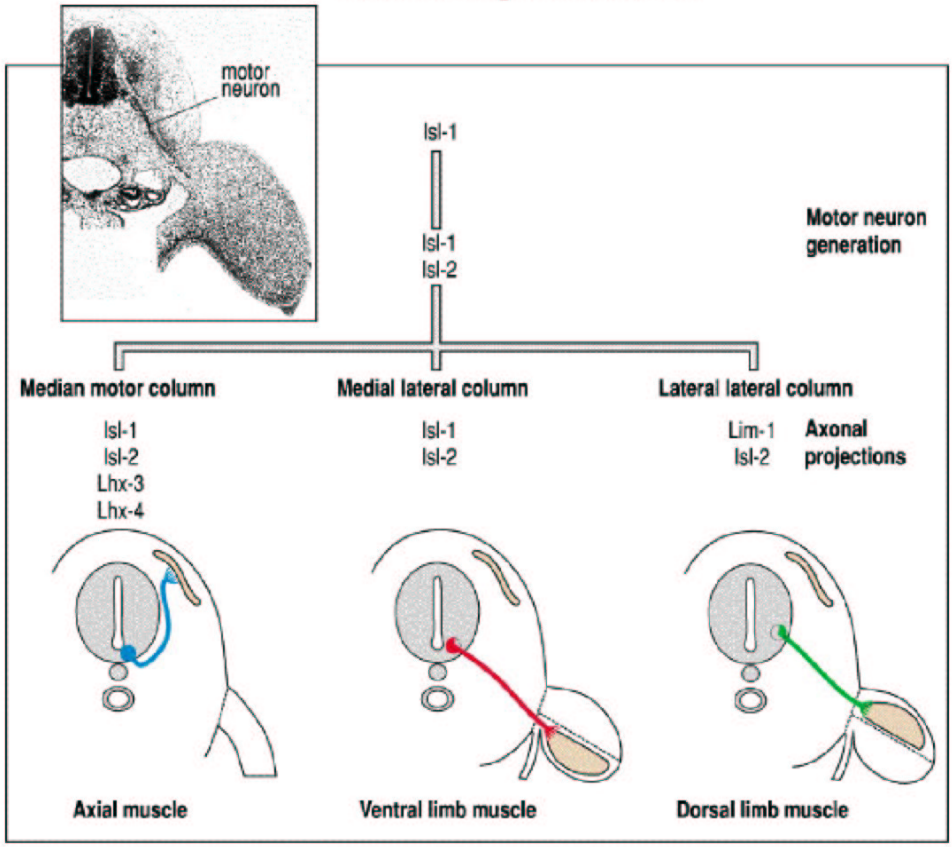
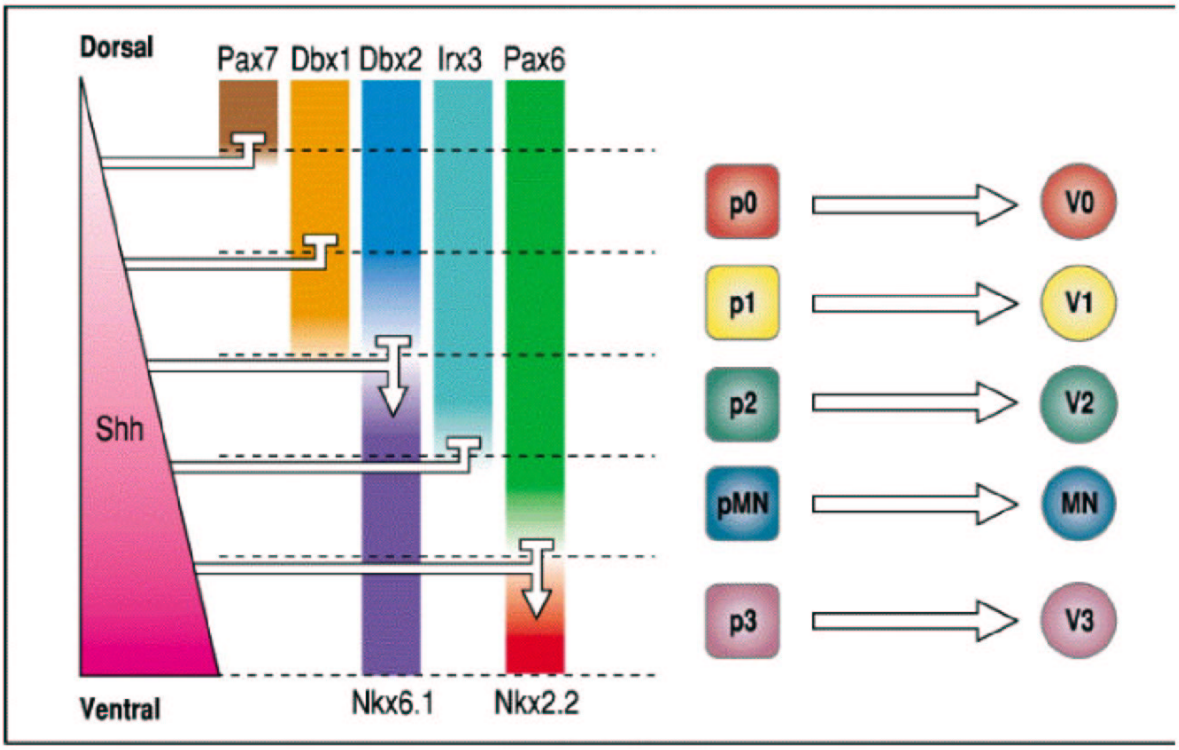
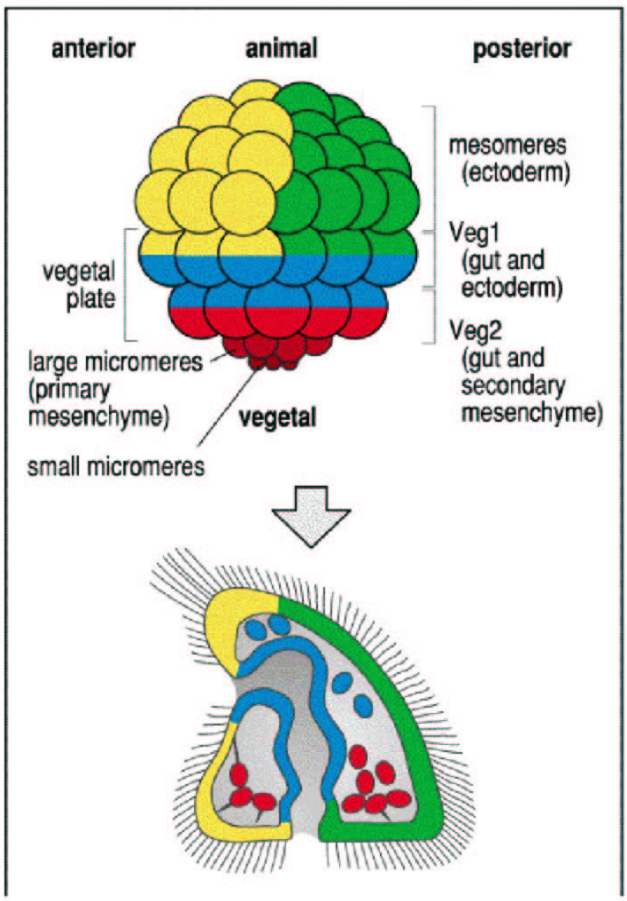
Motor neurone specification

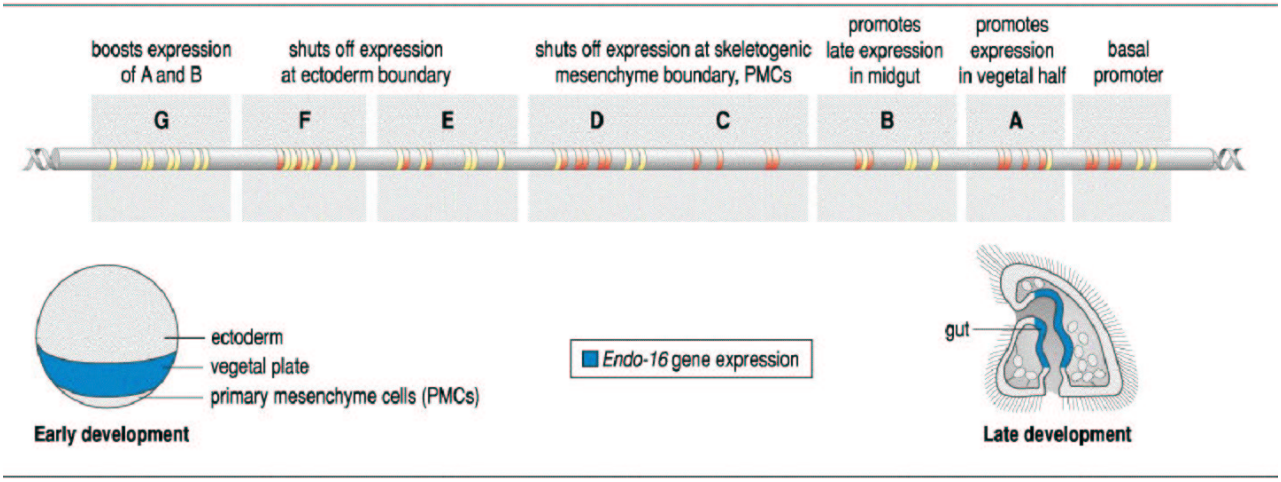


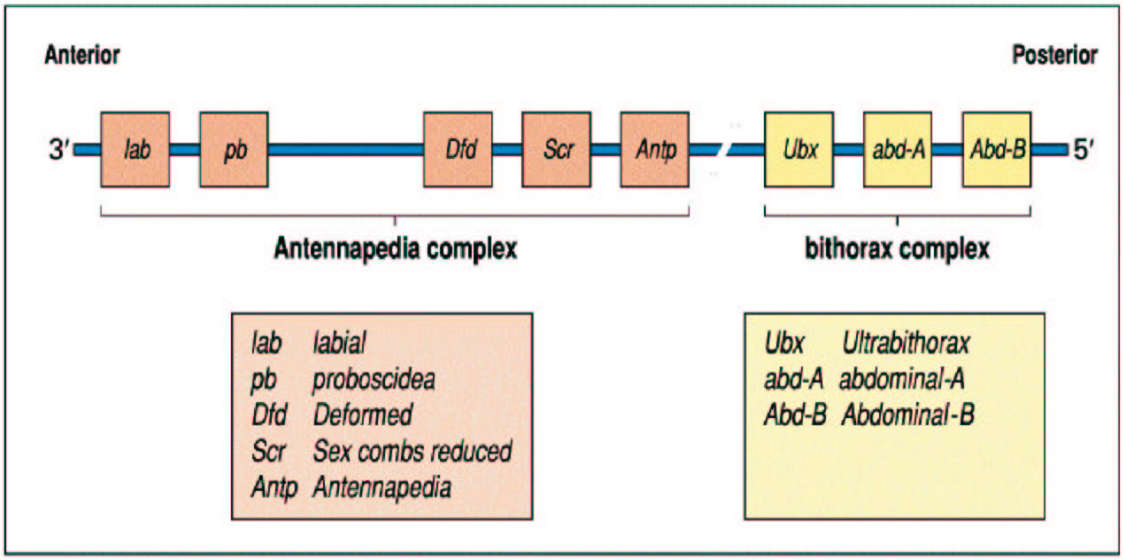
Specification of motor neurones and other neurones spinal cord

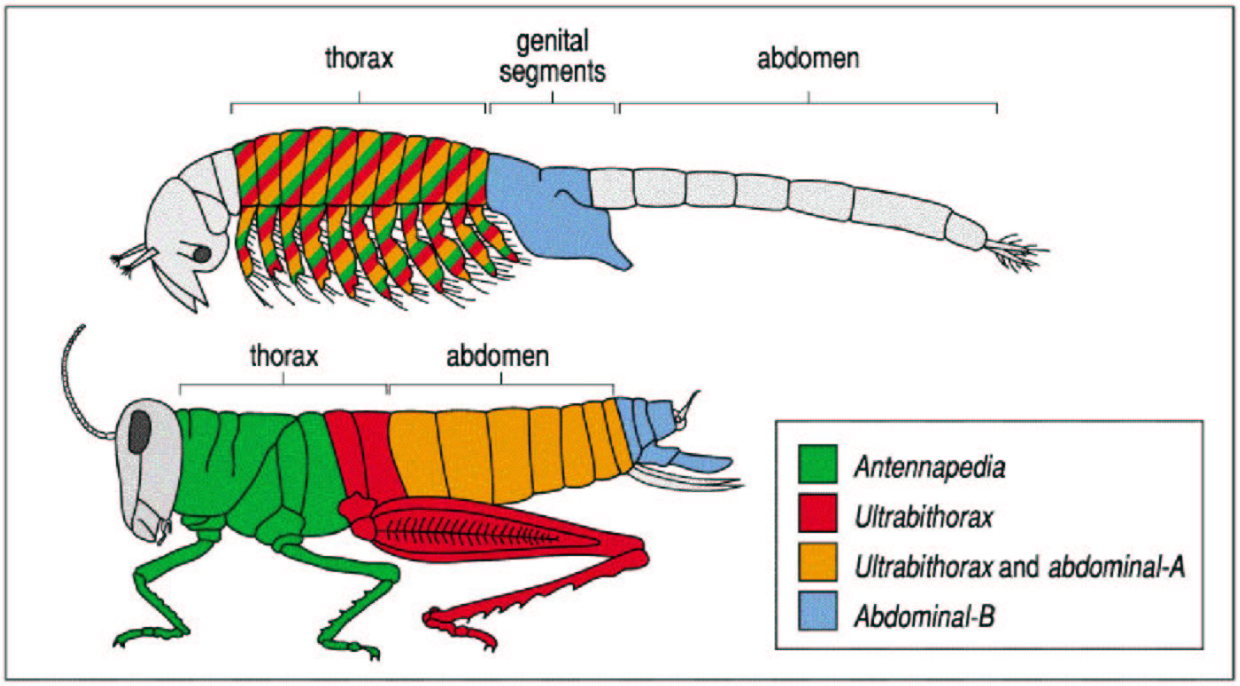


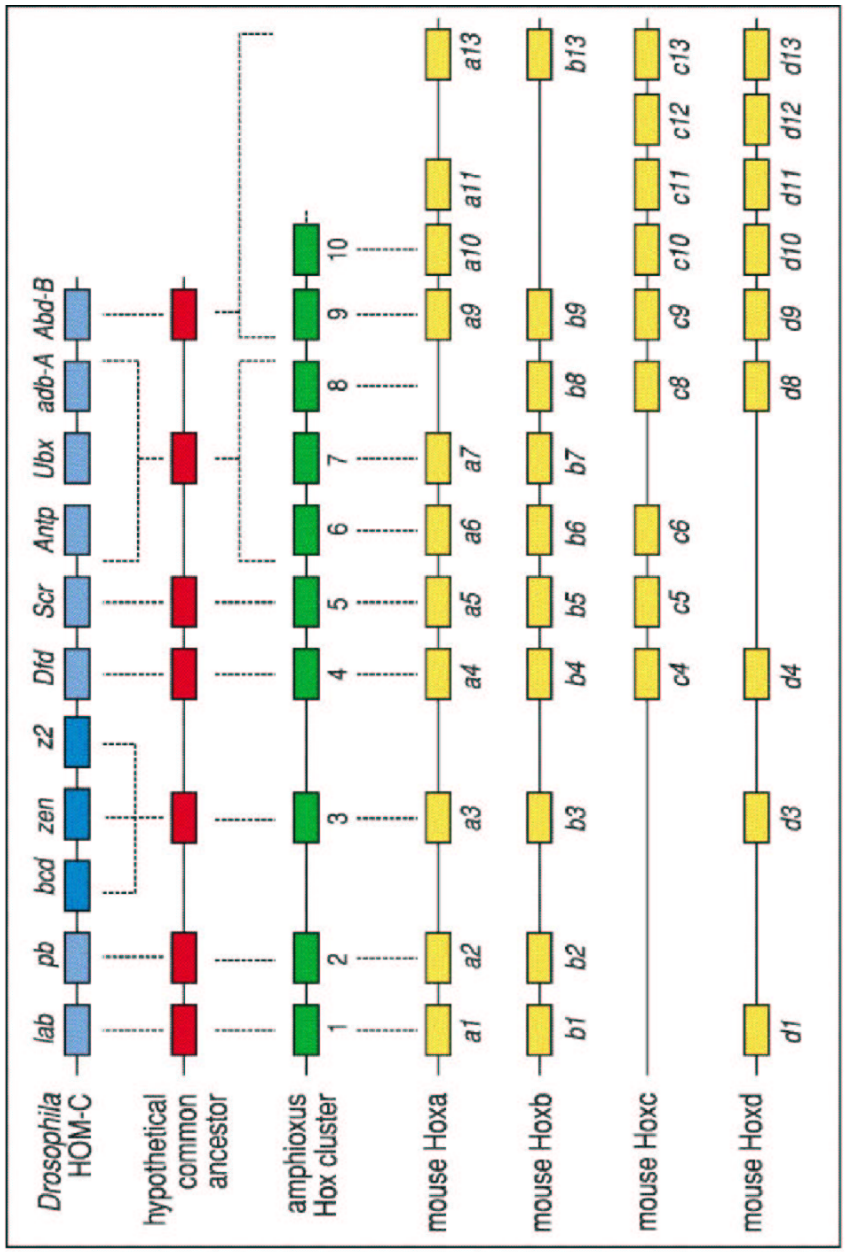


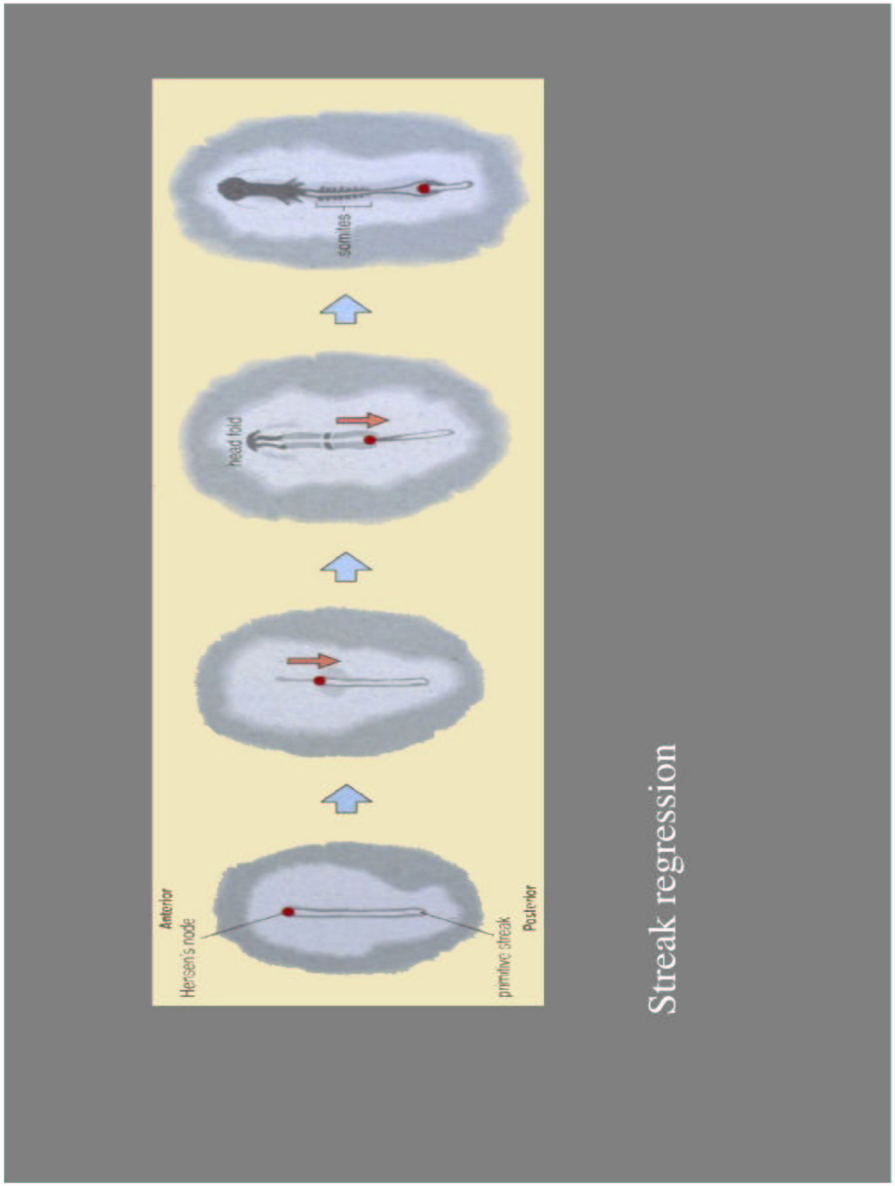
Cis-regulatory region of endo-16 sea-urchin

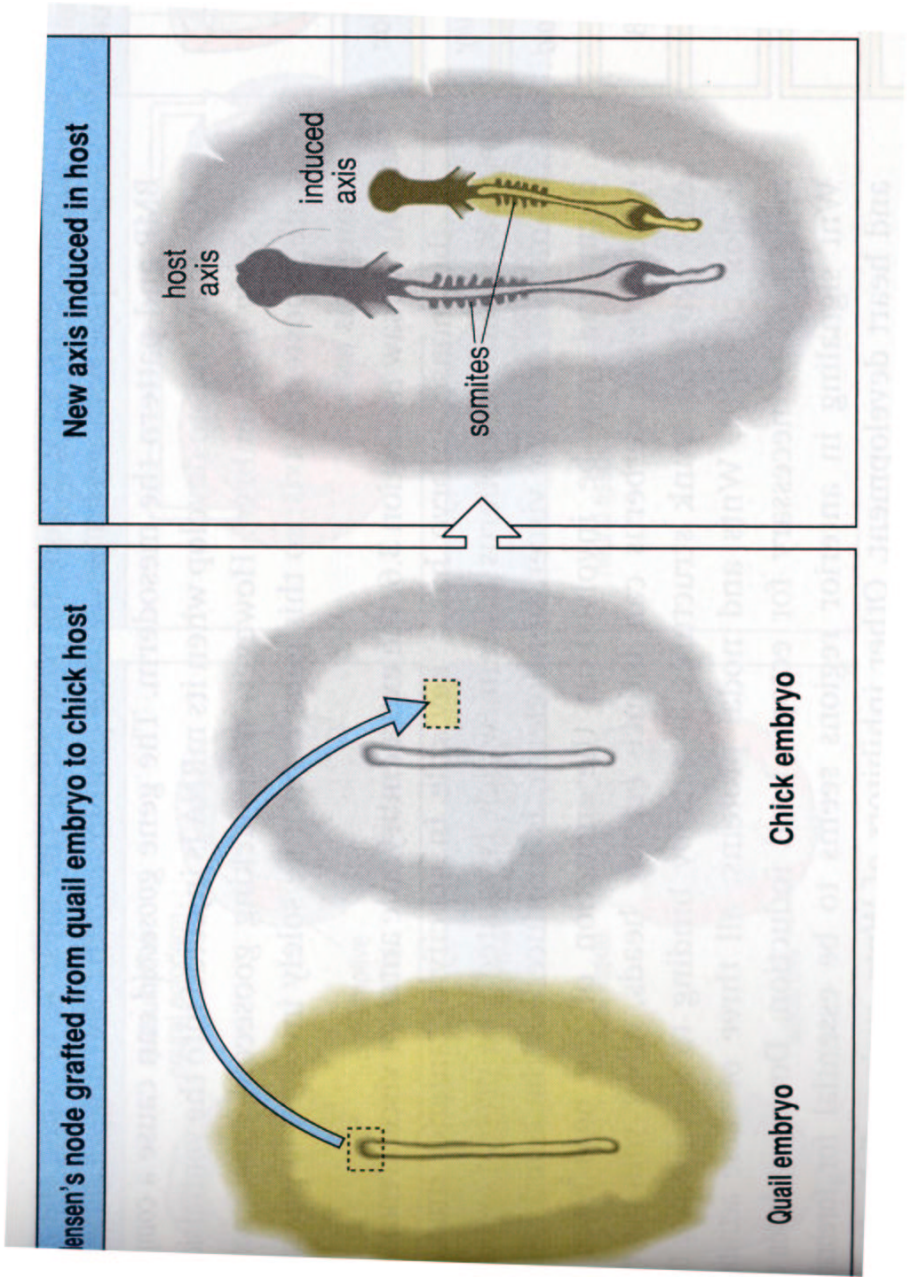


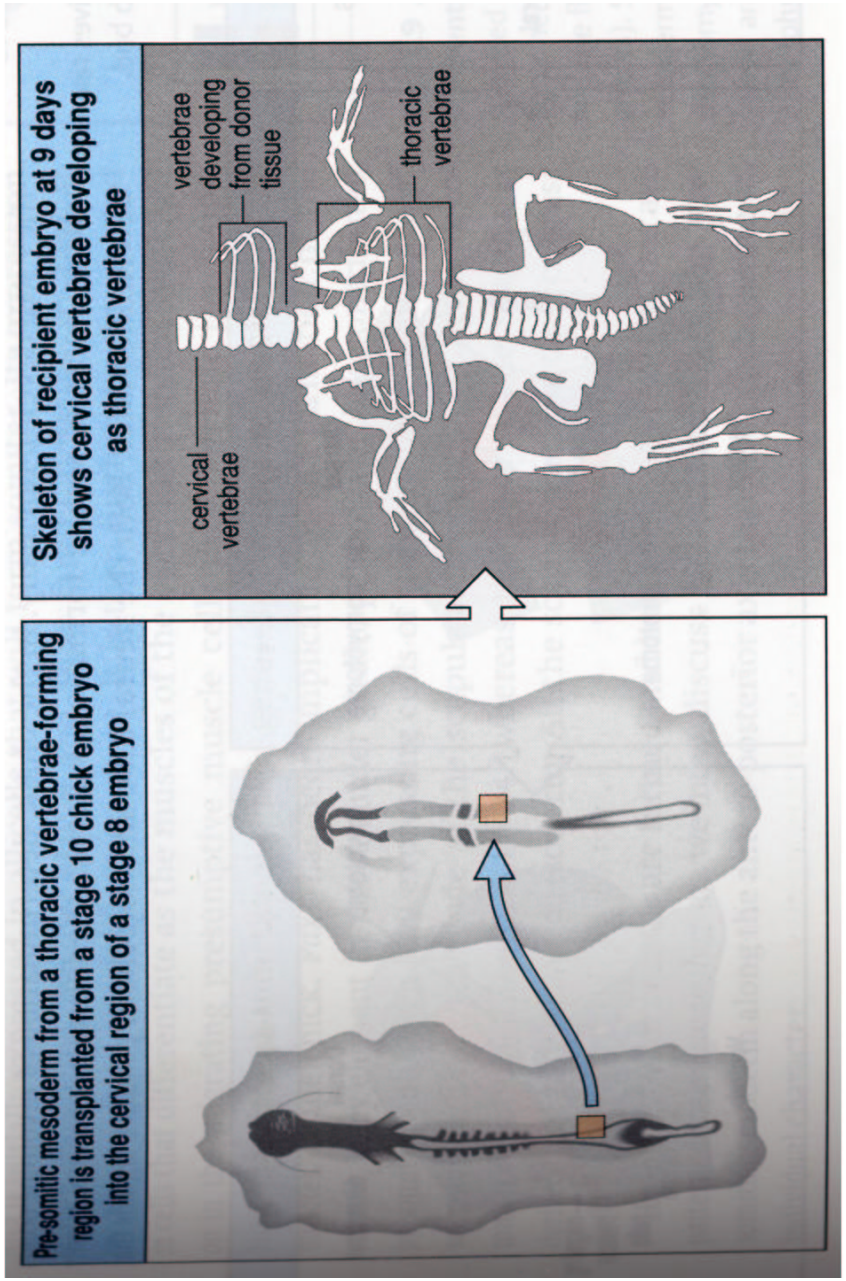

Anterior
Posterior
3′
lab
pb
Dfd
Scr
Antp
Ubx
abd-A
Abd-B
5′
Antennapedia complex
bithorax complex
lab labial
pb proboscidea
Dfd Deformed
Scr Sex combs reduced
Antp Antennapedia
Ubx Ultrabithorax
abd-A abdominal-A
Abd-B Abdominal-B

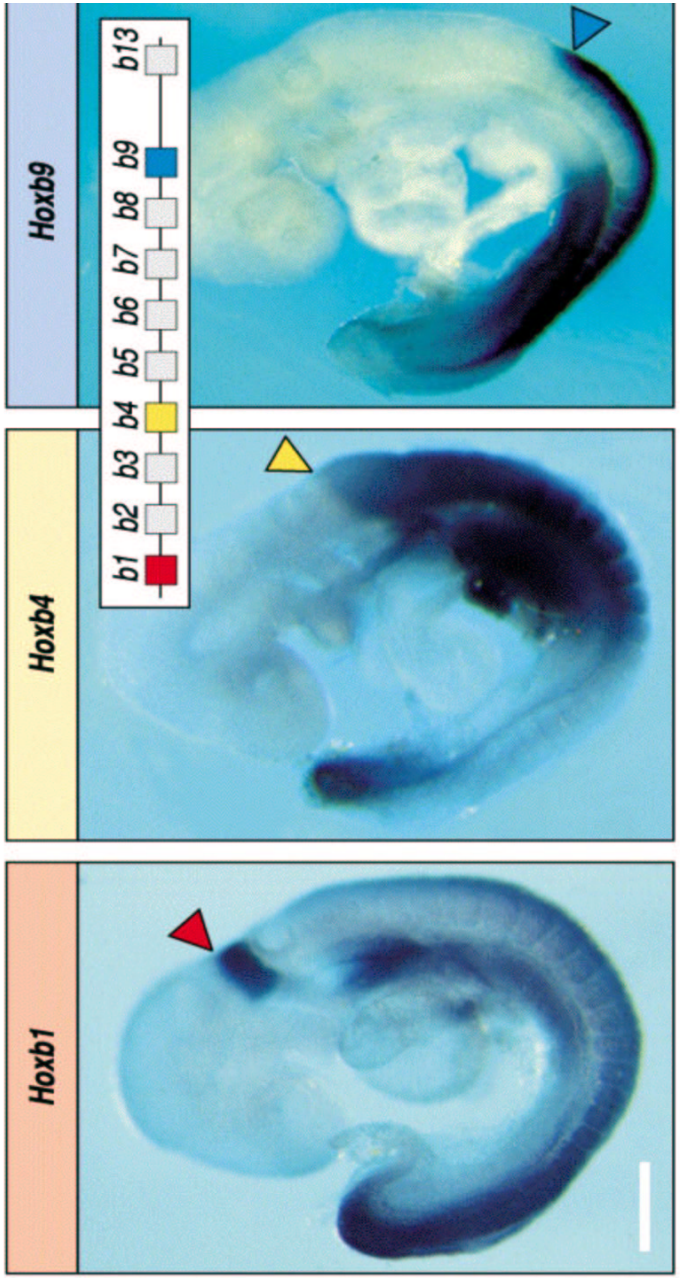

thorax
genital segments
abdomen
thorax
abdomen
Antennapedia
Ultrabithorax
Ultrabithorax and abdominal-A
Abdominal-B

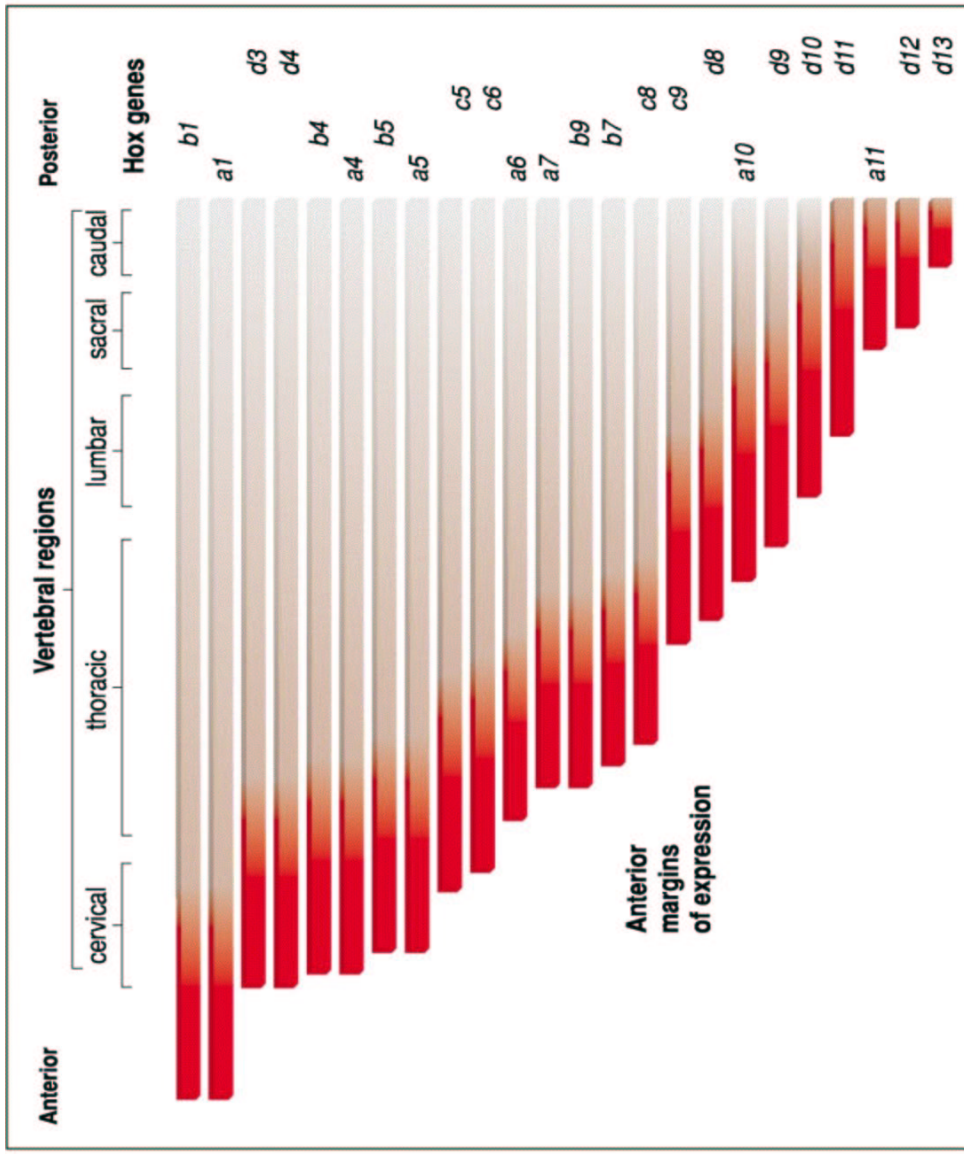

Drosophila HOM-C
lab pb bcd zen z2 Dfd Scr Antp Ubx adb-A Abd-B
hypothetical common ancestor
amphioxus Hox cluster
1 2 3 4 5 6 7 8 9 10
mouse Hoxa
a1 a2 a3 a4 a5 a6 a7 a9 a10 a11 a13
mouse Hoxb
b1 b2 b3 b4 b5 b6 b7 b8 b9 b13
mouse Hoxc
c4 c5 c6 c8 c9 c10 c11 c12 c13
mouse Hoxd
d1 d3 d4 d8 d9 d10 d11 d12 d13

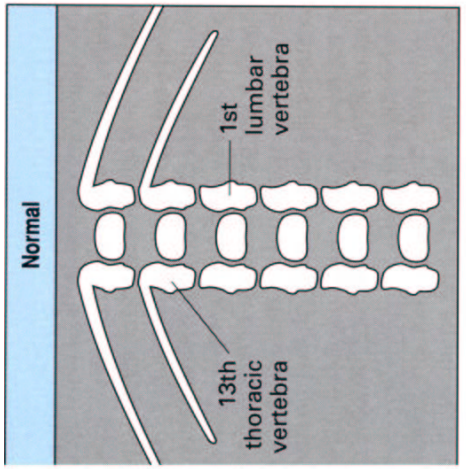

Anterior
Hensen's node
primitive streak
Posterior
head fold
somites
Streak regression

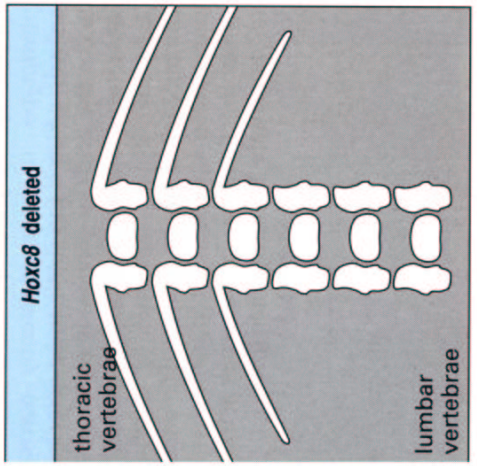

ensen's node grafted from quail embryo to chick host
Quail embryo
Chick embryo
New axis induced in host
host axis
induced axis
somites

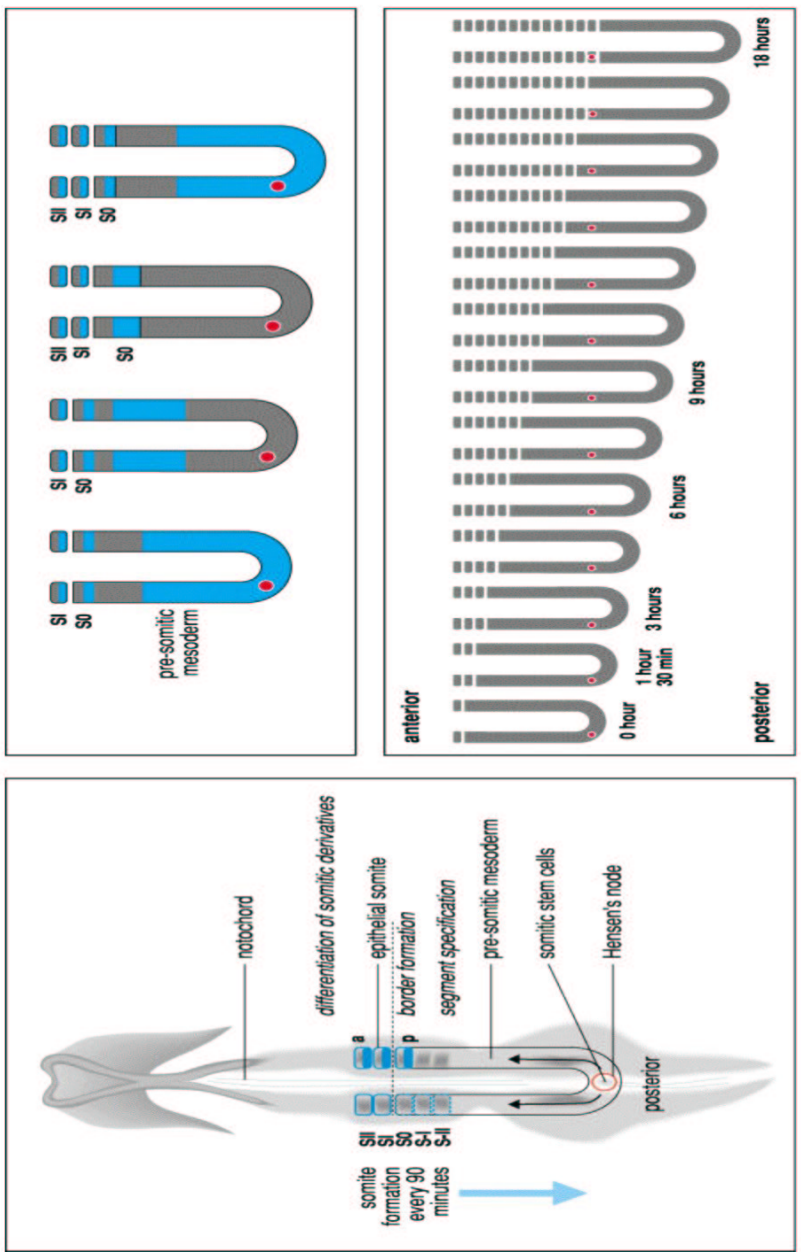

Pre-somitic mesoderm from a thoracic vertebrae-forming region is transplanted from a stage 10 chick embryo into the cervical region of a stage 8 embryo
Skeleton of recipient embryo at 9 days shows cervical vertebrae developing as thoracic vertebrae
vertebrae developing from donor tissue
thoracic vertebrae
cervical vertebrae

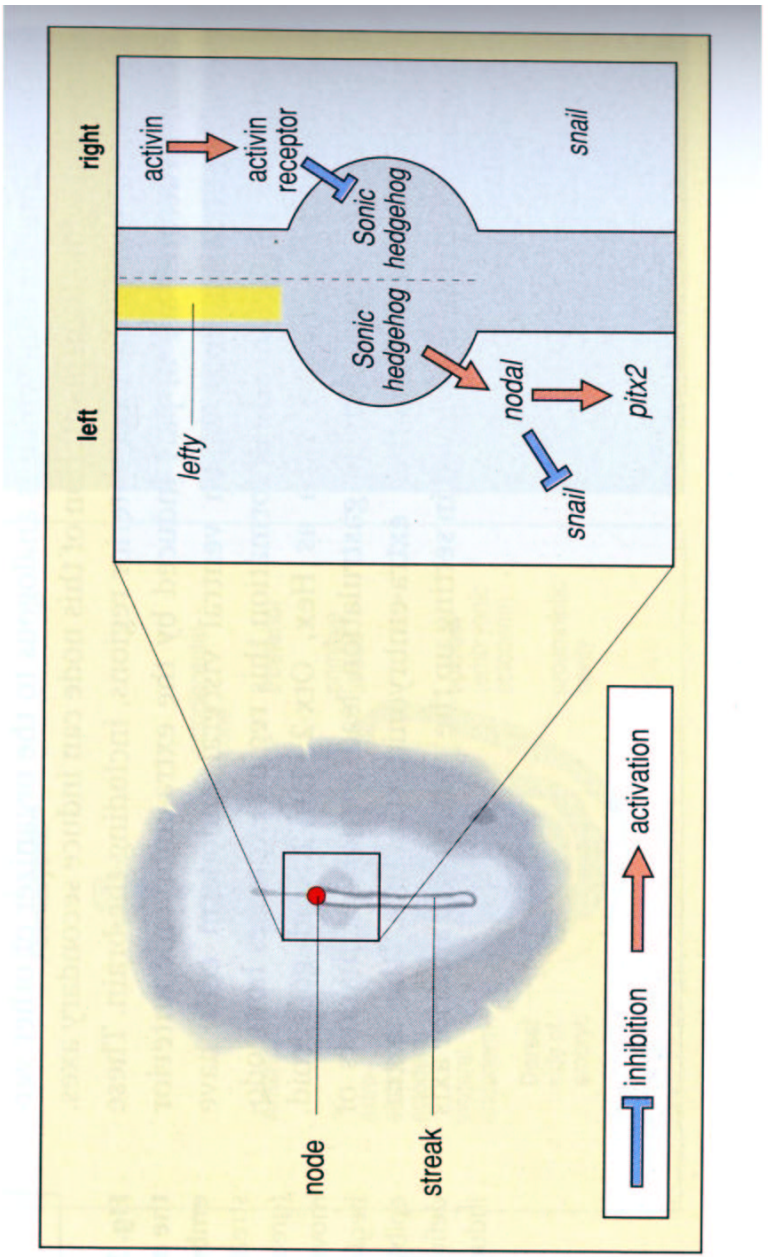

Hoxb9
Hoxb4
Hoxb1
b1 b2 b3 b4 b5 b6 b7 b8 b9 b13

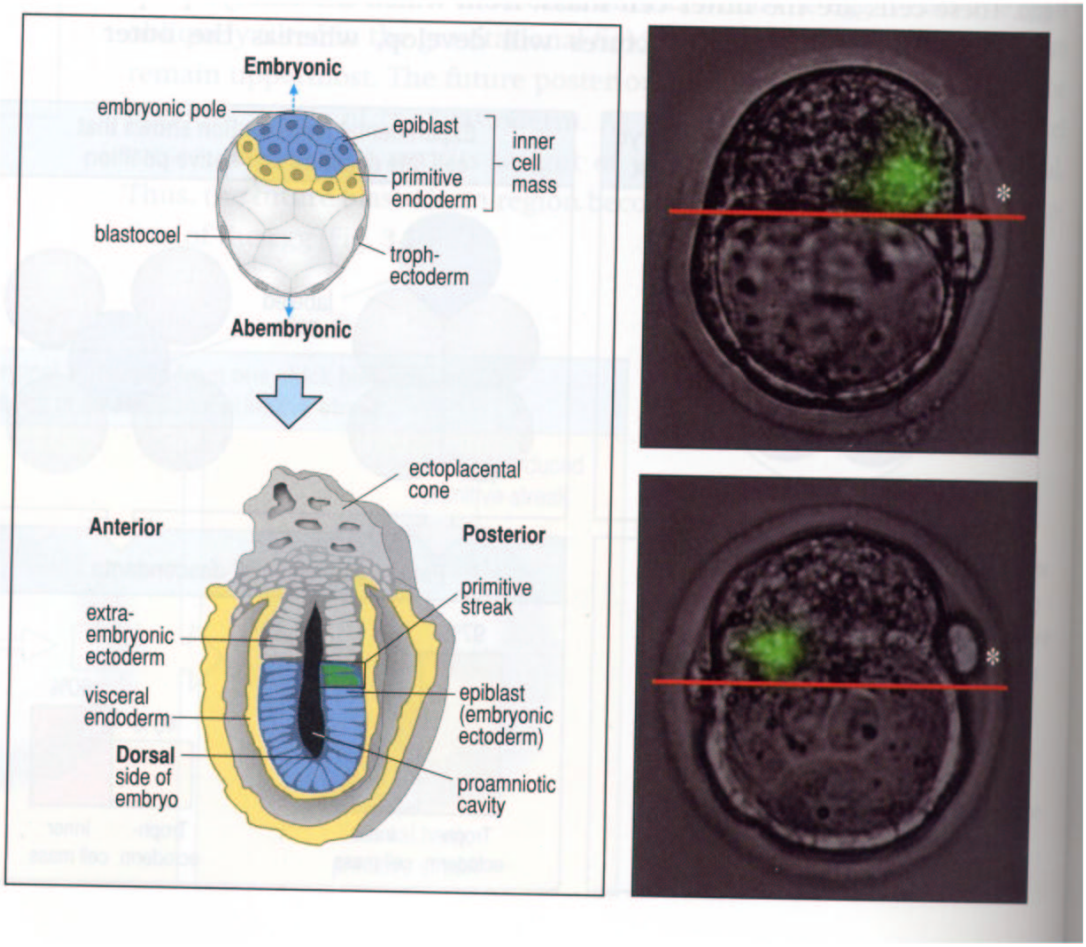

Anterior
Vertebral regions
cervical
thoracic
lumbar
sacral
caudal
Posterior
Hox genes
b1
a1
d3
d4
b4
a4
b5
a5
c5
c6
a6
a7
b9
b7
c8
c9
d8
a10
d9
d10
d11
a11
d12
d13
Anterior
margins
of expression

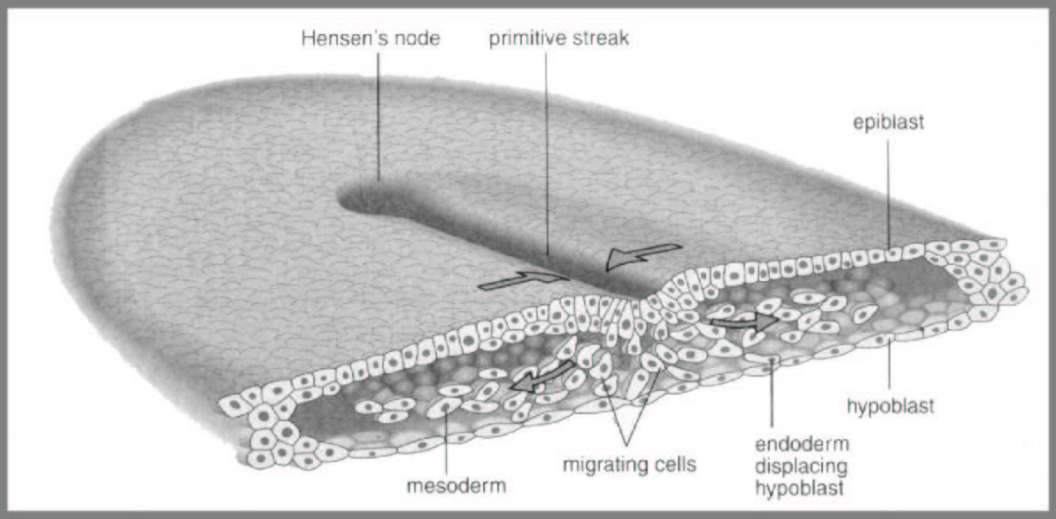

Normal
1st
lumbar
vertebra
13th
thoracic
vertebra


Hoxc8 deleted
thoracic
vertebrae
lumbar
vertebrae

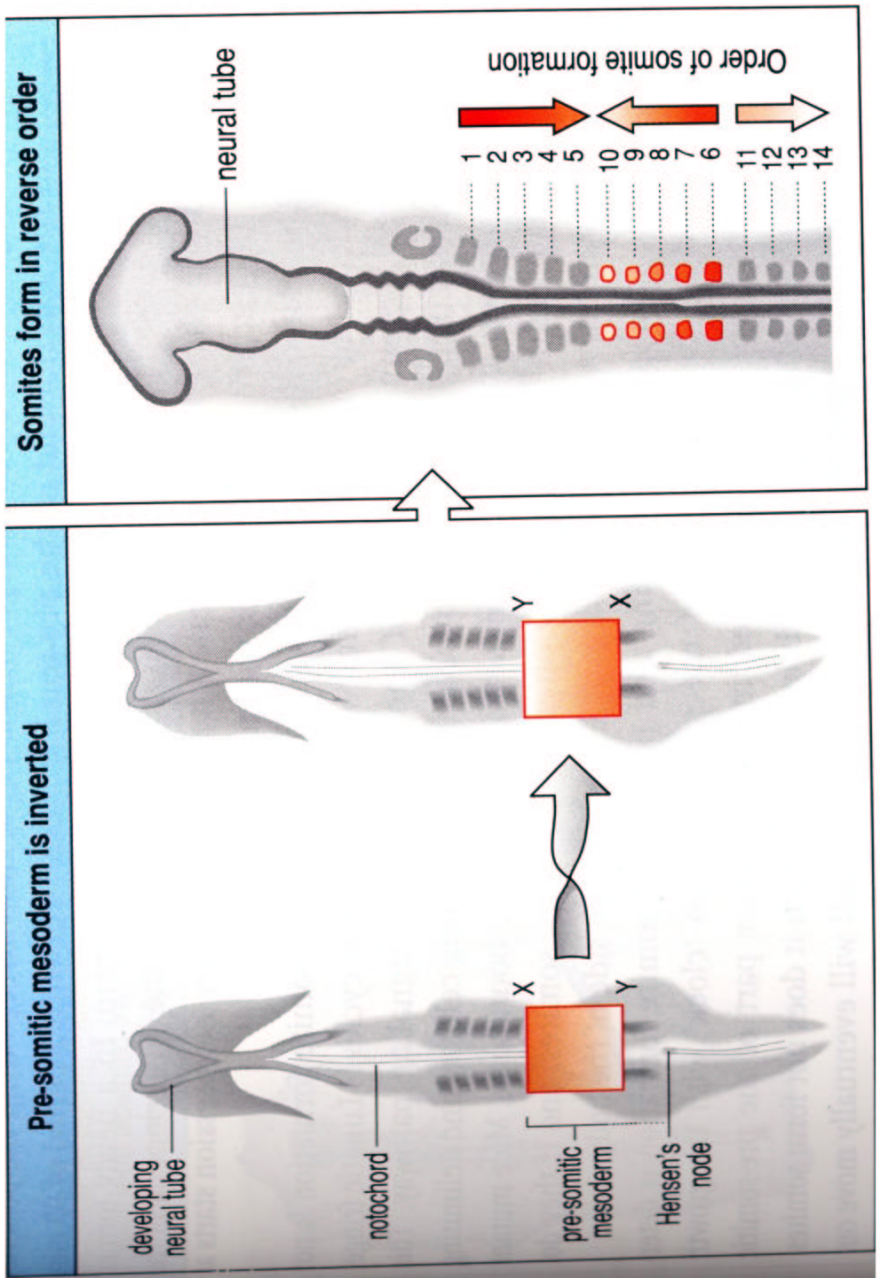

SI
S0
pre-somitic mesoderm
SII
SI
S0
anterior
posterior
0 hour
1 hour 30 min
3 hours
6 hours
9 hours
18 hours
notochord
differentiation of somitic derivatives
epithelial somite
border formation
segment specification
pre-somitic mesoderm
somitic stem cells
Hensen's node
posterior
somite formation every 90 minutes

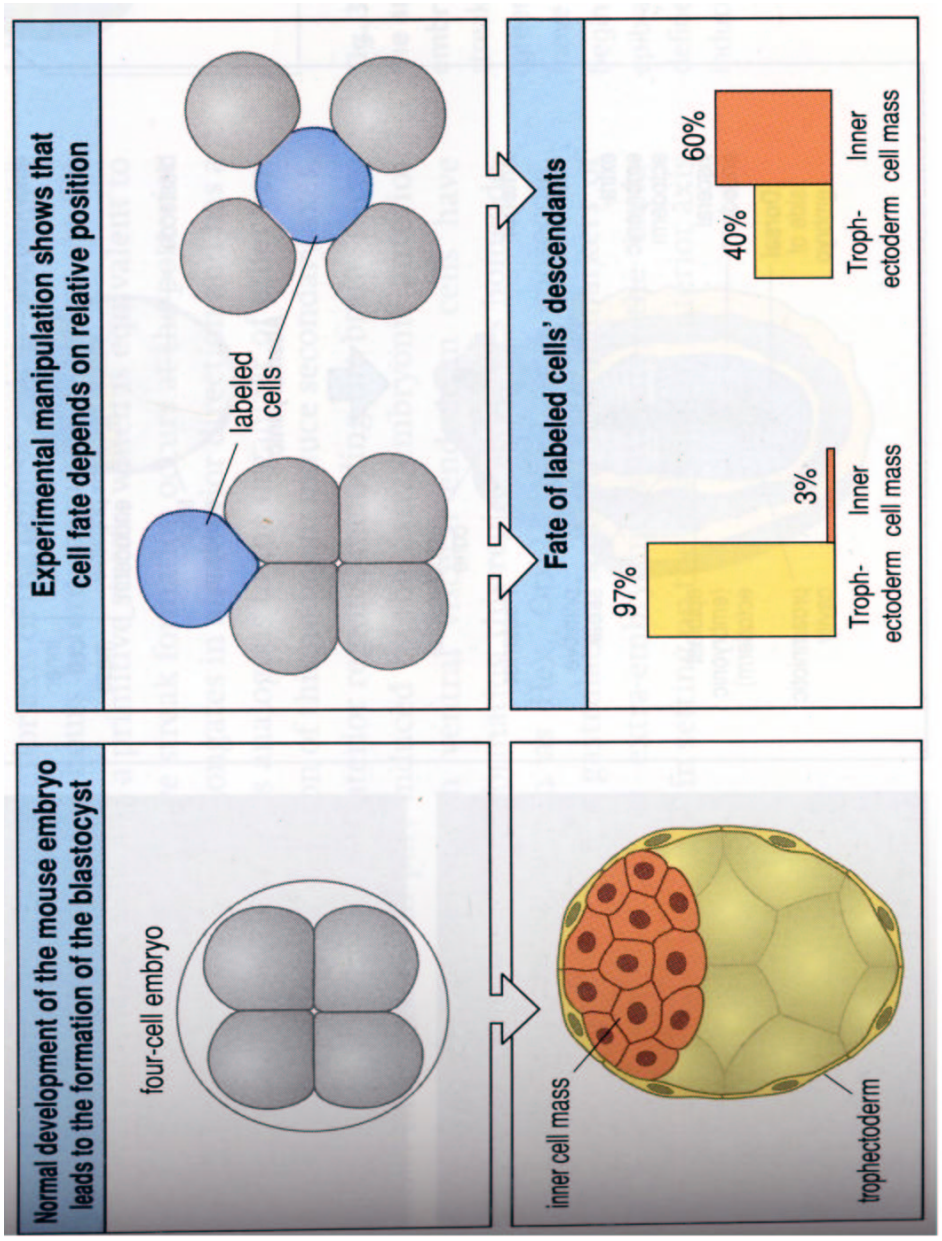

left
right
lefty
activin
activin receptor
Sonic hedgehog
Sonic hedgehog
nodal
snail
pitx2
snail
node
streak
inhibition
activation

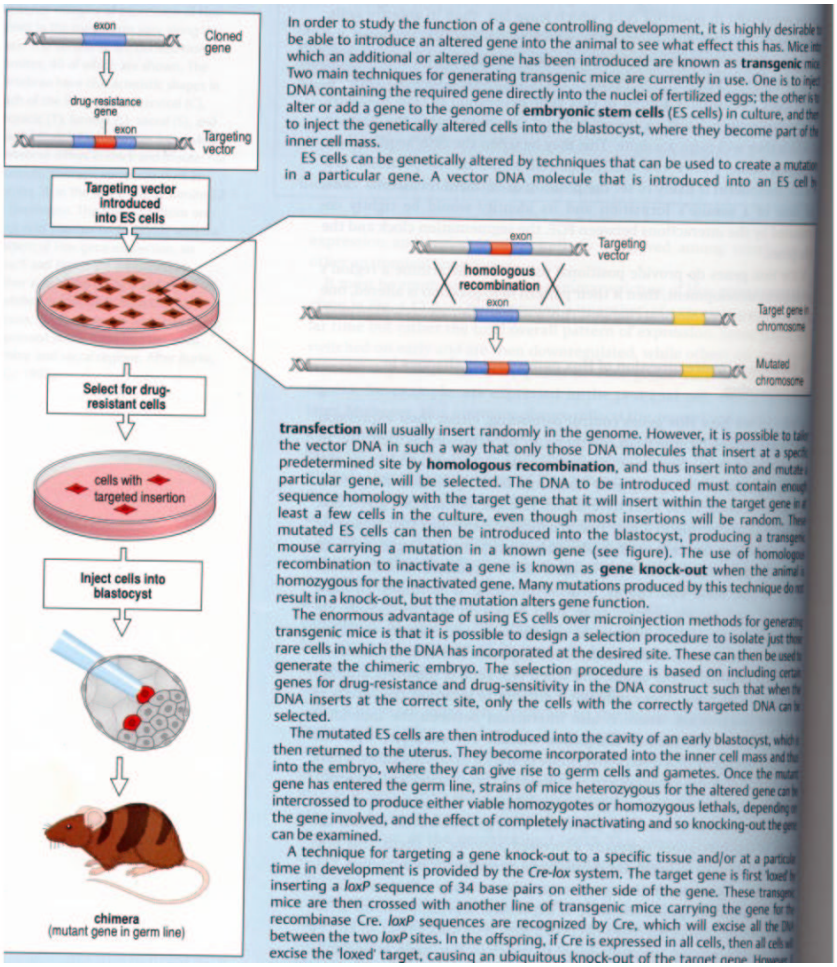

Embryonic
embryonic pole
epiblast
primitive endoderm
inner cell mass
blastocoel
troph-ectoderm
Abembryonic
ectoplacental cone
Anterior
Posterior
primitive streak
extra-embryonic ectoderm
visceral endoderm
epiblast (embryonic ectoderm)
Dorsal side of embryo
proamniotic cavity


Hensen's node
primitive streak
epiblast
hypoblast
endoderm displacing hypoblast
migrating cells
mesoderm

Overview


Pre-somitic mesoderm is inverted
developing neural tube
notochord
pre-somitic mesoderm
Hensen's node
X
Y
Y
X
Somites form in reverse order
neural tube
Order of somite formation
1 2 3 4 5 10 9 8 7 6 11 12 13 14


Normal development of the mouse embryo leads to the formation of the blastocyst
four-cell embryo
inner cell mass
trophectoderm
Experimental manipulation shows that cell fate depends on relative position
labeled cells
Fate of labeled cells' descendants
97%
3%
Troph-ectoderm
Inner cell mass
60%
40%
Troph-ectoderm
Inner cell mass



In order to study the function of a gene controlling development, it is highly desirable to be able to introduce an altered gene into the animal to see what effect this has. Mice into which an additional or altered gene has been introduced are known as **transgenic** mice. Two main techniques for generating transgenic mice are currently in use. One is to inject DNA containing the required gene directly into the nuclei of fertilized eggs; the other is to alter or add a gene to the genome of **embryonic stem cells** (ES cells) in culture, and then to inject the genetically altered cells into the blastocyst, where they become part of the inner cell mass.

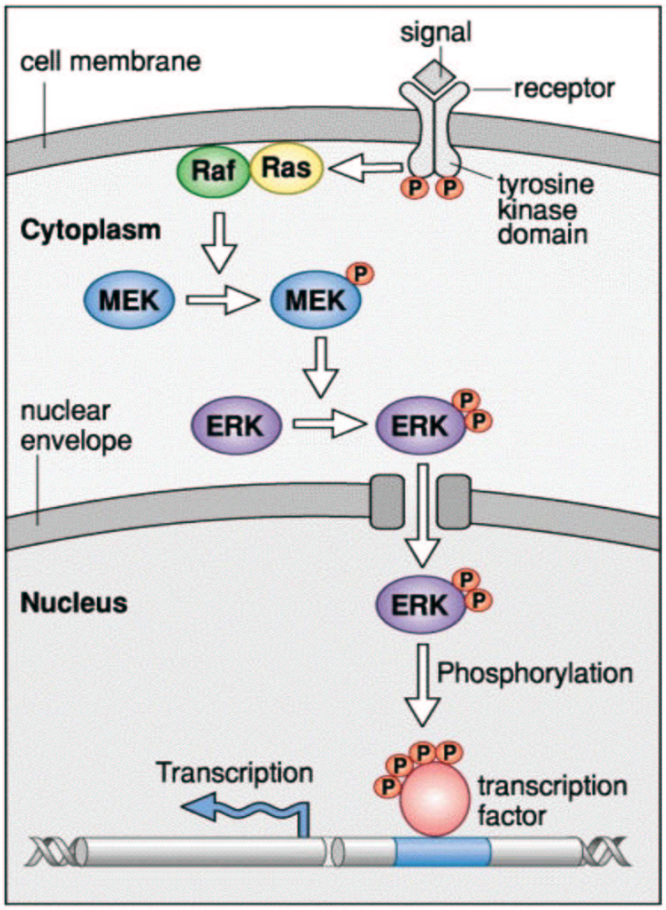
ES cells can be genetically altered by techniques that can be used to create a mutation in a particular gene. A vector DNA molecule that is introduced into an ES cell by

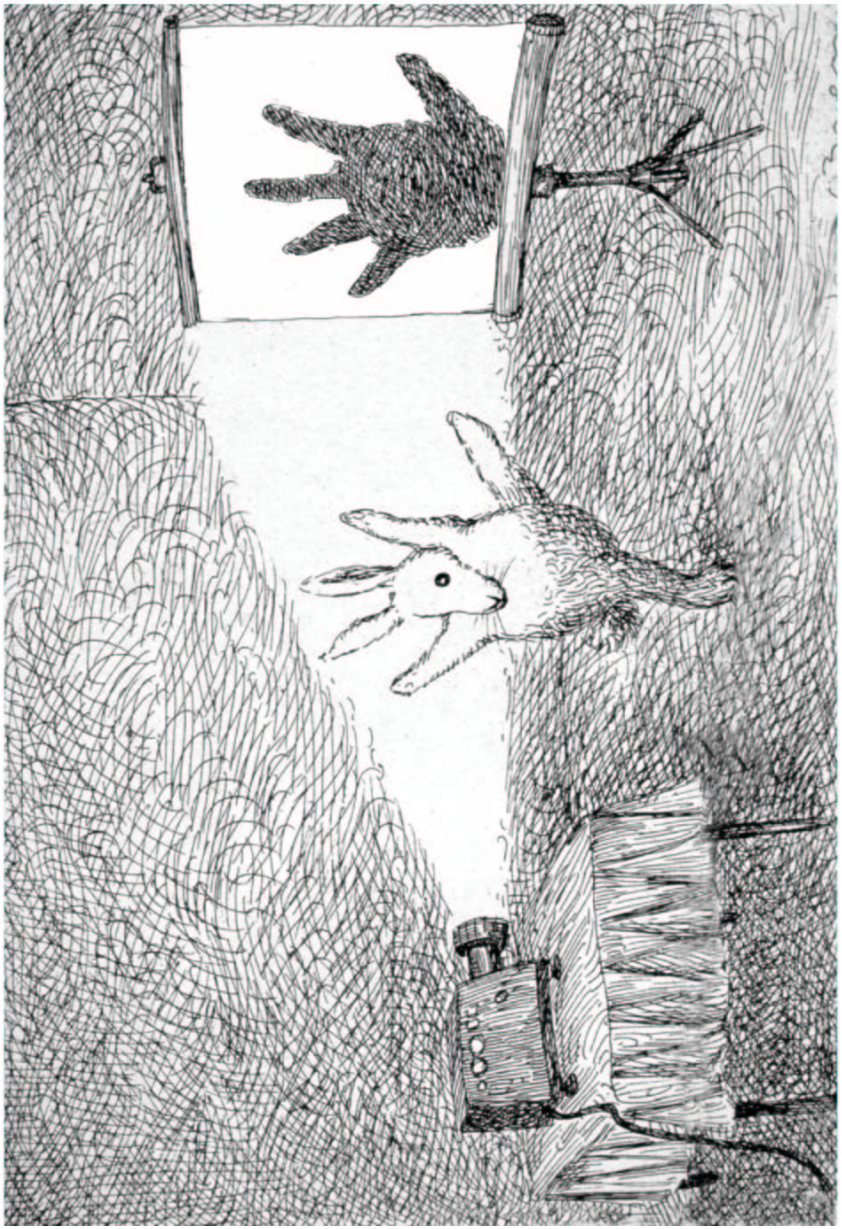
transfection will usually insert randomly in the genome. However, it is possible to tailor the vector DNA in such a way that only those DNA molecules that insert at a specific predetermined site by **homologous recombination**, and thus insert into and mutate a particular gene, will be selected. The DNA to be introduced must contain enough sequence homology with the target gene that it will insert within the target gene in at least a few cells in the culture, even though most insertions will be random. These mutated ES cells can then be introduced into the blastocyst, producing a transgenic mouse carrying a mutation in a known gene (see figure). The use of homologous recombination to inactivate a gene is known as **gene knock-out** when the animal is homozygous for the inactivated gene. Many mutations produced by this technique do not result in a knock-out, but the mutation alters gene function.

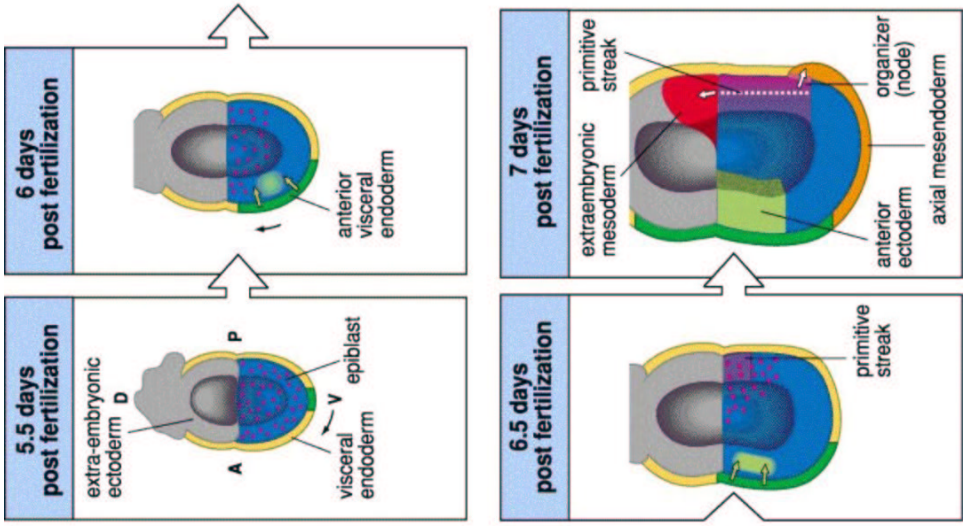
The enormous advantage of using ES cells over microinjection methods for generating transgenic mice is that it is possible to design a selection procedure to isolate just those rare cells in which the DNA has incorporated at the desired site. These can then be used to generate the chimeric embryo. The selection procedure is based on including certain genes for drug-resistance and drug-sensitivity in the DNA construct such that when the DNA inserts at the correct site, only the cells with the correctly targeted DNA can be selected.

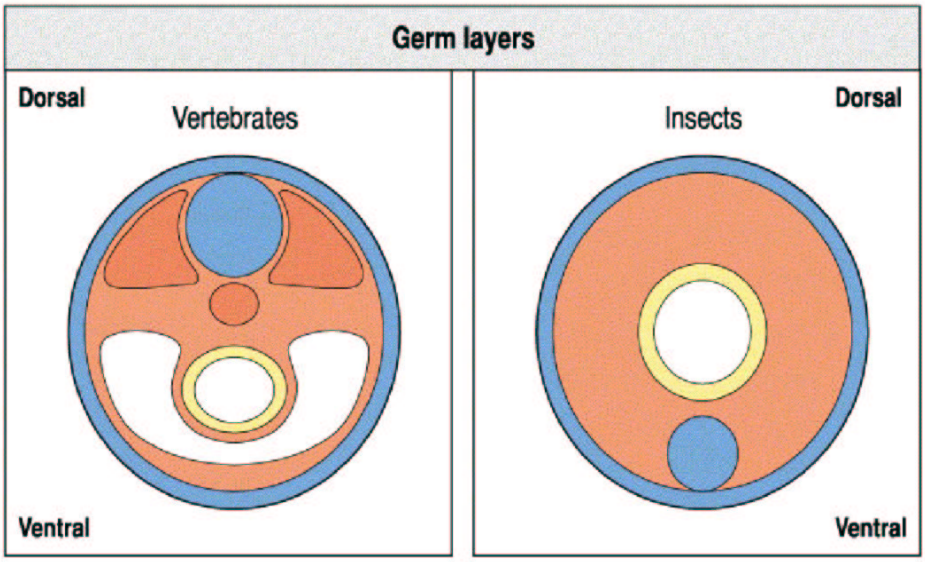
The mutated ES cells are then introduced into the cavity of an early blastocyst, which is then returned to the uterus. They become incorporated into the inner cell mass and thus into the embryo, where they can give rise to germ cells and gametes. Once the mutant gene has entered the germ line, strains of mice heterozygous for the altered gene can be intercrossed to produce either viable homozygotes or homozygous lethals, depending on the gene involved, and the effect of completely inactivating and so knocking-out the gene can be examined.

A technique for targeting a gene knock-out to a specific tissue and/or at a particular time in development is provided by the *Cre-lox* system. The target gene is first 'loxed' by inserting a *loxP* sequence of 34 base pairs on either side of the gene. These transgenic mice are then crossed with another line of transgenic mice carrying the gene for the recombinase Cre. *loxP* sequences are recognized by Cre, which will excise all the DNA between the two *loxP* sites. In the offspring, if Cre is expressed in all cells, then all cells will excise the 'loxed' target, causing an ubiquitous knock-out of the target gene. However

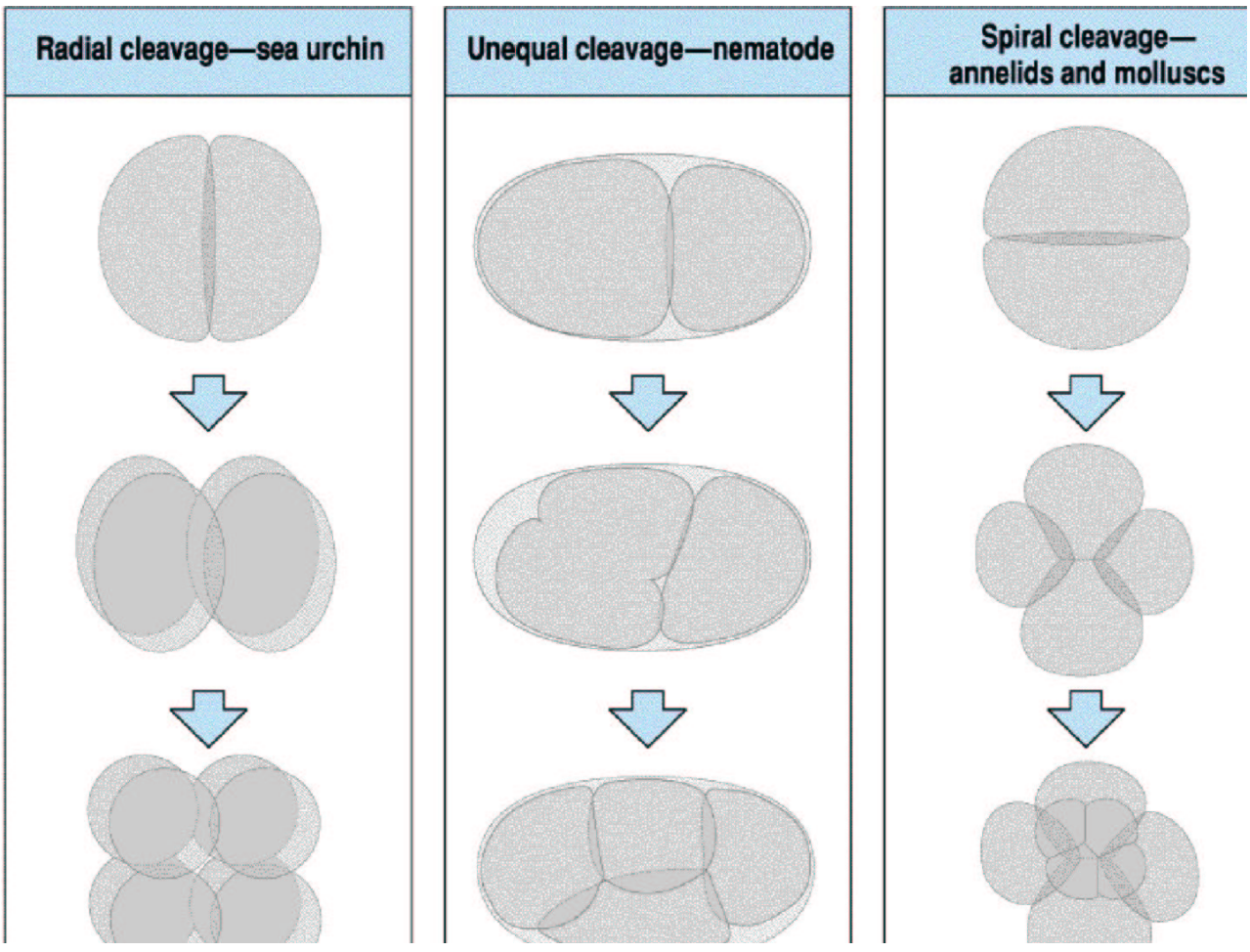




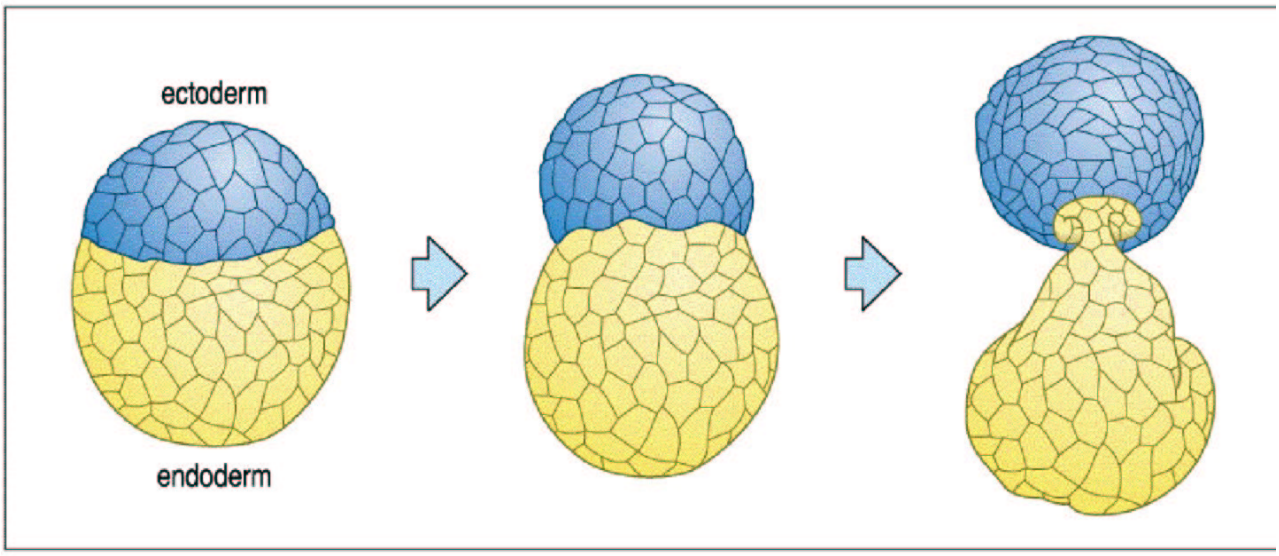

5.5 days post fertilization
extra-embryonic ectoderm
D
P
V
A
epiblast
visceral endoderm
6 days post fertilization
anterior visceral endoderm
6.5 days post fertilization
primitive streak
7 days post fertilization
extraembryonic mesoderm
primitive streak
organizer (node)
anterior ectoderm
axial mesendoderm



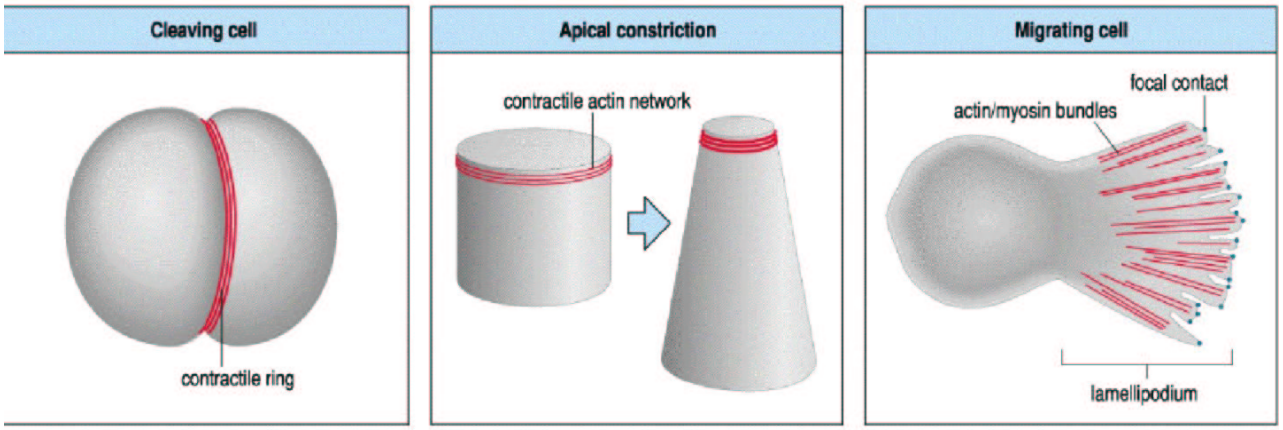
Germ layers	Organs	
Endoderm	gut, liver, lungs	gut
Mesoderm	skeleton, muscle, kidney, heart, blood	muscle, heart, blood
Ectoderm	skin, nervous system	cuticle, nervous system

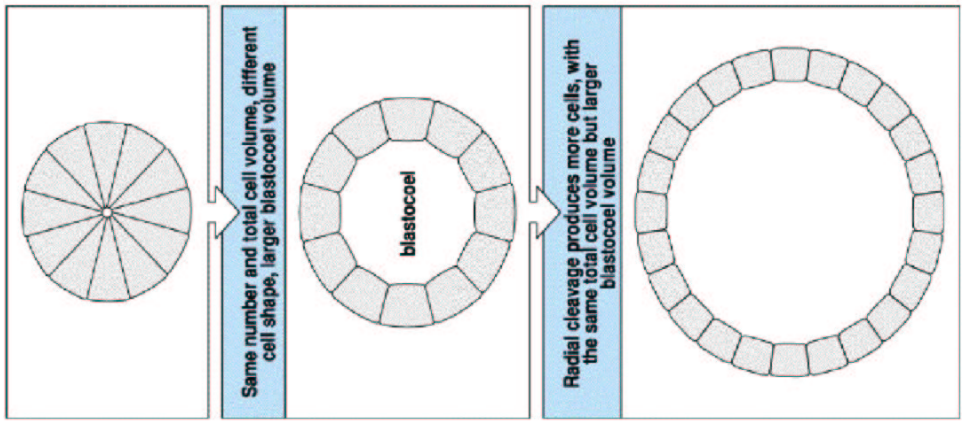


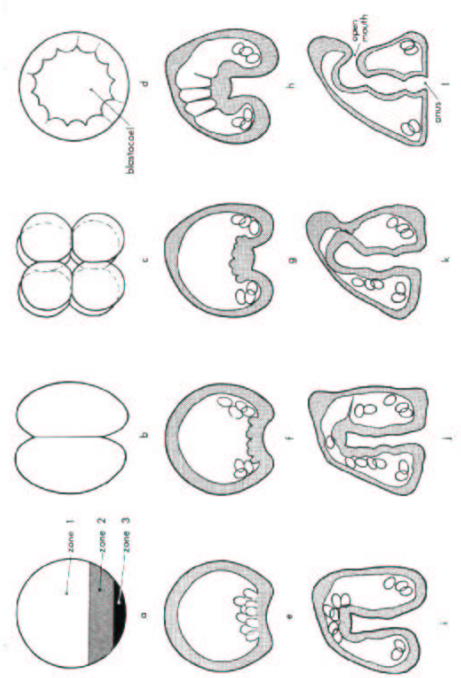
Differences in adhesion



Contractions

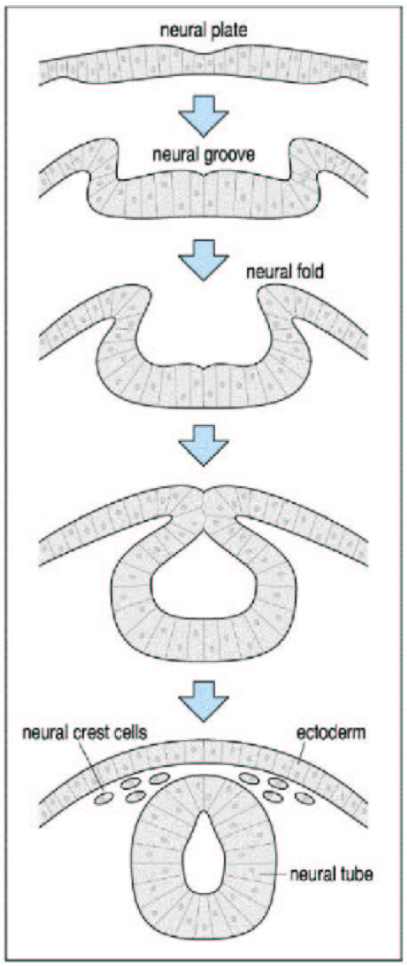




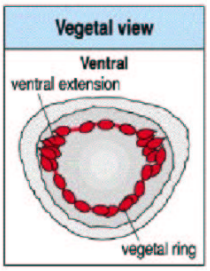


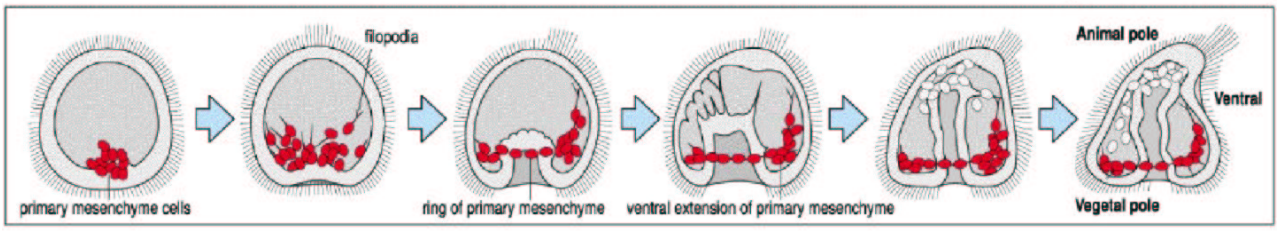
Sea urchin development

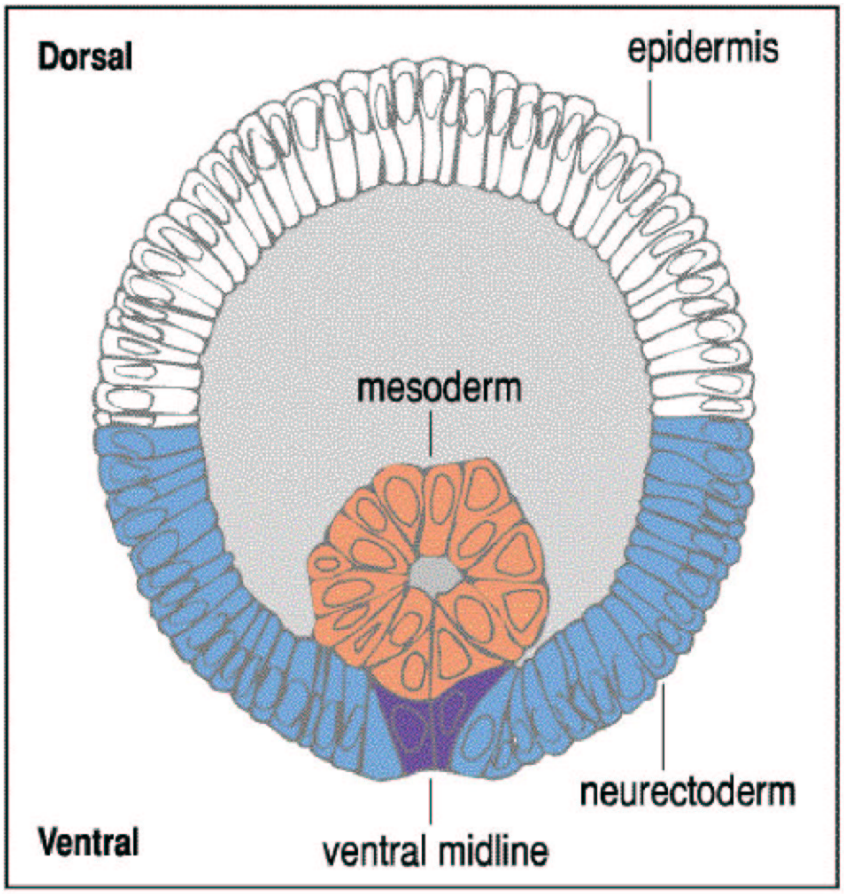
Neural tube formation



Sea urchin cell migration

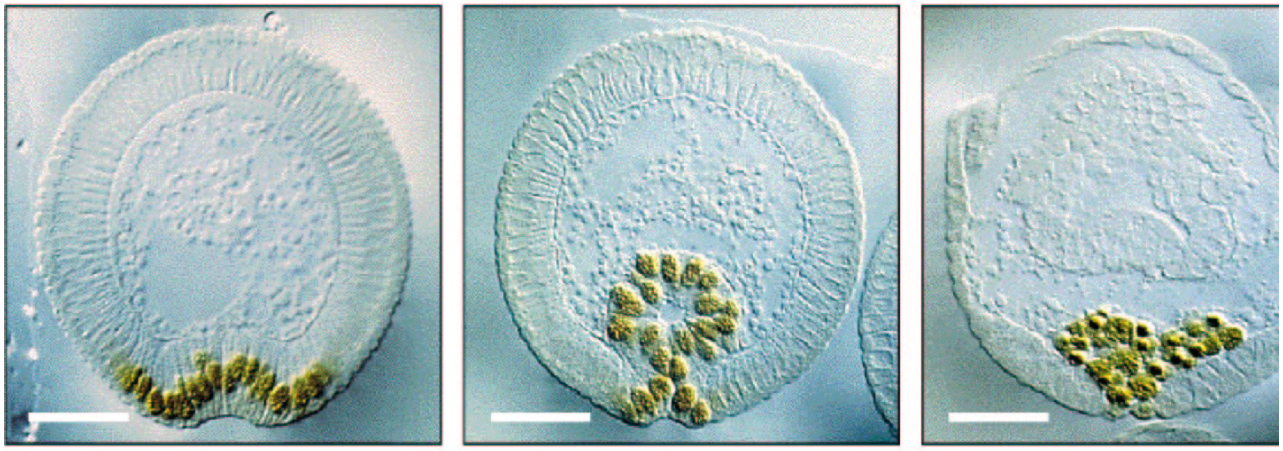




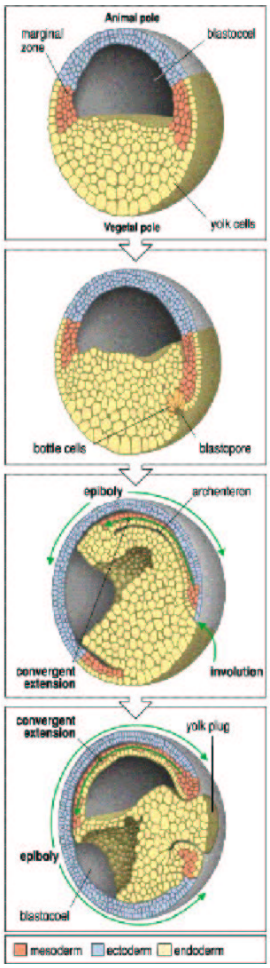


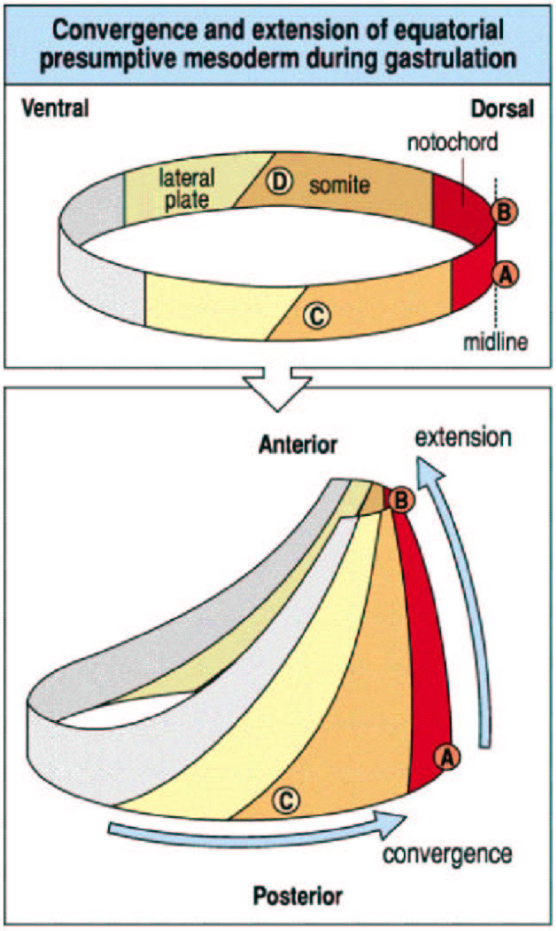
FLY GASTRULATION MESODERM

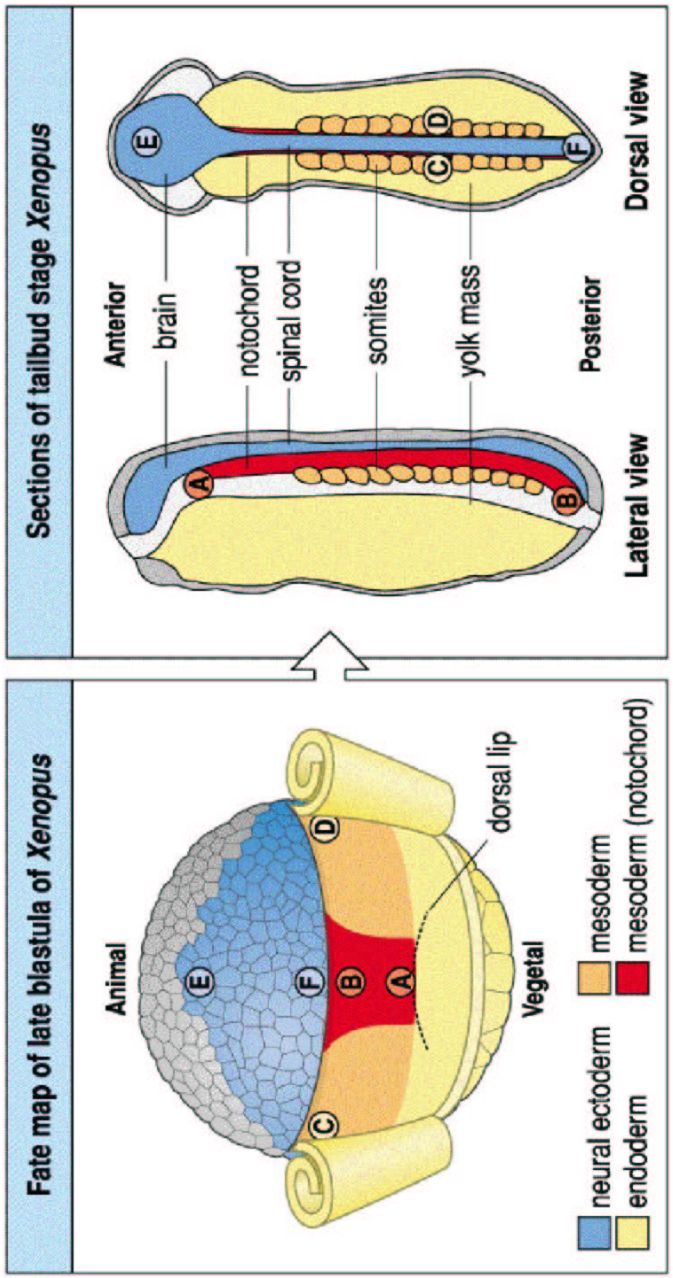
Fly gastrulation of mesoderm

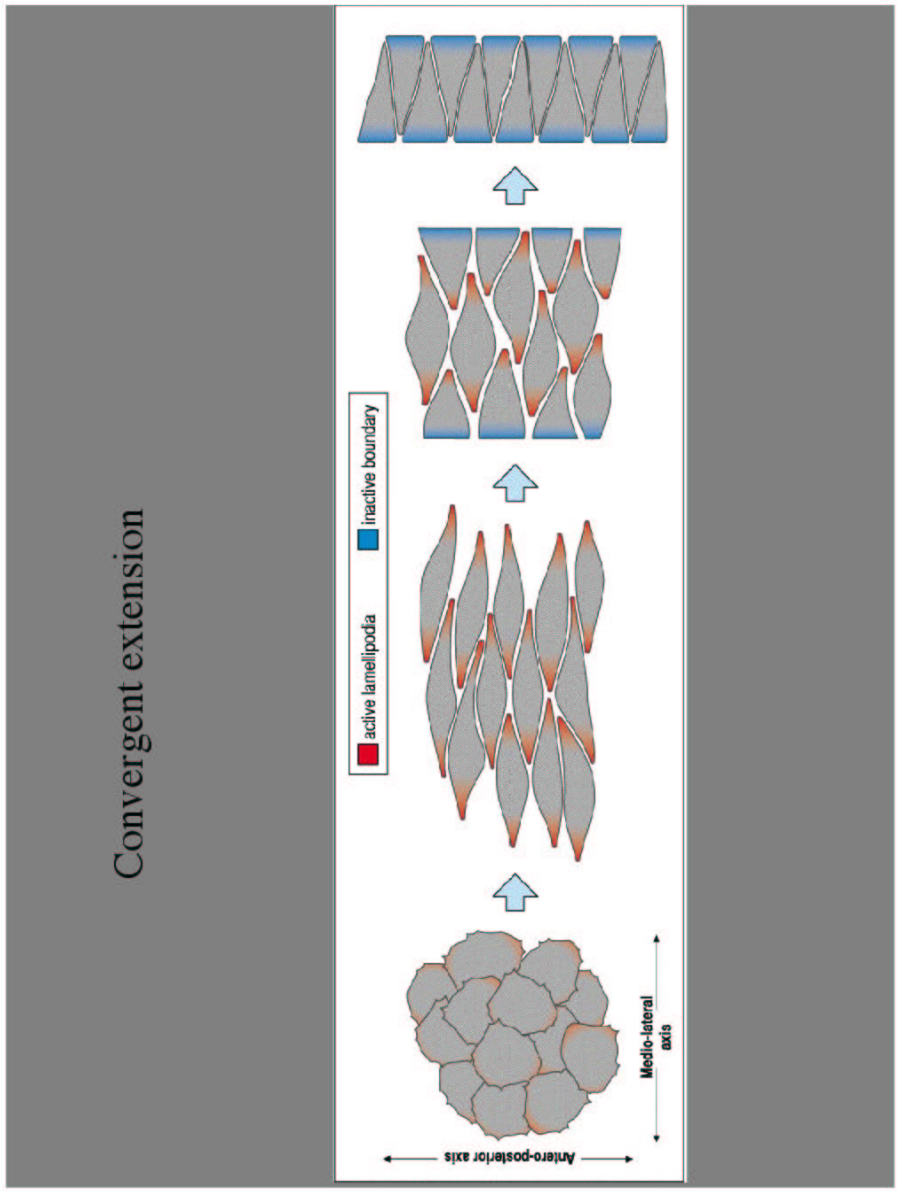


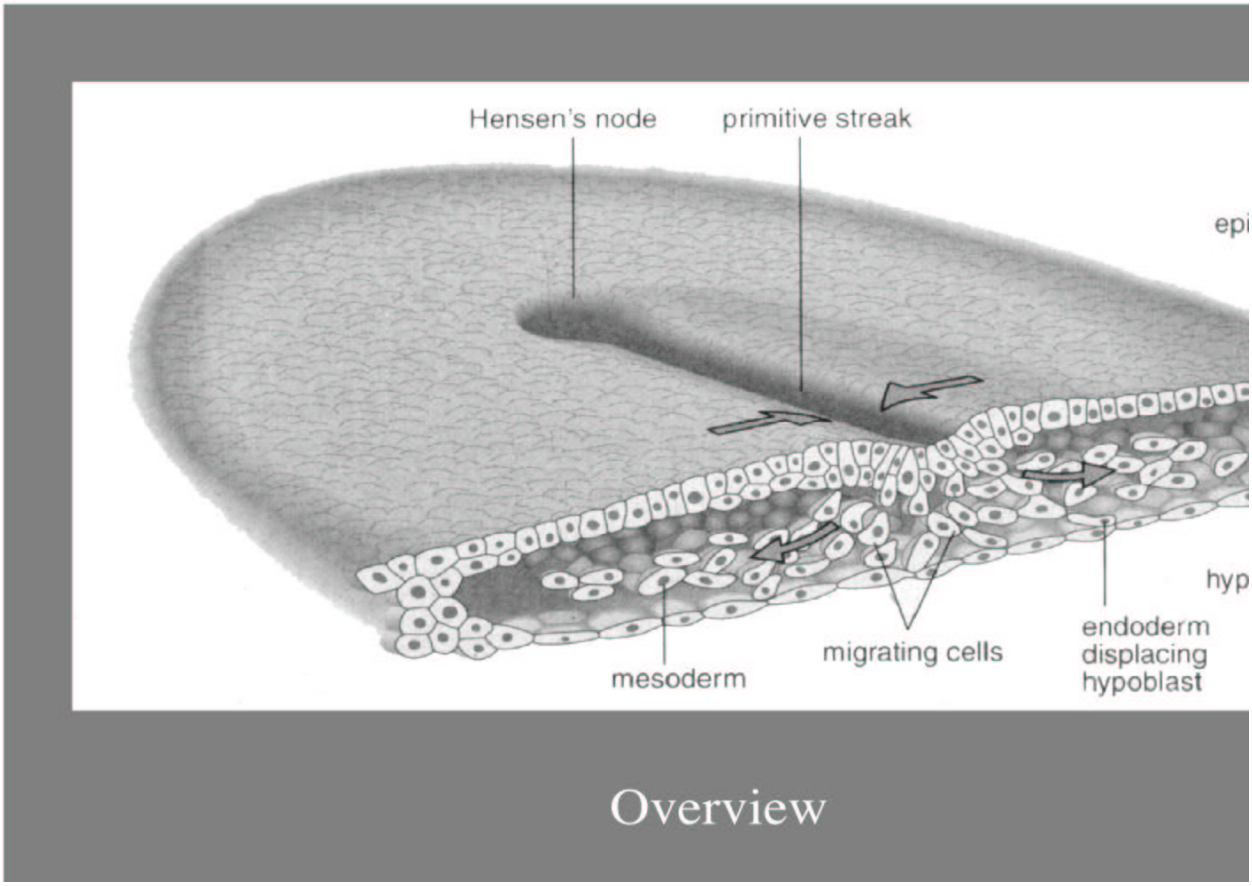
Gastrulation frog



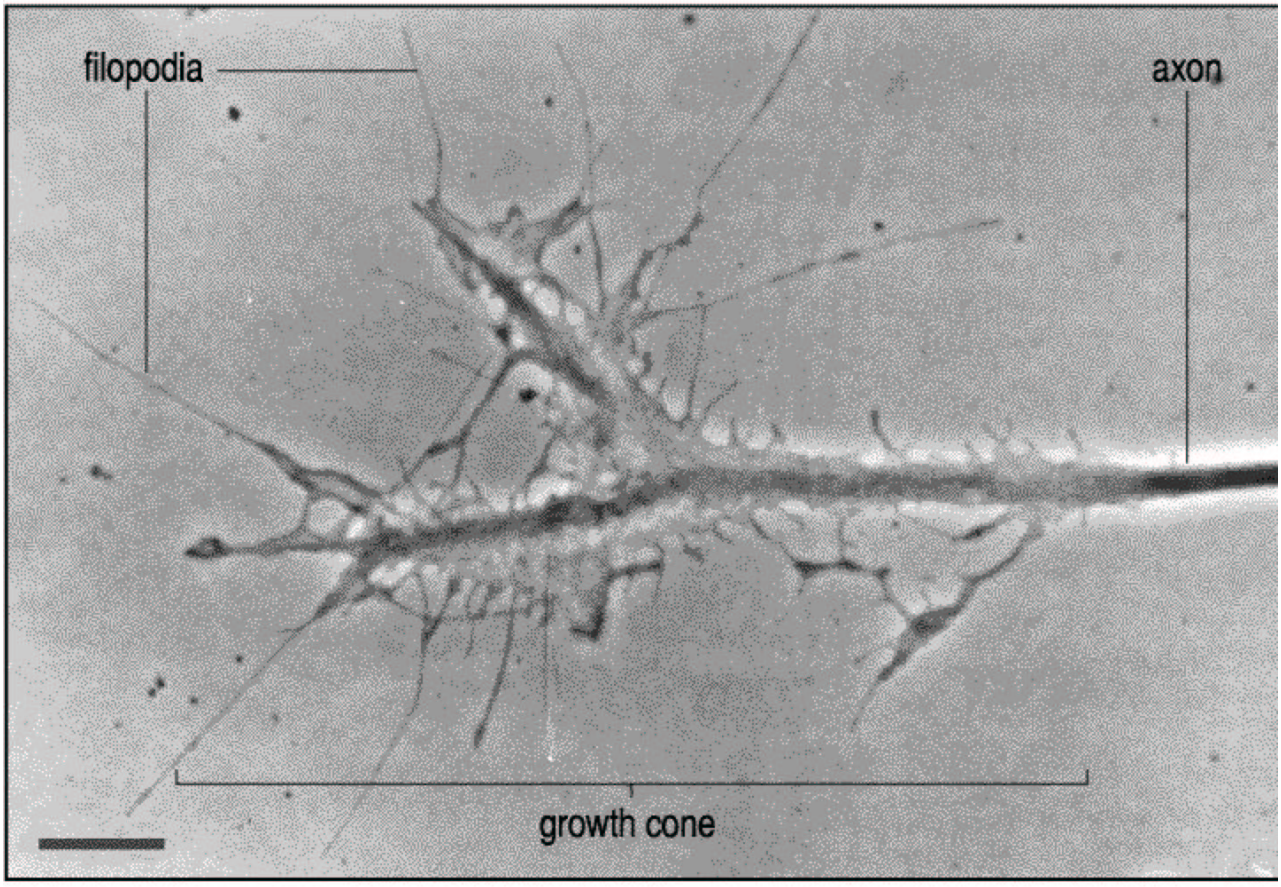


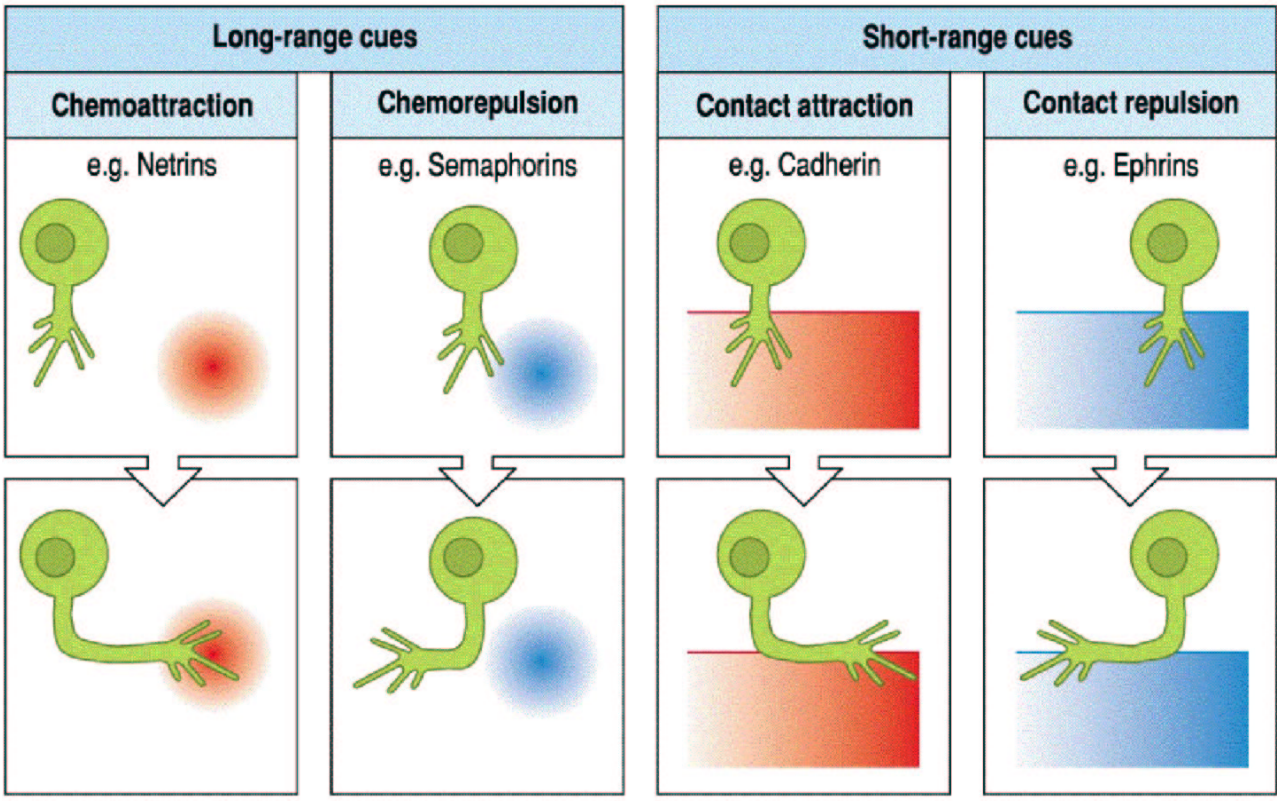

Fate map of late blastula of Xenopus
Animal
Vegetal
dorsal lip
neural ectoderm
endoderm
mesoderm
mesoderm (notochord)
Sections of tailbud stage Xenopus
Anterior
Posterior
brain
notochord
spinal cord
somites
yolk mass
Dorsal view
Lateral view


Convergent extension
active lamellipodia
inactive boundary
Antero-posterior axis
Medio-lateral axis

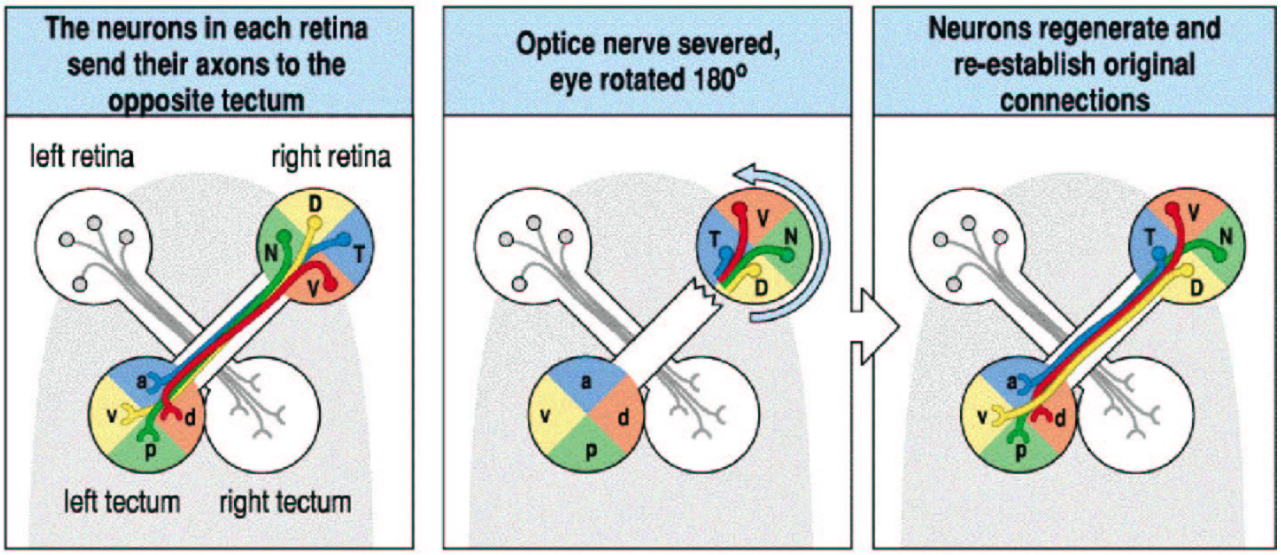

Hensen's node
primitive streak
epi
hyp
endoderm
displacing
hypoblast
migrating cells
mesoderm

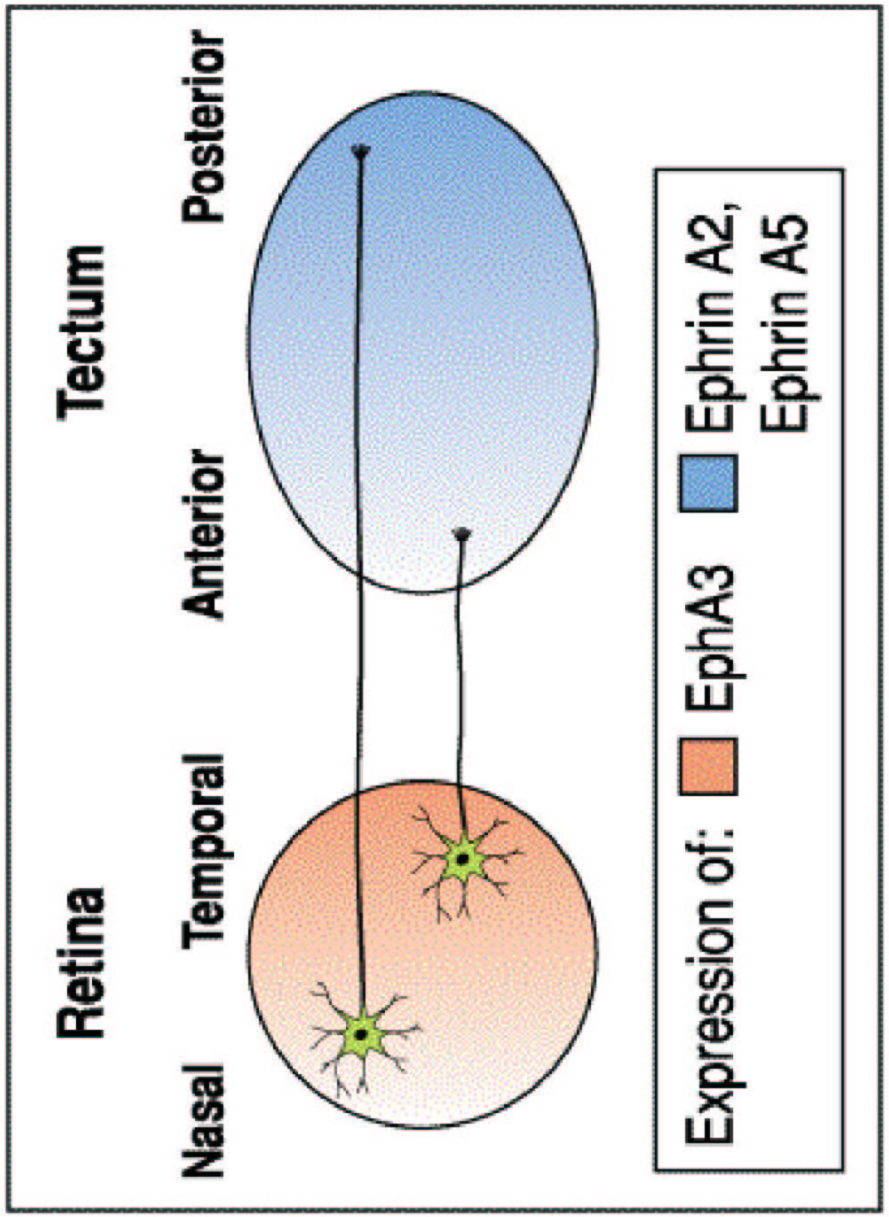
Overview

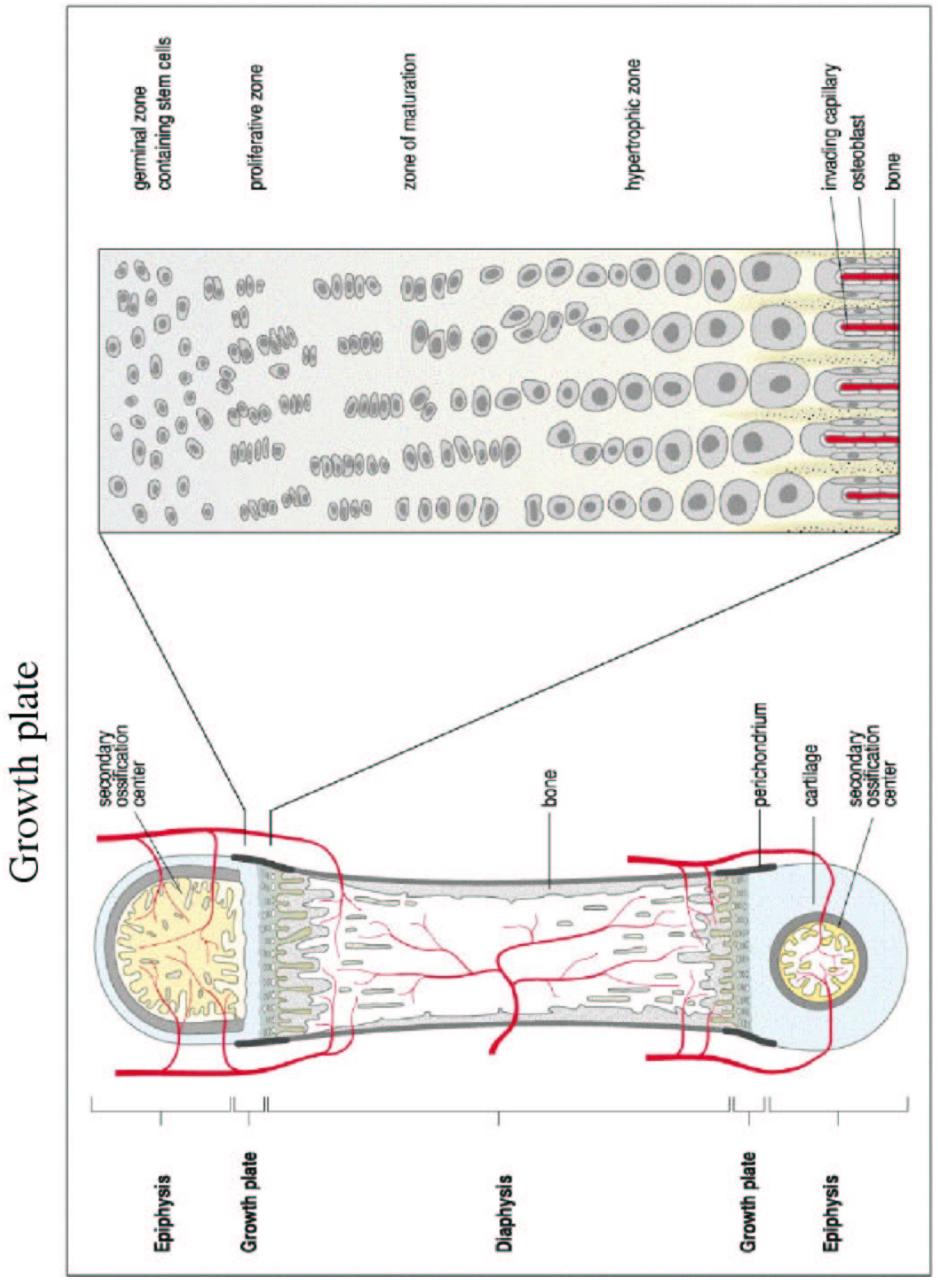

filopodia
axon
growth cone

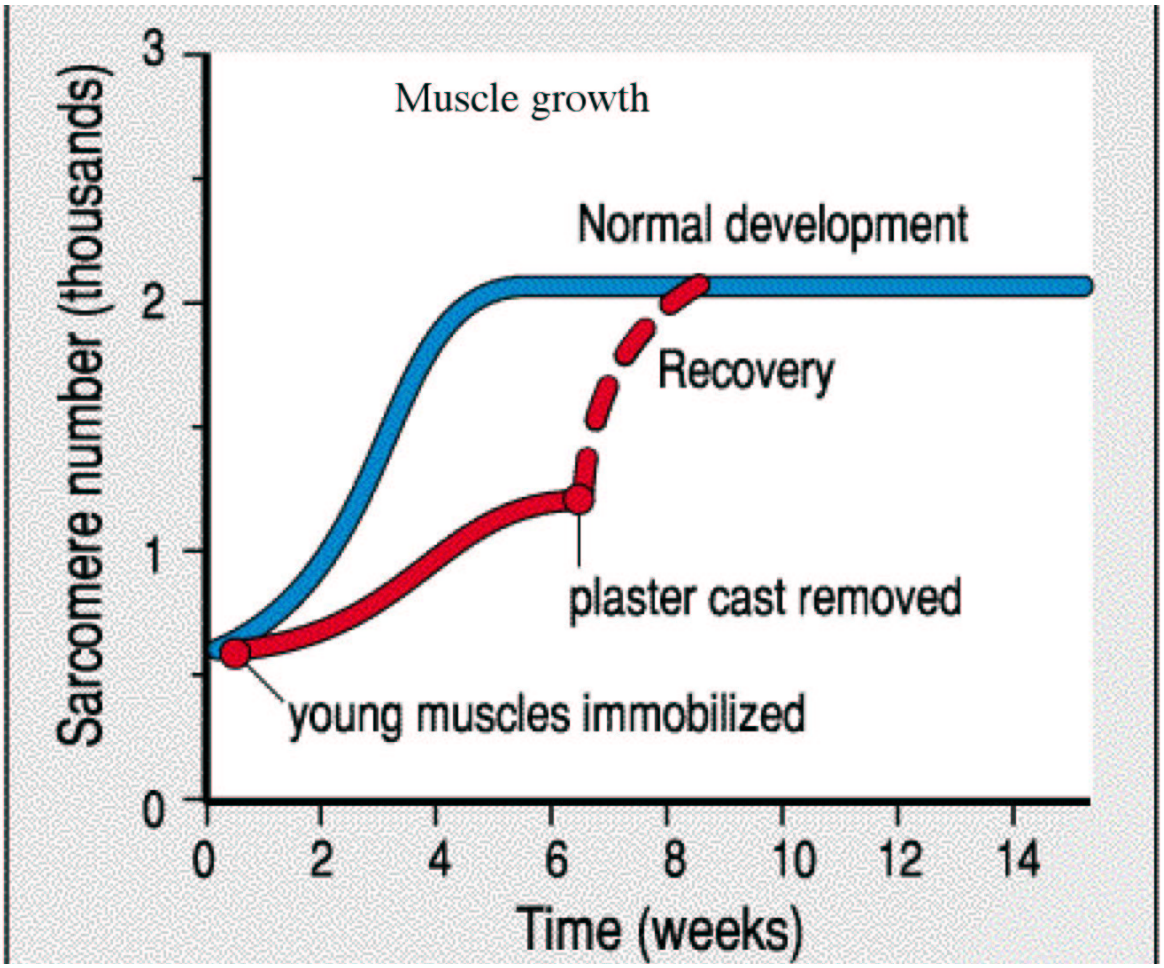


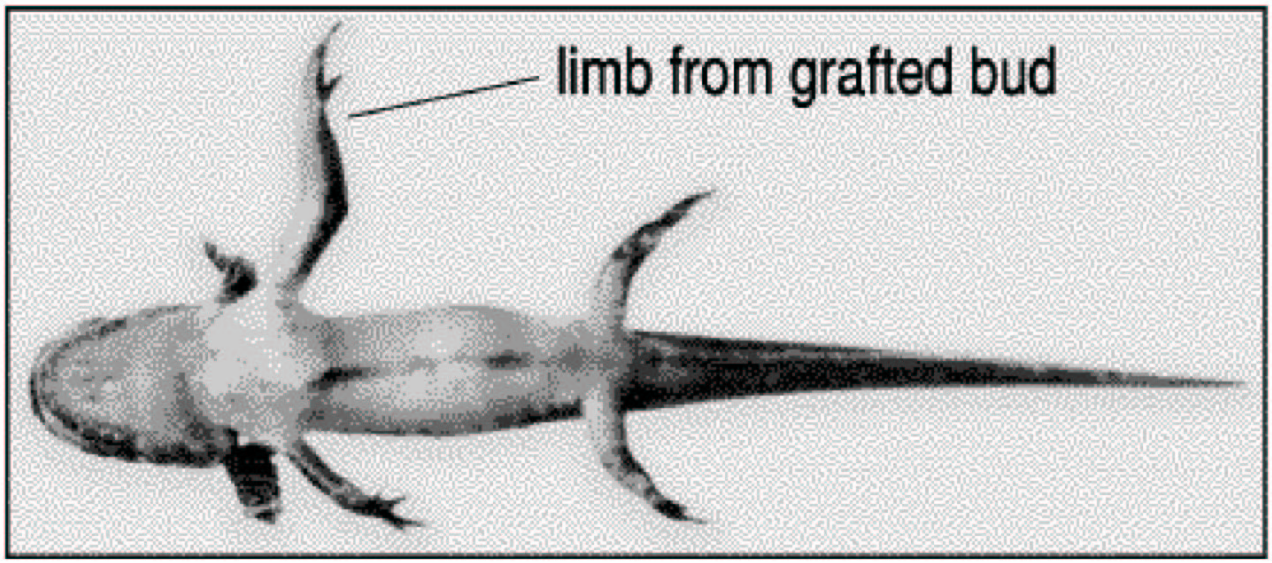
Retino-tectal connections




Retina
Tectum
Nasal
Temporal
Anterior
Posterior
Expression of:
EphA3
Ephrin A2,
Ephrin A5

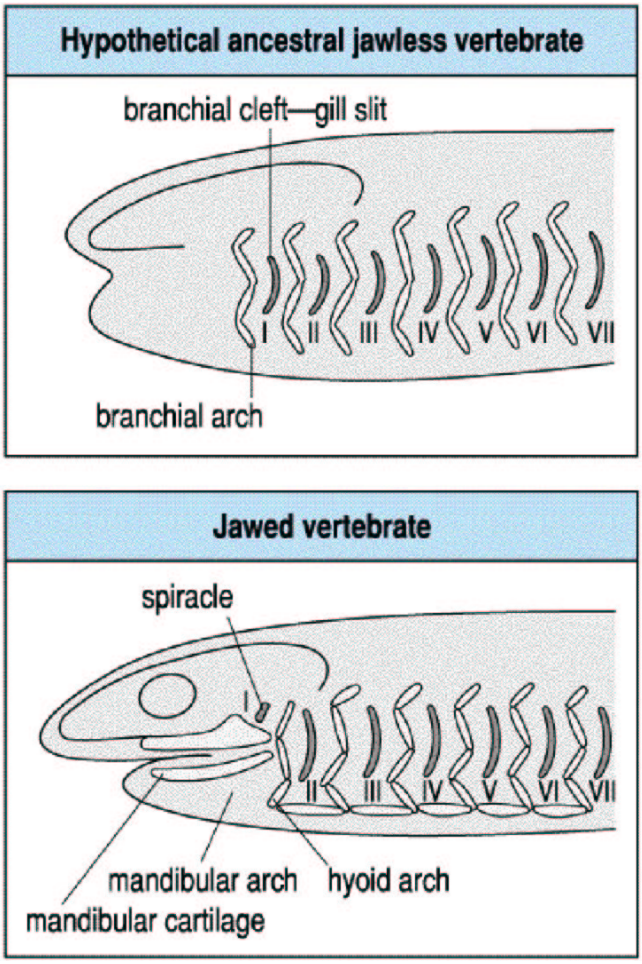

Growth plate
germinal zone containing stem cells
proliferative zone
zone of maturation
hypertrophic zone
invading capillary
osteoblast
bone
secondary ossification center
bone
perichondrium
cartilage
secondary ossification center
Epiphysis
Growth plate
Diaphysis
Growth plate
Epiphysis

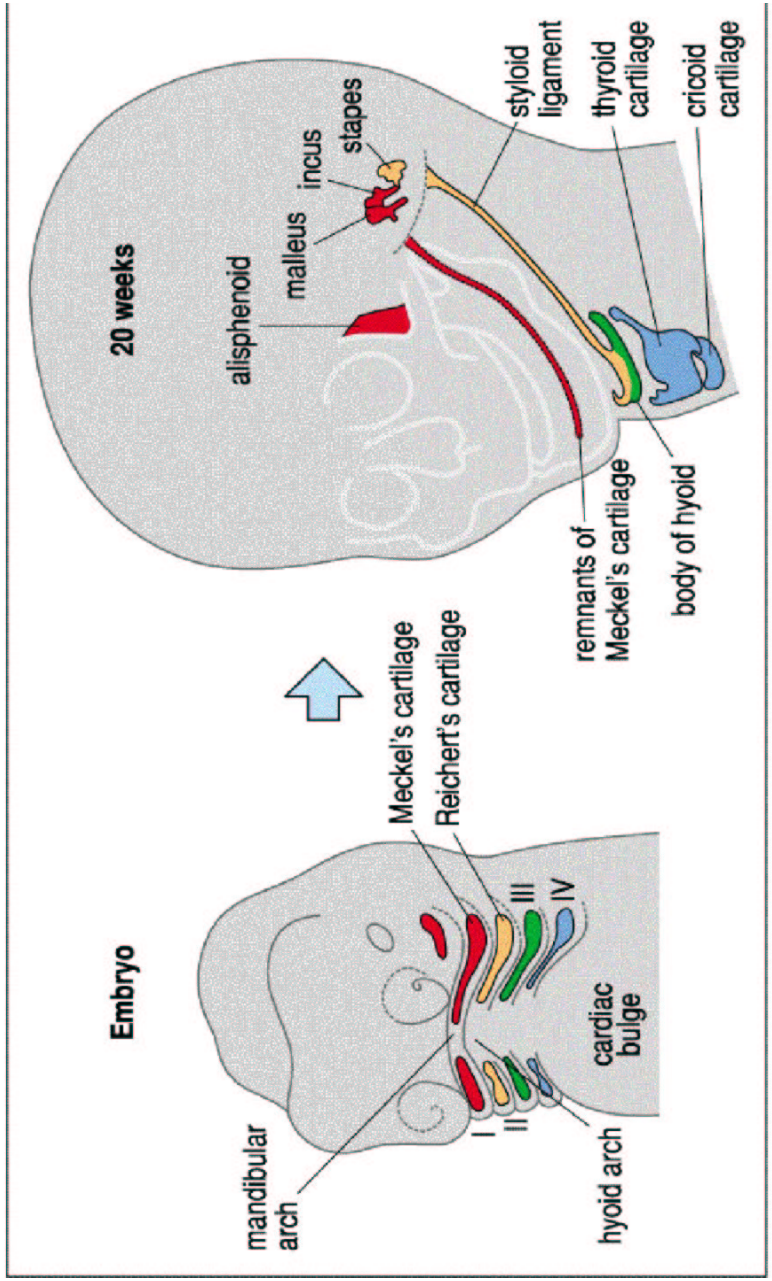

Muscle growth
Normal development
Recovery
plaster cast removed
young muscles immobilized
Sarcomere number (thousands)
Time (weeks)
0
1
2
3
0
2
4
6
8
10
12
14

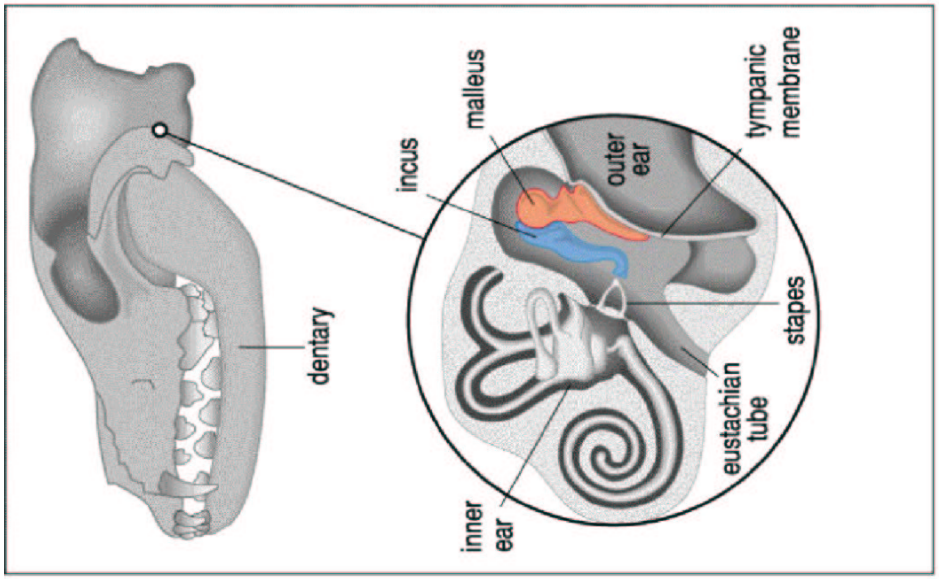

limb from grafted bud

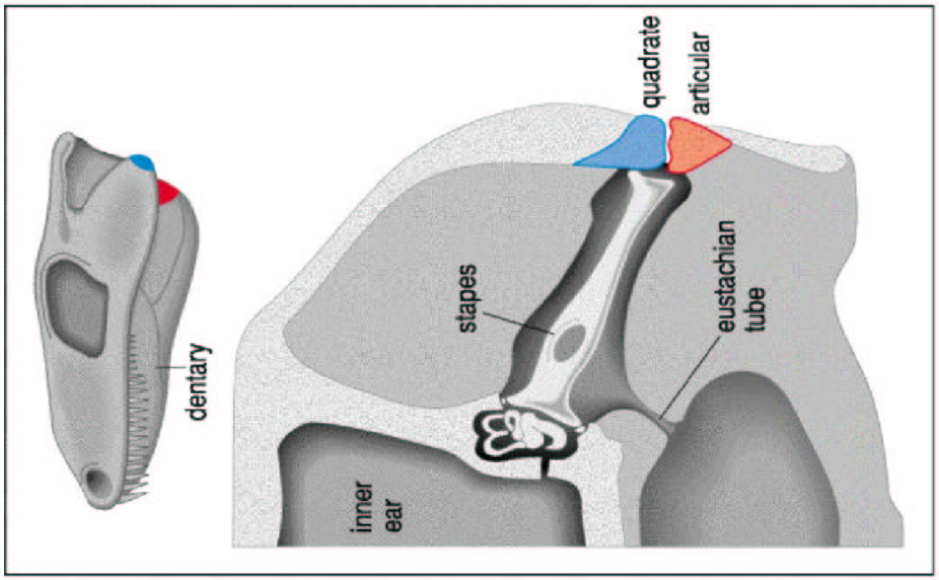
Ontogeny does not recapiulate phylogeny

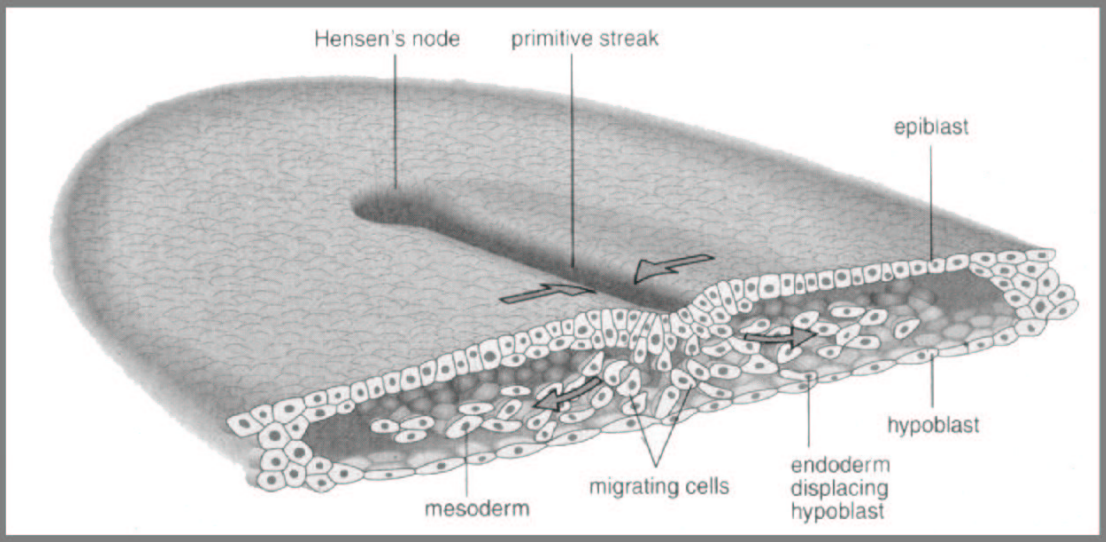
Evolution is a tinkerer




Embryo
mandibular arch
Meckel's cartilage
Reichert's cartilage
I
II
III
IV
cardiac bulge
hyoid arch
20 weeks
alisphenoid
malleus
incus
stapes
styloid ligament
thyroid cartilage
cricoid cartilage
remnants of Meckel's cartilage
body of hyoid


dentary
incus
malleus
outer ear
tympanic membrane
inner ear
eustachian tube
stapes


dentary
quadrate
articular
stapes
eustachian tube
inner ear


Hensen's node
primitive streak
epiblast
hypoblast
endoderm
displacing
hypoblast
migrating cells
mesoderm

Overview