# Japan-Lithuania Joint Life Sciences Symposium

# Role of the Activin Protein for Organs Formation in Vertebrate's Development

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25 September 2018
at Research Council of Lithuania

1. How many organs and tissues can form *in vitro* from *Xenopus*/mice multipotent
. cells using activin and/or RA ?

*Xenopus*; animal cap

mice: ES cells

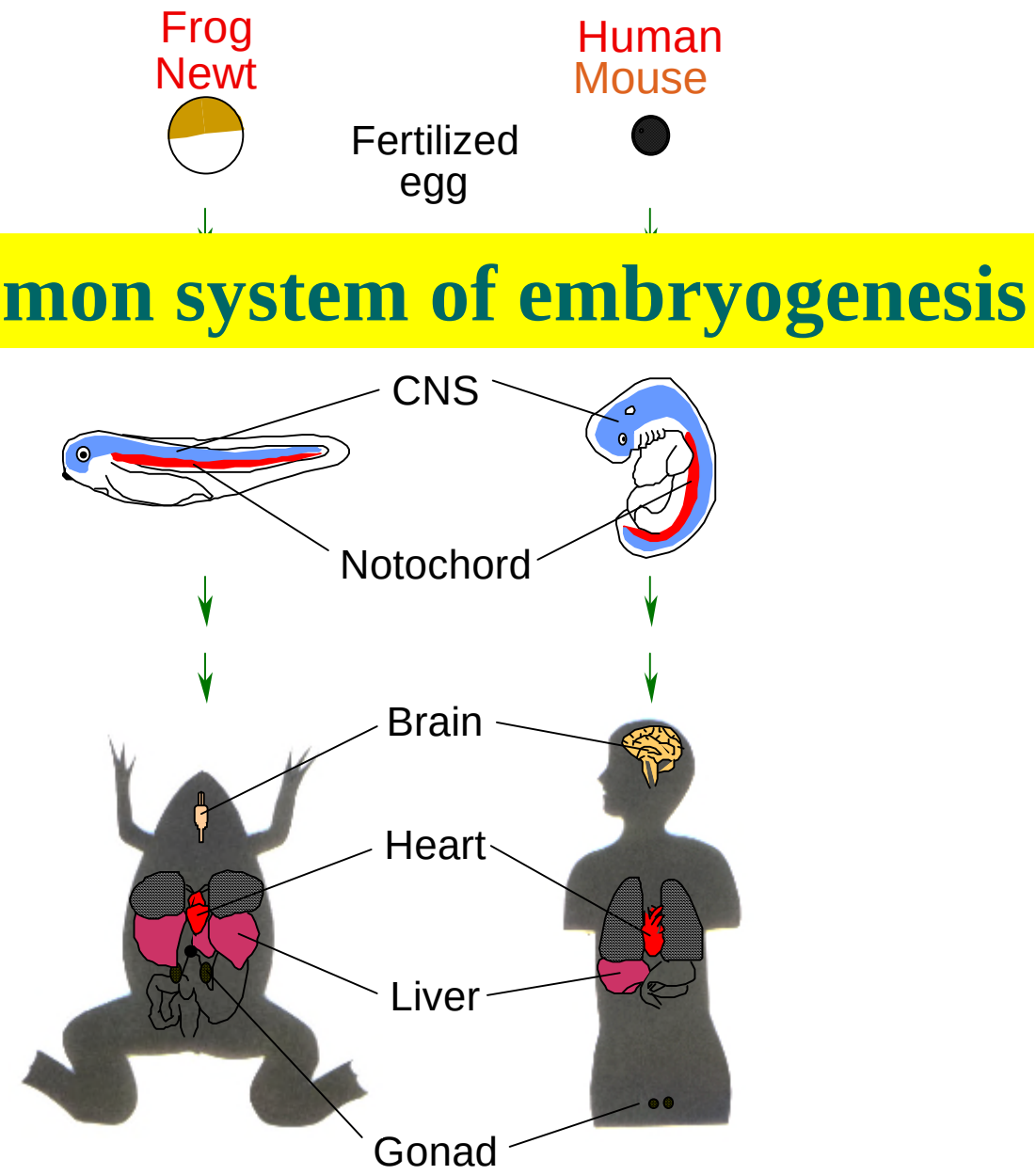
2. New regulation factor of activin signalling by MAN1

3. Approaches of human stem cells

1. How many organs and tissues can form *in vitro* from *Xenopus*/mice multipotent
. cells(animal cap/EScells) using
**activin** and/or RA ?

*Xenopus*; animal cap

mice; ES cells

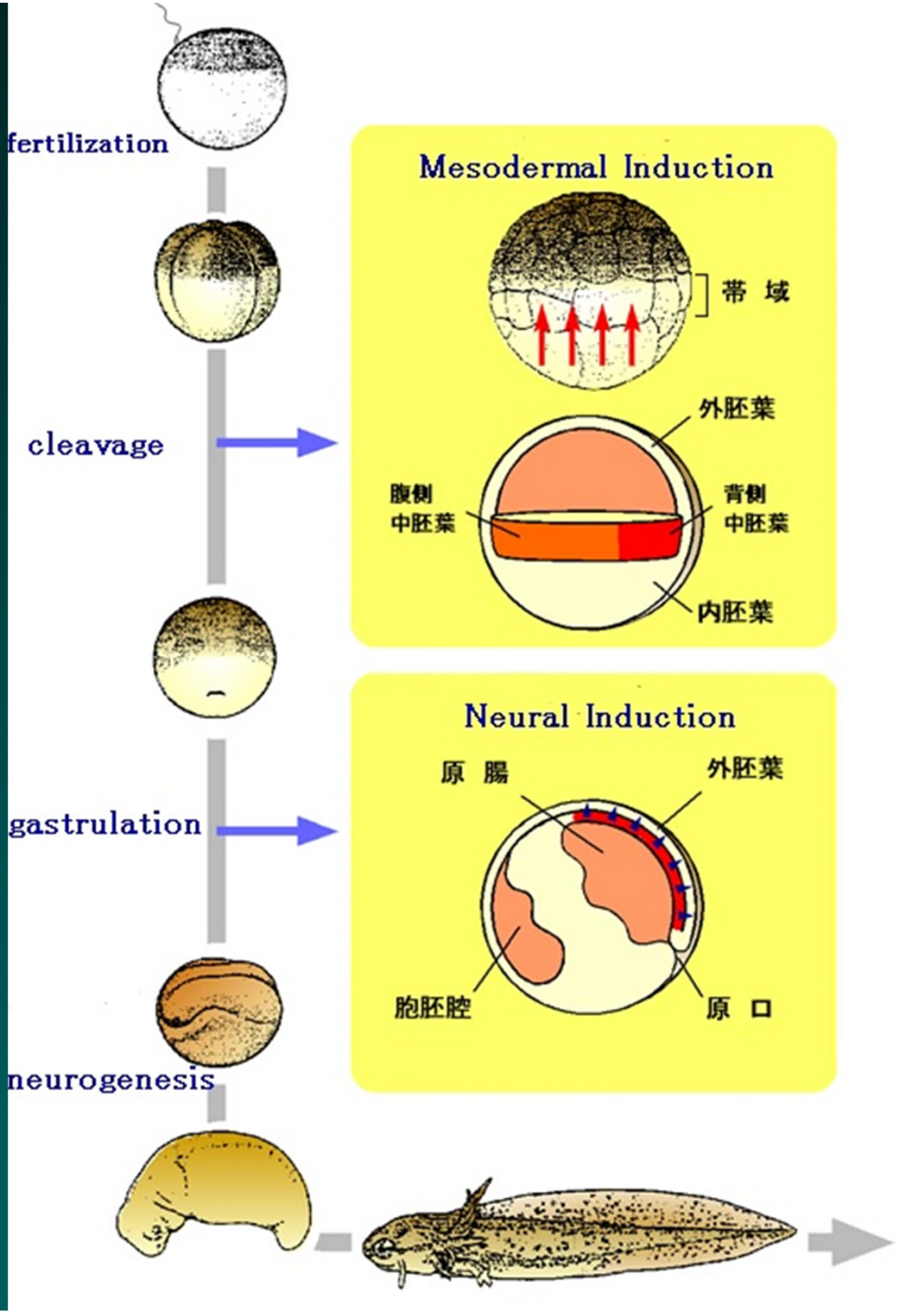

Frog
Newt
Human
Mouse
Fertilized
egg
Common system of embryogenesis
CNS
Notochord
Brain
Heart
Liver
Gonad
Common system of organogenesis

## Early Development :

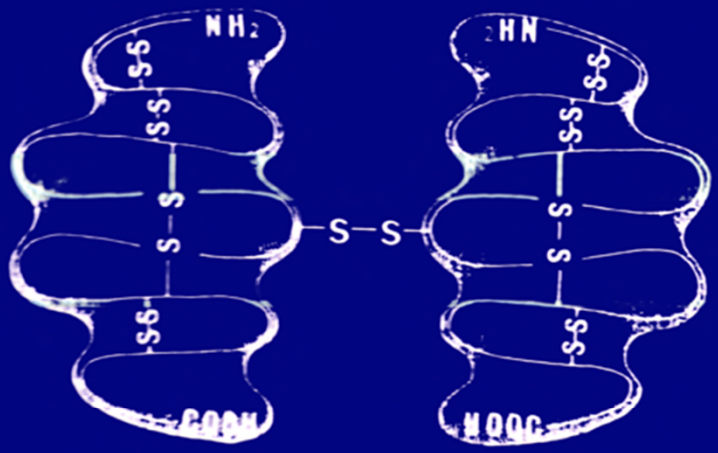
**Embryonic Induction in Amphibian Embryo was discovered by Spemann and Mangold at 1924.**

**Mesodermal and Neural Inductions are essential for body plan formation**

**Many scientists have started to identify the inducing factors**



# Schematic structure of activin A




Molecular weight : 25,OOO ( 12,5OO X 2 )

homodimer

Amino acids : 232 ( 116 X 2 )

SH = 18

**Activin is one of the proteins belong to TGF-beta family.**

**It takes 65 years to find out activin as a mesodermal inducing factor by M. Asashima at 1989.**

**During the 50 years, after the finding of organizer by Spemann and Mangold in 1924, “embryonic induction” has been the main theme in the field of developmental biology.**

**But nobody had succeeded to identify the inducing factor as a real substance.**

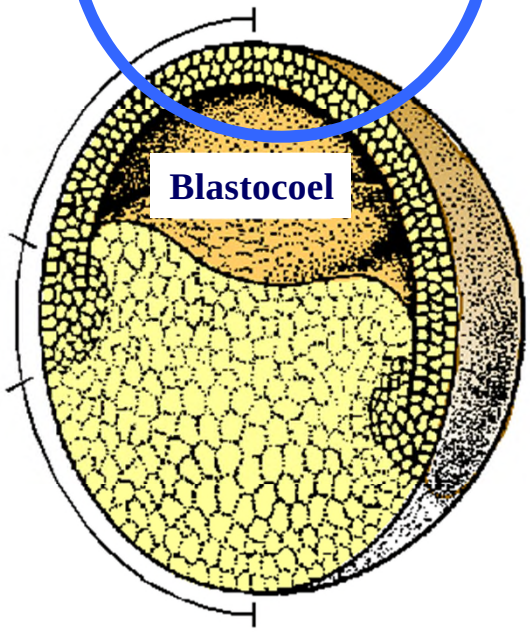
**From this new finding, new stream of the developmental biology has started.**

# Animal Cap Assay

Ectoderm

Mesoderm

Endoderm




Vegetal pole

Blastula (Sagittal section)

Animal cap

(multi or pluri- potential cells)

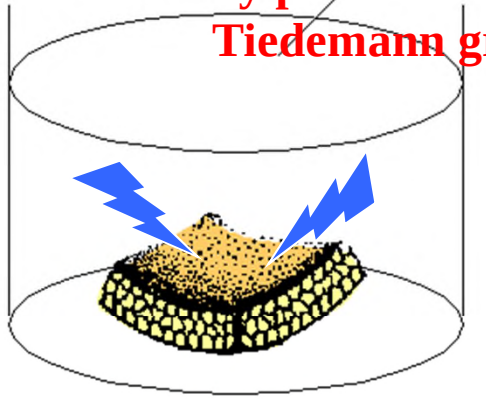
Animal pole

Saline

+

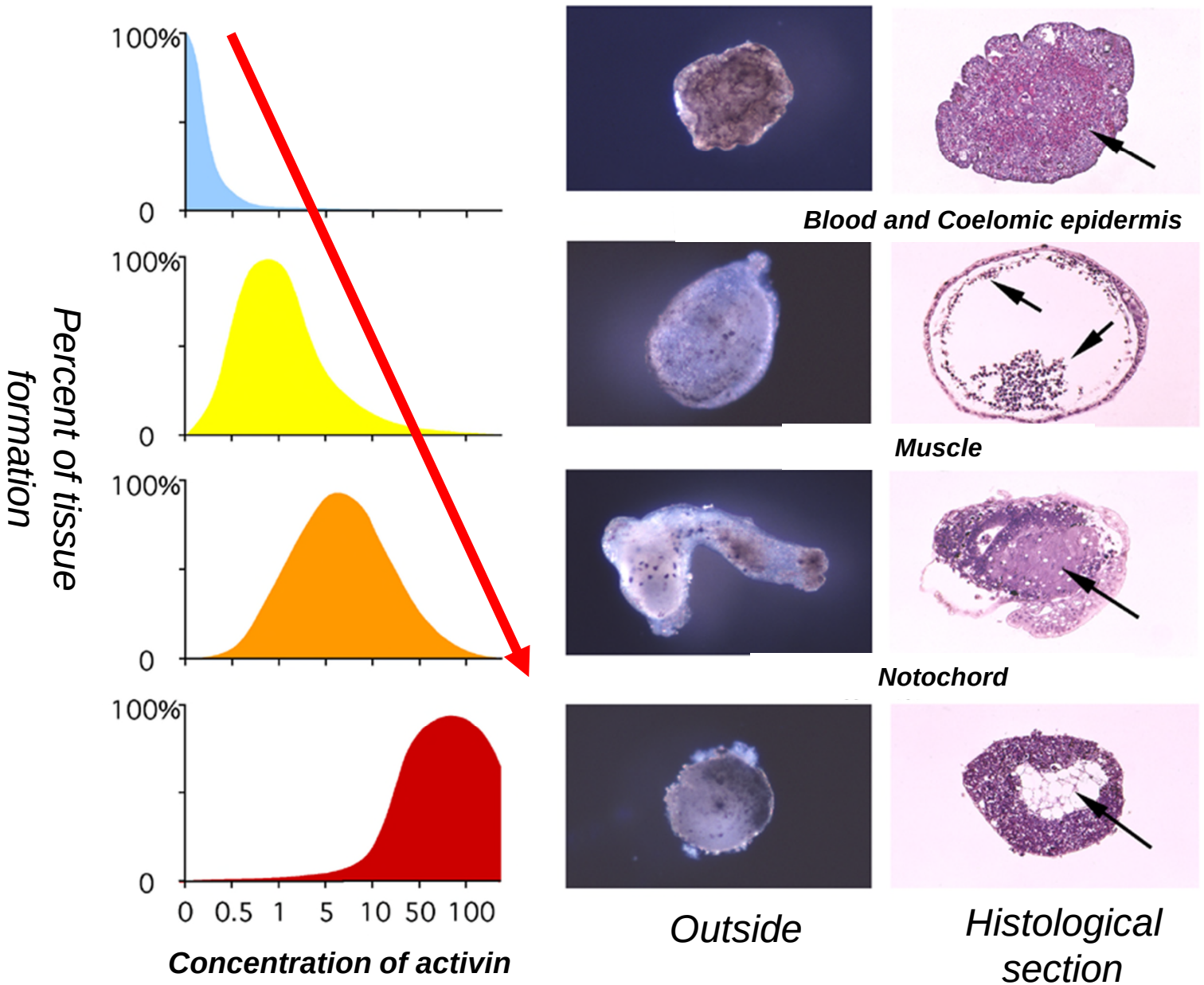
inducers

Ex.
(from chick embrys
by prof. H.
Tiedemann group.



Animal cap assay

# Dose dependent inducing activity by activin treatment on animal cap



# Muscle differentiation in early development

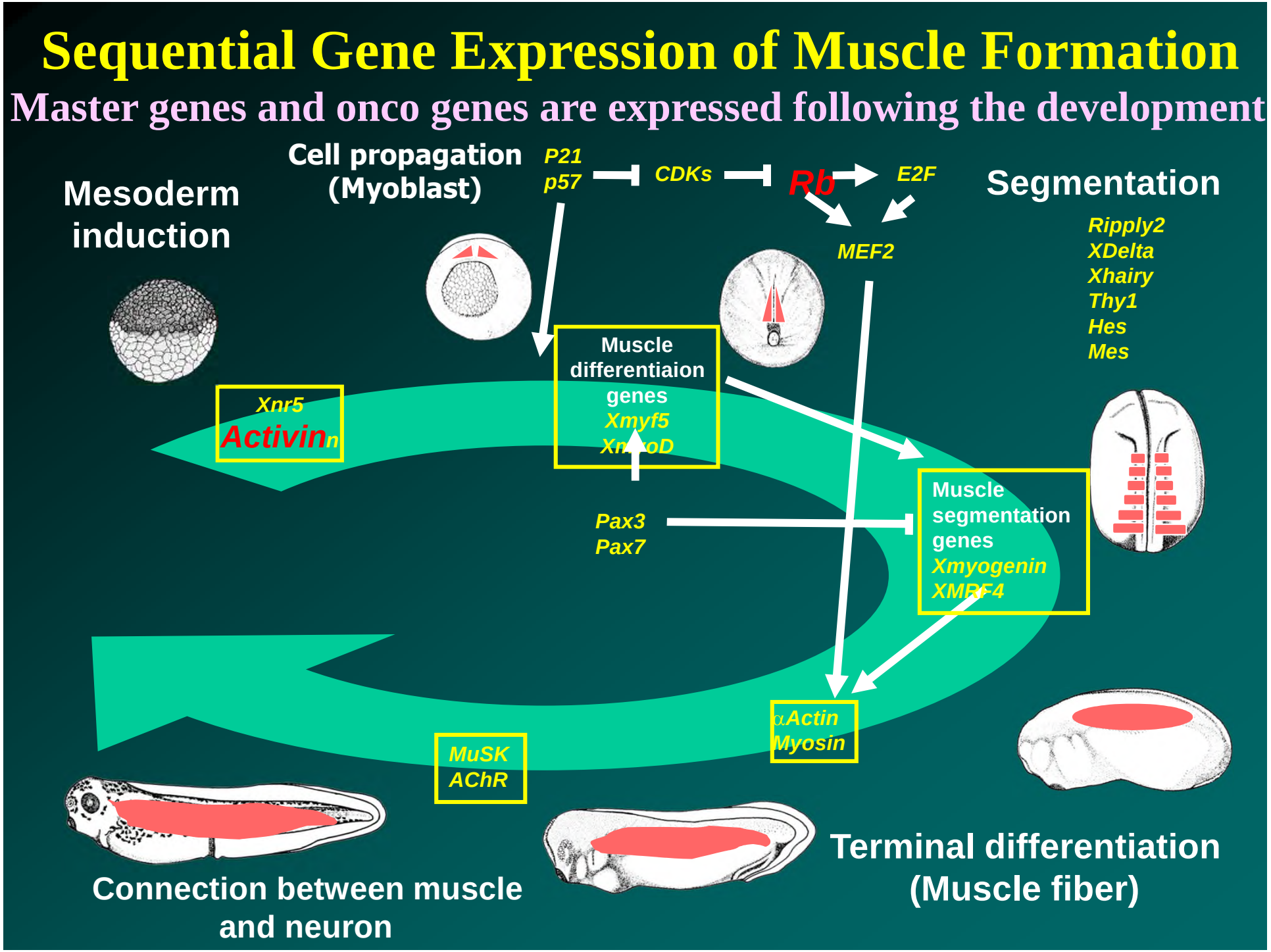
**Elongation and myogenesis of animal cap treated with activin**

**After treatment of activinA, the explant starts for muscle formation.**

5 ng/ml of Activin

# Sequential Gene Expression of Muscle Formation

## Master genes and onco genes are expressed following the development



# Activin-induced beating heart from animal cap

Treated with activin 100 ng/ml

7 days cultivation

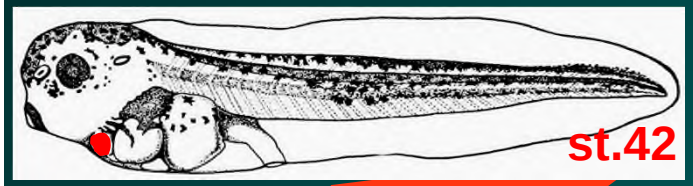
# *Xenopus* heart formation roadmap

*Nkx2.5*
*GATA4/5/6*
*MA41*
*XHMGA2*
*(dkk1)*
*(crescent)*


*XHAPLN3*
*Xhas2*
*Xversican*
*Tbx5*
*Tbx20*


*Notch1*
*Serrate1*
*Delta1*
*myocardin*
*(BMP2/4)*
*(wnt11)*

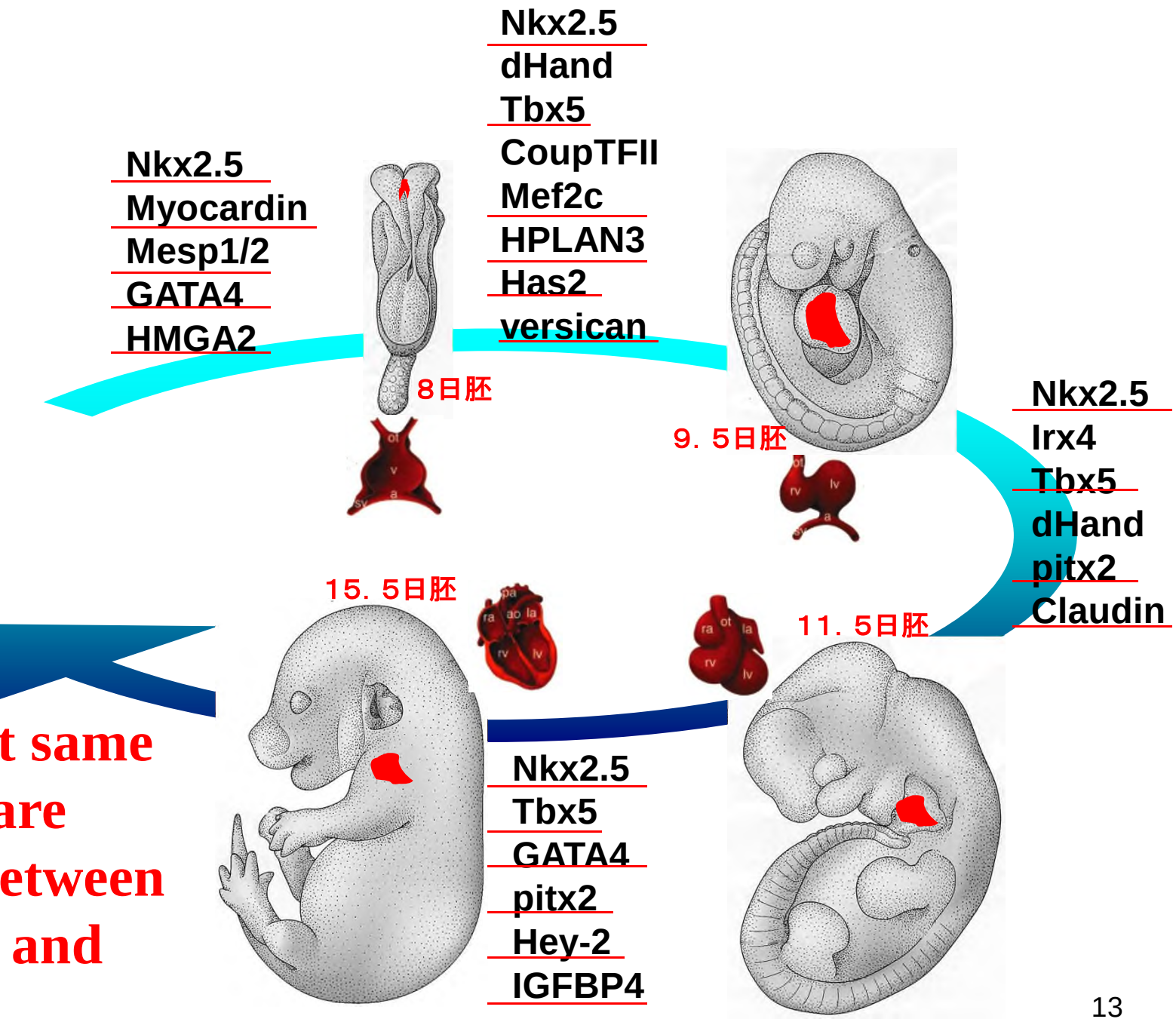
*islet1*
*hermes*
*Nkx2.3/2.10*
*Xclaudin-a/b*
*MA50*

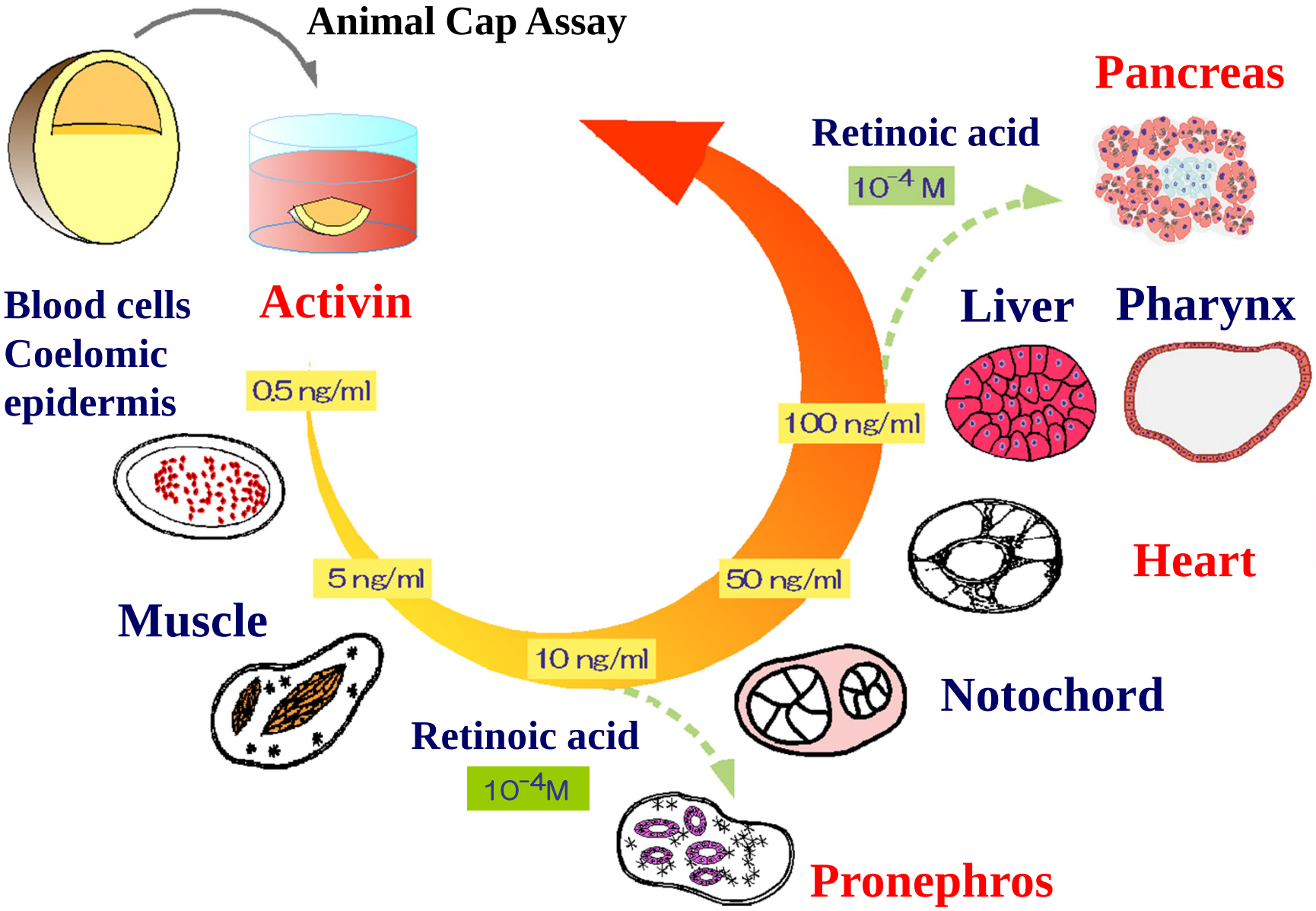

*ANF*

*XIGFBP4*

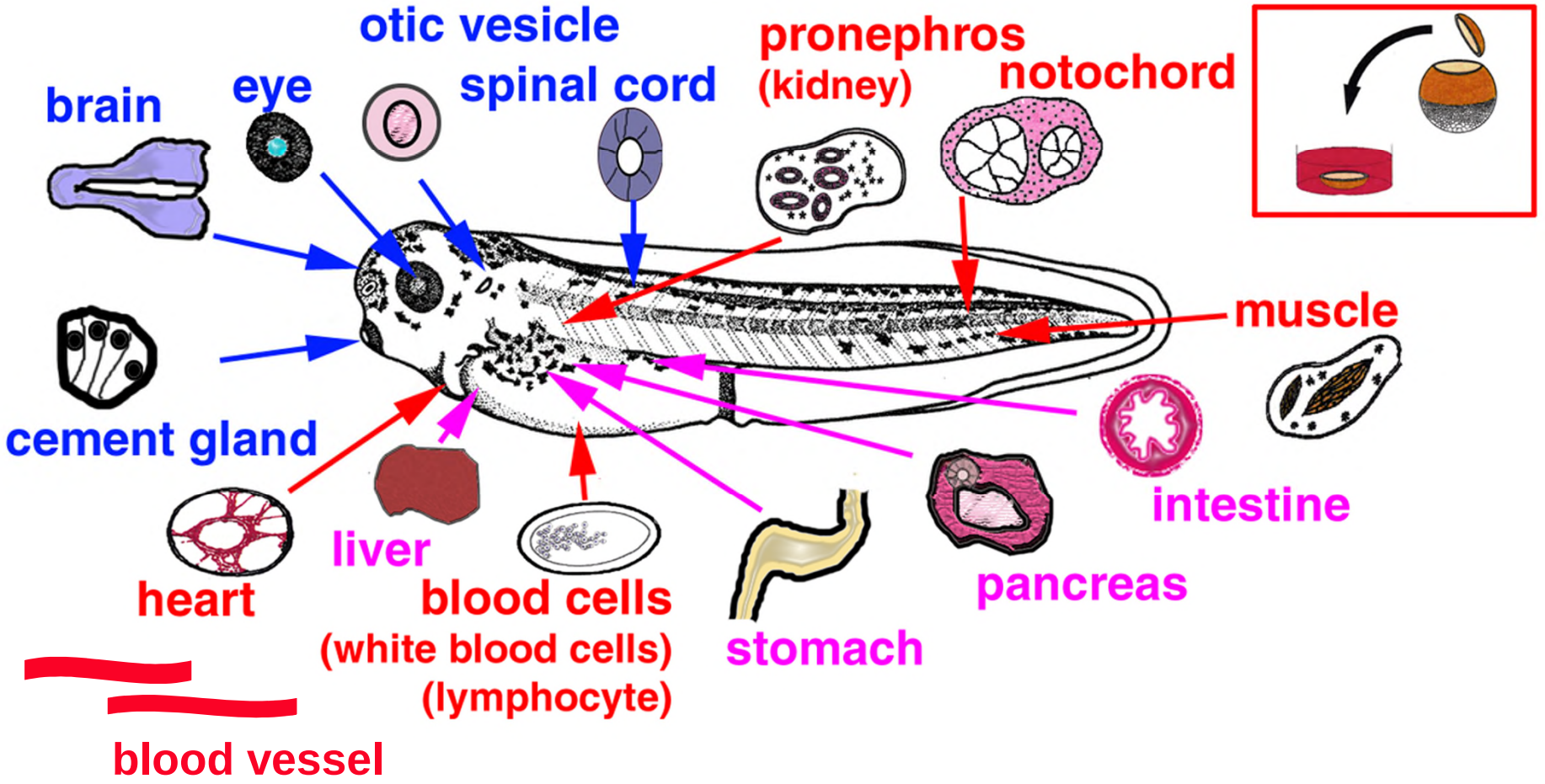

*MHCα/β*
*MLC2*
*cardiac actin*
*noggin2*
*cardiac troponin I/C/T*


Nkx2.5
Myocardin
Mesp1/2
GATA4
HMGA2
8日胚
Nkx2.5
dHand
Tbx5
CoupTFII
Mef2c
HPLAN3
Has2
versican
9．5日胚
Nkx2.5
Irx4
Tbx5
dHand
pitx2
Claudin
11．5日胚
15．5日胚
Nkx2.5
Tbx5
GATA4
pitx2
Hey-2
IGFBP4
Almost same genes are used between mouse and frog.

# Activin induces various tissues and organs from animal caps

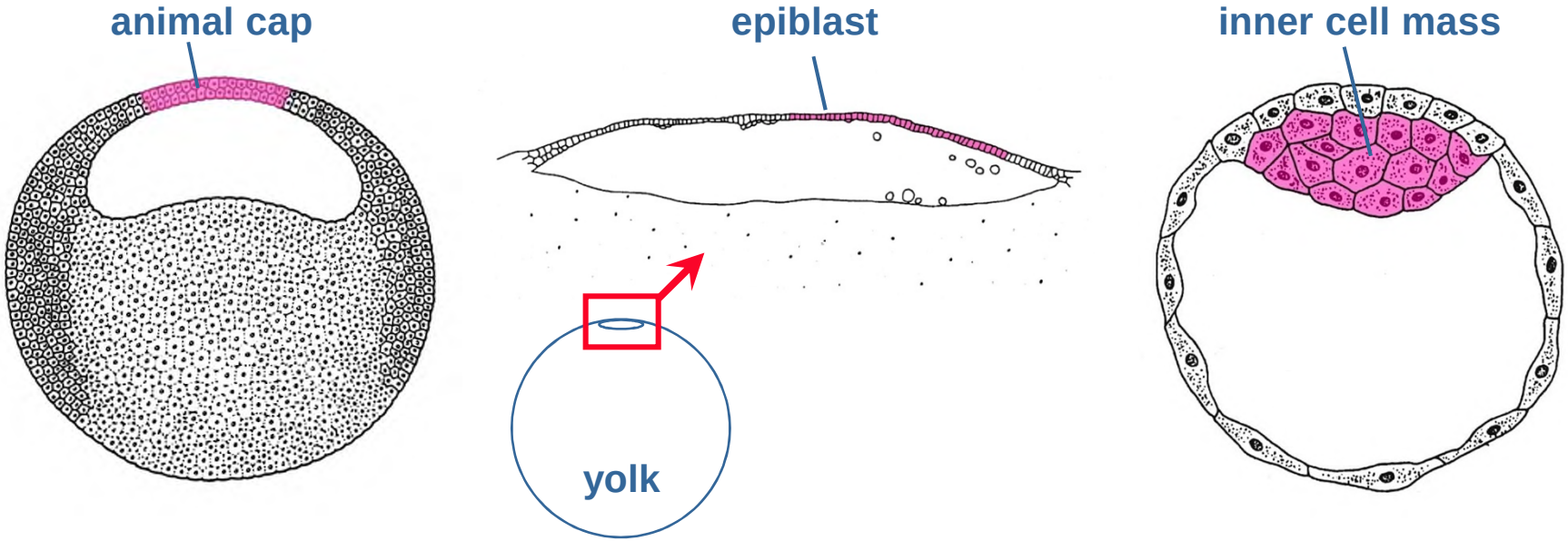


# Control of organogenesis *in vitro* using *Xenopus* animal cap




**Total over 20 organs and tissues (in Asashima Lab.)**

# Multi-potential cells at the blastula stage in vertebrates embryos



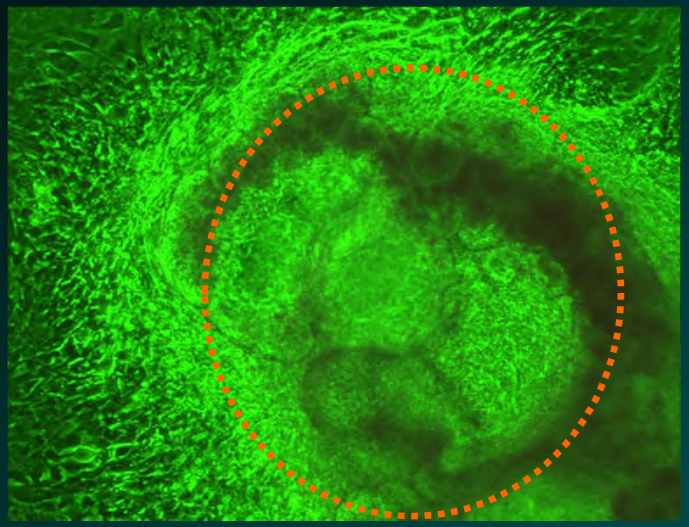

Xenopus (amphibia)

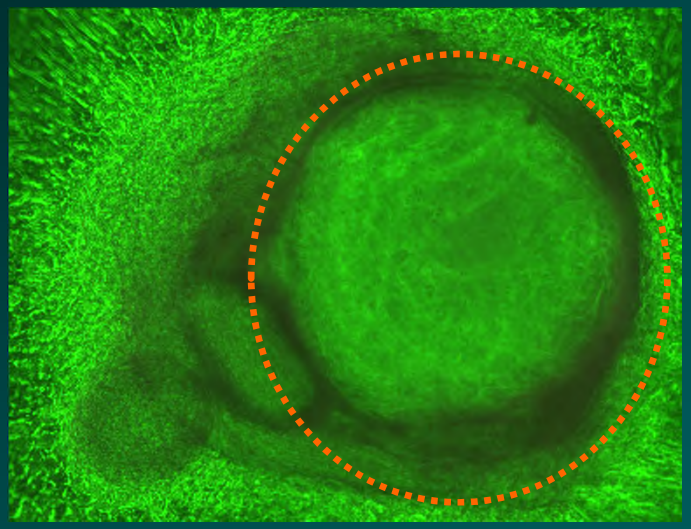
chicken (aves)

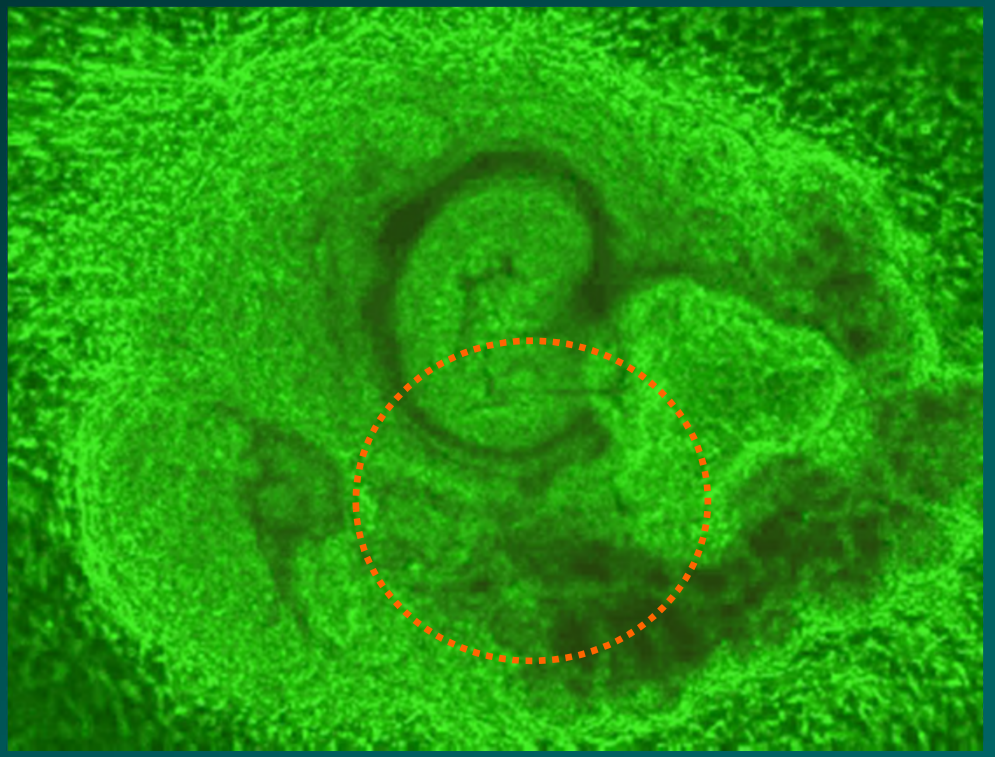
Mouse Human (mammal)

# Organogenesis from Mouse ES cells

# ES cells differentiation into gut-like and pancreas structures by the treatment with Activin and RAR agonist

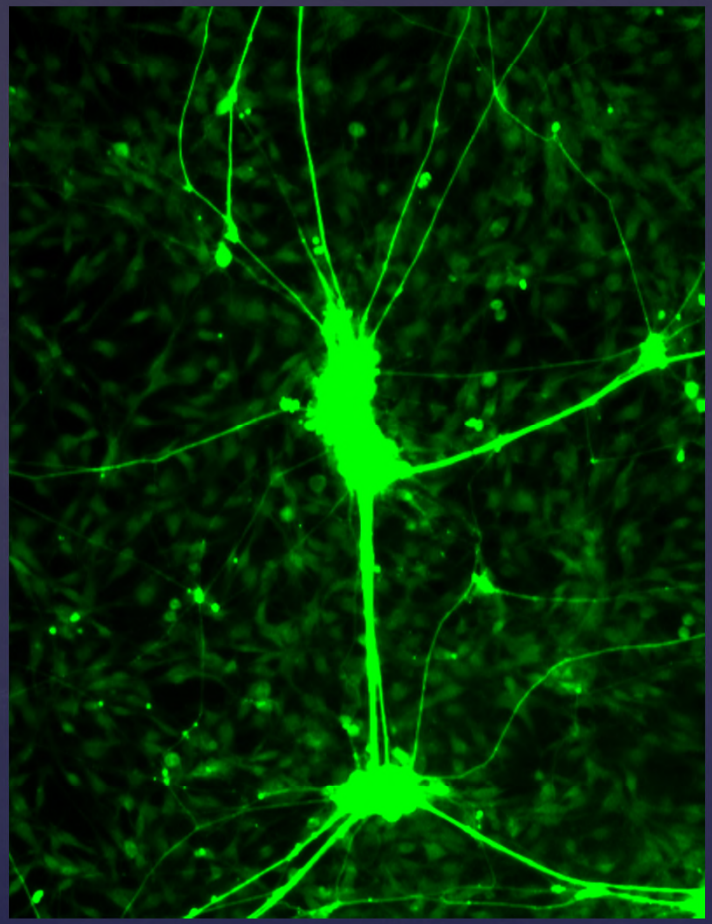




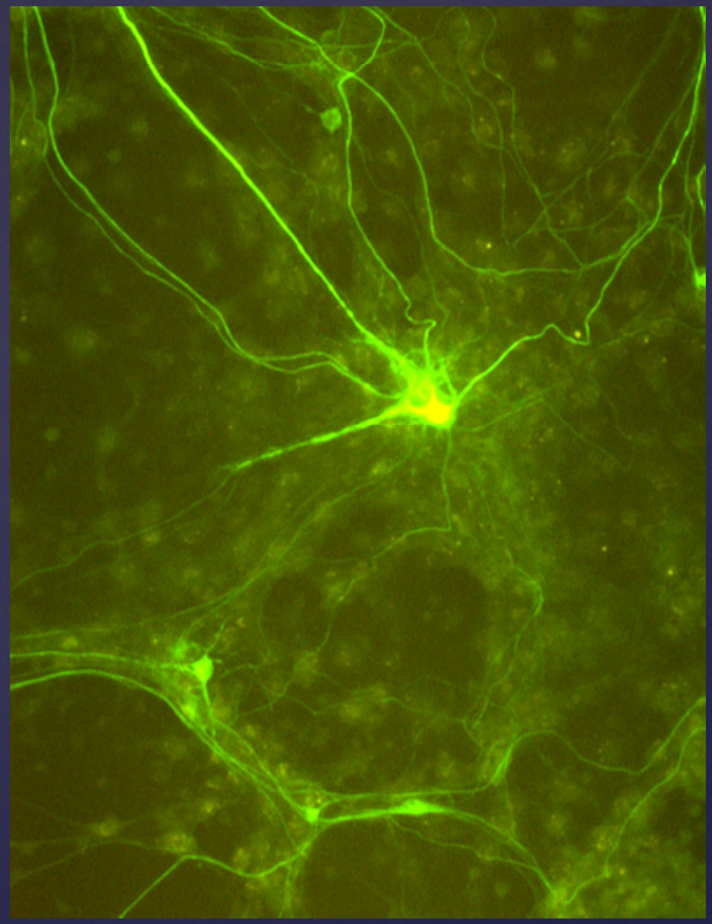


**Activin and RA are one of the key factors to induce the pancreas**

# Neural cells formation from mouse ES cells



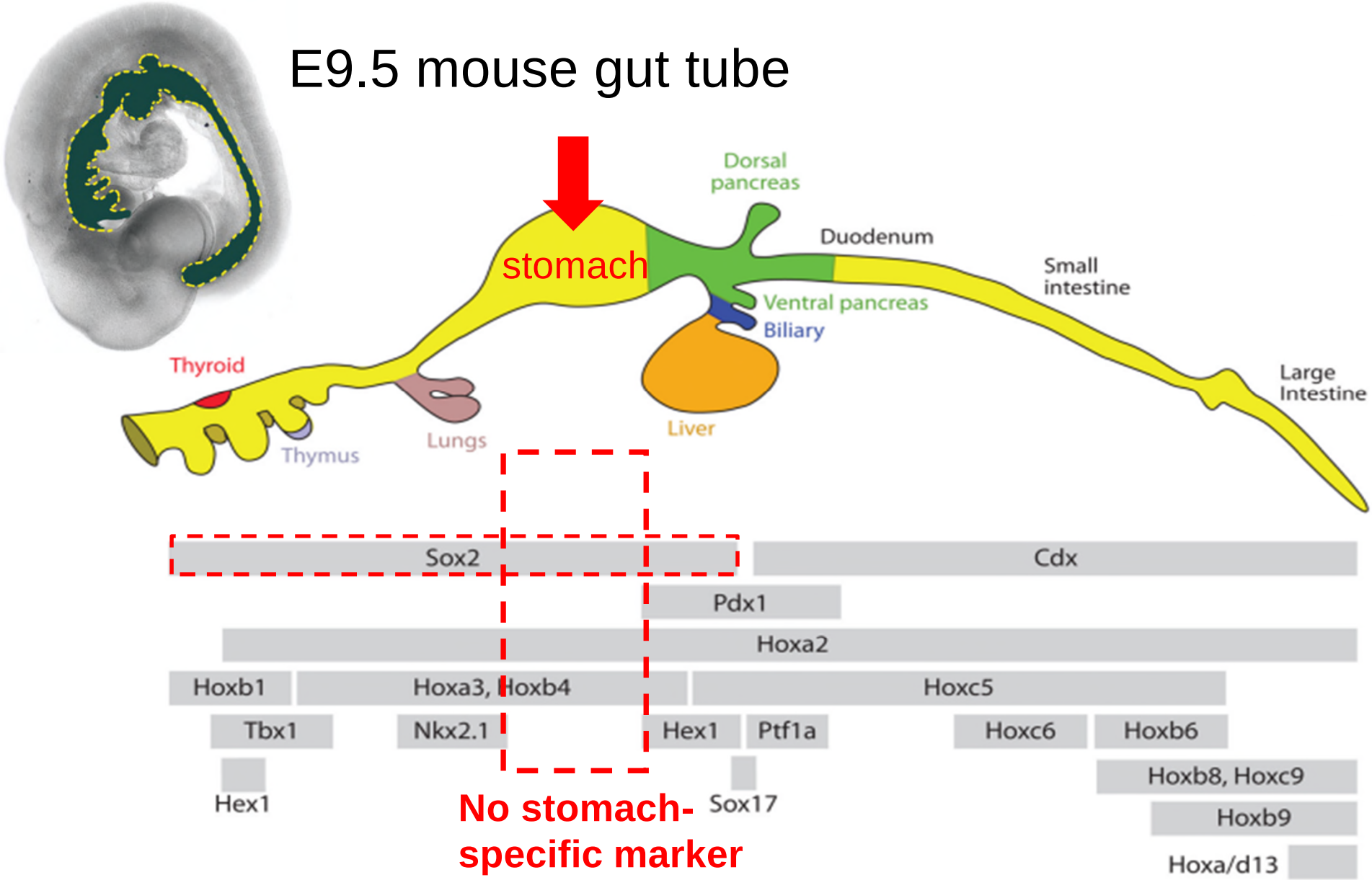
Anti-L-NF antibody (FITC)



Anti-H-NF antibody (FITC)

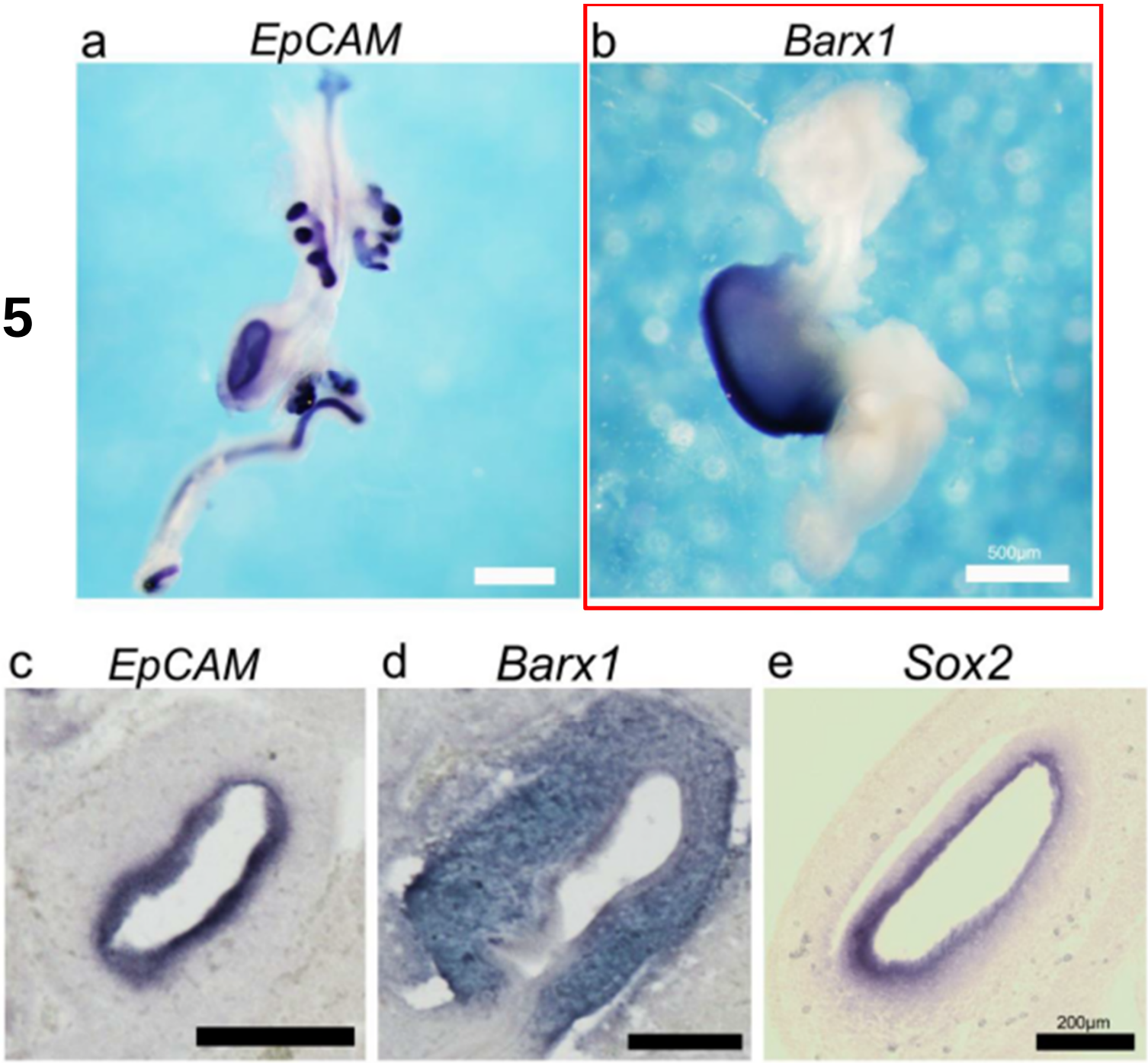
**Several kinds of neural cells are induced by changing of conditions**

# Differentiation of stomach tissue from ES cells



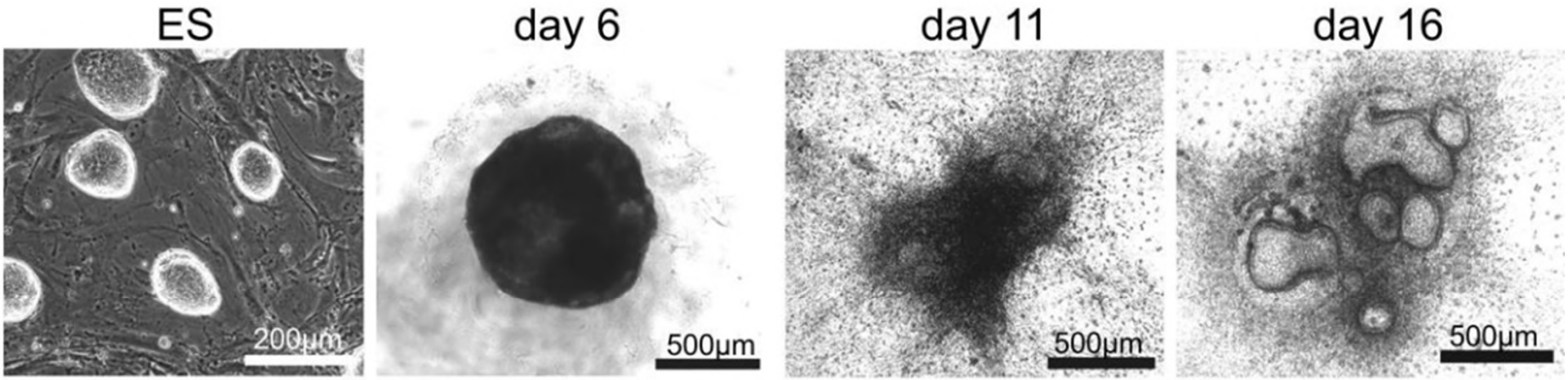

Zorn, AM and Wells, JM. *Annu Rev Cell Dev Biol*. (2009)

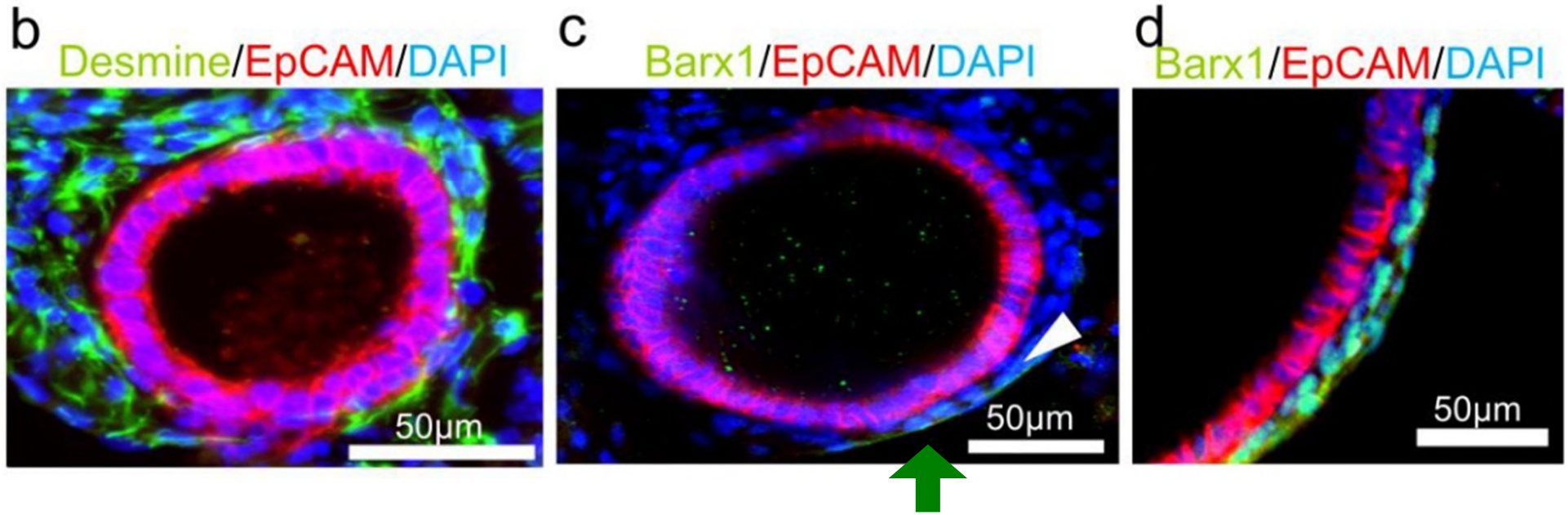
**E11.5**




Noguchi et al., Nature Cell Biology (2015)

# Generation of gustrointestine from mouse ES cells



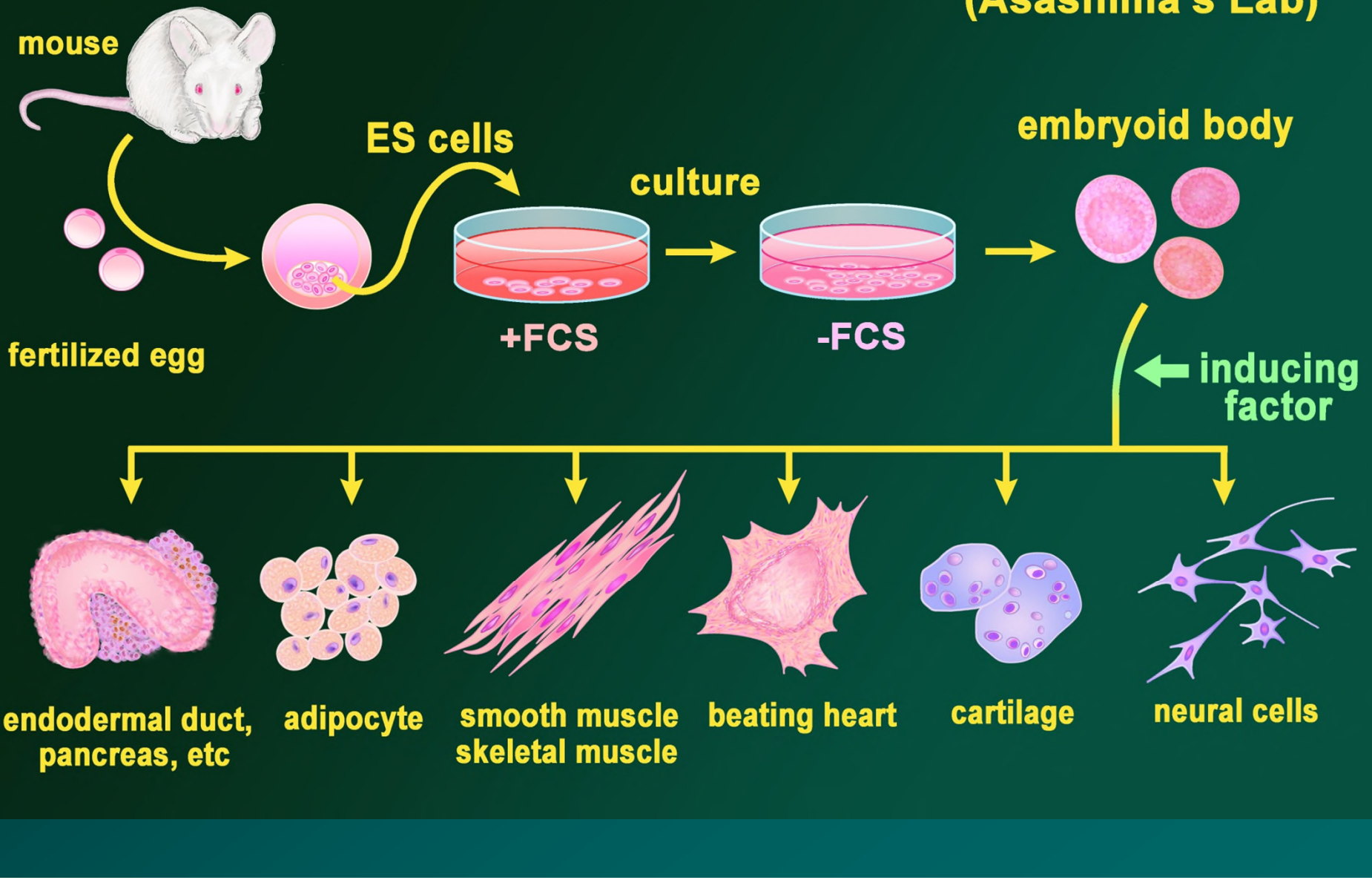




Very limited area was differentiated into stomach lineage

Noguchi et al., Nature Cell Biology (2015)

# *In vitro* induced organs from mice ES cells

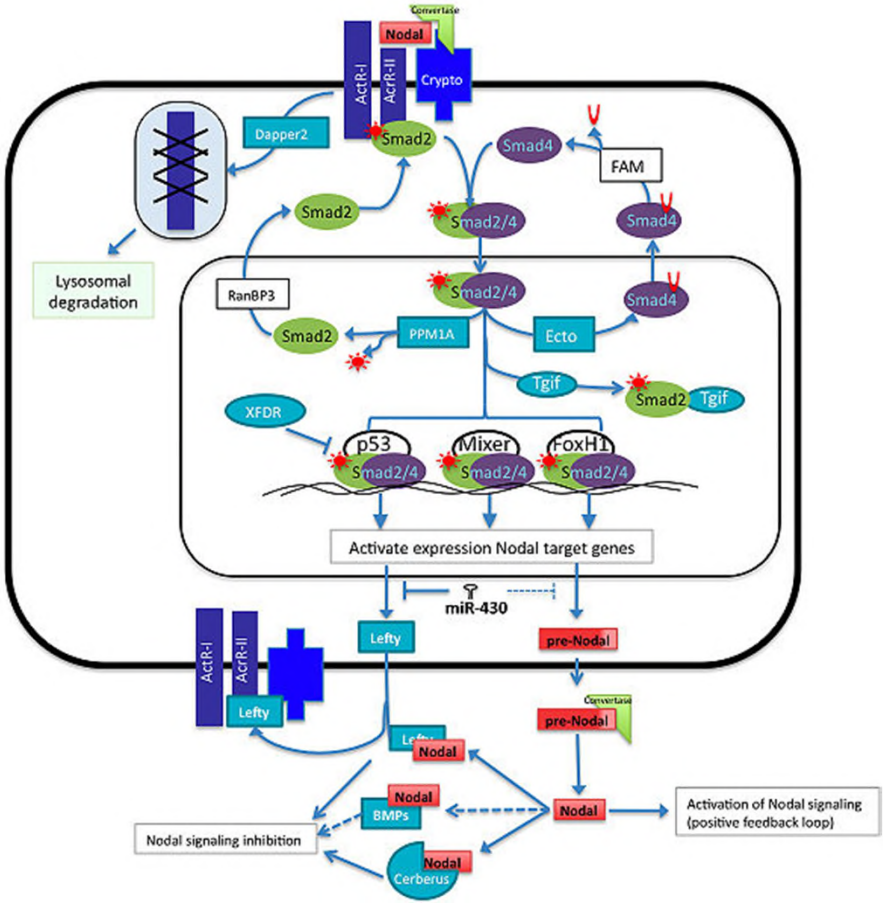
(Asashima's Lab)



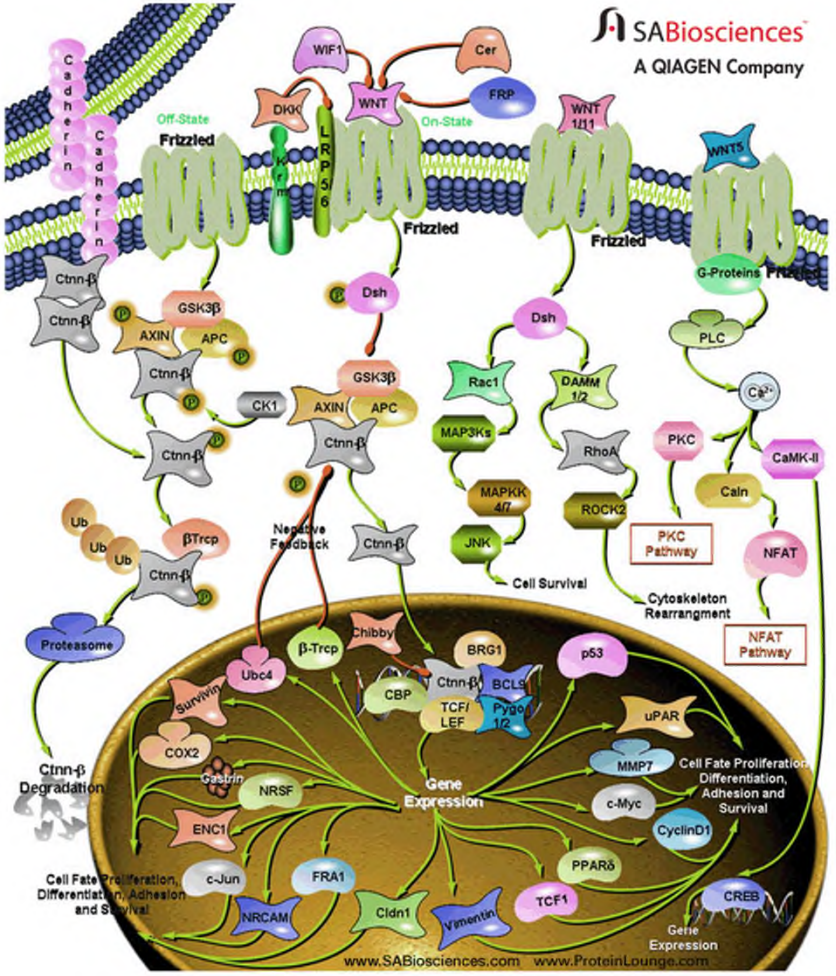
## 2. New regulation factor of activin signalling by MAN1

# Networks of signal transduction between Activin / Nodal and Wnt signalings

## Nodal/Activin signaling




## Wnt signaling



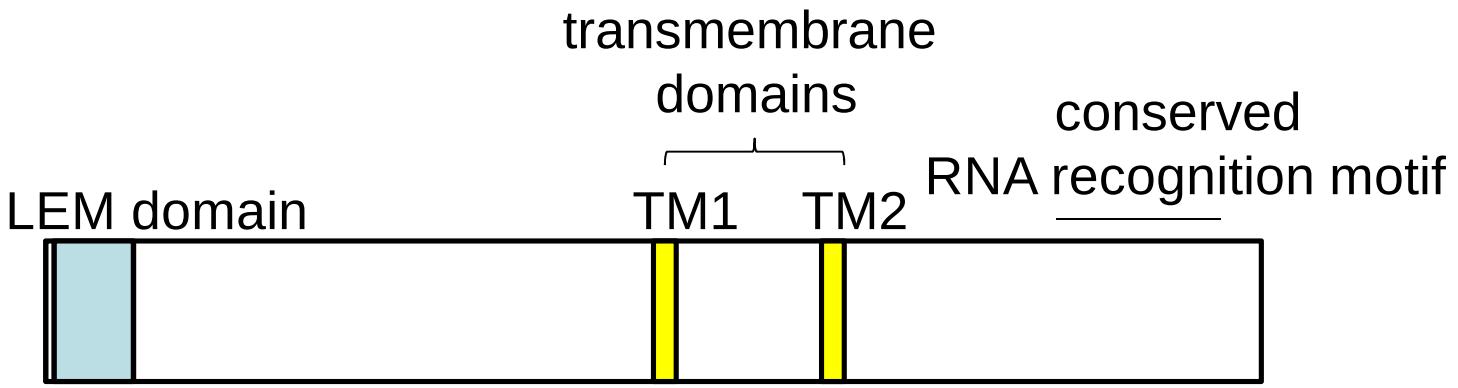
# New regulation factor of Activin

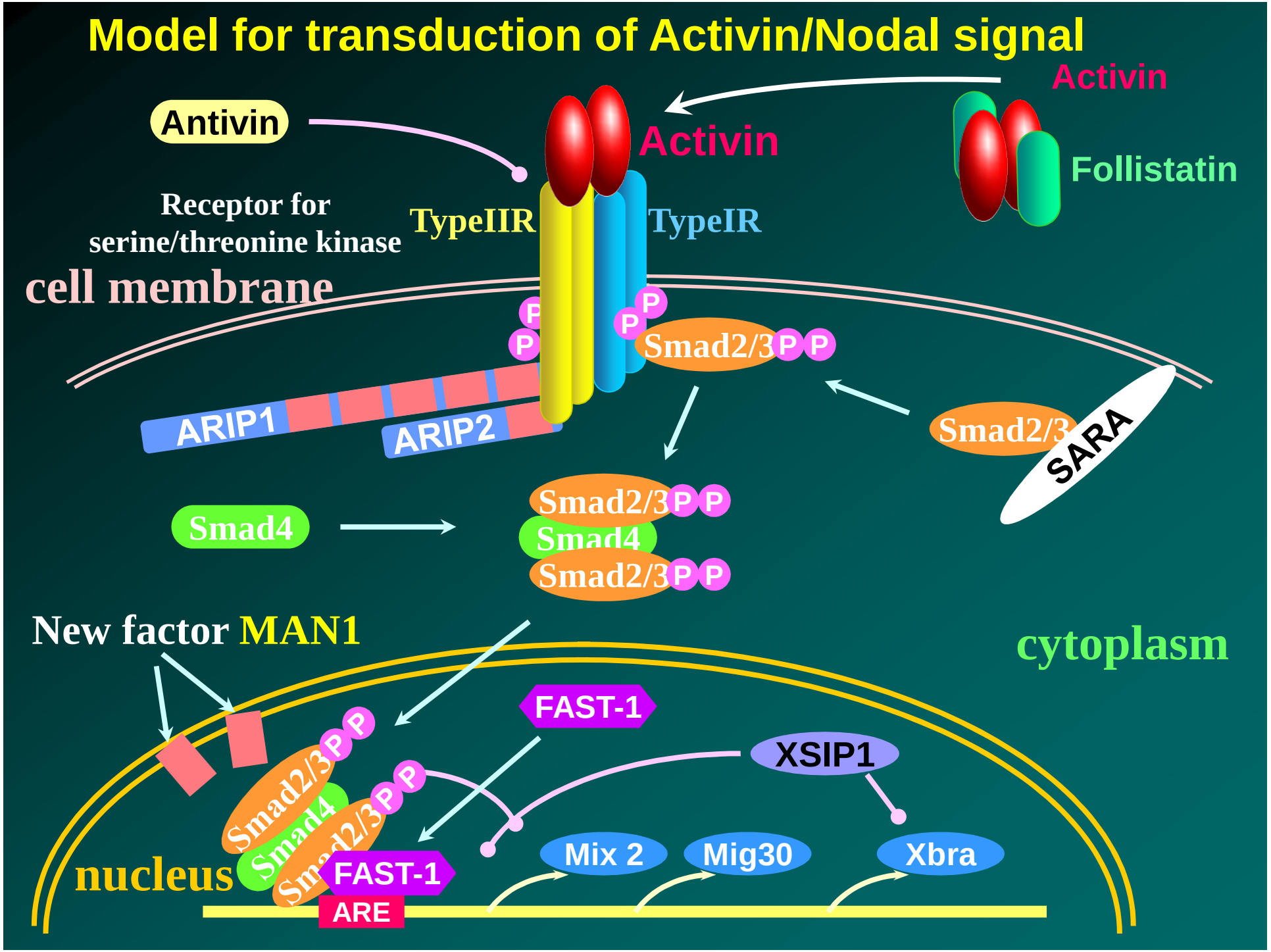
- **MAN1 (MAN antigens; Lemd3, LEM domain containing 3)**

  **…inner nuclear membrane protein**

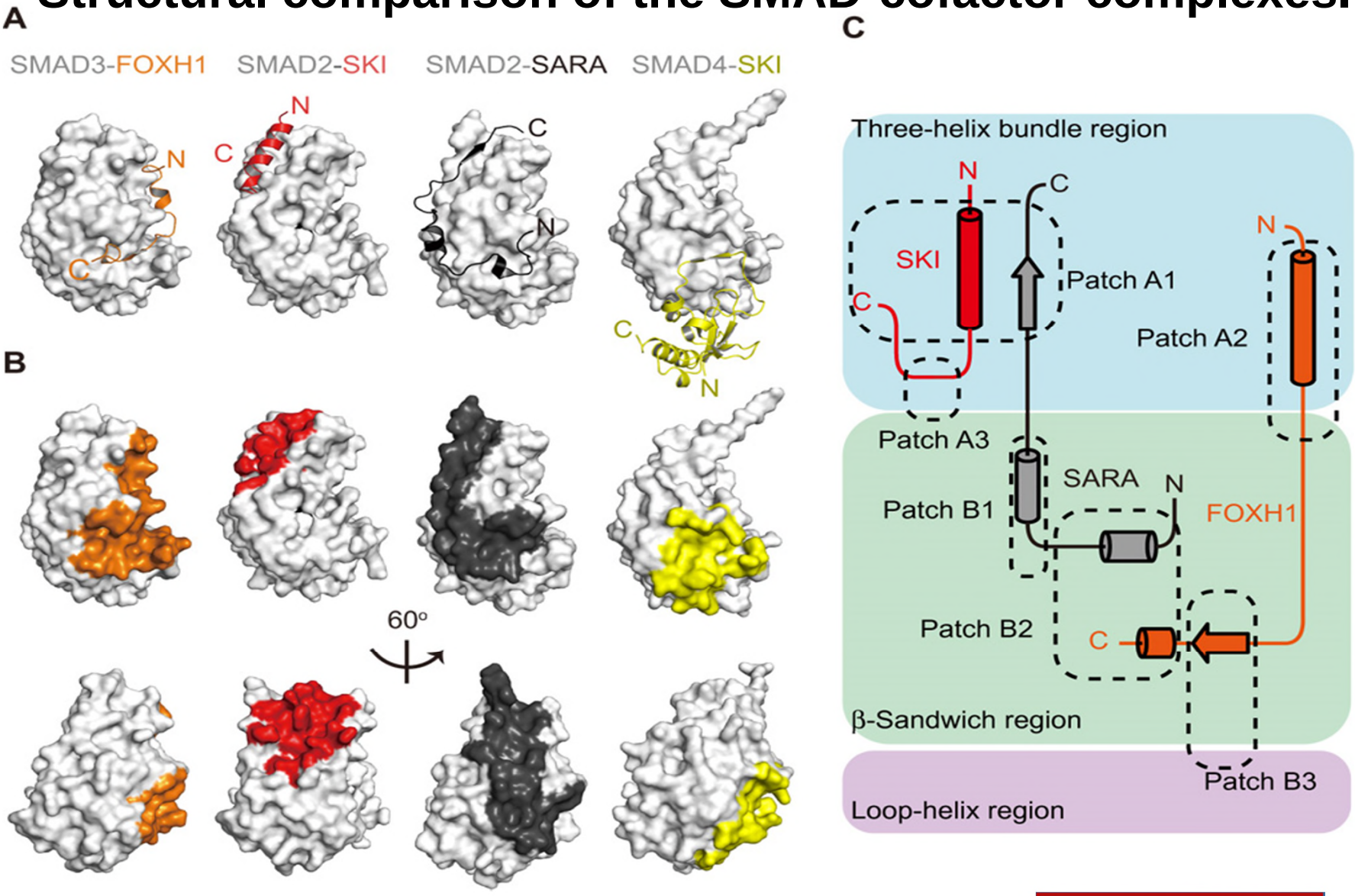
  **…one of antigens recognized by autoantibodies from a patients with a collagen vascular disease**

Lin et al., J. Biol. Chem. (2000).
Paulin-Vevasseur et al Chromosoma (1996)




Model for transduction of Activin/Nodal signal
Activin
Antivin
Activin
Follistatin
Receptor for
serine/threonine kinase
TypeIIR
TypeIR
cell membrane
P
P
P
P
Smad2/3PP
ARIP1
ARIP2
Smad2/3
SARA
Smad4
Smad2/3PP
Smad4
Smad2/3PP
New factor MAN1
cytoplasm
FAST-1
XSIP1
Smad2/3PP
Smad4
Smad2/3PP
nucleus
FAST-1
ARE
Mix 2
Mig30
Xbra

# Structural comparison of the SMAD-cofactor complexes.



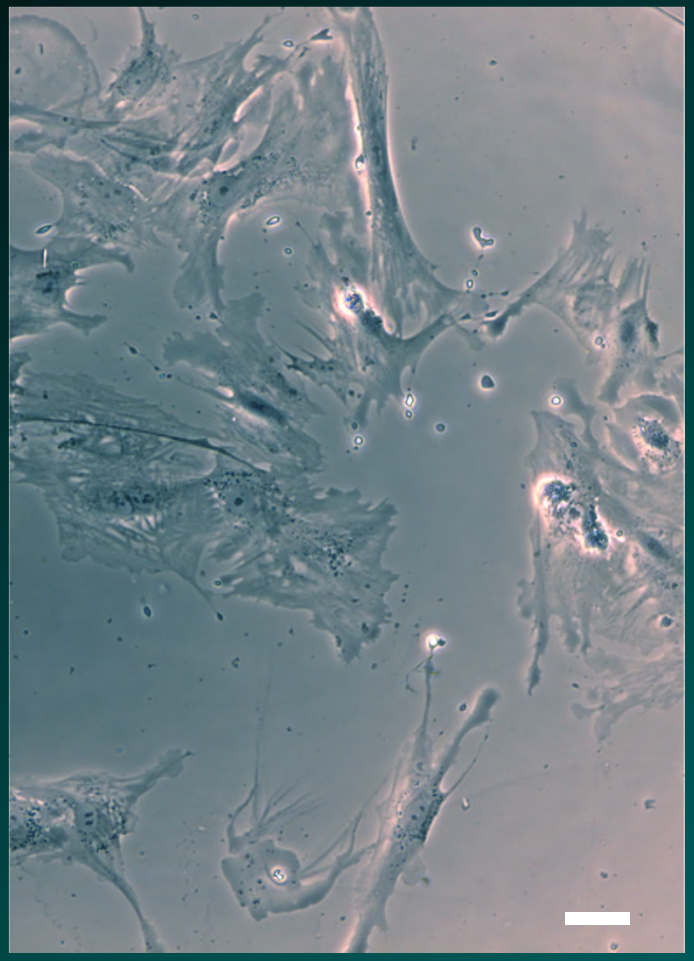

**Ken-ichi Miyazono et al., Sci. Signal. 2018;11:eaao7227**

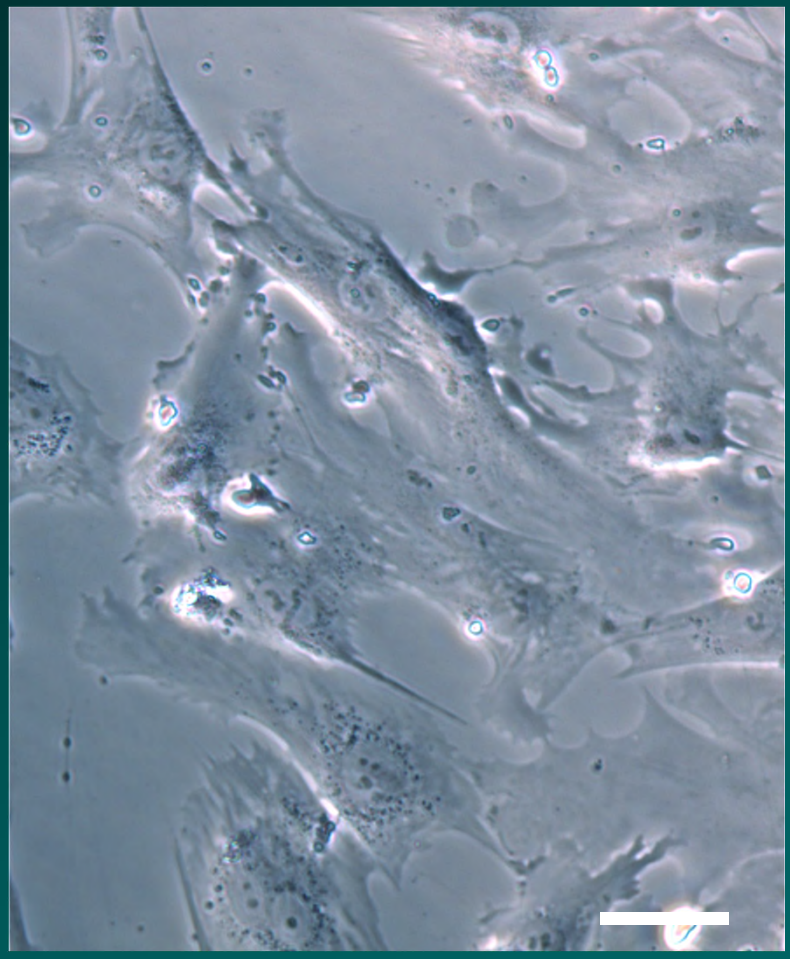


Science Signaling
AAAS

# 3. Approaches of human stem cells

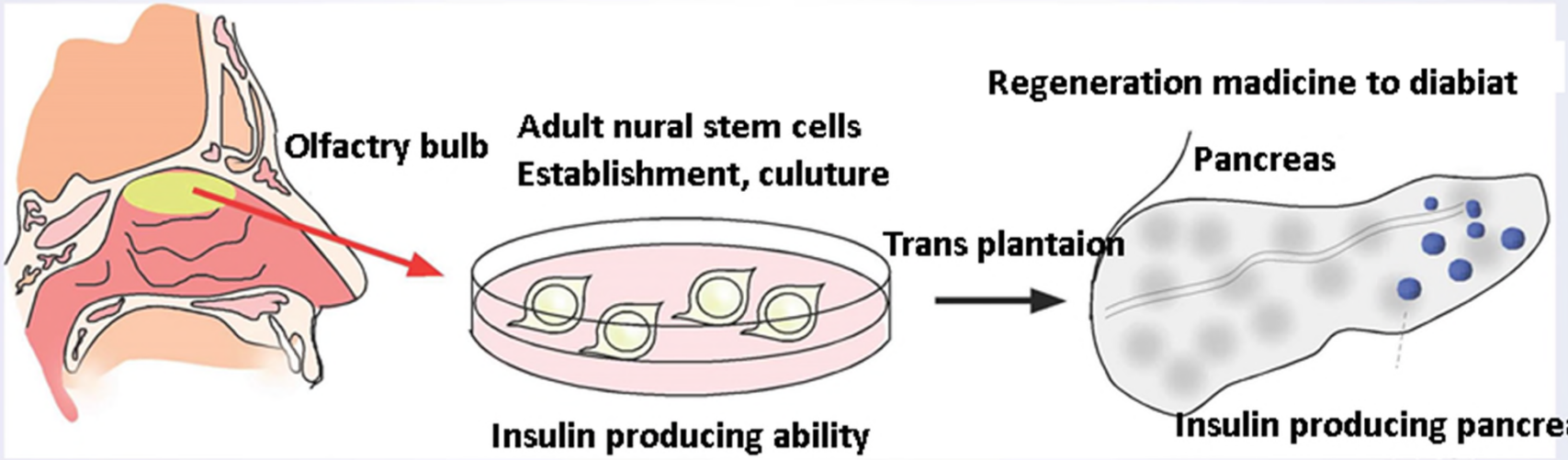
# Human adult stem cells





Our bodies have a lot of adult stem cells in many tissues and organs such as bone marrow, adipose tissue, brain, MSC etc.

# Establishment of neural stem cells from olfactry bulb




Extract neural stem cells from olfactory bulb in living state

Expand neural stem cell in dish and activate the insulin production ability

Transplantation adult neural progenitor cells into pancreas islet area by using own cells (rejection free)

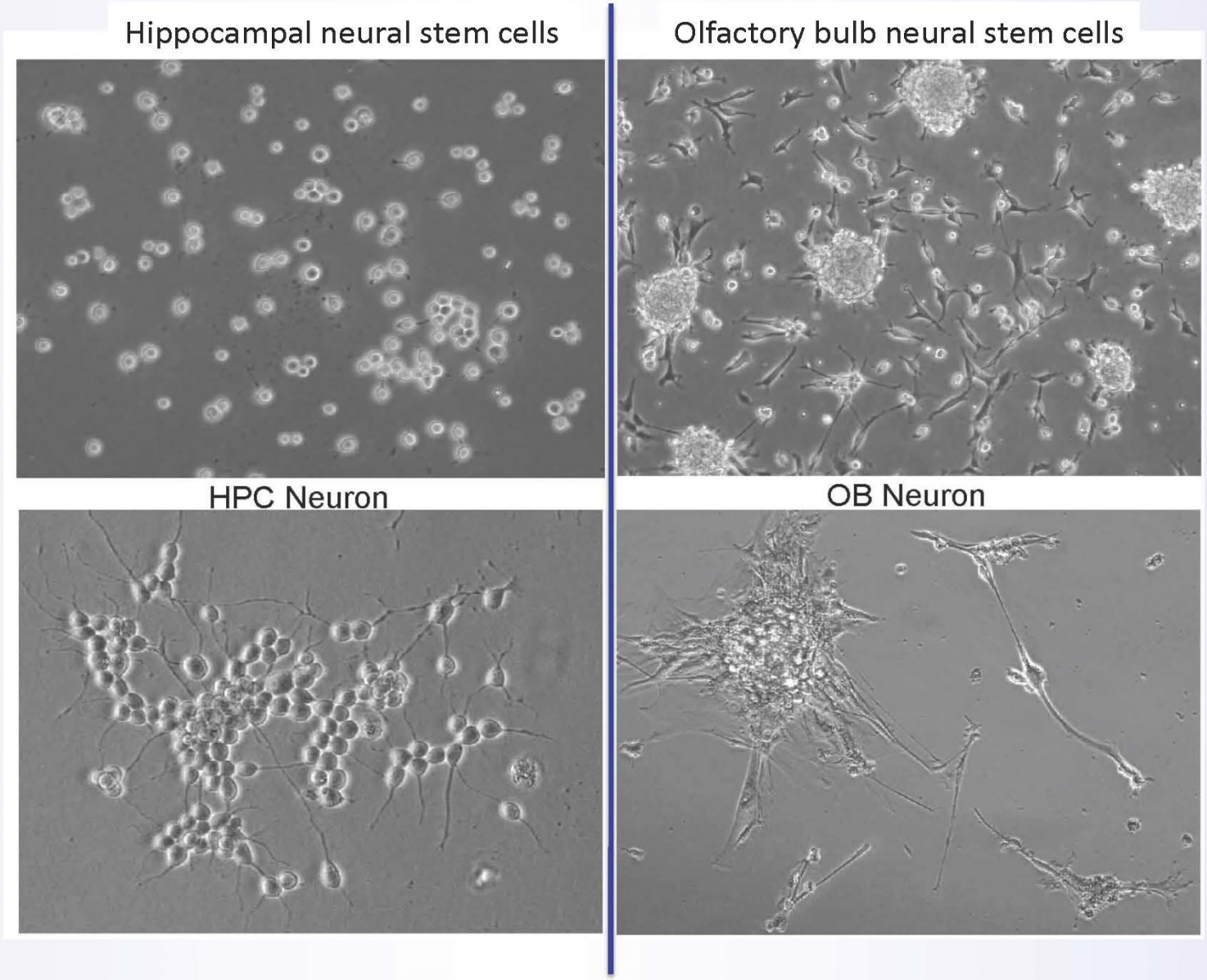
**These stem cells can differentiate into various kind nural cells.**

**Kuwabara et al.,Nat. neurosci.(2009),Wakabayashi et al(2016)JBC**

## Olfactory bulb and hippocampal neural stem cells

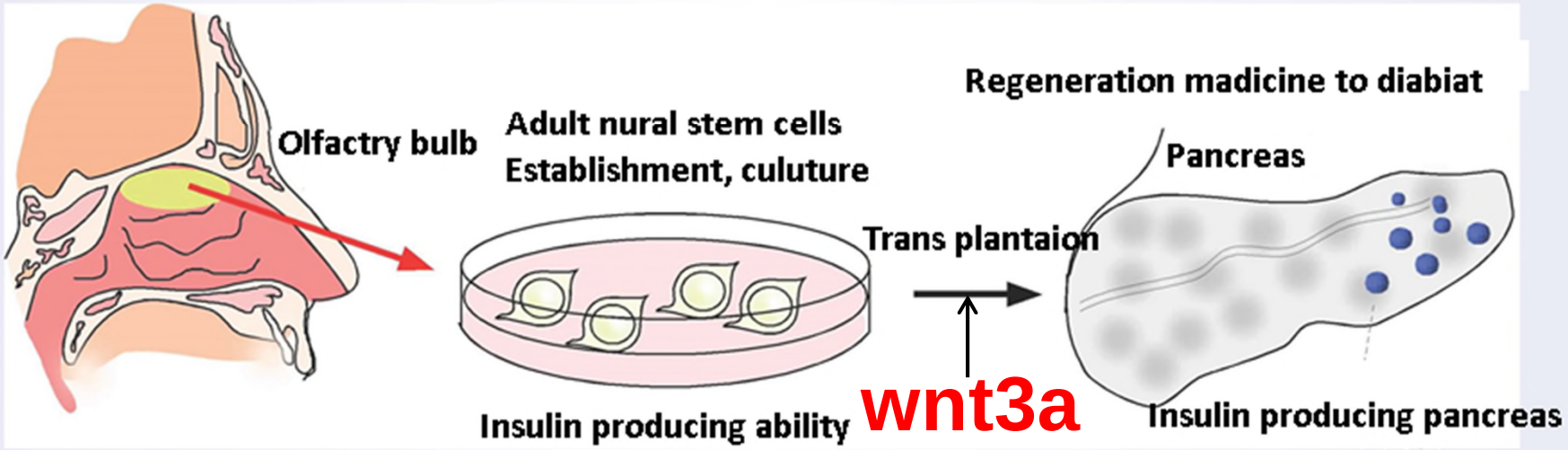
Stem cells

Induction of neural differentiation




**These stem cells can differentiate into several kind of neural cells.**

# Establishment of neural stem cells from olfactry bulb




Extract neural stem cells from olfactory bulb in living state

Expand neural stem cell in dish and activate the insulin production ability

Transplantation adult neural progenitor cells into pancreas islet area by using own cells (rejection free)

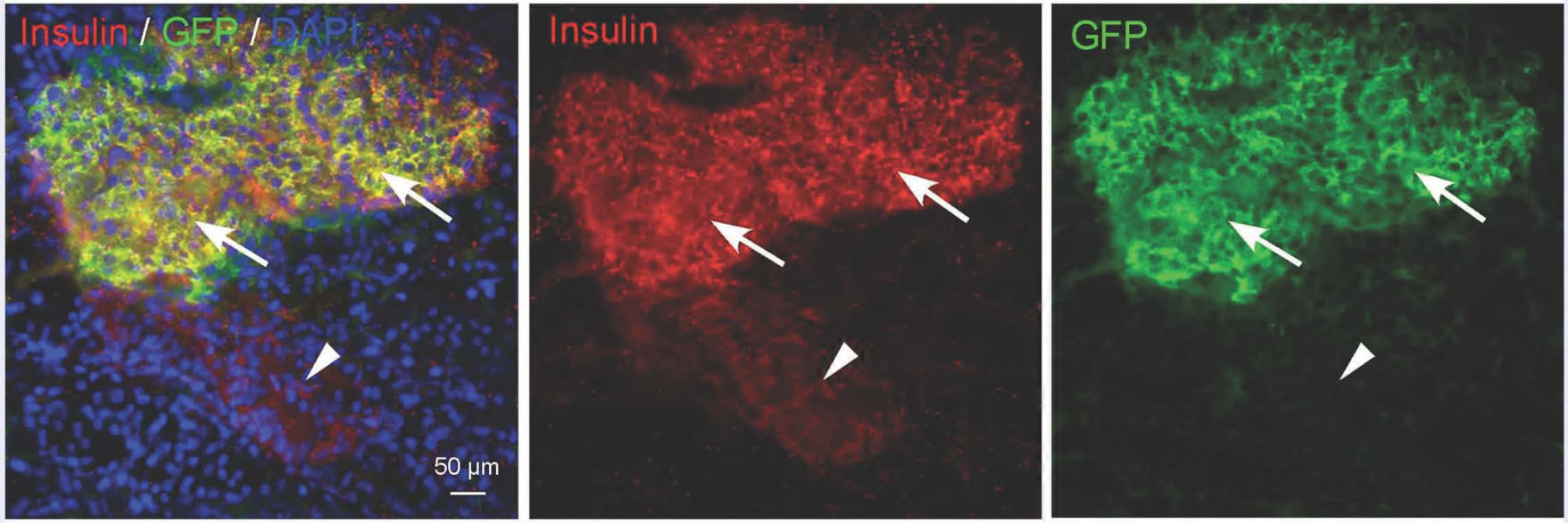
**These stem cells can produce insulin adding neural extract or Wnt 3a.**

**Kuwabara et al.,(2009)Nat.neurosci, Wakabayashi et al(2016)JBC**

# Transplanted OB-derived NSCs can produce insulin in vivo system

5 months later（GFP:transplanted NSCs）。GFP(-) original cells has no production insulin.

GFP(+) transplanted OB-derived NSCs actively produce insulin.



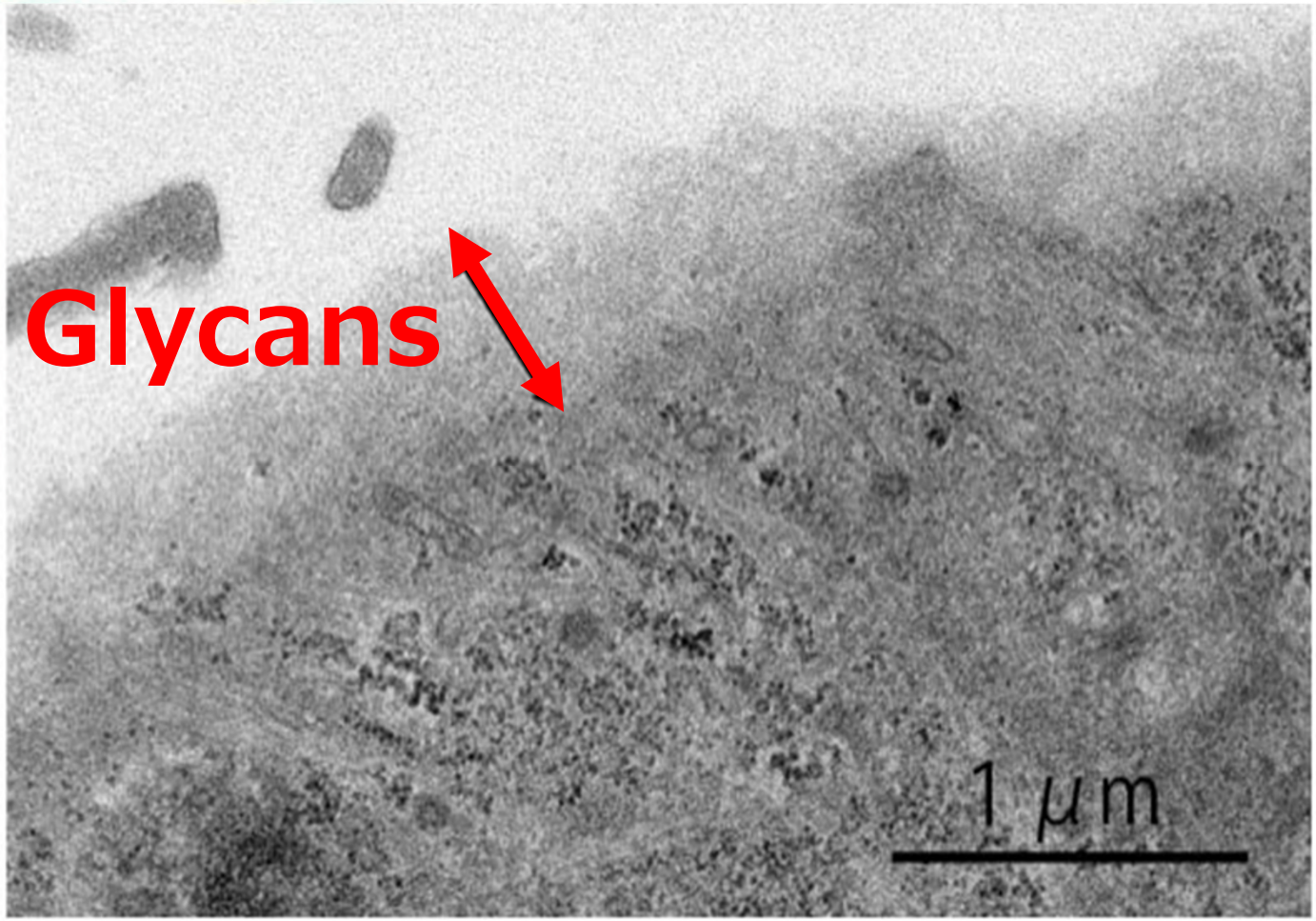

Control of transplantation　　Insulin produced cells　　GFP labelling explants

Kuwabara T. et al., Nature NeuroSci.(2009),Wakabayashi et al. JBC(2016)

National Institute of Advanced Industrial Science and Technology
AIST

# hES/iPSCs are covered with glycans

Electromicroscopic photograph of iPSCs




Collaboration with Dr. Yoshihiro Akimoto

# Development of new probes

## by glycan analysis of human ES/iPS cells

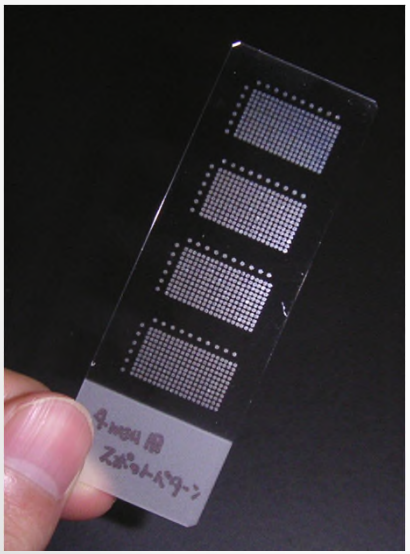
Extract crude membrane proteins from ES/iPS cells

↓

Label with fluorescent dye

↓

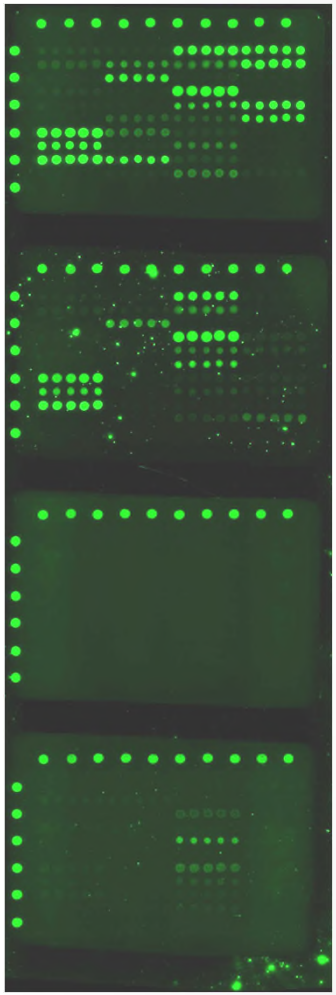
Apply onto "lectin microarray"



lectin microarray

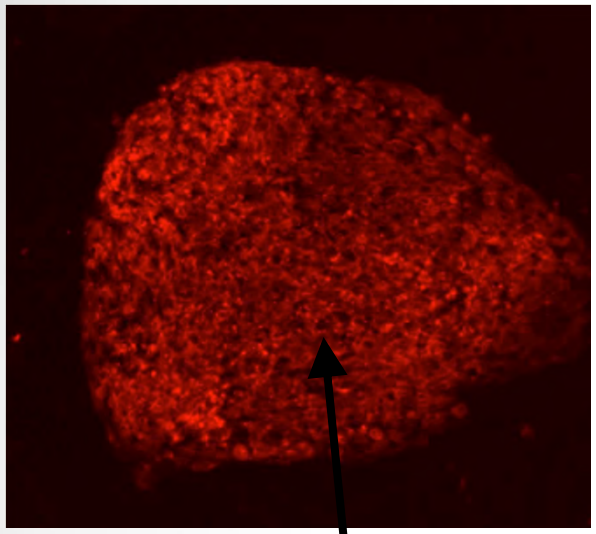
Lectins are proteins, which have various affinities to specific glycan structures.

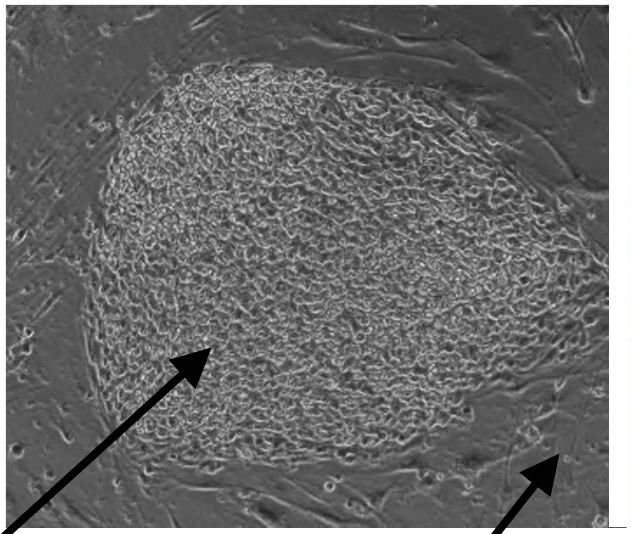
96 kinds of lectin protein probes are spotted on a slideglass.

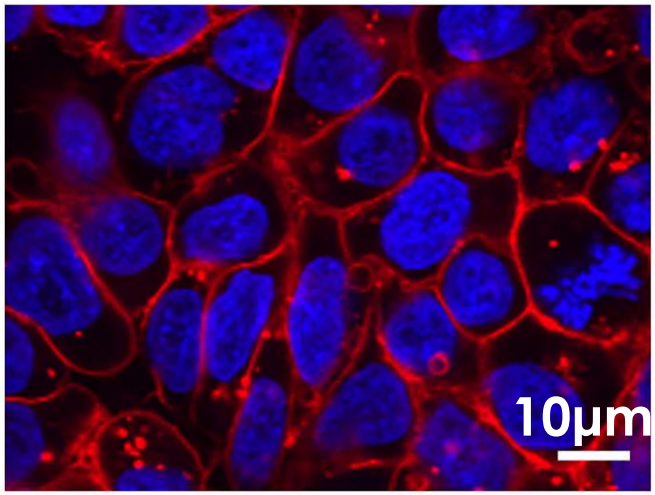


# rBC2LCN lectin binds to cell membrane of ES/iPS cells

Cy3 fluoresent dye-conjugated rBC2LCN








(Onuma et al., BBRC, 2013)

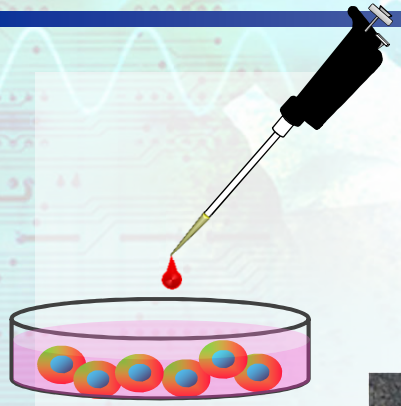
**EScell colony (201B7)**

**feeder cells (MEF)**

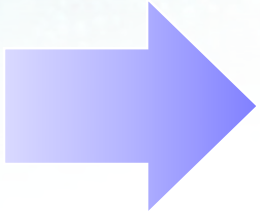
rBC2LCN lectin binds to cell membrane of human pluripotent stem cells.

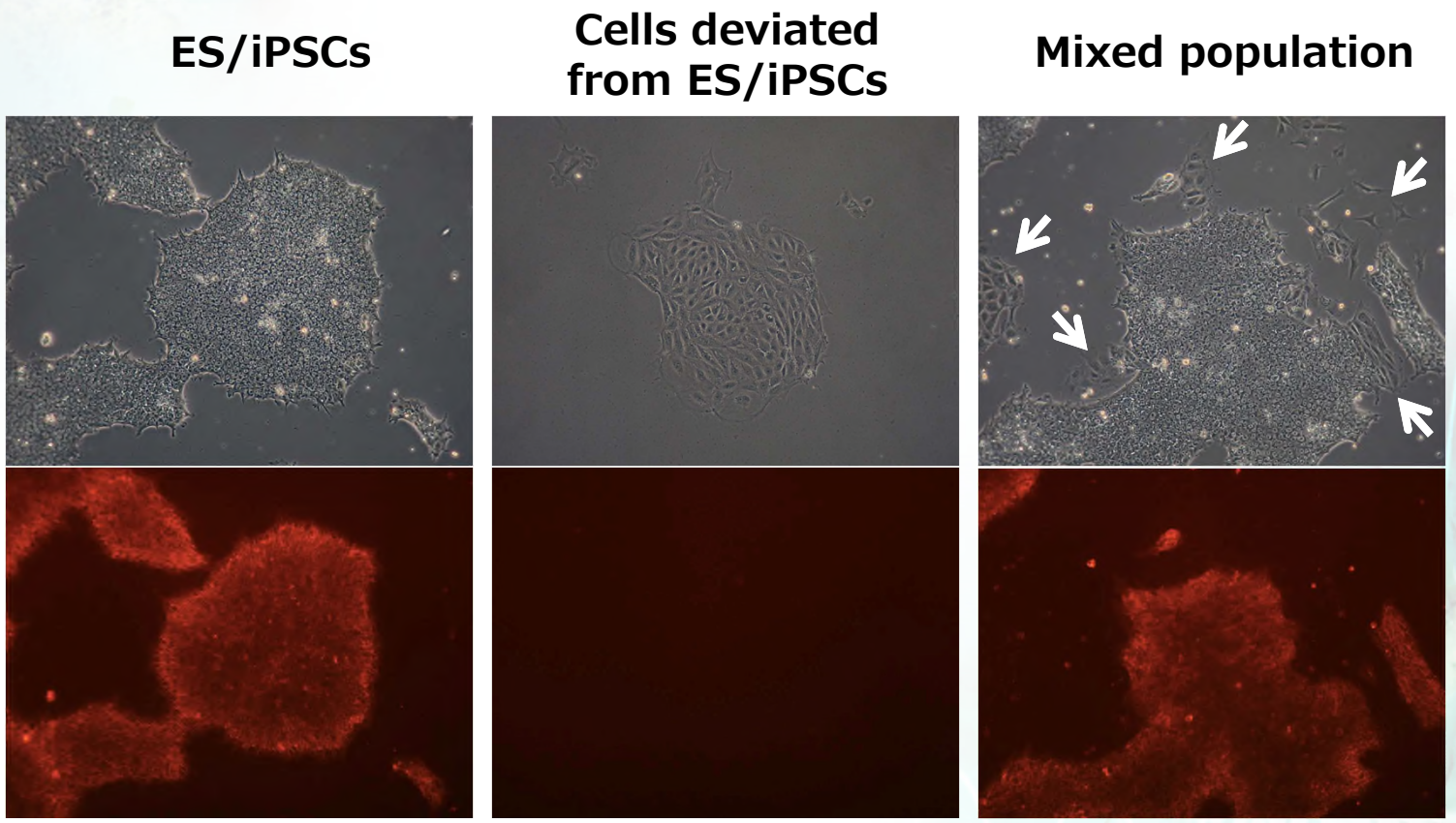
National Institute of Advanced Industrial Science and Technology
AIST

# Live staining of ES/iPSCs by rBC2LCN



## Add fluorescence-labeled rBC2LCN lectin into cell culture media






Onuma, Tateno et al. BBRC (2013)

**Point: No toxicity to cells**

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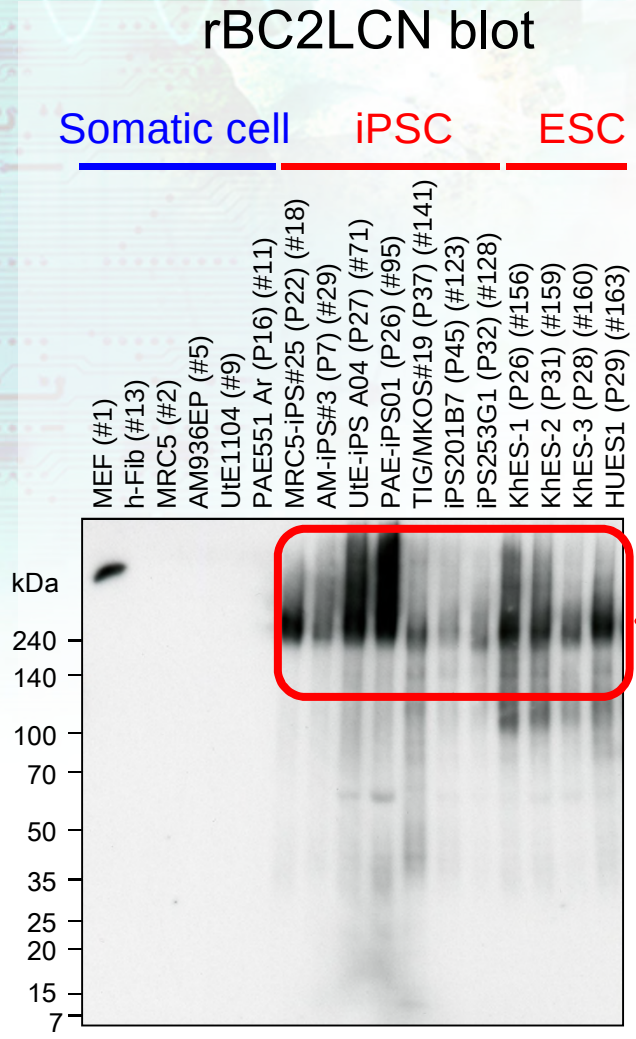
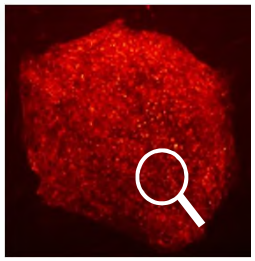
# H type3-podocalyxin is a ligand of rBC2LCN



Enlarge




Tateno et al. Stem Cells Transl Med 2013

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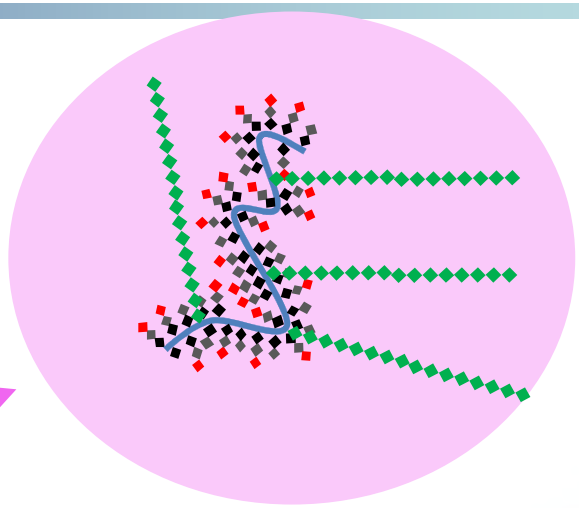
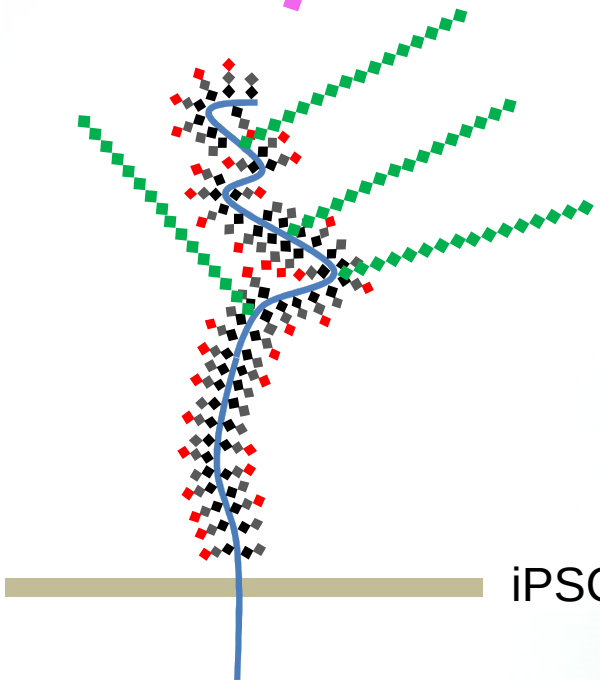
# H type3-podocalyxin is secreted into the cell culture medium from ES/iPSCs

Secrete into the cell culture medium



H type3-positive podocalyxin



iPSC/ESC cell surface

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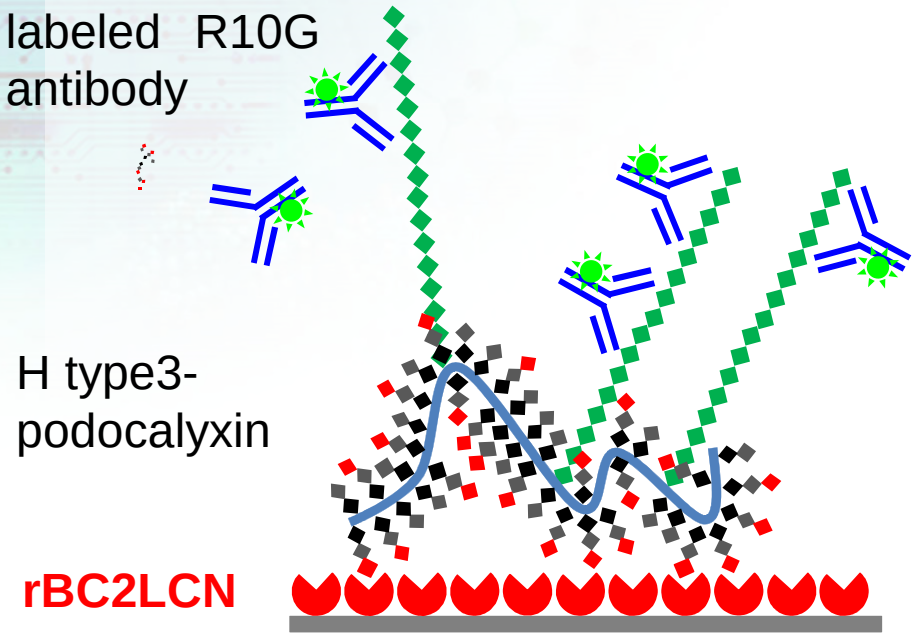
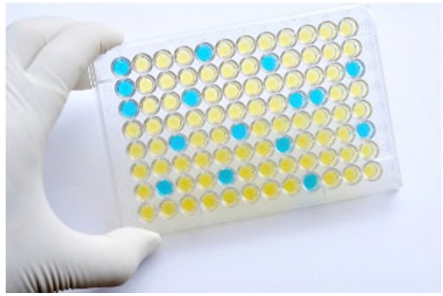
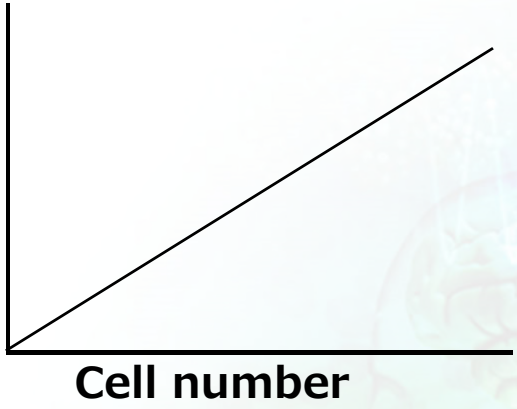
# Development of non-destructive method to detect undifferentiated cells including cancer using cell culture media

## Sandwich assay to detect H type3-positive podocalyxin




## Measurement of the number of iPSCs/ESCs






Tateno et al. Sci Rep 2014
Shimomura O.et al. Mol Cancer Ther.,2018

# Acknowlegements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**Thank you very much for your kind attention**