MZ - 104, UNIT - II

TOTIPOTENCY

Totipotency is defined as the ability of a single cell to divide and produce all the differentiated cells in an organism, including extraembryonic tissues. Totipotent cells formed during sexual and asexual reproduction include spores and zygotes. In some organisms, cells can dedifferentiate and regain totipotency. For example, a plant cutting or callus can be used to grow an entire plant. Mammalian development commences when an oocyte is fertilized by a sperm forming a single celled embryo, the zygote.

The zygote is totipotent, meaning that this single cell has the potential to develop into an embryo with all the specialized cells that make up a living being, as well as into the placental support structure necessary for fetal development. Thus, each totipotent cell is a self-contained entity that can give rise to the whole organism. This is said to be true for the zygote and for early embryonic blastomeres up to at least the 4-cell stage embryo.

Experimentally, totipotency can be demonstrated by the isolation of a single blastomere from a preimplantation embryo and subsequently monitoring its ability to support a term birth following transfer into a suitable recipient. This approach was pioneered in rats and has been realized in several mammalian species including nonhuman primates. The ability of isolated blastomeres from 2- and 4-cell stage, IVF produced embryos of the rhesus monkey to support term pregnancies and to produce live animals. As embryo development progresses to the 8-cell stage and beyond depending on the species, the individual blastomeres that comprise the embryo gradually lose their totipotency. It is generally believed that this restriction in developmental potential indicates irreversible differentiation and specialization of early embryonic cells into the first two lineages, the inner cell mass (ICM) that includes cells that will give rise to the fetus and the trophectoderm (TE), and an outer layer of cells that is destined to an extraembryonic fate.

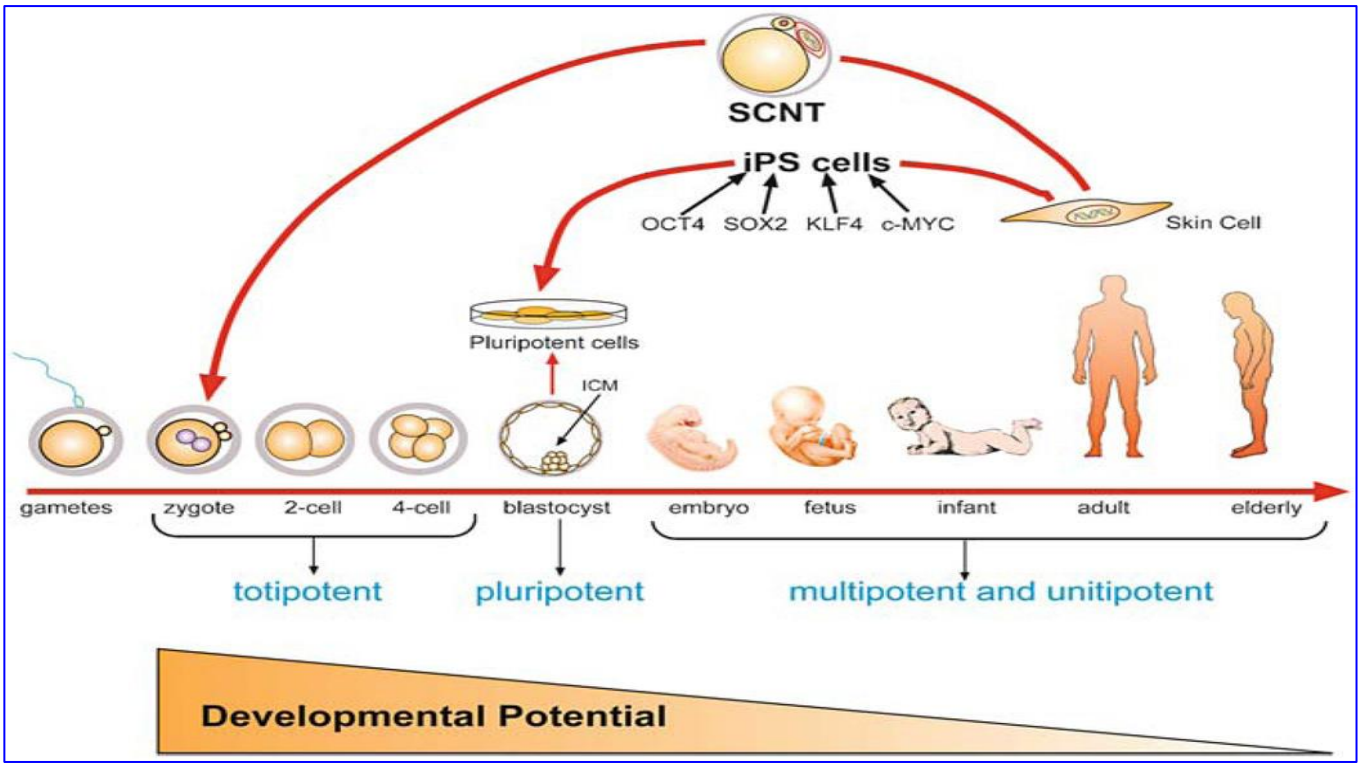

Fig 1: explains the Development and reprogramming. Ontogeny begins from a single cell, the zygote. The zygote and each blastomere of the early embryo are totipotent with the potential to develop into the whole organism. As development unfolds, the developmental potential of individual blastomeres gradually declines resulting subsequently in pluripotent, multipotent, unipotent and terminally differentiated somatic cells. However, developmental potential of somatic cells can be reinstated to the totipotent stage by SCNT or to the pluripotent state by direct reprogramming

A complication in assessing the state of potency of blastomeres isolated from more advanced stages of development is insufficient cytoplasmic volume. Thus, although the blastomeres may in fact be totipotent, embryonic development of relatively small isolated blastomeres arrests at or near the time of blastulation. The zygote and early blastomeres undergo several unusual mitotic or cleavage divisions that are not accompanied by a corresponding growth of cytoplasm, that is, there is no change in embryo size despite the presence of more cells or blastomeres and each individual blastomere becomes smaller. The embryonic genome at these early stages is transcriptionally quiescent and development is regulated by maternally inherited factors present at the time of fertilization in the oocyte. The transition in developmental regulation with activation of the embryonic genome and a complete loss of dependence on oocyte factors occurs before the blastocyst stage in a species-specific manner. Additionally, by the late morula or early blastocyst stage the embryo ceases cleavage divisions and resumes normal mitotic divisions with concomitant increases in cell volume during the S-phase.

The likelihood that early blastomeres retain totipotency for a major part of preimplantation development but experimentally we cannot prove it is directly supported by the fact that the addition of oocyte cytoplasm to a blastomere of the 8- to 16-cell stage embryo can restore, or perhaps more appropriately allow expression of, its full developmental potential. This approach, embryonic cell nuclear transfer, has been employed in the monkey to

demonstrate the totipotency of 8- to 16-cell stage blastomeres whereby reconstructed embryos when transferred to a recipient resulted in a term birth.

It is also known that conglomerates of embryonic cells at a later stage of development can develop into an organism. An experimental manipulation that supports this concept involves blastocyst splitting. Cutting the embryo into halves with an approximately equal distribution of TE and ICM cells can lead to the production of viable infants. Obviously, embryo splitting that creates demi embryos with highly distorted ratios of ICM to TE cells is inconsistent with the production of live births.

Pluripotency

It is "having more than one potential outcome." In cell biology, the definition of pluripotency has come to refer to a stem cell that has the potential to differentiate into any of the three germ layers: endoderm, mesoderm or ectoderm. Pluripotent stem cells can give rise to any fetal or adult cell type. However, a single cell or a conglomerate of pluripotent cells cannot develop into a fetal or adult animal because they lack the potential to organize into an embryo. In contrast, many progenitor cells that are capable of differentiating into a limited number of cell fates are described as multipotent. Somatic stem cells such as neural, bone marrow-derived, or hematopoietic cells would fit into this latter category.

At least some of the embryo's ICM cells are pluripotent, meaning that they can form virtually every somatic and germ cell type in the body. These ICM cells are self sustained and their pluripotency is maintained by endogenously expressed factors. In vivo, pluripotent cells within the ICM exist transiently; as the developmental program unfolds they differentiate into cells of the next embryonic or fetal stage. However, they can be isolated, adapted and propagated *in vitro* in an undifferentiated state as embryonic stem cells (ESCs). ESCs were first derived in 1981 from the ICM of the inbred mouse by Martin and Evans and Kaufman. In 1998, ESCs were successfully isolated from surplus, IVF-produced human embryos.

ESCs express specific markers or characteristics similar but not identical to the transient pluripotent cells of an embryo. This includes stage specific embryonic antigens, enzymatic activities such as alkaline phosphatase and telomerase, and "stemness" genes that are rapidly down-regulated upon differentiation, including *OCT4* and *NANOG*. Under specific conditions, ESCs can proliferate indefinitely in an undifferentiated state, suggesting that the transcriptional activity and epigenetic regulators capable of supporting pluripotency can be maintained *in vitro* in ESCs. However, when released from the influence of these culture conditions or following their introduction back into a host embryo, ESCs retain their ability to differentiate into any cell-type, just like ICM cells. Alternatively, they can differentiate *in vivo* in teratomas into cells representing the three major germ layers: endoderm, mesoderm and ectoderm or they can be directed to differentiate in vitro into any of the 200+ cell types present in the adult body. Since many human diseases result from defects in a single cell type, pluripotent human ESCs may become an unlimited source of any cell or tissue type for replacement therapy thus providing a possible cure for many devastating diseases.

Parenthetically, one of the challenges before clinical transplantation studies involving hESCs can begin concerns the immune response anticipated after transplantation. Human ESCs are routinely derived from IVF embryos and transplantation of such cells into genetically unrelated patients will incite an immune response and result in rejection. Histocompatibility is one of major unsolved problems in transplant medicine. Rejection of unmatched transplanted tissues is provoked by alloantigens present on graft tissues by the recipient's immune system.

The alloantigens or antigenic proteins on the surface of transplant tissues that mostly cause immune rejection are the blood group antigens (ABO) and the major histocompatibility complex (MHC) proteins, also designated in humans as human leukocyte antigens (HLA). Matching donor and recipient HLA types is important to reduce a cytotoxic T-cell response in the recipient, and subsequently improve the chances of survival of the transplant. However, tissue or organ transplantation from one individual to another is a daunting task due to the existence of two classes of HLA molecules (Class I, and II), each encoded by multiple genes and most importantly, each of these genes represented by multiple alleles. For example, there are 22 different alleles identified so far for the class I HLA-A gene and 42 alleles for HLA-B. Thus, due to HLA polymorphism, the chances of finding a donor–recipient match based on just a few HLA genes (HLA-A, -B, and -DR) could be one in several million. Therefore, the need for developing approaches for deriving histocompatible pluripotent cells is commonly recognized.

What is the difference between totipotent, pluripotent, and multipotent?

Totipotent cells can form all the cell types in a body, plus the extraembryonic, or placental, cells. Embryonic cells within the first couple of cell divisions after fertilization are the only cells that are totipotent. Pluripotent cells can give rise to all of the cell types that make up the body; embryonic stem cells are considered pluripotent. Multipotent cells can develop into more than one cell type, but are more limited than pluripotent cells; adult stem cells and cord blood stem cells are considered multipotent.

Cell potency is a cell's ability to differentiate into other cell types. The more cell types a cell can differentiate into, the greater its potency. Potency is also described as the gene activation potential within a cell, which like a continuum, begins with totipotency to designate a cell with the most differentiation potential,Pluripotency, multipotency, Oligopotency and Unipotency.

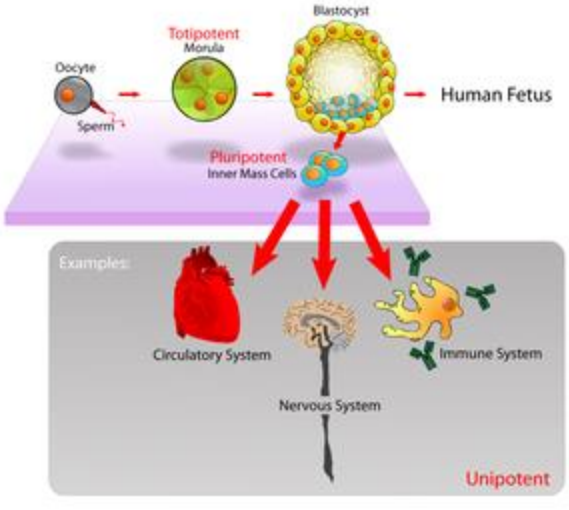

Fig: Pluripotent, embryonic stem cells originate as inner mass cells within a bastocyst. These stem cells can become any tissue in the body, excluding a placenta. Only the morula's cells are totipotent, able to become all tissues and a placenta.

Totipotency

Totipotency (Lat. *totipotentia*, "ability for all [things]") is the ability of a single cell to divide and produce all of the differentiated cells in an organism. Spores and zygotes are examples of totipotent cells. In the spectrum of cell potency, totipotency represents the cell with the greatest differentiation potential, being able to differentiate into any embryonic cell, as well as any extraembryonic cell. In contrast, pluripotent cells can only differentiate into embryonic cells.

A fully differentiated cell can return to a state of totipotency.The conversion to totipotency is complex and not fully understood. In 2011, research revealed that cells may differentiate not into a fully totipotent cell, but instead into a "complex cellular variation" of totipotency.Stem cells resembling totipotent blastomeres from 2-cell stage embryos can arise spontaneously in mouse embryonic stem cell cultures and also can be induced to arise more frequently *in vitro* through down-regulation of the chromatin assembly activity of CAF-1.

The human development model can be used to describe how totipotent cells arise.Human development begins when a sperm fertilizes an egg and the resulting fertilized egg creates a single totipotent cell, a zygote. In the first hours after fertilization, this zygote divides into identical totipotent cells, which can later develop into any of the three germ layers of a human (endoderm, mesoderm, or ectoderm), or into cells of the placenta (cytotrophoblast or syncytiotrophoblast). After reaching a 16-cell stage, the totipotent cells of the morula differentiate into cells that will eventually become either the blastocyst's Inner cell mass or the outer trophoblasts. Approximately four days after fertilization and after several cycles of cell division, these totipotent cells begin to specialize. The inner cell mass, the source of embryonic stem cells, becomes **pluripotent**.

Research on *Caenorhabditis elegans* suggests that multiple mechanisms including RNA regulation may play a role in maintaining totipotency at different stages of development in some species. Work with zebrafish and mammals suggest a further interplay between miRNA and RNA-binding proteins (RBPs) in determining development differences.

Primordial germ cells

In mouse primordial germ cells, genome-wide reprogramming leading to totipotency involves erasure of epigenetic imprints. Reprogramming is facilitated by active DNA demethylation involving the DNA base excision repair enzymatic pathway. This pathway entails erasure of CpG methylation (5mC) in primordial germ cells via the initial conversion of 5mC to 5-hydroxymethylcytosine (5hmC), a reaction driven by high levels of the ten-eleven dioxygenase enzymes TET-1 and TET-2.

Pluripotency

In cell biology, pluripotency (Lat. *pluripotentia*, "ability for many [things]") refers to a stem cell that has the potential to differentiate into any of the three germ layers: endoderm (gut, lungs, yolk sac), mesoderm (muscle, skeleton, blood vascular, urogenital, dermis), or ectoderm (nervous, sensory, epidermis), but not into extra-embryonic tissues like the placenta. However, cell pluripotency is a continuum, ranging from the completely pluripotent cell that can form every cell of the embryo proper, e.g., embryonic stem cells and iPSCs, to the incompletely or partially pluripotent cell that can form cells of all three germ layers but that may not exhibit all the characteristics of completely pluripotent cells.

Multipotency

Hematopoietic stem cells are an example of multipotency. When they differentiate into myeloid or lymphoid progenitor cells, they lose potency and become oligopotent cells with the ability to give rise to all cells of its lineage.

Multipotency is when progenitor cells have the gene activation potential to differentiate into discrete cell types. For example, a hematopoietic stem cell —and this cell type can differentiate itself into several types of blood cell like lymphocytes, monocytes, neutrophils, etc., but it is still ambiguous whether HSC possess the ability to differentiate into brain cells, bone cells or other non-blood cell types.

Research related to multipotent cells suggests that multipotent cells may be capable of conversion into unrelated cell types. In another case, human umbilical cord blood stem cells were converted into human neurons. There is also research on converting multipotent cells into pluripotent cells.

Multipotent cells are found in many, but not all human cell types. Multipotent cells have been found in cord blood, adipose tissue, cardiac cells, bone marrow, and mesenchymal stem cells (MSCs) which are found in the third molar.
MSCs may prove to be a valuable source for stem cells from molars at 8–10 years of age, before adult dental calcification. MSCs can differentiate into osteoblasts, chondrocytes, and adipocytes.

Oligopotency

In biology, oligopotency is the ability of progenitor cells to differentiate into a few cell types. It is a degree of potency. Examples of oligopotent stem cells are the lymphoid or myeloid stem cells. A lymphoid cell specifically, can give rise to various blood cells such as B and T cells, however, not to a different blood cell type like a red blood cell. Examples of progenitor cells are vascular stem cells that have the capacity to become both endothelial or smooth muscle cells.

Unipotency

In cell biology, a unipotent cell is the concept that one stem cell has the capacity to differentiate into only one cell type. It is currently unclear if true unipotent stem cells exist. Hepatoblasts, which differentiate into hepatocytes (which constitute most of the liver) or cholangiocytes (epithelial cells of the bile duct), are bipotent. A close synonym for *unipotent cell* is *precursor cell.*

N. **B.** Some abbreviations used are: SCNT=Somatic Cell Nuclear transfer; iPS = Induced pluripotent Stem cells; OCT4=Octamer binding transcription factor 4; NANOG=Nanog homeobox, transcription factor, hESCs=human embryonic stem cells

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