Before Section II of lecture:
Pls revise 'Gene expression' topic and complete the pre-lecture qns on pg 33

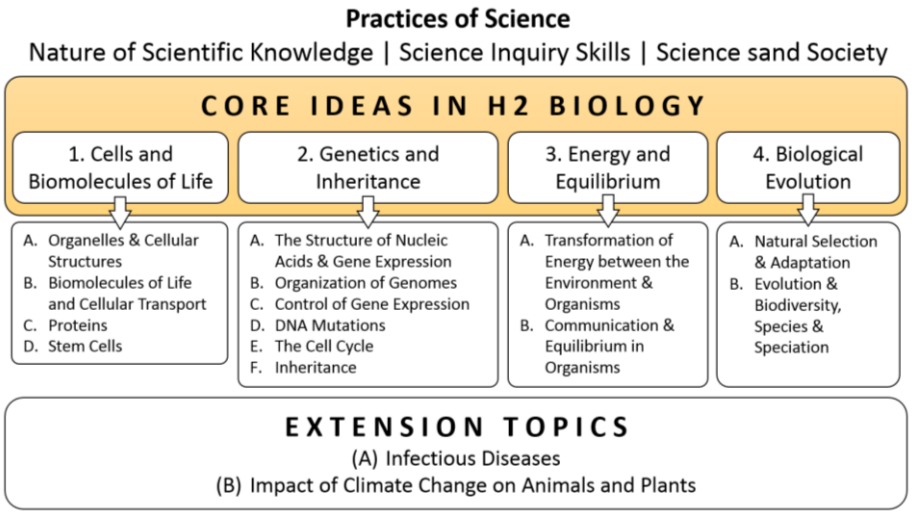
Tampines Meridian Junior College

JC2 H2/9744 Biology 2024

Core Idea 2B | 2C | 1D

8. Genetics & Inheritance (V)

Organization of Genome & Control of Gene Expression in Eukaryotes | Stem Cells



SYLLABUS OVERVIEW		
No.	**Overarching Idea**	**Topics**
1	**Core Idea 1** The Cell and Biomolecules of Life	Cell – The Basic Unit of Life
2		Biomolecules of Life and Cellular Transport
3	**Core Idea 3** Energy and Equilibrium	Transformation of Energy – Photosynthesis and Cellular Respiration
4	**Core Idea 2** Genetics and Inheritance	Genetics and Inheritance (I) – The Cell Cycle
5		Genetics and Inheritance (II) – DNA Replication and Gene Expression
6		Genetics and Inheritance (III) – DNA Mutations and their Consequences
7		Genetics and Inheritance (IV) – Molecular Techniques in DNA Analysis
8		Genetics and Inheritance (V) – Organization of Genome & Control of Gene Expression in Eukaryotes *[Includes Core Idea 1D: Stem Cells]*
9		Genetics and Inheritance (VI) – Organization and Inheritance of Viral Genomes
10		Genetics and Inheritance (VII) – Organization of Genome & Control of Gene Expression in Prokaryotes
11		Genetics and Inheritance (VIII) - Inheritance
12	**Core Idea 3** Energy and Equilibrium	Communication and Equilibrium in Multicellular Organisms
13	**Core Idea 4** Biological Evolution	Biological Evolution
14	**Extension Topic A** Infectious Diseases	Immunity and Infectious Diseases
15	**Extension Topic B** Impact of Climate Change on Animals & Plants	Climate Change – Causes and Impacts on Animals and Plants

NARRATIVES

An understanding of *Genetics and Inheritance* helps make sense of the transition from molecular to organismal levels. *Genetics and Inheritance* provides the molecular basis to the understanding of how variation in populations arises and this is important in the study of biological evolution. At the cellular level, expression of genes involves cellular structures such as the nucleus, endoplasmic reticulum and ribosome. Many essential products of gene expression are enzymes involved in biochemical pathways which control physiological functions. As such, mutation of genes may give rise to dysfunctional proteins which in turn could result in diseases. Sickle cell anemia and cancer are raised as examples of genetic diseases.

The following questions should help students frame their learning:

- How does the genetic make-up of an organism and the environment influence the organism's appearance, behavior and survival?
- How does the inheritance of genetic information ensure continuity of humans as a species?

Heritable information, in the form of DNA (and in some cases RNA), provides for continuity of life

Genetic information is stored in an organism's DNA; expression of genes results in the synthesis of functional products, such as rRNA, tRNA and proteins. These products play a role in intra- and extra-cellular biochemical pathways and influence the physiological processes in organisms.

Genomes contain heritable information necessary for continuity of life at all levels: cell, organism and system. This information is stored and passed on to subsequent generations via DNA. Reproduction can occur at the cellular or organismal level; each progeny needs to receive heritable genetic information from its parent(s).

An understanding of how eukaryotic, prokaryotic and viral genomes are organised has implications on how gene expression in organisms is controlled. Eukaryotic genomes are organised in a more complex manner (compared to prokaryotic genomes). DNA is wrapped around histone proteins and compacted to form linear chromosomes; the number of chromosomes varies between eukaryotic species. Structurally, linear chromosomes have centromeres and telomeres, and their DNA consists of coding and non-coding sequences with the latter being in larger proportions. Coding DNA is expressed to give functional products (e.g. proteins, rRNA, tNRA) while non-coding DNA, e.g. control elements and centromeres, are involved in regulation of gene expression and nuclear division respectively.

Expression of genetic information involves molecular mechanisms and gene regulation results in differential gene expression.

In a single organism, the genes contained in all the nuclei of somatic cells are exactly the same, but the cell types differ morphologically and functionally. The differences between cell types are not due to the different genes being present, but due to differential gene expression, i.e. the expression of different sets of genes by cells with the same genome.

Regulation of gene expression gives a cell control cover its structure and function. It allows cell differentiation to occur. It may be controlled by the way DNA is packed in chromatin and at the various steps of protein synthesis, i.e. from the transcription to post-translational modification of a protein. It is the basis for cellular differentiation and morphogenesis which gives an organism versatility and adaptability. Gene expression can be studied using fundamental techniques of molecular biology such as the polymerase chain reaction (PCR), gel electrophoresis, Southern blotting and nucleic acid hybridization *(covered in Topic 7)*.

Stem cells have the potential to divide and differentiate into different cell types.

Following fertilization, a single-cell zygote develops into a multicellular organism. The zygote can replicate its DNA, divide its nucleus, and divide into two genetically-identical cells. Cell potency describes a cell's ability to differentiate into other cell types. The zygote and cells formed from the first few cell divisions during embryonic development (up to the eight-cell stage) produce totipotent cells. Beyond the eight-cell stage, one of the two daughter cells remains undifferentiated, retaining the ability to divide indefinitely as a stem cell, while the other daughter cell differentiates. After the eight-cell stage, the cells begin to specialise into pluripotent stem cells. Pluripotent stem cells undergo further specialisation into multipotent cells, which can further differentiate to become unipotent stem cells.

Environment signals triggers the differentiation of a cell into a more specialised form. Cell differentiation involves changing or regulating the expression patterns of genes. Each specialised cell type in an organism

expresses a subset of all the genes that constitute the genome and this expression is regulated by various mechanisms resulting in differential gene expression of the same genome.

It is important to recognise that a cell is dynamic in nature and not a static structure. At any point of time, numerous activities are occurring in the cell. In a plant cell, photosynthesis and respiration can be occurring simultaneously. This causes the biochemical changes in the cytoplasm of the plant cell. If it is necessary to produce more chlorophyll pigments or increase the amount of cellulose, the rate of protein synthesis in those biochemical pathways will increase.

REFERENCES

Organisation and Control Eukaryotic Genome

1. Campbell Biology by Reece *et al*. (9th Edition)
2. Molecular Biology of The Cell by Alberts, Bray, Lewis, Raff, Roberts and Watson (4th or 5th Edition)
3. Essential Cell Biology by Alberts, Bray, Hopkins, Johnson, Lewis, Raff, Roberts & Walter (3rd edition)
4. http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/P/Promoter.html

Stem cells

5. Molecular Biology of the Cell by Bruce Alberts, Dennis Bray, Julian Lewis, Martin Raff and Keith Roberts
6. Application of Genetics, Cambridge Advanced Sciences by Gregory, J (2000)
7. Taylor, D J, Green, N P O, Stout, G W and Soper R (1997) Biological Science 1 and 2 (Third Edition)
8. Taylor, D (2001), Growth, Development and Reproduction Cambridge Modular Sciences (Second Edition)
9. Stem cell facts by International Society for stem cell research.

LEARNING OUTCOMES

Core Idea 2B: Organization of Genomes
The nuclear genomes of eukaryotes differ greatly in size, number of genes and gene density. The number of chromosomes differs between species and, in addition, certain organelles in eukaryotes possess small amounts of their own DNA. Eukaryotic genomes generally have a higher proportion of non-coding DNA to coding DNA.

Candidates should be able to:

a) Describe the structure and organisation of eukaryotic genomes (e.g. DNA/RNA, single-/double-stranded, number of nucleotides, packing of DNA, linearity/circularity and presence/absence of introns)

e) Describe the structure and function of non-coding DNA in eukaryotes (i.e. portions that do not encode protein or RNA, including introns, centromeres, telomeres, promoters, enhancers and silencers)
(Knowledge of transposons, satellite DNA, pseudo-genes and duplication of segments is not required)

Core Idea 2C: Control of Gene Expression
In eukaryotes, regulation of gene expression can occur at the chromatin level, transcriptional level, post-transcriptional level, translational level and/or even post-translational level.

Candidates should be able to:

b) Explain how differential (i.e. spatial and temporal) gene expression in eukaryotes can be regulated at different levels:
- **i)** chromatin level (histone modification and DNA methylation);
- **ii)** transcriptional level (control elements, such as promoter, silencers and enhancers, and proteins, such as transcription factors, including activators and repressors);
- **iii)** post-transcriptional level (processing of pre-mRNA in terms of intron splicing, polyadenylation and 5' capping);
- **iv)** translational level (half-life of RNA and initiation of translation); and
- **v)** post-translational level (biochemical modification and protein degradation).

Core Idea 1D: Stem Cells

This concept highlights the diversity in cell type and morphology in an organism. In an organism, all cells except the gametes are genetically identical. Yet, a liver cell, and a rod cell in the eye and an epithelial cell in the ileum differ significantly in terms of morphology and function due to differential gene expression. The same genome gives rise to a wide range of cells which further forms tissues, organs and systems in an organism.

The ability of stem cells to divide and self-renewal allows growth. Stem cells replace cells that die or are damaged. During embryogenesis, cell division and differentiation allow the development of an entire organism in utero (Latin term which means "in the womb") from a single-cell zygote.

Stem cells hold great potential as medical treatments. Hematopoietic (blood) stem cells are used in blood marrow transplants in cancer treatments. Skin stem cells are used to culture skin cells to treat patients with massive burns. Ethical debates over the use of stem cells are primarily concerned with the use of embryonic stem cells. Use of adult stem cells faces fewer of such ethical issues.

Candidates should be able to:

a) Describe the unique features of stem cells, including zygotic stem cells, embryonic stem cells and blood stem cells (lymphoid and myeloid), correctly using the terms:
- **i)** totipotency (e.g. zygotic stem cells)
- **ii)** pluripotency (e.g. embryonic stem cells)
- **iii)** multipotency (e.g. lymphoid and myeloid stem cells)

b) Explain the normal functions of stem cells in a living organism, including embryonic stem cells and blood stem cells (lymphoid and myeloid).

c) Discuss the ethical implications of the applications stem cells in research and medical applications and how human induced pluripotent stem cells (iPSCs) overcome some of these issues. (Procedural details how iPSCs are formed are not required.)

LECTURE OUTLINE

I. Organization of Eukaryotic Genome

1. Packaging of DNA into chromatin / chromosome
   - 1.1 Nucleosomes
   - 1.2 30nm chromatin fiber / solenoid
   - 1.3 Looped domains
2. Types of chromatin
3. Features of eukaryotic genome
   - 3.1 Coding DNA sequences
     - 3.1.1 Genes
     - 3.1.2 Gene families
   - 3.2 Non-coding DNA sequences
     - 3.2.1 Centromere
     - 3.2.2 Telomere
     - 3.2.3 Introns
     - 3.2.4 Regulatory sequences
     - 3.2.5 Repetitive DNA (not in syllabus, but knowledge needed)

II. Control of Eukaryotic Gene Expression

4. Overview and Key Concepts in the control of gene expression
   - 4.1 Overview
   - 4.2 Key concepts
5. How is gene expression controlled at different stages?
   - 5.1 Control at the chromosomal (DNA) level (chromatin remodeling)
     - 5.1.1 Histone modifications
     - 5.1.2 DNA methylation
   - 5.2 Control at the transcriptional level
     - 5.2.1 Eukaryotic RNA polymerases
     - 5.2.2 Regulation of transcriptional initiation
   - 5.3 Control at the post-transcriptional level
     - 5.3.1 Addition of 7-methylguanosine to the 5’ end of mRNA (5’ capping)
     - 5.3.2 Addition of poly(A) tail at the 3’ end of mRNA (3’ polyadenylation)
     - 5.3.3 RNA splicing
     - 5.3.4 Alternative RNA splicing
   - 5.4 Control at the translational level
     - 5.4.1 Regulation of mRNA degradation / half-life of mRNA
     - 5.4.2 Regulation of translational initiation
     - 5.4.3 Polyribosomes
   - 5.5 Control at the post-translational level
     - 5.5.1 Protein modifications/processing
     - 5.5.2 Protein degradation
6. Summary Map on the control of gene expression

III. Stem Cells

7. Stem Cells
   - 7.1 Unique features of stem cells
   - 7.2 Developmental potential of stem cells
     - 7.2.1 Totipotent stem cells
     - 7.2.2 Pluripotent stem cells
     - 7.2.3 Multipotent stem cells
   - 7.3 Types of stem cells and their normal functions in living organisms
     - 7.3.1 Embryonic stem cells (ES cells)
     - 7.3.2 Adult stem cells
     - 7.3.3 Comparison between embryonic and adult stem cells
   - 7.4 Stem cells in research and in medical applications
     - 7.4.1 What is stem cell therapy?
     - 7.4.2 Current uses of stem cell therapy
     - 7.4.3 Potential uses of stem cell therapy
   - 7.5 Ethical implications of stem cells in research and in medical applications
     - 7.5.1 Ethical implications
     - 7.5.2 Enforcement currently in place
     - 7.5.3 Alternative methods to obtain embryonic stem cells

ONLINE RESOURCES

Organization and Control of Eukaryotic Genome

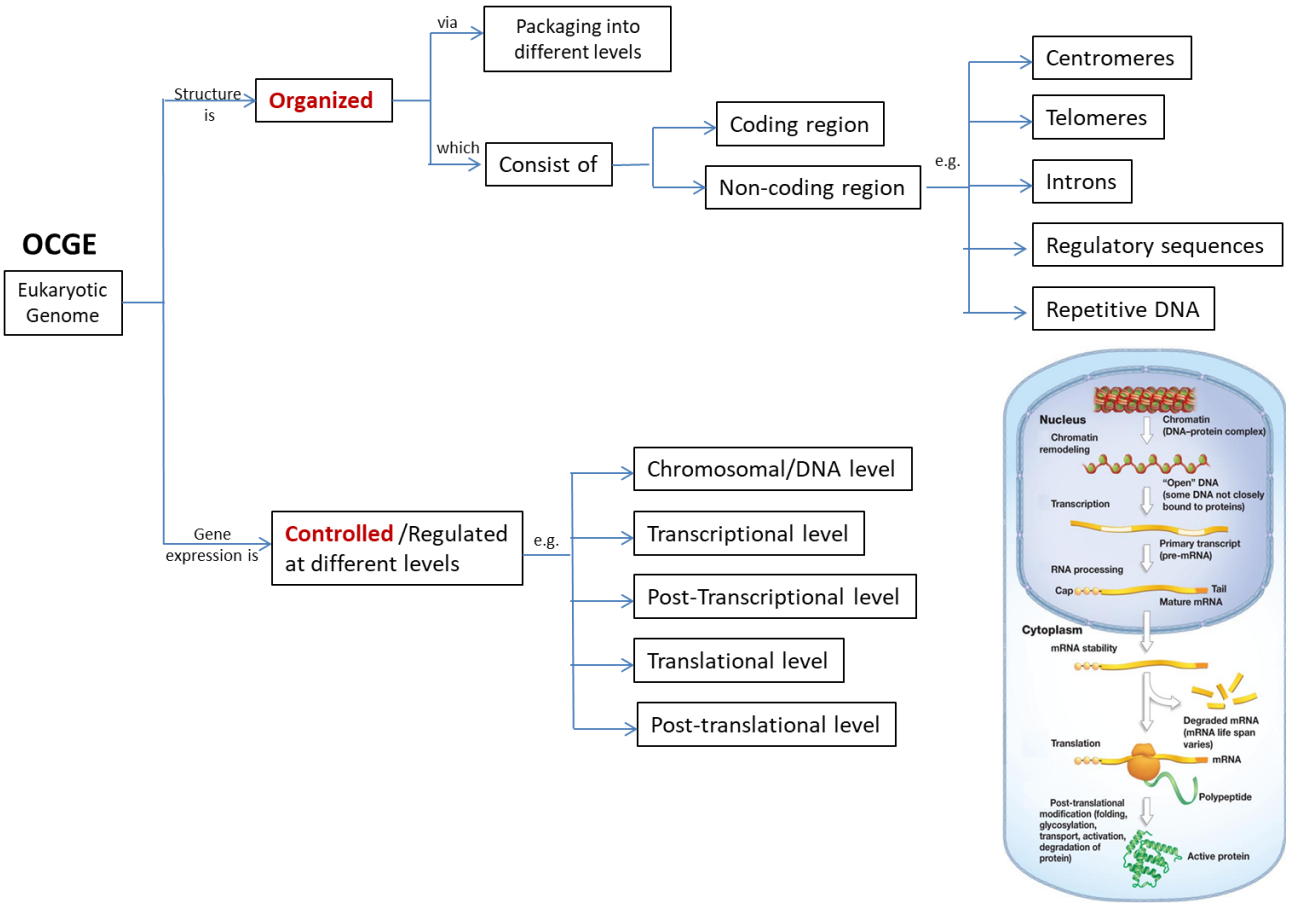
No.	Content	URL	Remarks
1	Packing of DNA into chromosome	http://www.dnaftb.org/29/index.html	• Excellent animation to visualise DNA being packaged into a metaphase chromosome
2	Telomeres and telomerase	https://learn.genetics.utah.edu/content/basics/telomeres/ http://www.wiley.com/legacy/college/boyer/0470003790/cutting_edge/telomeres/telomeres.htm https://www.youtube.com/watch?v=2NS0jBPurWQ	• About telomeres and telomerase • (Animation) End-replication problem and activity of telomerase
3	Control of gene expression in eukaryotes – Overview	https://www.youtube.com/watch?v=7XrhoNO1GKw	• Overview of some of the various stages at which protein production can be controlled in eukaryotes
4	Gene silencing	https://www.biointeractive.org/classroom-resources/role-mecp2-gene-silencing	• Animation of an example of a repressor protein silencing a gene
5	RNA splicing	https://www.youtube.com/watch?v=aVgwr0QpYNE	• How spliceosomes remove introns and splice exons together

Stem cells

No.	Content	URL	Remarks
1	Nature of stem cells	https://learn.genetics.utah.edu/content/stemcells/scintro/	
2	Animation on unlocking stem cell potential	https://learn.genetics.utah.edu/content/stemcells/scfuture/	Possible applications of stem cells
3	Animation on Culturing embryonic stem cells and Somatic Cell Nuclear Transfer (SCNT)	http://www.sumanasinc.com/webcontent/animations/content/stemcells_scnt.html	
4	Animation on induced pluripotent stem cells (iPS)	http://www.youtube.com/watch?v=i-QSurQWZo0	
5	Readings on Ethical issues in Stem Cell Research and Therapy	https://www.eurostemcell.org/embryonic-stem-cell-research-ethical-dilemma http://www.aish.com/ci/sam/48969936.html	

I. Structure and Organization of Eukaryotic Genome

Overview



1. Structure of Eukaryotic Genome

Packaging of DNA into chromatin / chromosome

- Each eukaryotic chromosome is a single **linear** DNA double helix, which undergo multiple levels of packaging, along with large amount of proteins.

- Eukaryotic chromosomes contain an enormous amount of DNA relative to their condensed length (2×10^8 nucleotide pairs in a human chromosome). On average, the total length of the 46 DNA molecules in a human cell, when extended, measure up to 2-meters, 20 thousand times longer than the cell diameter.

- The massive amount of DNA (in all 46 human chromosomes) is packed compactly into the nucleus through and elaborate, multi-level packaging of DNA (Fig. 1.1).

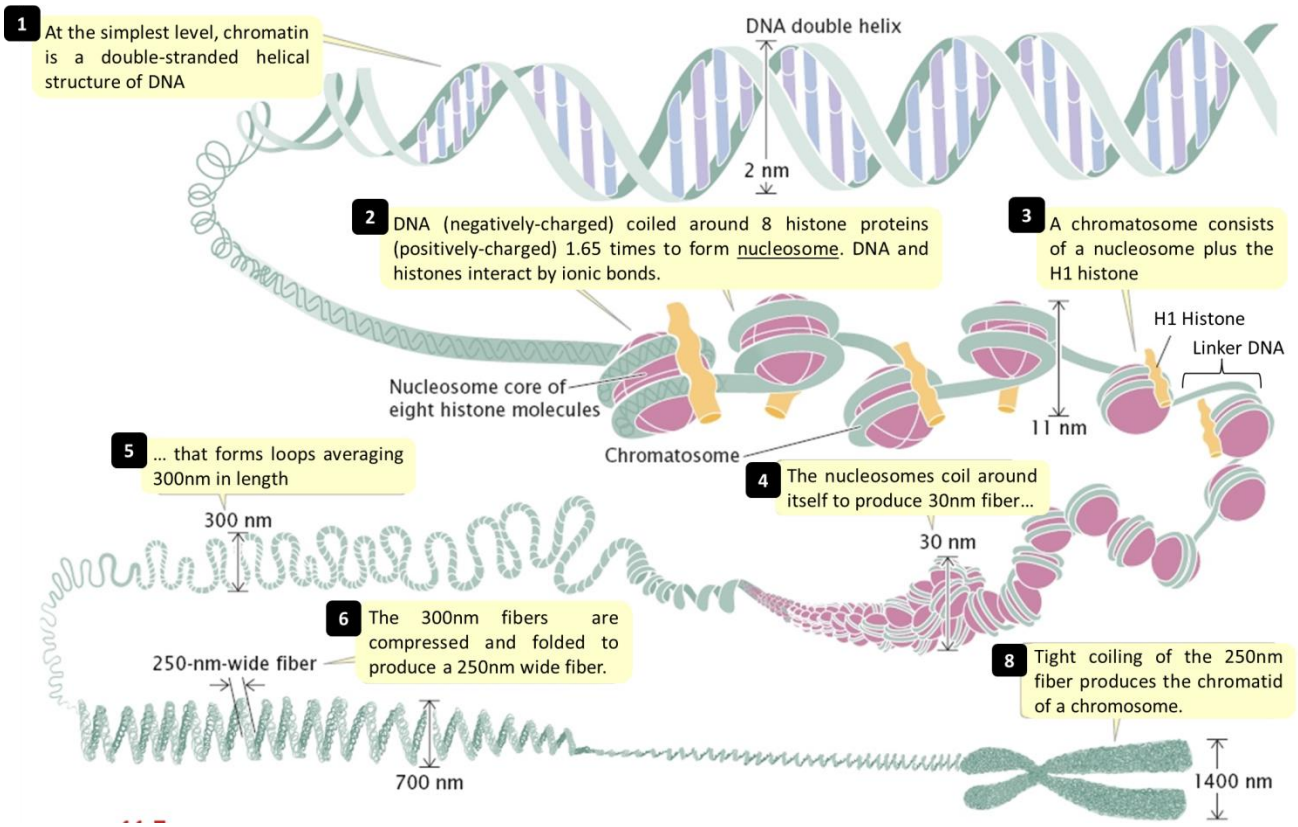


Fig. 1.1: Overview of compact eukaryotic DNA packaging which is achieved by the formation of highly organized complex of DNA and proteins, which is further extensively folded into chromatin and eventually chromosome.

- The extensive packaging of DNA in chromosomes results from three levels of folding:
  - Nucleosomes
  - 30-nm chromatin fiber / solenoid
  - Looped domains

1.1 Nucleosomes

- The first level of packaging is the formation of **nucleosome** (DNA-histone complex / nucleoprotein complex). It has the appearance of 'bead-on-a-string'.

- It involves the binding of **negatively charged DNA** to **positively charged histones** by **ionic bonds.**

  - DNA is negatively charged due to presence of phosphate groups.
  - Histone proteins are positively charged due to high proportion of **positively charged lysine** and **arginine** amino acids.

- Each nucleosome bead consists of **DNA** wound around a protein core consisting of **eight histone molecules** (octameric histones).

  - Two molecules each of four different types of histones – **H2A, H2B, H3 & H4** (Fig. 1.2a/c). The amino end of each histone extends outwards from the nucleosome (the histone tail).

  - A fifth histone molecule called **H1**, **attaches to linker DNA near the nucleosome**. Linker DNA refers to the length of DNA between the nucleosome beads, with varying length of a few base pairs to about 80-bp (60-bp in humans) (Fig. 1.2a). This **promotes interaction between adjacent nucleosomes**, allowing chromatin to undergo the next level of packaging into a 30-nm fiber (Fig 1.2b). Hence, H1 plays a crucial role in the **compaction of DNA**.

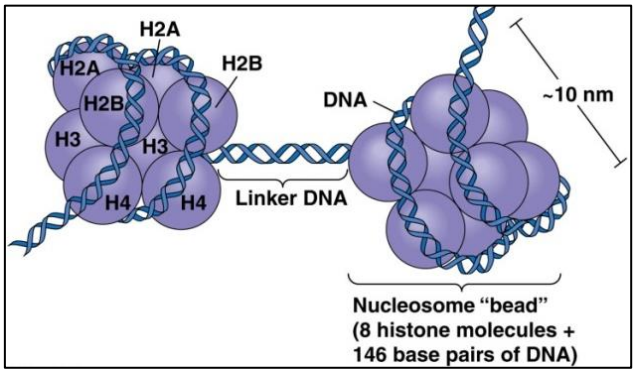


Fig. 1.2a: Nucleosome which is made up of DNA wound around an octameric histone, made up of 2 molecules of H2A, H2B, H3 and H4. The length of DNA between the nucleosomes are is known as the linker DNA.

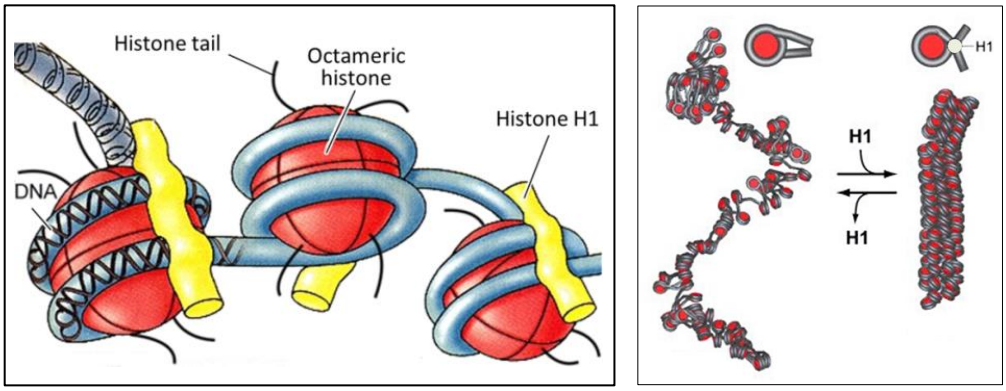


Fig. 1.2b: The binding of H1 histones allows further compaction of chromatin structure.

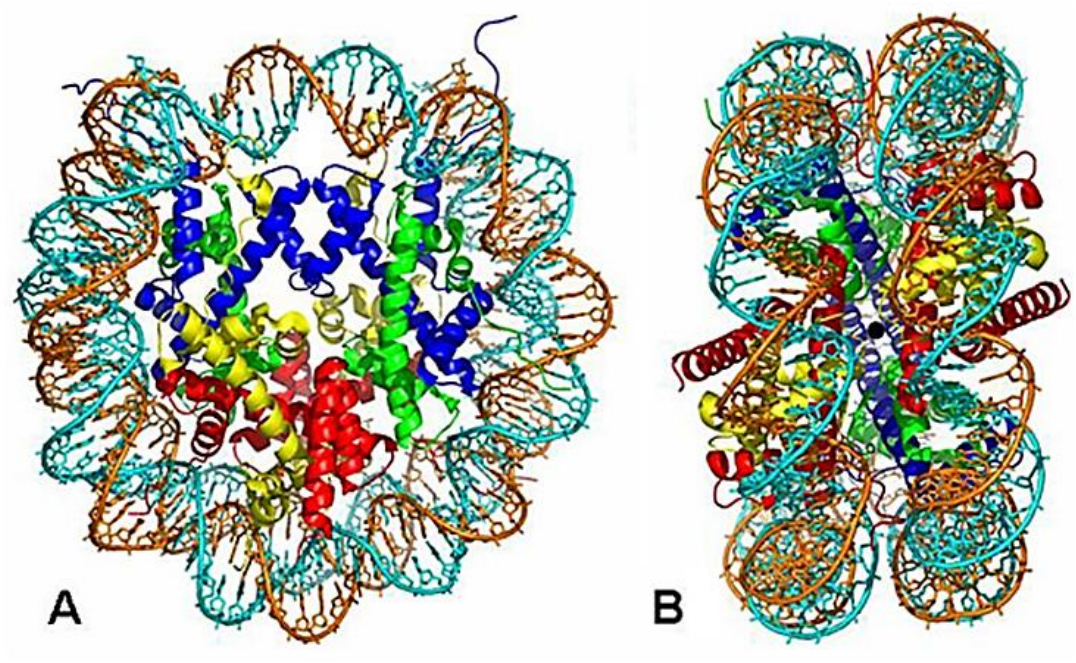


Fig. 1.2c: Computer imaging of a nucleosome. (A) top-down view (B) side view
**Label in diagrams (A) and (B) the position of DNA and histones.*

1.2 30-nm Chromatin Fiber / Solenoid

- The string of nucleosome beads **coil around itself** into a higher order helix with the aid of the H1 histone to form a **30-nm chromatin fiber / solenoid** (Fig. 1.3).

- There are **6 nucleosomes per turn** of the helix of the solenoid.

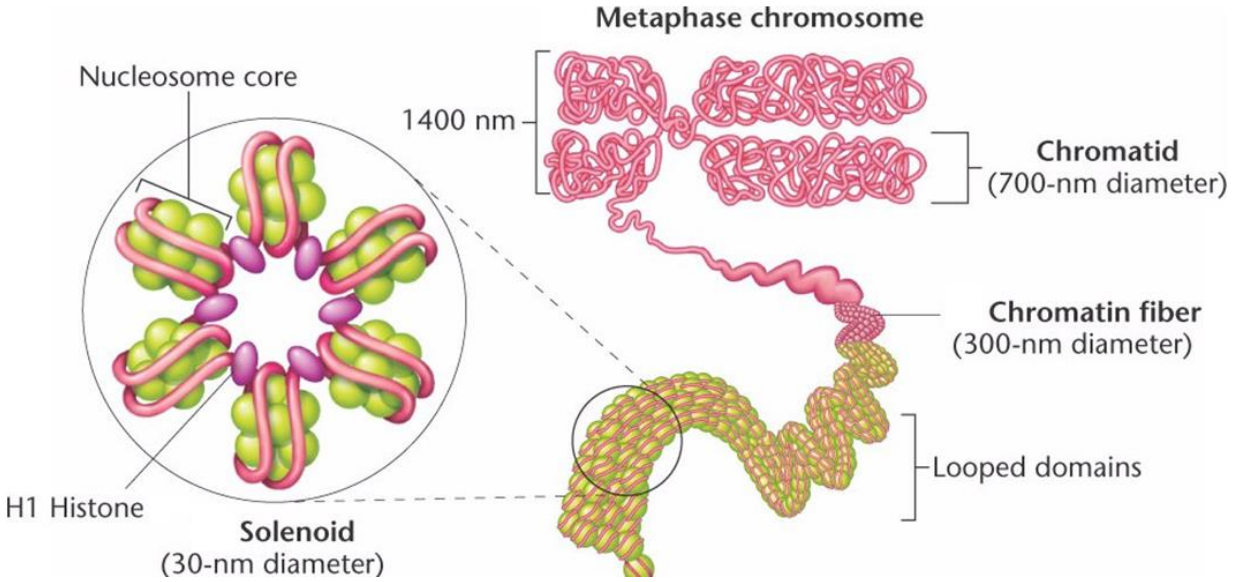


Fig. 1.3: A solenoid (30-nm fiber) is made from the coiling of nucleosome beads (6 beads per turn)

1.3 Looped Domains

- In the next level of packing, the 30-nm chromatin fiber attaches to multiple locations on a **chromosome scaffold** in a series of **looped domains** to form **300-nm fiber** (Fig. 1.4).
    - The chromosome scaffold is made of **non–histone proteins**. These non-histone proteins are non-integral part of chromosome that helps the compaction of 30-nm chromatin into metaphase chromosome.
- **Further condensation** and packing of the chromatin that occurs during mitosis forms the **metaphase chromosome**.

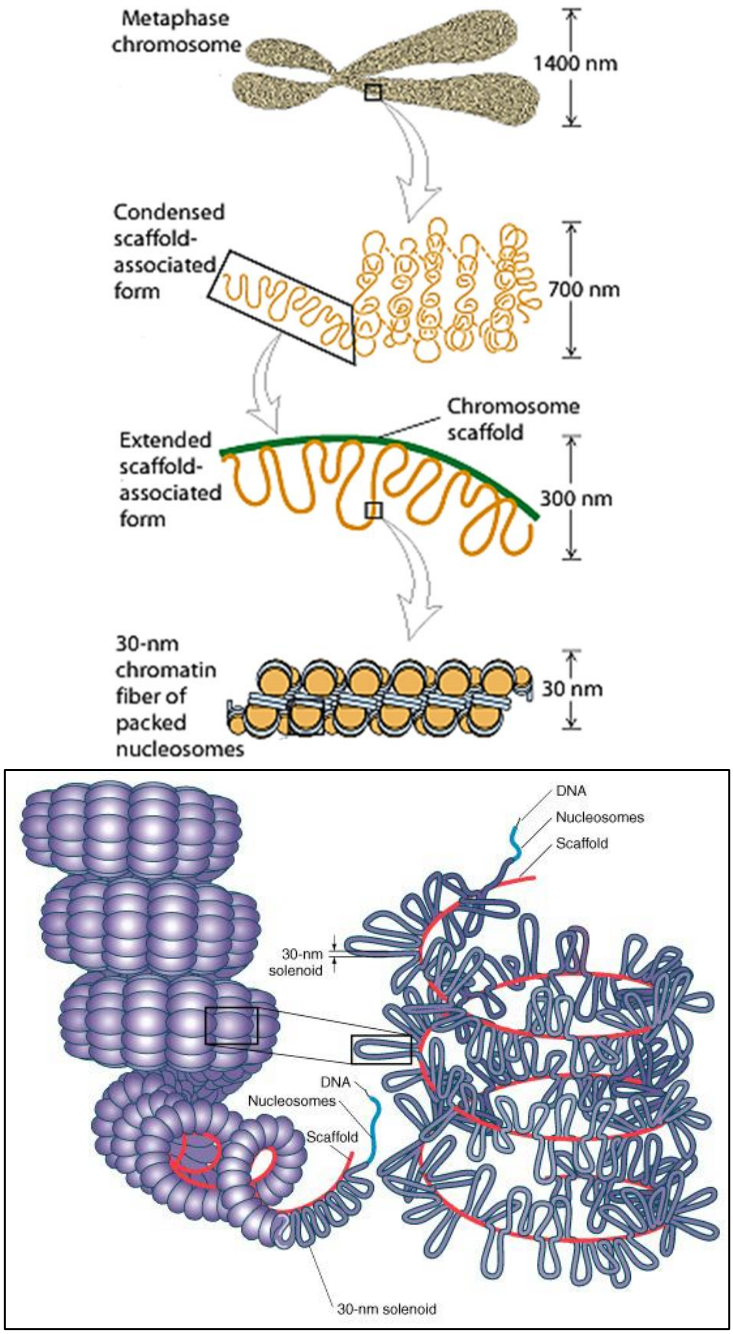


Fig. 1.4: Looped domains and the metaphase chromosome

2. Types of Chromatin

- During **interphase**, the centromeres and telomeres of chromosomes, as well as other chromosomal regions, exist in a highly condensed state similar to that seen in a metaphase chromosome (Fig. 2.1). This type of interphase chromatin, visible as clumps under a light microscope, is called **heterochromatin**, to distinguish it from the less compacted, more dispersed **euchromatin**.

- **Heterochromatin**

    - **Closely packed / highly condensed / compact**

    - Makes up ~10% of an interphase chromosome

    - Because it is highly compact, heterochromatic DNA is largely **inaccessible to the transcription machinery** (e.g. transcription factors, RNA polymerase), hence genes found within heterochromatin are **transcriptionally inactive**.

    - Also comprises centromeres *(Section 3.1.1)* and telomeres *(Section 3.1.2)*

- **Euchromatin**

    - **More diffused / less compacted** region of the interphase chromosome.

    - **Transcriptionally active** due to the looser packing of euchromatin which makes its DNA **accessible to the transcription machinery**, so genes present in euchromatin can be transcribed.

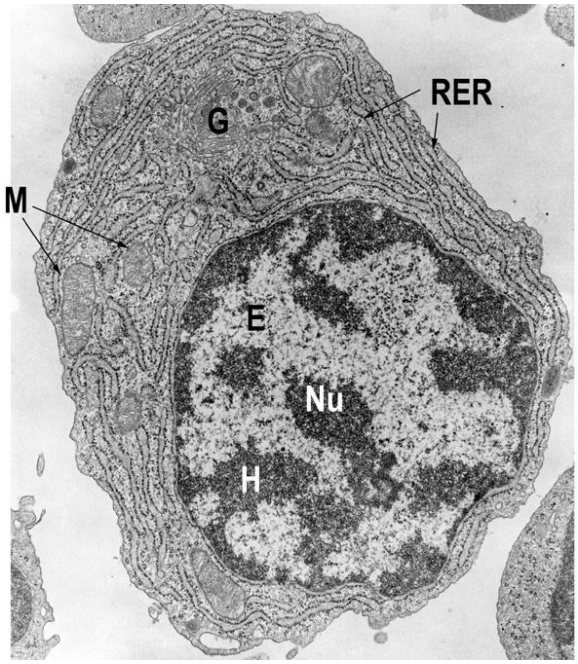


Fig. 2.1: Electron micrograph showing euchromatin (E) and heterochromatin (H).
M – mitochondria, G – Golgi body, RER – rough endoplasmic reticulum, Nu - nucleolus

- The chromosome is a dynamic structure that is condensed, loosened, modified and remodelled as necessary for various cell processes, including mitosis, meiosis and gene activities.

- Chemical modifications of histones affect the state of chromatin condensation and also have multiple effects on gene activity (*Section 5.1.1*).

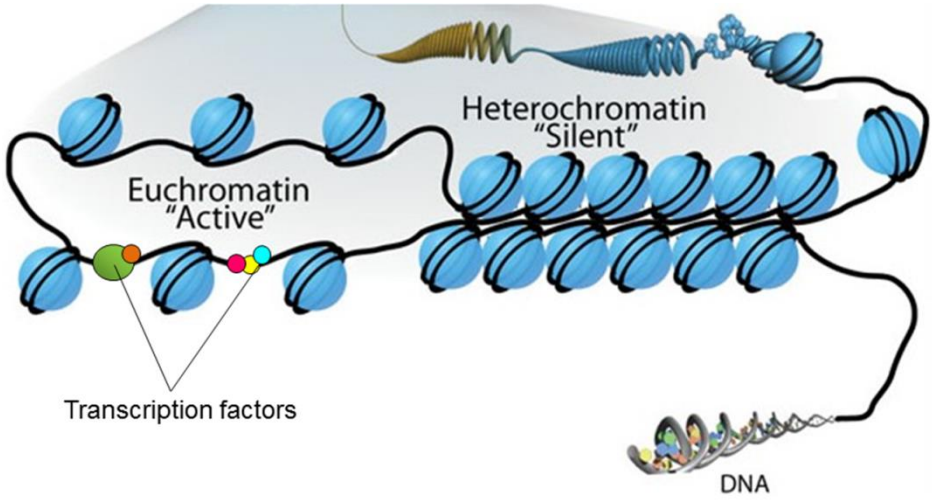


Fig. 2.2: The less compact euchromatin (left) allows transcription factors to access the promoter, thus genes found within euchromatin are transcriptionally active. The reverse is true for heterochromatin (right).

3. Features of Eukaryotic Genome

- The **genome** of an organism or species refers to the **complete DNA sequence of the organism/species**.

- The genome includes both **coding sequences** (*Section 3.1*) and **non-coding sequences** (*Section 3.2*) of the DNA (Fig. 3.1).

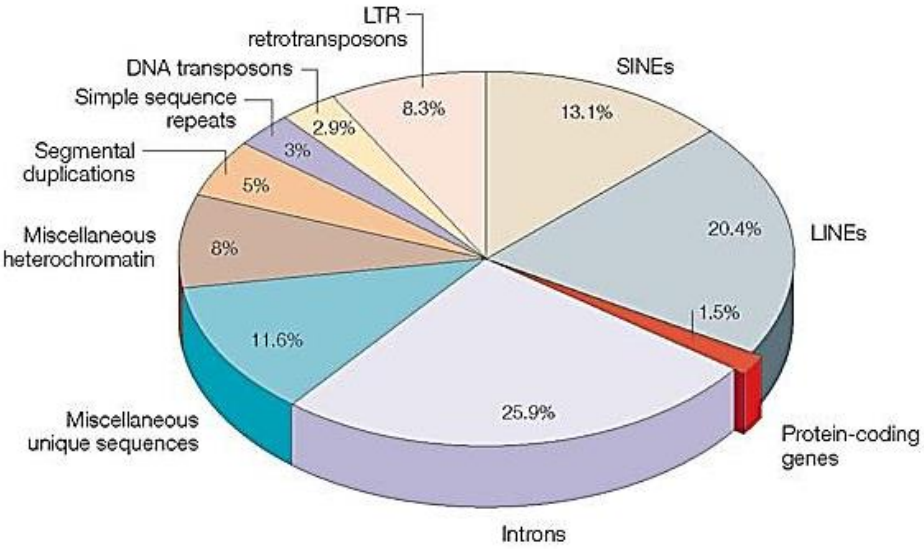


Fig. 3.1: Distribution of coding and non-coding sequences in the eukaryotic genome.
Except the terms "protein-coding genes" and "introns", you need NOT learn the other terms in this diagram.

3.1 Coding DNA Sequences

- Coding DNA sequences are the regions of DNA known as **genes** that **are transcribed** (into mRNA, tRNA or rRNA) **and/or translated** (into proteins).

3.1.1 Genes

- A **gene** is a **discrete unit of hereditary information** consisting of **specific nucleotide sequence in DNA** (or RNA in some viruses) which **codes for RNA or a specific sequence** of **amino acids** in a protein.

- Within a gene, there exists coding and non-coding sequences. Regions of a gene that code for the protein/RNA are known as **exons**. Other regions that do not code for the protein/RNA are known as **introns** (*Section 3.2.4*)

- Genes make up only a small portion of the eukaryotic genome (about 1.5% in humans) as most of the DNA does not encode protein.

3.1.2 Gene Families – Groups of identical or similar genes

- In the eukaryotic genome, many genes are unique sequences, with only one copy per haploid set of chromosomes. For example, there is only one gene for the ABO blood group in humans and it is present on chromosome 9.

- However, more than half the genome's coding DNA occur in multiple copies, which could be **clustered** (grouped together) **or dispersed in the genome**. Such genes, which are either identical or very similar, belong to multi-gene families. We define a **multi-gene family** as a **collection of identical or very similar genes**.

- Some multi-gene families consist of **<u>identical genes</u>**, usually **clustered in tandem** (side by side). This organization enables the cell to **rapidly produce** encoded RNA or proteins. Eg:

    - rRNA gene families

    - Histone gene family (Fig. 3.2) - cell rapidly produces sufficient levels of histone proteins to pack the newly-synthesised DNA during S phase of interphase

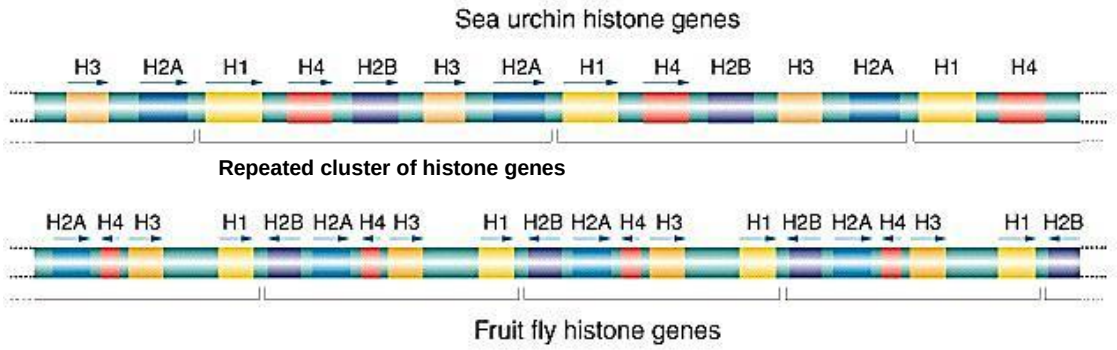


Fig. 3.2: The histone gene family in sea urchin and fruit fly are clustered and repeated in tandem.

- Some multi-gene families consist of many copies of genes that are **<u>non-identical but very similar</u>** in their nucleotide sequences. E.g:

    - Globin gene families (Fig. 3.3)

        - α-globin and β-globin gene families are two related families of non-identical repetitive gene sequences **dispersed** in the genome. They code for a group of proteins that include α and β polypeptide subunit of haemoglobin.

            - α-globin gene family is located on chromosome 16 in humans and encodes various forms of α-globin.
            - β-globin gene family is located on chromosome 11 and encodes various forms of β-globin.

        - The different forms of each globin subunit are **expressed at different times in development**, allowing haemoglobin to function effectively in the changing environment of the developing animal.

        - In human, the embryonic and fetal forms of haemoglobin (α_2 and γ_2 haemoglobin) have a higher affinity for oxygen than the adult forms (α_2 and β_2 haemoglobin), ensuring the efficient transfer of oxygen from mother to developing foetus during pregnancy.

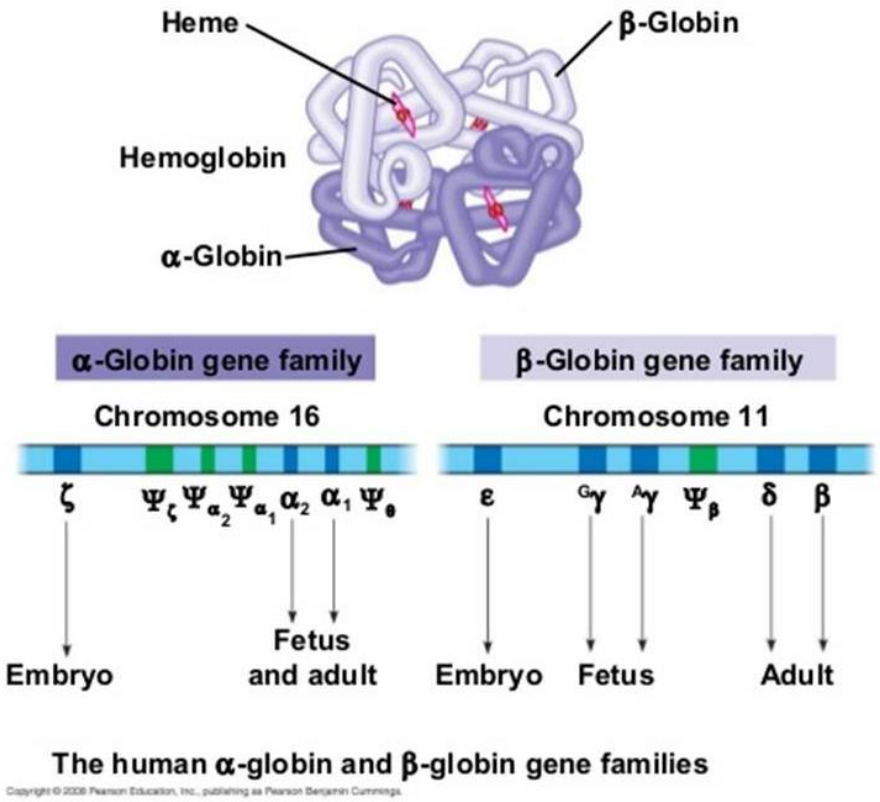


Fig. 3.3: The globin gene families.
The α-globin gene family (left) which consist of α-globin genes that are being expressed in different developmental stages (embryo, fetus and adult stages). The same goes for the β-globin gene gamily (right).

3.2 Non-Coding DNA Sequences

- The non-coding DNA sequences refer to sequences of the DNA molecule that **do not code for any protein or RNA** (makes up about 97% of the genome in humans).

- There are several types of non-coding sequences:
  3.2.1 Centromeres
  3.2.2 Telomeres
  3.2.3 Introns
  3.2.4 Regulatory sequences associated with genes (control elements, terminator)

3.2.1 Centromeres

- Centromere is **non-coding**, **repetitive DNA** sequences consisting of about 171 deoxyribonucleotide pairs in some organisms. The repetitive DNA sequence is known as **satellite DNA.** The sequences of the satellites vary slightly (Fig. 3.3A).

- The sequence of nucleotides of the centromere is **highly conserved**, i.e. the sequence has remained essentially unchanged throughout evolution.

- Centromere is easily visualized as the **most constricted, highly condensed region** of a metaphase chromosome.

- Functions of centromeres:

  - The site where **kinetochore proteins** attach, so that spindle fibres (microtubules) can bind to them for homologous chromosomes or sister chromatids to separate properly during nuclear division. (Fig. 3.3B)

  - The site where **cohesin proteins** attach, to bind two sister chromatids together prior to their separation during anaphase (Fig. 3.3C)

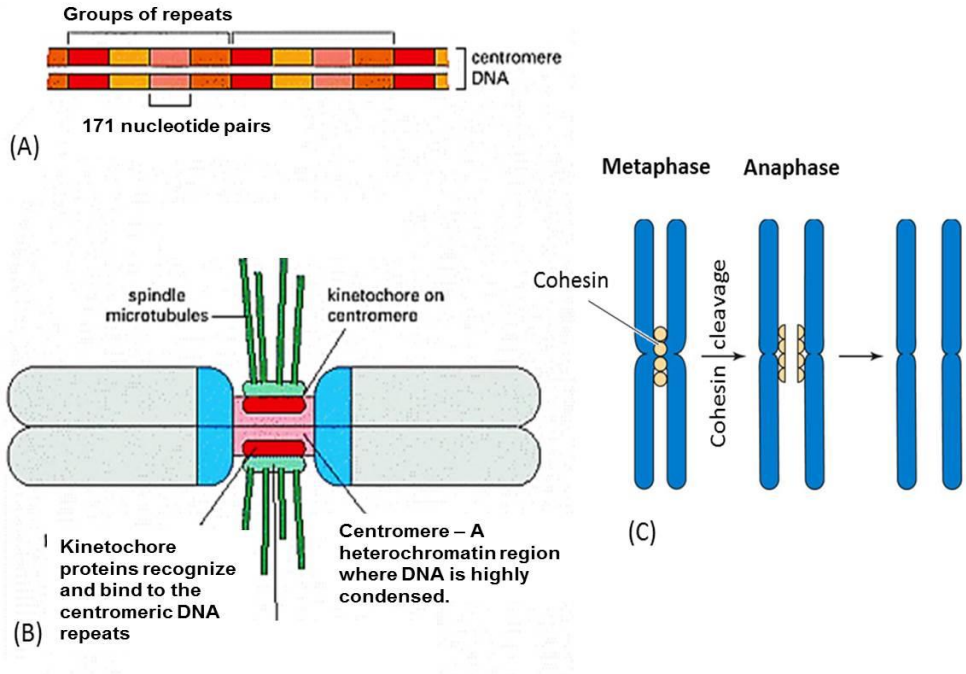


Fig. 3.3: (A) The repeating nature of centromeric DNA. (B) Assembly of kinetochore proteins on the centromere facilitates the binding of microtubules to the chromosome. (C) Cohesin proteins bound to the centromere allows sister chromatids to be joined together prior to anaphase.

3.2.2 Telomeres

- Telomeres are **short**, **repeating**, **non-coding DNA** sequence found at the **two ends of the DNA / chromosome** (Fig. 3.4).
- The number of telomeric repeats varies between about 100 and 10000.
- The exact sequence of the repeats in a telomere varies, depending on the species.
- The telomeres of **humans** consist of a repeat of six nucleotide sequences, **5' TTAGGG 3'**.

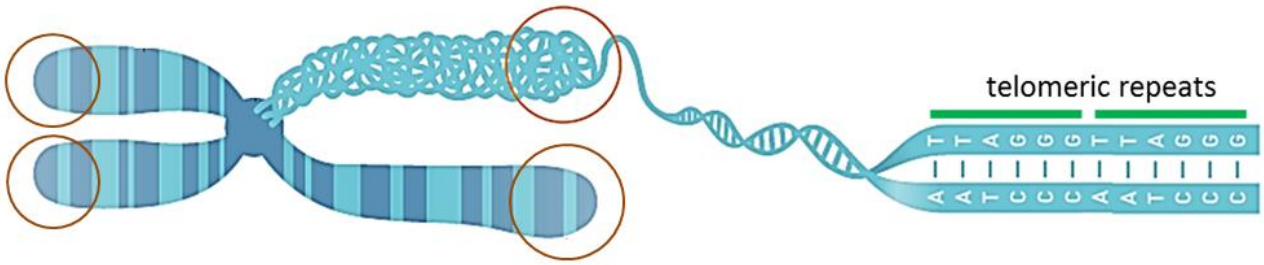


Fig. 3.4: Telomeric sequence is found at the ends of DNA molecule.

- Like centromeres, telomeres are **highly conserved sequences**.
- In some types of cells such as stem cells, the enzyme **telomerase** (Fig. 3.6) is present to synthesise telomeres.

(A) The DNA End Replication Problem

- The ends of **linear chromosomes** pose a problem for the usual DNA replication machinery.
    - For the leading strand, synthesis can continue to the end of its template. However, for the lagging strand, replacing the final RNA primer with deoxyribonucleotides at the 5' end of the daughter DNA strand is not possible.
    - This is because DNA polymerase can only add deoxyribonucleotides to the free 3'-OH end of a pre-existing polynucleotide but not to the 5' end.
    - This results in the shortening of DNA after each round of replication and is known as the End Replication Problem (Fig. 3.5).
- Therefore, a dividing cell that undergoes repeated rounds of replication will produce shorter and shorter DNA molecules (Fig. 3.5).

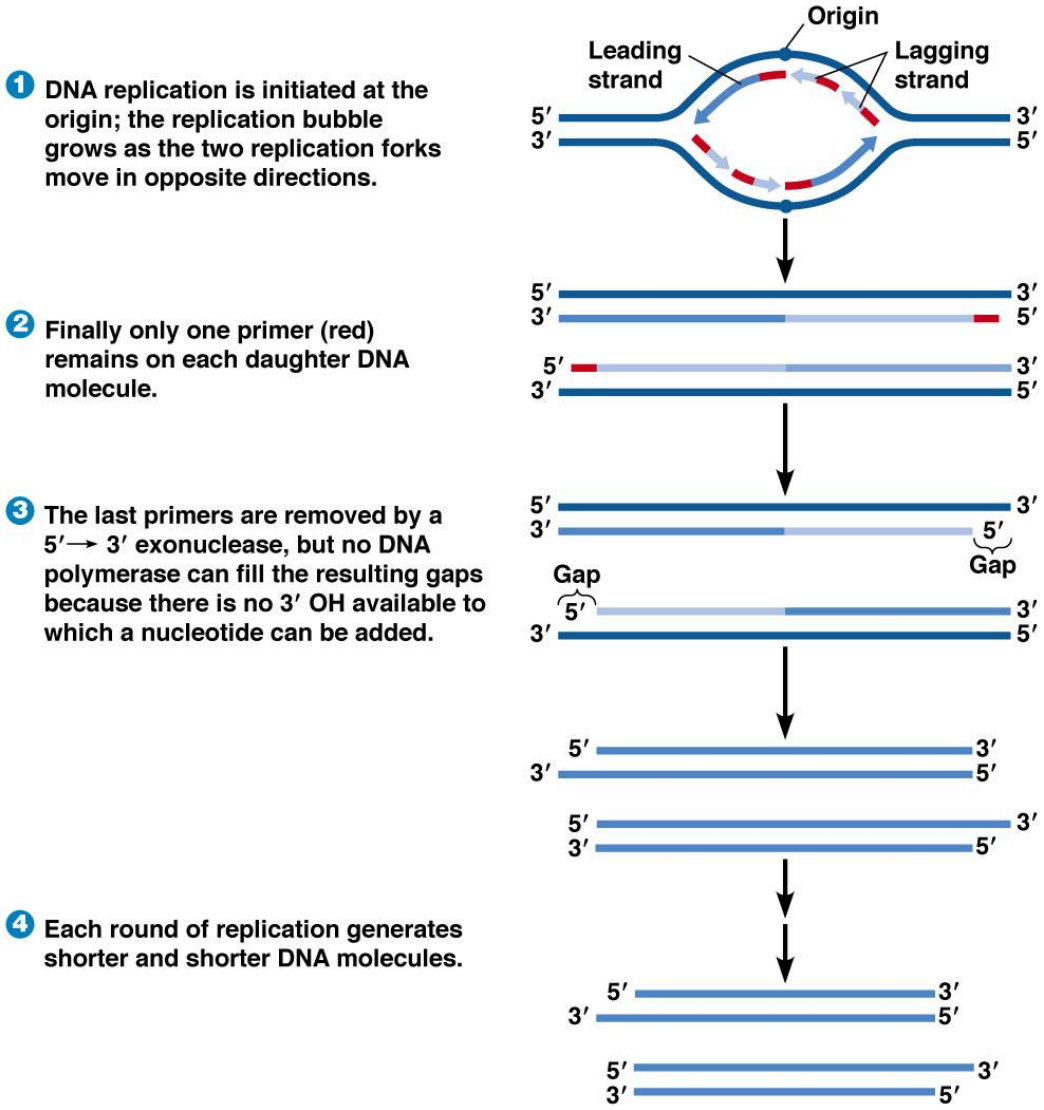


Fig. 3.5: The end replication problem results in shortening of DNA after each round of DNA replication.

- The shortening of telomeric DNA is observed in most dividing somatic cells and in cells cultured in the laboratory.

- As DNA in dividing somatic cells tends to be shorter in older individuals, it is proposed that the shortening of telomeres may be related to the ageing process of certain tissues. This is due to the **limited number of times** (~40-60 times; known as the **Hayflick limit**) that **each cell can divide before essential genes are lost**.

- When the telomere is shortened to a '**critical length'**, it undergoes cellular senescence (cell division completely stops) and **apoptosis** (programmed cell death).

- However, certain eukaryotic cells (e.g. stem cells, germ cells that give rise to gametes) are able to restore their shortened telomeres. This is due to the presence of enzyme **telomerase**, which catalyses the lengthening of telomeres.

Do prokaryotes face the same end replication problem? Why?

(B) Telomerase

- Telomerase is a **ribonucleoprotein** – a complex of RNA and proteins (Fig. 3.6).

- It has a **short RNA sequence** which provides an **AAUCCC template** to guide the insertion of the telomere repeat TTAGGG. *(This example is based on human telomerase and telomeres)*

- The protein component of telomerase is a **reverse transcriptase**, which synthesizes DNA from its RNA template.

- Telomerase is absent (i.e. the telomerase genes are switched off) in most cells, as most cells are differentiated cells which do not divide anymore (e.g. cardiac muscle cell).

- However, telomerase is generally present in
  - germ-line cells (cells that give rise to gametes)
  - stem cells, including embryonic stem cells
  - unicellular eukaryotes e.g. *Tetrahymena thermophila*
  - cancer cells

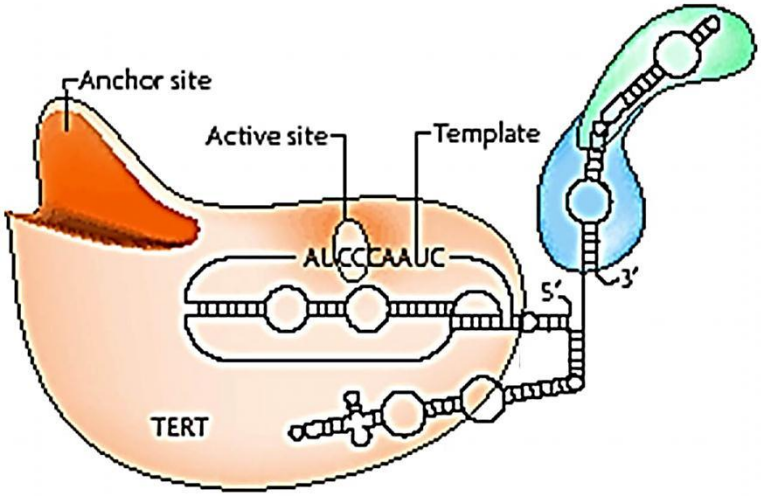


Fig. 3.6: Telomerase, a complex of proteins and RNA.
TERT = Telomerase Reverse Transcriptase
Anchor site = binds DNA

- How telomerase lengthens telomeres (Fig. 3.7)

  (1) End replication problem occurs, resulting in a **gap** at the **5' end of daughter strand**.

  (2) Telomerase **attaches** via part of its RNA sequence to **3' end of the parental strand** (also called 3' overhang) of the telomere by complementary base pairing.

  (3) The RNA sequence serves as **template** for the **extension** of new telomeres at the **3' end of the parental strand**. This is catalysed by the **reverse transcriptase** (TERT) of telomerase.

  (4) Once this strand is synthesized, the daughter strand is then extended the usual way in DNA replication involving primase, DNA polymerase and DNA ligase.

  (5) Telomerase advances along the DNA strand as more nucleotides are added to the **parental** strand.

  (6) After lengthening, in many eukaryotes, the telomeric DNA loops back on itself and the 3' overhang invades into the duplex part of the telomere (Fig. 3.8)

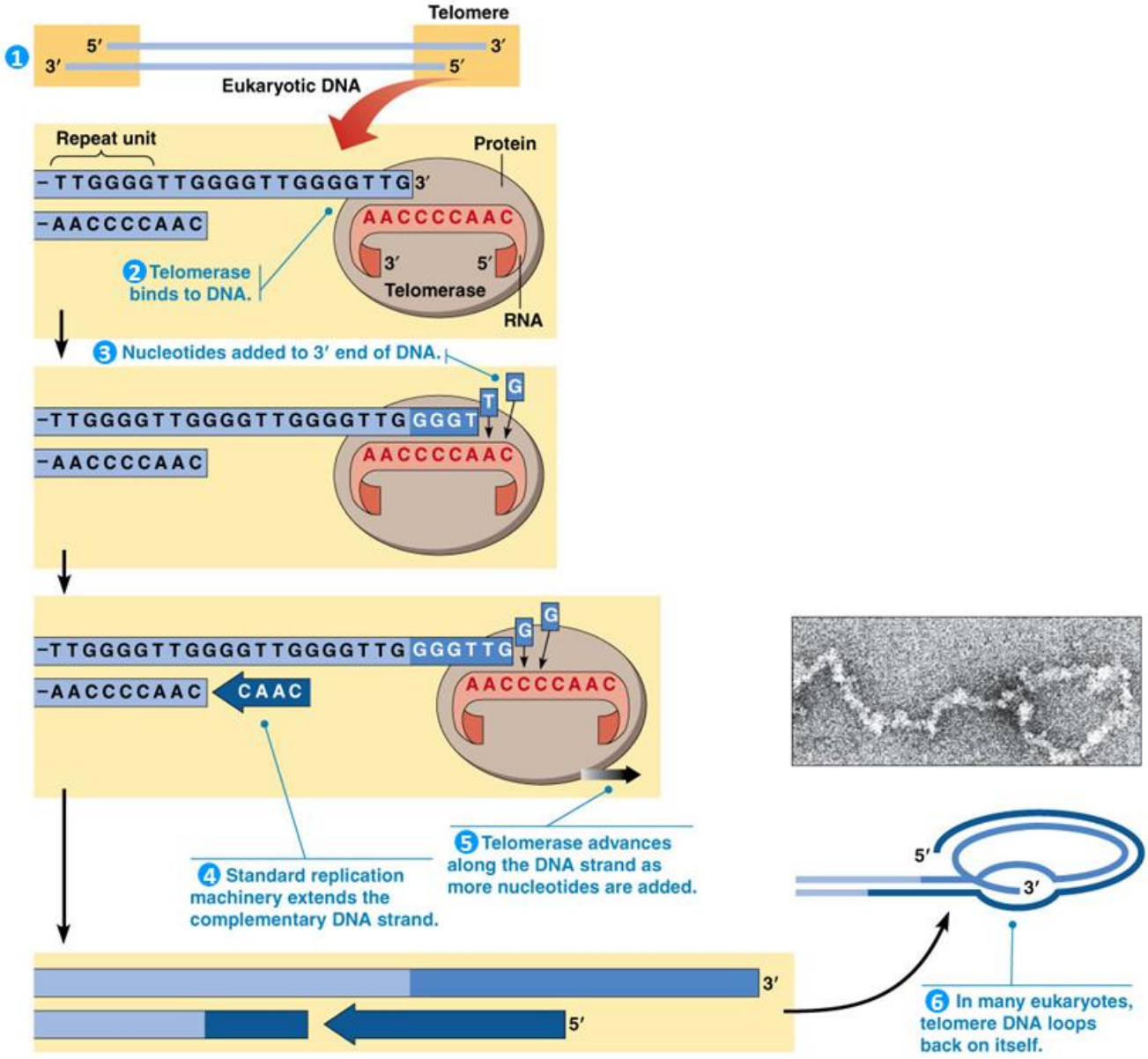


Fig. 3.7: Shows how telomerase (ribonucleoprotein) extends telomeres.

- **Telomerase and cancer**

  - Normal shortening of telomeres may protect organisms from cancer by limiting the number divisions that somatic cells can undergo.

  - However, researchers found high levels of telomerase activity throughout the cell cycle of cancer cells. This suggests that cancer cells can constantly lengthen their telomeres, allowing the cells to persist.

  - Hence, agents that prevent expression of the telomerase genes or inhibit telomerase activity are used in cancer treatment.

(C) Functions of Telomeres

- Telomeres protect the organism's genes from being lost with each round of DNA replication *(Section 3.2.2.1)*. Each time the DNA of a cell replicates, some of the telomere is lost (usually 25-200 base pairs per replication). Hence, it acts as a disposable **buffer zone** that **protects genes that are located near the ends of DNA molecules from being eroded**.

- The telomeric loop binds to specific proteins (Fig. 3.8) so as to **prevent two chromosomes ends from fusing** (e.g. by non-homologous end joining).

- The telomeric loop and the specific proteins associated with telomeric DNA (Fig. 3.8) also **prevent the staggered DNA ends from activating the cell's system that monitors DNA damage**. This system recognizes exposed single-stranded DNA ends as a defect and degrades the chromosome, resulting in **apoptosis**.

  - Apoptosis (a.k.a **programmed cell death**) – a well-orchestrated self-destruction process brought about by signals that trigger the activation of a cascade of proteins in the cell destined to die.

- Telomeres **slow down ageing** of the cell. It has been proposed that shortening of telomeres is associated with **aging process** of certain tissues and even to aging of the organism as a whole.

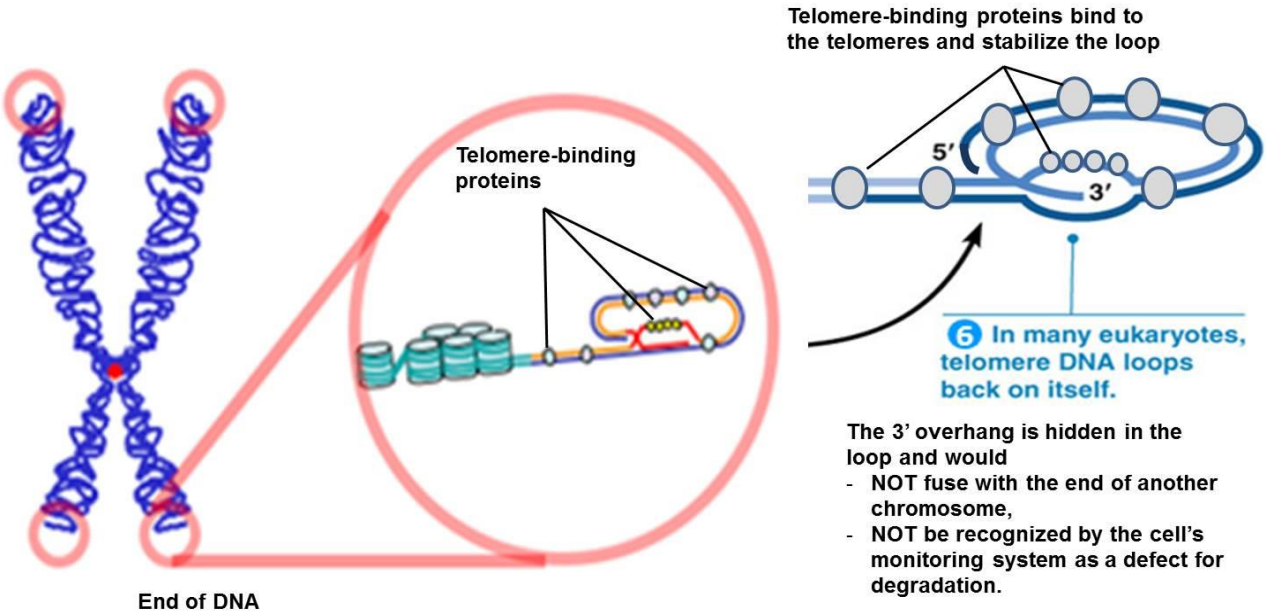


Fig. 3.8: A typical looping feature at the telomeric ends. Various telomere-binding proteins bind to the telomeric DNA to stabilize the loop where the 3' overhang is hidden.

3.2.3 Introns

- First discovered in 1977.
- Introns are **non-coding DNA sequences interspersed among coding sequences in a gene**.
- About 95% of human genes contain introns. The average human gene contains about 8 introns. The largest number of introns in a gene so far discovered is more than 75 in the human dystrophin gene.
- The average length of introns typically ranges between 65 to 20,000 nucleotides but some have been found to have enormous length of more than 200,000 base pairs. In comparison, about 80% of exons are less than 200 base pairs in length.

  (FYI: histone genes do not have introns, unlike other eukaryotic genes.)
- Introns in DNA are transcribed. Thus the primary mRNA transcript (also called pre-mRNA) also consists of both introns (interfering) and exons (expressed).
- These **introns are excised** and the **exons are spliced** together during RNA processing (Fig. 3.9). *(Refer to Section 5.3.3).*
- Small nuclear ribonucleoproteins (snRNP) and other proteins form a molecular complex called **spliceosome** which interacts with the **splice sites** at the **ends of an intron**. It cuts at specific sequence to release the intron and splice together the two exons that flank the intron.

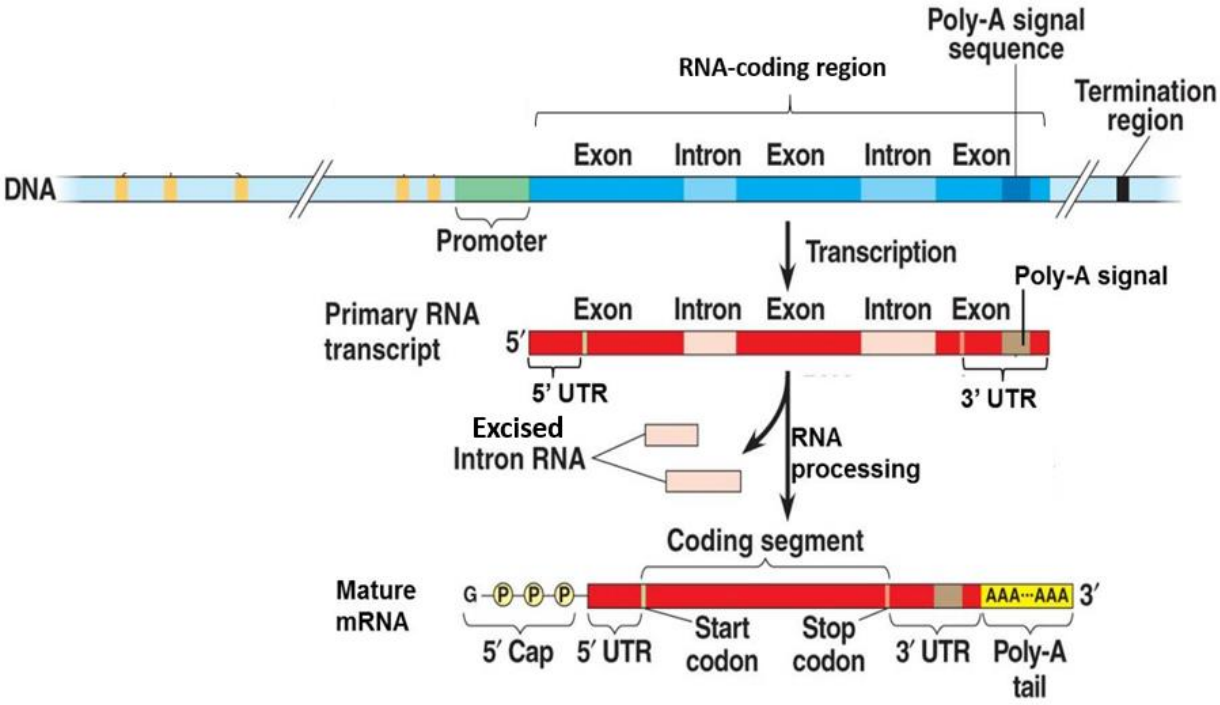


Fig. 3.9: Presence of introns between exons in primary transcript.
During RNA processing, the introns are removed and the exons are spliced together.
Hence, introns are segments of RNA-coding region that are not destined to be translated, while exons are segments of RNA-coding region that are destined to be translated.
(Exceptions: Part of first exon before the start codon is the 5'UTR (untranslated region) of mRNA, and part of last exon after the stop codon is the 3'UTR of mRNA)

- **Functional significance of introns:**

  Although, in general, introns do not code for proteins, scientists have discovered and are still discovering that the presence of introns is significant for the cell or organism. Outlined below are 4 findings:

  1) The presence of introns makes it possible for a **single gene to encode more than one kind of protein.**

     - This is because introns may **contain regulatory sequences** that **control gene expression** via **alternative RNA splicing** in different cell types. (*Section 5.3.4*)

     - As a result, the pre-mRNAs in different cell types are spliced differently to bring together different combinations of exons. This means that the mature mRNA have different nucleotide sequences, and hence will be translated to different polypeptides /proteins.

  2) In some organisms, introns may **regulate transcription initiation**.

     - E.g. – In yeast, intron-bearing genes are found to produce more mRNA and proteins than genes without introns.

     - E.g. – In a type of mice, gene expression increased when a specific intron is inserted in between the promoter and an intron-less growth hormone gene.

  3) Introns help to **buffer against mutation** on exons.
     (Link to JC2 topic – Biological Evolution)

     - For most genes, introns contain more nucleotides than exons. Since mutation is random, the probability of it happening at the introns would be higher than in the exons.

     - Mutations which occur within introns are not expressed and would not have any effect on the protein synthesized.
       (Exception: If mutation occurs at the splice site, such mutation may result in the inability for spliceosome to recognize the splice site. What is the implication?).

  4) Introns may **facilitate evolution of new and potentially useful proteins.**
     (Link to JC2 topic – Biological Evolution)

     - During meiosis, if crossing over between non-sister chromatids occurs at the **non-coding introns <u>within a gene</u>**, **new combinations of exons** can be created. This could produce **new alleles** (Fig. 3.10) that could be inherited by the offspring. The new alleles may code for **new functional proteins**.

     - This is an example of **exon shuffling**, which by definition is the recombination of exons that result in the formation of new alleles or genes.

     - Similarly, **unequal crossing over** at the **introns of <u>two different genes</u>** due to misaligned homologous chromosomes also reshuffles exons to give rise to **new genes**.

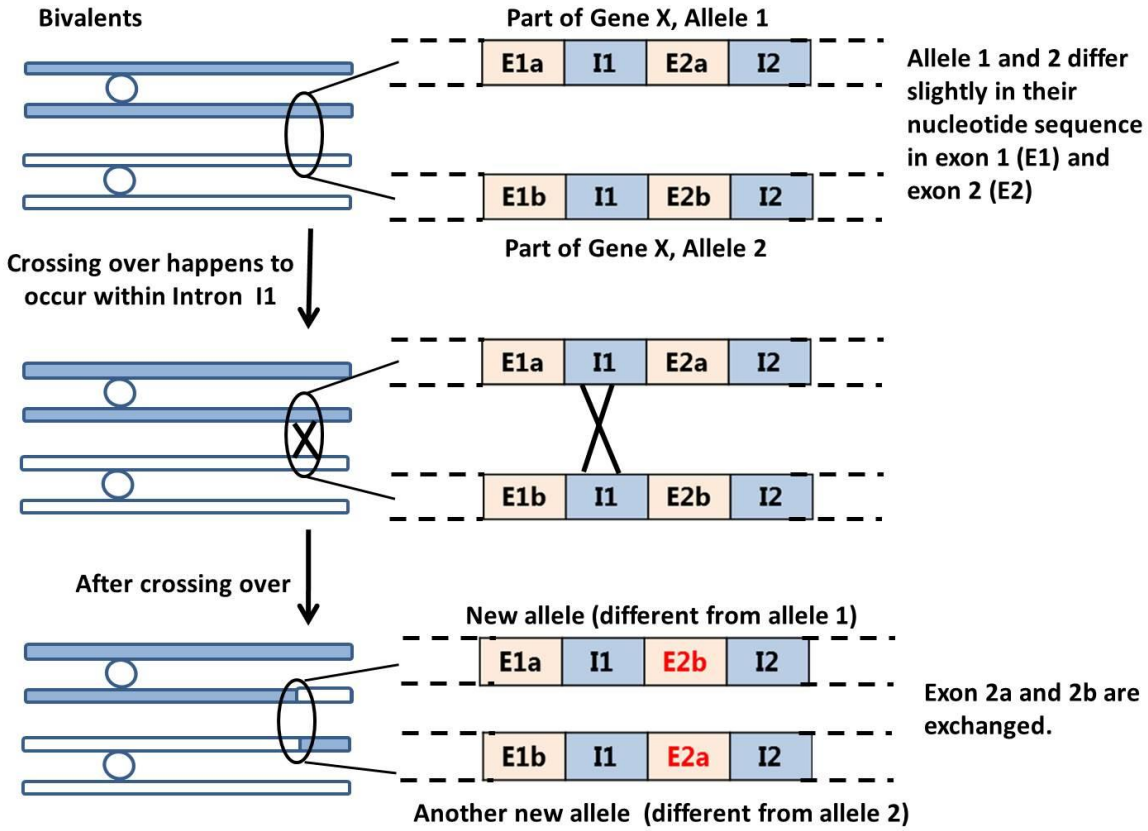


Fig. 3.10: Exon shuffling due to crossing over at an intron within a gene

3.2.4 Regulatory Sequences

- Regulatory sequences are **non-coding DNA** sequences involved in the **regulation of gene expression**.

- There are several types of regulatory sequences (Fig. 3.11)

(A) Control elements

- Segments of non-coding DNA that regulate transcription of a gene when bound by **transcription factors** (*Section 5.2.2*).

- Control elements can be further categorized into (1) promoter, (2) proximal control elements and (3) distal control elements

(1) Promoter

❖ Non-coding DNA sequence **upstream** of RNA-coding region where **general transcription factors** and **RNA polymerase** bind to initiate **basal level** of transcription.

❖ **Transcription factors are proteins** that bind to specific DNA sequences and thereby control transcription via interactions with RNA polymerase.

(2) Proximal control elements

❖ These are non-coding DNA sequences located **100-200bp upstream of the promoter**, where **specific transcription factors** bind to regulate the rate of transcription. Proximal control elements are usually **gene-specific** (Fig. 3.12).

❖ Examples: CCAAT box (~100bp upstream), CG-rich box (~200bp upstream), among many others.

(3) Distal control elements

❖ These are non-coding DNA sequences located **thousands of nucleotides upstream or downstream** of the promoter, where **specific transcription factors (activators or repressors)** bind to activate or repress transcription of **specific genes**.

❖ There are 2 types of distal control elements:

- **Enhancers** – Non-coding DNA sequences where **activators** (proteins) bind to **further increase** the rate of transcription of a gene.

- **Silencers** – Non-coding DNA sequences where **repressors** (proteins) bind to **repress** transcription of a gene.

(B) Terminator

- Non-coding sequence of DNA **downstream** of RNA-coding region that **signals end of transcription**.

Note: **'Upstream'** and **'downstream'** are terminologies used to describe the relative position of the nucleotide sequences within the DNA or RNA.

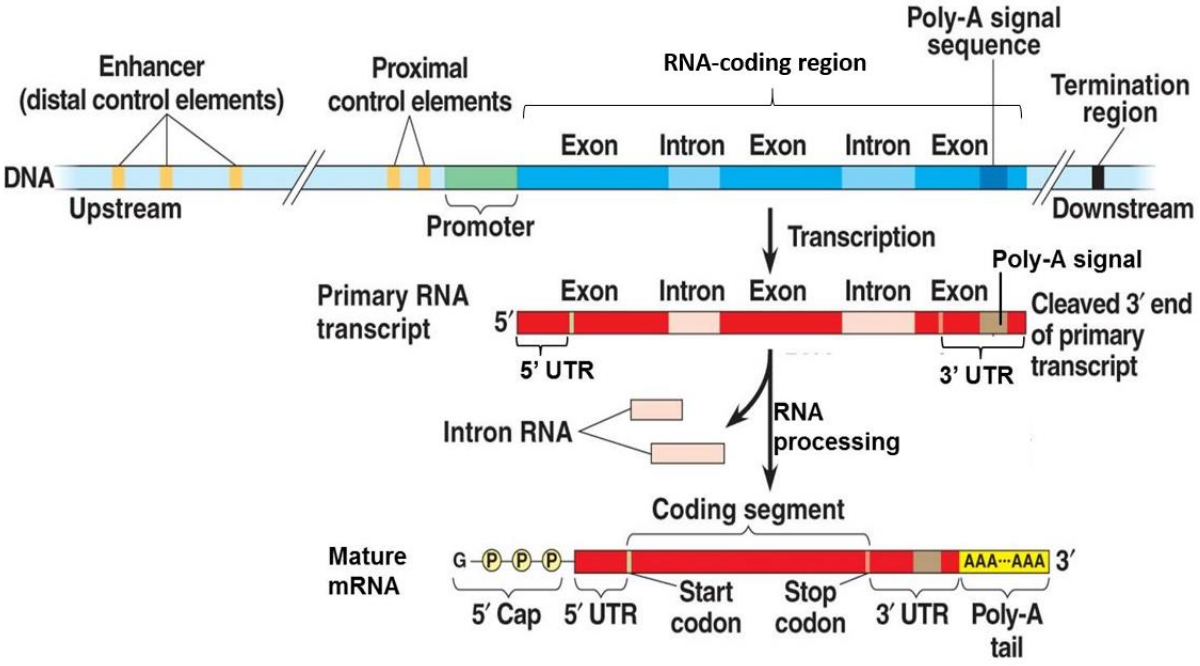


Fig. 3.11: Regulatory sequences in eukaryotic DNA, including promoter, terminator, proximal and distal control elements. Also shown is the primary RNA transcript which undergoes RNA processing to become mature mRNA.

3.2.5 Repetitive DNA *(Not in syllabus)*

- Repetitive DNA are nucleotide sequences that are present in multiple copies throughout the genome.

- Each type of repetitive DNA differs in length.

- There are two categories of repetitive DNA (Fig. 3.13):
  - Tandemly repetitive DNA (a.k.a. satellite DNA)
  - Interspersed repetitive DNA

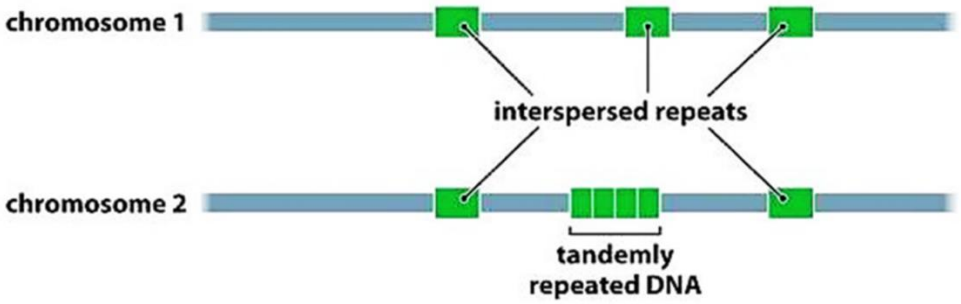


Fig. 3.13: Tandem repeats vs interspersed repeats

(1) *Tandem repeats (a.k.a. satellite DNA)*

- Tandem repeats are short DNA sequences repeated in series.

- Depending on the number of base-pairs in each repeat and the total DNA length, they can be further classified into:

Types of tandem repeats	No. of base-pairs per repeating unit	No. of repeating units (n)	Examples
Satellite DNA	5 – 300 depending on species	10^5 – 10^6 times	• Centromere • Telomere $(TTAGGG)_n$
Mini-satellite DNA (a.k.a. variable no. tandem repeats, **VNTR**)	10 – 400 (average about 20)	20 – 50 times	• $(AGTTCGCGTGA)_n$
Micro-satellite DNA (a.k.a. short tandem repeats, **STR**)	2 – 4 (mostly 2)	10 – 100 times	• $(GC)_n$ • $(TC)_n$

- In mammalian DNA, about 10-15% of the genome consists of tandemly repetitive DNA.

- Much of the satellite DNA appears to play a structural role at telomeres and centromeres of chromosomes.

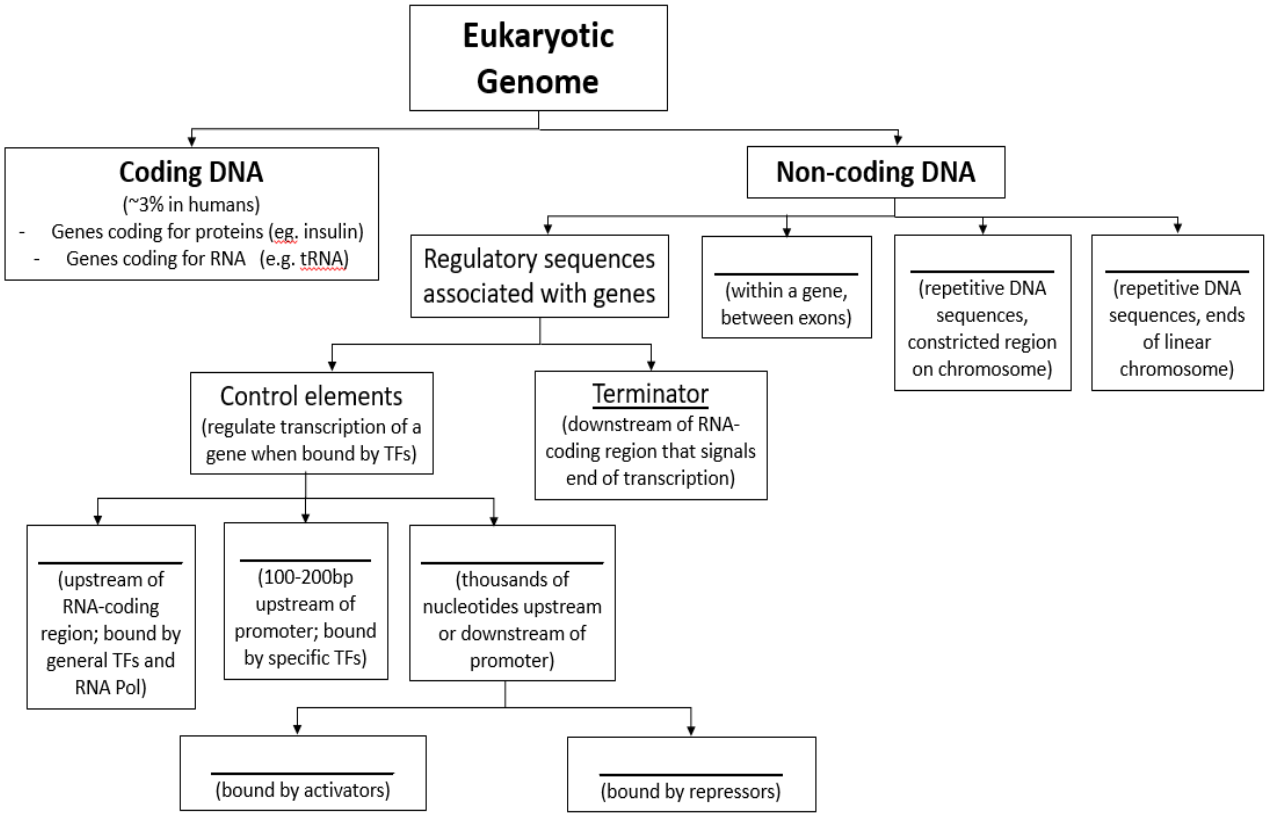
- VNTR and STR can be found on many chromosomes, and vary in length between individuals. Each VNTR and STR variant acts as an inherited unit, allowing them to be used for personal or parental identification. Hence, VNTR and STR analysis is useful in genetics and biology research, forensics, and DNA fingerprinting *(refer to topic on Molecular Techniques).*

(2) *Interspersed repeats*

- Interspersed repetitive DNA are repetitive DNA sequences **scattered** at multiple sites in the genome (even in introns).
- Each unit of repetitive sequences is about hundreds to thousands of base pairs long.
- The ‘dispersed’ copies are similar and usually not identical to each other.
- Each type appears to be derived from a transposable element (transposons), a mobile segment of DNA which is able to move around the genome from one place to another.
- Many of these elements leave copies of themselves when they move (‘copy-and-paste’), which explains how they propagate and become common throughout the genome.
- One common family of similar interspersed repetitive sequences are called **Alu elements.**
  - Each unit is about 300 nucleotides pairs long repeated at multiple sites in a genome.
  - These Alu elements are usually transcribed into RNA molecules with unknown roles in the cell.

CONSOLIDATION PRACTICE

With reference to Section 3.2, fill in the blanks below with the correct terms. An example has been done for you.



Pre-lecture Exercise (to complete before lecture on Section II):
How much do you remember from the Gene Expression topic in JC1? (You may use the diagrams below to help you.)

1) What is a gene?

……

……

2) What does gene expression mean?

……

……

3) (a) What stages are involved in the expression of a protein-coding gene?

……

(b) Where do the above-named stages take place in a prokaryotic vs eukaryotic cell?

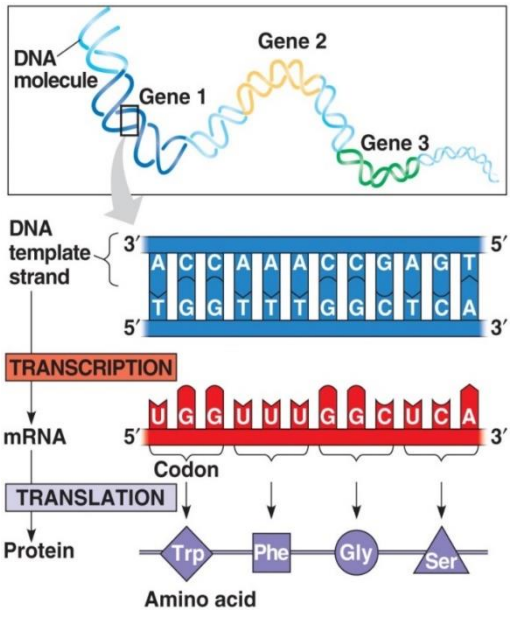
……

……

……

4) Besides the difference in size, what is one other structural difference between prokaryotic vs eukaryotic DNA?

……



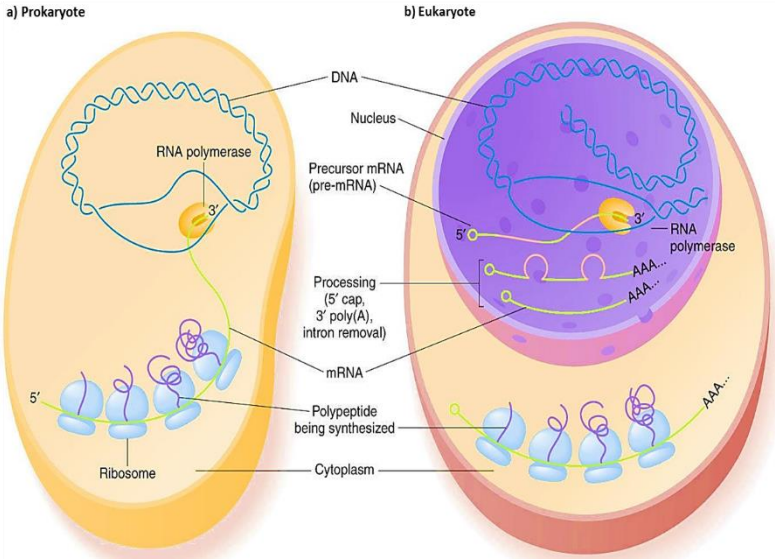


Fig B: Overview of gene expression in (a) prokaryotes and (b) eukaryotes.

4. Overview and key concept in the control of gene expression

"An oboe squawks loudly, several violins squeaks shrilly, and a tuba adds to a rumble to the noisy chaos. Then the conductor's baton rises, pauses and begins a series of elaborate movements, directing specific instruments to join in and others to raise or lower their volume at exact moments. Properly balanced and timed, discordant sounds are transformed into a beautiful symphony that enraptures the audience."

In a similar way, cells intricately and precisely regulate their gene expression. ***Not all the genes are expressed at the same time****, as for the orchestra, not all instruments play at the same time. Both prokaryotes and eukaryotes, must* ***alter their patterns of gene expression in response to changes in environmental conditions****. The unicellular and multicellular organisms must* ***continually turn genes on and off*** *in* ***response to signals from their external and internal environments*** *(e.g. signals from other cells, hormones etc.)*

Adapted from
Chapter 18 Campbell Biology 9th Ed, Reece et al.& Chapter 7 Molecular Biology of the Cell 4th Ed, Alberts et al.

4.1 Overview

- All organisms, both prokaryotes and eukaryotes, do not need to express all their genes all the time. They must regulate the types of genes (spatial gene expression) to be expressed at any given time (temporal gene expression). Both unicellular organisms and the cells of multicellular organisms must continually turn genes on and off.

- In contrast, genes that are required for the basic maintenance of cellular function are constitutively expressed in all cells of an organism, under normal conditions. These are called **housekeeping genes.** Examples: genes coding for histone and ribosomal proteins (*can you name other examples?)*.

Why regulate gene expression?

- Gene expression is regulated to **meet the needs of the cell or organism** at **any given time**. **Some genes are turned on only at a particular stage in the life** of the multicellular organism and are **permanently switched off thereafter**. This is known as **temporal gene expression**. Examples:

    - Cells at different stages of embryonic development have different development potential (i.e. totipotent, pluripotent or multipotent) as different sets of genes are expressed at different times *(Section 7.2).*

    - The genes for embryonic and fetal haemoglobin are expressed in the embryo and the fetus respectively. Close to birth, these genes are progressively turned off while the genes for adult haemoglobin are turned on.

    - Cells that actively undergo mitosis or meiosis would be actively expressing genes that code for proteins required for this process *(can you name some examples?)*

- In multicellular organisms which are made up of different types of cells, regulation of gene expression is also essential **for cell specialization / differentiation**. To perform its specific role**, each cell type expresses a unique set of genes** in its genome where **certain genes are expressed and others are not**. This is known as **spatial gene expression**.

    - The genome of almost all the cells in an organism is identical and remains constant throughout its lifetime.

- The differences between cell types are not due to the presence of different genes, but due to **differential gene expression**, the expression of different genes by cells with the same genome.

- A typical human cell expresses about 20% of its protein-coding genes at any given time. Differentiated cells, such as nerve cells, express an even smaller fraction of their genes.

- Example 1: Cells in the zygote to the 8-cells stage are totipotent as in these cells, a specific set of genes which control totipotency are switched on, while other genes that control differentiation are switched off. *(Section 7.2).*

- Example 2: In liver cells, genes that code for proteins which allow for liver functions are expressed, while other genes such as those required for nerve cell functions are not.

- Allows the organism **to respond to signals from** their **external and internal environments** in a **coordinated manner**. E.g.:

  - In plants, changes in day length trigger the synthesis of hormones responsible for flowering.

  - In young mammals, the presence of growth hormones (an internal signal) switches on various genes which are responsible for growth and development to take place.

There are different ways by which gene expression can be regulated. **Section 5** explains **how** this can occur.

4.2 Key Concepts

Questions	Concepts
Q1) In the SAME individual, does a lens cell in the eye have the same DNA as a liver cell? A1)	**Key Concept 1:** • All cells in a multicellular organism have the **same** genome. • The amount of DNA per cell is **constant** except for certain situations.
Q2) Since both liver cells and lens cells have the genes for making the proteins albumin (blood protein) and crystalline (protein in lens of eyes), why do these cells have different functions? A2)	**Key Concept 2:** • Not all the genes in a cell are expressed. Only housekeeping genes are expressed all the time. • **Differential gene expression** occurs at different stages of life and for specialization in different cell types. **Key Concept 3:** • Gene expression process can be **turned on or off**, **accelerated or slowed down.**

- The control of gene expression can occur at any stage in the **pathway from gene to functional proteins**. The most important point of control is the initiation of transcription (transcriptional level), as it minimizes wastage of resources.

- At each stage, activation or repression of gene expression may occur.

- Fig. 4.2 shows an overview of the various stages at which gene expression (synthesis of functional protein) can be controlled.

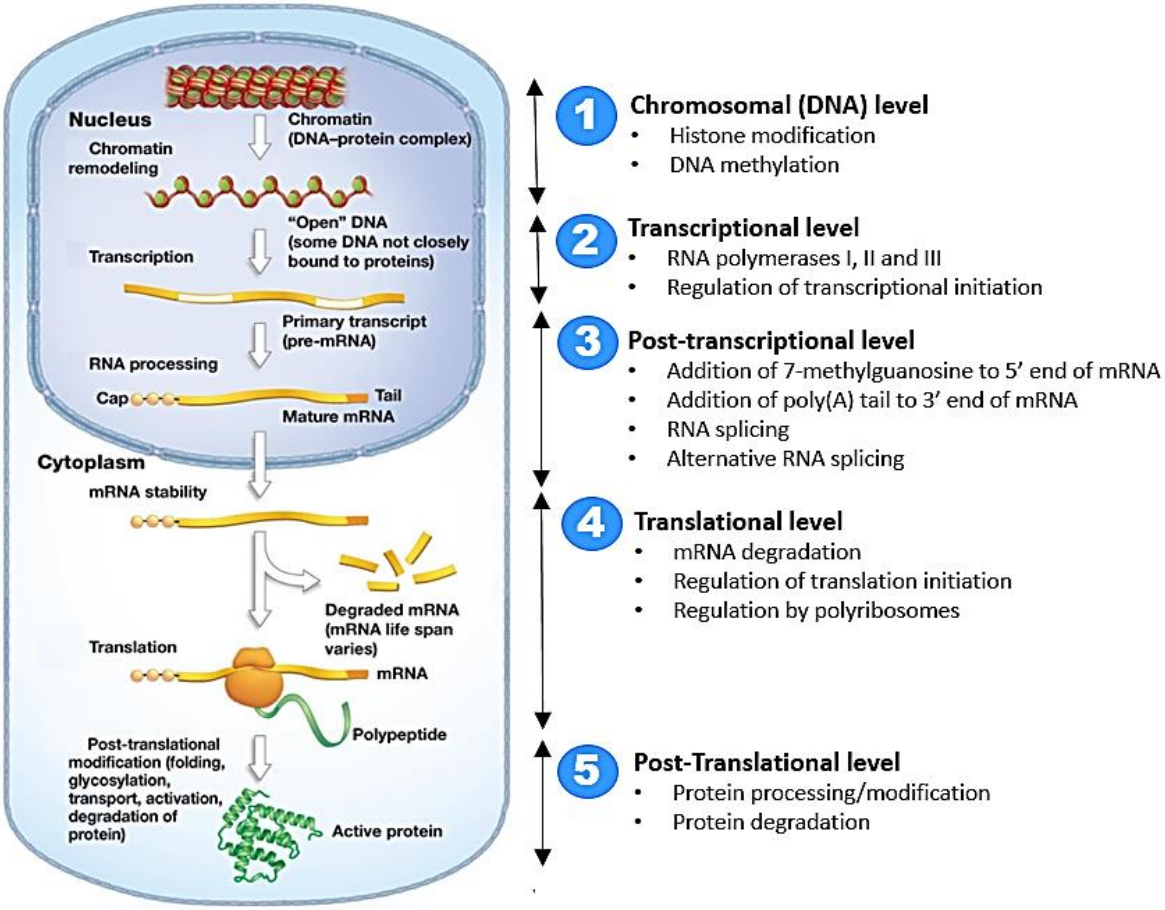


Fig. 4.2: Overview of the various stages at which gene expression can be controlled.

- Some key features of eukaryotic protein synthesis that differentiate it from prokaryotic protein synthesis include:

    - The presence of **introns** (non-coding sequences) that are **interspersed between exons** (coding sequences), thus requiring post-transcriptional RNA splicing prior to translation.

    - During initiation of transcription, the formation of the transcription initiation complex requires the assembly of proteins known as **transcription factors** other than RNA polymerase.

    - The product of transcription is a **pre-mRNA** or **primary transcript** that requires **post-transcriptional modifications** *(Section 5.3)* before translation occurs to produce a polypeptide.

    - There is a **relatively large number of control elements** (Fig. 3.11) associated with a eukaryotic gene. Control elements are sequences of non-coding DNA that regulate transcription of a gene by binding proteins known as transcription factors (*Section 3.2.4*).

5 How is Gene Expression Controlled at Different Stages?

- Spatial and temporal gene expression can be controlled at various stages in the pathway from gene to functional proteins (Fig. 4.2).

5.1 Control at the Chromosomal (DNA) Level – Chromatin Remodelling

- Chromatin remodelling (modification) plays a significant role in transcriptional regulation.
    - Genes in the **heterochromatin region**, which is more **highly condensed**, are usually **not expressed**.
    - The tightly packed nucleosomes decrease the accessibility of a gene's promoter by transcription factors and RNA polymerase, thus hindering gene expression.
- To make the gene accessible for transcription, **chromatin-remodelling proteins/enzymes** use the energy from ATP hydrolysis to **reposition the DNA** wrapped around the octameric histones, so that the **promoter** is **accessible to transcription factors** and **RNA polymerase**.
    - E.g. histone acetyltransferase - loosens the attachment of DNA to histones (Fig. 5.1)
    - E.g. remodelling proteins - dissociates octameric histones from DNA (Fig. 5.1)

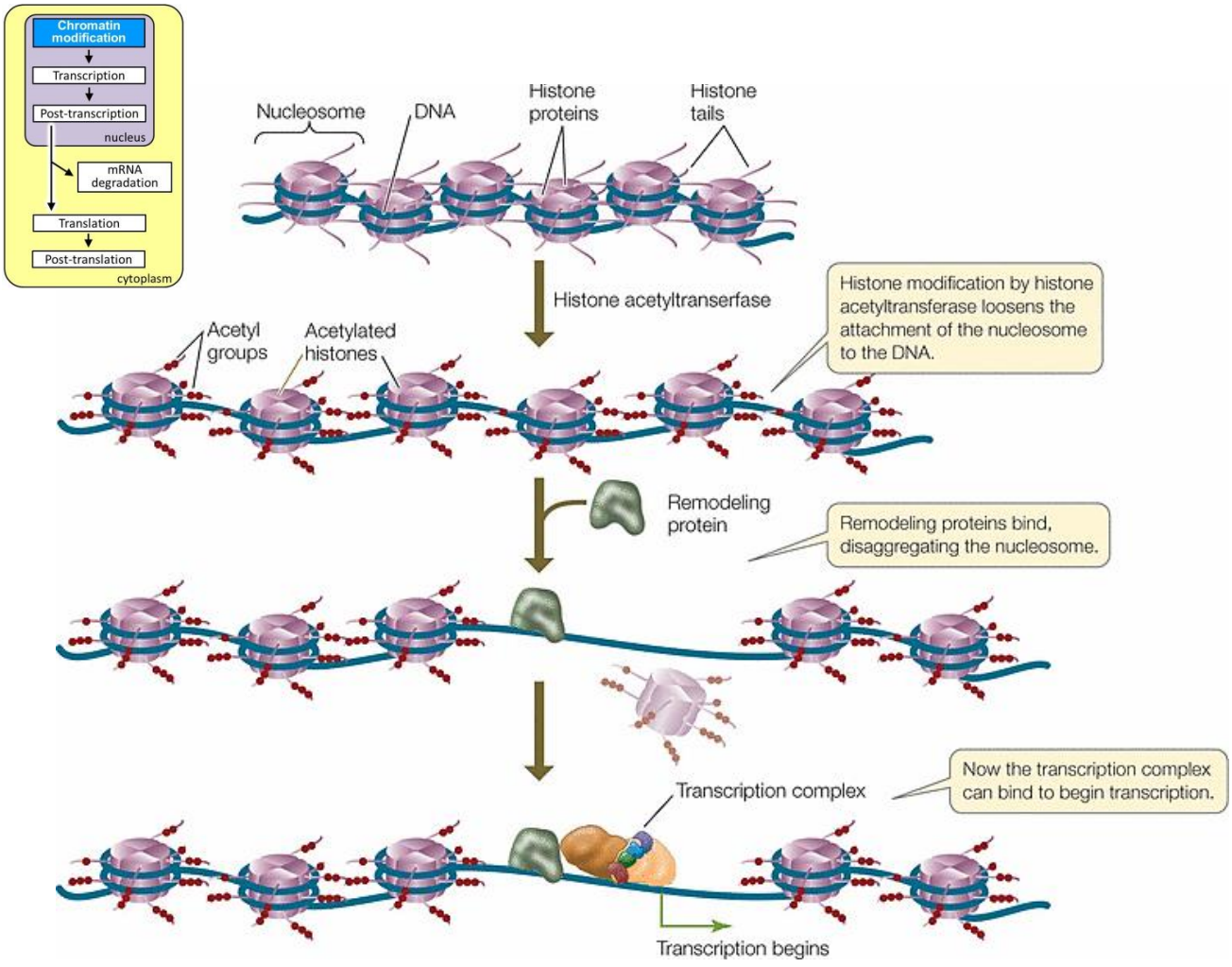
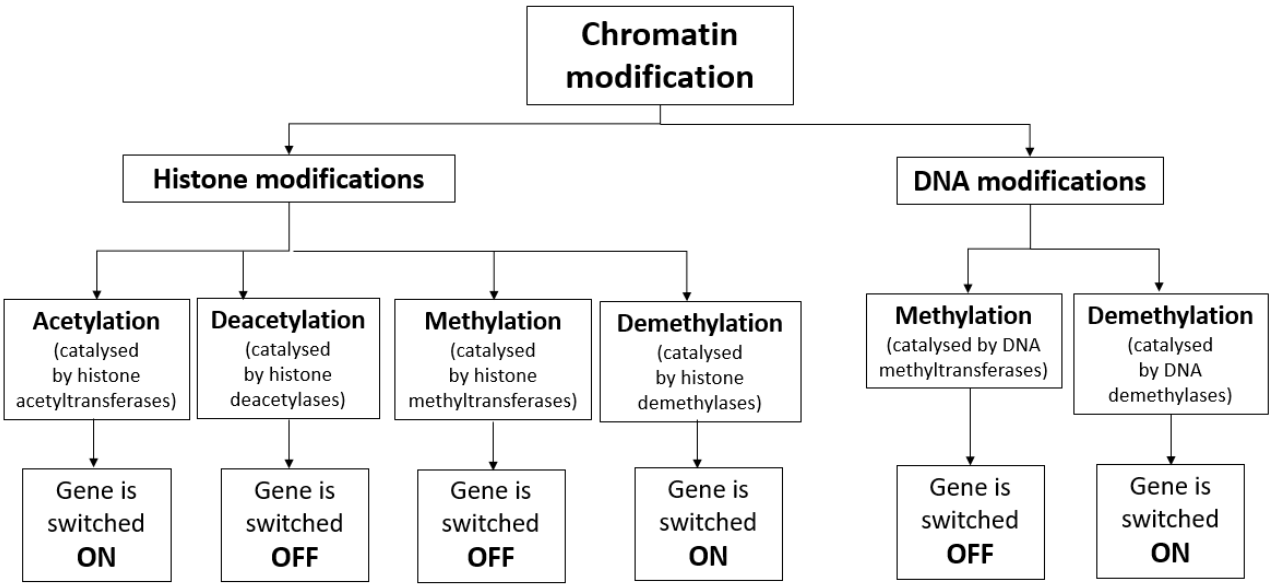


Fig. 5.1: Remodeling of the chromatin structure to allow access of transcription factors to the control elements.

- Conversely, other types of remodelling enzymes can also condense the chromatin to prevent transcription.
    - e.g. genes which are expressed during embryonic development are permanently switched off in this manner after the embryonic stage.

- Chromatin can be tightened or loosened by either modifying histones (*Section 5.1.1*) or DNA (*Section 5.1.2*):



5.1.1 Histone modifications

- The **N-terminus** of each histone molecule in a nucleosome, which **protrudes outward** from the nucleosome are known as **histone tails** (Fig. 5.2).

- These histone tails are accessible to various modifying enzymes (e.g. histone acetyltransferases, histone deacetylases and methyltransferases), which catalyse the addition or removal of specific chemical groups.

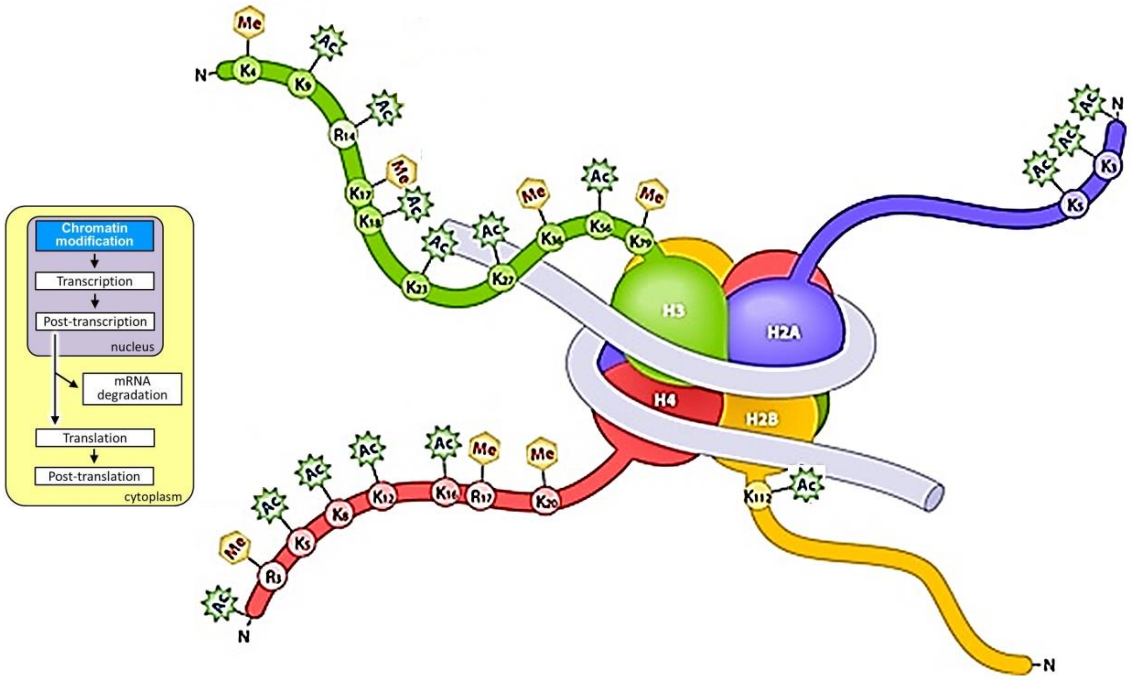


Fig. 5.2: Lysine (K) and arginine (R) residues on the histone tails are often chemically modified through the addition of acetyl group (–$COCH_3$, Ac) or methyl group (–CH_3, Me).

(A) Histone acetylation and deacetylation (Fig. 5.1, 5.3a)

Histone acetylation	Histone deacetylation
• In histone acetylation, **acetyl groups** ($CH_3\overset{O}{\overset{\|}{C}}-$) are attached to positively charged **lysine and arginine residues** in the histone tails.	• In histone deacetylation, acetyl groups are removed from the lysine and arginine residues in the histone tails.
• Catalysed by **histone acetyltransferase (HAT)** (a.k.a. histone acetylase). Some histone acetyltransferase are recruited by / requires the binding of transcriptional activators to function *(refer to Section 5.2.2.2)*	• Catalysed by **histone deacetylase (HDAC)**. Some histone deacetylase are recruited by / requires the binding of transcriptional repressors to function. *(refer to Section 5.2.2.2)*
• When the lysine/arginine residues are acetylated, their **positive charges** are **neutralized**.	• In deacetylated histones, the lysine/arginine residues are **positively charged** and can form **ionic bonds** with **negatively-charged DNA**.
• The histone tails are **not able to form ionic bonds** with the negatively-charged DNA and can also no longer bind to neighbouring nucleosomes.	
• The chromatin structure is **loosened**.	• This results in a **tightened nucleosome** and hence a **more compact** chromatin structure.
• As a result, proteins involved in transcription will have **easier access** to the DNA in an acetylated region. ○ General transcription factors and RNA polymerase can access the promoter ○ Specific transcription factors can access the proximal and distal control elements.	• As a result, proteins involved in transcription will have **no access** to the DNA in a deacetylated region ○ General transcription factors and RNA polymerase cannot access the promoter ○ Specific transcription factors cannot access the proximal and distal control elements.
• Gene expression is **activated**.	• Gene expression is **repressed** (i.e. prevented).

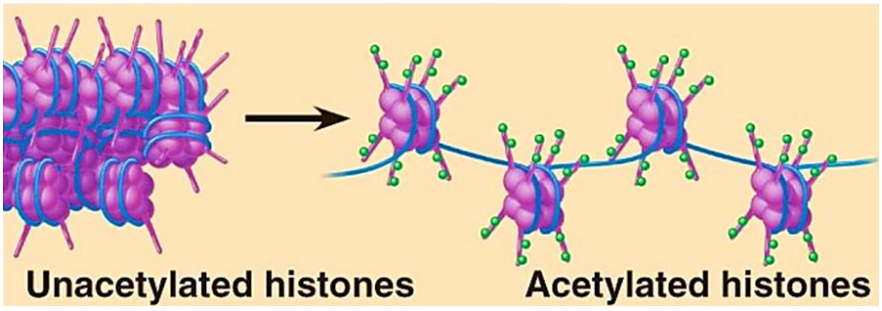


Fig. 5.3a: Acetylation of histone tails promotes loose chromatin structure that permits transcription**.**

(B) Histone methylation and demethylation (Fig. 5.3b)

Histone Methylation	Histone Demethylation
• Histone methylation is the **addition of a methyl group** (–CH_3) to the histone tails. • A reversible process catalysed by **histone methyltransferases** (HMT). • Methyl groups are non-polar (hydrophobic), hence they tend to interact with each other closely via **hydrophobic interactions**. • Histone methylation therefore promotes the **tightening** of nucleosomes and hence the condensation of chromatin. • This results in the methylated region being transcriptionally inactive. • Gene expression is **repressed**.	• Histone demethylation is the **removal of a methyl group** (–CH_3) from the histone tails. • A reversible process catalysed by **histone demethylases** (HDM). • Histone demethylation will **loosen** the chromatin structure. • The demethylated regions are thus transcriptionally active. • Gene expression is **activated**.

- Methyltransferases can add one, two or three methyl groups to lysine and arginine.

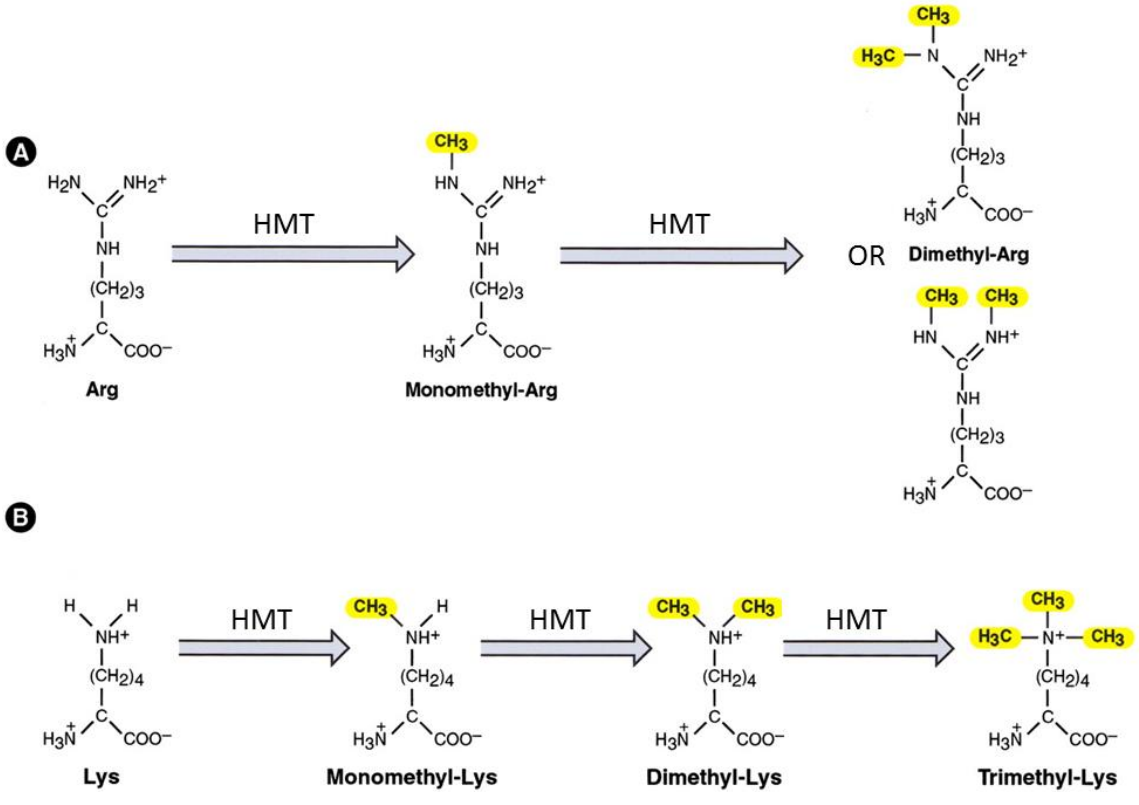


Fig. 5.3b: arginine (A) & Lysine (B) on the histone tails are methylated to promote gene silencing.
HMT = histone methyltransferase

CONSOLIDATION PRACTICE

Fill in the blanks below. Some examples have been done for you.

	Types of HISTONE modification	Effect on chromatin structure	Effect on gene expression
A	**Acetylation** • addition of acetyl groups ($CH_3\overset{O}{\overset{\|}{C}}-$)	Positive charges on histone tails are **…..** → unable to form **…..** with negatively-charged DNA → chromatin structure is **…..**	Promoter (DNA) is **…..** to RNA polymerase and transcription factors → Gene is switched **……..**
	Deacetylation • removal of acetyl groups ($CH_3\overset{O}{\overset{\|}{C}}-$)	Positive charges on histone tails are restored → form **ionic bonds** with negatively-charged DNA → chromatin structure is **more compact**	Promoter (DNA) is **not accessible** to RNA polymerase and transcription factors → Gene is switched **OFF**
B	**Methylation** • addition of a methyl group ($-CH_3$)	Methyl groups (non-polar) interact with each other via **…..** → tighter nucleosomes → chromatin structure is **…..**	Promoter (DNA) is **…..** to RNA polymerase and transcription factors → Gene is switched **……..**
	Demethylation • removal of a methyl group ($-CH_3$)	Chromatin structure is **loosened**	Promoter (DNA) is **more accessible** to RNA polymerase and transcription factors → Gene is switched **ON**

5.1.2 DNA methylation

- DNA methylation leads to **gene inactivation**.
    - The methyl group is often added to **cytosine** on a stretch of cytosine-guanine dinucleotide (CG), catalysed by **DNA methyltransferases** (Fig. 5.4).
    - It was reported that **methylated DNA recruits histone deacetylase** (HDAC) to further **promote chromatin compaction**.
    - DNA methylation **changes the structure of the DNA** such that it is **no longer complementary to transcription factors and RNA polymerase**.
    - Essential for **long-term inactivation of genes**, which occurs during normal cell differentiation of the embryo.
        - E.g. genes that are required for embryonic development (e.g. embryonic globin genes) are switched on at the embryonic stage but permanently inactivated by methylation thereafter.

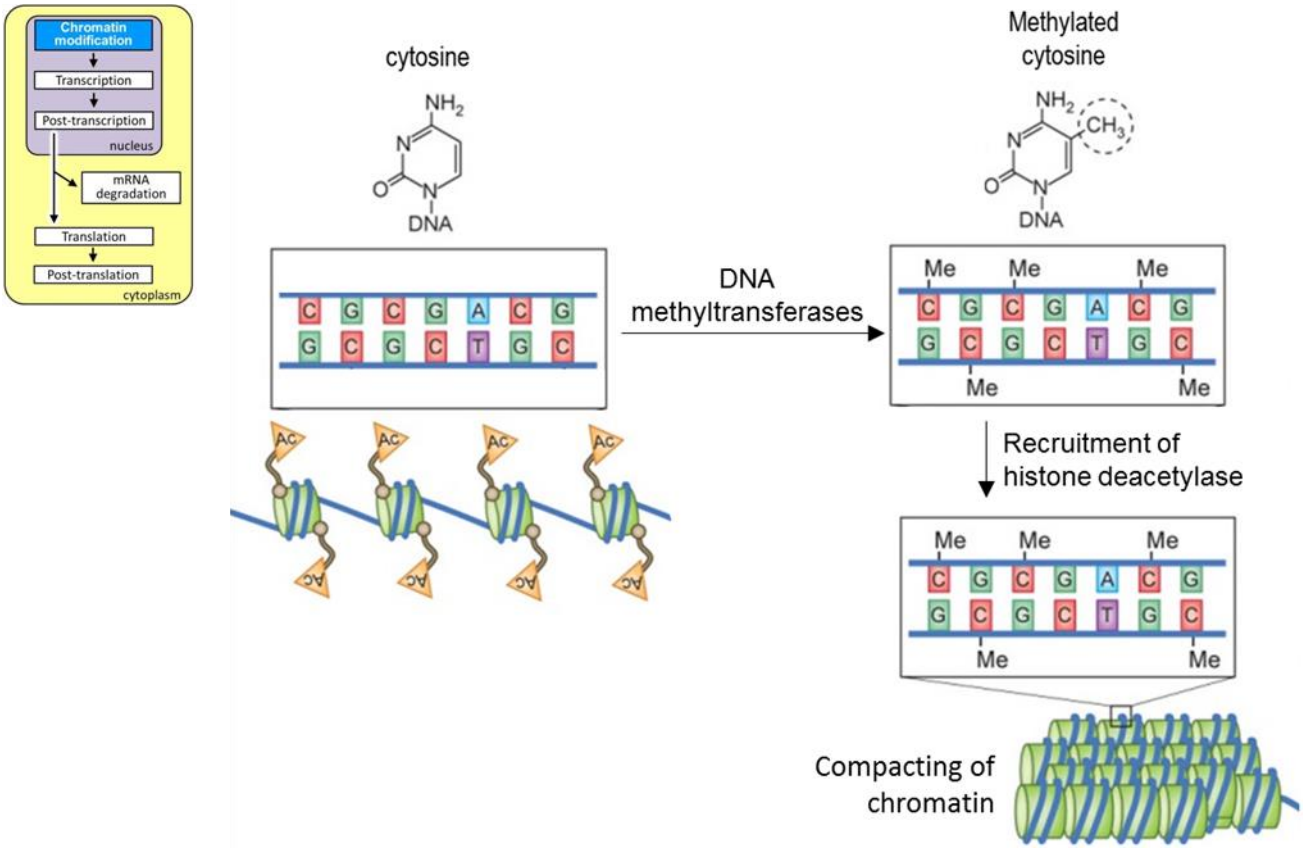


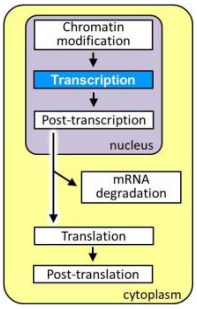
Fig. 5.4: Methyl groups are often added to cytosine bases in a stretch of CG dinucleotides. Methylated DNA recruits HDAC to remove acetyl groups on histone tails, leading to chromatin compaction, and hence gene inactivation.

- DNA demethylation (reverse process) is catalyzed by **DNA demethylases**. This loosens the chromatin structure, hence switching on the gene.

5.2 Control at the transcriptional level

5.2.1 Eukaryotic RNA polymerases

- In contrast to prokaryotes, which contain only a single type of RNA polymerase, eukaryotic nuclei have three types of RNA polymerases; namely RNA polymerase I, RNA polymerase II and RNA polymerase III.
- The types of polymerases present allow the cells to control the type of RNA transcribed.
- These 3 polymerases are structurally similar but they transcribe different types of genes:



Type of RNA polymerase	Location	Types of genes transcribed
RNA pol I	Nucleolus	• Most genes that code for ribosomal RNA (rRNA) e.g. 18S rRNA, 5.8S rRNA, 28S rRNA
RNA pol II	Nucleoplasm	1. Genes that code for proteins 2. Genes that code for small nuclear RNA (snRNA) that forms part of a spliceosome
RNA pol III	Nucleoplasm	1. Genes that code for transfer RNA (tRNA) 2. Gene that codes for 5S rRNA 3. Genes that code for various small RNAs

(Note: no need to memorise details)

5.2.2 Regulation of transcriptional initiation

- The regulation of transcription initiation in eukaryotes involves **proteins that bind to DNA** that either **facilitate or inhibit** the **binding of RNA polymerase**.

- Before looking at how eukaryotic cells control their transcription, let's review the various components required for transcription, organization of a typical eukaryotic gene as well as the initiation of transcription.

REVIEW

The components involved in the regulation of transcriptional initiation:

- Regulatory DNA sequences
  - **Promoter** containing the TATA box (site where RNA polymerase and general transcription factors bind to)
  - **Proximal control elements** (Upstream to and near promoter)
  - **Distal control elements** (either enhancers or silencers, which may be thousands of nucleotides upstream or downstream from the promoter)

- Proteins/enzymes
  - **General / Basal transcription factors** (bind to TATA box within promoter; forms part of the transcription initiation complex)
  - **RNA polymerase** (binds to TATA box within promoter, forms part of the transcription initiation complex)
  - **Specific transcription factors** (either activators which bind to enhancers to increase the rate of transcription, or repressors that bind to silencers to decrease the rate of transcription)

The organisation of a typical eukaryotic gene and its regulatory sequences (Fig. 5.5):

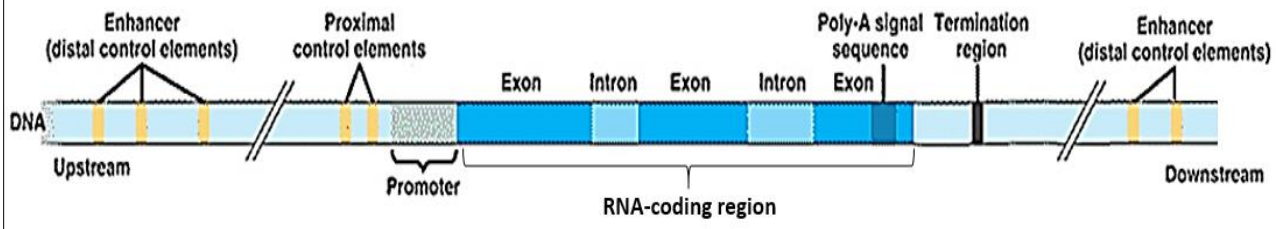


Fig. 5.5: Organization of a typical eukaryotic gene and its regulatory sequences.

How transcription is initiated in eukaryotic cells

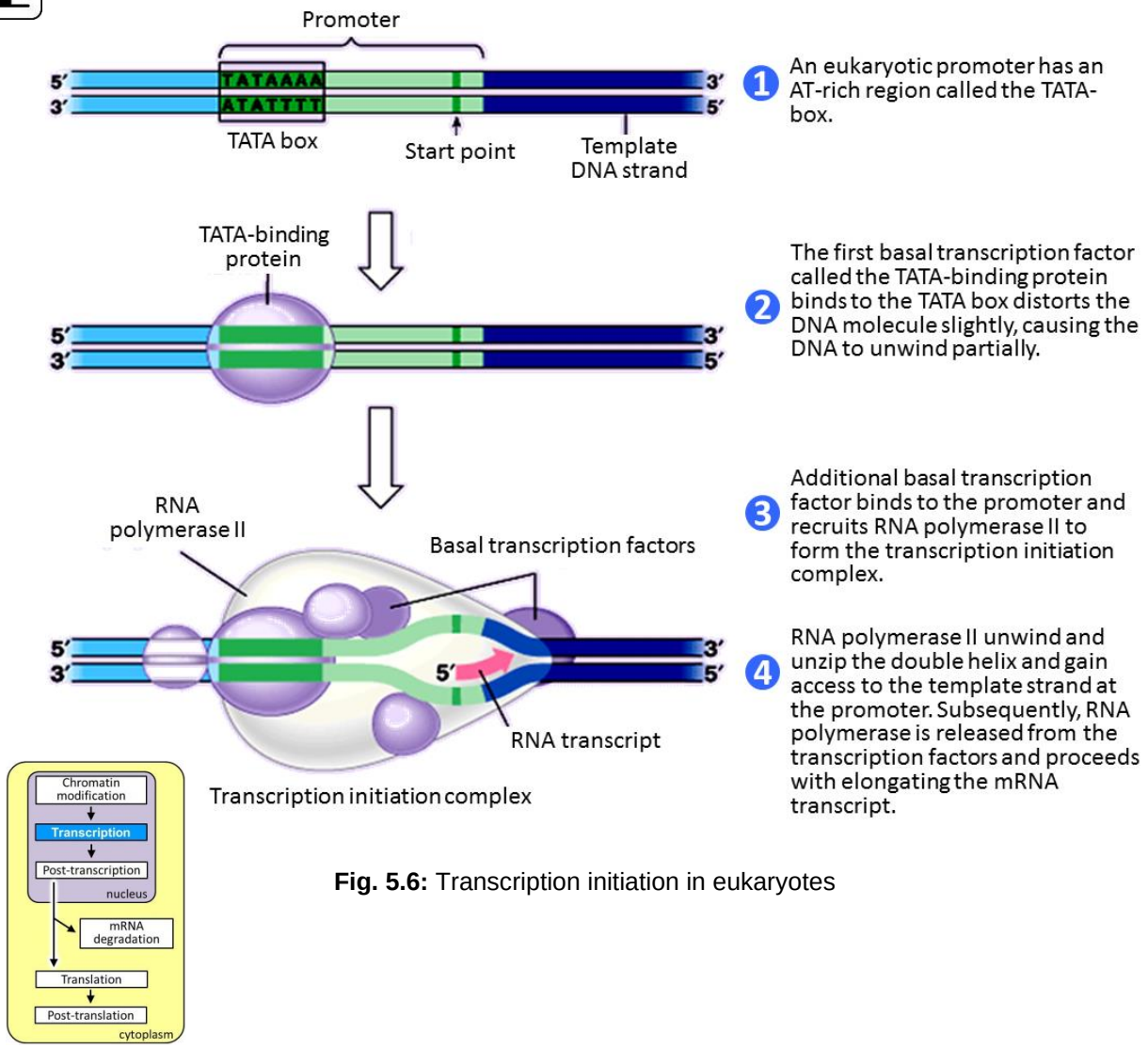


Fig. 5.6: Transcription initiation in eukaryotes

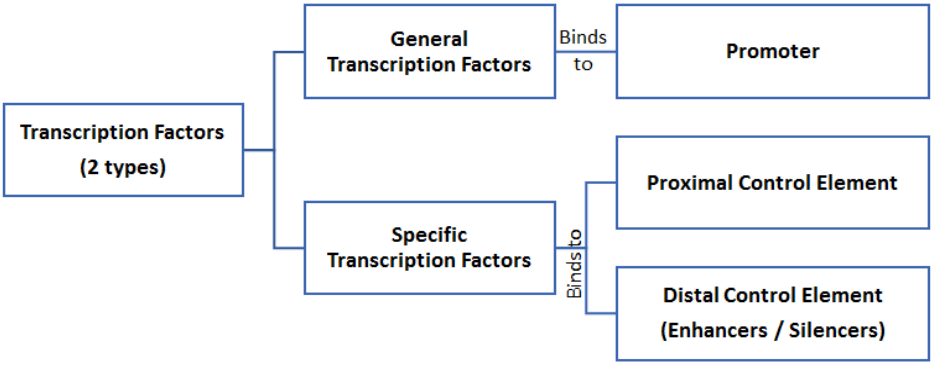
Why does TATA-binding protein bind only to TATA box and nowhere else in the entire genome?

- Initiation of transcription is the **most important control point** in gene expression.
- As seen in Fig. 5.6, the formation of transcription initiation complex on the promoter is required for transcription of the gene to occur.
- Associated with most eukaryotic genes are multiple **control elements** (non-coding DNA) that serve as **binding sites for specific transcription factors**.
- These control elements and the transcription factors they bind to are critical to the precise regulation of gene expression as seen in different cell types.

(A) Roles of transcription factors in the regulation of transcription initiation

- **Transcription factors** are a family of **proteins** that bind to specific DNA sequences (control elements) and thereby control transcription via interactions with RNA polymerase.

- There are 2 types of transcription factors: **general/basal transcription factors** and **specific transcription factors**, which bind to different control elements:



(1) General / Basal transcription factors

- Required for transcription of **all** protein-coding genes, therefore assemble at **all promoters** used by RNA polymerase II (Fig. 5.7).

- Serve the following functions:

  1) help to position RNA polymerase correctly at the promoter.

  2) aid in the partial unzipping of DNA double helix to allow transcription to begin.

  1) help to release the RNA polymerase from the promoter to enter into elongation mode once transcription begins.

- Interaction of general transcription factors and RNA polymerase II usually leads only to a **low rate of initiation** and production of a few RNA transcripts, many of which are incomplete.

- RNA polymerase II usually **falls off the template strand prematurely** before it even reaches the termination sequence to complete transcription.

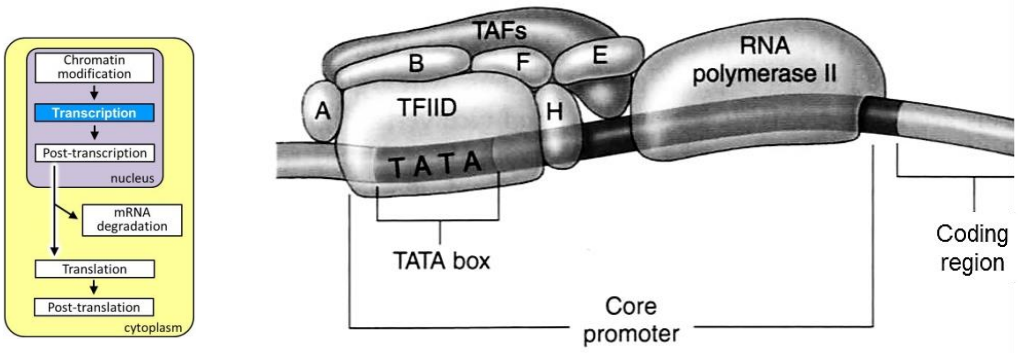


Fig. 5.7: The assembly of general transcription factors and RNA polymerase II at the promoter leads to the initiation of a basal level of transcription.

(2) Specific transcription factors

There are two groups of specific transcription factors which bind to different control elements:

- One group binds to the **proximal control element**, located near the promoter (they are simply known as **specific transcription factors**).

- Another group of specific transcription factors (known as **activators or repressors**) binds specifically to the **distal control elements** (**enhancers /silencers**).

I) Activators

- In eukaryotes, high level of transcription of particular genes at appropriate times depends on the interactions of **distal control elements** with specific transcription factors known as **transcriptional activators**.

- Transcriptional activators are **proteins** that to **increase the rate of transcription** (above basal level). They do so by binding to distal control elements known as **enhancers**.

- Transcriptional activators **bind to enhancers of specific genes** to increase rate of transcription through the following ways:

  i) They **recruit DNA-bending proteins** which cause the **bending of DNA.** The bending of DNA **brings the bound activators closer to the transcription initiation complex at the promoter**.

  This helps to stabilize the assembly of active transcription initiation complex (general transcription factors and RNA polymerase II) on the promoter and **ensures that RNA polymerase II completes transcription without falling off prematurely**. This increases the rate of transcription of the gene. (Fig. 5.8).

  ii) They **recruit histone acetyltransferase** (a.k.a. histone acetylase) to add acetyl groups to histone tails (Fig. 5.9 and Fig. 5.1). This loosens the chromatin structure by reducing the ionic bonding between positively-charged histones and negatively-charged DNA.

  iii) They **recruit other chromatin remodelling proteins** to the region, making it less compact and more accessible to other transcription factors and RNA polymerase II (Fig. 5.9 and Fig. 5.1)

  iv) They **regulate the frequency** at which **new RNA polymerase II reinitiates transcription**. This results in many copies of RNA produced (hence increasing gene expression).

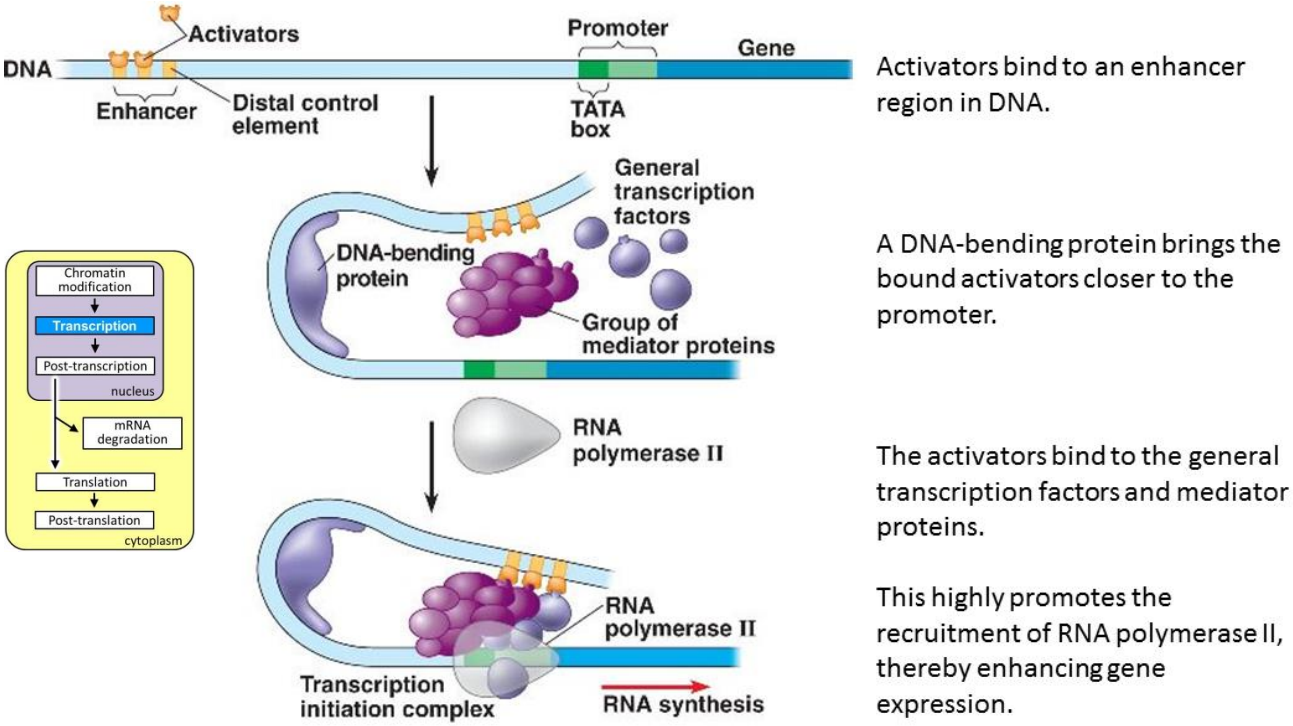


Fig. 5.8: Activators bind to an enhancer promotes the assembly of the transcription initiation complex.

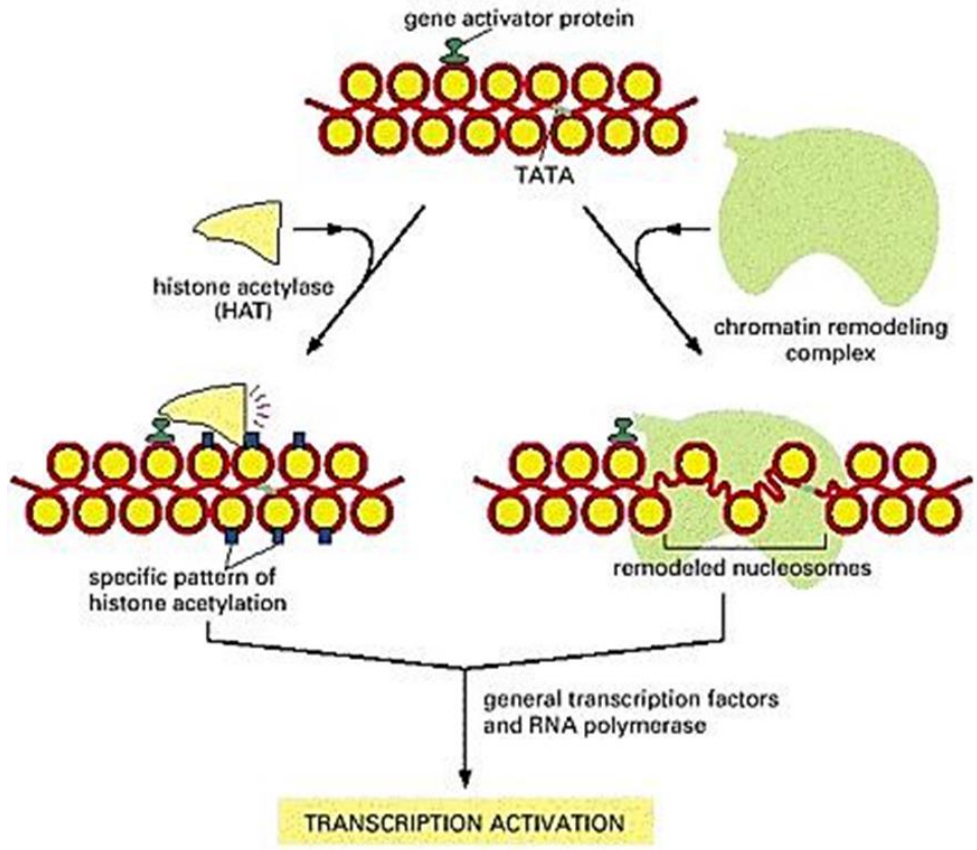


Fig. 5.9: Activator recruits HAT and remodelling proteins and thus rendering the DNA packaged in chromatin more accessible to general transcription factors and RNA polymerase II.

(2) Specific transcription factors (cont'd)

II) Repressors

- Transcriptional repressors are **proteins** which **interfere with transcription initiation** or **reduce the rate of transcription** when bound to distal control elements known as **silencers**.

- Transcriptional repressors **bind to silencers of specific genes** to prevent transcription in the following ways:

  1) They may **interact with a nearby activator**, preventing it from functioning (Fig. 5.10), such as preventing the activator from recruiting histone acetyltransferase.

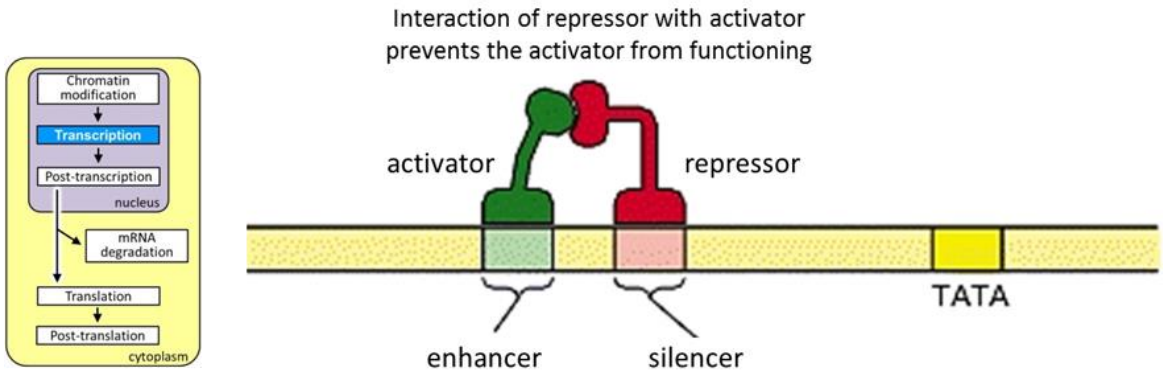


Fig. 5.10: Physical interaction between repressor and activator prevents the activator from functioning.

  2) They may also **interact with general transcription factors** bound at the promoter, preventing their assembly into transcription initiation complex (Fig. 5.11).

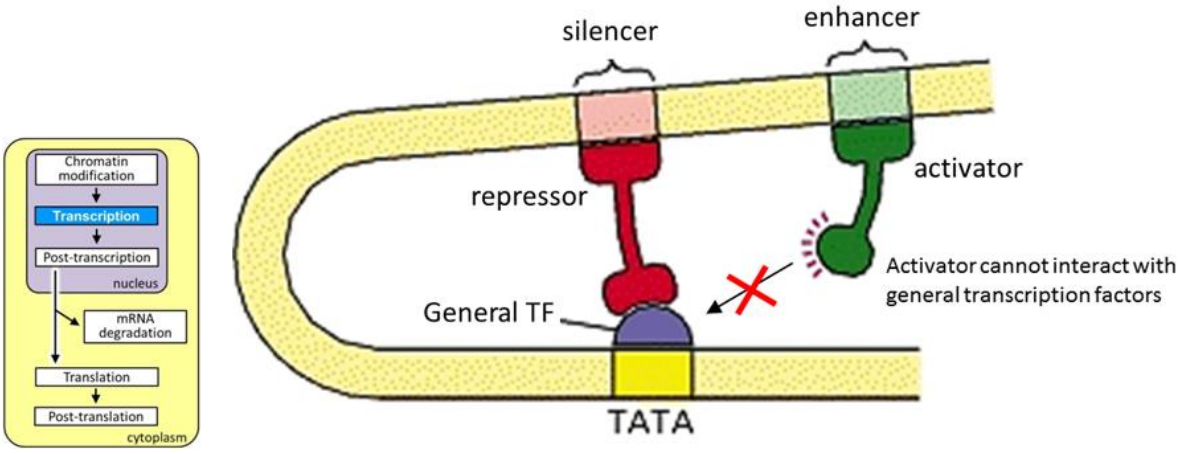


Fig. 5.11: Repressor may bind to general transcription factors and prevents activator from binding.

3) When an enhancer and a silencer share an overlapping DNA region, the binding of repressor to the silencer would **block the binding of activators to the enhancer**. (Fig. 5.12).

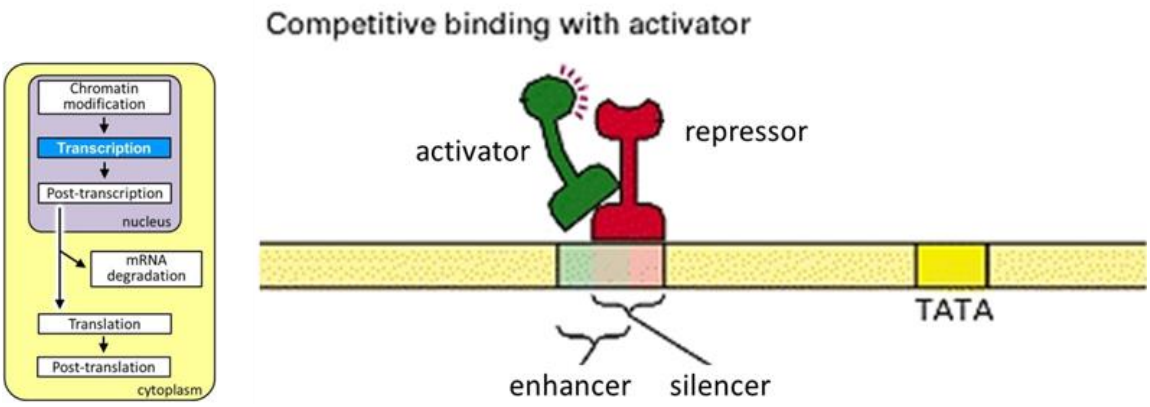


Fig. 5.12: Both activator and repressor compete for the same DNA region.

4) Some transcriptional repressors, when bound to silencers, **recruit histone deacetylase** to remove acetyl groups from histone tails at specific regions. **Chromatin remodelling proteins** may also be recruited to **tighten the chromatin structure** (Fig. 5.13).

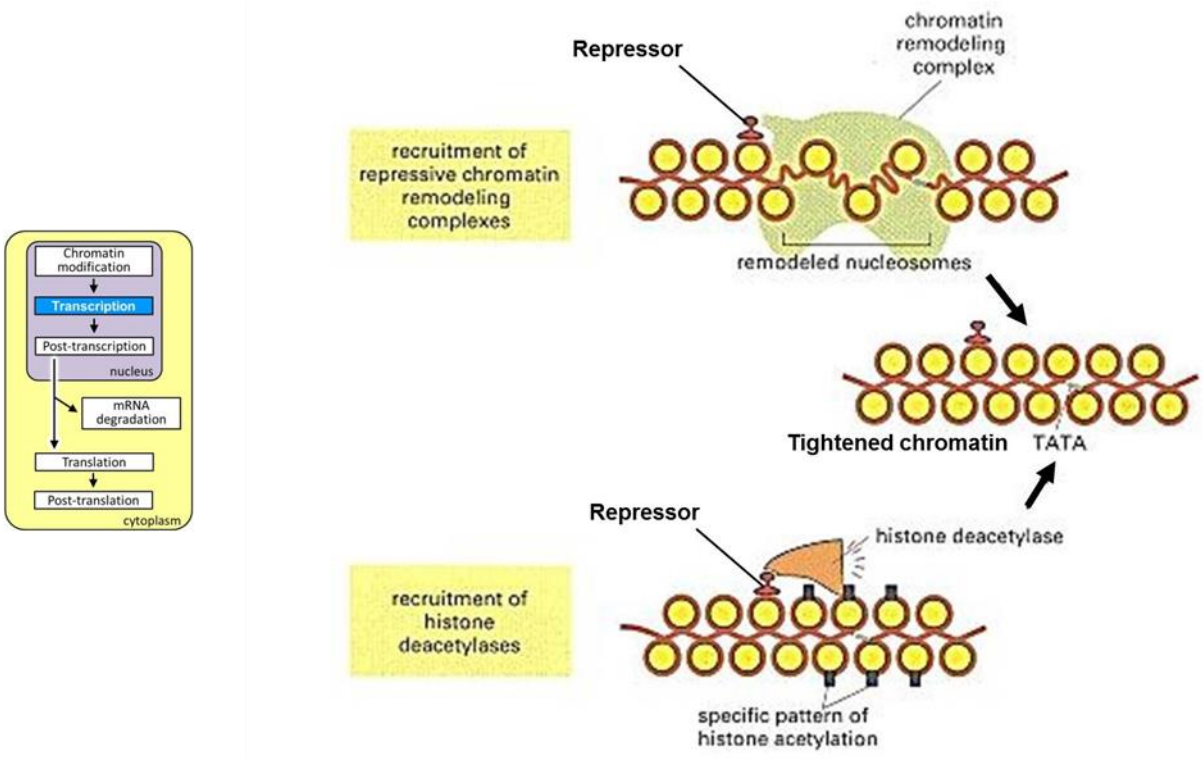


Fig. 5.13: The recruitment of histone deacetylase and chromatin remodelling proteins tightens the chromatin structure.

- In summary, binding of transcriptional activators to specific enhancers increases the rate of transcription beyond the basal level, whereas the binding of transcriptional repressors to specific silencers represses the transcription of genes.

(B) A specific combination of control elements determines differential gene expression in different cell types

- In humans, both liver cells and lens cells have the genes for making the proteins albumin and crystallin, but only liver cells make albumin (a blood protein) and lens cells make crystallin (a protein found in the lens of the eyes).

- This is because each cell type synthesizes its **unique set of specific transcription factors** that determines which genes are expressed.

- All the activators required for high-level expression of the albumin gene are present only in liver cells (Fig. 5.14a), whereas the activators needed for expression of the crystallin gene are present only in lens cells (Fig. 5.14b).

- In this example, although the albumin gene and crystallin gene may share a common control element (Fig. 5.14, middle segment of enhancer), the **expression of each gene depends on the unique combination of control elements to which specific activators or repressors bind**.

- Note: For simplicity, only the activators are shown in Fig. 5.14. The repressors may also influence transcription.

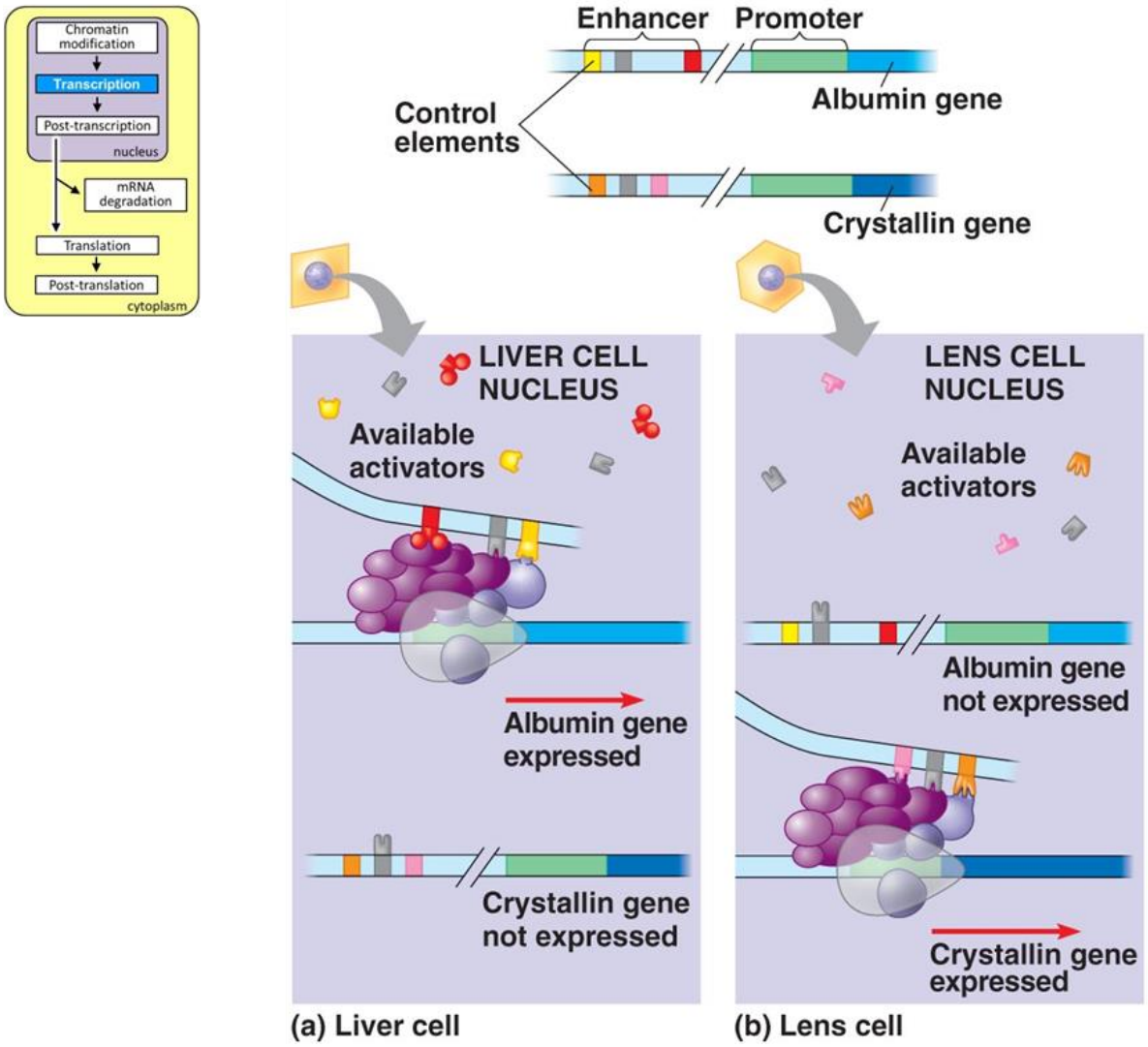


Fig. 5.14: Cell type-specific transcription where specific set of activators bind to unique combination of enhancers in the liver cell and lens cell respectively

(C) Summary of how gene expression can be increased at DNA and transcriptional levels (Fig. 5.15)

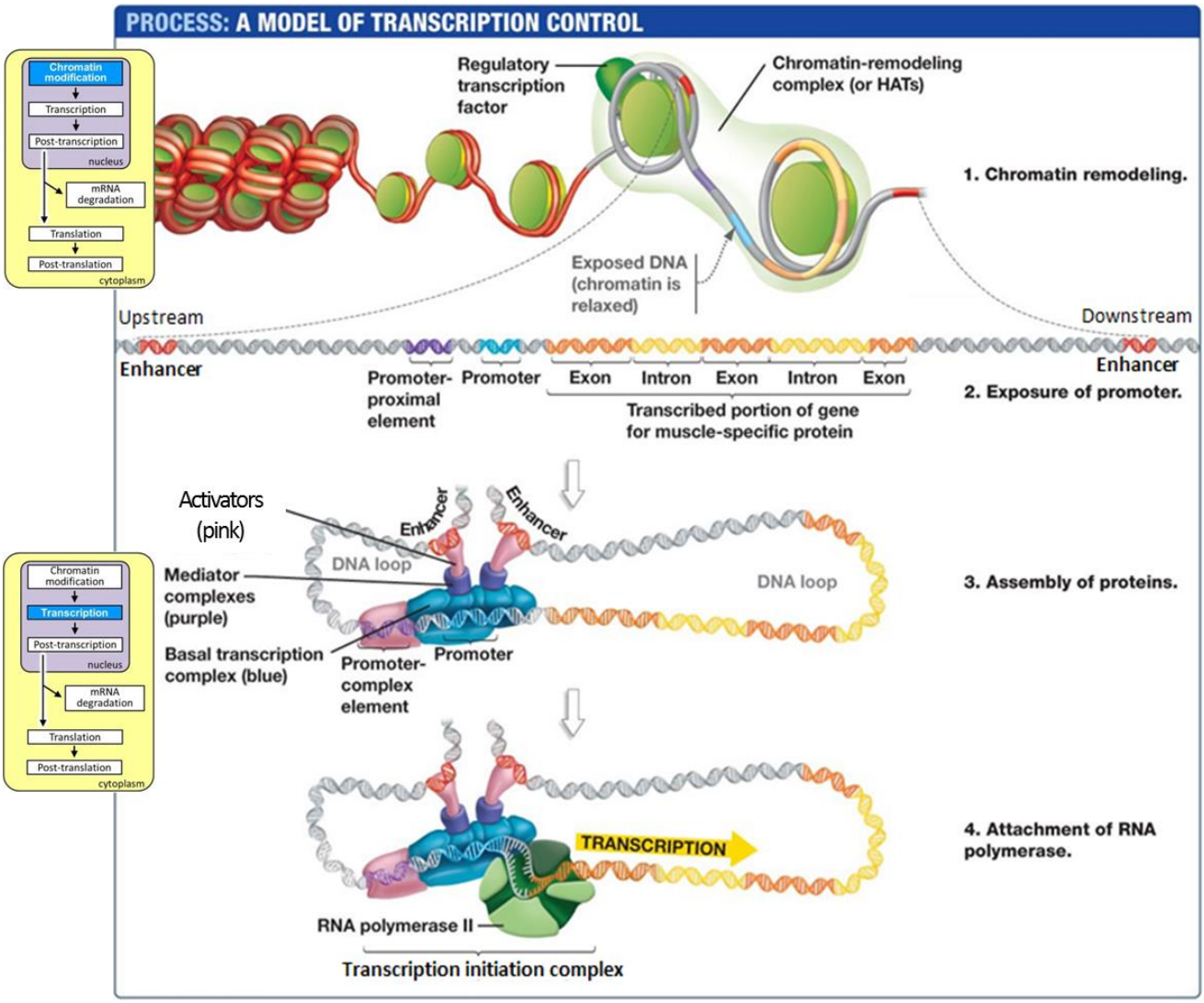


Fig. 5.15: Recruitment of HAT and chromatin remodelling proteins causes the loosening of the DNA from histones, which allows promoter and enhancers to be accessible by transcription factors and RNA polymerase.

STOP CHECK IN HERE

CONSOLIDATION PRACTICE

Fill in the blanks below with the correct terms.

Proteins Involved		DNA	Effects
• **General transcription factors** • **RNA polymerase**	binds to	**Promoter**	..... level of transcription
Specific transcription factors		Proximal control elements	Regulate transcription of gene
Specific transcription factors (activators)		**Distal control elements (Enhancers)**	..... rate of gene expression
Specific transcription factors (repressors)		**Distal control elements (Silencers)**	..... rate of gene expression

5.3 Control at the post-transcriptional level

- Following transcription in eukaryotes, **pre-mRNAs** (primary transcripts) produced are modified in various ways **in the nucleus** before being exported as **mature mRNAs** to the cytoplasm.

- In the process, both ends (5' and 3') of pre-mRNA are modified, introns are excised and exons are spliced together (Fig. 3.11).

5.3.1 Addition of 7-methylguanosine to the 5' end of mRNA (5' capping)

- The 5' end of the pre-mRNA transcript is capped with **7-methylguanosine (7-MG)**, **a modified form of a guanine** nucleotide. This guanine base has an additional methyl group at the 7th position on the guanine ring, hence the name (Fig. 5.16).

- The 7-methylguanosine (7-MG) is added to the pre-mRNA via a **5' to 5' linkage**.

Recall: How does this linkage differ from the way other nucleotides in mRNA are linked?

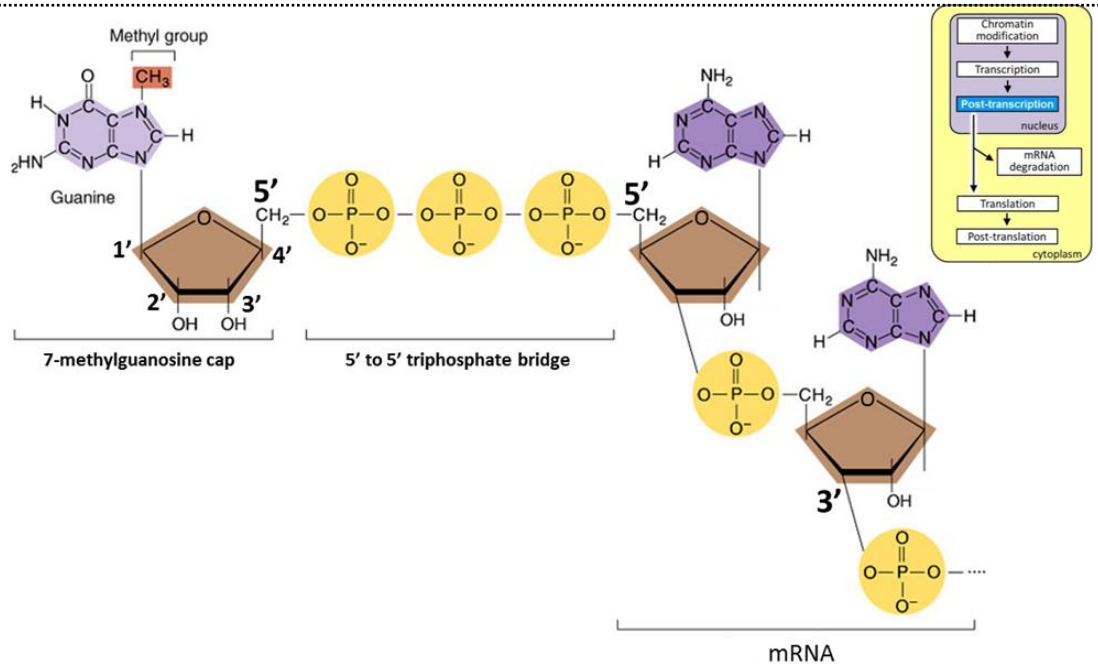


Fig. 5.16: Addition of a 7-mG cap to the 5' end of the pre-mRNA via a 5' to 5' linkage.

- The 5' cap has three important functions in regulating gene expression:

  (1) **Protect the mRNA from degradation** by hydrolytic enzymes (5' exonucleases) found in the cytosol. The slower the degradation of the mRNA, the longer it exists to be translated into polypeptide, hence increasing gene expression.

  The duration of existence of mRNA is expressed as its "half-life" – the time taken for 50% of the mRNA to be degraded. Half-life differs for different mRNA, from a few seconds to several months.

  (2) Facilitate the **export of mature mRNA** from **nucleus to cytosol**.

  (3) As a **site of attachment** for **translation initiation factors** to promote the binding of ribosomes to the 5' end of mRNA during translation.

5.3.2 Addition of poly(A) tail at the 3’ end of mRNA (3’ polyadenylation)

- The polyadenylation signal, AAUAAA, triggers the cleavage of the 3’ end of the pre-mRNA 10 –35 nucleotides downstream from it.

- **Poly(A) polymerase** then uses ATP to synthesize a stretch of 50–250 adenine nucleotides at the 3’ end of the pre-mRNA transcript, called the poly(A) tail (Fig. 5.17).

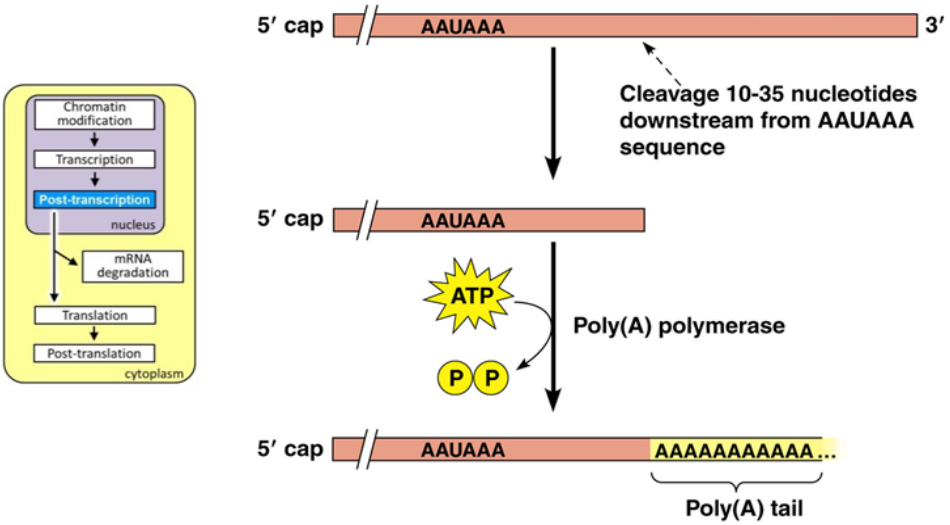


Fig. 5.17: The sequence AAUAAA signals the addition of poly(A) tail to 3’ end of mRNA.

- The poly(A) tail is a binding site for a group of proteins known as the poly(A)-binding proteins. These proteins cooperate with other factors to affect the export, stability and translation of mRNA.

- Hence, the 3’-poly(A) tail serves three important functions in regulating gene expression:

  (1) **Prolongs the half-life of mRNA** by buffering the mRNA against degradation by **3’ exonucleases**, i.e. it allows itself to be degraded first.

  (2) Facilitates the **export of mature mRNA** from **nucleus to cytoplasm**.

  (3) When bound by poly(A)-binding proteins, these proteins may **interact with translation initiation factors** bound at the 5’ end and help to **promote translation** (Fig. 5.18).

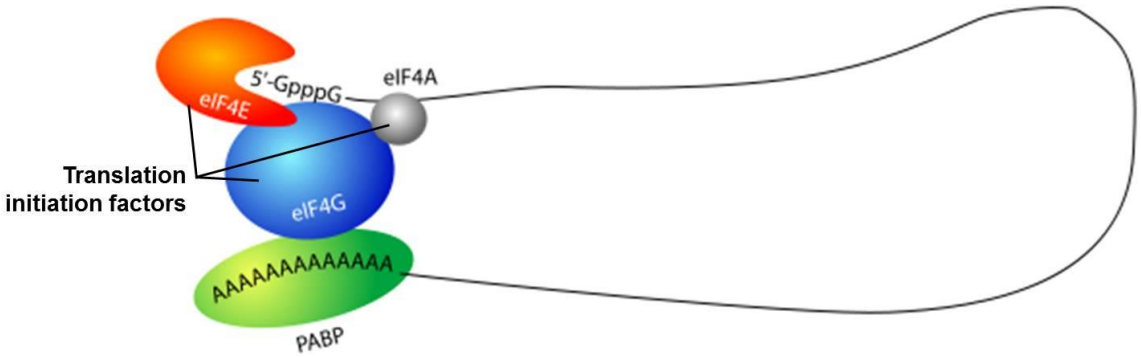


Fig. 5.18: How the poly(A) facilitate translation by binding to poly(A)-binding proteins (PABP) and translation initiation factors *(no need to learn the specific names)*.

5.3.3 RNA splicing

- **Pre-mRNA** consists of **both introns** (non-coding sequences) and **exons** (coding sequences).
- During RNA splicing, **all introns are excised** and the **exons are spliced** (joined) together.
- RNA splicing is necessary to facilitate the transport of mature mRNA from nucleus to the cytoplasm and to ensure that **only exons are translated to produce functional proteins**.
- The process of RNA splicing is carried out by **spliceosome**, an **RNA-protein complex** made up of **small nuclear ribonucleoproteins** (**snRNPs**).
- snRNPs are located in the nucleus and composed of **snRNAs** (small nuclear RNAs) and various **proteins**.
- The snRNAs (each about 150 ribonucleotides) play an important role in:
  - Splice site recognition
  - Spliceosome assembly
  - Catalysis of RNA splicing
- **How** is RNA splicing carried out? (Fig. 5.19):

  1) snRNPs contain **snRNA** which bind to **splice sites** at **each end of an intron** in pre-mRNA via **complementary base pairing.**

  2) The snRNPs then join with other proteins to form a larger complex called **spliceosome**.

  3) This causes the intron to form a loop, bringing the two ends of the intron together.

  4) The spliceosome cuts the ends of the intron to **excise the intron** and **splice (join)** the two **exons** that flank the intron.

  5) This forms the **mature mRNA** which comprises only exons.

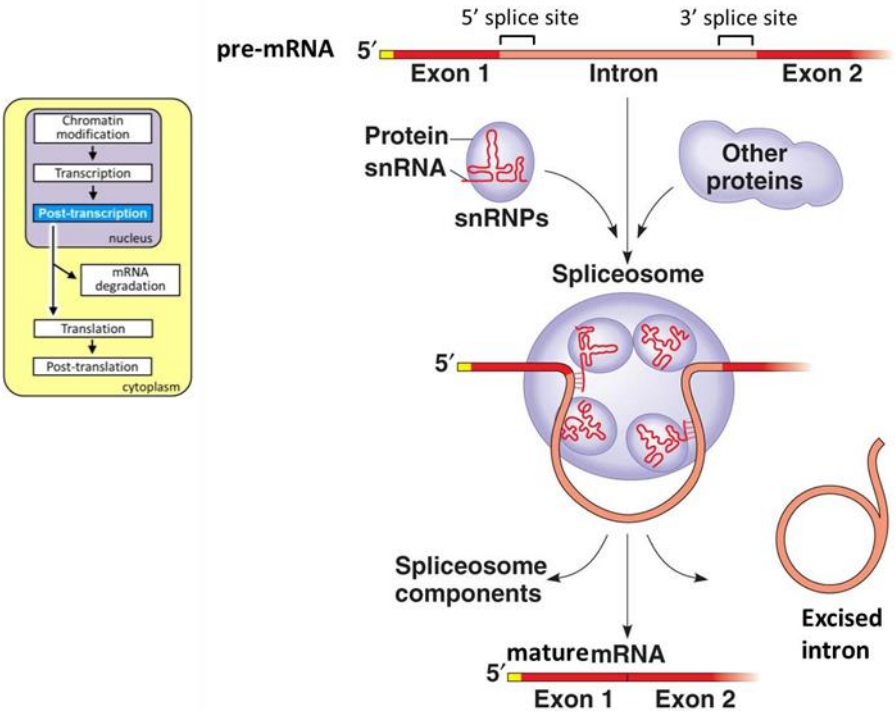


Fig. 5.19: RNA splicing by spliceosome (a ribonucleoprotein)

- **Different exons** may **code for different domains of a protein** with different functions, e.g. one domain may include the active site of an enzymatic protein while another may attach the protein to a cellular membrane. (Fig. 5.20).

  A protein domain = a part of a protein that can fold, function and exist independently of the rest of the protein.

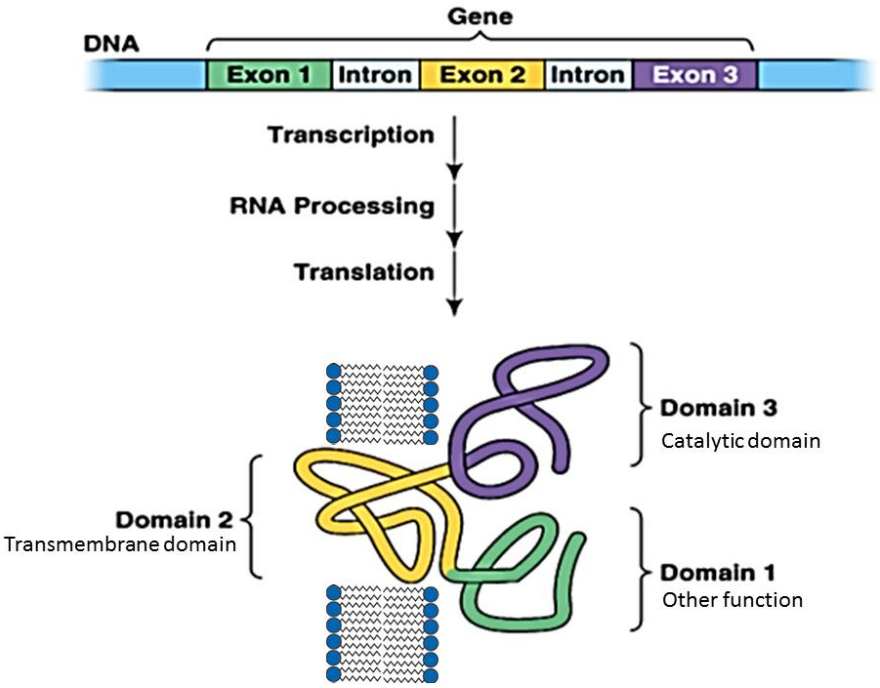


Fig. 5.20: A protein can have more than one domain, each encoded by an exon.

5.3.4 Alternative RNA splicing

- In alternative RNA splicing, the **same pre-mRNA** synthesized in **different cell types** have **all introns excised** but **different combinations of exons spliced together**.

- This gives rise to **different mature mRNA** sequences and hence **different proteins** (thus controlling the types of proteins being expressed in different cells).

    - For example, the exons of *troponin T* gene can be spliced in different ways in different tissues to produce different mature mRNAs, hence different proteins (Fig. 5.21).

- This process enables a **single gene** to **encode more than one kind of polypeptide**.

    - Example: A fruit fly gene called *Dscam* was found to have enough alternatively spliced exons to generate more than 38,000 different proteins! Clearly, alternative splicing expands the repertoire of a eukaryotic genome.

How is this possible?

- In alternate RNA splicing, **some exons are excised together with the introns**, and the remaining exons are spliced together (in sequential order, i.e. 1234, 135, 125, 245, 234, 345, but not 314, 534, 431) to produce different mature mRNAs

- The decision as to which exon is to be removed or included involves **regulatory sequence in the pre-mRNA** and **regulatory proteins (specific to each cell type)**. The binding of regulatory proteins to regulatory sequences within the pre-mRNA controls **intron-exon choices**. *(Details not required)*

- The resulting mature mRNA will have different nucleotide sequences and hence will be translated to different polypeptide sequences. These polypeptides are folded differently to produce different tertiary structures which may or may not perform similar functions.

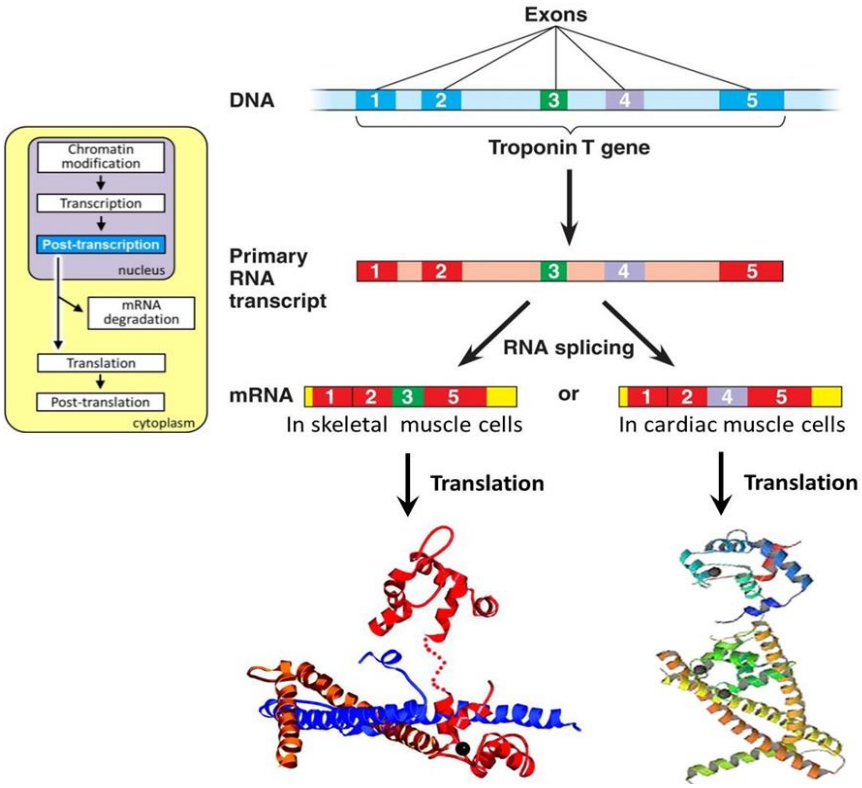


Fig. 5.21: The troponin gene encodes different but related troponin T proteins in different tissues (skeletal troponin and cardiac troponin). The protein isoforms is produced due to alternative splicing of the same primary RNA transcript.

5.4 Control at the translational level

5.4.1 Regulation of mRNA degradation / half-life of mRNA

- The life span (half-life) of mRNA molecules in the cytoplasm is important in determining the extent of protein synthesis in a cell. The longer the life span (half-life), the higher the gene expression.
- The level of expression of that gene can therefore be reduced by mRNA degradation.
- The first major step of mRNA degradation is the process of deadenylation (i.e. removal of the 3' poly(A) tail)
- Bacterial mRNA molecules are degraded by nucleases within a few seconds to a few minutes while mRNAs in multicellular eukaryotes can remain for hours, days or even weeks.
- How mRNA is degraded (Fig. 5.22):
    1. The 3' **poly(A) tail** is **shortened** by **deadenylase** enzyme.
    2. The shortening of the 3' poly(A) tail triggers the **removal of the 7-methylguanosine cap**, which then enables **5' exonucleases** to degrade the mRNA from the 5' end.
    3. The mRNA can also be degraded by **3' exonucleases** from the 3' end.

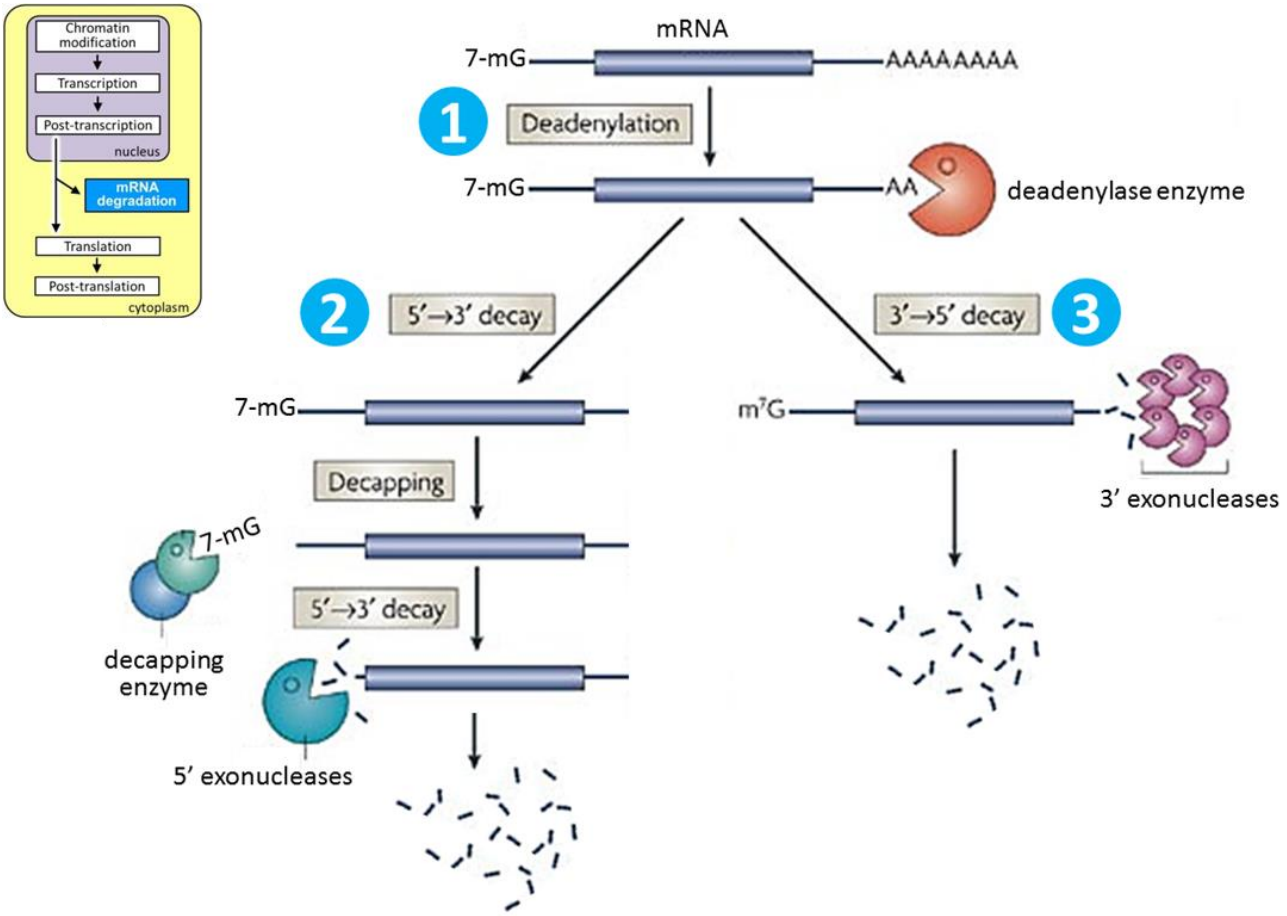


Fig. 5.22: mRNA degradation is triggered by shortening of poly(A) tail

5.4.2 Regulation of translational initiation

- Once mRNA is present for translation, translational initiation is often the most crucial step in determining protein synthesis. There are generally three mechanisms to control the initiation of translation.

(A) Role of translation initiation factors in regulating the translation of all mRNAs

- Translation of **all** the mRNA present in the cell may be regulated simultaneously through the **activation or inactivation of translation initiation factors** required to initiate translation.

- Translation initiation factors **bind** to the **5'-7mG cap** and the **5'-UTR** to **facilitate the binding of ribosome** (Fig. 5.23).

- Such a 'global' control of translation allows the cell to shut down translation if environmental conditions are poor (e.g. shortage of a certain amino acid) or delay translation until an appropriate time or conditions return (e.g. until after fertilization of an egg or when light condition returns for plants).

    - *Example 1* – An unfertilized ovum stores large amount of mRNA. Just after fertilization, translation is triggered by the sudden activation of translation initiation factors. The response is a burst of synthesis of proteins encoded by the stored mRNAs, leading to active mitotic divisions that form the embryo.

    - *Example 2* – Some plants or algae store mRNAs during periods of darkness. The return of light conditions triggers the reactivation of translation initiation factors.

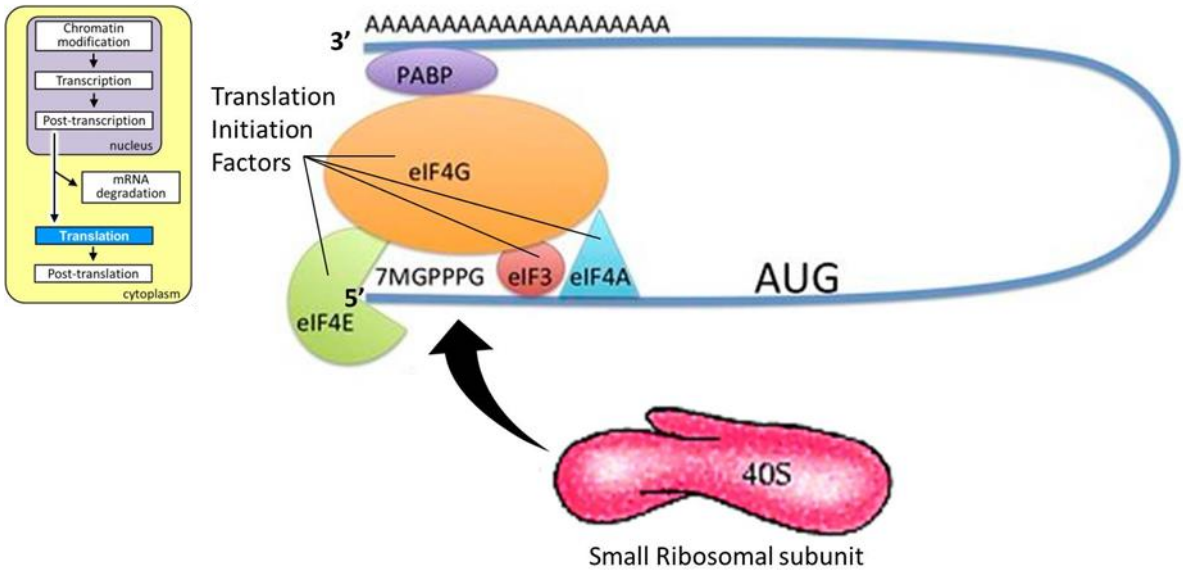


Fig. 5.23: The binding of poly(A)-binding proteins (PABP) facilitates the binding of the various translation initiation factors to the 5'-cap and the 5'-UTR, which in turns facilitates the binding of 40S small ribosomal subunit to initiate translation.

(B) Binding of specific translational repressors to the 5'-UTR prevents translation of specific mRNA

- To **reduce** protein synthesis, **specific translational repressor proteins** can bind to the **5' untranslated region** (5'-UTR) of **specific mRNAs**. This **prevents attachment of ribosomes**.

- Example: translation of ferritin mRNA *(this is an illustration of the concept. No need to learn the details)*

    - Ferritin protein is a ubiquitous intracellular protein that *stores* iron.

    - In the presence of **low** iron concentration, a specific regulatory protein called IRE-binding protein binds to the specific 5'-UTR called the iron-response element (IRE). This prevents the attachment of translation initiation factors and ribosomes and hence translation is inhibited. (Fig. 5.24a).

    - In the presence of **high** iron concentration, iron binds to IRE-binding protein, altering its shape, rendering it unable to bind to the IRE. Hence, the ribosome can access to the 5'-UTR to initiate translation (Fig. 5.24b)

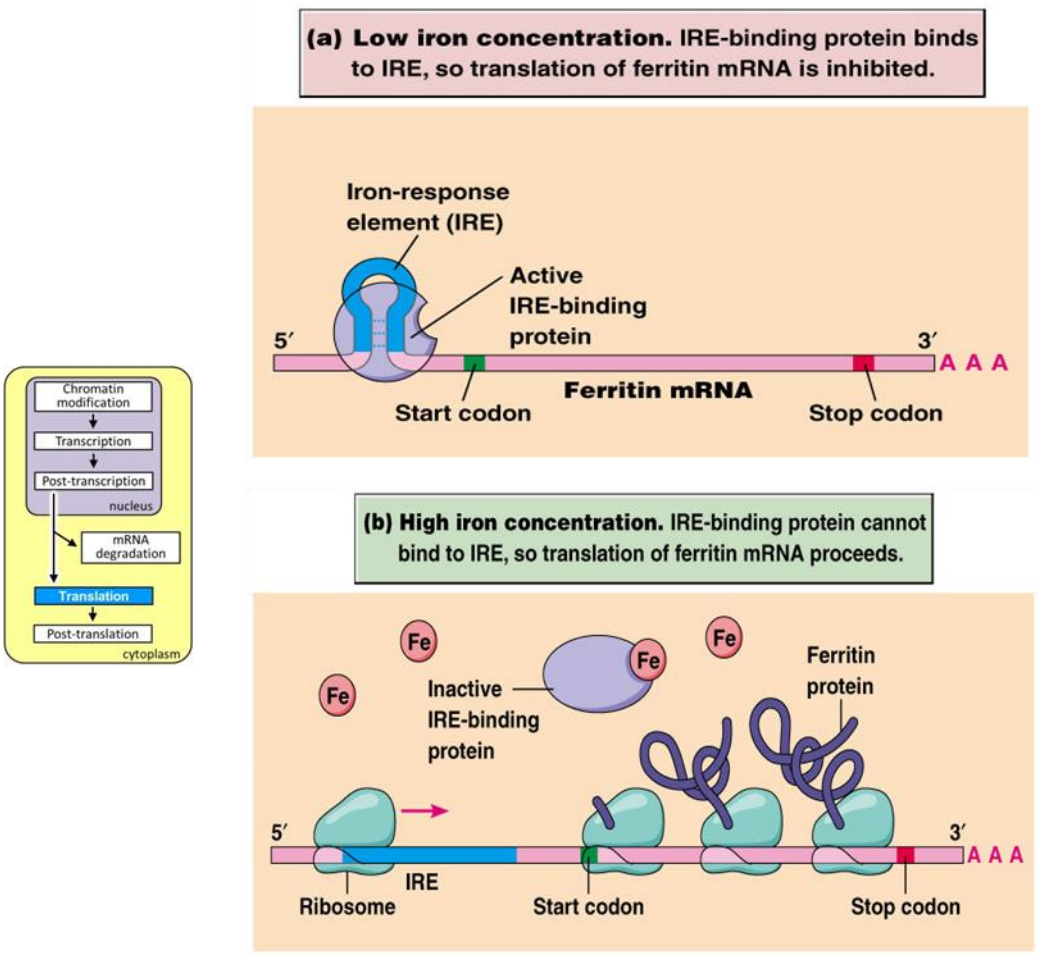


Fig. 5.24: Translation can be repressed by binding of regulatory proteins to the 5'-UTR.

(C) The length of poly(A) tail can determine the initiation of translation

- As previously mentioned (Fig. 5.18, 5.23), the 3' poly(A) tail serves as a binding site for poly(A)-binding proteins, which in turn facilitate the binding of translation initiation factors to the 5' end of mRNA.

- The length of the 3' poly(A) tail therefore determines if the initiation of translation is possible. Translation can only take place if the length of the 3' poly(A) tail is sufficient for translation to be initiated.

    - In the egg cell of many organisms, 3' poly(A) tails of stored mRNAs lack sufficient length to allow initiation of translation.

    - At appropriate time during embryonic development, cytoplasmic enzymes add more adenine nucleotides to allow translation to be initiated.

    - Conversely, translation initiation is prevented when cytoplasmic enzymes remove adenine nucleotides from the 3' poly(A) tails.

5.4.3 Polyribosomes

- Once a ribosome moves past the start codon, a second ribosome can attach to the mRNA, resulting in a number of ribosomes trailing along the mRNA called **polyribosomes** or polysomes (Fig 5.25).

- Polyribosomes are found in both bacterial and eukaryotic cells.

- Multiple ribosomes can translate an mRNA at the same time to make multiple copies of a polypeptide very quickly.

- They enable a cell to **increase the rate of gene expression** at the translational stage.

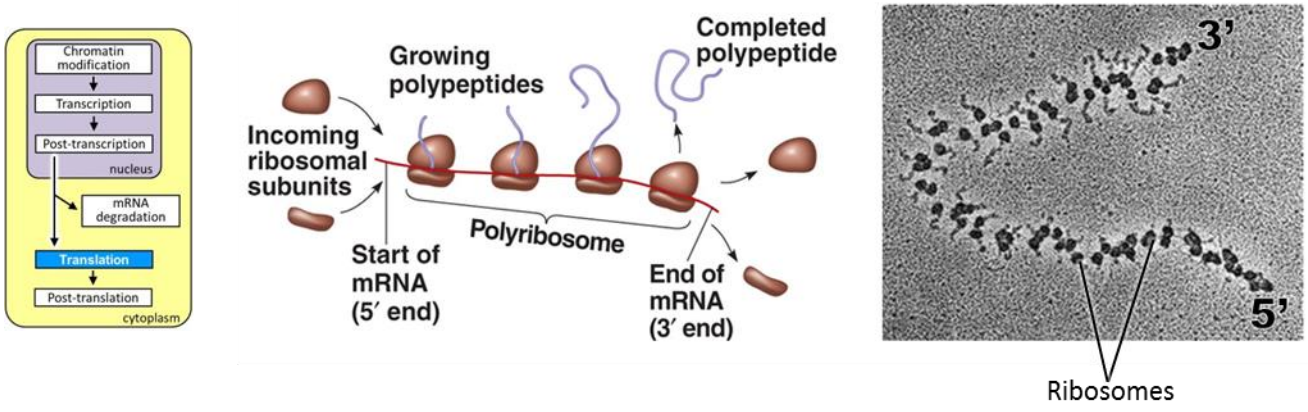


Fig. 5.25: (Left) Diagrammatic representation of a polyribosome.
(Right) Electron micrograph of a polyribosome.

5.5 Control at the post-translational level

- The final opportunities for controlling gene expression occur **after translation**.
- Many of these modifications occur extremely rapidly compared to the time required at other levels of control.

5.5.1 Protein modifications

- Regulation of gene expression might occur at **any of the steps** involved in eukaryotic polypeptide processing **to yield functional protein** or in transporting a protein to the Golgi apparatus such as the following:

1. **Cleavage of methionine** (first amino acid) in some proteins.
2. **Cleavage of polypeptide** to form the functional/active protein (Fig. 5.26).
3. Many proteins undergo **biochemical/covalent modifications** to become functional through:
   - ❖ **Phosphorylation** – addition of phosphate group
   - ❖ **Glycosylation** – addition of one or more sugar monomers (e.g. ABO antigens)
4. Cell surface proteins and many others must also be **transported to target destinations in the cell in order to function** (Fig. 5.27). For example, some of the membrane proteins found in the inner mitochondrial membrane are encoded by the genes in the nucleus. Once synthesized, they must be transported into the mitochondria and be embedded in the inner mitochondrial membrane.

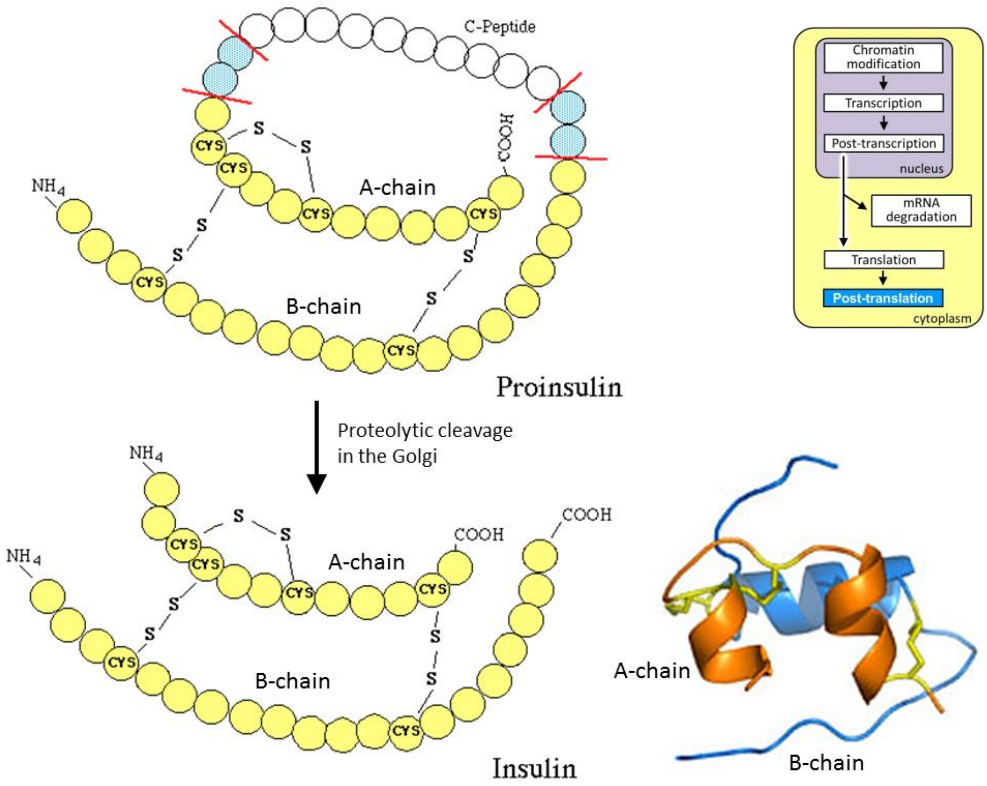


Fig. 5.26: Proinsulin formed in the RER undergoes proteolysis cleavage in the Golgi to form the functional insulin, which comprises of two chains linked via disulphide bonds.

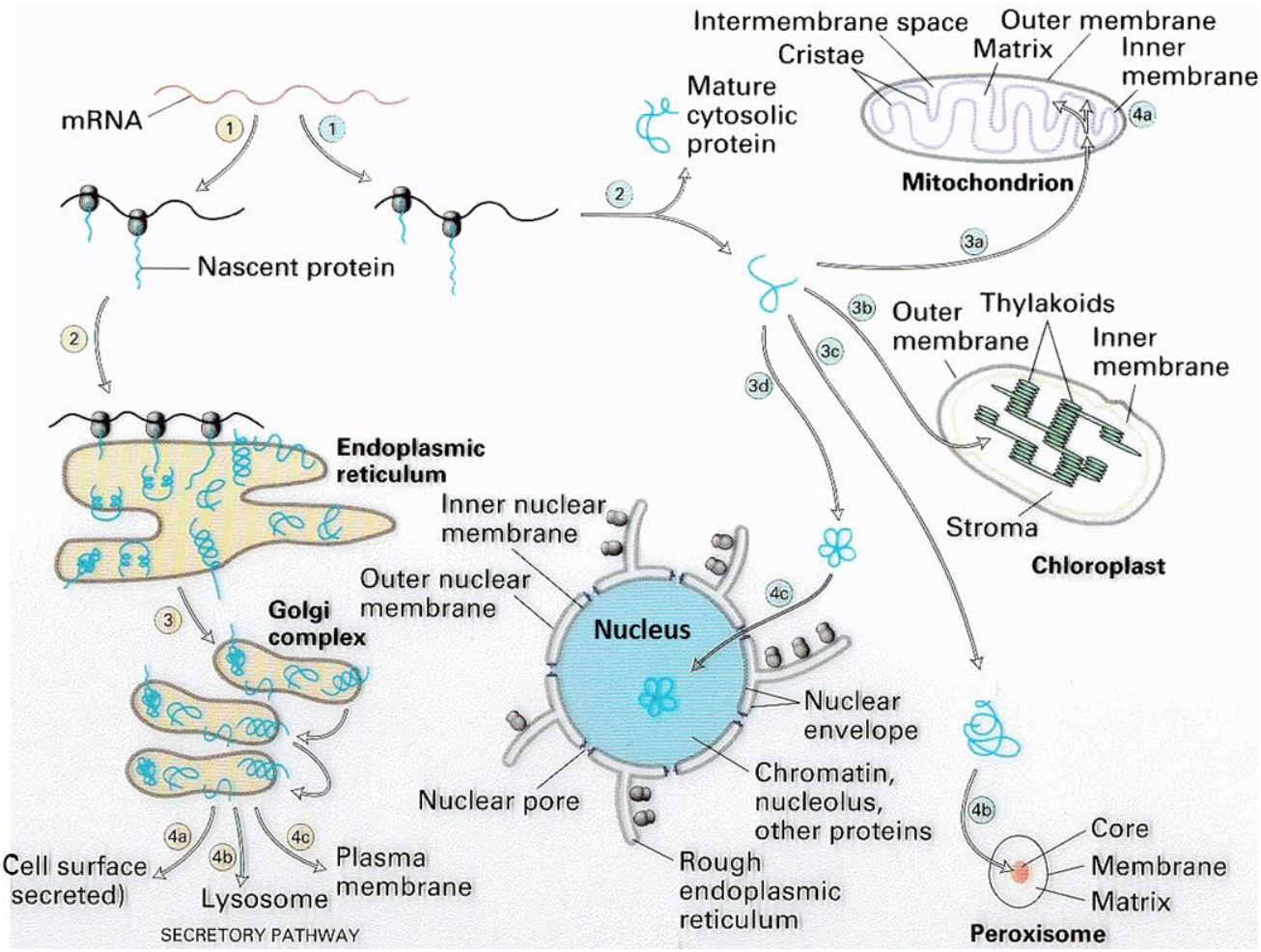


Fig. 5.27: Regardless of whether the protein is synthesized on the RER or in the cytosol, they must be transported to their target destination in order to function.

5.5.2 Protein degradation

- The length of time each protein functions in the cell is strictly regulated by selective degradation.
- Proteins intended for degradation are marked by the covalent attachment of small protein molecules (76 amino acids long) called **ubiquitin**.
- For **cytosolic proteins,** large protein complex called **proteasome** recognize the ubiquitin and degrade the tagged cytosolic protein (Fig. 5.28).
- For **membrane proteins** (e.g. ion channels), ubiquitination triggers **endocytosis** and the sorting of the proteins into **multivesicular bodies**, which then **fuse with lysosomes** that degrade the membrane proteins (Fig. 5.29).

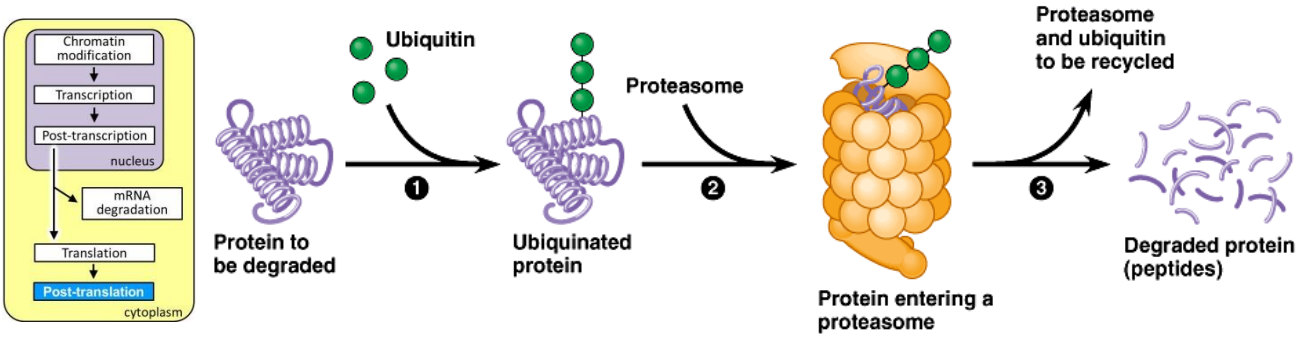


Fig. 5.28: Ubiquitin-dependent protein degradation in the proteasome

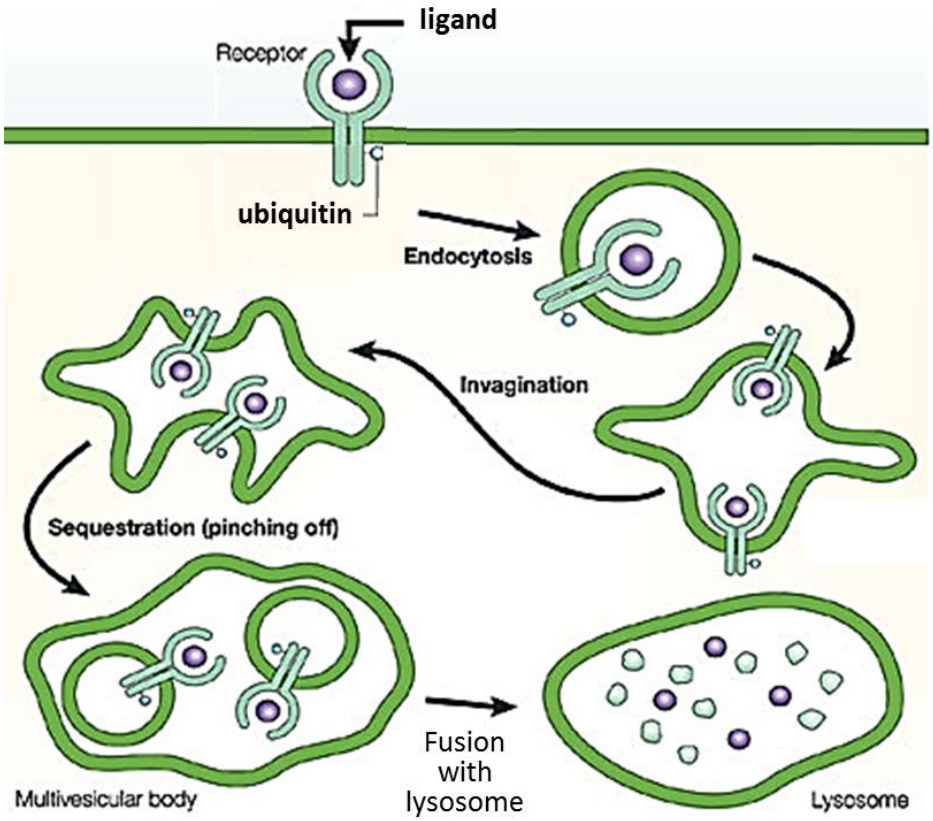


Fig. 5.29: Lysosome-dependent degradation of ubiquitin-tagged membrane proteins

- Protein degradation is affected by several features.

(1) *Presence of PEST sequence*

- Some proteins contain a region rich in proline, glutamic acid, serine and threonine (PEST). These regions are often recognized and tagged with ubiquitin. In proteins where the PEST region is removed, the protein's half-life is extended many times.

(2) *Type of amino acid at the N-terminal*

- Even though the first amino acid to be added to all protein is methionine, it is usually removed during post-translational modification, thereby exposing the next amino acid in line.
- The half-life of the protein depends on the type of residue at the N-terminal.
- When the same protein is modified to carry different amino acid at its N-terminal, its half-life is greatly affected.

Residue	**Half-life**	**Residue**	**Half-life**	**Residue**	**Half-life**	**Residue**	**Half-life**
Valine	100h	Leucine	5.5h	Serine	1.9h	Phenylalanine	1.1h
Methionine	30h	Alanine	4.4h	Asparagine	1.4h	Glutamic acid	1.0h
Isoleucine	20h	Histidine	3.5h	Lysine	1.3h	Arginine	1.0h
Proline	20h	Tryptophan	2.8h	Cysteine	1.2h	Glutamine	0.8h
Threonine	7.2h	Tyrosine	2.8h	Aspartic acid	1.1h		

(3) *Exposure of signals buried in the hydrophobic core*

- Signals for degradation may also be buried in the hydrophobic core. This is why partially folded or abnormal, mutant proteins may be prone to degradation. When such proteins exist in their normal state, the signals are hidden and the protein is thus long-lived.

6. Summary

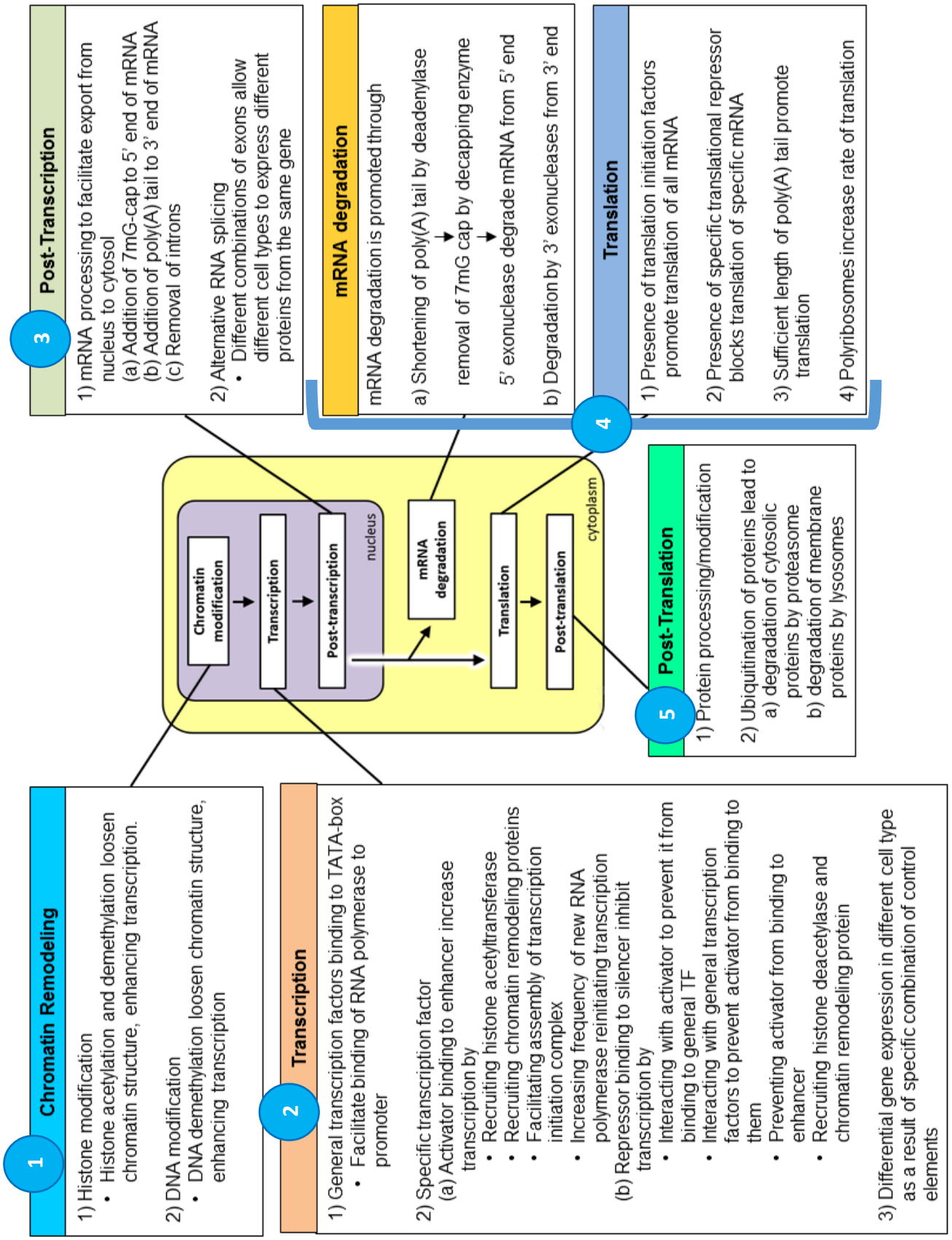


Fig. 6.1: A summary on the various stages at which gene expression can be controlled.
(Indicate whether the mechanisms summarized allowed for gene expression to occur. If yes, indicate if it increases or decreases the rate of transcription)

III. Stem Cells

You have learnt in Section I and II that the various differentiated cells take on different structures and functions due to the different sets of genes that are expressed (differential gene expression), producing different sets of proteins which allow cells to carry out their specialised functions. *(Refer to Sections 4.1 – 4.2)*.

The various types of differentiated cells are derived though a series of **mitosis** and **differentiation** process, from a fertilized egg (zygote). This is possible because different sets of genes are switched on and off during embryonic development to give rise to different cell types of different functions. And in this process of embryonic development, a unique group of unspecialised cells, known as stem cells can be obtained (Fig, 7.1).

In this section on stem cells, we will learn how stem cells obtained from various developmental stages are different, how **differential gene expression** (spatial and temporal) is crucial to development, and how each type of stem cell can be used for therapeutic purposes.

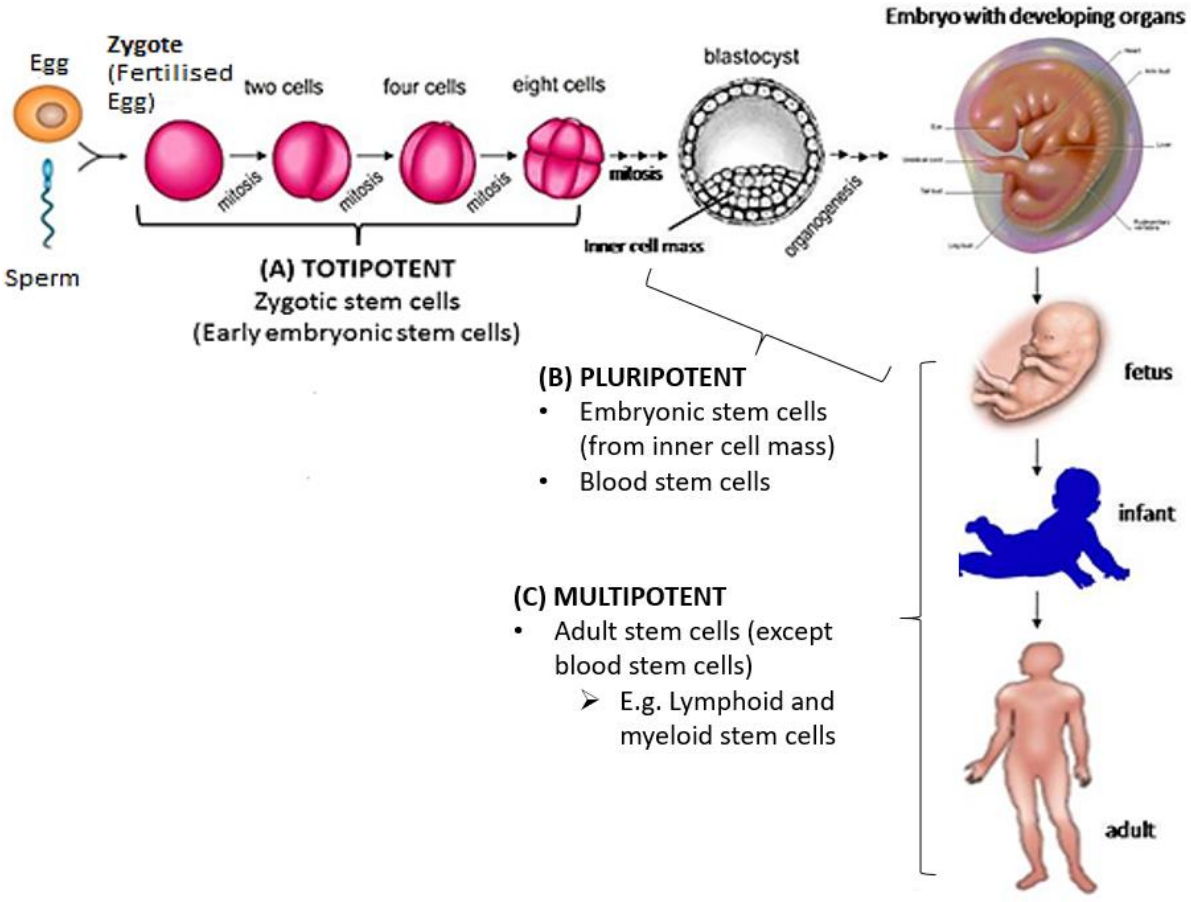


Fig. 7.1: The development of zygote to whole organism (human) and the different types of stem cells obtained from the different developmental stages

7. Stem Cells

- A stem cell is an **unspecialised cell** that can **divide and self-renew indefinitely** and has the **ability to differentiate into specialised cells** under **appropriate conditions** (Fig. 7.2).

- Thus stem cells are able to and must accomplish the dual task of self-renewal (replenish their own population) and the generation of specialised cells (e.g. red blood cells).

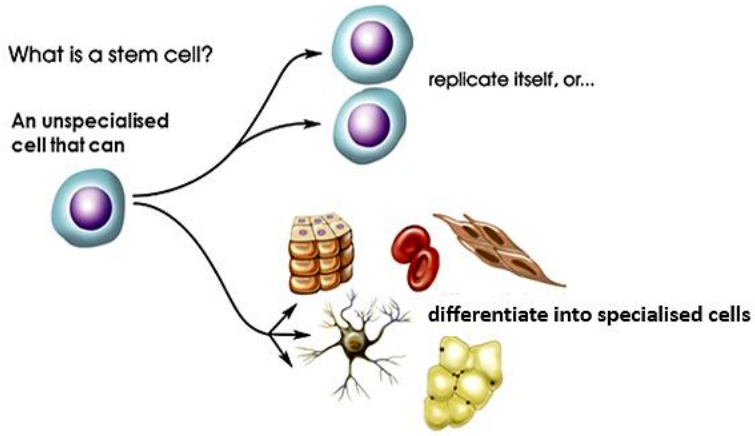


Fig. 7.2: Stem cells can either replicate itself or give rise to specialised cells.

7.1 Unique features of stem cells

1. **Unspecialised**

    - There is an **absence of tissue-specific structures** that allow it to perform specialised functions.

2. Can **continually divide and self-renew** (though cell division) for a long period of time.

    - Stem cells are able to **proliferate** (replicate many times), unlike normal cells.

    - The division of stem cells can be symmetrical or asymmetrical (Fig. 7.3):

        a) Symmetrical cell division: A stem cell can divide to generate either
        - ➢ two daughter cells that remain as stem cells, or
        - ➢ two daughter cells that are committed to differentiate into a specialised cell.

        b) Asymmetrical cell division: A stem cell can divide to generate one daughter cell that remains as a stem cell and one daughter cell that is committed to differentiate into a specialised cell.

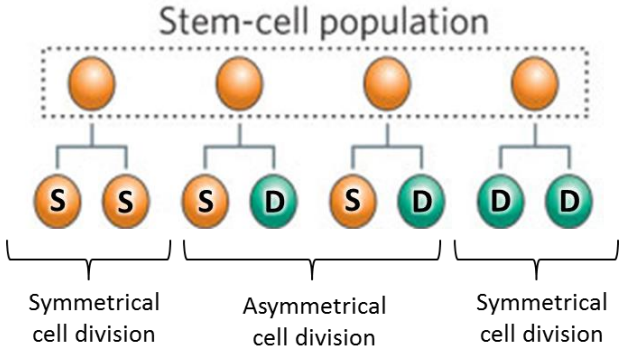


Fig. 7.3: Symmetrical and asymmetrical cell division.

3. Have the **ability to differentiate into specialised cell types under appropriate conditions**, triggered by intracellular and extracellular signals.

   - The intracellular signals include:
     - **Cytoplasmic factors (proteins)** encoded by **genes** in the genome of the cell. These factors send signals to the nucleus via **transcription factors** to turn on and off various genes.

   - The extracellular signals (Fig. 7.4A and 7.4B) are:
     - **Chemical molecules/growth factors** surrounding a cell, which may be secreted by neighbouring cells.
     - **Physical contact** with a neighbouring cells.

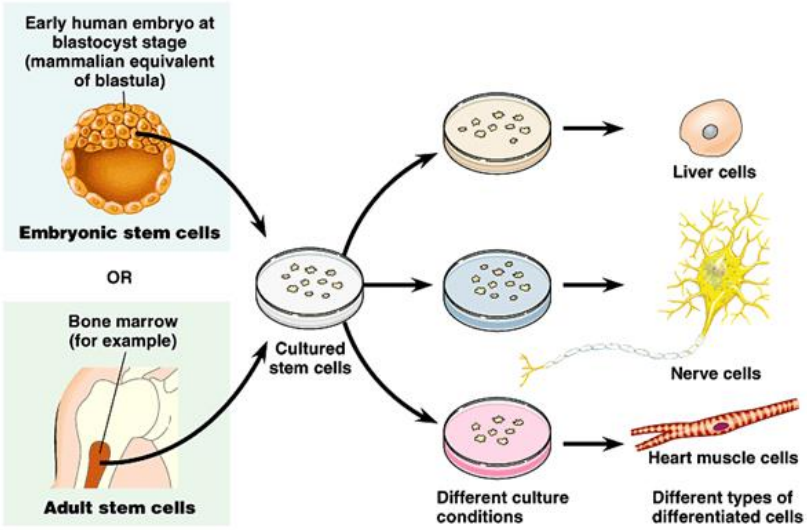


Fig. 7.4A: By varying the conditions of the cell cultures, stem cells can be coaxed to differentiate into different types of specialised cells *(in vitro).*

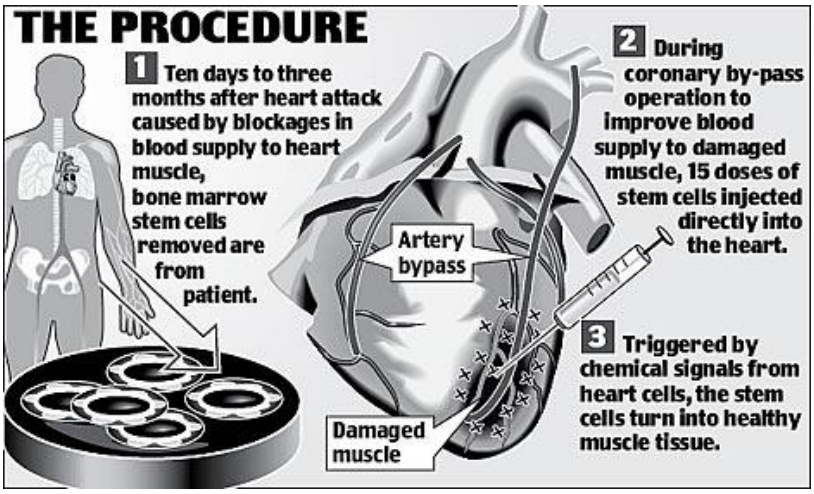
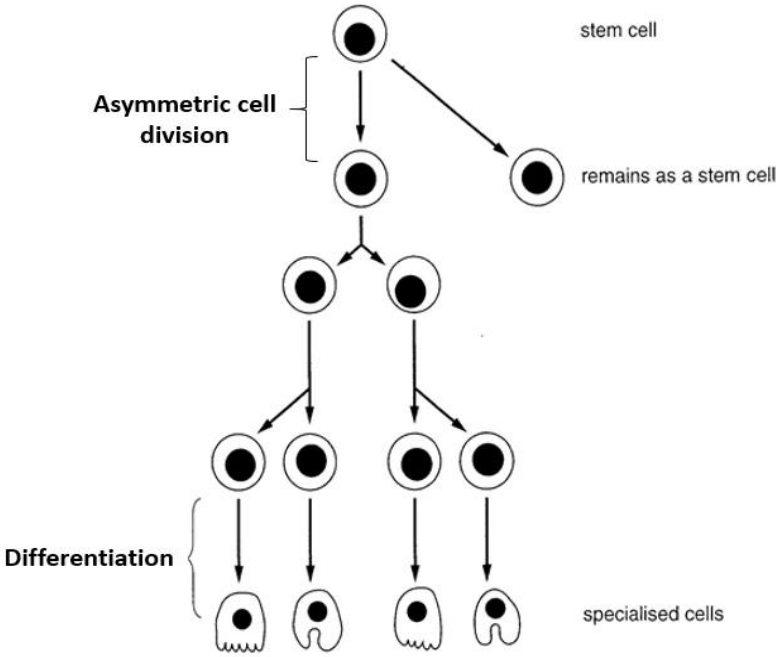


Fig. 7.4B: Using stem cells derived from patient's bone marrow to repair patient's heart – due to extracellular chemical signals secreted by the neighboring heart cells, the stem cells are coaxed to differentiate into healthy heart muscle tissues *(in vivo).*

STOP
Check With Guard Before Proceeding

The figure below shows a stem cell dividing asymmetrically, where one daughter cell remains as a stem cell and one daughter cell is committed to differentiate into a specialized cell.



1. Name 3 unique features of stem cells.

2. Are specialised cells genetically identical to the stem cells from which they originated from?

3. Outline **how** an embryonic stem cell (pluripotent) gives rise to a neuron (specialized cell) in the body. [Recall: OCGE]

7.2 Developmental potential of stem cells

- All stem cells have the **complete set of genetic information (genes)**.

- However, due to **differential gene expression**, different sets of genes are expressed during different stages of embryonic development.

    - For example, genes that are switched on during the 4-cell stage during embryonic development allow the cells in that stage to be totipotent (*Section 7.2.1)*. This set of genes that maintain totipotency however, are switched off during the blastocyst stage where the cells are pluripotent (*Section 7.2.2*). At the blastocyst stage, another set of genes that maintain pluripotency is switched on instead.

- Hence, stem cells derived from the different stages of embryonic development have different levels of **developmental potential**, namely
    - Totipotent (*Section 7.2.1*),
    - Pluripotent (*Section 7.2.2*) or
    - Multipotent (*Section 7.2.3*).

- Changes in development potential occur as the zygote develops to form the whole organism (Fig. 7.5).

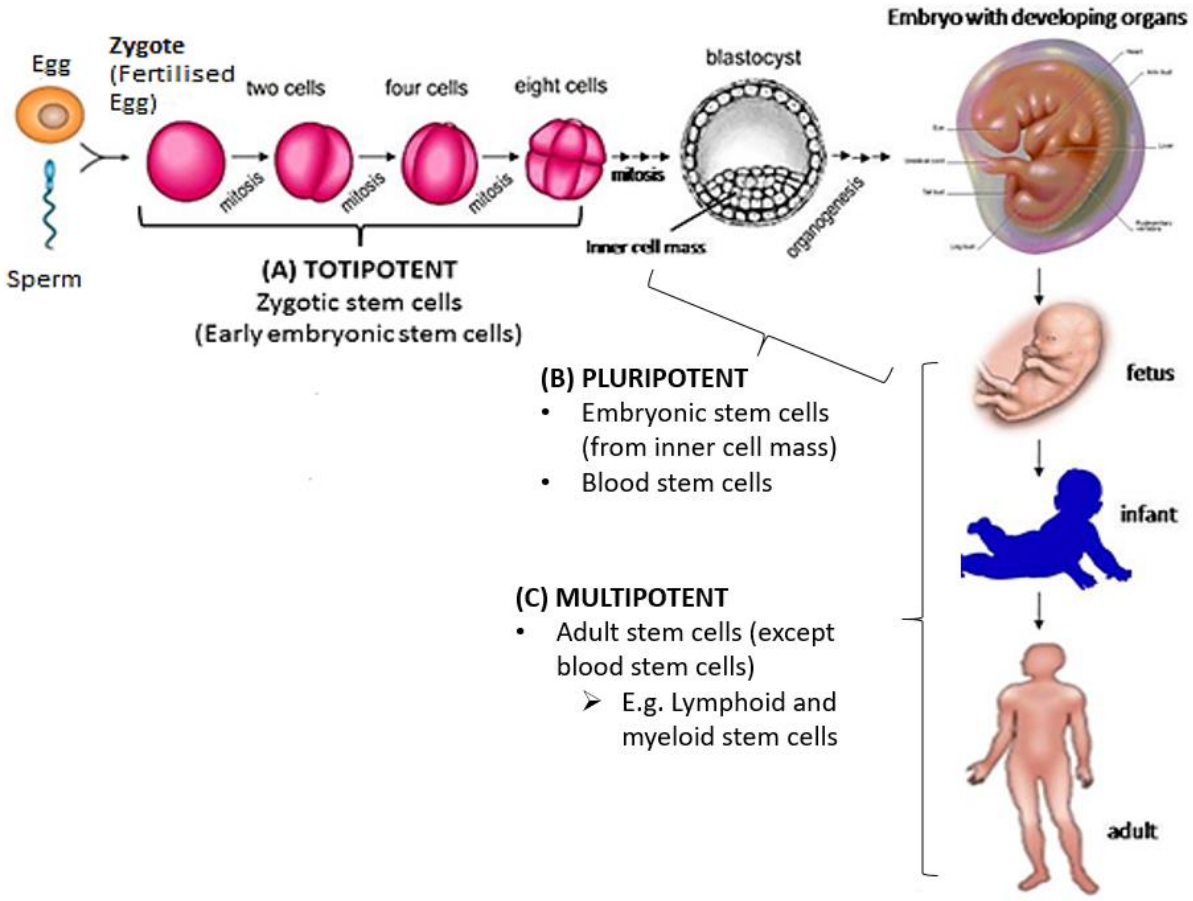


Fig. 7.5: Different types of stem cells with different developmental potential can be obtained from different developmental stages of human.

7.2.1 Totipotent stem cells

- Totipotent stem cells have the **ability to differentiate into any cell type** in the adult body, **including cells of the extraembryonic membranes** (E.g. placenta) (Fig. 7.6). Hence each cell has the ability to develop into the **whole organism.**

- They are **both pluripotent and multipotent**.

- Examples of totipotent stem cells (Fig. 7.6):

    - **Zygotic stem cells (<u>Early</u> embryonic stem cells)** → obtained from the zygote (fertilised egg), 2-cell, 4-cell to 8-cell mammalian embryo

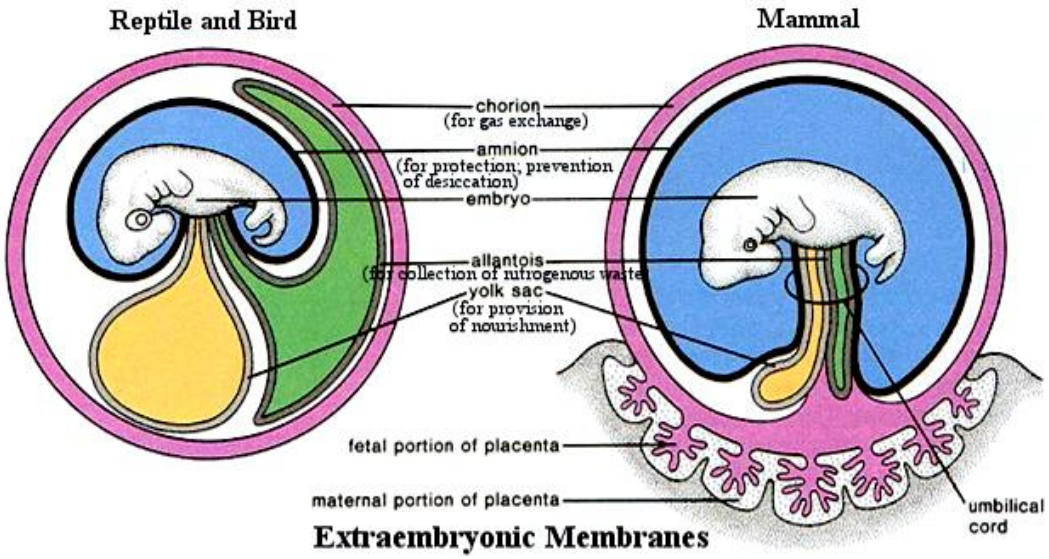


Fig. 7.6: Extraembryonic membranes are membranous structures that appear in parallel with the embryo in the embryonic development, which includes the chorion, amnion, allantois, yolk sac, and the placenta. They form from the embryo but do not become part of the individual after its birth.

7.2.2 Pluripotent stem cells

- Pluripotent stem cells have the **ability to differentiate into all cell types** in the adult body, **but not cells of the extraembryonic membranes.**
- They are multipotent but not totipotent.
- Pluripotent stem cells are isolated from **embryonic tissues** and can be **grown in culture**, but require special methods to prevent them from differentiating to specialised cells.
- Examples of pluripotent stem cells:
    - **Embryonic stem (ES) cells** → obtained from the **inner cell mass** of **blastocyst** (Fig. 7.5 and 7.6) *[Refer to Section 7.3.1 for more details]*
    - **Embryonic germ (EG) cells** → found in precursor of the gonads in aborted fetuses (Fig. 7.7).
    - **Embryonal carcinoma (EC) cells** → obtained from teratocarcinomas (a tumor that occasionally occurs in a gonad of a fetus that has an abnormal number of chromosomes, or from a rare germ cell tumour that occurs in the ovaries and testes) (Fig. 7.7).
    - **Hematopoietic stem cells** (*aka* **blood stem cells**) *[Refer to Section 7.3.2 for more details]*

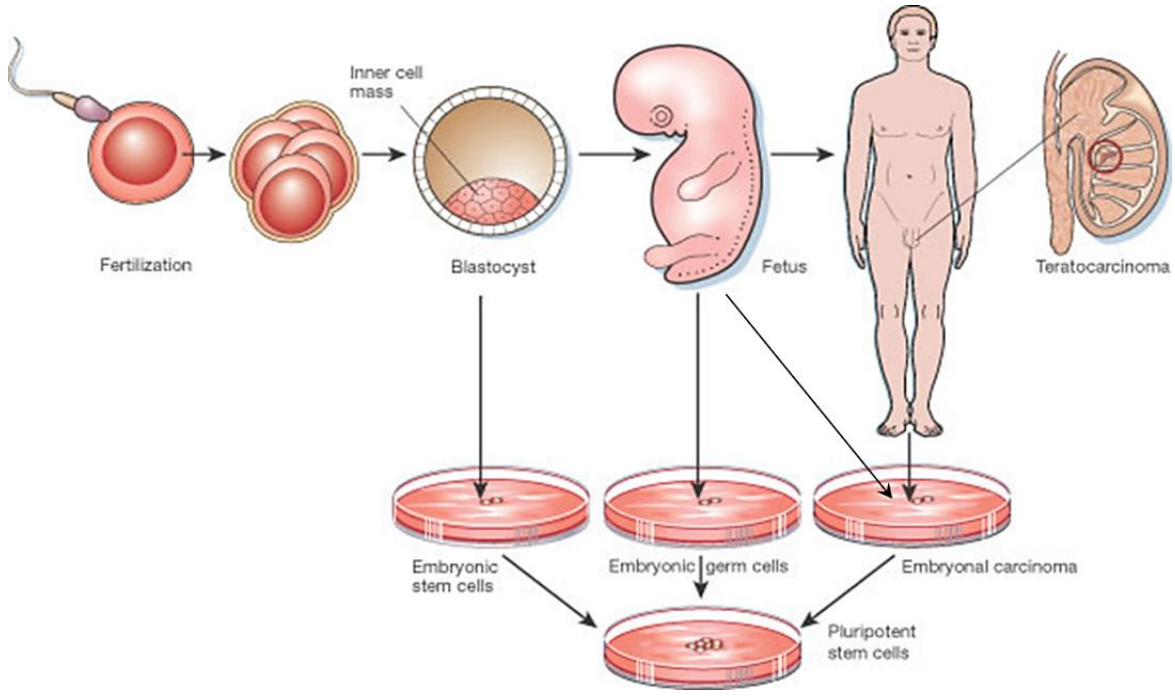
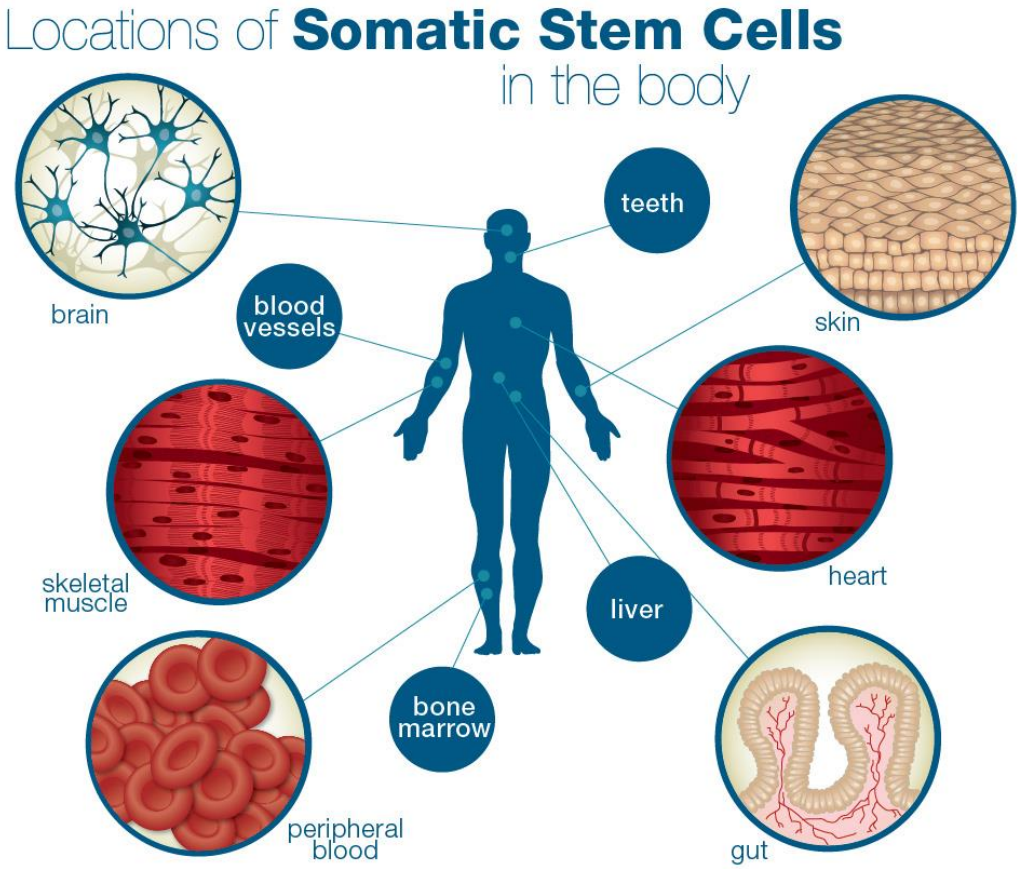


Fig. 7.7: Pluripotent stem cells from different developmental stages in human.

7.2.3 Multipotent stem cells

- Multipotent stem cells have the **ability to differentiate into a limited range of cell types**.
- They are **neither totipotent nor pluripotent**.
- In the body, they function to **replace** dead, damaged or worn out cells (normal function).
- Examples of multipotent stem cells:
    - **Adult stem cells** (except blood stem cells) → found in **most organs** of the body (Fig. 7.8)
        - E.g. lymphoid and myeloid stem cells *[Refer to Section 7.3.2 for more details]*



Adapted from: learn.genetics.utah.edu

Fig. 7.8: Example of locations where adult stem cells (*aka* somatic stem cells) can be obtained from the human body.

Summing up the 3 developmental potential of stem cells (Fig. 7.9)

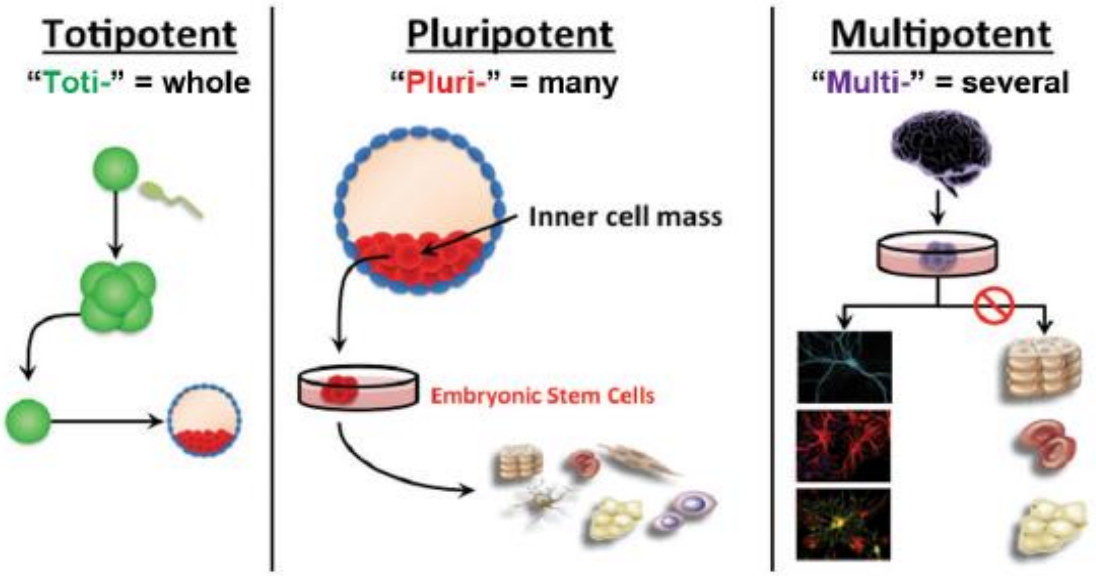


Fig. 7.9: Picture illustration of the developmental potential of stem cells

STOP
Check With Guard Before Proceeding

Test your understanding on the developmental potential of stem cells

Fill in the blanks in the table below.

	Stem cell types	Development potential	Examples
Decreasing level of developmental potential →			
	multipotent	Limited range of cell types	Adult stem cells (except blood stem cells) → e.g. lymphoid and myeloid stem cells

7.3 Types of human stem cells and their normal function in living organisms

- Fig. 7.10 below shows the various types of human stem cells. The developmental potential of the stem cells depends on when it was obtained during the developmental stages of the human.

- Major types of stem cells that are extensively studied and researched upon are:
  - Zygotic stem cells (*Section 7.3.1*)
  - Embryonic Stem Cells *(Section 7.3.1)*
  - Adult Stem Cells *(Section 7.3.2)*

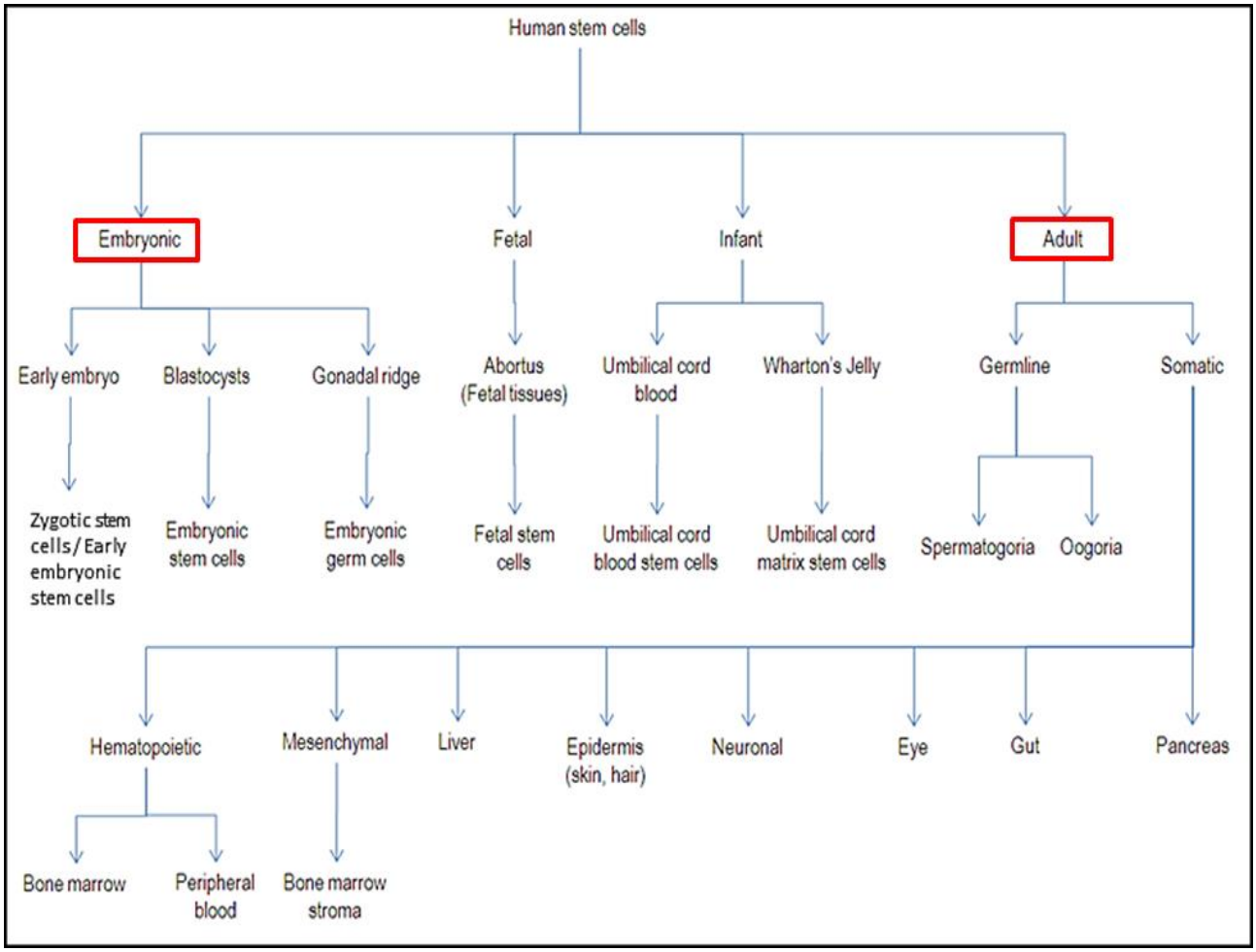


Fig. 7.10: Types of human stem cells

7.3.1 Embryonic stem cells

(A) Types of embryonic stem cells, characteristics and normal function

There are two types of embryonic stem cells:

(1) **Zygotic stem cells** (*a.k.a* **<u>Early</u> embryonic stem cells**)

- Derived from **zygote** and the **first 8 cells after cleavage** (Fig. 7.5).
- They are **totipotent.**
- **Function**:
  - ❖ Have the **ability to differentiate into all cell types** in the body of multicellular organisms, **including the cells of the extra-embryonic membranes** (*Section 7.2.1*).

(2) **Embryonic stem cells** (*a.k.a* **blastocyst stem cells**)

- Obtained from the **inner cell mass of the blastocyst** (Fig. 7.11A).
- They are **pluripotent**.
- **Function**:
  - ❖ Have the **ability to differentiate into all cell types** in the body of an organism **except cells of the extraembryonic membranes.**

    This is possible because the embryonic stem cells will give rise to cells in all **three germ layers –** ectoderm, mesoderm and endoderm. These germ layers are layers in an animal embryo, from which the tissues and organs of the body develop (Fig. 7.11B).

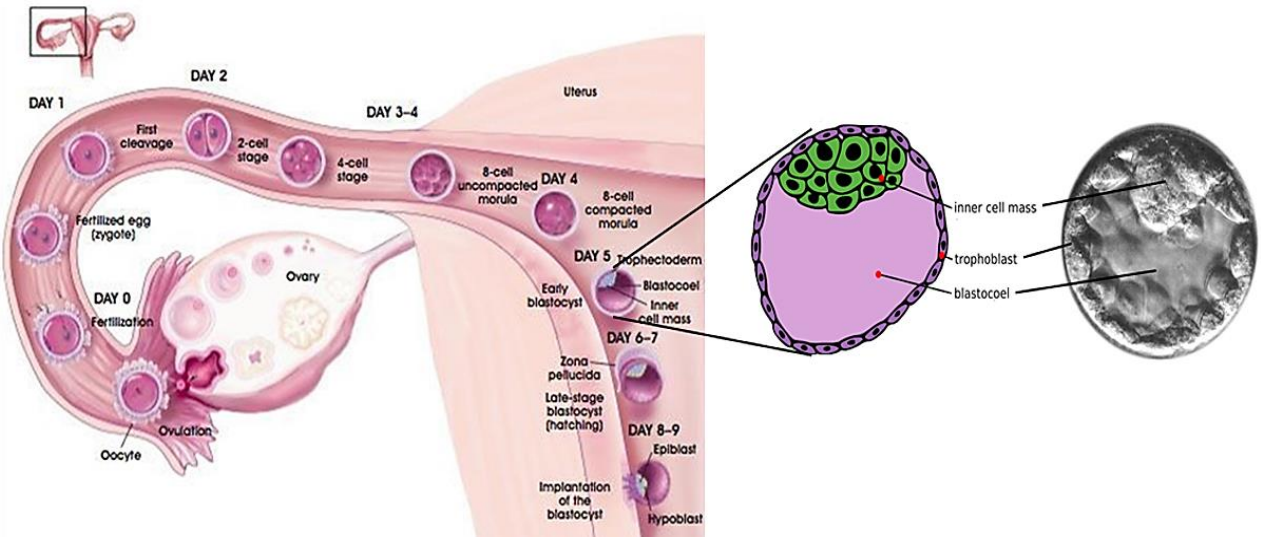


Fig. 7.11A: A blastocyst is a ball of cells produced 4 to 5 days after fertilization. The blastocyst is made up of the trophoblast (outer layer of cells that eventually forms the placenta), blastocoel (hollow cavity), and the inner cell mass (group of approximately 20 cells at one end of the blastocoel that develops into the embryo, and ultimately the fetus).

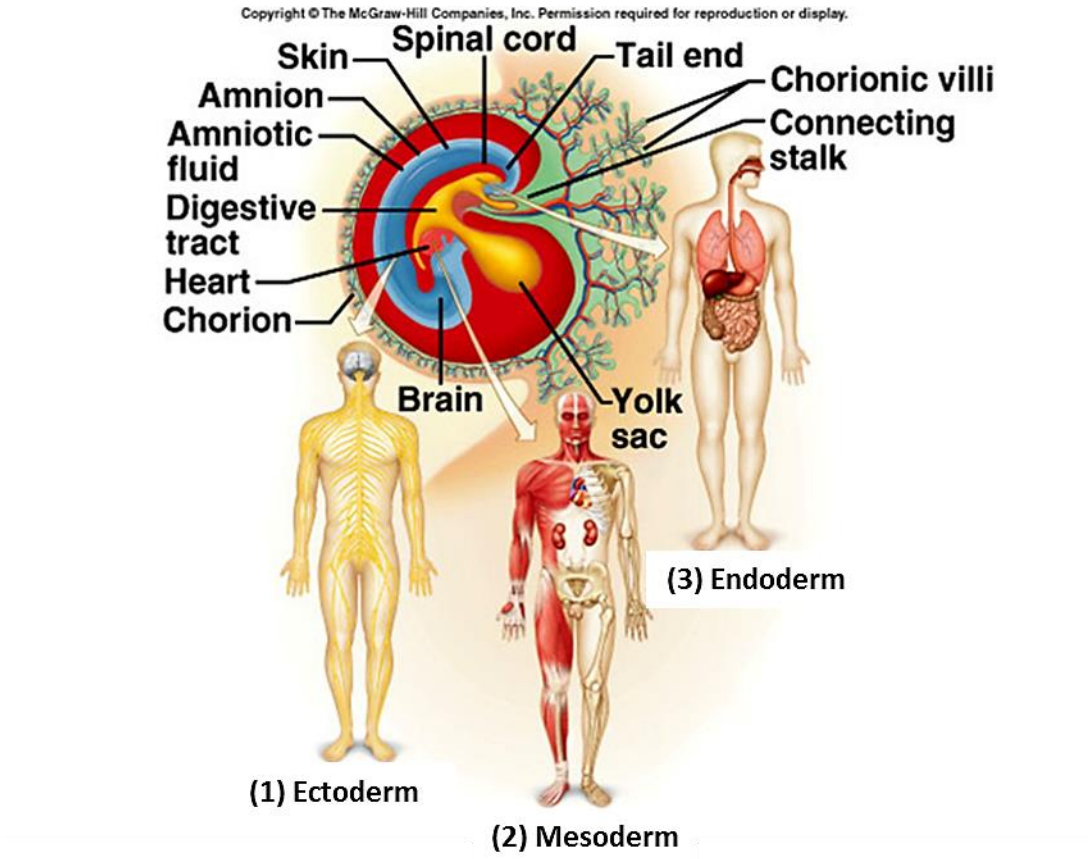
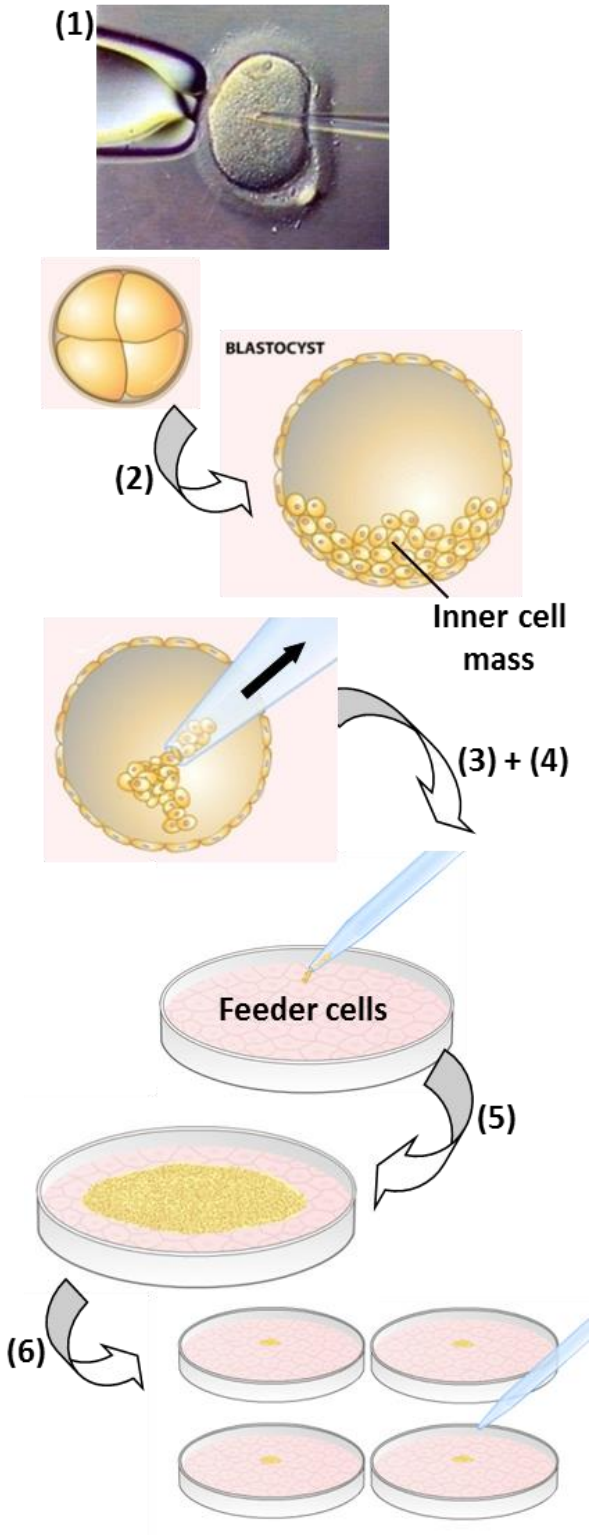


Fig. 7.11B: The blastocyst stem cells give rise to cells in the three germ layers.
(1) Ectoderm (external layer) gives rise to epidermal cells in the skin and the nervous system.
(2) Mesoderm (middle layer) gives rise to organs e.g. heart, connective tissues and blood cells.
(3) Endoderm (internal layer) gives rise to lining of digestive tract and its associated organs (e.g. pancreatic cells).

(B) Culturing embryonic stem cells (blastocyst stem cells)



(1) The ovum undergoes ***in vitro*** **fertilisation** with the sperm to form a **zygote**, which is transferred to a culture dish that contains a nutrient medium.

(2) On the petri dish, the zygote divides to form a **blastocyst.**

(3) The **inner cell mass** of the **blastocyst** is transferred into another **culture dish.**

(4) The inner surface of the culture dish is typically coated with a layer of mouse embryonic connective tissue cells (**feeder cells / feeder layer**) that have been treated.

The treated feeder cells provide the inner cell mass with a sticky surface for them to attach to, and release important **nutrients** and hormones into the **culture medium**. **Growth factors** that produce desired changes in the cells are added to the culture medium.

(5) Over the course of several days, the cells of the inner cell mass **proliferate.**

(6) Subculturing (replating some/all cells into fresh culture medium) is carried out. This serves to prolong the life and/or expand the number of cells in culture.

*Embryonic stem cells that have proliferated in the cell culture for six or more months without differentiating, and appear genetically normal, are referred to as an **embryonic stem cell line**.

Cell line consists of cells that can be maintained and grown in a dish outside of the body.

(C) Stimulating embryonic stem cells to differentiate into specialised cells

- Once embryonic stem (ES) cells are allowed to **clump together** to form **embryoid bodies** (aggregates of cells derived from ES cells)**,** they begin to **differentiate into specialised cells spontaneously**.

- **Chemical composition of the culture medium** has to be **changed** to **direct** the process of **differentiation** (Fig. 7.12).

- **Different growth factors** can be added to generate cultures of specific types of specialised cells.

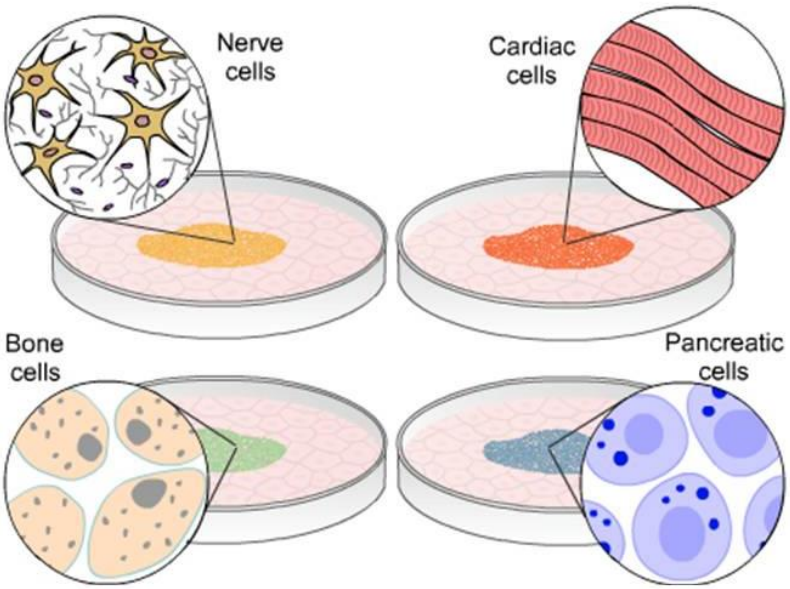


Fig.7.12: Under appropriate culture conditions, these ES cells divide or "self-renew", and the cell mass grows. By adding appropriate signaling molecules into the culture medium, the ES cells can be coaxed to differentiate into specific specialised cell types.

7.3.2 Adult stem cells

(A) Characteristics and normal functions of adult stem cells

- In a fully-developed individual, an adult stem cell is an **undifferentiated cell** found among differentiated cells in a **tissue or organ**, which can **renew** itself or **differentiate into specialised cell types** of a tissue or organ.

- They are **multipotent** (*except* blood stem cells which are pluripotent).

- **Normal function**:

  ❖ The primary role of adult stem cells in living organism is to **maintain and replace** dead, damaged or worn out cells in the tissue in which they are found.

  They reside in a specific area of each tissue called a "**niche**", and they exist in a **very small number** in each tissue. They remain quiescent (non-dividing) for many years. However, when **activated by disease or tissue injury**, they can differentiate into specialised cell types of the tissue in which they reside.

- They exhibit **plasticity** and are capable of **transdifferentiation** under certain conditions (Section 7.3.2C).

- If adult stem cells accumulate sufficient mutations, they may produce a clone of cancer cells.

(B) Examples of adult stem cells (1 – 5)

(1) Hematopoietic stem cells (*aka* **blood stem cells**) (Fig. 7.13)

  - Found in **bone marrow** and **umbilical cord blood.**

  - Function – constantly divide and differentiate into specialised cells to **replace all worn out blood cell types** in the body (Table 2). For e.g., the lifespan of an erythrocyte is 3 months.

Table 2: The various types of blood cells and their respective functions

Type of cell			Main Function	Typical conc. in human blood (cells per litre)
Erythrocytes (Red blood cells)			Transport O_2 and CO_2	5×10^{12}
Leucocytes (White blood cells)	Granulocytes	Neutrophils	Engulf & destroy invading bacteria	5×10^{9}
		Eosinophils	Destroy larger parasites & modulate allergic inflammatory responses	2×10^{8}
		Basophils	Release histamine in certain immune reactions	4×10^{7}
	Lymphocytes	B cells	Make antibodies	2×10^{9}
		T cells	Kill virus-infected cells & regulate activities of other leucocytes	1×10^{9}
	Monocytes		Become tissue macrophages which engulf & digest invading microorganisms, foreign bodies and damaged senescent cells	4×10^{8}
	Natural killer (NK) cells		Kill virus-infected cells and some tumour cells	1×10^{8}
Platelets (cell fragments arising of megakaryocytes in bone marrow)			Initiate blood clotting	3×10^{11}

- Recent research suggests that blood stem cells can migrate from bone marrow to various organs (E.g. liver) and give rise to hepatocytes (liver cells), demonstrating **pluripotency** (instead of multipotency).

- Hematopoiesis is the production of blood cell types. When hematopoiesis occurs, blood stem cells first divide to form the myeloid stem cells (*aka* myeloid progenitor cells) and lymphoid stem cells (*aka* lymphoid progenitor cells), which further differentiate into all types of blood cells.

  - **Myeloid stem cells** are **multipotent.** Its normal function is to differentiate into erythrocytes, neutrophils, basophils, eosinophils, monocyte/macrophages, and platelets.

  - **Lymphoid stem cells** are **multipotent**. Its normal function is to differentiate into natural killer cells, B lymphocytes and T lymphocytes.

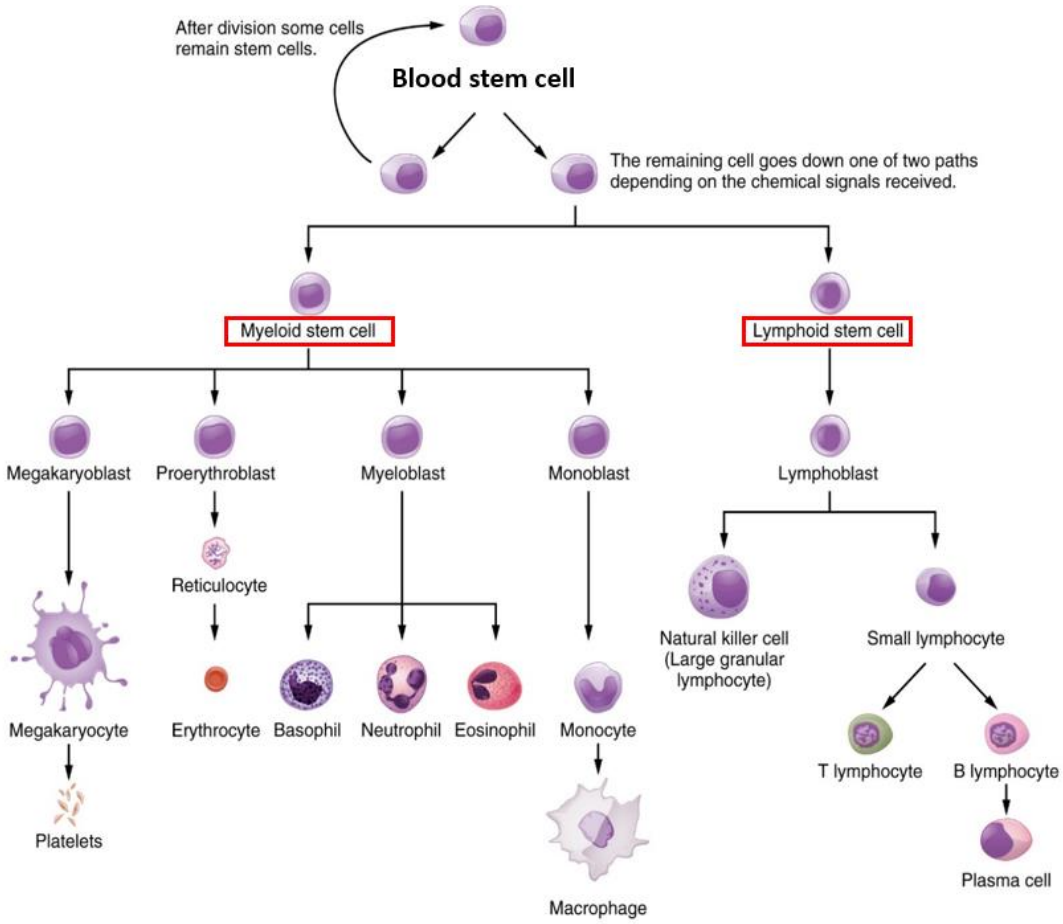


Fig. 7.13: Blood stem cells first divide to form the myeloid stem cells and lymphoid stem cells, which then differentiates into various blood cell types.

OTHER EXAMPLES OF ADULT STEM CELLS (FYI only)

(2) Bone marrow stromal cells (*aka* **mesenchymal stem cells**) (Fig. 7.14)

- Found in **bone marrow** and **umbilical cord blood and tissue.**

- Give rise to a variety of cell types:
  - ❖ Bone cells (osteocytes)
  - ❖ Cartilage cells (chondrocytes)
  - ❖ Fat cells (adipocytes)
  - ❖ Other kinds of connective tissue cells such as those in tendons

- Function – constantly divide and differentiate into specialized cells (as above) that are needed for **growth of bones and cartilage** during childhood and puberty.

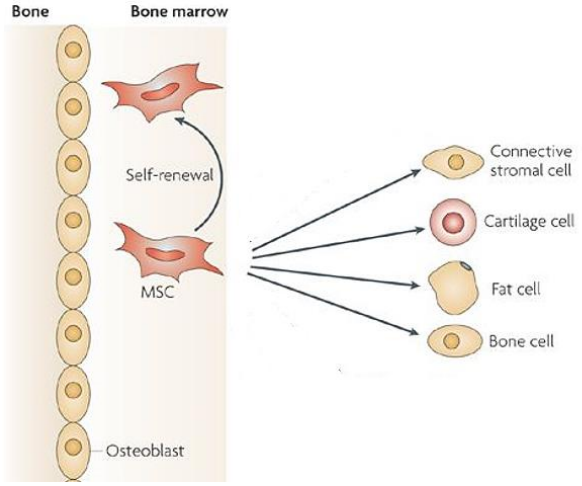


Fig. 7.14: Mesenchymal stem cells (MSC) gives rise to various specialised cell types.

(3) Neural stem cells (Fig. 7.15)

- Found in the **brain**.

- Give rise to 3 cell types:
  - ❖ Neurons (nerve cells) – Function to transmit nerve impulses. They stop dividing soon after birth.
  - ❖ Astrocytes (non-neuronal cell) – Star-shaped cells in the brain and spinal cord. They perform many functions, including the provision of physical support and nutrients to the nervous tissues.
  - ❖ Oligodendrocytes (non-neuronal cell) – Function to insulate the axons in the Central Nervous System, forming the myelin sheath.

- Function – divide and differentiate into specialised cells (as above) required for the **development of the nervous system** in developing mammals, as well as for **repair during brain injury.**

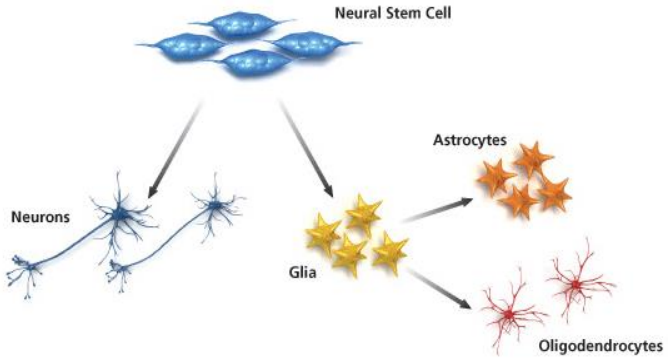


Fig. 7.15: Neural stem cells give rise to a variety of brain cells.

(4) Epithelial stem cells (Fig. 7.16)

- Found in the **lining** of the **digestive tract.**

- Give rise to the following cell types:
  - ❖ Absorptive cells – Cells on the surface of villi of the small intestine. They have microvilli to increase surface area to volume ratio for absorption of nutrients.
  - ❖ Goblet cells – Cells that are specialized for secretion of mucus in the intestines.
  - ❖ Paneth cells – Cells that secretes anti-bacterial proteins into the lumen, providing protection for the stem cells which line the intestinal walls.
  - ❖ Enteroendocrine cells – Cells that secrete a particular hormone that influences gastrointestinal secretion.

- Function – constantly divide and differentiate into specialized cells (as above) to **replace the lining of digestive tract**.

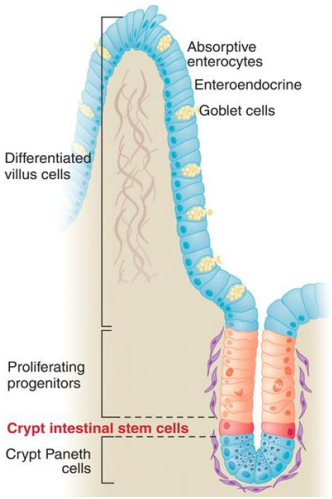


Fig. 7.16: Epithelial stem cells (crypt intestinal stem cells) give rise to various specialised cell types.

(5) Skin stem cells (Fig. 7.17)

- Found in the **basal layer** of the **epidermis** and at the **base** of **hair follicles**

- Function – constantly divide and differentiate to **replace cells of the epidermal layer**.
  - ❖ Epidermal stem cells - give rise to keratinocytes, which migrate to the surface of the skin and form a protective layer.
  - ❖ Follicular stem cells - give rise to both the hair follicle and to the epidermis.

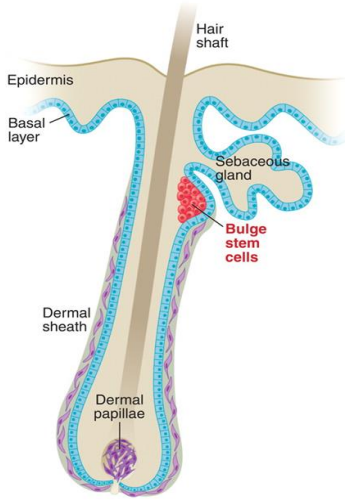


Fig. 7.17: Most follicular stem cells reside in an area called the bulge and give rise hair follicle and epidermis.

(C) Transdifferentiation of adult stem cells

- Transdifferentiation is the **conversion** of a cell type present in one tissue or organ **into a cell type from another tissue or organ** without going through a pluripotent cell state.

- Some adult stem cells exhibit a property called **plasticity**, which is the **ability to differentiate into specialised cells of other types of tissues** (that it normally does not differentiate into). This can occur naturally in response to injury.

- In the laboratory, these stem cells can be **ARTIFICIALLY INDUCED** to **transdifferentiate into other specialised cells** (Fig. 7.18)**.**

- Examples of stem cells exhibiting plasticity:

    - Blood stem cells may transdifferentiate into brain cells (e.g. neurons), skeletal muscle cells, and cardiac muscle cells.

    - Bone marrow stromal cells may transdifferentiate into cardiac muscle cells and skeletal muscle cells.

    - Brain stem cells may transdifferentiate into blood cells and skeletal muscle cells.

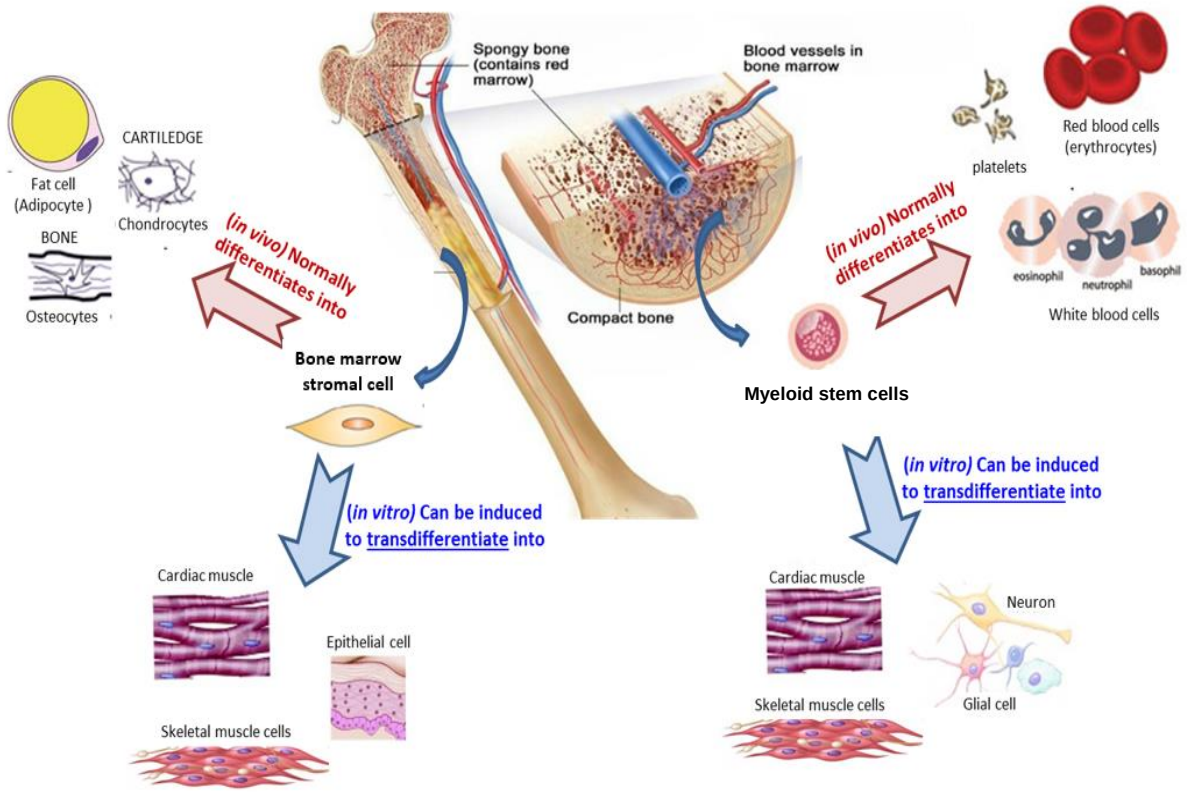


Fig. 7.18: Plasticity of adult stem cells.
For example, bone marrow stromal cells naturally gives rise to fat cells and bone cells etc.
But under suitable conditions and with appropriate chemicals, it can be induced to transdifferentiate into cardiac muscle cells and skeletal muscle cells instead.

7.3.3 Comparison between embryonic and adult stem cells

Features	Embryonic stem cells	Adult stem cells
Developmental potential	Pluripotent	Multipotent (except blood stem cells which are pluripotent)
Accessibility	Cell line can be easily cultured in lab, and hence easier to access and purify	Hard to access and purify from the tissue/organ, as adult stem cells are rare.
Degree of proliferation	Higher degree of proliferation	Lower degree of proliferation
Telomerase Activity	Higher telomerase activity due to a need for rapid cell division	Lower telomerase activity due lesser need for cell division
Probability of Immune rejection after transplantation	Higher chance of immune rejection, as ES cells are usually from a different individual.	Stem cells are isolated from the patient, and is then reintroduced back to the patient, hence lower chance of immune rejection

7.4 Stem cells in research and in medical application

The ability to continually divide and differentiate into different types of cells have made stem cells a significant focus of medical research and application. Imagine having the ability to return memory to an Alzheimer's patient, treat Parkinson's disease, replace skin that was lost during a terrible accident or enable a wheelchair-bound person to walk again. However, stem cells can't treat diseases until scientists learn how to manipulate stem cells to get them to develop into tissues or organs. As of today, many of these possible uses are still only potential uses waiting to be further studied and researched on.

7.4.1 What is stem cell therapy?

- The goal of stem cell therapy and research is to **understand the way stem cell divides and differentiates** in order to utilise it for **repair of a damaged tissue that cannot heal itself**.

- Stem cell therapy is accomplished by:

  (1) coaxing stem cells already in the body to produce new tissue, or

  (2) transplanting embryonic stem cells (pluripotent) into the damaged area and directing them to grow new, healthy tissue.

  (3) Harvesting adult stem cells (multipotent) from the patient and then transplanting these stem cells into the damaged area. These stem cells are then directed to grow into new, healthy tissue.

- Stem cell therapy involving (2) consists of the following steps (Fig. 7.19):

  1. **Embryonic stem cells** (ES cells) are harvested from the inner cell mass of the blastocyst (obtained from IVF clinic).

  2. The ES cells are **cultured and allow to propagate under specific conditions.**

  3. The stem cells are then triggered to **divide and differentiate** into the **desired specialised cell types** (e.g. neurons), **using appropriate growth factors and chemicals.**

  4. These specialised cell types are **transplanted** into the **damaged tissue of the patient** to carry out normal function (e.g. Parkinson's patient).

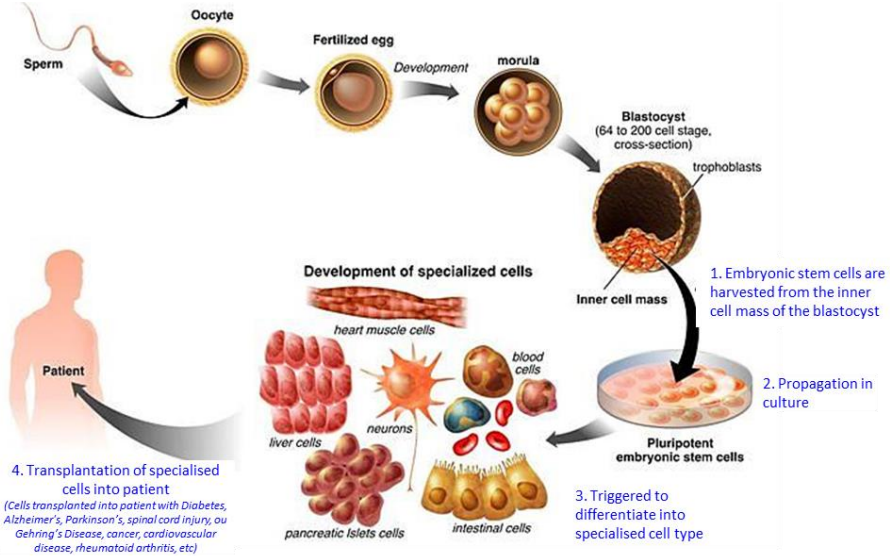


Fig 7.19: Stem cell therapy using embryonic stem cells (pluripotent) harvested from inner cell mass of blastocyst.

- Stem cell therapy involving (3) consists of the following steps (Fig. 7.20):

  1. **Adult stem cells** are harvested from the patient / another individual.

  2. The stem cells are **cultured and allow to propagate under specific conditions.**

  3. *(depends on treatment)* The stem cells are then triggered to **divide and differentiate** into the **desired specialised cell types, using appropriate growth factors and chemicals**.

  4. These stem cells / specialised cell types are **transplanted** into the **damaged tissue of the patient** to carry out normal function.

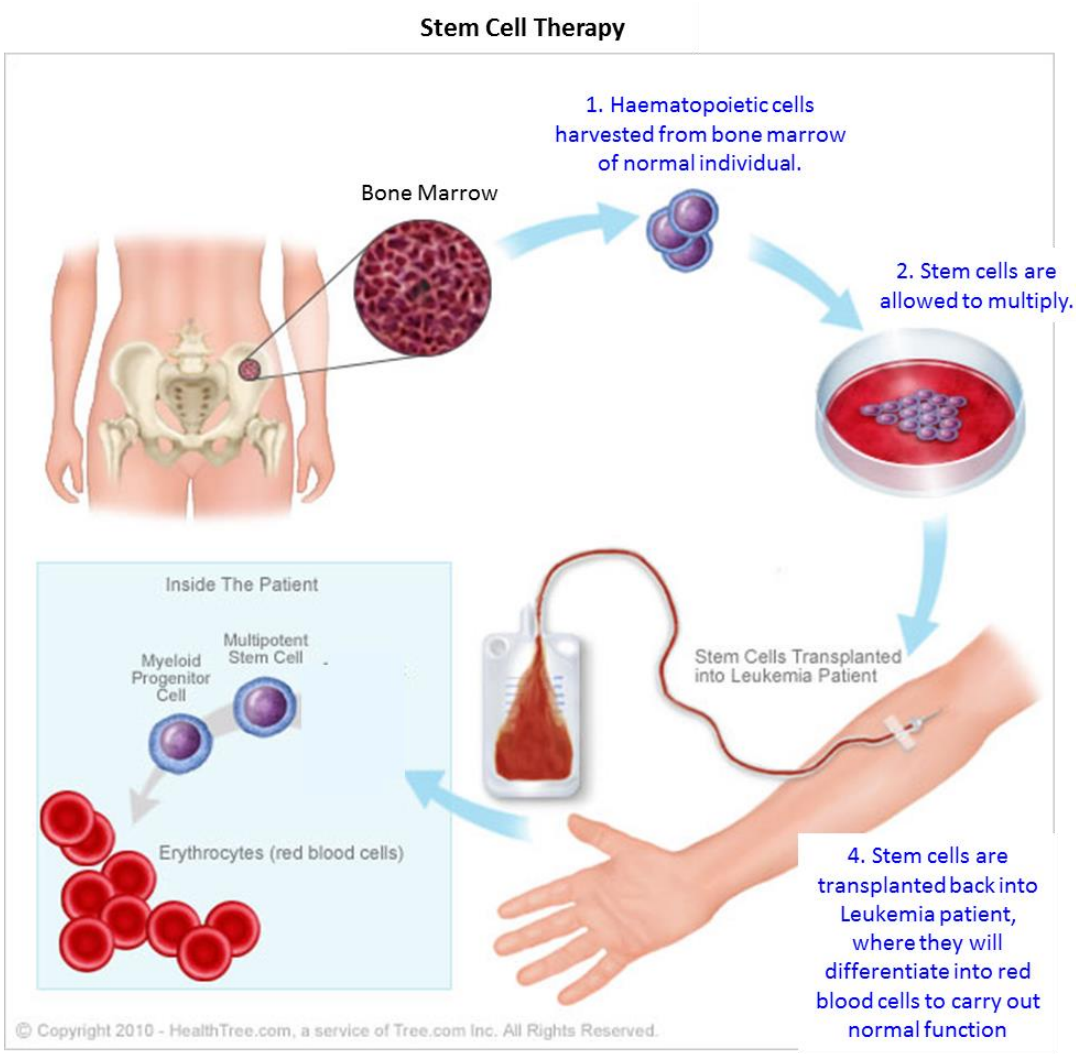


Fig. 7.20: Stem cell therapy using haematopoietic stem cells (blood stem cells) harvested from bone marrow of a normal individual. These stem cells are then transplanted into Leukemia patient. In the patient, these stem cells give rise to myeloid stem cells, which then differentiate into normal red blood cells.

- Stem cell therapy can be used in a wide range of treatment but it is important to understand that some of the uses may be established uses (i.e. it has proven to work; are currently use for medical treatment), but there are others that are simply potential uses (i.e. research is still ongoing and are currently not use for medical treatment).

7.4.2 Current uses of stem cell therapy

Some stem cells therapy has been use for medical treatment. These are established therapies which have been shown to be safe and effective:

- Blood stem cells from the bone marrow are currently the most frequently used stem cells for therapy.
  - Doctors have been using bone marrow transplant to transfer blood stem cells to patients.
  - More advanced techniques are being used to treat leukemia (Fig. 7.21A), lymphoma and several inherited blood disorders.
  - Umbilical cord blood is often another source of blood stem cells and in some cases, used as an alternative treatment to bone marrow transplant.

- Some bone, skin and corneal disease or injuries can be treated by grafting tissues (Fig 7.21B) that are derived from or maintained by stem cells.

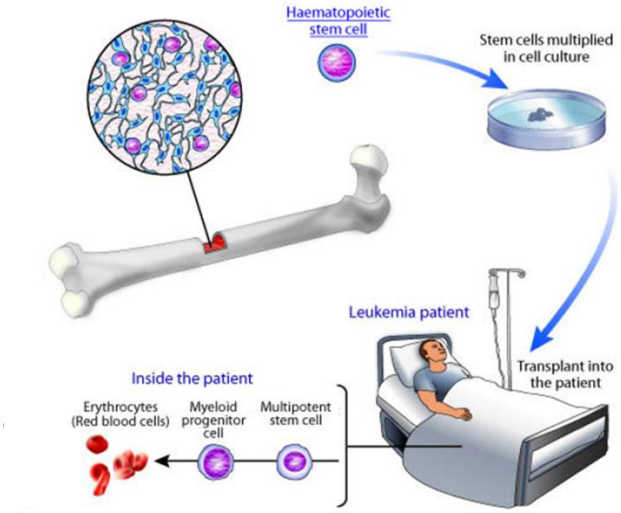


Fig. 7.21A: Treatment of leukemia by haematopoietic stem cells (blood stem cells) derived from bone marrow.

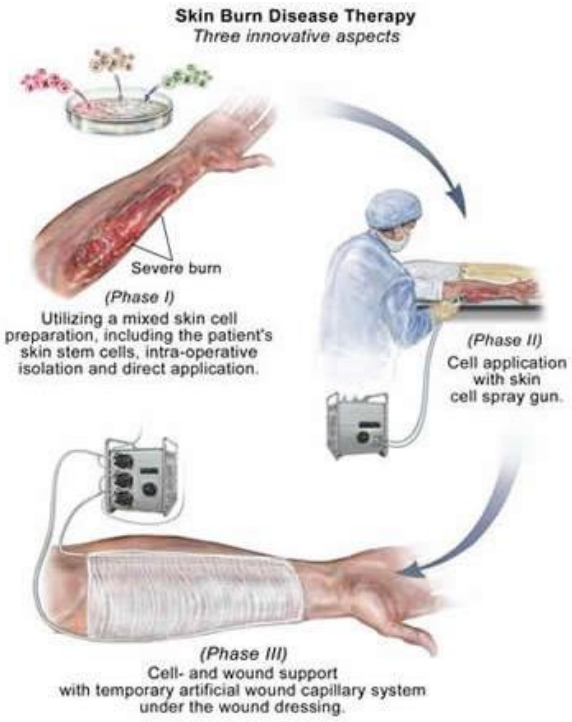


Fig. 7.21B: Skin grafting using patient's skin stem cells to treat severely burnt skin.

7.4.3 Potential uses of stem cell therapy

Other stem cell treatments, while promising are still at very early experimental stages. These therapies are still in the research stage and are not used for treatment as of today. For example:

- Directing mesenchymal stem cell found in the bone marrow to become bone, cartilage (Fig. 7.22A), fats, and possibly even muscle to treat a range of musculoskeletal abnormalities and cardiac disease.

- Using stem cell therapy to treat retinal diseases (Fig. 7.22B) and corneal epithelial regeneration (Fig. 7.22C).

- Introducing neural stem cells into the brain to replace the damaged neurons in Alzheimer's patient .

- Scientists were also able to modify immune functions of certain cells experimentally, which created the possibility of treating immune abnormalities such as graft-versus-host disease (a complication that occur after bone marrow transplant, where the donated bone marrow cells attack the recipient's body).

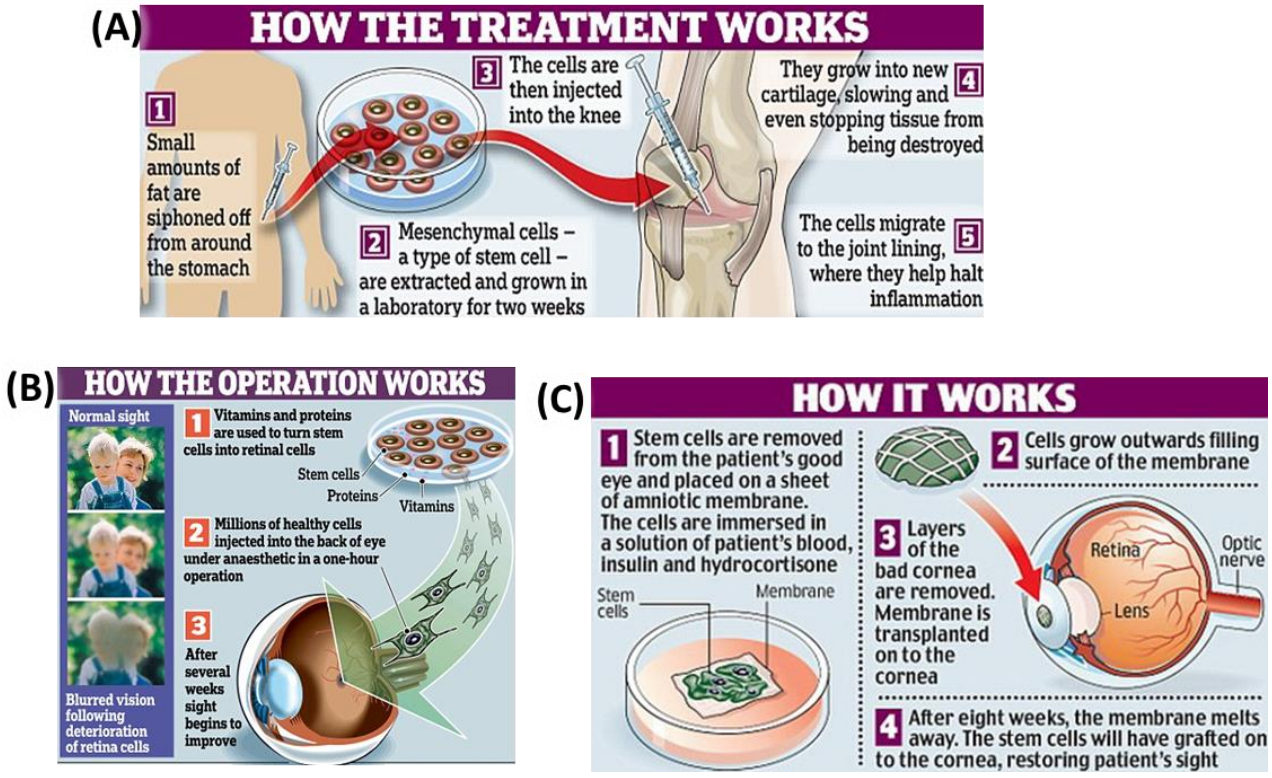


Fig. 7.22: (A) Possible stem cell therapy to treat musculoskeletal abnormalities.
(B) Possible stem cell therapy to treat retinal disease.
(C) Possible stem cell therapy to treat corneal epithelial regeneraton

7.5 Ethical implications of stem cells in research and medical applications and methods to overcome these ethical implications

7.5.1 Ethical implications

Though stem cell research leads to lifesaving treatment, the use of embryonic stem cells requires the **destruction of embryos**, raising several ethical and social issues:

- **Definition of when human life begins**
    - Embryonic stem cell research uses the cells from the inner cell mass of the blastocysts.
    - However, embryos with the inner cell mass removed do not continue to form fetuses, thus forfeiting a human life.
    - For those who consider human life begins at conception, destruction of an embryo is akin to destruction of a human life.

- **Value of an embryo**
    - Though embryos do not currently exhibit the properties of personhood, they will, if allowed to develop and fulfil their potential. Since embryos are potential persons, they ought to be accorded the moral respect and dignity that personhood warrants.
    - Hence, embryos cannot be treated as just mere body parts.

- **Purpose of Embryo Creation**
    - To many, the purpose of embryo creation is for procreation (for another human life).
    - Hence harvesting eggs that are meant for creation of a human life, for the purpose of research, defy many religious and ethical values.

Note that the ethical issues are related to the use of embryos. Use of adult stem cells faces fewer issues.

7.5.2 Enforcement currently in place

In view of the ethical concerns of many regarding the use of embryonic stem cells for research, enforcements are currently in place to regulate the use of these stem cells for research, which include:

- **Informed consent from couples who undergone *in-vitro* fertilization (IVF).**
    - After their IVF treatment, couples who want to donate their embryos for Stem Cell Research should have a full understanding of the nature of research and the commercial implications (e.g. whether they hold propriety rights in the tissue lines developed from embryonic cells), before giving informed consent.

- Donation of eggs by women should be **voluntary** and **without compensation,** as compensation may lead to exploitation of poor women and trading of reproductive tissues. However without compensation, women have been **unwilling to donate their eggs**.

7.5.3 Alternative methods to obtain embryonic stem cells (pluripotent)

- **Induced pluripotent stem cells (iPs cells)** are obtained from **differentiated somatic cells.**
  - In 2006, scientists Kazutoshi Takahashi and Shinya Yamanaka from Kyoto, Japan, managed to reprogram specialised mouse cells to form embryonic stem cells through the viral delivery of 4 stem cell-associated genes into the mouse dermal cells.
  - These differentiated cells are **induced through chemical signals** to **regain pluripotency** (Fig. 7.23A and B).
  - These signals which act as reprogramming factors, trigger the **expression of genes and factors** that enable the cells to be **pluripotent.**
  - Medical Research and the potential use of iPs cells in treatment are of great importance today because:

    (1) these iPS cells possess the **developmental potential (pluripotency) of the embryonic stem cells**.

    ❖ iPS cells are able to **divide and renew themselves continually**, hence the cell line can be maintained under lab conditions.

    ❖ iPS cells can be induced under appropriate conditions and chemicals to **differentiate into all types of cells, except the extraembryonic membranes**, hence it can be used for stem cell therapy.

    (2) iPS cell use avoids the ethical issues of embryonic stem cell research as embryos are not used.

    ❖ Since iPS cells are derived from differentiated somatic cells, they **do not lead to destruction of embryos** and hence avoid the ethical issues pertaining the use of embryos.

    (3) tissue rejection can be avoided.

    ❖ Somatic differentiated cells used can be derived from the patient, hence **chances of immune response in the patient may be lower** when used for therapy.

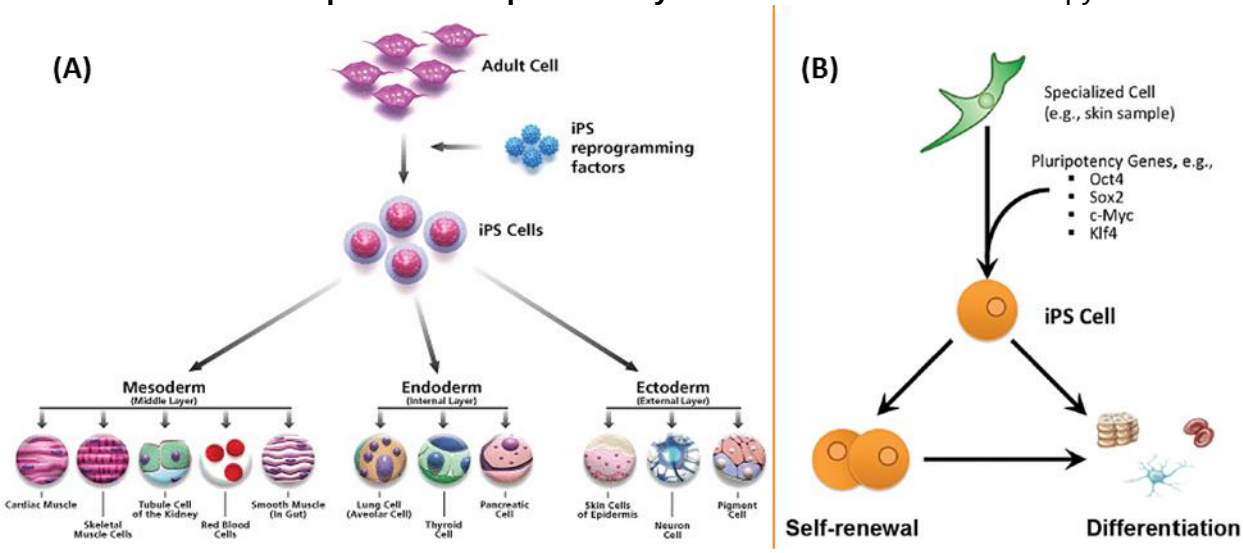


Fig. 7.23A & B: Induced pluripotent stem cells (iPS cells) are differentiated somatic cells that have been genetically reprogrammed to be pluripotent. These iPs cells are capable of self-renewal and can be induced to differentiate under appropriate conditions.

- Stem cells obtained from already **deceased embryos (cadaveric fetal tissue)**.
    - e.g. aborted embryos.
- Stem cells obtained from **living embryos by nondestructive biopsy**.
    - e.g. obtaining stem cells from the **8 cell stage**.
- Stem cells obtained from **somatic cell nuclear transfer (SCNT)**. (Fig. 7.24)
    - DNA of an unfertilized egg is replaced by DNA of a somatic cell with mutation on the gene for blastocyst implantation.
    - This results in the formation of embryo-like pluripotent stem cells artifacts with the inability to implant in the uterus.
    - Similar technique that produced *Dolly* the sheep.
    - To date, this technique is currently still scientifically impossible in humans and is also ethically controversial.
        - ❖ *Ethical issue:* Those who object SCNT believe that creating embryos with the intention of using them for research and destroying them in the process violates respect for human life.

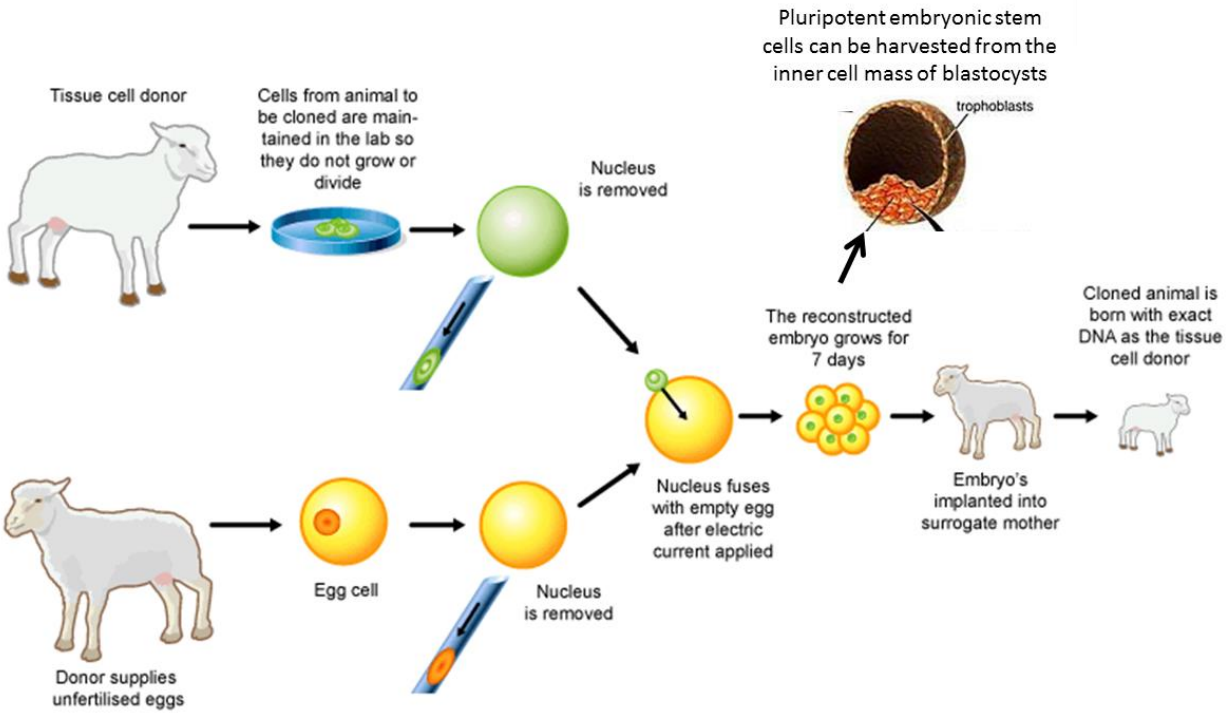


Fig. 7.24: Somatic cell nuclear transfer. This technique will allow scientist to harvest pluripotent ES cells from the inner cell mass or allow the blastocysts to continue developing into a whole organism (cloning)

While Section 7 clearly depicts the use of stem cells in curing disease, one needs to realise that there are bioethical issues to consider when using these treatment in reality. It is therefore important to consider the facts and explore alternative ways to hopefully derive a better and more acceptable method as time progresses.

The End