Cell differentiation and development of a multicellular organism.

Differentiation. Morphogenesis. Ontogenesis. Stem cells. Totipotency. Pluripotency. Ageing mechanisms of the body.

1. Give the definitions to the following terms: cell differentiation, morphogenesis, embryogenesis, ontogenesis, stem cells, totipotency, pluripotency.
2. Explain how the level of expression of various genes changes during cell differentiation and at different stages of development of a multicellular organism.
3. Describe the use of stem cells in medicine and cosmetology, analyze the advantages and disadvantages of these methods.
4. Analyze the various theories of aging in the body and the possible relationship of the aging process with stem cells and molecular biological processes.

Living organisms can be made of a single cell, such as bacteria and protists, or they can be multicellular, like plants, animals, and fungi. Unicellular organisms, like bacteria, are able to perform all life functions within one single cell. They can transport molecules, metabolize nutrients, and reproduce within this one cell.

Multicellular organisms need many different types of cells to carry out the same life processes. Each of these special types of cells has a different structure that helps it perform a specific function. Humans have many different types of cells with different jobs, such as blood cells that carry oxygen and nerve cells that transmit signals to all parts of the body

An animal or plant starts its life as a single cell—a fertilized egg. The entire set of transformations of an individual from the moment of inception to his death is called ontogeny. The development of a whole organism from a fertilized egg is a complex and multi-stage process called embryogenesis. In the process of embryonic development, many phenomena occur, which together can be characterized as an interconnected and mutually consistent process of reproduction, growth, differentiation and specialization of cells, the progenitor of which is 1 fertilized cell.

. Differentiation is the process of the emergence and development of structural and functional differences between initially homogeneous embryonic cells, as a result of which specialized cells, tissues and organs of a multicellular organism are formed. Cell differentiation is an essential part of the formation of a multicellular organism.

During development, this cell divides repeatedly to produce many different cells in a final pattern of spectacular complexity and precision. In a developing embryo, all these processes are happening at once, in a kaleidoscopic variety of different ways in different parts of the organism.

(1) *cell proliferation*, producing many cells from one,
(2) *cell specialization*, creating cells with different characteristics at different positions,
(3) *cell interactions*, coordinating the behavior of one cell with that of its neighbors,
(4) *cell movement*, rearranging the cells to form structured tissues and organs

Cell differentiation is the process by which cells become specialized in order to perform different functions. The processes of organizing differentiated cells into tissues and organs are called morphogenesis.

Features of cell differentiation include: The potential of differentiation gradually appears with the development of the individual. During embryonic development, the cells gradually change from "all-around" to "multi-energy", and finally to the "single-energy", which is the general rule of cell differentiation. In the process of individual development, multicellular biological cells have both temporal differentiation and spatial differentiation; cell differentiation is compatible with the state and speed of cell division, and differentiation must be based on division, that is, differentiation is inevitable with division, but the dividing cells do not

necessarily need differentiate. The higher the degree of differentiation, the worse the ability to divide; the cell differentiation is highly stable. Under normal physiological conditions, cells that have differentiated into a specific, stable type are generally impossible to reverse to undifferentiated state or become other types. In the general case, differentiation is irreversible, i.e. highly differentiated cells cannot transform into cells of another type. This phenomenon is called terminal differentiation and is inherent mainly in animal cells. Differences manifested in the process of differentiation are retained by the cells during reproduction, that is, they are hereditarily fixed (for example, liver cells, when multiplying, produce only liver cells, and muscle cells - only muscle cells, etc.).

Cell differentiation is plastic, and the differentiated cells re-enter the undifferentiated state or transdifferentiate into another type of cell under special conditions. Under certain conditions, the differentiated cells are also unstable, and their gene expression patterns can also undergo reversible changes and return to their undifferentiated state. This process is called dedifferentiation.

Differentiation mainly occurs in the embryonic period, as well as in the early stages of postembryonic development. In addition, differentiation occurs in some organs of the adult organism. Adult or 'somatic' stem cells are thought to be undifferentiated

Cell differentiation takes place in three situations:

- The *growth* of an immature organism into an adult.
- Normal *turnover* of cells such as blood cells in mature organisms.
- The *repair* of damaged tissues when specialized cells have to be replaced.

During the differentiation process, cells gradually become committed towards developing into a given cell type. Here, the state of commitment may be described as "specification" representing a reversible type of commitment or "determination" representing irreversible commitment.
Although the two represent differential gene activity, the properties of cells in this stage is not completely similar to that of fully differentiated cells. For instance, in the specification state, cells are not stable over a long period of time.

There are two mechanisms that bring about altered commitments in the different regions of the early embryo.
These include:

- Cytoplasmic localization
- Induction

Cytoplasmic Localization - This occurs during the earliest stage of embryo development. Here, the embryo divides without growth and undergoes cleavage divisions that produce blastomeres (separate cells). Each of these cells inherit a given region of the cytoplasm of the original cell that may contain cytoplasmic determinants (reuratory substances). Once the embryo becomes a morula (solid mass of blastomeres) it is composed of two or more differently committed cell populations. The cytoplasmic determinants may contain mRNA or protein a given state of activation that influence specific development.

Induction - In induction, a substance secreted by one group of cells causes changes in the development of another group. During early development, induction tends to be instructive in that tissue assumes a given state of commitment in the presence of the signal.
In induction, inductive signals also evoke various responses at varying concentrations which results in the formation of a sequence of groups of cells, each being in a different state of specification.

During the final phase of cell differentiation, there is formation of several types of differentiated cells from one population of stem cells of the precursor. Here, terminal differentiation occurs both in embryonic development as well as in tissues during postnatal life.

Stem cells have the ability to differentiate into specialized cells and can self-renew; dividing to give rise to new stem cells. Once the female egg has been fertilized, the cells formed after cell division contain DNA that is identical. Beginning with the totipotent and pluripotent stem cells which give rise to all of the specialized tissues in the body, the DNA sequence of the cells does not change. The genome is normally identical in every cell; the cells differ not because they contain different genetic information, but because they express different sets of genes. Individual development from one cell to a multicellular mature organism is the result of sequential, selective inclusion in different gene regions of chromosomes in different cells. This selective gene expression controls essential processes by which the embryo is constructed.

Cellular differentiation is often controlled by cell signaling. Many of the signal molecules that convey information from cell to cell during the control of cellular differentiation are called growth factors. Although the details of specific signal transduction pathways vary, these pathways often share the following general steps. A ligand produced by one cell binds to a receptor in the extracellular region of another cell, inducing a conformational change in the receptor. The shape of the cytoplasmic domain of the receptor changes, and the receptor acquires enzymatic activity. The receptor then catalyzes reactions that phosphorylate other proteins, activating them. A cascade of phosphorylation reactions eventually activates a dormant transcription factor or cytoskeletal protein, thus contributing to the differentiation process in the target cell.[22] Cells and tissues can vary in competence, their ability to respond to external signals.[23]

Signal induction refers to cascades of signaling events, during which a cell or tissue signals to another cell or tissue to influence its developmental fate.

Although differentiation is not thought to occur by permanent loss of genetic material, DNA can be modified in a way that affects gene expression. For instance, DNA and its associated histone proteins (together known as chromatin) can be chemically modified by a cell's own machinery. Chromatin modification can affect gene expression by changing the accessibility of genes to transcription factors, in either a positive or a negative manner.
Two major classes of such chemical modifications include DNA methylation and histone modification (methylation and/or acetylation). These changes are often described as epigenetic because they do not act to alter the primary DNA sequence but instead act at a level just above the DNA sequence. Although DNA methylation and histone modification are not genetic, cells have mechanisms to copy this epigenetic information during their division so that their daughter cells contain the same regulatory data.
Changes in chromatin modification play an important role in regulating gene expression during developmental cell-type specification as well. For example, chromatin-modifying proteins play an essential role in muscle cell differentiation via interactions with key muscle-promoting transcription factors MyoD and MEF. That is, these factors are thought to help recruit chromatin modifying factors, such as histone acetyltransferases and deacetylases. In so doing, MyoD and MEF alter access to their target sites upstream of muscle differentiation genes. For instance, MyoD binds histone acetyltransferases p300 and PCAF, and this activity is essential for muscle cell differentiation. This example provides evidence for a link among chromatin modifications, transcription factors, and, ultimately, cell-fate-specific changes in gene expression.
Chromatin modification can be stable over the life of an organism, thereby effectively permanently influencing gene expression. However, that is not to say that chromatin modification is irreversible. For instance, chromatin can become mismodified in certain cancers, suggesting that, although important, the change is not permanent. Moreover, chromatin modifications are usually erased and reset during the production of gametes, such that the adult program of intrinsic cues is replaced with a program more suited to embryonic development
In fact, embryonic cell types are known to contain a unique set of chromatin modifications that are different from those found in adult cell types (Bernstein *et al*., 2006; Meshorer *et al*., 2006). This has led to the tantalizing proposal that chromatin modification helps lock in changes in gene

expression that are required during development. The permanent silencing of the genes involved only in embryogenesis could then drive the development of cells toward more mature cell types. By blocking accessibility of transcription machinery, for example, chromatin modification could prevent the need for continued repression through active binding of a repressive transcription factor. Alternatively, the genes required for an adult cell type might contain chromatin modifications (especially histone acetylation) that cause the DNA to become open and, therefore, more accessible to the transcription machinery.

Interestingly, embryonic cell types have been found to contain a signature chromatin modification in the regions that regulate the expression of genes involved in early embryonic development (Bernstein *et al.*, 2006). Such regions were found to contain chromatin modifications with both silencing and promoting characteristics. The finding of these bivalent (two-directional) markers in association with genes important for embryonic development has led to the belief that embryonic cells exist in a special epigenetic state, wherein they can choose to remain embryonic (as in an embryonic stem cell) or to differentiate (as in normal development), and bivalent domains provide a means by which to quickly choose between the two options.

Together, these lines of evidence have led to an emerging hypothesis that cell-cell signaling and epigenetic changes converge to guide cell differentiation decisions both during development and beyond

Basic cell differentiation occurs after a sperm cell fertilizes an egg and the resulting zygote reaches a certain size. At that point the zygote starts developing different cell types and needs differentiated cells to take on the specialized functions. A cell capable of differentiating into any type of cell is known as "totipotent". For mammals, totipotent includes the zygote and products of the first few cell divisions. There are also certain types of cells that can differentiate into many types of cells. These cells are known as "pluripotent" or stem cells in animals (meristemic cells in higher plants).Embryonic stem cells (ES cells) have the potential to develop into different types of cells.

Stem cell differentiation involves the changing of a cell to a more specialized cell type, involving a switch from proliferation to specialization. This involves a succession of alterations in cell morphology, membrane potential, metabolic activity and responsiveness to certain signals. Differentiation leads to the commitment of a cell to developmental lineages and the acquisition of specific functions of committed cells depending upon the tissue in which they will finally reside. Stem cell differentiation is tightly regulated by signaling pathways and modifications in gene expression.

Stem cells can be categorized into groups depending on their ability to differentiate.

- Totipotent: can differentiate into all cell types;
- Pluripotent: can differentiate into almost all cell types;
- Multipotent: can differentiate into a related family of cell types;
- Oligopotent: can differentiate into a few different cells;
- Unipotent: can produce one cell type only.

Differentiation mainly occurs in the embryonic period, as well as in the early stages of postembryonic development. In addition, differentiation occurs in some organs of the adult organism. Adult or 'somatic' stem cells are thought to be undifferentiated. Their primary role is to self-renew and maintain or repair the tissue in which they reside. Examples *of stem and progenitor cells in mature organism include:*

***H*ematopoietic Stem Cells** - These are from the bone marrow and are involved in the production of red and white blood cells as well as the platelets.

Mesenchymal Stem Cells - Also from the bone marrow, these cells are involved in the production of fat cells, stromal cells as well as a given type of bone cell.

Epithelial **Stem Cells** - These are progenitor cells and are involved in the production of certain skin cells.
Muscle Satellite Cells - These are progenitor cells that contribute to differentiated muscle tissue.

The rapid development of stem cell biology has provided us with a strong support for further understanding of the precise molecular regulation mechanisms in the development of organisms, as well as new treatments for cancer, cardiovascular and cerebrovascular diseases, neurodegenerative diseases, diabetes, and other diseases. It brought hopes to neurological diseases. Therefore, before the therapeutic potential of stem cells is widely applied to the clinic, it is necessary to have a deeper understanding of the characteristics and regulatory mechanisms of stem cell proliferation and differentiation that determine the stem cell fate, to survive and proliferate through the endogenous cells.
Stem cells with the potential for the treatment of a wide range of degenerative disorders may be obtained from a variety of sources but, for practical reasons, some of them are more likely to find earlier clinical application than others. The main types that have been studied in the context of stem cell therapy are embryonic stem cells, fetal stem cells and adult stem cells.
Embryonic stem cells and, to a lesser extent, fetal stem cells have the potential to repair many types of tissue because they are totipotent Embryonic stem cells can be greatly increased in number in culture as cell lines *in vitro* and may be immuno-priviledged. These attributes mean that they can be used to treat multiple patients. However, their use has been confounded by serious ethical issues and the very real likelihood that, being immortal, they will form tumors after they have been transplanted into patients . Undoubtedly, however, these barriers to widespread application will be overcome in the future. Stem cells themselves do not serve any single purpose but are important for several reasons.
First, with the right stimulation, many stem cells can take on the role of any type of cell, and they can regenerate damaged tissue, under the right conditions.

Aging is an unavoidable physiological consequence of the living animals. Mammalian aging is mediated by the complex cellular and organismal processes, driven by diverse acquired and genetic factors[1]. Aging is among the greatest known risk factors for most human diseases[2-5], and of roughly 150000 people who die each day across the globe, about two thirds die from age-related causes

There are two major theories of organismal aging: program and damage-based

Program theories

Programmed aging theories, sometimes referred to as active or adaptive aging theories, suggest that there is a deliberate deterioration with age because a limited life span results in evolutionary benefits

For many years, programmed aging has been debated and some studies have substantiated this hypothesis. For example, have suggested that there are mechanisms that preserve the integrity of spores of aging diploid yeast cells. Through these mechanisms, aging diploid cells that are induced to sporulate appear to lose all age-associated damage to a point that is no longer detectable, though the assumption that these findings can be extrapolated to higher organisms has been put into question Yet, though development and morphogenesis can be easily understood as programmed, as they are the end-result of a determined sequence of molecular and cellular events designed to produce a given phenotype, aging is mostly thought of as decay. If aging is indeed programmed, the purposes of such program remain unclear. Some have suggested that aging may constitute an altruistic plan, by eliminating post-reproductive age individuals, who would compete for resources, by avoiding overpopulation and by promoting adaptation through a succession of generations . The supporters of this view underscore that the

similarities between the biochemical pathways that regulate aging in organisms such as yeasts, flies and mice, together with evidence consistent with programmed death in salmon and other organisms, hint at the possibility that programmed aging can occur in higher eukaryotes. Moreover, this plan could be the result of "aging genes". Nonetheless, if this was the case, than certainly such mechanisms would be susceptible to inactivation, and, despite many gene mutations have been described as life-extending mutations none has been reported that abolishes the process of aging. It should be noted that, in some model organisms, genes have been demonstrated to play a pivotal role in aging. In fact, the first described mutation to yield a significant extension in the lifespan of *Caenorhabditis elegans* was in the *age-I* gene, which was shown to result in a 65% increase in mean lifespan and a 110% increase in maximum lifespan of this organism. Since then, many mutations that result in lifespan extension in *C. elegans* have been identified, most of which involving genes that are homologs of the of components of the insulin/IGF (insulin-like growth factor) pathway , namely, *daf-2/daf-16* and *sir2.1* , which, interestingly, have been shown to interact to extend lifespan in *C. elegans* .

Composed mostly of post-mitotic cells, *C. elegans* is one of the most widely studied model organisms. With a lifespan ranging from days to a few weeks, it has been noted that, under caloric restriction (CR) and/or crowded conditions, *C. elegans* can enter an alternative stasis-like developmental pathway, called *dauer* . This pathway consists of a developmental arrest, leading to an increased adult phase. This arrest suggests that, at least partially, aging and development are coupled in *C. elegans*, as well as in other invertebrates. However, in addition to the severity of the restriction (30–70% fewer calories than the control group), the degree of lifespan lengthening in *C. elegans* depends on numerous factors, namely, age at onset of restriction. Though providing some key insights into longevity, invertebrates are, nevertheless, distant animal models and are likely unrepresentative of human biology and physiology.

The endocrine system has also been viewed as involved in "telling the time". Because the levels of hormones such as growth hormone (GH) and its corresponding downstream target insulinlike growth factor I (IGF-1) decline with age, the idea that such changes cause aging has been suggested a few decades ago, and, in rats, deficiency in growth hormone production (loss of function mutations at the *Pit-I* locus) has been linked to lifespan extension and delayed immune aging. Due to the fact that the brain regulates the endocrine system, the neuroendocrine theory of aging has emerged as the main hormone-based theory of aging, and, not surprisingly, many anti-aging products aim at restoring the levels of specific hormones in older people. Some studies have supported the idea that the insulin pathway is associated with human longevity, as individuals with mutated *Prop-I* gene – a pituitary transcription factor whose mutation causes dwarfism – may live longer and patients with GH and IGF-1 deficiencies have shown signs of early aging, despite actually living longer. Some have proposed that such mechanisms could be activated by decreasing cellular replication or that it may operate on the basis of antioxidant regulation. Whatever the mechanism, it is now clear that the early assumption that the aging process is driven by hormone changes that occur with age is unsubstantiated. If anything, the decrease in GH/IGF-1 signaling increases lifespan, not the contrary and, more broadly, hormonal changes may regulate aging as an indirect consequence of the developmental program. The imbalance on chemical processes caused by differential gene expression and hormonal changes may contribute to aging, but, so far, such assertions remain in the realm of speculation. Furthermore, the significant lifespan differences observed in numerous species, under identical conditions, seems to indicate that there is no pre-determined timeline for aging. Thus, under certain conditions, it may be possible to prolong or to curtail lifespan, leading to the hypothesis that aging is not predetermined, but rather the end-result of a "wear-and-tear" mechanism.

(2) Damage theories

Evolutionary biologists may argue that aging occurs due to the absence of natural selection at the post-reproductive stage of life . Hence, aging is not programmed; instead, it is the absence of selection for maintenance Although such aging theories are subjectively appealing, as they convey a cure for aging, the accumulation of damage is a spontaneous entropy-driven

process, and, as such, its kinetics can be genetically and environmentally modulated, resulting in the wide range of life-spans we observe

Among the damage theories, a prevailing idea is that of oxidative damage. Reactive oxygen species (ROS) – partially reduced intermediates of oxygen that can be radical or non-radical molecules – are generated during metabolism through a number of inter-related reactions and are considered to lead to the cumulative DNA, protein and lipid damage observed over a lifetime. Approximately 2–3% of oxygen taken up is chemically reduced by the addition of single electrons. Incomplete reduction of oxygen can generate a variety of biologically relevant ROS such as, hydrogen peroxide, the anion radical superoxide and the hydroxyl radical. The electron transport chain in the mitochondria, the nicotinamide adenine dinucleotide phosphate oxidases (NADPH oxidase) and the 5-lipoxygenase as the three major sources of ROS in living cells . Multiple studies have highlighted the relatively haphazard molecular damage that ROS cause to lipids, proteins and nucleic acids and exposure to ROS have been demonstrated to trigger specific mechanisms aimed at neutralizing their effects

Adult stem cells, also known as somatic stem cells, are found throughout the body in every tissues and organs after development and function as self-renewing cell pools to replenish dying cells and regenerate damaged tissues throughout life. However, adult stem cells appear to age with the person. As stem cells age, their functional ability also deteriorates. Specifically, this regenerative power appears to decline with age, as injuries in older individuals heal more slowly than in childhood. For example, healing of a fractured bone takes much longer time in elderly than in young individuals. There is a substantial amount of evidence showing that deterioration of adult stem cells in the adult phase can become an important player in the initiation of several diseases in aging. The following is some of the examples of aging-associated effects on stem cells.

Neural stem cells (NSCs) are multipotent and self-renewing cells and located primarily in the neural tissues. In response to a complex combination of signaling pathways, NSCs differentiate into various specific cell types locally in the central nervous system (CNS), like neurons, astrocytes, and oligodendrocytes. NSCs in humans maintain brain homeostasis and it continuously replenishes new neurons, which are important for cognitive functions. However, there is now strong evidence for the aging-associated cognitive deficits, such as olfactory dysfunction, spatial memory deficits, and neurodegenerative disorders, which are caused by deterioration of NSC proliferation and differentiation and enhanced NSC senescence as a consequence of aging

Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into cells of mesenchyme tissues, including osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (muscle cells) and adipocytes (fat cells). MSCs were first isolated from the bone marrow of guinea pigs in 1970's and after that it was isolated from almost every organ in mice including fat, liver, spleen, pancreas, kidney, lung, muscle, and brain. Human MSCs have also been isolated from umbilical cord tissue and cord blood, placenta, bone and joints. However, the major sources of MSCs are the bone marrow-derived MSCs (BM-MSCs) and the adipose tissue-derived MSCs (A-MSCs); and they are currently the most studied MSCs. Aging also affects MSCs in humans and in animal models as indicated by the decrease in the bone marrow MSC pool and also shifts their lineage differentiation from one that usually favors osteoblastic differentiation to one that prefers adipogenic differentiation, which is largely responsible for the gradual and aging-associated shift of hematopoietic (red) marrows to fatty (yellow) marrows, and which also contributes significantly to the etiology of senile osteoporosis. It is also evident that with increasing donor age, MSCs from both bone marrow and adipose tissues have been shown to have reduced capacity to handle oxidative stress. During the aging process, oxidative stress leads to hyperactivity of pro-growth pathways, such as insulin/IGF-1 and mTOR pathways, and the subsequent accumulation of toxic aggregates and cellular debris ultimately lead to apoptosis, necrosis, or autophagy. In addition, in some non-skeletal tissues, particularly

the hematopoietic system, MSCs is a key niche component for hematopoietic cells. Aging of MSCs has been shown to be detrimental with respect to this important function.

Adult skeletal muscle stem cells (satellite cells) have a remarkable capacity to regenerate. Similarly, their regeneration capacity declines with aging, although it is not clear whether this is due to extrinsic changes in the environment and/or to cell-intrinsic mechanisms associated to aging. This impaired regenerative capacity of skeletal muscle during aging is due to accumulation of the altered progeny, which leads to progressive deterioration of tissue structure and function, manifesting after injury or in response to the depletion of memory B cells and naive T cells in the hematopoietic system in the elderly.

Hematopoietic stem cells (HSCs) are the blood-forming stem cells through the process of hematopoiesis. They are located in the red bone marrow within marrow cavity of most bones. HSCs also produce immune cells of the body. Since blood cells are responsible for constant maintenance and immune protection of every cell type of the body, the constant production of billions of new blood cells each day by HSCs is very important for mammal life. HSC-derived monocytes can give rise to osteoclasts, macrophage and granulocyte. Osteoclasts are giant cells with numerous nuclei that work in synergy with osteoblasts through complicated bone coupling mechanisms to maintain bone homeostasis. All these activities of HSCs are carefully modulated by a complex interplay between cell-intrinsic mechanisms and cell-extrinsic factors produced by the microenvironment; and aging altered this fine-tuned regulatory network, leading to aberrant HSC cell cycle regulation, degraded HSC function, and hematological malignancy.

There are several potential mechanisms that are believed to contribute to the aging-associated stem cell dysfunction; and they probably are in part responsible for many aging-associated diseases. The functions of aged stem cells become impaired as the result of cell-intrinsic pathways and surrounding environmental changes. With the sharp rise in the aging-associated diseases, the need for effective regenerative medicine strategies for the aged is more important than ever.

Literature:

1. Alberts et al, pp. 1145-1262.

2. Lodish et al, pp. 24-29, 975-1021.