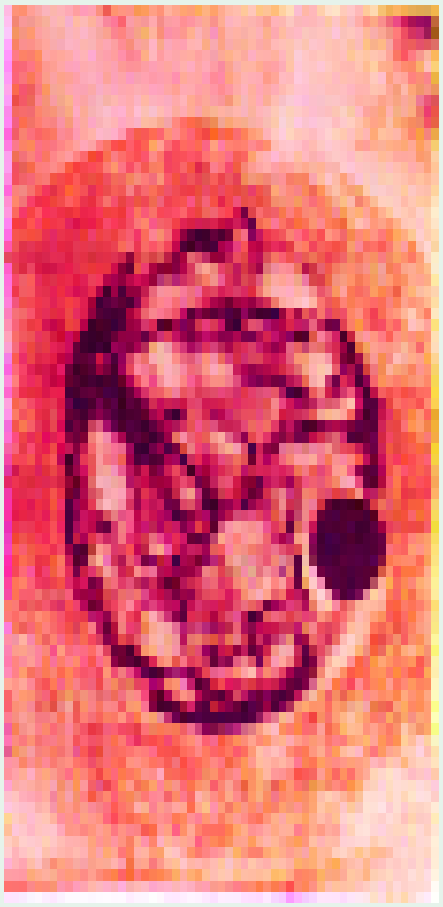
CHAPTER

12

Nucleus



Interphase nucleus.

The nucleus (L.,*nux* = nut) is the heart of the cell. It is here that almost all of the cell's DNA is confined, replicated and transcribed. The nucleus, thus, controls different metabolic as well as hereditary activities of the cell. A synonymous term for this organelle is the Greek word **karyon**. Nucleus serves as the main distinguishing feature of eukaryotic cells, *i.e.*, this is the true nucleus as opposed to the nuclear region, prokaryon or nucleoid of the prokaryotic cells (see Chapter 3). The following statement of **Vincent Allfrey** (1968) completely qualifies the central position of the nucleus in the affairs of an eukaryotic cell :

"The cell nucleus, central and commanding, is essential for the biosynthetic events that characterize cell type and cell fraction; it is a vault of genetic information encoding the past history and future prospects of the cell, an organelle submerged and deceptively serene in its sea of turbulent cytoplasm, a firm and purposeful guide, a barometer exquisitely sensitive to the changing demands of the organism and its environment. This is our subject — to be examined in terms of its ultrastructure, composition and function."

HISTORICAL

Nuclei were first discovered and named by **Robert Brown** in 1833 in the plant cells and were quickly recognized as a constant feature of all animal and plant cells. Nucleoli were described by **M.J. Schleiden** in 1838, although first noted by **Fontana** (1781). The term nucleolus was coined by **Bowman** in 1840. In 1879, **W. Flemming** coined the term chromatin for chromosomal meshwork. **Strasburger** (1882) introduced the terms cytoplasm and nucleoplasm. The existence of a mem-

brane delimiting the nucleus was first demonstrated by **O.Hertwig** in 1893. In 1934, **Barbara McClintock** recognized and named nucleolar organizers in the chromosomes. In 1950, **Callan** and **Tomlin**, first observed the nucleopores in the nuclei of amphibian oocytes. The role of nucleus in heredity was firmly established by the grafting experiments of **Hammerling** (1953) with *Acetabularia*. Ultrastructure of nuclear envelope, pore complexes and nuclear lamina were worked out by **Kirschner** *et al.*, (1977), **Schatten** and **Thoman** (1978), etc.

NUCLEO - CYTOPLASMIC INTERRELATIONSHIP

The evidences for nucleo-cytoplasmic communication as a factor in cell maintenance and development have been known before the rediscovery of Mendel's "genes". In the late nineteenth century, **Verworm**, **Balbiani** and others showed that following microsurgery, nucleated halves of various protozoans survived and grew, whereas the enucleated halves degenerated and died. Later, in the 1930s it was shown that insertion of nuclei into enucleated amoebae restored pseudopodial activity, feeding behaviour and growth. It was also shown that the nucleus was essential for the growth and regeneration of the morphologically complicated ciliate *Stentor*. In a classical series of experiments, spanning between 1934 and 1954, on the unicellular alga *Acetabularia*, **Hammerling** demonstrated by means of interspecific nuclear transplants, that morphological features, notably the shape of cap, were determined by the nucleus. He also showed that even after removal of the nucleus, the cell was able to continue morphogenesis for a time and proposed that the cytoplasm contained a store of morphogenetic material (later on rec-

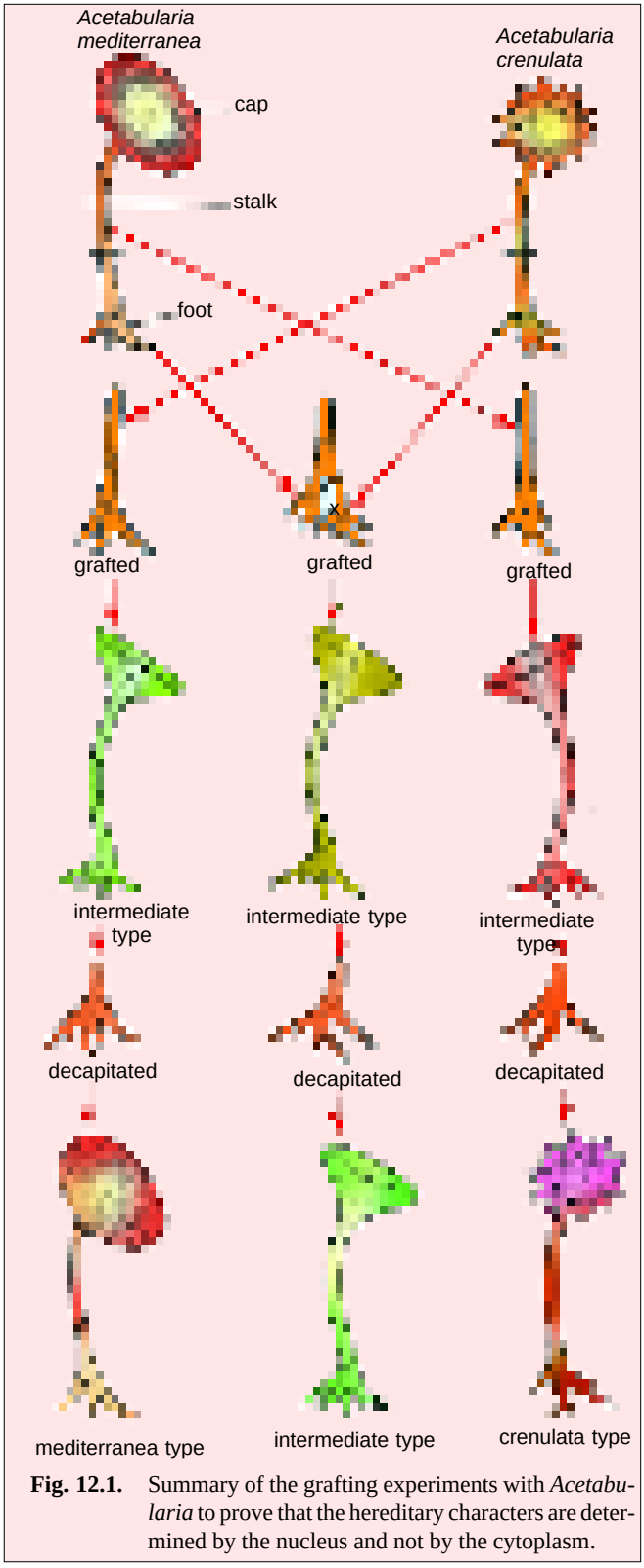


Fig. 12.1. Summary of the grafting experiments with *Acetabularia* to prove that the hereditary characters are determined by the nucleus and not by the cytoplasm.

ognized as mRNA molecules) that had been produced by the nucleus. Let us closely examine the Hammerling's classical nuclear transplantation experiments :

Hammerling's expe-riment. The body of an alga *Acetabularia* is about six centimeters long and is differentiated into a foot, a stalk and a cap. The cap has a characteristic shape for each species and is easily regenerated if removed. The single nucleus is situated in the rhizoid portion. *Acetabularia crenulata* has a cap, with about 31 rays, the tips of which are pointed, but *Acetabularia mediterranea* has about 81 rays with rounded tips. If the cap, stalk or even the nucleated portion of the rhizoid is removed, the remaining portion of the alga has the capacity to regenerate into a whole plant. The enucleated part loses the regeneration capacity after a few decapitations, but the nucleated portion always maintains this ability. When the stalk of one species is grafted on to the nucleated rhizoid of the other, an **intermediate type** of cap is formed. On decapitation, a second cap develops which resembles the cap of species which provides the nucleus (Fig. 12.1). When the nuclei of both the species are present in the same cytoplasm, an intermediate type of cap develops. Such experiments have clearly established that the nucleus is the storehouse for and the control tower of, all hereditary information.

OCCURRENCE AND POSITION

The nucleus is found in all the eukaryotic cells of the plants and animals. However, certain eukaryotic cells such as the mature sieve tubes of higher plants and mammaliam erythrocytes contain no nucleus. In such cells nuclei are present during the early stages of development. Since mature mammalian red blood cells are without any nuclei, they are called red blood "corpuscles" rather than cells (*L. corpus* = body, especially dead body or corpse).

The prokaryotic cells of the bacteria do not have true nucleus, *i.e.*, the single, circular and large DNA molecule remains in direct contact with the cytoplasm. The position or location of the nucleus in a cell is usually the characteristic of the cell type and it is often variable. Usually the nucleus remains located in the centre. But its position may change from time to time according to the metabolic states of the cell. For example, in the embryonic cells the nucleus generally occupies the geometric centre of the cell but as the cells start to differentiate and the rate of the metabolic activities increases, the displacement in the position of the nucleus takes place. In certain cells such as the glandular cells the nucleus remains located in the basal portion of the cell.

MORPHOLOGY

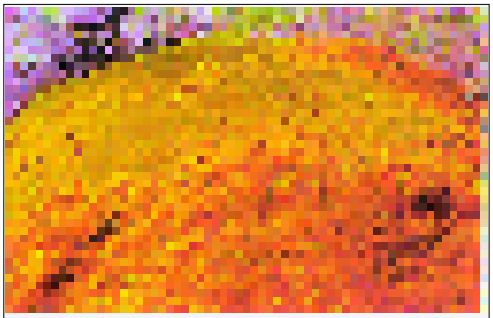
Number

Usually the cells contain single nucleus but the number of the nucleus may vary from cell to cell. According to the number of the nuclei following types of cells have been recognised :

1. Mononucleate cells. Most plant and animal cells contain single nucleus, such cells are known as **mononucleate cells.**

2. Binucleate cells. The cells which contain two nuclei are known as **binucleate cells**. Such cells occur in certain protozoans such as *Paramecium* and cells of cartilage and liver.

3. Polynucleate cells. The cells which contain many (from 3 to 100) nuclei are known as **polynucleate cells**. The polynucleate cells of the animals are termed as **syncytial cells**, while the polynucleate cells of the plants are known as **coeno-cytes**. The most common example of the syncytial



The nucleus surrounded by nuclear membrane. The pits are nuclear pores.

cells are the osteoblast (polykaryocytes of the bone morrow) which contain about 100 nuclei per cell and striated muscle fibres each of which contains many hundred nuclei. The siphonal algae *Vaucheria* contains hundreds of nuclei and certain fungi are the best example of the coenocytic plant cells.

Shape

The shape of the nucleus normally remains related with the shape of the cell, but certain nuclei are almost irregular in shape. The spheroid, cuboid or polyhedral cells (isodiametrical cells) contain the **spheroid** nuclei. The nuclei of the cylindrical, prismatic or fusiform cells are **ellipsoid** in shape. The cells of the squamous epithelium contain the **discoidal** nuclei. The leukocytes, certain infusoria, glandular cells of some insects and spermatozoa contain the irregular shaped nuclei. Nuclei of cells of silk glands of silk worm have finger-like extensions that greatly increase their surface area (Fig. 12.2).

Size

Generally nucleus occupies about 10 per cent of the total cell volume. Nuclei vary in size from about 3 µm to 25 µm in diameter, depending on cell type and contain diploid set of chromosomes. The size of the nucleus is directly proportional to that of the cytoplasm. **R. Hertwig** has given the following formula for the deduction of the size of the nucleus of a particular cell.

$$NP = \frac{V_n}{V_c - V_n}$$

Where NP is the nucleoprotein index, V_n is the volume of the nucleus and V_c is the volume of the cell.

Moreover, the size of the nucleus is related with the number of the chromosomes or ploidy. The haploid cells contain small-sized nuclei in comparison to the nuclei of the diploid cells. Likewise the polyploid cells contain larger nuclei than the diploid cells. Thus, the size of the nucleus of a cell depends on the volume of the cell, amount of the DNA and proteins and metabolic phase of the cell.

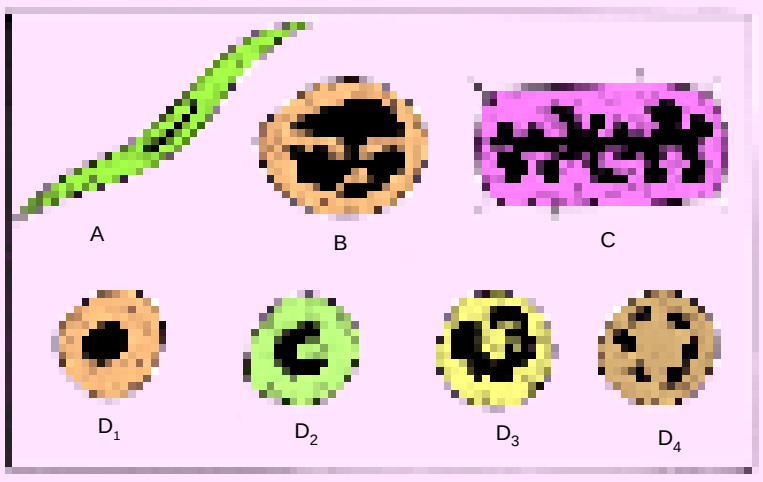


Fig. 12.2. Different shapes of the nucleus in animal cells. A—Elongated in muscle cell; B—Lobed in a human neutrophil cell; C—Branched in a silk spinning cell of an insect larva; D1 to D4—Variable shape in leucocytes.

ISOLATION TECHNIQUES

The number of nuclei can be measured easily in a chamber similar to that used for blood counts. For the isolation of nuclear envelope, nuclei are first of all separated from the rest of the cell. This is normally accomplished by disrupting tissue in homogenizers wherein the clearance is such that nuclei are not broken but the plasma membrane and endoplasmic reticulum are severely disrupted. Nuclei can then be harvested by differential centrifugation. They are then lysed by sonication and their envelopes separated on density gradient centrifugation.

Alternatively, DNAase digestion followed by extraction with salts releases envelopes which again can be banded on sucrose or cesium chloride gradients (see **Thorpe**, 1984).

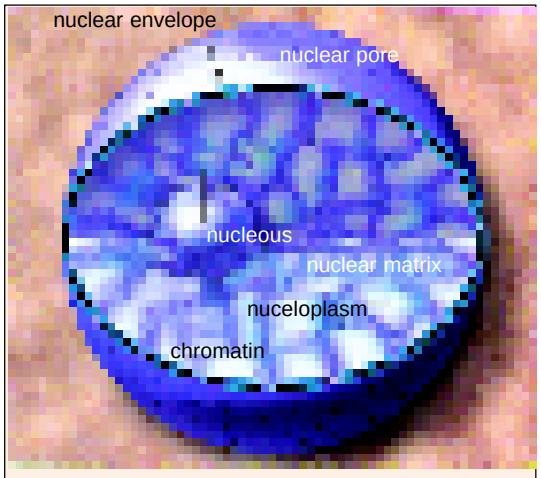
The chemical organization of the nucleus has been investigated by two main approaches. The first, which is essentially biochemical, consists of isolating a large enough number of nuclei to permit analysis by biochemical methods. The second approach, which is essentially cytologic, uses the cytophotometric and radio-autographic methods. A nucleolar fraction may be obtained by treating the nuclei with highly ionic solutions and digesting the chromatin with DNAse (**Penman** *et al.*, 1966). The amount of DNA in an eukaryotic nucleus can be determined by microspectrophotometry, a technique that measures with precision the amount of Feulgen staining material each nucleus contains.

ULTRASTRUCTURE

The nucleus is composed of following structures : 1. The nuclear membrane or karyotheca or nuclear envelope; 2. The nuclear sap or nucleoplasm; 3. The chromatin fibres; and 4. The nucleolus.

1. Nuclear Envelope

The nuclear envelope (or perinuclear cisterna) encloses the DNA and defines the nuclear compartment of interphase and prophase nuclei. It is formed from two concentric unit membranes, each 5–10 nm thick. The spherical **inner nuclear membrane** contains specific proteins that act as binding sites for the supporting fibrous sheath of intermediate filaments (IF), called **nuclear lamina**. Nuclear lamina has contact with the chromatin (or chromosomes) and nuclear RNAs. The inner nuclear membrane is surrounded by the **outer nuclear membrane**, which closely resembles the membrane of the endoplasmic reticulum, that is continuous with it. It is also surrounded by less organized intermediate filaments (Fig. 12.3). Like the membrane of the rough ER, the outer surface of outer nuclear membrane is generally studded with ribosomes engaged in protein synthesis. The proteins made on these ribosomes are transported into space between the inner and outer nuclear membrane, called **perinuclear space**. The perinuclear space is a 10 to 50 nm wide fluid-filled compartment which is continuous with the ER lumen and may contain fibres, crystalline deposits, lipid droplets or electron-dense material (see **Thorpe**, 1984).



The cell nucleus.

Nuclear lamina. It is also called **fibrous lamina**, **zonula nucleum limitans**, **internal dense lamella**, **nuclear cortex** and **lamina densa**.

The nuclear lamina is a protein meshwork which is 50 to 80 nm thick (**DeRobertis** and **De Robertis, Jr.**, 1987) or 10 to 20 nm thick (**Alberts** *et al.*, 1987). It lines the inside surface of the inner nuclear membrane, except the areas of nucleopores, and consists of a square lattice of intermediate filaments. In mammals, these intermediate filaments are of three types : **lamins A**, **B** and **C** having M.W. 74,000, 72,000 and 62,000 daltons, respectively. The lamins form dimers that have a rod-like domain and two globular heads at one end. Under appropriate conditions of pH and ionic strength, the dimers spontaneously associate into filaments that have a diameter and repeating structure similar to those of cytoplasmic filaments.

The nuclear lamina is a very dynamic structure. In mammalian cells undergoing mitosis, the transient phosphorylation of several serine residues on the lamins causes the lamina to reversibly

disassemble into tetramers of hypophosphorylated lamin A and lamin C and membrane associated lamin B. As a result, lamin A and C become entirely soluble during mitosis, and at telophase they become dephosphorylated again and polymerize around chromatin. Lamin B seems to remain associated with membrane vesicles during mitosis, and these vesicles in turn remain as a distinct subset of membrane components from which nuclear envelope is reassembled at telophase. Inside an interphase nucleus, chromatin binds strongly to the inner part of the nuclear lamina which is believed to interfere with chromosome condensation. In fact, during meiotic chromosome condensation, the nuclear lamina completely disappears by the pachytene stage of prophase and reappears later during diplotene in oocytes, but does not reappear at all in spermatocytes.

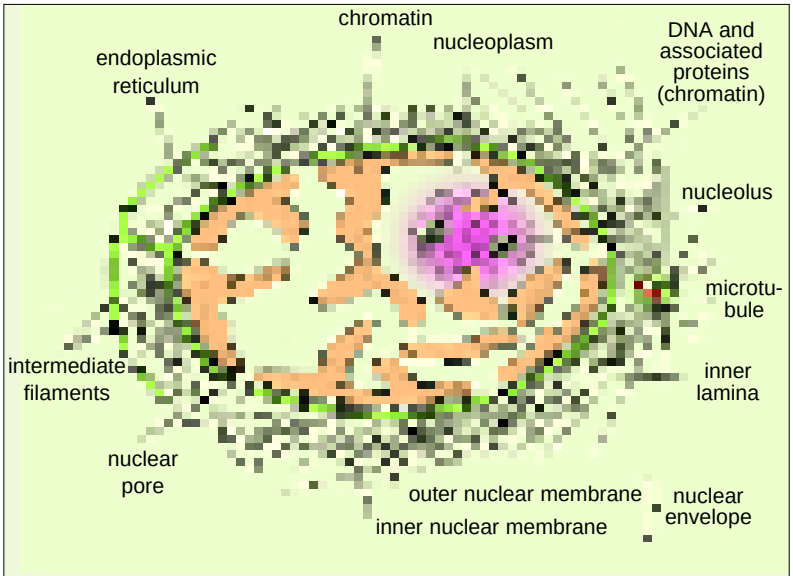


Fig. 12.3. Cross-section of a typical cell nucleus showing ultrastructure (after Alberts *et al.*, 1989).

The lamins may play a crucial role in the assembly of interphase nuclei. For example, when cells are left for a long time in colchicine (drug which arrests cells in metaphase), the lamins assemble around individual chromosomes, which then surrounded by nuclear envelopes give rise to micronuclei containing only one chromosome. A similar phenomenon occurs during normal amphibian development. In the first few cleavages of amphibian development, the nuclear envelope initially forms around individual chromosomes, forming several vesicles that then fuse together to form a single nucleus. This suggests that chromatin is the nucleating centre for the deposition of a nuclear lamina and envelope.

Nucleus of a cultured human cell that has been stained with fluorescently labeled antibodies to reveal the nuclear lamina (red) which lies on the inner surface of the nuclear envelope.

Nuclear pores and nucleocytoplasmic traffic. The nuclear envelope in all eukaryotic forms, from yeasts to humans, is perforated by **nuclear pores** which have the following structure and function :

1. Structure of nuclear pores.

Nuclear pores appear circular in surface view and have a diameter between 10nm to 100 nm. Previously it was believed that a diaphragm made of amorphous to fibrillar material extends across each pore limiting free transfer of material. Such a diaphragm called **annulus** has been observed in animal cells, but lacking in plant cells. Recent electron microscopic studies have found that a nuclear pore has far more complex structure, so it is called **nuclear pore complex**. Each pore complex has an estimated molecular weight of 50 to 100 million daltons. Negative staining techniques have demonstrated that pore complexes have an eight-fold or octagonal symmetry. More recent computerized image-processing techniques of **Unwin** and **Mulligan** (1982) have shown that the pore complex consists of two "**rings**" (**R** or annuli) at its periphery with an inside diameter of 80 nm, a large particle that forms a **central plug** (**C**) and radial '**spokes**' (**S**) that extends from the plug to the rings (Fig. 12.5 B and C). **Particles** (**P**) are anchored to cytoplasmic ring and are thought to be inactive ribosomes. The '**hole**' in the centre of the pore complex is an aqueous channel through which water-soluble molecules shuttle between the nucleus and the cytoplasm. This hole often appears to be plugged by a large central granule (**central plug**) which is believed to consist of newly made ribosomes or other particles caught in transit.

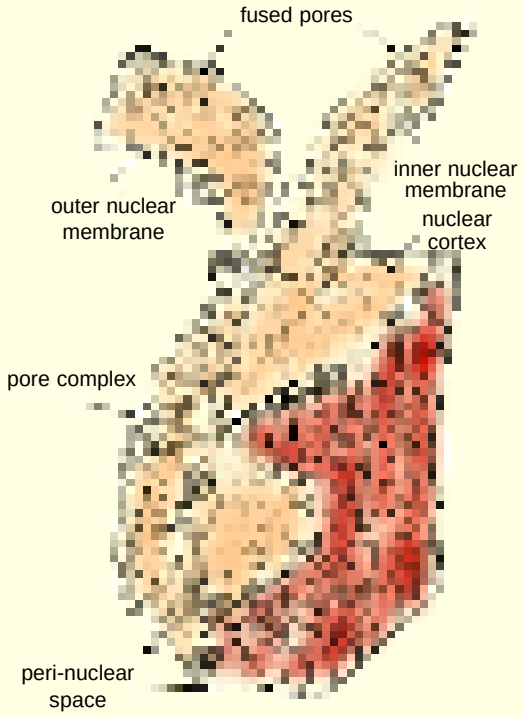


Fig. 12.4. A model of the nuclear surface complex as proposed by **Schatten** and **Thoman** (1978) (after Thorpe, 1984).

The pore complex perforates the nuclear envelope bringing the lipid bilayers of the inner and outer nuclear membrane together around the margins of each pore. Despite this continuity, which would seem to provide a pathway for the diffusion of membrane components between the inner and outer membranes, the two membranes remain chemically distinct.

Quite recently, following two proteins have been found to be associated to the nuclear pores : one is an integral membrane protein, a glycoprotein of 120,000 daltons that may anchor the annuli to the lipid bilayer (**Gerace** *et al.*, 1982). The second protein is a 63,000 dalton protein (that has covalently bound acetyl neuraminic acid) located on the cytoplasmic side of the electron-dense material that occludes the nuclear pores (**Davis** and **Blobel**, 1986). This protein may be involved in the transport of materials through the nuclear pores.

2. Number of nuclear pores (Pore density). In nuclei of mammals it has been calculated that nuclear pores account for 5 to 15 per cent of the surface area of the nuclear membrane. In amphibian oocytes, certain plant cells and protozoa, the surface occupied by the nuclear pores may be as high as 20 to 36 per cent. The number of pores in the nuclear envelope or **pore density** seems to correlate with the transcriptional activity of the cell (Table 12-1). Thus, pore densities as low as ~3 pores/μm^2 are seen in nucleated red blood cells and lymphocytes (which are inactive in transcription). These cells are highly differentiated but metabolically inactive and they are non- proliferating cells. The majority of

proliferating cells have pore densities between 7 and 12 pores/μm^2. Among cells of a third type, differentiated but highly active, pore densities are often 15 to 20 pores/μm^2. Liver, kidney and brain cells fall into this category. Still higher pore densities are found in specialized cells, such as salivary gland cells (~40 pores/μm^2) and the oocytes from *Xenopus laevis* (~50 pores/μm^2), both of which are very active in transcription.

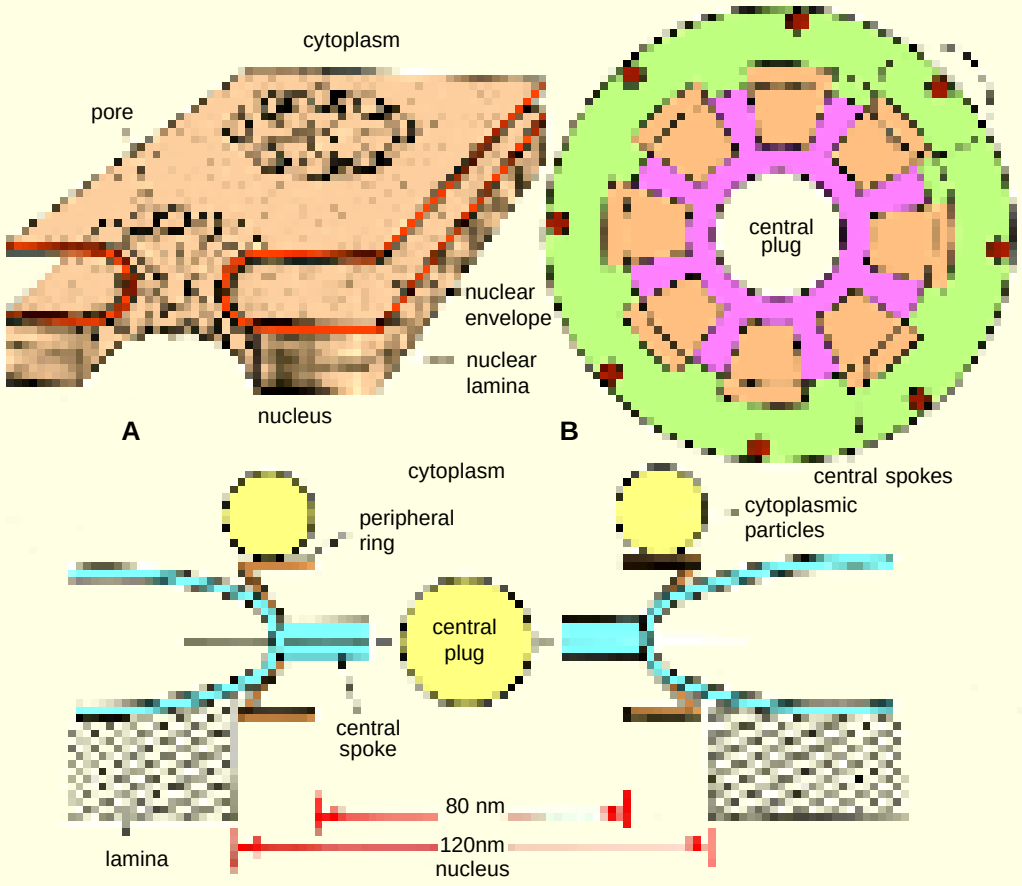
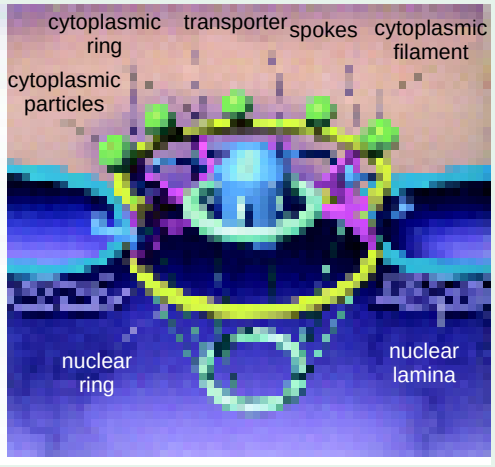


Fig. 12.5. Nuclear pore. A—A part of nuclear envelope with 80 nm pores occupied by pore complexes of octogonal radial symmetry; B—Nuclear pore in top view; C—A nuclear pore in cross section (after De Robertis and De Robertis, Jr., 1987).

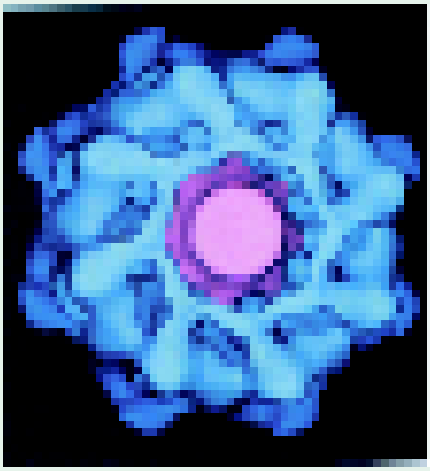
Table 12-1. **Pore densities and nuclear pores per nucleus in various cell types (source : Thorpe, 1984).**

Cell type	Pore density (Pores/μm^2)	Pores per nucleus
1. Human lymphocytes	3.2	405
2. Leopard frog embryo	5.6	1729
3. Human embryonic lung	8.5	2788
4. African green monkey kidney	8.6	4277
5. Newt heart	7.6	12,707
6. *Xenopus laevis* oocyte	51	37.7×10^6
7. *Triturus alpestris* oocyte	50	57×10^6

3. Arrangement of nuclear pores on nuclear envelope. In somatic cells, the nuclear pores are evenly or randomly distributed over the surface of nuclear envelope. However, pore arrangement in other cell types is not random but rather range from **rows** (*e.g.*, spores of *Eqisetum*) to **Clusters** (*e.g.*, oocytes of *Xenopus laevis*) to **hexagonal** (*e.g.*, Malpighian tubules of leaf hoppers) packing order (see **Thorpe**, 1984).



(a) A three dimensional representation of a vertebrate nuclear pore complex.



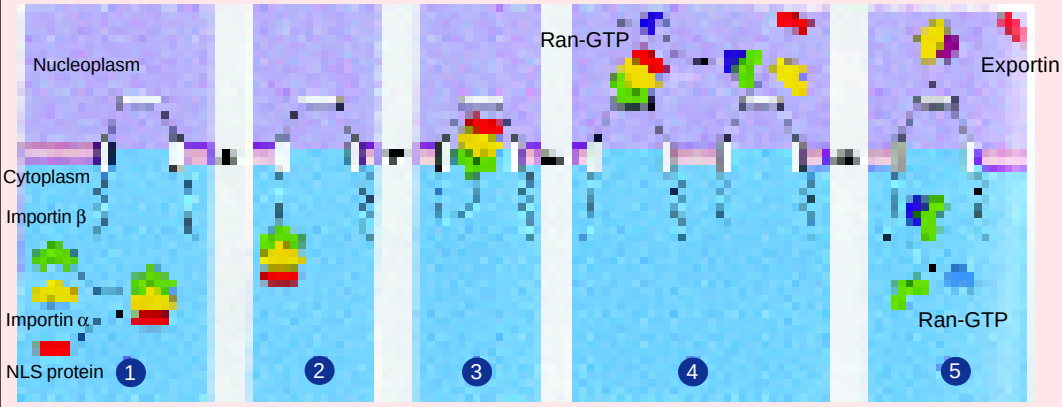
(b) A three dimensional model based on high resolution electron microscopy showing nuclear pore complex.

4. Nucleo-cytoplasmic traffic. Quite evidently there is considerable trafficking across the nuclear envelope during interphase. Ions, nucleotides and structural, catalytic and regulatory proteins are imported from the cytosol (cytoplasmic matrix); mRNA, tRNA and ribosome subunits are exported to the cytosol (cytoplasmic matrix) (see **Reid** and **Leech**, 1980). However, one of the main functions of the nuclear envelope is to prevent the entrance of active ribosomes into the nucleus.

The pore appears to function like a close fitting diaphragm that opens to just the right extent when activated by a signal on an appropriate large protein (having a diameter up to 20 nm). Recently, it has been investigated that the nuclear-specific proteins (called **karyophilic proteins**) have in their molecular structure some type of signals, called **karyophilic signals** or **nuclear import signals**, that enable them to accumulate selectively in the nucleus. For example, **nucleoplasmin** is an abundant, pentameric nuclear protein having distinct head and tail domains. Nucleoplasmins are actively transported through the nuclear pores, probably while still in their folded form. The karyophilic signal for such a nuclear import apparently resides in the tail domains and such an active nuclear transport requires energy which is derived from ATP hydrolysis. Similar signals are also noted in a short sequence (126-132 amino acids) of **simian virus 40 T** antigen molecule. These short sequences when attached to bigger molecules (even to metal particles such as gold) allow these bigger molecules to enter the nucleus via the nuclear pores.

5. Rate of transport through the nuclear pores. As we have already described, the nuclear envelope of a typical mammalian cell contains 3000 to 4000 pores (about 11 pores/ μm^2 of membrane area). If the cell is synthesizing DNA, it needs to import about 10^6 histone molecules from the cytoplasm every 3 minutes in order to package newly made DNA into chromatin, which means that on an average each pore needs to transport about 100 histone molecules per minute. Further, if the cell is growing rapidly, each nuclear pore needs to export about three newly assembled ribosomes per minute to the cytoplasm, since ribosomes are produced in nucleus but function in the cytoplasm. The export of new ribosomal subunits is particularly problematic since these particles are about 15 nm in diameter and are much too large to pass through the 9 nm channels of nuclear pores, it is believed that they are

specifically exported through the nuclear pores by an active transport system. Similarly, mRNA molecules complexed with special proteins to form ribonucleoprotein particles, are thought to be actively exported from the nucleus.



Importing proteins from the cytoplasm into the nucleus. The protein bearing a nuclear localization signal (NLS) binds to the heterodimeric receptor (importin α/β) (step 1) forming a complex that associates with a cytoplasmic filament (step 2). The protein complex is moved through the nuclear pore (step 3) and into the nucleoplasm where it interacts with Ran-GTP and dissociates (step 4). The importin β subunit is transported back to the cytoplasm in association with Ran-GTP (step 5), where the Ran-GTP is hydrolyzed. Ran-GDP is subsequently transported back to the nucleus and importin α back to the cytoplasm.

Lastly, nuclear pores are not the only avenues for nucleocytoplasmic exchanges (Fig. 12.6). For example, small molecules and ions readily permeate both nuclear membranes. Larger molecules and particles may pass through the membrane by formation of small pockets and vesicles that traverse the envelope and empty on the other side.

2. Nucleoplasm

The space between the nuclear envelope and the nucleolus is filled by a transparent, semi-solid, granular and slightly acidophilic ground substance or the matrix known as the **nuclear sap** or **nucleoplasm** or **karyolymph**. The nuclear components such as the chromatin threads and the nucleolus remain suspended in the nucleoplasm.

The nucleoplasm has a complex chemical composition. It is composed of mainly the nucleoproteins but it also contains other inorganic and organic substances, *viz*., nucleic acids, proteins, enzymes and minerals.

1. Nucleic acids. The most common nucleic acids of the nucleoplasm are the DNA and RNA. Both may occur in the macromolecular state or in the form of their monomer nucleotides.

2. Proteins. The nucleoplasm contains many types of complex proteins. The nucleoproteins can be categorized into following two types :

(i) Basic proteins. The proteins which take basic stain are known as the basic proteins. The most important basic proteins of the nucleus are **nucleoprotamines** and the **nucleohistones**.

The nucleoprotamines are simple and basic proteins having very low molecular weight (about 4000 daltons). The most abundant amino acid of these proteins is **arginine** (pH 10 to 11). The protamines usually remain bounded with the DNA molecules by the salt linkage. The protamines occur in the spermatozoa of the certain fishes. The nucleohistones have high molecular weight, *e.g*., 10,000 to 18,000 daltons. The histones are composed of basic amino acids such as **arginine**, **lysine** and **histidine**. The histone proteins remain associated with the DNA by the ionic bonds and they occur in the nuclei of most organisms. According to the composition of the amino acids following types of

histone proteins have been recognised, *e.g.*, histones rich in lysine, histones with arginine and histones with poor amount of the lysine.

(ii) Non-histone or Acidic proteins. The acidic proteins either occur in the nucleoplasm or in the chromatin. The most abundant acidic proteins of the euchromatin (a type of chromatin) are the **phosphoproteins**.

3. Enzymes. The nucleoplasm contains many enzymes which are necessary for the synthesis of the DNA and RNA. Most of the nuclear enzymes are composed of non-histone (acidic) proteins. The most important nuclear enzymes are the **DNA polymerase**, **RNA polymerase**, **NAD synthetase**, **nucleoside triphosphatase**, **adenosine diaminase**, **nucleoside phosphorylase**, **guanase**, **aldolase**, **enolase**, **3-phosphoglyceraldehyde dehydrogenase** and **pyruvate kinase**. The nucleoplasm also contains certain cofactors and coenzymes such as **ATP** and **acetyl CoA**.

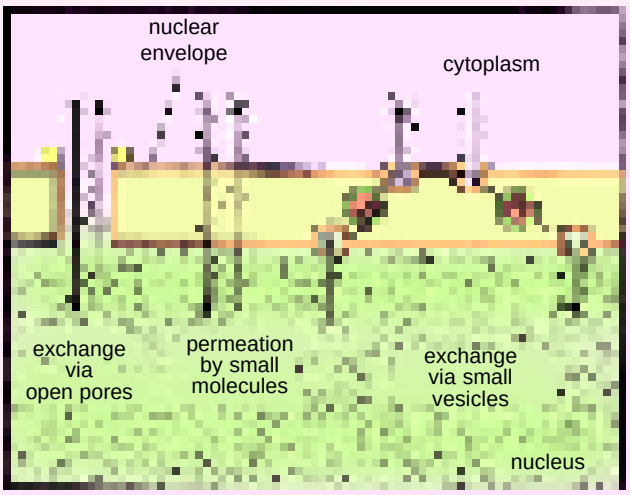


Fig. 12.6. Various avenues for transport of material from the nucleus to the cytosol (cytoplasmic matrix) (after Sheeler and Bianchi, 1987).

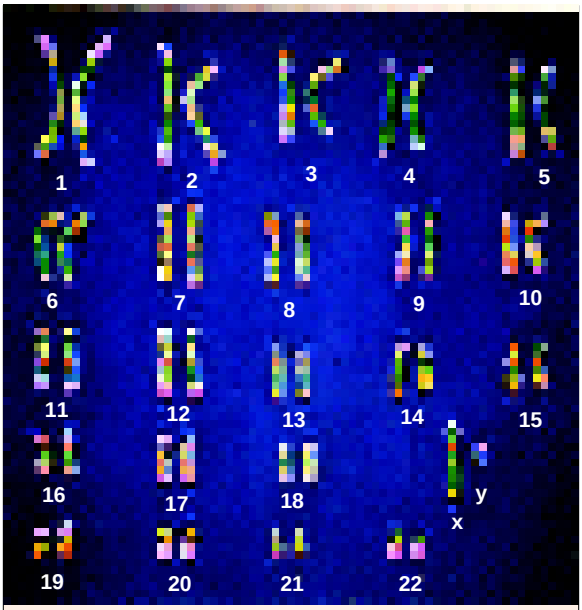
4. Lipids. According to **Stoneburg** (1937) and **Dounce** (1955), the nucleoplasm contains small lipid content.

5. Minerals. The nucleoplasm also contains several inorganic compounds such as phosphorus, potassium, sodium, calcium and magnesium. The chromatin comparatively contains large amount of these minerals than the nucleoplasm.

3. Chromatin Fibres

The nucleoplasm contains many thread-like, coiled and much elongated structures which take readily the basic stains such as the basic fuchsin. These thread-like structures are known as the **chromatin** (*Gr.*, *chrome*=colour) **substance** or **chromatin fibres**. Such chromatin fibres are observed only in the interphase nucleus. During the cell division (mitosis and meiosis) chromatin fibres become thick ribbon-like structures which are known as the **chromosomes**.

Chemically, chromatin consists of DNA and proteins. Small quantity of RNA may also be present but the RNA rarely accounts for more than about 5 per cent of the total chromatin present. Most of the protein of chromatin is histone, but "nonhistone" proteins are also present. The protein : DNA weight ratio averages about 1:1. Histones are



The 23 pairs of chromosomes in the human karyotype.

constituents of the chromatin of all eukaryotes except fungi, which, therefore, resemble prokaryotes in this respect (see **Sheeler** and **Bianchi**, 1987).

The fibres of the chromatin are twisted, finely anastomosed and uniformly distributed in the nucleoplasm. Two types of chromatin material have been recognised, *e.g.*, heterochromatin and euchromatin.

A. Heterochromatin. The darkly stained, condensed region of the chromatin is known as heterochromatin. The condensed portions of the nucleus are known as **chromocenters** or **karyosomes** or **false nucleoli**. The heterochromatin occurs around the nucleolus and at the periphery. It is supposed to be metabolically and genetically inert because it contains comparatively small amout of the DNA and large amount of the RNA.

B. Euchromatin. The light stained and diffused region of the chromatin is known as the euchromatin. The euchromatin contains comparatively large amount of DNA.

4. Nucleolus

Most cells contain in their nuclei one or more prominent spherical colloidal acidophilic bodies, called **nucleoli**. However, cells of bacteria and yeast lack nucleolus. The size of the nucleolus is found to be related with the synthetic activity of the cell. Therefore, the cells with little or no synthetic activities, *e.g.*, sperm cells, blastomeres, muscle cell, etc., are found to contain smaller or no nucleoli, while the oocytes, neurons and secretory cells which synthesize the proteins or other substances contain comparatively large-sized nucleoli. The number of the nucleoli in the nucleus depends on the species and the number of the chromosomes. The number of the nucleoli in the cells may be one, two or four. The position of the nucleolus in the nucleus is eccentric.

A nucleolus is often associated with the **nucleolar organizer (NO)** which represents the secondary constriction of the nucleolar organizing chromosomes, and are 10 in number in human beings (Fig. 12.7). In corn, *Zea mays* chromosome 9 and 6 contain 'darkly staining knobs' or nucleolar organizers (**Heitz** and **McClintock**, 1930s). Nucleolar organizer consists of the genes for 18S, 5.8S and 28S rRNAs. The genes for fourth type of r RNA, *i.e.*, 5S rRNA occur outside the nucleolar organizer.

1. Chemical composition of nucleolus. Nucleolus is not bounded by any limiting membrane; calcium ions are supposed to maintain its intact organization. Chemically, nucleolus contains DNA of nucleolar organizer, four types of rRNAs, 70 types of ribosomal proteins, RNA binding proteins (*e.g.*, nucleolin) and RNA splicing nucleoproteins (U_1, U_2.....U_{12}). It also contains phospholipids, orthophosphates and Ca^{2+} ions. Nucleolus also contains some enzymes such as acid phosphatase, nucleoside phosphorylase and NAD^+synthesizing enzymes for the synthesis of some co-enzymes, nucleotides and ribosomal RNA. **RNA methylase** enzyme which transfers methyl groups to the nitrogen bases, occurs in the nucleolus of some cells.

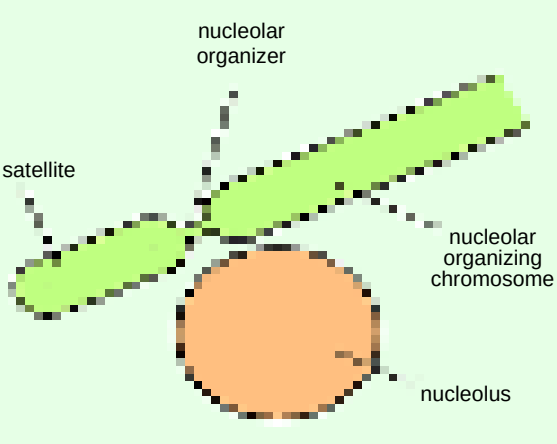


Fig 12.7. A satellited chromosome and an attached nucleolus.

2. Ultrastructure and function of nucleolus. Nucleolus are the sites where biogenesis of ribosomal subunits

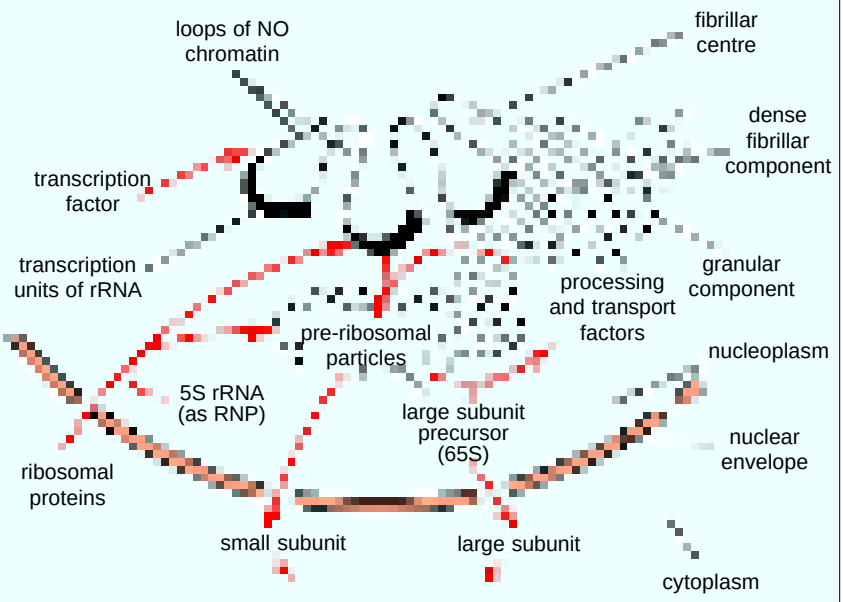


Fig. 12.8. Three different regions of the nucleolus and their involvement in ribosome assembly.

(*i.e.*, 40S and 60S) takes place. In it three types of rRNAs, namely 18S, 5.8S and 28S r RNAs, are transcribed as parts of a much longer precursor molecule (45S transcript) which undergoes processing (RNA splicing, for example) by the help of two types of proteins such as nucleolin and U3 sn RNP (U3 is a 250 nucleotide containing RNA, sn RNP represents small nuclear ribonucleoprotein). The 5S r RNA is transcribed on the chromosome existing outside the nucleolus and the 70 types of ribosomal proteins are synthesized in the cytoplasm. All of these components of the ribosomes migrate to the nucleolus, where they are assembled into two types of ribosomal subunits which are transported back to the cytoplasm. The smaller (40S) ribosomal subunits are formed and migrate to the cytoplasm much earlier than larger (60S) ribosomal subunits; therefore, nucleolus contains many more incomplete 60S ribosomal subunits than the 40S ribosomal subunits. Such a time lag in the migration of 60S and 40S ribosomal subunits, prevents functional ribosomes from gaining access to the incompletely processed heterogeneous RNA (hn RNA; the precursor of m RNA) molecule inside the nucleus (see **Alberts** *et al.*, 1989).

Different stages of formation of ribosomes are completed in three distinct regions of the nucleolus. Thus, their **initiation**, **production** and **maturation** seem to progress from centre to periphery. Following three regions have been identified in the nucleolus (Fig. 12.8) :

(i) Fibrillar centre. This pale-staining part represents the innermost region of nucleolus. The RNA genes of nucleolar organizer of chromosomes are located in this region. The transcription (*i.e.*, ribosomal RNA synthesis) of these genes is also initiated in this region.

(ii) Dense fibrillar component. This region surrounds the fibrillar centre and RNA synthesis progresses in this region. The 70 ribosomal proteins (rps) also bind to the transcripts in this region.

(iii) Cortical granular components. This is the outermost region of the nucleus where processing and maturation of pre-ribosomal particles occur.

3. Mitotic cycle of nucleolus. The appearance of nucleolus changes dramatically during the cell cycle. During meiosis as well as during mitosis the nucleolus disappears during prophase. 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 (NOs). For example, in humans the r RNA genes are located near the tips of each of the 5 different chromosomes (*i.e.*, paired autosomes 13,14,15,21 and 22; **Franke**, 1981). Accordingly, 10 small nucleoli are formed after mitosis in a human diploid 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.

Now let us see what happens to the RNA and protein components of the disintegrated nucleolus during mitosis? It seems that at least 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 (**Anastassova-Kristeva**, 1977).

REVISION QUESTIONS

1. Describe the nuclear envelope and the structure of its pores. What similarities occur between nuclear envelope and endoplasmic reticulum ?
2. Describe the ultrastructure of nucleus.
3. Discuss the cytochemistry of nucleus.
4. How many types of proteins and nucleic acids are found in the nucleus ?
5. Describe the structure and function of the nucleolus. How is it formed ?
6. Does amount of DNA has any correlation with the ploidy of the cell? What ?