

The Nucleus: DNA, Chromatin And Chromosomes

Professor Alfred Cuschieri

Department of Anatomy, University of Malta.



Objectives

By the end of this unit the student should be able to:

1. List the major structural components of the nucleus
2. Name the fundamental properties of DNA
3. Explain the role of proteins in the organization of DNA, chromatin and chromosomes
4. Outline the structure and functions of nuclear pores
5. Name the types of molecules that pass between the nucleus and the cytoplasm
6. Identify the components of the nucleolus, their composition and major functions

Practical skills objectives

The student should be able to:

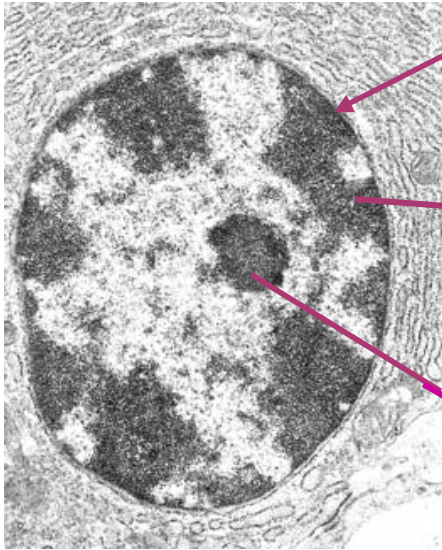
- Identify the ultrastructural components of the nucleus and nucleolus in electron micrographs
- Distinguish between eu- and hetero- chromatin
- Identify variations in shape, size and texture of nuclei in histological sections
- Interpret the significance of prominent nucleoli in cells.

Recommended Reading

"The World of the Cell" Becker WM, Kleinsmith LJ, Hardin J

● Chapter 16: The structural basis of cellular information: DNA, chromosomes and the nucleus

Components and functions of the nucleus.



The nuclear envelope consists of two membranes enclosing a space, surrounds the nucleus, and is traversed by nuclear pores

- Controls movement of molecules between nucleus and cytoplasm

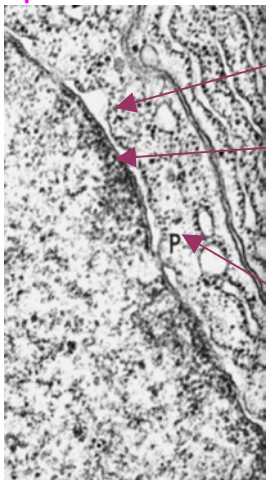
Chromatin and nuclear matrix

- Formed of the genetic material (DNA) and associated proteins

The nucleolus

- Produces ribosomal RNA

The nuclear envelope consists of two membranes enclosing a space



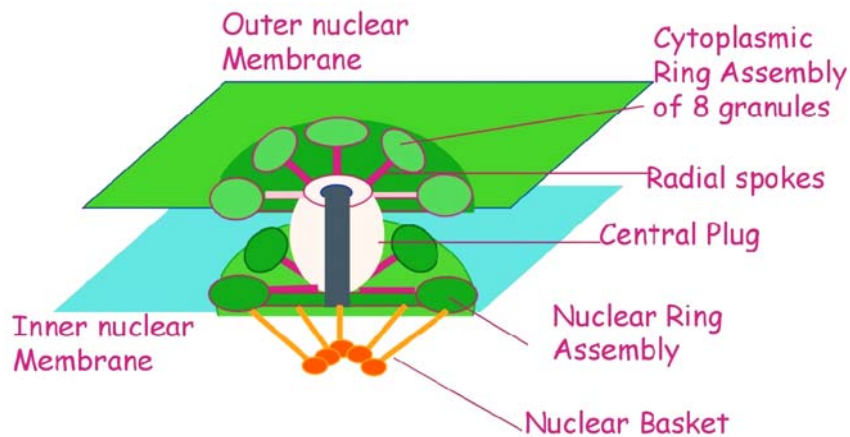
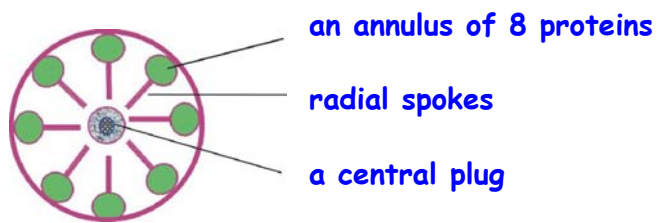
The cytoplasmic surface of the outer membrane has numerous ribosomes and is continuous with RER

The nuclear surface of the inner membrane is associated with a nuclear lamina for the attachment of chromatin

At the nuclear pore (P) the two membranes are fused

Nuclear pores:

- Are membrane-bound channels
- 60-90 nm diameter
- Regulate passage of substances between nucleus and cytoplasm
- Vary in number according to the cell's function and stage of development
- Consist of proteins forming a distinct complex as shown in the diagram:



Nuclear pore complexes can be isolated by chemical treatment

1. Differential centrifugation separates the nuclei
2. NaHCO_3 makes the chromatin soluble
 - the remaining nuclear envelopes are termed nuclear ghosts
3. Differential centrifugation separate the nuclear ghosts
4. Detergents are used to dissolve the lipid membranes
 - the isolated pore complexes (protein) remain
5. Centrifugation isolates the pore complexes

The pore complexes have a distinct structure consisting of a rosette of 8 granules.



Passage of Substances Through Nuclear Pores

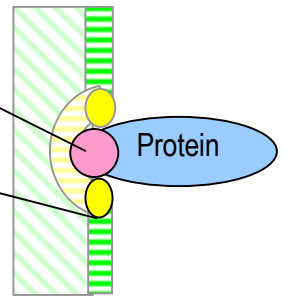
The molecules that pass from the cytoplasm into the nucleus are mainly:

- (a) Ions;
- (b) Nucleotides for DNA synthesis;
- (c) Proteins for binding to DNA

The molecules that pass from the nucleus into the cytoplasm are m-RNA and r-RNA bound to protein as RNA-protein complexes.

Protein molecules require a special mechanism to pass through the nuclear pores:

- a signal at the **-COOH** end of the protein
- a signal **receptor** at the pore boundary
- membrane-bound **ATPase** to releases energy for the process



A fibrous lamina is situated on the nuclear surface of the nuclear envelope:

- It consists of proteins.
- It has a woven fibrillar structure
- It serves to anchor the chromatin to the nuclear envelope

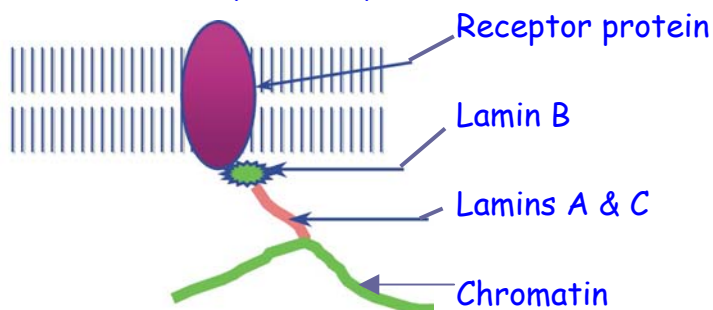
The main proteins of nuclear envelope are:

1. Pore proteins:

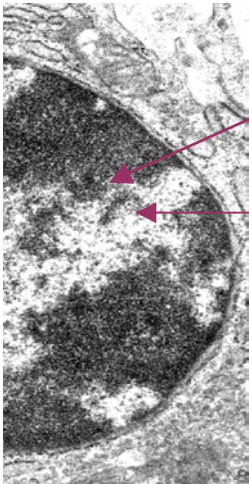
- Large pore proteins (known as gp210)
 - Anchor the pore complex to membrane
- Small pore protein (known as p62)
 - Regulate transport through the pore

2. Lamins:

- Consist of 3 types: Lamins A, B, C
- Form a meshwork of filaments (similar to intermediate filaments) in the nuclear lamina
- Bind chromatin to receptor proteins in the inner membrane of the nuclear envelope. They are attached in the following sequence:



The nucleus consists of fibrillar material termed chromatin



Chromatin in nuclei exists as:

a. **heterochromatin** - closely packed or condensed fibrils, where DNA is inactive.

b. **euchromatin** - loosely packed fibrils, where active transcription occurs

The amount of euchromatin reflects the state of activity of the nucleus. Nuclei of cells that are actively synthesising proteins have a large amount of euchromatin (e.g. neurons renewing their organelles; plasma cells producing antibodies). Cells that are not actively synthesizing protein have heterochromatic

nuclei (e.g. circulating lymphocytes are inactive cells in transit; sperm cells have very closely packed heterochromatin)

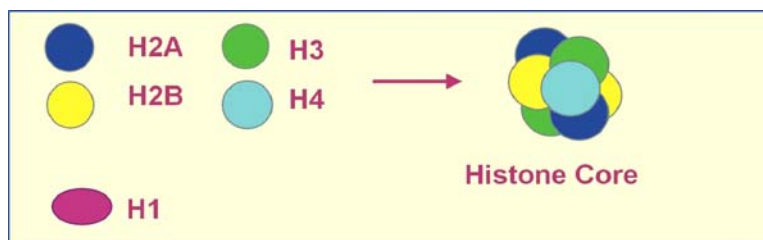
The nuclear material consists of

- DNA
- Histone protein
- Non-histone chromosome protein
- RNA

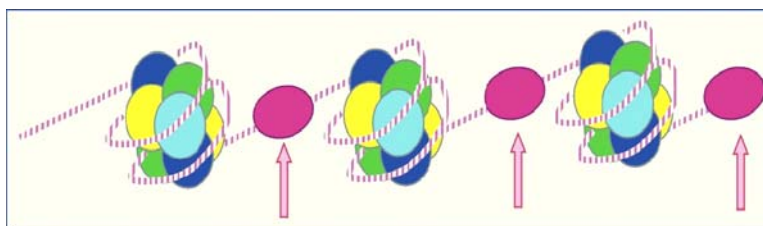
DNA and proteins are organised in a highly regular pattern to form nucleosomes and chromatin.

The proteins regulate the packing of DNA. Proteins constitute over 80% of the total mass of nuclear material, while DNA forms 15 to 20%.

There are 5 histone proteins associated with DNA



Two molecules each of histones H2A, H2B, H3 and H4 form a histone core.



DNA is wound around the histone cores - two turns of DNA around each core form a nucleosome. The DNA of each nucleosome contains 146 nucleotide pairs. The stretch of DNA between one nucleosome and the next is called linker DNA. H1 histone is associated with the linker DNA. Nucleosomes can be isolated from chromatin by treatment with a hypotonic solution in an aqueous medium.

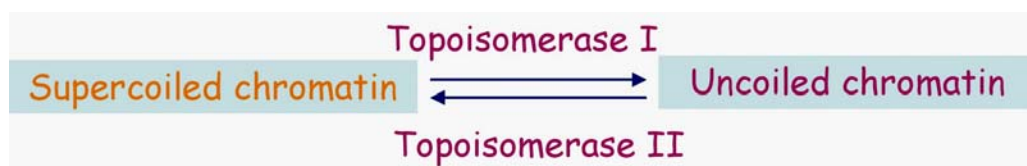
H1 histones are responsible for the primary coiling of DNA

When chromatin is exposed to dextran sulphate, it is depleted of histones. The histone-depleted chromatin forms free loops of DNA emanating from the chromatin mass.

Further coiling of chromatin fibres is responsible for different levels and types of packaging.

This supercoiling is dependent on the non-histone chromosome proteins. (NHCP)

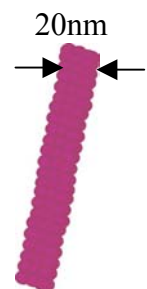
Among the large variety of NHCP are two enzymes which control the supercoiling are topoisomerase II and topoisomerase I.



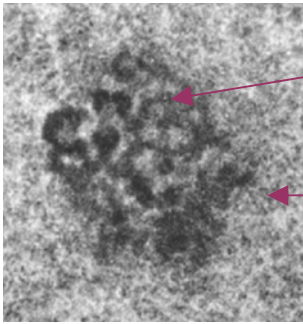
Complex coiling of nucleosome chains form chromatin fibrils.

Chromatin fibres have a diameter of 20-30 nm diameter.

Note that the chromatin in electron micrographs has a fibrillar structure. Each fibril is a chromatin fiber with a diameter of 20-30nm.



The nucleolus consists of:



- A dense fibrillar part, consisting of DNA, carries the message for synthesis of r-RNA
- A pale granular part, consisting of r-RNA is combined with protein to form ribonucleoprotein particles

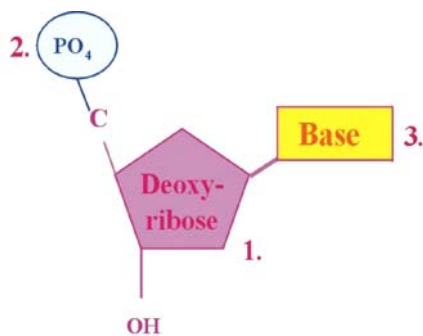
The presence of a prominent nucleolus indicates that the cell is actively synthesizing protein. A prominent nucleolus is present in protein-secreting cells such as those of the pancreas, plasma cells, developing blood cell precursors and others

The **nuclear matrix** is a residual network of protein fibrils remaining after:

- Removal of nuclear envelope (using detergent)
- Removal of histones (using high salt concentration)
- Removal of DNA (using nuclease)

The DNA Molecule Consists of a Long Nucleotide Chain.

Each nucleotide consists of 3 elements:

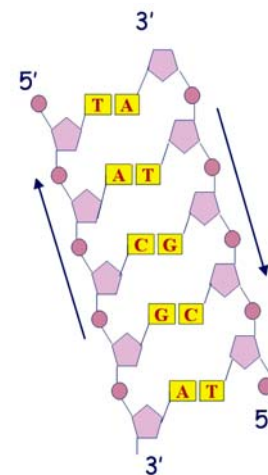


1. A pentose sugar molecule (deoxyribose)
2. A phosphate group that links one nucleotide to another
3. A base that could be one of the following:

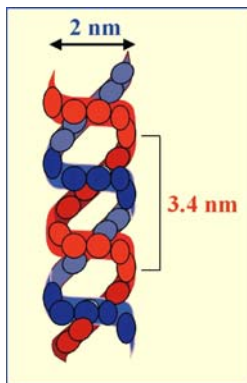
- Adenine
- Cytosine
- Thymine
- Guanine

Some Features of the DNA Molecule

- DNA is a very long molecule consisting of two strands of nucleotides.
- The two polynucleotide strands run in opposite directions
- Each strand has a polarity indicated by 3' and 5' ends
- Specific base pairing links the two strands:
Adenine always pairs with Thymine;
Cytosine always pairs with Guanine



Characteristics Of DNA - Shape and size:



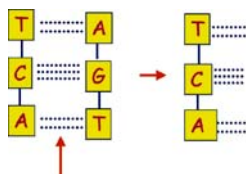
The DNA molecule:

- * has the form of a double helix
- * has a diameter of 2 nm.
- * one spiral turn = 3.4 nm length contains 10 base pairs.

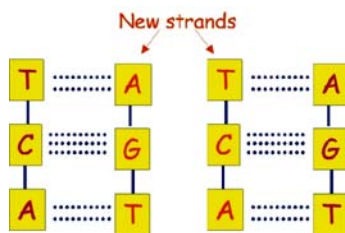
Note: 1 nanometre (nm) = 10^{-9} m.

DNA has 3 basic properties

1. DNA REPLICATES ITSELF



DNA makes an identical copy of itself .
This function is possible because of **specific base pairing**.

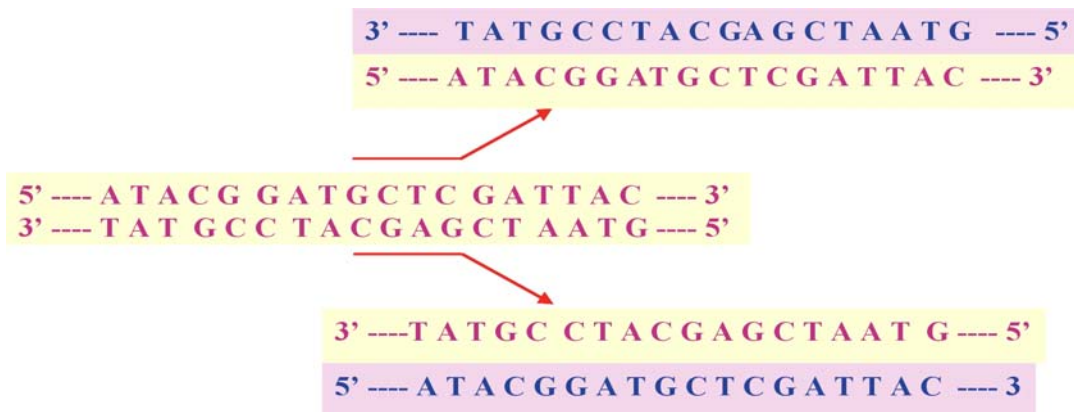


The two DNA strands separate by breaking open the H-bonds linking the bases. Each DNA strand serves as a template for the synthesis of a complementary chain of nucleotides. Specific base pairing ensures that the sequence of bases is preserved on both molecules of DNA.

DNA replication is the basis of propagation of life.

DNA replication involves two main steps:

1. The two strands of DNA separate:

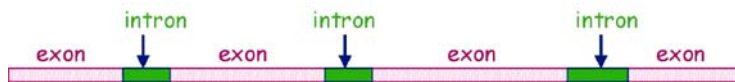


2. Each strand serves as a template for synthesis of a complementary strand.
 The two resulting daughter molecules of DNA are identical to the original DNA molecule.

2. DNA CARRIES THE GENETIC MESSAGE.

- ☞ A stretch of DNA coding for one particular protein constitutes a **gene**.
- ☞ Each gene is specific for one specific protein.
- ☞ **Each** protein is responsible for a particular function in a cell

In a gene the coded message is contained in **exons** (expressed sequences), which are usually interrupted by **introns** (intervening sequences) that are not parts of the gene message.

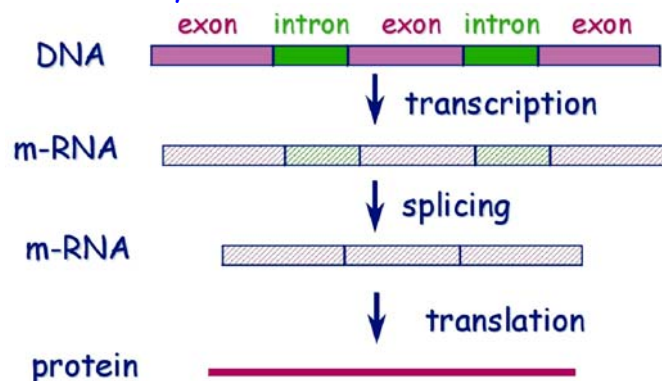


The number of introns varies from 0 to 80 in different genes, but is the same for a particular gene. Introns are like blank spaces separating paragraphs in a written message. They intervene but do not interrupt the message. They also serve a purpose.

The Genetic Message Is First Transcribed and Then Translated

- **Transcription** is the process of synthesis of messenger RNA using the coded message in a stretch of DNA (a gene) as template. It occurs in the nucleus.
- **Translation** is the process of synthesis of a specific protein using m-RNA as a template. It occurs in the ribosomes in the cytoplasm.

Note that both exons and introns are transcribed. The introns are then spliced off the m-RNA. Only the exons are translated.



Translation Requires 3 Types of RNA

i. Messenger-RNA (m-RNA)

- Serves as a template for the formation of a specific protein.
- Is specific - There is one m-RNA for each protein.

ii. Ribosomal-RNA (r-RNA)

- Is required for the assembly of ribosomes.
- Ribosomes are the machines that synthesize proteins based on the m-RNA template. Ribosomes are non-specific i.e. they are used in the synthesis of all proteins.

iii. Transfer-RNA (t-RNA)

- Transfers the appropriate amino-acid to a growing protein chain
- There is one t-RNA for each amino-acid

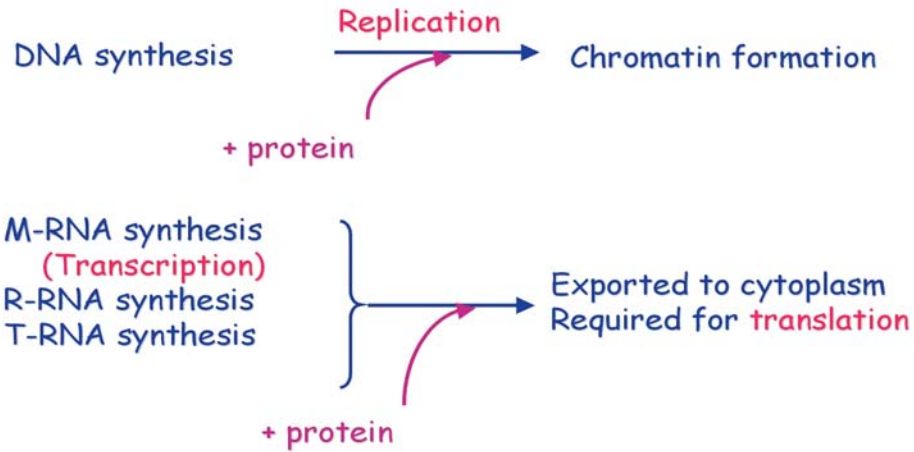
3. DNA IS UNIVERSAL

- Whatever the living organism - bacteria, plants, animals or man - the basic structure of DNA is the same.
- Only the sequence of bases differs and consequently the genetic message; the resulting proteins and their specific functions are also different.
- Because DNA is universal, DNA from one organism can be grafted on to the DNA of another organism. This is the basis of genetic engineering.

Note the main processes that occur within the nucleus:

- **Replication** - the process of **synthesis of DNA**, which occurs prior to mitosis or meiosis
- **Transcription** - the process of **synthesis of m-RNA**, a copy (transcript) of the genetic message in one gene
- **Synthesis of r-RNA** - important components of the ribosomes
- **Synthesis of t-RNA** - to be used in the cytoplasm for translation of proteins

DNA and RNA are always coupled with protein in the nucleus



The proteins that form part the nucleus are synthesized in the cytoplasm and imported into the nucleus.
