

riboswitches, as they are called, undergo a change in their folded conformation that allows them to alter the expression of a gene involved in production of that metabolite. Thus riboswitches act by means of a feedback mechanism similar to the alternative RNA structures that regulate attenuation in the *trp* operon. Most riboswitches suppress gene expression by blocking either termination of transcription or initiation of translation. Like the repressors that function in conjunction with operons, riboswitches allow cells to adjust their level of gene expression in response to changes in the available levels of certain metabolites. Given that they act without the participation of protein cofactors, riboswitches are likely another legacy from an ancestral RNA world (page 454).

REVIEW

1. Describe the cascade of events responsible for the sudden changes in gene expression in a bacterial cell following the addition of lactose. How does this compare with the events that occur in response to the addition of tryptophan?
2. What is the role of cyclic AMP in the synthesis of β -galactosidase?
3. What is a riboswitch?

12.2 | Control of Gene Expression in Eukaryotes: Structure and Function of the Cell Nucleus



A primary difference between prokaryotic and eukaryotic organisms is the presence of a nucleus in eukaryotic cells. Moreover, most eukaryotic organisms have much larger genomes, which are present as double-stranded DNA molecules arranged in linear chromosomes that can be easily visualized during mitosis (as in Figure 12.22*b*). Before discussing regulation of gene expression in eukaryotes, we will first discuss the consequences of compartmentalizing genomes within nuclei and how large genomes are packaged into DNA-protein complexes called **chromatin**. The DNA in prokaryotic organisms is not packaged into chromatin; as a result, DNA-binding proteins such as RNA polymerase, repressors, and CRP-cAMP complexes can directly bind to their preferred binding sites. In contrast, DNA-binding proteins in eukaryotes have to find their preferred binding sites in the context of a complicated DNA-protein complex.

Considering its importance in the storage and utilization of genetic information, the nucleus of a eukaryotic cell has a rather undistinguished morphology (Figure 12.5). 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 435); and (3) the *nucleoplasm*, the fluid substance in which the solutes of the nucleus are dissolved.

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

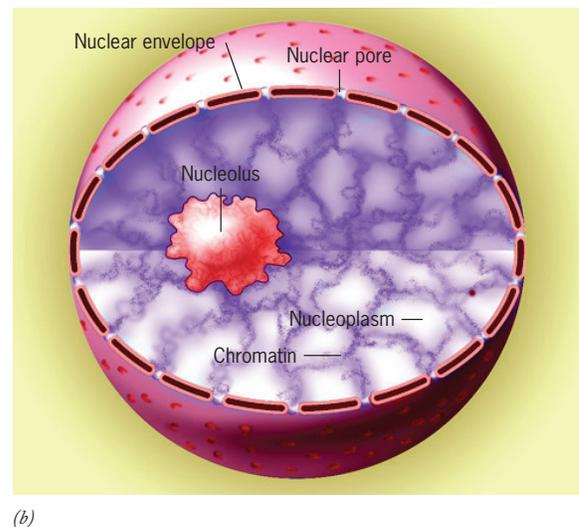
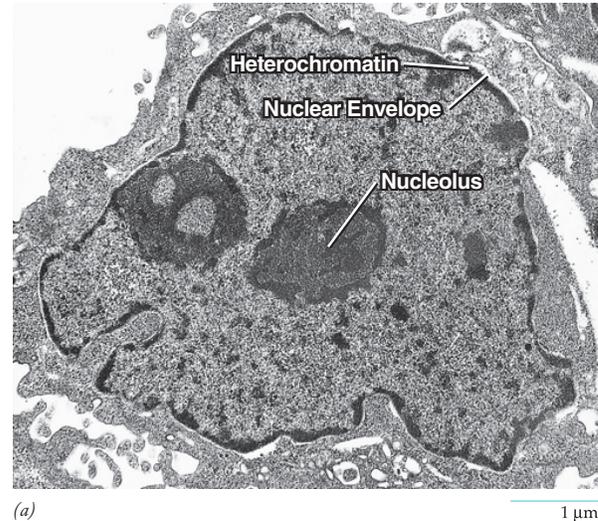
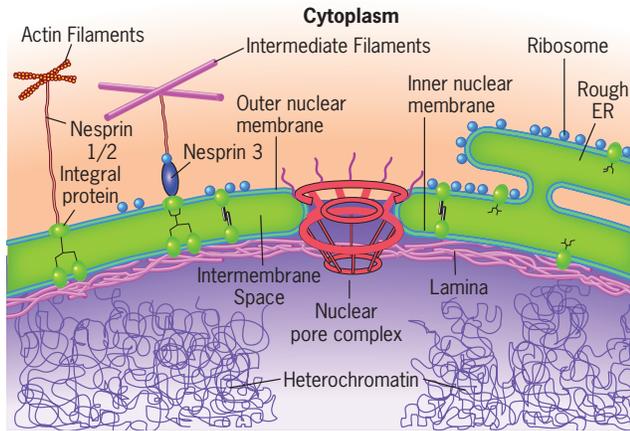
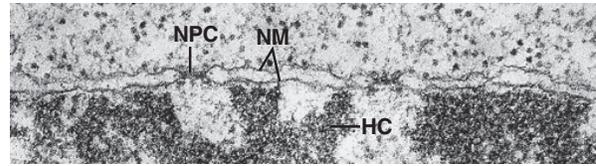


Figure 12.5 The cell nucleus. (a) Electron micrograph of an interphase HeLa cell nucleus. Heterochromatin (page 498) is evident around the entire inner surface of the nuclear envelope. Two prominent nucleoli are visible, and clumps of chromatin can be seen scattered throughout the nucleoplasm. (b) Schematic drawing showing some of the major components of the nucleus. (A: FROM WERNER W. FRANKE, INT. REV. CYTOL. (SUPPL.) 4:130, 1974., WITH PERMISSION FROM ELSEVIER.)



(a)

membranes arranged parallel to one another and separated by 10 to 50 nm (Figure 12.6). Together these membranes contain upwards of 60 distinct transmembrane proteins, including a number of species that link the outer nuclear membrane with elements of the cytoskeleton (Figure 12.6a). The inner and outer nuclear membranes are fused at sites forming circular pores that contain complex assemblies of proteins. The average mammalian cell contains several thousand nuclear pores. The outer membrane is generally studded with ribosomes and is continuous with the membrane of the rough endoplasmic

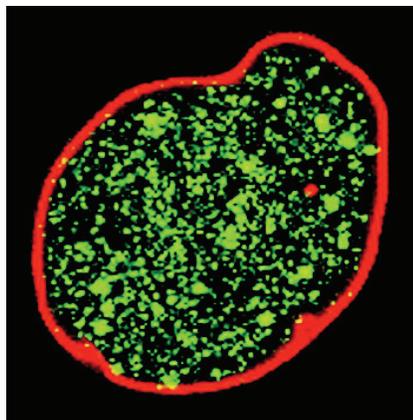


(b)

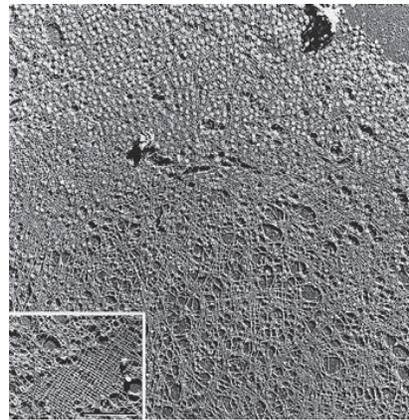
Figure 12.6 The nuclear envelope. (a) Schematic drawing showing the double membrane, nuclear pore complex, nuclear lamina, and the continuity of the outer membrane with the rough endoplasmic reticulum (ER). Both membranes of the nuclear envelope contain their own distinct complement of proteins. The actin filaments and intermediate filaments of the cytoskeleton are connected to the outer nuclear membrane by fibrous proteins (Nesprins). (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. 91:475, 1981, FIG. 8. REPRODUCED WITH PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS.)

reticulum. The space between the membranes is continuous with the ER lumen (Figure 12.6a).

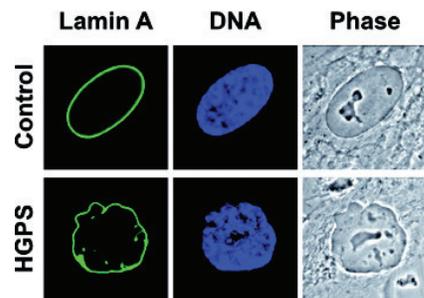
The inner surface of the nuclear envelope of animal cells is bound by integral membrane proteins to a thin filamentous meshwork, called the **nuclear lamina** (Figure 12.7). The nuclear lamina provides mechanical support to the nuclear enve-



(a)



(b)



(c)

Figure 12.7 The nuclear lamina. (a) Nucleus of a cultured human cell that has been stained with fluorescently labeled antibodies against lamin A/C to reveal the nuclear lamina (red), which lies on the inner surface of the nuclear envelope. A protein that is proposed to be part of a nuclear matrix (or nuclear scaffold) appears 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, FIG. 2. REPRODUCED WITH PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS; B: FROM U. AEBI, J. COHN, L. BUHLE, AND L. GERACE, NATURE 323:561, 1986. REPRINTED BY PERMISSION FROM MACMILLAN PUBLISHERS LIMITED; C: FROM ANNA MATTOUT ET AL., COURTESY OF YOSEF GRUENBAUM, CURR. OPIN. CELL BIOL. 18:338, 2006, WITH PERMISSION FROM ELSEVIER.)

lope, serves as a site of attachment for chromatin fibers at the nuclear periphery (Figure 12.6*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). The disassembly of the nuclear lamina prior to mitosis is induced by phosphorylation of the lamins.

Mutations in one of the lamin genes (*LMNA*) are responsible for a number of diverse human diseases, including a rare form of muscular dystrophy (called EDMD2) in which muscle cells contain exceptionally fragile nuclei. Mutations in *LMNA* have also been linked to a disease, called Hutchinson–Gilford progeria syndrome (HGPS), that is characterized by premature aging and death during teenage years from heart attack or stroke. Figure 12.7*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 12.7*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 alters the way the gene transcript is spliced, leading 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 Nuclear Pore Complex and Its Role in Nucleocytoplasmic Trafficking 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, mRNAs, tRNAs, and ribosomal subunits that are manufactured in the nucleus must be transported through the nuclear envelope in the opposite direction. Some components, such as the snRNAs of the spliceosome (page 450), 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 12.8*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 12.8*c*, in which granular material presumed to consist of a ribosomal subunit is seen squeezing through one of these pores.

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 a doughnut-shaped structure called the **nuclear pore complex (NPC)** that straddles the nuclear envelope, projecting into both the cytoplasm and nucleoplasm. The NPC is a huge, supramolecular complex—15 to 30 times the mass of a ribosome—that exhibits

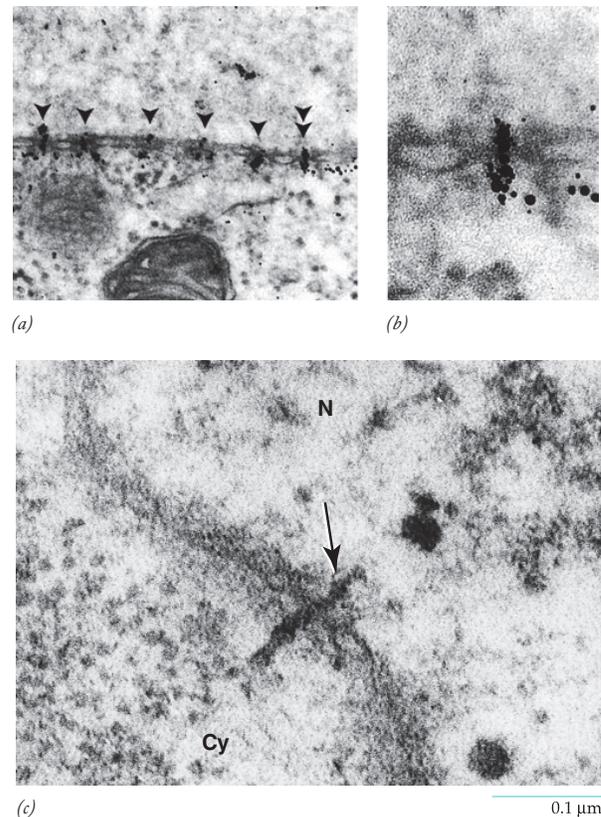
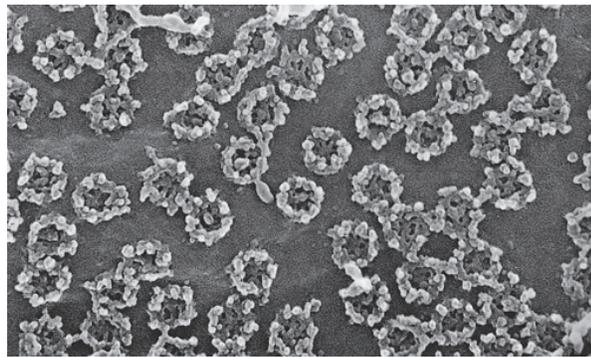
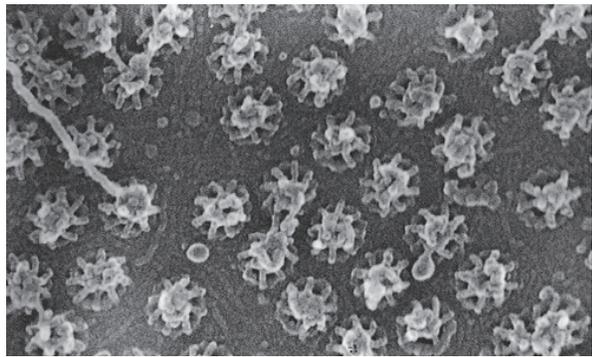


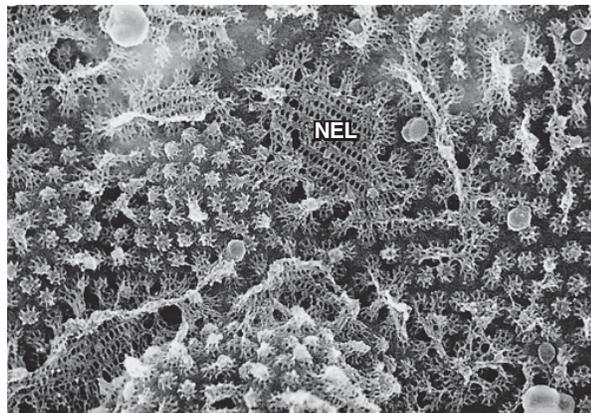
Figure 12.8 The movement of materials through the nuclear pore. (a) Electron micrograph of the nuclear–cytoplasmic border of a frog oocyte taken minutes after injection with gold particles that had been coated with a protein normally found in the nucleus. These particles pass through the center of the 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 a 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. FELDHER; C: FROM BARBARA J. STEVENS AND HEWSON SWIFT, *J. CELL BIOL.* 31:72, 1966, FIG. 23. REPRODUCED WITH PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS.)



(a) 0.2 μm



(b)



(c) 0.6 μm

Figure 12.9 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, FIGURES 1–3. REPRODUCED WITH PERMISSION OF THE ROCKEFELLER UNIVERSITY PRESS.)

octagonal symmetry due to the eightfold repetition of a number of structures (Figure 12.10). 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 multiple copies—8, 16, or 32 in number—in keeping with the octagonal symmetry of the structure.

The structure of the NPC is seen in the electron micrographs of Figure 12.9 and the models of Figure 12.10. At the heart of the NPC is a central channel, which is surrounded by a ring of nucleoporins whose rearrangements can change the diameter of the opening from about 20 to 40 nm. 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

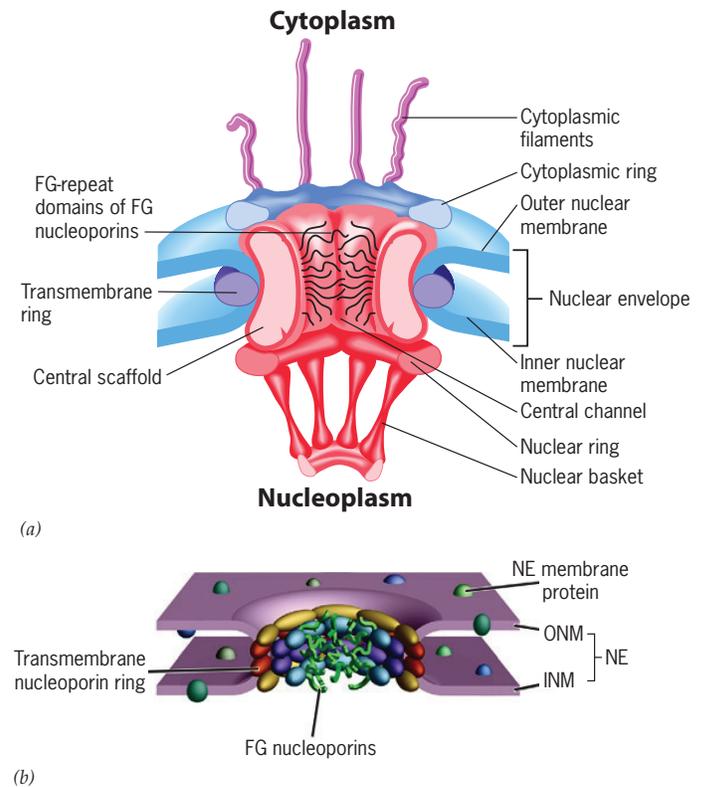


Figure 12.10 A model of a vertebrate nuclear pore complex (NPC). (a) Schematic representation of a vertebrate NPC as it is situated within the nuclear envelope. This elaborate structure consists of several parts, including a scaffold and transmembrane ring that anchors the complex to the nuclear envelope, a cytoplasmic and a nuclear ring, a nuclear basket, and eight cytoplasmic filaments. The FG-containing nucleoporins line a central channel with their disordered FG-containing domains extending into the opening and forming a hydrophobic meshwork. (b) Three-dimensional reconstruction of a portion of a nuclear pore complex showing the localization of individual nucleoporin molecules within the structure. The FG nucleoporins are shown in green. Several transmembrane nucleoporins span the pore membrane, forming an outer ring that anchors the NPC to the nuclear envelope. (B: FROM JAVIER FERNANDEZ-MARTINEZ AND MICHAEL P. ROUT, *CURR. OPIN. CELL BIOL.* 21: 604, 2009, WITH PERMISSION FROM ELSEVIER.)

period of seconds to minutes. Recent studies suggest that this dynamic exchange of nucleoporins may play a role in activating the transcription of chromatin that is associated with the NPC.

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 57) 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 central channel. The FG domains form a hydrophobic meshwork or sieve that blocks the free 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 localize to the nucleus. Conversely, if this NLS is fused to a non-nuclear protein, such as serum albumin, and injected into the cytoplasm, the modified protein becomes concentrated in the nucleus. Thus, targeting of proteins to the nucleus 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 316). 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 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 12.11*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 12.11*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 12.11*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 nu-

clear pore complex. The receptor–cargo complex then moves through the nuclear pore (step 3, Figure 12.11*a*) by engaging in a series of successive interactions with the FG domains of the FG-containing nucleoporins, allowing passage of the receptor–cargo complex through the NPC.

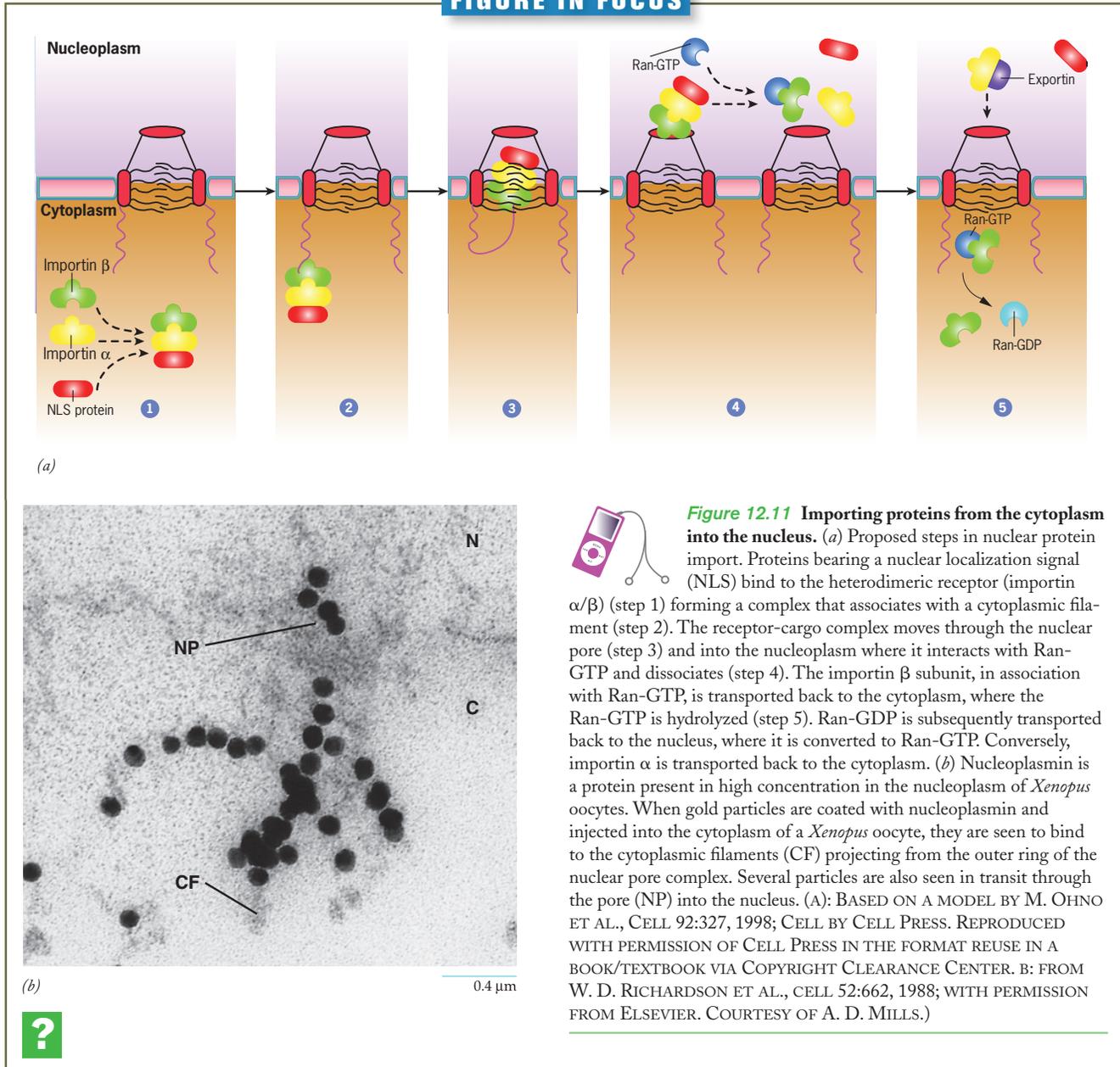
Once the bound cargo proceeds through the NPC, a GTP-binding protein called **Ran** drives release of the transported protein into the nuclear compartment. Like other GTP-binding proteins, such as Sar1 (page 296) and EF-Tu (page 471) discussed in earlier chapters, Ran can exist in an active GTP-bound 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 15.21*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 12.11*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 out of 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 12.11*a*, Ran-GTP promotes the *assembly* of export 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.

RNA Transport Most of the traffic out of the nucleus consists of various types of RNA molecules—mRNAs, rRNAs,

FIGURE IN FOCUS



snoRNAs, miRNAs, and tRNAs—that are synthesized in the nucleus and function in the cytoplasm or are modified in the cytoplasm and return to function in the nucleus. These RNAs move through the NPC as ribonucleoproteins (RNPs).

As with protein transport, RNA transport involves the association of transport receptors that ferry mRNP complexes through nuclear pores. 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. Numerous studies have demonstrated a functional link between pre-mRNA splicing and mRNA ex-

port; 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?