Study of generation of neural tissue initated by Spemann and Mangold

Xenopus development

- In xenopus fertilised egg: Dark hemisphere - animal pole, white hemisphere - vegetal pole. Predicts future anterior-posterior development.
- Neurulation occurs approx. 5 hours after gastrulation, forming a function tadpole within four days
- The animal-vegetal axis is transformed into anteroposterior axis, with the development of the dorsoventral axis.
    - Dorsal blastopore first appear on the dorsal side, the notochord will ingress into the dorsal pore and migrate along the dorsal midline
    - **Spemann's grafting experiment:** Cells taken from blastopore grafted into ectodermal tissue will induce blastopore formation, dorsal pore is therefore also called Spemann organiser
- Specification: Cells are unspecified during early development, become specified later in development
    - **Experimental support:** grafting of early neuroectoderm into host, graft become epithelium, grafting in later stages, graft retains neural plate form.

- Neural specific gene expression
    - Foxg1 - expressed in the anterior forebrain
    - Hoxb9 - expressed in the posterior spinal cord
    - Sox2 - expressed in the neural plate
    - Snai2 - expressed by neural crest cells
- In-situ hybridisation is used to visualise the expression of these genes

BMP signalling: Notochord gene expression: In-situ hybridisation showed expression of chordin, noggin, follistatin around the notochord (organiser) induce neural tissue formation, antagonising BMP4 effect

- **Experiment:** The induction effect of these induction proteins is supported by culturing epithelium in CNF containing media, result in induction to neuroepithelium
- **Experiment:** Oligonucleotide morpholino transient inhibition of CNF induce a total loss of nervous system. Inhibition of chordin only result in loss of secondary axis after grafting, showing primary induction effect of chordin.
- Chordin acts by antagonising BMP activity
    - BMP induce epidermal fate, binding to type I and type II receptors.
    - BMP induce expression of downstream transcription factors Vent1 and Msx1, inhibit neural plate induction genes (hoxb9, foxg1)
    - Chordin bind and sequestrate BMP ligand, prevent epidermal fate adoption
    - **In-situ hybridisation** shows BMP4 not expressed in dorsal region in early embryo but co-expressed in later stages.
    - **Dominant-negative** approach editing BMP4 receptors induce formation of neural plate tissues, suggesting BMP is the epidermalising factor inducing epidermis formation from default neural fate.

Wnt signalling: Chordin injection not enough to induce head formation

- Wnt bind to Frz receptors, activate downstream transcription factor Hoxb9, which inhibit forebrain development (foxg1)
- Expression of Cerebus, FrzB, Dkk1 in the anterior endomesoderm (anteriormost part of notochord) inhibit Wnt
- FrzB bind to Wnt, sequestration.
- **Experiment:** FrzB anterior expression is visualised with in-situ hybridisation in xenopus

- **Experiment:** Chordin + FrzB injection inhibit epidermal fate and promote head formation in xenopus

Anterior expressed Cerebus, Dkk1, FrzB and dorsally expressed CNF, the antagonists create two gradients with high ventral BMP and high posterior Wnt concentration, allows patterning of the nervous system in xenopus.