Informacje różne

- Egzamin pisemny na początku czerwca
- Podręcznika brak

Lizabeth Allison - **Fundamental Molecular Biology**

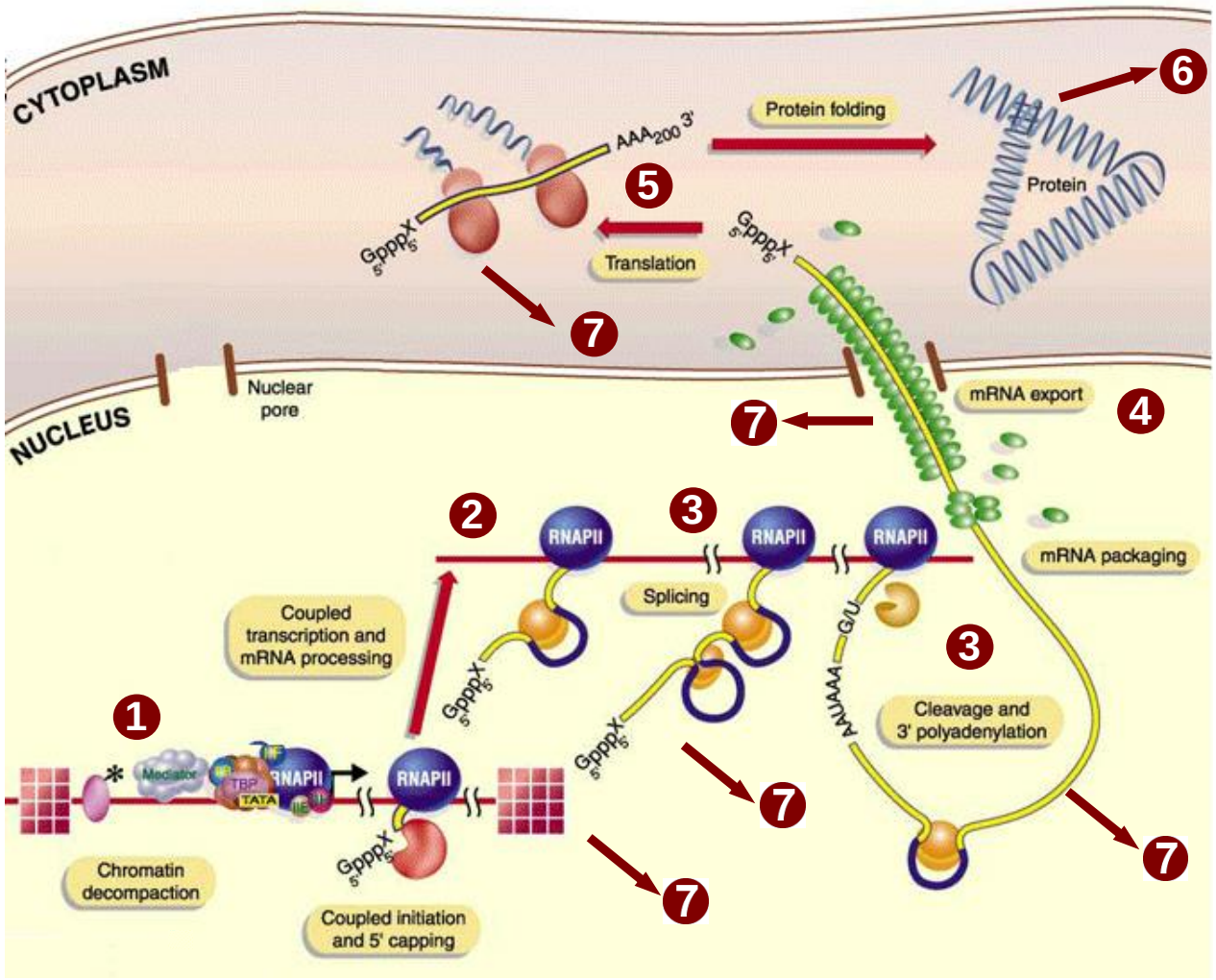
- Wykłady na stronie IGIBu

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

- dydaktyka, - Fakultety i wykłady monograficzne, - RGE, - materiały dla studentów

- Listy na 3 wykładach by poprawić w USOSIE
- Skreślanie z wykładu - teraz, a nie przed egzaminem

REGULATION OF GENE EXPRESSION - 1



1) chromatin

2) transcription

3) RNA processing

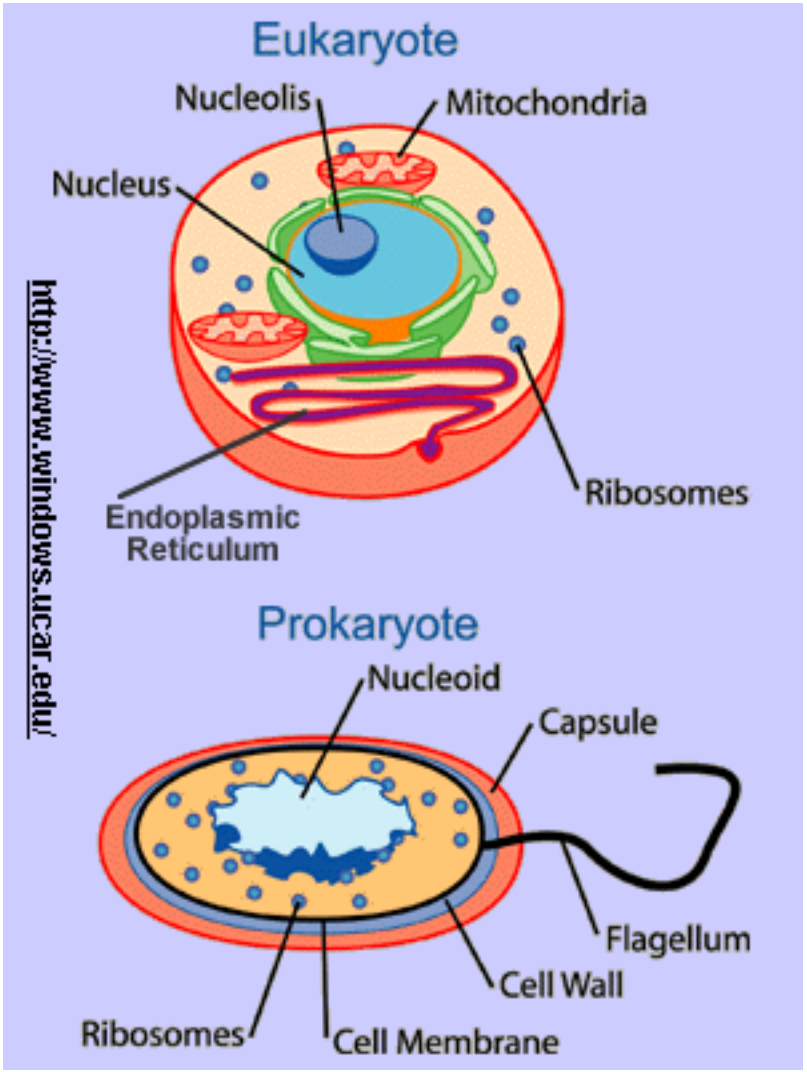
4) RNA export

5) translation (mRNA)

6) protein stability

7) RNA degradation

BACTERIAL vs EUKARYOTIC CELL



10-100 µm

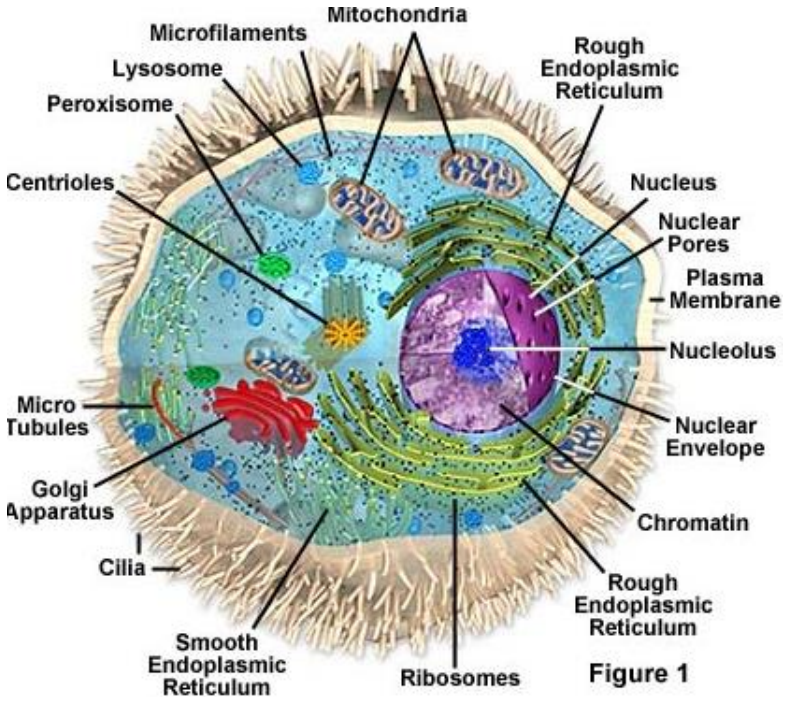


Figure 1

1-10 µm

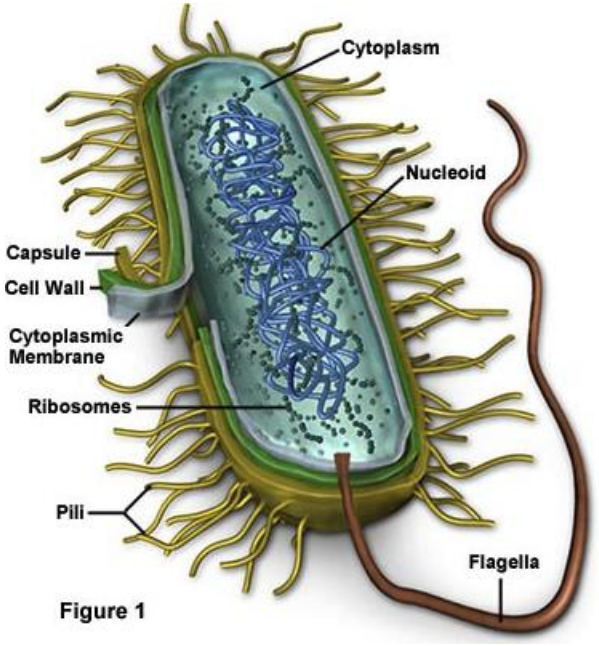
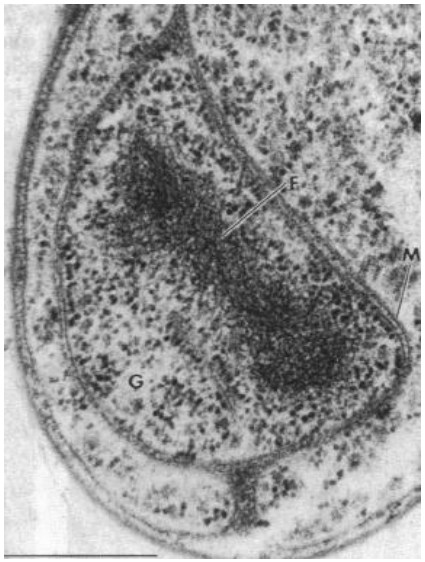
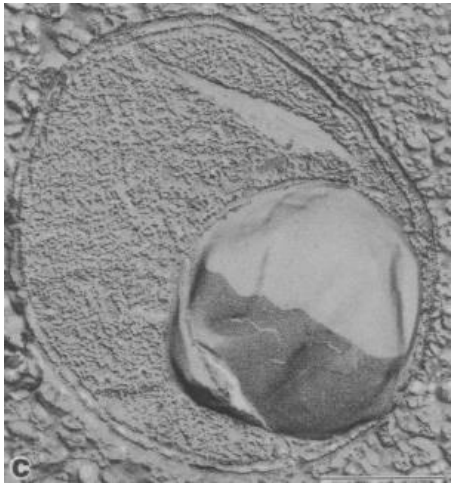


Figure 1

COMPARTMENTALIZED BACTERIA

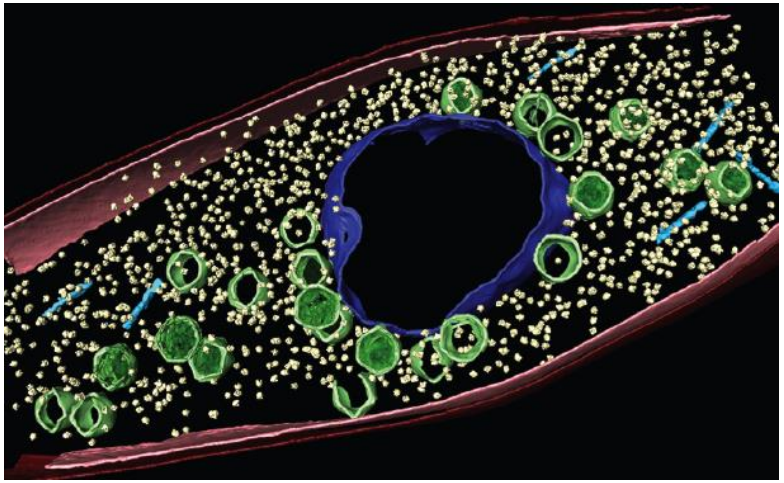
Eubacterium *Gemmata obscuriglobus* has a membrane-bound nucleoid





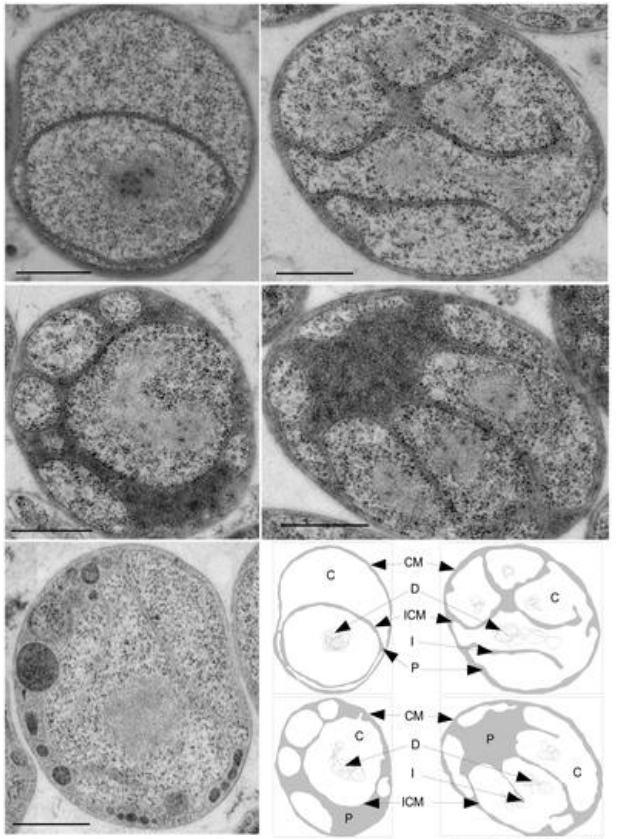
Fuerst and Webb, PNAS, 1991

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



Chaikeeratisak et al, Science, 2017

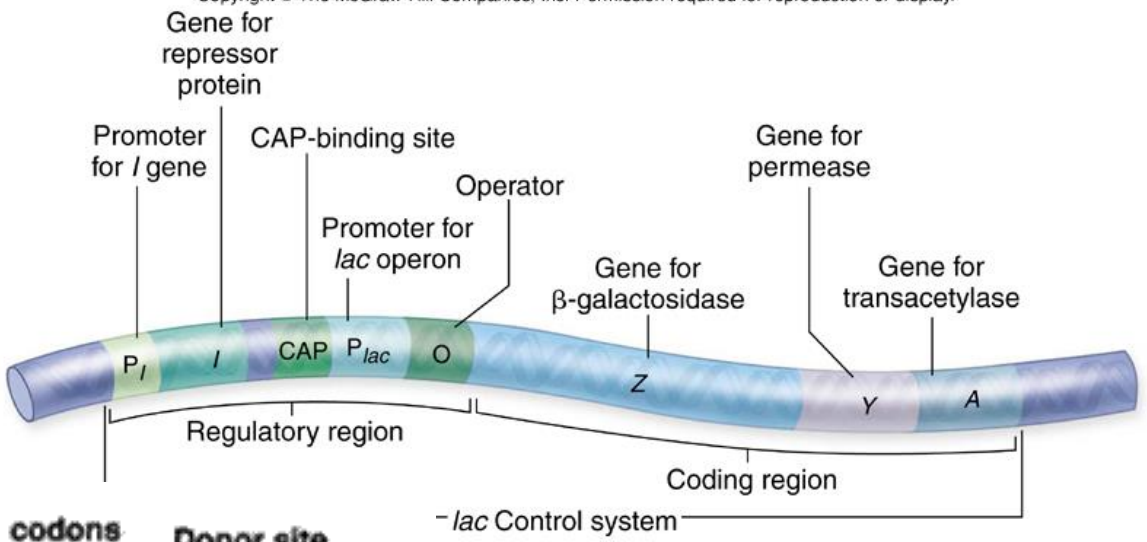
Planctomycetes-Verrucomicrobia-Chlamydiae Superphylum have membrane coat-like proteins



Santarella-Mellwig et al, PLOS Biology, 2010

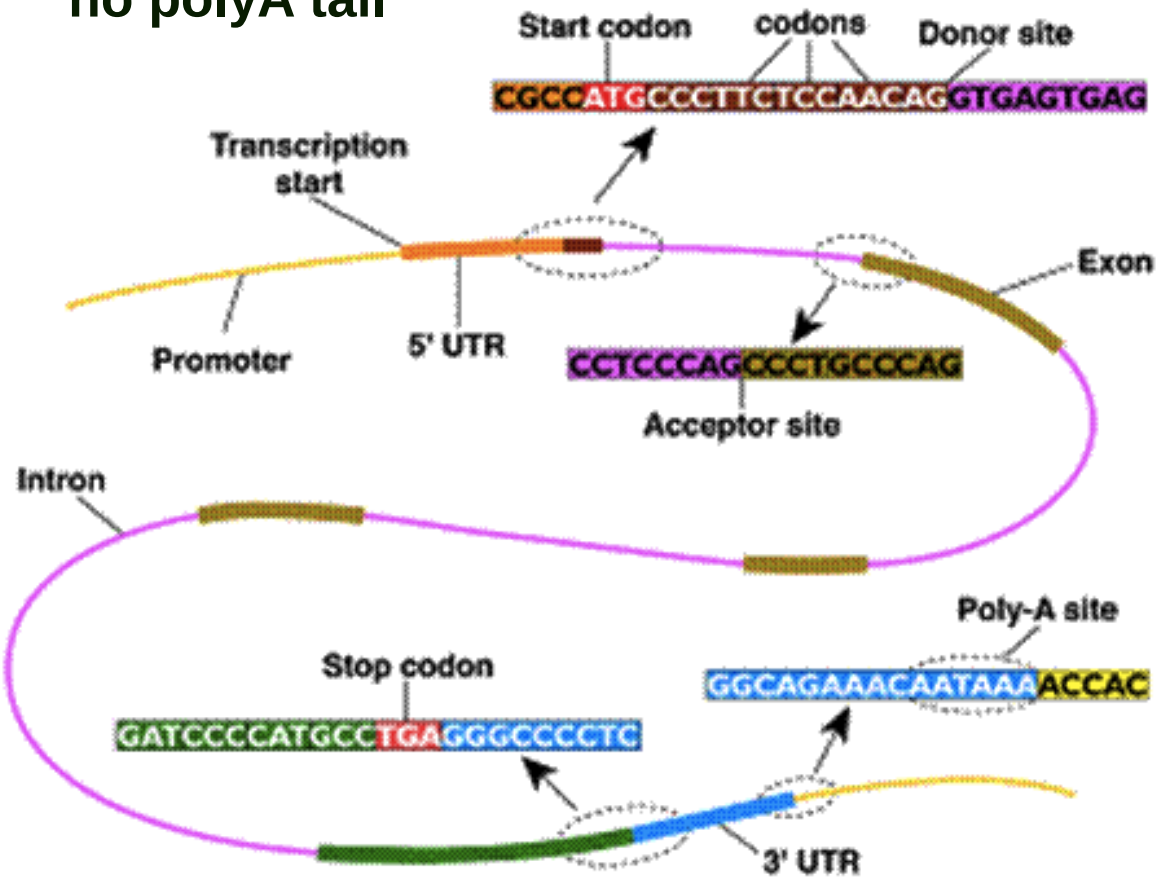
CM, cytoplasmic membrane (+cell wall)
ICM, intracytoplasmic membrane
P, paryphoplasm
I, invaginations of the ICM; D, DNA; V, vesicle

GENE STRUCTURE



in Bacteria:

- **operons**
- **polycistronic**
- **no 5’ cap, no introns, no polyA tail**

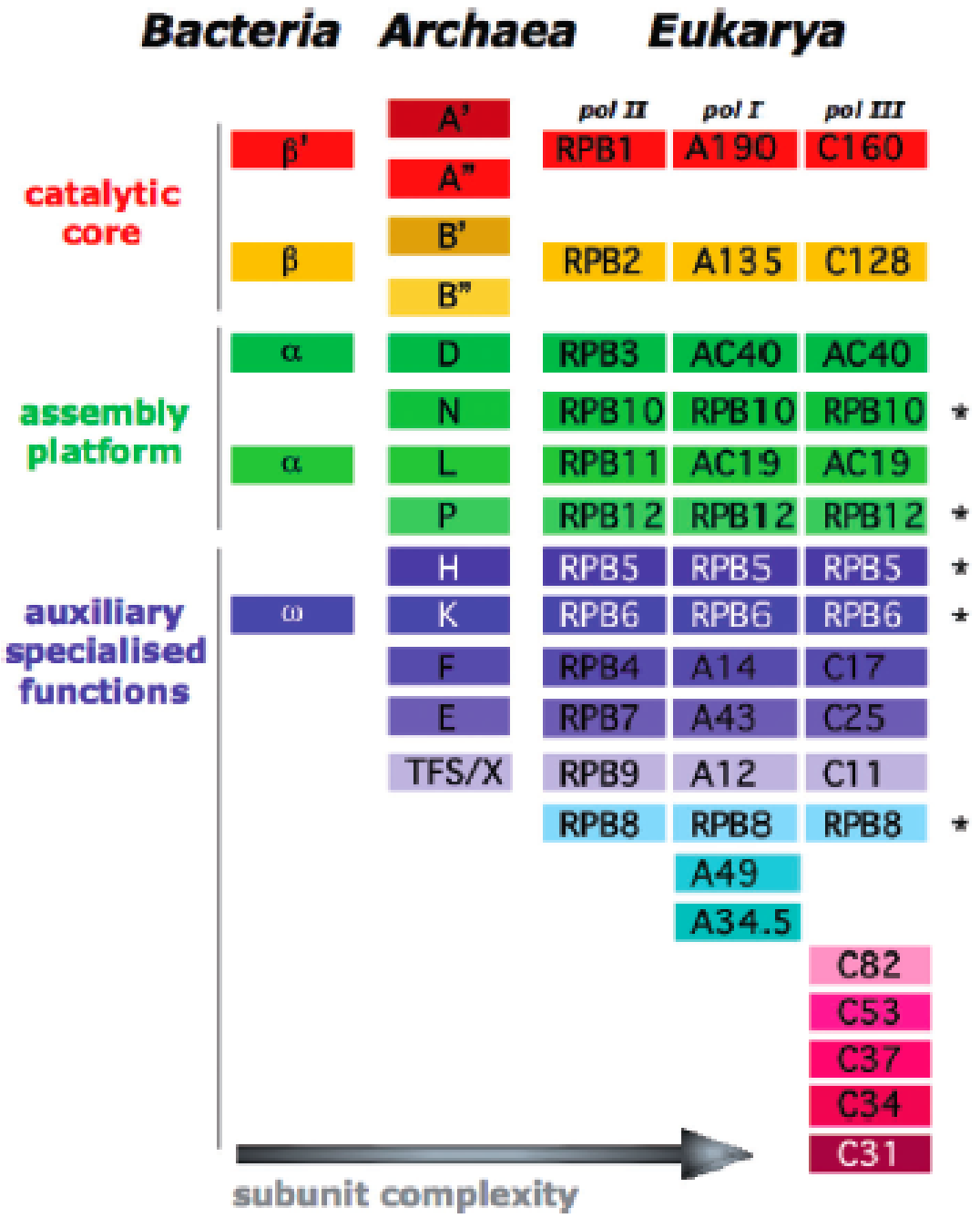


in Eukarya:

- **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

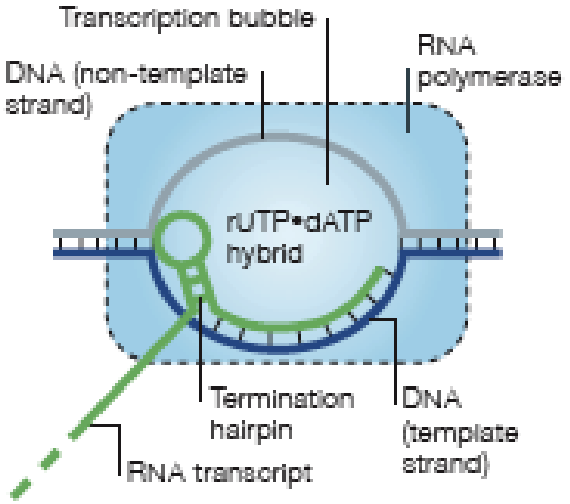
RNA POLYMERASES



Werner, Mol Microbiol, 2007

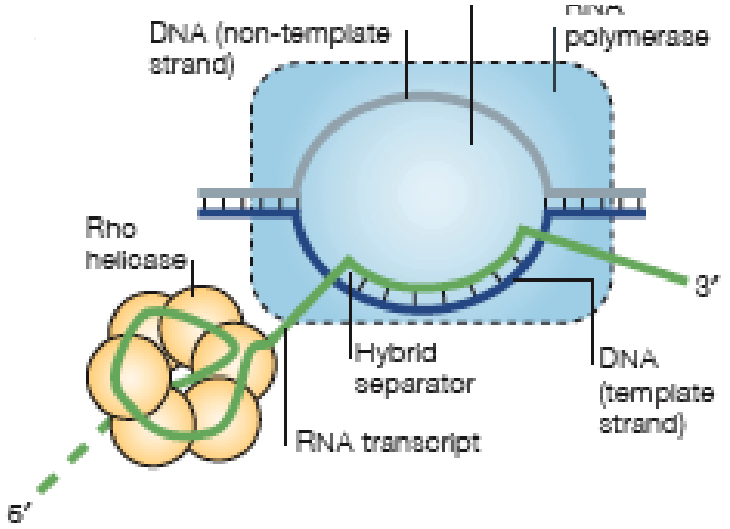
TRANSCRIPTION TERMINATION

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

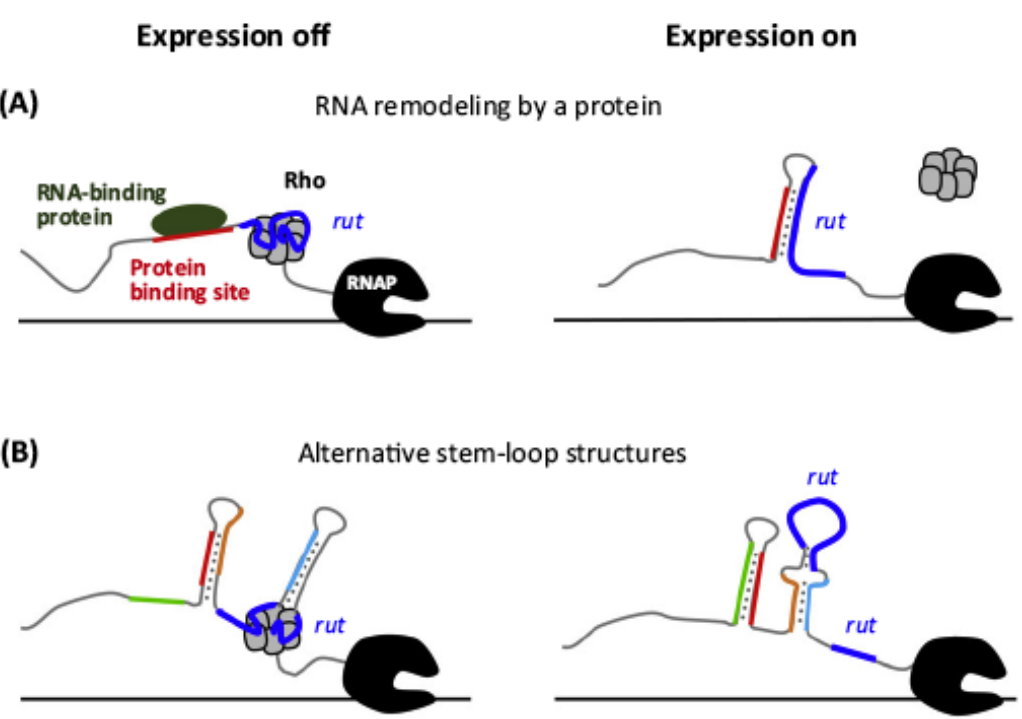
Rho-dependent termination

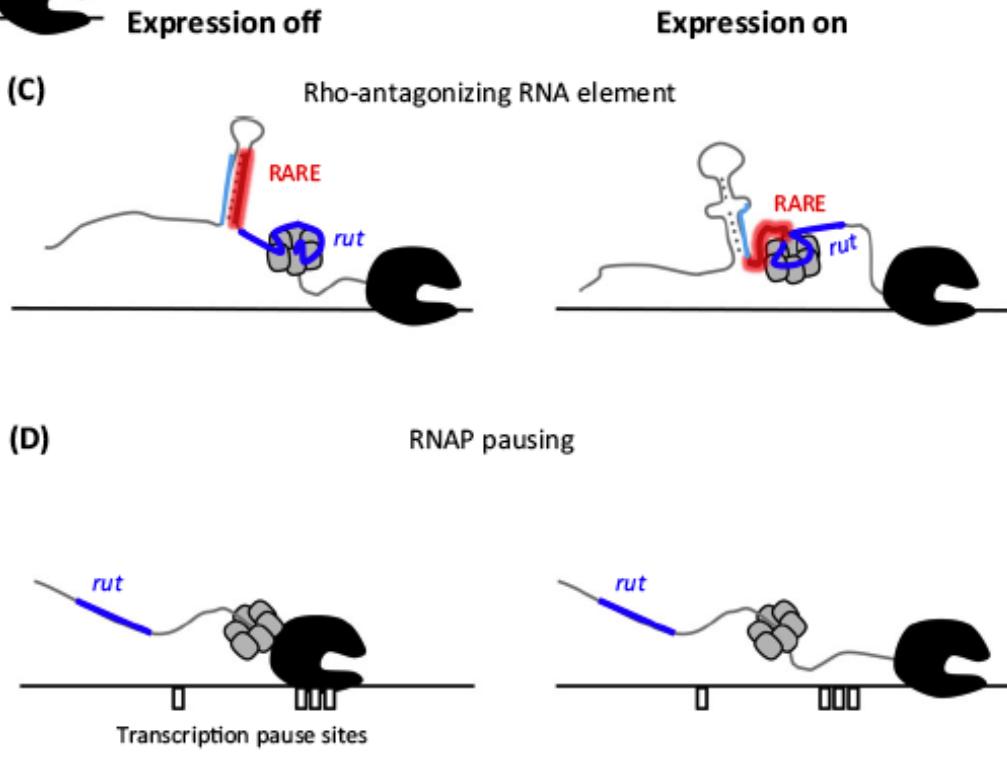


- mediated by a hexameric helicase Rho
- binds to the nascent transcript
- translocates along the RNA
- catches up with RNAP and stops at pause sites
- changes RNA conformation resulting in DNA-RNA hybrid destabilisation

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

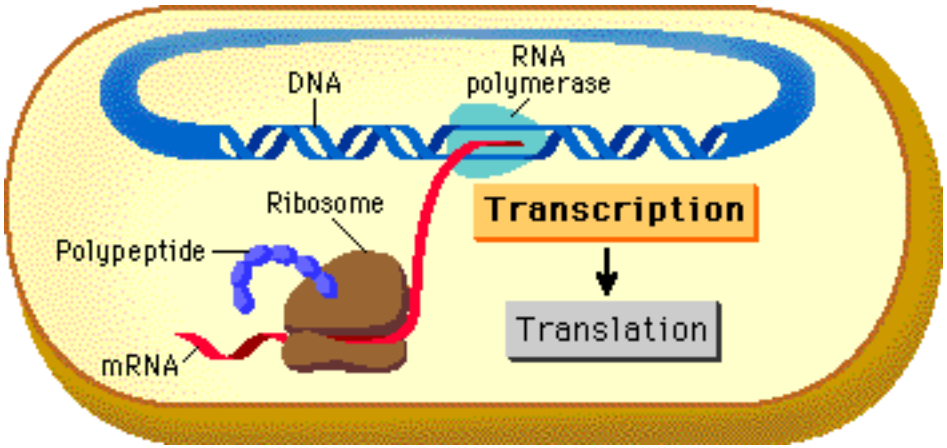
REGULATED Rho-DEPENDENT TRANSCRIPTION TERMINATION

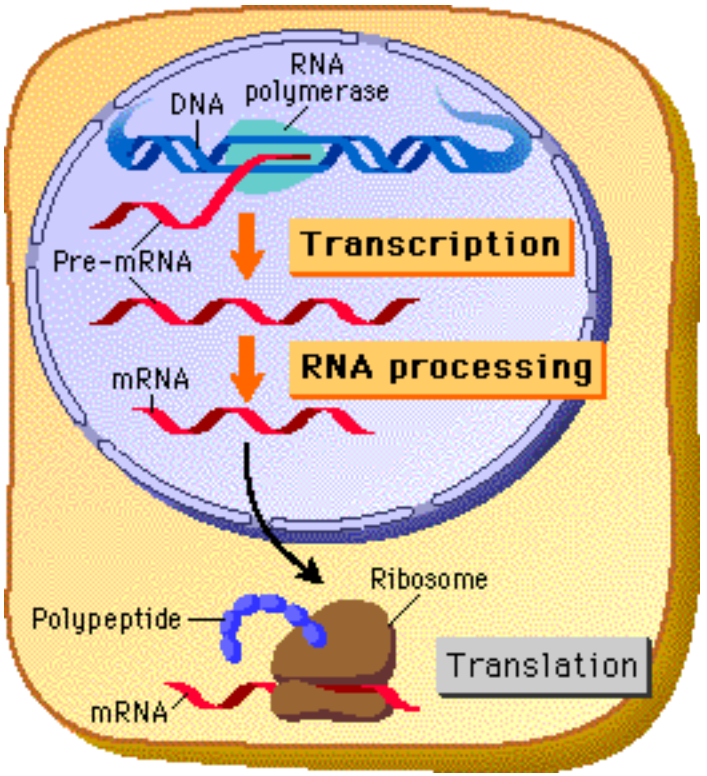


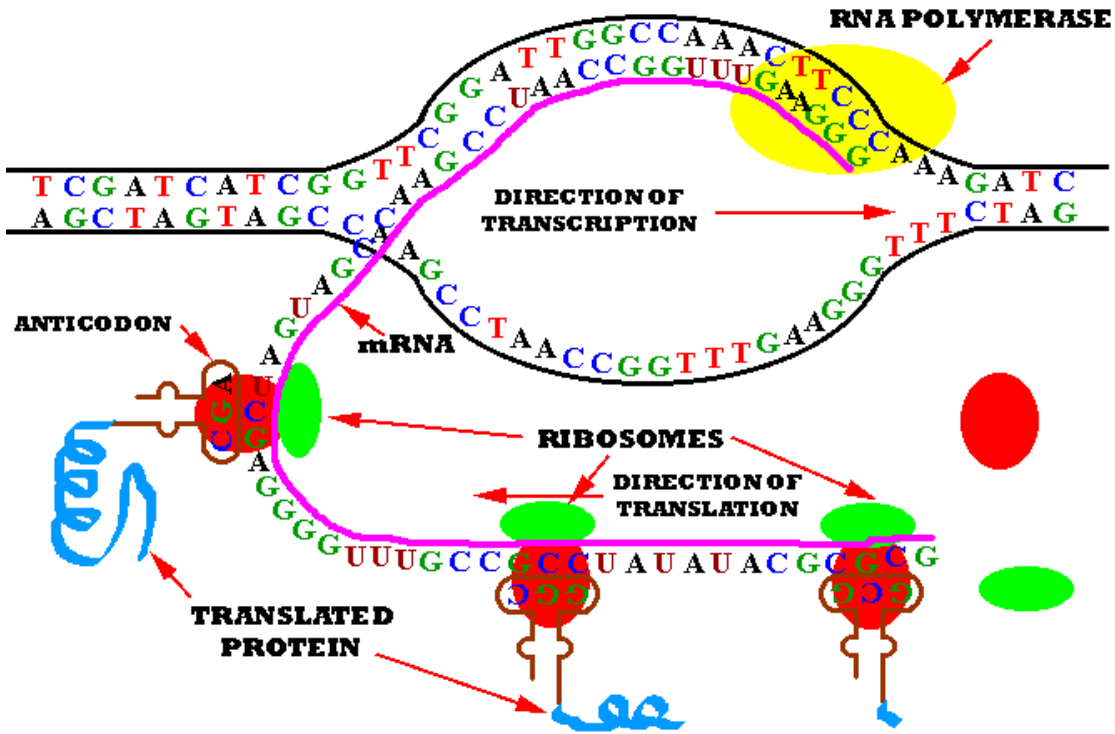


Kriner et al, TiBS, 2016

GENE EXPRESSION: BACTERIA vs EUKARYA
TRANSCRIPTION AND TRANSLATION

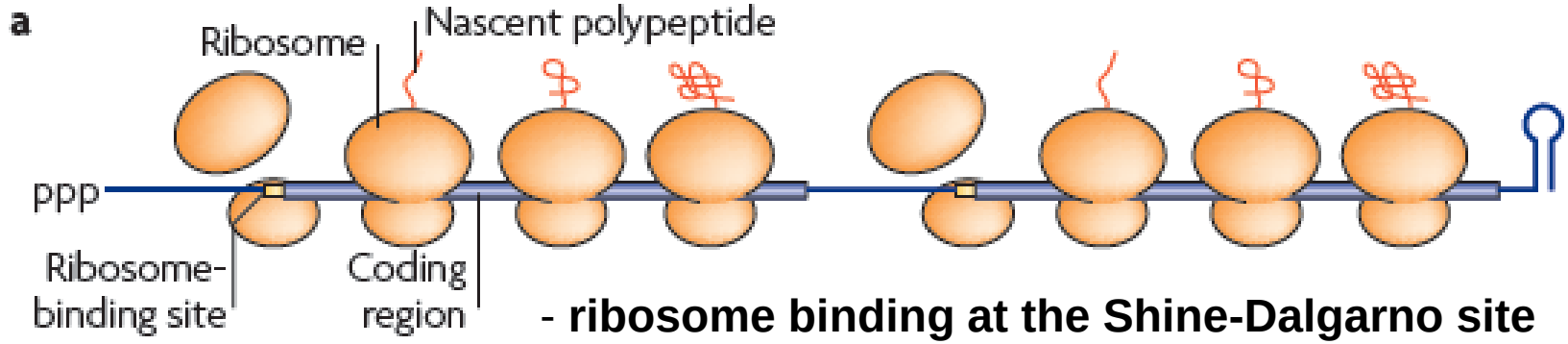




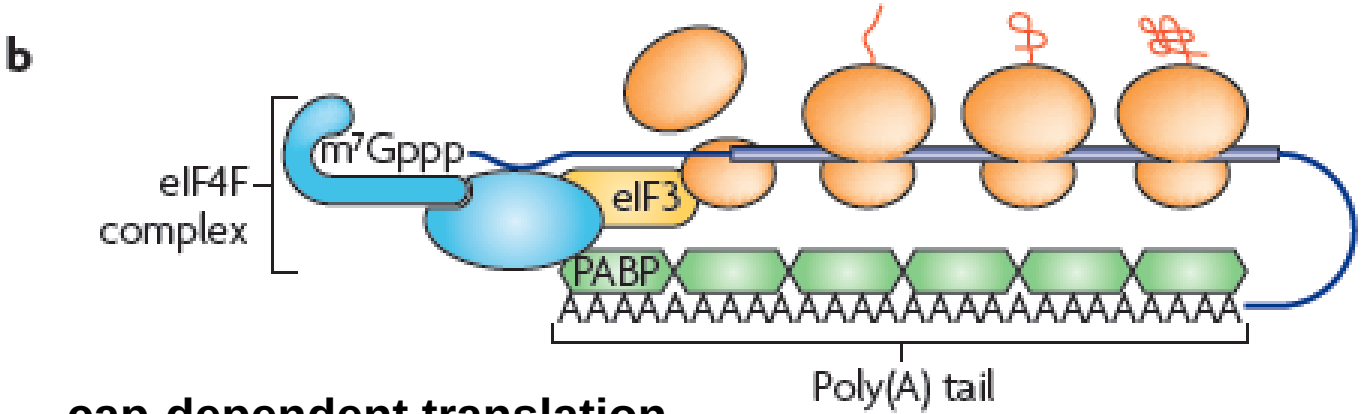


http://www.phschool.com/science/biology_place/biocoach/transcription/tctlpreu.html

mRNA STRUCTURE AND TRANSLATION
BACTERIA vs EUKARYA



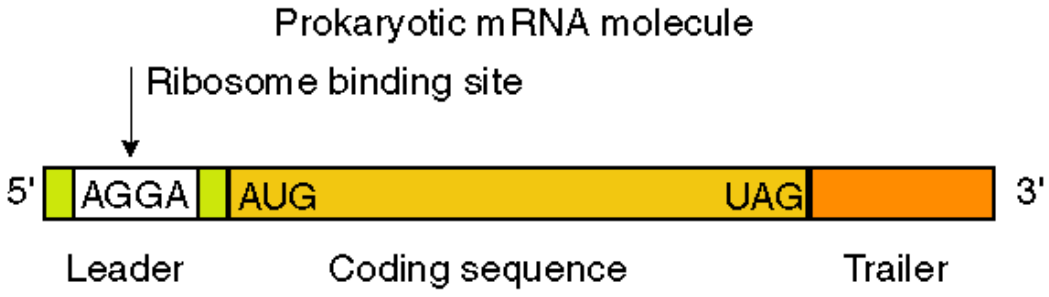
- ribosome binding at the Shine-Dalgarno site
- no ribosome scanning



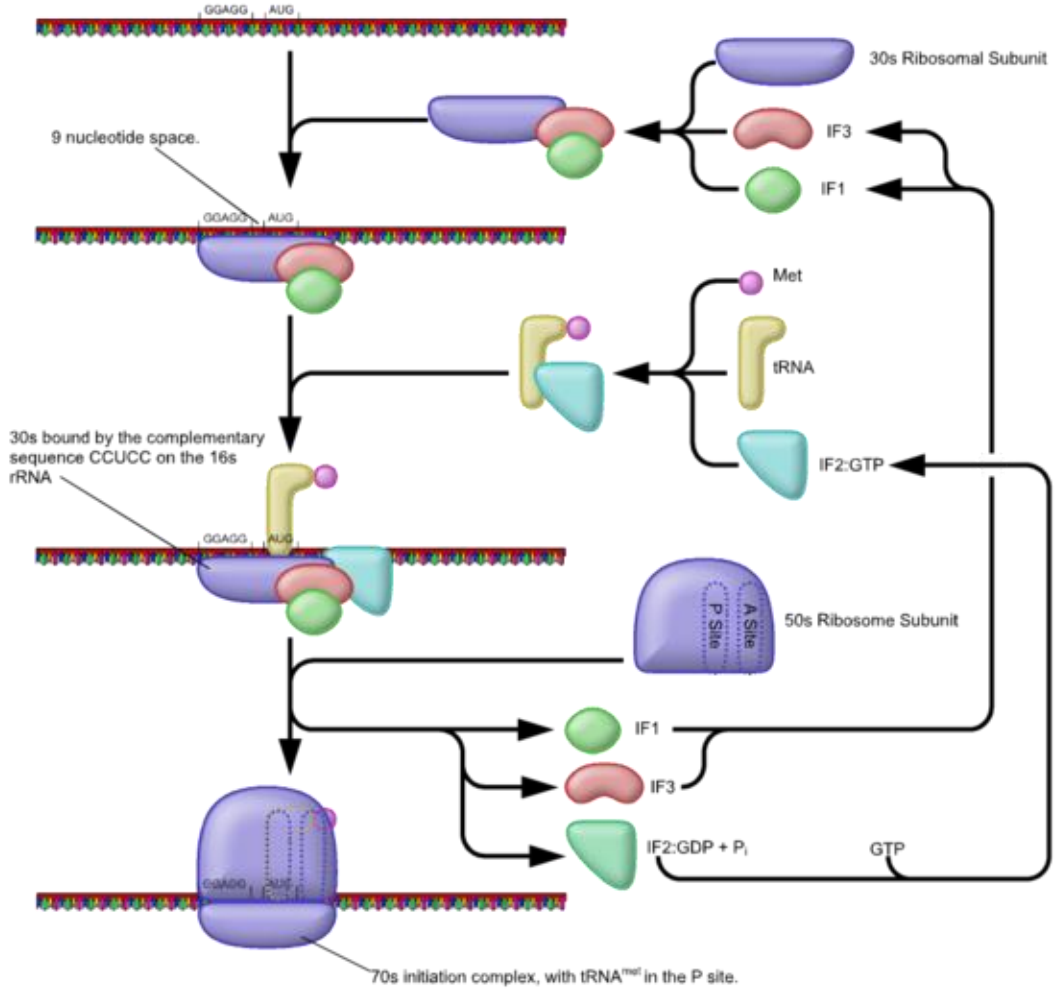
- cap-dependent translation
- ribosome scanning for translation initiation

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

TRANSLATION in BACTERIA

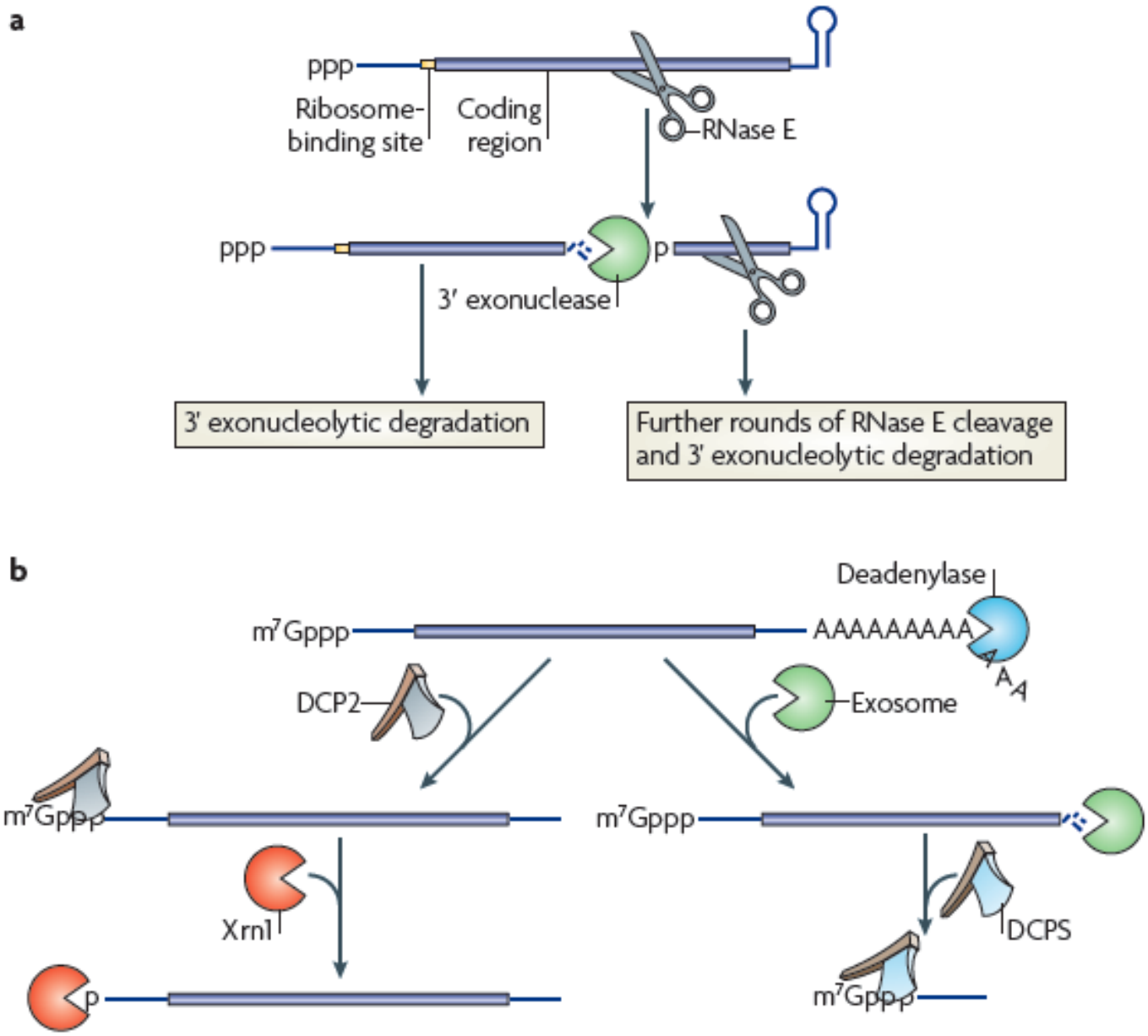


Shine-Dalgarno sequence upstream of AUG start codon helps to recruit the ribosome by interacting with the complementary region in the 3' end of 16S rRNA



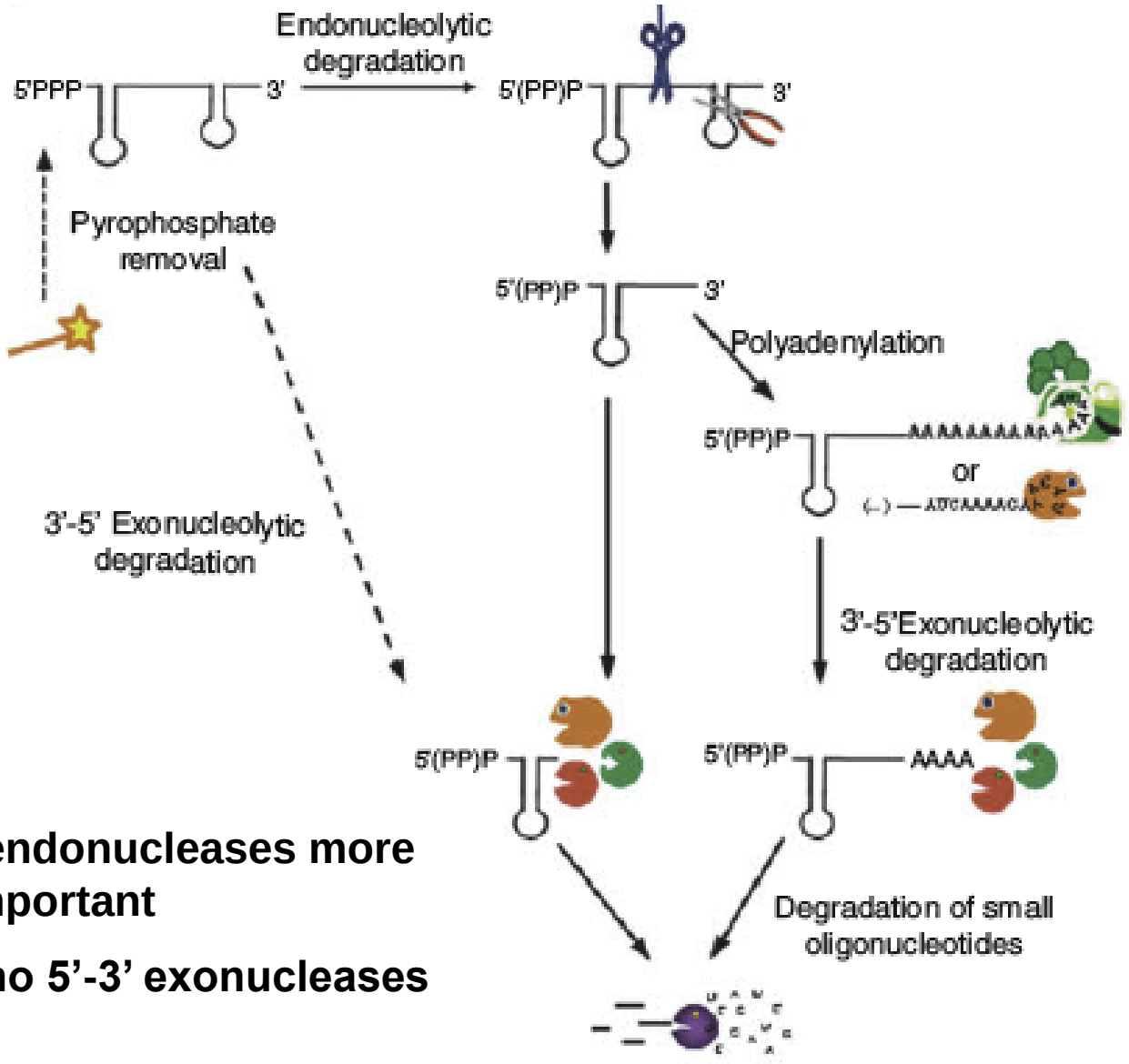
see the movie at:
http://pubs.acs.org/cen/multimedia/85/ribosome/translation_bacterial.html

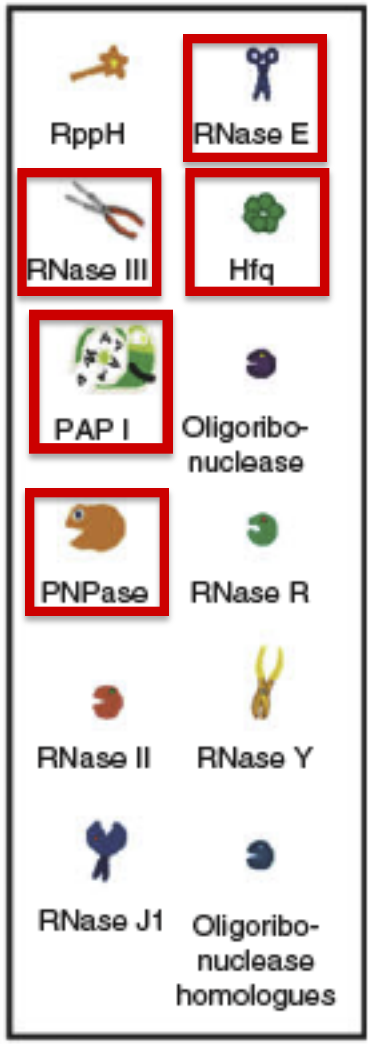
mRNA DECAY BACTERIA vs EUKARYA



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

mRNA DECAY in BACTERIA *E. coli*



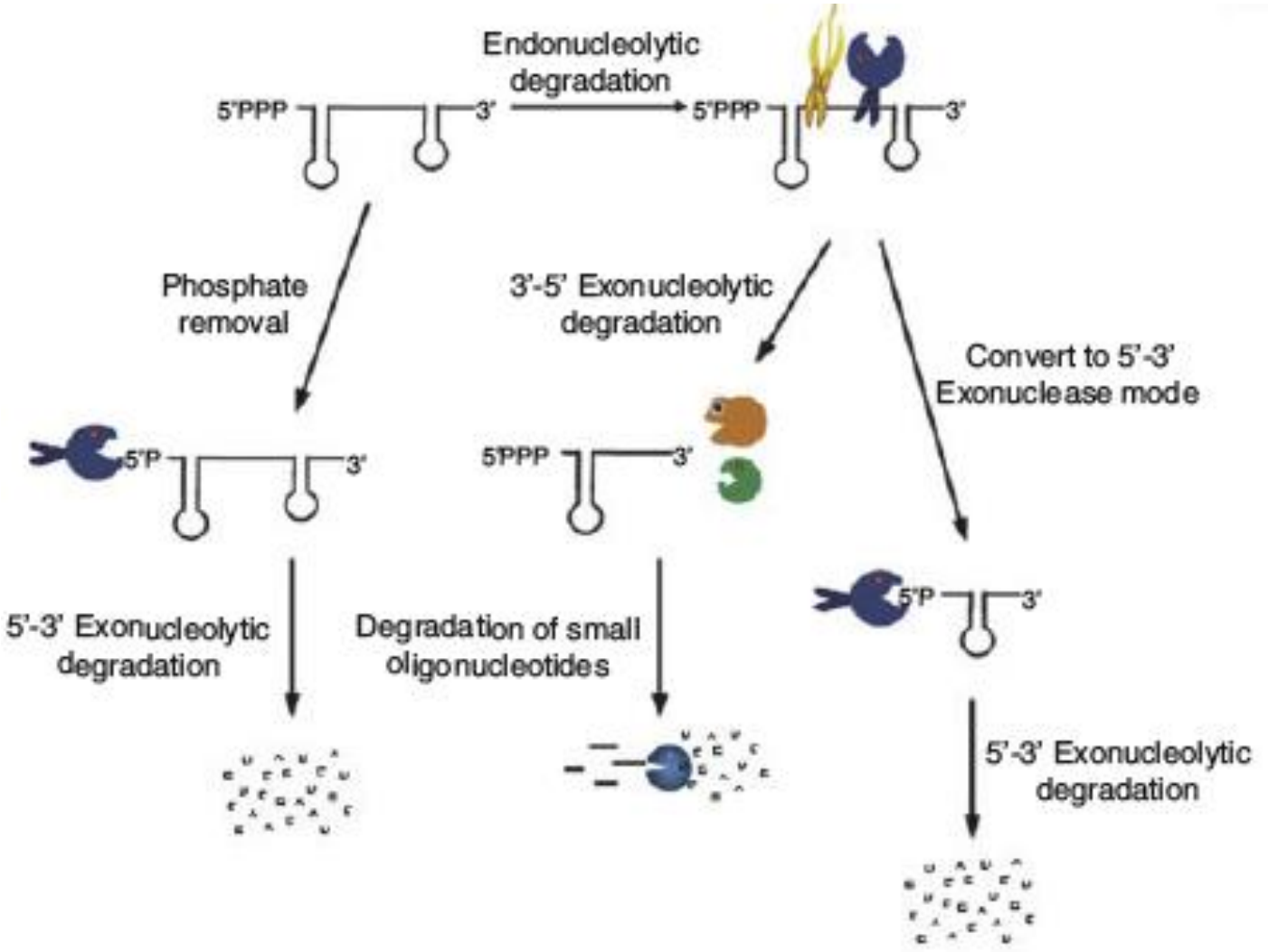


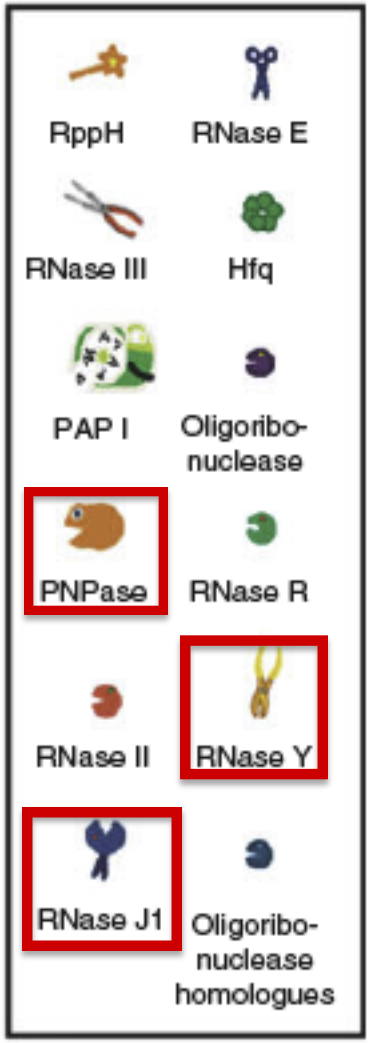
- **endonucleases more important**
- **no 5'-3' exonucleases**

- **no RNase J1 (5' exo and endo)**
- **no RNase Y**

Silva et al, WIRERNA 2011

mRNA DECAY in BACTERIA *B. subtilis*





- no PAP I
- no RNase E

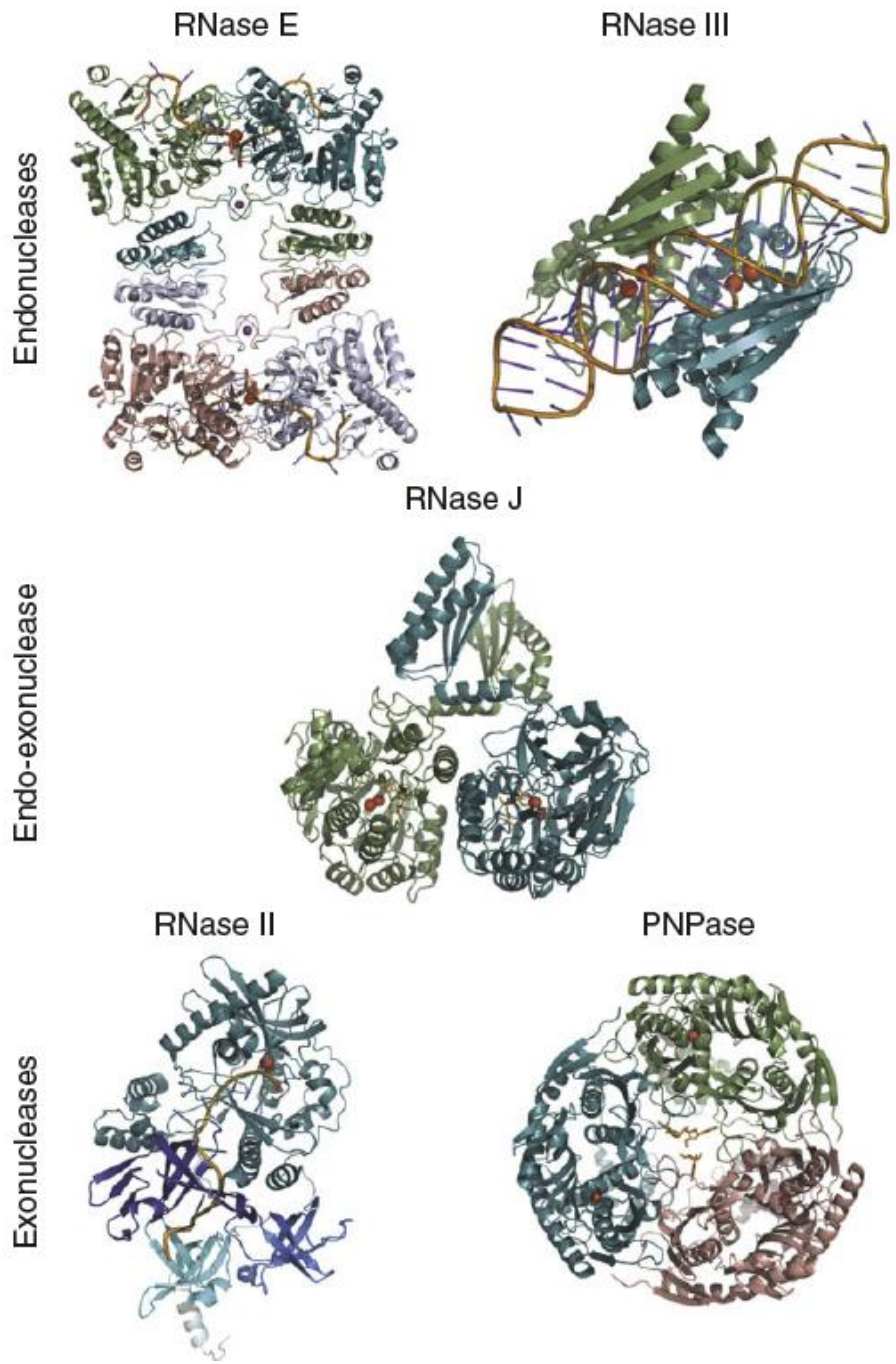
Silva et al, WIRERNA 2011

RNA ENZYMES
BACTERIA vs EUKARYA

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
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
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

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

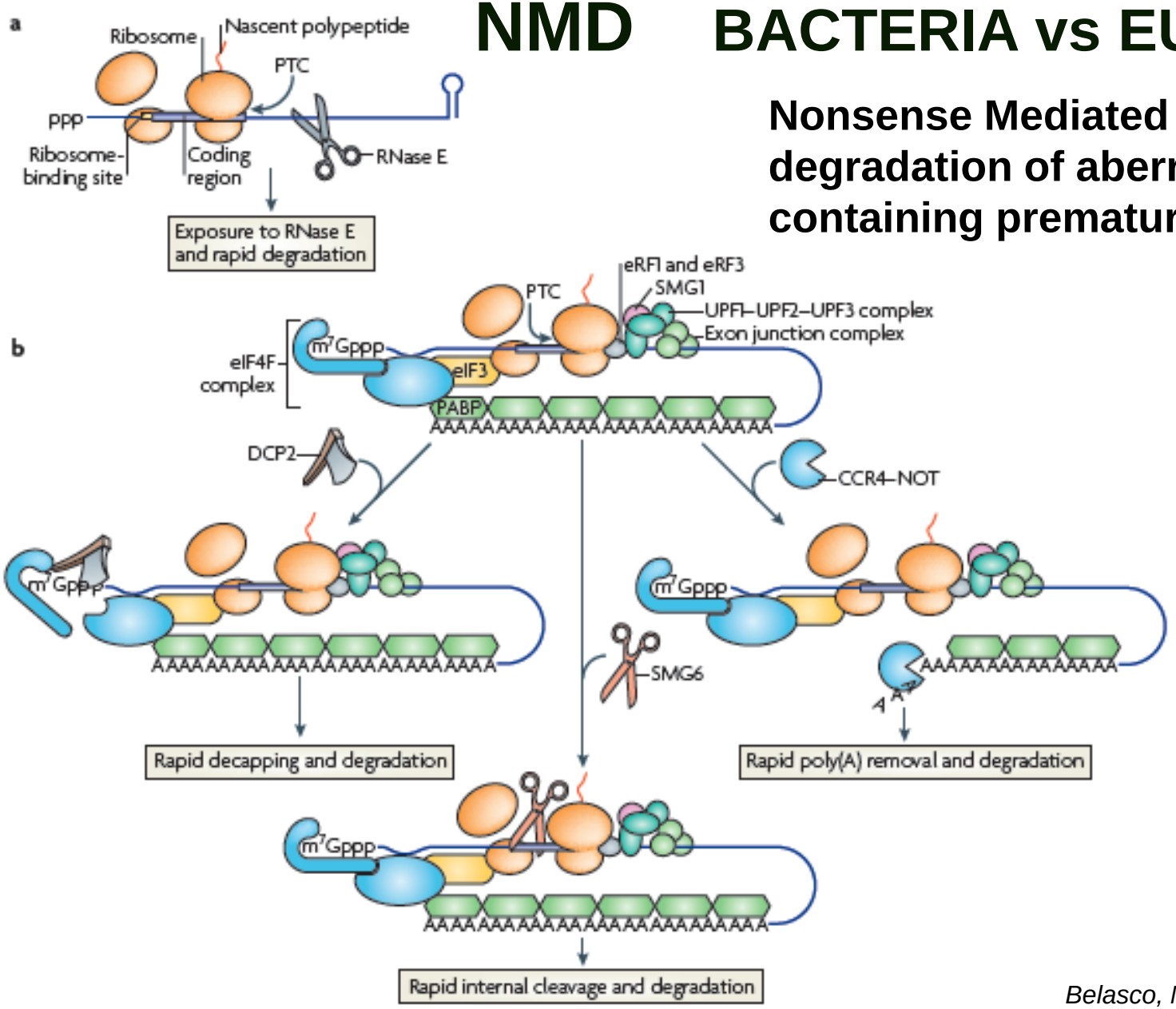


STRUCTURES of BACTERIAL RNA ENZYMES in COMPLEX with SUBSTRATES

Silva et al, WIRERNA 2011

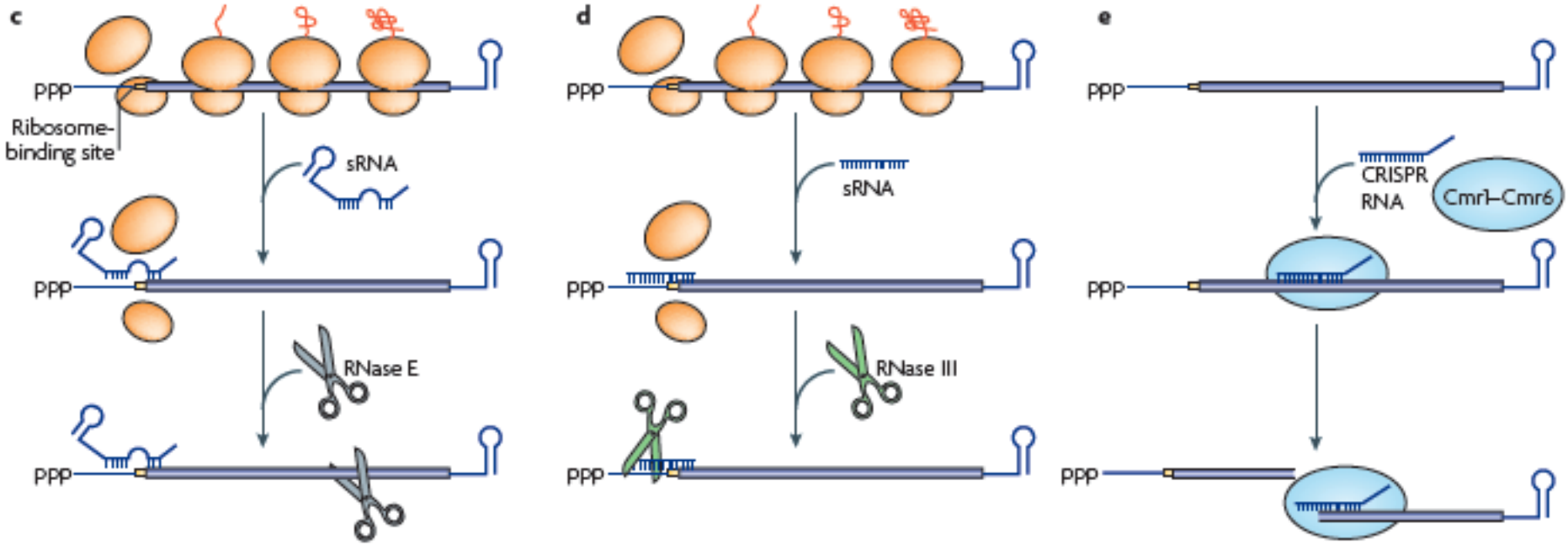
SPECIALIZED mRNA DECAY: NMD BACTERIA vs EUKARYA

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

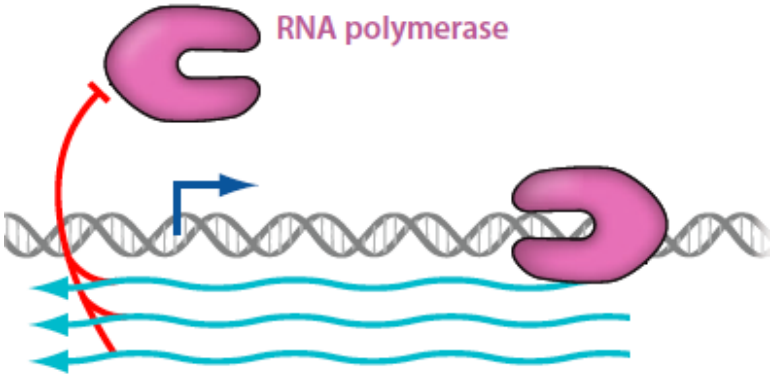


Belasco, Nat.Rev.Mol.Cell.Biol, 2012

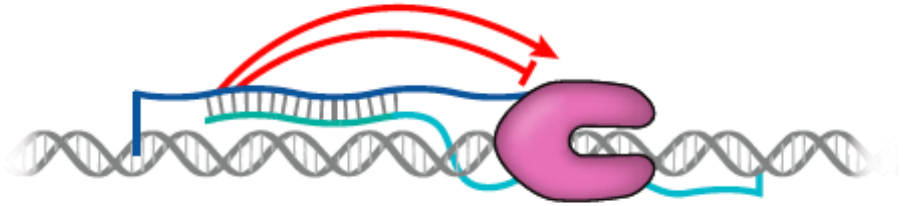
REGULATION of GENE EXPRESSION by sRNAs in BACTERIA



Transcription interference



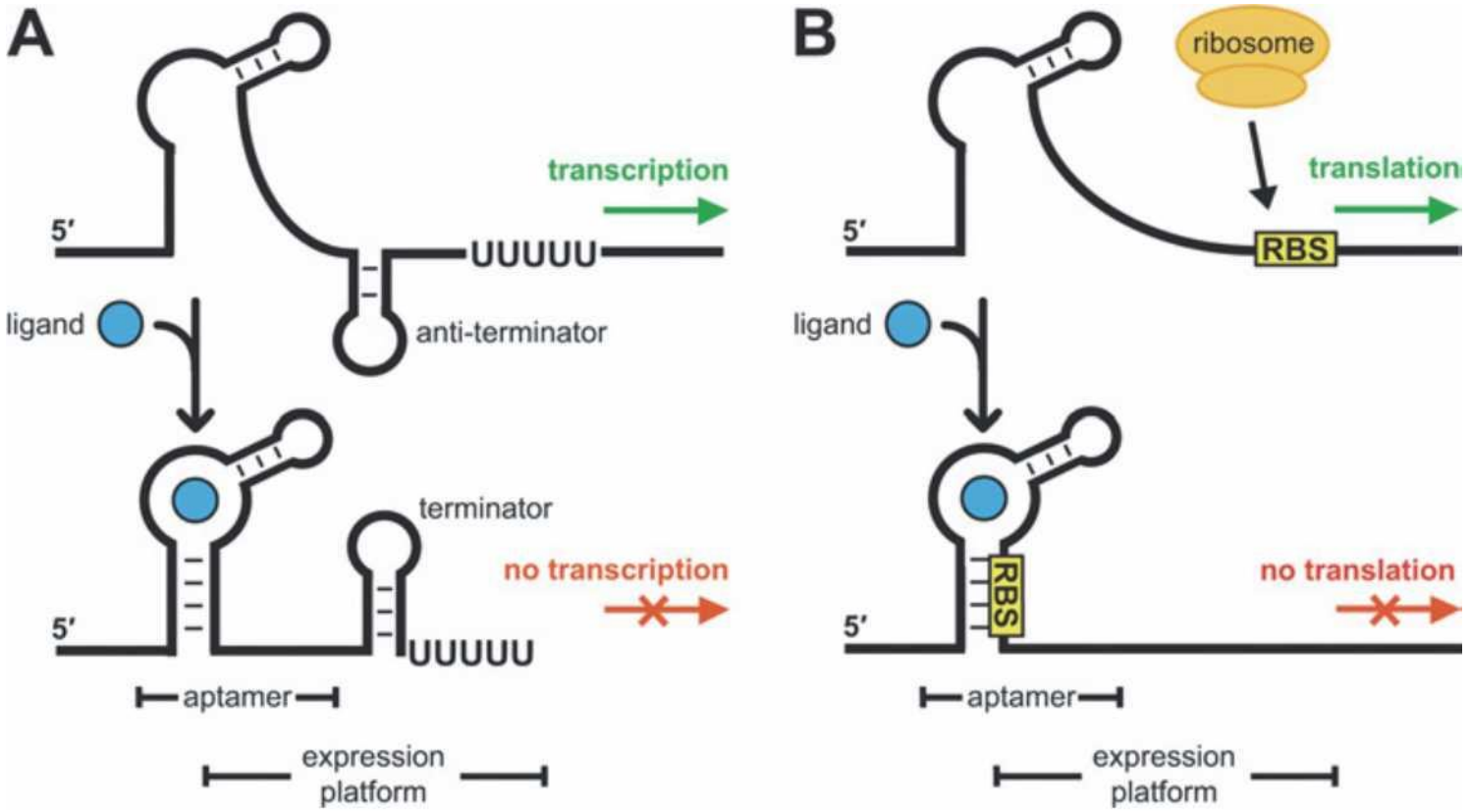
Transcription attenuation



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

RIBOSWITCHES 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



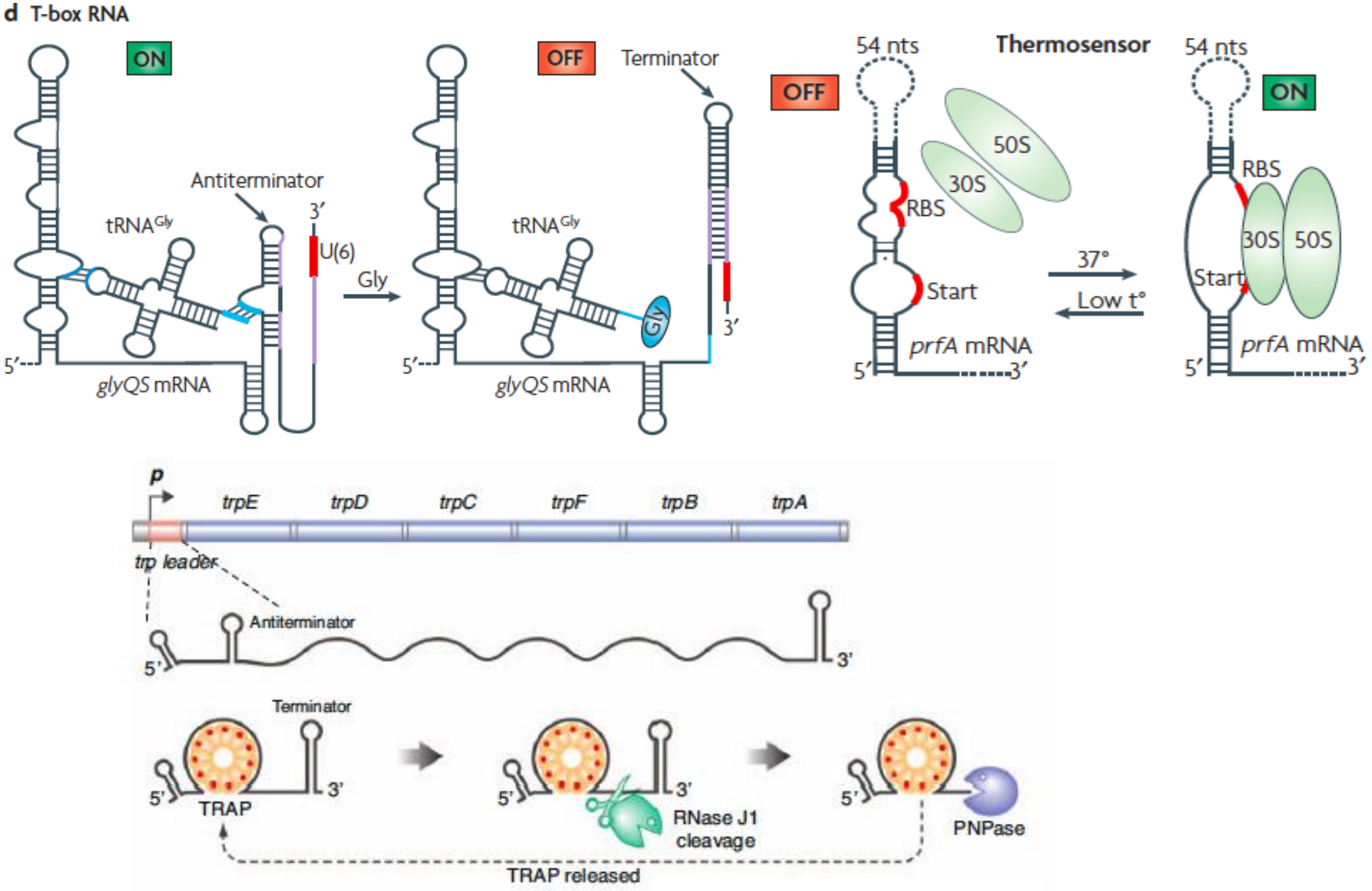
http://www.umich.edu/~rnapeopl/WalterSummaryRiboswitch.htm

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

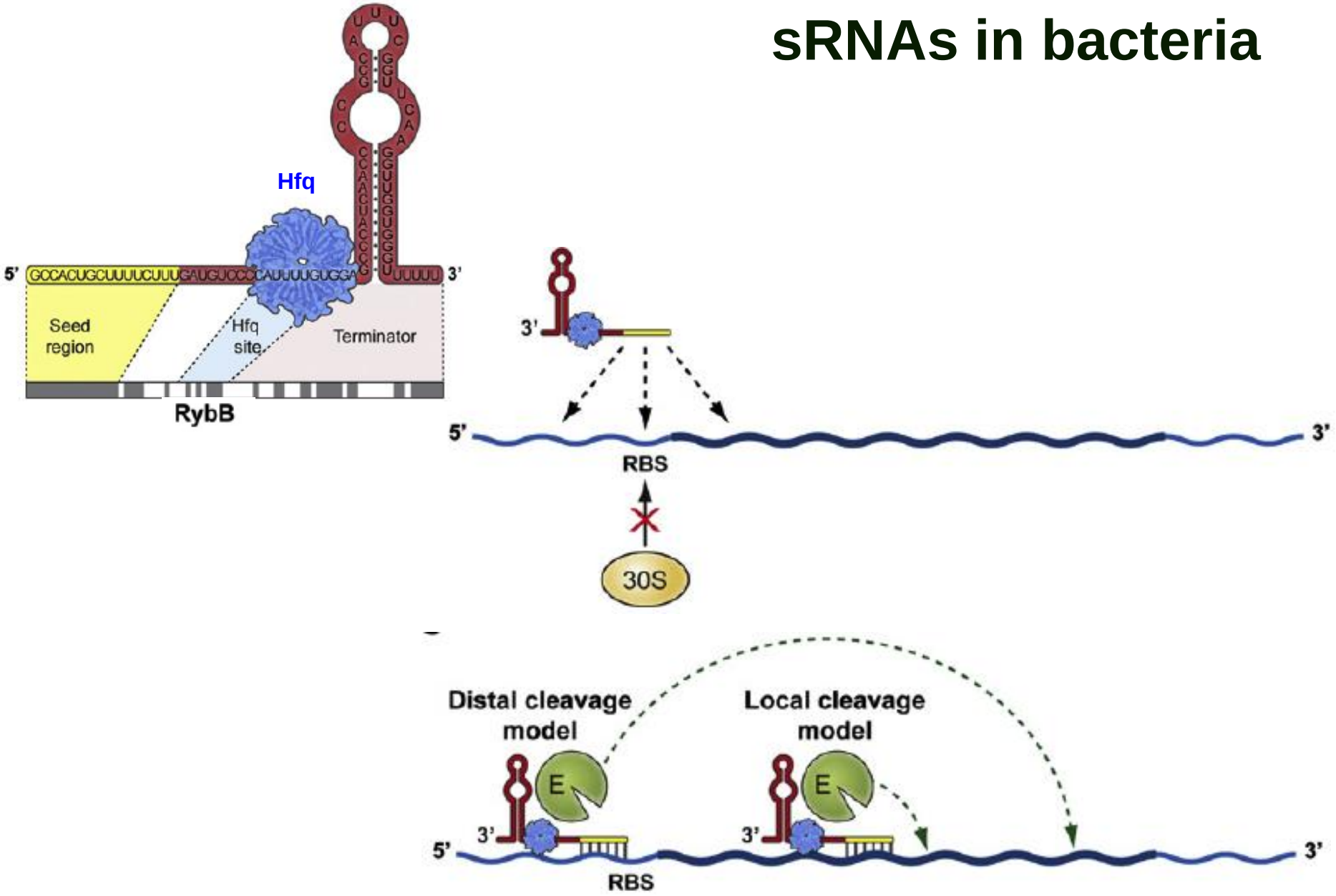
Serganov and Patel, Nat.Rev.Genet, 2007

RIBOSWITCHES



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

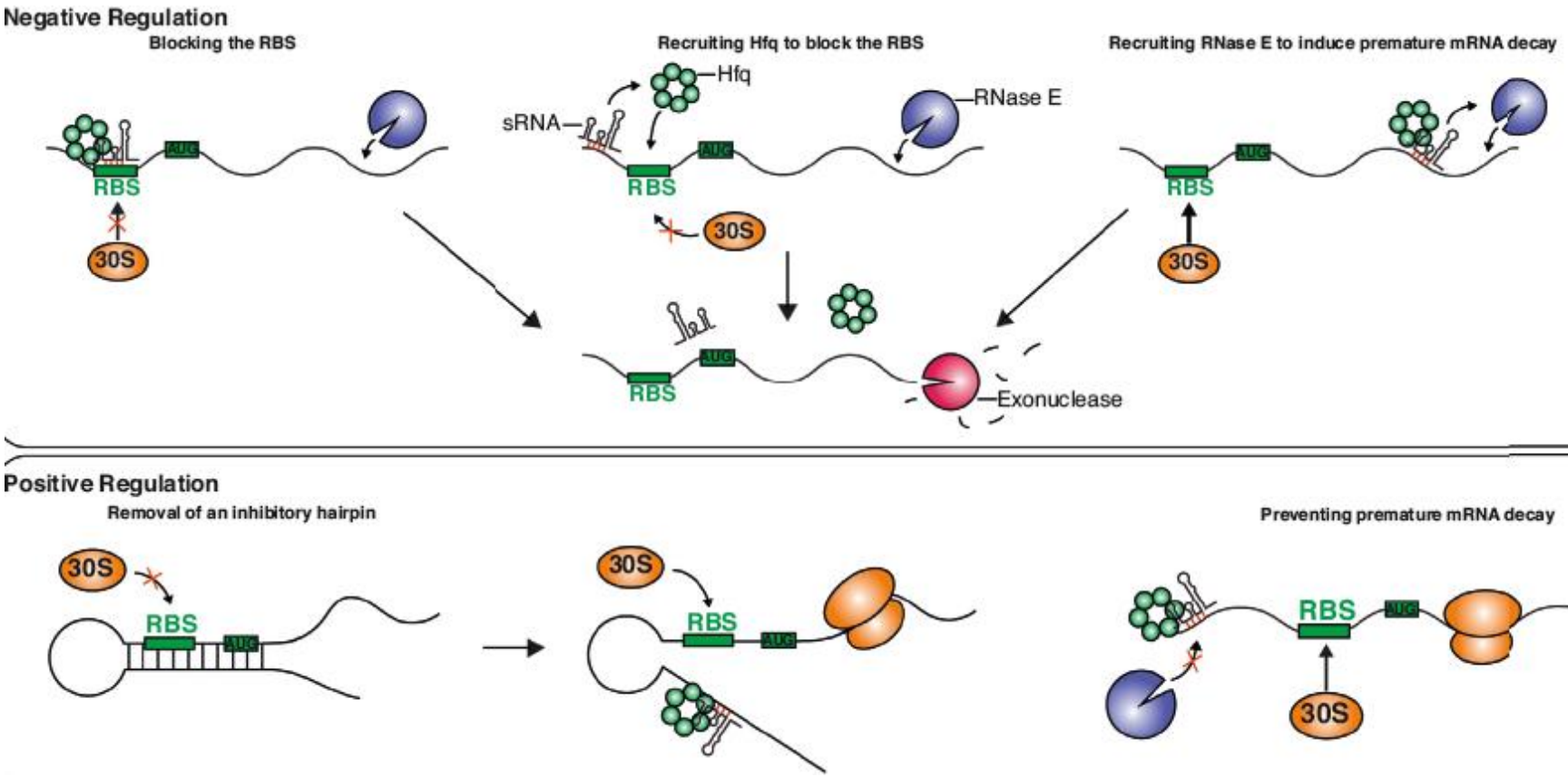
sRNAs in bacteria



Storz et al, MCell, 2011

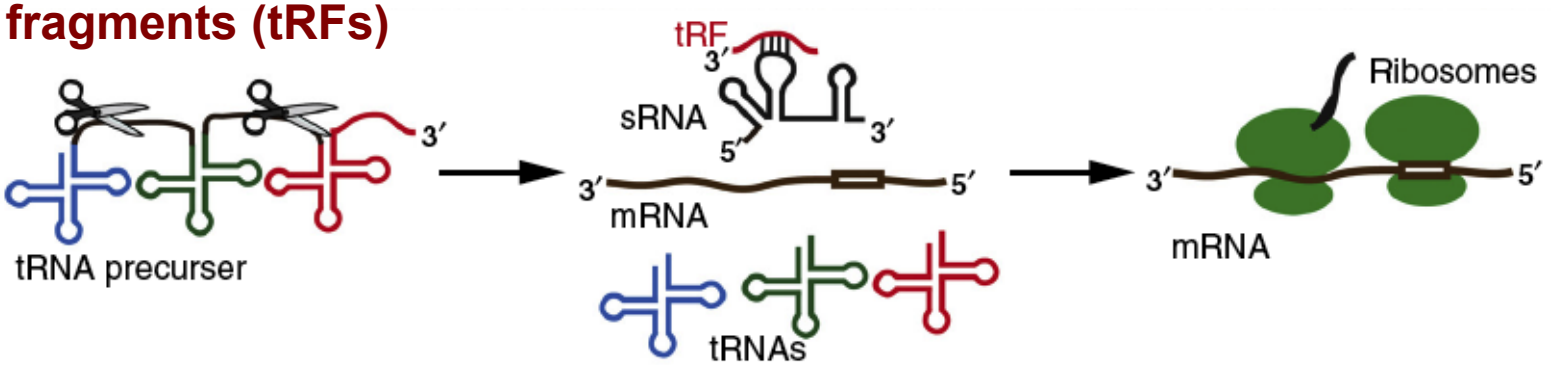
sRNAs in bacteria

Regulation of translation initiation and/or mRNA decay



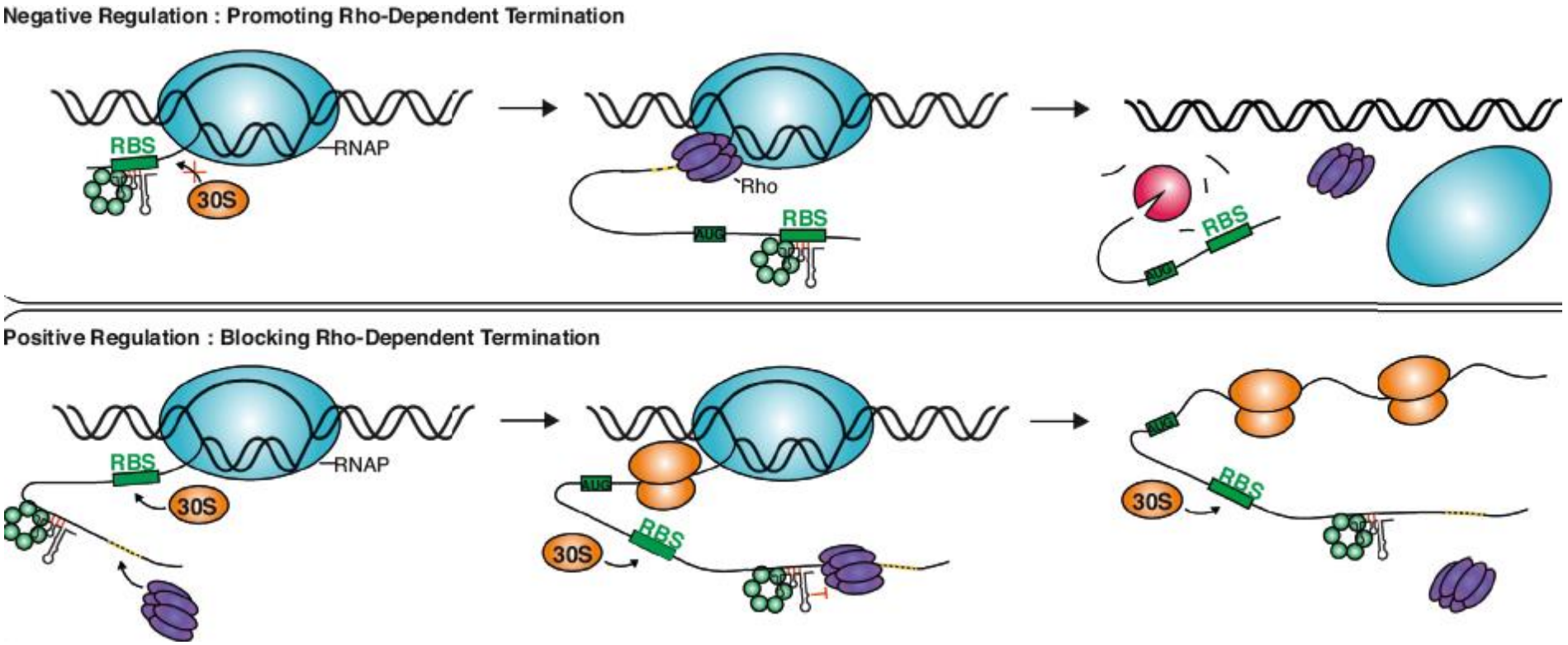
Kavita et al, Curr Op Micro, 2018

… by tRNA fragments (tRFs)



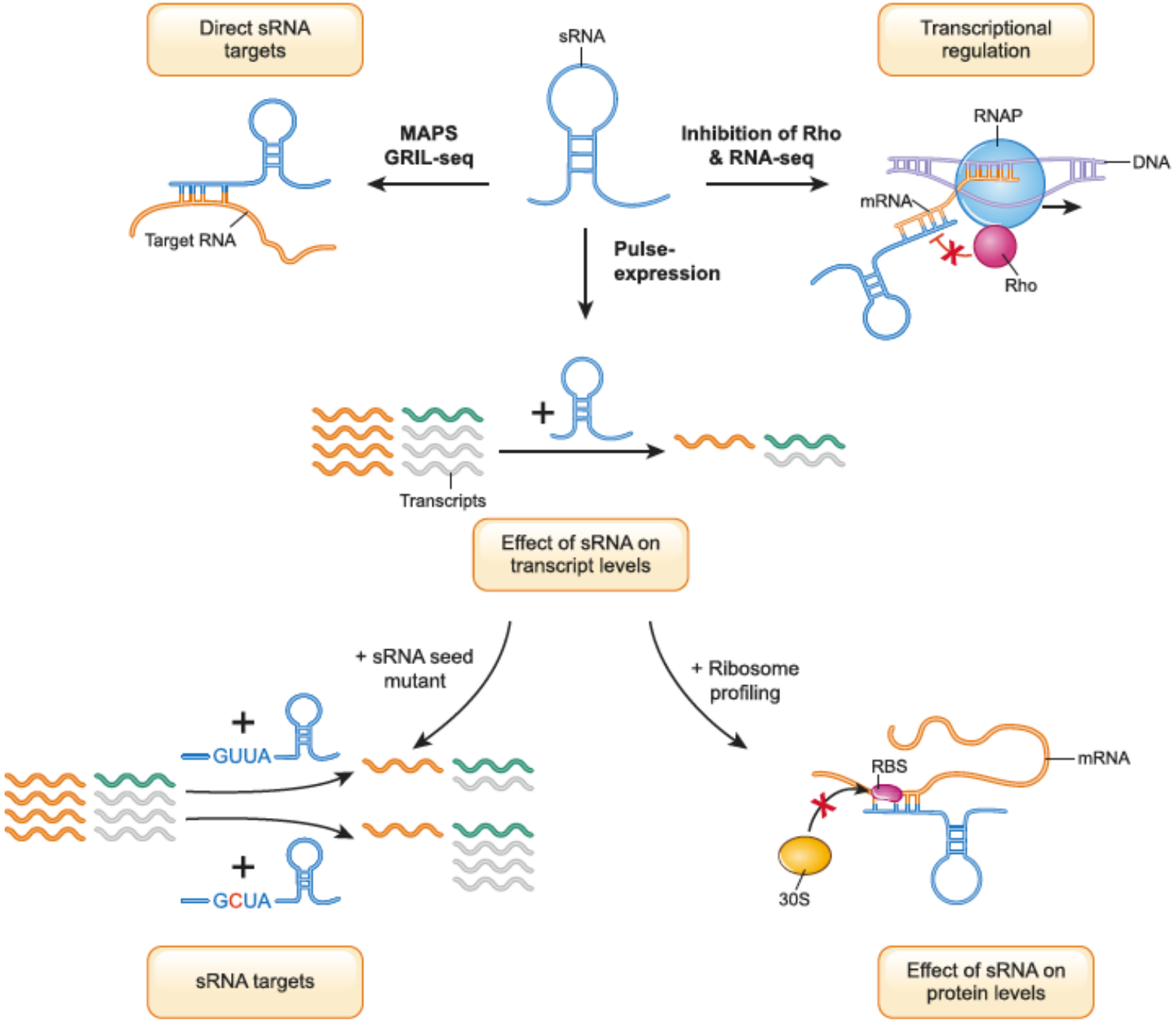
sRNAs in bacteria

Regulation of Rho-dependent transcription termination



Kavita et al, Curr Op Micro, 2018

sRNA functions and targets



Hor et al, MCell, 2017

BACTERIAL POLYADENYLATION

• Two bacterial 3' terminal polymerases:

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

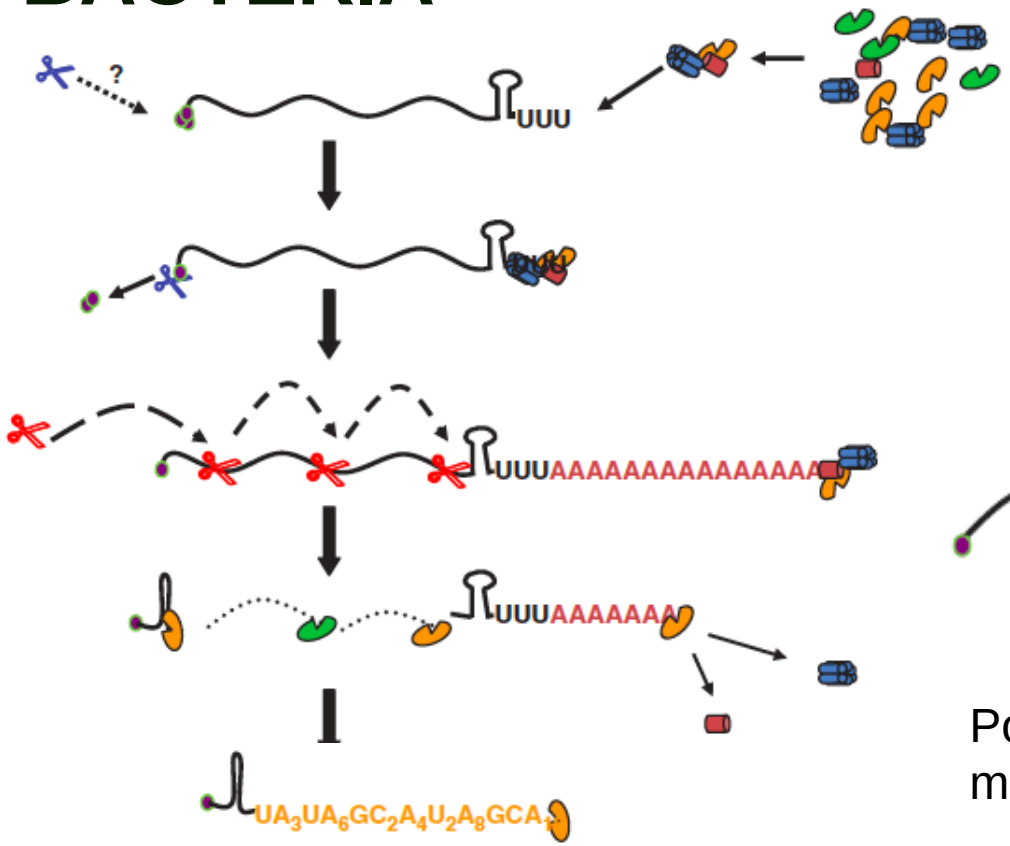
• poly(A) tails shorter (10-60 nts), occur for 2-60% of molecules of a given transcript

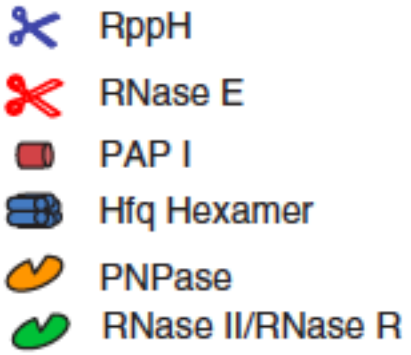
• polyadenylation sites are diverse, no consensus

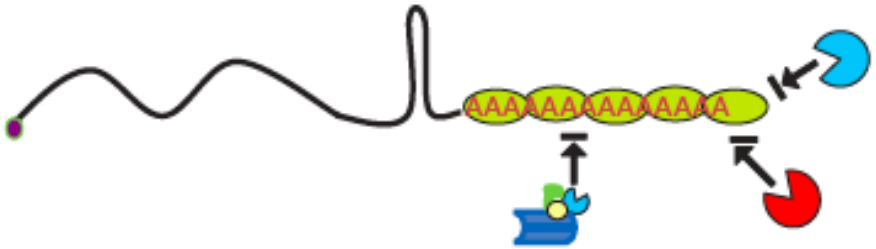
E. coli	mRNA	*lpp, rpsO, ompA, secG, rmf, pcnB, trxA*
	rRNA	16S rRNA, 23S rRNA
	nc RNA	6S RNA, 4.5S RNA, RNA I, SoK, SraK, SraL, GlmY, SsrA, RnpB
	tRNA	*cysT, hisR, leuX, trpT, leuU, tyrT, tyrV*
B. subtilis	mRNA	*mpB, rpsD, cry1Aa*
	rRNA	23S rRNA
	tRNA	$tRNA^{Cys\text{-}LeuU}$
Streptomyces	mRNA	*redD,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

Mohanty and Kushner WIRERNA, 2010

POLYADENYLATION-ASSISTED RNA DECAY in BACTERIA







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

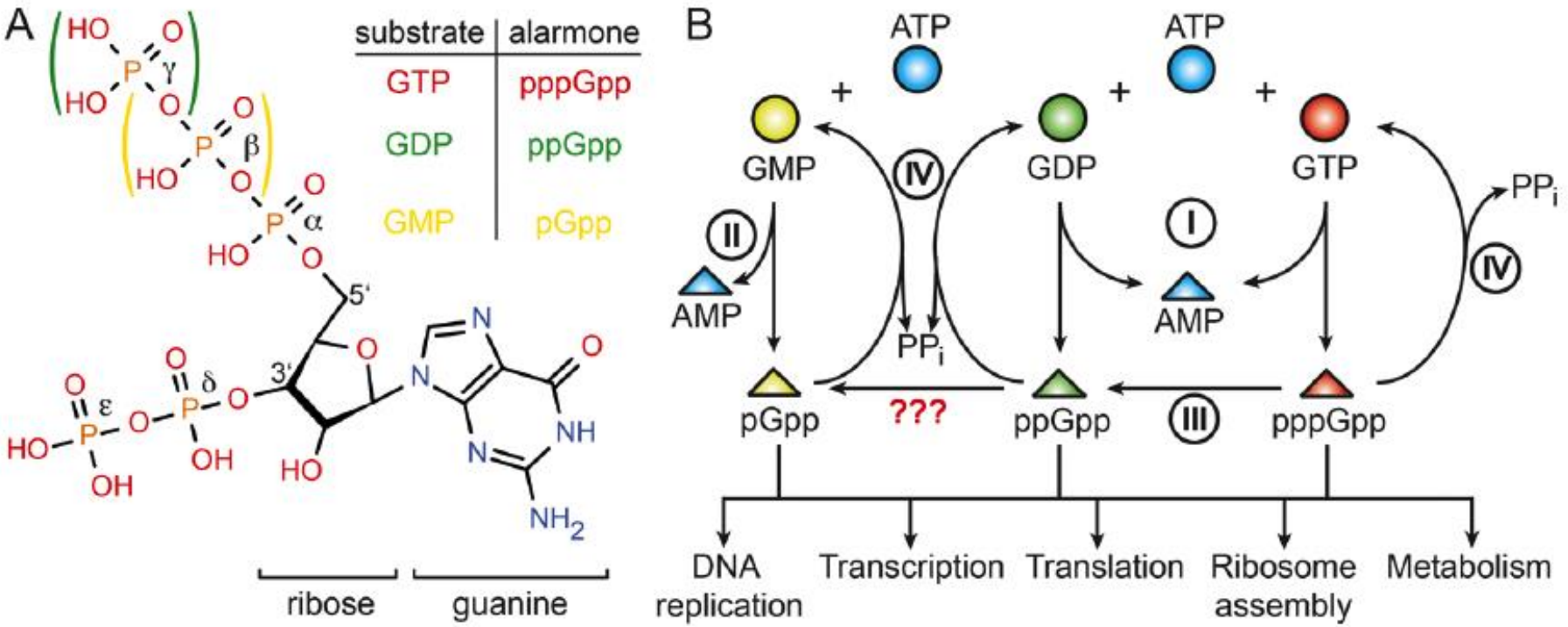
Hfq-mediated polyadenylation by PAP I in *E. coli*

- 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 follow endonucleolytic cleavage by RNaseE

Mohanty and Kushner WIRERNA, 2010

REGULATION by (p)ppGpp alarmones

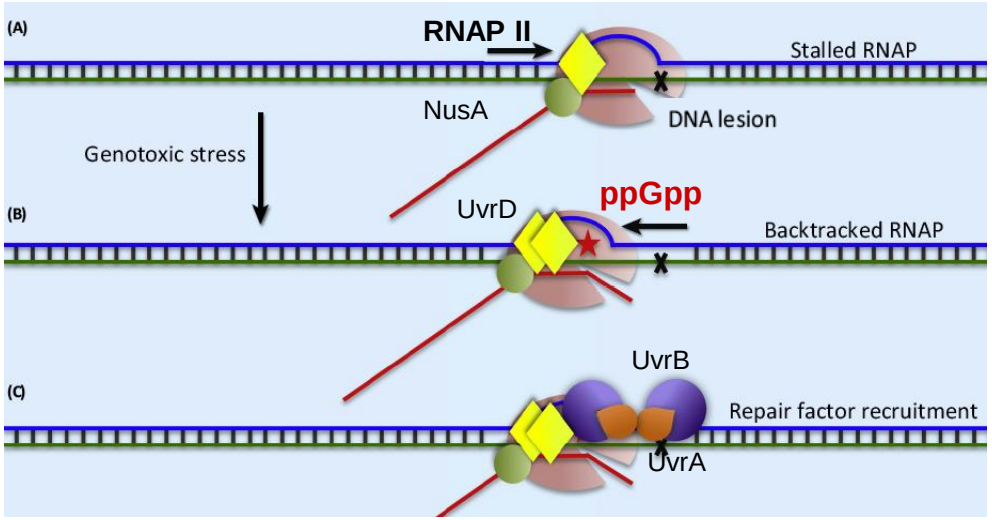
Regulation of different stress response pathways



Steinchen and Bange, *Mol Microbiol*, 2016

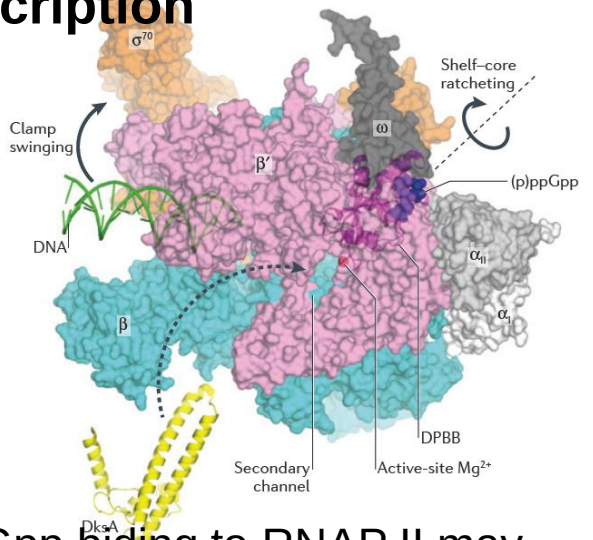
REGULATION by (p)ppGpp alarmones

Transcription-coupled repair



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

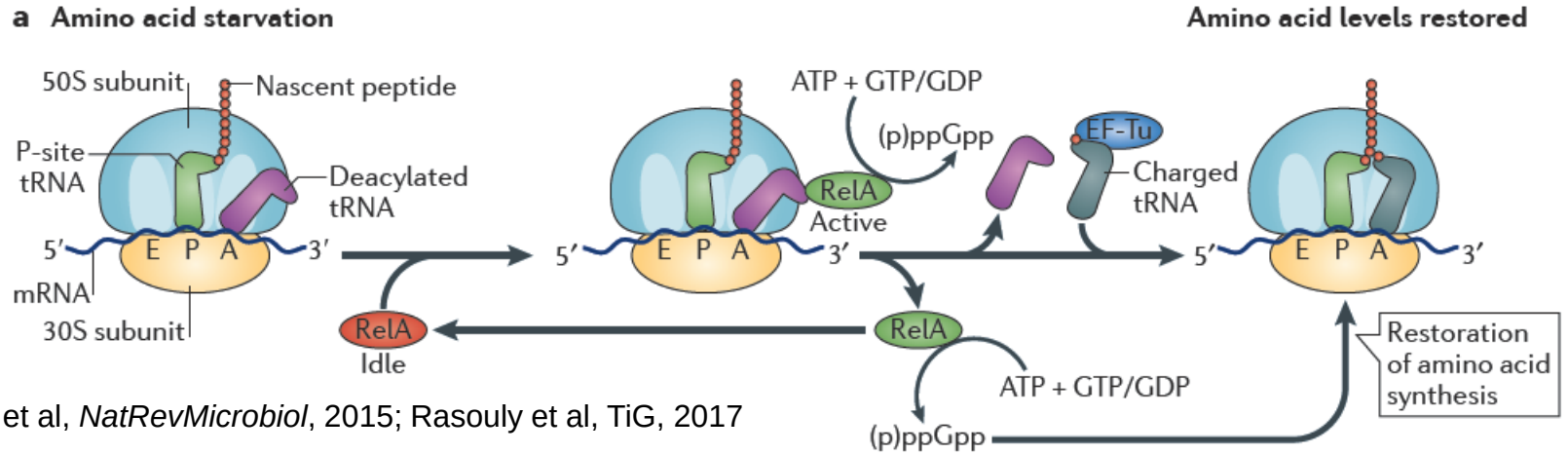
Transcription



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

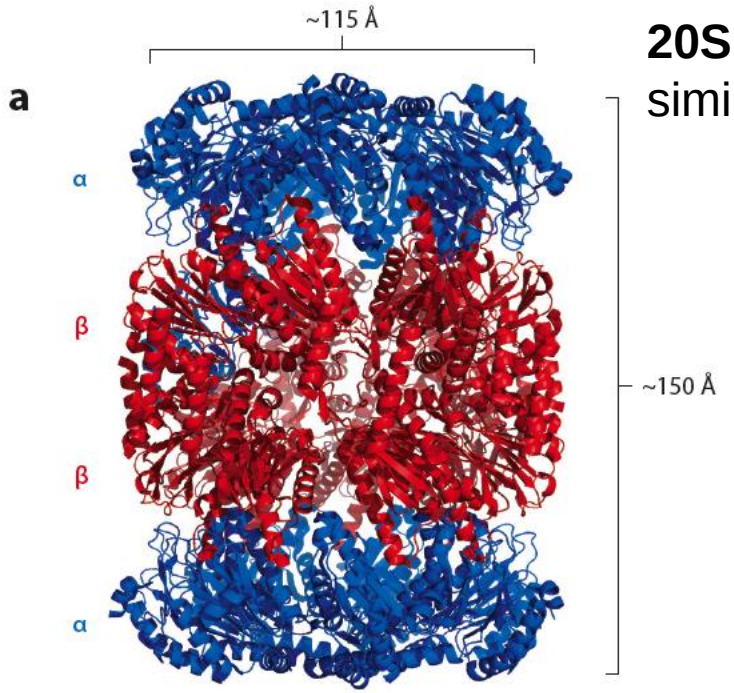
Translation

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

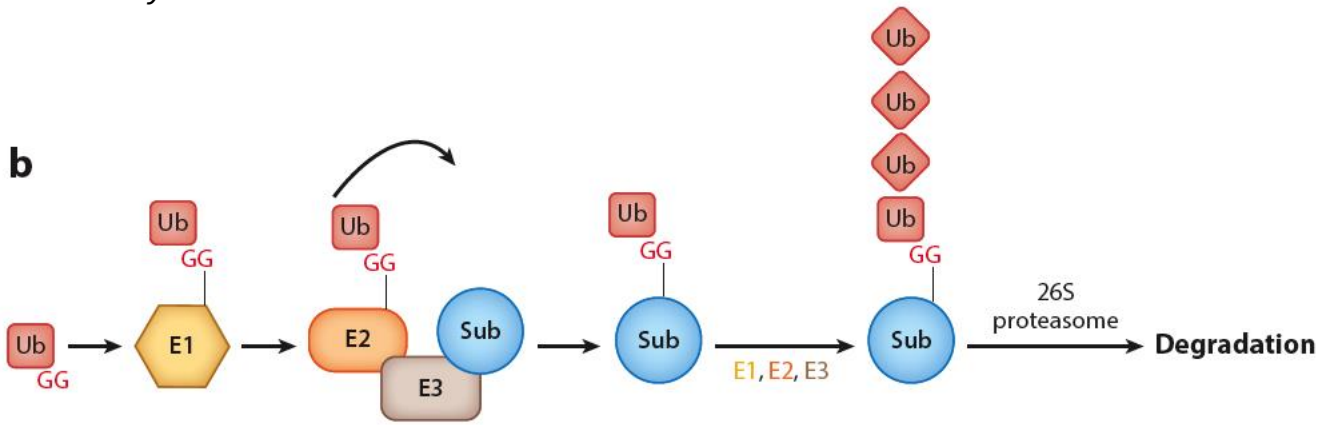
PROTEIN DEGRADATION: PROTEASOME



Mycobacterium tuberculosis

20S core particle
similar the eukaryotic and archaeal 20S

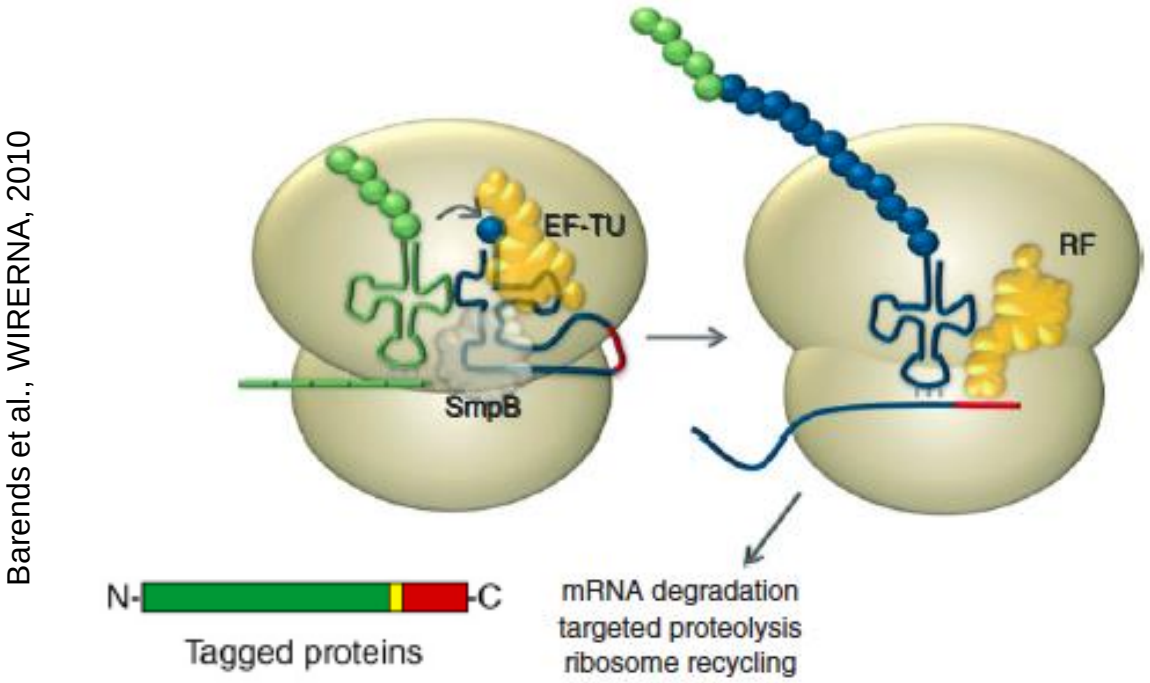
Other proteases:
AAA$^+$ Clp ATP-dependent proteases
ClpXP, ClpAP, Lon, HflB and Tsp



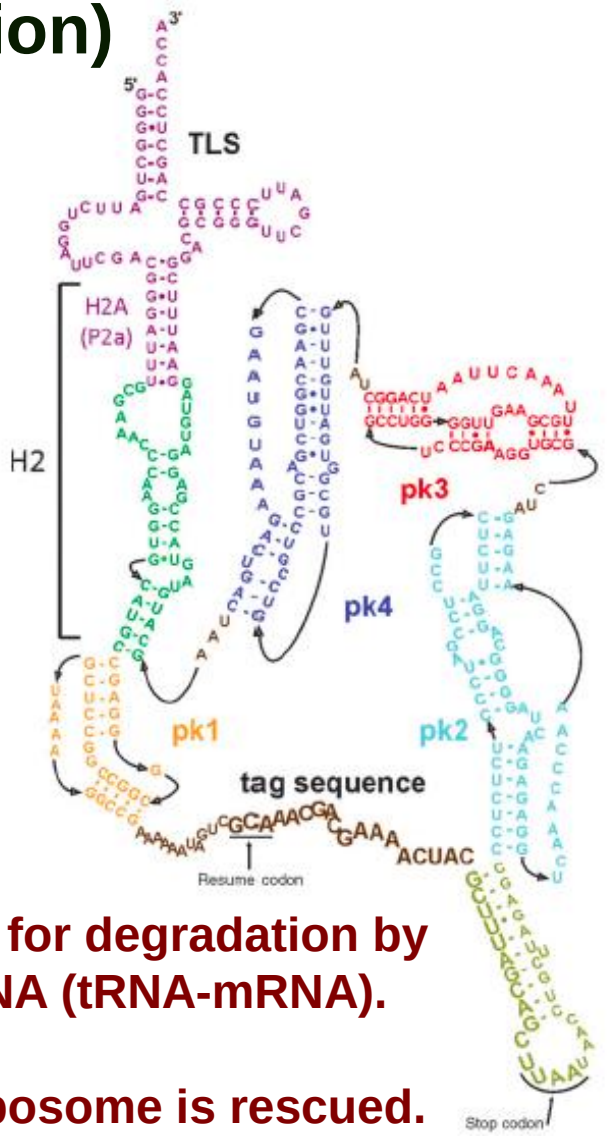
Jastrab and Darwin, Annual Rev Micro, 2016

PROTEIN DEGRADATION in BACTERIA by tmRNA TAGGING (*trans*-translation)

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

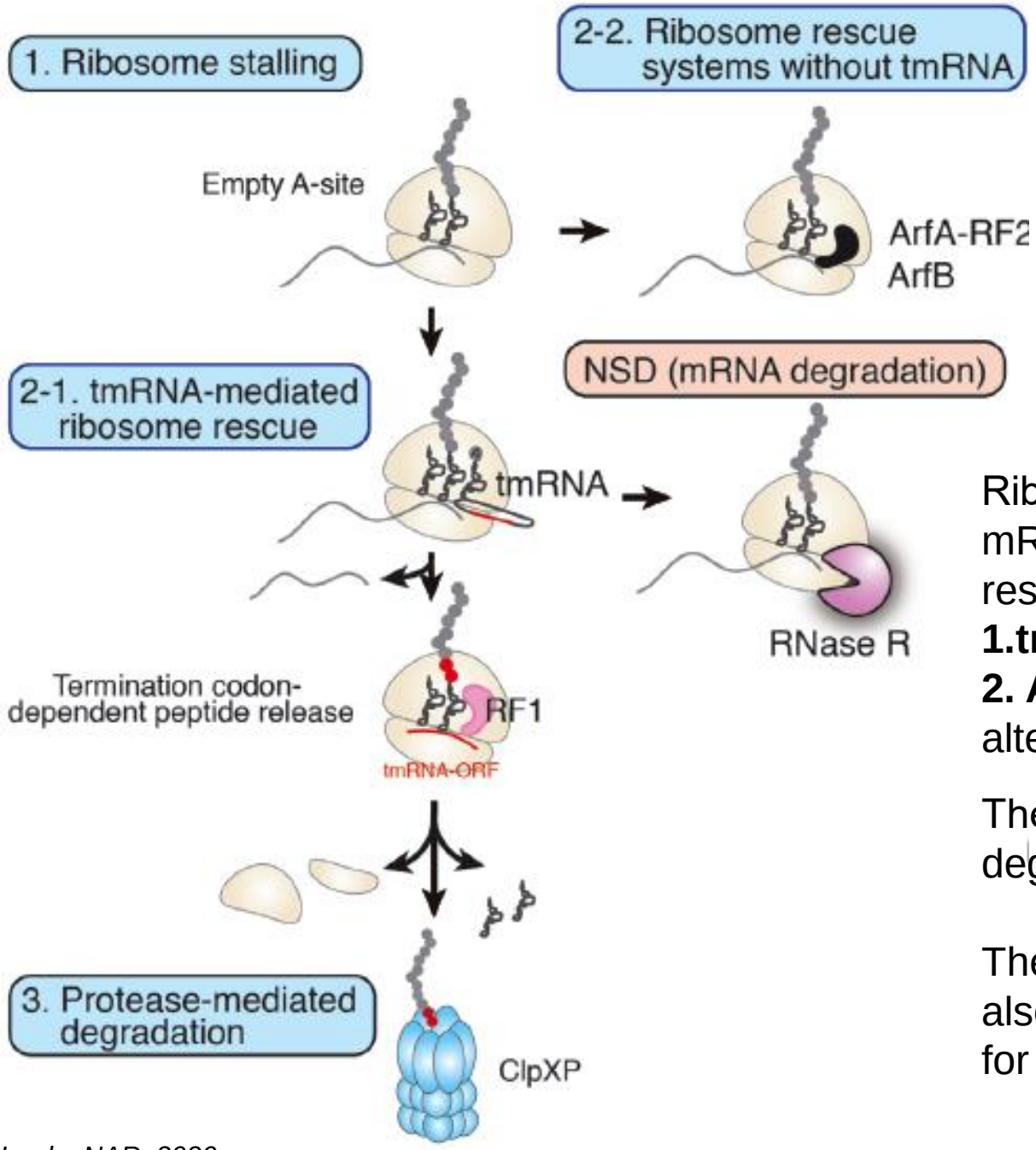


Barends et al., WIRERNA, 2010



- **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, 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



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

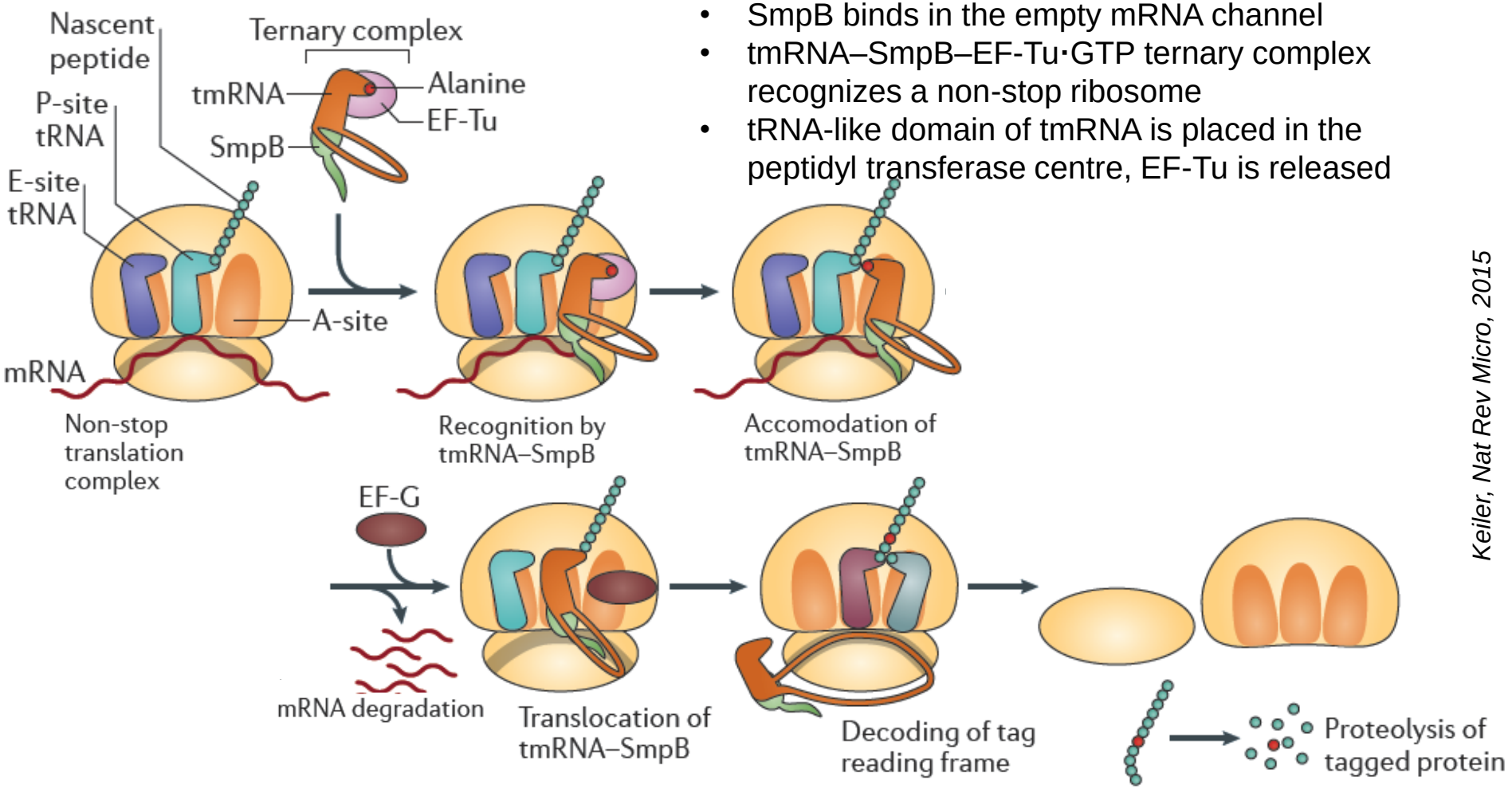
These recruit 3’-5’ **RNaseR** that degrades nonstop mRNAs

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

Inada, NAR, 2020

Ribosome rescue by *trans*-translation

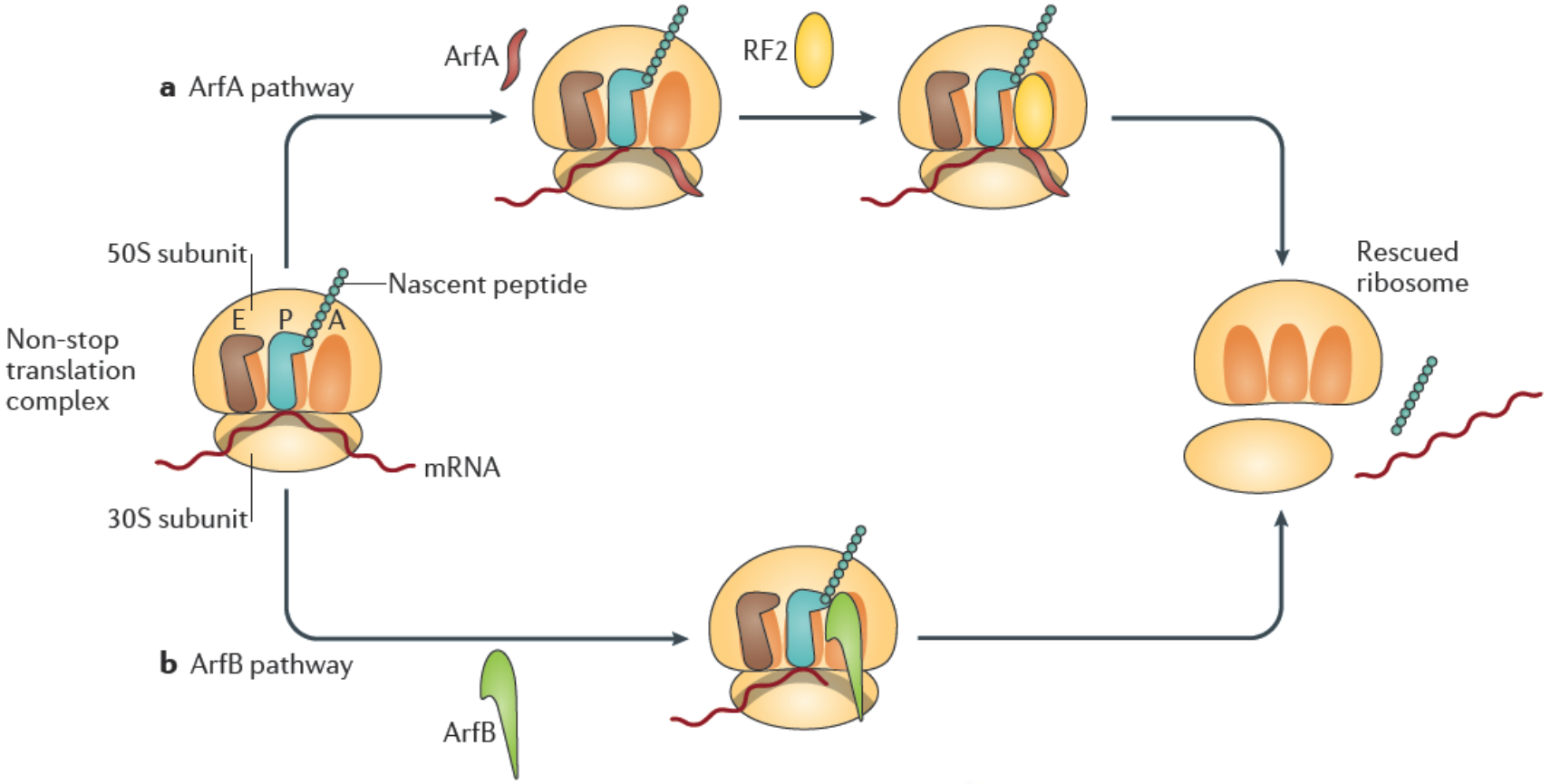
- SmpB binds in the empty mRNA channel
- tmRNA–SmpB–EF-Tu·GTP ternary complex recognizes a non-stop ribosome
- tRNA-like domain of tmRNA is placed in the peptidyl transferase centre, EF-Tu is released



Keiler, Nat Rev Micro, 2015

- Nascent polypeptide is transferred to the alanine attached to the tmRNA, peptidyl-tmRNA-SmpB is translocated via EF-G to the P-site.
- The tag reading frame of the tmRNA is placed in the mRNA channel, original mRNA is removed from the ribosome and degraded.
- The tag reading frame is translated, translation is terminated on its stop codon, then ribosome and the tagged protein are released, the peptide is rapidly degraded by proteases.

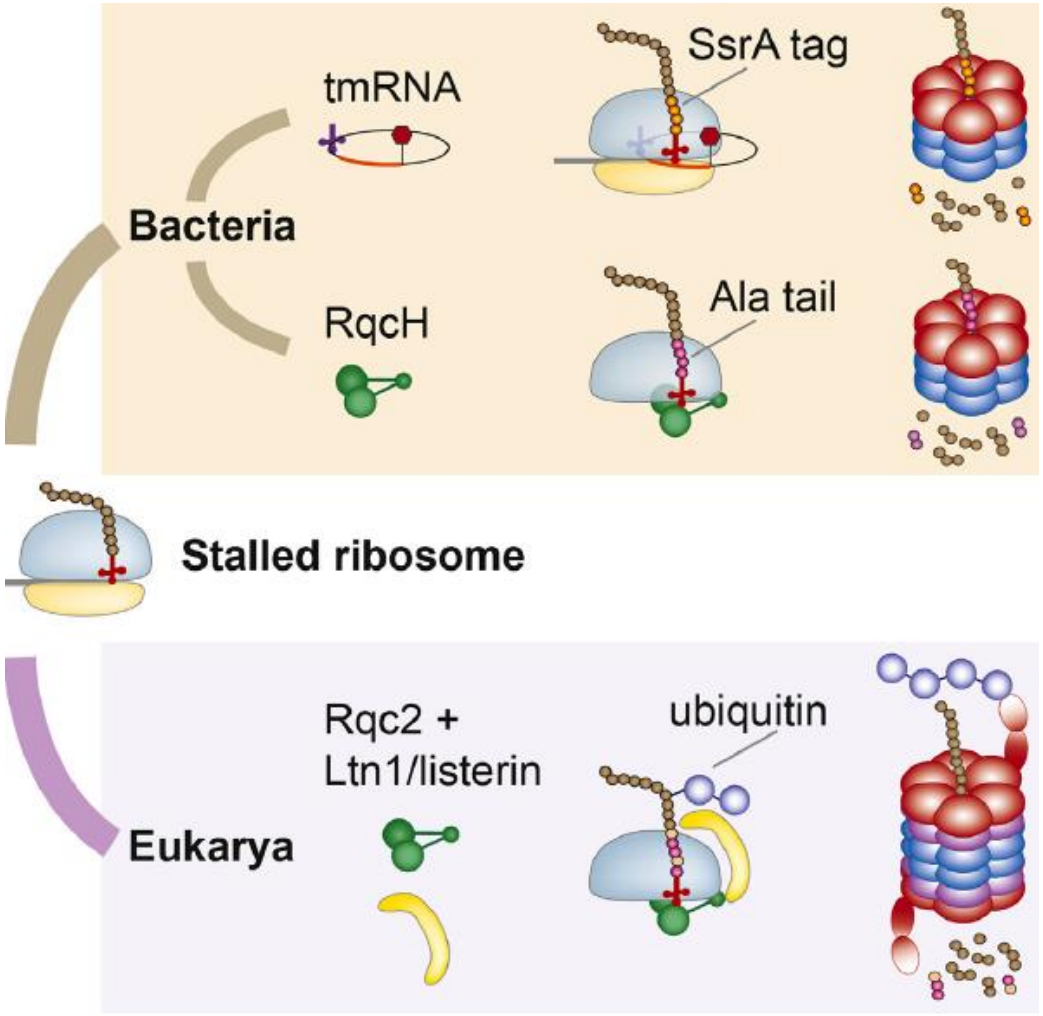
Ribosome rescue by ArfA and ArfB
(alternative rescue factors as a back-up system)



- **ArfA** recognizes the non-stop complex, binds in or near the empty mRNA channel.
- This facilitates binding of peptide chain release factor RF2 to site A.
- RF2 catalyses hydrolysis of the peptidyl-tRNA bond and releases the ribosome, mRNA and nascent peptide, without their degradation.
- **ArfB** binds in the empty mRNA channel, its GGQ motif directly catalyses hydrolysis of the peptidyl-tRNA bond. The ribosome, nascent peptide and mRNA are released.

Keiler, Nat Rev Micro, 2015

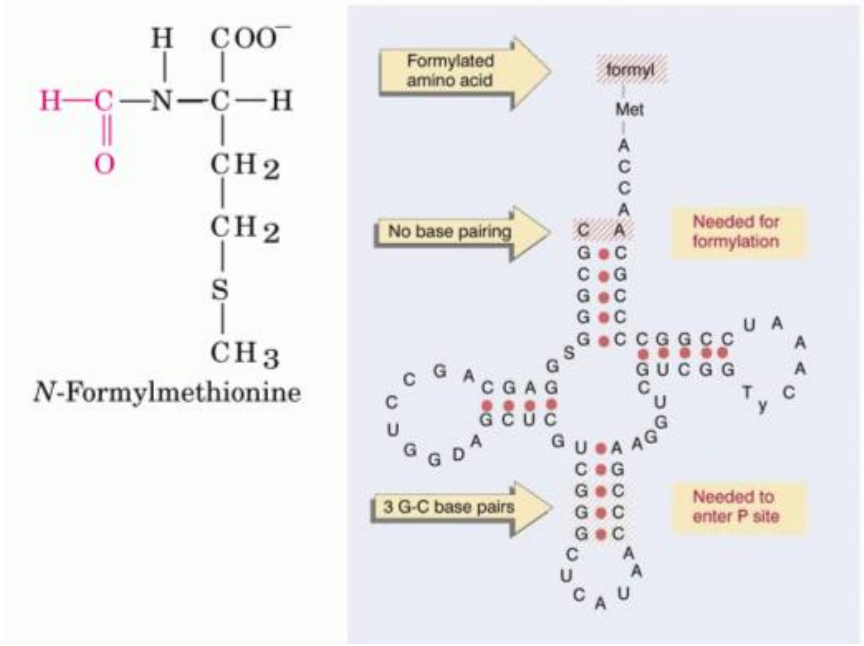
Prokaryotic ribosome-associated quality control (RQC)



- partially redundant with the tmRNA/SsrA 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

$tRNA^{Met}$ 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

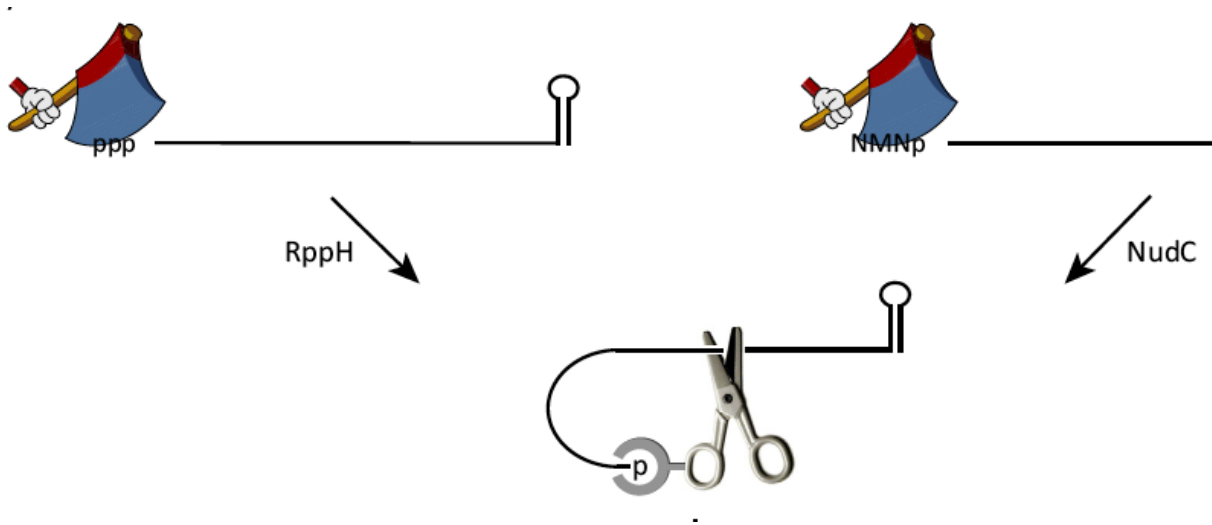
RNA MODIFICATION in BACTERIA

m^{6}A: enzymes unknown; function unknown

m^{5}C: not confirmed

NAD+ 5’ cap:

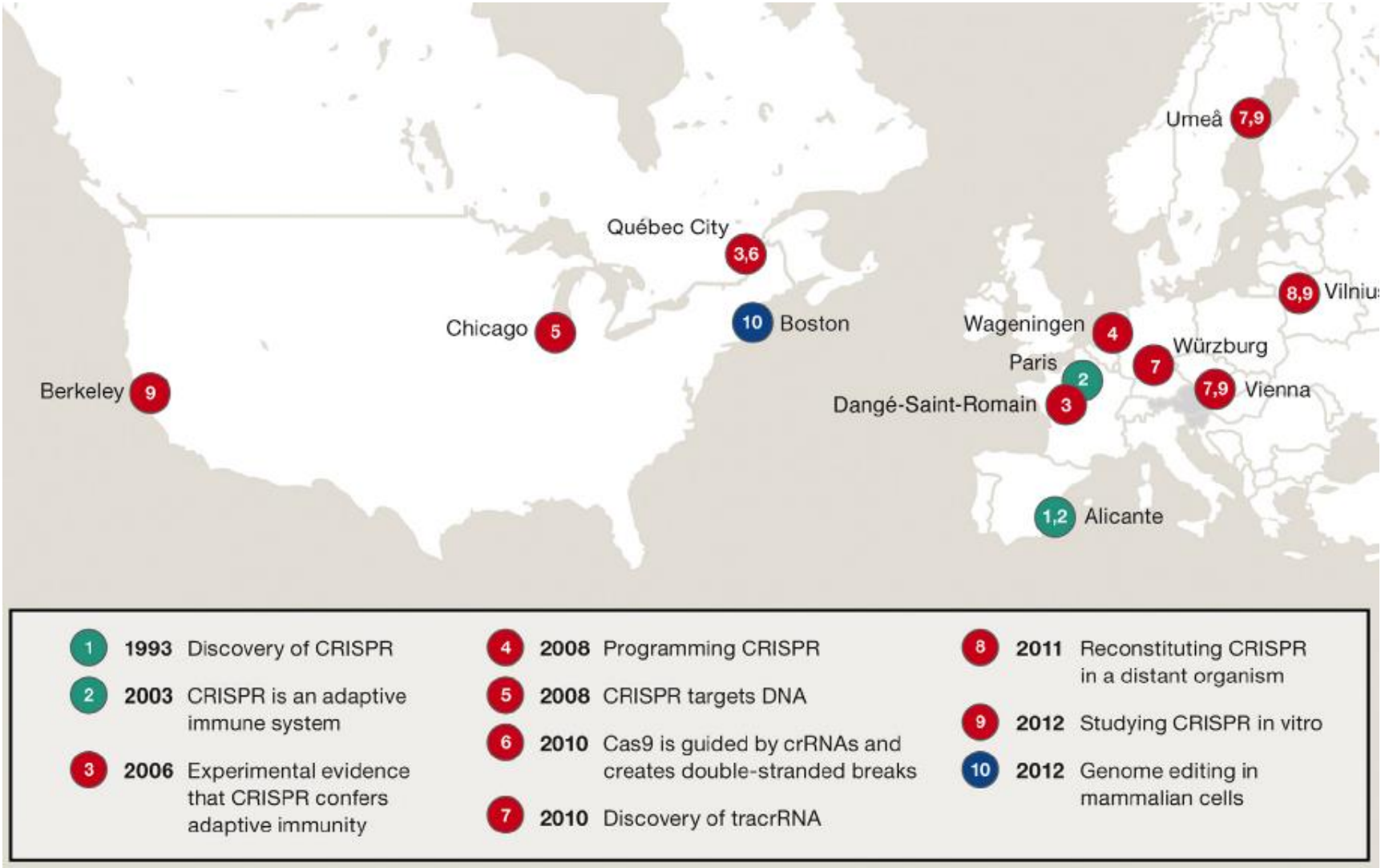
• a small fraction of RNAs, predominantly shorter (<200 nts, regulatory sRNAs and some mRNAs), carry NAD+ 5’ cap
• probably added co-transcriptionally by RNA polymerase, maybe also post-transcriptionally by transferases or DNA/RNA ligases
• removed by NudC
• function unknown, probably stabilize mRNAs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



Other RNA caps:
- coA
- Np$_n$N
(e.g. Np$_4$A alarmone related to stress)

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

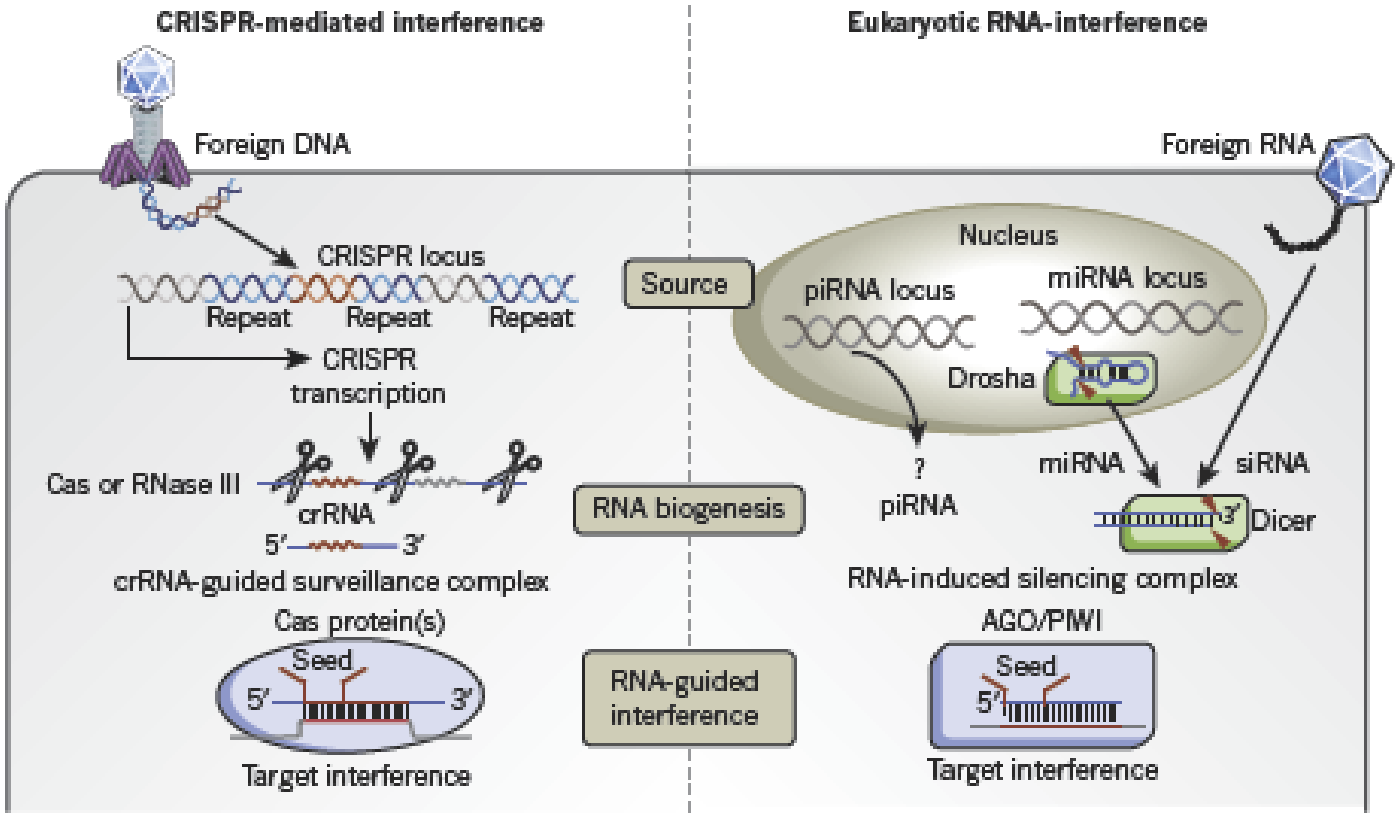
CRISPR/Cas history



Lander, Cell, 2016

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

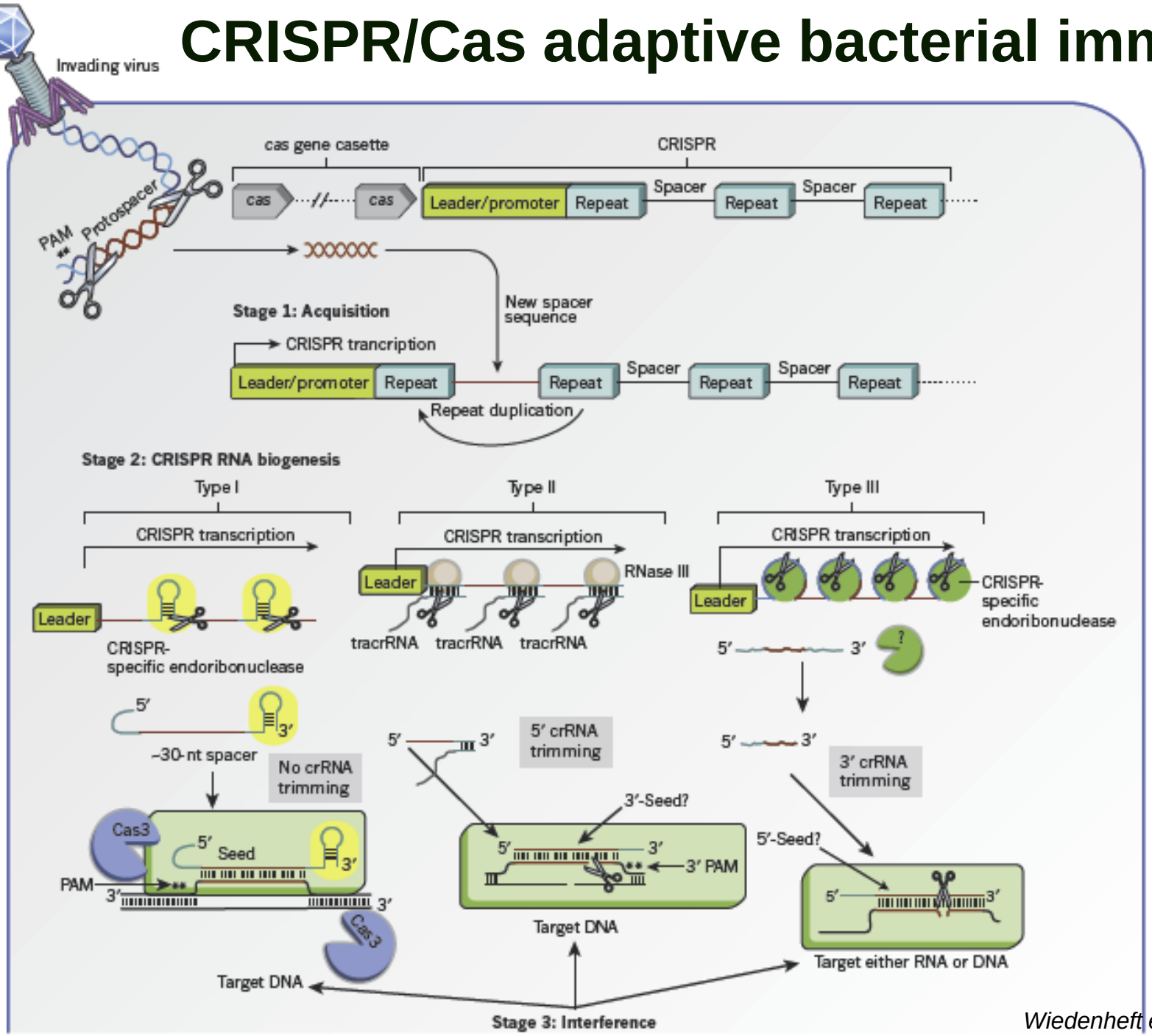
CRISPR Clustered Regularly Interspaced Short Palindromic Repeat
Cas- CRISPR associated



- **CRISPR: 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**

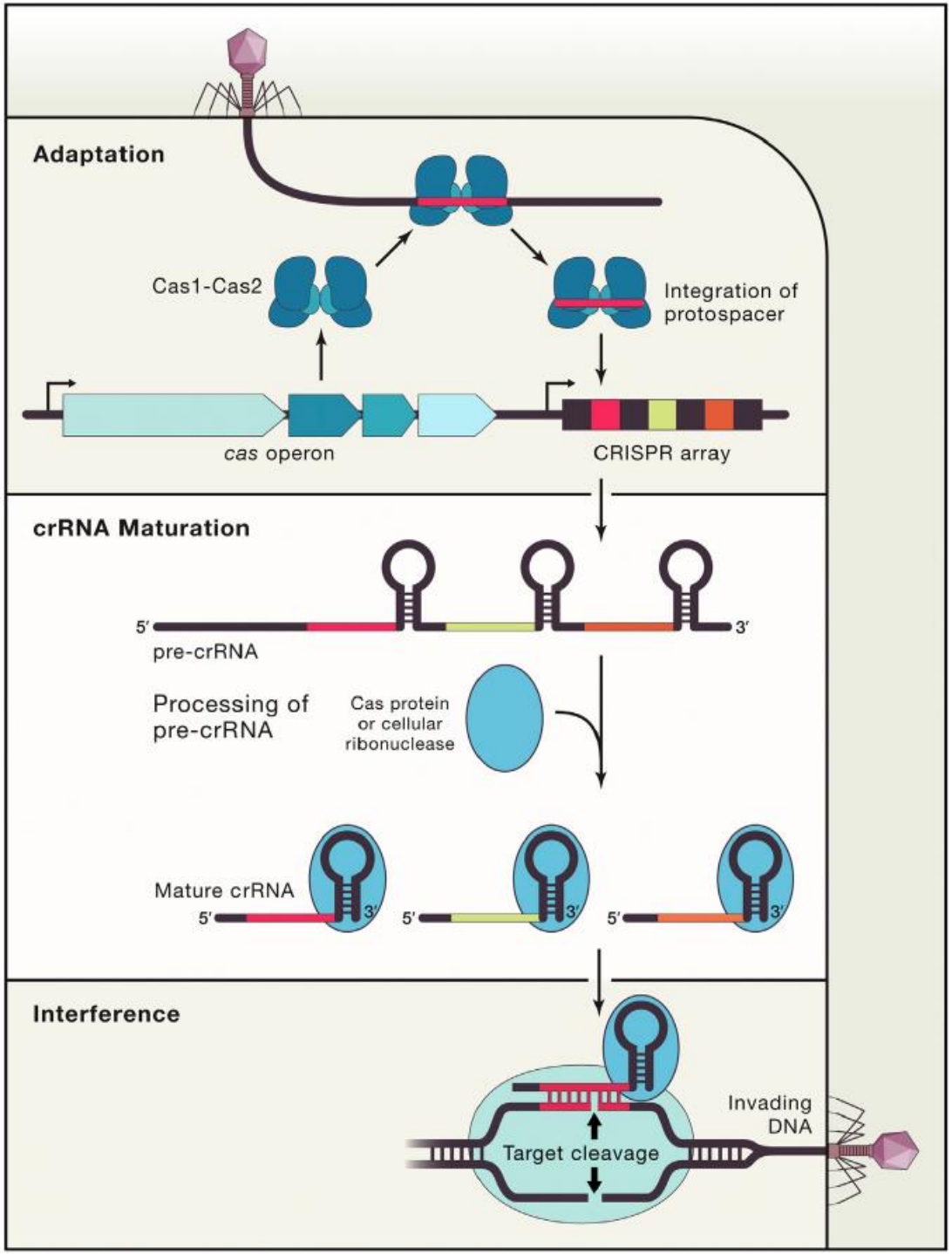
Wiedenheft et al, Nature, 2012

CRISPR/Cas adaptive bacterial immunity



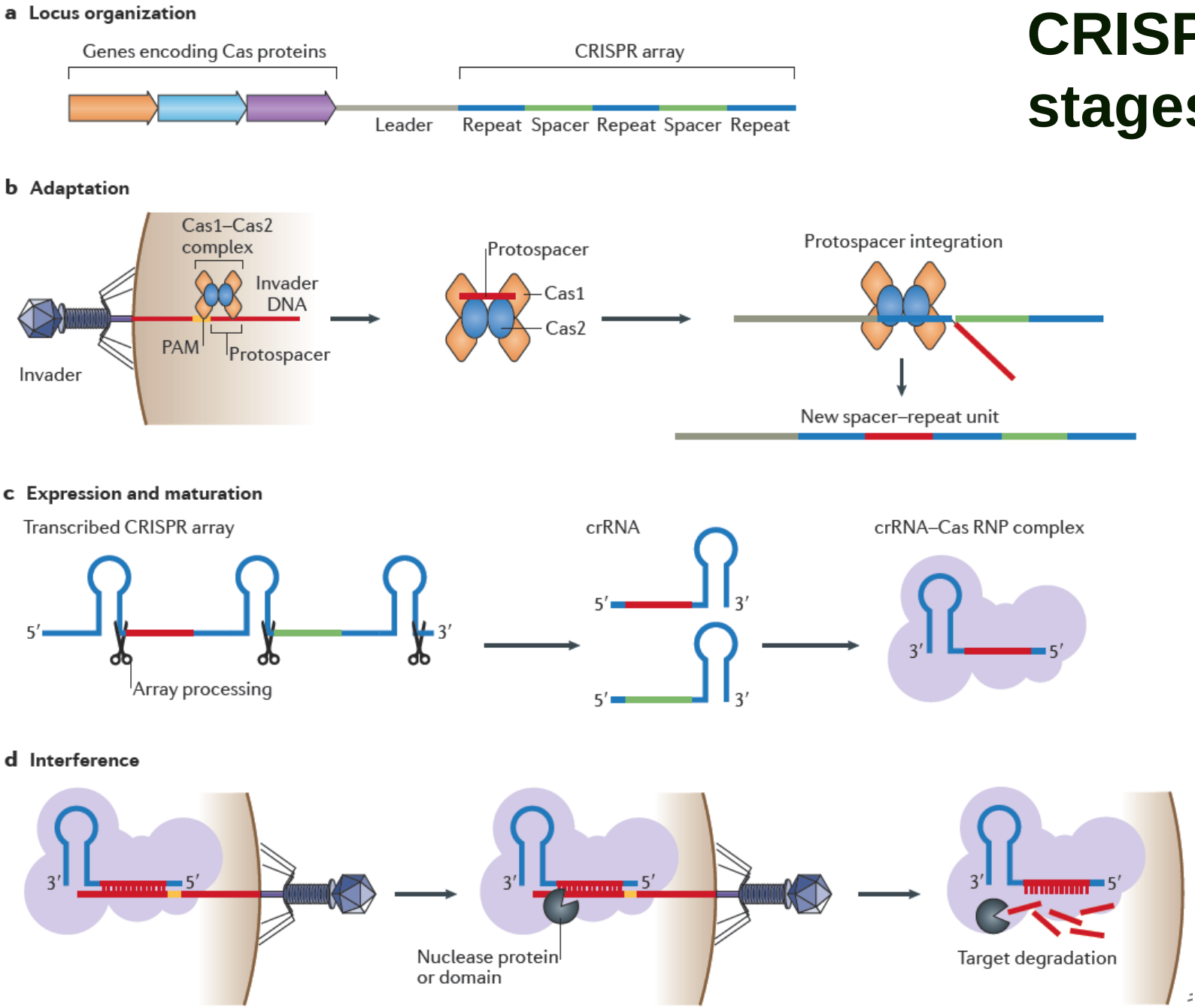
Wiedenheft et al, Nature, 2012

CRISPR/Cas stages



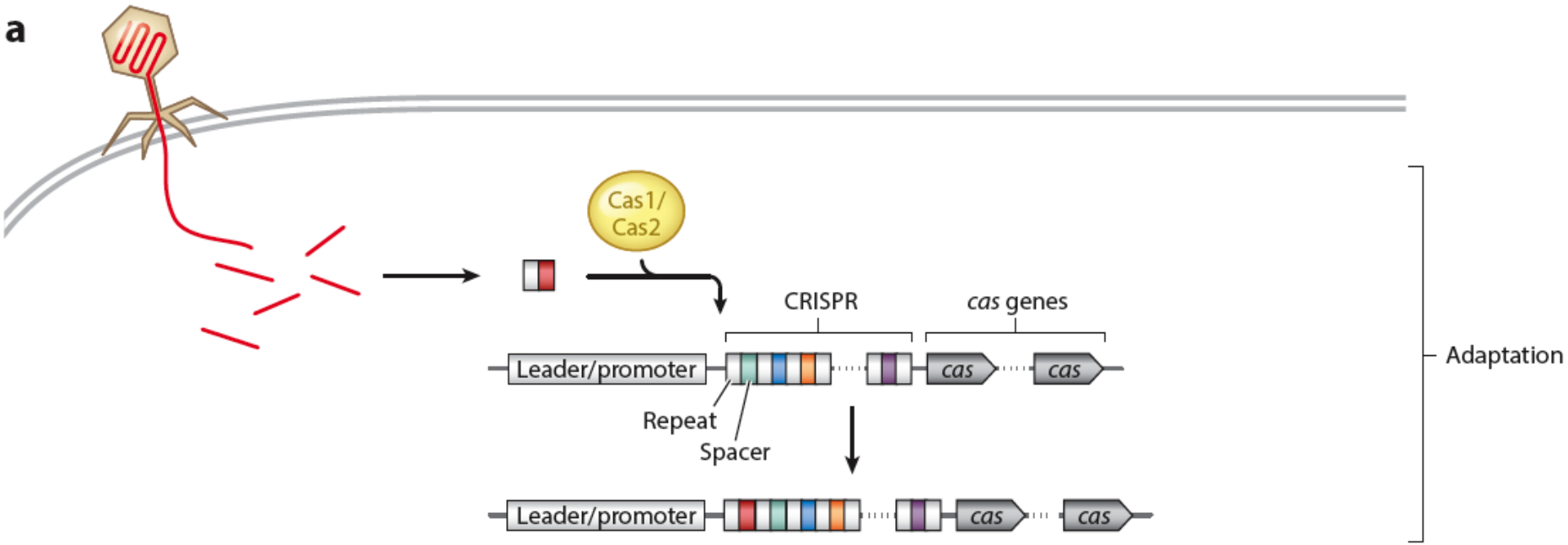
Hille et al, Cell, 2018

CRISPR/Cas stages



Amitai and Sorek, NatRevMicro, 2016

CRISPR/Cas: adaptation and spacer acquisition



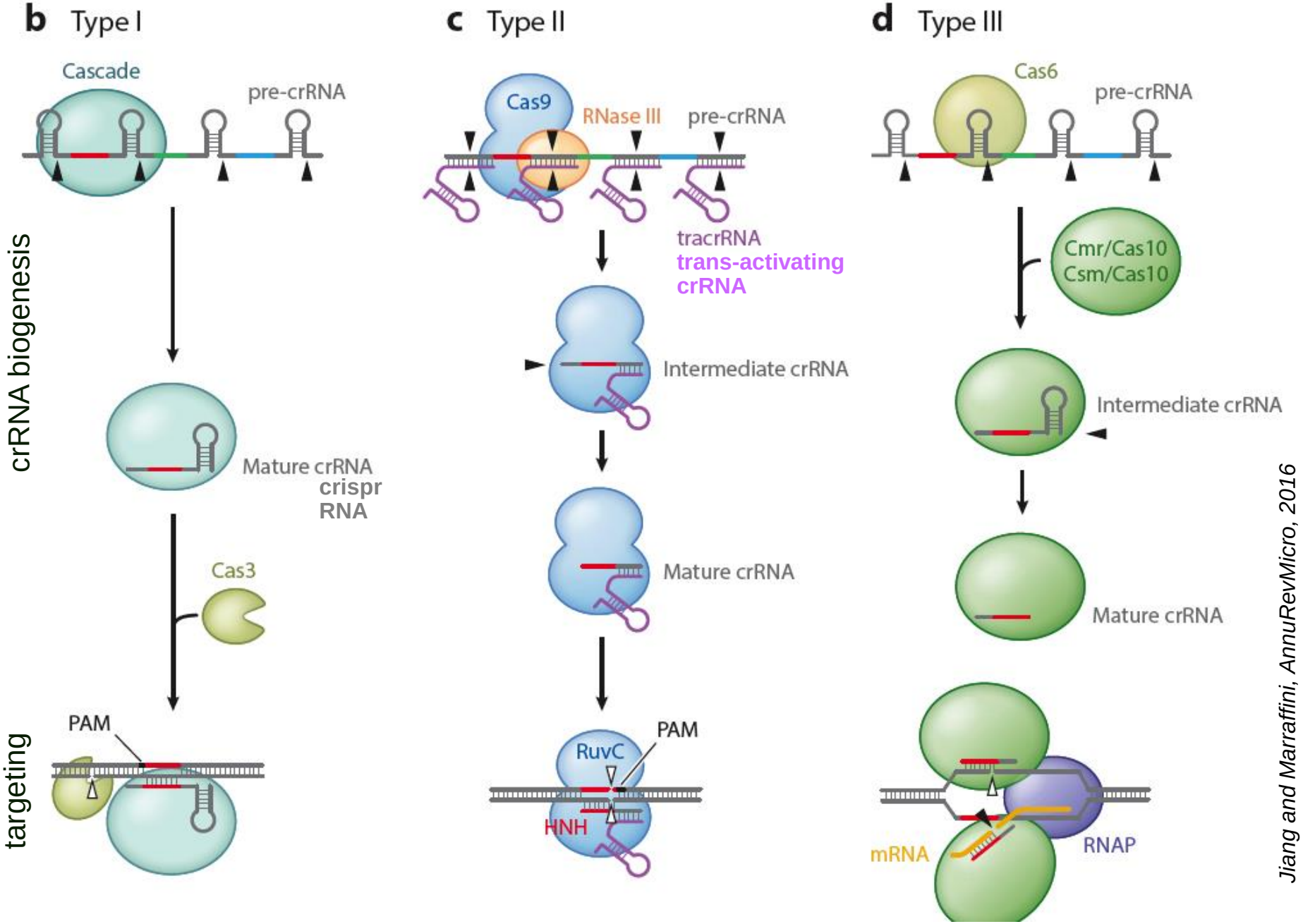
PAM protospacer-adjacent motif in type I immunity

- 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

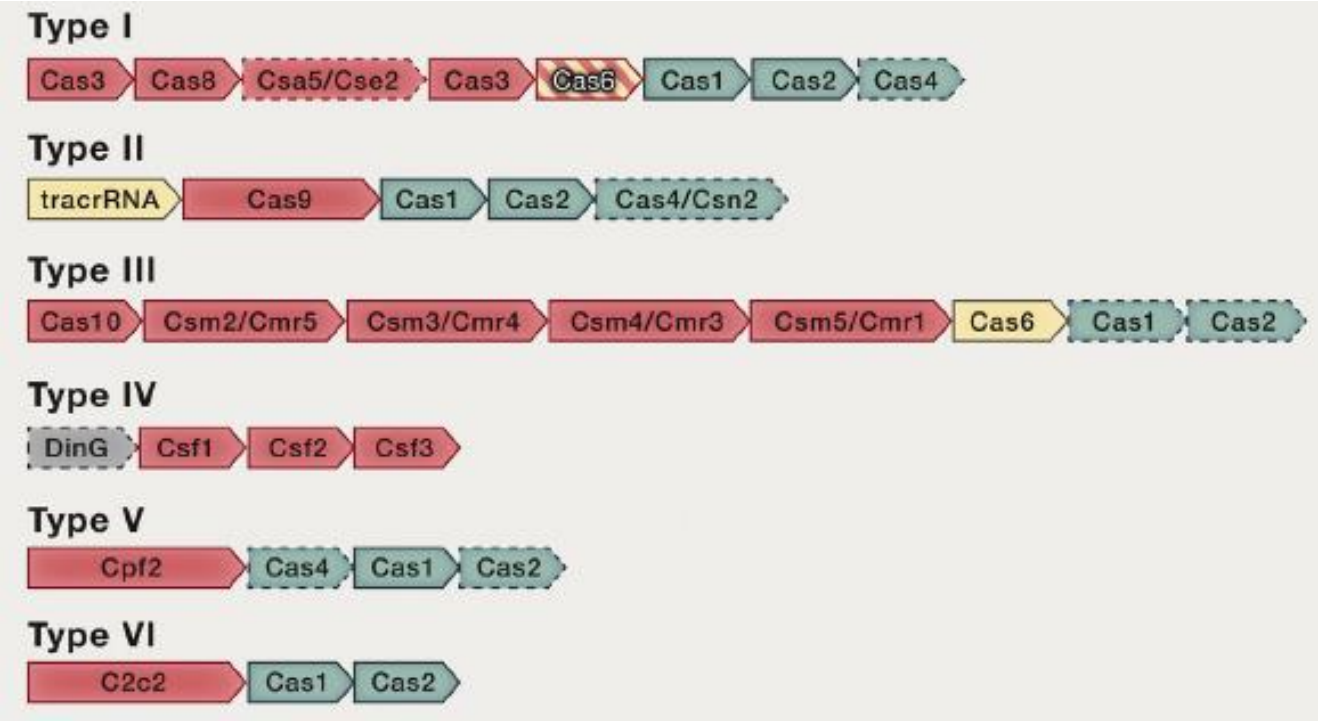
Class	Type	Subtype	Hallmarks	Example effector	Example organism	Studies Cited
Class 1	Type I		multisubunit effector complex; Cas3	Cascade	*E. coli*	Brouns et al., 2008
	Type III	III-A	multisubunit effector complex; Csm effector module; DNA targeting	Cas10-Csm	*S. epidermidis*	Marraffini and Sontheimer, 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



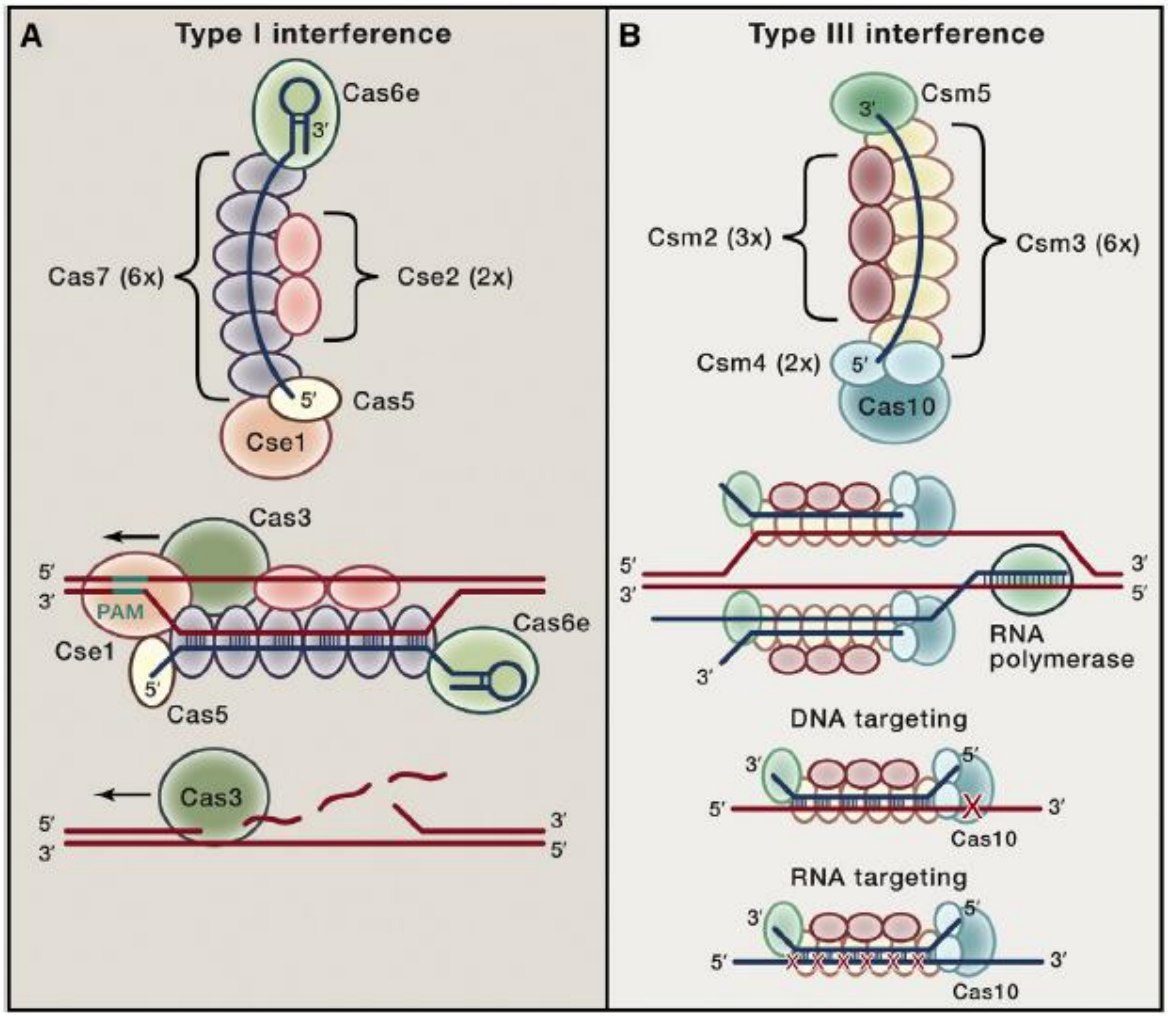
Wright et al, Cell, 2016

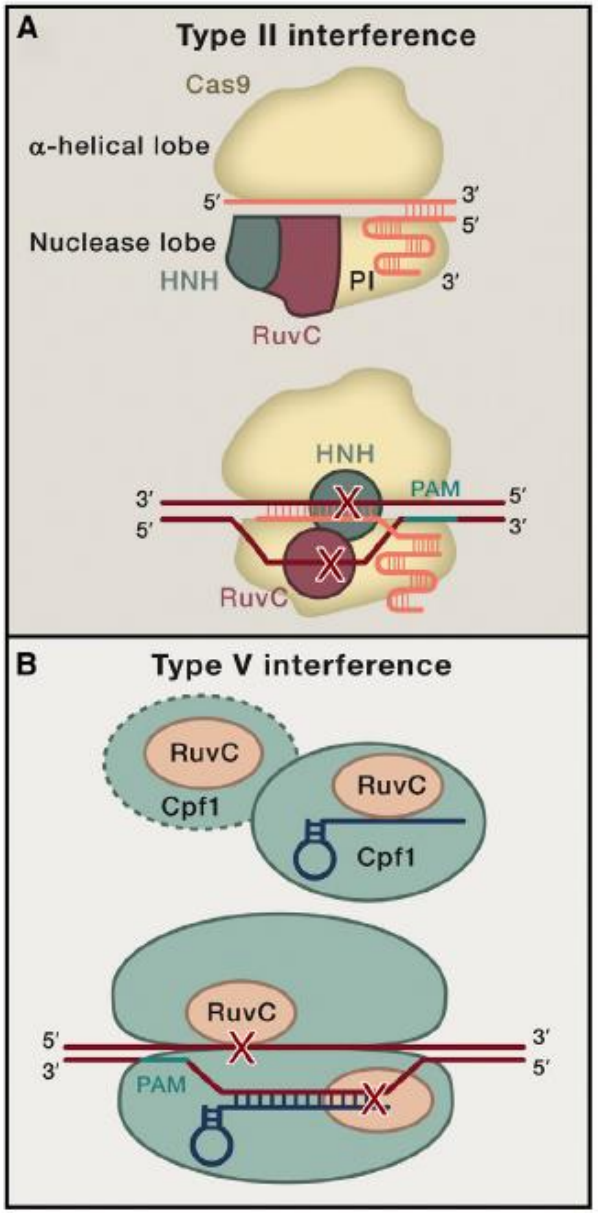
CRISPR/Cas types

targets DNA

targets RNA and actively transcribed DNA

target DNA





Wright et al, Cell, 2016

Global RNA biology in bacteria

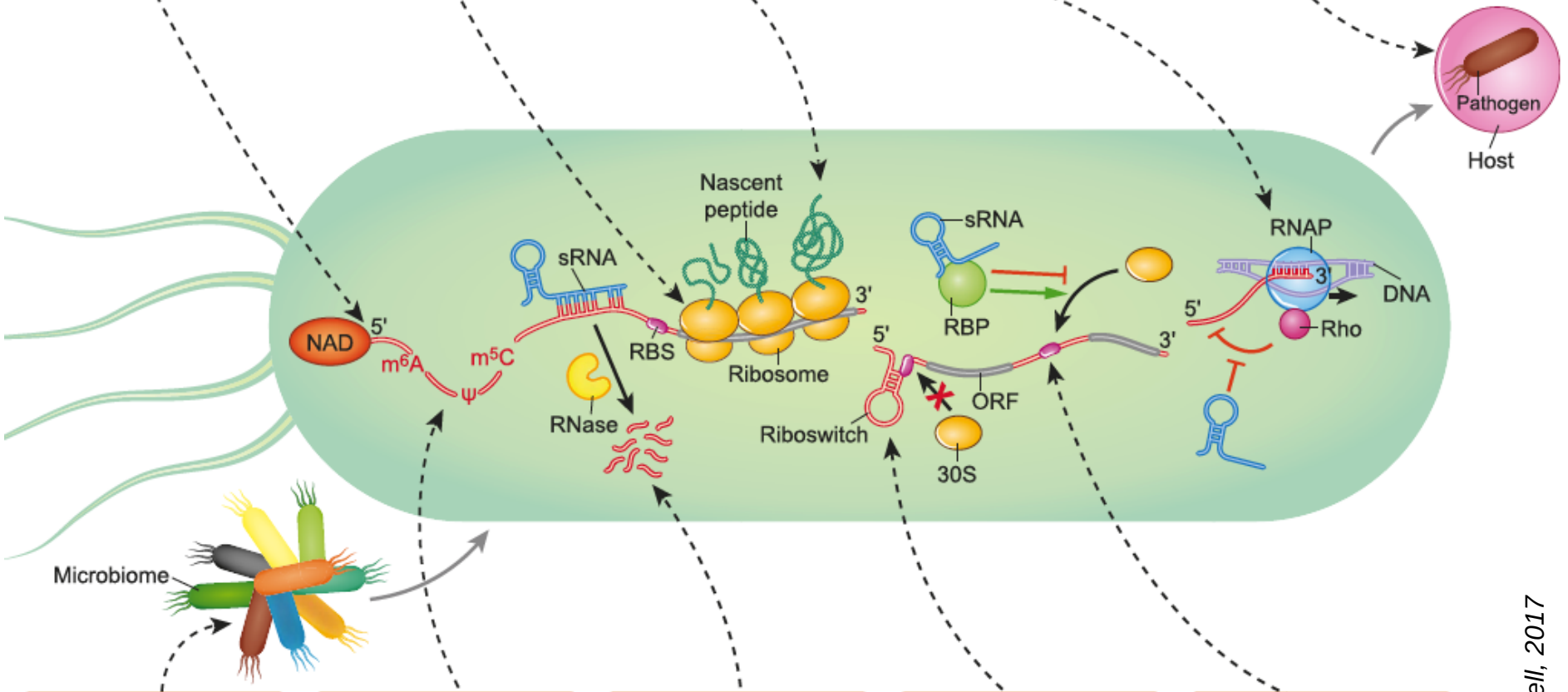
Knowledge of nearly all TSSs facilitates the annotation of new transcripts.

Transcript mapping reveals previously unknown conditional, alternative and orphan operons.

Quantifying ribosome density on cellular mRNAs provides an overview of translation and the proteome.

Assaying the global effects of Rho inhibition provides insight into transcription termination mechanisms, including effects of sRNAs.

Simultaneous RNA profiling of pathogen and host uncovers gene expression changes in both organisms during infection.



Antibiotic-responsive elements detected in whole microbiomes, including uncultured microorganisms.

Discovery of new RNA modifications and their locations in transcripts.

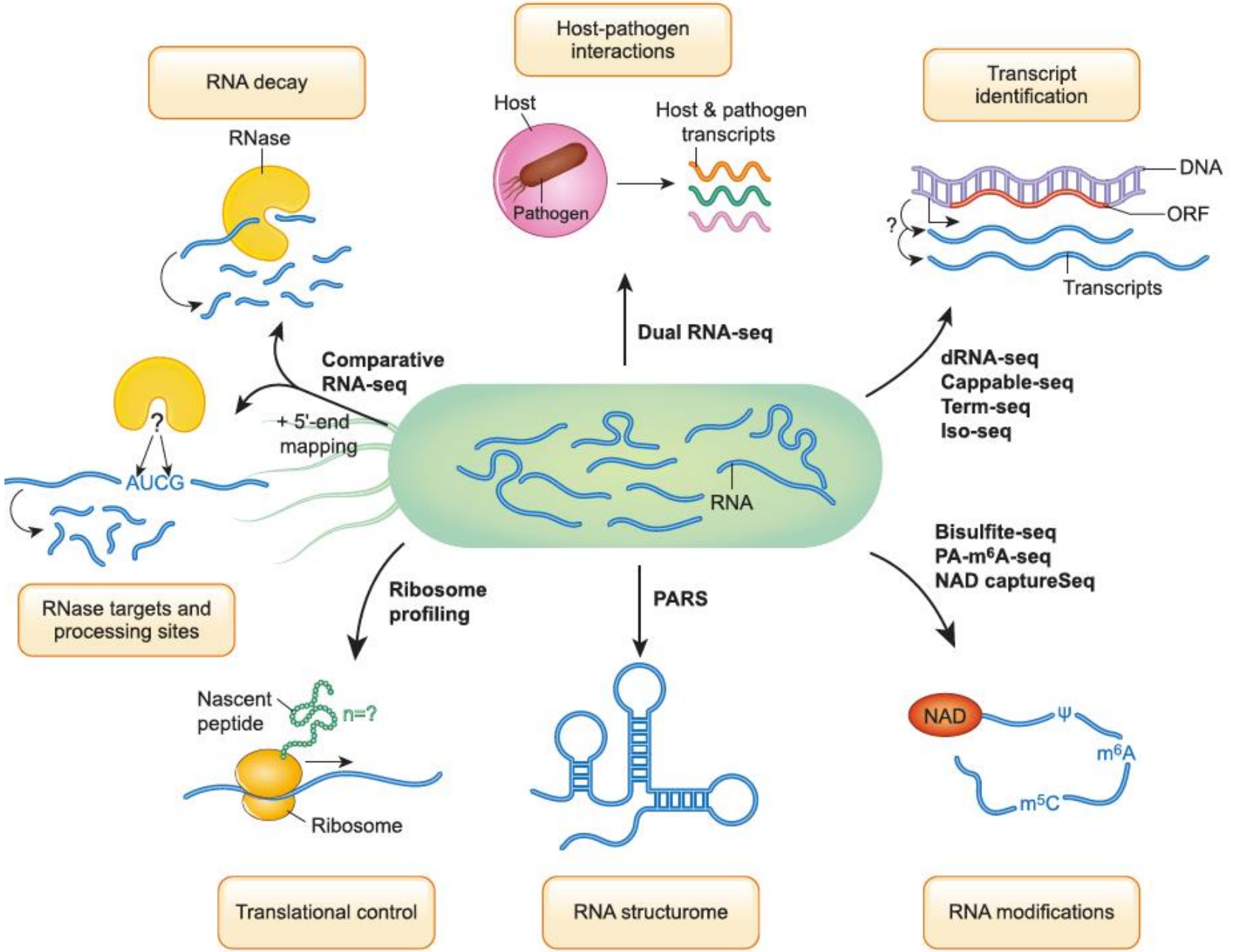
Analysis of RNase function reveals previously unknown cleavage motifs in cellular RNA.

Transcriptome-wide RNA structure probing reveals functions of *cis*-riboregulators such as riboswitches or RNA thermometers.

Techniques to study sRNAs and their targets reveal extensive post-transcriptional regulatory networks.

Hor et al, MCell, 2017

Global RNA biology in bacteria



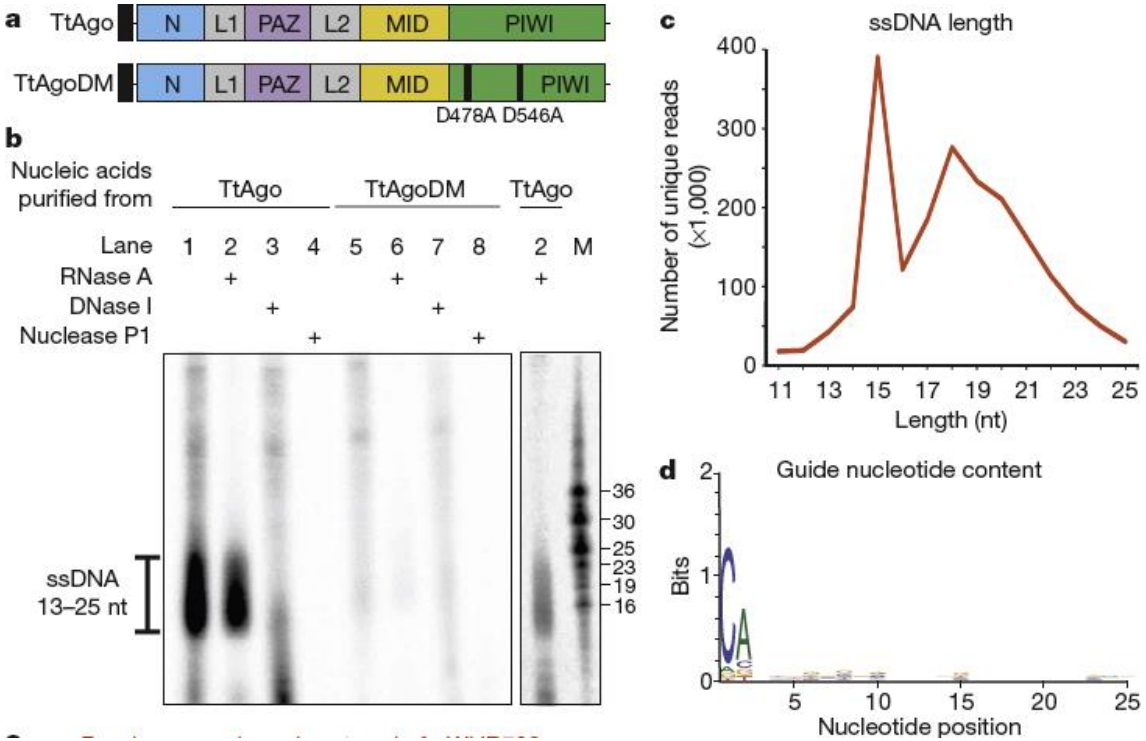
Hor et al, MCell, 2017

LETTER

doi:10.1038/nature12971

DNA–guided DNA interference by a prokaryotic Argonaute

Daan C. Swarts[1]*, Matthijs M. Jore[1]*, Edze R. Westra[1], Yifan Zhu[1], Jorijn H. Janssen[1], Ambrosius P. Snijders[2], Yanli Wang[3], Dinshaw J. Patel[4], José Berenguer[5], Stan J. J. Brouns[1] & John van der Oost[1]



DNA-guided DNAi as a host defence system

- *Thermus thermophilus* TtAgo interacts with 13-25 nt DNA guides (plasmid derived)
- sDNAs guide TtAgo to cleave complementary foreign DNA

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

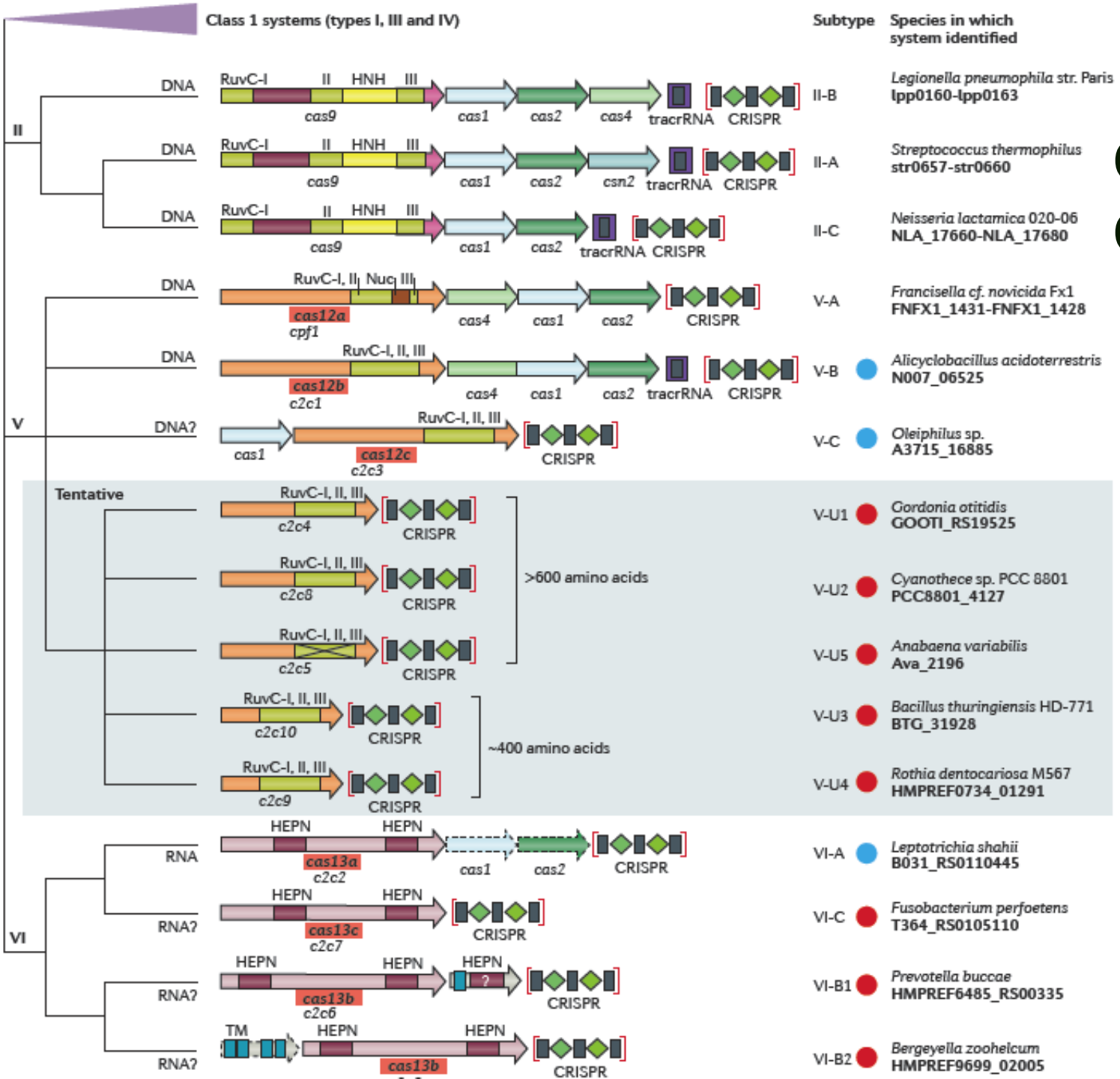
- polyadenylation-assisted RNA degradation

(occurs also in Eukaryotes)

- no cap-dependent translation or ribosome scanning

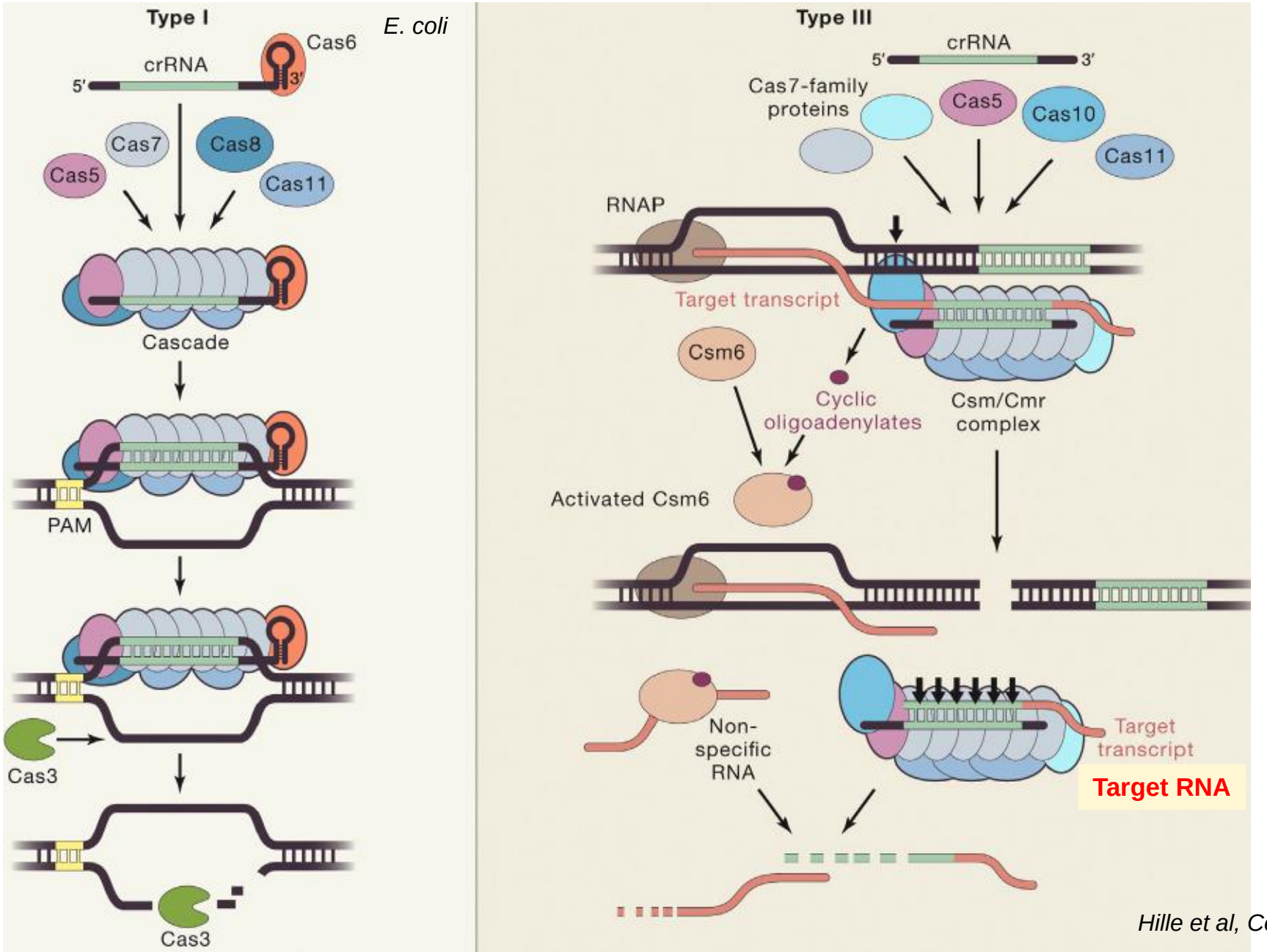
- tmRNA tagging for protein degradation

CRISPR/ Cas types



These last slides about CRISPR-Cas are only for those who are interested

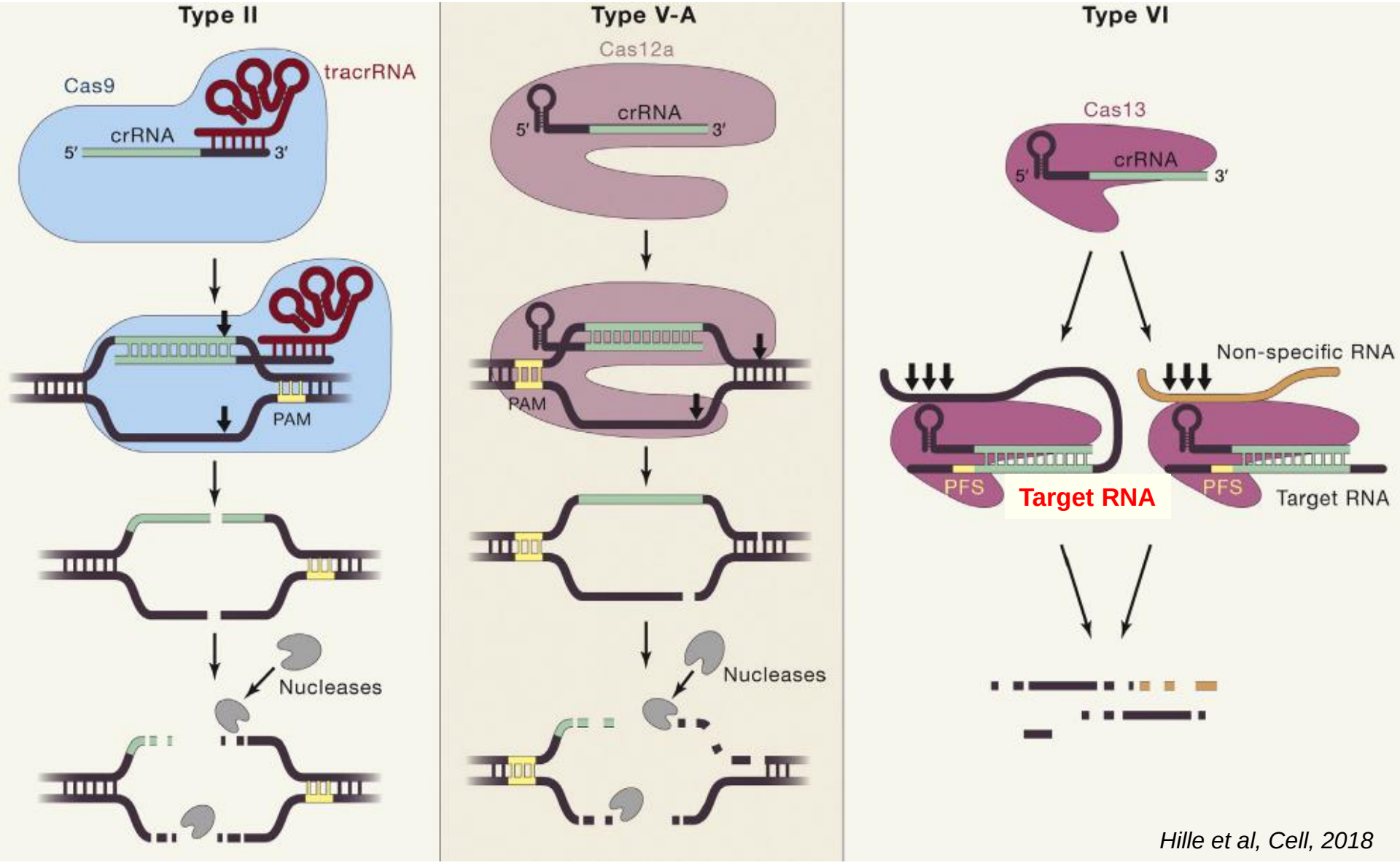
Interference of Class 1 CRISPR/Cas



Hille et al, Cell, 2018

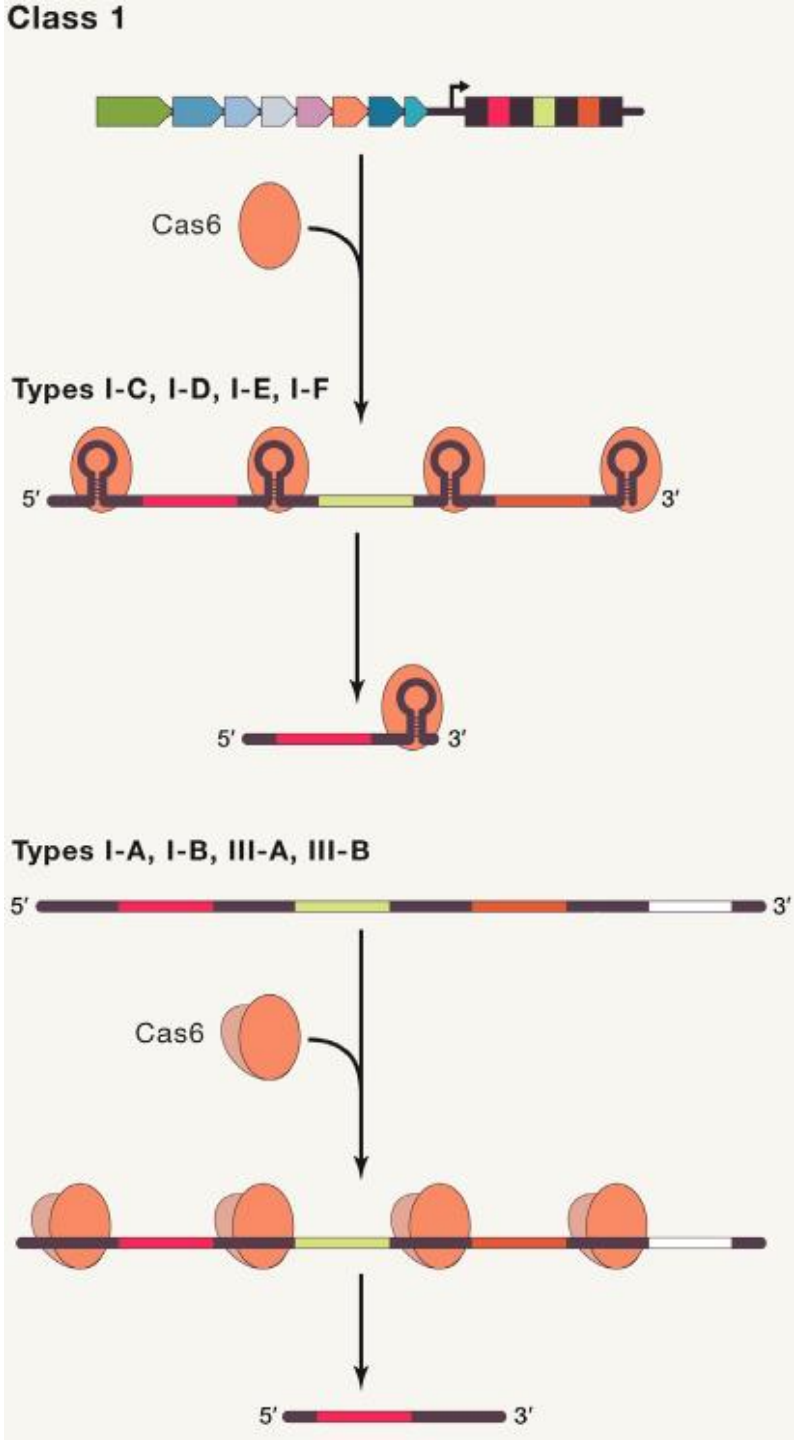
Interference of Class 2 CRISPR/Cas

One protein effector: Cas9, Cas12a or Cas13



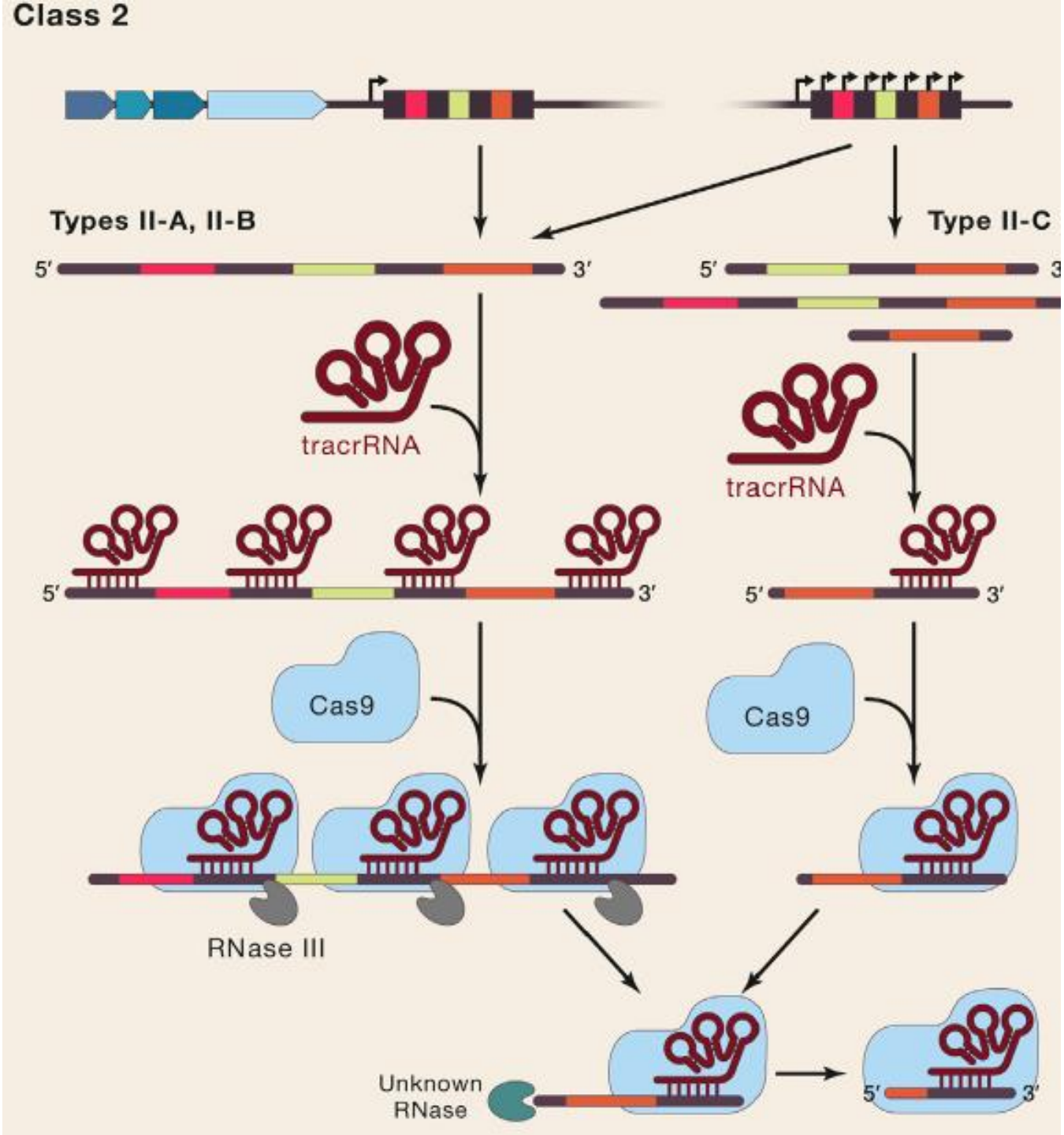
Hille et al, Cell, 2018

Class 1 crRNA maturation



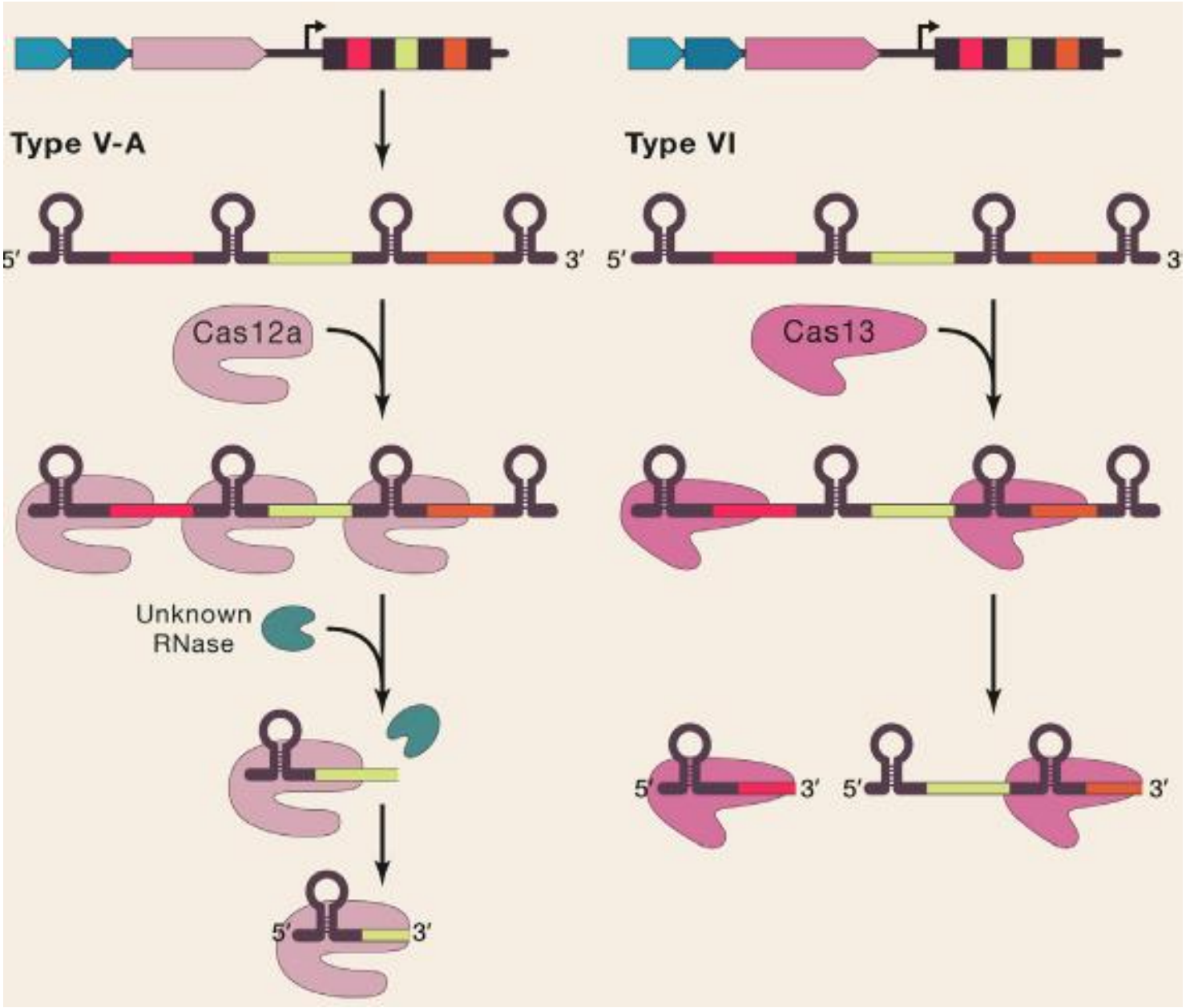
Hille et al, Cell, 2018

Class 1 crRNA maturation



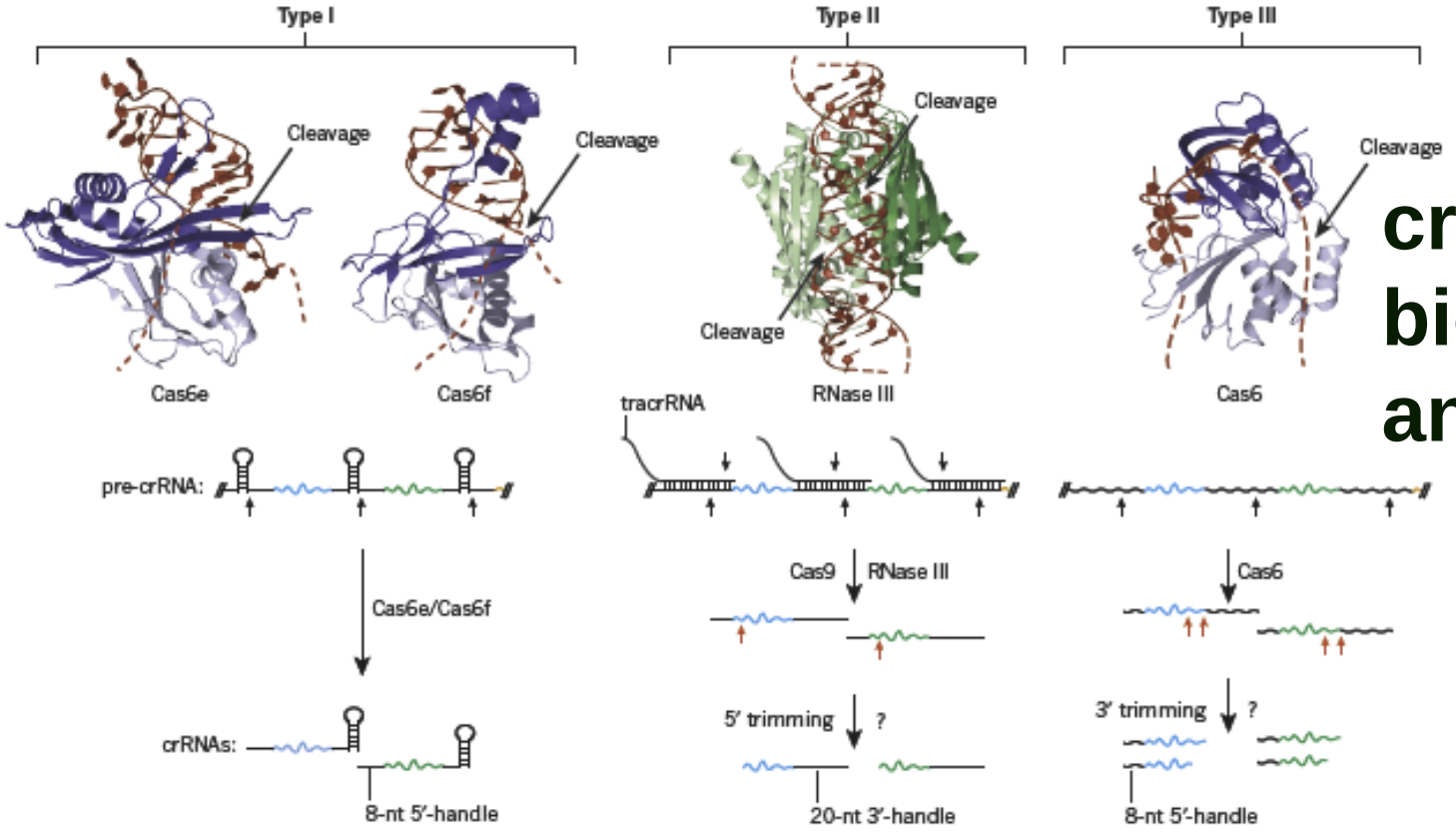
Hille et al, Cell, 2018

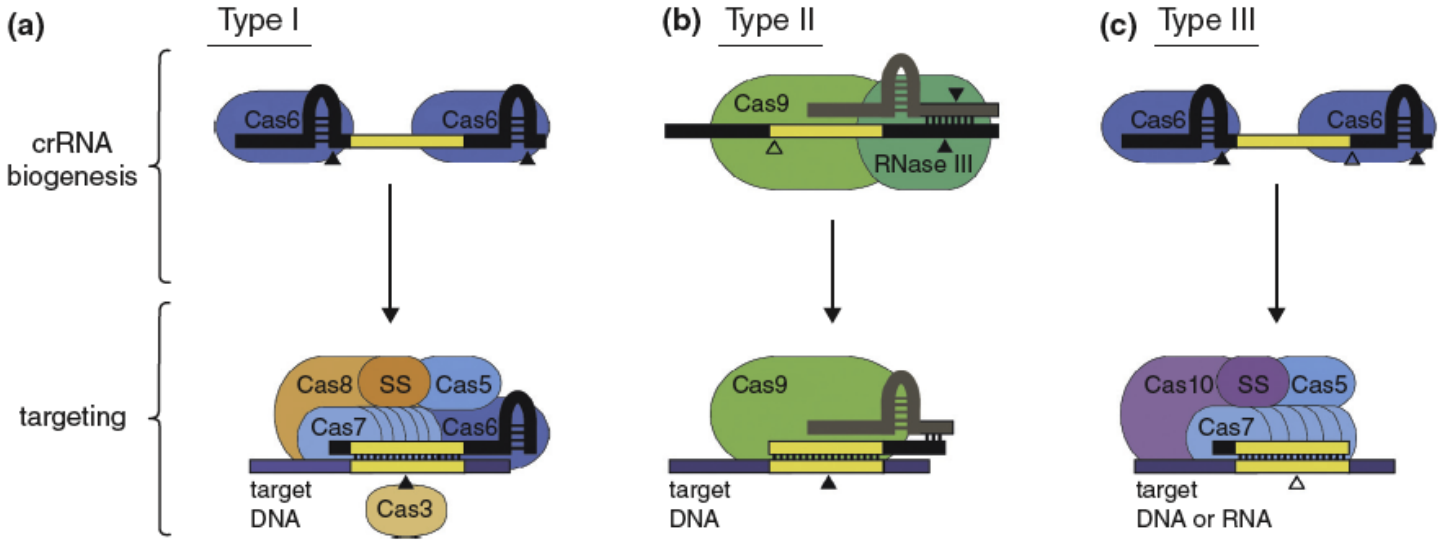
Class 1 crRNA maturation



Hille et al, Cell, 2018

crRNA biogenesis and function





Wiedenheft et al, Nature, 2012;
Hatoum-Aslan and Marraffini, Curr. Op. Microbiol. 2014