7. Embryonic induction, Primary organizer, Differentiation & Competence

Embryonic induction

Study of fate-maps provides us with the knowledge of the fate or final location of various areas of the early embryo after normal embryonic development but fate-maps do not give us information on the potentialities for differentiation of the cellular components of various parts of the developing embryo. In developmental biology such knowledge is considered to be essential and hence is of great importance and is referred to as **determination.**

To gain an insight on whether the presumptive areas of a blastula are pre-determined to form specific parts or not, different methods of experimental analysis have been devised. Amongst these, the most common experimental approach has been **transplantation.** In this experimental method, from embryos of different stages small portions are cut and transplanted either at a different location in the same embryo or in another embryo. When transplantation is done in a different location of the same embryo then it is referred to as **autoplastic implantation.** If, however, the transplantation is done in some other embryo but of the same species then it is called **homoplastic implantation.** Further, if transplantation is performed between embryos of two related species belonging to the same genus then it is called **heteroplastic implantation.** However, if transplantation is done on a similar stage embryo of a distantly related species then it is called **xenoplastic implantation.** The embryo from which the part to be transplanted is removed is referred to as the **donor** and the embryo which receives this part is called the **host.**

Transplantation is a complex experimental procedure and in comparison to invertebrates it is more difficult in vertebrates. But in the frog and salamander, embryologists have obtained successful transplantational results. For suitable illustration of the concept of determination the pioneering and pathfinding transplant experiments performed by **Hans Spemann** in 1918 are described here. To study determination of the presumptive ectodermal region, that is, epidermal and neural areas of the embryo, **Spemann** performed **heteroplastic** transplant experiments between the embryos of two related species of salamanders, namely, *Triturus cristatus* and *Triturus taeniatus* (Fig. 7.1). In these experiments a small piece of the epidermal region of an early **gastrula** was

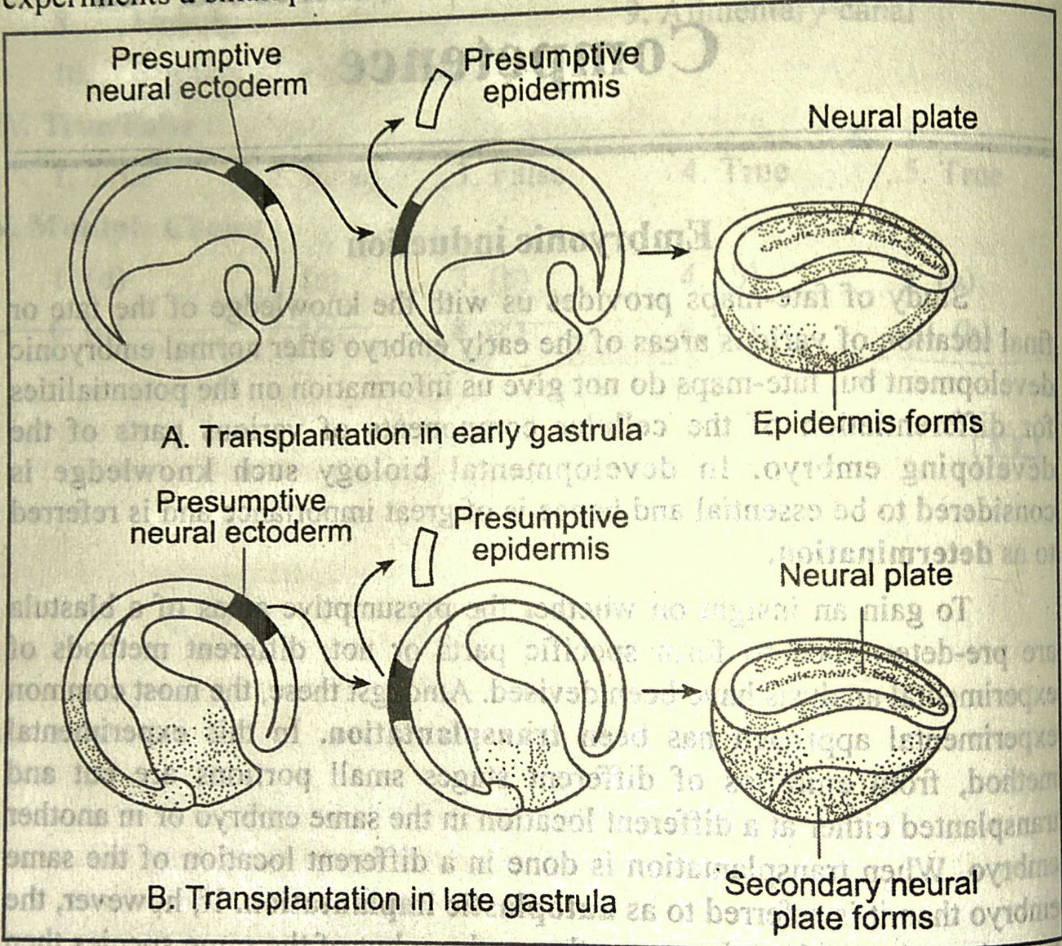

Fig. 7.1. ***Determination of ectoderm during newt gastrulation presumptive neural ectoderm from one newt embryo is transplanted into a region in another embryo that normally becomes epidermis. (A) When the transfer is done in the early gastrula, the presumptive neural tissue develops into epidermis and only one neural plate is seen. (B) When the same experiment is performed on late-gastrula tissues, the presumptive neural cells form neural tissue, thereby causing two neural regions to form on the host. (After Saxen and Toivonen, 1962).***

transplanted into the neural area of another embryo of the same stage. Consequently, according to the available new situation, the transplanted material initially developed into the neural plate and then became the part of neural canal of the host. The transplanted piece differentiated only into the neural tissue. Likewise, when a piece of neural area of an embryo is transplanted into the epidermal area of another embryo, then according to the available situation it differentiates into the epidermis.

From the above experiments it is evident that at the embryonic stages when transplantation was done the fate of the presumptive nervous system and the presumptive epidermis is not determined. Thus it can be concluded that besides the normal presumptive fate called the **prospective significance,** under experimental conditions the various regions of an embryo also have the capacity to differentiate differently. This capacity is referred to as the **prospective potency.**

Besides the above described transplant experiments with early gastrula, when similar type of experiments were done with embryos at the end of gastrulation *i.e.* late gastrula then completely opposite results were obtained. When the neural region of a donor embryo was transplanted into a different location of a late gastrula then in contrast to the given location, it always differentiated into the usual brain or spinal cord. From these experiments it can be concluded that at the end of gastrulation, in the presumptive neural region, etc. of the embryo, the capacity to differentiate according to location is lost.

It is obvious that between the early gastrula stage and late gastrula stage some fundamental changes in the developing embryo take place in which the prospective potency of the presumptive neural and epidermal region etc. is curtailed to become equal to the prospective significance, that is, a stabilization of the various regions of the embryo occurs (Table 7.1). It is this stabilization of embryonic region which is termed as **determination.** After the completion of the determination process, the different parts of the embryos are considered as **determined.** The causes of the determination are described as *Embryonic Induction.*

Table 7.1. Results of tissue transplantation during early-and late-gastrula stages in the newt

Donor region	Host region	Differentiation of donor tissue	Conclusion
Early gastrula			
Prospective neurons	Prospective epidermis	Epidermis	Dependent (conditional) development
prospective epidermis	Prospective neurons	Neurons	Depedent (conditional) development
Late gastrula			
Prospective neurons	Prospective epidermis	Neurons	Independent (autonomous) development (determined)
Prospective epidermis (determined)	Prospective neurons	Epidermis	Independent (autonomous) development

From the transplantation experiments performed in context to the determination process it was evident that the determination of the pieces of ectoderm does not occur due to intrinsic factors but the differentiation of the ectodermal parts is dependent upon the given environment or location in which they are situated. In this chain of experiments, the following experiments also established that for the determination of the development of the neural plate, contact of the ectoderm with the roof of the archenteron is necessary, that is, only those parts of the ectoderm differentiate into the neural plate and neural tissue which are in contact with the roof of the archenteron.

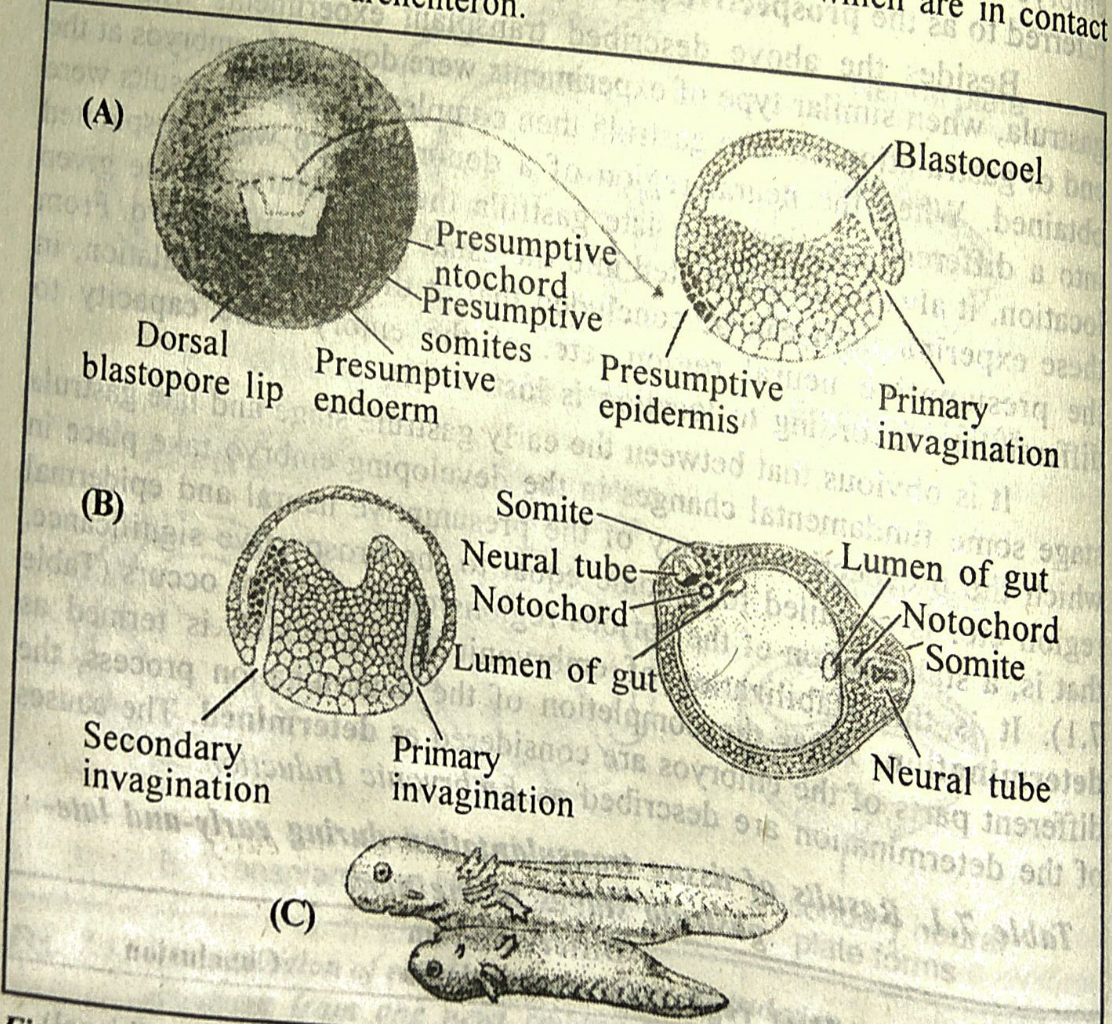

Fig. 7.2. Self-differentiation of the dorsal blastopore lip tissue. (A) Dorsal blastopore lip from early gastrula is transplanted into another early gastrula in the region that normally becomes ventral epidermis, (B) Tissue invaginates and forms a second archenteron and then a second embryonic axis. Both donor and host tissues are seen in the new neural tube, notochord, and somites, (C) Eventually, a second embryo forms that is joined to the host.

In this series, **Hilde Mangold (1924)** performed two extremely important heteroplastic transplantation experiments with the embryos of two species of salamanders. She transplanted a piece of the dorsal lip of the blastopore of an early gastrula of *Triturus cristatus* on the lateral lip of blastopore of the same stage embryo of *Triturus taeniatus*. It was noticed that a major part of the transplanted piece invaginated inside through the blastopore of the host but a narrow strip remained on the surface of the embryo. When the host embryo developed further it was observed that at the site of transplantation a complete set of additional organs developed which in relative terms was almost like a **secondary embryo** (Fig. 7.2). It is obvious that all these additional set of organs would not have developed if the host-embryo did not possess cells of the transplanted piece. From this experiment the conclusion could be drawn that the differentiation of the second set of organs was due to some specific influence of the transplanted material. Such type of effect which influences the differentiation of organs in an embryo is referred to as **embryonic induction** and the part which is the source of this induction is called the **inductor.** Another example of induction is Hensen's node (Fig. 7.3).

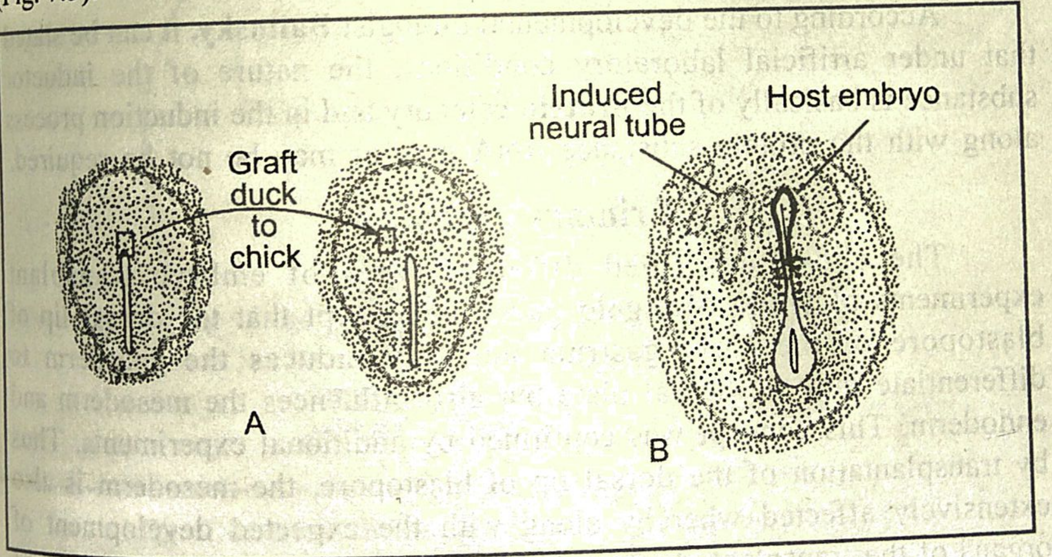

Fig. 7.3. Induction of a new embryonic axis by Hensen's node. (A) Hensen's node tissue is removed from a duck embryo and implanted into a host chick embryo, (B) An accessory neural tube is induced at the graft site. (After Waddington, 1933).

Nature of induction :

From the description of embryonic induction it is evident that the induction of the neural plate is caused by **chordamesoderm** present right below it. However, the question arises—what is the fundamental nature of induction ? In other words, what are the factors present in the inductor which are responsible for induction ? For gaining an insight on the problem, a series of investigative experiments were conducted which are sequentially and briefly described as follows :

In the initial investigation, the constituents of the dorsal lip of blastopore of the early gastrula were destroyed or killed by various methods and then transplanted into a living embryo. It was observed that even in the dead constituents, the ability for induction is not lost. It was thus concluded that the basic cause of induction, was some chemical substance synthesized and secreted by the inductor.

The next logical question was on the possible nature of the chemical inductor/inductors. As a result of detailed and extensive investigations, a series of different types of chemicals were described which were capable of embryonic induction and could act as organizers. Amongst these some of the more important ones are : lipoids, steroids, organic acids like the muscle adenylic acid = adenosine monophosphate, thymonucleic acid = DNA, linolenic acid, stearic acid, etc. Ribonucleic acid (RNA) and proteinaceous chemicals have also been shown to cause induction under artificial situations, that is, laboratory conditions.

According to the developmental biologist **Balinsky,** it can be stated that under artificial laboratory conditions, the nature of the inductor substance is basically of the **protein** category and in the induction process along with the protein substance RNA may or may not be required.

Primary organizer

The above described different types of embryo transplant experiments of **Hilde Mangold** gave the concept that the dorsal lip of blastopore of the early gastrula not only induces the ectoderm to differentiate into the neural plate but also influences the mesoderm and endoderm. This concept was confirmed by additional experiments. Thus by transplantation of the dorsal lip of blastopore, the mesoderm is also extensively affected whereby along with the expected development of organs of the transplanted piece, because of this induction, the organs of the host also differentiate and as a result almost a **secondary embryo** develops.

Appreciating the ability of the transplanted dorsal lip of blastopore to develop into an entire embryo, **Hans Spemann (1938)** referred the dorsal lip of blastopore as the **primary organizer** or **organizer.** The organizer of the gastrula, is represented by the **grey crescent** in the uncleaved egg cell or ovum. The contention that in the grey crescent of the ovum are present the specific characteristics of the organizer has also been confirmed by experimental proof.

Although the presence of the primary organizer was first of all gained in the tailed urodelan amphibians but very soon it was discovered that in the early gastrula stages of several other vertebrates the dorsal lip of the blastopore and the roof of the archenteron played a significant role in embryonic development. Different experimental evidences have specifically demonstrated that in vertebrates, in general, the **cauda-mesoderm** induces the differentiation of the nervous system and the sense organs. A ventral signal is released throughout the vegetal region of the embryo. This induces the marginal cells to become mesoderm. A signal specify the marginal cells to become posterior mesoderm. On the dorsal side (away from the point of sperm entry), a signal is released by the vegetal cell. This dorsal signal induces the formation of the Spemann organizer in the overlying marginal zone cells. (After De Robertis et al., 1972) (Fig. 7.4).

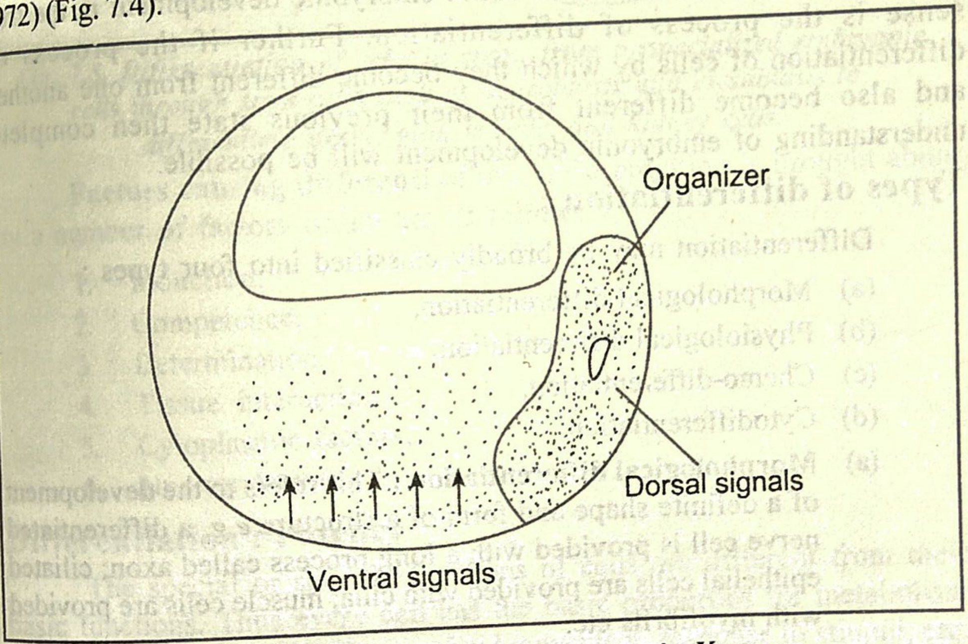

Fig. 7.4. Mode for mesoderm induction in Xenopus.

In normal embryonic development, the various developing parts are harmoneously grouped and interlinked to each other, that is, they are not haphazardly present jumbled together. In other words, it can also be said that this harmoneous state is due to **regional specificity** exhibited by the various inductors. An inductor regulates or exerts the regional specificity on the induced parts or organs. For example, the anterior part of the roof of archenteron induces the differentiation of the cephalic or head structures and this is called the **head inductor;** the rest of the archenteric roof induces the organs of the posterior trunk and of the tail-bud and is accordingly called the **spino-caudal inductor.** The head inductor in turn is further divided into two inductors : the **archencephalic inductor** wich induces the anterior brain, eyes and nose, and the **deuterocephalic inductor** which induces the differentiation of the posterior brain and the ear vesicles.

Differentiation

It is difficult to exactly define the term **differentiation** and according to **Balinsky (1970)** at least two definitions of the term can be considered.

In a primary broad sense, **differentiation** is *the process in which the cells or parts of an organism besides becoming different from one another also become altered from their earlier state.* For example, during embryonic development at the time of the induction of the neural plate by the roof of the archenteron, the cells not only become different from those of the presumptive epidermis but also become different from the part of the blastoderm from where they had basically originated. Hence, in this context it can be stated that the entire embryonic development in the true sense is the process of differentiation. Further if the process of differentiation of cells by which they become different from one another and also become different from their previous state then complete understanding of embryonic development will be possible.

Types of differentiation :

Differentiation may be broadly classified into four types :

(a) Morphological differentiation,

(b) Physiological differentiation,

(c) Chemo-differentiation,

(d) Cytodifferentiation.

(a) **Morphological differentiation :** This refers to the development of a definite shape and form of a structure *e.g.* a differentiated nerve cell is provided with a long process called axon; ciliated epithelial cells are provided with cilia; muscle cells are provided with myofibrils etc.

(b) **Physiological differentiation :** This is more or less equal to the condition when the early embryonic cells attain special ability to do a particular function. This can also be called as functional differentiation. The nerve cells are specialized to conduct impulses.

(c) **Chemodifferentiation :** This comprises the change in the chemical composition of a cell or an organ. Chemical differentiation brings about the morphological and physiological differentiation. The chemical changes are invisible. They are under the direct control of genes.

(d) **Cytodifferentiation :** The process by which cells gradually specialize to undergo change in shape and function is called cytodifferentiation.

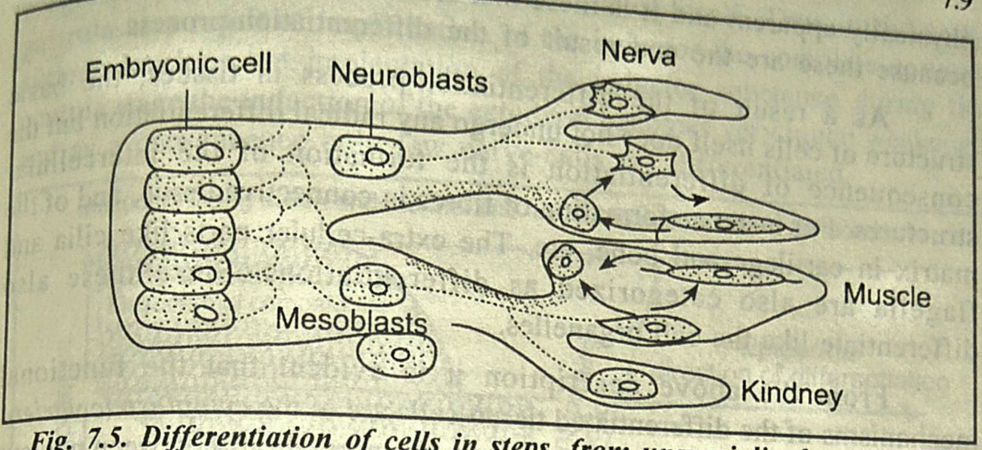

Fig. 7.5. Differentiation of cells in steps, from unspecialized embryonic cells through stem cells such as neuroblasts and mesoblasts to differentiate nerve, glia, muscle, and kidney cells.

Factors causing differentiation : Differentiation is brought about by a number of factors which are as follows :

1. Induction,
2. Competence,
3. Determination,
4. Tissue interactions,
5. Cytoplasmic factors,
6. Nuclear factors.

Differentiation : Potency

The special or specific functions of cells are different from their basic functions. Thus every cell has the basic properties for metabolism (respiration, synthesis, etc.), amoeboid movement, response to stimuli, etc. This fundamental functional capacity is present in both undifferentiated and differentiated cells. However, besides this, differentiated cells also possess specific functional properties which others do not have. The functional specificity of differentiated cells is referred to as **potency.** For example, only nerve cells have the specific functional property of transmitting nerve impulses at an extremely fast rate. Similarly, only hepatic cells are capable of secreting bile and only in pigment cells (melanocytes and melanophores), the pigment substance is synthesized (Fig 7.5).

Differentiation : Mechanism

The specific functional quality of differentiated cells is dependent upon the specific mechanisms present in them. These mechanisms can be of different types, like the structural organelles in cells; for example, *myofibrils* of muscle cells, the *cilia* of the nasal epidermis, the long *axons* of nerve cells, etc. It is evident, that all these structural features are physically apparent and it is these that are referred to as *differentia-tions* because these are the end result of the differentiation-process.

As a result of the differentiation-process in tissues, the basic structure of cells itself does not undergo any radical differentiation but the consequence of differentiation is the formation of the intercellular structures. For instance formation of fibres in connective tissue, and of the matrix in cartilage and bone, etc. The extra-cellular parts like cilia and flagella are also categorized as differentiations. Since these also differentiate like the cell organelles.

From the above description it is evident that the functional mechanisms of the differentiated tissue-cells are of the *cytoplasmic variety.* Due to these mechanisms a change from the normal ratio between nucleus and cytoplasm is brought about. It is generally agreed that in fully differentiated cells, the nucleus remains relatively unchanged but the quantity of the cytoplasm increases. The basic constituents of the nucleus are the chromosomes (which are made up of DNA) and the major constituents of the cytoplasm are the proteins. Hence for obtaining an understanding of the mechanism of differentiation the relative ratio between DNA and protein has also been investigated and as expected it has been found out that there are relative differences present between the DNA and protein content of the differentiated cells.

Competence

In context to embryonic induction, detailed and extensive research with inductors and the areas which are induced has been conducted and it has been revealed that for the induction of the development of the nervous system, the presence of presumptive notochord and presumptive mesodermal myotomal somites just below the presumptive neural tissue is essential. The next series of experiments also indicated that induction of the anterior region of the brain is brought about by the roof of the archenteron and as a consequence of induction, the development of the nervous system is completed.

In the above experiments, the inducted tissue was either the ectoderm or the presumptive ectoderm of the gastrula and different embryonic inductors induced it to develop into the nervous system. In relation to this, these experiments have also revealed that the reactive ability of the presumptive ectoderm of the embryo is maximal in the early gastrula but which during the course of gastrulation progressively declines and by the beginning of neurulation is completely lost.

From this series of experiments it has also been established that besides the loss of percentage inductive ability there also occurs a progressive quantum decrease in differentiation itself. Thus in the early gastrula as a result of implantation of the inductor the complete nervous system develops but implantation of the inductor substance during the neurula stage the induction of the ectoderm becomes very much weakened and as a consequence only few nerve-cells are differentiated.

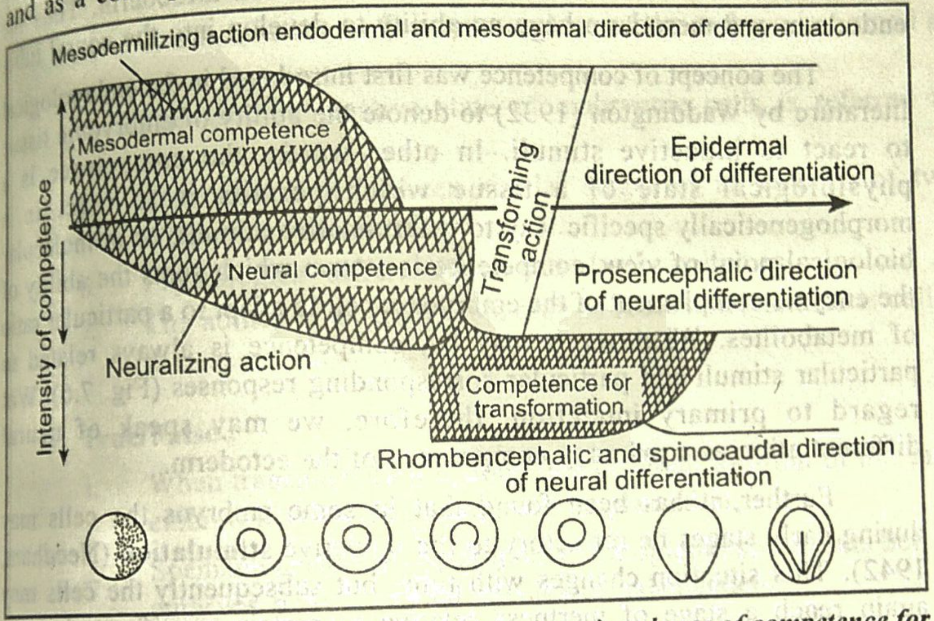

Fig. 7.6. ***Diagrammatic presentation of the successive phases of competence for endodermal and mesodermal differentiation, prosencephalic neural differentiation, and rhombencephalic and spinocaudal neural differentiation of the presumptive ectoneuroderm. In the absence of any inductive action, the ectoderm develops exclusively in an epidermal, direction (After Berrill, 1971).***

The experiments on the induction phenomenon also showed that for proper interaction with the inductor and as a consequence to differentiate into the neural tissue, the reacting cells of the embryo must also be in a suitably reactive state. This specific reactive state of embryonic cells is referred to as **competence** or competence is the ability of an embryonic area to react to a stimulus.

It can be thus said that the ability of *neural induction* is present specifically in the ectoderm but this capacity is present only for a limited period. If during this period induction of the presumptive ectoderm to differentiate into nervous system does not occur, then it will differentiate into the epidermis since for initial differentiation of the epidermis any special induction or inductor is not necessary.

The competence is a time limited phenomenon with a beginning and an end. As the age of the embryo advances, the competence of the various structures gets gradually reduced. For example, the ectoderm of a tadpole cannot develop into the neural tube in a later stage. The ectoderm is competent enough to develop into the neural tube under the influence of chorda mesoderm. But the endoderm and the mesoderm can not develop into the neural tube under the influence of chorda mesoderm. Thus the endoderm and mesoderm have no ability to develop into the neural tube.

The concept of competence was first introduced in the embryological literature by Waddington (1932) to denote the ability of embryonic tissue to react to inductive stimuli. In other words, the competence is a physiological state of a tissue which permits it to react in a morphogenetically specific way to determinative stimuli, or in molecular biological point of view, competence is a term which sums the ability of the enzyme complement of the embryonic cell to adapt to a particular ratio of metabolites. Whatever it may be, competence is always related to particular stimuli and particular corresponding responses (Fig. 7.6). Wht regard to primary induction, therefore, we may speak of neural differentiation as a **primary competence** of the ectoderm.

Further, it has been found that in some embryos the cells may during early stages be refractory to the inductive **stimulation** (Needham, 1942). This situation changes with time, but subsequently the cells may again reach a stage of inertness relative to certain specific embryonic inductors. Embryonic cells thus may exist in two temporarily distinct states of incompetence. Lovtrup (1974) has introduced the names **precompetence** and **postcompetence** for these phases.

QUESTIONS

I. Long Questions :

1. Give a detailed account of embryonic induction.
2. Illustrate differentiation. Describe various types of differentiation and factors causing it.
3. Explain in detail the competence giving suitable examples.

II. Short Questions (5-7 Lines) :

1. What is competence ?
2. What is primary organizer ?
3. Define prospective potency.
4. Describe various types of transplantation.
5. Illustrate regional specificity.
6. Define potency.
7. Descri...