Early Development of Vertebrates

July 17, 2008
Vertebrate Development - Overview

Models: chick & mouse

Chick

Discoidal cleavage: formation of a three-layered blastodisc
Primitive streak formation
Gastrulation and primitive streak regression
Axis formation

Mouse

Rotational cleavage: formation of the blastocyst
Implantation and formation of embryonic and extraembryonic tissues
Gastrulation and derivation of germ layers
Patterning the axes
## Cleavage Patterns

**Holoblastic (complete cleavage)**

<table>
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**Meroblastic (incomplete cleavage)**

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Disocoidal Meroblastic Cleavage

**Blastodisc** cytoplasm $\sim 2-3$ mm

Equatorial and vertical cleavages produce 5-6 cell layers

- vertical layers eventually reduced to single layer - **epiblast**
Three-Layered Blastodisc

- 5 – 6 cell layer blastodisc sheds cells in the center
  - single layer remains = area pellucida (epiblast)

- edges – area opaca

- marginal zone – important for determining cell fate

- some area pellucida cells detach; form poly-invagination islands
Three-Layered Blastodisc - 2

<table>
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<tr>
<th>MIDSAGITTAL</th>
<th>VENTRAL</th>
<th>DORSAL</th>
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<tr>
<td>(B) Stage XII</td>
<td><img src="image" alt="Diagram" /></td>
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<td>Primary hypoblast cells</td>
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- poly-invagination islands combine; form the **primary hypoblast**

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- sheets of tissue from posterior marginal zone (**Koller’s sickle**) migrate anteriorly
  - forms the **secondary hypoblast**
- space between epiblast and hypoblast forms **blastocoel**
- epiblast cells at the midline thicken, accumulate, move anteriorly
- form primitive streak

- primitive streak moves anteriorly by intercalation and convergent extension
- a depression forms within the primitive streak: the **primitive groove**
- primitive groove serves as an opening through which cells gastrulate
Primitive Streak Formation

Primitive streak

Area opaca
Area pellucida
Thickening area of blastoderm
Anterior
Margin
Koller’s sickle
Posterior
Area opaca
Area pellucida
Primitive streak taking shape

VENTRAL       DORSAL

Primitive streak
**Primitive Streak - 2**

**Hensen’s node** – anterior thickening; leading edge of the primitive streak
- primitive pit
- primitive groove
  - equivalent of the dorsal lip of the blastopore (amphibians)
- cells pass through groove individually
  - epithelial to mesenchyme

**Primitive streak defines axes:**
- posterior to anterior extension
- cells enter dorsal to ventral
- separates left from right
Hensen’s node extends 60 – 75% length of area pellucida; then regresses - creates posterior dorsal axis

Posterior forms anal region

NOTE – avian embryos exhibit distinct anterior-to-posterior gradient of developmental maturity
Migration of Endodermal and Mesodermal Cells

Prechordal plate & notochord
Notochord & somites
Intermediate mesoderm
Lateral plate mesoderm
Hensen’s node
Primitive streak
Epiblast
Blastocoel
Hypoblast
Endoderm

FGF8 expressed in primitive streak - repels migrating cells away
FGF4 produced by chordamesoderm - attracts migrating mesoderm cells

Deep lateral migrating cells form endoderm - displace hypoblast
Shallower cells form mesodermal mesenchyme
Chick Gastrulation

24 h – primitive streak at full extension
25 h – two somite stage
Primitive Streak Regression

Posterior border of pellucida area

0.0 10.5 20.5
Hours

Regression of primitive streak

Lengthening of notochord
Chick - Primitive Streak Initiation

The role of **Gravity:**
- ovum rotates ~ 20 h in reproductive tract
  - lighter yolk components shift to lie beneath one side of blastoderm
  - contents? – probably maternal determinants
  - portion becomes the **Posterior Marginal Zone (PMZ)**
    - primitive streak forms here

*PMZ acts as an equivalent to the amphibian Nieuwkoop center*
(expresses Vg1 and Nodal)

PMZ initiates primitive streak;
- also prevents other regions from initiating their own primitive streaks

Hensen’s node forms just anterior to the PMZ

Hensen’s node: the equivalent to amphibian dorsal blastopore lip
- gastrulation initiation site
- cells become chordamesoderm
- cells can organize a second embryonic axis when transplanted
Left side requires active:
1) Nodal (paracrine factor)
2) Pitx2 (transcription factor)

Nodal blocks *snail* (cSnR) expression
Nodal activates *Pitx2* expression

Activin inhibits *Shh* expression
Activin stimulates *Fgf8* expression

What limits Activin expression to the right side??
Early Development in Mammals

Mammalian development is difficult to study.

- zygotes are very small; ~ 100 μm diameter
- produced in relatively low numbers
- development inside another body makes observation very difficult

Although mammalian eggs are isolecithal and contain very little yolk, their embryos act as if they are sitting on top of a large imaginary ball of yolk
- i.e. gastrulate like fish, reptiles, and birds
Mammalian Fertilization

- Mammalian cleavage is among the slowest.
- 1st cleavage ~ 1 day after fertilization.
- Subsequent cleavages 12 – 24 h apart.
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Rotational Cleavage

(A) ECHINODERM AND AMPHIBIAN

(B) MAMMAL
Mouse Cleavage

Rotational cleavage
- asynchronous
- no MBT

Compaction – 8-cell stage
- tight junctions between outside cells
  seal off inside of sphere
Mouse Cleavage

Morula – 16-cell stage
- small group of internal cells; **inner cell mass (ICM)**
  - ICM will form the embryo proper
- larger group of external cells; **trophoblast (trophectoderm)**
  - trophoblast will form extraembryonic structures
  - secretes hormones causing uterus to retain fetus
- **cavitation** – trophoblast secretes fluid into morula (via Na$^+$ pumps)
  - creates blastocoel
  - hydrostatic pressure pushes ICM to one end

Blastocyst – unique to mammals
Blastocyst Hatching & Implantation

**Zona pellucida** prevents adhesion to uterine wall
(premature adhesion = ectopic pregnancy)

Trophoblast attaches to uterine wall
- forms the **chorion** – embryonic portion of the placenta

Trophoblast secretes proteases
- digests uterine ECM
- blastocyst implants

ICM – forms the embryo proper
- also, the **yolk sac, allantois, and amnion**

Note – ICM cells are pluripotent
(source of embryonic stem cells)
Human Embryo and Placenta

50 days gestation

chorion

amnion

yolk sac

allantois (not visible)
Derivation of Mammalian Tissues

Blastocyst – contains trophoblast and ICM
Derivation of Mammalian Tissues

**Hypoblast** (a.k.a. primitive endoderm or visceral endoderm) forms an extraembryonic membrane – the yolk sac.

**Epiblast** – forms embryo proper

**Hypoblast** (a.k.a. primitive endoderm or visceral endoderm)
- forms an extraembryonic membrane – the yolk sac

Two layers together form: **bilaminar germ disc**
Derivation of Mammalian Tissues

Epiblast splits to form **embryonic epiblast** and **amnionic ectoderm**

- **amnionic ectoderm** lines cavity; cavity fills with **amnionic fluid**

Trophoblast forms **cytotrophoblast** and **syncytiotrophoblast**
Cytotrophoblast:
- adheres to endometrium
- proteolyze uterine wall
- secretes paracrine factors to attract maternal blood vessels
- displaces vascular tissue; lines blood vessels with trophoblast cells

Syncytiotrophoblast:
- digests uterine tissue

Extraembryonic endoderm gives rise to yolk sac

Extraembryonic mesoderm and trophoblast give rise to blood vessels and umbilical cord
Trophoblast forms the **chorion**: embryonic contribution to the placenta - also induces uterine cells to produce the **decidua** (maternal portion)
Human Embryo and Placenta

50 days gestation

- chorion - note blood vessels
- chorionic villi

amnion

To fetus
From fetus
Umbilical arteries
Umbilical vein

Intervillus space

Chorion
Trophoblast cells
Maternal portion of placenta
Maternal vein
Maternal artery

To mother
From mother
Mammalian Gastrulation

day 15 of gestation

- Extraembryonic mesoderm
- Syncytiotrophoblast
- Amnionic cavity
- Primitive groove
- Yolk sac
- Bilaminar germ disc
- Epiblast
- Hypoblast
Mammalian Gastrulation

- endoderm displaces hypoblast

Gastrulation begins at posterior region with node formation

- mesoderm follows endoderm
Anterior-Posterior Patterning

1) Gradients of Wnts, BMPs, FGFs form: posterior - high anterior - low

2) BMP and Wnt antagonists (e.g. Chordin) expressed by the node, notochord, and head mesoderm

3) Retinoic acid gradient: posterior - high anterior - low

Summary

Posterior regions determined by BMPs, Wnts, FGFs, RA

Anterior determined (in part) by blocking posterior signals

Next, Hox genes determine A-P axis patterning
**Hox (Homeotic) Genes**

Drosophila homeotic selector genes determine segment identity.

Mammals contain 4 sets of Hox complexes: Hoxa - Hoxd.

Order on chromosome and order of expression are similar.

3’ Hox genes are expressed more anteriorly than 5’ Hox genes.
Hox genes are expressed along the dorsal axis from the anterior boundary of the hindbrain through the tail.

Generally, the level of the body along the A/P axis is determined by the most posterior Hox gene expressed.
Hox Expression Along the Dorsal Axis

e.g. expression of different Hox genes specify different vertebrae type; in mice:
  7 cervical (neck)
  14 thoracic (rib)
  6 lumbar (abdominal)
  4 sacral (hip)
  variable number of tail

Hox knockouts (all paralogous copies) = shifts in vertebra identity

Many Hox genes are sensitive to retinoic acid
  - RA gradient (high posterior)
  - controlled by differential synthesis and degradation
Effects of Retinoic Acid

(A) Normal development

- RA gradient from posterior to anterior
- many Hox genes sensitive to RA

Changing RA shifts Hox gene expression
- e.g. excess RA: last cervical vertebra takes on thoracic (posterior) identity

(B) Retinoic acid

Gain
- Excess RA
  - C0
  - C2
  - C3
  - C4
  - C6
  - C7

Loss
- RA receptor
  - C1
  - C3
  - C4
  - C5
  - C6
  - C7
Dorsal-Ventral Axis

Establishment of the dorsal-ventral axis in mammals is not well defined.
- the hypoblast forms on the side of the ICM exposed to blastocyst fluid
- the dorsal axis forms from ICM cells in contact with the trophoblast and amnionic cavity

Whether first cleavages determine this pattern is unknown.
Right-Left Axis

**Right-Left Asymmetry**

<table>
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<td>lungs</td>
</tr>
<tr>
<td>spleen</td>
<td>liver</td>
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**Two levels of regulation**

**Organ-specific:**
- *situs inversus viscerum (iv)* gene (dynein – motor protein)
- mutations cause randomized L-R asymmetry for each organ
- causes problems (sometimes fatal)

**Global:**
- *inversion of embryonic turning (inv)* gene
- mutations cause all asymmetrical organs to be reversed
- usually not a large problem

Activation of **Nodal** and **Pitx2** on the left side of the lateral plate mesoderm

**Mechanism:** frog – Vg1 placement
chick – suppression of *sonic hedgehog (Shh)*
mouse – asymmetric distribution of Shh, etc.
Left-Right Axis Mechanism (Mouse)

ciliated cells of the node

Nodal vesicular parcels (NVP) contain Shh and RA
- if parcels are not secreted
  L-R asymmetry fails to establish

Cilia powered by **dynein ATPase**

**iv** gene codes for a dynein protein