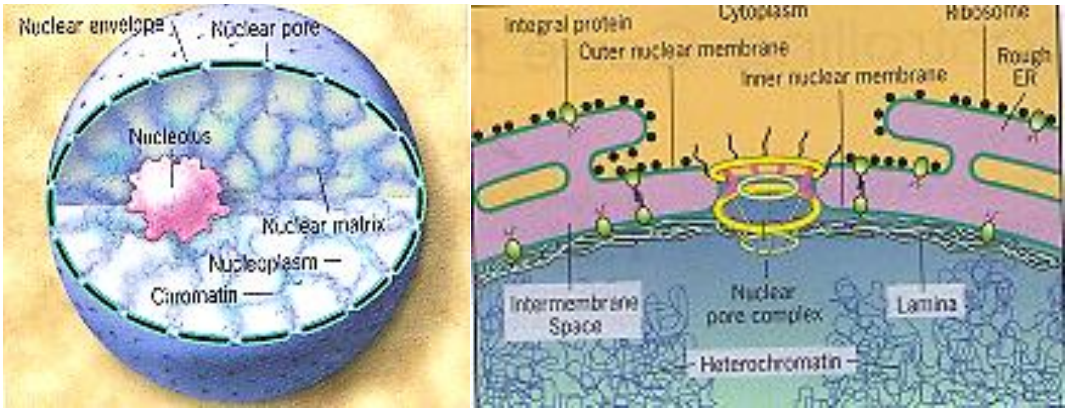
NUCLEUS & ORGANIZATION OF CHROMATIN

The Nucleus of a Eukaryotic cell

Considering its importance in the storage and utilization of genetic information, the nucleus of a eukaryotic cell has a rather undistinguished morphology. The intents of the nucleus are present as a viscous, amorphous mass of material enclosed by a complex **nuclear envelope** that forms a boundary between the nucleus and cytoplasm. Included within the nucleus of a typical interphase (nonmitotic) cell are (1) the chromosomes, which are present as highly extended nucleoprotein fibers, termed **chromatin***;* (2) one or more **nucleoli**, which are irregularly shaped electron-dense structures that function in the synthesis of ribosomal RNA and the assembly of ribosomes (3) the **nucleoplasm**, the fluid substance in which the solutes of the nucleus are dissolved; and (4) the **nuclear matrix**, which is a protein-containing fibrillar network.

The Nuclear Envelope

The separation of a cell's genetic material from the surrounding cytoplasm may be the single most important feature that distinguishes eukaryotes from prokaryotes, which makes the appearance of the nuclear envelope a landmark in biological evolution. The **nuclear envelope** consists of two cellular membranes arranged parallel to one another and separated by 10 to 50 nm. The membranes of the nuclear envelope serve as a barrier that keeps ions, solutes, and macromolecules from passing freely between the nucleus and cytoplasm. The two membranes are fused at sites forming circular pores that contain complex assemblies of proteins. The average mammalian cell contains several thousand nuclear pores. The outer membrane is generally studded with ribosomes and is continuous with the membrane of the rough endoplasmic reticulum. The space between the membranes is continuous with the ER lumen.



The inner surface of the nuclear envelope of animal cells bound to a thin filamentous meshwork, called the **nuclear lamina** by integral membrane proteins. The nuclear lamina provides mechanical support to the nuclear envelope, serves as a site of attachment for chromatin fibers at the ear periphery, and has a poorly understood role in DNA replication and transcription. The filaments of nuclear lamina are approximately 10 nm in diameter and are composed of polypeptides, called ***lamins***. Mutations in one of the lamin genes *(LMNA)* are responsible for a number of diverse human diseases, including a rare form of muscular dystrophy (called EDMD2) in which muscle cells contain exceptionally fragile nuclei. Mutations in ***LMNA*** have also been linked to a disease, called Hutchinson-Gilford Progeria Syndrome (HGPS) that is characterized by premature aging and death during teenage years from heart attack or stroke.

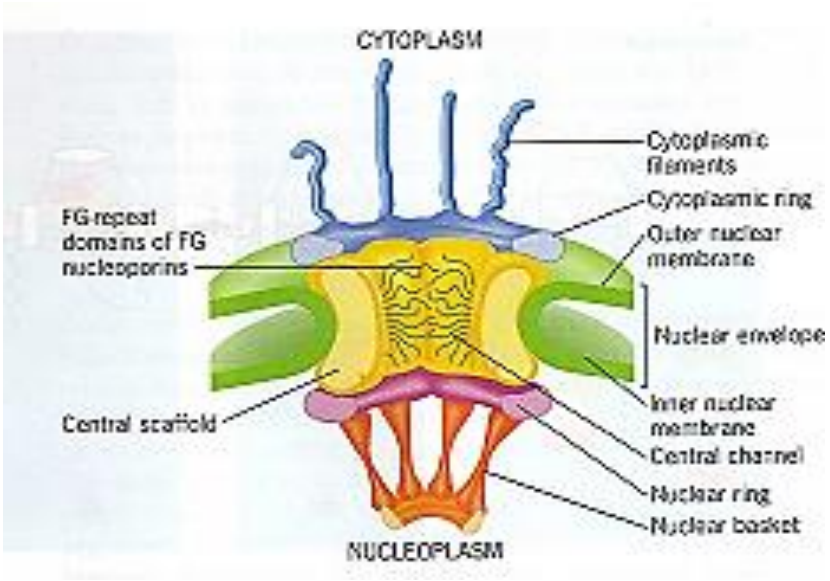
The Structure of the Nuclear Pore Complex and its Role in Nucleocytoplasmic Exchange

The nuclear envelope is the barrier between the nucleus and cytoplasm, and nuclear pores are the gateways across that barrier. Unlike the plasma membrane, which prevents passage of macromolecules between the cytoplasm and the extracellular space, the nuclear envelope is a hub of activity for the movement of RNAs and proteins in both directions between the nucleus and cytoplasm. The replication and transcription of genetic material within the nucleus require the participation of large numbers of proteins that are synthesized in the cytoplasm and transported across the nuclear envelope. Conversely the mRNAs, tRNAs, and ribosomal subunits that are manufactured in the nucleus must be transported through the nuclear envelope in the opposite direction.

Some components, such as the snRNAs of the spliceosome, move in both directions; they are synthesized in the nucleus, assembled into RNP particles in the cytoplasm, and then shipped back to the nucleus where they function in mRNA processing.

Nuclear pores contain an intricate structure called the **nuclear pore complex (NPC)** that appears to fill the pore like a stopper, projecting into both the cytoplasm and nucleoplasm. The NPC is a huge, supramolecular complex—15 to 30 times the mass of a ribosome—that exhibits octagonal symmetry due to the eightfold repetition of a number of structures. Despite their considerable size and complexity, NPCs contain only about 30 different proteins, called **nucleoporins**, which are largely conserved between yeast and vertebrates. Each nucleoporin is present in at least eight copies, in keeping with the octagonal symmetry of the structure. The NPC is not a static structure, as evidenced by the finding that many of its component proteins are replaced with new copies over a time period of seconds to minutes.

Among the nucleoporins is a subset of proteins that possess, within their amino acid sequence, a large number of phenylalanine glycine repeats (FG). The FG repeats are clustered in a particular region of each molecule called the FG domain. Because of their unusual amino acid composition, the FG domains possess a disordered structure that gives them an extended and flexible organization. The FG repeat-containing nucleoporins are thought to line the central channel of the NPC with their filamentous FG domains extending into the heart of the 20-to 30-nm-wide channel. The FG domains form a hydrophobic meshwork or sieve that blocks the diffusion of larger macromolecules between the nucleus and cytoplasm.



In 1982, Robert Laskey and his co-workers at the Medical Research Council of England found that nucleoplasmin, one of the more abundant nuclear proteins of amphibian oocytes, contains a stretch of amino acids near its C-terminus that functions as a **nuclear localization signal (NLS).** This sequence enables a protein to pass through the nuclear pores and enter the nucleus. Another key player, a GTP-binding protein called **Ran**, like other GTP-binding proteins, such as **Sari** and **EF-Tu** is also reported recently**.**

Chromosomes and Chromatin

Chromosomes seem to appear out of nowhere at the beginning of mitosis and disappear once again when cell division has ended. The appearance and disappearance of chromosomes provided early cytologists with a challenging question about the nature of the chromosome in the nonmitotic cell.

Packaging the Genome

An average human cell contains about 6.4 billion base pairs of DNA divided among 46 chromosomes (the value for a diploid, unreplicated number of chromosomes). Each unreplicated chromosome contains a single, continuous DNA molecule; the larger the chromosome, the longer the DNA it contains. Given that each base pair is about 0.34 nm in length, 6 billion base pairs would constitute a DNA molecule fully 2 m long. How is it possible to fit 2 meters of DNA into a nucleus only 10 |xm (1 X 10"^{5} m) in diameter and, at the same time, maintain the DNA in a state that is accessible to enzymes and regulatory proteins? Just as important, how is the single DNA molecule of each chromosome organized so that it does not become hopelessly tangled with the molecules of other chromosomes? The answers lie in the remarkable manner in which a DNA molecule is packaged.

Nucleosomes: The Lowest Level of Chromosome Organization

Chromosomes are composed of DNA and associated protein, which together is called **chromatin**. The orderly packaging of eukaryotic DNA depends on **histones**, a remarkable group of small proteins that possess an unusually high content of the basic amino acids arginine and lysine. Histones are divided into five classes,

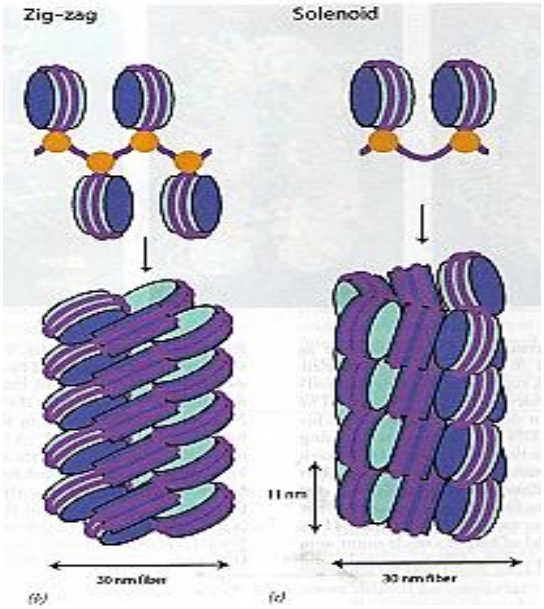
which can be distinguished by their arginine/lysine ratio (**H1, H2A, H2B, H3, H4**). The amino acid sequences of histones, particularly H3 and H4, have undergone very little change over long periods of evolutionary time. The H4 histones of both peas and cows, for example, contain 102 amino acids, and their sequences differ at only 2 amino acid residues. Why histones are so highly conserved? One reason is histones interact with the backbone of the DNA molecule, which is identical in all organisms. In addition, nearly all of the amino acids in a histone molecule are engaged in an interaction with another molecule, either DNA or another histone. As a result, very few amino acids in a histone can be replaced with other amino acids without severely affecting the function of the protein.

In the early 1970s, it was found that when chromatin was treated with nonspecific nucleases, most of the DNA was converted to fragments of approximately 200 base pairs in length. In contrast, a similar treatment of HAWDNA (i.e., DNA devoid of proteins) produced a randomly sized population of fragments. This finding suggested that chromosomal DNA was protected from enzymatic attack, except at certain periodic sites along its length. It was presumed that the proteins associated with the DNA were providing the protection. In 1974, using the data from nuclease digestion and other types of information, Roger Kornberg, then at Harvard University, proposed an entirely new structure for chromatin. Kornberg proposed that DNA and histones are organized into repeating subunits, called **nucleosomes**. We now know that each nucleosome contains a **nucleosome core particle** consisting of 146 base pairs of supercoiled DNA wrapped almost twice around a disk-shaped complex of eight histone molecules. The histone core of each nucleosome consists of two copies each of histones H2A, H2B, H3, and H4 assembled into an **octamer**, as discussed below. The remaining histone—type HI—resides outside the nucleosome core particle. The HI histone is referred to as a **linker histone** because it binds to part of the **linker DNA** that connects one nucleosome core particle to the next. Fluorescence studies indicate that H1 molecules continuously dissociate and reassociate with chromatin. Together the HI protein and the histone octamer interact with about 168 base pairs of DNA. H1 histone molecules can be selectively removed from the chromatin fibers by subjecting the preparation to solutions of low ionic strength. When Hl-depleted chromatin is observed under the electron microscope, the nucleosome core particles and naked linker DNA can be seen as separate elements, which together appear like '**beads on a string**'.

Understanding of DNA packaging has been greatly advanced in recent years by dramatic portraits of the nucleosome core particle obtained by X-ray crystallography. The eight histone molecules that comprise a nucleosome core particle are organized into four heterodimers: two H2A-H2B dimers and two H3-H4 dimers. Dimerization of histone molecules is mediated by their C-terminal domains, which consist largely of a helices folded into a compact mass in the core of the nucleosome. In contrast, the N-terminal segment of each core histone (and also the C-terminal segment of H2A) takes the form of a long, flexible tail that extends past the DNA helix and into the surroundings.

Histone modification is not the only mechanism to alter the histone character of nucleosomes. In addition to the four "conventional" core histones discussed above, several alternate versions of the H2A and H3 histones are also synthesized in most cells. The importance of these histone variants, as they are called, remains largely unexplored, but they are thought to have specialized functions. Another variant, **H2A.X**, is distributed throughout the chromatin, where it replaces conventional H2A in a fraction of the nucleosomes. H2A.X becomes phosphorylated at sites of DNA-strand breakage and may play a role in recruiting the enzymes that repair the DNA. Two other core histone variants - **H2A.Z** and **H3.3** - can be incorporated into nucleosomes of genes as they become activated and may play a role in promoting the transcription of that genetic locus.

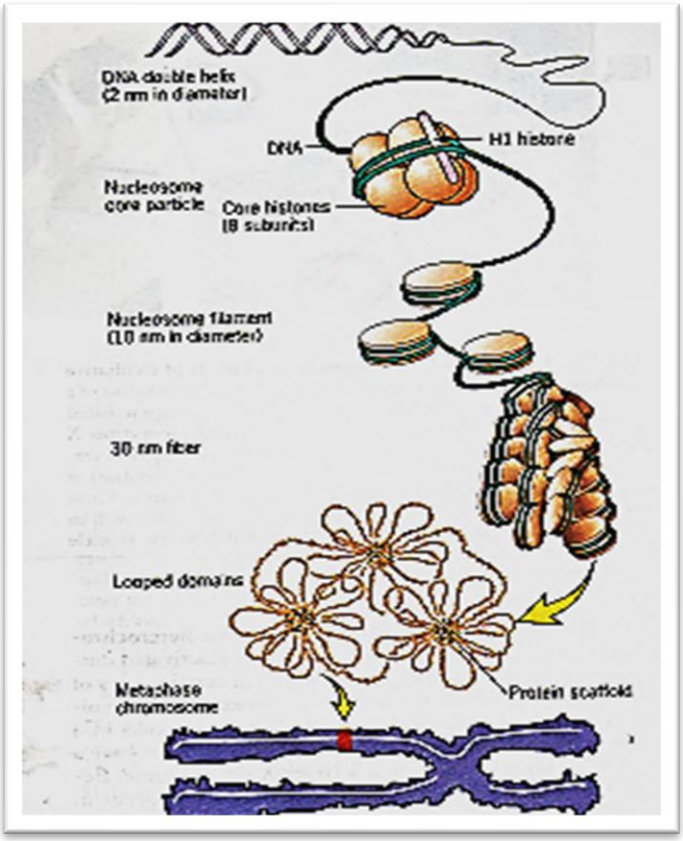
DNA and core histones are held together by several types of noncovalent bonds, including ionic bonds between negatively charged phosphates of the DNA backbone and positively charged residues of the histones. The two molecules make contact at sites where the minor groove of the faces inward toward the histone core, which occurs at approximately 10 base-pair intervals. In between these points of contact, the two molecules are seen to be separated by considerable space, which might provide access to the DNA for transcription factors and other DNA-binding proteins. For many years, histones were thought of as inert, structural molecules but, as we will see in following sections, these small proteins play critically important roles in determining the activity of the DNA with which they are associated. It has also become evident that chromatin a dynamic cellular component in which histones, regulatory proteins, and a myriad variety of enzymes move in and out of



nucleoprotein complex to facilitate the complex tasks of DNA transcription, compaction, replication, recombination, *AND* repair. The assembly of nucleosomes is the first important step in the compaction process. With a nucleotide - nucleotide spacing of 0.34 nm, the 200 base pairs of a single 10-nm nucleosome would stretch nearly 70 nm if fully extended. Consequently, it is said that the packing ratio of the DNA of nucleosomes is approximately 7:1.

Higher Levels of Chromatin Structure

A DNA molecule wrapped around nucleosome core particles of 10-nm diameter is the lowest level of chromatin organization. Chromatin does not, however, exist within the cell in this relatively extended, "beads-on-a-string" state. When chromatin is released from nuclei and prepared at physiologic ionic strength, a fiber of approximately 30-nm thickness is observed. Despite more than two decades of investigation, the structure of the 30-nm fiber remains a subject of debate. Two models (**zig zag** and **solenoid**) in which the nucleosomal filament is coiled into the higher-order, thicker fiber is shown in figure. The models differ in the relative positioning of nucleosomes within the fiber. Recent research favors the **zig zag** model in which successive nucleosomes along the DNA are arranged in different stacks and alternating nucleosomes become interacting neighbors. Regardless of how it is accomplished, the assembly of the 30-nm fiber increases the DNA-packing ratio an additional 6-fold or about 40-fold altogether



Maintenance of the 30-nm fiber depends on the interaction between histone molecules of neighboring nucleosomes. Linker histones and core histones have both been implicated in higher-order packaging of chromatin. If, for example, HI linker histones are selectively extracted from compacted chromatin, the 30-nm fibers uncoil to form the thinner, more extended beaded filament. Adding back HI histone leads to restoration of the higher-order structure. Core histones of adjacent nucleosomes may interact with one another by means of their long, flexible tails. Structural studies indicate, for example, that the N-terminal tail of an H4 histone from one nucleosome core particle can reach out and make extensive contact with both the linker DNA between nucleosome particles and the H2A/H2B dimer of adjacent particles. These types of interactions are thought to mediate the folding of the nucleosomal filament into a thicker fiber. In fact, chromatin fibers prepared with H4 histones that lack their tails are unable to fold into higher-order fibers.

The next stage in the hierarchy of DNA packaging is thought to occur as the 30-nm chromatin fiber is gathered into a series of large, supercoiled loops, or domains, that may be compacted into even thicker (80-100 nm) fibers. The DNA loops are apparently tethered at their bases to proteins that are part of an organized nuclear scaffold or matrix. Included among these proteins is a type II topoisomerase that presumably regulates the degree of DNA supercoiling. The topoisomerase would also be expected to untangle the DNA molecules of different loops should they become intertwined. Normally, loops of chromatin fibers are spread out within the nucleus and cannot be visualized, but their presence can be revealed under certain circumstances. For instance,

when isolated mitotic chromosomes are treated with solutions that extract histones, the histone-free DNA can be seen to extend outward as loops from a protein scaffold.

The mitotic chromosome represents the ultimate in chromatin compactness; 1 u.m of mitotic chromosome length typically contains approximately 1 cm of DNA, which represents a packing ratio of 10,000:1. An overview of the various levels of chromatin organization, from the nucleosomal filament to a mitotic chromosome, is depicted in Figure.

Heterochromatin and Euchromatin

After mitosis has been completed, most of the chromatin in highly compacted mitotic chromosomes returns to its diffuse interphase condition, lately 10 percent of the chromatin, however, generally remains in a condensed, compacted form throughout interphase. This compacted densely stained chromatin is seen at the periphery of the nucleus. Chromatin that remains compacted during interphase is called **heterochromatin** to distinguish it from **euchromatin,** which returns to a dispersed state. When a radioactively labeled RNA precursor such as [^{3}H] uridine is given to cells that are subsequently fixed, sectioned, and autoradiographed, the clumps of heterochromatin remain largely unlabeled, indicating that they have relatively little transcriptional activity. The state of a particular region of the genome, whether it is euchromatic or heterochromatic, is stably inherited from one cell generation to the next. Heterochromatin is divided into two classes. **Constitutive heterochromatin** remains in the compacted state in all cells at all times and, thus, represents DNA that is permanently silenced. In mammalian cells, the bulk of the constitutive heterochromatin is found in the region that flanks the telomeres and centromere of each chromosome and in a few other sites, such as the distal arm of the Y chromosome in male mammals. The DNA of constitutive heterochromatin consists primarily of repeated sequences and contains relatively few genes. In fact, when genes that are normally active move into a position adjacent to heterochromatin (having changed position as the result of transposition or translocation), they tend to become transcriptionally silenced, a phenomenon known as **position effect**.

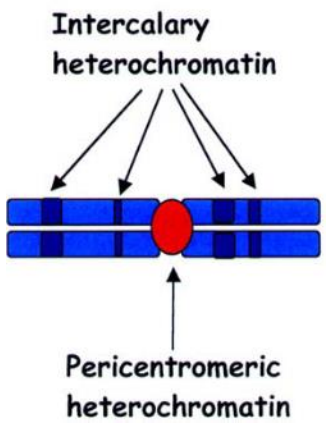
It is thought that heterochromatin contains components whose influence can spread outward a certain distance, affecting nearby genes. The spread of heterochromatin along the chromosome is apparently blocked by specialized barrier sequences **(boundary elements)** in the genome. Constitutive heterochromatin also serves to inhibit genetic recombination between homologous repetitive sequences. This type of recombination can lead to DNA duplications and deletions.

Heterochromatin has been associated with several functions, from gene regulation to the protection of the integrity of chromosomes; some of these roles can be attributed to the dense packing of DNA, which makes it less accessible to protein factors that usually bind DNA or its associated factors

Unlike the constitutive variety, **facultative heterochromatin** is chromatin that has been specifically inactivated during certain phases of an organism's life or in certain types of differentiated cells. An example of facultative heterochromatin can be seen by comparing cells of a female mammal to those of a male. The cells of males have a tiny Y chromosome and a much larger X chromosome. Because the X and Y chromosomes have only a few genes in common, males have a single copy of most genes that are carried on the sex chromosomes. Although cells of females contain two X chromosomes, only one of them is transcriptionally active. The other X chromosome remains condensed as a heterochromatic clump called a **Barr body** after the researcher who discovered it in 1949. Formation of a Barr body ensures that the cells of both males and females have the same number of active X chromosomes and thus synthesize equivalent amounts of the products encoded by X-linked genes.

Properties of heterochromatin

1 Heterochromatin is condensed
2 Heterochromatin DNA is late replicating
3 Heterochromatin DNA is methylated
4 In heterochromatin, histones are hypo-acetylated
5 Histones from heterochromatin are methylated on lysine 9
6 Heterochromatin is transcriptionally inactive
7 Heterochromatin does not participate in genetic recombination
8 Heterochromatin has a gregarious instinct



X Chromosome Inactivation

Based on her studies of the inheritance of coat color in mice, the British geneticist Mary Lyon proposed the following in 1961:

1. Heterochromatinization of the X chromosome in female mammals occurs during early embryonic development and *leads to the inactivation of the genes on that chromosome.*
2. Heterochromatinization in the embryo is a random process *in the sense that the paternally derived X chromosome* and the maternally derived X chromosome stand an equal chance of becoming inactivated *in any given cell.* Consequently, at the time of inactivation, the paternal X can be inactivated in one cell of the embryo, and the maternal X can be inactivated in a neighboring cell. Once an X chromosome has been inactivated, its heterochromatic state is transmitted through many cell divisions, so that the same X chromosome is inactive in all the descendants of that particular cell.
3. Reactivation of the heterochromatinized X chromosome occurs in germ cells prior to the onset of meiosis. Consequently, both X chromosomes are active during oogenesis, and all of the gametes receive a euchromatic X chromosome.

Maternally and paternally derived X chromosomes may contain different alleles for the same trait and thus adult females are in a sense **genetic mosaics**, where different alleles function in different cells. X-chromosome mosaicism is reflected in the patchwork coloration of the fur of some mammals, including calico cats (Lyon hypothesis)

The Histone Code and Formation of Heterochromatin

Cells contain a remarkable array of enzymes that are able to add chemical groups to or remove them from specific amino acid residues in the histone tails. The past few years has seen the emergence of a hypothesis known as the **histone code**, which postulates that the state and activity of a particular region of chromatin depend on the specific modifications, or combinations of modifications, to the histone tails in that region. In other words, the pattern of modifications adorning the tails of the core histones contains encoded information governing the properties of those nucleosomes. Studies suggest that histone tail modifications act in two ways to influence chromatin structure and function.

1. The modified residues serve as docking sites to recruit a specific array of nonhistone proteins, which then determine the properties and activities of that segment of chromatin. Each of the proteins bound to the histones is capable of modulating some aspect of chromatin activity or structure.
2. The modified residues alter the manner in which the histone tails of neighboring nucleosomes interact with one another or with the DNA to which the nucleosomes are bound. Changes in these types of interactions can lead to changes in the higher order structure of chromatin.

Structure of a Mitotic Chromosome

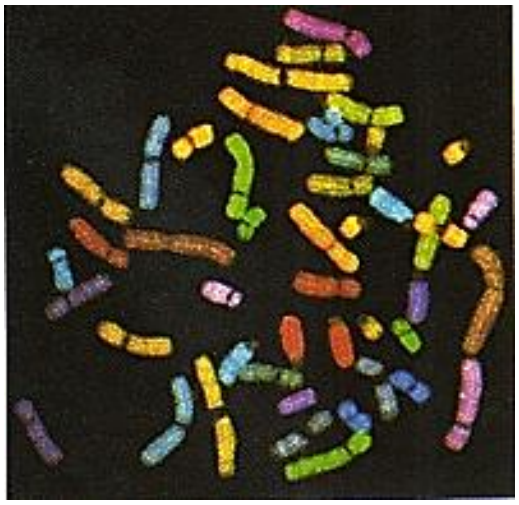
The relatively dispersed state of the chromatin of an interphase cell favors interphase activities, such as replication and transcription. In contrast, the chromatin of a mitotic cell exists in its most highly condensed state, which favors the delivery of an intact 'package' of DNA to each daughter cell. Mitotic chromosomes have also proven useful to biologists and physicians because they contain a complete set of genetic material of a cell and are made readily visible by simple techniques. When a chromosome undergoes compaction during prophase, it adopts a distinct and predictable shape determined primarily by the length of the DNA molecule in chromosome and the position of the centromere. The individual chromosomes as cut out of a photograph 1, can be matched up into homologous pairs (23 in humans) and ordered according to decreasing size. A preparation of this is called a **karyotype.** The chromosomes shown in the type of Figure 2 have been prepared using a staining procedure that gives the chromosomes a cross-banded appearance. The pattern of these bands is highly characteristic for each chromosome of a species and provides a basis to identify chromosomes and compare them from one species to the next. Karyotypes are routinely prepared from cultures of blood cells and used to screen individuals for chromosomal abnormalities. As discussed in the accompanying Human Perspective, extra, missing, or grossly altered chromosomes can be detected in this manner.

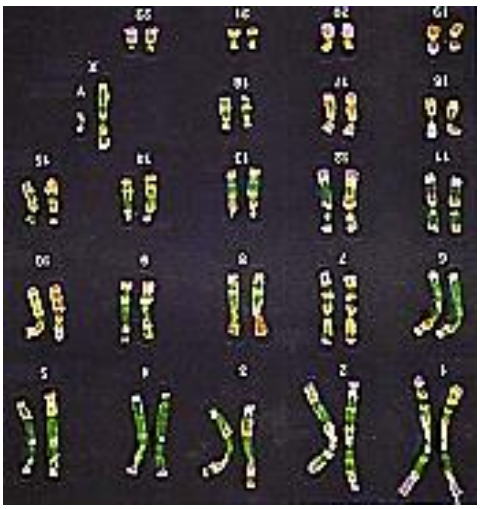
Telomeres

Each chromosome contains a single, continuous double-stranded DNA molecule. The tips of each DNA molecule are composed of an unusual stretch of repeated sequences that, together with a group of specialized proteins, forms a cap at each end of the chromosome called a **telomere.** Unlike most repeated sequences that vary considerably from species to species, the same telomere sequence are found throughout the vertebrates, and similar sequences are found in most other organisms. This similarity in sequence suggests that telomeres have a conserved function in diverse organisms. A number of DNA-binding proteins have been identified that bind specifically to the telomere sequence and are essential for telomere function. The protein that is bound to the chromosomes plays a role in regulating telomere length in yeast. As recently discovered, the short **factitive** DNA sequence of the telomeres also serves as a **template** for the synthesis of noncoding RNAs whose functions have become a focus of current interest.

If cells were not able to replicate the ends of their DNA, the chromosomes would be expected to become shorter and shorter with each round of cell division. This predicament has been called "the **end-replication problem**." The primary mechanism by which organisms solve the "end-replication problem," came to light in 1984 when Elizabeth Blackburn and Carol Greider of the University of California, Berkeley, discovered a novel enzyme, called **telomerase**, which can add new repeat units to the 3' end of the overhanging strand. Once the 3' end of the strand has been lengthened, a conventional DNA polymerase can use the newly synthesized 3' segment as a template to return the 5' end of the complementary strand to its previous length.

If telomeres are such an important factor in limiting the number of times that a cell can divide, one might expect telomeres to be a major factor in normal human aging. Although the subject is controversial, there is scientific support for this idea. For example, studies have reported that older persons whose white blood cells have shorter telomeres are more likely to have cardiovascular disease or to contract serious infections than persons of comparable age whose blood cells have longer telomeres. Another series of investigations have found that patients with Werner's syndrome, an inherited disease that causes patients to age much more rapidly than normal, is



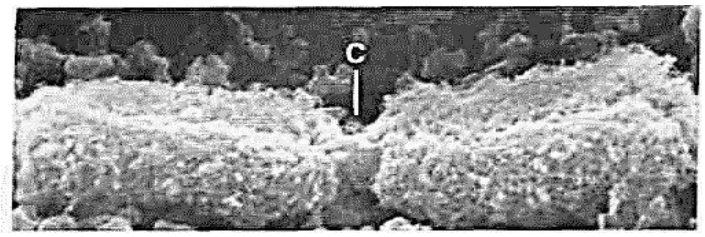


characterized by abnormal telomere maintenance. It has even been reported that women who are under chronic stress as a consequence of caring for seriously ill children have shorter telomeres and reduced telomerase activity.

According to current consensus, telomere shortening plays a key role in protecting humans from cancer by limiting the number of divisions of a potential tumor cell. Malignant cells, by definition, are cells that have escaped the body's normal growth control and continue to divide indefinitely. How is it that malignant tumor cells can divide repeatedly without running out of telomeres and bringing on their own death? Unlike normal cells that lack detectable telomerase activity, approximately 90 percent of human tumors consist of cells that contain an active telomerase enzyme. It is speculated that the growth of tumors is accompanied by intense selection for cells in which the expression of telomerase has been reactivated. The vast majority of tumor cells fail to express telomerase and die out, whereas the rare cells that express the enzyme are "immortalized." This does not mean that activation of telomerase, by itself, causes cells to become malignant. Unlimited cell division is only one property of cancer cells.

Centromeres

Each chromosome contains a site where the outer surfaces are markedly indented. The site of the constriction marks the **centromere** of the chromosome. In humans the centromere contains a tandemly repeated, 171-base-pair DNA sequence (called **a-satellite DNA***)* that extends for at least 500 kilobases. This stretch of DNA associates with specific proteins that distinguish it from other parts of the chromosome. For instance, centromeric chromatin contains a unique H3 histone variant, called CENP-A, which replaces conventional H3 in a certain fraction of the centromeric nucleosomes. During the formation of mitotic chromosomes, the CENP-A-containing nucleosomes become situated on the outer surface of the centromere where they serve as the platform for the assembly of the kinetochore. The kinetochore, in turn, serves as the attachment site for the microtubules that separate the chromosomes during cell division. Chromosomes lacking CENP-A fail to assemble a kinetochore and are lost during cell division.



It has been suggested in previous chapters that DNA sequences responsible for essential cellular functions tend to be conserved. It came as a surprise, therefore, to discover that centromeric DNA exhibited marked differences in nucleotide sequence, even among closely related species. This finding suggests that the DNA sequence itself is not an important determinant of centromere structure and function, a conclusion that is strongly supported by the following studies on humans. Approximately 1 in every 2000 humans is born with cells that have an excess piece of chromosomal DNA that forms an additional diminutive chromosome, called a **marker chromosome**. In some cases, marker chromosomes are devoid of a-satellite DNA, yet they still contain a primary constriction and a fully functional centromere that allows the duplicated marker chromosomes to be separated normally into daughter cells at each division. Clearly, some other DNA sequence in these marker chromosomes becomes ‘selected’ as the site to contain CENP-A and other centromeric proteins. The centromere appears at the same site on a marker chromosome in all of the person's cells, indicating that the property is transmitted to the daughter chromosomes during cell division. In one study, marker chromosomes were found to be transmitted stably through three generations of family members.

Epigenetics:

Epigenetics is the study of heritable changes in gene activity that are not caused by changes in the DNA sequence; it also can be used to describe the study of stable, long-term alterations in the transcriptional potential of a cell that are not necessarily heritable. Unlike simple genetics based on changes to the DNA sequence (the genotype), the changes in gene expression or cellular phenotype of epigenetics have other causes. As described earlier, a-satellite DNA is not required for the development of a centromere. In fact, dozens of unrelated DNA sequences have been found at the centromeres of marker chromosomes. It is not the DNA that indelibly marks the site as a centromere but the CENP-A-containing chromatin that it contains. These findings raise a larger issue. Not all inherited traits are dependent on DNA sequences. Inheritance that is not encoded in DNA sequence is referred to as **epigenetic** as opposed to **genetic**. The inactivation of the X chromosome

discussed earlier is another example of an epigenetic phenomenon: the two X chromosomes can have identical DNA sequences, but one is inactivated and the other is not. Furthermore, the state of inactivation is transmitted from each cell to its daughters throughout the life of the person. However, unlike genetic inheritance, an epigenetic state can usually be reversed; X chromosomes, for example, are reactivated prior to formation of gametes. Inappropriate changes in epigenetic state are associated with numerous diseases. There is also evidence to suggest that differences in disease susceptibility and longevity between genetically identical twins may be due, in part, to epigenetic differences that appear between the twins as they age. Biologists have discussed epigenetic phenomena for decades, but have struggled to understand (1) the mechanisms by which epigenetic information is stored and (2) the mechanisms by which an epigenetic state can be transmitted from cell to cell and from parent to offspring. In this discussion, we will focus primarily on one type of epigenetic phenomenon: the state of a cell's gene activity. Consider a stem cell residing at the base of the epidermis. Certain genes in these cells are transcriptionally active and others are repressed, and it is important that this characteristic pattern of gene activity is transmitted from one cell to its daughters. However, not all of the daughter cells continue life as stem cells; some of them take on a new commitment and begin the process of differentiation into mature epidermal cells. This step requires a change in the transcriptional state of that cell. Recent attention has focused on the histone code as a critical factor in both the determination of the transcriptional state of a particular region of chromatin and its transmission to subsequent cellular generations.

When the DNA of a cell is replicated, histones associated with the DNA as part of the nucleosomes are distributed randomly to the daughter cells along with the DNA molecules. As a result, each daughter DNA strand receives roughly half of the core histones that were associated with the parental strand. The other half of the core histones that become associated with the daughter DNA strands are recruited from a pool of newly synthesized histone molecules.

Highly Repeated DNA Sequences

The highly repeated fraction, which consists of sequences present in at least 10 copies per genome, constitutes anywhere from about 1 to 10 percent of the total DNA. Highly repeated sequences are typically short (a few hundred nucleotides at their longest) and present in clusters in which the given sequence repeats itself over and over again without interruption. A sequence arranged in this end-to-end manner is said to be present *in tandem.* Highly repeated sequences fall into several overlapping categories, including satellite DNAs, minisatellite DNAs, and microsatellite DNAs.

1. Satellite DNAs.
Satellite DNAs consist of short sequences (about five to a few hundred base pairs in length) that form very large clusters, each containing up to several million base pairs of DNA. In many species, the base composition of these DNA segments is sufficiently different from the bulk of the DNA that fragments containing the sequence can be separated into a distinct "satellite" band during density gradient centrifugation (hence the name *satellite* DNA). Satellite DNAs tend to evolve very rapidly, causing the sequences of these genomic elements to vary even between closely related species.

2. Minisatellite DNAs.
Minisatellite sequences range from about 10 to 100 base pairs in length and are found in sizeable clusters containing as many as 3000 repeats. Thus, minisatellite sequences occupy considerably shorter stretches of the genome than do satellite sequences. Minisatellites tend to be unstable, and the number of copies of a particular sequence often increases or decreases from one generation to the next as the result of unequal crossing over. Consequently, the length of a particular minisatellite locus is highly variable in the population, even among members of the same family. Because they are so variable (or **polymorphic***)* in length, minisatellite sequences form the basis for the technique of ***DNA fingerprinting***, which is used to identify individuals in criminal or paternity cases

3. Microsatellite DNAs.
Microsatellites are the shortest sequences (1 to 5 base pairs long) and are typically present in small clusters of about 10 to 40 base pairs in length, which are scattered quite evenly through the genome. DNA replicating enzymes have trouble copying regions of the genome that contains these small, repetitive sequences, which causes these stretches of DNA to change in length through the generations. Because of their variable lengths within the population, microsatellite DNAs have been used to analyze the relationships between different

human populations, as illustrated in the following example. Many anthropologists argue that modern humans arose in Africa. If this is true, then members of different African populations should exhibit greater DNA sequence variation than human populations living on other continents because genomes of African populations have had longer to diverge. The argument for African genesis has received support from studies on human DNA sequences.

The Nuclear Matrix

When isolated nuclei are treated with nonionic detergents and high salt (e.g., 2 M NaCl), which remove lipids and nearly all of the histone and nonhistne proteins of the chromatin, the DNA is seen as a halo surrounding a residual nuclear core. If the DNA fibers are subsequently digested with DNase, the structure that remains possesses the same shape as the original nucleus but is composed of a network of thin protein-containing fibrils crisscrossing through the nuclear space. This insoluble fibrillar network has been named the nuclear matrix. The nuclear matrix serves as more than a skeleton to maintain the shape of the nucleus or a scaffold on which to organize loops of chromatin, it also serves to anchor much of the machinery that is involved in the various activities of the nucleus, including transcription, RNA processing, and replication.