Transport of macromolecules into and out of the cell nucleus

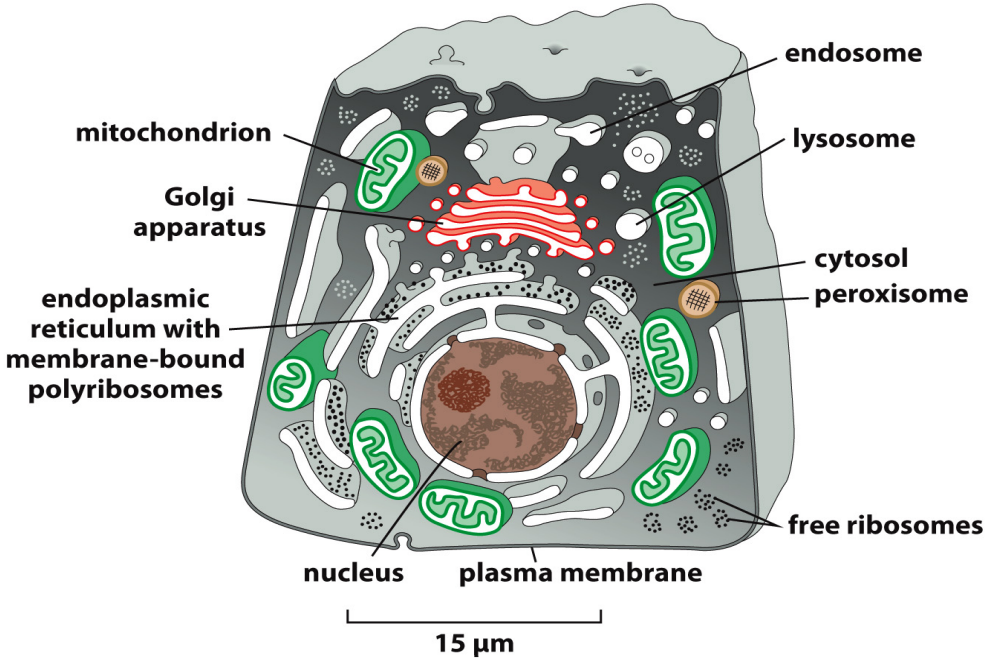
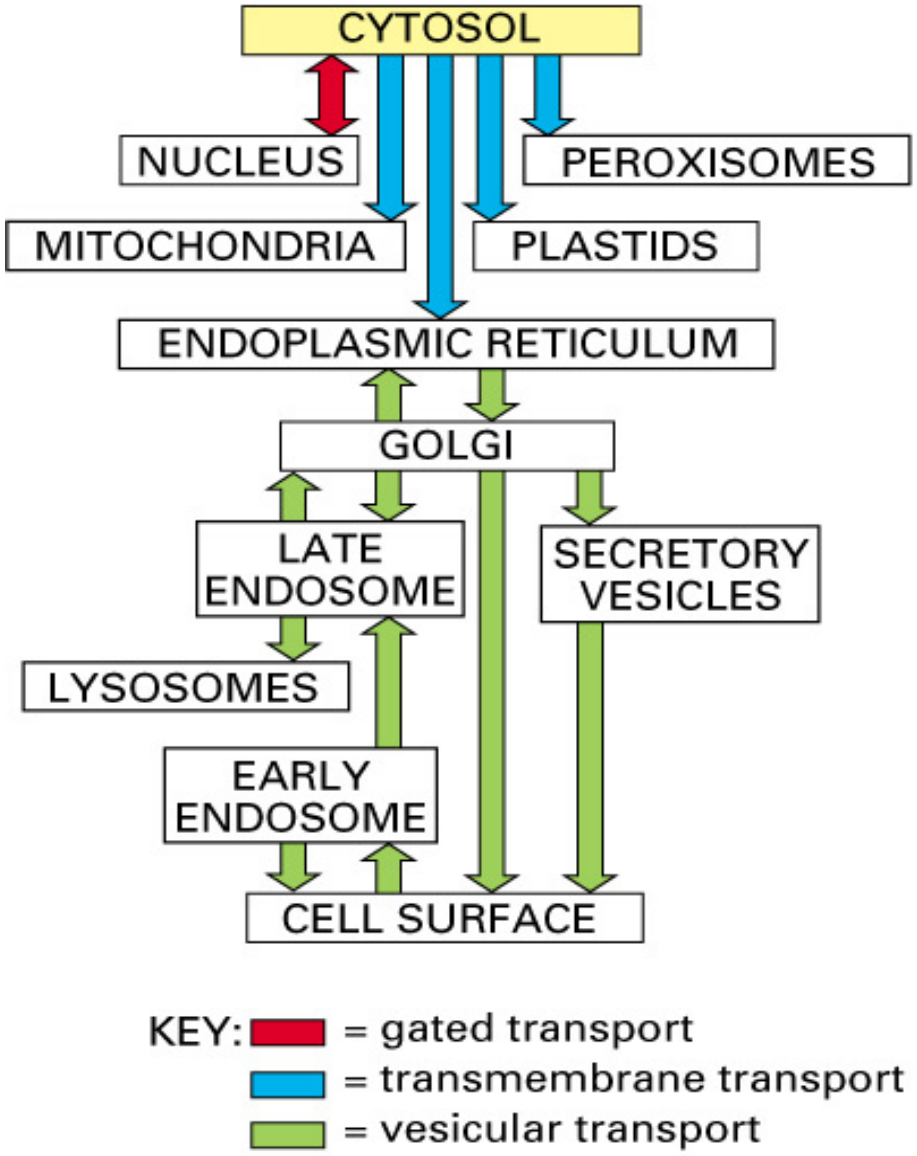
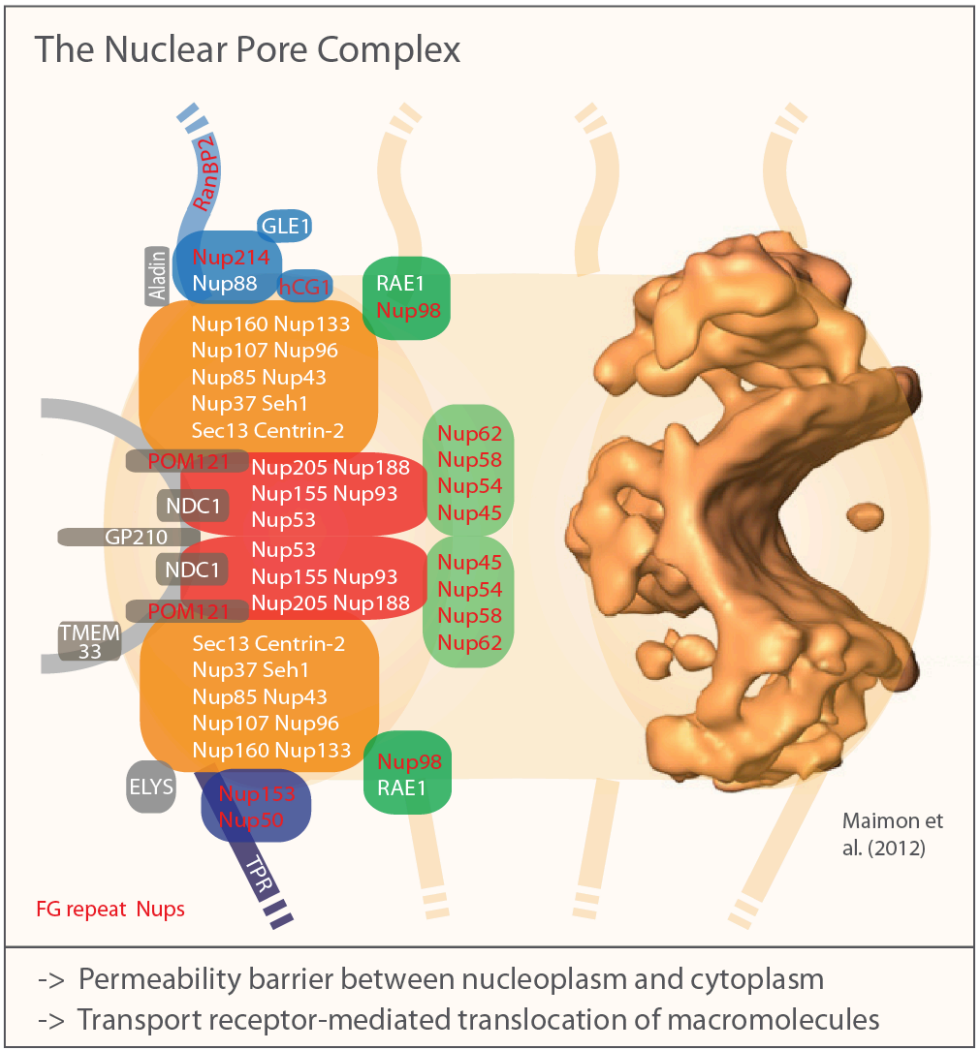


Figure 12-1 Molecular Biology of the Cell 6e (© Garland Science 2015)



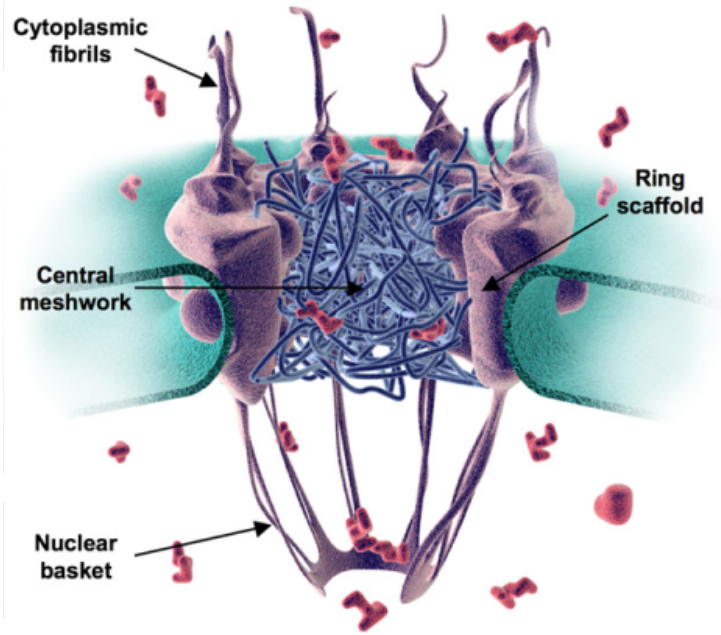
NPC structure

Rothballer and Kutay (2012) Cell SnapShot



-> Permeability barrier between nucleoplasm and cytoplasm
-> Transport receptor-mediated translocation of macromolecules

Patel *et al.* (2007) Cell



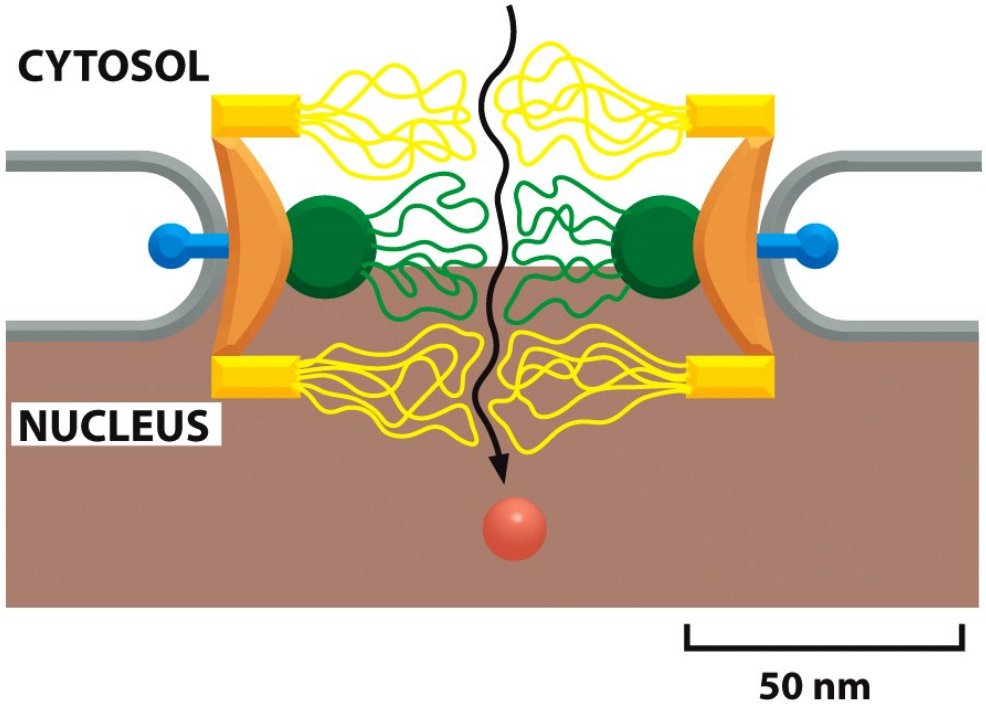
- octagonal symmetry, 100 MDa
- composed of about 30 different nucleoporins (nups)

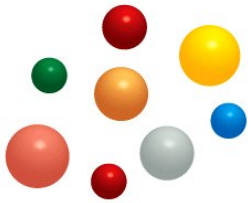
FG repeat domain nups:

1. binding sites for nuclear transport receptors
2. define permeability characteristics of NPC

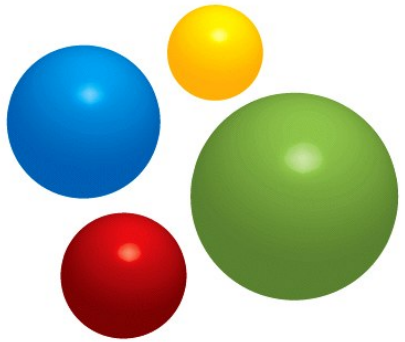
Diffusion limit of NPCs

diffusion limit: ≈ 40 kDa, 9 nm





size of molecules
that enter nucleus
by free diffusion



size of macromolecules
that enter nucleus
by active transport

Transport Substrates

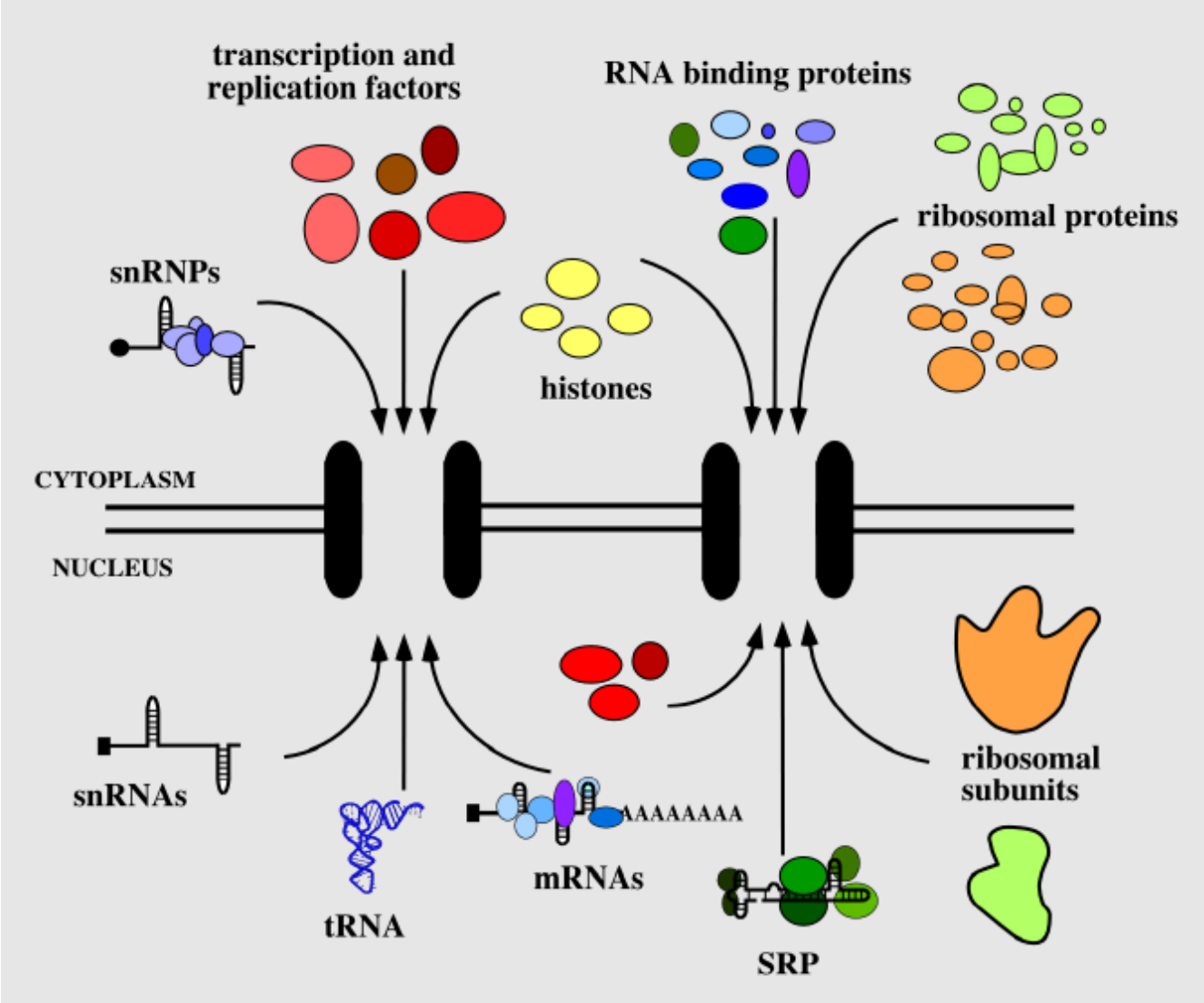
Import of proteins
- DNA replication, e.g. histones
- transcription, mRNA-processing
- ribosomal proteins

Import of snRNPs
- pre mRNA-splicing

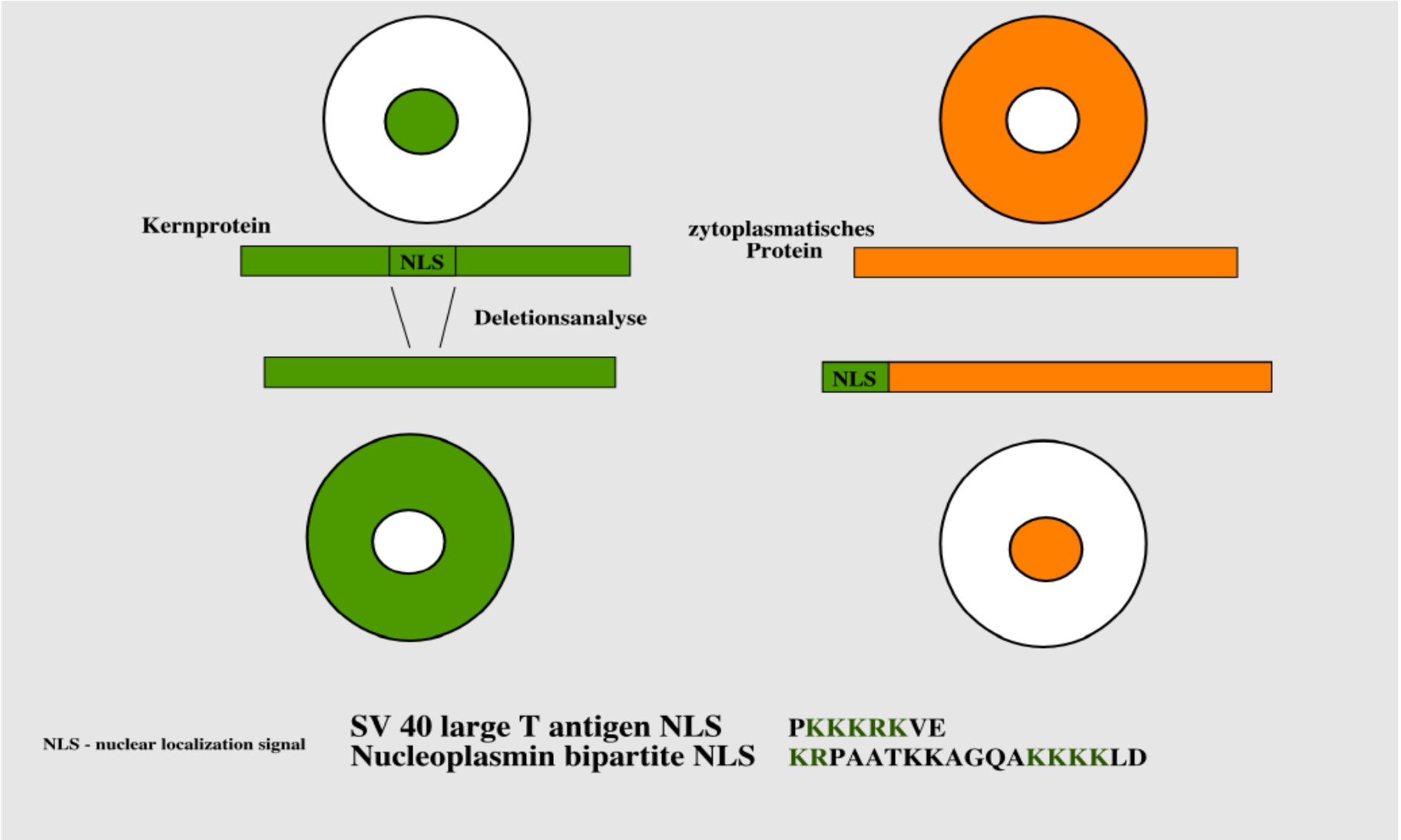
Export of RNA
- tRNAs
- microRNAs

Export of RNPs
- snRNAs
- mRNAs
- ribosomal subunits

Export of proteins
- e.g. many regulatory proteins



Identification of nuclear localization signals (NLSs)



Identification of nuclear localization signals

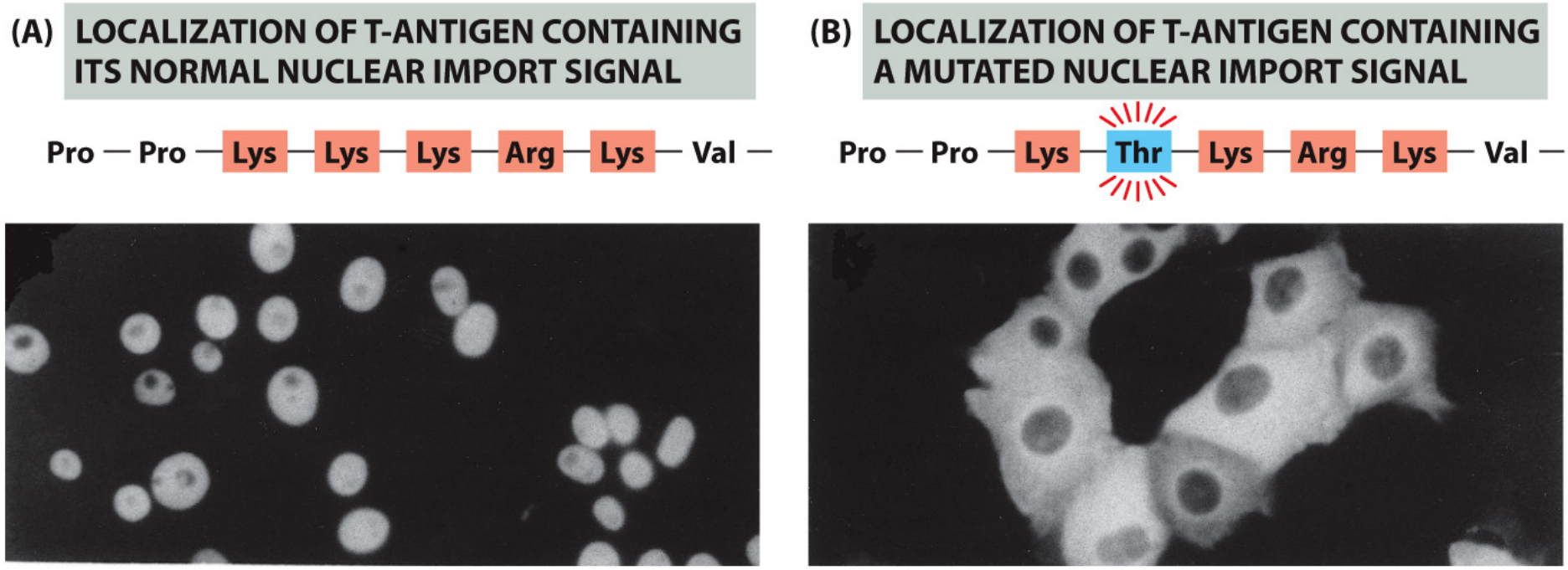


Figure 12-9 Molecular Biology of the Cell 6e (© Garland Science 2015)

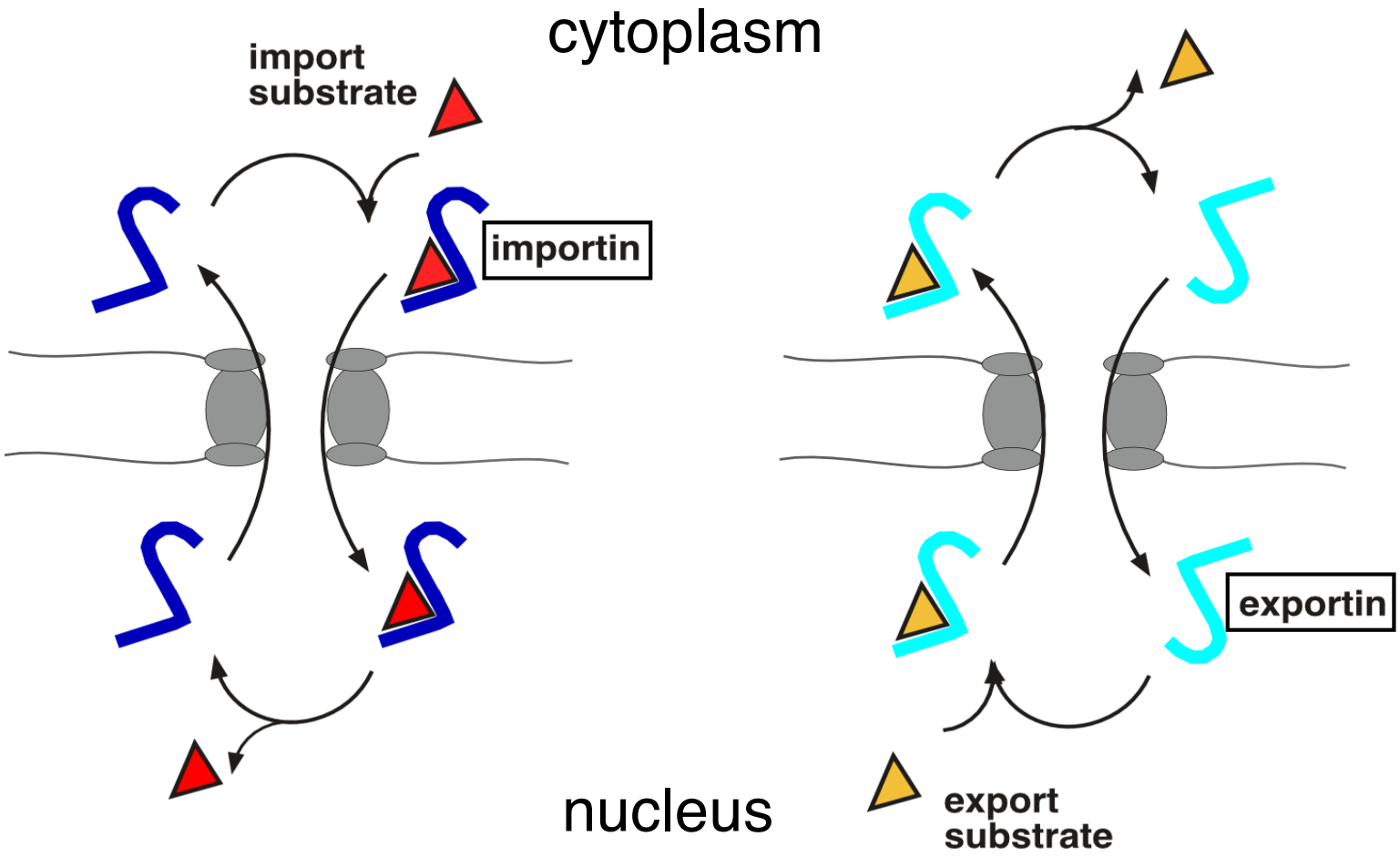
There are different types of import and export signals

NLS
Nulcear
Localization
Signal

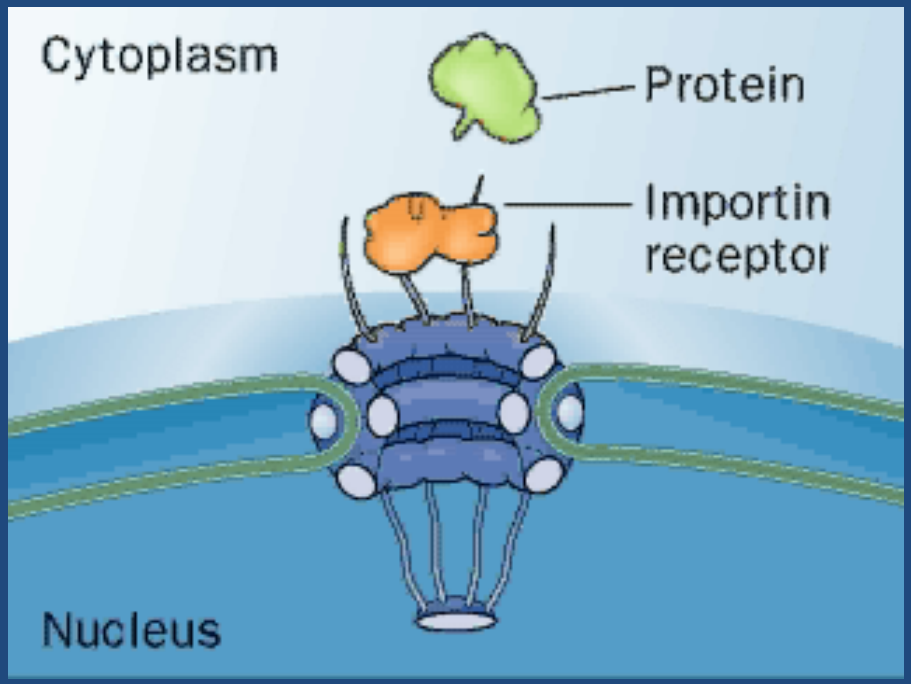
NES
Nuclear
Export
Signal

Transport signal	Example	Features
Classical monopartite NLS	**SV40 T antigen**	**PKKKRKVE**
Classical bipartite NLS	nucleoplasmin	KRPAATKKAGQAKKKKL
M9 domain (PY-NLS)	hnRNPA1	…NQSSNFGPMKGGNFGGRSSGPY
BIB domain	rpL23a	HKKKKIRTSPTFRRPKTLRLRRQPKYP RKSAPRRNKL
RS domain	SR proteins	Phosphorylated RS domains
Nuclear Export Signal	**HIV Rev**, PKI	**L-X_{2-3}-(L,I,M,F,M)-X_{2-3}-L-X-(L,I,V)** Φ^1-$(x)_{2-3}$-Φ^2-$(x)_{2-3}$-Φ^3-x-Φ^4

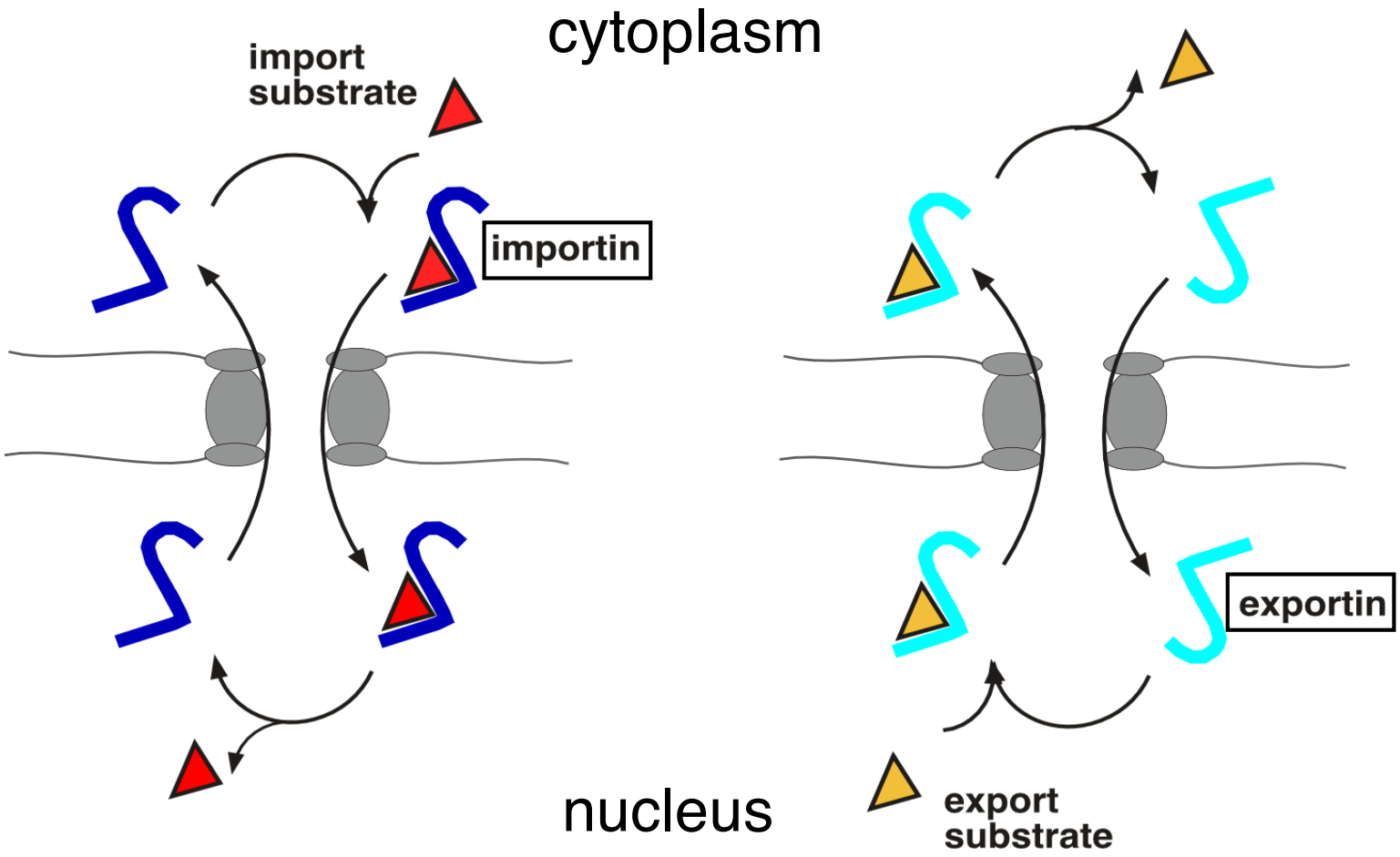
Shuttling nuclear transport receptors – Importins and Exportins



- Nuclear transport receptors recognize import or export signals
- Allow for translocation of cargo through the NPC

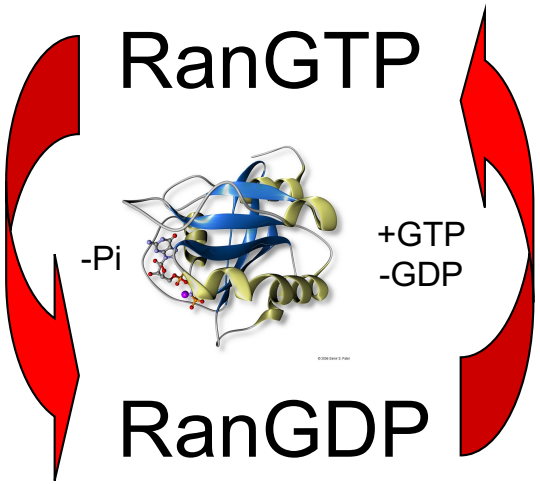

Cytoplasm
Protein
Importin
receptor
Nucleus

Shuttling nuclear transport receptors – Importins and Exportins



How is transport compartment-specific cargo binding and dissociation achieved (directionality of transport)?

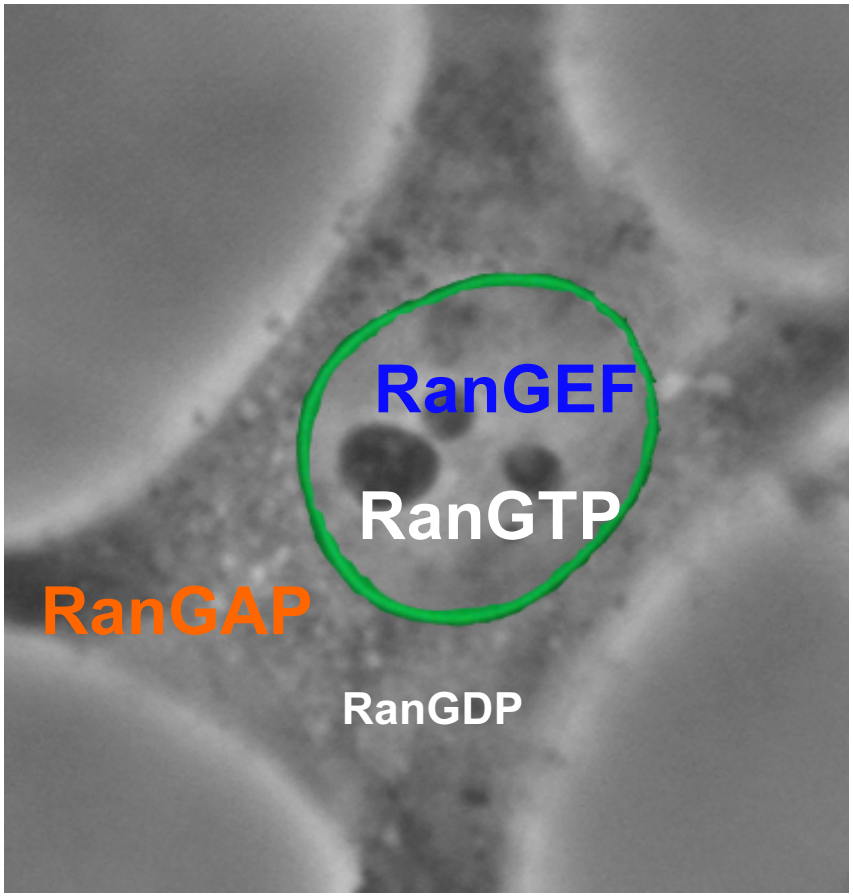
The RanGTPase system



RanGAP

RanGEF
(RCC1)

Ran GTPase Activating Protein
Ran Guanyl Nucleotide Exchange Factor



⇒ **asymmetric distribution of RanGTP in the cell**

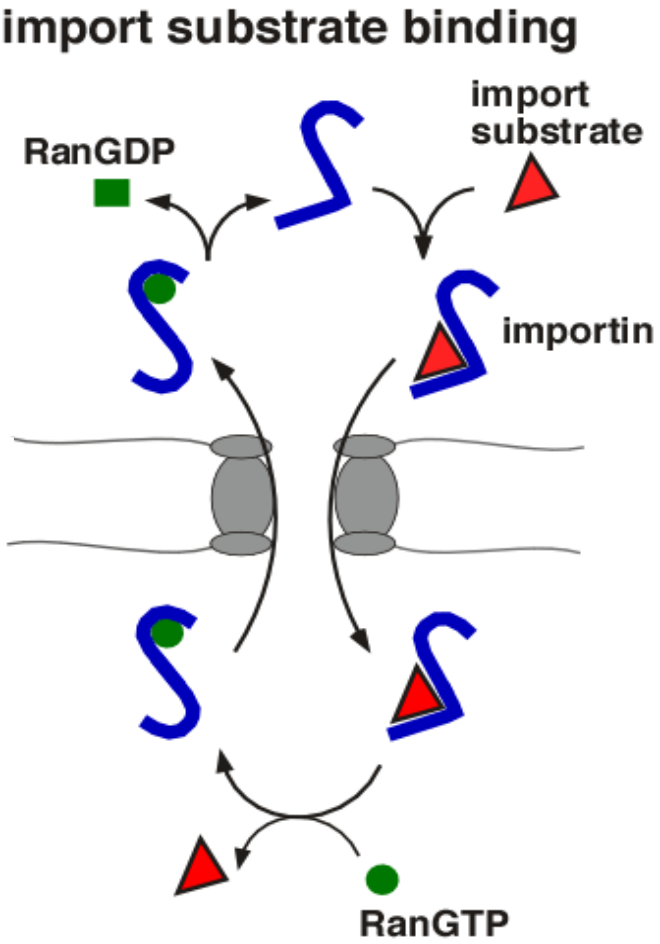
nucleus: RanGTP concentration high
cytoplasm: RanGTP concentration low

The GTPase Ran – the conformational switch

RanGTP and RanGDP possess different conformations

RanGTP binding to importins causes import substrate dissociation

Cytoplasm: low [RanGTP] maintained by RanGAP

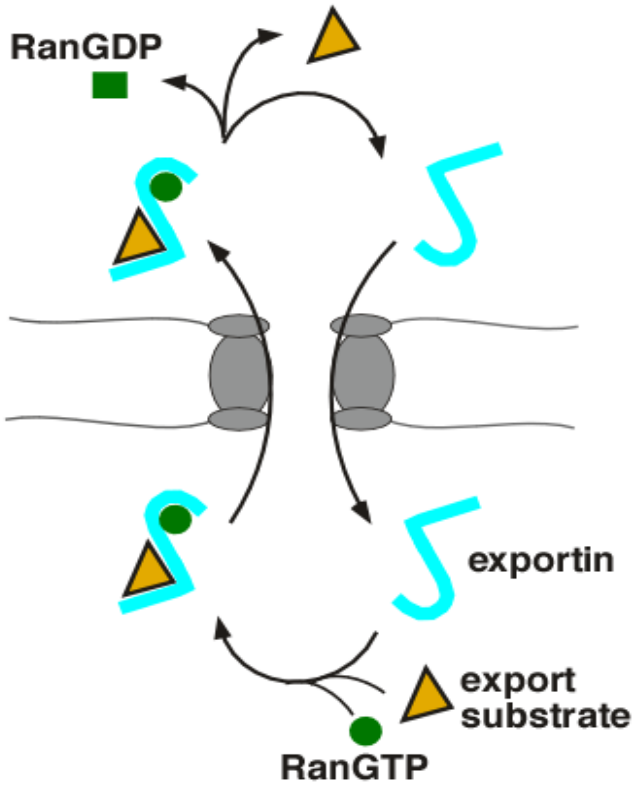


Nucleus: high [RanGTP] maintained byRanGEF

RanGTP stimulates binding of export substrates to exportins

Cytoplasm: low [RanGTP] maintained by RanGAP

export substrate dissociation



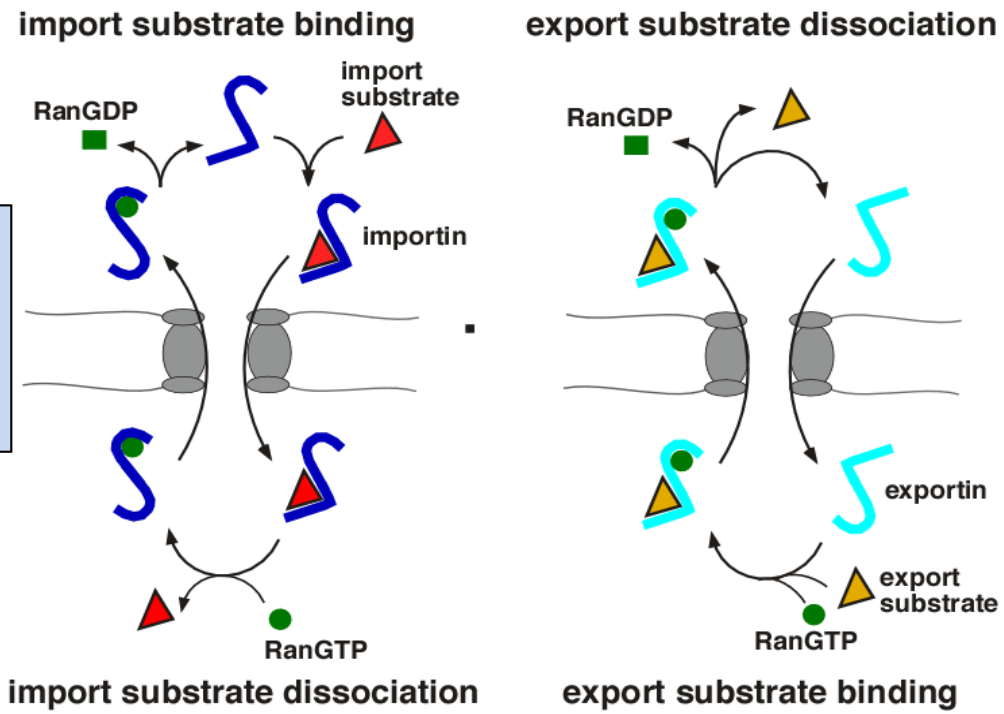
export substrate binding

Nucleus: high [RanGTP] maintained byRanGEF

The RanGTPase system imposes directionality to nuclear transport

Cytoplasm: low [RanGTP] maintained by RanGAP

Importins:
- high affinity for RanGTP
- RanGTP dissociates import substrate from importins



Exportins:
- low affinity for RanGTP
- RanGTP and export substrate bind cooperatively to exportin

Nucleus: high [RanGTP] maintained byRanGEF

nuclear transport receptors:
- shuttle continuously between nucleus and cytoplasm
- interact with NPC (FG repeat nups) and can drive their own import and export
- RanGTP-binding controls transport substrate association
- protein superfamily

Nuclear import and export by shuttling transport receptors

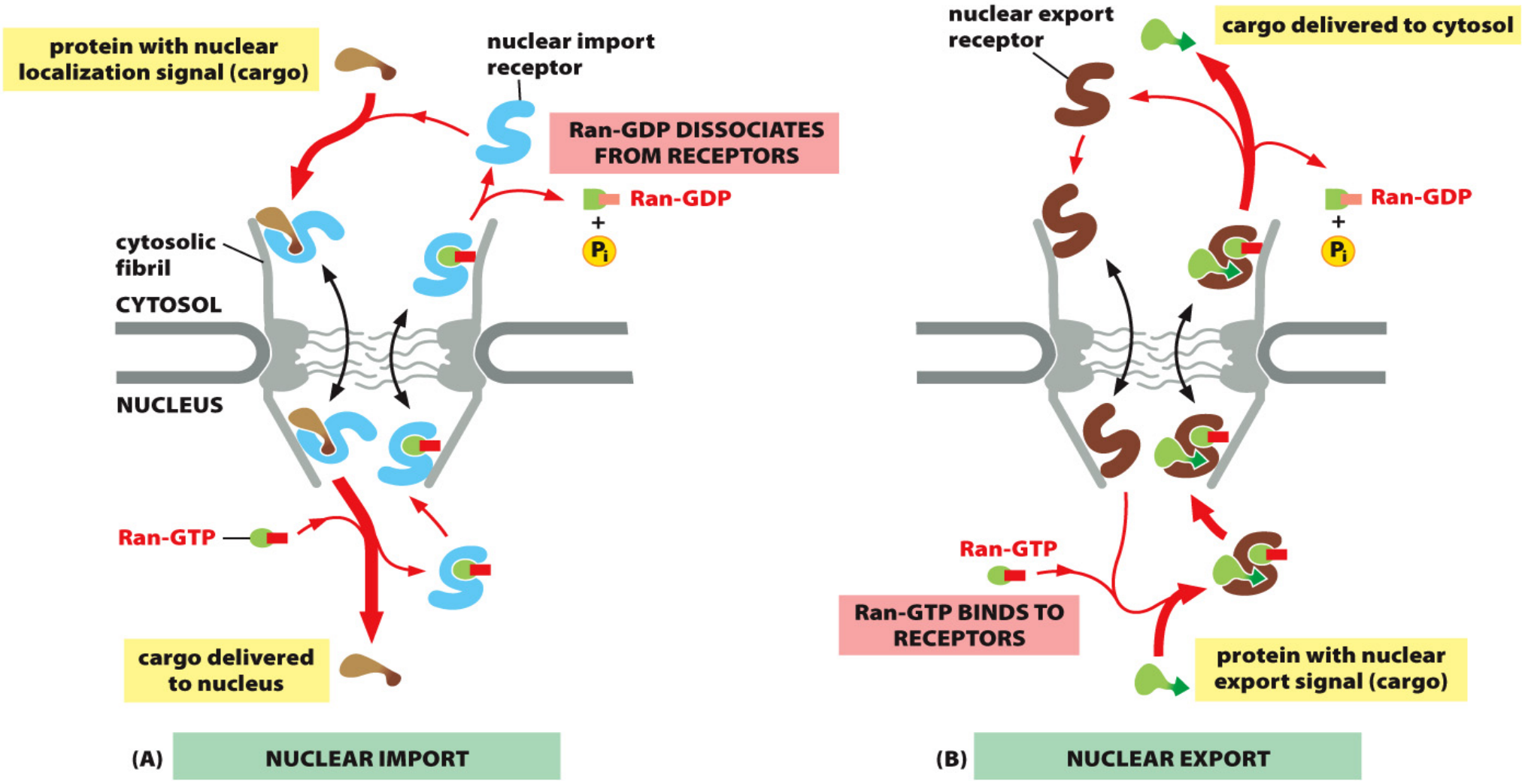


Figure 12-13 Molecular Biology of the Cell 6e (© Garland Science 2015)

Different types of transport receptors bind different types of transport signals

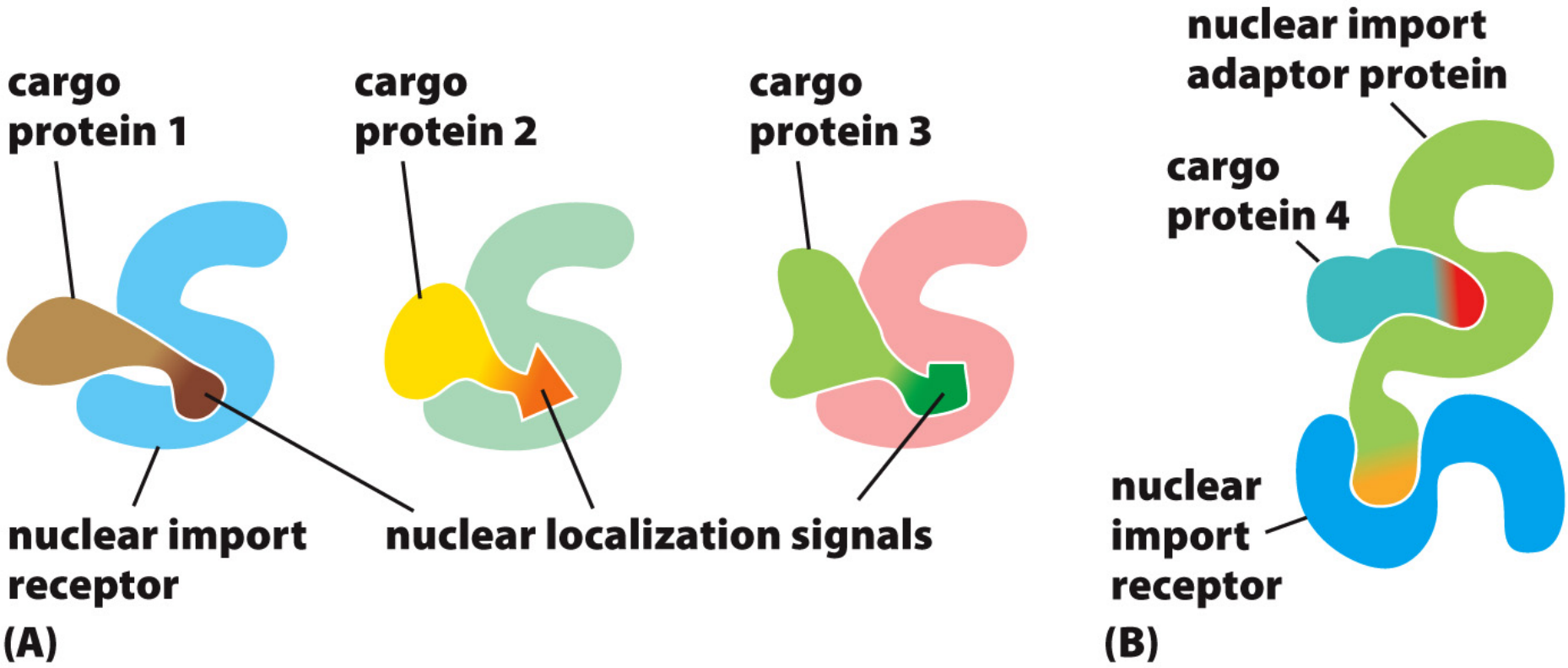
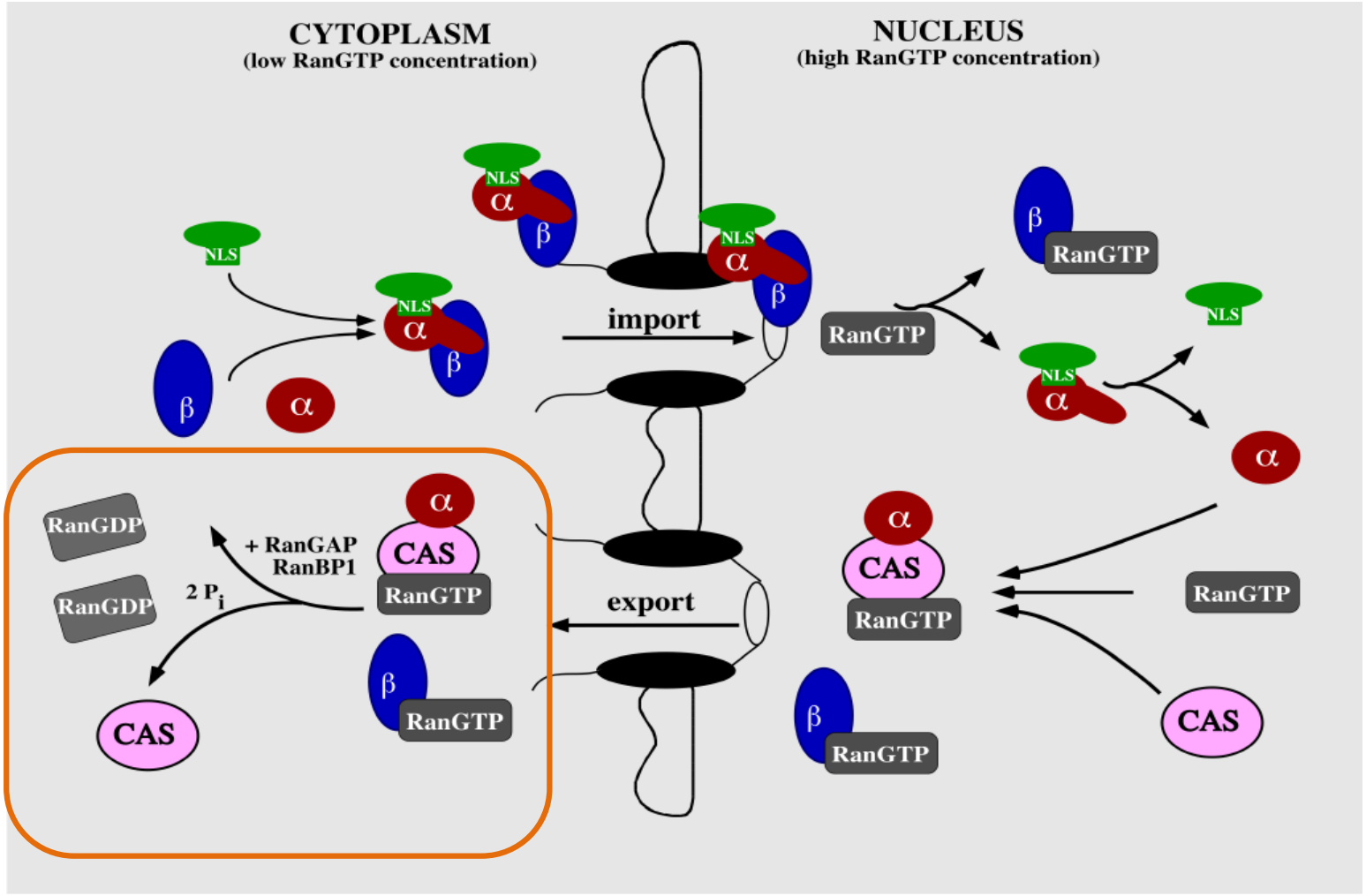


Figure 12-11 Molecular Biology of the Cell 6e (© Garland Science 2015)

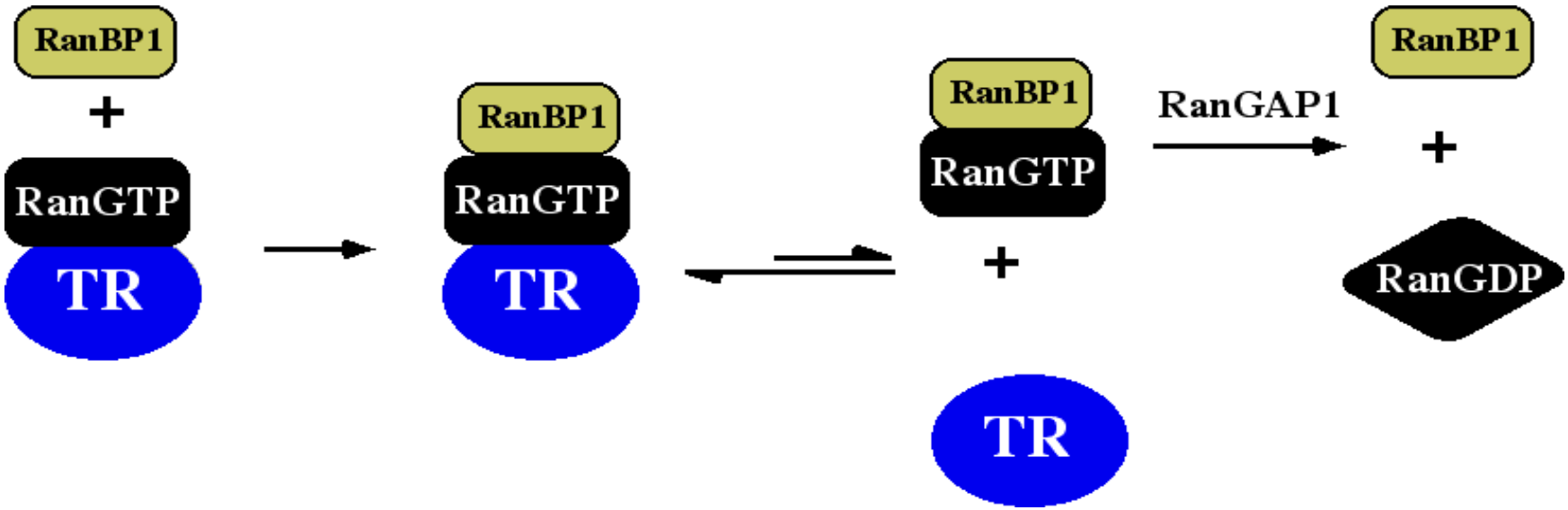
A full transport cycle
(example NLS protein import)



How is RanGTP dissociated from nuclear transport receptors?

Dissociation of RanGTP from nuclear transport receptors (TR)

The Ran-binding protein family (RanBP1, RanBP2)



Cytoplasmic RanBP1-family members help to dissociate RanGTP from transport receptors (TR) and present RanGTP to RanGAP for conversion into RanGDP

Dissociation of RanGTP from nuclear transport receptors (TR)

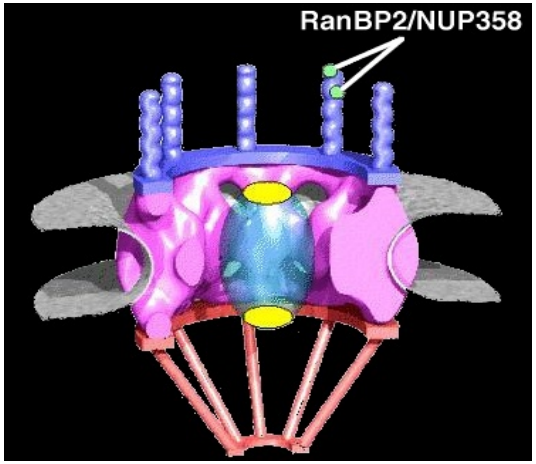
The Ran-binding protein family (RanBP1, RanBP2)

RanBP1 23 kDa

- cytoplasmic

RanBP2 358 kDa

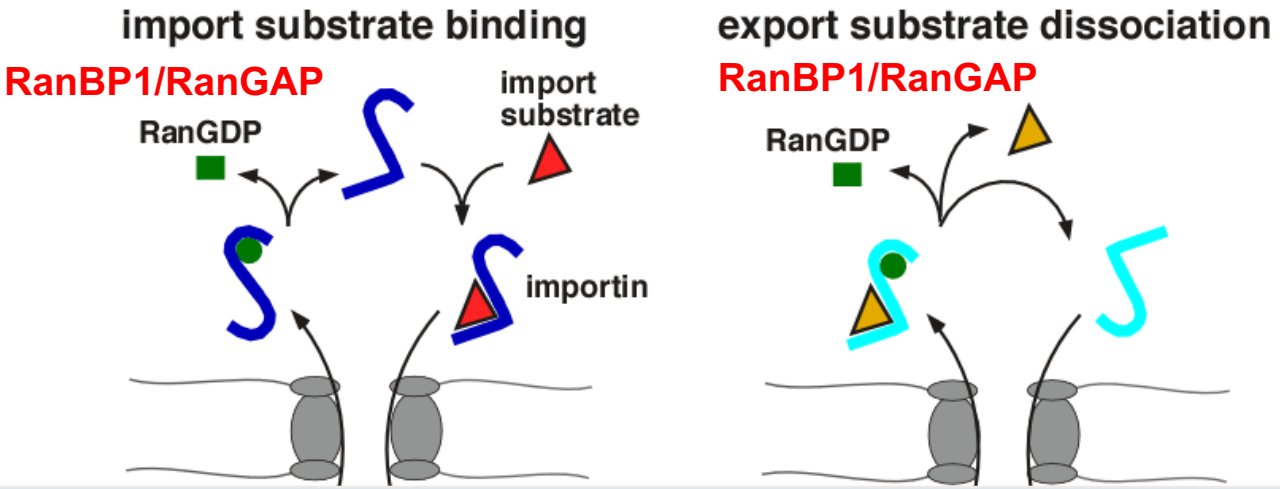
- nucleoporin



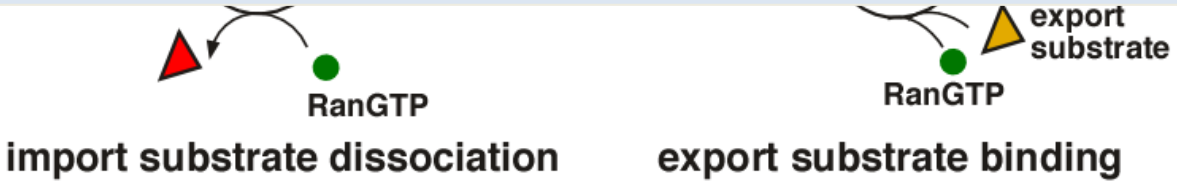
RanBD (RBD)- Ran binding domain

Dissociation of RanGTP from nuclear transport receptors (TR)

Cytoplasm: low [RanGTP] maintained by RanGAP



With each round of transport at least one molecule of RanGTP is exported from the nucleus



Nucleus: high [RanGTP] maintained byRanGEF

How is Ran replenished to the cell nucleus?

With each round of transport at least one molecule of RanGTP is exported from the nucleus

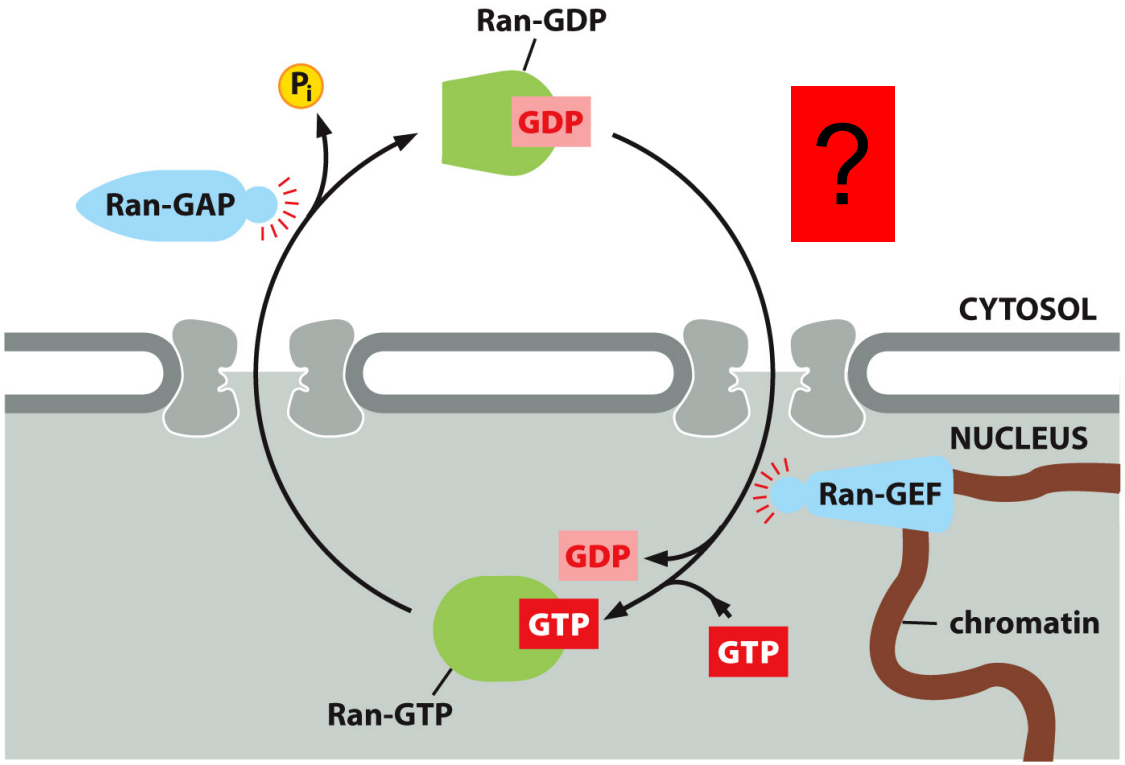
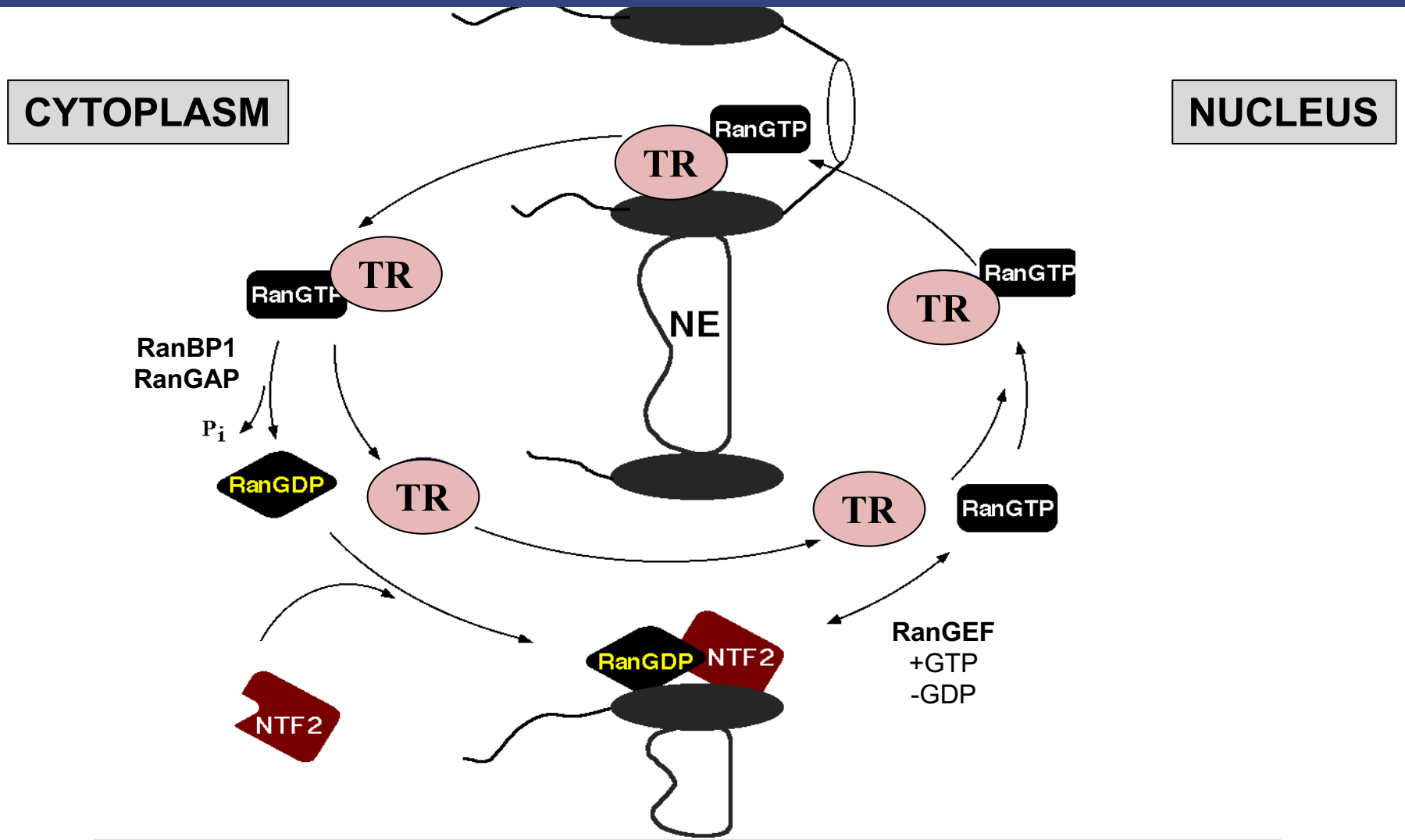


Figure 12-12 Molecular Biology of the Cell 6e (© Garland Science 2015)

Import of RanGTP into the nucleus by NTF2 (nuclear transport factor 2)



- NTF2 does not belong to the importin/exportin family
- nuclear export of NTF2 does not take along a RanGTP molecule

Components of the RanGTPase system

Factor	Interaction RanGTP/ GDP	Localization	Function
Ran	n.a.	**Nuc**/Cyt	Directionality of nuclear transport
Transport receptor superfamily	RanGTP	Nuc/ NPC/ Cyt	Translocation of macromolecules through the NPC
RanGAP	RanGTP	Cyt/ NPC	Stimulation of GTP hydrolysis on Ran
RanGEF (RCC1)	nucleotide-free form of Ran	Nuc	Nucleotide exchange
RanBP- family: RanBP1 RanBP2	RanGTP	 Cyt NPC (cyt. side)	 Export complex dissociation Export complex dissociation
NTF2	RanGDP	Cyt/ NPC	Nuclear import of Ran

EduApp Question 1

EduApp Question 2

The energy requirement of nuclear transport

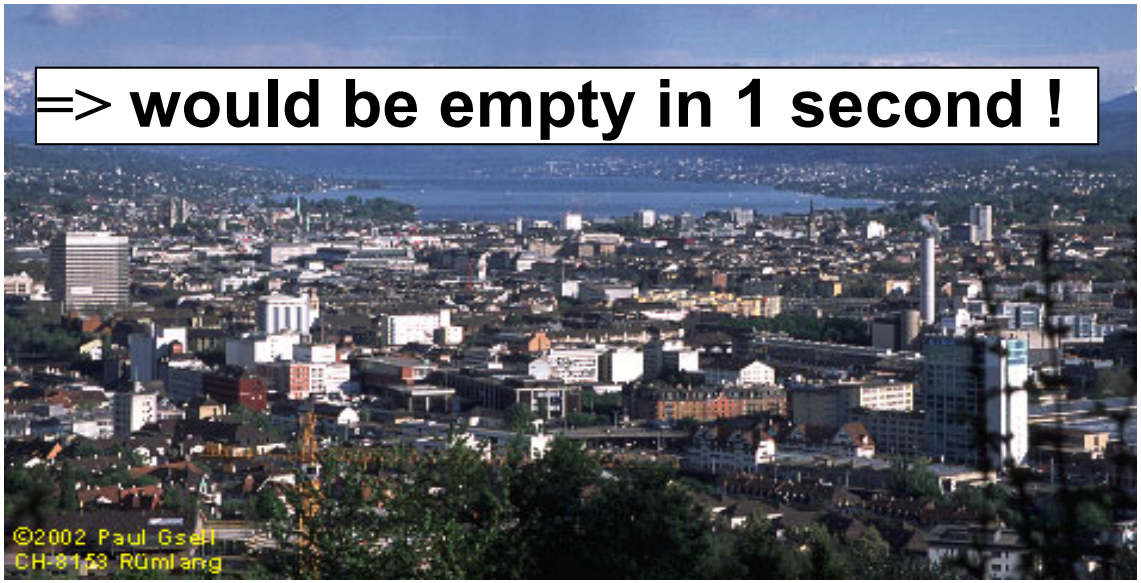
Transport through NPCs is very fast

assume 100 molecules/NPC/second (can be up to 1000 molecules/sec !)

≈ 3000-4000 NPC/nucleus

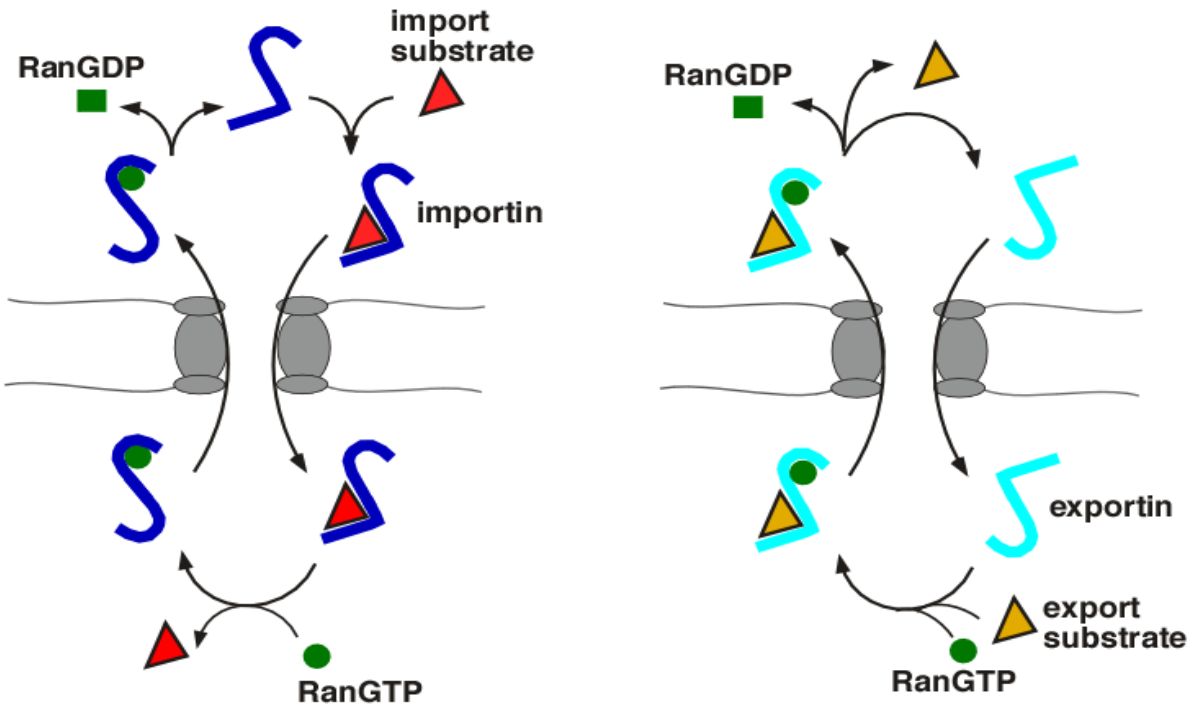
=> 300000-400000 molecules/nucleus/sec

city of Zürich:
400 000 inhabitants:



The energy requirement of nuclear transport

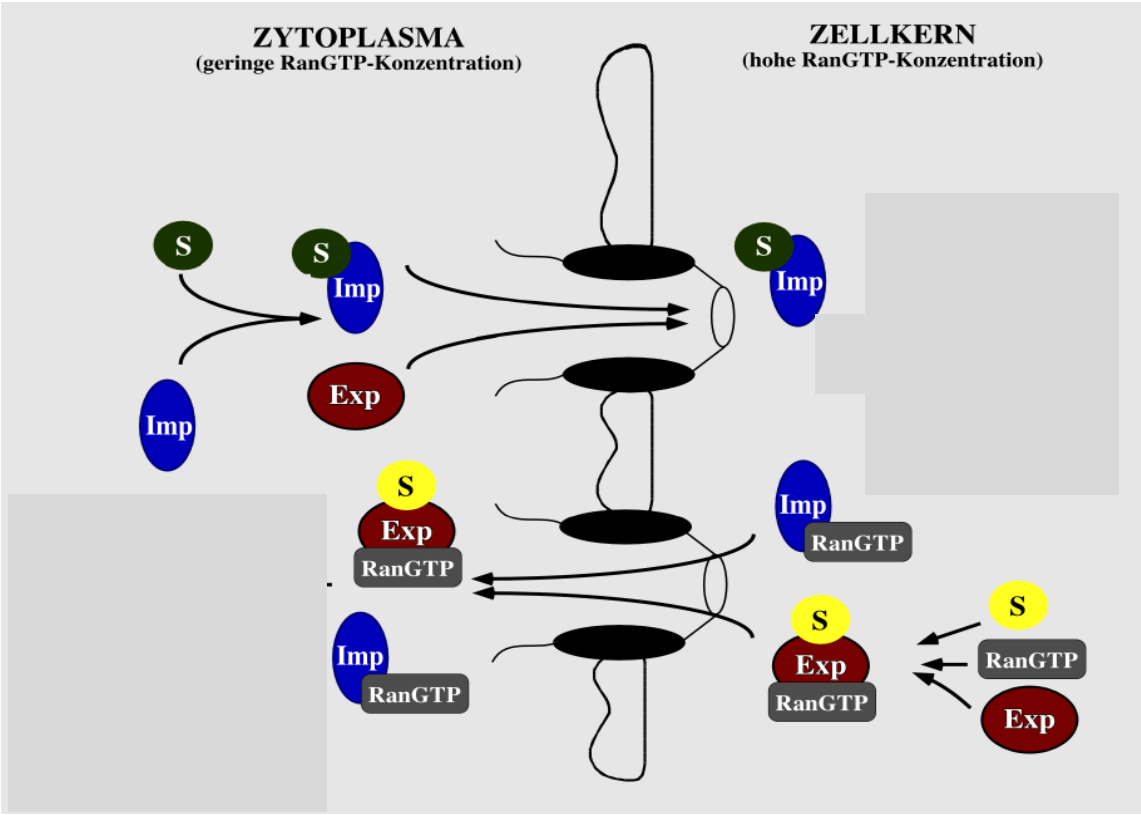
Translocation through the NPC is by facilitated diffusion (energy-independent)



Single round import is not linked to energy consumption
Multiple round import requires energy (GTP hydrolysis on Ran)

The energy requirement of nuclear transport

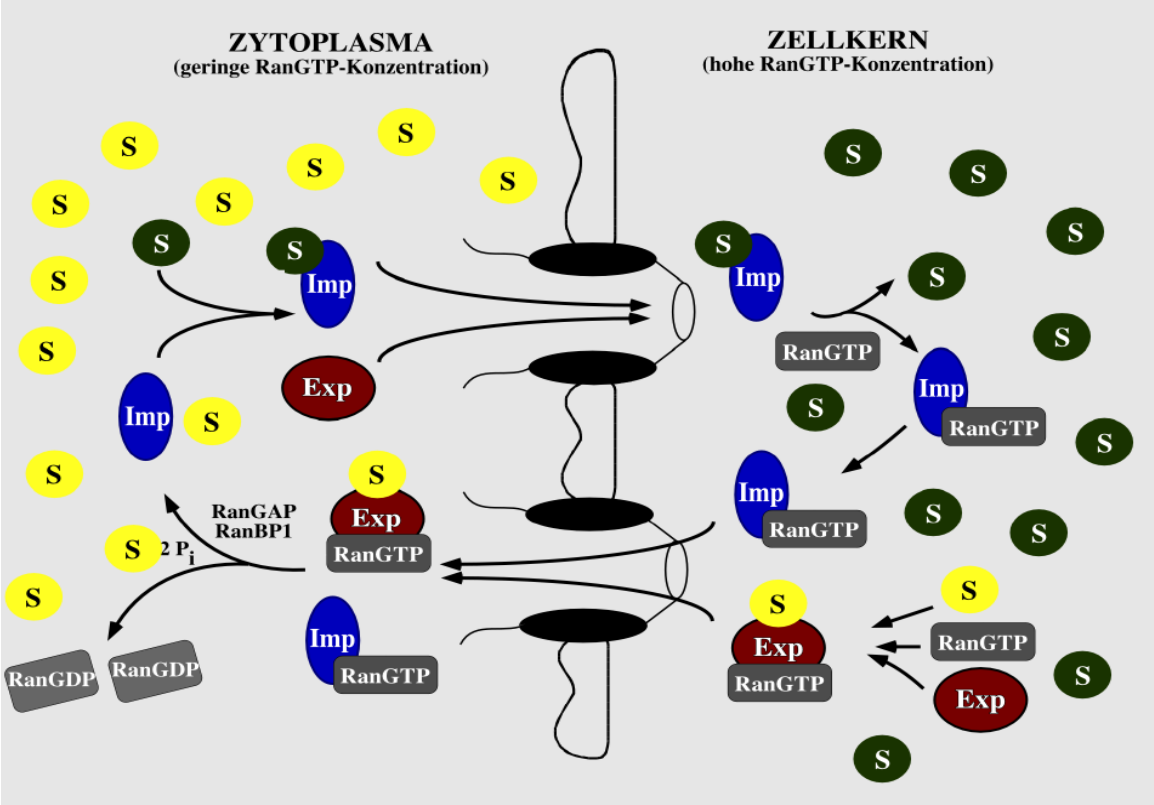
Translocation through the NPC is by facilitated diffusion (energy-independent)



Single round import is not linked to energy consumption

The energy requirement of nuclear transport

Coupling of the transport reaction to the RanGTP gradient allows for accumulation of cargo against a concentration gradient



Multiple round import requires energy (GTP hydrolysis on Ran)

Summary - Translocation through the NPC

- facilitated diffusion, energy-independent

- RanGTP gradient determines directionality

- single round transport is energy-independent

- RanGTP hydrolysis provides energy for transport against a gradient of chemical activity

Nuclear transport can be regulated by controlling access to the transport machinery

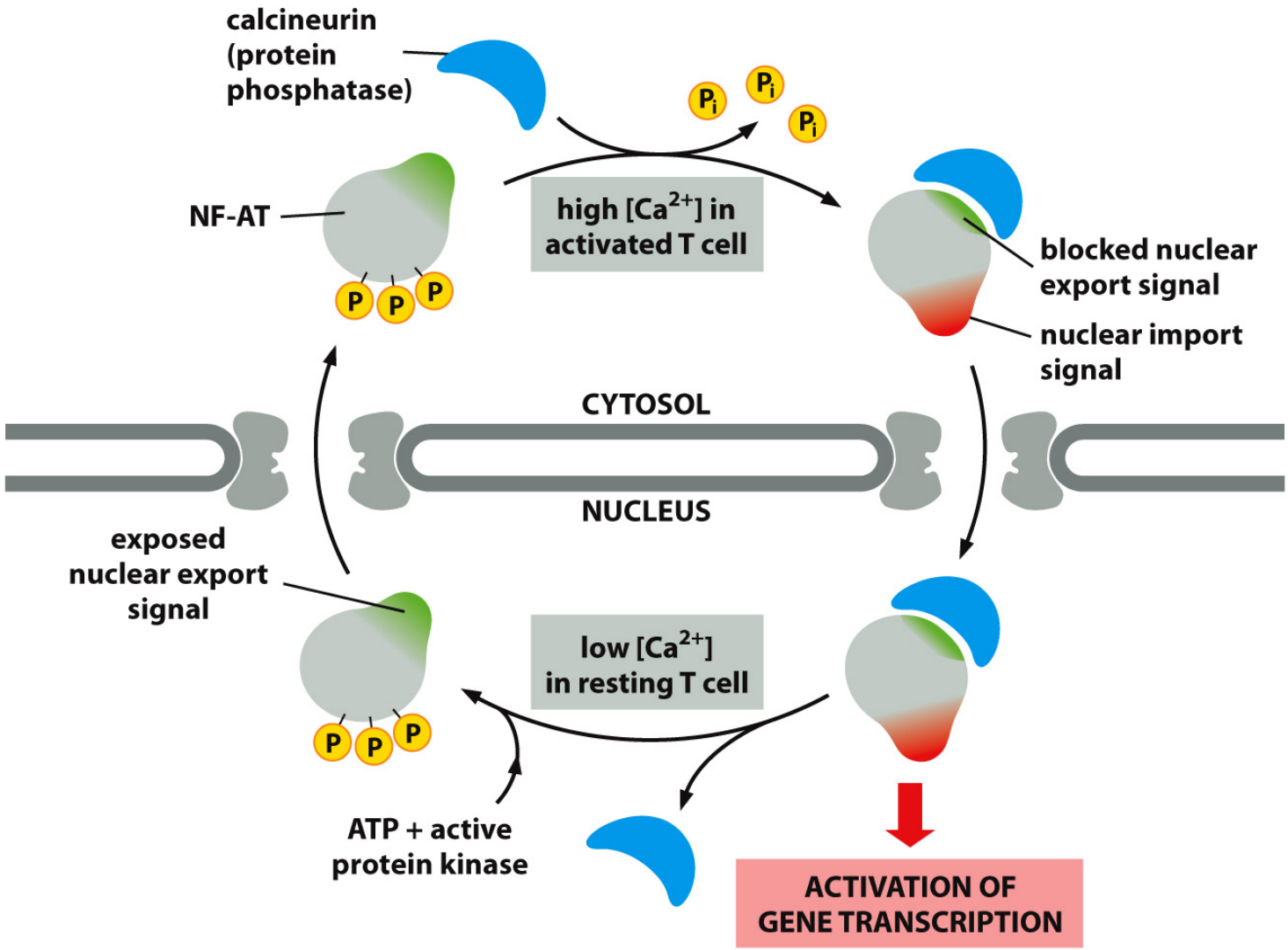


Figure 12-15 Molecular Biology of the Cell 6e (© Garland Science 2015)

Nuclear transport can be regulated

Regulated nuclear import of NF-AT