CELL DETERMINATION AND DIFFERENTIATION

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In the earlier section we have seen that fate maps show the areas of embryo that will produce different tissues and organs in the adult and cell fate describes what a cell will become in the future during the normal course of development. We have also learnt that the fate of a particular cell can be traced by labelling it and subsequently observing what structure it will become a part of. The **developmental potential** or the **potency** of a cell describes the range of different cell types that it can give rise to. The zygote and very early blastomeres formed in it by cleavage are **totipotent**. This means that each of the very early blastomere of the zygote has the potential to develop into a complete organism as was demonstrated by experiments conducted by Dreisch (Refer again to Fig 10.4). Totipotency is not seen commonly after the first few divisions in the blastula. As development proceeds, the developmental potential of individual cells decreases until their fate is "**determined".** This means that each cell becomes committed to form a part of a specific structure and so its fate is determined for following a particular path of development. **Differentiation** on the other hand, is the process during which the cells stop dividing and acquire the unique structure and functional properties of a particular cell type. This is thus, the last stage in the process when an undifferentiated cell undergoes a series of events to become a specialized cell type like a muscle cell or nerve cell or skin cell etc.

10.6.1 Cell Determination

You are aware that blood cells differ vastly in morphology and function from each other and from other specialised cells like the muscle cells, though all of them arise from the same germ layer namely, mesoderm (refer to Fig 10.10 again). However, before these differences arise there is a period when these cells in the mesoderm do not look different from their neighbouring cells despite the fact that their fate has been already determined. This means that they are **committed** to follow a specific path of development to form particular types of cells

The process of commitment takes place in two steps:

a) **Specification**: The fate of the cell is known to have been specified when it is capable of differentiating autonomously even if it is placed in an environment that is neutral like a culture medium in a petri dish (Fig.10.14 a). However, at this stage the commitment of the cell is flexible and it can be influenced by its environment to become another type of cell rather than what it **was specified** or fated to be (see Fig.10.14 b).

b) **Determination**: this step is after **specification** of the cell to form a particular type of cell. In the stage of determination of the cell the fate of the cell becomes inflexibly or irreversibly specified or determined to form a particular type of cell. As a result, the cell will still autonomously differentiate into its original specified fate and its differentiation into a

particular type of cell will be independent of its environment or its position in the embryo it (see Fig.10.14 c).

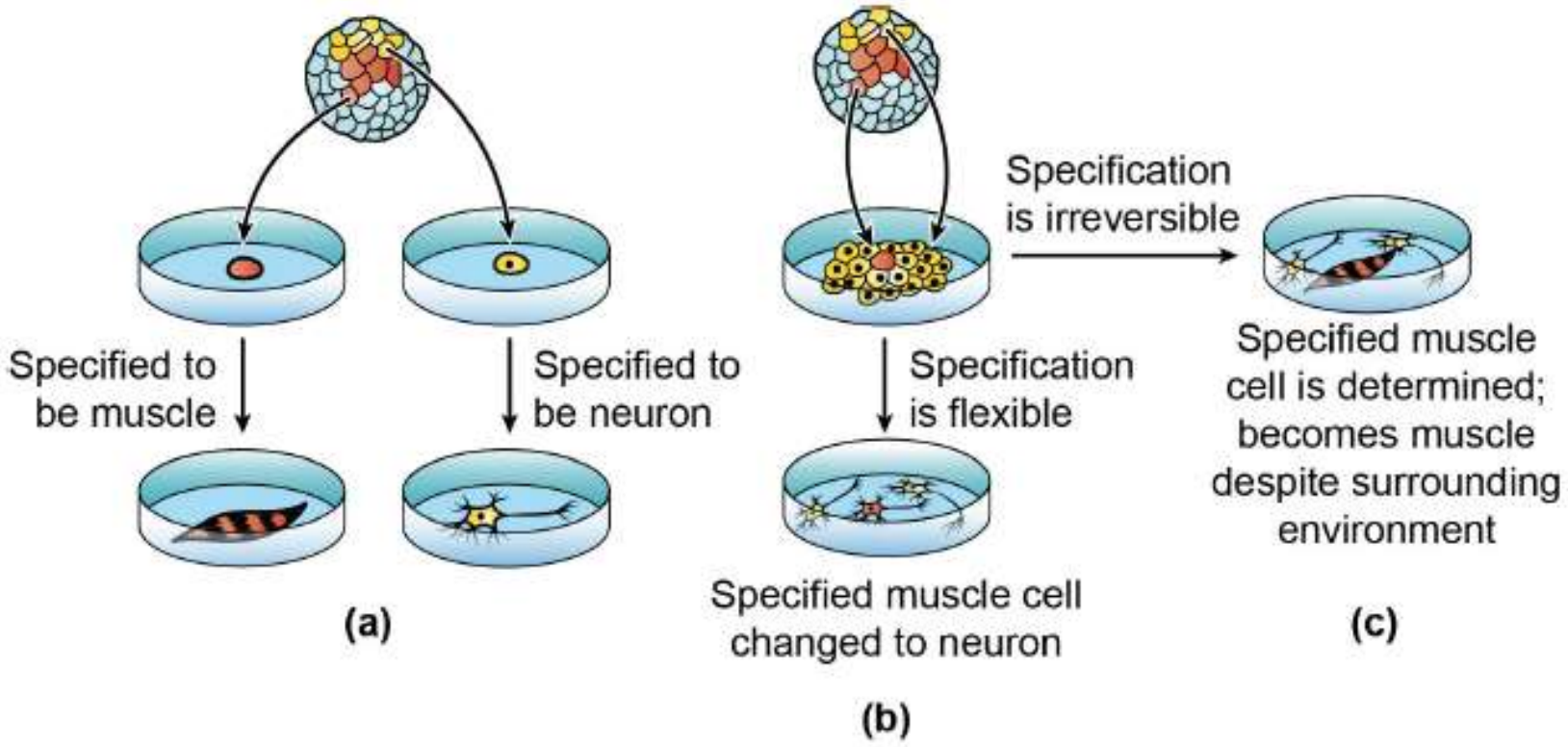

Fig.10.14: Two differently positioned cells that are fated to form muscle and neuron cells have been taken and isolated from the blastula and grown in a petri dish. a) blastomeres that are specified for muscle cells differentiate into muscle cells and those specified for neuronal cells differentiate into neurons; b) If the blastomere specified but not determined for forming muscle cells is placed in a cluster of neuronal tissue it differentiates into neural cells; c) if the blastomere is already in the determined phase then it will differentiate into muscle cells even if it is placed with neuronal tissue.

10.6.2 Cell Differentiation

Cytological studies in the early 20th century established the fact that all the somatic cell arising from the fertilized egg contained the same chromosomal complement. This fundamental concept is known as **genomic equivalence**. In this section we will learn how this concept has been proved to be true by the help of some path breaking experiments. This concept of genomic equivalence has raised several questions. For instance if all the cells contained the same genomic complement then how do cells become different from each other? Furthermore, why do only some cells like the red blood cells (RBC) make haemoglobin proteins which are never produced in other cells of the body? Or why insulin hormone is produced and secreted only from certain cells of the pancreas and never in the kidneys or in the brain? By 1960s, based on experimental evidence, the concept of **differential gene expression** came into existence to provide answer to these and several other questions and to explain how similar looking cells with the same type of genetic complement differentiate into different types of cells.

Genomic Equivalence

The best test of whether all somatic cells contain the same complement of genes as the fertilized egg from which they have arisen is to check if the nuclei of the differentiated somatic cells still have the ability or potency to generate all types of cells. If indeed this is the case then a nucleus taken from one type of cell in the body should be totipotent (having the ability to produce all types of cells) and if transplanted into an activated enucleated (nucleus removed) cell should be able to give rise to all the cells of the body.

In the 1950s, Robert Briggs and Thomas King conceptualized and conducted the experiments for determining the totipotentency of the nucleus of early embryonic cells .In their experiment they first combined the technique of enucleation and activation of an oocyte of the leopard frog *Rana pipens*. The oocyte was activated by pricking it with a sterile needle which provided it with the necessary stimulus to undergo all the cytoplasmic and biochemical rearrangements associated with fertilization, including the completion of second meiotic division at the animal pole of the cell. Puncturing the oocyte at this point let the spindle and chromosomes flow out of the cell (enucleation). The nucleus from a donor cell was then removed and by using a micropipette

was inserted into this activated, enucleated cell. Briggs and King demonstrated that blastula (early stage embryo) nuclei when transferred into activated enucleated oocytes could direct the development of a completely formed tadpole (Fig.10.18). They also found that while the nuclei at the blastula stage were totipotent, there was a dramatic decrease in the nuclear potency of cells at later stages. For instance, when nuclei from somatic cells of the tail bud region of tadpoles were used in a similar experiment, normal development did not occur. Thus it was concluded that nucleus from developing embryonic cells appeared to lose the ability to direct development as they underwent determination and differentiation. Further work continued using nuclear transplantation studies and in 1962 John Gurdon a PhD student in Cambridge University showed that if the nucleus of a fertilised frog egg was replaced with a nucleus from the cell of the tadpole intestine, the egg could develop into a new frog. Though the success rate was low, it proved that the nucleus of a mature cell still contained the genetic information needed to build all cell types. This was a major landmark in animal development though the acknowledgment of the importance of his work came much later. It was 40 years after his first experiments with nuclear transformation that the Nobel Prize was awarded in 2012 to Gurdon along with Shinya Yamanake, whose lab induced pluripotent cells.

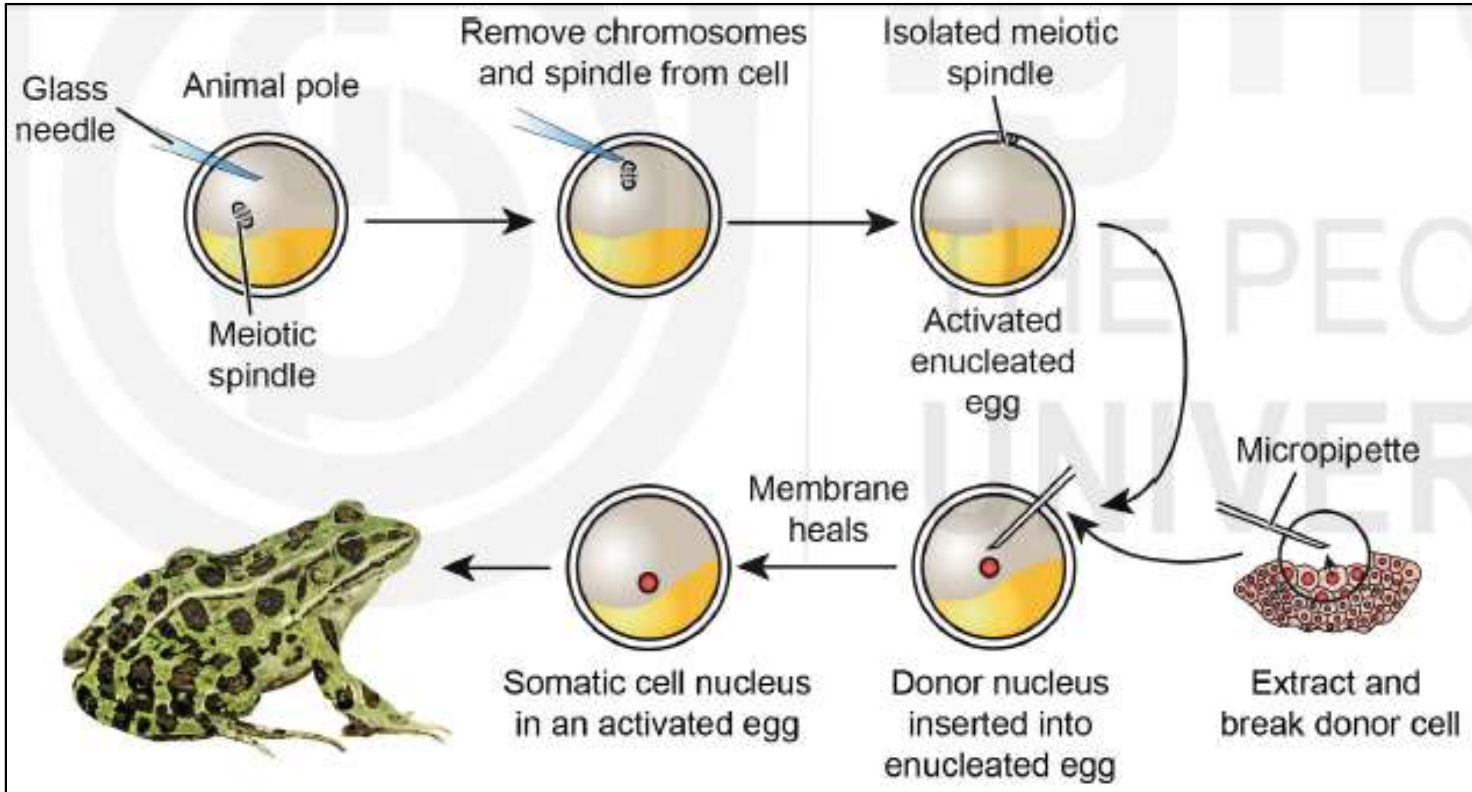

Fig.10.18: Procedure used in enucleation and transplantation of a nucleus from a somatic cell in *Rana pipens*.

Fig.10.19: Cloned Sheep Dolly

Nuclear transplantation techniques were used by experimental embryologists to create clones of several species over the years. In 1997 Ian Wilmut and his colleagues demonstrated that somatic cells still had the potency to produce all the cells of the body. The result was Dolly (Fig.10.19), a sheep that was cloned by using the nucleus taken from the mammary gland of an adult sheep and transplanting it into the egg taken from another strain of sheep. This was done in a culture and later the eggs containing the transplanted nucleus were implanted into the uteri of pregnant sheep. Of the 434 sheep oocytes only one survived to become Dolly. DNA analysis however, confirmed that Dolly's cells had indeed been derived from the donor nucleus. This was the final proof of

genomic equivalence of somatic cells and that the genome is conserved during differentiation of cells. Since the first cloning experiments, cloning of adult mammals has been done in mice, rats, guinea pigs, rabbits, dog, cat, cows and horses. Figure 10.20 shows the cloning of rats using two different types of rats by using similar techniques that were used for cloning Dolly

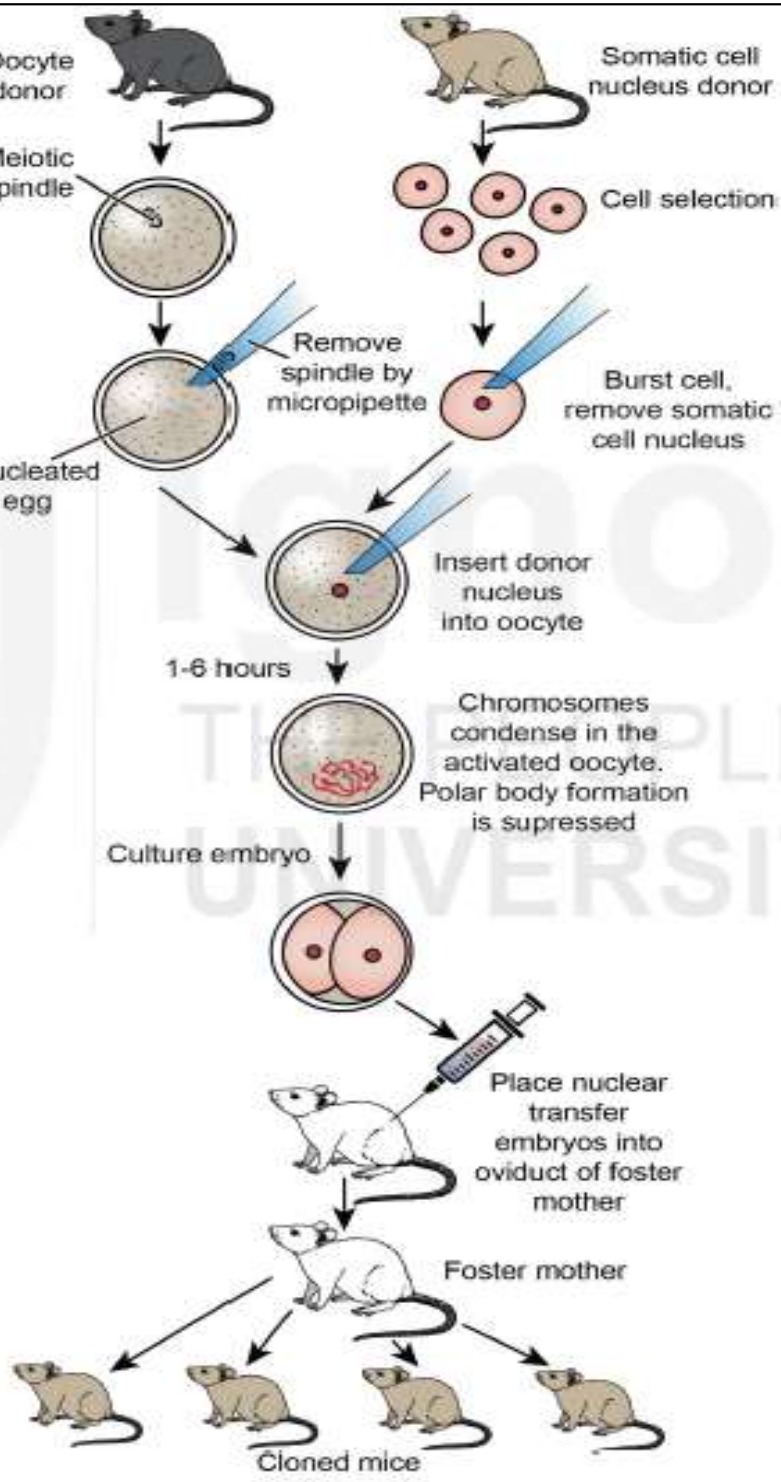

Fig. 10.20: Cloning of mice by employing similar techniques that were used to clone the sheep of Dolly.

You would certainly have heard about stem cells and their use in medical research. Cell mechanisms of determination and differentiation are the basis of this research. Stem cells refer to cells present in embryos and adult that retain their ability to differentiate into many kinds of cells. In humans, stem cells have been found in bone marrow, brain, in some muscles, skin and liver. These cells can normally differentiate into a limited number of cell types. Because of this they are referred to as multipotent cells and not totipotent. Under normal conditions our bodies use the stem cells to regenerate and replace tissue such as skin, blood, liver etc. Stem cell research hopes to exploit the multipotent characteristics of cell in order to regenerate damaged and diseased tissue and organs. Research is going on with some success in stem cell therapy to regrow damaged spinal cord tissue, replace diseased heart muscle tissue and skin tissue and in search of cures for cancer which is really a disease of abnormal cell division and differentiation patterns.