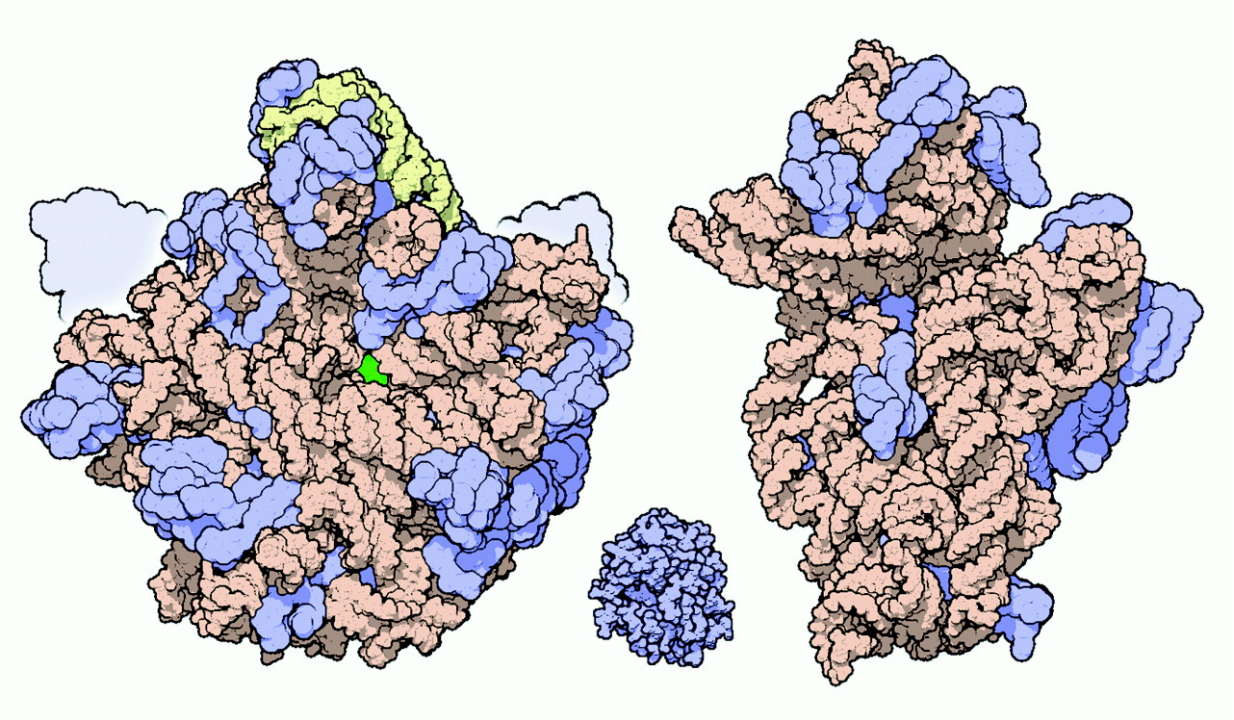
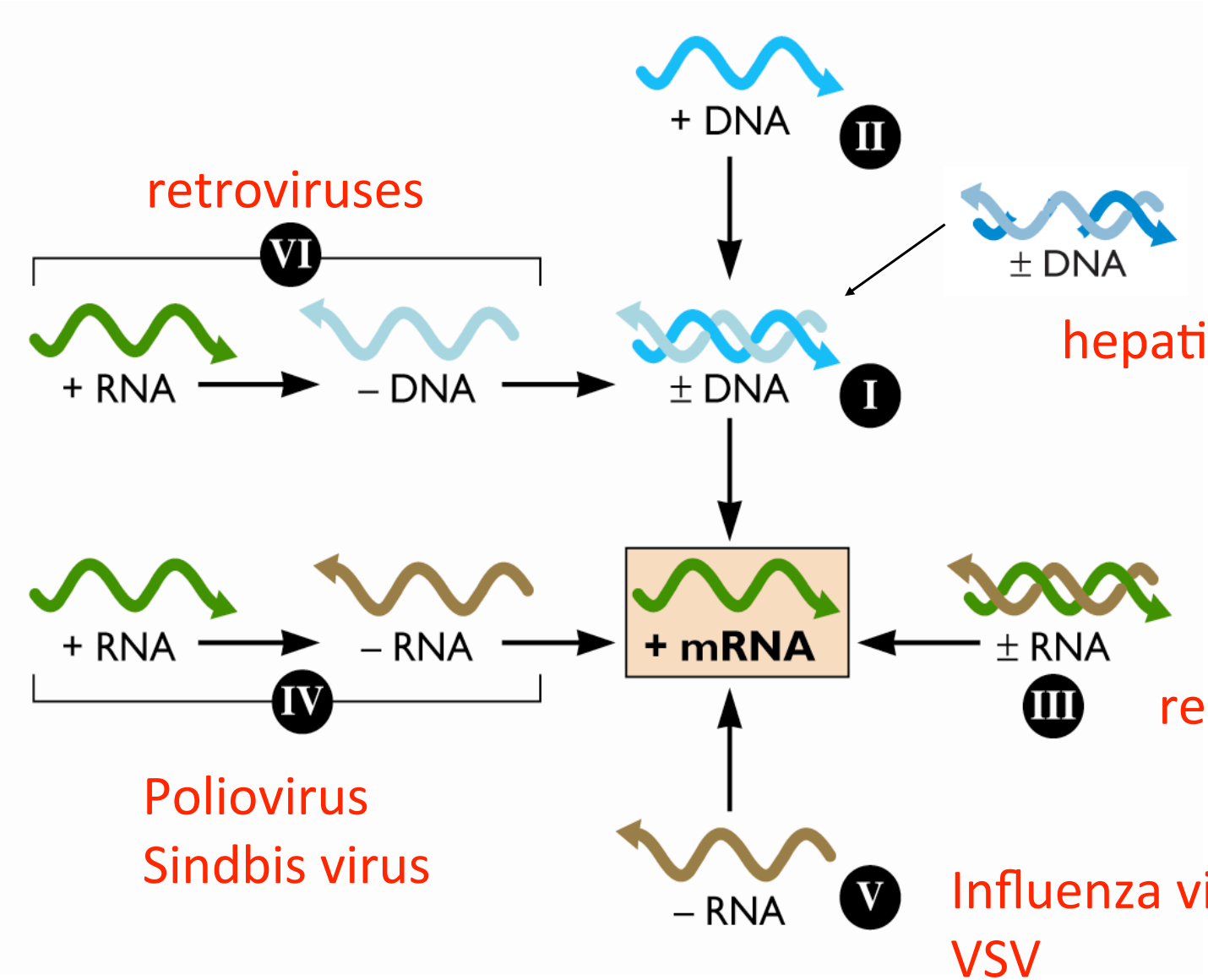
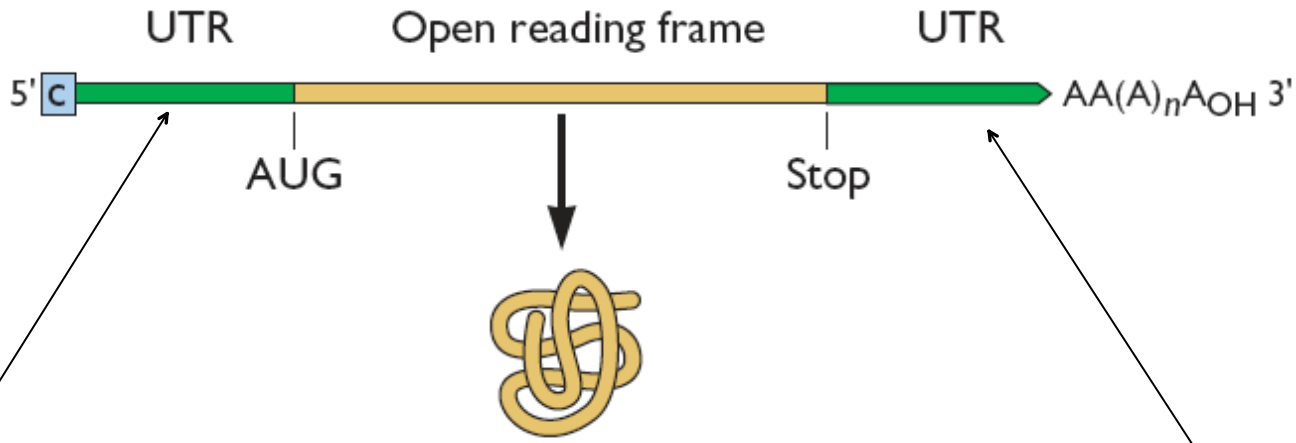
TWiV 238
Lost in translation




retroviruses
+ DNA
II
± DNA
hepatitis B virus
VI
+ RNA
– DNA
± DNA
I
+ mRNA
+ RNA
– RNA
IV
± RNA
III
reovirus
Poliovirus
Sindbis virus
– RNA
V
Influenza virus
VSV



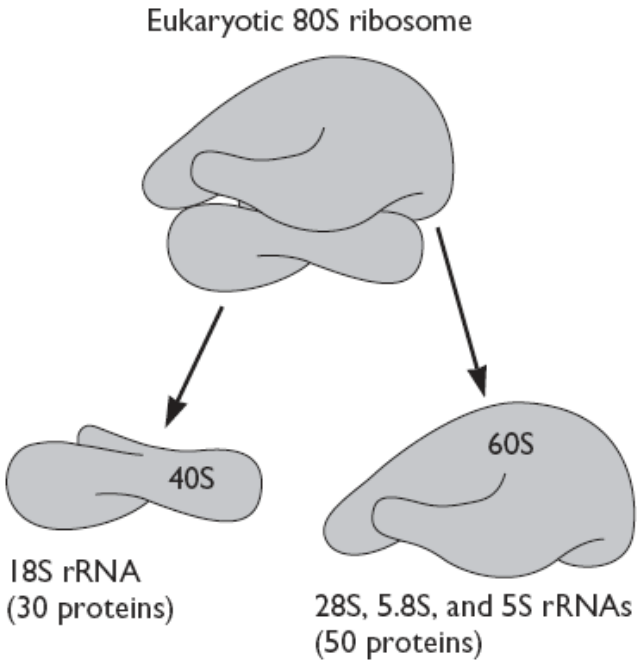
5’-untranslated region

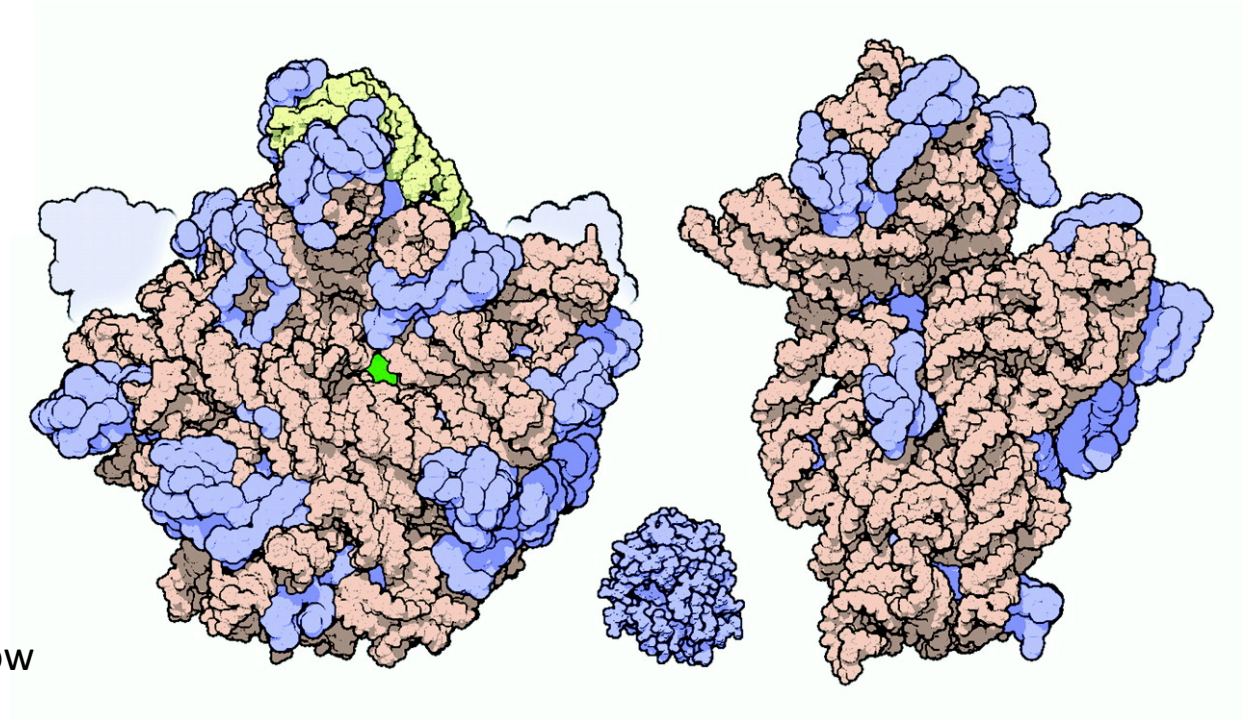
- 3 – >1,000 nt in length, typically 50 – 70 nt
- Often contains RNA secondary structures; must be unwound to allow passage of ribosome
- Length and secondary structure influence translation efficiency

3’-untranslated region

- Can regulate translation initiation, translation efficiency, mRNA stability
- **poly(A) tail**, necessary for efficient translation

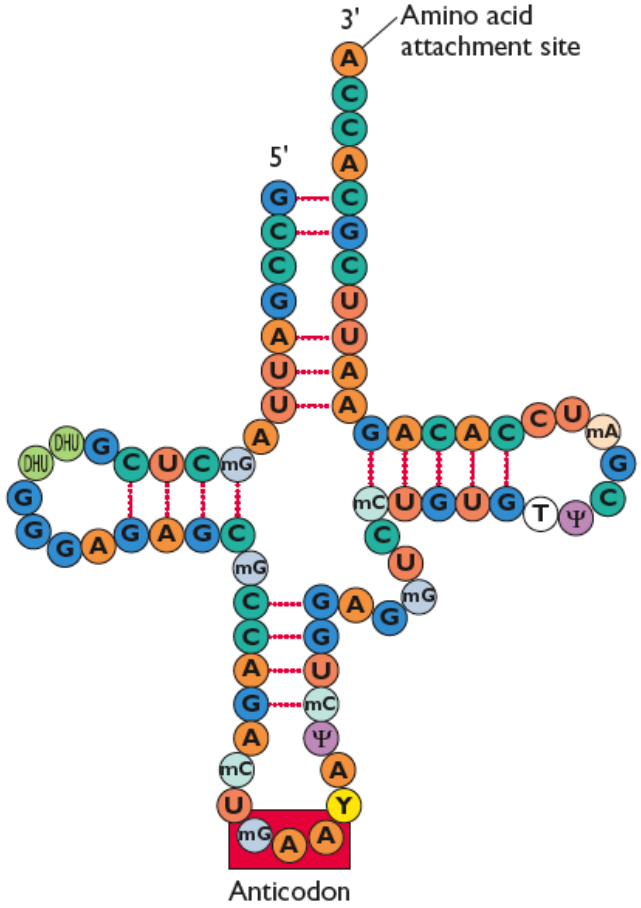
Translational machinery: Ribosomes

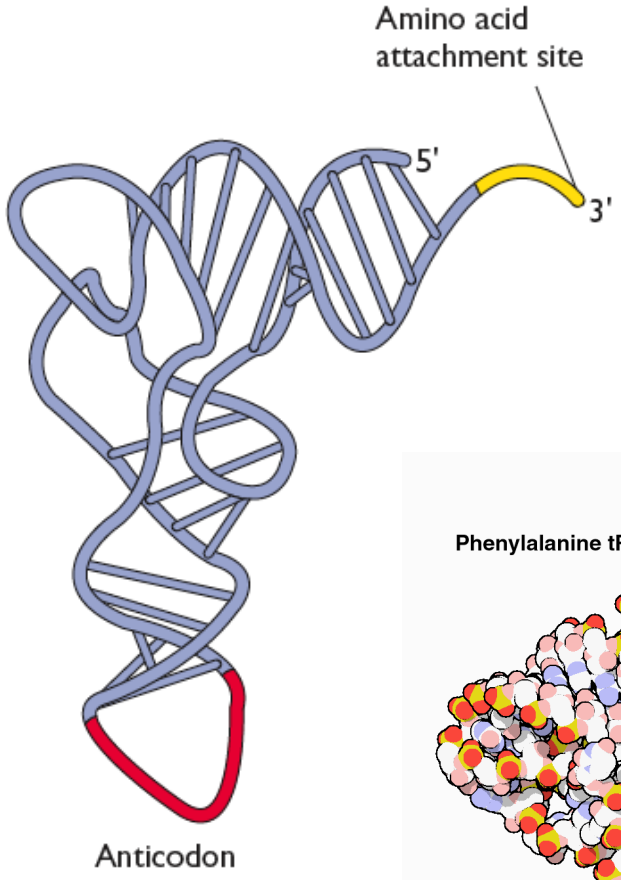


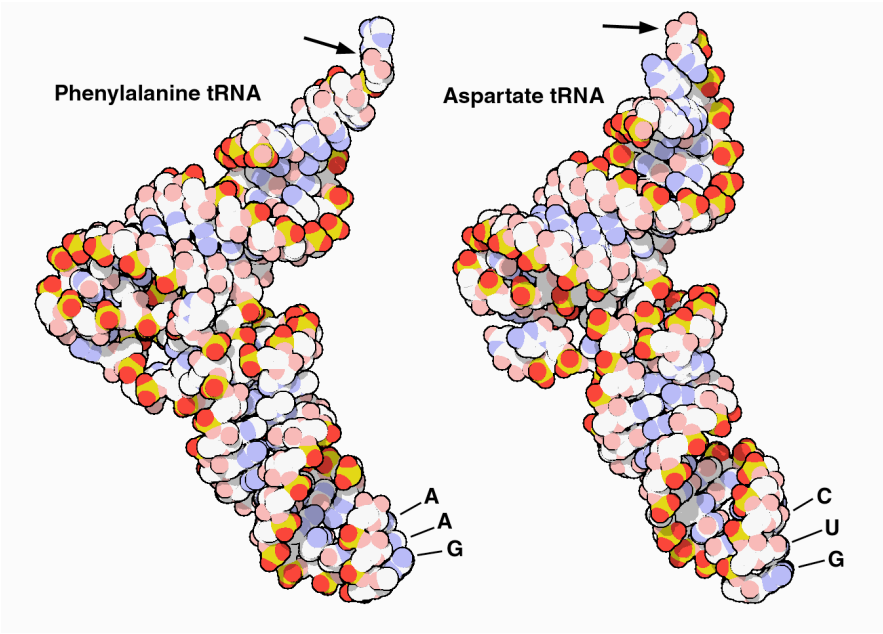


RNA=pink, yellow
Protein=blue

Translational machinery: tRNAs



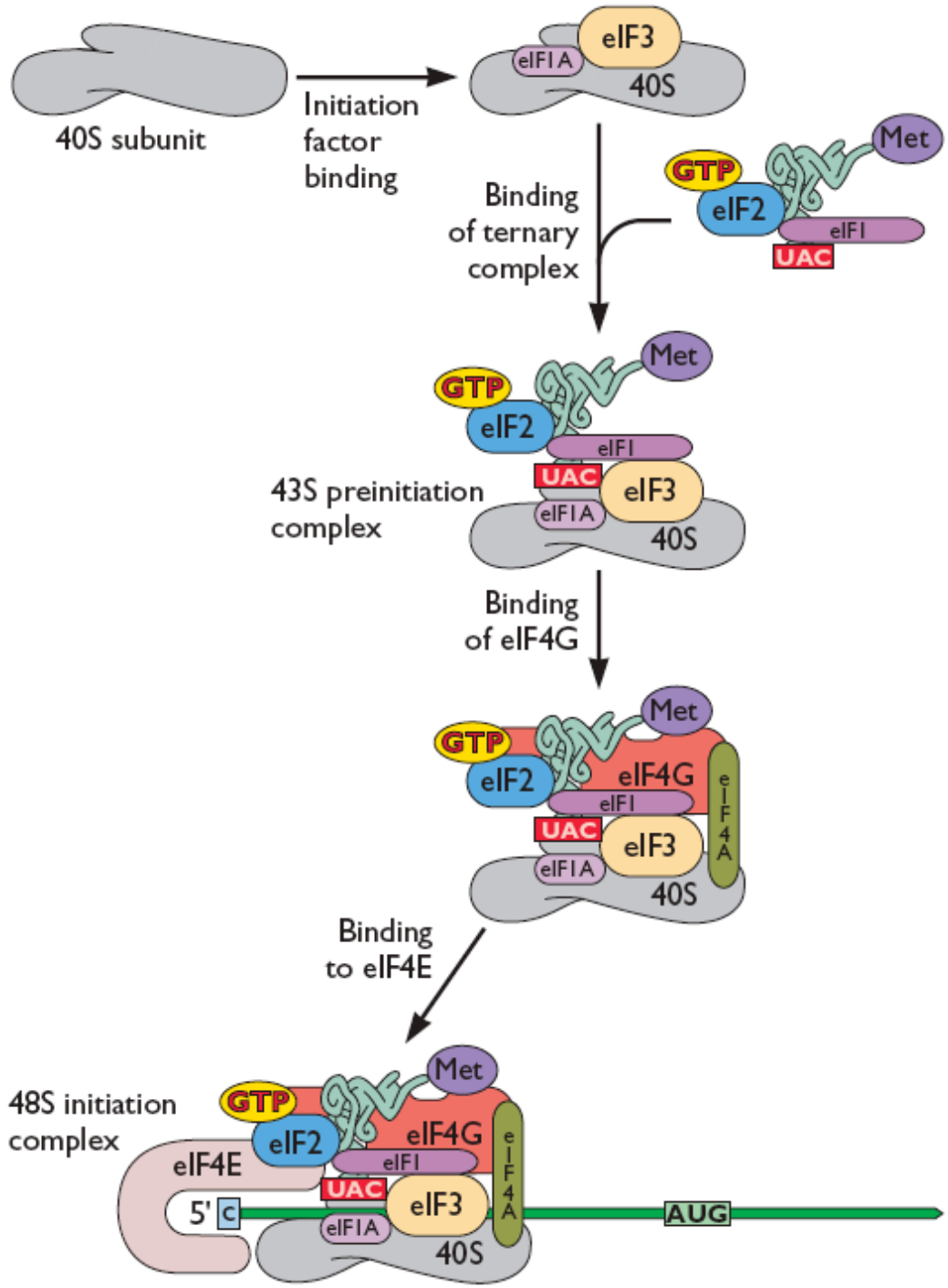


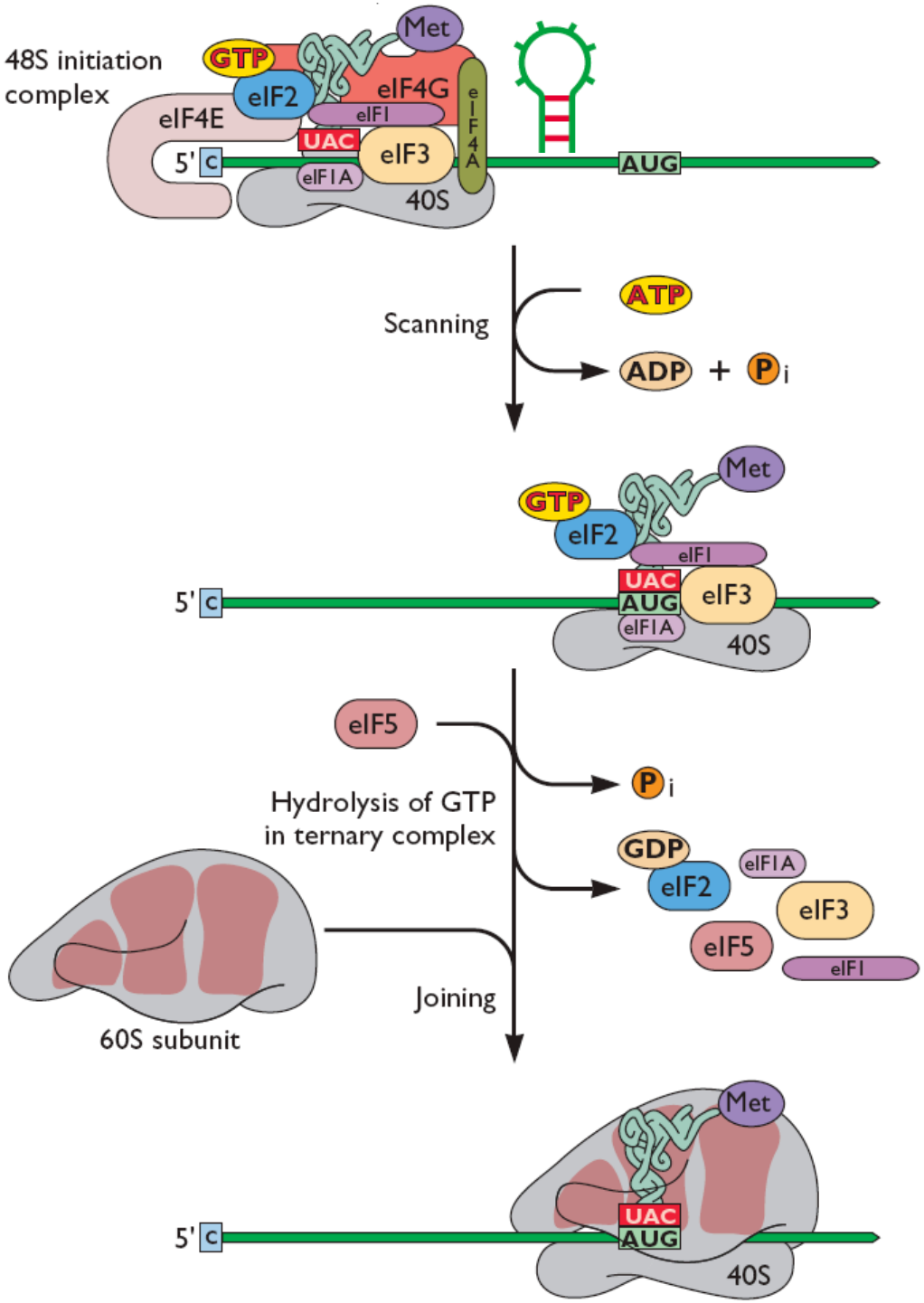


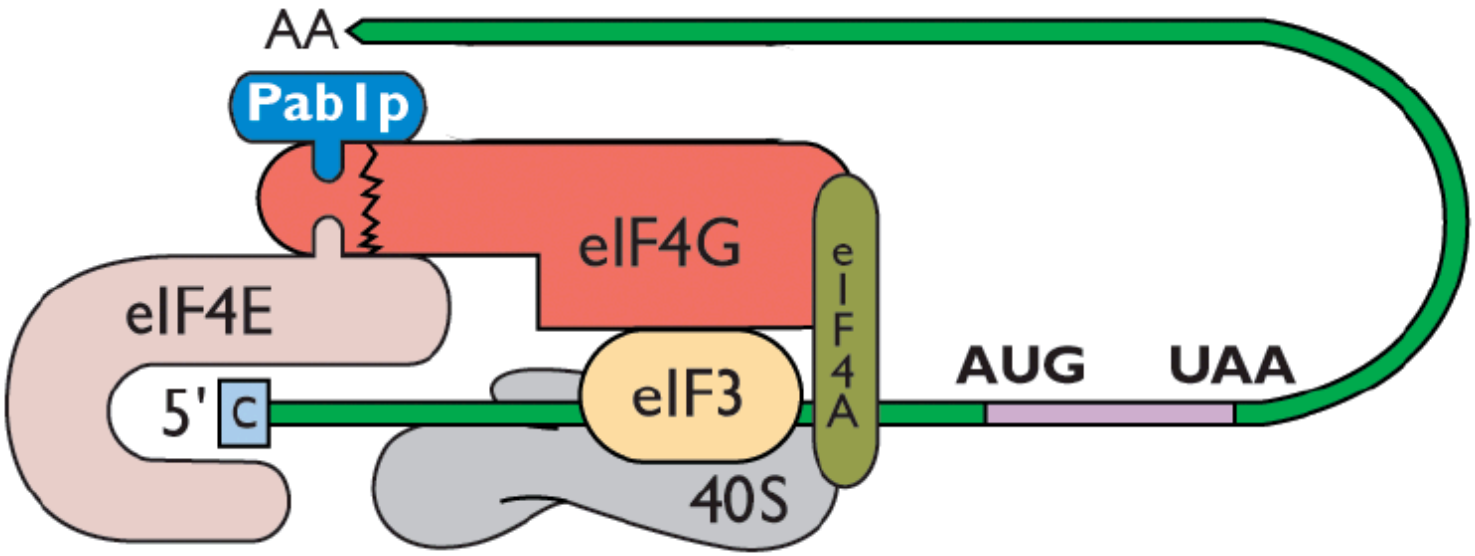
Translational machinery

- Ribosomes
- tRNAs
- initiation proteins (eIF)
- elongation proteins (eEF)
- termination proteins (eRF)

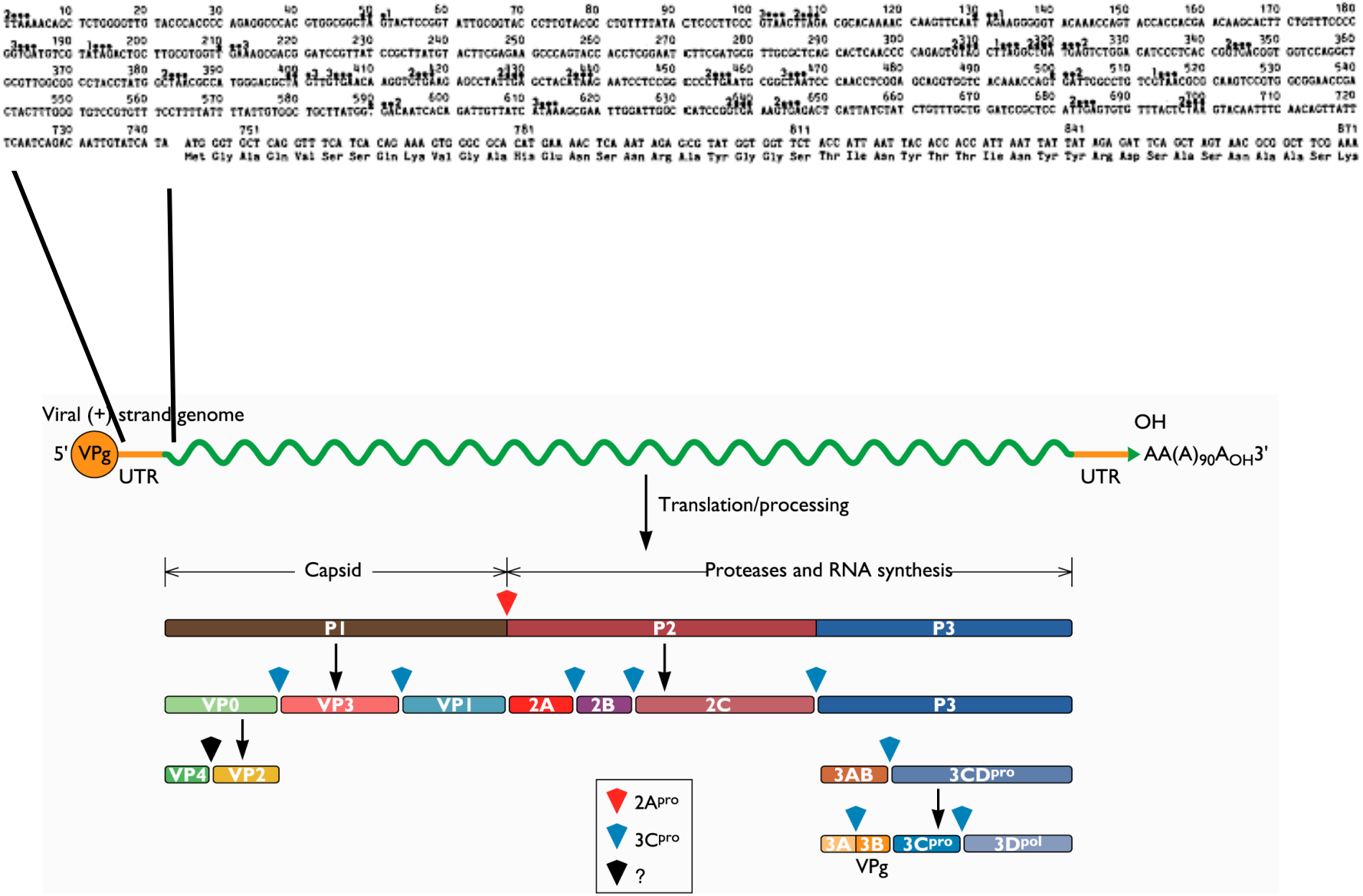
5’-end dependent initiation

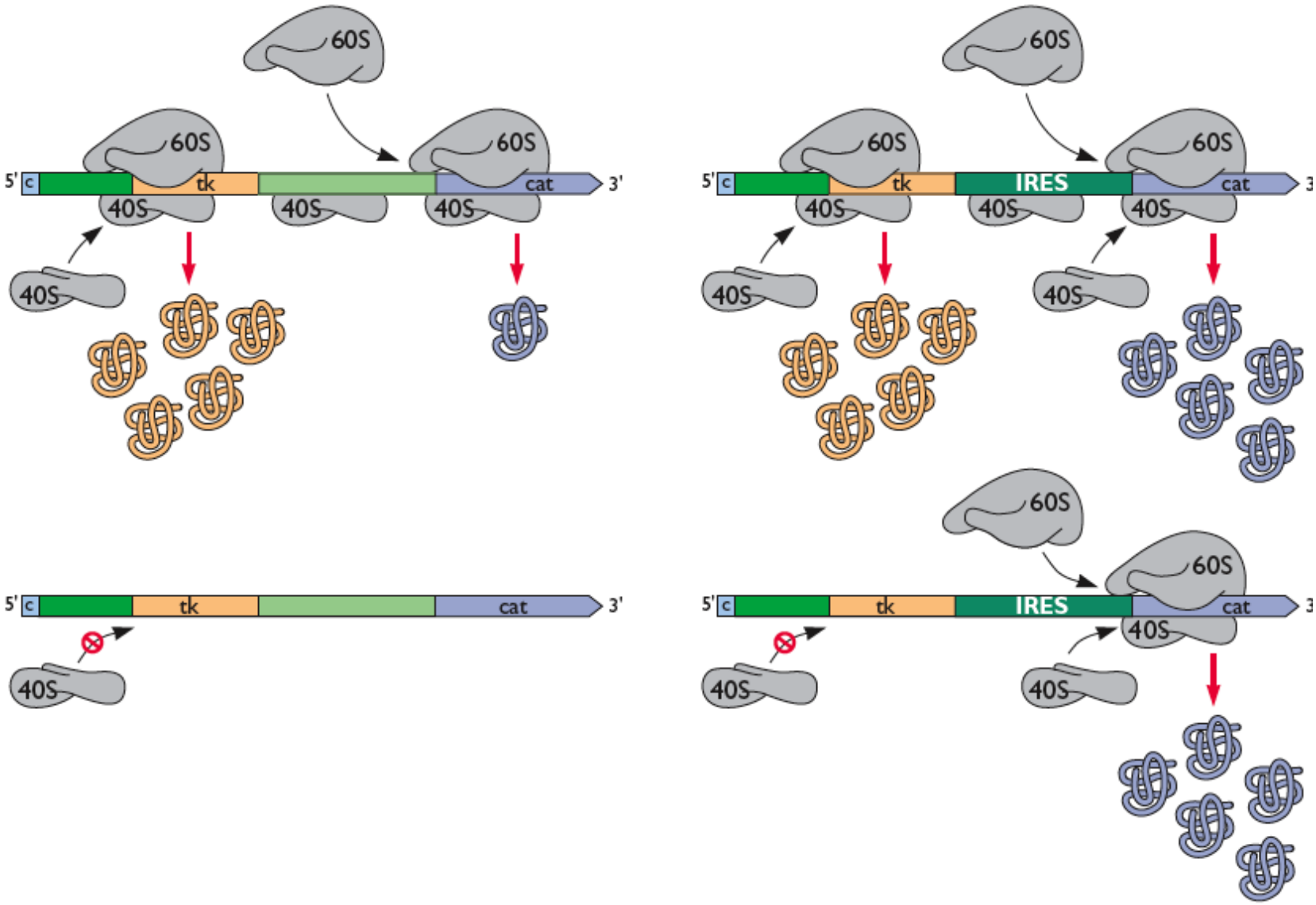



48S initiation complex
GTP
Met
eIF2
eIF4G
eIF1
eIF4E
UAC
eIF3
eIF4A
5'
C
eIF1A
40S
AUG
Scanning
ATP
ADP + Pi
GTP
Met
eIF2
eIF1
UAC
AUG
eIF3
5'
C
eIF1A
40S
eIF5
Pi
Hydrolysis of GTP in ternary complex
GDP
eIF1A
eIF2
eIF3
eIF5
eIF1
60S subunit
Joining
Met
UAC
AUG
5'
C
40S

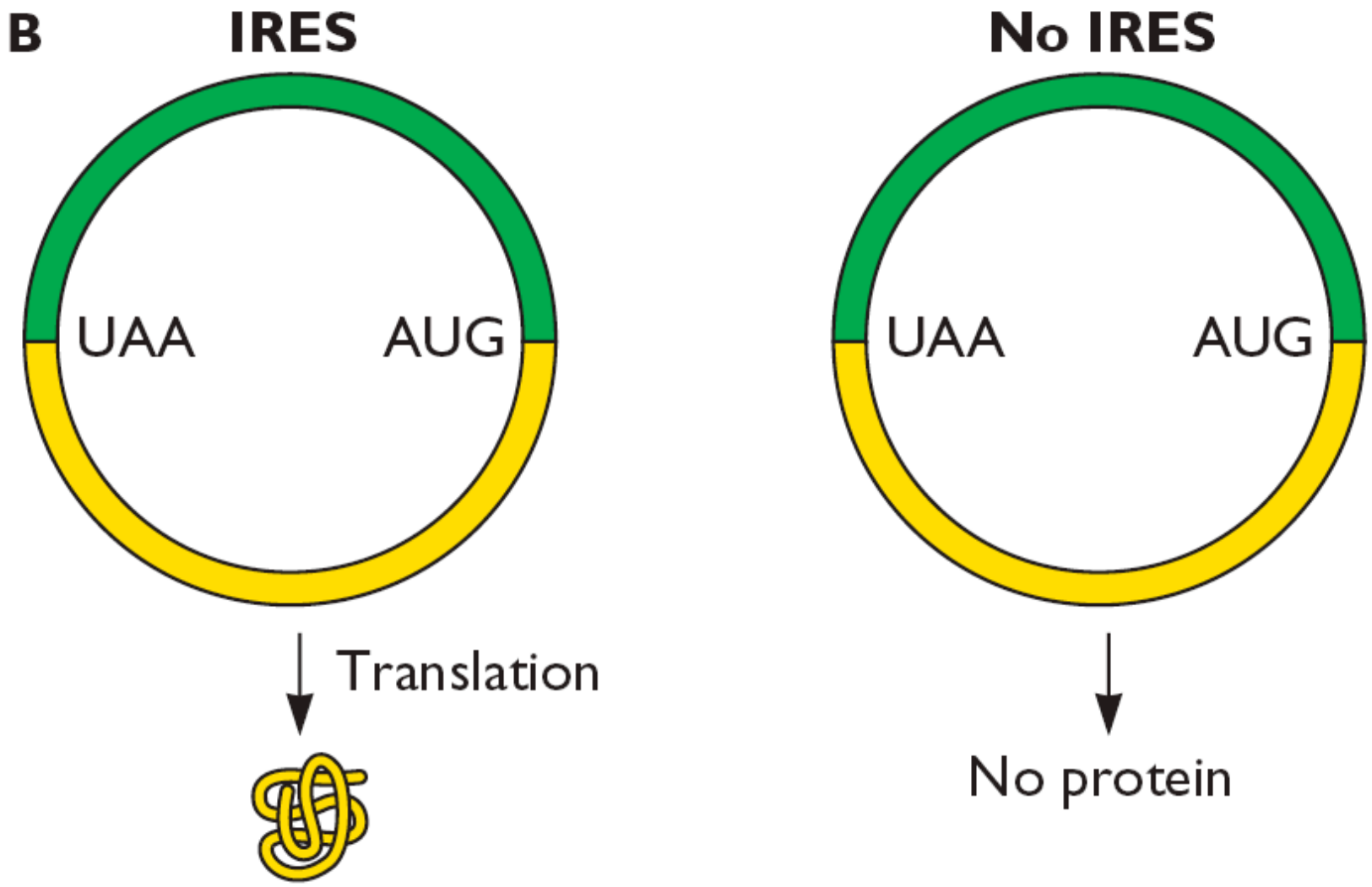

AA
Pab I p
eIF4G
eIF4E
5'
C
eIF3
eIF4A
AUG
UAA
40S

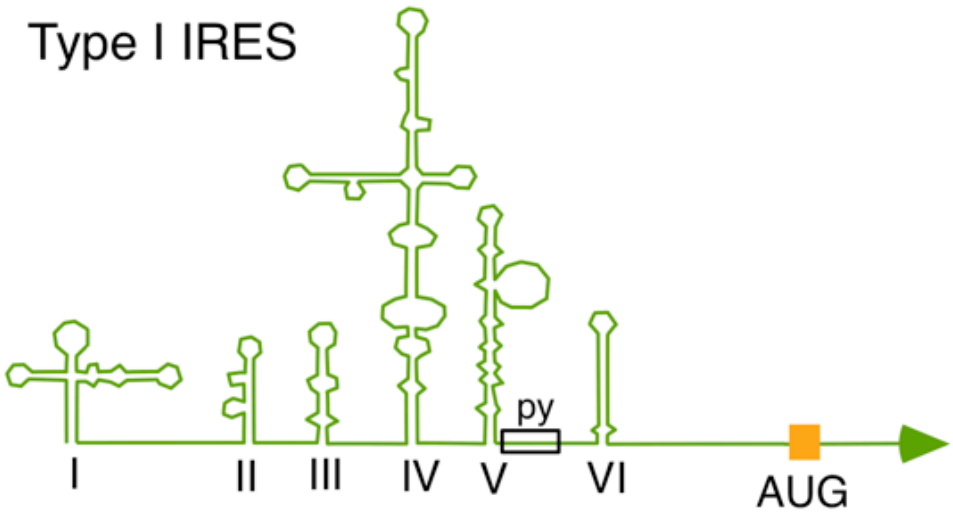
Internal initiation

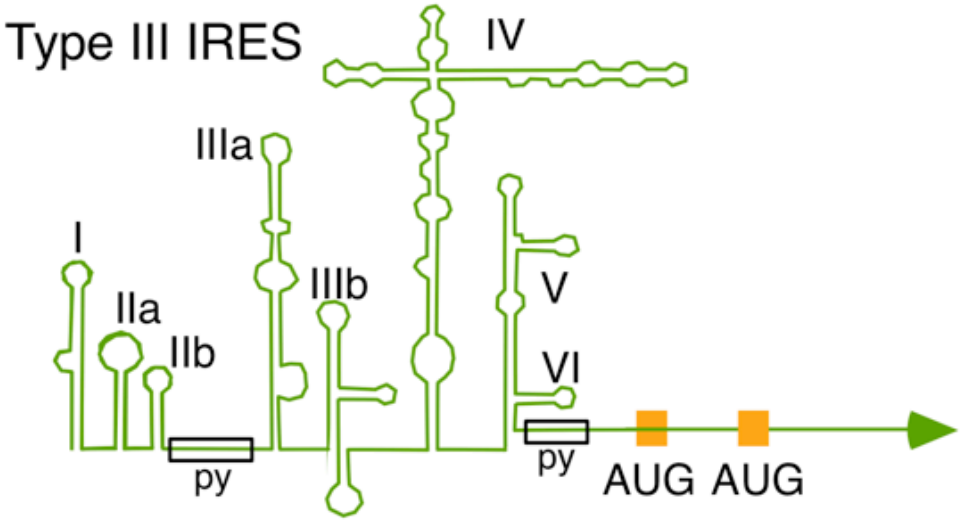


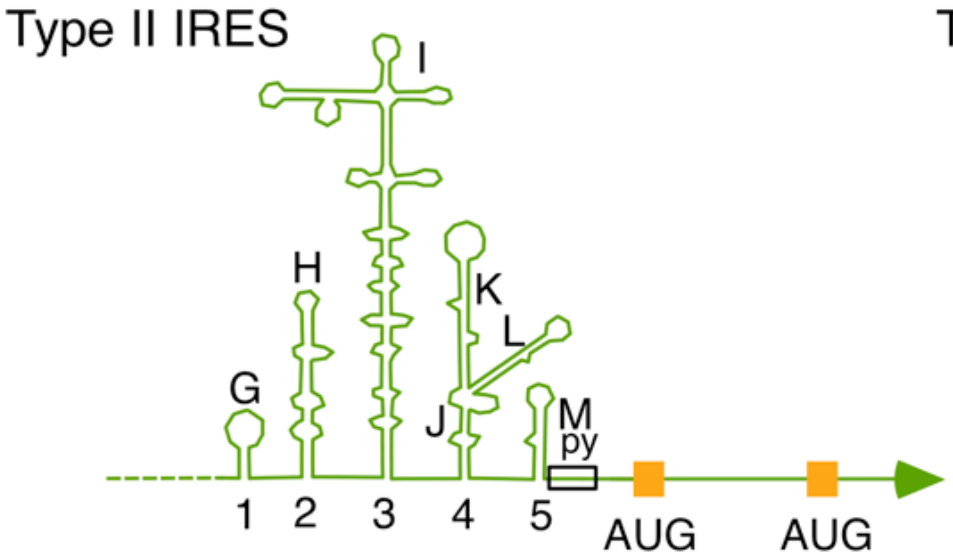


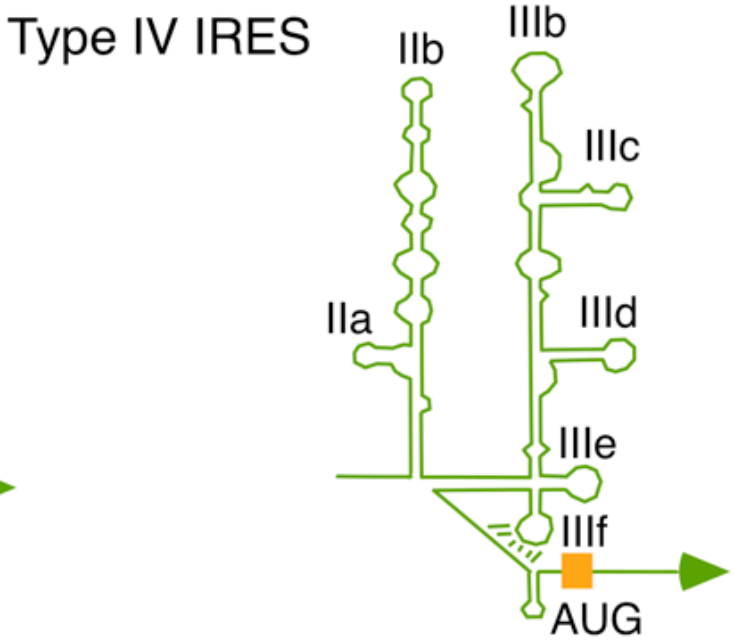
IRES = internal ribosome entry site


B
IRES
UAA
AUG
Translation
No IRES
UAA
AUG
No protein

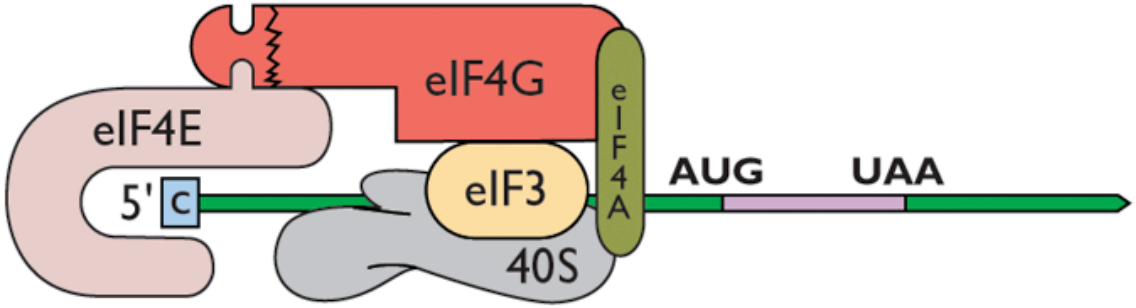

Type I IRES
I
II
III
IV
V
py
VI
AUG


Type III IRES
IV
IIIa
I
IIa
IIb
IIIb
V
VI
py
py
AUG
AUG


Type II IRES
I
H
K
L
G
J
M
py
1
2
3
4
5
AUG
AUG

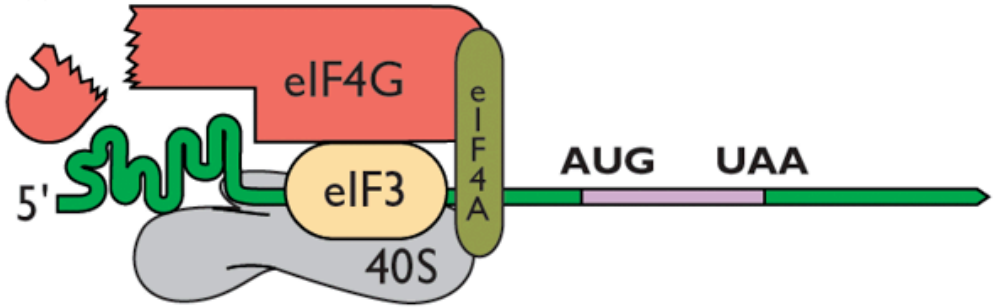

Type IV IRES
IIb
IIIb
IIIc
IIa
IIId
IIIe
IIIf
AUG

Cap



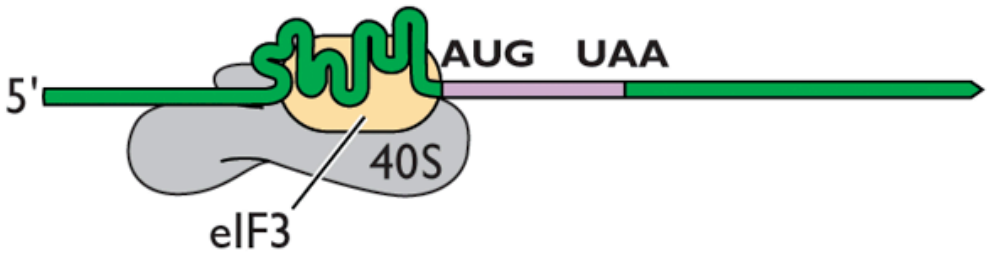
all eIFs

Type I, II IRES



all eIFs except eIF4E

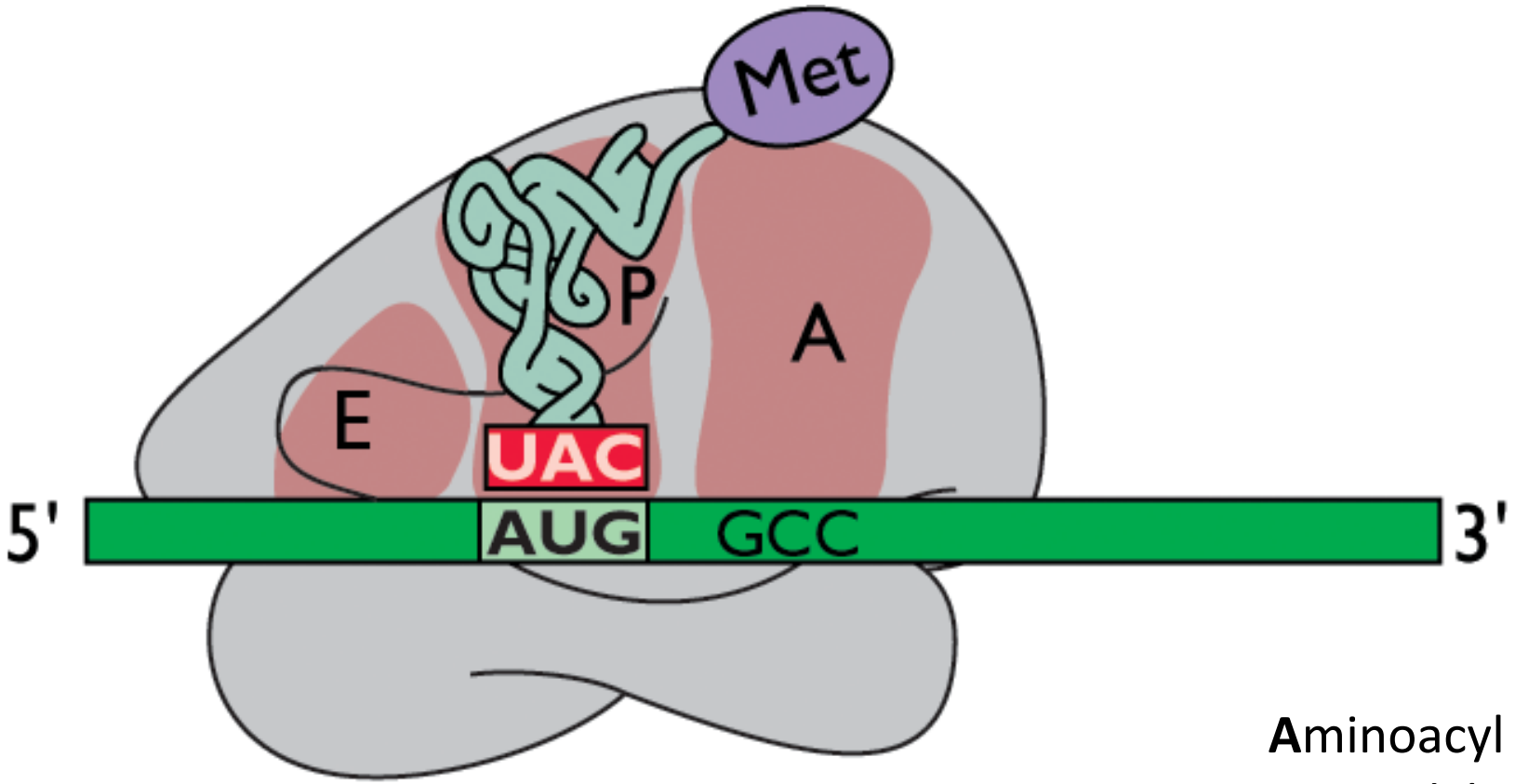
Hepatitis C virus IRES



eIF2, eIF3

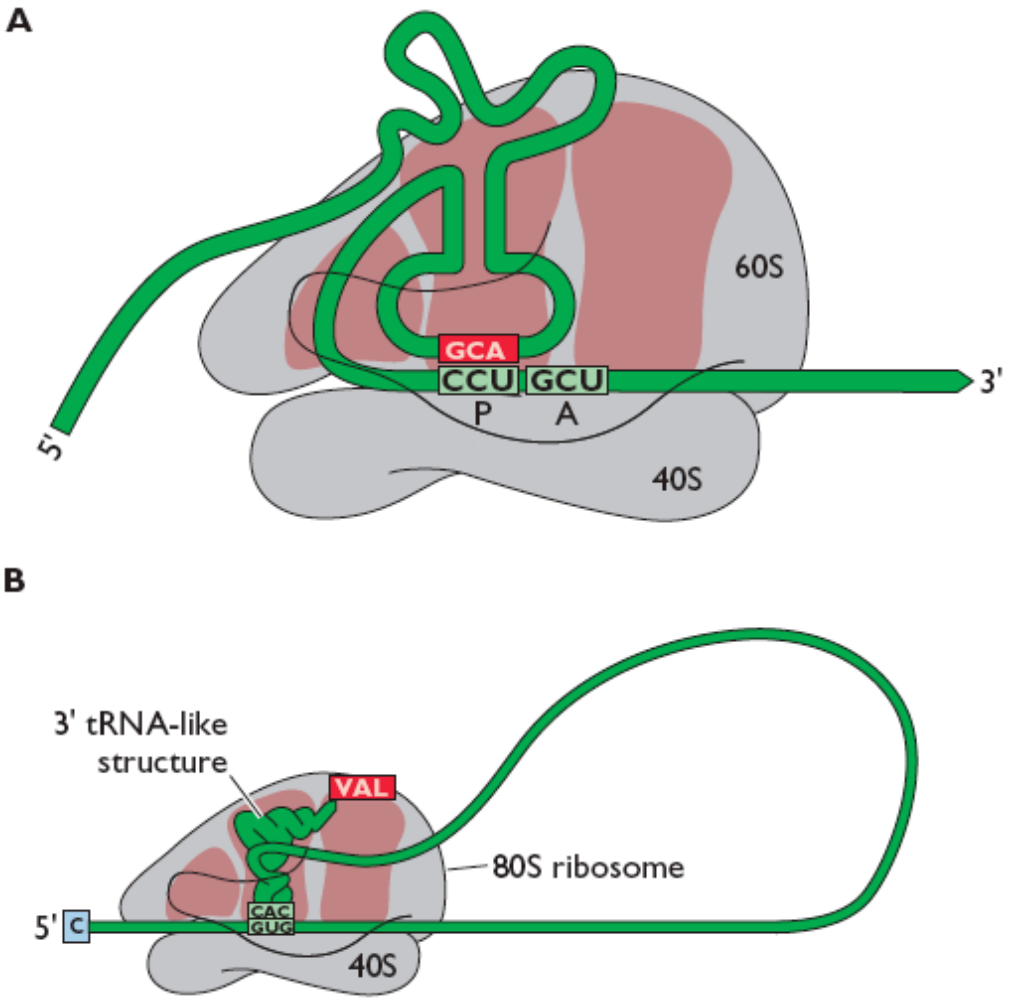
- Viral IRESes
  - Picornaviruses
  - Flaviviruses (hepatitis C virus)
  - Pestiviruses (bovine viral diarrhea virus, classical swine fever virus)
  - Retroviruses (SIV, MMLV, HTLV, FLV)
  - Insect picorna-like viruses (cricket paralysis virus, Plautia stali intestine virus)

- Cellular IRESes
  - BiP
  - c-Myc
  - *Antennapaedia*
  - Ornithine decarboxylase
  - Fibroblast growth factor
  - Vascular endothelial growth factor
  - protein kinase $p58^{PITSLRE}$

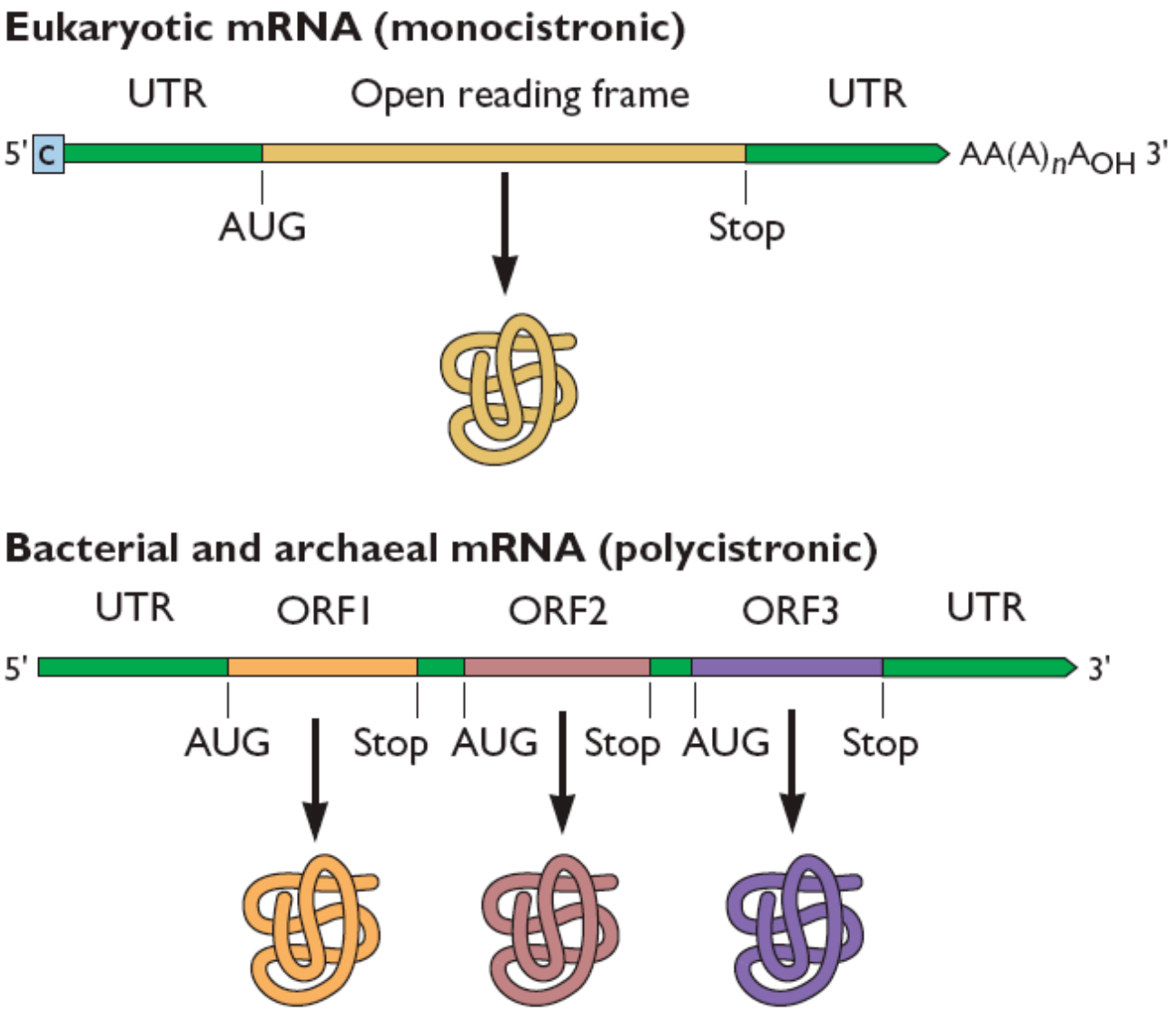


Aminoacyl
Peptidyl
Exit

Methionine-independent initiation



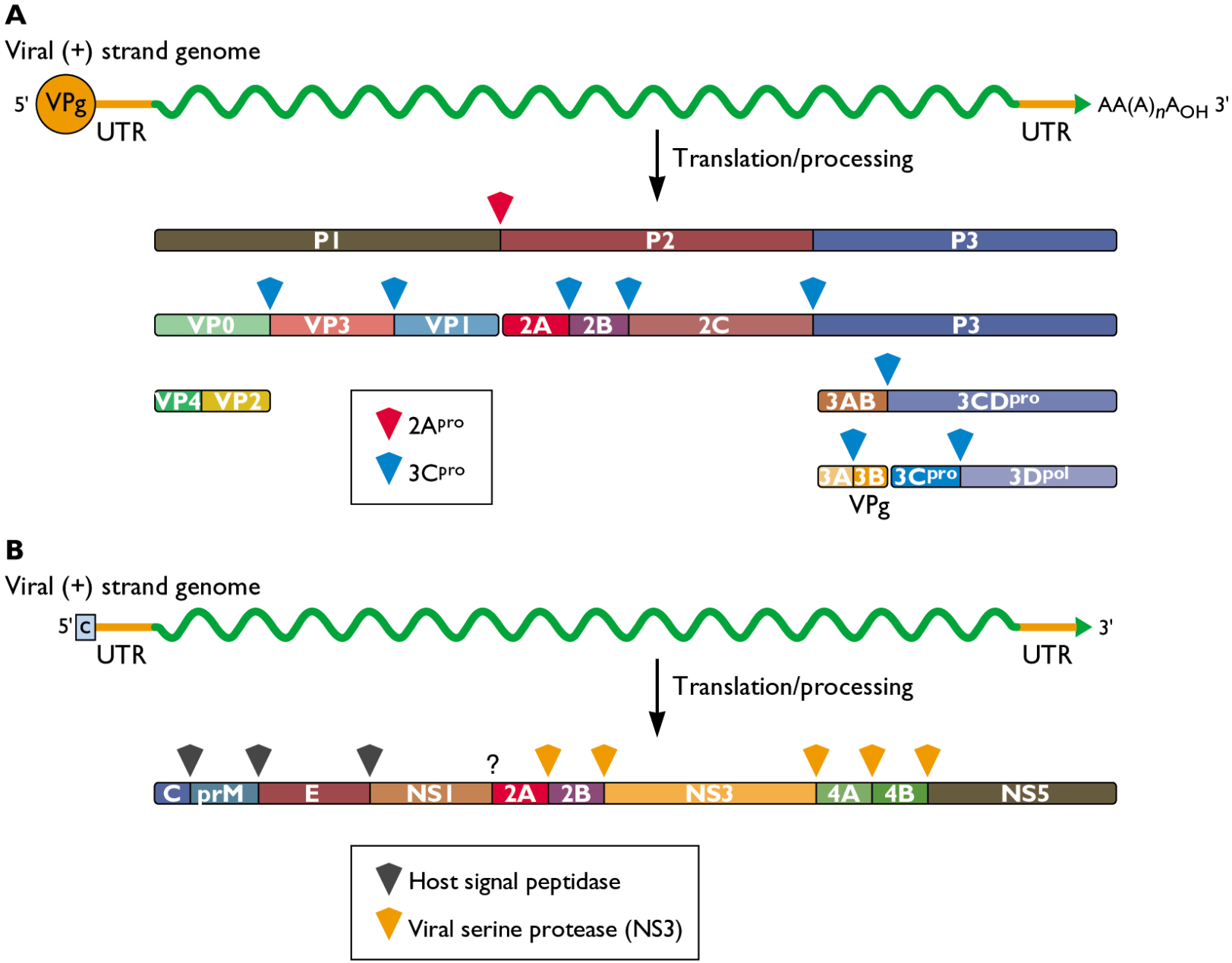
- Can assemble 80S ribosomes without any eIFs or Met-tRNAi
- RNA mimics tRNAi


Eukaryotic mRNA (monocistronic)
UTR
Open reading frame
UTR
5'
C
AA(A)nAOH 3'
AUG
Stop
Bacterial and archaeal mRNA (polycistronic)
UTR
ORF1
ORF2
ORF3
UTR
5'
3'
AUG
Stop
AUG
Stop
AUG
Stop

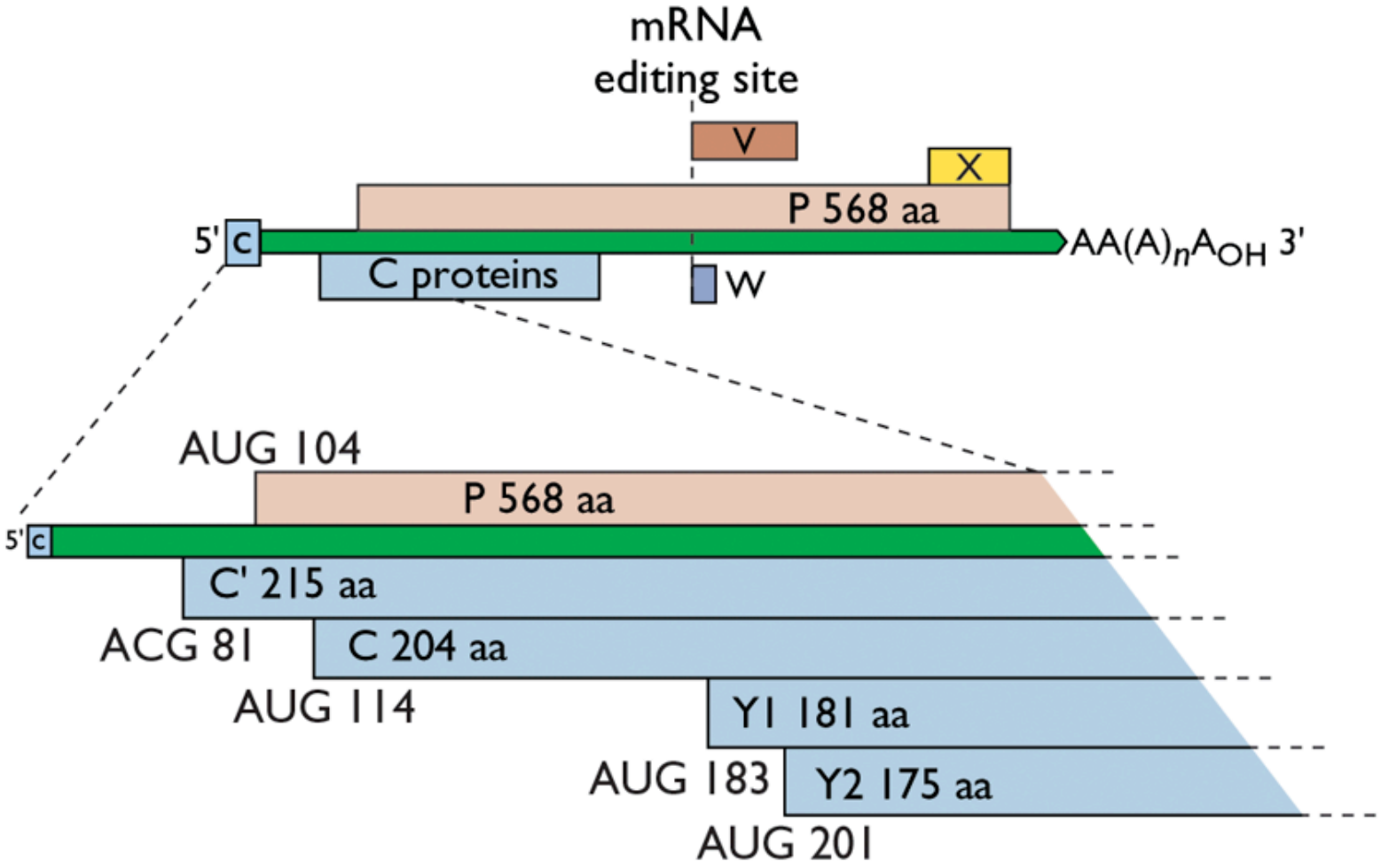
Decoding the viral genome

- Polyprotein (*Picornaviridae, Flaviviridae, Togaviridae, Arenaviridae, Bunyaviridae, Retroviridae*)
- Subgenomic mRNAs (Rhabdoviridae, *Paramyxoviridae, Togaviridae*)
- Segmented genome (*Orthomyxoviridae*, *Reoviridae*)
- Internal initiation (IRES) (*Picornaviridae, Flaviviridae)*
- Leaky scanning (*Retroviridae, Paramyxoviridae)*
- Re-initiation of translation (*Orthomyxoviridae, Herpesviridae*)
- Suppression of termination (*Retroviridae, Togaviridae)*
- Ribosomal frameshifting (*Retroviridae)*

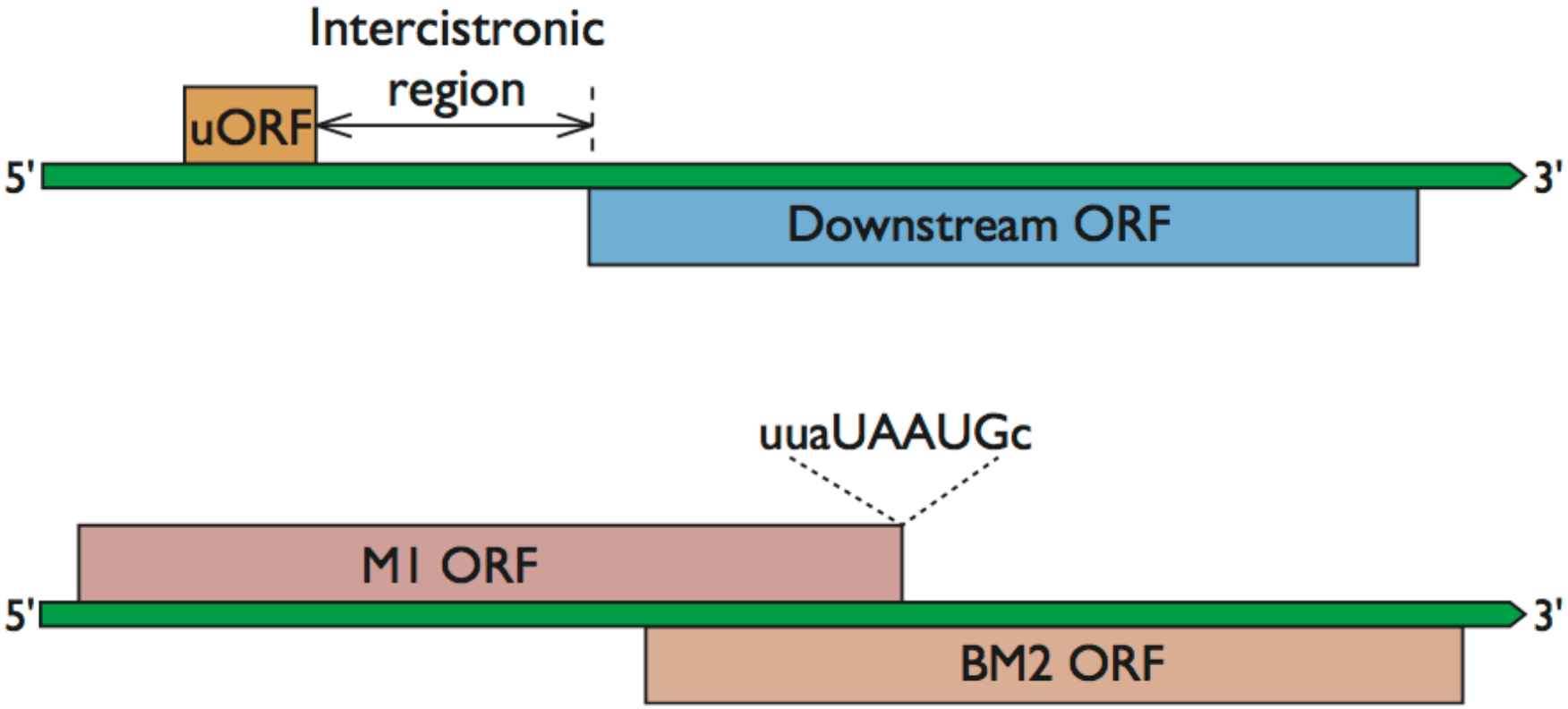
Polyprotein synthesis



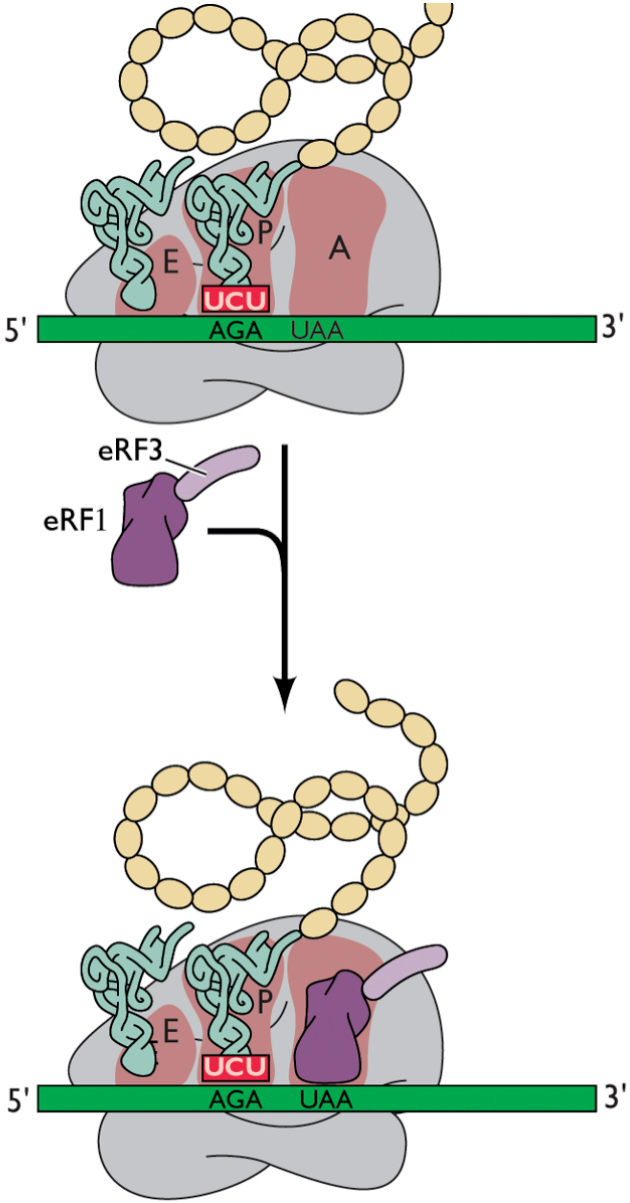
Leaky scanning



Re-initiation of translation



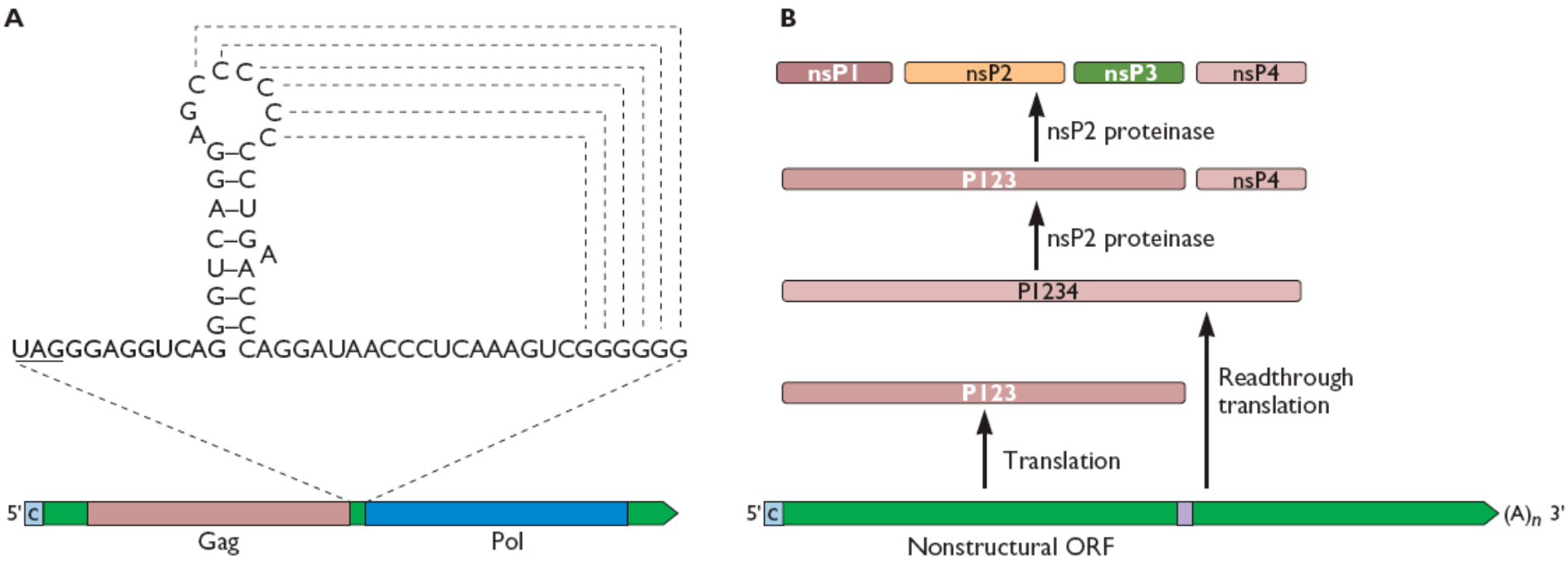
Suppression of termination



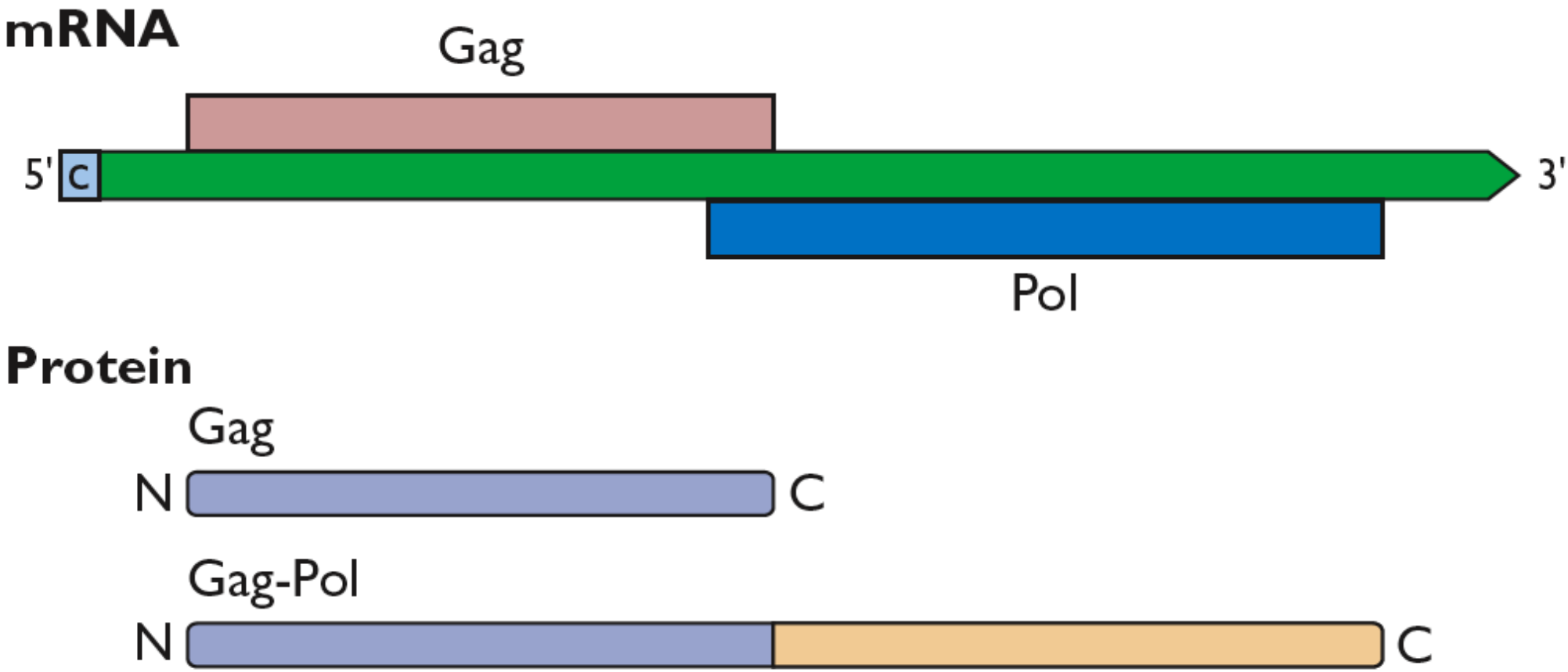
eRF1 and eRF3 recognize all 3 stop codons (UAA, UAG, UGA

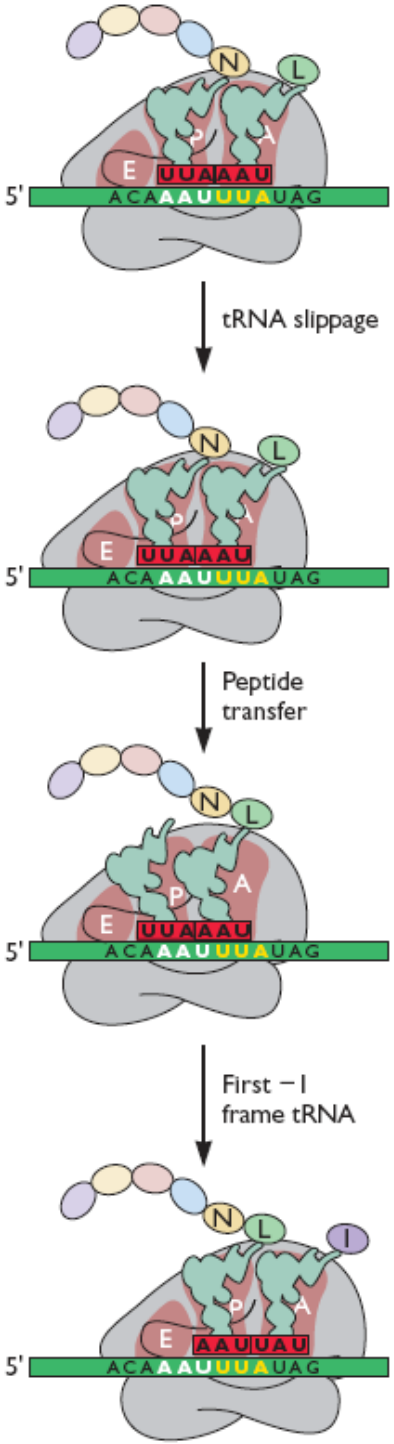
But occasionally stop codons may be recognized by a charged tRNA

Suppression of termination



Ribosomal frameshifting



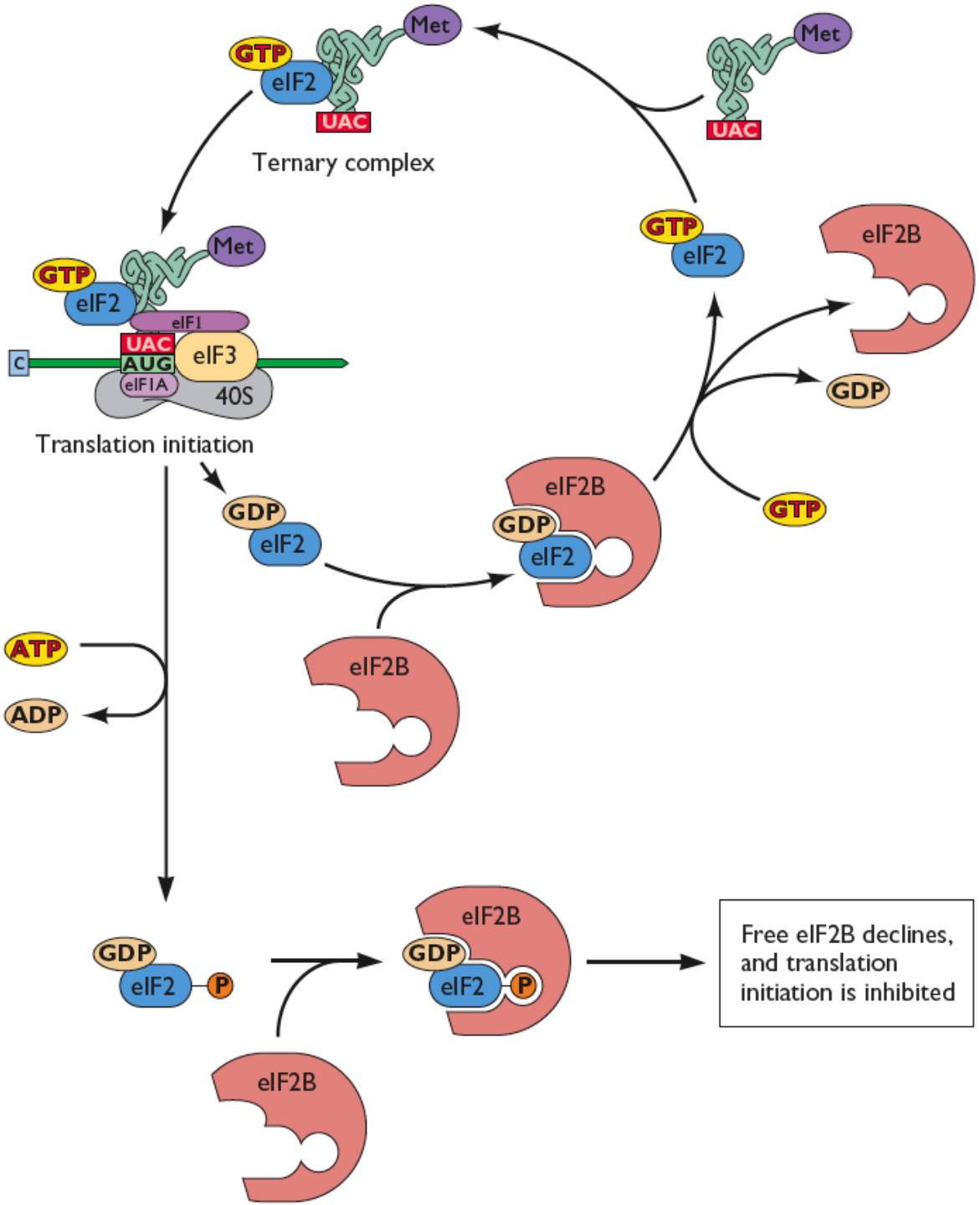

N
L
P
A
E
UUAAAU
5'
ACAAAUUUAUAG
tRNA slippage
N
L
P
A
E
UUAAAU
5'
ACAAAUUUAUAG
Peptide
transfer
N
L
P
A
E
UUAAAU
5'
ACAAAUUUAUAG
First −1
frame tRNA
N
L
I
P
A
E
AAUUAU
5'
ACAAAUUUAUAG

Regulation of translation in virus-infected cells

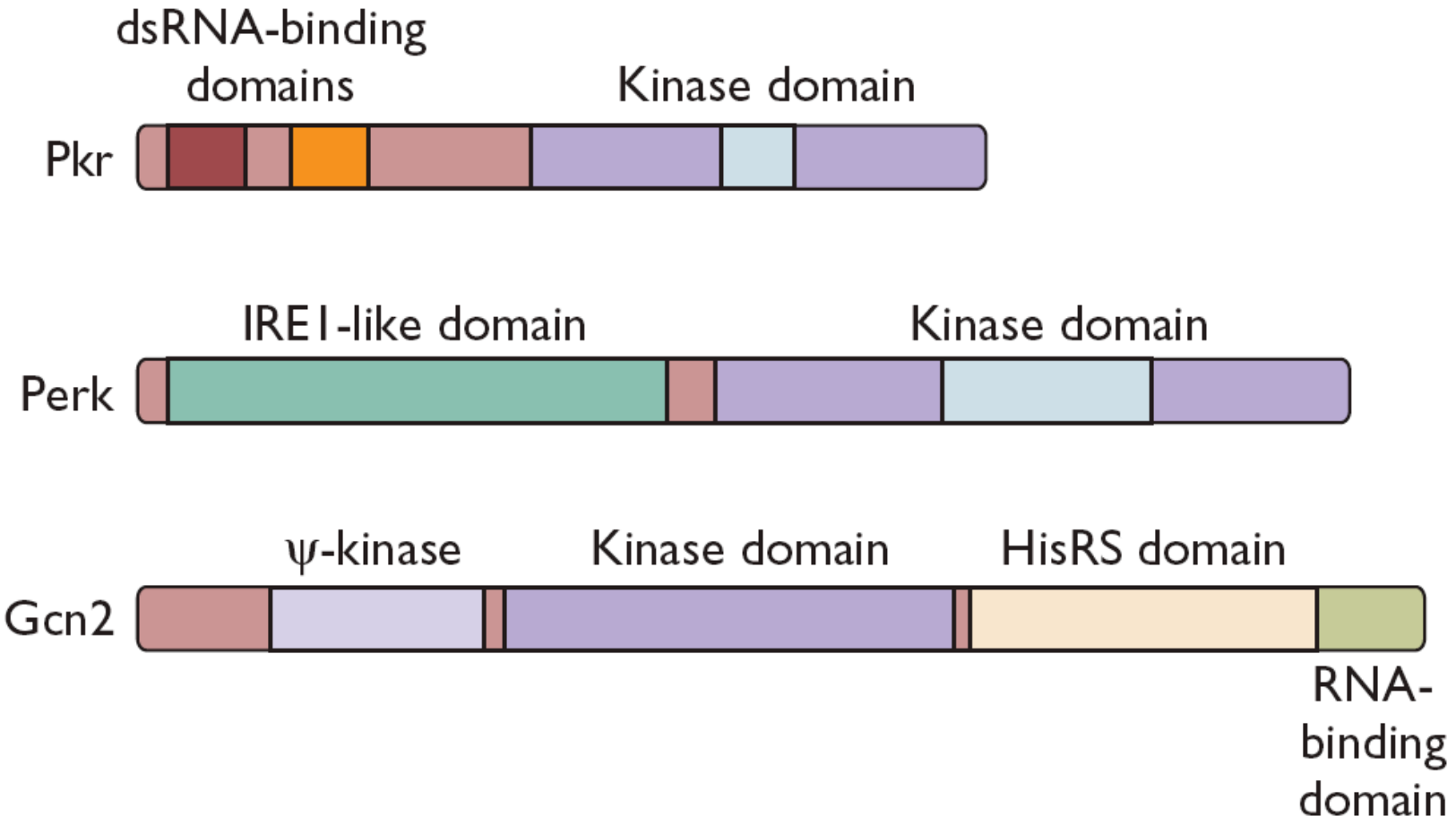
- Initiation
- Elongation
- Termination

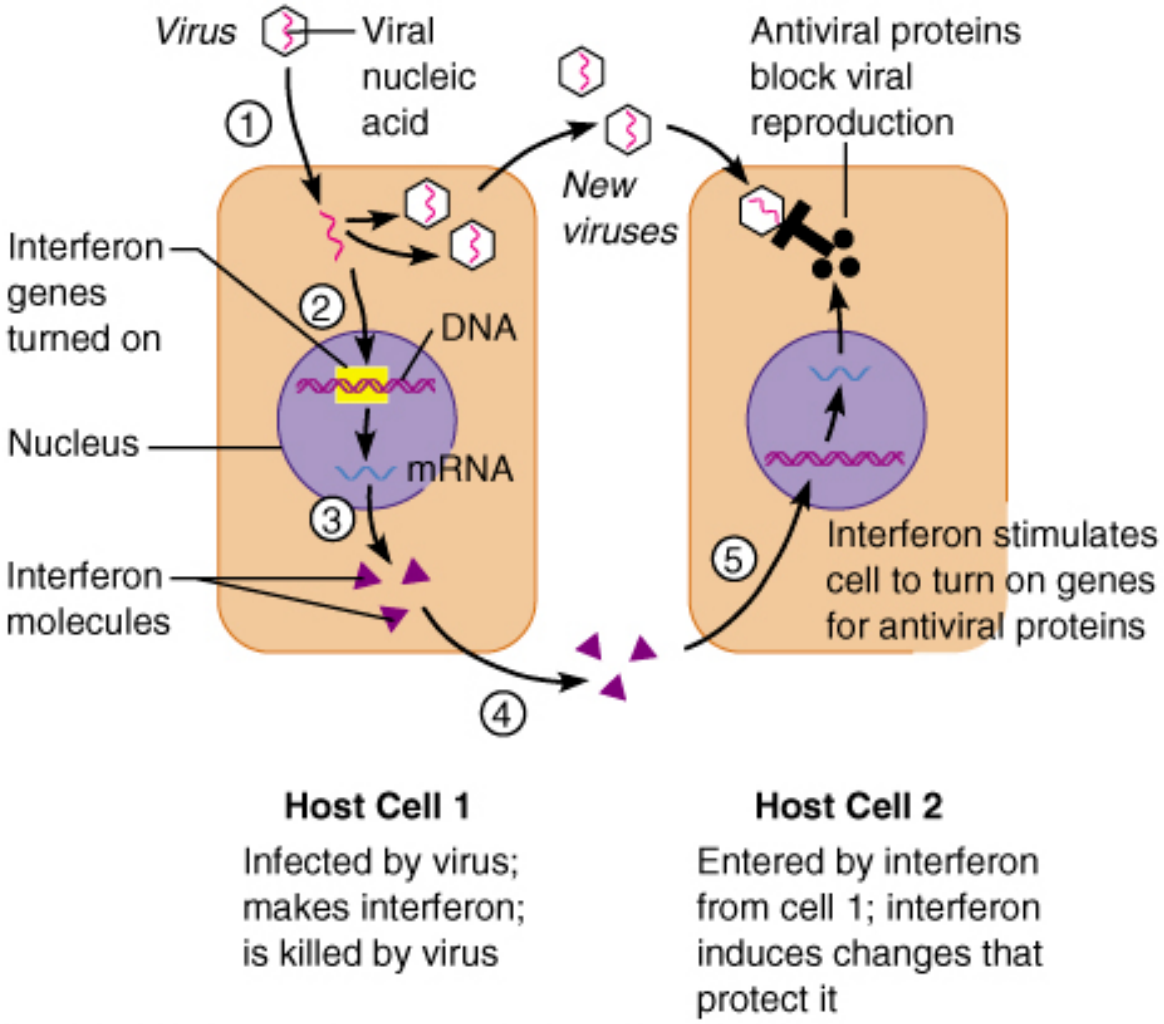
	Viruses	Viral functions	Effects on target
eIF4E	Caliciviruses and TMV	VPg	Binds eIF4E and recruits factors to viral mRNA
	Enteroviruses	Unknown	Induces host miR-141 and suppresses *eIF4E* mRNA translation
4EBP1	VSV	M protein	Dephosphorylate 4EBP1 (via inactivation of AKT–mTOR)
	Reoviruses	p17	
	SV40	Small t antigen	Dephosphorylates 4EBP1 (PP2A dependent)
	HSV-1	US3	Phosphorylate 4EBP1 (via TSC2 inactivation)
	HCMV	UL38	
	KSHV	v-GPCR	Phosphorylate 4EBP1 (via PI3K–AKT–mTOR pathway activation)
	EBV	LMP2A	
	Adenoviruses	E4 ORF1 and E4 ORF4	
	HCV	NS5A	Phosphorylates 4EBP1 (via FKBP38 binding to activate mTOR)
	HPV	E6	Phosphorylates 4EBP1 (via PDK1 activation and TSC2 degradation)
eIF4G	Enteroviruses	2A protease	Cleave eIF4G
	Caliciviruses	3C protease	
	Retroviruses	Protease	
	FMDV	Leader protease	
	Influenza viruses	Polymerase NS1	Binds eIF4G and promotes viral-mRNA translation
	Adenoviruses	100K	Binds eIF4G, dephosphorylates eIF4E (via competitive displacement of MNK1) and promotes ribosome shunting on viral mRNAs
	Rotaviruses	NSP3	Binds eIF4G and competitively displaces PABP
	HSV-1	ICP6	Binds eIF4G and increases its interaction with eIF4E
	Enteroviruses	IRES	Interacts with eIF4G to recruit the 40S ribosomal subunit
eIF4A	HSV-1	vhs	Binds eIF4A and either eIF4H or eIF4B, and this targets vhs endoribonuclease activity to mRNAs, accelerating mRNA turnover
	HCMV	UL69	Binds eIF4A (consequence unknown)
eIF5B	Enteroviruses	3C protease	Cleaves eIF5B
PABP	Enteroviruses	3C and 2A proteases	Cleave PABP
	Caliciviruses	3C-like protease	
	Lentiviruses	Protease	
	Rubella virus	Capsid	Binds PABP and suppresses translation
	Influenza viruses	NS1	Binds PABP (consequence unknown)
	HSV-1	ICP27	Binds PABP and stimulates translation of a viral mRNA subset
		ICP27 and UL47	Cause nuclear PABP accumulation
	HCMV	UL69	Binds PABP (consequence unknown)
	KSHV	SOX and K10	Bind PABP and causes its nuclear accumulation
	Bunyaviruses	NSS protein	Causes nuclear PABP accumulation
	Rotaviruses	NSP3	Displaces PABP from eIF4G, and interacts with RoXaN to cause nuclear PABP accumulation
eIF3	Measles virus	N protein	Binds eIF3g and impairs translation
	Rabies virus	M protein	Binds eIF3h and impairs translation
	SARS CoV and IBV	Spike protein	Binds eIF3f and impairs translation
	Caliciviruses (including noroviruses)	VPg	Binds eIF3 and recruits factors to viral mRNA
	CaMV	RISP	Binds eIF3a and eIF3c, binds the 60S ribosomal subunit L24 and recruits ribosomes for re-initiation
		TAV	Binds and activates TOR, and recruits RISP
	HCV, CSFV and HIV	IRES	Interacts with eIF3 and recruits translation machinery
eIF2	HSV-1	US11	Inhibits PKR
		gB	Inhibits PERK
		γ34.5	Regulates eIF2α phosphatase
	EBV	SM	Inhibits PKR
		EBER RNAs	Bind PKR and prevent its activation
	HCMV	TRS1 and IRS1	Bind dsRNA and prevent PKR activation
	KSHV	v-IRF2	Binds PKR and prevents its activation
	VacV	E3L	Binds dsRNA and PKR
		K3L	Acts as a pseudosubstrate for PKR and PERK
	Adenoviruses	VA RNA	Binds PKR and prevents its activation
	ASFV	DP17L	Dephosphorylates eIF2α by recruiting PP2A
	HCV	NS5A	Inhibits PKR
		E2	Acts as a pseudosubstrate for PKR and PERK
		IRES	Inhibits PKR
	Influenza viruses	NS1	Sequesters dsRNA and prevents PKR activation
	Reoviruses	σ3	
	HPV	E6	Binds GADD34–PP1α to dephosphorylate eIF2α
eEF1A and eEF1B	TMV	VPg	Binds eEF1A so that it is redistributed to viral replication compartments
	SARS CoV	N protein	Binds eEF1A and impairs translation
	HIV-1	Gag	Binds eEF1A and impairs viral mRNA translation to stimulate packaging
	HSV-1	UL13	Phosphorylate eEF1Bα
	HCMV	UL97	
	EBV	BGLF4	
eRF1	HIV-1	Reverse transcriptase	Binds eRF1, modulates termination and re-initiation, and protects viral mRNAs from nonsense-mediated decay
	HCMV	uORF2	Binds eRF1 and inhibits translation at its own stop codon to regulate translation of the downstream HCMV ORF
Ribosome	HCV, CSFV and HIV	IRES	Binds the 40S ribosome in conjunction with eIF3
	CrPV	IRES	Binds the 40S ribosome to mediate initiation factor-independent translation
	FCV and influenza B virus	TURBS	Base-pairs with 18S ribosomal RNA to promote re-initiation
	KSHV	ORF57	Binds PYM to recruit 40S ribosomes to viral mRNAs

4EBP, eIF4E-binding protein; ASFV, African swine fever virus; CaMV, cauliflower mosaic virus; CrPV, cricket paralysis virus; CSFV, classical swine fever virus; dsRNA

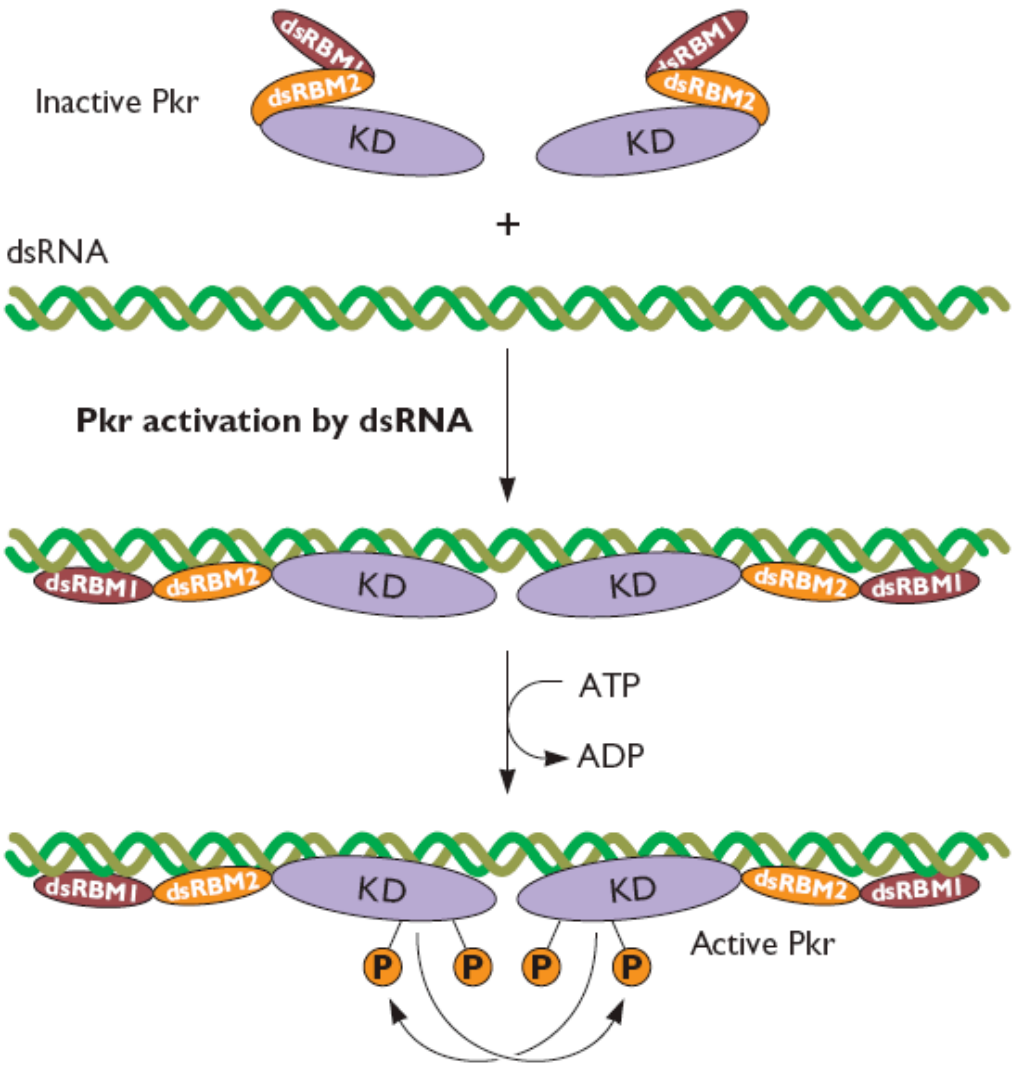

Met
GTP
eIF2
UAC
Ternary complex
Met
UAC
Met
GTP
eIF2
eIF1
UAC
AUG
eIF3
C
eIF1A
40S
Translation initiation
GTP
eIF2
eIF2B
GDP
eIF2B
GDP
eIF2
GDP
GTP
ATP
ADP
eIF2B
GDP
eIF2
P
eIF2B
GDP
eIF2
P
eIF2B
Free eIF2B declines, and translation initiation is inhibited

eIF2α kinases

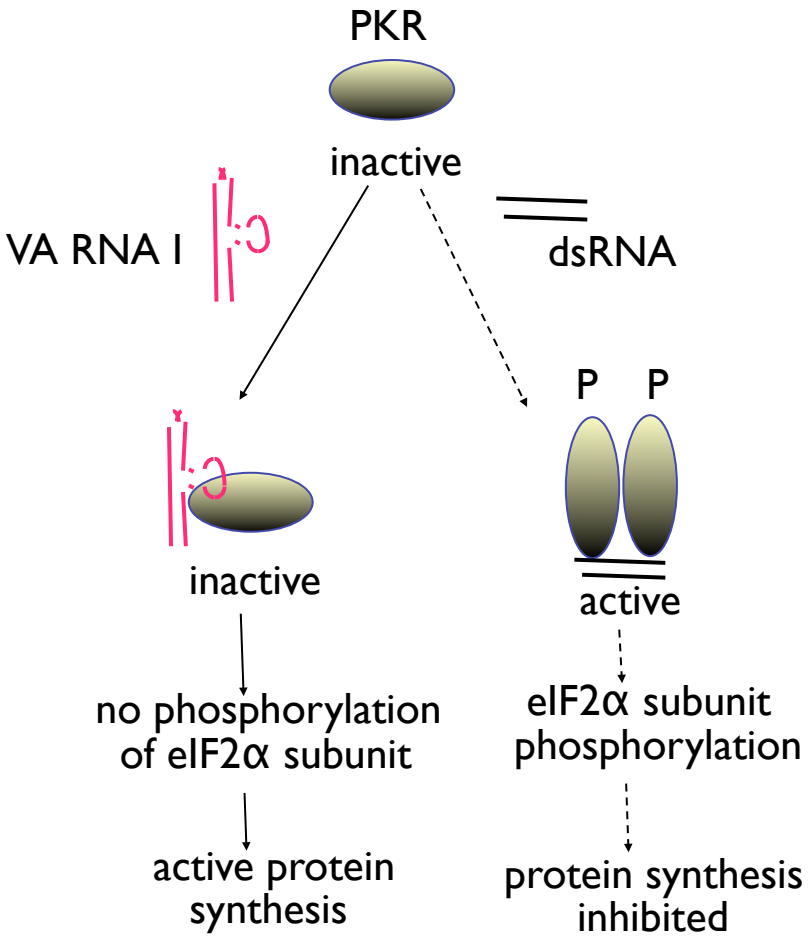



Virus
Viral nucleic acid
1
Interferon genes turned on
2
DNA
Nucleus
mRNA
3
Interferon molecules
4
New viruses
Antiviral proteins block viral reproduction
5
Interferon stimulates cell to turn on genes for antiviral proteins
Host Cell 1
Infected by virus; makes interferon; is killed by virus
Host Cell 2
Entered by interferon from cell 1; interferon induces changes that protect it

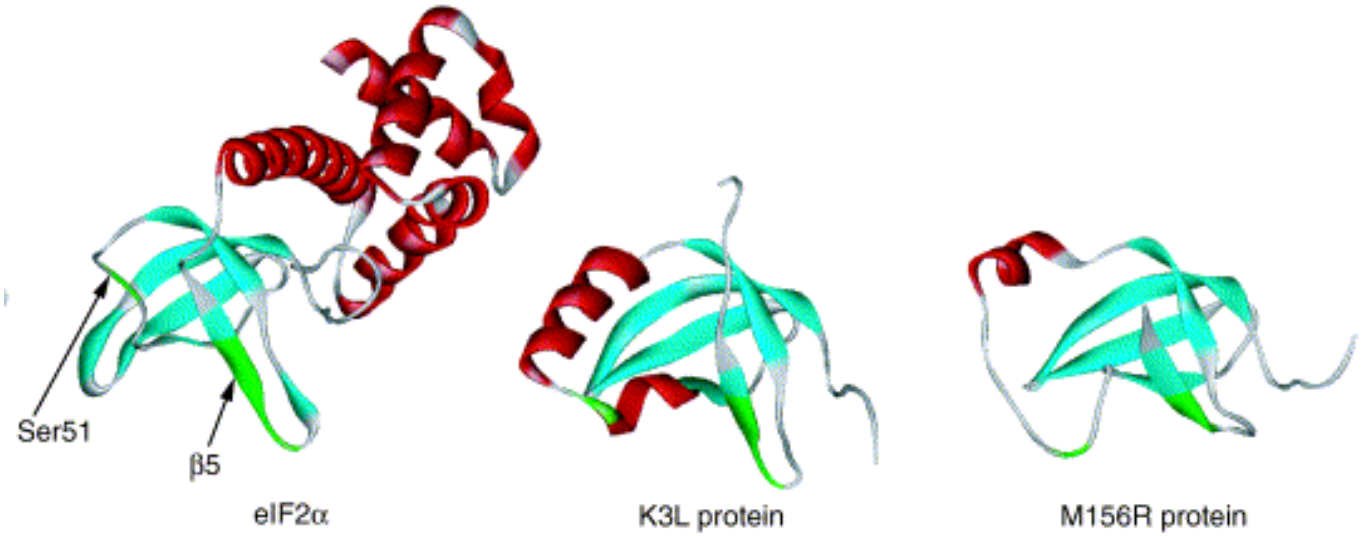
PKR


Inactive Pkr
dsRBM1
dsRBM2
KD
KD
+
dsRNA
Pkr activation by dsRNA
dsRBM1
dsRBM2
KD
KD
dsRBM2
dsRBM1
ATP
ADP
dsRBM1
dsRBM2
KD
KD
dsRBM2
dsRBM1
P
P
P
P
Active Pkr

Adenovirus VA RNA I prevents activation of PKR



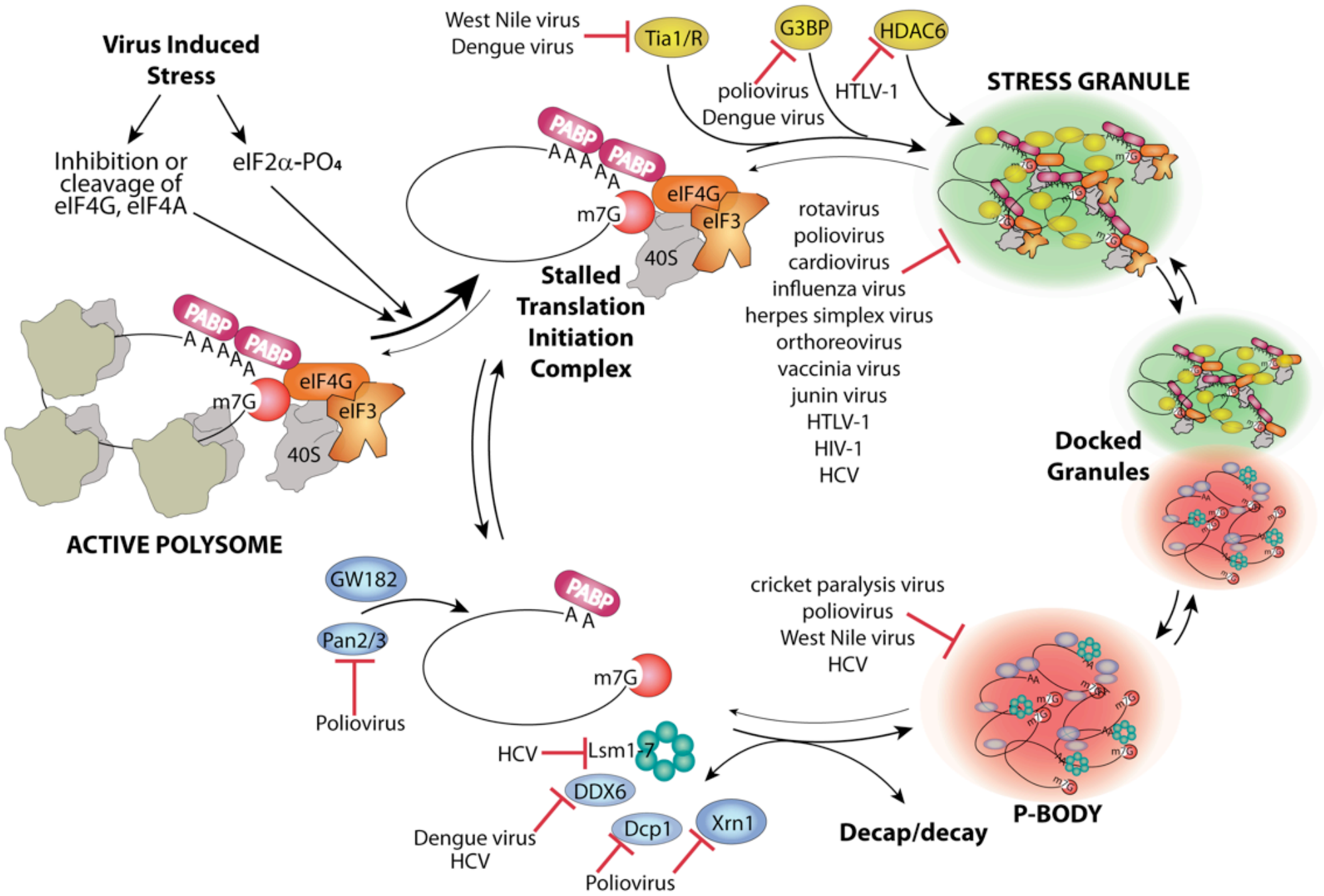
Vaccinia virus encoded pseudosubstrates mimic eIF2α



Targets of viral inhibitors of eIF2α phosphorylation

Target	Virus	Inhibitor	Mechanism
dsRNA	Herpes simplex virus	Us11	Binds and sequesters dsRNA
	Influenza virus	NS1	Binds and sequesters dsRNA
	Reovirus	σ3	Binds and sequesters dsRNA
	Vaccinia virus	E3L	Binds and sequesters dsRNA
Pkr	Adenovirus	VAI RNA	Blocks activation by dsRNA
	Epstein-Barr virus	EBER	Blocks activation by dsRNA
	Human immunodeficiency virus type 1	TAR RNA	Blocks activation by dsRNA
	Herpes simplex virus	Us11	Binds Pkr
	Kaposi sarcoma herpesvirus	vIRF-2	Binds Pkr
	Baculovirus	PK2	Inhibits dimerization
	Hepatitis C virus	NS5A	Inhibits dimerization
	Human immunodeficiency virus type 1	Tat	Reduces Pkr expression
eIF2α	Hepatitis C virus	E2	Pseudosubstrate, blocks Pkr-eIF2α interaction
	Human immunodeficiency virus type 1	Tat	Pseudosubstrate, blocks Pkr-eIF2α interaction
	Vaccinia virus	K3L	Pseudosubstrate, blocks Pkr-eIF2α interaction
Phosphatase	Herpes simplex virus	γ34.5	Binds phosphatase, directs to eIF2α
	Simian virus 40	T antigen	Downstream of eIF2α?
Pact	Herpes simplex virus	Us11	Binds Pact

[a]RBM, RNA-binding motif.


Virus Induced Stress
Inhibition or cleavage of eIF4G, eIF4A
eIF2α-PO4
West Nile virus
Dengue virus
Tia1/R
G3BP
HDAC6
poliovirus
Dengue virus
HTLV-1
STRESS GRANULE
PABP
AAAA
m7G
eIF4G
eIF3
40S
Stalled Translation Initiation Complex
rotavirus
poliovirus
cardiovirus
influenza virus
herpes simplex virus
orthoreovirus
vaccinia virus
junin virus
HTLV-1
HIV-1
HCV
Docked Granules
ACTIVE POLYSOME
GW182
Pan2/3
Poliovirus
cricket paralysis virus
poliovirus
West Nile virus
HCV
HCV
Lsm1-7
DDX6
Dengue virus
HCV
Dcp1
Xrn1
Poliovirus
Decap/decay
P-BODY