Global Focus on Knowledge Lecture Series
2006 Winter Semester: “Science of Life”

Life Science: from the Perspective of Developmental Biology

The 1st Lecture Oct.16 (Mon)

From an egg to an adult body-The Morphogenetic Mechanism

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Developmental Biology

How are diverse cell groups formed from a single cell?
What helps the system of multicellular organisms to be formed stably?
Diverse life forms live on the Earth. It is important to know the morphology or natural history of those organisms.

The “evolutionary tree” inserted here was omitted according to copyright issue.
There are as many types of development as the number of organism species. (more than 10 million species)

Selection of model organisms

Example

Typical species:
- Round worms: Nematode
- Insects: Drosophila
- Fish: Zebrafish, Killifish
- Amphibians: Xenopus, Newt
- Mammals: Mouse
- Plants: Thaliana, etc.

- Research on development mechanisms of each organism
- Comparison of each organism’s development (comparative embryology)
“All the animals are born from an egg.”
(Ex ovo omnia)

William Harvey
A.D.1578-1657

Harvey indicated the importance of the egg in animal development.
An ovum is a single heterogeneous cell. Substances are precisely positioned due to the polarity and gradient.

“The picture of mating xenopuses” is omitted according to copyright issue.

“Substances in ovum are unevenly distributed.”

“The picture of unequal distribution in ovum” is omitted according to copyright issue.

Pairing of *Xenopus laevis*

Ovary of xenopus
Polarity and gradient are made during oogenesis while ovums endocytose or produce substances. Polarity and gradient are important notions in morphology.

Along with maturation of oocyte, yolk proteins (vitellogenin etc.) supplied from blood vessel and various maternal factors newly produced are accumulated with polarity.
Oogenesis of Vertebrates

- primordial germ cell
- stem cell
- mitosis
- first ooblast
- meiosis (4 times)
- first oocyte
- lampbrush chromosome:
  - growth of oocyte
  - homologous recombination of chromosome
- follicle cell intakes oocyte
- accumulation of yolk in oocyte
- germinal vesicle
- pigment granule
- yolk (scutellum)
- follicle cell
- matured oocyte

Egg formation of Xenopus
Each ovum has polarity and gradient before fertilization.
As an embryo develops, polarity causes a change in gradient. This causes gene expression and cell differentiation.
Elucidation of development mechanism ①

**Directionality**

① What makes parts of the body anterior, posterior, right, left, ventral, or dorsal?

② Observe how various organs and tissues develop.

↓

Observe how typical parts of the body differentiate in a living creature.

From whole to parts (formation of each organ and tissue)
From parts to whole (head region • tail region • individual)

⇒ Integrity of individual body
Elucidation of development mechanism

Experiment Method

① Observation of normal development (morphology change • gene expression)

② Isolation & identification of various factors (protein etc.) and genes.

③ Artificially treat the embryo, and discover the mechanism via changes in the embryo
  • embryo manipulation by microscopic operation
  • mechanism analysis by gene transfer or inhibition, etc.

Frogs and newts were used in classical embryology experiments since they were easy to handle.
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)
"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)

Hans Spemann (1869-1941)

Hilde Mangold (1898-1924)
色素の多いスジイモリの囊胚（側面）

クシイモリの原口上脇部をスジイモリの腹方常域移植

宿主の背側の第一次神経板

宿主の腹側に移植片の誘導作用でできた第二次神経板

右の胚の中央部で横断した切片

移植手術後約一週間の胚（誘導で第二次胚を形成）
The Experiment of Spemann and Mangold
— transplant of an upper blastopore’s lip (1924)
1924-impact of Spemann & Mangold’s discovery of organizer

- Proved “embryo development by cellular interaction”
- Indicated efficiency of positivistic method (embryo manipulation)
- **Demonstrated the central part of the embryo that controls body formation**

The inevitable question here

“On what does this interaction depend?”

Focuses on proteins and nucleic acids along with development of physical chemistry and biochemistry
Main stream is to research life process by search of substance.
Hypotheses on concentration gradient of inducing factors

Double Potential Hypothesis

Double Gradient Hypothesis

1977 Misuzu Shobo
“Illustrations of embryogenesis” inserted here was omitted according to copyright issue.
Common System

- CNS
- Notochord

Common Organ

- Brain
- Heart
- Liver
- Gonad
Omnipotent stem cell of vertebrate embryo

Xenopus laevis
newt
(amphibian)

chicken
(avian)

mouse
human
(mammal)
Morphology change from egg to tadpole

Embryo induction

Cell differentiation

Morphogenesis

etc.
Primary development and embryo induction of amphibian

- **fertilization**
- **cleavage**
- **formation of archenteron**
- **Formation of nerve**

**mesoderm induction**
- ventral mesoderm
- dorsal mesoderm
- endoderm

**nerve induction**
- archenteron
- ectoderm
- blastula cavity
- blastopore
Development of Xenopus embryo

cleavage (view from vegetal pole) to neurula period
left: *Xenopus tropicalis*, right: *Xenopus laevis*
Animal Cap Assay

Animal cap assay

- Animal pole
- Saline
- Inducing factor

ectoderm ← animal hemisphere
mesoderm ← spectrum
endoderm ← vegetal hemisphere

vegetal pole

blastopore (vertical section)

animal cap assay
Structure of Activin A

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

Homodimer

Amino acids: 232 (116 x 2)

$SH = 18$
Concentration dependent mesoderm differentiation of activin-treated animal cap

Formation of each tissue:
- Unorganized epidermis
- Ventral mesoderm tissue (blood cell, epidermis)
- Muscle
- Notochord
- Outer vision
- Tissue section

Concentration of activin for animal cap treatment (ng/ml) vs. formation of each tissue.
Extending movement and muscle differentiation of activin-treated animal cap
Can induce various tissues depending on activin concentration.

- Notochord-related genes
- Blood cell-related genes
- Nucleus
- Blood cell

Activin

Cell membrane

High signal

Low signal

Animal cap

Cultures in high or low concentration

Activin solution
Organs and tissues formed from activin-treated animal cap

activin solution

[liver]

[heart]

[pharynx]

[pronephros]

[+ retinoic acid $10^{-4}M$]

[+ notochord]

[muscle]

[blood cell coelomic epidermis]

[0.5 ng/ml]

[5 ng/ml]

[50 ng/ml]

[100 ng/ml]
① What makes parts of the body anterior, posterior, right, left, ventral, or dorsal?

ex1: Position of sperm penetration determines ventral-dorsal axis
ex2: Formations of head-tail axis, ventral-dorsal axis, left-right axis
Cleavage of *Xenopus laevis* fertilized egg

View from animal pole (1 cell period ~ 128 cell period)
Gastrulation of Xenopus Embryo

Horder TJ. Int J Dev Biol, vol 45, p105-Fig.3, 2001
Experiment to make head part and tail part of larva in vitro

1. Activin treatment: 100 ng/ml for 1 h
2. Sandwich culture
3. Short time (0–6 h)
4. Long time (12–24 h)
5. Result: Trunk and tail
6. Head
Head structure and trunk-and-tail structure induced artificially from undifferentiated cell of *xenopus*
Organ section

Normal embryo

head

trunk

tail

Sandwich transplant

head

trunk

tail
Reproduction of morphogenesis by activin gradient

Xenopus blastula

Animal cap

Concentration of activin A

Free

Low

Middle

High

Undifferentiated cell mass

Smooth and oval

Elongated

Fragile and ragged

Combination of activin-treated ectoderm

Head form

Trunk-tail form
Reproduction of morphogenesis by activin gradient

- outer image of tadpole-like structure induced artificially
Reproduction of morphogenesis by activin gradient

Tissue section of tadpole-like structure artificially induced

Cell mass untreated by activin (comparison)

Most organs and tissues are formed (notochord, muscle, eye, brain, enteron, etc.)
Control of fundamental body plan by activin A, Con A and retinoic acid

- **Con A (1 mg/ml)**
  - retinoic acid
  - brain
  - spinal cord
  - muscle
  - eye
  - ear
  - notochord
  - cement gland

- **activin A (0.5 ng/ml)**
  - Con A
  - retinoic acid
  - 300 μg/ml
  - notochord
  - ventro-lateral mesoderm

- **activin A (10 ng/ml)**
  - muscle
  - muscle
  - pronephric tube

- **activin A (100 ng/ml)**
  - pharynx
  - pancreas
  - 10^{-4} M
② Focus on development of various tissues and organs

-an example of organ formation research using kidneys
Kidney development

**pronephros** (1 nephron) tadpole

**mesonephros** (30 nephron) adult frog

**metanephros** (about 1 million nephrons) Human, etc.
Formation of pronephros from an animal cap in vitro

A. late blastula
B. culture 4 days
C. activin A: 10 ng/ml
D. retinoic acid: $10^{-4}$ M

process steps:
1. animal cap
2. culture 3 hours

image description:
- Panel A: early stage of development
- Panel B: cells after 3 hours culture
- Panel C: cells after 4 days culture
- Panel D: mature pronephros formation
Formation of pronephric structure in vitro (A+B) and in vivo (C+D)
The Result of DNA Microarray Analysis of Pronephros
(In Vitro-Induced Pronephros of Xenopus laevis)

\[ \log_{10} \left( \frac{\text{Cy5}}{\text{Cy3}} \right) \]

- \text{Cy5} = \text{activin} 10\text{ng/ml}
- \text{Cy3} = \text{activin} + \text{RA}

Decrease

Increase
Kidney development and gene expression in amphibians (frog)
Kidney development and gene expression in mammals (human, mouse)
Gene groups expressed during kidney development

Stages of development

9 15 20 23 28 31 33 35 37

Marker gene gastrula neurula larva

kidney tubule iter

Xpax-8
Xwnt-4
Delta-1
Notch-1
XTRAP-γ
MLK-2
XSMP-30
3G8 (antibody)

kidney tubule

Xlim-1
Id2
Xpax-2
XC3H-3b
NDRG1
Na⁺-K⁺ ATPase α Subunit
Xsal-3
Dullard
xCIC-K
Id4
Xlcaax-1
Xfz8
Gremlin
3A6 (antibody)

iter

4A6 (antibody)

glomerus

XWT1
Kidney of a mouse embryo

Nishinakamura R. et al., Development, vol 128, p3110-Fig.4, 2001
Knockout of the SALL gene (gene responsible for kidney development in a frog) would cause a kidney defect in a mouse.

Cases of human babies without kidneys were reported. (a.k.a. Townes-Brocks syndrome over a long time)
Cause of the syndrome was discovered to be a defect in the SALL gene.

Function of the SALL gene in kidney development was discovered in frogs. SALL was found to be responsible for kidney development of all vertebrates.
Organs and tissues developed from undifferentiated xenopus cell (animal cap) in vitro (at Asashima Lab.)
Abbreviations:
- ARIP1
- ARIP2
- SARA
- Smad2
- Smad4
- FAST-1
- XSIP1
- Mix 2
- Mig30
- Xbra

Signaling Pathway:
1. Activin binds to Type II receptors.
2. Type II receptors recruit Type I receptors.
3. Activin-receptor complex phosphorylates Smad2.
4. Smad2 binds to Smad4.
5. Smad2-Smad4 complex is translocated to the nucleus.
6. Smad2-Smad4 complex activates transcription of target genes.
7. Antivin and follistatin inhibit Activin signaling.
8. FAST-1 and XSIP1 regulate Activin signaling.

Locations:
- Cell membrane
- Nucleus
Morphogenesis and cavity

- the issue of post-genomes
Cavity formation is important in animal development. Cells move toward the cavity to produce a new cavity. Simple form develops into a complex form.
Blastula of a sea urchin

Vegetal pole cells emboly toward the blastocoele.

Gastrulation in Xenopus Embryo

Horder TJ. Int J Dev Biol, vol 45, p105-Fig.3, 2001
Formation of neural tube

More complex cavity formation and neural formation

neural fold

neural groove

ectoderm

epidermis (cephalic ectoderm)

diencephala

optic vesicle

retina (optic cap)

diencephala

lenses (being induced)
More complex cavity formation and neural formation

Development of the central nervous system in a mammal

Front part of the neural tube differentiates into several regions to form different parts of the brain structure.
“The illustration of cavity and neural formation”
inserted here was omitted according to copyright issue.

“The illustration of cavity and eye formation”
inserted here was omitted according to copyright issue.
Broadening Developmental Biology

- Astrobiology
  - development in space
  - Krinostat experiment
- Molecular imaging
  - single myocardium cell
  - cell memory
- Artificial cell and single cell
- Molecular biology
  - Microfabrication (new technology)
- Brain/neuroscience
  - memory and learning
  - hippocampus & activin
- Mathematical model
- Regeneration science
  - regeneration medicine
  - organ formation from an undifferentiated cell in vitro (22 organs)
  - heart
- Developmental biology
  - understanding of life
  - molecular mechanism of normal development
  - embryology
  - relationship with chemistry
  - relationship with mathematics
  - relationship with complex systems
  - modeling process of development
- Animal development

Note: The diagram indicates the broadening of developmental biology by connecting various fields and technologies.