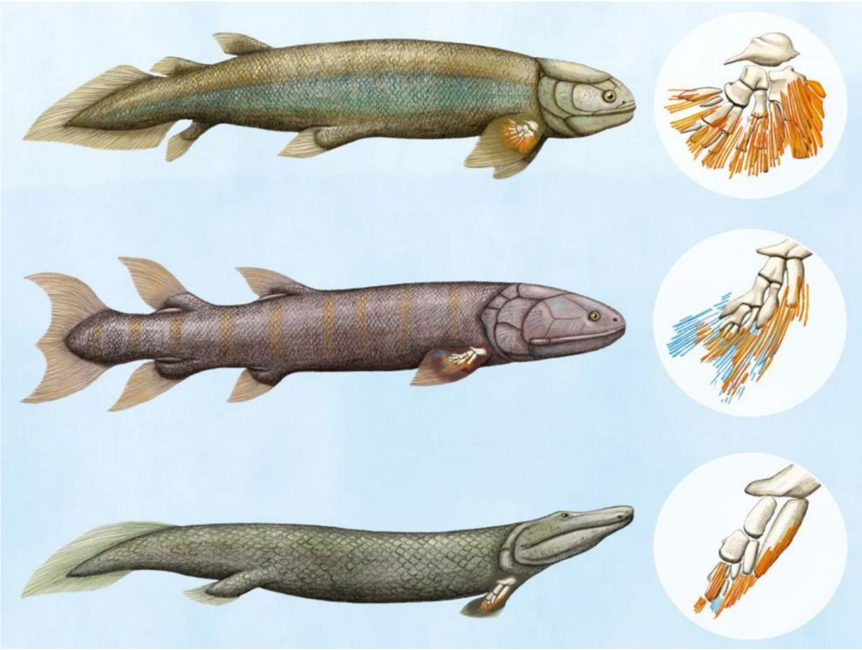


Image: Stewart et al., PNAS 2019

The two-pair appendage plan predates the origin of tetrapods and dates back over 430 MYA

Why is this plan so conserved?

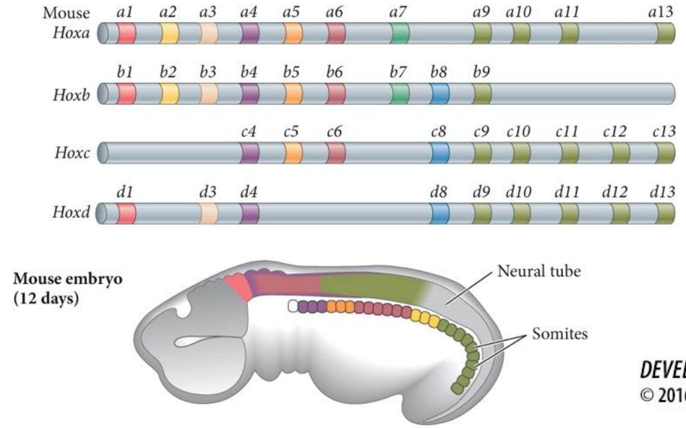
Developmental constraints in the evolution of paired appendages

The number and location of paired appendages has been **remarkably constrained**.

Adding new limb pairs would require inserting new LPM competent zones.
This requires **global re-organization** of the Hox patterning. This is virtually impossible, since Hox domains control other organ placement and development (*e.g.,* the nervous system, heart, digestive system).

Large-scale changes would disrupt essential body plan features, thus they are strongly selected against.

Hox genes control regionalization of the entire body and placement of most organ systems



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RA and FGF signaling are critical for development of other organs as well

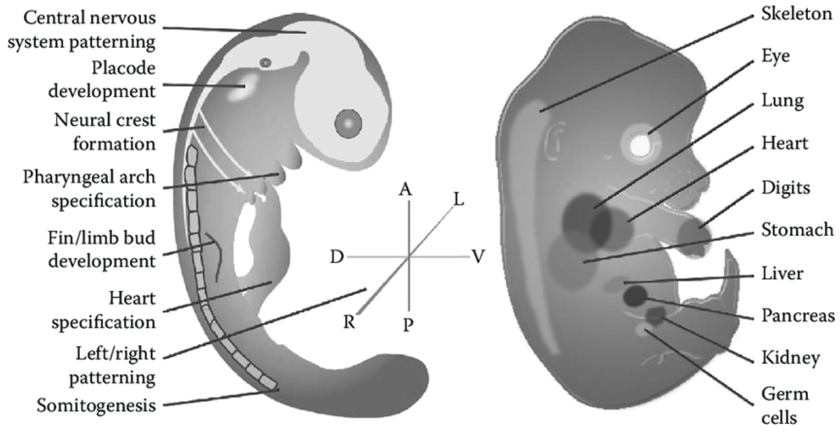

Image: Carvalho and Schubert, 2013

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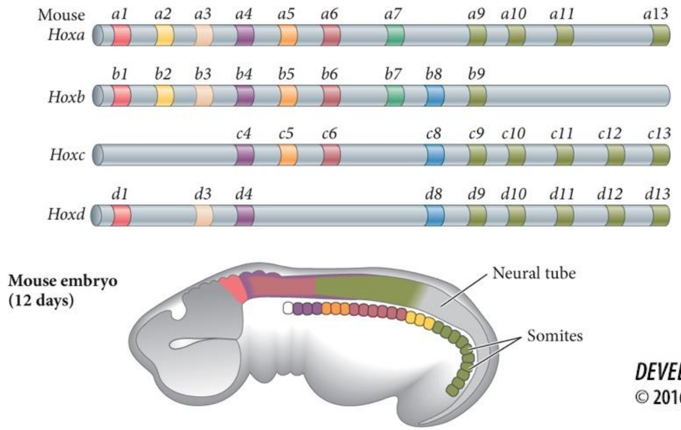

DEVELOPMENTAL BIOLOGY 11e, Figure 12.23

Functional integration of developmental programmes constrains their evolution

The "Developmental Burden" concept:
Once a developmental system becomes tightly integrated with other essential developmental processes, it becomes very difficult for evolution to modify it without causing widespread dysfunction.

"Developmental burden" means that limb initiation is deeply integrated with axial patterning, RA/FGF gradients, and LPM organogenesis—so altering limb number would disrupt many essential systems, making limb-number evolution highly constrained.

Breaking the code

Normal limbs (cartilage, bone)
Additional pair of limbs

Image: Lozovska et al., Nat Commun 2024

nature communications

Article | Open access | Published: 20 March 2024

Tgfbr1 controls developmental plasticity between the hindlimb and external genitalia by remodeling their regulatory landscape

Anastasiia Lozovska, Artemis G. Korovesi, André Dias, Alexandre Lopes, Donald A. Fowler, Gabriel G. Martins, Ana Nóvoa & Moisés Mallo

… In the absence of Tgfbr1, the pericloacal mesoderm generates an extra pair of hindlimbs at the expense of the external genitalia…The hindlimb and the genital primordia share many of their key regulatory factors…

External genitalia and hindlimb buds have likely evolved from an ancestral common primordium. They share ancestral developmental modules. Destabilizing genitalia identity can **reactivate limb-like developmental programs.**

Summary: Positional control of limb development

Local initiation of the limb bud
What factors determine the location of limb buds?
Hox-code, LPM competence, and inductive activity

Inhibition of additional limb buds
What factors suppress additional limb buds' development?
Hox-code and lack of inductive activity

Evolution of paired appendages and developmental burden
What has constrained the evolution of the limb bud number?
Ancestral two appendage fields, integration of the developmental programs (burden) with axial patterning

Image: https://artprojectsforkids.org/

Developmental gene programs contstrain the evolution of possible developmental outcomes

Questions for self-control

1. Where along the vertebrate body axis are limb buds initiated, and which embryonic tissues contribute to their formation?

2. What molecular mechanisms determine the anterior–posterior positions of forelimb and hindlimb buds in vertebrates?

3. Limbs are formed at two specific positions in the vertebrate animal's trunk. Why are limb buds not normally induced outside the forelimb and hindlimb fields?

4. Why do tetrapods and all gnathostomes consistently have only two pairs of appendages?

5. Explain the concept of *Developmental Burden* and illustrate it using the molecular programs that control limb position.

Additional literature

Hox code and RA:

Nishimoto S, Wilde SM, Wood S, Logan MPO. RA Acts in a Coherent Feed-Forward Mechanism with *Tbx5* to Control Limb Bud Induction and Initiation. Cell Reports (2015) 12(5): 879-891. DOI: https://doi.org/10.1016/j.celrep.2015.06.068

Wang Y, Hintze M. et al., Permissive and instructive Hox codes govern limb positioning. eLife (2024) 13: RP10059. DOI: https://doi.org/10.7554/eLife.100592.1

Royle SR, Tabin CJ, Young JJ. Limb positioning and initiation: An evolutionary context of pattern and formation. Developmental Dynamics (2021) 250: 1264–1279. DOI:https://doi.org/10.1002/dvdy.308

Petit F, Sears K, Ahituv N. Limb development: a paradigm of gene regulation. Nat Rev Genet (2017) 18, 245–258. DOI: https://doi.org/10.1038/nrg.2016.167

Evolution:

Onimaru K, Shoguchi E, Kuratani S, Tanaka M. Development and evolution of the lateral plate mesoderm: comparative analysis of amphioxus and lamprey with implications for the acquisition of paired fins. Developmental Biology (2011) 359(1): 124-136. DOI: https://doi.org/10.1016/j.ydbio.2011.08.003

Shubin N, Tabin C, Carroll S. Fossils, genes and the evolution of animal limbs. Nature (1997) 388, 639–648. DOI: https://doi.org/10.1038/41710

Genitalia to limb transformation:

Lozovska, A., Korovesi, A.G., Dias, A. et al. Tgfbr1 controls developmental plasticity between the hindlimb and external genitalia by remodeling their regulatory landscape. Nat Commun (2024) 15: 2509. DOI: https://doi.org/10.1038/s41467-024-46870-z