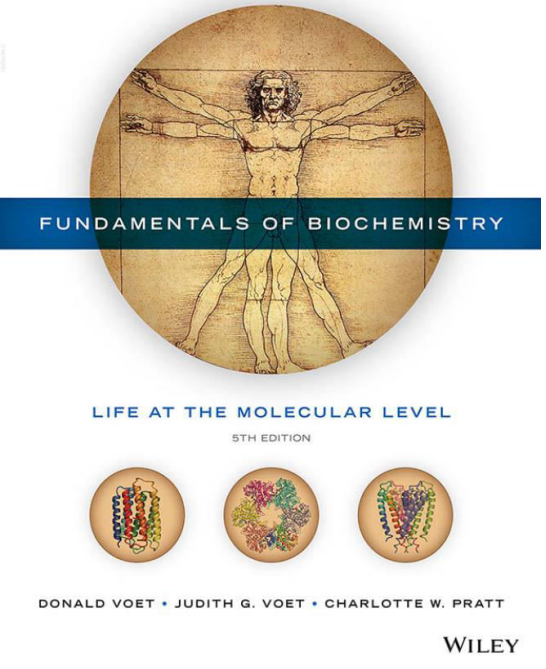
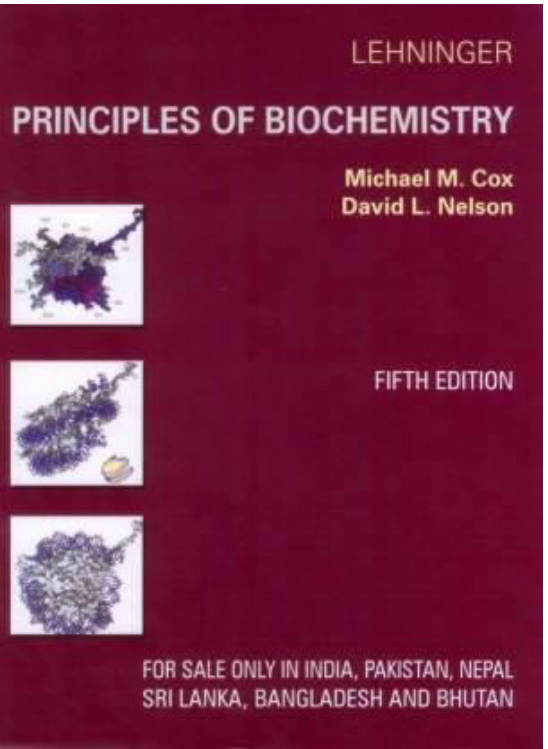
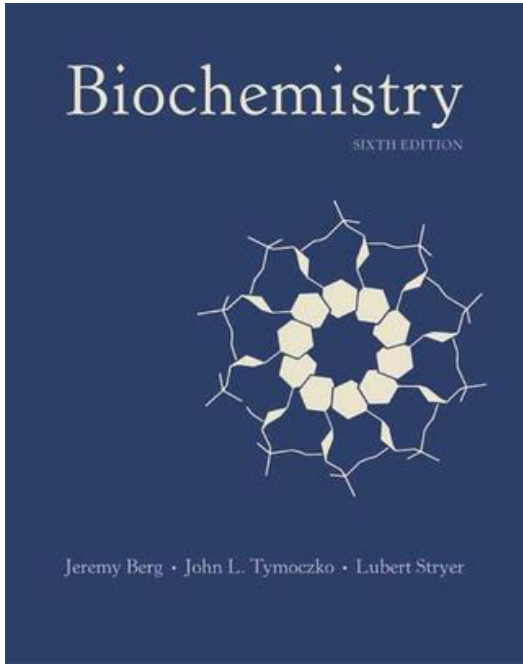


Subcellular localization of enzymes



Dr. Akhilendra Pratap Bharati
Assistant Professor
Department of Life Science and Biotechnology



Secretory Enzymes

- Several distinct pathways through the cytoplasm have been identified.

- **A biosynthetic pathway** can be discerned in which **proteins are synthesized in the endoplasmic reticulum**, **modified during passage** through the Golgi complex, and transported from the Golgi complex to various destinations, such as the plasma membrane, a lysosome, or the large vacuole of a plant cell.

- This route is also referred to as the **secretory pathway**, because many of the proteins synthesized in the endoplasmic reticulum are destined to be discharged (secreted or exocytosed) from the cell.

- Secretory activities of cells can be divided into two types:

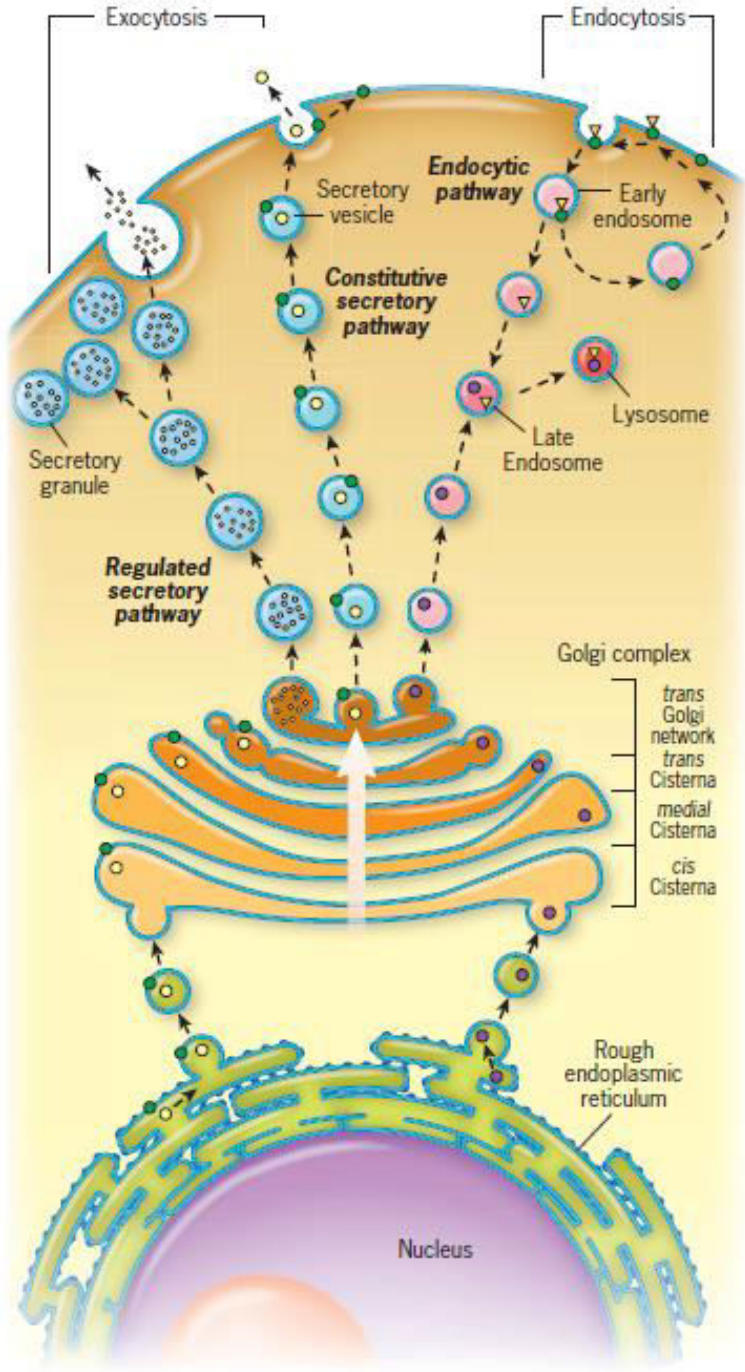
    1. Constitutive
    2. Regulated

Constitutive secretion

During constitutive secretion , materials are transported in secretory vesicles from their sites of synthesis and **discharged into the extracellular space in a continual manner**. Most cells engage in constitutive secretion, a process that contributes not only to the formation of the extracellular matrix but to the formation of the plasma membrane itself.

Regulated secretion

During regulated secretion, materials are **stored as membrane-bound packages** and **discharged only in response to an appropriate stimulus**. Regulated secretion occurs, for example, in **endocrine cells that release hormones**, in **pancreatic acinar cells that release digestive enzymes**, and in **nerve cells that release neurotransmitter**s. In some of these cells, materials to be secreted are stored in large, densely packed, membrane-bound secretory granules.



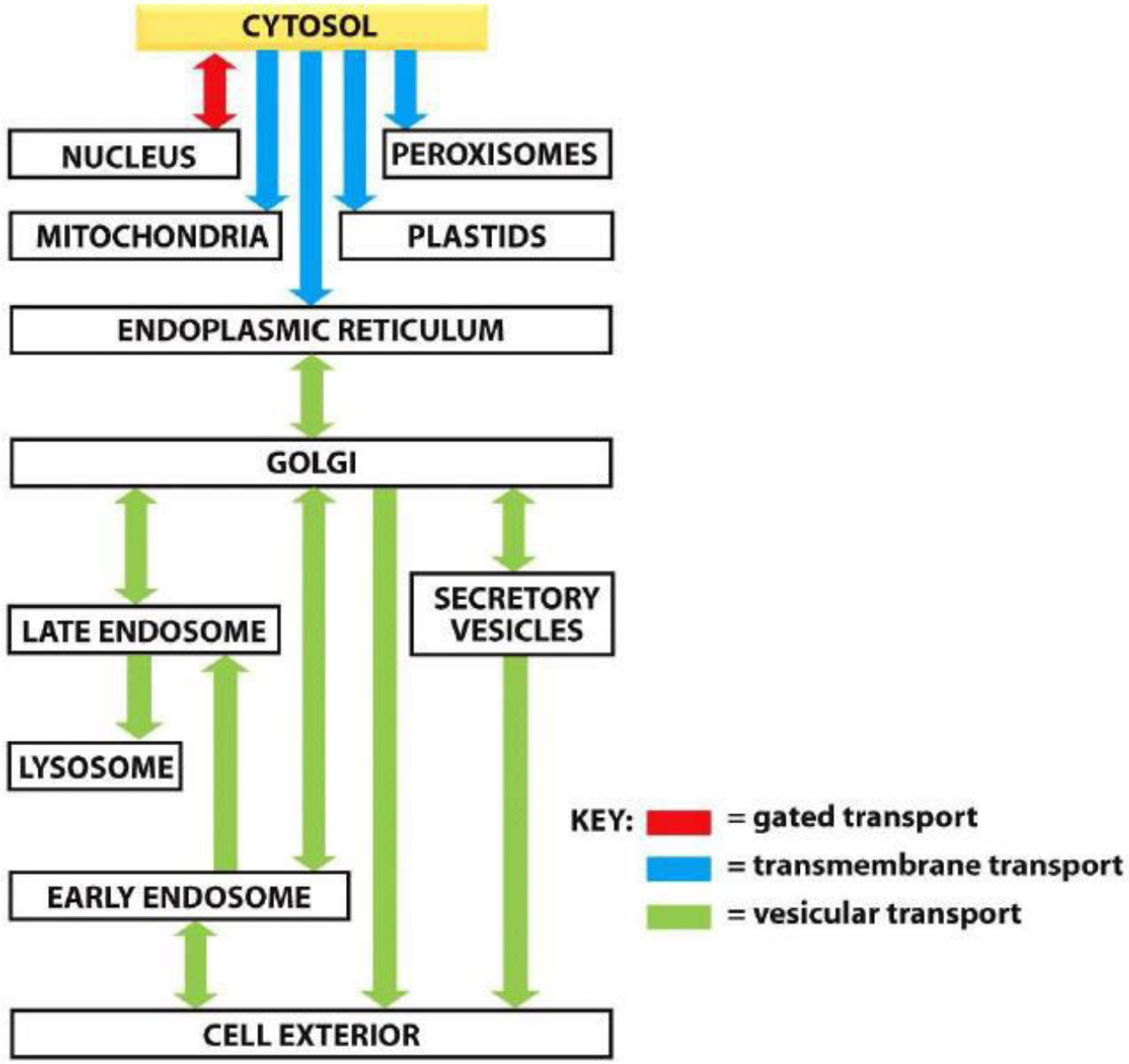
Proteins can move between compartments in different ways

Three fundamentally different ways by which proteins move from one compartment to another-

In gated transport, the protein traffic between the cytosol and nucleus occurs through the nuclear pore complexes acting as selective gates- topologically similar

In transmembrane transport, membrane-bound protein translocators directly transport specific proteins across a membrane from the cytosol into a space that is topologically distinct

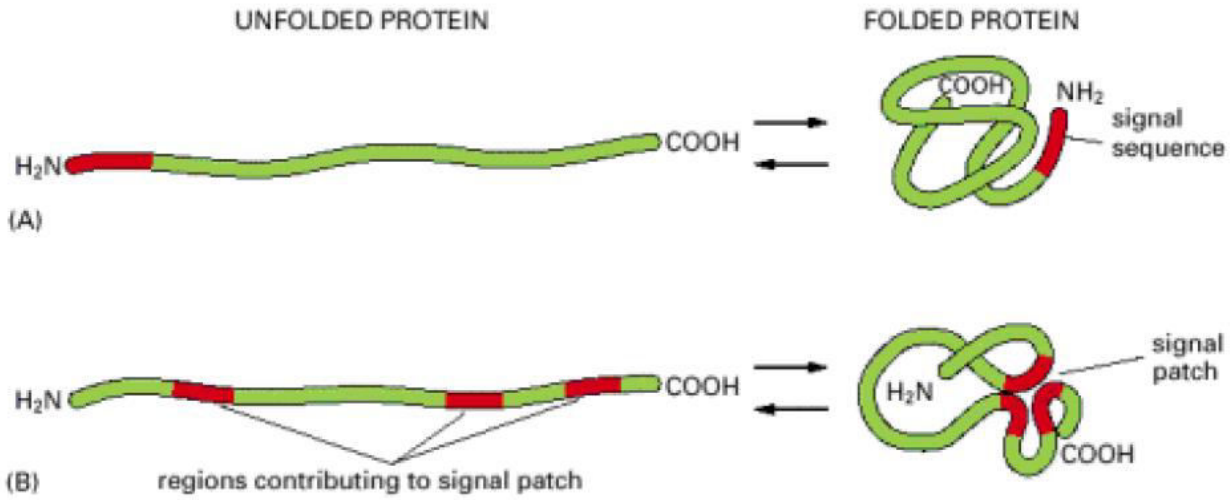
In vesicular transport, membrane-enclosed transport intermediates—which may be small, spherical transport vesicles or larger, irregularly shaped organelle fragments—ferry proteins from one compartment to another which are topologically similar



Signal sequences direct proteins to the correct cellular address

There are at least two types of sorting signals in proteins

- One type resides in a continuous stretch of amino acid sequence, typically 15–60 residues long – signal peptide
- Other type consists of a specific three-dimensional arrangement of atoms on the protein's surface that forms when the protein folds up – signal patch



Signal sequences are used to direct proteins from the cytosol into the ER, mitochondria, chloroplasts, and peroxisomes, and they are also used to transport proteins from the cytosol to the nucleus (vice versa) and from the Golgi apparatus to the ER

Signal Sequence

FUNCTION OF SIGNAL SEQUENCE	EXAMPLE OF SIGNAL SEQUENCE
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Leu-Ala-Leu-Lys-Leu-Ala-Gly-Leu-Asp-Ile-
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into plastid	$^{+}H_3N$-Met-Val-Ala-Met-Ala-Met-Ala-Ser-Leu-Gln-Ser-Ser-Met-Ser-Ser-Leu-Ser-Leu-Ser-Ser-Asn-Ser-Phe-Leu-Gly-Gln-Pro-Leu-Ser-Pro-Ile-Thr-Leu-Ser-Pro-Phe-Leu-Gln-Gly-
Import into peroxisomes	-Ser-Lys-Leu-COO^{-}
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Return to ER	-Lys-Asp-Glu-Leu-COO^{-}

The importance of each of these signal sequences for protein targeting has been shown by experiments in which the peptide is transferred from one protein to another by genetic engineering techniques. Placing the N-terminal ER signal sequence at the beginning of a cytosolic protein, for example, redirects the protein to the ER

Signal peptidase removes the signal sequence after localization

Most membrane enclosed organelles cannot be constructed from scratch: They enclosed Information in the organelle Itself

When a cell reproduces by division, it has to duplicate its membrane-enclosed organelles

Each daughter cell inherits from its mother a complete set of specialized cell membranes

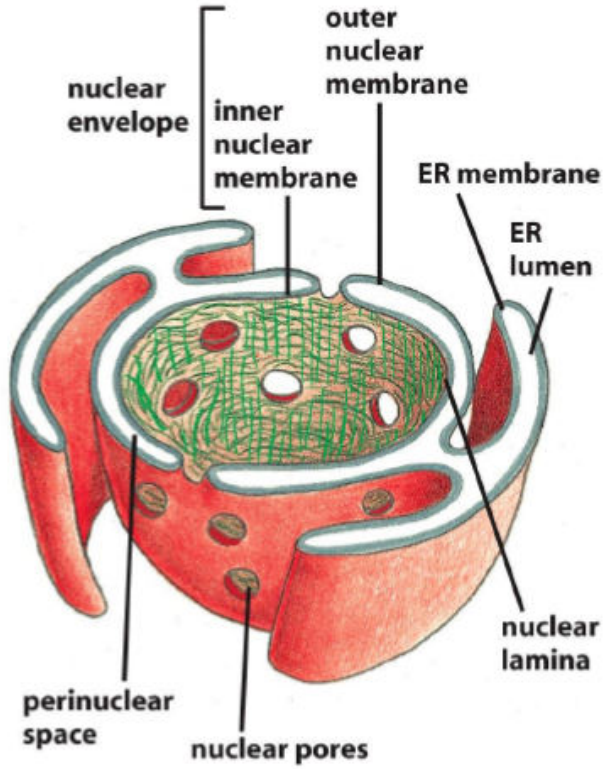
This inheritance is essential because a cell could not make such membranes from scratch

A new ER could not be made without an existing ER. The same is true for mitochondria, plastids, and peroxisomes

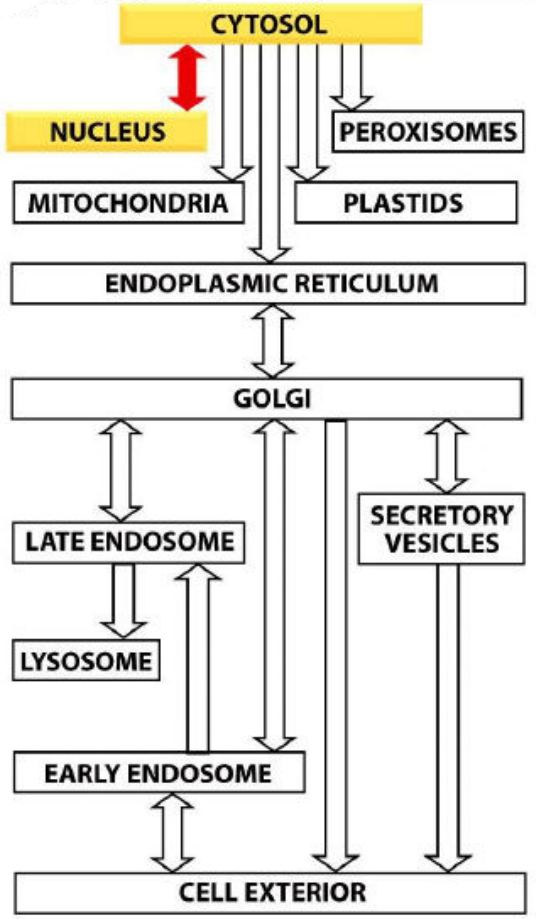
Thus, it seems that the information required to construct a membrane-enclosed organelle does not reside exclusively in the DNA that specifies the organelle's proteins. Epigenetic information is also required, and this information is passed from parent cell to progeny cell in the form of the organelle itself

The Transport of Molecules between the Nucleus and Cytosol

The nuclear envelope encloses the DNA and defines the nuclear compartment



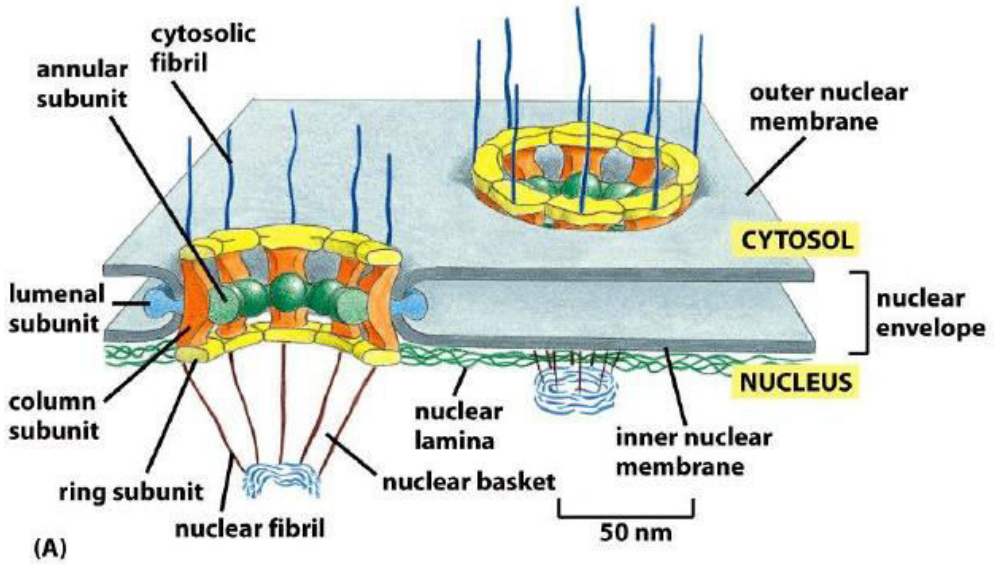
The double-membrane envelope is penetrated by nuclear pore complexes and is continuous with the endoplasmic reticulum.

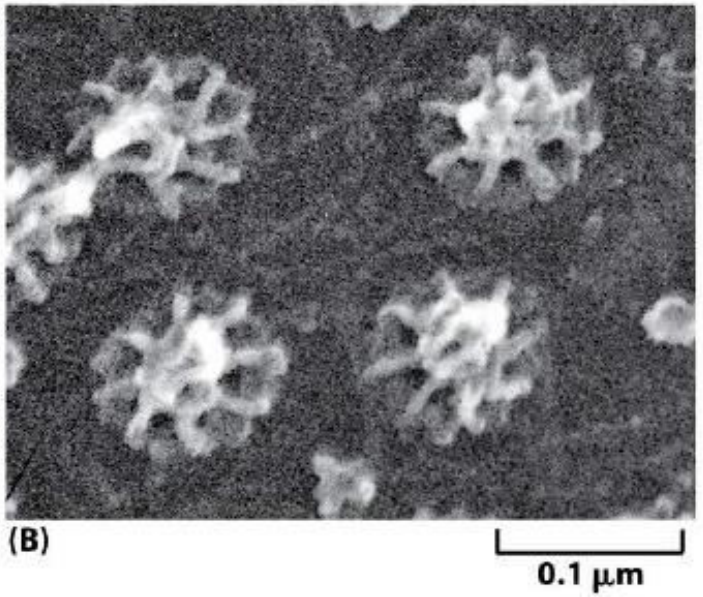


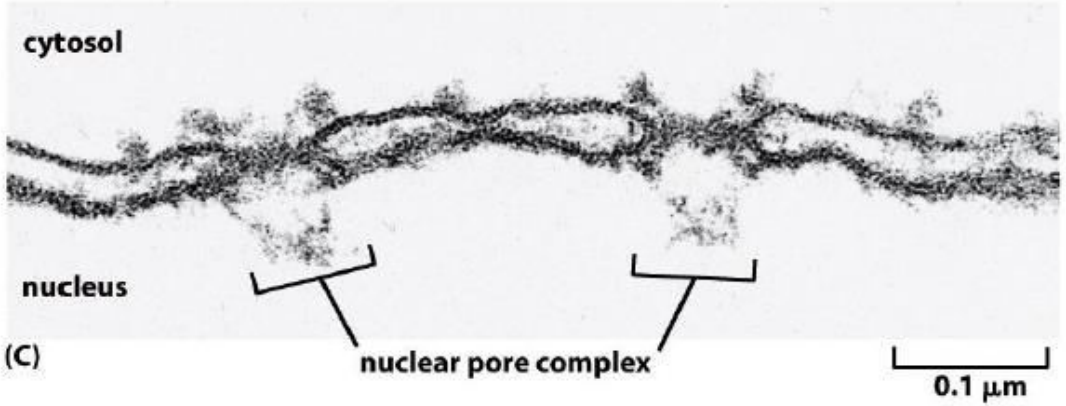
Bidirectional traffic occurs continuously between the cytosol and the nucleus. -- histones, DNA and RNA polymerases, gene regulatory proteins, and RNA-processing proteins—are selectively imported into the nuclear compartment from the cytosol

Nuclear pore complex

Nuclear pore complexe has an estimated molecular mass of about 125 million and is thought to be composed of more than 30 different proteins, called nucleoporins, that are arranged with a striking octagonal symmetry

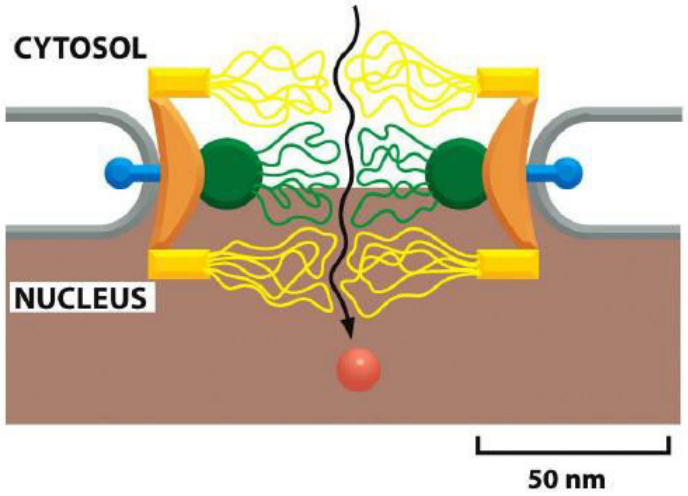






The nuclear envelope of a typical mammalian cell contains 3000 – 4000 pore complexes

Free diffusion through Nuclear pore complex



size of molecules
that enter nucleus
by free diffusion

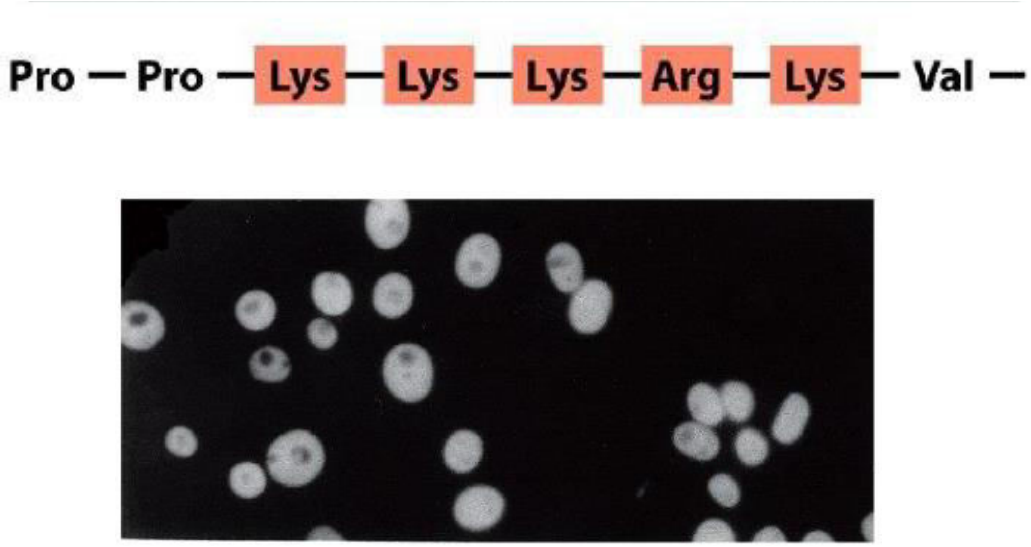
size of macromolecules
that enter nucleus
by active transport

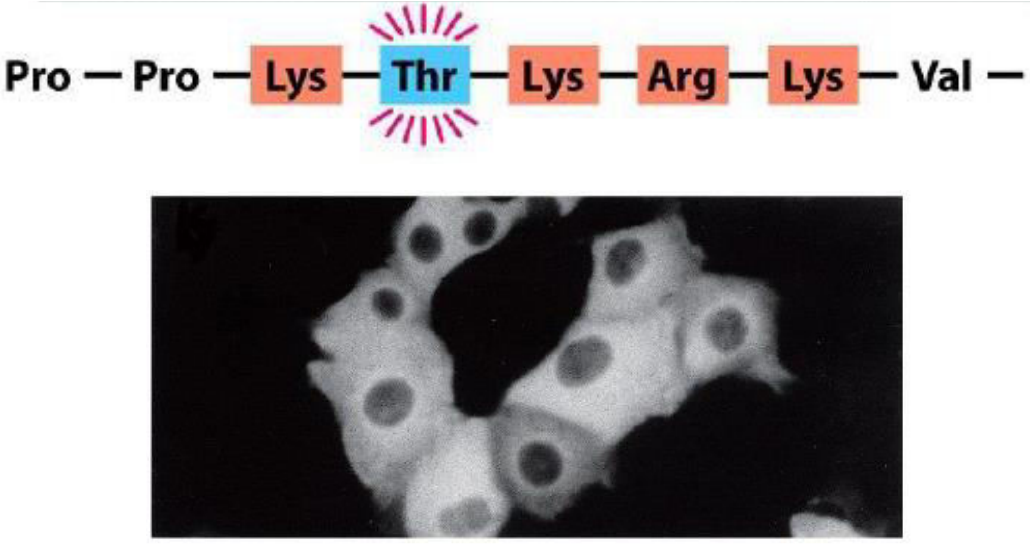
The nuclear pore complex contains a pathway for free diffusion equivalent to a water-filled cylindrical channel about 9 nm in diameter and 15 nm long

Small molecules (5000 daltons or less) diffuse in so fast that the nuclear envelope can be considered to be freely permeable to them. A protein of 17,000 daltons takes 2 minutes to equilibrate between the cytosol and the nucleus, whereas proteins larger than 60,000 daltons are hardly able to enter the nucleus at all

Nuclear localization signal direct nuclear protein to the nucleus

The selectivity of this nuclear import process resides in nuclear localization signals (NLSs), which are present only in nuclear proteins



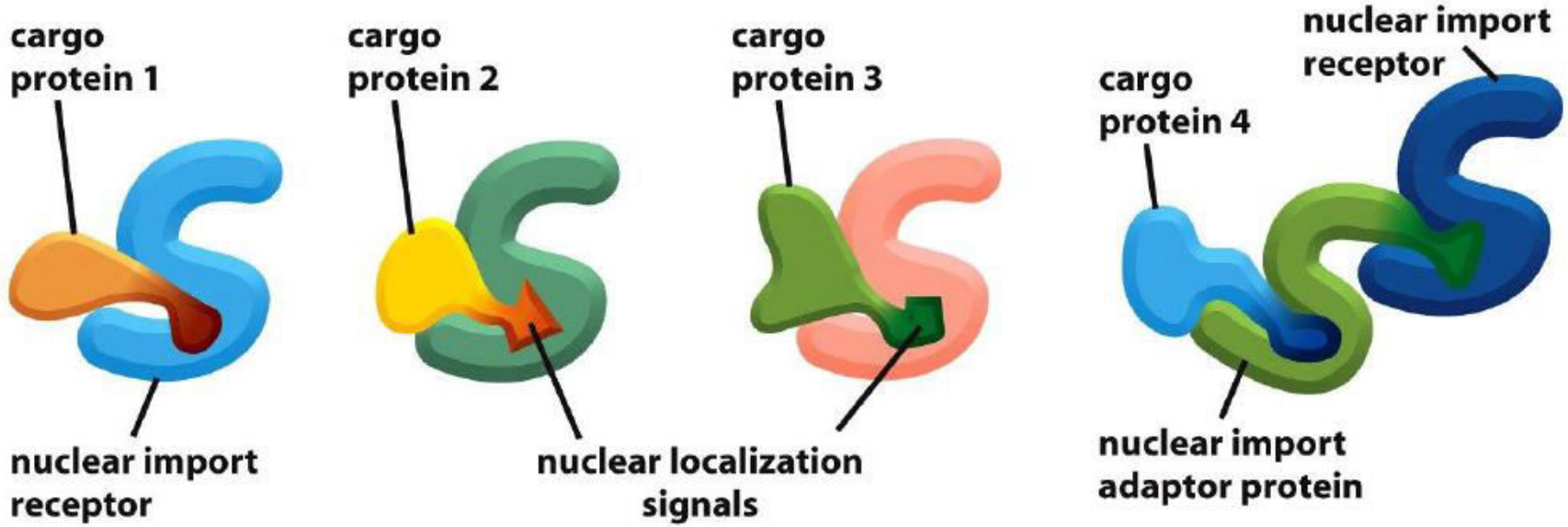


In many nuclear proteins they consist of one or two short sequences that are rich in the positively charged amino acids lysine and arginine, the precise sequence varying for different nuclear proteins

The signals characterized this far can be located almost anywhere in the amino acid sequence and are thought to form loops or patches on the protein surface

Nuclear import receptor

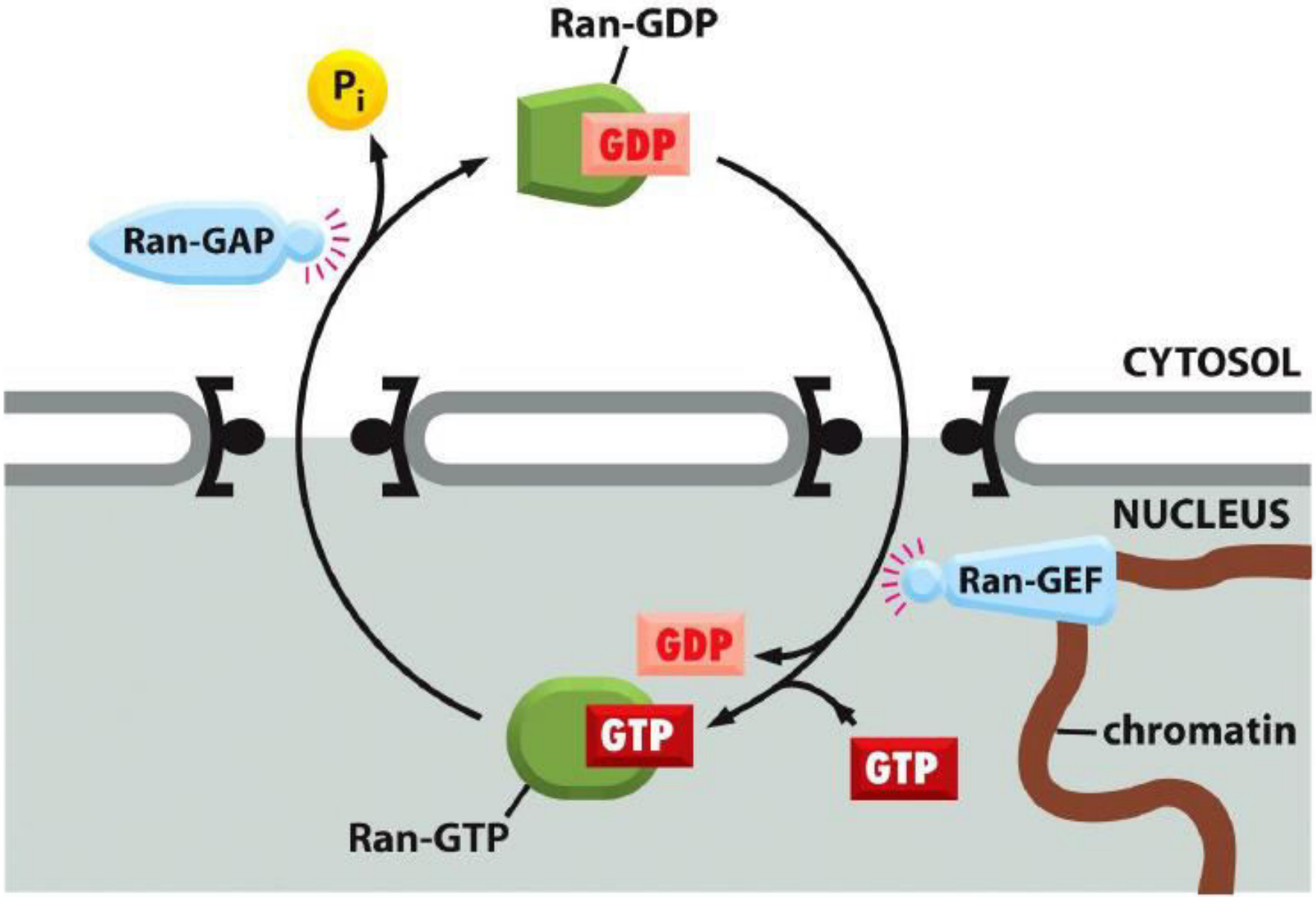
To initiate nuclear import, most nuclear localization signals must be recognized by nuclear import receptors, which are encoded by a family of related genes – specific receptor for specific cargo – repeated binding and dissociation of receptor + cargo with FG repeats of NPC



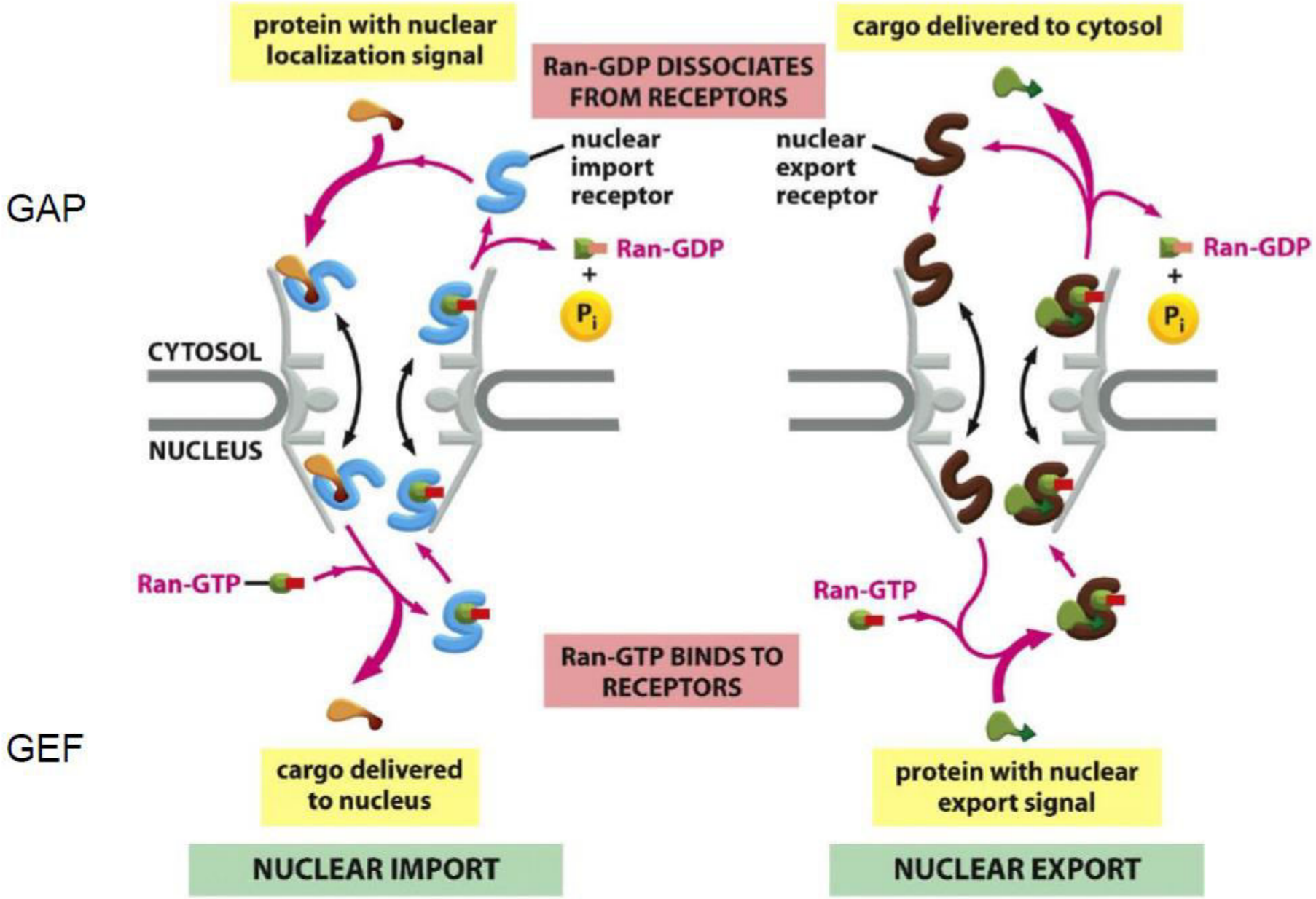
Nuclear export works like nuclear import, but in reverse

The transport system relies on nuclear export signals on the macromolecules to be exported, as well as on complementary nuclear export receptors

The Ran GTPase drives directional transport through nuclear pore complex



A model for how GTP hydrolysis by Ran provides directionality nuclear transport



Ran-GTP receptor-cargo interaction