Chapter 13
The Nucleus

The nucleus is the hallmark of eukaryotic cells; the very term eukaryotic means having a "true nucleus".

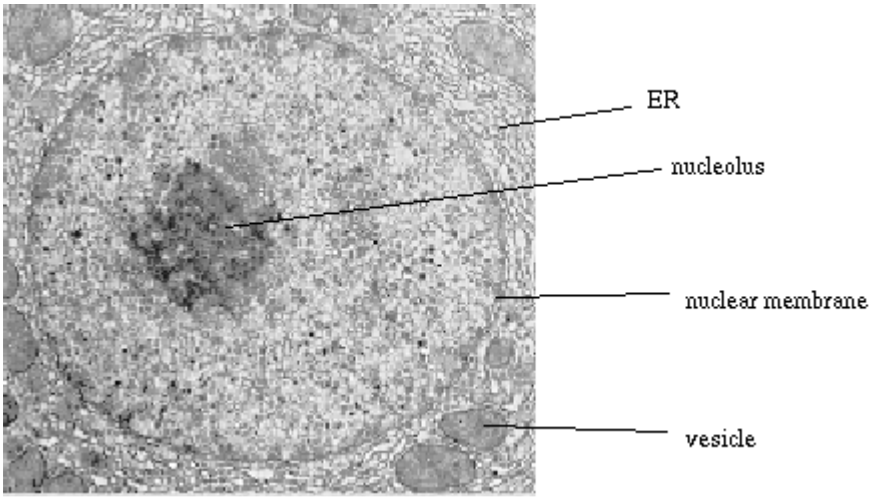

Fig.13.1. The EM of the Nucleus of a Eukaryotic Cell

13.1. The Nuclear Envelope Eukaryotic Cells

The nucleus is enveloped by a pair of membranes enclosing a **lumen** that is continuous with that of the **endoplasmic reticulum.** The inner membrane is stabilized by a meshwork of **intermediate filament proteins** called **lamins**. The nuclear envelope is perforated by thousands of **nuclear pore complexes** (**NPC**s) that control the passage of molecules in and out of the nucleus.

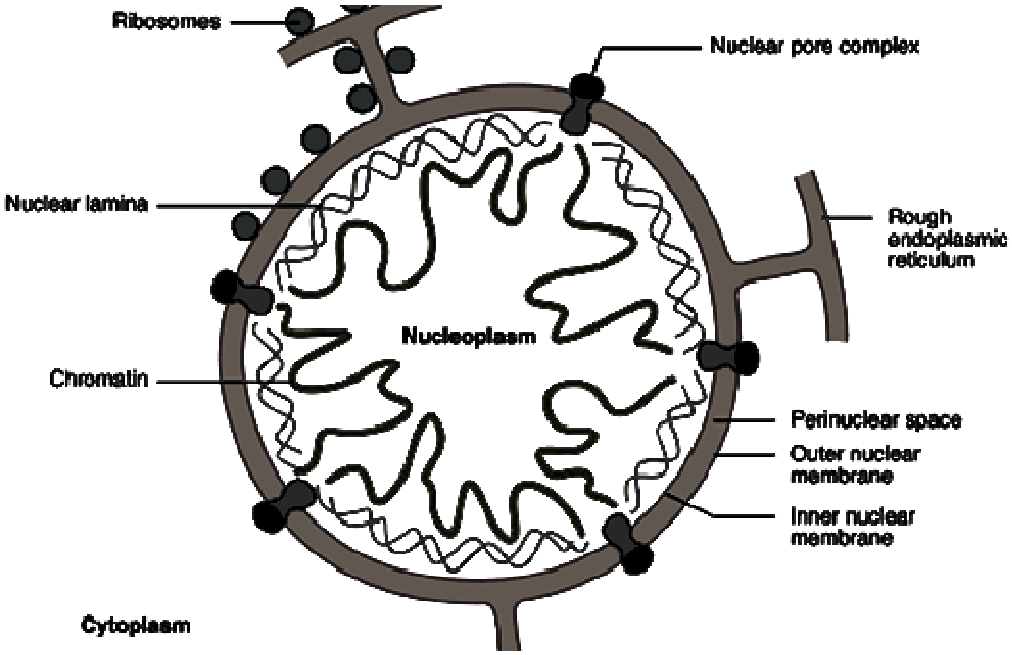

Fig.13.2. The Nuclear Envelope Eukaryotic Cells

13.2. Chromatin

The nucleus contains the **chromosomes** of the cell. Each chromosome consists of a single molecule of DNA complexed with an equal mass of proteins. Collectively, the DNA of the nucleus with its associated proteins is called **chromatin**. Most of the protein consists of multiple copies of 5 kinds of **histones**. These are basic proteins, bristling with positively charged **arginine** and **lysine** residues. (Both Arg and Lys have a free amino group on their **R group**, which attracts protons (H^+) giving them a positive charge.) Just the choice of amino acids you would make to bind tightly to the negatively-charged **phosphate groups** of DNA. Chromatin also contains small amounts of a wide variety of **nonhistone proteins**. Most of these are **transcription factors** (e.g., the **steroid receptors**) and their association with the DNA is more transient.

13.3. Nucleosomes

Two copies of each of four kinds of histones H2A, H2B, H3 and H4 form a core of protein, the **nucleosome** core. Around this is wrapped about 147 base pairs of DNA. From 20–60 bp of DNA link one nucleosome to the next. Each **linker** region is occupied by a single molecule of **histone 1** (**H1**).

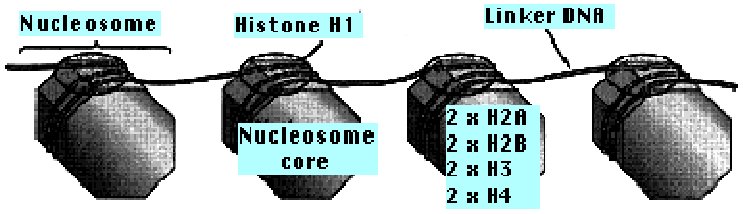

Fig.13.3. Nucleosomes

The binding of histones to DNA does not depend on particular nucleotide sequences in the DNA but does depend critically on the amino acid sequence of the histone. Histones are some of the most conserved molecules during the course of evolution. Histone H4 in the calf differs from H4 in the pea plant at only 2 amino acids residues in the chain of 102.

The formation of nucleosomes helps somewhat, but not nearly enough, to make the DNA sufficiently compact to fit in the nucleus. In order to fit 46 DNA molecules (in humans), totalling over 2 meters in length, into a nucleus that may be only 10 μm across requires more extensive folding and compaction. Interactions between the exposed "tails" of the core histones causes nucleosomes to associate into a compact fibre 30 nm in diameter. These fibres are then folded into more complex structures whose precise configuration is uncertain and which probably changes with the level of activity of the genes in the region.

13.4. Histone Modifications

Although their amino acid sequence (primary structure) is unvarying, individual histone molecules do vary in structure as a result of chemical modifications that occur later to individual amino acids. These include adding **acetyl** groups (CH_3CO-) to lysines,

phosphate groups to serines and threonines, methyl groups to lysines and arginines. Although 75–80% of the histone molecule is incorporated in the core, the remainder — at the N-terminal — dangles out from the core as a "tail". The chemical modifications occur on these tails, especially of H3 and H4. Most of theses changes are reversible. For example, acetyl groups are **added** by enzymes called **histone acetyltransferases** (**HAT**s)(not to be confused with the "HAT" medium used to make monoclonal antibodies!) and **removed** by **histone deacetylases** (**HDACs**). More often than not, acetylation of histone tails occurs in regions of chromatin that become active in gene **transcription**. This makes a kind of intuitive sense as adding acetyl groups neutralizes the positive charges on Lys thus reducing the strength of the association between the highly-negative DNA and the highly-positive histones.

But there is surely more to the story. Acetylation of Lys-16 on H4 prevents the interaction of their "tails" needed to form the compact 30-nm structure of inactive **chromatin**. But this case involves interrupting protein-protein not protein-DNA interactions. Methylation, which also neutralizes the charge on lysines (and arginines), can either stimulate or **inhibit** gene transcription in that region. Methylation of lysine-4 in H3 is associated with **active** genes while methylation of lysine-9 in H3 is associated with **inactive** genes. (These include those **imprinted genes** that have been **permanently inactivated** in somatic cells. And adding phosphates causes the chromosomes to become more — not less — compact as they get ready for mitosis and meiosis. In any case, it is now clear that histones are a dynamic component of chromatin and not simply inert DNA-packing material.

13.5. Histone Variants

We have genes for 8 different varieties of histone 1 (H1). Which variety is found at a particular linker depends on such factors as the type of cell, where it is in the **cell cycle**, and its stage of differentiation. In some cases, at least, a particular variant of H1 associates with certain transcription factors to bind to the **enhancer** of specific genes turning off expression of those genes. Some other examples of histone variants include

1. H3 is replaced by **CENP-A** ("centromere protein A") at the nucleosomes near **centromeres**. Failure to substitute CENP-A for H3 in this regions blocks centromere structure and function.
2. H2A may be replaced by the variant H2A.Z at the boundaries between euchromatin and heterochromatin.
3. All the "standard" histones are replaced by variants as **sperm** develop.

In general, the "standard" histones are incorporated into the nucleosomes as new DNA is synthesized during **S phase** of the cell cycle. Later, some are replaced by variant histones as conditions in the cell dictate.

13.6. Euchromatin versus Heterochromatin

During **interphase**, little can be seen of chromatin structure (except for special cases like the **polytene chromosomes** of *Drosophila* and some other flies). However, the density of the chromatin (that is, how tightly it is packed) varies throughout the nucleus. The dense regions are called **heterochromatin** and less dense regions are called **euchromatin**.

(a) Heterochromatin

Heterochromatin is found in parts of the chromosome where there are few or no genes, such as centromeres and telomeres is densely-packed; greatly enriched with **transposons** and other "junk" DNA; replicated late in **S phase of the cell cycle;** and has reduced crossing over in **meiosis.** Those genes present in heterochromatin are generally inactive; that is, not **transcribed** and show **increased methylation** of the cytosines in CpG islands of the DNA; **decreased acetylation** of histones and **increased methylation of lysine-9 in histone H3**, which now provides a binding site for **heterochromatin protein 1** (HP1), which blocks access by the **transcription factors** needed for gene transcription.

(b) Euchromatin

Euchromatin is found in parts of the chromosome that contain many genes is loosely-packed in loops of **30-nm fibres**. These are separated from adjacent **hetero**chromatin by **insulators**. The loops are often found near the **nuclear pore complexes**. (This would seem to make sense making it easier for the gene transcripts to get to the cytosol, but there is evidence that as gene transcription proceeds, the active DNA actually moves into the interior of the nucleus.) The genes in euchromatin are active and thus show **decreased methylation** of the cytosines in CpG islands of the DNA; **increased acetylation** of histones and **decreased methylation** of lysine-9 in histone H3.

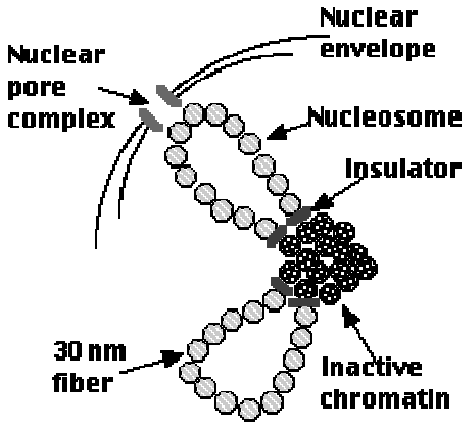

Fig.13.4. Euchromatin

The diagram represents a hypothetical model of how euchromatin and heterochromatin may be organized during **interphase** in a vertebrate cell.

13.7. Nucleosomes and Transcription

Transcription factors cannot bind to their **promoter** if the promoter is blocked by a nucleosome. For at least some genes, one of the first functions of the assembling **transcription factors** is to slide the nucleosome father along the DNA molecule exposing the gene's promoter so that the transcription factors can then bind to it. The actual transcription of protein-coding genes is done by **RNA polymerase II** (RNAP II). In order for it to travel along the DNA to be transcribed, a complex of proteins removes the nucleosomes in front of it and then replaces them after RNAP II has transcribed that portion of DNA and moved on.

13.8. The Nucleolus

During the period between cell divisions, when the chromosomes are in their extended state, 1 or more of them (10 in human cells) have loops extending into a spherical mass called the nucleolus. Here are synthesized three (of the four) kinds of **RNA molecules** (28S, 18S, 5.8S) used in the assembly of the large and small subunits of **ribosomes**. 28S, 18S, and 5.8S ribosomal RNA is transcribed (by **RNA polymerase I**) from hundreds to thousands of tandemly-arranged **rDNA genes** distributed (in humans) on 10 different chromosomes. The rDNA-containing regions of these 10 chromosomes cluster together in the nucleolus. (In **yeast**, the 5S rRNA molecules — as well as transfer RNA molecules — are also synthesized (by **RNA polymerase III**) in the nucleolus.) Once formed, rRNA molecules associate with the dozens of different ribosomal **proteins** used in the assembly of the large and small subunits of the ribosome. But all proteins are synthesized in the cytosol — and all the ribosomes are needed in the cytosol to do their work — so there must be a mechanism for the transport of these large structures in and out of the nucleus. This is one of the functions of the nuclear pore complexes.

13.9. Nuclear Pore Complexes (NPCs)

The nuclear envelope is perforated with thousands of pores. Each is constructed from a number (30 in yeast; probably around 50 in vertebrates) different proteins called **nucleoporins**.

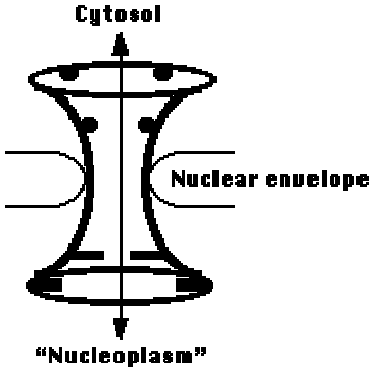

Fig.13.5. Nuclear Pore Complexe

The entire assembly forms an aqueous channel connecting the cytosol with the interior of the nucleus ("nucleoplasm"). When materials are to be transported through the pore, it opens up to form a channel some 25 nm wide — large enough to get such large assemblies as ribosomal subunits through. Transport through the nuclear pore complexes is **active**; that is, it requires energy, many different carrier molecules each specialized to transport a particular cargo and docking molecules in the NPC.

(a) Import into the Nucleus

All proteins are synthesized in the cytosol and those needed by the nucleus must be imported into it through the NPCs. Probably each of these proteins has a characteristic sequence of amino acids — called a **nuclear localization sequence** (NLS) — that targets it for entry. They include all the **histones** needed to make the nucleosomes, all the **ribosomal proteins** needed for the assembly of ribosomes, all the **transcription factors** (e.g., the **steroid receptors**) needed to turn genes on (and off) and all the **splicing**

factors needed to process pre-mRNA into mature mRNA molecules; that is, to cut out intron regions and splice the exon regions.

(b) Export from the Nucleus

Molecules and macromolecular assemblies exported from the nucleus include the **ribosomal subunits** containing both rRNA and proteins, **messenger RNA** (mRNA) molecules (accompanied by proteins), **transfer RNA** (tRNA) molecules (also accompanied by proteins) and **transcription factors** that are returned to the cytosol to await re-use. Both the RNA and protein molecules contain a characteristic **nuclear export sequence** (NES) needed to ensure their association with the right carrier molecules to take them out to the cytosol.