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*M.Sc. Zoology (Semester IV)*
*Elective Paper: Cell and Molecular biology*

*Unit: 3.4*

# Mechanism of nuclear cytoplasmic exchange

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## Nuclear cytoplasmic exchange

Transportation between the cytoplasm and the nucleoplasm is critical for survival of the cells. It is a tightly regulated process .

Several million macromolecules selectively pass between nucleus and cytoplasm every minute.

The nuclear envelope is a physical barrier which regulates the traffic between nucleoplasm and cytoplasm.

It is a phospholipid bilayer membrane which consists of two layers; inner and outer membrane .

Inner and outer membranes are separated by the perinuclear space. The cytoplasm is connected to the nucleoplasm via nuclear pores.

Although small size of molecules (less than 30 kDa) freely move through the nuclear pore, bigger molecules need the help of special carrier proteins.

## Nuclear Transport Cycle

Transportation of macromolecules including protein or RNAs between nucleoplasm and cytoplasm occurs through nuclear pore complex (NPC) in the nuclear envelope.

NPC is highly selective and bidirectional transporter for various cargo molecules. There are four important factors for the nuclear transport:

1. *NPCs and Nucleoporins (NUPs),*

2. *Nuclear localization signals (NLSs) or nuclear export signals (NESs)* in cargo molecules.

3. *Ran proteins* that allows for active transport and directionality,

4. *Karyopherins (importin/exportin/transportin)* that can recognize cargo molecules,

## 1. Nuclear pore complexes and Nucleoporins

NPC is a complex basket-like structure with huge molecular mass of 120 MDa in humans.

It made up of NUPs with 100–150 nm in diameter and 50–70 nm in thickness according to the species.

Each NPC has a central hole (~30 nm in diameter and ~50 nm in long) that connects between the nucleoplasm and the cytoplasm.

Main structures of NPC include the inner pore ring, the nuclear and cytoplasmic rings, the nuclear basket, and the cytoplasmic basket.

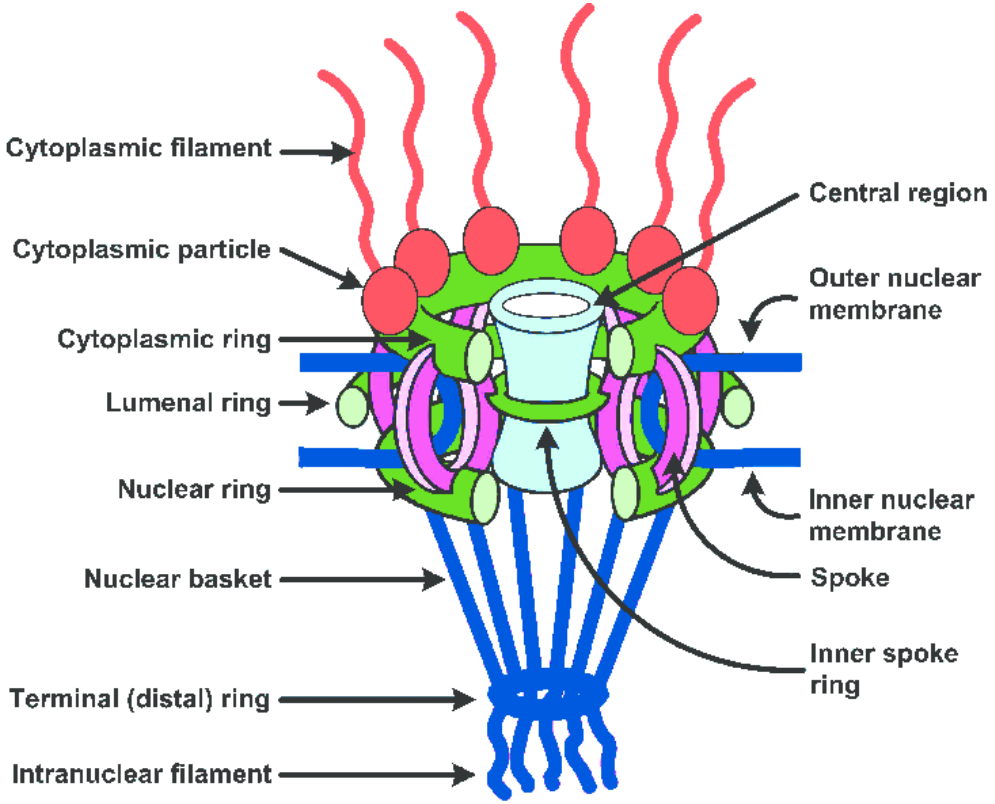



Figure 1: The NPC structure

## Nucleoporins

NPC is composed of multiple copies of 30–50 different NUPs and 500–1,000 NUPs are integrated into the NPC structure.

NUPs serve as an architectural scaffold and a permeability regulator.

Some NUPs have intrinsically disordered domains rich in repeating amino acid sequences such as FXFG (Phe-XPhe-Gly), Phe-Gly (FG), or GLFG (Gly-Leu-Phe-Gly), which act as docking sites for karyopherins (importin or exportin).

These repeating sequences line the central hole and regulate the passage of cargo molecules.

Such as NUP62 complex which consists of NUP62, NUP58, and NUP54 has been shown to include FG-repeats.

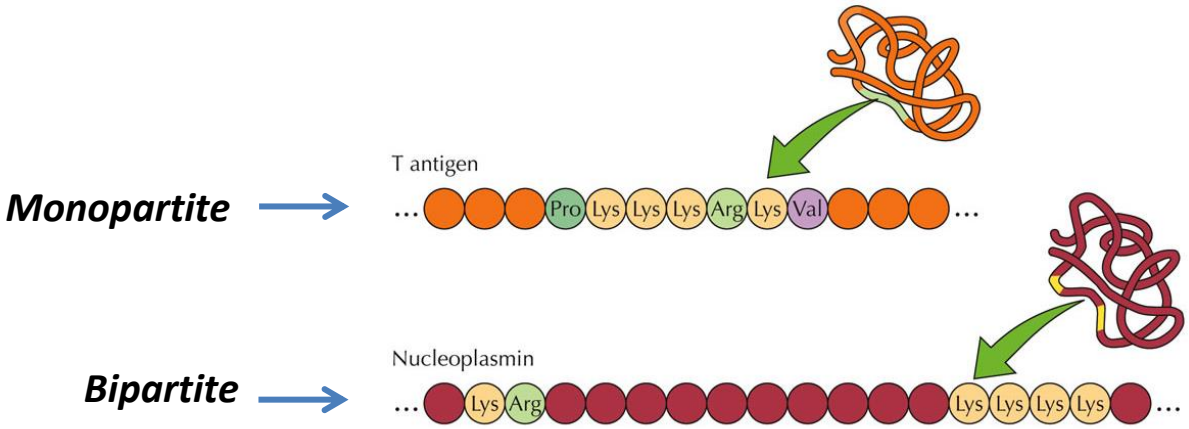
## 2. Nuclear localization signals (NLSs) or nuclear export signals (NESs)

### Nuclear localization signals (NLSs)

The proteins required for nuclear functions such as DNA polymerases, RNA polymerases, transcription factors and many others are targeted to the nucleus by specific amino acid sequences called NLS. The NLS is recognized by **nuclear transport receptors** that direct the transport of the proteins through the NPCs.

The first NLS was characterised by Alan Smith and colleagues in 1984 from simian virus 40 (SV40) T- antigen as a classical monopartite sequence “PKKKRKV”.

Classical NLSs contain monopartite or bipartite signals.




Bipartite NLSs have two clusters of basic amino acids sequence. The prototypical bipartite NLS is nucleoplasminfound in Xenopus laevis (**KR**PAATKKAGQA**KKKK**).

## Nuclear export signals (NES)

Some proteins remain within the nucleus following their import from the cytoplasm, but many others shuttle back and forth between the nucleus and he cytoplasm.

Proteins are targeted for export from the nucleus by specific amino acid sequences called nuclear export signals (NES).

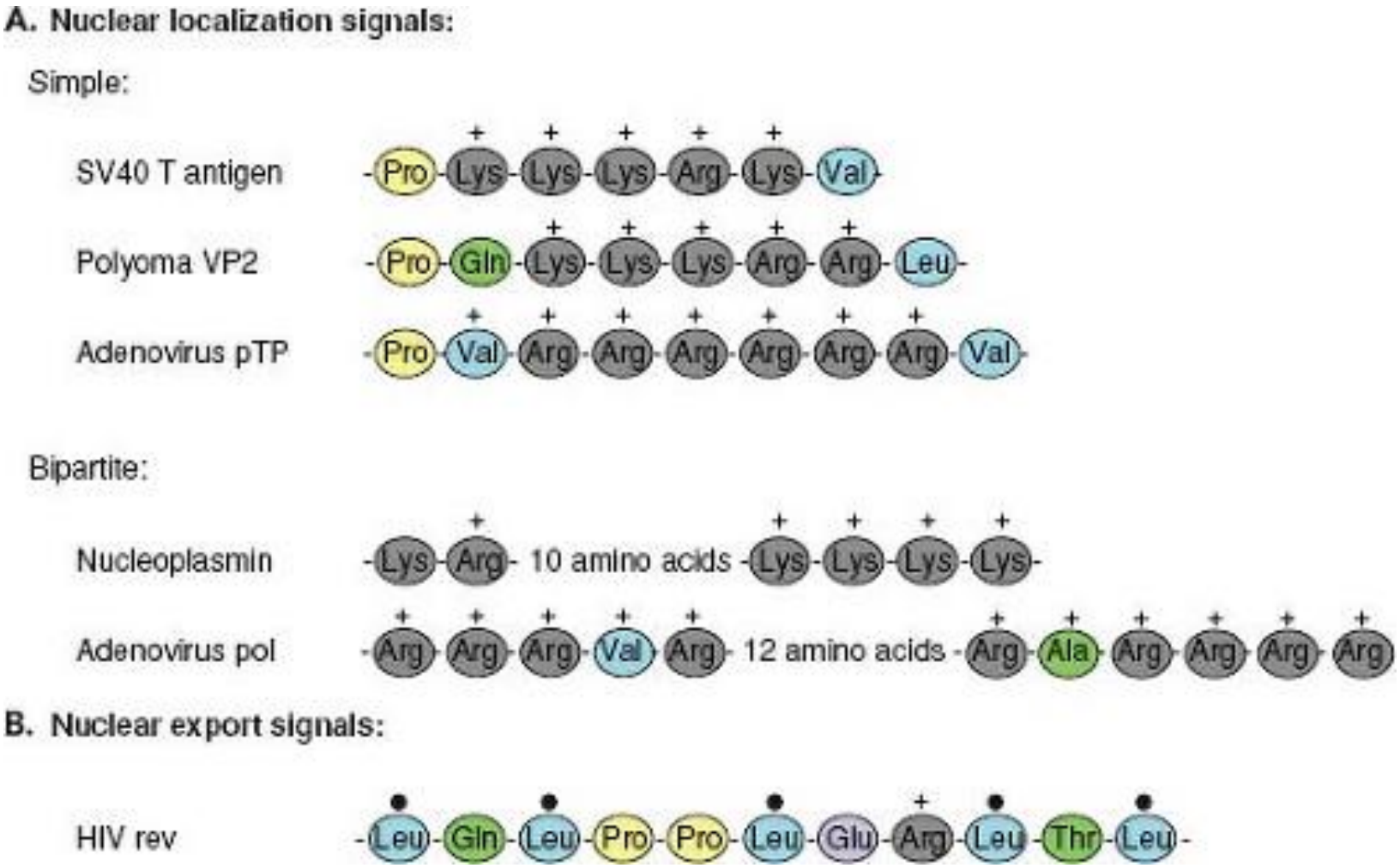
The NES contains hydrophobic amino acids.
The consensus sequence for NESs is Φ1-X(2-3)-Φ2-X(2- 3)-Φ3-X-Φ4 motif (Φ: represents hydrophobic residues L, I, F, M, or V and X: any amino acid).

NES is recognised by receptors within the nucleus called exportin, which directs protein transport through the NPC to the cytoplasm.

Different exportins have their specific cargo molecules. For example, CAS (exportin-2) transports importin-α.

CRM1 (exportin-1) is a ubiquitous nuclear export receptor containing hydrophobic residues.

# Examples of NLS and NES



## 3. Karyopherin: importin and exportin

Karyopherins, also known as *importins* or *exportins*, are a superfamily of **nuclear transport receptors** that facilitate the translocation of proteins, RNAs, and ribonuclear particles across the NPC in a Ran GTP hydrolase-dependent process.

More than 20 karyoperins in human have been reported. Among them, 11 karyopherins (importin-β, importin-β2, importin-4, importin-5, importin-7, importin-8, importin-9, importin-11, transportin-SR, importin-13, exportin-4) are involved in import or bidirectional transport of cargo molecules.

Eight exportins (exportin-1–exportin-7, exportin-t) are involved in the export of cargo molecules.

Each karyopherin has its own specific cargo molecules. For example, exportin-2 contributes to the export of importin-α and exportin-t to the export of t-RNA.

Karyopherins can also export nucleotide motif including tRNA, miRNA, rRNA, viral RNA, and uridine-rich small nuclear RNAs. However, structure of mRNAs is highly diverse unlike other RNAs, so that they can be transported by processing and assembly into ribonucleoproteins.

## 4. Ran Proteins

The activity of karyopherins are regulated by interaction of a protein called Ran. Ran gives the nuclear transport directionality thro ugh the nuclear pore.

Ran is a GTP binding protein whose confirmation and activity is regulated by GTP binding and hydrolysis.

RanGTP is about 100-fold more abundant in the nucleoplasm than in the cytoplasm, which is possible due to the fact that Ran's guanine-exchange factor (Ran GEF) is located in the nucleoplasm

Similarly, the Ran GTPase activating protein (Ran GAP) is located in the cytoplasm.

The activity of Ran GEF and Ran GAP ensures the unequal distribution of Ran/GTP across the nuclear envelope, with high concentration of Ran/GTP in the nucleus which provides directionality of transport across nuclear envelope.

## Distribution of Ran/GTP across the nuclear envelope

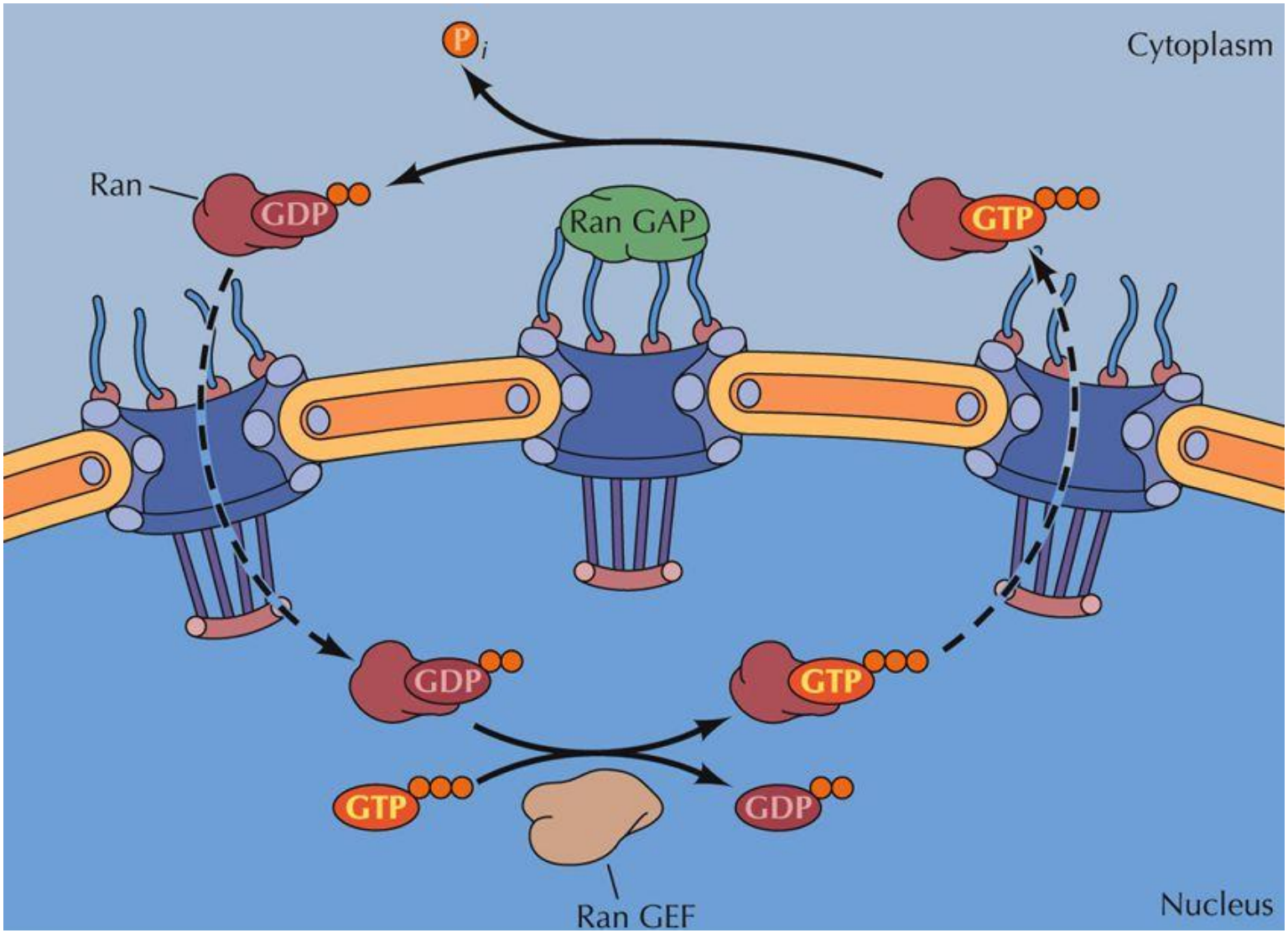



Figure 2: The activity of Ran GEF and Ran GAP causes the unequal distribution of Ran/GTP across the nuclear envelope. Ran/GTP is high in the nucleus.

## Import cycle

First step of the nuclear transport cycle is the formation of importin-cargo complex.

Importins can bind cargo molecules after recognition of their NLSs. Depending on types of NLSs, different importins are involved.

After the formation of importin(s)-cargo complex, importin is specifically recruited to NPC in the nuclear pore and then the complex can pass through the nuclear pore.

In the nucleoplasm, binding of RanGTP to importin-cargo complex facilitates dissociation of cargo, and then RanGTP bound importin is exported to the cytoplasm.

Recycling of importin-Ran/GTP complex is mediated by a nuclear export receptors.

In the cytoplasm GTP is hydrolysed to GDP. This releases the importin so that it can bind to a new cargo protein in the cytoplasm and participate in the another round of transport

## Import cycle

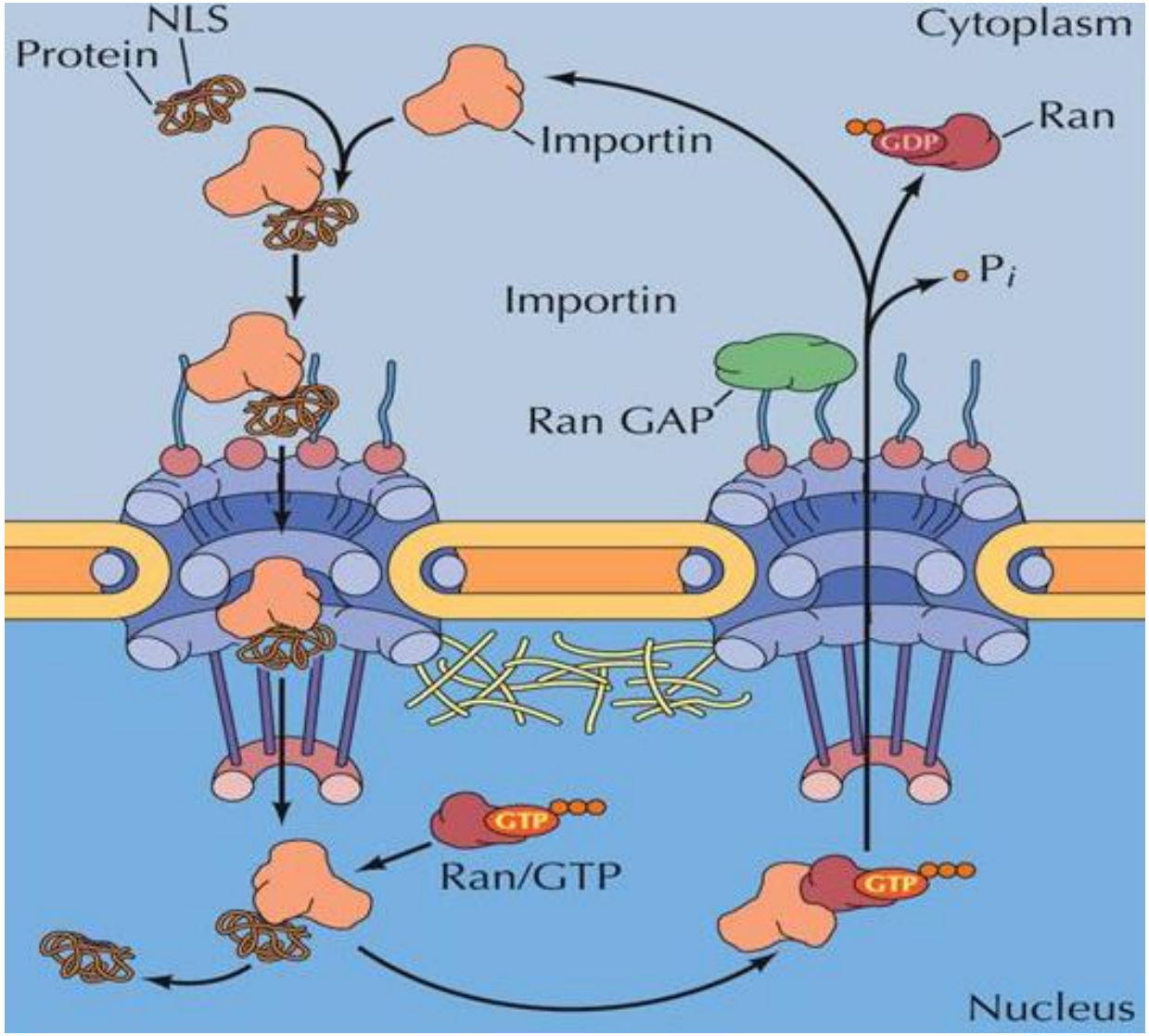



Figure 3: Protein import through nuclear pore complex

## Export cycle

Cargos containing NES are recognized by exportins. The NES-bound exportin forms a complex with RanGTP.

RanGTP is 100-fold more abundant in the nucleoplasm than in the cytoplasm.

Binding of RanGTP to exportins induces the high affinity of exportin for its cargo molecule.

The exportin-cargo-RanGTP complex specifically binds the docking site of NPCs and then passes through nuclear pore.

RanGAP in the cytoplasm hydrolyzes RanGTP in the complex, which leads to the dissociation of the complex .

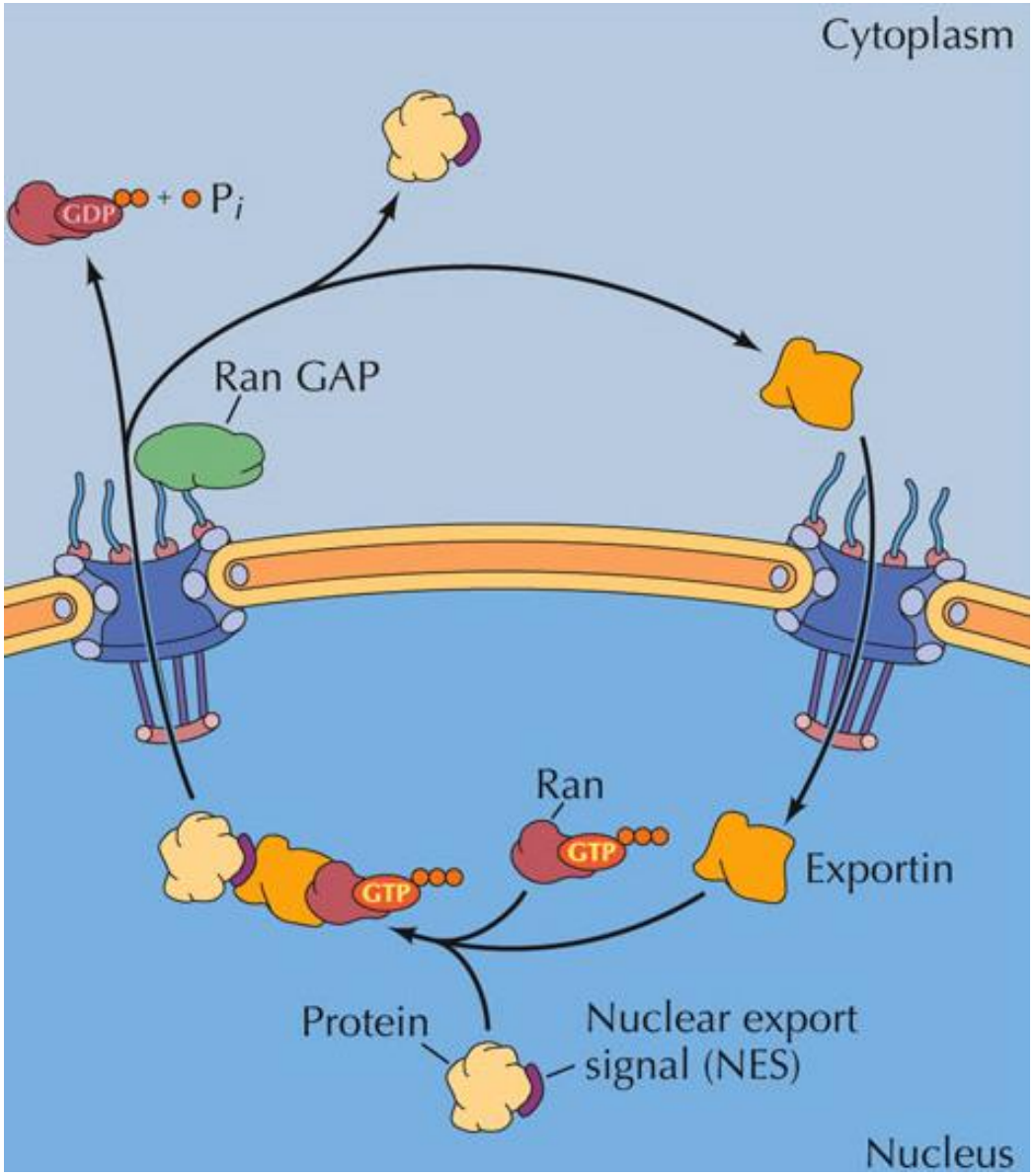



Figure 4: Nuclear Export

# Regulation of Nuclear Protein Import

The transport of proteins to the nucleus is highly regulated event. Such as transcription factors (TFs) import and export into the nucleus is a well established mechanism to control these protein activities.

In one such mechanisms, TFs associates with cytoplasmic protein that mask their NLS and therefore, they remain in cytoplasm. For example, under unstimulated condition NF-κB binds with IκB (inhibitory protein) in the cytoplasm. IκB mask NF-κB NLS and it remains in cytoplasm. In stimulated condition, IκB is phosphorylated and degraded, allowing NF-κB to enter nucleus and activate transcription of target genes

Another example is of transcription factor Pho4 whose NLS is masked by phosphorylation. When this TF is required inside the nucleus , the dephosphorylation happens and it is imported into the nucleus

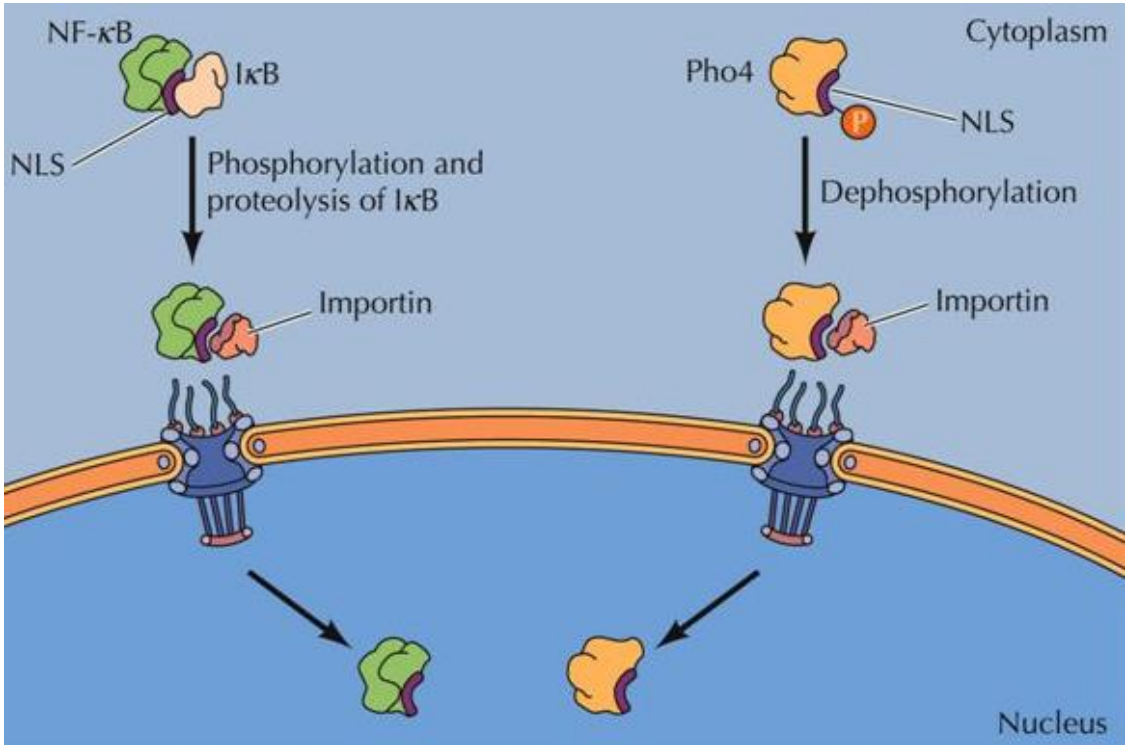



Figure 5: Regulation of nuclear import of transcription factor

## Transport of RNAs

All RNAs are transported across the nuclear envelope as ribonucleoprotein complexes (RNPs). The importins and exportins transports tRNAs, rRNAs, miRNAs and snRNAs in a Ran/GTP dependent manner.

rRNAs associates with ribosomal proteins and other factors in nucleolus and exported to cytosol by Crm1.

tRNA and miRNA precursors are exported by exportin-t and exportin-5, respectively.

mRNA are exported by a distinct mechanism that does not involve karyopherins and is independent of Ran.

Pre mRNAs are associated exporter complex in the nucleus in concert with the completion of splicing and polyadenylation. Exporter complex directs mRNAs through NPCs.

Directionality of the process is established by a RNA helicase localised to the cytoplasmic face of the NPC. The mRNA /exporter complex is remodeled by the 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.

# Transport of RNAs

Many small RNAs such as snRNAs and snoRNAs functions within the nucleus as a component of RNA processing machinery.

snRNAs are initially transported to cytoplasm, where they associate with proteins to form snRNPs and then returns to the nucleus.

Crm1 and other transport Receptor protein that bind 5’ 7-methylguanosine caps of snRNAs are involved in the export of snRNAs to the cytoplasm.

In contrast, the sequences present on the snRNP proteins are responsible for the transport of snRNPs from the cytoplasm to the nucleus

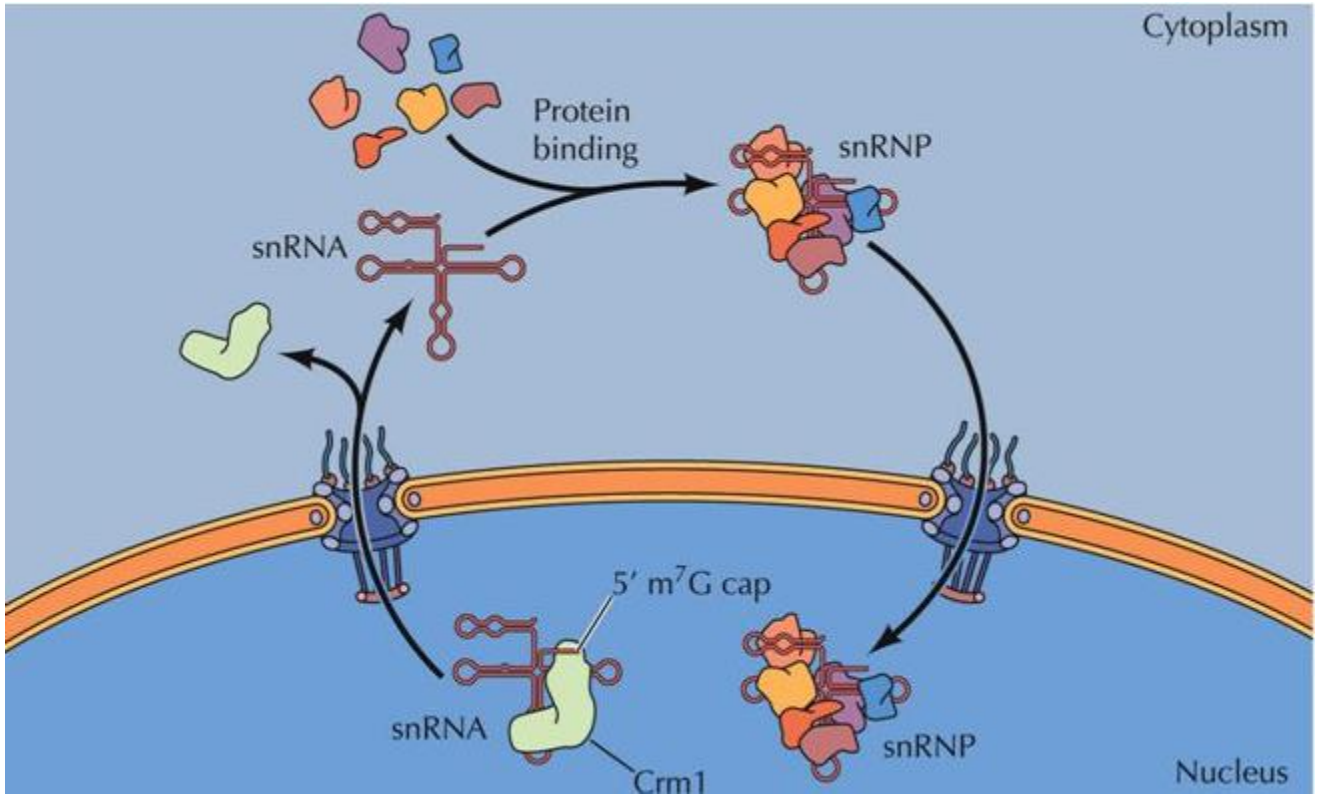



Figure 6: Transport of snRNAs between nucleus and cytoplasm

## References

***Genes to Cells***, 01 Oct 2000, 5(10):777-787
***International Journal of Molecular Sciences,*** 2018, *19*(5), 1445
***The Cell,*** A molecular Approach, Sixth Edition

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