

NUCLEUS

Introduction: The nucleus is a membrane-bound organelle that contains genetic material (DNA) of eukaryotic organisms. As such, it serves to maintain the integrity of the cell by facilitating transcription and replication processes.

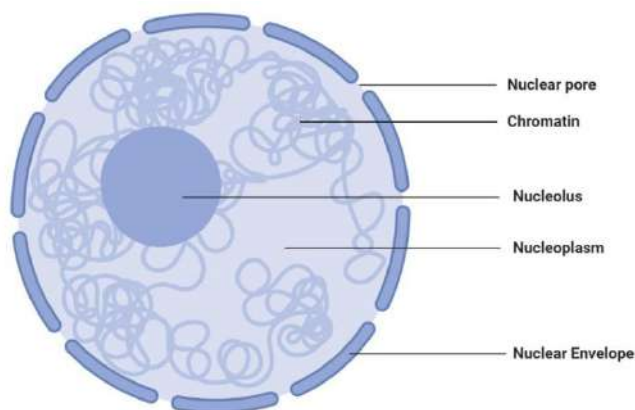
It's the largest organelle inside the cell taking up about a tenth of the entire cell volume. This makes it one of the easiest organelles to identify under the microscope.

Structure and Organization of the Nucleus

As the organelle that contains the genetic material of a cell, the nucleus can be described as the command center of a cell. As such, the nucleus consists of a number of structured elements that allow it to perform its functions. In general, the nucleus has a spherical shape. However, it may appear flattened, ellipsoidal or irregular depending on the type of cell. For instance, the nucleus of columnar epithelium cells appears more elongated compared to those of other cells. The shape of a nucleus, however, may also change as the cell matures.

The structure of a nucleus encompasses the nuclear membrane, nucleoplasm, chromosomes, and nucleolus.

A. Nuclear Membrane or Envelope



a) The nuclear membrane is a double-layered structure that encloses the contents of the nucleus. The outer layer of the membrane is connected to the endoplasmic reticulum.

b) Like the cell membrane, the nuclear envelope consists of phospholipids that form a lipid bilayer.

c) The envelope helps to maintain the shape of the nucleus and assists in regulating the flow of molecules into and out of the nucleus through nuclear pores. The nucleus communicates with the remaining of the cell or the cytoplasm through several openings called nuclear pores.

d) Such nuclear pores are the sites for the exchange of large molecules (proteins and RNA) between the nucleus and cytoplasm.

e) A fluid-filled space or perinuclear space is present between the two layers of a nuclear membrane.

B. Nucleoplasm

a) Nucleoplasm is the gelatinous substance within the nuclear envelope.

b) Also called karyoplasm, this semi-aqueous material is similar to the cytoplasm and is composed mainly of water with dissolved salts, enzymes, and organic molecules suspended within.

c) The nucleolus and chromosomes are surrounded by nucleoplasm, which functions to cushion and protect the contents of the nucleus.

d) Nucleoplasm also supports the nucleus by helping to maintain its shape. Additionally, nucleoplasm provides a medium by which materials, such as enzymes and nucleotides (DNA and RNA subunits),

can be transported throughout the nucleus. Substances are exchanged between the cytoplasm and nucleoplasm through nuclear pores.

C. Nucleolus

- a) Contained within the nucleus is a dense, membrane-less structure composed of RNA and proteins called the nucleolus.
- b) Some of the eukaryotic organisms have a nucleus that contains up to four nucleoli.
- c) The nucleolus contains nucleolar organizers, which are parts of chromosomes with the genes for ribosome synthesis on them. The nucleolus helps to synthesize ribosomes by transcribing and assembling ribosomal RNA subunits. These subunits join together to form a ribosome during protein synthesis.
- d) The nucleolus disappears when a cell undergoes division and is reformed after the completion of cell division.

D. Chromosomes

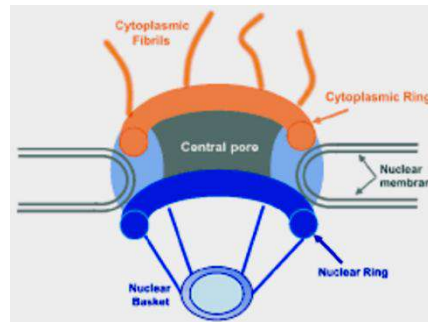
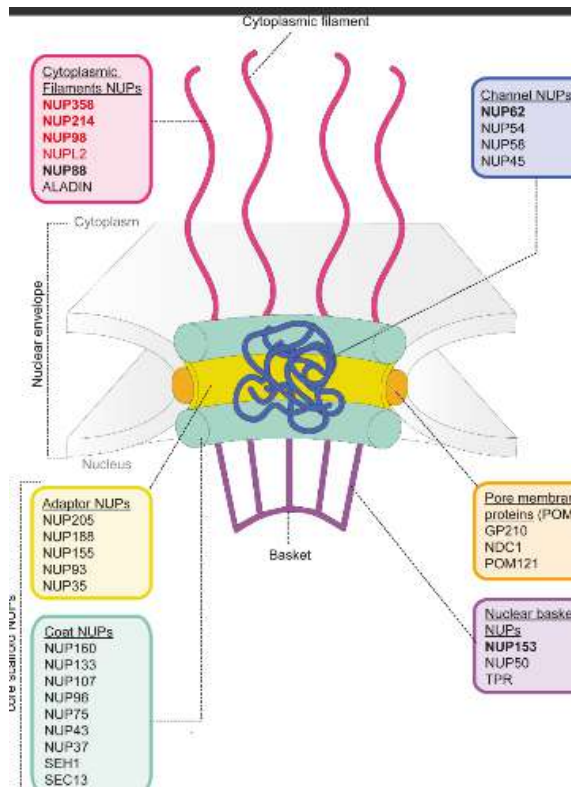
- a) The nucleus is the organelle that houses chromosomes.
- b) Chromosomes consist of DNA, which contains heredity information and instructions for cell growth, development, and reproduction.
- c) Chromosomes are present in the form of strings of DNA and histones (protein molecules) called chromatin.
- d) When a cell is “resting” i.e. not dividing, the chromosomes are organized into long entangled structures called chromatin.
- e) The chromatin is further classified into heterochromatin and euchromatin based on the functions. The former type is a highly condensed, transcriptionally inactive form, mostly present adjacent to the nuclear membrane. On the other hand, euchromatin is a delicate, less condensed organization of chromatin, which is found abundantly in a transcribing cell.

Besides the nucleolus, the nucleus contains a number of other non-membrane-delineated bodies. These include Cajal bodies, Gemini of coiled bodies, polymorphic interphase karyosome association (PIKA), promyelocytic leukemia (PML) bodies, paraspeckles, and splicing speckles.

Nuclear pore

Definition:

A nuclear pore is a part of a large complex of proteins, known as a nuclear pore complex that spans the nuclear envelope, which is the double membrane surrounding the eukaryotic cell nucleus. There are approximately 1,000 nuclear pore complexes (NPCs) in the nuclear envelope of a vertebrate cell, but it varies depending on cell type and the stage in the life cycle.



Structure:

- The human nuclear pore complex (hNPC) is a 110 megadalton (MDa) structure.
- The proteins that make up the nuclear pore complex are known as nucleoporins; each NPC contains at least 456 individual protein molecules and is composed of 34 distinct nucleoporin proteins.
- About half of the nucleoporins typically contain solenoid protein domains—either an alpha solenoid or a beta-propeller fold, or in some cases both as separate structural domains.
- The other half show structural characteristics typical of "natively unfolded" or intrinsically disordered proteins, i.e. they are highly flexible proteins that lack ordered tertiary structure. These disordered proteins are the FG nucleoporins, so called because their amino-acid sequence contains many phenylalanine—glycine repeats.

Significance:

Nuclear pore complexes allow the transport of molecules across the nuclear envelope. This transport includes RNA and ribosomal proteins moving from nucleus to the cytoplasm and proteins (such as DNA polymerase and lamins), carbohydrates, signaling molecules and lipids moving into the nucleus. It is notable that the nuclear pore complex (NPC) can actively conduct 1000 translocations per complex per second.

Nuclear transport

Introduction:

Nucleoporin-mediated transport is not directly energy requiring, but depends on concentration gradients associated with the RAN cycle. Ran (RAS-related Nuclear protein) is a 25 kDa small G protein that is essential for the translocation of RNA and proteins through the nuclear pore complex.

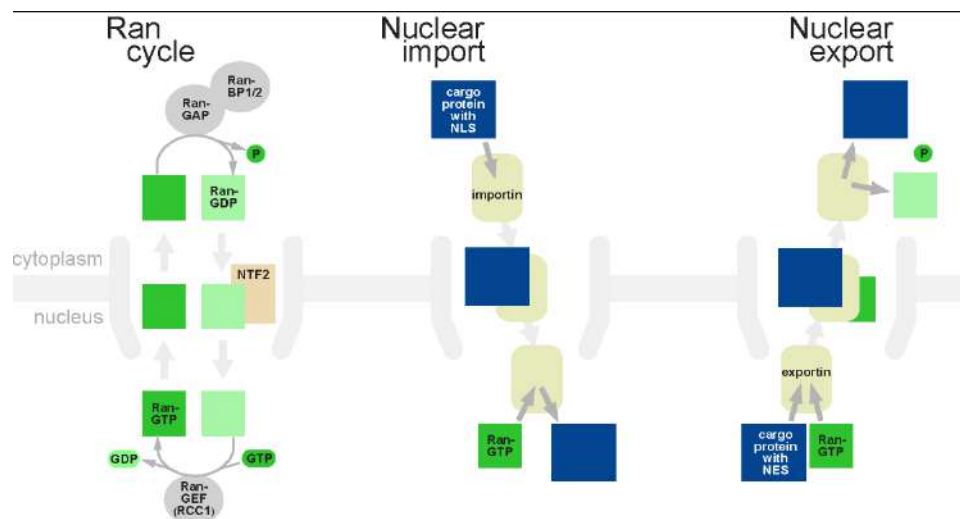
The RAN Cycle:

- Ran exists in the cell in two nucleotide-bound forms: GDP-bound and GTP-bound.
- RanGDP is converted into RanGTP through the action of RCC1, the nucleotide exchange factor for Ran. RCC1 is also known as RanGEF (Ran Guanine nucleotide Exchange Factor).
- Ran's intrinsic GTPase-activity is activated through interaction with Ran GTPase activating protein (RanGAP), facilitated by complex formation with Ran-binding protein (RanBP).

- d) GTPase-activation leads to the conversion of RanGTP to RanGDP, thus closing the Ran cycle.

Import of Proteins

- Any cargo with a nuclear localization signal (NLS) exposed will be destined for quick and efficient transport through the pore. Several NLS sequences are known, generally containing a conserved sequence with basic residues such as PKKKRKV. Any material with an NLS will be taken up by importins to the nucleus.
- The classical scheme of NLS-protein importation begins with Importin- α first binding to the NLS sequence, which then acts as a bridge for Importin- β to attach.
- The importin β —importin α —cargo complex is then directed towards the nuclear pore and diffuses through it.
- Once the complex is in the nucleus, RanGTP binds to Importin- β and displaces it from the complex.
- Then the cellular apoptosis susceptibility protein (CAS), an exportin which in the nucleus is bound to RanGTP, displaces Importin- α from the cargo. The NLS-protein is thus free in the nucleoplasm.
- The Importin β -RanGTP and Importin α -CAS-RanGTP complex diffuses back to the cytoplasm where GTPs are hydrolyzed to GDP leading to the release of Importin β and Importin α which become available for a new NLS-protein import round.



Export of Proteins

- Some molecules or macromolecular complexes need to be exported from the nucleus to the cytoplasm, as do ribosome subunits and messenger RNAs. Thus there is an export mechanism similar to the import mechanism.
- In the classical export scheme, proteins with a nuclear export sequence (NES) can bind in the nucleus to form a heterotrimeric complex with an exportin and RanGTP (for example the exportin CRM1).
- The complex can then diffuse to the cytoplasm where GTP is hydrolysed and the NES-protein is released.
- CRM1-RanGDP diffuses back to the nucleus where GDP is exchanged to GTP by RanGEFs.
- This process is also energy dependent as it consumes one GTP. Export with the exportin CRM1 can be inhibited by Leptomycin B.

Chromatin

Definition:

Chromatin is a complex of DNA and protein found in eukaryotic cells. Its primary function is packaging long DNA molecules into more compact, denser structures. This prevents the strands from becoming tangled and also plays important roles in reinforcing the DNA during cell division, preventing DNA damage, and regulating gene expression and DNA replication.

During mitosis and meiosis, chromatin facilitates proper segregation of the chromosomes in anaphase; the characteristic shapes of chromosomes visible during this stage are the result of DNA being coiled into highly condensed chromatin.

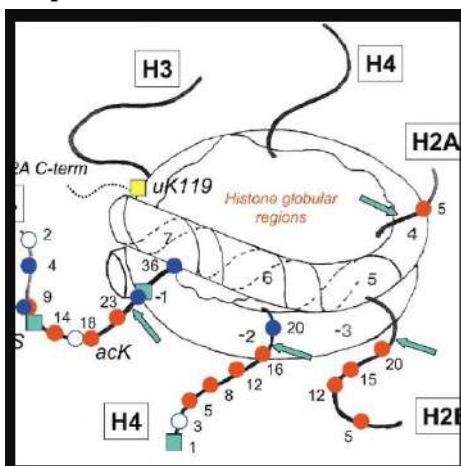
Proteins:

Histones and nonhistones are two major types of proteins associated with DNA in chromatin. Both types of proteins play an important role in determining the physical structure of the chromosome.

A. Histones:

- The histones are the most abundant proteins in chromatin. They are small basic proteins with a net positive charge that facilitates their binding to the negatively charged DNA. Five main types of histones are associated with eukaryotic nuclear DNA: H1, H2A, H2B, H3, and H4. Weight for weight, there is an equal amount of histone and DNA in chromatin.
- The amino acid sequences of histones H2A, H2B, H3, and H4 are highly conserved, evolutionarily speaking, even between distantly related species. Evolutionary conservation of these sequences is a strong indicator that histones perform the same basic role in organizing the DNA in the chromosomes of all eukaryotes.
- Histones play a crucial role in chromatin packing.

Importance of Histone N-terminal tails:



Histone N-terminal tails are central to the processes that modulate nucleosome structure and function. The N-terminal tails of the four core histones are targets for posttranslational modifications such as acetylation, methylation, and phosphorylation that correlate with changes in gene activity. Contribution of histone tails to nucleosome structure and nucleosome arrays has been demonstrated by several researchers, but the mechanisms involved in acetylation-mediated transcriptional regulation remain to be elucidated.

Removal of the H3 and H4 N-terminal tails, as well as their acetylation, provokes a dramatic change in the linking-number difference of the (H3-H4)₂ tetrameric particle, with a release of up to 70% of the negative supercoiling previously constrained by this structure. The (H3-H4)₂ tetramers containing tailless or hyperacetylated histones shows a striking preference for relaxed DNA over negatively supercoiled DNA.

B. Nonhistones:

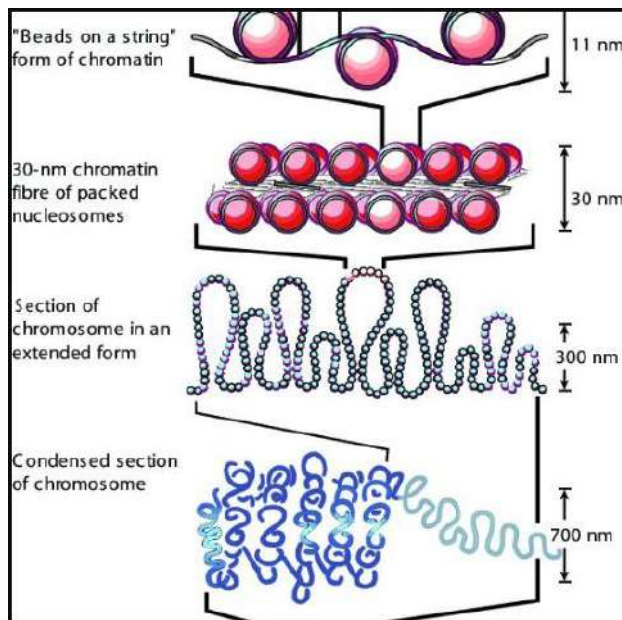
- Nonhistones are all the proteins associated with DNA, apart from the histones. Nonhistones are far less abundant than histones. Many nonhistones are acidic proteins—proteins with a net negative charge.

- b) Nonhistones include proteins that play a role in the processes of DNA replication, DNA repair, transcription (including gene regulation), and recombination. Each eukaryotic cell has many different nonhistones in the nucleus.
- c) In contrast to the histones, the nonhistone proteins differ markedly in number and type from cell type to cell type within an organism, at different times in the same cell type, and from organism to organism.

Chromatin Packing:

Introduction: A diploid human cell, for example, has more than 1,400 times as much DNA as does E. coli. Without the compacting of the bp of DNA in the diploid cell (two genome copies), the DNA of the chromosomes of a single human cell would be more than 2 meters long (about 6.5 feet) if the molecules were placed end to end. Several levels of packing enable chromosomes that would be several millimeters or even centimeters long to fit into a nucleus that is a few micrometers in diameter.

Levels of Chromatin Packing:



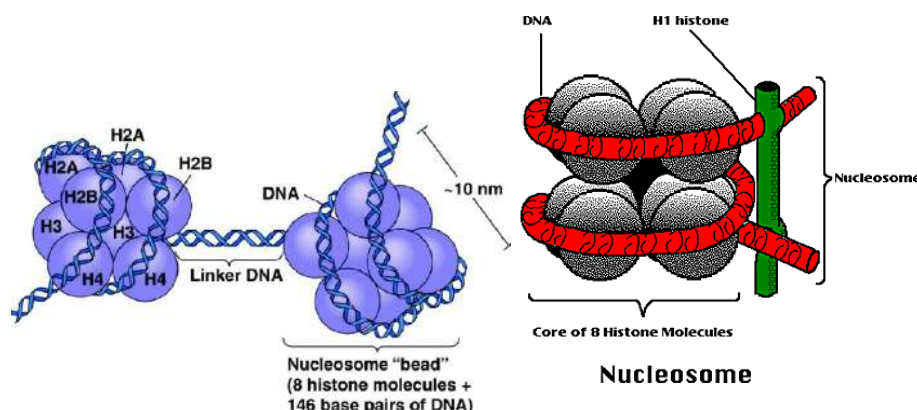
1. 11 nm fiber:

a) With the electron microscope, different chromatin structures are seen. The lowest-level structures are seen while reconstituting purified DNA and histones in vitro, and the higher-level structures reflect the extra degrees of packaging necessary to compact the DNA in vivo. The least compact form seen is the 11-nm chromatin fiber, which has a characteristic “beads-on-a-string” morphology.

b) The beads have a diameter of about 10 nm. The beads are called **nucleosomes**, the basic structural units of eukaryotic chromatin. A nucleosome is about 11 nm in diameter and consists of a core of eight histone proteins—two each of H2A,

H2B, H3, and H4—around which a 147-bp segment of DNA is wound about 1.65 times.

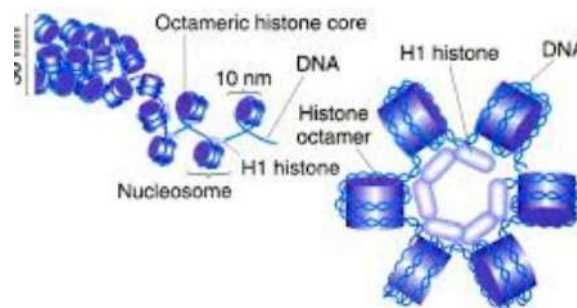
- c) This configuration serves to compact the DNA by a factor of about six.
- d) Individual nucleosomes are connected by strands of linker DNA. The length of linker DNA varies within and among organisms. Human linker DNA, for example, is 38–53 bp long.



2. 30 nm fiber:

- The next level of chromatin condensation is brought about by histone H1. A single molecule of H1 binds both
- to the linker DNA at one end of the nucleosome and to the middle of the DNA segment wrapped around core histones.
- The binding of H1 causes the nucleosomal DNA to assume a more regular appearance with a zigzag arrangement. The nucleosomes themselves then compact into a structure about 30 nm in diameter called the 30-nm chromatin fiber.
- One possible model for the 30-nm fiber—the solenoid model—has the nucleosomes spiraling helically.
- Another, more recent, model proposes that the 30-nm fiber is an irregular zigzag of nucleosomes.

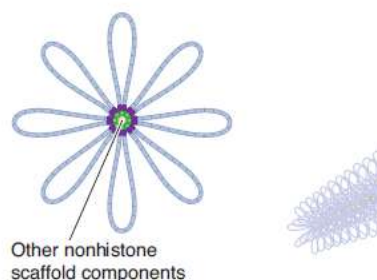
A model of chromatin structure 30 nm fibers



3. 300 nm fiber:

- Chromatin packing beyond the 30-nm chromatin filaments is less well understood. Current models derive from 1970s-vintage electron micrographs of metaphase chromosomes depleted of histones. The photos show 30–90-kb loops of DNA attached to a protein “scaffold” with the characteristic X shape of the paired sister chromatids. If the histones are not removed, looped domains of 30-nm fibers are seen. An average human chromosome has approximately 2,000 looped domains.
- Each looped domain is held together at its base by nonhistone proteins that are part of the chromosome scaffold. Stretches of DNA called scaffold associated regions, or SARs, bind to the nonhistone proteins to determine the loops.
- These loops are assumed to be arranged in a spiral fashion around the central chromosome scaffold.
- In cross section, the loops would be seen to radiate out from the center like the petals of a flower. Overall, this packing produces a chromosome that is about 10,000 times shorter, and about 400 times thicker, than naked DNA.

a) Fiber loops of 30-nm chromatin fibers attached to chromosome scaffold



4. 700 nm fiber:

- a) The final level of packaging is characterized by the 700 nm structure seen in the metaphase chromosome.
- b) The condensed piece of chromatin has a characteristic scaffolding structure that can be detected in metaphase chromosomes. This appears to be the result of extensive looping of the DNA in the chromosome.

Euchromatin and Heterochromatin:

The degree of DNA packing changes throughout the cell cycle. The most dispersed state is when the chromosomes are about to duplicate (beginning of S phase of the cell cycle), and the most highly condensed is within mitosis and meiosis.

Two forms of chromatin are defined, each on the basis of chromosome-staining properties.

A. Euchromatin: It is the chromosomes or regions of chromosomes that show the normal cycle of chromosome condensation and decondensation in the cell cycle. Visually, euchromatin undergoes a change in intensity of staining ranging from the darkest in the middle of mitosis (metaphase stage) to the lightest in the S phase. Most of the genome of an active cell is in the form of euchromatin.

Typically, (1) euchromatic DNA is actively transcribed, meaning that the genes within it can be expressed; and (2) euchromatin is devoid of repetitive sequences.

B. Heterochromatin: This, by contrast, is the chromosomes or chromosomal regions that usually remain condensed— more darkly staining than euchromatin—throughout the cell cycle, even in interphase. Heterochromatic DNA often replicates later than the rest of the DNA in the S phase.

Genes within heterochromatic DNA are usually transcriptionally inactive.

There are two types of heterochromatin:-

a. Constitutive heterochromatin is present in all cells at identical positions on both homologous chromosomes of a pair. This form of heterochromatin consists mostly of repetitive DNA and is exemplified by centromeres and telomeres.

b. Facultative heterochromatin, by contrast, varies in state in different cell types, and at different developmental stages—or sometimes, from one homologous chromosome to another. This form of heterochromatin represents condensed, and therefore inactivated, segments of euchromatin.

The Barr body, an inactivated X chromosome in somatic cells of XX mammalian females, is an example of facultative heterochromatin

EUCHROMATIN VERSUS HETEROCHROMATIN

Location:	Found in the nucleus's inner body.	Found near the edge of the nucleus.
Type:	Only one type is found in the cell's nucleus.	Two types are found in the cell's nucleus: facultative heterochromatin and constitutive heterochromatin.
Presence:	Found in both eukaryotes and prokaryotes.	Found in eukaryotes only.
Function:	Allows genetic changes to take place and the genes to be transcribed.	Allows gene expression to be regulated and maintains the genome's structural integrity.
Condensation or decondensation:	During periods of the cell cycle, condensation and decondensation of the DNA is alternated.	Except when DNA is replicated, heterochromatin stays condensed during each period of the cell cycle.
Stickiness:	Not sticky.	Sticky.
Replication of DNA:	Replicates early	Replicates late
Transcriptional activity:	Consists of regions that are transcriptionally active	Few or none of the regions are transcriptionally active
Genetic activity:	May be exposed to chromosomal crossover so it is genetically active	Prevents chromosomal crossover so it is genetically inactive
Amount of DNA contained:	Contains a low amount of DNA compared to heterochromatin.	Contains a high amount of DNA.
Intensity when stained:	Stained lightly.	Stained dark.
Packaging intensity:	Packaged loosely.	Packaged tightly.