Chapter 9: Protein Trafficking

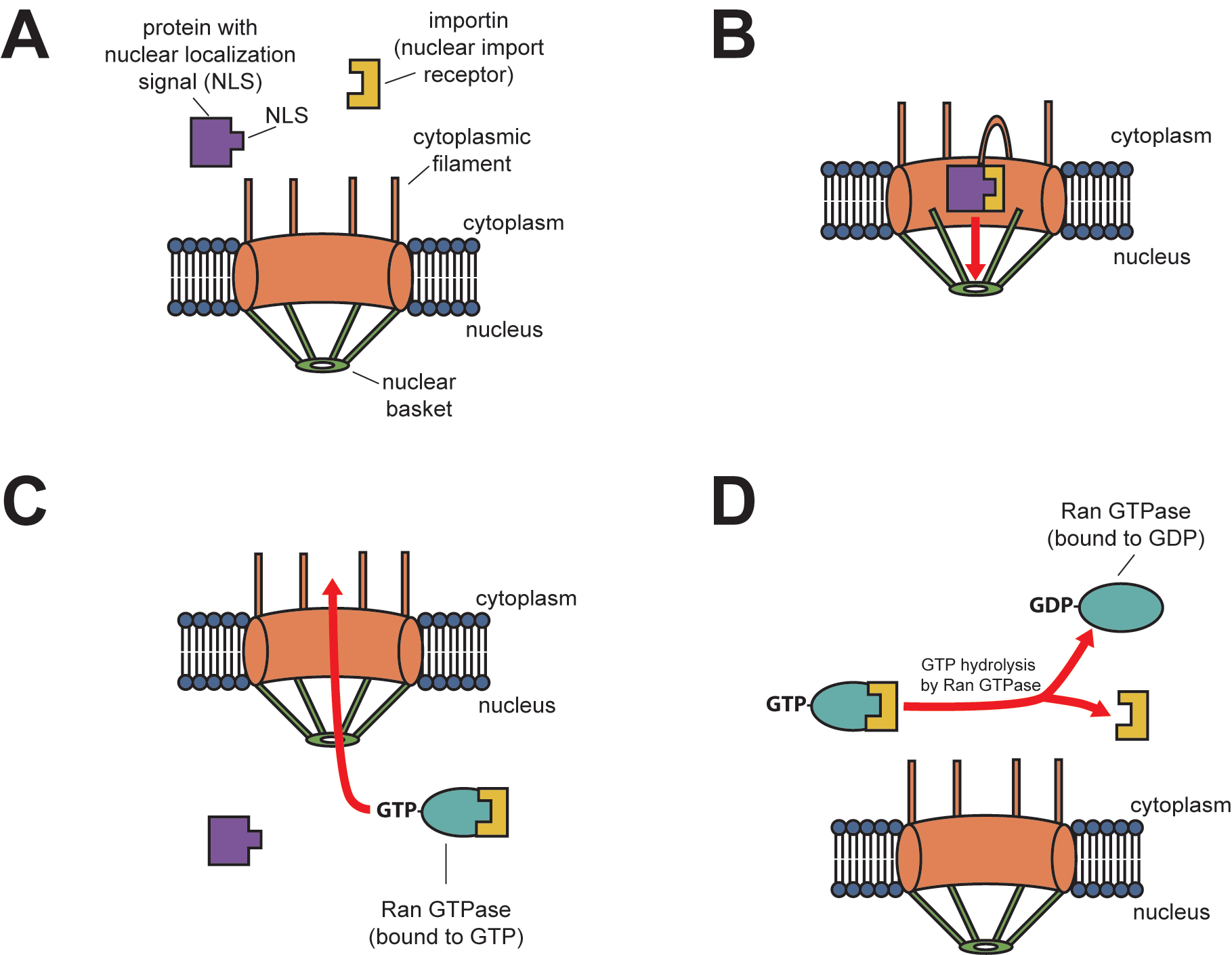
After transcription and translation, proteins may need to be shipped, or “trafficked”, to specific locations within the cell. When a ribosome begins translating an mRNA, the cell still needs instructions on where to send the new protein so that it may carry out its ultimate function. Those instructions are in **signal sequences**, specific amino acid sequences within the translated protein that have specific properties based on the biochemistry of the particular amino acids. Most signal sequences are found at the N-terminus of proteins; however, signal sequences can also be found at the C-terminus and in internal locations within the protein.

# Signal Sequences

Signal sequences direct the protein from the cytoplasm to a particular organelle. For eukaryotes, there are specific signal peptides that can direct the protein to the nucleus, to the mitochondria, to the endoplasmic reticulum and other organelles. The signal sequences are specifically recognized by receptors that are soluble or membrane-bound. Receptors are soluble for import into the nucleus, but for delivery to the mitochondria, ER or other organelles receptors are located within the membranes of cellular compartments. These receptors help guide the insertion of the protein into or through the membrane. Almost all protein synthesis in eukaryotes is carried out in the cytoplasm using mRNA generated from the genomic DNA. An exception are a few proteins in the chloroplasts and mitochondria that are self-generated using their own DNA and ribosomes). Proteins found in any other compartment or embedded in any membrane must have been delivered to that compartment by its signal sequence.

## Nuclear localization

The nucleus is one organelle where proteins need to be transported for proper cell function and growth. Examples of the proteins needed in the nucleus include DNA and RNA polymerases, transcription factors, and histone proteins. These and other nuclear proteins have a signal sequence known as the **NLS**, or **nuclear localization signal**. This is a well-studied pathway that involves a set of importin adapter proteins and the nuclear pore complex (Figure 1). Transport into the nucleus is particularly challenging because it has a double membrane that is contiguous with the endoplasmic reticulum membrane). Although there are other mechanisms for making proteins that are embedded in the nuclear membrane, the primary mechanism for import and export of large molecules into and out of the nucleus itself is the **nuclear pore complex**. The complex is very large and is composed of over 50 different proteins (nucleoporins, sometimes called “nups”). The nucleoporins are assembled into a large open octagonal pore through the nuclear membranes. As Figure 1 indicates, there are antenna-like fibrils on the cytoplasmic face, and these help to guide proteins from their origin in the cytoplasm to the nuclear pore, and on the nuclear side there is a basket structure. The protein must bear a nuclear localization signal (NLS) for appropriate targeting to the nuclear pore complex. Additionally, translation of the protein is completed in the cytoplasm and the protein is folded *before* nuclear import. While in the cytoplasm, an importin-α protein binds to the NLS of a nuclear protein, and also binds to a protein called importin-β that is recognized and bound by the nuclear pore complex. The importin proteins are **nuclear transport receptors** that are soluble, not membrane-bound, allowing binding of the cargo protein (the protein gaining entry to the nucleus) and transport of the complex through the nuclear pore.



**Figure 9-1: Transport through the nuclear pore.** A) The NLS is recognized by the importin nuclear transport receptor. B) The complex is moved into the nucleus. C) Ran-GTP facilitates the dissociation of the complex. D) Ran-GTP and other associated proteins are exported and Ran-GTP is hydrolyzed to Ran-GDP in the cytoplasm to release importin.

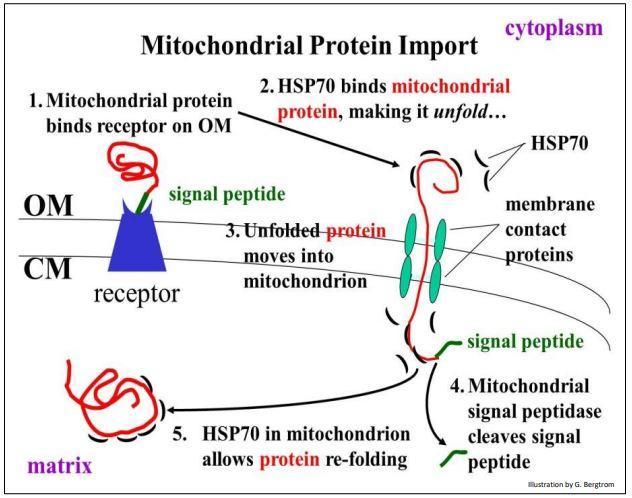
Once the complex of the cargo protein containing the NLS and the **importin** proteins/nuclear transport receptors are moved into the nucleus, binding of Ran-GTP, a small GTPase, causes the complex of proteins to dissociate (Figure 1c). The imported cargo protein is released in the nucleus. The importins are also released in the nucleus, but they are exported back out through the nuclear pore to be reused with another protein targeted for the nucleus. The Ran-GTP is also a part of the export complex (Figure 1d), once in the cytoplasm, the hydrolysis of GTP to GDP by Ran (activated by Ran-GAP, a cytoplasmic protein) provides the energy to dissociate the cargo (e.g. mRNA) from the importin. The Ran-GDP then binds to importins, re-enters the nucleus, and the GDP is exchanged for GTP, repeating the cycle of nuclear import for proteins with a nuclear localization sequence.

## Mitochondrial localization

Recall that mitochondria contain their own genome and translational machinery. Thus, they transcribe RNAs and translate proteins of their own. However, genes in the nucleus encode many of the proteins found in mitochondria. Similar to the transport of proteins into the nucleus, mitochondrial protein transfer is post-translational. This means that mitochondrial proteins formed in the cytoplasm have already folded, assuming a tertiary structure. The folded protein exposes an N-terminal signal sequence on its surface that is recognized and bound to a receptor protein embedded in the outer mitochondrial membrane. The receptor protein spans both the mitochondrial outer membrane (OM) and cristal membrane (CM).

Binding of the cargo protein to the receptor protein delivers the protein to membrane contact proteins that also span both mitochondrial membranes. The membrane contact proteins act as a channel, or pore, through which the mitochondrial protein will cross into the mitochondrial matrix, called a **translocation channel**. Mitochondria have two such translocation channels: translocation channel of the outer membrane (**TOM**) and translocation channel of the inner membrane (**TIM**). Mitochondrial proteins destined for the intermembrane space pass through TOM, while those mitochondrial matrix proteins pass through both TOM and TIM.

For the protein to cross through this translocation channel, it must be unfolded, or linearized, during import and then refolded upon entry into the mitochondria. But there is a problem: the folded protein cannot cross the membrane by itself! The entry of a fully translated mitochondrial protein from the cytoplasm requires a so-called chaperone protein, in this case, the HSP70 (heat-shock 70) protein. HSP70 controls unfolding of the mitochondrial protein as it passes into the mitochondrial matrix. Upon removal of the signal peptide by a mitochondrial signal peptidase, another HSP70 molecule resident in the mitochondrion facilitates refolding of the protein into a biologically active shape.

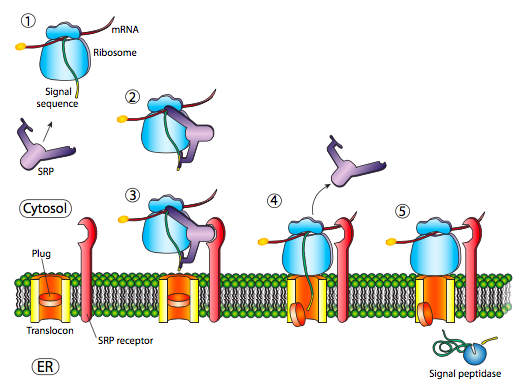


**Figure 9-2: Transport of proteins into the mitochondria.** Proteins imported into the mitochondria are recognized by their signal sequence and imported in their linearized/unfolded form through the translocation channels. The chaperone protein HSP70 controls the unfolding and refolding of the protein once it is imported.

## Transport to the endoplasmic reticulum

Functional proteins destined for the endoplasmic reticulum (smooth and rough), the Golgi apparatus, the lysosome, the cell membrane or to be secreted from the cell, are targeted differently than those previously discussed. These proteins are targeted to the rough endoplasmic reticulum early in translation so that they complete translation in the RER, and then be packaged into vesicles for transport through the Golgi and then to their final location. Here, in addition to an **ER N-terminal signal sequence** which directs the ribosome to the RER, the position of secondary internal signal sequences (sometimes called signal patches or **stop sequences**) helps to determine the disposition of the protein as it enters the ER. The ER signal sequence is a series of hydrophobic amino acids that will become lodged in the hydrophobic portion of the ER membrane once delivered.

The initial insertion requires recognition of the ER signal sequence by **SRP**, the signal recognition particle. The SRP is a GTPase and exchanges its bound GDP for a GTP upon binding to a protein’s ER signal sequence. It binds both the ER signal sequence and the ribosome during translation of the protein, which is a different mechanism from the ones used for nuclear and mitochondrial import, which are imported post-translationally. The SRP usually binds as soon as the signal sequence is available, and when it does so, it arrests translation until it is docked to the ER membrane. The SRP with its attached protein and ribosome then docks to a receptor (called the **SRP receptor**) embedded in the ER membrane and extending into the cytoplasm. Incidentally, this is the origin of the “rough” endoplasmic reticulum: the ribosomes studding the ER are attached during translation to the ER cytoplasmic surface by the nascent polypeptide it is producing and an SRP. The SRP receptor can exist on its own or in association with a **translocon**, which is a translocation channel. The SRP receptor (SR) is also a GTPase, and is usually carrying a GDP molecule when unassociated. However, upon association with the translocon it exchanges its GDP for a GTP. These GTPs are essential because when the SRP binds to the SRP Receptor, both GTPase activities are activated and the resulting release of energy dissociates both from the translocon and the nascent polypeptide. This relieves the block on translation imposed by the SRP, translation restarts, and the new protein is pushed on through the translocation channel in a linearized, or unfolded state as it is being synthesized. Once the N-terminal signal sequence has completely entered the lumen of the ER, it reveals a recognition site for **signal peptidase**, a hydrolytic enzyme that resides in the ER lumen and whose purpose is to snip off the signal peptide. Note that the N-terminus of the ER targeted protein is delivered through the translocation channel first, thereby allowing proper removal of the hydrophobic N-terminal signal sequence prior to protein folding (Fig 9-3).

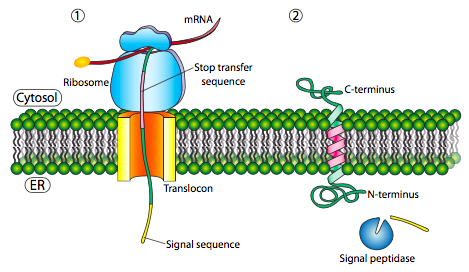


**Figure 9-3: SRP and its receptor SR mediate movement of proteins through the ER membrane.** The SRP recognizes the signal sequence and binds to it and the ribosome, temporarily arresting translation. The SRP-polypeptide-ribosome complex is bound by its receptor, SR, which positions the complex on a translocon. Once the ribosome and polypeptide are docked on the translocon, the SRP dissociates, and translation resumes, with the polypeptide moving through the translocon as it is being synthesized.

If the only signal sequence present is the N-terminal signal sequence, then the remainder of the protein is synthesized and pushed through the translocation channel and a soluble protein is deposited in the ER lumen, as shown in Figure 9-3. What about proteins that are embedded in a membrane? Transmembrane proteins have internal signal sequences (sometimes called signal patches). Depending on their relative locations, they may be considered either **start-transfer** or **stop-transfer sequence**, where “transfer” refers to translocation of the peptide through the translocation channel (Figure 9-4).

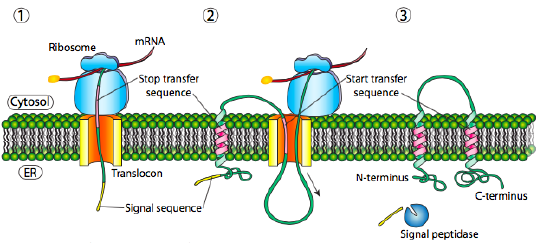
If there is a significant stretch of mostly-uninterrupted hydrophobic residues, it would be considered a stop-transfer signal, as that part of the proteican get stuck in the translocation channel (and subsequently the ER membrane) forcing the remainder of the protein to remain outside the ER. This generates a protein that inserts into the membrane once, with its N-terminus in the ER lumen and the C-terminus in the

cytoplasm, called a **single-pass protein**. In a **multi-pass transmembrane protein**, there could be several start- and stop- transfer hydrophobic signal patches.



**Figure 9-4: Single-pass transmembrane protein insertion.** (1) The signal sequence has allowed the ribosome to dock on a translocon and newly made polypeptide is threaded through until the stop-transfer sequence. (2) The hydrophobic stop transfer sequence gets “stuck” in the membrane, forcing the rest of the polypeptide to stay in the cytoplasm as it is translated.

Building on the single-pass example, if there were another signal patch after the stop-transfer sequence, it would act as a start-transfer sequence, attaching to a translocon and allowing the remainder of the protein to be moved into the ER. This results in a protein with both N- and C- termini in the ER lumen, passing through the ER membrane twice, and with a cytoplasmic loop sticking out (Fig 9-5).



**Figure 9-5: Insertion of double-pass transmembrane protein**. After the initial stop transfer sequence is embedded in the membrane, a start transfer sequence instructs the remainder of the peptide to be fed through the translocon, resulting in a protein with both N-terminus and C-terminus facing the lumen.

It is important to note that there are other configurations of single-pass and multiple-pass membrane proteins possible. The examples shown are not indicative of all the potential membrane protein configurations.

Associated Online Videos:

[Nuclear Import](https://www.youtube.com/watch?v=rrvTbix4FtQ)

[Mitochondrial Import](http://vcell.ndsu.nodak.edu/animations/mito-pt/index.htm)

[ER import](https://www.youtube.com/watch?v=2aguTUg1sJk)