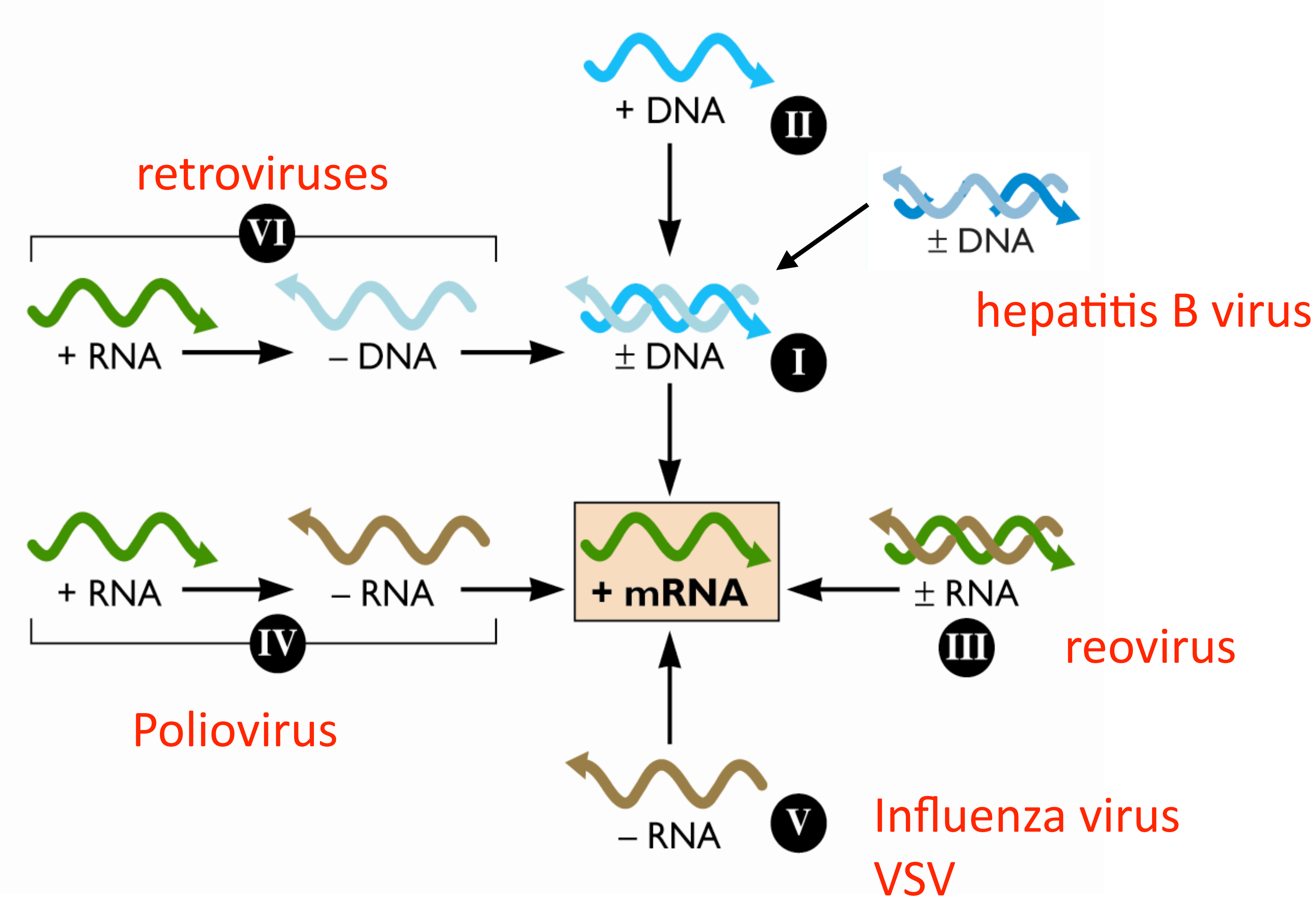
Lost in Translation

Lecture 10
Biology W3310/4310
Virology
Spring 2016

Translation is that which transforms everything
so that nothing changes.
—GÜNTER GRASS



retroviruses

hepatitis B virus

reovirus

Poliovirus

Influenza virus
VSV

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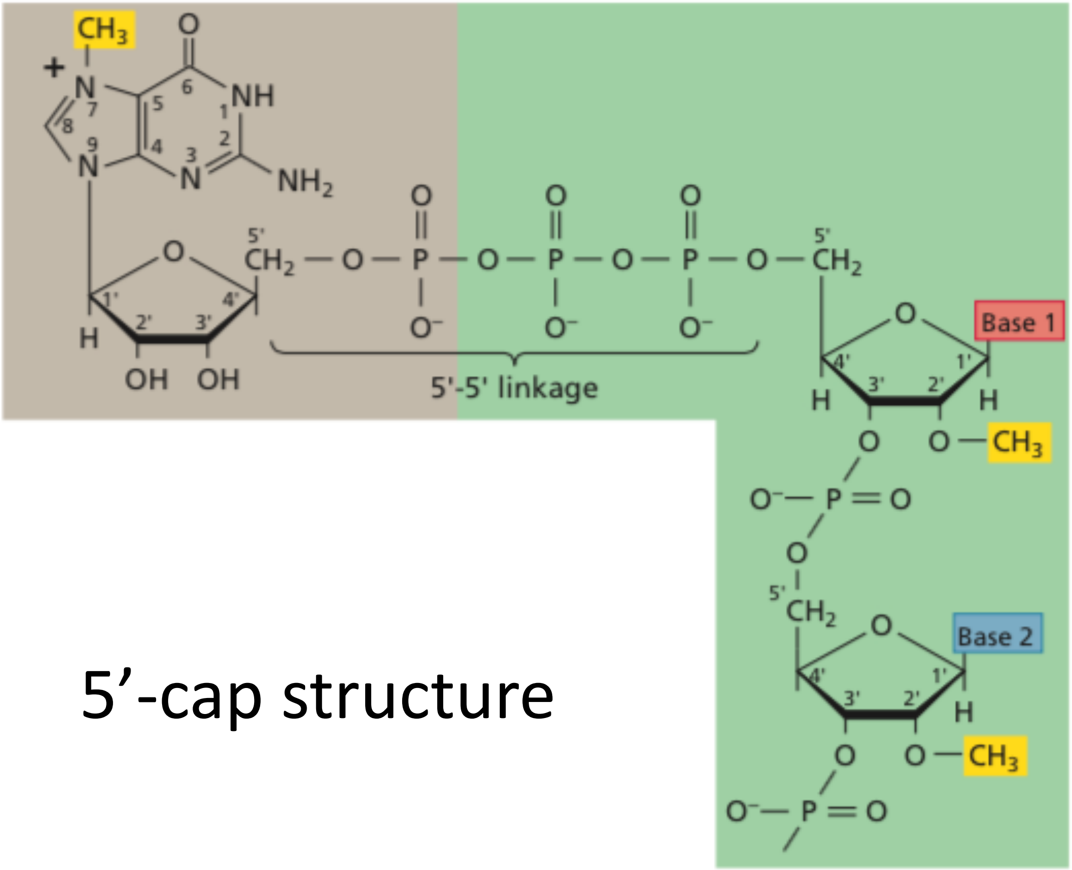
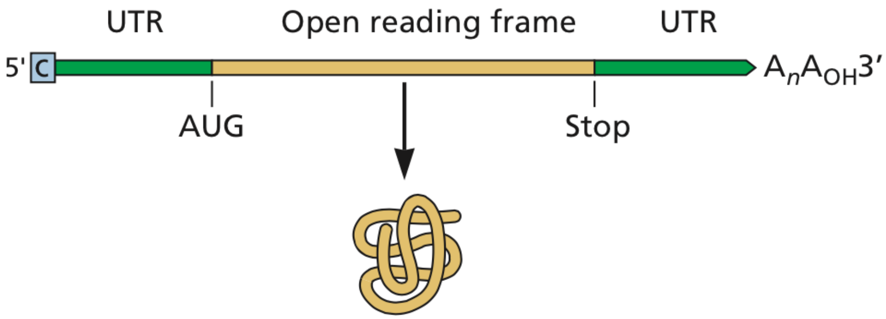
5’-cap structure





- Most eukaryotic mRNAs except organelle mRNAs and certain viral mRNAs
- 5'-7-methylguanosine (m7G) joined to second nucleotide of mRNA by 5'-5' phosphodiester linkage
- Directs pre-mRNAs to processing and transport pathways, regulates mRNA turnover, required for efficient translation by 5'-end dependent mechanism

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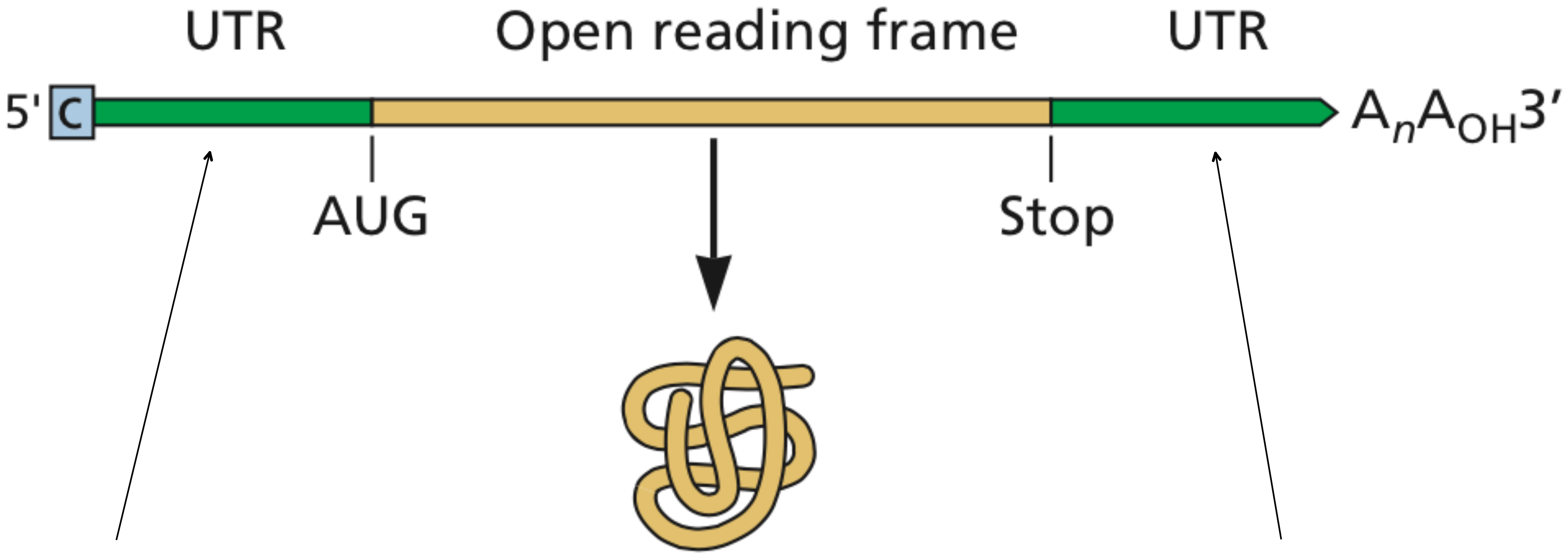


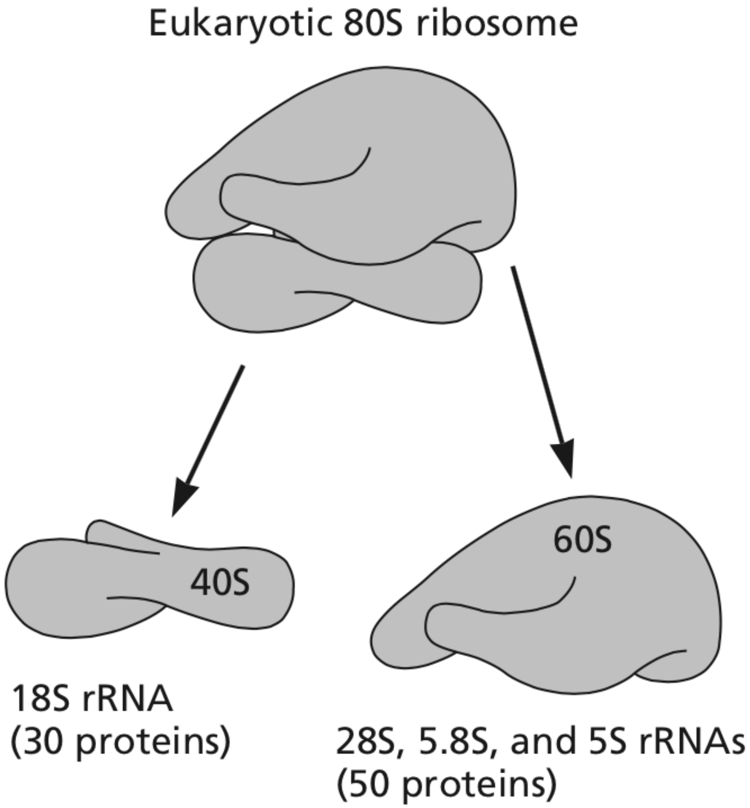
5’-untranslated region

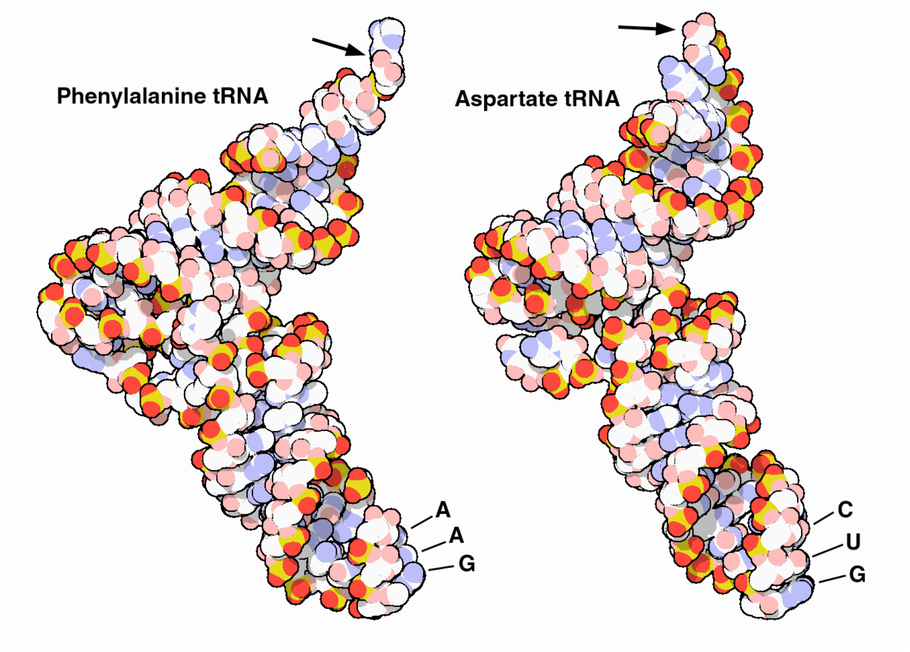
- 3 – >1,000 nt in length, typically 50 – 70 nt
- Often contains RNA secondary structures; must be unwound to allow passage of ribosome
- Length and secondary structure influence translation efficiency

3’-untranslated region

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

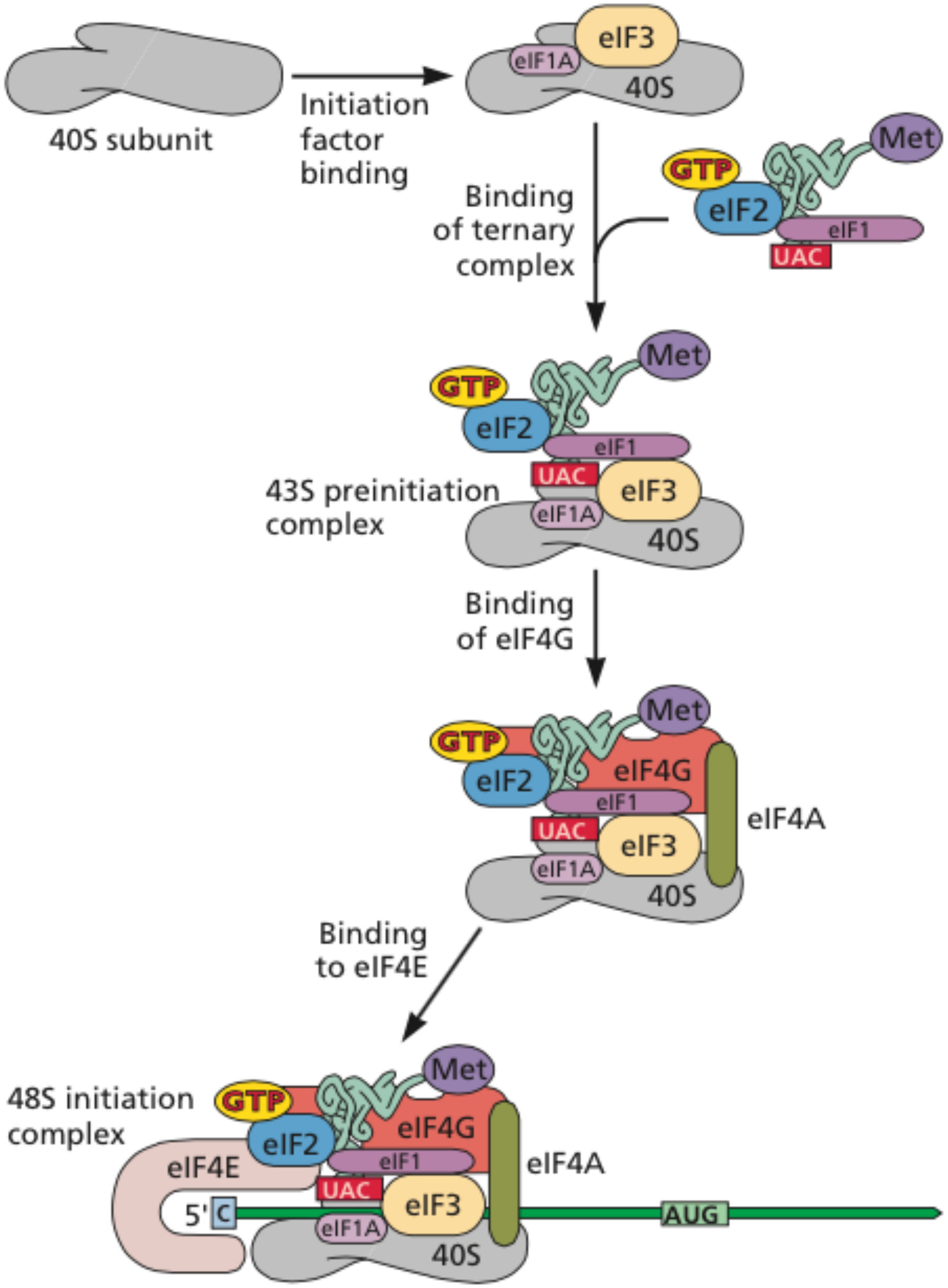
Translational machinery





- Initiation proteins (eIF)
- Elongation proteins (eEF)
- Termination proteins (eRF)

5’-end dependent initiation



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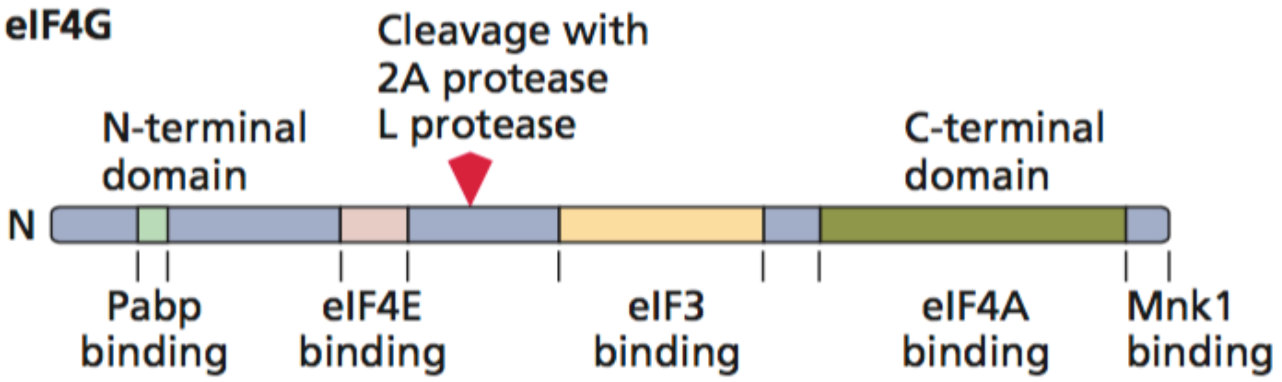
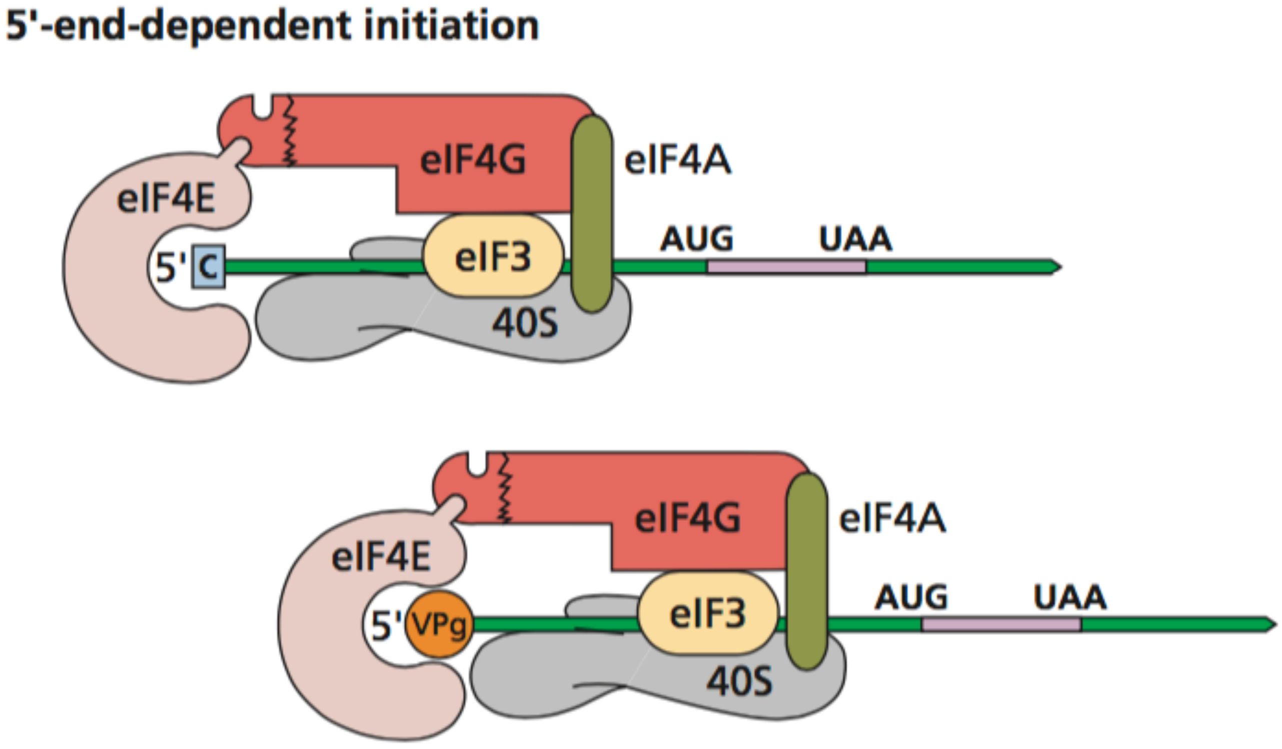
5’-end dependent initiation


eIF4G


5'-end-dependent initiation

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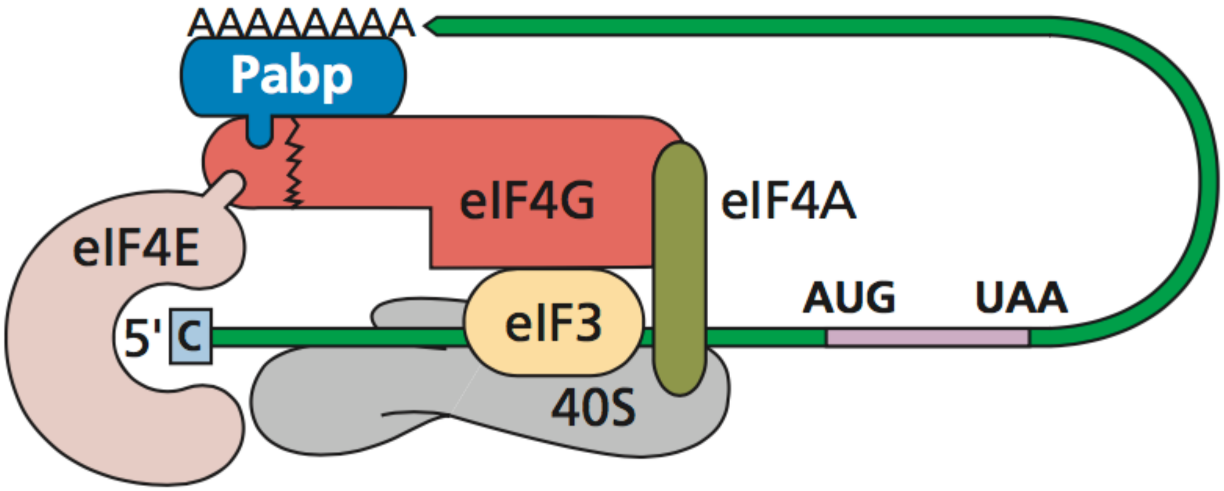
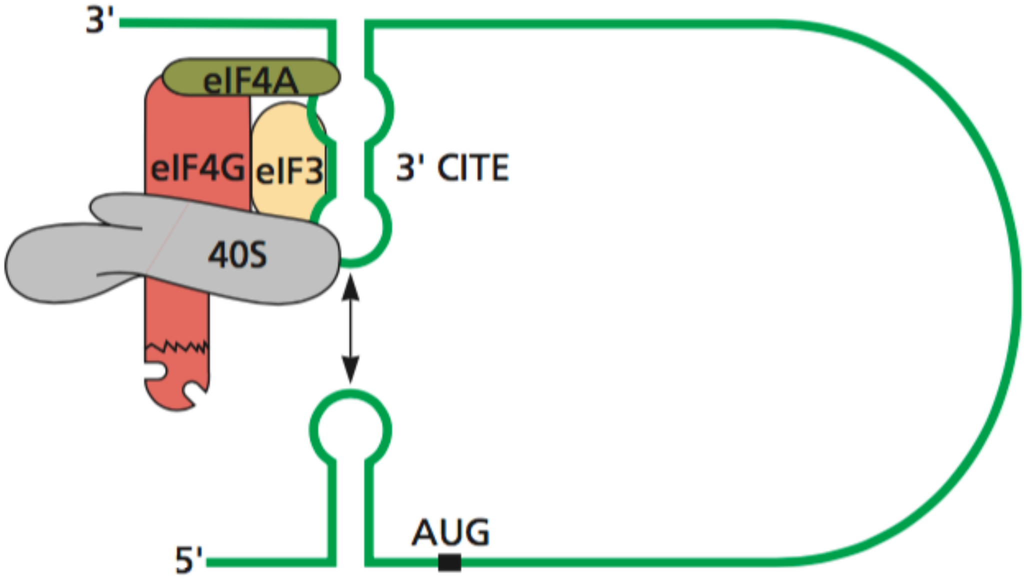
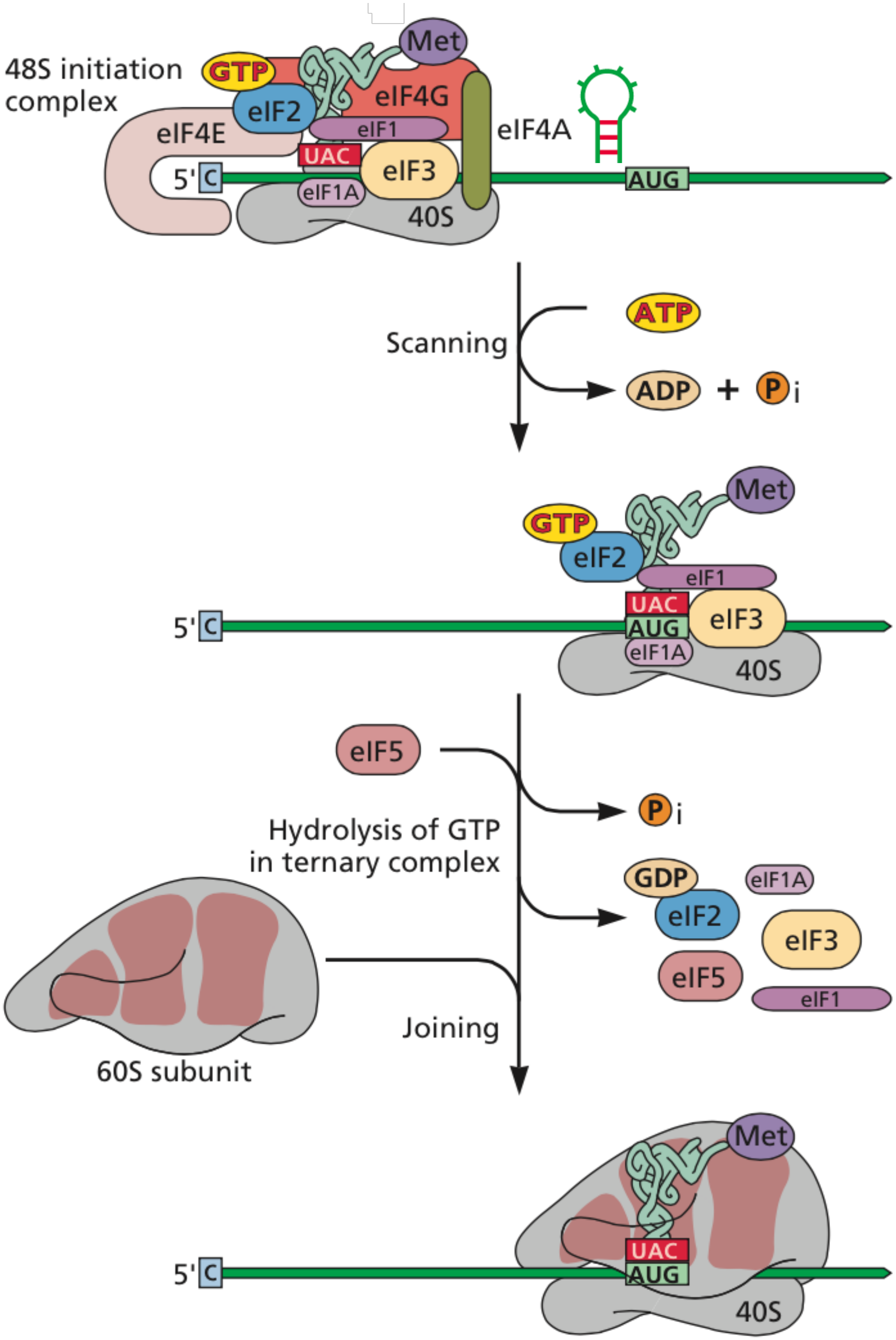
Juxtaposition of mRNA ends





Pea enation mosaic virus
Barley yellow dwarf virus



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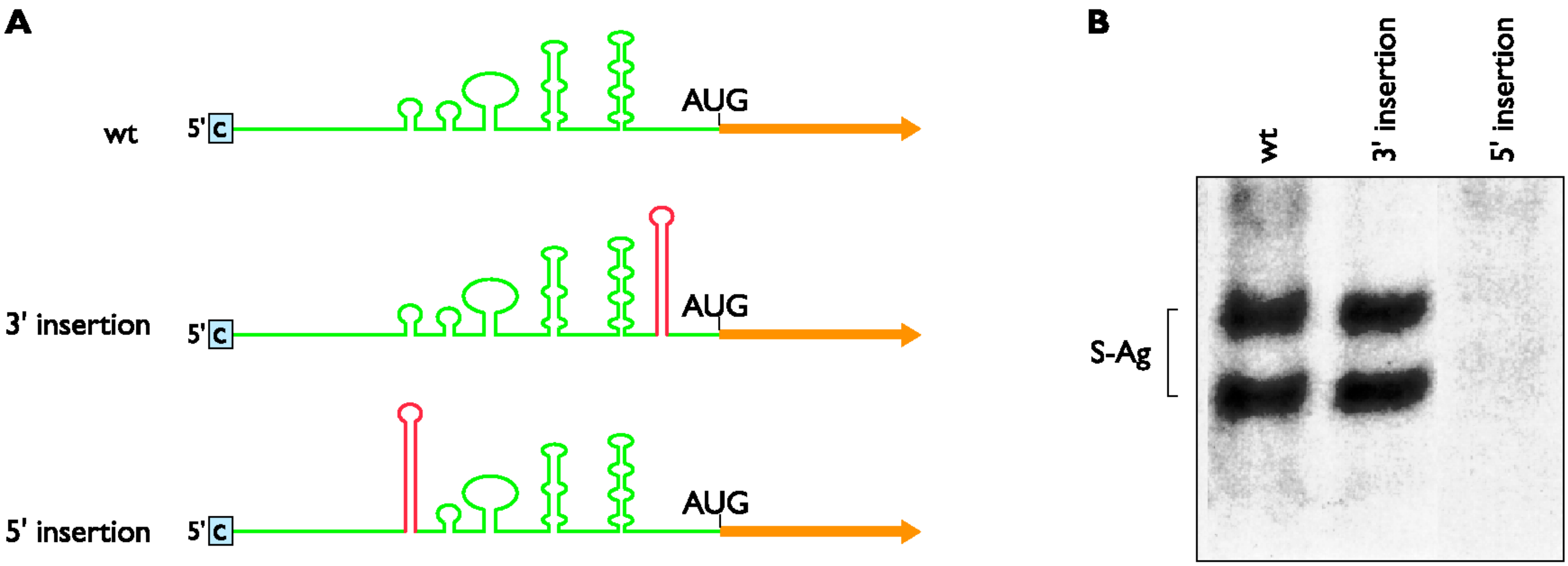
Which statement about the 5’-cap on mRNA is incorrect?

1. It consists of m7G joined to second nucleotide of mRNA by an unusual 5'-5' phosphodiester linkage
2. It is present on most cellular mRNAs
3. It is required for efficient translation by 5’-end dependent initiation
4. It binds the cap-binding protein eIF4E
5. It is found on mRNA but not pre-mRNA

Other mechanisms for decoding have been discovered in virus-infected cells

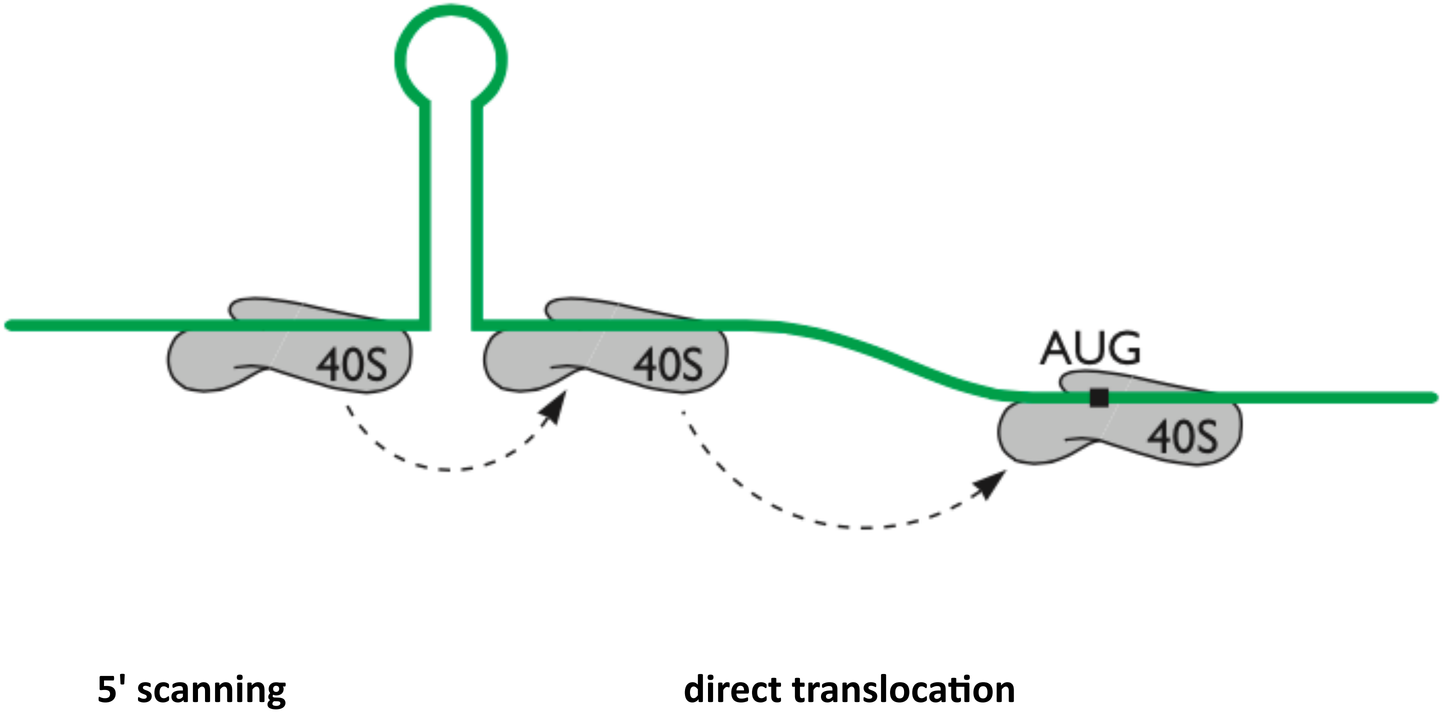
Ribosome shunting



-80 kcal/mole

Ribosome shunting

- –35s mRNAs of plant caulimoviruses
- –Late adenovirus mRNAs
- –P/C mRNA of Sendai virus



Shunting is predicted to decrease dependence of mRNAs for eIF4F during initiation by reducing the need for mRNA unwinding

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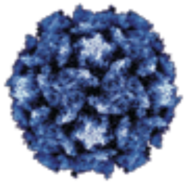
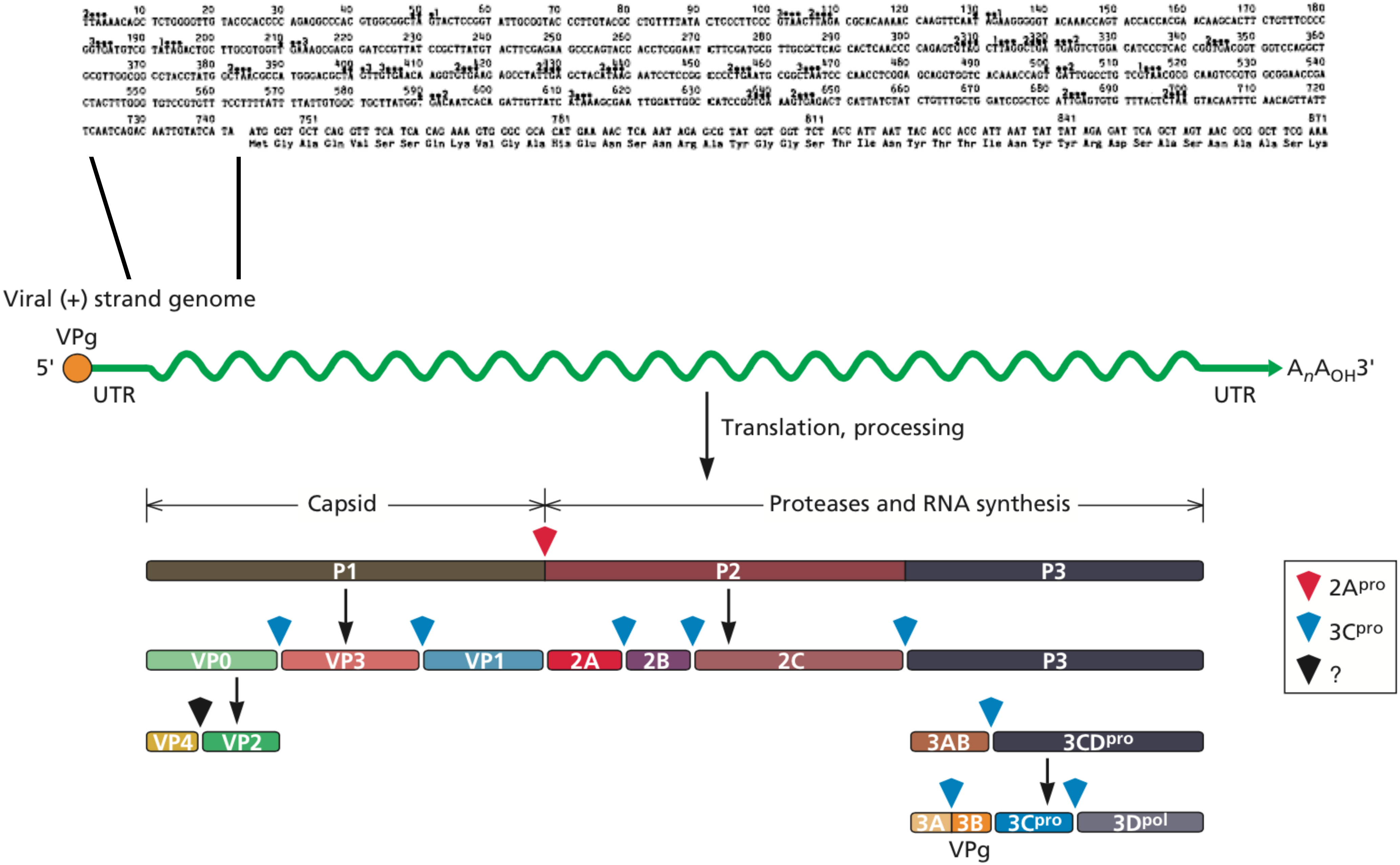
Internal initiation





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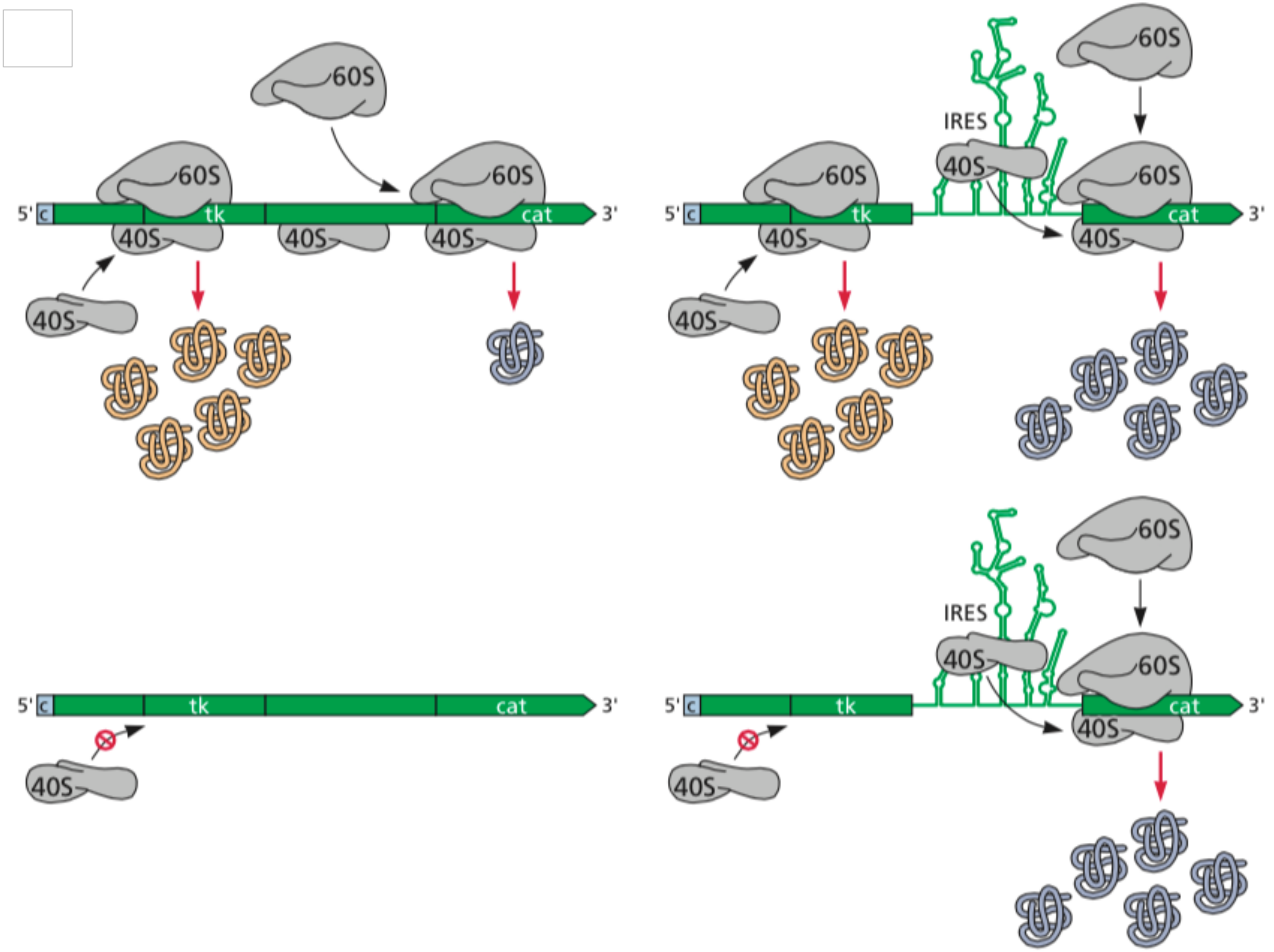
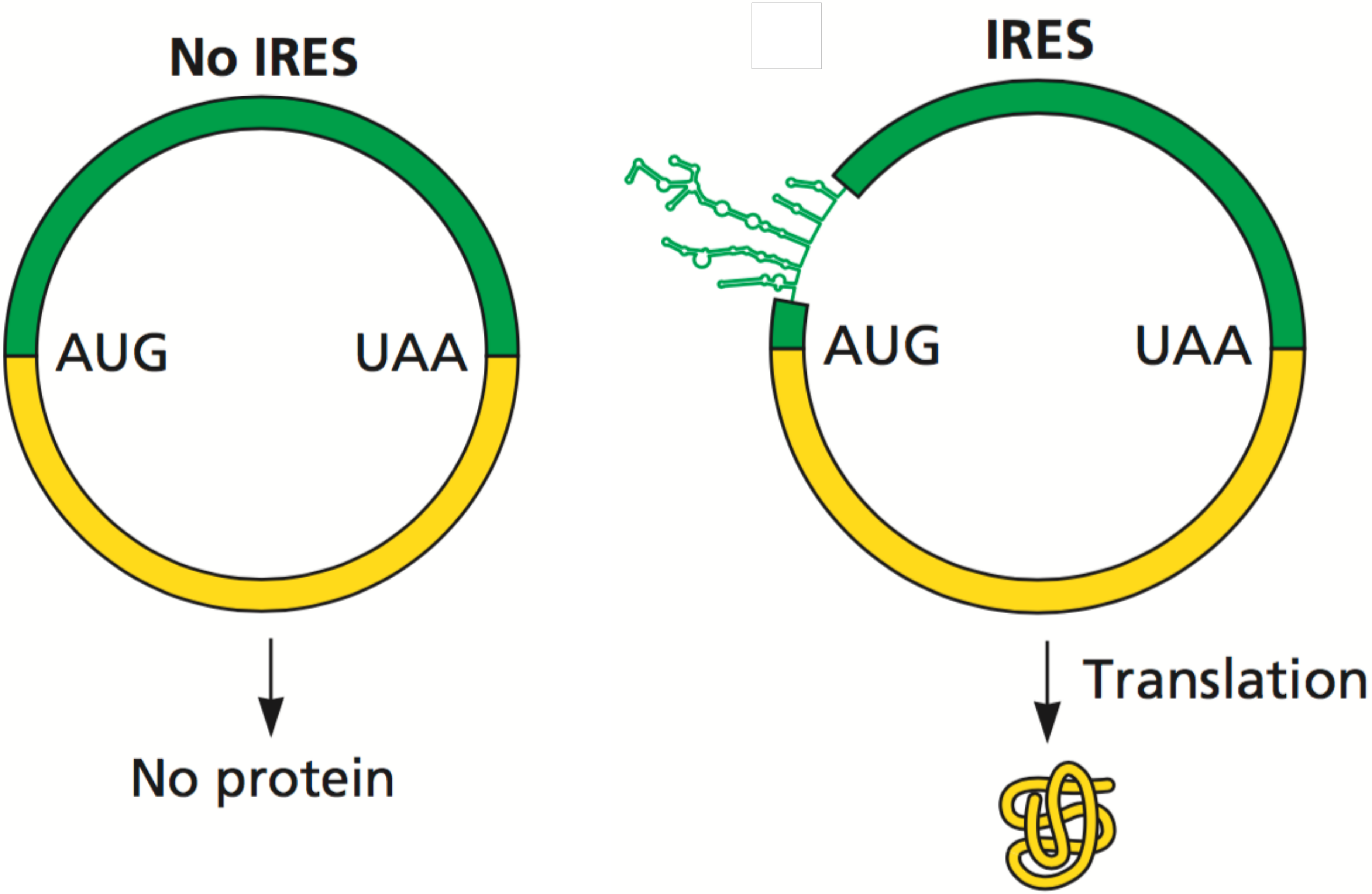
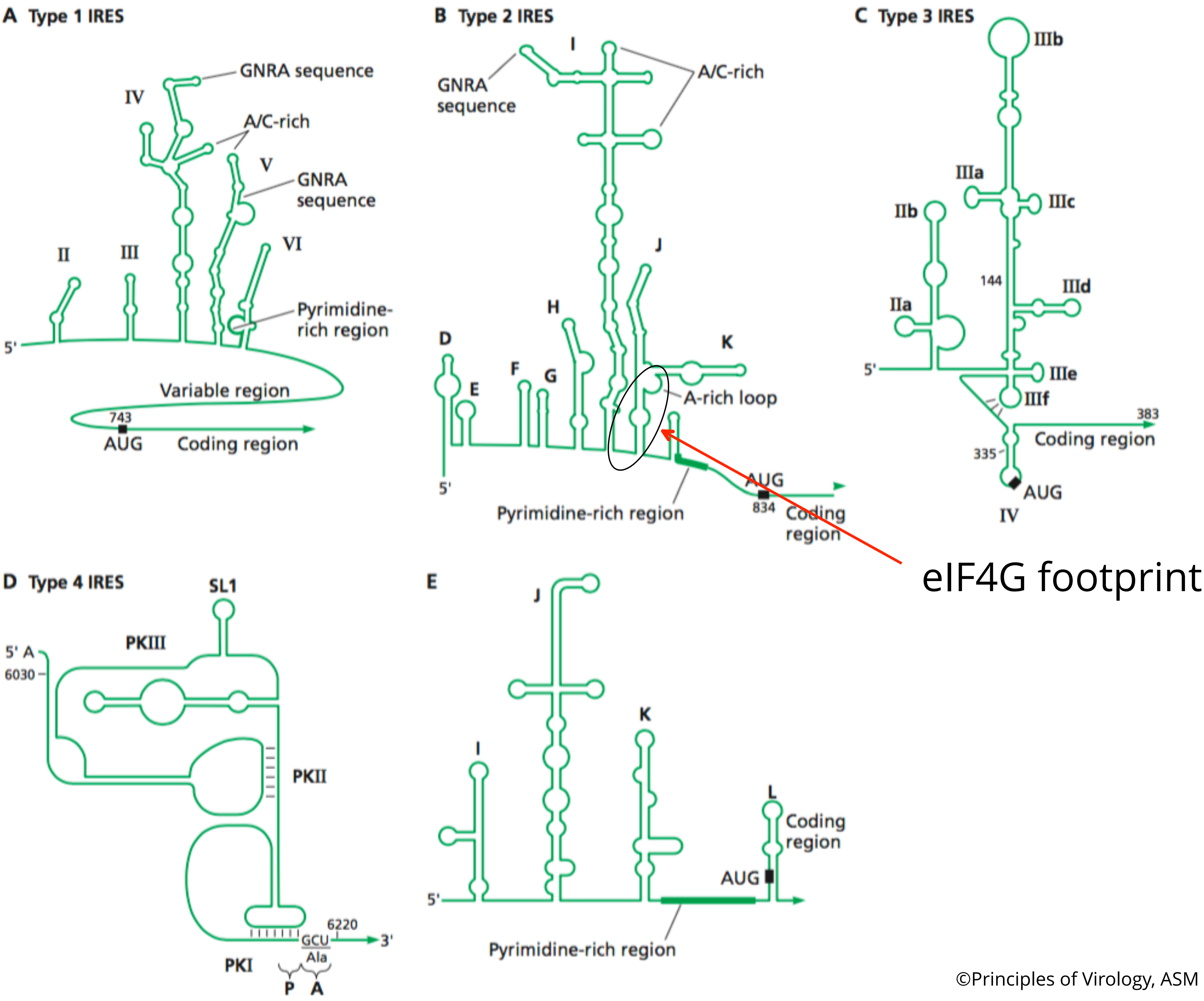


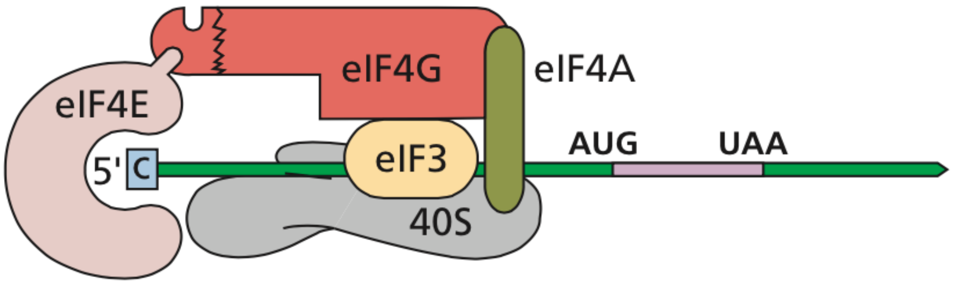
IRES = internal ribosome entry site


No IRES
AUG
UAA
No protein
IRES
AUG
UAA
Translation



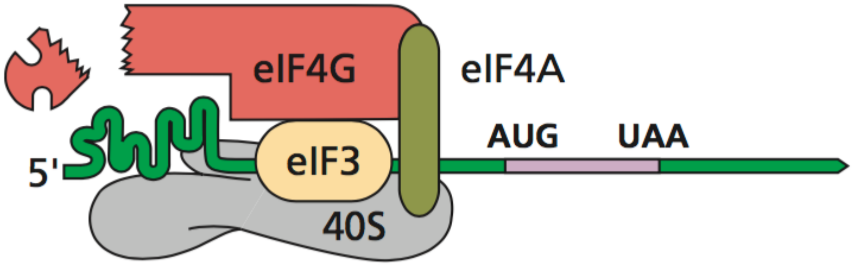
eIF4G footprint

5'-end-dependent initiation



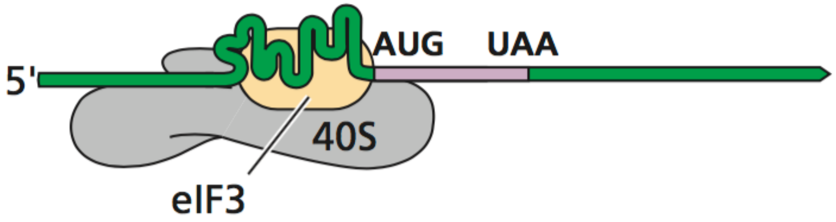
all eIFs

Type 1 or 2 IRES



all eIFs except eIF4E

Hepatitis C virus IRES



eIF2, eIF3

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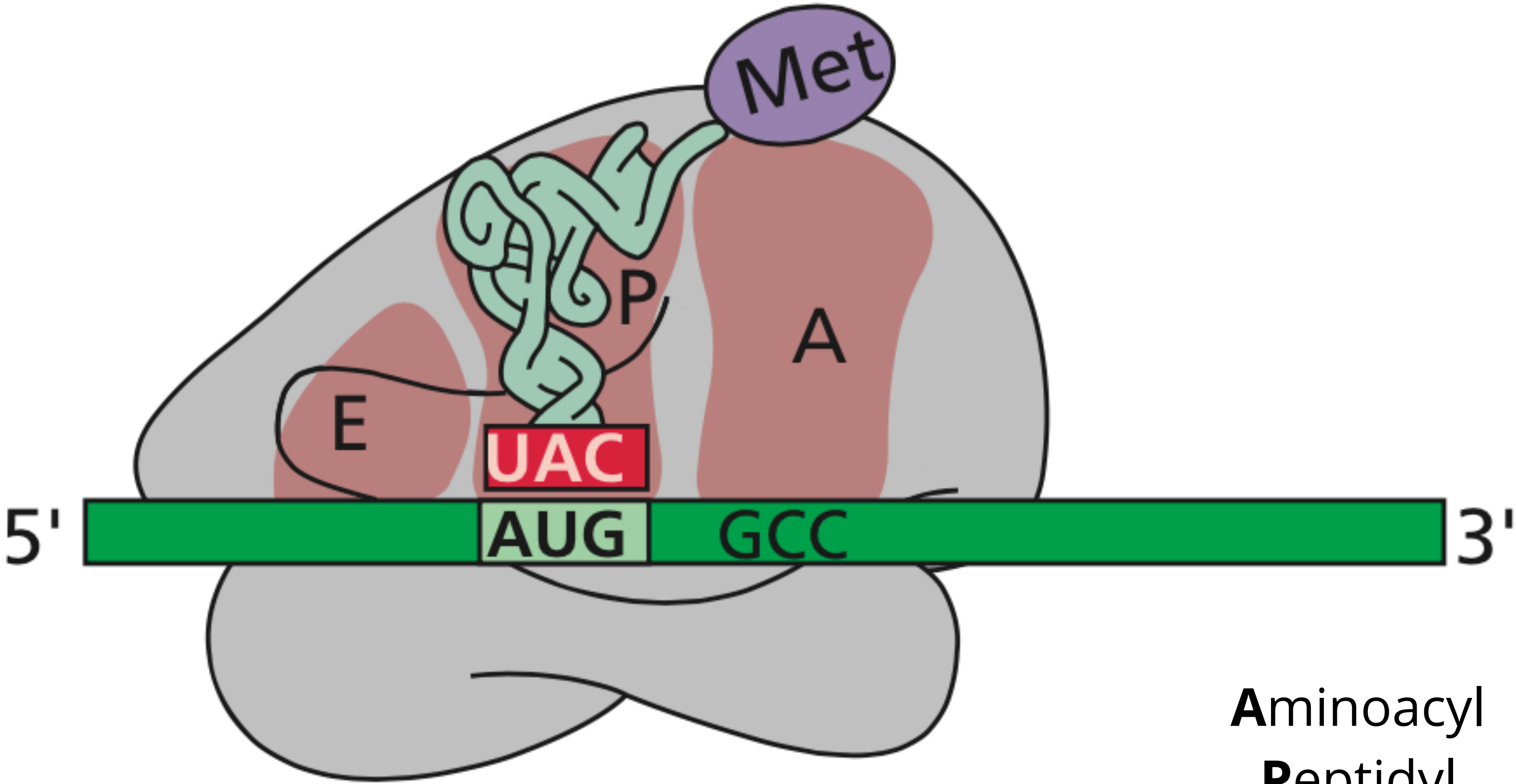
What do ribosome shunting and internal ribosome initiation have in common?

1. Cap recruitment of 40S subunit
2. Both involve RNA secondary structures
3. Ribosome scanning through the entire 5’-UTR
4. Both require cap-binding protein eIF4E
5. All of the above



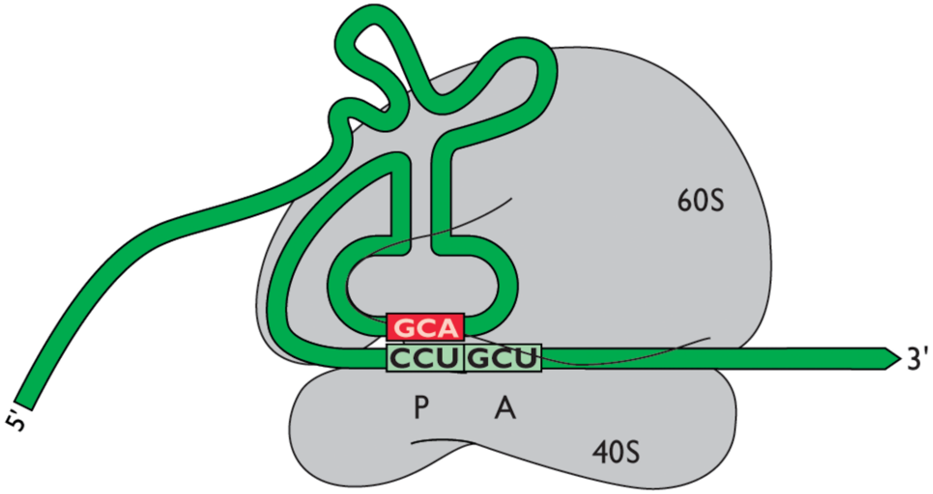
Aminoacyl

Peptidyl

Exit

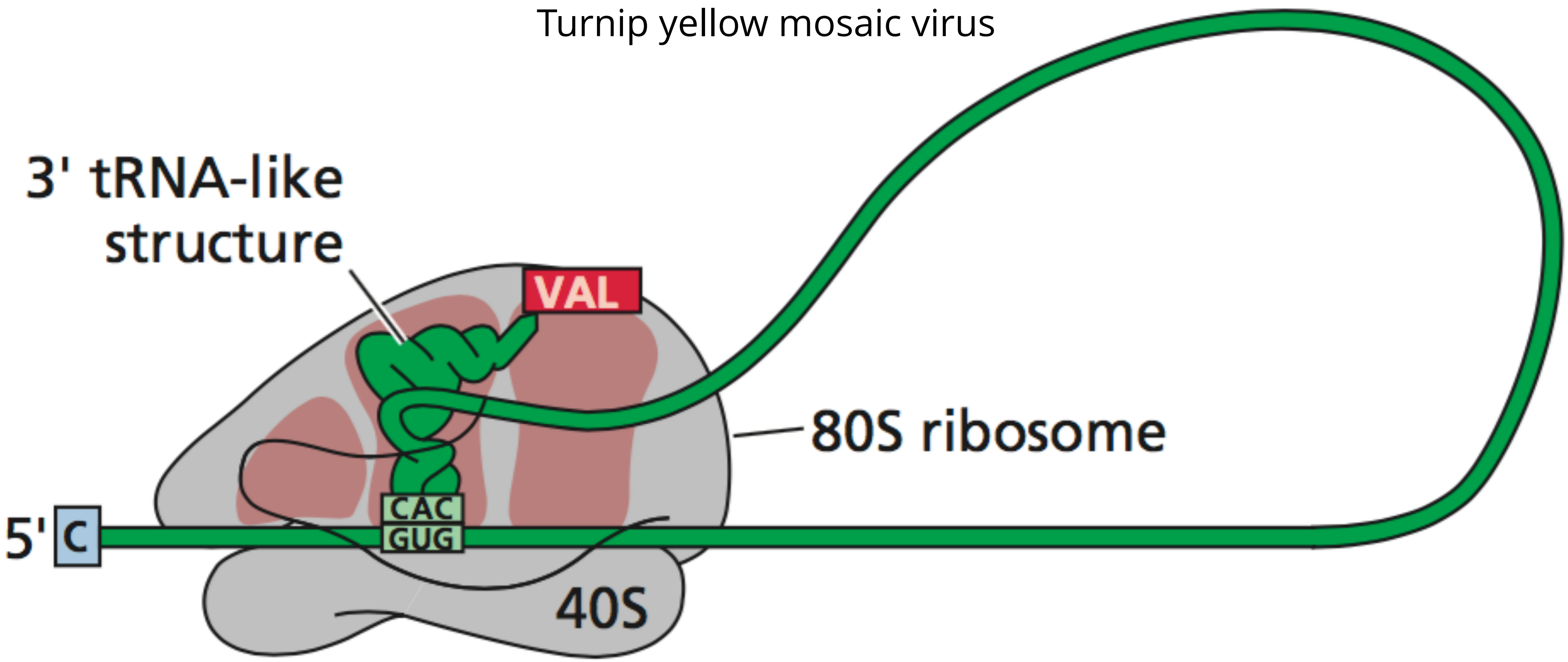
Methionine-independent initiation

Cricket paralysis virus



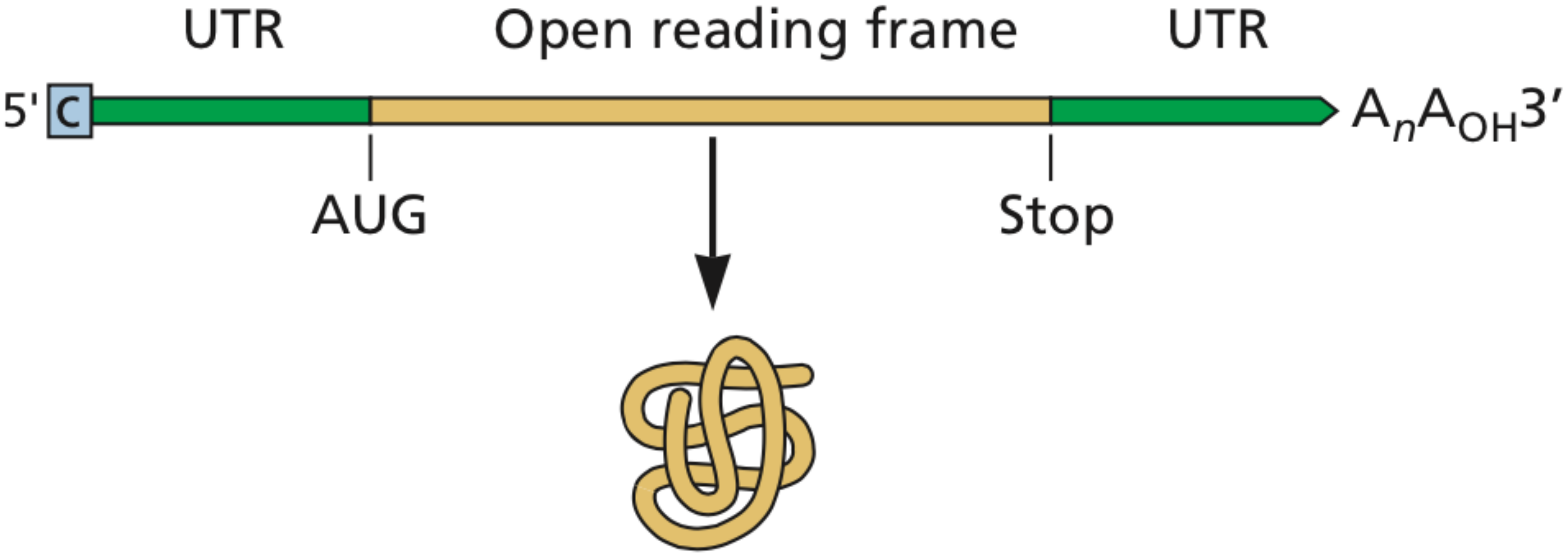
- Can assemble 80S ribosomes without any eIFs or Met-tRNAi
- RNA mimics tRNAi

Methionine-independent initiation

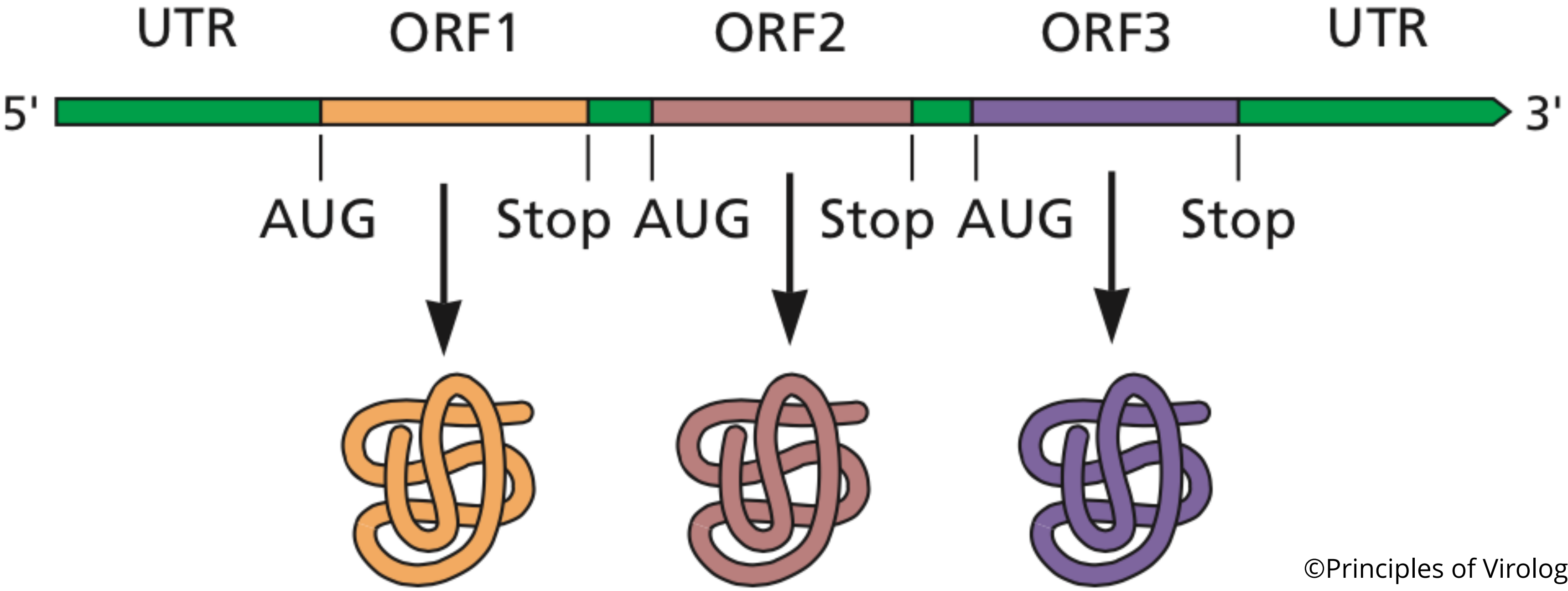


- Can assemble 80S ribosomes without any eIFs or Met-tRNAi
- RNA mimics tRNAi

Eukaryotic mRNA (monocistronic)



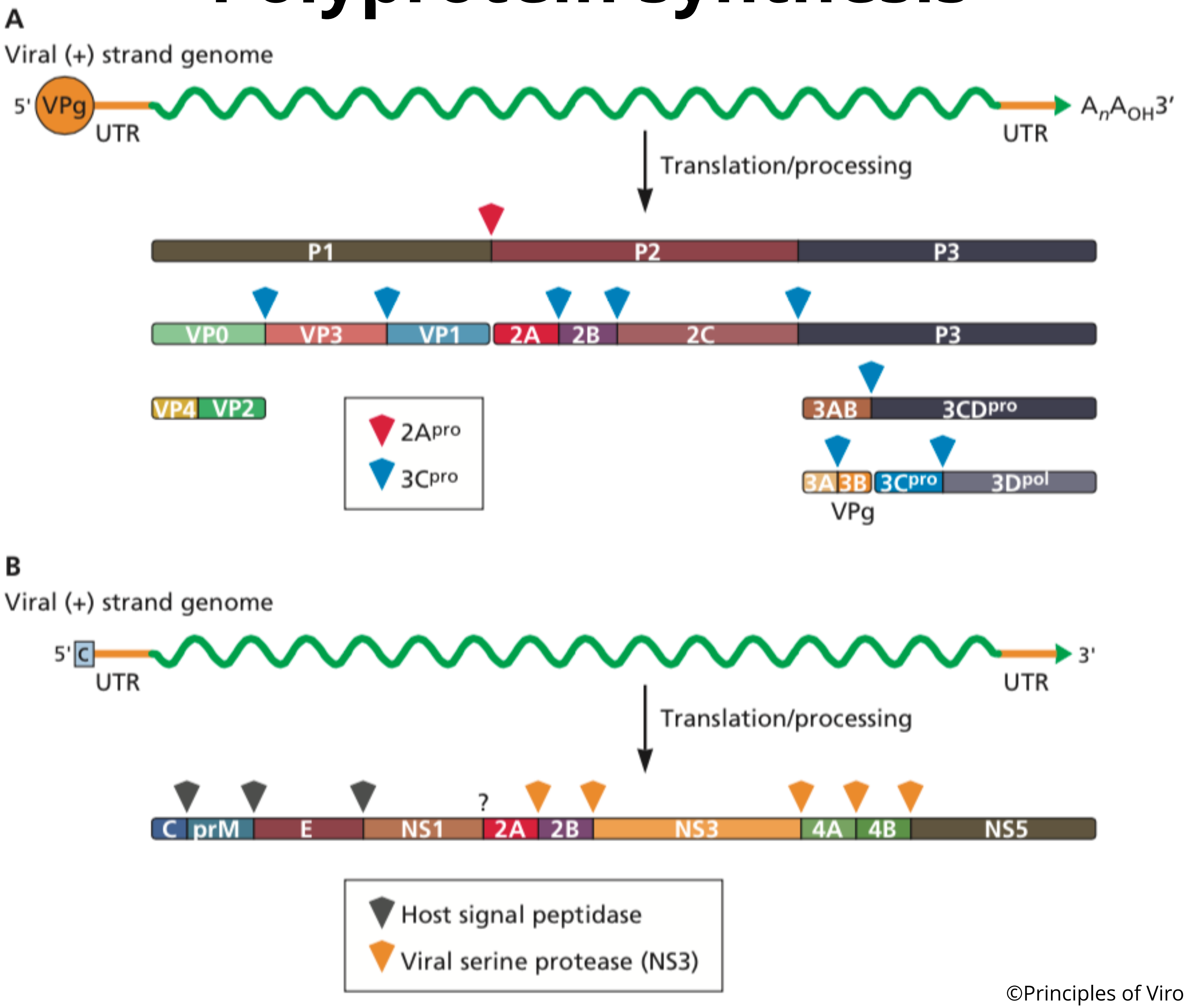
Bacterial and archaeal mRNA (polycistronic)



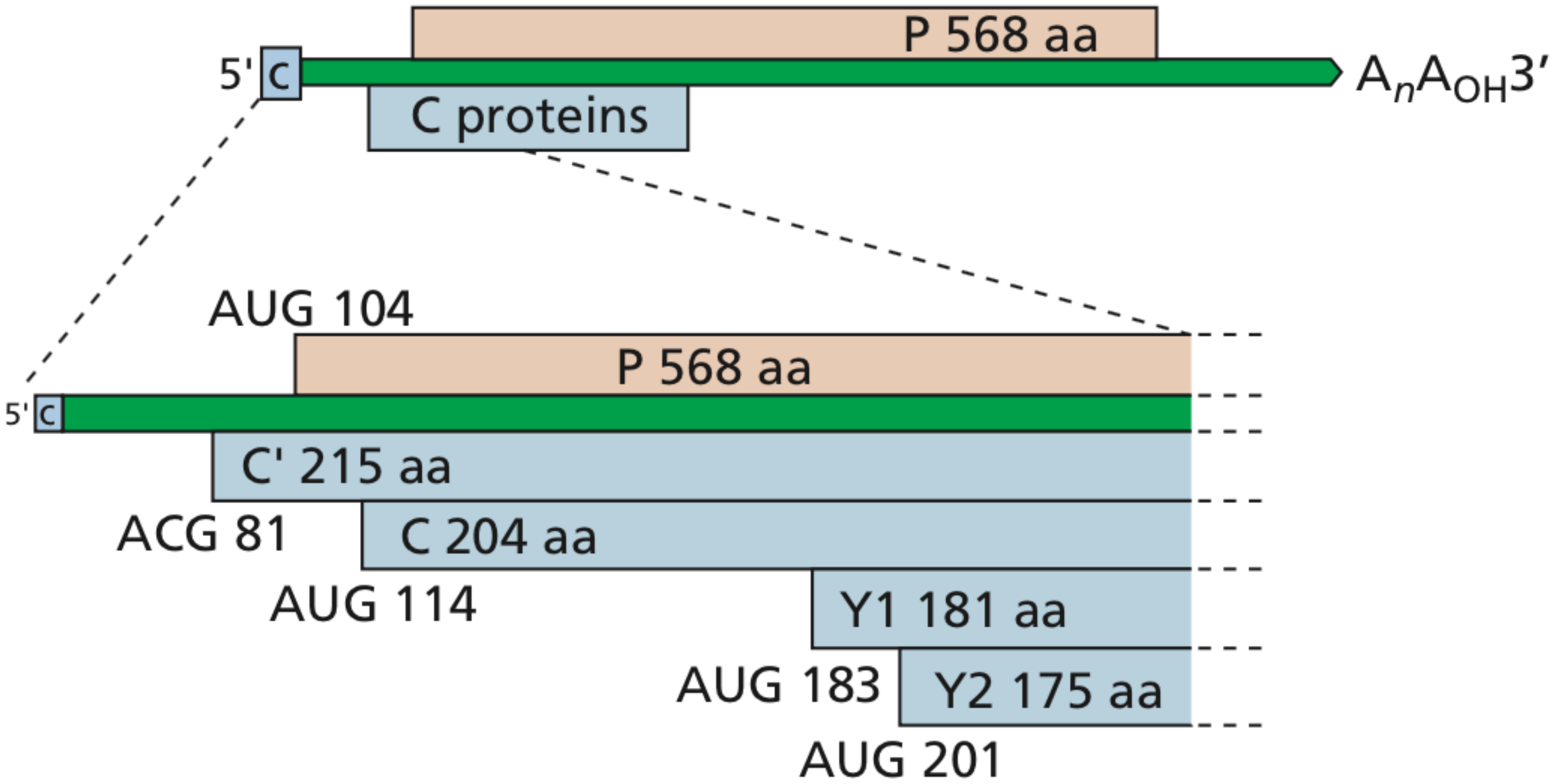
Maximizing coding capacity of the viral genome

- Polyprotein (*Picornaviridae, Flaviviridae, Togaviridae, Arenaviridae, Bunyaviridae, Retroviridae*)
- Subgenomic mRNAs (Rhabdoviridae, *Paramyxoviridae, Togaviridae*)
- Segmented genome (*Orthomyxoviridae*, *Reoviridae*)
- RNA Splicing (*Orthomyxoviridae, Adenoviridae, Polyomaviridae)*
- 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



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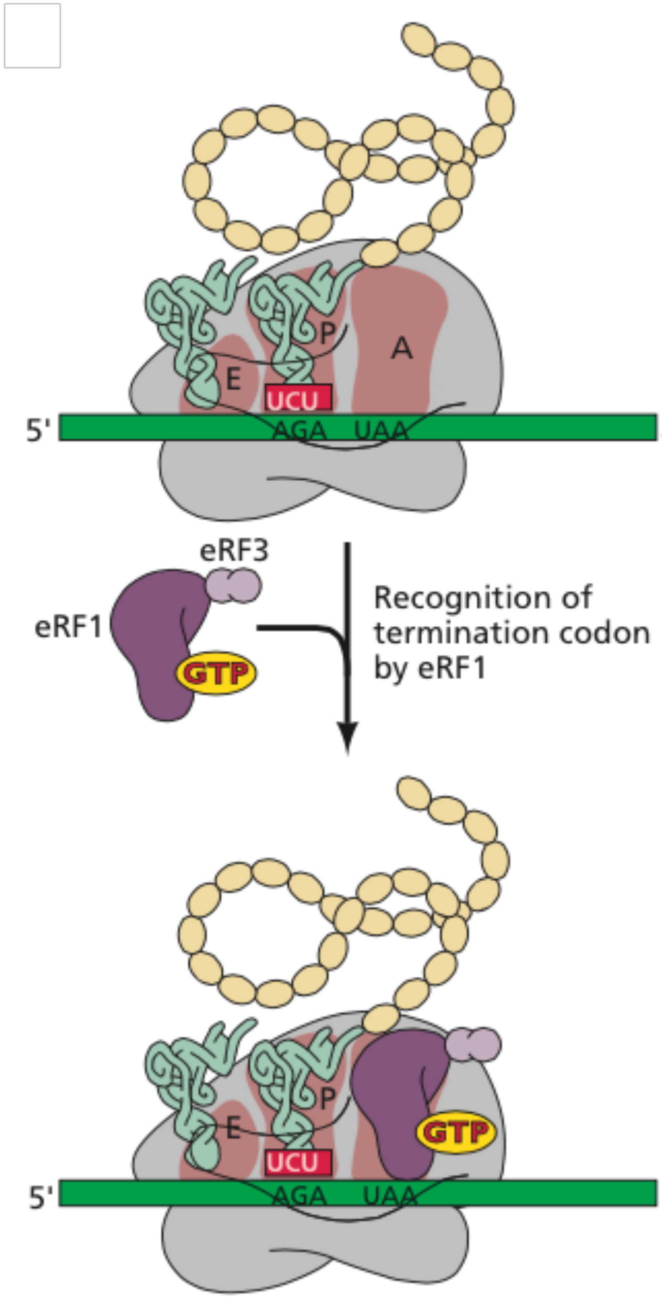
Suppression of termination



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

Stop codons may be recognized by charged tRNA - misreading, or charged suppressor tRNA (e.g. selenocysteine for UGA)

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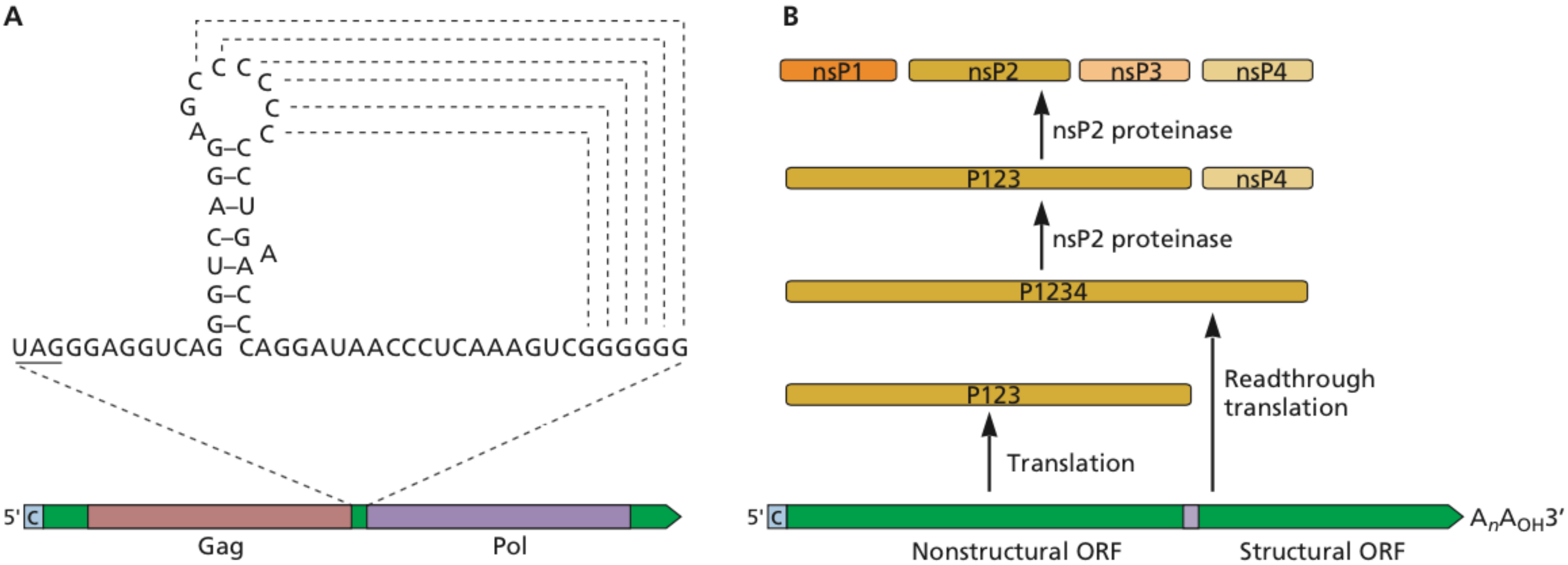
Suppression of termination



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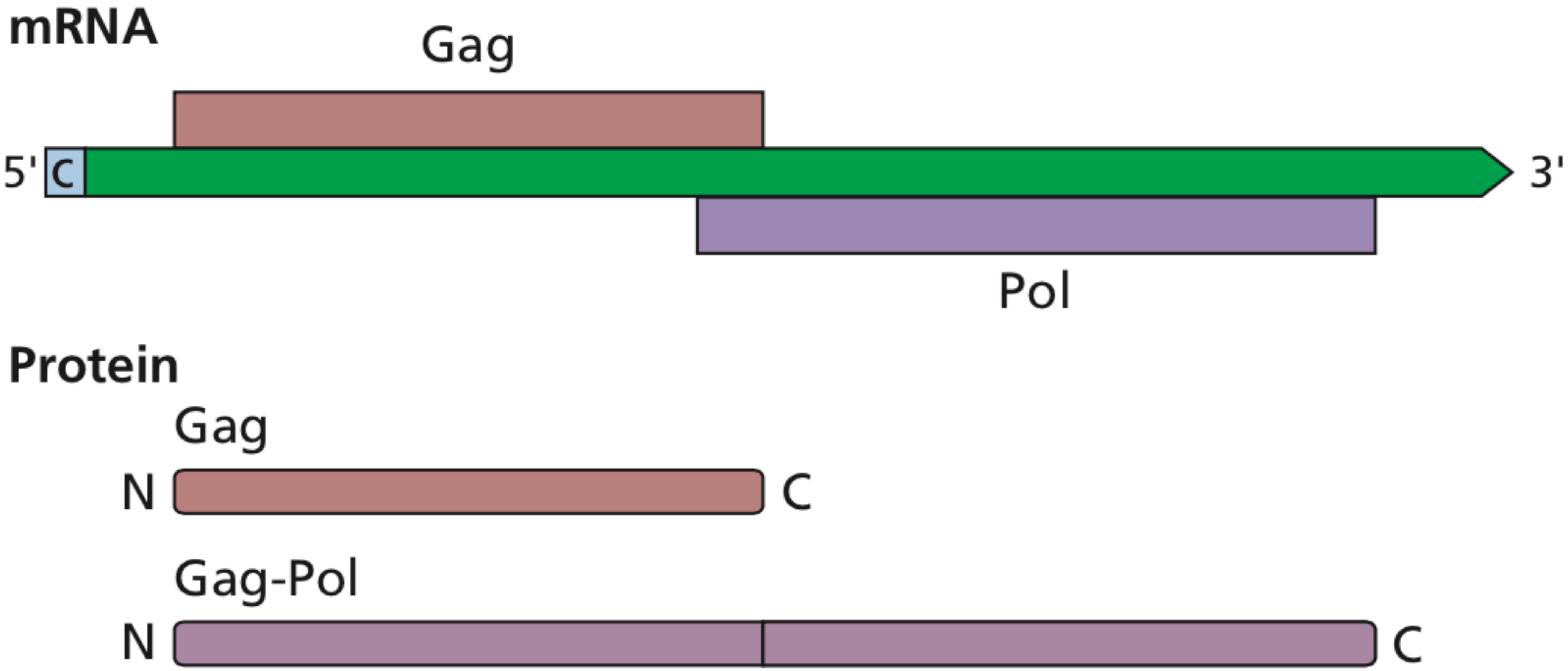
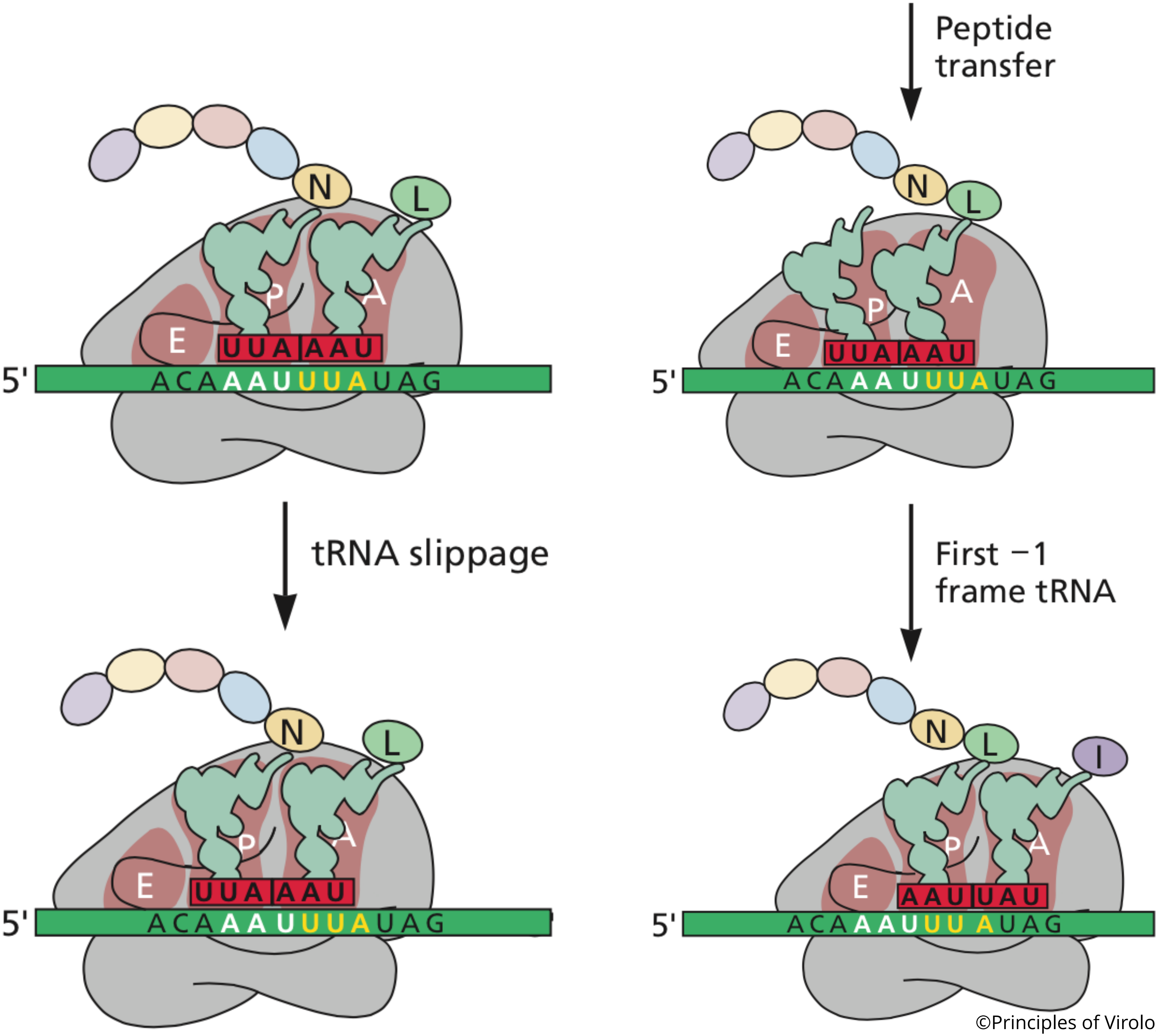
Ribosomal frameshifting




Peptide transfer
N
L
P
A
E
UUA
AAU
5'
ACAAAUUUAUAG
tRNA slippage
First −1 frame tRNA
I
AAU
UAU

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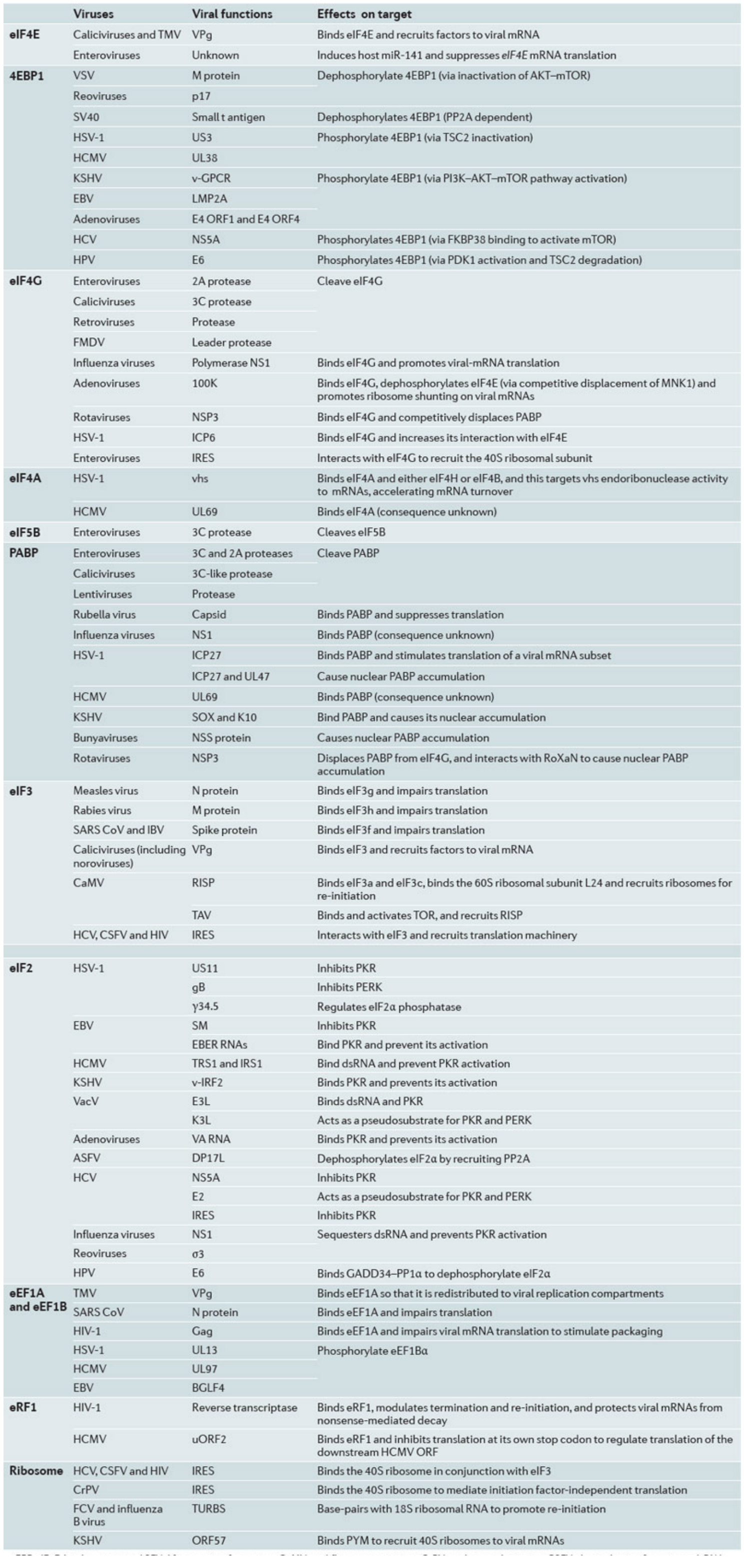
Compared with a polycistronic mRNA, a monocistronic mRNA:

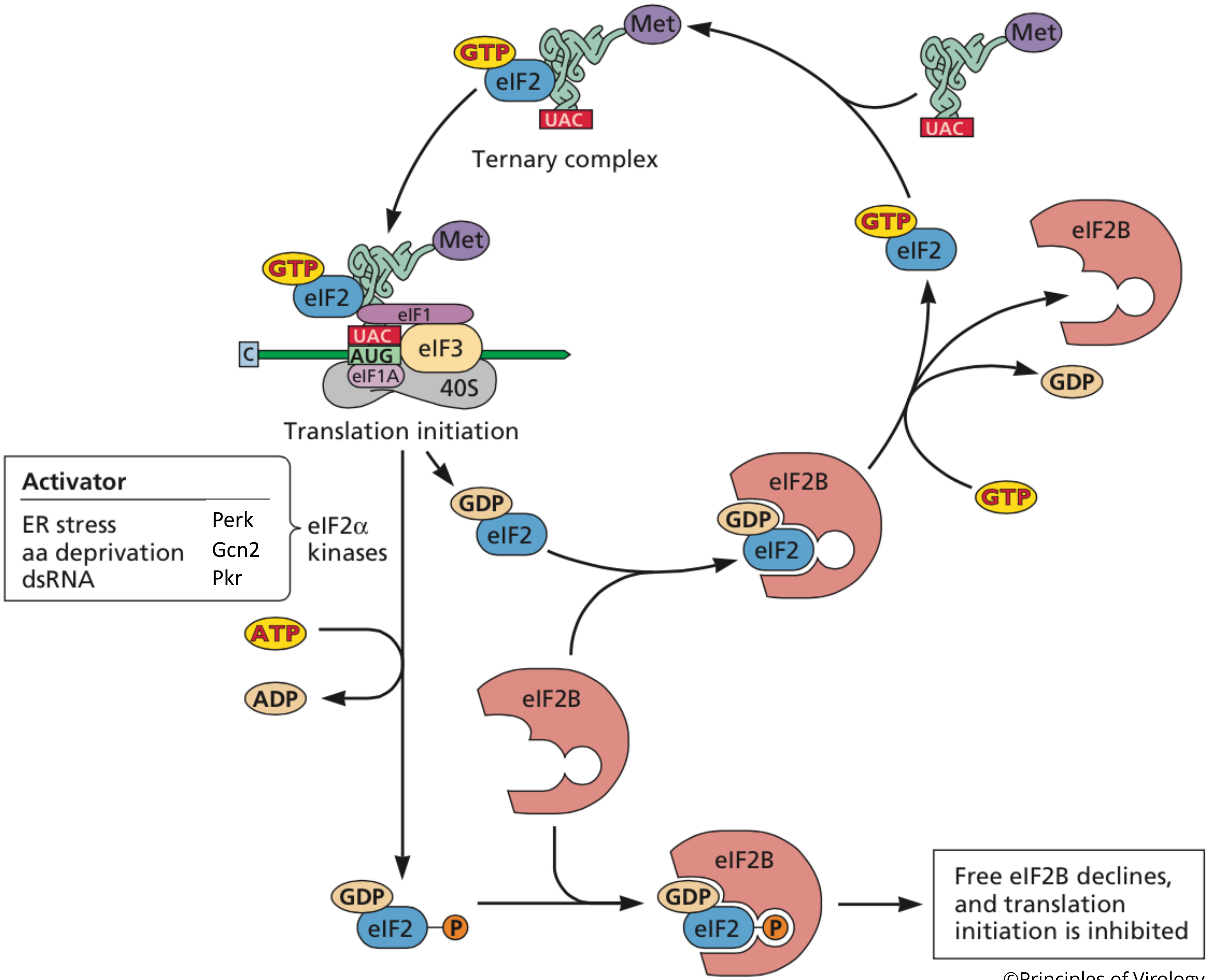
1. Does not require an AUG start codon
2. Does not bind the 40S ribosomal subunit
3. Has only one open reading frame
4. Is only found in viruses and bacteria
5. All of the above

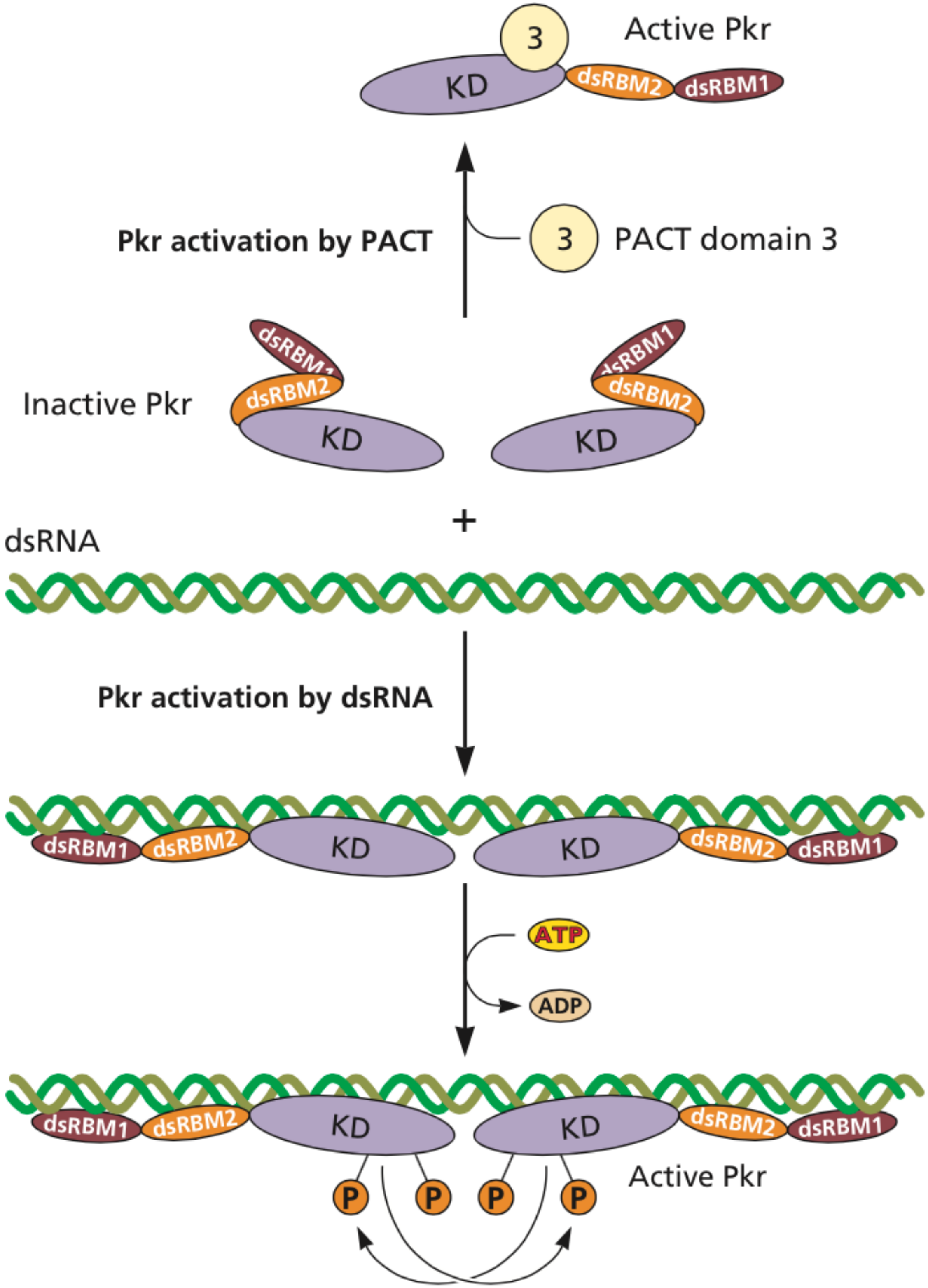
Regulation of translation in virus-infected cells

- 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

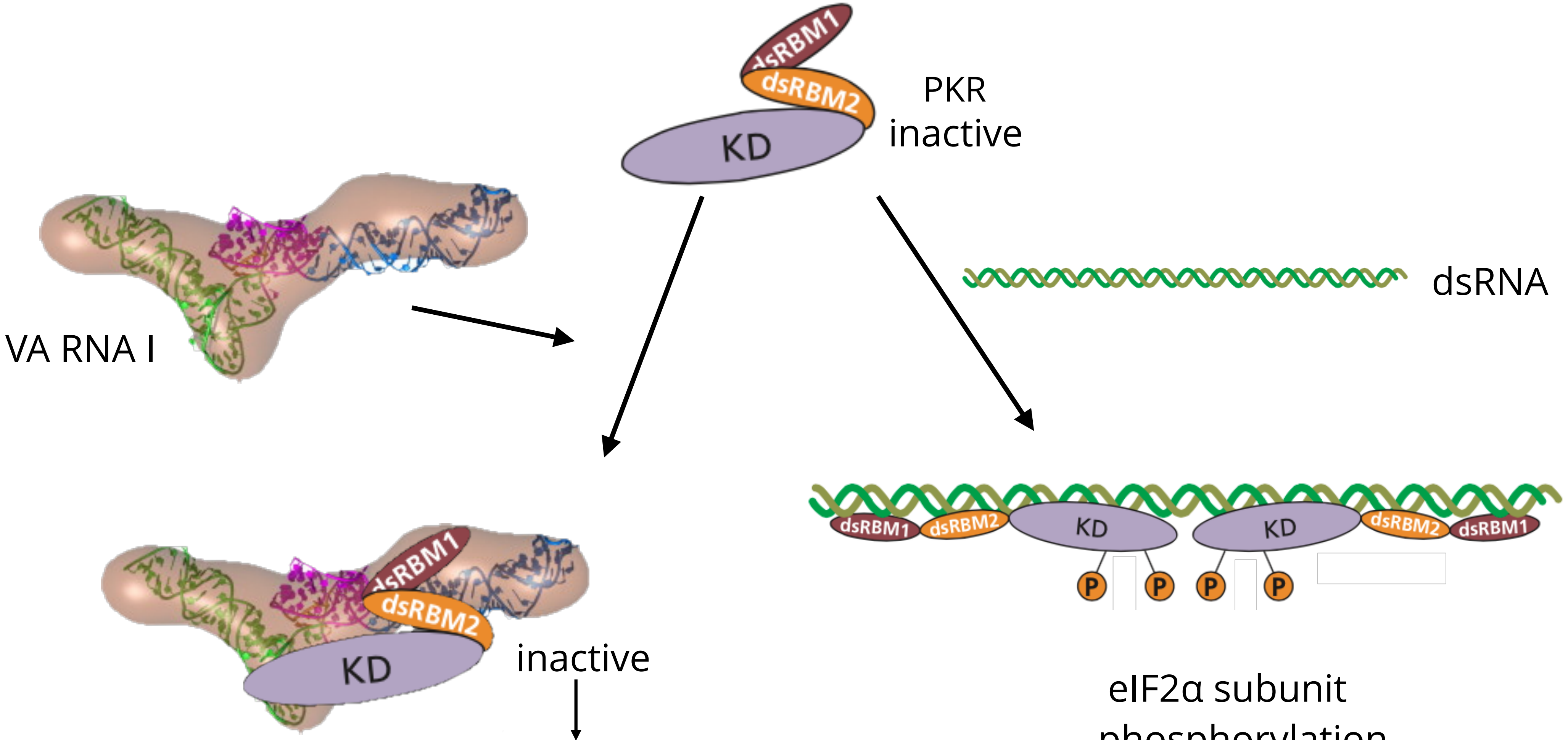

GTP
eIF2
Met
UAC
Ternary complex
Met
UAC
GTP
eIF2
Met
eIF1
UAC
C
AUG
eIF3
eIF1A
40S
Translation initiation
GTP
eIF2
eIF2B
GDP
Activator
ER stress
aa deprivation
dsRNA
Perk
Gcn2
Pkr
eIF2α kinases
GDP
eIF2
eIF2B
GDP
eIF2
GTP
ATP
ADP
eIF2B
GDP
eIF2
P
eIF2B
GDP
eIF2
P
Free eIF2B declines, and translation initiation is inhibited


3
Active Pkr
KD
dsRBM2
dsRBM1
Pkr activation by PACT
3
PACT domain 3
Inactive Pkr
dsRBM1
dsRBM2
KD
dsRBM1
dsRBM2
KD
+
dsRNA
Pkr activation by dsRNA
dsRBM1
dsRBM2
KD
KD
dsRBM2
dsRBM1
ATP
ADP
dsRBM1
dsRBM2
KD
KD
dsRBM2
dsRBM1
Active Pkr
P
P
P
P

PKR and cellular antiviral response

- PKR induced and activated by virus infection
- Leads to inhibition of host translation, apoptosis
- Different viral mechanisms have evolved to inactivate the PKR pathway

Adenovirus VA RNA I prevents activation of PKR



PKR
inactive

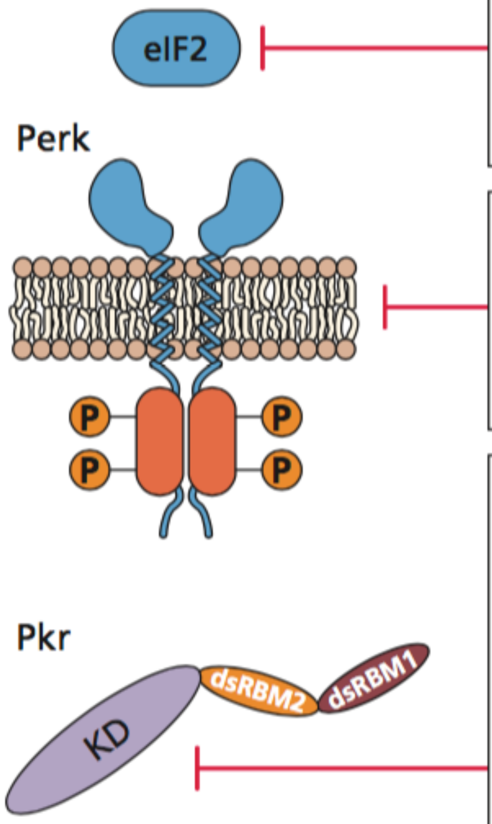
VA RNA I

dsRNA

inactive
↓
no phosphorylation
of eIF2α subunit
↓
active protein
synthesis

eIF2α subunit
phosphorylation
↓
protein synthesis
inhibited

Viral proteins and RNAs that counter inactivation of eIF2



Vaccinia virus K3L
(pseudosubstrate)

Herpes simplex virus γ34.5
(phosphatase regulatory subunit)

Herpes simplex virus gB

Vaccinia virus K3L

Hepatitis C virus E2

dsRNA-binding proteins:
Herpes simplex virus US11
Influenza virus NS1
Reovirus σ3
Vaccinia virus E3L

Antagonists of RNA:
Adenovirus VA RNA I
Epstein-Barr virus EBER

Antagonists of protein:
Vaccinia virus K3L
Sendai virus C
Hepatitis virus NS5A
Adenovirus E1b55K, E4Orf6

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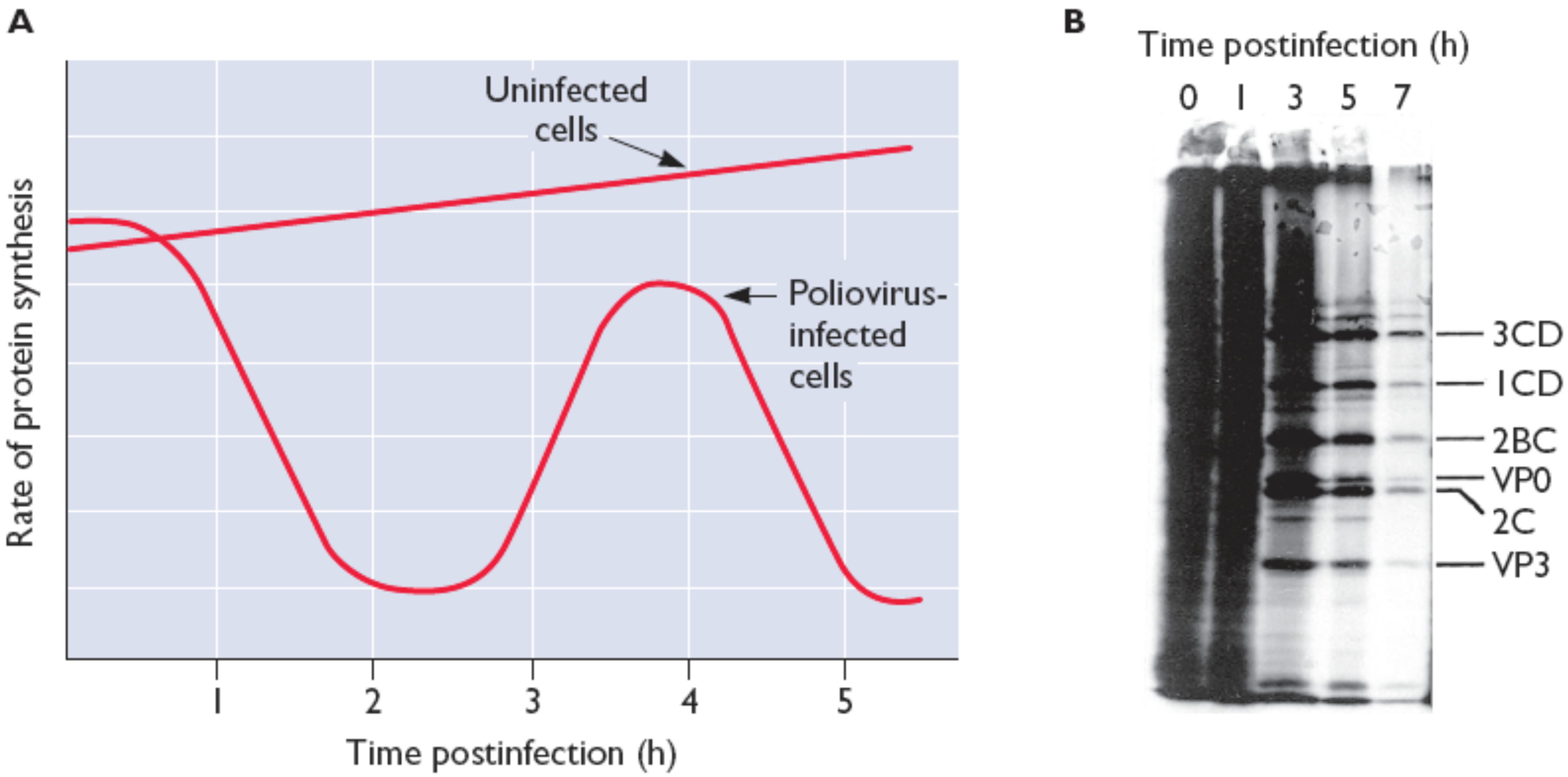
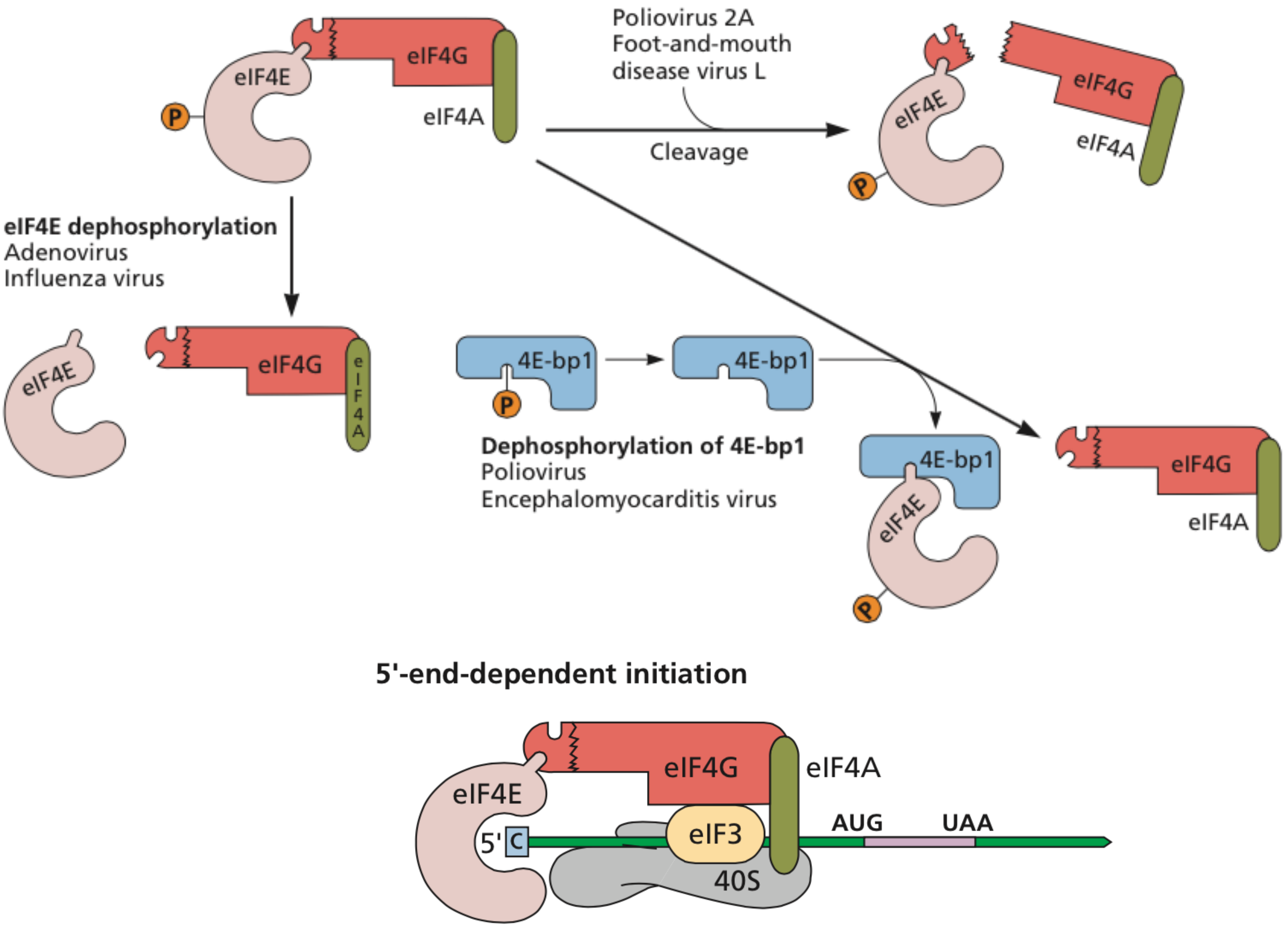
PKR is an interferon-induced enzyme that is activated by _____, leading to phosphorylation of ______ and inhibition of translation.

1. GDP, eIF2alpha
2. dsRNA, eIF2alpha
3. dsRNA, eIF2B
4. ssRNA, eIF2alpha
5. None of the above


A
Rate of protein synthesis
Uninfected cells
Poliovirus-infected cells
1
2
3
4
5
Time postinfection (h)
B
Time postinfection (h)
0
1
3
5
7
3CD
1CD
2BC
VP0
2C
VP3

Modulation of cap recognition



Poliovirus 2A
Foot-and-mouth
disease virus L

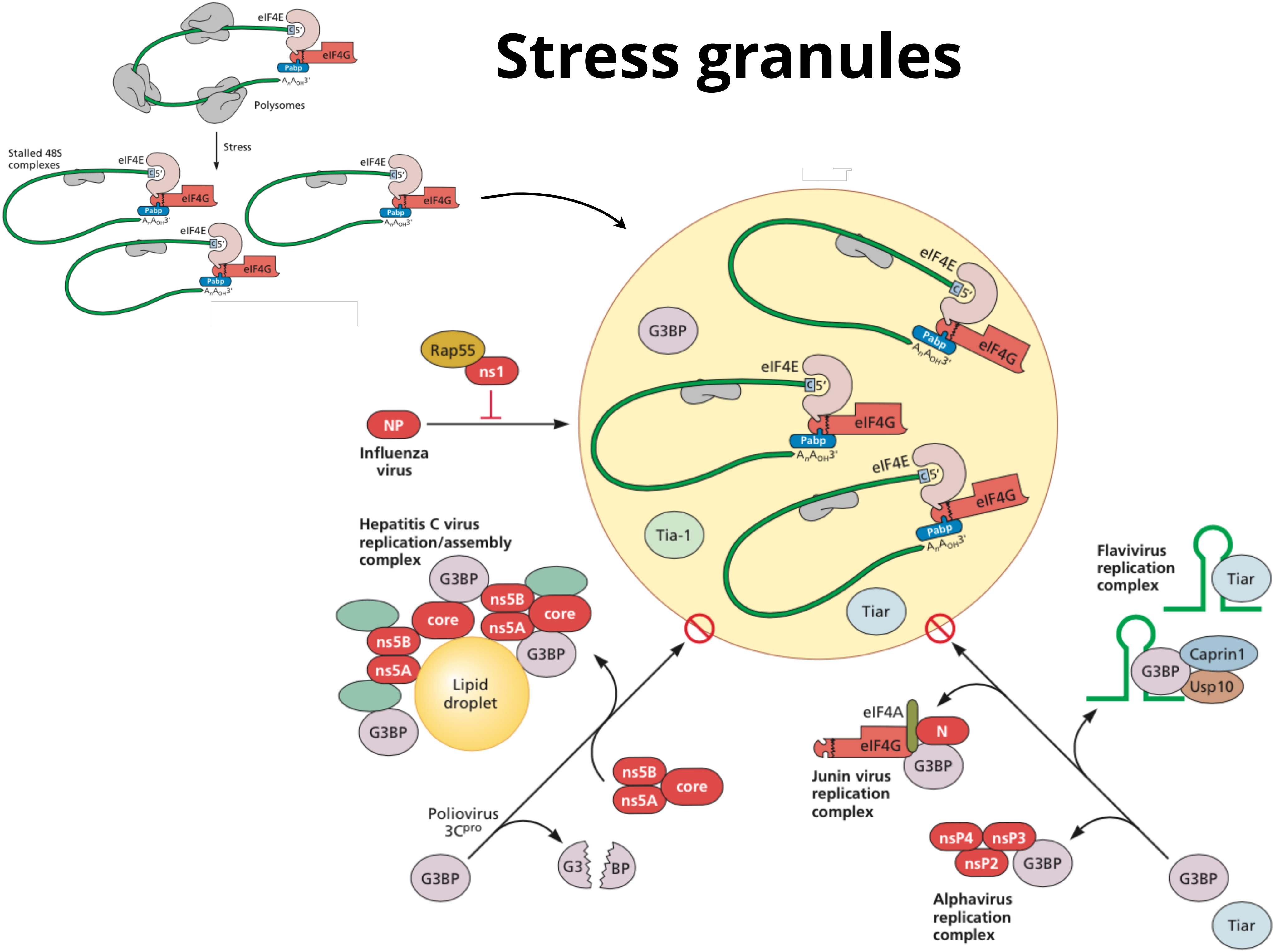
Cleavage

eIF4E dephosphorylation
Adenovirus
Influenza virus

Dephosphorylation of 4E-bp1
Poliovirus
Encephalomyocarditis virus

5'-end-dependent initiation

Stress granules



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