学術俯瞰講義

奥深さと美しさによる全体像

可能性が生まれる

発生生物学からみた
生命科学
浅島　誠
東京大学大学院総合文化研究科 教授

分子モーターから観た
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廣川　信隆
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ゲノムから見た
生命科学
黒岩　常祥
東京大学名誉教授

主題科目／テーマ講義

生命の科学

構造と機能の調和

10月16日→1月29日

月曜日 5時限
駒場キャンパス

16時20分▶17時50分
18号館ホール

Global Focus on Knowledge Lecture Series

2006 Winter Semester: “Science of Life”

Life Science-from the Perspective of Developmental Biology

No.1 Oct.16 Mechanism of formation from an egg to an adult body

No.2 Oct.23 Biological information system and networking

No.3 Oct.30 Mechanism of organ formation

Professor Makoto Asashima

Graduate School of Arts and Sciences, University of Tokyo

Organ formation in Development

The development of an organism

A single cell（fertilized egg) proliferates, and differentiates into various tissues and organs to form an individual body.
To form a well-controlled individual body

↓

Organ formation could be understood in the whole process of embryo development.

Development

Formation of structure and function

Determination of directions of the whole embryo

Differentiation of 3 germ layers and induction of each of them

Determination of locations

Induction between tissues

Formation of each organ

Formation of the whole individual

What cause these?

↓

Analysis of mechanism

Understanding phenomena

An overview of vertebrate embryo development (example: in amphibians)

Flow of direction in determination, and organ formation in an embryo

（in case of amphibians）

time

polarity of an unfertilized ovum

determination of an animal/vegetal pole

induction between ectoderm and endoderm

determination of ventral-dorsal axis（sperm penetration）

differentiation of mesoderm（mesoderm induction

determination of the anterior-posterior axis（right-left axis）

gastrolulation

differentiation of notochord

formation of archenteron

differentiation of nerve tissu（neural induction）

to ord induces nerve from the epidermis）

both sides of the notochord

mesomere

middle mesoderm

visceral plate mesoder

lateral plate mesoderm

body wall plate mesoc

formation of a neural tube

formation of segments

determination of location

differentiation of each organ by induction betw

fertilized egg

Sperm penetrates the ovum by fertilization.

Cortical layer rotates

Ventral

Dorsal

posite side of sperm penetration spot becomes the dorsal side.

Determination of ventral-dorsal axis

Process of cleavage

morula~blastula

Vegetal polar endoderm induces animal-polar ectoderm becomes mesoderm

ectoderm

mesoderm

endoderm

inducti on

blastula~ archigastrula

section

ventral mesoderm

induction

dorsal mesoderm

Nieuwkoop center

organizer

The Nieuwkoop center is formed in dorsal mesoderm and induces dorsal mesoderm into the organizer.

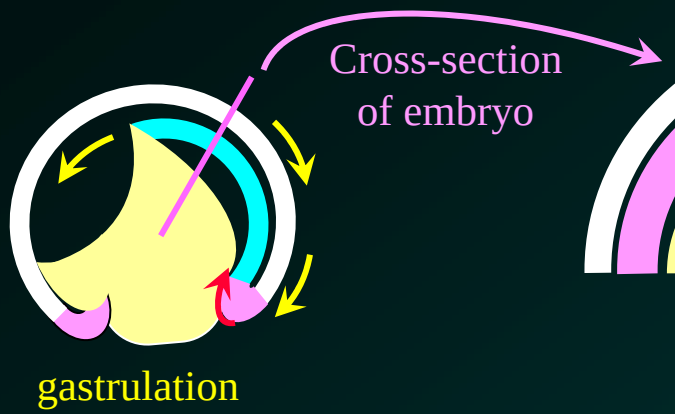
notoc hord

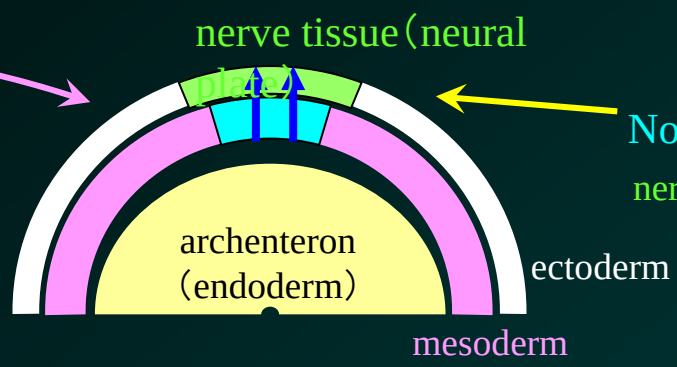
blastopora

Gastrulation starts from the dorsal mesoderm . Central region of embodying mesoderm differentiates into the notochord.

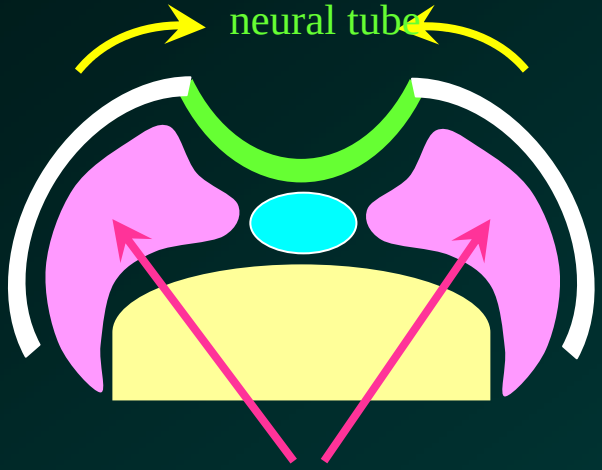
Determination of central axis

archigastrula～neurula

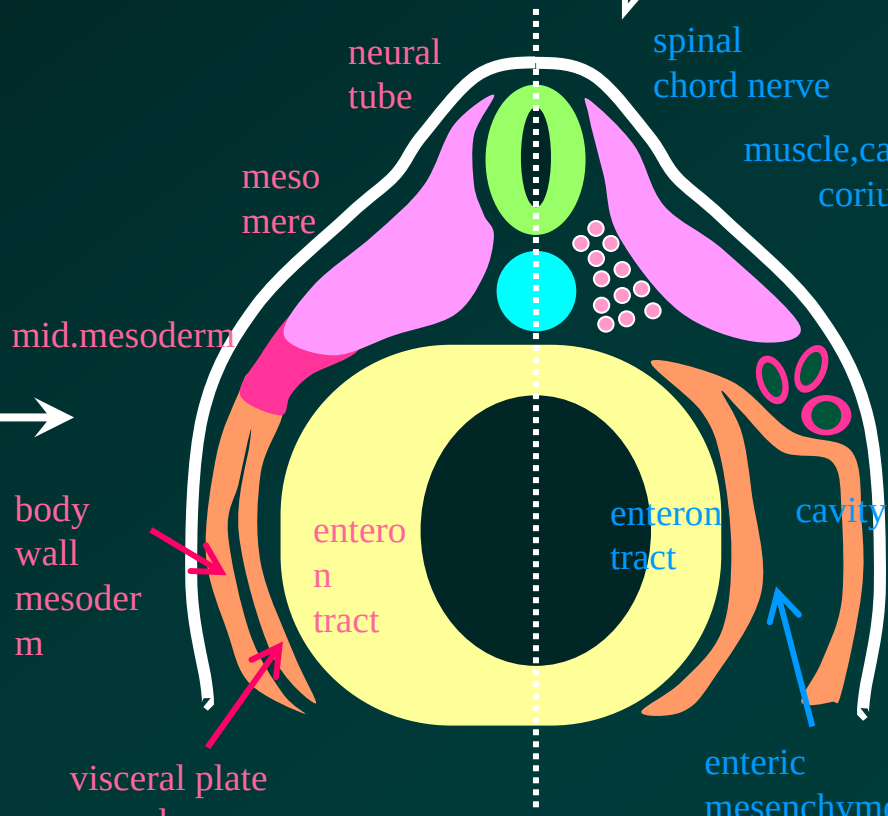




differentiation



Somite and lateral plate differentiate from mesoderm at both sides of the notochord.



Example of research on organ formation

①

Structure of the whole individual and interaction in organ formation

individual
↑ ↓
organ
↑ ↓
tissue
↑ ↓
cell

②

Interaction between tissues during organ development

③

Function of various genes in organ formation

- Mechanisms of organ formation can be discovered by considering phonomena at each scale, and by thinking cross-sectionally.
  （①,②,③ cannot be perfectly separated）
- It is important to understand the relationship between structure and function.

① The interaction between the structure of the whole individual and organ formation

Formation of the segment structure
and information concerning location in the embryo

Segment：a structure common to many animals

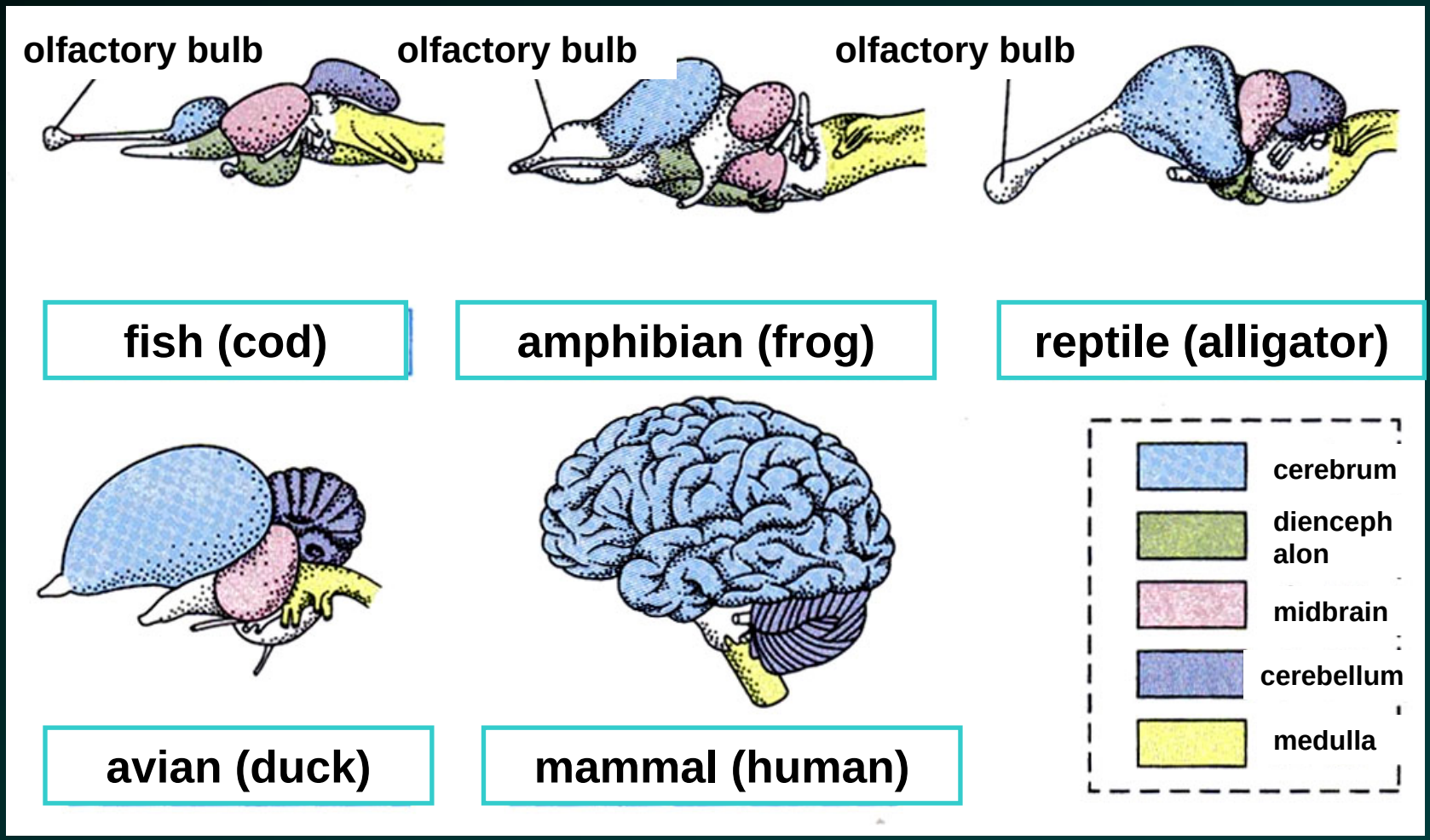
An animal body has a segment structure responsible for keeping positional information

example；

- Determination of cervical spine・spinal chord・lumbar spine・regions of brain・position of legs and arms of vertebrates
- Determination of head・trunk・tail, position of limbs and wings of invertebrates

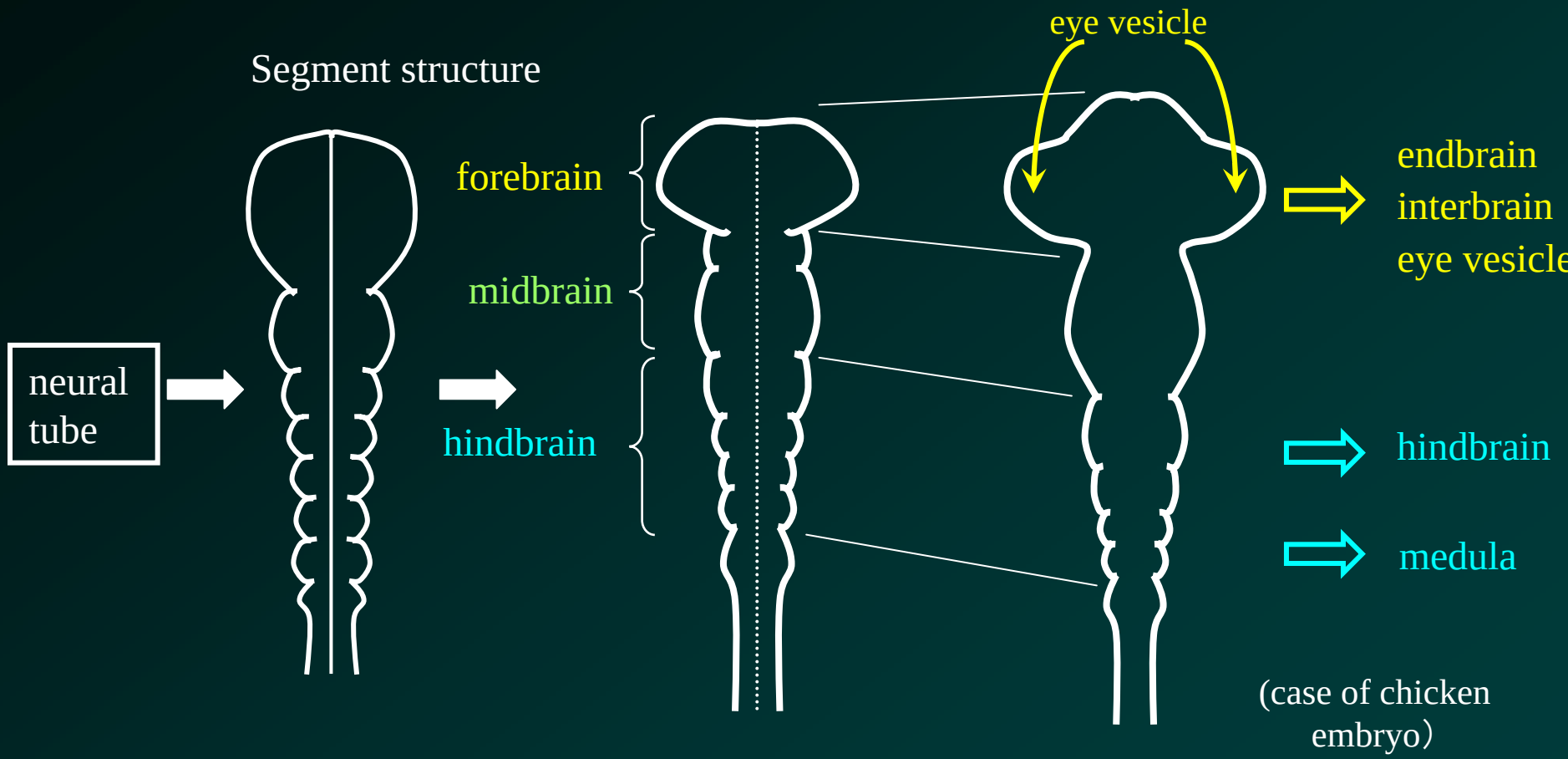
“Brain segments” & “somites” are especially important.

The structure of a vertebrate's brain



Commonly seen functional segment structures

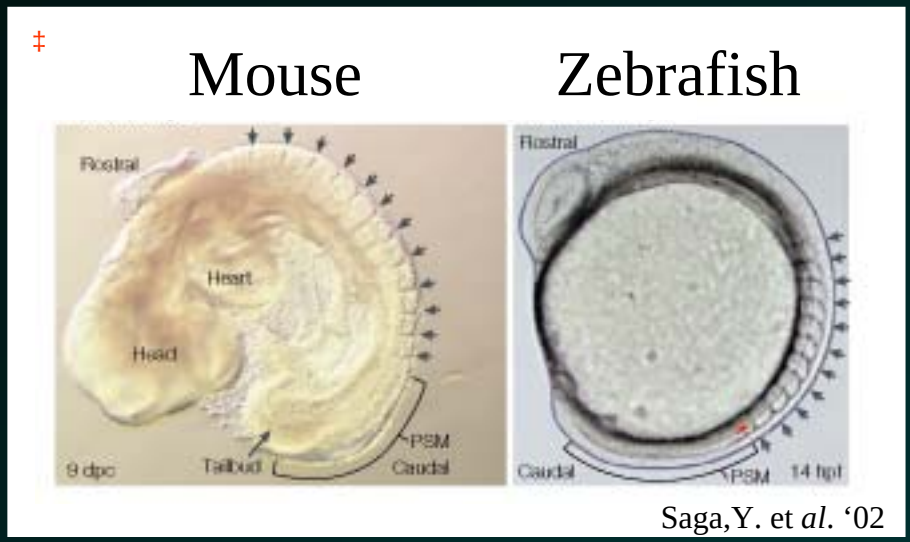
Segment structure of the brain



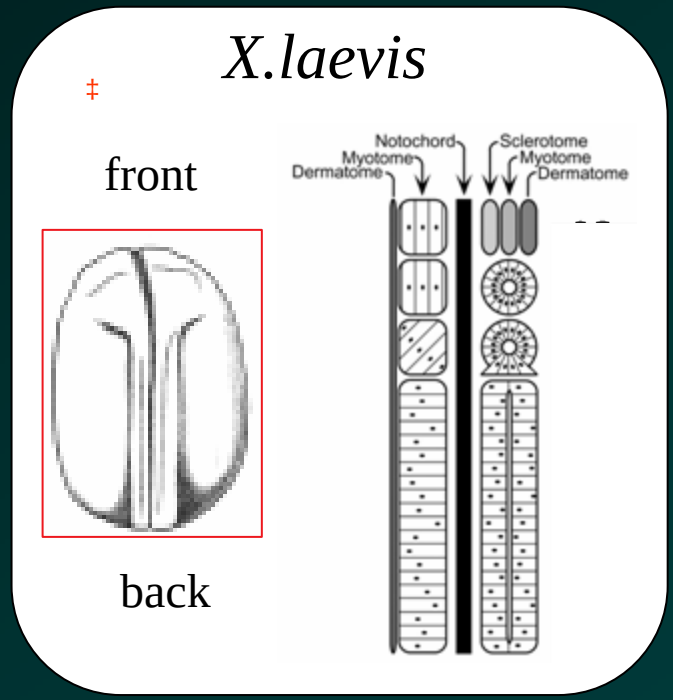
As a neural tube develops, constriction occurs, and the brain is segmented.

Segments are formed as a result of complications in the “cavities”

Somites



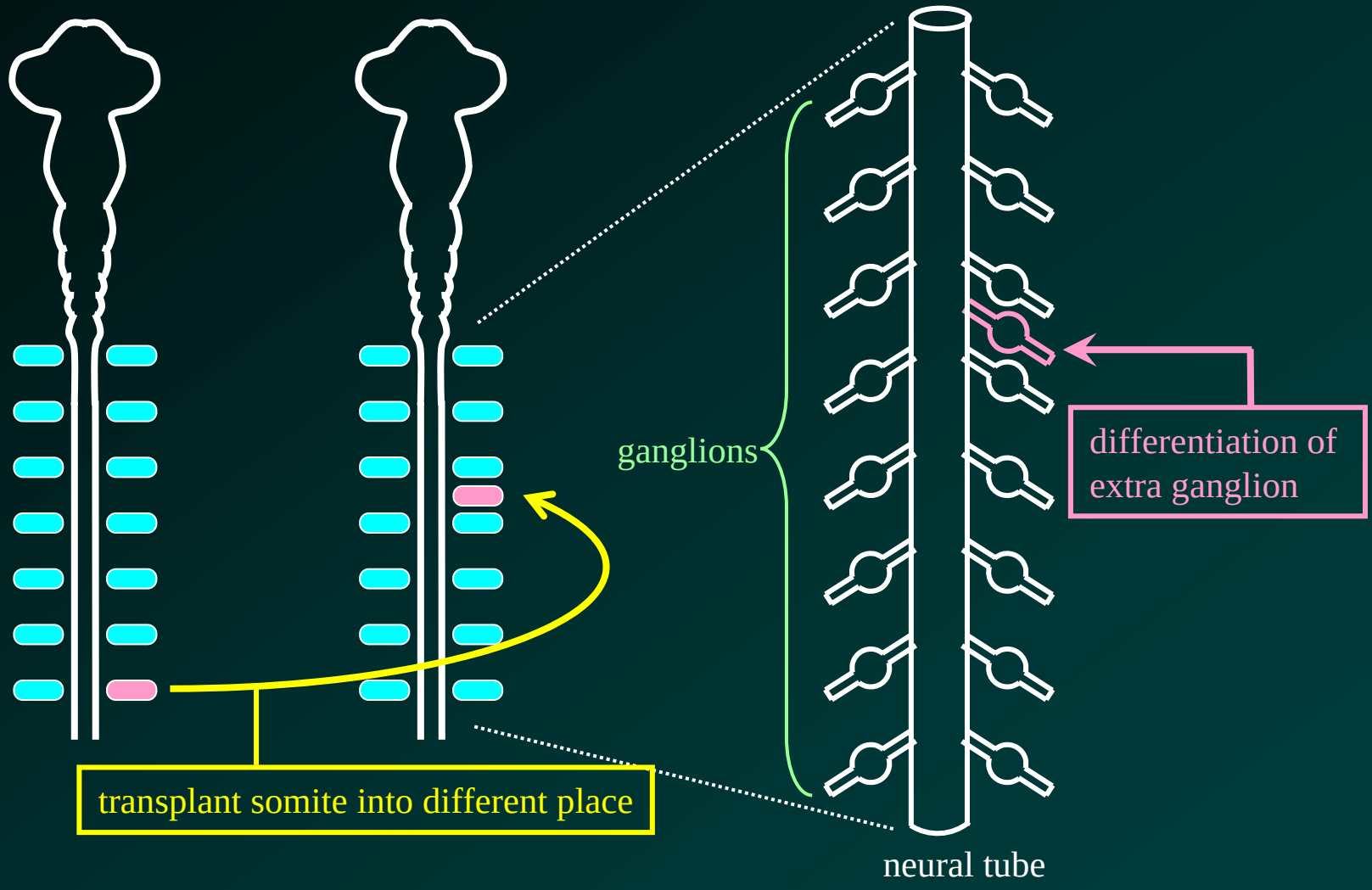
Saga,Y. et *al*. '02



(left 2) Saga Y. et al., Nat Rev Genet, vol 2, p836-Fig.1, 2001 (right) Jen WC. et al., Development, vol 124, p1171-Fig1, 1997

- Repeated structures formed in an early stage of development
→ basis of segmented structures such as the spinal chord, muscles, and the nervous system
- Somites of vertebrates are formed cyclically one by one along with the growth of the embryo.

Somites contain location information



When somites are transplanted into different parts,
extra ganglions differentiate from the neighboring neural tube.

Slight disarray in which location information is functionall covered by a flexible change in neuron projection.

"The illustration of neuron projection" inserted here was omitted according to copyright issue.

When segments are partially reversed, neuron projection works to project them to an alternate location.

Segments of insects and mammals are regulated by the Hox gene family

"The illustration of
Hox gene family regulation"
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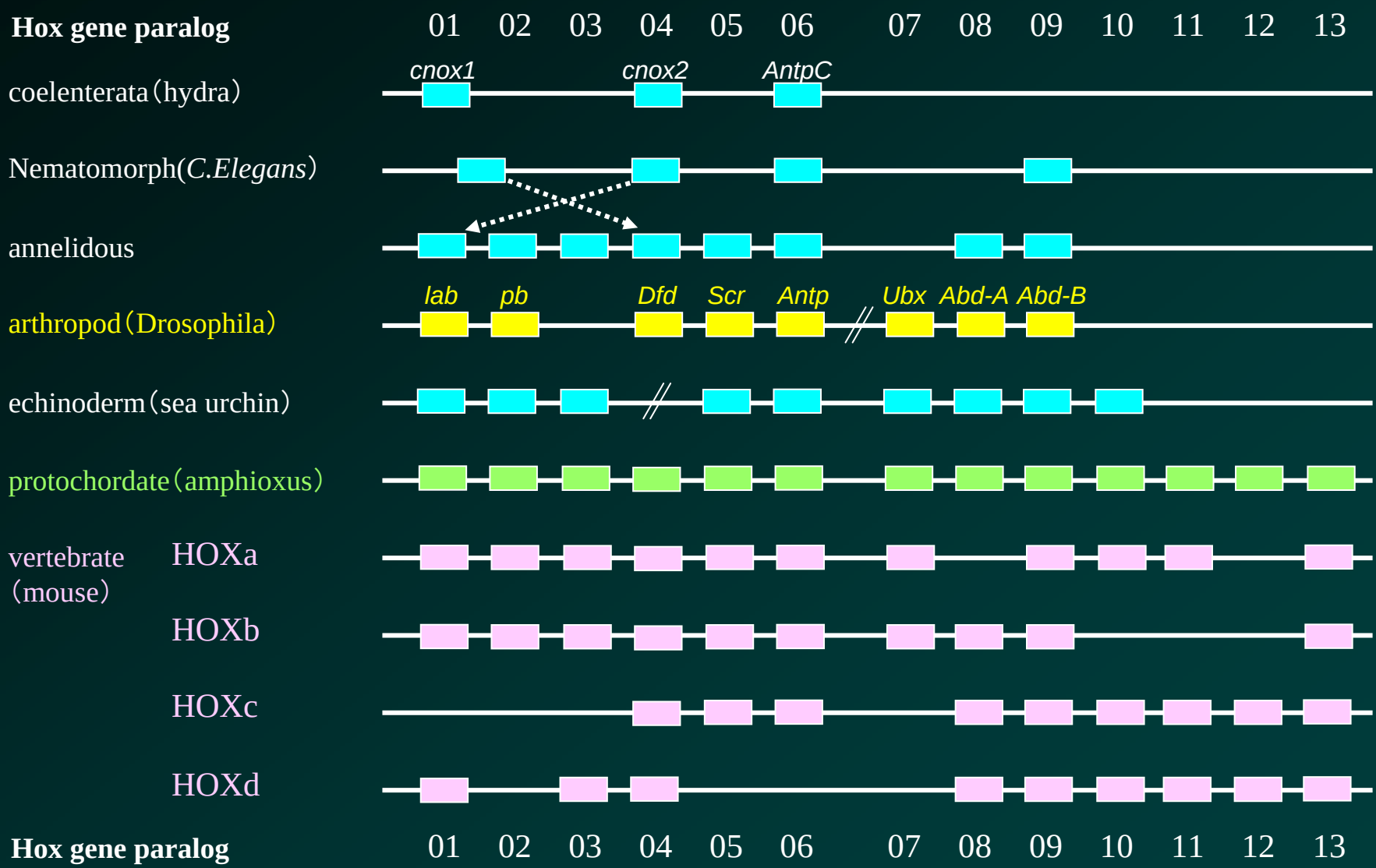
Hox gene groups that determine segment structures are common to many animals



Disarray in segment formation changes the positions of the adnex

T2 T3

"The illustration for expression pattern of homeotic gene" inserted here was omitted according to copyright issue.

Mutant of drosophila whose T3 structure changed to T2 and whose wings increased to 4

"The photo of mutant drosophila" inserted here was omitted according to copyright issue.

These mutations are called "homeotic mutations".

The effect of retinoic acid treatment on embryo developmen

Normal mouse embryo

"The picture of
Retinoic acid treated mouse embryo"
inserted here was omitted
according to copyright issue.

Front structure（jawbone）is lost in retionic acid treatment

Retinoic acid changes location information.

The effect of retinoic acid treatment on embryo development②

"The picture of
Retinoic acid treated tadpole"
inserted here was omitted
according to copyright issue.

Double limbs are formed when the tadpole's tail is cut and treated with retinoic acid.

Retinoic acid changes location information.

Regions where limbs differentiate are determined by somites

"The illustration for limb forming regions" inserted here was omitted according to copyright issue.

The pictures inserted here was omitted according to copyright issue.

FGF10 expresses in the embryo.

Extra limb is formed if FGF10 expresses at an abnormal location

formation of fore limb

FGF10, FGF8, Wnt2b/8c, Wnt3a determine where the limbs are forr

formation of hind limb

Location information determining types of fore and back limbs

“The explanation for

location information determining types of limbs”

inserted here was omitted

according to copyright issue.

Fore limbs are located by Tbx5, hind limbs are located by Tbx4

Limbs are induced at the middle position by abnormal expression of a chimera structure when fore and hind limbs are formed.

Location information determining types of fore and back limbs

Expression of Tbx5 at fore limb

Tbx5 differentiates at fore part
Tbx4 differentiates at hind part
of middle limb

Expression of Tbx4 at hind limb

The pictures inserted here was omitted according to copyright issue.

Limb induced between fore limb and hind limb by FGF

Front part differentiated into wings
Hind part differentiated into limbs

Limbs developed by interactions between AER and mesenchyme

The illustration for limbs development inserted here was omitted according to copyright issue.

Removal of AER
→ tip is not formed

Transplant of extra AER
→ 2 tips are formed

Exchange hind limb mesenchyme and fore limb mesenchyme
→ hind limb is formed
（types of limb are dependent on mesenchyme）

Removal of mesenchyme
→ tip is not formed

Replacement of AER by FGF bead
→ normal wing

AER：apical ectodermal ridge

Location information which determines types of fingers

The illustration for fingers determining location information

inserted here was omitted

according to copyright issue.

Finger types scoot down when inter-digital tissue is removed.

The same result occurs when BMP is inhibited by beads soaked with Noggin

Many abnormal fingers are formed when location information of the inter-digital tissue is disturbed. ↑

Finger types are determined by the concentration of BMP in inter-digital tissue.

Abnormal development of limbs by Hox gene mutation

‡

Fore limbs of a mouse

Hoxa-11, Hoxd-11 Inhibited mouse

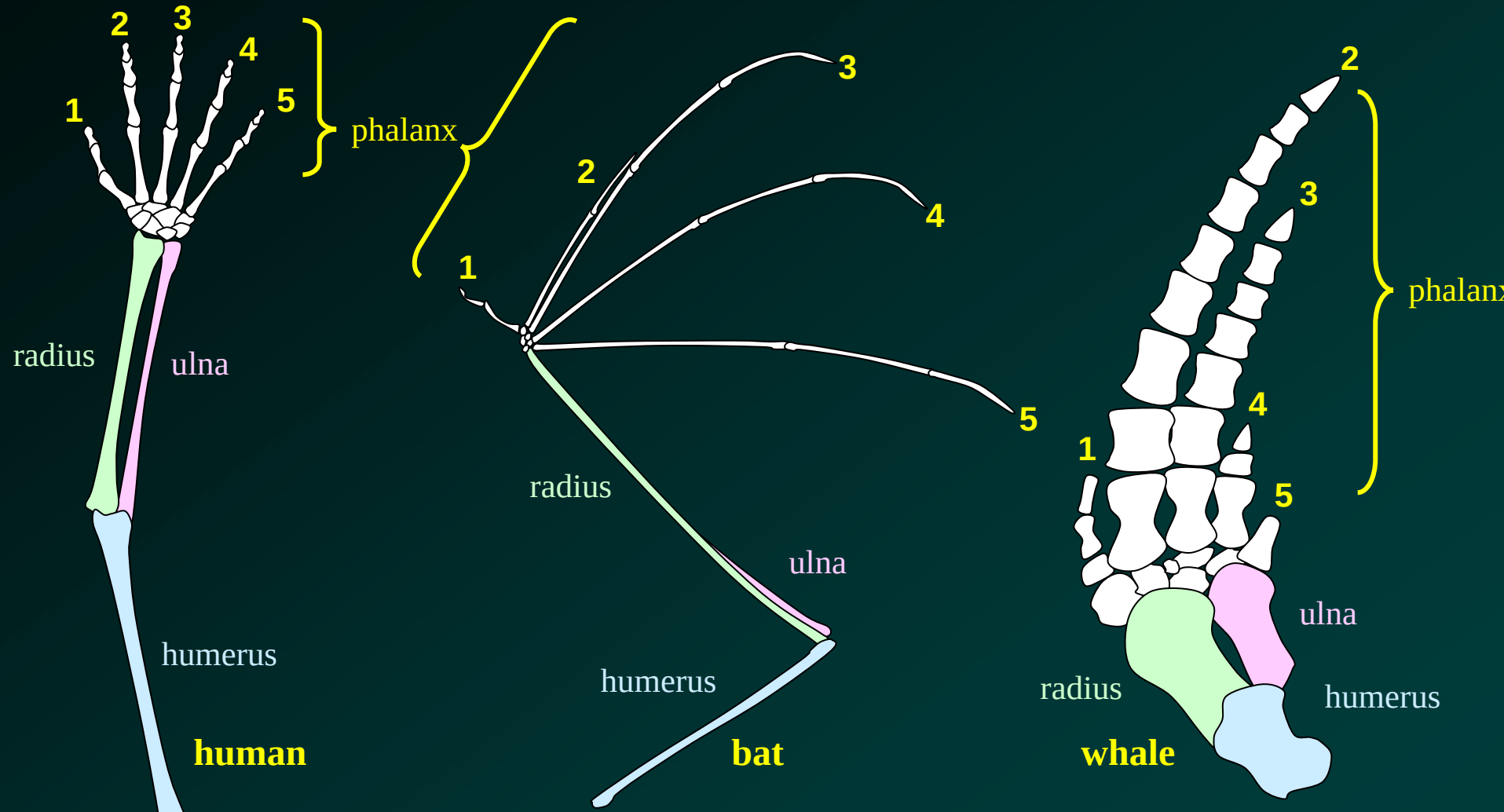
The illustration for abnormal development of limbs inserted here was omitted according to copyright issue.

Abnormal fingers by HOXD-13 mutation

↑ hypothesis on Hox gene correspondence concerning the regional specificity of fore limbs

The Hox gene family regulates location information of the body.

Common structures in arms and legs



Form of vertebrates' limbs are different, but there are corresponding common structures.

These organisms form diverse structures typical to each species using same gene groups or common signal transduction system during development.

② The interaction between tissues during organ development

example: the interaction between epithelia and mesenchyme

“Induction” between tissues

example:

- induction between ectoderms・endoderms → induce mesoderm
- induction between ectoderms（epithelia）・mesoderms（notochord） → induce nervous syste

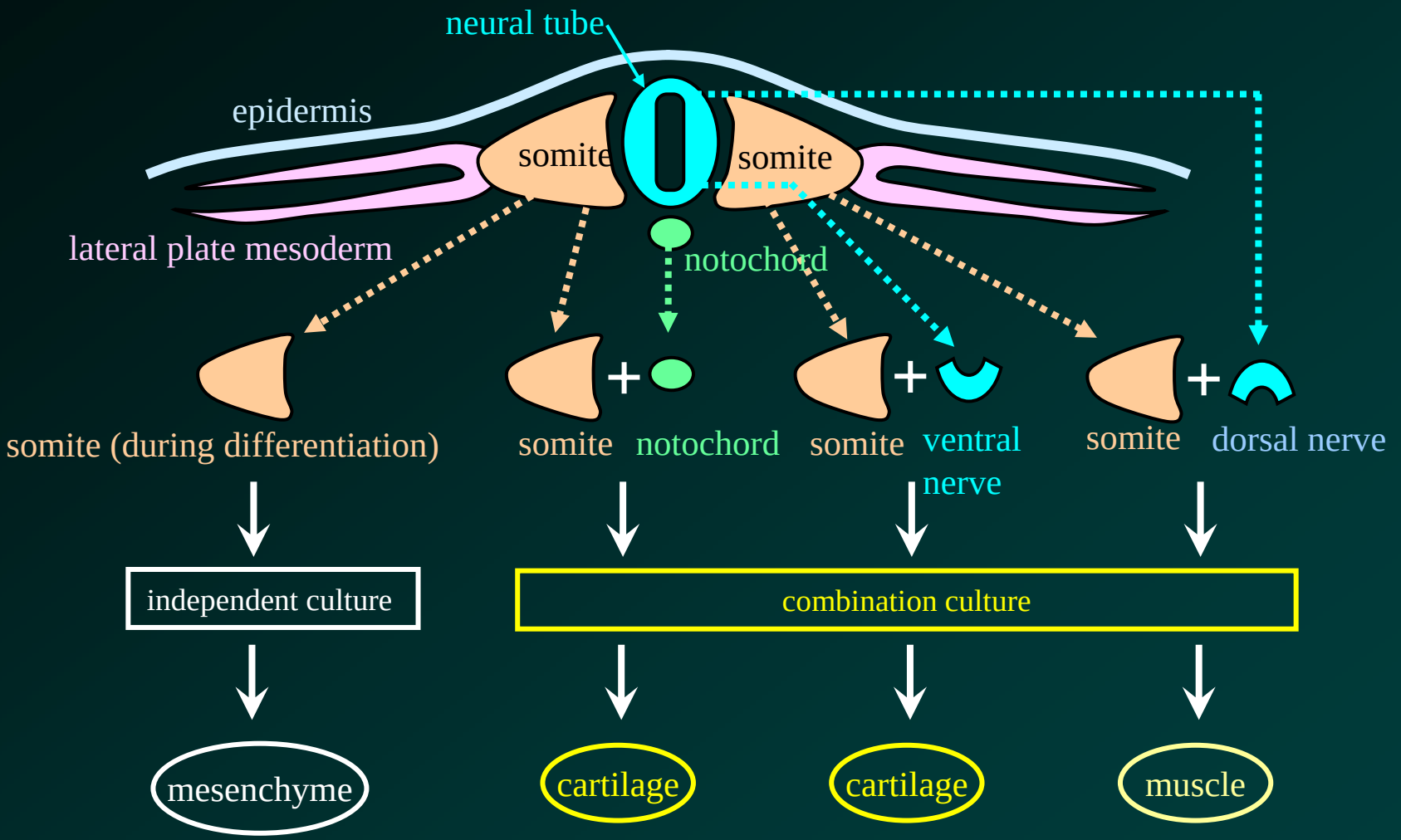
↓

“Induction” is the effect from the tendency of neighboring tissues to differentiate.

↓

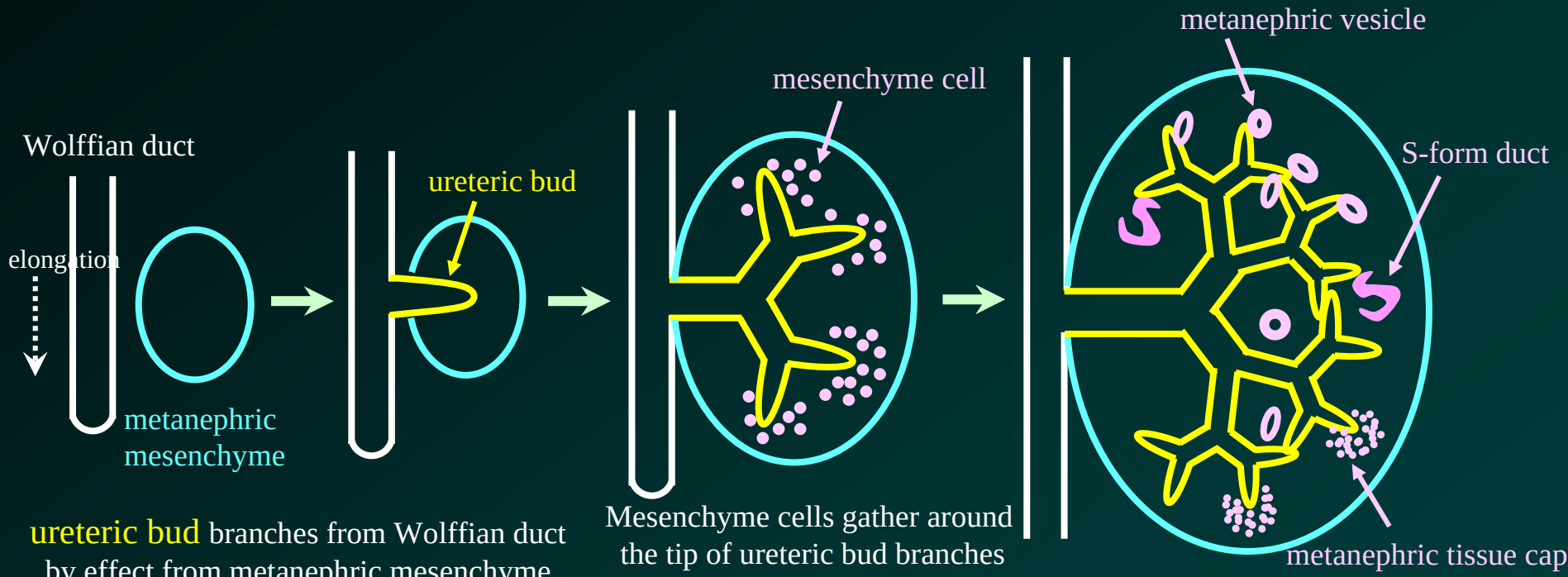
Interactions between epithelia (endoderm) and mesenchyme (mesoderm) play an important role in forming the structures of organs

How somite differentiates is determined by induction from neighboring tissues.



When somite tissues are cultured with other tissues, the directions of their differentiation change by the combination of tissues

A kidney induced by interactions between the epithelia and mesenchyme



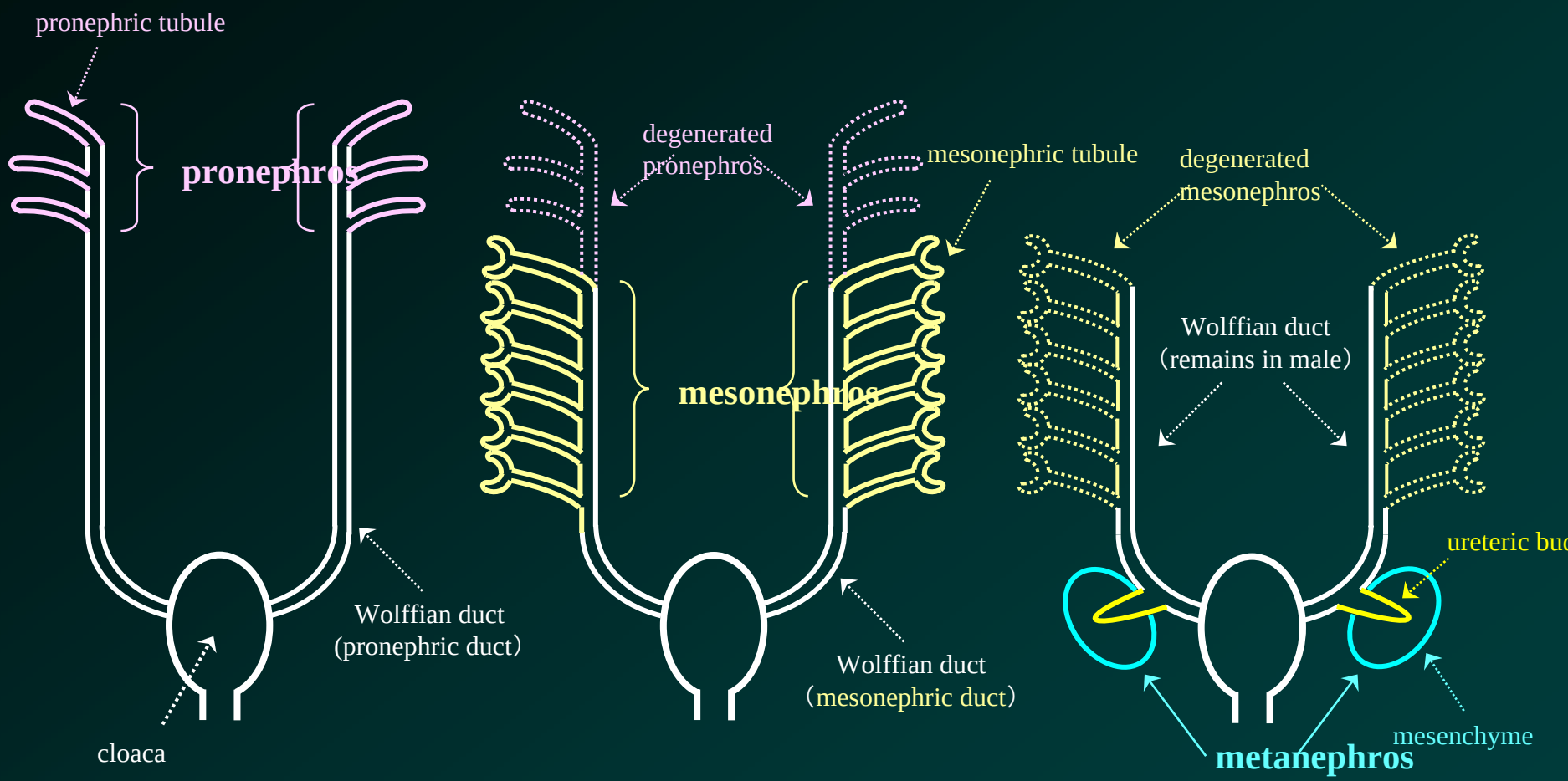
ureteric bud branches from Wolffian duct by effect from metanephric mesenchyme

Mesenchyme cells gather around the tip of ureteric bud branches

Kidney tubule, glomerulus, etc. differentiate from mesenchyme by inducing signal from the tip of ureteric bud.

Mammals' (including humans') kidneys are metanephros.
They are formed by interactions between the ureter bud projected from the Wolffian duct and metanephric mesenchyme.

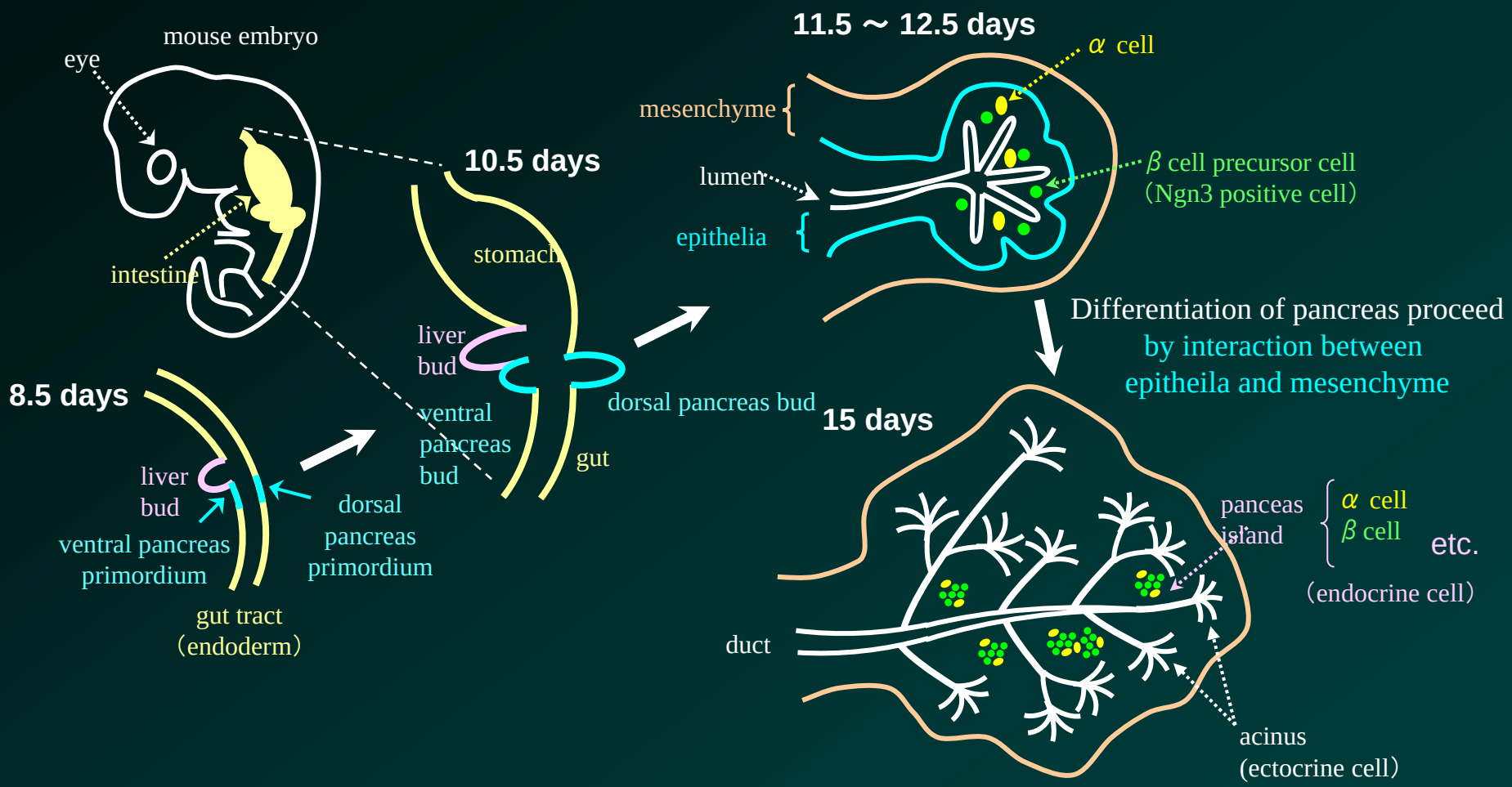
The structure of a vertebrate's kidney



The structures of mammals' metanephros and amphibians' pronephros differ.

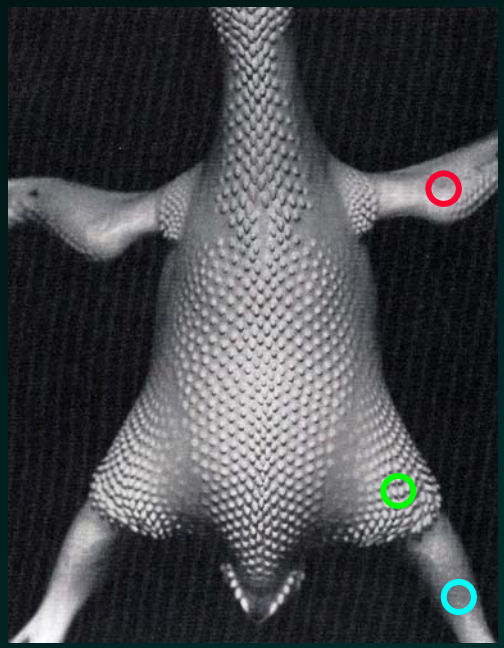
The differences lie in interaction with metanephric mesenchyme

A pancreas is induced by interaction between the epithelia and mesenchyme.



The structure of pancreas glands is formed by interactions between intestinal epithelia and neighboring mesenchyme.

Interaction between the epithelia and mesenchyme in bird's wings



mesenchyme

Epithelia of wings

induced structures

Corium of wings

Corium of thigh

Corium of limbs

eather in wings

eather in thigh

Ssquama and nail of limbs

When the epithelia of wings is coordinated with the corium mesenchyme of different parts, a structure corresponding to corium is induced in epithelia.

③ The function of genes in organ development

- A method of research on organ formation
- The mechanism of organ formation

To analyze the regulating mechanism of organ formation and body development

Functional analysis of genes in nuclear genome and coded proteins

↓

Methods of function analysis for genes and proteins

1. Analyze expression pattern

（test to see if the genes are functioning）

2. Overexpress genes and check the effects

（inject mRNA into the cell to observe genetic transformation by a virus vector）

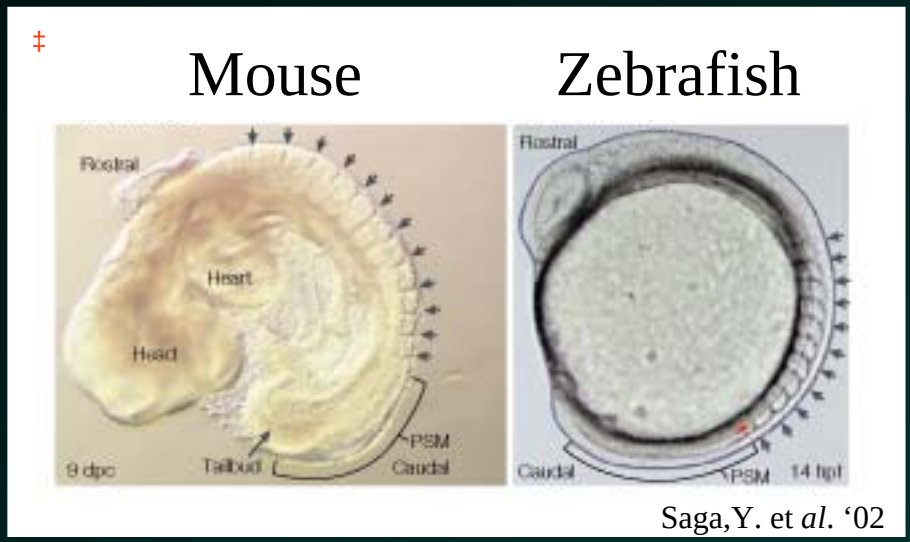
3. Inhibit expression of genes and check the effects

（knockout，knockdown by RNAi，dominant negative, etc.）

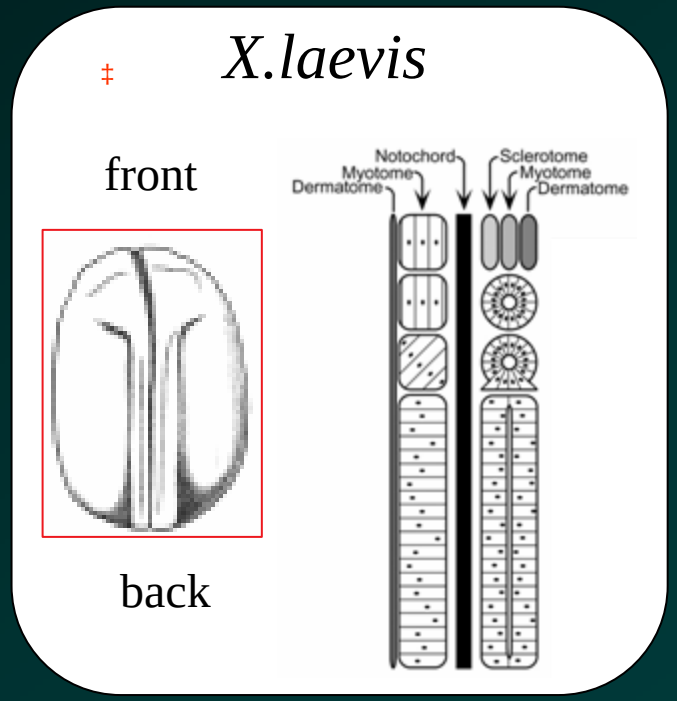
The molecular mechanism of organ formation①

On the molecular mechanism of somite formation and a method for its research

Somites



Saga,Y. et *al*. '02



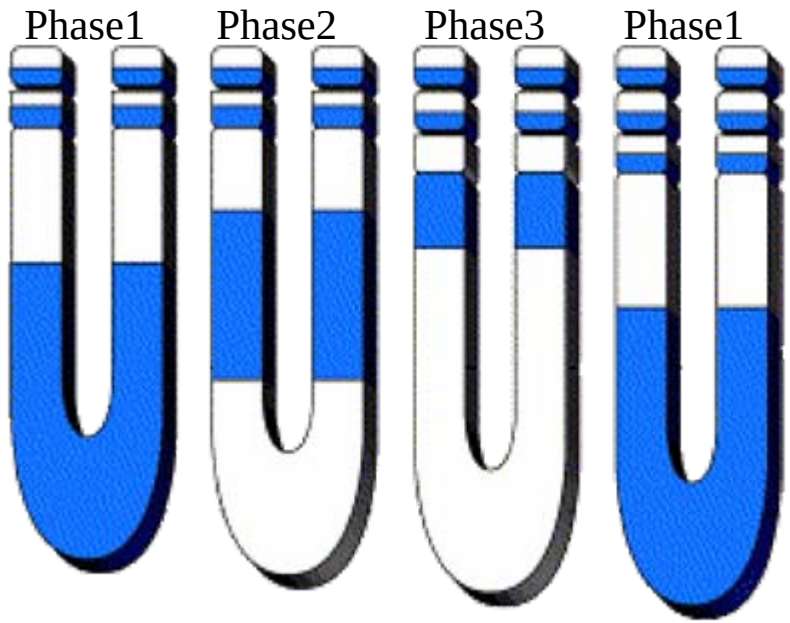
(left 2) Saga Y. et al., Nat Rev Genet, vol 2, p836-Fig.1, 2001 (right) Jen WC. et al., Development, vol 124, p1171-Fig1, 1997

- A repeated structure formed in an early stage of development

→ basis of segmented structures such as the spinal chord, muscles, and the nervous system

- The somites of vertebrates are formed cyclically one by one along with the growth of the embryo.

Thee molecular mechanism of somite formation



Clock

Gene groups whose expression change cyclically

Notch signal

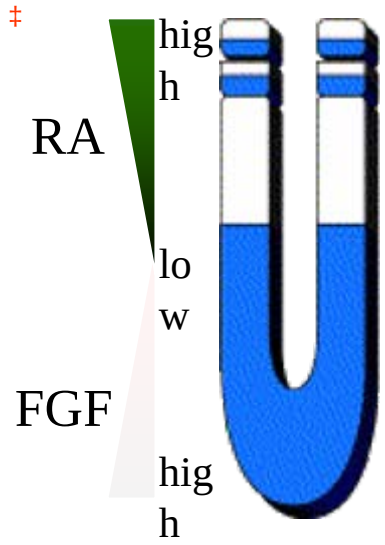
Wnt signal

Current Opinion in Genetics & Development

Bessho Y. et al., Curr Opin Genet Dev., vol 13, p380-Fig.1, 2003

- Expressions of some gene groups change cyclically during embryo growth.
- The forefront of these gene groups appear at the location at which the somite separates.

The molecular mechanism of somite formation



Wavefront

Gene groups that make the gradient at back and front of embryo

FGF signal

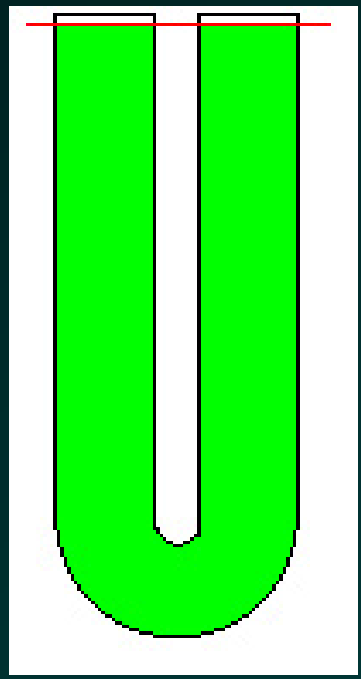
RA signal

Bessho Y. et al., Curr Opin Genet Dev., vol 13, p380-Fig.1, 2003

- There are gene groups that make the gradient at the back and front of the embryo.
- The boundaries of these gene groups are related to the locations of the somites.

The molecular mechanism of somite formation

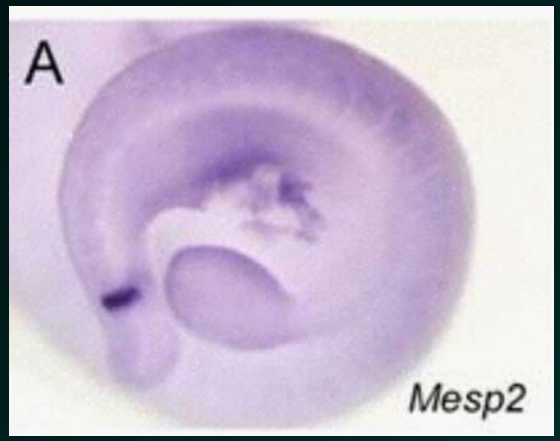
Clock and wavefront model



The clock (green) changes cyclically, and the wavefront (red line) is located on boundary of the gradients. Where the clock and the wavefront touch each other, the locations of the somite can be determined.

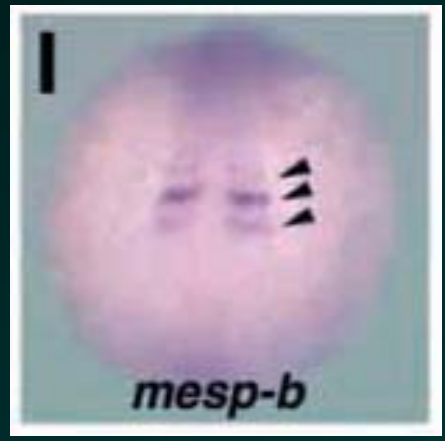
The location of somites by Mesp family

- Mouse



Nakajima et al, 2006

- Zebrafish



Sawada et al, 2000

- *X.laevis*

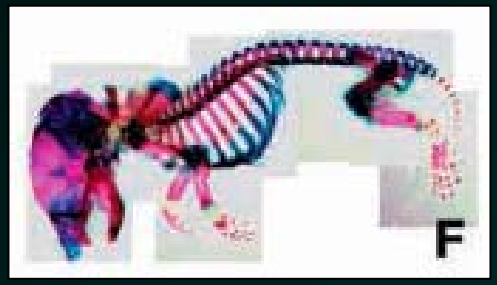
Sparrow et al, 1998

• Gene groups responsible for somite formation (Mesp family) are isolated.

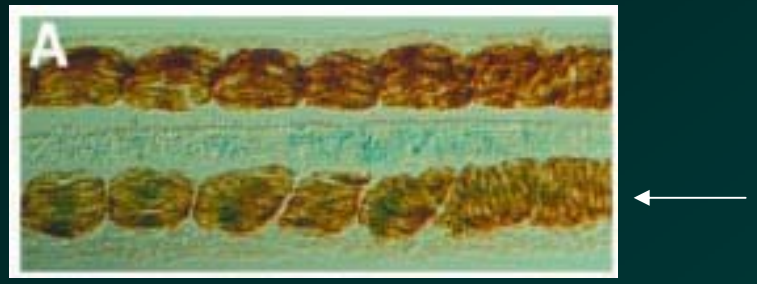
• Genes in the Mesp family are expressed in stripes at the locations of the somites.

The location of somites by the Mesp family

Mouse normal embryo



X.Laevis normal embryo



Mesp2-knockout mouse

Nomura-kitabayashi et al, 2002

Embryo with overexpression of *Thylacine-1*

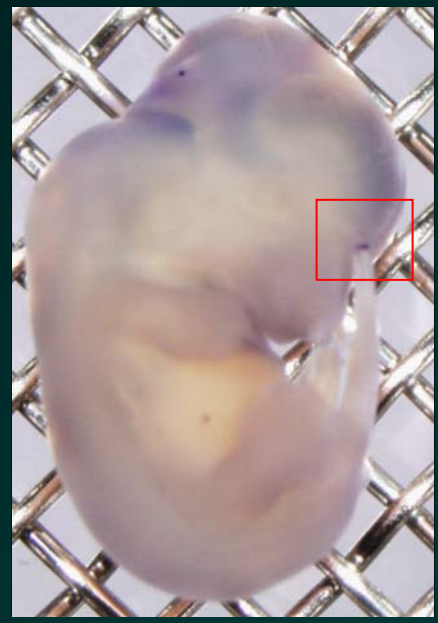
Sparrow et al, 1998

Gain-of-function and loss-of-function of genes in the Mesp family caused the abnormal formation of somites.
→Genes of the Mesp family seem to determine locations where the somites are formed.

Isolation of a new gene family

Xenopus bowline

Xenopus Ledgerline



Mouse ripply1

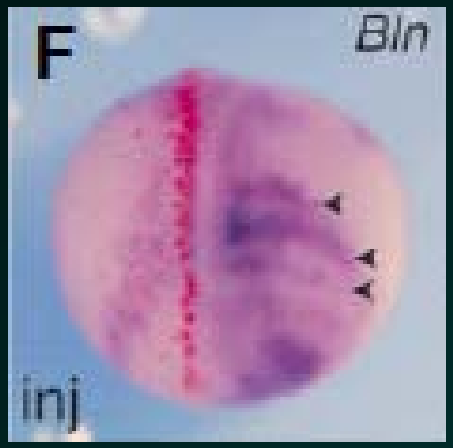
• Recently, new gene families（*bowline* and *Ledgerline* from *the X.laevis*, and the *ripply1* gene from the Mouse and Zebrafish）were isolated.

• These genes were expressed at the locations of somite formation as in the case of the Mesp family.

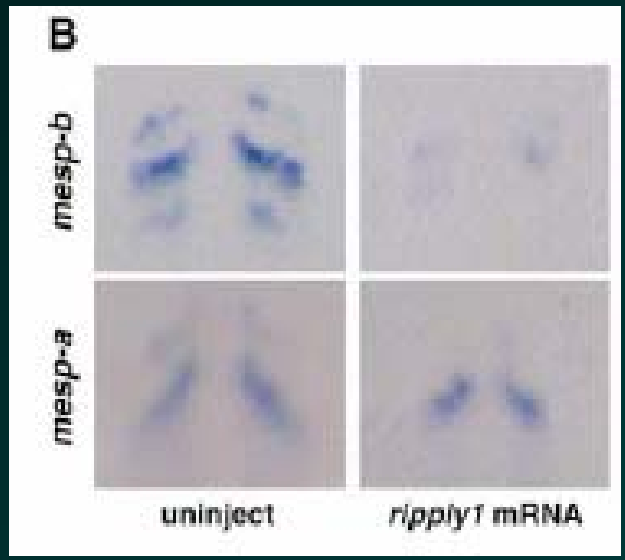
Isolation of a new gene family

Embryo with overexpression of *Zebrafish ripply1*

Embryo with overexpression of *bowline*



Kondow et al, 2006



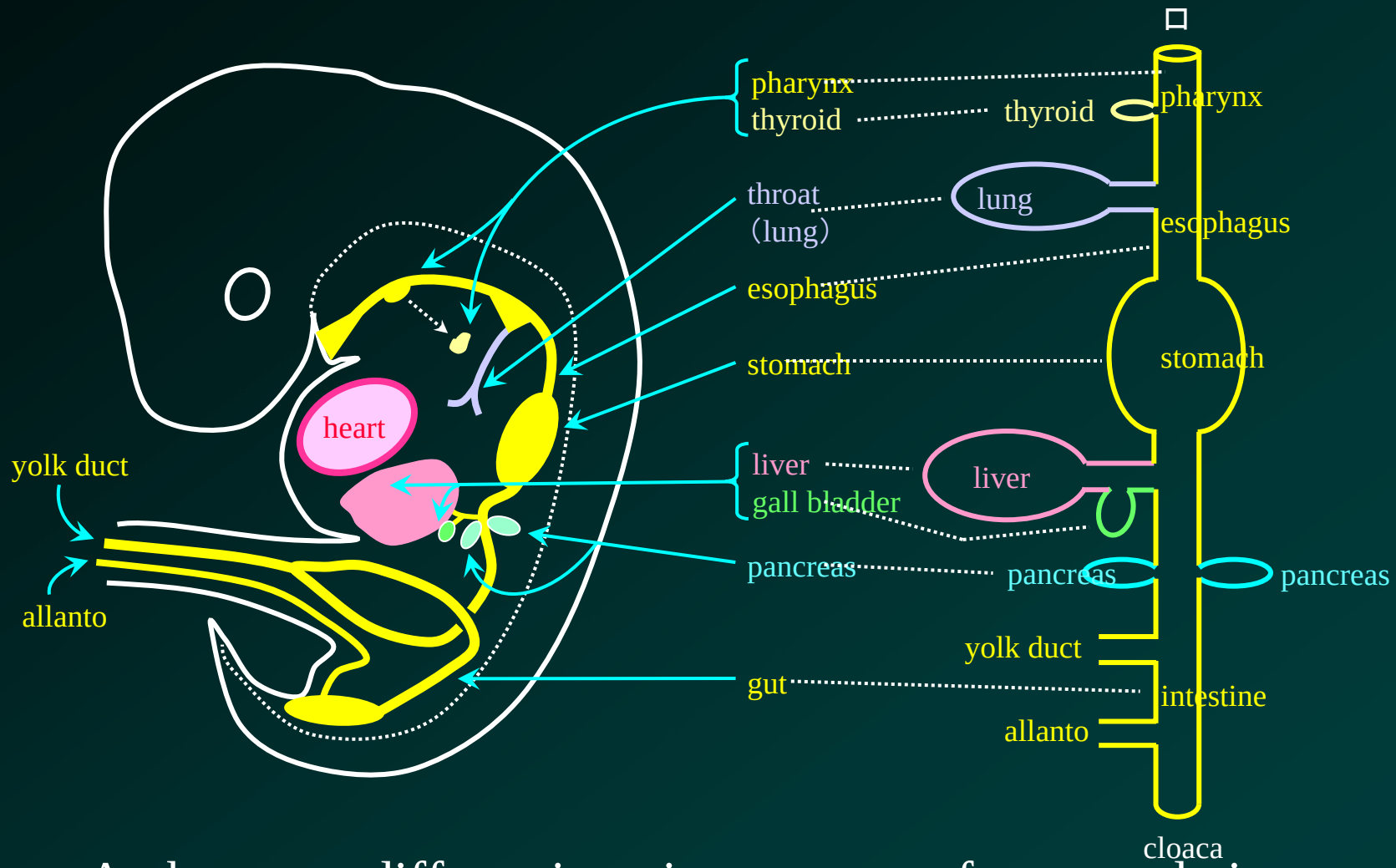
Kawamura et al, 2005

• The effects of *bowline* and *ripply1* on the expression of a Notch signal and the Mesp family genes were discovered.
→Research on functions of these newly-found gene groups would uncover mechanism of metamere formation.

The molecular mechanism of organ formation②

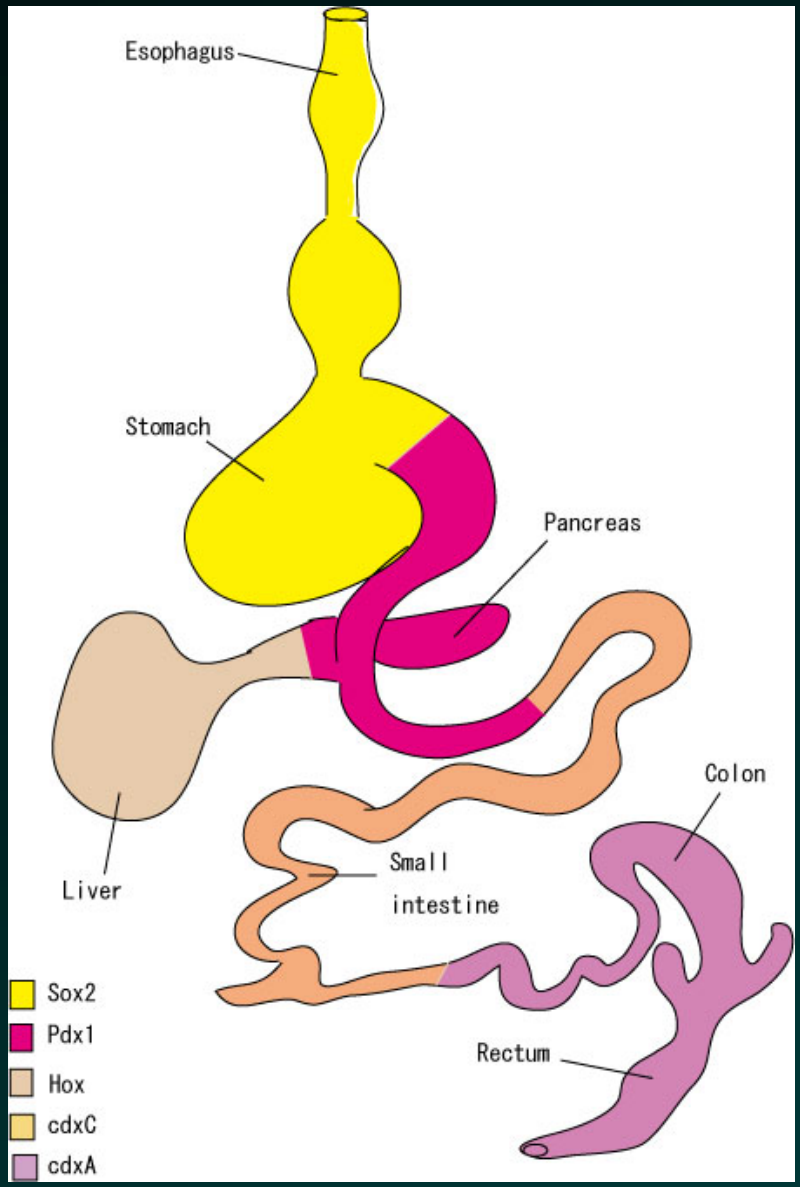
The molecular mechanism of enteron formation
and associated research

The mechanism of enteron / pancreas / liver differentiation



- Archenteron differentiates into enteron after gastrulation.
- Structures with specific functions differentiate at each region by induction to epithelia of mesenchyme.

The molecular mechanism of enteron / pancreas / liver differentiation



Genes expressed specifically at each region

- esophagus, stomach: Sox2
- duodenum, pancreas: Pdx1
- liver: Hox
- small intestine: cdxC
- colon, rectum: cdxA

↓

Specific genes work for differentiation of each region (enteron, liver, pancreas)

Stomach of a 12th day embryo
cSP/ECPg

Differentiation of enteron in a chicken embryo

‡

Cross section

epithelia

cavity

mesenchyme

6th day

12th day

1.5th day

3rd day

esophagus

stomach

ventriculus

small intestine

colon

future stomach endoderm

future intestine endoderm

epithelial development of each region and

substances produced from epithelia

become flat and multi-layered

gland formation

pepsinogen

(ECPg)

(cSP, Sox2)

keratinization

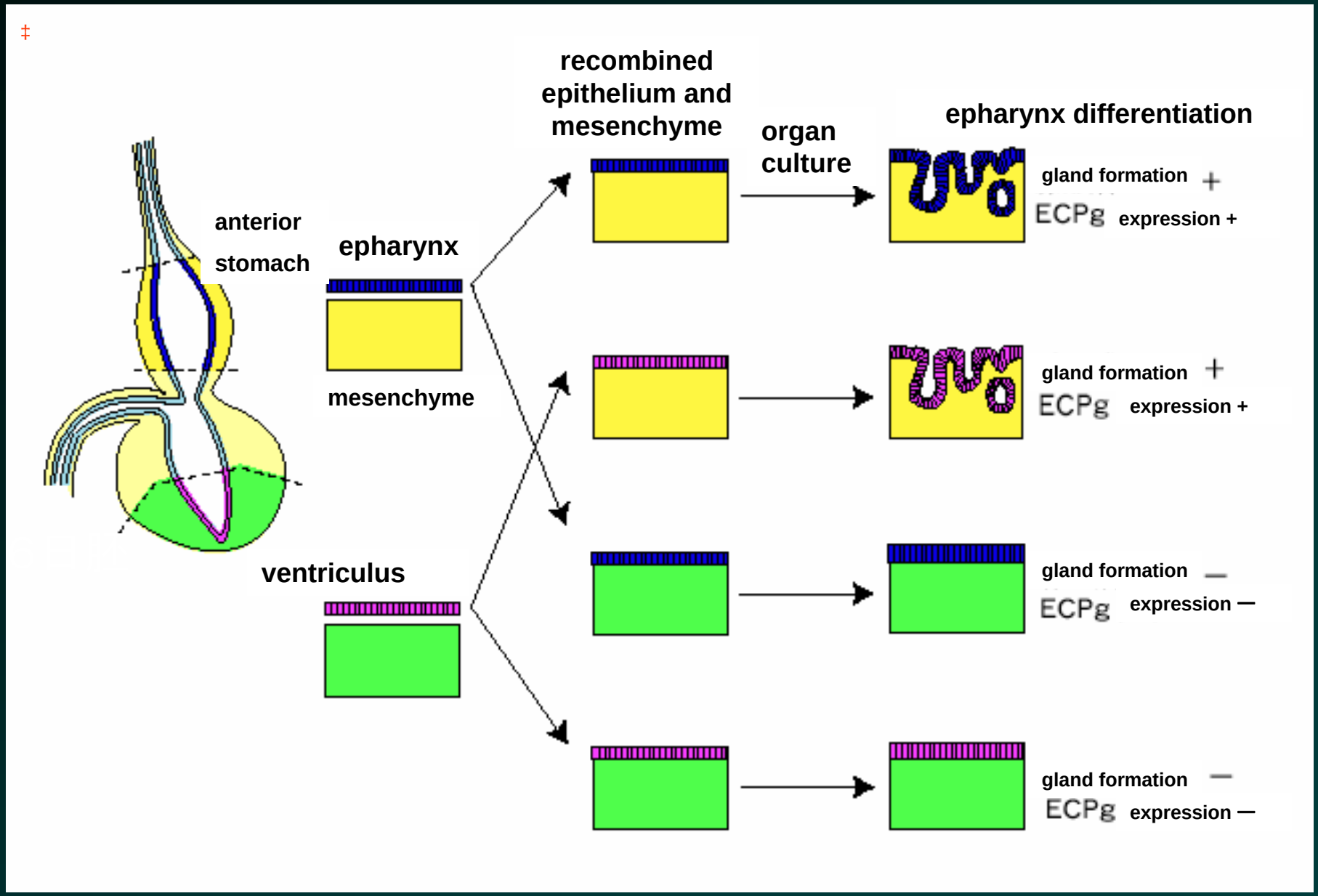
mucus

villus formation

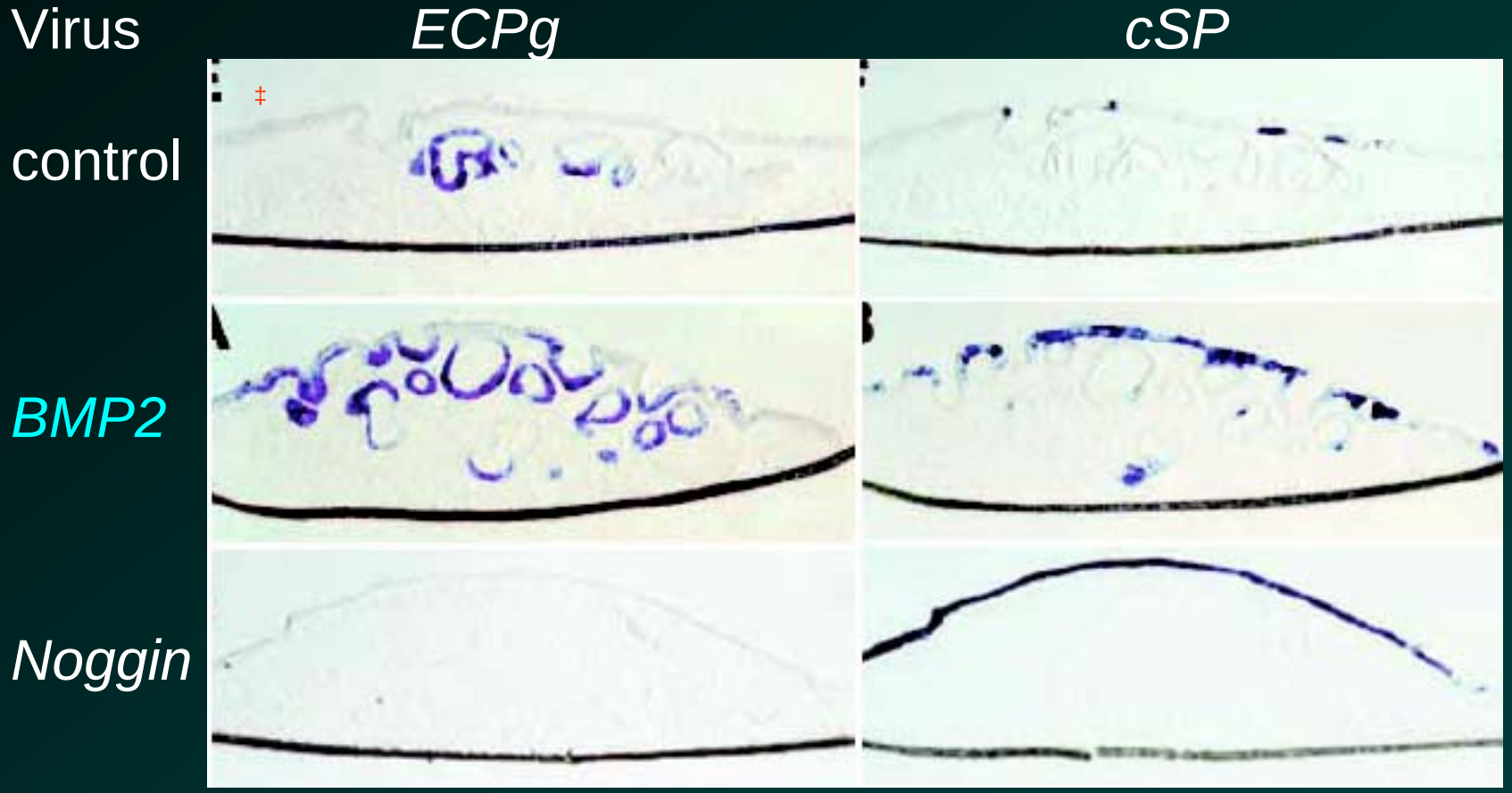
sucrase

IFABP, CdxA

Prospective fate of epithelium is “induced” by mesenchyme.



Effect of overexpression of BMP2 and Noggin on stomach differentiation

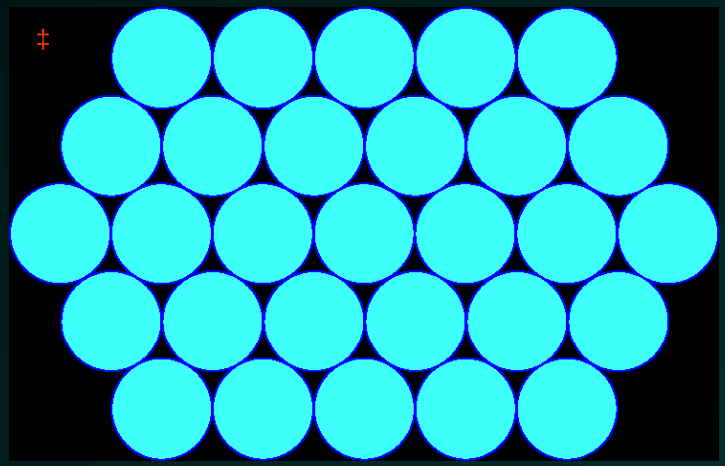


- *The BMP2* gene expresses specifically in the anterior stomach before and after gland formation.
- Overexpression of *BMP2* in mesenchyme promotes formation of gland and expression of pepsinogen.

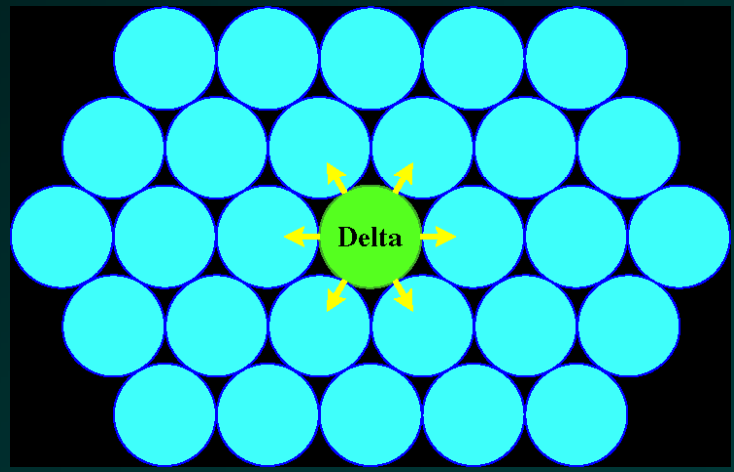
→ BMP2 regulates secretory gland structure of anterior stomach

Characteristics of a Notch signal

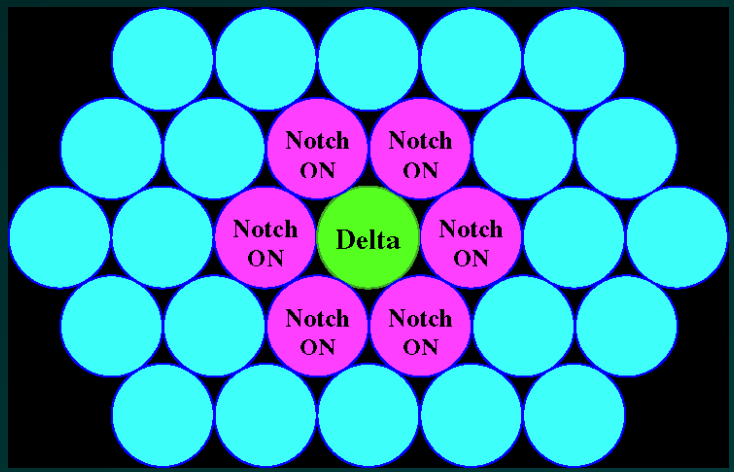
Even cell mass



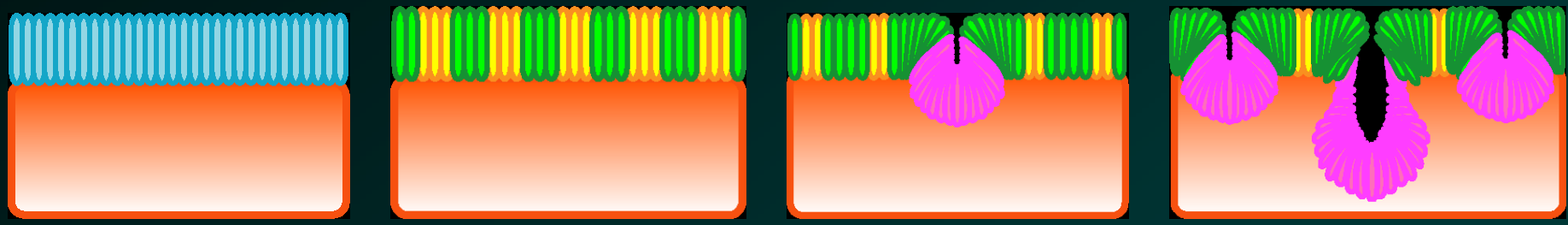
Expression of ligand



Signal transduction to surrounding cells



Control model of anterior stomach epithelial cell differentiation by a Notch signal



① st.28　② st.29-　③ st.29　④ st.29~

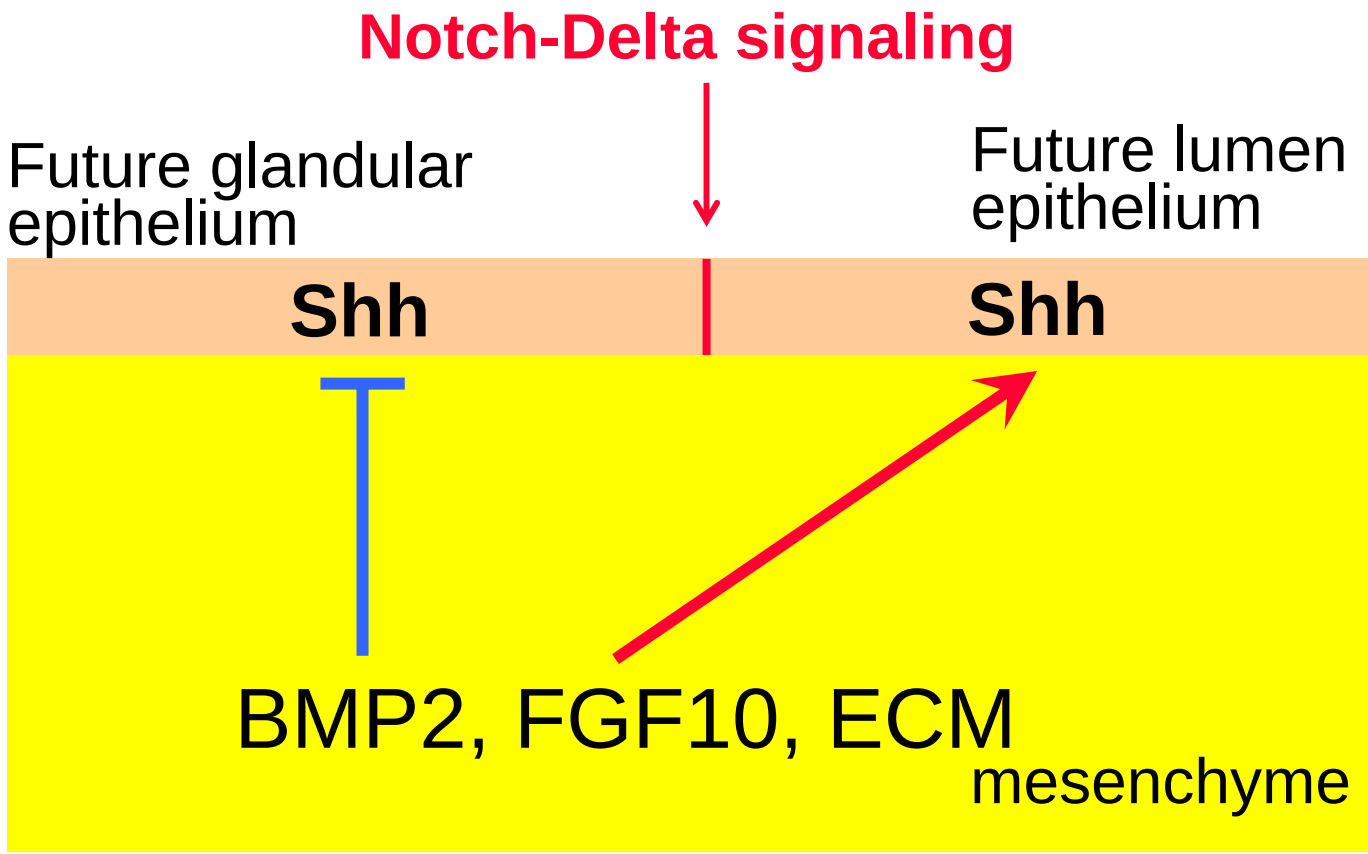
① Anterior stomach epithelia consists of an even cell mass of undifferentiated epithelial cells.

② As differentiation of lumen epithelial cells begin, a Notch1 signal is activated with Delta1, and glandular epithelial precursor cells are preserved.

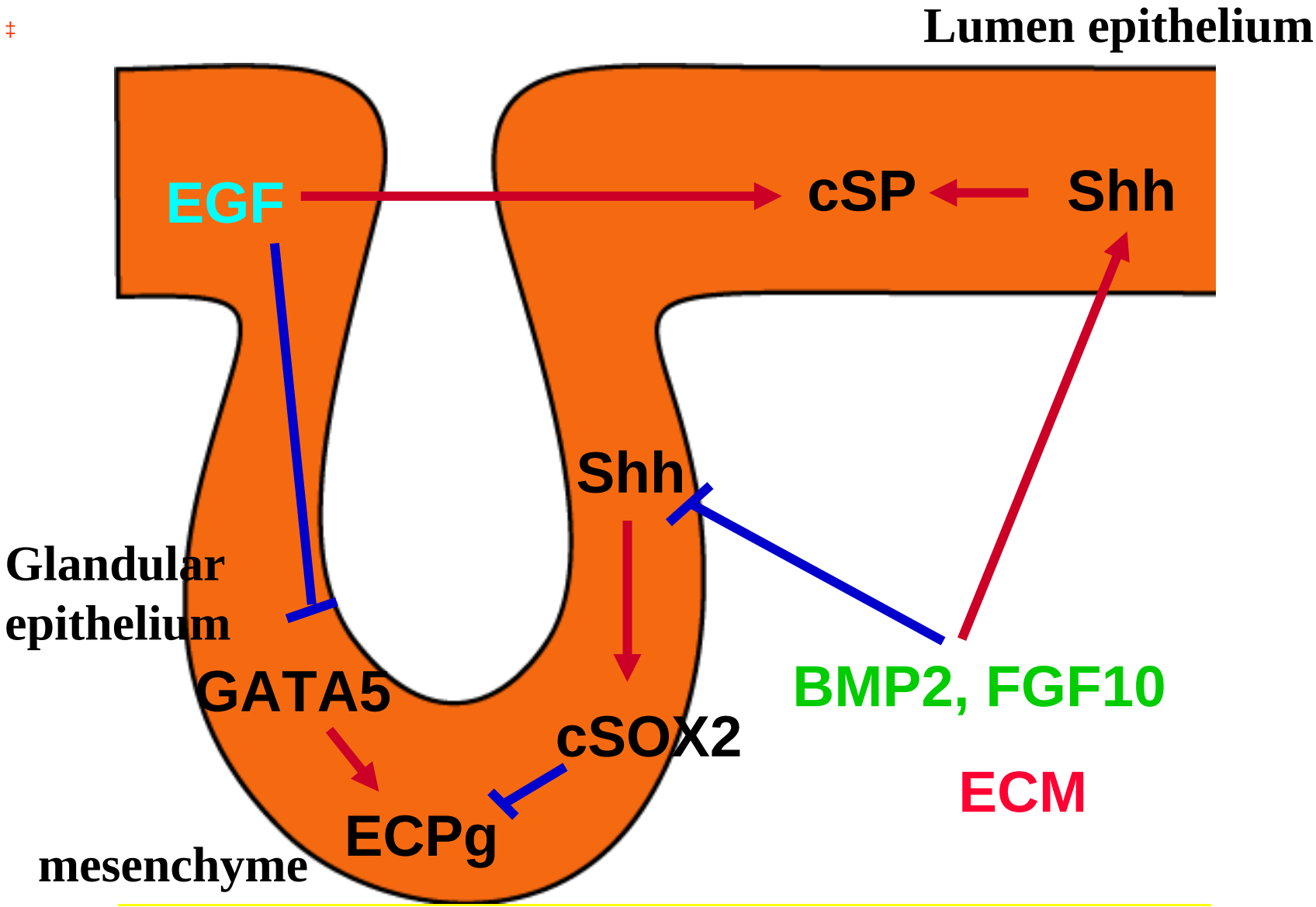
③ Glandular epithelial precursor cells differentiate into glandular epithelial cells when the Notch1 signals disappear, and glands are formed.

④ The Notch1 signal fades continuously, and new glands are formed as the anterior stomach gets bigger.

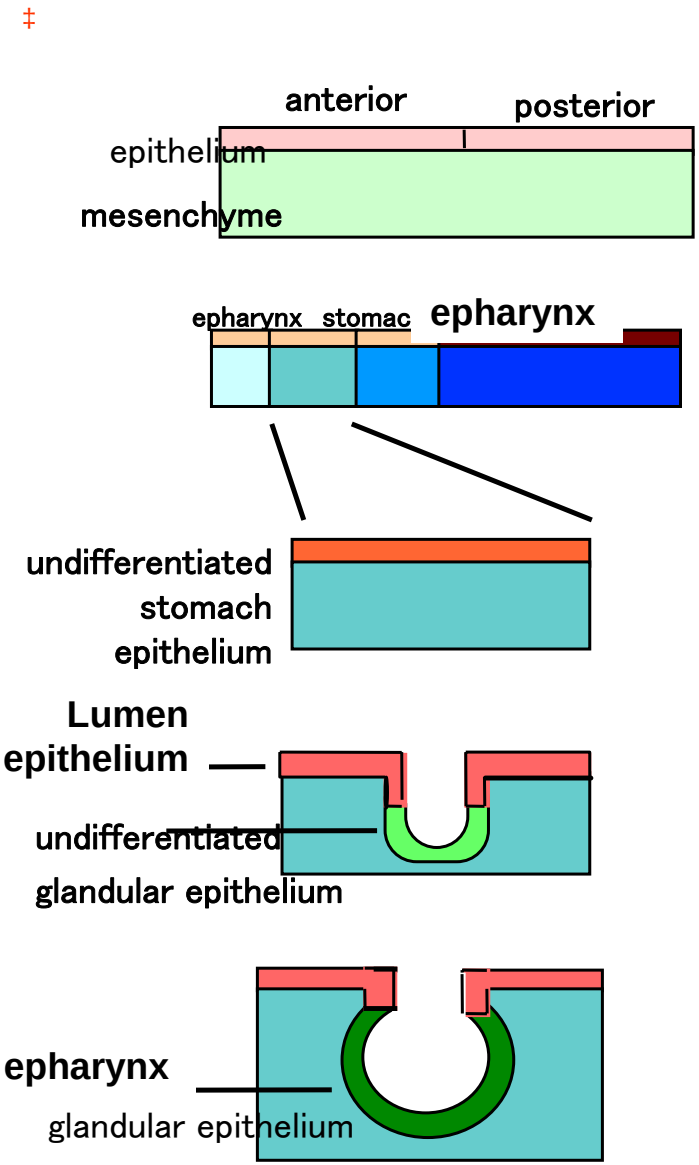
Genes related to differentiation of the stomach epithelium（1）



Genes related to differentiation of the stomach epithelium（2）



Summary of gene expressions in the formation and functional development of stomach glands



Gene groups that determine characteristics of gastrointestinal epithelia — Shh, cGATA5, HNF-3β

Establish anterior and posterior regions — anterior: cSox2; posterior: CdxA

Key gene to determine of anterior stomach region

Differentiation from undifferentiated epithelium to glandular epithelium and lumen epithelium

Notch-Delta signal、Sonic hedgehog

Morphogenseis of glands and cell differentiation

BMP2, FGF10, EGF

Functional differentiation of glandular epithelium (expression of ECPg gene) — GATA transcriptional factors, cSox2,

Differentiation of lumen epithelium (expression of cSP gene) — Smad transcriptional factors

Regionalization in the gastrointestinal tract

Regionalization in the anterior stomach

The molecular mechanism of organ formation③

The molecular mechanism of heart development
and associated research

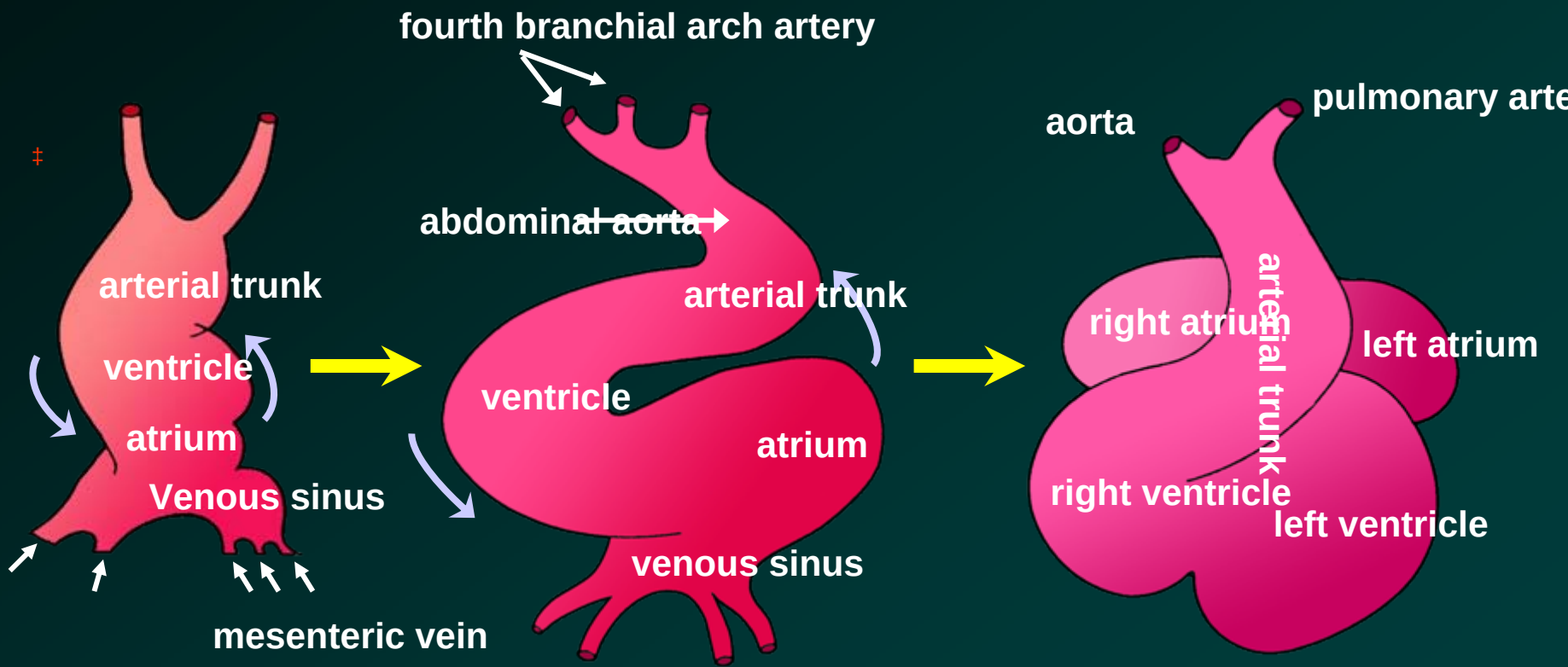
Mechanisms in heart development

"The picture of
heart development"
inserted here was omitted
according to copyright issue.

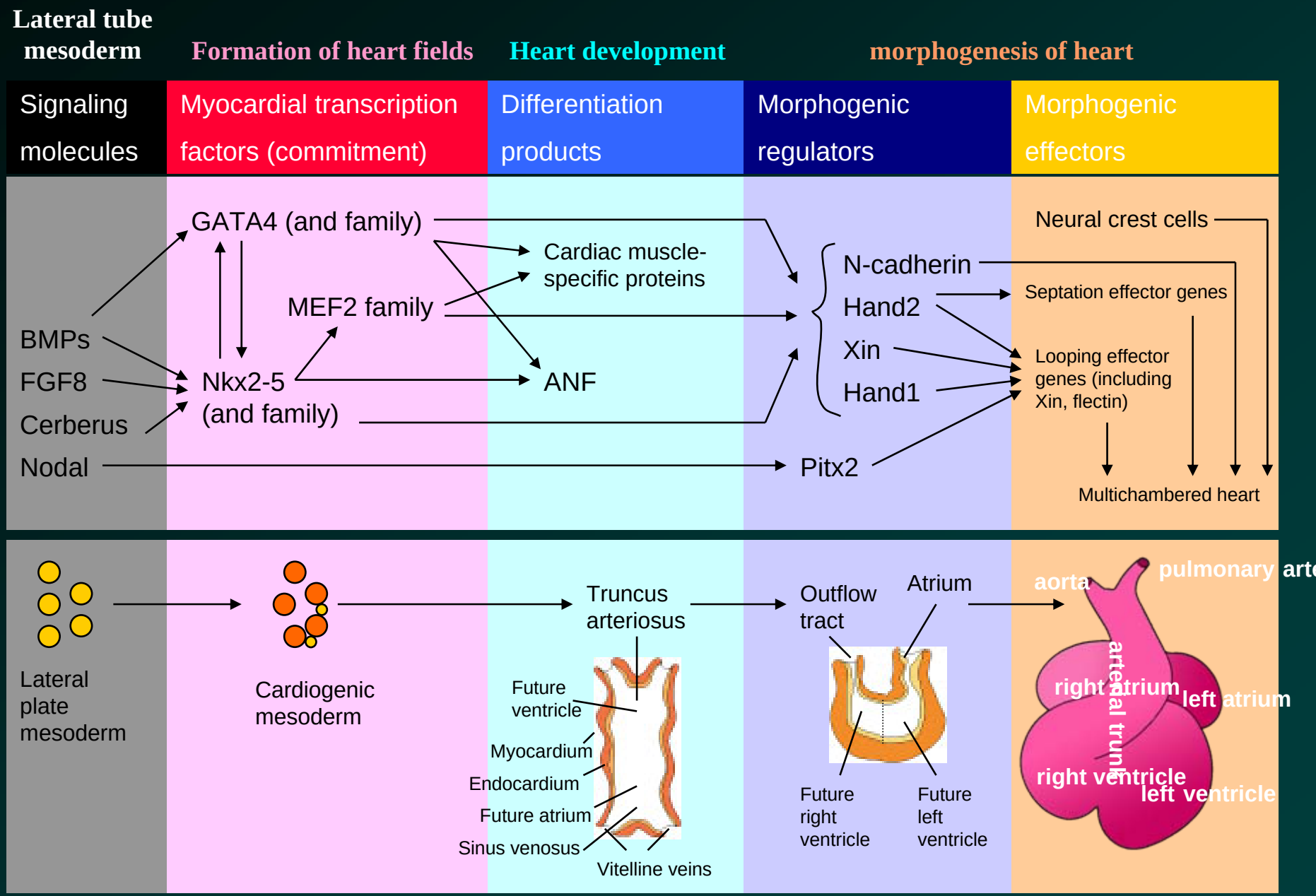
immunostaining
- brown ; ventricular myosin, ventricle marker
- blue ; atrial myosin, atrium marker

The tubular structure forms a loop, and eventually forms an interventricular septum.

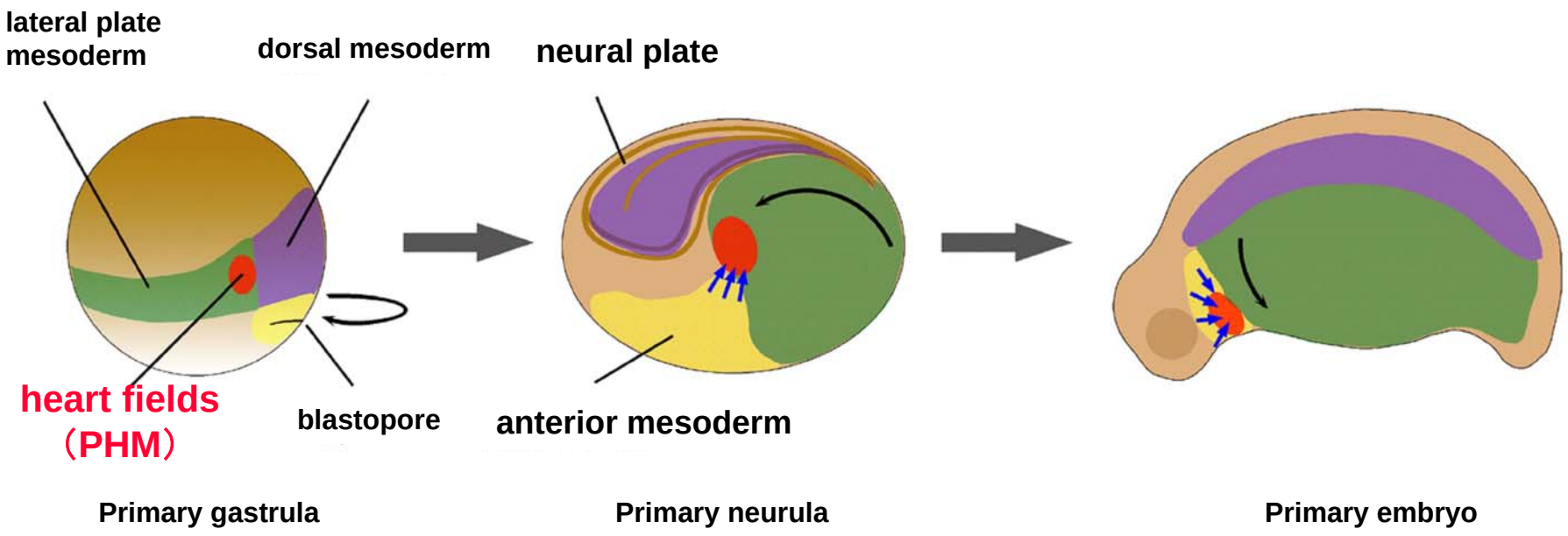
Formation of the human heart loop（ventral side）



The molecular mechanism in heart development

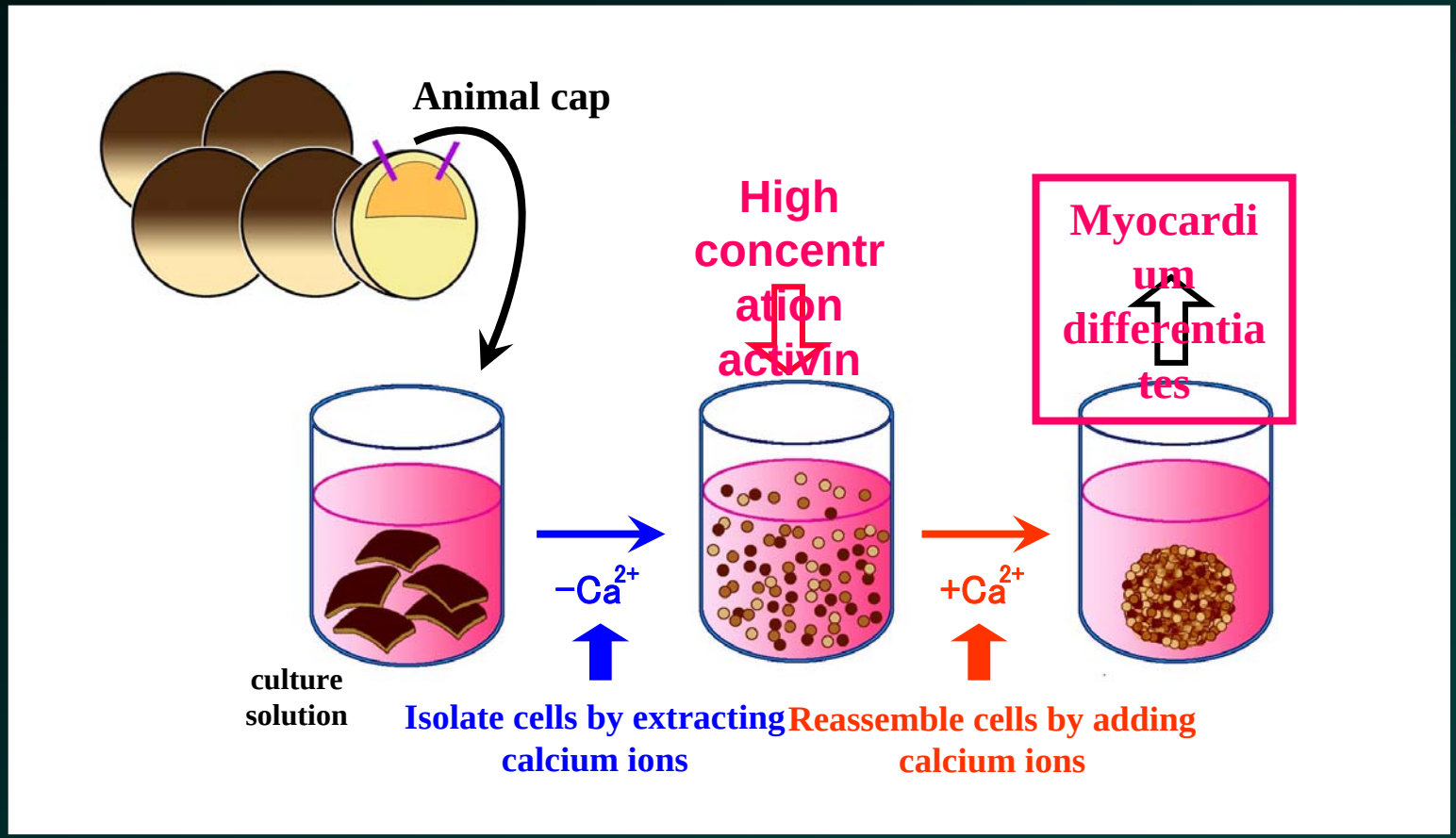


Heart development in a Xenopus embryo



Heart fields (PHM) migrate, and are induced by an anterior endoderm.

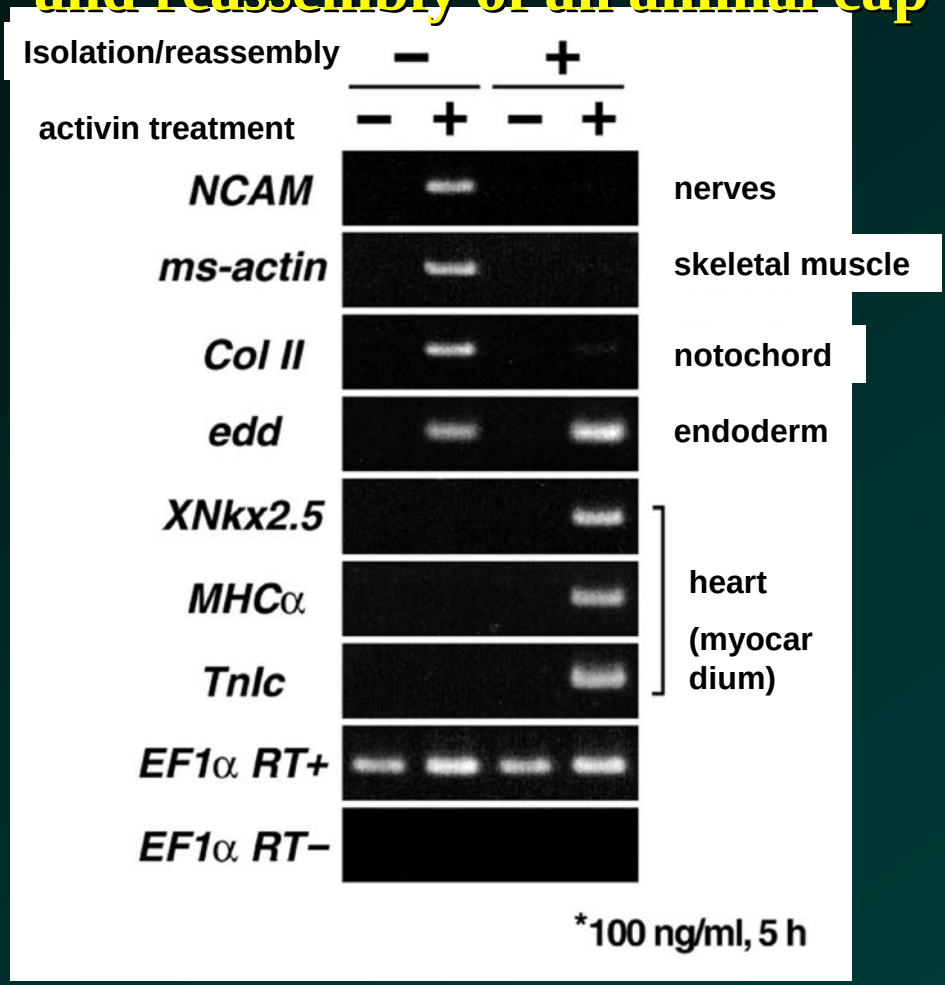
Induction system of the heart: experiment with undifferentiated Xenopus cells



When isolated animal caps of Xenopus blastopore are reassembled after high concentration activin treatment and are incubated, an autonomously pumping heart-like structure is induced.

Pumping “heart” made from an animal cap

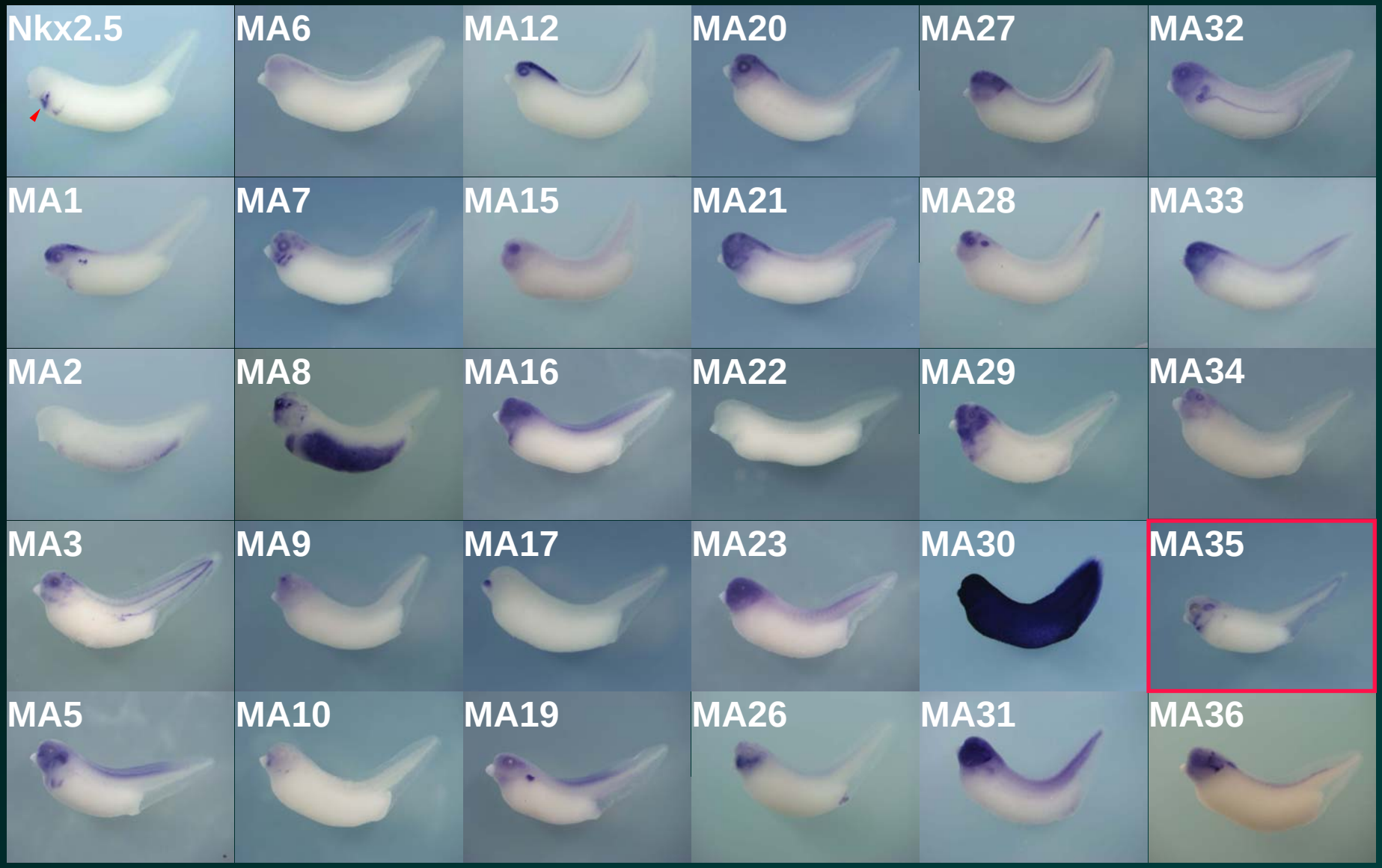
Evaluation of a heart-inducing system formed by isolation and reassembly of an animal cap



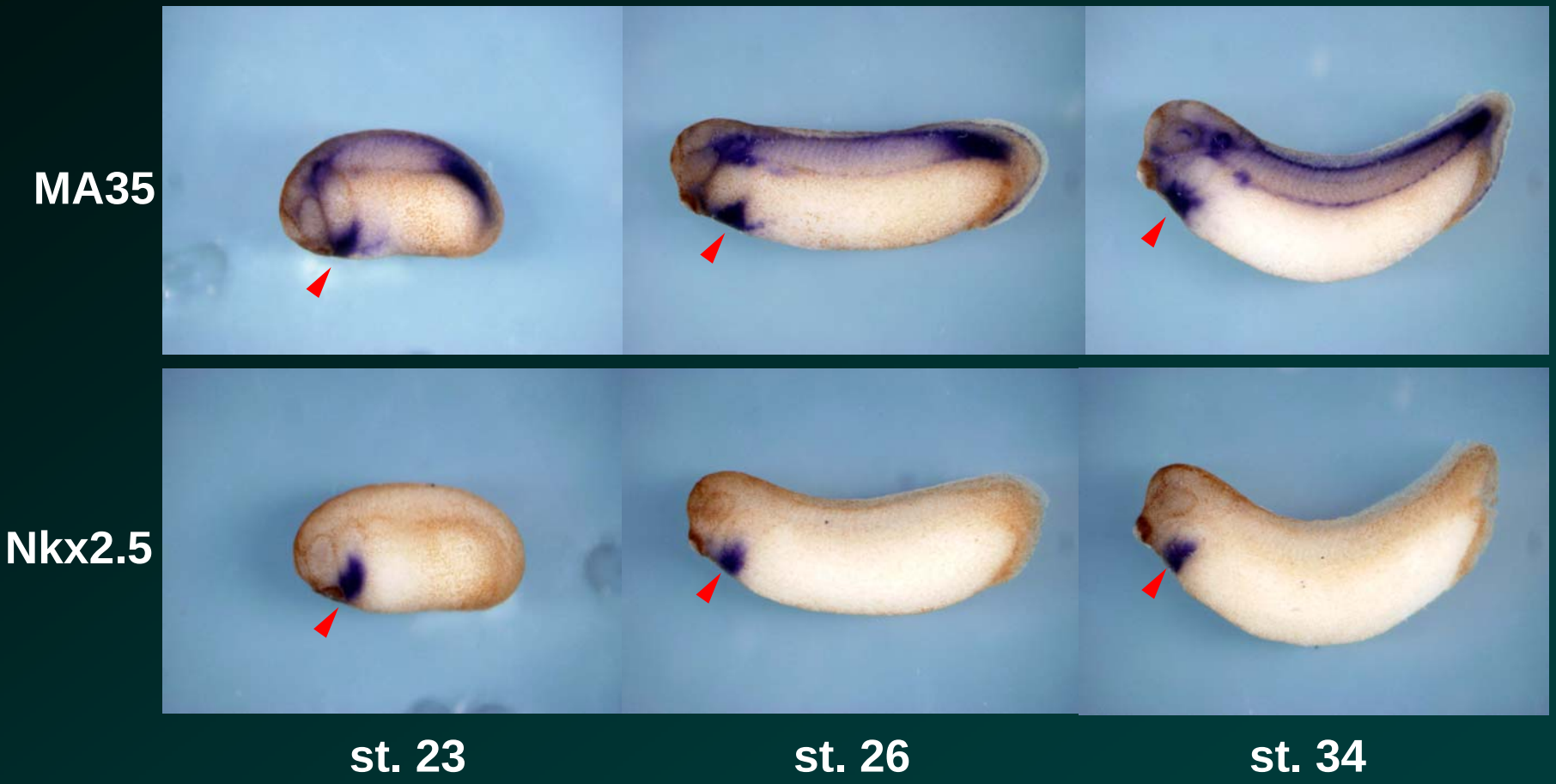
- Without isolation/reassembly, the expression of the mesoderm marker increases.
- With isolation/reassembly, the expression of endoderm and heart marker increases.

→ This heart induction system using an animal cap reproduces a real heart-formation mechanism.

Research on genes which are specific to the heart



MA35 gene expresses in the heart



Inhibiting experiment with MA35①

Control

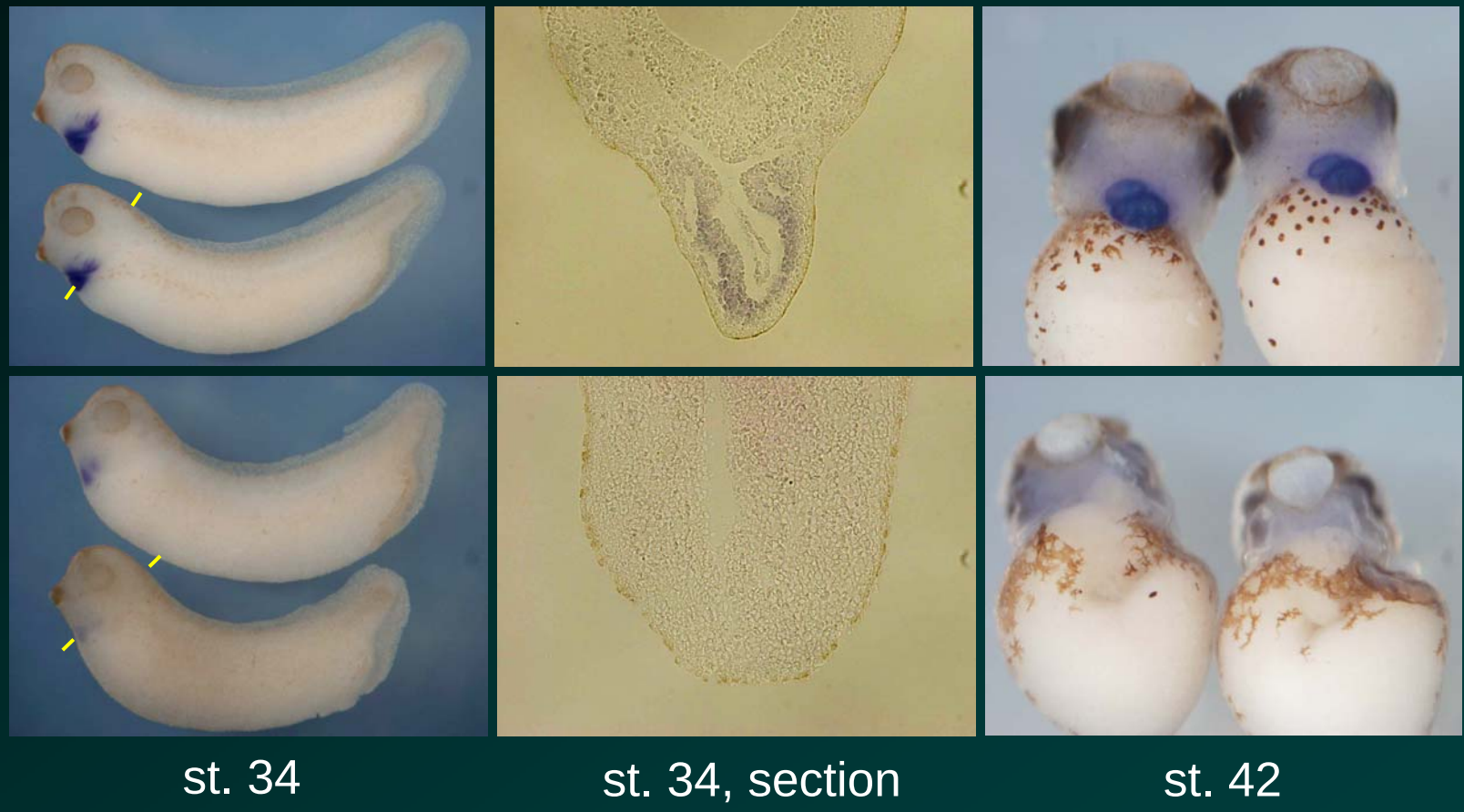
MO injected (function inhibited embryo）

Heart is not formed when the function of MA35 is inhibited.

→ MA35 is essential for heart development.

Inhibiting experiment with MA35②

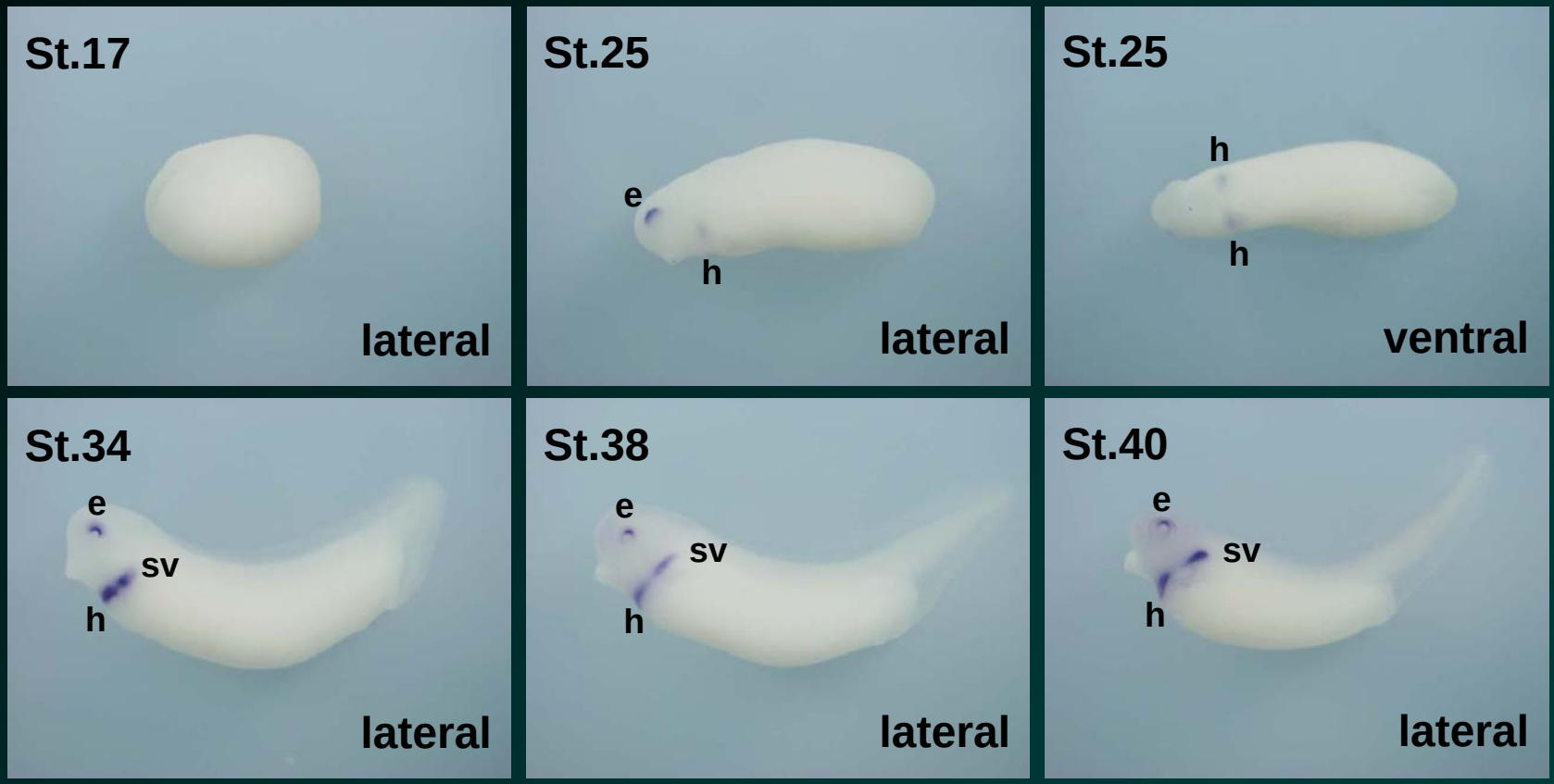
Heart marker gene



Expression of the heart marker decreases when the function of MA35 is inhibited.

→ MA35 is essential for heart development.

Regions of *XTbx5* expression in heart development

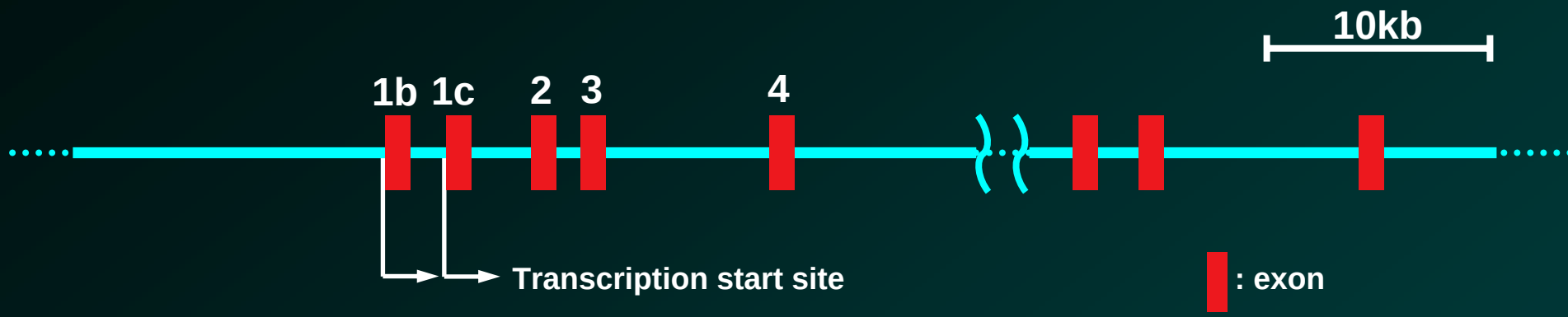


e：future retinoid region　h：future atrium・ventricle region　sv：future venous sinus region

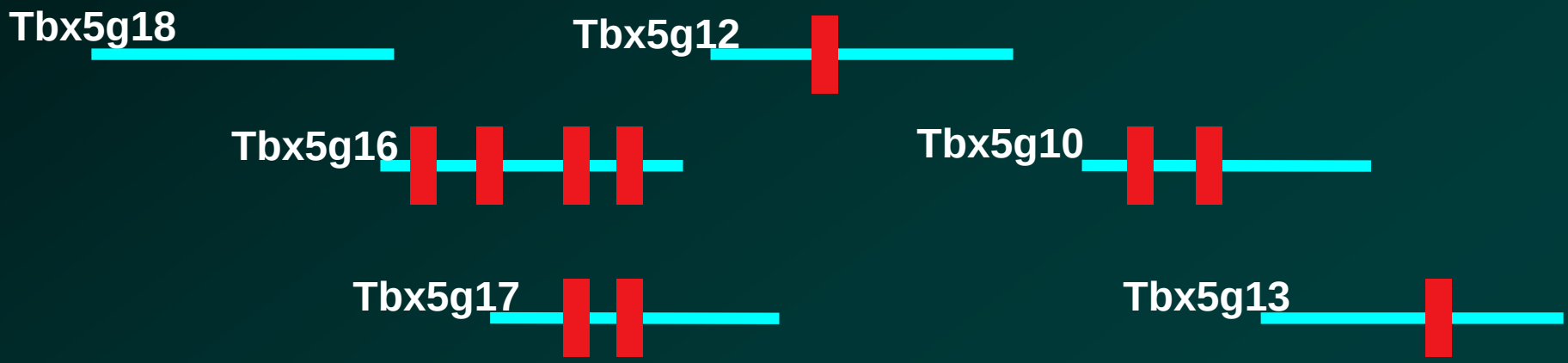
XTbx5 is the gene that expresses in heart and venous sinus regions.

→ Research on modulation mechanism of *XTbx5* during heart and vessel development.

The structure of the *XTbx5* gene



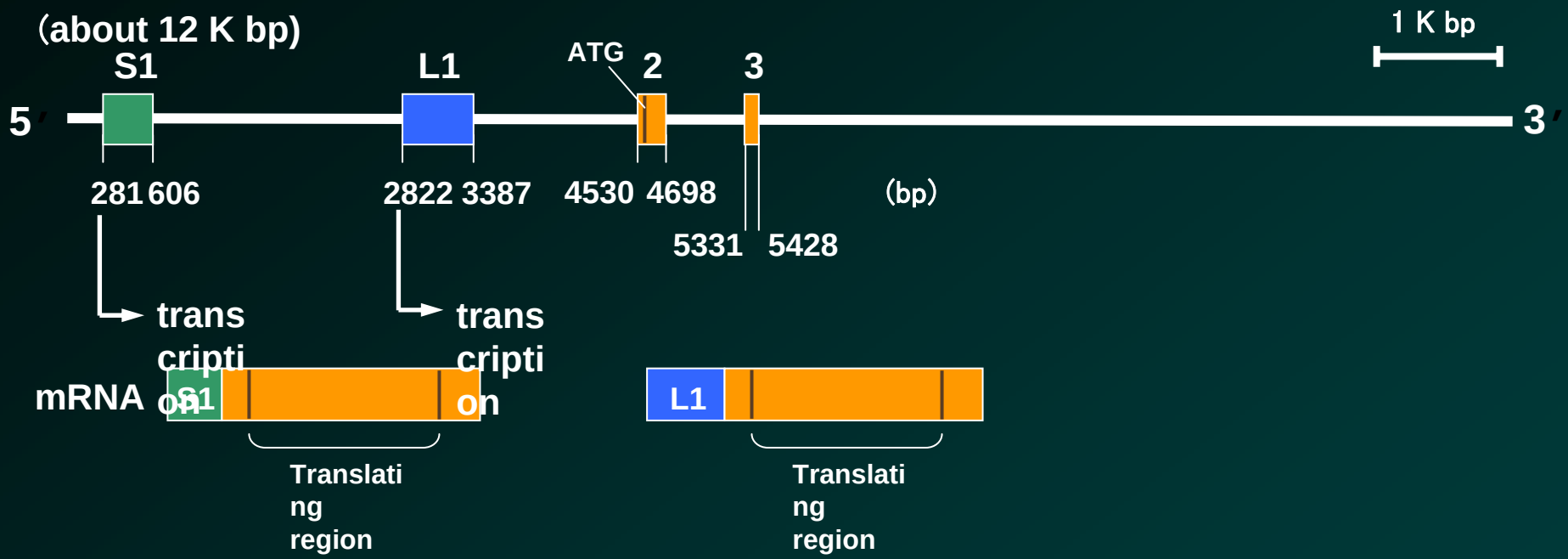
Isolated genomic clones

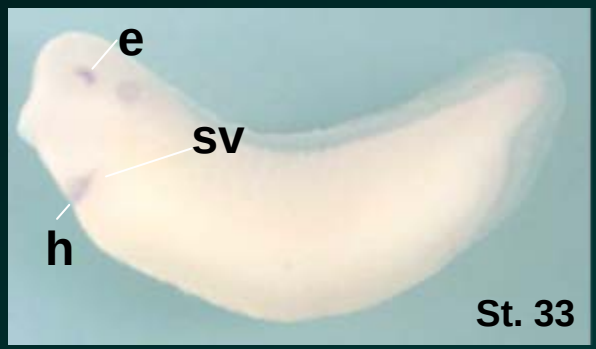


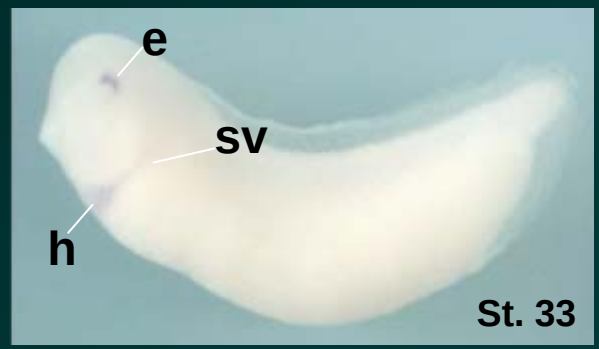
The structure of the upper sequence of the *XTbx5* genome

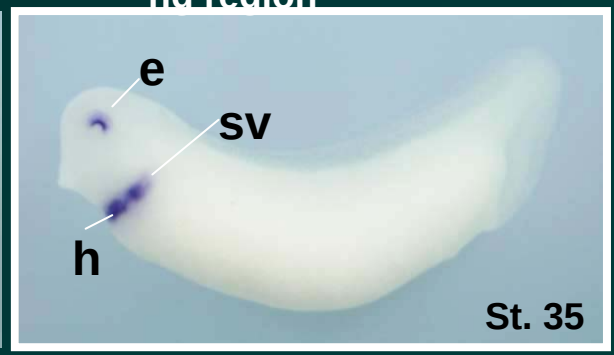
Isolated genome segment

(about 12 K bp)



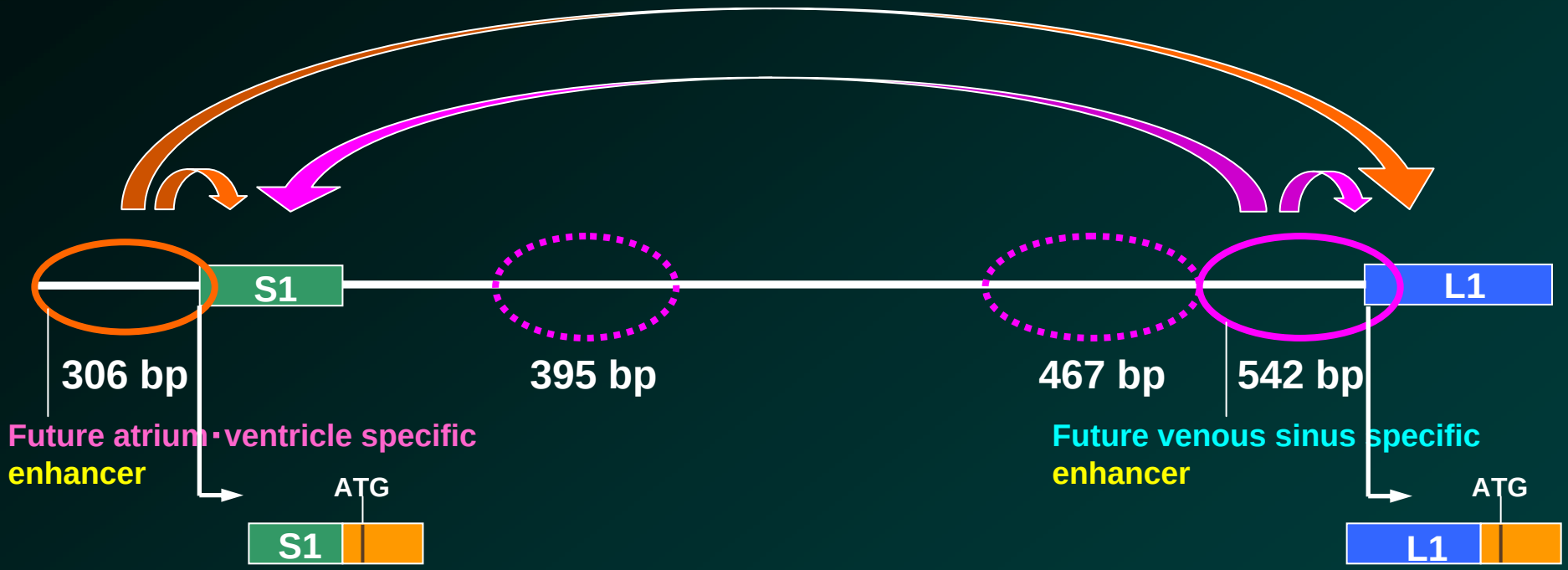




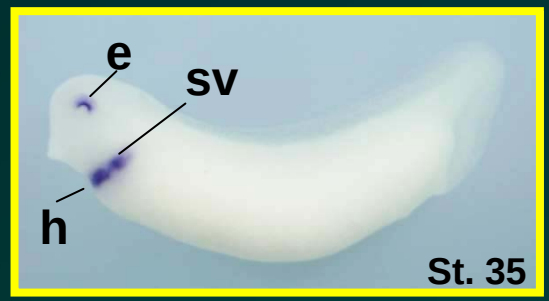


e：future retinoid region h：future atrium・ventricle region
sv：future venous sinus region

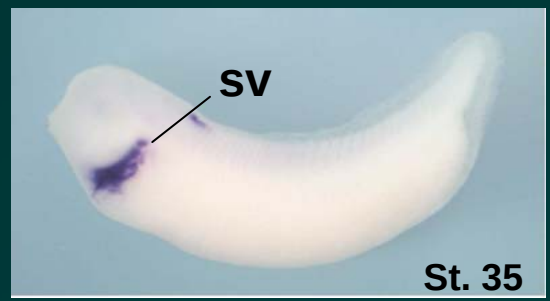
The interaction between heart and vessel development is regulated by control between genes



e：future retinoid region
h：future atrium・ventricle region



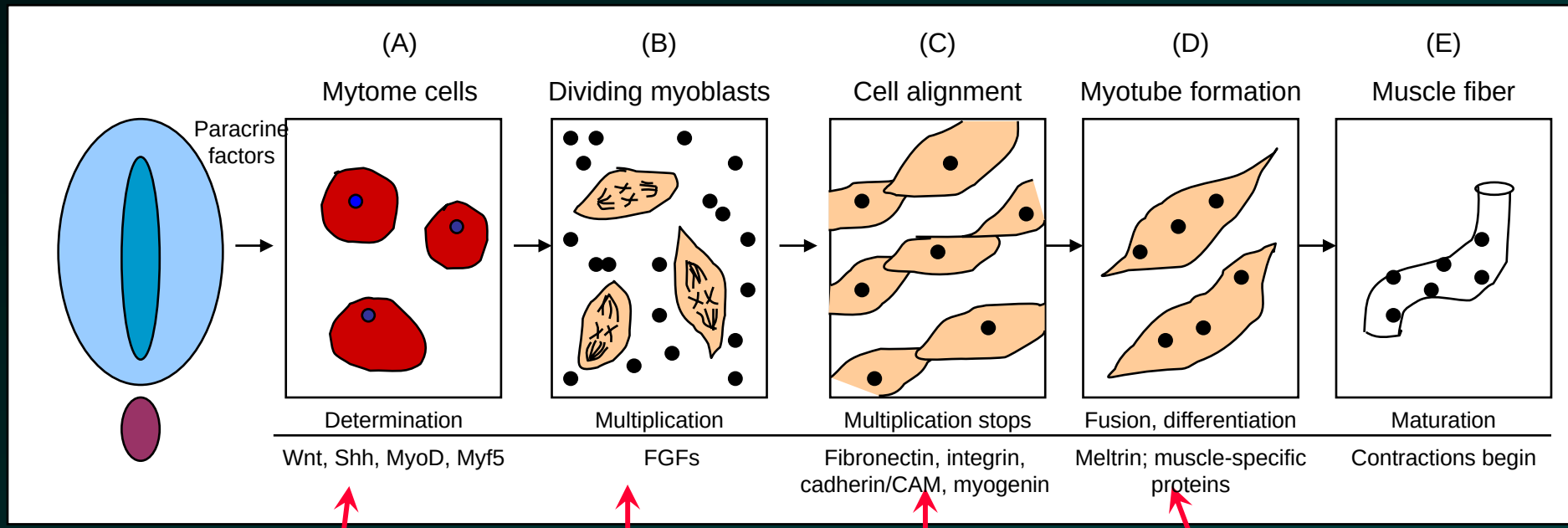
Expression of inner



The molecular mechanism of organ formation④

The molecular mechanism of other organ formation

The molecular mechanism of muscle differentiation



Sarcomere differentiation
Wnt, Shh,
MyoD, Myf5

Division of myoblasts
FGFs

Cell alignment
Fibronectin
integrinm
cadherin
myogenin

Cell fusion
（multinucleation）
Meltrin

The mechanism of skeletal differentiation

“The illustration for skeletal differentiation”

inserted here was omitted

according to copyright issue.

Bone develops from mesenchyme（mesoderm, and matures through ossification by cartilage formation and accumulation of minerals, and penetration of vessels.

Skeletal differentiation is regulated by various homeobox genes and BMP, and region-specific bones are correctly formed.

A supply of calcium is essential for skeletal differentiation （chicken）

“The photo of a chicken embryo with abnormal bone” inserted here was omitted according to copyright issue.

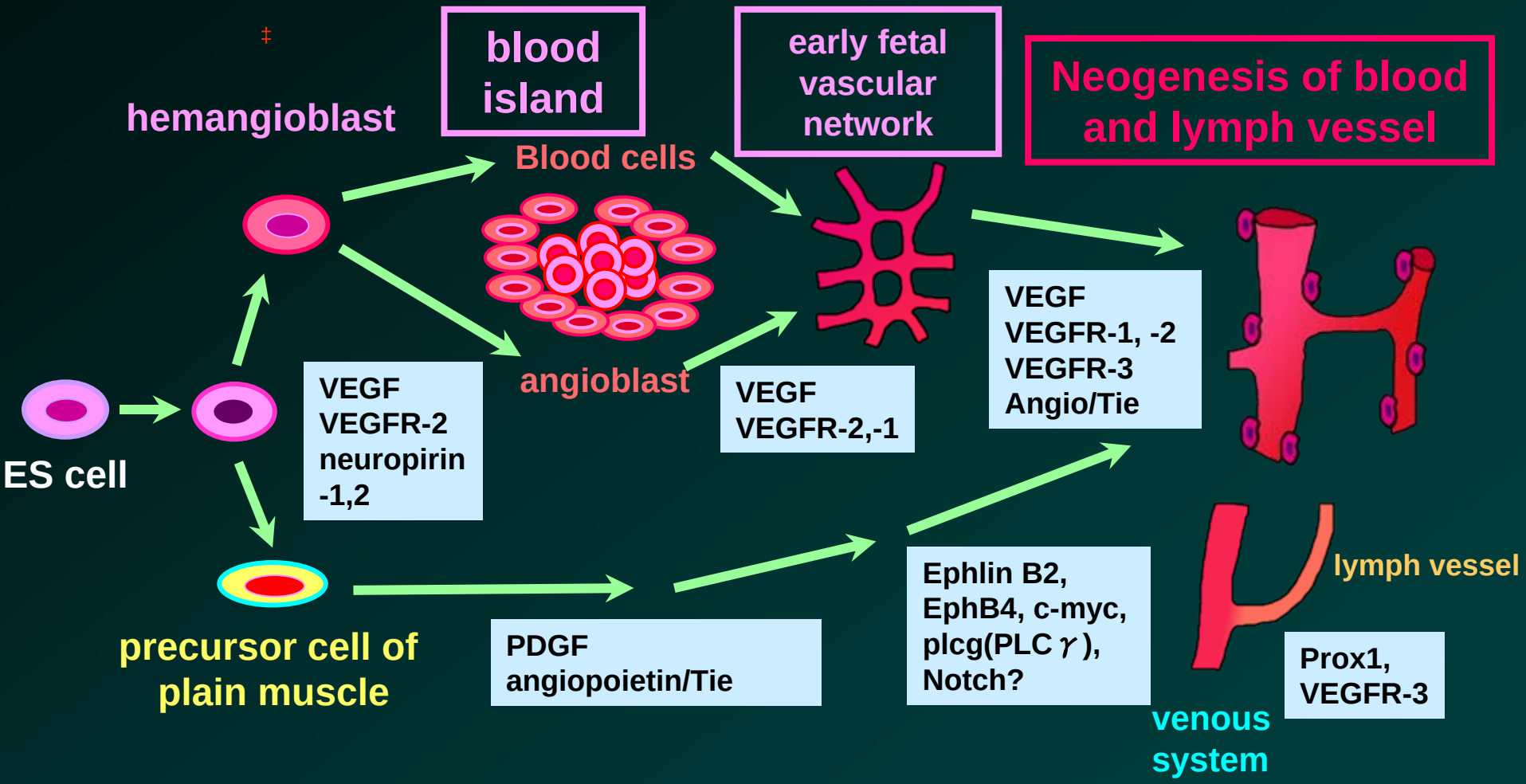
Abnormal bone development

normal

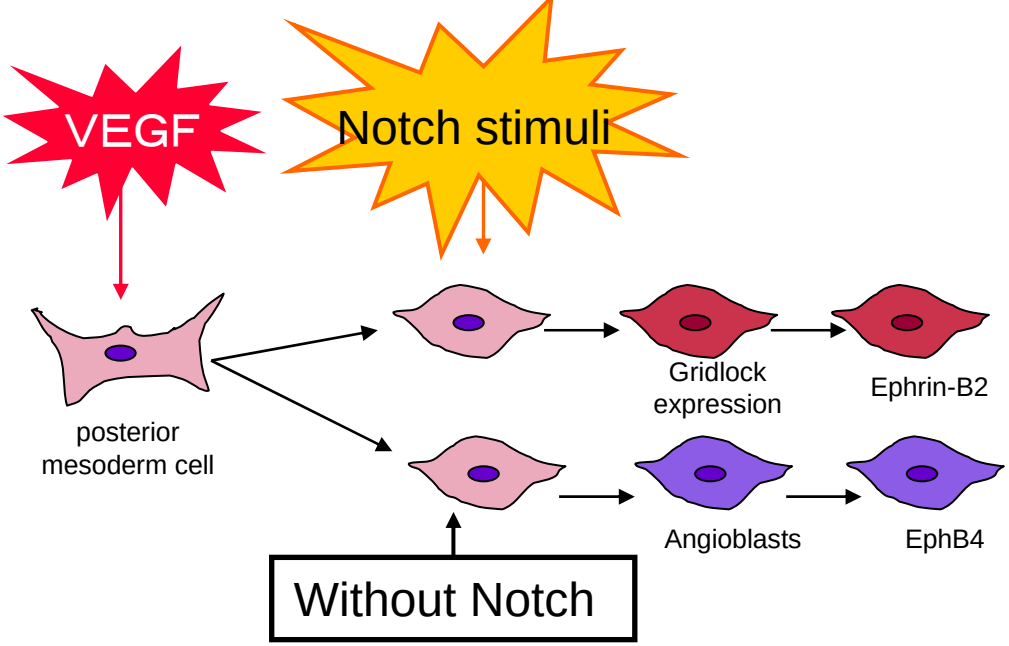
（skeletal tissues are dyed using alizarin red）

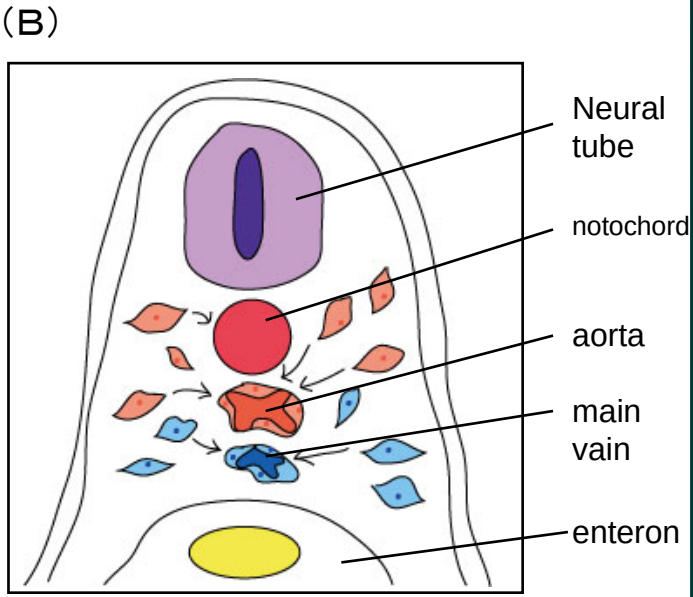
When a chicken embryo is incubated outside of its shell, abnormal ossification takes place for lack of calcium supply.（The positions are correct, but ossification has stopped.）

Development mechanism of blood & lymph vessels



Differentiation of blood vessel cells are monitored by VEGF signal and Notch signal





Mesoderm cells differentiate into blood vessels using VEGF stimul、

Existence of a Notch signal determines whether to differentiate into an artery or a vein.

Move toward appropriate positions as arteries and veins differentiate.

The molecular mechanism of blood cell differentiation

"The illustration for blood cell differentiation" inserted here was omitted according to copyright issue.

Overall organ formation

Organ development and apotosis

Normal embryo

"The photo of

a functionally inhibited embryo"

inserted here was omitted

according to copyright issue.

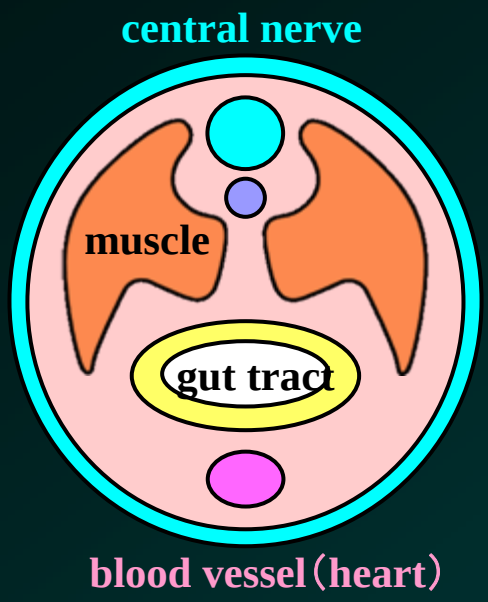
caspase-9
Functionally inhibited embryo

As the result of apotosis i
the brain structure is abno

caspase-9; the gene that causes apotosis

Appropriate regulation of apotosis is important for healthy organ formation

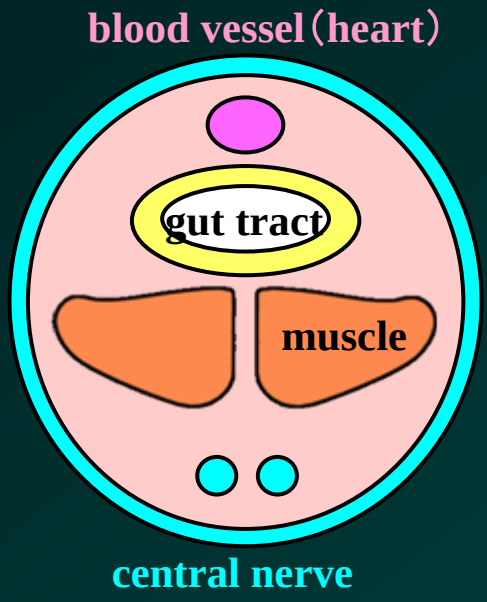
similarities of structure and ventral-dorsal reversal in vertebrates and insects



vertebrates

dorsal

ventral



insects

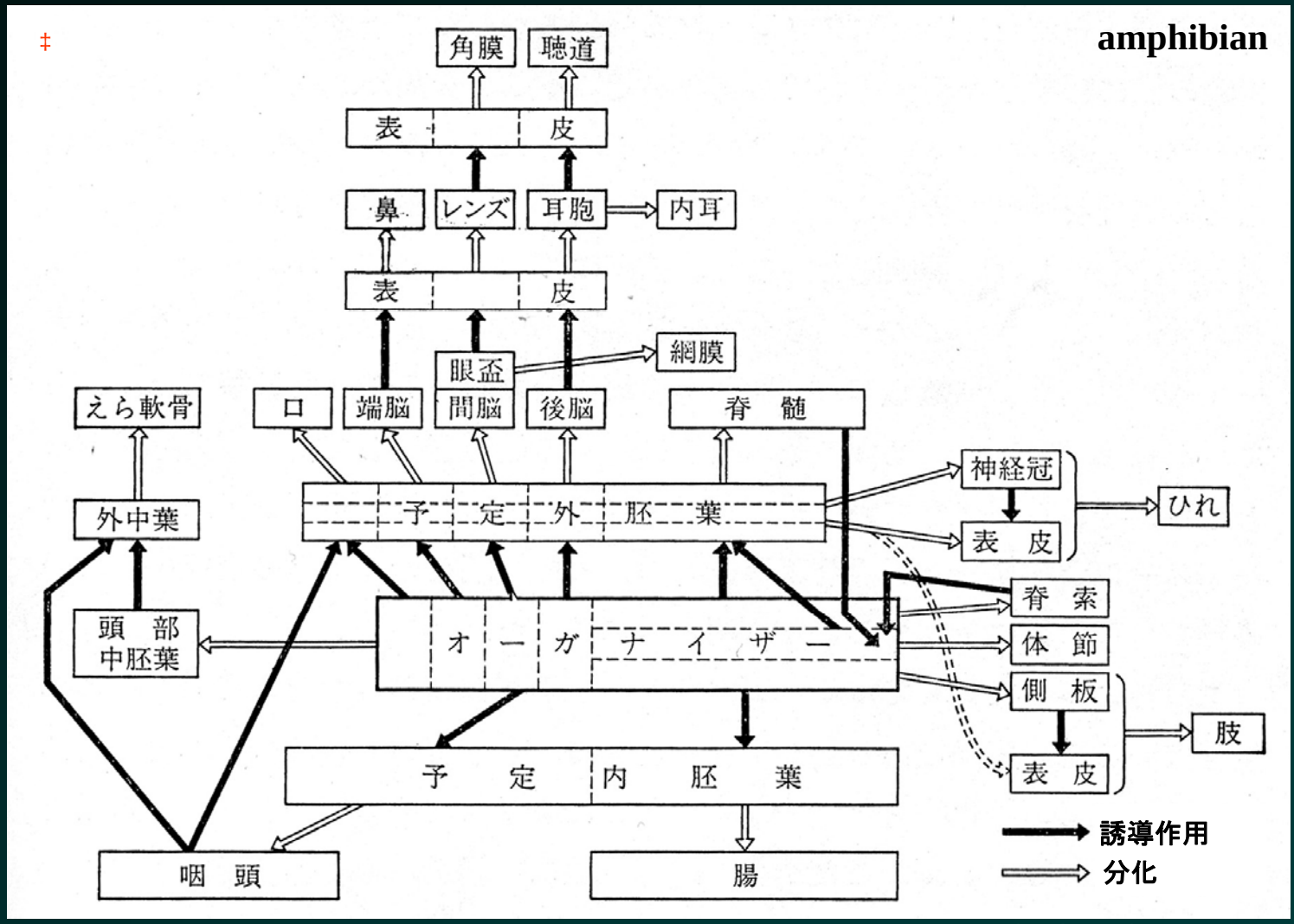
germ layers	vertebrates	insects
endoderm	gut tract, liver, lung	gut tract
mesoderm	muscle, heart, blood cell, bone, kidney	muscle, heart, blood cell
ectoderm	skin, nerve system	epidermis, nerve system

organ differentiation

Organs with similar functions differentiate from same structure of 3 germ layers.

However, dorsal side of vertebrates correspond to ventral side of insects.

Organ and tissue induction in vertebrates



1983 Idemitsu Shoten

There are many stages of “induction” in organ development.