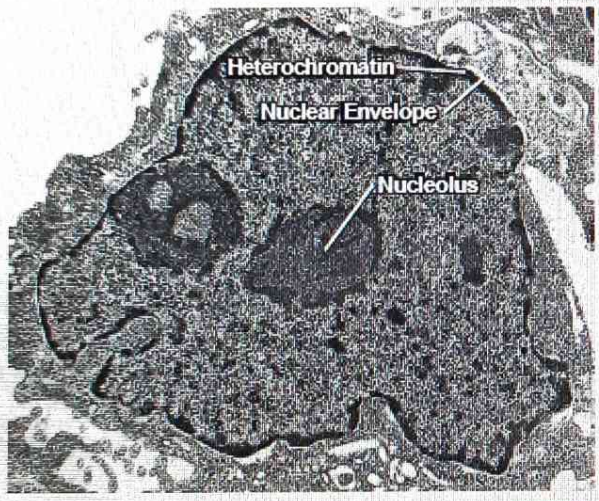
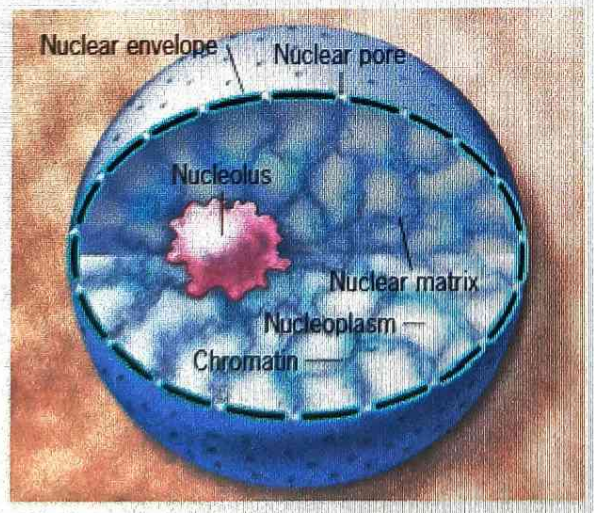
12.1 THE NUCLEUS OF A EUKARYOTIC CELL

Considering its importance in the storage and utilization of genetic information, the nucleus of a eukaryotic cell has a rather undistinguished morphology (Figure 12.1). The contents 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 (i.e., 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 (discussed on page 428); (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.



(a)

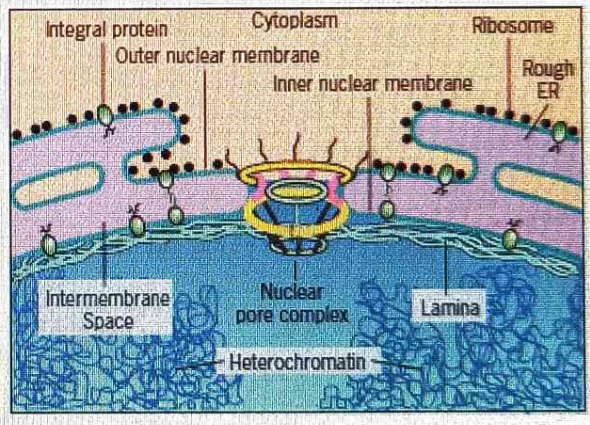


(b)

FIGURE 12.1 The cell nucleus. (*a*) Electron micrograph of an interphase HeLa cell nucleus. Heterochromatin (page 485) is evident around the entire inner surface of the nuclear envelope. Two prominent nucleoli are visible, and clumps of chromatin can be seen scattered throughout the nucleoplasm. (*b*) Schematic drawing showing some of the major components of the nucleus. (*a*: FROM WERNER W. FRANKE, INT. REV. CYTOL. (SUPPL.) 4:130, 1974.)

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 (Figure 12.2*a*). 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.



(a)

(b)

FIGURE 12.2 The nuclear envelope. (*a*) Schematic drawing showing the double membrane, nuclear pore complex, nuclear lamina, and the continuity of the outer membrane with the rough endoplasmic reticulum (ER). Both membranes of the nuclear envelope contain their own distinct complement of proteins. (*b*) Electron micrograph of a section through a portion of the nuclear envelope of an onion root tip cell. Note the double membrane (NM) with intervening space, nuclear pore complexes (NPC), and associated heterochromatin (HC) that does not extend into the region of the nuclear pores. (*b*: FROM WERNER W. FRANKE ET AL. J. CELL. BIOL. 91:47s, 1981; BY COPYRIGHT PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS.)

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 (Figure 12.2*a*).

The inner surface of the nuclear envelope of animal cells is bound by integral membrane proteins to a thin filamentous meshwork, called the **nuclear lamina** (Figure 12.3). The nuclear lamina provides mechanical support to the nuclear envelope, serves as a site of attachment for chromatin fibers at the nuclear periphery (Figure 12.2*b*), and has a poorly understood role in DNA replication and transcription. The filaments of the nuclear lamina are approximately 10 nm in diameter and composed of polypeptides, called *lamins*. Lamins are members of the same superfamily of polypeptides that assemble into the 10-nm intermediate filaments of the cytoplasm (see Table 9.2). As in the cytoplasm, the integrity of the intermediate filaments that make up nuclear lamina is regulated by phosphorylation and dephosphorylation. The disassembly of the nuclear lamina prior to mitosis is induced by phosphorylation of the lamins by a specific protein kinase.

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. Figure 12.3*c* shows the misshapen nuclei from the cells of a patient with HGPS, demonstrating the importance of the nuclear lamina as a determinant of nuclear architecture. It is interesting to note that the phenotype depicted in Figure 12.3*c* has been traced to a synonymous mutation, that is, one that generated a different codon for the same amino acid. In this case, the change in DNA sequence altered the way the gene transcript was spliced, which led to production of a shortened protein, causing the altered phenotype. This example illustrates how the sequence of a gene serves as a "multiple code:" one that directs the translation machinery and others that direct the splicing machinery and protein folding.

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

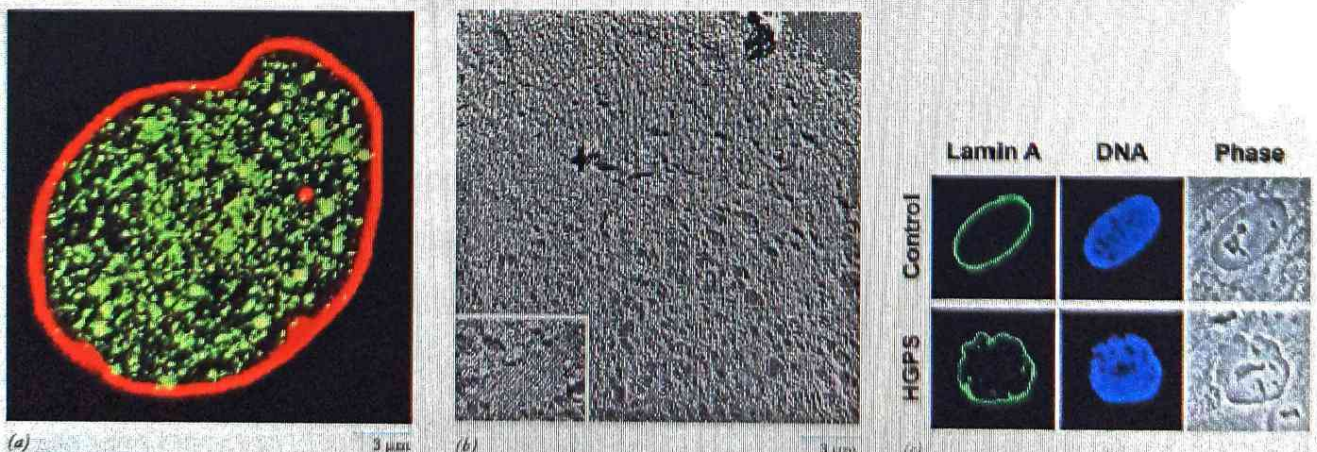
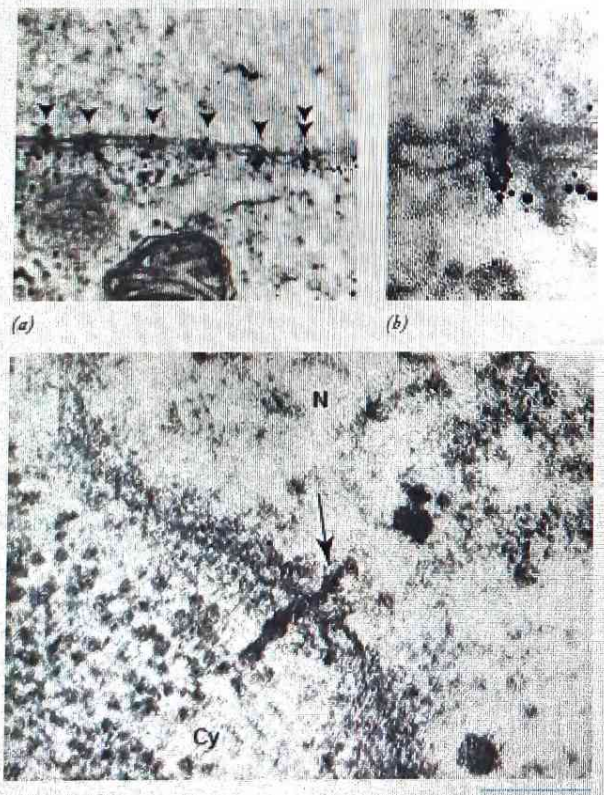


FIGURE 12.3 The nuclear lamina. (*a*) Nucleus of a cultured human cell that has been stained with fluorescently labeled antibodies to reveal the nuclear lamina (red), which lies on the inner surface of the nuclear envelope. The nuclear matrix (page 499) is stained green. (*b*) Electron micrograph of a freeze-dried, metal-shadowed nuclear envelope of a *Xenopus* oocyte that has been extracted with the nonionic detergent Triton X-100. The lamina appears as a rather continuous meshwork comprising filaments oriented roughly perpendicular to one another. Inset shows a well-preserved area from which nuclear pores have been mechanically removed. (*c*) These micrographs show the nucleus within a fibroblast that had been cultured from either a patient with HGPS (bottom row) or a healthy subject (top row). The cells are stained for the protein lamin A (left column), for DNA (middle column), or shown in a living state under the phase contrast light microscope (right column). The cell nucleus from the HGPS patient is misshapen due to the presence in the nuclear lamina of a truncated lamin A protein. (A: FROM H. MA, A. J. SIEGEL, AND R. BEREZNEY, J. CELL BIOL. 146:535, 1999; BY COPYRIGHT PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS; B: REPRINTED WITH PERMISSION FROM U. AEBI, J. COHN, L. BUHLE, AND L. GERACE, NATURE 323:561, 1986; © COPYRIGHT 1986, MACMILLAN MAGAZINES LIMITED; C: FROM ANNA MATTOUT ET AL., COURTESY OF ROBERT D. GOLDMAN, CURR. OPIN. CELL BIOL. 18:338, 2006.)

spliceosome (page 444), 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. To appreciate the magnitude of the traffic between the two major cellular compartments, consider a HeLa cell, which is estimated to contain about 10,000,000 ribosomes. To support its growth, a single HeLa cell nucleus must import approximately 560,000 ribosomal proteins and export approximately 14,000 ribosomal subunits every minute.

How do all of these materials pass through the nuclear envelope? In one early approach, a suspension of tiny gold particles was injected into cells and passage of the material through the nuclear envelope was observed with the electron microscope. As illustrated in Figure 12.4*a*,*b*, these particles move from the cytoplasm into the nucleus by passing single-file through the center of the nuclear pores. Electron micrographs of cells fixed in the normal course of their activities have also shown that particulate material can pass through a nuclear pore. An example is shown in Figure 12.4*c*, in which granular material presumed to consist of a ribosomal subunit is seen squeezing through one of these pores.



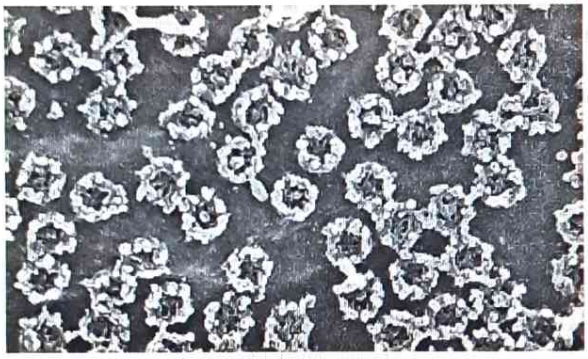
(*a*) (*b*) (*c*)

FIGURE 12.4 The movement of materials through the nuclear pore. (*a*) Electron micrograph of the nuclear–cytoplasmic border of a frog oocyte taken minutes after injection with gold particles that had been coated with a protein normally found in the nucleus. These particles are seen to pass through the center of the nuclear pores (arrows) on their way from the cytoplasm to the nucleus. (*b*) At higher magnification, the gold particles are seen to be clustered in linear array within each pore. (*c*) Electron micrograph of a section through the nuclear envelope of an insect cell showing the movement of granular material (presumed to be a ribosomal subunit) through a nuclear pore. (A,B: COURTESY OF C. M. FELDHERR; C: FROM BARBARA J. STEVENS AND HEWSON SWIFT, J. CELL BIOL. 31:72, 1966; BY COPYRIGHT PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS.)

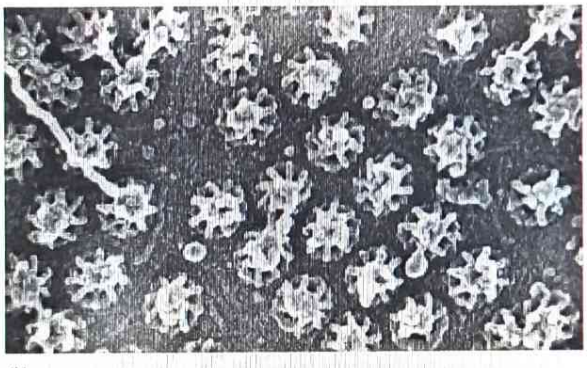
Given the fact that materials as large as gold particles and ribosomal subunits can penetrate nuclear pores, one might assume that these pores are merely open channels, but just the opposite is true. Nuclear pores contain an intricate structure called the **nuclear pore complex** (**NPC**) that appears to fill the pore like a stopper, projecting into both the cytoplasm and nucleoplasm. The structure of the NPC is seen in the electron micrographs of Figure 12.5 and the model of Figure 12.6. 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 (Figure 12.6). 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, by their single letter names). 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 (page 56) 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 (greater than about 40,000 Daltons) 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. The best studied, or "classical" NLSs, consist of one or two short stretches of positively charged amino acids. The T antigen encoded by the virus SV40, for example, contains an NLS identified as -Pro-Lys-Lys-Lys-Arg-Lys-Val-. If one of the basic amino acids in this sequence is replaced by a nonpolar amino acid, the protein fails to become localized in the nucleus. Conversely, if this NLS is fused to a nonnuclear protein, such as serum albumin, and injected into the cytoplasm, the modified protein becomes concentrated in the nucleus. Thus, targeting of proteins to the nu-



(a) 0.2 μm



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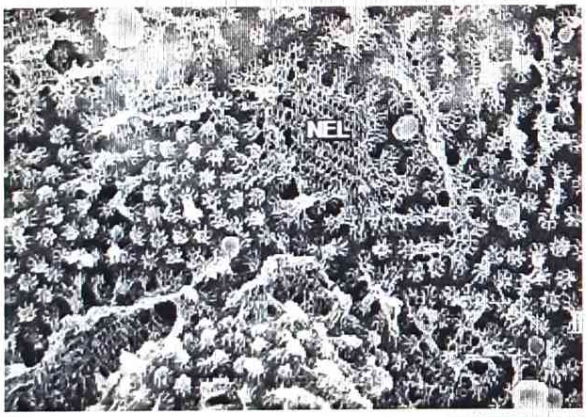


(c) 0.6 μm

FIGURE 12.5 Scanning electron micrographs of the nuclear pore complex from isolated nuclear envelopes of an amphibian oocyte. (*a*) The cytoplasmic face of the nuclear envelope showing the peripheral cytoplasmic ring of the nuclear pore complex. (*b*) The nuclear face of the nuclear envelope showing the basket-like appearance of the inner portion of the complex. (*c*) The nuclear face of the envelope showing the distribution of the NPCs and places where intact patches of the nuclear lamina (NEL) are retained. In all of these micrographs, isolated nuclear envelopes were fixed, dehydrated, dried, and metal-coated. (FROM M. W. GOLDBERG AND T. D. ALLEN, J. CELL. BIOL. 119:1431, 1992; BY COPYRIGHT PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS.)

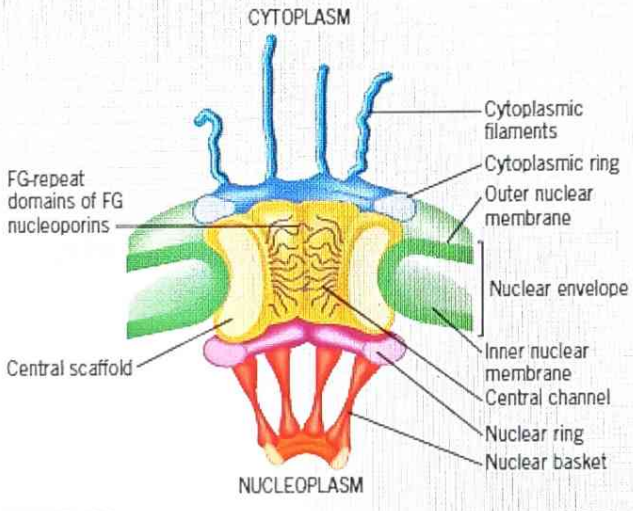
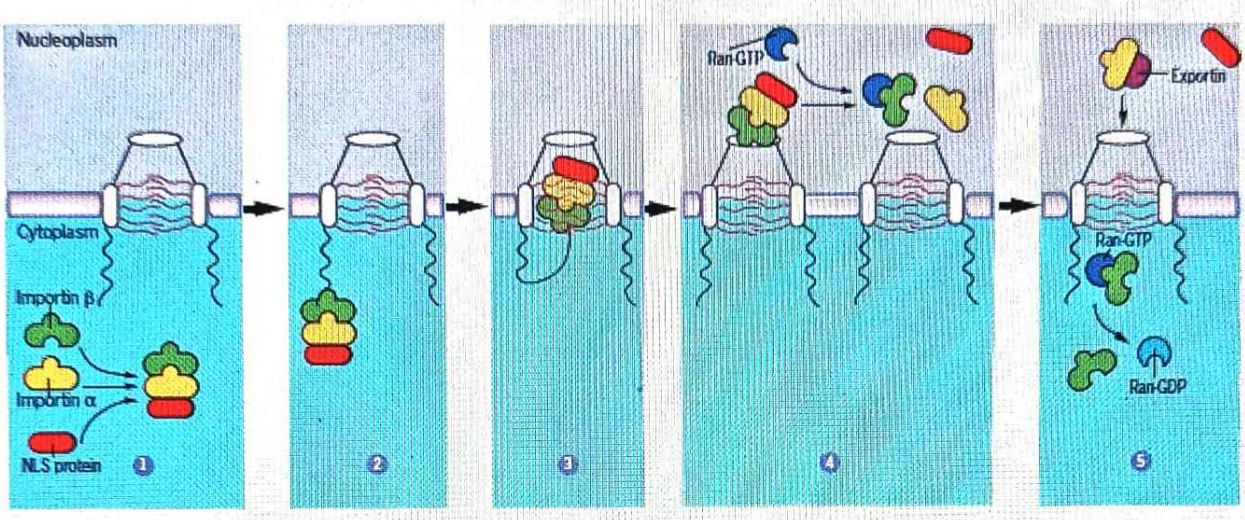


FIGURE 12.6 A model of a vertebrate nuclear pore complex (NPC). Three-dimensional representation of a vertebrate NPC as it is situated within the nuclear envelope. This elaborate structure consists of several parts, including a scaffold that anchors the complex to the nuclear envelope, a cytoplasmic and a nuclear ring, a nuclear basket, and eight cytoplasmic filaments. The FG-containing nucleoporins line the channel with their disordered FG-containing domains extending into the opening and forming a hydrophobic meshwork.

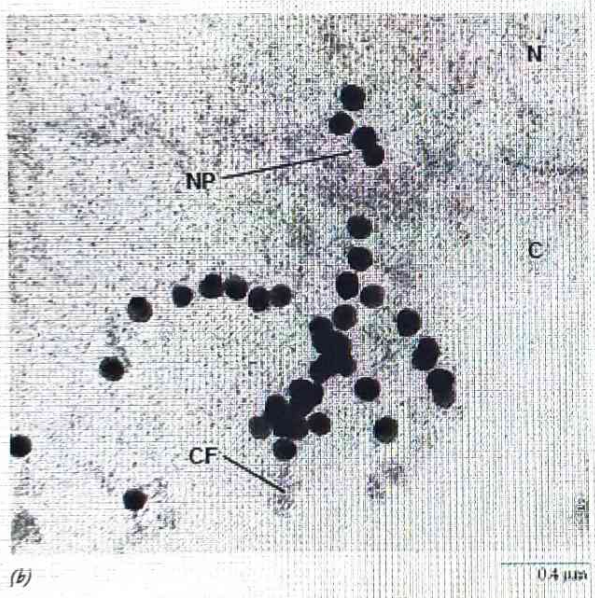
cleus is similar in principle to trafficking of other proteins that are destined for segregation within a particular organelle, such as a mitochondrion or a peroxisome (page 309). In all of these cases, the proteins possess a specific "address" that is recognized by a specific receptor that mediates its transport into the organelle.

The study of nuclear transport has been a very active area of research, driven by the development of in vitro systems capable of selectively importing proteins and RNPs into the nucleus. Using these systems, researchers can identify which proteins are required for the nuclear import of a particular macromolecule. These efforts have identified a family of proteins that function as mobile *transport receptors*, ferrying macromolecules across the nuclear envelope. Within this family, *importins* move macromolecules from the cytoplasm into the nucleus and *exportins* move macromolecules in the opposite direction.

Figure 12.7*a* depicts some of the major steps that occur during the nuclear import of a protein, such as nucleoplasmin, that contains a classical NLS. Import begins as the NLS-containing cargo protein binds to a heterodimeric, soluble NLS receptor, called *importin* α/β, that resides in the cytoplasm (step 1, Figure 12.7*a*). The transport receptor is thought to escort the protein cargo to the outer surface of the nucleus where it likely docks with the cytoplasmic filaments that extend from the outer ring of the NPC (step 2). Figure 12.7*b* shows a number of gold particles bound to these filaments; these particles were coated with an NLS-containing nuclear protein that was being transported through the nuclear pore



(a)



(b)

FIGURE 12.7 Importing proteins from the cytoplasm into the nucleus. (*a*) Proposed steps in nuclear protein import, as described in the text. 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 receptor-cargo complex moves through the nuclear pore (step 3) and into the nucleoplasm where it interacts with Ran-GTP and dissociates (step 4). The importin β subunit, in association with Ran-GTP, is transported back to the cytoplasm, where the Ran-GTP is hydrolyzed (step 5). Ran-GDP is subsequently transported back to the nucleus, where it is converted to Ran-GTP. Conversely, importin α is transported back to the cytoplasm. (*b*) Nucleoplasmin is a protein present in high concentration in the nucleoplasm of *Xenopus* oocytes. When gold particles are coated with nucleoplasmin and injected into the cytoplasm of a *Xenopus* oocyte, they are seen to bind to the cytoplasmic filaments (CF) projecting from the outer ring of the nuclear pore complex. Several particles are also seen in transit through the pore (NP) into the nucleus. (A: BASED ON A MODEL BY M. OHNO ET AL., CELL 92:327, 1998; B: FROM W. D. RICHARDSON ET AL., CELL 52:662, 1988; BY PERMISSION OF CELL PRESS, COURTESY OF A. D. MILLS.)

complex. The receptor–cargo complex then moves through the nuclear pore (step 3, Figure 12.7*a*) by engaging in a series of successive interactions with the FG domains of the FG-containing nucleoporins. These interactions are thought to "dissolve" portions of the FG-rich meshwork that fills the interior of the channel, allowing passage of the receptor-cargo complex through the NPC.

Now that we've gotten the bound cargo through the NPC and into the nuclear compartment, we need to introduce another key player, a GTP-binding protein called **Ran**. Like other GTP-binding proteins, such as Sar1 (page 290) and EF-Tu (page 464) discussed in earlier chapters, Ran can exist in an active GTP-bound form or an inactive GDP-bound form. Ran's role in regulating nucleocytoplasmic transport is based on a mechanism in which the cell maintains a high concentration of Ran-GTP in the nucleus and a very low concentration of Ran-GTP in the cytoplasm. The steep gradient of Ran-GTP across the nuclear envelope depends on the compartmentalization of certain accessory proteins (see Figure 15.19*b* for further discussion). One of these accessory proteins (named *RCC1*) is sequestered in the nucleus where it promotes the conversion of Ran-GDP to Ran-GTP, thus maintaining the high nuclear level of Ran-GTP. Another accessory protein (named RanGAP1) resides in the cytoplasm where it promotes the hydrolysis of Ran-GTP to Ran-GDP, thus maintaining the low cytoplasmic level of Ran-GTP. Thus the energy released by GTP hydrolysis is used to maintain the Ran-GTP gradient across the nuclear envelope. As discussed below, the Ran-GTP gradient drives nuclear transport by a process that depends only on receptor-mediated diffusion; no motor proteins or ATPases have been implicated.

We can now return to our description of the classical NLS import pathway. When the importin–cargo complex arrives in the nucleus, it is met by a molecule of Ran-GTP,

which binds to the complex and causes its disassembly as indicated in step 4, Figure 12.7*a*. This is the apparent function of the high level of Ran-GTP in the nucleus: it promotes the disassembly of complexes imported from the cytoplasm. The imported cargo is released into the nucleoplasm, and one portion of the NLS receptor (the importin β subunit) is shuttled back to the cytoplasm together with the bound Ran-GTP (step 5). Once in the cytoplasm, the GTP molecule bound to Ran is hydrolyzed, releasing Ran-GDP from the importin β subunit. Ran-GDP is returned to the nucleus, where it is converted back to the GTP-bound state for additional rounds of activity. Importin α is transported back to the cytoplasm by one of the exportins.

Ran-GTP plays a key role in the escort of macromolecules from the nucleus, just as it does in their import from the cytoplasm. Recall that Ran-GTP is essentially confined to the nucleus. Whereas Ran-GTP induces the disassembly of imported complexes, as shown in step 4 of Figure 12.7*a*, Ran-GTP promotes the *assembly* of exported complexes. Proteins exported from the nucleus contain amino acid sequences (called *nuclear export signals*, or *NESs*) that are recognized by transport receptors that carry them through the nuclear envelope to the cytoplasm. Most of the traffic moving in this direction consists of various types of RNA molecules—especially mRNAs, rRNAs, and tRNAs—that are synthesized in the nucleus and function in the cytoplasm. In most cases, these RNAs move through the NPC as ribonucleoproteins (RNPs).

Transport of an mRNP from the nucleus to cytoplasm is associated with extensive remodeling; certain proteins are stripped from the mRNA, while others are added to the complex. Transport of mRNPs does not appear to require Ran but does require the activity of an RNA helicase located on the cytoplasmic filaments of the NPC. It is speculated that the helicase provides the motive force to move the mRNA into the cytoplasm. Numerous studies have demonstrated a functional link between pre-mRNA splicing and mRNA export; only mature (i.e., fully processed) mRNAs are capable of nuclear export. If an mRNA still contains an unspliced intron, that RNA is retained in the nucleus.

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: What is the nature of the chromosome in the nonmitotic cell? We are now able to provide a fairly comprehensive answer to this question.

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 μm (1×10^{-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 (Table 12.1). 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 are histones 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 *naked* DNA (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 nucleo-

TABLE 12.1 Calf Thymus Histones

Histone	Number of residues	Mass (kDa)	%Arg	%Lys	UEP* ($\times 10^6$ year)
H1	215	23.0	1	29	8
H2A	129	14.0	9	11	60
H2B	125	13.8	6	16	60
H3	135	15.3	13	10	330
H4	102	11.3	14	11	600

*Unit evolutionary period: the time for a protein's amino acid sequence to change by 1 percent after two species have diverged.

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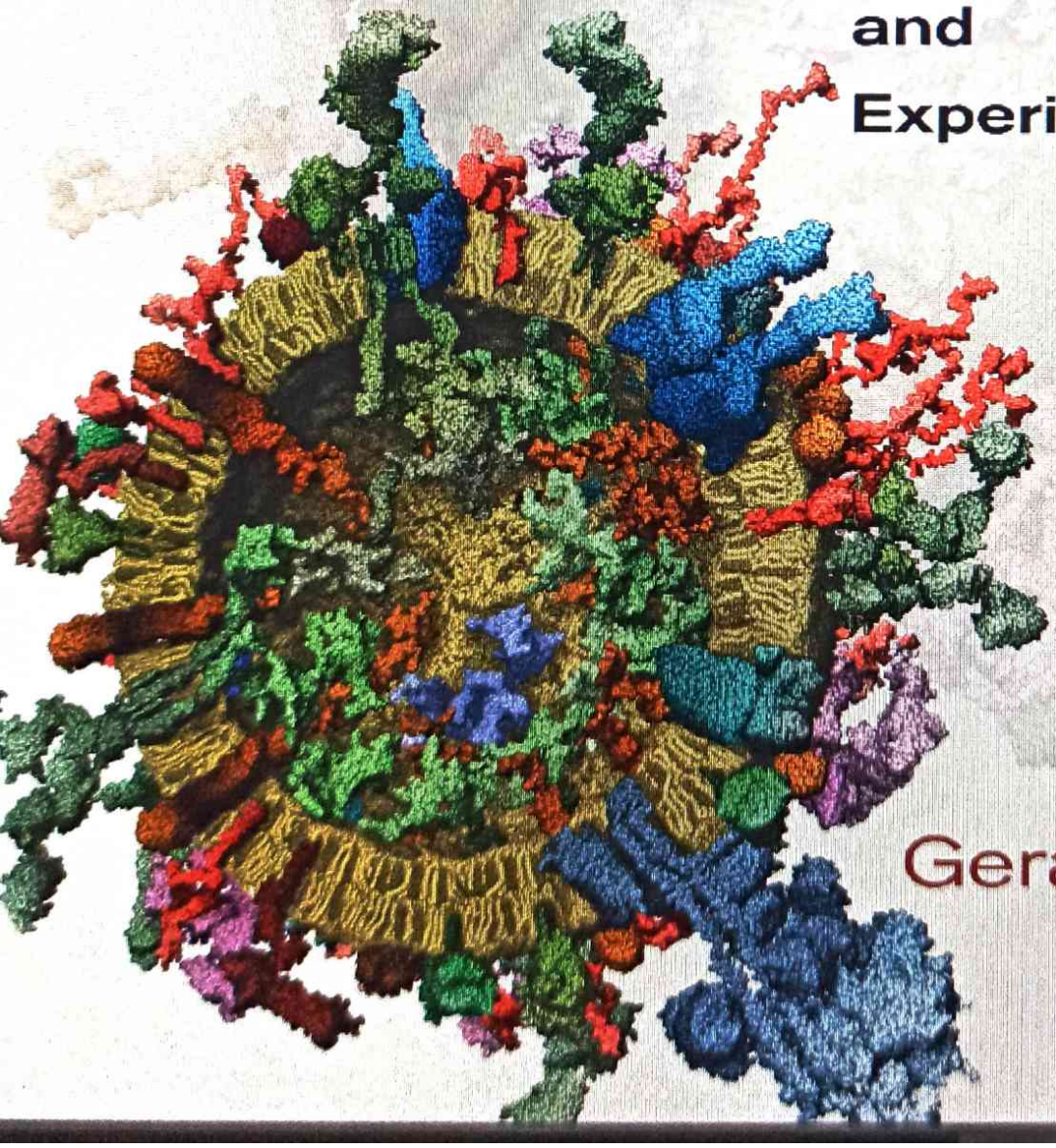
H2A	129	14.0	9	11	60
H2B	125	13.8	6	16	60
H3	135	15.3	13	10	330
H4	102	11.3	14	11	600

*Unit evolutionary period: the time for a protein's amino acid sequence to change by 1 percent after two species have diverged.

6th edition

Cell and Molecular Biology

Concepts and Experiments



Gerald Karp

12

The Cell Nucleus

Robert Brown for the first time in 1833 discovered a prominent body within the cell and termed it **nucleus.** A synonymous term for this organelle is the Greek word **karyon.** A German biologist, **J. Hammerling,** demonstrated in 1934 that the nucleus determines the characters of the cell and ultimately the characters of the individual. He conducted certain experiments using two species of a green alga, *Acetabularia.* The two species, namely *A. crenulata* and *A. mediterranea* used in this experiment differ in the shape of their caps. While in *A. crenulata* the cap has loose rays, in *A. mediterranea* an umbrella-like cap is found. The nucleus in both the species is situated in rhizoid at the bottom of stalk. If cap is cut off, it will develop again and its shape will be that of the original type. However, if after removing the caps, stalk of one species, is grafted on rhizoid (containing the nucleus) of the other species, shape of cap will be determined by nucleus and not by stalk (Fig. 12.1). If the nucleus belongs to *A. crenulata*, shape of cap will be of the *crenulata* type and if the nucleus comes from *A. mediterranea*, cap will be of *mediterranea* type. When both nuclei are present, shape of cap will be intermediate. This experiment demonstrated clearly for the first time that characters of an individual are controlled by nucleus of the cell or cells. This controlling attribute of the nucleus is now well established and is known to be due to the presence of chromosomes in this organelle.

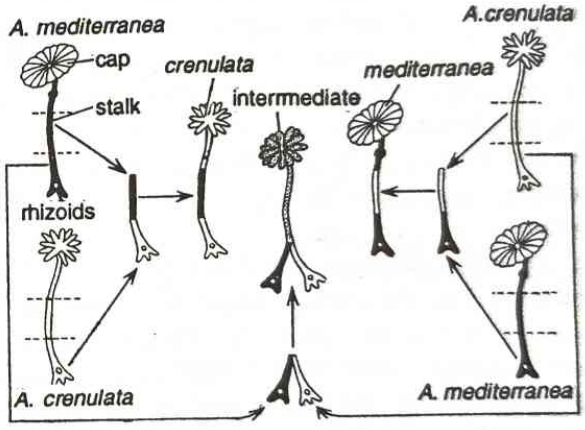


Fig. 12.1. Hammerling's experiment in *Acetabularia* showing relative roles of nucleus and cytoplasm (redrawn from Swanson: *The Cell,* 1971).

On the basis of presence or absence of well-defined nucleus, living organisms were earlier classified into two groups by molecular biologists. These groups are (i) **prokaryotes**, the individuals which do not have a well-organized nucleus and will therefore include viruses, bacteria and blue-green algae; and (ii) **eukaryotes**, which would include the remaining types, which have a well-organized nucleus. However, according to a phylogenetic classification proposed in 1996, living organisms are classified into the following three gropus ~~(i)~~ **bacteria** ; ~~(ii)~~ **archaea** and ~~(iii)~~ **eucarkya** (see Chapter 1 for more details of this new phylogenetic classification). The nuclear

equivalent of a prokaryotic organism is known as **prokaryon** or more commonly as **nucleoid** rather than a nucleus. The 'prokaryon' or 'nucleoid' does not have a true chromosome; it is not enclosed in a nuclear envelope and does not divide by regular mitosis. The nuclei may even be absent in some specialized cells of eukaryotes. For instance mature mammalian red blood cells are also without any nuclei. This is why they are often called as red blood **corpuscles** rather than cells.

A nucleus may be described as having three important parts, namely, **nuclear membrane** or **nuclear envelope, nucleolus** and **chromosomes.** The fluid, in which nucleolus and chromosomes are present and which is enclosed in nuclear membrane, is called **nucleoplasm**.

Nuclear Envelope

Nuclear boundary of interphase and prophase nuclei is called nuclear membrane or nuclear envelope. It breaks down at the end of prophase and is reformed at the end of the nuclear division. It consists of a double membrane having two unit membranes (Fig. 12.2). The space between two unit membranes varies in width and is known as **perinuclear space**. Outer membrane is continuous with the endoplasmic reticulum.

Each unit membrane is 7.5 nm (1 nm = 10 Å) in diameter and perinuclear space may vary from regular 15 nm wide spaces to irregular cavities several hundred times wide. Outline of nuclear envelope is smooth and interrupted by pores which appear circular in surface view. Diameter of these pores varies from 30 nm to 100 nm. In sections. it is obvious that at the boundary of these pores, outer and inner unit membranes are joined. These pores provide direct contact between nucleus and cytoplasm and allow import and export of protein and RNA (particularly export of messenger RNA, which is synthesized in the nucleus and then reaches cytoplasm for protein synthesis). The inner nuclear membrane is lined by the **nuclear lamina**, which is composed of A and B type **lamins** (specialized type of intermediate filament proteins). Similarly, the outer membrane is surrounded by a network of intermediate filaments, which are not as well organized as the nuclear lamina (Fig. 12.3).

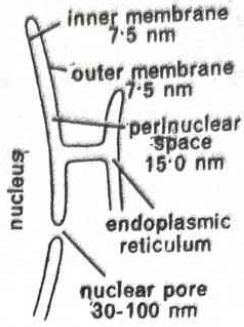


Fig. 12.2. Nuclear membrane showing double structure.

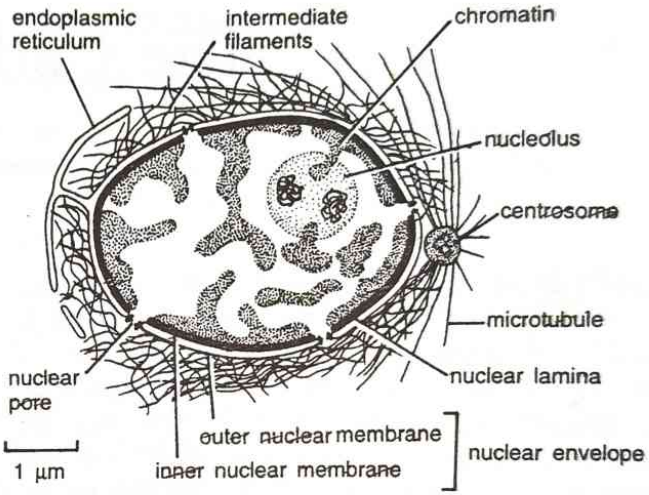


Fig. 12.3. Structure of nuclear envelope.

Disassembly and reassembly of nuclear envelope

As mentioned above, the nuclear envelope disassembles at the onset of mitosis and is reassembled at the end of mitosis. The mechanism of assembly involves (i) the attachment of vesicles to the chromatin, followed by (ii) the fusion of vesicles to form a double membrane system. The binding of vesicles to the chromatin requires both chromatin and membrane bound proteins, but does not require ATP. On the other hand, the fusion of membrane bound vesicles to form the nuclear membrane does require ATP and GTP hydrolysis, that is also required in membrane fusion events during exocytosis and endocytosis (for details, consult Chapter 16). The vesicles used for assmebly of nuclear envelope form a subset of **ER-derived vesicles**, and are distinct from the majority of other

ER-derived vesicles (COPII). Lamin-depolymerization and polymerization during disassembly and reassembly of the nuclear envelope also invloves reversible phosphorylation.

Nuclear pore complex (NPC)

Nuclear pore occupies a central position among the major cellular structures, but still remains one of the least understood structures. However, during the late 1980s and early 1990s, significant progress was made towards a better understanding of the structure and function of the nuclear pore. During this period, new pore proteins were identified (particularly in yeast), the genes for several of these proteins were cloned, a number of mutants in these pore proteins were isolated and detailed mechanism of nucleocytoplasmic traffic was proposed. Further, the pore was reconstituted *in vitro*, a number of **'signal sequences'** and one or more **'signal sequence receptors'** were identified, and a new **'basket-like structure'** was found attached to the inner side of the nuclear pore. (Consult review by Douglass J. Forbes in *Ann. Rev. Cell Biol.*, 1992 for detailed account).

The nuclear pore is a large complex structure of 125 million daltons or 30 times the size of a eukaryotic ribosome. The pore is 120nm in diameter and 50nm in thickness. It consists of four separate elements : (i) the **scaffold,** which includes majority of the pore, (ii) the central hub or **transporter,** which carries out active transport (both import and export) of proteins and RNAs, (iii) short **thick filaments** attached to the cytoplasmic side of the pore and (iv) a newly discovered **basket** attached to the nucleoplasmic side of the pore.

The scaffold is a stack of three closely apposed rings, namely the **cytoplasmic ring,** the **nucleoplasmic ring** and a **central ring** of thick spokes. Each ring has a eight-fold symmetry. The spokes of central ring are attached to the transporter on the inner side, and to the nucleoplasmic and cytoplasmic rings on the outer side. Interspersed between the spokes are **aqueous channels**, 9nm wide, which allow diffusion of proteins and metabolites between the nucleus and the cytoplasm.

The transporter is a proteinaceous ring, 36–38 nm in diameter and consists of two **irises** of eight arms each. The two irises are assumed to be stacked atop one another and open sequentially, each like

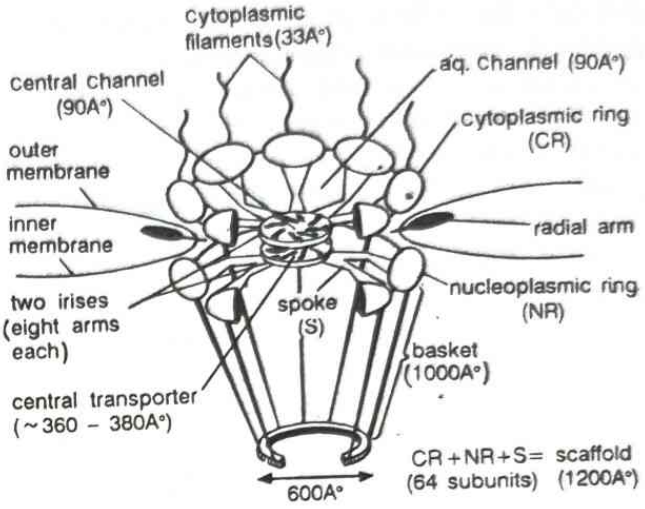


Fig. 12.4. Detailed structure of the nuclear pore, showing iris and transporter.

the diaphragm of a camera, to let a nuclear protein or RNA pass through from the nucleus to the cytoplasm. On the cytoplasmic side of the pore, thick fibres (3.3nm in diameter) extend into the cytoplasm. On the nuclear side, a large basket like structure is found, which consists of eight filaments (each 100 nm long), extending from nucleoplasmic ring of the pore and meeting a smaller ring (60 nm in diameter) within the nucleus. This basket may play an important role in RNA export. The detailed structure of nuclear pore complex is shown in Figure 12.4.

Nucleocytoplasmic transport

Nuclear pore complexes (NPCs) are the sites of exchange of macromolecules between the cytoplasm and the nucleus. Each NPC has a mass of 125 megadaltons in higher eukaryotes and contains about 100 different polypeptides called **Nup (nuclear pore proteins** or **nucleoporins**). Following are some of the characteristic features of many of these nucleoporins : (i) modification of O-linked N-acetyl glucosamine; (ii) presence of short degenerate repeats (e.g. FXFG repeats in Nup153p and p62, and GLFG repeats in Nup98p; in FXFG and GLFG repeats, each letter stands for an amino acid; X means any amino acid; consult Chapter 3). The nuclear pore complex has a passive diffusion channel, 9nm in diameter, permitting diffusion of many but not all small proteins including cytochrome c. Several small proteins like histones (<9nm) and other larger proteins (>9nm and upto 25nm) pass through NPC by an active transport

process. The active process is facilitated by (i) energy, (ii) signal sequence and (iii) saturability, so that the process is mediated by carrier molecules.

The transport through NPCs involves both import to and export from the nucleus. All nuclear proteins are imported from the cytoplasm where they are synthesized and all tRNAs/mRNAs are exported to the cytoplasm, where they are used for protein synthesis. There are also molecules that are first exported to the cytoplasm and then reimported. For instance, some of the small nuclear RNAs (snRNAs) involved in RNA splicing (see Chapter 39) include U1, U2, U4, and U5. They are exported out of the nucleus after transcription. In the cytoplasm, they assemble with Sm core proteins and undergo a number of modifications such as cap hypermethylation involving conversion of the cap $m^7GpppN5'$ to $m^{2,2,7}GpppN$. These partly mature U snRNPs re-enter the nucleus and associate with proteins specific for U snRNPs. In case of U4, the U4 snRNP re-enters the nucleus and associates with U6 RNA. Thus the snRNPs complete their assembly by export followed by reimport in the nucleus. The situation is reverse in case of ribosomal proteins. They enter the nucleus, get incorporated into ribosomal subunits and are then re-exported as parts of ribosome subunits. It has been estimated in HeLa cells, that 100 ribosomal proteins and 3 ribosomal subunits travel through each pore each minute. This demonstrates that transport through NPC is a major activity. The details of components of the transport machinery and the mechanism involved in this transport will be briefly discussed in this section. For a detailed account, the readers may consult two recent reviews (Science, 15 March, 1996, pages 1513-1518; 24 April, 1997, pages 779-787).

Signals for transport across the pore. The import and export of proteins and RNPs across the NPCs are facilitated by the presence of signal sequences. A number of these signal sequences that have been studied and characterized are listed in Table 12.1. Some of these sequences, described as 'classical' **nuclear localization sequences (NLSs)**, are characterized by one or more clusters of basic amino acids. The two examples of NLSs include signal sequences in large antigen of SV40 and the bipartite NLS of **nucleoplasmin**. It has been shown that both these NLSs use the same receptor. While NLSs are used for import, similar export signal sequences are also known, which are described as **nuclear export signal (NES)**. These NES sequences have already been found in atleast following three cases : (i) human immunodeficiency virus (HIV) Rev protein, (ii) PKI, an inhibitor of protein kinase A and (iii) transcription factor IIIA (TFIIIA) used for transcribing 5SRNA genes, tRNA genes, etc. The only solitary example of a signal sequence that directs both import and export by shuttling between the nucleus and the cytoplasm is the M9 domain of hnRNP protein, such as RNP protein A1, also called hnRNPA1 (a human protein involved in mRNA export).

The export of RNA from the nucleus is actually characterized by the following three features — (i) the mRNA needs to be released from the retention process which allows the precursor mRNA to stay in the nucleus for post-transcriptional processing events; (ii) RNAs are exported in the

Table 12.1. **Signal sequences involved in protein transport across the nuclear pore complex (NPC)**

	Function	Signal (length)*	Amino acid sequence*
1.	Import	SV40 large T antigen NLS (7)* Nucleoplasmin bipartite NLS (16)*	PKKKRKV KRPAAIKKAGQAKKKK
2.	Export	HIV-10Rev NES (9)* TF III A NES (19)* PKI NES (10)*	LPPLERLTL QPPDASKADPALPVLENLTLK L ALKLAGLDI
3.	Targets import receptor to nucleus	IBB domain of importin (41)*	RMRKFKNKGKDTAEL RRRRVEVSVELR KAKK DEQILKRRNV
4.	Confers rapid shuttling	hRNPA1 M9 (38)*	NQSSNFGP MKGGNFGG RSSGPYGGGG QYFA KP RNQGGY

* length represents number of amino acid residues; figures in parentheses represent numbers of amino acid residues.

form of RNPs (ribonucleoproteins), where associated proteins (e.g. Rev with NES domain or TFIIIA) actually carry the signals; (iii) many signals may be involved in the export of large RNPs making the study of the mechanism difficult. The only well defined example of RNA export thus is U snRNAs with hypermethylated cap ($m^{2,2,7}$ GpppN), which binds to the proteins (**cap binding proteins complex** or **CBC**) involved in export, thus forming RNPs.

Import of nuclear proteins. The following four factors are known to be required in nuclear protein import: (i) **importin-α** (also called NLS receptor; called SRP1p in yeast) (ii) **importin-β** (also called p97 or PTC97), (iii) the small GTPase **Ran**, and (iv) **pp15** (also called p10 or NTF2). Importin is also called **karyophorin**. The import actually involves several stages including the following — the protein to be imported has an NLS which helps in binding of protein to importin α – β heterodimer, the latter thus acting as a receptor, the receptor site being present in subunit importin α. An importin β binding (IBB) domain in importin– α subunit helps not only in interaction with importin– β, but also facilitates translocation through NPC to the nucleus. The NLS-protein-receptor complex docks to the nuclear pore complex via improtin– β and is subsequently translocated through the pore by an energy-dependent mechanism. It also requires Ran and pp15. The energy required for translocation across the NPC comes atleast partly from GTP hydrolysis, which is faciliated by Ran (a GTPase). The Ran GTPase needs the nuclear protein, RCC1 (a Ran activating protein). The function of pp15 will be known in future only. Since the distance to be travelled by NLS-protein-receptor complex is atleast 100nm and one GTP can help movement (through motor protein kinesin or the myosin power stroke) to only 8-10nm, it is estimated that the movement of a NLS-protein receptor complex would need 10 GTP molecules. After the translocation, the constituents of NLS-protein-receptor complex become separated, of which the protein remains in the nucleus and importin– β and importin– α are recylced to the cytoplasm, the former rather rapidly and the latter rather slowly

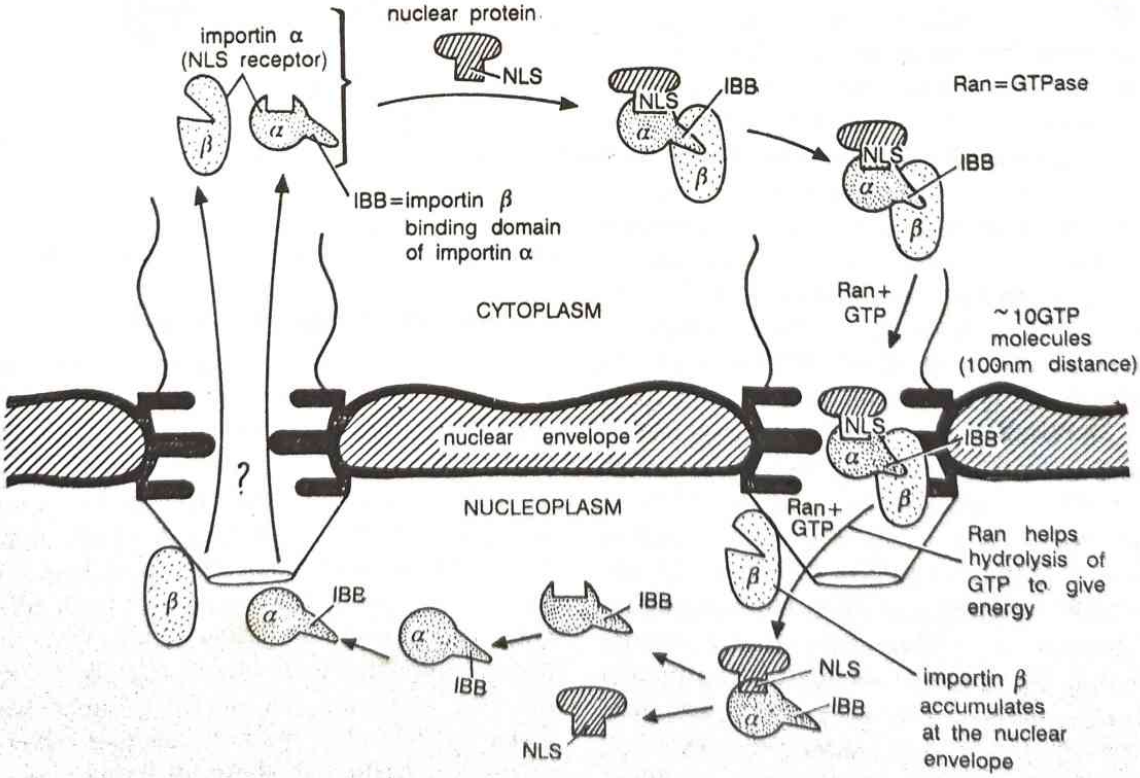


Fig. 12.5. Steps involved in the import of nuclear proteins (redrawn from Science, 15 March 1996)

(BC-27)

due to the requirement of dissociation from NLS (Fig. 12.5).

Export of RNA from the nucleus. RNA export from the nucleus across NPC is also mediated by some signal sequences in the proteins that associate with RNA to form RNPs. The RNA to be exported may contain response elements. The best studied such protein is HIV-1 **Rev** protein. An intron in unspliced HIV-1 RNAs contains **RRE (Rev response element)**, where several Rev molecules can bind and allow export of unspliced RNA.

Rev protein has a **RNA binding domain** and an **'activation domain'** the latter being a nuclear export sequence (NES). When coupled with a heterologous protein, the NES of Rev protein directs the rapid export of the fusion product. When NES directs export of a protein the bound protein is also exported automatically. A search for a receptor for NES suggested that human proteins hRip or Rab and yeast protein Riplp are the possible candidates for such a receptor (Rip = Rev-interacting protein; Rab = Rev activation domain binding protein). These proteins seem to be related to nucleoporins, but their role in export is not yet fully understood, although atleast two models are available. In one model sequential interaction of Rev-NES first with Rip and then with nucleoporins takes place, while in the second model Rev-Rip complex is translocated across NPC through interactions between Rip and other nucleoporins (Fig. 12.6).

Export and reimport of RNAs. Two examples of export followed by reimport of RNA are 5S rRNA and U SnRNAs. Two proteins (TFIIIA and ribosomal protein L5) seem to be involved as mediators in export of 5S RNA. TFIIIA seems to contain a sequence similar to Rev NES. Similarly for export of U SnRNAs, a nuclear monomethyl **cap binding protein complex (CBC)** seems to be involved. CBC contains two proteins (CBP80 containing NES like sequence and another nucleoplasmic protein CBP20). Another human protein hnRNPA1 and several other hnRNP proteins are also involved in export. They shuttle rapidly between the nucleus and the cytoplasm. The protein hnRNPA1 remains bound to polyA-RNA. A short domain M9 of hnRNPA1 confers the shuttling behaviour. More information about these shuttling proteins will become available in future.

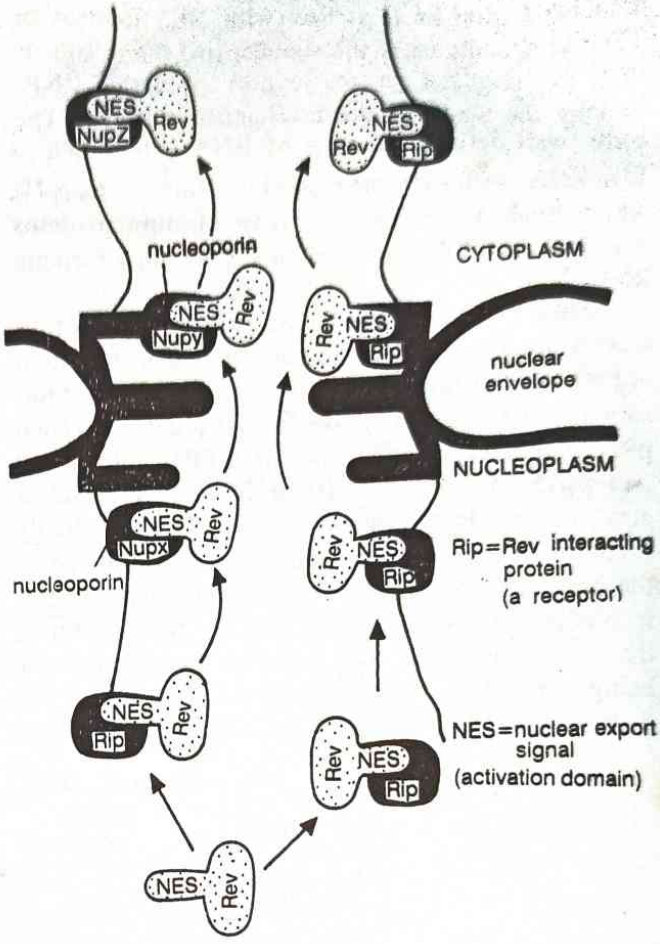


Fig. 12.6. Two models for Rev-mediated export of RNA from the nucleus (redrawn from Science, 15 March 1996)

Evolutionery origin of nucleus

There are atleast two contrasting theories available for evolutionary origin of the nucleus : (i) **Karyo genic hypothesis**. According to this hypothesis the nucleus and its surrounding membranes were gradually acquired through some unspecified segregating process. (ii) **Endokaryotic hypothesis**. According to this hypothesis, the nucleus, like other eukaryotic organelles (e.g. mitochondria and chloroplasts) enclosed in double membrane, has been derived through capture by an engulfing species. In this case, the guest (the nucleus), rather than being under the control of the host, has taken over control of the host. This hypothesis is simple and explains the origin of nucleus, chloroplasts and mitochondria

by the same mechanism, rather than two separate mechanisms needed, one for the origin of the nucleus and the other for the origin of chloroplasts and mitochondria.

Nucleolus

In higher organisms, every cell nucleus has a spherical, colloidal body called nucleolus, which is associated with a particular nucleolar organizing chromosome (Fig. 12.7). A special region in this chromosome is known as the **nucleolar organizing region (NOR)** to which usually the nucleolus remains associated (Fig. 12.8). Quite often, more than one nucleoli in the same nucleus may also be observed. These several nucleoli may subsequently coalesce and give rise to a larger single nucleolus.

While chromatin mainly consists of DNA, nucleolus mainly consists of RNA, acidic dyes and basic dyes; phospholipids and alkaline phosphatase are also found. Nucleolar DNA had also been reported in a number of cases. This DNA is believed to represent the nuclolar organizer.

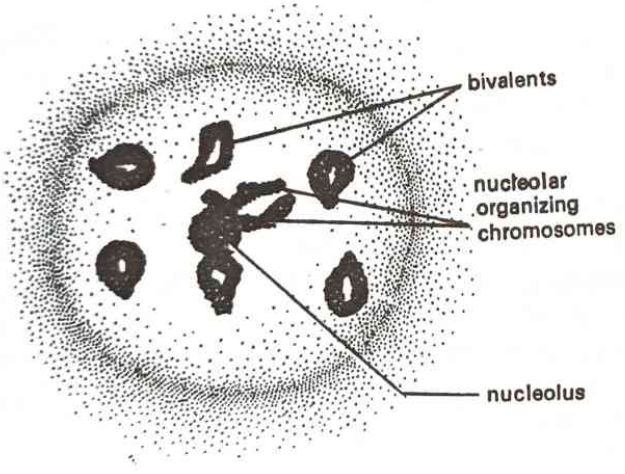


Fig. 12.7. A diakinesis cell showing attachment of a bivalent with nucleolus.

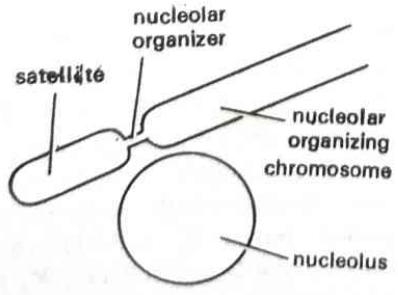


Fig. 12.8. A satellited chromosome and an attached nucleolus.

The nucleolus was first described in 1781 by **Fontana**. Ever since then, vast literature has been published on the nucleolus which has been adequately covered in a monograph entitled *"The Nucleolus and Ribosome Biogenesis"* published in 1985. This monograph was written by the distinguished French molecular biologist, **Asen Hadjiolov**, who suddenly passed away in April 1996. Since the publication of the above monograph, considerable work has been done, which was adequately covered in a special issue of the journal *Chromosoma* brought out in the year 1997 in the memory of Dr. Hadjiolov. The present status of he biology of nucleolus is briefly summarized here.

Nucleologenesis

Nucleolus can be seen as a very conspicuous structure in the interphase nucleus. It disappears during mitosis and reappears at the next interphase. The process by which the nucleolus is formed, is described as nucleologenesis. During prometaphase to early telophase, when the nucleolus remains disappeared, a number of **non-ribosomal nucleolar proteins** (e.g. **B23, fibrillarin, nucleolin** and **p52**) as well as **U3 snoRNA** are found in (i) the peripheral regions of chromosomes and in the (ii) **nucleolus derived foci (NDF)** found as cytoplasmic particles 1-2μ in diameter; the number of these NDFs can reach as many as 100 per cell at mid- to late-anaphase, but later their number declines to few or none at telophase.

The decline in the number of NDF approximately coincides with the appearance of **prenucleolar bodies (PNBs)** and reforming of the nucleoli. The **nucleolar shuttling phosphoprotein, Nopp 140**, however, did not follow the above pattern of localization. It remains dispersed throughout the cytoplasm during pro-metaphase to early telophase, and is not found in NDF. These observations suggest that the NDF and prenucleolar bodies are the precursors of the newly formed nucleoli, but the forces that bind them together to form nucleoli are still unknown.

Nucleologenesis can also be induced in cultured cells by experimental treatment. It has been shown that the nucleolus disapperars upon hypotonic shock of the interphase cells and reappears when

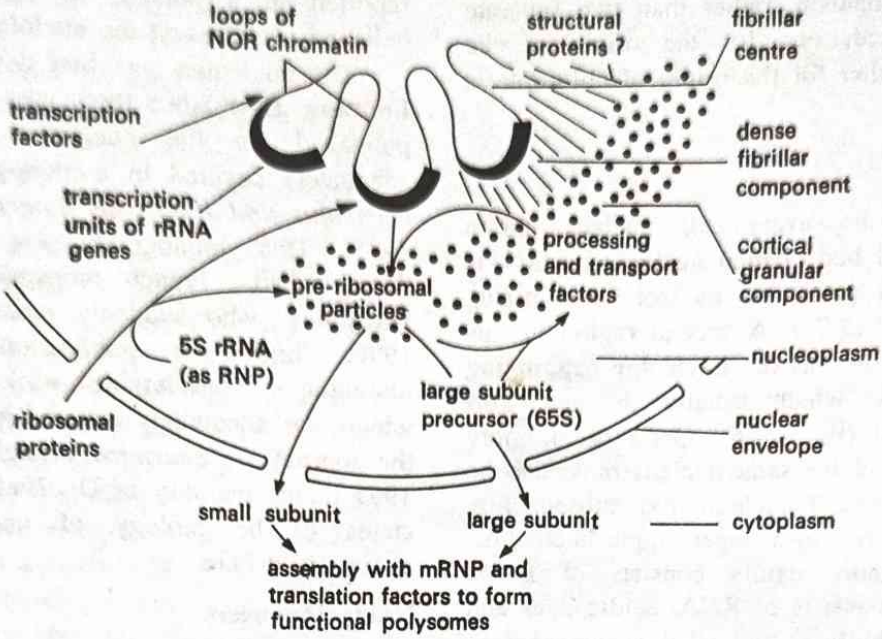


Fig. 12.9. Three regions of the nucleolus showing their roles in ribosomal RNA (rRNA) synthesis.

these cells are returned to isotonic medium, although the cells continue to remain at interphase. Actinomycin D also blocks the formation of nucleoli from prenucleolar bodies (PNBs), suggesting that the nucleolus formation requires active transcription of ribosomal RNA (rRNA) genes by RNA polymerase I.

Nucleolus and the ribosome biogenesis

The nucleolus is the site of ribosome biosynthesis, where the synthesis of ribosomal RNA (rRNA) and the assembly of ribosome takes place. It has been shown that the genes for ribosomal RNA are clustered at the nucleolar organizer region in the form of tandemly repeated ribosomal DNA (rDNA) units (see details in Chapter 49). The initiation, production and maturation of ribosomes in the nucleolus seem to proceed from centre to the periphery in the following three distinct regions: (i) **Fibrillar centre (FC)**, where rRNA genes of NOR (nucleolar organizing region) are located; the transcription of rRNA genes (RNA synthesis on DNA template) also takes place in this region. (ii) **Dense fibrillar component (DFC)**, which surrounds the fibrillar centre and where RNA synthesis progresses. The 80S ribosomal proteins (rps) also bind to the transcripts in this region. (iii) **Cortical granular component (GC)**, which is the outermost region and where processing and maturation of pre-ribosomal particles occurs. These three regions of the nucleolus and their roles in ribosome formation are shown in Figure 12.9.

The transcription for the synthesis of pre-rRNA actually occurs in FC and particularly at its edge in a region described as **transcription zone (txn)**. In this region are observed the structures described as **'christmas trees'** by **O. Miller**. The ribosomal precursors (pre-rRNAs) move from one compartment to the next for different stages of processing and are subsequently transported to the cytoplasm to make mature ribosomes.

Small nucleolar RNAs (snoRNAs) and rRNA processing

Like small nuclear RNAs (U snRNAs; e.g. U1, U2, U4, U5, U6, etc.) found in the nucleus, small nucleolar RNAs (U snoRNAs; e.g. U3, U7, U8, U14, etc.) are found in the nucleolus. During the last more than a decade, the list of snoRNAs has grown to over 50, which can be grouped into two major categories (excluding 7-2/MRP); (i) those derived from evolutionarily conserved DNA sequences called boxes C and D (**box C** = 5'-RUGAUGA-3', **box D** = 5'-CUGA-3') present at the 5' and 3' ends respectively, and (ii) those with an **ACA motif** at the 3' end Most of the snoRNAs of box C/D class are encoded within introns of

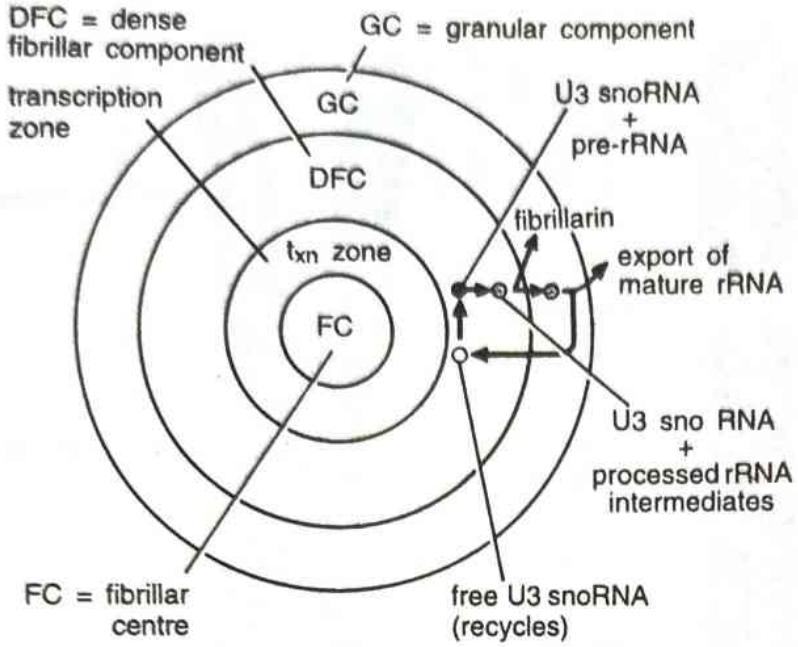
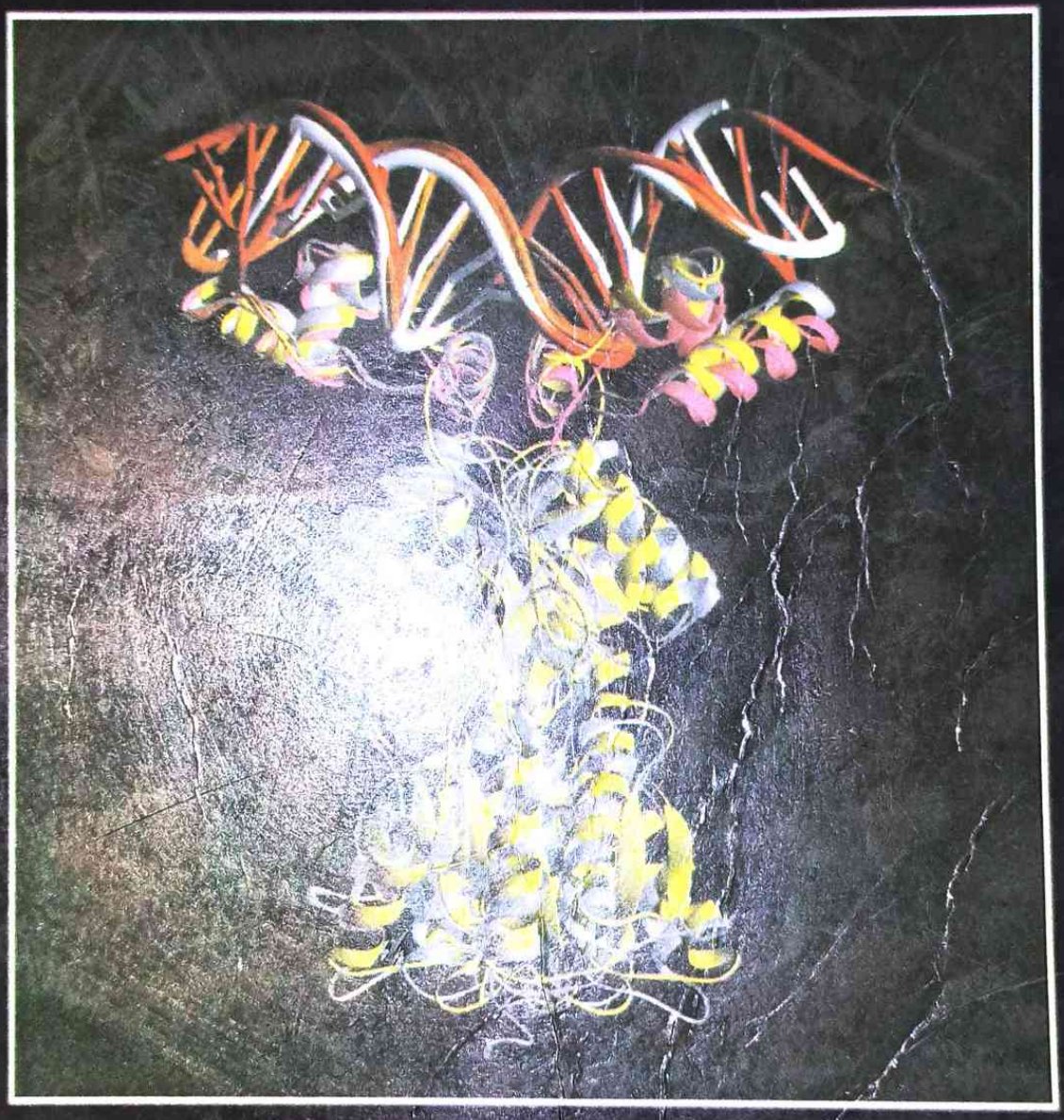


Fig 12.10. A cycle involving association and dissociation of U3 snoRNA with pre-rRNA in the nucleolar regions

other genes and are important for rRNA methylation and rRNA processing . It is believed that U3 snoRNA associates with the nascent pre-rRNA and accompanies it, when it passes through the different domains of the nucleolus during its processing. This U3 snoRNA recycles from the granular component (GC) to the dense fibrillar component (DFC) for association with another nascent pre-rRNA (Fig. 12.10).

While some of the snoRNAs are ubiqutous (e.g. U3 and U14), there are others, which are described either only in vertebrates or only in yeast. Seven snoRNAs (U3, U8, U14, U22, SnR10, SnR30, RNA component of RNAase MRP) are now known to be required in the processing of pre-rRNA. Of these U3 and U14 are bound to ETS and/or ITS1, which are removed by early processing (the precursor rRNA consists of 5'-ETS1-18S-ITS1-5.8S -ITS2-28S-ETS2-3'; ETS = external transcribed spacer; ITS = internal transcribed spacer; for structure of rRNA genes consult Chapter 49). It is not known, however, whether, these snoRNAs act as chaperones to help in correct folding of the pre-rRNA, so that the correct sites are exposed for cleavage or whether the snoRNAs themselves are involved directly in cleavage reactions.

CELL AND MOLECULAR BIOLOGY



P. K. GUPTA