at sites of fusion between the inner and outer nuclear membranes. Protein filaments extend from both the cytoplasmic and nuclear rings, forming a distinct basketlike structure on the nuclear side.

Selective transport of proteins to and from the nucleus

Several million macromolecules selectively pass between the nucleus and the cytoplasm every minute. The basis for selective traffic across the nuclear envelope was first understood for proteins imported from the cytoplasm to the nucleus. Such proteins are responsible for all aspects of genome structure and function; they include histones, DNA polymerases, RNA polymerases, transcription factors, splicing factors, and many others. These proteins are targeted to the nucleus by specific amino acid sequences called **nuclear localization signals**, which are recognized by **nuclear transport receptors** that direct the transport of the proteins through the nuclear pore complex.

◆ Many viruses must gain entry to the nucleus in order to replicate. Following infection of a cell, retroviruses, such as HIV, reverse transcribe their genomic RNA to synthesize a DNA provirus in the cytoplasm. HIV has evolved special mechanisms for the transport of proviral DNA to the nucleus where it can be transcribed.

The first nuclear localization signal to be mapped in detail was characterized by Alan Smith and colleagues in 1984. These investigators studied simian virus 40 (SV40) T antigen, a virus-encoded protein that initiates viral DNA replication in infected cells (see Chapter 6). As expected for a replication protein, T antigen is normally localized to the nucleus. The signal responsible for its nuclear localization was first identified by the finding that mutation of a single lysine residue prevents nuclear import, resulting instead in the accumulation of T antigen in the cytoplasm. Subsequent studies defined the T antigen nuclear localization signal as the seven-amino-acid sequence Pro-Lys-Lys-Lys-Arg-Lys-Val. Not only was this sequence necessary for the nuclear transport of T antigen but its addition to other, normally cytoplasmic, proteins was sufficient to direct their accumulation in the nucleus.

Nuclear localization signals have since been identified in many other proteins. Some of these sequences, like that of T antigen, are short stretches rich in basic amino acid residues (lysine and arginine). Often, however, the amino acids that form the nuclear localization signal are close together but not immediately adjacent to each other. For example, the nuclear localization signal of nucleoplasmin (a protein involved in chromatin assembly) consists of two parts: a Lys-Arg pair followed by four lysines located ten amino acids farther downstream (**Figure 9.9**). Both the Lys-Arg and Lys-Lys-Lys-Lys sequences are required for nuclear targeting, but the ten amino acids between these sequences can be mutated without affecting nuclear localization. Because this nuclear localization sequence is composed of two

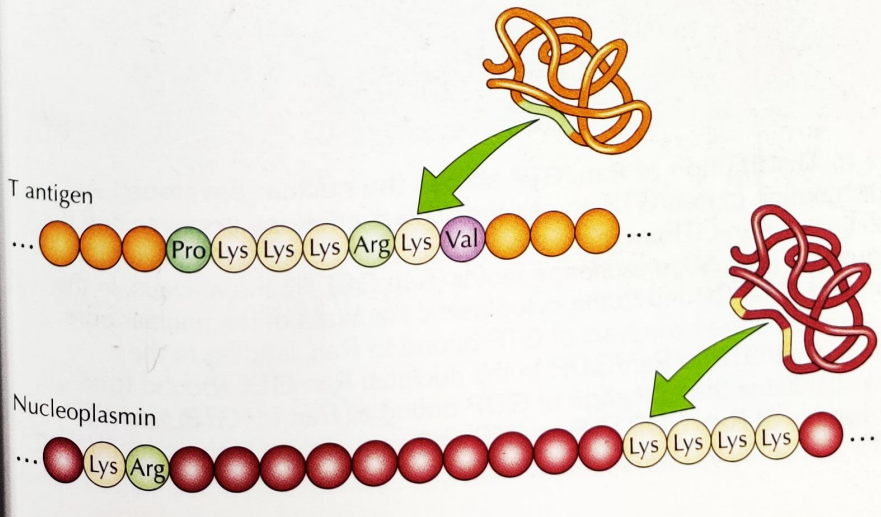

FIGURE 9.9 Nuclear localization signals The T antigen nuclear localization signal is a single stretch of amino acids. In contrast, the nuclear localization signal of nucleoplasmin i bipartite, consisting of a Lys-Arg sequence, followed by a Lys-Lys-Lys-Lys sequence loca ten amino acids farther downstream.

separated elements, it is called bipartite. Similar bipartite motifs appear to function as the localization signals of many nuclear proteins; thus they may be more common than the simpler nuclear localization signal of T antigen. While many nuclear localization signals consist of these basic amino acid residues—often termed the basic or "classical" nuclear localization signal—the amino acid sequences and structures of other nuclear localization signals vary considerably. Some are far apart in the amino acid sequence and depend on normal folding of the protein for their activity.

Nuclear localization signals are recognized by nuclear transport receptors called **importins** because they carry proteins into the nucleus. The activity of the importins is regulated by interaction with a protein called **Ran**, which controls the directionality of movement through the nuclear pore. Ran is one of several types of small GTP-binding proteins whose conformation and activity are regulated by GTP binding and hydrolysis. Other examples of small GTP-binding proteins include Ras (see Figure 8.39), several of the translation factors involved in protein synthesis (see Figure 8.14), Arf and Rab (discussed in Chapter 10), and Rac, Rho and Cdc42 (discussed in Chapter 15). For Ran, enzymes that stimulate GTP hydrolysis to GDP are localized to the cytoplasmic side of the nuclear envelope, whereas enzymes that stimulate the exchange of GDP for GTP are localized to the nuclear side (**Figure 9.10**). Consequently, there is an unequal distribution of Ran/GTP

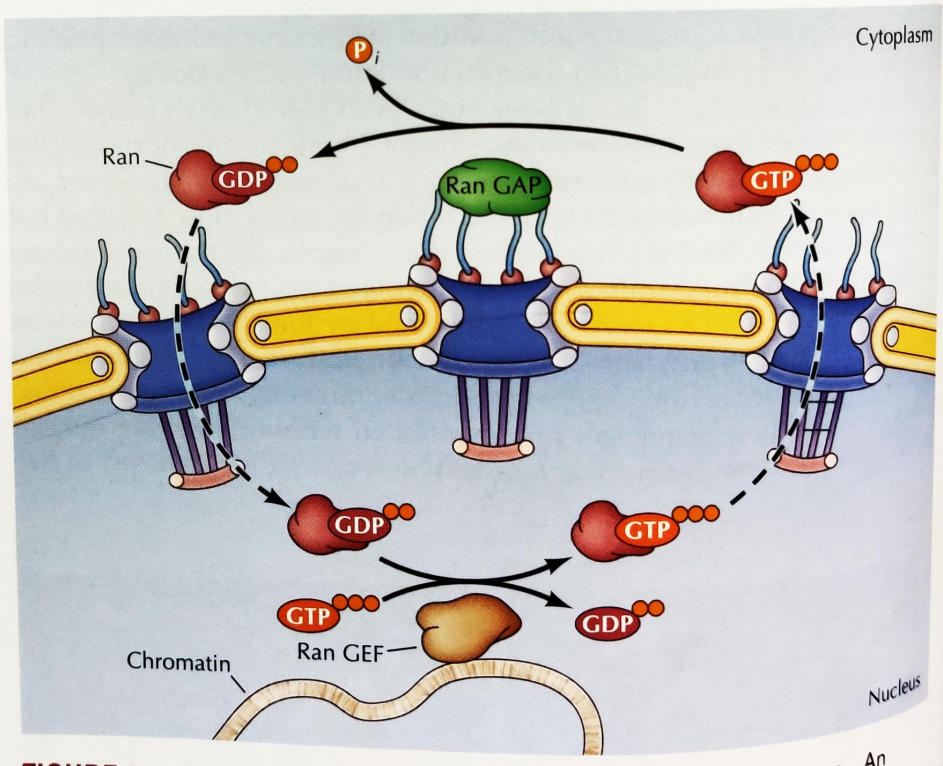

FIGURE 9.10 Distribution of Ran/GTP across the nuclear envelope An unequal distribution of Ran/GTP across the nuclear envelope is maintained by the localization of Ran GTPase-activating protein (Ran GAP) in the cytoplasm and Ran guanine nucleotide exchange factor (Ran GEF) in the nucleus. In the cytoplasm, Ran GAP (bound to the cytoplasmic filaments of the nuclear pore complex) stimulates the hydrolysis of GTP bound to Ran, leading to the conversion of Ran/GTP to Ran/GDP. In the nucleus, Ran GEF (bound to chromatin) stimulates the exchange of GDP bound to Ran for GTP, leading to the conversion of Ran/GDP to Ran/GTP. Consequently, a high concentration of Ran/GTP is maintained within the nucleus.

across the nuclear envelope, with a high concentration of Ran/GTP in the nucleus. This high concentration determines the directionality of nuclear transport of cargo proteins.

Protein import through the nuclear pore complex begins when a specific importin binds to the nuclear localization signal of a cargo protein in the cytoplasm (Figure 9.11). This importin/cargo complex then binds to proteins in the cytoplasmic filaments of the nuclear pore complex, and transport proceeds via the interaction of importins with proteins that line the central channel of the pore. Once the cargo/importin complex reaches the nuclear side of the envelope, Ran/GTP (present in high concentration in the nucleus) binds to importin. This causes a change in the conformation of the importin, which disrupts the cargo/importin complex, displacing the cargo protein and releasing it into the nucleus.

The importin-Ran/GTP complex is then exported back through the nuclear pore complex. In the cytoplasm the GTP is hydrolyzed to GDP. This releases the importin so that it can bind to a new cargo protein in the cytoplasm and participate in another round of transport. The Ran/GDP formed in the cytoplasm is then transported back to the nucleus by its own import receptor (a protein called NTF2), where Ran/GTP is regenerated.

WEBSITE ANIMATION 9.1

Protein Import and Export through the Nuclear Pore Complex *Proteins are targeted to or from the nucleus by virtue of having nuclear localization signals or nuclear export signals, respectively, allowing the proteins to be recognized by receptors that direct their transport through the nuclear pore complex.*

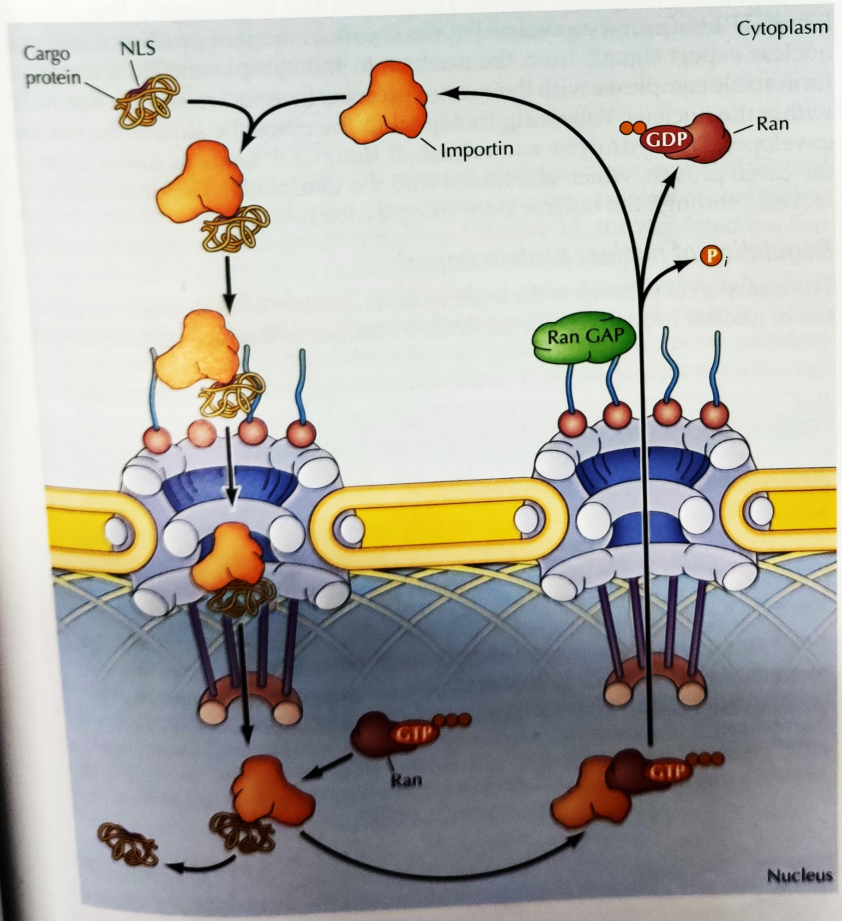

FIGURE 9.11 Protein import through the nuclear pore complex Transport begins when the nuclear localization sequence (NLS) of a cargo protein is recognized by an importin. The importin/cargo complex binds to nuclear pore proteins in the cytoplasmic filaments and is transported through the pore. At the nuclear side of the envelope, Ran/GTP binds to the importin, disrupting the importin/cargo complex and releasing the cargo protein into the nucleus. The importin-Ran/GTP complex is re-exported through the nuclear pore and the GTPase-activating protein (Ran GAP) in the cytoplasm hydrolyzes the GTP on Ran to GDP, releasing the importin.

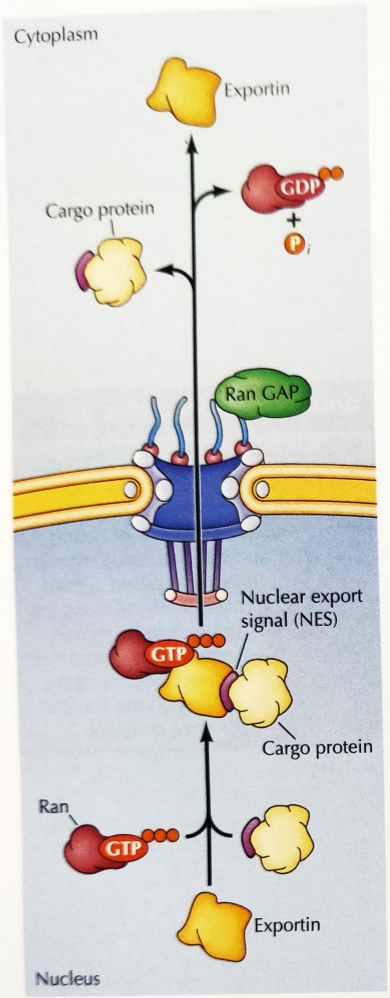

FIGURE 9.12 Nuclear export Complexes between cargo proteins bearing nuclear export signals (NES), exportins, and Ran/GTP form in the nucleus. Following transport through the nuclear pore complex, Ran GAP stimulates the hydrolysis of bound GTP, leading to formation of Ran/GDP and release of the cargo protein and exportin into the cytoplasm.

Some proteins remain within the nucleus following their import from the cytoplasm, but many others shuttle back and forth between the nucleus and the cytoplasm. Some of these proteins act as carriers in the transport of other molecules, such as RNAs; others coordinate nuclear and cytoplasmic functions (e.g., by regulating the activities of transcription factors). Proteins are targeted for export from the nucleus by specific amino acid sequences, called **nuclear export signals**. Like nuclear localization signals, nuclear export signals are recognized by receptors within the nucleus—**exportins**, which direct protein transport through the nuclear pore complex to the cytoplasm. Like importins, many exportins are members of a family of nuclear transport receptors known as **karyopherins** (Table 9.1).

Exportins bind to Ran, which is required for nuclear export as well as for nuclear import (Figure 9.12). However, Ran/GTP promotes the formation of stable complexes between exportins and their cargo proteins, whereas it dissociates the complexes between importins and their cargos. This effect of Ran/GTP binding on exportins dictates the movement of proteins containing nuclear export signals from the nucleus to the cytoplasm. Thus exportins form stable complexes with their cargo proteins in association with Ran/GTP within the nucleus. Following transport to the cytosolic side of the nuclear envelope, GTP hydrolysis and release of Ran/GDP leads to dissociation of the cargo protein, which is released into the cytoplasm. Exportins are then recycled through the nuclear pore complex for reuse.

Regulation of nuclear protein import

The transport of proteins to the nucleus is yet another level at which the activities of nuclear proteins can be controlled. Transcription factors, for example,

TABLE 9.1 Karyopherins with Known Substrates

Karyopherin	Substrates
Import	
Kapα/Kapβ1 dimer	Proteins with a basic amino acid nuclear localization signal (e.g., nucleoplasmin)
Snurportin/Kapβ1	snRNPs (U1, U2, U4, U5)
Kapβ1 alone	Cdk/cyclin complexes
Kapβ2 (transportin)	mRNA binding proteins, ribosomal proteins
Importin7/Kapβ1 dimer	Histone H1, ribosomal proteins
Export	
Crm1	Proteins with a leucine-rich nuclear export signal, snurportin, snRNAs
CAS	Kapα
Exportin-t	tRNAs
Exportin5	miRNAs

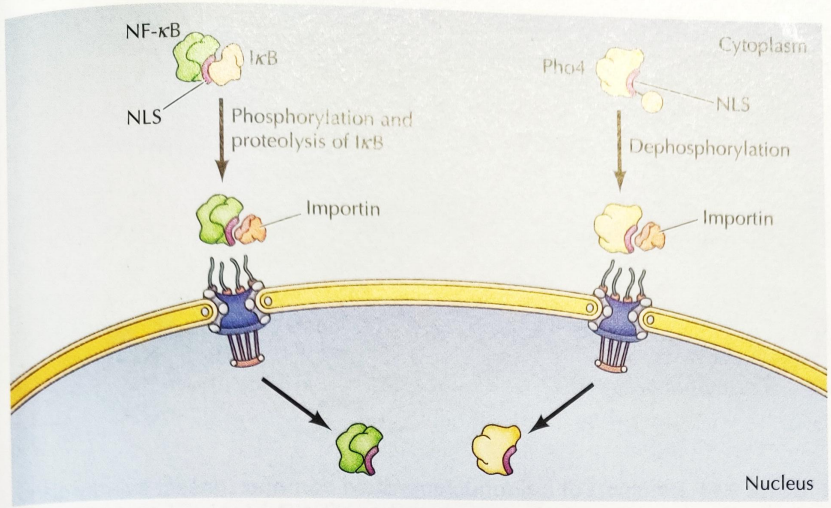

FIGURE 9.13 Regulation of nuclear import of transcription factors The transcription factor NF-κB is maintained as an inactive complex with IκB, which masks its nuclear localization sequence (NLS) in the cytoplasm. In response to appropriate extracellular signals, IκB is phosphorylated and degraded by proteolysis, allowing the import of NF-κB to the nucleus. The yeast transcription factor Pho4 is maintained in the cytoplasm by phosphorylation in the vicinity of its nuclear localization sequence. Regulated dephosphorylation exposes the NLS and allows Pho4 to be transported to the nucleus.

are functional only when they are present in the nucleus, so regulation of their import to, and export from, the nucleus is a novel means of controlling gene expression. As will be discussed in Chapter 15, the regulated nuclear import of both transcription factors and protein kinases plays an important role in controlling cell behavior by providing a mechanism through which signals received at the cell surface can be transmitted to the nucleus.

In one mechanism of regulation, transcription factors (or other proteins) associate with cytoplasmic proteins that mask their nuclear localization signals; because their signals are no longer recognizable, these proteins remain in the cytoplasm. A good example is provided by the transcription factor NF-κB, which is activated in response to a variety of extracellular signals in mammalian cells (**Figure 9.13**). In unstimulated cells, NF-κB is found as an inactive complex with an inhibitory protein (IκB) in the cytoplasm. Binding to IκB masks the NF-κB nuclear localization signal, thus preventing NF-κB from being transported to the nucleus. In stimulated cells, IκB is phosphorylated and degraded by ubiquitin-mediated proteolysis (see Figure 8.44), allowing NF-κB to enter the nucleus and activate transcription of its target genes.

The nuclear import of other transcription factors is regulated directly by their phosphorylation rather than by association with inhibitory proteins (see Figure 9.13). For example, the yeast transcription factor Pho4 is phosphorylated at a serine residue adjacent to its nuclear localization signal. Phosphorylation at this site inhibits Pho4 by interfering with its nuclear import. Under appropriate conditions, regulated dephosphorylation of this site activates Pho4 by permitting its translocation to the nucleus.

Transport of RNAs

Whereas many proteins are selectively transported from the cytoplasm to the nucleus, most RNAs are exported from the nucleus to the cytoplasm. Since

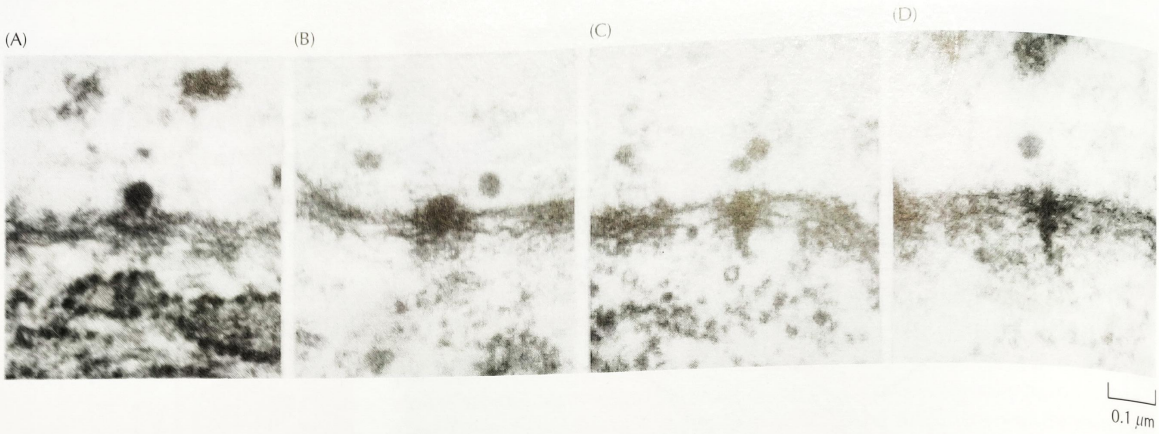

FIGURE 9.14 Transport of a ribonucleoprotein complex Insect salivary gland cells produce large ribonucleoprotein complexes (RNPs), which contain 35–40 kb of RNA and have a total mass of approximately 30 million daltons. This series of electron micrographs shows the attachment of such an RNP to a nuclear pore complex (A) and the unfolding of the RNA during its translocation to the cytoplasm (B–D). (From H. Mehlin et al., 1992. *Cell* 69: 605.)

proteins are synthesized in the cytoplasm, the export of mRNAs, rRNAs, tRNAs, and microRNAs (miRNAs) is a critical step in gene expression in eukaryotic cells. Like protein import, the export of RNAs is a selective process mediated by transport receptors that interact with the nuclear pore complex.

All RNAs are transported across the nuclear envelope as ribonucleoprotein complexes (RNPs) (**Figure 9.14**). Karyopherin importins and exportins (see Table 9.1) transport tRNAs, rRNAs, miRNAs and small nuclear RNAs in a Ran/GTP–dependent manner. Ribosomal RNAs are first associated with both ribosomal proteins and specific RNA processing proteins in the nucleolus, and nascent 60S and 40S ribosomal subunits are then separately transported to the cytoplasm (see Figure 9.32) by a mechanism involving the karyopherin Crm1. Their export from the nucleus is mediated by nuclear export signals present on proteins within the subunit complex. tRNAs and miRNA precursors are exported from the nucleus by exportin-t and exportin5, respectively, which bind directly to the RNAs.

mRNAs are exported by a distinct mechanism that does not involve karyopherins and is independent of Ran (**Figure 9.15**). Pre-mRNAs are associated with a set of at least 20 proteins throughout their processing in the nucleus and eventual transport to the cytoplasm, which is mediated by a distinct mRNA exporter complex. The exporter complex is recruited to pre-mRNAs in the nucleus in concert with the completion of splicing and polyadenylation. It then transports the mRNAs through the nuclear pore complex. Directionality of the process is established by a RNA helicase localized to the cytoplasmic face of the nuclear pore complex. The mRNA/exporter complex is remodeled by the

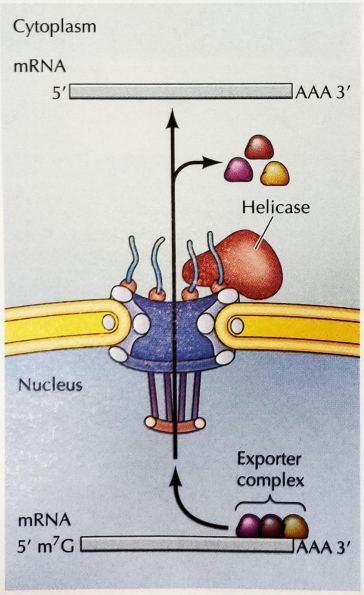

FIGURE 9.15 mRNA export Following processing, mRNAs are bound by an exporter complex, which mediates their transport through the nuclear pore complex. A helicase associated with the cytoplasmic face of the nuclear pore complex then releases the mRNA into the cytoplasm.

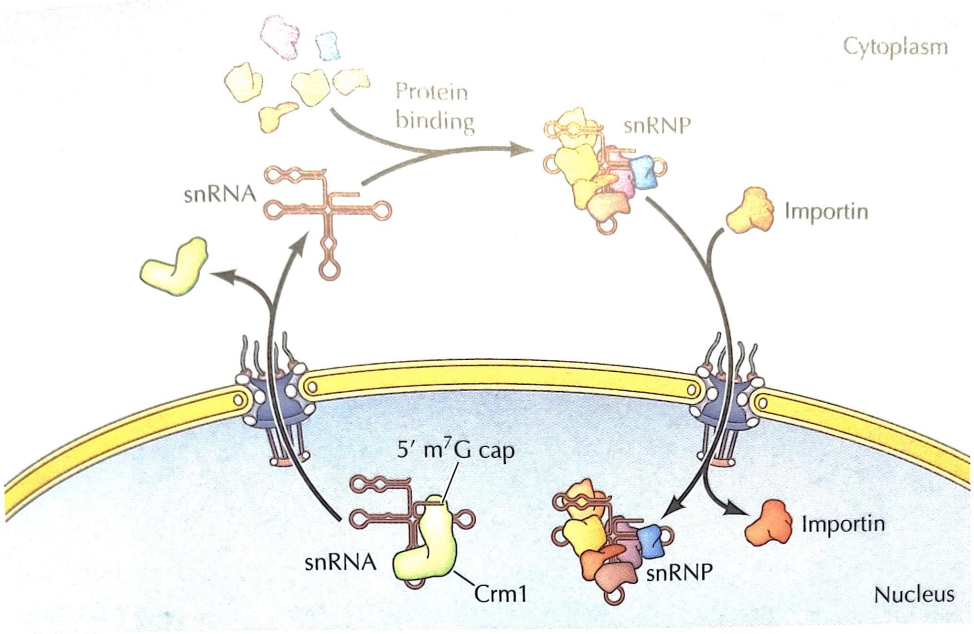

FIGURE 9.16 Transport of snRNAs between nucleus and cytoplasm Small nuclear RNAs (snRNAs) are initially exported from the nucleus to the cytoplasm by an exportin (Crm1) that recognizes the 5′ 7-methylguanosine cap. In the cytoplasm, the snRNAs associate with proteins to form snRNPs, which are recognized by an importin and transported back to the nucleus.

helicase as it reaches the cytoplasmic side of the pore. This releases the mRNA into the cytoplasm and prevents its transport back into the nucleus.

In contrast to mRNAs, miRNAs, tRNAs, and rRNAs, which function in the cytoplasm, many small RNAs (snRNAs and snoRNAs, discussed later in the chapter) function within the nucleus as components of the RNA processing machinery. snRNAs are initially transported from the nucleus to the cytoplasm, where they associate with proteins to form functional snRNPs and then return to the nucleus (**Figure 9.16**). Crm1 and other transport receptor proteins that bind to the 5′ 7-methylguanosine caps of snRNAs are involved in the export of the snRNAs to the cytoplasm. In contrast, sequences present on the snRNP proteins are responsible for the transport of snRNPs from the cytoplasm to the nucleus.

Internal Organization of the Nucleus

The nucleus is more than a container in which chromatin, RNAs, and nuclear proteins move freely in an aqueous solution. Instead, the nucleus has an internal structure that organizes the genetic material and localizes nuclear functions. Chromosomes occupy distinct regions of the nucleus and are organized such that the transcriptional activity of a gene is correlated with its position. In addition, the nucleus contains a variety of discrete subnuclear structures that are associated with multiple aspects of gene expression and DNA repair.

Chromosome organization and gene expression

Chromatin becomes highly condensed during mitosis to form the compact metaphase chromosomes that are distributed to daughter nuclei (see Figure 5.18). During interphase, some of the chromatin (**heterochromatin**) remains highly condensed and is transcriptionally inactive; the remainder of the chromatin (**euchromatin**) is decondensed and distributed

◆ Ciliated protozoa
types of nuclei: a poly
ronucleus that contai
tionally active genes,
more diploid transcrip
tive micronuclei that p
sexual reproduction.