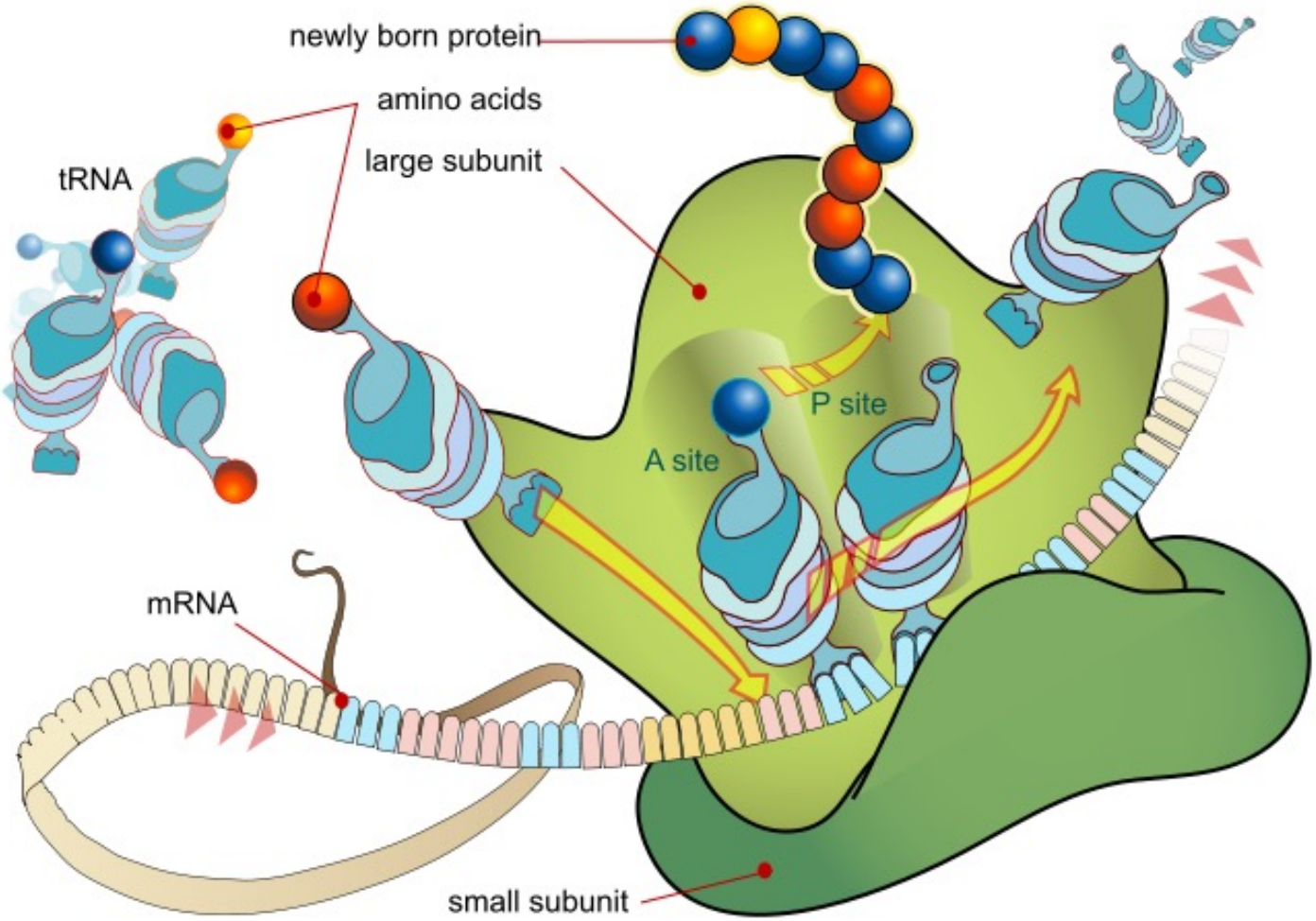
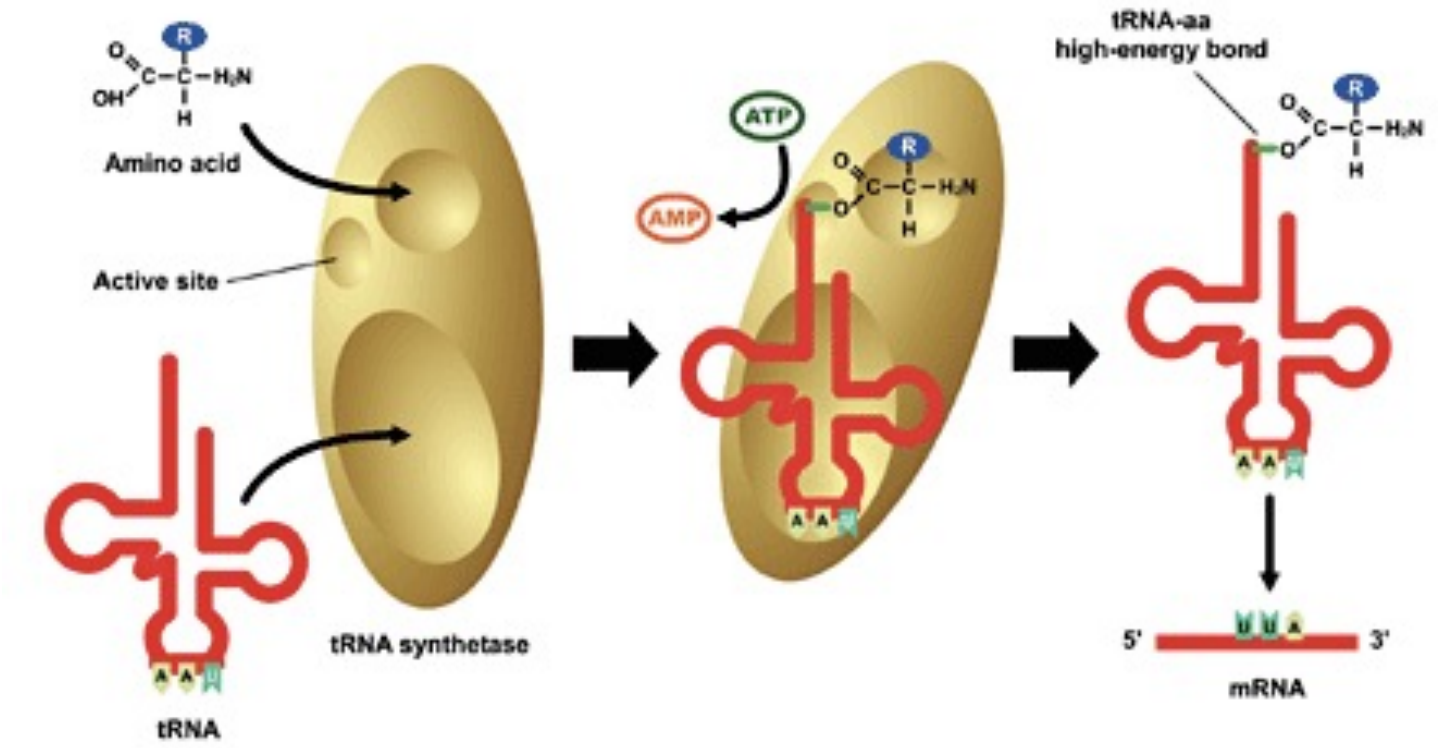
TRANSLATION

How to make proteins?



tRNA charging by tRNA sythetases



tRNA charging occurs in two steps:

1. **AA + ATP → Aminoacyl-AMP + PP**
2. **Aminoacyl-AMP + tRNA →Aminoacyl-tRNA + AMP**

Is catalyzed by aminoacyl-tRNA synthetases

There are at least 20 aa-tRNA synthetases, one for each amino acid

Aminoacylation accuracy is very important for translation fidelity

aa-tRNA synthetases

One synthetase for each amino acid
a single synthetase may recognize multiple tRNAs for the same amino acid

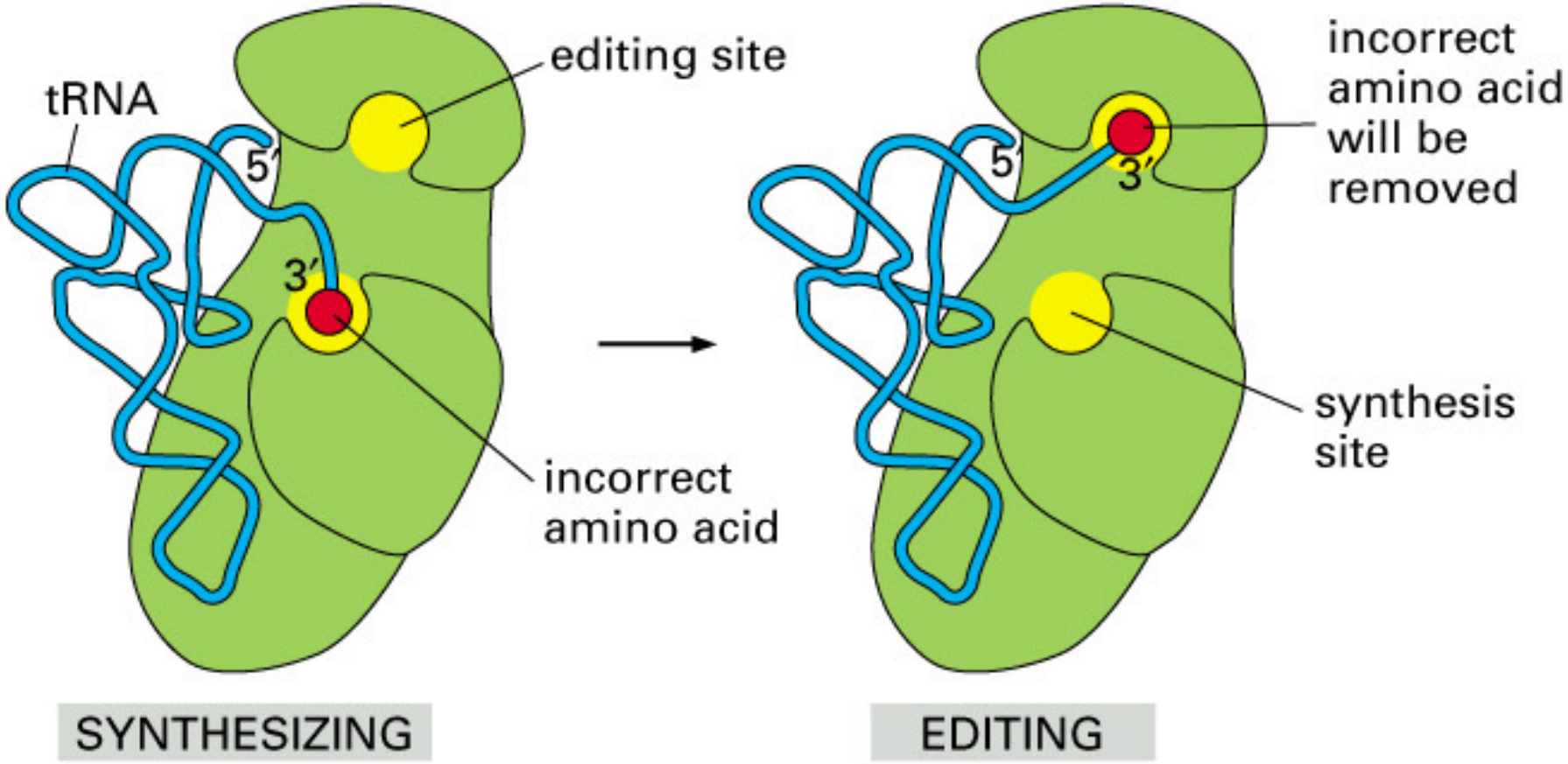
Two classes of synthetases

- bind to the acceptor stem and the anticodon loop of tRNA
- have different 3-dimensional structures
- differ in tRNA side they recognize and how they bind ATP

Class I - monomeric, acylates the **2' OH** on the terminal ribose
Arg, Cys , Gln, Glu, Ile, Leu, Met, Trp Tyr, Val

Class II - dimeric, acylate the **3' OH** on the terminal ribose
Ala, Asn, Asp, Gly, His, Lys, Phe, Ser, Pro, Thr

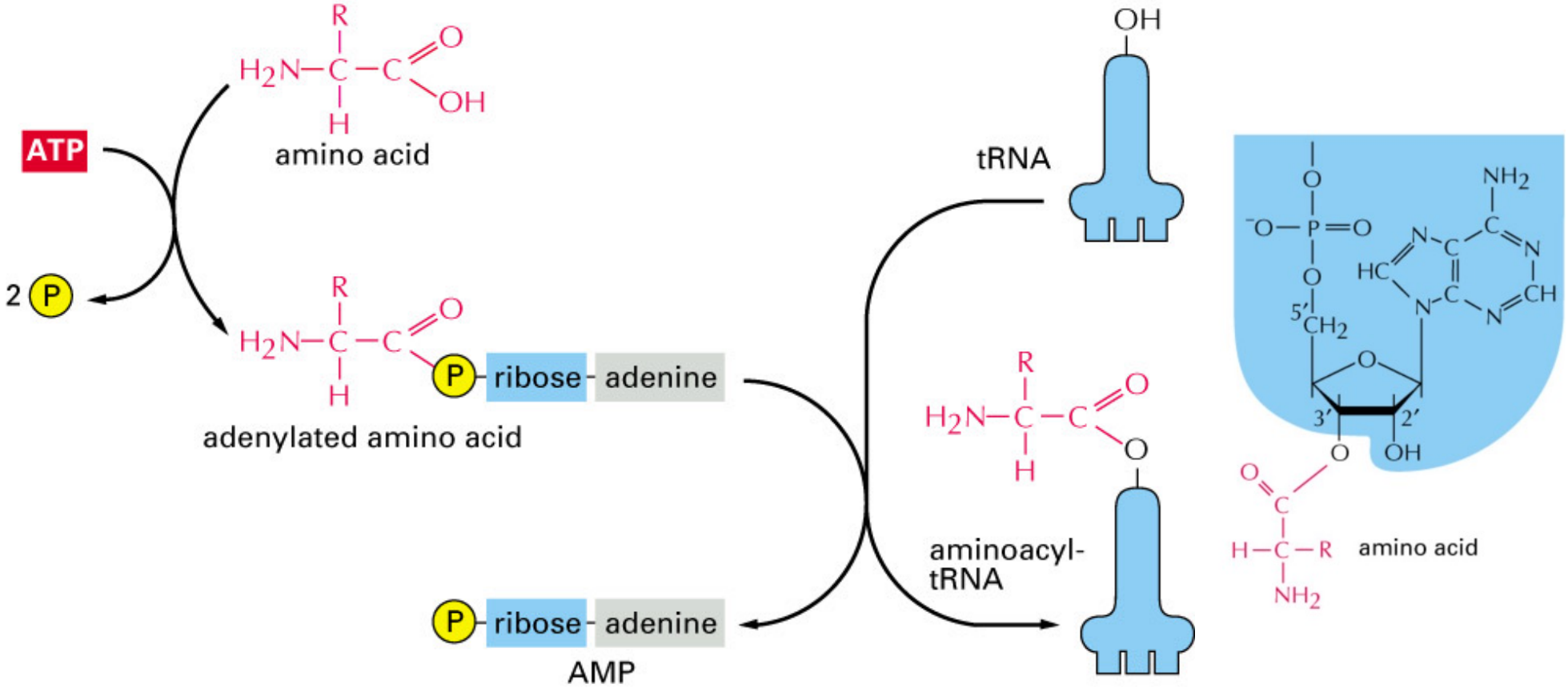
High fidelity of aa-tRNA synthetases



- Accuracy is achieved by two active sites: one that charges tRNA (synthesis site) and one that hydrolyzes mischarged aa-tRNAs (editing site)
- Isoleucine IleRS discriminates 50 000-fold for Ile over Val

  (Ile and Val differ by one methylene group)

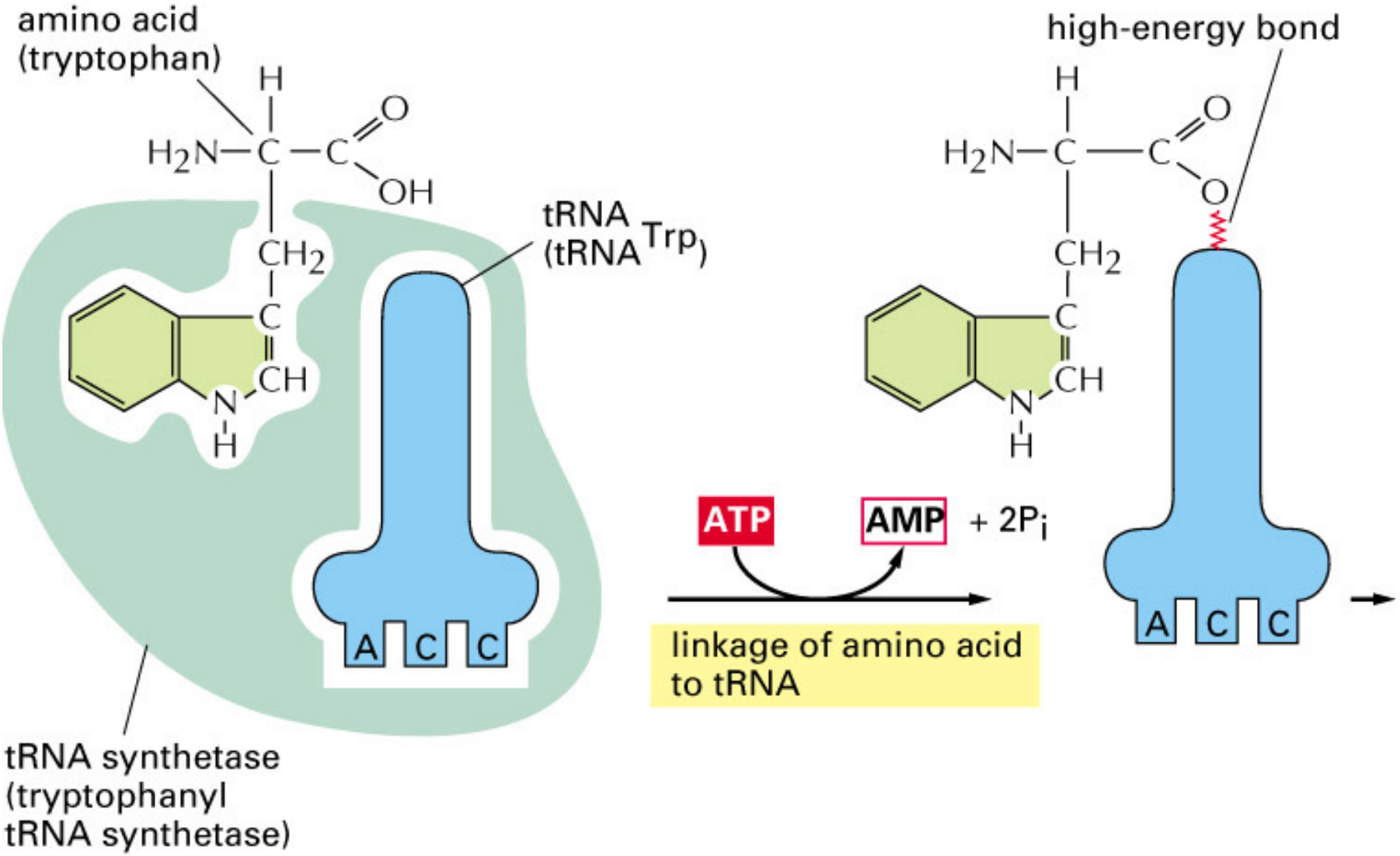
tRNA charging by tRNA sythetases



Translation fidelity

Two levels of control to ensure incorporation of the proper amino acid:

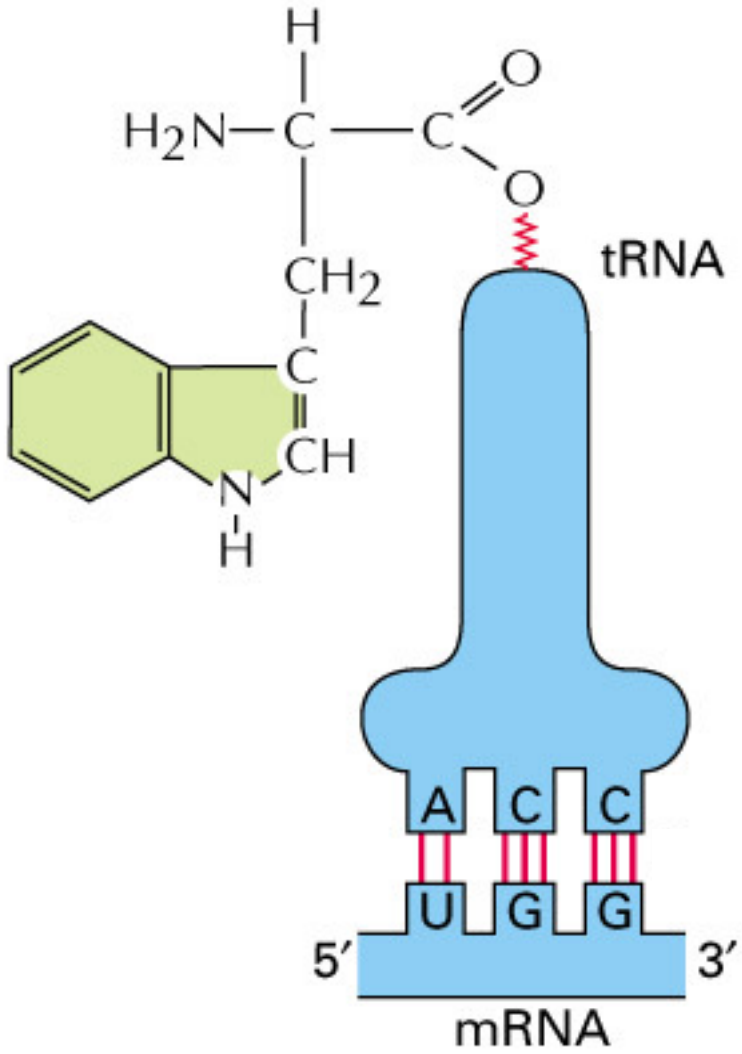
1. charging of the proper tRNA



Translation fidelity

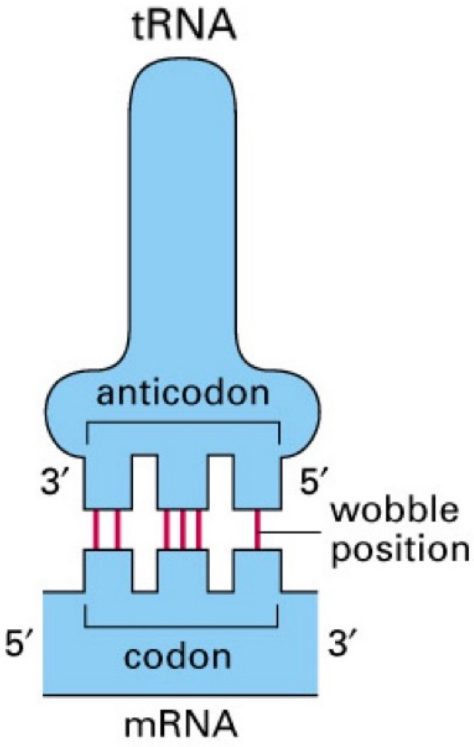
2. Matching cognate tRNA to mRNA

Incorporation of the correct aa-tRNA is determined by base-pairing between the tRNA anticodon and mRNA



Wobble position

- **Allows for more flexibility, broader specificity and genetic code degeneracy, that one tRNA can recognize more than one codon**
  There are 64 codons but only 40 tRNAs
- **Important for tRNA charging by synthetases** (via structure)
- **Minimizes the damage that can be caused by misreading of the code**
- **Helps faster dissociation of tRNA from mRNA**
- **Modification of the wobble position affect translation elongation and fidelity, especially during stress**



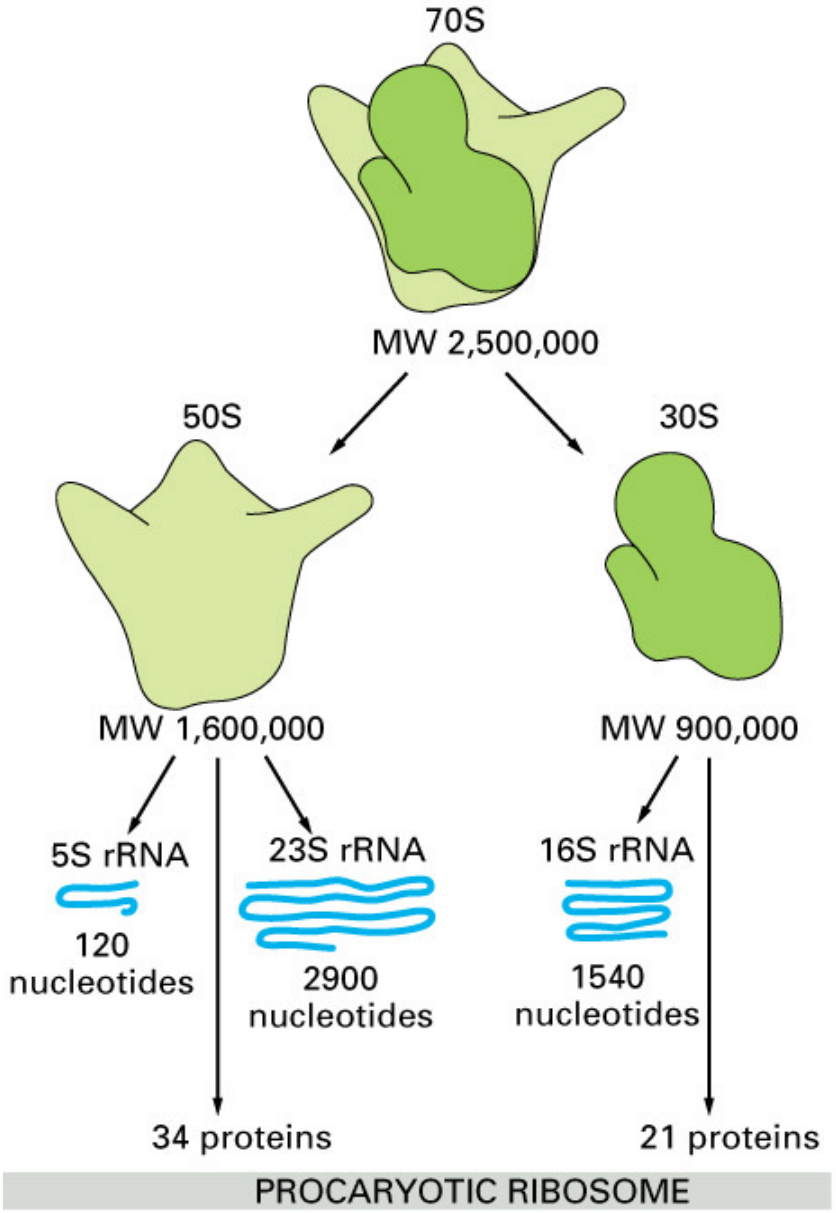
wobble codon base	possible anticodon bases
U	G or I
C	G or I
A	U
G	C

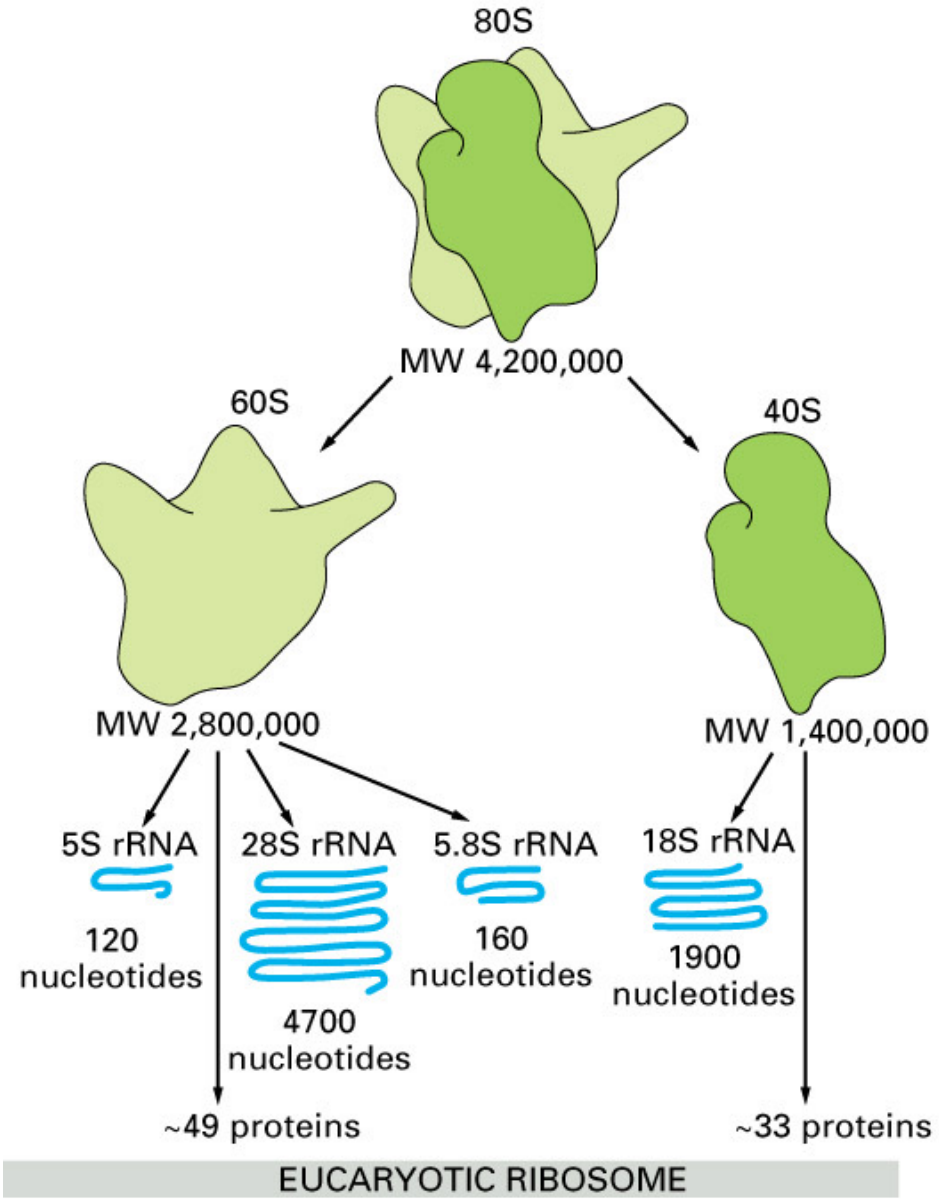
tRNA charging: the second genetic code

- tRNA structure
- the charging reaction
- aa-tRNA synthetases and tRNA recognition
- proofreading mechanism

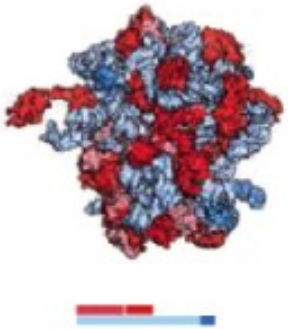
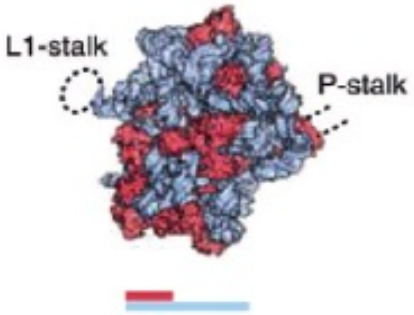
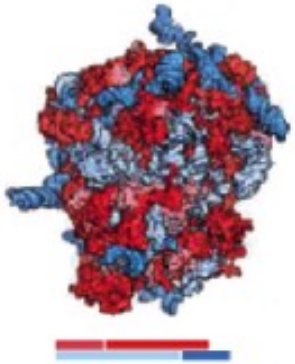
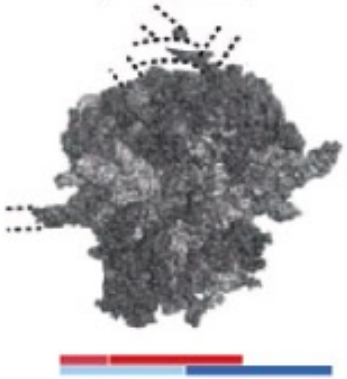
GCA GCC GCG GCU	AGA AGG CGA CGC CGG CGU	GAC GAU	AAC AAU	UGC UGU	GAA GAG	CAA CAG	GGA GGC GGG GGU	CAC CAU	AUA AUC AUU	
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	
A	R	D	N	C	E	Q	G	H	I	
UUA UUG CUA CUC CUG CUU	AAA AAG	AUG	UUC UUU	CCA CCC CCG CCU	AGC AGU UCA UCC UCG UCU	ACA ACC ACG ACU	UGG	UAC UAU	GUA GUC GUG GUU	UAA UAG UGA
Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
L	K	M	F	P	S	T	W	Y	V	

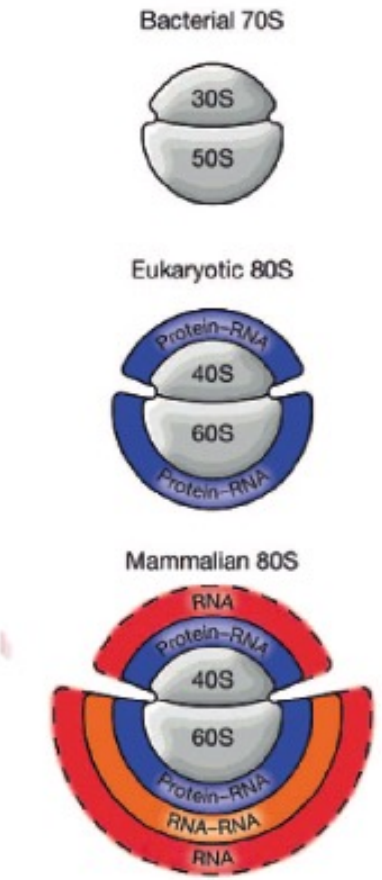
The Ribosome





The Ribosome

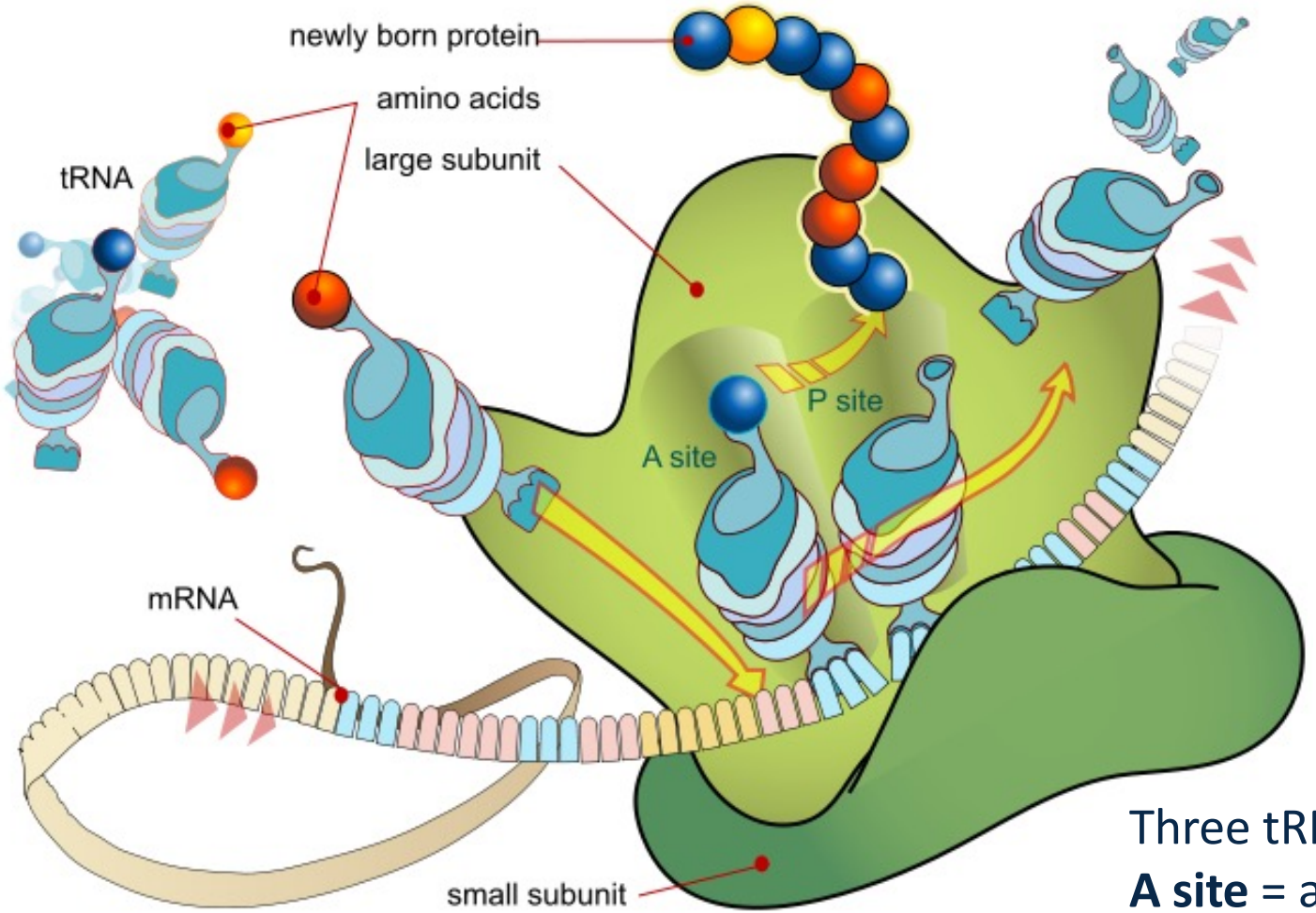
Bacteria (*T. thermophilus* or *E. coli*)	The common core	Lower eukaryotes (*S. cerevisiae*)	Higher eukaryotes (*H. sapiens*)
			
2.3 MDa	2.0 MDa	3.3 MDa	4.3 MDa
54 proteins 3 rRNA	34 proteins 3 rRNA	79 proteins 4 rRNA	80 proteins: 4 rRNA
Large subunit (50S): 33 proteins 23S rRNA—2,904 bases 5S rRNA—121 bases	Large subunit: 19 proteins 23S rRNA—2,843 bases 5S rRNA—121 bases	Large subunit (60S): 46 proteins 5.8S rRNA—158 bases 25S rRNA—3,396 bases 5S rRNA—121 bases	Large subunit (60S): 47 proteins 5.8S rRNA—156 bases 28S rRNA—5,034 bases 5S rRNA—121 bases
Small subunit (30S): 21 proteins 16S rRNA—1,542 bases	Small subunit: 15 proteins 16S rRNA—1,458 bases	Small subunit (40S): 33 proteins 18S rRNA—1,800 bases	Small subunit (40S): 33 proteins 18S rRNA—1,870 bases



An additional protein/rRNA layer in eukaryotic ribosomes with the increasing complexity

Schmidt, PhD thesis 2017

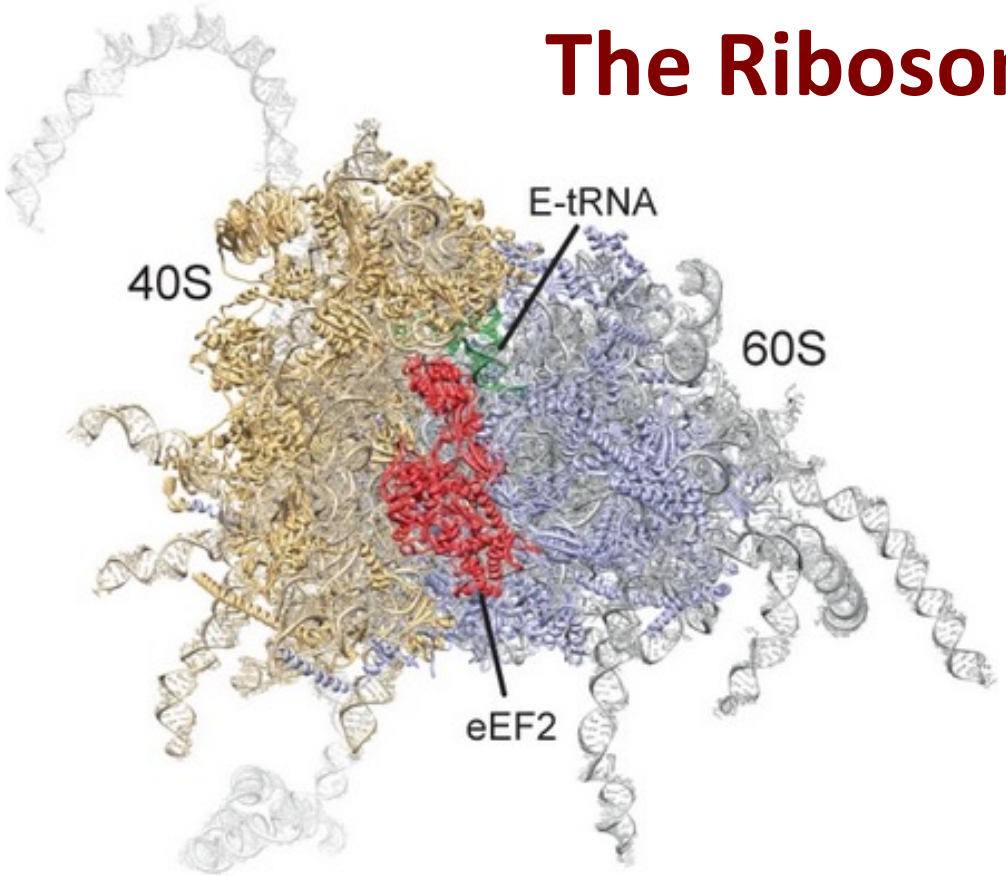
The Ribosome

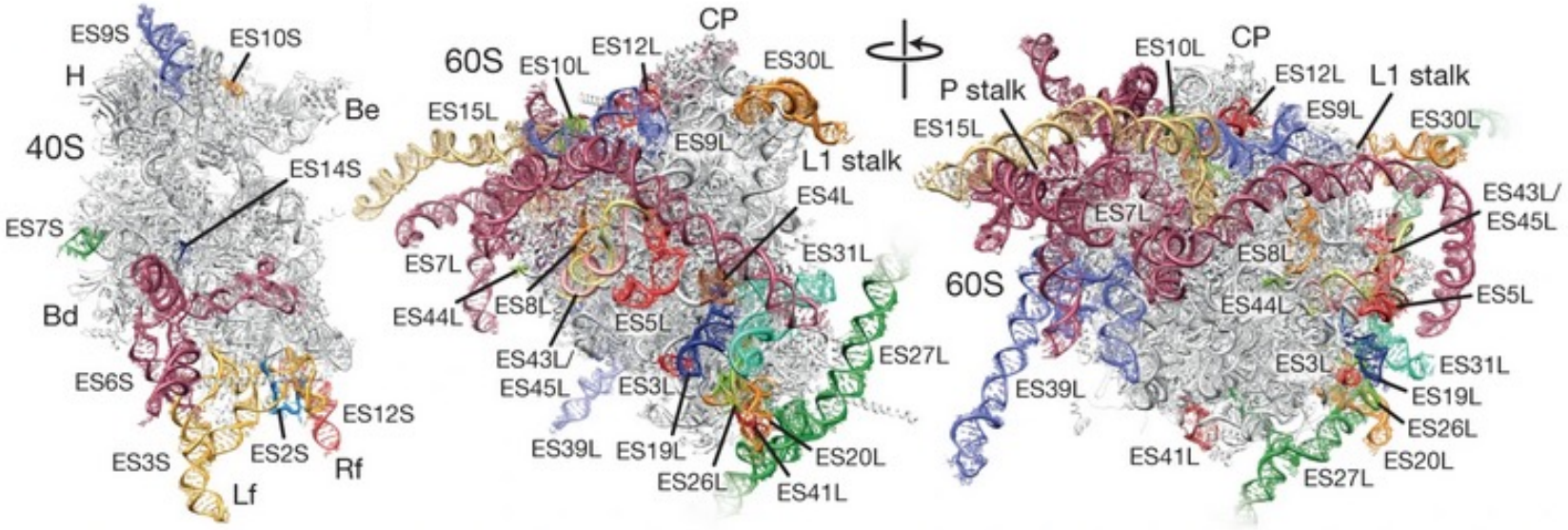


Three tRNA binding sites
A site = amino-acyl tRNA binding site
P site = peptidyl-tRNA binding site
E site = exit site

The Ribosome

Cryo-EM structure of the human 80S ribosome

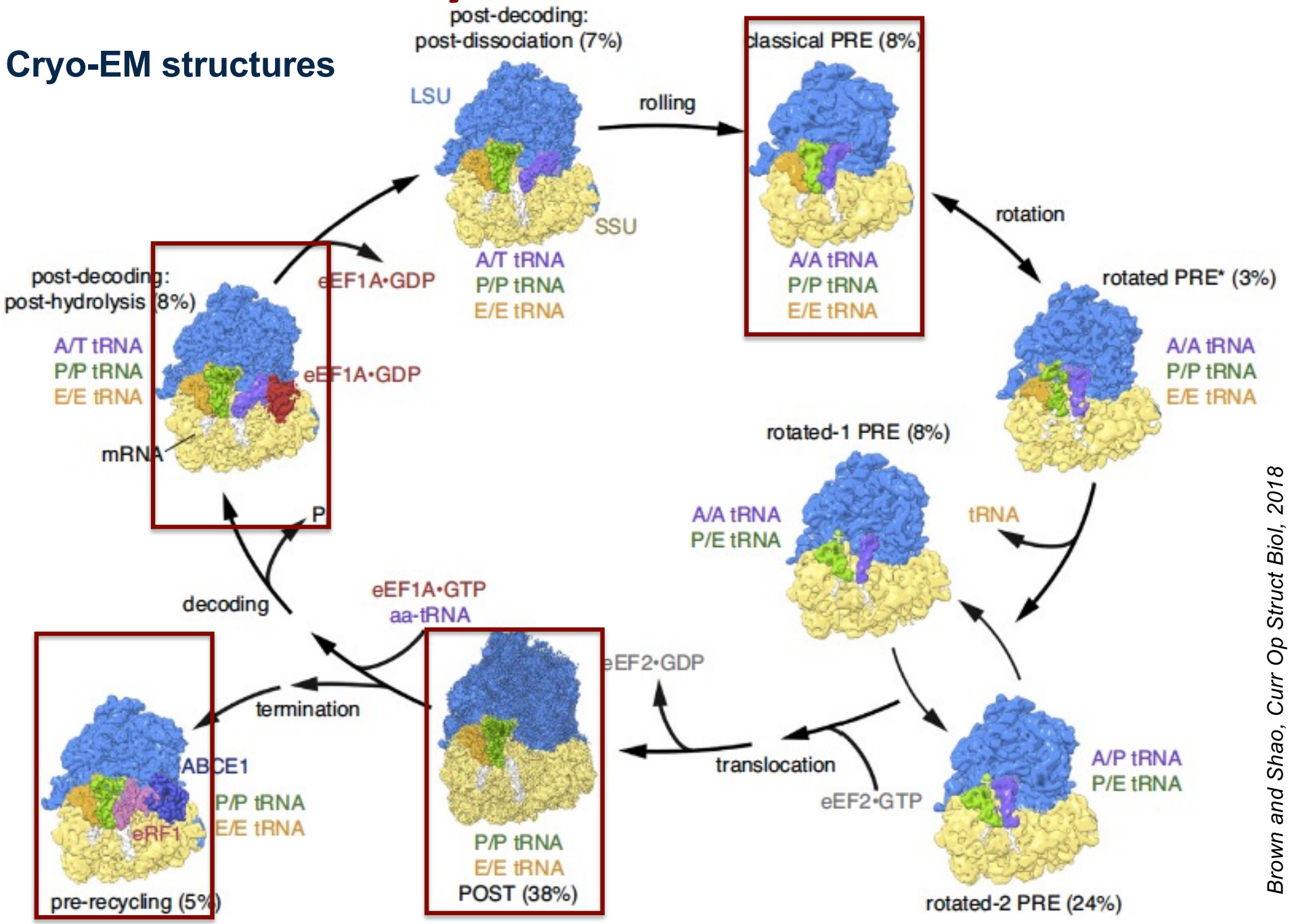




Anger et al, Nature, 2013

Translation cycle

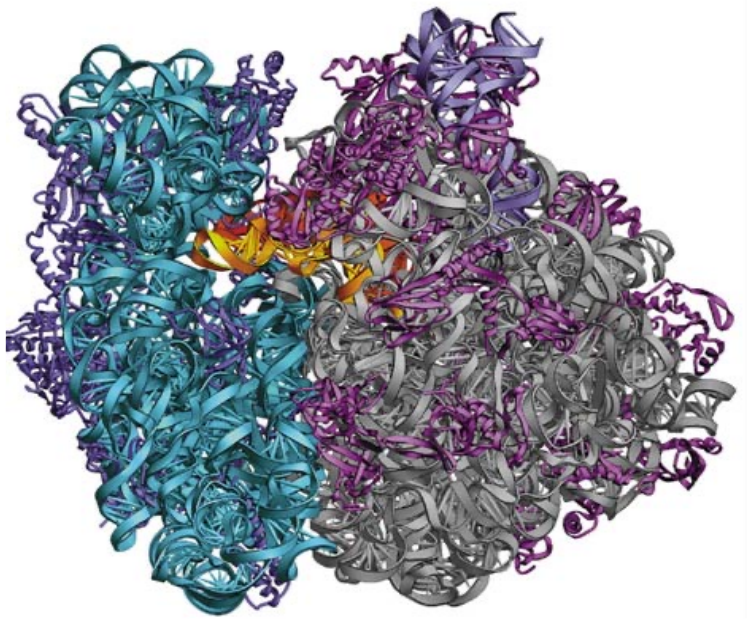
Cryo-EM structures



Brown and Shao, Curr Op Struct Biol, 2018

Ribosome is a ribozyme with a peptidyl transferase (PT) activity

- No ribosomal protein with a PT activity
- Drugs (chloramphenicol) that inhibit PT bind to the 25S rRNA (PT loop)
- Mutations that provide resistance to these drugs map to the PT loop
- Nearly all (99%) of proteins can be stripped from the large subunit and it still retains the PT activity
- Only RNA chains are close enough to the PT center (X-ray structure)
- Ribosomal proteins are important for ribosome stability and integrity, but NOT for catalysis



Catalysis

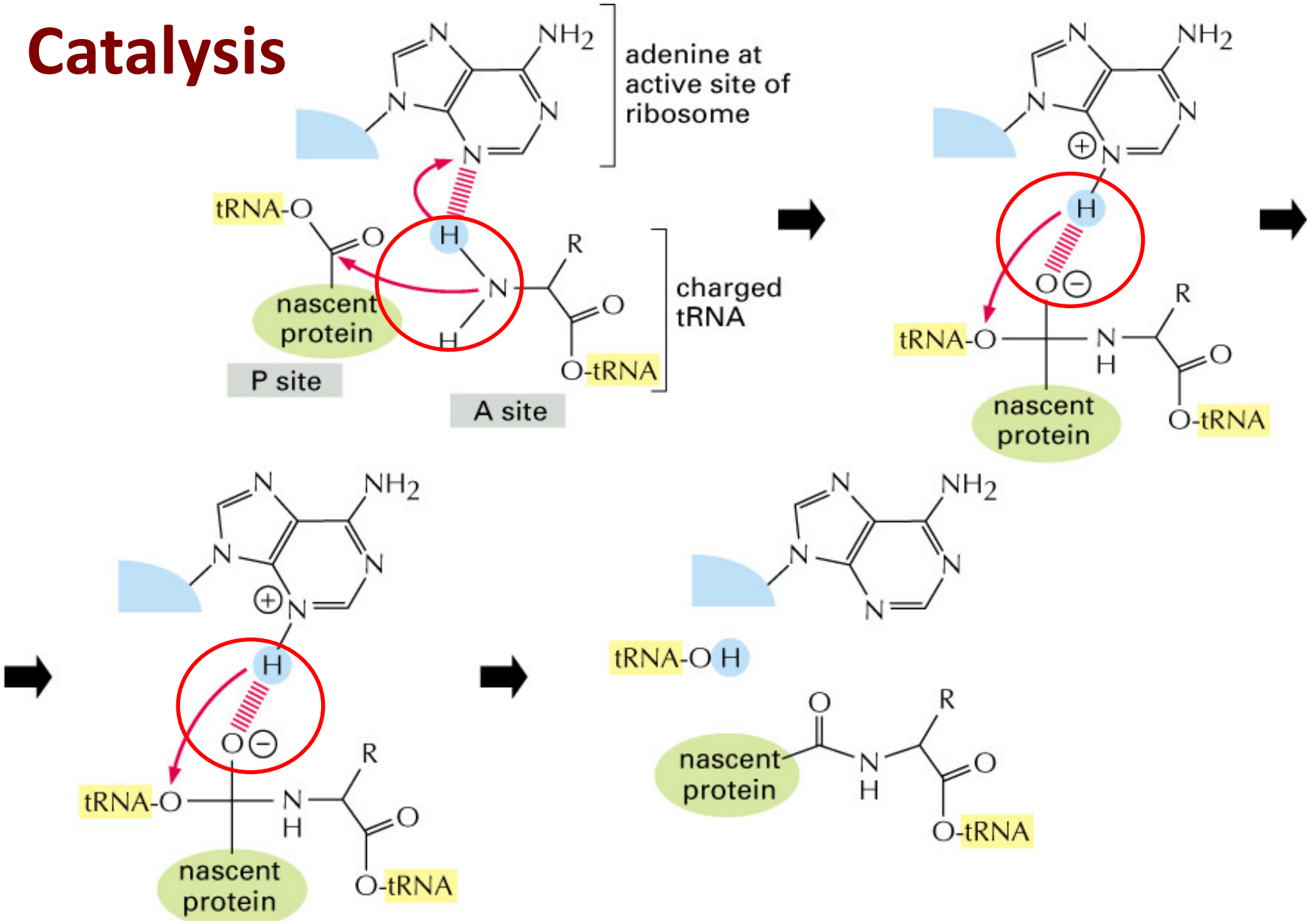
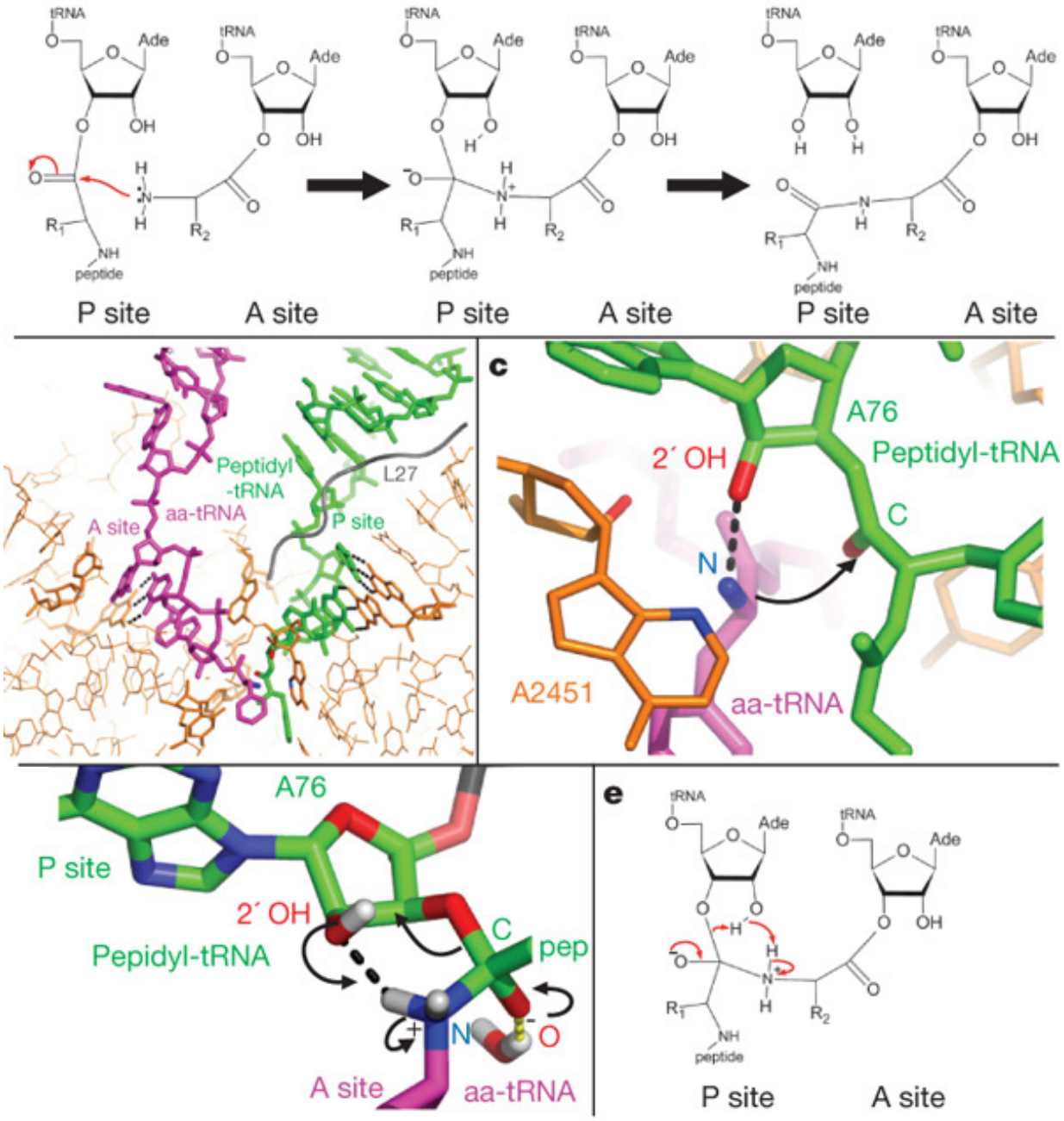


Fig **Peptide bond formation is catalyzed by the large subunit rRNA. Mechanism: α-amino group of aa-tRNA nucleophillically attacks the ester carbon of the peptidyl-tRNA to form a new peptide bond.**

Peptide bond formation



Schmeing and Ramakrishnan, Nature, 2009

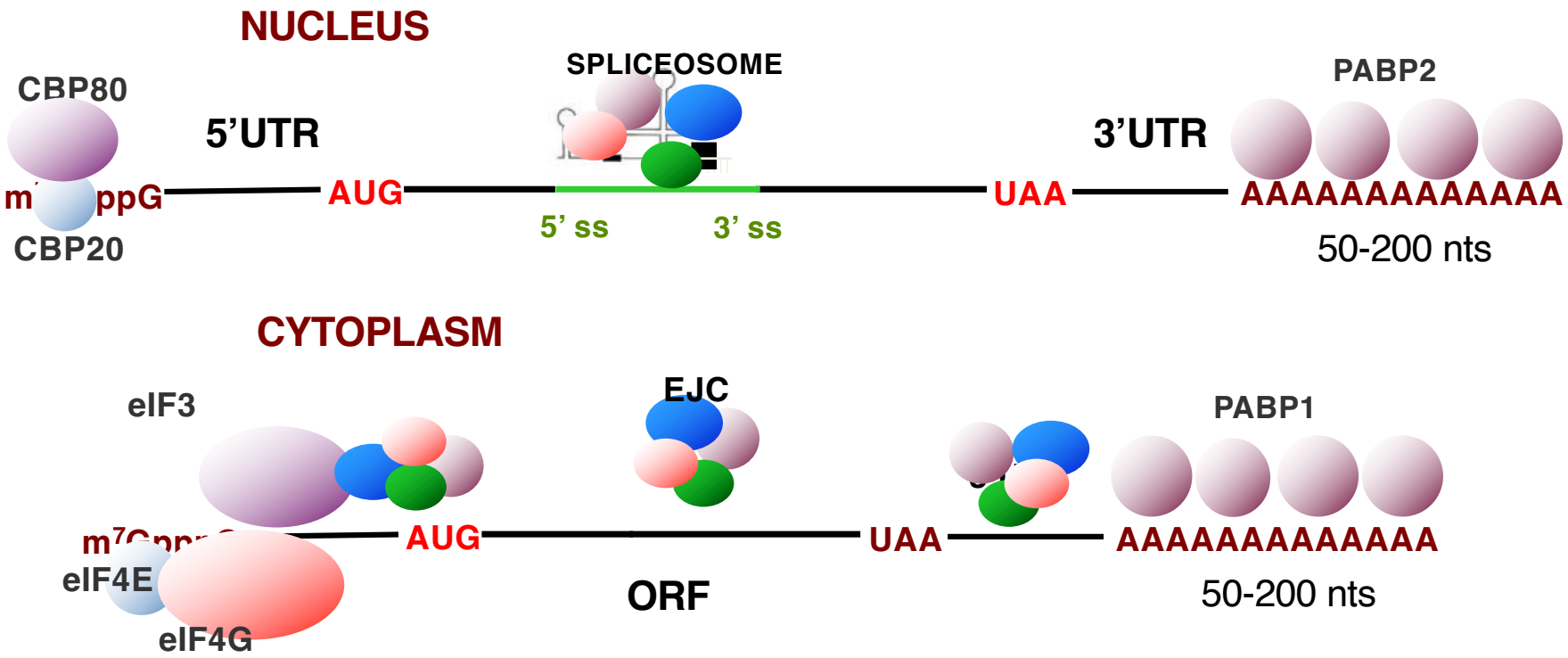
Catalysis

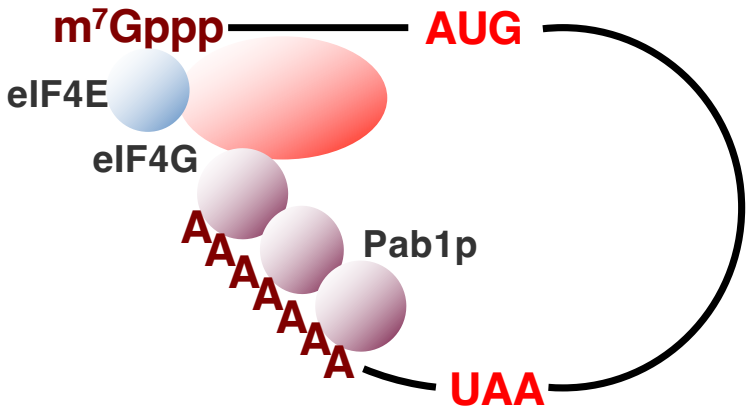
See the movie by Martin Schmeing and Rebecca Voorhees in the Venki Ramakrishnan lab at the LMB Cambridge, UK

https://www.youtube.com/watch?v=qOojy0QBF8g

Schmeing and Ramakrishnan, Nature, 2009

Eukaryotic mRNA



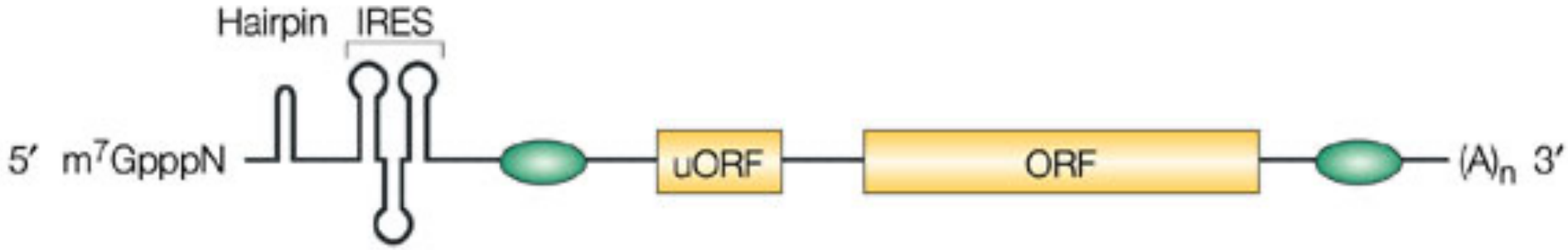


mRNA $t_{1/2}$ = few minutes to 2 hours (yeast) to >90 hours (mammals)

ORF- Open **R**eading **F**rame
encodes a protein

UTR- Un**T**ranslated **R**egion

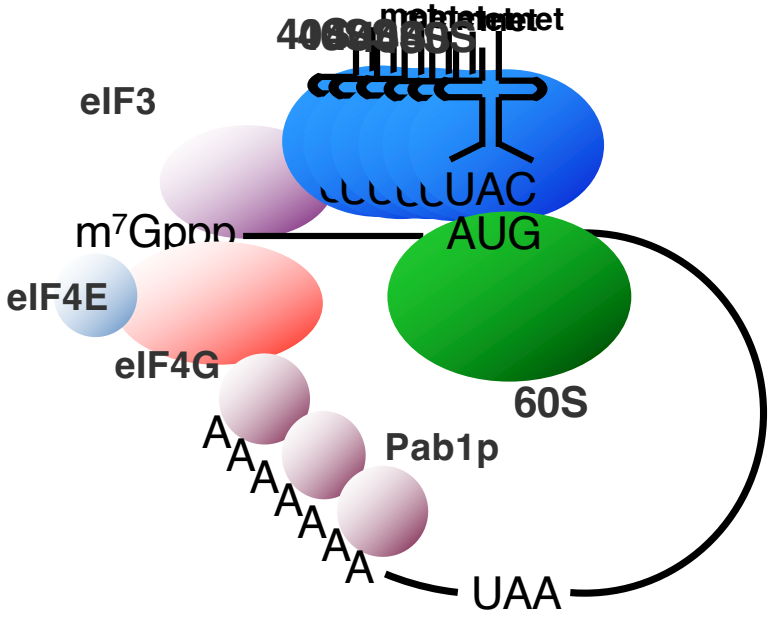
Eukaryotic mRNA



uORF- upstream **ORF**
- regulates the efficiency of ribosome re-initiation
- affects mRNA stability (via NMD)
- regulates gene expression via biding of protein factors
- its translation may generate regulatory cis-acting peptide
- regulates gene expression during stress

IRES – Internal **R**ibosome **E**ntry **S**ite
- a structured RNA region within 5’ UTR
- allows for cap-independent translation and initiation of translation inside RNA
- often used by viral mRNAs and a few cellular mRNAs (some of them can also utilize the scanning cap-dependent mechanism, this may be regulated by the intracellular concentration of eIF4G)

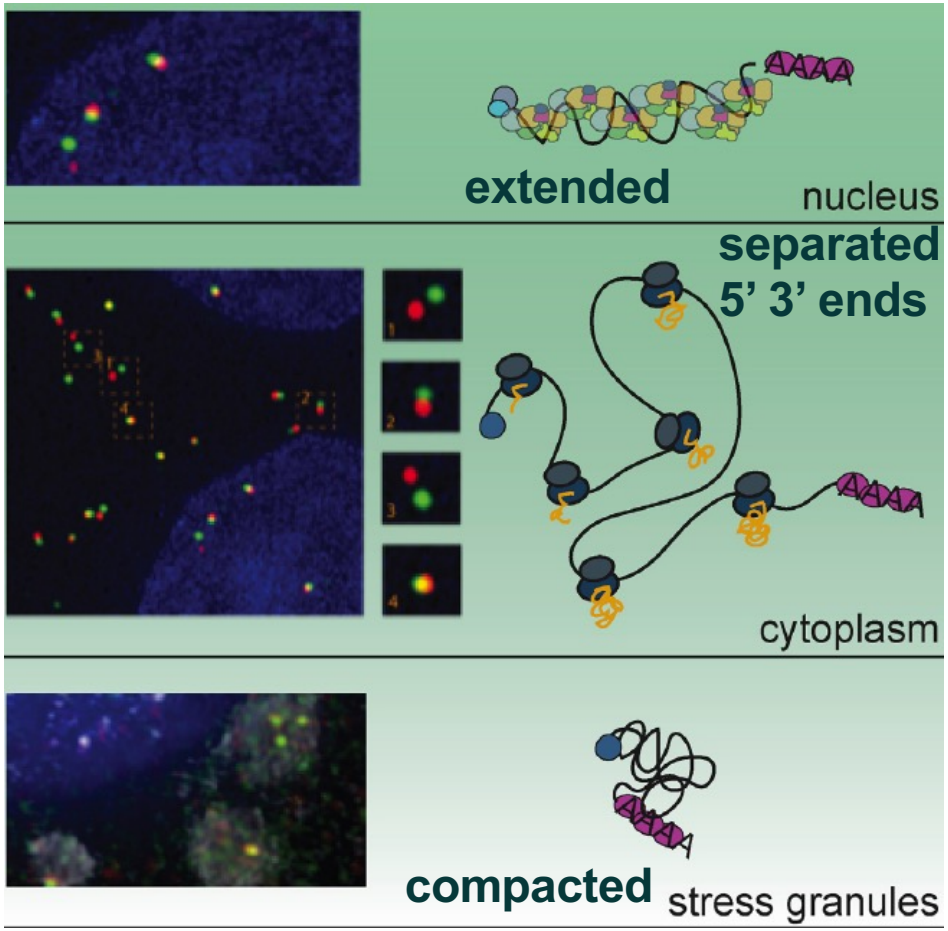
CAP-dependent translation by scanning



- eIF4E interacts with m7G cap to form translationally active mRNA
- circular mRNA protects agains degradation and stimulates translation
- eIF4E/eIF4G/PAB recruits small ribosomal subunit
- tRNA-bound 40S scans mRNA to locate START

Translating mRNAs: circular or not?

Single-molecule mulit-color smFISH using fluorescent probes for different mRNA regions



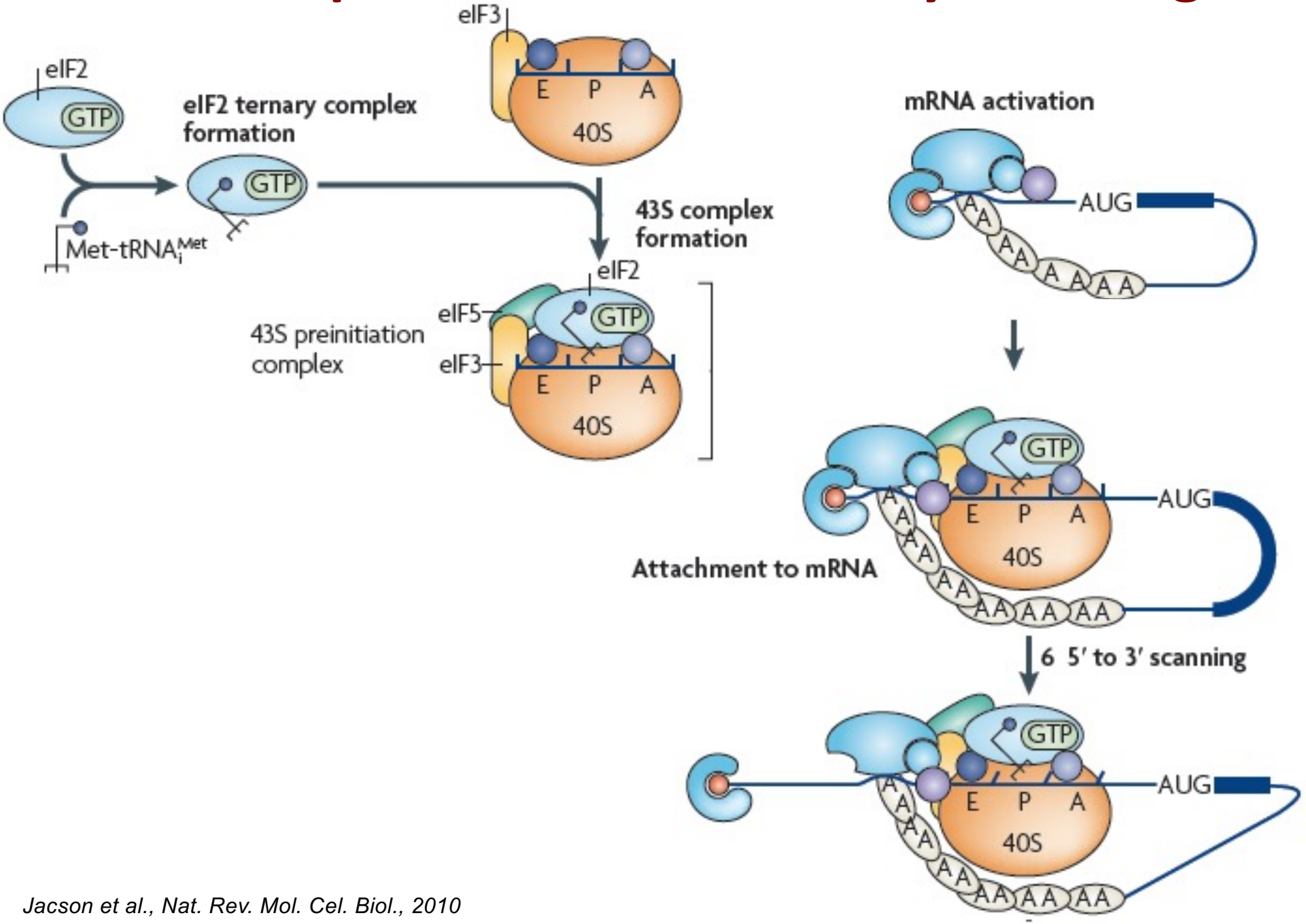
Translation does not occur in a stable circularized mRNA conformation

Adivarahan et al, Mol Cell, 2018

Pre-translational mRNPs form linear rods

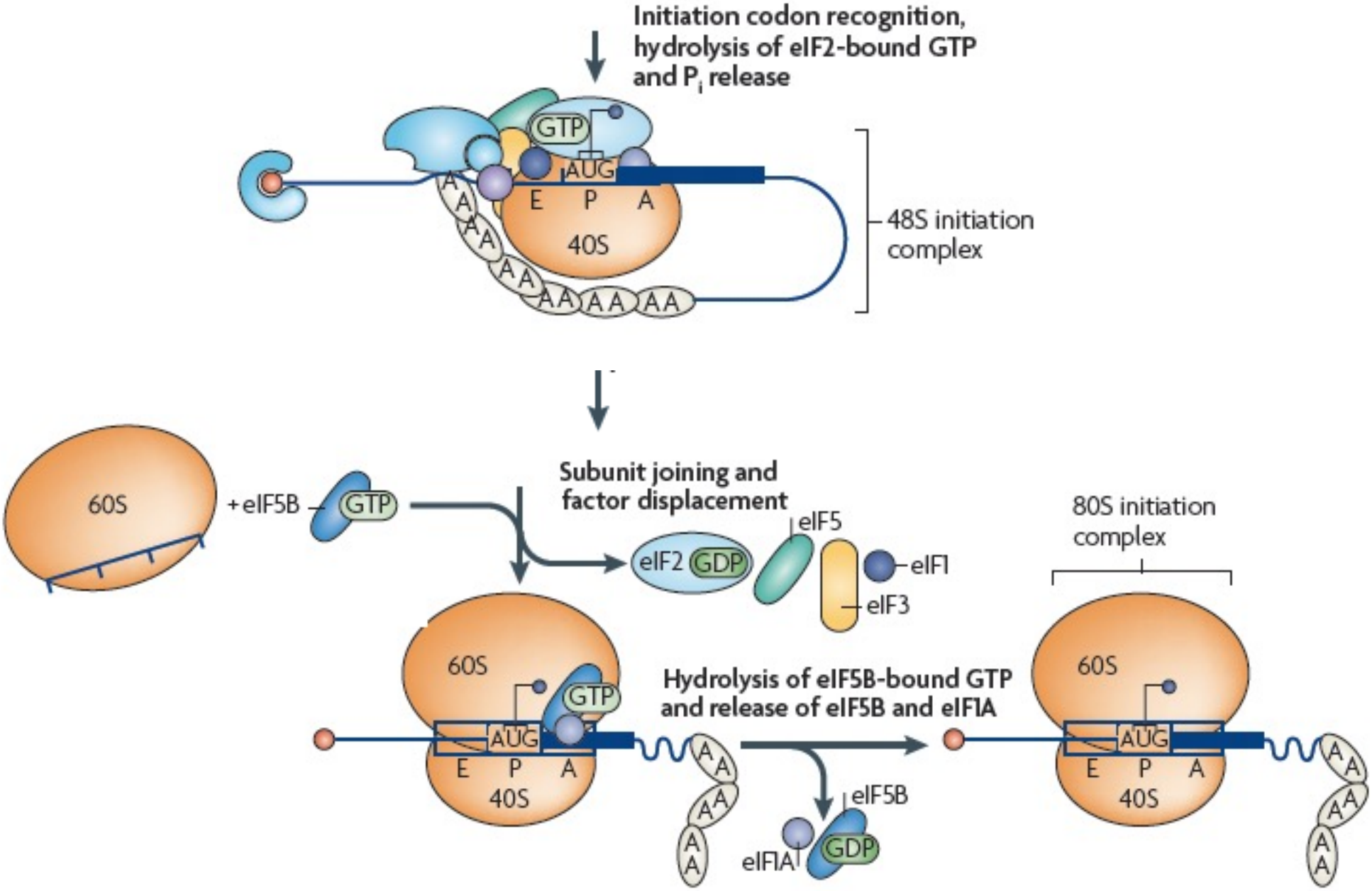
Metkar et al, Mol Cell, 2018

CAP-dependent translation by scanning



Jacson et al., Nat. Rev. Mol. Cel. Biol., 2010

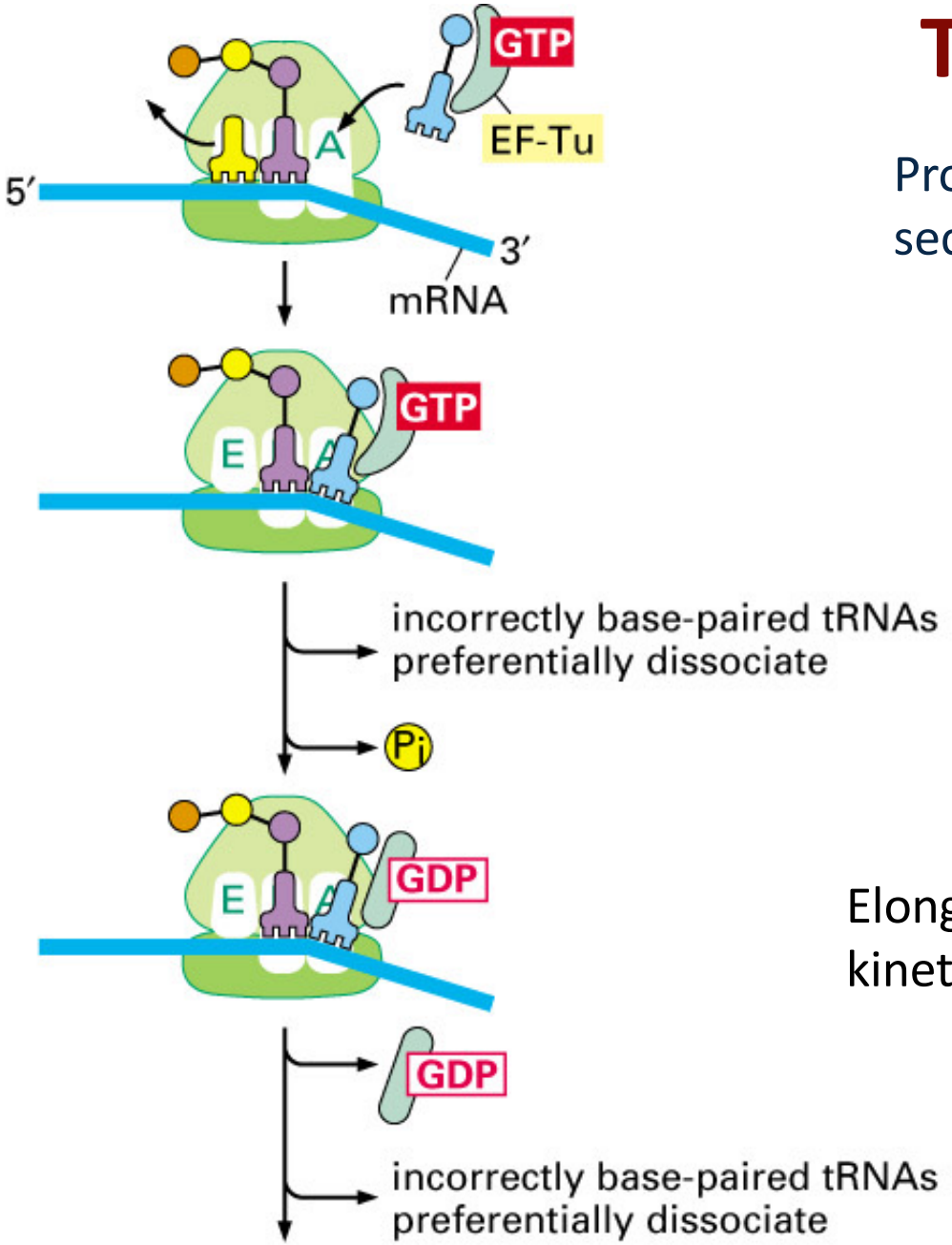
CAP-dependent translation by scanning



Jacson et al., Nat. Rev. Mol. Cel. Biol., 2010

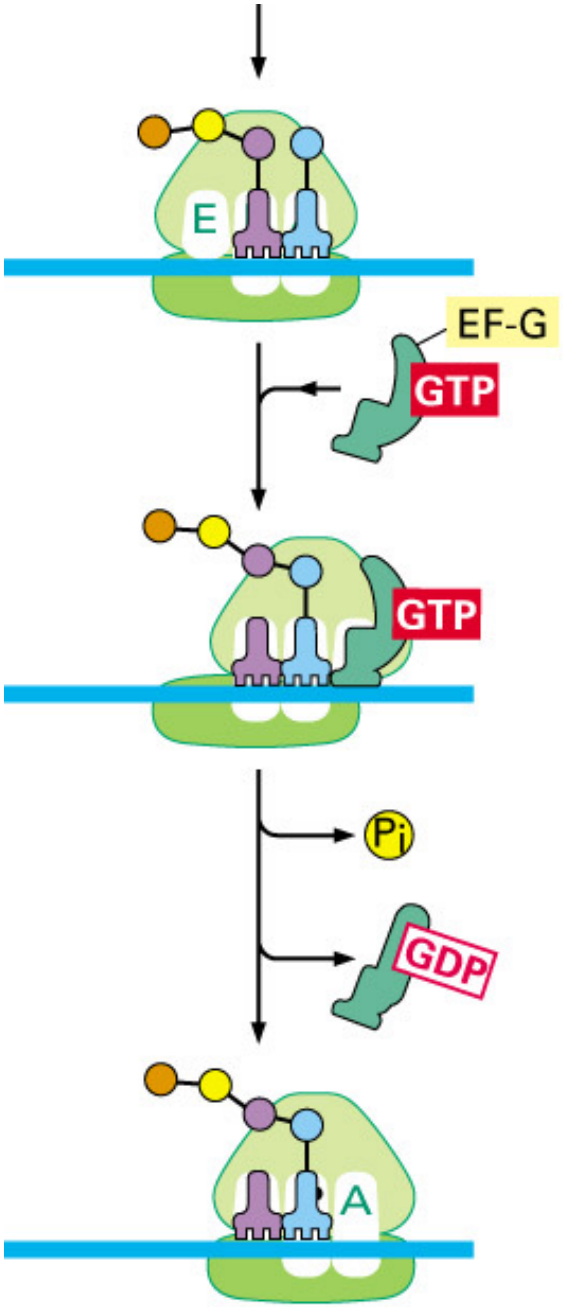
Translation cycle

Proper reading of the anticodon - the second translation quality control step



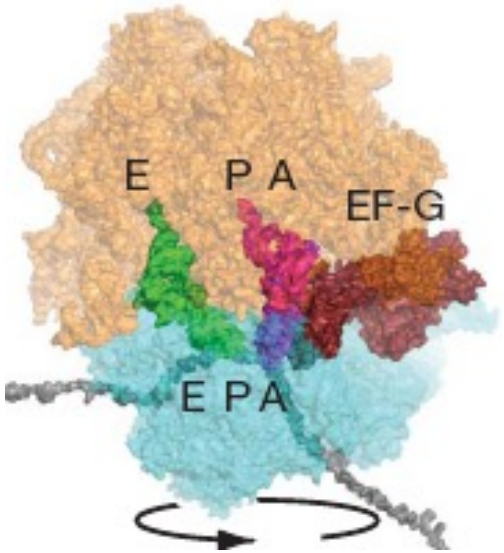
Elongation factors introduce a two-step kinetic proofreading

Translation cycle



A second elongation factor EF-G/EF-2 drives the translocation of the ribosome along the mRNA

GTP hydrolysis by EF-1 and EF-2 drives protein synthesis forward

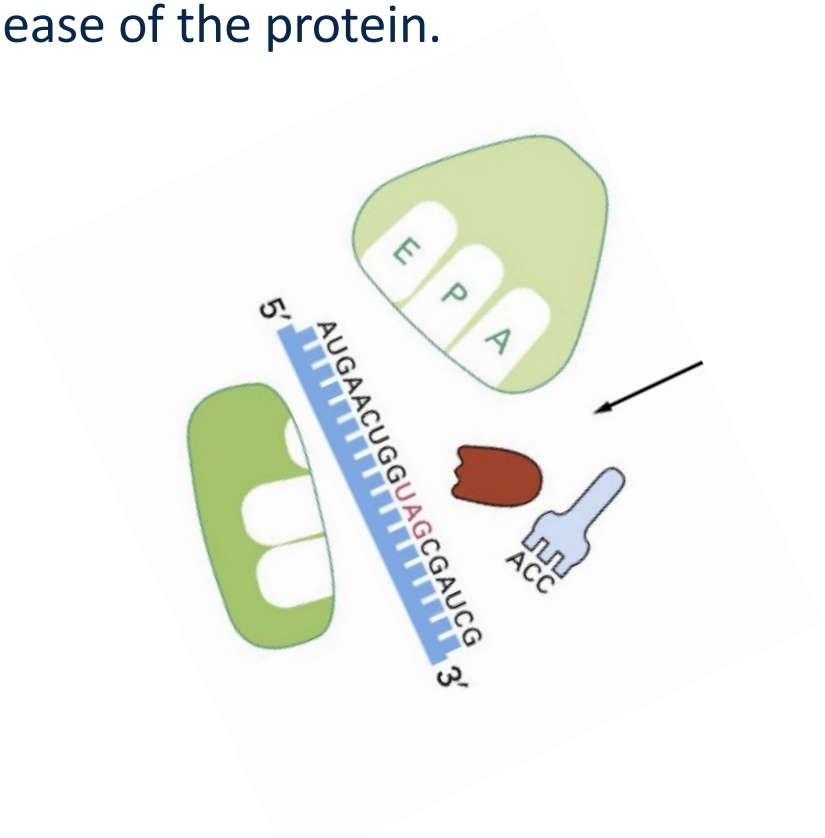


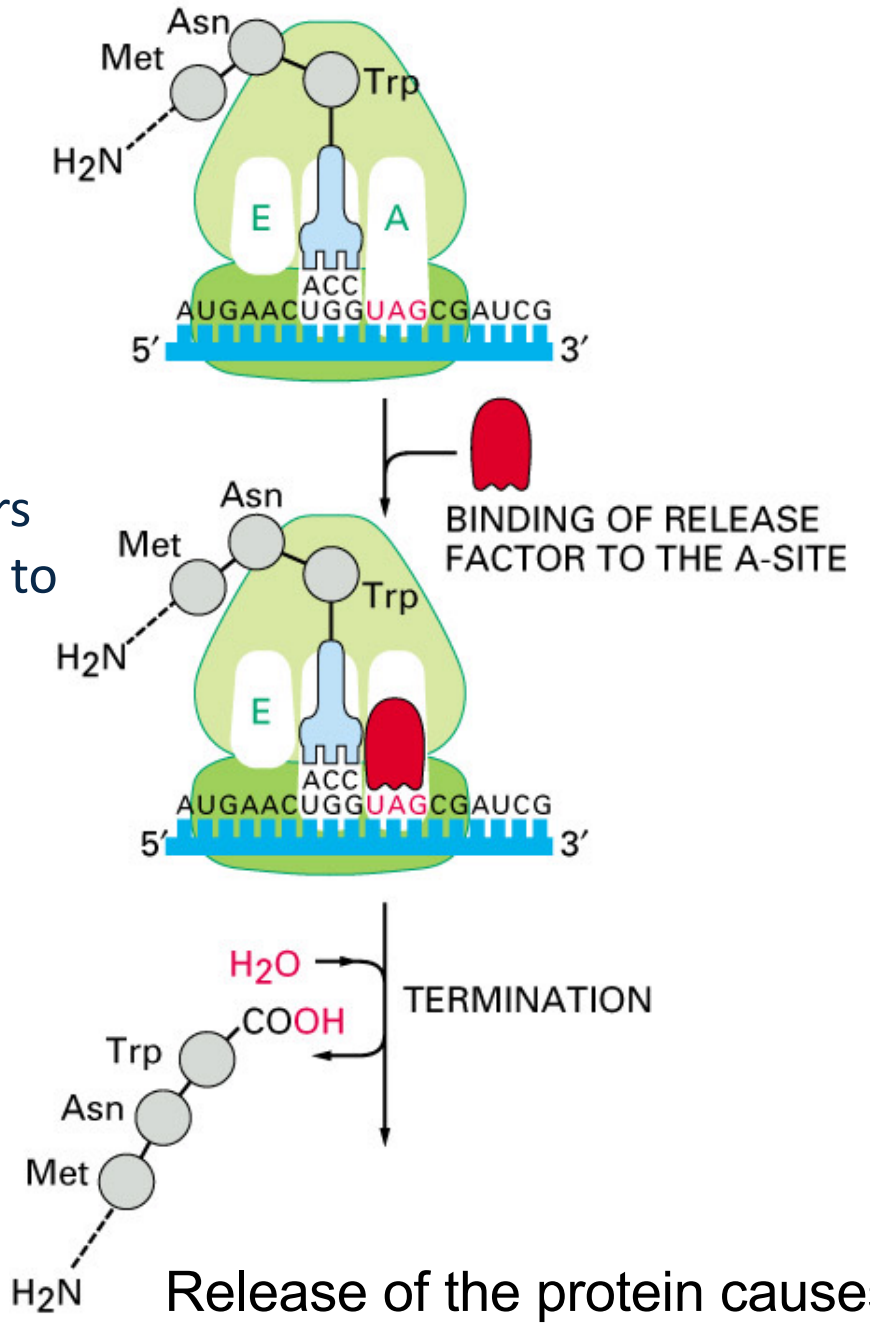
Schmeing and Ramakrishnan, Nature, 2009

Translation cycle

Termination of translation is triggered by stop codons

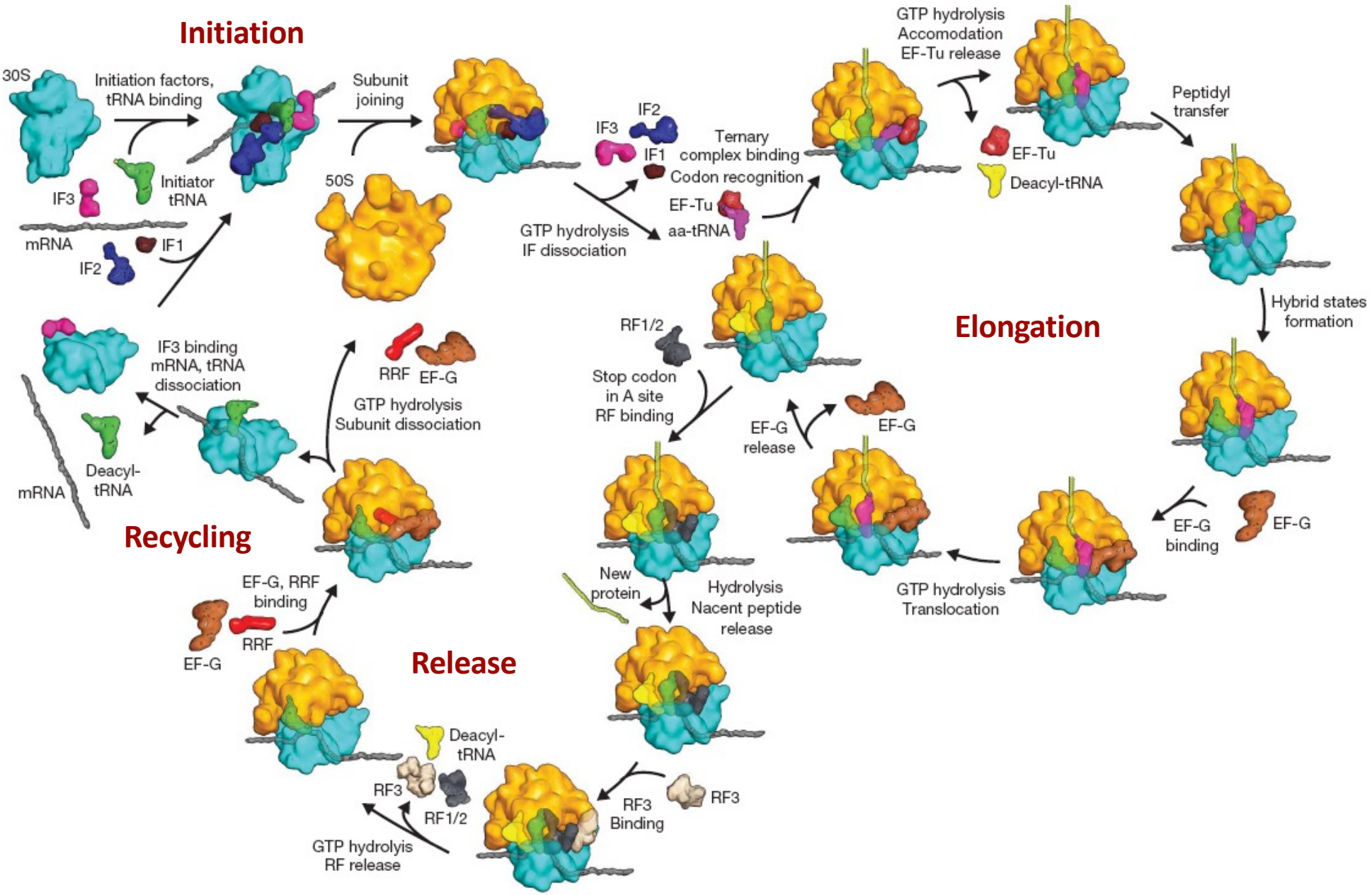
Release factor enters the A site and triggers hydrolysis the peptidyl-tRNA bond leading to release of the protein.





Release of the protein causes the ribosome disassociation

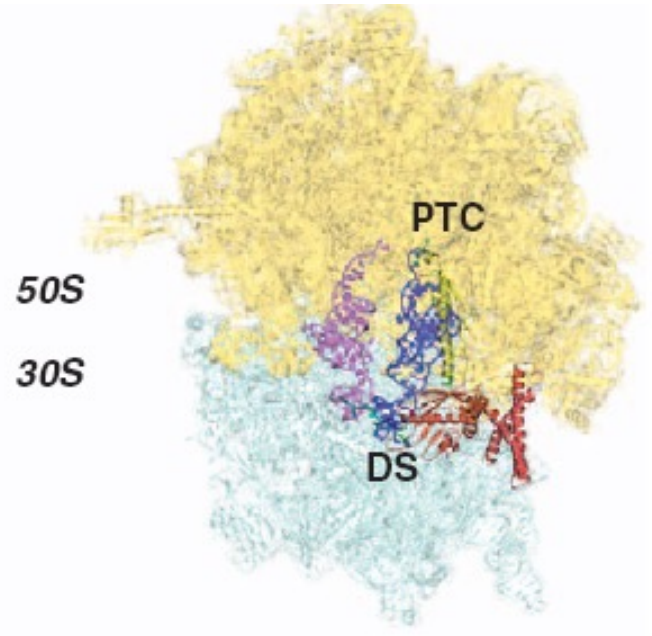
Translation cycle



Schmeing and Ramakrishnan, Nature, 2009

Translation termination

Release Factor is a molecular mimic of a tRNA



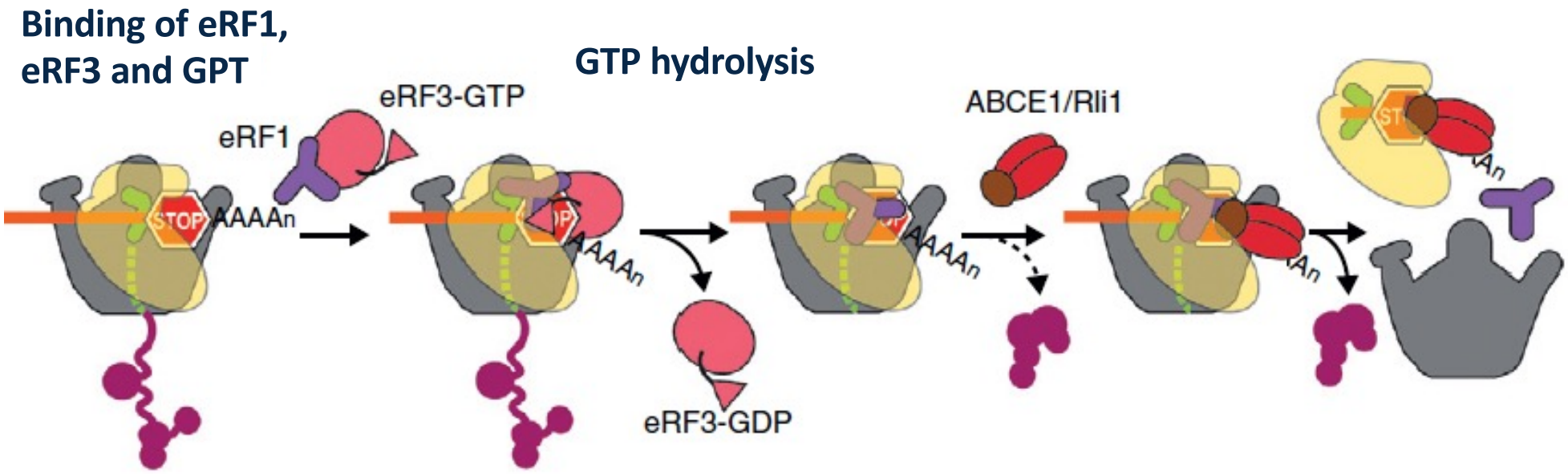
Crystal structure of the 70S–RF2 complex

Termination factors

Prokaryotes
RF-1 = UAA, UAG
RF-2 = UAA, UGA
RF-3 = GTPase

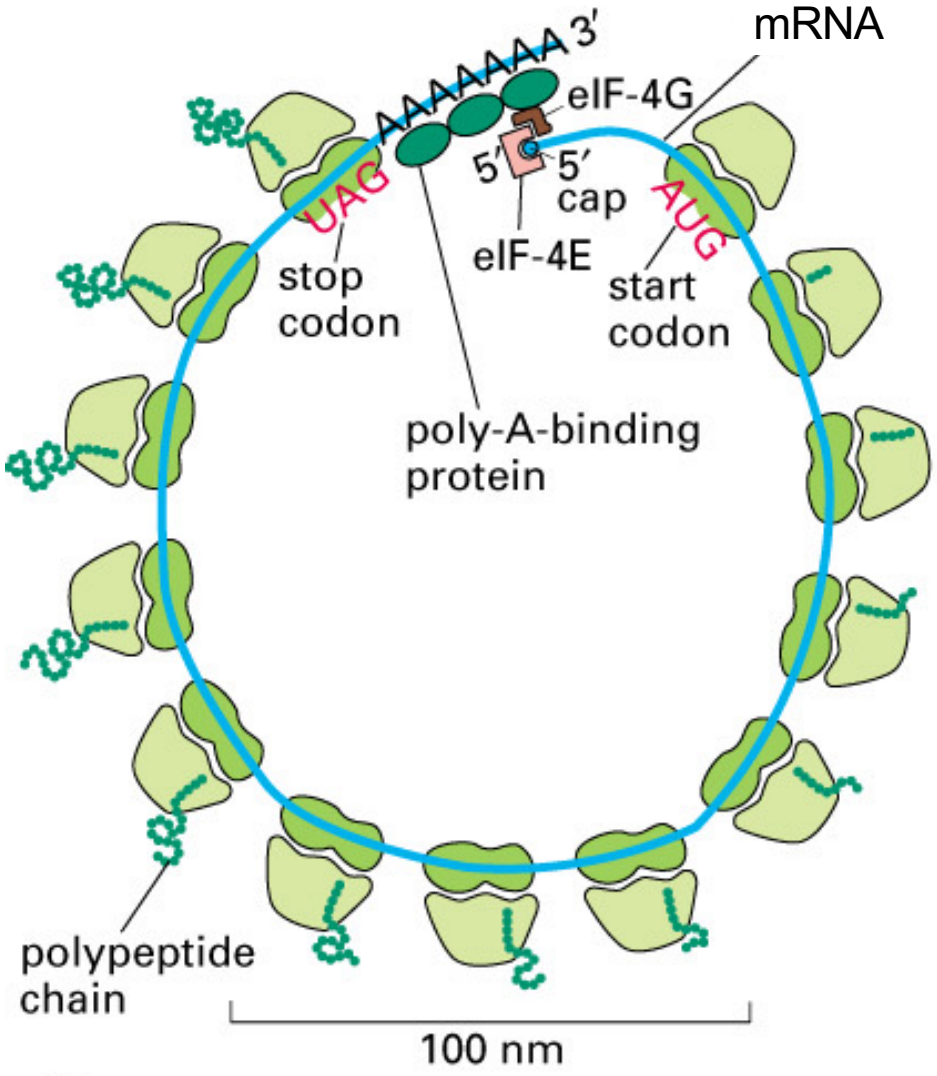
Eukaryotes
eRF1 = UAA, UAG, UGA
-
eRF3 = GTPase

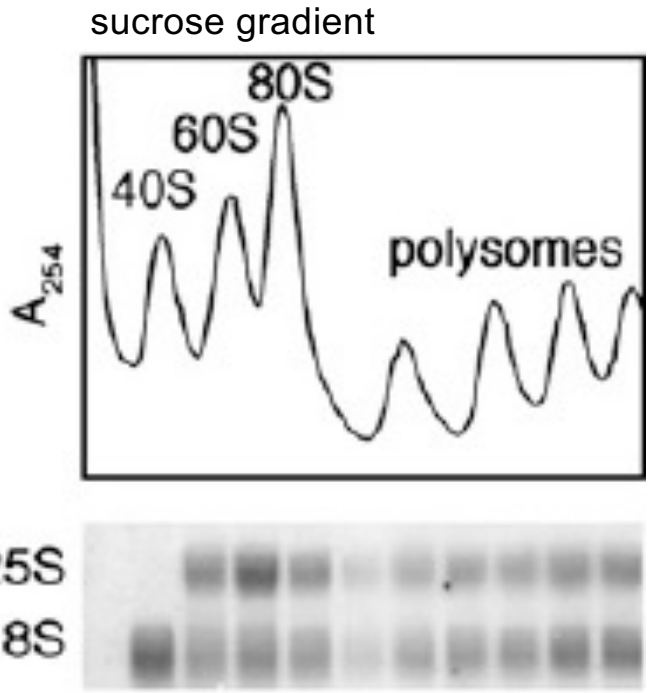
Translation termination

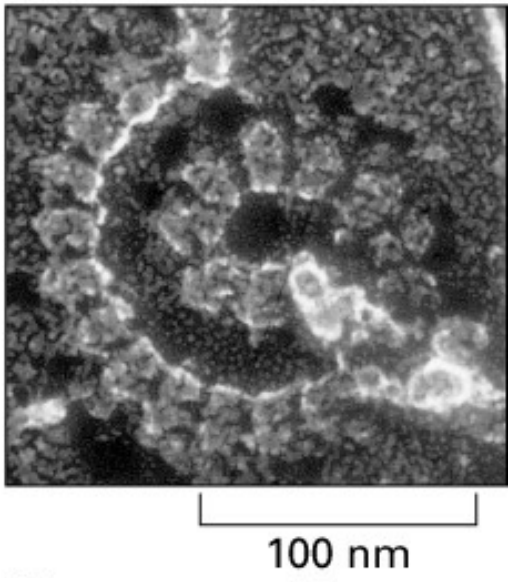


Franckenberg et al., Curr Op Struct Biol 2012

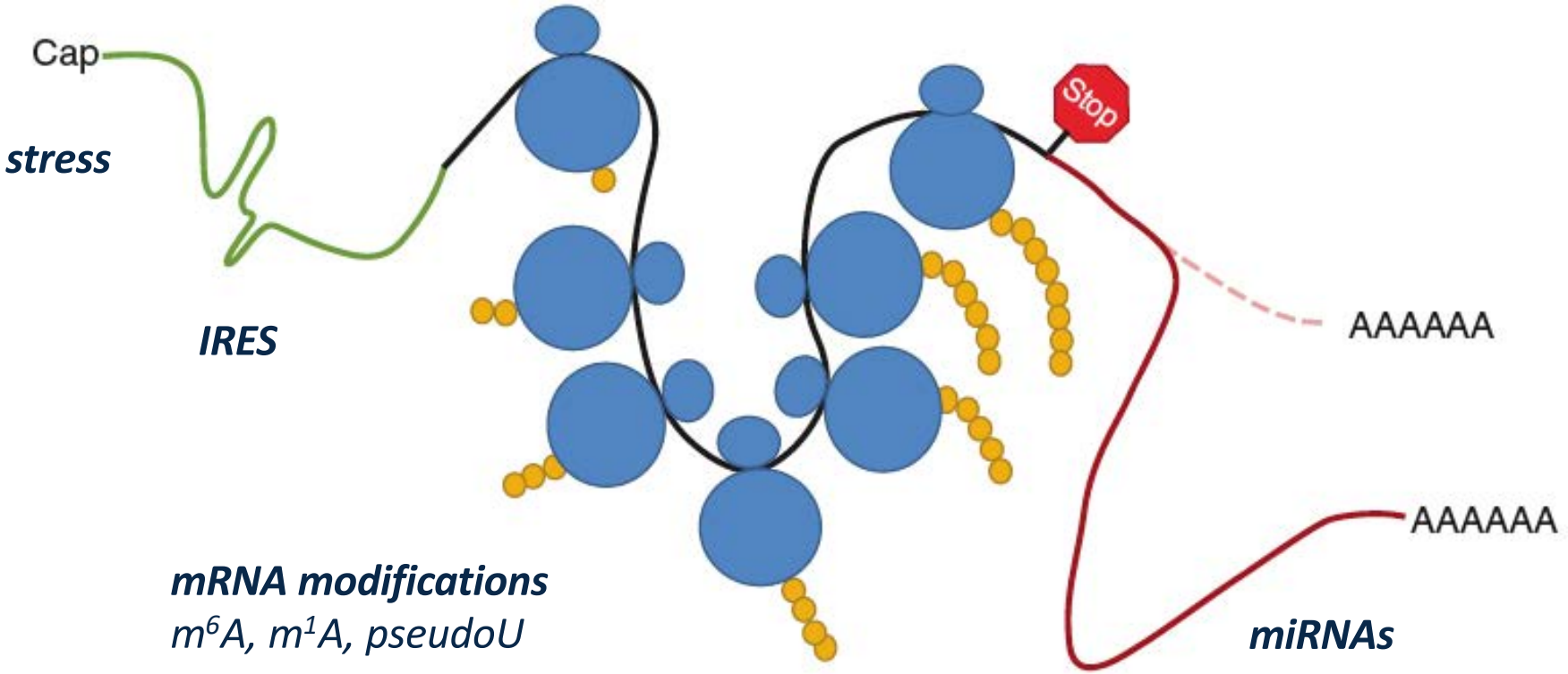
mRNAs translation on polyribosomes







Translation regulation



5′ UTR	ORF	3′ UTR
Translation initiation rates	Ribosome pausing	Protein/RNA *trans* factors
RNA structure	Premature termination	Alternative polyadenylation
Protein/RNA *trans* factors	Slow elongation rates	Improper termination
	Protein/RNA *trans* factors	

Heck and Wilusz, CSH PerpspBiol, 2018

Translation regulation

by STRESS via kinase cascade (mTOR)
nutrients, DNA damage, heat/cold shock, hypoxia, oxidative strss

General control of translation initiation

Nutrient availability

(amino acids/carbohydrate)

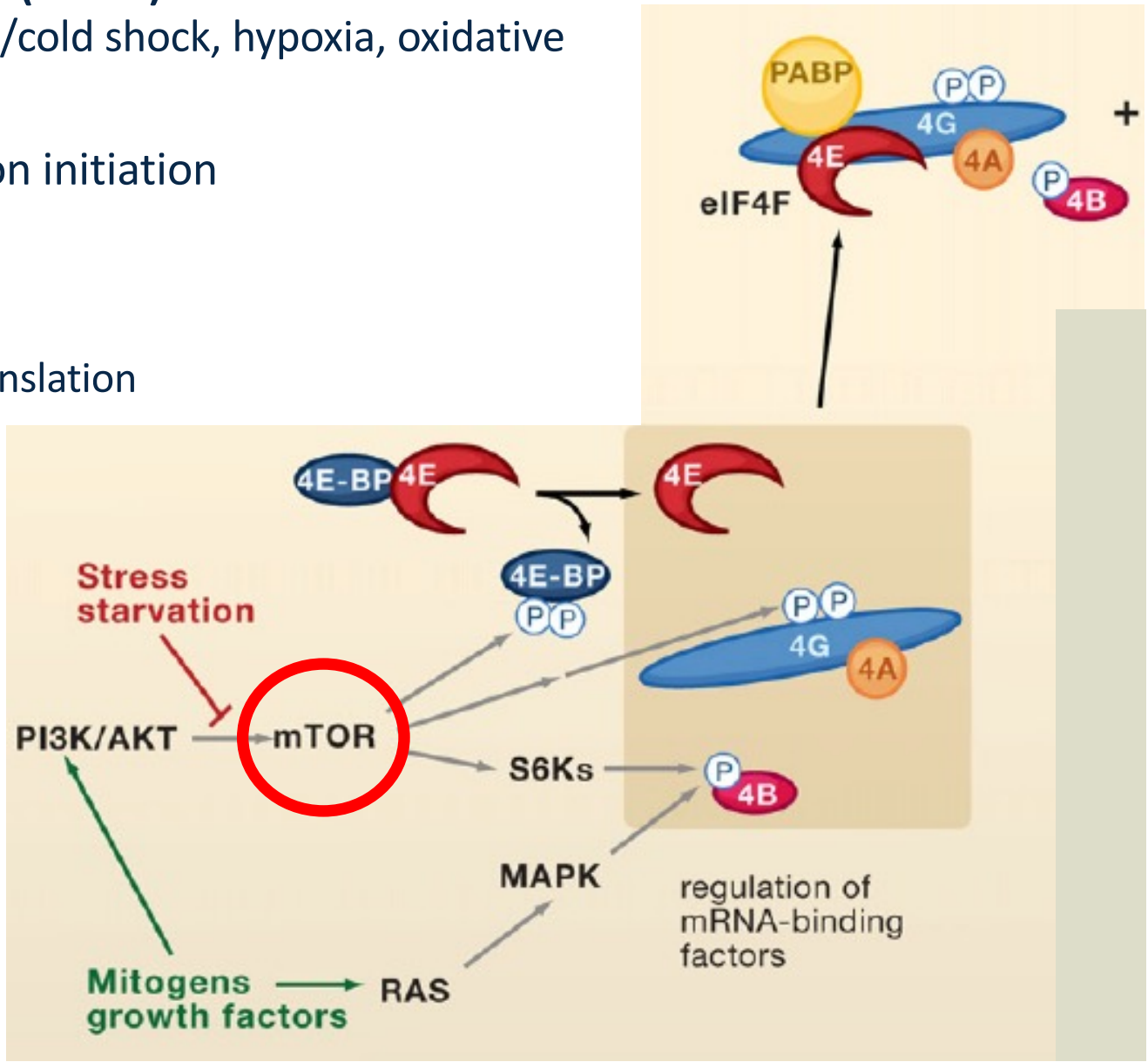
low nutrient downregulates translation

Growth factor signals

stimulation of cell division

upregulates translation

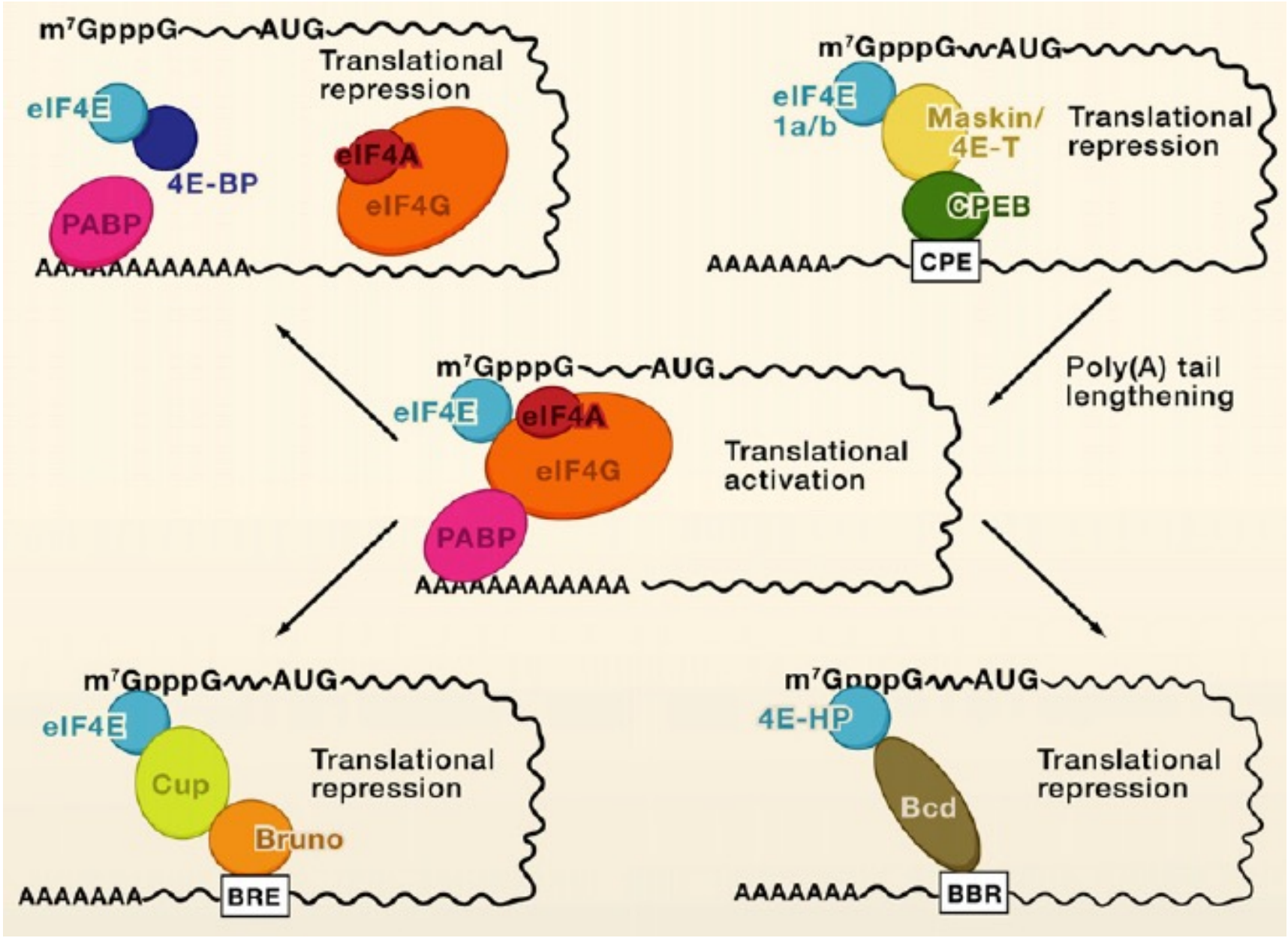
Phosphorylation of eIF2
Phosphorylation of eIF4 binding proteins
eIF4E availability



Sonenberg and Hinnebusch, Cell, 2009

Translation regulation

by RBP and 3’ UTR



Sonenberg and Hinnebusch, Cell, 2009

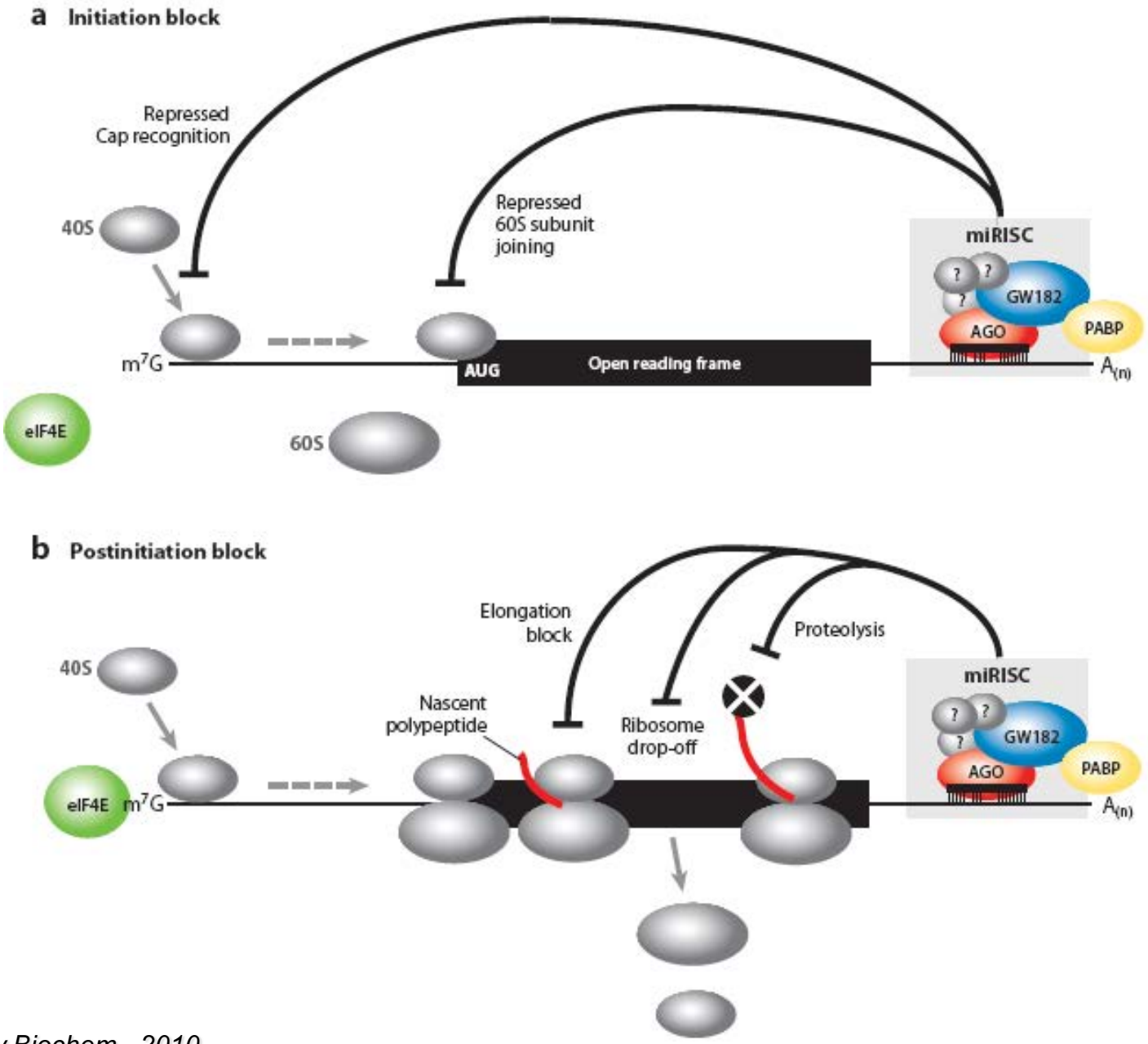
3’ UTRs: facts and gossips

- **are usually much longer than 5’ UTRs**
- **contain many regulatory protein-binding sequences**
- **regulate mRNA stability**
- **direct mRNAs to appropriate sites in the cell**
- **affect the efficiency of translation**
- **control timing of translation**
- **size in yeast:** **20** (min)**- 300** (av)**- 1000** (max) **nts**

  size in humans: **20** (min) **– 1000** (av)**- 10000** (max) **nts**

Translation regulation

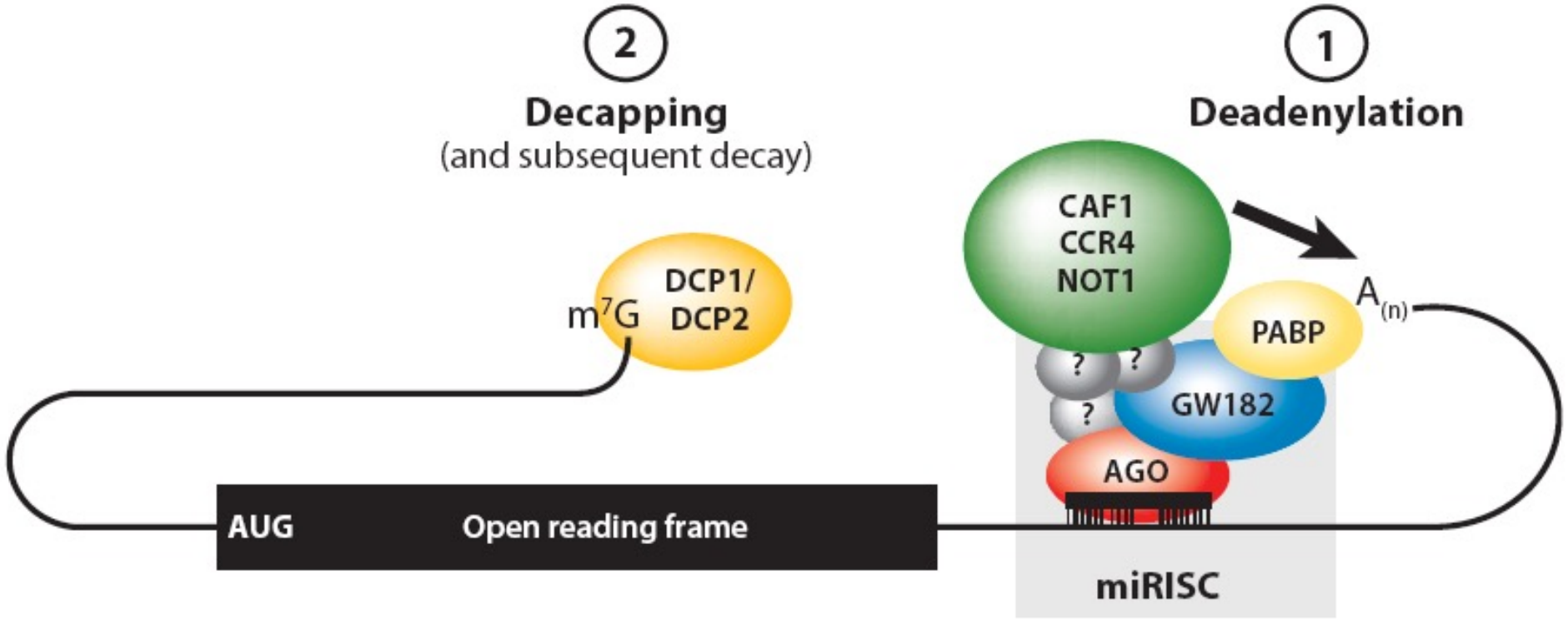
by miRNAs and 3'UTR



Fabian et al., Annu.Rev.Biochem., 2010

Translation regulation

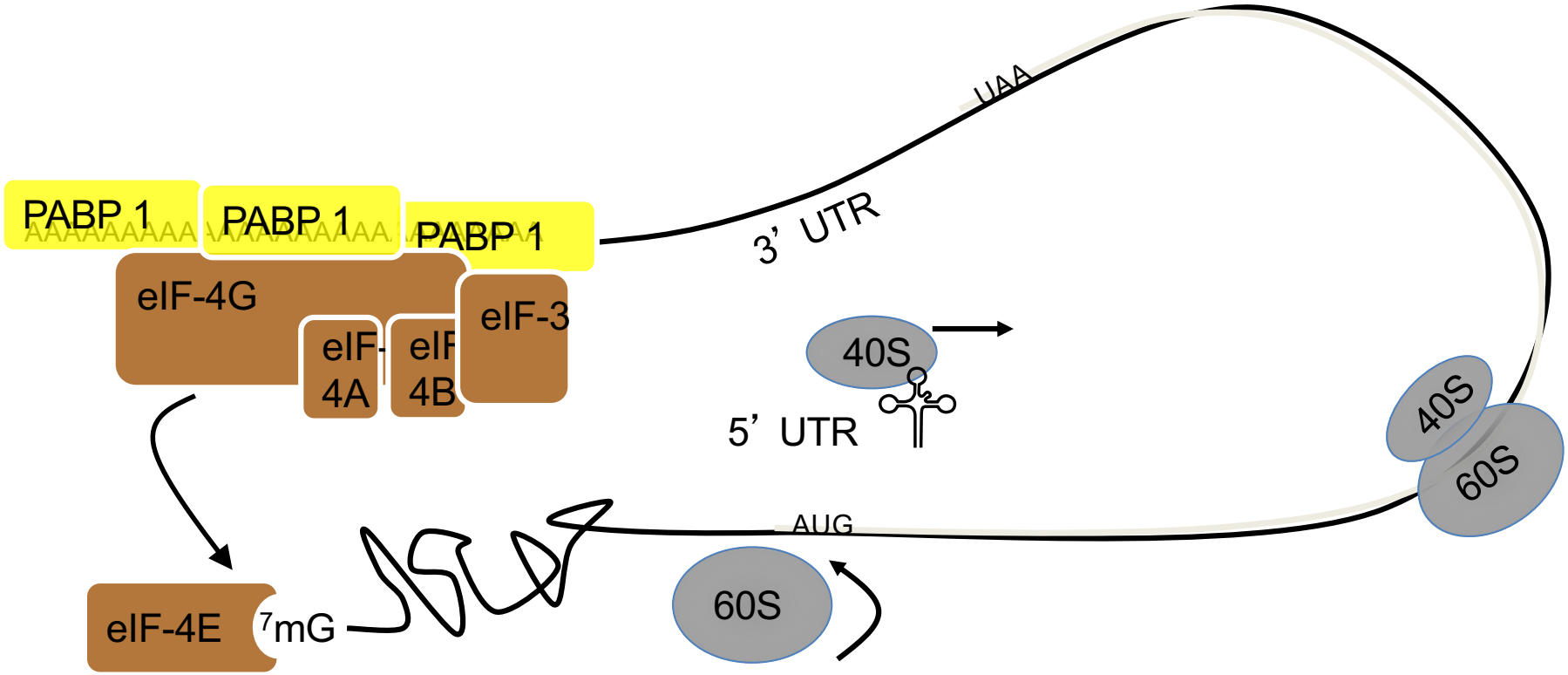
by miRNAs and mRNA degradation



Fabian et al., Annu.Rev.Biochem., 2010

Translation regulation

by 5' UTR



5' UTRs play contribute to translation efficiency of several cell cycle regulated proteins

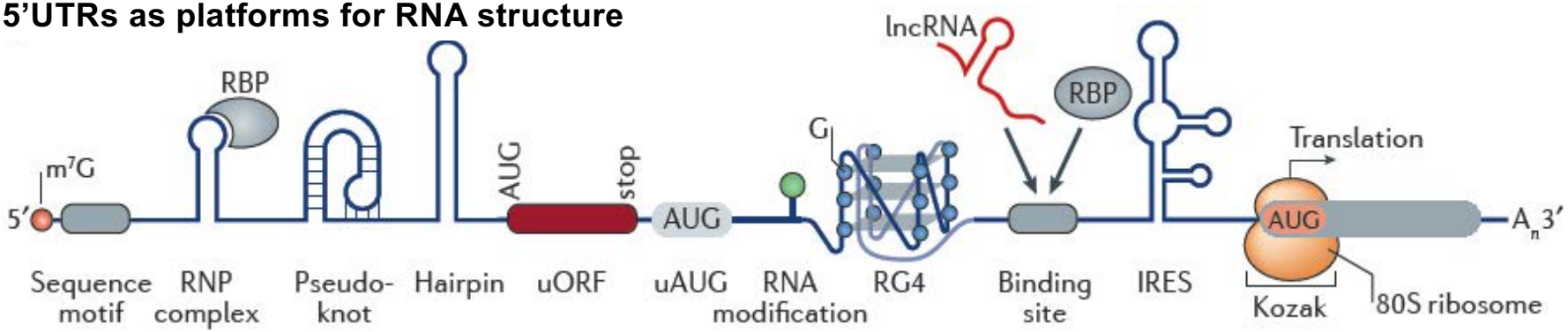
eIF-4E can increase translation of poorly translated mRNAs (e.g. of growth factors) with GC-rich secondary structures in long 5' UTRs (>1,000 nucleotides).

eIF-4E is an proto-oncogene, its over-expression causes malignant transformations.

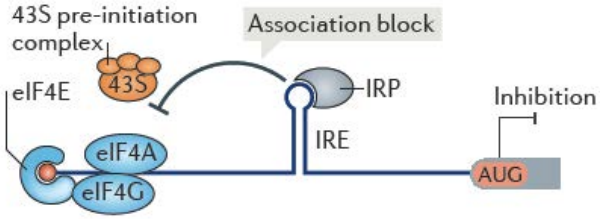
by 5’ UTR

Translation regulation

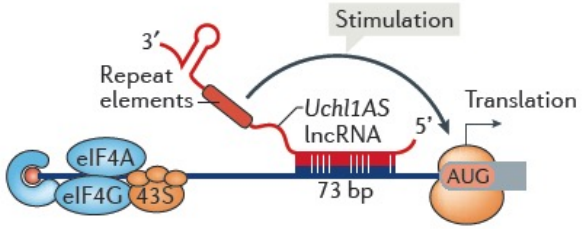
5’UTRs as platforms for RNA structure



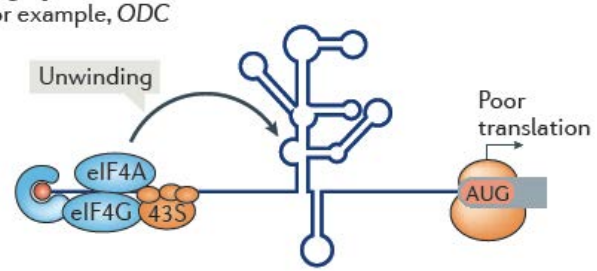
Stem–loop–RBP complex
for example, ferritin



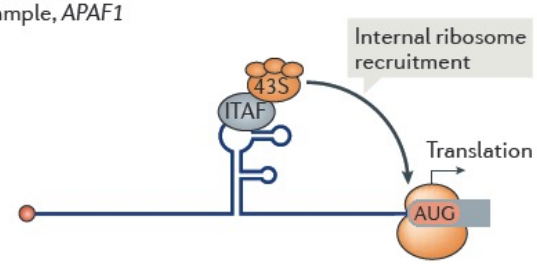
lncRNA association
for example, *Uchl1*



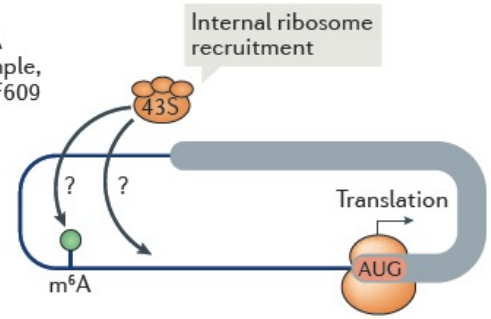
Highly structured 5′ UTR
for example, *ODC*



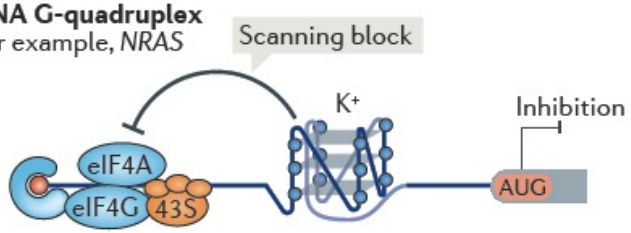
Cellular IRES
for example, *APAF1*



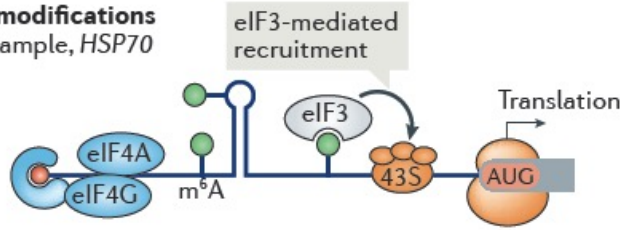
circRNA
for example, circ-ZNF609



RNA G-quadruplex
for example, *NRAS*

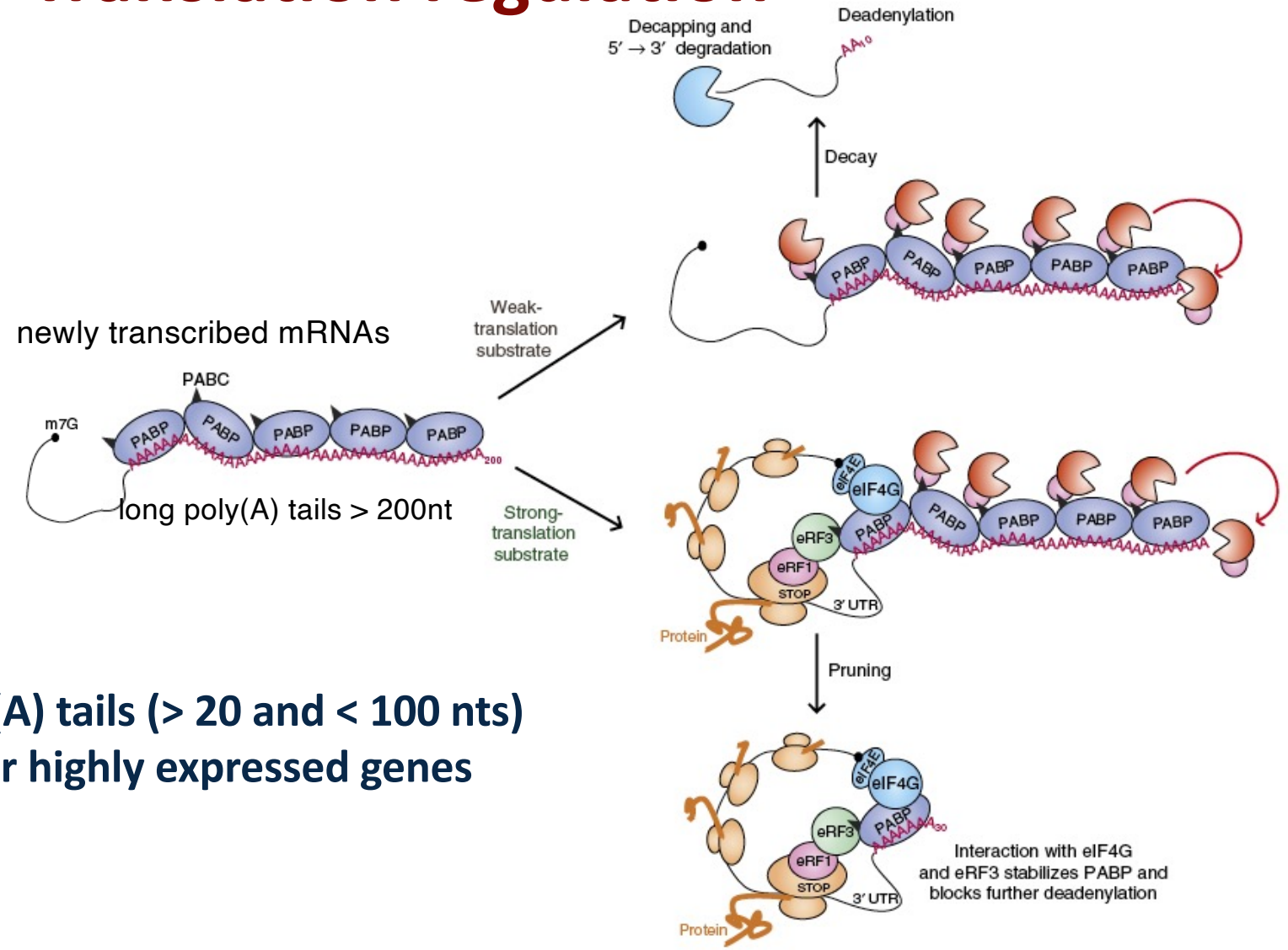


RNA modifications
for example, *HSP70*



Leppek et al.,
Nat Rev Cell Mol Biol, 2017

Translation regulation



Shorter poly(A) tails (> 20 and < 100 nts) are typical for highly expressed genes

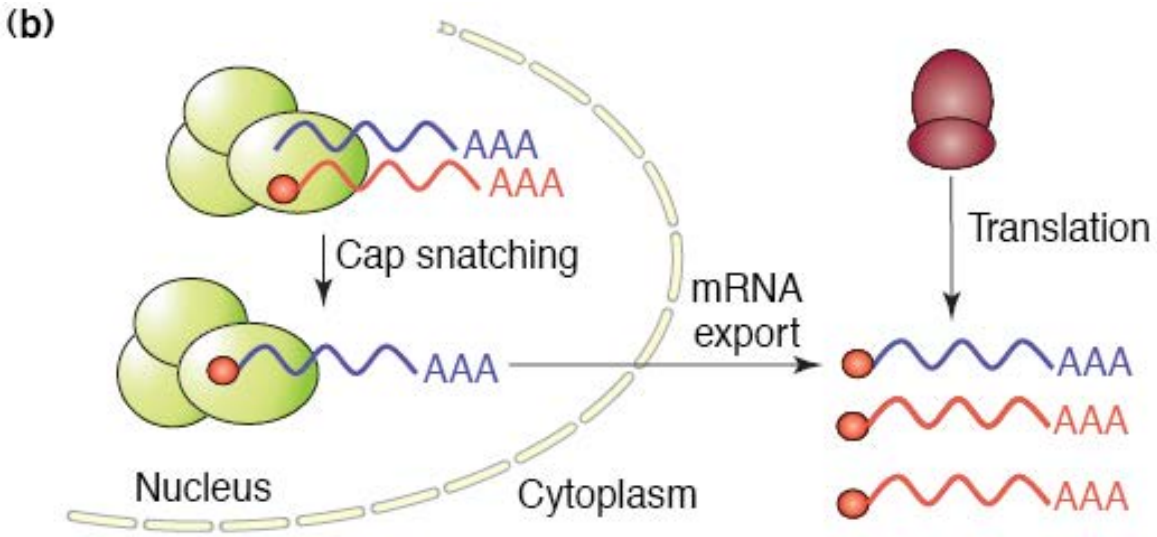
Strong translation mRNAs: PABP-eIF4G interaction stabilizes PABP binding to poly(A) allowing for poly(A) **pruning** to a defined length.
Weak translation mRNAs: not protected by translation, poly(A) tails are shortened by deadenylases recruited to PABC, which triggers **decapping** and 5'-3' **decay**

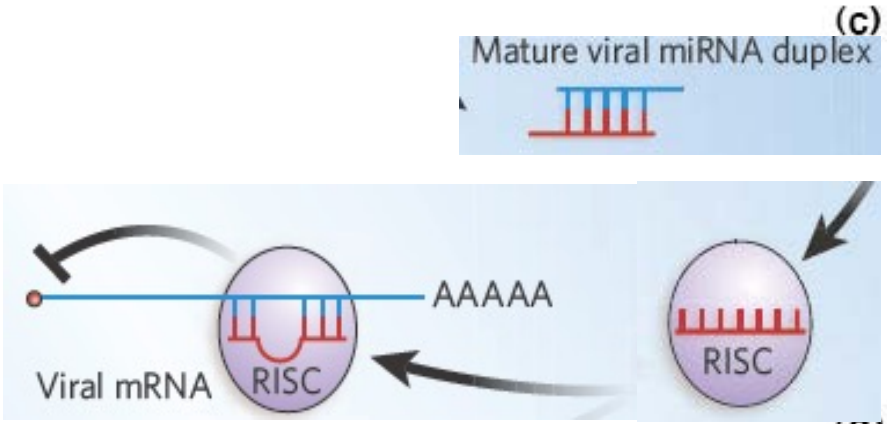
Lima et al, Nat Struct Mol Biol 2017

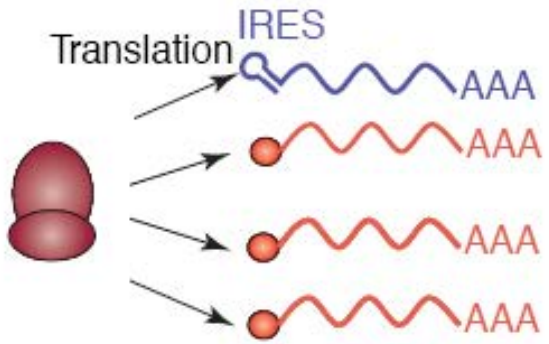
Translation regulation

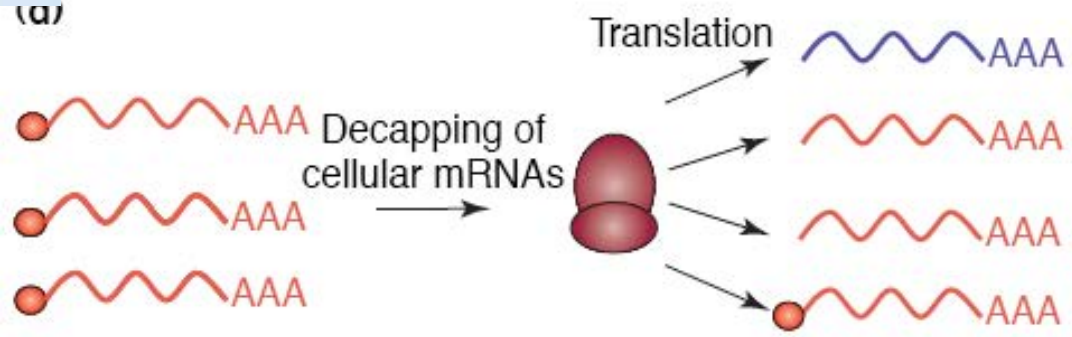
by viruses

- cap snatching
- IRES-dependent translation
- destroying cellular mRNAs
- inhibition of translation via viral miRNAs



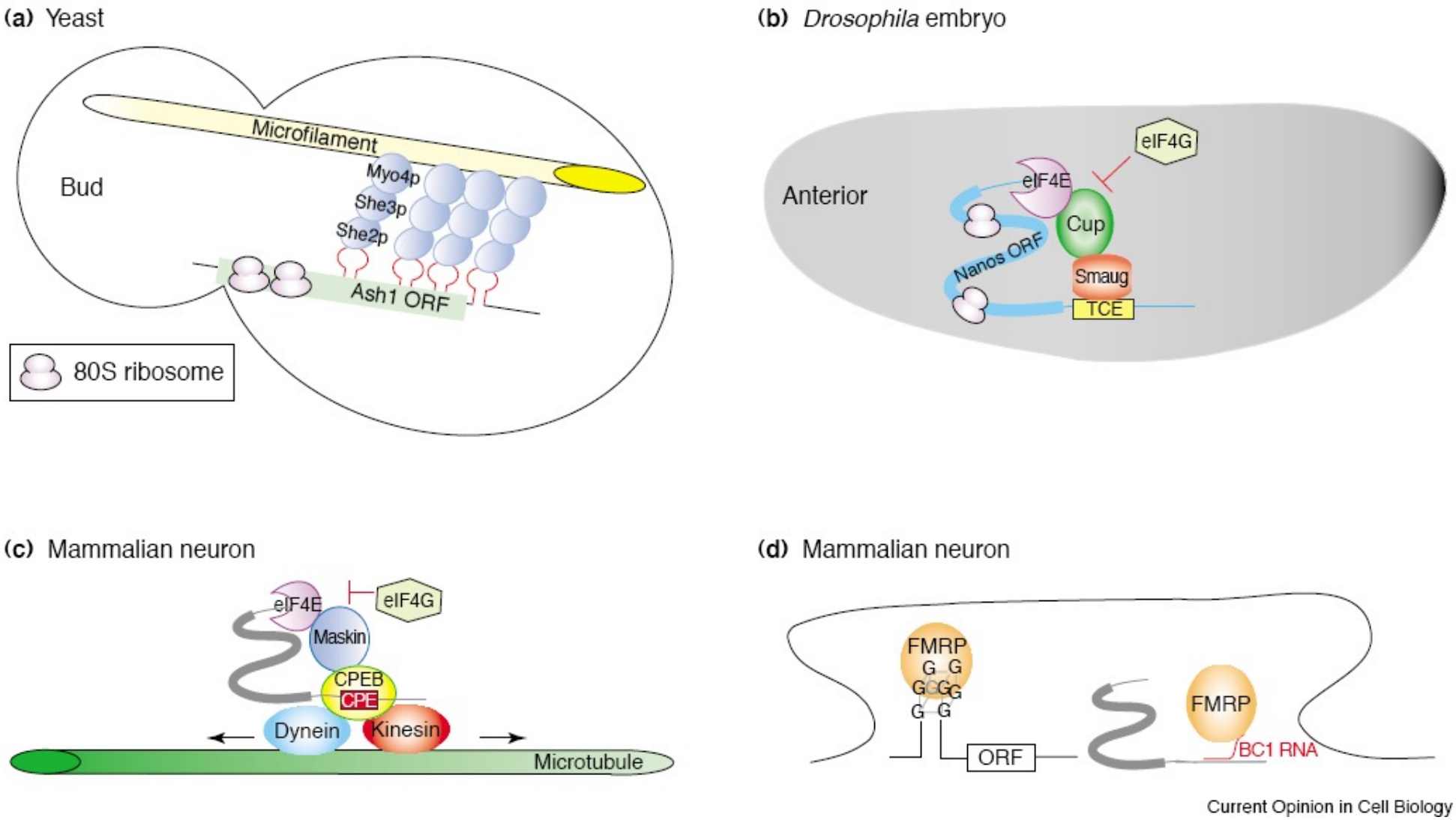






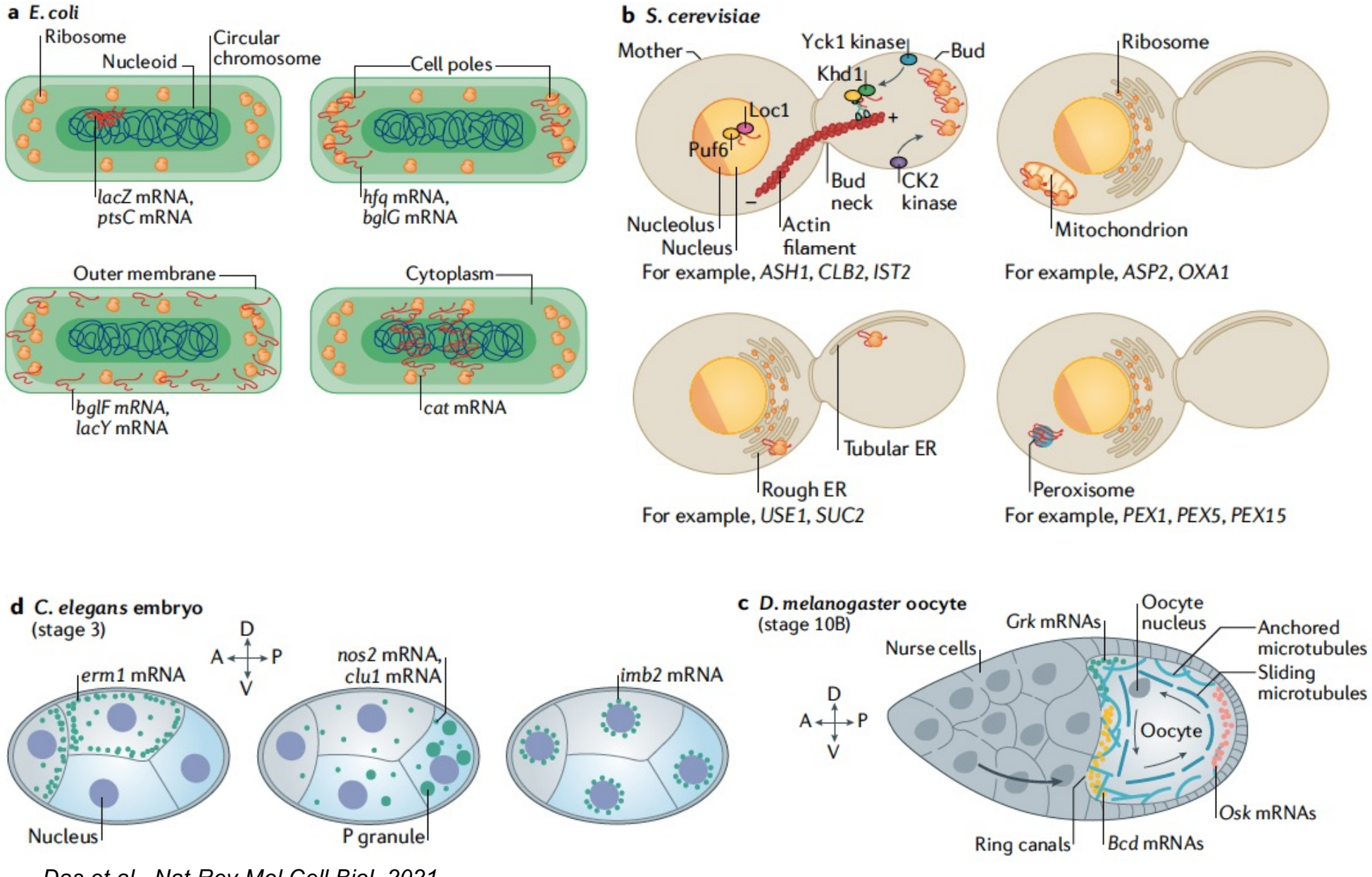
Cougot et al., TiBS, 2004;
Cullen, Nature, 2009

Localized translation



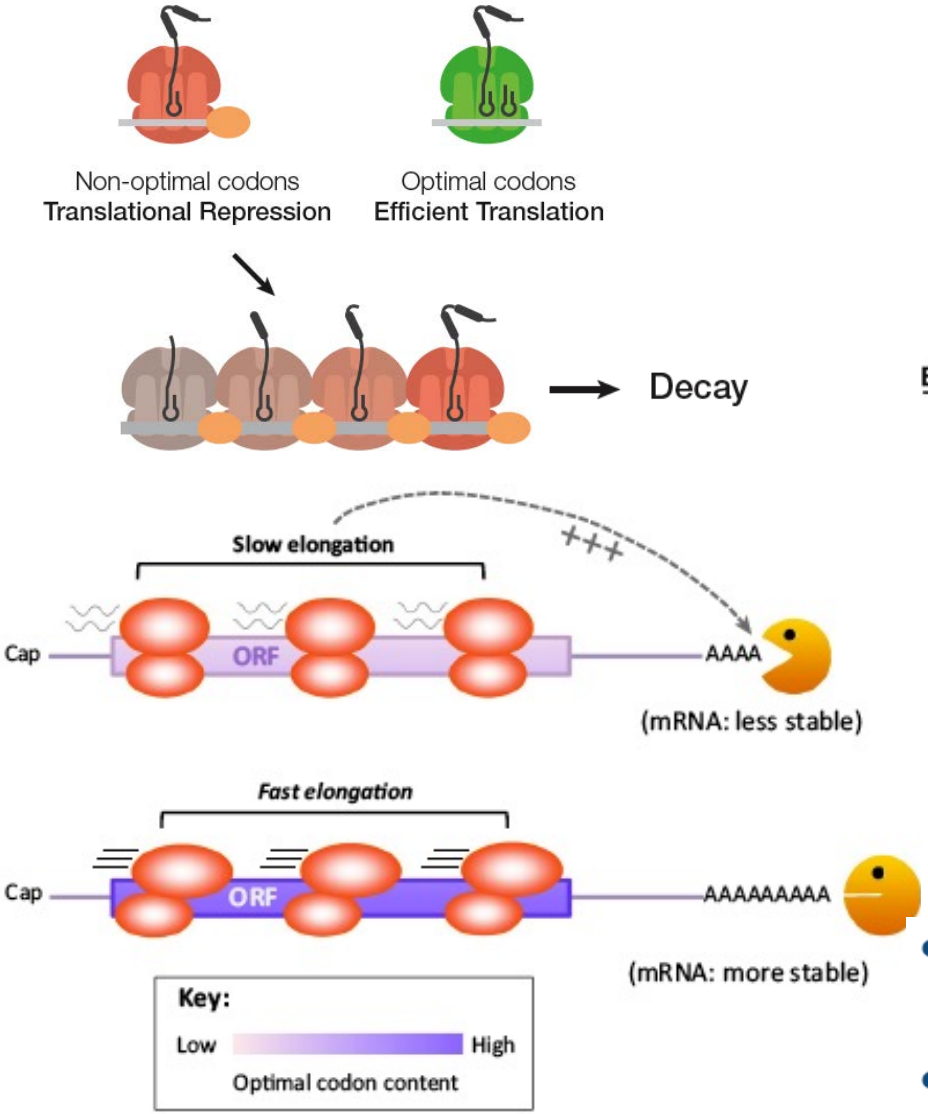
Huand and Richter, Cur. Op. Cell Biol., 2004

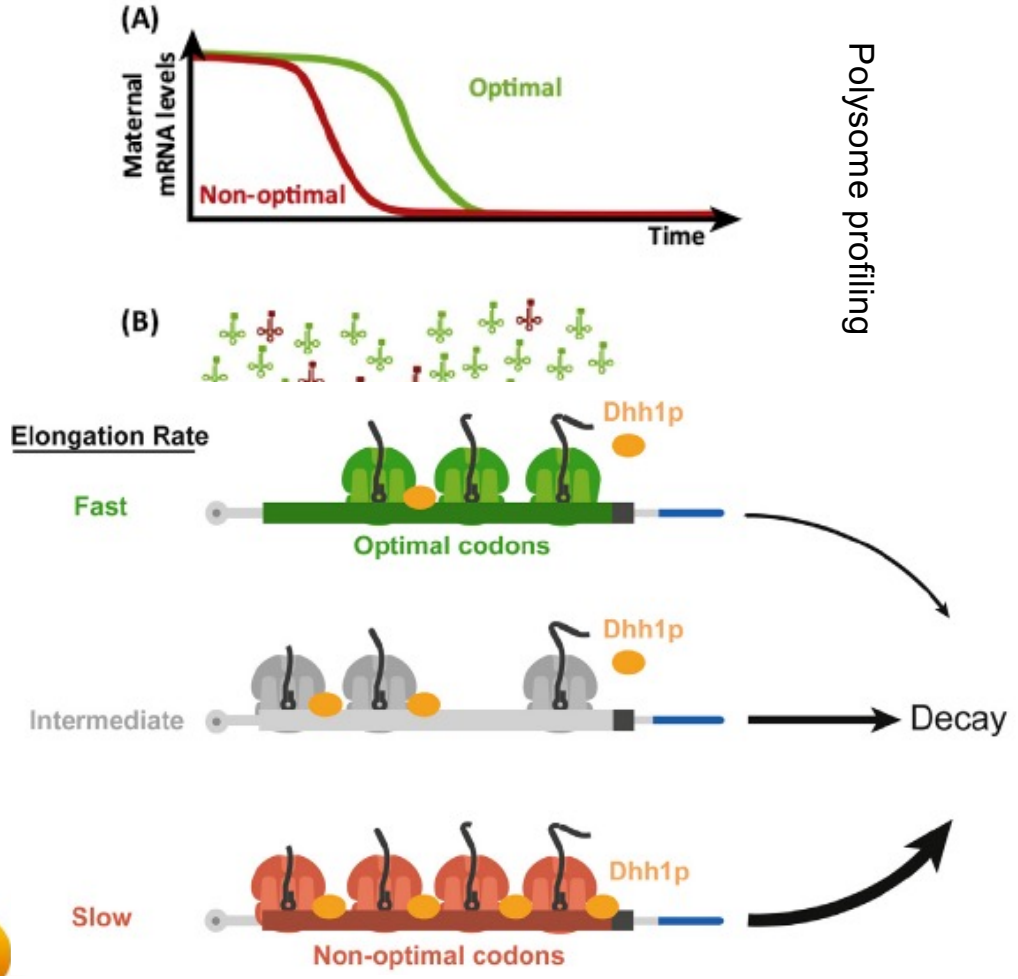
Localized translation



Das et al., Nat Rev Mol Cell Biol, 2021

Codon optimality, mRNA stability, translation

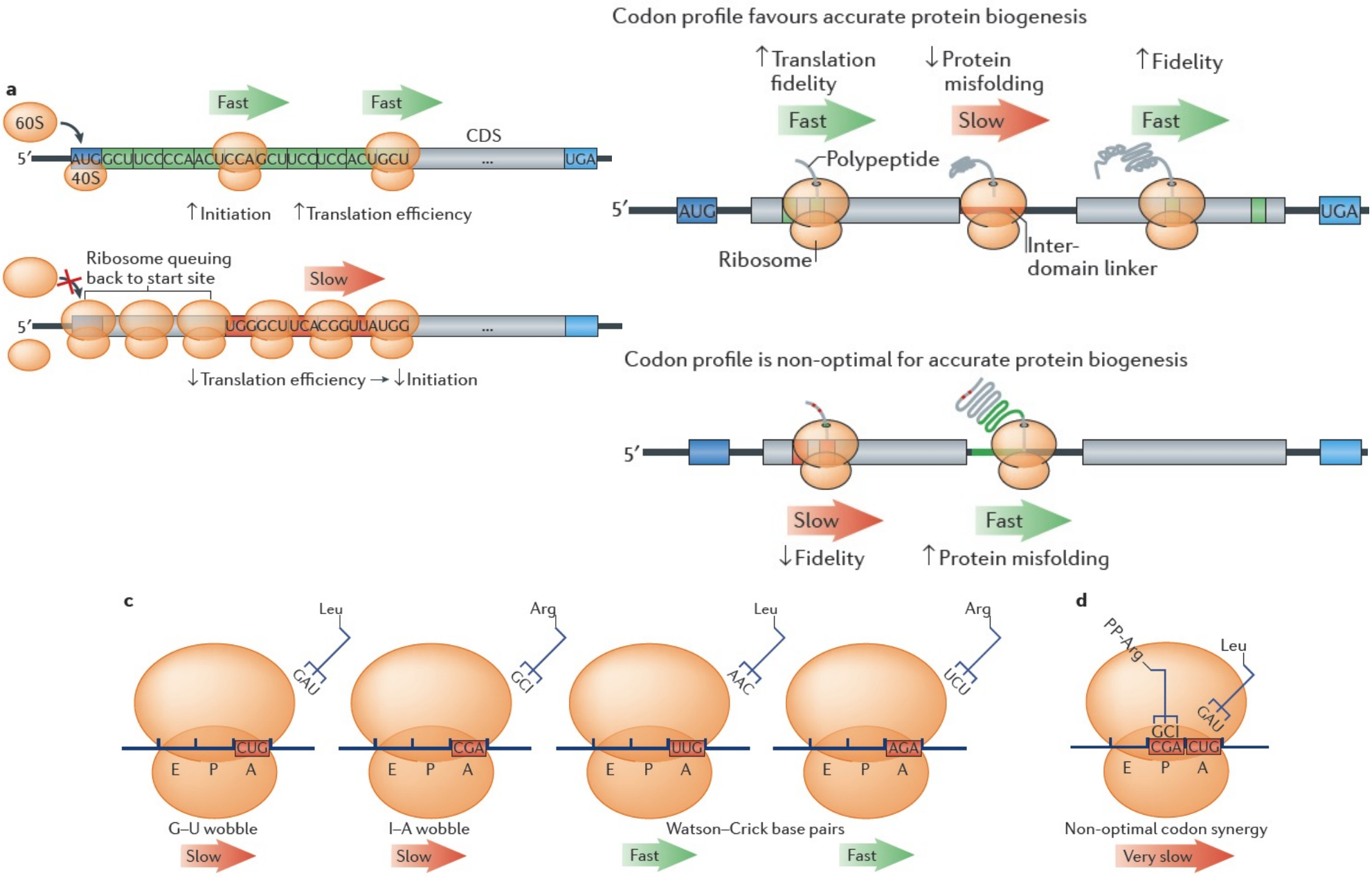




- The codon-dependent rate of translational elongation impacts mRNA stability
- The DEAD-box protein Dhh1p associates with the translating mRNP
- Dhh1p couples translation to mRNA decay by sensing codon optimality

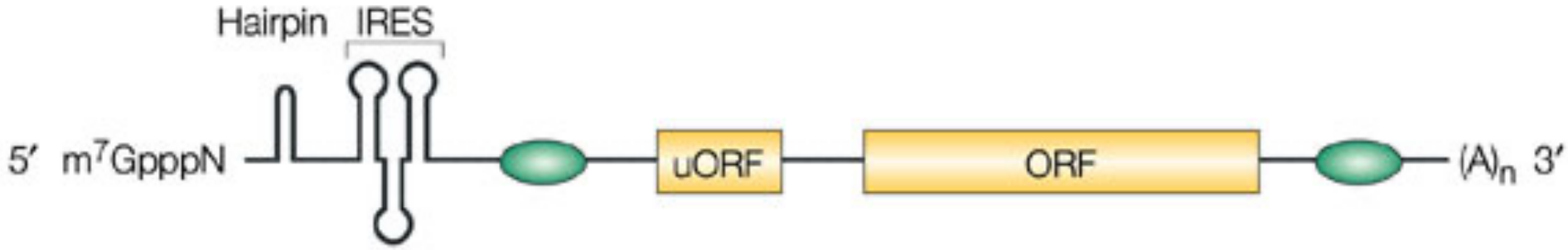
Chen and Coller, TiG, 2016; Chen and Shyu TiBS, 2016
Radhakrishnan, et al., Cell 2016

Codon optimality, mRNA stability, translation



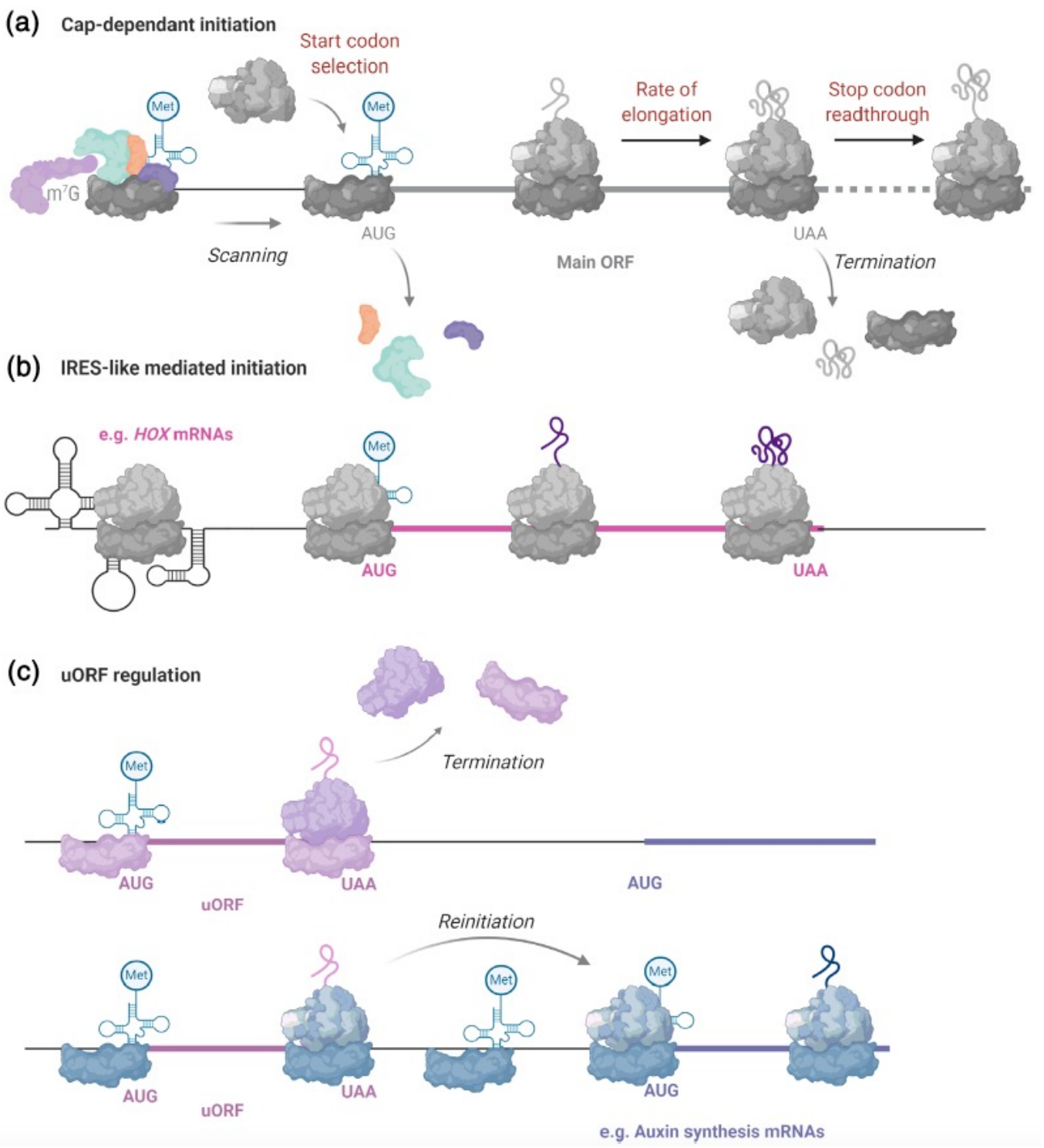
Hanson and Coller, NarRevMolCellBiol, 2017

Eukaryotic mRNA, alternative options



uORF- upstream **ORF**
- regulates the efficiency of ribosome re-initiation
- often represses expression of the main ORF
- affects mRNA stability (via NMD)
- regulates gene expression via biding of protein factors
- its translation may generate regulatory cis-acting peptide
- regulates gene expression during stress

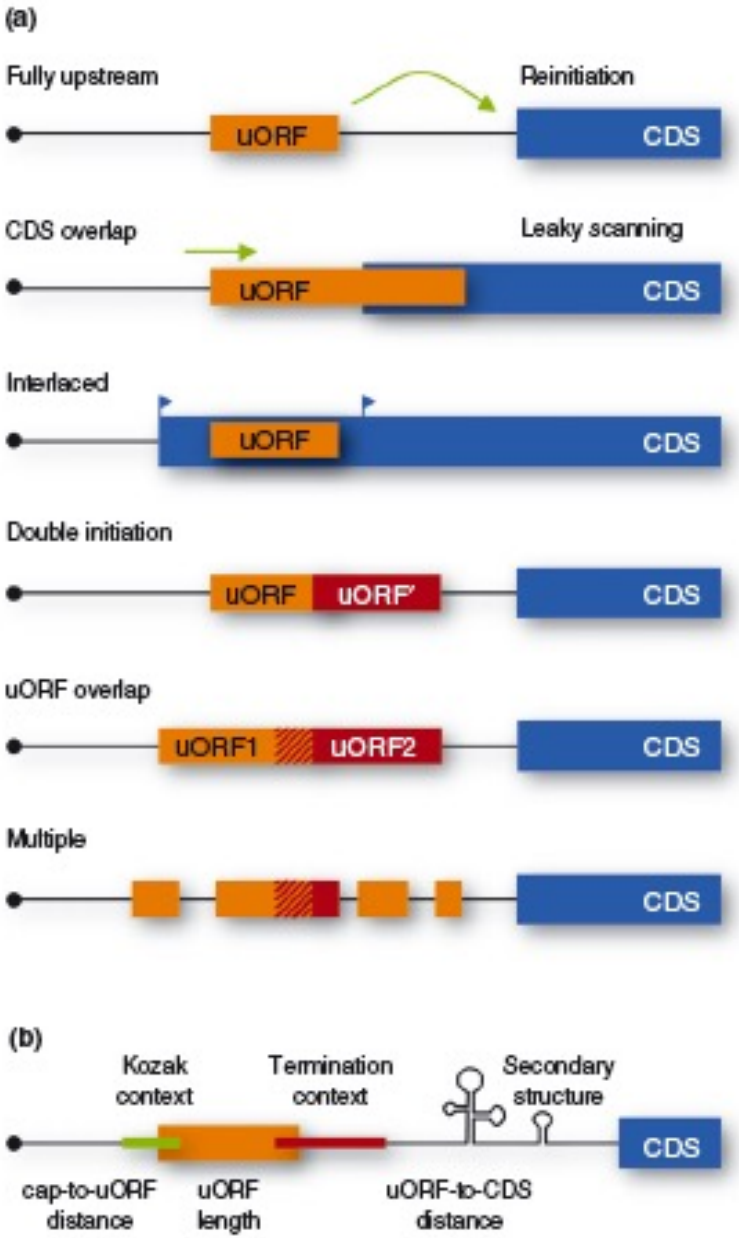
IRES – Internal **R**ibosome **E**ntry **S**ite
- a structured RNA region within 5’ UTR
- allows for **cap-independent translation** and initiation of translation inside RNA
- often used by viral mRNAs and a few cellular mRNAs (some of them can also utilize the scanning cap-dependent mechanism, this may be regulated by the intracellular concentration of eIF4G)



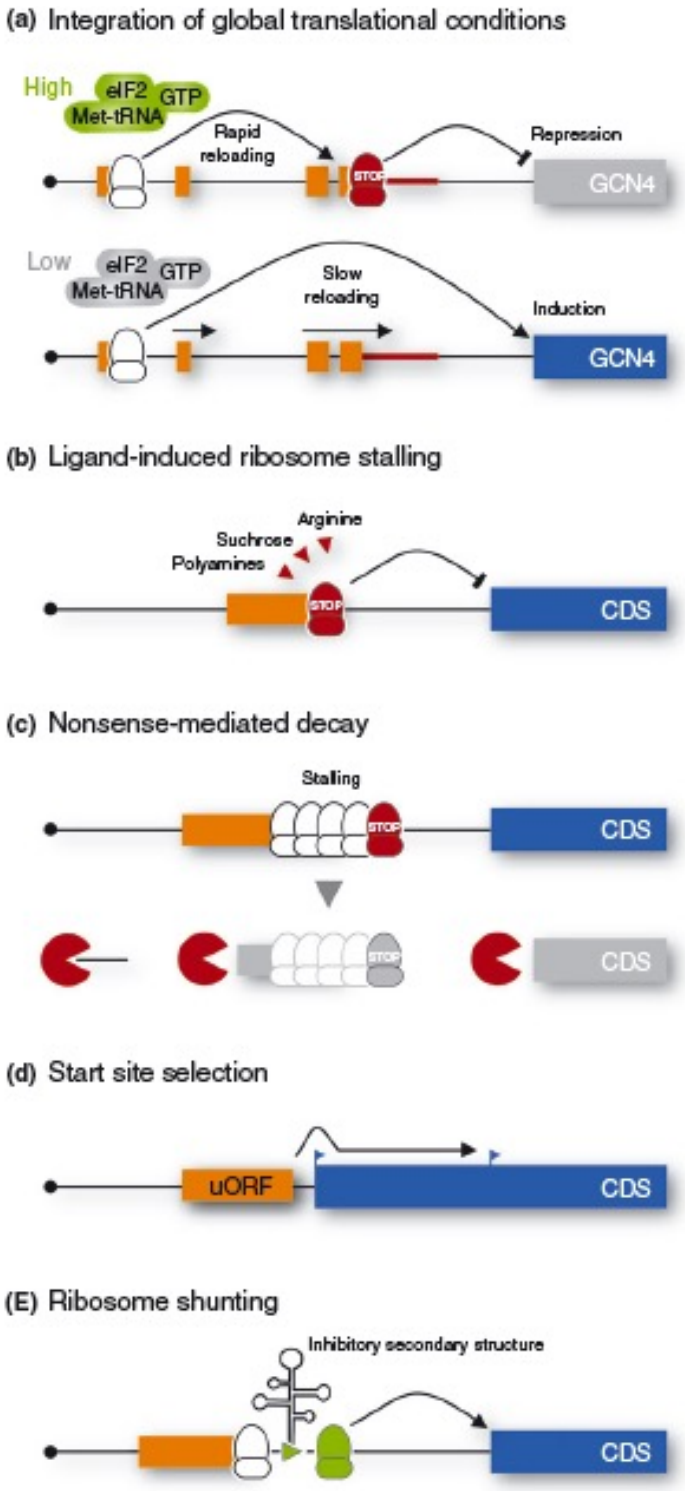
Alternative translation

Norris et al, WIREsRNA 2021

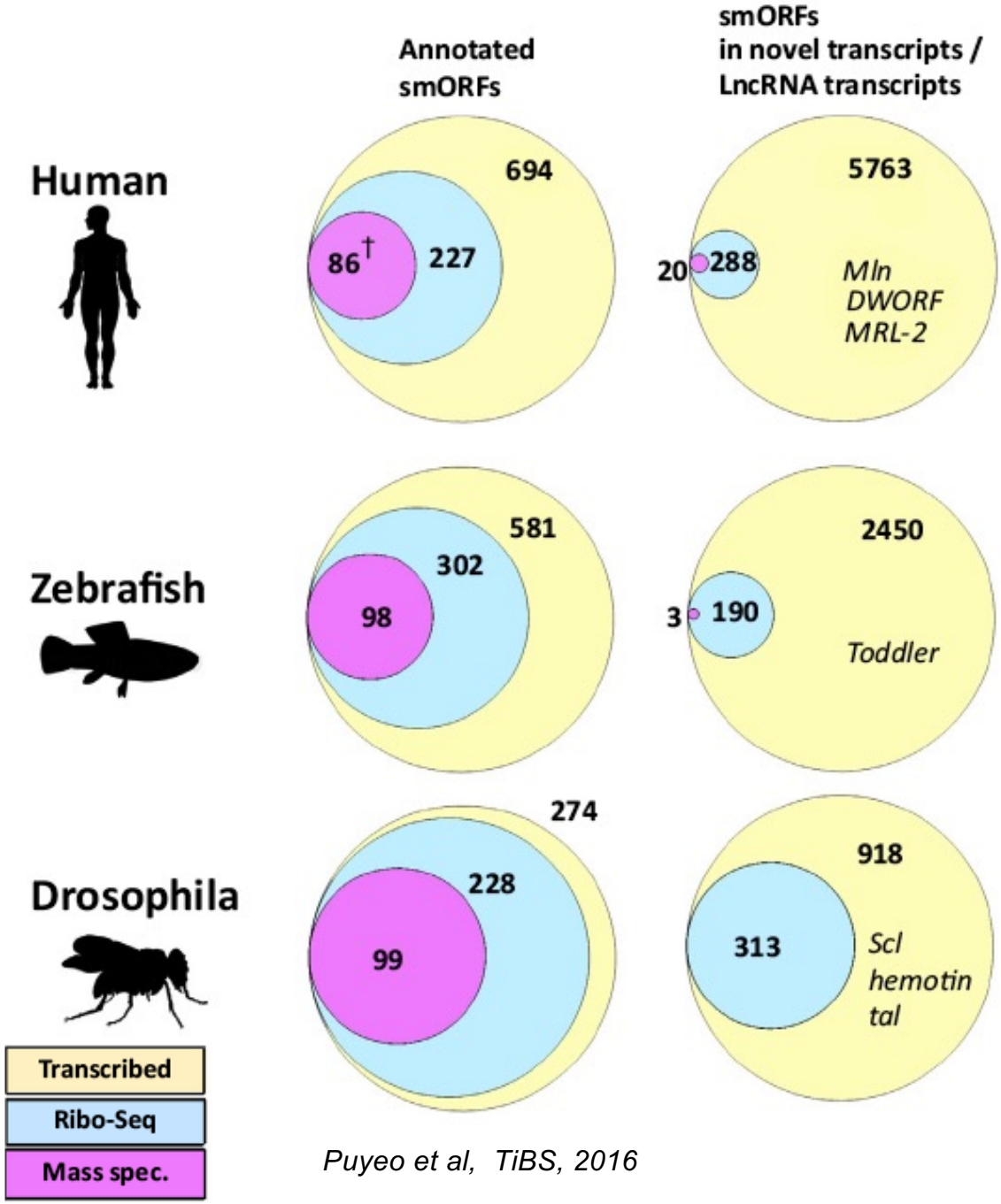
uORFs



Wethmar WIREsRNA, 2014



sORFs, sPEPs, smORFs = small ORFs

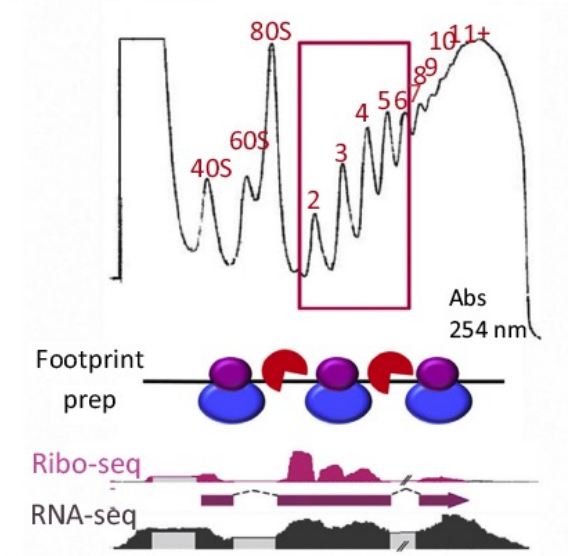


Puyeo et al, TiBS, 2016

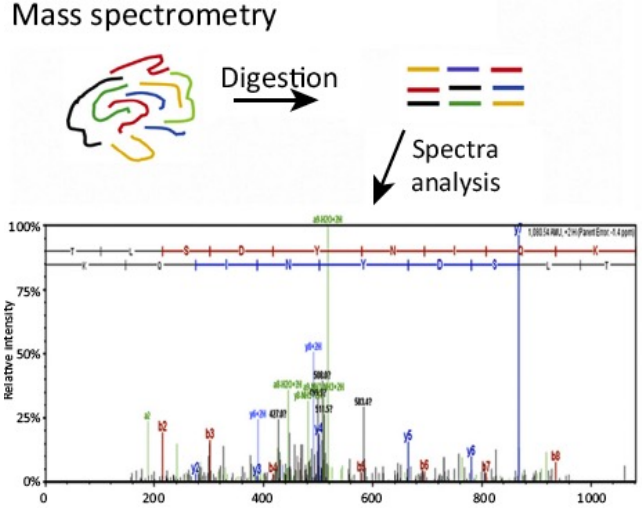
Bioinformatics approaches
Sequence composition analysis
Conservation

Biochemical approaches

Ribosome profiling



Mass spectrometry



sORFs

sPEPs

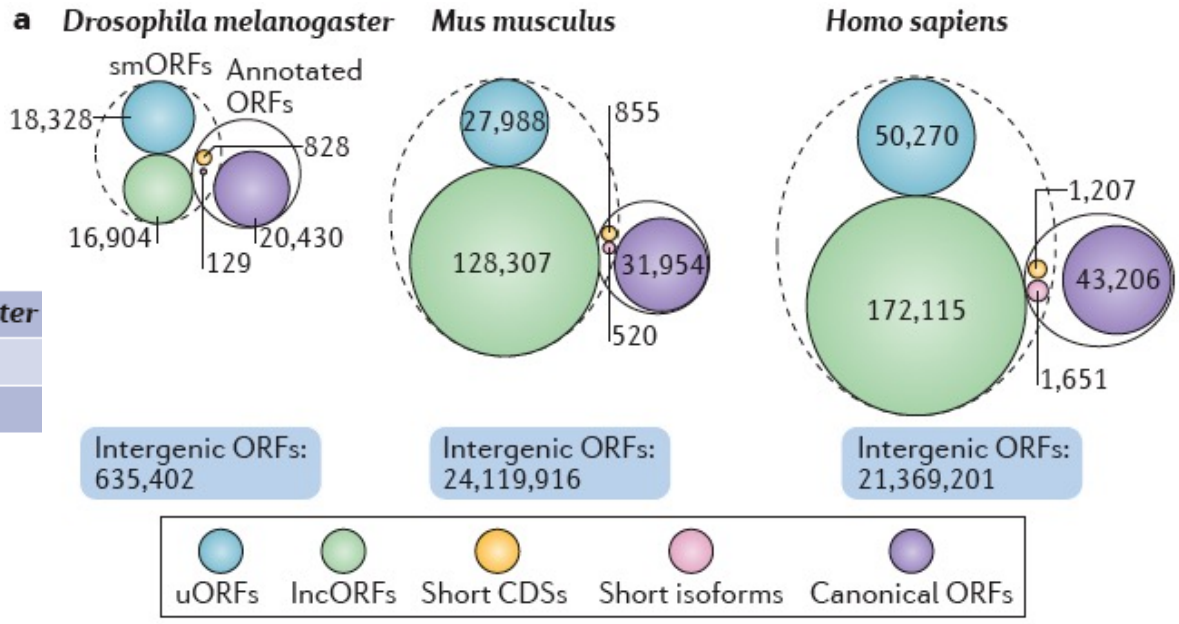
ORF class	RNA type	Median size (codons)	Translation[15]	Conservation	Coding features	Function
Intergenic ORFs	None	22	None	None[6,8]	Non-canonical AA	None
uORFs	5′ UTRs of mRNAs	22	Low	None[8,30]	• Nonrandom AA • No domains	• Non-coding • Translation regulation
lncORFs	lncRNAs	24	Low	None[8,10]	• Nonrandom AA • No domains	Non-coding or coding
Short CDSs	Short mRNAs	79	High	Class	• Positively charged AA • Transmembrane α-helices	• Coding • Regulators of canonical proteins
Short isoforms	Spliced mRNAs	79	High	Kingdom	• Canonical AA • Protein domain loss	• Coding • Small interfering peptides
Canonical ORFs	mRNAs	491	High	Kingdom	• Canonical AA • Multiple protein domains	• Coding[42] • Structural, enzymatic, regulatory

Transcribed smORFs

Untranslated region · ORFs · DNA · Other coding sequences · RNA splicing · Ribosome profiling signal

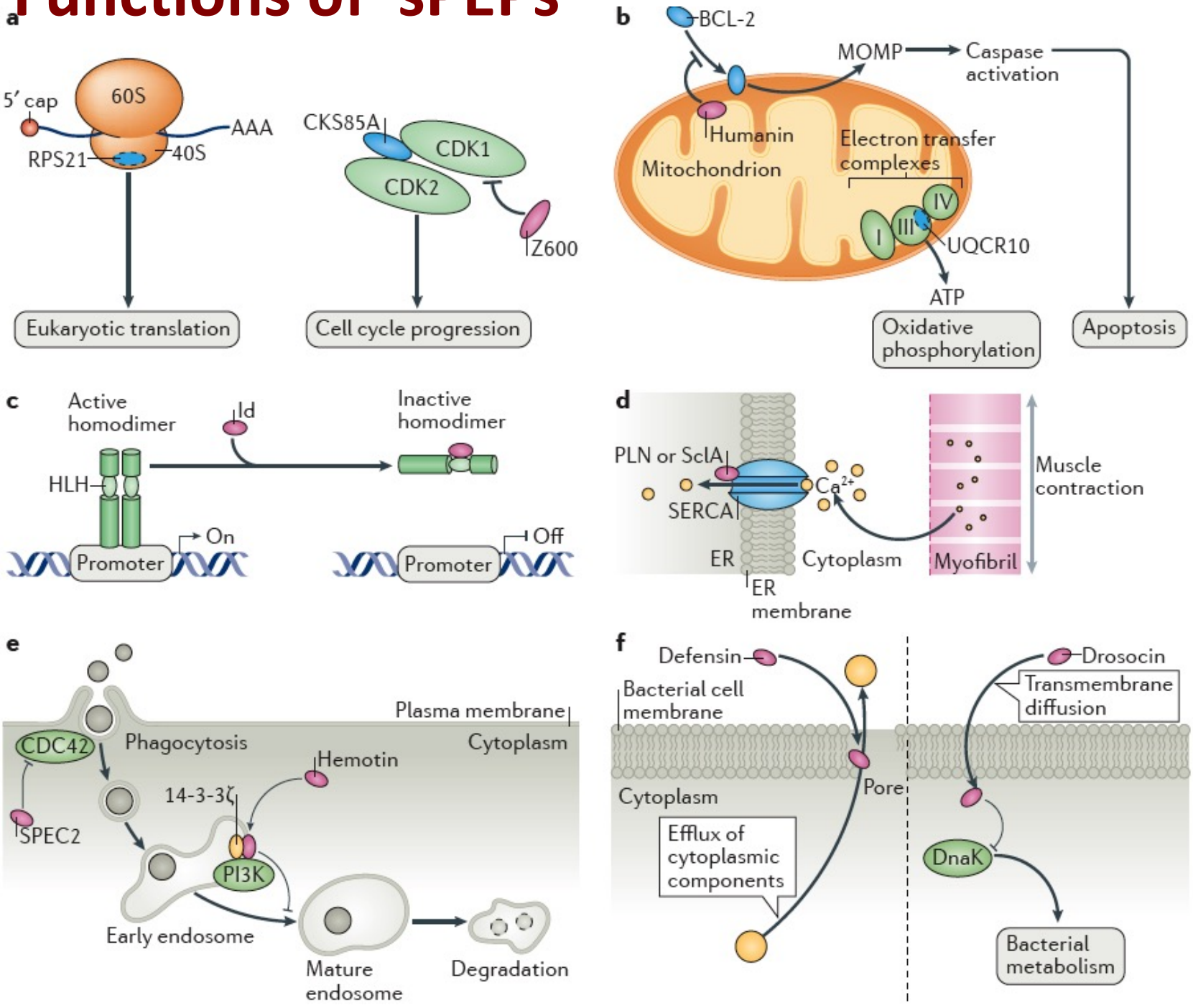
Median length (codons)

uORFs	lncORFs	Short CDSs	Canonical ORFs	
20	25	79	490	*D. melanogaster*
22	23	81	424	*M. musculus*
23	24	78	421	*H. sapiens*



Couso and Patraquim
Nat Rev Mol Cell Biol, 2017

Functions of sPEPs



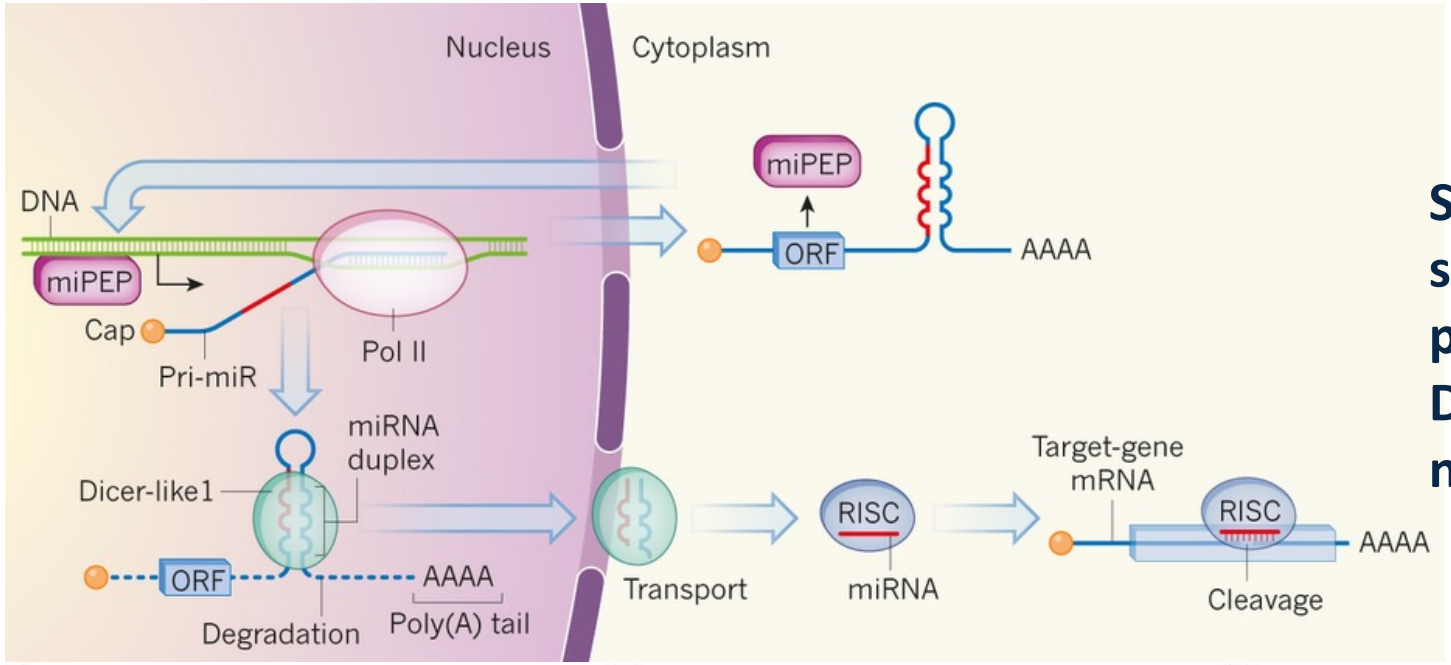
Couso and Patraquim
Nat Rev Mol Cell Biol, 2017

Functional sPEPs

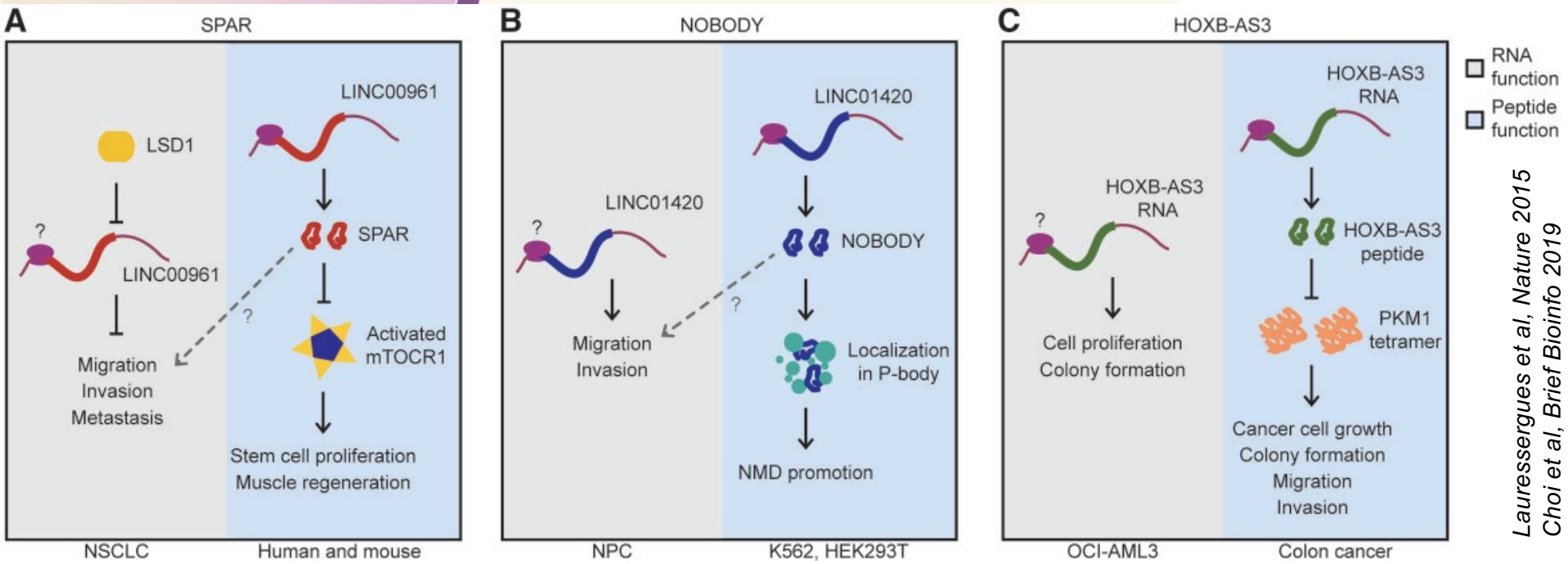
Species	Genes or transcripts	Number of residues in sPEP	Notes
Upstream sPEPs			
Arabidopsis thaliana	*GBF6*	28	Expression of the CDS is modulated by sucrose levels through a conserved sPEP
	SAMDC	52	Expression of the CDS is regulated by polyamines binding to the nascent upstream sPEP; orthologous to human *SAMDC*
	XPL1	26	Expression of the CDS is regulated by phosphocholine binding to the sPEP
Saccharomyces cerevisiae	*CPA1*	25	The sPEP reduces expression of the CDS through ribosomal stalling and blocking translation in response to increased arginine levels
Humans	*ASS1*	44	The sPEP regulates expression of *ASS1* in a *trans*-suppressive manner
	EPHX1	17 and 26	Expression of *EPHX1* is inhibited by *trans*-acting sPEPs that are encoded by two uORFs through interactions with the translation machinery
	HR	34	The sPEP is implicated in the regulation of *HR*; 13 causative mutations of Marie Unna hereditary hypotrichosis have been identified within the second uORF
	MKKS	63 and 50	Both sPEPs localize to the mitochondrial membrane and are predicted to function independently of MKKS
	NR3C1	93	The sPEP localizes to the cell membrane and regulates expression of the glucocorticoid receptor in a *trans*-acting manner through interaction with unknown cellular factors
	SAMDC	6	Expression of the CDS is regulated by polyamines binding to the nascent upstream sPEP; orthologous to *A. thaliana SAMDC*
Intergenic sPEPs			
A. thaliana	*PLS*	36	The sPEP is required for correct auxin–cytokinin homeostasis to modulate root growth and leaf vascular patterning
	ROT4	53	The sPEP is involved in regulation of leaf shape by reducing cell proliferation in lateral organs
Drosophila melanogaster	*Ilp8*	150	The sPEP provides a signal that promotes the delay of metamorphosis in response to conditions that that alter growth in imaginal discs
	HSPC300	75	The sPEP is a component of the WAVE–SCAR complex and is important in nervous system development for axonogenesis and neuromuscular synapse morphogenesis; *HSPC300* is orthologous to *brk1*
	pgc	71	The sPEP is essential for repressing Ser2 phosphorylation in the carboxy-terminal domain of RNA polymerase II in newly formed pole cells (which are the early germline progenitors) and thus has a fundamental role in germ-cell specification
	tal	11 and 32	The sORFs encode three peptides of 11 residues and one peptide of 32 residues that are essential for embryonic development and that are required for formation of epithelial architecture; *tal* is orthologous to *Mlpt*
	RanGAP	28 and 29	Both sPEPs are involved in the regulation of Ca^{2+} trafficking; alterations result in irregular muscle contractions
Overlapping sPEPs			
Humans	*TYRP1*	24	The sPEP is co-expressed from the *TYRP1* transcript
	CASP1	151	The sPEP is expressed from the intestinal carboxyl esterase gene and is recognized by human leukocyte antigen-B7-restricted renal cell carcinoma-reactive T cell clone
	AltPrP	73	The sPEP is co-expressed from the prion protein transcript in brain homogenates, primary neurons and peripheral blood mononuclear cells; it localizes to the mitochondria
	AltATXN1	185	The sPEP is co-expressed from the *ATXN1* transcript and is expressed in the cerebellum; it colocalizes and interacts with the ATXN1 protein in the nucleus
	AltMRVI1	134	The sPEP colocalizes to the nucleus and interacts with BRCA1

Andrews and Rothnagel, Nat Rev Genet, 2014

sPEPs and lncRNAs

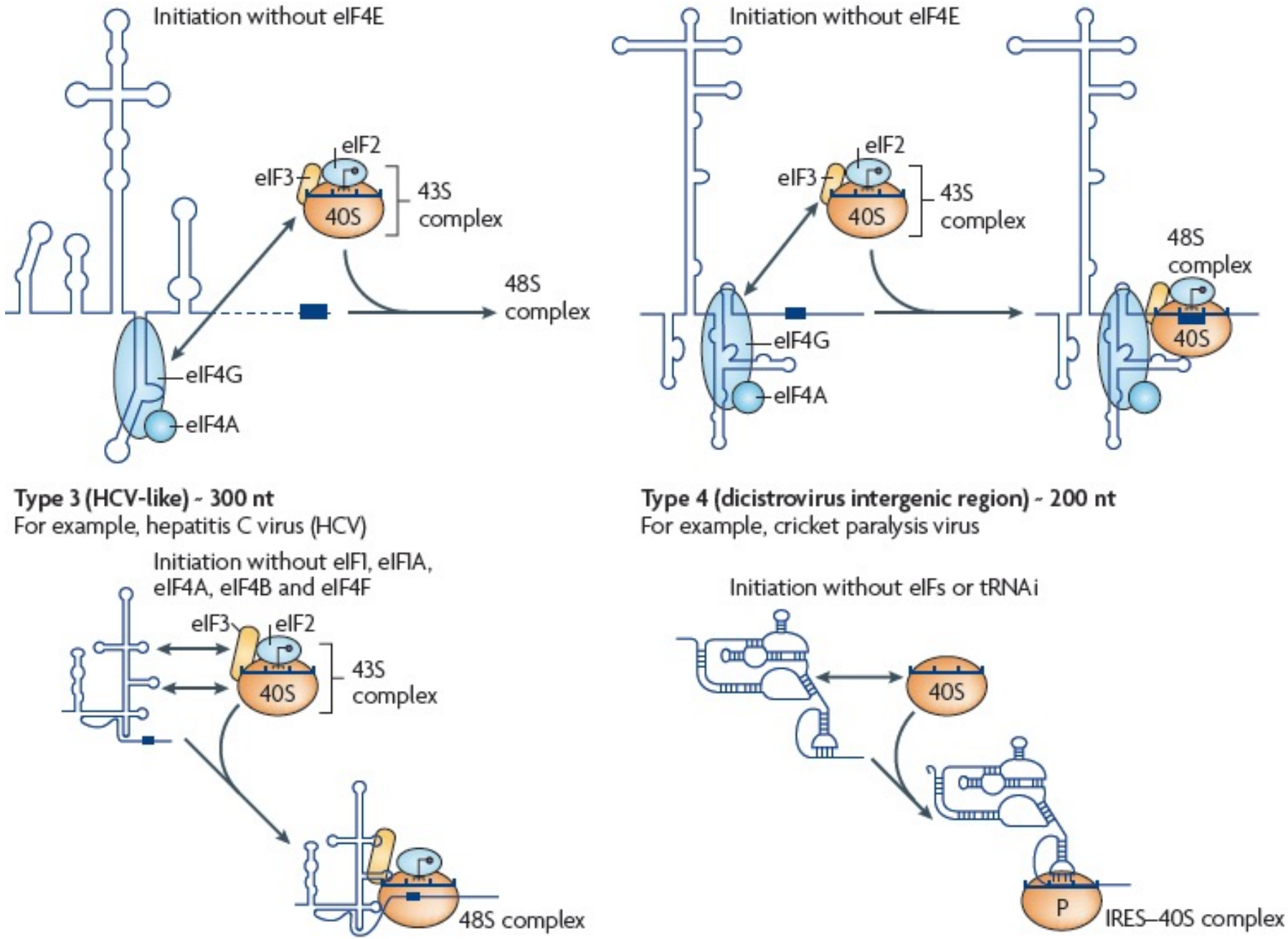


Some ncRNA code for sPEP with a functional potential.
Dual function as a ncRNA and a peptide.



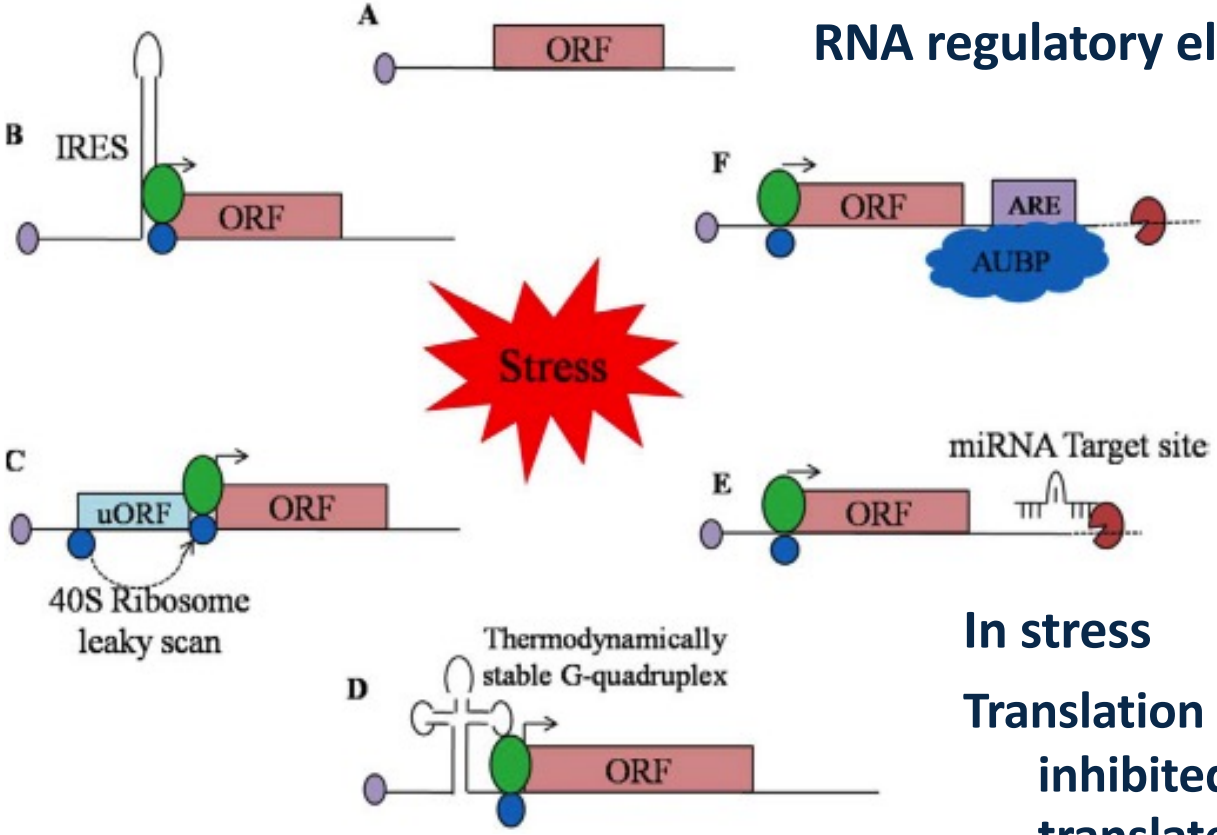
Lauressergues et al, Nature 2015
Choi et al, Brief Bioinfo 2019

IRES



Jacson et al., Nat. Rev. Mol. Cel. Biol., 2010

IRES, uORFs, UTRs in stress response



RNA regulatory elements in UTRs in stress

In stress

Translation of house-keeping proteins is inhibited but selected proteins are translated more efficiently via

IRES - cap-independent translation

uORFs - 40S leaky scanning initiation

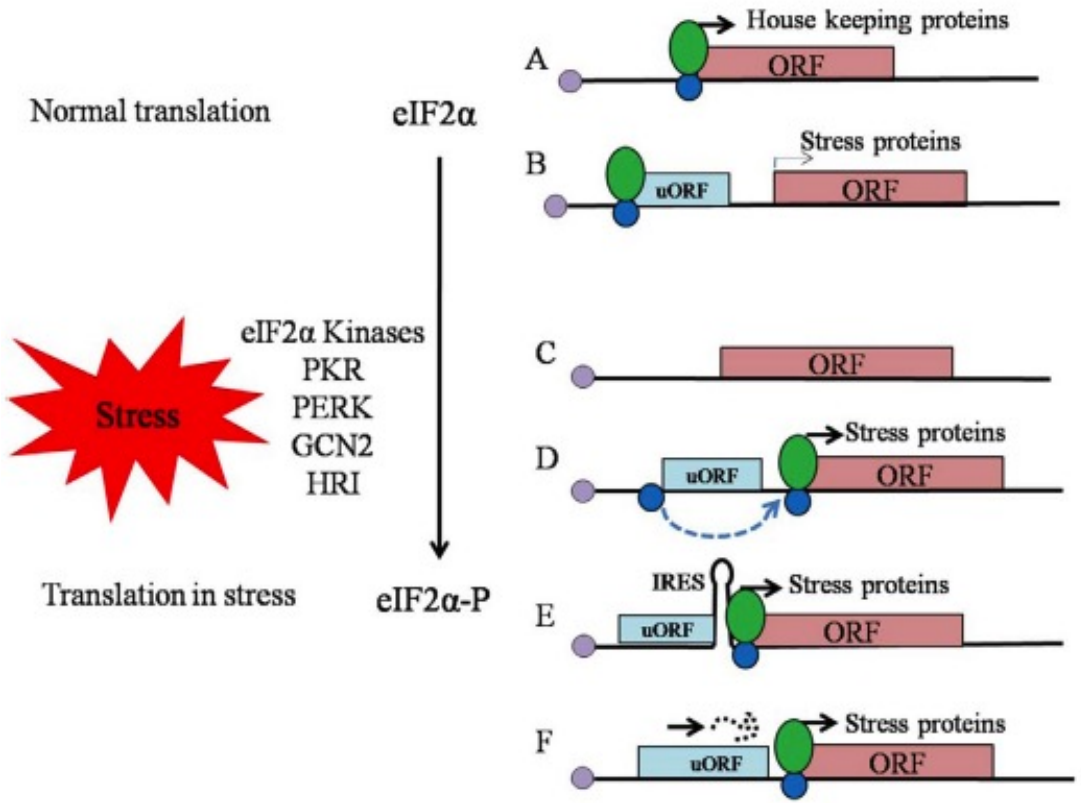
stable RNA structures

miRNAs

AUBPs (AU-rich BP)

Sajjanar et al, J Termal Biol, 2017

IRES, uORFs, UTRs in stress response

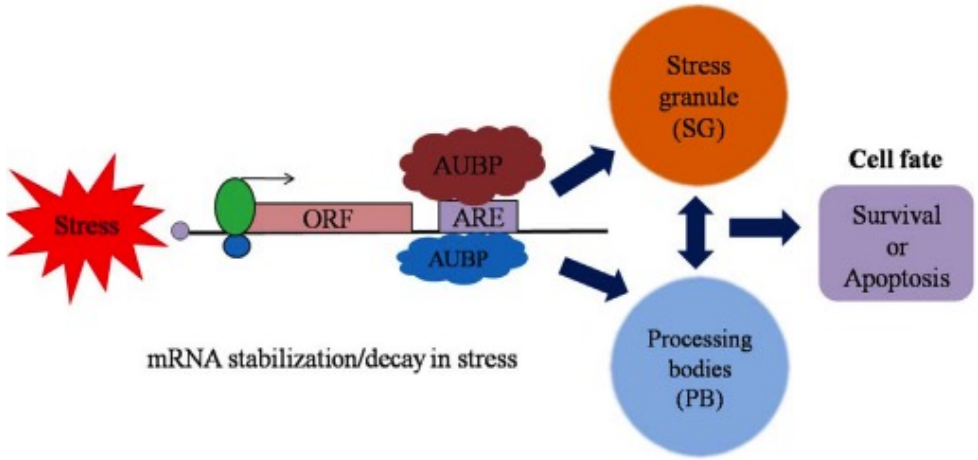


A. Cap-dependent translation of the main ORF, normal conditions (eIF2α)

B-F. Or under stress (eIF2α-P)

B. uORF reduces translation of the main ORF under stress, but...

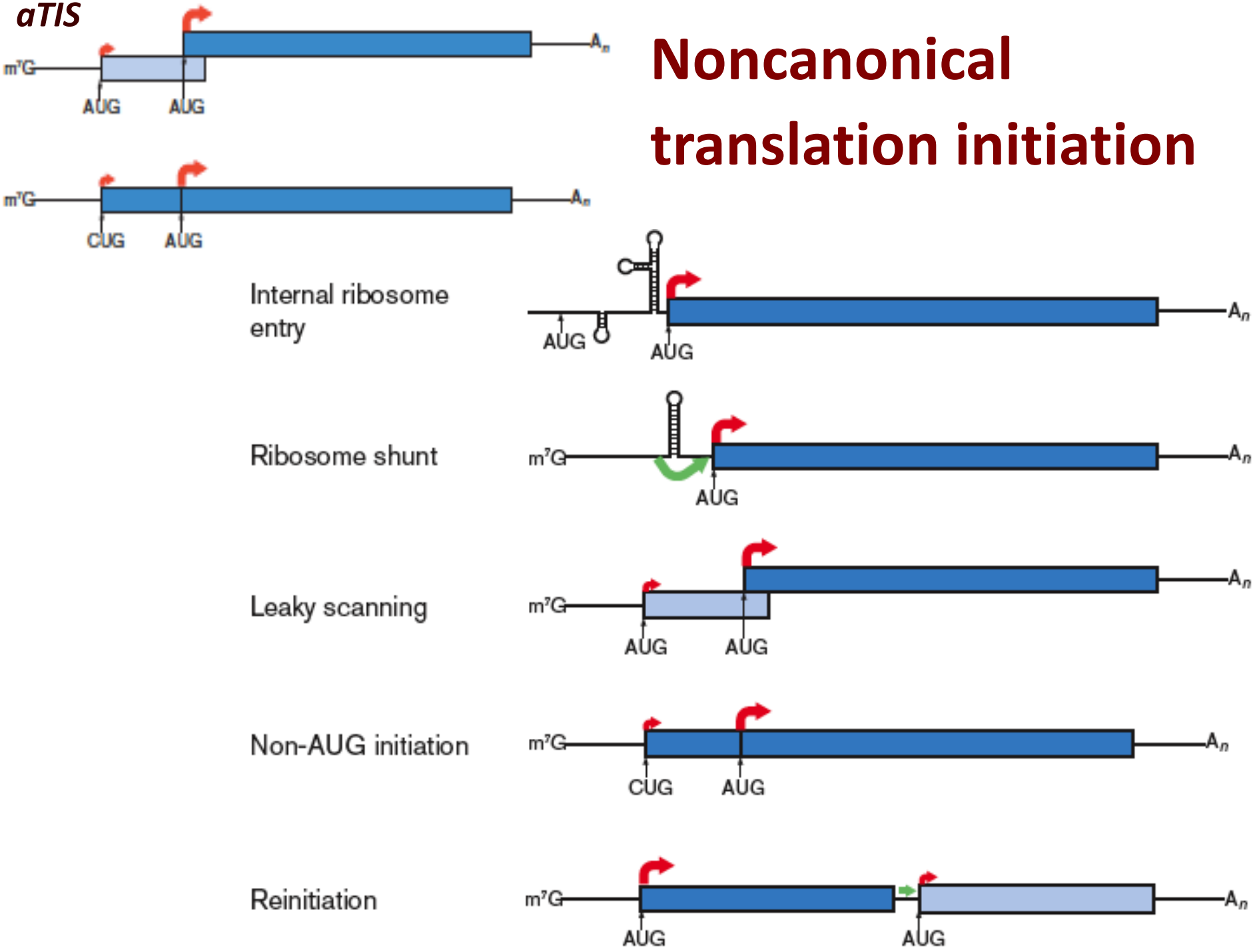
D-F. Translation of the main ORF in stress can be also stimulated by uORF by re-initiation (d), IRES (e) or a peptide encoded by uORF (f)



mRNA stability can be regulated in stress by AUBPs (AU-rich BP)

Sajjanar et al, J Termal Biol, 2017

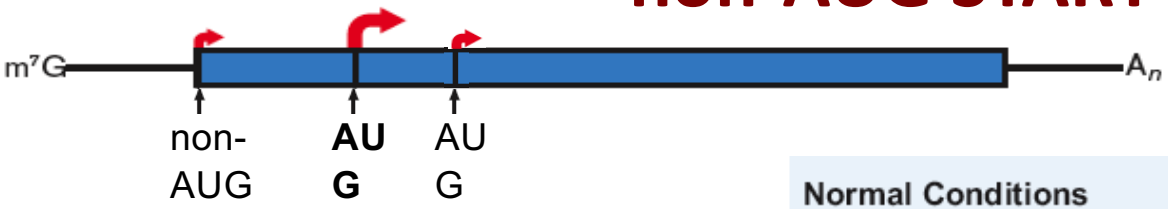
Noncanonical translation initiation



Firth and Brierley, J. Gen. Vir., 2012

Noncanonical translation initiation

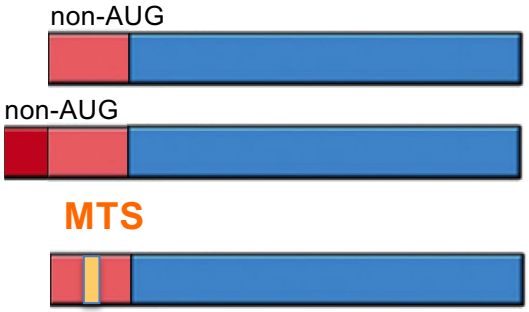
non-AUG START codon



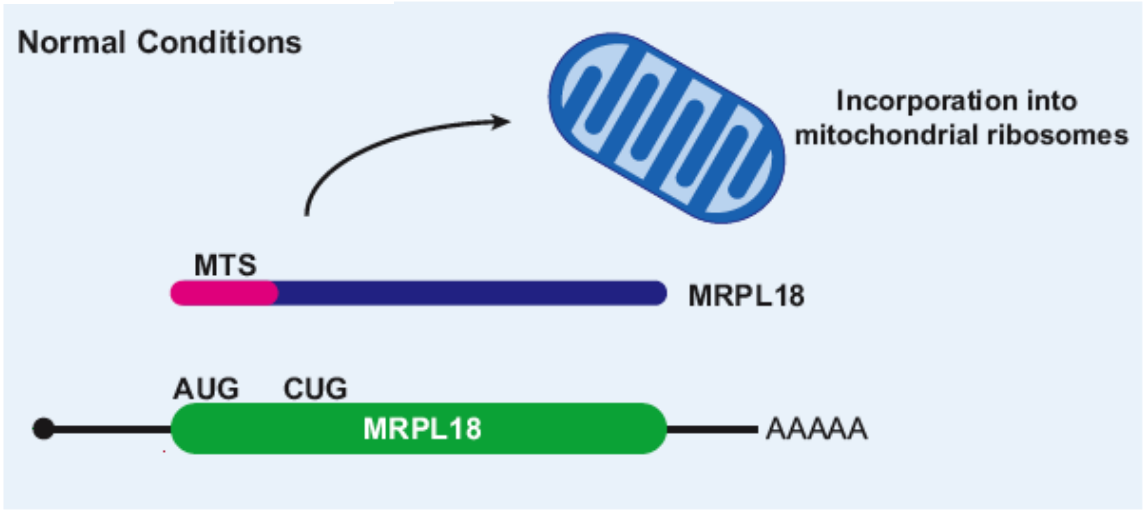
Protein isoforms

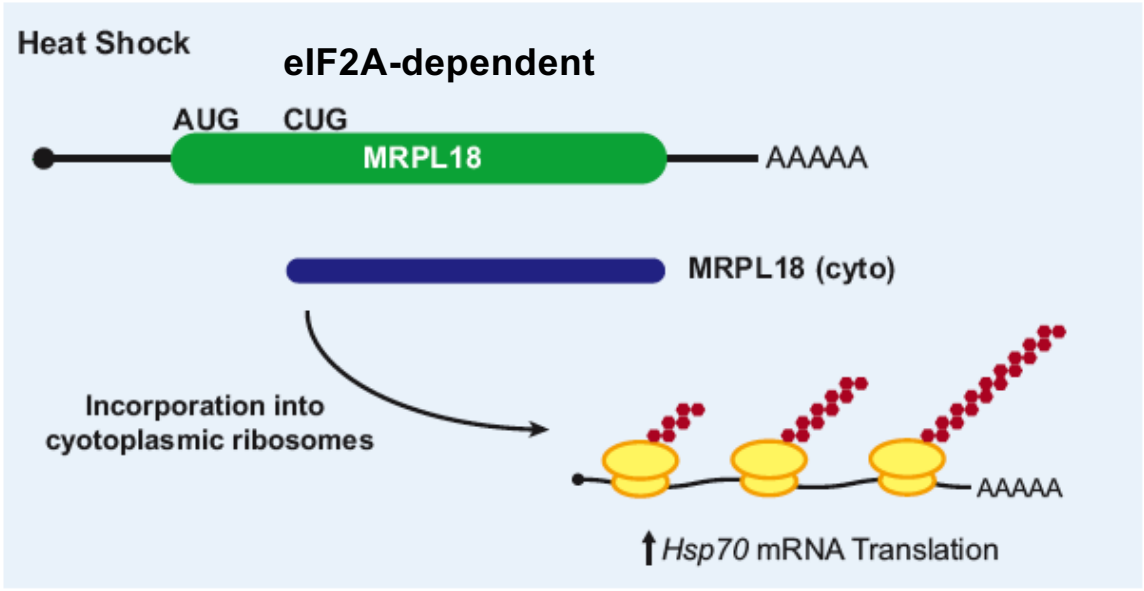
canonical

N-terminally extended



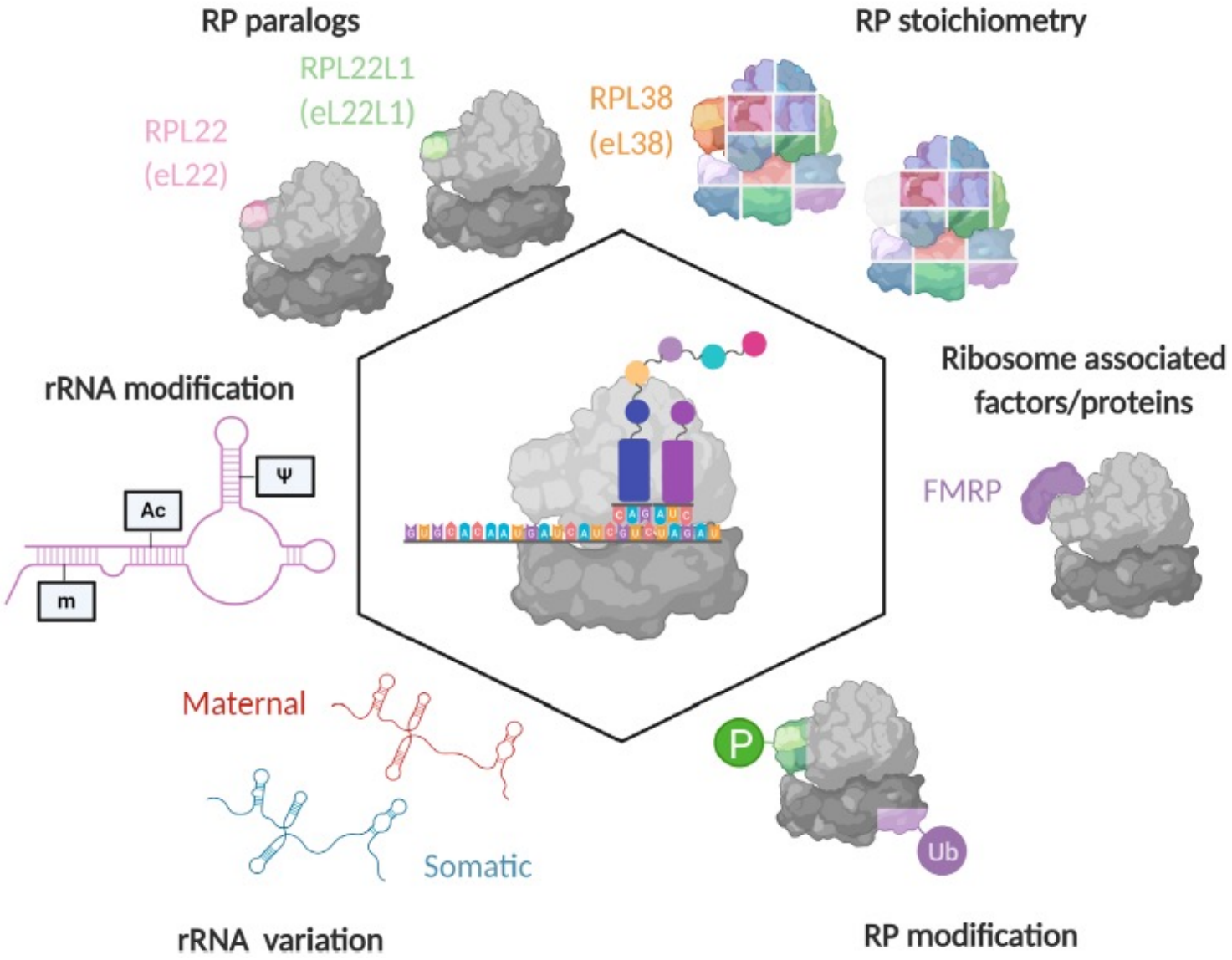
N-terminally truncated





Kearse and Wilusz, Gene Dev, 2017

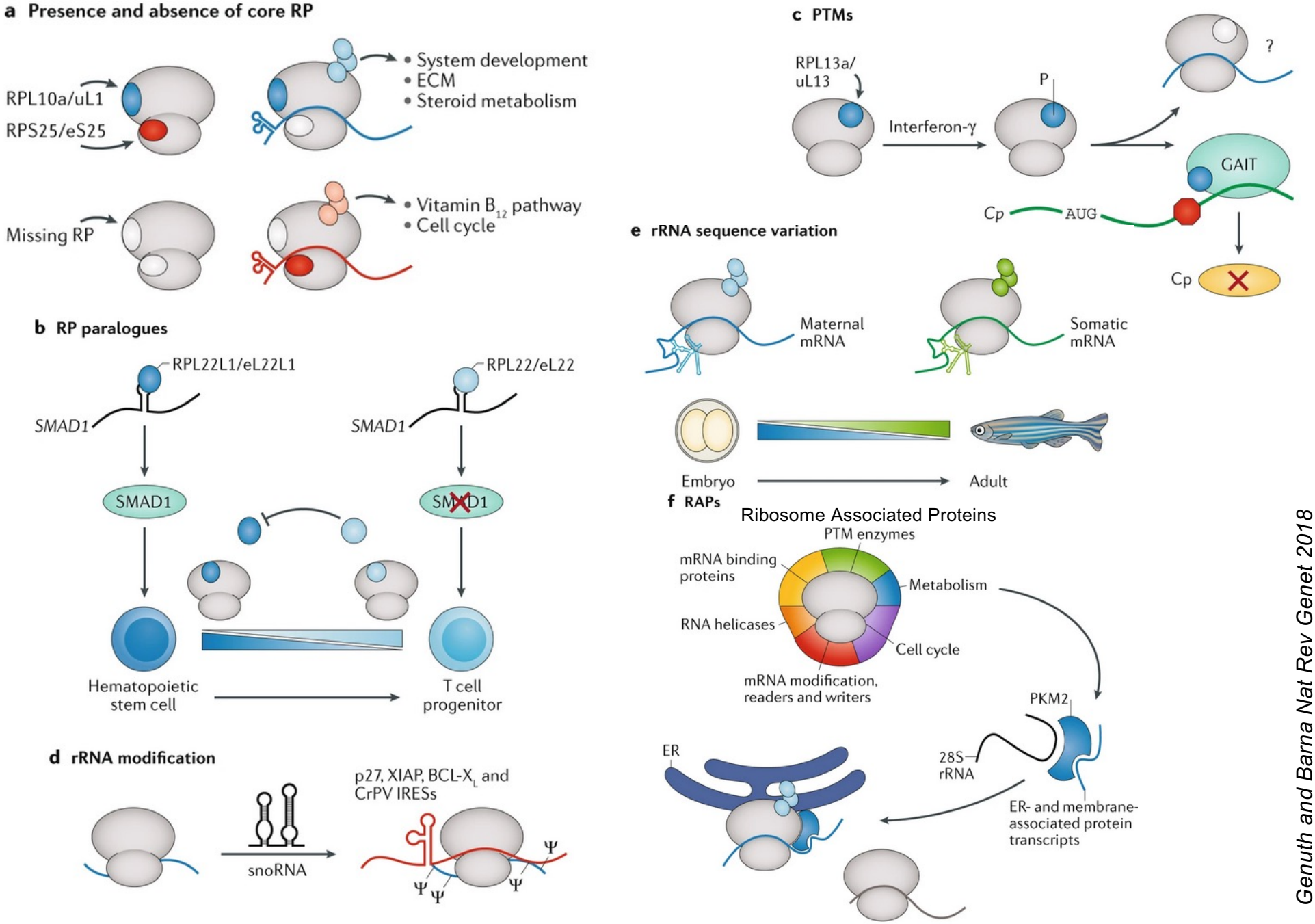
Alternative/specialized ribosomes



Specialized ribosomes and specific ribosomal protein paralogs may control translation of specific proteins

Norris et al, WIREsRNA 2021

Alternative/specialized ribosomes



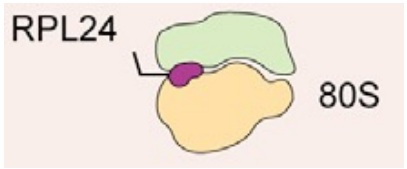
Genuth and Barna Nat Rev Genet 2018

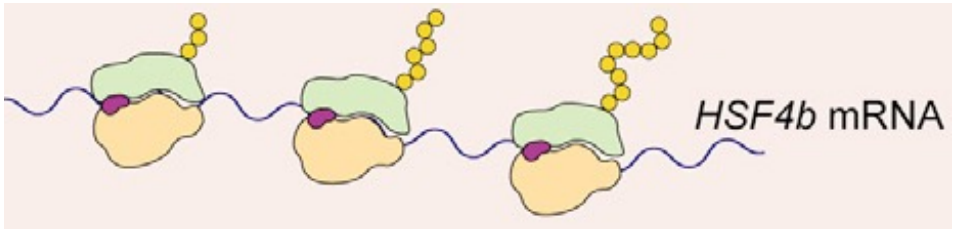
Alternative/specialized ribosomes

Ribosome protein composition following nutritional shift in yeast (RPL10 RPS1A/B, RPL8A and RPL8B)

Specific ribosomal protein paralogs control translation of mitochondrial proteins in yeast (RPL1b, RPL2b, or RPS26b)

Ribosomes containing m^{6}A modified 18S rRNA by METTL5 preferably translate 5'TOP mRNAs (mRNAs with 5' terminal oligopyrimidine motifs) **via promoting RPL24 and 18S rRNA interaction and 80S assembly**

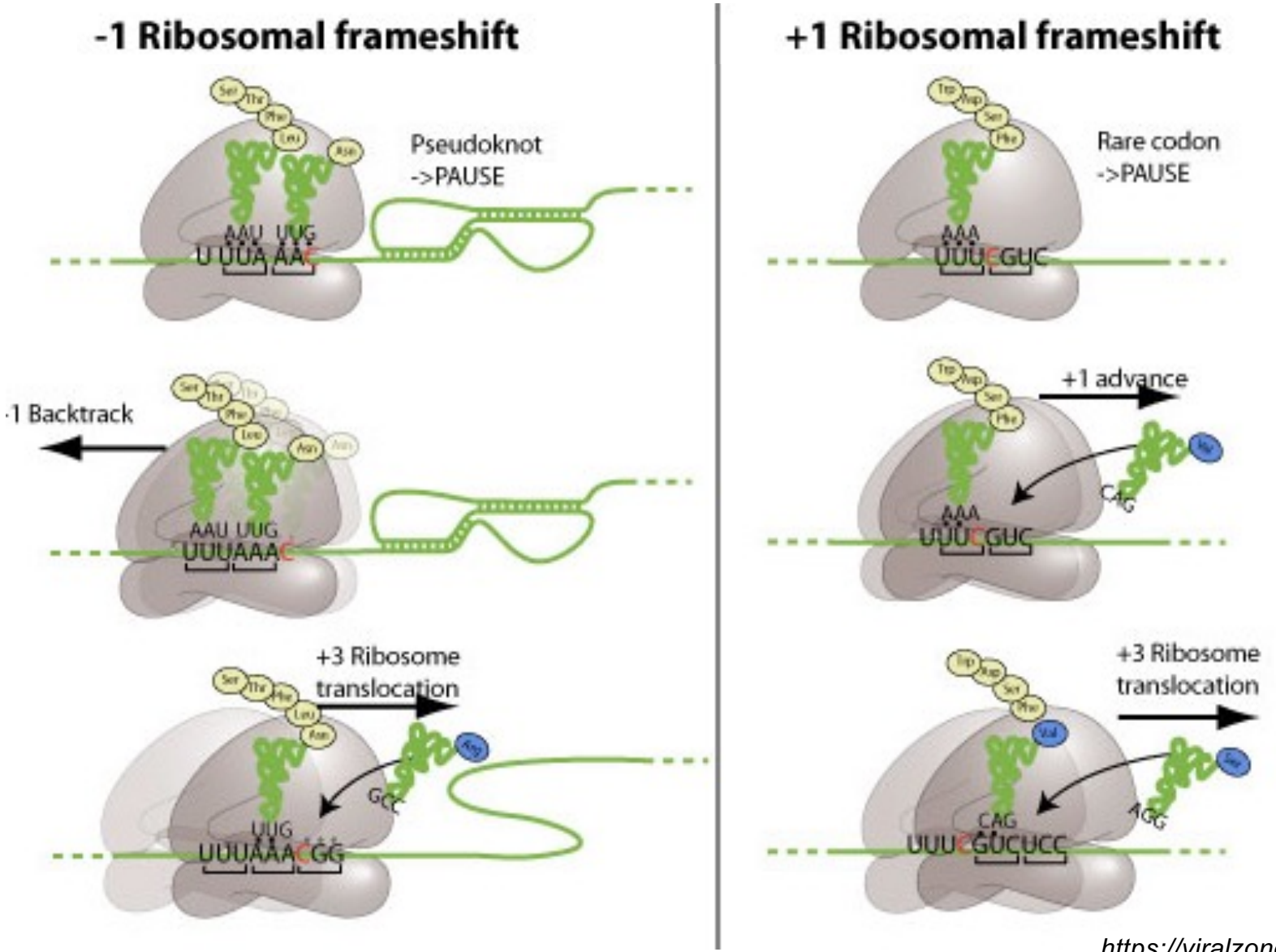




A male germ-cell-specific ribosome controls male fertility. RibosomeST, assembled with the male germ-cell-specific protein RPL39L, Ribosome^{Core} assemble with RPL39. RibosomeST predominantly cotranslationally regulates folding of a subset of male germ-cell-specific proteins that are essential for sperm formation.

Ribosomal frameshifting

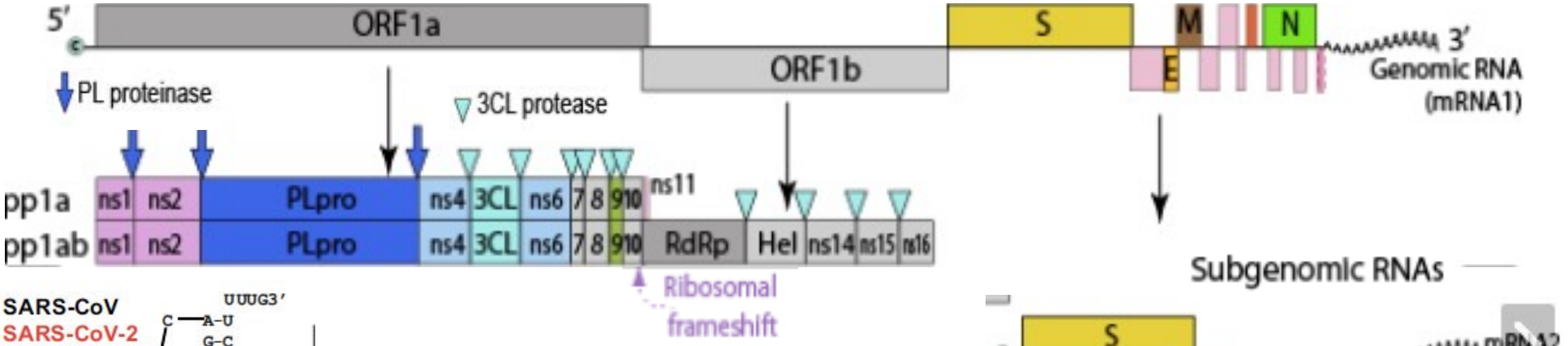
triplet code - three potential reading frames

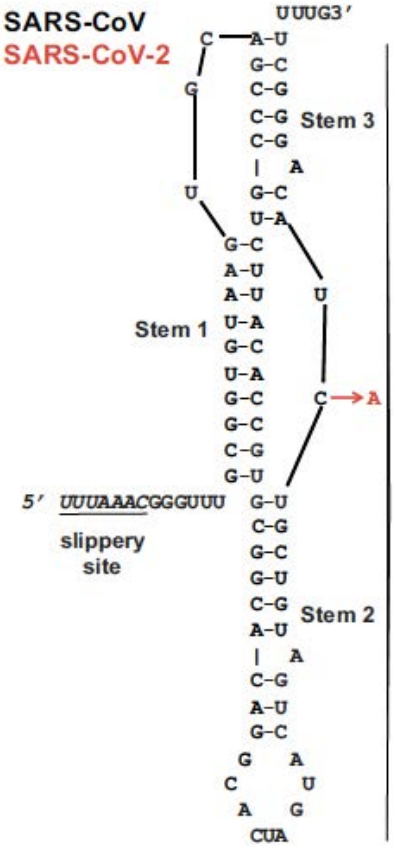


https://viralzone.expasy.org/860

-1 Programmed Ribosomal Frameshifting

-1PRF SARS-CoV-2

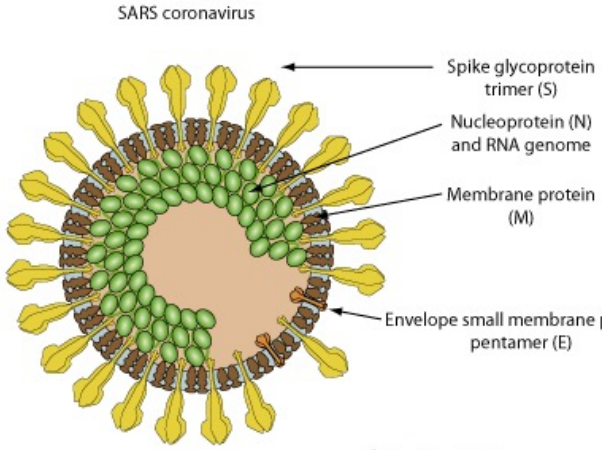


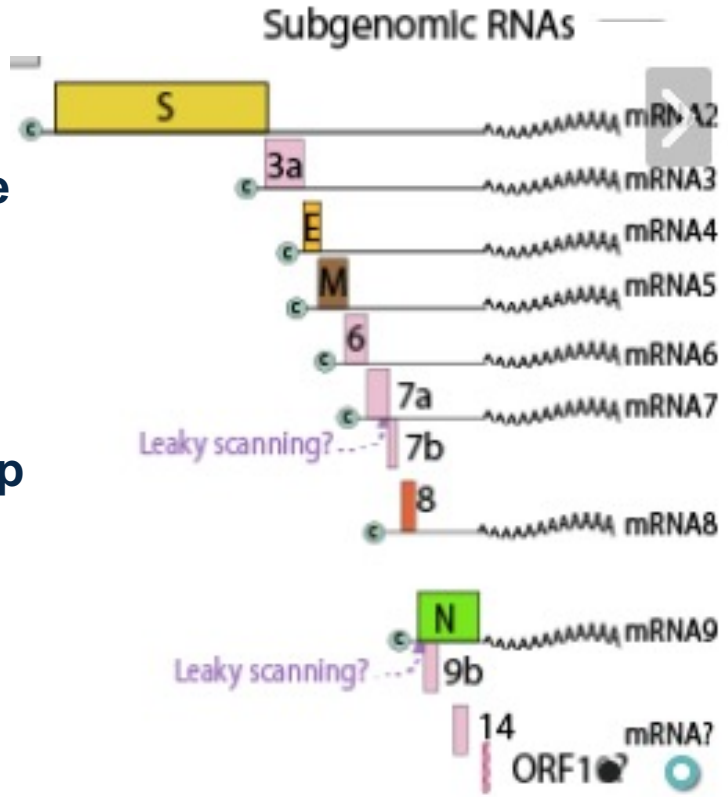


3-stemmed pseudoknot

ssRNA (+) genome, 27-32kb size
capped, polyadenylated
ORF1b translated by -1PRF
- **slippery site**
- **attenuator hairpin**

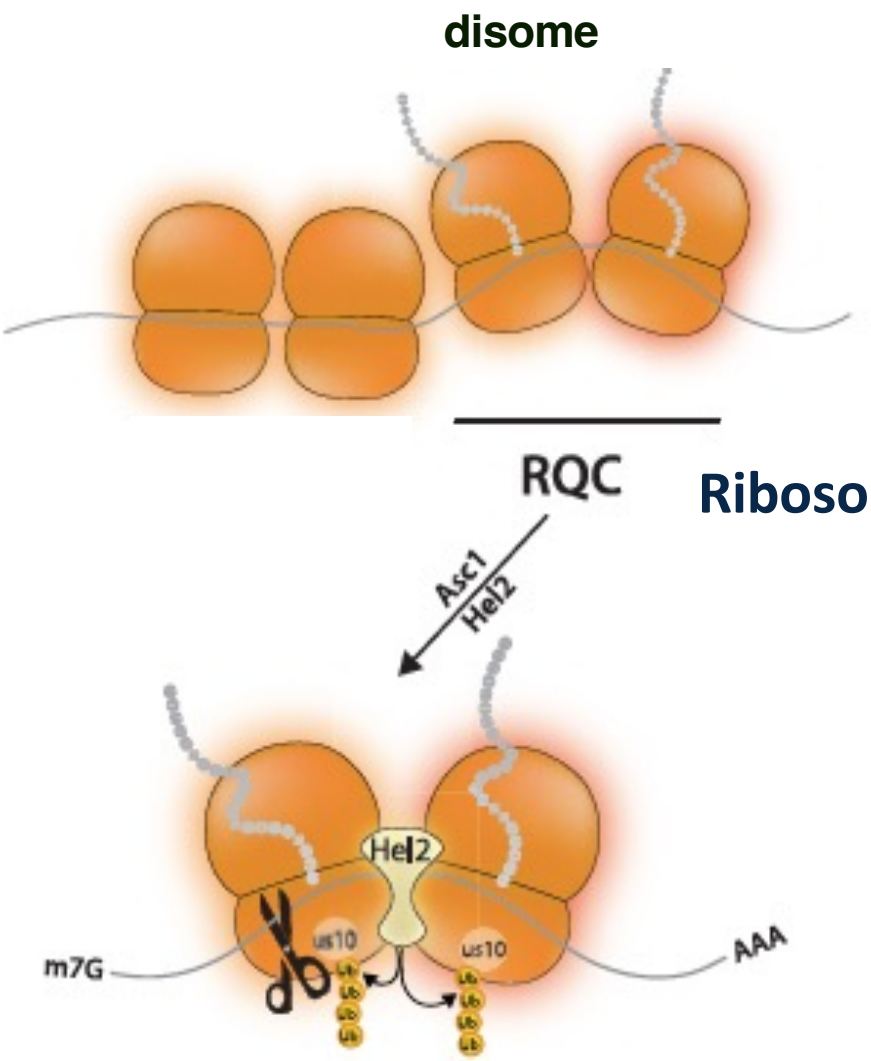
Processing of pp1ab gives RdRp





https://viralzone.expasy.org/785

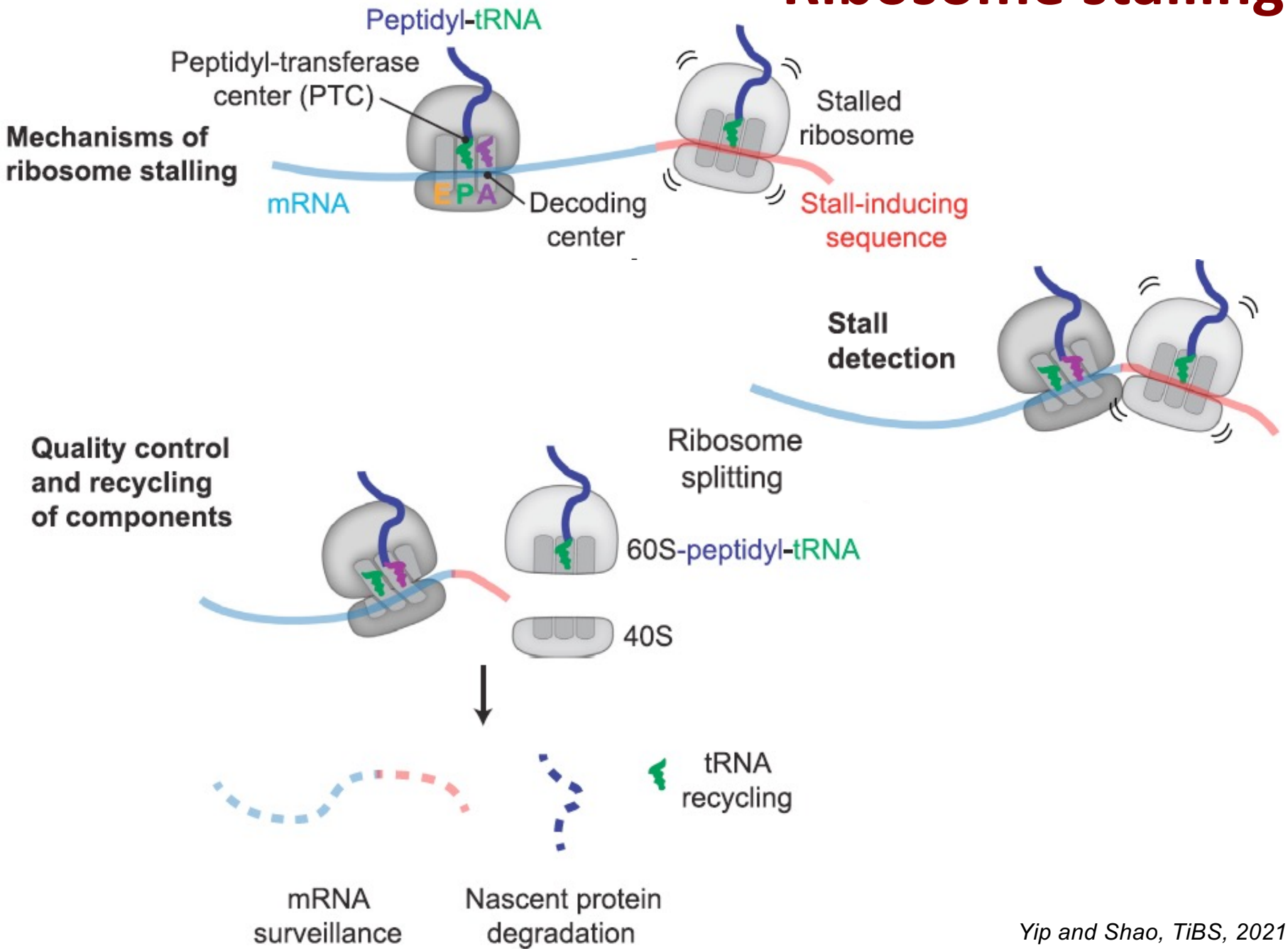
Ribosome pausing
Ribosome stalling
Ribosome collision
Disomes



Ribosome Quality Control

Collart and Weiss, NAR, 2020

Ribosome stalling



Yip and Shao, TiBS, 2021

TAKE-HOME MESSAGE

- **Eukaryotic translation:**

- is 5’-cap dependent

- uses a scanning mechanism

- energy is delivered by GTP hydrolysis (all steps)

- occurs on polysomes

- **The ribosome is the ribozyme**
- **Translation fidelity is ensured by charging the proper tRNA and recognition of cognate tRNA::mRNA,**
- **Translation is regulated by general and specific mechanisms, including stress, growth factors, miRNAs, viruses** (IRES), **metabolites**
- **Alternative processes- non-canonical initiation, frameshifting, leaky-scanning, stop codon readthrough**