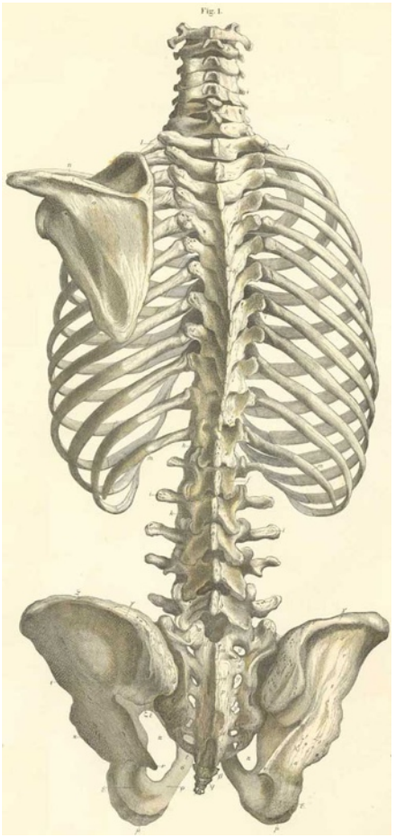
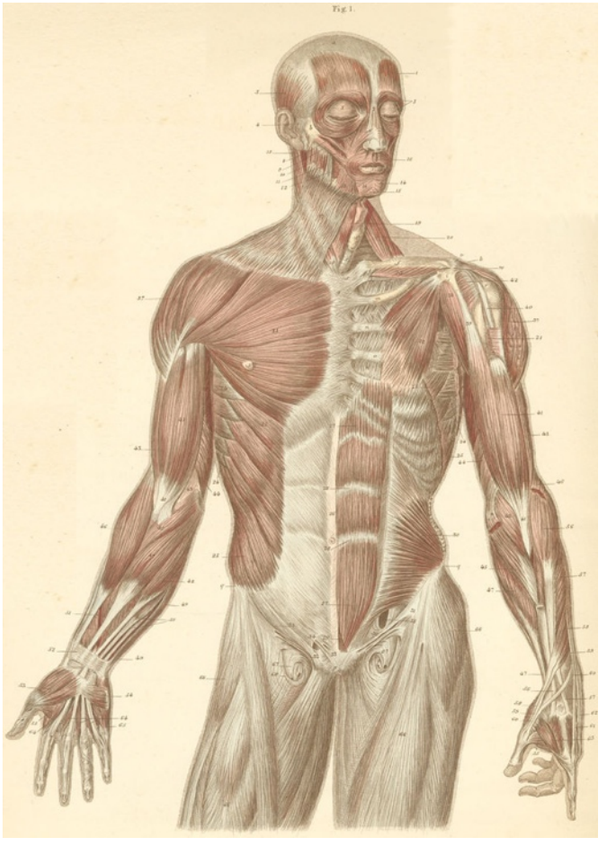
Patterning the vertebrate body axis

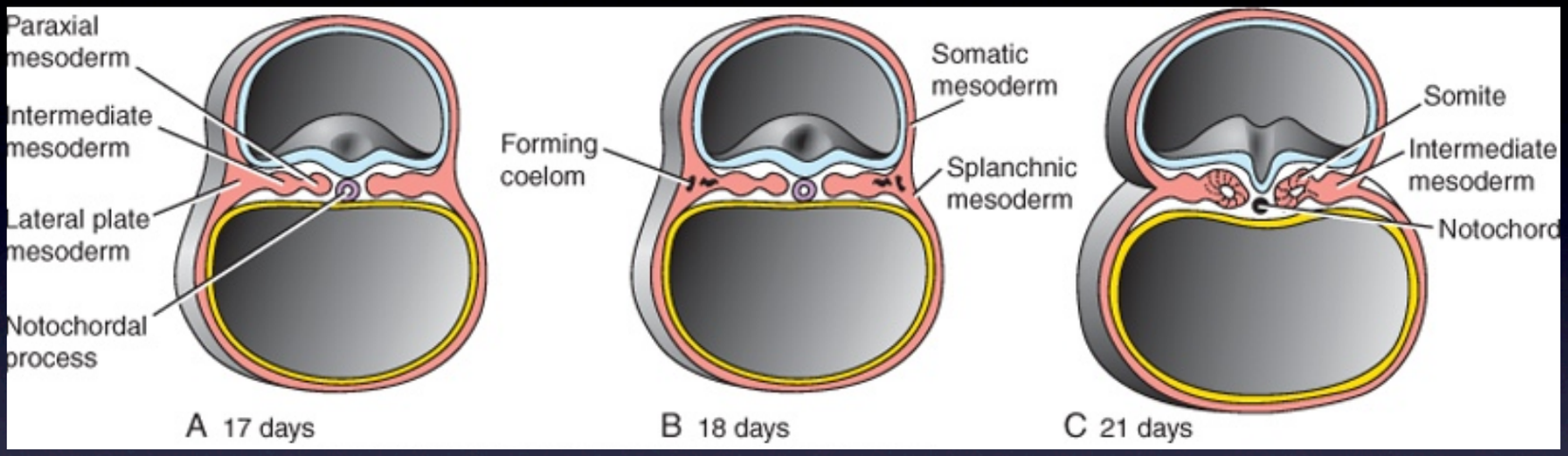


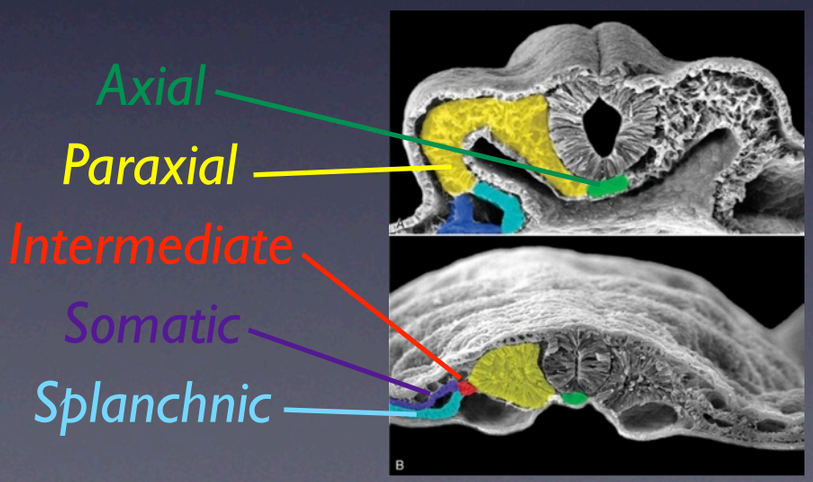


HD 3 Ed Laufer

February 24, 2009

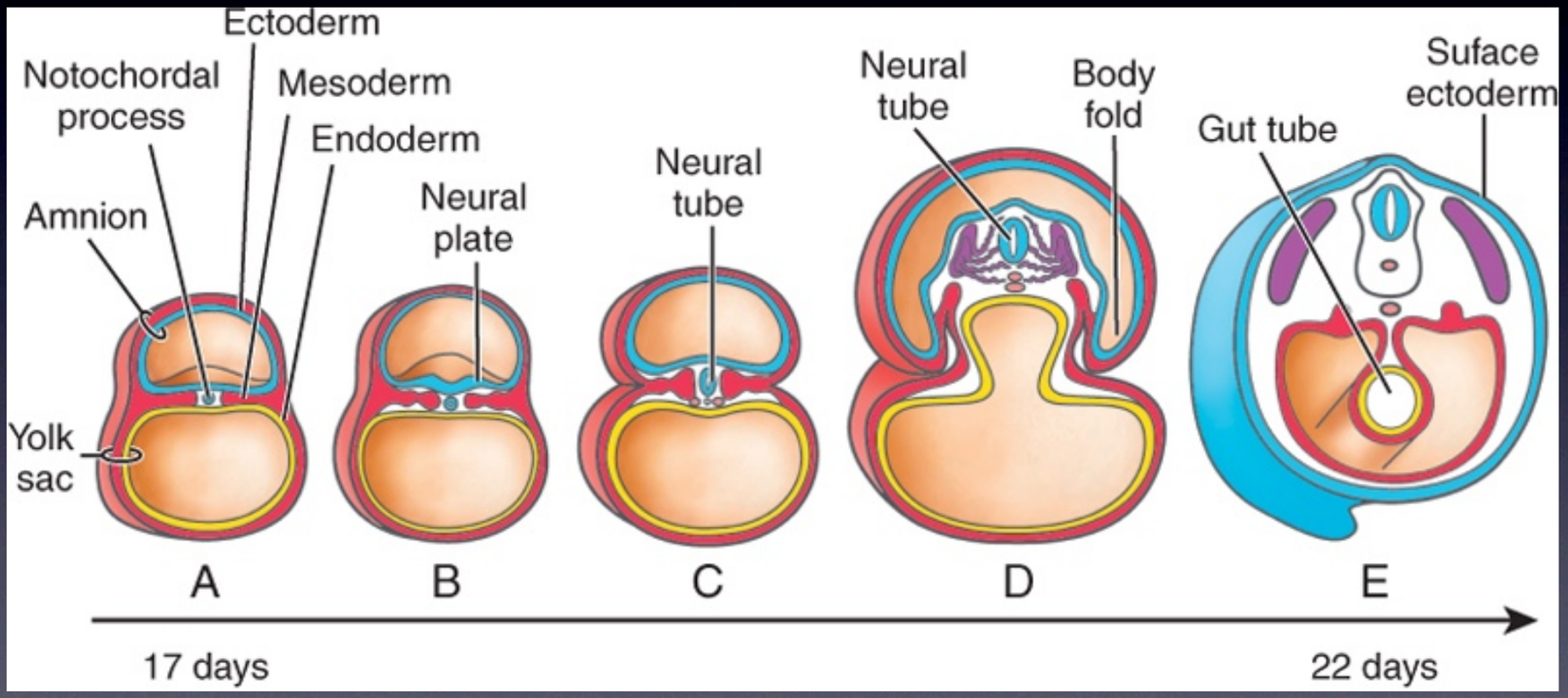
Regional differentiation of mesoderm



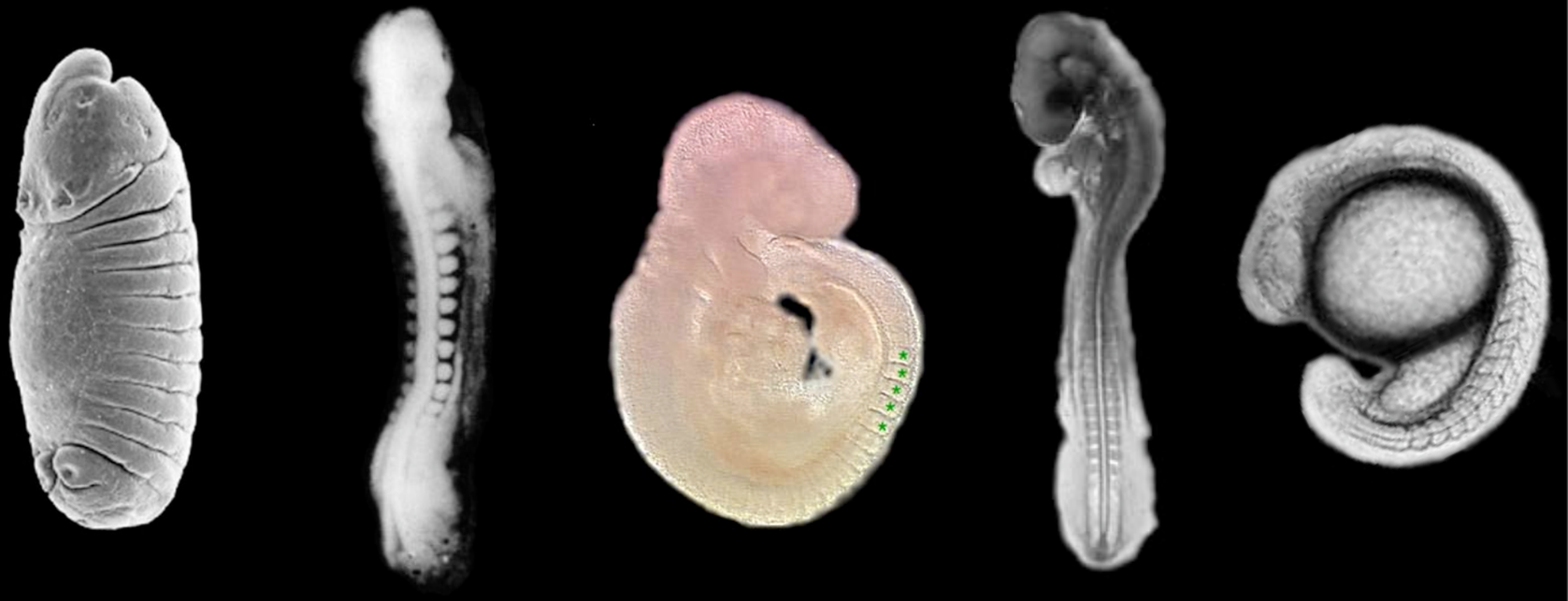


Chick embryo

Morphological changes at early post-gastrulation stages

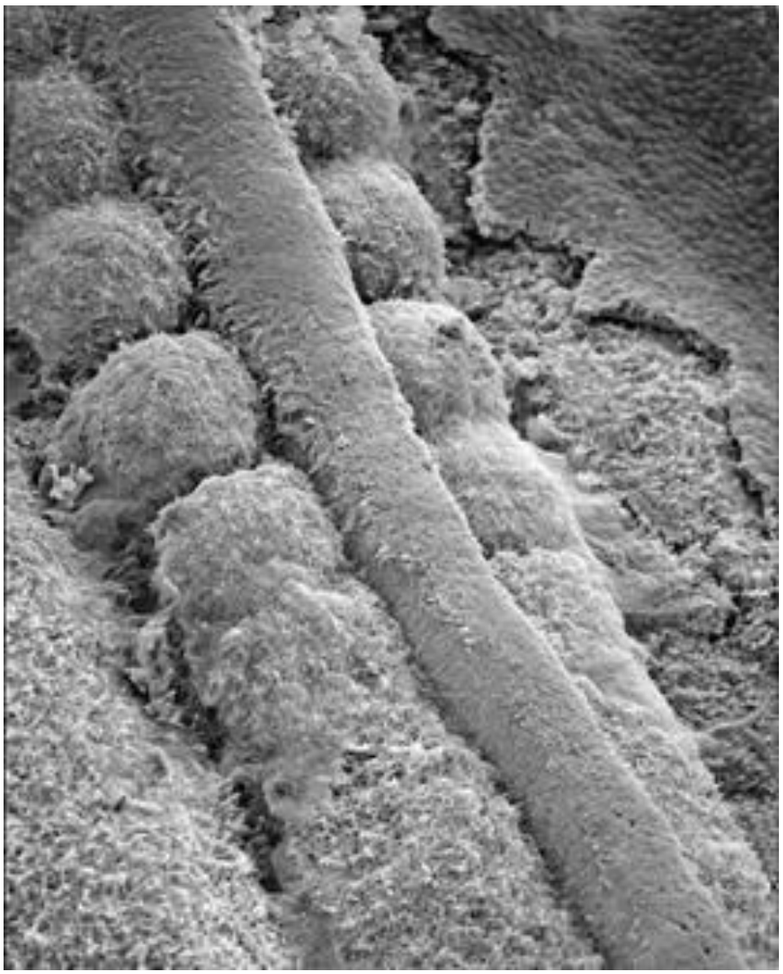


Segments organize the body plan during embryogenesis



Drosophila Human Mouse Chick Zebrafish

Axial segments originate from somites



Gilbert (2003)

<u>Somites are precursors of:</u>

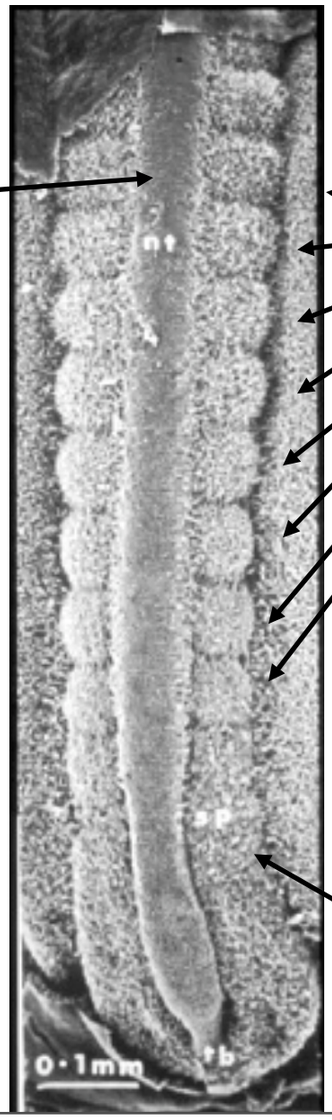
Axial skeleton (ribs, vertebrae)

Trunk/Limb muscles

Dermis

Axial tendons

Segmentation by sequential addition



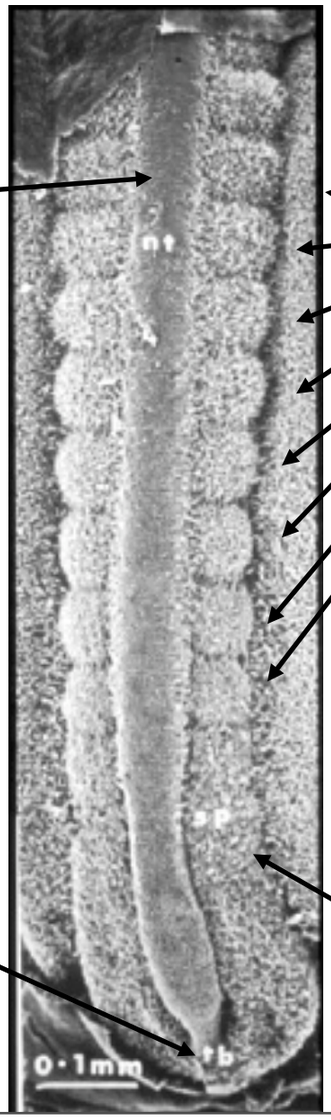
Neural tube

somites

Presomitic mesoderm
(unsegmented)

Dorsal view of segmenting paraxial mesoderm from chick

Segmentation by sequential addition



Neural tube

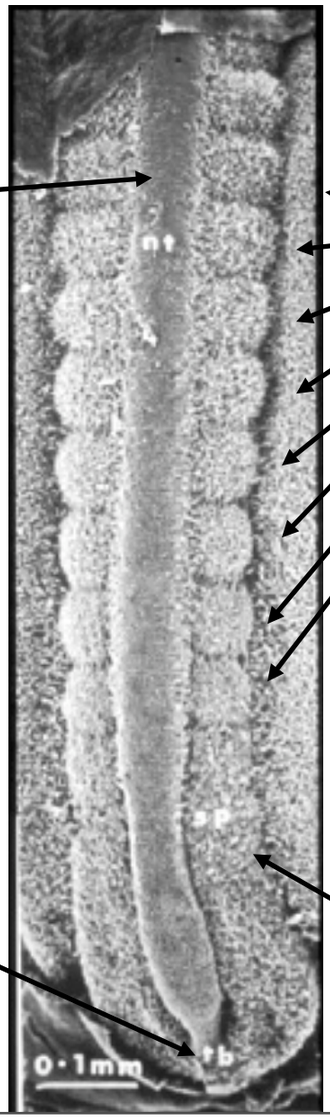
somites

A caudal growth zone generates new unpatterned tissue.

Presomitic mesoderm (unsegmented)

Dorsal view of segmenting paraxial mesoderm from chick

Segmentation by sequential addition



Neural tube

somites

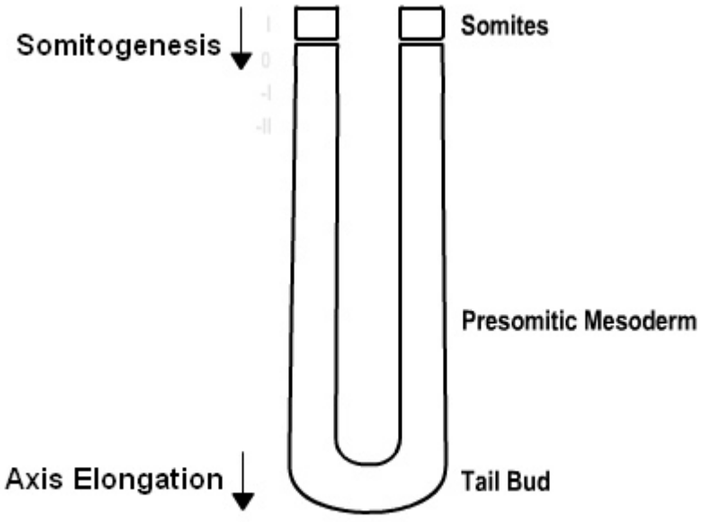
A caudal growth zone generates new unpatterned tissue.

Older, more rostral tissue, is allocated into segments in a rostrocaudal progression.

Presomitic mesoderm (unsegmented)

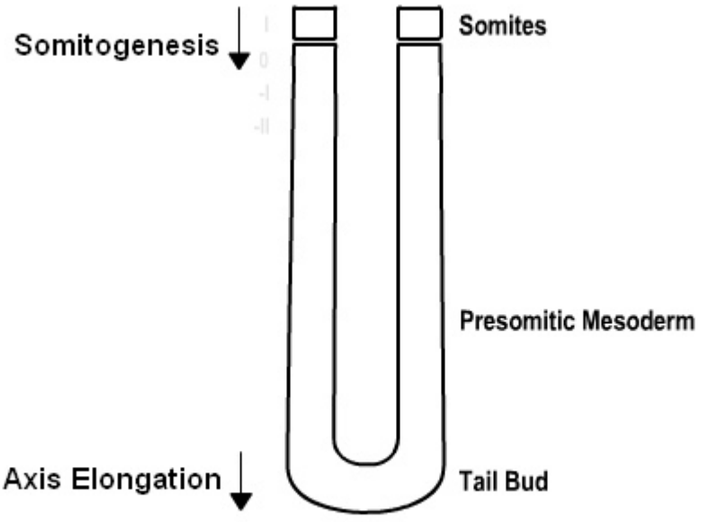
Dorsal view of segmenting paraxial mesoderm from chick

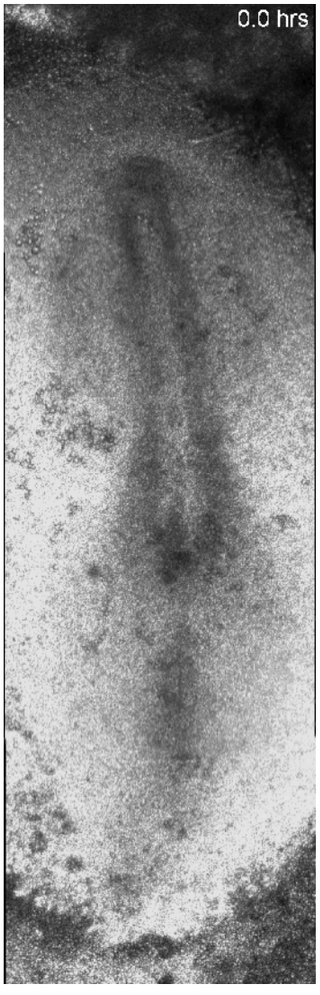
Rhythmic production of somites from the presomitic mesoderm (PSM)



2-day old chick embryo

Rhythmic production of somites from the presomitic mesoderm (PSM)





2-day old chick embryo

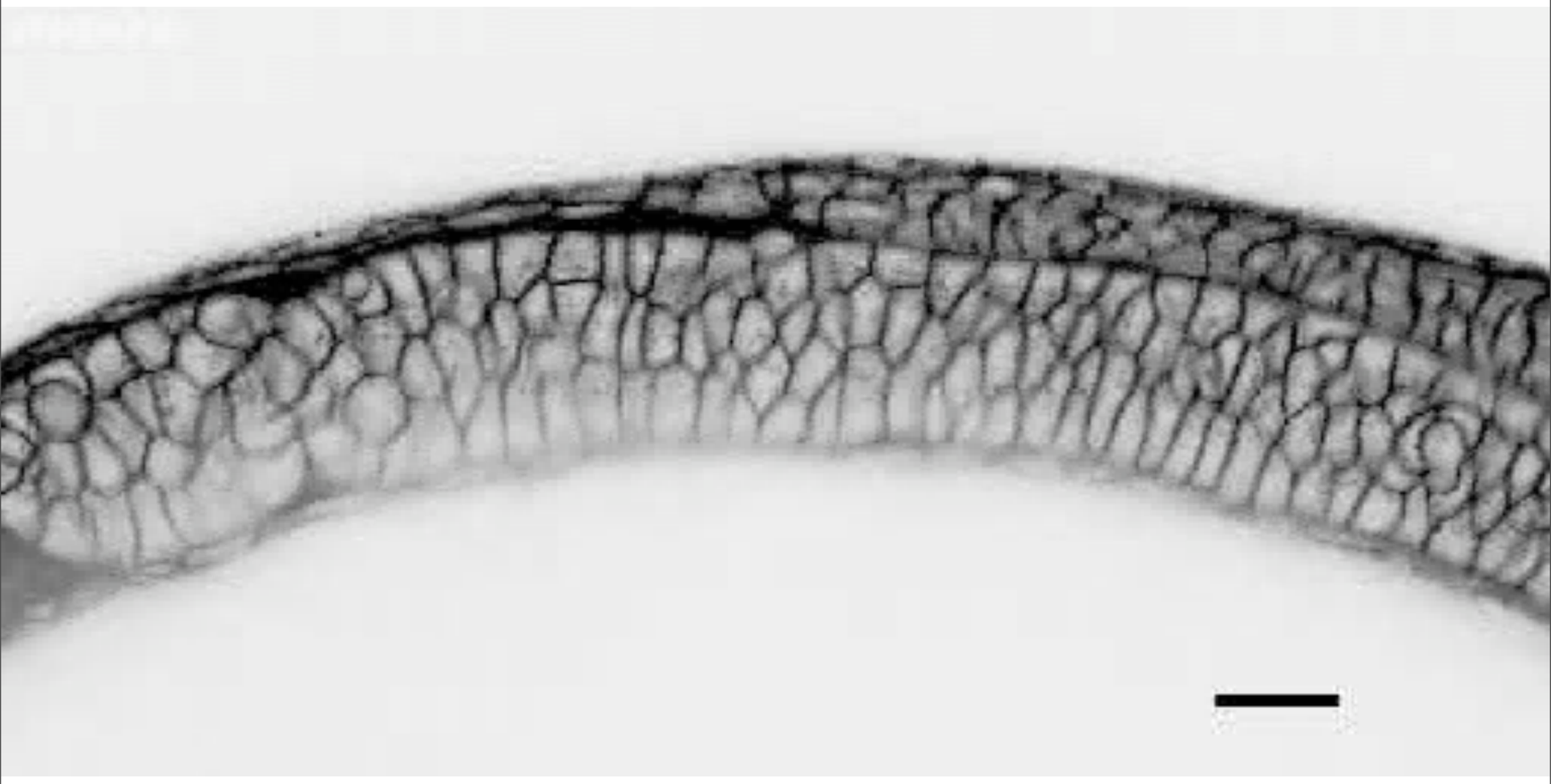
Lateral view of Segmentation

Rostral

Caudal

Olivier Pourquie

Lateral view of Segmentation

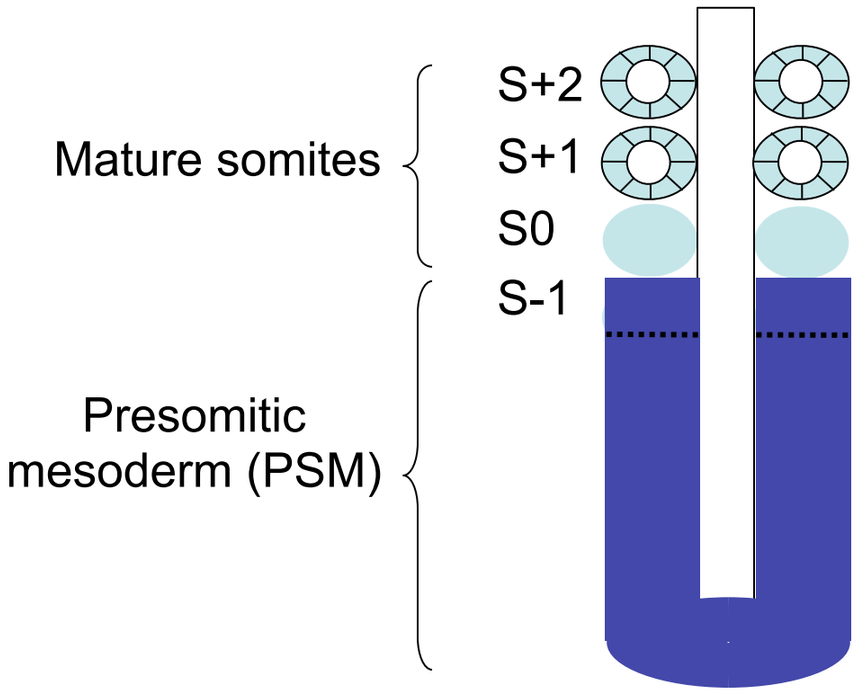


Rostral

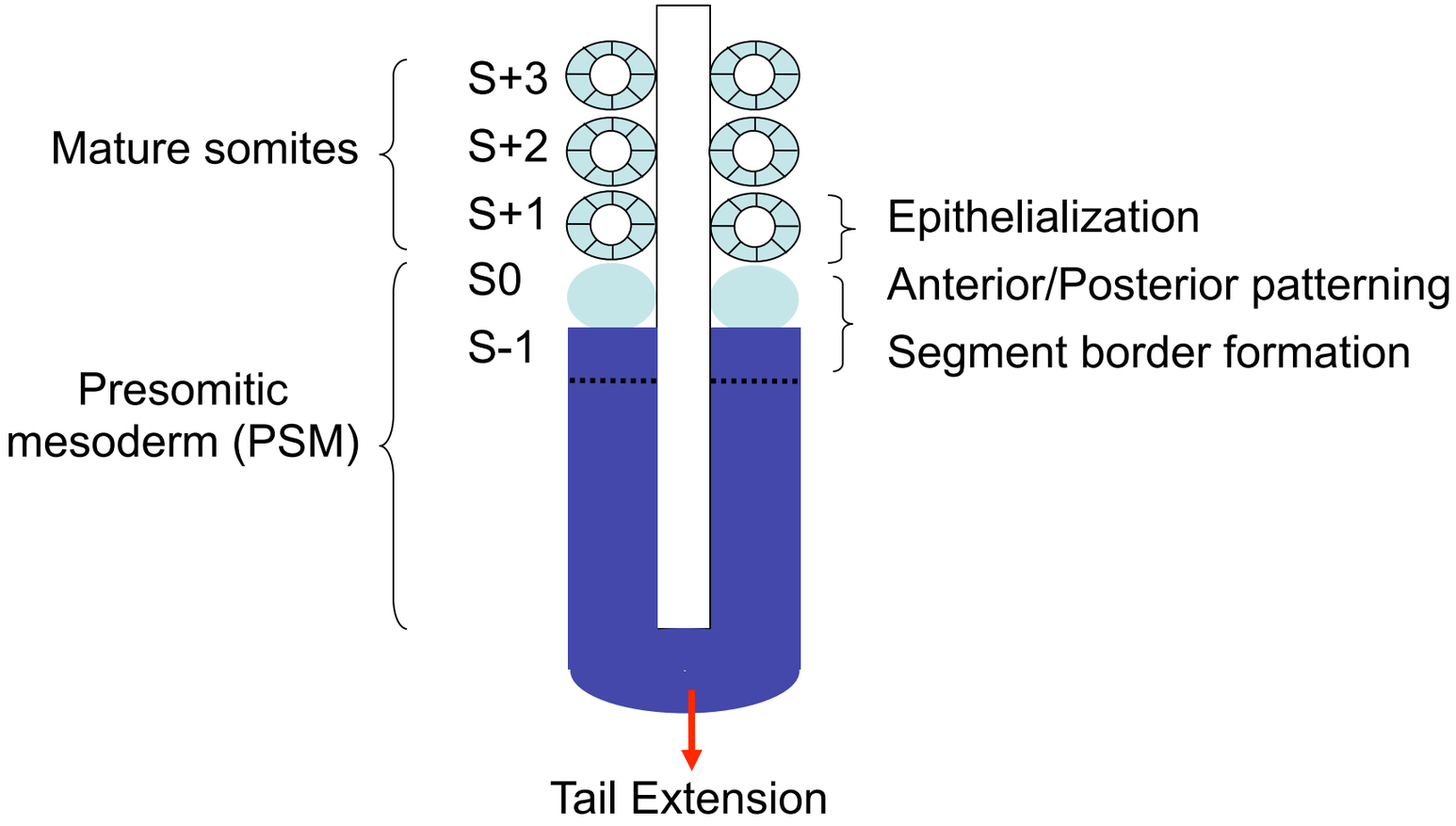
Caudal

Olivier Pourquie

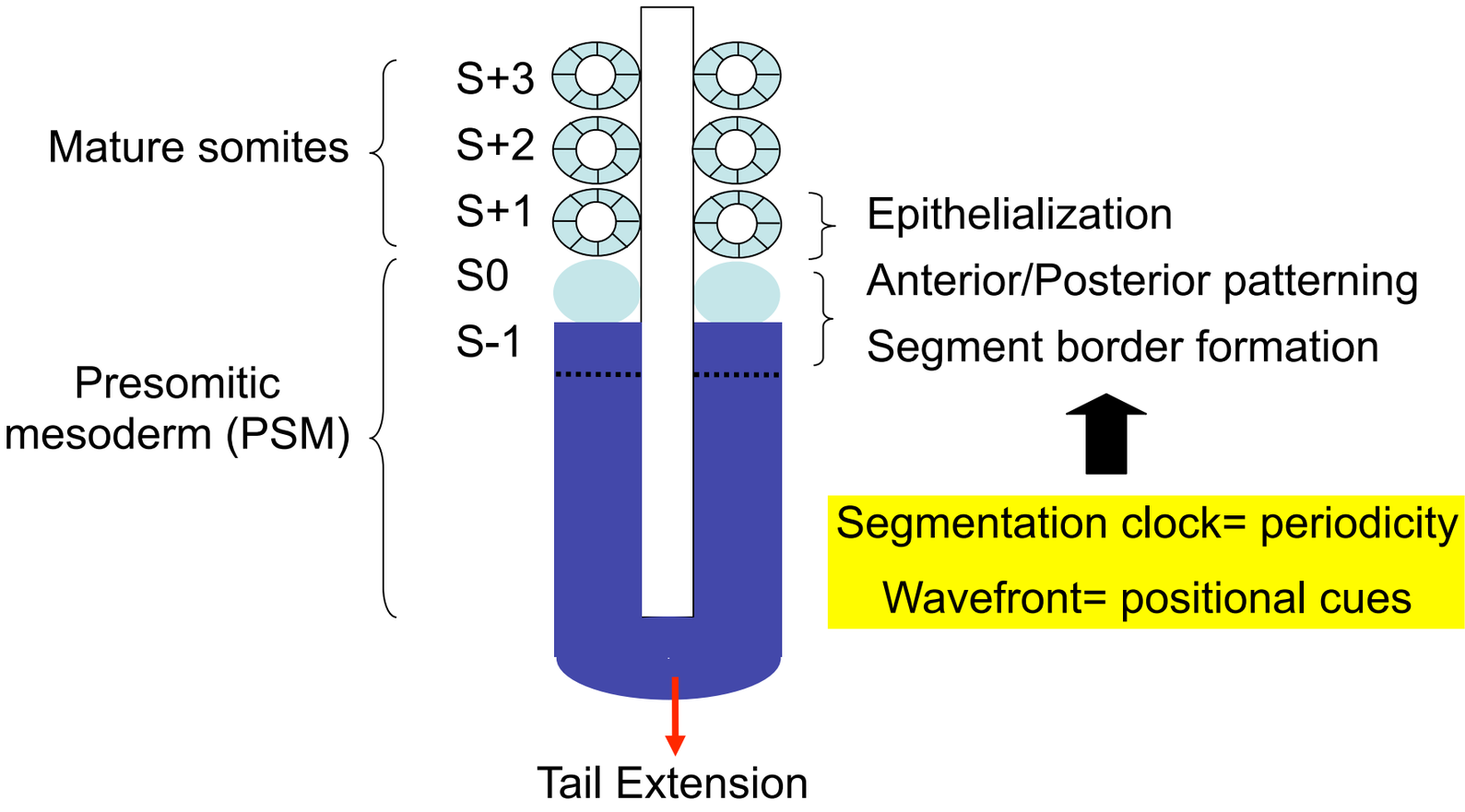
Somitogenesis is a reiterated process



Somitogenesis is a reiterated process

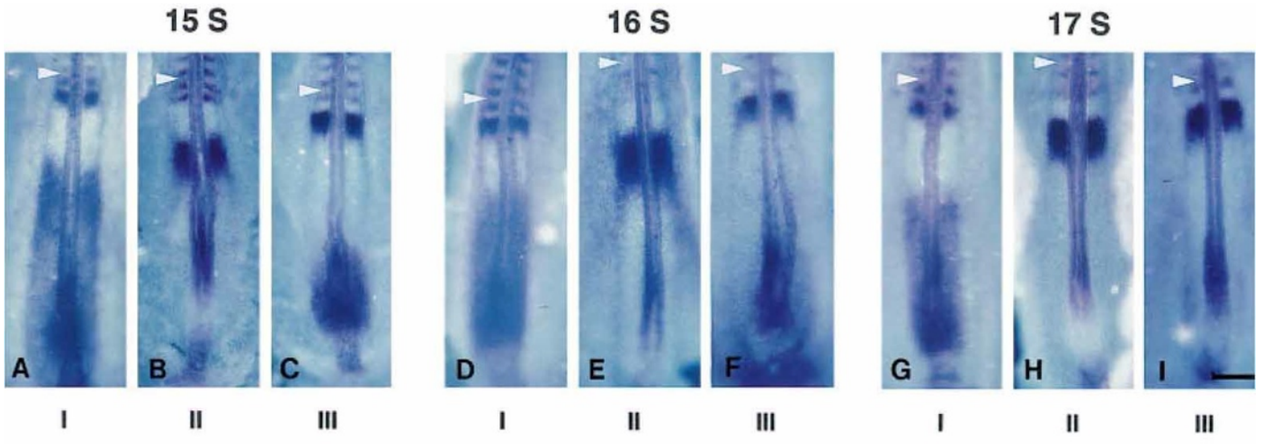


Somitogenesis is a reiterated process



Oscillatory Genes Regulate Somitogenesis

Expression
of the
hairy gene
in chick



Palmerim et al.,1997
(Pourquié Lab)

Ish-Horowicz lab
animation

Oscillatory Genes Regulate Somitogenesis

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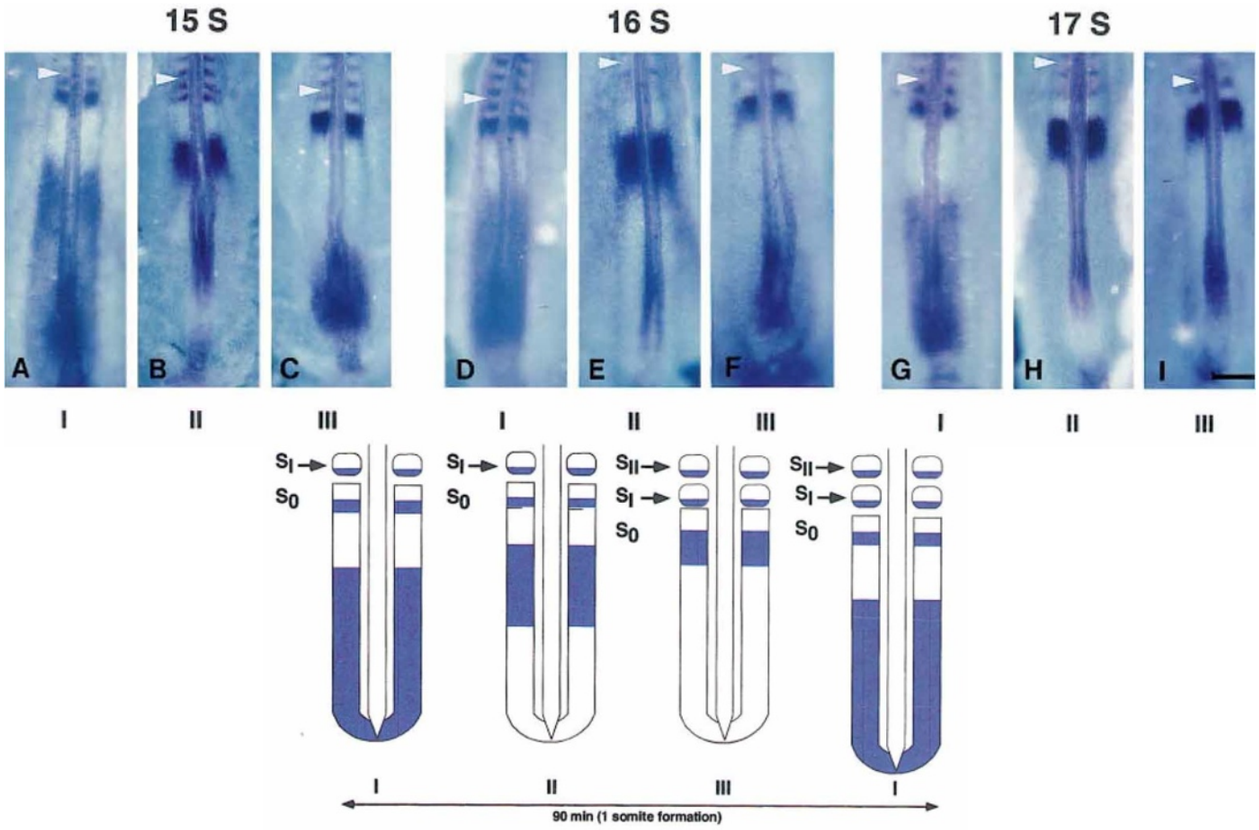

Palmerim et al.,1997
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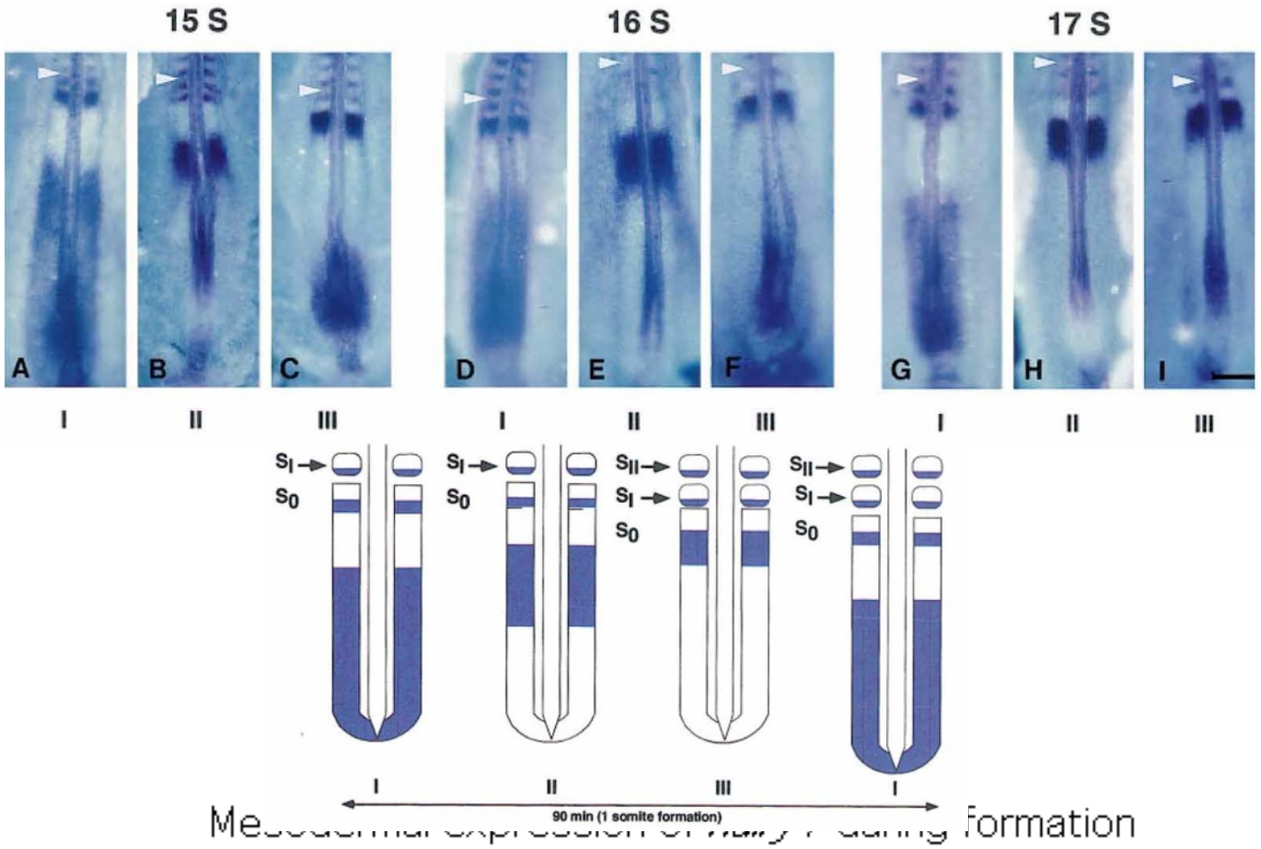
animation

Oscillatory Genes Regulate Somitogenesis

Expression of the *hairy* gene in chick

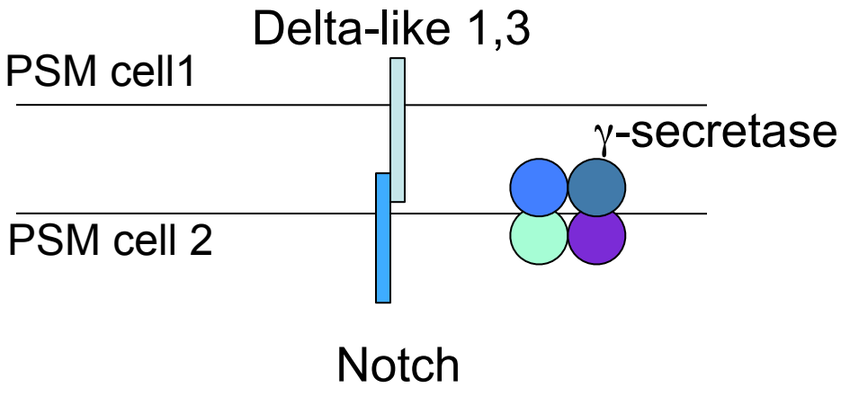


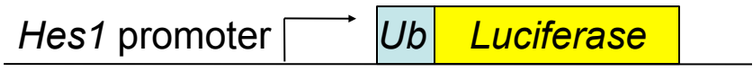
Palmerim et al.,1997 (Pourquié Lab)

Mesodermal expression of *hairy 1* during formation of the first 20 somites in the chick embryo: a clock-and-wavefront model simulates the observed pattern.
hairy 1 RNA in blue, central nervous system in grey, head to the right

Ish-Horowicz lab animation

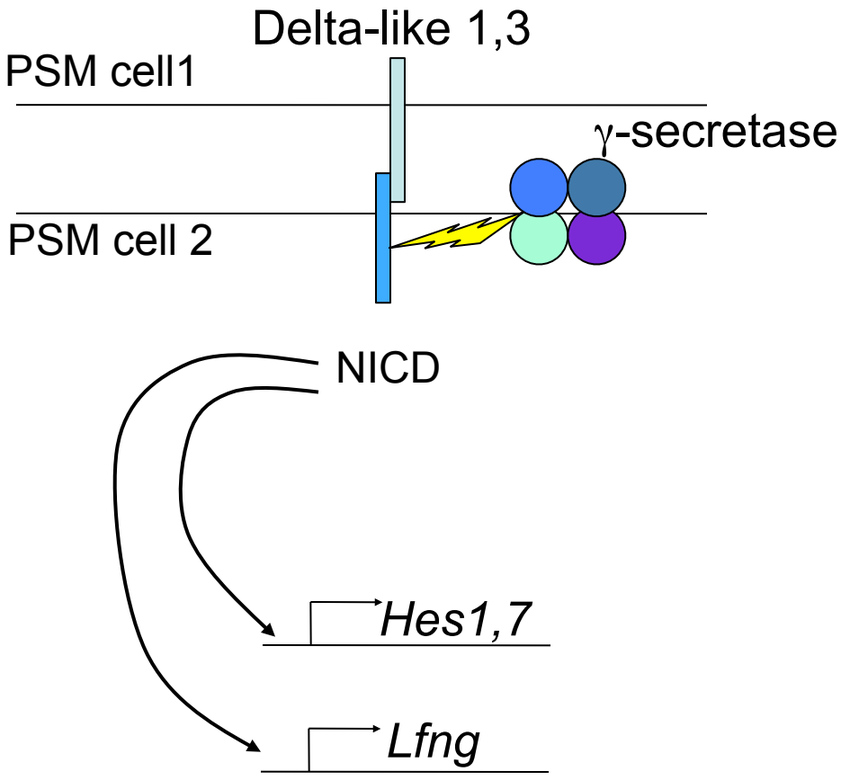
The Notch pathway underlies a "Segmentation Clock" in the PSM

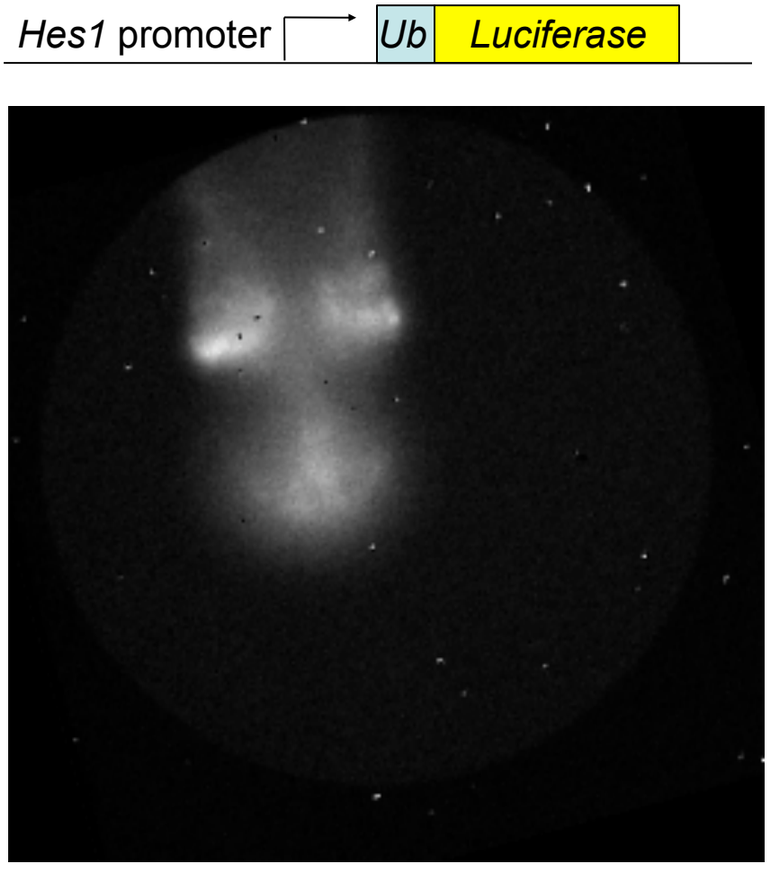




Masamiza (2006) PNAS

The Notch pathway underlies a "Segmentation Clock" in the PSM





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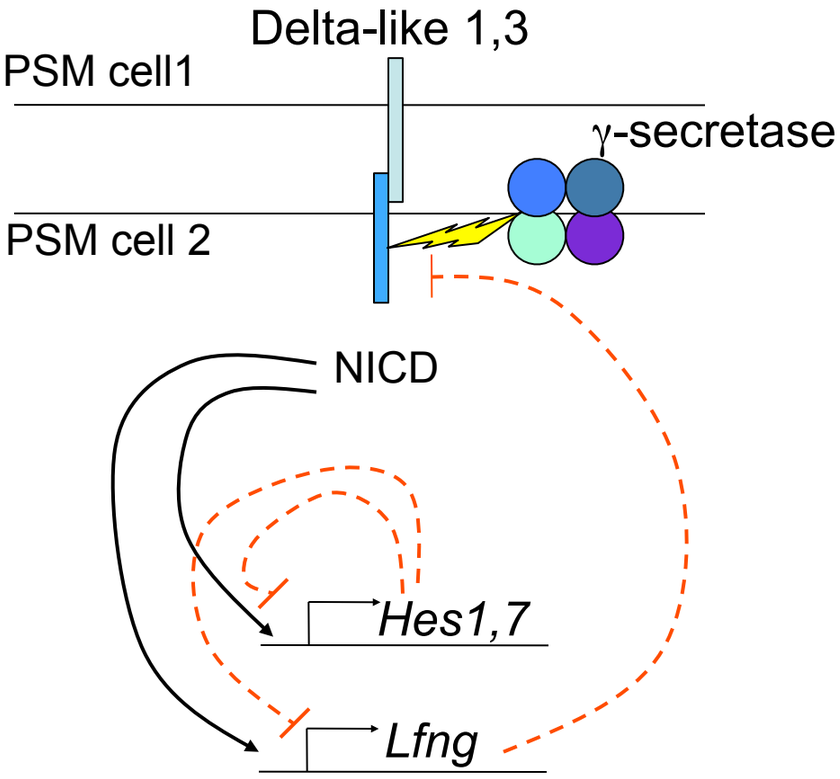

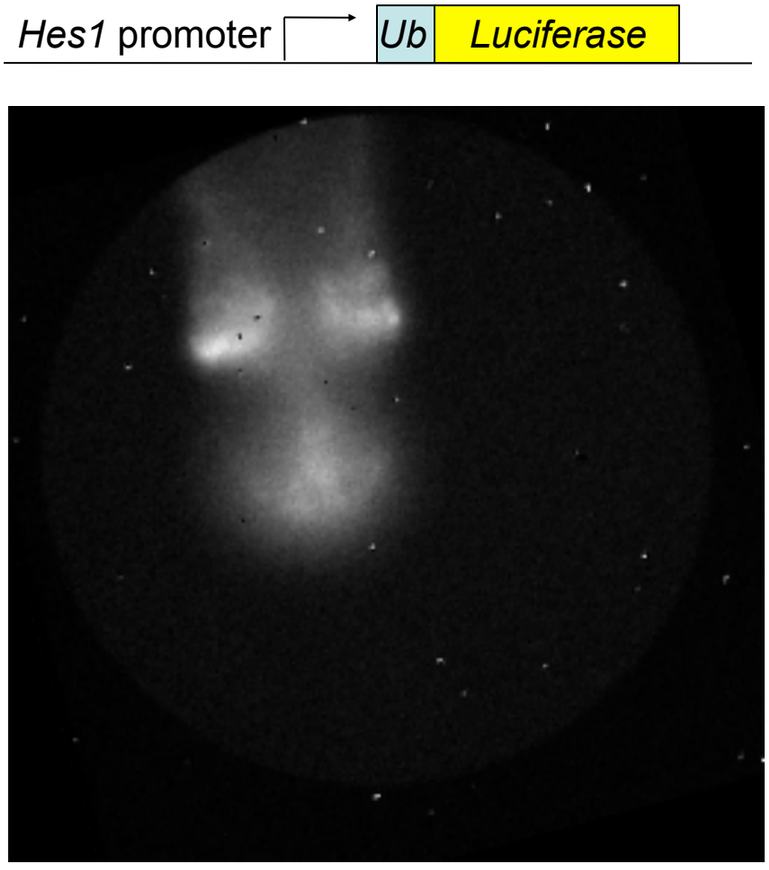

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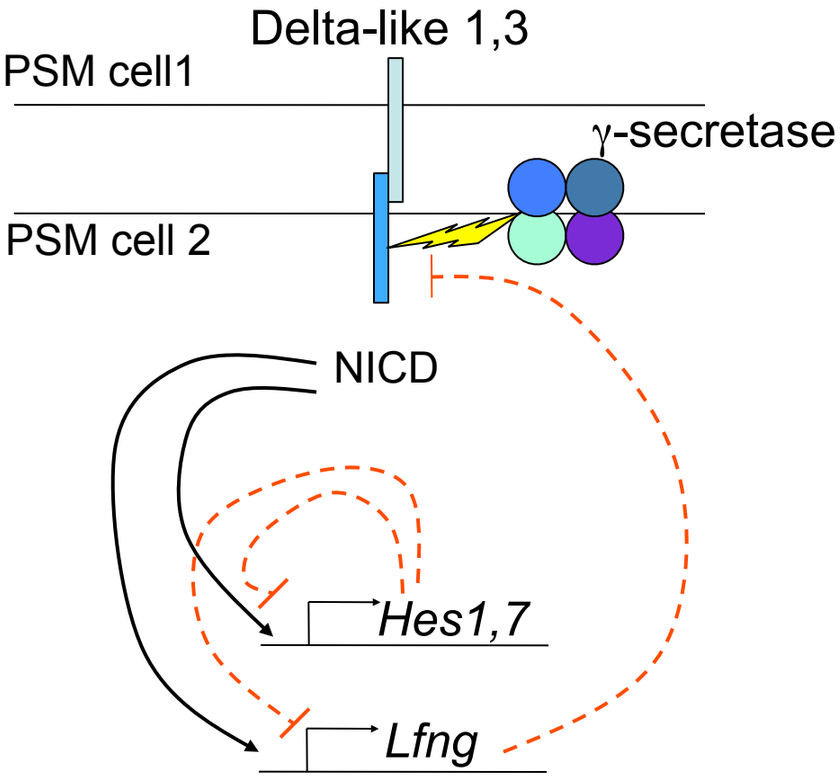
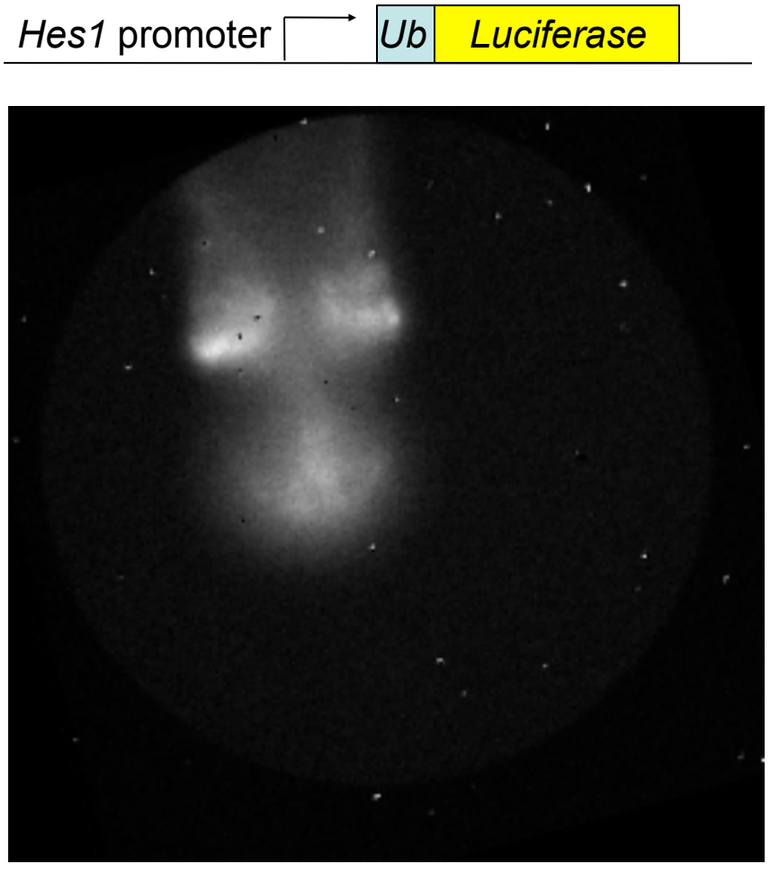




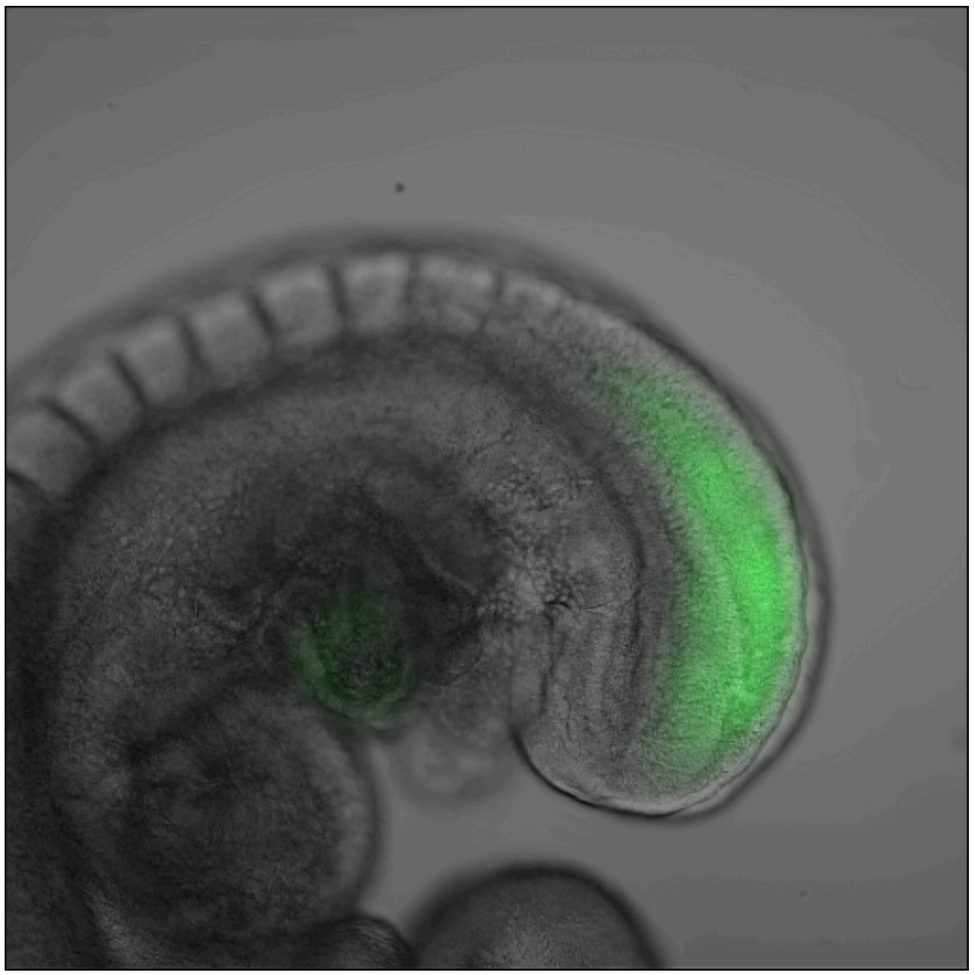
Masamiza (2006) PNAS

The vertebrate segmentation clock

Alexander Aulehla
and Olivier Pourquie

Lfng promoter-YFP
10 hour movie
Two photon time
lapse imaging

The vertebrate segmentation clock



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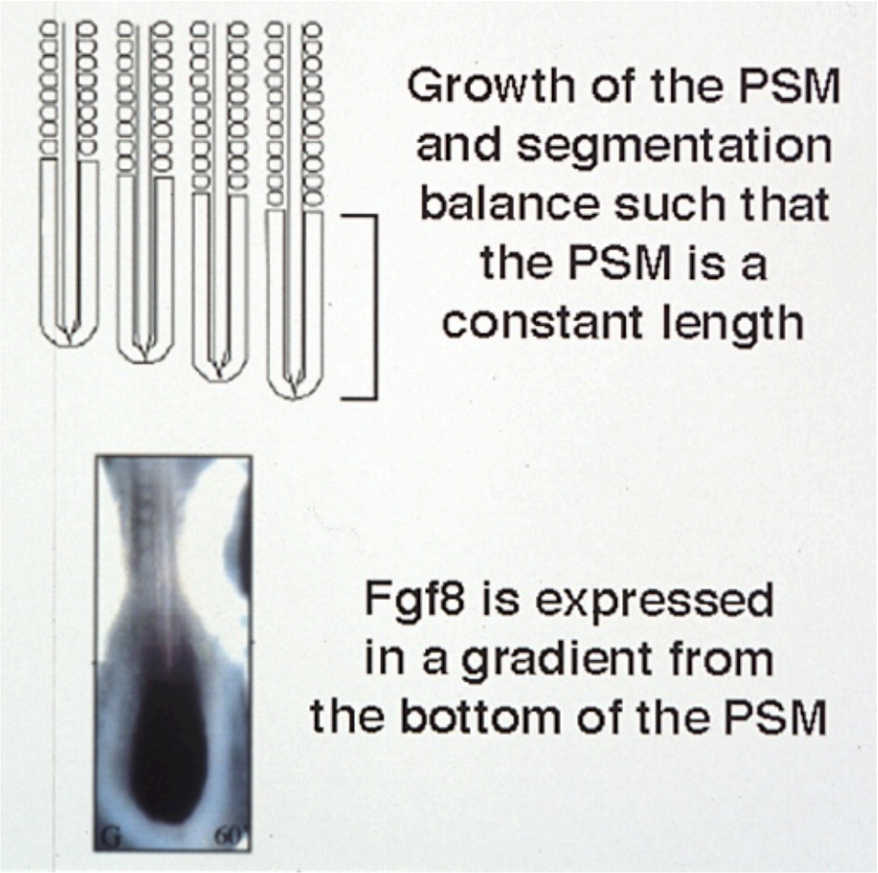
Why does the signaling oscillator only lead to segmentation at the rostral end of the PSM?



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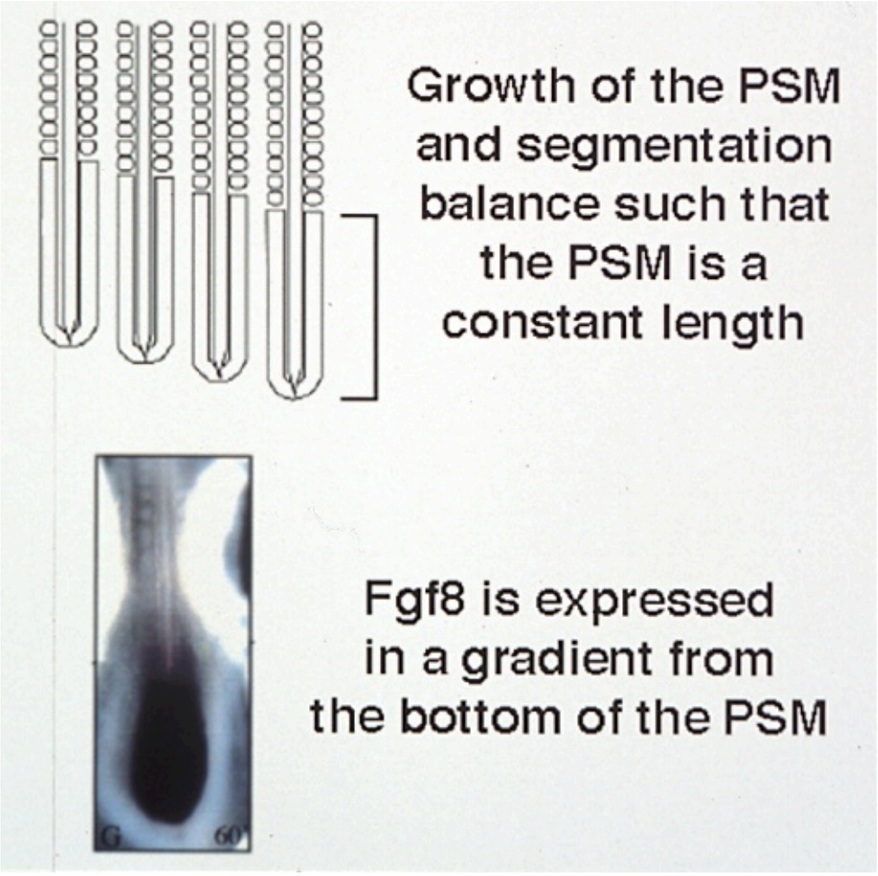
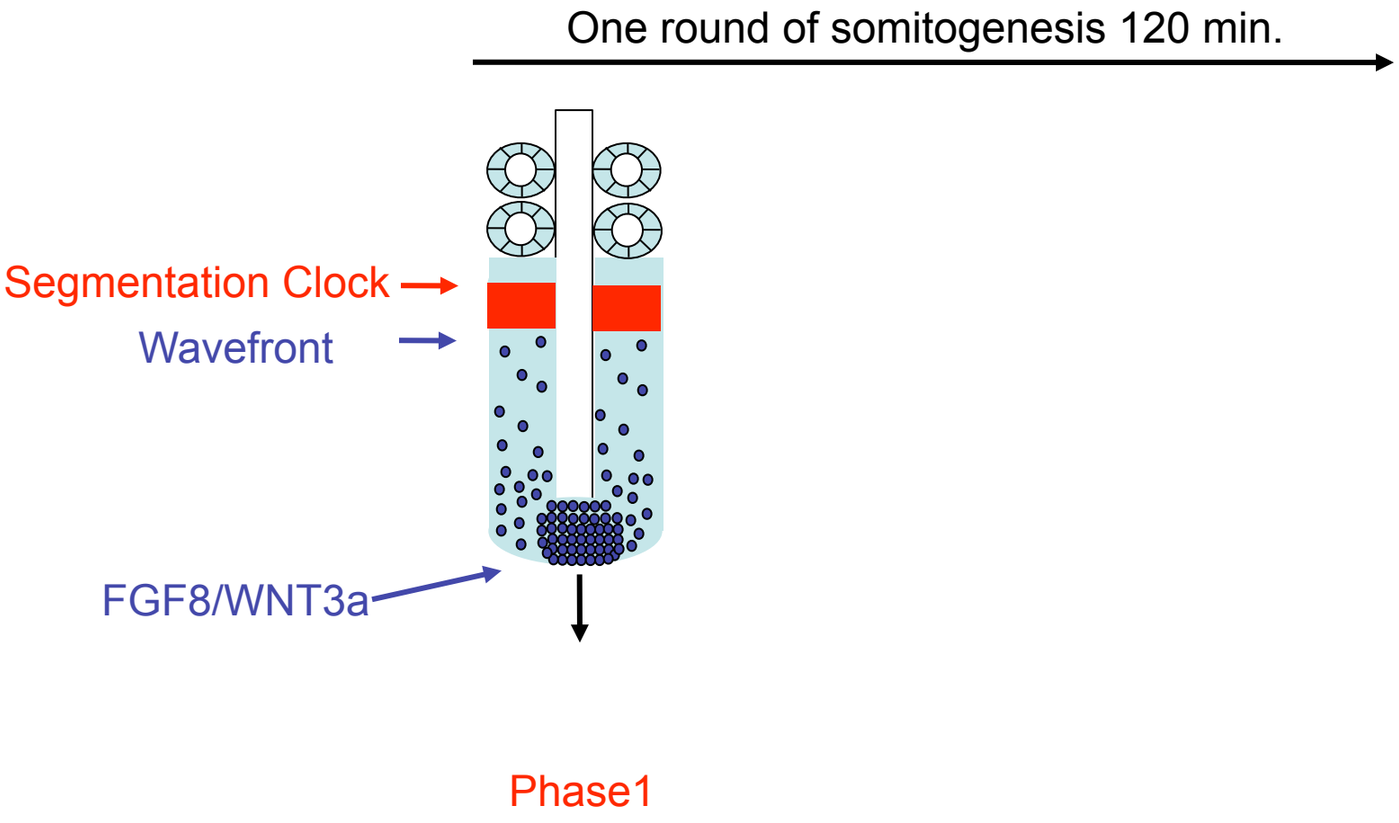


...because there is an inhibitory FGF/Wnt signal produced at the caudal end of the embryo

The “Wavefront” sets up positional information



Cells in the PSM periodically exhibit high levels of Notch signaling (each round of Notch signaling more caudal cells activate Notch before their cranial neighbors, in a “wave”).

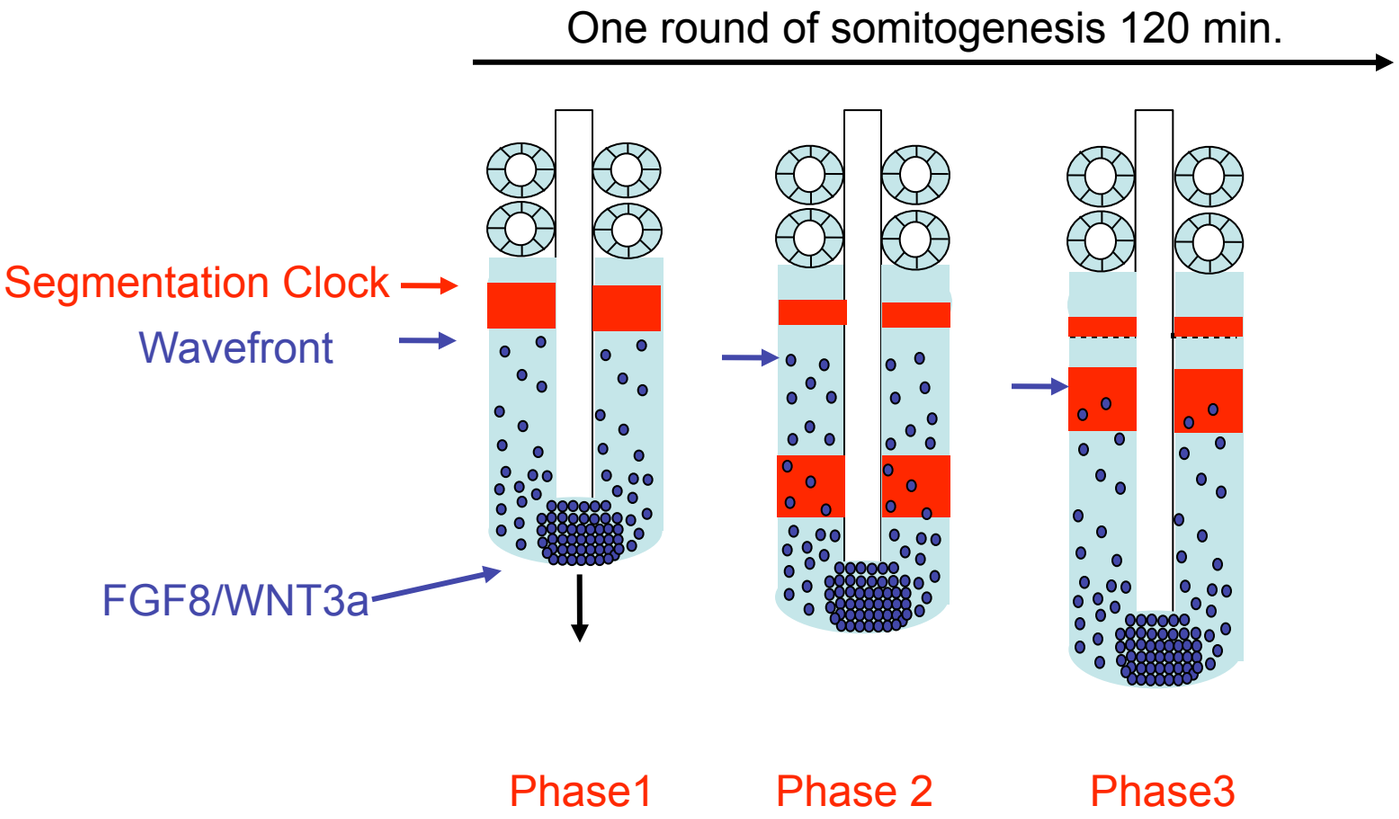
In response to Notch signaling the cells epithelialize with their neighbors and form spherical somites.

This response is blocked by Fgf produced at the caudal end of the PSM.

Thus only the most cranial cells, in the PSM, beyond the range of Fgfs, respond to Notch activity during each wave.

Continued growth at the caudal end results in more PSM cells and also moves the source of Fgf further back, allowing the next somite to form.

The “Wavefront” sets up positional information



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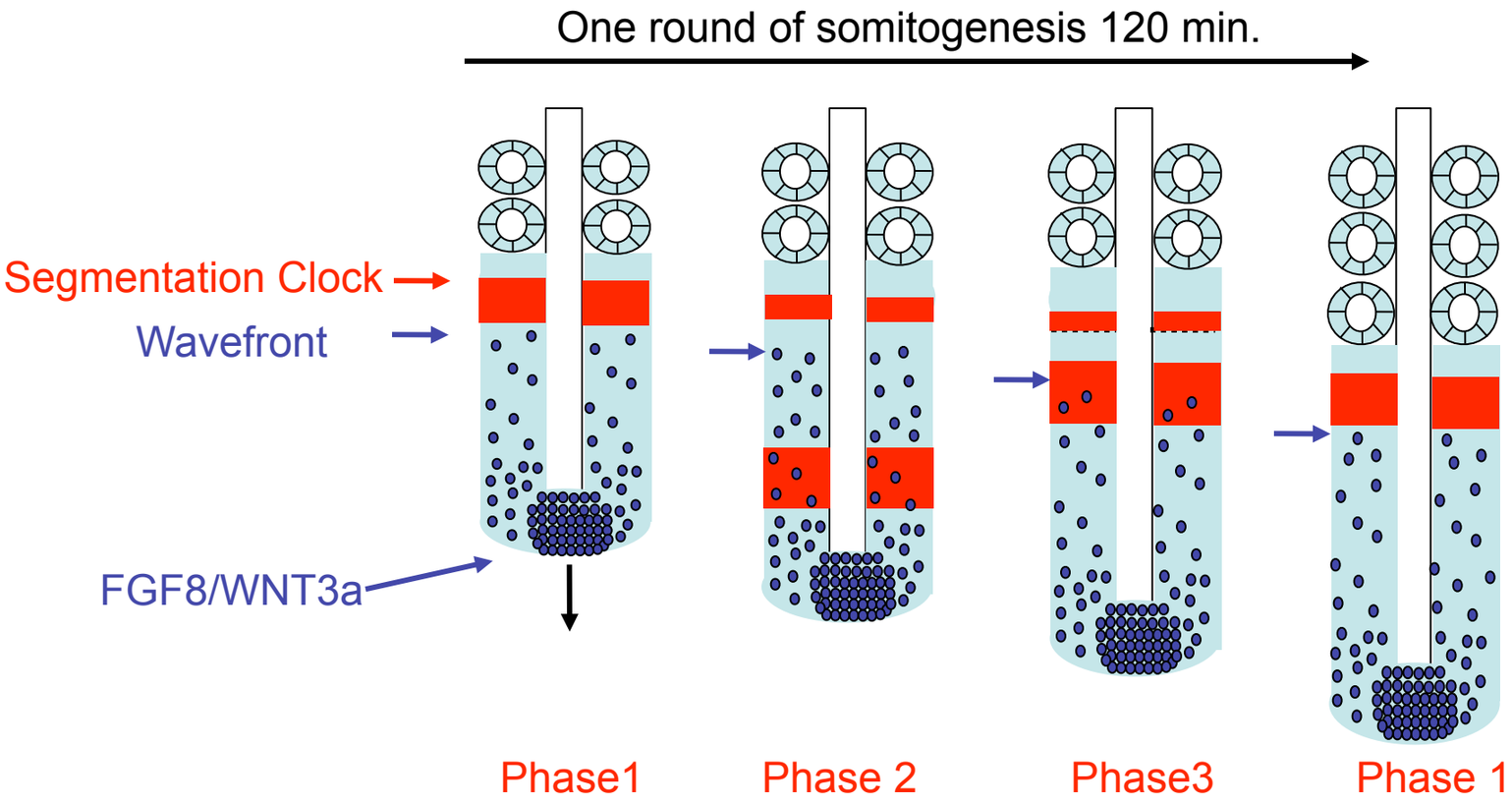

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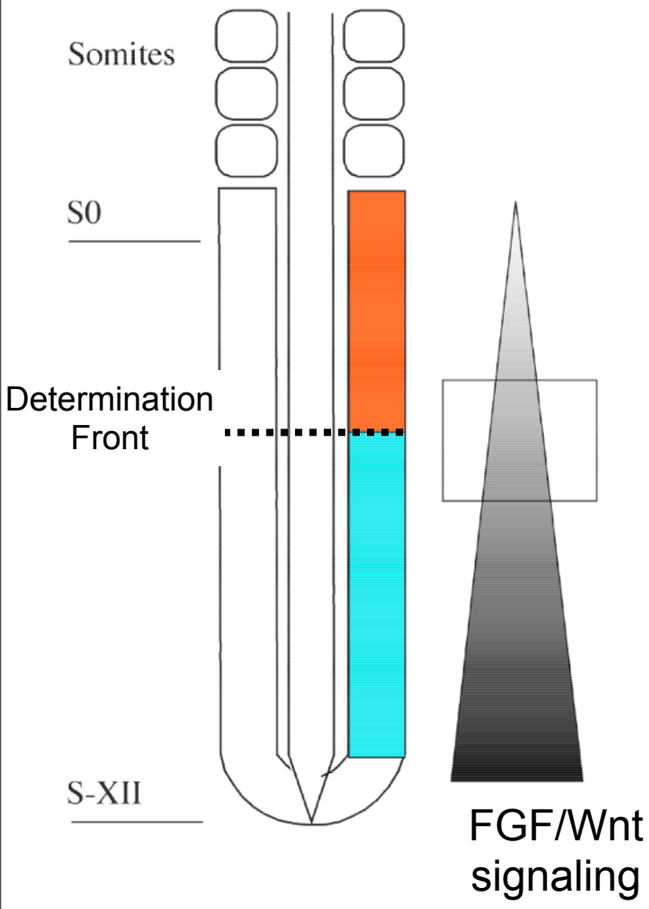
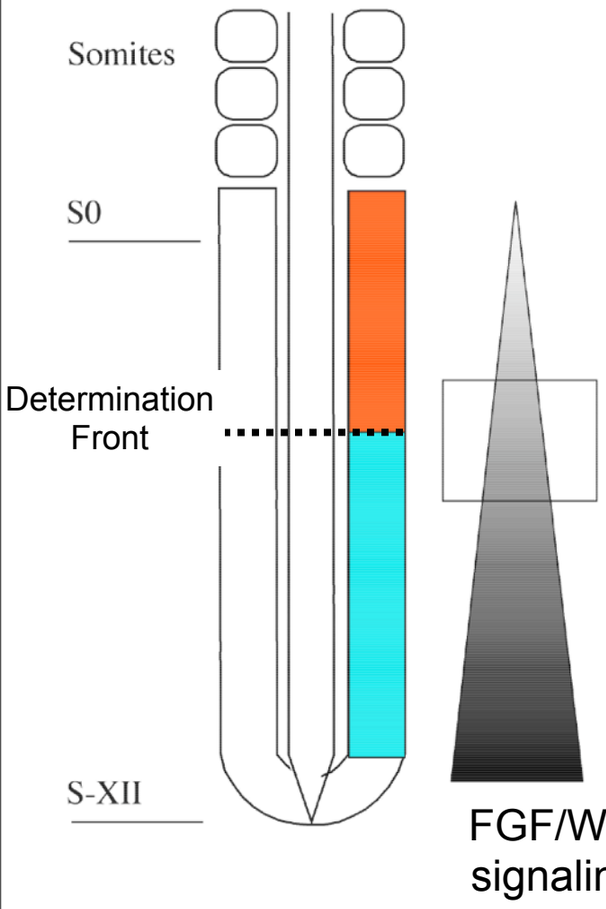
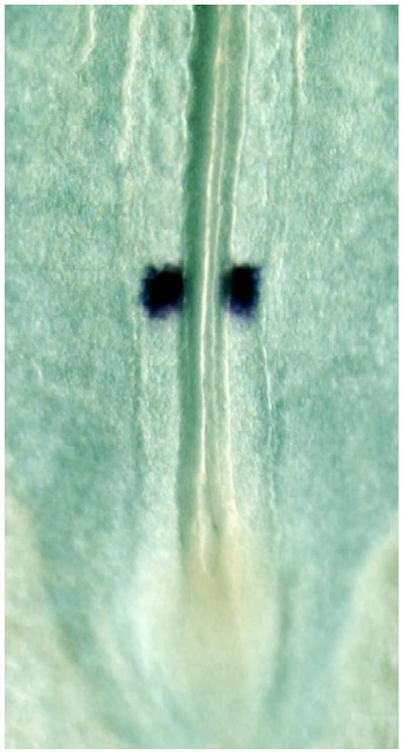
The wavefront/determination front controls the spatial response to the segmentation clock



The wavefront/determination front controls the spatial response to the segmentation clock





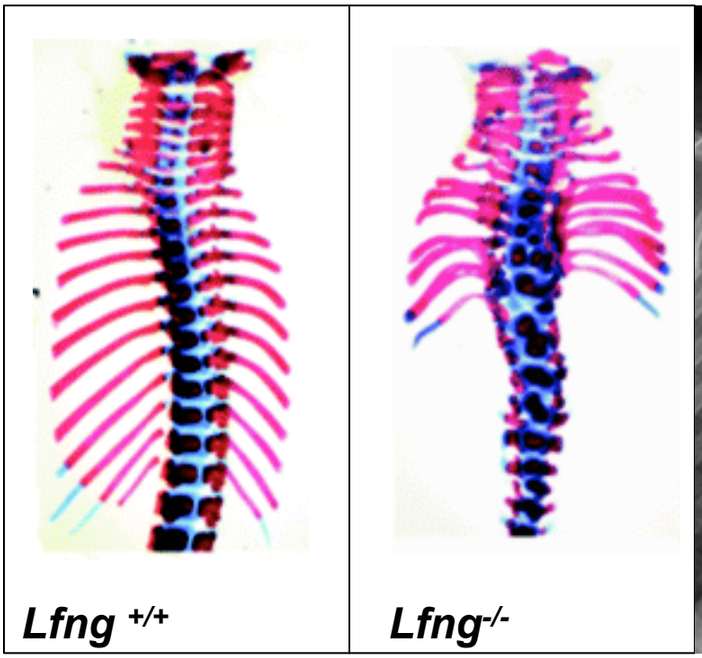
Differentiation
(Myf5, Pax1)

Segmentation (Mesp2)

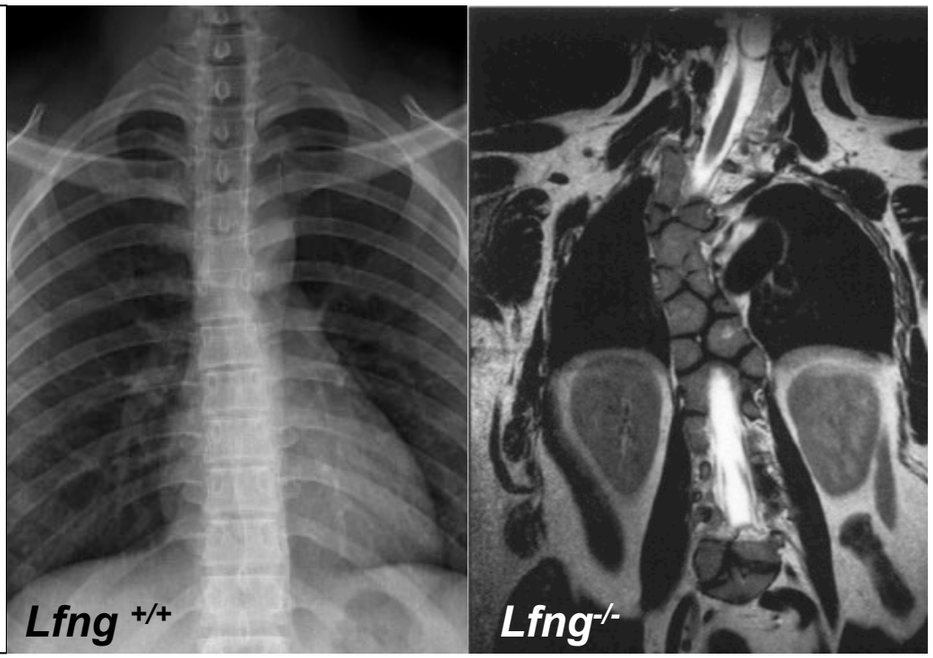
Posterior identity
(Brachyury Sprouty)

Mutations in the Notch pathway/segmentation clock lead to congenital scoliosis

Mouse



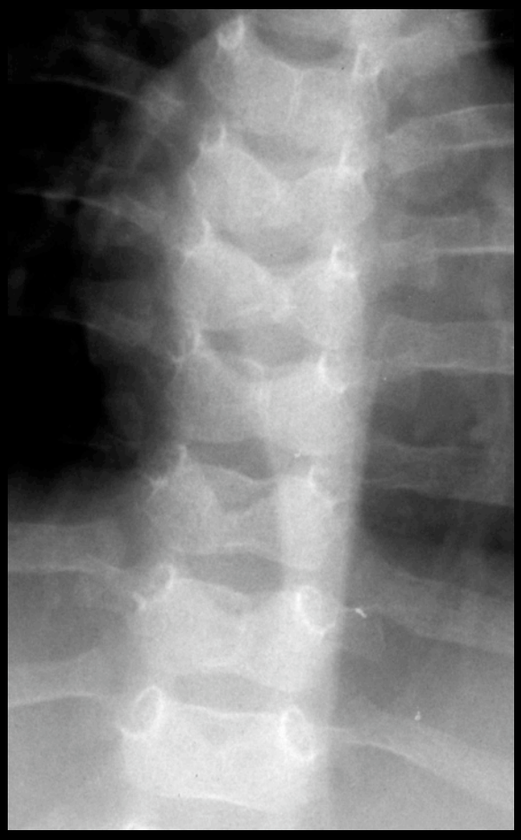
Human



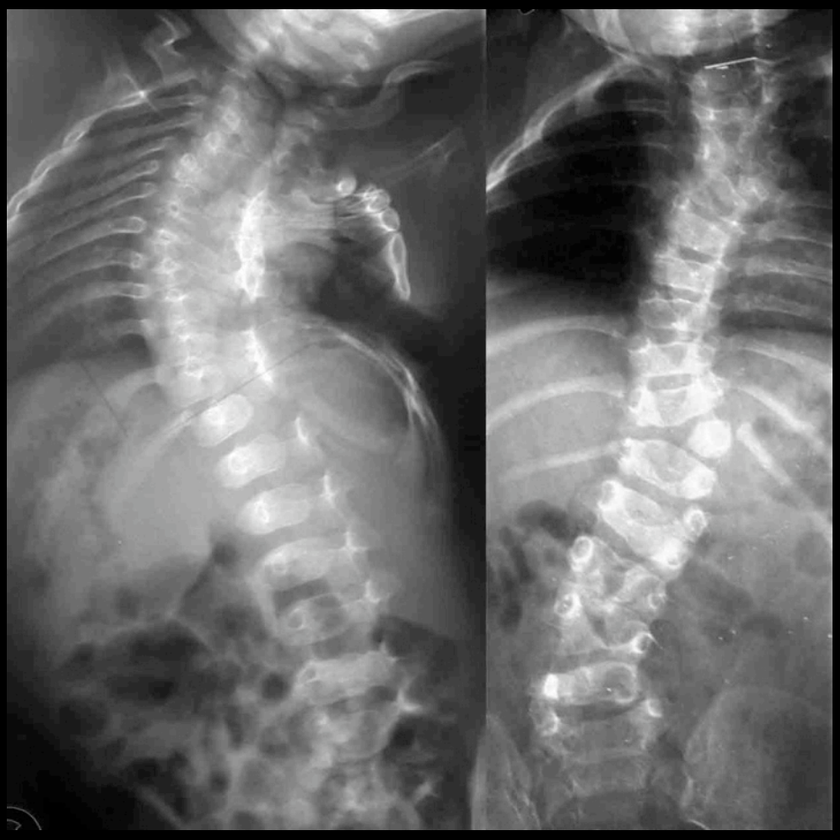
SPONDYLOCOSTAL DYSOSTOSIS, AUTOSOMAL RECESSIVE 3; SCDO3; OMIM 609813

Bulman Nature Genetics (2000)

Additional Congenital Vertebral Defects

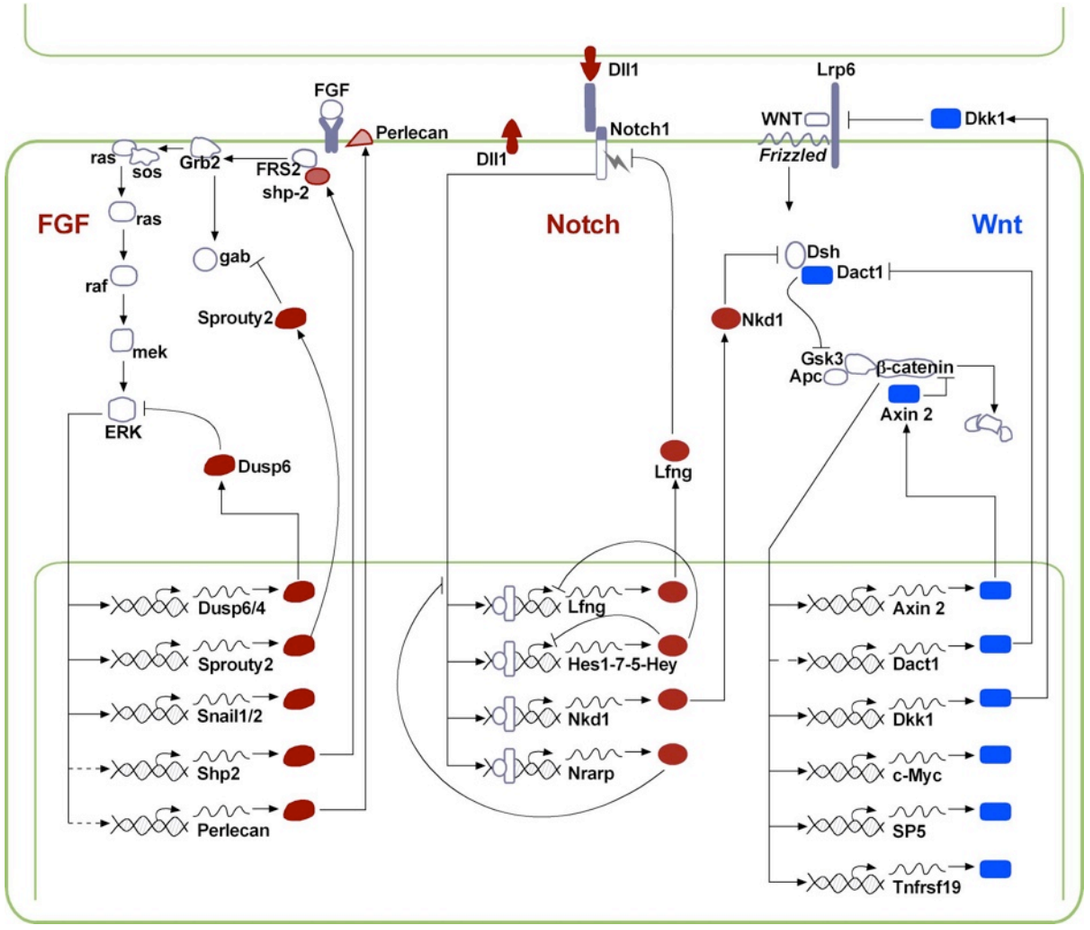


JAG1-Alagille syndrome (OMIM 118450)
(64% display vertebral defects)
From Nancy Spinner and Ian Krantz

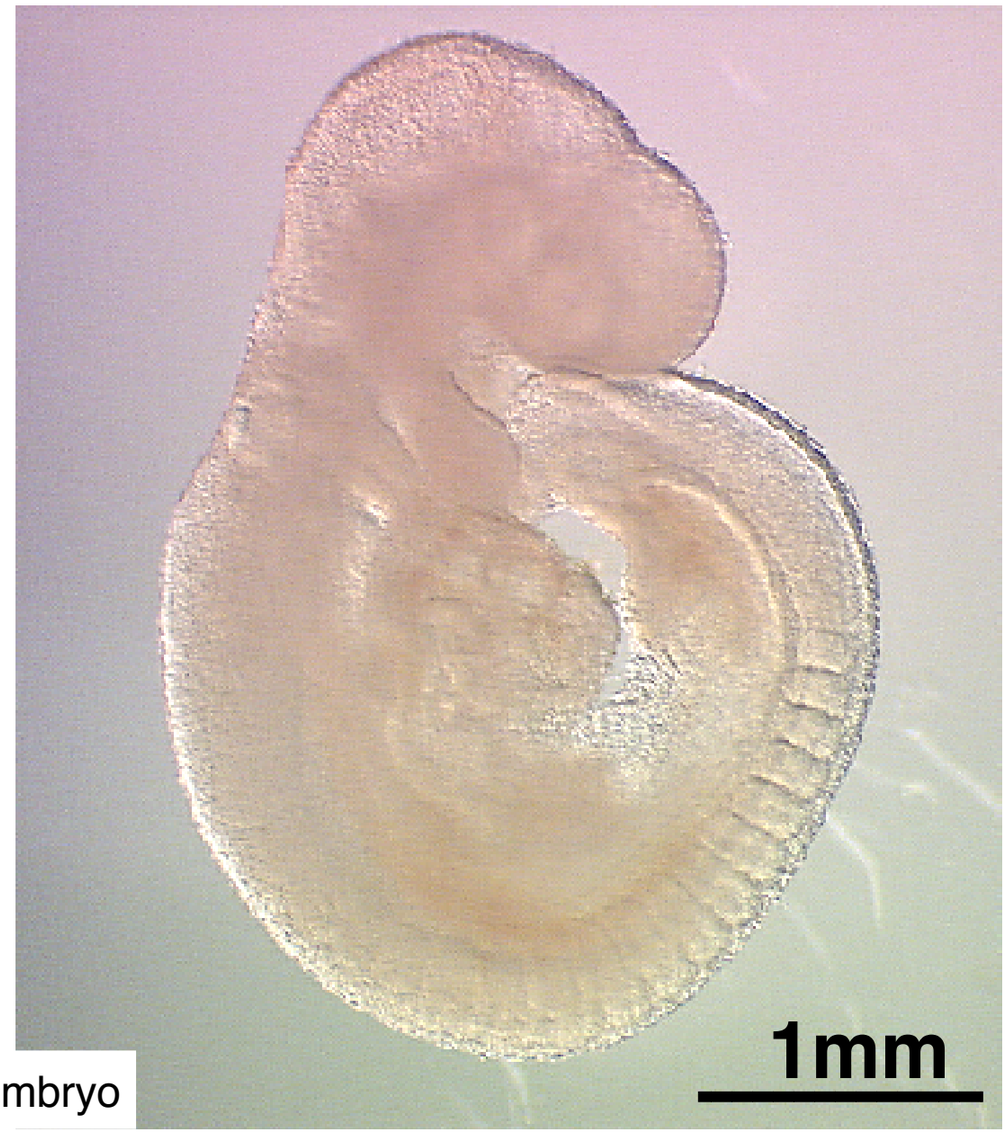


Congenital scoliosis & kyphosis
Uncloned, etiology not known
Erol et al., 2004

Coordinated temporal regulation of signaling modules of the FGF, Notch and Wnt pathways underlies the segmentation clock



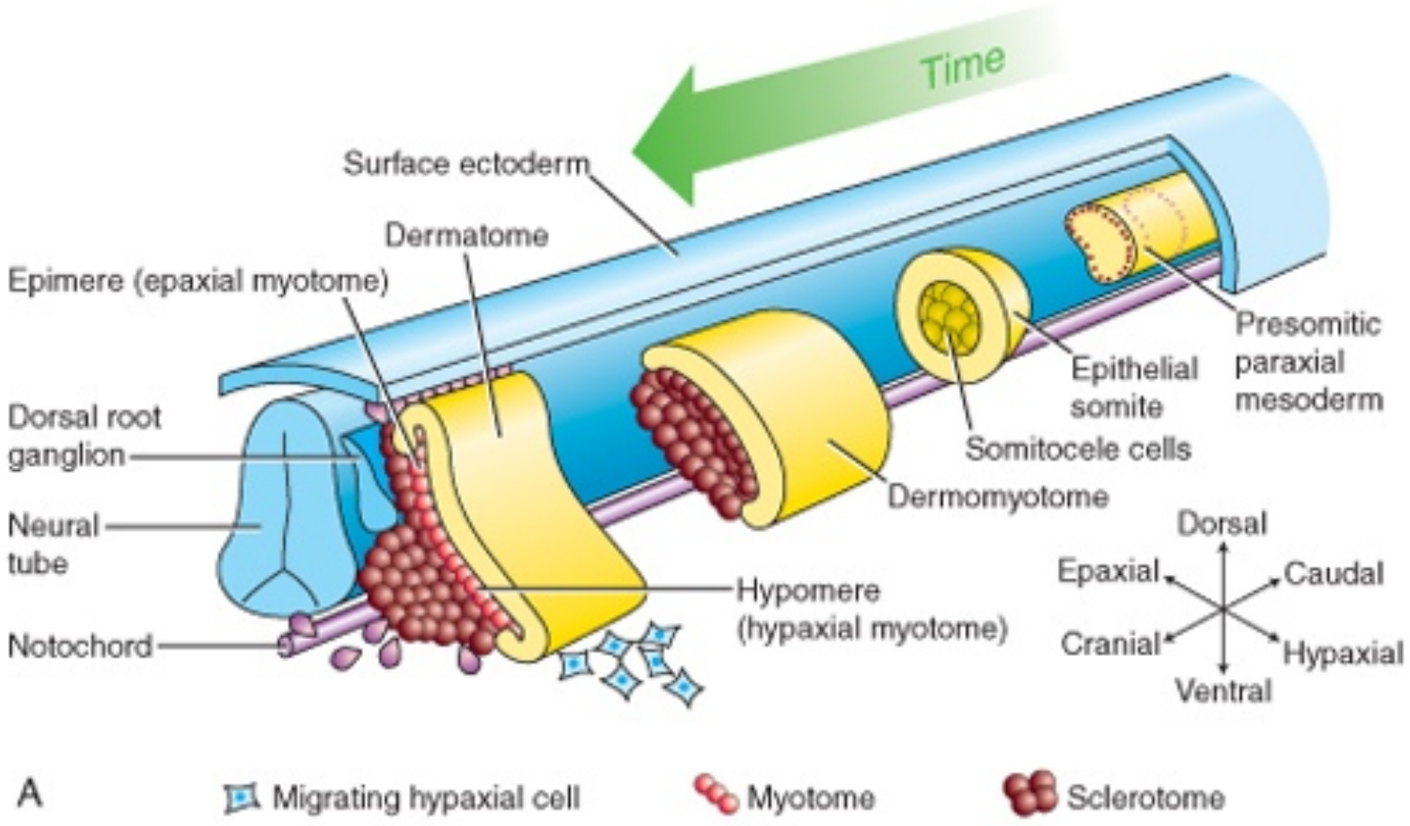
Somite differentiation



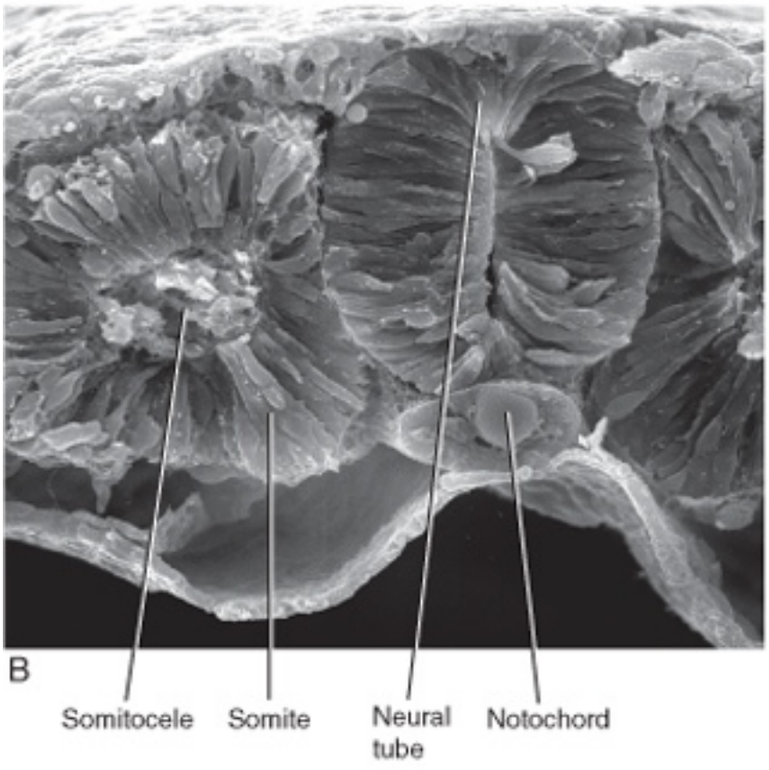
Day 9.5 mouse embryo

This is a sagital view of a day 9.5 mouse embryo, approximately half way through its gestation period. Here's the head, trunk and turn around the tail end of the embryo. The entire trunk musculoskeletal system arise from these beads-like segmented structures called somites. Somite is not only a hallmark of all vertebrate embryos, its segmented organization is reflected in the adult body, e.g. the vertebra and ribs.

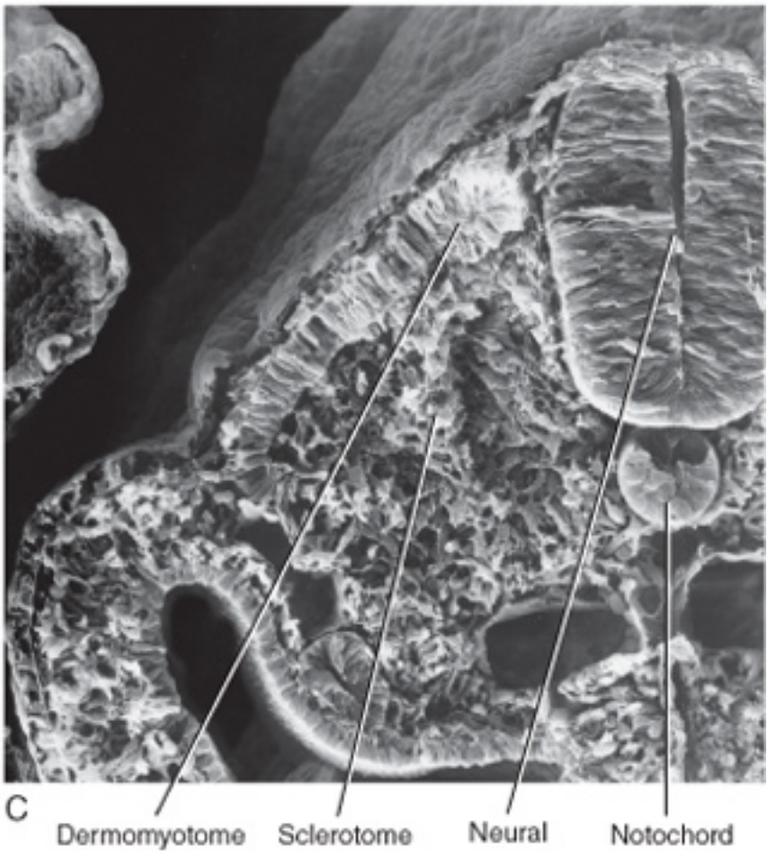
Somites differentiate progressively: multiple stages are present in the embryo at any time

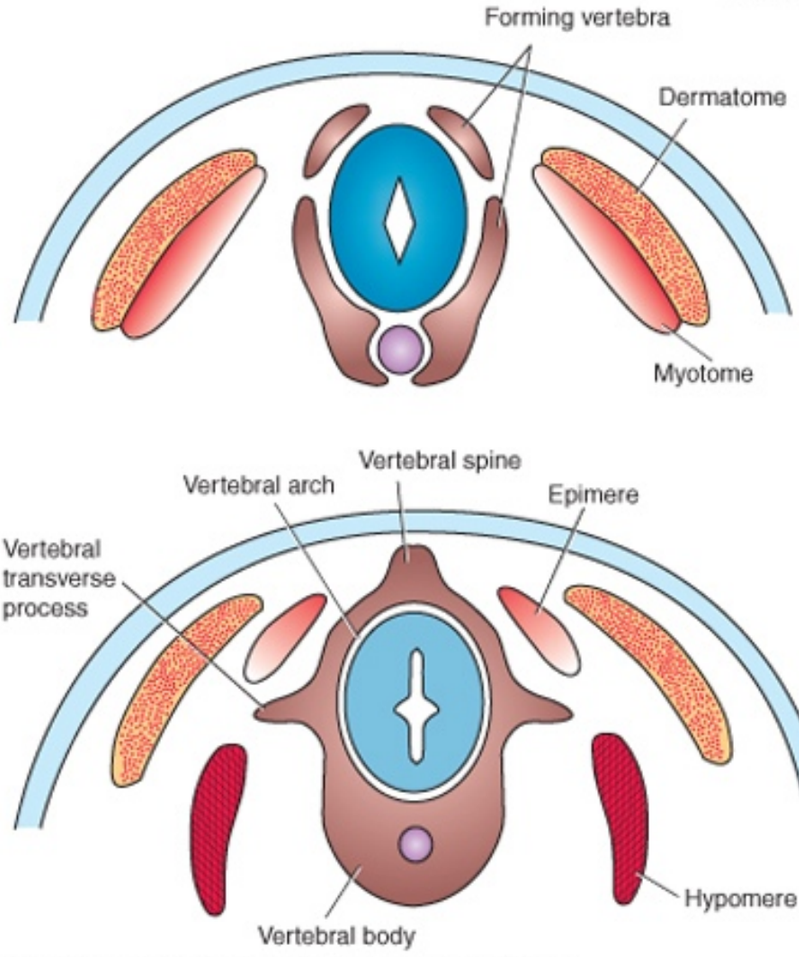


Somites differentiate progressively



Epithelial somite





enwolf et al: Larsen's Human Embryology, 4th Edition.

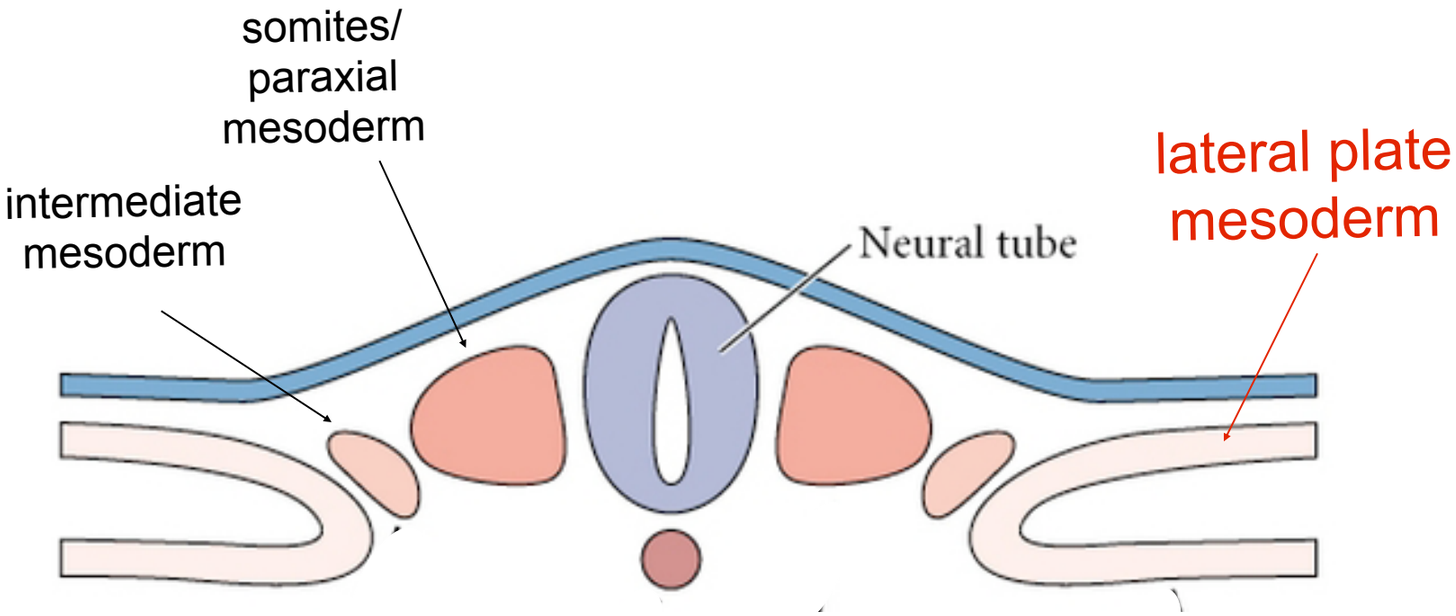
The sclerotome forms the vertebrae, the myotome forms muscles

By the end of the fifth week, prospective muscles are found divided into two parts:

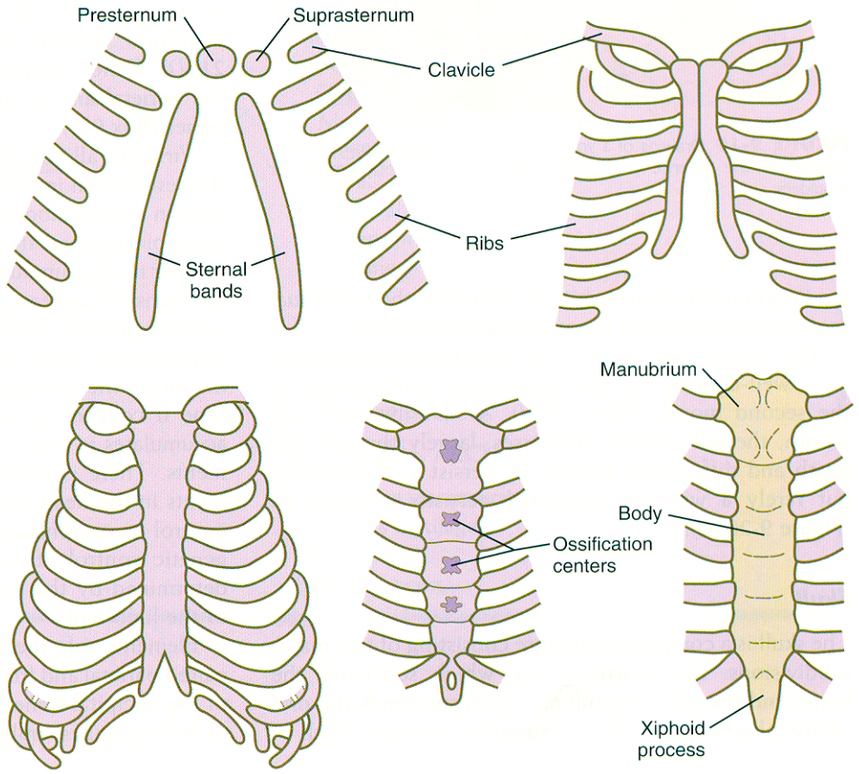
Epimere - back muscles (from dorsomedial lip of myotome)
Hypomere - body wall and limb muscles (from ventromedial lip of myotome)

Ribs and Sternum:

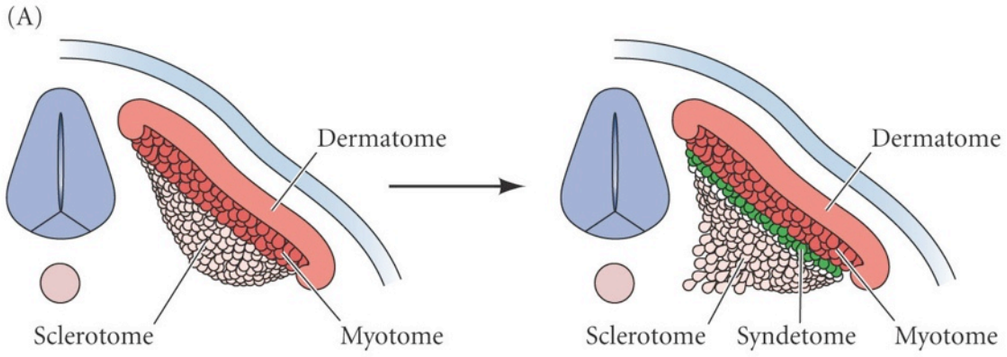
Unlike the rest of the axial skeleton (vertebrae and vertebral ribs, the sternum derives from lateral plate mesoderm



Two sternal bands condense bilaterally in the lateral plate mesoderm in the ventral body wall, migrate around the developing embryo and fuse at the midline to become the manubrium, sternebrae and xiphoid process



4th Somitic Compartment: The Syndetome



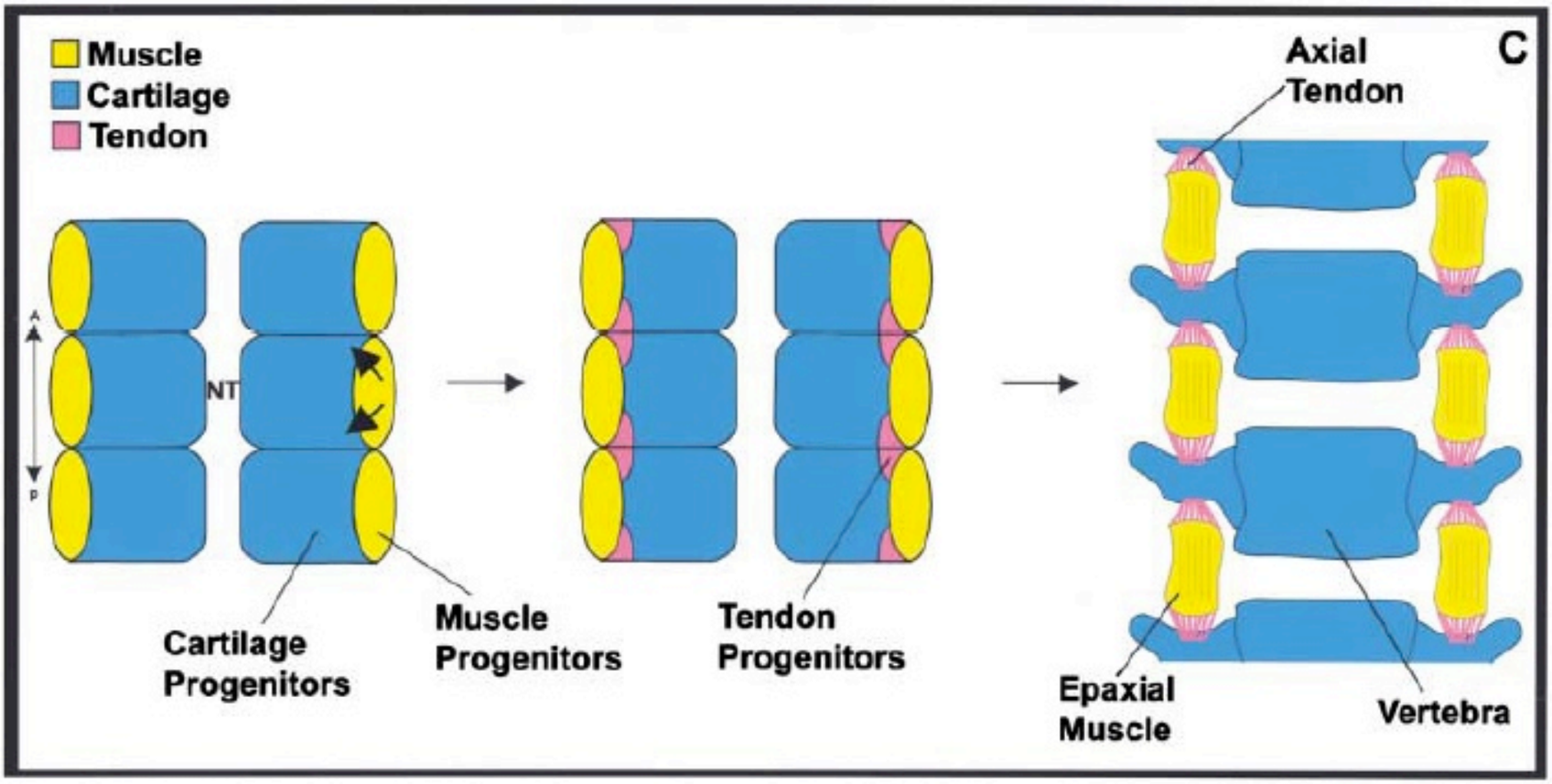
Defined by expression of ***scleraxis (Scx)***.

This population arises between myotome (after involution under dermomyotome) and sclerotome.

From sclerotomal compartment.

Gives rise to the tendons.

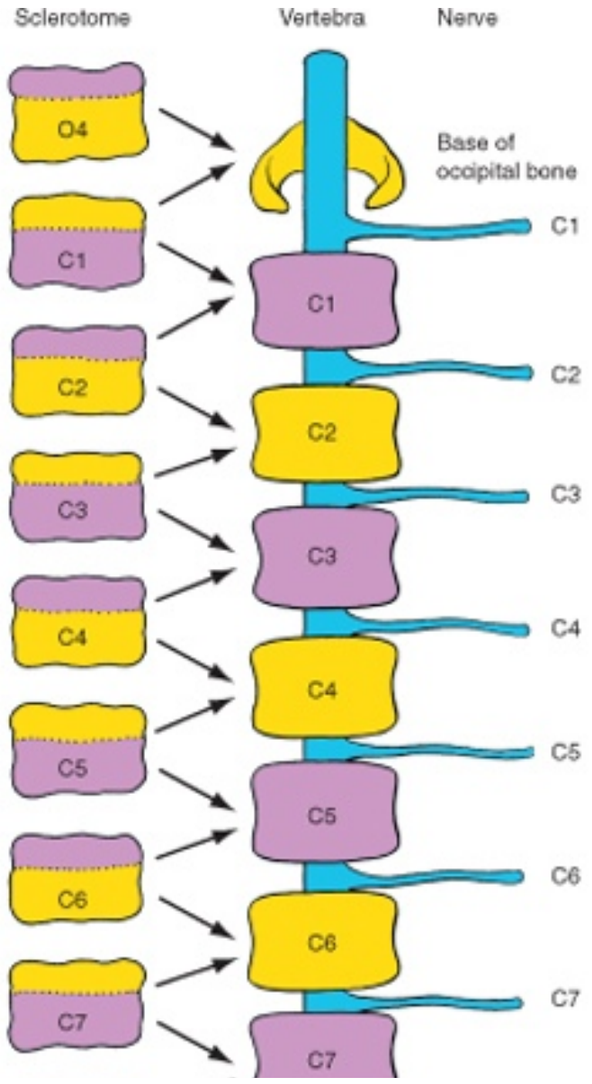
Places tendons in correct position in the axial skeleton…



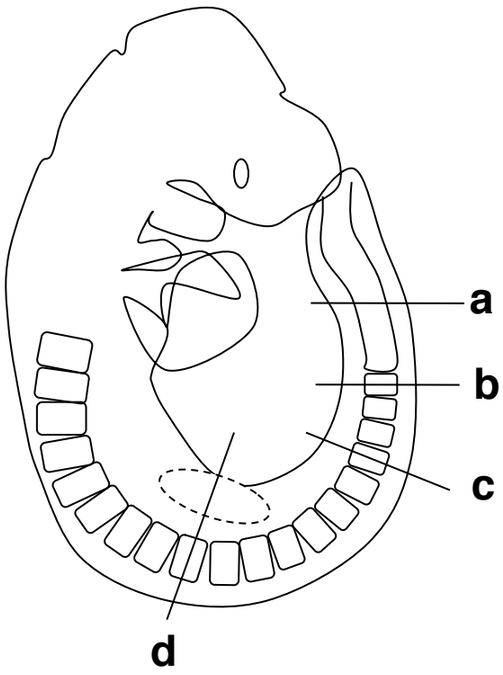
Resegmentation:

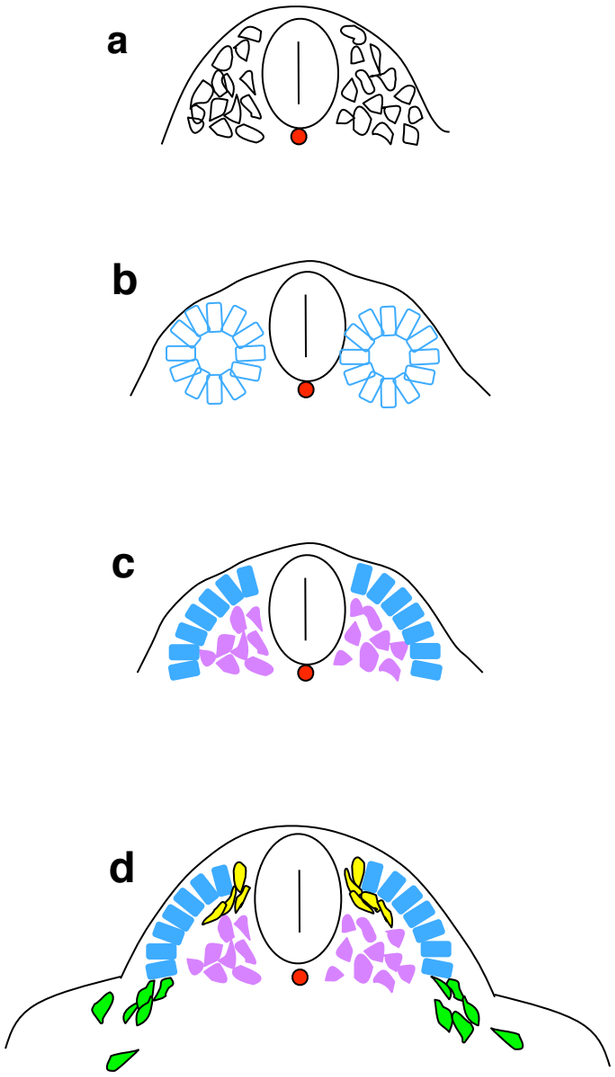
Cells from the caudal half of one somite and cells from the cranial half of the adjacent caudal somite form one vertebral body.

This allows the nerves of each segment to project out of phase from the vertebral bodies



The stereotypic pattern of somite development





presomitic mesoderm (psm) cells

somite formation
epithelialization

dermomyotome

sclerotome

medial myotome

central myotome

lateral myotome

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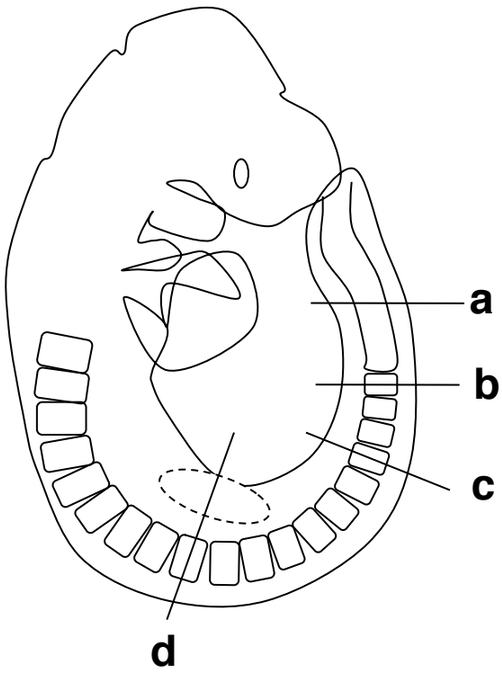

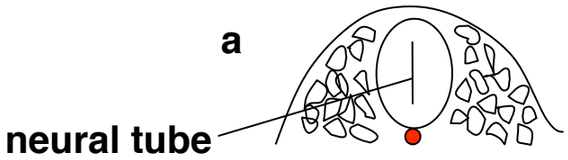

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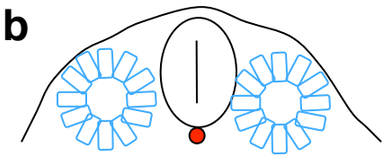

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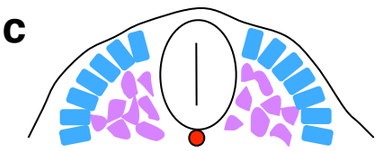

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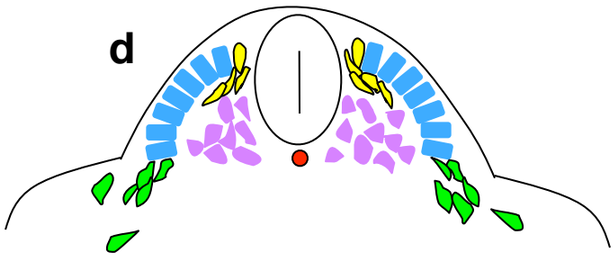

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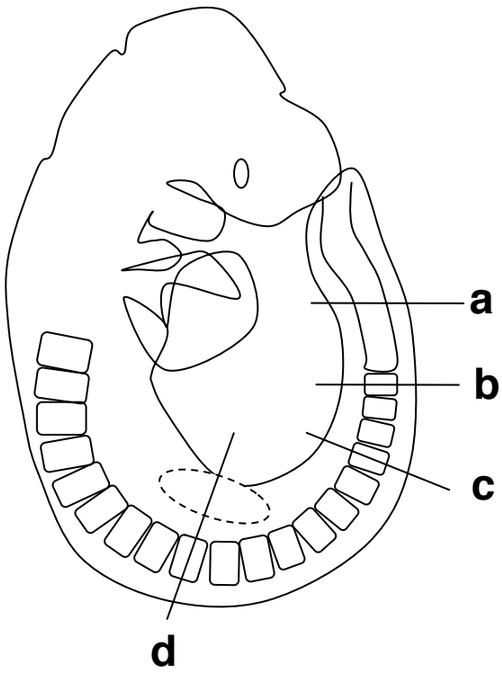

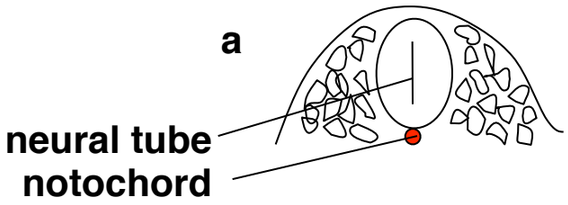

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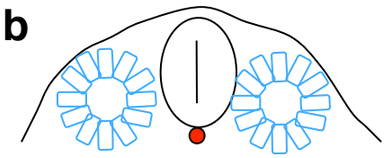

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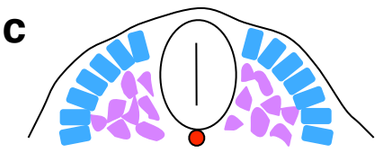

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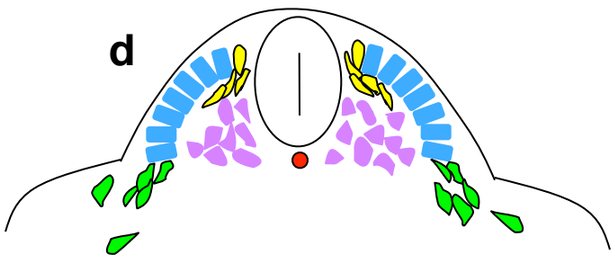

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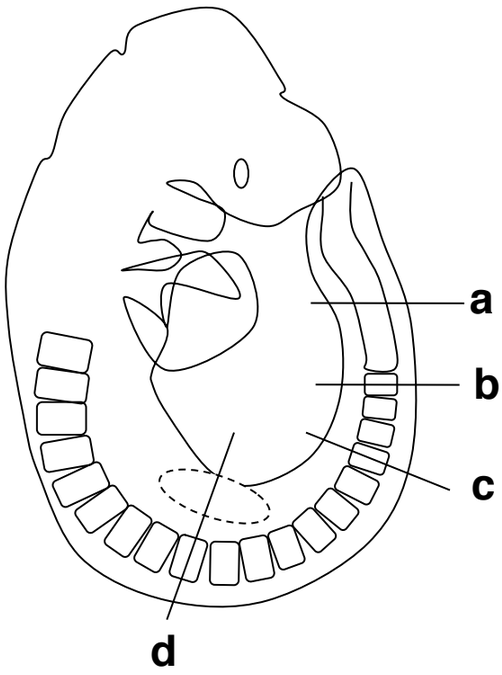

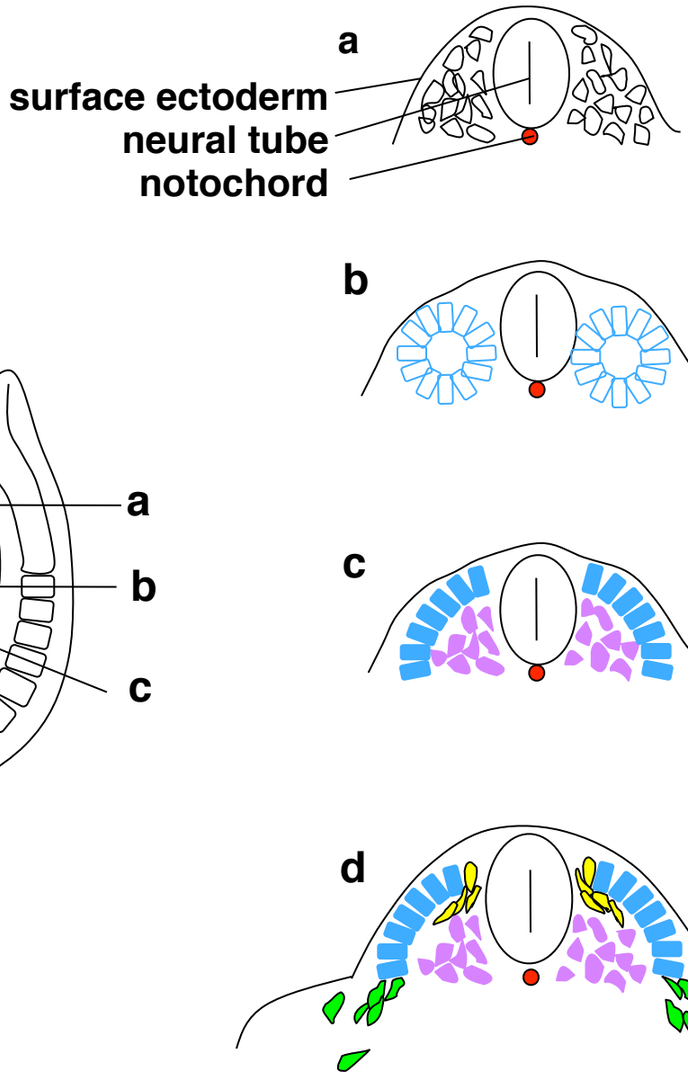

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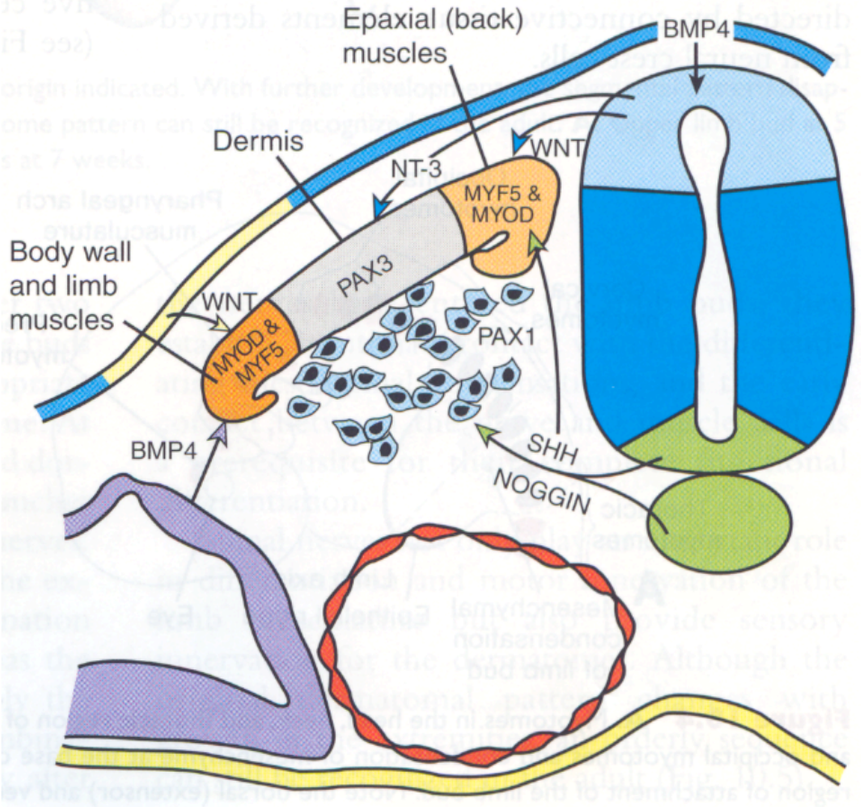
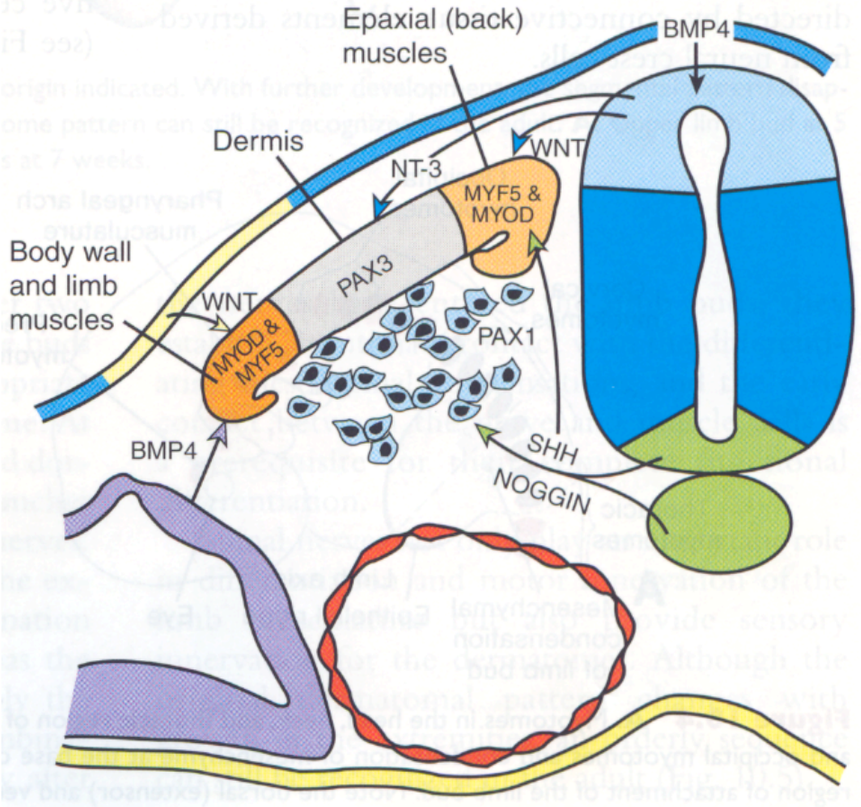


Inductive interactions subdivide the somite

- Shh and noggin (BMP antagonist), secreted by the notochord and floor plate cause the ventral part of the somite to form sclerotome (Pax1 txn factor).



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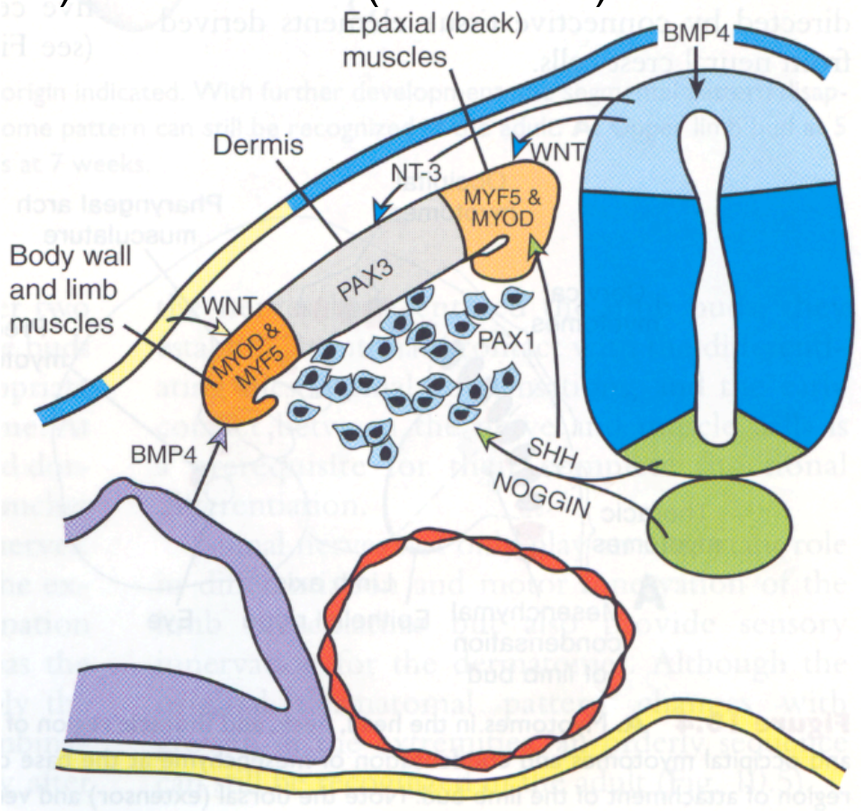

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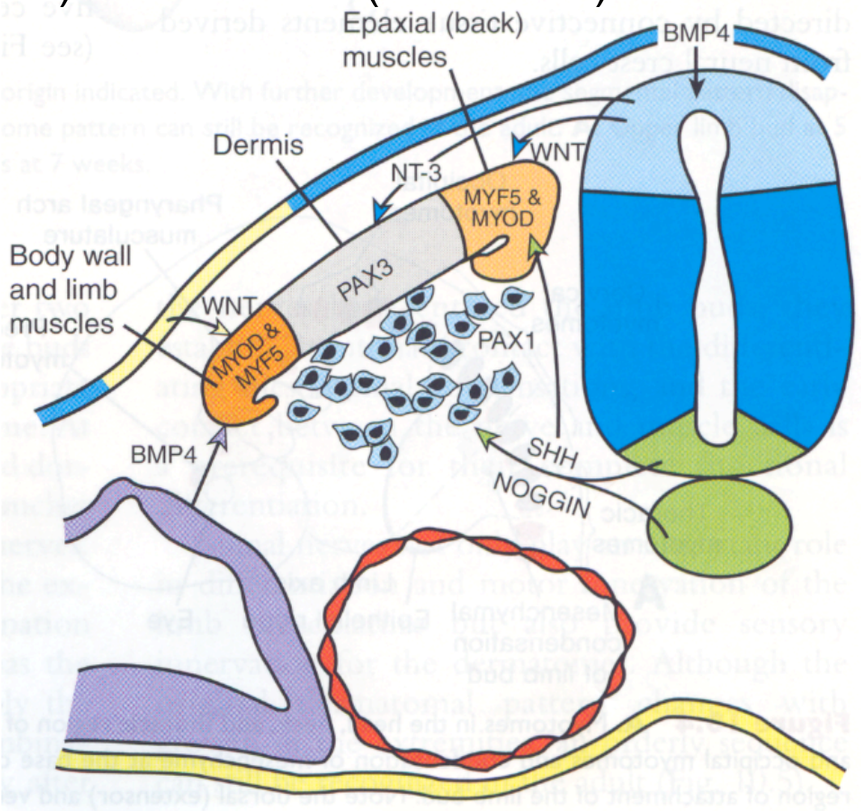

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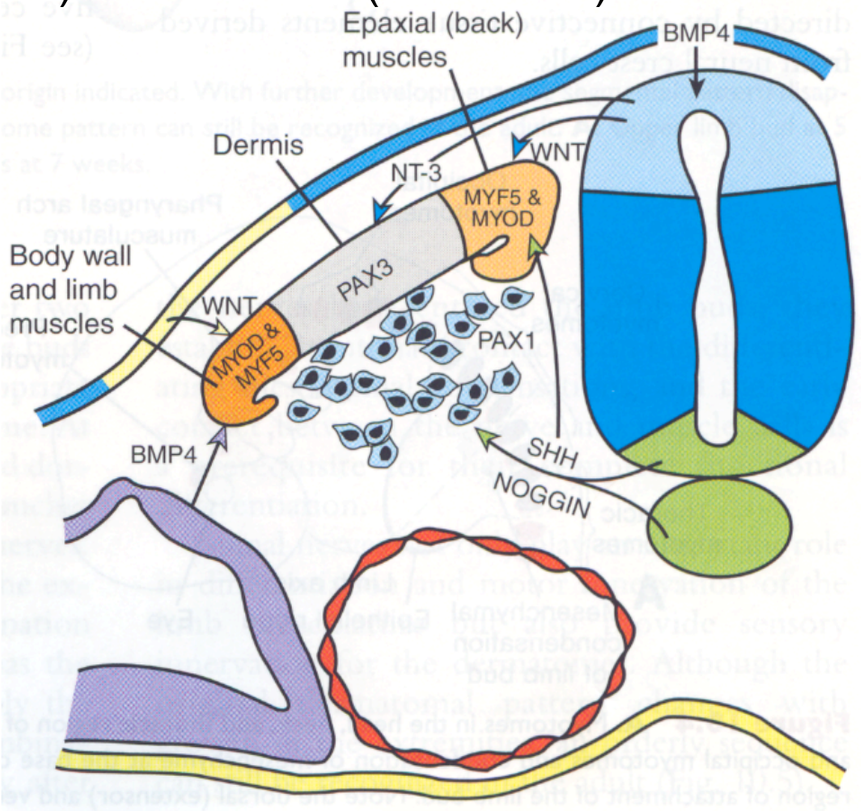

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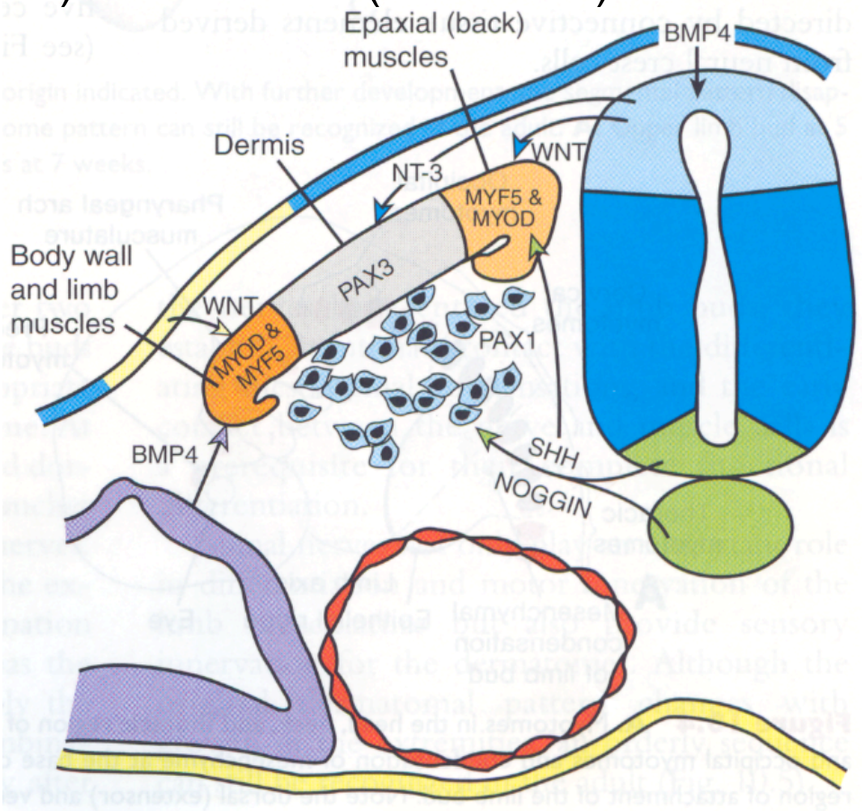
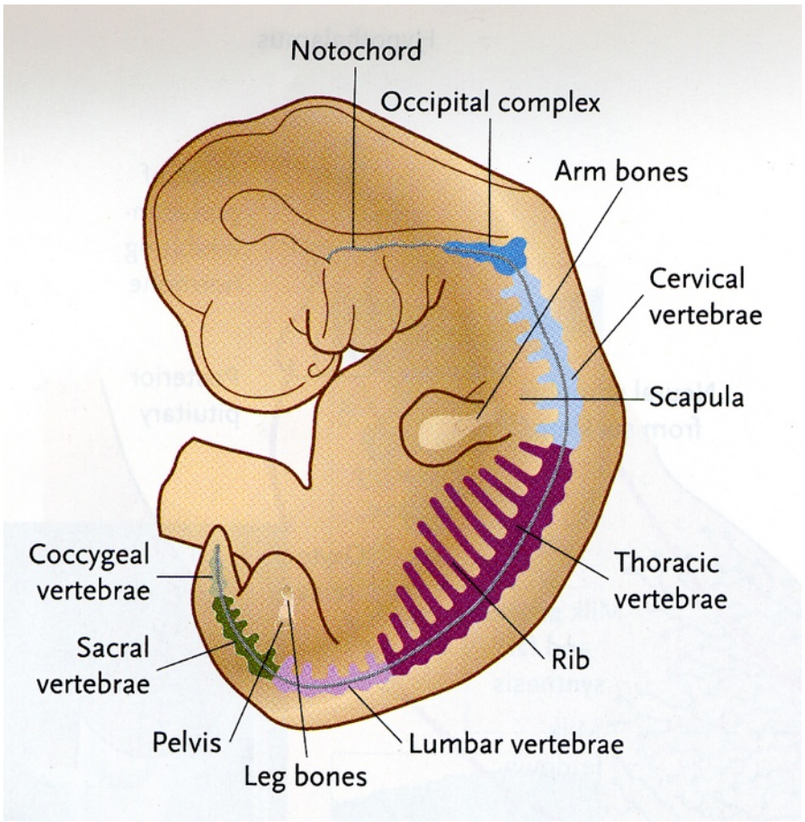
- NT-3 directs dermatome differentiation.

- Hypaxial (limb and body wall) muscles are formed from dorsolateral portion of the somite in response to Wnt and BMP signaling.



Patterning the axial skeleton - specification of the somite along the anteroposterior (AP) axis

Although the basic cellular differentiation pattern of somites at different axial positions is very similar, unique vertebral structures form along the craniocaudal axis, indicating that somites acquire specific identities according to their axial position.



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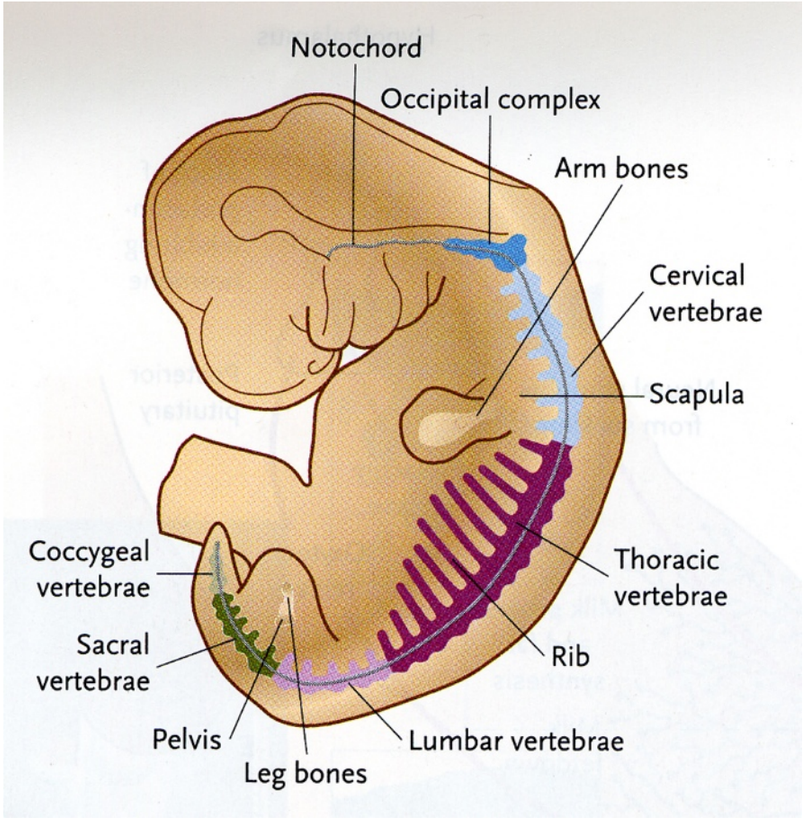


Axial identity is regulated by Hox gene expression

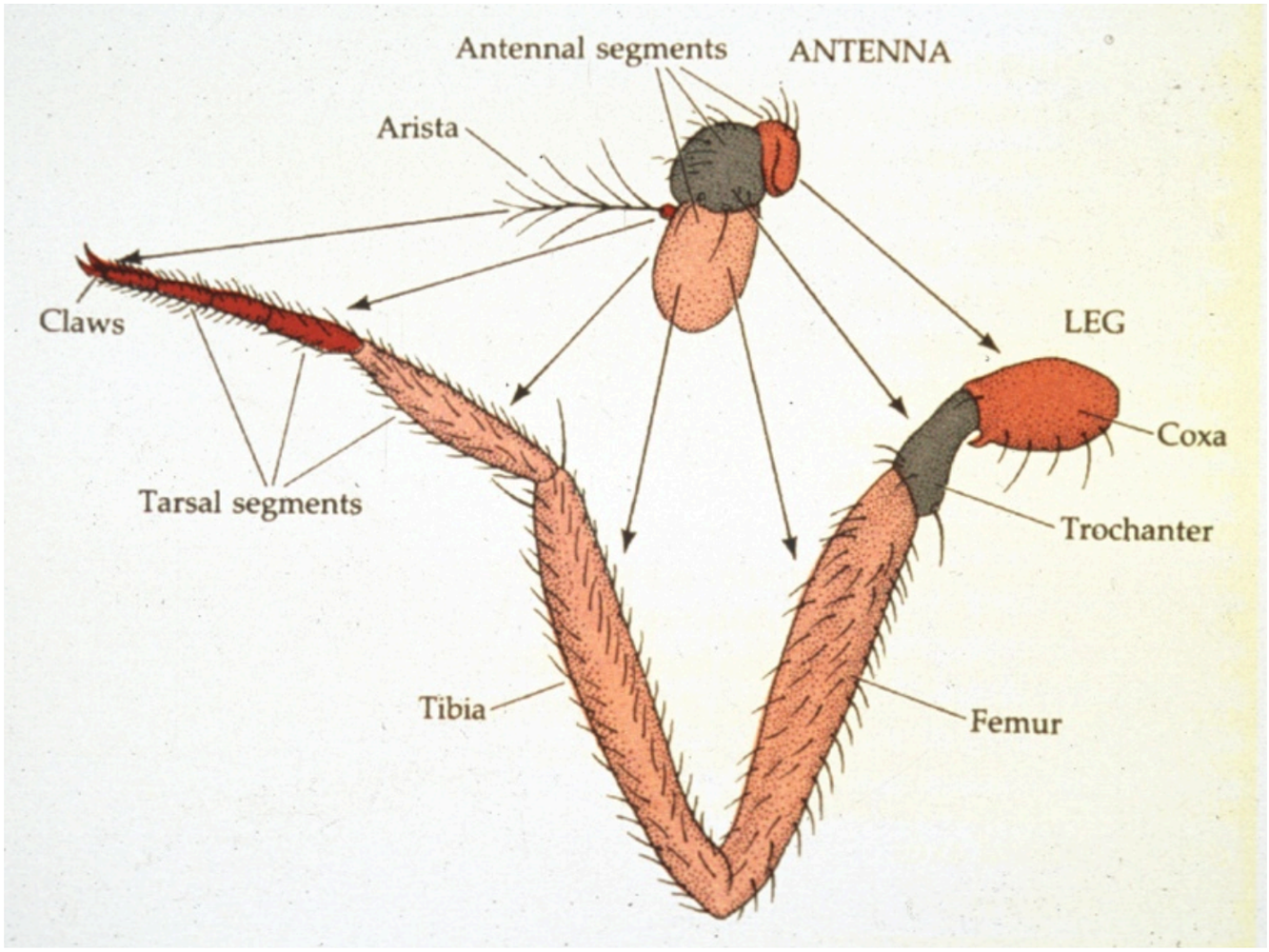
What are Hox genes?

A little bit of background...

Wild type fruit fly
T2 has a wing
T3 has a haltere (rudimentary wing)

(1) A fruit fly carrying a “homeotic mutation”:
the third thoracic segment develops like the second thoracic segment

(2) The fly leg and antenna are homologous structures

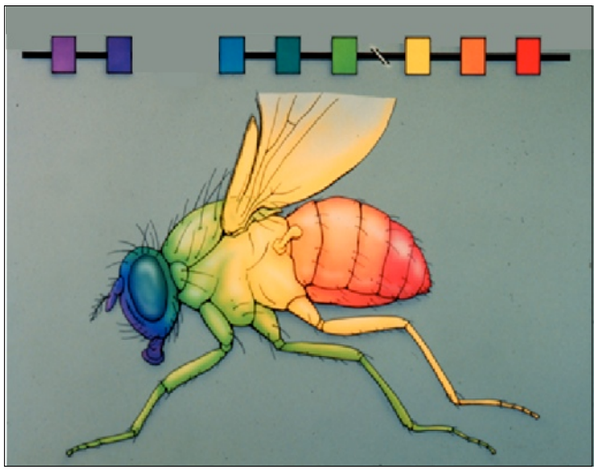


Homeotic mutant with legs in place of antennae

“Antennapedia”

The homeotic genes are arranged in a cluster that reflects the order of the body parts they regulate

Drosophila

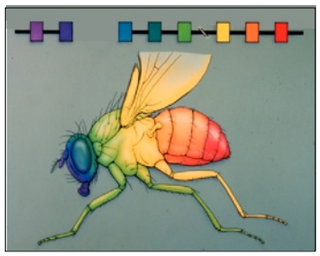


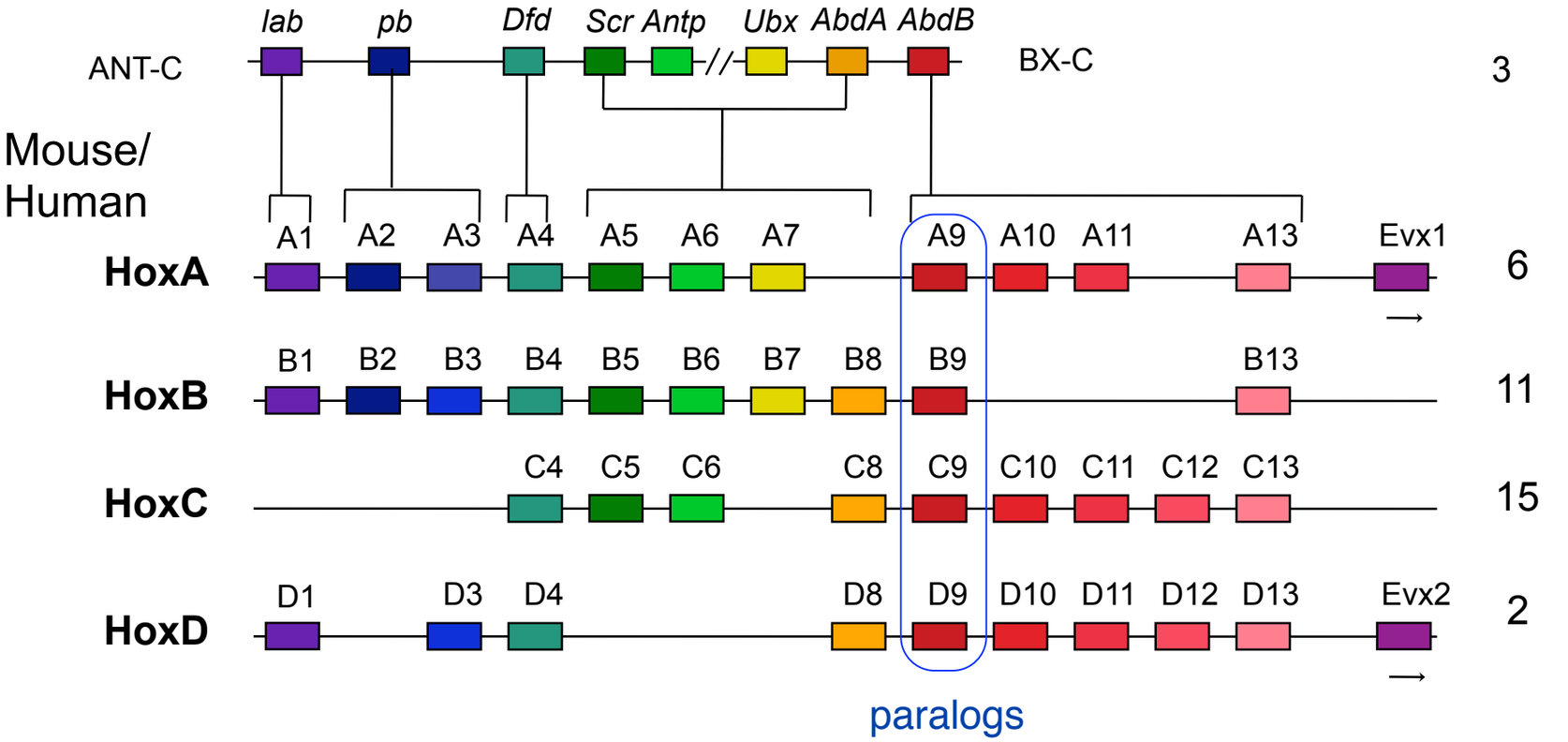
Chromosome

They encode transcription factors that contain a conserved DNA binding motif, the homeobox.

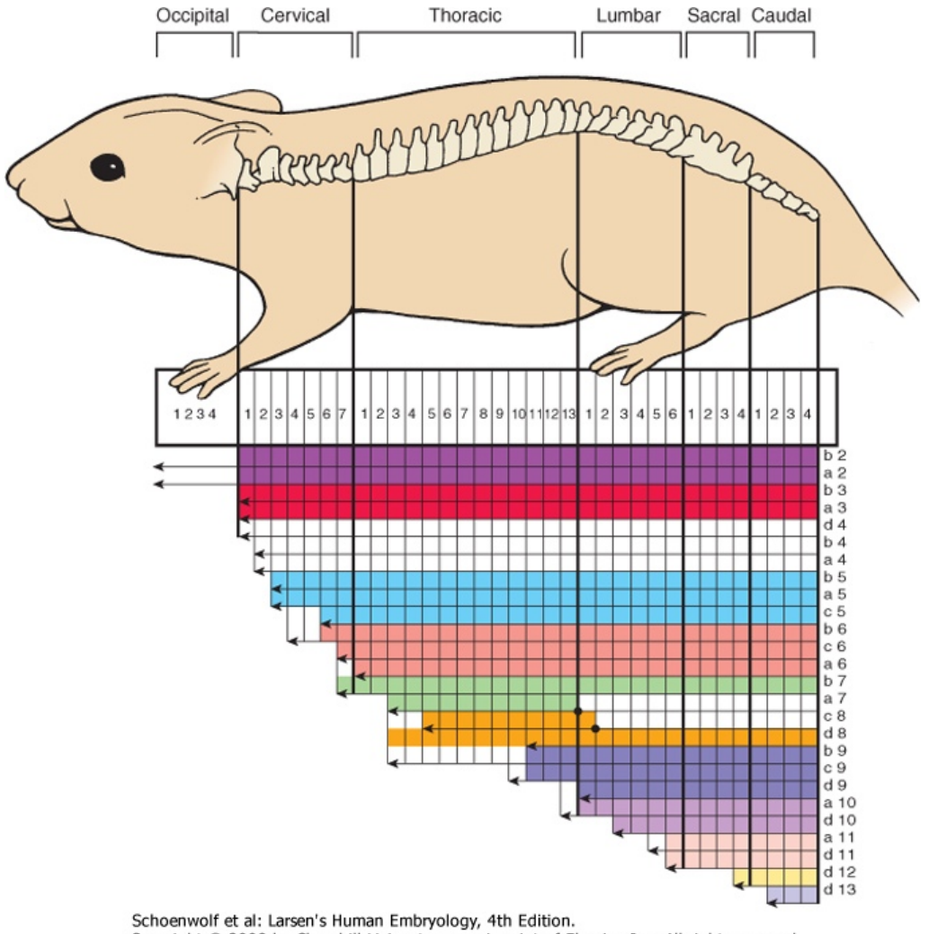
Vertebrates have homologous genes, arrayed along four chromosomes: The paralogous Hox gene clusters

Drosophila





Vertebrate Hox axial expression patterns also reflect their chromosomal order



Schoenwolf et al: Larsen's Human Embryology, 4th Edition.

Expression is progressively induced during gastrulation, and the expression patterns are nested in a chromosomal and morphological progression. This is called "colinearity".

The combination of Hox genes expressed in a tissue forms a Hox code.

Note that Hox10 paralogs have an anterior (rostral) expression boundary at the thoracic/lumbar border, and Hox11 at lumbar/sacral

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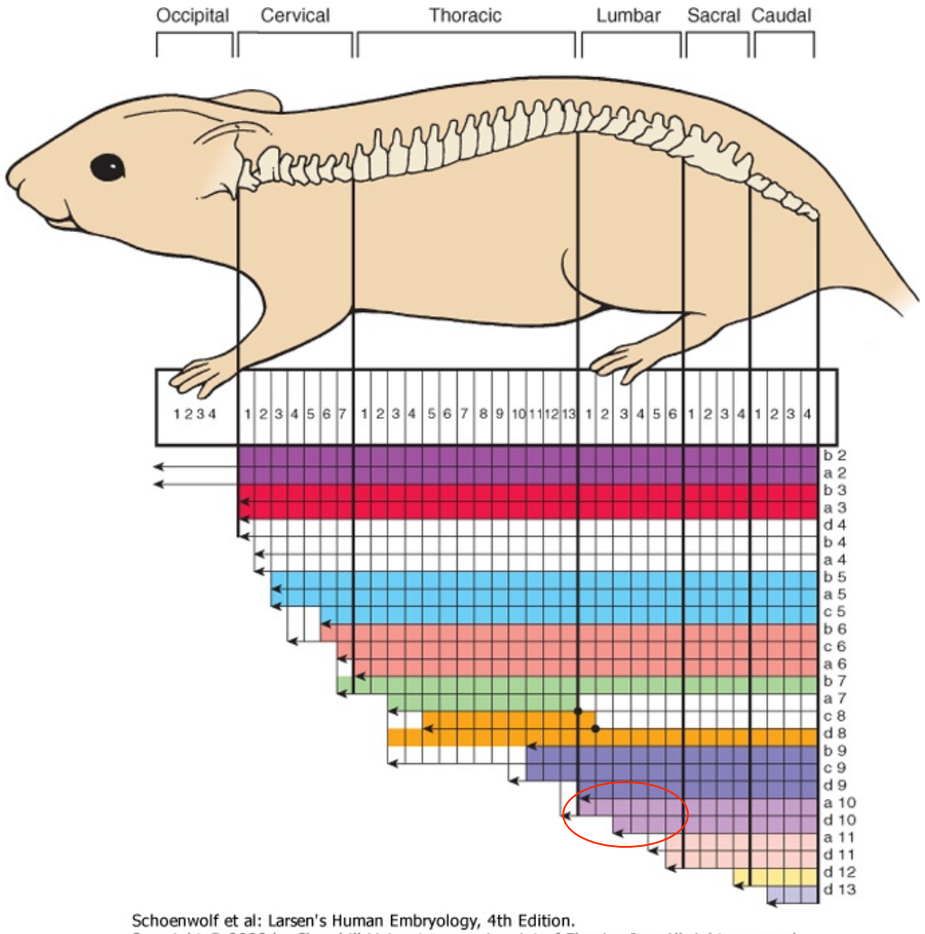

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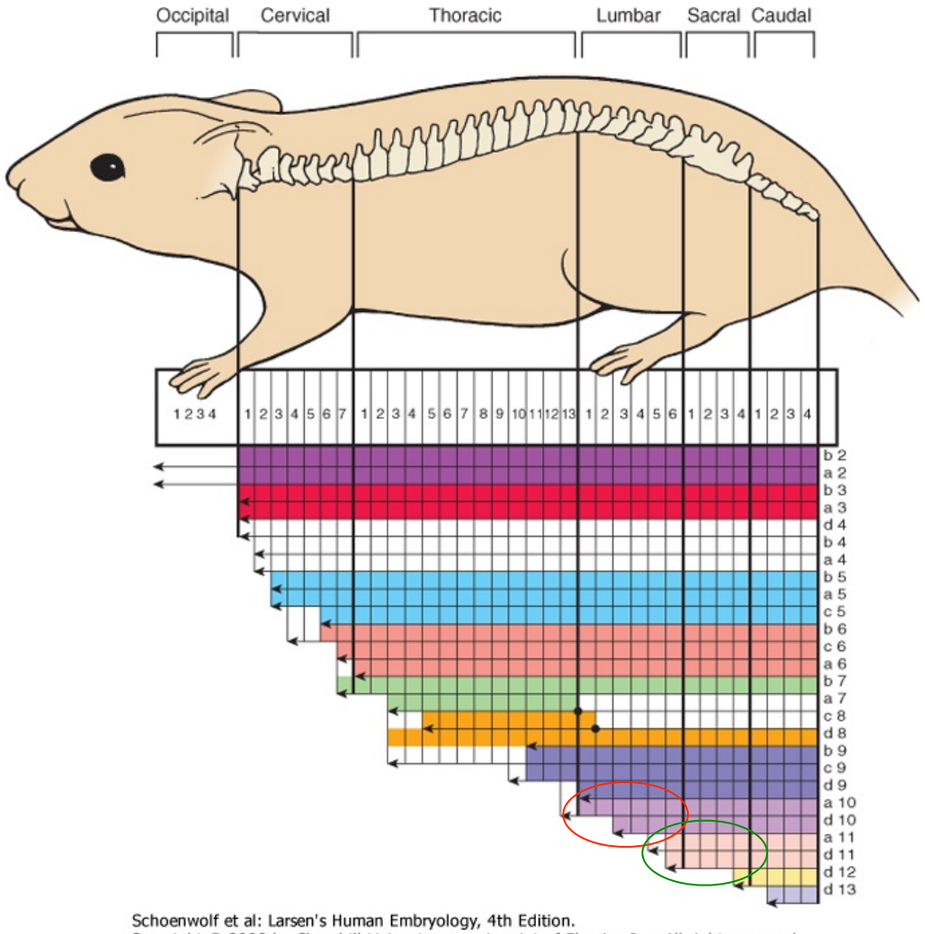

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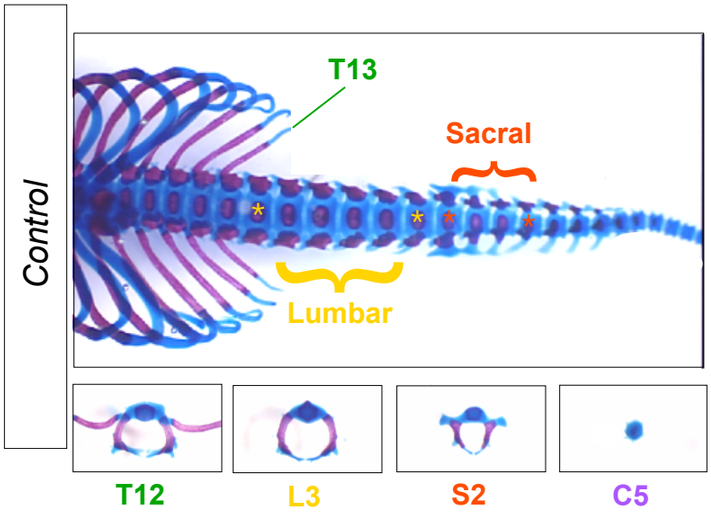
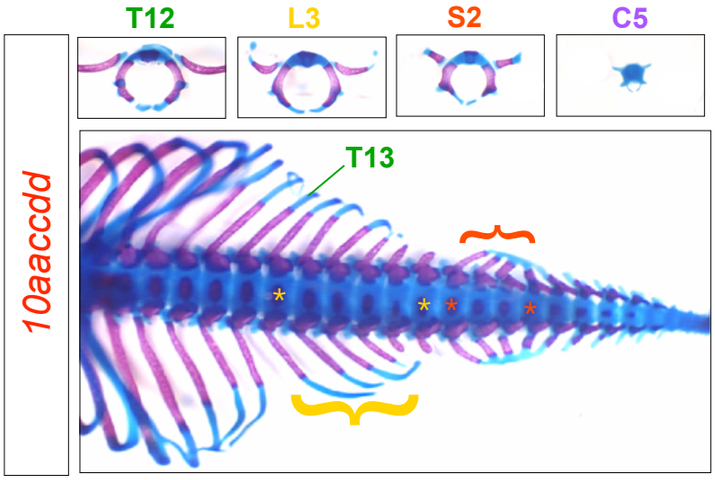
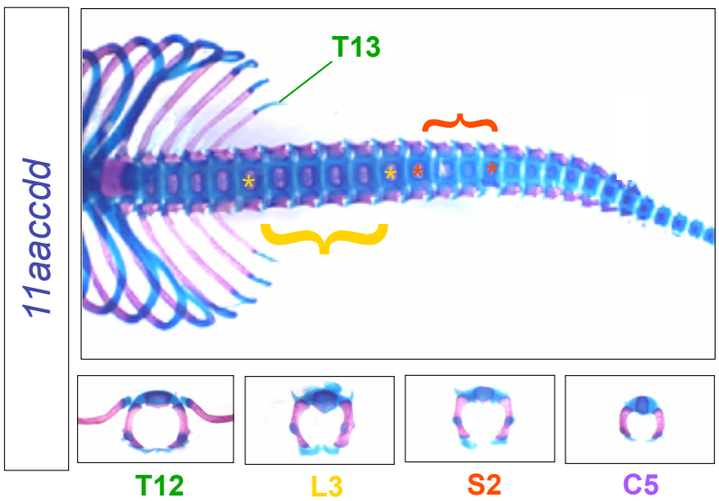
Loss of paralogous function results in complete loss of regional identity in axial column



‘Segment transformation’
Lumbar --> Thoracic

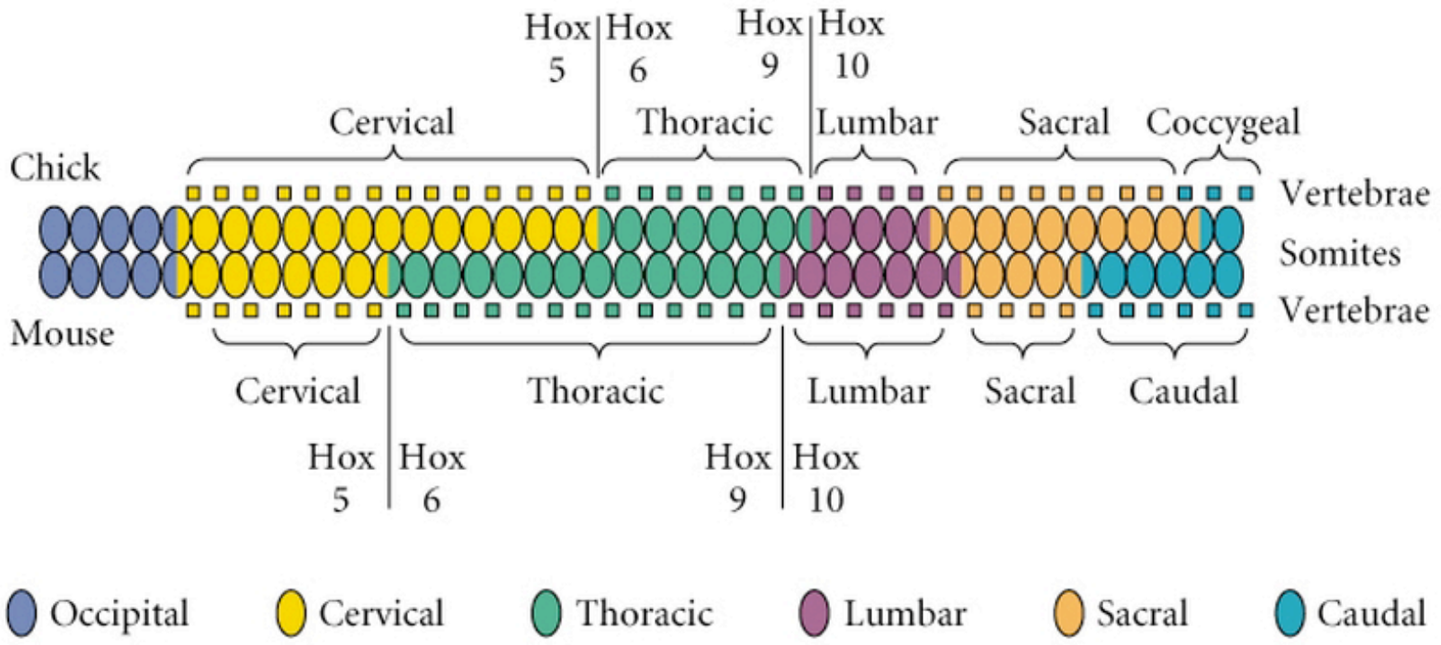




‘Segment transformation’
Sacral --> Lumbar

Wellik and Capecchi, *Science,* 2003

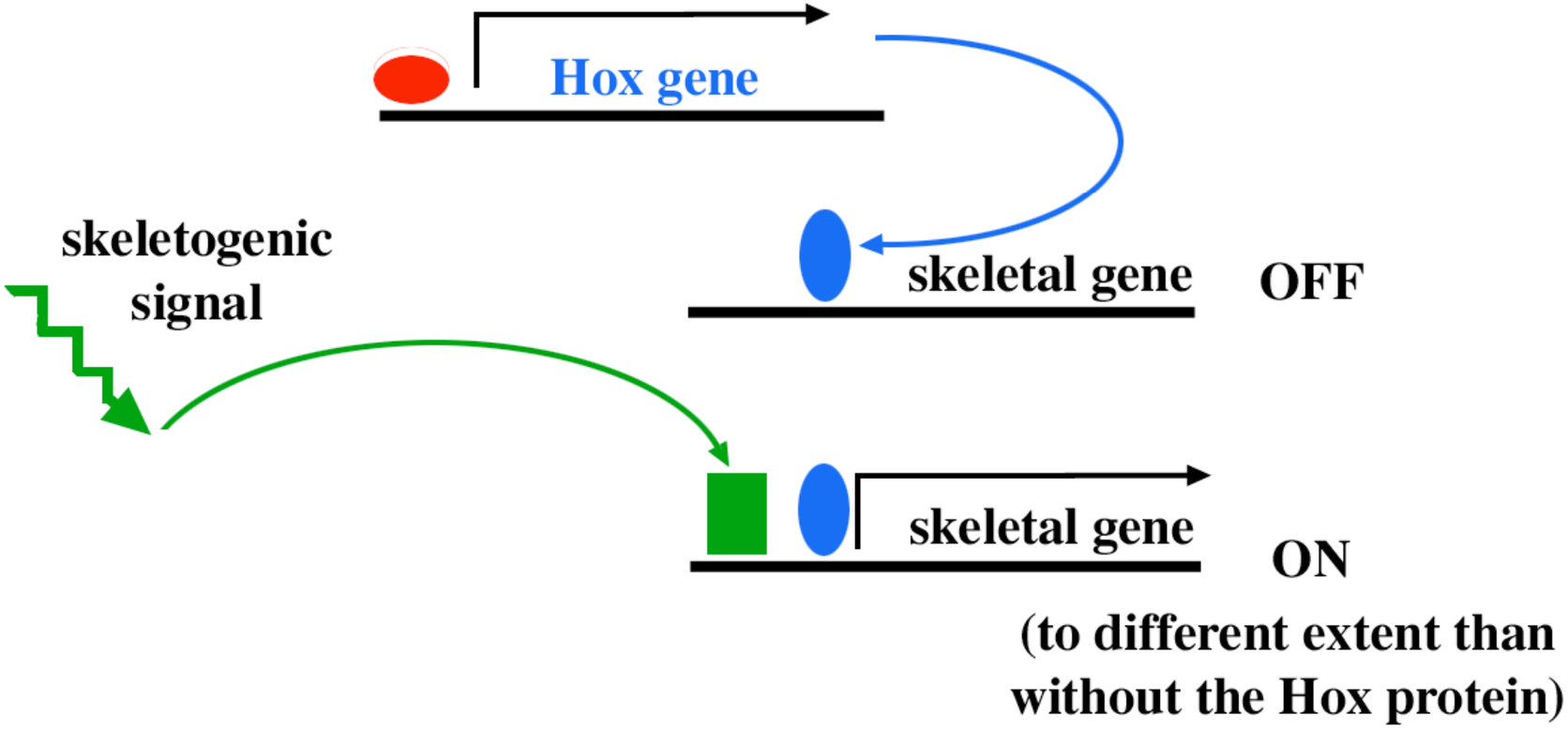
Hox expression is conserved and boundaries correlate with changes in vertebral morphology



Hox genes do not specify cartilage/bone directly

Rather they modulate the way skeletal progenitors respond to other signals.

Similar to inductive signals, they are used repeatedly to modulate pattern in many developing tissues.



Key Ideas (I):

The axial body plan is organized into repeating mesodermal structures called somites

Somites form progressively from presegmental mesoderm at the caudal end of the growing embryo, through a “clock and wavefront” mechanism that involves Notch, FGF and Wnt signaling

Malfunctioning of this signaling system cause vertebral segmentation defects

Somites are subdivided by inductive signals emanating from the surrounding embryonic structures

Somites differentiate into axial skeleton, muscles, dermis and tendons

Key Ideas (II):

Cells at different cranial-caudal and different dorsal-ventral locations in the embryo express different combinations of transcription factors "telling" cells where they are and hence modulating the structures they produce

Along the cranial-caudal ("anterior-posterior") axis, Hox genes provide a combinatorial code for cell fate

The homeobox is a highly conserved DNA-binding domain of the Hox proteins

There are 4 clusters of Hox genes (A->D) with a total of 13 gene families (low numbered Hox genes are expressed more cranially)

Hox genes are also used in adult cells as transcription factors that regulate growth and differentiation, but not pattern