Early Development and Axis Formation in Amphibians

July 15, 2008
The Amphibian Model

Common vertebrate (e.g. *Rana, Xenopus*)
Manipulable, observable (i.e. large eggs and embryos)

1) How are the body axes established?
   - How do relatively homogeneous cells (i.e. zygotes) establish polarity (complexity from simplicity)?

2) How are the germ layers determined?
   - How do zygotes send their cellular progeny (i.e. blastomeres) down pathways toward different fates?

3) How is development organized in a regulative system?
Amphibian Development - Overview

Fertilization, cortical rotation

Cleavage

Gastrulation

Axis and germ layer determination

The "Organizer"

Inductions:

- Mesoderm
- Dorsal/organizer
- Ectoderm

Axes Summary
Cortical Rotation

Fertilization – animal hemisphere

Microtubular network originates at fertilization point
### Holoblastic (complete cleavage)

<table>
<thead>
<tr>
<th>Yolk classification</th>
<th>Species</th>
<th>Cleavage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolecithal</td>
<td>echinoderms, amphioxis</td>
<td>Radial</td>
</tr>
<tr>
<td></td>
<td>annelids, molluscs, flatworms</td>
<td>Spiral</td>
</tr>
<tr>
<td>Mesolecithal</td>
<td>tunicates</td>
<td>Bilateral</td>
</tr>
<tr>
<td></td>
<td>mammals, nematode</td>
<td>Rotational</td>
</tr>
<tr>
<td></td>
<td>amphitans</td>
<td>Displaced radial</td>
</tr>
</tbody>
</table>

### Meroblastic (incomplete cleavage)

<table>
<thead>
<tr>
<th>Yolk classification</th>
<th>Species</th>
<th>Cleavage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telolecithal</td>
<td>cephalopod molluscs</td>
<td>Bilateral</td>
</tr>
<tr>
<td></td>
<td>fish, reptiles, birds</td>
<td>Discoidal</td>
</tr>
<tr>
<td>Centroletic</td>
<td>most insects</td>
<td>Superficial</td>
</tr>
</tbody>
</table>
Cell cycles regulated by mitosis-promoting factor (MPF)
- no G phases

... until *mid-blastula transition*
- promoters demethylated (i.e. de-repressed)
- transcription factors formed in vegetal cytoplasm
- embryonic control of development
Mechanics of Gastrulation

Formation of the dorsal lip
- vegetal rotation
- invagination of bottle cells
- involution of marginal zone cells

![Diagram of gastrulation process](image)
Mechanics of Gastrulation - 2

Formation of the dorsal lip
- vegetal rotation
- invagination of bottle cells
- involution of marginal zone cells

(B) Bottle cells
(C) Blastopore
(D) Cells crawl to animal pole

motive force
Xenopus Gastrulation

Animal pole (AP)
- Blastocoel
- Superficial cells
- Deep cells
- Bottle cells

Vegetal pole

Dorsal mesoderm
- Archenteron
- Mesoderm
- Endoderm

Dorsal blastopore lip
Blastocoel displaced

Ectoderm
- Archenteron
- Notochord
- Dorsal blastopore lip
- Lateral blastopore lip
- Yolk plug

Mesenchyme
- Ectoderm
- Notochord
- Dorsal blastopore lip
- Ventral mesoderm

Ventral blastopore lip
Anterior endomesoderm
Endoderm

Dorsal lip of blastopore

AP
Cell Movements during Gastrulation

- invagination
- involution
- epiboly (animal cap)
- intercalation and convergent extension
- migration
Xenopus Gastrulation - Blastopore

Mesenchyme
Ectoderm
Notochord
Dorsal blastopore lip
Ventral mesoderm

NIMZ
Yolk plug
Blastopore lip

A)

Dorsal lip

i

ii

Dorsal lip

iv

Ventral lip

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Determination of Amphibian Axes

If one blastomere received no gray crescent material - “belly piece” – blood, mesenchyme, gut cells - *no dorsal structures* (e.g. notochord, somites)

The gray crescent area is critical for proper development

Gray crescent = future *dorsal lip of the blastopore*
Dorsal Lip Transplant

Doral lip = "Organizer"
- organizes secondary D-V axis
- induced ventral cells to change fates
The Organizer

Transplantation experiments established the organizing properties of the *dorsal blastopore lip*, which ...

1) Self-differentiates (all others conditionally specified)
2) Establishes the dorsal-ventral axis
3) Specifies multiple tissues, including...
   - dorsal mesoderm, which includes...
     - head mesoderm (prechordal plate)
     - chordamesoderm (notochord)
4) Dorsalizes surrounding mesoderm into paraxial mesoderm
5) Induces the neural tube
6) Initiates the movements of gastrulation

How is the dorsal lip specified?
Xenopus cell fate depends mostly on whether the cells are located in the superficial or deep layers in the blastula.

- superficial layer on surface: ectoderm and endoderm precursors
- deep layers (mostly): mesodermal precursors

**Gastrulation** – moves cell layers into proper conjunction for induction/specification
Fate and Axis Determination

1) Anterior-posterior, dorsal-ventral, and left-right axes are specified by events triggered at fertilization and realized during gastrulation.

2) Mesoderm is determined by transcription factors and paracrine factors from the vegetal region.

3) Of all tissues in the Xenopus pre-gastrula, only the dorsal lip of the blastopore has its fate determined.

4) The dorsal lip will determine the fates of notochord and head endomesoderm.

Induction and Determination
Mesoderm Induction

Experimental evidence

Isolated animal or vegetal fragments do not produce mesoderm......
Mesoderm Induction

.. but, when animal cap is placed in conjunction with endoderm = **mesoderm induction**

Factors from endoderm direct marginal ectoderm cells to mesoderm
Mesoderm Induction

Vegetal mesoderm inducers:
- **VegT** - transcription factor
- **Vg1** - paracrine factor

Mesodermal fate depends on the origin of vegetal inducing cells:
- ventral vegetal cells induce mesenchyme, blood
- intermediate – muscle, kidney
- dorsal (organizer) – notochord, somites
Dorsal Signal: β-Catenin

β-catenin

A) anchor for cadherins

B) nuclear transcription factor (in Wnt pathway)
   (in sea urchins, specifies micromeres, endomesoderm)
   (in Xenopus, specifies dorsal structures; e.g. organizer)

β-catenin is initially distributed throughout the embryo,
- but accumulates only in dorsal cells.
- concentrated in the Nieuwkoop center and organizer

Dorsalization of β-catenin accomplished by
  a) protecting β-catenin in dorsal area,
  b) degrading β-catenin everywhere else.

Mechanism - cortical rotation ...
Disheveled/\(\beta\)-Catenin/Cortical Rotation

\(\beta\)-catenin induces cells to dorsal fates

- **\(\beta\)-catenin** is initially distributed throughout the oocyte
- **Glycogen synthase kinase 3 (GSK3)** is also distributed throughout oocyte
  - GSK3 marks \(\beta\)-catenin for degradation
  - **Dishevelled (Dsh)** blocks GSK3 activity
    - Dsh localizes in the cytoplasmic cortex at the vegetal pole
**Disheveled/β-Catenin/Cortical Rotation**

- **β-catenin, GSK3** distributed throughout
- **Dishevelled** - blocks GSK3-mediated β-catenin degradation
  - anywhere Dsh exists, β-catenin survives

**Diagram:**
- **Fertilization**
- **Sperm**
- **Dishevelled protein**
- **Cortical rotation**
- **Fast transport**
- **Slow transport**
- **Dsh** – Dishevelled
- **GBP** – GSK3 binding protein
- **Kinesin** - motor protein
Disheveled/GSK3/ β-Catenin

At the blastula stage, β-catenin is located exclusively in the future dorsal region.

GSK3 mediates β-catenin destruction ...... but Disheveled and GBP block GSK3 activity;
- resulting in β-catenin present only in the marginal area opposite the point of sperm entry (i.e. future dorsal lip)
Organizer Induction

β-catenin acts with Tcf3 (a transcription factor); stimulates expression of dorsalizing genes:

**Siamois** – TF; activates Xlim, goosecoid (dorsal determinants)

**Goosecoid** protein – TF responsible for organizer properties

Goosecoid also plays a part in specifying dorsal mesoderm; however, additional vegetal factors are needed:

**Vegetal TGF-β signals**
Mesoderm/Organizer Induction

(A) Stage 8
Ventral

β-catenin

VegT, Vg1

(B) Stage 9
Dorsal

Xnr

(C) Stage 10

Gradient of BMP4, Xwnt8

Organizer

Xnr – *Xenopus* nodal related genes

β-catenin → Nodal related high → Organizer

VegT, Vg1 → Nodal related low → Ventral mesoderm

Xnrs: TGF-β genes
Determination of Ectoderm

(A) TRANSPLANTATION IN EARLY GASTRULA

Early cells are uncommitted - exhibit regulative development

(B) TRANSPLANTATION IN LATE GASTRULA

Later cells are determined - exhibit autonomous development
Amphibian Development - Overview

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- Cleavage
- Gastrulation

**Axis and germ layer determination**

- The “Organizer”
- Inductions:
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  - Ectoderm

**Axes Summary**

(Photos by Harland lab/UC Berkeley)
Establishment of Axes - Summary

**D-V axis** – set up at fertilization

**A-P axis** – established by gastrulation movements across the dorsal lip of the blastopore

**L-R axis** – Nodal expression on Left, not Right
**Left – Right Axis**

Left–Right axis established by *Xenopus nodal-related* (Xnr1)

**Nodal** expression:
- common to all vertebrates
- expressed on the **left side**!

Xnr1 expression is limited to the left side in a process involving cortical rotation and Vg1

Block Xnr1 expression = random gut coiling, heart looping

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**A)** wild-type

**B)** Xnr1−/−

heart loops to the left

gut coiling – counter-clockwise