Various information

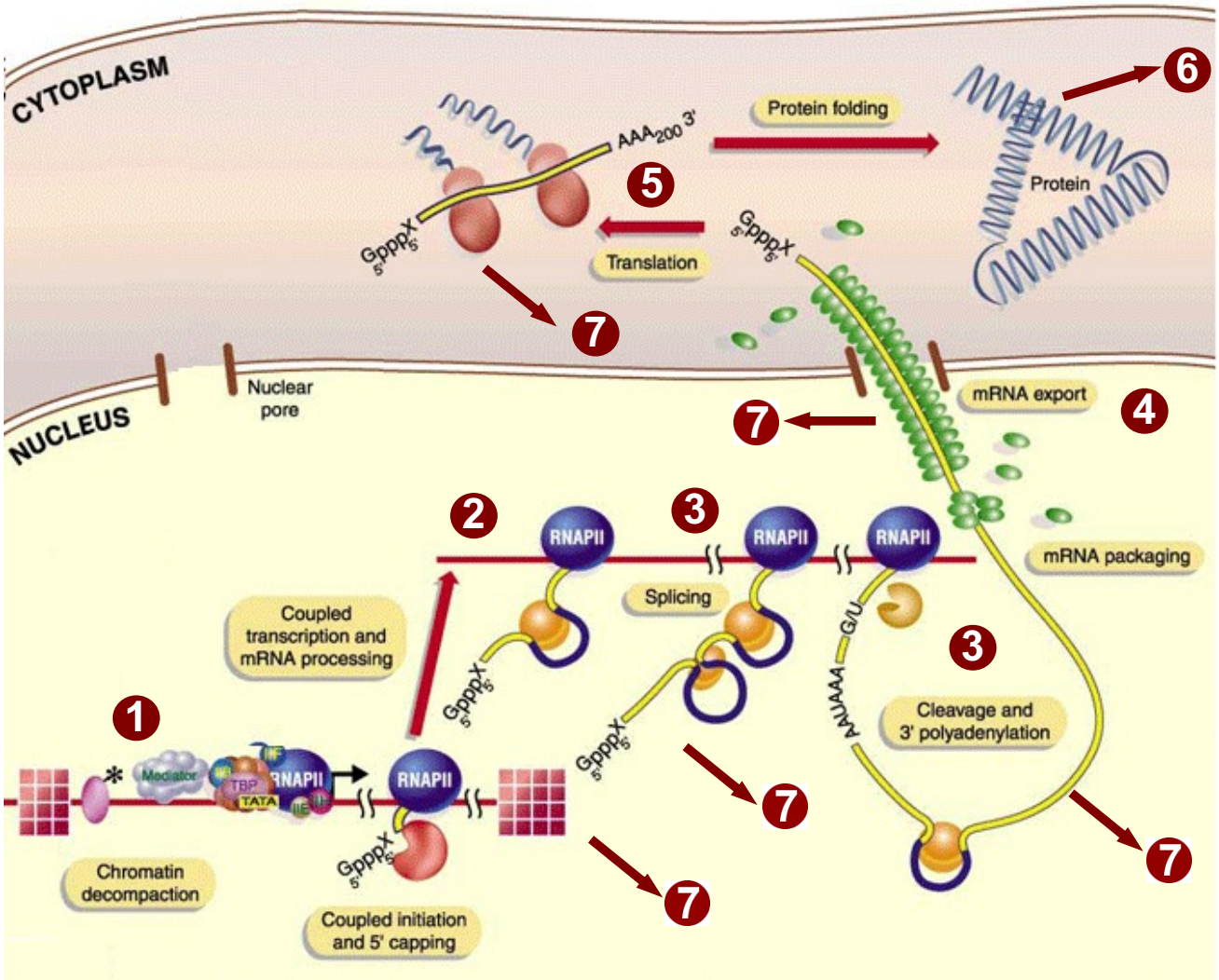
- Test exam at the begining of June
- No textbook

  Lizabeth Allison - **Fundamental Molecular Biology**
- Lectures (pdf) on IGIB webpage

  www.igib.uw.edu.pl/index.php/start2/start/

  - dydaktyka, - Fakultety i wykłady monograficzne, - RGE, - materiały dla studentów
- Resignation – better now or soon than before the exam

REGULATION OF GENE EXPRESSION



1) chromatin

2) transcription

3) RNA processing

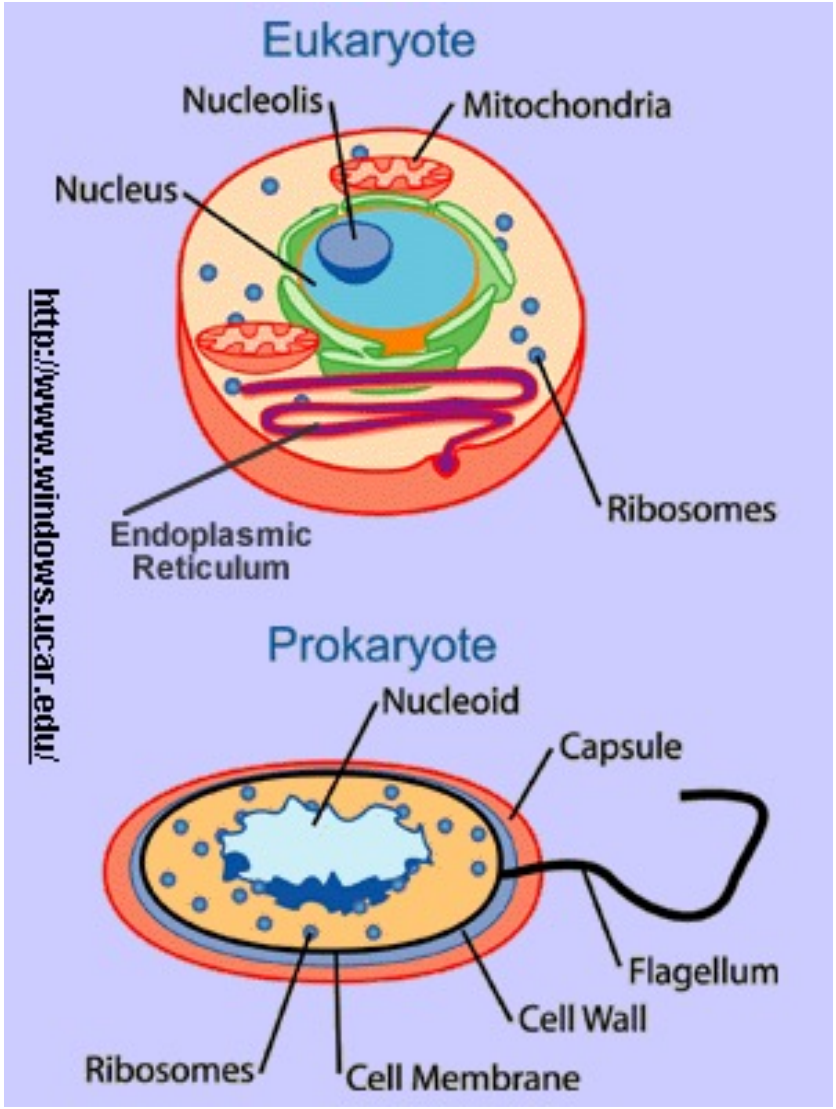
4) RNA export

5) translation (mRNA)

6) protein stability

7) RNA degradation

BACTERIAL vs EUKARYOTIC cell



10-100 µm

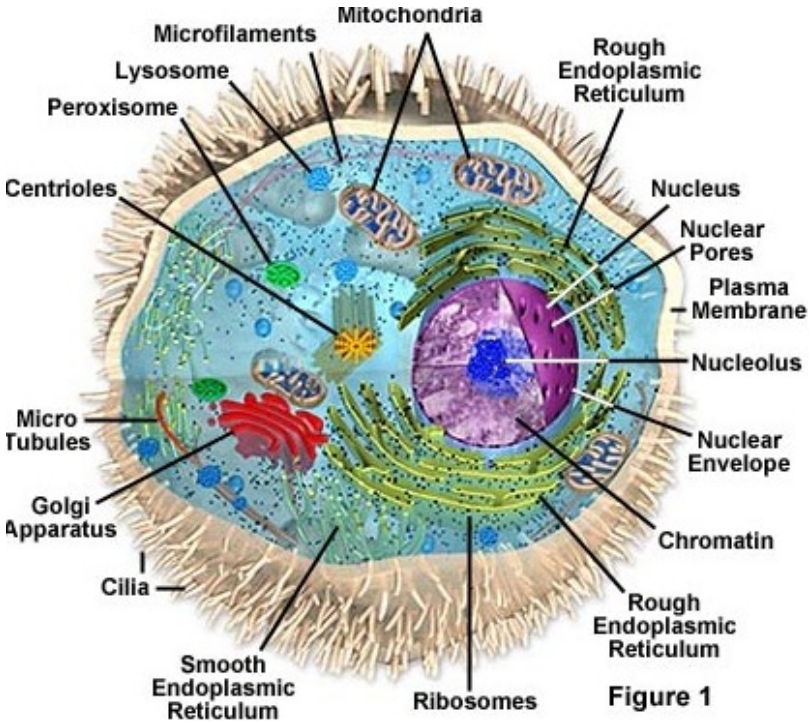


Figure 1

1-10 µm

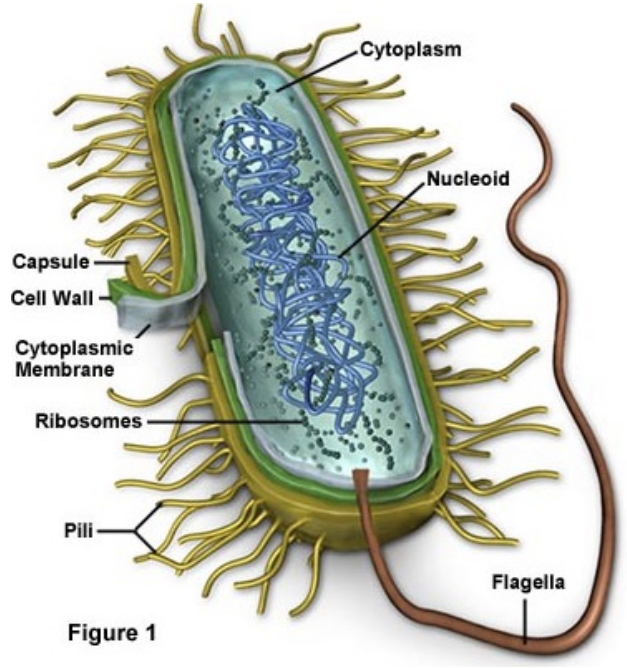
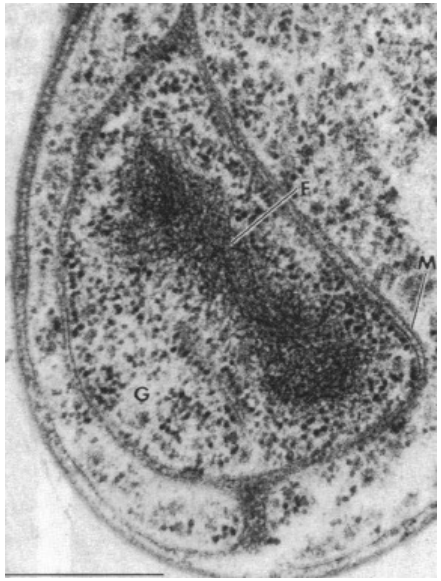
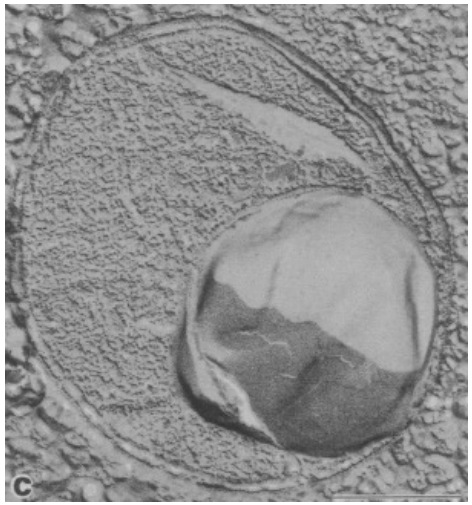


Figure 1

Compartmentalized bacteria

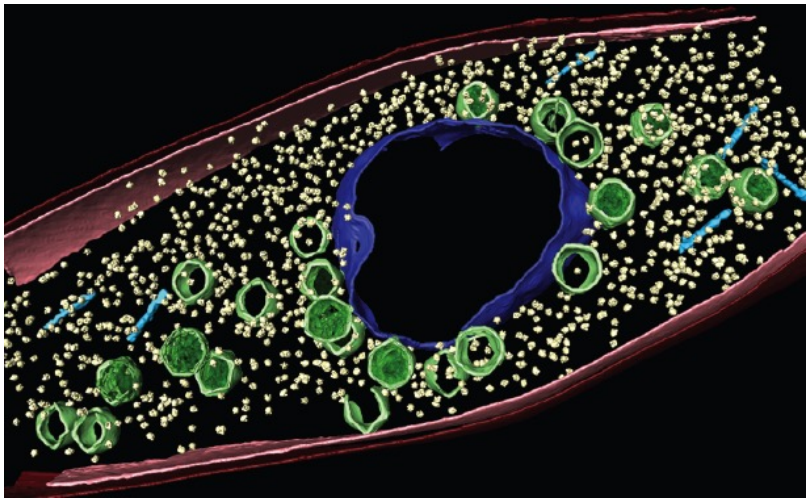
Eubacterium *Gemmata obscuriglobus* has a membrane-bound nucleoid





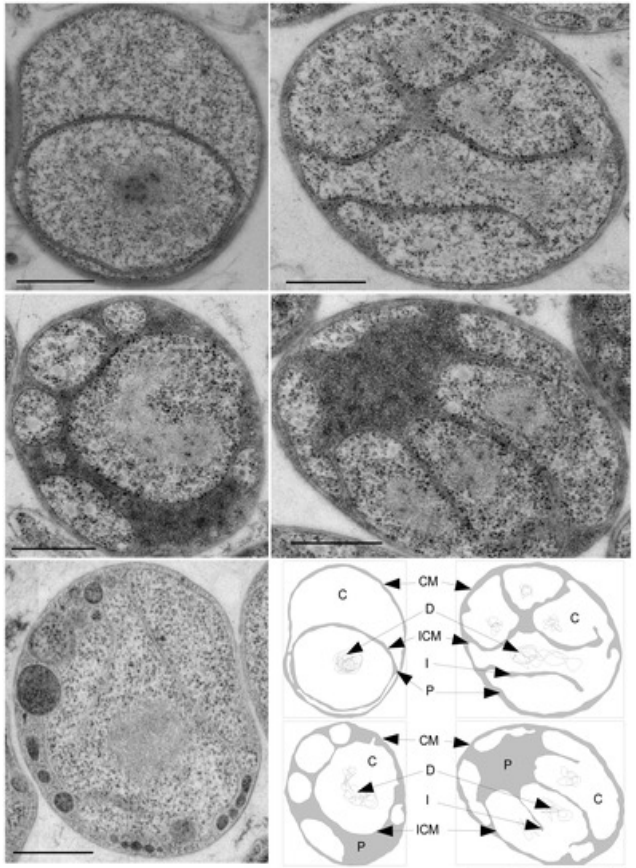
Fuerst and Webb, PNAS, 1991

Nucleus-like structure during viral replication in *Pseudomonas chlororaphis*



Chaikeeratisak et al, Science, 2017

Planctomycetes-Verrucomicrobia-Chlamydiae Superphylum have membrane coat-like proteins



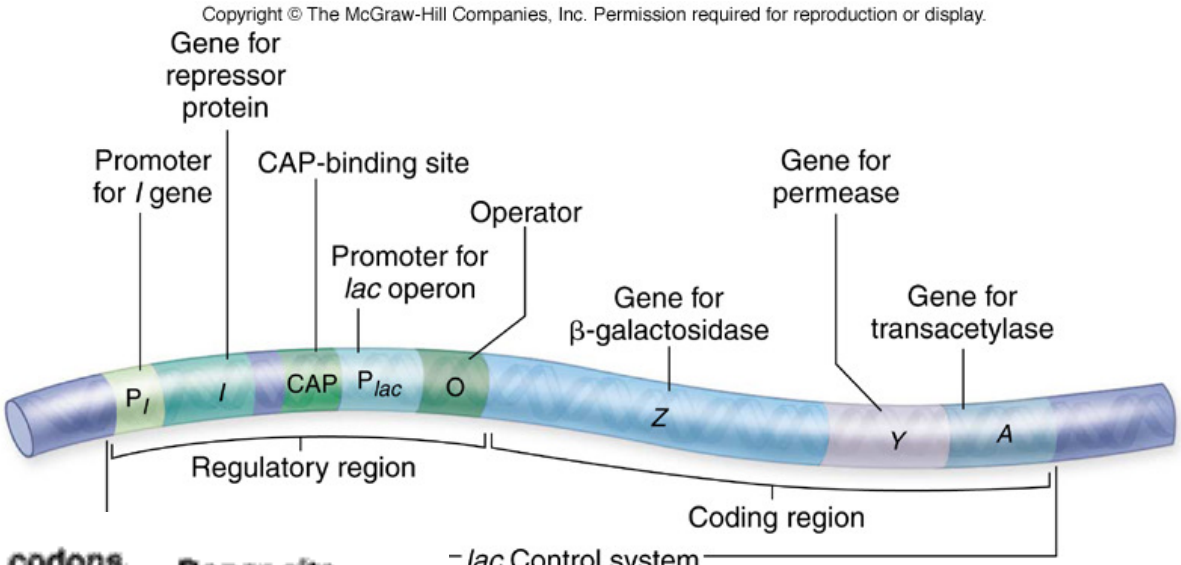
Santarella-Mellwig et al, PLOS Biology, 2010

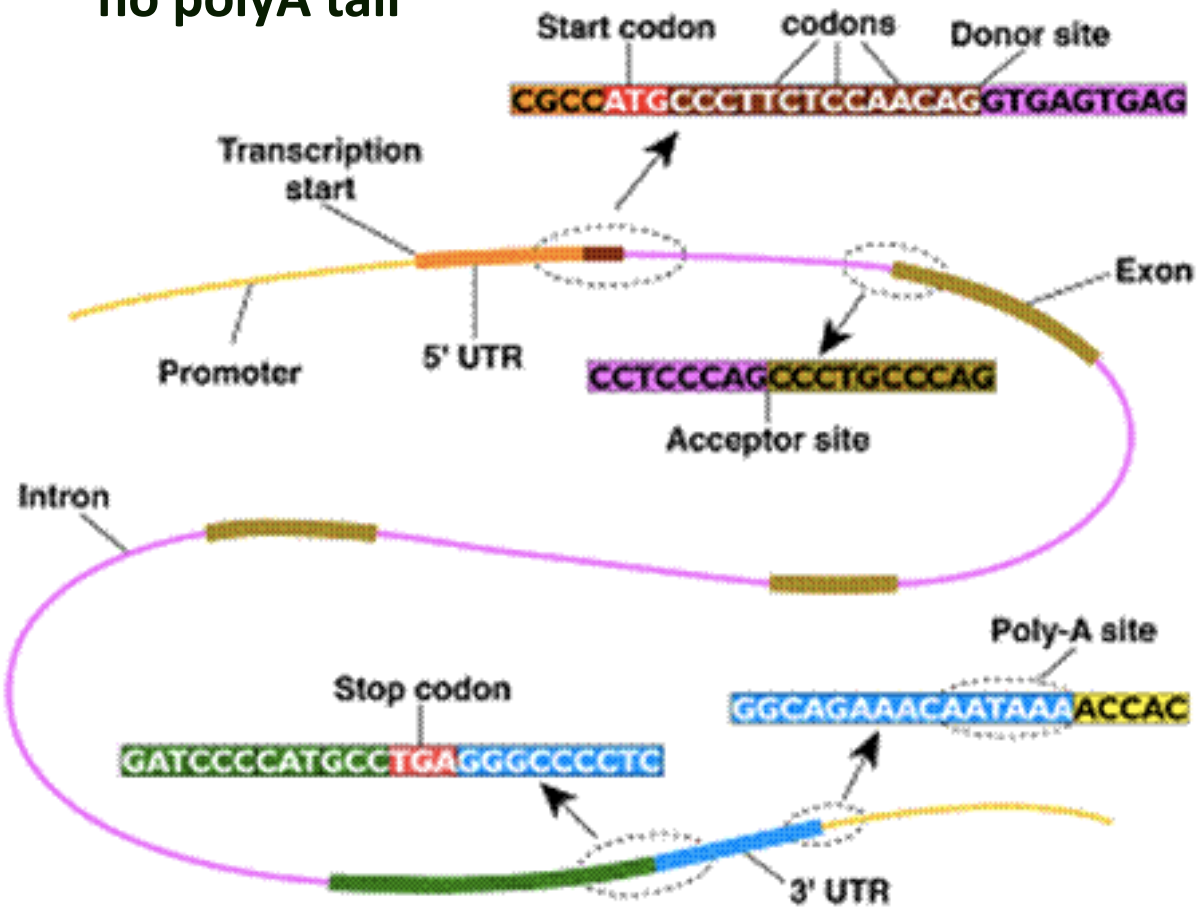
CM, cytoplasmic membrane (+cell wall)
ICM, intracytoplasmic membrane
P, paryphoplasm
I, invaginations of the ICM; D, DNA; V, vesicle

Gene structure

Bacteria

- operons
- polycistronic
- have 5’ and 3’ UTRs
- no 5’ cap, no introns, no polyA tail



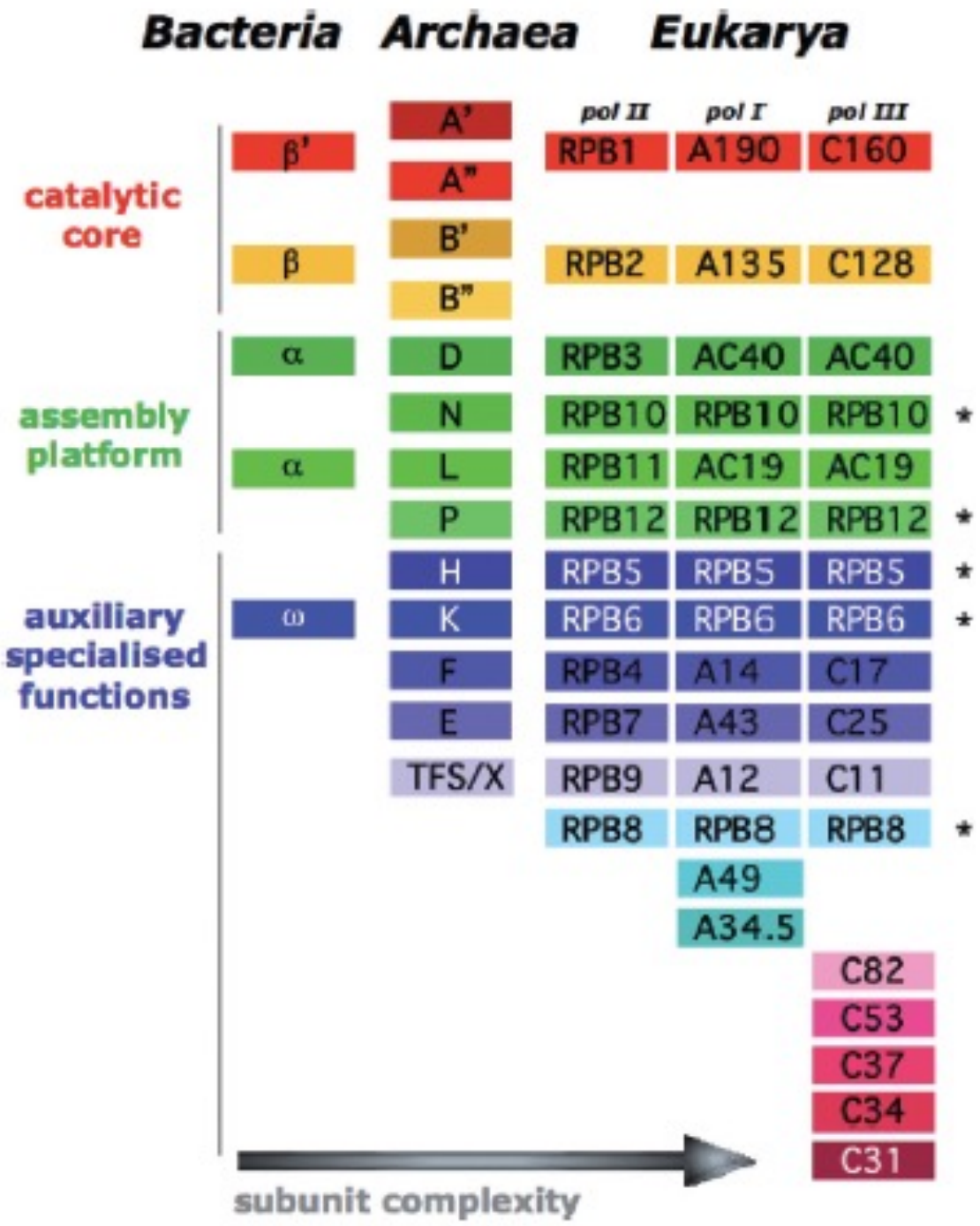


Eukaryotes

- **usually monocistronic** (policistronic also exist)
- **contain 5’ and 3’ UTRs** (untranslated region)
- **processing events:**

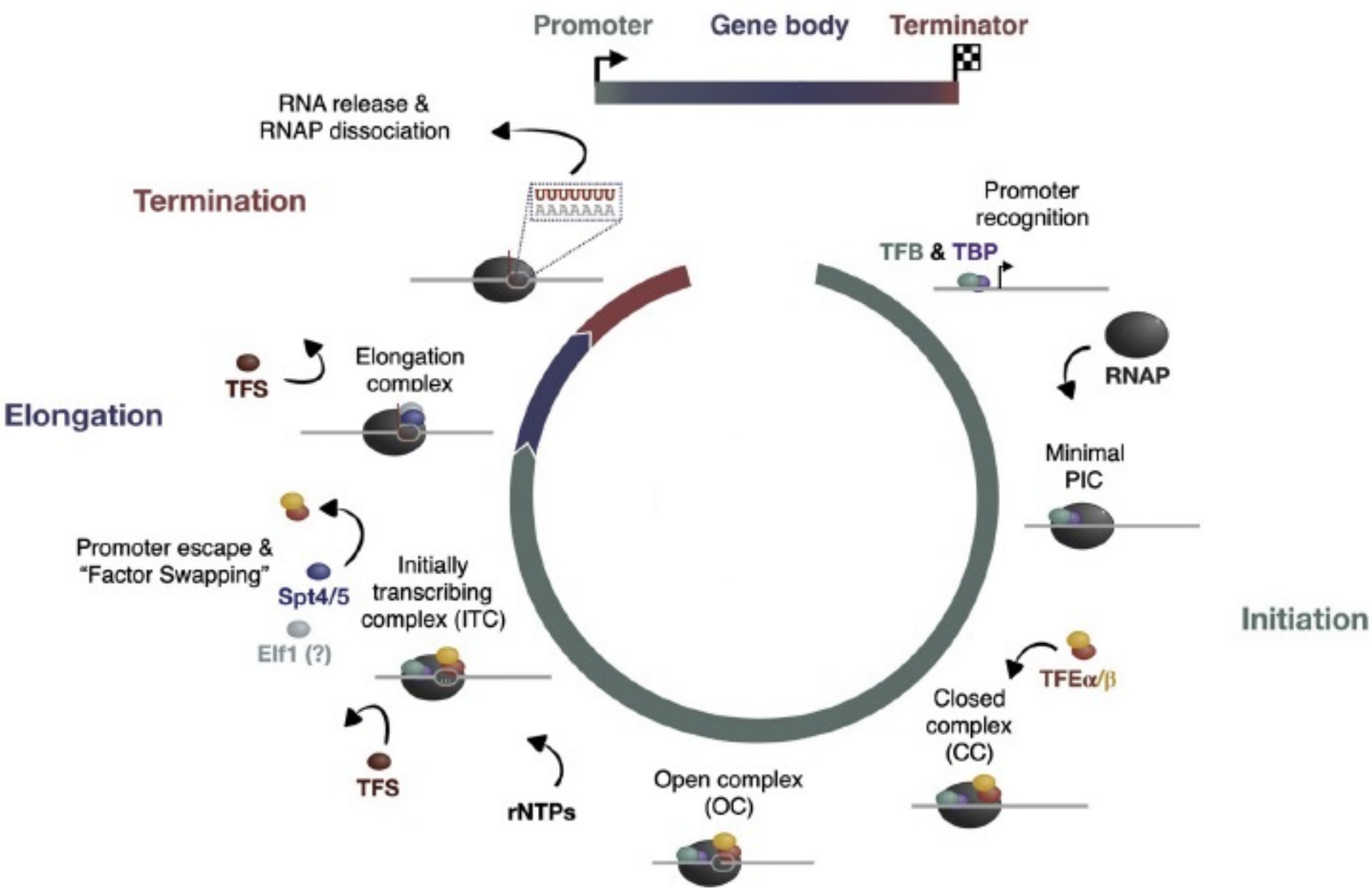
capping (Pol II transcripts)
splicing
editing
3’ end formation - cleavage and polyadenylation

RNA polymerases

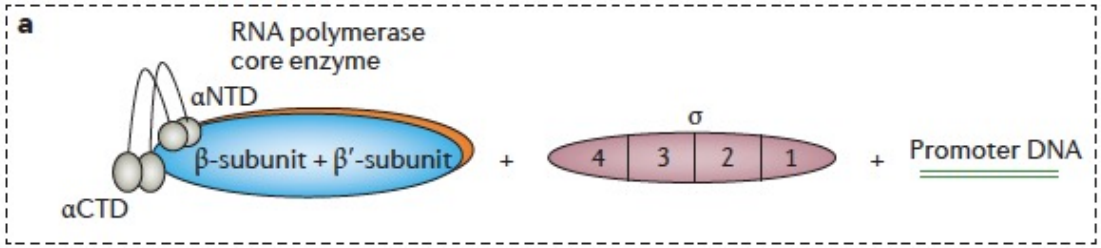


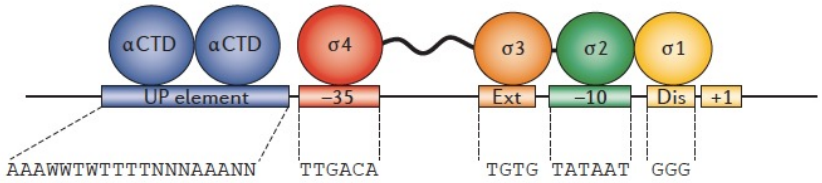
Werner, Mol Microbiol, 2007

Transcription

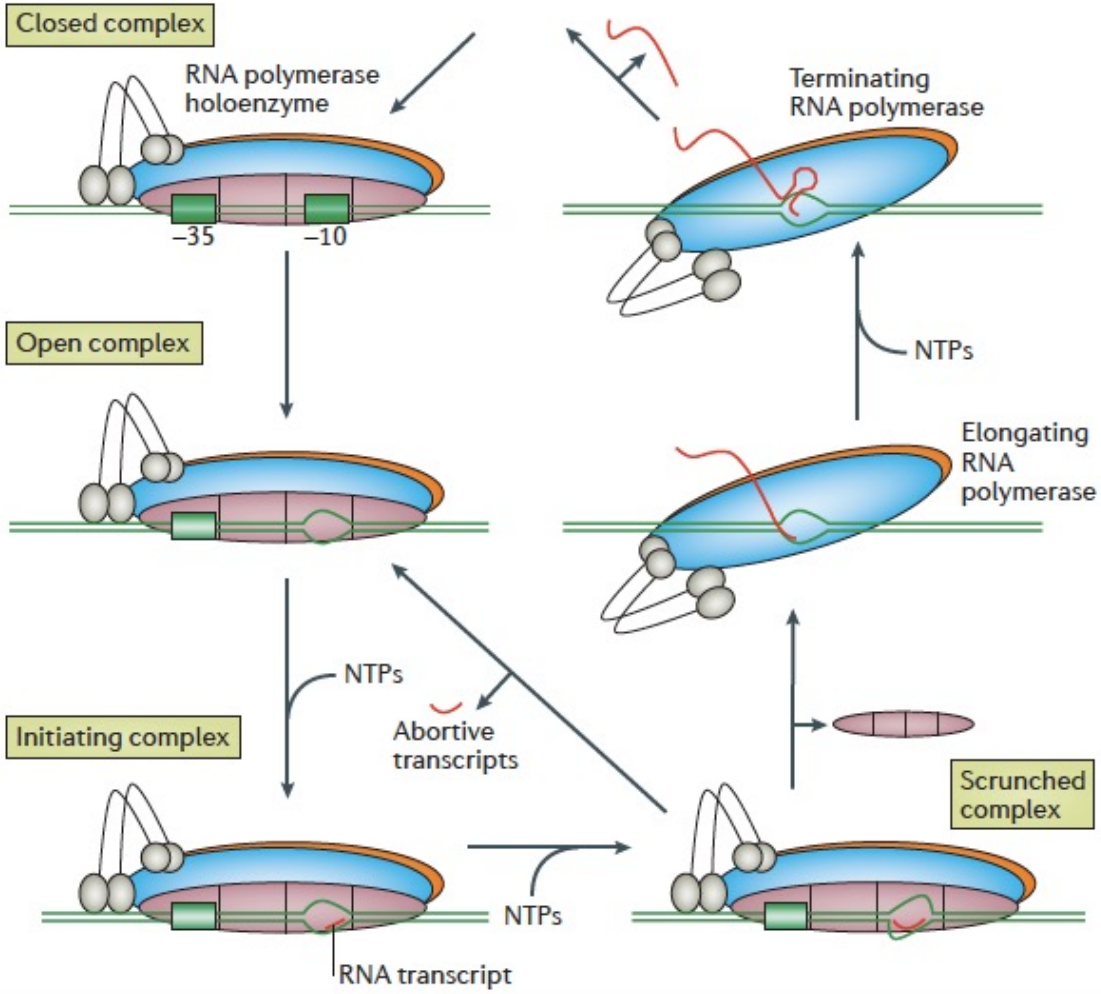


Transcription initiation and elongation





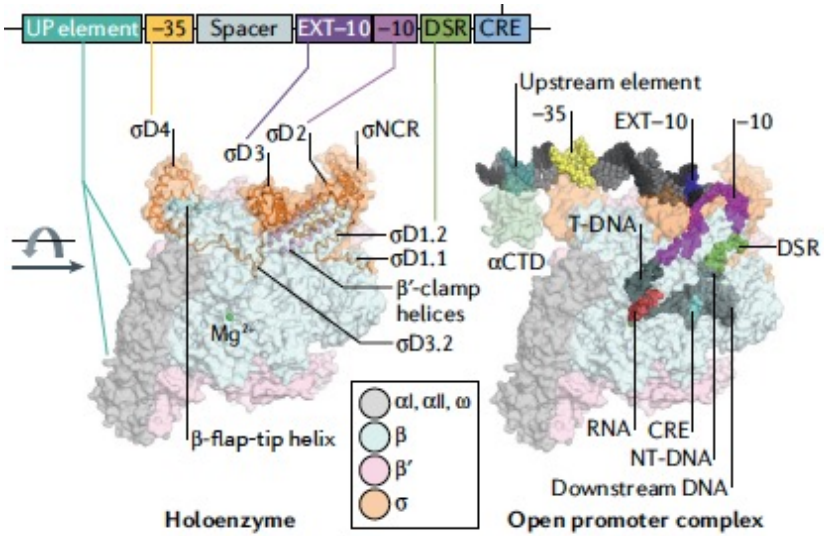
bacterial promoter



- The core RNAP binds a promoter specificity factor σ generating the holoenzyme.
- The holoenzyme recognizes and melts promoter DNA to form the open promoter complex.
- In the presence of rNTPs initiating complex is formed, which either produces abortive transcripts or transitions to elongating RNAP after σ dissociates causing promoter escape.

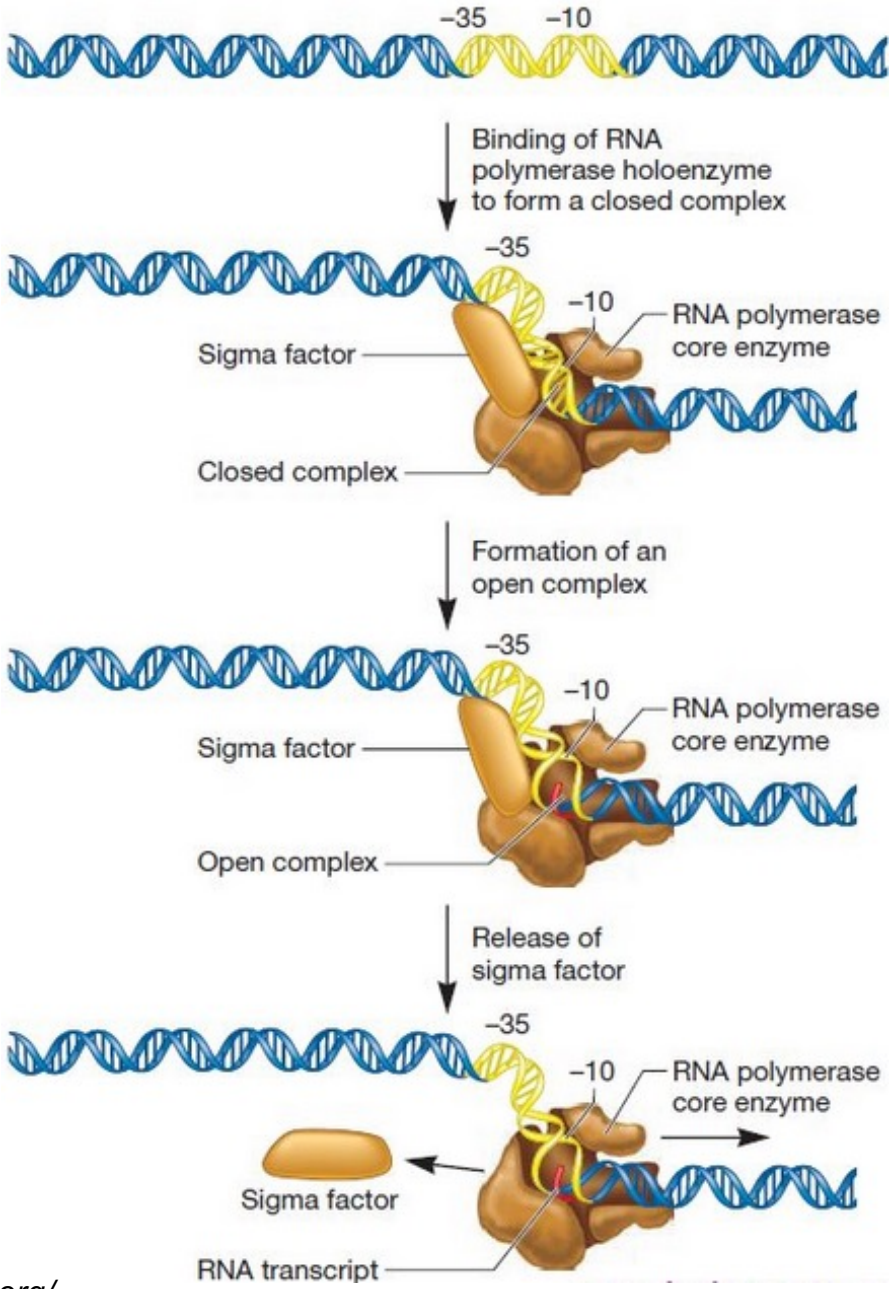
Browning and Busby , Nat. Rev. Microbiol, 2016

Transcription initiation and elongation



RNAP holoenzyme structure.
Recognition of the housekeeping promoter.

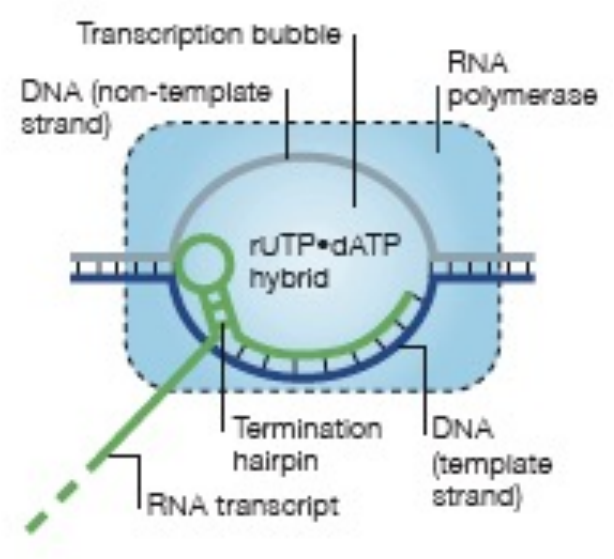
Regulation of bacterial RNAP
- repression or activation at promoters by transcription factors
- DNA methylation at promoter



Chen et al., , Nat. Rev. Microbiol, 2020; https://microbiologynotes.org/

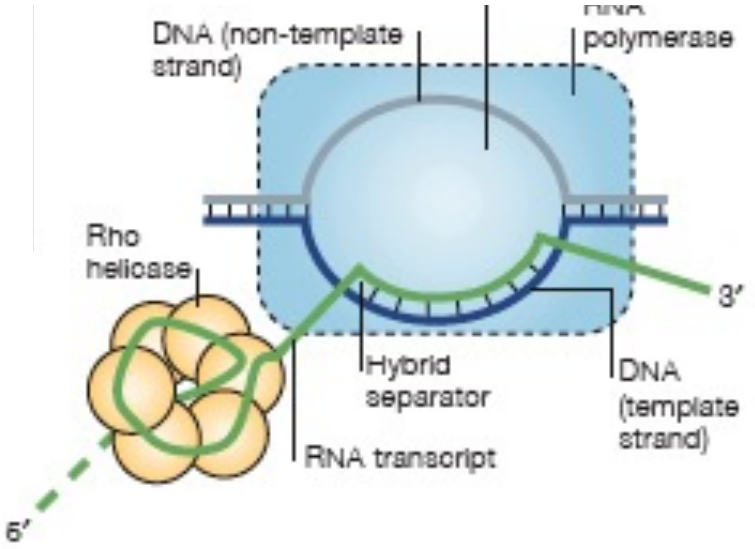
Transcription termination

Intrinsic termination



- caused by a stem-loop structure followed by the run of Us
- RNAP pauses on the stem-loop
- RNA-DNA hybrid is unwound
- transcription bubble collapses
- RNAP dissociates

Rho-dependent termination

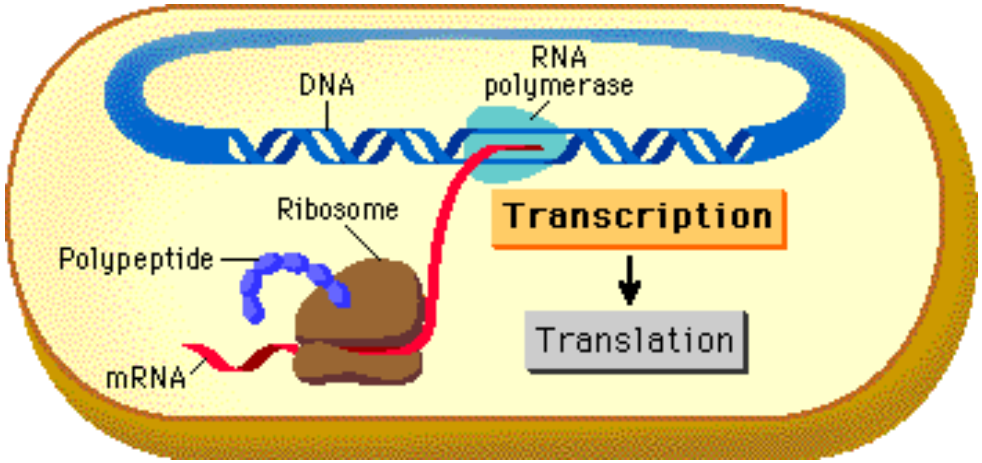


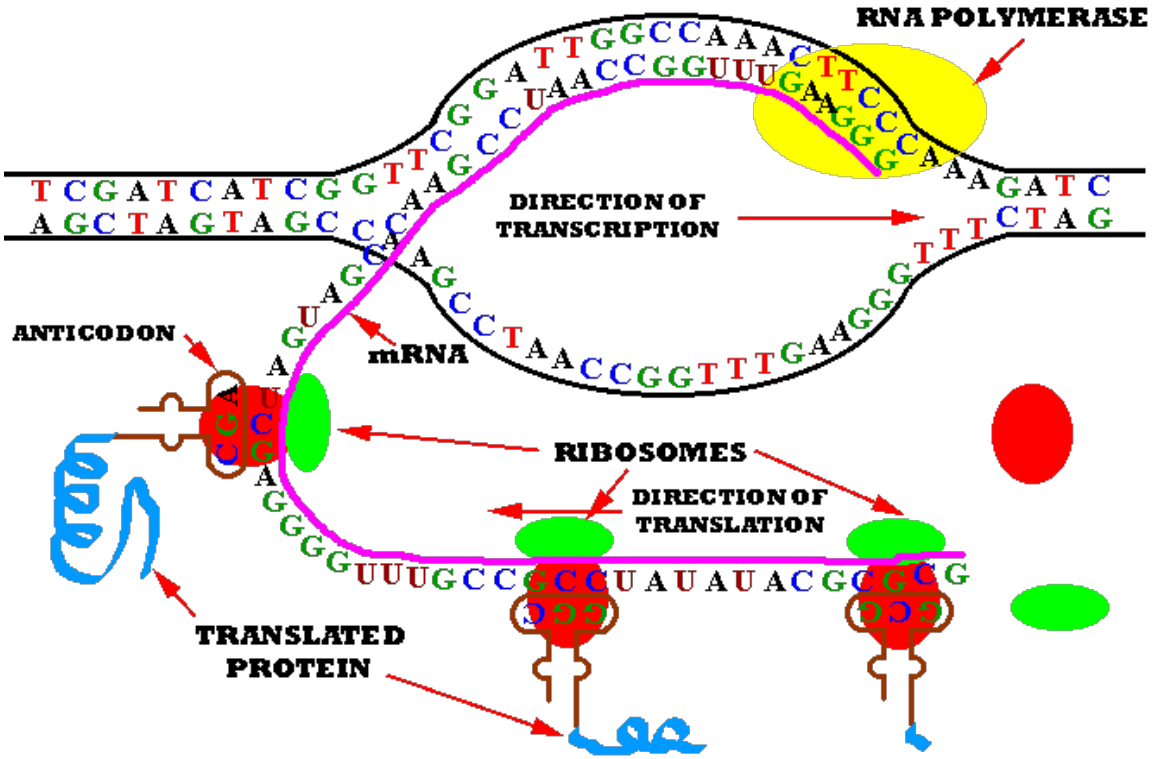
- mediated by a hexameric helicase Rho
- Rho binds to the nascent transcript
- translocates along the RNA (ATPase activity)
- catches up with RNAP and stops at pause sites
- changes RNA conformation resulting in DNA-RNA hybrid destabilisation
- dislodges paused RNAP

Greive and von Hippel, Nat. Rev. Mol. Cell Biol., 2005

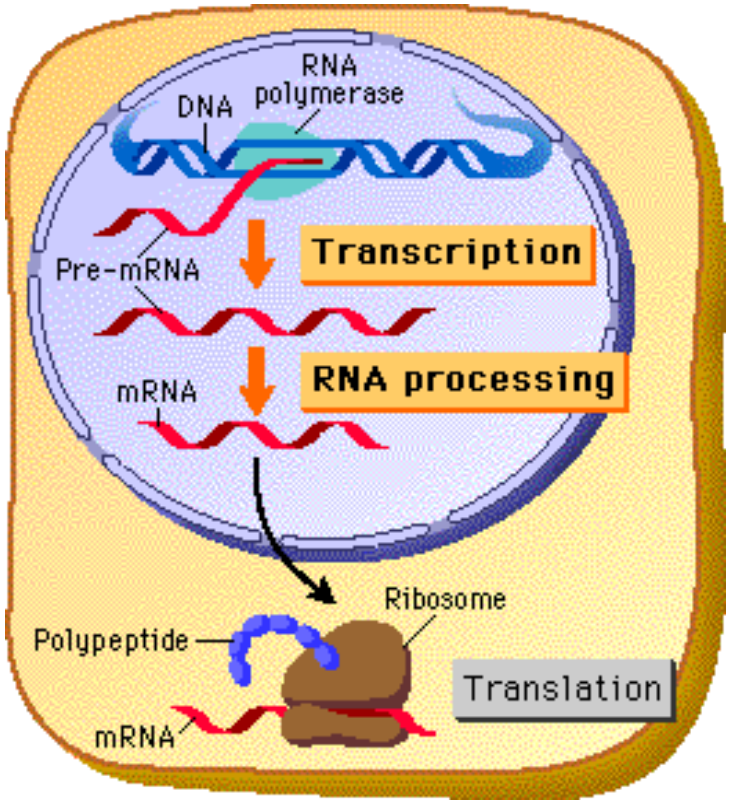
Gene expression: transcription and translation

Bacteria



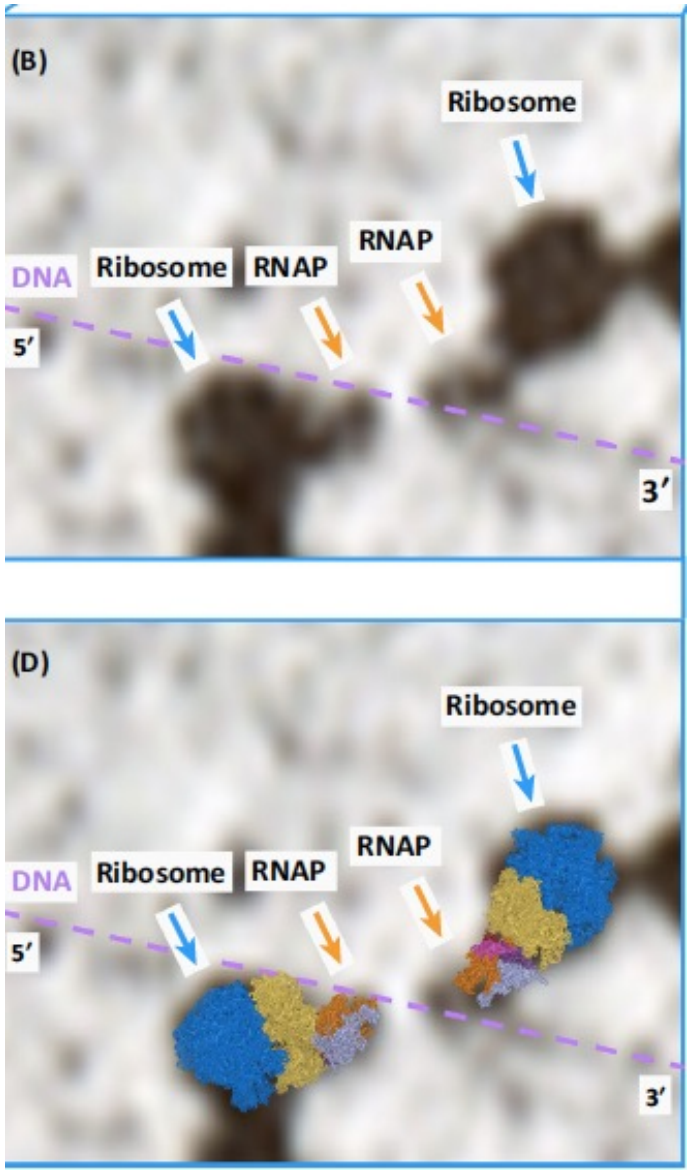


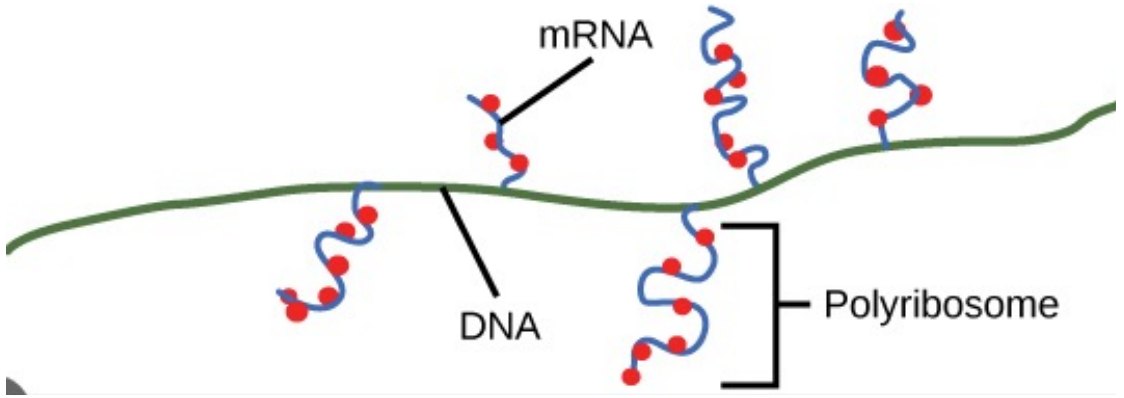
Eukaryotes

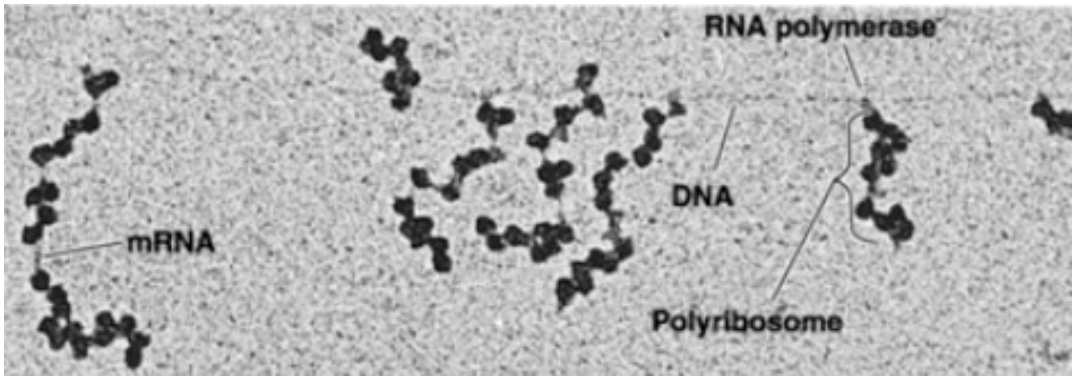


http://www.phschool.com/science/biology_place/biocoach/transcription/tctlpreu.html

Coupled transcription-translation (CTT)



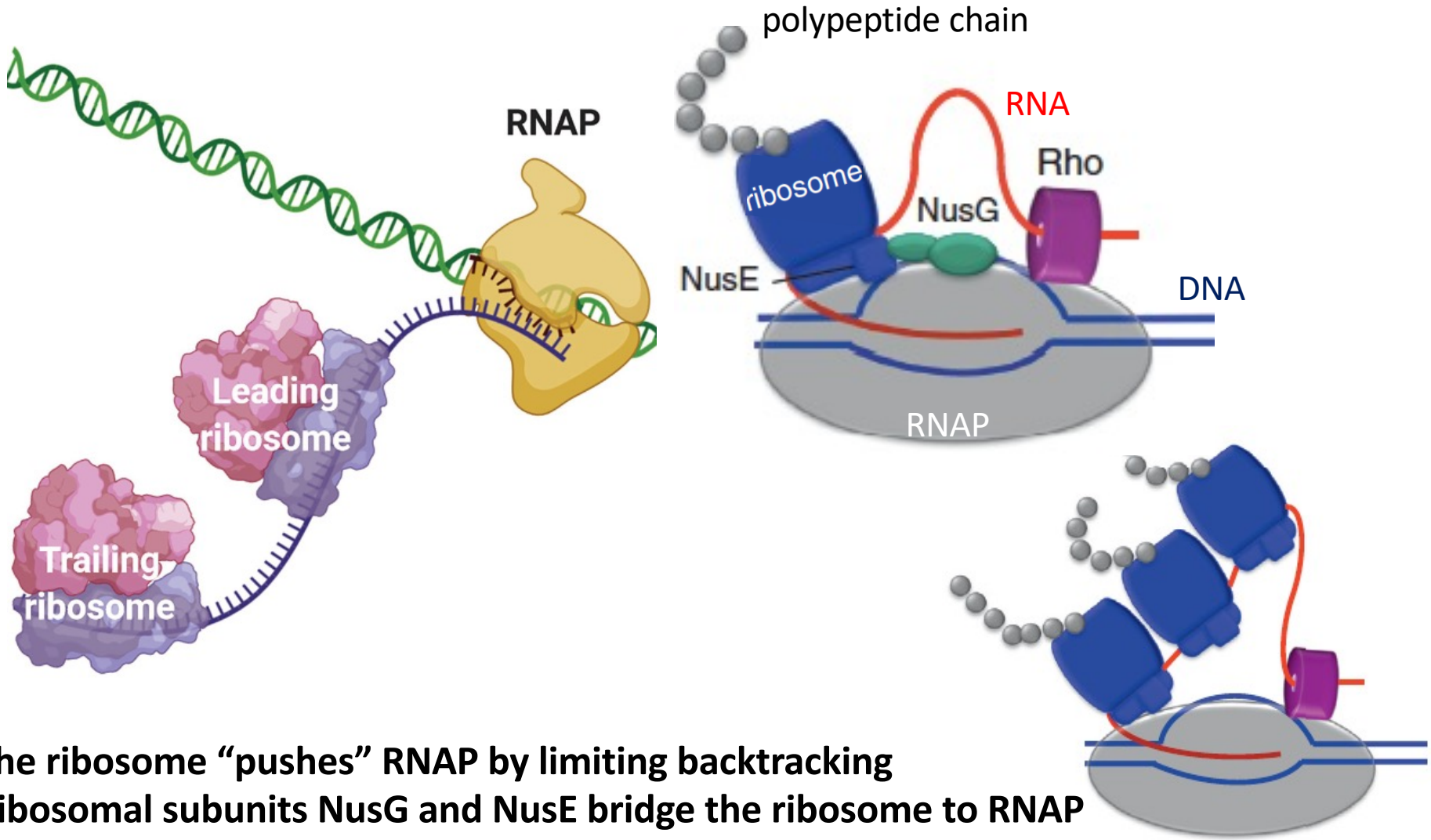




- **The pioneering ribosome physically associates and kinetically coordinates RNAP**
- **This allows for co-transcriptional regulation, translation-based attenuation and RNA quality control**

https://www.youtube.com/watch?v=DWB9FFgKtiE

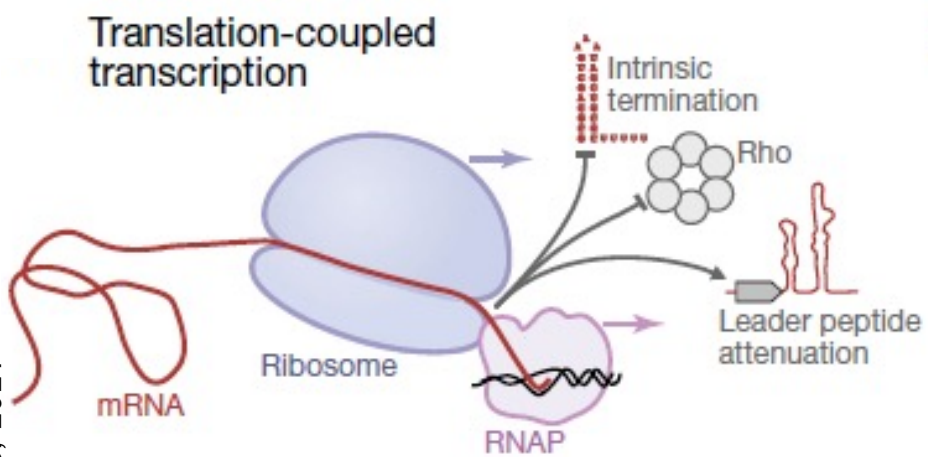
Coupled transcription-translation (CTT)

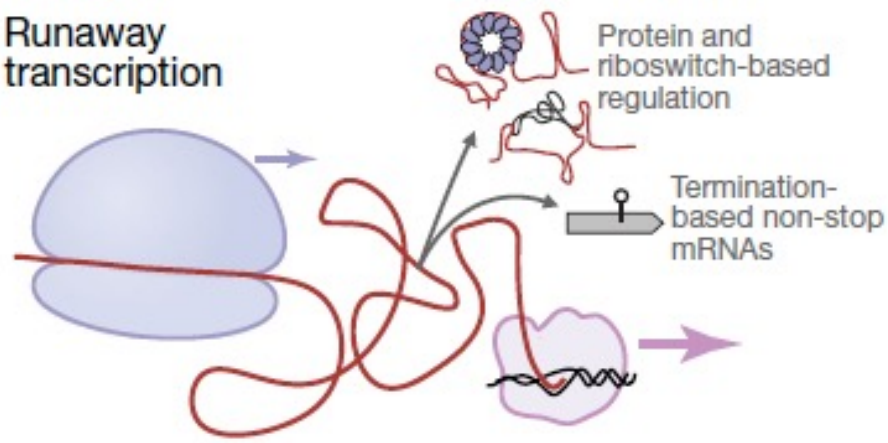


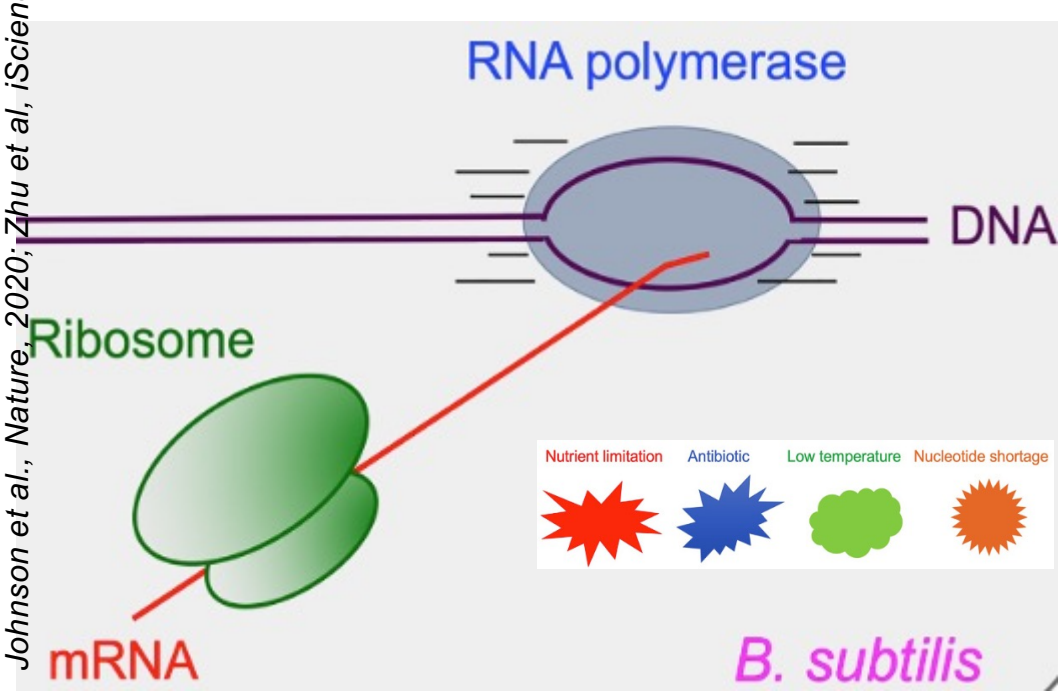
McGary and Nudler, Curr Opp Micro, 2013

- **The ribosome "pushes" RNAP by limiting backtracking**
- **Ribosomal subunits NusG and NusE bridge the ribosome to RNAP**
- **NusG and NusE act as transcription antiterminators and prevent Rho-dependent transcription termination**
- **disruption of coupling leads to loss of transcription processivity and triggers Rho-mediated premature transcription termination**

Uncoupled transcription-translation in *Bacillus subtilis* (not uniquely)







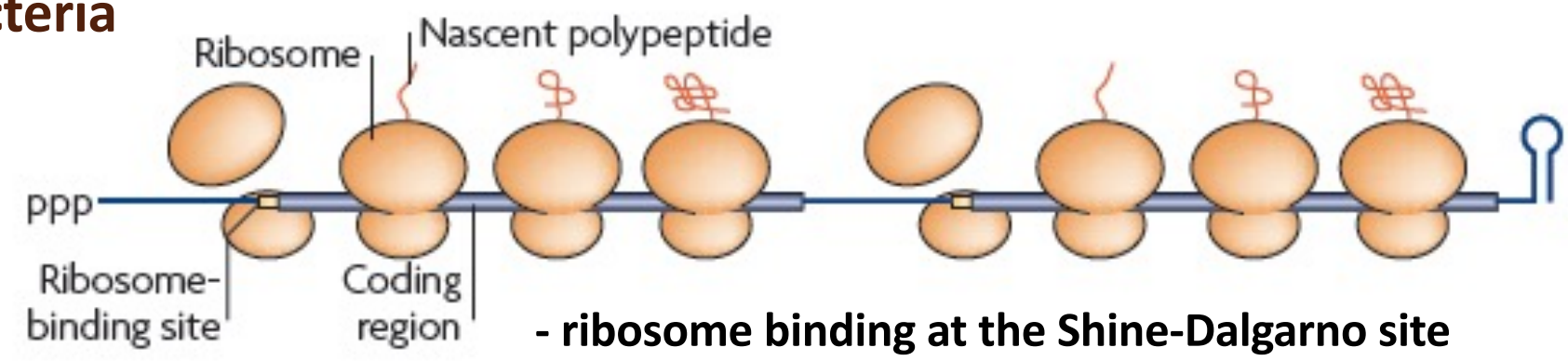
Johnson et al., Nature, 2020; Zhu et al, iScience, 2021

Transcription-translation kinetics

- **the speed of transcription elongation is much faster than of translation elongation ("runaway transcription")**
- **RNAP is ahead of the ribosome and insensitive to translation**
- **Rho-dependent transcription termination is not important for nascent mRNA translation**
- **transcription regulation/attenuation is based on riboswitches and proteins and less dependent on translation**

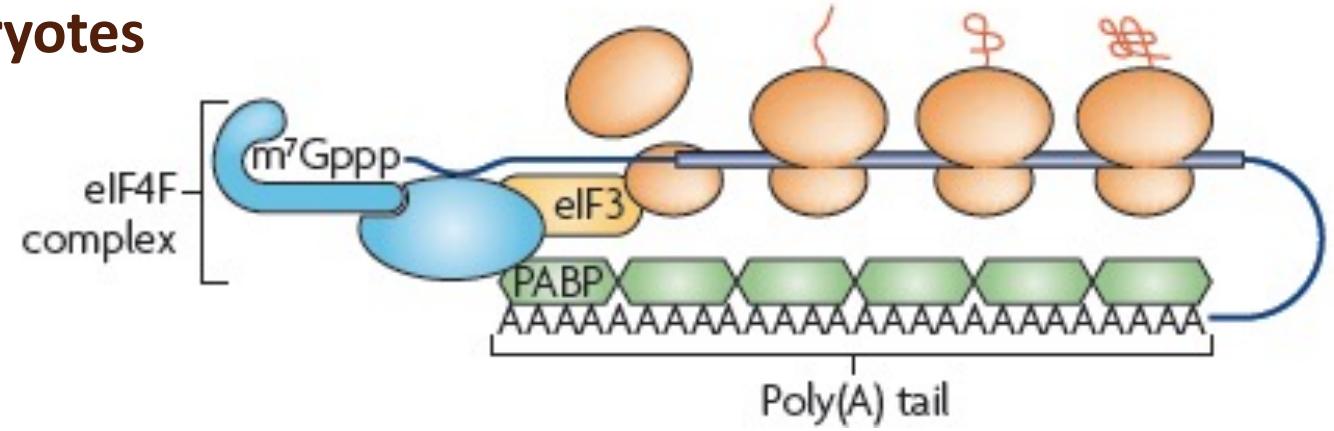
mRNA structure and translation

Bacteria



- ribosome binding at the Shine-Dalgarno site
- no ribosome scanning

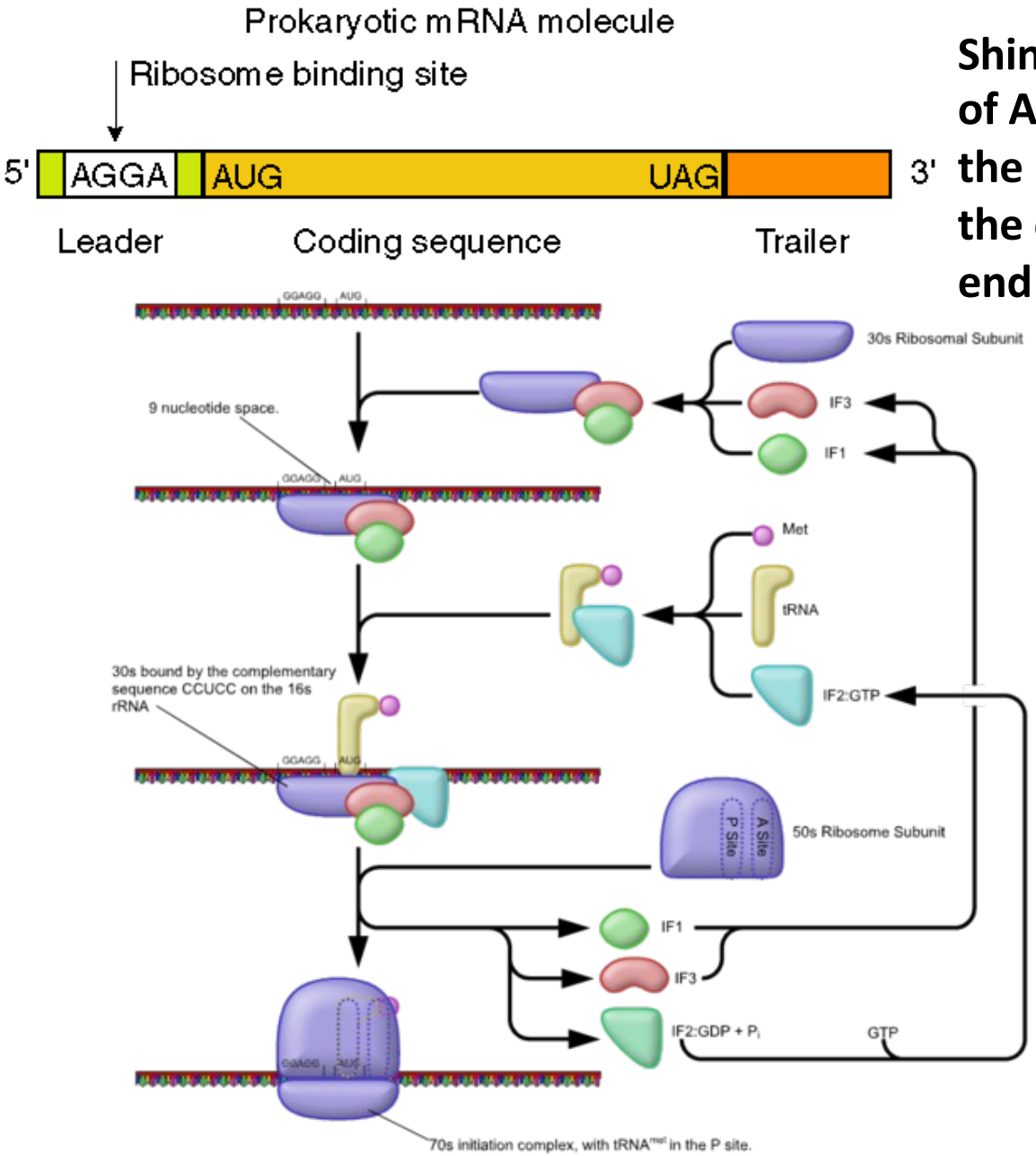
Eukaryotes



- cap-dependent translation
- ribosome scanning for translation initiation

Belasco, Nat.Rev.Mol.Cell.Biol, 2012

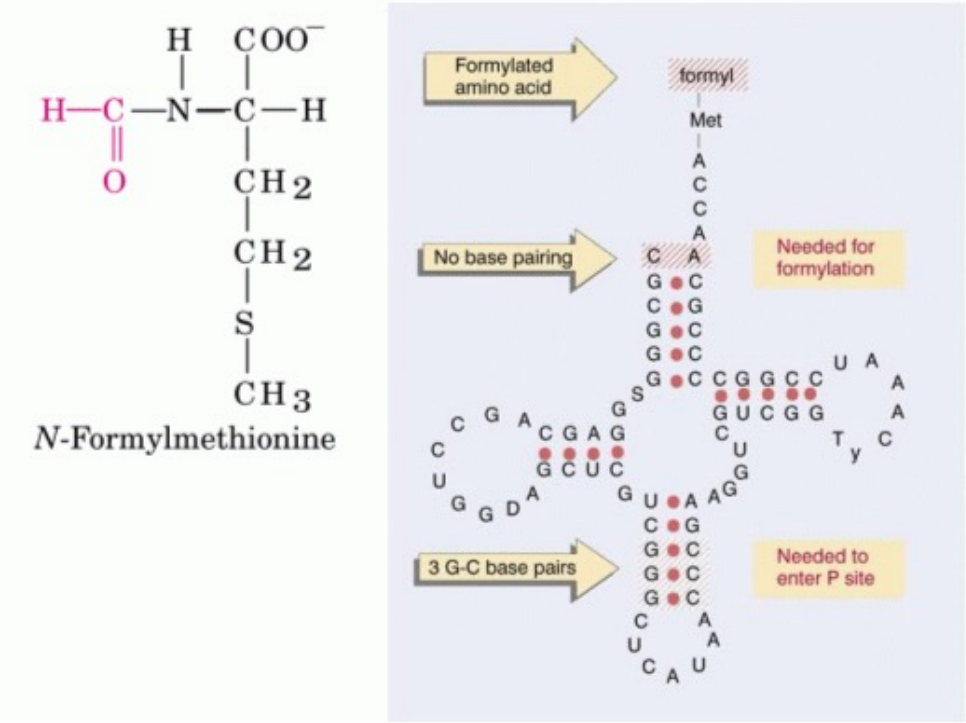
Translation in bacteria



Shine-Dalgarno sequence upstream of AUG start codon helps to recruit the ribosome by interacting with the complementary region in the 3' end of 16S rRNA

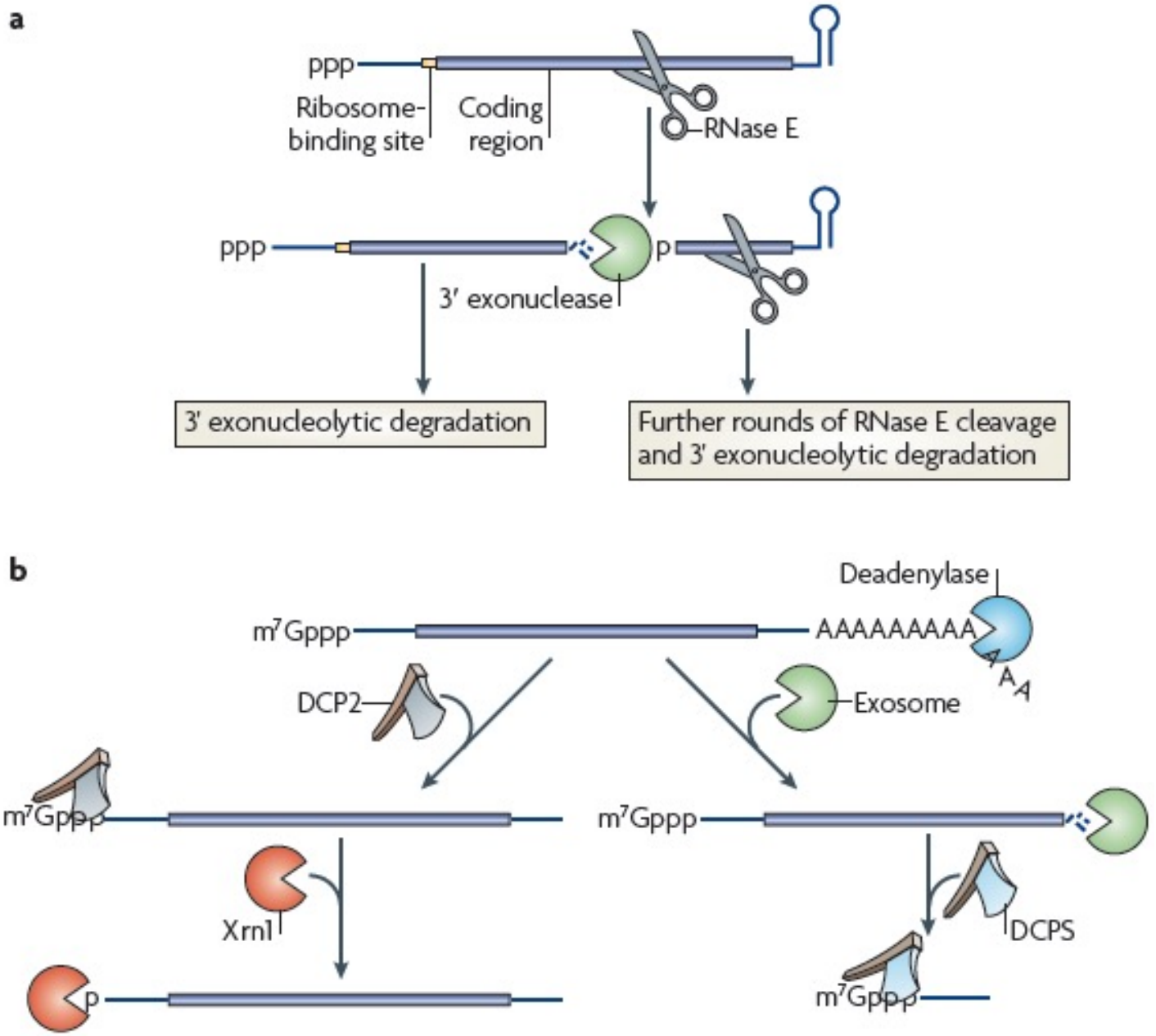
see a movie at:
https://www.youtube.com/watch?v=4V0suv7fk3s

$tRNA^{Met}$ versus $tRNA^{fMet}$



- **$tRNA^{fMet}$ - intitator tRNA in bacteria and organells** (mitochondria, chloroplasts)
- formyl group can be removed posttranslationally by methionine aminopeptidase following deformylation by peptide deformylase
- fMet uses specific tRNA (3'-5' UAC anticodon)
- in Eukariota and Archaea normal $tRNA^{Met}$ is used

mRNA decay



Belasco, Nat.Rev.Mol.Cell.Biol, 2012

RNA enzymes

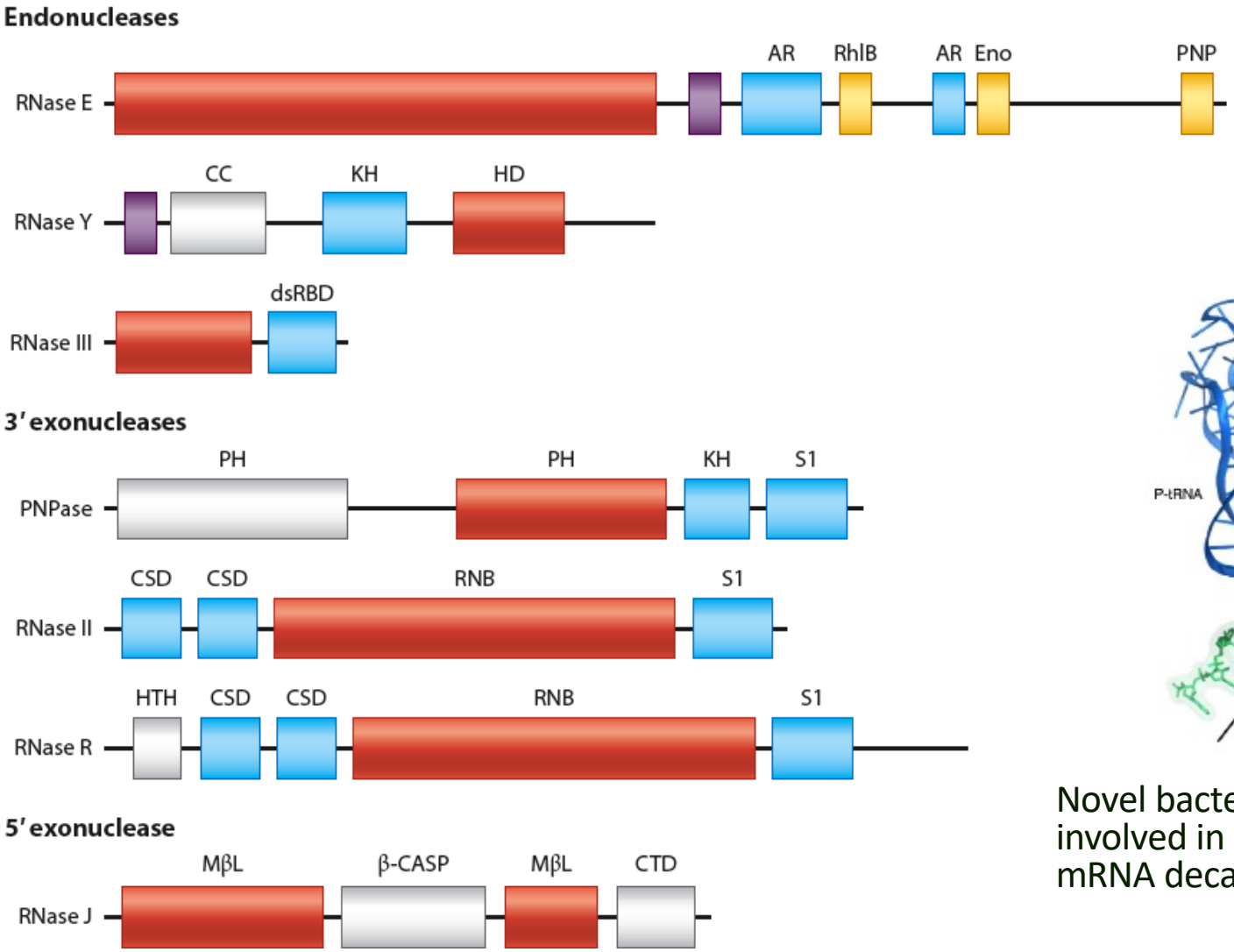
Bacteria vs Eukaryotes

Table 1 | **Enzymes of broad importance for cytoplasmic mRNA decay**

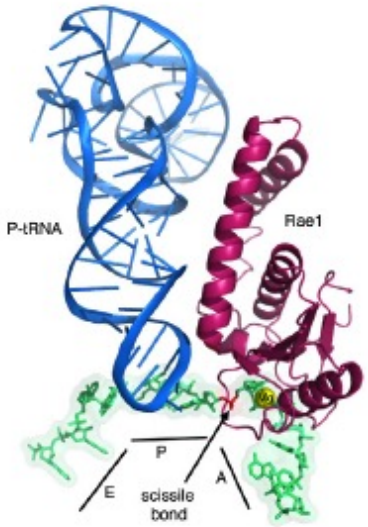
Kingdom	Enzyme	Specificity and/or function
Endonucleases		
Bacteria	RNase E* and RNase G*	Single-stranded RNA
	RNase III	Double-stranded RNA
	RNase J	Single-stranded RNA
	RNase Y	Single-stranded RNA
	Cmr complex	mRNA–CRISPR RNA duplexes
Eukaryotes	Argonaute	mRNA–siRNA or mRNA–miRNA duplexes that are fully paired
	SMG6	PTC-containing mRNAs
5′-end modification		
Bacteria	RppH	Pyrophosphate removal
Eukaryotes	DCP2	Decapping of RNA polynucleotides
	DCPS	Decapping of RNA oligonucleotides
3′-end modification		
Bacteria	Poly(A) polymerase (PcnB)	Polyadenylation
	Polynucleotide phosphorylase	Heteropolymeric tail addition
Eukaryotes	CCR4–NOT	Deadenylation
	PAN2–PAN3	Deadenylation
	PARN	Deadenylation
	Cid1* and ZCCHC11*	Oligouridylation
3′ exonucleases		
Bacteria	Polynucleotide phosphorylase	Single-stranded 3′ end
	RNase R	Single-stranded 3′ end
	RNase II	Single-stranded 3′ end
	Oligoribonuclease	RNA oligonucleotides
Eukaryotes	Exosome	3′ end not protected by PABP
5′ exonucleases		
Bacteria	RNase J	Monophosphorylated 5′ end
Eukaryotes	XRN1	Monophosphorylated 5′ end

Belasco, Nat.Rev.Mol.Cell.Biol, 2012

Bacterial exo- and endo-nucleases



Huiet al, Annu Rev genet, 2015

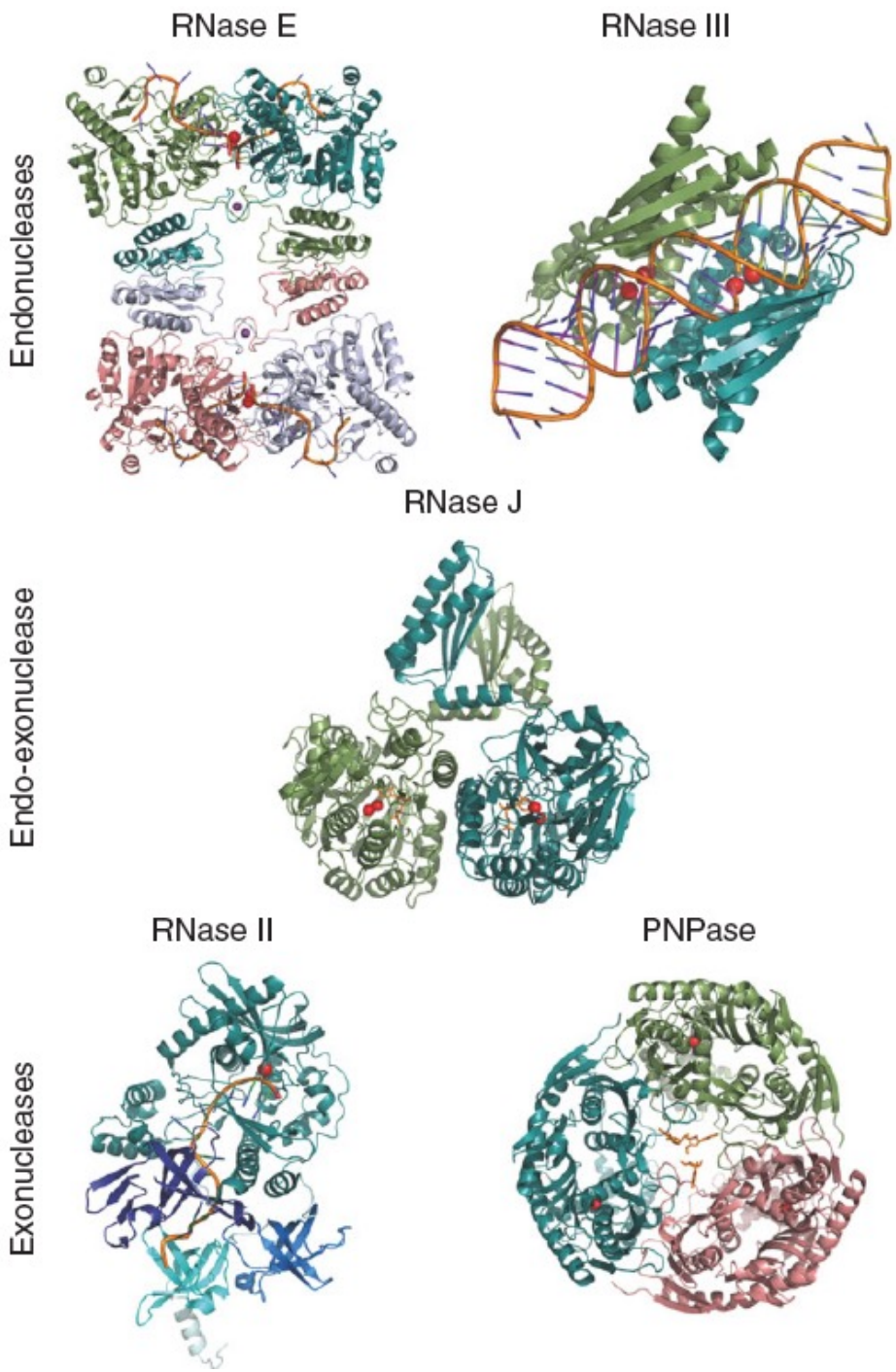


Novel bacterial endonuclease Rae1 involved in ribosome-dependent mRNA decay in *Bacillus subtilis*

Leroy et al, EMBO, 2017

Prokaryotic RNases

Family	RNases	Characteristics
Exonucleases 3'→ 5'		
RNR	RNase II	nonspecific processive, degrades only ssRNA, mRNA decay
	RNase R	nonspecific processive, degrades ssRNA and dsRNA, mRNA decay
DEDD	RNase D	distributive, small RNA and stabile RNA processing
	RNase T	
	Oligoribonuclease	specific for oligoribonucleotides
RBN	RNase BN/Z	distributive exonuclease 3'- 5' and endonuclease, tRNA processing
PDX	PNPase	phosphorolytic processive, degradosome subunit, KH/S1 RNA BD domains, degrades ss/dsRNA
	RNase PH	phosphorolytic distributive
Exonucleases 5'→3'		
*RNAse J1/J2		present in *Bacillus subtilis*, specific for 5' monoP ssRNA, mRNA decay
Endonucleases		
RNase III		dsRNA specific, rRNA, tRNA, mRNA processing, mRNA degradation
RNase E		degradosome subunit, mRNA decay; rRNA tRNA and RNaseP RNA processing
RNase G		similar to RNase E
RNase I		nonspecific, mRNA degradation
RNase H		specific for RNA:DNA hybrid
RNase P		tRNA 5' end processing
RNase Z		tRNA 3' end processing
Rae1/YacP		ribosome-dependent mRNA decay in *Bacillus subtilis*
*RNAse J1/J2		mRNA decay in *Bacillus subtilis*
RNase Y		mRNA decay in *Bacillus subtilis*
MazF/EndoA		toxin, mRNA degradation in stress conditions, sequence specific
RNAse M5		5S rRNA maturase in *Bacillus subtilis*



Structures of bacterial RNA enzymes in complex with substrates

Silva et al, WIREsRNA 2011

Degradation of bacterial mRNAs

Degradosome - major complex involved in mRNA decay in bacteria, functions as dimer

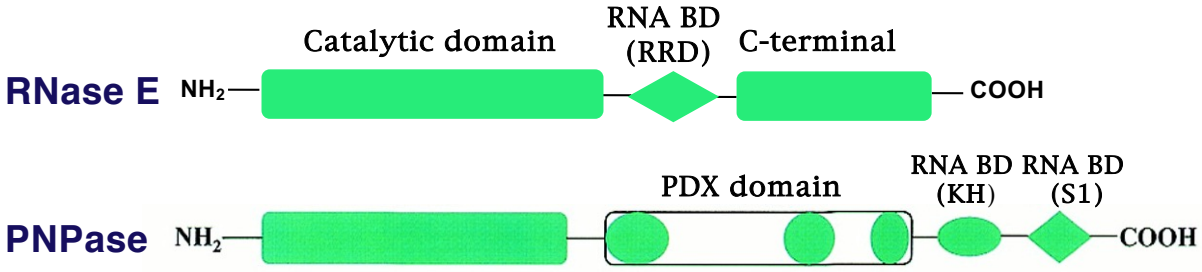
RNase E 5'-phosphate -dependent endoribonuclease, N-terminal nucleolytic domain, C-terminal protein binding domain, central RNA binding domain (BD)

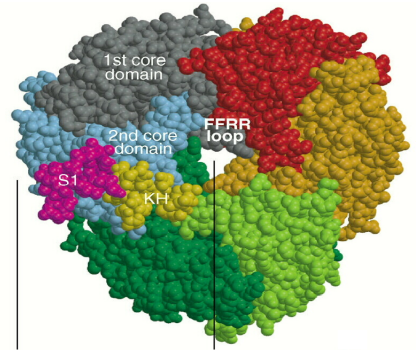
PNPase phosphorolytic processive exonuclease 3' - 5', KH and S1 RNA BD

RhlB ATP-dependent helicase, DEAD box, stimulate degradation of structured RNA regions

Enolase glycolytic enzyme

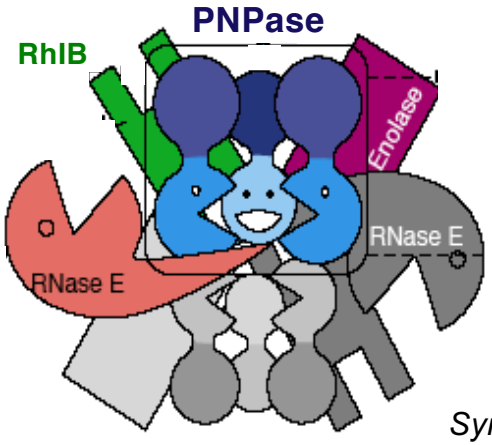
additional DnaK/GroEL chaperons , poliphosphate kinase, poly(A) polymerase, S1 ribosomal protein





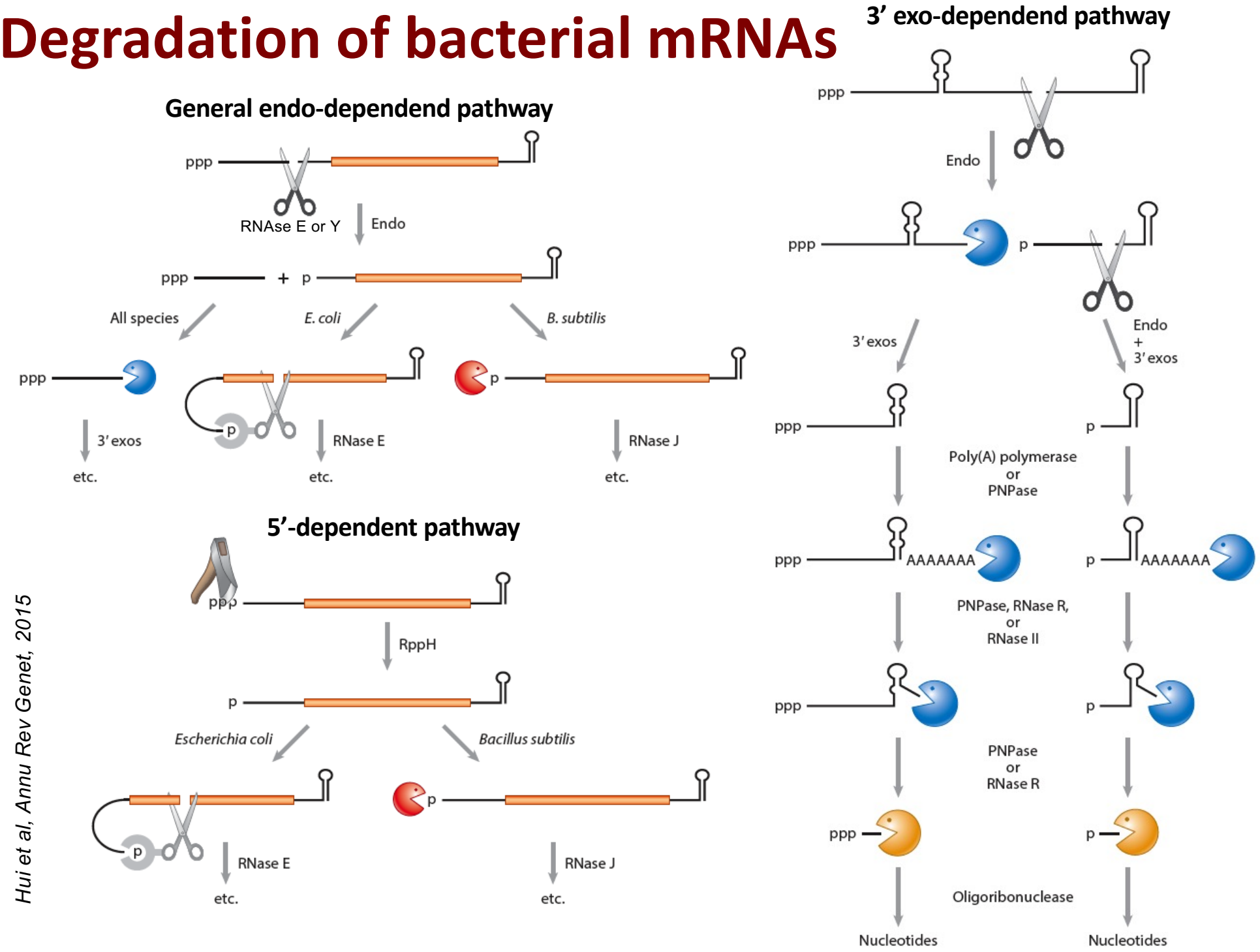
PNPase trimer

Symmons et al, Structure, 2000



Symmons et al, TiBS, 2002

Degradation of bacterial mRNAs

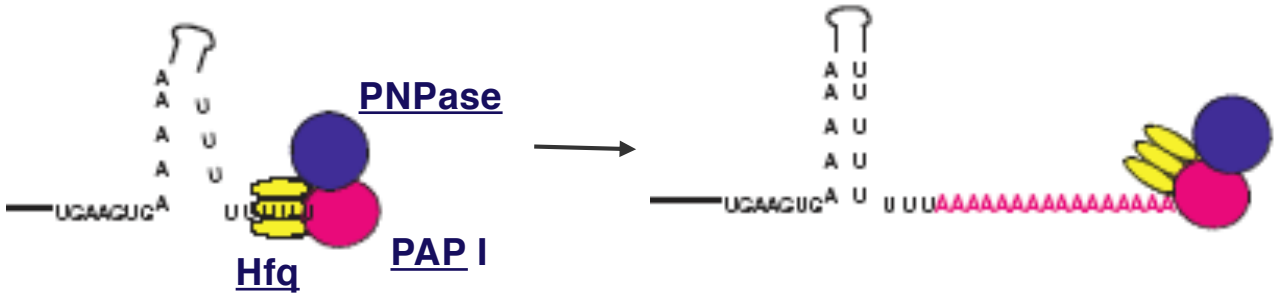


Hui et al, Annu Rev Genet, 2015

Degradation of bacterial mRNAs

3’ end stem-loop structure of transcripts targeted for degradation becomes often polyadenylated by PAP (poly(A) polymerase) and PNPase (polynucleotide phosphatase), with the help of Hfq (hexameric RNA chaperone).

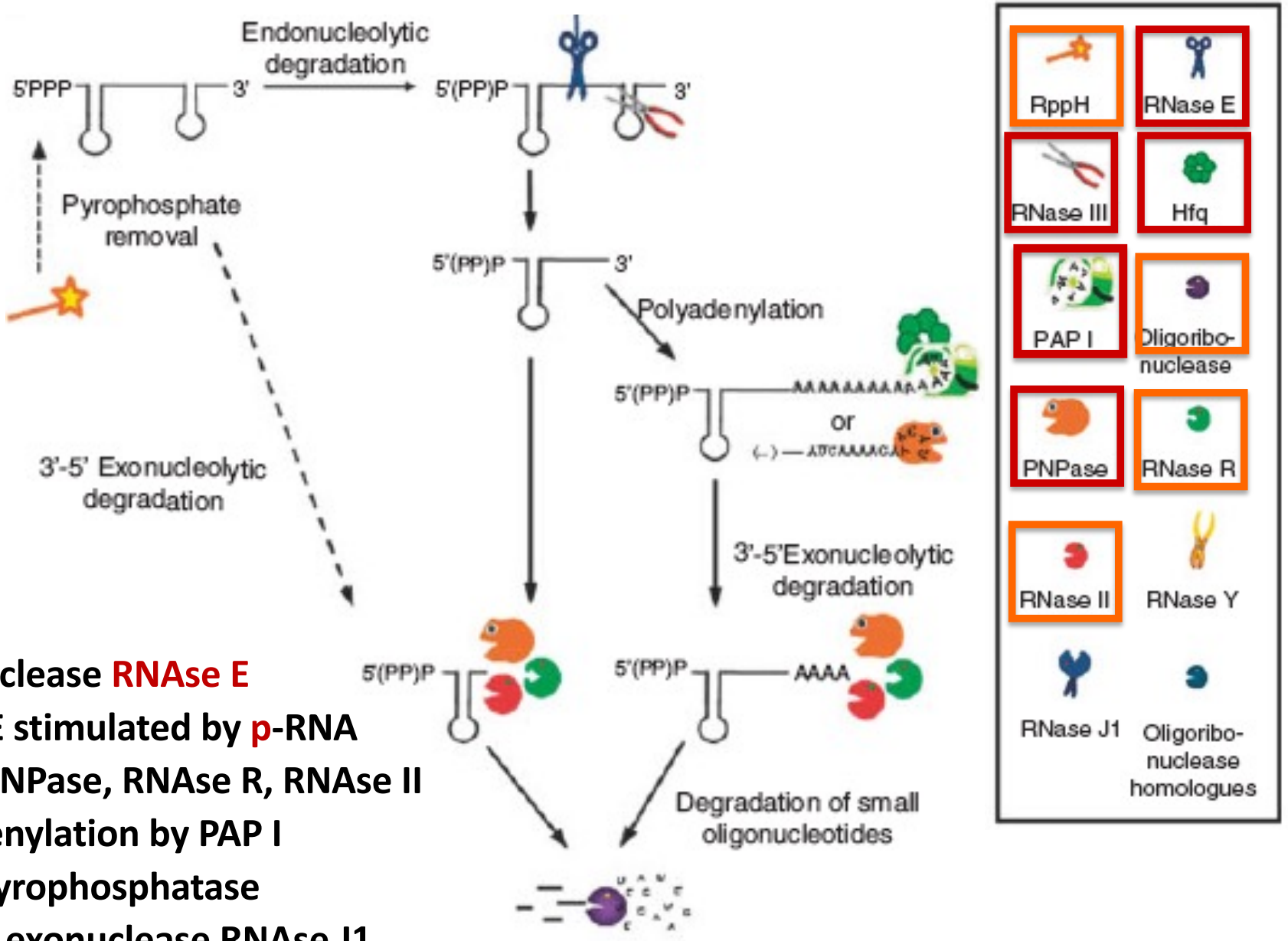
RNase E cleavage initiates degradation by 3’ - 5’ exonucleases, mainly RNase II, RNase R and PNPase.



Mohanty et al, Mol. Microbiol., 2004

Symmons et al, TiBS, 2002

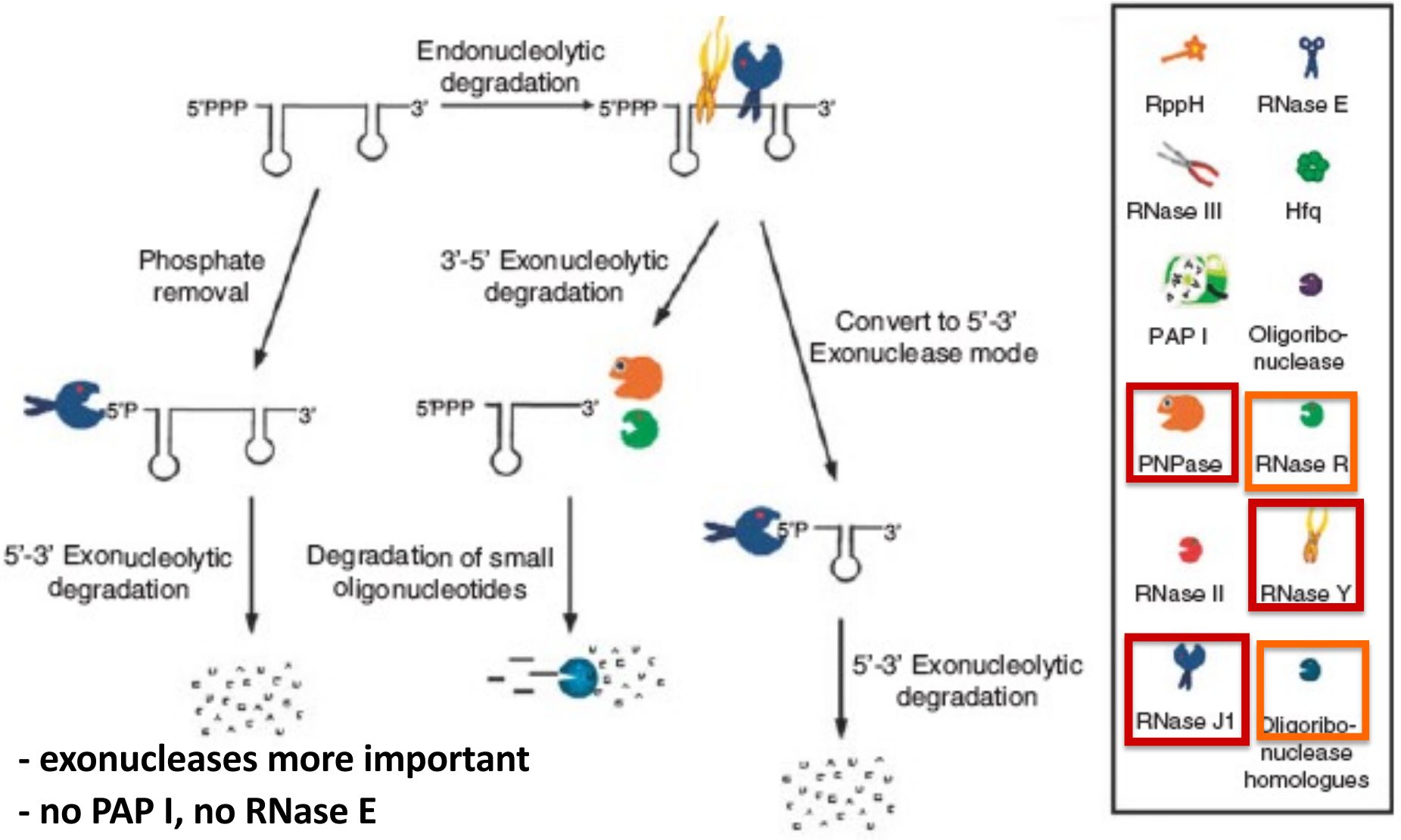
mRNA decay in bacteria *E. coli*



- endonuclease RNAse E
- RNase E stimulated by p-RNA
- 3' exo PNPase, RNAse R, RNAse II
- polyadenylation by PAP I
- RppH pyrophosphatase
- no 5'-3' exonuclease RNAse J1
- no endo RNase Y

Silva et al, WIREsRNA, 2011

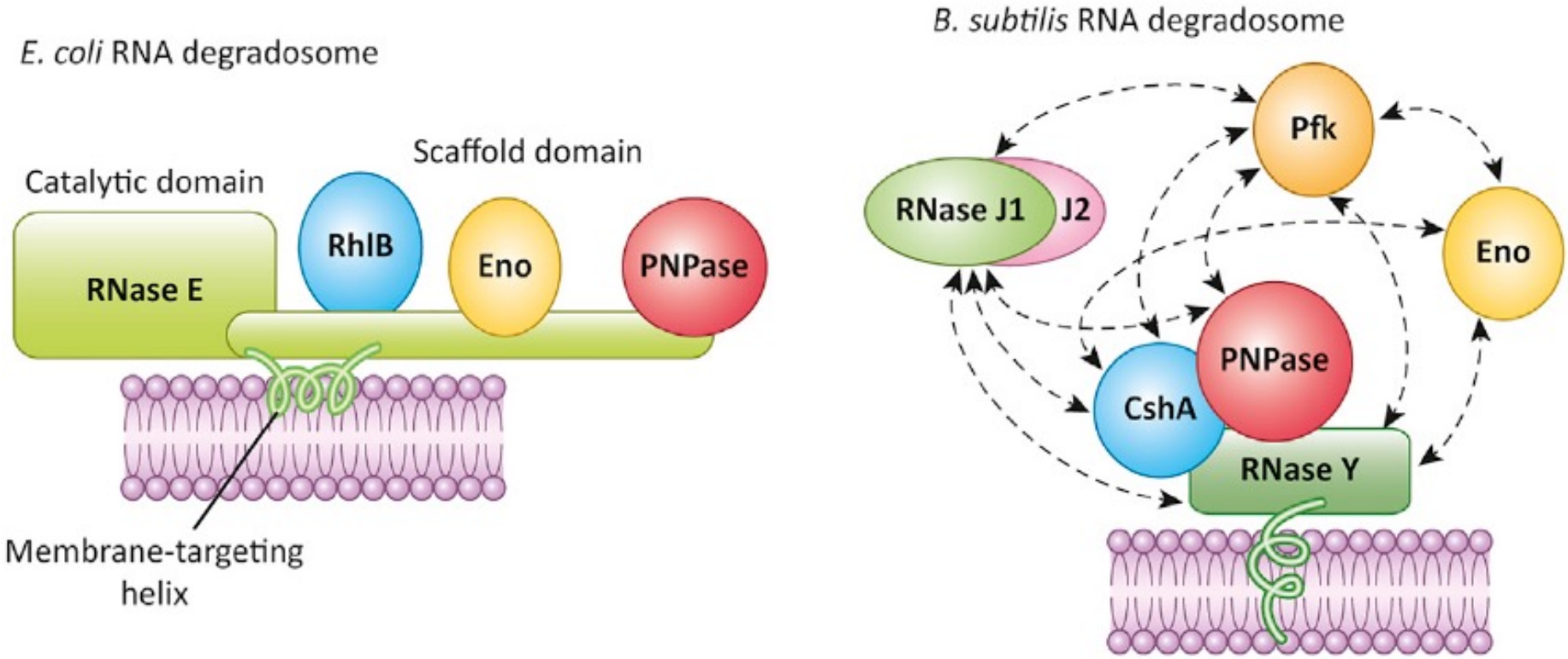
mRNA decay in bacteria *B. subtilis*



- exonucleases more important
- no PAP I, no RNase E
- PNPase RNase R
- 5'-3' exonuclease RNase J1 (5' exo + endo)
- endo RNase Y

Silva et al, WIREsRNA, 2011

Bacterial RNA degradosomes

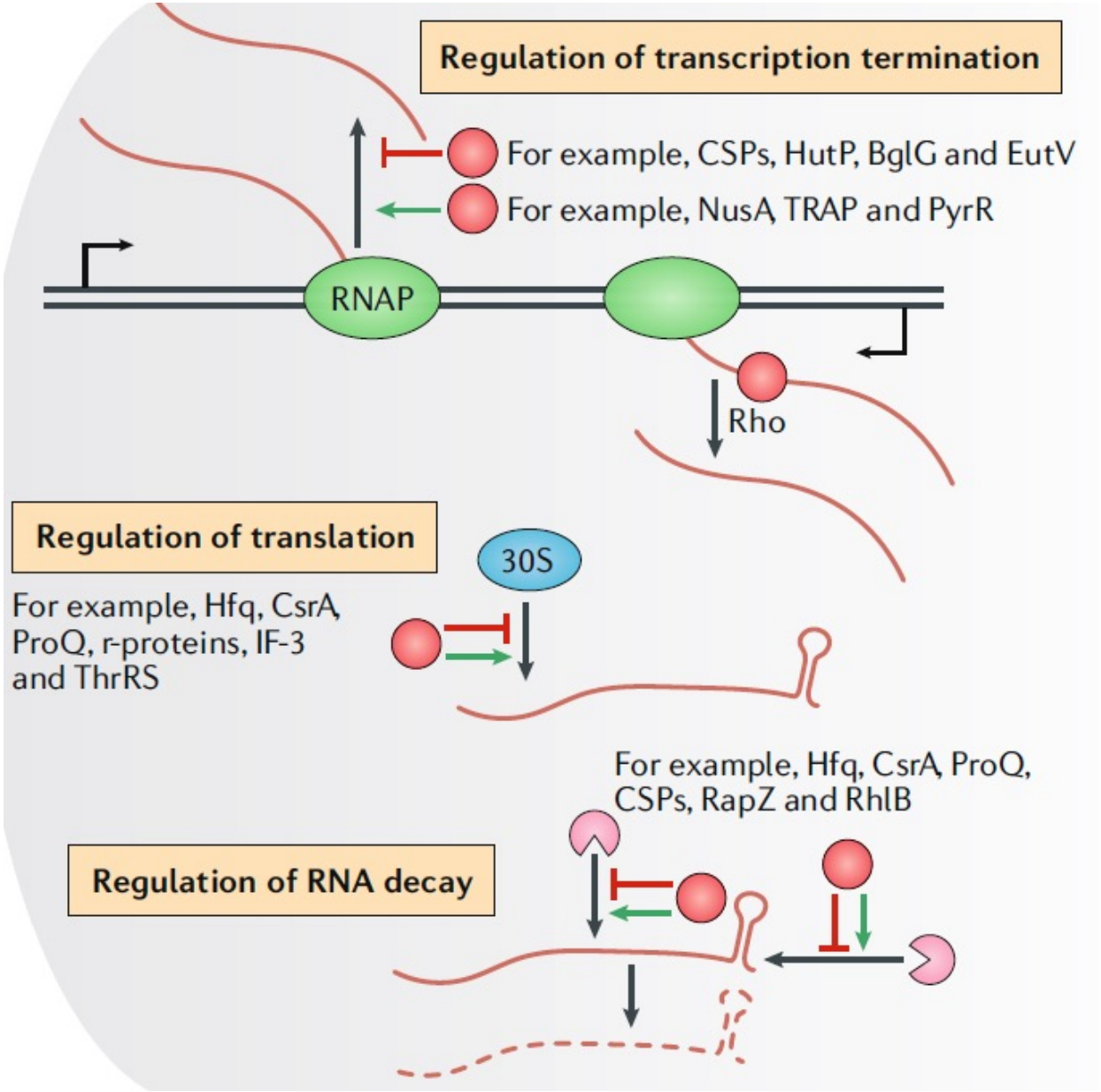


Degradosomes vary in different prokaryotic organisms

They are regulated by autoregulation, protein post-translational modifications, binding partners, organization in foci, targeting to bacterial membranes

Tejada-Arranz et al, TiBS, 2019

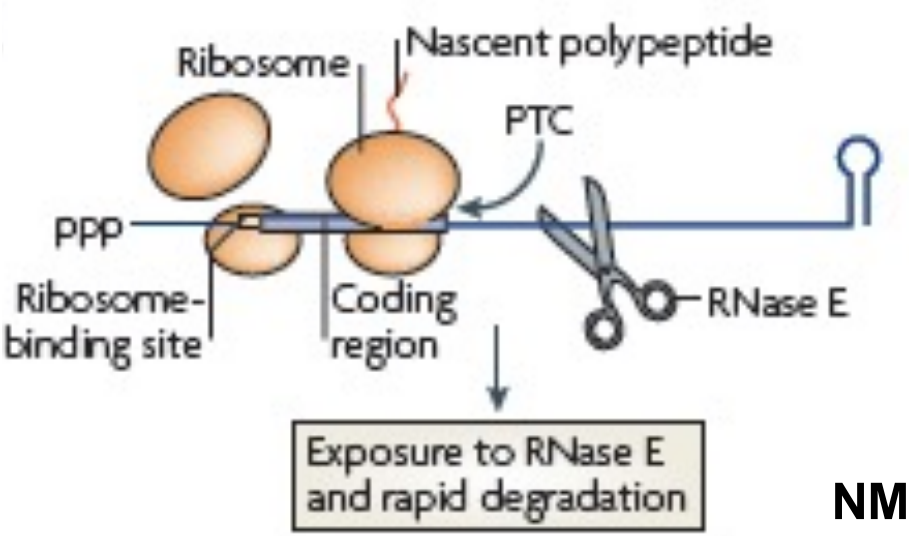
Regulation by RNA Binding Proteins (RBPs)



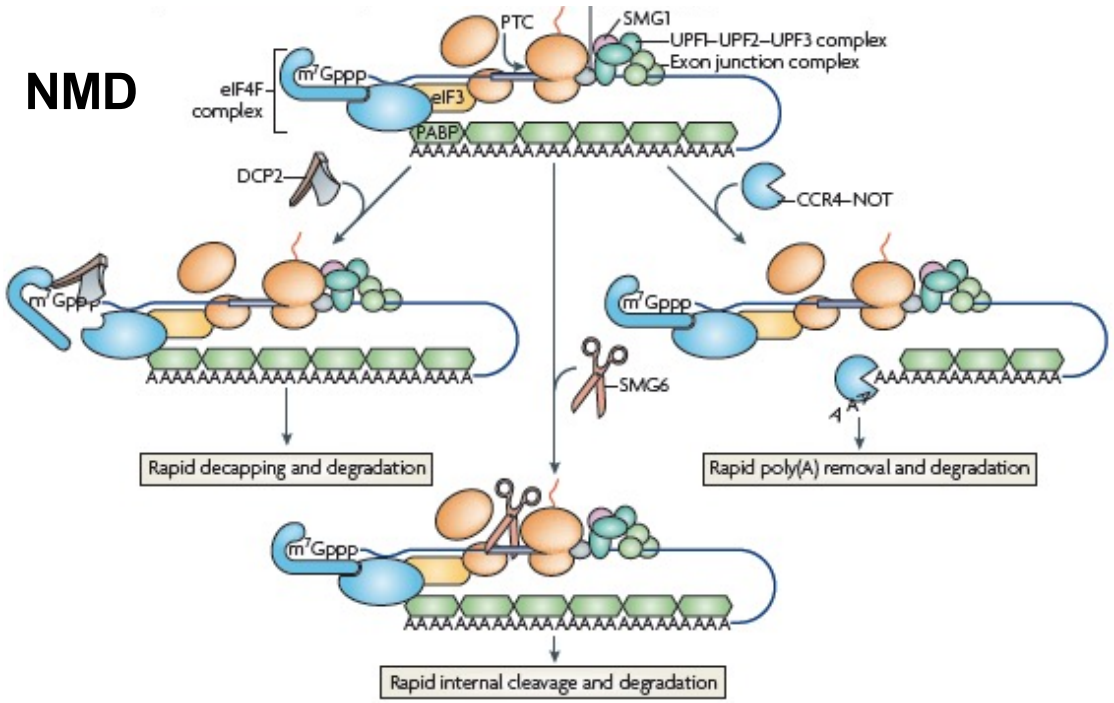
Holmqvist and Vogel, Nat Rev Micro 2018

Specialized mRNA decay

BACTERIA vs EUKARYA

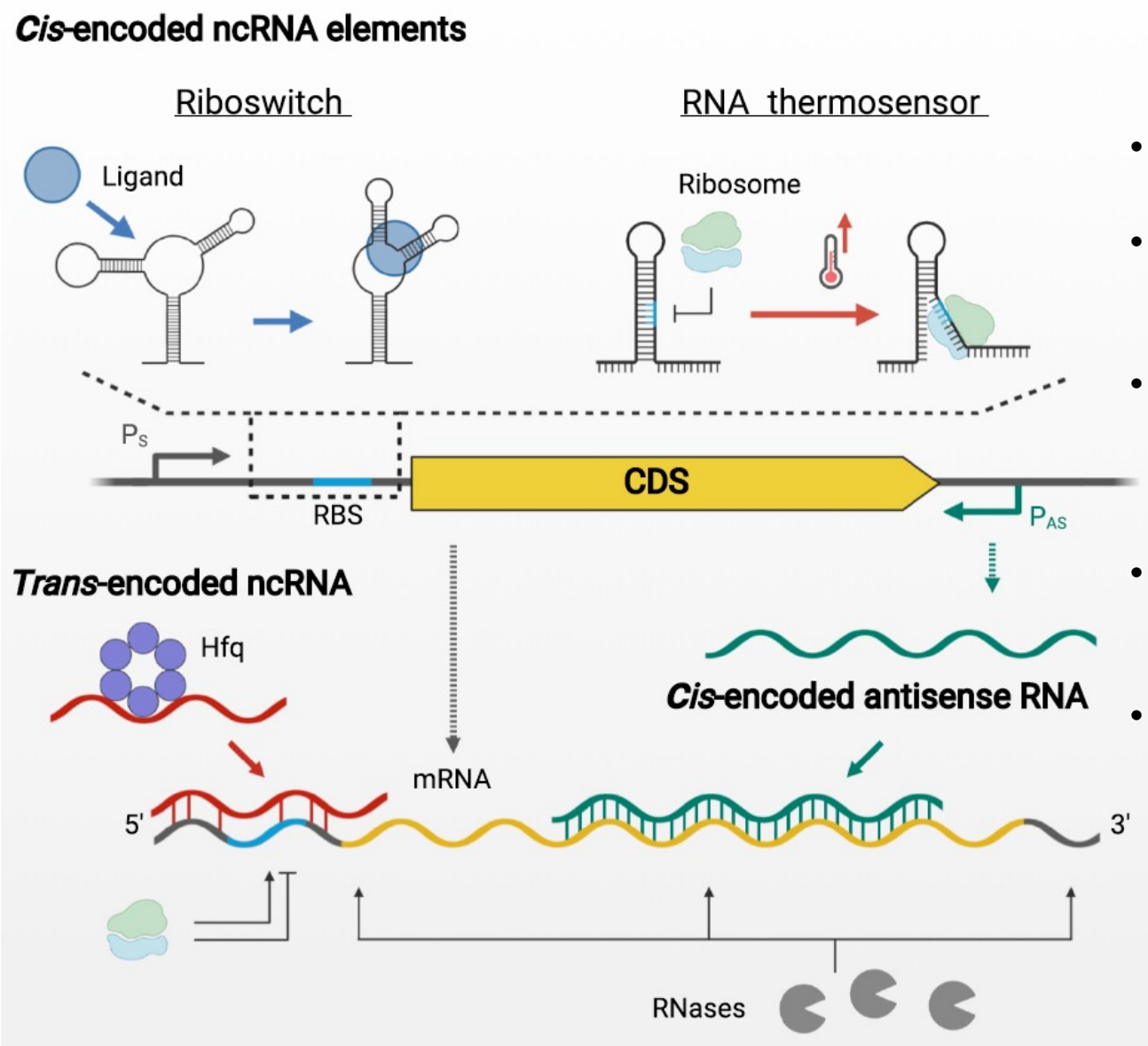


Nonsense Mediated Decay: degradation of aberrant mRNAs containing premature STOP codon



Belasco, Nat.Rev.Mol.Cell.Biol, 2012

sRNAs in bacteria

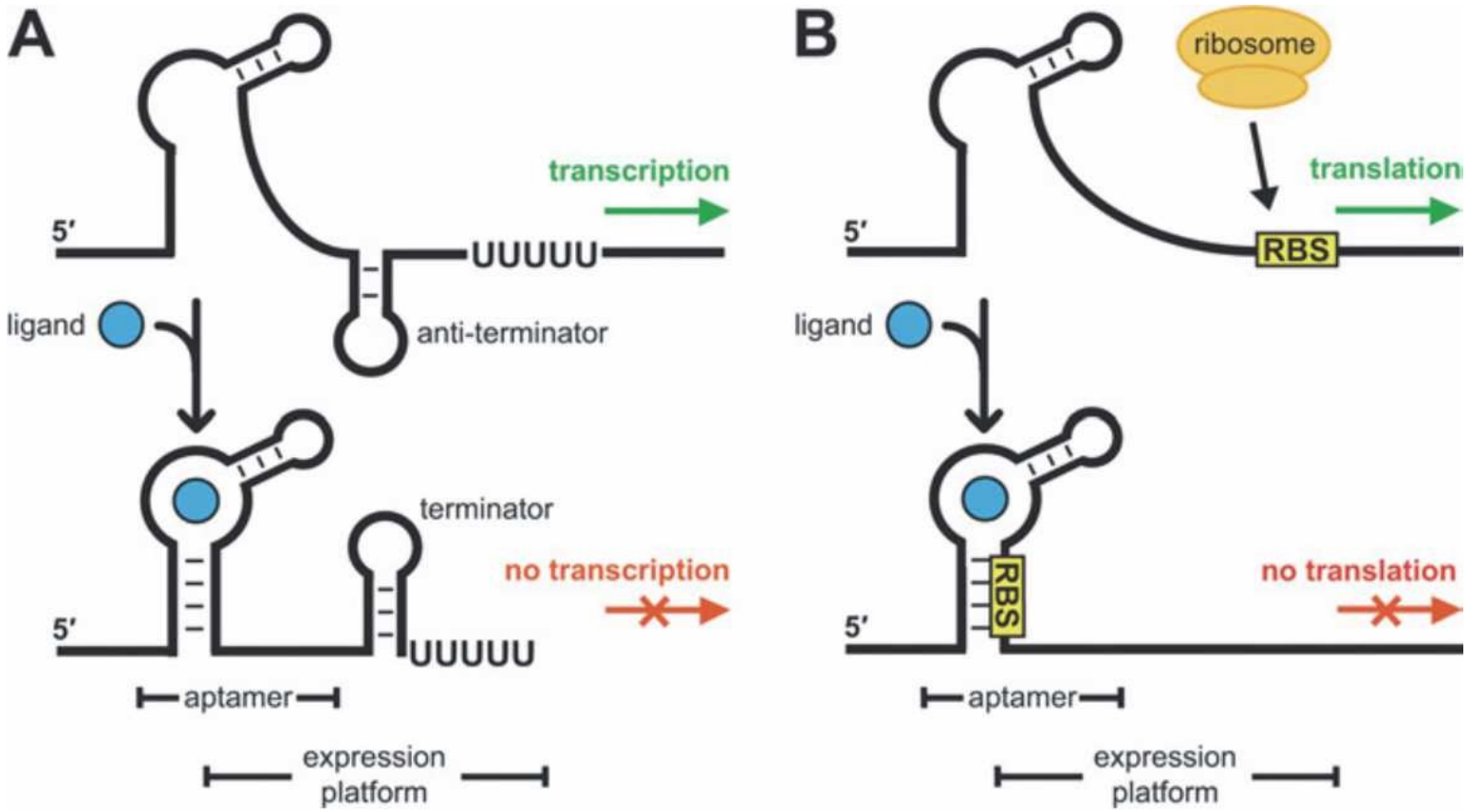


- ***Cis*-encoded ncRNAs** are located in mRNA 5'-UTRs
- **Riboswitches** bind ligands, which modulates mRNA transcription or translation
- **RNA thermosensors** change secondary structure in different temperatures, which affects translation
- ***Cis*-encoded antisense RNAs** base-pair to target mRNA and induce degradation by RNases
- **Transencoded ncRNAs** interact by imperfect base-pairing to target mRNA 5'-UTRs, assisted by RNA chaperone proteins (e.g. Hfq). This either affects mRNA stability (via degradation), or translation (via RBS accessibility)

Eichner et al, TiMicro, 2022

Riboswitches are more common in bacteria

- RNA elements that undergo structural change in response to binding of a regulatory small effector molecule
- usually act in cis to regulate the transcript in which they are encoded
- used to sense cellular metabolism



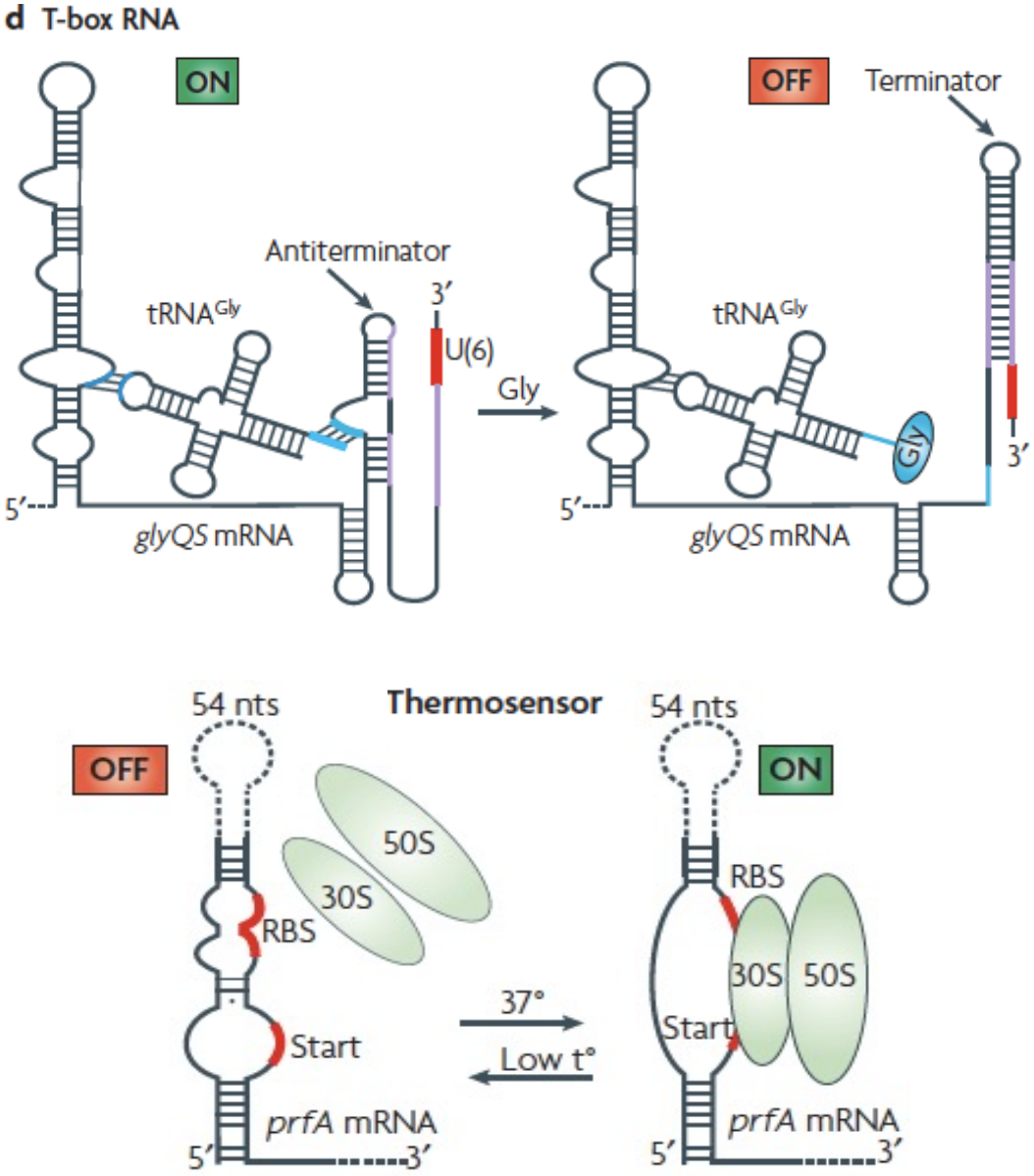
http://www.umich.edu/~rnapeopl/WalterSummaryRiboswitch.htm

Types of riboswitches

RNA switches						
Thermosensors			Gene control		Variable	Phages, bacteria, eukaryotes
sRNAs			Gene control	Hfq	>85	Bacteria
T-boxes			Gene control	tRNA	190	Mostly Gram+ bacteria
Metabolites	Coenzymes	TPP	Gene control	TPP	100	Bacteria, archaea, eukaryotes (fungi, plants)
		FMN	Gene control	FMN	120	Bacteria
		AdoCbl	Gene control	AdoCbl	200	Bacteria
		SAM-I	Gene control	SAM	105	Mostly Gram+ bacteria
		SAM-II	Gene control	SAM	60	α- and β-proteobacteria
		SAM-III (S_{MK})	Gene control	SAM	80	Gram– bacteria
	Amino acids	Lysine	Gene control	Lysine	175	γ-proteobacteria, *Thermotogales*, *Firmicutes*
		Glycine (I+II)	Gene control	Glycine	110	Bacteria
	Nucleobases	Guanine	Gene control	Guanine, hypoxanthine	70	Gram+ bacteria
		Adenine	Gene control	Adenine	70	Bacteria
		$preQ_1$	Gene control	$preQ_1$	35	Bacteria
Magnesium		*mgtA*	Gene control	Mg^{2+}	70	Gram– bacteria

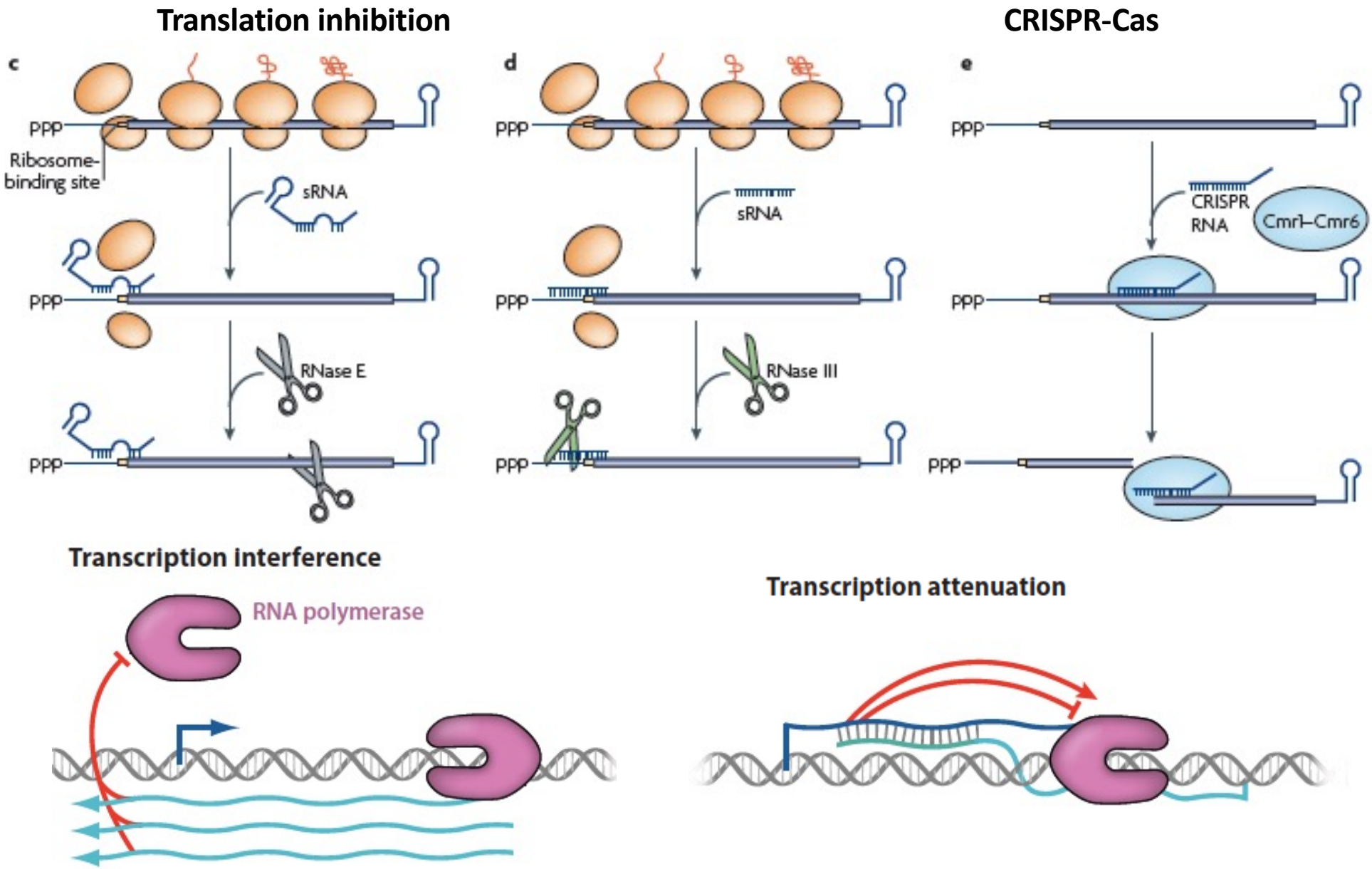
Serganov and Patel, Nat.Rev.Genet, 2007

Riboswitches



Condon and Bechhofer, Cur.Op.Microbiol., 2011; Serganov and Patel, Nat.Rev.Genet, 2007

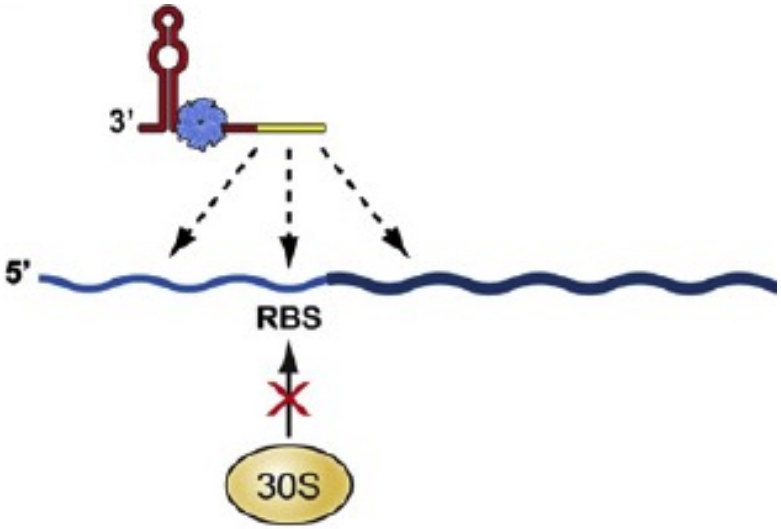
Regulation by sRNAs in bacteria



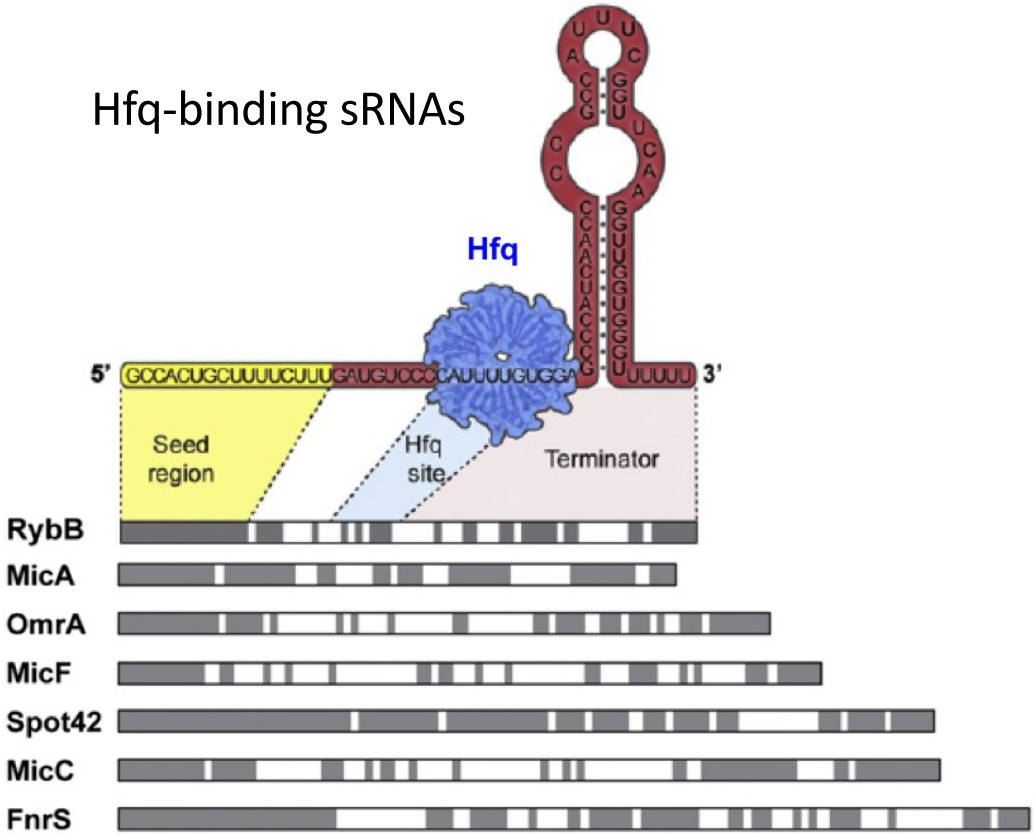
Belasco, Nat.Rev.Mol.Cell.Biol, 2012; Thomason and Storz, Ann.Rev.Genet, 2010

sRNAs in bacteria

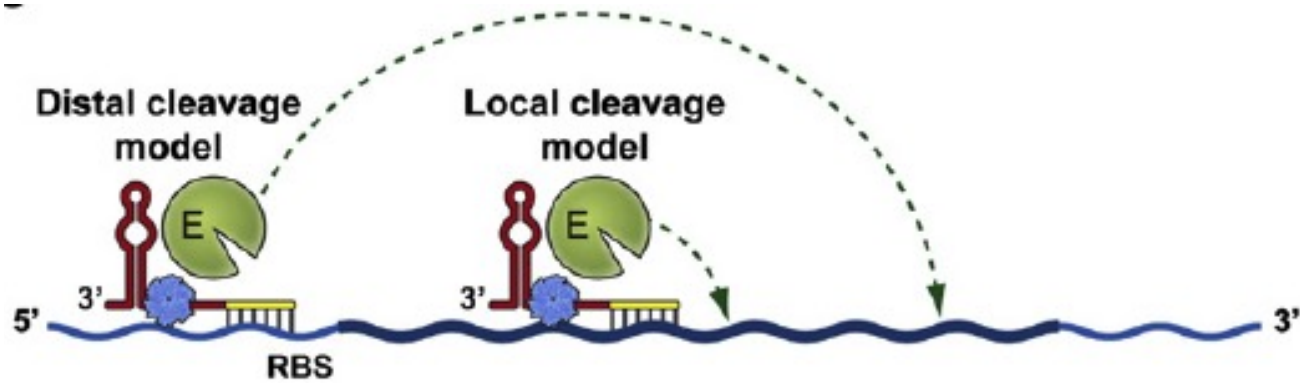
sRNA/Hfq block RBS
and inhibit translation



Hfq-binding sRNAs



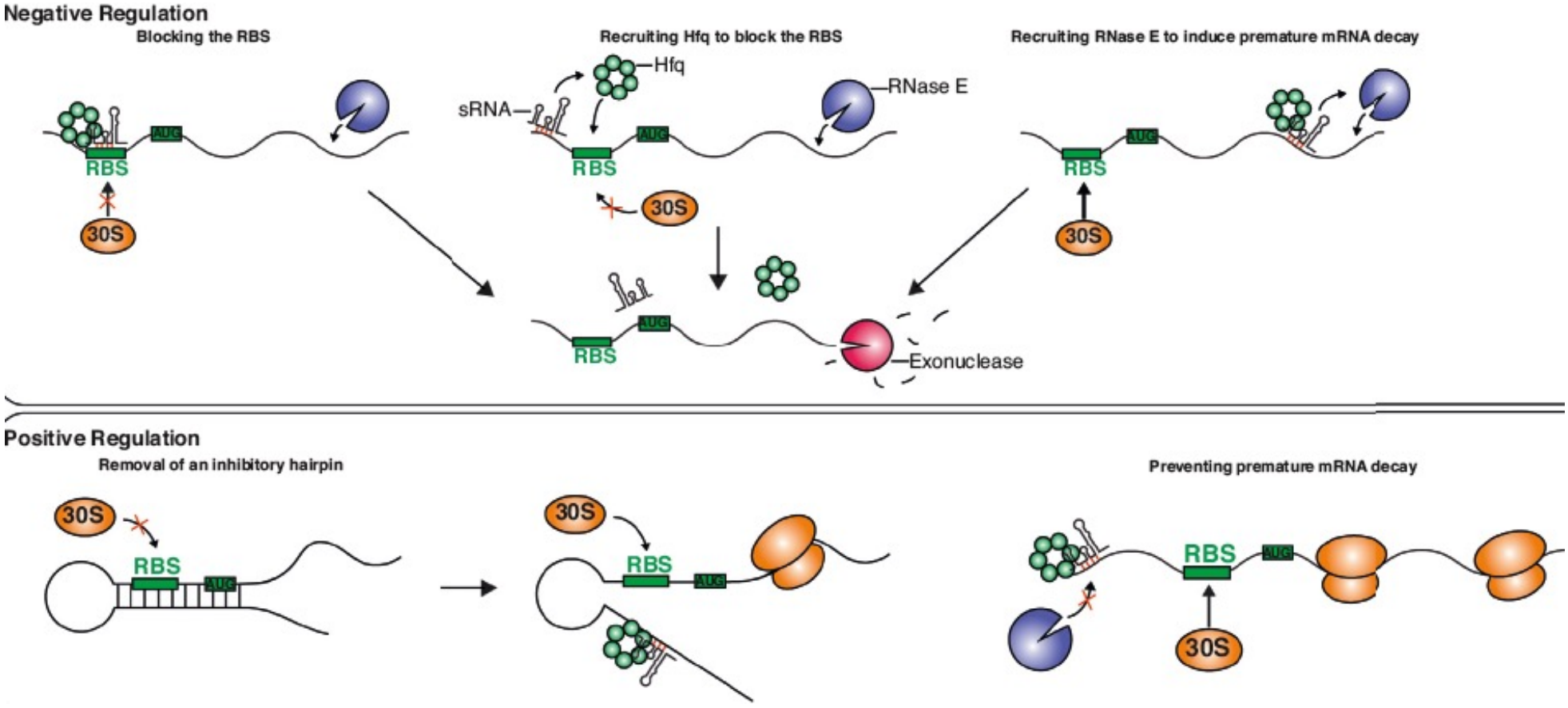
sRNA/Hfq base-pair with target mRNA and
direct RNase-E –mediated degradation



Storz et al, MCell, 2011

sRNAs in bacteria

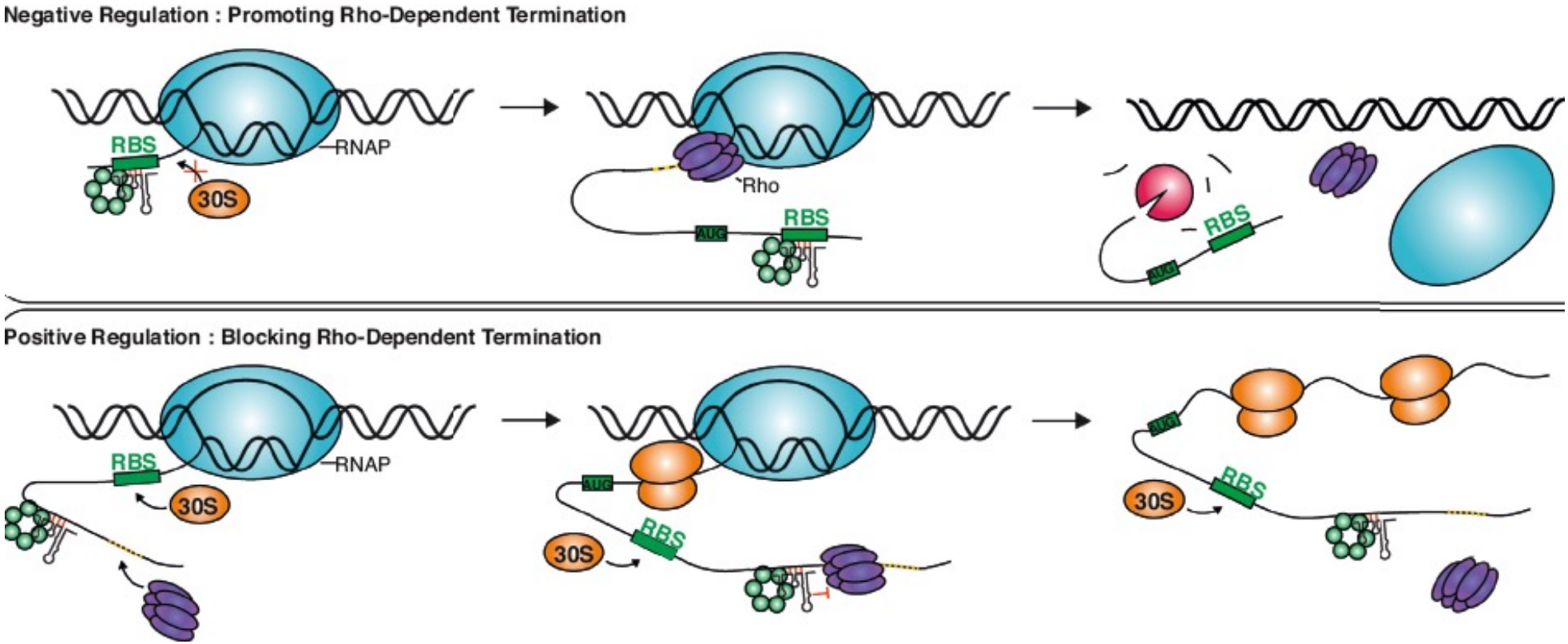
Regulation of translation initiation and/or mRNA decay



Kavita et al, Curr Op Micro, 2018

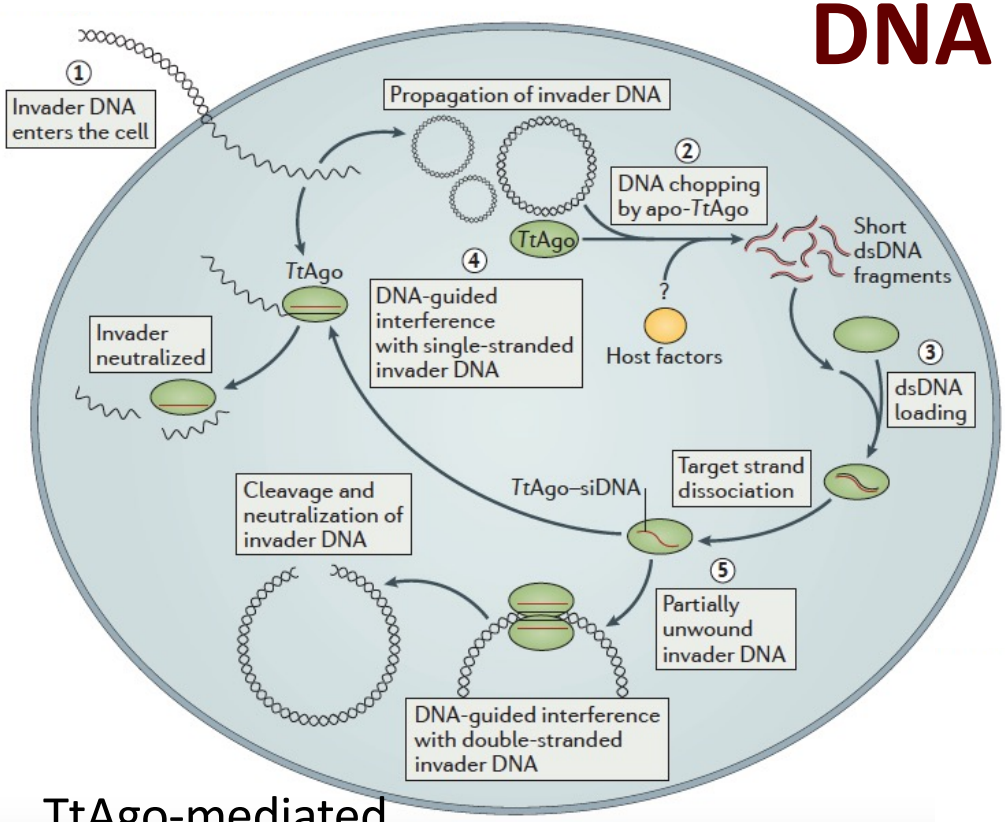
sRNAs in bacteria

Regulation of Rho-dependent transcription termination

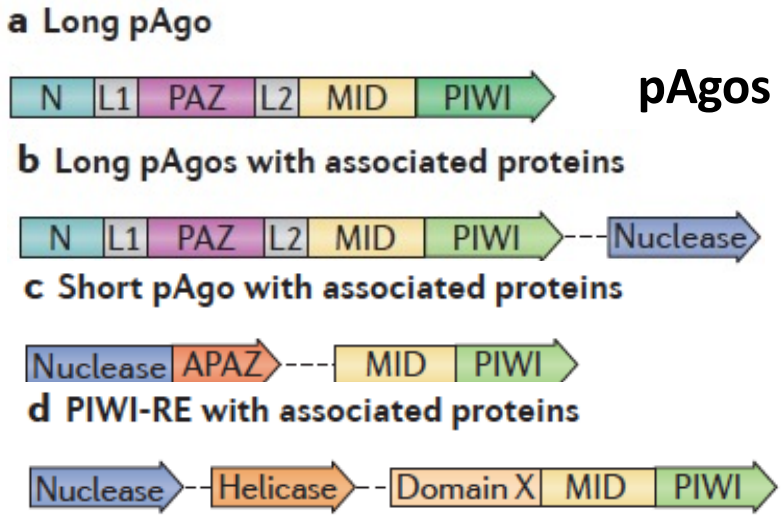


Kavita et al, Curr Op Micro, 2018

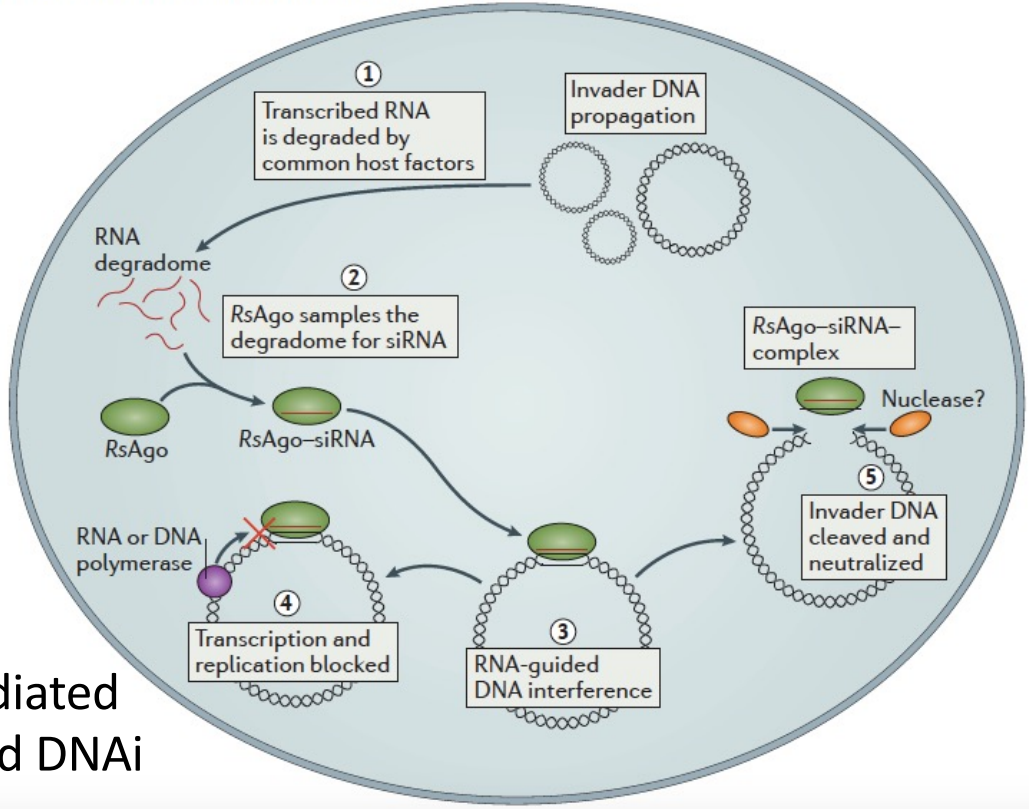
DNA interference in bacteria



TtAgo-mediated
DNA-guided DNAi



DNAi host defence systems:
pAgos interact with sDNA/sRNA that guide pAgos to cleave complementary foreign DNA



RsAgo-mediated
RNA-guided DNAi

Hegge et al, Nat Rev Micro, 2017

Bacterial polyadenylation

- Two bacterial 3' terminal polymerases:

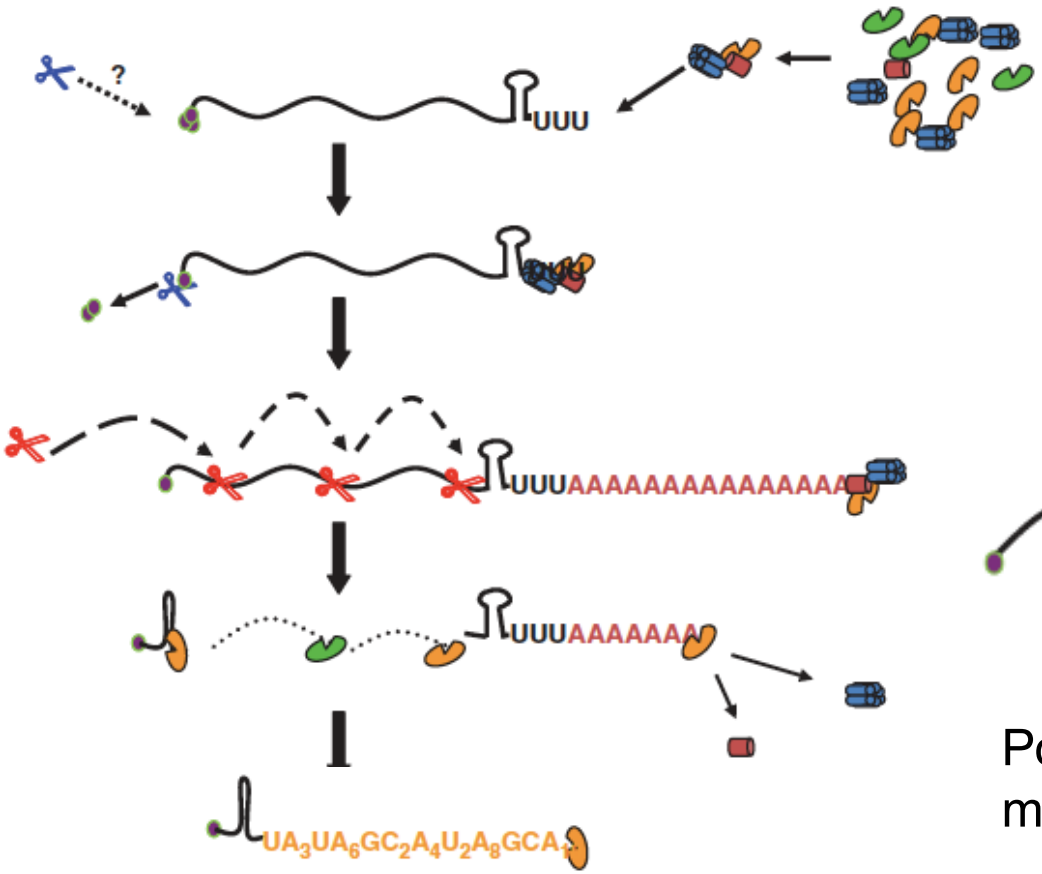
PAP I - Poly(A) (*E. coli*) and **PNPase -** Polynucleotide (*E. coli*, *B. subtilis*)

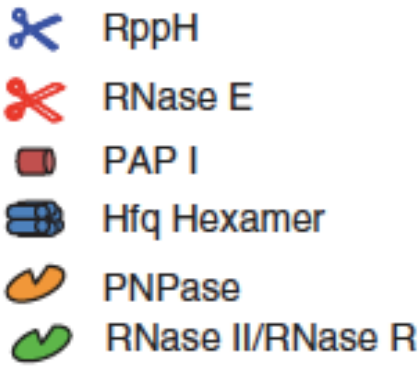
- poly(A) tails shorter (10-60 nts), occur for 2-60% of molecules of a given transcript
- polyadenylation sites are diverse, no consensus

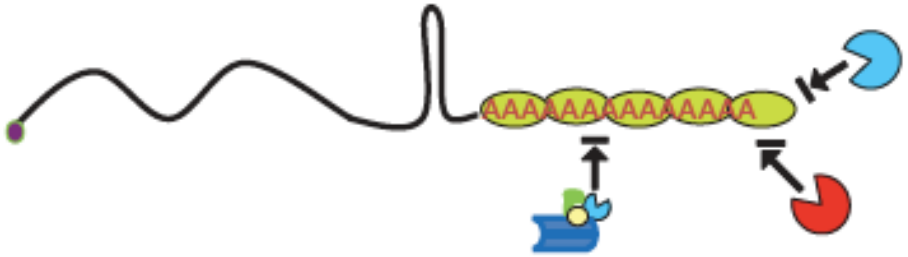
E. coli	mRNA	*lpp, rpsO, ompA, secG, rmf, pcnB, trxA*
	rRNA	16S rRNA, 23S rRNA
	nc RNA	6S RNA, 4.5S RNA, RNA I, SoK, SraK, SraL, GlmY, SsrA, RnpB
	tRNA	*cysT, hisR, leuX, trpT, leuU, tyrT, tyrV*
B. subtilis	mRNA	*mpB, rpsD, cry1Aa*
	rRNA	23S rRNA
	tRNA	$tRNA^{Cys\text{-}LeuU}$
Streptomyces	mRNA	*redD, actII-orf4, pnp, clpP, leuA*
	rRNA	16S rRNA, 23S rRNA
Synechocystis	mRNA	*rbcL*
	rRNA	23S rRNA
	tRNA	$tRNA^{Fmet}$

Mohanty and Kushner WIREsRNA, 2010

Polyadenylation-assisted RNA decay







Potential PolyA BP: Hfq, CspE, RP S1 may protect poly(A) tails

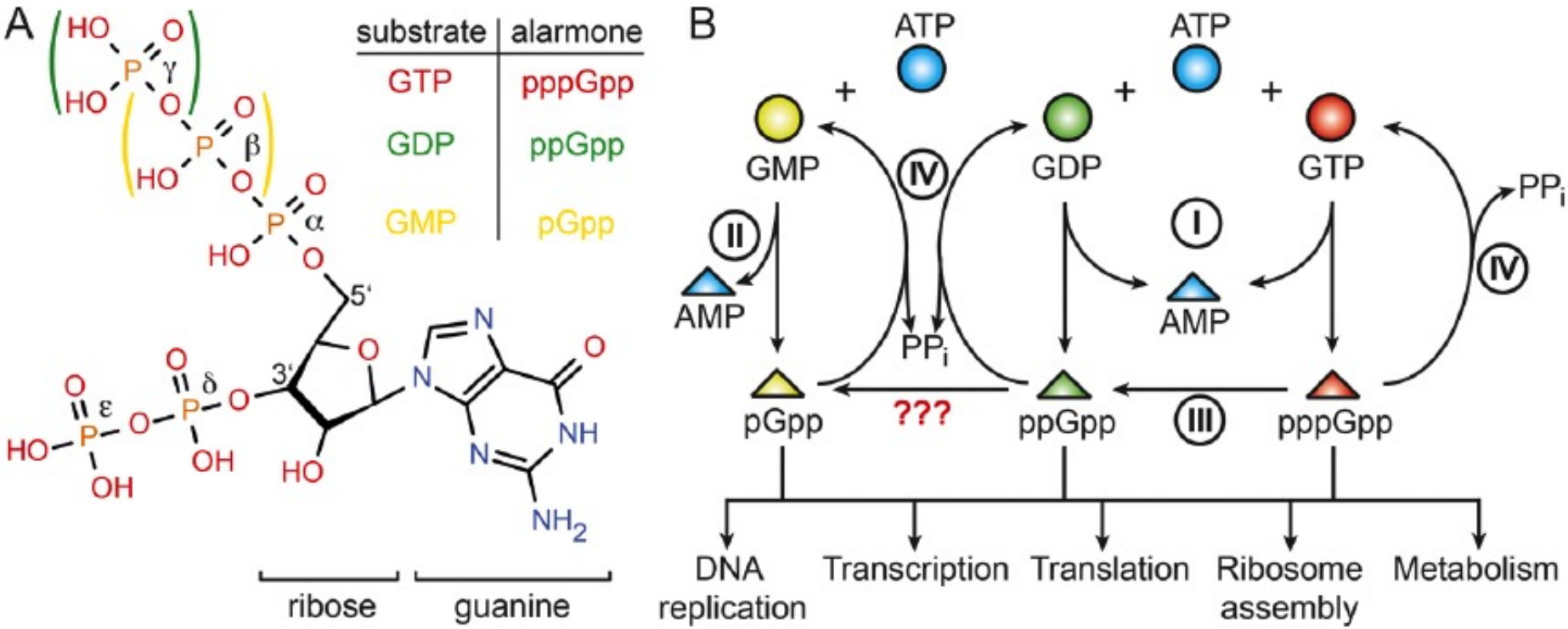
Hfq-mediated polyadenylation by PAP I in *E. coli*

- Hfq binds to the base of A/U-rich region of the Rho-independent terminator causing stem melting
- Hfq associates with PAP I and PNPase helping poly(A) tail addition
- PNPase degrades mRNA from the 3' end, additional 3'-5' degradation after endonucleolytic cleavage by RNase E

Mohanty and Kushner WIRERNA, 2010

Regulation by (p)ppGpp alarmones

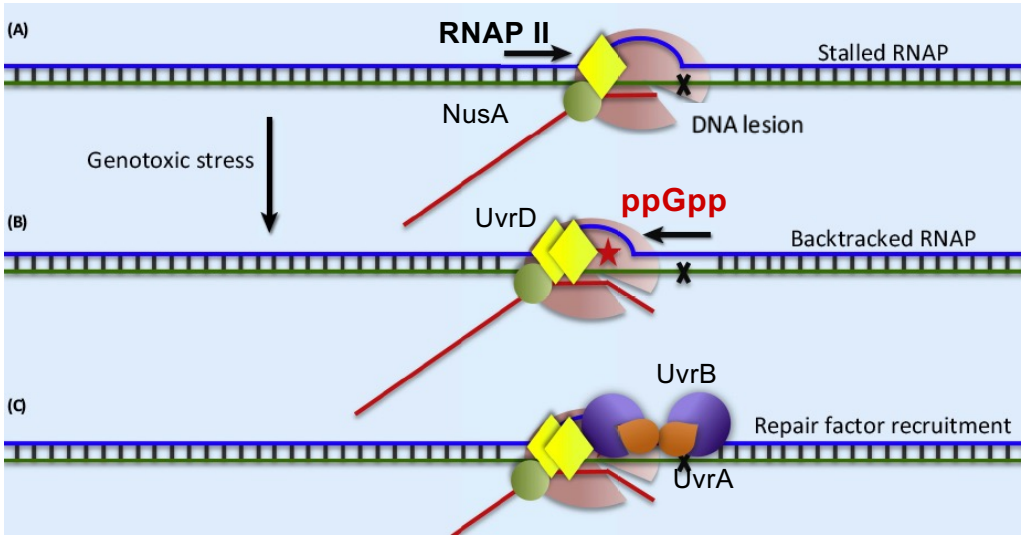
Regulation of different stress response pathways



Steinchen and Bange, Mol Microbiol, 2016

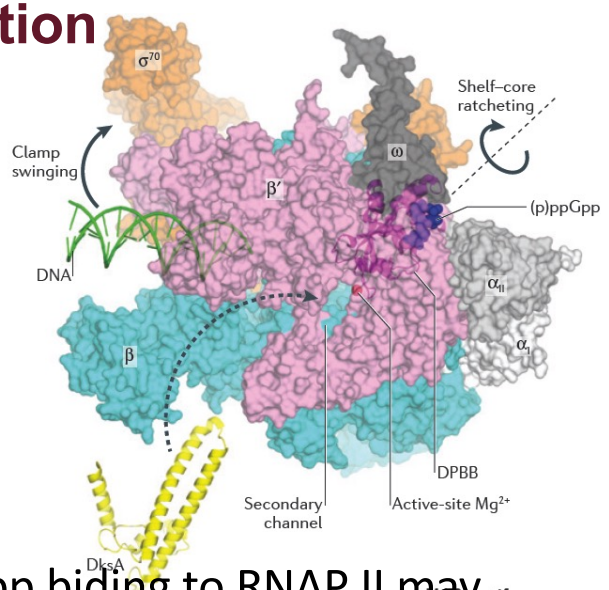
Regulation by (p)ppGpp alarmones

Transcription-coupled repair



RNAP II stalled on DNA lesion is backtracked by ppGpp binding, which facilitates recruitment of NER factors

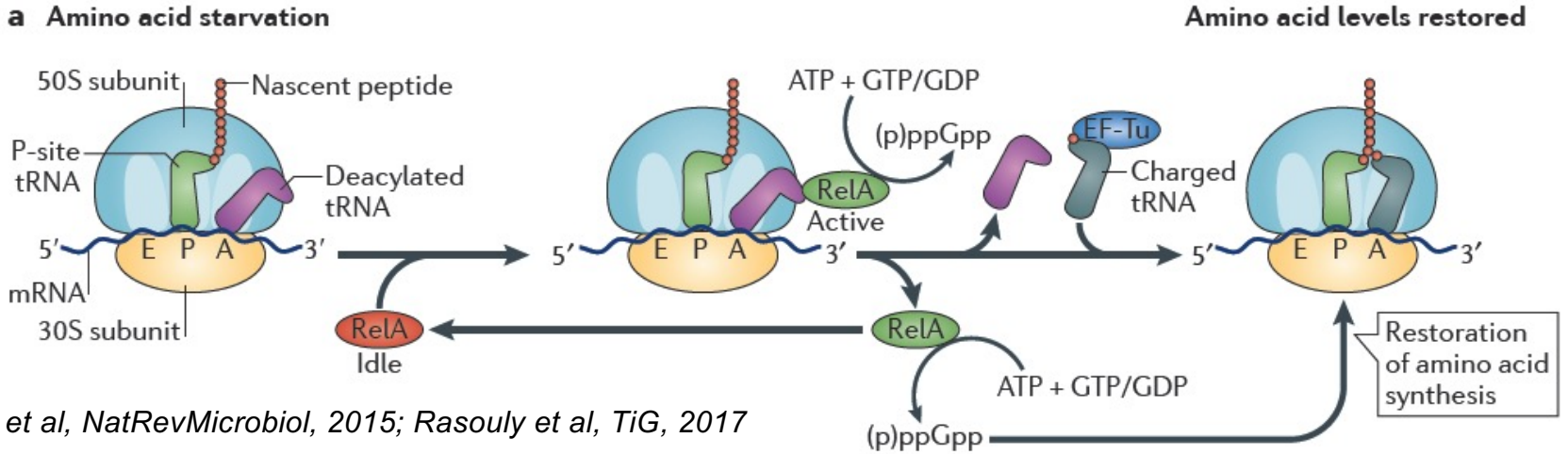
Transcription



(p)ppGpp biding to RNAP II may regulate its efficiency by inducing allosteric signal to the catalytic Mg^{2+}

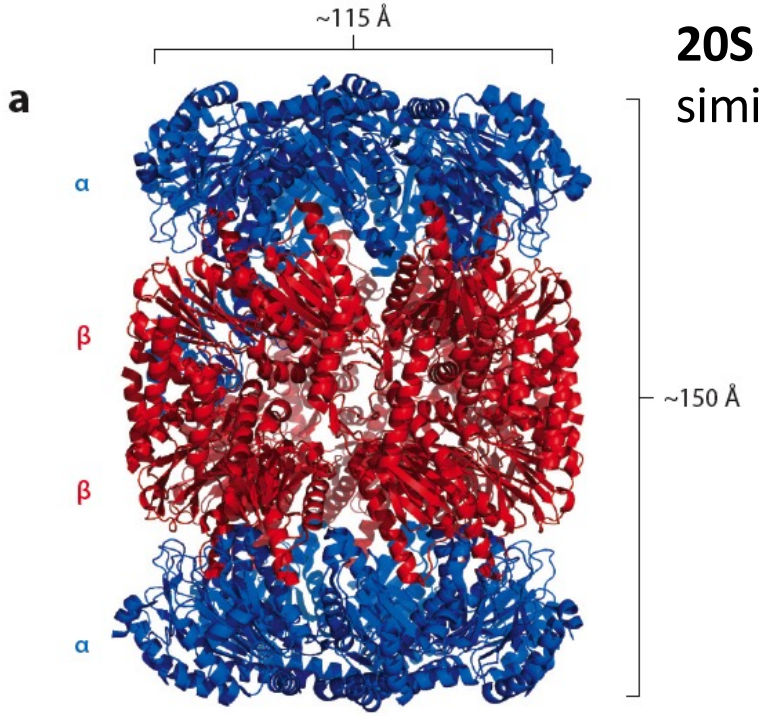
Translation

Starvation generates deacetylated tRNAs that induce RelA-mediated synthesis of (p)ppGpp which directs amino acid synthesis



Hauryliuk et al, NatRevMicrobiol, 2015; Rasouly et al, TiG, 2017

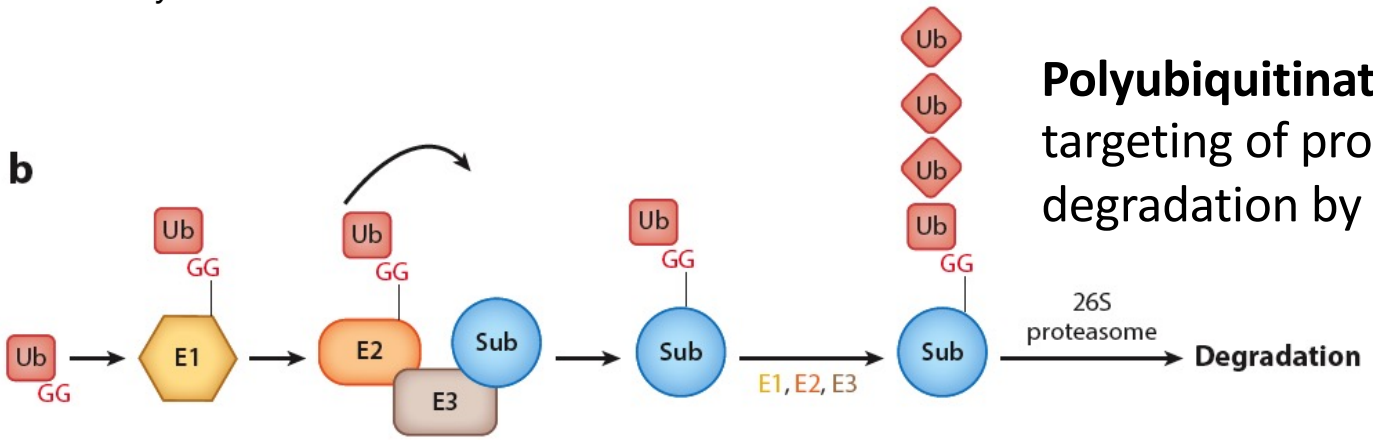
Protein degradation: proteasome



Mycobacterium tuberculosis

20S core particle
similar the eukaryotic and archaeal 20S

Other proteases:
AAA$^+$ Clp ATP-dependent proteases
ClpXP, ClpAP, Lon, HflB and Tsp

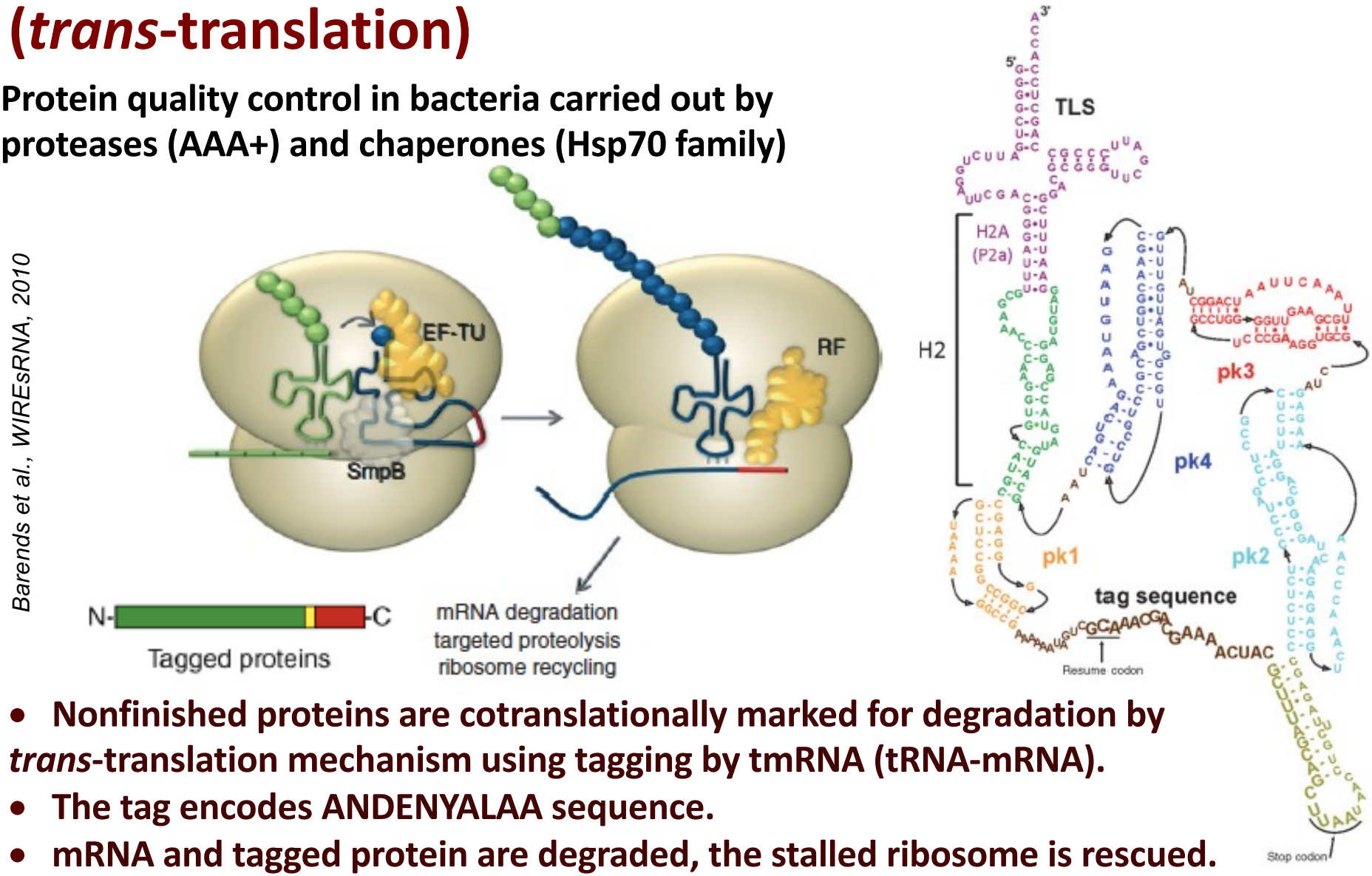


Polyubiquitination - mediated targeting of proteins for degradation by proteasome

Jastrab and Darwin, Annual Rev Micro, 2016

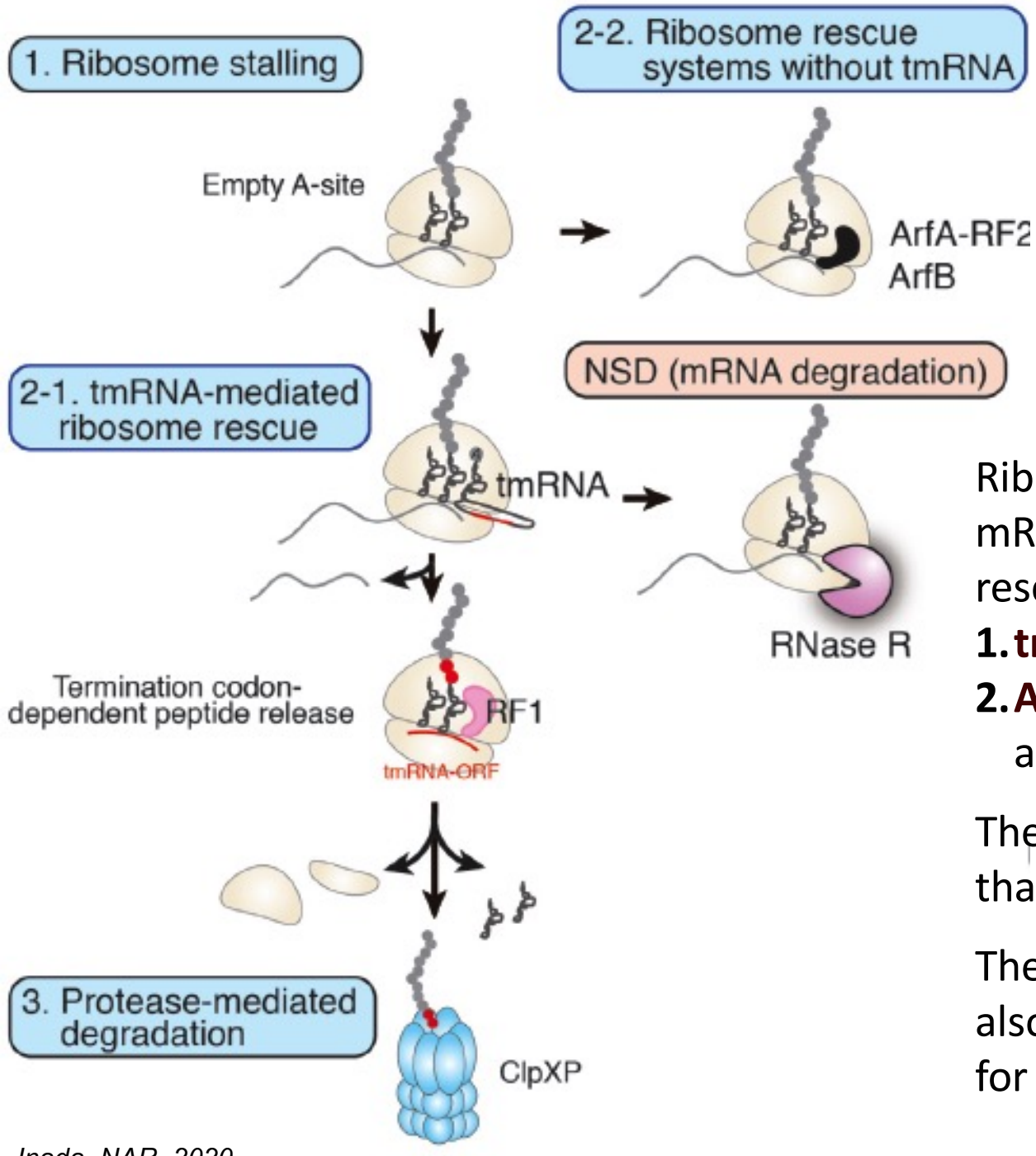
Protein degradation by tmRNA tagging (*trans*-translation)

Protein quality control in bacteria carried out by proteases (AAA+) and chaperones (Hsp70 family)



- **Nonfinished proteins are cotranslationally marked for degradation by *trans*-translation mechanism using tagging by tmRNA (tRNA-mRNA).**
- **The tag encodes ANDENYALAA sequence.**
- **mRNA and tagged protein are degraded, the stalled ribosome is rescued.**
- **tmRNA interacts with SmpB, RP S1, EF-Tu and alanyl-tRNA synthetase.**
- **This mechanism operates for example in stress for misfolded proteins.**

Stalled ribosome rescue systems



Inada, NAR, 2020

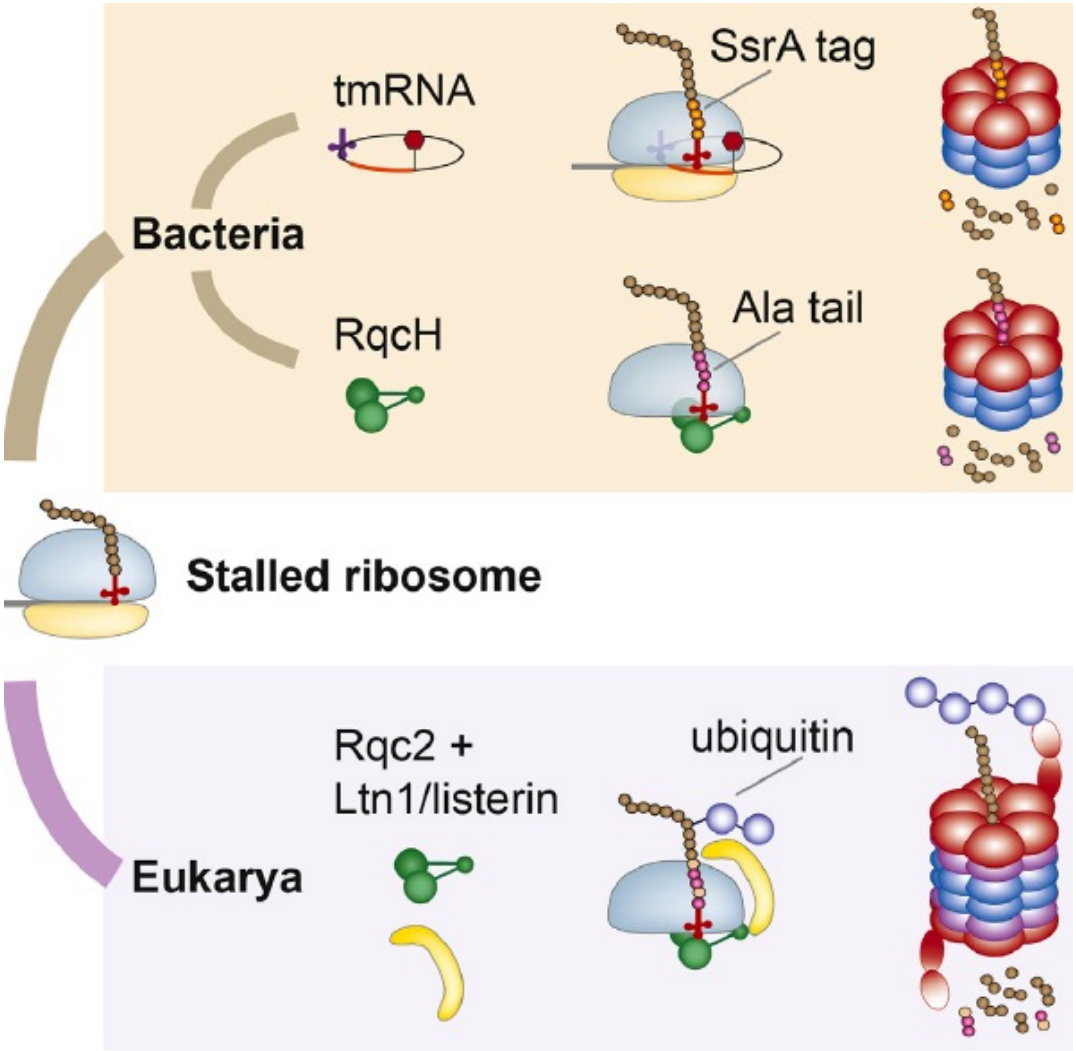
Ribosomes stalled at 3’ end of mRNAs lacking stop codon are rescued by:

1. **tmRNA-SmpB complex**
2. **ArfA/RF2 and ArfB**
   alternative rescue factors

These recruit 3’-5’ **RNase R** that degrades nonstop mRNAs

The tmRNA-SmpB mechanism also targets peptide byproducts for degradation by proteases

Ribosome-associated quality control (RQC)



- partially redundant with the **tmRNA** mechanism
- **RqcH** extends substrates with C-terminal Ala tails that act as degrons
- **RQC** protects cells against translation inhibition and environmental stresses

Lytvynenko et al, Cell, 2019

RNA modification in bacteria

tRNA, rRNA: **as in other organisms**

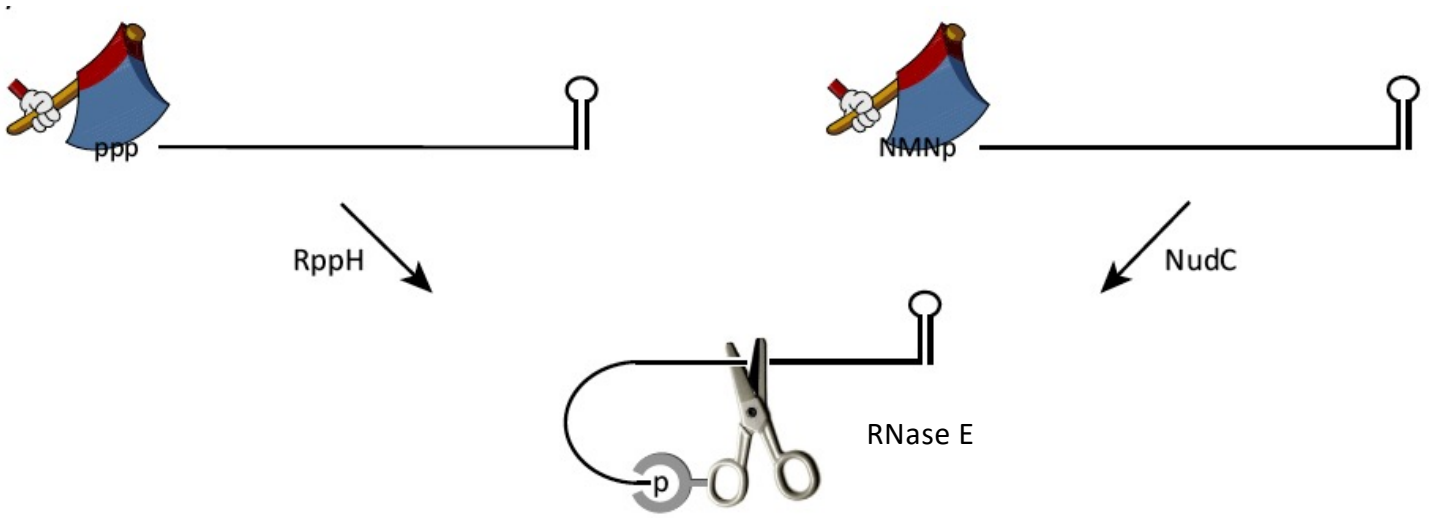
mRNA:

m^6A: enzymes unknown; function unknown

m^5C: not confirmed

NAD 5' cap:

- a small fraction of RNAs, predominantly shorter (<200 nts, regulatory sRNAs and some mRNAs), carry NAD 5' cap
- probably added co-transcriptionally by RNAP, maybe also post-transcriptionally by transferases or DNA/RNA ligases
- removed by NudC
- function unknown, probably stabilize mRNAs from degradation by RppH and RNase E



Luciano and Belasco, TiBS, 2015; Jaschke et al, Curr Op Micro 2016

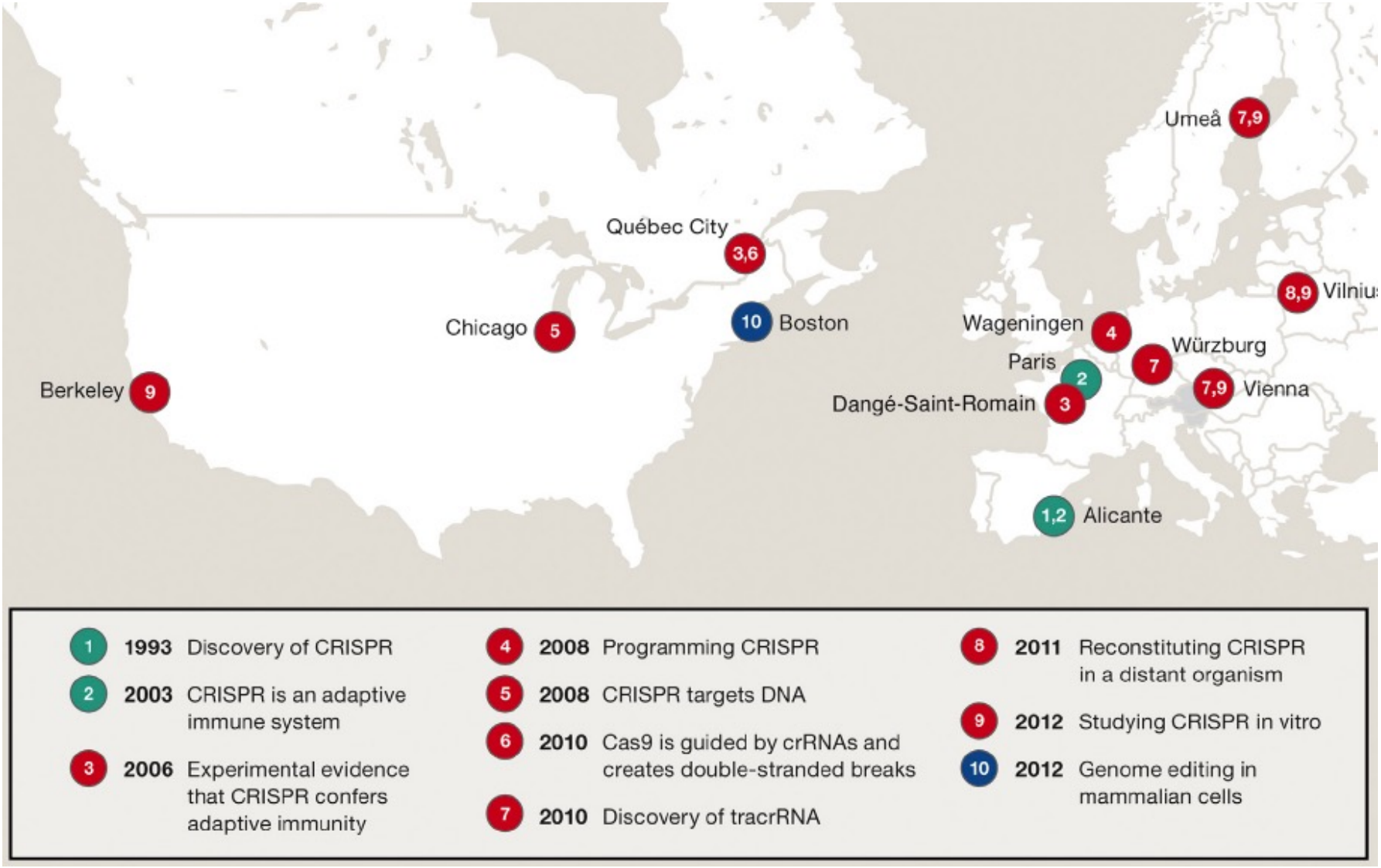
Other RNA caps:

- coA

- Np_nN

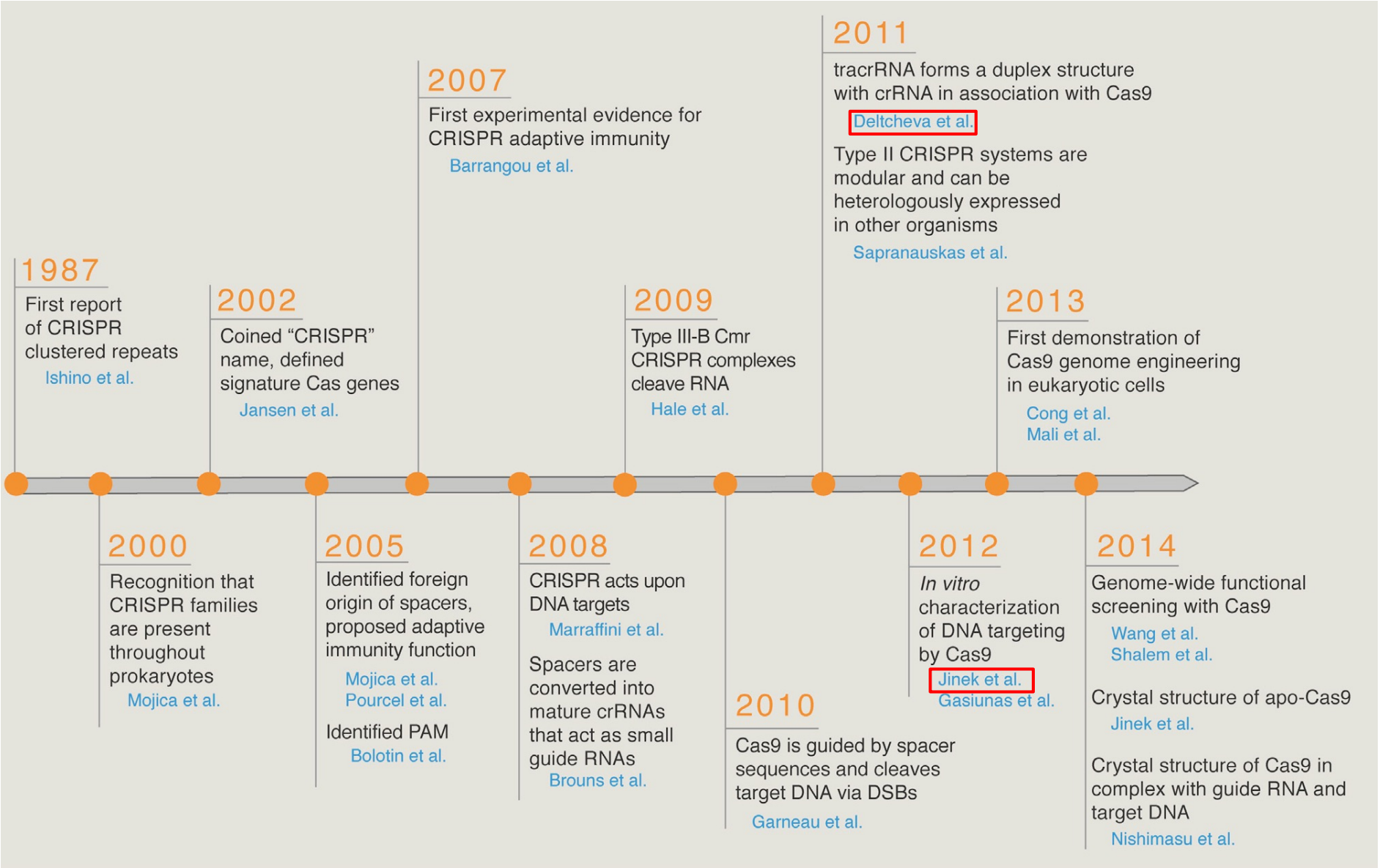
(e.g. Np_4A alarmone related to stress)

CRISPR-Cas history



Lander, Cell, 2016

CRISPR-Cas history



CRISPR-Cas: CRISPR-based genome editing

Nobel 2020

Emmanuelle Charpentier
Max Planck Institute

Jenifer Doudna
University of California

CRISPR RNA maturation by *trans*–encoded small RNA and host factor RNase III

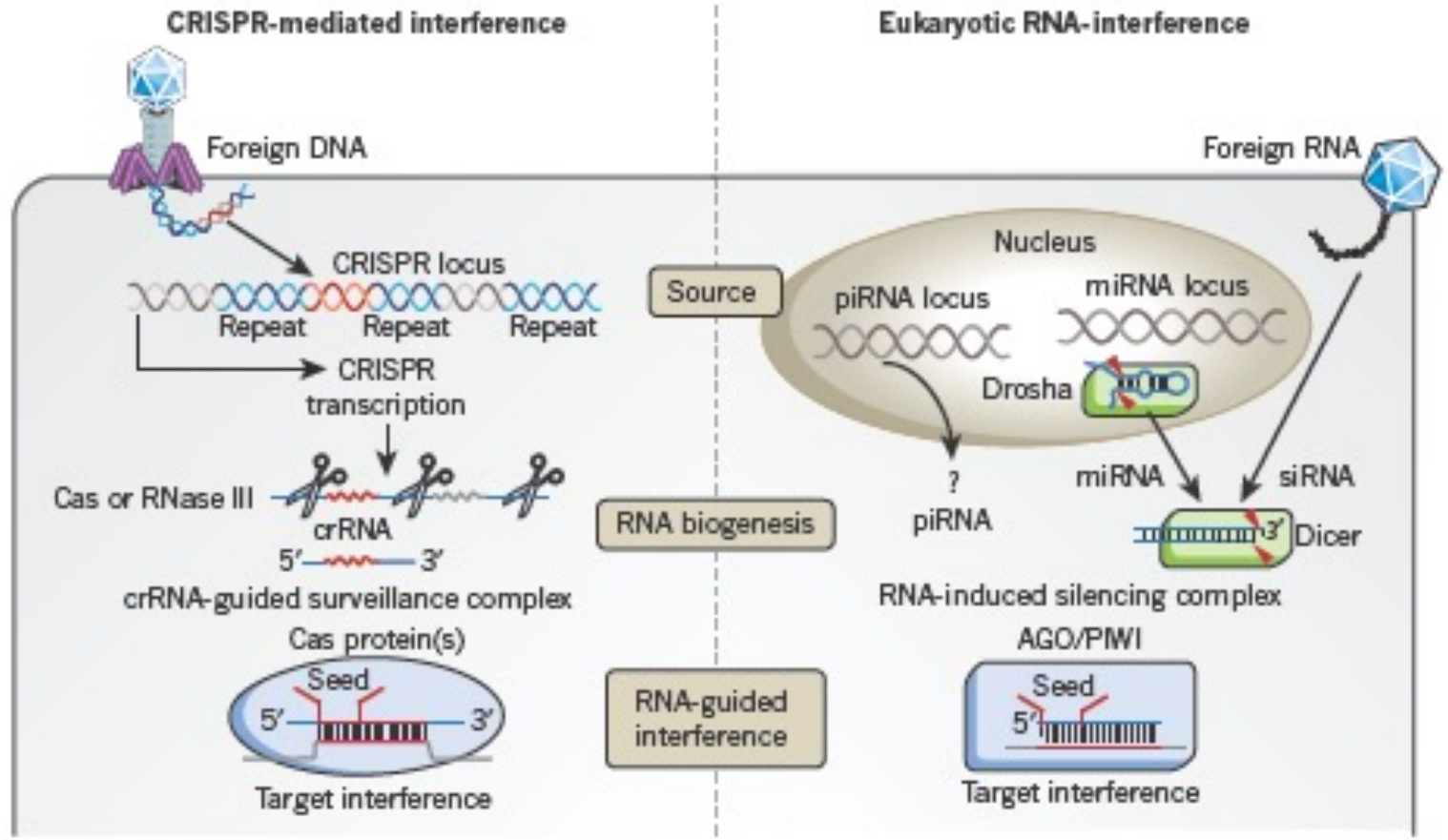
Elitza Deltcheva[1,2], Krzysztof Chylinski[1,2]*, Cynthia M. Sharma[3]*, Karine Gonzales[2], Yanjie Chao[3,4], Zaid A. Pirzada[2], Maria R. Eckert[2], Jörg Vogel[3,4] & Emmanuelle Charpentier[1,2]

A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

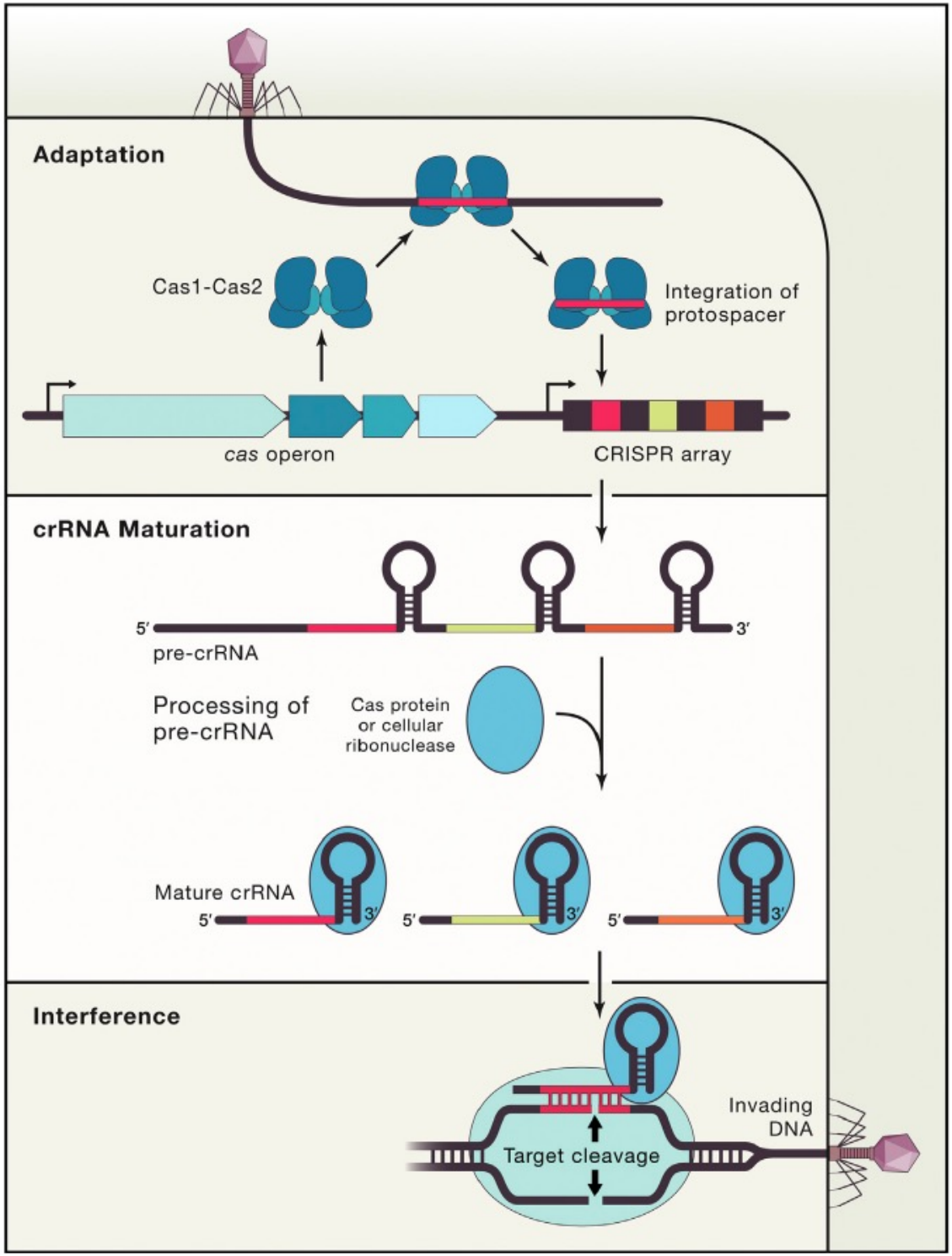
Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Hauer,[2]† Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡

CRISPR-Cas adaptive bacterial immunity
RNA-guided RNAi in Bacteria and Archaea

CRISPR - Clustered Regularly Interspaced Short Palindromic Repeat
Cas - CRISPR associated



Wiedenheft et al, Nature, 2012

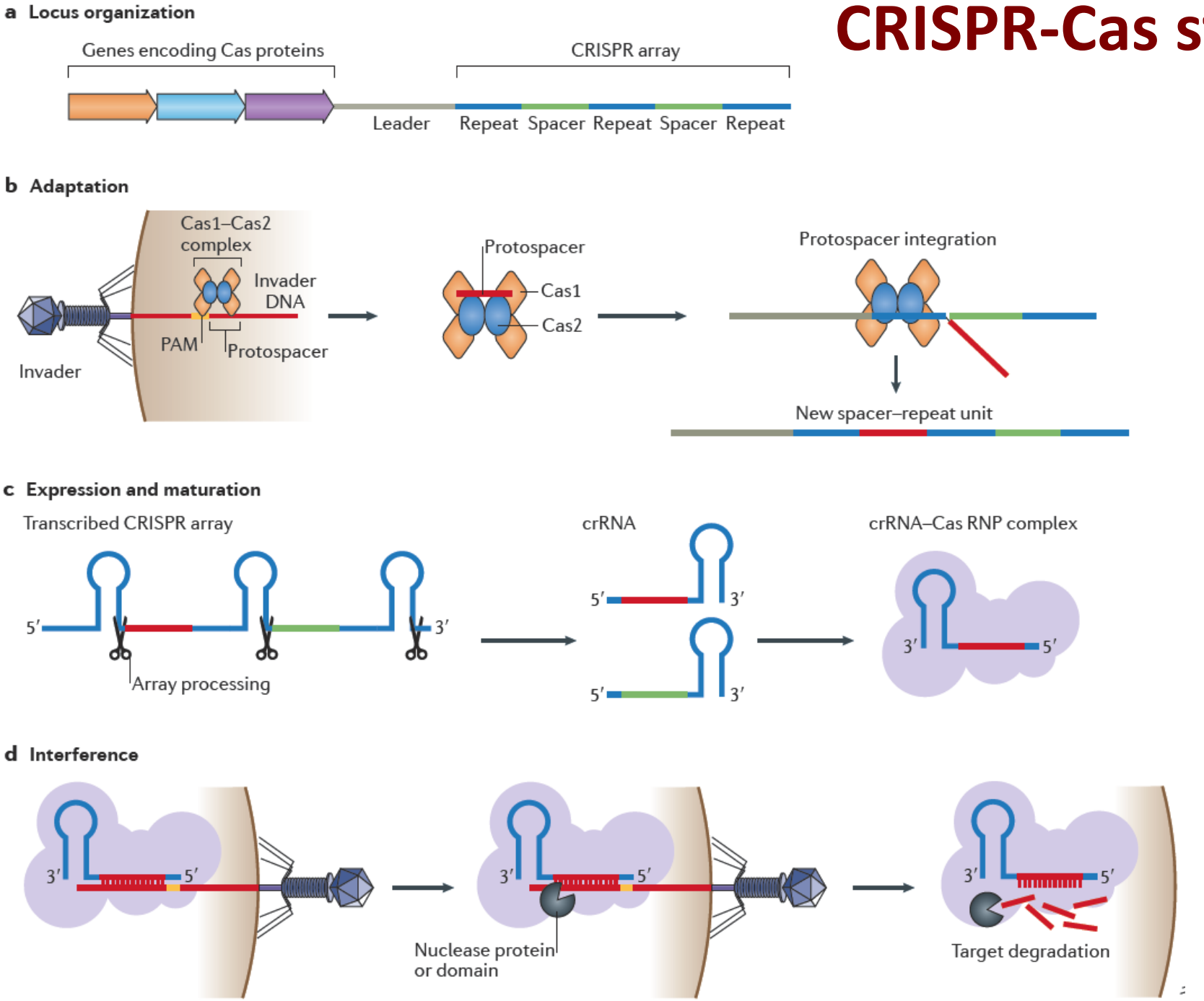


CRISPR-Cas stages

- foreign DNA is integrated into the CRISPR locus
- long CRISPR transcripts are processed by Cas or RNase III nuclease
- short crRNAs assemble into surveillance complexes
- target invading DNAs or RNAs recognized by crRNA „seed” are destroyed

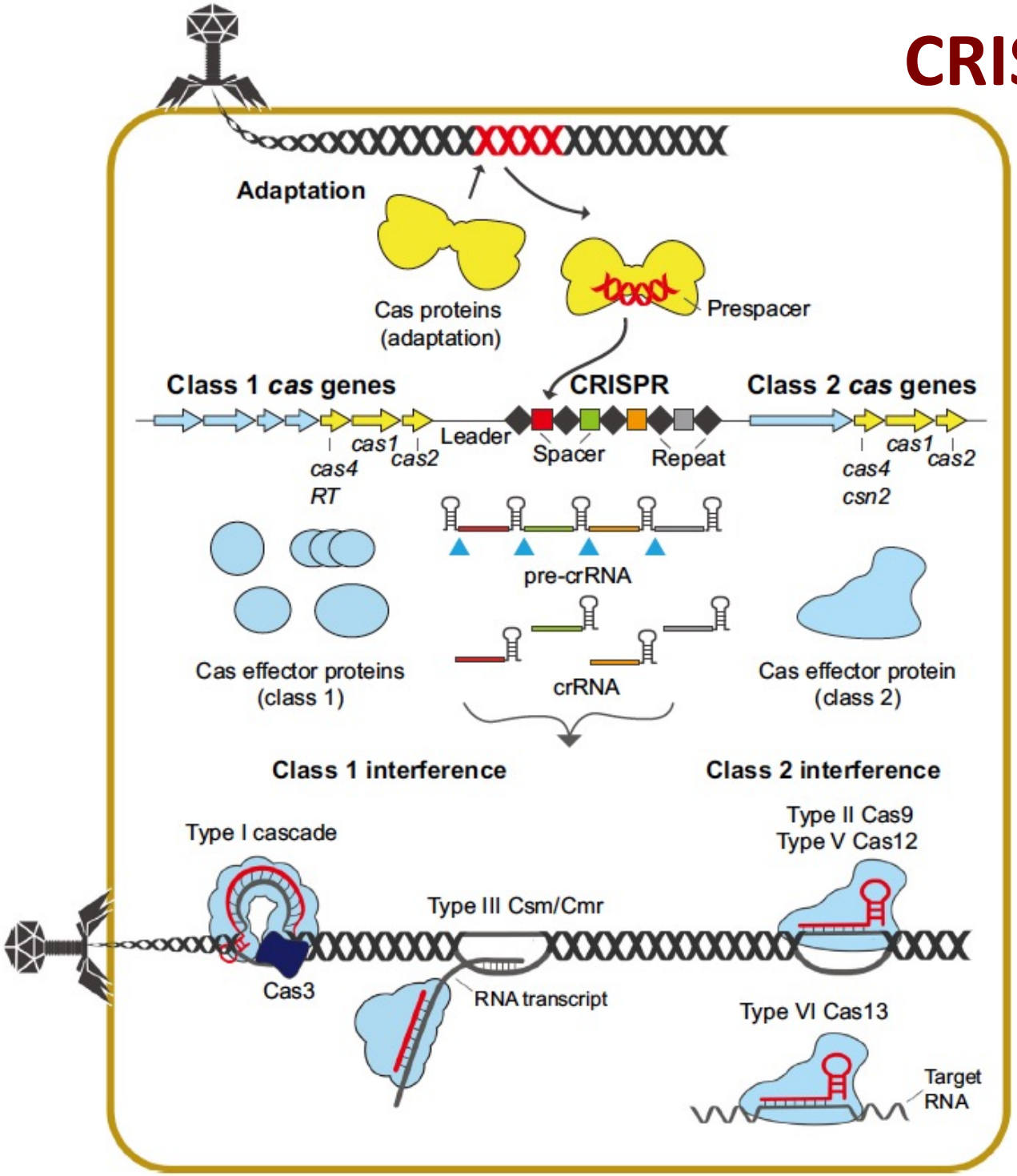
Hille et al, Cell, 2018

CRISPR-Cas stages



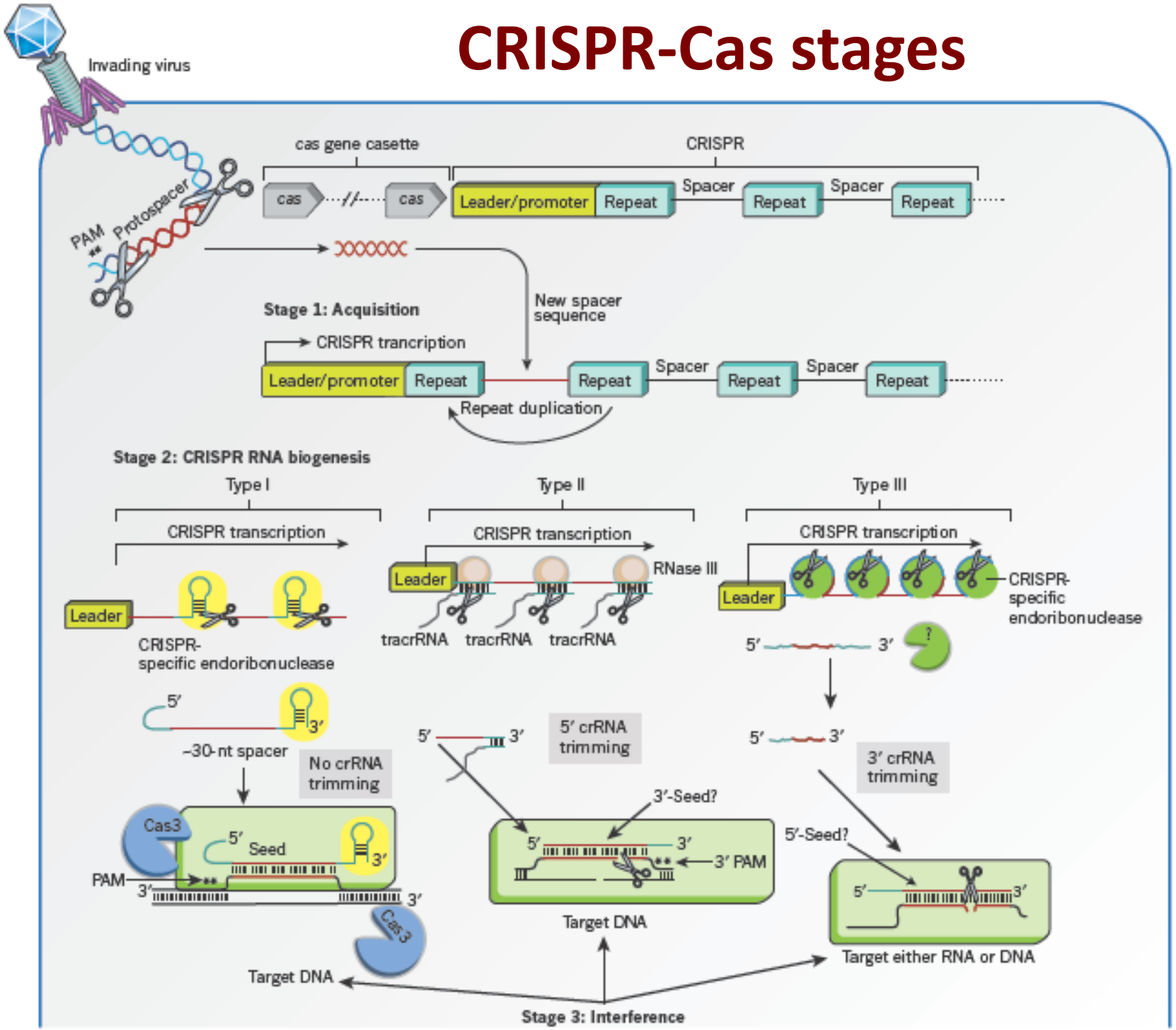
Amitai and Sorek, NatRevMicro, 2016

CRISPR-Cas stages



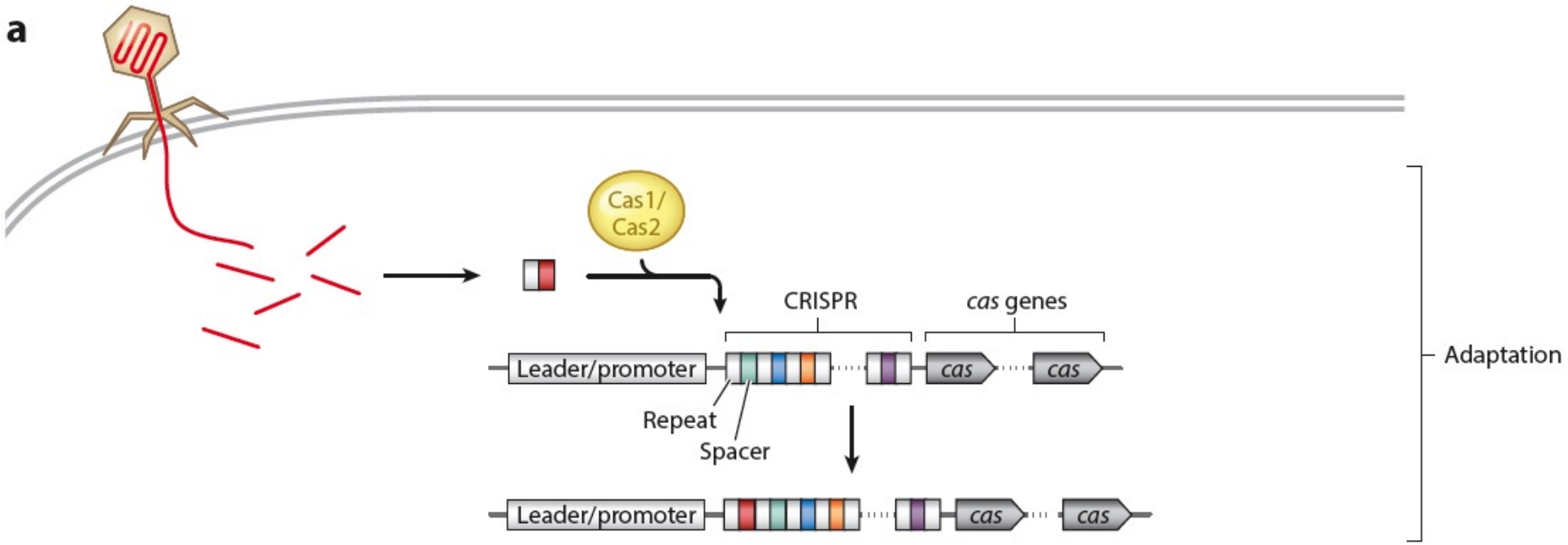
Lee and Sashital, TiBS, 2022

CRISPR-Cas stages



Wiedenheft et al, Nature, 2012

CRISPR-Cas: adaptation and spacer acquisition



PAM protospacer-adjacent motif in type I immunity

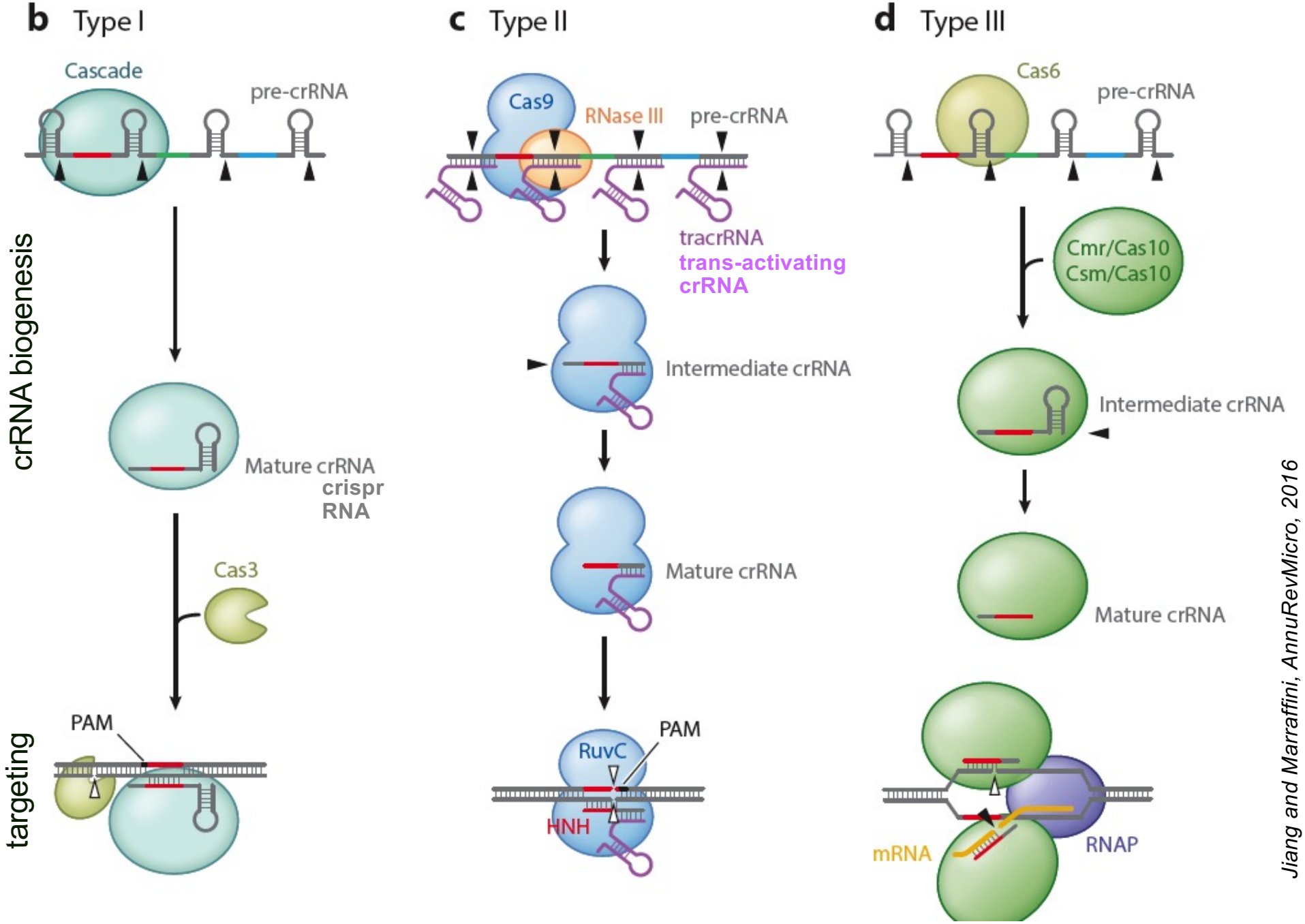
- usually tri-nucleotide (AWG in *E. coli*) recognized by the Cascade complex (CasA in *E. coli*)

- probably allows tolerance to self

(prevents autoimmunity against spacer DNA sequences complementary to crRNAs they encode)

Jiang and Marraffini, AnnuRevMicro, 2016

CRISPR-Cas: crRNA biogenesis, targeting



Jiang and Marraffini, AnnuRevMicro, 2016

CRISPR-Cas types

Table 1. Classification and Examples of CRISPR Systems

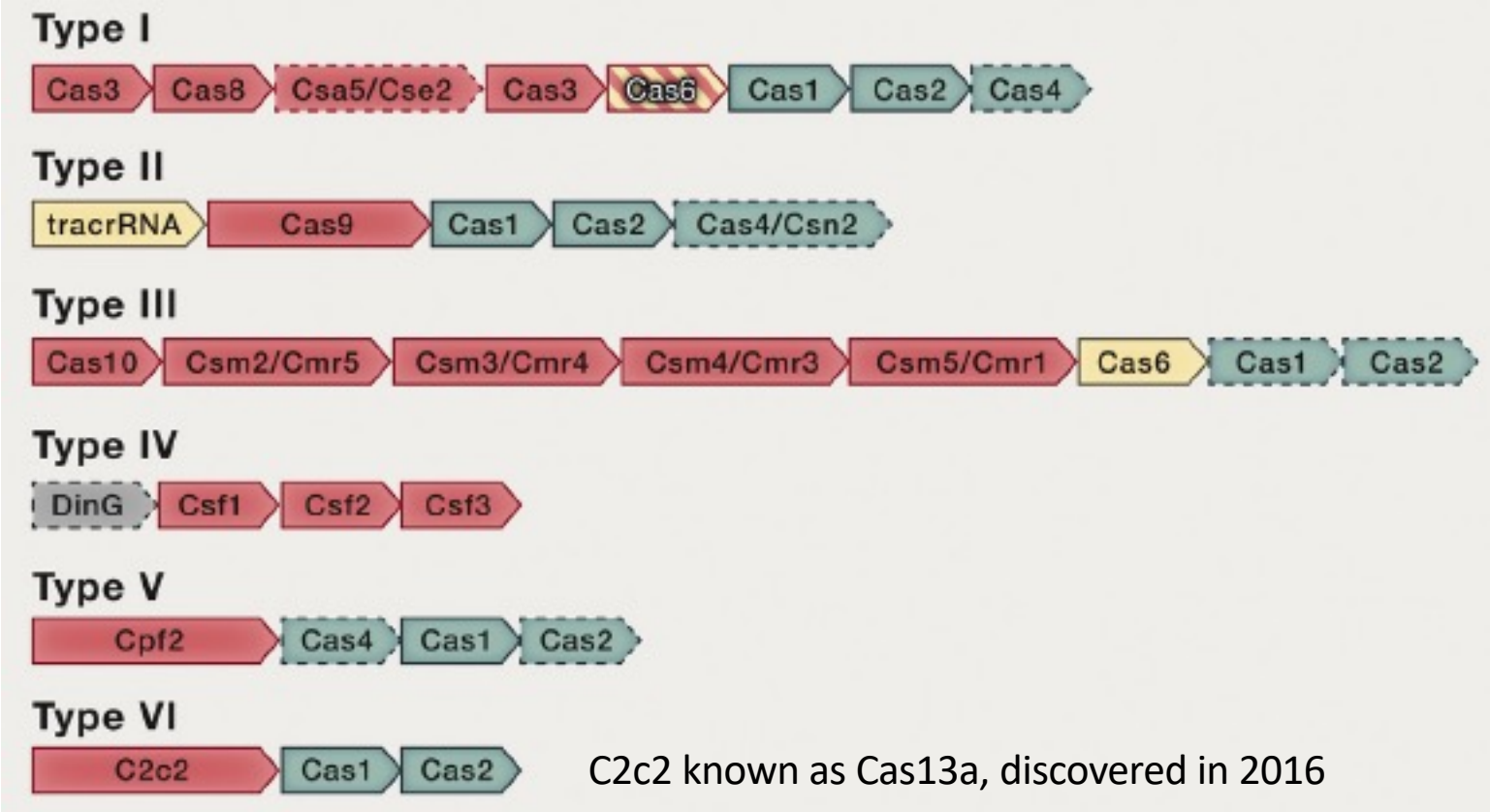
Class	Type	Subtype	Hallmarks	Example effector	Example organism	Studies Cited
Class 1	Type I		multisubunit effector complex; Cas3	Cascade	*E. coli*	Brouns et al., 2008
	Type III	III-A	multisubunit effector complex; Csm effector module; DNA targeting	Cas10-Csm	*S. epidermidis*	Marraffini and Sontheimer, 2008
		III-B	multisubunit effector complex; Cmr effector module; RNA targeting	Cmr	*P. furiosus*	Hale et al., 2009
Class 2	Type II		single protein effector; tracrRNA	Cas9	*S. thermophilus*	Bolotin et al., 2005; Barrangou et al., 2007; Sapranauskas et al., 2011; Gasiunas et al., 2012
					S. pyogenes	Deltcheva et al., 2011; Jinek et al., 2012; Cong et al., 2013; Mali et al., 2013
	Type V		single protein effector; single-RNA guided	Cpf1	*F. novicida*	Zetsche et al., 2015

Class	Class 1 Multi-subunit crRNA-effector complex			Class 2 Single-subunit crRNA-effector complex		
Type	Type I	Type III	Type IV	Type II	Type V	Type VI
Effector complex	Cascade	Csm and Cmr	n.d.	Cas9	Cpf1, C2c1, C2c3	C2c2
Target	dsDNA	ssRNA/ ssDNA	n.d.	dsDNA	dsDNA	ssRNA

Lander, Cell, 2016; Tamulaitis, TiMicro 2016

CRISPR - Cas types

Gene organization



C2c2 known as Cas13a, discovered in 2016

involved in interference

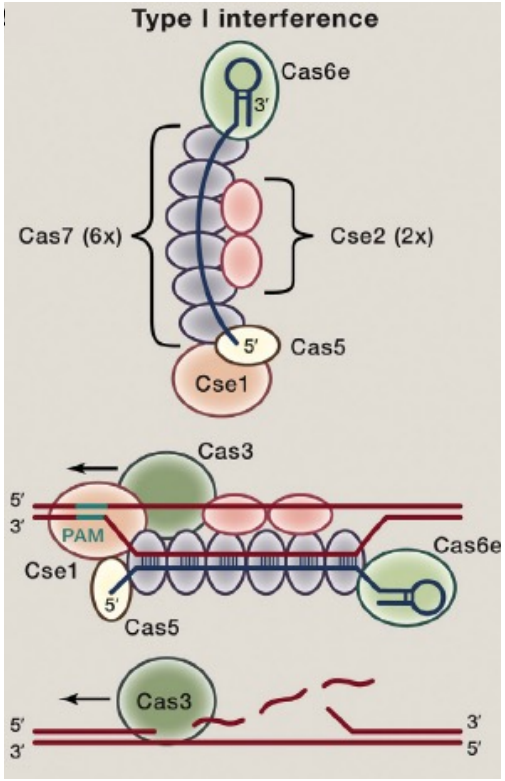
involved crRNA biogenesis

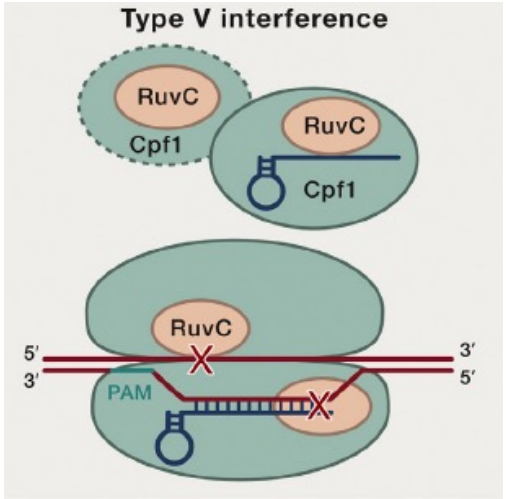
involved in adaptation

dashed lines- present in some subtypes

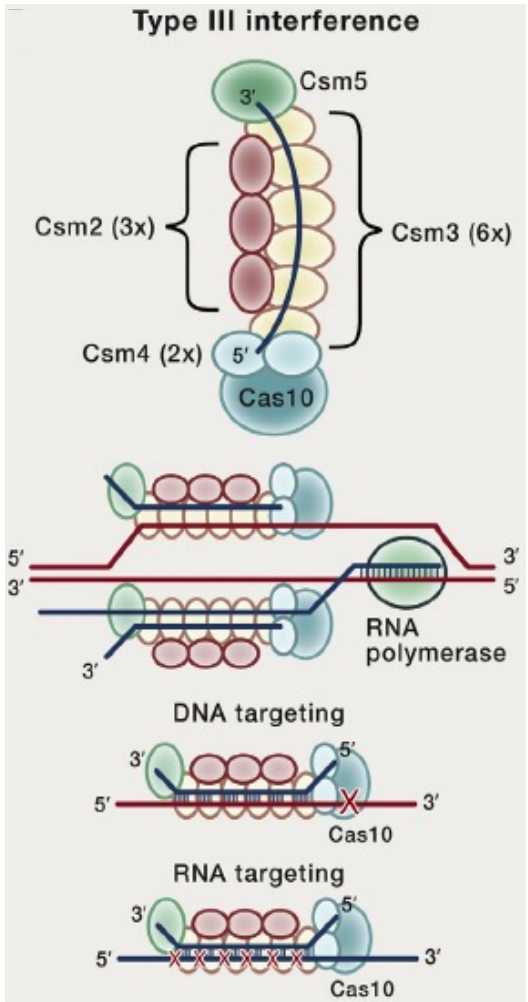
Wright et al, Cell, 2016

CRISPR-Cas interference types

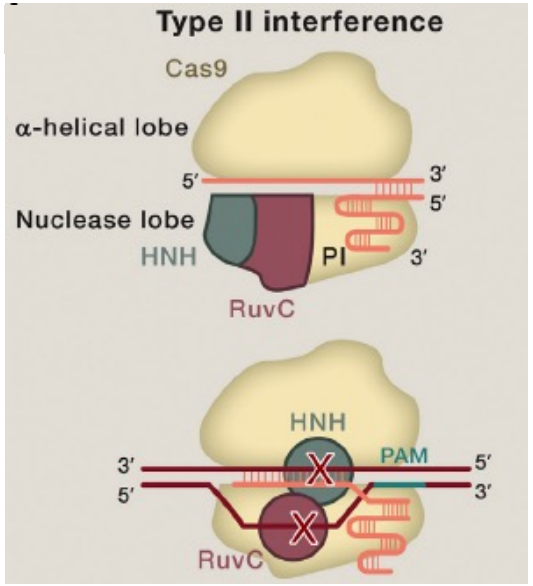


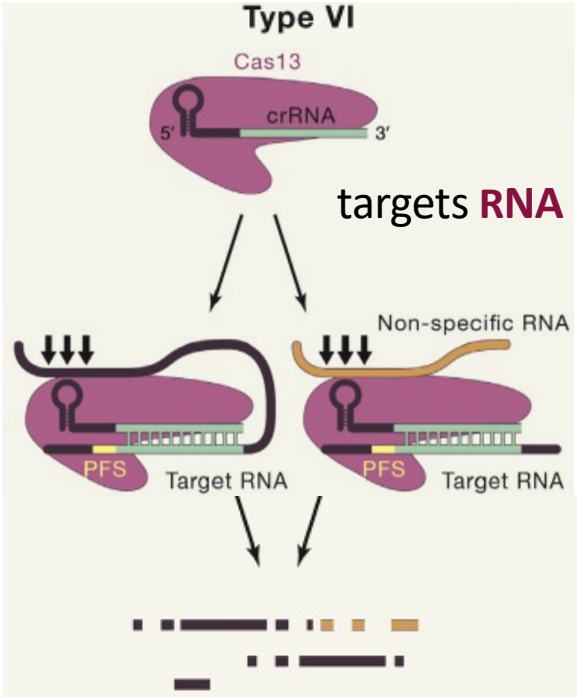


target **DNA**



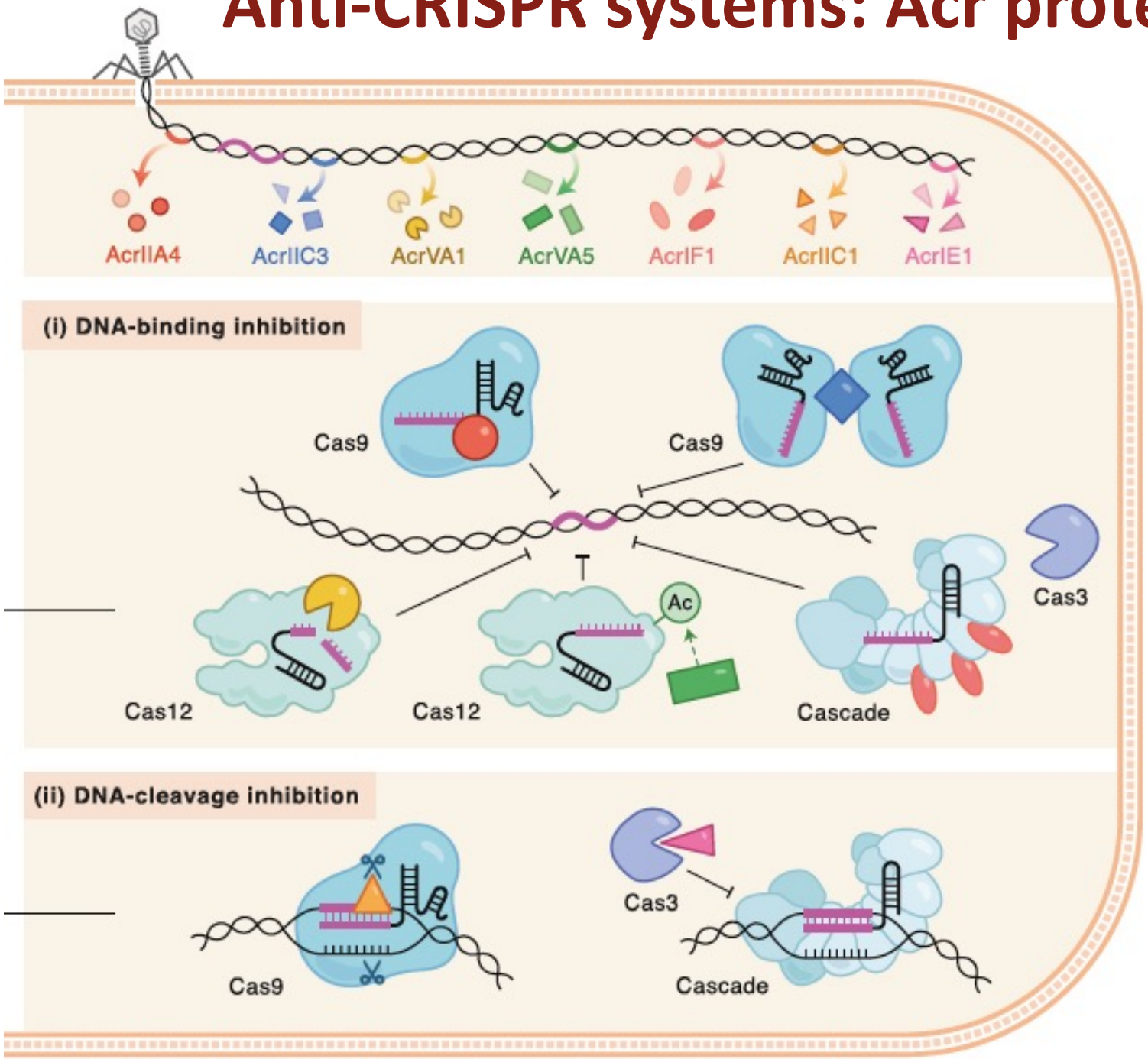
targets nascent **RNA** and actively transcribed **DNA**





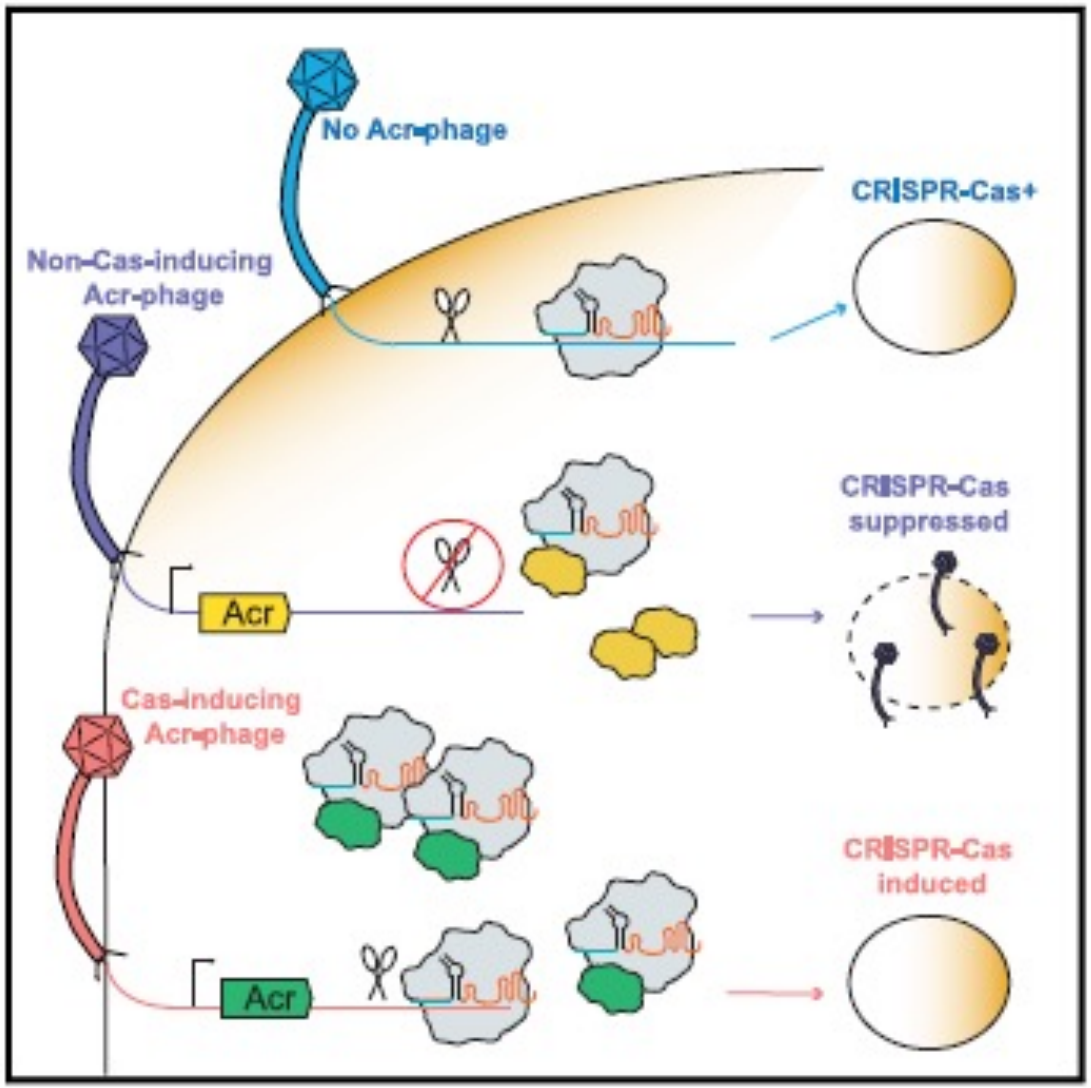
Wright et al, Cell, 2016; Hille et al, Cell, 2018

Anti-CRISPR systems: Acr proteins



Marino et al, Nature Methods, 2020

Anti-CRISPR proteins trigger a burst of CRISPR-Cas expression that enhances phage defence



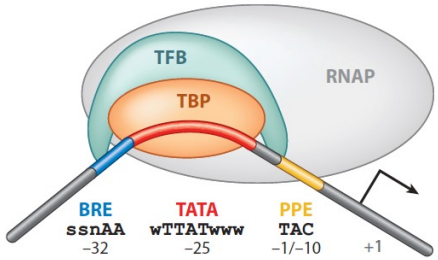
Highlights

- Phage-encoded anti-CRISPRs (Acrs) induce CRISPR-Cas9 expression
- Cas induction is rapid and occurs within the time frame of a single phage infection
- Cas induction reduces Acr-phage lysis and lysogeny
- Tracr-L regulation is an "anti-anti-CRISPR" strategy to combat Acr-phages

Worman et al, Cell Rep 2024

Archaea

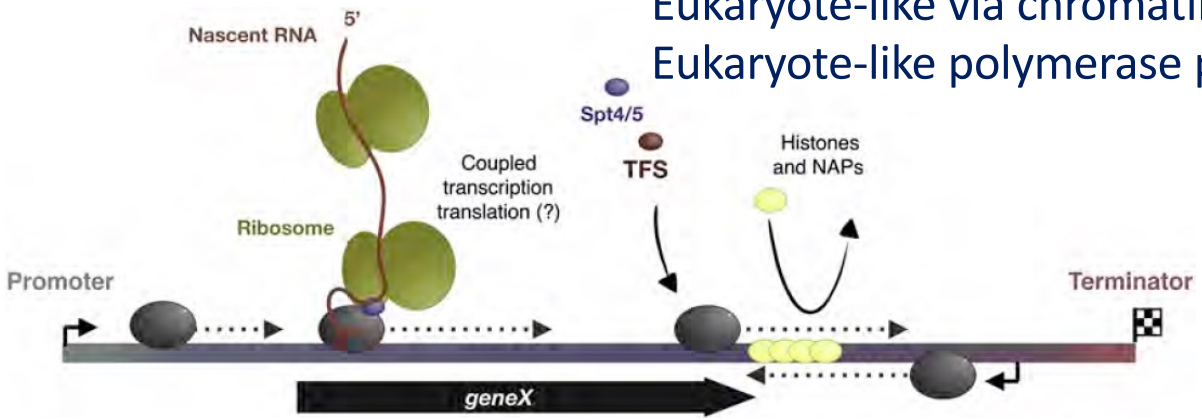
Transcription



- DNA template: eukaryote-like, chromatinized via histones
- RNAP and PIC: bacterial-like but related to eukaryotic Pol II
- TFs: bacterial-like, eukaryote-like or hybrid
- Transcription cycle: bacterial-like
- Termination: bacterial-like
- TCC: Spt4/5 TF couple transcription and translation via interaction with NusE (RP S10)
- Transcription regulation: both

  Eukaryote-like via chromatin and histone-like proteins

  Eukaryote-like polymerase pausing



mRNA structure

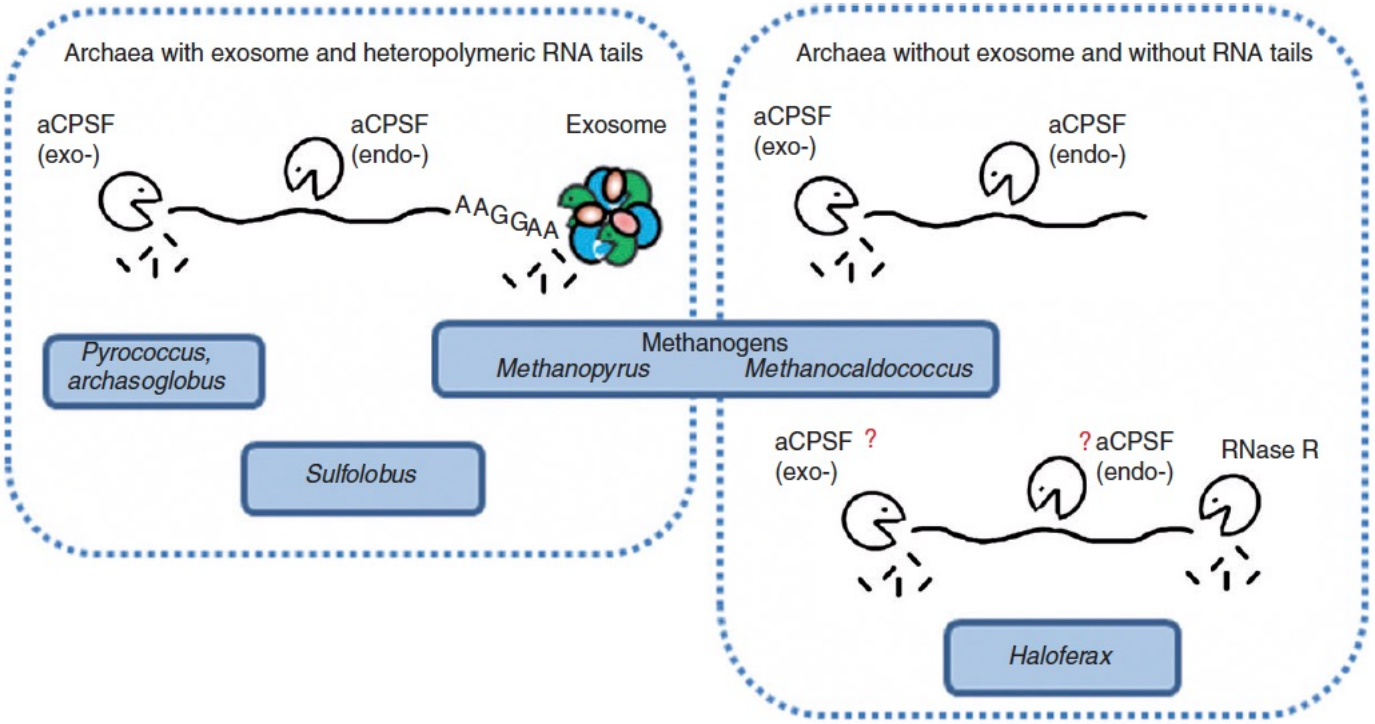
- Gene structure: bacteria-like, many genes organized in polycistronic operons
- mRNAs: no caps, no introns
- No 5'UTRs or very short 5'UTRs, no poly(A) tail
- Polyadenylation: bacterial-like but not by PAP I or PNPase (no homologs)

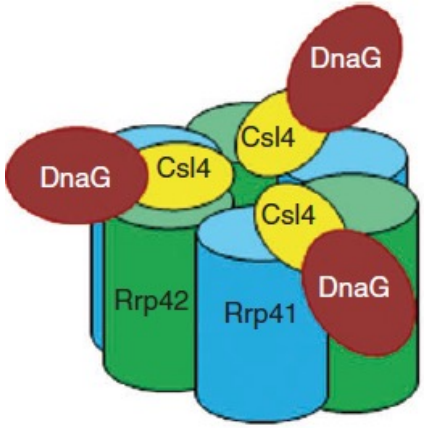
Instead of PNPase archaeal **exosome** adds destabilizing heteropolymeric adenosine-rich tails to RNA 3' ends in hyperthermophiles and some methanogens, but not in halophiles

Martinez-Pastor et al, Annu Rev Genet, 2017; Blombach et al, JBC, 2019; Mohanty and Kushner, WIREsRNA, 2010

Archaea

RNA Decay: exosome and exonucleases (e.g. beta-CASP)





Archaeal exosome
Structurally and functionally similar to bacterial PNPase

sRNAs and ncRNAs

- sRNAs: bacterial-like
- snoRNAs: eukaryote-like
- CRISPR-Cas: : bacterial-like type I and III
- ncRNAs: eukaryote-like antisense and pervasive transcription

Evguenieva-Hackenberg et al, WIREsRNA, 2014; Martinez-Pastor et al, Annu Rev Genet, 2017; Blombach et al, JBC, 2019

Archaea

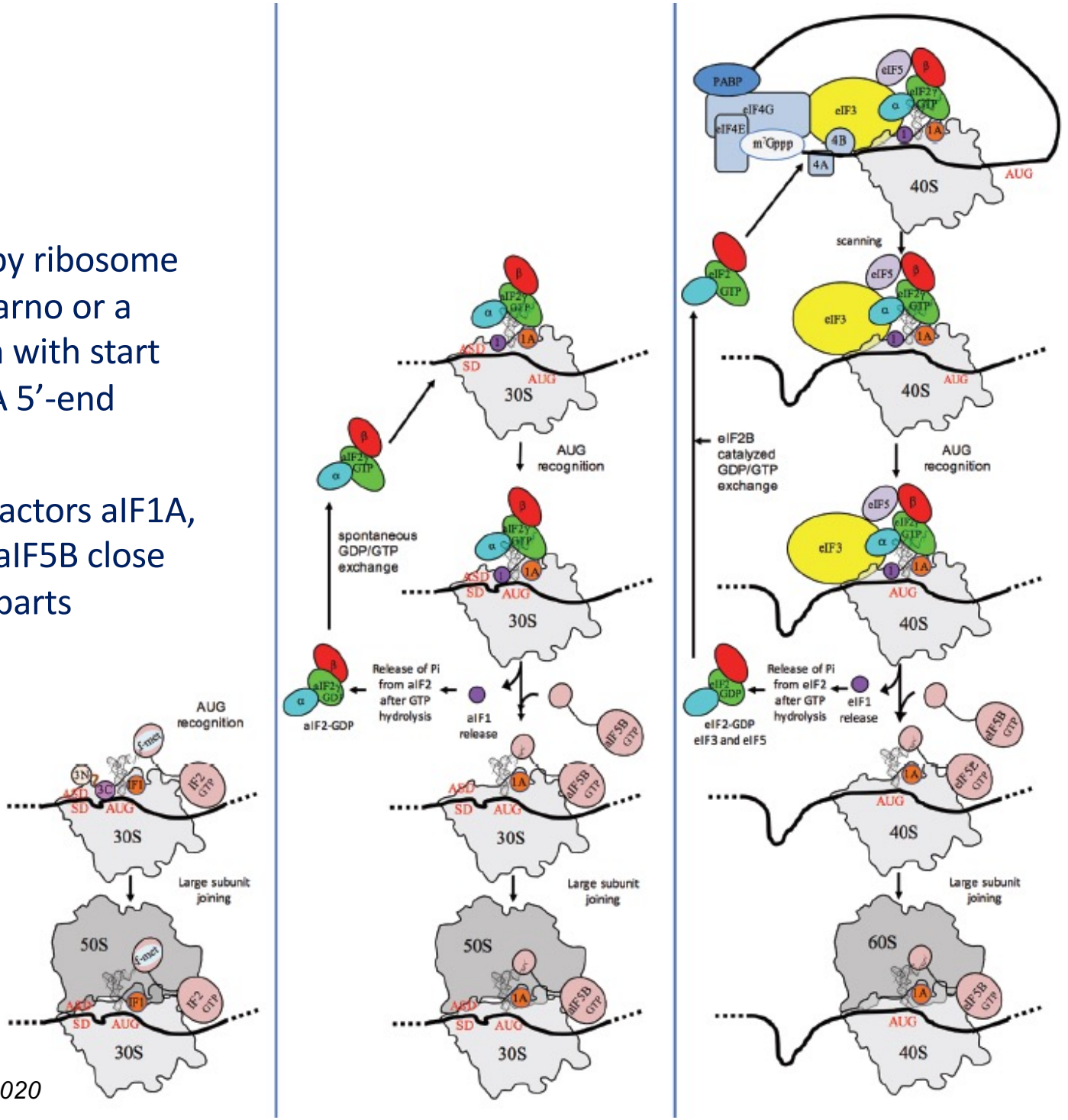
Translation

- Bacterial-like

Translation initiation by ribosome binding to Shine-Dalgarno or a leaderless mechanism with start codons close to mRNA 5’-end

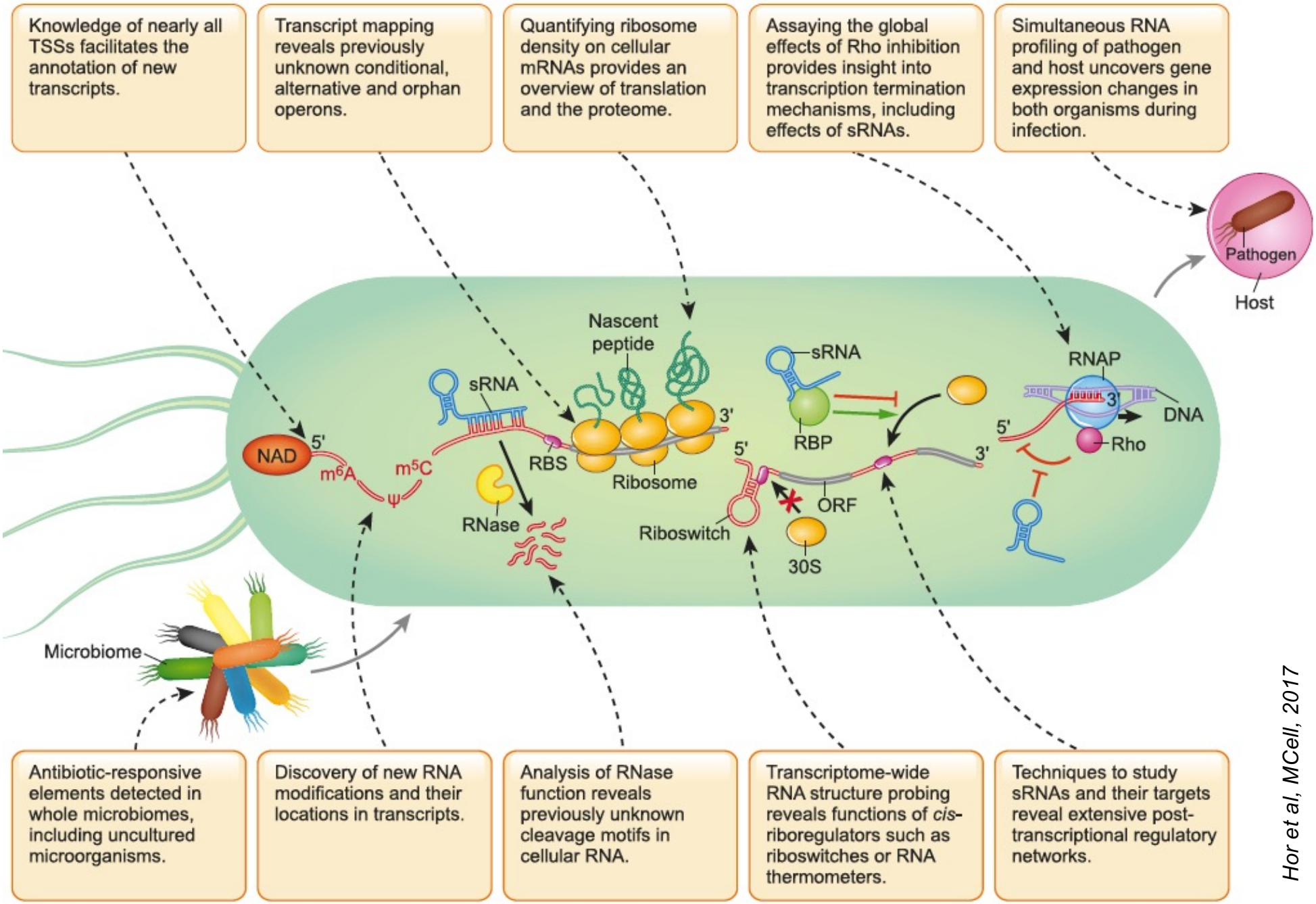
- Eukarote-like

Translation initiation factors aIF1A, e/aIF1, e/aIF2, and e/aIF5B close to eukaryotic counterparts



Schmitt et al, Frontiers Micro, 2020

Global RNA biology in bacteria



Hor et al, MCell, 2017

TAKE-HOME MESSAGE

Elements specific for bacterial gene expression:

- no compartmentalization

- transcription and translation are coupled

- polycistronic transcription units

- one RNA polymerase

- no 5’ cap, no introns (no splicing), no regular poly(A)

- endonucleases play more important role in mRNA decay

- polyadenylation-assisted RNA degradation

(occurs also in Eukaryotes)

- no cap-dependent translation or ribosome scanning

- tmRNA tagging for protein degradation