VIII. THE CELL NUCLEUS

VIII.1. CELL CYCLE

Introduction

Cellular proliferation is essential for normal growth and development. The most obvious example of the importance of regulated cell division events is embryogenesis. After fertilization, the zygote nucleus is formed, and the process of embryonic development begins. Over a 40 week gestation-period, millions of mitotic cell divisions occur, cellular differentiation takes place, and a human being is formed. However, there are numerous other examples for which rapid cellular division is necessary for human survival. For example, cellular proliferation must occur if humans are to repair wounds and mount effective immune responses. In addition, every day the body must replace millions of cells that are lost from normal wear and tear. If these normal repair mechanisms are blocked, by radiation damage, for example, a person will die within a few days.

Cells divide by the process of mitosis. However, mitosis, or M phase, occupies only a very small segment of the cell cycle. Most of a cell's existence is spent outside M phase in a period known as interphase. It is during interphase that cell growth, DNA synthesis, and the duplication of other important cellular constituents occur.

For a human to survive, the proliferation of cells must be tightly regulated. If a cell is able to evade the normal mechanisms that control cell growth a cancer is formed, and the effects on the person's health are devastating. Therefore, the regulation of cellular growth must be unerring. Complex mechanisms are used to govern cellular proliferation. Some molecules, such as growth factors, stimulate cellular growth and division, whereas others, such as the protein products of tumor suppresor genes, are thought to down regulate cell division events.

The General Strategy of the Cell Cycle

The **cell cycle** is defined as the period that extends from the time a cell comes into existence, as a result of cell division, until the instant that cell divides to give rise to two daughter cells.

The duration of the cell cycle varies greatly from one cell type to another. Fly embryos have the shortest known cell cycles, each lasting as little as 8 minutes, while the cell cycle of a mammalian liver cell can last longer than a year. In rapidly growing cultures of human cells, the cell cycle generally lasts for approximately 20 to 24 hours. In the human body, the cell cycle can be relatively short, as with cultured cells, or it can be much longer. For example, liver hepatocytes are thought to divide only once or twice a year.

The stages of cell cycle -The cell cycle is traditionally divided into several distinct phases, of which the most dramatic is **mitosis**, the process of nuclear division, leading up to the moment of cell division itself.

In most cells the whole of M phase takes only about an hour, which is only a small fraction of the total cycle time. The much longer period that elapses between one M phase and the next is known as **interphase.** Under the microscope this appears, as an uneventful interlude in which the cell simply grows in size. But other techniques reveal that interphase is actually a busy time for the proliferating cell, during which elaborate preparations of cell division are occurring in a closely ordered sequence. In particular, it is during interphase that the DNA in the nucleus is replicated.

Replication of the nuclear DNA usually occupies only a portion of interphase, called the **S phase** of the cell cycle (S = synthesis).The interval between the completion of mitosis and the beginning of DNA synthesis is called the **G_1 phase** (G = gap), and the interval between the end of DNA synthesis and the beginning of mitosis is called the **G_2 phase**. G_1 and G_2 provide additional time for growth: if interphase lasted only long enough for DNA replication, the cell would not have time to double its mass before it divided. During G_1 the cell monitors its environment and its own size and, when the time is ripe, takes a decisive step that commits it to DNA replication and completion of a division cycle. The G_2 phase provides a safety gap, allowing the cell to ensure that DNA replication is complete before it plunges into mitosis. G_1, S, G_2, and M are traditional subdivisions of the **standard cell cycle.** But there are some cells that whose cell cycle does not conform to this standard scheme.

Because cells require time to grow before they divide, the standard cell cycle is generally quite long -12 hours or more for fast growing tissues in a mammal, for example. Although the lengths of all phases of the cycle are variable to some extent, by far the greatest variation, in most of the commonly studied types of cells, occurs in the duration of G_1. Cells in G_1, if they have not yet committed themselves to DNA replication, can pause in their progress around the cycle and enter a specialized resting state, often called G_0 (G zero), where they can remain for days, weeks, or even years before resuming proliferation.

In conditions that favour growth, the total protein content of a typical cell increases more or less continuously throughout the cycle. Likewise, RNA synthesis continues at a steady rate, except during M phase, when the chromosomes are apparently too condensed to allow transcription. When the pattern of synthesis of individual proteins is analyzed, the vast majority are seen to be synthesized throughout the cycle. For most of the constituents of the cell, therefore, growth is a steady, continuous process, interrupted only briefly at M phase, when the nucleus and then the cell divide into two.

DNA synthesis and the visible events of mitosis are not the only discrete processes occurring against this background of continuous growth. The centrosome, for example, has to be duplicated in preparation for mitosis, so as to form the two poles of the mitotic spindle. And production of a few key proteins is switched on at a high rate at a specific stage of the cycle. Histones, which are required for the formation of new chromatin, are made at high rate only in S phase, and the same is true for some of the enzymes that manufacture deoxyribonucleotides and replicate DNA.

The turning on and off of genes and the starting and stopping of processes such as DNA synthesis and mitosis are the overt consequences of a much less easily observed series of sudden transitions in the state of the cell-cycle control.

The Control of the Cell Cycle

The cell cycle it is regulated at certain critical points of the cycle by feedback from the processes that are being performed.

The cell-cycle control system is a cyclically operating biochemical device constructed from a set of interacting proteins that induce and coordinate the essential *downstream processes* that duplicate and divide the cell's contents ("downstream" in this context meaning simply that they occupy a subordinate position in the hierarchy of cell-cycle control). In the standard cell cycle the control system is regulated by brakes that can stop the cycle at specific *checkpoints*. The brakes are important because they allow the cell-cycle control system to be regulated by signals from the environmental controls generally act on the control system at one or other of

two major checkpoints in its cycle -one in G_1, just before entry into S phase; the other in G_2, at the entry to mitosis. In higher eukaryotic cells signals that arrest the cycle usually act at the G_1 control point. This checkpoint is called *start* in yeast, and in mammalian cells we call it simply the G_1 checkpoint. When circumstances forbid cell division, it is at this point in the cycle that many cells halt. In a continuously cycling cell the G_1 checkpoint is the point where the cell-cycle control system triggers a process that will initiate S phase, and the G_2 checkpoint is where it triggers a process that will initiate M phase.

The Cell-Cycle Control System Is a Protein -Kinase-Based Machine

The mechanisms that drive the cell past the G_2 checkpoint into M phase is the similar to the mechanism who operates at the G_1 checkpoint, but the components are different.

The cell-cycle control system is based on two key families of proteins. The first is the family of **cyclin- dependent protein kinases (Cdk** for short), which induce downstream processes by phosphorylating selected proteins on serines and threonines. The second is a family of specialized activating proteins, called **cyclins** that bind to Cdk molecules and control their ability to phosphorylate appropriate target proteins.

The cyclic assembly, activation, and disassembly of cyclin-Cdk complexes are the pivotal events driving the cell cycle. Cyclins are so called because they undergo a cycle of synthesis and degradation in each division cycle of the cell. There are two main classes of cyclins**: mitotic cyclins**, which bind to Cdk molecules during G_2 and are required for entry into mitosis, and **G_1 cyclins**, which bind to Cdk molecules during G_1 and are required for entry into S phase.

In the mammalian cells there are at least two different Cdk proteins, one for each checkpoint.

In outline, the events that drive the cell into mitosis are as follows:

Mitotic cyclin accumulates gradually during G_2 and binds to Cdk to form a complex known as **M-phase-promoting factor (MPF).** This complex is at first inactive, but through the action of other enzymes that phosphorylate and dephosphorylate it, it is converted to an active form. The ultimate activation of MPF is almost explosive. This is belived to be due to a positive feedback mechanism whereby active MPF increases the activity of the enzymes that activate MPF: thus the concentration of active MPF builds up at an accelerating pace until a critical flashpoint is reached, whereupon a flood of active MPF triggers the downstream events that propel the cell into mitosis. MPF is inactivated equally suddenly by the degradation of mitotic cyclin at the metaphase-anaphase boundary, enabling the cell to exit from mitosis.

Each step of Cdk activation or inactivation marks a **cell-cycle transition** and presumably has an effect on the cell-cycle control system it self, initiating reactions that will eventually lead it to trigger the next downstream process. The mechanism operating at the G_1 checkpoint is much less well understood than that at the G_2 checkpoint, but the principles are believed to be similar: just as the assembly of MPF ultimately triggers the events of mitosis, so the assembly of a related complex comprising a Cdk protein and G_1 cyclin is thought to drive the cell past the G_1 checkpoint, triggering the events that lead to DNA replication. The downstream events induced by the activation of Cdk at the G_1 and G_2 checkpoints are completely different. The particular proteins that are phosphorylated by activated Cdk protein are therefore thought to depend on the cyclin component of the complex.

According to how cell go through the cell cycle, cells can be:

1. Static cell populations consist of cells that no longer divide, such as cells of the central nervous system, and skeletal muscle cells. Those cells are stopped in G_1.

2. Stable cell populations consist of cells that divide episodically and at slow rates to maintain normal tissue or organ structure and that may be stimulated by injury to become more mitotically active.

VIII.2. THE CELL INTERPHASE NUCLEUS

According to the cell cycle there are two types of nucleus:
- the nucleus in interphase (metabolic state)
- the nucleus in division (genetic state)

VIII.2.1.MORPHOLOGICAL CHARACTERISTICS OF INTERPHASE NUCLEUS

a) The position of the nucleus

- usually the nucleus occupies a central position inside the cell.
- in cell that accumulates the reserve substances, like in fat cell, the nucleus is squeezed at the cell periphery. The nucleus has an eccentric position.
- in cell that accumulates granules of secretion, as in glands cells, the nucleus has a basal position.

b) The shape. The shape of the nucleus corresponds in general to the form of the shape that contains that nucleus. Ex: spherical cell has a spherical nucleus, columnar cell has an ovoid nucleus, and longitudinally shaped cell (muscle cell) has a flattened shaped nucleus. There are also cells with irregular shaped nucleus like polimorphonuclear leucocytes.

c) The number. Most human cells contain a nucleus (mononucleated cells). A notable exception is adult erythrocyte which lacks a nucleus. Erythrocyte precursors in the bone are nucleated cells.

There are also cells with more nuclei (multinucleated cells): striated muscular fibre, osteoblasts, and in pathological cases like in cancer.

d) The dimensions. The nucleus size and volume vary with the cellular type. The dimensions also depend on the cell metabolic activity and the age of the cell. The dimensions are between 4-25µ. Ex: the spermatozoon's nucleus has 4µ and the ovum nucleus has 20µ.

VIII.2.2.THE ULTRASTRUCTURE OF THE INTERPHASE NUCLEUS

The sequestering of nearly the entire cellular DNA in the nucleus marks the major difference between eukaryotic and prokaryotic cells.

The nucleus of a nondividing cell, also called an interphase cell, consists of the following components:

- ***membranous nuclear envelope***
- ***nuclear skeleton***
- ***chromatin***
- ***nucleolus (nucleoli)***
- ***nucleoplasm***

VIII.2.2.1. The nuclear envelope

The nuclear envelope encloses the DNA and defines the nuclear compartment. It is formed from two concentric membranes that are continuous with the endoplasmic reticulum. Although the inner and outer nuclear membranes are continuous, the two membranes maintain distinct protein compositions.

The **inner nuclear membrane** contains specific proteins that act as binding sites for the feltlike nuclear lamina that supports it. The inner membrane is surrounded by the **outer nuclear membrane**, which closely resembles the membrane of the rough endoplasmic reticulum. Like the membrane of the rough endoplasmic reticulum, the outer nuclear membrane is studded with ribosomes engaged in protein synthesis. The proteins made on these ribosomes are transported into the space between the inner and outer nuclear membranes (the perinuclear space), which is continuous with the ER lumen.

Bidirectional traffic occurs continuously between the cytosol and the nucleus. The many proteins that function in the nucleus-including histones, DNA, and RNA polymerases, gene regulatory proteins, and RNA-processing proteins-are selectively imported into the nuclear compartment from the cytosol where they are made. At the same time, tRNAs and mRNAs are synthesized in the nuclear compartment and then exported to the cytosol. Like the import process, the export process is selective; mRNAs, for example, are exported only after they have been properly modified by RNA-processing reactions in the nucleus. In some cases the transport process is complex: ribosomal proteins are made in the cytosol, imported into the nucleus-where they assemble with newly made ribosomal RNA into particles- and then exported again to the cytosol as part of a ribosomal subunit; each of these steps involves selective transport across nuclear envelope.

The nuclear envelope in all eukaryotes, from yeasts to humans, is perforated by **nuclear pores**. Each pore is formed by a large, elaborate structure known as the **nuclear pore complex**, which has an estimated molecular mass of about 125 million and is thought to be composed of more than 100 different proteins, arranged with a striking octagonal symmetry.

The pore complex penetrates the double membrane of the nuclear envelope bringing together the lipid bilayers of the inner and outer nuclear membranes at the boundaries of each pore. Although this would seem to allow exchange of components (proteins, phospholipids) between those two membranes, evidence now indicates that these two membranes remain chemically distinct. Therefore, the protein components of the nuclear pore complex must provide a barrier preventing bulk exchange between these two membranes, in addition to providing a channel of communication between the nuclear compartment and the cytoplasm of the cell.

Measurements of nuclear pore complexes have demonstrated that they are highly organized structures, with an outside diameter of approximately 100 nm and an internal channel of 9 to 10 nm in diameter.

In cross-section the nuclear pore complex appears composed of three parts: (1) a column component which forms the bulk of the pore wall; (2) an annular component, which extends "spokes" toward the center of the pore and (3) a luminal component, which is formed by a large transmembrane glycoprotein that is thought to help anchor the complex to the nuclear membrane. In addition, fibrils protrude from both the cytosolic and nuclear sides of the complex. On the nuclear side the fibrils converge to form cagelike structures.

In general the more active the nucleus is in transcription, the grater the number of pore complexes its envelope contains. The nuclear envelope of a typical mammalian cell contains 3000 to 4000 pore complexes. If the cell is synthesizing DNA, it needs to import about 10^6 histone molecules from the cytosol every 3 minutes in order to package newly made DNA into chromatin, which means that, on average, each pore complex needs to transport about 100 histone molecules per minute. If the cell is growing rapidly, each pore complex also needs to transport about 6 newly assembled large and small ribosomal subunits per minute from the nucleus, where they are produced, to the cytosol, where they are used.

The properties of transport through the nuclear pores have been addressed by injection of radiolabeled compounds into the cytosol and examining the rate of their appearance into the nucleus. Such experiments have demonstrated that the nuclear pores are freely permeable to ions and small molecules, including proteins with a diameter smaller than 9nm (60kDa or less relative molecular mass, M_r). Nonnuclear proteins larger than 9 nm in diameter (> 60 kDa) are excluded from nuclear transit. However, nuclear resident proteins that are synthesized in the cytoplasm and are larger than 60kDa are readily transported into the nucleus, indicating that there must be mechanisms for the selective transport of molecules across the nuclear envelope.

The selectivity of the nuclear protein import resides in **nuclear localization signals,** which are present only in nuclear proteins. The signals have been precisely defined in many nuclear proteins using recombinant DNA technology. They can be located almost anywhere in the amino acid sequence and generally consist of a short sequence (typically from four to eight amino acids) that varies for different nuclear proteins but is rich in the positively charged amino acids lysine and arginine and usually contains proline.

Macromolecules are actively transported into and out of the nucleus through nuclear pores. The initial interaction of a nuclear protein with the nuclear pore complex requires one or more cytosolic proteins that bind to the nuclear localization signals and help direct the nuclear protein to the pore complex, where it appears to bind to the fibrils that project from the rim of the complex. The nuclear protein then moves to the center of the pore complex, where it is actively transported across the nuclear envelope by a process that requires ATP hydrolysis.

The proteins and structures involved in the active transport process are not known. A diverse set of related cytosolic proteins is required for the initial binding of nuclear proteins to the complex. These proteins, called **nucleoporins**, contain a simple sugar (N-acetylglucosamine) that aided their identification through the use of lectins and specific antibodies.

VIII.2.2.2. Nuclear skeleton - nuclear lamina

The nuclear lamina lies adjacent to the inner surface of the nuclear envelope, between the membrane and the marginal heterochromatin.

The fibrous (nuclear) lamina, a thin, electron-dense protein layer, has a supporting or "nucleoskeletal" function. If the membranous component of the nuclear envelope is disrupted by exposure to detergent, the fibrous lamina remains, and the nucleus retains its shape.

The **nuclear lamina** is a meshwork of intermediate filaments. It is typically 10-20 nm thick and is interrupted in the region of nuclear pores to provide a passageway for macromolecules entering and leaving the nucleus. In mammalian cells the nuclear lamina is composed of **lamins (A, B, C)**, which are homologous to other intermediate filament proteins but differ from them in at least four ways: (1) Their central rod domain is somewhat longer. (2) They contain a nuclear transport signal that directs them from the cytosol, where they are made, into the nucleus. (3) They assemble into a two-dimensional sheetlike lattice, which is thought to require their association with other proteins. (4) The meshwork they form is unusually dynamic and rapidly disassembles at the start of mitosis and reassembles at the end of mitosis; the disassembly and reassembly are mediated by the phosphorylation and dephosphorylation of several serine residues on the lamins.

The nuclear lamina is thought to give shape and stability to the nuclear envelope, to which it is anchored by the attachment to both the nuclear pore complexes and the inner nuclear membrane. As the chromatin is also thought to interact directly with the nuclear lamina, the lamina provides a structural link between the DNA and the nuclear envelope.

VIII.2.2.3. Nuclear matrix - nucleoplasm

Definition: Nucleoplasm is the material enclosed by the nuclear envelope exclusive of the chromatin and the nucleolus.

When isolated nuclei are subjected to extraction in high-ionic-strength, neutral-detergent-containing buffers to remove most of the internal components, a fibrous network of proteins, and the nuclear matrix remains intact and roughly maintains the outward appearance of the nucleus.

Composition: The **nuclear matrix** is defined biochemically as a structure containing 10% of the total nuclear proteins, 30% of the nuclear RNA, 1% to 3% of the total DNA, 3% of the nuclear phospholipids.

Electron microscopy of nuclear matrix preparations show that they comprise mainly fibrilar elements that remain associated with identified nuclear structure, such as the nuclear pore complex and lamina.

Functions: Although defined structurally and biochemically, the function of the nuclear matrix is unclear. Perhaps its most obvious role would be to provide organization and structure to the internal nuclear compartment. Newly replicated DNA and the enzymatic components necessary for DNA synthesis are associated with the matrix, suggesting a role in the organization of the DNA replication machinery. Recent evidence indicates that genes that are being transcribed and the products of their transcription (ex. heterogeneous nuclear RNA) are enriched in nuclear matrix preparations. Localization of RNA transcripts in the nucleus using us fluorescent -labeled nucleic acid probes has shown that the RNAs follow tracks in the nuclear compartment, with a more intense fluorescent signal seen near the nuclear borders. Thus, following transcription, RNAs do not diffuse within the nucleoplasm, but are possibly bound to nuclear matrix fibers as they are spliced to form mature mRNAs bound for the cytoplasm. These experiments support the concept that the nuclear matrix plays an important role in the organization of the nuclear compartment. However, the nature of the individual nuclear matrix components and their interactions remain to be elucidated.

VIII.2.2.4. The nucleolus

Definition: The nucleolus is a ribosome -producing machine.

Structure

When interphase cells are examined under the microscope, the most prominent feature observed in the nucleus is a dense structure, termed the **nucleolus.** Consequently, it was so closely scrutinized by early cytologists that an 1898 review could list some 700 references. By the 1940s cytologists had demonstrated that the nucleolus contains high concentrations of RNA and proteins, but its major function in ribosomal RNA synthesis and ribosome assembly was not discovered until the 1960s.

Some of the details of nucleolar organization can be seen in the electron microscope. Unlike the cytoplasmic organelles, the nucleolus is not bounded by a membrane; instead, it seems to be constructed by the specific binding of unfinished ribosome precursors to one another to form a large network. In typical electron micrograph four partially segregated regions can be distinguished:

- (1) a pale -staining **fibrilar center,** which contains DNA that is not being actively transcribed;
- (2) a **dense fibrilar component**, which contains RNA molecules in the process of being synthesized;

- (3) a **granular component,** which contains maturing ribosomal precursor particles;
- (4) a **nucleolar matrix**, which is a fibrous network that may participate in the organization of nucleolus.

The size of the nucleolus reflects its activity. Its size therefore varies greatly in different cells and can change in a single cell. It is very small in some dormant plant cells, but can occupy up to 25% of the total nuclear volume in cells that are making unusually large amounts of protein. The differences in size are due largely to differences in the amount of the granular component, which is probably controlled at the level of ribosomal gene transcription: electron microscopy of spread chromatin shows that both the fraction of activated ribosomal genes and the rate at which each gene is transcribed can vary according to circumstances.

Functions

The continuous transcription of multiple gene copies ensures an adequate supply of the runes, which are immediately packaged with ribosomal proteins to form ribosomes. This packaging occurs in the nucleus, in a large, distinct structure called **nucleolus.** The nucleolus contains large loops of DNA emanating from several chromosomes, each of which contains a cluster of rRNA genes. Each such gene cluster is known as a **nucleolar organizer** region. Here the rRNA genes are transcribed at a rapid rate by RNA polymerase I. The beginning of the rRNA packaging process can be seen in electron micrographs of these genes: the 5' tail of each transcript is encased by a protein-rich granule. These granules, which do not appear on other types of RNA transcripts, presumably reflect the first of the protein-RNA interactions that take place in the nucleolus.

The intact 45S transcript is first package into a large complex containing many different proteins imported from the cytoplasm, where all proteins are synthesized. Most of the 80 different polypeptide chains that will make up the ribosome, as well as the 5S rRNAs, are incorporated at this stage. Other molecules are needed to process the 45S rRNA and to guide the assembly process. Thus the nucleolus also contains other RNA-binding proteins and certain small ribonucleoprotein particles that are believed to help catalyze the construction of ribosomes. These components remain in the nucleolus when the ribosomal subunits are exported to the cytoplasm in finished form. An especially notable component is **nucleolin**, an abundant, well-characterized RNA-binding protein that seems to coat only ribosomal transcripts; this protein stains with silver in the characteristic manner of the nucleolus itself.

As the 45S rRNA molecule is processed, it gradually loses of its RNA and protein and then splits to form separate precursors of the large and small ribosomal subunits.

Within 30 minutes, the first mature small ribosomal subunits, containing their 18S rRNA, emerge from the nucleolus and appear in the cytoplasm. Assembly of the mature large ribosomal subunit, with its 28S, 5.8S, and 5S rRNAs, takes about an hour to complete. The nucleolus therefore contains many more incomplete large ribosomal subunits than small ones. The last steps in ribosome maturation occur only as these subunits are transferred to the cytoplasm. This delay prevents functional ribosomes from gaining access to the incompletely processed mRNA molecules in the nucleus.

The nucleolus is reassembled on specific chromosomes after each mitosis

The appearance of the nucleolus changes dramatically during the cell-division cycle. As the cell approaches mitosis, the nucleolus first decreases in size and then disappears as the

chromosomes condense and all RNA synthesis stops, so that generally there is no nucleolus in a metaphase cell. When ribosomal RNA synthesis restarts at the end of mitosis (in telophase), tiny nucleoli reappear at the chromosomal locations of the ribosomal RNA genes.

In humans the ribosomal RNA genes are located near the tips of each 5 different chromosomes (the pairs of chromosomes 13, 14, 15, 21, 22). Correspondingly, 10 small nucleoli form after mitosis in a human cell, although they are rarely seen as separate entities because they quickly grow and fuse to form the single large nucleolus typical of many interphase cells.

RNA and protein components of the disassembled nucleolus during mitosis, it seems that some of them become distributed over the surface of all of the metaphase chromosomes and are carried as cargo to each of the two daughter cell nuclei. As the chromosomes decondense at telophase, these "old" nucleolar components help reestablish the newly emerging nucleoli.

VIII.2.2.5. The chromatin

The genome of the cell is sequestered in the nuclear compartment. The nucleus contains almost of the genetic information of the cell in the form of DNA. DNA is composed of four nucleotides: two are purines that have a double -ring structure (adenine and guanine), and two are pyrimidines that have a single -ring structure (thymine and cytosine). The basic structure of DNA, derived in 1953 by Watson and Crick, is that of two polynucleotide chains that are held together by hydrogen bonds between adenine and thymine (A-T base pairing) and guanosine and cytosine (G-C base pairing). The two chains are antiparallel or complementary and are coiled into a double helix of approximately 2 nm in diameter. The information is stored in "words" consisting of three nucleotides, termed the **codons**, of the genetic code. The DNA within the cell nucleus is associated with a variety of nuclear proteins, and the DNA-protein complex is referred to as **chromatin**. Proteins associated with the DNA can be divided into two general categories: a) the nonhistones chromosal proteins and b) the histones.

a) **Nonhistone proteins** are heterogeneous class of polypeptides that includes:

- structural proteins (the high-mobility group of proteins; HMG);
- regulatory proteins (those that appear to have a direct role in gene regulation);
- enzymes needed for nuclear function (RNA polymerases, DNA polymerases).

b) **Histones** are found only in eukaryotic cells and are by far the most abundant proteins present in the nucleus. Their total mass in chromatin is about equal to that of the DNA. Histones are relatively small proteins that are rich in positively charged amino acids (arginine and lysine), which gives them an overall strong positive charge (basic) that enables them to bind tightly with the negatively charged (acidic) DNA molecules. There are five types of histones, designated H1, H2A, H2B, H3, and H4. Four of the histones, H2A, H2B, H3, and H4 are termed the **nucleosomal histones**, as they are responsible for formation of the inner core of a DNA-protein complex called **the nucleosome.**

The **nucleosome** is the basic unit of chromatin fiber and gives chromatin the beads-on-a-string appearance in electron micrograph. Examination of the structure of histone -DNA chromatin complexes has relied on digestion of the chromatin with nonspecific nuclease. These studies have shown that the basic structure of chromatin can be resolved into a repeating unit, called the nucleosomal bead. Each nucleosome bead is formed from an octamer of proteins containing two copies each of the H2A, H2B, H3, and H4 histones, around which is wrapped about 150 nucleotide pairs of DNA. This is the amount of DNA that will make two complete turns around the octamer core of nucleosomal histones, forming a chromatin fiber that is approximately 11 nm in diameter.

Because it contains the simplest arrangement of DNA and protein, the 11-nm chromatin fiber can be considered the basic unit of chromatin packaging in the nucleus. Only a small portion of the DNA is found packaged as an 11-nm fiber in an interphase cell and is probably limited to those regions of DNA that are actively transcribing gene sequence. When nuclei are treated very gently and examined by electron microscopy, most of the chromatin is found in a fiber that measures 30nm in diameter. This 30-nm chromatin fiber is thought to represent the packaging of the nucleosomes by the remaining histone, H1. One model that accounts for the formation of the 30-nm chromatin fiber is the cooperative binding of H1 molecules to nucleosomal DNA. Each histone H1 molecule binds through the central region of the molecule to a unique site on the nucleosome and extends to contact sites on adjacent nucleosomes. This cooperative binding would compact the nucleosomes such that they are pulled together into regular-repeating arrays, forming the 30-nm chromatin fiber.

Chromatin inside a eukaryotic cell nucleus in interphase has been divided into two classes based on its state of condensation. Chromatin that is highly condensed and considered to be transcriptionally inactive is referred to as **heterochromatin**. In electron micrograph of interphase nuclei, the heterochromatin is generally concentrated in a band around the periphery of the nucleus and around the nucleolus. The amount of heterochromatin present in the nucleus is correlated with the transcriptional activity of the cell. That is, little heterochromatin is present in transcriptionally active cells, whereas nuclei of mature spermatozoa, a transcriptionally inactive cell, contains predominantly highly condensed chromatin. In a typical eukaryotic cell, about 90% of the chromatin is thought to be transcriptionally inactive. This amount of inactive chromatin is much more than can be accounted for as the highly condensed heterochromatin. Therefore, heterochromatin is thought to be a special class of inert chromatin that may have specialized functions. For example, the DNA near the centromere region is composed of repetitive DNA, and these sequences appear to constitute a major portion of the heterochromatin DNA. The remaining 10% of chromatin that is transcriptionally active is found in a more extended dispersed conformation and is called **euchromatin**. Euchromatin is responsible for proving the RNA molecules that exit the nucleus and encode the proteins of the particular cell type.

Chromosomes are visible as distinct units in the light microscope when the chromatin is extensively condensed at mitosis. As a 30-nm fiber, the chromatin could not account for the degree of DNA condensation in methaphase chromosomes. Consequently, higher-order packaging units are required to achieve this state. From studies examining the appearance of specialized chromosomes, it is thought that regions of the chromosome are present as extended loops of the 30-nm chromatin fiber held together at base of the loop by a specific protein-DNA complex. The model of chromatin condensation presented in the figure shows that to account for the size of the typical human chromosome (~1,4 μm) in its most condensed state, the extended loop structures of the chromatin must be condensed again, possibly by drawing in the loop domains to form a tightly wound helical formation . Thus, to achieve the compaction necessary to fit the $\sim 10^7$ base pair of the DNA in the individual chromosomes into the ~ 1.4-μm chromosomes seen at metaphase there must be at least 4 orders of packaging of the DNA above the 2 -nm double helical chain of the DNA molecule.

The human genome contains 46 chromosomes in each diploid cell; one of the sex determined chromosomes and 22 pairs of autosomal chromosomes. Cytological methods, involving staining of fully condensed metaphase chromosomes with various stains or dyes, have been useful for the identification of individual chromosomes. For example, staining metaphase chromosome with the Giemsa reagent results in a very characteristic pattern of bands on each

chromosomal unit, termed G banding. Once the chromosomes have been stained, they can be examined under the microscope. The display of the chromosomes prepared in such a manner is referred to as the **karyotype** of the organism. The normal human female karyotype is 46,XX; and the male is 46,XY. With the aid of these methods is have been possible to correlate a variety of human syndromes with abnormalities in chromosomes number, (ex. Down syndrome which is characterize by the presence of an additional chromosome 21) or abnormalities that result in the loss or movement of a particular region of an individual chromosome in the genome (ex.-cri du chat syndrome, which results from the loss of a portion of the small arm of chromosome 5). Thus, karyotype examination provides a powerful tool for the recognition of chromosomal abnormalities associated with particular genetic diseases and is a particularly useful technique for prenatal diagnosis for such disorders.

The Barr body can be used to identify the sex of the fetus.

Some chromosomes are repressed in the interphase nucleus and exist only in the tightly packed heterochromatic form. One X chromosome of the female is an example of such of chromosome. This fact can be used to identify the sex of the fetus. This chromosome was discovered in 1949 by Barr and Bertram in nerve cells of female cats, where it appears as a well stained round body, now call the Barr body, adjacent to the nucleolus. In cells of the oral mucous membrane, the Barr body is located adjacent to the nuclear envelope.

VIII.3. THE CHROMOSOMES

The nucleus of a typical human cell is about 5-8 μm in diameter and contain about 2 meters of DNA. DNA bound to nucleosomes undergoes additional folding and packing to form the 30 nm fiber, the folding mechanism and structures of this and the larger structures are not well understood. Higher orders of chromatin coiling include the formation of transcriptionally active DNA (euchromatin) into loops that condenses. The complex task of packaging DNA is accomplished by specialized proteins that bind to and fold the DNA, generating a series of coils and loops that provide increasingly higher levels of organization and prevent the DNA from becoming an unmanageable tangle. DNA is compacted in a way that leaves it accessible to all of the enzymes and other proteins required for transcription, DNA replication, and DNA repair.

At the time of cell division, chromatin becomes condensed into the chromosomes. The length of a metaphase chromosome varies between 1.5 μ - 8μ.The state of condensation of the chromosomes thus varies according to the cell cycle which goes through a series of stages. But, chromosomes are almost always depicted as they shown, and chromosomes are in fact in a highly condensed state that normally occurs only for a brief period, in division. At all other times, the chromosomes are extended as long, thin, tangled threads of DNA in the nucleus and cannot be distinguished in the light microscope. Future packaging during the early phase of cell division causes chromosomes to become visible by light microscopy after staining.

Chromosomes can be clearly visualized with the optical microscope, so they were the subject after their discovery in 1876 and by 1910 it became clear that genetic phenomena could be explained in terms of chromosomes behavior. In the past, chromosomes were routinely stained with Giemsa stain; however, with the recent development of in situ hybridization techniques, the fluorescent in situ hybridization (FISH) procedure is now more often used to visualize a chromosomal spread. These spread are observed with fluorescence microscopes, and computer-controlled cameras are then used to capture images of the chromosomes pairs.

Human cells, with the exception of the germ cells, each contain two copies of each chromosome, one inherited from the mother and one from the father; the maternal and paternal chromosomes of a pair are called homologous chromosomes. The only nonhomologous chromosomes pairs are the sex chromosomes in males, where a Y chromosome is inherited from the father and an X chromosome from the mother.

The morphology of the chromosomes

The morphology of the chromosomes is best studied during metaphase and anaphase (phases of division), which are the periods of maximal contraction. Each long DNA replication two chromatids held together by complexes of cohesion proteins make up each chromosome.

Chromosome in metaphase consists of the following structural elements considered obligatory: chromatids, telomere, centromer.

Chromatides are two identical longitudinal subunits in size and shape, each containing one DNA molecule.

Telomere are the rounded ends of chromatids that play a role in maintaining their chromosome structure and individuality .Telomere is found at each of the two ends of a chromosomes. Telomeres contain repeated nucleotides sequences that enable the ends of chromosomes to be replicated. They are important structures because they are the terminators of the DNA replication bifurcation and have genes for the synthesis of ribosomal and transport RNAs, and are also involved in the mechanisms of apoptosis.

Centromer represents the place of union of the two sister chromatids at primary constriction. Primary constriction is the area at which chromatids are more narrowed. Based on conventional techniques, primary constrictions are poorly stained. Chromosomal shape is determined by the position of the centromere. This is the region of the chromosomes that becomes attached to the mitotic spindle. The centromere lies within a thinner segment of the chromosome, the primary constriction. The region flanking the centromere frequently contains highly repetitive DNA and may stain more intensely with basic dyes (heterochromatin). During mitosis, a protein complex called **kinetochore** is formed at the centromere and attaches the duplicated chromosomes to the spindle.

The structural elements present only on certain chromosomes are:

- secondary constrictions,

- satellites.

Secondary constrictions are morphological elements present only in certain pairs of human chromosomes with localization:

- terminal, in the distal region of the short arms, for acrocentric chromosomes, act as a nucleolar organizer

- proximal, in the proximal region of long chromosome arms for chromosome 1, 9, 16 pairs.

Satellites are located in the distal portion of the short arm of the acrocentric chromosomes and are round or oval in shape.

The number and morphology of the chromosomes are characteristic of each species. In humans, somatic cells (cells of most tissues) contain 46 chromosomes, are called diploids. Sperm cells and mature oocytes (germ cells) are haploid (contain only 23 chromosomes, with half the diploid number of chromosomes), each pair having been separated during meiosis. The chromosome diploid set is 2n, and the haploid set is n. The 46 chromosomes in human diploid cells are available in 23 pairs. Within a pair, the chromosomes are identical in size and shape, encode the same genes, but are different in origin, one originally derived from the mother and one derived from the father. The members of each chromosomal pair are called homologous

because, although from different parents, they contain forms (alleles) of the same genes. Cells of most tissues (somatic cells) are considered diploid because they contain pairs of chromosomes. There are 22 pairs of autosomal chromosomes and a pair of sexual chromosomes (heterosomes or gonosomes) in the somatic cells: the pairs XX to female and XY in male.

Microscopic analysis of chromosomes usually begins with a cultured cells arrested in mitotic metaphase by colchicine or other compounds that disrupt microtubules (structural component of the mitotic spindle). After processing and staining the cells, the condensed chromosomes of one nucleus are photographed by light microscopy and rearranged to produce a karyotype in which stained chromosomal regions (bands) can be analyzed.

The identification of human chromosomes is based on their morphology, using strictly coded criteria in international standardization conferences. Based on the chromosome length, the centromer position, the existence of secondary satellites and constrictions, the 23 pairs of chromosomes were classified into seven groups from A to G. These are the ***human karyotype***:

Group A comprises large chromosome pairs 1-3.

Group B comprises pairs of large chromosomes 4-5.

Group C comprises pairs of medium chromosomes 6-12. This group also includes chromosome X.

Group D comprises medium-sized chromosome pairs of 13-15.

Group E comprises pairs of small chromosomes 16-18.

Group F comprises small chromosomes, pairs 19-20.

Group G comprises the smallest chromosomes pairs 21-22. In this group is included also the chromosome Y.

A variety of molecular probes that are now commercially available are used in cytogenetic testing to diagnose disorders caused by chromosomal abnormalities. Karyotypes are also used for prenatal determination of sex in fetuses and for prenatal screening for certain genetic diseases.

Medical Application

Karyotyping is important for many prenatal diagnoses, in which chromosomal analysis of cultured cells from the fetus or amnion can detect certain genetic anomalies. As with karyotypes of adults, missing or extra chromosomes and chromosomal alterations are readily seen. New methods of chromosomal staining and molecular technique such as fluorescence in situ hybridization (FISH) are continuously being developed and used for cytogenetic diagnosis.

In humans, chromosomes 21 is a very small chromosomes and the one most likely to be “overlooked” at the metaphase/anaphase checkpoint. Failure of these homologous chromosomes to separate in the first meiotic division also occurs with greater frequency in older oocytes (or sperm progenitor cells). A gamete retaining this chromosomes pair forms a viable zygote after fertilization, but the developing trisomy 21 individual has morphologic and cognitive impairments associated with Down syndrome.

VIII.4. FUNCTIONS OF THE INTERPHASIC NUCLEUS

During the interphase, the nucleus is called the "metabolic nucleus" because it commands, controls and coordinates the metabolic activity of the cell. Proteins are involved in organizing all cellular structures and performing all cellular functions.

During the whole of his life, the cell has two important functions: protein synthesis and division. The mechanism involved in these processes is accomplished in successive stages and has been called the ,,central dogma of molecular biology".

The stages of information transmission are in order: replication (DNA duplication), transcription (RNA synthesis), and translation (protein synthesis)

VIII.4.1. DNA REPLICATION (DNA DUPLICATION)

A first approach to the DNA replication mechanism was achieved by Chargaff's studies on the composition of the nitrogen bases. In the process of analyzing DNA molecules from a wide variety of organisms, Chargaff has discovered a remarkable pattern: adenine and thymine bases have always been found to be in equal quantities, just like cytosine and guanine bases. This model became known as the ,,Chargaff rule"

Although Chargaff has guessed that this regularity has to reflect a fundamental property of DNA, it has not discovered its exact importance. The importance of the relationships between adenine and thymine, cytosine and guanine became evident in 1953 when Watson and Crick published a two-dimensional DNA structure model. This model was based on DNA analysis by X-ray diffraction by Franklin and Wilkins; they have suggested to Watson and Crick the molecular arrangement of spiral or helix DNA. Watson and Crick have discovered that the two strands of the DNA helix can be held together by hydrogen bonds between the nitrogen bases of the two opposite strands. In this double helix the phosphate saccharide ends of the two strands have opposite polarity, one being oriented in the direction 5 '→ 3' and the other in the direction 3 '→ 5'. The nitrogen bases in the two strands are oriented towards the inside of the DNA molecule and can thus interact with each other. The most remarkable feature of this model was the discovery that the hydrogen bonds that stabilize the helix can be made between adenine from a chain and the thymine in the other or between cytosine and guanine. Since the sequence of the bases in a chain determines the sequence of the opposite strand, the two chains are complementary. The deepest implication of Watson and Crick's model was that it showed a simple molecular explanation of the ability of cells to duplicate their genetic information. It has been pointed out that from the two DNA strands, each can serve as a matrix for copying the opposite strand based on the complementarity of the nitrogen bases. Because each molecule of DNA synthesized by this mechanism contains an old DNA strand and a newly synthesized one, the process has been called semiconservative replication

The replication mechanism is realized in successive stages and requires the presence of a specialized enzyme machine.

1. The initiation of the synthesis takes place at a specific point in the DNA molecule called ***origin*** that will lead to the synthesis of a ***replicon***. Prokaryotes have only one origin and one replicon, and thousands of origins and thousands of replicons are founds in eukaryotes. The origin of replication is a specific nucleotide sequence called ***autonomous replication sequence*** or ***autonomous replication sequence (ARS).***

2. Synthesis begins by separating the parental DNA strands and forming the replication fork, so named because of its shape in Y. The DNA molecule is very thin and thus the helix separation will not encounter any frictional resistance. In addition, the hydrogen bonds that stabilize the molecule are very weak so that the energy required for separation is virtually negligible. The synthesis of the new strands occurs bidirectionally, starting from a single origin with the formation of two replication forks in opposite directions to the origin.

3. The synthesis of the new strands is accomplished by DNA polymerase that uses the old strands as matrix. The DNA polymerase role is to add the nucleotide to the OH-3 'group of deoxyribose. The synthesis of the new strands takes place in a single direction 5 '→ 3' for this reason, the new strand 5 '→ 3' is continuously synthesized and is referred to as the ***leading strand***, and the 3 '→ 5' chain is synthesized slowly and discontinuously being called ***lagging strand***. In the case of the lagging strand, the synthesis speed is slowed because DNA polymerase must synthesize fragments also in the 5 '→ 3' direction. These fragments are called Okazaki fragments and have a length of 2000 nucleotides at prokaryotes and 100-200 nucleotides at eukaryotes.

Replication machine

DNA replication involves a large number of enzymes but also proteins that do not exhibit enzymatic activity. Enzymes and proteins involved in replication form a multiproteic structure called ***replisome*** that assembles at the replication fork only at the time of initiation of DNA synthesis.

1. The ***primosome*** is an enzymatic complex that moves along the fork, separates the two chains, and attaches the RNA primers to synthesize the Okazaki fragments of the lagging strand. Is made of:

- *DNA helicases* separate the strands of double helix chains and continue to move on a single strand. With two strands with different orientations, there will also be two helicases which cut the double helix chain strands.

- *DNA primase* initiates DNA synthesis by attaching short RNA sequences consisting of 5-8 ribonucleotides called ***primers.***

2. ***DNA polymerases***. Enzymes that play a role in the synthesis of new strands on parental chains (template) are DNA polymerases. At prokaryotes were called DNA polymerase I, II, III, and at eukaryotes α, β, γ. At eukaryotes, DNA polymerase α is moving in the 5 '→ 3' direction and attaches the nucleotides continuously to the leading strand and discontinously (Okazaki fragments) on the lagging strand. In addition to synthetic activity, DNA polymerase has two subunits with nuclease activity:

a) After synthesis of Okazaki fragments, DNA polymerase digests RNA primers and replaces them with complementary deoxynucleotides.

b) The DNA polymerase also has the role of correcting the replication mechanism errors that may occur in the DNA molecule by introducing a non-complementary base.

3. ***DNA ligase.*** After Okazaki fragments were synthesized and their primers were digested, DNA ligase unites the fragments and performs the continuity of the lagging strand.

4. ***Single strand DNA binding proteins (SSB proteins)*** are proteins that bind to single stranded DNA and are designed to stabilize DNA strands in loose regions and prevent double-stranded structure repair, thus favoring catenary function. They are part of the auxiliary proteins (chaperone proteins).

Corrective systems of replication

Replication occurs at a high polymerization rate: 1000 pairs of nucleotides / s in bacteria and 100 pairs of nucleotides / s in eukaryotes. This can lead to the occurrence of numerous replication errors that are usually corrected by corrective systems that *ensure fidelity of replication.*

Corrective systems act sequentially:

a) nucleotide selection for the formation of complementary base pairs is ensured by DNA polymerase efficiency; if the nucleotide selection was altered and the inserted nucleotide is not complementary, the a new correction system is involved.

b) recognizing the incorrect base and eliminating it from the 3'-5 'exonuclease. Replacement of the excised region with the correct sequence is performed by DNA polymerase.

c) linking fragments with restoring the chain continuity is accomplished by DNA ligase.

VIII.4.2. TRANCRIPTION

The flow of genetic information follows the pathway:

DNA ---------- RNA (transcription) …….. Protein (translation).

Transcription is the process of producing a strand of RNA from a strand of DNA. Similar to the way DNA is used as a template in DNA replication, it is again used as a template during transcription. The information that is stored in DNA molecules is rewritten or ,,transcribed" into a new RNA molecule.

The information coded in DNA cannot act directly as a template for protein synthesis but must be first transcribed into messenger RNA. Messenger RNA carries information specifying amino acid sequences of proteins from DNA to ribosomes. In addition to this informational RNA, other RNAs are required for the very complex process of translating.

These other nucleic acids are:

- Transfer RNA (tRNA); serves as translator molecule in protein synthesis; translate mRNA codons into aminoacids;
- Ribosomal RNA (rRNA); plays catalytic (ribosome) roles and structural roles in ribosomes;

In prokaryotes, transcription and translation occurs, both in the cytoplasm, whereas in eukaryotes these processes are spatially separated (transcription occurs in the nucleus and translation into cytoplasm). The transcription of DNA takes place throughout the interphase and is blocked in mitosis.

Transcription device

Through transcription, the sequence of base of the DNA is transcribed into the reciprocal sequence of bases in a strand of RNA. Through transcription, the information of DNA molecule is passed onto the new strand of RNA which can then carry the information to where proteins are produced. RNA molecules used for this purpose are known as messenger RNA (mRNA).

An enzyme called ,,RNA polymerase" is responsible for separating the two strands of DNA in a double helix. As it separates the two strands, RNA polymerase builds a strand of mRNA by adding the complementary nucleotides (A, U, G, C) to the template strand of the DNA.

In ***prokaryotes*** there is a single RNA polymerase that is responsible for the synthesis of the three types of RNA.

In ***eukaryotes*** there are three types of RNA-polymerases involved in transcription:

- RNA polymerase I is found in nucleolus and catalyses RNA synthesis;
- RNA polymerase II is found in the nucleus and participates in mRNA synthesis;
- RNA polymerase III is found in the nucleus and synthesizes the RNA and ribosomal units 5S, 18S and 28S.

In the transcription process are involved other auxiliary proteins (transcription factors), regulatory proteins (regulating the process), as well as a number of transcription control elements found in the DNA structure.

Stages of transcription

Since transcription occurs only in the 5 '→ 3' direction, only one of the two DNA strands will act as a template and will form the newly synthesized RNA.

1. *The initiation step* comprises the recognition by the RNA polymerase of the gene to be transcribed, as well as the location from which the information begins to be copied, called the start site or promoter. The promoter is a DNA sequence that contains the information for initiating RNA synthesis and determines which of the two DNA strands will be transcribed. The recognition sequence of this *promoter* is the "TATA box" region (sequence in which the thymine changes with adenine). After binding of the RNA polymerase to the promoter, the double helix of DNA is dissected, the nucleotide sequences being thus exposed

2. *The elongation step* in which the RNA polymerase advances by despairing the DNA helix and subsequently exposing other bases for "reading". Thus, the RNA chain extends with a 5 '→ 3' nucleotide. The assembly of the transcribed DNA portion and the gene-attached RNA molecules is called the "transcription unit" and appears on the electron microscope as a fern leaf

3. *Termination phase.* Is the ending of transcription, and occurs when RNA polymerase crosses a stop (termination sequence in the gene. The mRNA strand is complete, and it detaches from DNA.

Posttranscriptional metabolism

The newly synthesized RNA by transcription is called primary transcribed RNA. Before leaving the nucleus, the primary transcribed RNA molecules will undergo some changes:

a) addition to the 5 'end of a G-methylated nucleotide, a process called "5'-capping", with an important role in initiating protein synthesis, but also to provide protection for the RNA molecule that is transcribed.

b) addition to the 3 'end of a poly-A chain consisting of 100-200 nucleotides with adenine.

Some genes are responsible for the production of other forms of RNA that play a role in translation, including transfer tRNA and rRNA.

- ***Exons*** code for amino acids and collectively determine the amino acid sequence of the protein product. It is these portions of the gene that are represented in final mature mRNA molecule.
- ***Introns*** are portions of the gene that do not code for amino acids, and are removed (spliced) from the mRNA molecule before translation.

Gene control regions

- **Start site.** A start site for transcription
- **A promoter.** A region a few hundred nucleotides ,,upstream" of the gene (toward the 5' end). It is not transcribed into mRNA, but plays a role in controlling the transcription of the gene. Transricption factors bind to specific nucleotide sequences in the promoter region and assist in the binding of RNA polymerases.
- **Enhancers.** Some transcription factors (called activators) bind to regions called ,,enhancers" that increase the rate of transcription. These sites may be thousands of nucleotides from the coding sequences or within an intron. Some enhancers are conditional and only work in the presence of the other factors as well as transcription factors.

- **Silencers.** Some transcription factors (called represors) bind to regions called „silencers” that depressed the rate of transcription.