Overview of Next Three Lectures

1. 1st Class: Introduction to Gastrulation
   1. Basic concept
   2. Germ layer formation

2. 2nd Class: Interaction of Signals
   1. Nodal and its relatives
   2. FGF and its relatives
   3. Other minor players

3. 3rd Class: Formation of Body Axis
   1. Body axis formation
   2. Breaking the asymmetry

Conclusions from Nieuwkoop’s Experiment

1. Mesoderm is induced when endoderm and ectoderm are juxtaposed.

2. Signals arise from endoderm and received by ectoderm; i.e., the ectoderm is induced to form mesoderm.

3. The signal from the endoderm is secreted.

4. Signal only acts over a short distance.

5. Ectoderm is only competent for this signal for a short period during development.

Follow-ups of Nieuwkoop’s Experiments

- Ectoderm
- Endoderm
- Dorsal mesoderm
- VENTRAL mesoderm
- Notochord muscle
- Blood, LPM vessels, kidney

Two Signal Model

1. Mesoderm is induced when endoderm and ectoderm are juxtaposed.

2. There must be more than one signal from the endoderm (Dorsal vs Ventral).
**Two step model for mesoderm induction**

**Criteria for Mesodermal Inducer Based on Nieuwkoop’s Studies**

1. Gene or protein must be expressed and active in the correct time and place.
2. Protein must be secreted and act at a distance.

**Animal Cap Assays**

Every molecule ever identified in 30+ years of screening that fits this criteria is either:

1. A member of the FGF superfamily
2. A member of the TGF superfamily

**So, what did they find?**

**Is FGF an Endogenous Inducing Signal?**
**Brief Overview of FGF**

1. First mesodermal inducer identified.
2. Predominantly induces ventral mesoderm.
3. DN-FGFR abolishes the majority of mesoderm.
4. Only FGF independent mesoderm is prechordal mesoderm.

**FGF is Required for Formation of Posterior and Ventral Mesoderm**

**Problems with FGF as a Sole Signal**

1. In regards to mesoderm formation or patterning, no gene has yet to be identified that is solely FGF inducible.
2. FGF can only induce posterior mesoderm.

**TGF Beta Family**

Two subgroups:
1. Induce all mesoderm: Nodal, activin, and Vg-1
2. Induce ventral mesoderm: BMPs (BMP2, 4, 7)

**TGF Beta Signaling Cascade**

Vertebrates have 7 Type I receptors (Alk) and 5 Type II receptors.

**Diverse Ligand/Receptor Pairs**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Type</th>
<th>Expression</th>
<th>Loci</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP1</td>
<td>1</td>
<td>High</td>
<td>WT</td>
<td>cDNA</td>
</tr>
<tr>
<td>BMP2</td>
<td>1</td>
<td>Low</td>
<td>WT</td>
<td>cDNA</td>
</tr>
<tr>
<td>BMP4</td>
<td>2</td>
<td>High</td>
<td>WT</td>
<td>cDNA</td>
</tr>
<tr>
<td>BMP7</td>
<td>3</td>
<td>Low</td>
<td>WT</td>
<td>cDNA</td>
</tr>
<tr>
<td>Nodal</td>
<td>4</td>
<td>High</td>
<td>WT</td>
<td>cDNA</td>
</tr>
</tbody>
</table>
TGF SIGNALING DURING DEVELOPMENT

1. In early development, Nodal expresses from the endoderm.
2. Dorsal most mesoderm express antagonists of Nodal.
3. Localized antagonist expression create a gradient of Nodal signaling.

REGULATION OF NODAL BY ANTIVIN/LEFTY

1. Antivin/Lefty is a divergent member of the TGF superfamily.
2. Lacks the α-helix required for dimerization.
3. Main function is downregulating Nodal.

REGULATION OF CHORDIN/BMP BY TSG/TOLLOID

1. Twisted gastrulation (TSG) is BMP binding protein function as an antagonist.
2. TSG activity is modulated by Tolloid mediated proteolytic cleavage.
3. Cleaved TSG function as a permissive cue for BMP signaling in the presence of Chordin.

TOLLOID MEDIATED CLEAVE OF TSG

CERBERUS

1. Secreted protein widely expressed in involuting endoderm.
2. Multivalent antagonist can bind to Xnr5, Xwnt8, and BMP4.
3. By inhibiting trunk mesoderm development, Cerberus promote head development.

Is FGF AND Nodal the Endogenous Inducing Signal? Is it something altogether different?
What is the organizer?

Life cycle of Xenopus

Early development of Xenopus

Sperm entry point and Cortical rotation in Xenopus

1. A large centriole enters with the sperm nucleus.
2. Microtubules form and trigger a 30° rotation of the cortex.

UV induced defects in Axis Formation can be rescued

Axis Formation is Sensitive to UV

The rotation can be blocked by
1. UV irradiation
2. Interference with microtubule polymerization

The rotation can be restored by
1. Centrifugation
2. Upside down incubation
Dorsal side of Xenopus is determined by sperm entry point.

Where is Nieuwkoop Center?

Nieuwkoop Center

1. Dorsal vegetal blastomeres which can induce an ectopic dorsal mesoderm when transplanted.
2. They form anterior gut endoderm.
3. In later stages, these cells lose their potentials.
4. Important for inducing the organizer.

Nieuwkoop Center can induce secondary axis.

Nieuwkoop Center and the Canonical Wnt Pathway

1. Wnts can mimic Nieuwkoop Center; i.e. inject ligands into ventral endoderm get secondary axis formation.
2. Dominant negative GSK3, beta-catenin or plakoglobin injections into ventral endoderm get secondary axis formation.
3. Maternal depletion of maternal beta-catenin mRNA leads to a ventralized embryo.

Canonical Wnt signaling.
The Organizer Forms on the Dorsal Side of the Embryo

Spemann and Mangold’s Organizer Grafts

The organizer expresses many antagonists

Two Signal Emanate From the Endoderm

Specification ≠ Fate

Hypothesis: Must exist region a third signal that modifies mesodermal cell types.

Two step model for mesoderm induction
Three waves of mesodermal induction signals

1. Signal from the ventral endoderm that induces ventral mesoderm.
2. Signal from the dorsal endoderm (Nieuwkoop center) that induces dorsal mesoderm.
3. Signal from the dorsal mesoderm (Organizer) that refines the gradient.

The 3-Signal Model by Smith and Slack

3-Signal Model Circa 2009

Interaction of Nodal/Wnt

Goosecoid as a common target for both Nodal and Wnt