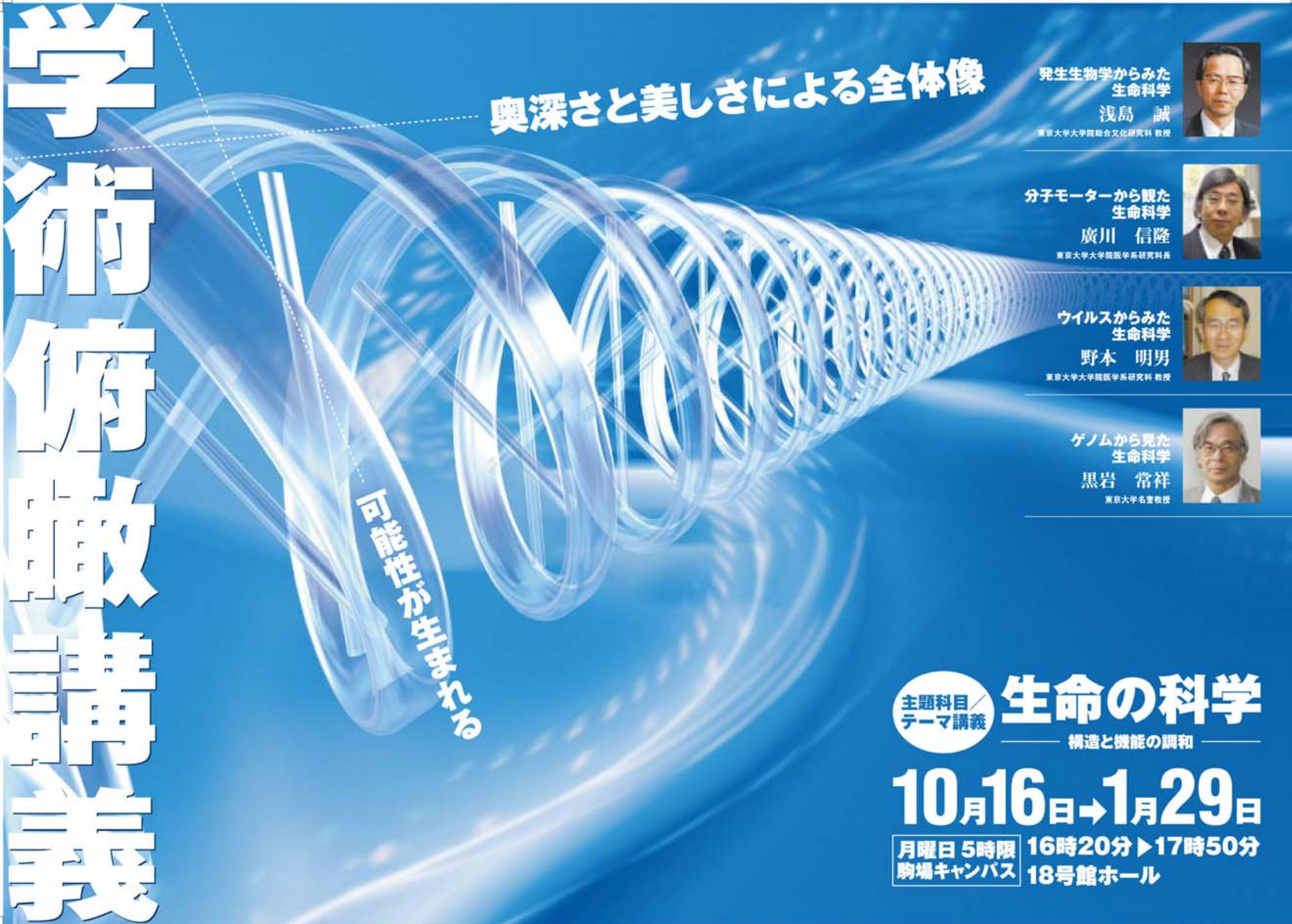


学術俯瞰講義
奥深さと美しさによる全体像
可能性が生まれる
発生生物学からみた生命科学
浅島 誠
東京大学大学院総合文化研究科 教授
分子モーターから観た生命科学
廣川 信隆
東京大学大学院医学系研究科長
ウイルスからみた生命科学
野本 明男
東京大学大学院医学系研究科 教授
ゲノムから見た生命科学
黒岩 常祥
東京大学名誉教授
主題科目／テーマ講義
生命の科学
構造と機能の調和
10月16日→1月29日
月曜日 5時限
駒場キャンパス
16時20分▶17時50分
18号館ホール

Global Focus on Knowledge Lecture Series

2006 Winter Lecture: “Science of Life”

Life Science: from the Perspective of Developmental Biology

1st lecture Oct.16（Mon）Mechanism of formation from an egg to an adult

2nd lecture Oct.23（Mon）Biological information system and networking

3rd lecture Oct.30（Mon）Mechanism of Organ Formation

4th lecture Nov. 6（Mon）Science of Regeneration

Professor Makoto Asashima

Graduate School of Arts and Sciences, University of Tokyo

What is “Regeneration”?

- Reconstruction of defected cell・tissue・organ
- Phenomena characteristic of organisms
- Three aspects in research on regeneration.

① physiological regeneration　Duration of homeostasis (hair, nail, blood cells etc.)

Example: red blood cell → 2million cells are formed and destroyed per second

② damage recovery　damage → repair → recovery（regeneration）

Example: ・recovery from injury, regeneration of the liver
・regeneration observed after newt limb amputation
（regenerating ability of the animal）

③ regeneration in vitro

New method in regeneration science to make organs and tissues from undifferentiated cells under artificial control, applying the knowledge of ①, ②

↓

New field of science to study formation of organs and tissues
which might lead to regenerative medicine
as a remedy for injuries and sicknesses which cannot be treated by drugs.

Regeneration and stem cells

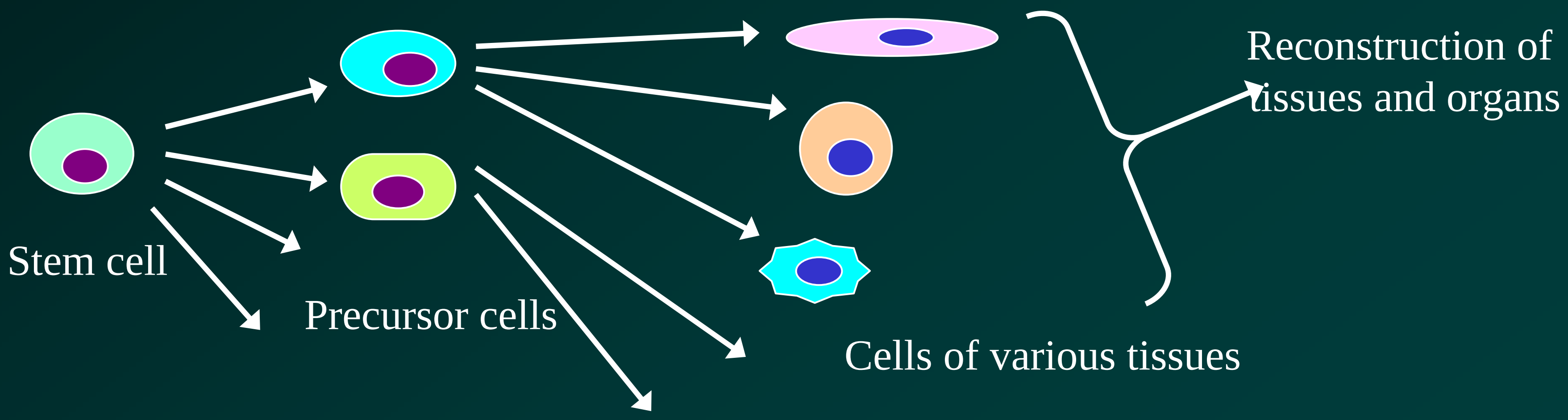
Stem Cells:

Cells which maintain an undifferentiated state, and have the ability to differentiate into various cells（omnipotency）

⇩

Cells which can be a base for restructuring defected tissues and organs.

- All tissues are rich with stem cells even after maturation.
  （skin，hair roots，intestines，muscles and brain have stem cells.）
- Stem cells' differential ability varies by tissues, organs, and species.



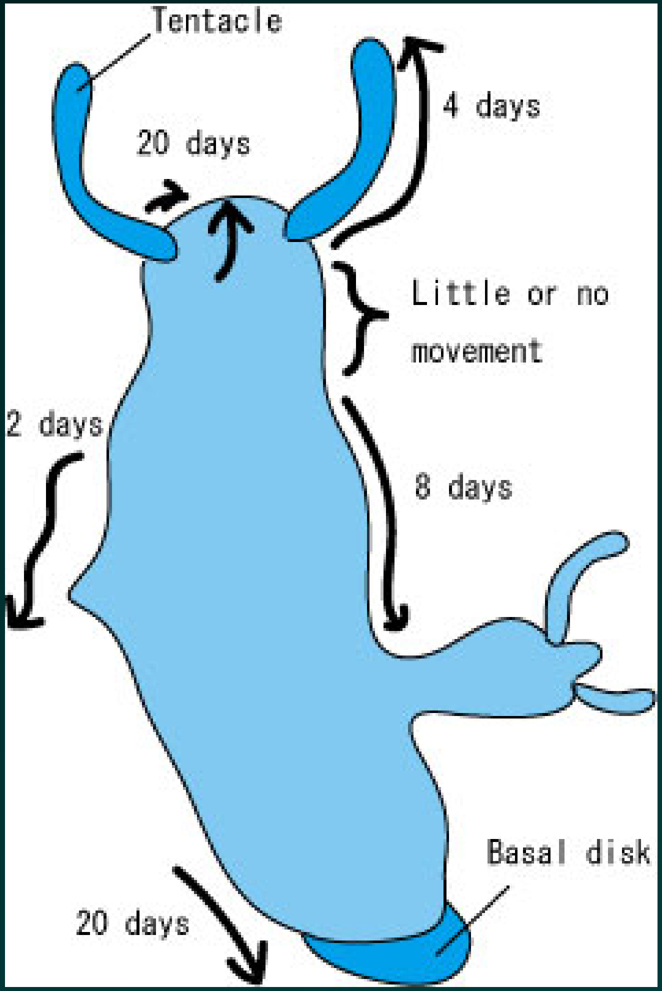
Regeneration in various animals and research on them

hydra（coelenterate ）
planarian（platyhelminth）
enchytraeus （annelida）
cricket（insects）
newt・axolotl（vertebrates）, etc.

The regeneration of coelenterate

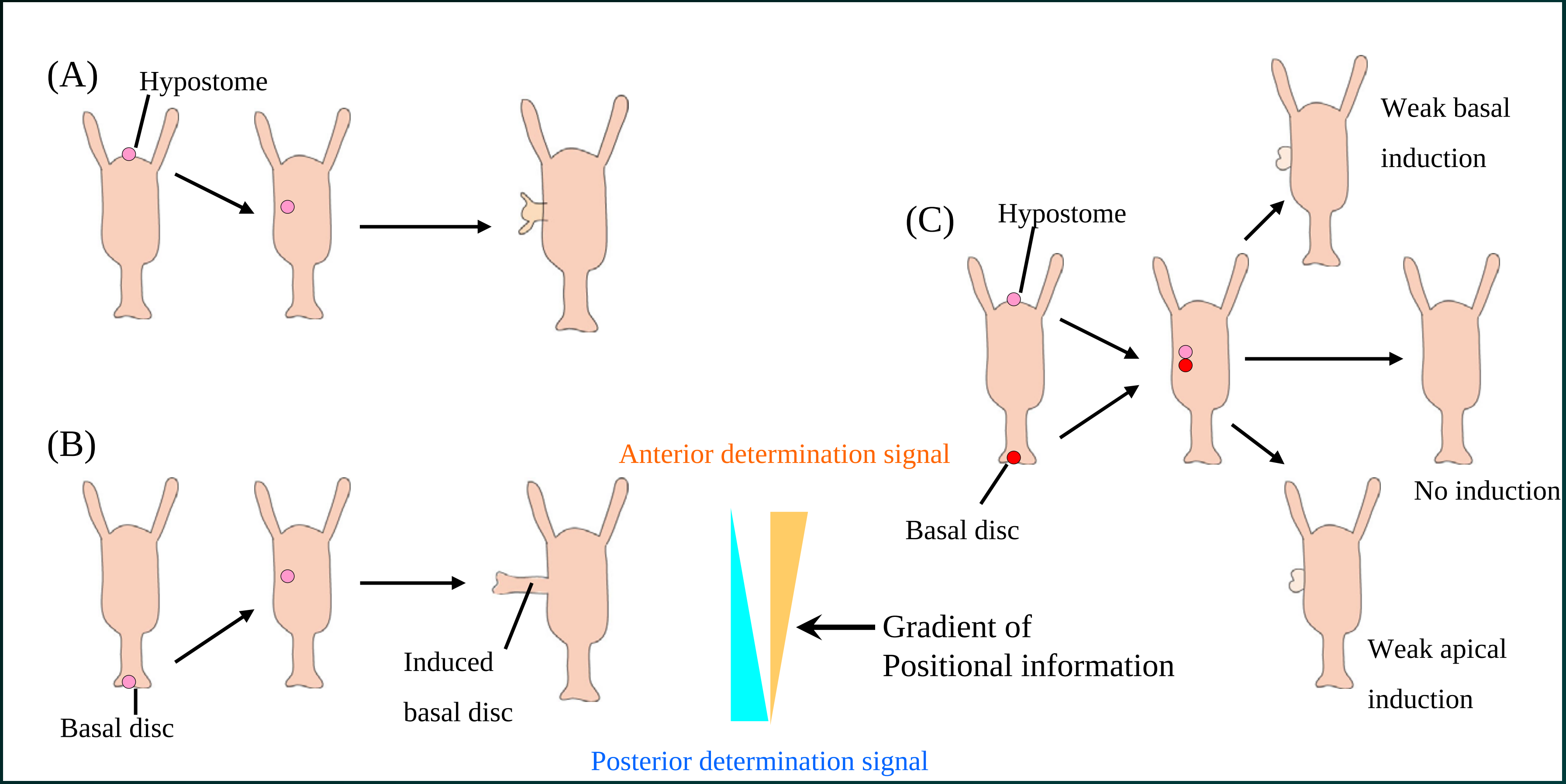
hydra

The hydra proliferates by budding



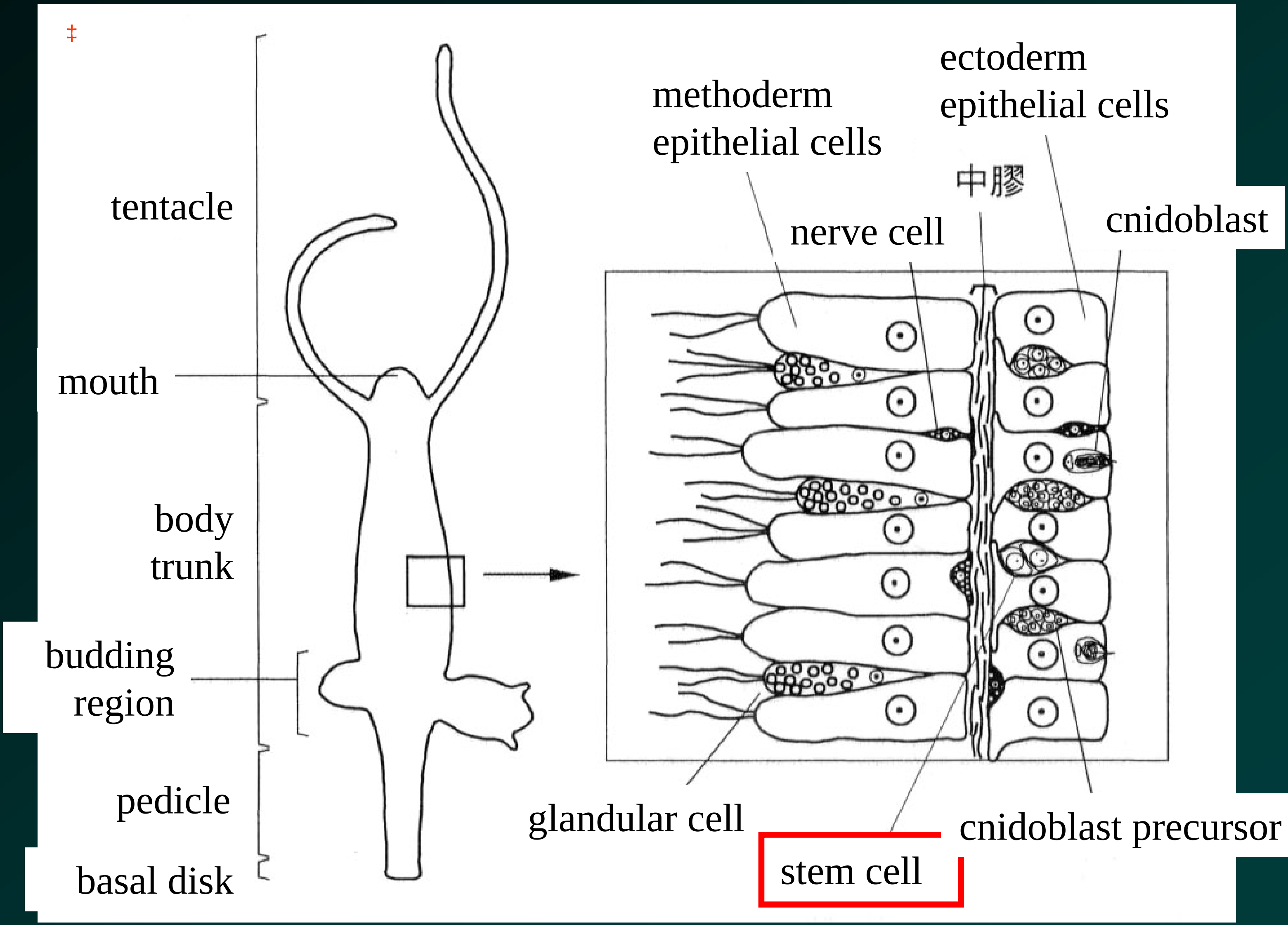
Hydra buds make a new individual.

In hydra budding, positional information depends on the transplanted region.



Head region buds when hypostome is tranplanted.
Foot region buds when basal disc is transplanted.

Stem cells of the hydra spread across the body

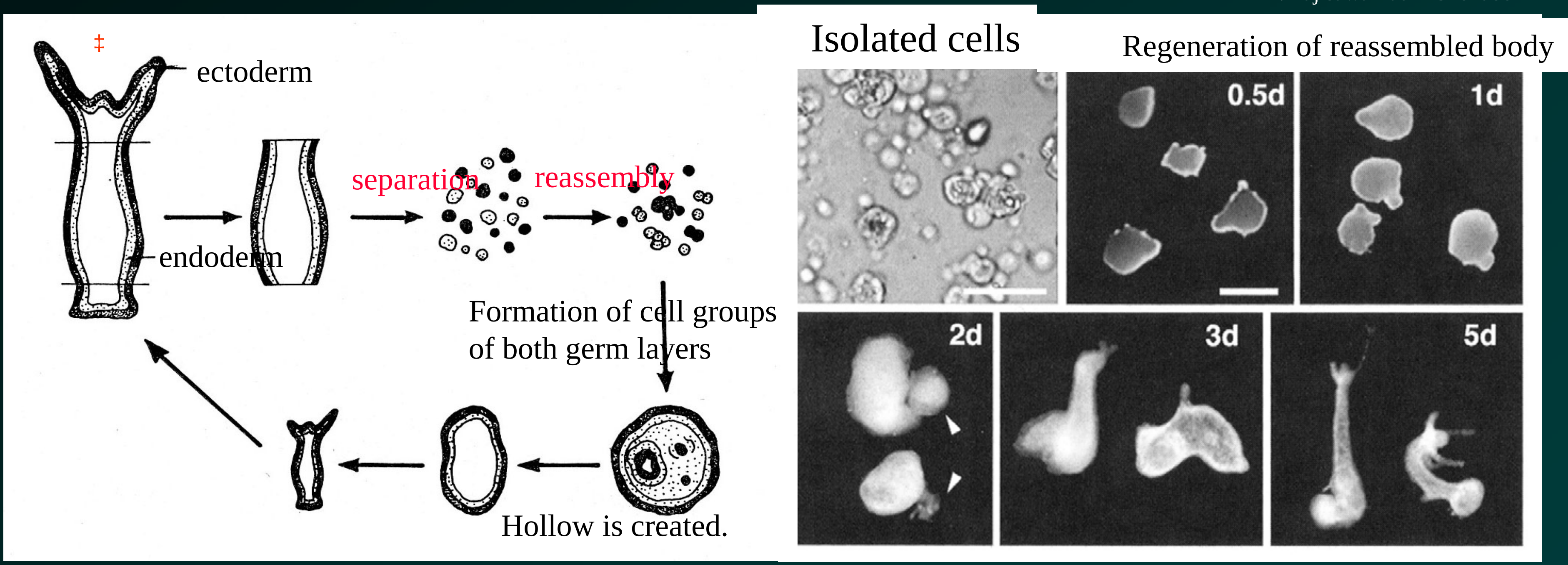


T.Fujisawa, 2001 Shokabo

Isolation, reassembly and regeneration in hydra

1983 Idemitsu Shoten

T. Fujisawa 2001 Shokabo

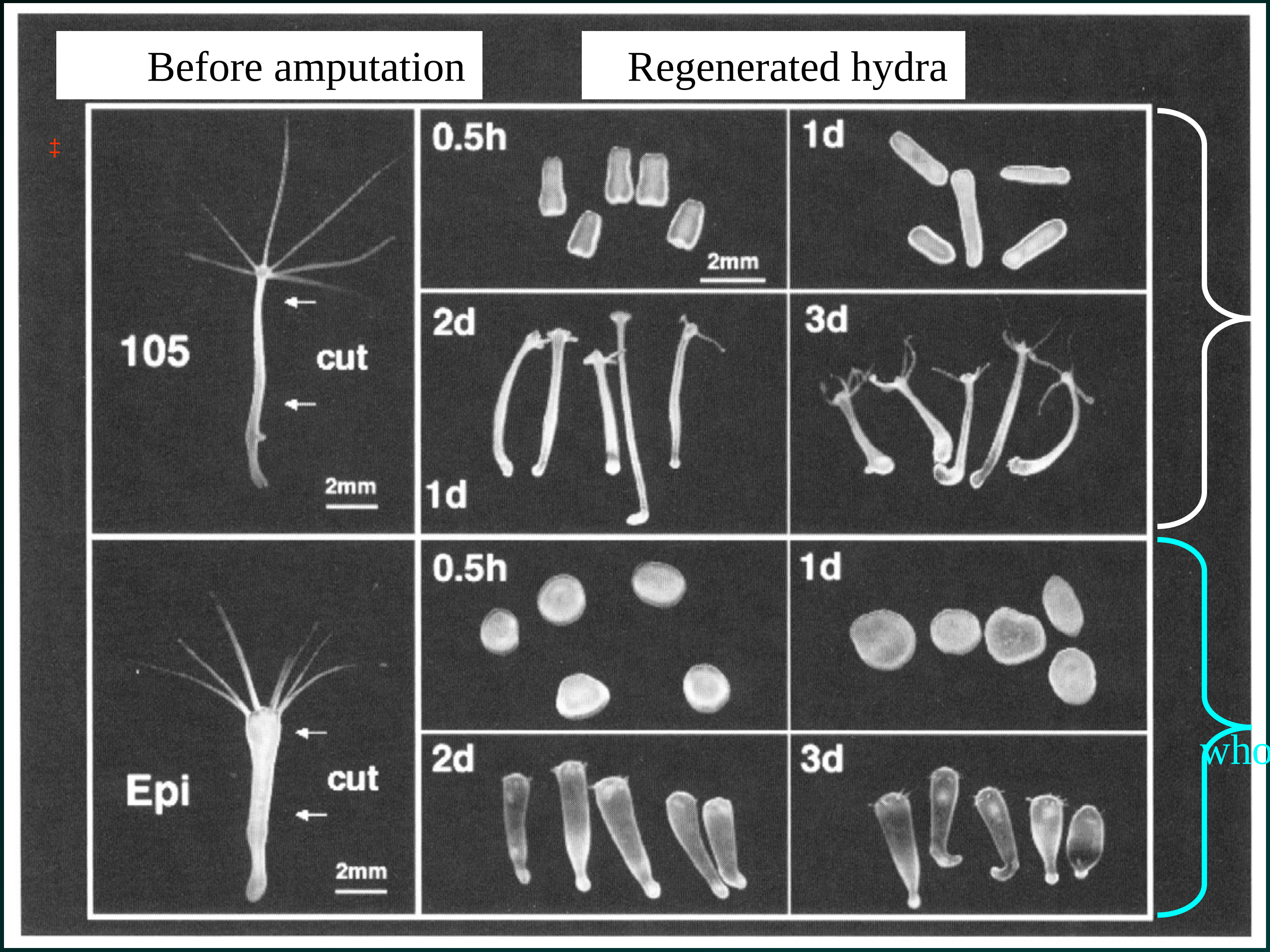


Cell isolation and reconstruction of a hydra

Isolated cells of the hydra regenerates again into an individual body if reassembled.

Reproduction of morphology is independent of stem cells

T. Fujisawa 2001 Shokabo



Regeneration of normal hydra

Regeneration of hydra whose stem cells are destro
(Structures like nerves do not regenerate.)

The morphology of a hydra can be reproduced only from epithelial cells.

However, stem cells are needed to retrieve perfect structure and functions.

What determines directionality of hydra morphology

T. Fujisawa 2001 Shokabo

Expression of a peptide Hym-30
is responsible for head region regeneration

Formation of head and foot region is regulated by a kind of peptide made from 10～20 amino acid residues

Regulation of foot region： Hym-323, Hym-346
↓
Functions from early stage of foot region regeneration.

Regulation of head region： Hym-301
↓
Functions during head region regeneration.
Relevant to tentacle formation.

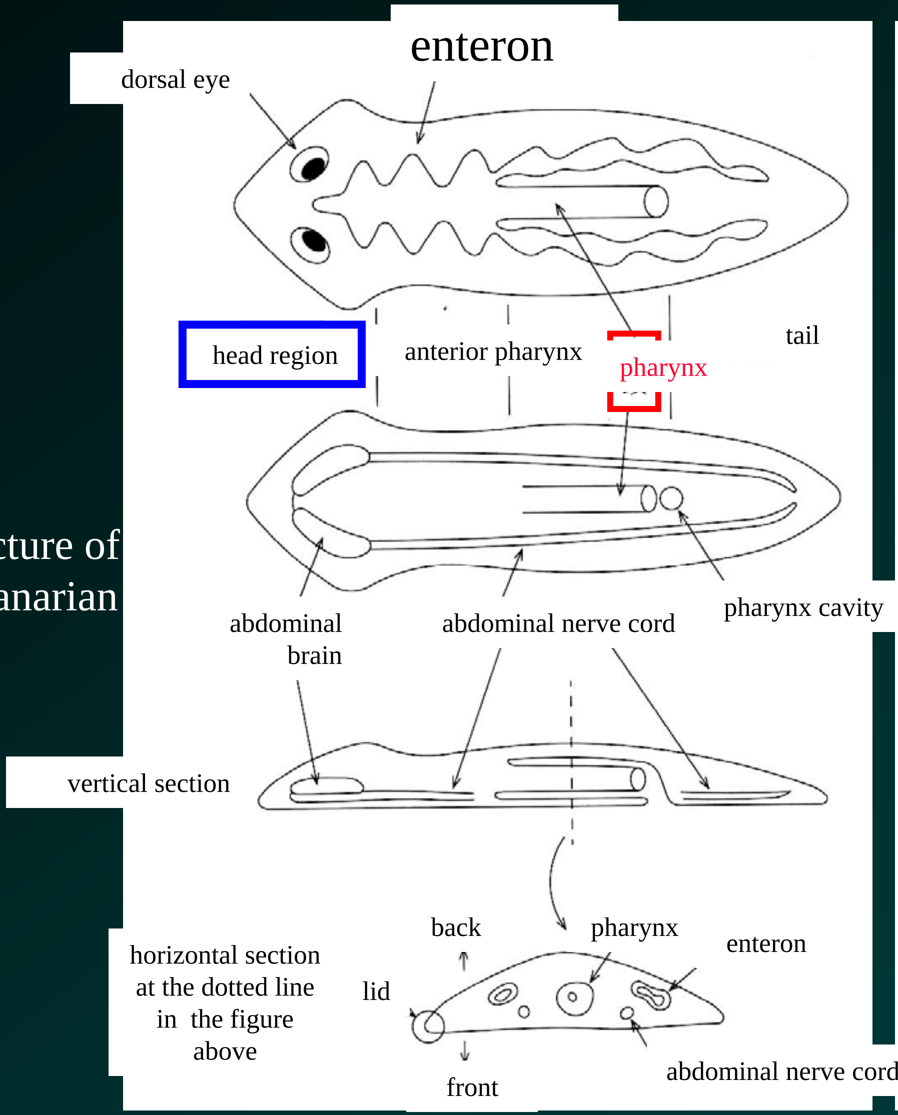
Regeneration mechanism of a hydra has many mysteries.

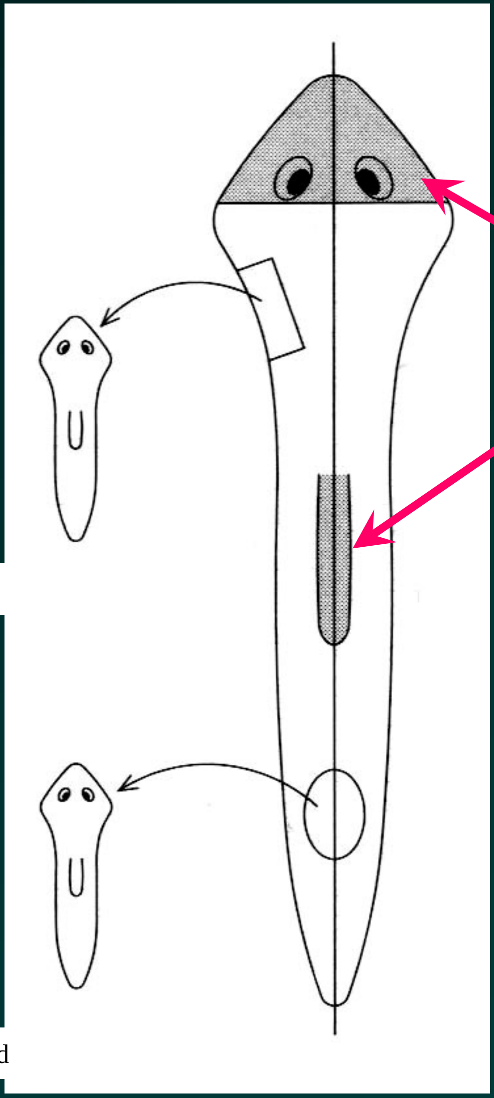
The regeneration of platyhelminth

planarian

Planarian has an extremely high capacity for regeneration

Structure of a planarian



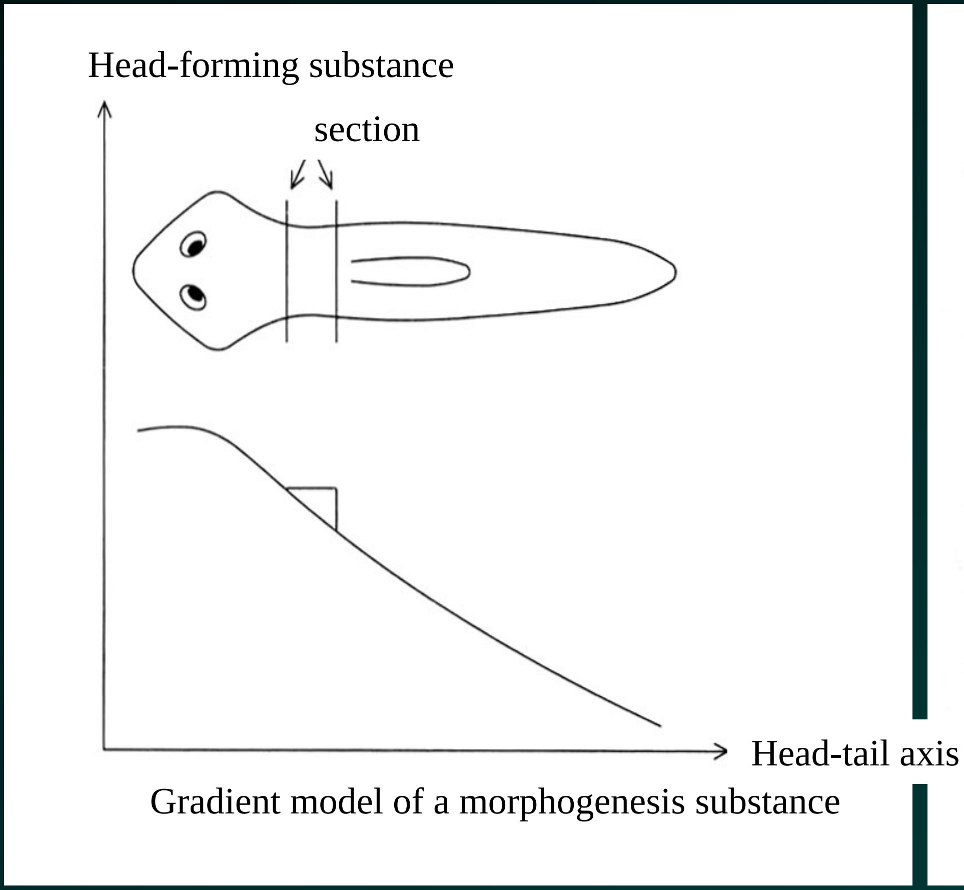


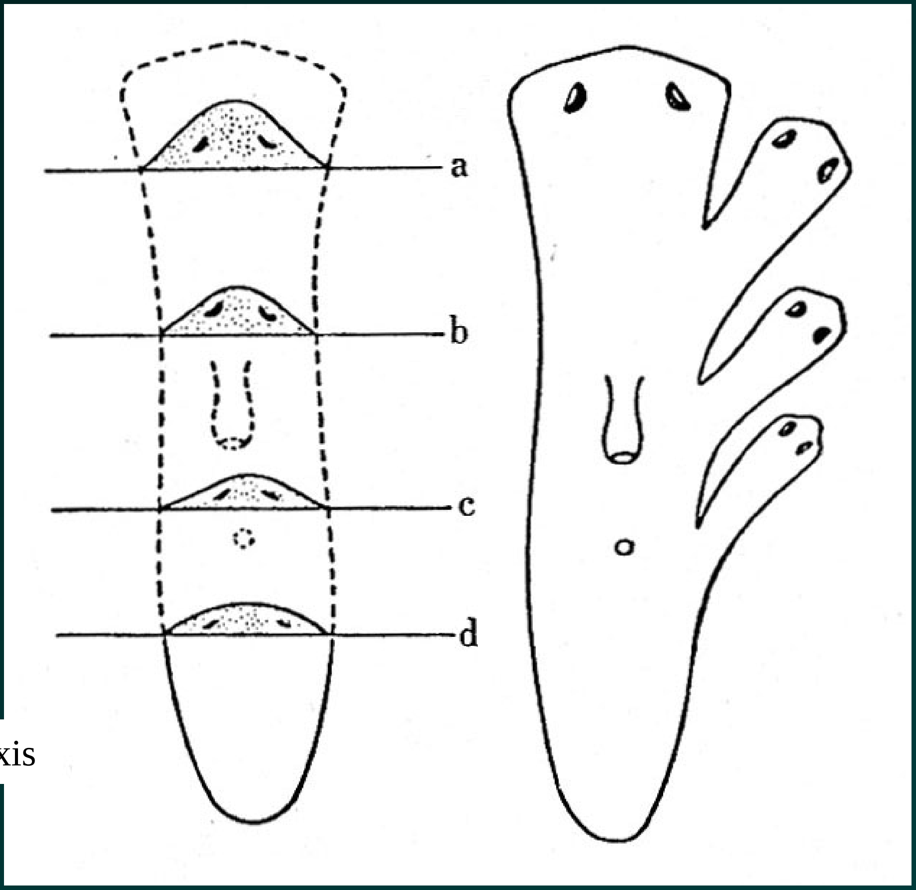
K. Watanabe 2001 Shokabo

A planarian can regenerate a whole body except for the anterior part of the head region and pharynx (regions with stem cells) from a fragment with dorsabdominal parts.

Positional information in planarian regeneration①

A gradient model which determines the anterior-posterior axis of planarian





K. Watanabe 2001 Shokabo

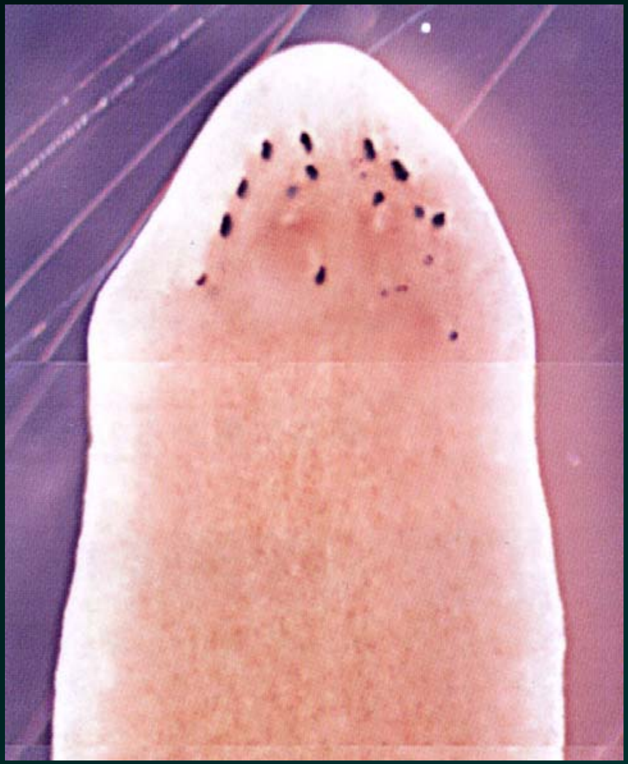
1983 Idemitsu Shoten

The substances and mechanisms that determine the head-tail axis are little known.

When the sides of planarian are cut in the direction of the head, new heads are formed as many as the number of cuts.

Stem cell defects in a planarian

2001 Shokabo



Multi-eyed planarian
with stem cell defects

The stem cells in the body are essential for regeneration of a planarian.

The process of regeneration is the partial repetition of morphogenesis during development.

Mutants with stem cell defects are observed in discovering the regeneration mechanism of a planarian.

↓

Issues:
What determines the omnipotency of stem cells?
What is the effect of cellular interaction during regeneration.

The regeneration of annelida

Example: *Enchytraeus japoneisis*

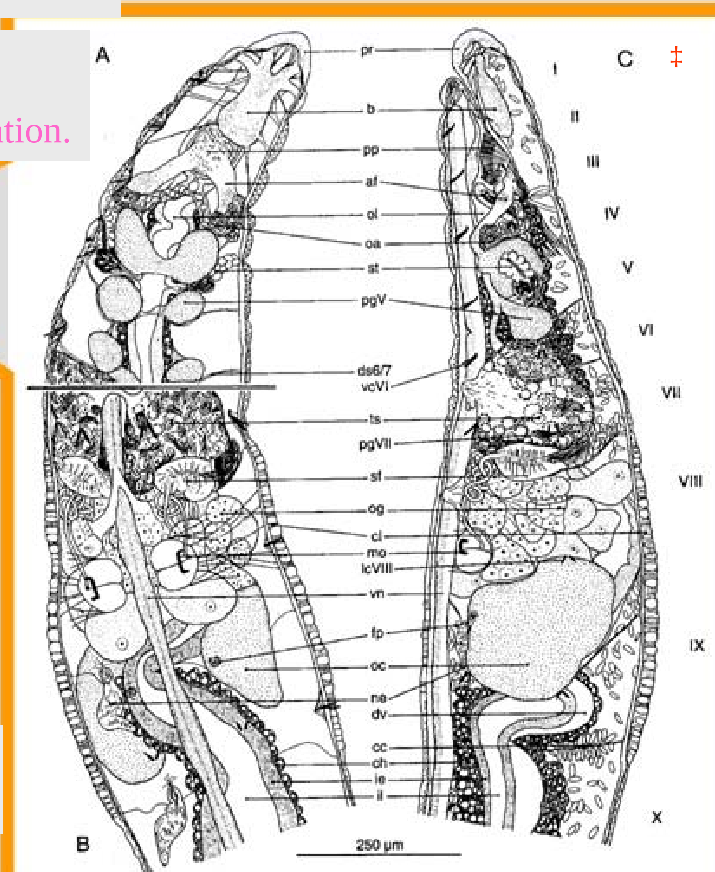
Enchytraeus japonensis has a high capacity for regeneration.

Enchytraeus japonensis

- The white angle worm is smaller than 1cm
- There are hundreds of species in the world

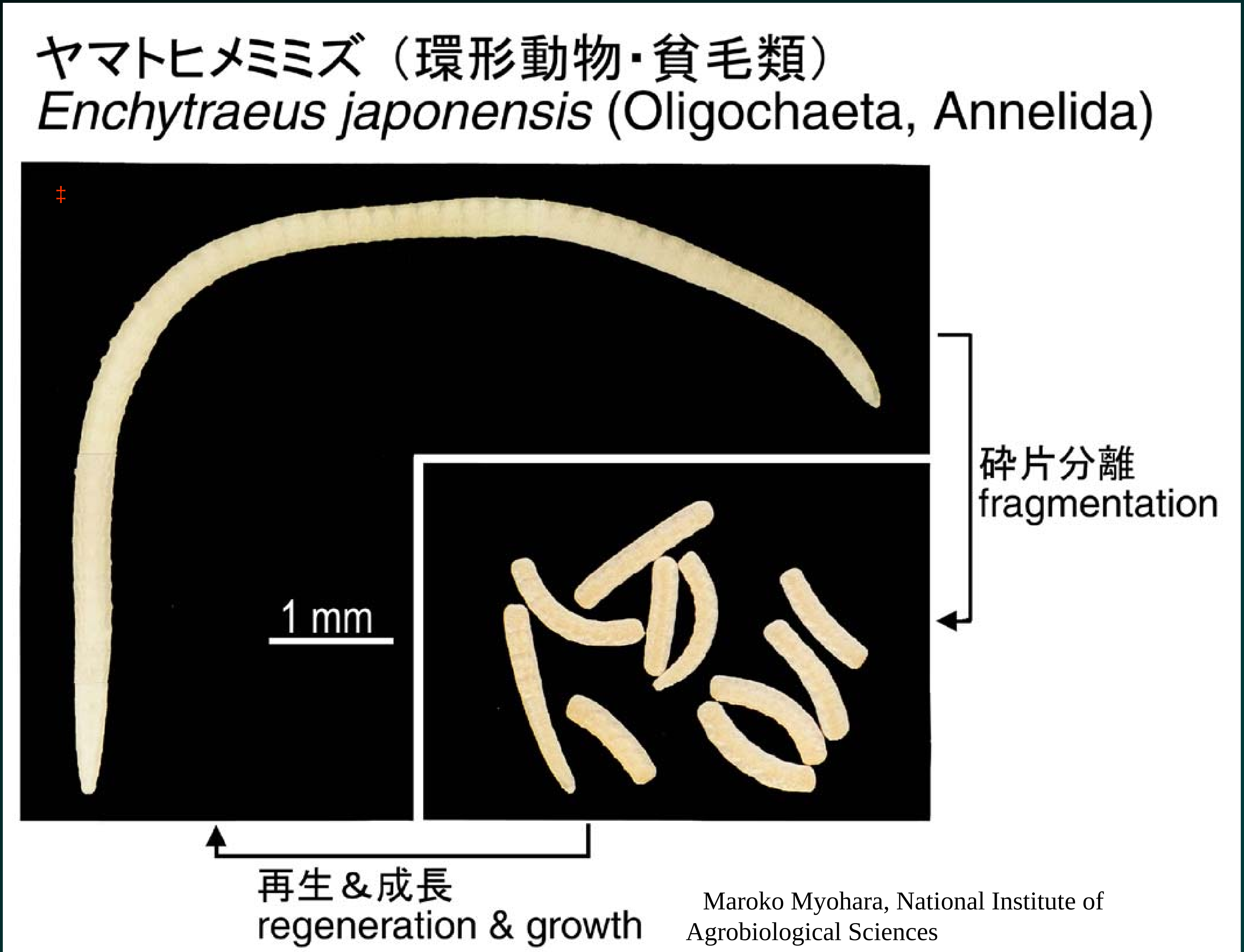
8 species, including *japonensis*, reproduce asexually by fragment separation.

The inner structure of the body is complicated.



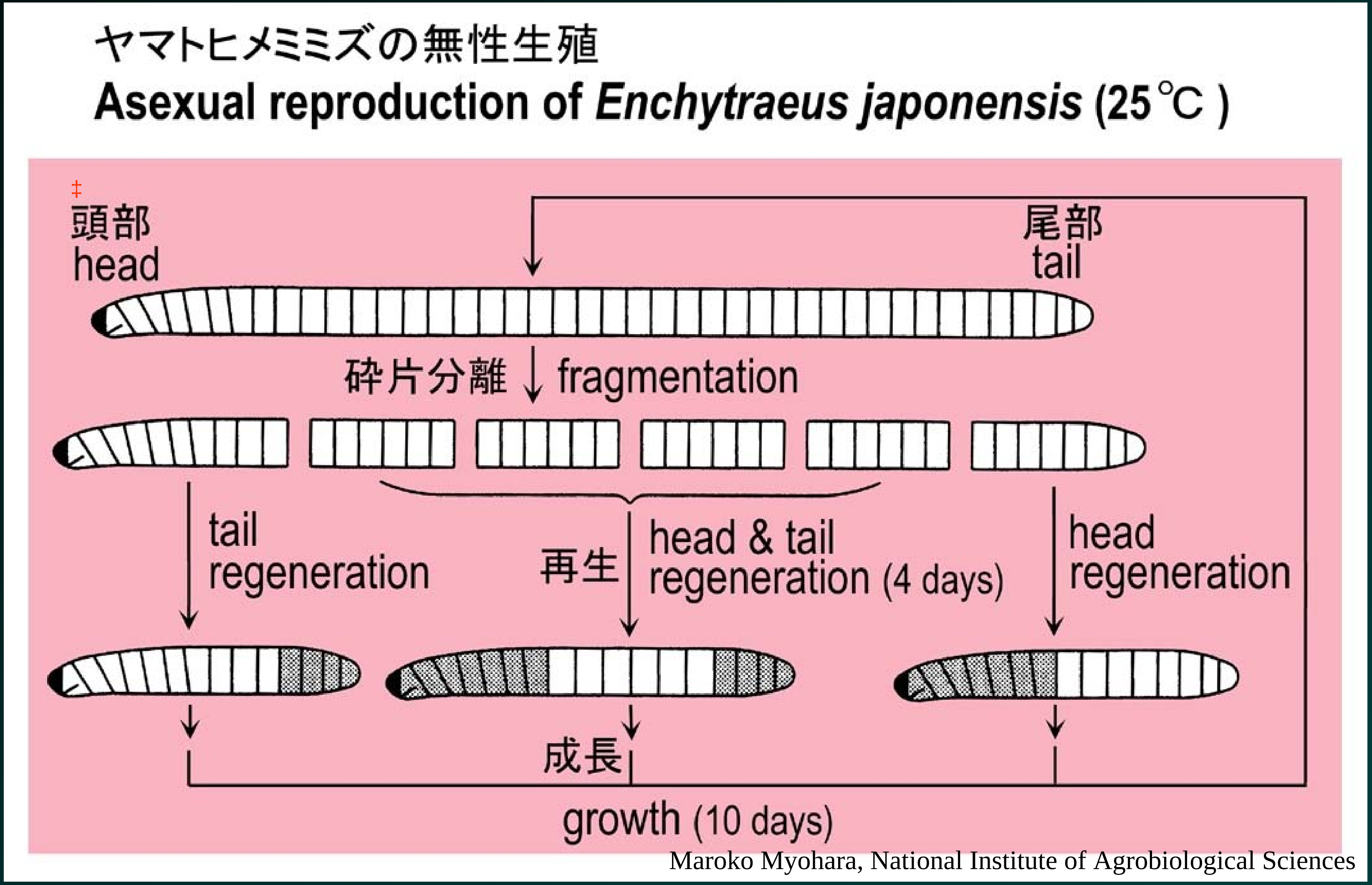
Maroko Myohara, National Institute of Agrobiological Sciences

Regeneration of the *Enchytraeus japonensis* by fragmentation



Maroko Myohara, National Institute of Agrobiological Sciences

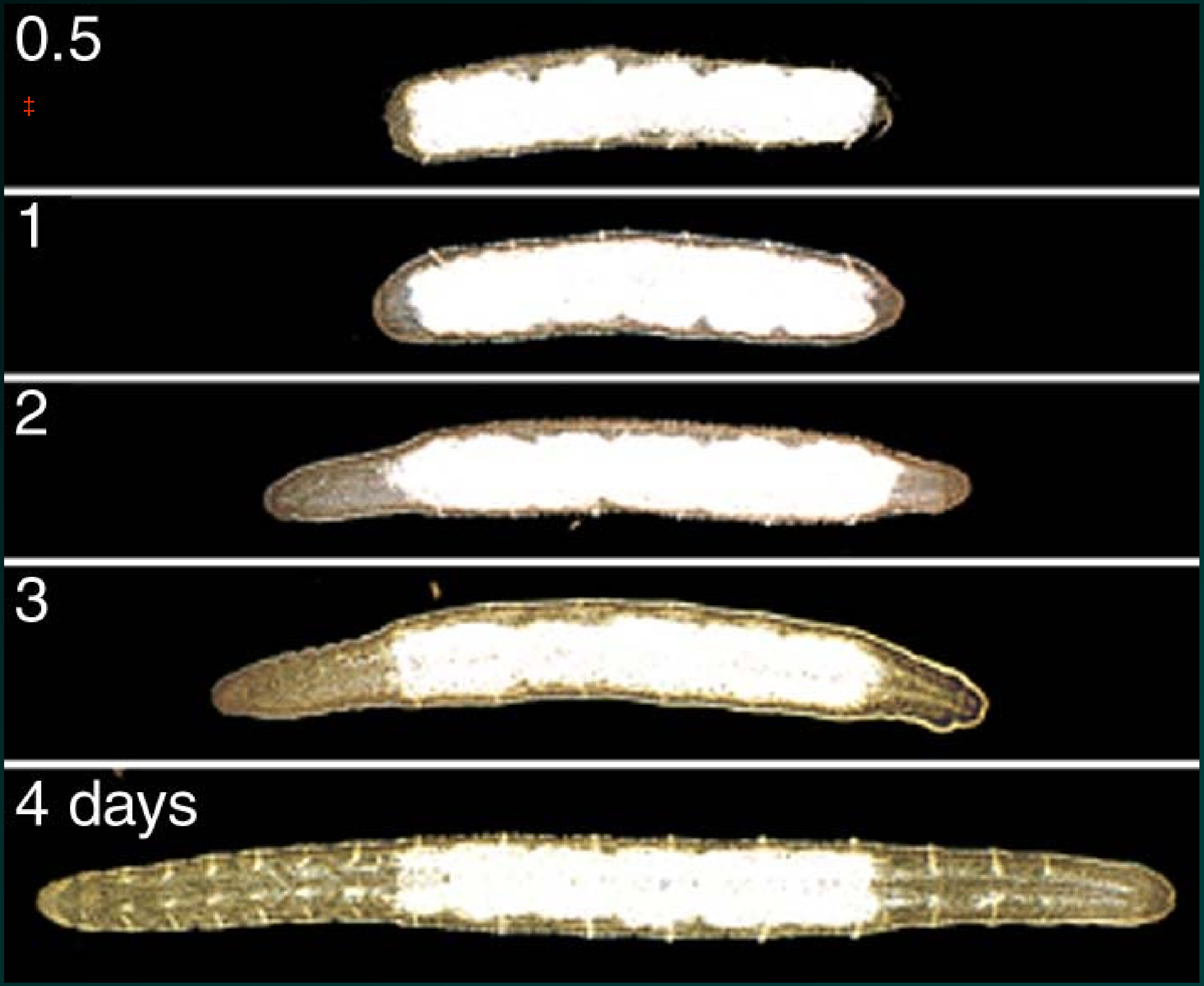
Asexual reproduction of *Enchytraeus japonensis*



A mechanism for reproducing clones by "self cutting"

→ "Regeneration" is part of normal reproduction.

Regeneration of *Enchytraeus japonensis*①

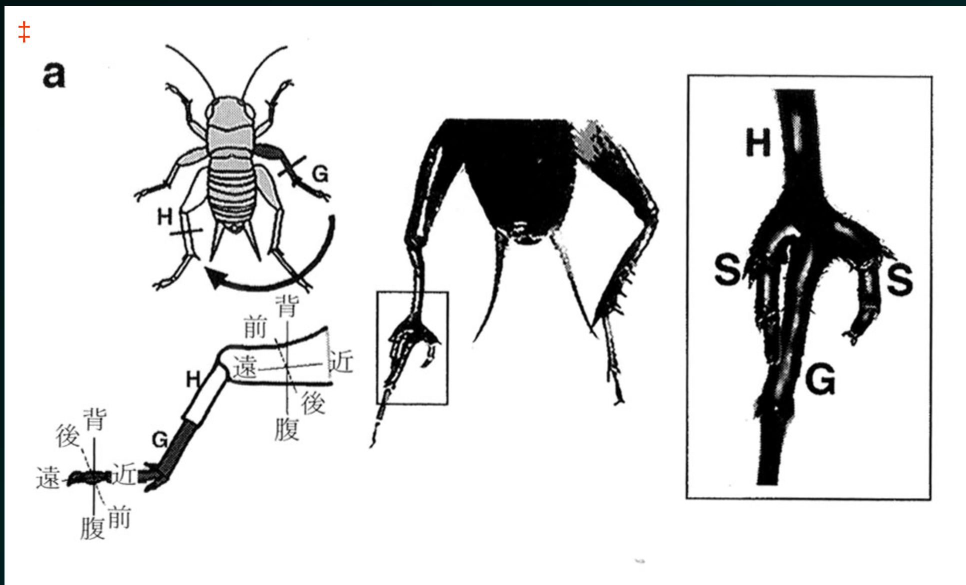


Maroko Myohara, National Institute of Agrobiological Sciences

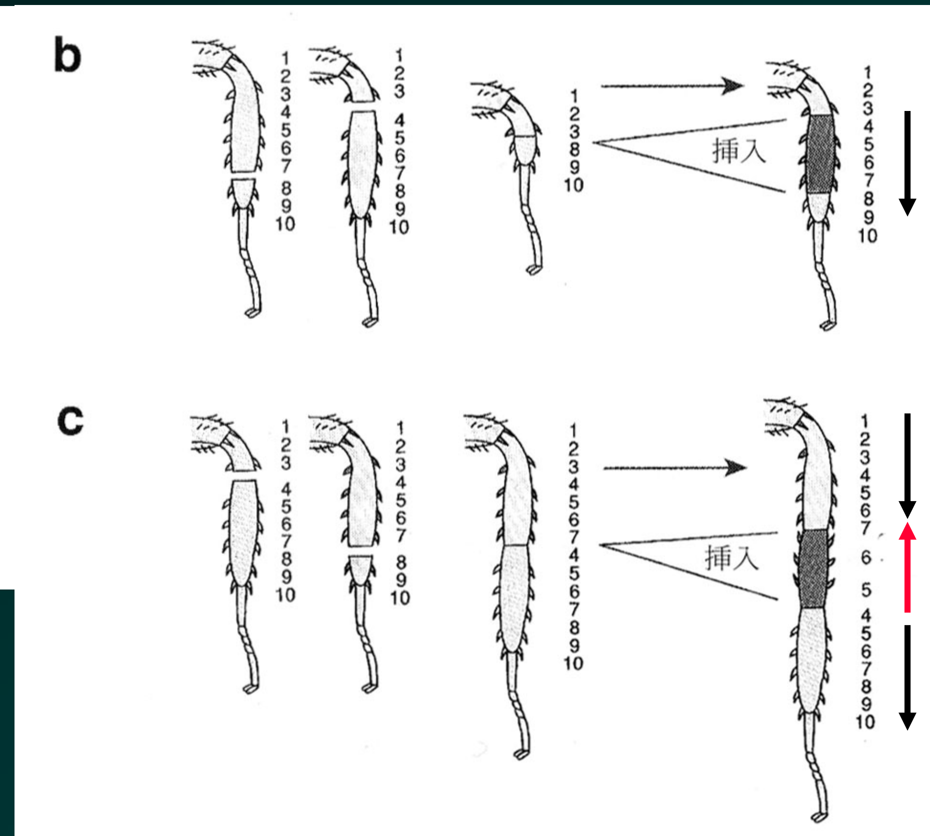
The regeneration of an arthropod

Cricket

The limbs of a cricket have clear positional information.



Taro Mito, 2001 Shokabo

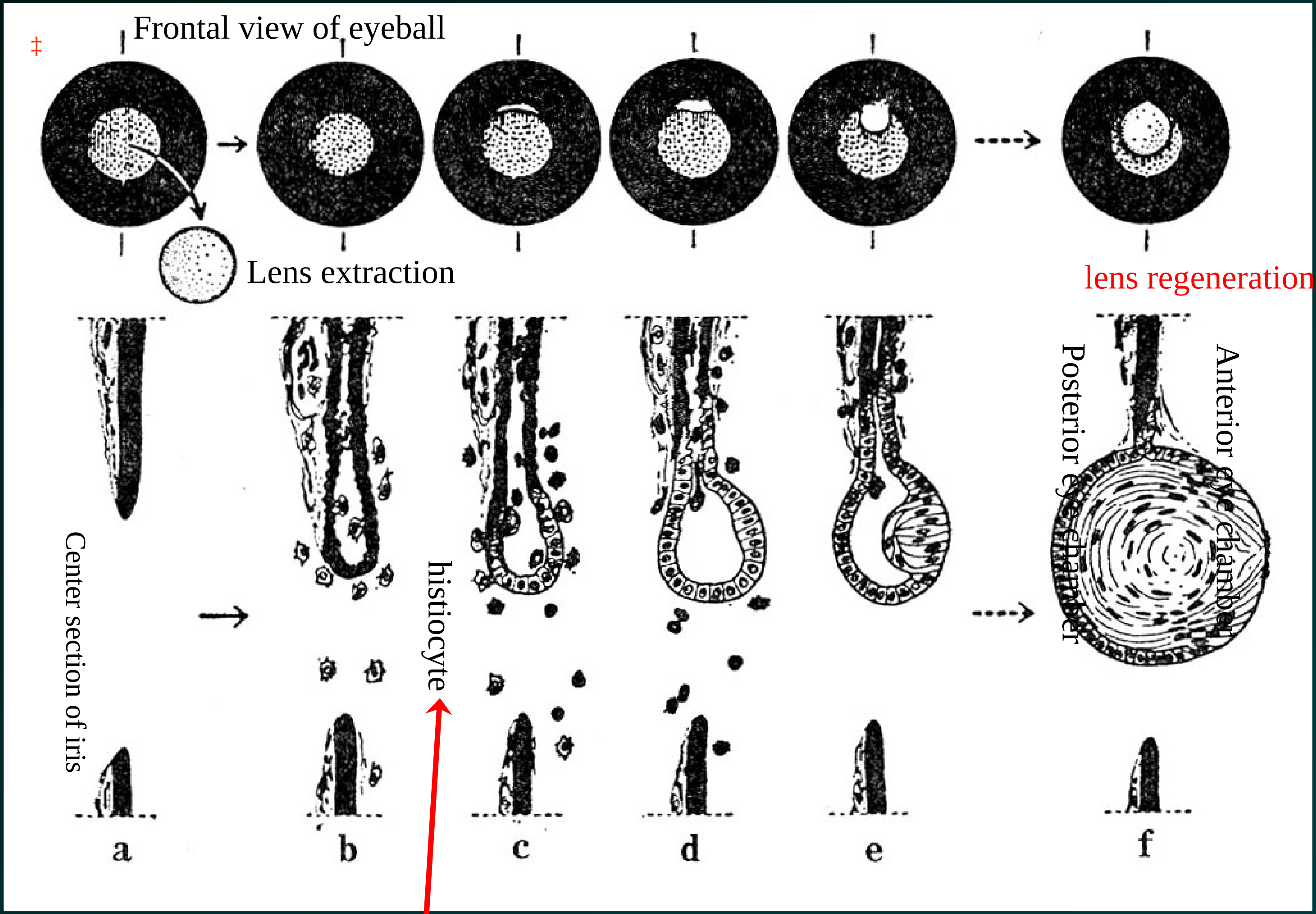


- The limbs of a cricket regenerate.
- When a fore limb is cut and transplanted upside down to a cut section of the back limb, an extra limb is formed by the disruption of positional information.
- When limbs in the same region are cut and transplanted, the direction of the regenerating region is controlled by repeating positional information at both sides of the limb.

The regeneration of vertebrates

newt・axolotl

The lens regeneration of a newt



Phagocytized melanin granules by a histiocyte

1983, Idemitsu Shoten

The lens of a newt regenerate from an upper(dorsal) iris pigment epithelial cell.

Limb regeneration in a newt

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

The limbs of a newt regenerate very well.

A urodele (newt) regenerates, but salientian (frog) does not regenerate well.

Changes in tissues during limb regeneration of a newt

"The picture of newt limbs"

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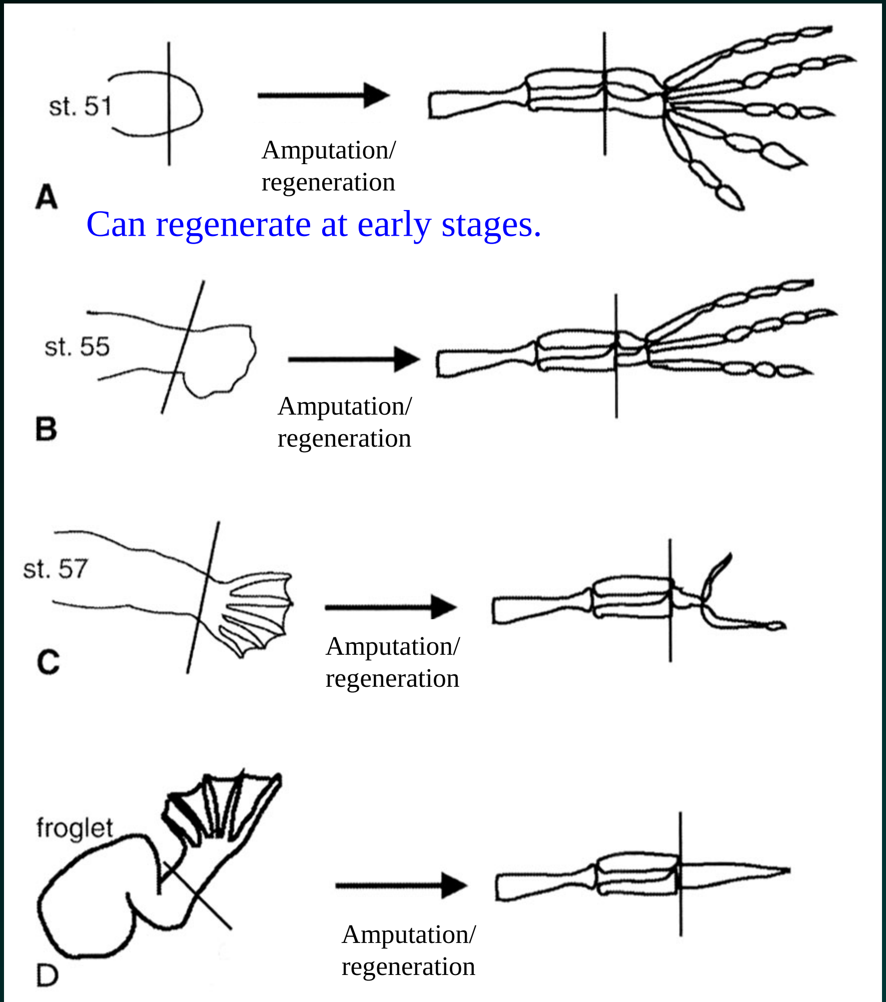
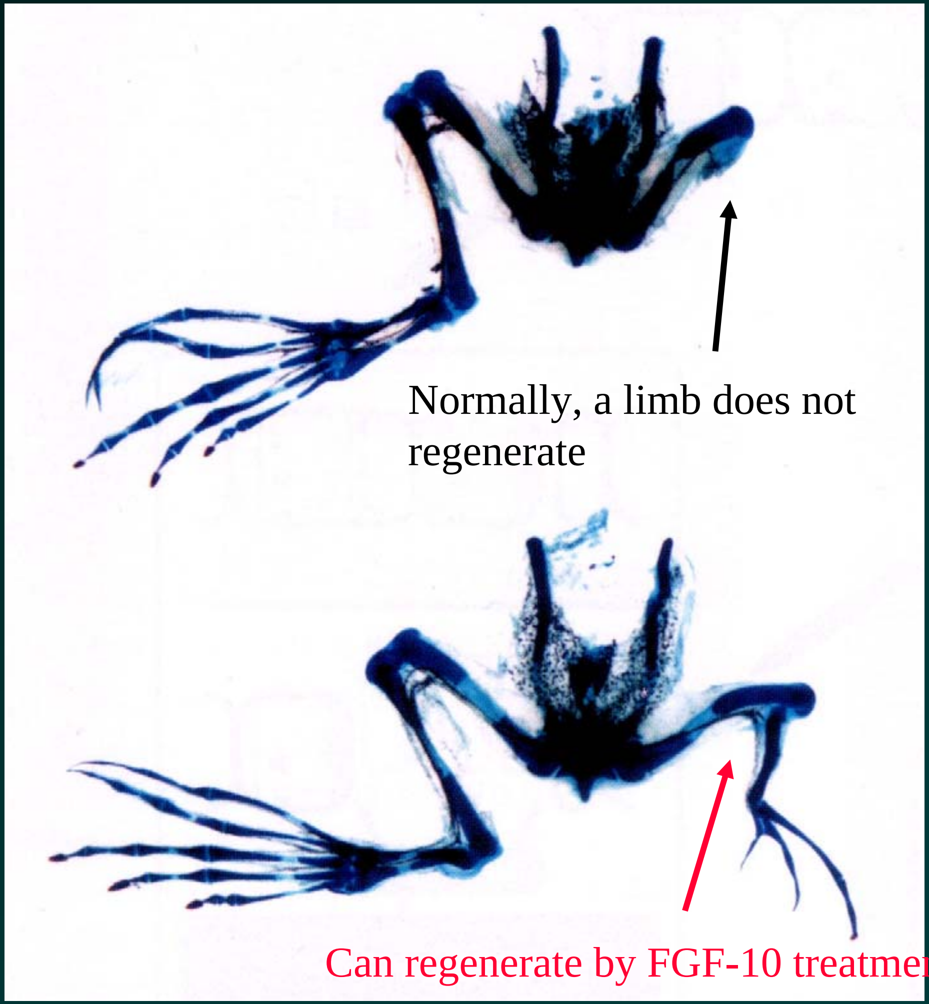
Regeneration of limbs in a frog





Shokabo 2001

The regenerating ability of frogs lowers as development proceeds.

The regeneration of frog limbs can be stimulated by FGF-10 treatment.

Various animals develop from the combinations of similar genes.

What is the difference between animals that regenerate and those that do not?

↓

unknown

Hypotheses:
- Functions and regulations of genes are different.
- Locations and numbers of stem cells are different.

Issues in research on regeneration in model animals

- Elucidating the roles and behaviors of stem cells in regeneration.
- Elucidating a mechanism for determining positional information during regeneration.
- Identification of working factors in regeneration and their functions

Tissue and stem cell regeneration in mammals

Tissue stem cells are found in
skin · hair · intestinal epithelia · muscles · nerves, etc.

Can humans regenerate their injured bodies?

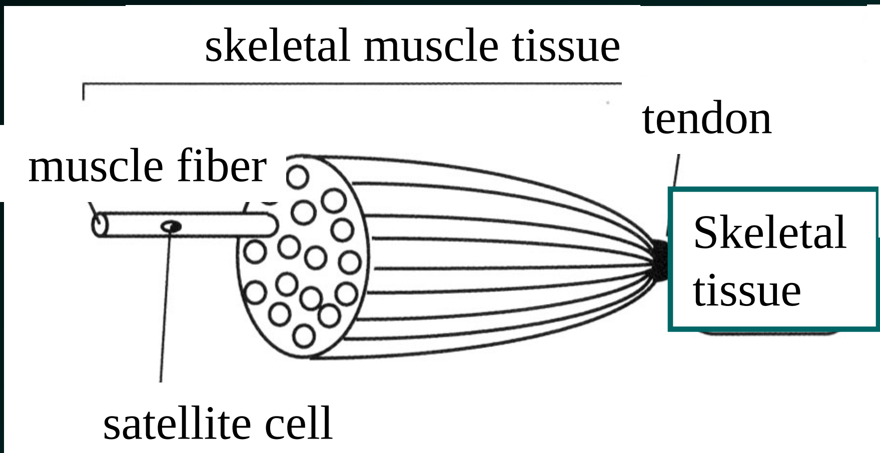
① Superficial wounds can be cured.
② Homeostasis is maintained by the constant turnover of cells

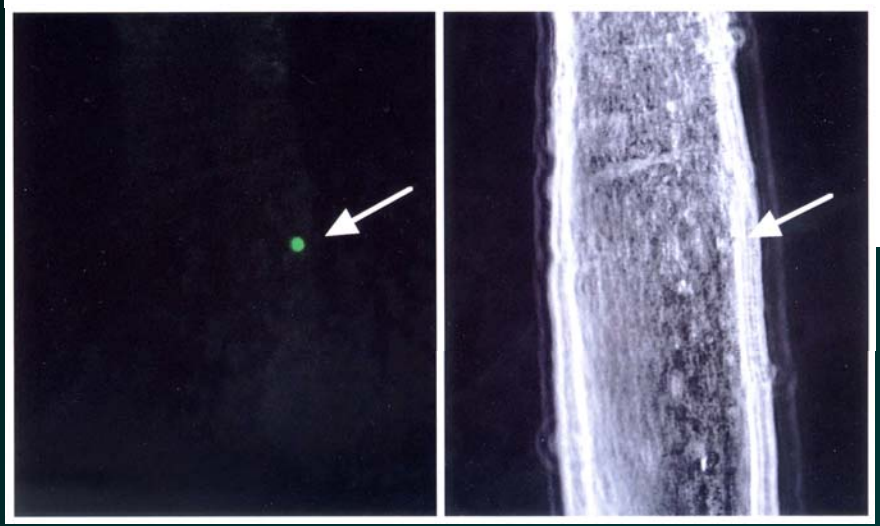
Example:
- Blood cells are destroyed and produced constantly.
- The turnover of skin cells is constant.
- Hair keeps falling out and growing out.
- Epithelia of the small intestine turn over frequently.
- Muscle recovers when damaged.

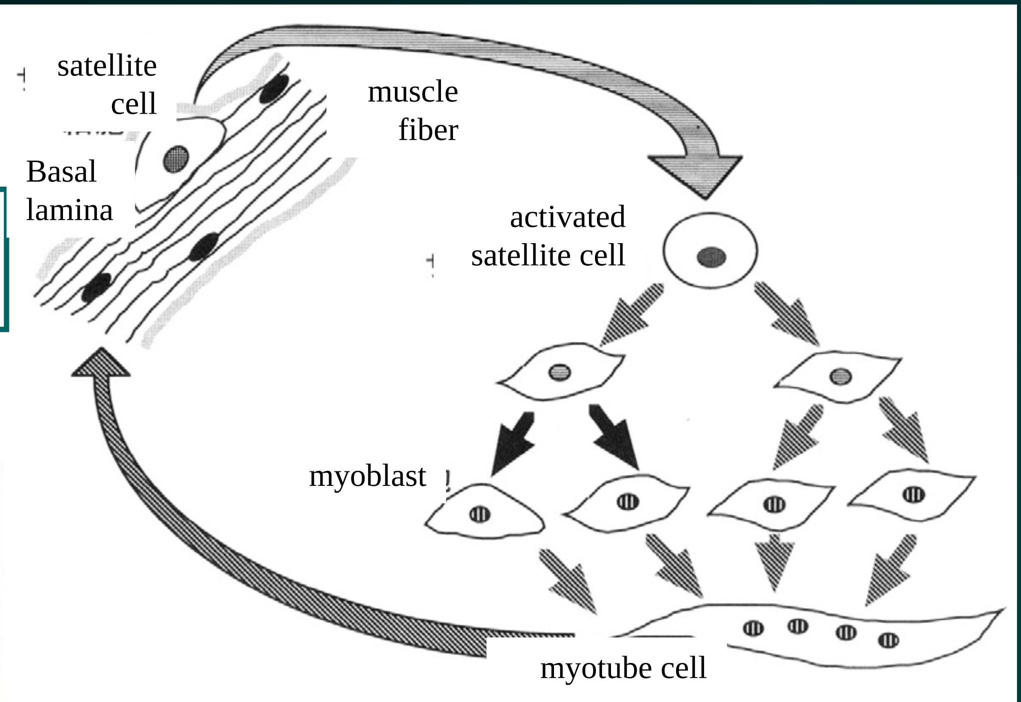
Tissue stem cells control the mechanism of cell metabolism.

Tissue stem cells have been discovered recently in the brain and the eye.

Muscle tissue stem cells





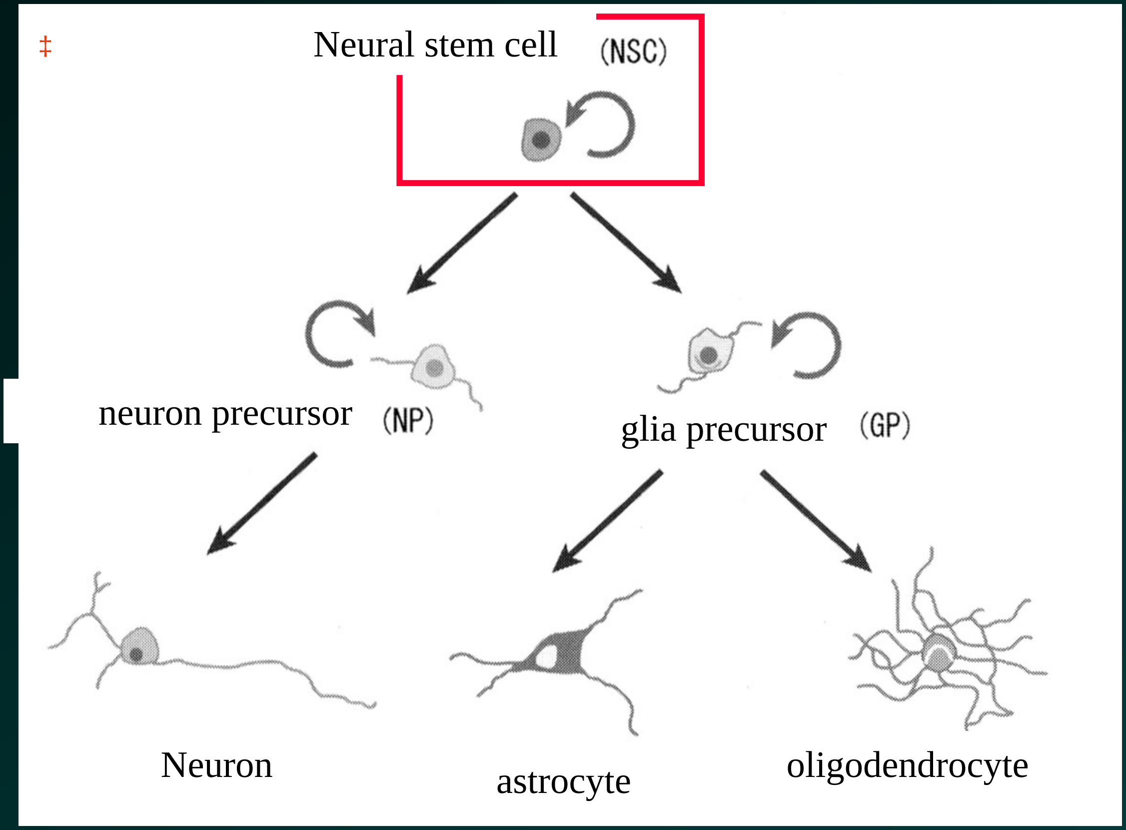


Stem cells called satellite cells are found in muscles, which are responsible for muscle defect recovery.

A. Hashimoto, 2001 Shokabo

Neural stem cells in the brain

An adult brain has stem cells.



Y. Yoshizaki 2001 Shokabo

Neural stem cells can differentiate into any cells in the nervous system.

Future applications such as using artificially differentiated dopaminergic neurons in a remedy for Parkinson’s disease are considered.

Cells that can be found in a tissue stem cell in an eyeball

endo
drug

erentiated

"The picture of eyeball tissue"
inserted here was omitted
according to copyright issue.

retin
epith

d
s

epithelia

nerve retina

Visual recovery

CMZ: ciliary marginal zone

An eyeball has cells which divides, and can be used as stem cells for the retina.

Use of eyeball stem cells to be incubated in vitro, then transplanted back to the eye to cause the recovery of eyesight.

Regenerative medicine using stem cells

Promotion of healing with cell transplantation
Recovery of serious defects is difficult

↓

To make up for organs which cannot recover normally, can't we use human cells to construct organs?

↓

The new concept
"Regenerative medicine by in vitro organ formation"

Paradigm change in organ formation research

before

Observe and analyze phenomena during normal organ development

↓

after

Develop artificial reproducing system of organ formation, and discover mechanisms of organ formation by analyzing this system.

Develop a method of artificial organ formation.

Classification of stem cells and problems in research

Stem cells： Cells which maintain an undifferentiated state, and have the ability to differentiate into various cells（omnipotency）

Embryo stem cell（ES cell）

- Omnipotent.
- Ethical problems regardijng ES cells taken from human embryos
- Cause cancer when transplanted（teratoma）

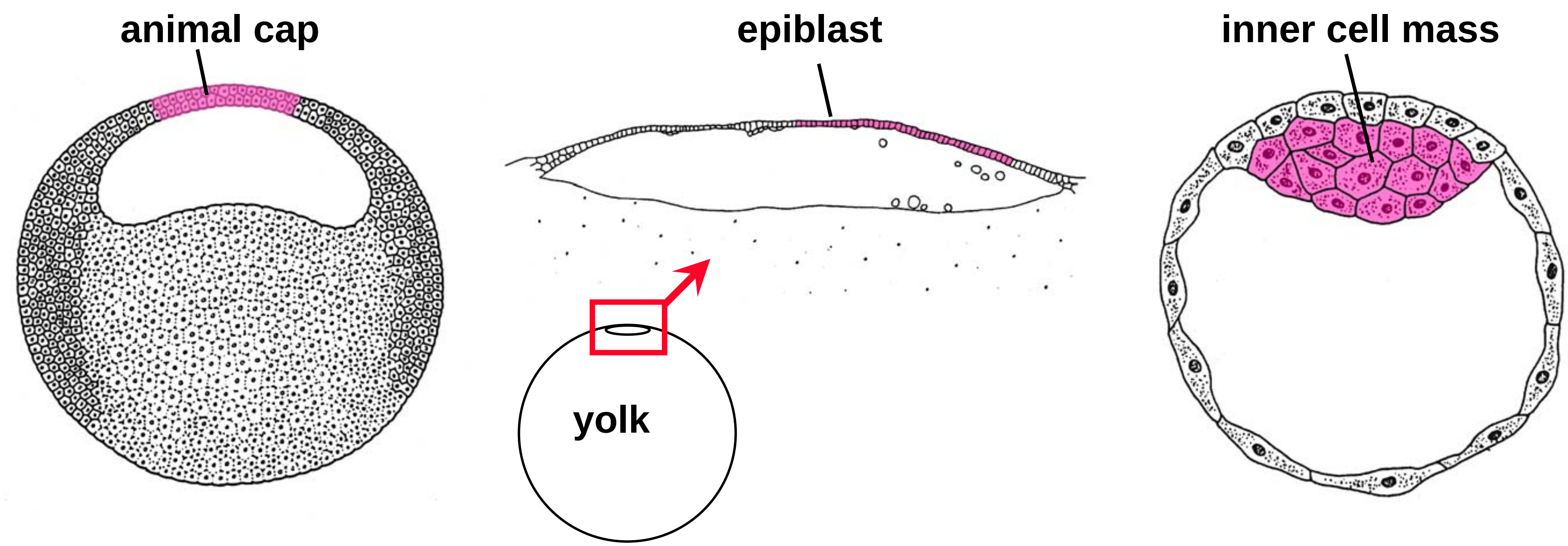
Somatic stem cell（adult stem cell, tissue stem cell）

- omnipotent
- without ethical problems
- low ability to proliferate

Analyze organ formation using mouse ES cells, and apply discoveries to tissue stem cells.

Find a way of research to clear away ethical problems

Omnipotent stem cells in vertebrate embryos



Xenopus
nest
(amphibians)

chicken
(avians)

rat
human
(mammals)

Research on organ formation is conducted using these stem cells （undifferentiated cells)

Typical flow of organ formation research

① Establishment of an in vitro induction system, to each tissues and organs from undifferentiated cells

② Comparison with normal tissues and organs（histological & molecular biological analysis）

③ Function recovery experiment by transplant

④ Isolation and function analysis of newly found genes responsible for organ/tissue formation

Furthermore,

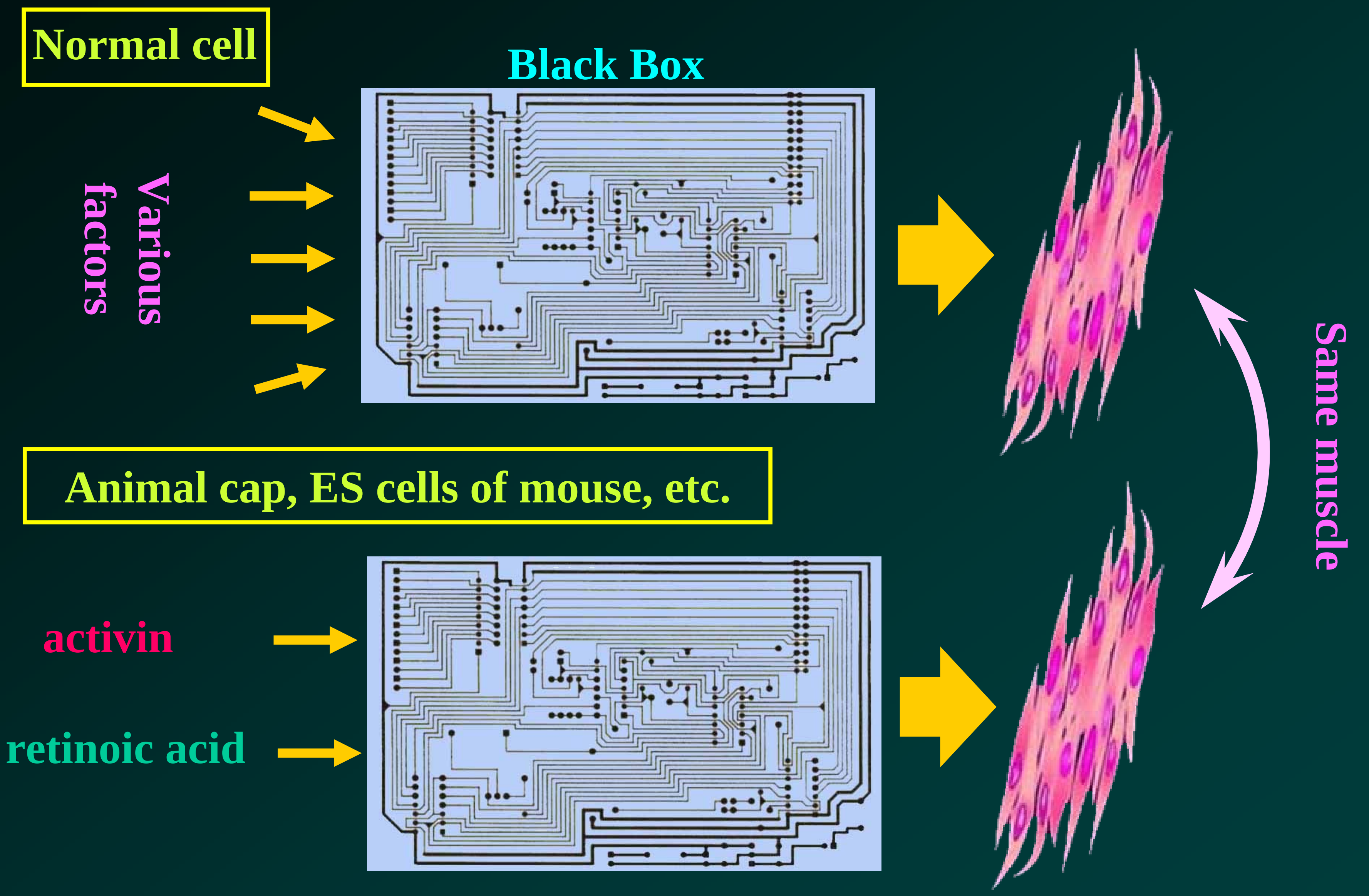
Elucidation of organ/tissue formation mechanism and application of it to organ/tissue formation method

Reformation and development of induction system and incubation to discover systematic organ incubation

（application to medicine）

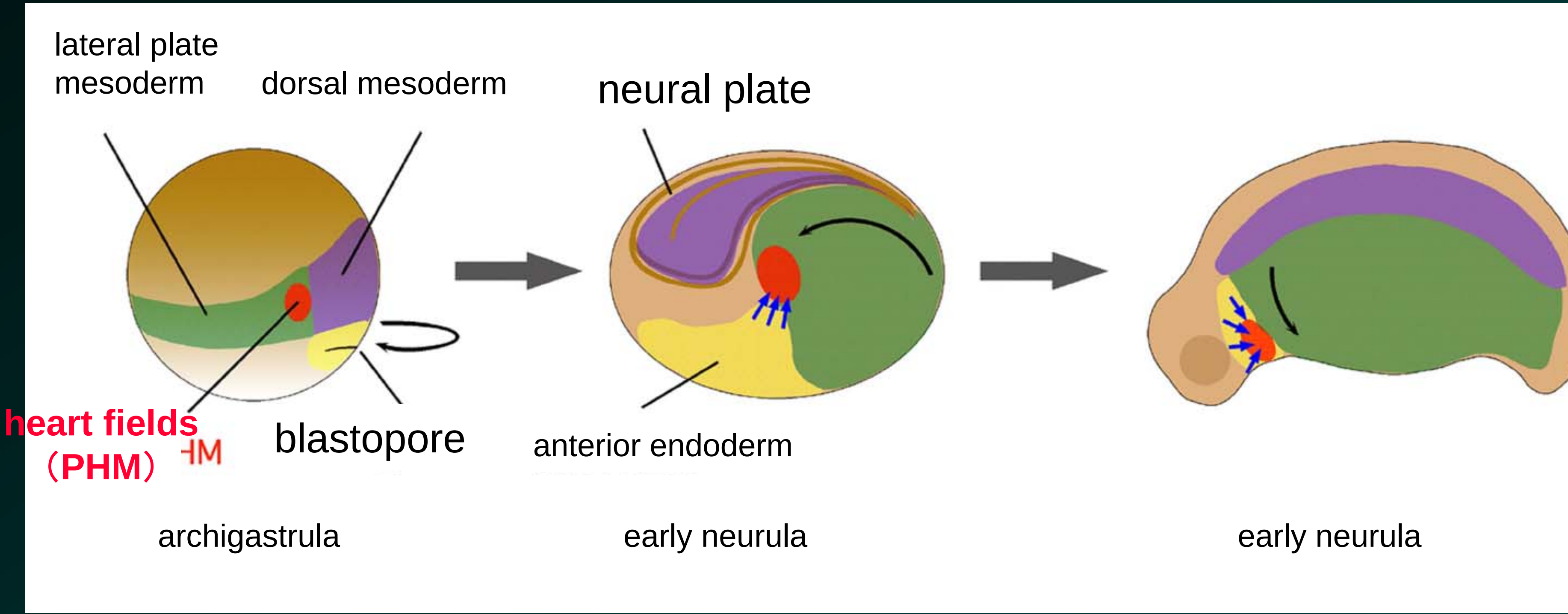
Research in organ formation using undifferentiated Xenopus cells

Organ formation model



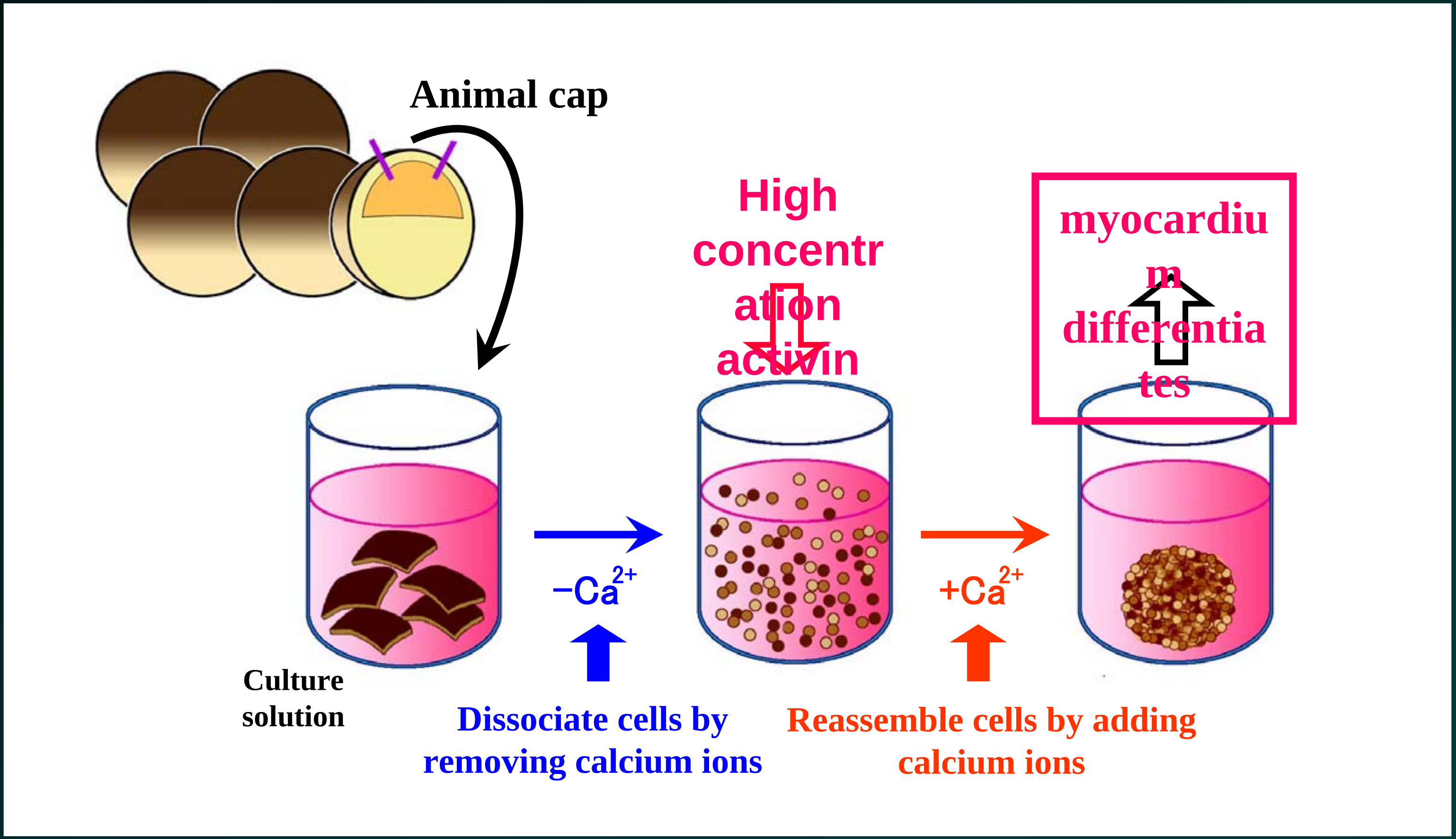
Examination of a heart system induced using undifferentiated Xenopus cells

Heart development in a Xenopus embryo



Heart fields (PHM) migrate, and can be induced by an anterior endoderm

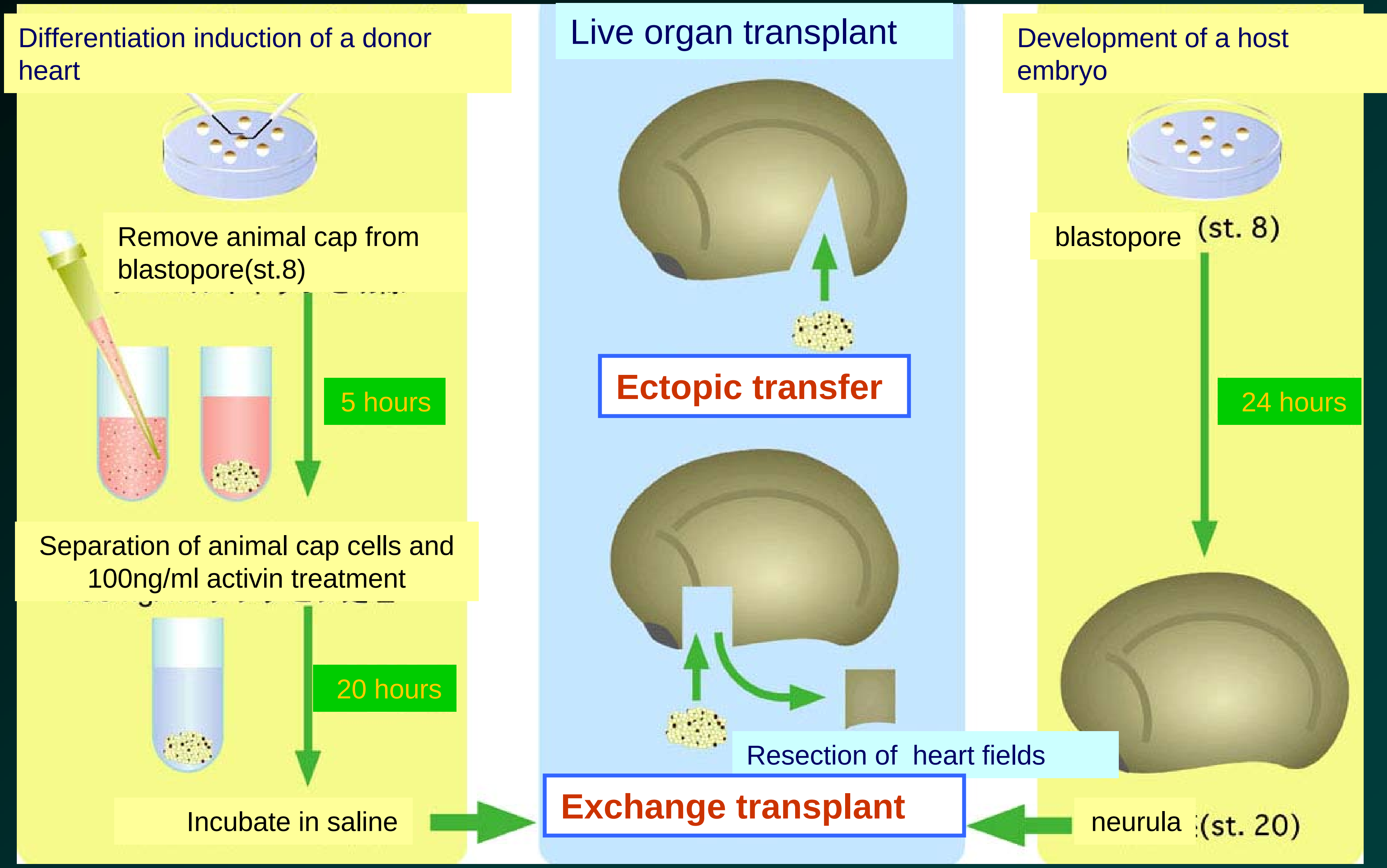
Heart induction system examined using undifferentiated Xenopus cells



Isolate animal cap from Xenopus blastula,
treat with high concentration of activin, reassemble, and incubate.
An autonomously pumping heart-like structure is induced.

Pumping "heart" made from an animal cap

Experiment transplanting heart fields induced from undifferentiated Xenopus cells

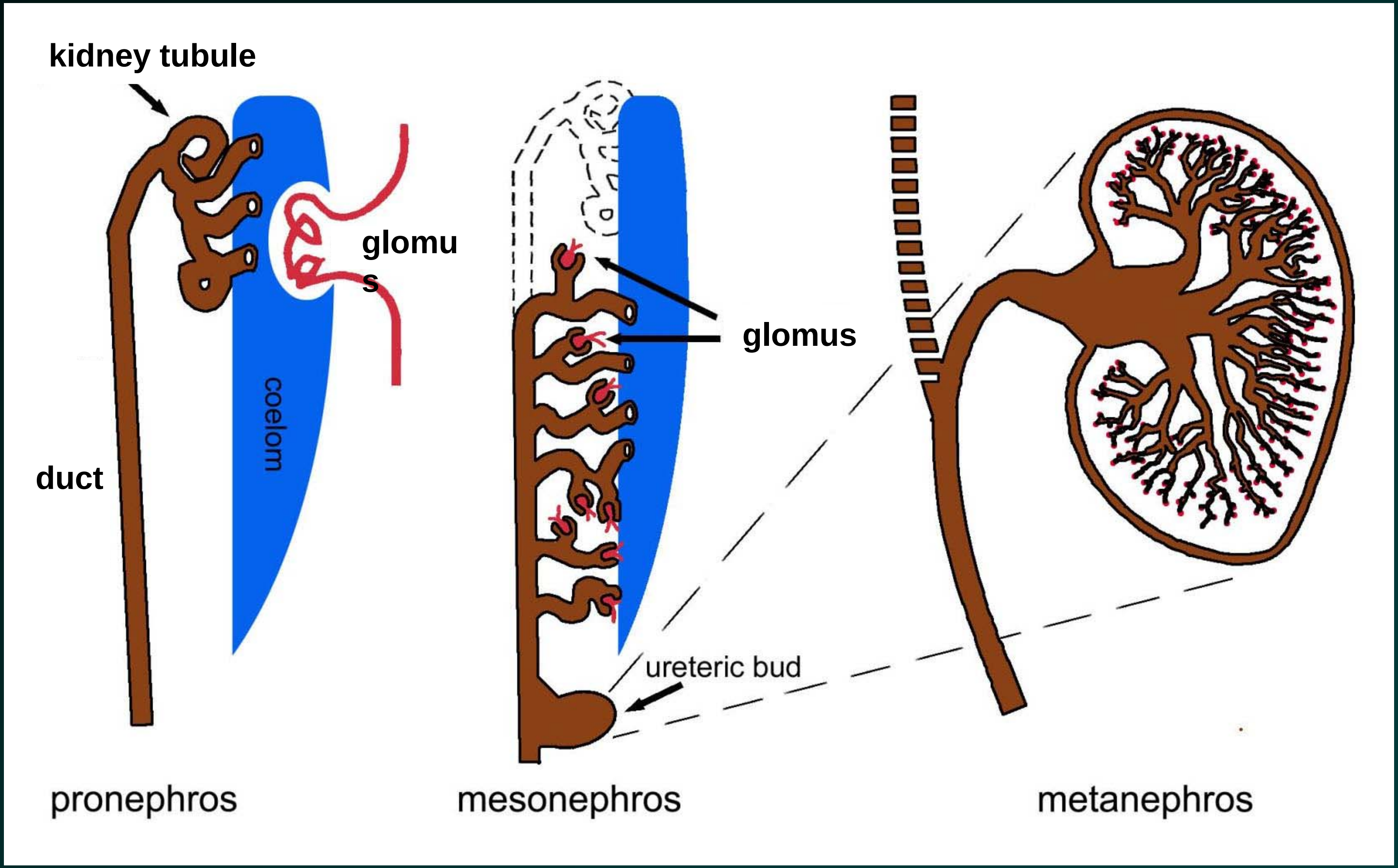


An embryo transplanted with pumping tissue induced from an animal cap

Ectopic heart-transplanted larva

Kidney system induced using undifferentiated Xenopus cells

Differentiation in a kidney

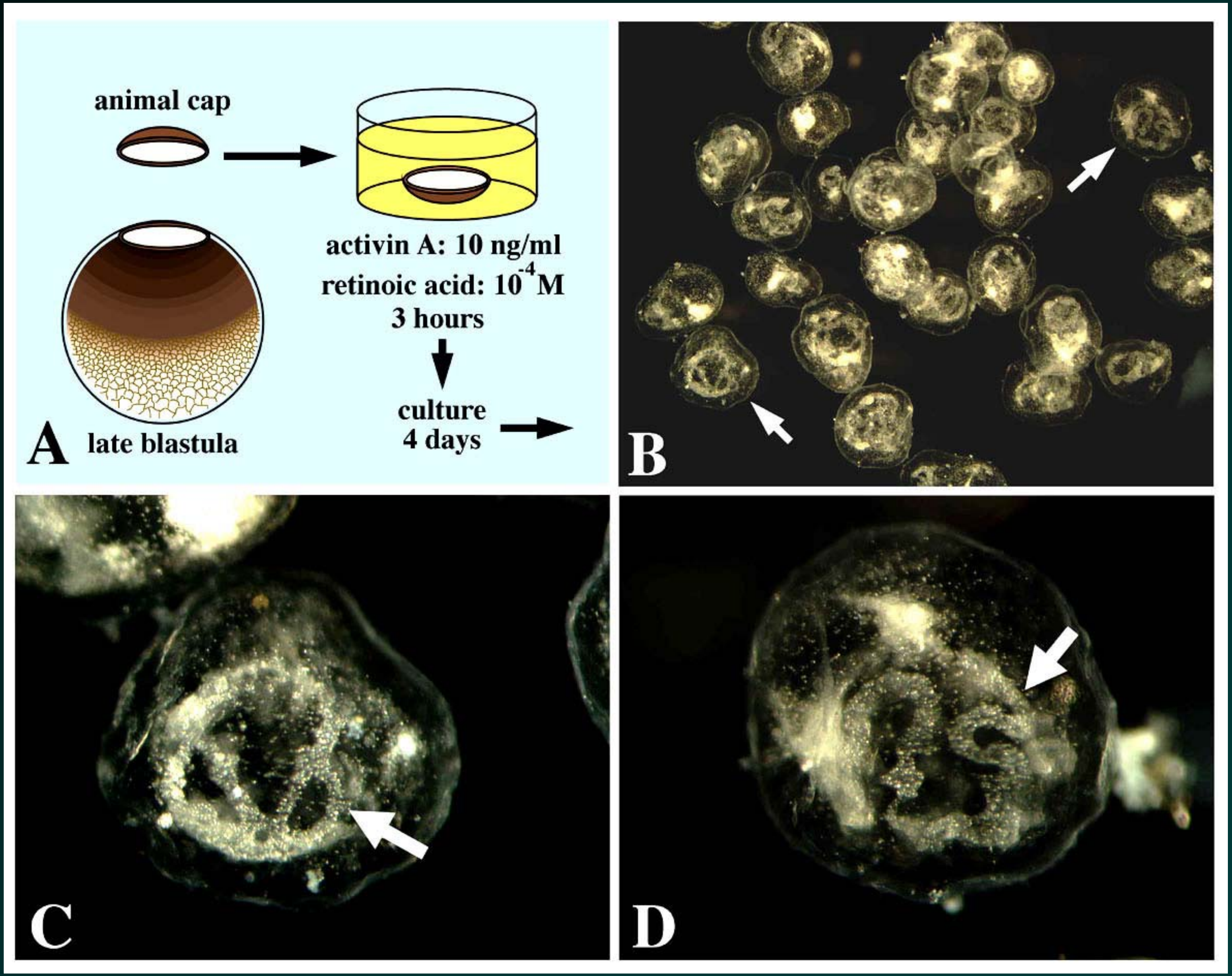


pronephros
（1 nephron）
tadpole

mesonephros
（30 nephrons）
adult frog

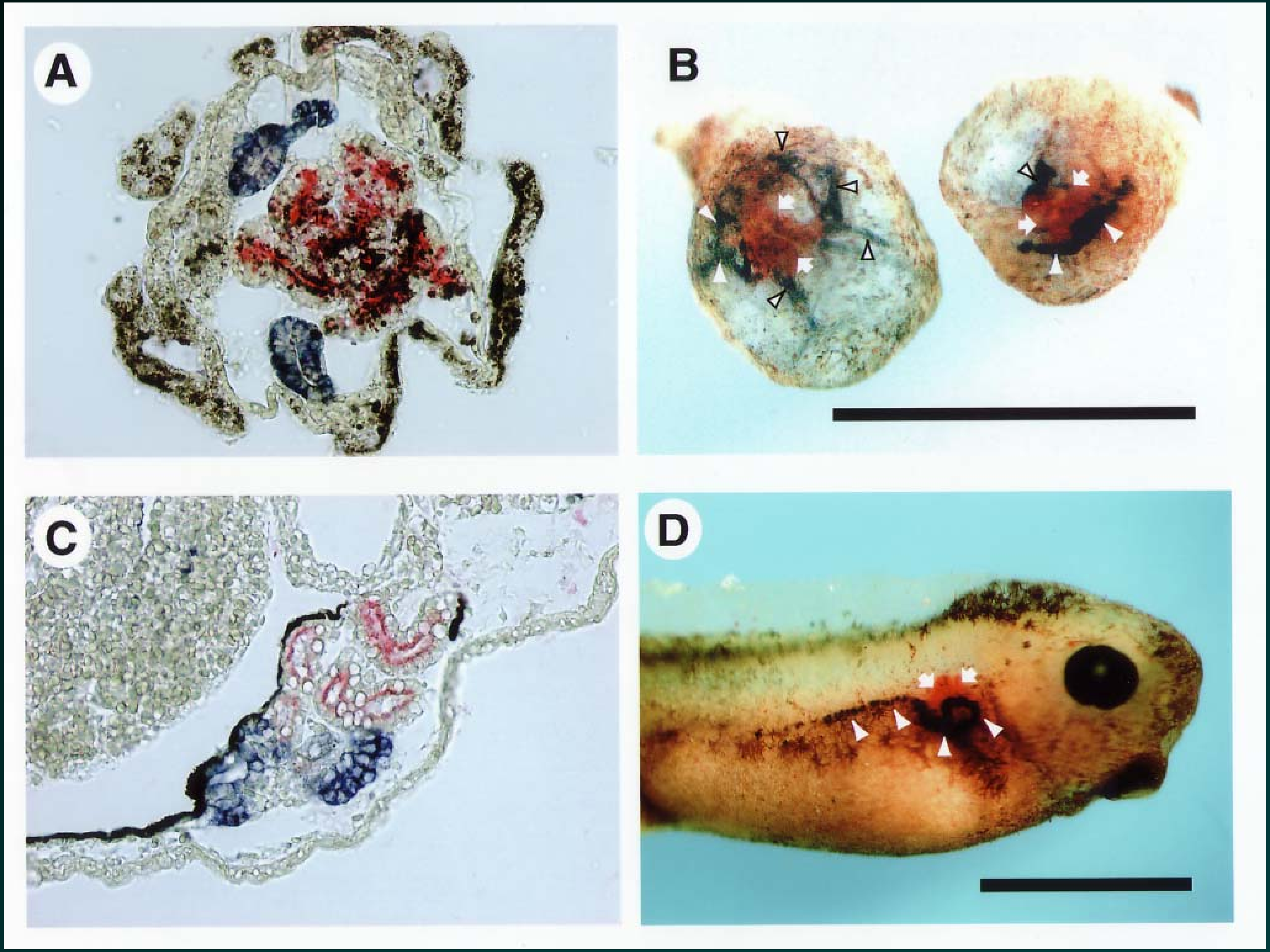
metanephros
（a million nephrons）
human, etc.

The induction of pronephros from undifferentiated Xenopus cells①



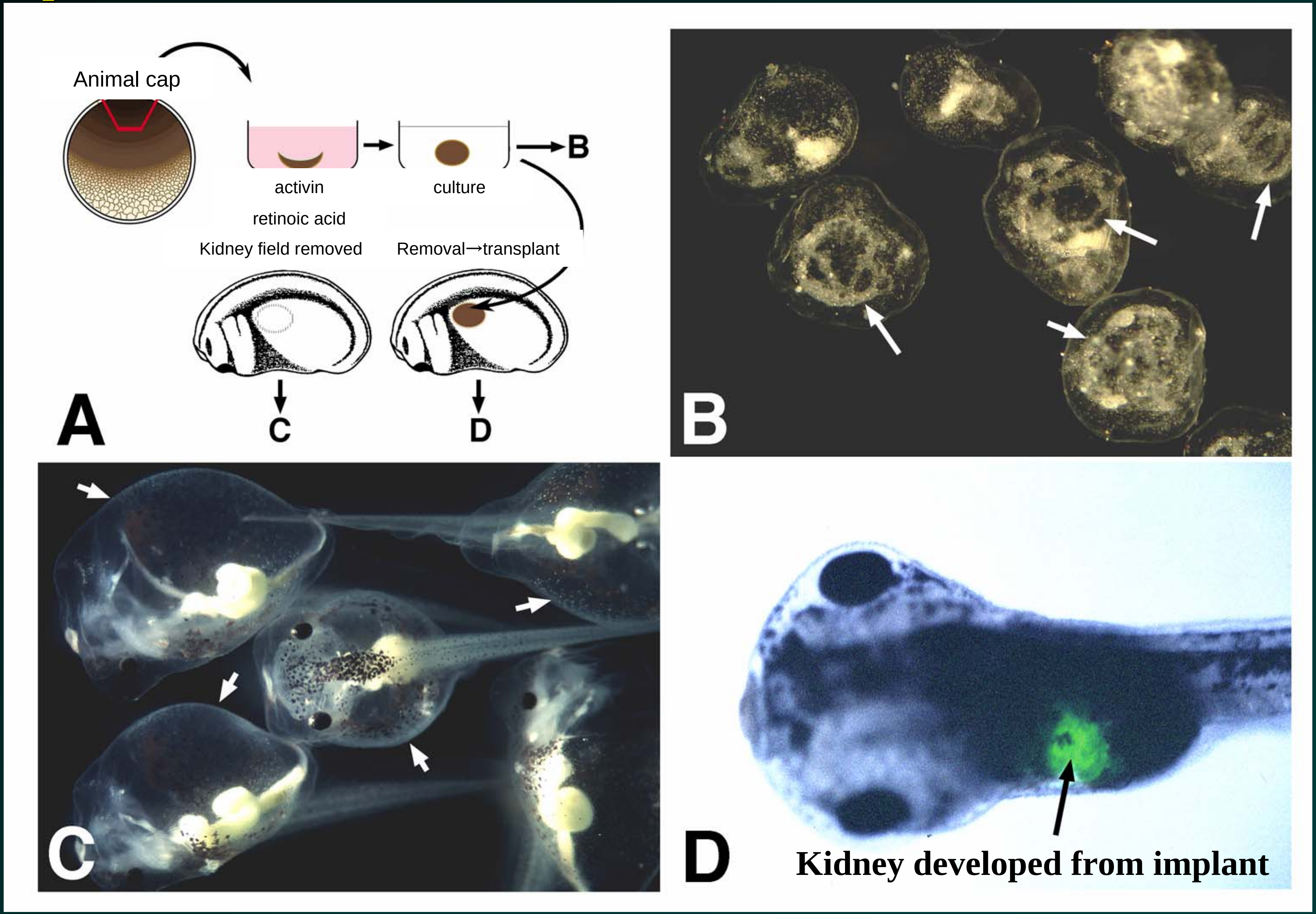
Pronephros are formed when an animal cap is treated with activin and retinoic acid.

The induction of pronephros from undifferentiated Xenopus cells②



Pronephros induced from an animal cap were dyed by kidney-specific antibodies in the same way as in a normal pronephros.

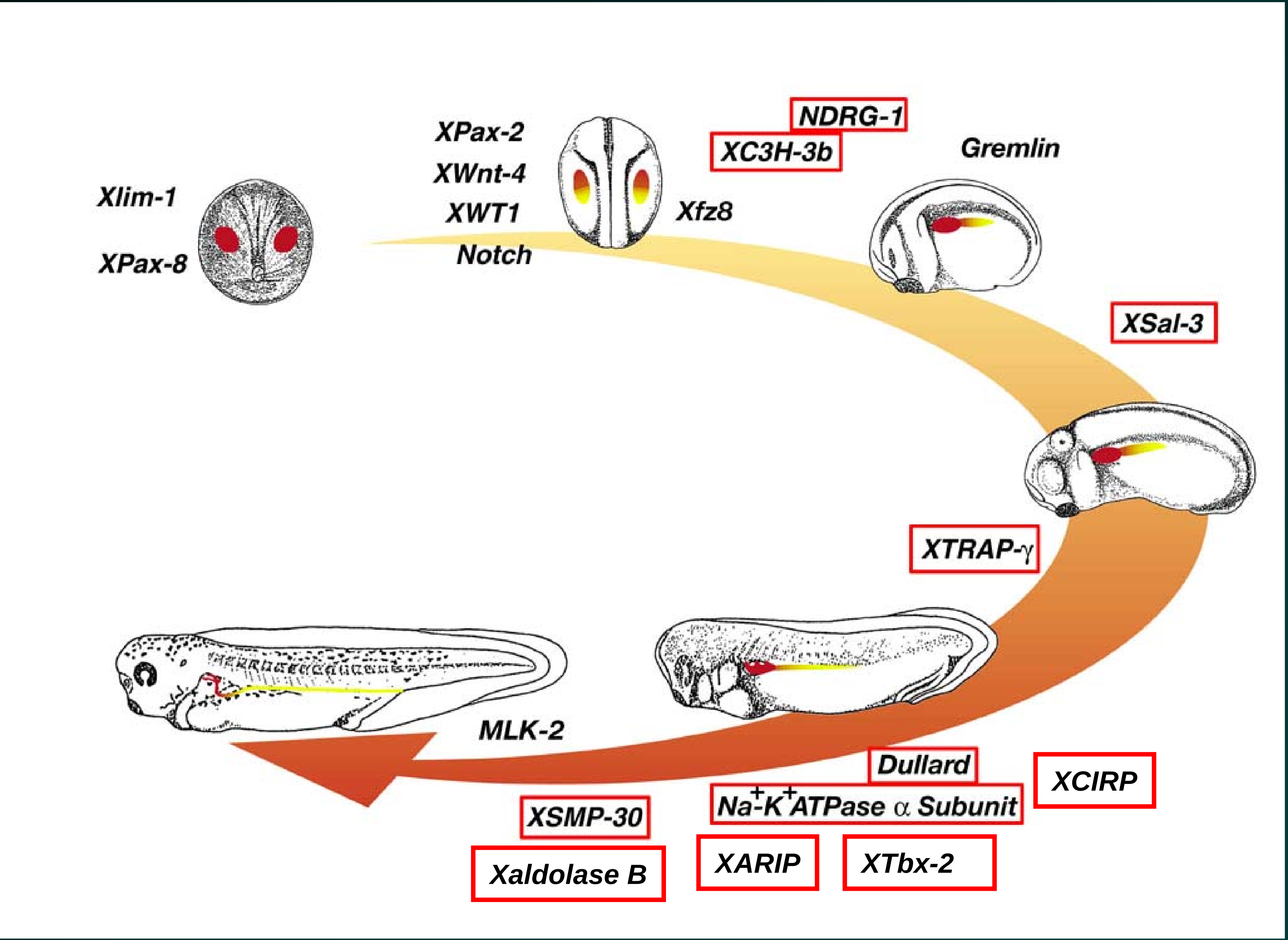
An experiment transplanting kidney fields induced from undifferentiated Xenopus cells



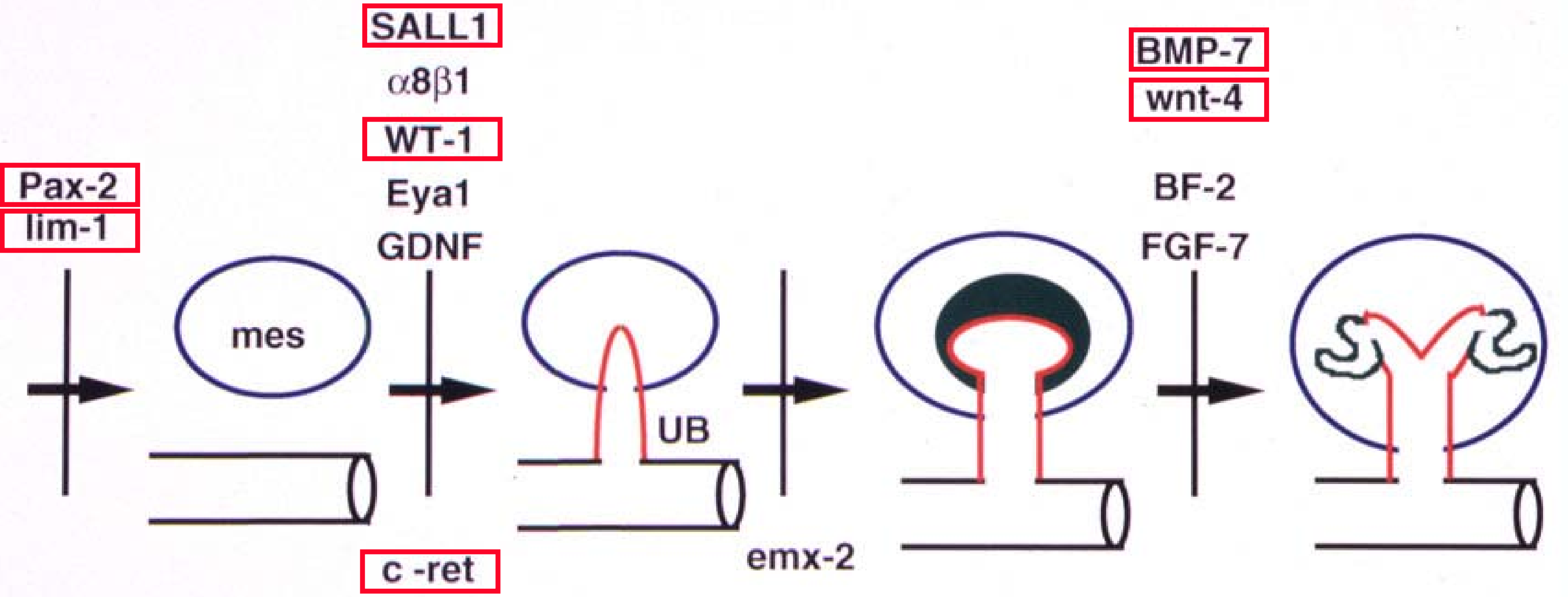
Pronephros with a normal function was formed when pronephros fields induced from an animal cap were transplanted.

→ This induction system reproduces the formation of normal kidney fields.

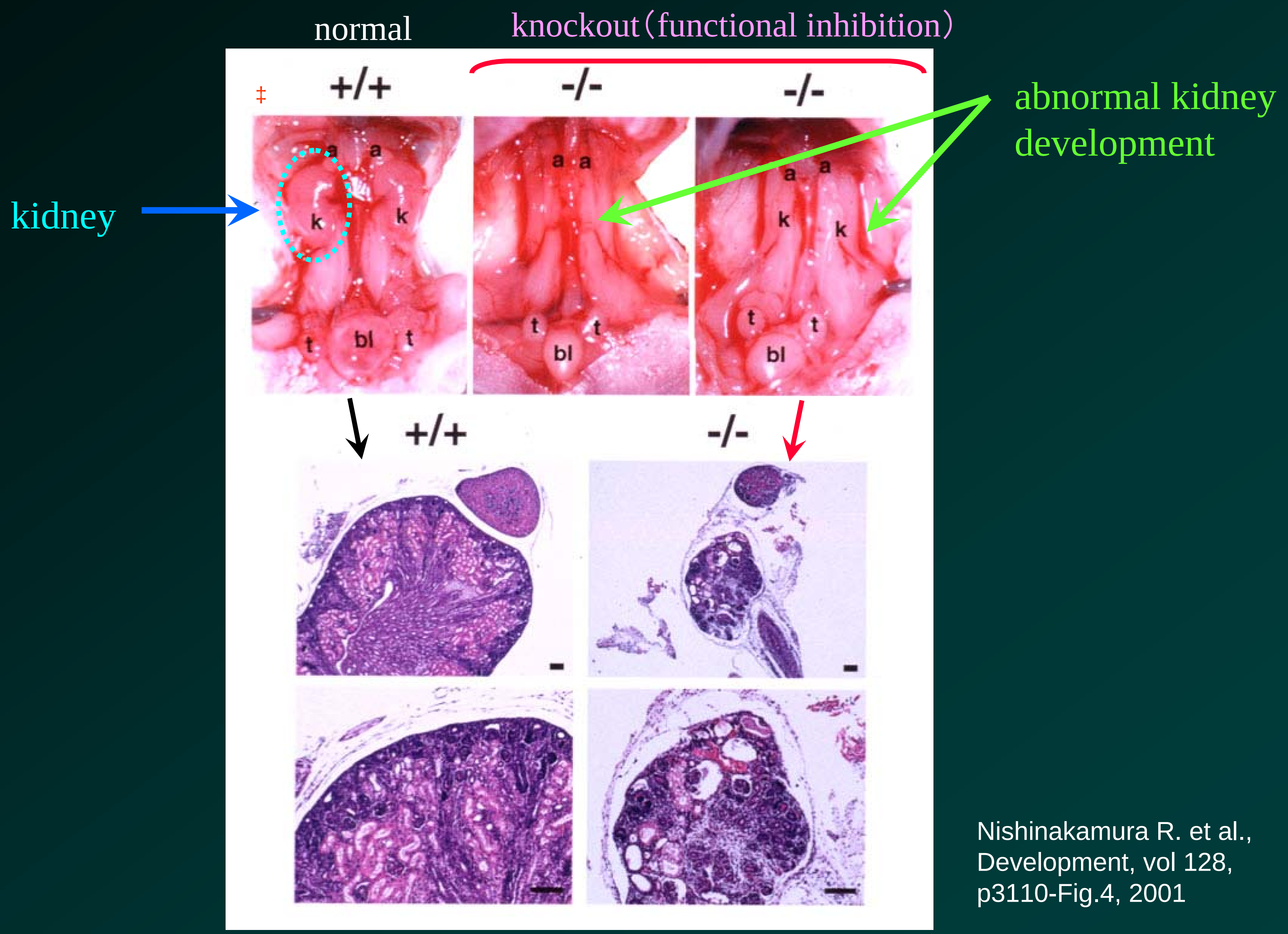
Gene expression in the development of a Xenopus pronephros



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



An abnormal kidney formed in a SALL-knockout mouse

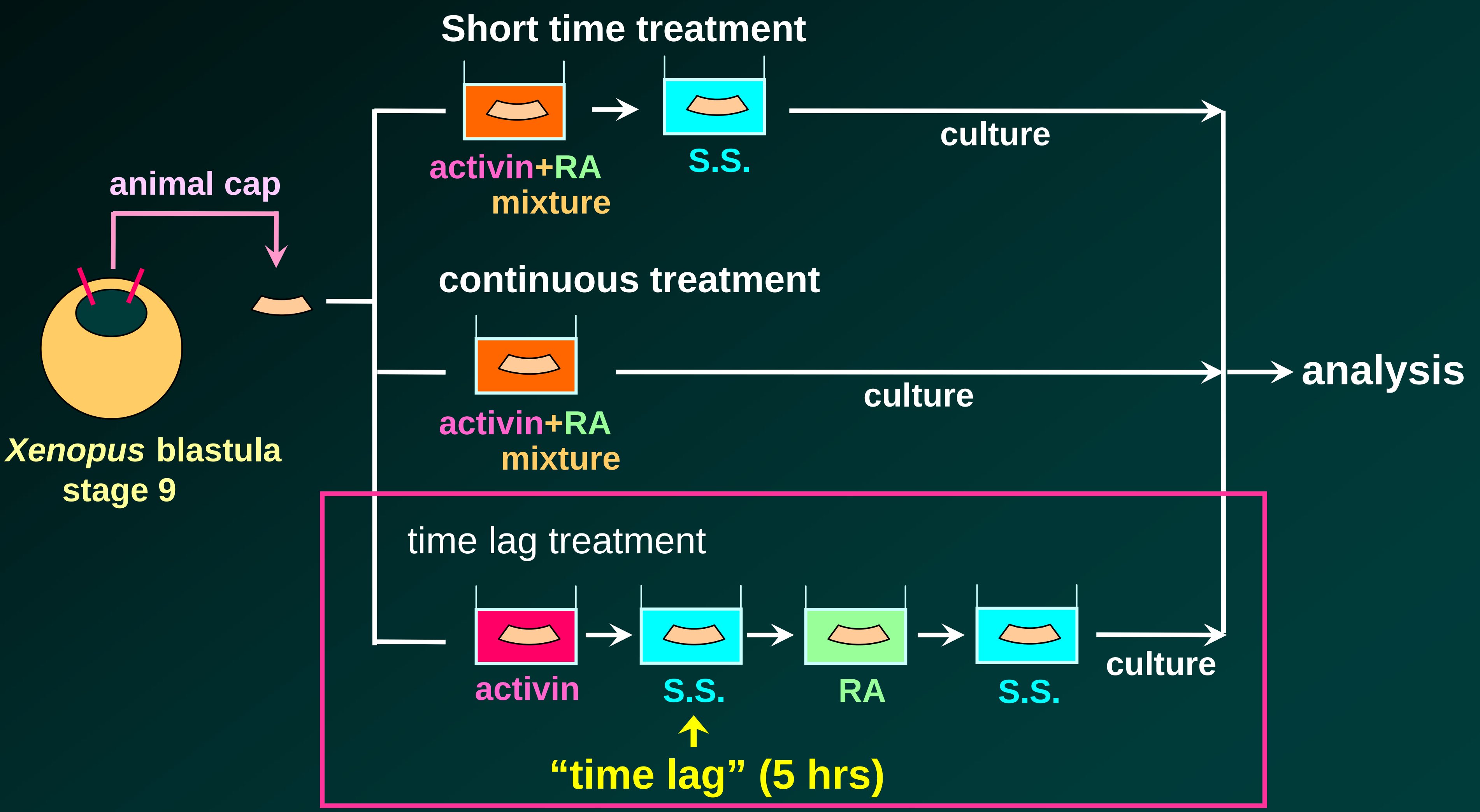


Nishinakamura R. et al., Development, vol 128, p3110-Fig.4, 2001

SALL gene whose function in Xenopus pronephros is also important in the kidney formation of a mouse

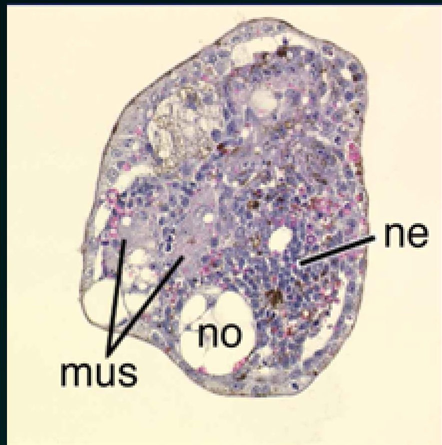
Pancreas induction system examined with undifferentiated Xenopus cells

A system made by introducing an animal cap into the pancreas using activin and retinoic acid

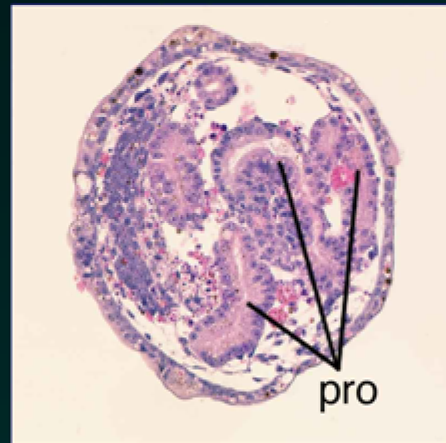


Pancreatic tissues can frequently be induced by time lag treatments.

Images of animal cap tissues treated with activin and retinoic acid



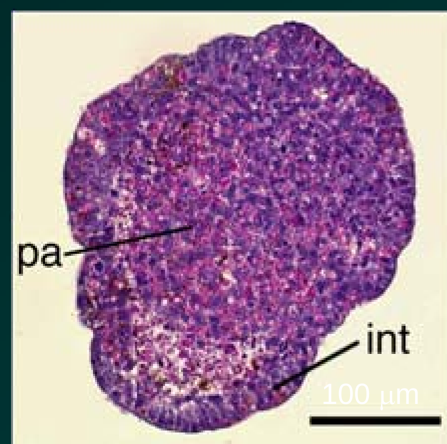
activin 100 ng/ml



activin 100 ng/ml
retinoic acid 10^{-4} M

untreated

activin 400 ng/ml



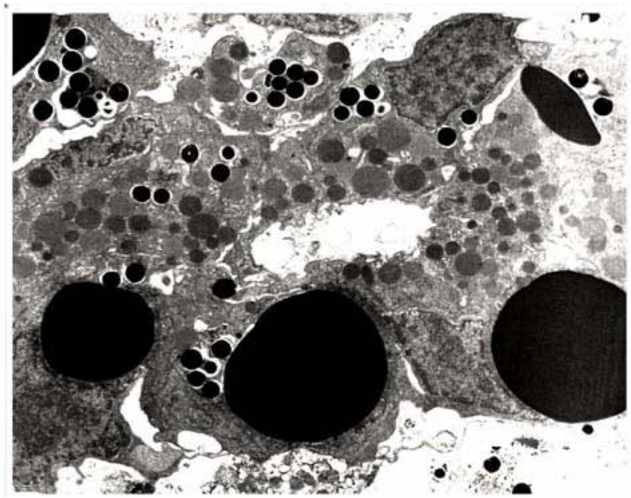
activin 400 ng/ml
retinoic acid 10^{-4} M

mus : muscle
ne : nerve
no : notochord
pro : pronephros
edd : endoderm mass
int : gut epithelium
pa : pancreas

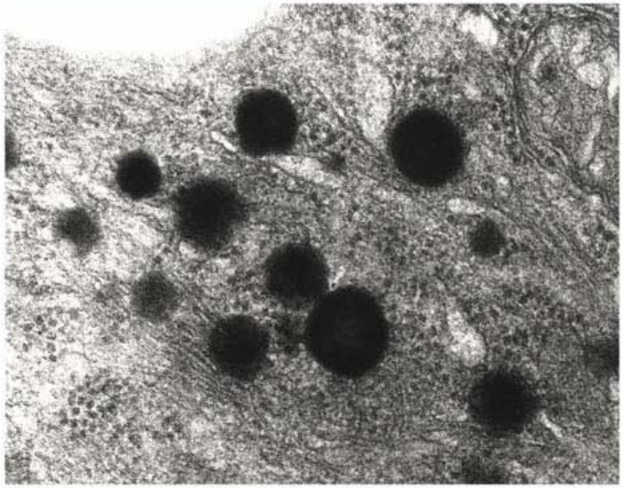
The pancreas is induced with 400 ng/ml activin＋ 10^{-4}M retinoic acid.

Microstructure of a pancreas induced from an animal cap

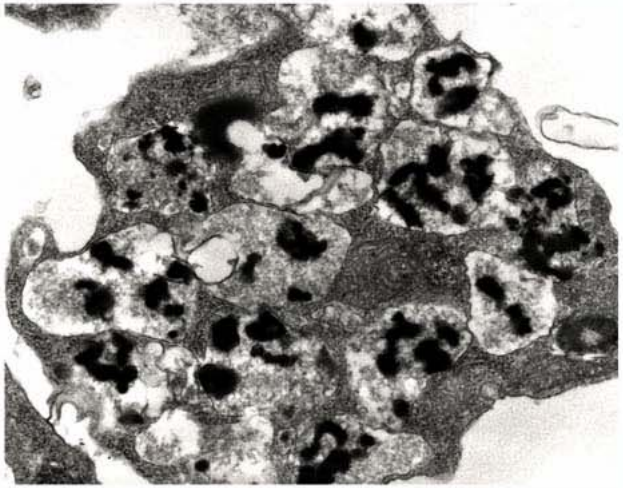
Images of each region observed by an electron microscope



Pancreatic exocrine gland



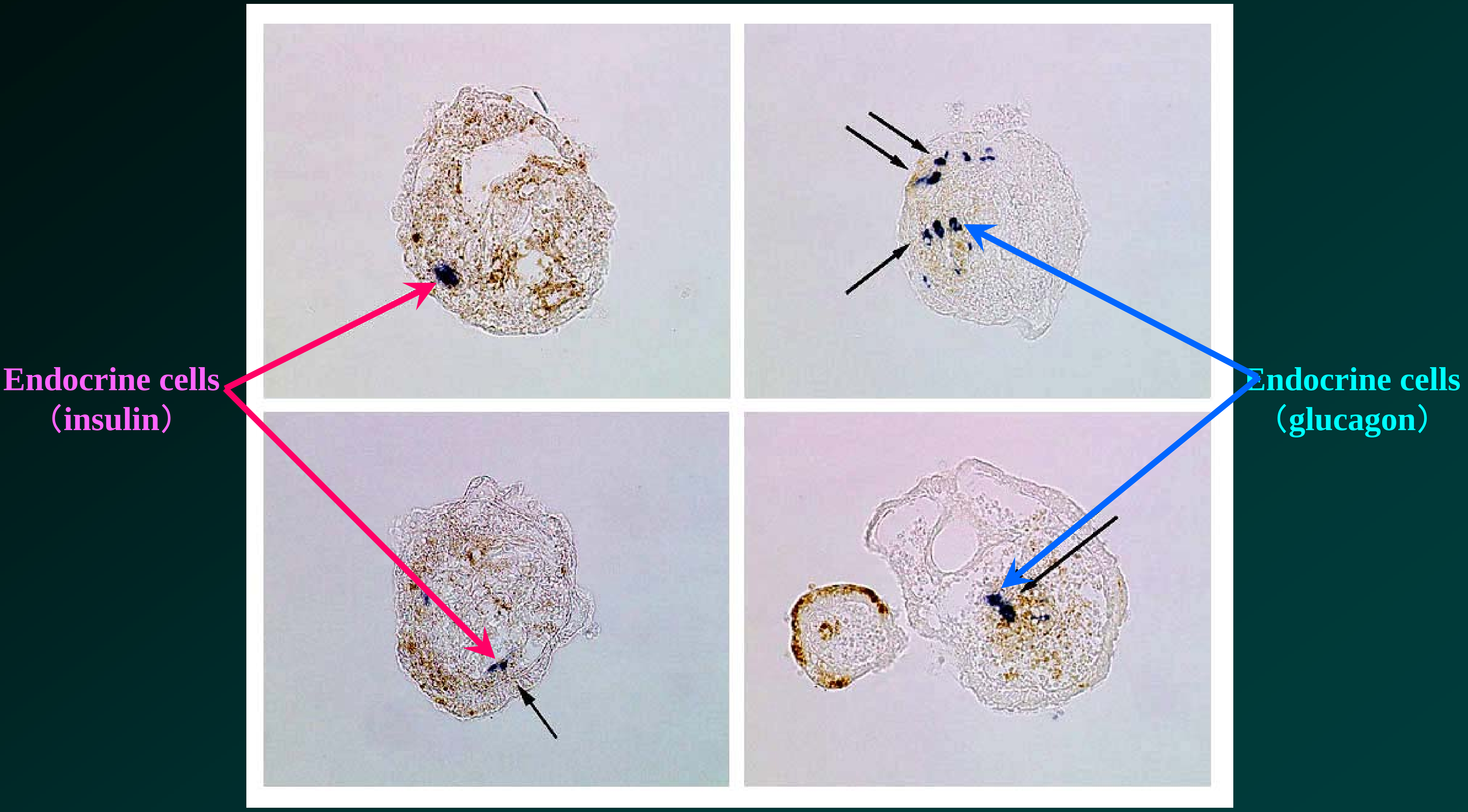
Pancreatic endocrine gland
（glucagon secreting cells）



Pancreatic endocrine gland
（insulin secreting cells）

Pancreatic tissues induced from an animal cap
have the same structure as normal pancreatic tissues.

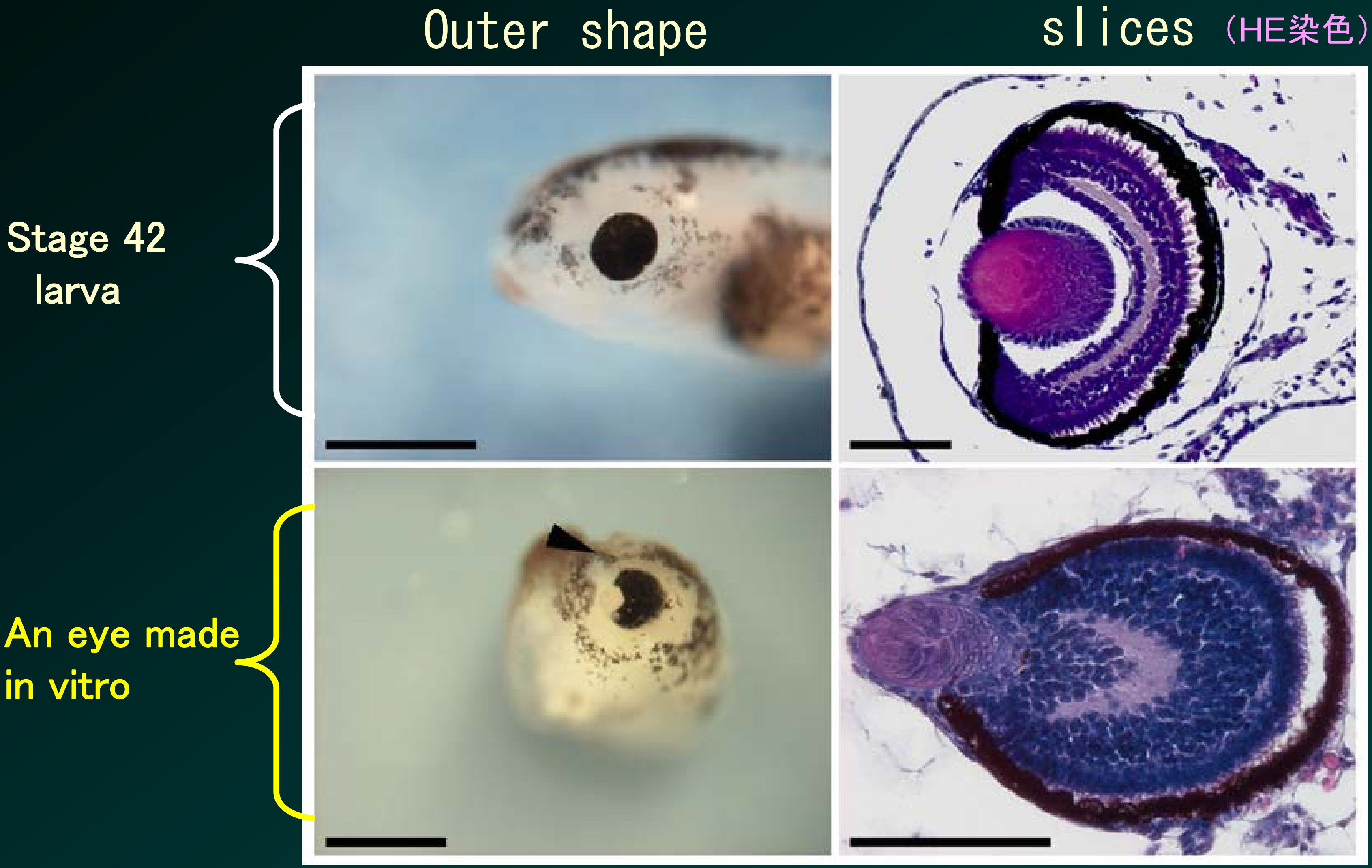
Antibody dyeing of a pancreas induced from an animal cap



Induced pancreatic tissues produced insulin and glucagon.

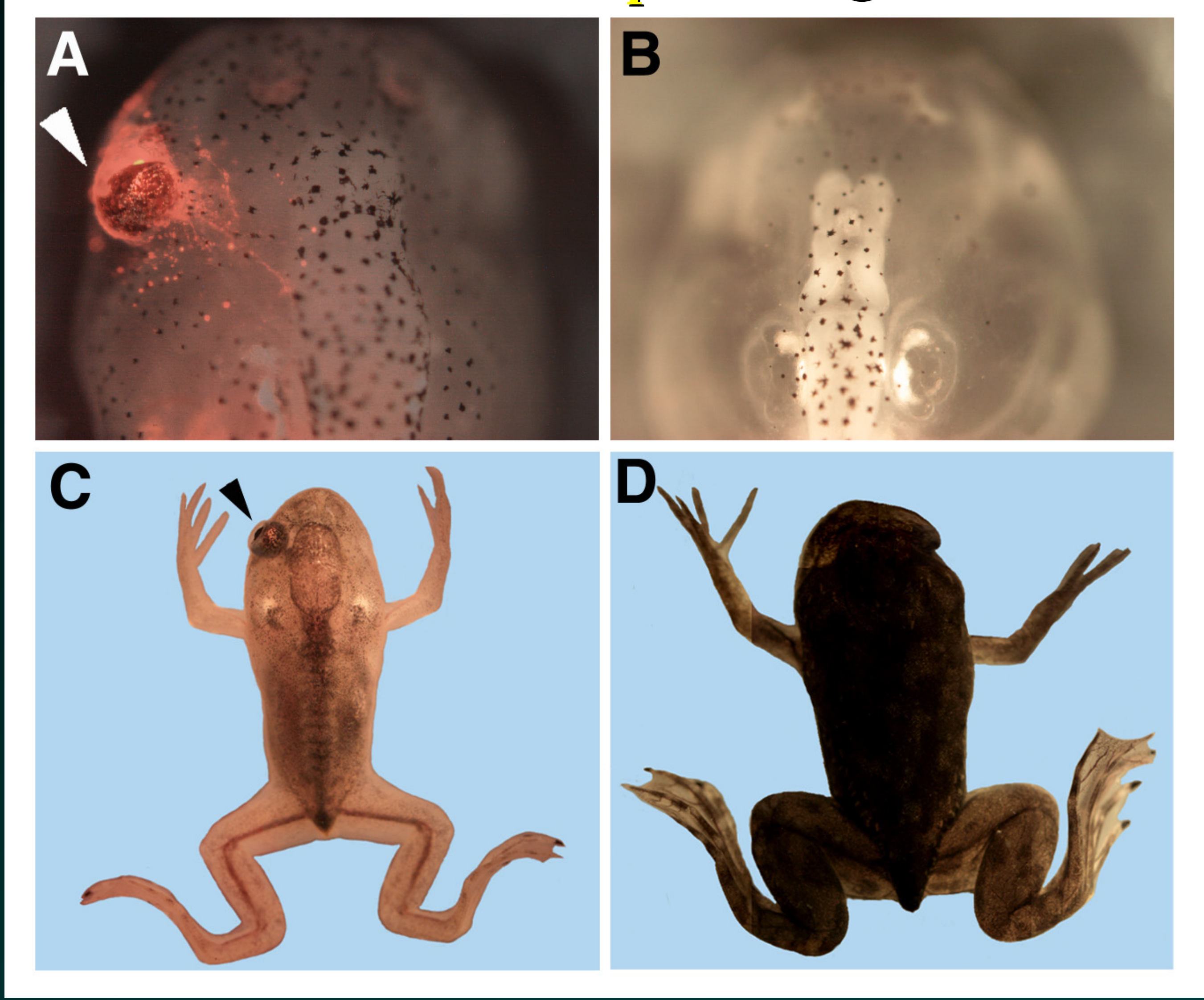
An eye induction system
examined with undifferentiated Xenopus cells

Eye induced from undifferentiated Xenopus cells : an examination of tissue slices



Eye induced in vitro has the same structure as a normal eye.

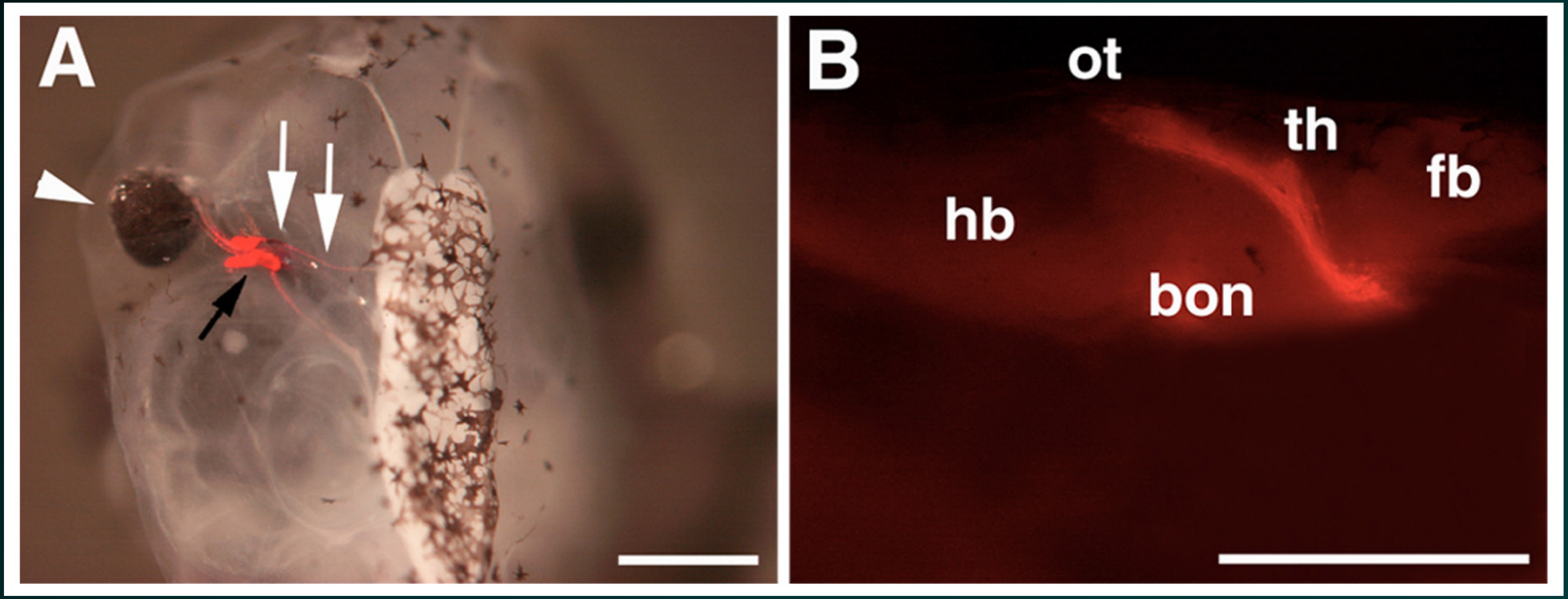
An experiment transplanting eyes induced from undifferentiated Xenopus cells①



The body color of the frog with an eye implant is light because it can sense light.

→ The transplanted eye is functioning!

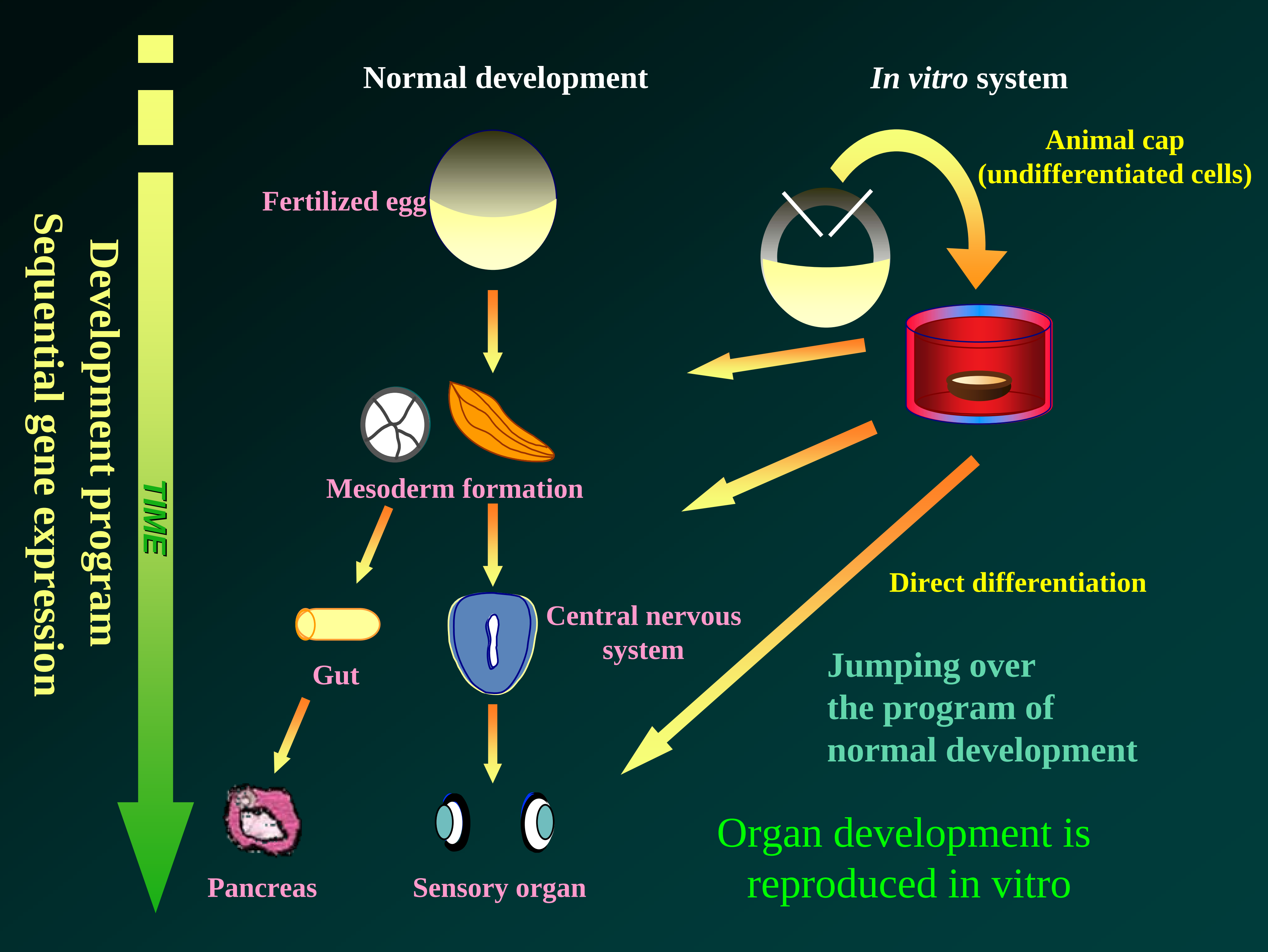
An experiment transplanting eyes induced from undifferentiated Xenopus cells②



Axon dyed by staining a tracer

The optic nerves of a transplanted eye are correctly projected onto the mesocephalic tectum.

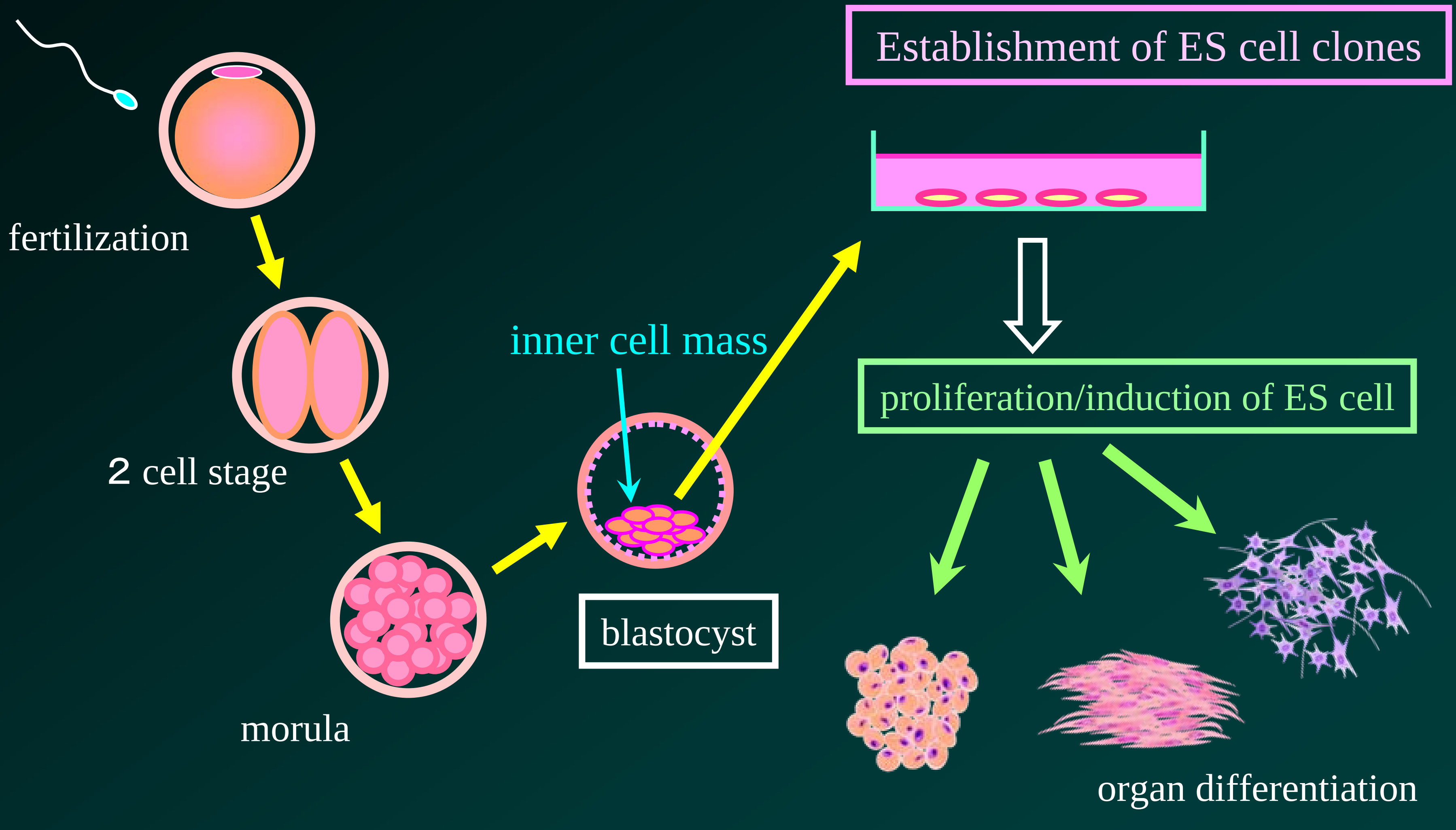
→ The transplanted eye is functioning.


Development program
Sequential gene expression
TIME
Normal development
In vitro system
Fertilized egg
Animal cap
(undifferentiated cells)
Mesoderm formation
Gut
Central nervous
system
Direct differentiation
Jumping over
the program of
normal development
Pancreas
Sensory organ
Organ development is
reproduced in vitro

Research on organ formation using mouse ES cells

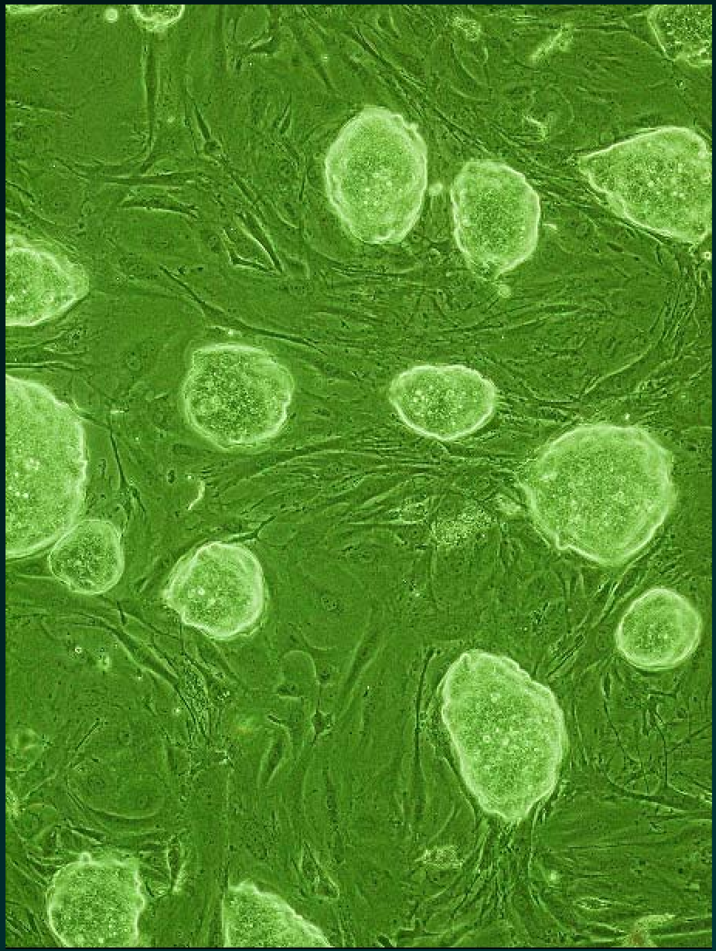
How similar is the differentiative potential of undifferentiated frog cells and mouse ES cells?

Early embryo development of mouse and ES cells



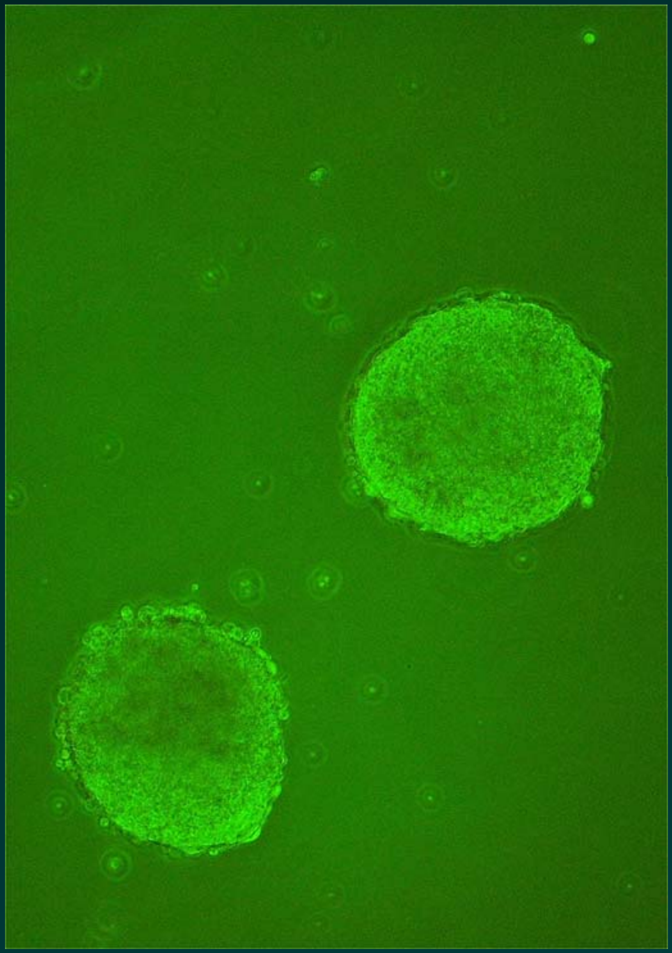
A colony of mouse ES cells and an embryoid

Colony of ES cells.



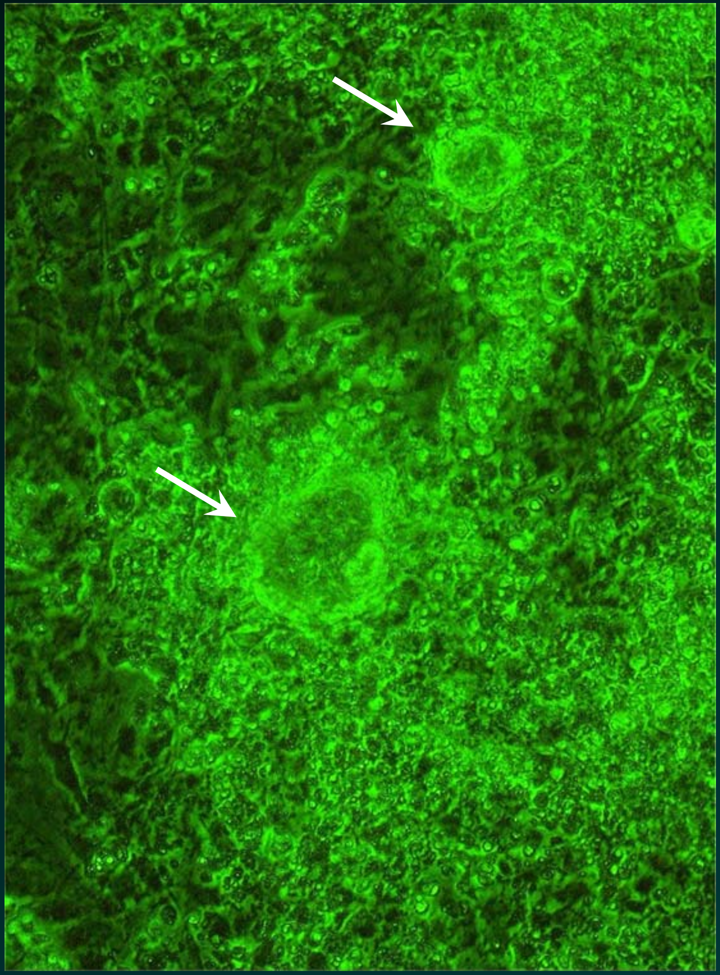
(15% FCS, +LIF)

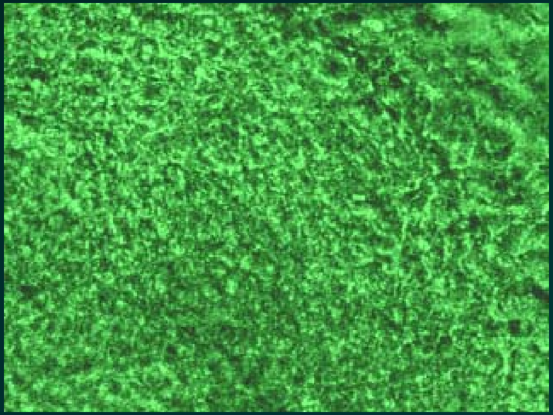
embryoid



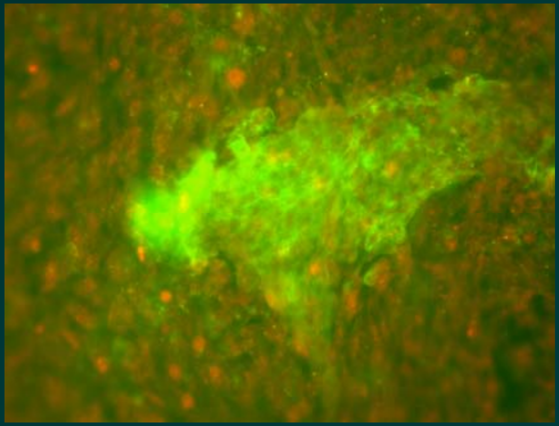
(15% KSR, -LIF)

Induction of myocardium from mouse ES cells using retinoic acid-induced PA024



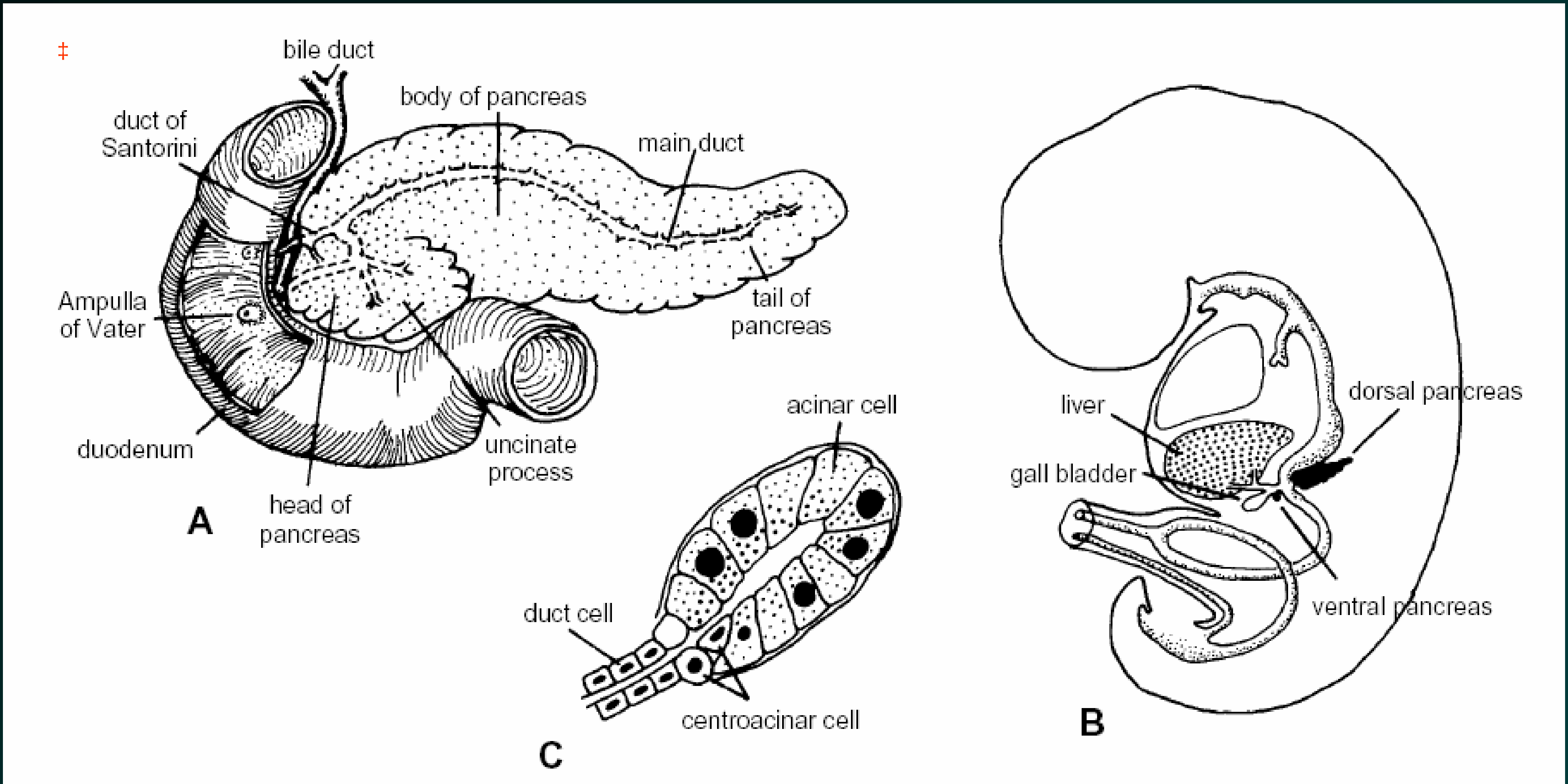


Spontaneous contractile movement of differentiated cadiomyoblast was observed 1-2 days after the treatment.



Staining of anti-cardiac muscle-specific toroponin I Antibody (FITC; green) and nucleus (PI, red)

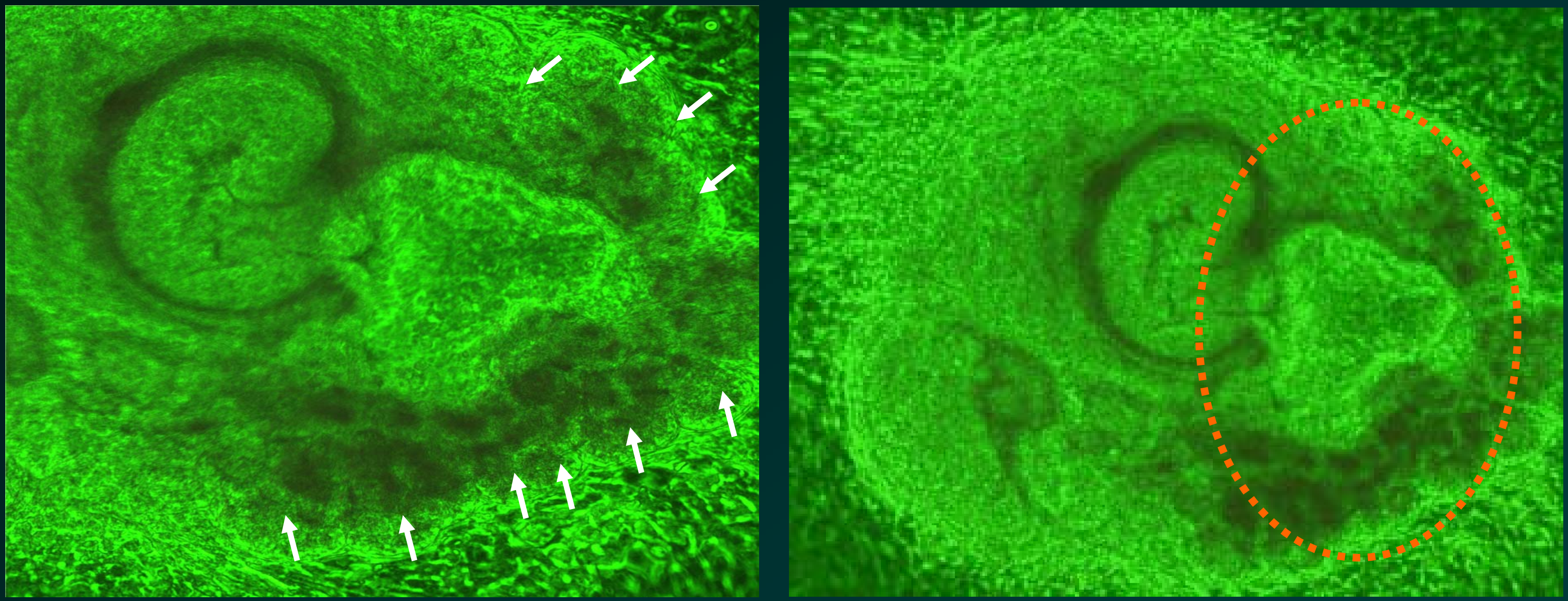
Development of the pancreas in a mouse



Slack JM. et al. Development, vol 121, p1570-Fig1, 1995

The pancreas is formed next to the duodenum (enteric tube).

Induction of a pancreas from mouse ES cells using activin and retinoic acid

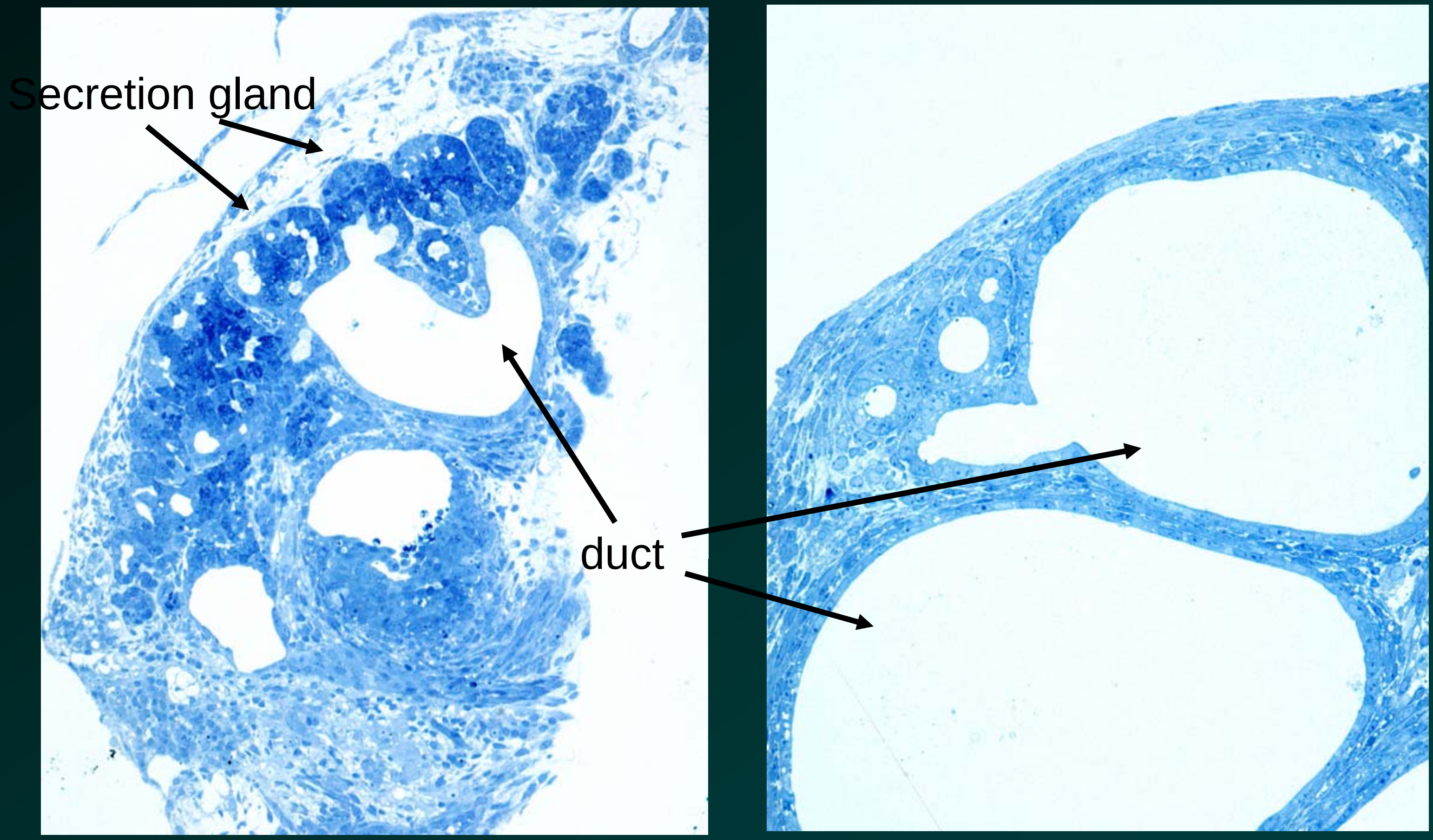


arrows：glandular structure of pancreas

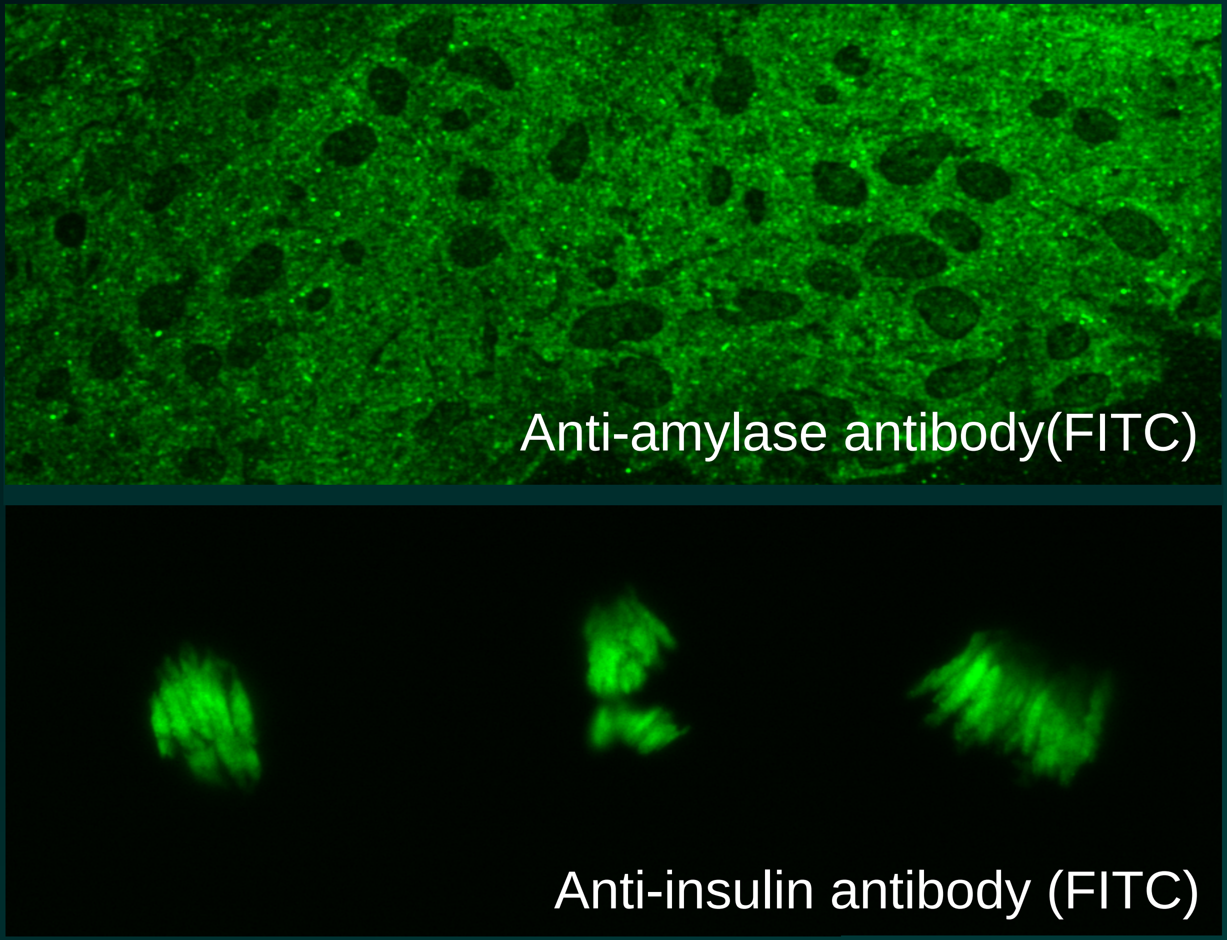
Glandular structure of pancreas and enteron were induced at the same time

Pancreas formation is reproduced in normal development.

Images of pancreatic tissues induced from mouse ES cells

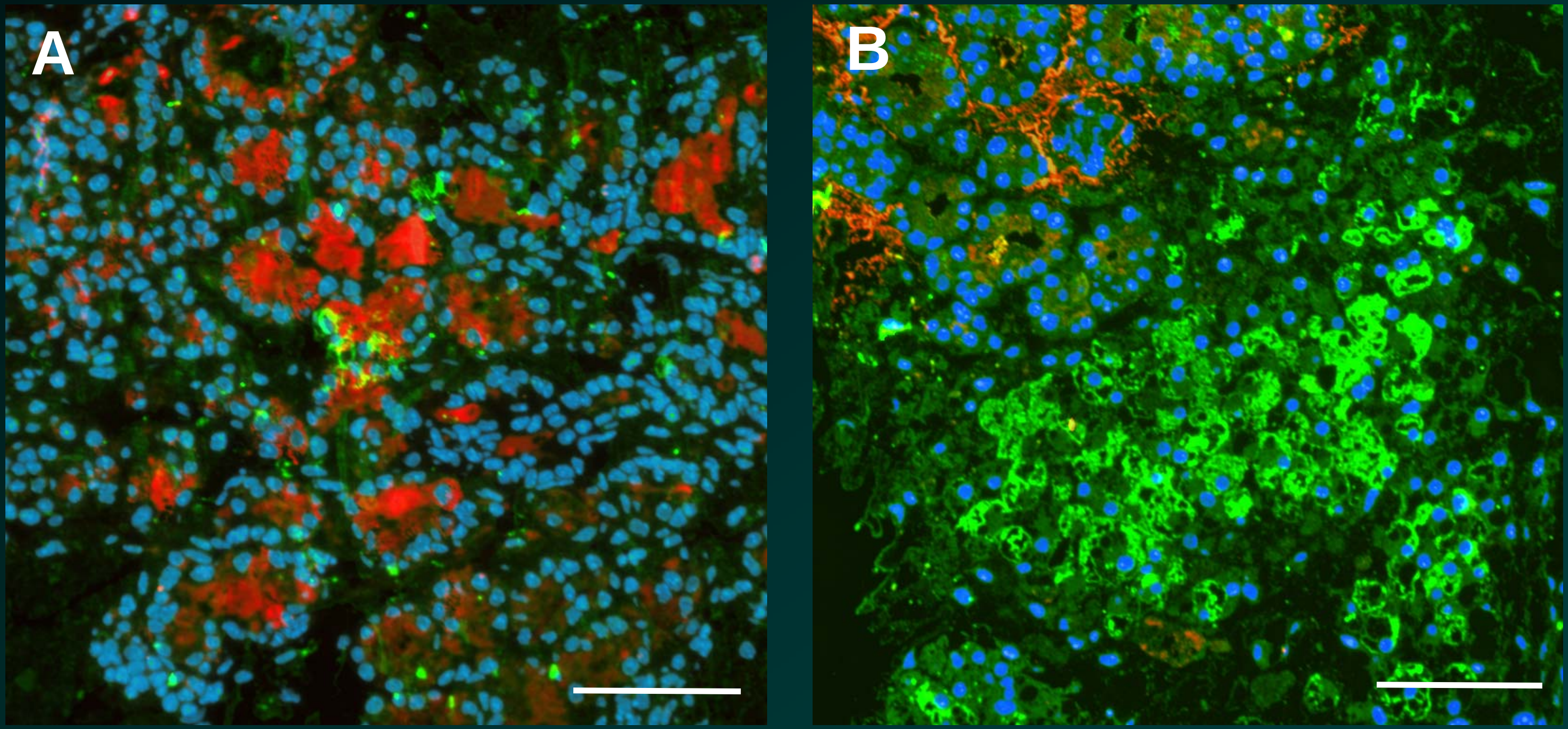


Immunostaining of a pancreas induced from mouse ES cells



Insulin production specific to pancreatic endocrine glands were observed.

Effects of concentration control of activin and retinoic acid upon the induction of pancreatic tissues

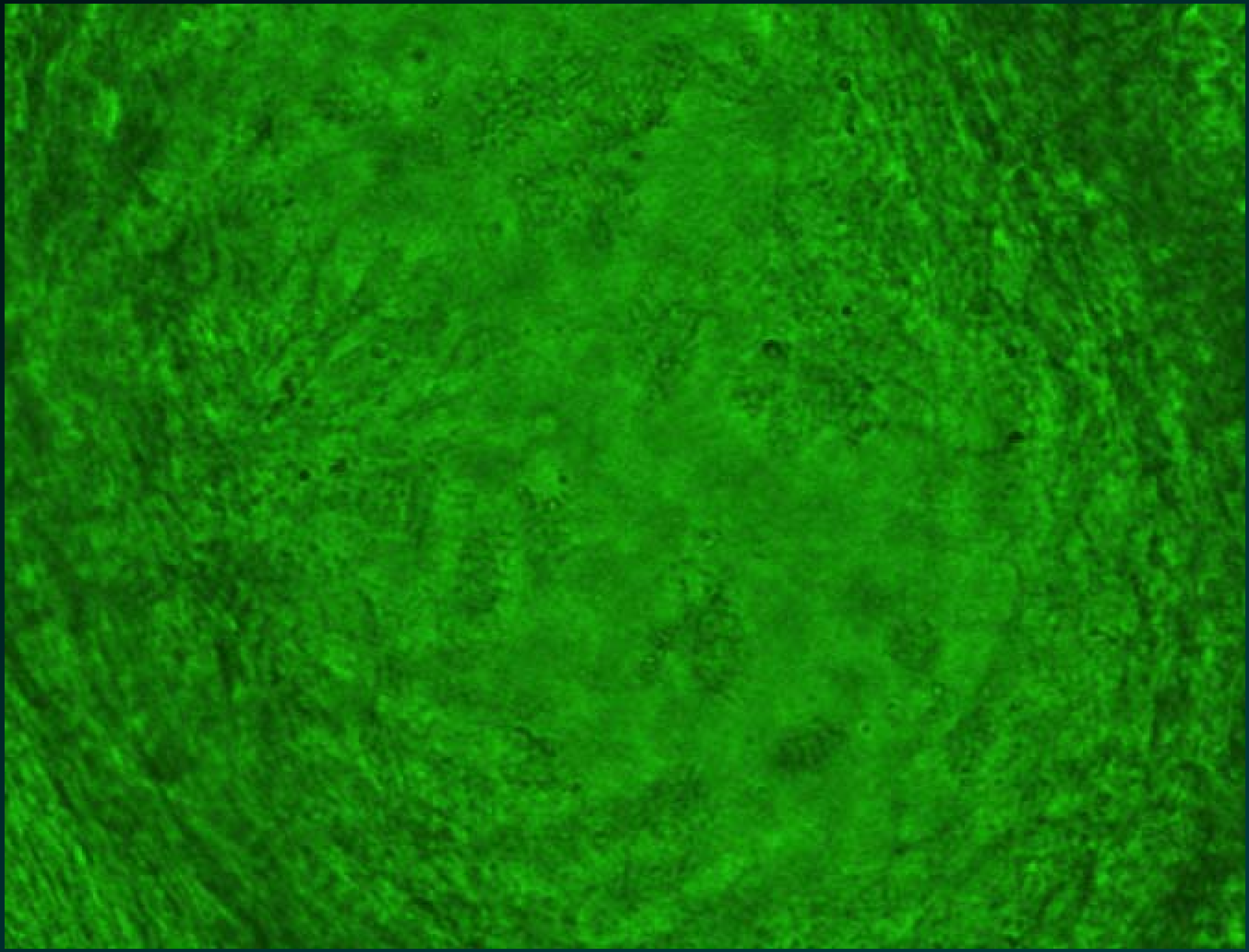


Endocrine cells

Ectocrine cells (bar = 50 mm)

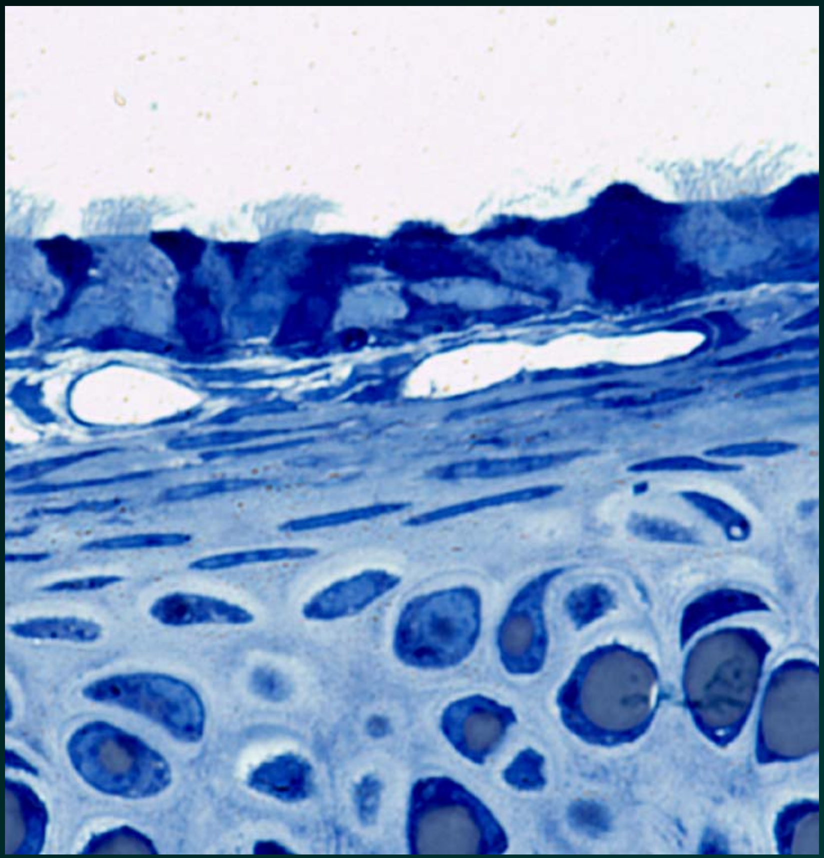
nucleus / insulin / amylase

Cilia cells induced from mouse ES cells

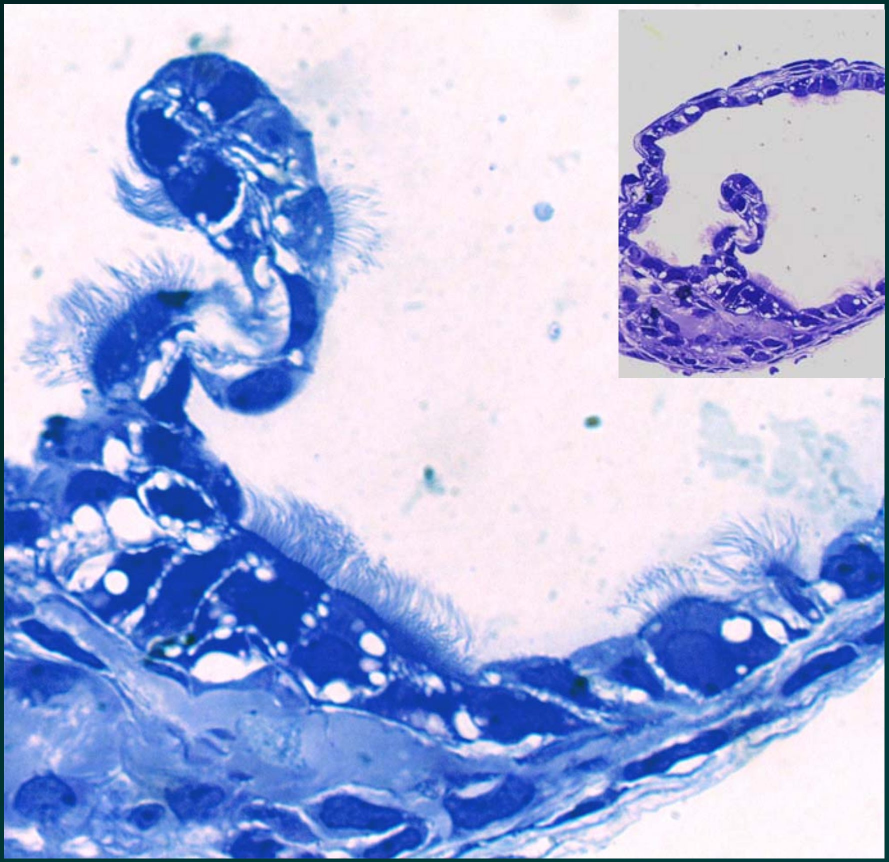


Cilia cells work in the cilia epithelium of trachea, salpinx, brain ventricle.

Tracheal epithelium induced from mouse ES cells

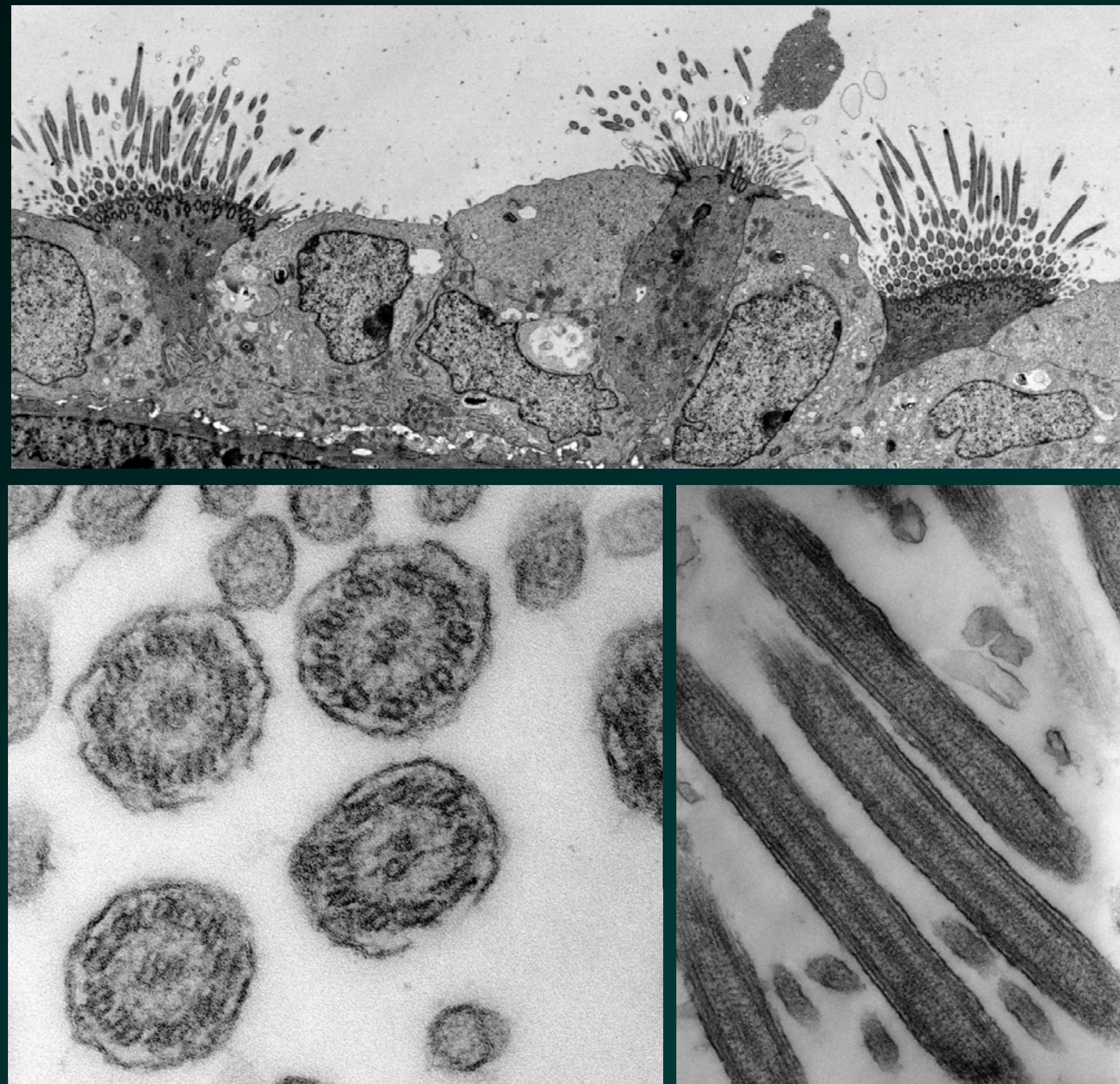


Tracheal epithelium of an adult mouse



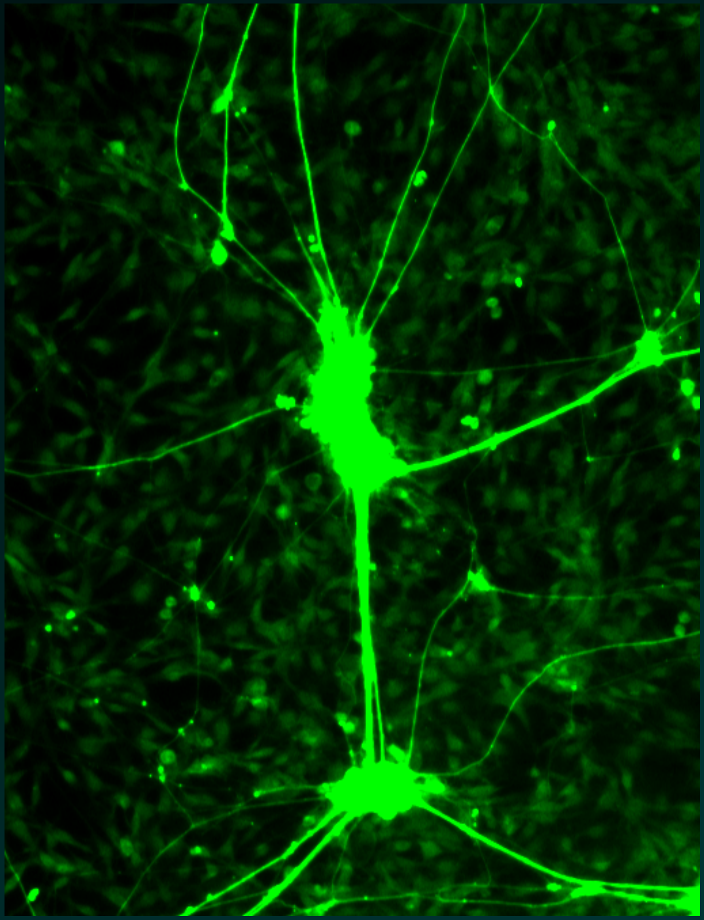
Tracheal epithelium-like structure induced from ES cells

Cilia cells induced from mouse ES cells

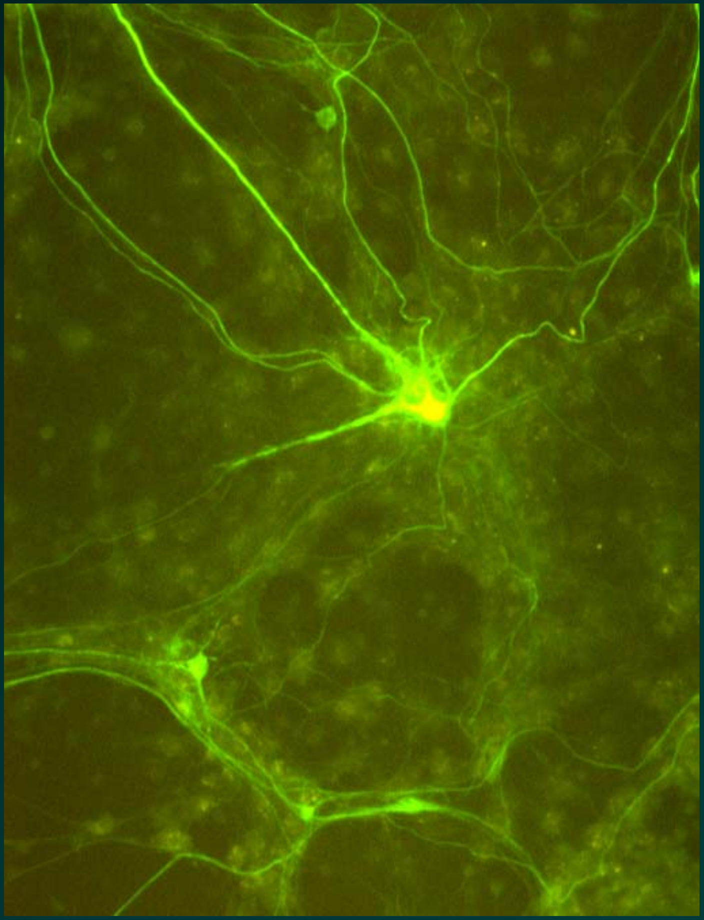


Cilia has a 9+2 structure.

Nerve cells induced from mouse ES cells



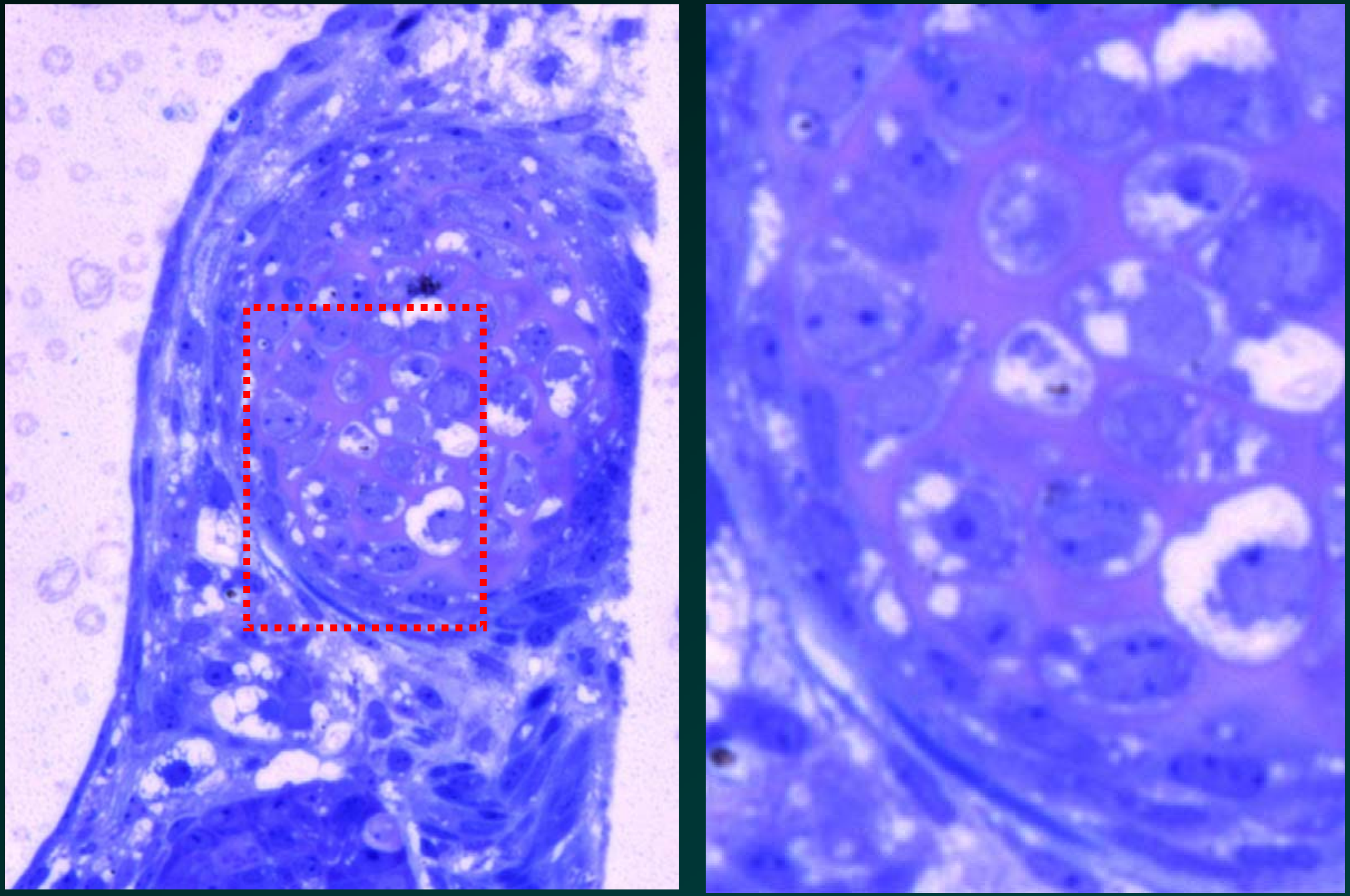
Anti-L-NF antibody (FITC)

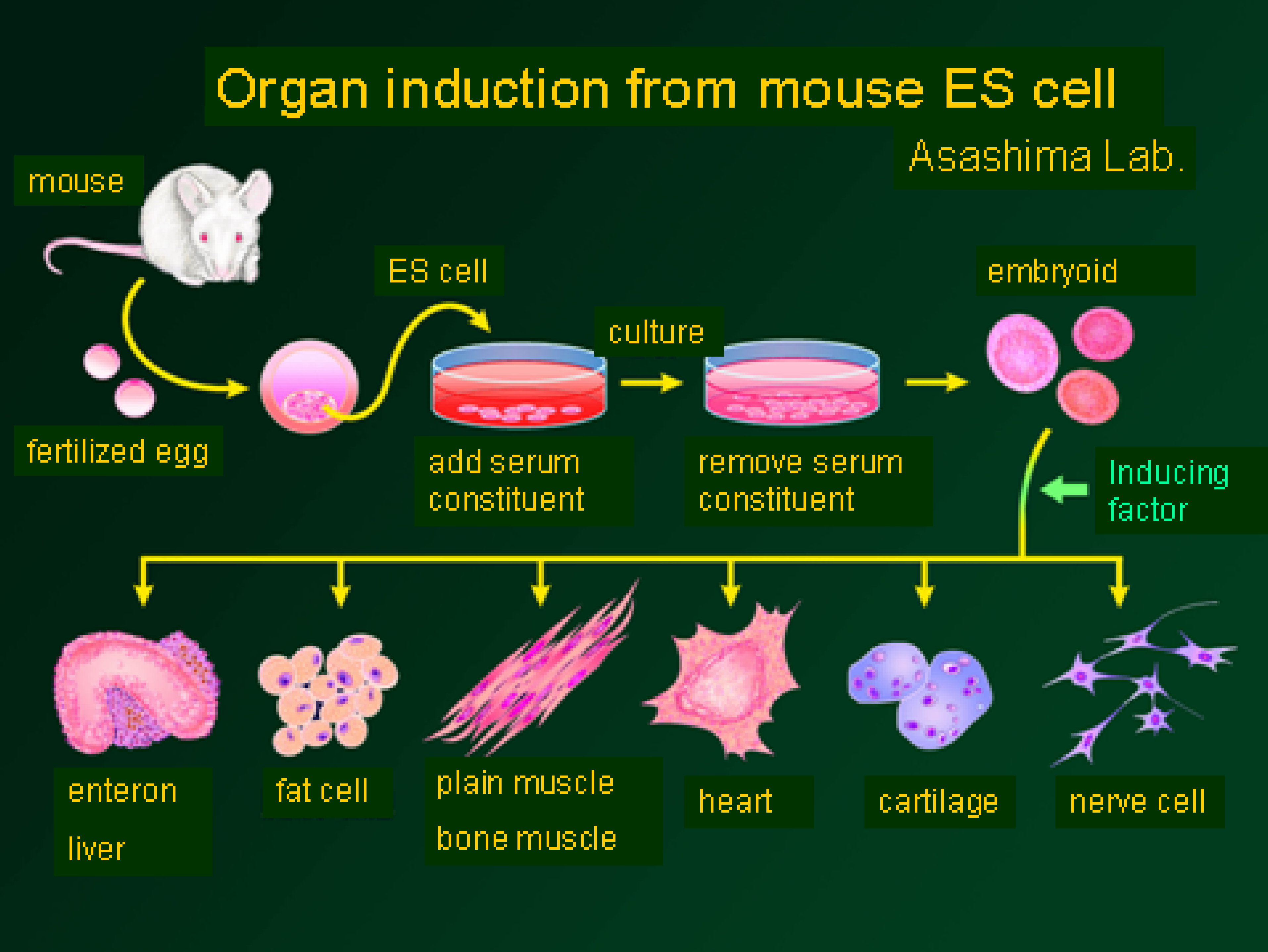


Anti-H-NF antibody (FITC)

Nerve cells are induced by retinoic acid and a FGF2 treatment.

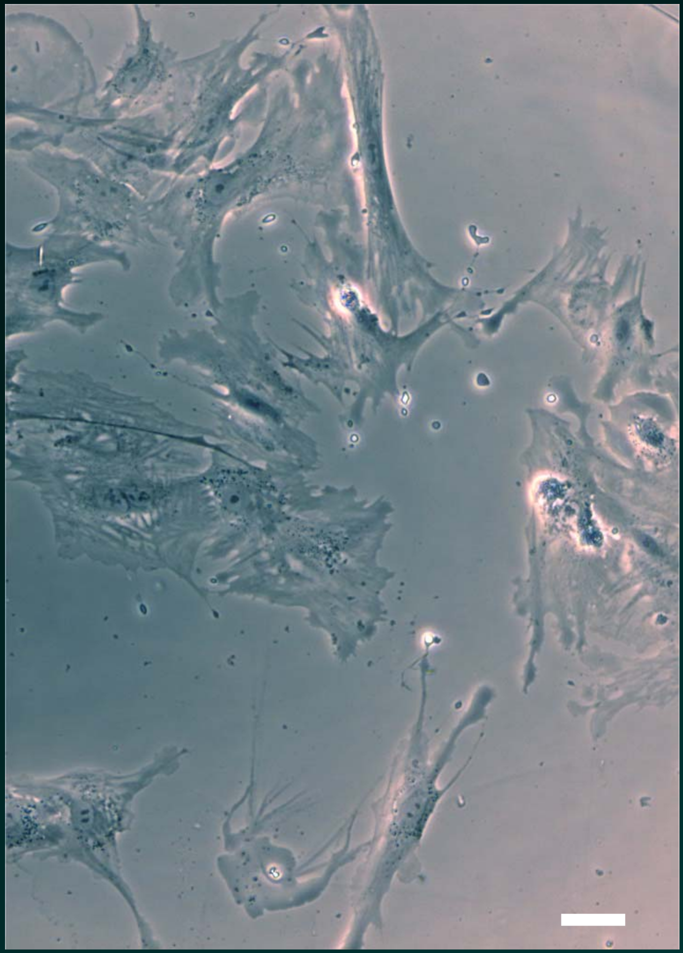
Cartilage cells induced from mouse ES cells

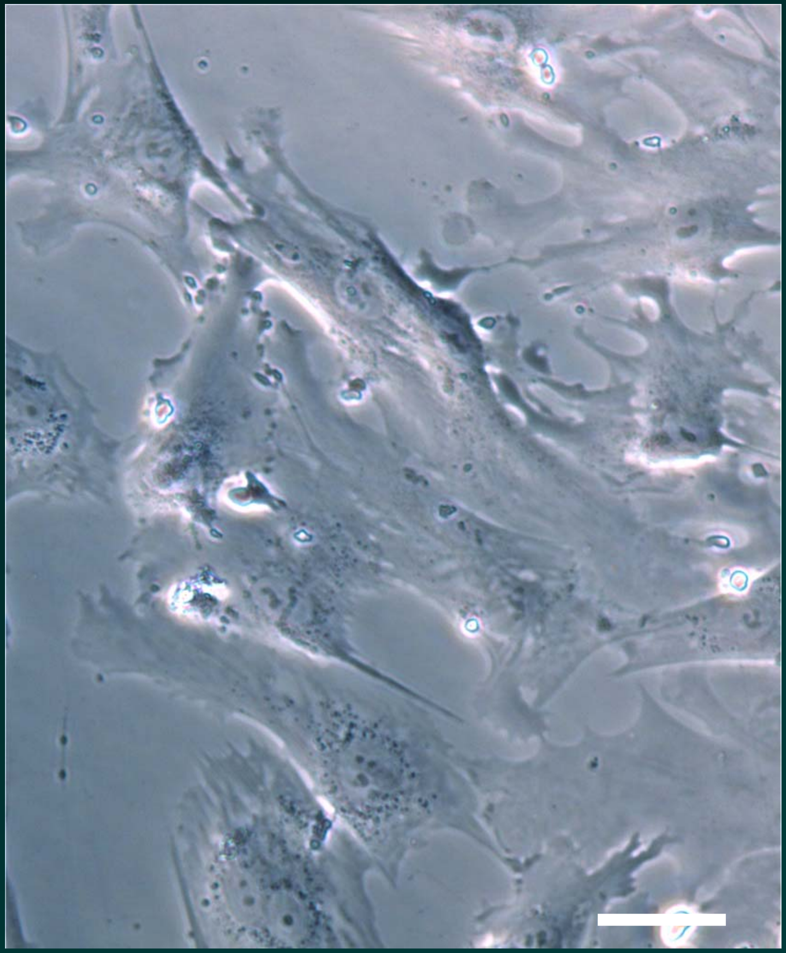



Organ induction from mouse ES cell
Asashima Lab.
mouse
fertilized egg
ES cell
culture
embryoid
add serum constituent
remove serum constituent
Inducing factor
enteron
liver
fat cell
plain muscle
bone muscle
heart
cartilage
nerve cell

One research area using tissue stem cells

The tissue stem cells of humans



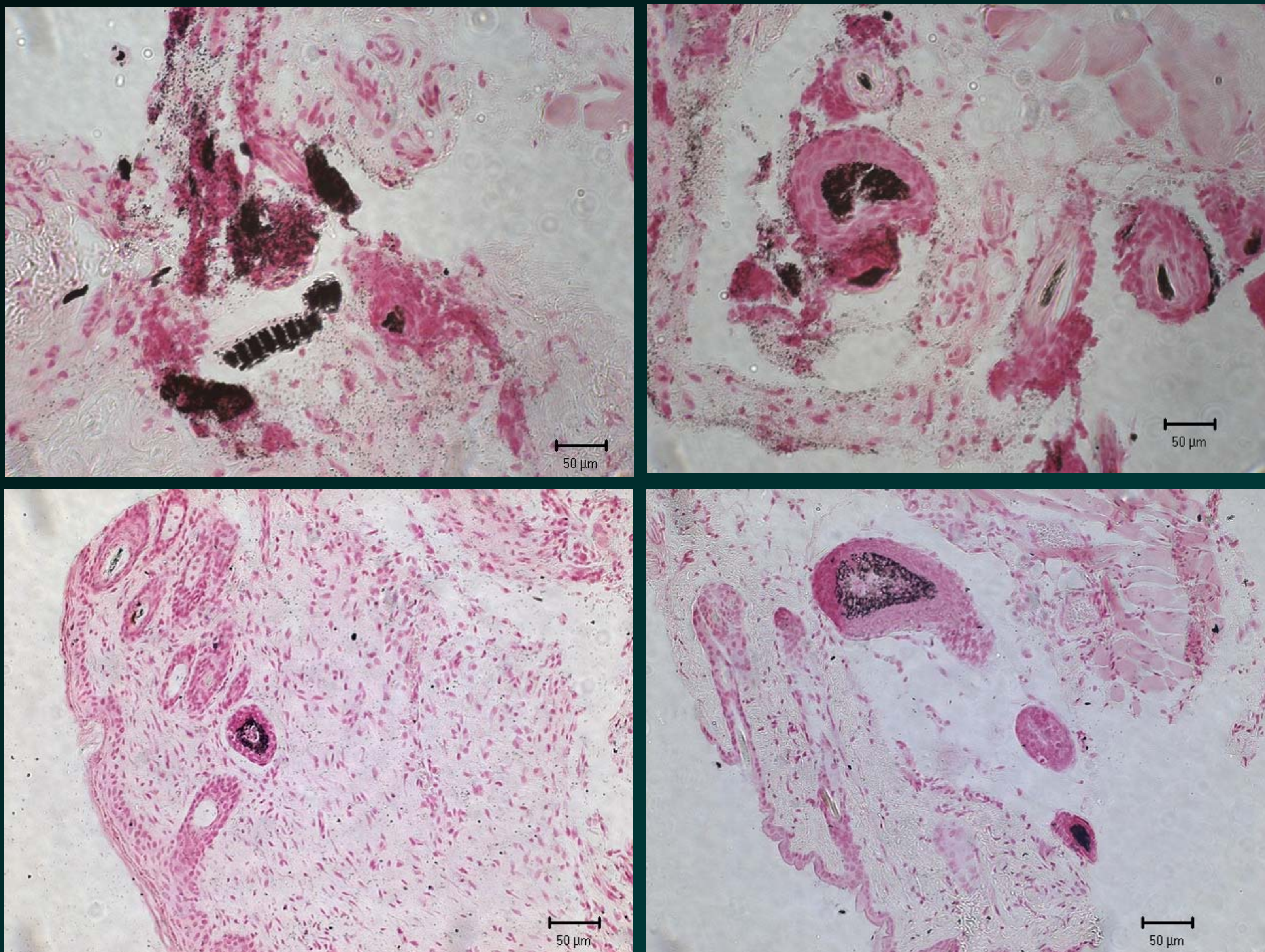


Is knowledge of ES cells applicable to human tissue stem cells?

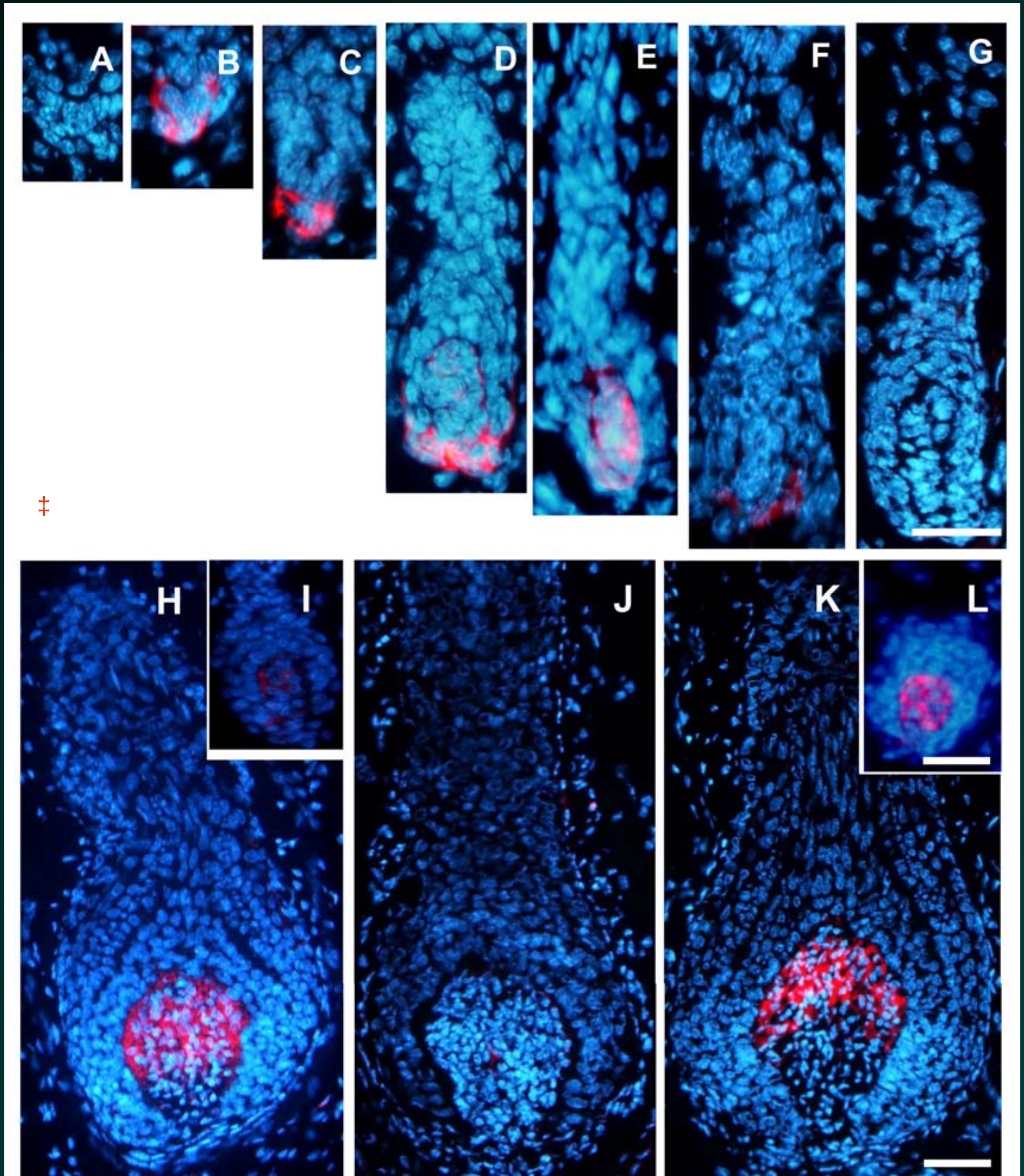
→ Research should proceed energetically.

Formation of skin hair from mouse ES cells and mesenchymal cells

Formation of skin hair from mouse ES cells and mesenchymal cells

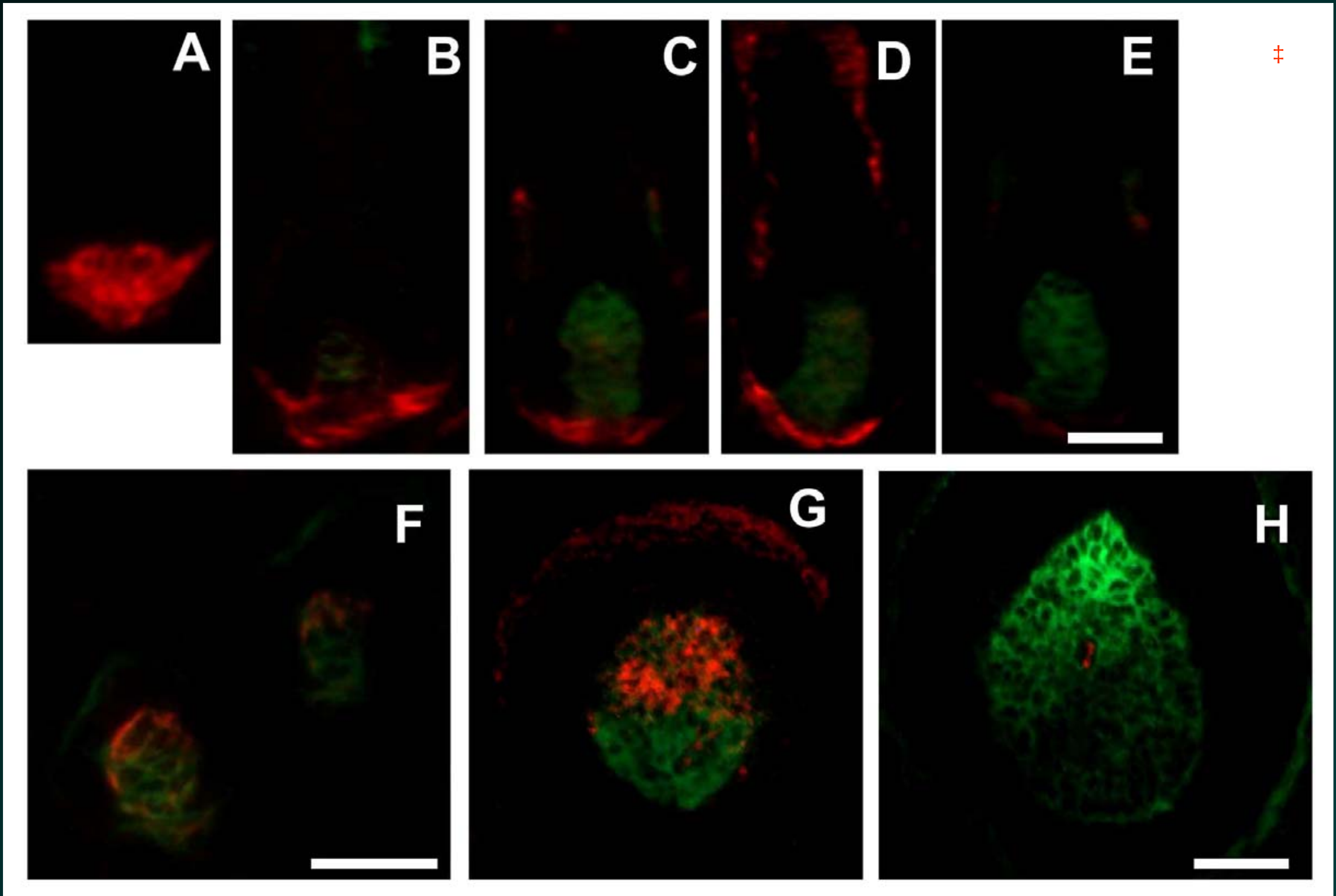


Formation of hair follicles and localization of undifferentiated cells in a mouse’s skin



Ito Y. et al., J Invest Dermatol. 2006 Dec 21; [Epub ahead of print], p2-Fig.1(c-n)

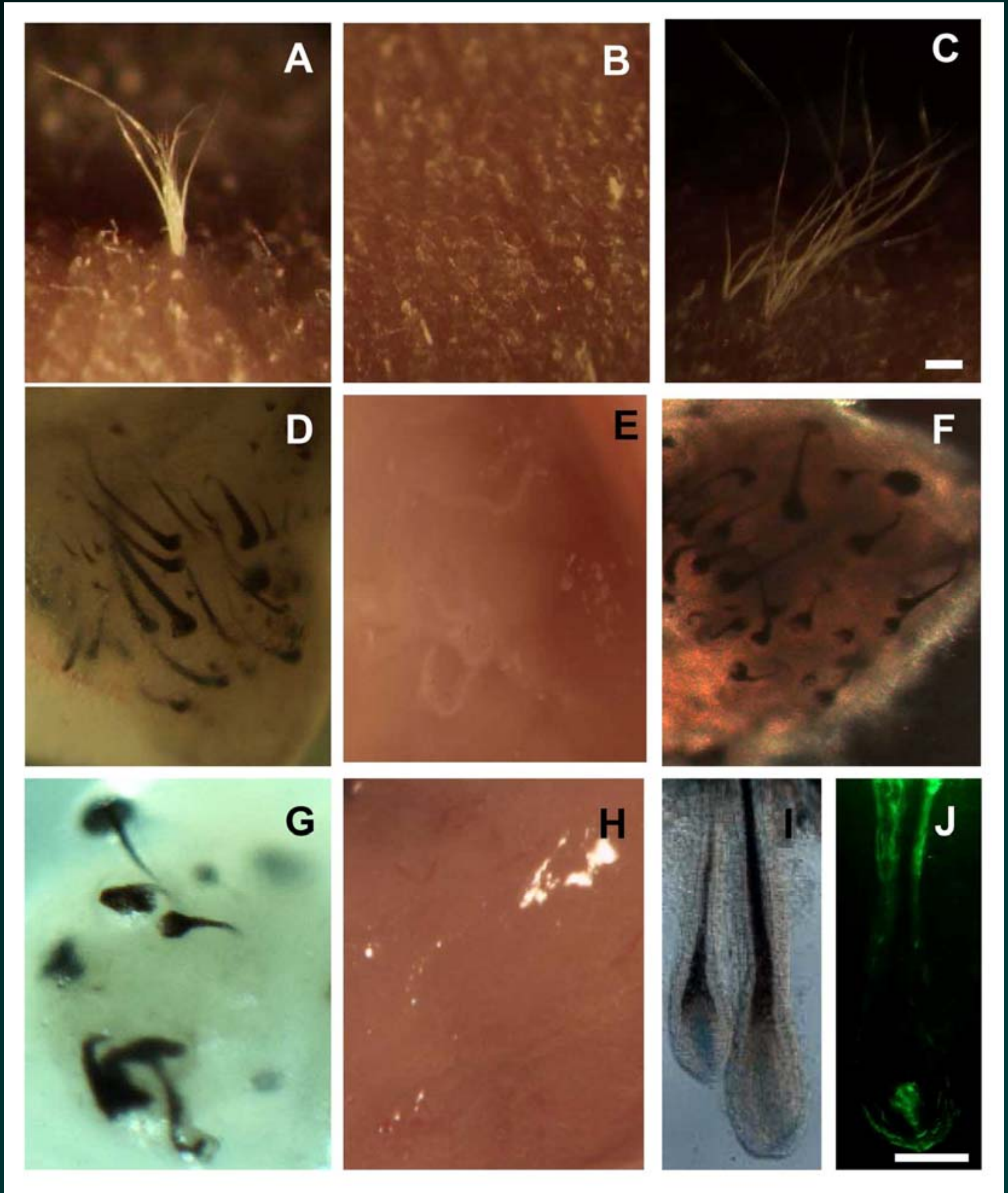
Changes in the hair follicles in a mouse' s skin after plucking



Ito Y. et al., J Invest Dermatol. 2006 Dec 21; [Epub ahead of print], p3-Fig.2(a-h)

Changes after transplanting undifferentiated hair follicle cells to the mouse's skin

‡



Ito Y. et al., J Invest Dermatol. 2006 Dec 21; [Epub ahead of print], p5-Fig.5

Examples illustrating the history of human regeneration medicine

（1） skin transplants

（2） regeneration of bone and cartilage

— using scafolds

（3） cornea transplants （partial）

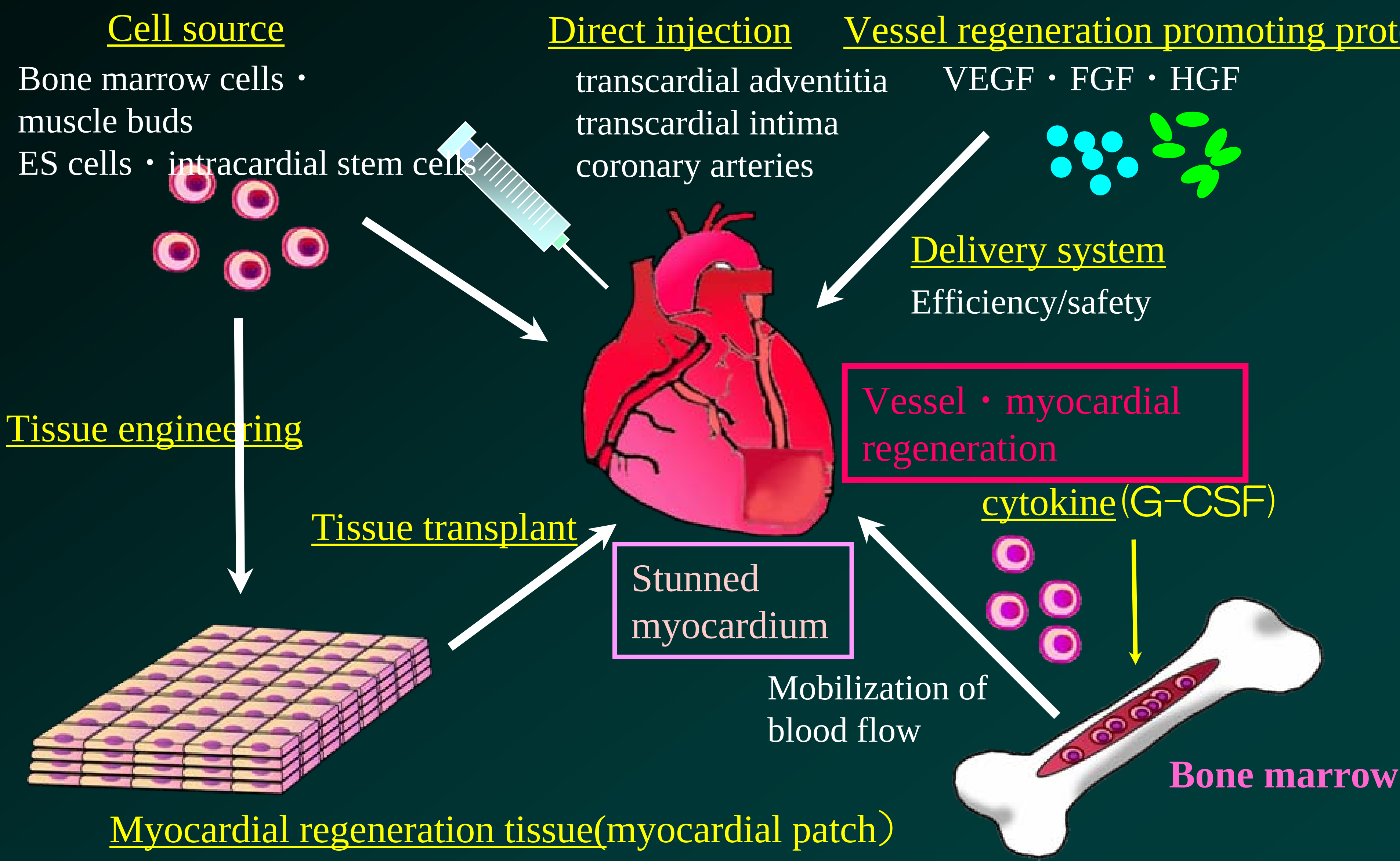
（4） blood vessel regeneration （HGF,VEGF, etc.）

（5） tooth bud regeneration, etc.

Artificial skin

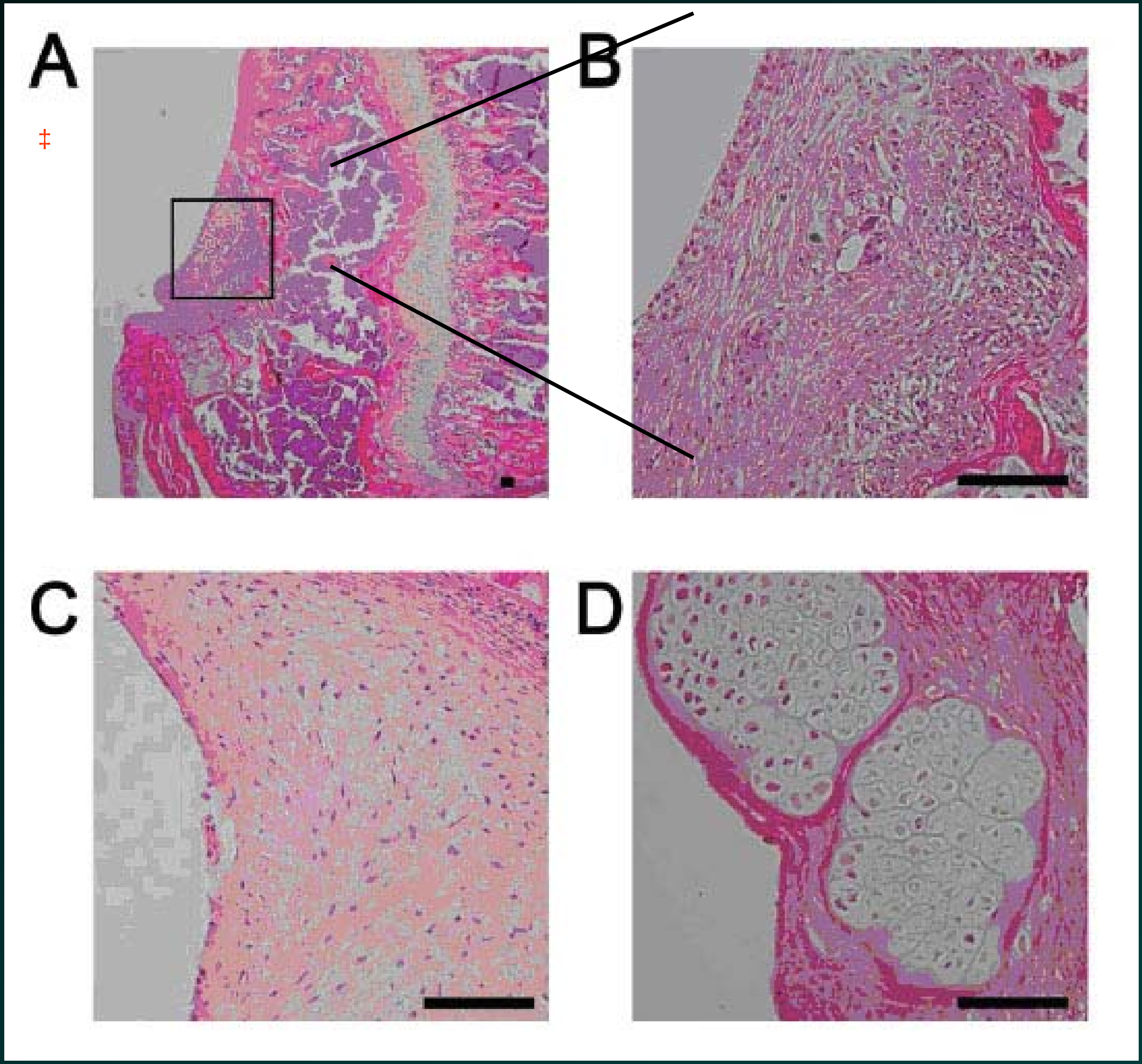
"The picture of artificial skin" inserted here was omitted according to copyright issues.

Regenerative heart medicine



T. Shimizu, 2005, The Japanese Society for Regenerative Medicine

Cartilage regeneration
by human mesenchymal stem cell（hMSC）transplants



A, B
Untreated defected cartilage
（Control）

C, D
Defected cartilage with
hMSC transplants

Yuge L. et al., Stem Cells Dev, vol 15, p926-Fig4-(A-D), 2006

（transplant experiment of hMSC in a mouse）

Future research