学術講義

生きた事が生まれる

生命の科学

発生生物学からみた
生命科学

浅島 誠

分子モーターから見た
生命科学

廣川 信隆

ウイルスからみた
生命科学

野木 明男

ゲノムから見た
生命科学

黒岩 常祥

10月16日⇒1月29日

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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

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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.

- 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

Understanding phenomena → Analysis of mechanism

What cause these?
An overview of vertebrate embryo development
(example: in amphibians)
Flow of direction in determination, and organ formation in an embryo

- polarity of an unfertilized ovum
- determination of an animal/vegetal pole
- determination of ventral-dorsal axis (sperm penetration)
- determination of the anterior-posterior axis (right-left axis)
- gastrolulation
- differentiation of notochord
- formation of archenteron
- differentiation of nerve tissue (neural induction)
- both sides of the notochord (neural induction)
- formation of a neural tube
- formation of segments
- determination of location

- induction between ectoderm and endoderm
- differentiation of mesoderm (mesoderm induction)
- determination of location
- differentiation of each organ by induction between ectoderm and endoderm
Sperm penetrates the ovum by fertilization. Cortical layer rotates. Opposite side of sperm penetration spot becomes the dorsal side.

Determination of ventral-dorsal axis

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

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

Determination of central axis
**archigastrula~neurula**

Cross-section of embryo

Neural plate rises up and forms neural tube.

Notochord induces nerve to ectoderm.

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

- **archenteron (endoderm)**
- **ectoderm**
- **mesoderm**

**Differentiation**

- **nerve tissue (neural)**
- **mid.mesoderm**
- **body wall mesoderm**
- **visceral plate mesoderm**
- **enteronic tract**
- **enteric mesenchyme**
- **cavity**
- **spinal chord nerve**
- **muscle, cartilage, corium, etc.**
- **Kidney, etc.**
Example of research on organ formation

① Structure of the whole individual and interaction in organ formation

② Interaction between tissues during organ development

③ Function of various genes in organ formation

- Mechanisms of organ formation can be discovered by considering phenomena 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
As a neural tube develops, constriction occurs, and the brain is segmented. Segments are formed as a result of complications in the “cavities”.

Segment structure of the brain

- **forebrain**
- **midbrain**
- **hindbrain**
- **eye vesicle**
- **endbrain**
- **interbrain**
- **medula**

(case of chicken embryo)
Somites

- 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.

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
When somites are transplanted into different parts, extra ganglions differentiate from the neighboring neural tube.
Slight disarray in which location information is functionally covered by a flexible change in neuron projection.

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

“The illustration of neuron projection” inserted here was omitted according to copyright issue.
Segments of insects and mammals are regulated by the Hox gene family

“The illustration of Hox gene family regulation” inserted here was omitted according to copyright issue.
Hox gene groups that determine segment structures are common to many animals.

Hox gene paralogs:
- Coelenterata (hydra)
- Nematomorph (C. Elegans)
- Annelidous
- Arthropod (Drosophila)
- Echinoderm (sea urchin)
- Protochordate (amphioxus)
- Vertebrate (mouse)

Hox gene paralogs:
- HOXa
- HOXb
- HOXc
- HOXd

Gene groups:
- cnox1
- cnox2
- AntpC
- lab
- pb
- Dfd
- Scr
- Antp
- Ubx
- Abd-A
- Abd-B

Hox gene groups that determine segment structures are common to many animals.
Disarray in segment formation changes the positions of the adnex.

These mutations are called "homeotic mutations."
The effect of retinoic acid treatment on embryo development

Normal mouse embryo

“The picture of
Retinoic acid treated mouse embryo”
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Front structure (jawbone) is lost in retionic acid treatment
Retinoic acid changes location information.
The effect of retinoic acid treatment on embryo development②

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

Retinoic acid changes location information.

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

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

formation of fore limb

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

Limb induced between fore limb and hind limb by FGF

Front part differentiated into wings
Hind part differentiated into limbs

The pictures inserted here was omitted according to copyright issue.
Limbs developed by interactions between AER and mesenchyme

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

AER: apical ectodermal ridge

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
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

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.

Hoxa-11, Hoxd-11 Inhibited mouse
Forms 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:

\[
\begin{align*}
\text{induction between } & \text{ectoderm} \cdot \text{endoderm} \rightarrow \text{induce mesoderm} \\
\text{induction between } & \text{ectoderm (epithelia)} \cdot \text{mesoderm (notochord)} \rightarrow \text{induce nervous system}
\end{align*}
\]

“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.
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

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 formation

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

- 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.

(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
Thee molecular mechanism of somite formation

• 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.

Bessho Y. et al., Curr Opin Genet Dev., vol 13, p380-Fig.1, 2003
The molecular mechanism of somite formation

Wavefront
Gene groups that make the gradient at back and front of embryo
FGF signal
RA signal

- 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.

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

- 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

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.
• 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*

- 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 formation ②

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)
Differentiation of enteron in a chicken embryo

Cross section

- epithelia
- cavity
- mesenchyme

6th day

12th day

- esophagus
- stomach
- ventriculus
- small intestine
- colon
- future stomach endoderm
- future intestine endoderm

1.5th day

3rd day

- gland formation
- pepsinogen (ECPg)
- (cSP, Sox2)
- keratinization
- mucus
- villus formation
- sucrase
- IFABP, CdxA

Epithelial development of each region and substances produced from epithelia become flat and multi-layered.

Future stomach endoderm and future intestine endoderm will develop into flat and multi-layered epithelia.

Substances produced from epithelia include pepsinogen, keratinization, mucus, villus formation, sucrase, IFABP, and CdxA.

Differences between regions:
- Gland formation
- Esophagus
- Stomach
- Ventriculus
- Small intestine
- Colon

- Endoderm development in each region
- Flat and multi-layered epithelia

Diagram showing the differentiation process from 1.5th day to 12th day.
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
Anterior stomach epithelia consists of an even cell mass of undifferentiated epithelial cells.

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

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

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)

Notch-Delta signaling

Future glandular epithelium

BMP2, FGF10, ECM

mesenchyme

Future lumen epithelium

Shh
Genes related to differentiation of the stomach epithelium (2)

Glandular epithelium

GATA5

ECPg

cSOX2

BMP2, FGF10

EGF

cSP

Shh

Lumen epithelium

mesenchyme

ECM
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 anterior stomach region
- cSox2

Differentiation from undifferentiated epithelium to glandular epithelium and lumen epithelium
- Notch-Delta signal, Sonic hedgehog

Morphogenesis of glands and cell differentiation
- BMP2, FGF10, EGF

Functional differentiation of glandular epithelium (expression of ECPγ gene)
- GATA transcriptional factors, cSox2, Smad transcriptional factors
The molecular mechanism of organ formation

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.

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

#### Lateral tube mesoderm

- **Signaling molecules**
  - BMPs
  - FGF8
  - Cerberus
  - Nodal

#### Formation of heart fields

- **Myocardial transcription factors (commitment)**
  - GATA4 (and family)
  - MEF2 family
  - Nkx2-5 (and family)
- **Differentiation products**
  - Cardiac muscle-specific proteins
  - ANF

#### Heart development

- **Morphogenetic regulators**
  - N-cadherin
  - Hand2
  - Xin
  - Hand1
  - Pitx2

#### Morphogenesis of heart

- **Neural crest cells**
- **Septation effector genes**
- **Looping effector genes (including Xin, flectin)**
- **Multichambered heart**

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#### Diagram:

- **Lateral plate mesoderm**
- **Cardiogenic mesoderm**
- **Outflow tract**
- **Atrium**
- **Truncus arteriosus**
- **Future atrium**
- **Future right ventricle**
- **Future left ventricle**
- **Sinus venosus**
- **Vitelline veins**
- **Myocardium**
- **Endocardium**
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

MA35

Nkx2.5

st. 23

st. 26

st. 34
Control MO injected (function inhibited embryo)

Inhibiting experiment with MA35

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

→ MA35 is essential for heart development.
Inhibiting experiment with MA35

Heart marker gene

Nkx2.5  Nkx2.5  cTnl

Control (normal embryo)

MO inj. (function inhibited embryo)

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

$XTbx5$ 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

- $Tbx5g18$
- $Tbx5g16$
- $Tbx5g17$
- $Tbx5g12$
- $Tbx5g10$
- $Tbx5g13$
The structure of the upper sequence of the $XTbx5$ genome

Isolated genome segment

(about 12 K bp)

S1

L1

ATG

2

3

281 606

2822 3387

4530 4698

5331 5428

(bp)

mRNA

Translation region

S1 probe

L1 probe

Translating region

e : future retinoid region

h : future atrium • ventricle region

sv : future venous sinus region

St. 33

St. 33

St. 35
The interaction between heart and vessel development is regulated by control between genes.
The molecular mechanism of organ formation

The molecular mechanism of other organ formation
The molecular mechanism of muscle differentiation

(A) Mytome cells
Paracrine factors

(B) Dividing myoblasts
Multiplication
FGFs

(C) Cell alignment
Multiplication stops
Fibronectin, integrin, cadherin/CAM, myogenin

(D) Myotube formation
Fusion, differentiation
Meltrin; muscle-specific proteins

(E) Muscle fiber
Maturation
Contractions begin

Sarcomere differentiation
Wnt, Shh, MyoD, Myf5

Division of myoblasts
FGFs

Cell alignment
Cell fusion (multinucleation)

Myogenin
Meltrin
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)

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.)

“The photo of a chicken embryo with abnormal bone” inserted here was omitted according to copyright issue.
Development mechanism of blood & lymph vessels

**Hemangioblast**

ES cell

**Blood island**

- VEGF
- VEGFR-2
- Neuropirin-1,2

**Angioblast**

- PDGF
- Angiopoietin/Tie

**Blood cells**

**Early fetal vascular network**

- VEGF
- VEGFR-1, -2

**Neogenesis of blood and lymph vessel**

- VEGFR-3
- Angio/Tie

- EphB2, EphB4, c-myc, plcγ (PLCγ), Notch?

**Lymph vessel**

- Prox1, VEGFR-3

**Venous system**

- VEGF
- VEGFR-1, -2

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

Mesoderm cells differentiate into blood vessels using VEGF stimuli. 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
Appropriate regulation of apoptosis is important for healthy organ formation. Caspase-9, the gene that causes apoptosis, is involved in this process. 

As the result of apoptosis, the brain structure is abnormal in functionally inhibited embryos. 

“The photo of a functionally inhibited embryo” inserted here was omitted according to copyright issue.
similarities of structure and ventral-dorsal reversal in vertebrates and insects

Organs with similar functions differentiate from same structure of 3 germ layers. However, dorsal side of vertebrates correspond to ventral side of insects.
There are many stages of “induction” in organ development.