Graduate School of Arts and Sciences, University of Tokyo

The final lecture series

40 years researching mechanisms of animal development

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Graduate School of Arts and Sciences, University of Tokyo

2007.3.3 at lecture room #900 Komaba campus

My study in biology started from ibis.

Starting point of biology is to learn from nature and organisms.

Watching and inspecting organisms in the field is an emotional experience.

Picture is worth a thousand words.

(field work at the same place for 40 years)

coexistence of collection and protection

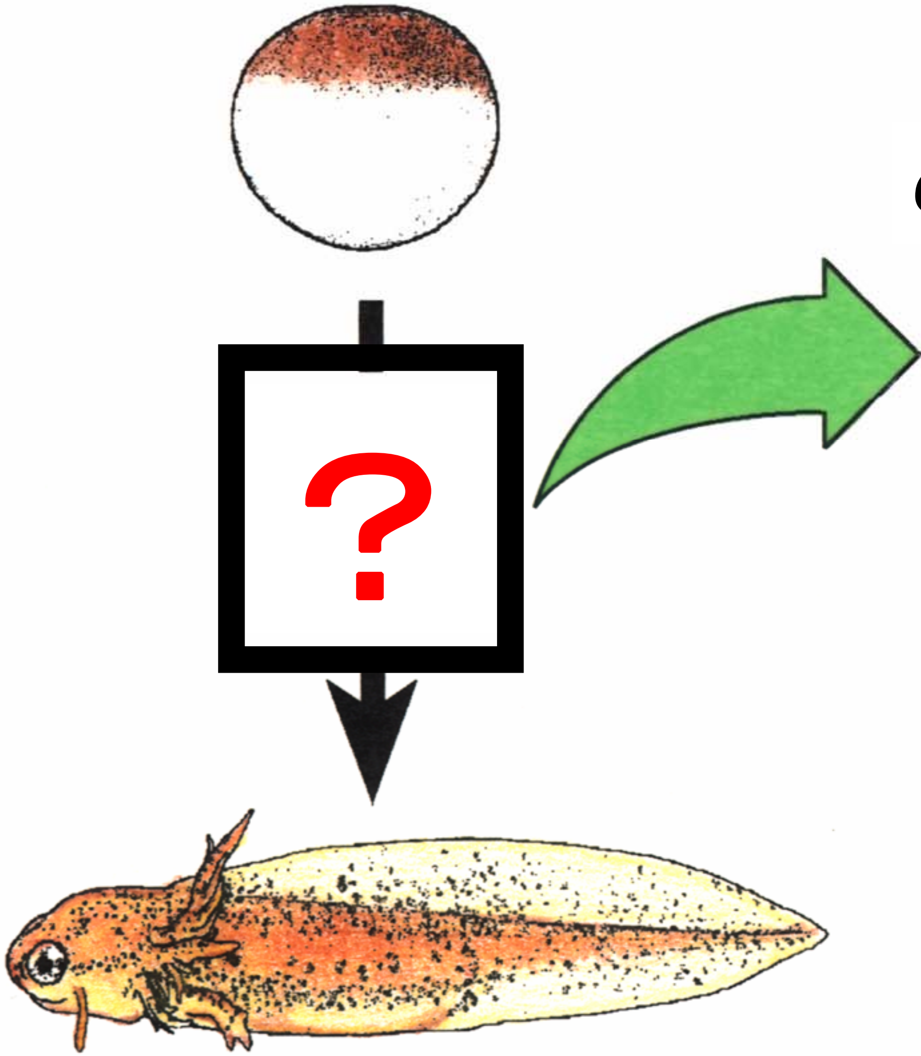
We collect and research amphibians for 40 years in north Niigata, but population has not changed since. They are rather increasing. Collecting animals and returning them to the original same site is difficult, but it is a good lesson for students.

Things learned from newt collecting

1. **Newt cancer is cured during hibernation.**
   → low temperature therapy of cancer
2. **Mating displays of newts**
   → A couple is made only when they love each other.
3. **Skin patterns of stomach are inherited.**
   → Birth regions can be identified from skin patterns.
4. **In waters of severe cold winter, newts move in a newt ball.**
   (Assembly of 500~1500 newts.)
5. **Limbs, eyes, and lenses of newt are reformed after removal**
   → strong capacity for regeneration
6. **Can newts predict earthquakes?**

⇒ Being able to see these phenomenon in the nature, we find the beauty, fascinating aspects, and wonders in living organisms.

Morphogenesis from an egg to a tadpole



embryonic induction

cell differentiation

morphogenesis

.
.
.

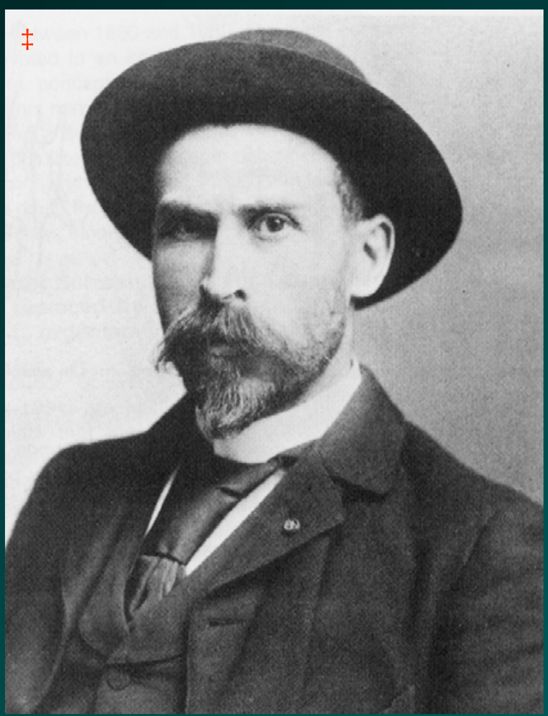
etc.

The Father of Experimental Embryology: Roux

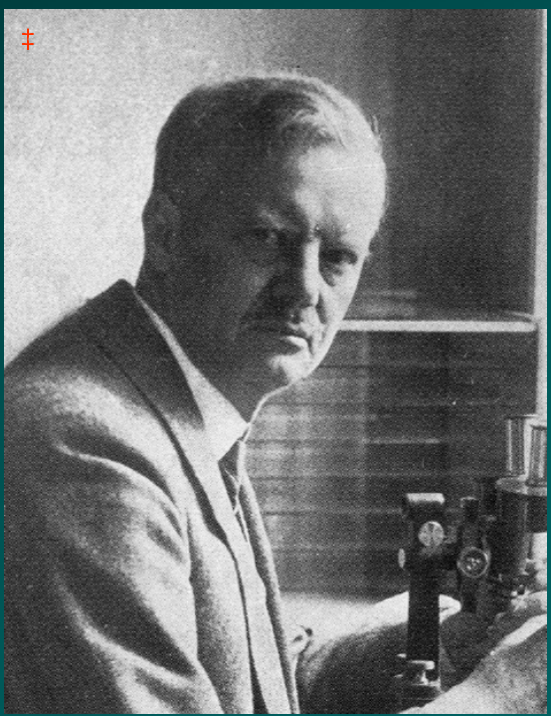
manipulated animal embryo(egg) and started positivistic experimental embryology.

Discoverers of Embryo Induction: Spemann and Mangold

discovered the organizer which is the center of morphology formation and found “induction”in embryo development for the first time→ **Established the modern developmental biology**



Wilhelm Roux (1850-1924)



Hans Spemann (1869-1941)

Hilde Mangold (1898-1924)

"Int J Dev Biol, vol 40, No.1, 1996" p10-Fig.2(Klaus Sander), p60-Fig.1 (Viktor Hamburger), p54-Fig.8 (Peter E. Faessler)

Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren

Von

H.Spemann und Hilde Mangold

Mit 25 Textabildungen

Sonderdruck aus

dem Archiv für

Mikroskopische Anatomie

Und

Entwicklungsmechanik

Herauagegeben von

Wilhelm Roux

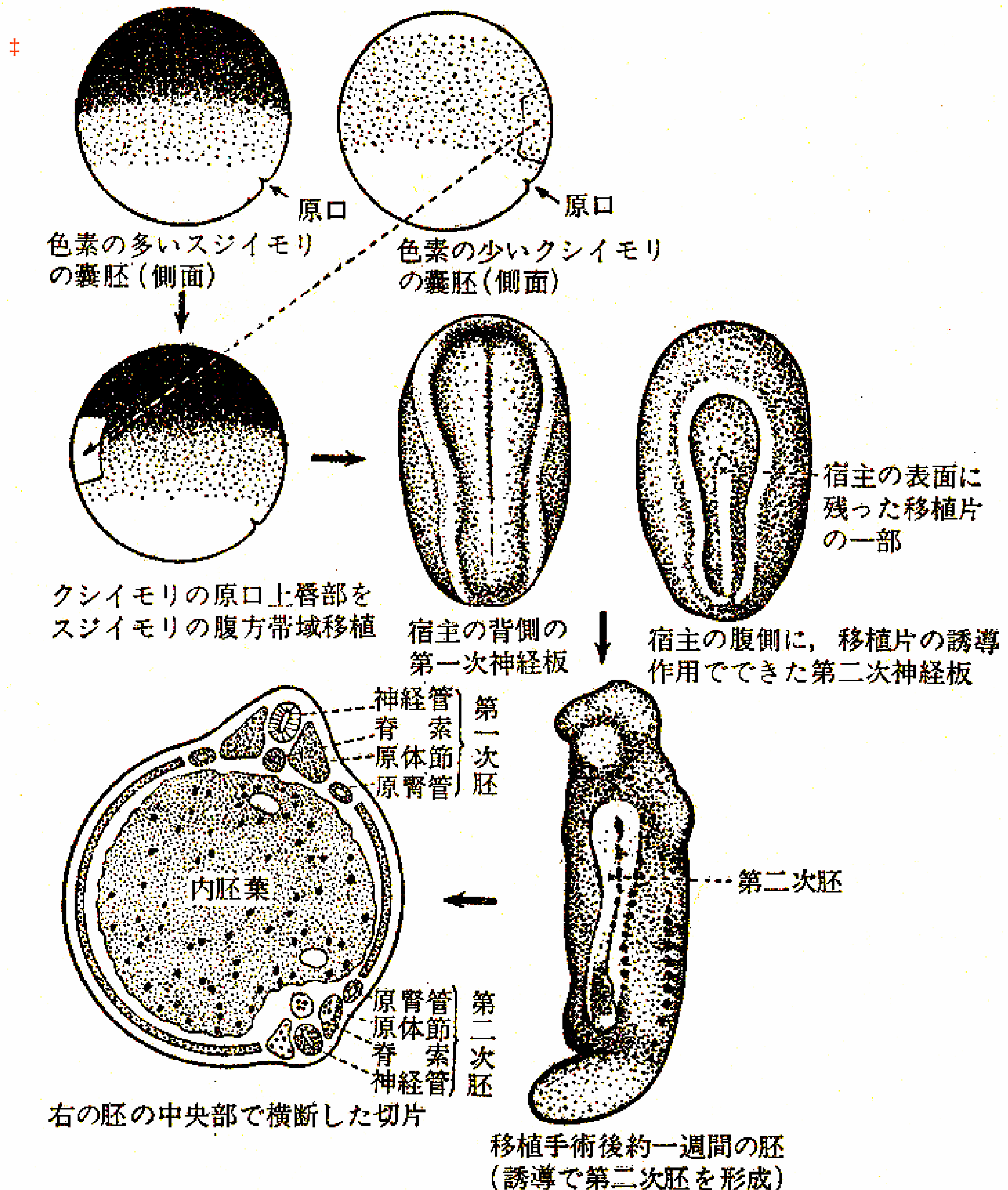
unter Mitwrikung von **H.Braus** und **H. Spemann**

100.Band 3./4. Heft

Berlin

Julius Springer

1924

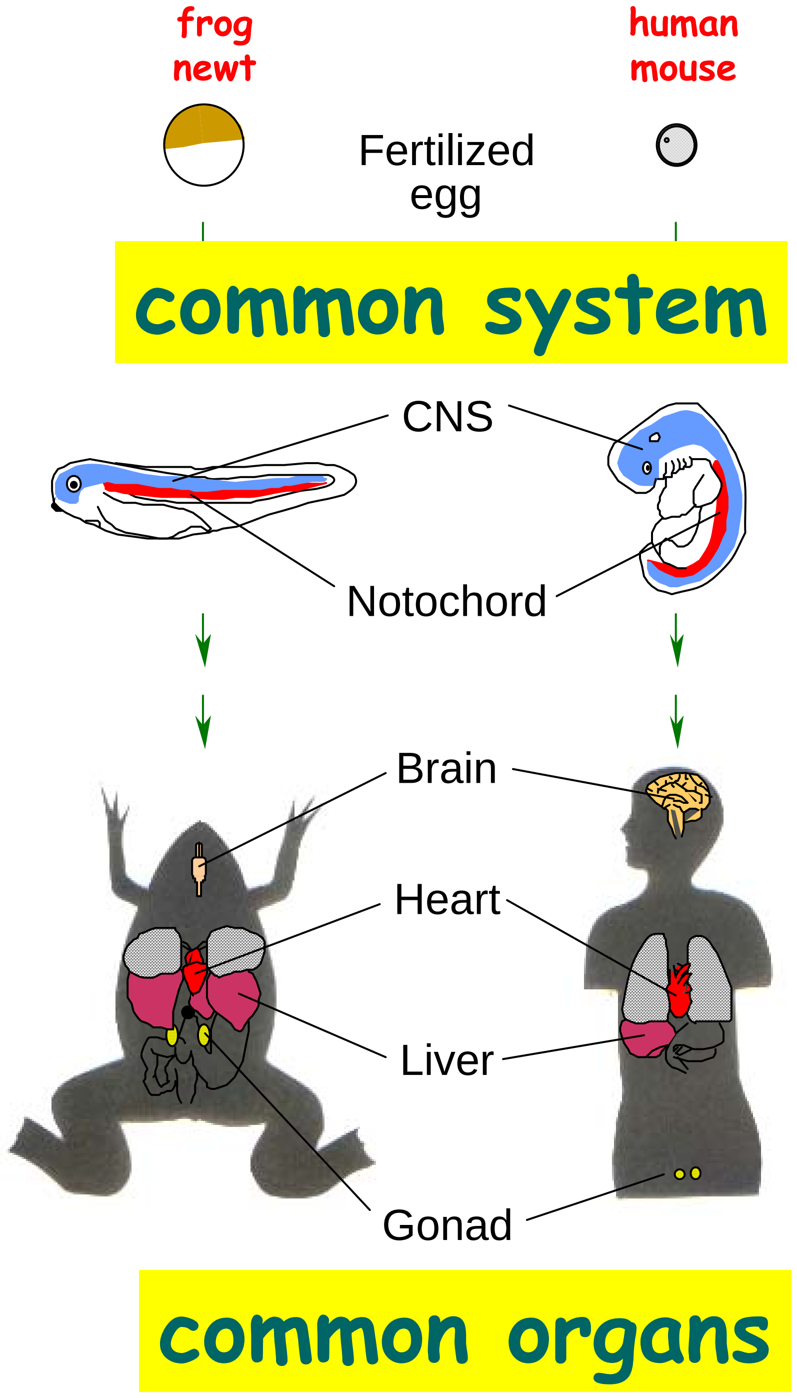


Takeo Mizuno･Makoto Asashima “Easy-to-Understand Biology IB･II New Edition” p161-fig.45, 2001, Buneido

The “picture of Spemann” inserted here was omitted according to copyright issues.

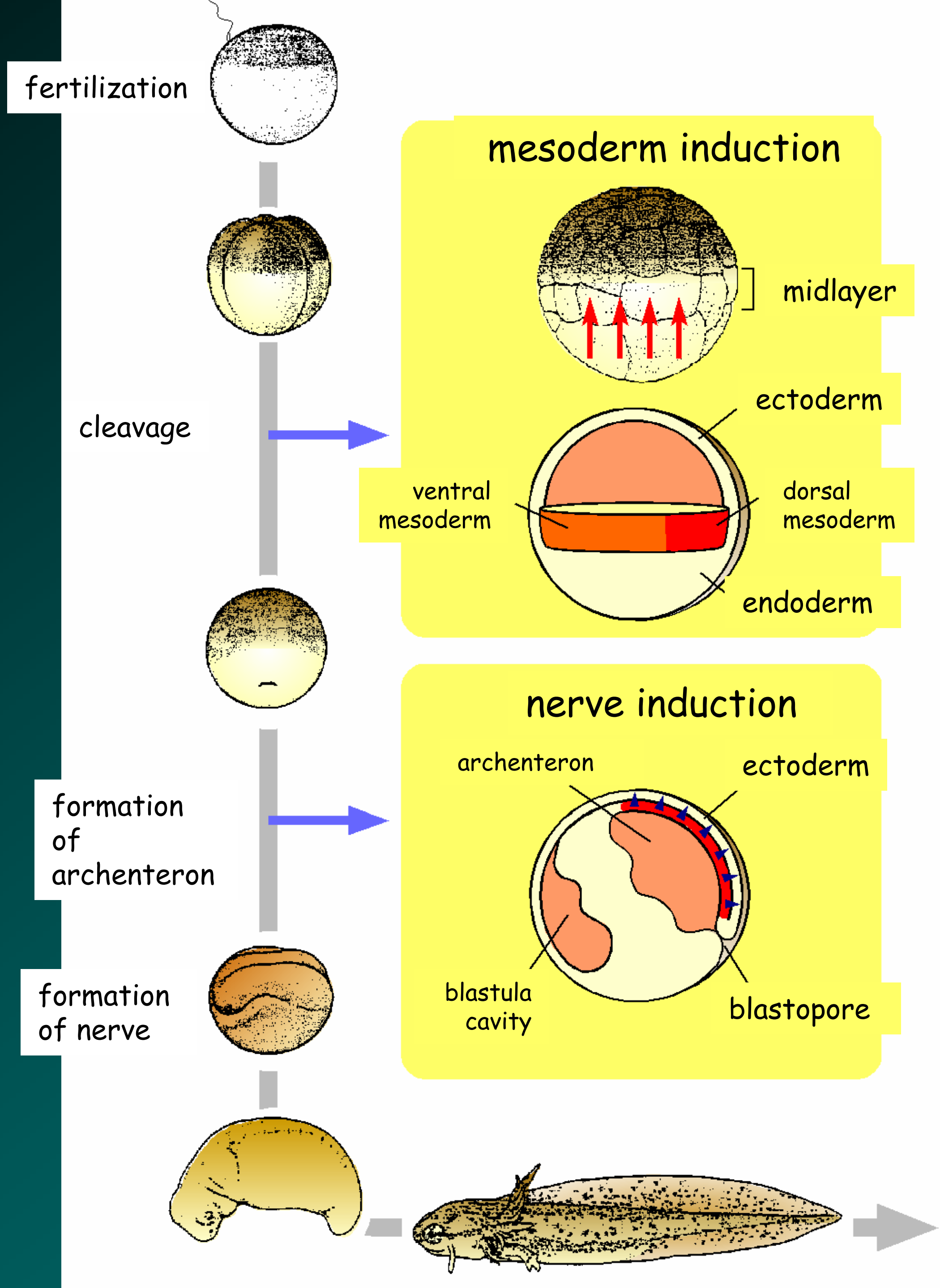
The “picture of Spemann’s Nobel prize” inserted here was omitted according to copyright issues.

1935 Nobel prize
in physiology and medicine



frog
newt
human
mouse
Fertilized egg
common system
CNS
Notochord
Brain
Heart
Liver
Gonad
common organs

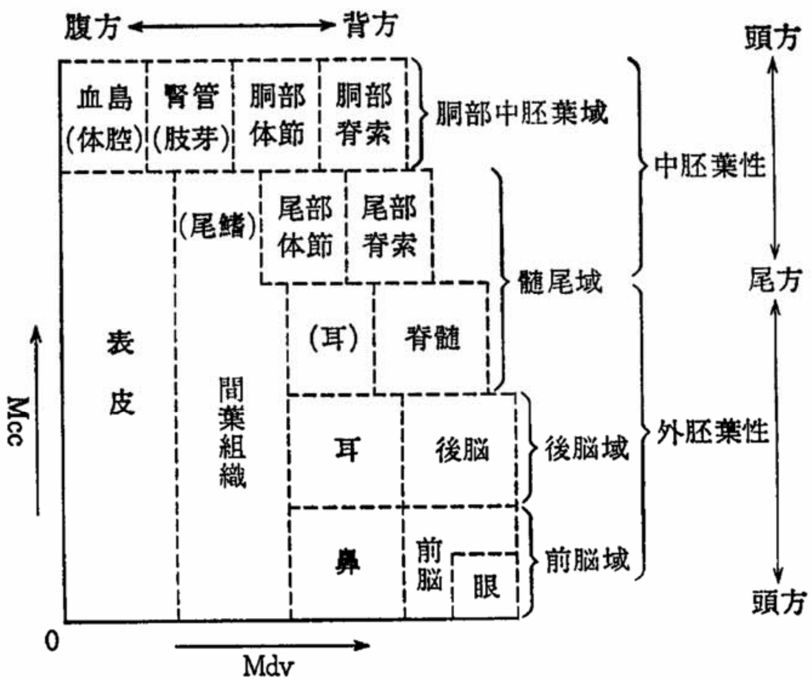
Primary development and embryo induction of amphibian



Hypotheses on concentration gradient of inducing factors during animal morphogenesis

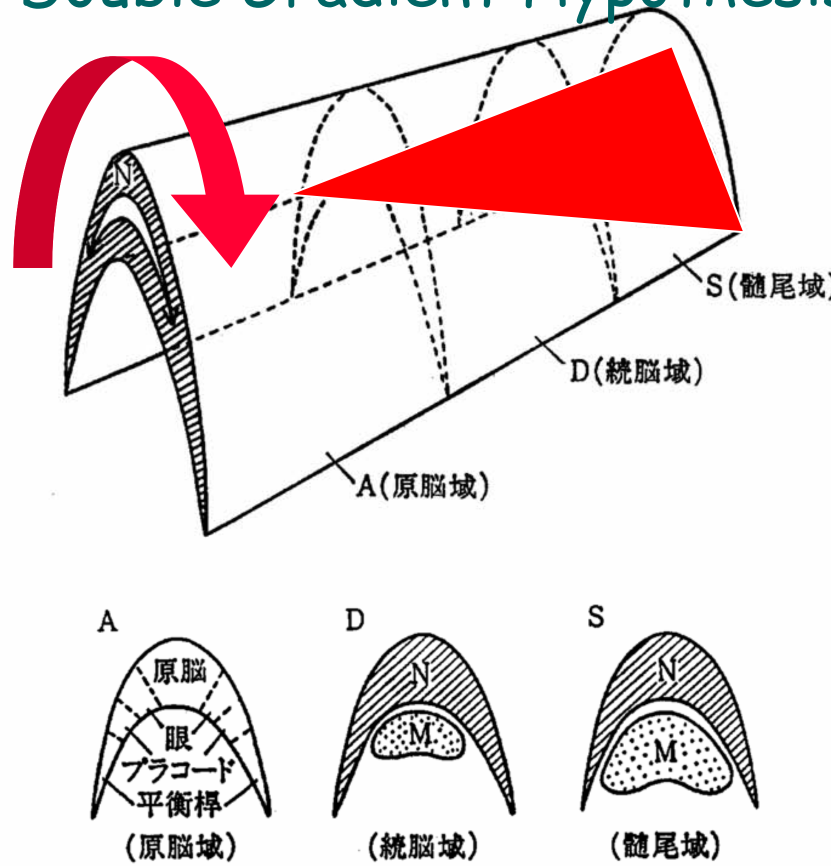
‡

Double Potential Hypothesis



重複ポテンシャル論による Mdv, Mcc が予定外胚葉に働いた際に生ずる各種分化過程の模式図.
(Yamada 1956, a. b.)
Mdv＝背腹規定能
Mcc＝頭尾規定能

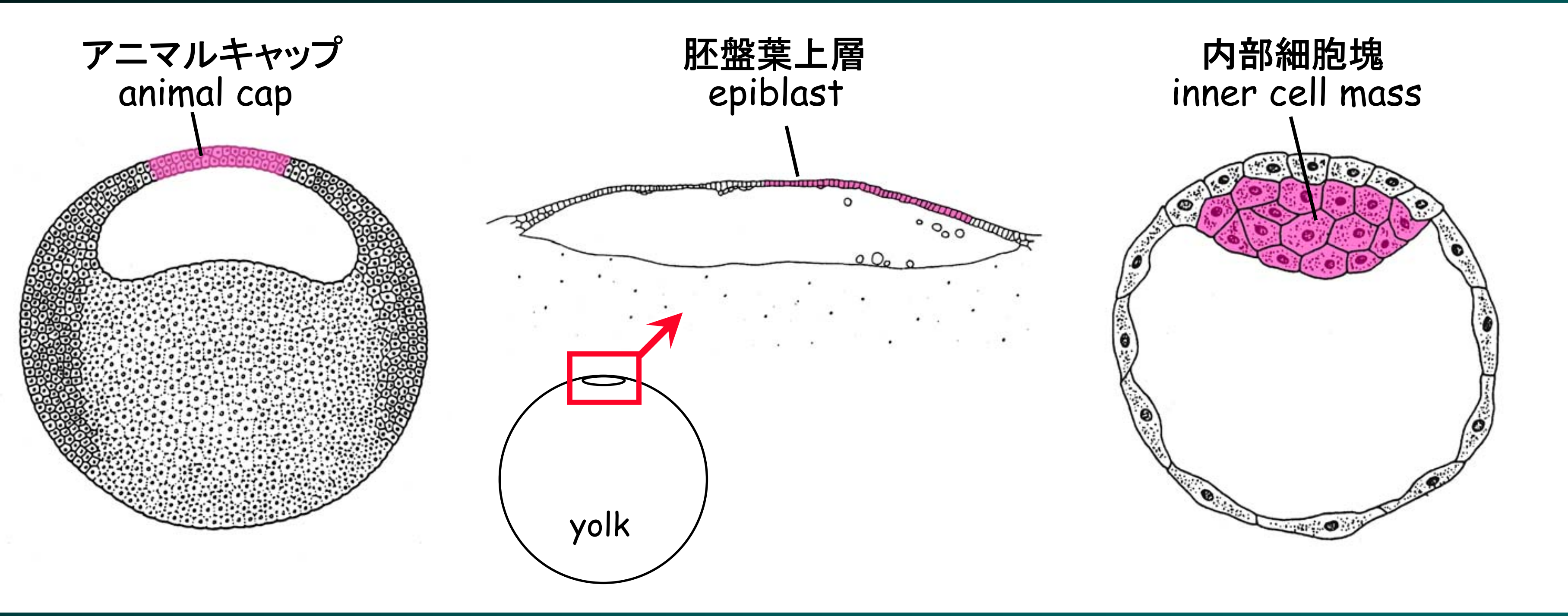
Double Gradient Hypothesis



二勾配説の模式図. M, 中胚葉化活性；N, 神経化活性；A, 頭端部誘導；D, 続脳部誘導；S, 髄尾部誘導.
(Toivonen & Saxén, 1955 より)

Osamu Nakamura･Izumi Kawakami “Organizer”, p33-fig.1-13, p34-fig, 1-14 1977 Misuzu Shobo

Omnipotent stem cell of vertebrate embryo

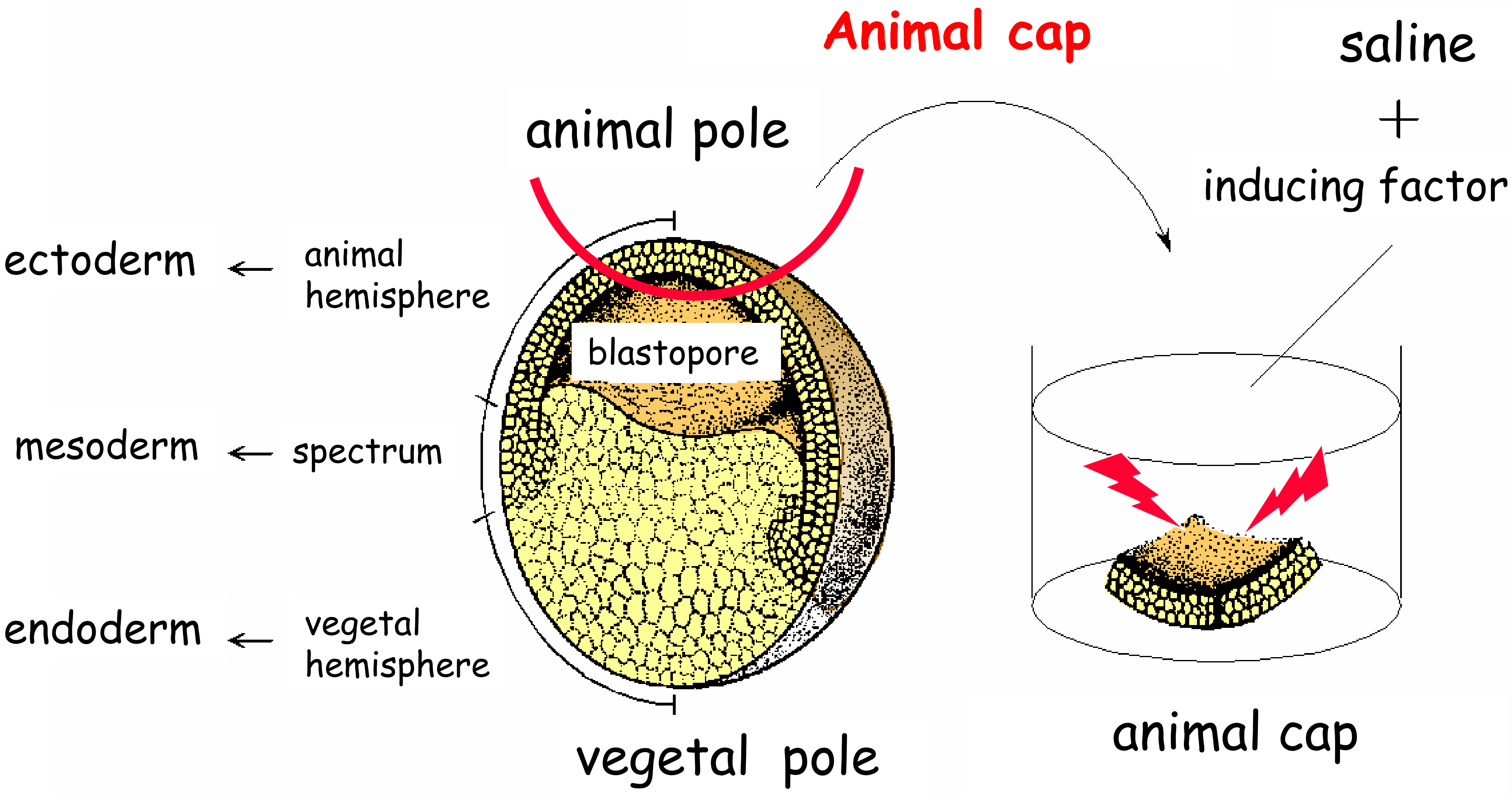


Xenopus laevis
newt
(amphibian)

chicken
(avian)

mouse
human
(mammal)

Animal Cap Assay



blastopore (vertical section)

Until 1950s, more than half of researches on development were on induction.
However, in 1981 meeting of " International Society of Developmental Biologists", presentations directly on induction were only 2. (This seems abnormally few.)
Anyway, research on induction has obviously declined.
Importance of the theme is clear still,
so why had researchers lose their interest in this in such a short time?

Setsuto Okada
"Blueprint of the body ~from planarian to humans~"
Iwanami books 358

History of researches on organizer and embryonic induction is, to stretch the point a bit, the document of sweat and effort. In spite of its unfortunate history, functions of organizer during development and induction remain important still.

Maybe, we cannot expect someone to confront issues on organizer and embryonic induction, and get them understood in some kind of experiment ever after in the future.

Research from totally different aspect or on a different concept may somehow discover functions of organizer, and find some sort of resolution.

Setsuto Okada
"Blueprint of the body ~from planarian to humans~"
Iwanami books 358

Germany, Free University of Berlin, Lab of microbiology

Newt collecting when I was the researcher in the Lab. of Microbiology at Free University of Berlin

From International Journal of Developmental Biology (Vol. 40, 113-122, 1996)

Book covers of

H.Spemann"Experimentelle Beitrage Zu Einertheorie Der Entwicklung"

O.Nakamura and I. Kawakami "Organizer"

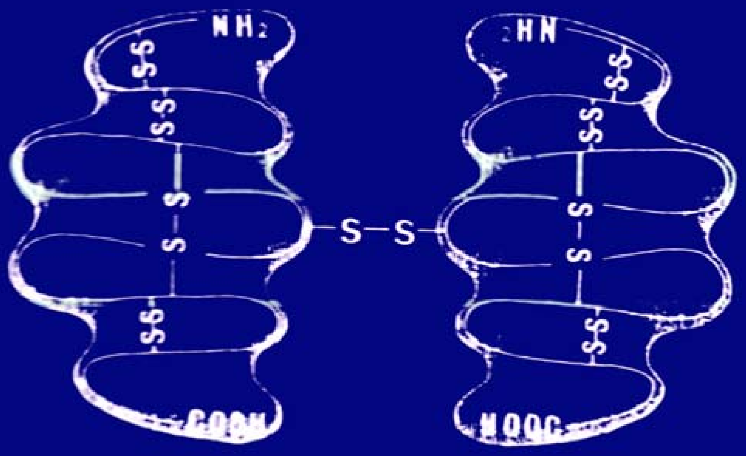
O.Nakamura and S.Toivonen "Organizer"

inserted here were omitted according to copyright issues.

1936 1977 1978

In this time,
researches on induction were at the climax of misfortune.

Structure of Activin A



Molecular weight : 25,000 (12,500 X 2)

homodimer

Amino acids : 232 (116 X 2)

SH = 18

Theses presenting that activin is the inducing factor of mesoderm

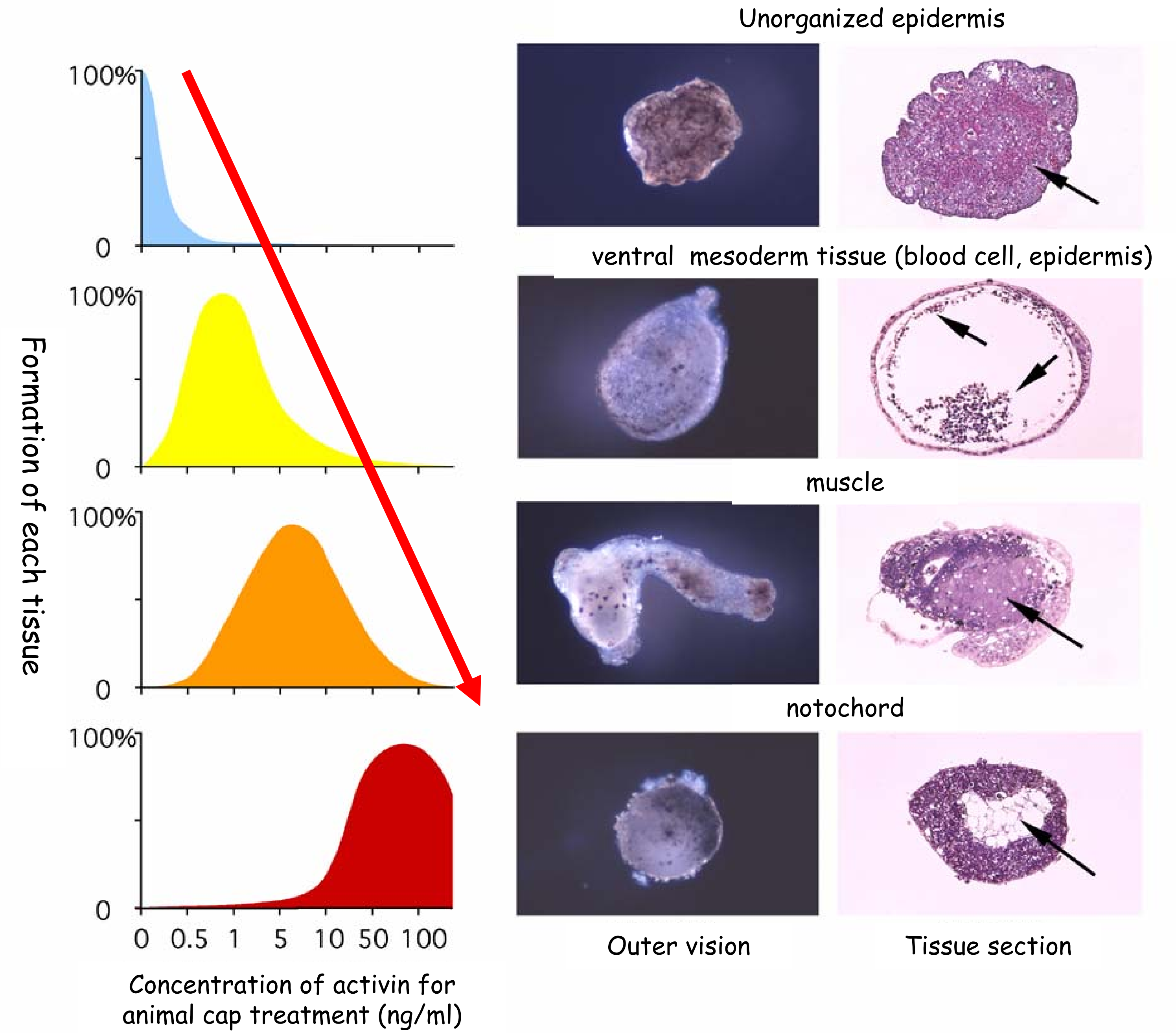
1. 1989 Sept. Asashima et al. MI factor (Japan)
2. 1990 Feb. Asashima et al. MI factor (Japan)
3. 1990 June J. C. Smith et al. MIF factor (U.K.)
4. 1990 June A. J. M. Van den Eijnden et al. MIF factor (The Netherland)
5. 1990 Aug. D. A. Melton et al. PIF factor (U.S.A.)
6. 1990 Aug. H. Tiedemann, Asashima Vegetalizing factor (Germany, Japan)

The inducer, searched for about 75 years in the world, was discovered to be the protein called activin.

However, I still think my cynical attitude in this writing was not meaningless. This is because quite a few actors (genes!) in induction are listed up in recent, revived researches. Needless to say, **it is almost clear that growth factor works in mesoderm induction, and products of homeogenes work in nerve induction (by organizer). Especially, functions of activin in the former stand out.**

Setsuto Okada
"Blueprint of the body ~from planarian to humans~"
Iwanami books 358

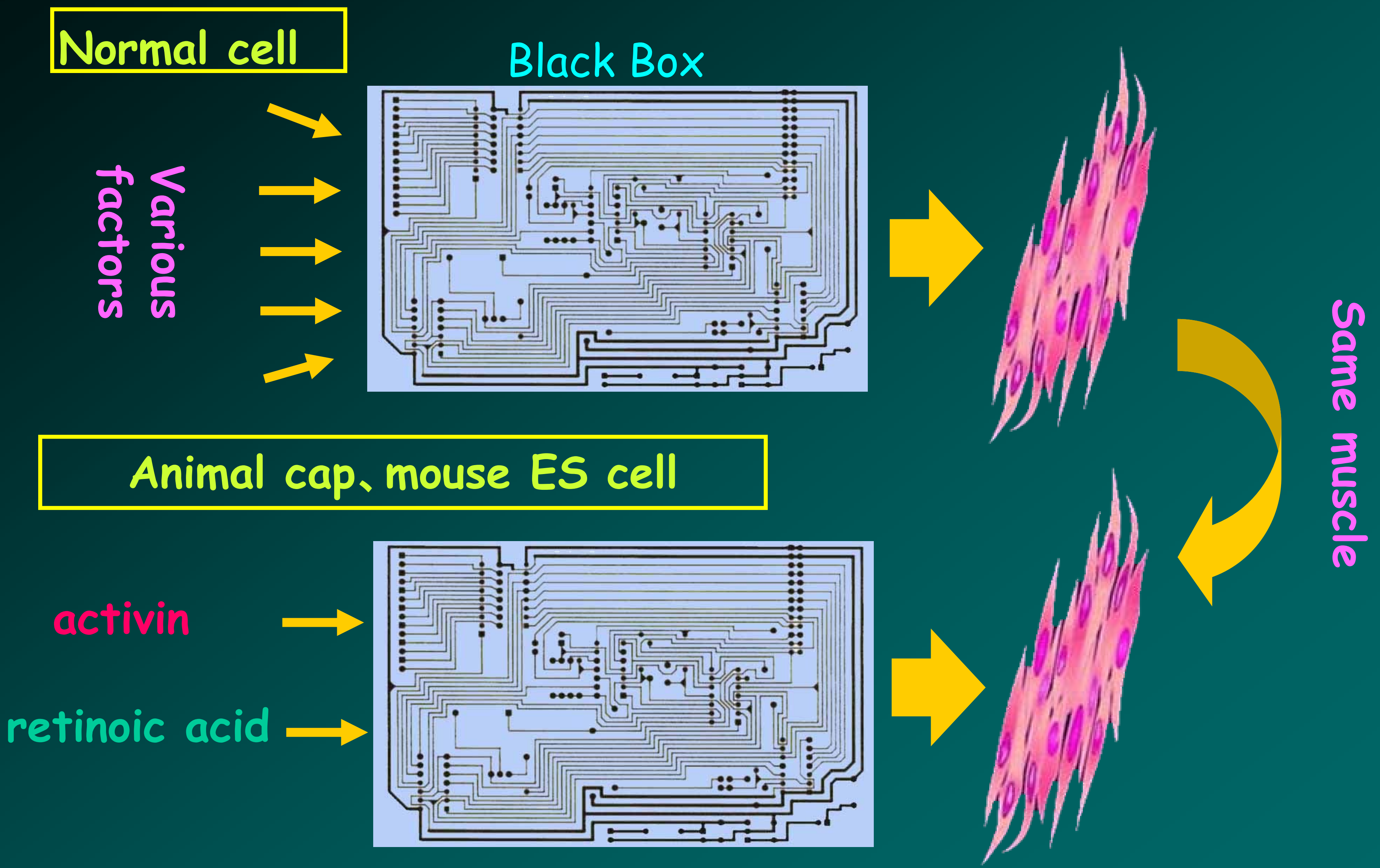
Concentration dependent mesoderm differentiation of activin-treated animal cap



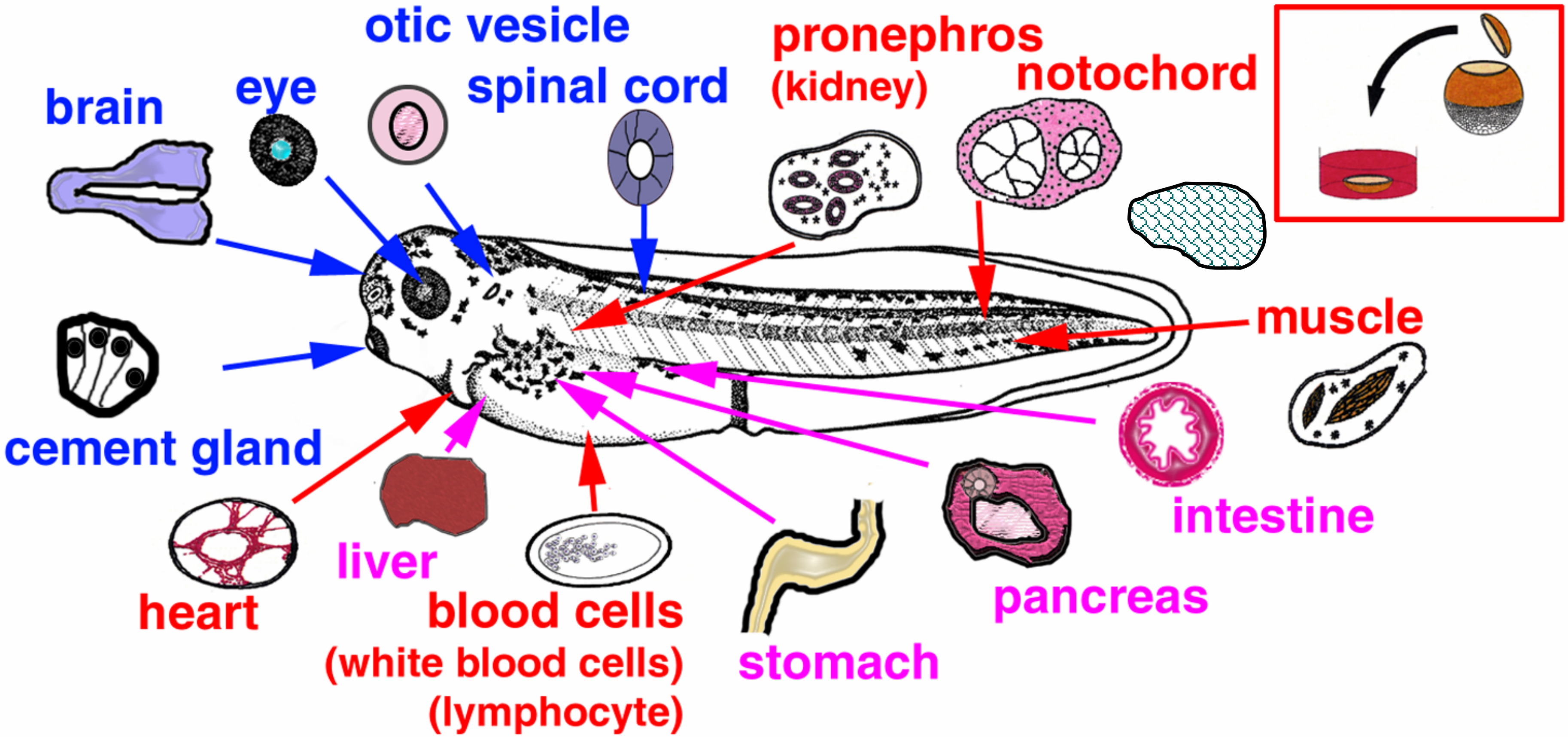
Extending movement and muscle differentiation of activin-treated animal cap

The fact that this activity is responsible for genes for muscle development to be expressed in order was discovered.

Organ formation models



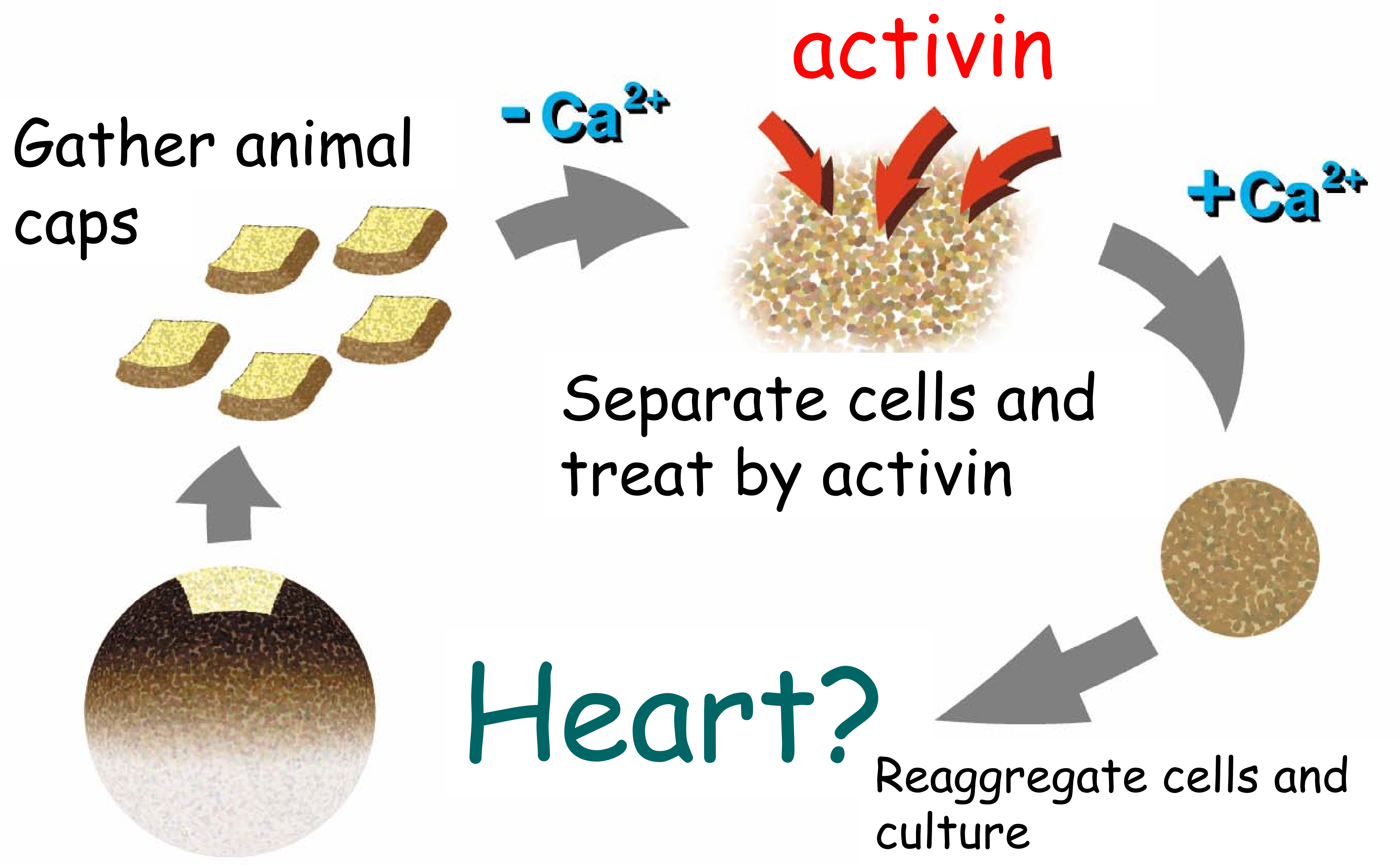
Control of organogenesis *in vitro* using *Xenopus* animal cap



22 organs and tissues were made from undifferentated cell

Heart formation from amphibian undifferentiated cell (animal cap) and transplantation

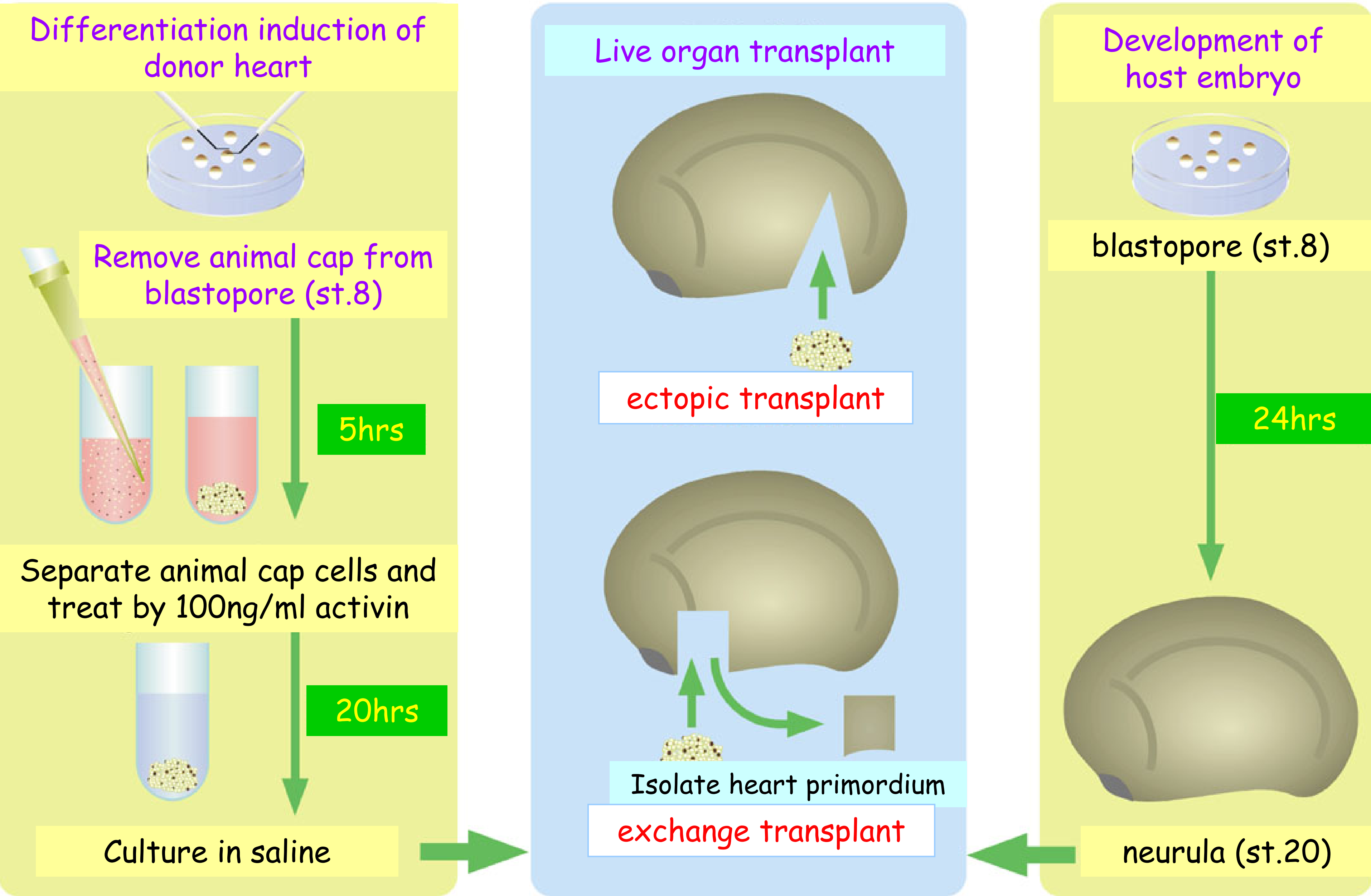
Make heart from Xenopus animal cap



Pumping of the heart made from animal cap

The heart formed by 100 ng/mL activin treatment

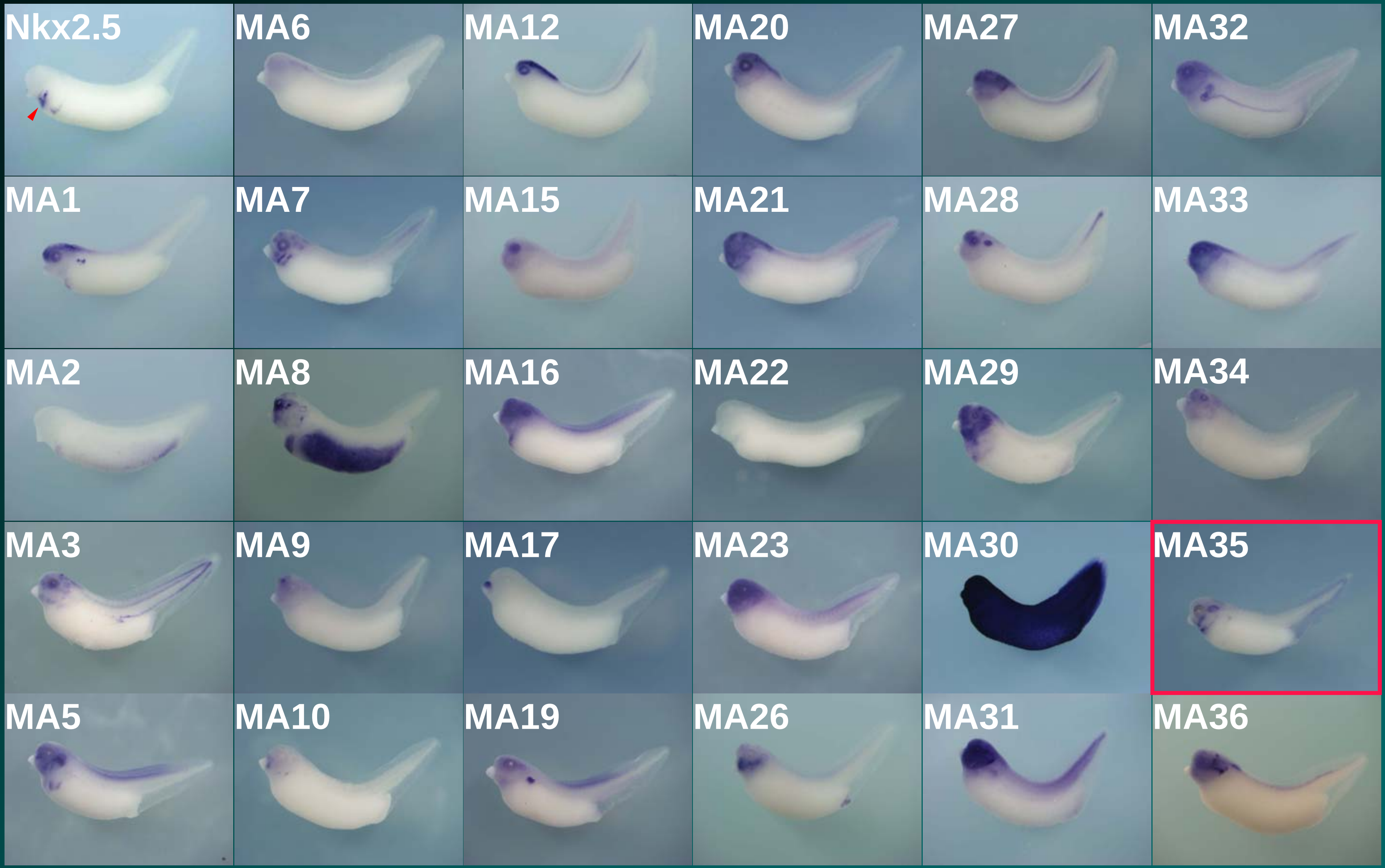
Live organ transplant of heart primordium induced in vitro



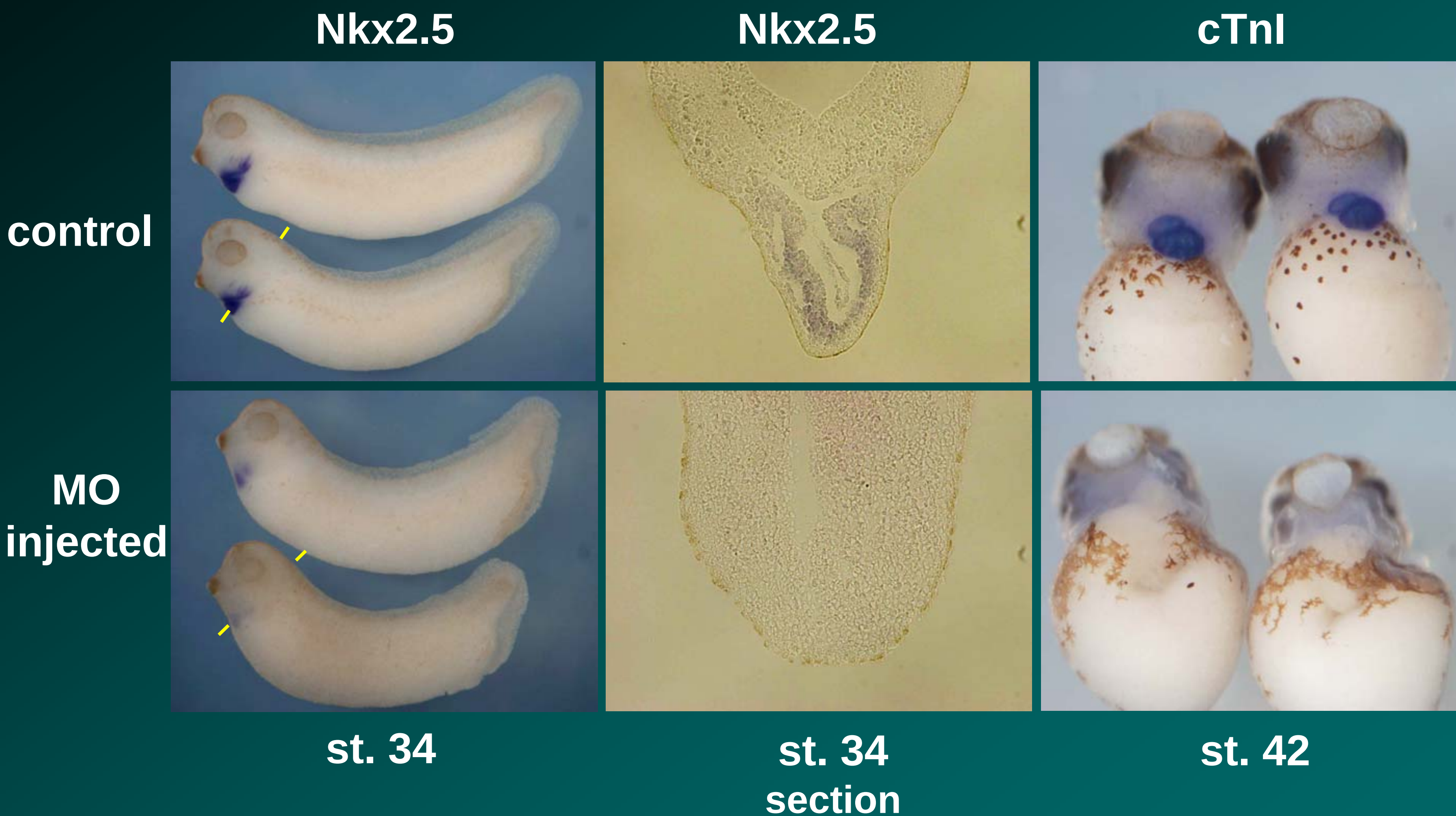
Embyo transplanted with a pumping tissue induced from animal cap

Heart ectopically transplanted larva

Search for heart related gene by *in situ* hybridization



Heart deficiency caused by functional inhibition of MA35 gene ①



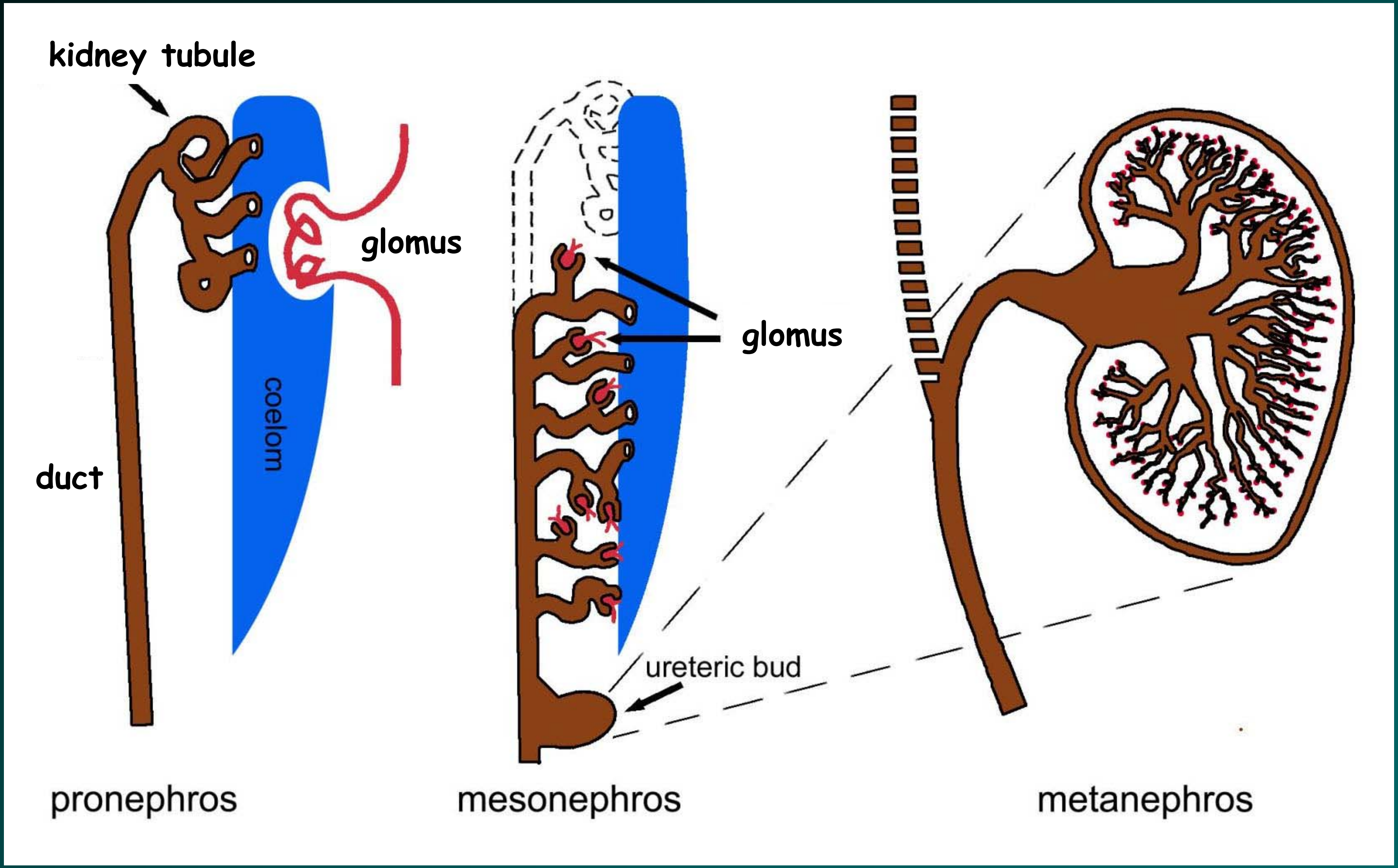
Heart deficiency caused by functional inhibition of MA35 gene ②

control（normal embryo）

MA35 inhibited embryo （heart deficient）

Kidney forming from amphibian undifferentiated cell and transplant

Differentiation in a kidney

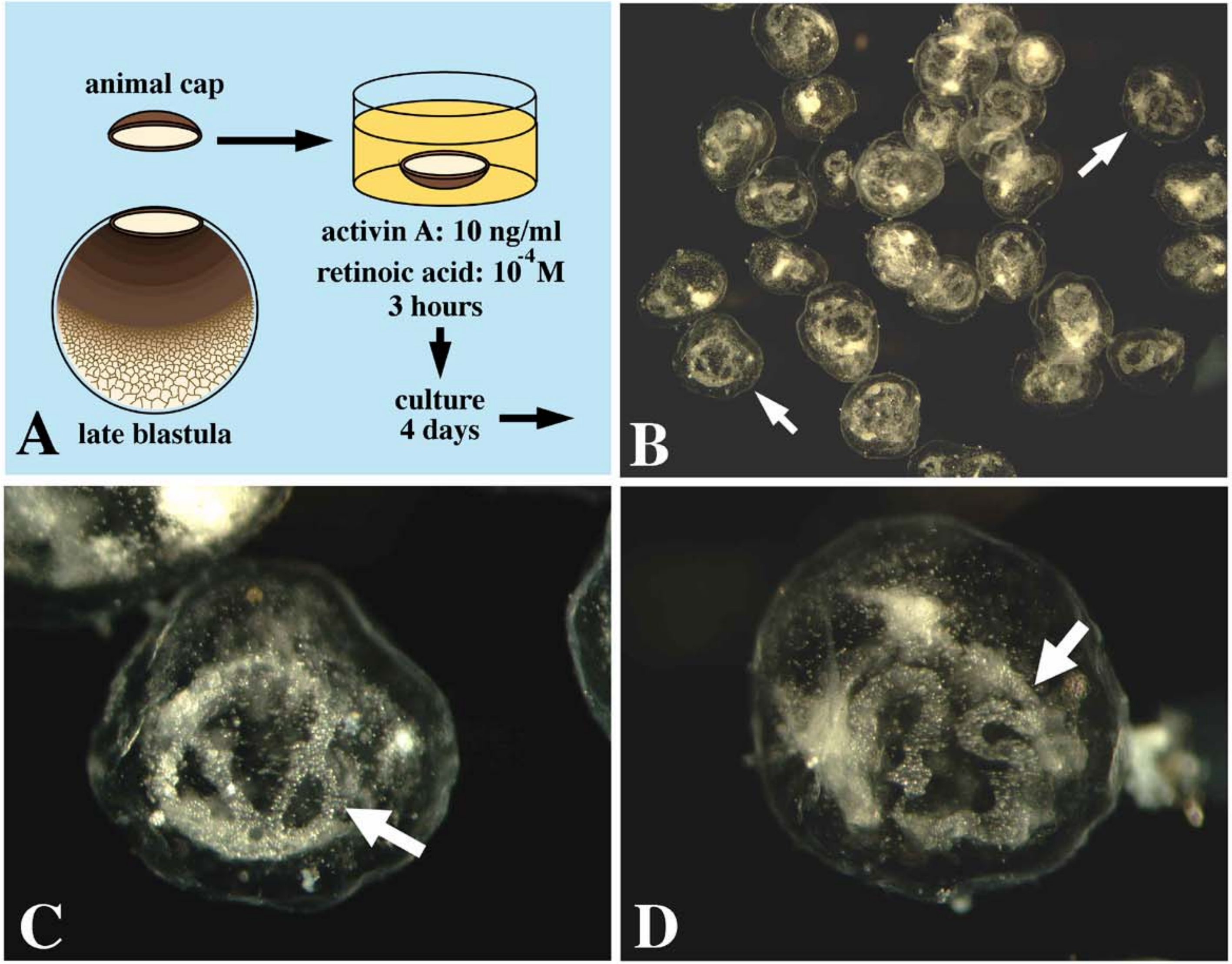


pronephros
(1 nephron)
tadpole

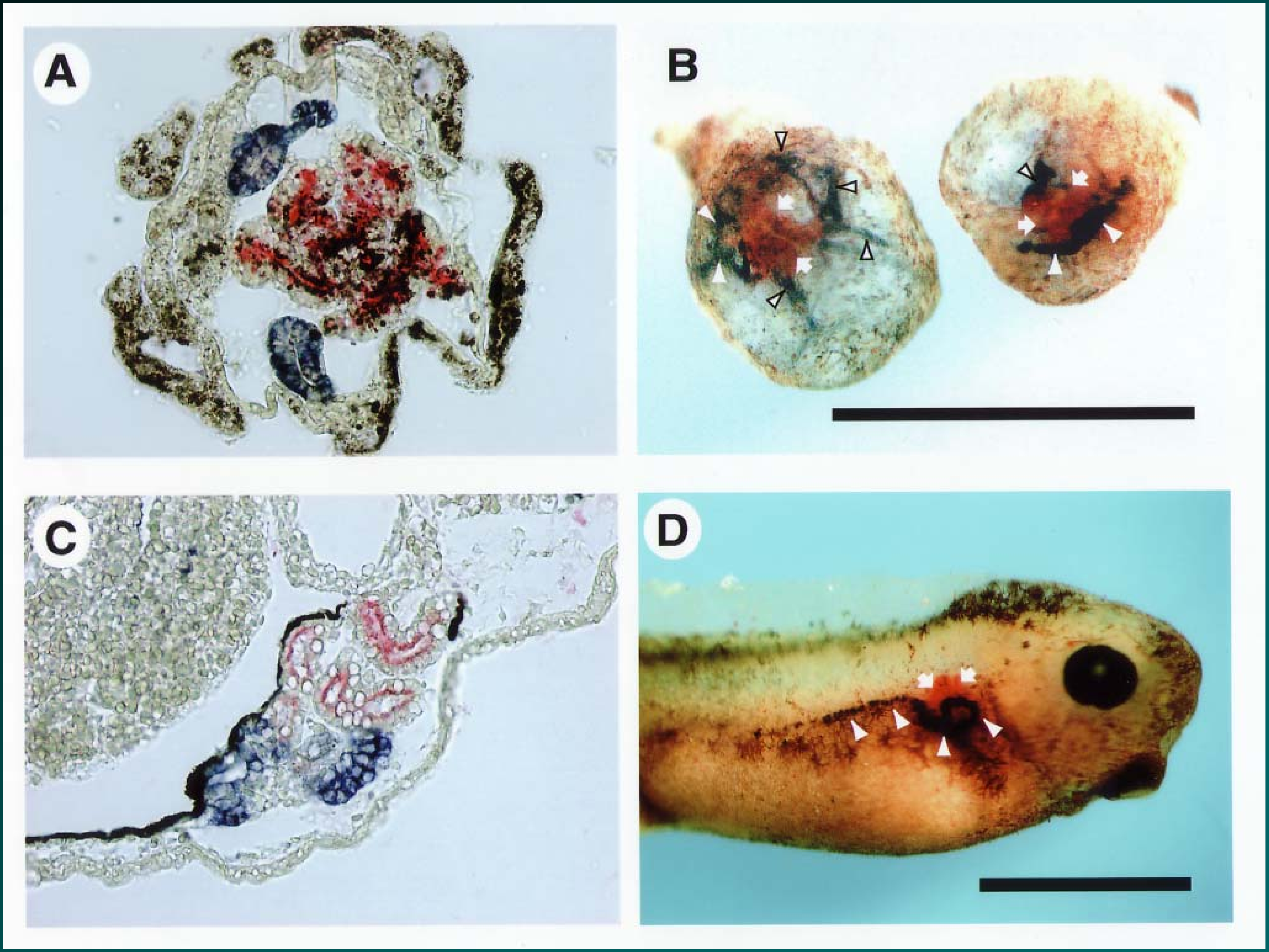
mesonephros
(30 nephrons)
adult frog

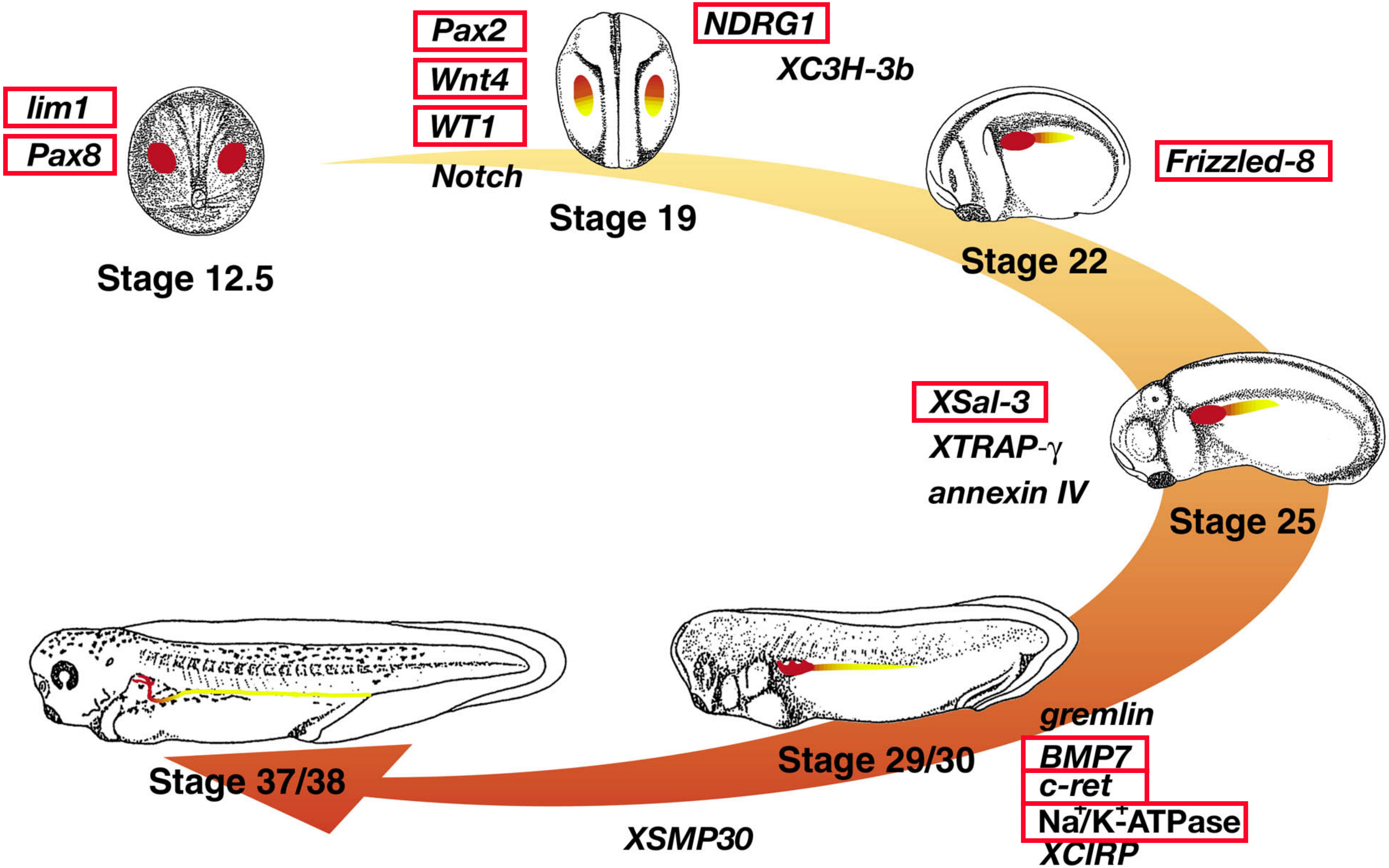
metanephros
(a million nephrons)
human, etc.

Pronephros forming from animal cap in vitro



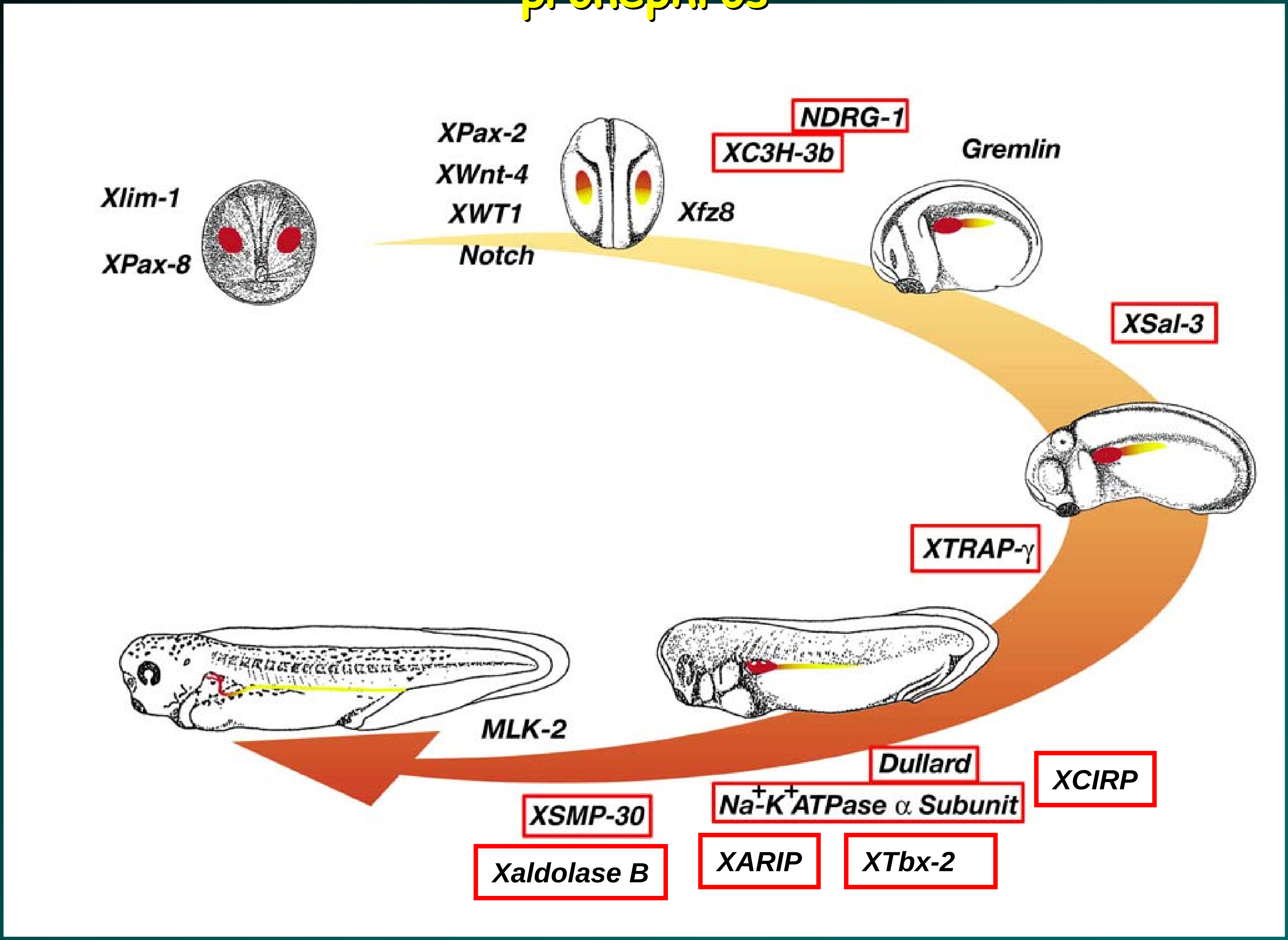
Forming of pronephros structure in vitro (A+B) and in vivo (C+D)



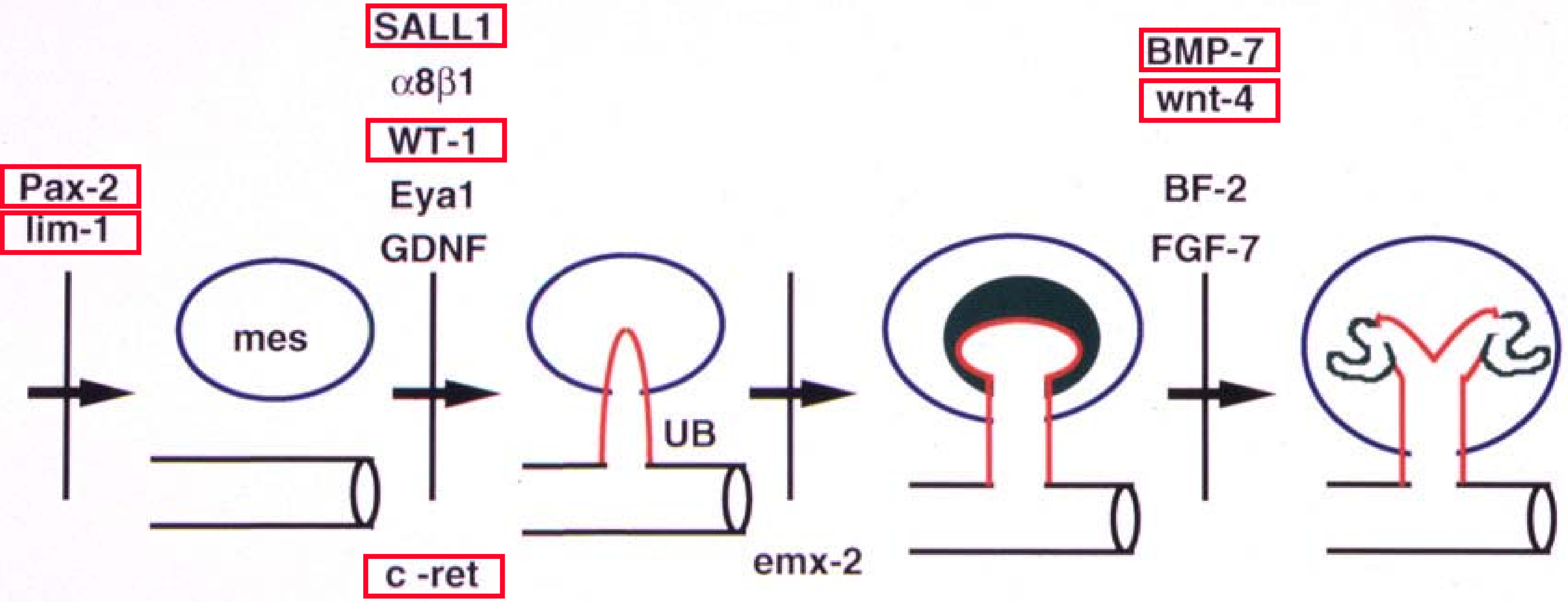


Kidney development and gene expression in amphibian (frog)

Gene expression in the development of a Xenopus pronephros



Kidney development and gene expression in mammals (human, mouse)

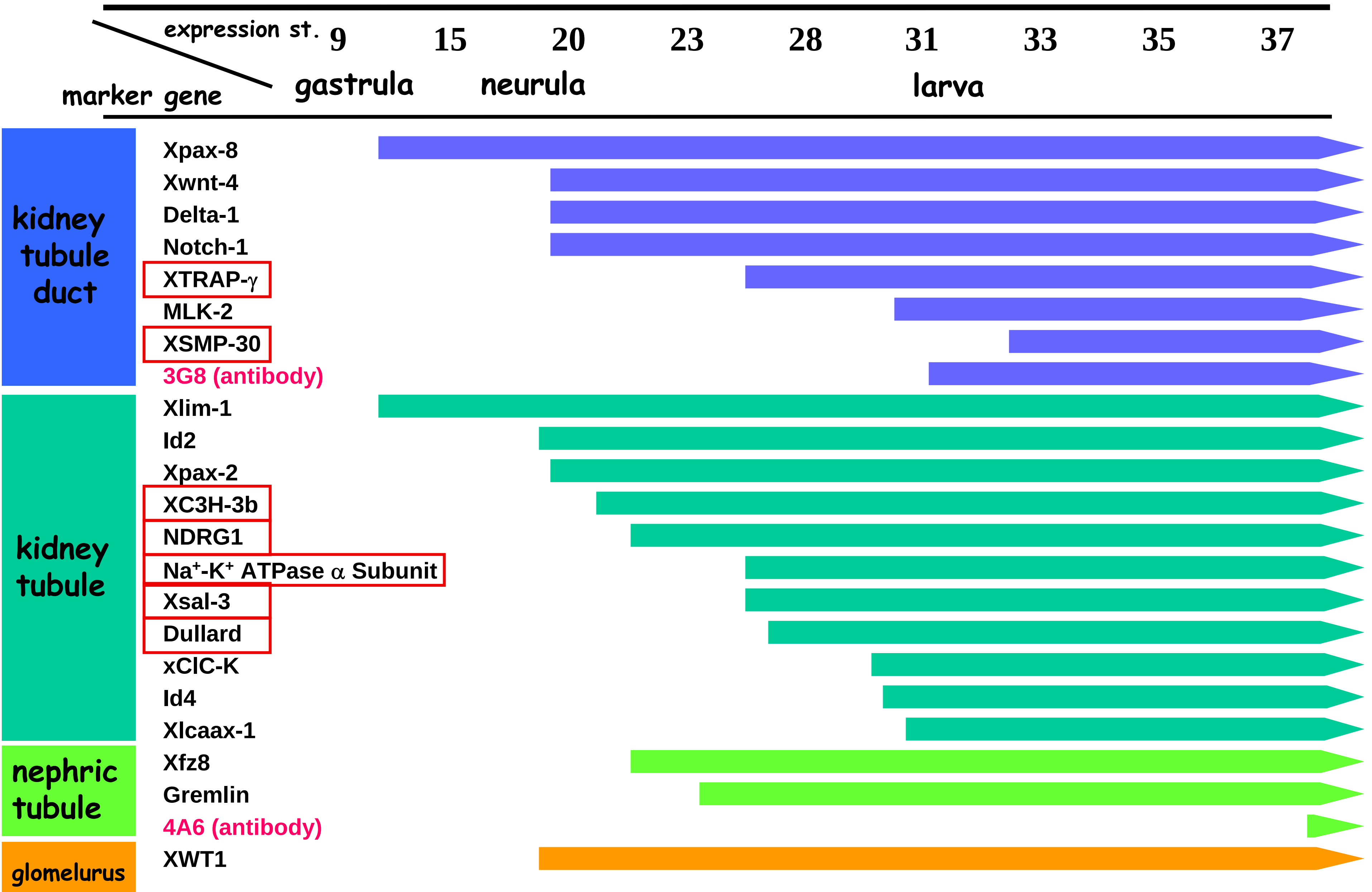


The article from "Asahi-Shimbun"

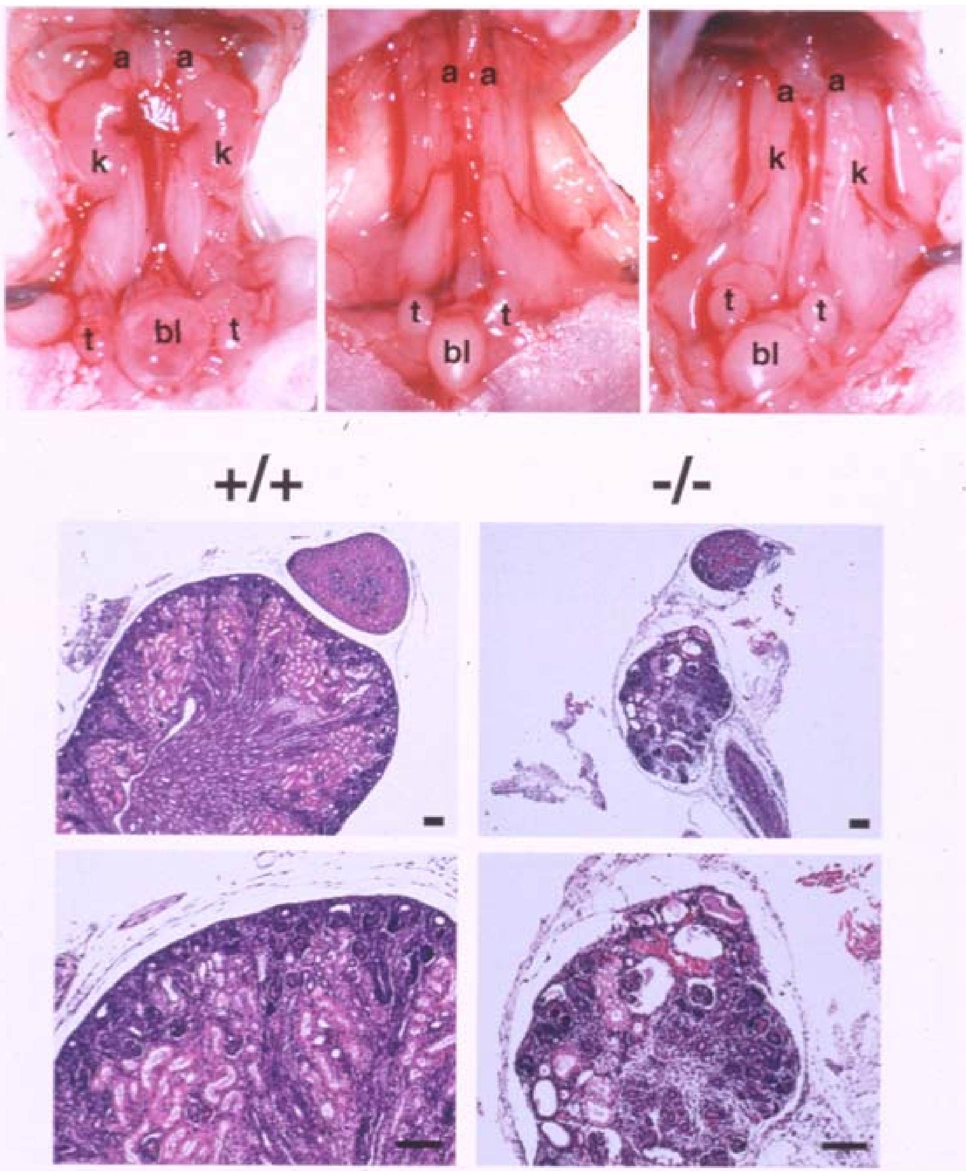
inserted here was omitted according to copyright issues.

Asahi-Shimbun
2007.2.14

Gene groups expressed during kidney development

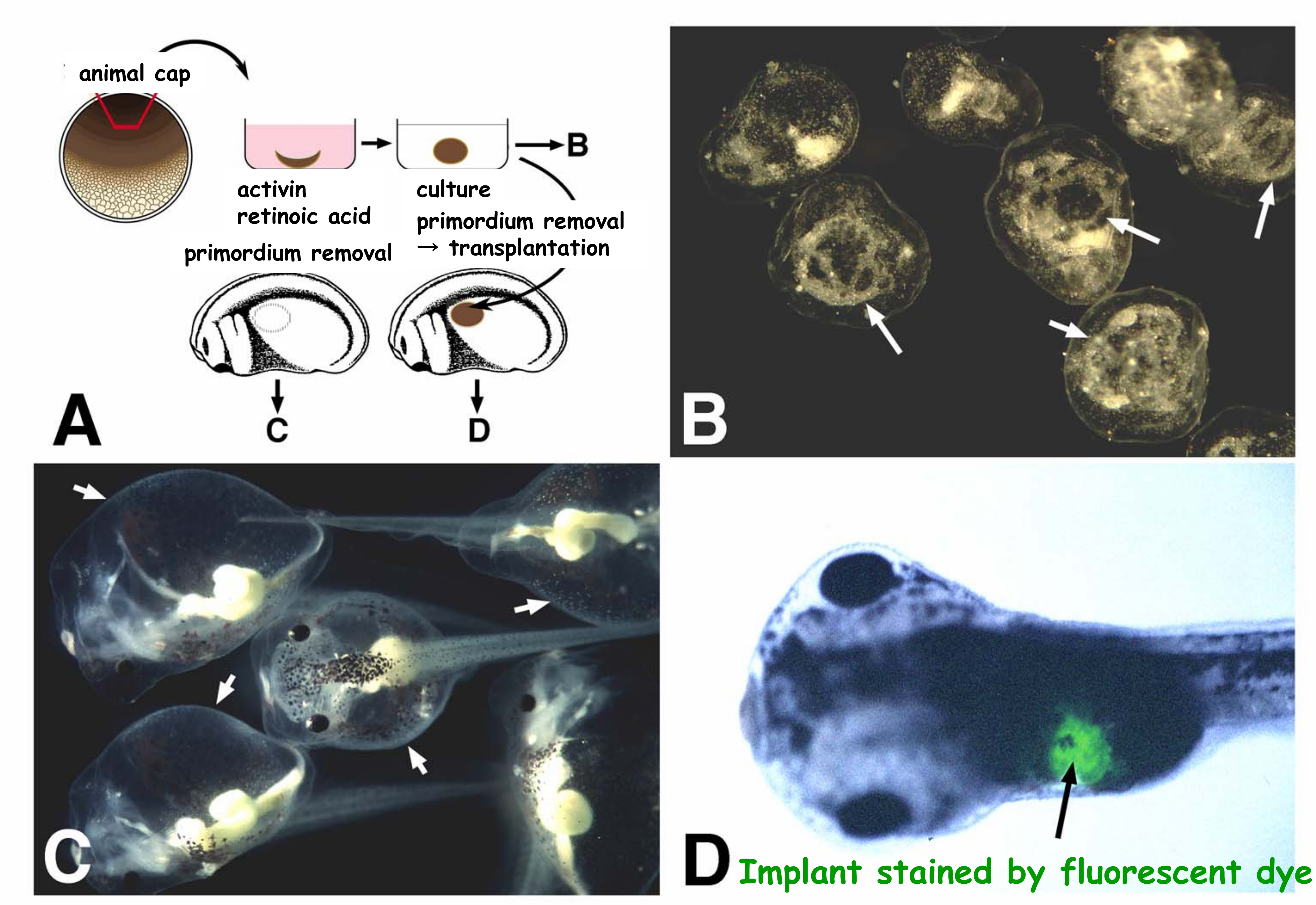


‡ mouse embryo kidney



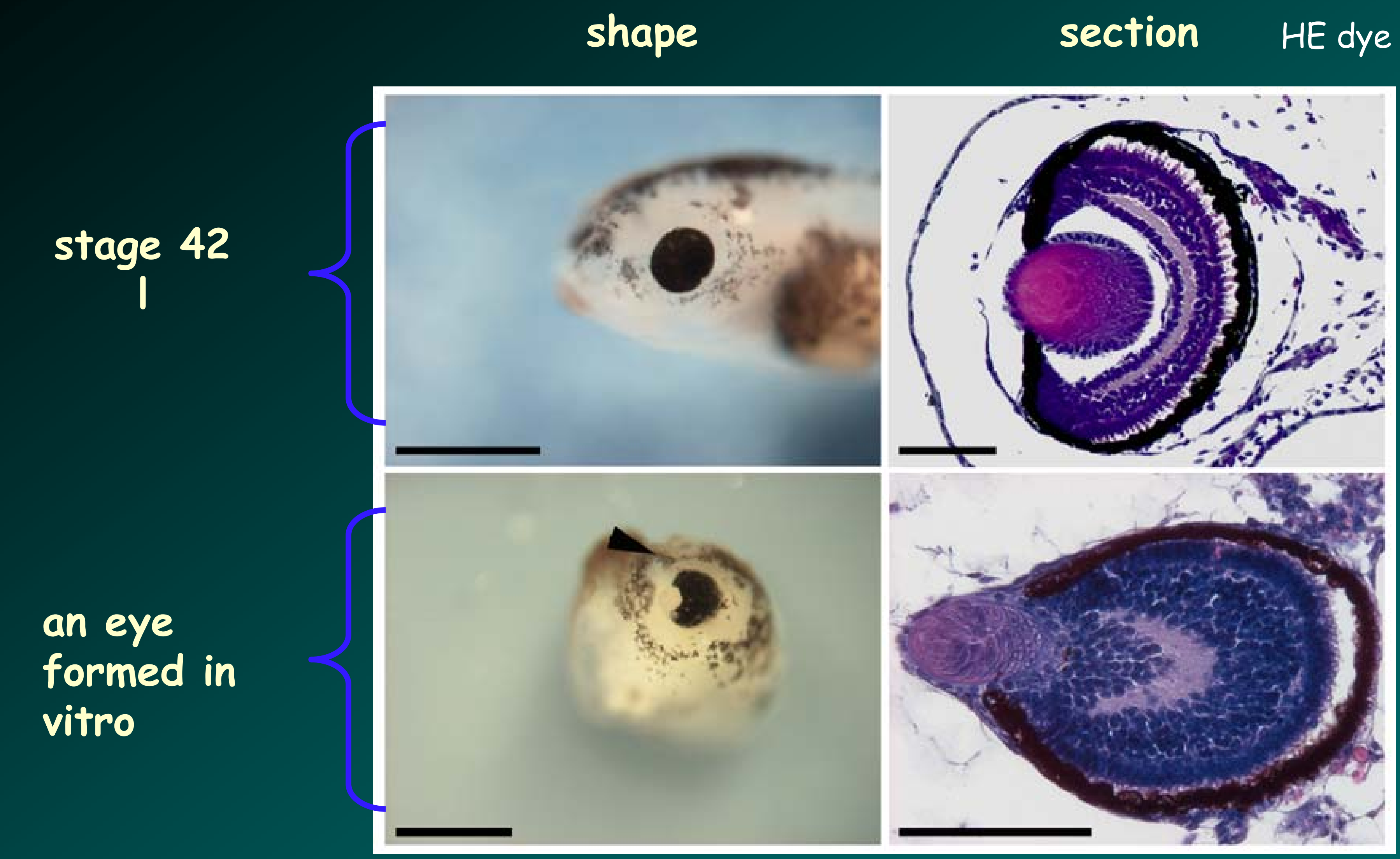
Nishinakamura R. et al., Development, vol 128, p3110-Fig.4, 2001

Transplanting experiment of kidney formed in vitro



Eye formation and transplant from undifferentiated amphibian cell

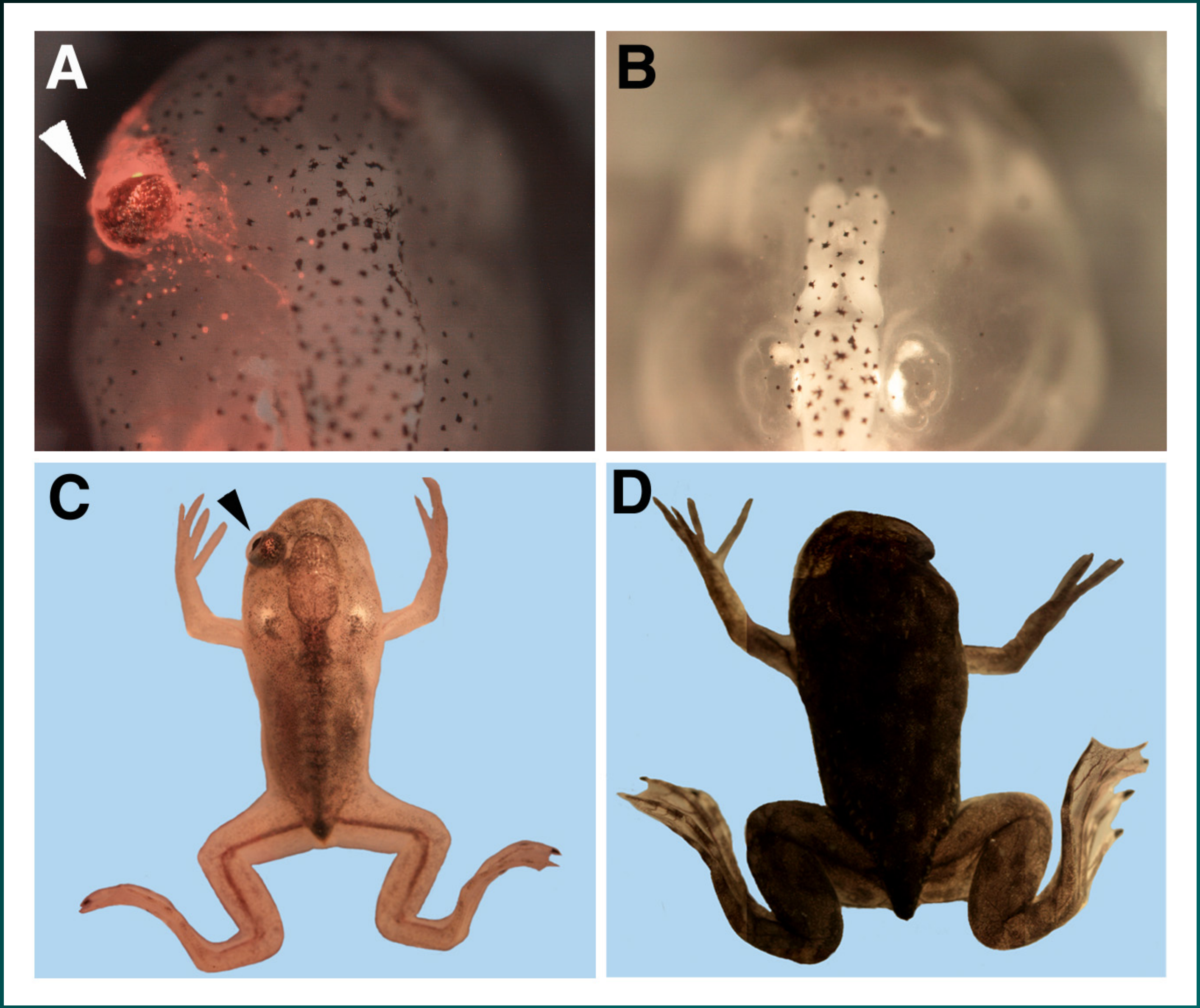
Eye induced from undifferentiated Xenopus cell : observation of tissue



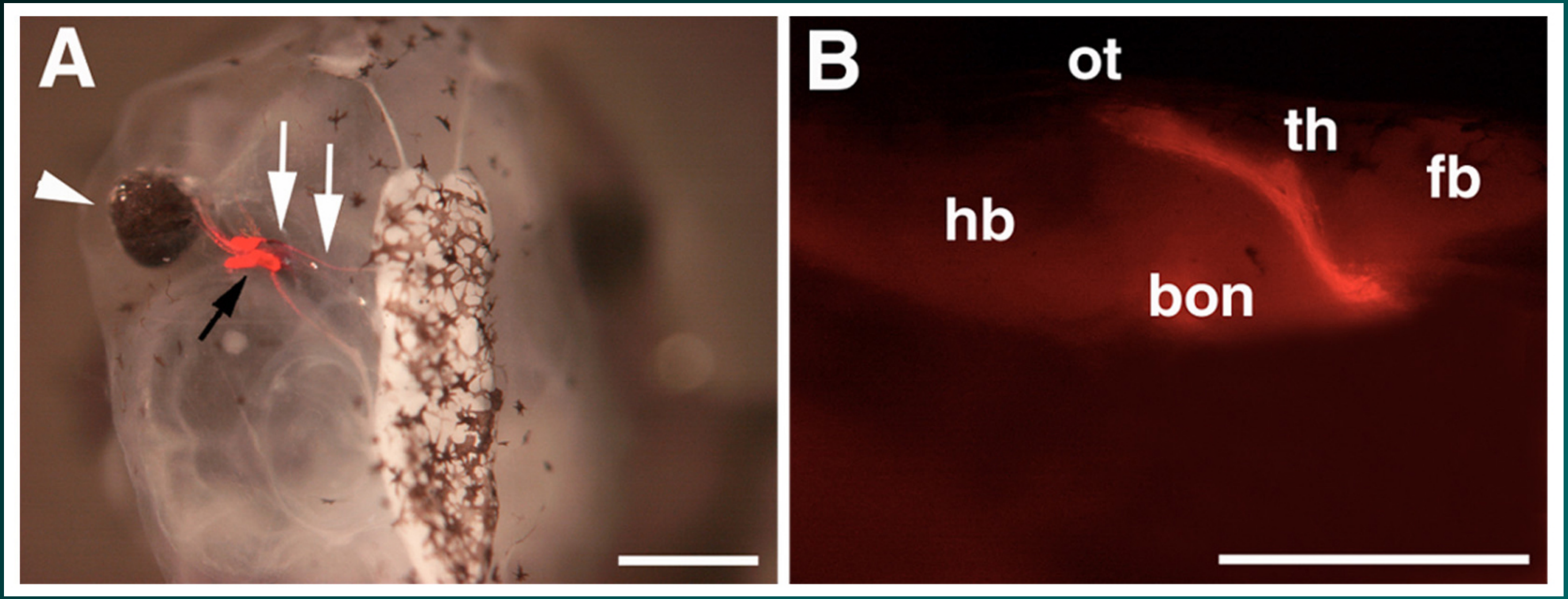
Eyes made in vitro have the same structure as an amphibian

Transplant eye formed in vitro

Remove both eyes



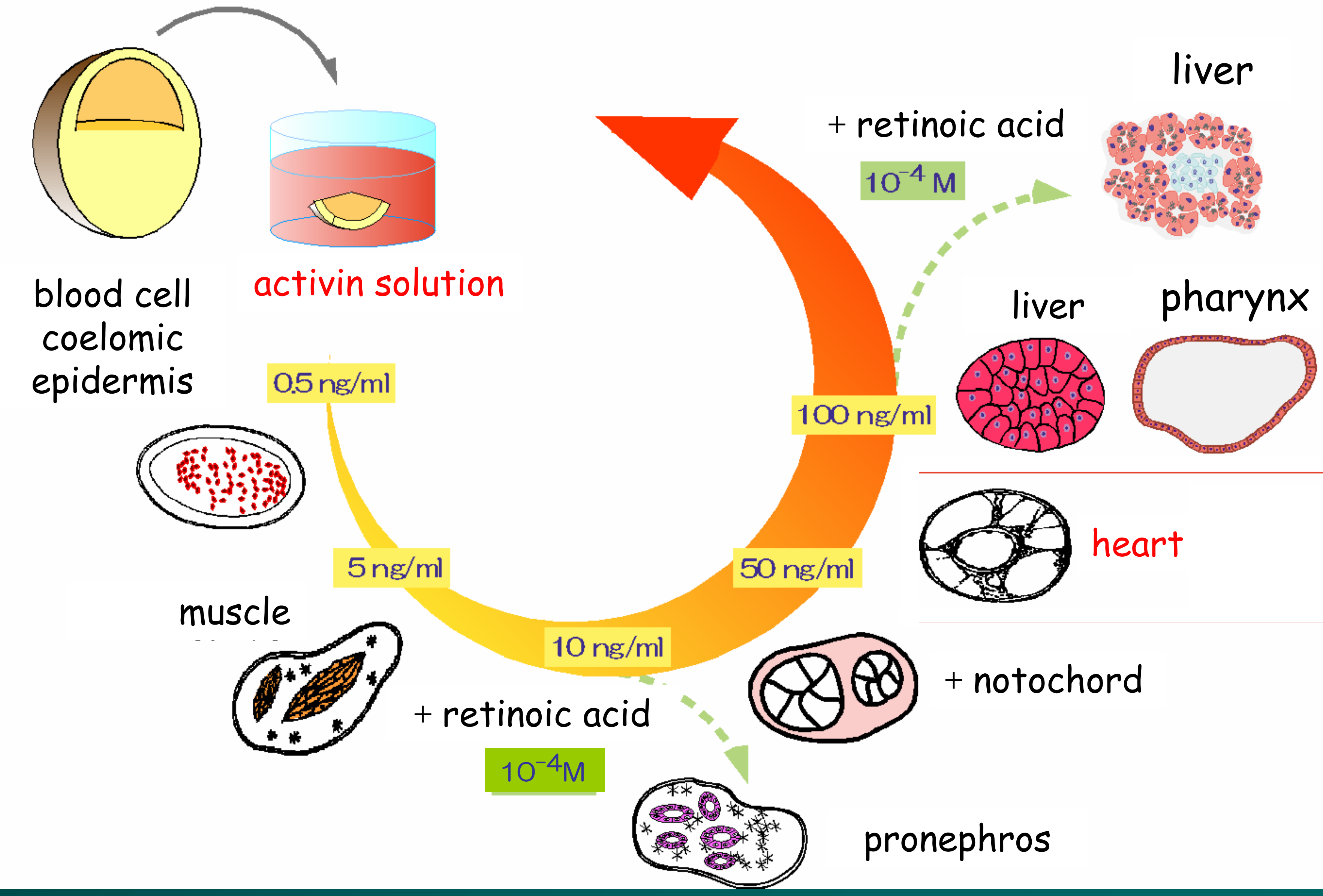
Connection of nerves between transplanted eye and optic tecta



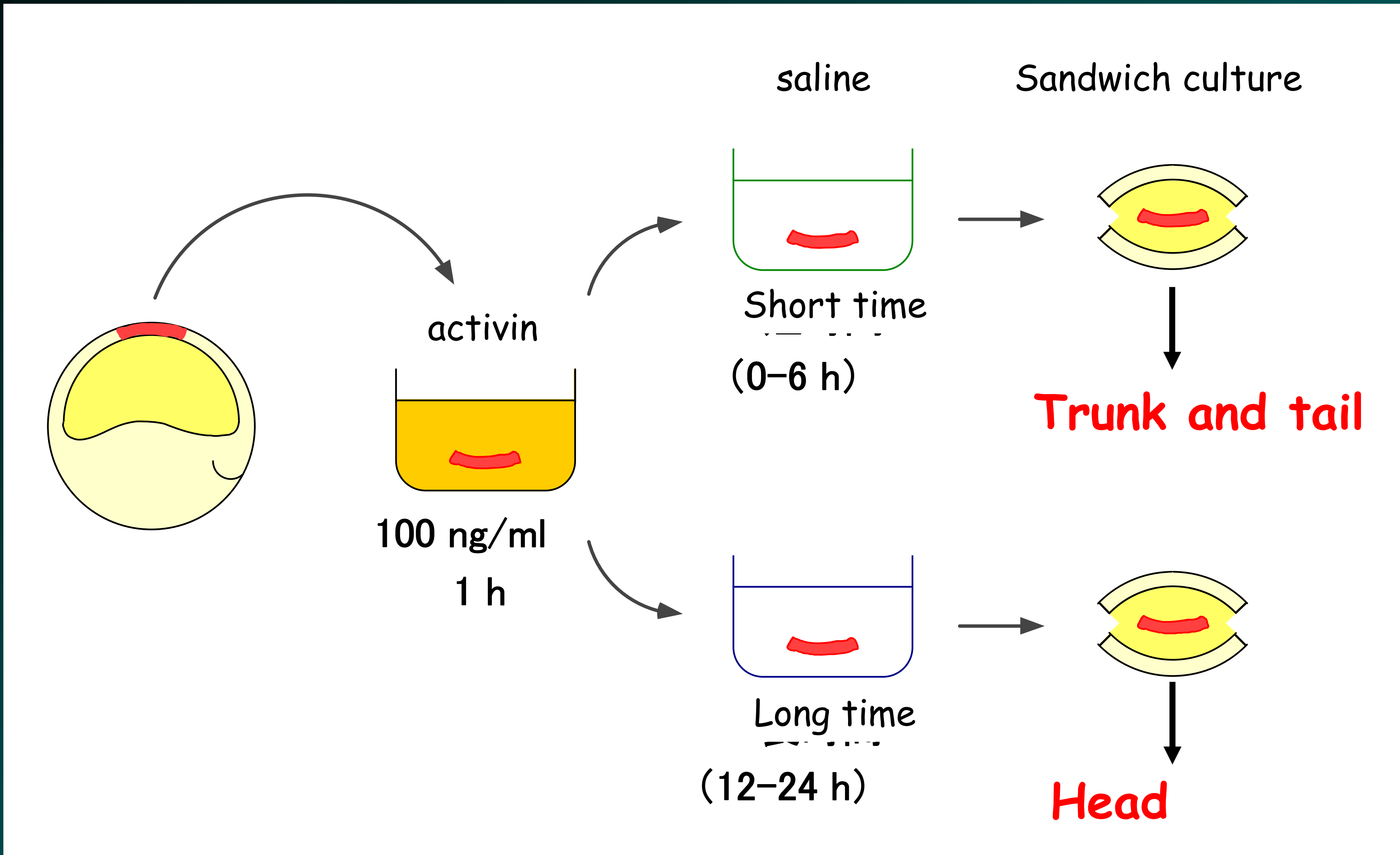
Upper view

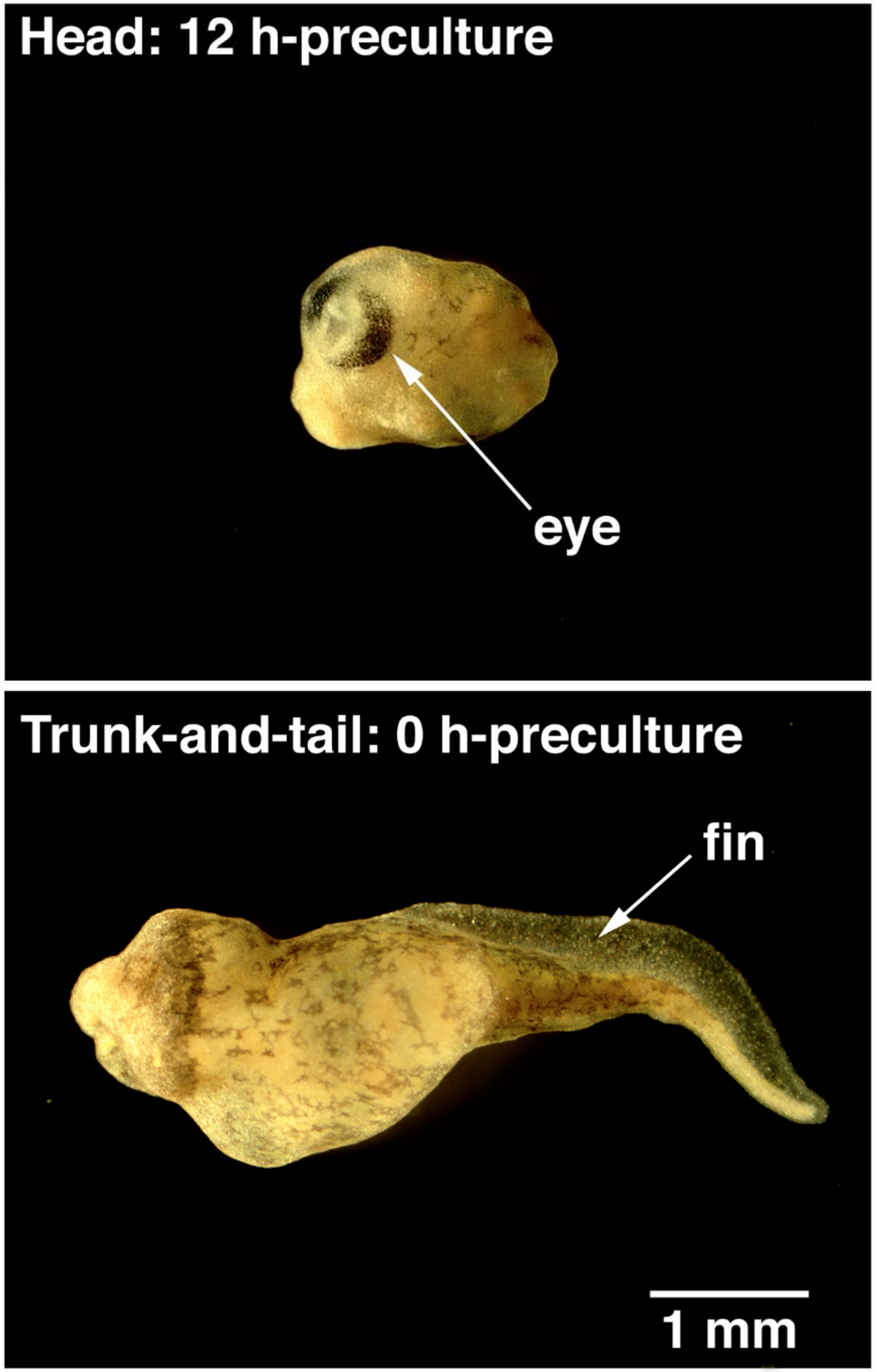
Side view

Organs and tissues formed from activin-treated animal cap

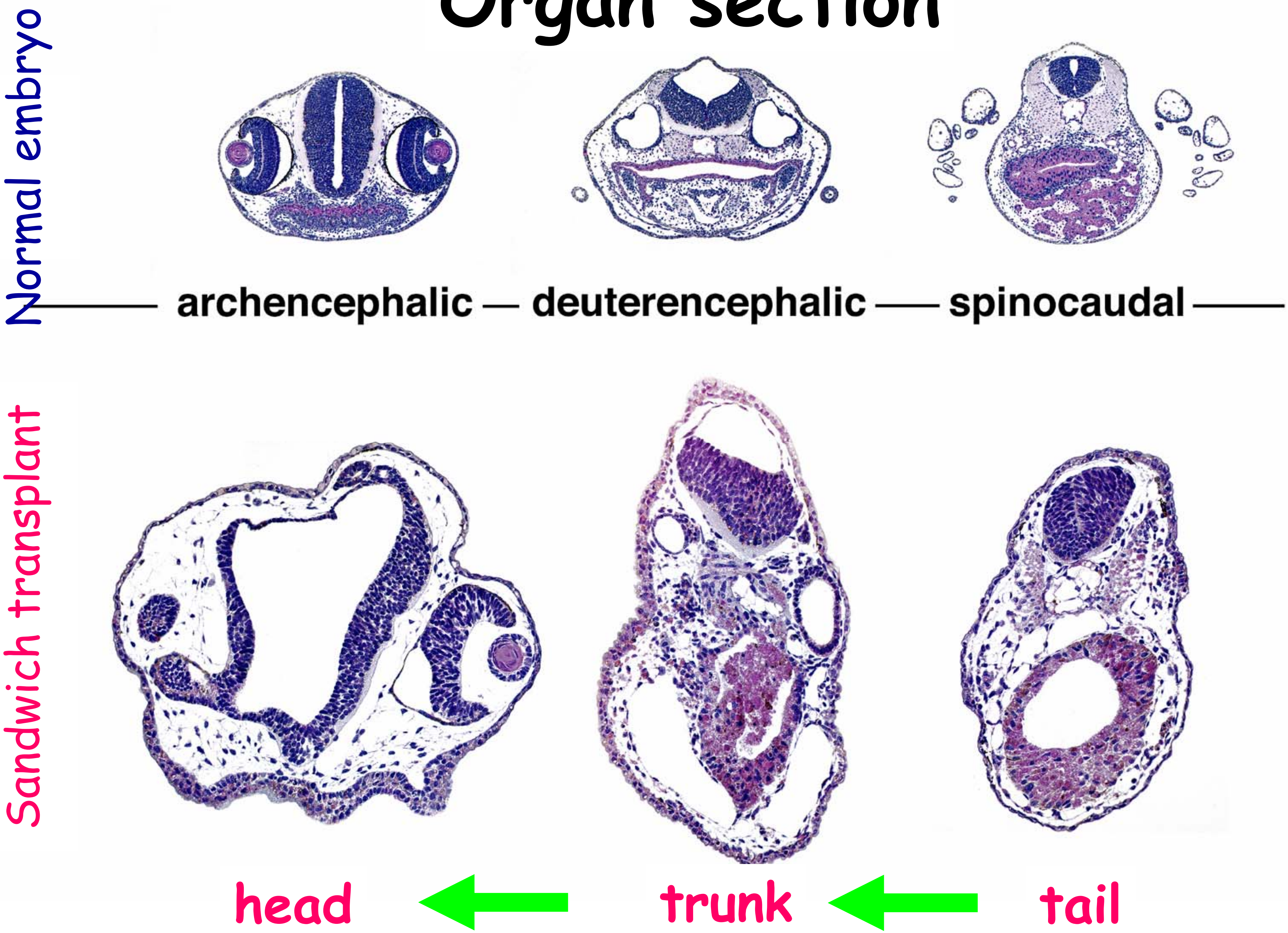


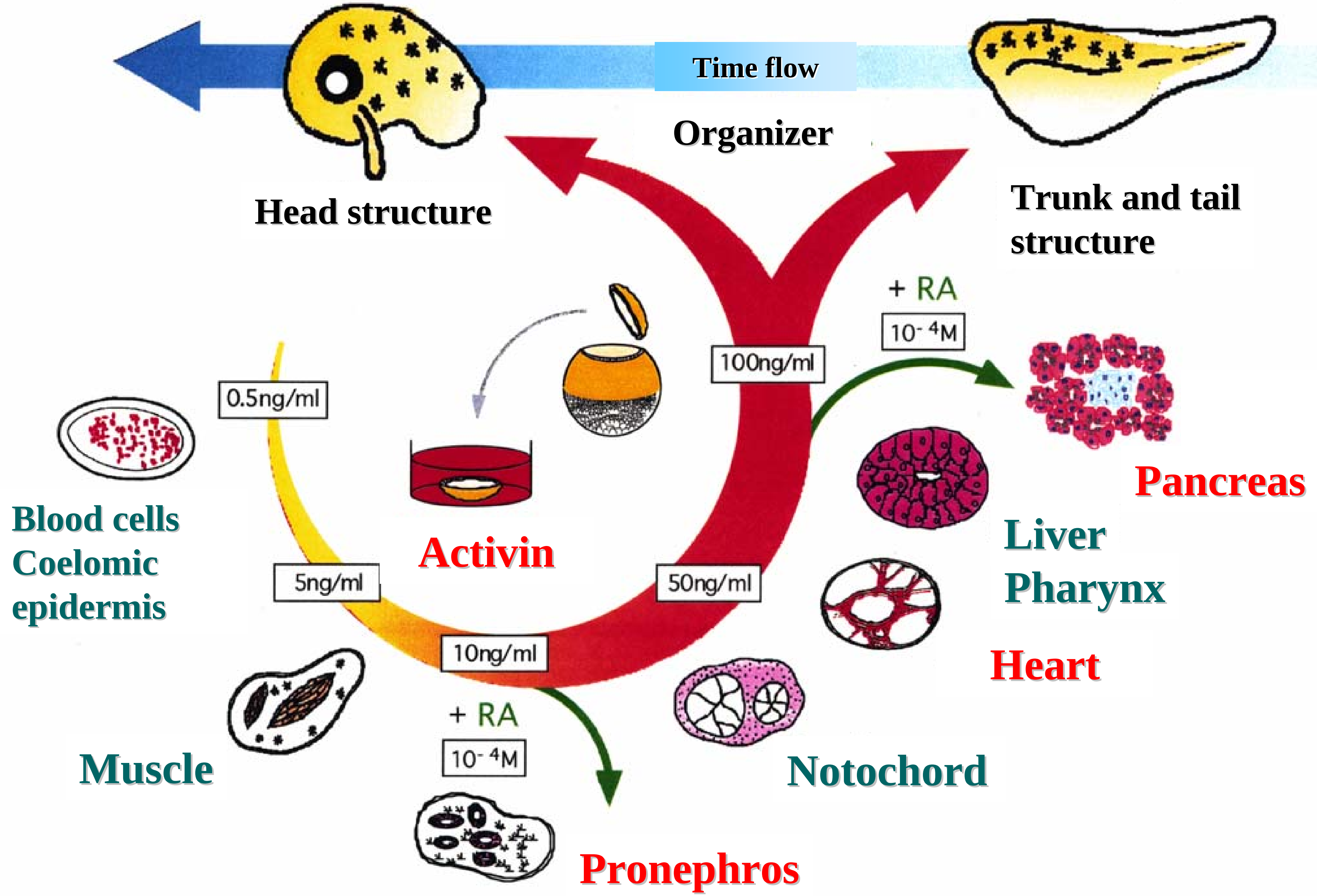
Experiment to make head part and tail part of larva in vitro



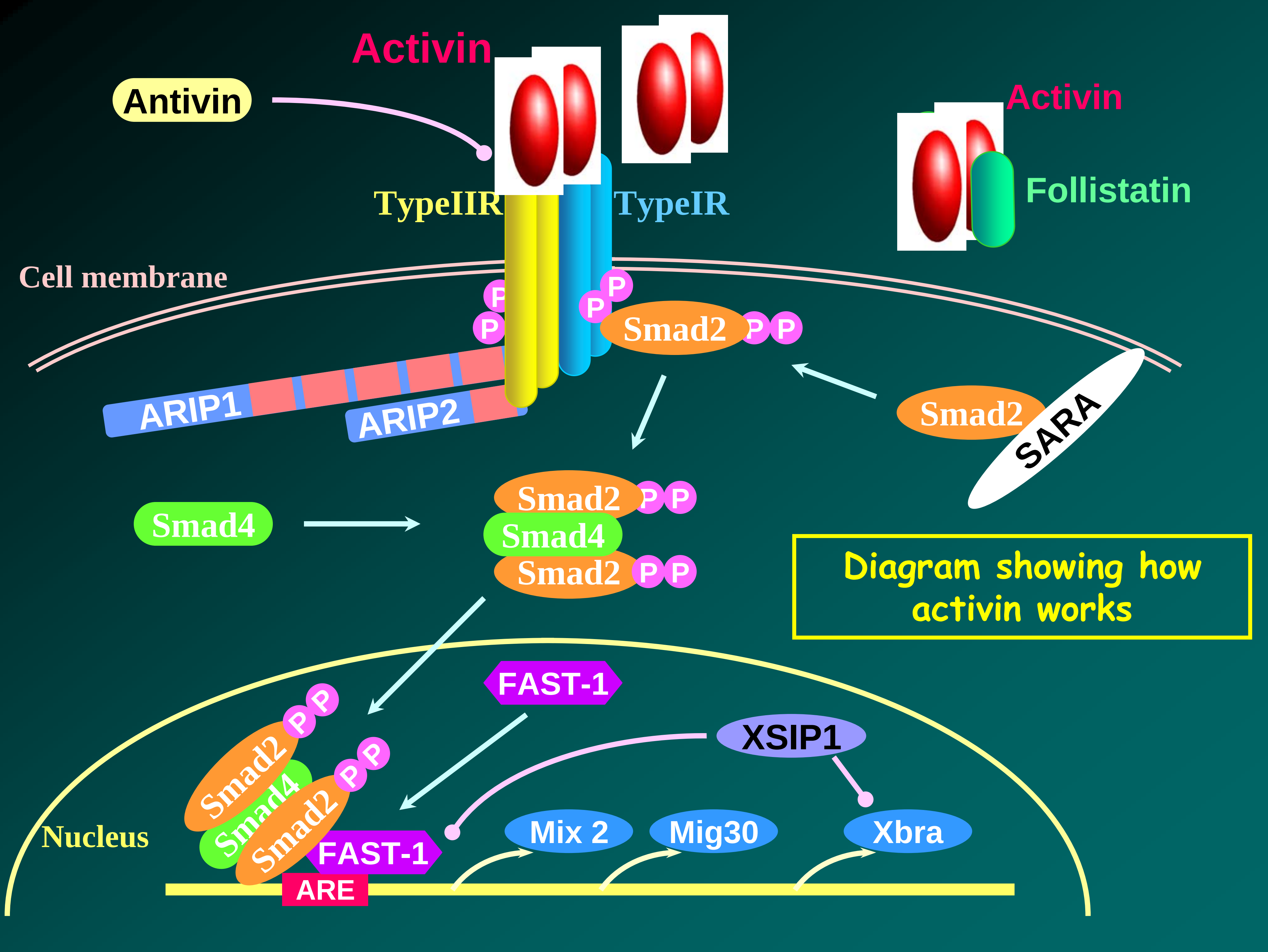

Head: 12 h-preculture
eye
Trunk-and-tail: 0 h-preculture
fin
1 mm

Organ section



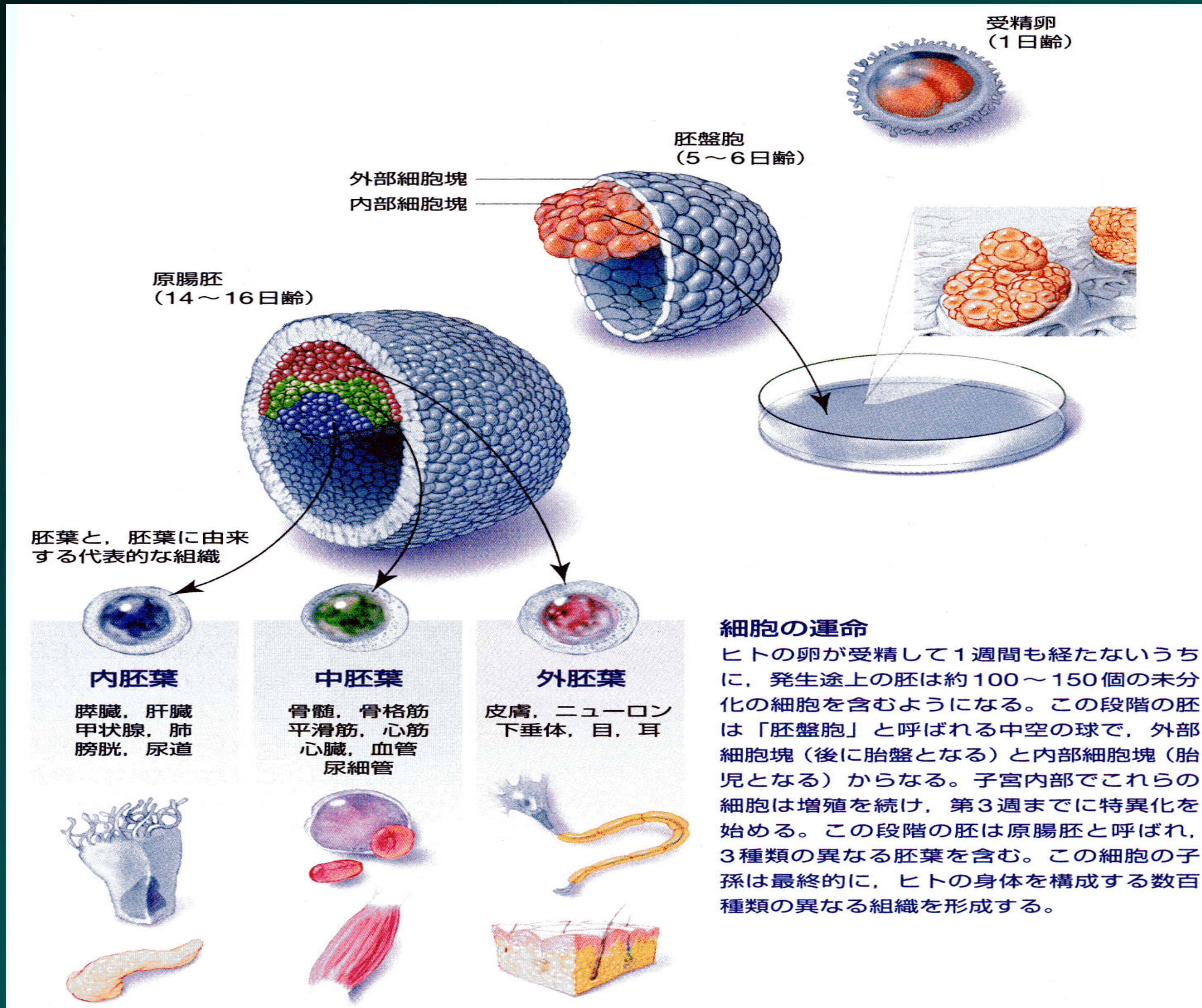


Time flow
Organizer
Head structure
Trunk and tail structure
+ RA
10-4M
100ng/ml
0.5ng/ml
Pancreas
Blood cells
Coelomic
epidermis
Activin
Liver
Pharynx
5ng/ml
50ng/ml
10ng/ml
Heart
+ RA
10-4M
Muscle
Notochord
Pronephros

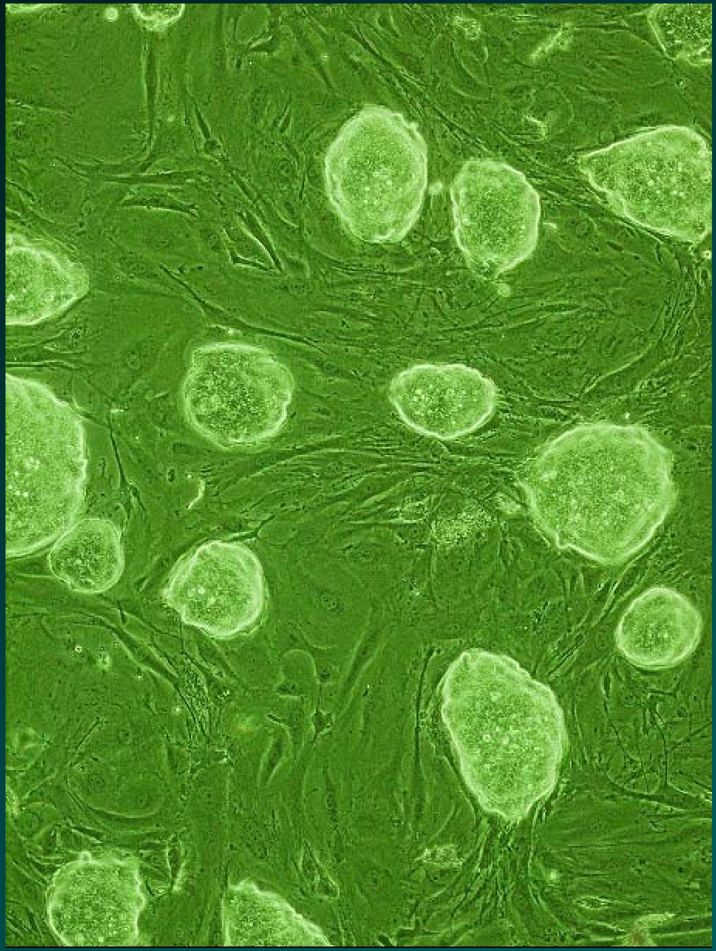

Activin
Antivin
TypeIIR
TypeIR
Activin
Follistatin
Cell membrane
P
P
P
P
Smad2
P
P
ARIP1
ARIP2
Smad2
SARA
Smad2
P
P
Smad4
Smad4
Smad2
P
P
Diagram showing how activin works
FAST-1
XSIP1
Smad2
P
P
Smad4
Smad2
P
P
FAST-1
ARE
Nucleus
Mix 2
Mig30
Xbra

Organ formation from undifferentiated mouse cell (ES cell)

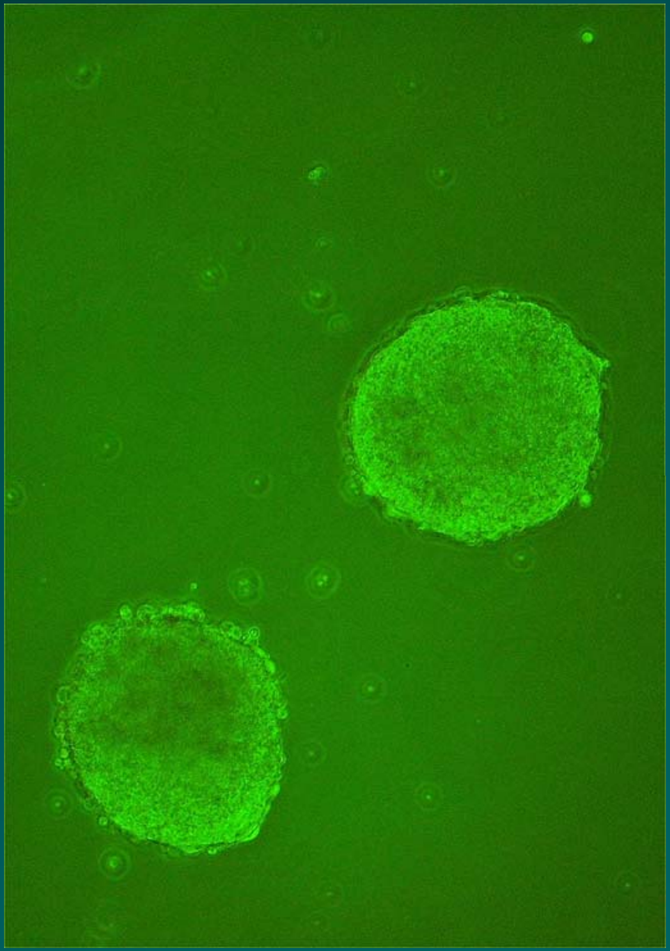
Embryonic stem cell（ES cell）



mouse ES cell and embryoid

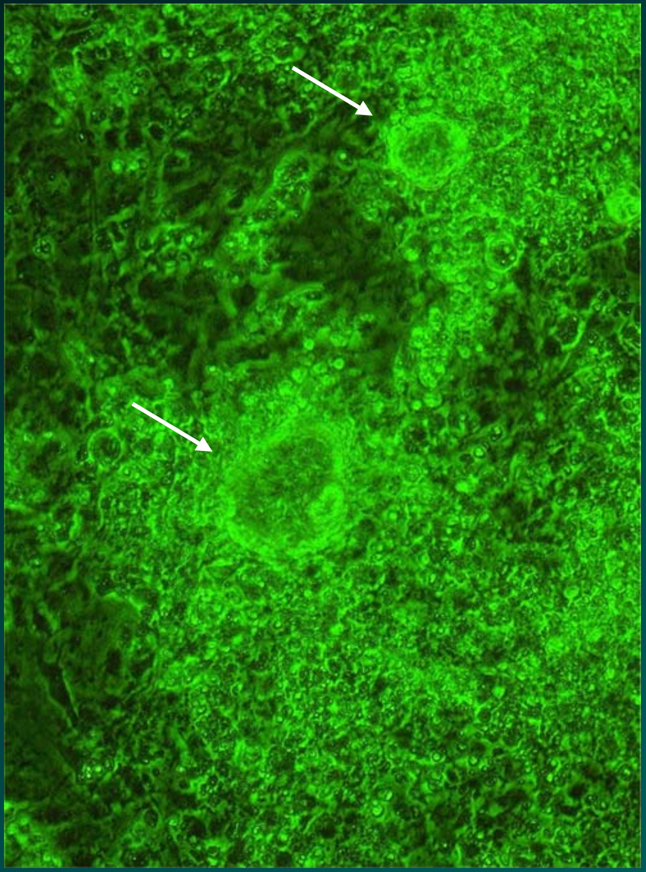


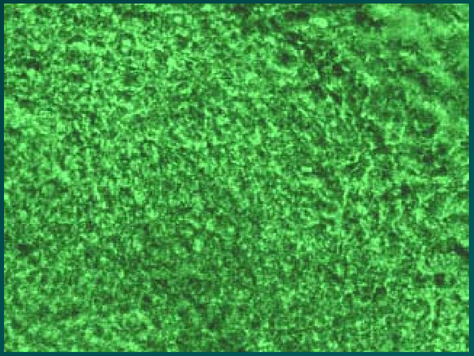
(15% FCS, +LIF)
With blood serum and LIF



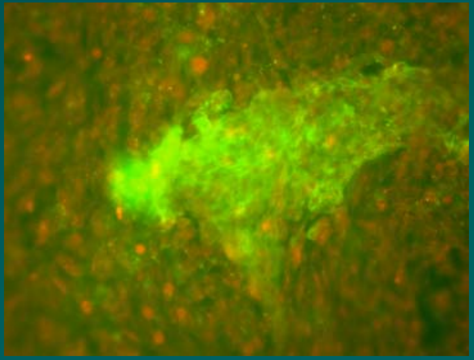
(15% KSR, -LIF)
Without serum and LIF

Myocardium formation from mouse ES cell



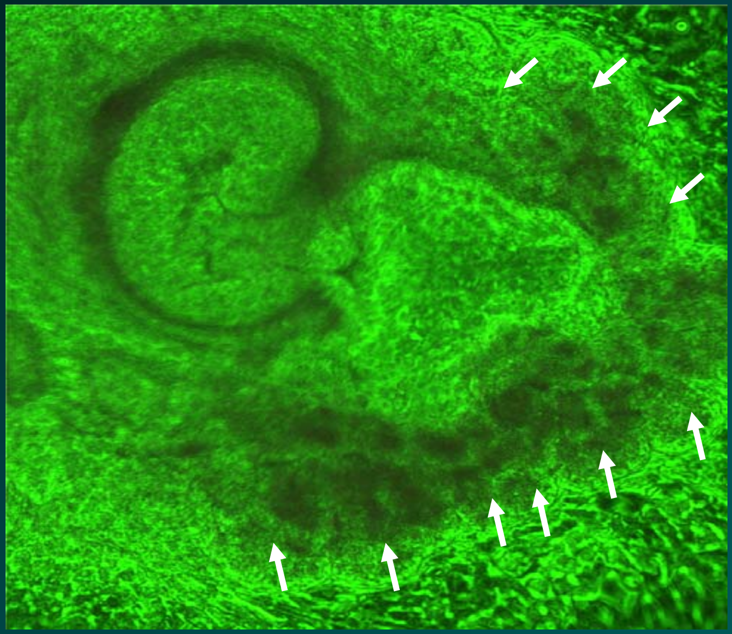


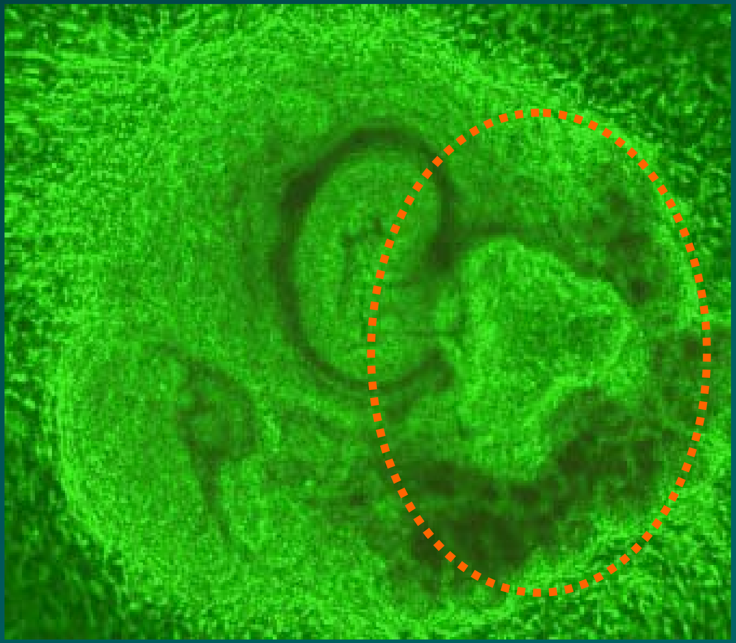
Induction of myocardium cell by RA024 treatment
（1-2 days culture）



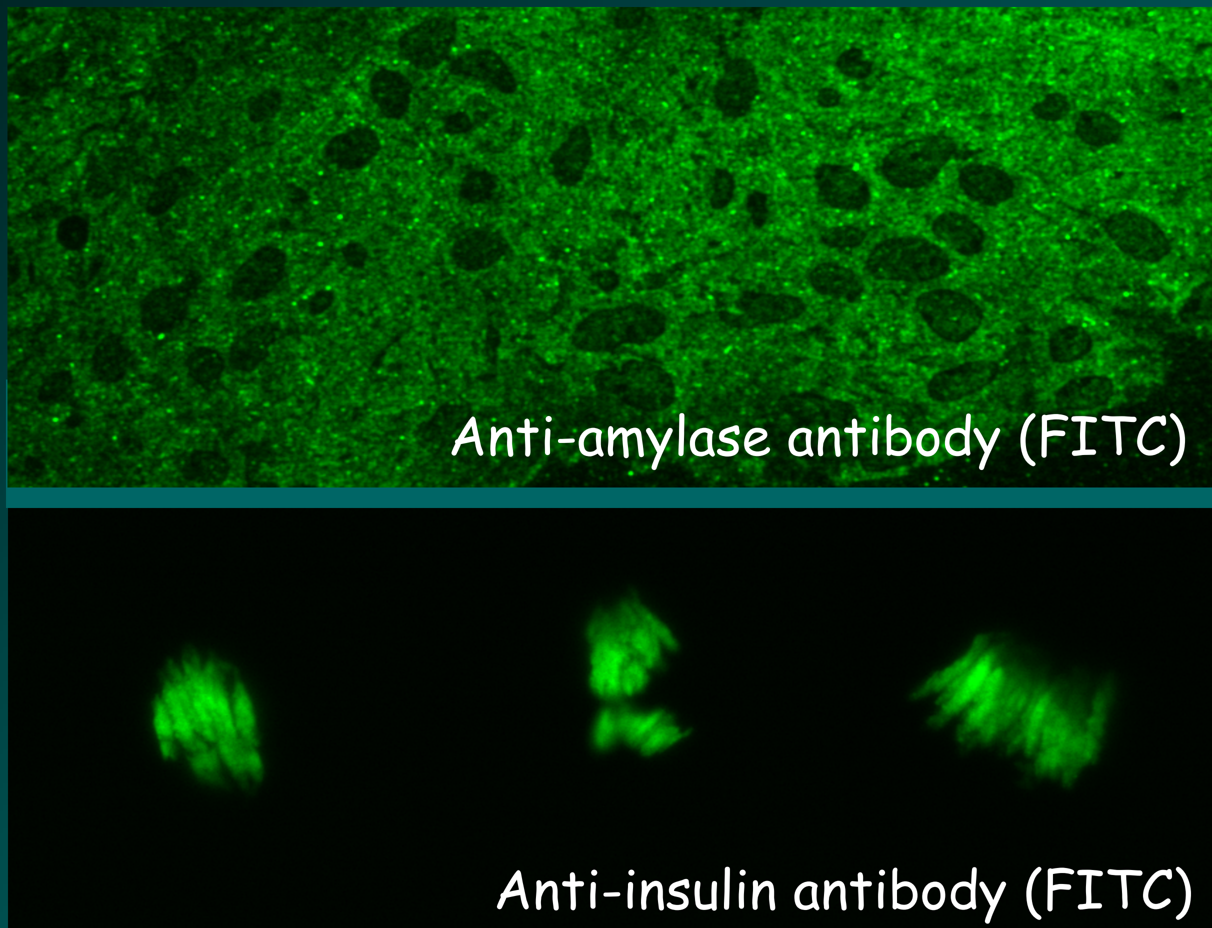
Dye by troponin antibody (myocardium specific marker

Pancreas formation from mouse ES cell

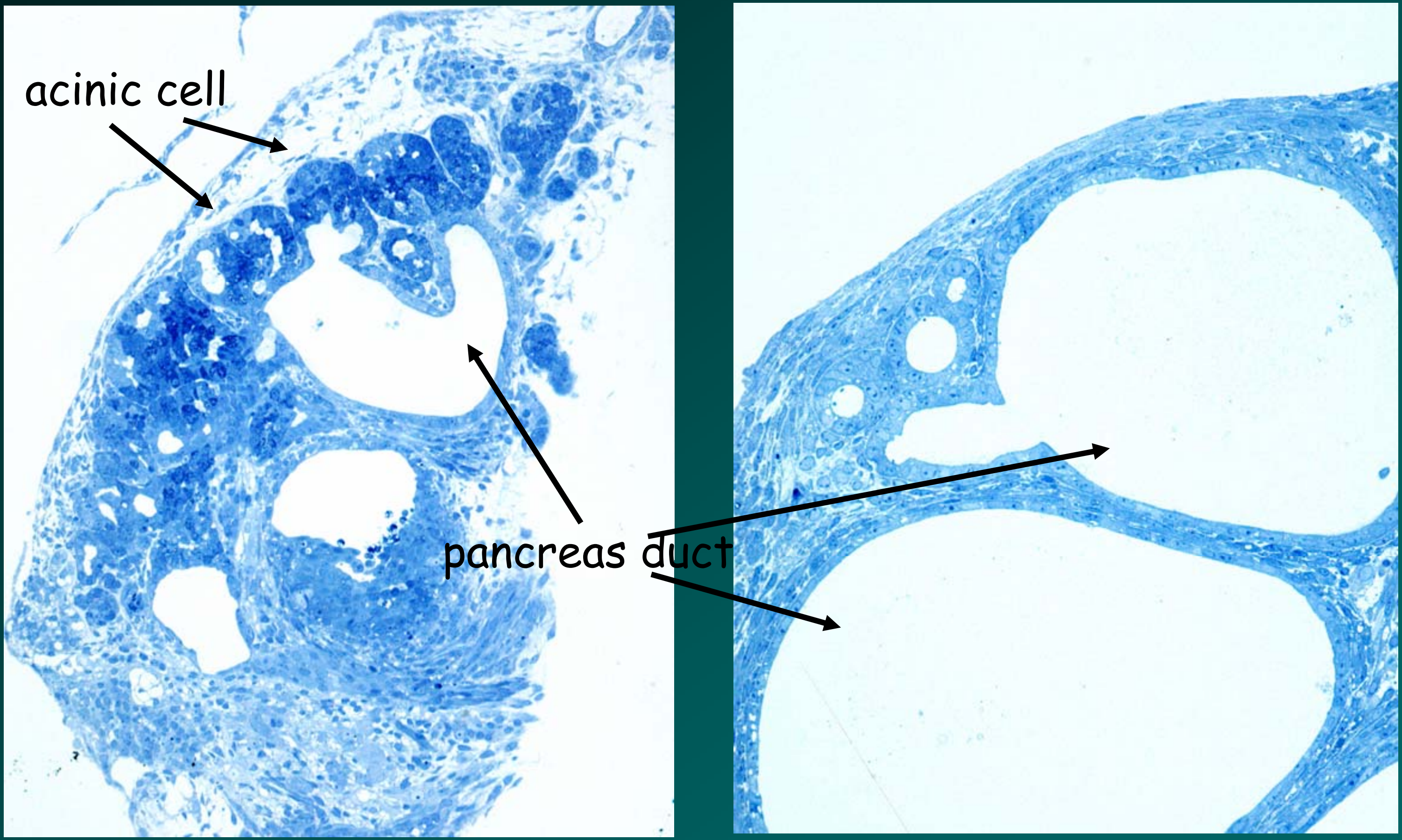




Secretion of insulin and amylase from the pancreas formed from mouse ES cell

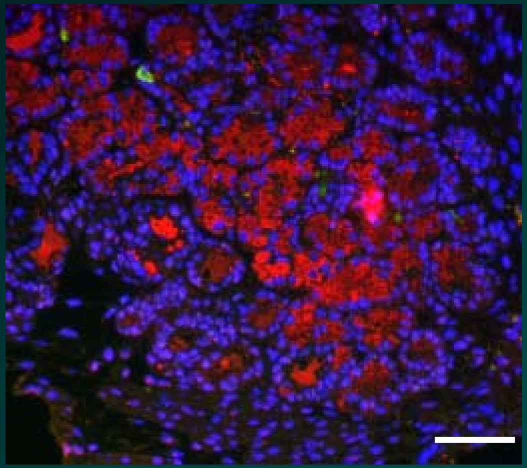


Tissue slice of pancreas during development from ES cell

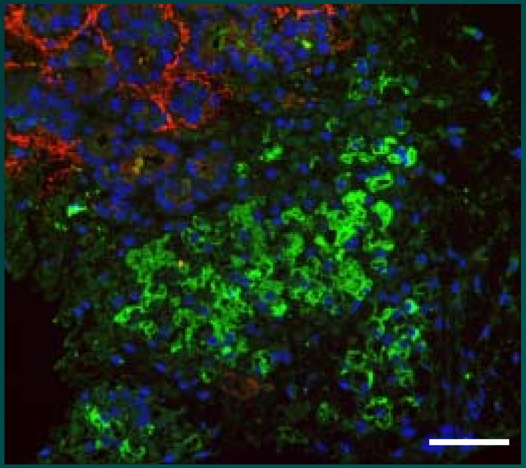


Double antibody dye by insulin and amylase

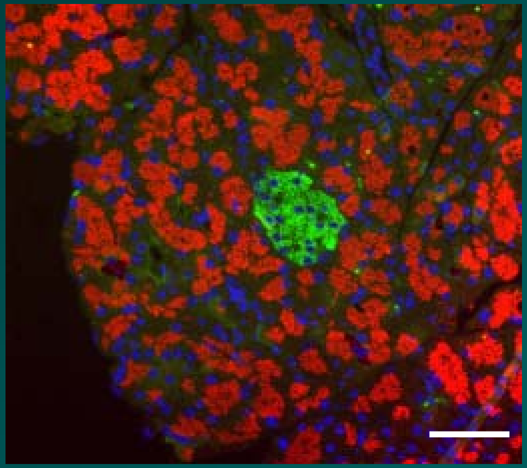
amylase /
Insulin C peptide /
DAPI
(bar = 50 μm)



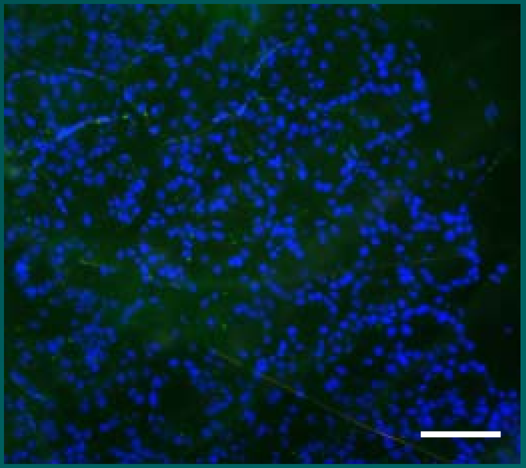
0.1 mM RA +
10 ng/ml activin



0.1 mM RA +
25 ng/ml activin

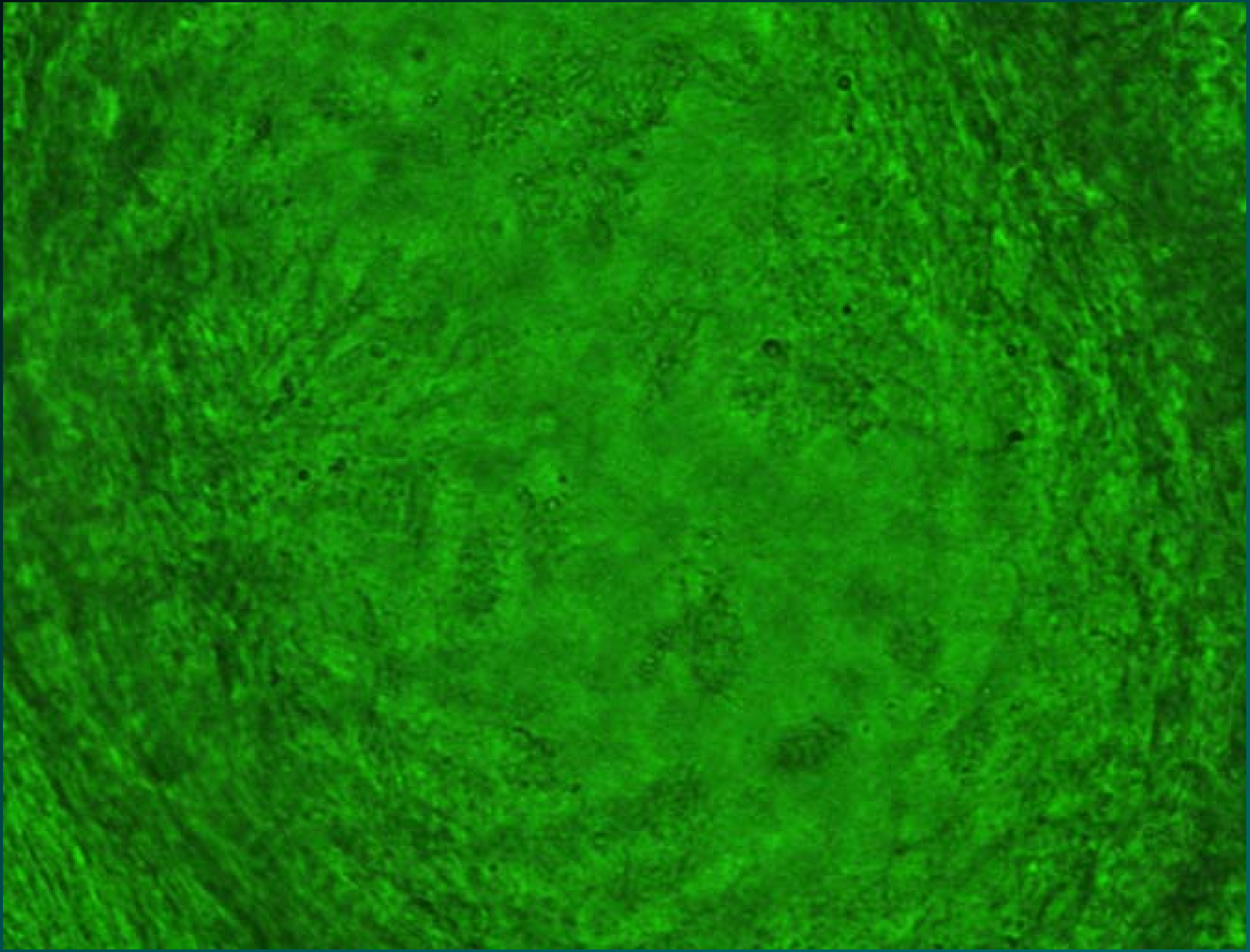


mouse pancreas

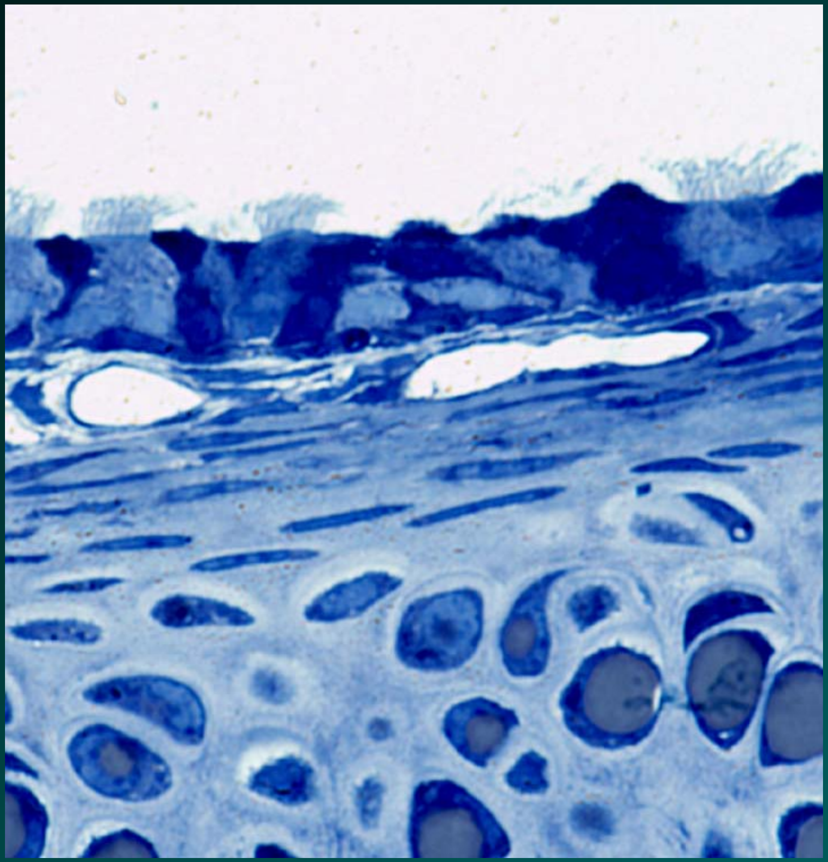


control

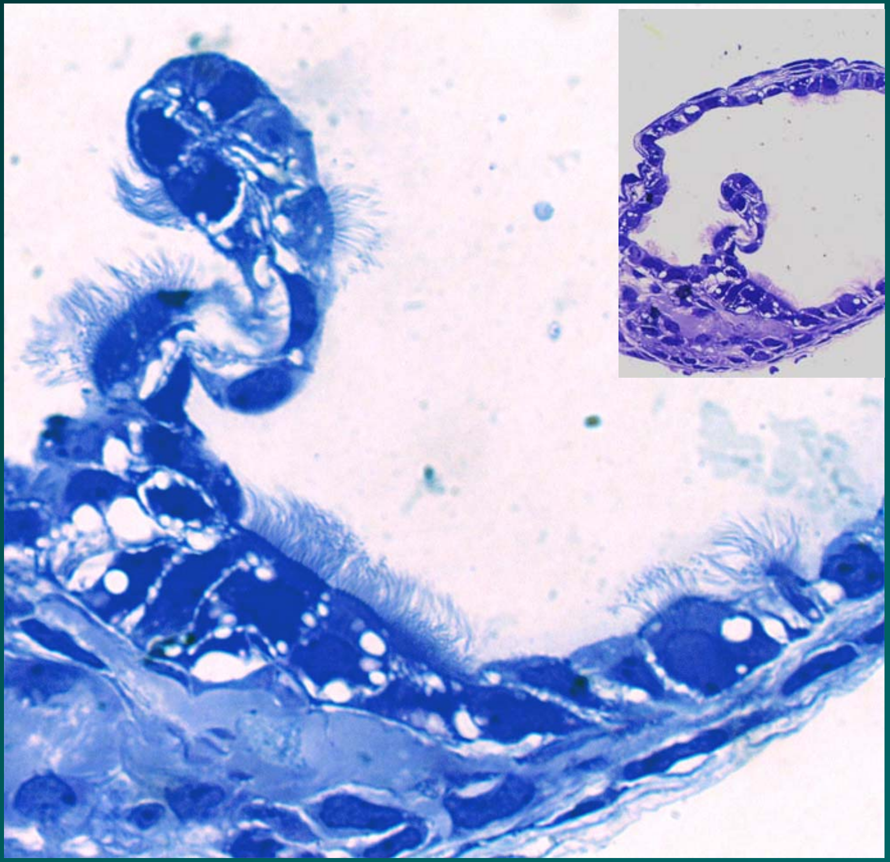
Trachea formation from mouse ES cell



Comparison between structures of trachaea from mouse ES cell and normal trachaea

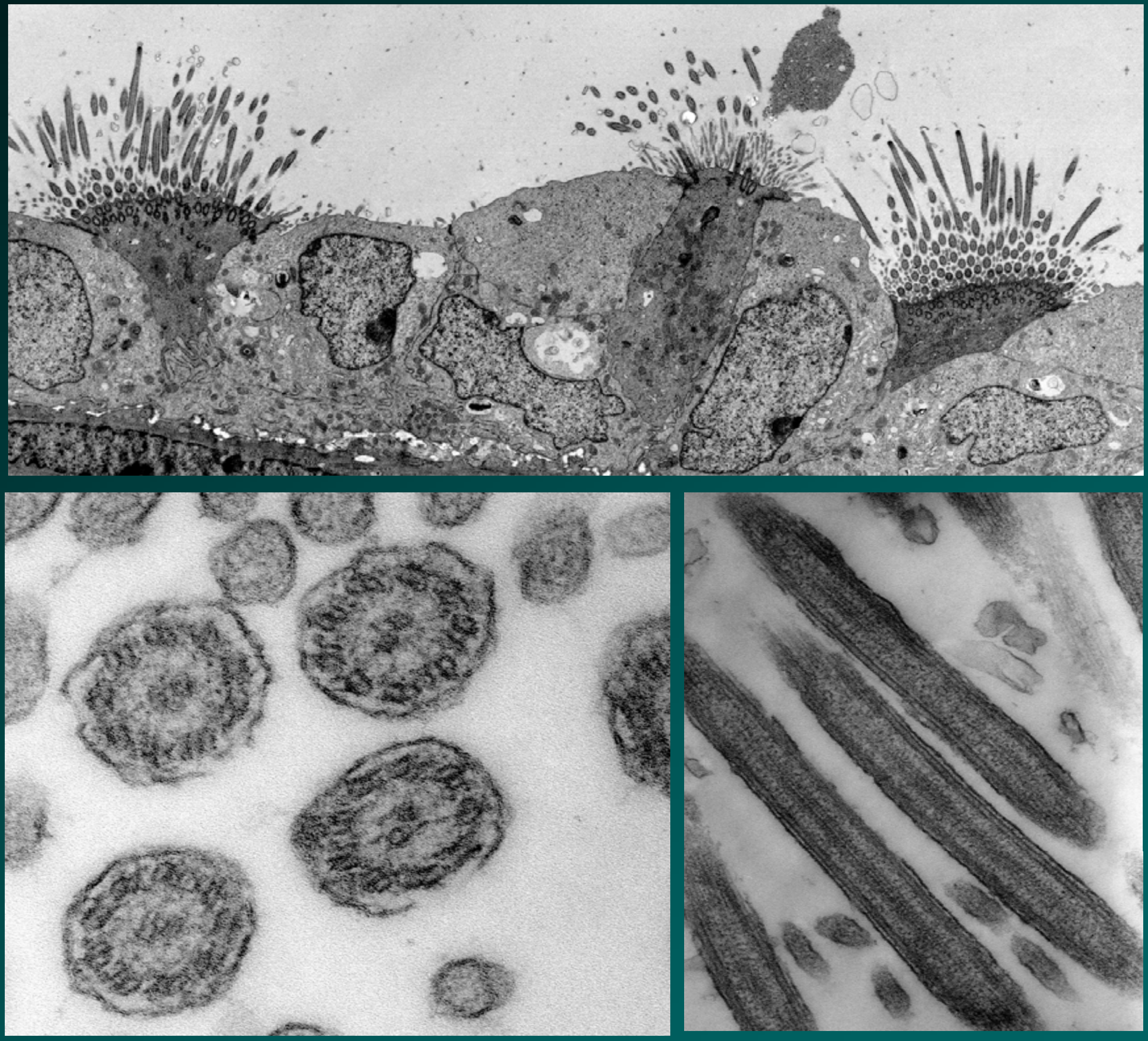


Normal mouse trachaea



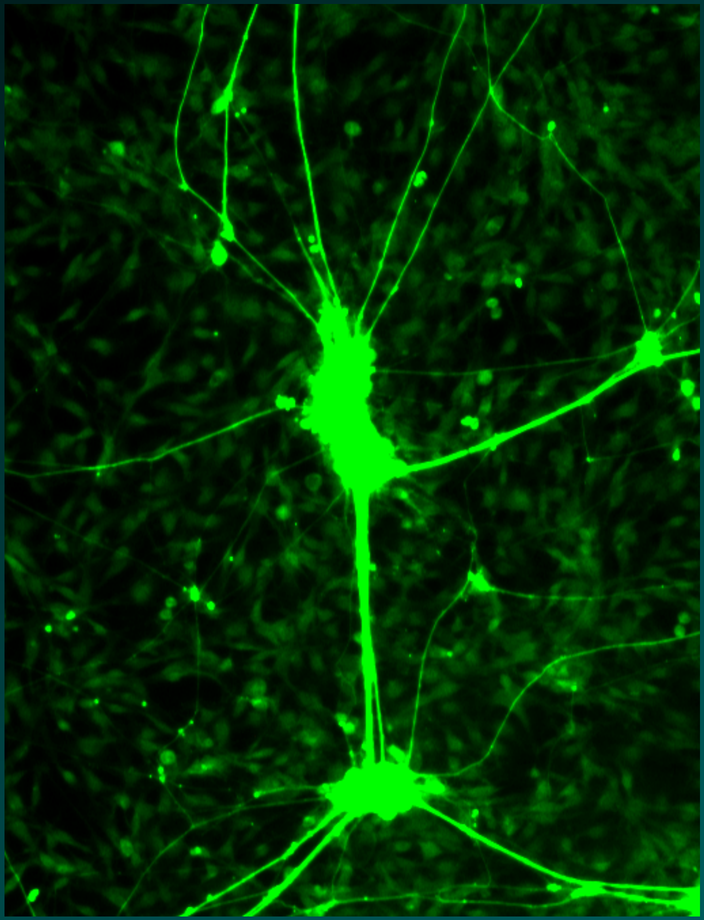
Trachaea from mouse ES cell

Cilium formation from mouse ES cell

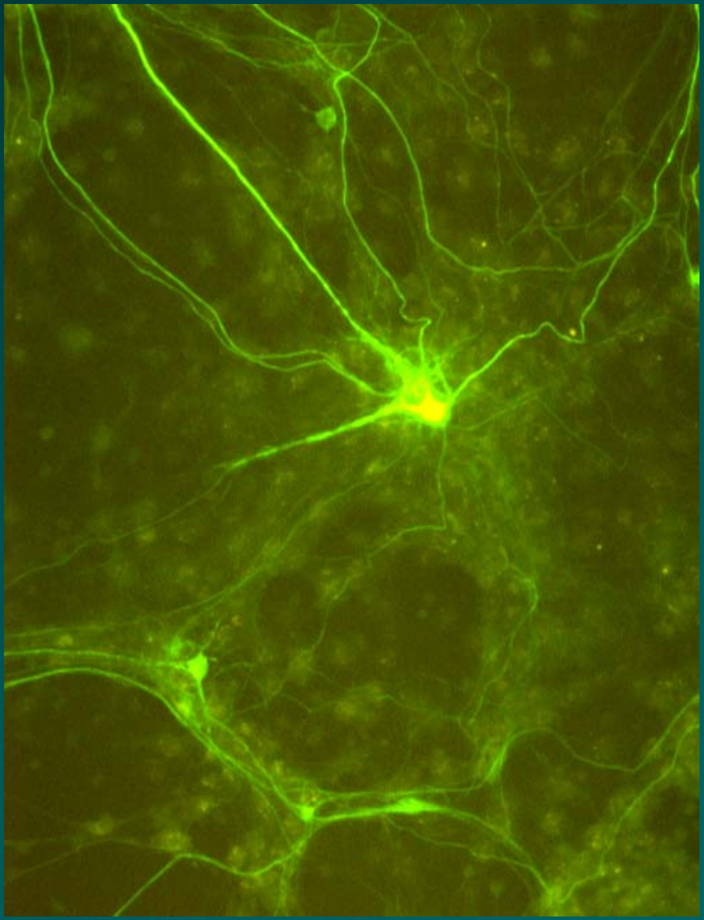


9+2 structure typical in cilium cell is seen

Nerve cell formation from mouse ES cell



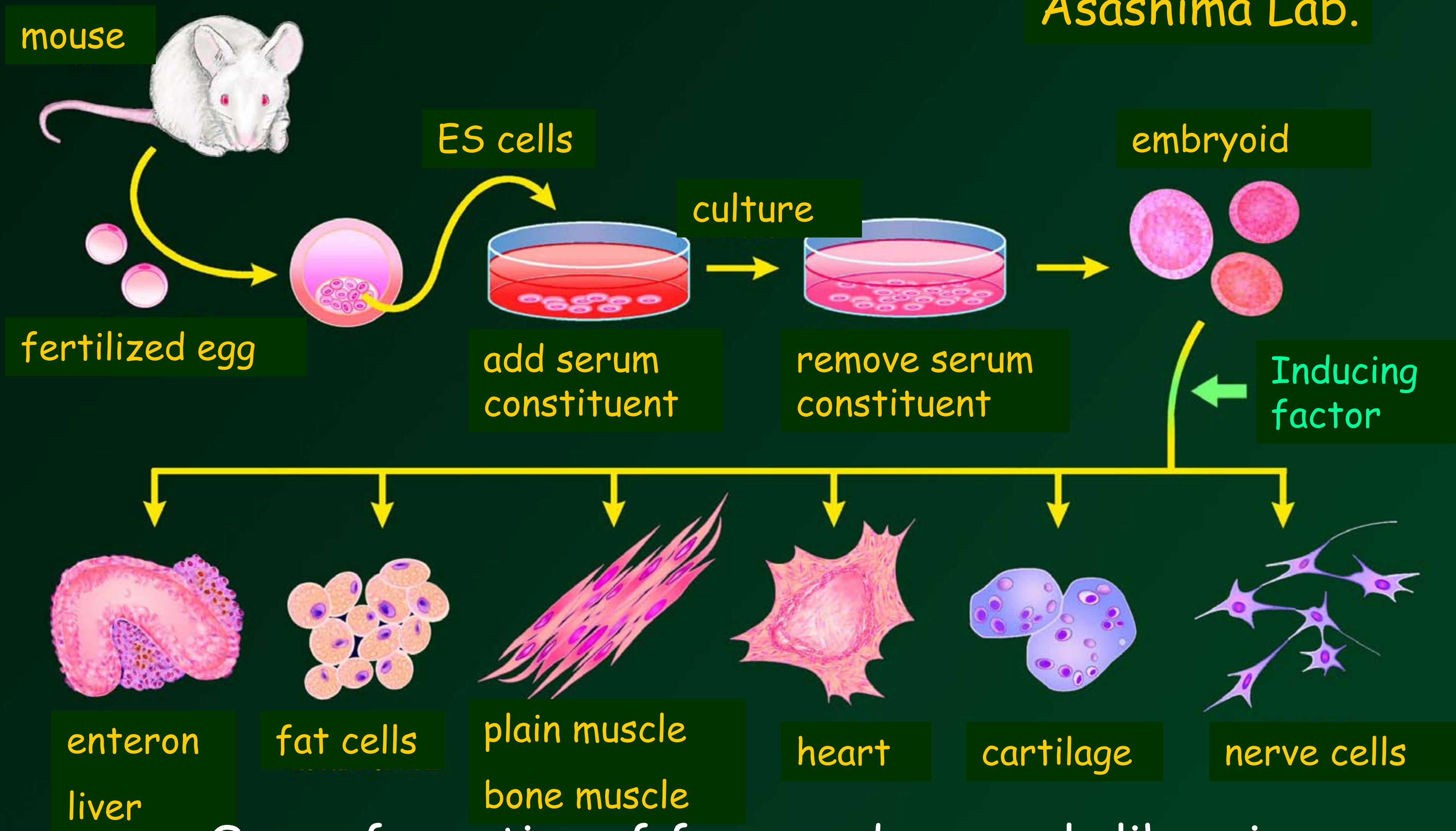
anti-L-NF antibody (FITC)



anti-H-NF antibody (FITC)

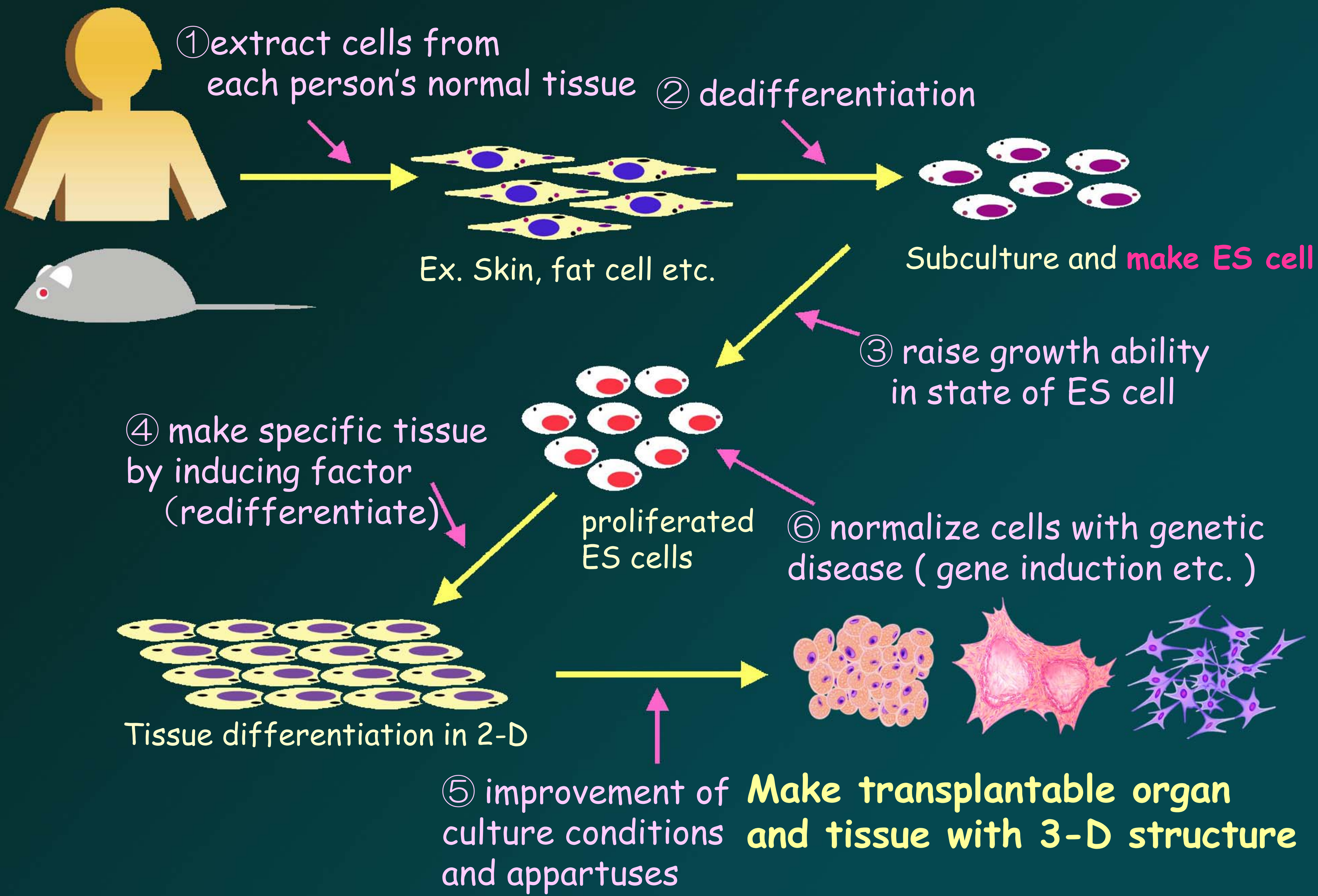
Organ induction from mouse ES cells

Asashima Lab.



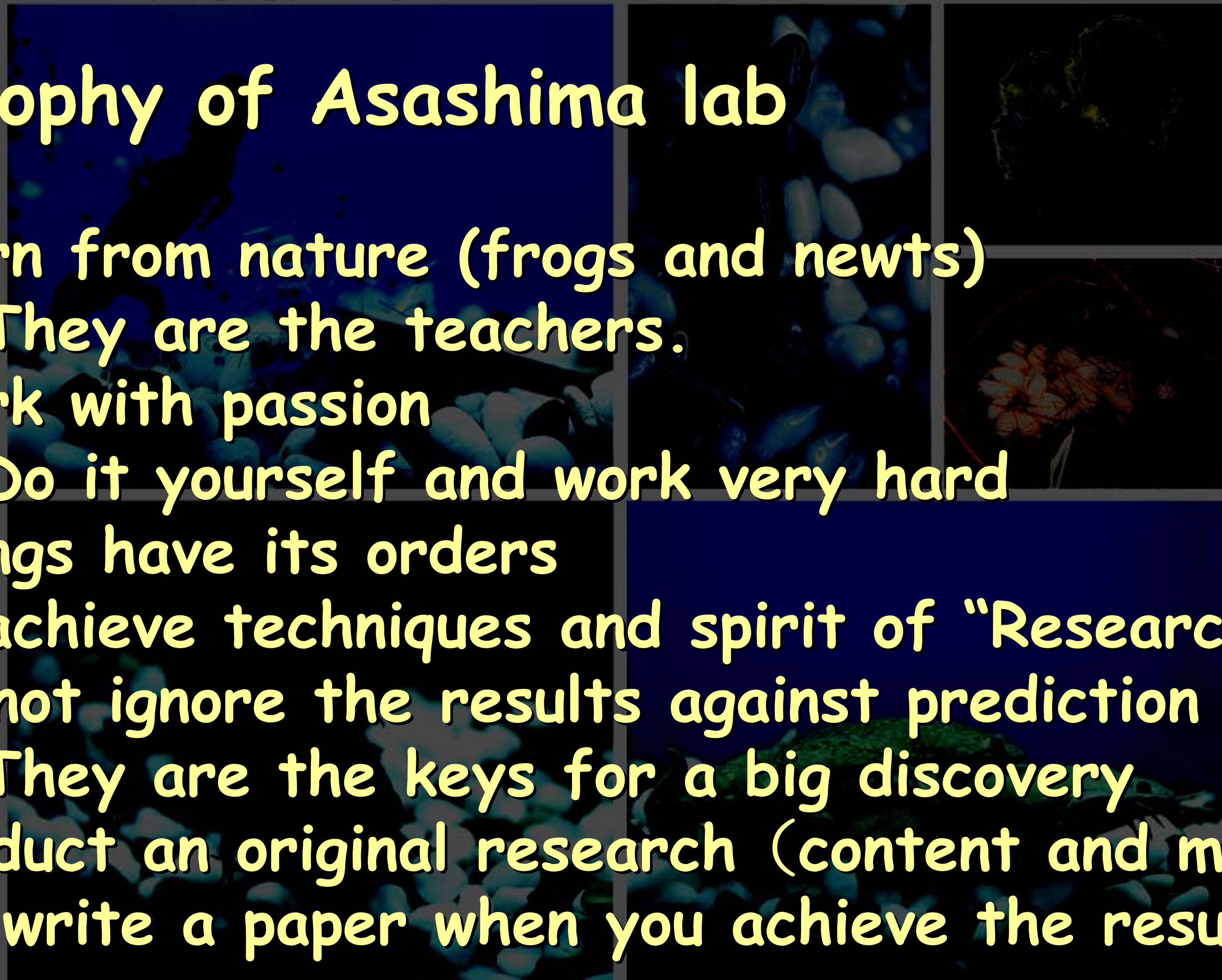
Organ formation of frogs and mammals like mice are regulated by similar systems.

Future plan


①extract cells from
each person's normal tissue
② dedifferentiation
Ex. Skin, fat cell etc.
Subculture and make ES cell
③ raise growth ability
in state of ES cell
④ make specific tissue
by inducing factor
（redifferentiate)
proliferated
ES cells
⑥ normalize cells with genetic
disease (gene induction etc.)
Tissue differentiation in 2-D
⑤ improvement of
culture conditions
and appartuses
Make transplantable organ
and tissue with 3-D structure

Philosophy of Asashima lab

1. Learn from nature (frogs and newts)
   —They are the teachers.
2. Work with passion
   —Do it yourself and work very hard
3. Things have its orders
   —achieve techniques and spirit of "Research first"
4. Do not ignore the results against prediction
   —They are the keys for a big discovery
5. Conduct an original research (content and method) and write a paper when you achieve the result



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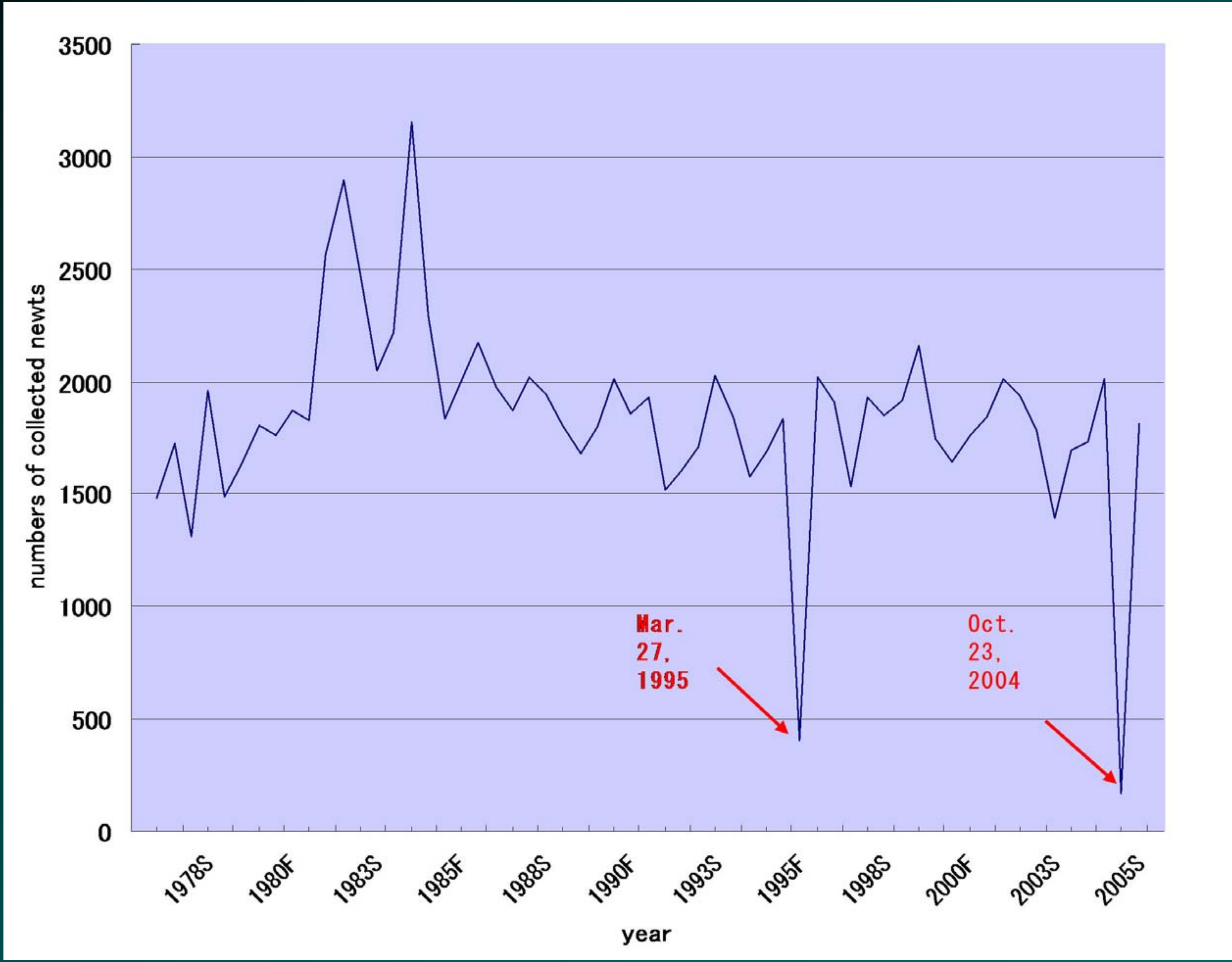
Again, I am extremely grateful.

At last, I would like to thank young students in my lab who voluntarily planned and supported my final lecture.

Makoto Asashima

Numbers of newts collected in Murakami-shi, Niigata



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