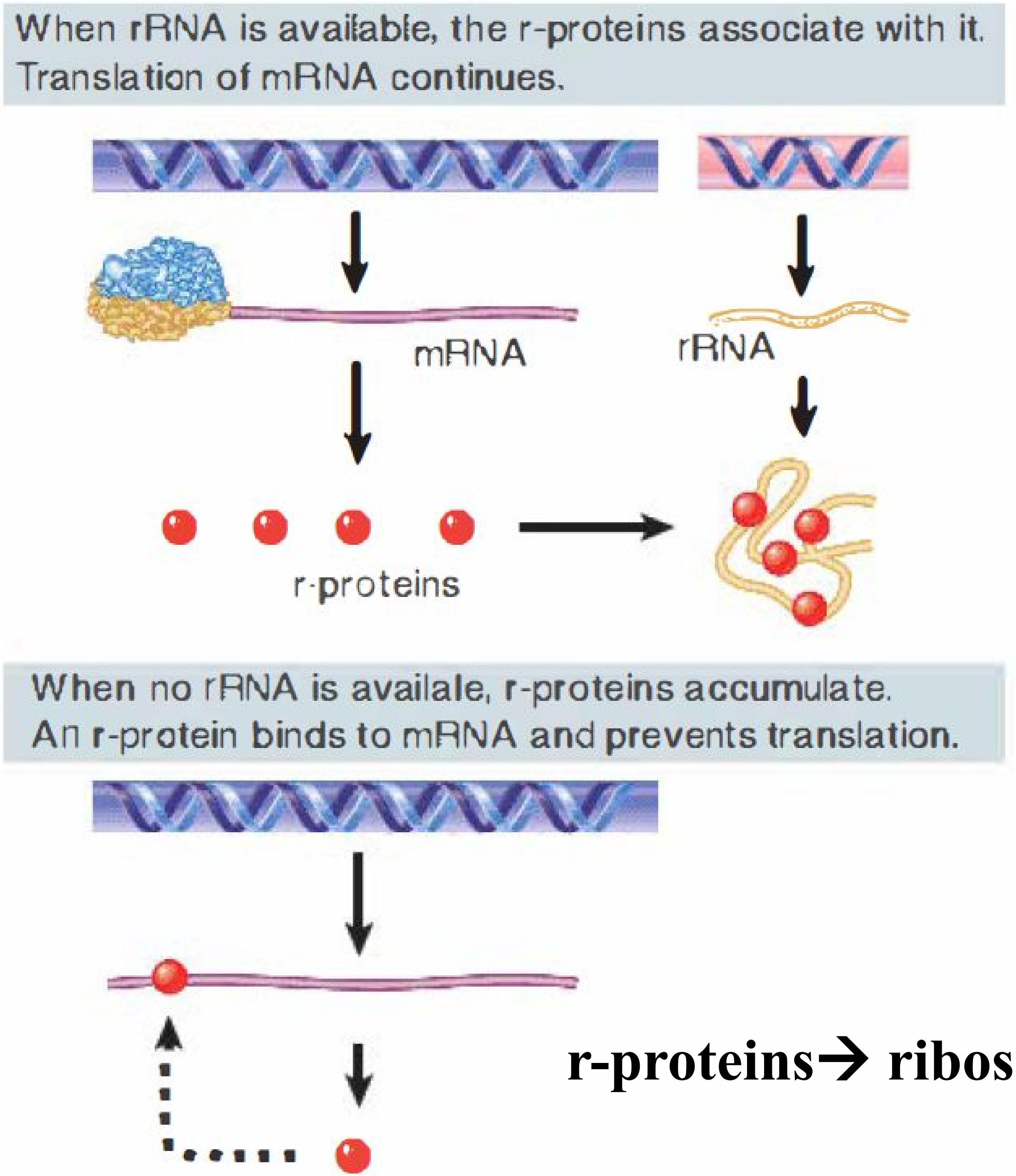
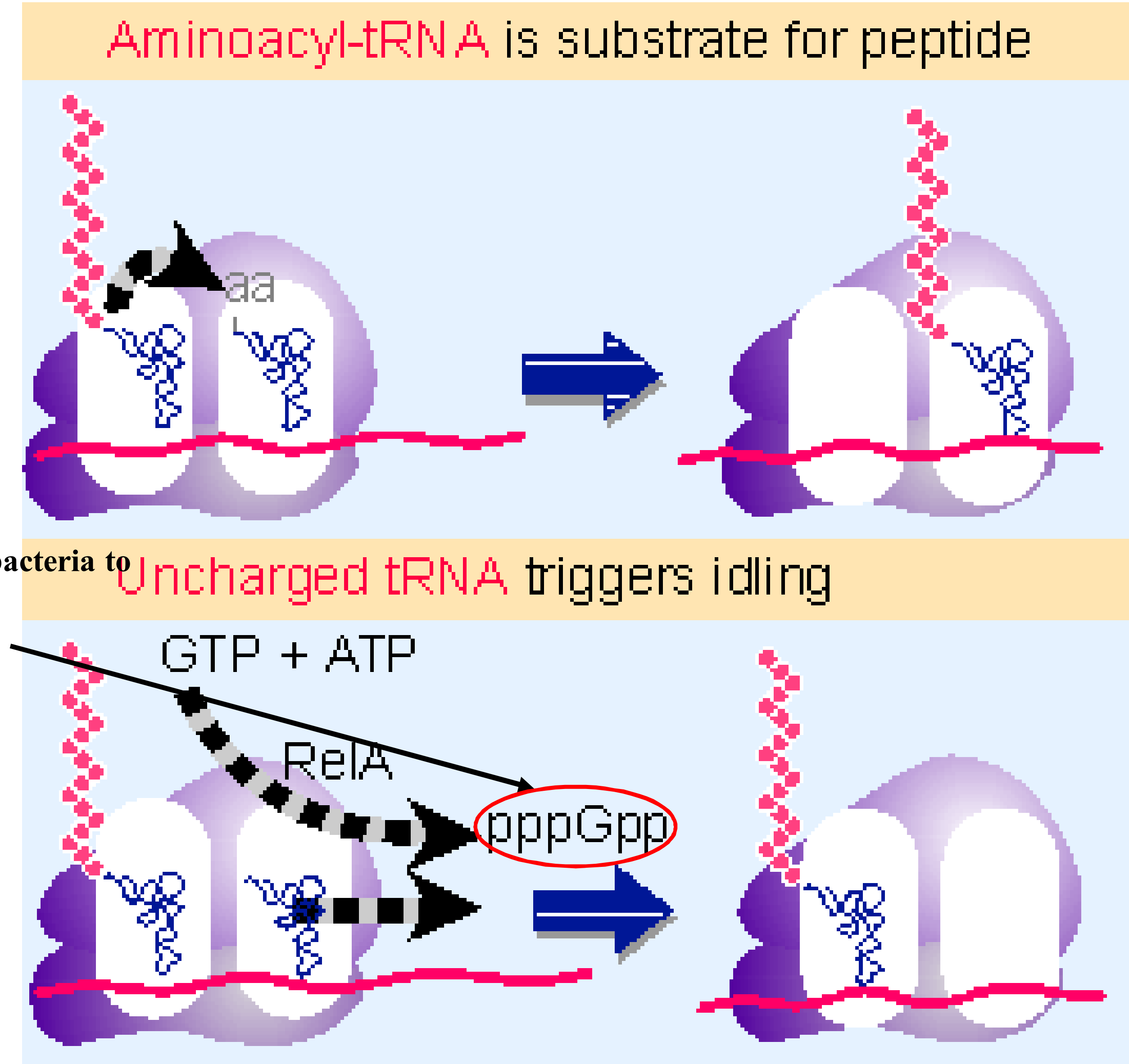


r-proteins→ ribosomal proteins

FIGURE 26.42 Translation of the r-protein operons is autogenously controlled and responds to the level of rRNA.

<u>Stringent</u> response: when bacteria find themselves in such poor growth conditions that they lack a sufficient supply of amino acids to sustain protein synthesis, they shut down a wide range of activities.

Small molecule used by bacteria to regulate gene expression



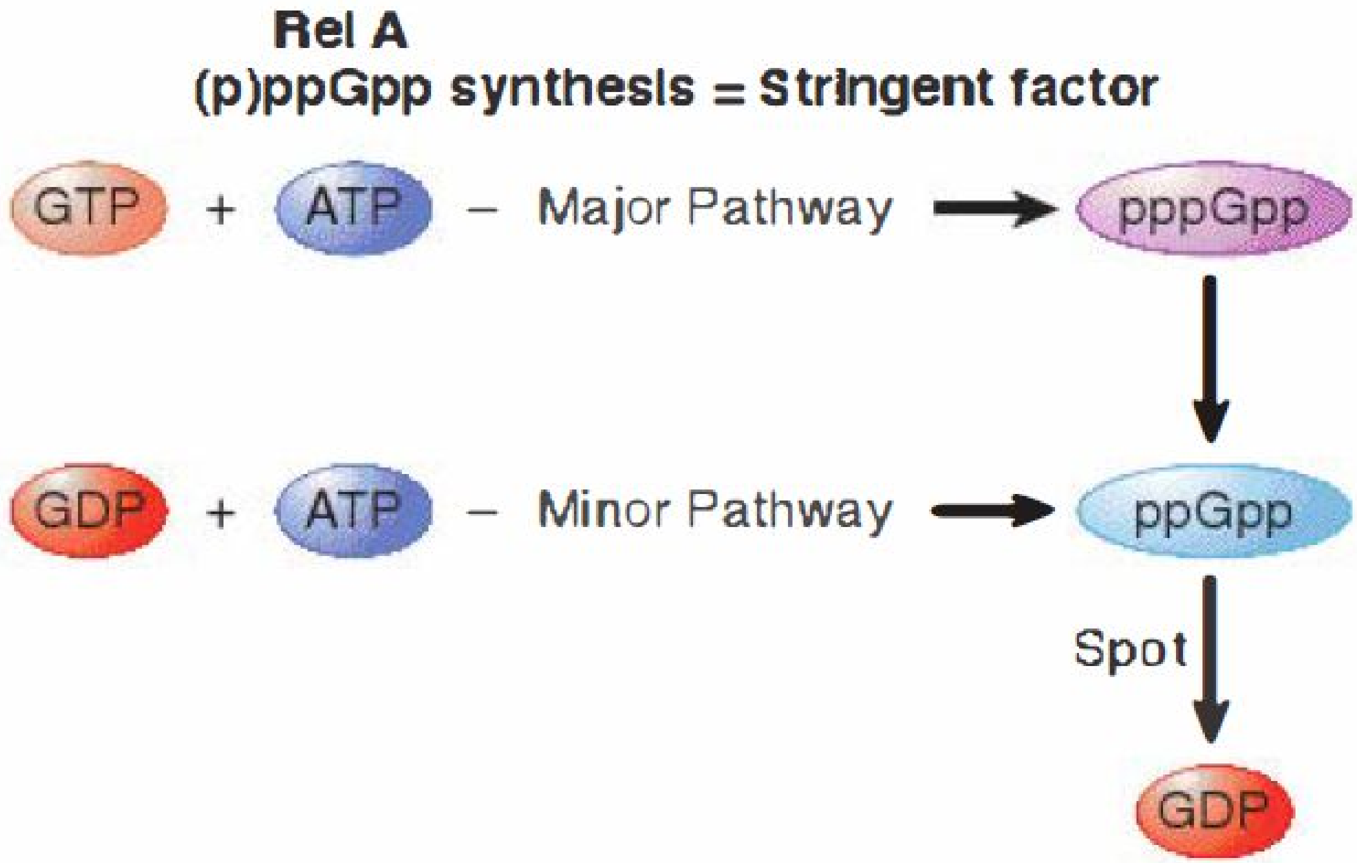
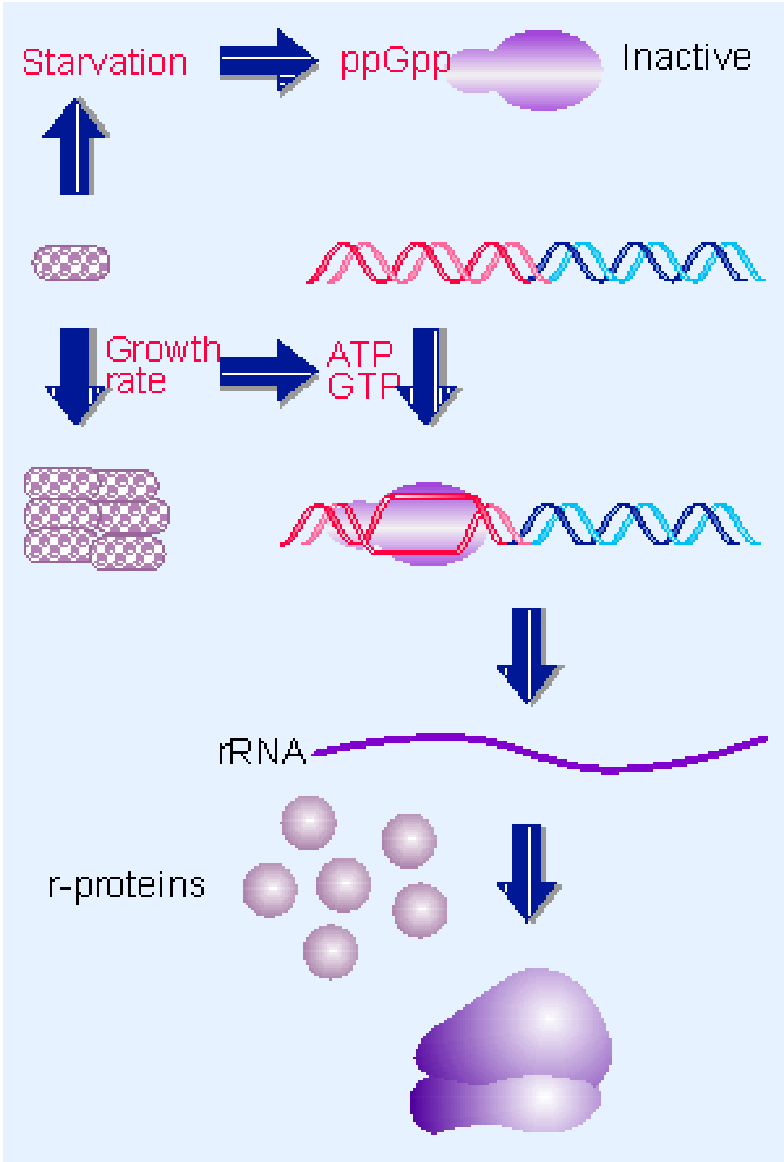
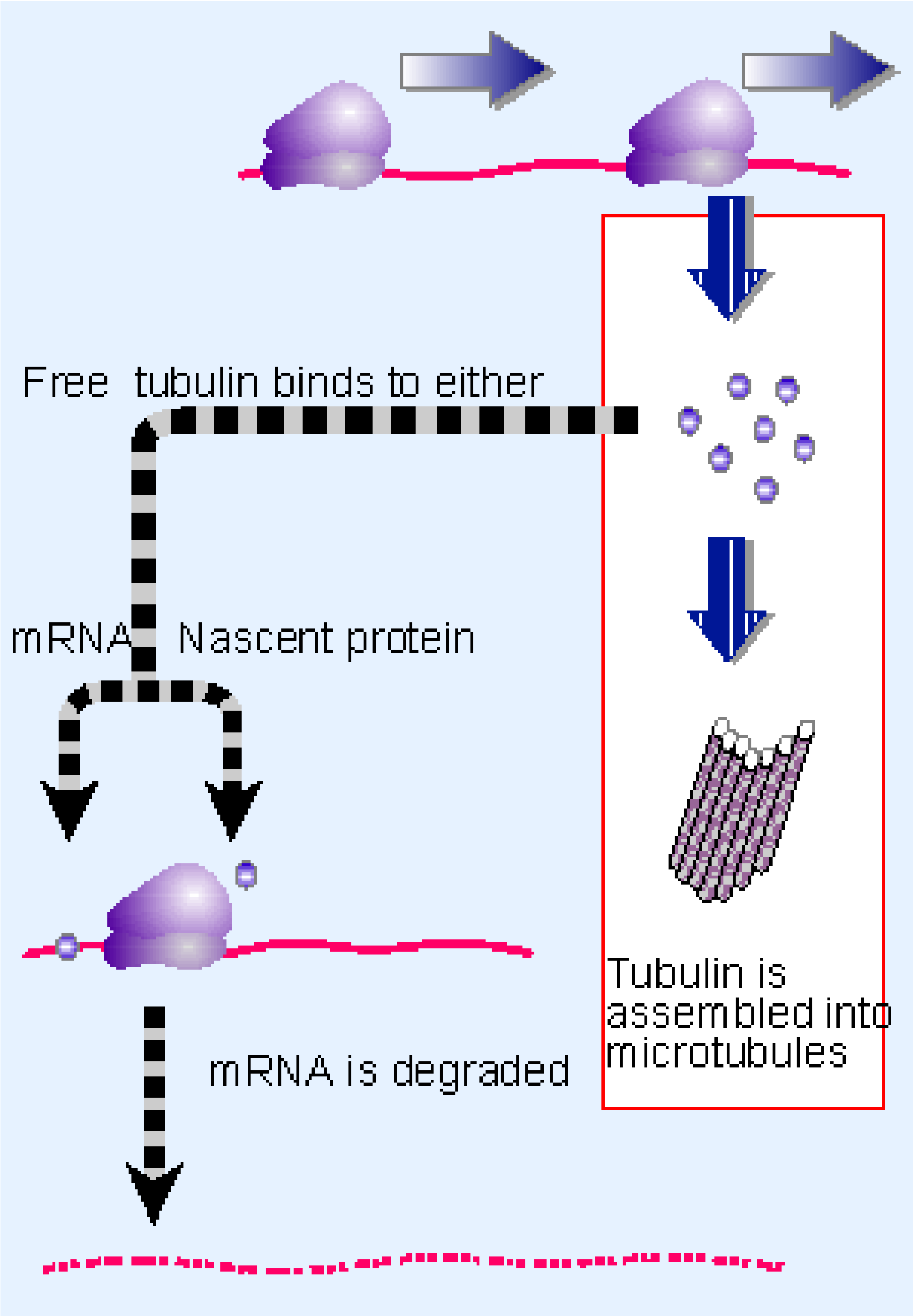


FIGURE 26.40 Stringent factor catalyzes the synthesis of **pppGpp and** ppGpp; ribosomal proteins can dephosphorylate **pppGpp** to **ppGpp. ppGpp** is degraded when it is no longer needed.



Nucleotide levels control initiation of rRNA transcription.



Tubulin is one of several members of a small family of globular proteins. The most common members of the tubulin family are α-tubulin and β-tubulin, the proteins that make up microtubules.

Tubulin is assembled into microtubules when it is synthesized. Accumulation of excess free tubulin induces instability in the tubulin mRNA by acting at a site at the start of the reading frame in mRNA or at the corresponding position in the nascent protein.

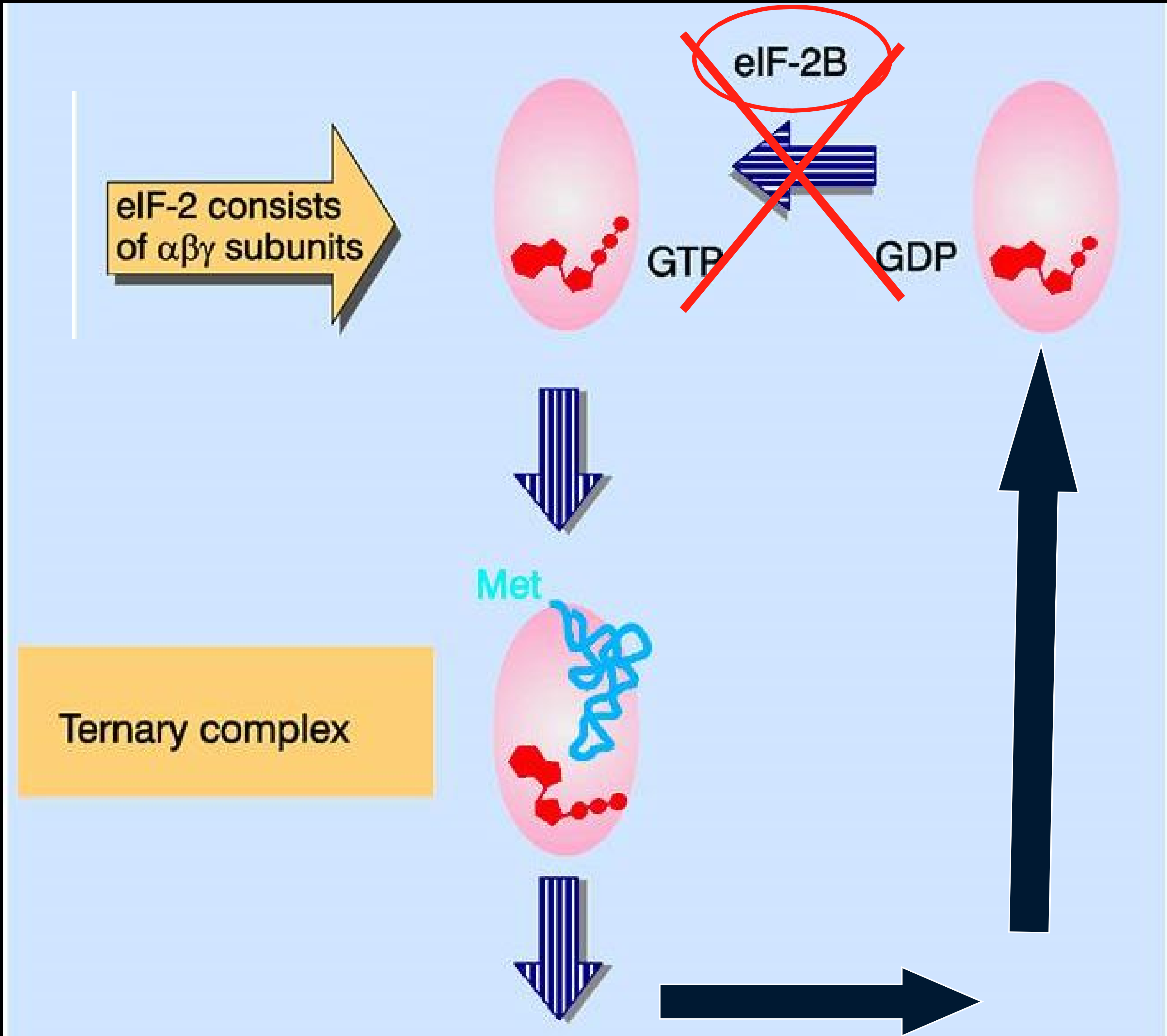
Regulation of translation

- General
- Specific

Initiation Factors		Activity
prokaryotes	*eukaryotes*	
IF3	eIF-1	Fidelity of AUG codon recognition
IF1	eIF-1A	Facilitate Met-tRNAiMet binding to small subunit
	eIF-2	Ternary complex formation
	eIF-2B (GEF)	GTP/GDP exchange during eIF-2 recycling
	eIF-3 (12 subunits)	Ribosome antiassociation, binding to 40S
	eIF-4F (4E, 4A, 4G)	mRNA binding to 40S, RNA helicase activity
	eIF-4A	ATPase-dependent RNA helicase
	eIF-4E	5' cap recognition
	eIF-4G	Scaffold for of eIF-4E and -4A
	eIF-4B	Stimulates helicase, binds with eIF-4F
	eIF-4H	Similar to eIF4B
	eIF-5	Release of eIF-2 and eIF-3, GTPase
IF2	eIF5B	Subunit joining
	eIF-6	Ribosome subunit antiassociation

eIF2

- 3 subunits: α, β, γ
- β subunit helps the GTPase activity and regulates tRNAi-eIF2 γ binding
- α subunit is a translation regulator. It is phosphorylated (ser 51) by different kinases as stress response.


eIF-2B
eIF-2 consists
of αβγ subunits
GTP
GDP
Met
Ternary complex

eIF4F

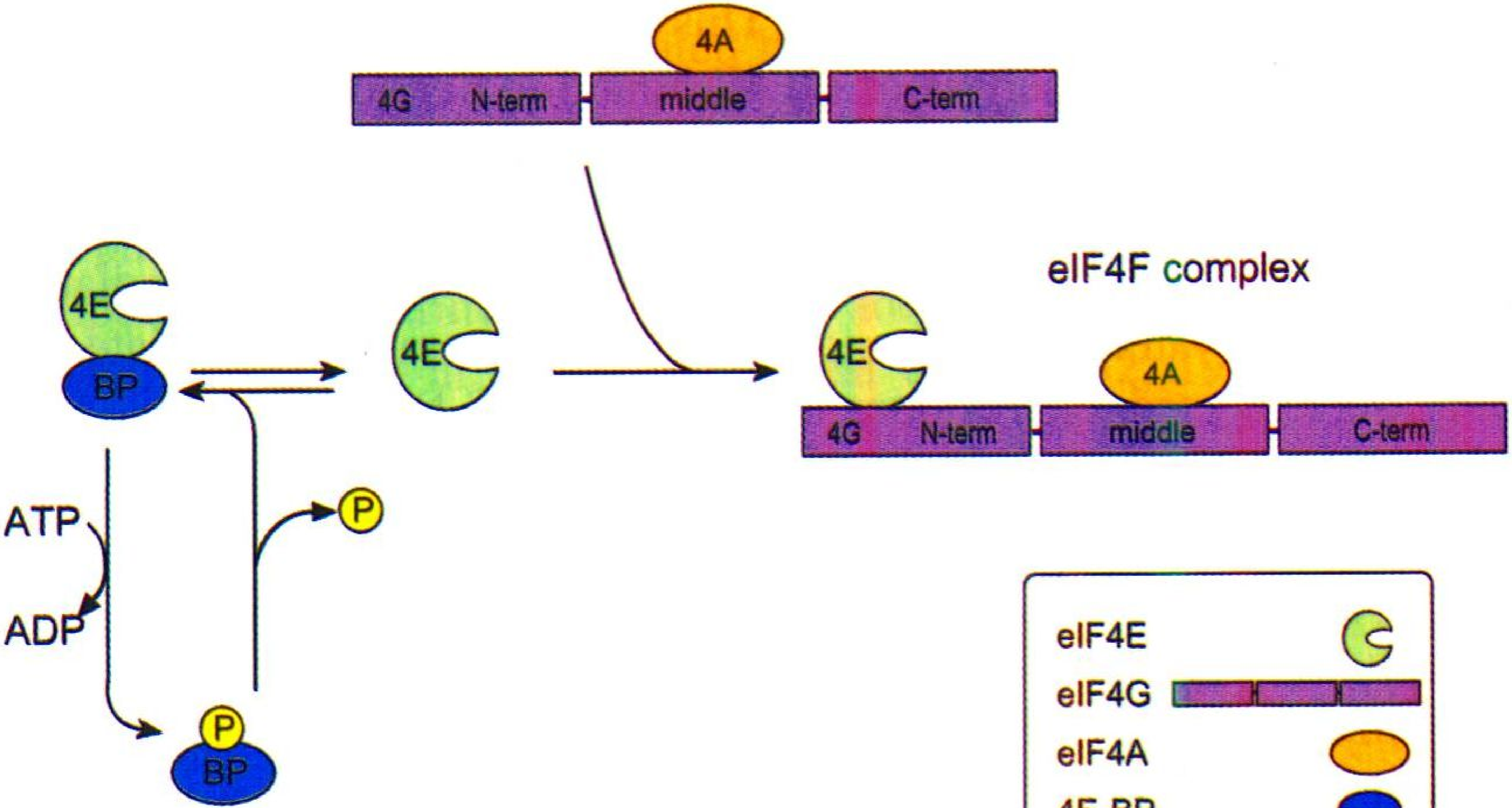
- **Made by 3 subunits**

  <u>eIF4A</u>: elicase, helped by eIF4B

  <u>eIF4E</u>: cap binding protein, regulated by phosphorylation and interaction with eIF4E-BP

  <u>eIF4G</u>: adaptor, interacts with many factors

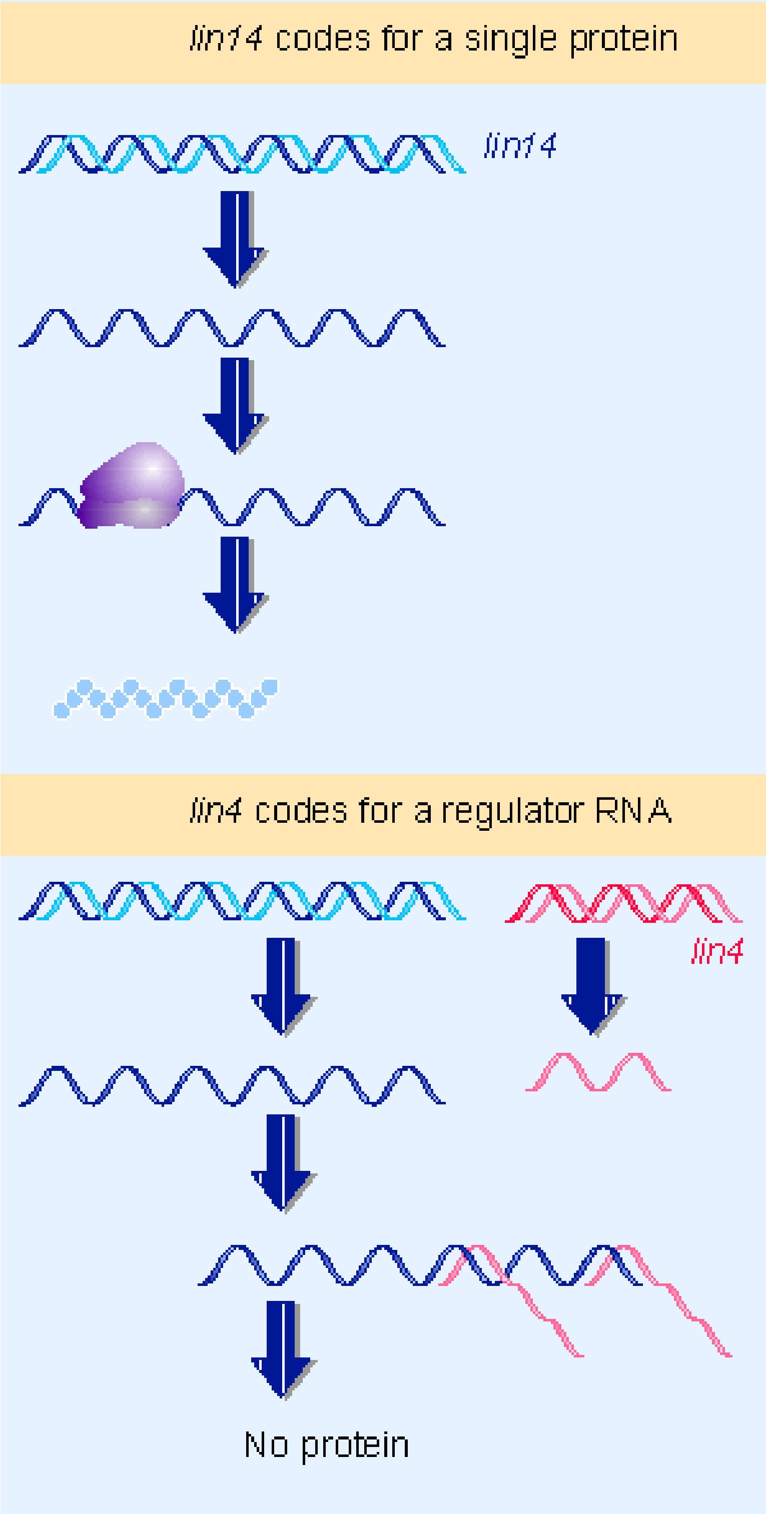
eIF4F



4A
4G N-term
middle
C-term
eIF4F complex
4E
BP
4E
4E
4A
4G N-term
middle
C-term
ATP
ADP
P
P
BP
eIF4E
eIF4G
eIF4A
4E-BP
phosphate group
P

Regulation of translation

- General
- Specific



<u>lin-14</u> encodes a protein whose activity is required for specifying the division timings of a specific group of cells during postembryonic development in the nematode *Caenorhabditis elegans*.

dsRNA

- Viral origin
- Hexogen (artificially inserted in cells)

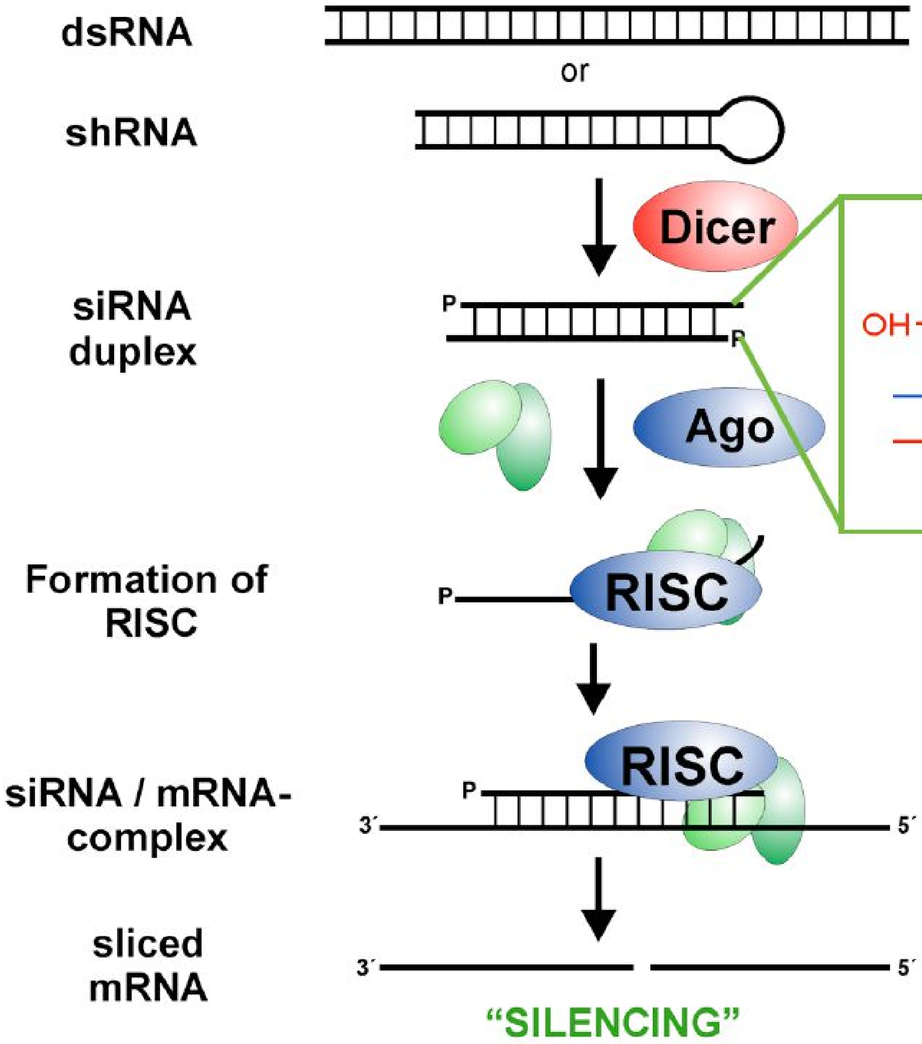
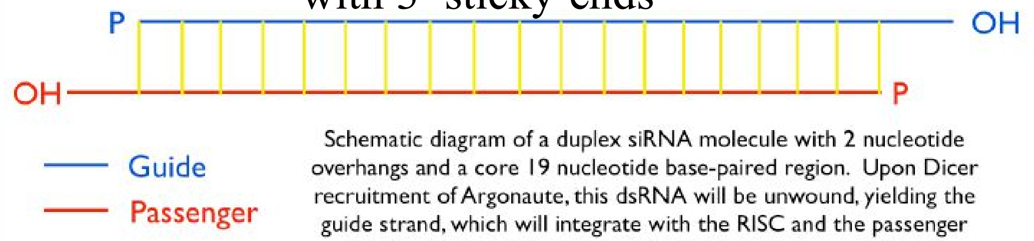


Diagram illustrating the major steps in siRNA biogenesis and subsequent siRNA-mediated gene silencing.

21 to 23 nucleotides with 3' sticky ends



Schematic diagram of a duplex siRNA molecule with 2 nucleotide overhangs and a core 19 nucleotide base-paired region. Upon Dicer recruitment of Argonaute, this dsRNA will be unwound, yielding the guide strand, which will integrate with the RISC and the passenger strand, which will be subsequently degraded by exonucleases.

RNA silencing
(post-transcriptional gene silencing)

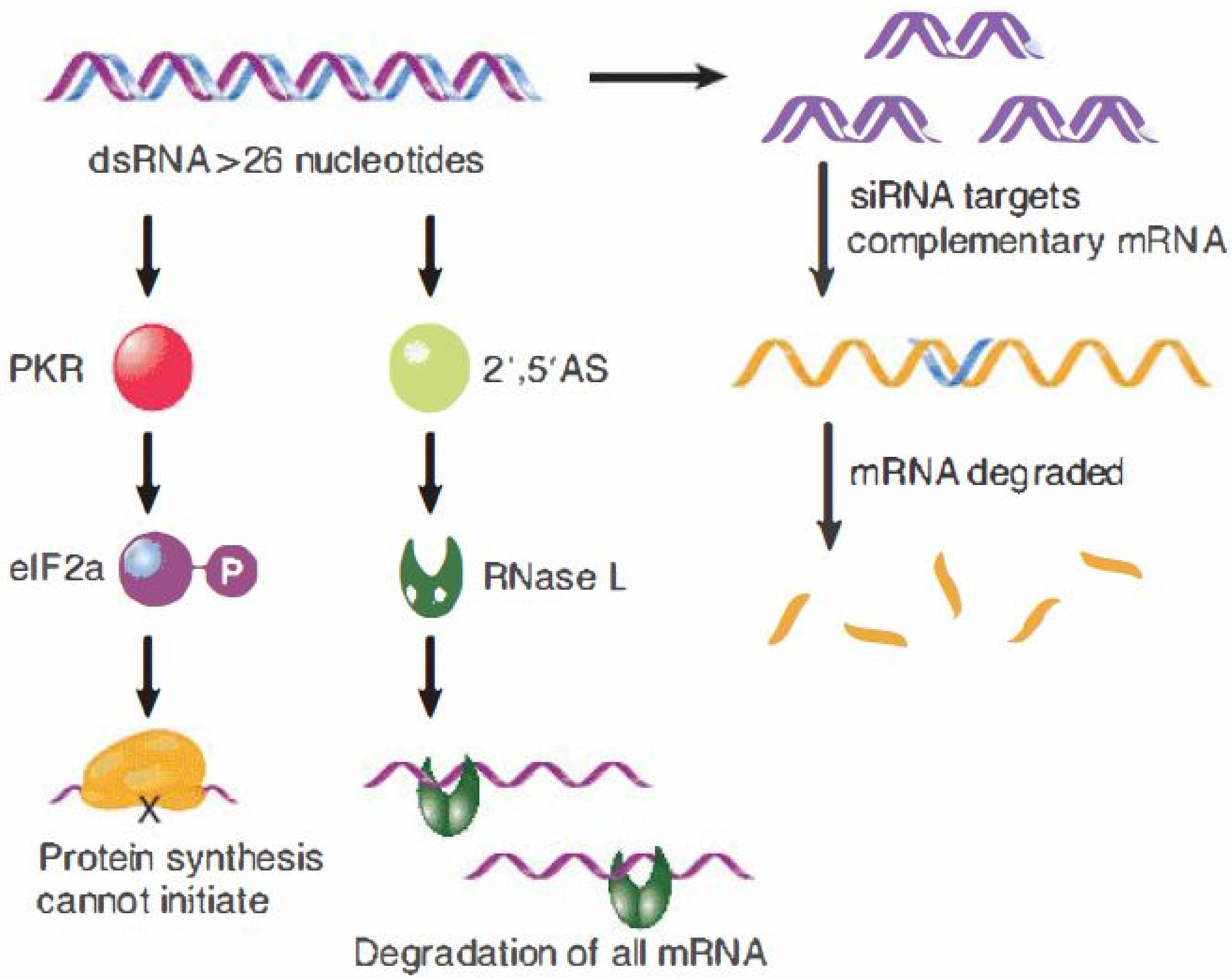
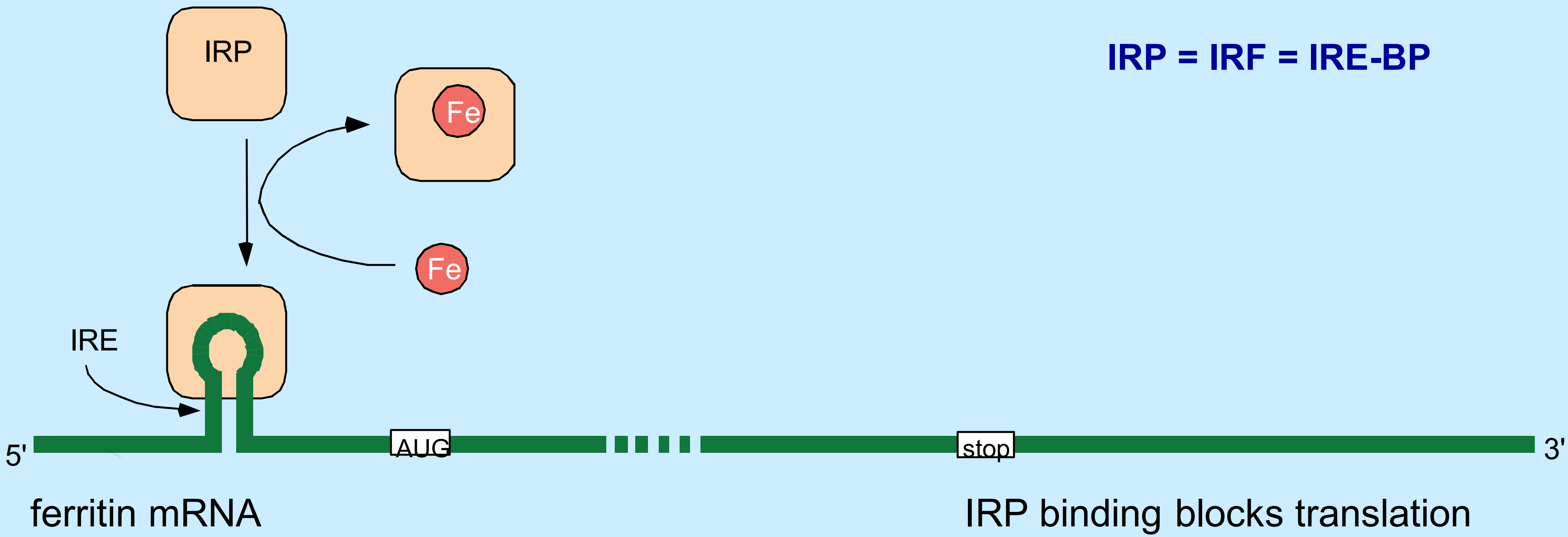


FIGURE 30.13 Long dsRNA inhibits protein synthesis and triggers degradation of all mRNA in mammalian cells, as well as having sequence-specific effects.

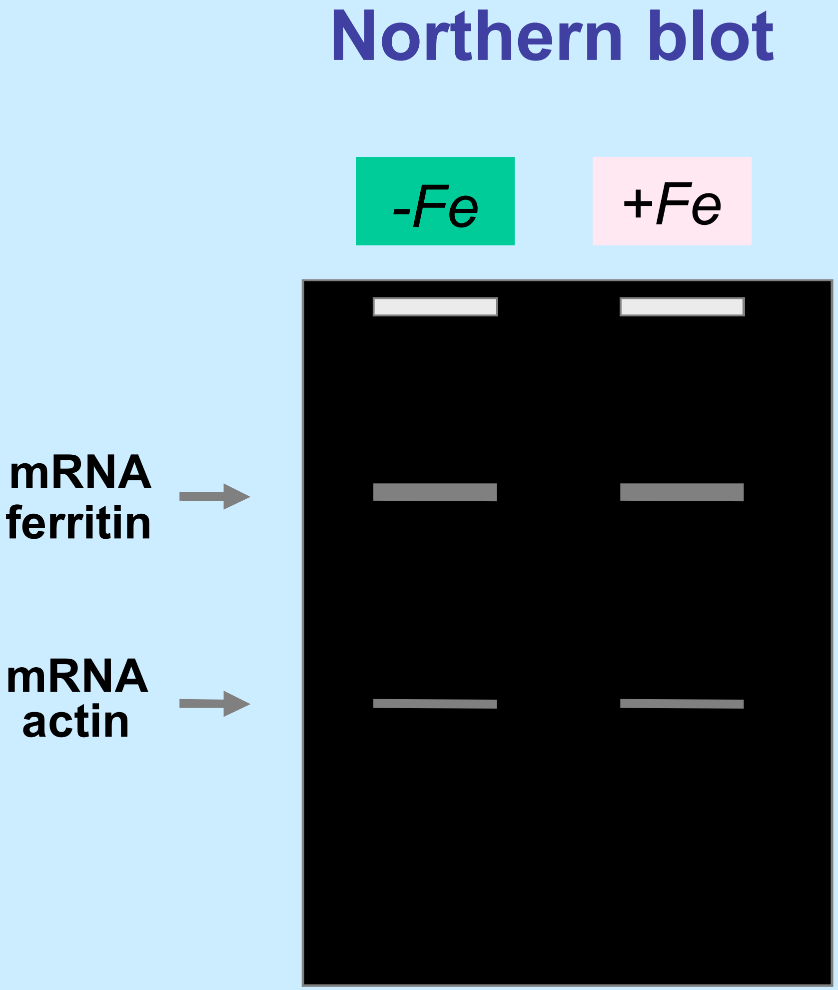
Translational control of ferritin synthesis in animal cells

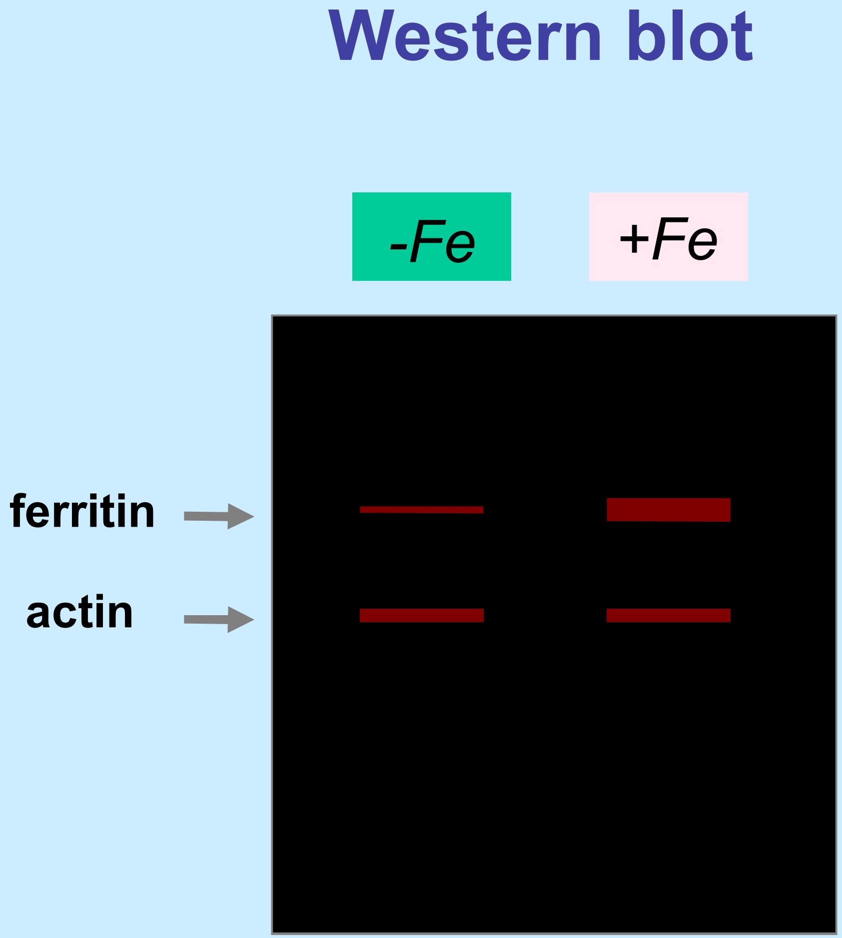


IRP = IRF = IRE-BP

Ferritin is a globular protein complex consisting of 24 protein subunits and is the main *intracellular iron storage protein* in both prokaryotes and eukaryotes, keeping it in a soluble and non-toxic form.

Translational control proof



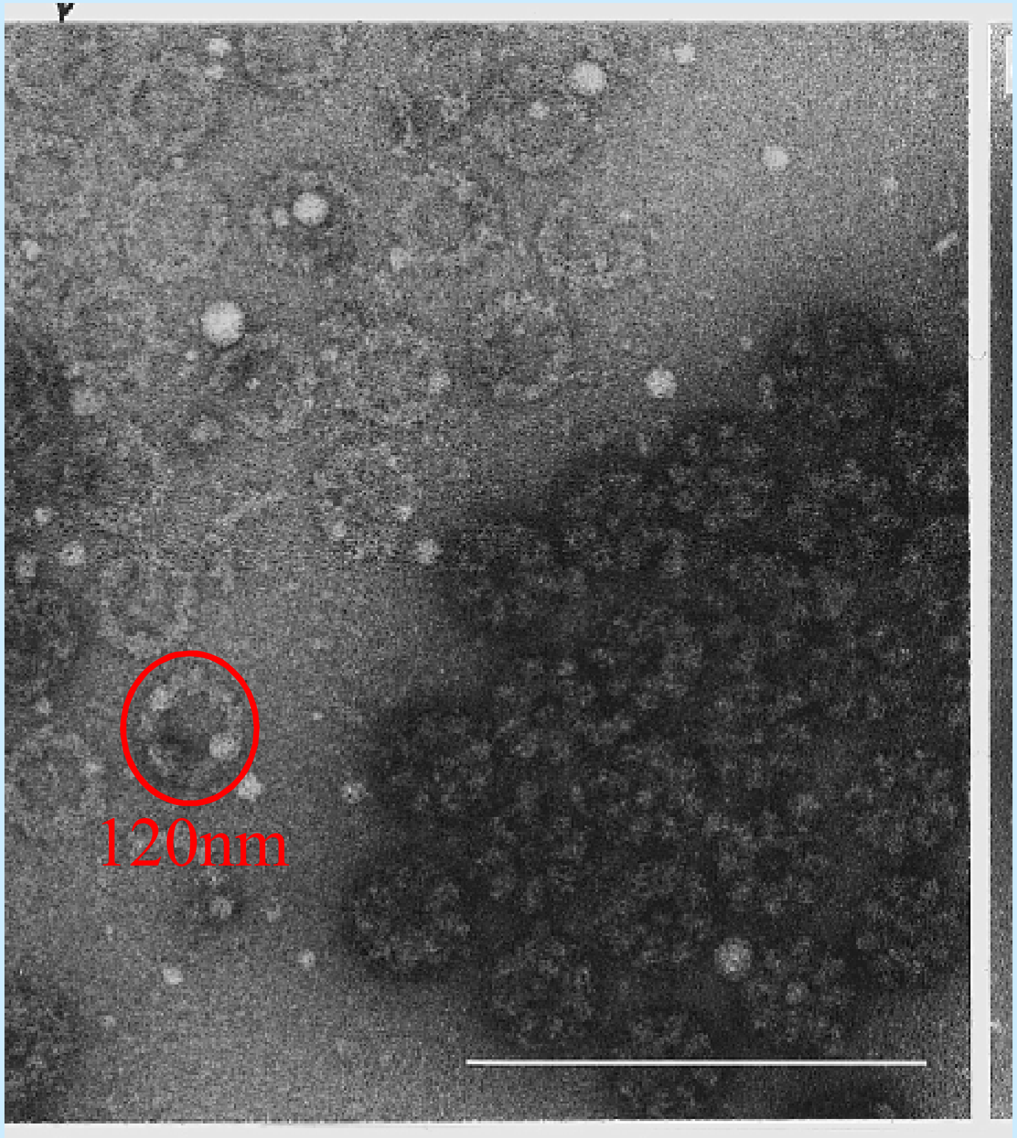


Nucleus-cytoplasm transport

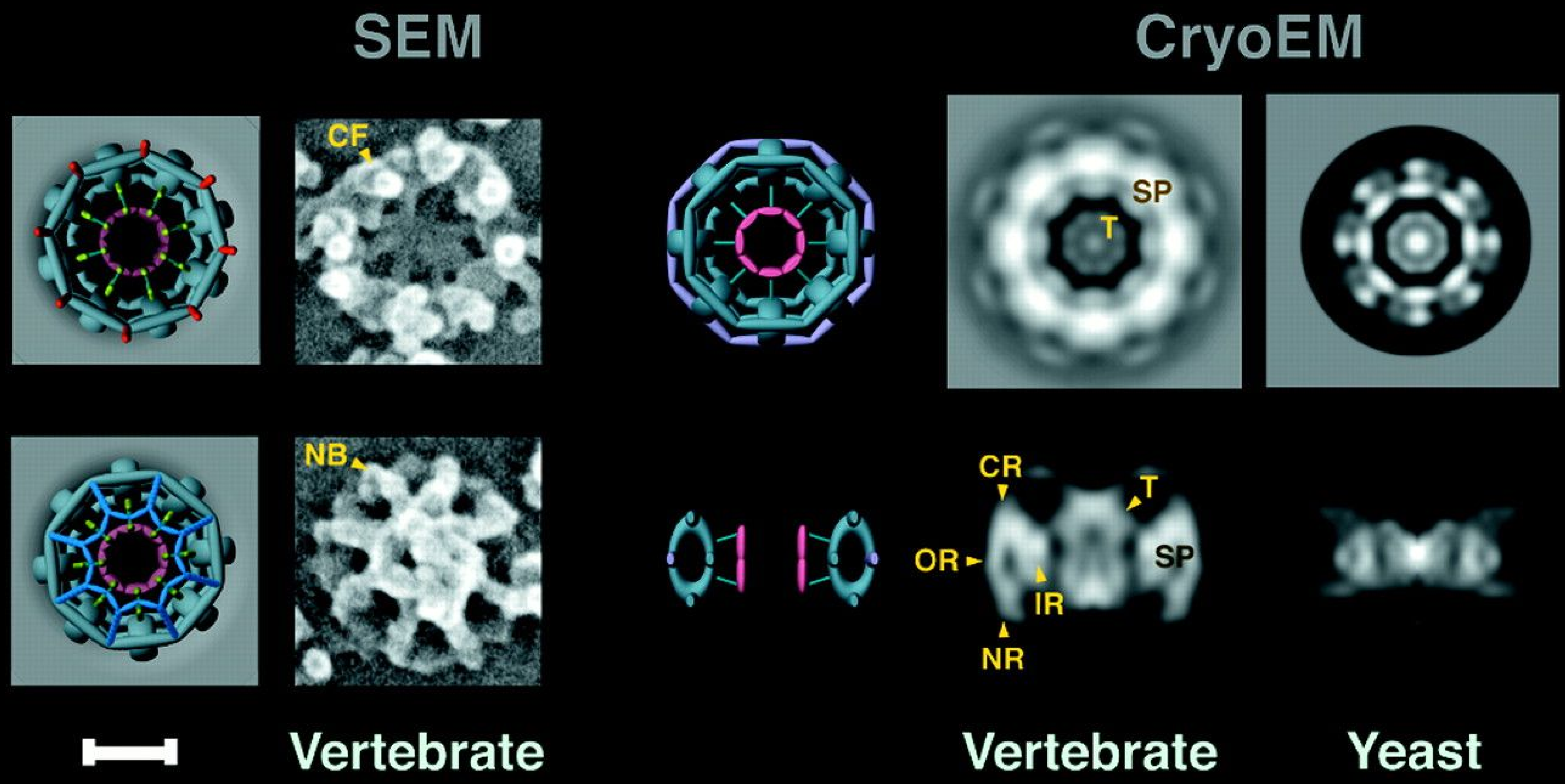
- ***Involves RNA and proteins***
- ***It is bidirectional***
- ***Uses the nuclear pore complexes***

Direction	Substrate	Passages /pore/min
Import	Histones	100
	Nonhistone proteins	100
	Ribosomal proteins	150
Export	Ribosomal subunits	~5
	mRNA	<1

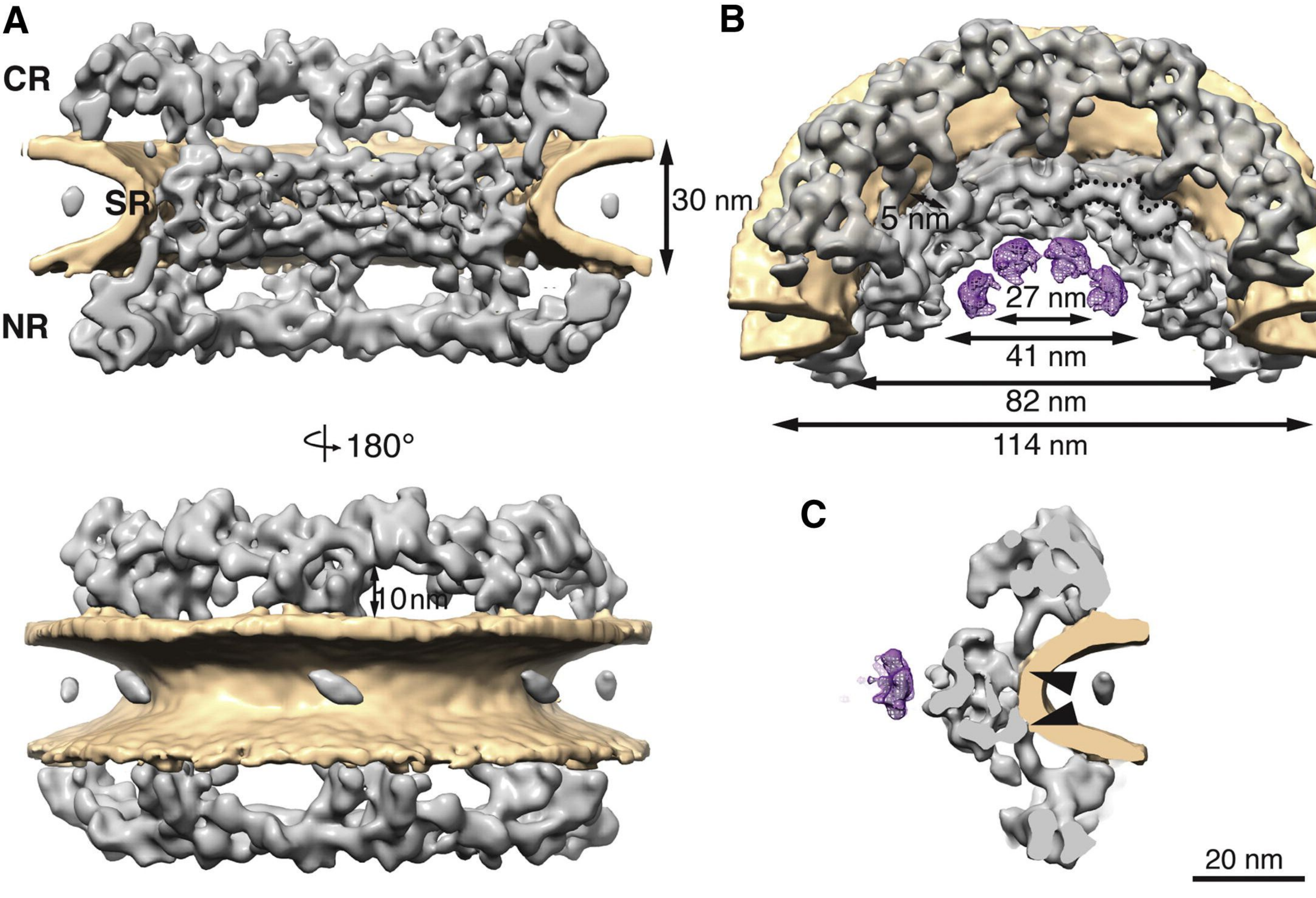
Nuclear pores are used for import and export.

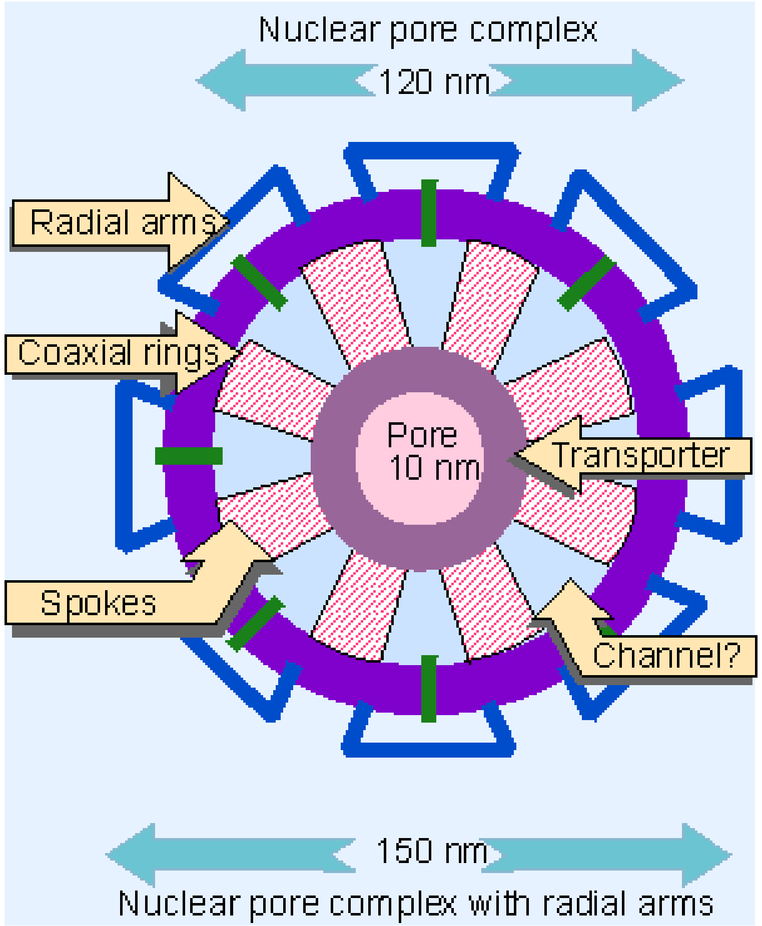


Nuclear pores appear as annular structures by electron microscopy. The bar is 0.5 mm. Photograph kindly provided by Ronald Milligan.

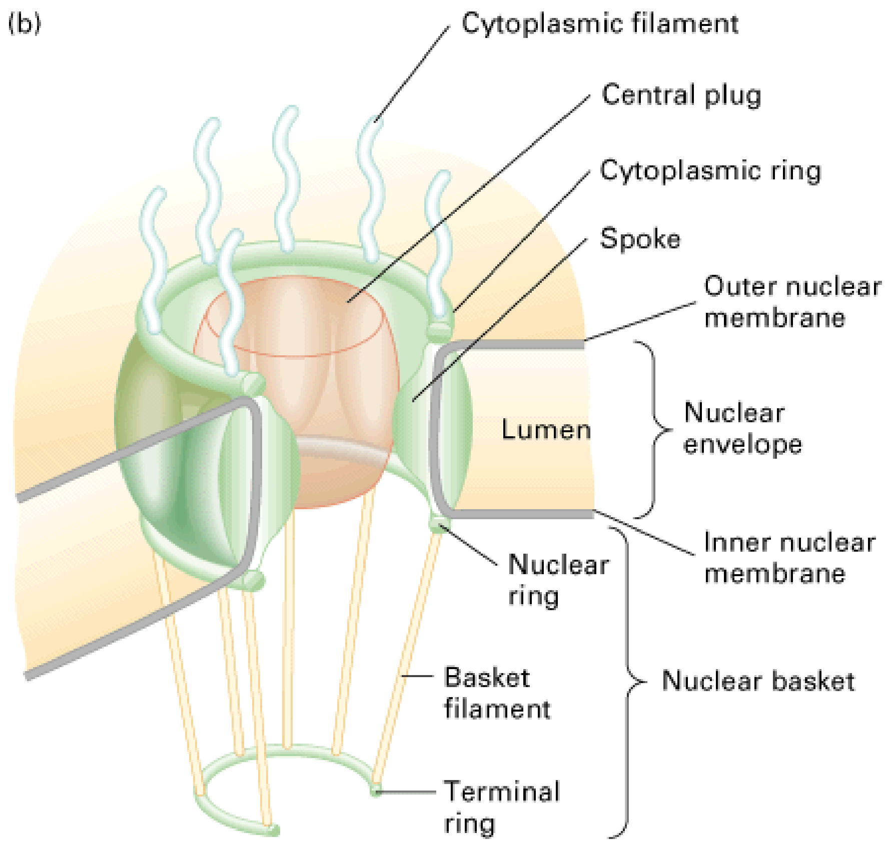


SEM
CryoEM
CF
SP
T
NB
CR
T
OR
SP
IR
NR
Vertebrate
Vertebrate
Yeast

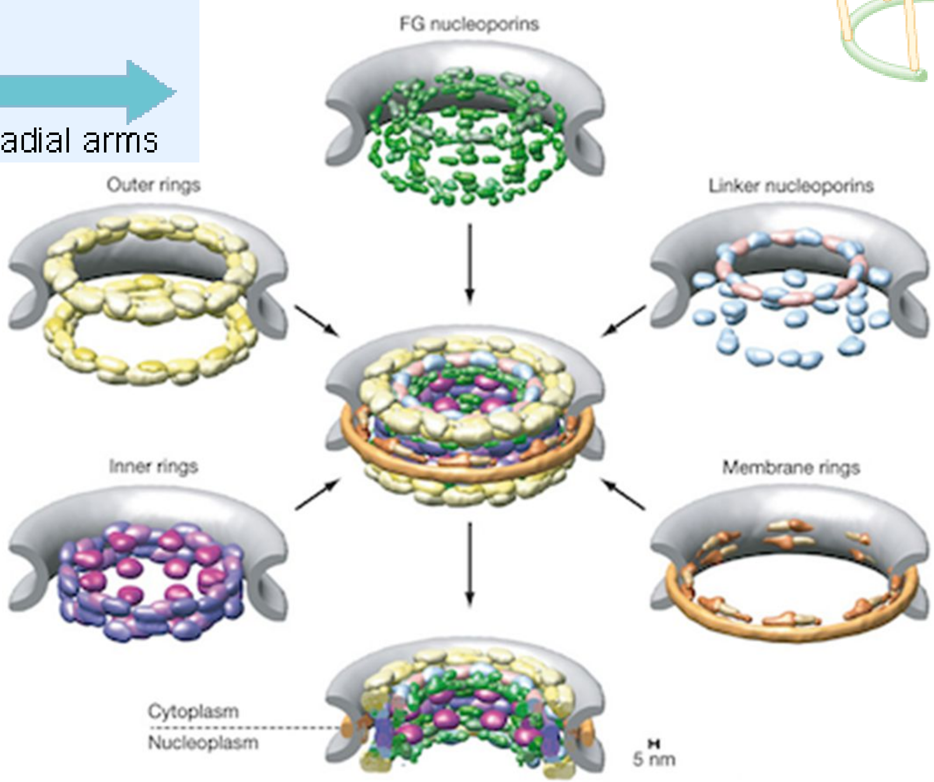

A
CR
SR
30 nm
NR
180°
10nm
B
5 nm
27 nm
41 nm
82 nm
114 nm
C
20 nm



Nuclear pore complex
120 nm
Radial arms
Coaxial rings
Pore
10 nm
Transporter
Spokes
Channel?
150 nm
Nuclear pore complex with radial arms

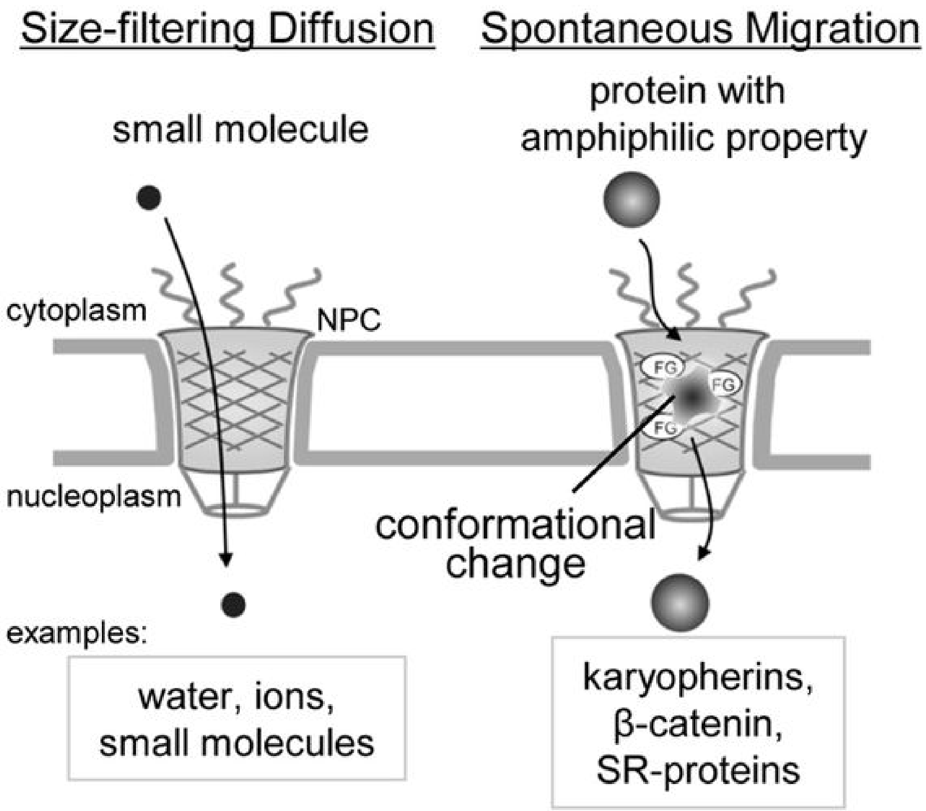


(b)
Cytoplasmic filament
Central plug
Cytoplasmic ring
Spoke
Outer nuclear membrane
Lumen
Nuclear envelope
Inner nuclear membrane
Nuclear ring
Basket filament
Nuclear basket
Terminal ring



FG nucleoporins
Outer rings
Linker nucleoporins
Inner rings
Membrane rings
Cytoplasm
Nucleoplasm
5 nm

A

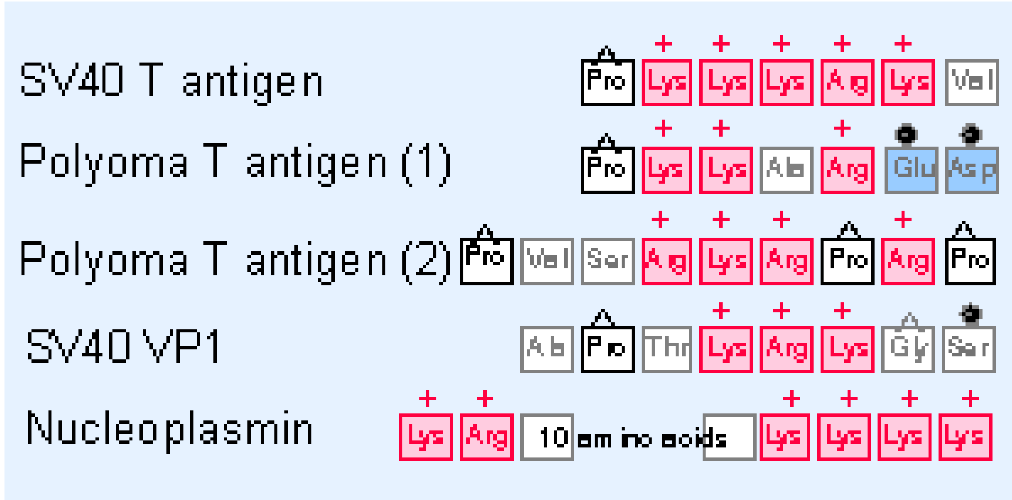


B

	Size-filtering diffusion	Spontaneous migration	Karyopherin-mediated transport
Size limitation	+	−	−
Change of surface hydrophobicity	−	+	(−)
Transporter	−	−	+
Transport against concentration gradient	−	−	+
Examples	water, ions, small molecules	karyopherins, β-catenin, SR-proteins	NLS/NES-proteins

Nucleus-cytoplasm transport

- ***NLS*** *(nuclear localization sequence) = amino acidic sequence needed for import to nucleus*
- ***NES*** *(nuclear export sequence) = amino acidic sequence needed for export to cytoplasm*



A. *Classical* Nuclear Localization Signal

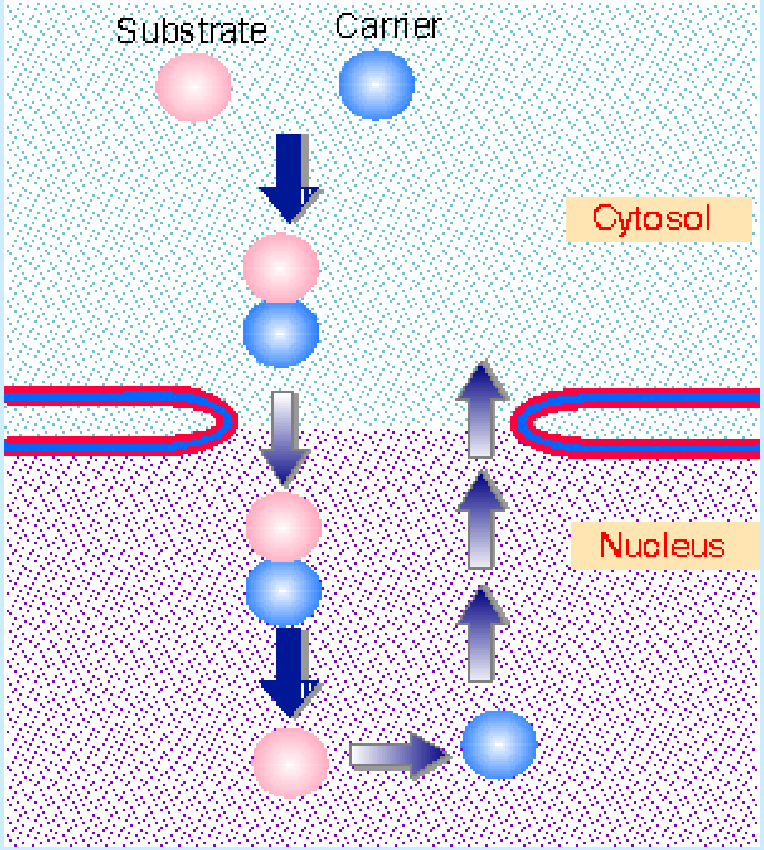
SV40 T antigen:	^{126} **PKKKRKV** ^{132}
Human c-MYC:	^{320} **PAAKRVKLD** ^{328}
Nucleoplasm:	^{155} **KR....$X_{(10)}$....KKKK** ^{170}

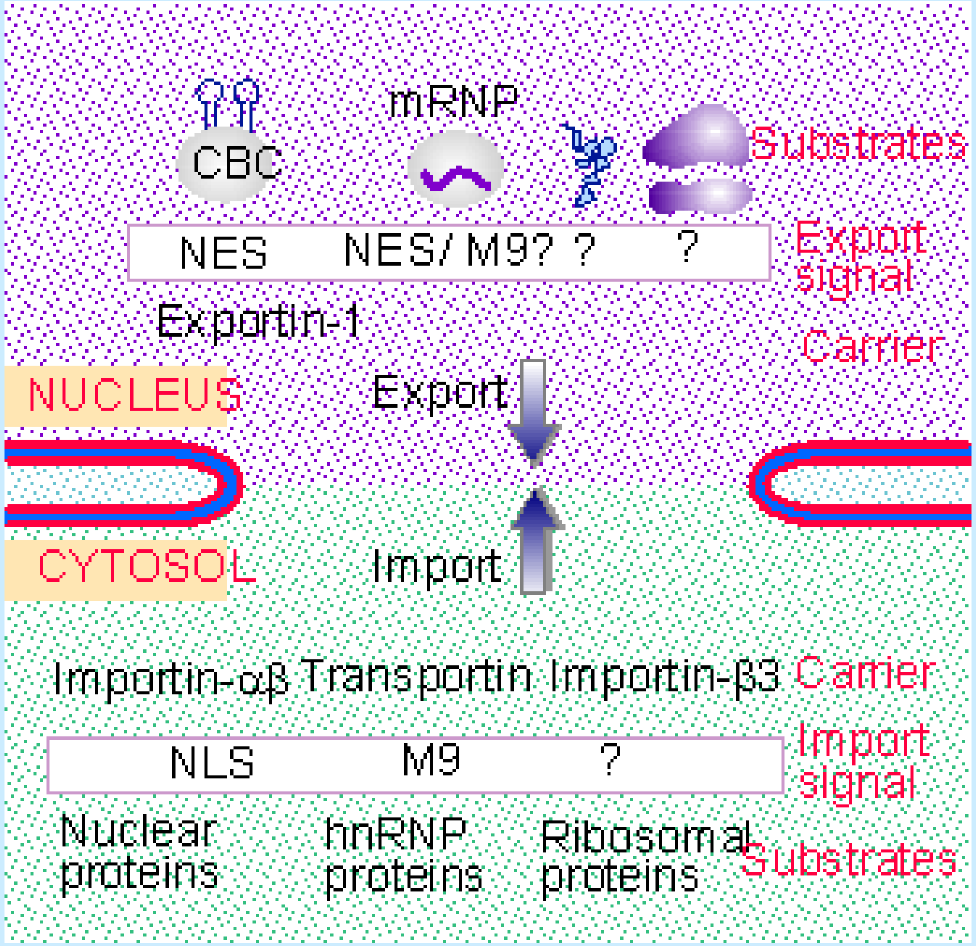
B. Transport Signals of cargos *up*-regulated by Phosphorylation

EBNA-1 NLS:	^{379} **KRPRSPSS** ^{386}
HBV core:	^{149} **VRRRDR...$X_{(17)}$...SPRRRR** ^{180}
SV40 T antigen:	^{110} **PSS...$X_{(7)}$...S...$X_{(5)}$ ...PKKKRKV** ^{132}
STAT1-dsNLS:	^{378} **RK...$X_{(30)}$ ...KEQKNAGTR...$X_{(283)}$ ...Y** ^{701}
ERK5-NLS:	^{520} **KRRRR...$X_{(8)}$ ...KRR** ^{535}
ERK1/2-NTS:	^{232} **LDQLNHILGILGSPSQEDL** ^{250}

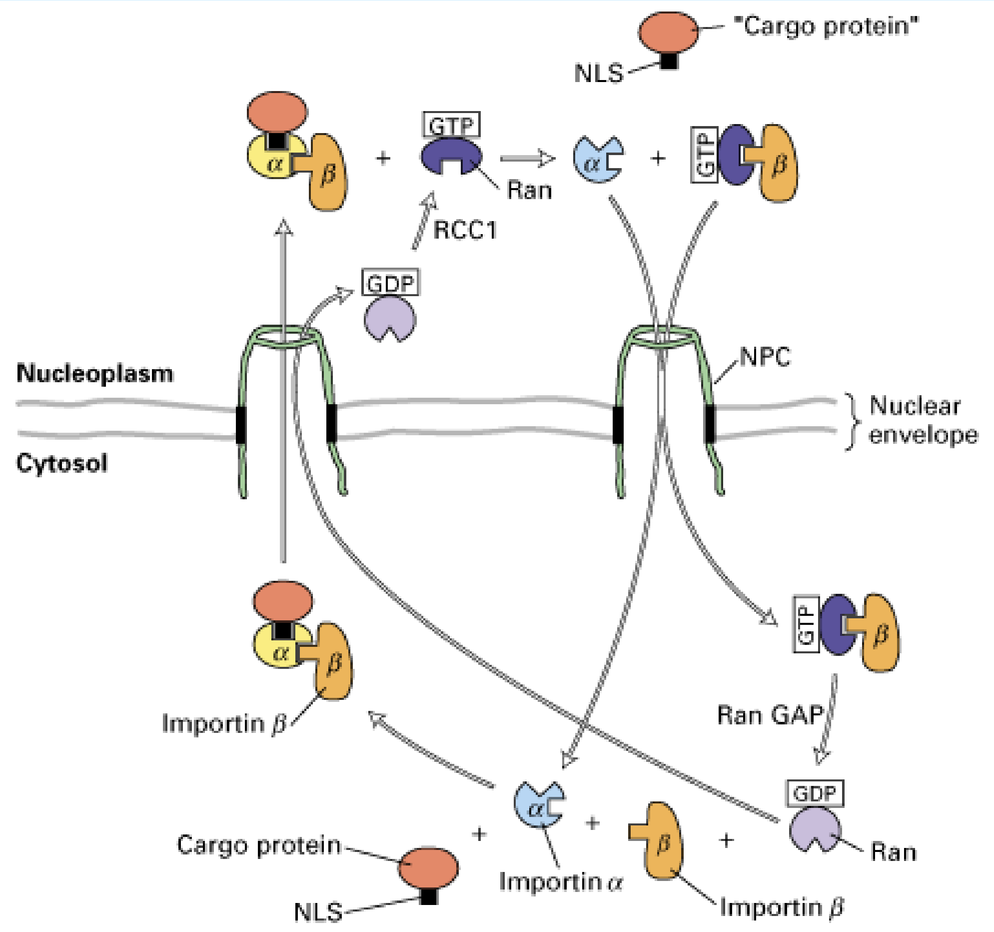
C. Transport Signals of cargos *down*-regulated by Phosphorylation

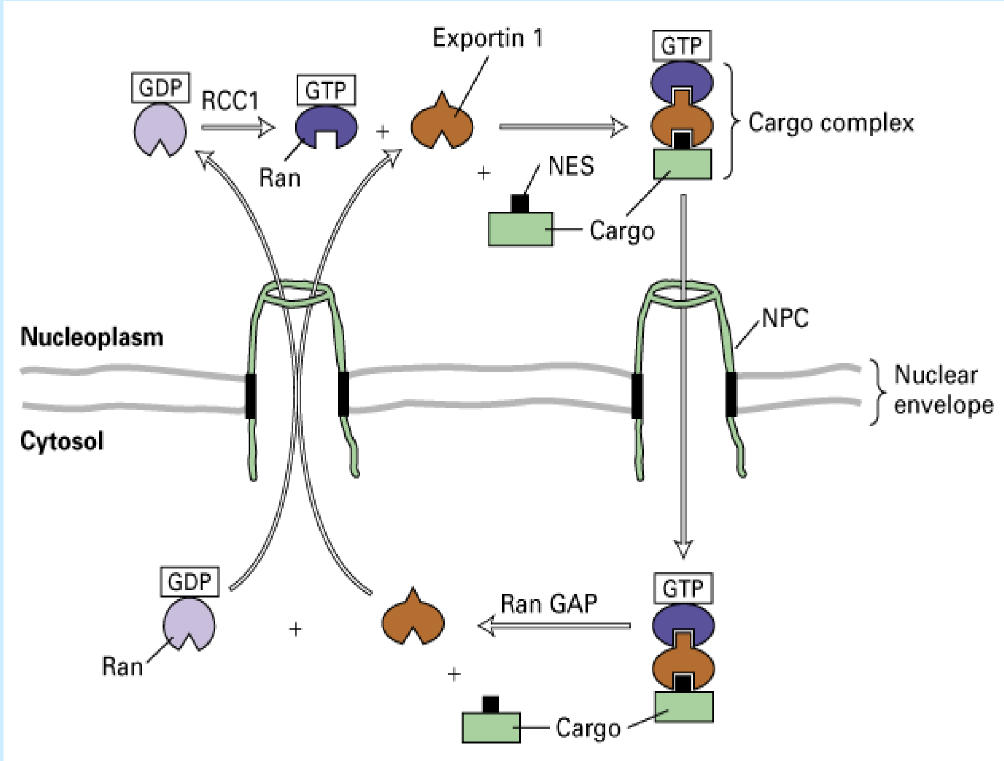
Msn2p:	^{575} **SSLRRKS...$X_{(34)}$...RRPSYRRKSMTSRRSS** ^{633}
NFATc1:	^{241} **RSSRPASPCNKRKYS** ^{641}
Pho4-NLS:	^{140} **SANKVTKNKSNSSPYLNKRKGKPGPDS** ^{166}
PTHrP-ncNLS:	^{66} **RYLTQETNKVETYKEQPLKTPGKKKKGKP** ^{94}
Swi6:	^{157} **ELGSPLKKLKIDT** ^{169}





A carrier protein binds to a substrate, moves with it through the nuclear pore, is released on the other side, and must be returned for reuse. There are multiple pathways for nuclear export and import.


"Cargo protein"
NLS
GTP
Ran
RCC1
GDP
α
β
NPC
Nucleoplasm
Nuclear envelope
Cytosol
Importin β
Ran GAP
Cargo protein
NLS
Importin α
Importin β
Ran


Exportin 1
GDP
RCC1
GTP
Ran
Cargo complex
NES
Cargo
NPC
Nucleoplasm
Nuclear envelope
Cytosol
Ran GAP
Ran
Cargo