

# Chapter 8

## Embryonic Induction

### 8.1. Basic Concepts

#### (a) Determination of the Primary (1°) Organ Rudiments

The movements which occur during gastrulation are irreversible, and create new shapes and forms. Therefore, these movements are referred to as **morphogenetic movements**. These movements of gastrulation are not a function of the embryo as a whole, but rather a function of the collective parts of the embryo. Recall that **fate maps** reveal what the various presumptive tissues will eventually become. **Transplantation experiments** are used to study inducing factors.

#### (b) Some Definitions

1. Transplantation is the placement of tissue from an embryo into a suitably prepared wound.
2. Autoplastic transplantation is taking a piece of an embryo and moving it to another location on the **same embryo**.
3. Homoplastic transplantation is moving a piece of tissue from one embryo to another embryo of the **same species**.
4. Heteroplastic transplantation is moving a piece of tissue from one embryo to another embryo of different species belonging to the **same genus**.
5. Xenoplastic transplantation is moving a piece of tissue from one embryo to another embryo of a **different genus**.

The embryo from which the transplant is taken is the **donor** and the animal into which the transplant is made is the **host**. It is desirable to be able to distinguish between the host tissue and donor tissue. This is done by differential cell size, staining properties or pigmentation, and can be done by artificially staining the donor tissue.

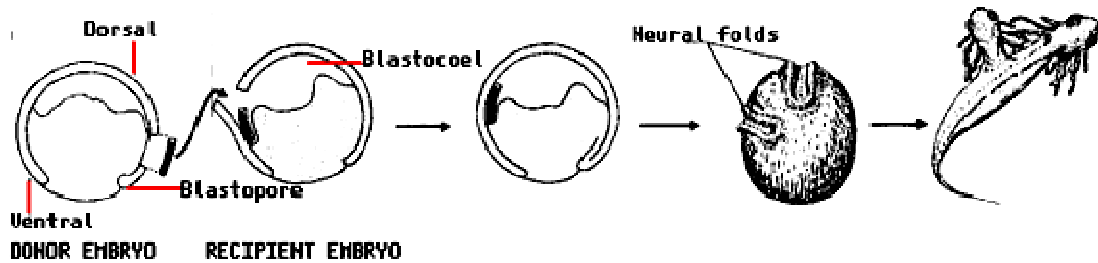
### 8.2. Spemann Experiments

Spemann (1921) performed a Heteroplastic transplantation between two species of the newt, genus *Triturus*. He transplanted a piece of presumptive neural ectoderm of one **early gastrula** embryo into the prospective epidermis region of another. The transplanted tissue developed into epidermis. Conversely, when a piece of prospective epidermis was transplanted into the presumptive neural ectoderm, it developed into part of the neural tube. Therefore, the fates of presumptive nervous tissue or presumptive epidermis are **not fixed** at the **early gastrula** stage.

Completely different results are observed if the donor embryo used is in a **late stage of gastrulation** instead of an early stage of gastrulation. If a piece of the neural plate of a **late gastrula** is transplanted into another part of the embryo it will develop into nervous tissue. If a piece of prospective epidermis is transplanted into the region of the neural plate it develops into an epidermal patch within the nervous system. The fate of the tissues becomes progressively narrowed during **gastrulation**.

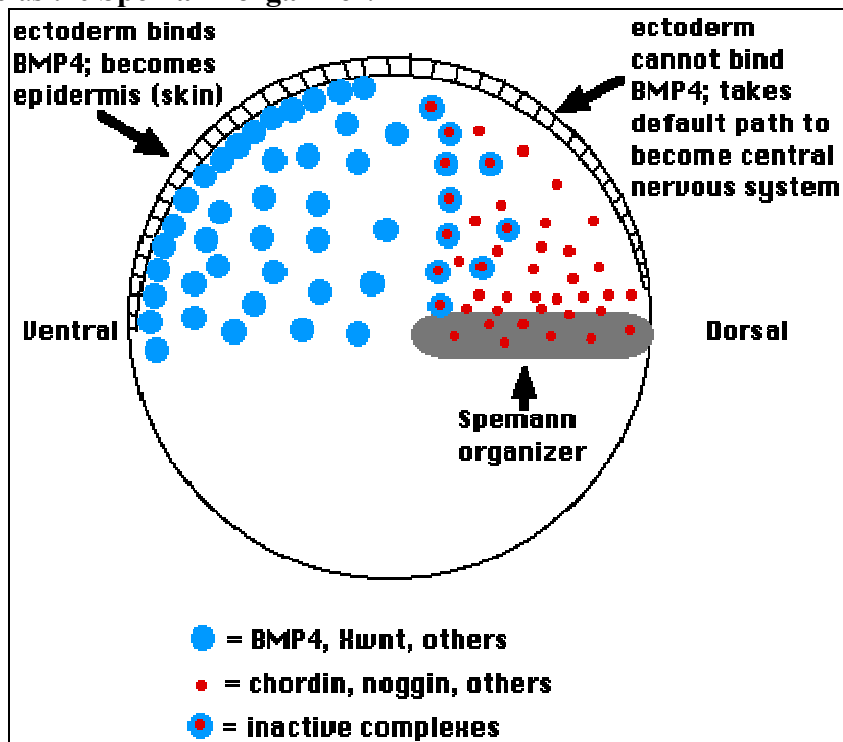
Spemann & Mangold (1924) transplanted a dorsal lip of an unpigmented newt gastrula (*Triturus cristatus*) into the ventral side of a pigmented newt gastrula (*T. taeniatus*). This

experiment won the 1935 Nobel Prize in Medicine or Physiology. Even in this inappropriate region involution occurred. In addition, **axis induction** occurred, where a **secondary axis** forms, having a gut, neural tube, notochord and somites. Because of the pigmentation difference between the species, it could be seen that much of the secondary axis is produced from host tissue. Therefore, the graft influenced the surrounding cells to develop into certain organs. This "influence" is called **embryonic induction**. The inducer is the part which is the source of the influence. The secondary axis is always parallel to the primary axis. Therefore, the host dictates secondary axis orientation. Transplant experiments with avian Henson's Nodes also give a parallel secondary axis. From this experiment and others it was shown that the **induction of the neural system** is due to the underlying tissue, namely the **chordamesoderm** (presumptive notochord and somite mesoderm).



*Fig. 8.1. Embryonic Induction*

Spemann called the **dorsal lip of the blastopore** the **primary organizer**, because of its ability, when transplanted, to initiate the development of a secondary embryo. Now, it is also referred to as the **Spemann organizer**.



*Fig. 8.2. The Spemann Organizer*

The presence of a primary organizer was discovered in **urodele amphibians**. Since then, it has been found in most vertebrate groups (all those studied) that the chordamesoderm (i.e. archenteron roof) is capable of inducing the nervous system and sense organs. The various parts which are induced to develop do so in an orderly manner, because the inducers are regionally specific.

1. The head inductor (anterior part of the chordamesoderm) induces head organs to form.
2. The trunk inductor (posterior part of the chordamesoderm) induces trunk organs and the tail bud to form.
3. The anterior part of the chordamesoderm passes over the dorsal lip of the blastopore first and the posterior part passes over last.

Therefore, the **dorsal lip** of an **early gastrula** stage embryo is a **head inductor** (and induces head organs to form).

### **8.3. The dorsal lip of a late gastrula is a trunk inductor**

During early gastrulation, primary neural induction is **planar**, with signals traveling within the plane of the marginal zone (of the amphibian fate map). During late gastrulation signals are **vertical**, traveling from the chordamesoderm to the neural ectoderm. The relative importance of these two inductions varies among amphibian species.

Kenter and Menton (1987) found the *Xenopus* ectoderm cells express N-CAM (a **neural cell adhesion molecule**) as an early response to neural induction. Therefore, in normal embryos N-CAM is expressed in the neural plate, but not in the epidermis. Isolated ectoderm will not express N-CAM, but will if it is exposed to mesoderm.

In 1933, **Holtfreter** (a collaborator of Spemann) produced **axolotl exogastrulae**, by putting them into hypertonic salt solutions after removing their fertilization membranes. There is as much N-CAM in the exogastrula ectoderm as in normal neuroectoderm, because this induction was a planar (as opposed to vertical) signal. The ectoderm forms no recognizable neural structures.

### **8.4. Mesoderm induction occurs prior to primary embryonic induction**

The inductive event between the chordamesoderm and neural tissue was originally believed to be the first to occur and was thus termed **primary induction**. However, the induction of the **mesoderm** has been found to occur before the neural induction. The types of cells can be divided into an animal hemisphere, vegetal hemisphere and equatorial or marginal cells. These names reflect their positions on the blastula.

**Nieuwkoop** (1969) cultured explants from these 3 regions, either alone or in combination, with these results:

<b>Explant</b>	<b>Tissue formed</b>	
animal	forms	epidermis
marginal	forms	mesoderm
vegetal	forms	endoderm
animal+vegetal	forms	mesoderm

Marginal cells develop into mesoderm because an inducer is secreted by the adjacent vegetal cells. **Gurdon et al** (1985), using molecular markers, and **Mohun & Wilson** (1986), with genetic transcription experiments, confirmed this idea.

In another experiment, embryos were cultured, either intact or disaggregated. When treated with a medium which makes the blastomeres lose their adhesive qualities the embryo collapses into a heap. The cells in the heap remain contiguous but no longer adhere to each other. Muscle-specific actin mRNA's (*which is unique to mesoderm*) were detected in the collapsed heap of cells. If the heap of cells are separated so they are not in physical proximity no muscle actin mRNA is transcribed. This indicates that proximity but not adhesive cell contact is necessary for mesoderm induction in *Xenopus*.

### **8.5. Identity of Mesoderm Inducing Substance**

Various molecules from diverse types of embryonic or adult cells appear to induce mesoderm. (examples: chick embryos, carp swim bladder, guinea pig bone marrow and cultured mammalian cells). The common factor is that they contain growth factors. Growth factors are released from one cell type and cause changes in other cell types. This property fits with what is expected for an inducing substance. Isolation of the inducing substances has been difficult because they are probably present in the embryo in minute quantities.

### **8.6. Analysis of the Nature of Induction**

Saxen (1961) placed a piece of millipore filter between a piece of the dorsal lip and a piece of presumptive ectoderm in *Triturus*. The presumptive ectoderm was induced to form nervous structures. Therefore, physical contact is not necessary between an organizer and the induced tissue. It was found that other tissues have inductive ability:

1. The neural plate itself and parts of the nervous system are inductors.
2. The notochord and somite mesoderm retain their ability to act as inductors long after the neurula stage.
3. Almost any adult tissue can induce neural plate formation, as well as other organ rudiments.
4. Tissues of animals of different phyla can serve as inductors. Extracts from guinea pig bone marrow, liver cells and cultured human tumor cell lines have inductive ability.
5. Therefore, the inductive substance is either widely distributed or a variety of substances can serve as the inductor. The latter is the case.

E. Wehmeier (1934) found that an aqueous extract from muscle tissue caused strong induction. Further, it was found that numerous organic substances cause induction (AMP (*adenosine monophosphate*), DNA, fatty acids and the dye, methylene blue).

Holtfreter (1947) found that exposure of presumptive ectoderm to acid or alkaline conditions for a short time caused them to be induced to form neural tissue.

### **8.7. Neural Induction by the Secreted Polypeptide Noggin**

Lamb *et al.* (1993) working in Richard Harland's lab, researchers have purified a polypeptide, **noggin**, which induces neural tissue. DNA which codes for noggin also induces neural tissue. Noggin has been found in the Spemann Organizer. More specifically, noggin induces cement glands and anterior brain, but not hind brain and

spinal cord. Human and *Xenopus* noggins have 80% amino acid identity. Both human and *Xenopus* noggin can induce neural tissue in *Xenopus*.

**Noggin** and a similar messenger, **chordin**, appear to work by **disinhibition**. That is, they interfere with an inhibitory signal, which normally blocks the default program. It has been postulated that **BMP-4 (bone morphogenetic protein 4)** is a natural **inhibitor of dorsal axis formation**.

**Noggin** and **chordin**, which are secreted by the organizer, inactivate BMP-4 signalling. This **allows dorsal development**. When isolated **marginal cells** are placed into a medium containing **noggin** they become **dorsalized**, which is demonstrated by the synthesis of muscle actin mRNA. This mRNA is abundant in skeletal muscle, which is derived from dorsal mesoderm, but not lateral or ventral mesoderm.

## **8.8. Secondary Induction**

Many other cell types originate as a result of inductive interaction throughout development of the organism; this is **secondary induction**. Inductive interactions have been classified as either **instructive or permissive**. In an instructive interaction, the inducing tissue apparently gives precise information to commit cells to a new pathway of development. Using the chick, Cairns and Saunders (1954) transplanted mesoderm from different regions of the leg or wing into foreign regions and showed that the overlying ectoderm developed according to the origin of the mesoderm. If a piece of thigh mesoderm is grafted under the ectoderm of the wing, the developing wing ectoderm will form thigh feathers above the grafted mesoderm. Such an interaction presumes that the responding tissue is relatively undetermined. Besides having a definite normal fate, called **prospective significance**, the parts of the embryo have the ability to develop into other parts, under experimental conditions. This ability of parts of an early embryo to develop into more than one type of tissue is called **prospective potency**. The narrowing of prospective potencies, which fixes the fate of the embryonic tissues, is called **determination**. After determination has occurred in a tissue that tissue is said to be determined. It now has a more narrow prospective potency.

Instead of a single instructive event precipitating the formation of the lens from the head ectoderm, recent work examining the induction of the amphibian lens suggests that induction of this tissue is a multi-step process, and there appears to be a series of inductive events that progressively bias the head ectoderm to form lens. In **permissive** interactions, responding cells are already determined and poised to differentiate; they simply require a signal from the inducing tissue to allow them to express their potential. The pancreas is a good example. It develops as an outgrowth of the gut, but its development requires a contribution from the mesoderm. If pancreatic mesoderm and endoderm are separated and cultured separately in a 9-day mouse embryo, development ceases. If they are recombined in culture, the endoderm does differentiate into normal exocrine and endocrine cells of the pancreas.

Rutter and his colleagues (1964) found that substituting a different type of mesenchyme, from the salivary gland, would cause pancreatic endoderm differentiation. This endoderm differentiation was also observed using somites (which normally produce muscle and cartilage cells) and young pureed embryos (embryo extract). Therefore, at the time the pancreatic endoderm is observable, it is apparently already determined to the extent that a relatively non-specific cue will complete the differentiation process.