Nearly a century ago, Ramon y Cajal initiated an extensive series of anatomical studies which revealed the extraordinary diversity, organizational complexity, and precision of connections between cells in the nervous system. Over the past decade striking advances in dissecting the molecular and cellular bases of neural development have been made. The following lectures are aimed to understand neural development.

Formation and patterning of the nervous system

II. Neural Patterning - patterning of neural progenitors along the dorsoventral and anteroposterior axis.
III. Neurogenesis - differentiation of neural progenitors into postmitotic neurons and glia.
IV. Understanding neural patterning in the context of neurogenesis
NEURAL INDUCTION: the delineation of ectodermal cells to neural fate.

NEURULATION: the process in which the ectoderm of the future brain and spinal cord - the neural plate - develops folds (neural folds) and forms the neural tube.

Neural induction constitutes the initial step in the generation of the vertebrate nervous system, whereby embryonic ectodermal cells are exposed to signals that will instruct the cells to become neural progenitor cells unless exposed to signals that divert them to alternative fates. Historical perspective.
In amphibians, neural tissue forms on the dorsal side of the embryo, whereas epidermis forms on the ventral. Experiments, first performed in newt embryos and then in frog, showed that when the dorsal lip of the blastopore of gastrula stage embryos was transplanted to the ventral ectoderm, recipient ectoderm cells surrounding the site of transplantation formed a fully developed secondary nervous system. The secondary body axis arose from non-pigmented tissue – therefore from the host embryo. This discovery proved that the graft had the capacity not only to induce neural tissue but also to completely establish a new axis.

Mangold and Spemann’s “organizer” experiment

Similar inductive capacity was later demonstrated for amniotic organizing centers:

- Chick - Waddington and Schmidt, 1933
- Mouse - Beddington, 1994

Collectively, these findings led to the idea that the organizer region is a local source of inductive signals that impose neural fate on the surrounding dorsal ectoderm at gastrula stages.

In frogs, the mechanism by which the organizer region induces neural fate in amphibians was studied using both whole embryos and by in vitro culture of explants (animal cap assays).

**What is the neural inducer?**

- Noggin (example of how cloned)
- Chordin
- Follistatin
- Xnr3
- Cerebrus
Strategy used to identify genes that induce neural tissue

- UV irradiation blocks rotation
- Ventralized embryo

Inject UV irradiated embryos with mRNAs and try to rescue normal development
- “Expression cloning”

Smith and Harland, 1992, 1993

Expression cloning

- Promoter enables transcription of mRNAs in vitro
- Pooled mRNAs are injected into UV-irradiated eggs

Inject UV irradiated embryos with mRNAs and try to rescue normal development
- “Expression cloning”

Smith and Harland, 1992, 1993
Noggin expression rescues UV-irradiated embryos.

Increasing amounts of noggin mRNA

Neural tissue is induced in the absence of mesoderm even at gastrula stages when mesoderm induction by activin is no longer effective.

How do neural inducers function?

Noggin, follistatin and chordin all display similar direct neural activity (that is, molecules that induce the formation of neural tissue in the ectoderm without the concomitant formation of mesoderm).

It was thought that these molecules act in an inductive manner to induce the formation of neural tissue.

Three lines of evidence indicated a more indirect action:

1. Dissociation of naive ectodermal explants

2. A surprising result from analysis of the role of activin signaling...

Melton and colleagues study mesoderm induction by activin [block activin using a DN receptor ActRII]
Generating a dominant negative activin receptor.

Expression of a dominant negative activin receptor turns the entire ectoderm into neural tissue.

- No mesoderm is made
- The entire ectoderm becomes neural tissue!

What does this mean about the role of induction? And how does Noggin work?

3. The homology of chordin to a Drosophila gene known as short gastrulation (sog), which had been shown to antagonize the BMP homologue decapentaplegic (Dpp). BMP4 is expressed throughout the ventral ectoderm but is excluded from the organizer.

BMP4 can induce dissociated ectoderm cells to differentiate as skin rather than neurons.

BMPs are members of the TGFβ superfamily.
BMPs and activin bind to related receptors.

Nodal-TGFβ Activin and BMP ligands interact with type I and type II receptors.

Dominant negative activin receptor blocks signaling through all TGFβ family ligands.

Truncated type II receptors can form heterodimers with activin or BMP receptors and block signaling through both pathways.

Noggin exerts its organizer activity by binding directly to BMP4, blocking the binding of BMP4 to BMP receptors, and antagonizing BMP4 function.

Noggin antagonizes BMP4 to allow ectodermal cells to achieve their "default" state, which is neural.
Other molecules with organizer activity also act as BMP antagonists.

The BMP signaling pathway and the specification of ectodermal cell fates.

Summary of Xenopus data:

- BMP inhibition by signals secreted from the organizer sufficient for neural induction.

Variations on the theme, is neural default conserved in other vertebrates?

1. Is the organizer required for neural induction?
   - Like the frog organizer, the chick and mouse node can induce ectopic neural cells, demonstrating that it is sufficient.
   - However, genetic studies in mouse, i.e., absence of HNF3b or the Arkadia protein, mouse embryos fail to generate the node. However, these embryos develop a neural plate.
   - Moreover, if the gastrula organizer is removed from chick, zebrafish, and mouse embryos neural plate forms.

Variations on the theme, is neural default conserved in other vertebrates?

1. The organizer is not required for neural induction.
2. Neural induction is initiated before gastrulation.

At blastula stages, before the onset of gastrulation, the chick embryo is patterned along the mediolateral axis, i.e., epidermal markers Dlx5 and GATA2, early neural markers SOX3.
In the chick embryo neural induction is initiated before gastrulation. Lateral epiblast cells isolated from blastula stage embryos generate epidermal cells and medial epiblast cells generate neural cells when grown as explants in vitro.

Variations on the theme: Is neural default conserved in other vertebrates?

- 1. The organizer is not required for neural induction.
- 2. In the chick embryo neural induction is initiated before gastrulation.
- 3. BMP antagonists are not required for neural induction. In mouse, neural plate is formed in embryos lacking functional Follistatin, Noggin and Chordin, or both Noggin and Chordin. But these molecules appear to play a role maintaining neural fate and regulating the size of the neural plate (evidence from chick explants of node and zebrafish mutants).

Other molecular pathways involved in specifying neural fate

- 1. FGF signaling
- 2. Wnt signaling

FGF and Wnt signaling in chick neural cell fate specification

As in Xenopus, during gastrulation prospective neurons in medial epiblast express FGF. Thus, FGF signaling in medial epiblast acts to rescue neural fate in BMP inhibited medial neural plate explants.

Wnt-signaling in chick neural cell fate specification

In lateral epiblast there is a low level of FGF3 expression, however these cells take on an epidermal fate. What are the signals that prevent lateral cells from responding to FGF signals? Wnt.

Wnt3A and Wnt8C are expressed in lateral but not medial epiblast cells both in vitro and in vivo.
FGF and Wnt signaling in chick neural cell fate specification

In the presence of WNT signaling medial epiblast cells maintain BMP and epidermal fate, even in the presence of FGF. At low concentrations of WNTs, BMP antagonists such as Noggin promote neural fate. Blocking FGF signaling inhibits neural cell fate even in the presence of Noggin.

A conserved mechanism of neural induction?

Conserved aspects:
- BMP signals block neural and promote epidermal fate.
- Bmp RNA expression is excluded from prospective neural cells and is present in prospective epidermal cells.

Diverse aspect:
- The time at which cells receive signals that initiate the exclusion of Bmp expression from prospective neural cells and the molecular nature of these signals seem to be different in amphibians and chicks.

Embryonic stem cells and the default model for neural induction

Neural differentiation of embryonic stem cells in vitro: a road map to neurogenesis in the embryo

Abranches et al., PLOS 4, 1-14
Figure 1: ES-cell derived NPs culture analyses

Figure 2. Chemical inhibition of Notch activity by secretase inhibitor LY411575.

Figure 3: Timing and production of neurons and glia in rosette cultures

Figure 4. NS cell potential of the in vitro neuroepithelial rosette cultures

Figure 5. Validation of microarray results.

Figure 6. Clustering analysis of differentially expressed genes.