Mosaic versus regulative development

Mosaic development depends upon localized cytoplasmic factors while regulative development depends upon cell-cell (group) interactions.

In some invertebrates, cell fate is often specified at the single cell level, not in groups of cells, and does not rely upon positional information.

This process results from asymmetric cell division to distribute the cytoplasm unequally and allow determination of different cell fates.

Most organisms, both mosaic and regulative development.

Cell lineage of *Caenorhabditis elegans* (nematodes)

The complete cell lineage of *Caenorhabditis elegans* is invariant.

The larva of 558 cells grows to an adult of 959 cells (plus germ cells). 113 undergo programmed cell death.

No polarity exists in the unfertilized egg, but sperm entry controls the first cleavage (very different).

A cap of actin microfilaments form at the anterior end and P granules become localized to the posterior. Microfilaments participate the location of P granules.

After fertilization, egg enter unequal cleavage. Fig 6.3

The AB cell becomes the anterior and P cell (contain P granules) is posterior. P granules are ribonucleoprotein that specify the germ cells, it regulate translation.

P granule movement is the result (not a cause) of posterior determination, by cytoskeleton. Fig 6.3

P granule move depend on sperm entry.


Fig 6.1 Phylogentic tree showing relationship between the organisms considered in this book.
Cell lineage of Caenorhabditis elegans

Axes depend on asymmetric division & cell interactions. Before fertilization, there is no evidence of any asymmetry in the nematode egg.

The first division gives a large anterior AB cell and a small P1 cell. (P1, a stem cell, produces a P* cell and another.) The first cleavage, defines the future A/P axis, with the large AB cell marking the anterior and smaller P1 cell the posterior.

During first three divisions, P cells give rise to a number of lineages but the P4 cell then gives rise to only germ cells.

The AB cell divides into anterior ABa (neurons, epidermis plus pharynx mesoderm) and the posterior ABp (neurons, epidermis and specialized cells).

P1 divides into P2 and EMS. EMS divides to make MS (mesodermal pharynx) and E (gut).

P2 becomes P3 and C (epidermis & muscle).

P3 becomes P4 and D (muscle).

All the cells undergo further invariant divisions, and by about 100 minutes after fertilization, gastrulation begins.

Fig 6.3 Localization of P granules after fertilization

Sperm entry region is posterior

Egg nucleus is anterior

Fusion sperm and egg nucleus

P granules move to posterior

P granules localized in posterior. Small cell contain P granules.

Two cell stage

All the P granules are in the P4 cell, it only germline only

Stain DNA

2010/10/1
**Maternal gene in nematodes: A/P axis**

Maternal gene par-1 encode PAR-1 protein, which localized in the future posterior region after fertilization. PAR-1 and P granules are contribute to the development of A/P axis (polarity). The distribution of PAR-1 and P granules are regulated by microtubule-organizing center at the posterior of the fertilized egg. The center arise from the aster of microtubules contributed by sperm nucleus. Asymmetry cleavage (first) → PAR-1 unequal distribution

Egg (PAR-1 and P granules) → After fertilization → microtubules move → posterior → egg division → asymmetric distribution

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**C. elegans: cell-cell interactions (not groups) for D/V axis develop**

From second cleavage, Cell-cell interactions specify cell fate in the early nematode embryo. Cell fate is invariant but experiments reveal that cell-cell interactions are crucial. Fig 6.4

D/V change → R/L change
ABp must contact the P2 cell (or it becomes an ABa cell).

D/V determined in cleavage 2
R/L determined in cleavage 3 (Fig 6.5)
ABp and ABa contain the same substance, it may form the equal division. Different contact, may induced different differentiation.

EMS → ventral part development (gut)
AB → Bilateral development

---

**Asymmetric division and cell-cell interaction specify cell fate in the early nematode embryo**

AB and P1 cell are unequal division, but ABa and ABp are equal division. (positional signal different → different fate)

ABa and ABp must be by interactions with adjacent cell (P1 cell). For removing P1, a normal product of ABa, but no ABp cell. P2 cell responsible for specifying ABp cell.

Maternal gene glp-1 and apx-1 involve of the P2 induced ABp cell. glp-1 encodes a transmembrane receptor. Its mRNA is uniformly distributed but translation is repressed in the P cell and is restricted to the AB cell. At two cell, glp-1 protein only expression in AB cell. After 2nd cleavage, P2 expresses apx-1 protein on its surface which activates Glp-1 receptor. This causes ABa and ABp descendants to respond to signals from the P2 cell differently. Fig 6.6

Other maternal gene skin in EMC cell (higher than AB cell). Mutant skin gene, EMC only produce muscle.
Induction → posterior development

Fig 6.6 An early inductive specifics ABp

ABa and ABp are symmetric division form AB cell. But ABp received signal from P2 cell.

1. Maternal gene glp-1 and apx-1 involve of the P2 induced ABp cell.
2. glp-1 encodes a transmembrane receptor. Its mRNA is uniformly distributed but translation is repressed in the P cell and is restricted to the AB cell.
3. At two cell, glp-1 protein only expression in AB cell. After 3' cleavage produced ABA and ABp also has expressed.
4. After 2nd cleavage, P2 expresses apx-1 protein on its surface which activates Glp-1 receptor. This causes ABA and ABp descendants to respond to signals from the P2 cell differently.

5. Other maternal gene skin in EMC cell (higher than AB cell). Mutant skin gene, EMC only produce muscle.

Gut development
Depend on induction
Only 4-cell stage EMS did not formed the gut.
Remove P2 cell, did not formed the gut. But P2 + EMS can produced gut
Cell-cell interaction play an important role of the development of gut.

Cell differentiation (Fig 6.7 Cell fate is linked to the pattern of cell division)
It is closely linked to cell division
MS cell: from the sequence p-a-a-p-p → apoptosis.
For asymmetry cell division. Unequal cleavage produced different concentration of some protein → different effect.
Each division produces an anterior (a) and a posterior (p) cell.

At 80-cell (gastrula stage), a fate map can be made for the nematode embryo (Fig.6.8)

At this stage, cell from different lineages that contribute to the same tissue or organ

a: anterior
l: left
r: right
p: posterior
Experiments Show that Cell-Cell Interactions Are Required for the EMS Cell to Form Intestinal Lineage Determinants

(A) Isolated shortly after its formation, the EMS blastomere can produce gut-specific granules. If left in place for longer periods, it can not

(B) If the EMS cell is recombined with either or both derivatives of the AB blastomere, it will not form gut-specific granules.

(C) If recombined with the P2 blastomere, the EMS cell give rise to gut specific structures

Gene control graded temporal information in nematode development

Adult nematode: 550 cells, and 4 larval stages.
Critical development: cell lineage and position, gene control at right time and right place.
Temporal information is important in the nematode.
Heterochronic mutants alter the timing of developmental events. Fig 6.10
(Mutation that alter the timing of developmental events are called heterochronic mutant)

Nematodes: Homeobox genes

A small cluster of homeobox genes specify cell fate along the A/P axis.
Four homeobox genes, similar to the HOM/HOX genes, plus an additional less related homeobox gene, make up the C. elegans Hox cluster. Fig 6.9
They are expressed in different positions along the A/P axis.
Expressed during embryogenesis, their primary role is in larval development.

Less effect

Adult nematode: 550 cells, and 4 larval stages.
Critical development: cell lineage and position, gene control at right time and right place.
Temporal information is important in the nematode.
Heterochronic mutants alter the timing of developmental events. Fig 6.10
(Mutation that alter the timing of developmental events are called heterochronic mutant)

In-14 (from lateral hypodermal T-cell lineage) mutants affect the T.ap lineage(epidermis, neuron

Lin-14 mutant → disturbance in the timing of cell division → change in the patterns of cell lineage

Gain-of-function (gf) mutations result in retarded development (happens later than normal).
Loss-of-function (lf) mutations result in precocious development (happens earlier than normal).
Normal, T cell generates (T.ap; T blast cell) → epidermis, neurons and support cells in first (L1) and second (L2) larval stages; Later larval stages (L3 and L4), some of T cell descendants divide to other structure.

The concentration of lin-14 protein is post-transcriptionally regulated in an interesting and unusual way. The translation of lin-14 mRNA can be repressed by lin-4 RNA, which complexes with lin-14 mRNA. Lin-4 mRNA ↑ (during the later larval stages) → temporal gradient in lin-14 protein; so mutation of lin-4 → gain of function mutations in lin-14

Gene control graded temporal information in nematode development

Gene control timing of development: by control the concentration of some substance, causing it to decrease with time Fig. 6.11

Temporal gradient control development Lin-14 mRNA → translate to protein, when protein to high, inhibited by lin-4 mRNA (formation of complex)

gf: gain-of-function mutation (high level lin-14) go on induction
If: loss-of-function mutation (low level lin-4) loss inhibition
Cell lineage of Ascidians (tunicates)

Ascidians, also known as tunicates, are marine animals that belong to the phylum Urochordata. Ascidian larvae appear to be similar to vertebrate neurulas (notochord, neural tube, and muscles).

Notochord cell

The egg and early embryo are regulative (part) and mosaic, but after a few cleavages, the cells are mostly determined. Fig 6.20

Ascidians embryogenesis is cleavage pattern (mosaic development), cytoplasmic factors specifying cell fate, cell interaction are plays relative part.

Muscle may be specified by localized cytoplasmic factors

Before fertilization, the yellow granules are uniformly distributed through the egg. The cells that acquire yellow cytoplasm—the myoplasm—during cleavage are those that will give rise to the muscle cells of the larval tial.

Egg → fertilization → yellow pigment granules (YPG) → rearrangement → myoplasm → move to vegetal and lateral by microtubule move (two stage) → form of crescent → posterior end, gastrulation start. The crescent marks the future posterior end. During cleavage, the myoplasm becomes confined to particular cells for posterior end.

Cytoplasmic Rearrangement in the Fertilized Egg of Styela partita

Yellow pigment granules (YPG)

(A) Before fertilized, the yellow cortical cytoplasm surrounds the gray yolk inner cytoplasm

(B) After sperm entry (in vegetal oocyte), the yellow cortical cytoplasm and the clear cytoplasm derived from the breakdown of the oocyte nucleus contract vegetally toward the sperm

(C) As the sperm pronucleus migrates animaly toward the newly formed egg pronucleus, the yellow and clear cytoplasms move with it

(D) The final positions of the yellow cytoplasm marks the location where cells give rise to tail muscles.
Bilateral Symmetry in the Egg of the Tunicate Styela partita

(A) Uncleavaged egg. The regions of cytoplasm destined to form particular organs are labeled here and coded by color throughout the diagrams. (mosaic development)

(B) 8-cell embryo, showing the blastomeres and the fates of various cells. The embryo can be viewed as two 4-cell halves; each division on the right side of the embryo has a mirror image division on the left (C,D) views of later embryos from the vegetal pole.

The dashed line show the plane of bilateral symmetry.

Two B4.1 cell contain myoplasm → primary muscle, endoderm
The blastomeres adjacent to B4.1, developed secondary muscle cells.
The myoplasm (yellow pigmented in Styela embryos) gives rise to the muscles of the larval tail by apparent mosaic development. It may be regulated or induced by autonomous specification by a morphogenetic factor. Morphogenetic factor → affect cell → cell cleavage → affect differentiate
This experiments provided the involvement of cytoplasmic determinants in muscle differentiation.

Good correlation between muscle development and yellow cytoplasm, these observations alone do not establish that something in the yellow cytoplasm causes differentiation of the cells into muscles. Only one factor did not induced muscle, must be combination of many signal. It is regulative development.

Autonomous specification by a morphogenetic factor. The macho-1 mRNA message is localized to the muscle-forming tunicate cytoplasm. In situ hybridization shows the macho-1 message found first in the vegetal pole cytoplasm, then migrating up the presumptive posterior surface of the egg and becoming localized in the B 4.1 blastomere

Cell-autonomous: If only the mutant cell exhibit the mutant phenotype and are not rescued by the normal cells, the gene is acting cell-autonomously; a gene product not influencing other cells. (only one mutant → affect phenotype; directly)
Notochord development requires induction.
A-lineage cells give notochord, from 32 cell; B-lineage cells give notochord from 110 cells.
FGF can induce notochord formation.
Notochord induced by vegetal region, between 32 to 110 cell stage. However, the notochord is induced by vegetal cells and involves the homologue of the Brachyury (T) gene (Regulative development).

The lineage of the ascidian notochord.

Types of cell movement during gastrulation:
- Invagination
- Involution
- Ingression
- Delamination
- Epiboly


Comparison of Normal Tunicate Embryos and Embryos from which Posterior Vegetal Cytoplasm has been Removed:
Wild type
Posterior vegetal cytoplasm removed
Can not form tail and muscle.

Gastrulation in Tunicates:
A-C: cross sections and D-F: scanning electron micrographs from the vegetal pole.
A-D: the invagination of the ectoderm.
B-E: involution of the mesoderm.
C-F: epiboly of the ectoderm.
SLIME MOLDS AND WATER MOLDS

Slime molds (see photograph) and water molds are protistans that superficially resemble fungi. Like fungi, the slime molds and water molds are not photosynthetic. In addition, many of the slime molds and water molds have bodies formed from thread-like structures called hyphae, which many fungi possess as well. However, several characteristics differentiate slime molds and water molds from fungi, including the fact that fungi have cell walls composed of chitin, while slime molds and water molds do not. Slime molds and water molds play an important role in the recycling of nutrients by digesting decaying organic material. The slime molds and water molds, like the other protistans, have complex life cycles.

Cell lineage of the Cellular Slime Mold (Dictyostelium discoideum)

Cellular slime molds are eukaryotes that diverged earlier than plant and animals and share properties with each (animals: cell movements during morphogenesis; plants: cellulose cell walls).

Slime mold alternates between a unicellular and a multicellular phase of life cycle. Fig 6.23

“Dicty” is unicellular but aggregates to form a multicellular “slug” (~100,000 cells) during famine. The slug forms a fruiting body with a mass of spore cells on top of a stalk.

Aggregation: chemotactic mechanism
Gene (smlA) control aggregation size.

Patterning of the slime mold slug involves cell sorting and positional signaling

In slug, anterior end: prestalk; posterior end: prespore cell

Prestalk cell: migration down through the prespore region → push the prespore region upward

Possible mechanism:
1. Some information → positional information → form morphogen gradient → the slug relative position is specified
2. Differentiation of cells at random → sort out to give the normal pattern

Prestalk AB expressed ecmA and ecmB and arranged in a funnel-shaped region near the tip of slug.
Pst B only expressed ecmB, it near prestalk and prespore, and move downward at the culmination stage.
Pst O/ACL is anterior-like cell (expressed ecm A). Pst O/ACL become pst O cell then pst A cell

The arrangement of cell types seems to be due to some form of cell sorting (may not positional signaling).

Pattern from a diversity of cell types. Which expressed different gene (ecmA and ecmB) and code for extracellular matrix protein

Prestalk AB expressed ecmA and ecmB and arranged in a funnel-shaped region near the tip of slug.
Pst B only expressed ecmB, it near prestalk and prespore, and move downward at the culmination stage.
Pst O/ACL is anterior-like cell (expressed ecm A). Pst O/ACL become pst O cell then pst A cell

The arrangement of cell types seems to be due to some form of cell sorting (may not positional signaling).
Chemical signals direct cell differentiation in the slime mold

Extracellular cyclic AMP can induce prespore cell differentiation. Chemotaxis Developing cell release → differentiation inducing factor (DIF) → increase cytoplasmic enzyme ↑ → negative feedback → destroy DIF. DIF is graded, posterior region is highest concentration.

Summary: slime mold aggregation and differentiation

- Free-living myxamoebae aggregate to form a multicellular 'slug'
- Specification of prestalk and prespore cells within the slug
- Slug migrates with prestalk cells at anterior end
- Slug stops moving and develops into a fraying body with a cellular stalk and a head of spores

Nucleosome and Chromatin Structure

- Where gene regulation takes place
  - Opening of chromatin
  - Transcription
  - Translation
  - Protein stability
  - Protein modifications
**Transcriptional Regulation**

Strongest regulation happens during transcription

Best place to regulate:
- No energy wasted making intermediate products

However, slowest response time
- After a receptor notices a change:
  1. Cascade message to nucleus
  2. Open chromatin & bind transcription factors
  3. Recruit RNA polymerase and transcribe
  4. Splice mRNA and send to cytoplasm
  5. Translate into protein

**Promoter and Enhancers**

- Promoter necessary to start transcription
- Enhancers can affect transcription from afar

**Transcription Factors Binding to DNA**

Transcription regulation:
- Certain transcription factors bind DNA
- Binding recognizes DNA substrings:
  - Regulatory motifs

**Regulation of Genes**

Transcription Factor (Protein)

RNA polymerase (Protein)

DNA

Regulatory Element

Gene
Regulation of Genes

Transcription Factor (Protein)

RNA polymerase

DNA

Regulatory Element

Gene

Example: A Human heat shock protein

TATA box: positioning transcription start
TATA, CCAAT: constitutive transcription
GRE: glucocorticoid response
MRE: metal response
HSE: heat shock element

The Cell as a Regulatory Network

- Genes = wires
- Motifs = gates

If C then D
If B then NOT D
If A and B then D
If D then B

Make D

Make B
A spiral pattern of cleavage is seen in some molluscs. The animal cells are displaced in a spiral arrangement. (clockwise = dextral; counterclockwise = sinistral)

Two common features of mollusc development are:

- first and second unequal cleavages give 3 small cells and 1 big cell (the D blastomere becomes posterior-dorsal embryo).
- By the 64 to 128 cell stage, cell fates can be mapped. Handedness is maternally specified and dextral spiral is genetically dominant to sinistral.
- The body axes are defined by early cleavages and cytoplasmic determinants are clearly important.

Early development of the Annelids is similar to the Molluscs (spiral cleavage).

- The teloplasm is segregated into the D blastomere which divides into the teloblasts, the source of mesodermal and ectodermal segmented structures.
- Centrifugation experiments alter the distribution of the teloplasm.
- Two sets of 5 teloblasts, one on each side of the embryo, behave as stem cells and undergo repeated asymmetric cell divisions with one cell maintaining the parental identity.
- This generates a string of daughter blast cells, the first-formed giving rise to the most anterior segments.
Sea urchins and starfish: Good model for developmental study. Transparency and easy handling. Sea urchin is a simple regulative development model. Sea urchin 1-3 cleavage is symmetric, after 4 cleavage is asymmetric, it produced four small micromeres at one pole of the egg (the vegetal). 1-2 cleavages divide along animal-vegetal axis. 3 cleavage is equatorial and divides the embryo into animal and vegetal halves. During the 4th cleavage, 4 small animal cells divide equally but the 4 large vegetal cells undergo asymmetric division to generate 4 vegetal micromeres and 4 macromeres which multiply into 1000 ciliated cells enclosing the blastocoel. Gastrulation starts with entry of the primary mesenchyme about 40 cells (mesoderm) which lays down skeletal rods, followed by the endoderm and secondary mesenchyme which together stretch across the blastocoel to form the digestive tract. Maternal factors specify the animal/vegetal axis and likely specify the vegetal organizer in the micromeres. Sea urchins have a large capacity to regulate along the A/V axis with signals similar to that of the frog D/V axis.

Echinoderm

Invertebrate: Sea Urchin

Radial holoblastic cleavage (isolecithal)
The 4th cleavage, very different from the first three. In animal pole, four cell divide to 8 blastomeres and with the same volume (the 8 cells also called mesomeres). In vegetal pole, undergoes an unequal cleavage to four large cells (macromeres) and four small cells (micromeres). The animal mesomeres divide equatorially to produced two tiers: an1 and an2. The vegetal macromeres divide a small cluster beneath the large tier. 128 cells blastula. Meridionally
Sea Urchin: blastula formation

The blastula stage of sea urchin development begins at the 128 cells. Blastulation: The cells form a hollow sphere surrounding a central cavity (blastocoel). Every cell contact with proteinaceous fluid of the blastocoel (inside) and with the hyaline layer on the outside. About 9th or 10th cleavage, cells become specified and they end develop cilia. Ciliated blastula → rotate within fertilization envelop (E→F) → vegetal pole of blastula become thickened (forming vegetal plate) → then animal pole synthesizes and secret hatching enzyme → digest fertilization envelope → embryo is a free swimming hatched blastula.

Fate maps and the determination of sea urchin blastomeres

Fate maps and the determination of sea urchin blastomeres.

Fate map and cell lineage of the sea urchin.

Fate map of the zygote

Late blastula with ciliary tuft and flattened vegetal plate

blasta

Primary mesenchyme cells

Secondary mesenchyme cells

Skeletal rods

Imagining endoderm

Stomodeum

Anus

Skeletal rods

Mouth

Prism-stage larva

Pluteus larva
Formation of syncytial cables by primary mesenchyme cells of sea urchin

SEM of spicules formed by the fusing of primary mesenchyme cells into syncytial cables

C: SEM of primary mesenchyme cells enmeshed in the extracellular matrix of early gastrula.
D: Gastrula-stage mesenchyme cell migration

The extracellular matrix fibrils of the bastocoel lie parallel to the animal-vegetal axis

Ingression of primary mesenchyme cells

Invagination of the vegetal plate

SEM of external surface of the early gastrula

Entire sequence of gastrulation in sea urchin
The sea urchin egg is polarized along the animal-vegetal axis

Defined the animal-vegetal polarity in the ovary.

Pigment in vegetal region. Animal-vegetal axis is stable, it develop to different region.

Cytoplasmic difference along the animal-vegetal axis

Important development signals are produced by micromeres in the vegetal region.

Fig 6.13 Development of isolated sea urchin blastomers

The D/V axis is related to the plane of the first cleavage

D/V axis, mouth is ventral side
Not identified in the egg. It is labile up to as late as the 16 cell stage. Not at the first cleavage.

The D/V axis lies 45° clockwise from the first cleavage plane as viewed from the animal pole (Fig 6.14)
Cleavage clockwise is related with cytoskeleton.

Fig 6.16 Regulation in sea urchin development

The fate map is finely specified by regulation

At 60cell, 4 region (different color) along animal-vegetal axis

Large and small micromeres: to mesoderm (formed the primary mesenchyme → skeleton)

Micromeres is maternally determined.

Vegetal plate (Veg 1 and 2): to endoderm, mesoderm (secondary mesenchyme → muscle, connective tissue) and some ectoderm.

Mesomeres (ectoderm): to anterior and posterior region

Sea urchin embryo is regulative development, not cleavage pattern.

Half embryo still developed a adult.

Fig 6.16
The vegetal region acts as an organizer

Experiment proof: Vegetal region play an important role of organization

Micromeres ↓ Signal ↓ Veg 2 ↓ Skeleton-forming organizer

New micromeres can induce a new gastrulation

Micromeres can induce animal half to form a gut, and ectoderm correctly

Maternal gene gradient may regulated the D/V axis development

Ability of the Micromeres to Induce a Secondary Axis in Sea Urchin Embryos

(A) Transplanted micromeres

(B) Primary mesenchyme

(C) Skeleton forming organizer

Ability of the Micromeres to Induce Presumptive Ectodermal Cells to Acquire Other Fates

Different regulatory region control sea urchin develop

Drosophila: even-skipped contains many binding sites for transcription factor, these site are encode regulatory modules

In sec urchin: endo-16 as modular; In blastula, vegetal region (presumptive endoderm) → secreted glycoprotein endo-16 (unknow function)...

Endo-16 control by 2200 base pair (at least 30 target sites, 13 different transcription can bind).

Endo-16 regulatory region likely pair-rule gene in Drosophila.

Modular organization of the endo-16 gene regulatory region. A-G are modular subregions.
<table>
<thead>
<tr>
<th>Animal-vegetal axis</th>
<th>Dorso-ventral axis</th>
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<tr>
<td>localized maternal factors in egg related to plane of first cleavage but is stable up to the 16-cell stage</td>
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<tr>
<td>animal-vegetal axis</td>
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<tr>
<td>organizes specified in vegetal region (furcillaria) Wnt pathway signaling and β-catenin accumulation</td>
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