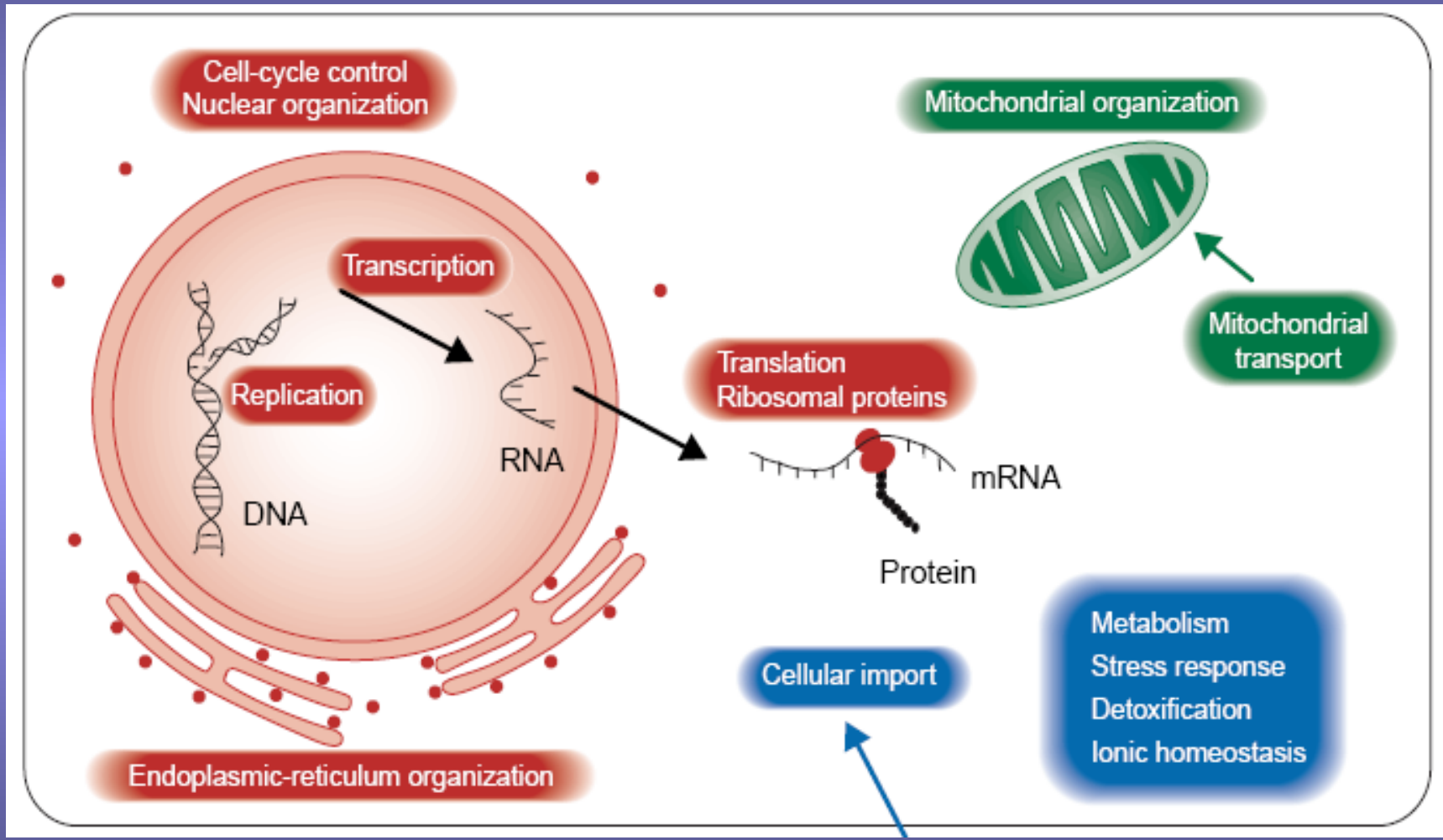
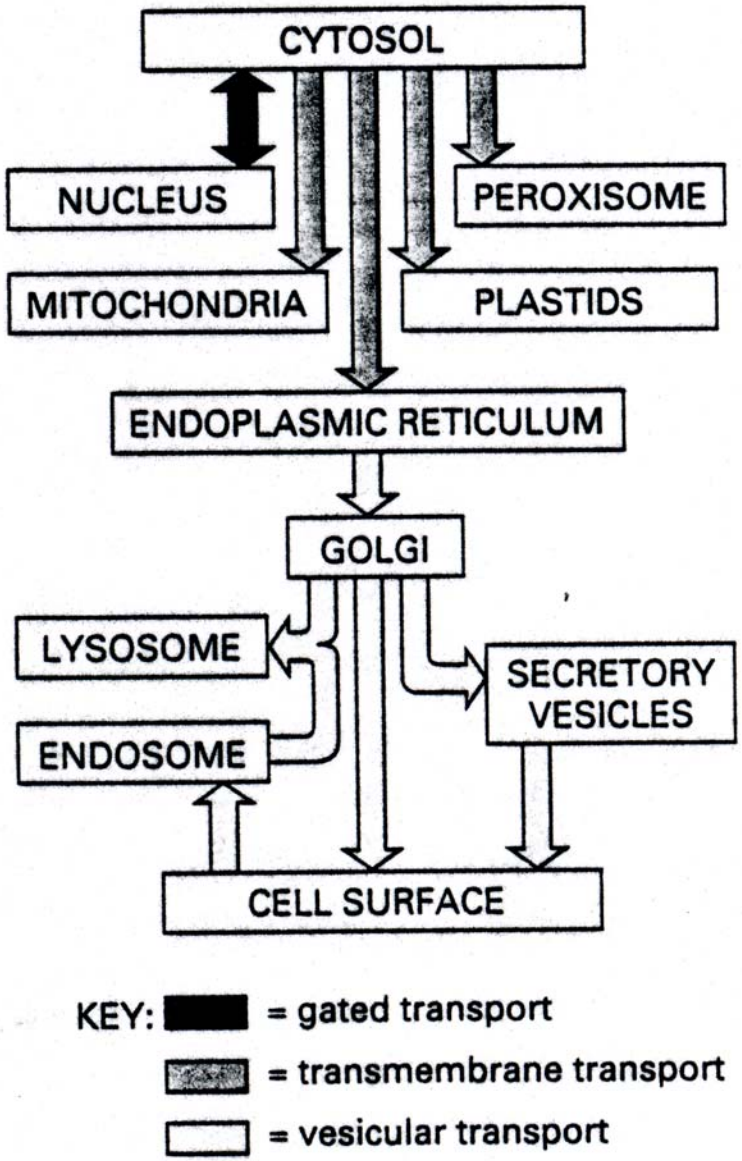
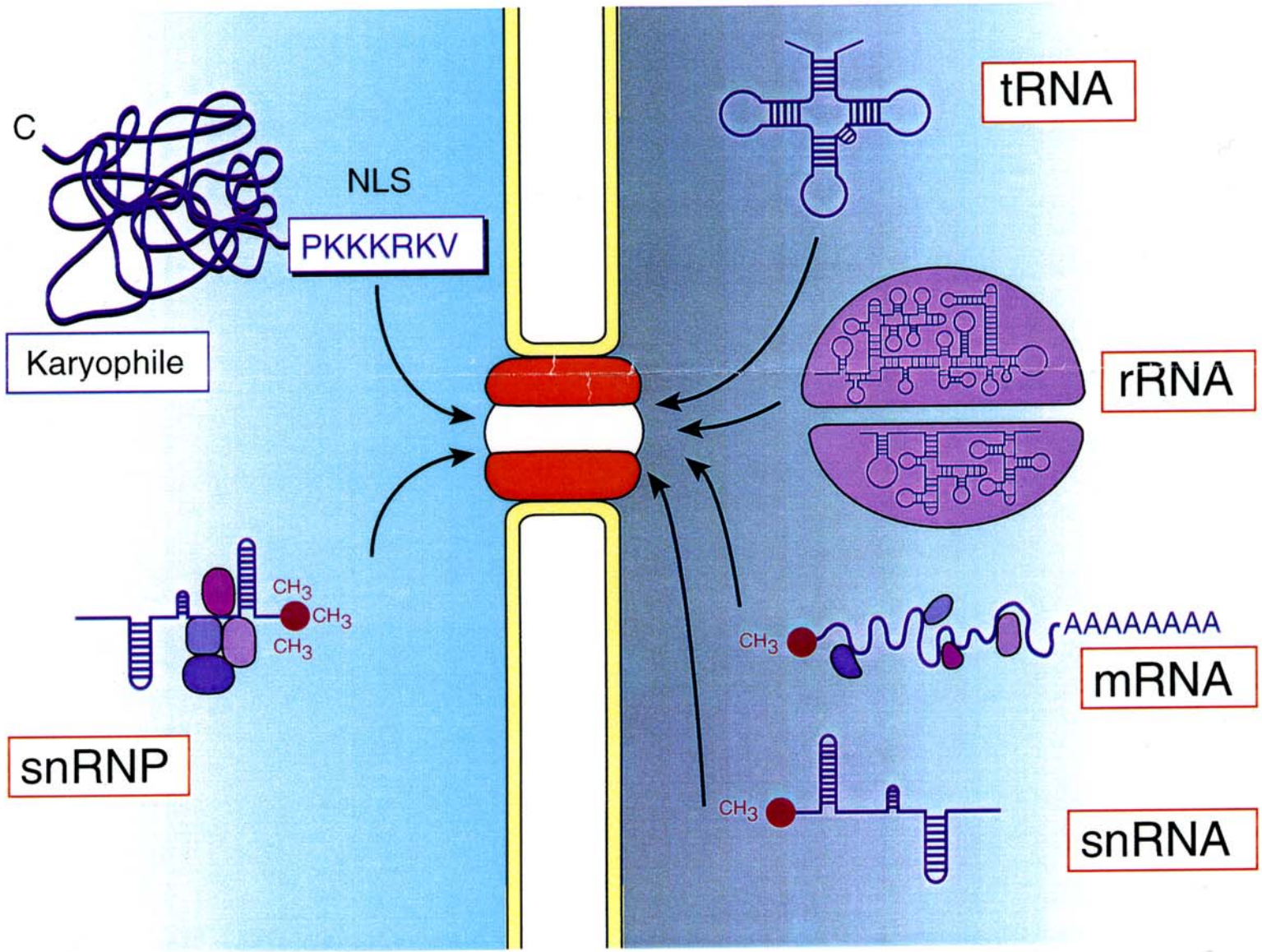
Nucleocytoplasmic transport

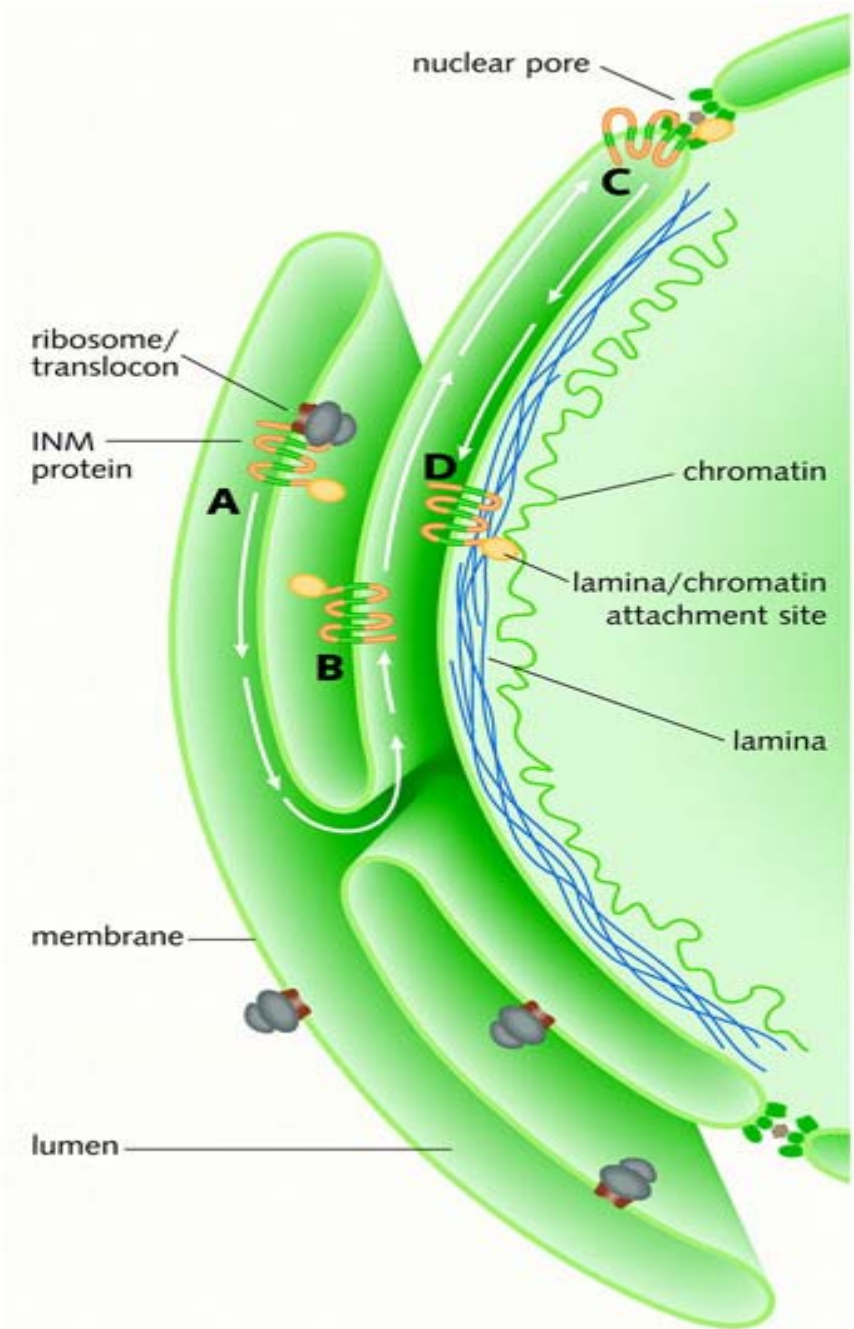
Origin of eukaryotic cell nuclei by endosymbiosis of Archaea in Bacteria

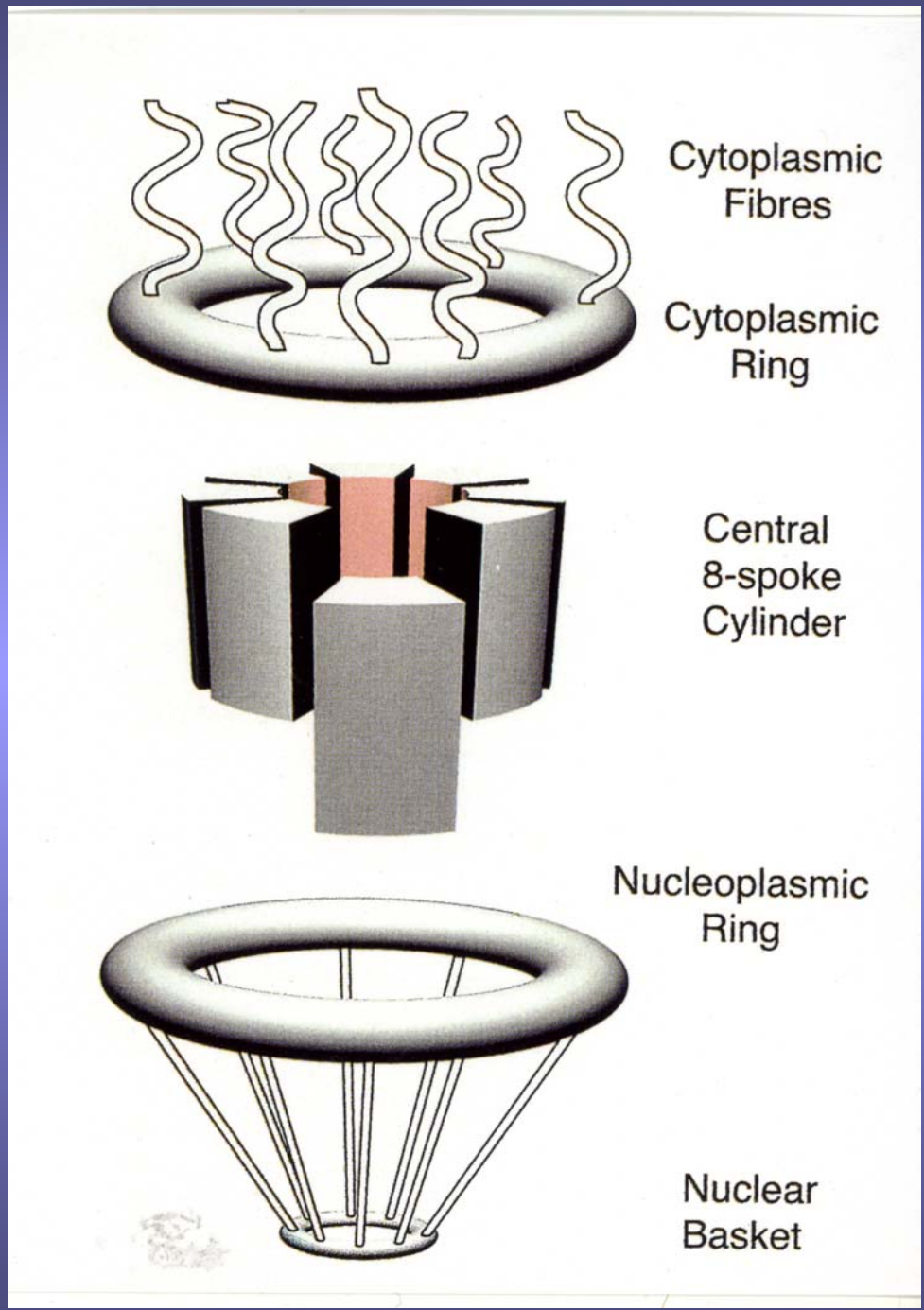


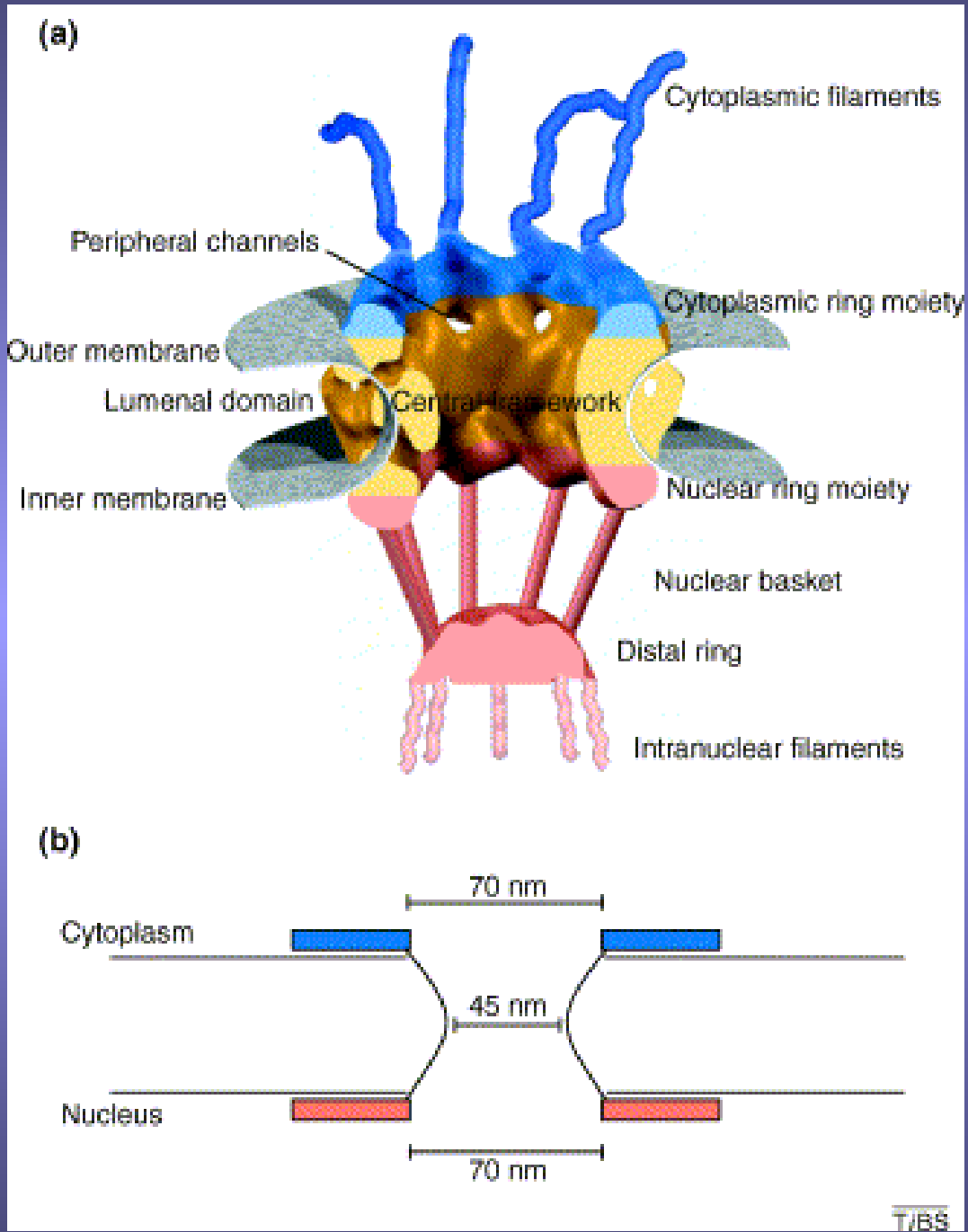
Horiike *et al.* Nat. Cell Biol., 2001, 3, 210-214.


CYTOSOL
NUCLEUS
PEROXISOME
MITOCHONDRIA
PLASTIDS
ENDOPLASMIC RETICULUM
GOLGI
LYSOSOME
SECRETORY VESICLES
ENDOSOME
CELL SURFACE
KEY:
= gated transport
= transmembrane transport
= vesicular transport

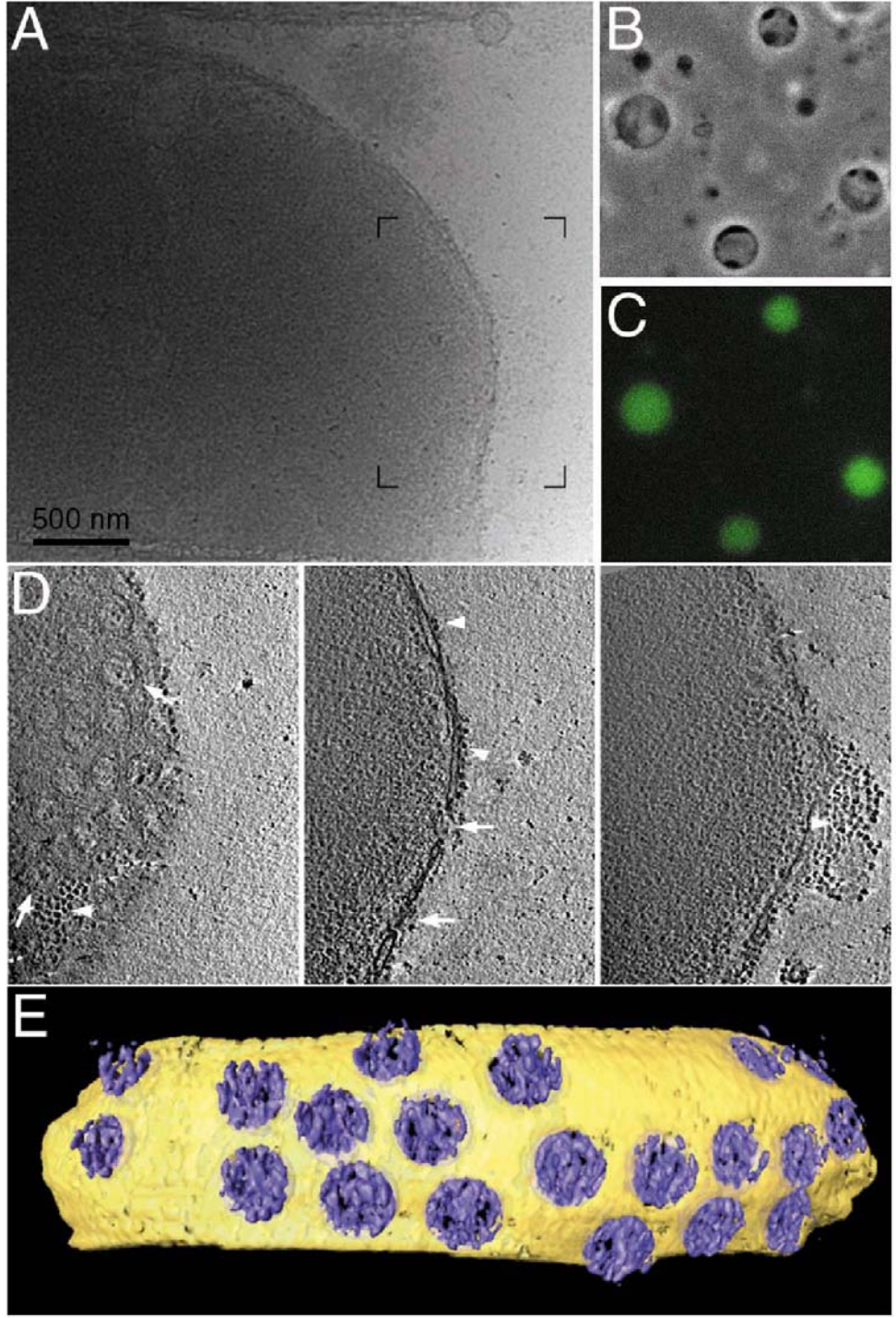

C
NLS
PKKKRKV
Karyophile
tRNA
rRNA
CH3
CH3
CH3
snRNP
CH3
AAAAAAAA
mRNA
CH3
snRNA


nuclear pore
C
ribosome/
translocon
INM
protein
A
D
chromatin
lamina/chromatin
attachment site
B
lamina
membrane
lumen

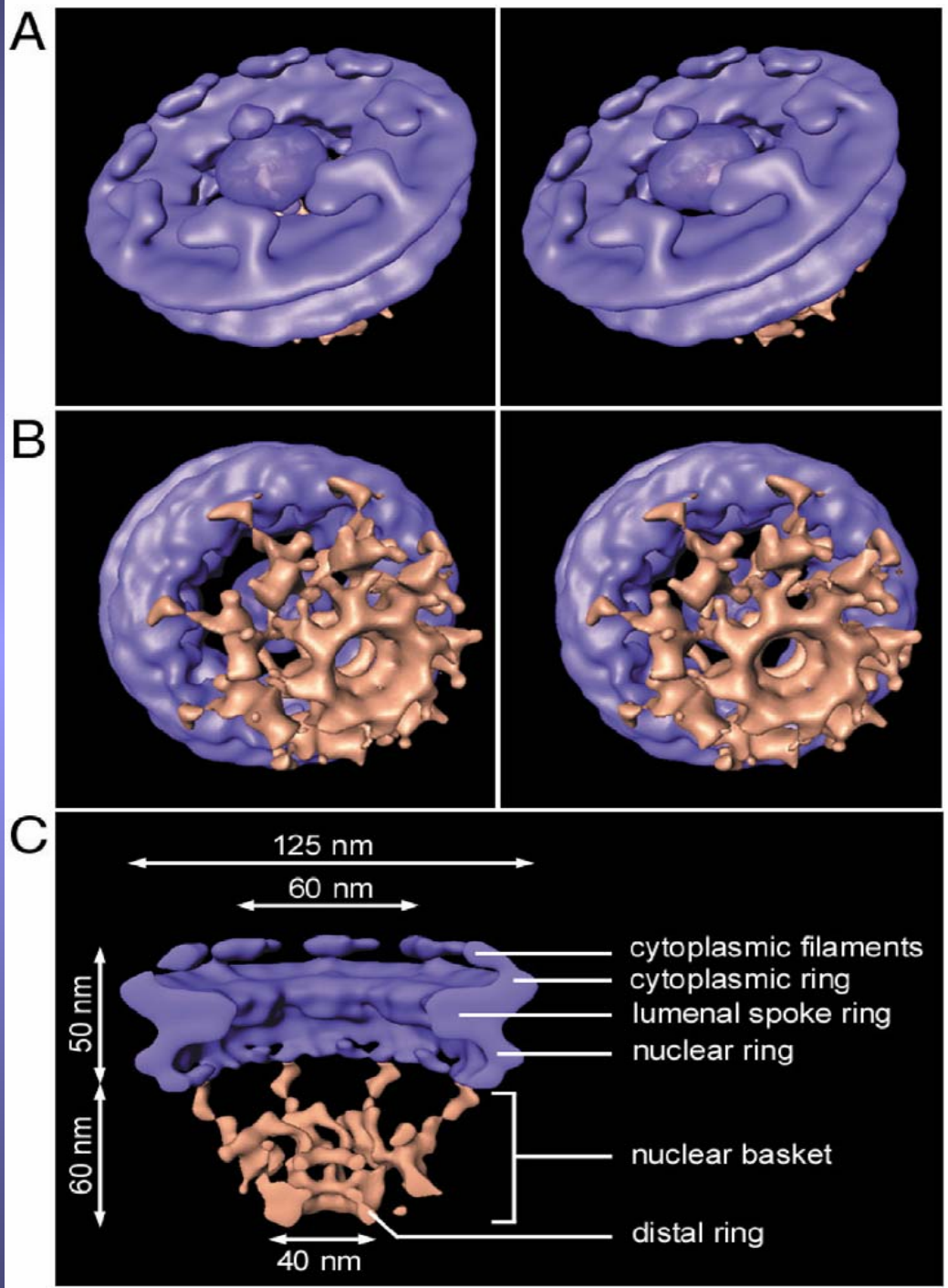

Cytoplasmic Fibres
Cytoplasmic Ring
Central 8-spoke Cylinder
Nucleoplasmic Ring
Nuclear Basket


(a)
Cytoplasmic filaments
Peripheral channels
Cytoplasmic ring moiety
Outer membrane
Lumenal domain
Central framework
Inner membrane
Nuclear ring moiety
Nuclear basket
Distal ring
Intranuclear filaments
(b)
70 nm
Cytoplasm
45 nm
Nucleus
70 nm
TiBS

Nuclear Pore Complex structure and dynamic revealed by cryoelectron tomography (in a close-to-life state)

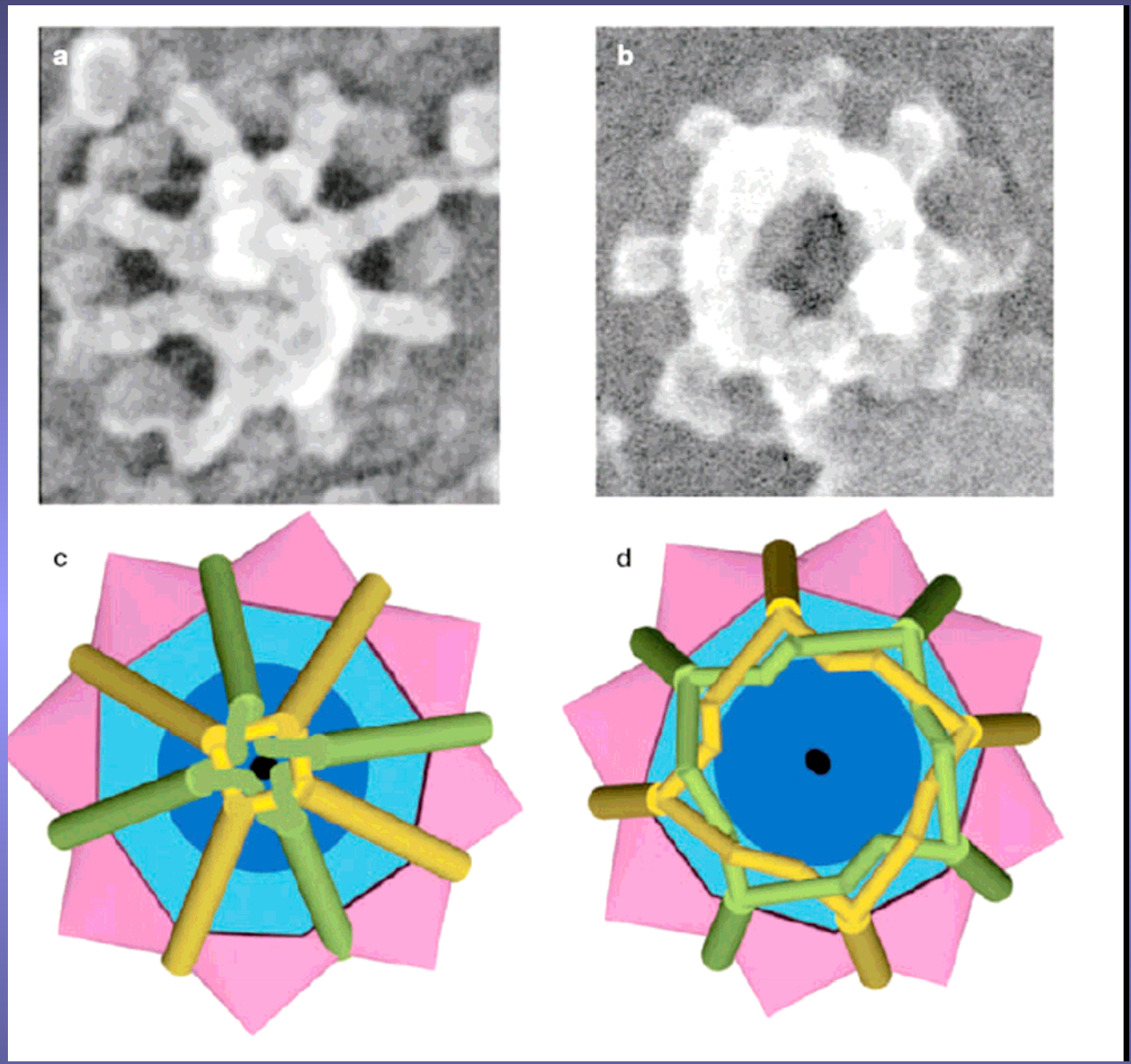


Beck *et al.* Science, 2004, 306, 1387-90.



stereo view

stereo view



a. ‘resting’ or closed state

b. ‘active’ or open states

Powers M.and M Dasso. Nat. Cell Biol., 2004, 6, 82-86.

Nuclear localization signals (NLS) , a "classical" NLS :

a. **monopartite**, simple sequence of 3-5 basic amino acid residues

- SV40 T antigen PKKKRKVE
- c-myc PAAKRVKLD
- p53 PQPKKKPL
- v-jun/c-jun KSRKRKL

b. **bipartite**, a signal consisting of basic dipeptyde upstream from a simple basic sequence

- nucleoplasmin KRPAATKKAGQAKKKKLD
- androgen receptor RKCYEVGMMKGGIRKDR
- topoisomerase I RKEEKVRASGDAKIKKE

c. **nucleolar localization sequences** (an entry and retention signal)

- HIV Tat GRKKRRQRRR
- HSP70F KRKHKKDISQNKRAVRR
- HTLV-1p27 PKTRRRPRRRSQRKRPP

These sequences have been identified based on one or more of three basic criteria:

- mutation or deletion of the NLS leads to cytoplasmic localization of the protein in question.

- the NLS is active in nuclear targeting as a peptide covalently coupled to a normally cytoplasmic localized protein.

- the NLS is able to target a normally cytoplasmic localized protein to the nucleus when encoded in a fusion protein.

NLS ARE FUNDAMENTALLY DIFFERENT FROM OTHER SIGNAL OR TARGETING SEQUENCES IN THAT THEY ARE NOT CLEAVED DURING TRANSPORT.

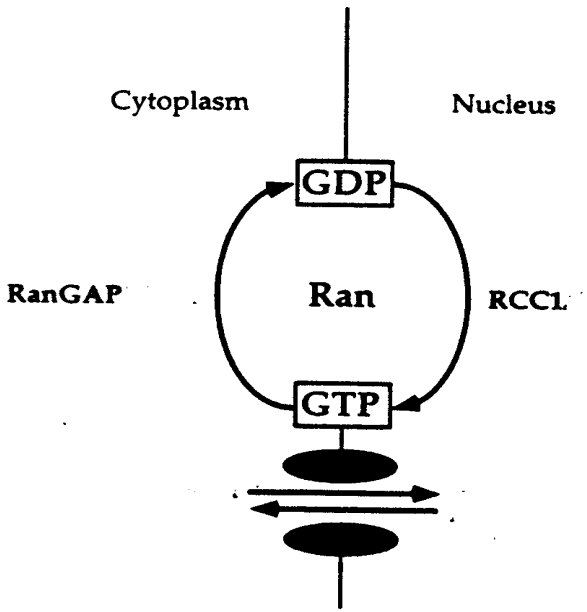
THE NUCLEAR IMPORT OF PROTEIN DEPENDS ON RAN PROTEIN

Ran protein is small GTPase, detectable in the cytosol and the nucleus

Rans regulators:

Ran's GTP exchange factor, termed **RCC1**, located in the nucleus

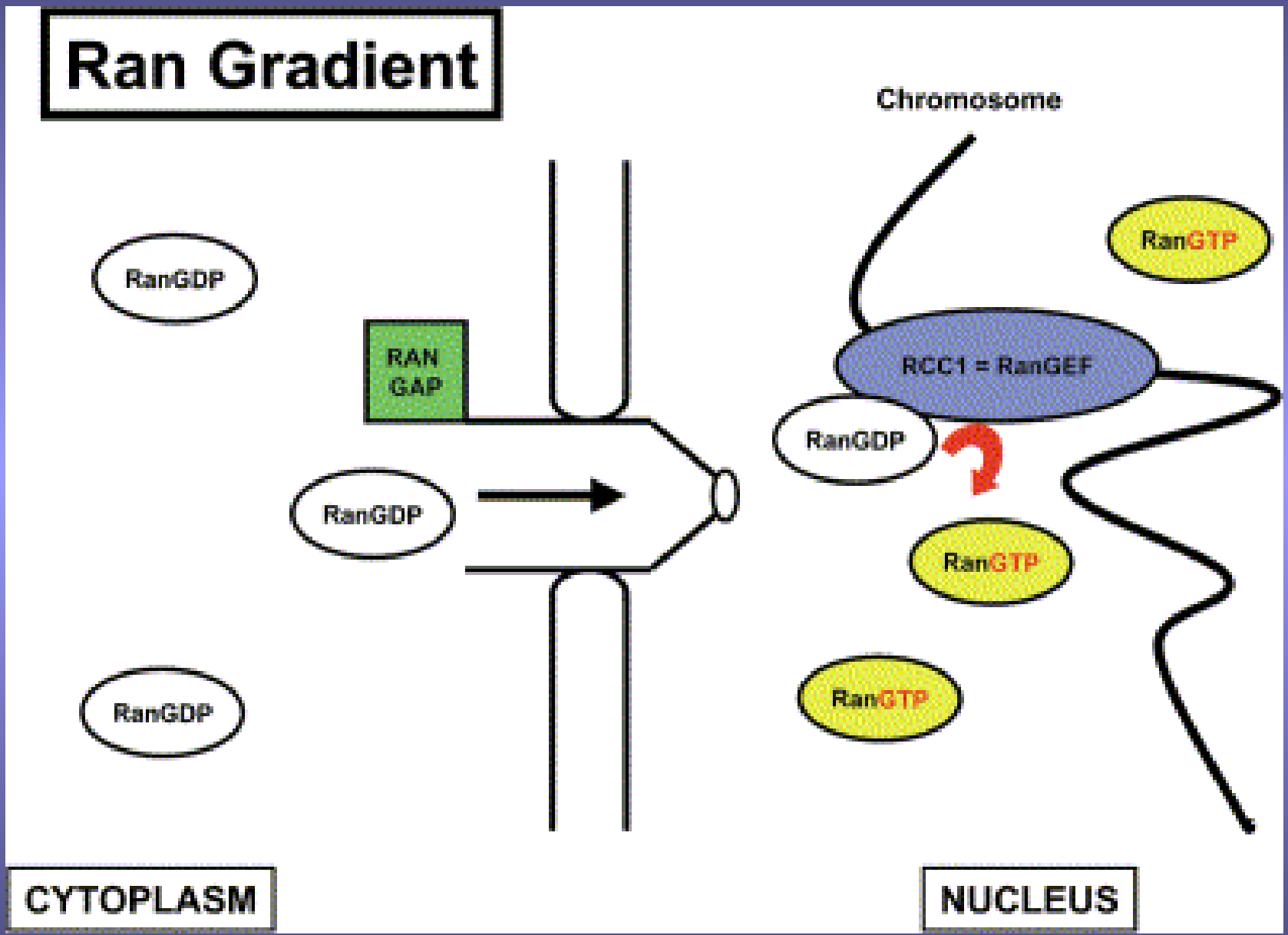
Ran's GTPase-activating protein, **Ran-GAP**, localized to the cytoplasm

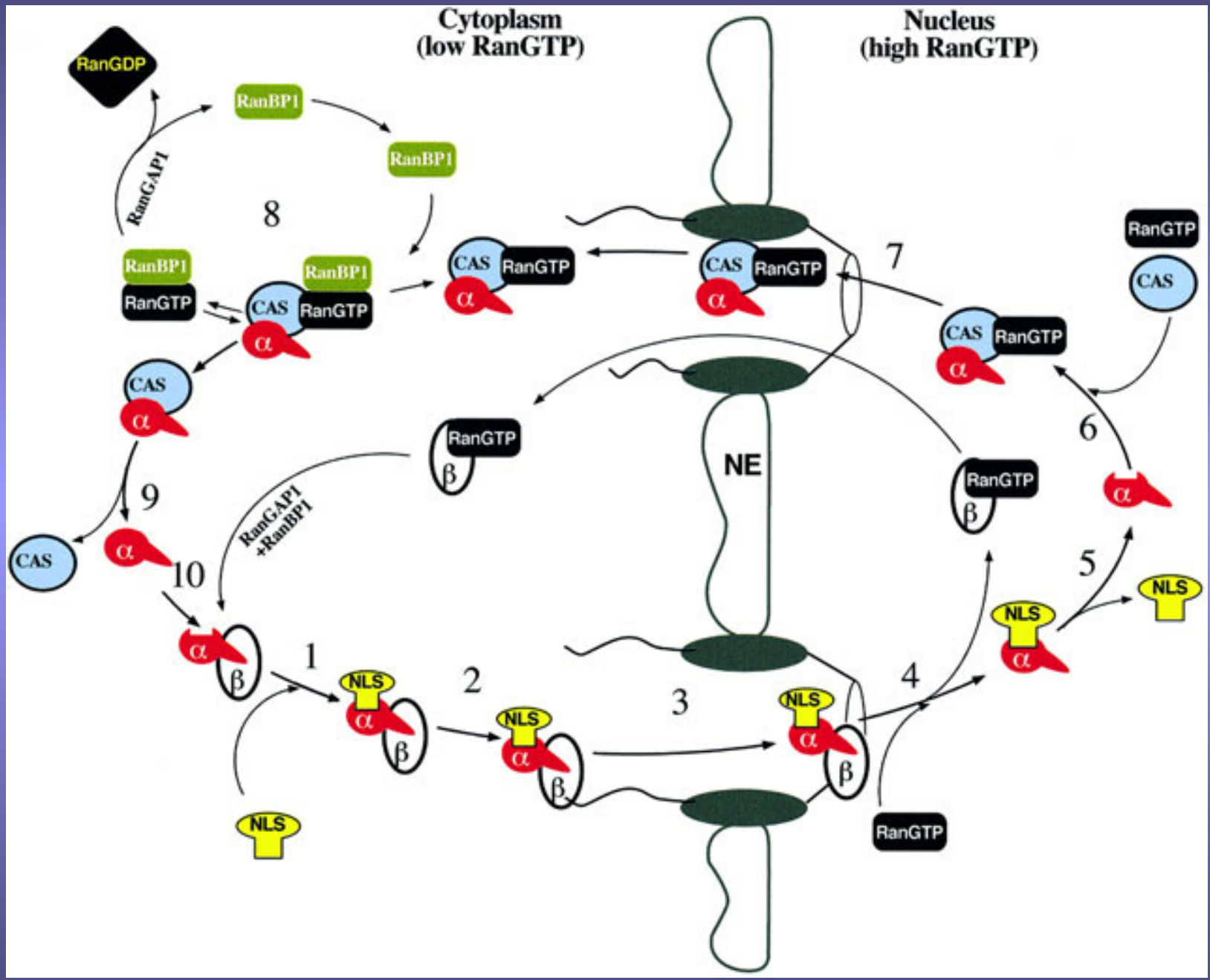


Localization of Ran Effectors

The compartmentalization of its regulators indicates that Ran moves between the cytoplasm and the nucleus.

Ran Gradient




Cytoplasm
(low RanGTP)
Nucleus
(high RanGTP)
RanGDP
RanBP1
RanBP1
RanGAP1
8
RanBP1
RanGTP
RanBP1
CAS
RanGTP
CAS
RanGTP
7
RanGTP
CAS
CAS
RanGTP
CAS
RanGTP
6
NE
RanGTP
RanGTP
9
RanGAP1
+RanBP1
CAS
10
5
NLS
NLS
1
2
3
4
NLS
NLS
NLS
NLS
RanGTP

The best understood function of importin α is to serve as an adaptor that links classical NLS-containing proteins to importin β, which in turn, docks the ternary complex at the NPC (to FxFG domains of nucleoporins) and facilitates its translocation into the nucleus.

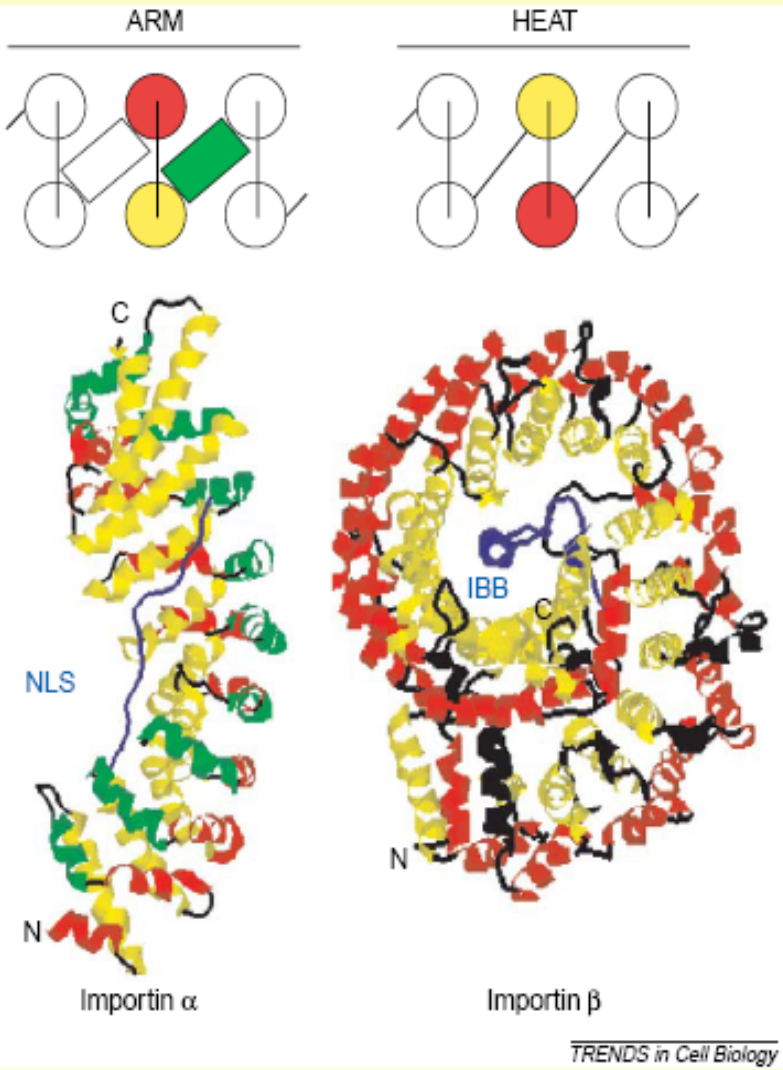
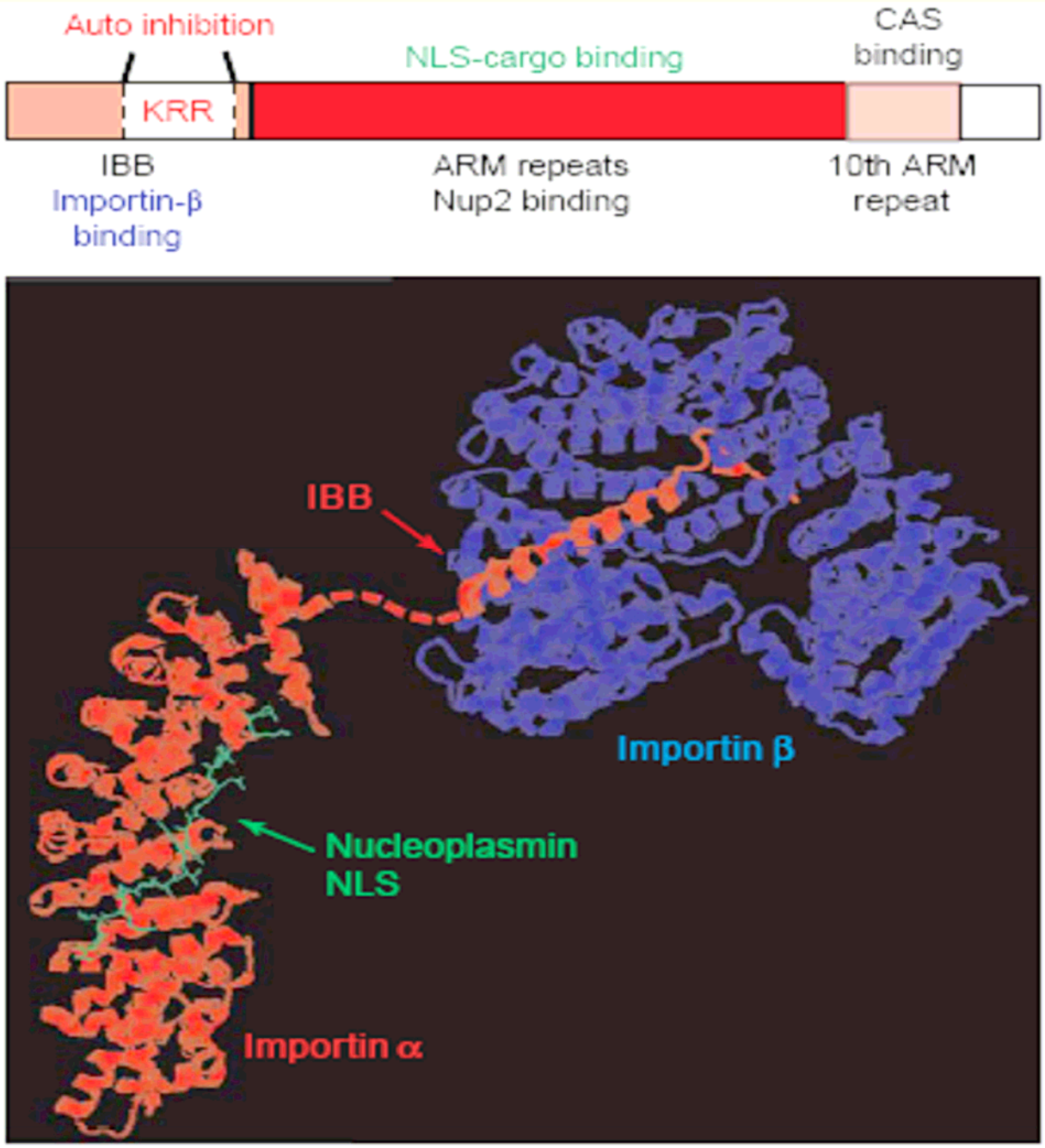
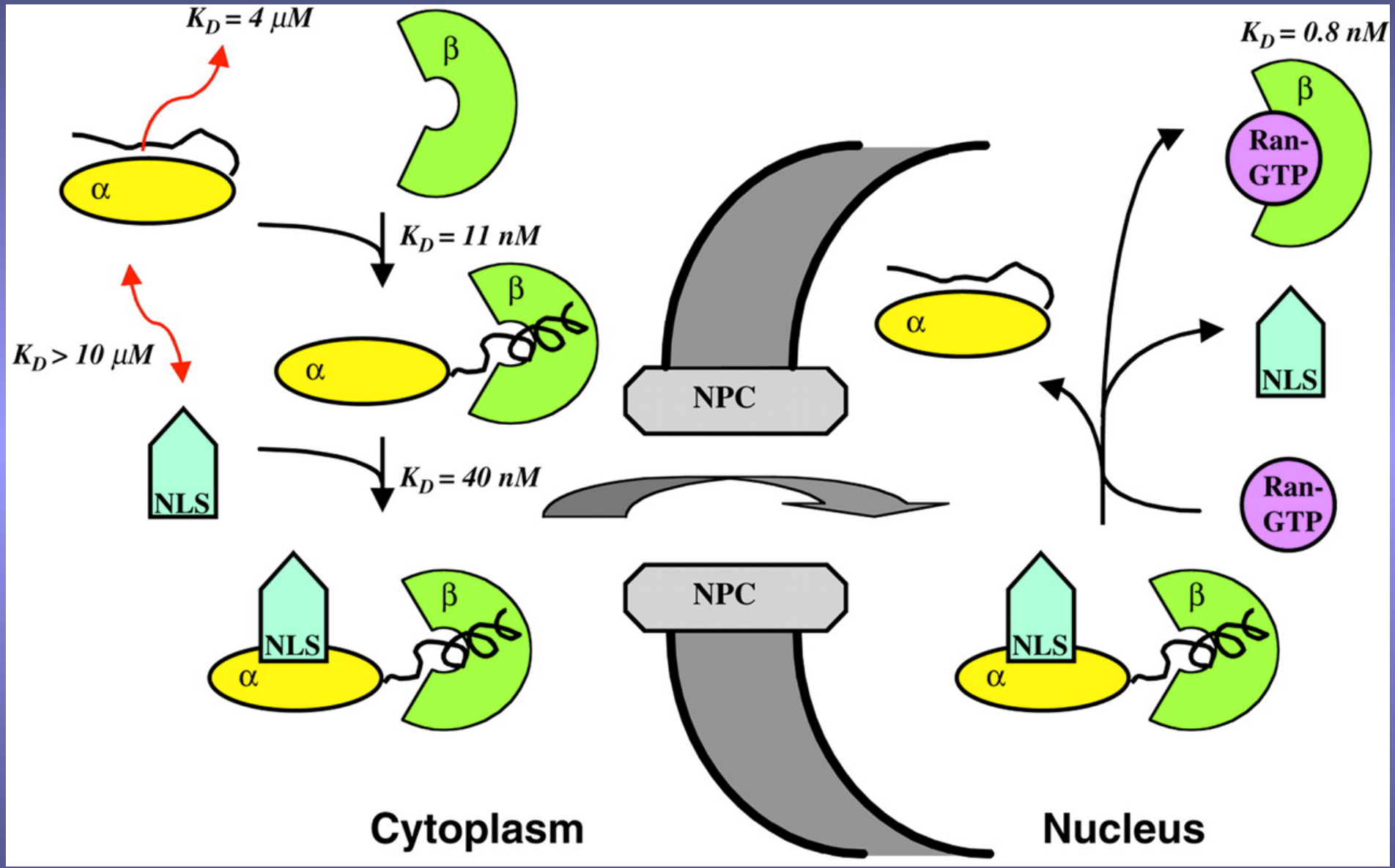
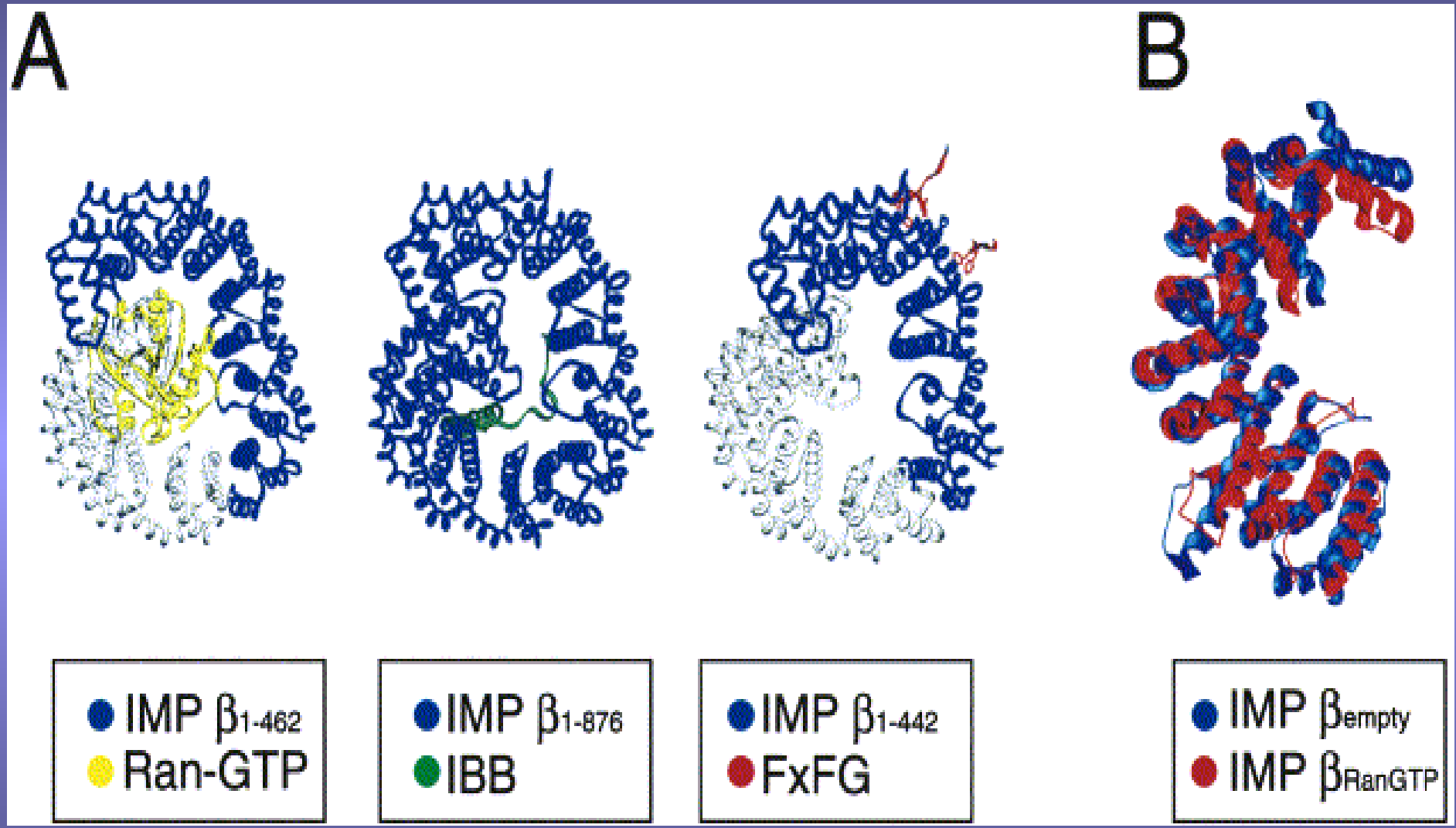


Figure I. Importin α and importin β consist of tandem arrays of armadillo (ARM; α) and HEAT (β) helical repeat motifs. At the top of the figure, the H1, H2 and H3 helices of importin α are shown in green, red and yellow, respectively, and the A and B helices of importin β are indicated by red and yellow, respectively [73]. The tandemly repeated sequences of ARM and HEAT motifs occur in a wide variety of eukaryotic proteins. The H1, H2 and H3 helices of the ARM protein importin α (left) are shown in green, red and yellow, respectively. The A and B helices of the HEAT protein importin β (right) are indicated by red and yellow, respectively; bound ligands are shown in blue. The structures shown are those of yeast importin α bound to the bipartite nuclear localization signal of nucleoplasmin (PDB 1ee5 [12]) and human importin β bound to the importin-β-binding domain of importin α (PDB 1qgk [71]). The structures are oriented with their N-termini at the bottom and their C-termini at the top.



TRENDS in Cell Biology


K_D = 4 μM
β
α
K_D = 11 nM
β
α
K_D > 10 μM
NLS
K_D = 40 nM
NPC
α
K_D = 0.8 nM
β
Ran-GTP
NLS
Ran-GTP
NLS
β
α
NPC
NLS
β
α
Cytoplasm
Nucleus


A
B
IMP β1-462
Ran-GTP
IMP β1-876
IBB
IMP β1-442
FxFG
IMP βempty
IMP βRanGTP

Nuclear export signal NES (leucine-rich sequence)

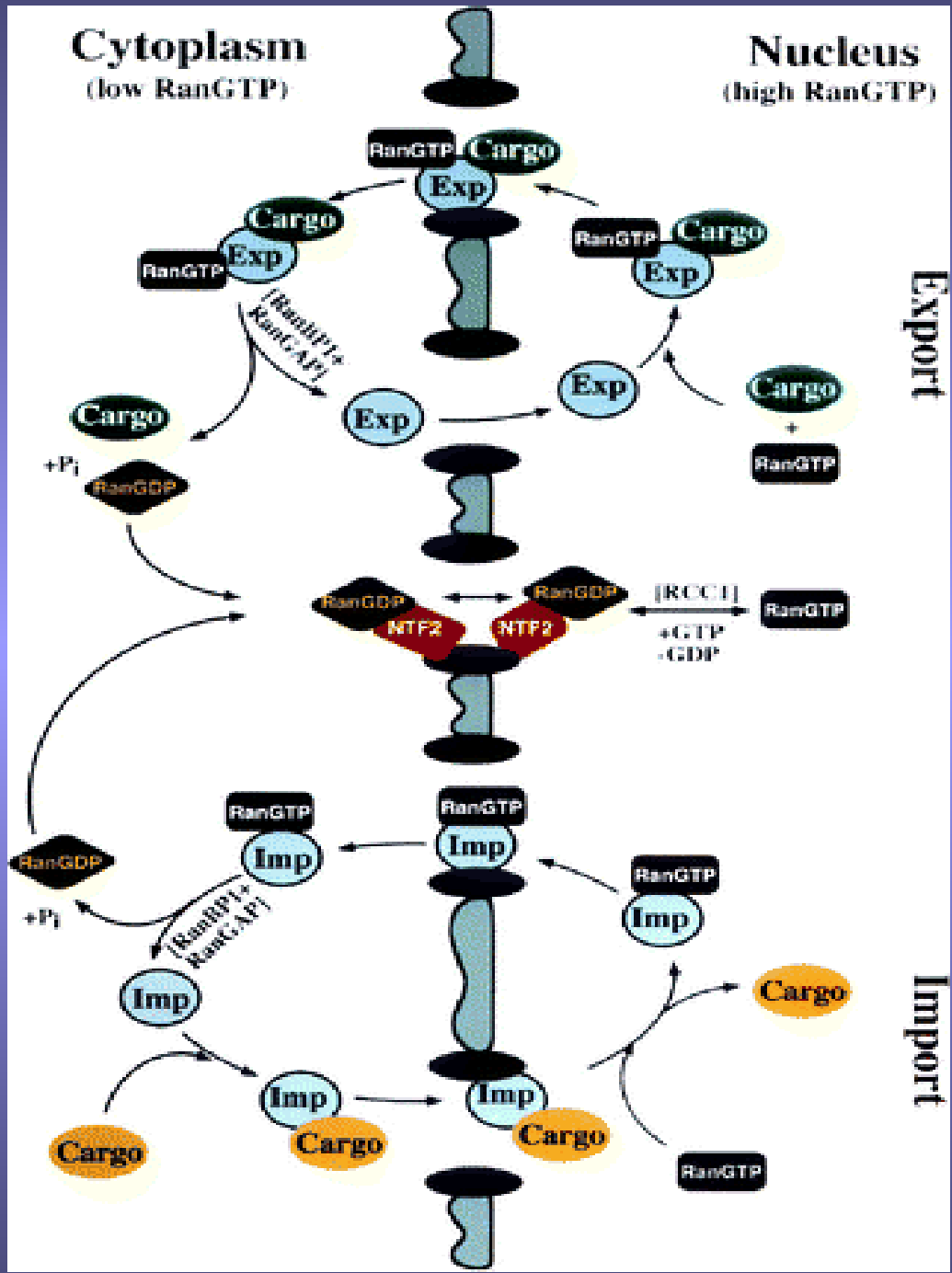
- carry by proteins and RNP to be able exit the nucleus

- consensus: **LxxxLxxLxL (V,I)**

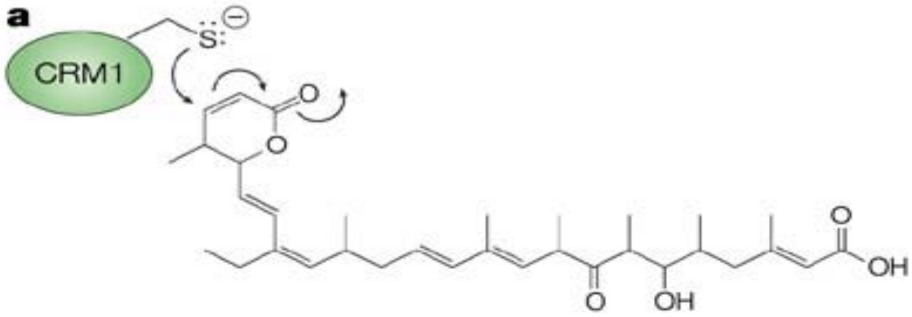
- exportin 1 – NES dependent export substrate receptor

- it have been identified in a variety of cellular and virial protein:

- MAPKK ALQKKLEELELD
- Rev LOLPPLERLTLD
- α-actin ALPHAILRLDLA

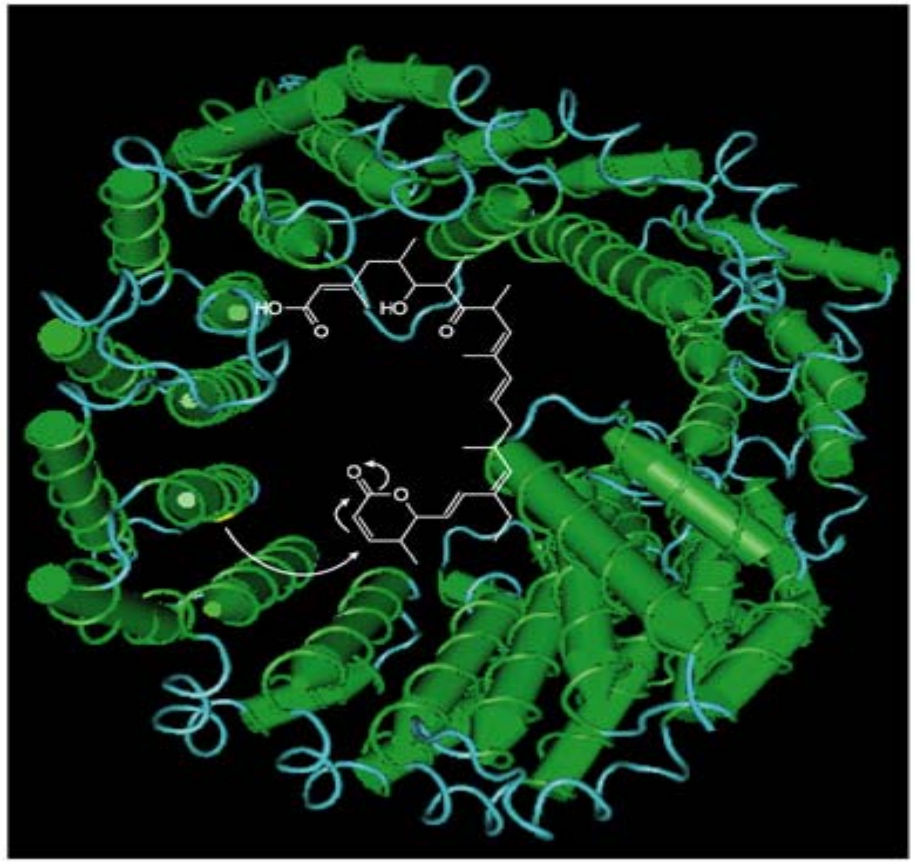

Cytoplasm
(low RanGTP)
Nucleus
(high RanGTP)
RanGTP
Cargo
Exp
Export
[RanBP1+ RanGAP]
+Pi
RanGDP
NTF2
[RCC1]
+GTP
-GDP
Imp
Import

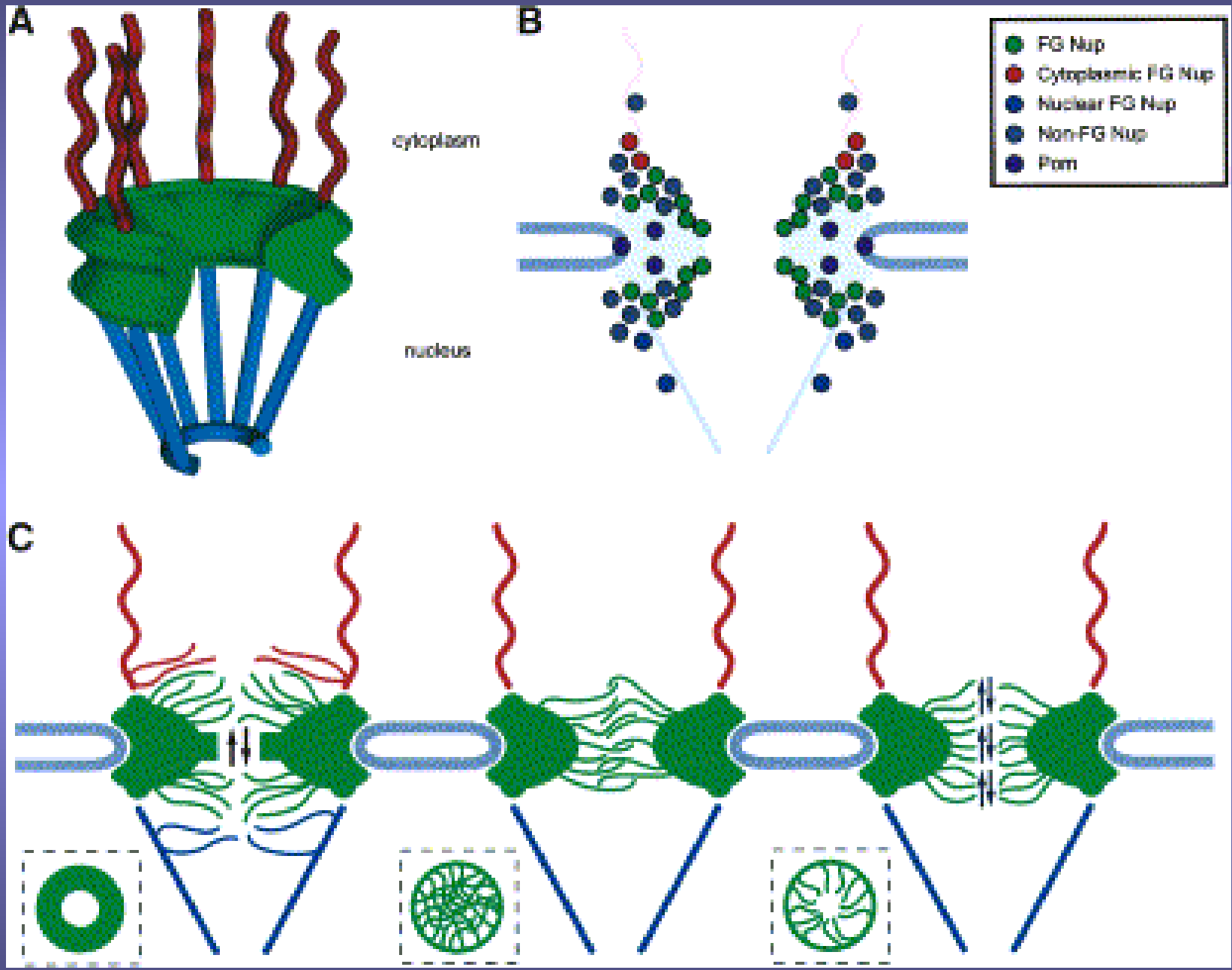
a

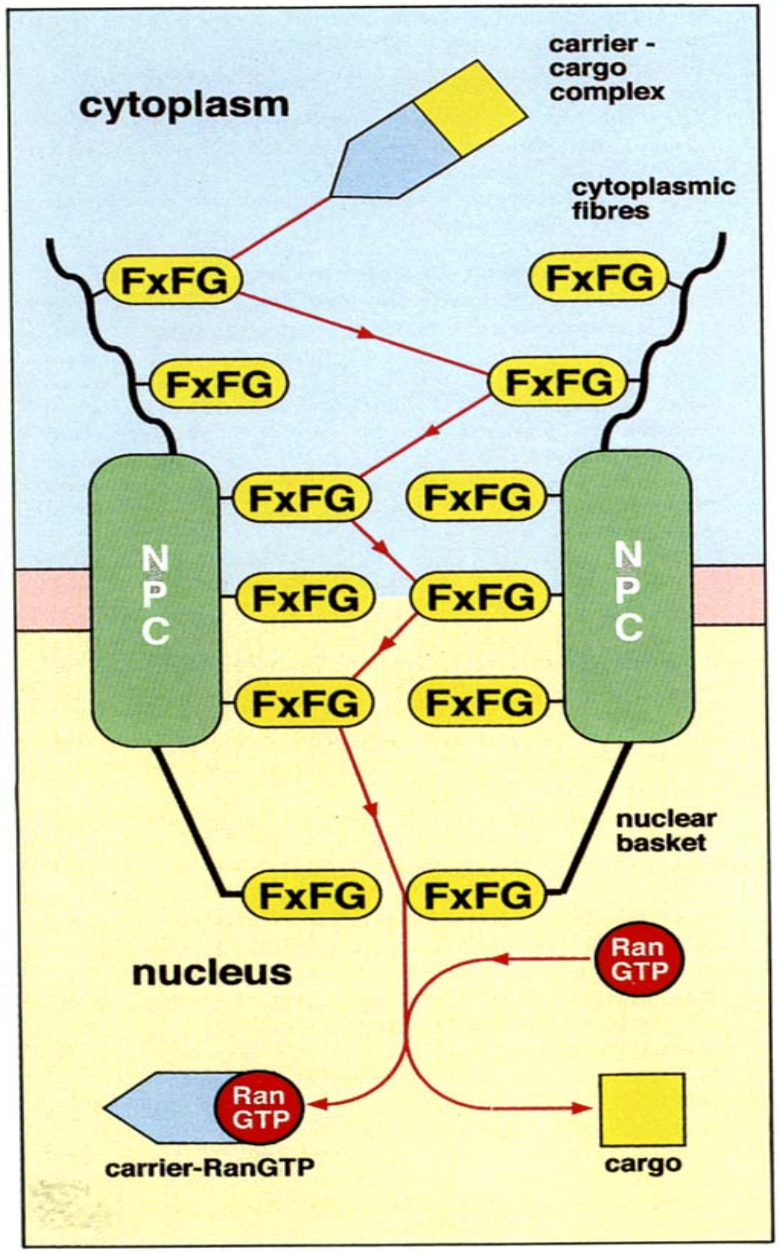


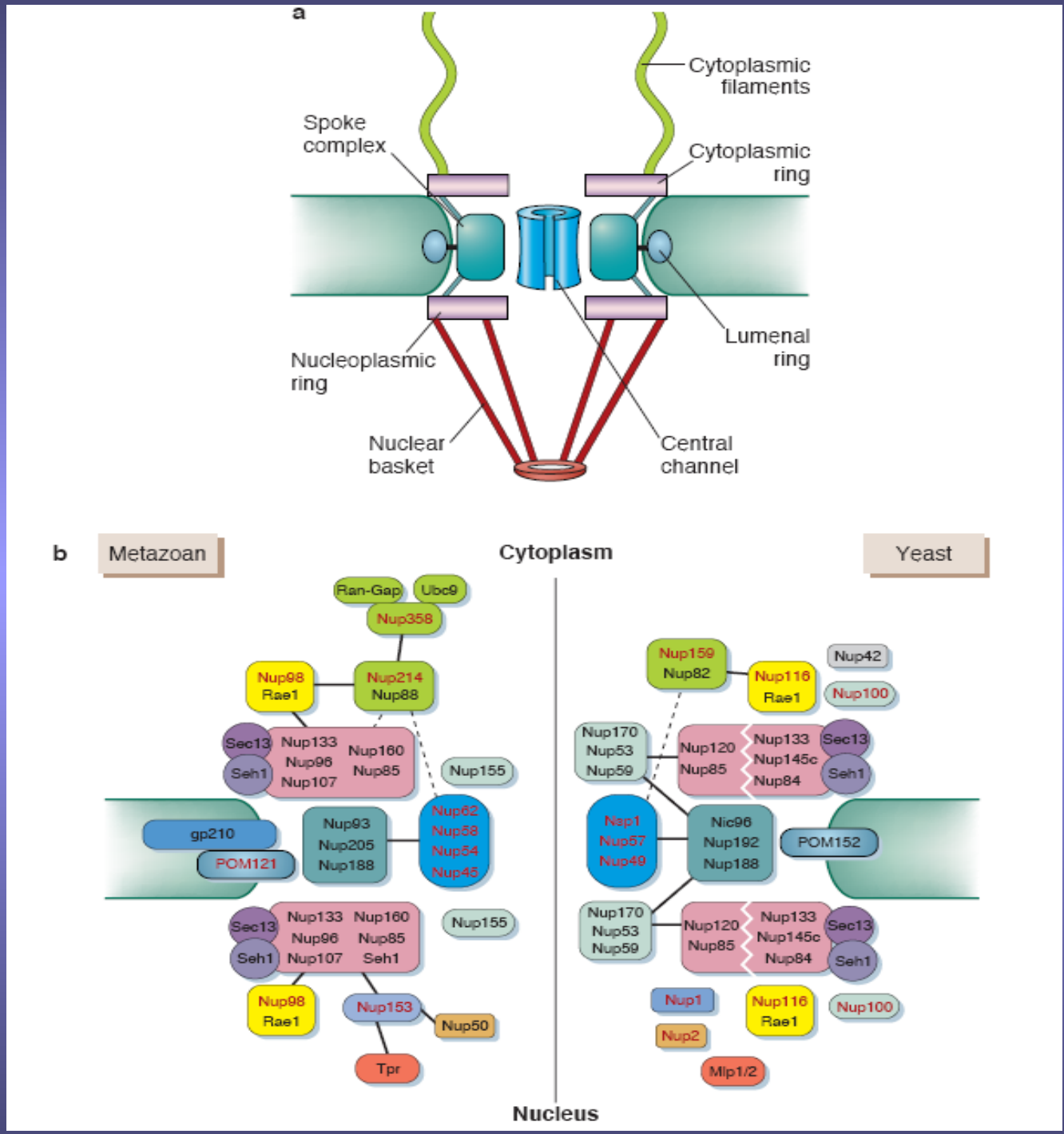
Leptomycin B

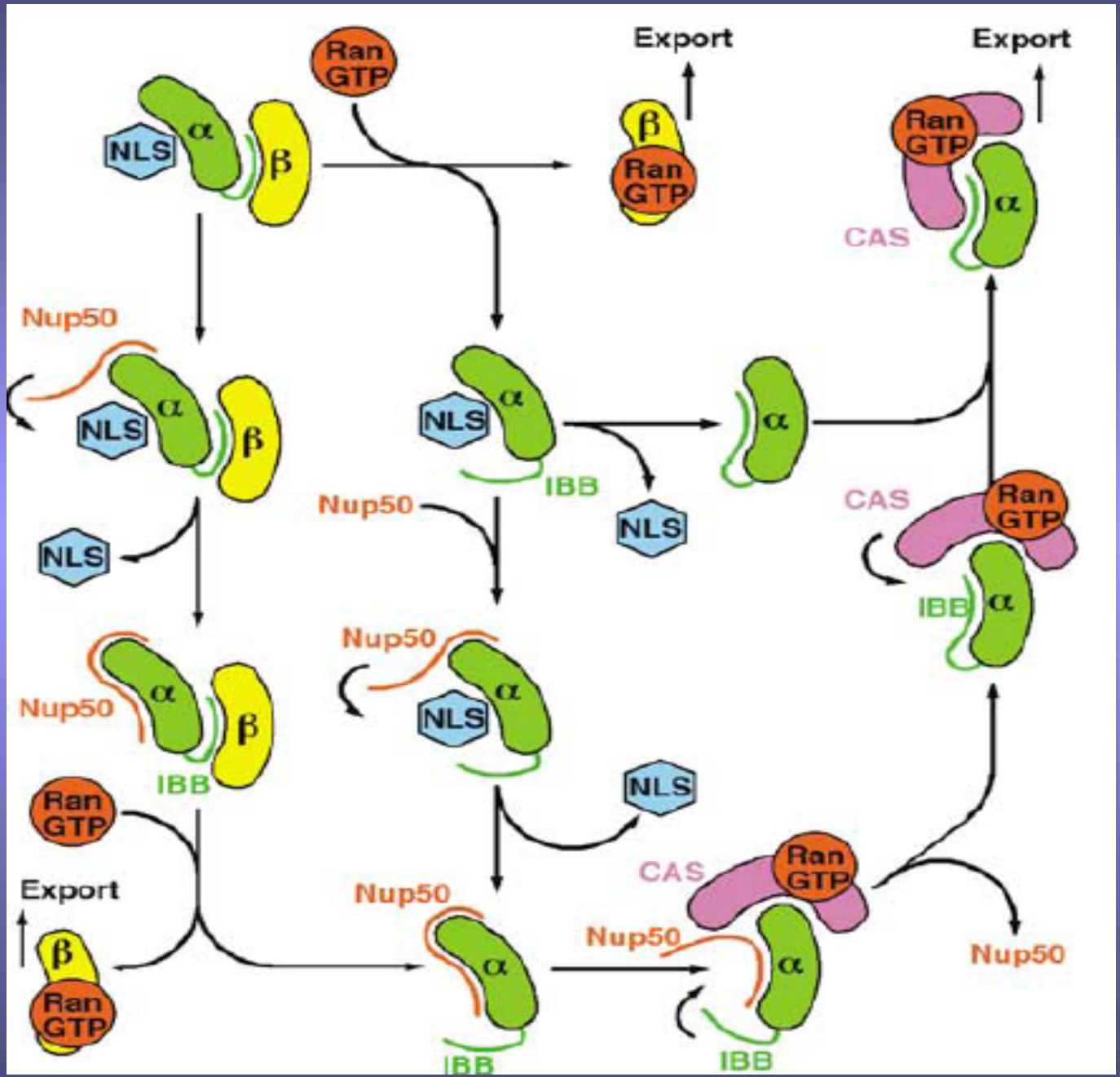
b

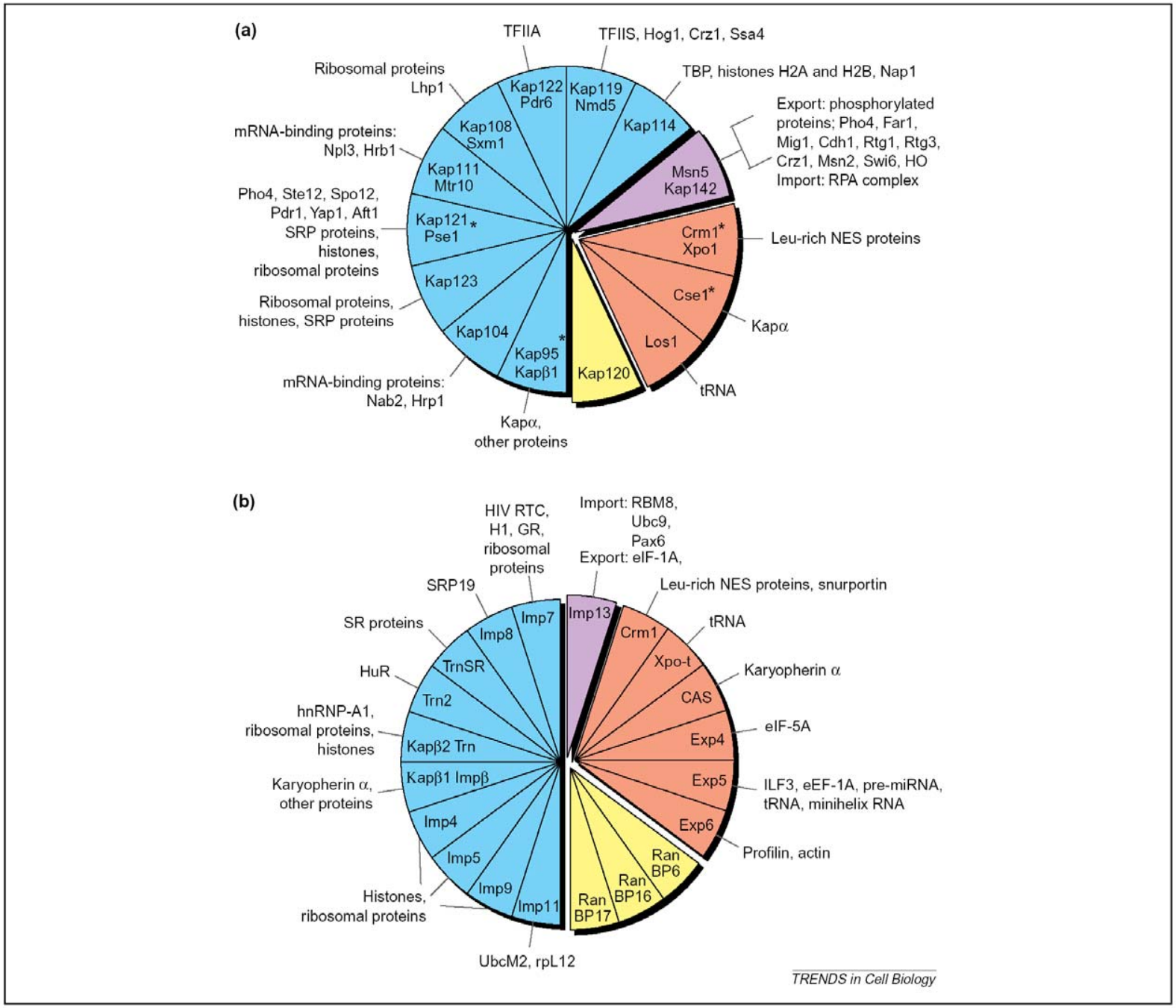



A
cytoplasm
nucleus
B
FG Nup
Cytoplasmic FG Nup
Nuclear FG Nup
Non-FG Nup
Pom
C


carrier -
cargo
complex
cytoplasm
cytoplasmic
fibres
FxFG
FxFG
FxFG
FxFG
FxFG
FxFG
FxFG
FxFG
FxFG
FxFG
FxFG
FxFG
N
P
C
N
P
C
nuclear
basket
nucleus
Ran
GTP
Ran
GTP
carrier-RanGTP
cargo

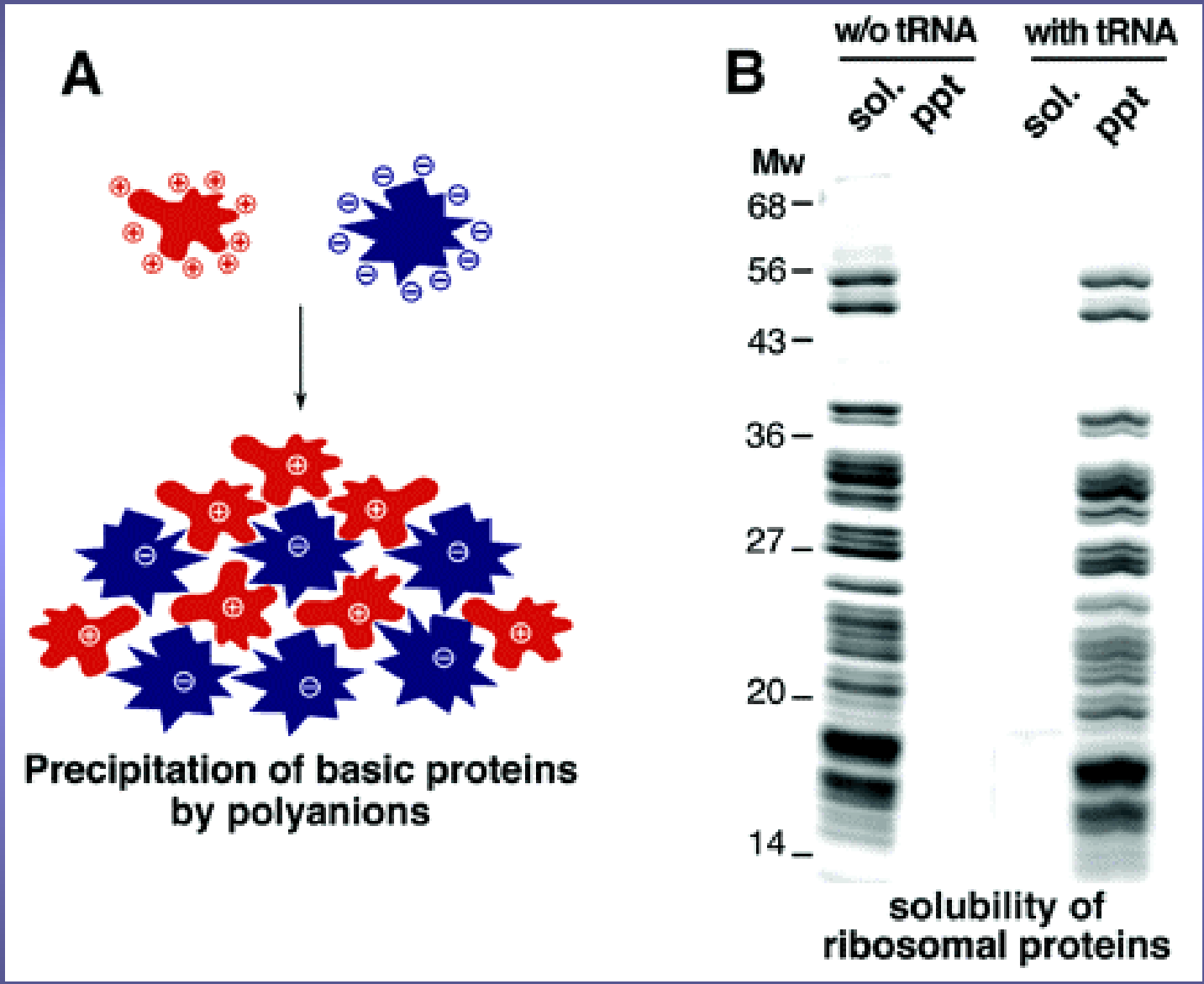

a
Cytoplasmic filaments
Spoke complex
Cytoplasmic ring
Lumenal ring
Nucleoplasmic ring
Nuclear basket
Central channel
b
Metazoan
Cytoplasm
Yeast
Ran-Gap
Ubc9
Nup358
Nup98 Rae1
Nup214 Nup88
Sec13
Seh1
Nup133 Nup96 Nup107
Nup160 Nup85
Nup155
gp210
POM121
Nup93 Nup205 Nup188
Nup62 Nup58 Nup54 Nup45
Nup159 Nup82
Nup116 Rae1
Nup42
Nup100
Nup170 Nup53 Nup59
Nup120 Nup85
Nup133 Nup145c Nup84
Sec13
Seh1
Nap1 Nup57 Nup49
Nic96 Nup192 Nup188
POM152
Nup1
Nup2
Mlp1/2
Nup153
Nup50
Tpr
Nucleus


Ran GTP
Export
Export
NLS
α
β
Ran GTP
CAS
Nup50
NLS
α
β
NLS
α
IBB
NLS
Nup50
NLS
Nup50
α
β
IBB
CAS
Ran GTP
IBB
α
Nup50
α
NLS
Ran GTP
NLS
Export
β
Ran GTP
Nup50
α
IBB
CAS
Ran GTP
Nup50
α
IBB
Nup50

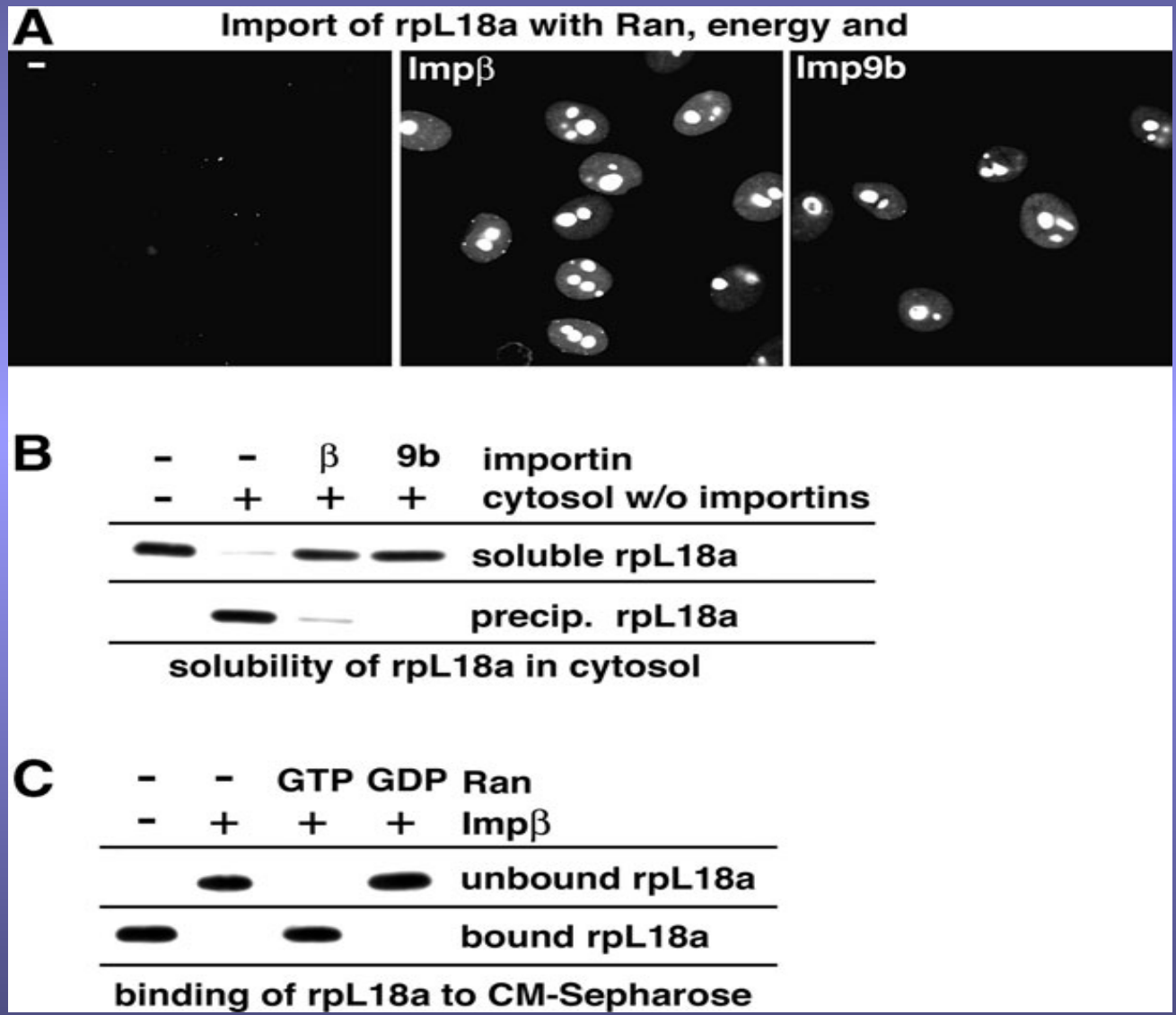


In HeLa cells ~20% of the total cellular protein interacts with members of the impβ family

Importins fulfil a dual function as nuclear import receptors and “basic chaperones” for exposed basic domains.


A
Precipitation of basic proteins
by polyanions
B
w/o tRNA
with tRNA
sol.
ppt
sol.
ppt
Mw
68
56
43
36
27
20
14
solubility of
ribosomal proteins

Importins are the only cytoplasmic factors that are capable of keeping very basic polypeptides such as ribosomal proteins or histons soluble in the cytosol at the physiological, i.e. low micromolar, concentration.



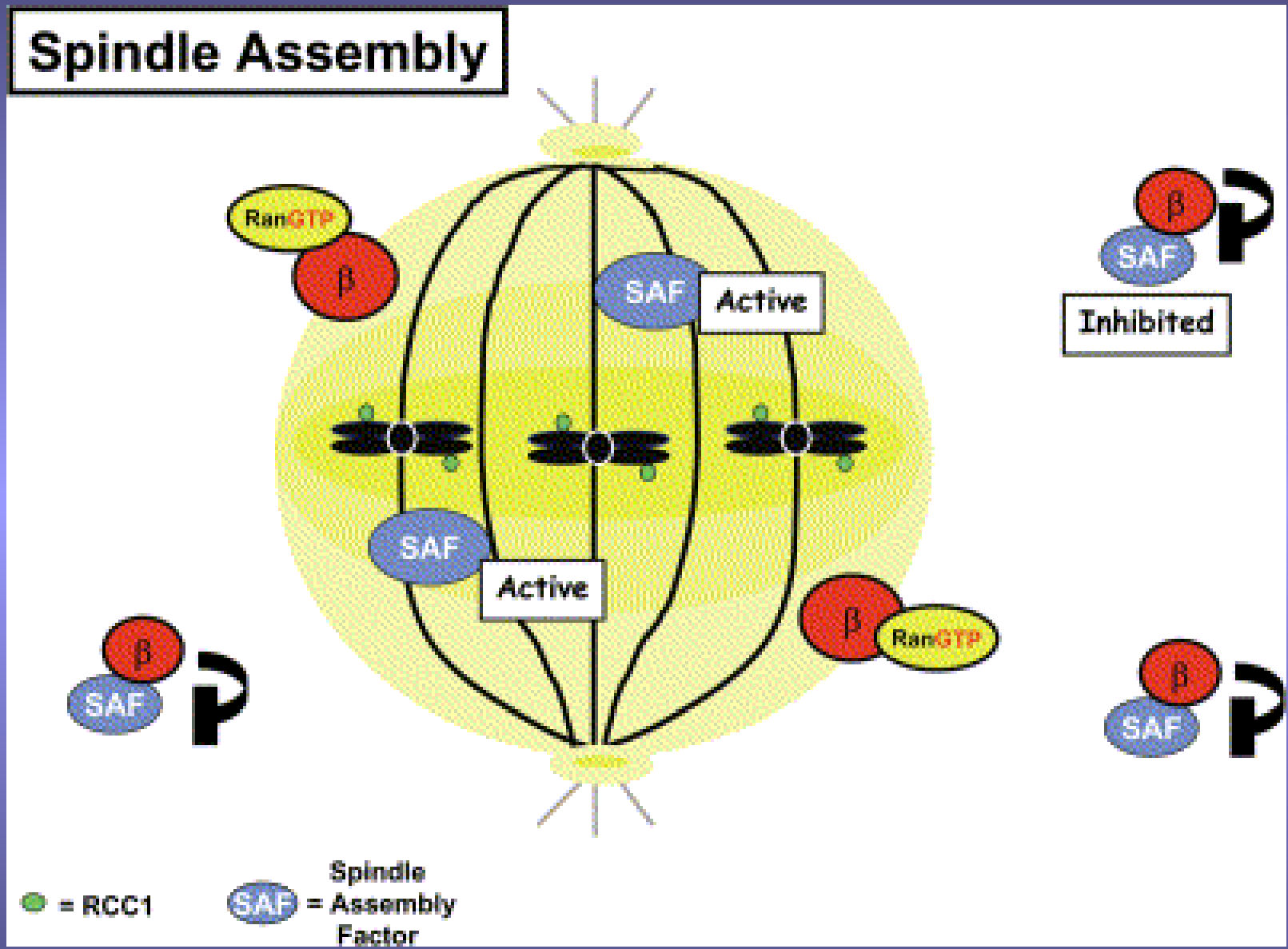
Jakel *et al.* EMBO J., 2002, 21, 377-386.

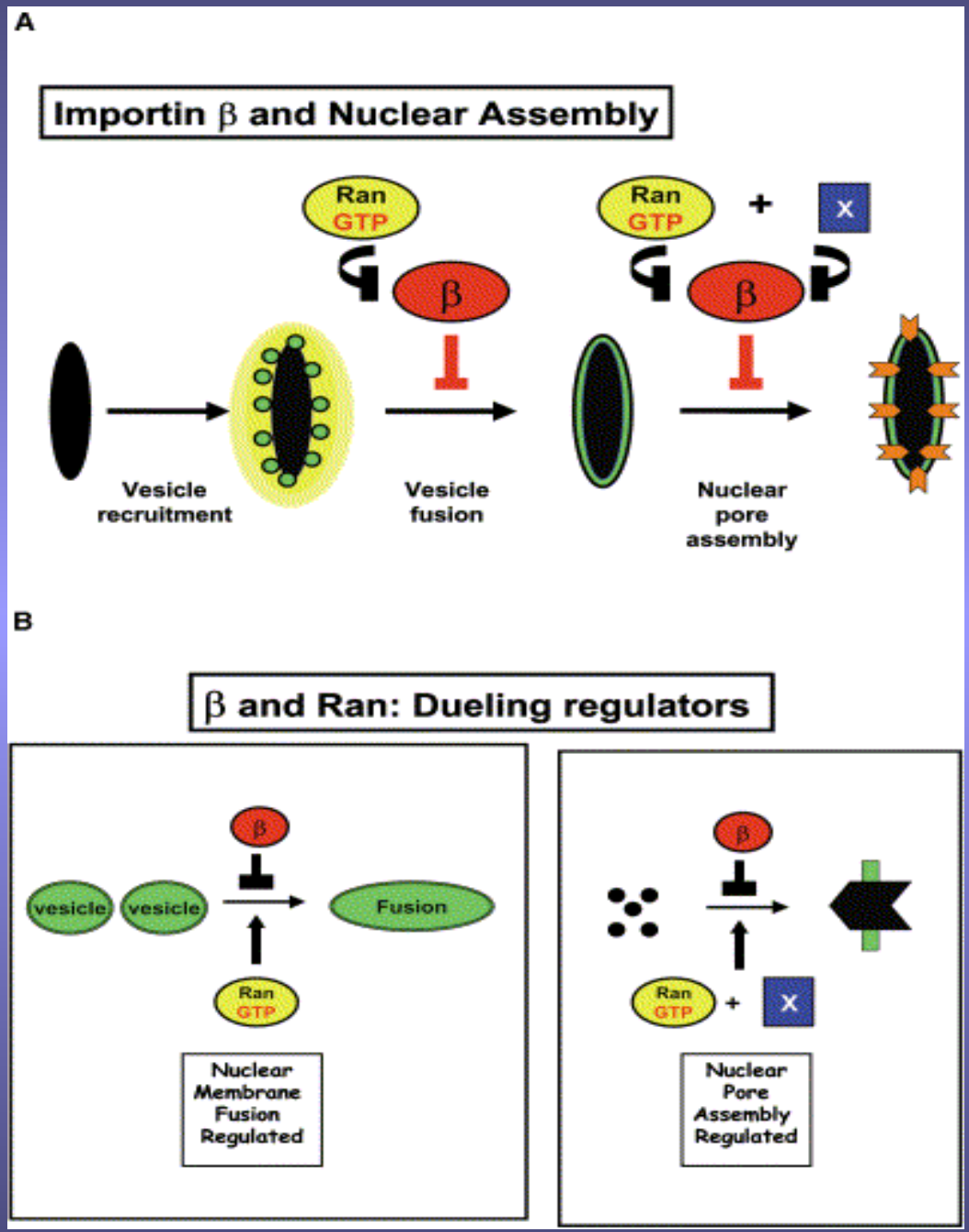
The total nuclear import receptor concentration in the cytosol might amount to 10 μM or >1mg/ml. Importins are thus concentrated similarly to abundant HSPs (which bind folding and assembly intermediates with exposed hydrophobic regions).

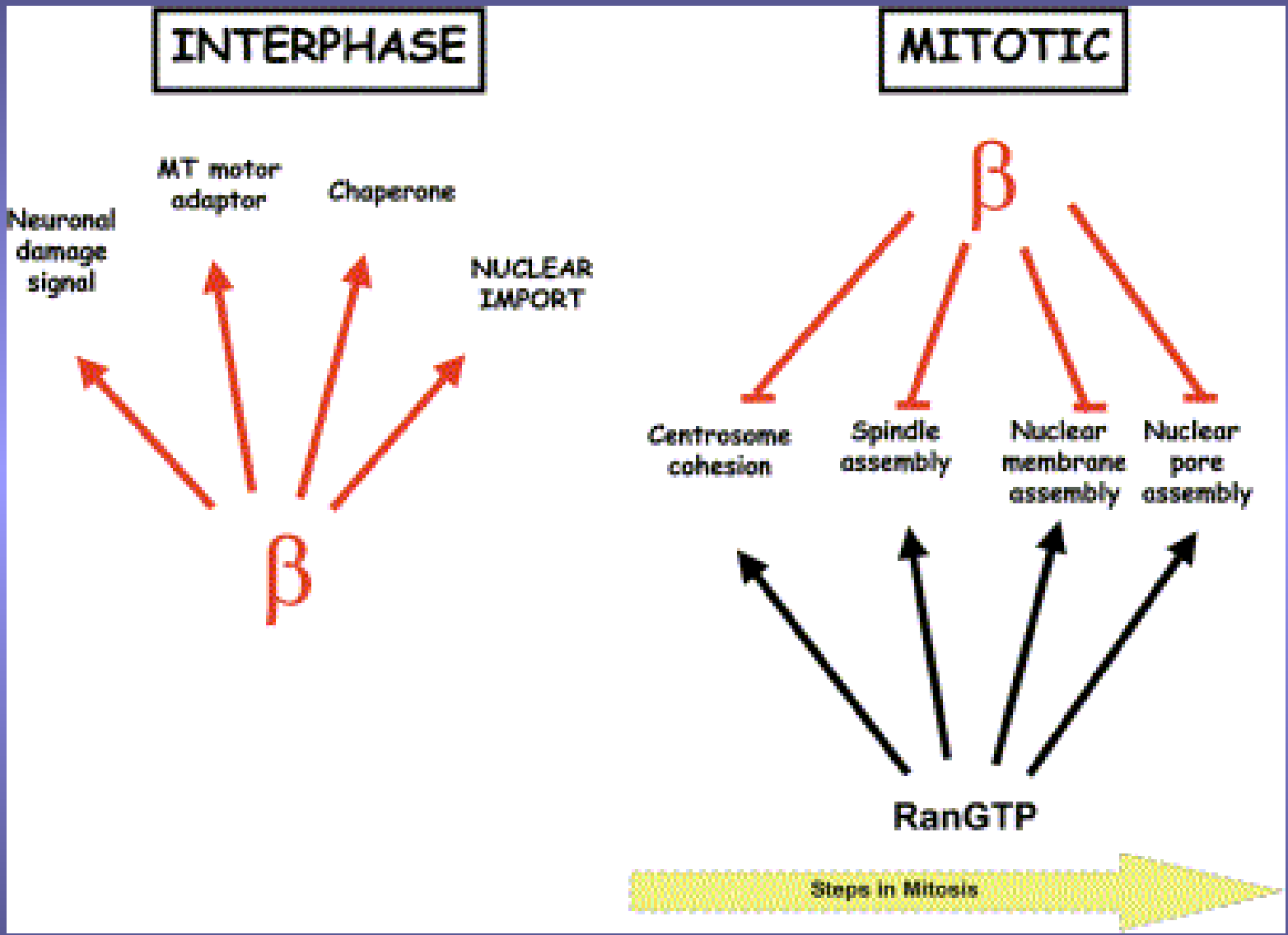
The anti-aggregation activity of importins should have been useful even before nuclear pores were functional and might thus represent the most ancient part of the nuclear transport machinery.

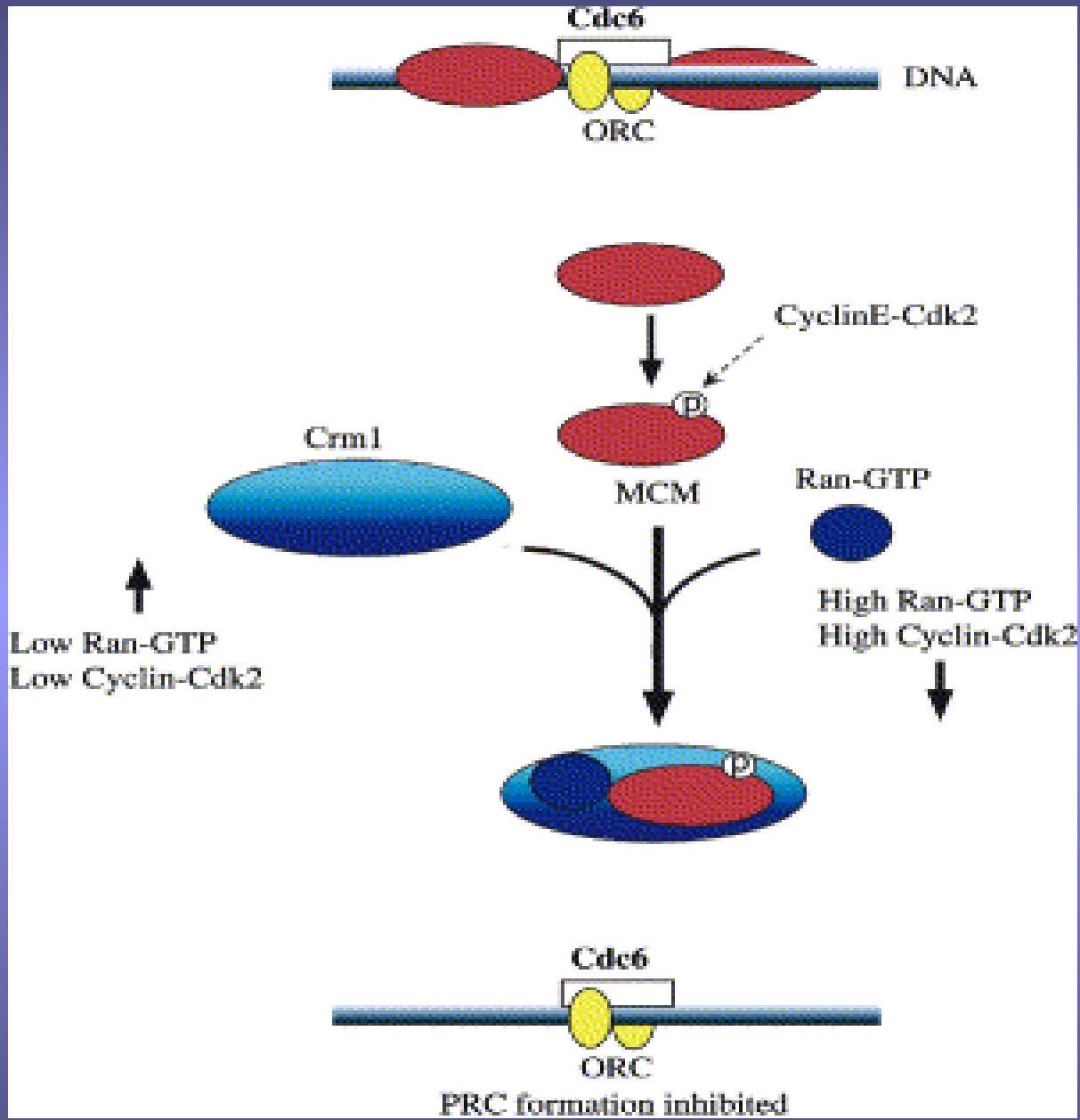
In the cell cycles of animal cells, the nucleus is disassembled at M-phase: what happens to the nucleocytoplasmic transport machinery under these condition?

Spindle Assembly

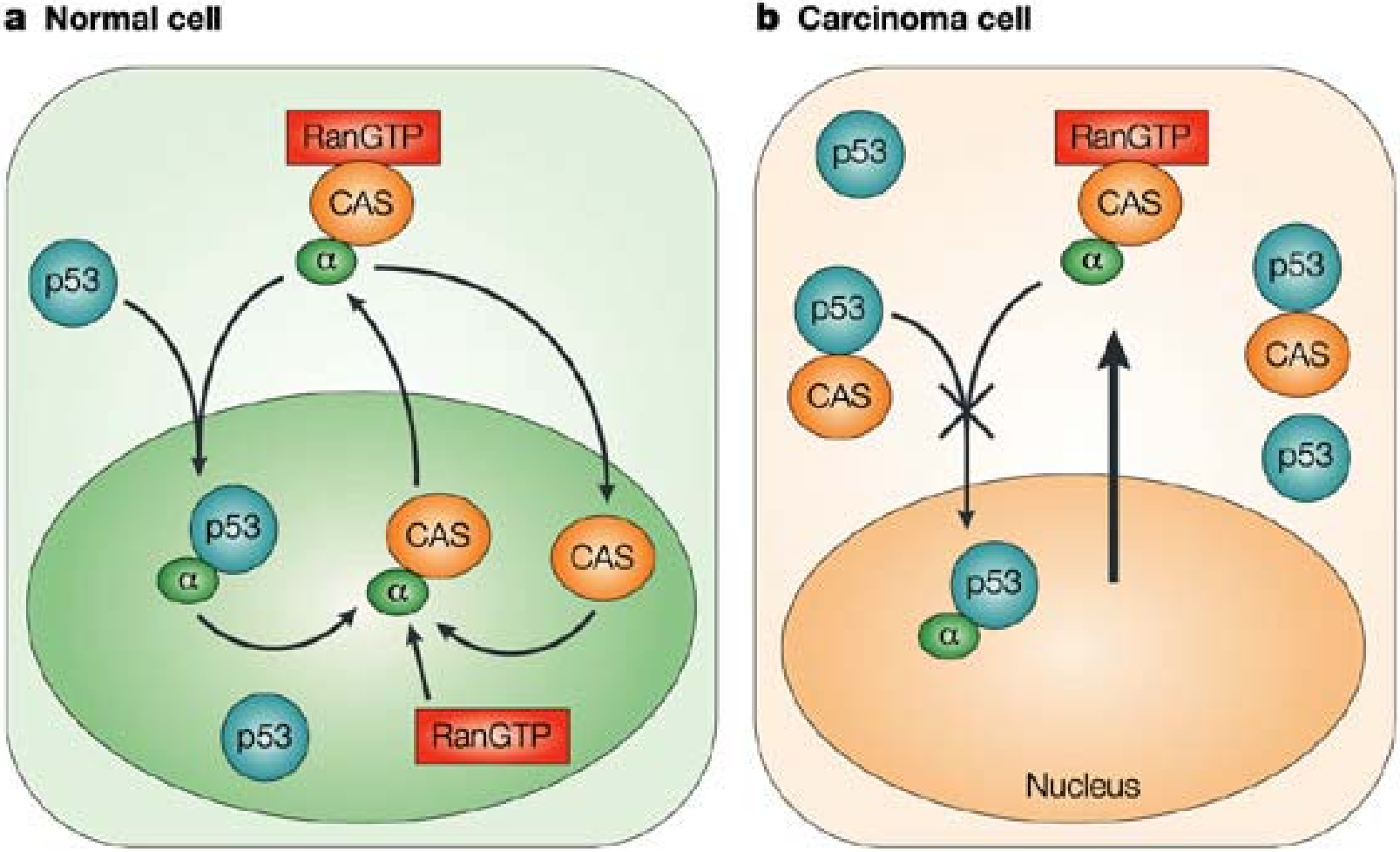



A
Importin β and Nuclear Assembly
Ran
GTP
β
Ran
GTP
+
X
β
Vesicle
recruitment
Vesicle
fusion
Nuclear
pore
assembly
B
β and Ran: Dueling regulators
β
vesicle
vesicle
Fusion
Ran
GTP
Nuclear
Membrane
Fusion
Regulated
β
Ran
GTP
+
X
Nuclear
Pore
Assembly
Regulated


INTERPHASE
MT motor adaptor
Chaperone
Neuronal damage signal
NUCLEAR IMPORT
β
MITOTIC
β
Centrosome cohesion
Spindle assembly
Nuclear membrane assembly
Nuclear pore assembly
RanGTP
Steps in Mitosis



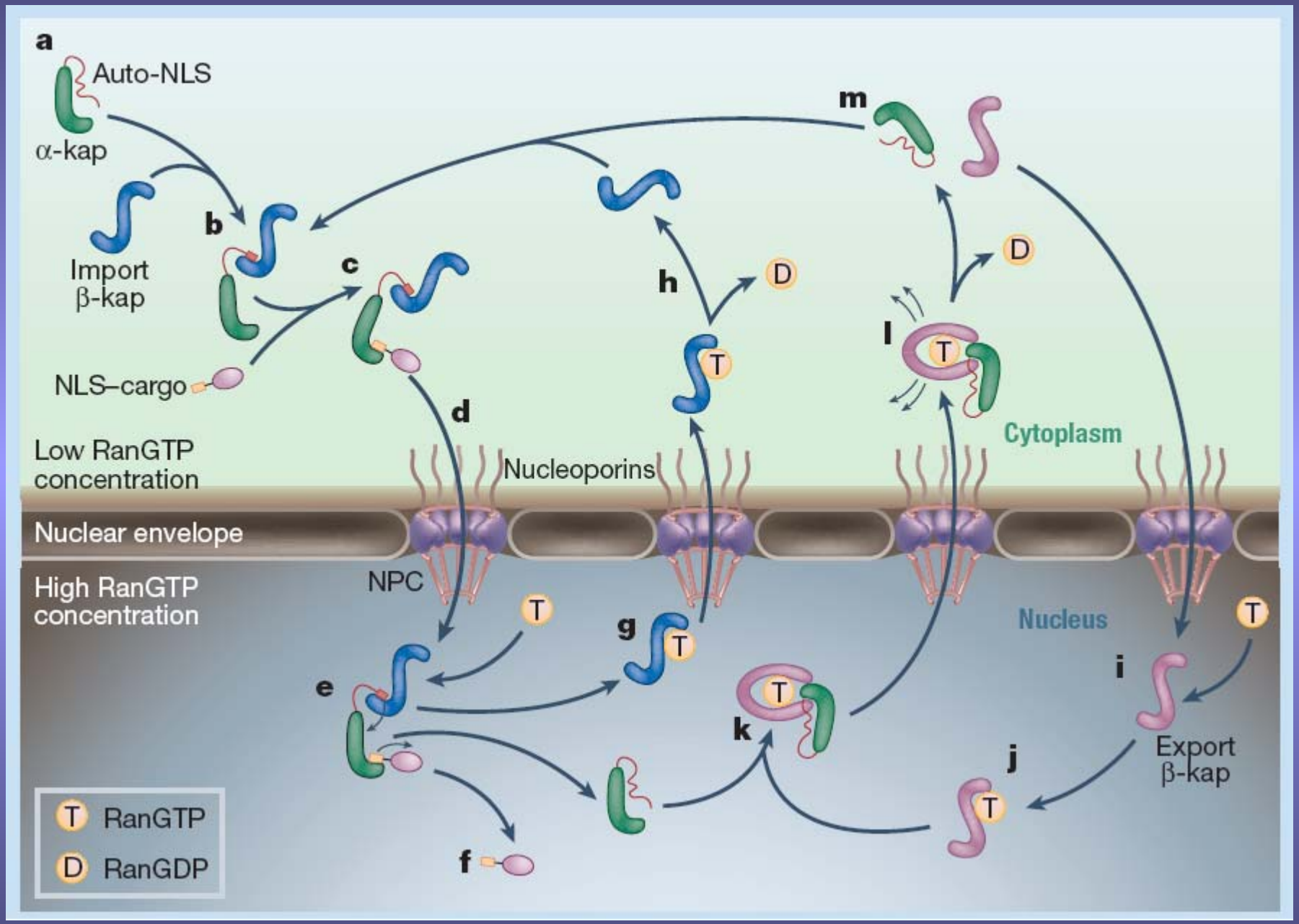
Yamaguchi and Newport. Cell, 2003, 113, 115-125.



Nature Reviews | Cancer

Ran is a master regulator of nuclear physiology

- in interphase, it flags the position of the nucleus and provides directionality to nucleocytoplasmic transport
- in ana- and metaphase it regulates microtubule nucleation around chromatin
- in telophase it is required for nuclear envelope and NPC formation
- in S-phase it blocks re-replication


a
Auto-NLS
α-kap
Import β-kap
b
c
NLS–cargo
d
Low RanGTP concentration
Nucleoporins
Nuclear envelope
NPC
High RanGTP concentration
e
f
g
h
D
T
m
l
Cytoplasm
Nucleus
i
Export β-kap
j
k
T RanGTP
D RanGDP