Chapter 4. INTERPHASE NUCLEUS

DEFINITION

The nucleus is a specific organelle found in eukaryotic cells, delimited by the nuclear envelope, which separates its content from the rest of the cytoplasm. It contains the nucleoplasm, in which primarily reside chromatin and one or more nucleoli. It is the vital center of the cell and controls all cell activities through DNA. The DNA, an essential component of chromatin, carries the genes of the hereditary heritage (or genome).

I. STRUCTURE AND ULTRASTRUCTURE

1. UNDER THE LIGHT MICROSCOPE

Under the light microscope, the interphase nucleus often appears spherical and of variable size. It is typically found as a single instance per cell; however, there are some multinucleated cells containing multiple nuclei (e.g., bone cells or osteoclasts), and others are anucleate, having lost their nucleus during maturity (e.g., red blood cells or erythrocytes).

2. UNDER THE ELECTRON MICROSCOPE

Observation of the nucleus under the transmission electron microscope (TEM) or scanning electron microscope (SEM), using various techniques (contrast enhancement, cryofracture, negative staining combined with three-dimensional image processing), allows for the detailed examination of the ultrastructural organization of the nuclear envelope, chromatin, nucleoplasm, and nucleolus.

2.1. Nuclear Envelope

2.1.1. Nuclear Membranes

The nuclear envelope is a specialized portion of the endoplasmic reticulum **(lecture notes p17)**. It consists of two membranes, each 6nm thick, with a trilamellar structure that is asymmetrical and fluid mosaic-like. They are separated by a perinuclear space or cavity, 10 to 50nm thick, which is continuous with the cavity of the endoplasmic reticulum.

The outer nuclear membrane bears ribosomes on its cytosolic side, and the inner nuclear membrane is associated on its nucleoplasmic side with a thin, electron-dense layer called the lamina densa. This latter corresponds to a network of intermediate filaments composed of lamins. It provides rigid structural support to the nuclear envelope and serves to anchor chromatin at the periphery of the nucleus.

The nuclear envelope helps maintain the shape of the nucleus but primarily ensures the protection of genetic material. Similar to the rough endoplasmic reticulum (RER), it is involved in the synthesis of certain resident proteins of the nuclear envelope. Additionally, like the smooth endoplasmic reticulum (SER), it serves as a storage site for calcium ions (Ca^{2+}).

2.1.2. Nuclear Pore Complex

The nuclear envelope is a selective barrier where there are regions of interruption, known as nuclear pores **(Figures 1, 2).** Their number varies depending on the cell type and, more importantly, the physiological activity of the cells. They form a complex structure, referred to as the nuclear pore complex (NPC).

The NPC is composed of two large rings, each 120 nm in diameter, the cytosolic ring and the nucleoplasmic ring, which enclose a central opening or central transporter of 30 nm in diameter. Each of the two rings is formed by an assembly of eight radial arms that protrude into the central opening, thereby delineating eight lateral channels. A third smaller ring is located in the nucleoplasm. There appears to be a very stable interconnection between the NPCs and the lamina densa, which serves as their anchor.

The passive transport of soluble molecules occurs at the level of the lateral channels, while the active transport of larger molecules takes place through the central channel or transporter. The nuclear envelope also facilitates the import and export of various molecules through the NPCs and the double membranes **(Figure 3)**. This enables control and regulation of exchanges between the cytosol and the nucleoplasm.

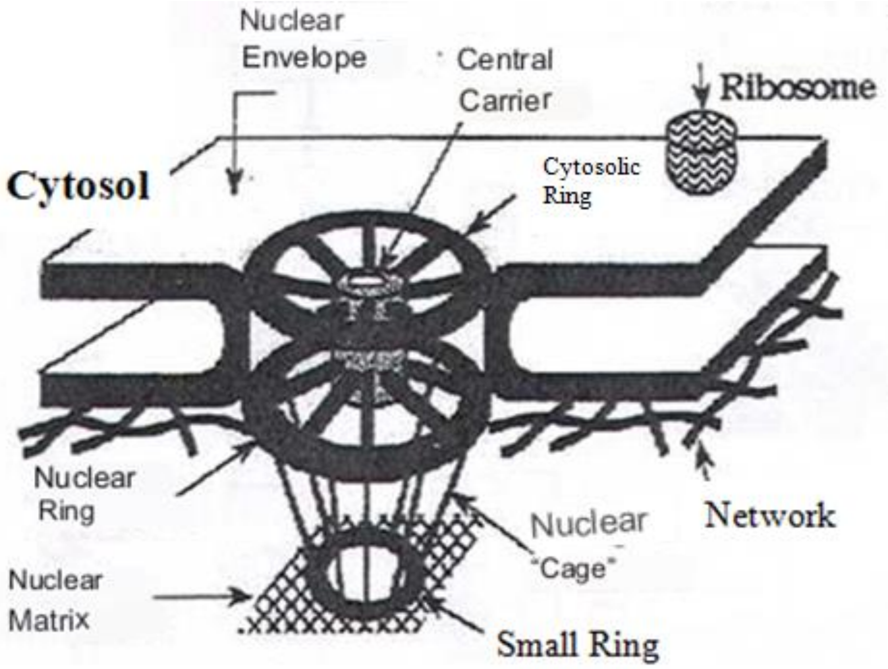

Figure 1: Nuclear Pore Complex: Perspective View.

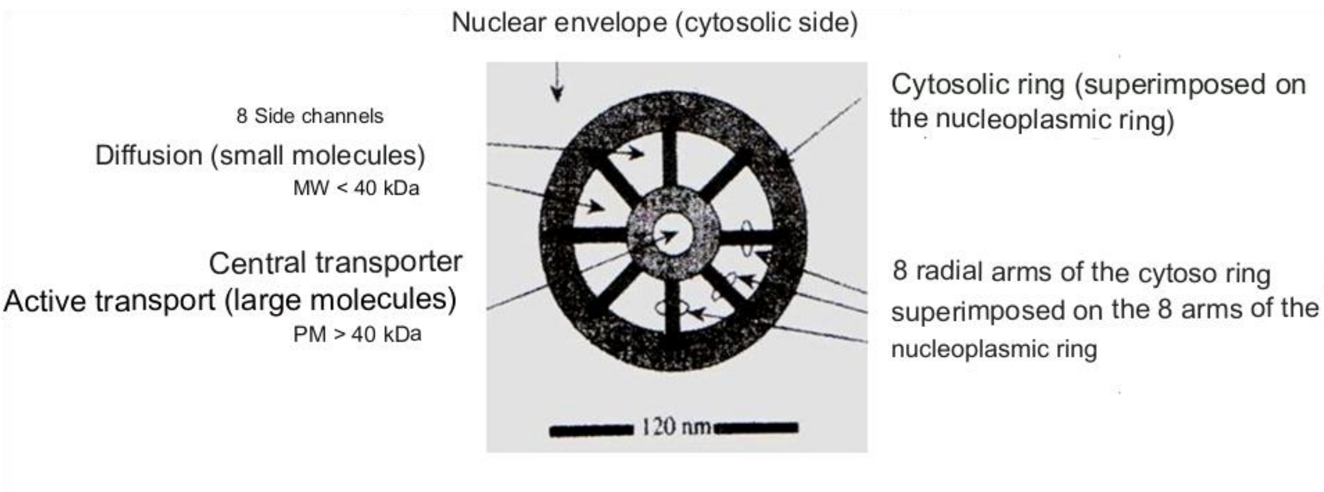

Figure 2: The nuclear pore complex: front view from the cytosol.

II.ANALYSIS OF VARIOUS COMPONENTS

The UCD (Ultracentrifugation) allows for the isolation of the nuclear fraction (pellet 1). The disruption of the nuclear envelope following the action of ultrasonics or osmotic shocks, followed by several centrifugation steps, allows for the separate isolation of subfractions within the nucleus: the nuclear envelope, nucleoplasm, chromatin, and nucleolus.

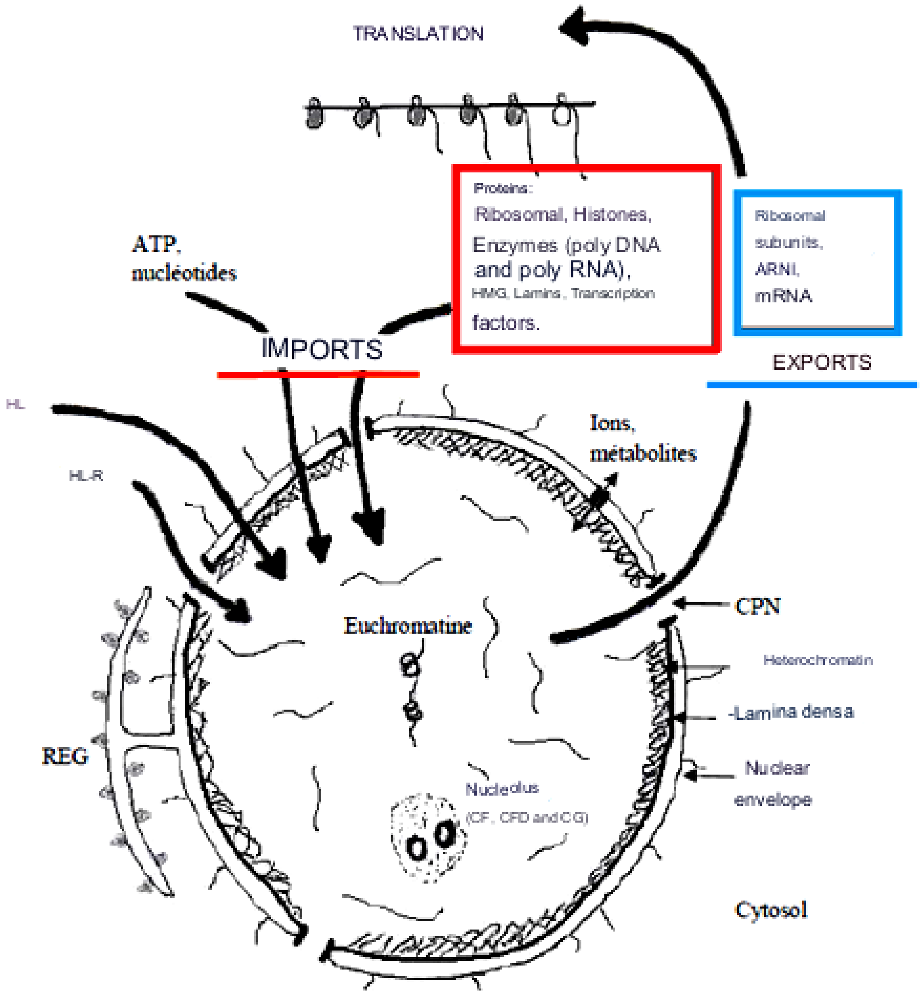

Figure 3: structural and dynamic characteristics of the interphase core.
CPN: nuclear pore complex. CF: fibrillar center. CFD: dense fibrillar component. HL: fat-soluble hormone.
HL-R: cytosolic receptors, REG: granular endoplasmic reticulum, HMG:high mobility group.

1.Nucleoplasm

It is a viscous gel equivalent to the hyaloplasm **(Figure 4)**, Inside it, there is a nuclear matrix composed of thin protein fibrils in a network arrangement with granules at the intersection of its meshes, a sort of nuclear scaffold that maintains the nucleus's shape and acts as a framework upon which chromatin is organized. This matrix serves as an anchor for DNA and RNA in replication, transcription, and maturation processes.

The nucleoplasm also contains various molecules, including enzymatic or structural proteins, mRNA and tRNA, various types of ions like Ca2+, Na+, K+, and Mg2+, nucleotides, ribosomal subunits, and a submembranous protein network with a mesh-like organization that surrounds the small nucleoplasmic ring of the NPC.

Figure 4: Organization of the nuclear matrix in TEM.

2. Chromatin

Chromatin plays a role in cell division and growth. Under TEM, it appears in two forms: heterochromatin and euchromatin **(lecture notes p77).**

2.1. Heterochromatin

It is condensed, electron-dense chromatin, primarily located at the periphery of the nucleus on the nucleoplasmic side of the inner nuclear membrane and in contact with the lamina densa. It is also found to some extent around the nucleolus, referred to as perinucleolar heterochromatin.

2.2. Euchromatin

It is decondensed, light, diffuses, or dispersed in the nucleoplasm. The application of autoradiography, which uses radioactive precursors, has shown that RNA synthesis takes place in euchromatin. Therefore, euchromatin is the active form of chromatin, and heterochromatin is the inactive form.

After its isolation, spreading, and the application of the negative staining technique, it can be observed under high magnification in TEM that it consists of two types of fibers: A-fiber with a diameter of 10 to 11nm, organized in a bead-like structure, constituting euchromatin, and B-fiber with a diameter of 25 to 30nm, much more condensed, forming heterochromatin.

2.3. Chemical Composition

Chromatin contains 30 to 35% DNA; 30 to 40% of basic proteins called histones (H1, H2A, H2B, H3, and H4); 10 to 25% of acidic proteins, and 5 to 10% of RNA (being synthesized) associated with DNA in euchromatin (active chromatin).

2.4. MolecularOrganization

2.4.1. A-Fiber

La fibreThe A-fiber or euchromatin exhibits an organization resembling a string of beads, with the beads representing nucleosomes. Thus, the A-fiber corresponds to the nucleosomal fiber (Figure 5a).

A nucleosome (Figure 6), a disk measuring 10 to 11nm in diameter and 6nm in thickness, consists of an octamer of histones, which includes pairs of H2A, H2B, H3, and H4. The DNA located at the periphery wraps around the histone octamer. A fifth histone, H1, intervenes to lock the DNA by binding to each nucleosome near the site where the DNA helix enters and exits the octamer. In its presence, the DNA forms 2 complete turns. Nucleosomes are connected to each other by internucleosomal DNA. The DNA surrounding the histones and internucleosomal DNA together comprise approximately 200 base pairs of nucleotides.

2.4.2. B-Fiber

Histone H1, composed of a globular part and arms corresponding to the amino- and carboxy-terminal ends, also plays a role in the stacking of nucleosomes. It binds to a unique site on a nucleosome through its globular portion, and it is assumed that its arms extend to contact other sites on the histone octamer of adjacent nucleosomes. The nucleosomes are thus brought together in a repetitive and regular row, forming a helical structure, resulting in a higher-order structure known as a solenoid **(Figure 5b).** This is why it's referred to as the solenoid fiber corresponding to the B-fiber **(Figure 6).**

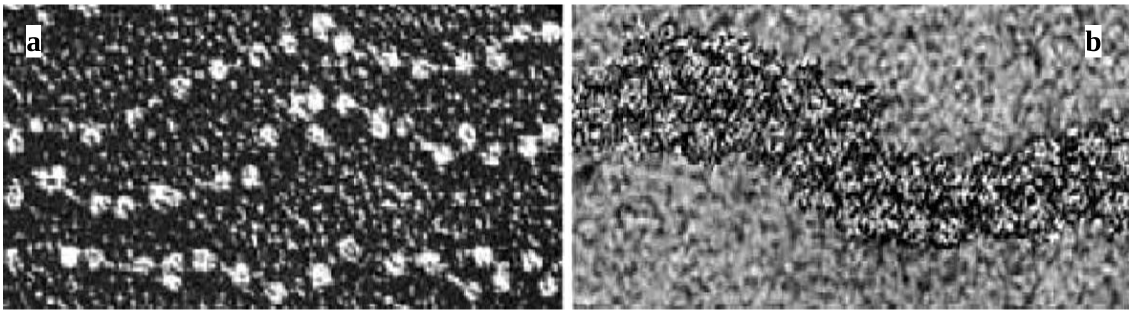

Figure5: Chromatin in bead-like necklace or A-fiber (a) and solenoid fiber or B-fiber (b).

2.5. Chromosome

Chromatin and chromosome **(handout p.85)** represent two different morphological states of the same genetic material. During mitosis, chromatin condenses more and becomes increasingly complex due to the involvement of acidic proteins that form a basic scaffold around which the solenoid fiber forms loops that condense further, reaching their maximum during metaphase. At this stage, the chromosome is 50,000 times shorter than the uncoiled DNA molecule **(Figure 6).**

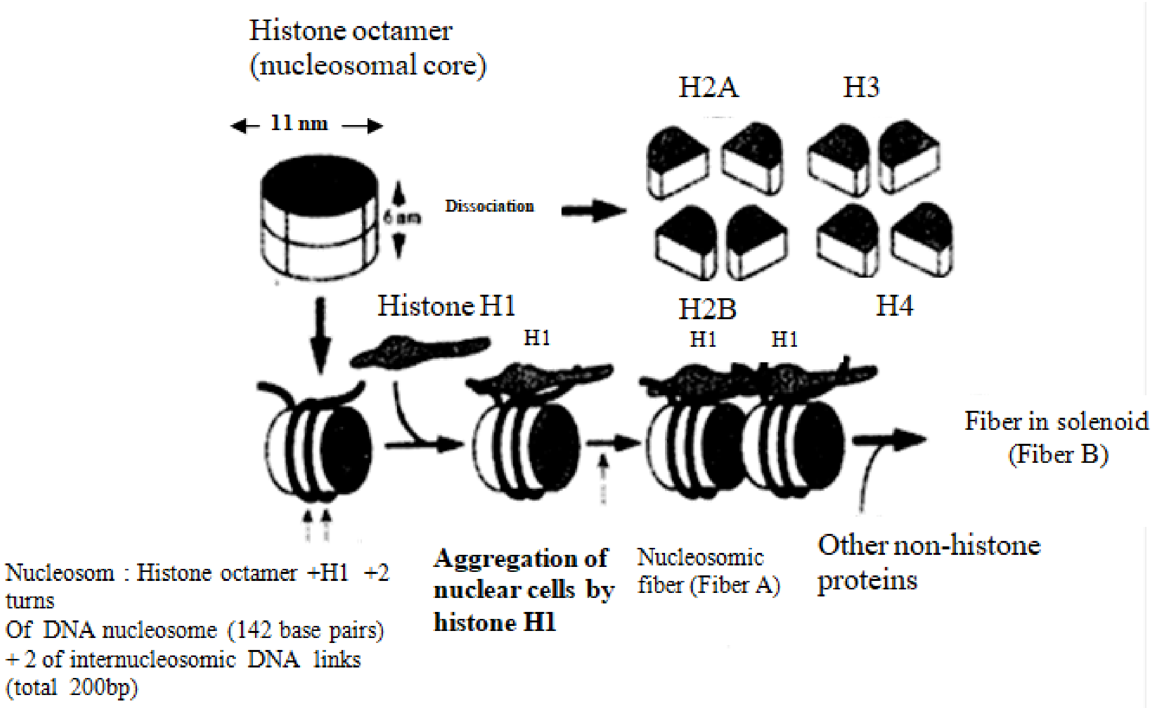

Figure 6: The nucleosome, the elementary unit of chromatin.

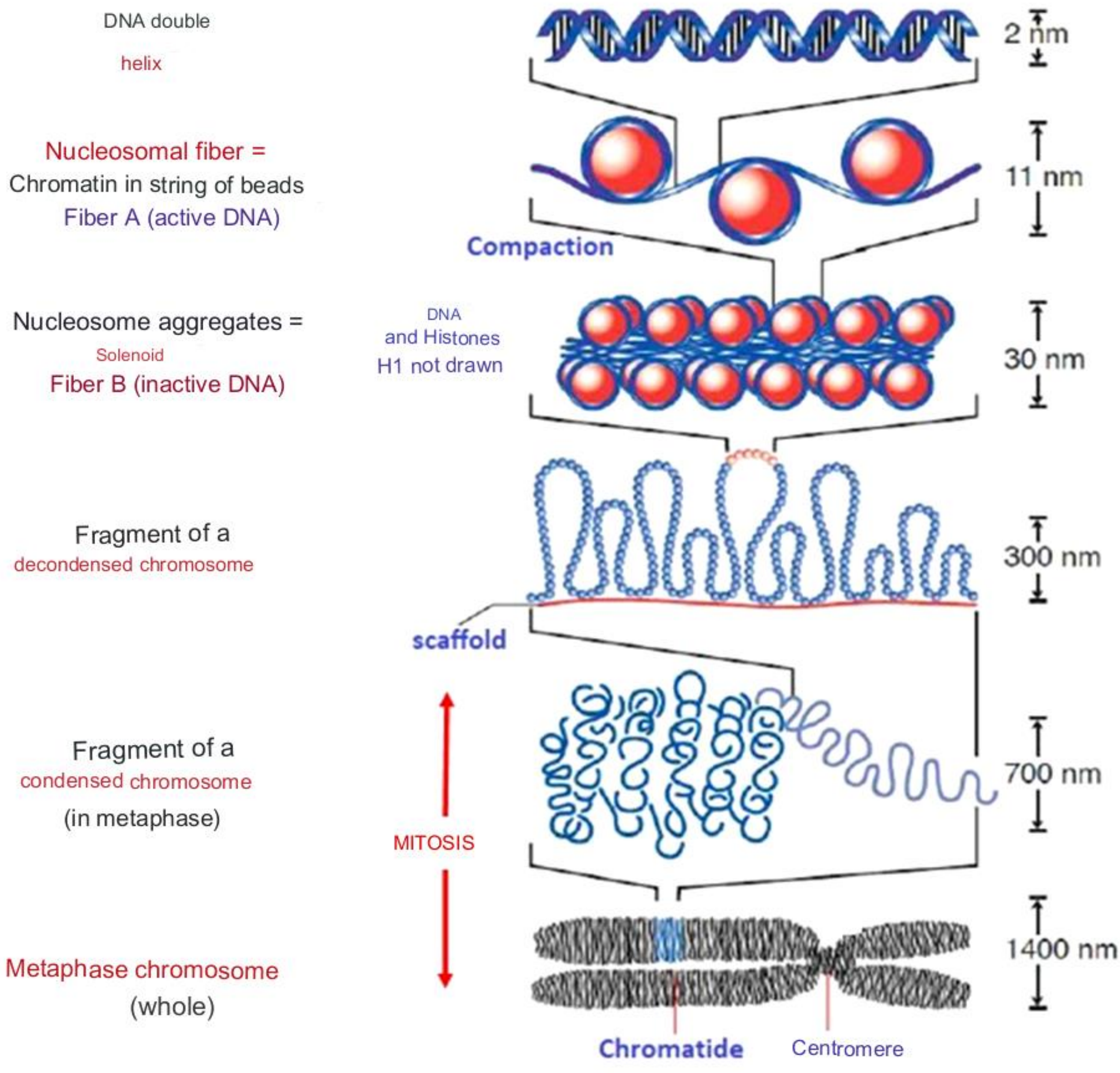

Figure 7: The different degrees of compaction of nuclear DNA

3. Nucleolus

It is a more or less spherical structure located within the nucleus, not bounded by a membrane **(handout p.77).** There is a defined number for each cell type, typically 1 or 2 nucleoli per nucleus or several (e.g., growing oocytes); it can be absent (e.g., spermatozoa). It disappears during prophase and re-forms during telophase, persisting throughout the entire interphase

3.1. Ultrastructure

In the TEM, the nucleolus exhibits three relatively distinct parts **(Figure 8)**:

- Fibrillar center (FC) generally located at the center of the nucleolus (there may be several FCs).
- Dense fibrillar component (DFC) surrounding the fibrillar center(s).
- Granular component (GC) located on the periphery of the two previous parts.

However, the arrangement of these three parts can vary depending on the cell type.

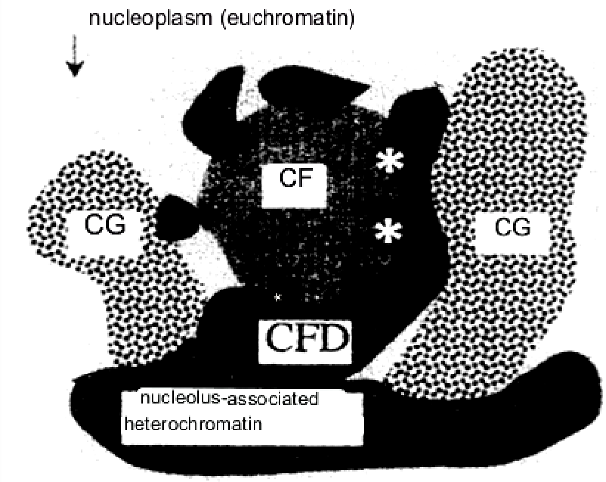

Figure 8: Ultrastructure of the nucleolus.

3.2. Chemical Composition

- CF contains the intercalary sequences of non-transcribed nucleolar DNA.

- CFD contains the transcribed sequences of active nucleolar DNA, the rRNA45S transcripts, various proteins (ribosomal proteins L 'Large' and S 'Small') associated with rRNA45S transcripts, histones, and numerous enzymes.

- CG contains maturing rRNA associated with ribosomal proteins L and S, enzymes, catalysts involved in maturation such as RNase and ribonucleoproteins or RNPs (RNA + protein), small and large ribosomal subunits, and synthesis.

3.3. Organisation

The nucleolus contains large loops of DNA that come from several chromatin fibers or chromosomes, each of which contains the amplified 45S rRNA gene. This gene is repeated multiple times in tandem (redundant gene) and is oriented in a single direction, separated each time by a non-transcribed DNA sequence. Each loop is called a "nucleolar organizer" **(Figure 9).**

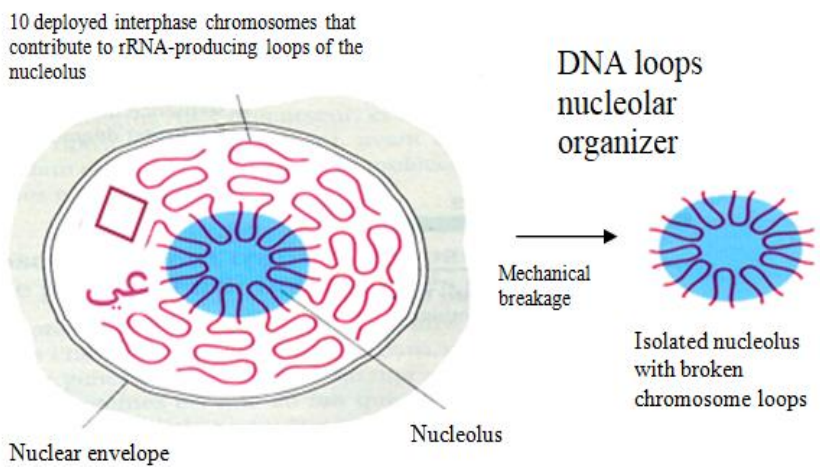

Figure 9:Diagram of a human cell showing chromatin loops containing rRNA genes from 10 interphase chromosomes.

3.4. Roles

3.4.1. Biogenesis of Ribosomal Subunits

The fundamental function of the nucleolus is the biogenesis of ribosomal subunits, and the nucleolus is the site of formation for these structures. This activity involves two steps:

- **Transcriptional Step**: In the dense fibrillar zone (at the boundaries between CF and CFD), all the genes of the 45S rRNA from all nucleolar organizers in the nucleolus start transcribing 45S rRNA (from 3' to 5') thanks to RNA polymerases **(Figure 10).** These transcripts of 45S rRNA are associated with ribosomal proteins L and S (see course notes p. 87), which have migrated into the nucleus after their synthesis in the cytosol. The transcripts of 45S rRNA associated with their L and S proteins pass into the granular zone (CG) while undergoing maturation (fragmentation) under the action of RNases and RNPs, forming various mature rRNAs (28S rRNA, 18S rRNA, and 5.8S rRNA).

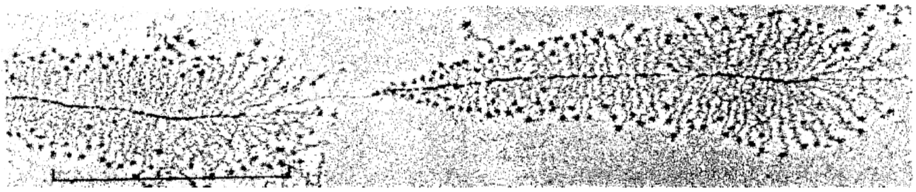

Figure10:Ultrastructure of the amplified rRNA gene.

- Assembly of ribosomal subunits:

The assembly of mature rRNAs associated with their proteins L and S takes place in the granular zone: rRNA 18S and 30 to 33 proteins S constitute the small ribosomal subunit 40S. rRNAs 28S and 5.8S and 40 to 50 proteins L, along with an rRNA 5S synthesized in the nucleoplasm from euchromatin, constitute the large ribosomal subunit 60S. The two subunits separately exit the nucleus through the nuclear pores into the cytosol, where they associate with mRNA for protein synthesis **(Figure 11).**

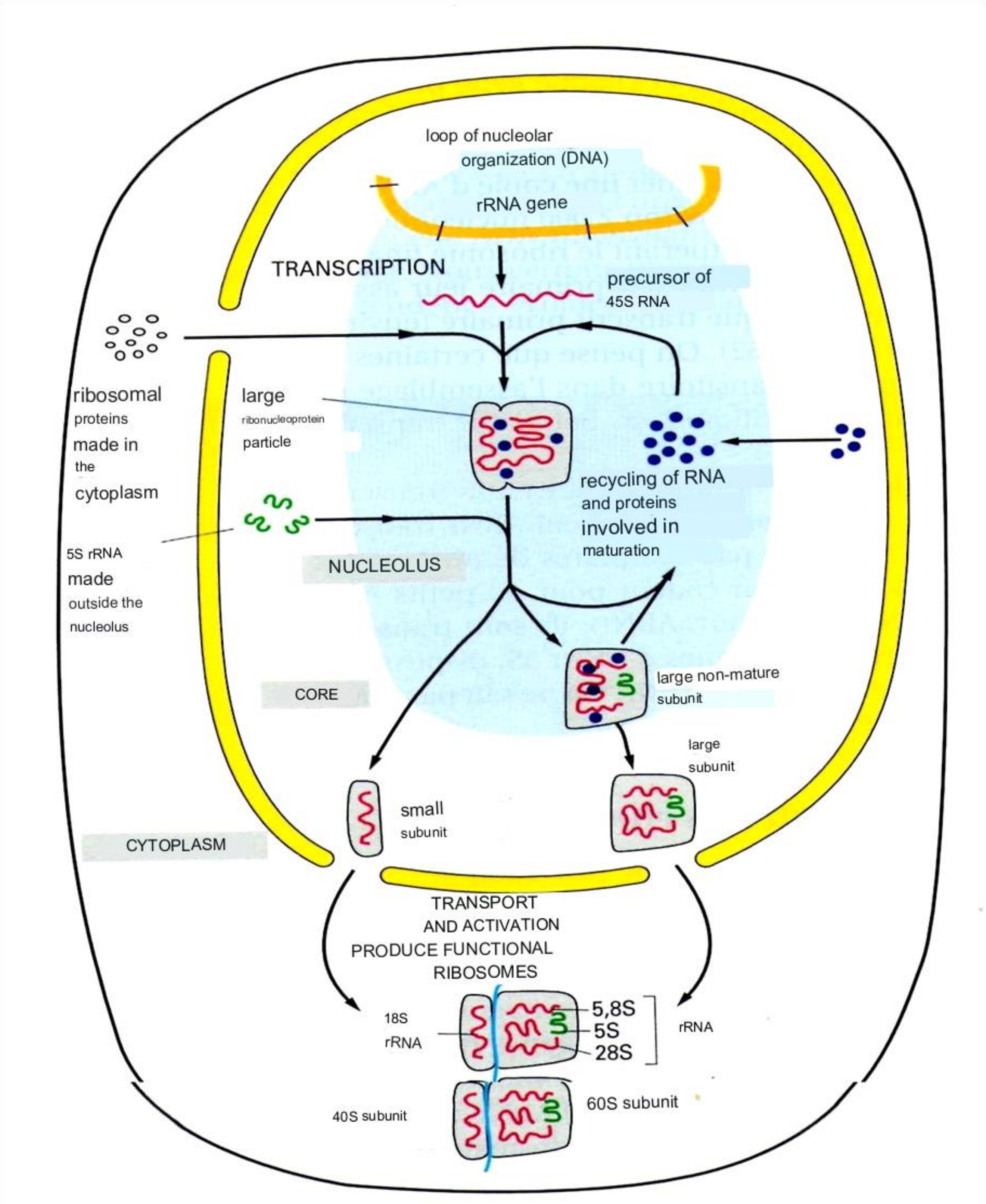

Figure11:Function of the nucleolus in ribosome synthesis.

To learn more

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