Neural induction

A group of cells is instructed to develop into the nervous system

Neural induction

• Chapter 19, pgs 430-434
• During embryo development, where does the nervous system come from?
  – Embryo anatomy
  – gastrulation
• Evidence for neural induction
  – fate vs. specification maps
  – the Spemann Organizer experiment
• Defining the organizer:
  – how to design experiments
  – molecules that induce neural tissue
• Neural is the default state for ectoderm
The basic vertebrate body plan

- All tissues come from one of three germ layers:
  - Ectoderm: skin, nervous system
  - Mesoderm: skeleton, muscle, kidney, heart, blood
  - Endoderm: gut, liver, lungs

- Nervous system is dorsal
- hollow neural tube
  - forebrain anterior, spinal cord posterior

Discovery of induction

- Cell interactions are very important
- Induction: one tissue directs the development of neighboring tissue
- Gastrulation:
  - cells move inside; through blastopore
- Spemann/Mangold discovery of the ORGANIZER.
  - Hilde Mangold and Hans Spemann, 1924
  - Fig. 19.2. Transplantation of dorsal lip of blastopore can induce a second body axis.
  - Small patch of tissue can change trunk tissue into a new embryo, complete with brain and spinal cord.

- Implications:
  - pattern of the body is regulated by special signaling centers
  - nervous system is induced by dorsal lip of blastopore
- What is the organizer?
The discovery of the noggin gene

- **Strategy to clone neural inducer**
  - Smith, et al., 1993
  - Lamb, et al., 1993

- **One cDNA from organizer was able to rescue neural formation**

- **Named: Noggin**
  - induces head formation
  - encodes secreted protein

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Formulating a hypothesis

- **Hypothesis**: one explanation for observations
  - A story that explains the known facts
  - **Important**: cause-and-effect explanation
  - Needs to be consistent with all observations
  - Often more than one hypothesis: “competing hypotheses”

- **Testing hypotheses**: first make predictions

- **Prediction**:
  - **IF** hypothesis is true, **THEN**… this experiment should give a certain result.
A hypothesis for noggin function

- Formulate a hypothesis:

Testing a hypothesis

- Three types of evidence
- Correlation: SHOW IT
- Loss-of-function: BLOCK IT
- Gain-of-function: MOVE IT
Correlation: SHOW IT

- **Co-relation:**
  - two events occur together (space or time)
  - Example: predictions about neural inducer
    - Where: neural inducer should be transcribed in organizer
    - When: neural inducer should be synthesized at beginning of gastrulation (late blastula)
    - If noggin is not present at the right place and time, it can be eliminated as a candidate for neural inducer

- **Correlation experiment:**
  - How can we determine where or when noggin is synthesized?
  - mRNA detection: in situ hybridization
Noggin pattern of expression

- Detection of noggin mRNA in late blastula frog embryo
- Label appears only in organizer region

How are antibodies made?

- Isolate material from cells or embryo
  - Isolate membranes from starved cells,
  - Or purify a specific protein
- Inject into animal, immune system reacts by producing large amounts of antibody that binds to antigen
- 1-2 months later, collect blood and isolate serum
- Purify antibodies specific for antigen
  - Example: antibody against choline acetyl transferase
Antibody labeling
• Direct immunofluorescence
• Antibodies only bind if specific antigen (protein) is present!

Correlation: SHOW IT
• Correlation is not Cause
  – only suggests one event causes the other
  – leads to a more specific hypothesis:
    • Noggin is secreted by the organizer and induces ectoderm to form neural tissue
• Careful! Correlations are weakest type of evidence
  – Very useful for suggesting hypotheses
• Other possible hypotheses:
  – neural induction causes synthesis of noggin
  – Unknown signal induces both noggin and neural
  – Simple coincidence?
• More rigorous tests are needed
Loss-of-function: BLOCK IT

- Experiments can provide loss-of-function evidence
  - block, interfere, prevent, remove, knockout, ablate
- Example of loss-of-function experiment:
  - injection of an antibody that specifically binds noggin protein into neural region of late blastula
  - antibody will bind noggin, prevent it from functioning

NE

Inject antibody:
Noggin bound, cannot act

Normal
No Neural

Loss-of-function: BLOCK IT

- Stronger than correlation, but still limited power
- Example:
  - What if noggin protein needed to keep cells alive? No induction in dead cells.
- Necessity = Requirement
  - Event or molecule is necessary for event to occur
- But:
  - just because something is necessary doesn’t mean that it is sufficient
- Sufficient = Enough to do the job “alone”
  - How can Sufficiency be demonstrated?
Gain-of-function: MOVE IT

- **Demonstration of sufficiency:**
  - Force event or molecule at new time or place
  - move, transplant, over-express, activate, induce
- **Example:**
  - Purify noggin protein
    - In test tube: make mRNA from cloned cDNA; translate mRNA into protein
  - Animal cap assay:
    - isolated animal cap is specified to be epidermis
    - incubate animal cap with noggin protein
      - result: induction of neural tissue
- **Conclusion:** noggin is **sufficient** to induce neural tissue

Necessary or Sufficient?

- Let’s invent examples of:
- **Necessary but not Sufficient**
  - “BLOCK IT” worked, but “MOVE IT” did not.
- **Sufficient but not Necessary**
  - “BLOCK IT” did not work, but “MOVE IT” did.
- **Necessary and Sufficient**
  - “BLOCK IT” and “MOVE IT” worked.
Analyzing experiment and results

Be able to define each of the following, and give an example:

- Observation
- Hypothesis
- Prediction
- Experiment
- Correlation
- Evidence
  - Loss-of-function
  - Gain-of-function

- Necessity
- Sufficiency
- Necessary but not sufficient
- Sufficient but not necessary
- Necessary and sufficient

Organizer expresses many specific genes

Transcription factors

- Lim-1: mutant mice

Secreted signals

- Chordin: BMP4 blocking
Organizer mutants

- Example: Lim-1 transcription factor
- Required for organizer function in mice
  - knock out of Lim1 gene blocks head development!
  - Shawlot and Behringer, 1995

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+/- +/- +/-
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**“Animal cap” assay: Default is neural**

**Animal cap**

- Experiment:
  - 1. Culture animal cap
    - Result: epidermis
  - 2. Dissociate animal cap cells, culture
    - Result: neural

**Community effect**

**Implication:**

dissociated cells
missing some signal

Whole explant=
epidermal fate
• keratin+
• NCAM-

Dissociated cells=
neural fate
• keratin-
• NCAM+
**Epidermal promoting signal: BMP4**

- **Finding the signal:** kitchen-sink approach
  - dissociate cells, add factors, look for epidermal markers
  - BMP4: bone morphogenetic protein 4
    - member of TGFβ family of secreted growth factors

**Animal cap**

**BMP4**

<table>
<thead>
<tr>
<th>Neural fate:</th>
<th>Epidermal fate:</th>
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<tbody>
<tr>
<td>keratin-, NCAM+</td>
<td>keratin+, NCAM-</td>
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**Chordin inhibits BMP4 to induce neural tissue**

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Mechanism of BMP4 inactivation

- **BMP4**
- **Noggin**
- **Both**

Neural induction

Chordin $\rightarrow$ BMP4 $\rightarrow$ Epidermis

(Default: Neural!)