CHAPTER 2

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The Molecular and Biochemical Basis of an Organism

INTRODUCTION

The multiple forms of life that exists on the Earth and the variability amongst them is known as biodiversity. In recent years, biodiversity has been deteriorating due to the human quest for development and advancement of their life. In order to sustain human life on this planet, it is inevitable that we ensure greater diversity of life forms and ecosystems and promote conservation. Life on earth is dependent on the chemical element carbon that forms the basis for the biochemical processes in an organism. However, all the chemicals that form the basis of life are formed by molecules which are aggregates of atoms linked by chemical bonds. There are different types of bonds that make the chemicals unique with regard to structure and reactivity. The biomacromolecules such as carbohydrates, lipids, nucleic acids, and proteins are the important chemicals that provide cellular fuel, form the basis of enzymes, structural and cellular components, and storage of genetic information.

Protein synthesis is a critical process in the body in which a cell manufactures proteins. These proteins are essential for a variety of functions in the body ranging from molecular level such as cell signaling to the macro level such as muscle development. At the cellular level, protein synthesis is a multi-step process through which appropriate protein molecules are produced from amino acids and they are stored or exported out of the cell to meet the body's requirements. Various cellular components including the nucleus, endoplasmic reticulum, and Golgi bodies are involved in the process of protein synthesis. Deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and ribosomes are the central players in this process.

All living organisms consist of monomeric units called cells; some contain a single cell (unicellular) and others contain many units (multicellular). In

multicellular organisms, for example in human, many trillions of cells are grouped into tissues. Several types of tissues work together in the form of organs with specialized functions. These specialized tissues contain unique cells and they are called as stem cells having capable of self renewing and differentiating into a variety of specialized cells such as neuron, immune cell, liver cell, muscle fibers, bone cell, etc. A lot of importance is attached to stem cells because of their potential use in treating various diseases that affects mankind. Medical researchers believe that solving the clues of division and differentiation of stem cells can be used to replace the loss of cells, for example, in neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, cardiovascular diseases, bone marrow transplants, skin transplants, etc. Understanding how the cell works are critical to devising strategies to combat diseases.

2.1 BIODIVERSITY

Biodiversity is the variety of life on earth. It is the variability among living organisms from all sources including, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.

Usually three levels of biodiversity are discussed- genetic, species, and ecosystem diversity:

1. Genetic Diversity is all the different genes contained in all individual plants, animals, fungi, and microorganisms. It occurs within a species as well as between species.
2. Species Diversity is the difference within and between the populations of a species and between different species.
3. Ecosystem Diversity is all the different varieties of habitats, biological communities, ecological processes as well as variations within individual ecosystems.

Importance of biodiversity

Biodiversity is part of our daily lives and livelihood, and includes resources upon which families, communities, nations and future generations depend. Having a different array of living organisms allows other organisms to take advantage over the resources provided.

Humans have always depended on the Earth's biodiversity for food, shelter, and health. Biological resources that provide goods for human use include:

1. **Food-** species that are hunted, fished, and gathered, as well as those cultivated for agriculture, forestry, and aquaculture.
2. **Shelter and warmth-** timber and other forest products and fibers such as wool and cotton.
3. **Medicines-** both traditional medicines and those synthesized from biological resources and processes.

Threats to biodiversity

Biodiversity is facing a lot of challenges and loss of biodiversity is a significant global issue. Species are becoming extinct at the fastest rate known in geological history and most of these extinctions have been tied to human activity.

The following reasons are the important cause for the loss of biological diversity:

1. *Habitat loss and destruction* is usually as a direct result of human activity and population growth, is a major force in the loss of species, populations, and ecosystems.
2. *Alterations in ecosystem composition* such as the loss or decline of a species, can lead to a loss of biodiversity.
3. *The introduction of exotic (non-native) species* can disrupt entire ecosystems and impact populations of native plants or animals. These invaders can adversely affect native species by eating them, infecting them, competing with them, or mating with them.
4. *The over-exploitation* (over-hunting, over-fishing, or over-collecting) of a species or population can lead to its downfall.
5. Human-generated *pollution and contamination* can affect all levels of biodiversity.
6. *Global climate change* can alter environmental conditions. Species and populations may be lost if they are unable to adapt to new conditions or relocate.

2.2 CHEMISTRY OF LIFE

All matter is composed of simple units called atoms. Each atom consists of a positively charged nucleus made up of protons and neutrons, surrounded by electrons bearing negative charges.

Same kinds of atoms combine to form an **element.** Both living and nonliving matter is composed of elements. Six of the elements that occur in nature- carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur (C, H, N, O, P, S)- are important for life and make up 98% of the body weight of organisms.

- *Posidonia oceanica* is a sea grass that grows in the Mediterranean Sea. It is vital to the marine ecosystem because, the environment frees up to 20 litres of oxygen per day, produces and exports biomass to the surrounding ecosystems, provides a breeding ground for fish and other marine species, acts as a barrier and prevents coastal erosion, etc. In the current global development, this ecosystem is also under threat due to chemicals, construction of ports, trawling, etc. Therefore, it has become essential to understand its habitat. Engineers have developed a hydroacoustic technology with the help of single beam echosounder, a multi-beam sonar, and a side scan sonar so that scientists can better examine the spatial classification of seafloor types and their vegetations. Such engineering technology enables the scientists to study large areas with very high resolution, unlike the traditional approach of visual sampling which is very limited.
- There is a growing interest in the field of "bio-inspired engineering" or "biomimetics" which deals with the study natural biomaterials and structures with unique properties which can be used to develop specialized and multi-functional biocompatible materials. This can be possible only by examining and studying the genetic and molecular diversity of species in our ecosystems.

A **compound** is a substance formed when two or more elements are chemically joined. Water, salt, and sugar are examples of compounds. When the elements are joined, the atoms lose their individual properties and have different properties from the elements which they are composed. When atoms interact with one another they form different chemical compounds by the formation of **chemical bonding (strong** attractive forces). An atom is said to be **stable** if the outer most electron orbit is completely filled (**Doublet configuration**- with 2 electrons in the outer most shell or with **Octet configuration**- with 8 electrons in the outer most shell).

Types of Bonding

Two types of bonds are formed between atoms, they are:

(1) Ionic Bonding.

(2) Covalent bonding.

Ionic Bonding: When an atom donates an electron and the other atom accepts the donated electron then a bonding arises between these two atoms called as **Ionic bond**. Atoms that donate electron will have more number of protons and hence attains a positive charge and called as **Cations**. Atoms receiving the electron will have more electrons hence attains a negative charge and called as **Anions.**

Example: NaCl (sodium chloride) – Na^+, Cl^-

Sodium with electronic configuration (2, 8, 1) has one electron in the outermost shell, while chorine (2, 8, 7) has only seven electrons in the outermost shell. So to obtain stable configuration, sodium donates an electron to chlorine and thus, both sodium and chlorine attains octet configuration and attains stability there by forming an ionic bond (Fig. 2.1).

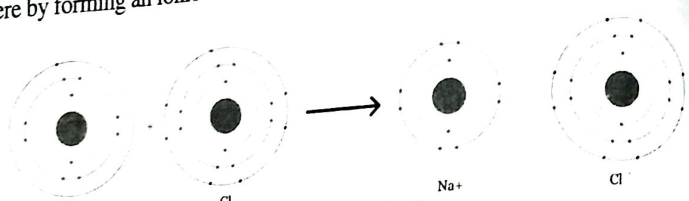

Fig. 2.1 *Electronic configuration of sodium chloride*

Covalent Bonding: This bonding involves sharing of valence electrons between two atoms to form a stable configuration. There may be sharing of two or three pairs of electrons which result in the formation of **double and triple bonds** in a molecule which fixes the atoms involved in the bond formation in a stable plane.

Example: Hydrogen gas forms the simplest covalent bond in the diatomic hydrogen molecule (Fig. 2.2). Hydrogen atom has one electron in the outermost valence shell thus another hydrogen atom join to form covalent bond by sharing its valence electron thereby attaining a doublet stable configuration.

Non-covalent Bonds: These are the weak forces that are seen in the biological system (refer to chapter 1). Non-covalent interactions are weaker

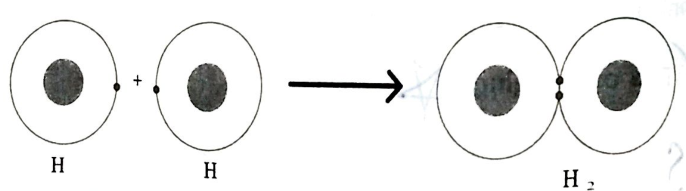

Fig. 2.2 *Electronic configuration of hydrogen*

than the covalent bonds. There are four types of non-covalent interactions:

1. Hydrogen bonds
2. Ionic bonds
3. Van der Waals forces
4. Hydrophobic interactions.

$C_m(H_2O)_n$

These interactions are critical in maintaining the shapes of nucleic acids, proteins and other biological macromolecular structures.

Example: The non-covalent interactions hold together the two strands of DNA in the double helix. They stabilize secondary and tertiary structures of proteins.

$C_m(H_2O)_n$

2.3 BIOCHEMISTRY AND HUMAN BIOLOGY

$(CH_2O)_n$

Biochemistry is the branch of science that deals with the study of chemical processes in living organisms. It governs all living processes and living organisms. Living things require millions of chemical reactions to survive. These chemical reactions are generally grouped under ***Metabolism***, which include ***Anabolism***- building up of molecules and ***Catabolism***- break down of molecules.

Biochemistry gives rise to incredible complexity of life. It deals with the **organic molecules** in the living system. Organic molecules always contain *carbon* and are large molecules with many atoms linked by covalent bonds.

Biomacromolecules

Although a cell is mostly with water, the rest of it contains carbon based molecules. These molecules usually are large and termed as *Polymers*. Polymers contain arranged units of smaller units called as *Monomers*. Biologists term them as *Biomacromolecules* that includes proteins, carbohydrates, lipids, nucleic acids, and other biomolecules.

(A) Carbohydrates $C_m(H_2O)_n$

A **carbohydrate** is an organic compound with the empirical formula $C_m(H_2O)_n$; that is, consists only of carbon, hydrogen, and oxygen, with hydrogen: oxygen atom ratio of 2:1 (as in water). The carbohydrates (saccharides) are divided into four types - ***monosaccharides, disaccharides*** (Fig. 2.3), ***oligosaccharides***, *and* ***polysaccharides*** (Fig. 2.4). The word *saccharide* comes from the Greek word, meaning "sugar".

DNA polymerase & synthesis
- Addition of bases of new strand from template strand.

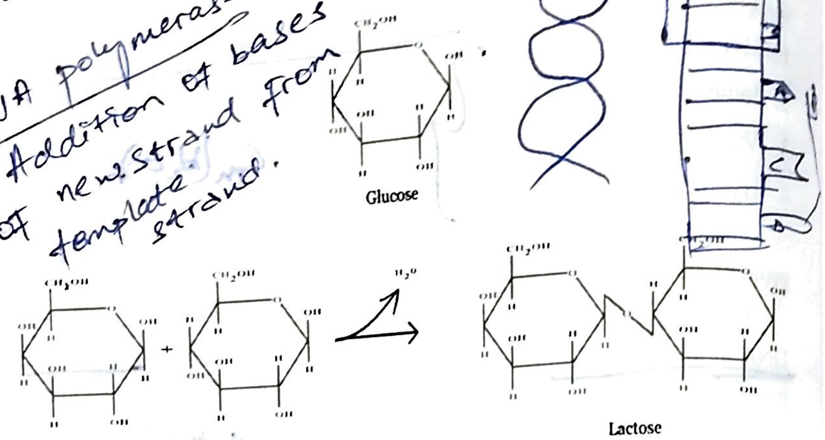

Fig. 2.3 *Biochemical structures of monosaccharides (glucose and galactose) and a disaccharide (lactose)*

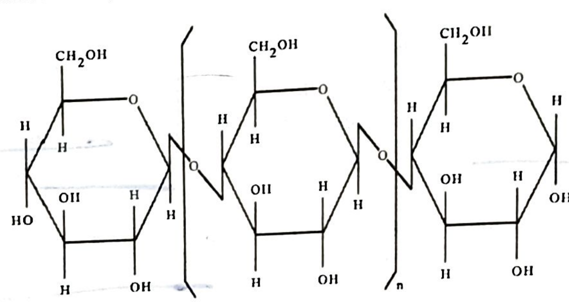

Fig. 2.4 *Biochemical structure of polysaccharide (cellulose)*

(1) Monosaccharides

These are called as simple sugars with general formula $(CH_2O)_n$ where n is three or more and are the main fuel that cells use for cellular work

Examples: Glucose, Fructose, and Galactose ($C_6H_{12}O_6$),

(2) Disaccharides

Two monosaccharides joined together are called a disaccharide. They are composed of two monosaccharide units bound together by a covalent bond known as a *glycosidic linkage* formed via a dehydration reaction, resulting in the loss of a hydrogen atom from one monosaccharide and a hydroxyl group from the other.

Examples: Sucrose, lactose.

(3) Oligosaccharides and polysaccharides

When a few (around three to six) monosaccharides are joined together, it is called an *oligosaccharide.* Many monosaccharides joined together make a *polysaccharide* (Fig. 2.4). They can be joined together in one long linear chain, or they may be branched. These are also said as complex carbohydrates.

Example: Cellulose

Org → non-polar

(B) Lipids

The lipids are a large and diverse group of naturally-occurring organic compounds that are soluble in non-polar organic solvents (e.g. ether, chloroform, acetone, and benzene) and generally insoluble in water. They are all esters of moderate to long chain fatty acids including *fats, waxes, steroids, and oils.*

Importance of lipids: Cell membrane is bilayered with a type of a lipid called as *phospholipids.* Phospholipids have a head that is polar and attract water (hydrophilic) and have 2 tails that are non-polar and do not attract water (hydrophobic).

(i) Fatty Acids: They are building blocks of lipids. Fatty acids are composed of a chain of methylene groups with a carboxyl functional group at one end. The fatty acid chains are usually between 10 and 20 carbon atoms long. The fatty "tail" is non-polar **(Hydrophobic)** while the carboxyl "head" is a little polar **(Hydrophilic)**.

Fatty acids can be **saturated** - they have as many hydrogens bonded to their carbons as possible or **unsaturated** - with one or more double bonds connecting their carbons, hence fewer hydrogens.

(ii) Triglycerides: Monomer of lipids containing three fatty acids linked by a molecule of glycerol. These are energy storage molecules.

(iii) Steroids: A **steroid** is a type of organic compound that contains a specific arrangement of four cycloalkane rings that are joined to each other.

Examples: cholesterol, the sex hormones estradiol and testosterone.

(iv) Waxes: Waxes are composed of a single, highly complex alcohol joined to a long chain fatty acid in a typical ester linkage. Waxes are important structural lipids often found as protective coatings on the surfaces of leaves, stems, hair, skin, etc. They provide effective barriers against water loss and in some situations make up the rigid architecture of complex structures such as the honeycomb of the beehive.

(C) Proteins

Proteins are essential parts of organisms and participate in virtually every process within cells. They are polymers of different types of amino acids linked by a peptide bond. Twenty different amino acids are linked in different order to build various types of proteins. Proteins are used to build cells, act as hormones and enzymes, provide structural integrity, and do much of the work in a cell.

(D) Nucleic acids

Nucleic acids are biological molecules essential for life and include **DNA** (deoxyribonucleic acid) and **RNA** (ribonucleic acid). They are made up of polymers of *nucleotides*. Each nucleotide consists of a base, sugar, and phosphate molecule.

DNA differs from RNA by the absence of 'O' in the 'OH' group of sugar molecule and thus, called as **deoxyribose** sugar. It has four bases adenine, guanine, thymine, and cytosine while in RNA the thymine is replaced by uracil.

Importance of Biomacromolecules

Biological Macromolecule	Importance
Carbohydrates	Dietary energy, Cellular fuel
Lipids	Long-term storage of energy, Constituents of biological membrane, Steroid hormones, etc.,
Proteins	Enzymes, structure of biological components, cellular transport, and many diverse functions
Nucleic Acids	Genetic information storage

- The knowledge of biochemistry is **essential** for the biochemical engineers in using bioreactors for the manufacture of foodstuffs, pharmaceuticals, and waste-water treatment.
- In brewery and fermentation, biochemistry is **essential** to study the chemical reactions involving microorganisms as production and processing agents. Biochemistry is useful in industries pertaining to food, medicine, agriculture, and biotechnology. Vaccine production and biopharmaceuticals require biochemical engineers.

2.4 PROTEIN SYNTHESIS

Protein synthesis is the process in which cells build proteins. Both prokaryotic cell and eukaryotic cell synthesize different types of proteins. It's a multi-step

process involving transcription, post-transcriptional modifications, translation, and post-translational modifications to yield the desired protein.

Before understanding the process of protein synthesis, a brief knowledge on DNA and RNA is essential:

DNA (Deoxyribose Nucleic Acid): DNA is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms. The main role of DNA molecules is the long-term storage of genetic information.

Frederick Griffith in 1928 showed that the DNA was the cell's genetic material. Rosalind Franklin took diffraction X-ray photographs of DNA crystals. Watson and Crick in the 1950's built the first model of DNA.

Structure of DNA: In 1953, Watson and Crick proposed a model to explain the arrangement of molecules in DNA. The model is characterized by the following features:

- DNA is formed of two **polynucleotide chains**, spirally coiled to form a **double helix.**
- Each chain is formed of many units called **nucleotides**- building blocks of DNA.
- A nucleotide is formed of three components, namely a phosphoric acid, a deoxyribose sugar and a nitrogenous base. The bases are Adenine (**A**), Guanine (**G**), Cytosine (**C**) and Thymine (**T**).
- Deoxyribose sugar and nitrogenous base comprise to form a **Nucleoside**, with addition of a phosphoric acid is called as **Nucleotide**.
- Adenine and guanine belong to a group of compounds called **Purines**, Thymine and cytosine belong to another group called as **Pyrimidines**. Purines are linked or bonded with pyrimidines.
- In a DNA molecule, adenine always pairs with thymine with two hydrogen bonds while guanine pairs with cytosine with three hydrogen bonds, the latter being more stronger interaction. The amount of adenine is equal to the amount of thymine and the amount of guanine is equal to the amount of cytosine. This is called as **Chargaff's Rule.**
- The two chains of a DNA are complementary to each other. One end of the chain is called 3' end and the other end is called 5' end. The strands are antiparallel in nature.
- The two complementary chains are twisted around each other to form a double helix. One turn of the helix is about 34Å and contains ten paired nucleotides with a distance of 3.4Å between each pair of bases. The width of the DNA molecule is 20Å.

DNA Replication: DNA replication is a biological process that occurs in all living organisms and copies their DNA; it is the basis for biological inheritance. The process starts with one double-stranded DNA molecule and produces two identical copies of the molecule. There are number differences between the mode of prokaryotic and eukaryotic DNA replications. In a cell, DNA replication generally begins at specific locations in the genome, called "origins".

Steps involved:

- Unwinding of DNA at the origin.
- Break of hydrogen bonds by unzipping of the strands by various enzymes and proteins.
- Addition of bases and synthesis of new strand from the template strand by DNA polymerase.

Ribonucleic acid (RNA): RNA is a nucleic acid containing ribose sugar. It is found in large amounts in the cytoplasm and fewer amounts in the nucleus. RNA is single stranded, sometimes may be folded back upon itself to form double stranded coiled form. The purines and pyrimidines amount are not equal, and thymine is replaced by another base called uracil (U). Three types of RNA are present:

(a) Messenger RNA (mRNA) constitutes 3-5% of total RNA content.

(b) Transfer RNA (tRNA) constitutes 15-17% of total RNA content.

(c) Ribosomal RNA (rRNA) constitutes 80% of total RNA content.

(a) Messenger RNA (mRNA)

Messenger RNA is a ribonucleic acid which carries the genetic information for protein synthesis from DNA to the cytoplasm. The mRNA constitutes of 3-5% of the total cellular RNA. The mRNA is synthesised as a complementary strand upon the chromosomal or genomic DNA. The mRNA carries the message in the form of *triplet codes*. It carries the information for a specific protein and is made up of several hundreds to few thousands nucleotides long.

One end of mRNA is called the 5'end and the other is the 3'end. At the 5'end, a cap region is present which helps the mRNA to bind to with ribosomes (Fig. 2.5). At the 3'end, a poly A tail is found, which contains 200-250 adenylate (A) nucleotides. These are called post-transcriptional modifications which can also provide stability to the mRNA. AUG generally represents translation initiation codon (methionine) and UAA represents translation termination codon in the mRNA.

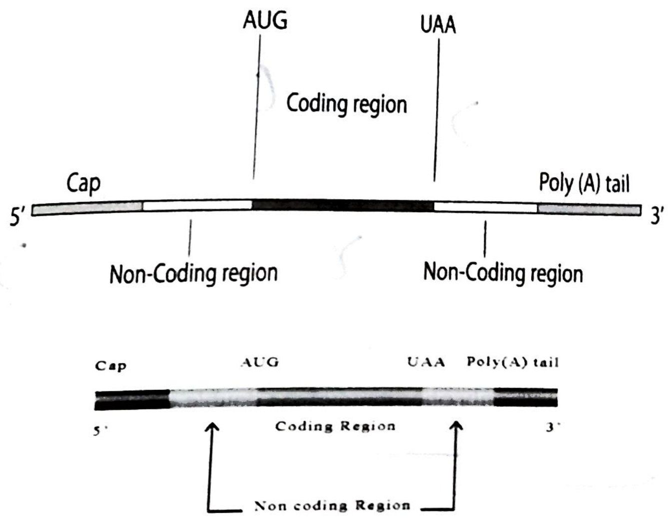

Fig. 2.5 *Coding and non-coding regions of Mrna*

(b) Transfer RNA (tRNA)

Transfer RNA is a type of RNA which transports amino acids to the site of protein synthesis. It is single stranded and constitutes about 15-17% of the total cellular RNA. Each tRNA can transport only one amino acid. There are 60 types of tRNA. As there are 20 different amino acids, certain amino acids are carried by more than one tRNA. It contains of 75-80 nucleotides.

The tRNA assigns a **clover leaf** like structure (Fig. 2.6). It contains four arms namely:

(a) Acceptor arm or amino acid-binding site

(b) D arm or enzyme site

(c) TΨ*C arm or Ribosome site

(d) Anticodon arm or Recognition site

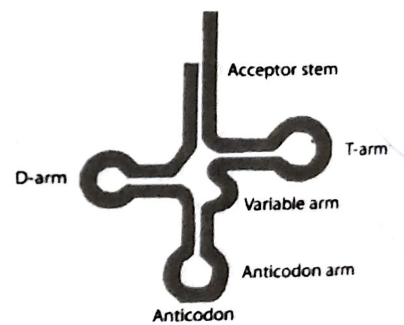

Fig. 2.6 *Schematic representation of a tRNA*

The anticodon loop contains codon complementary to the mRNA sequence. The respective anticodon makes the respective amino acid to bind at the acceptor arm. The D and T arm possess unusual bases like pseudouridine and dihydrouridine.

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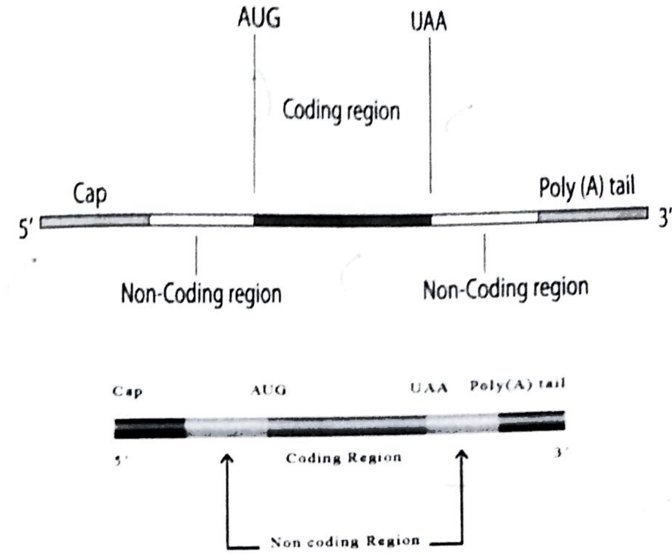

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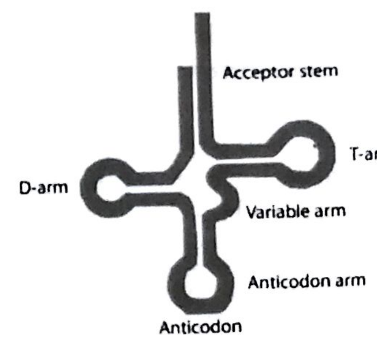

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(c) Ribosomal RNA (rRNA)

Ribosomal RNA is always associated with the ribosomes in the cytoplasm and it is around 100-3000 nucleotides in size. It constitutes about 80% of the total cellular RNA content. It is classified in to 7 types according to the sedimentation coefficient. They are 2.8S, 18S, 5.8S, 5S, 23S, 16S, 55S RNA.

In addition to the above RNAs, there are other smaller RNAs such as snRNA (small nuclear RNA), snoRNA (small nucleolar RNA), microRNA, siRNA (small interference RNA). They are non-coding RNAs and are involved regulating gene expression in eukaryotes.

Transcription: It is a process of synthesizing RNA molecule from a complementary strand of DNA. Since RNA is single stranded, the synthesis has to take place with only one strand of the double stranded DNA molecule. This strand is called the *antisense Strand* and other strand of DNA is called as the *sense Strand.*

Transcription involves three steps:

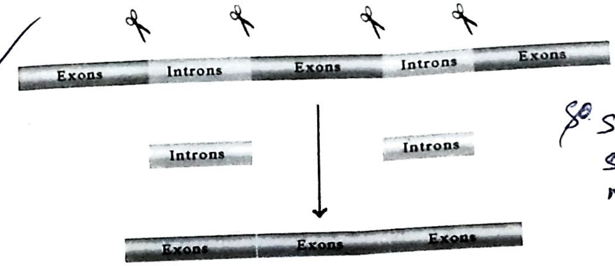
- Initiation
- Elongation
- Termination

Initiation: The RNA polymerase binds to the double stranded DNA by recognizing a sequence on it for attachment; this is called as promoter sequence or **TATA Box**. After the binding of RNA polymerase, the DNA molecule unzips itself by *20 base pairs*. The region which has to be transcribed following the promoter sequence is called as **downstream region** and sequence before the promoter is called as **upstream sequence**. The site (nucleotide) where transcription starts is called transcription initiation site and it is denoted as +1.

Elongation: As the unwinding of DNA takes place the RNA polymerase adds complementary bases and synthesizes RNA in the 5' ⟶ 3' direction. It continues until a gene is completely transcribed.

Termination: When the RNA polymerase reaches a stop signal in the DNA sequence, the RNA polymerase stops its work and unbinds itself from the DNA and mRNA falls off from the complex leading to the stop in transcription. After the synthesis of RNA from DNA by transcription, the resultant RNA is called as precursor mRNA (pre-mRNA). This contains both coding sequences **(exons)** and non-coding sequences **(introns)**. Thus, the introns have to be removed or spliced from the pre-mRNA (Fig. 2.7).

Splicing is a mechanism of removing of introns and joining of exons that occurs in spliceosome. A spliceosome contains a complex of small nuclear



So sn RNA
sno
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Fig. 2.7 *Mechanism of pre-mRNA splicing*

RNA and protein subunits. After the splicing, the RNA is said to be processed or **mature RNA** and it leaves the nucleus and enters the cytoplasm for translation.

Concept of Genetic code: Transcription involves formation of mRNA from the DNA and translation involves formation protein from the mRNA. Proteins are chain of amino acids; each amino acid is coded by a combination of three of the 4 bases. This triplet base is called as **codon (genetic code)**. There are 4 bases so total of 4 x 4 x 4= 64 codons. Totally only twenty amino acids are present but there are 64 codons and obviously, there are extra codons.

Properties of genetic code:

- Genetic code is universal among living organisms.
- Each amino acid is coded by more than one codon.- Redundancy
- No codon codes for more than one amino acid. – Ambiguous

Four codons among the 64 codons have the function to determine the start and end of the translation process (Table 2.1).

Table 2.1 *List of codons coding for the respective amino acids.*

First Base	Second Base				Third Base
	U	C	A	G	
U	UUU Phenylalanine	UCU Serine	UAU Tyrosine	UGU Cysteine	U
	UUC Phenylalanine	UCC Serine	UAC Tyrosine	UGC Cysteine	C
	UUA Leucine	UCA Serine	UAA Stop	UGA Stop	A
	UUG Leucine	UCG Serine	UAG Stop	UGG Tryptophan	G

C	CUU Leucine	CCU Proline	CAU Histidine	CGU Arginine	U
	CUC Leucine	CCC Proline	CAC Histidine	CGC Arginine	C
	CUA Leucine	CCA Proline	CAA Glutamine	CGA Arginine	A
	CUG Leucine	CCG Proline	CAG Glutamine	CGG Arginine	G
A	AUU Isoleucine	ACU Threonine	AAU Asparagine	AGU Serine	U
	AUC Isoleucine	ACC Threonine	AAC Asparagine	AGC Serine	C
	AUA Isoleucine	ACA Threonine	AAA Lysine	AGA Arginine	A
	AUG (Start) Methionine	ACG Threonine	AAG Lysine	AGG Arginine	G
G	GUU Valine	GCU Alanine	GAU Aspartate	GGU Glycine	U
	GUC Valine	GCC Alanine	GAC Aspartate	GGC Glycine	C
	GUA Valine	GCA Alanine	GAA Glutamate	GGA Glycine	A
	GUG Valine	GCG Alanine	GAG Glutamate	GGG Glycine	G

a) Start codon: AUG

b) Stop codons: UAG, UGA, UAA

Translation involves the transfer of the genetic code in an mRNA molecule into the amino acid sequence of a polypeptide (Fig. 2.8). It occurs in the cytoplasm. This involves four components:

- mRNA
- ribosomes (with large and small subunits)
- tRNA
- amino acids.

Translation mechanism comprises of three steps involving **(a) Initiation, (b) Elongation,** and **(c) Termination.**

(A) Initiation of Translation

- The mRNA binds to the smaller unit of the ribosome (30S). The attachment is made at the first codon of mRNA which is AUG coding

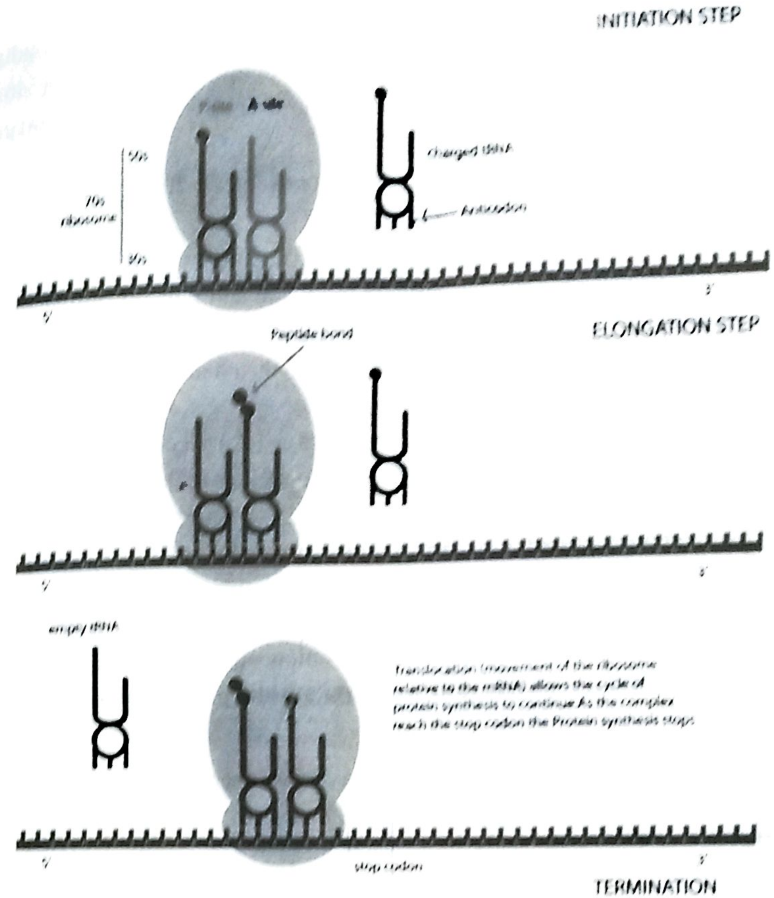


Fig. 2.8 Schematic representation of translation process

for amino acid methionine. Thus, the complex is called as *mRNA-30S complex.*

- The tRNA having the anticodon UAC complementary to AUG in mRNA attaches itself to the initiation codon and transports methionine to the 30S subunit. This complex of mRNA, tRNA and 30S is called *pre-initiation complex.* Later, 50S subunit joins to forms the complex, *Initiation Complex.*
- The complex has two slots for the entry of tRNA. They are **P site** (peptidyl site) and **A site** (aminoacyl site). The first tRNA attaches to the P site.

(B) Elongation and (C) Termination

- It refers to the addition of amino acids one by one to the first amino acid methionine, as per the sequence of the codons in mRNA.

- The second codon in the mRNA is recognized and the corresponding tRNA enters the A site and the respective amino acid is brought along with it. The tRNA with respective amino acid is called as **charged tRNA.**
- The formation of peptide bond occurs between the first amino acid and the second amino acid.
- As the complex moves and reads the next codon in the mRNA the first tRNA leaves the complex through the P site and falls off, followed the second tRNA occupying the P site and entry of new tRNA at the A site and corresponding amino acid is added to the chain and peptide bond is formed.
- This process continues as the *stop codon UAA/UGA/UAG* is reached, then the chain terminates and the process stops leaving behind the synthesized protein. The protein formed is a sequence of amino acids linked by peptide bond. This is called as termination.
- Proteins can further undergo post-translational modifications such glycosylation, phosphorylation, acetylation, sumoylation which may be required for their intracellular localization, activity, or other purposes.

Difference between Eukaryotic and prokaryotic protein synthesis:

Prokaryotes	Eukaryotes
The ribosomal subunits are 40S and 30S to form 70S.	In contrast, it contains 60S and 40S to form 80S.
The initiation codon varies and multiple start sites are seen.	In eukaryotes only AUG serves as the initiation codon
The initiating amino acid is methionine	Formylated form of methionine (N-Formyl methionine)

- Nano-bioelectronics is a multi-disciplinary research area comprising biotechnology, nanofabrication, and imaging. It examines the properties of biomolecules by nanotechnology and uses biomolecules to add novel functions to nanoelectronic devices. The ultimate goal is to incorporate biomaterials like DNA as structural framework in nanoelectronic devices. The ability of *DNA's self-assembly* is combined with electronic properties of nanotubes to overcome the problem of organizing nanotubes into nanoscale electronic circuits.
- *Role of Technology in Human Genome Project:* Human genome project's goal was to sequence the human DNA. The final sets of scientific papers were published in 2006 even though the working draft was released in 2000. The Department of Energy, USA was able to facilitate this process by developing technologies involving the expertise of biologists, chemists, engineers, and mathematicians. The high-throughput machine, DNA sequencer, was crucial to the success of the genome project. GRAIL (Gene Recognition and Assembly Internet Link) was one of the initial computer programs for identifying genes in DNA and DNA sequence analysis. The automation process of DNA sequencing led to cutting costs and hastening the DNA sequencing process.

2.5 STEM CELLS AND THEIR APPLICATIONS

Stem cells are unspecialized cells having the ability of self-renewal through cell division for long period. These stem cells have the potential to develop into specialized cells such as blood cells, muscle cells, neurons, myocytes, bone cells, hepatocytes etc.

Importance of stem cells

Stem cells can replicate indefinitely so they are serving as internal repair system for body to replace dead or damaged cells.

When a stem cell divides, one of the daughter cells has to remain unspecialized (like a parent stem cell) and the another daughter cell has become specialized cell type such as brain cell, blood cell under certain physiological condition (Fig. 2.9).

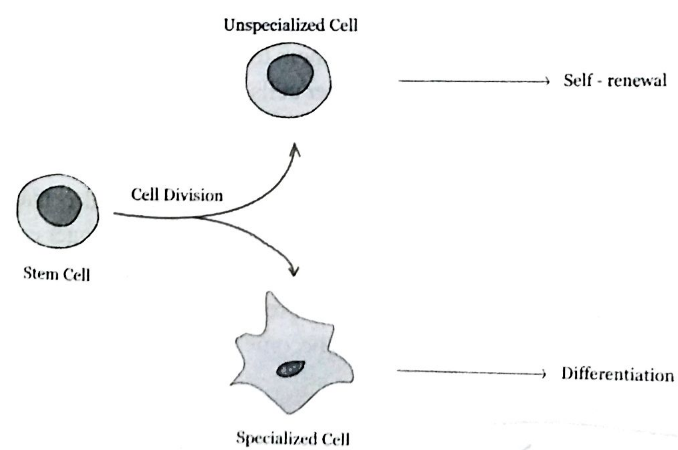


Fig. 2.9 *Unique properties of stem cells*

This is a promising area to know how an organism develops from a single cell with different types of potential properties. Since stem cells are having unique regenerative abilities, they offer new potentials for treatment of various diseases those results from dysfunction of a single type of cell.

Unique properties of stem cells

Stem cells are having three unique properties (Fig. 2.10). They are:

1. **Self-renewal:** Stem cells are immortal, unlimited in number and are capable of replicating/proliferating for a long period. They have potential to divide and maintain long term self-renewal.

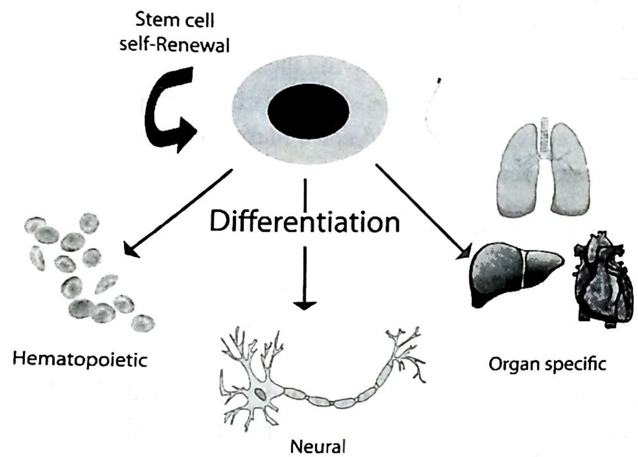


Fig. 2.10 *Differentiation of stem cells into other types of cells*

2. **Stem cells are unspecialized:** Stem cells do not have any tissue-specific or organ-specific structure that allows them to perform specified functions. During cell division, one of the daughter cells remains to be unspecialized.
3. **Stem cells give rise to specialized cells through the process of differentiation:** Several internal signals (include genes coding for cell structure and function) and external signals (include physical contact with neighboring cells, certain chemicals secreted by other cells) trigger each step of differentiation process. For example, stem cells can be differentiated into cartilage cells (chondrocytes) upon the presence of transforming growth factor-beta (TGF-β). At each step, cells become more specialized (Fig. 2.10).

Sources of stem cells

Embryonic stem cells: In embryogenesis, eggs are fertilized by sperm *in vitro* which is known as *in vitro* fertilization. As a result, zygote is developed

which undergoes series of cell division, and produces blastocyst. In early blastocyst stage (5 -7 days), a group of approximately 30 cells called inner cell mass (ICM) is surrounded by an outer layer. The outer layer is called as trophoblast which provides nutrient to the embryo and develops into a large part of the placenta. ICM can give rise to all types of cells or tissues except trophoblast.

Adult stem cells: Adult stem cells are tissue-specific, undifferentiated cells found in differentiated tissues or organs including brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testes (Fig. 2.11). Their main role is to play in tissue repair and tissue maintenance.

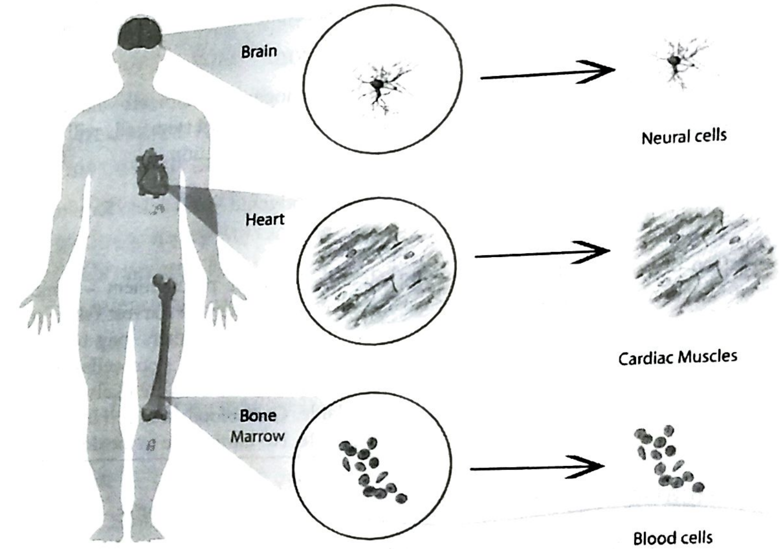


Fig. 2.11 *Sources of adult stem cells*

Adult type stem cells can be derived from various pregnancy-related tissues such as umbilical cords, placentas, and amniotic fluids.

In adults, stem cells are present within various tissues and organ systems such as bone marrow, liver, epidermis, retina, skeletal muscle, intestine, brain, dental pulp.

Cadavers can also be a source of adult stem cells. For example, neural stem cells have been removed from specific areas in post-mortem human brains as late as 20 hours following death.

Differentiation between embryonic and adult stem cells

Embryonic stem (ES) cells	Adult stem cells
1. Flexible- i.e., ES cells appear to have the potential to make almost all types of cells except trophoblast.	Less flexible- i.e., difficult to reprogram to form other tissue types.
2. Immortal- one ES cell line can potentially provide an endless supply of cells with defined characteristics.	Mortal with finite life time when cultured.
3. Availability - embryos can be obtained from in vitro fertilization and nuclear transplantation source.	Limited quantity - can sometimes be difficult to purify and obtain in large numbers.
4. Difficult to differentiate uniformly and homogeneously into a target tissue.	adult stem cells are already somewhat present in specialized tissues, inducement may be simpler.
5. Immunogenic - ES cells from a random embryo donor are likely to be rejected after transplantation.	Not immunogenic - recipients who receive the products of their own stem cells will not experience immune rejection.
6. Tumorigenic - Capable of forming tumors or promoting tumor formation	Non-tumorigenic - tend not to form tumors.
7. Isolation of embryos leads to destruction of developing human life and also very hard to isolate	Relative ease of procurement - some adult stem cells are easy to harvest (skin, muscle, marrow, fat), while others may be more difficult to obtain (brain stem cells). Umbilical and placental stem cells are likely to be readily available. Cord blood stem cell banks are now available. No harm done to the donor.

Classification of stem cells

The stem cells can be classified into several types based on their potency or plasticity. Potency or plasticity can be defined as the ability of the stem cell from one tissue to generate the specialized cell type(s) of another tissue.

Unipotent stem cells can form only one type of specialized cell type. For example, brain stem cells differentiate into only brain cells.

Multipotent stem cells can form multiple types of cells. For example, mesenchymal stem cells derived from bone marrow can differentiate into osteoblasts, adipocytes, chondrocytes, myocytes, and neuron-like cells.

Pluripotent stem cells can differentiate into almost all types of cell lineages. For example, cells (ICM) from blastocyst can differentiate into three germ cell layers (ectoderm, mesoderm and endoderm) but do not contribute for trophoblast.

Totipotent stem cells can differentiate into all cell types including cells of the trophoectoderm lineage. For example, fertilized egg and early cleavage stage of blastomeres.

Human embryonic stem cell isolation and culturing

Cell culture is a process of growing cells in the laboratory conditions. For generating human embryonic stem cells (hESCs), cells (ICM) from **blastocyst** are transferred into a culture dish containing a nutrient broth known as **culture medium.** The cells attach, proliferate and spread on the surface of the dish. The inner surface of the culture dish is typically coated with mouse embryonic skin cells that have been UV treated so they will not divide. This coating layer of cells is called a **feeder layer** (Fig. 2.12), which provides a sticky surface to which hESCs can attach. Also, the feeder cells release factors (growth factors, cytokines) into the culture medium. Thus, feeder layer can act as niche for promoting self-renewal or suppress of differentiation of hESCs. In each cycle of sub-culturing, the cells are referred to as a **passage**. hESCs can proliferate for six or more months without differentiation but can also maintain their pluripotent properties.

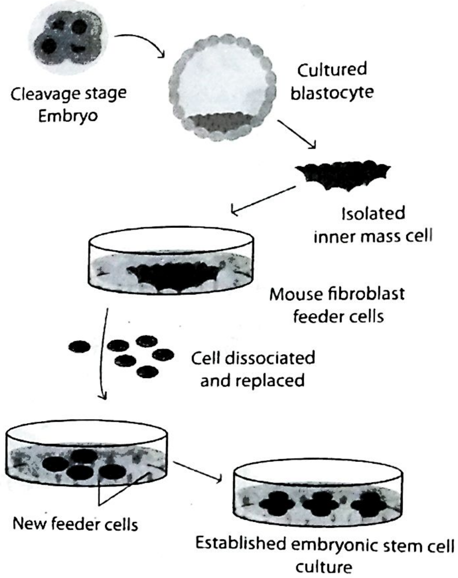
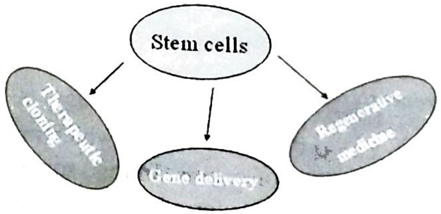


Fig. 2.12 *Culturing of human embryonic stem cells*

Applications:

Stem cell technology has a broad range of applications; here is an overview:



Therapeutic cloning

Somatic cell nuclear transfer (SCNT) involves extracting the nucleus of a cell and putting the nucleus into an egg which has been enucleated. Then, the egg is allowed to divide and grow. In therapeutic cloning, the growing egg is used as a source of stem cells, which are undifferentiated cells that can grow into a wide variety of different types of cells. In reproductive cloning, the egg is implanted into surrogate mother to grow a baby (Fig. 2.13). Dolly, a first female domestic sheep was the first mammal to be cloned from an adult somatic cell, using the process of SCNT.

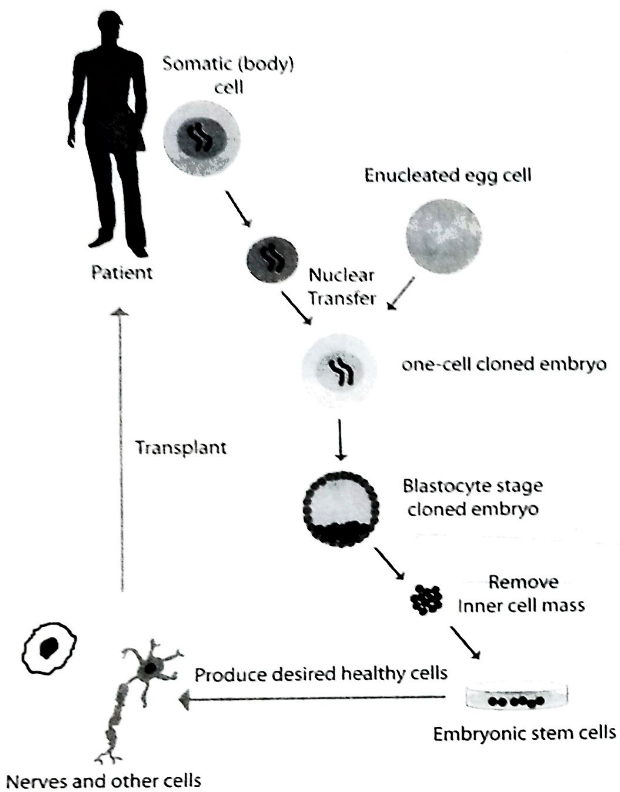
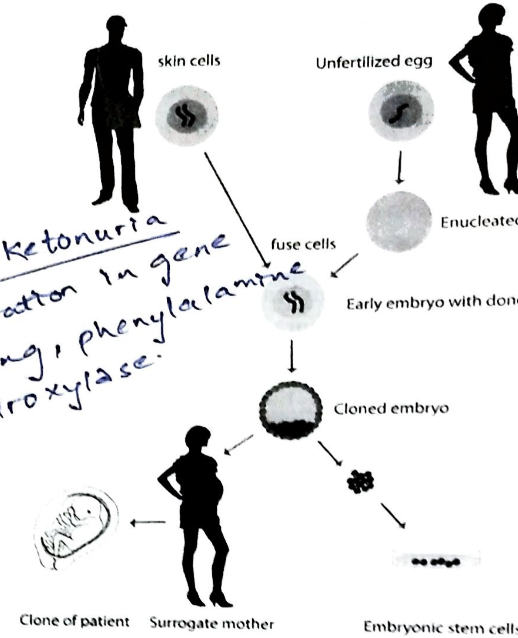


Fig. 2.13 *Therapeutic and reproductive cloning*

Therapeutic cloning can produce stem cells which may be used to treat various disorders or replacement of organs. In this, somatic cell is obtained from a patient and the nucleus is transferred into enucleated egg cell which

will develop into an embryo. At blastocyst stage of the embryo, the inner cell mass is isolated and cultured to differentiate into particular cell type which will be replaced into the patients as a transplant (Fig. 2.14).



phenylketonuria
- mutation in gene coding, phenylalanine hydroxylase.

induced fit
interaction b/w enzymes & its substrate is generally weak. Interaction is called induced fit.

Fig. 2.14 *Theoretical concept of therapeutic cloning*

Regenerative medicine

Regenerative medicine is the process of creating functional tissues to repair or replace tissue or organ function lost due to damage, or congenital defects. This field holds the promise of regenerating damaged tissues and organs in the body by stimulating previously irreparable organs to heal themselves. Regenerative medicine also empowers scientists to grow tissues and organs in the laboratory and safely implant them when the body cannot heal itself.

Benefits of Stem Cell Research in curing diseases: Stem cell research can potentially help treating a range of medical problems. It could lead us closer to cure:

Parkinson's disease: A degenerative disorder of the central nervous system due to the lack of a brain chemical called dopamine. To cure this disease, the patients are injected with stem cells to multiply nerve cells that release dopamine.

Muscular dystrophy is a group of inherited disorders that involve muscle weakness and loss of muscle tissue, which get worse over time due to weakened heart and lung muscles. Patients are given injections of healthy stem cells and they are able to walk faster.

Diabetes (Type 1): Autologous (originating from your own body) stem cell therapy becomes a promising tool to treat diabetes I. It reduces hyperglycemia and its associated complications. Recent evidence suggests that it also reduces hypoglycemic (low blood sugar) events that can result in death if not treated promptly. A study showed patients receiving injections with adult stem cells were able to go as long as four years without having to rely on insulin shots.

Polio: Poliomyelitis is a viral disease that can affect nerves and can lead to partial or full paralysis. Signals from brain are no longer sent to muscles in the leg. Stem cells are injected to encourage new spinal neurons to grow and help new muscle to grow.

Bone tissue engineering: Bone tissue engineering is an emerging interdisciplinary field for the development of viable substitutes that restore and maintain the function of human bone tissues. Spinal fusion, augmentation of fracture healing, and reconstruction of bone defects resulting from trauma, tumor, infections, biochemical disorders, or abnormal skeletal development are clinical situations in which surgical intervention is required. The types of graft materials available to treat such problems essentially include autologous bone (from the patient), allogeneic bone (from a donor), and a wide range of natural or synthetic biomaterials such as metals, ceramics, polymers, and composites. In one approach, mesenchymal stem cells are seeded on scaffolds (that provide structure and shape) along with signaling molecules. The goal is for the cells to attach to the scaffold, and thus resulting proliferation and

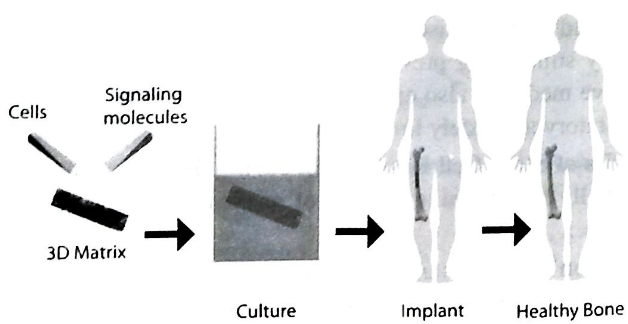


Fig. 2.15 *Scaffolds-guided bone regeneration*

differentiation of cells into normal, healthy bone. When bone grows, the scaffold degrades. At one stage there will be only bone and no more scaffold materials. The signaling molecules [for example, bone morphogenetic proteins (BMPs)] can also be adhered to the scaffold or incorporated directly into the scaffold material. BMPs promote mesenchymal stem cells towards osteoblasts (bone cells). Finally the scaffolds are implanted into the defect to induce and direct the growth of new bone (Fig. 2.15).

2. Gene therapy

Gene therapy is the insertion of genes into an individual's cells and tissues to treat a disease. The hereditary diseases in which a defective gene (nucleotide mutation, for example, sickle cell anemia) is replaced with a functional one. A "corrected" gene is inserted into the genome to replace an "abnormal," disease-causing gene. Gene therapy clinical trials are focused on cancer, infectious diseases, heart disease, and inadequate blood flow to the limbs, arthritis, and Alzheimer's disease.

Gene therapy involves two major strategies for delivering therapeutic transgenes into human recipients:

1. The first is to "directly" infuse the gene into a person by viral mediated transfer. Viruses that have been altered to prevent them from causing disease are often used as the vehicle for delivering the gene into certain human cell types. This is similar to transduction process how ordinary viruses infect cells. Some viruses commonly used as gene-delivery vehicles can only infect cells that are actively dividing. This limits their usefulness in treating diseases of the heart or brain, because these organs are largely composed of non-dividing cells.
2. The second strategy involves the use of living cells to deliver therapeutic transgenes into the body. In this method, the delivery cells often a type of stem cell, a lymphocyte, or a fibroblast, are removed from the body, and the therapeutic transgene is introduced into them via the vehicles. The genetically modified cells are tested and then allowed to grow and multiply and, finally, are infused back into the patient.

- A new computer vision technology has been developed to aid scientists investigating stem cells as to whether the dividing stem cell will further divide or become part of a future developing organ. It will be helpful in observing each and every stem cell and sort them into different categories.

- **Cell density of stem cells in cell growth dishes determine further differentiation of these cells. To give a uniform density, electronic control of seeding density of stem cells have been achieved using thin electrode films of poly(3,4-ethylenedioxythiophene) (PEDOT): Tosylate.**

Conclusions

Stem cells can now be artificially grown and transformed into specialized cell types with characteristics consistent with cells of various tissues. They can be taken from a variety of sources, including umbilical cord and bone marrow. Embryonic stem cell lines and autologous embryonic stem cells generated through therapeutic cloning have been proposed as promising candidates for future therapies. Adult stem cells are currently tested and used in medical therapies including tissue engineering. There are research and medical ethics associated with rules and regulations to be followed when stem cells are used for biomedical applications in human.

Chapter highlights

- The varieties of life on the Earth forming biodiversity are critical to sustenance of life on this planet. It can be genetic diversity, species diversity, and ecosystems diversity. Biodiversity is facing challenges and losses due to human population growth and their life style that may interfere and harm human way of life.
- All living and non-living beings are made up of atoms which then form molecules. The atoms within molecules are held by chemical bonds that are either weak or strong.
- In all living organisms, biochemical reactions called as metabolism occur that are essential for the survival of cells, structure, transport, cell signaling, cell division, etc.. Metabolism involves both anabolism and catabolism of biomacromolecules such as carbohydrates, lipids, proteins, and nucleic acids.
- Protein synthesis involves synthesis of proteins by the cells through the multi-step processes of transcription, post-transcriptional modification, translation and post-translational modifications.
- DNA replication is followed by messenger RNA (mRNA) production which carries the message of DNA from the nucleus to the cytoplasm. Transfer RNA (tRNA) carries the amino acids to ribosomal RNA (rRNA) to produce polypeptides according to the message on mRNA.

- Stem cells are unspecialized cells capable of developing into specialized cells such as neuron, liver cell, muscle fiber, etc. They can self-renew and differentiate into specialized cells. Embryonic and adult stem cells can be used for therapeutic cloning, gene therapy, and in regenerative medicine. Medical researchers believe that stem cells can be a source for curing a number of diseases afflicting human population.