Informacje różne

- Egzamin pisemny na początku czerwca
- Podręcznika brak

Lizabeth Allison - **Fundamental Molecular Biology**

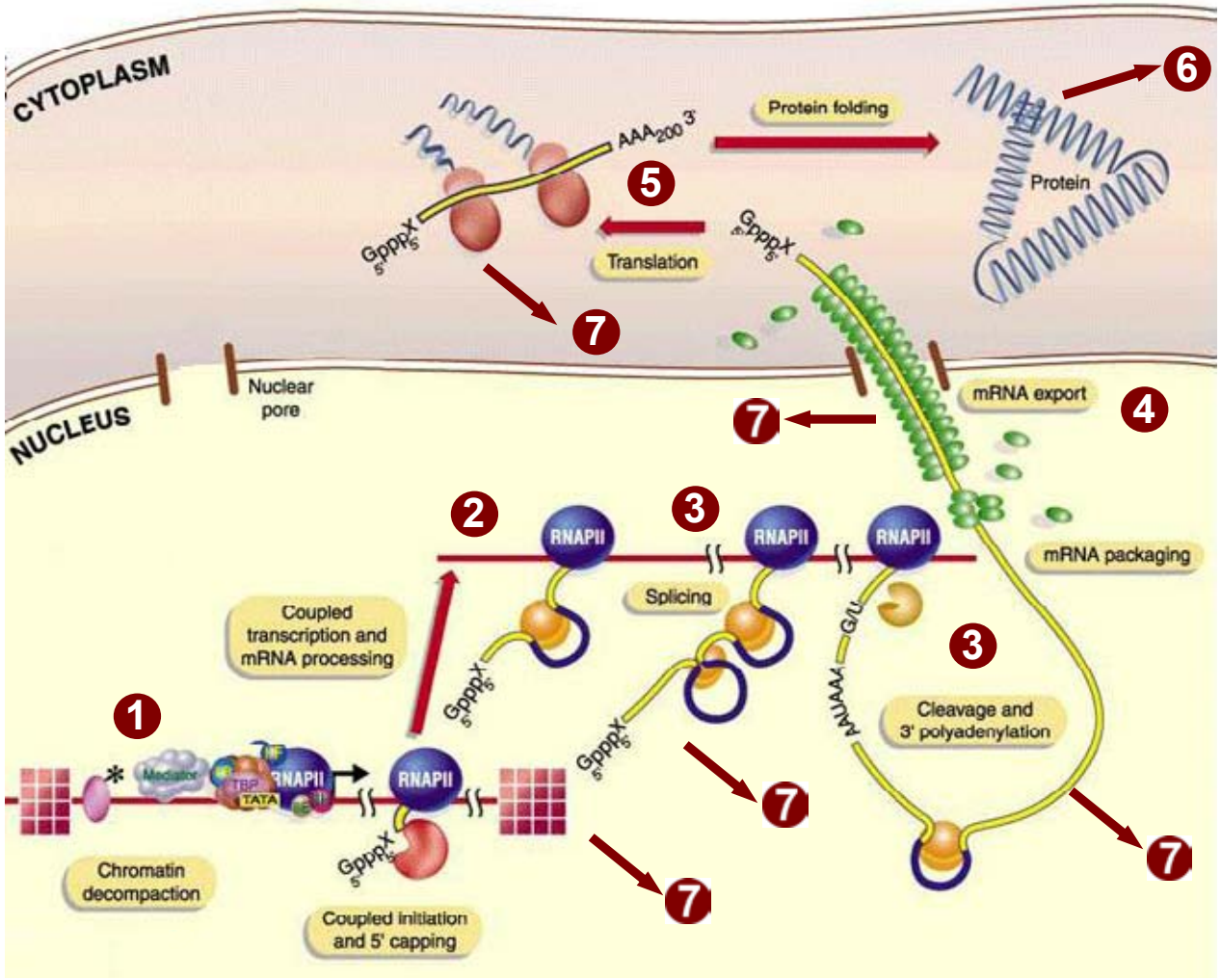
- Wykłady na stronie IGIBu

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

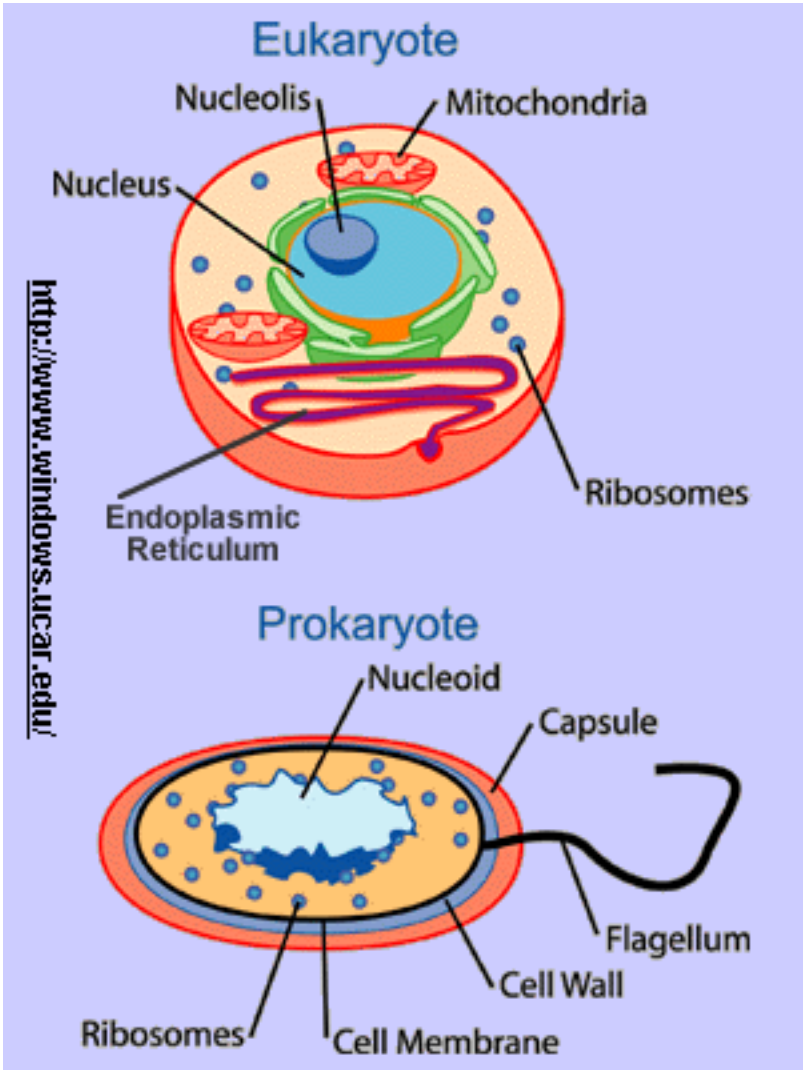
4) RNA export

5) translation (mRNA)

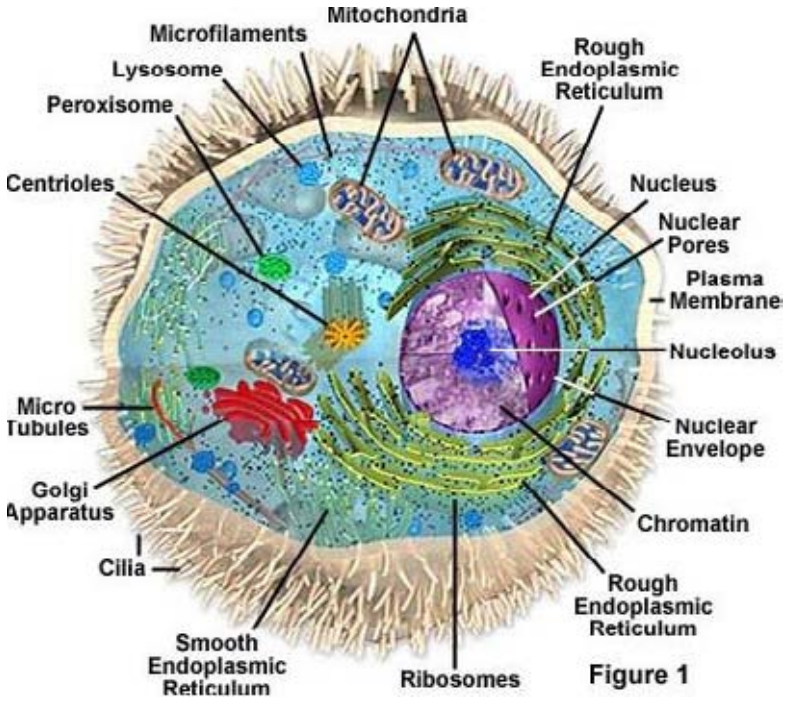
6) protein stability

7) RNA degradation

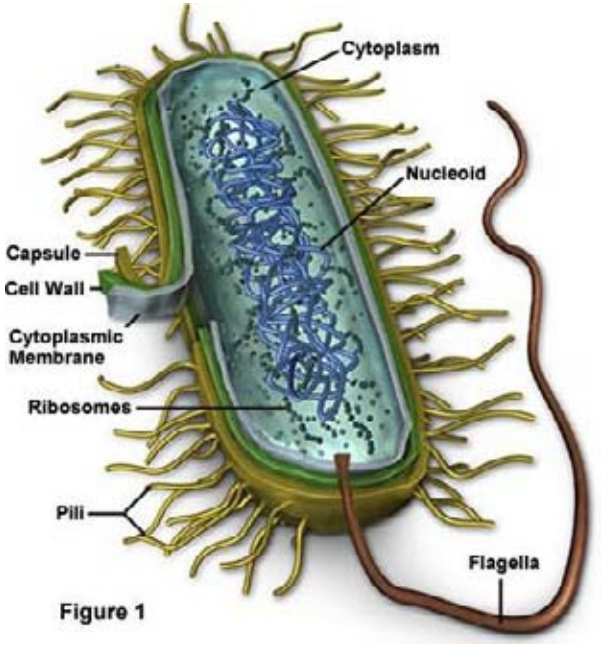
BACTERIAL vs EUKARYOTIC CELL



10-100 µm



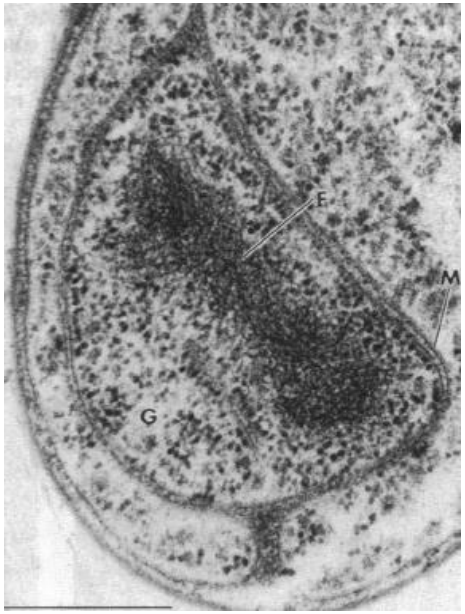
1-10 µm

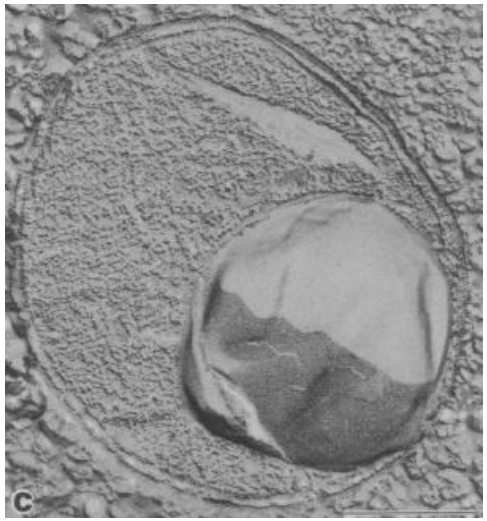


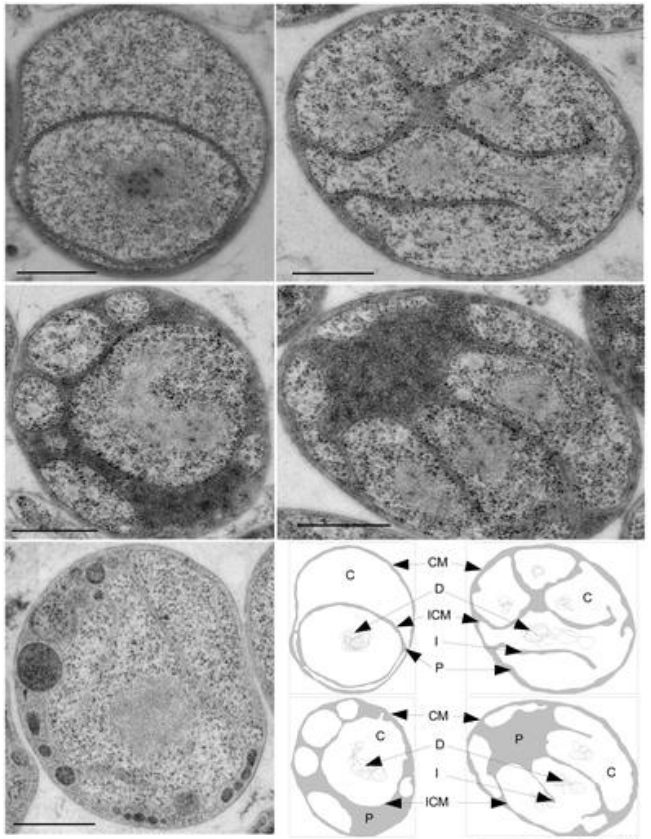
COMPARTMENTALIZED BACTERIA

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

Eubacterium *Gemmata obscuriglobus* has a membrane-bounded nucleoid





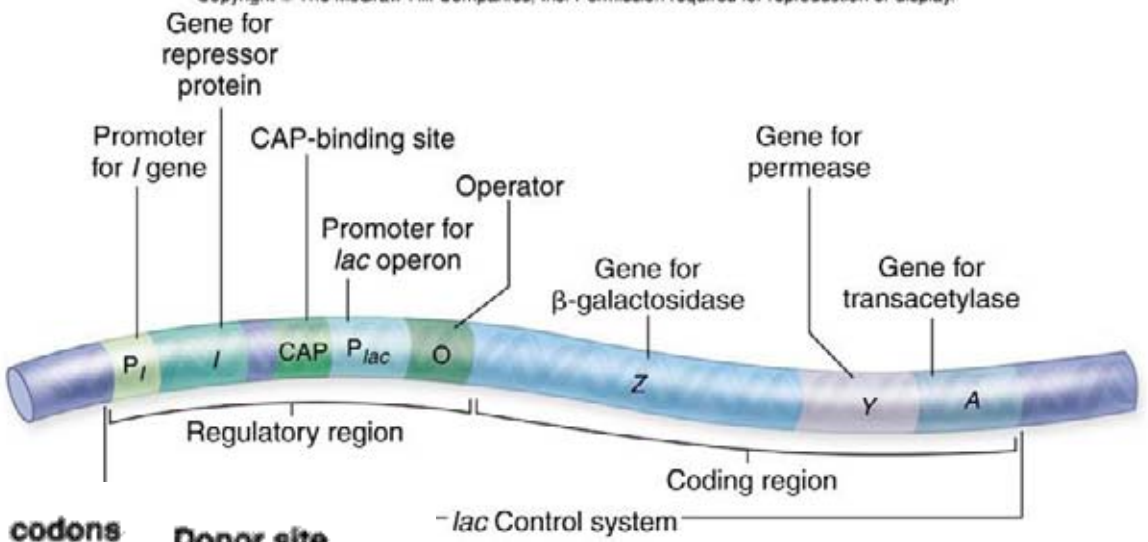


CM, cytoplasmic membrane (+cell wall)
ICM, intracytoplasmic membrane
P, paryphoplasm
I, invaginations of the ICM; D, DNA; V, vesicle

Fuerst and Webb, PNAS, 1991;

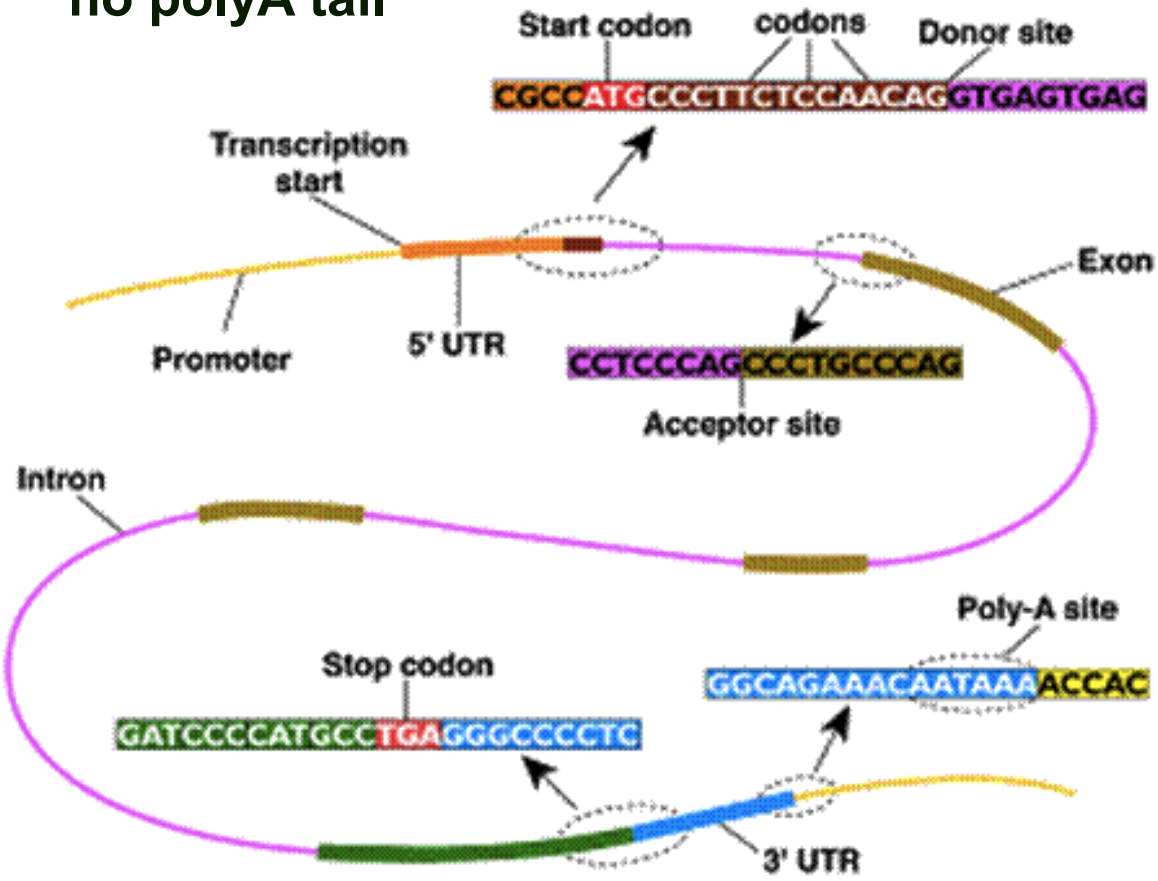
Santarella-Mellwig et al, PLOS Biology, 2010

GENE STRUCTURE



in Bacteria:

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

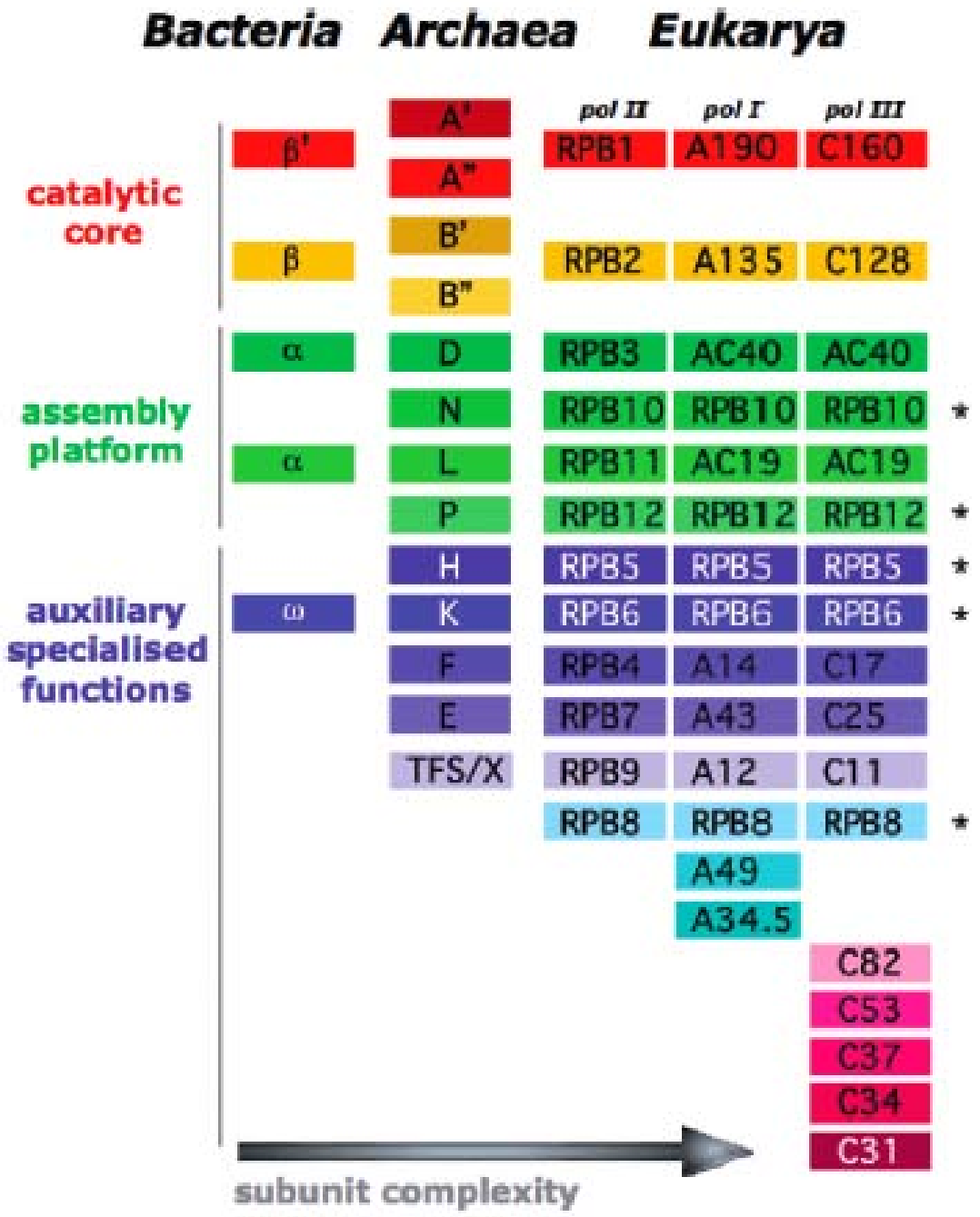


in Eukarya:

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

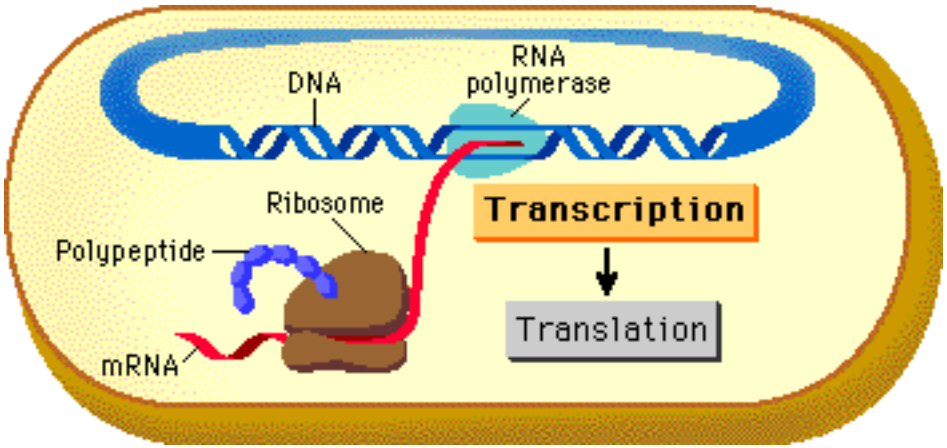
capping (Pol II transcripts)
splicing
editing
3' end formation - cleavage and polyadenylation

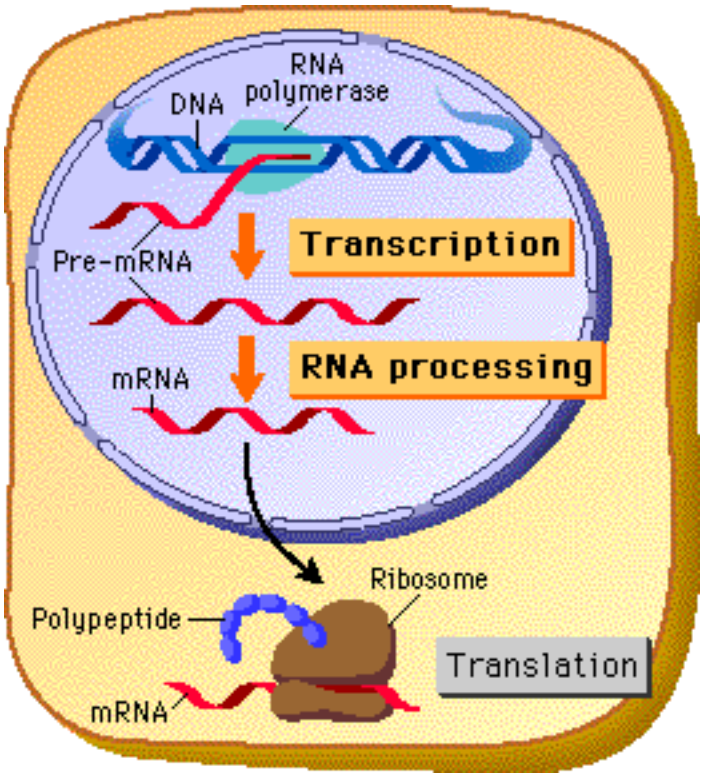
RNA POLYMERASES

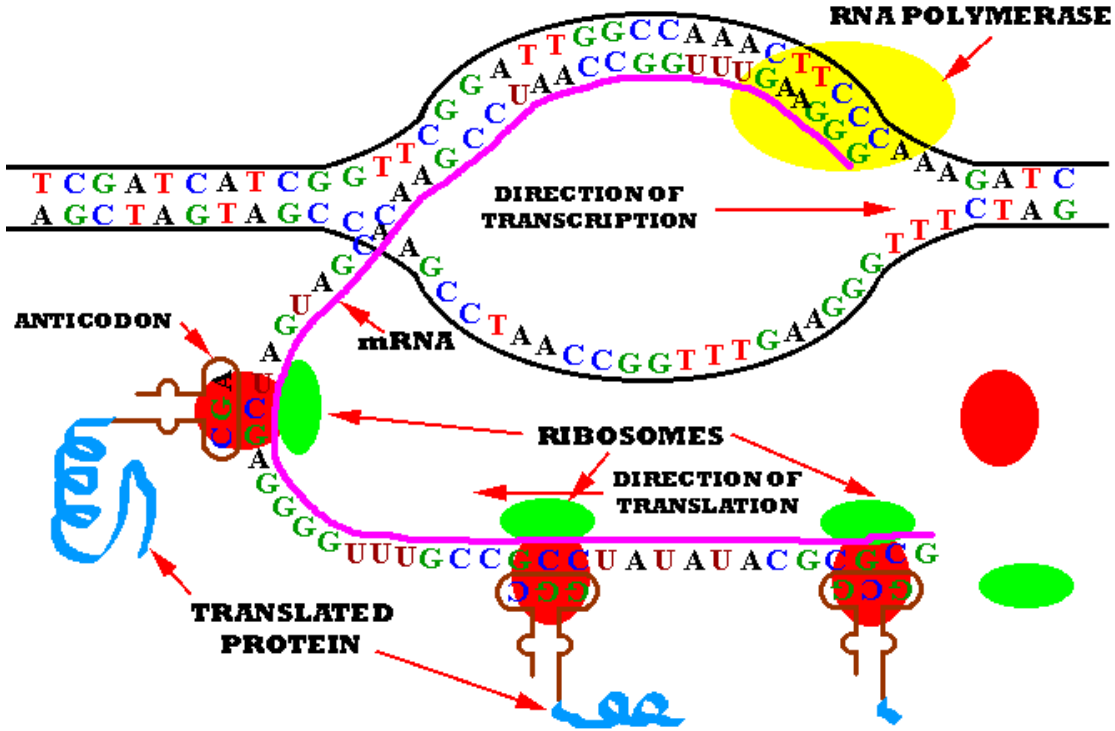


Werner, Mol Microbiol, 2007

GENE EXPRESSION: BACTERIA vs EUKARYA
TRANSCRIPTION AND TRANSLATION

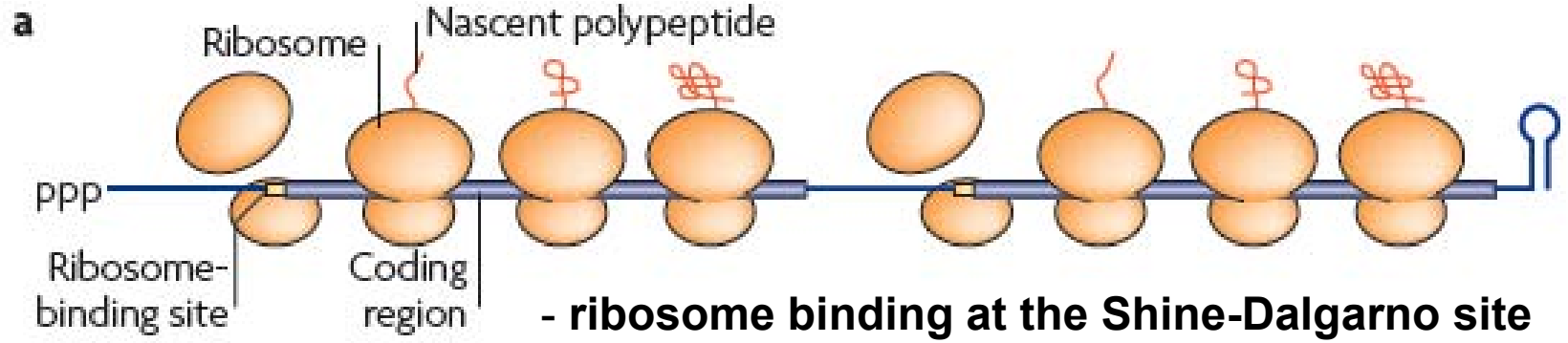




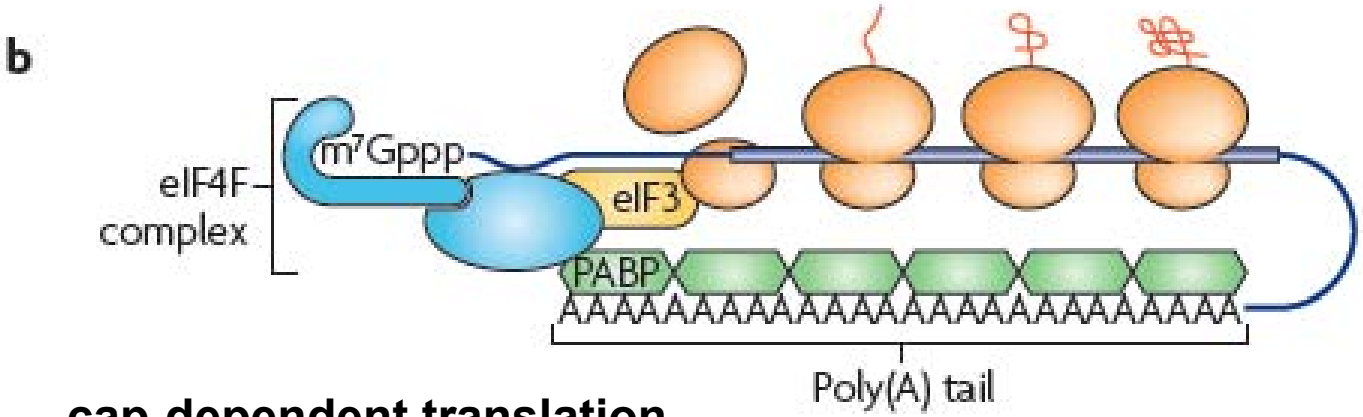


http://www.phschool.com/science/biology_place/biocoach/transcription/tctlpreu.html

mRNA STRUCTURE AND TRANSLATION
BACTERIA vs EUKARYA



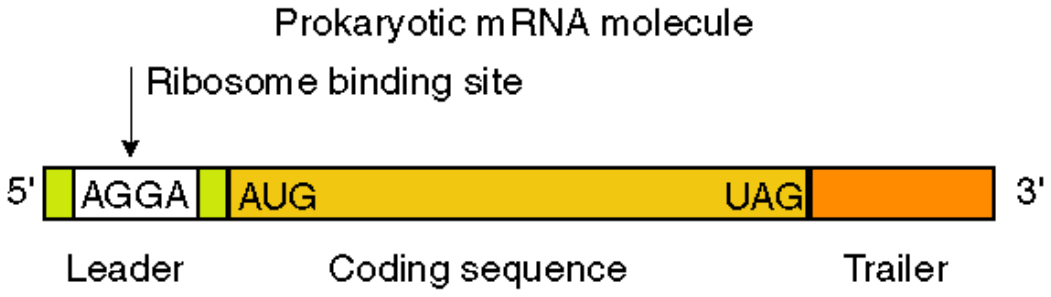
- ribosome binding at the Shine-Dalgarno site
- no ribosome scanning



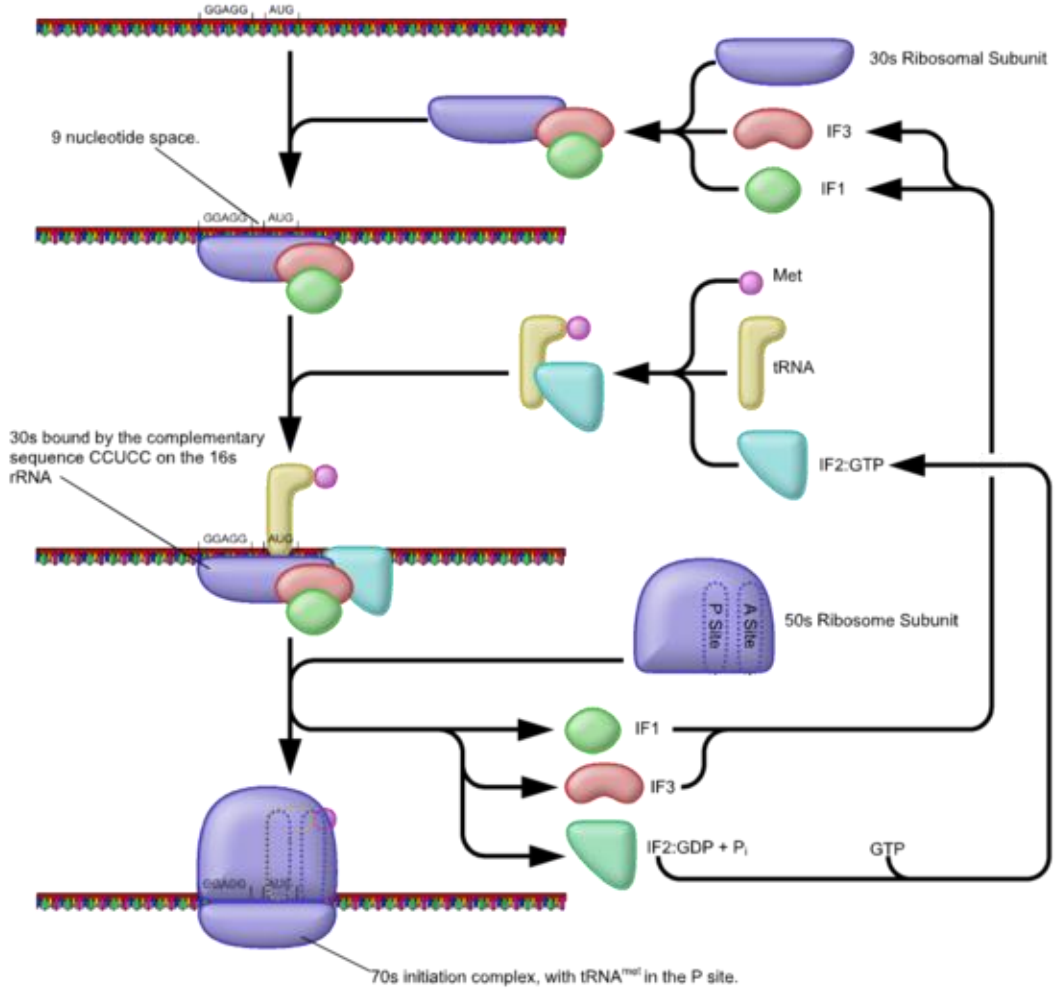
- cap-dependent translation
- ribosome scanning for translation initiation

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

TRANSLATION in BACTERIA

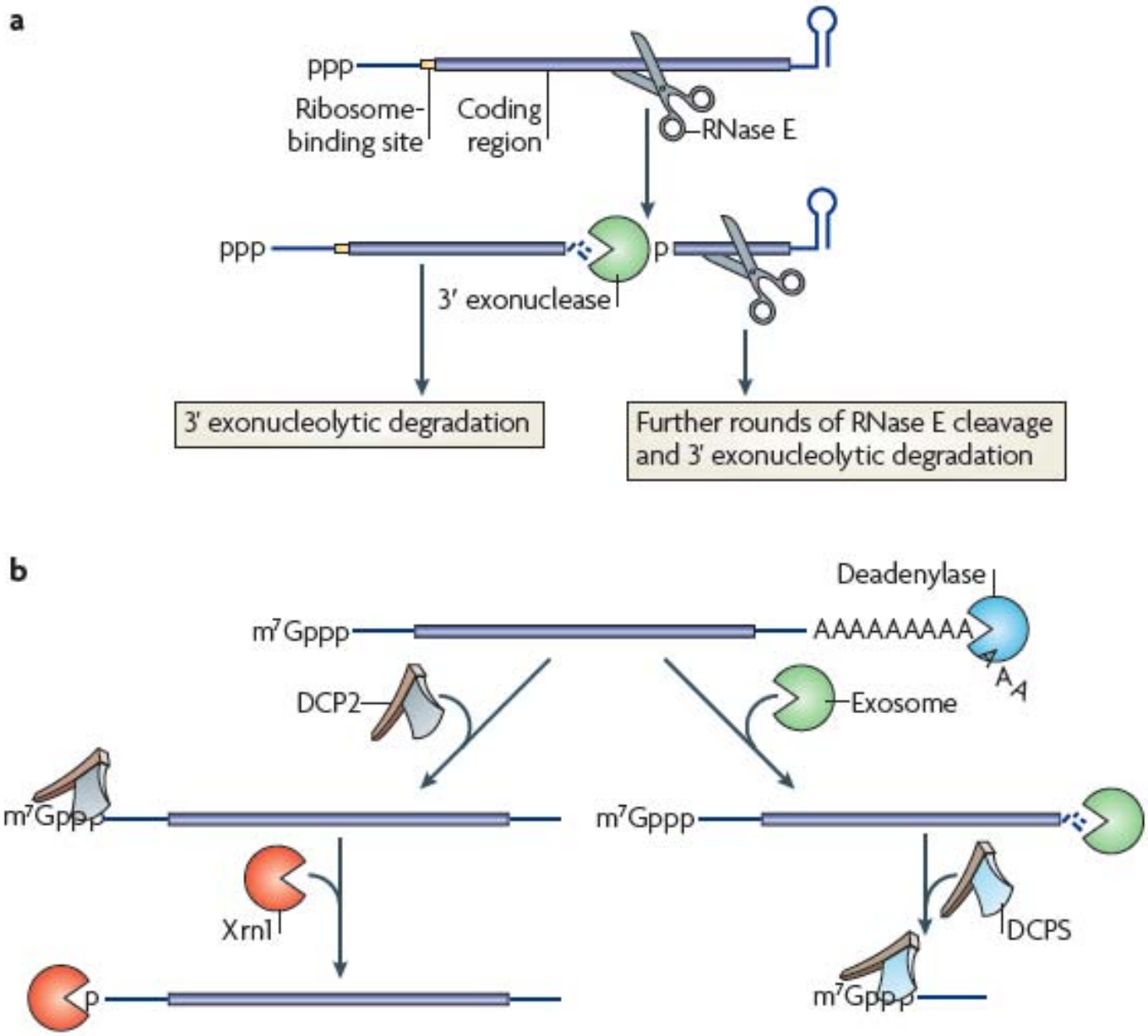


Shine-Dalgarno sequence upstream of AUG start codon helps to recruit the ribosome by interacting with the complementary region in the 3' end of 16S rRNA



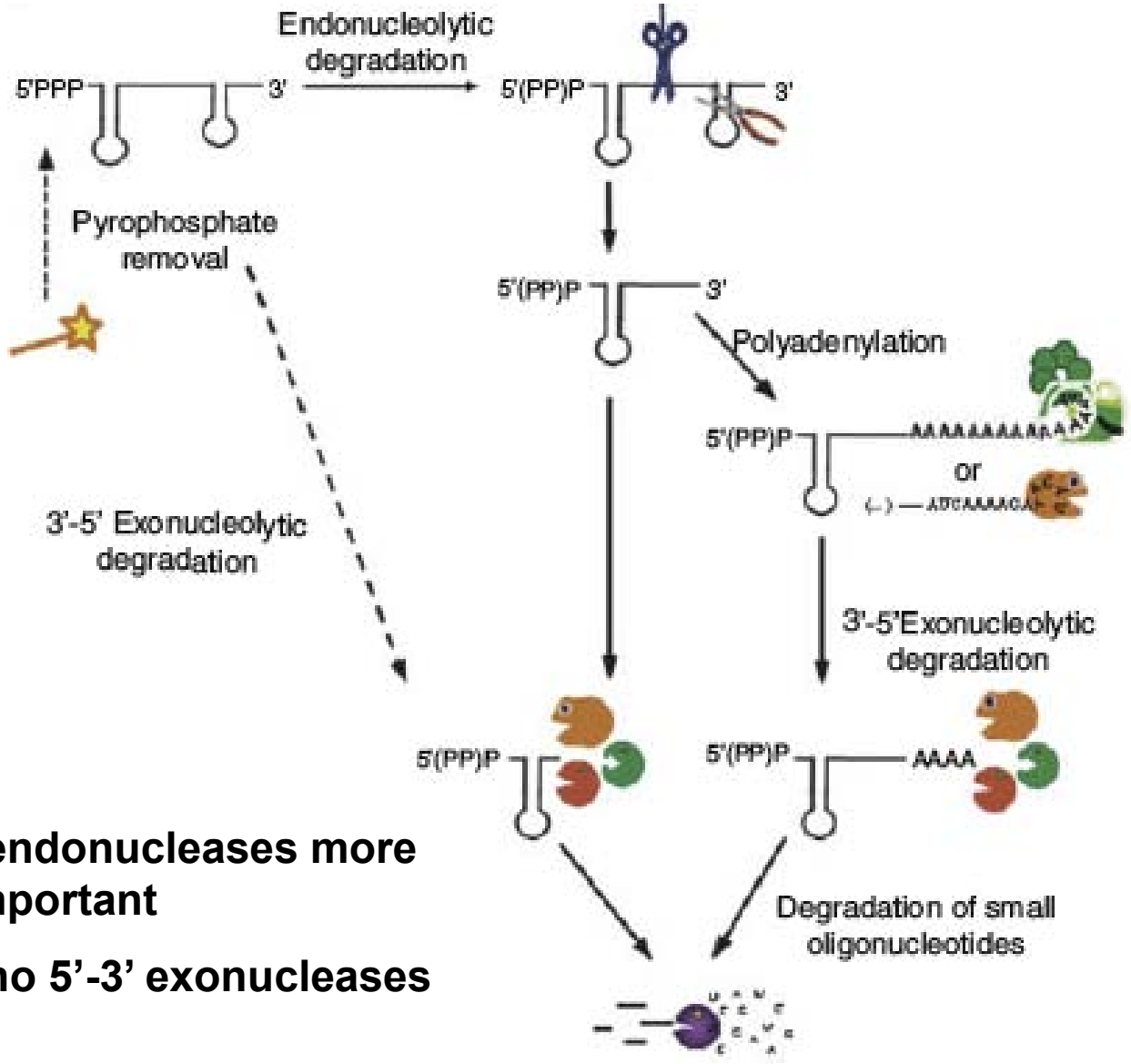
see the movie at:
http://pubs.acs.org/cen/multimedia/85/ribosome/translation_bacterial.html

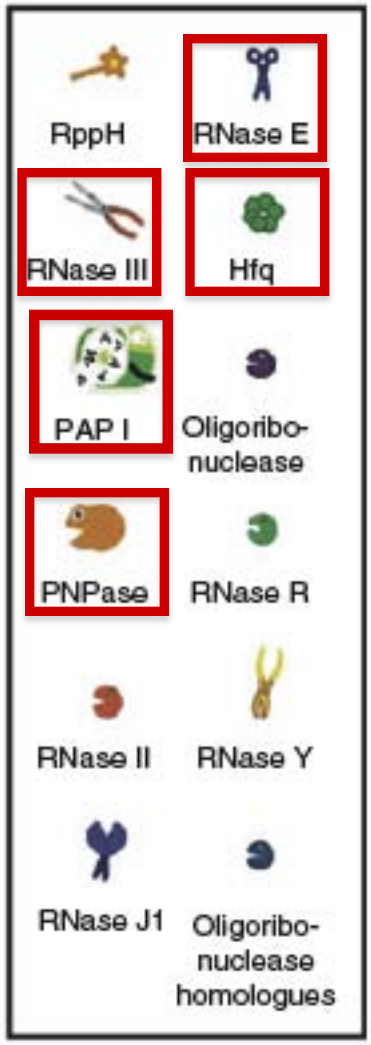
mRNA DECAY BACTERIA vs EUKARYA



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

mRNA DECAY in BACTERIA *E. coli*





- endonucleases more important

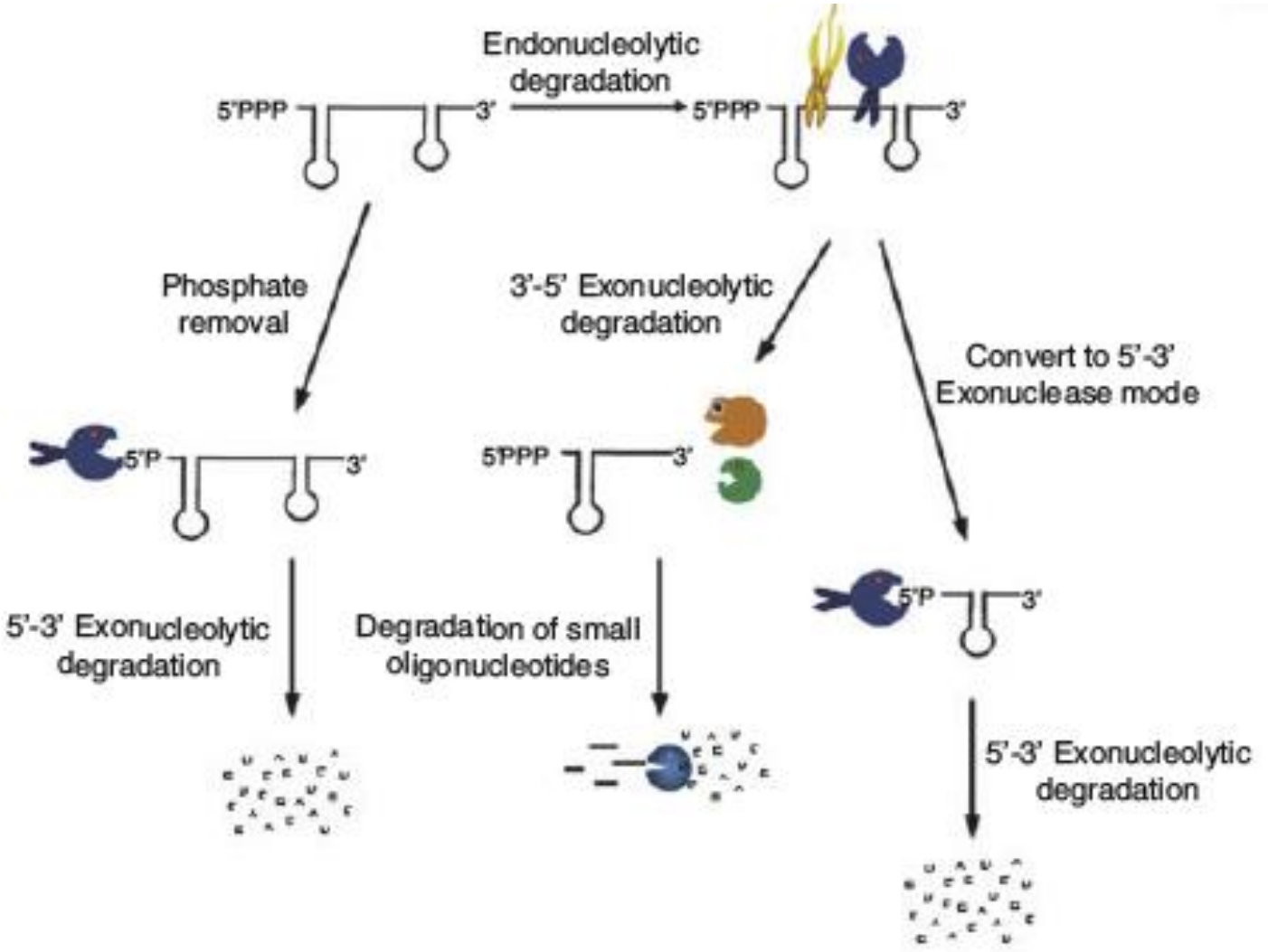
- no 5'-3' exonucleases

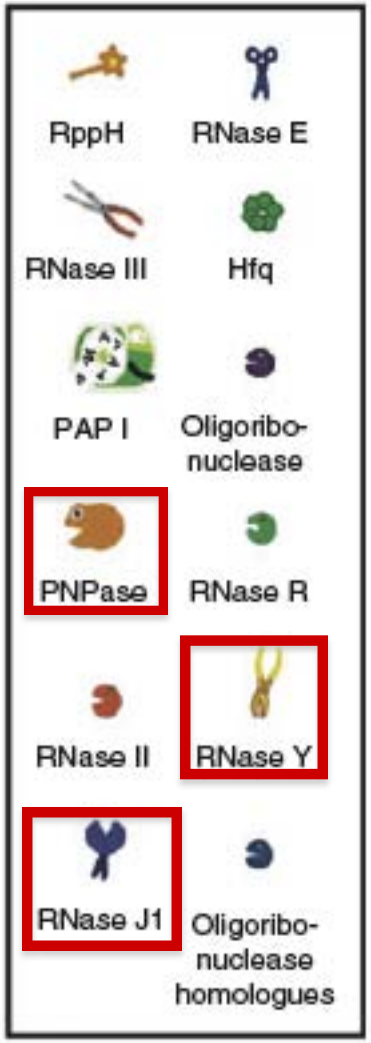
- no RNase J1 (5' exo and endo)

- no RNase Y

Silva et al, WIRERNA 2011

mRNA DECAY in BACTERIA *B. subtilis*





- no PAP I
- no RNase E

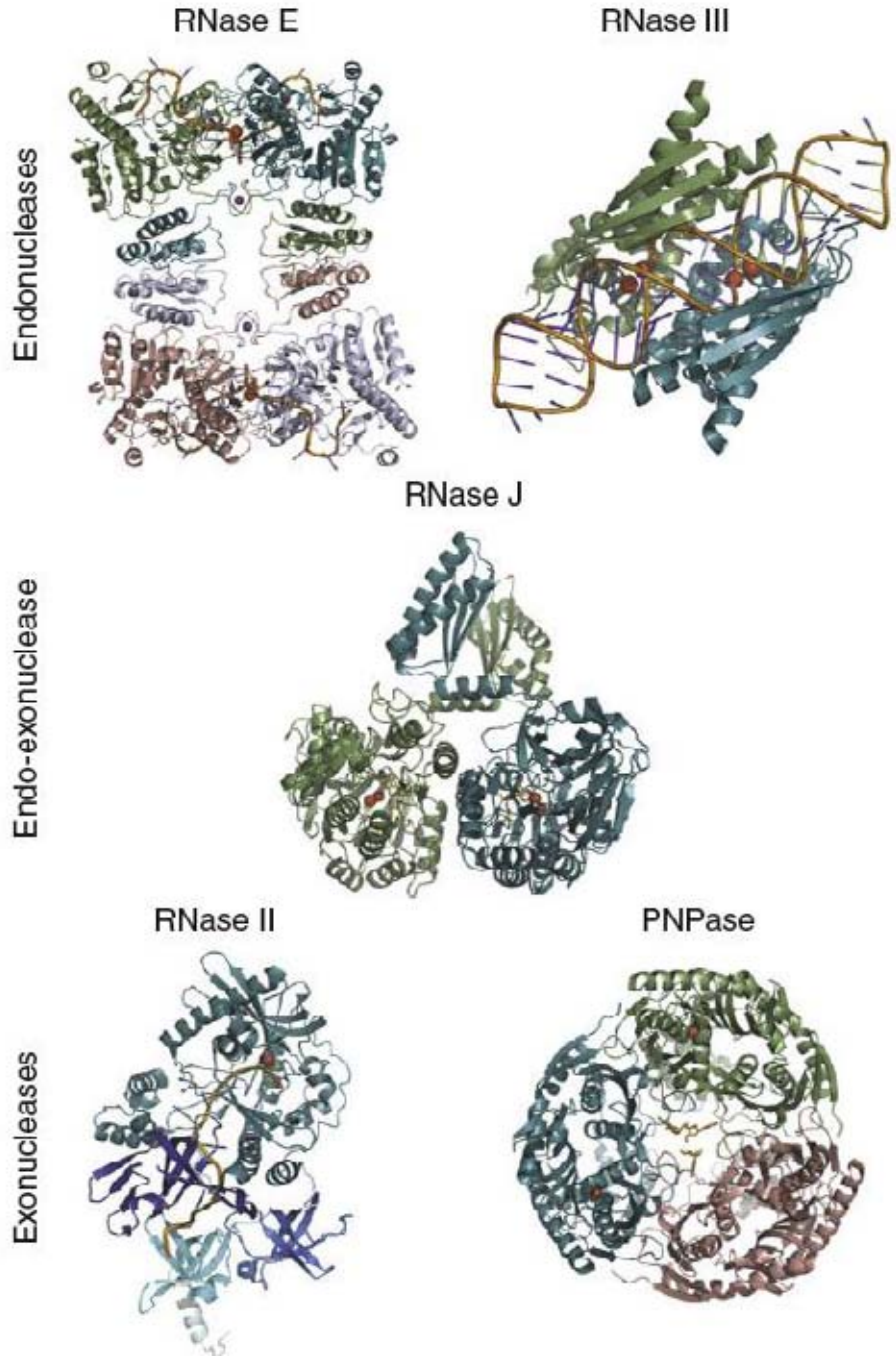
Silva et al, WIRERNA 2011

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

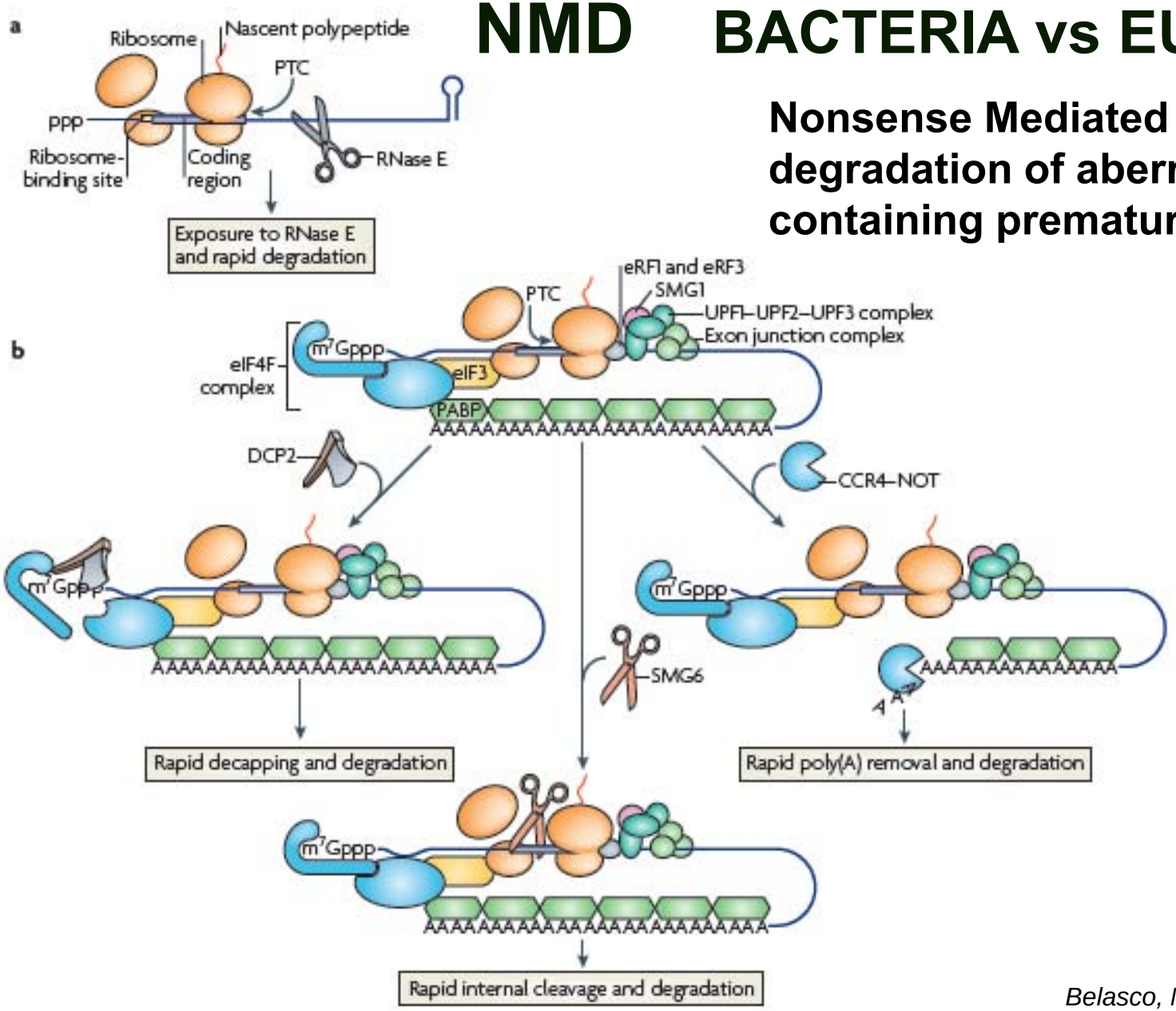


STRUCTURES of BACTERIAL RNA ENZYMES in COMPLEX with SUBSTRATES

Silva et al, WIRERNA 2011

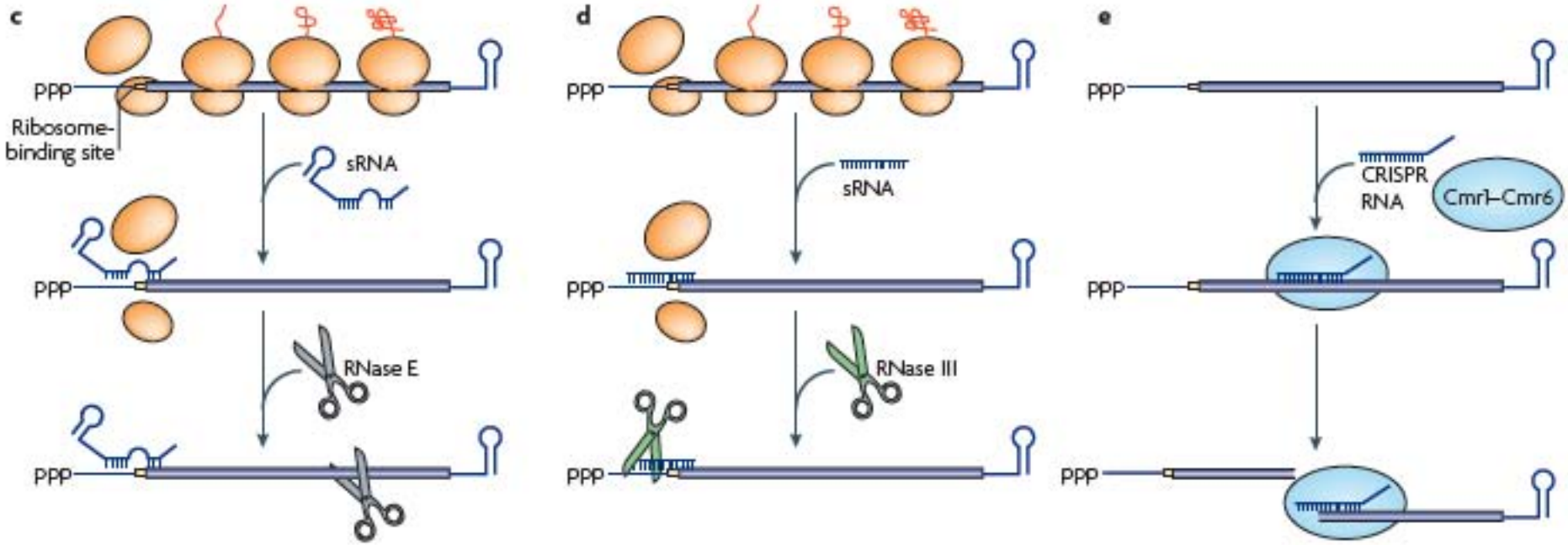
SPECIALIZED mRNA DECAY: NMD BACTERIA vs EUKARYA

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

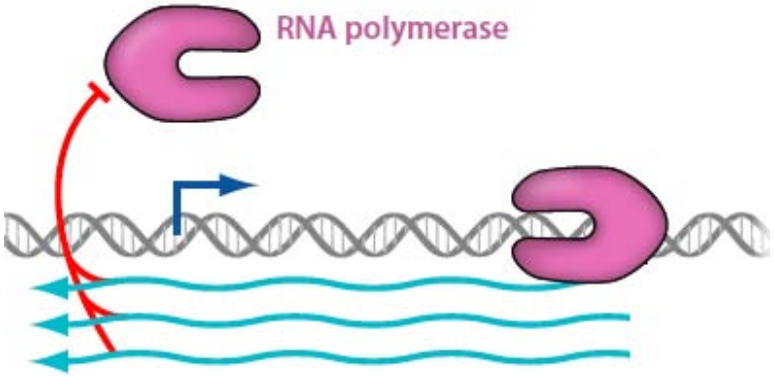


Belasco, Nat.Rev.Mol.Cell.Biol, 2012

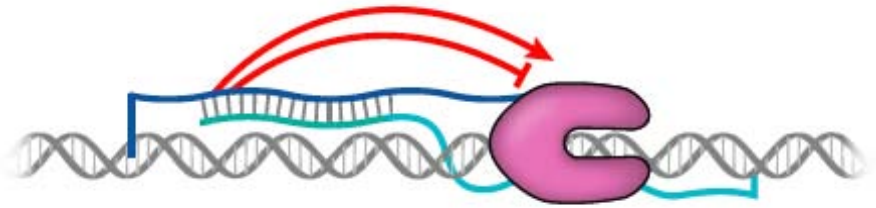
REGULATION of GENE EXPRESSION by sRNAs in BACTERIA



Transcription interference



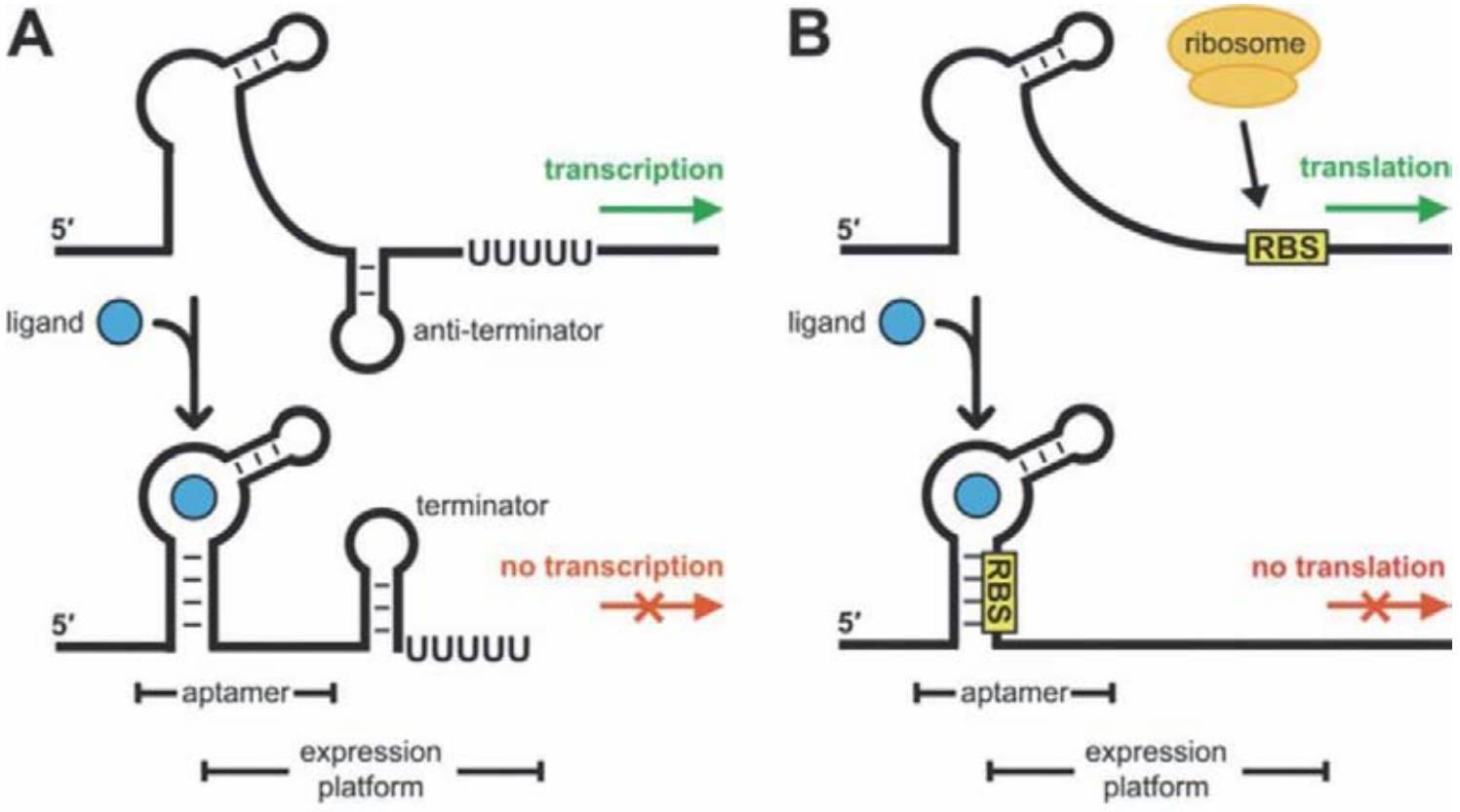
Transcription attenuation



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



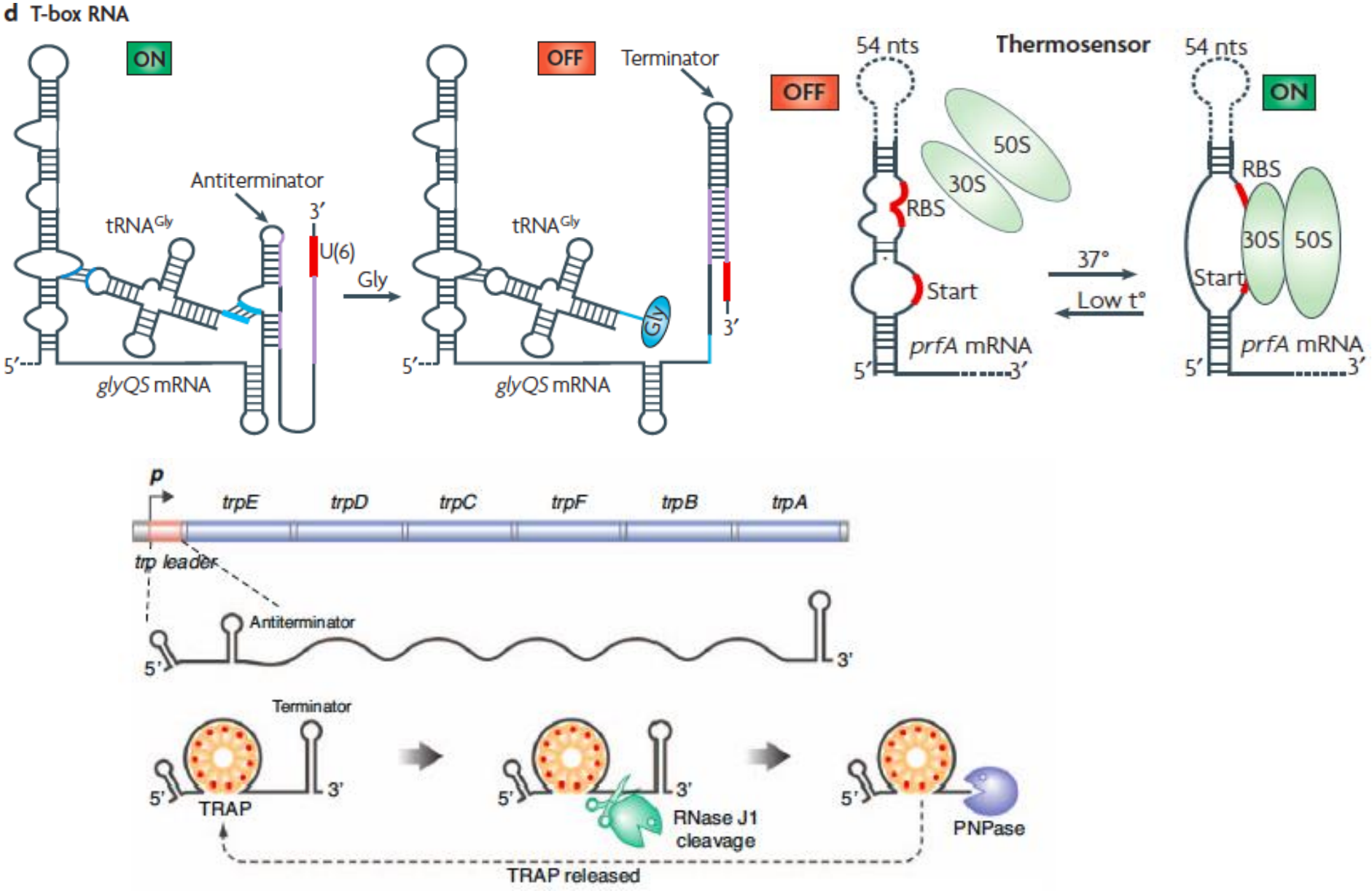
http://www.umich.edu/~rnapeopl/WalterSummaryRiboswitch.htm

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 protcobactcria, *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

BACTERIAL POLYADENYLATION

• Two bacterial 3' terminal polymerases:

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

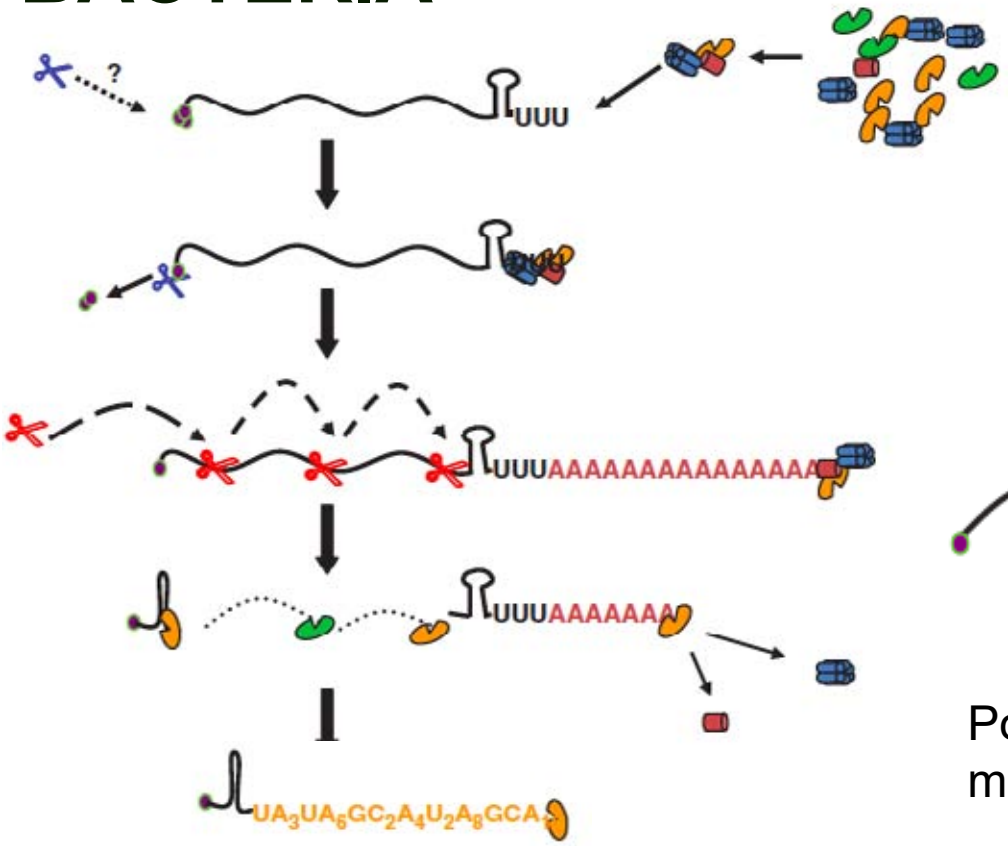
• poly(A) tails shorter (10-60 nts), occur for 2-60% of molecules of a given transcript

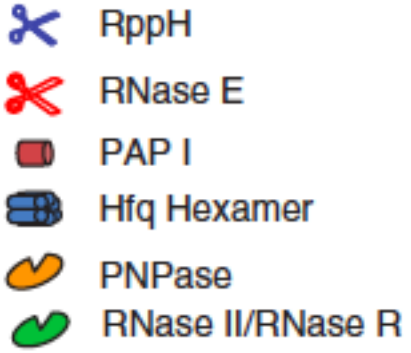
• polyadenylation sites are diverse, no consensus

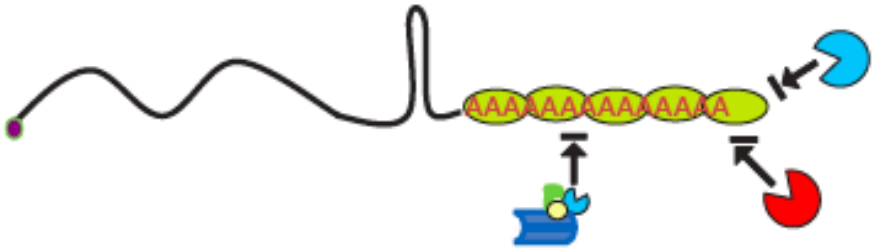
E. coli	mRNA	*lpp, rpsO, ompA, secG, rmf, pcnB, trxA*
	rRNA	16S rRNA, 23S rRNA
	nc RNA	6S RNA, 4.5S RNA, RNA I, SoK, SraK, SraL, GlmY, SsrA, RnpB
	tRNA	*cysT, hisR, leuX, trpT, leuU, tyrT, tyrV*
B. subtilis	mRNA	*mpB, rpsD, cry1Aa*
	rRNA	23S rRNA
	tRNA	$tRNA^{Cys\text{-}LeuU}$
Streptomyces	mRNA	*redD,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

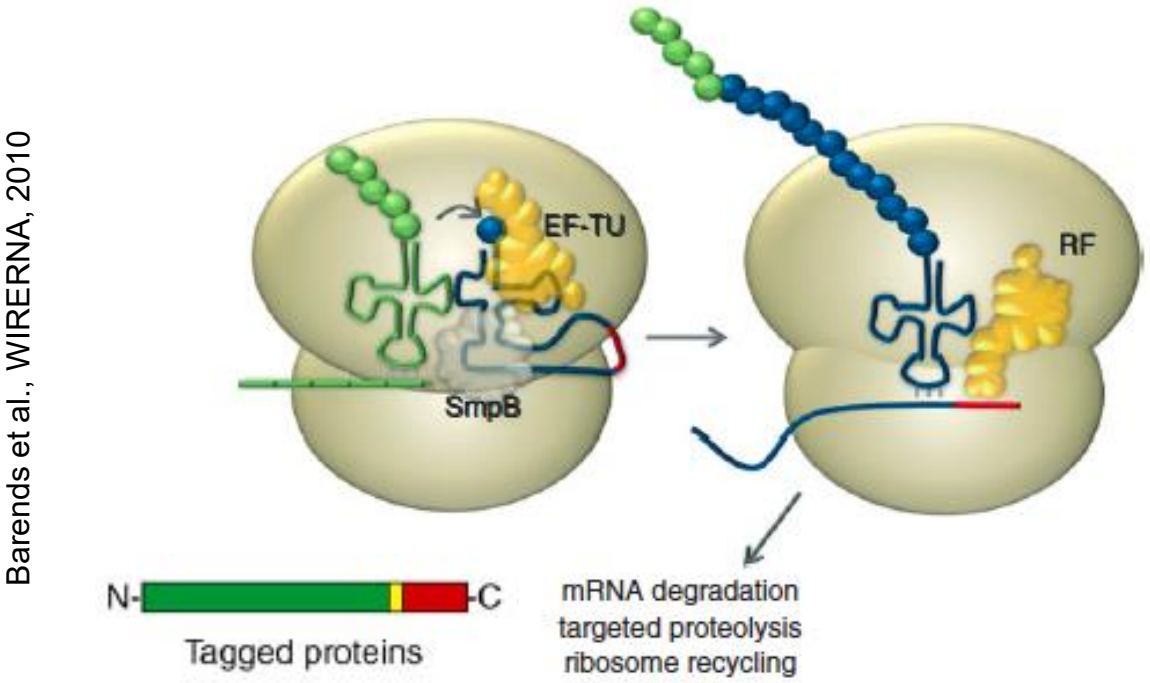
Hfq-mediated polyadenylation by PAP I in *E. coli*

- 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

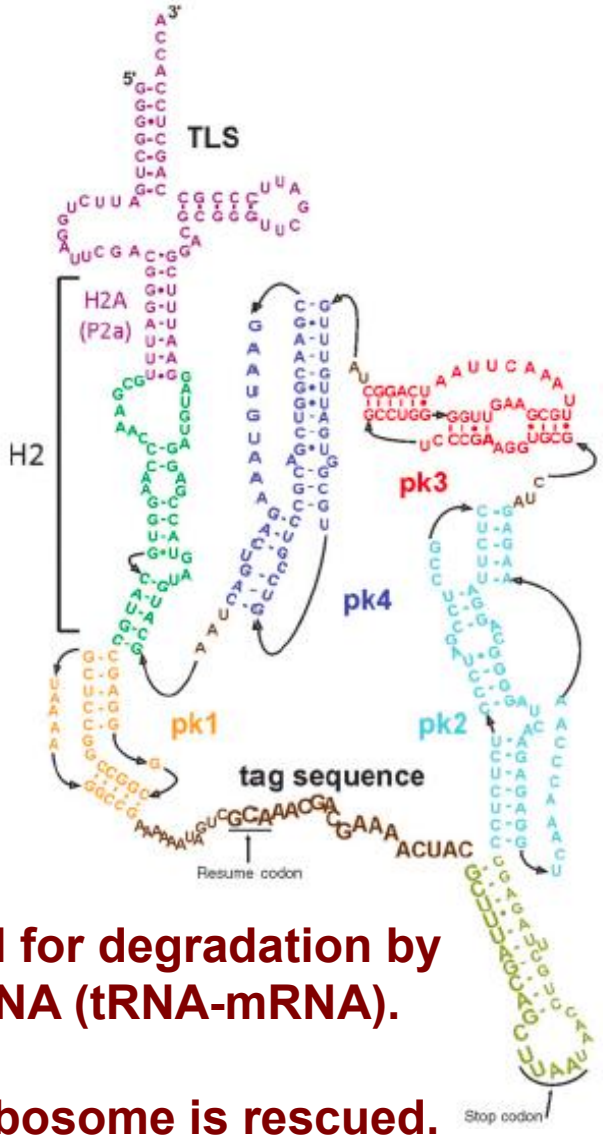
Mohanty and Kushner WIRERNA, 2010

PROTEIN DEGRADATION in BACTERIA by tmRNA TAGGING

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

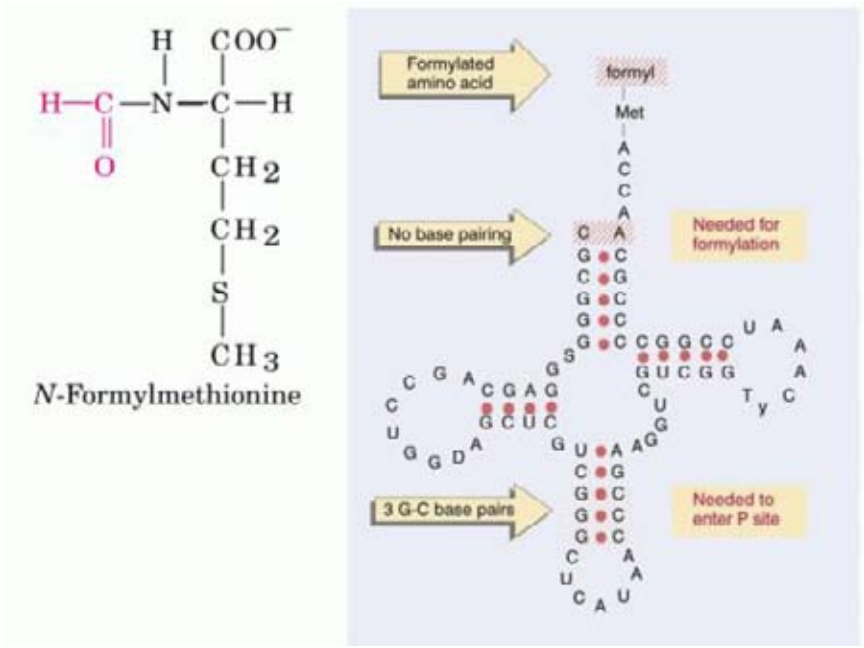


Barends et al., WIRERNA, 2010



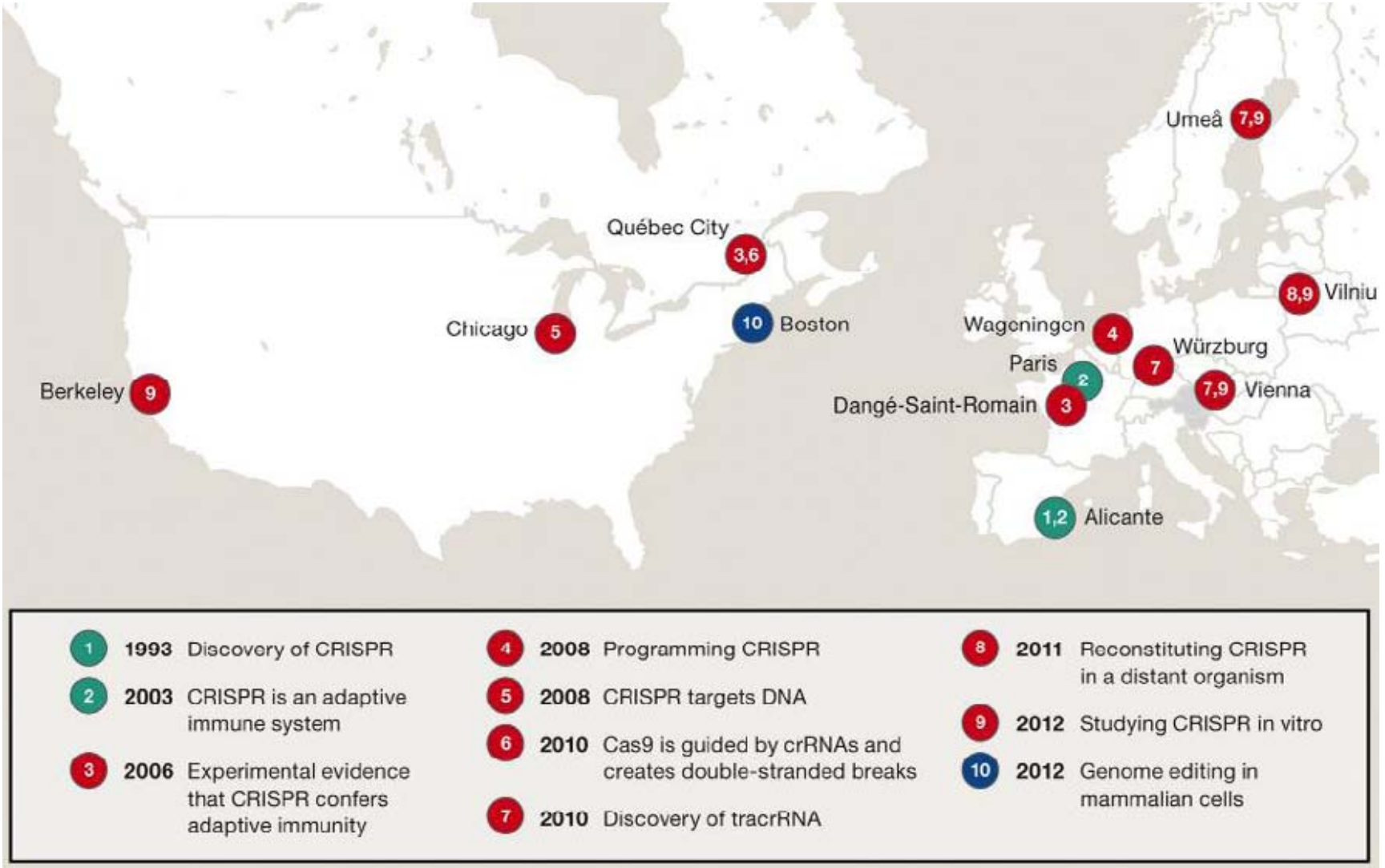
- **Nonfinished proteins are cotranslationally marked for degradation by trans-translation mechanism using tagging by tmRNA (tRNA-mRNA).**
- **The tag encodes ANDENYALAA sequence.**
- **mRNA and tagged protein are degraded, stalled ribosome is rescued.**
- **tmRNA interacts with SmpB, RP S1, EF-Tu and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

$tRNA^{Met}$ 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

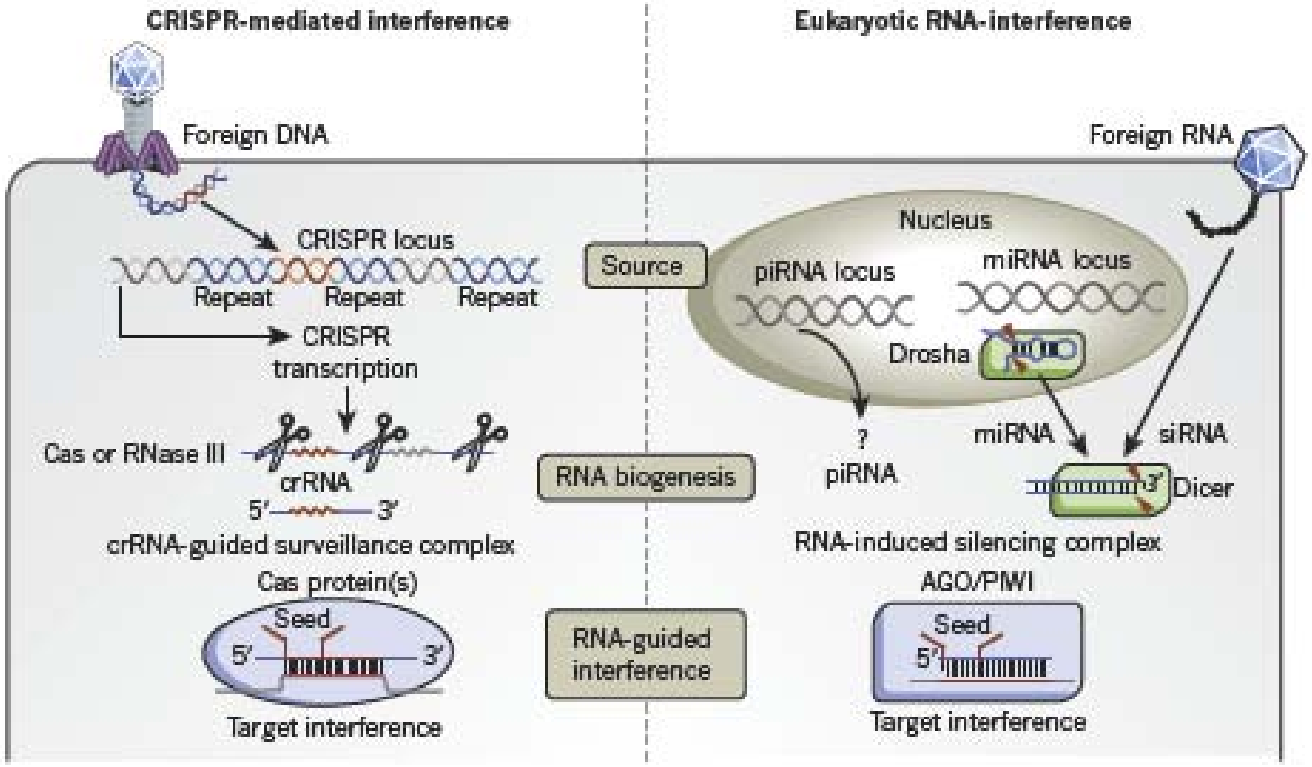
CRISPR/Cas history



Lander, Cell, 2016

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

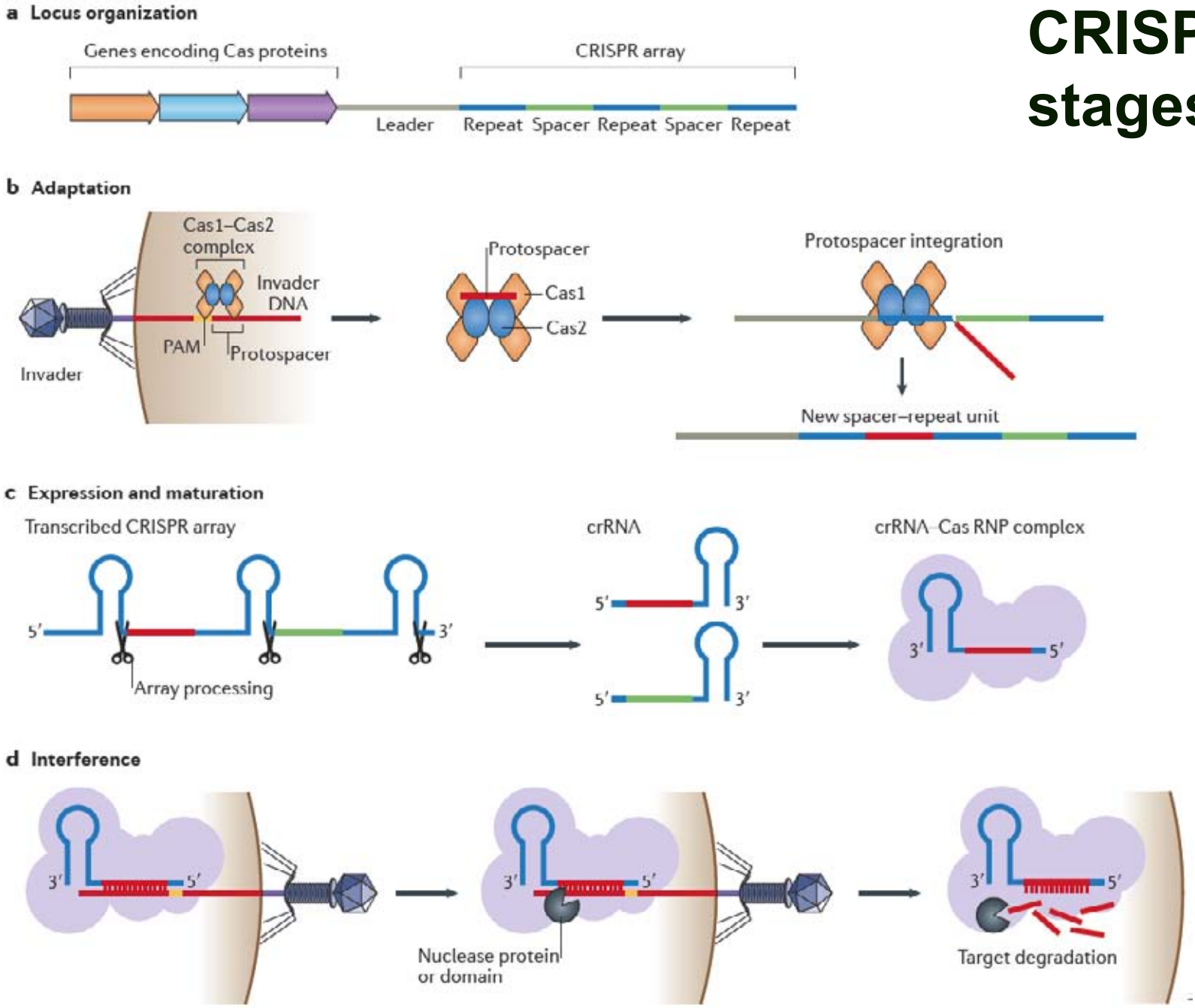
CRISPR Clustered Regularly Interspaced Short Palindromic Repeat
Cas- CRISPR associated



- **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**

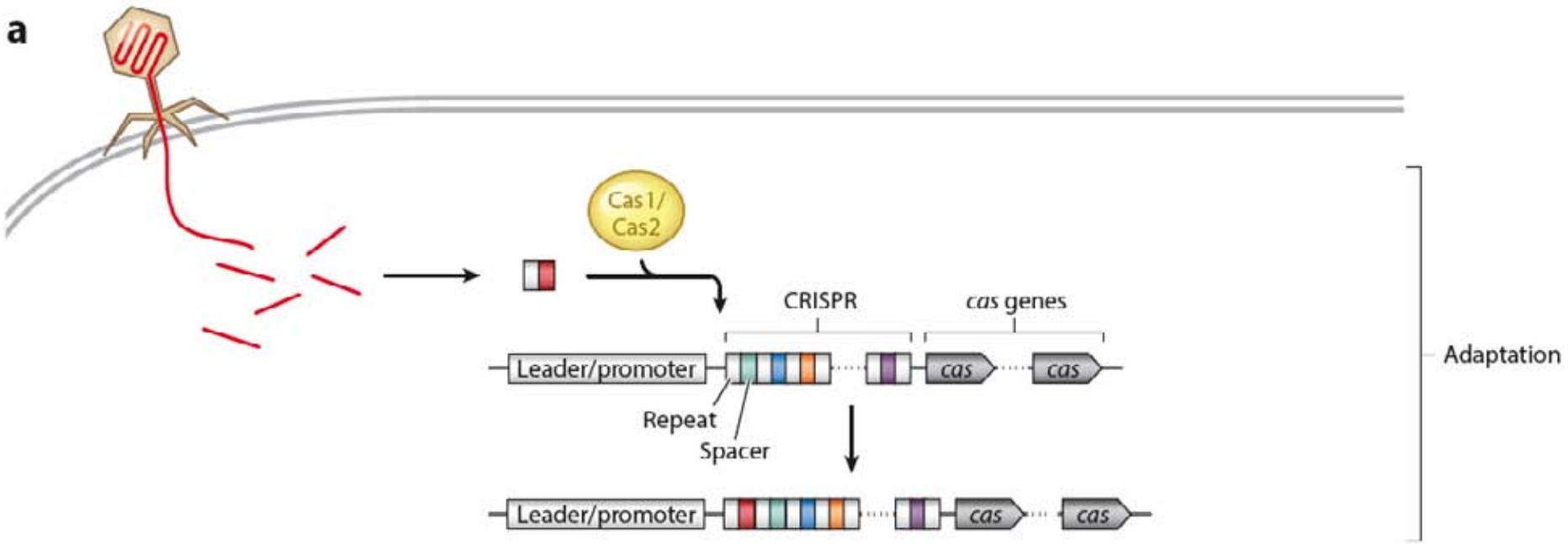
Wiedenheft et al, Nature, 2012

CRISPR/Cas stages



Amitai and Sorek, NatRevMicro, 2016

CRISPR/Cas: adaptation



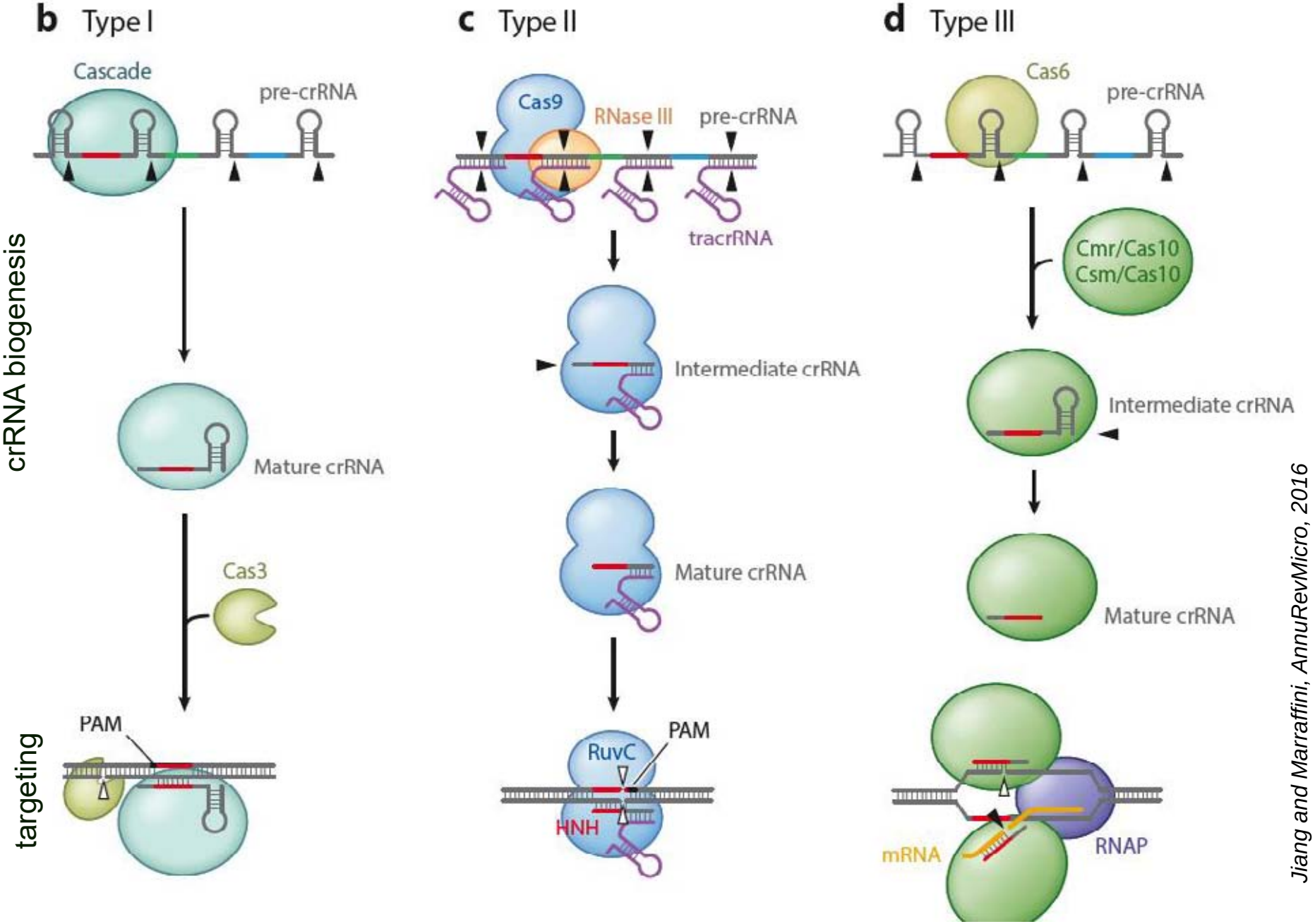
PAM protospacer-adjacent motif in type I immunity

- 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)

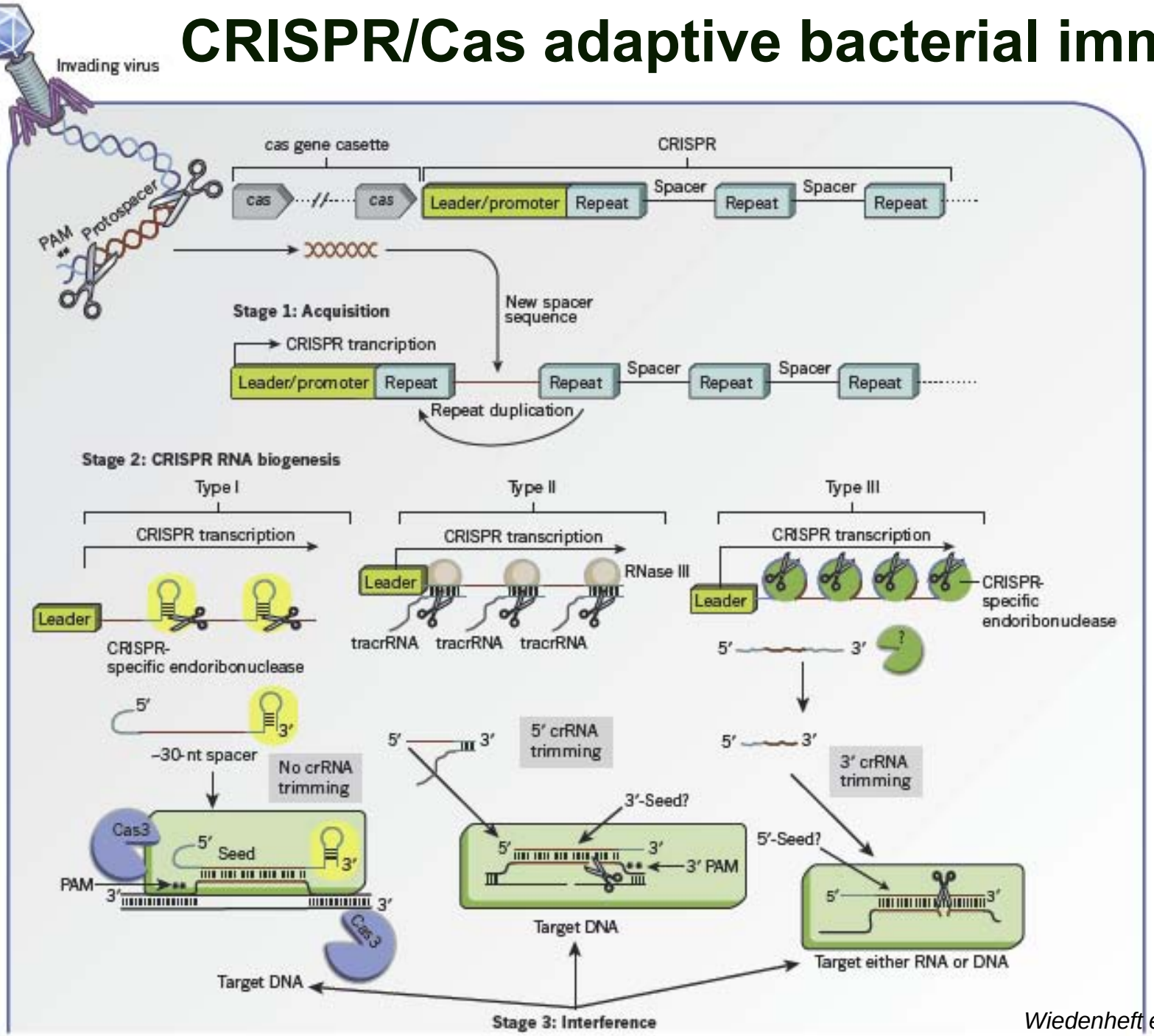
Jiang and Marraffini, AnnuRevMicro, 2016

CRISPR/Cas: crRNA biogenesis, targeting



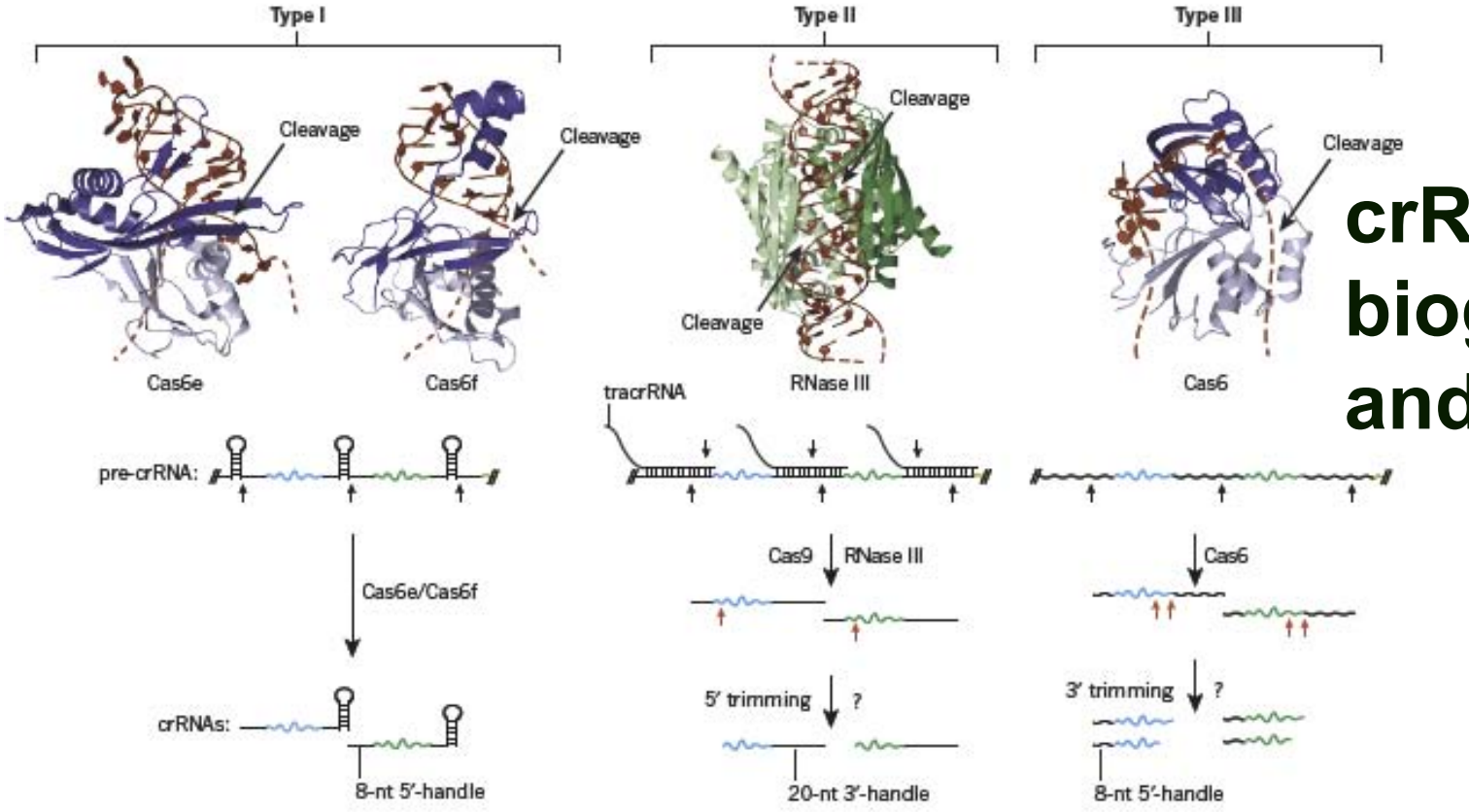
Jiang and Marraffini, AnnuRevMicro, 2016

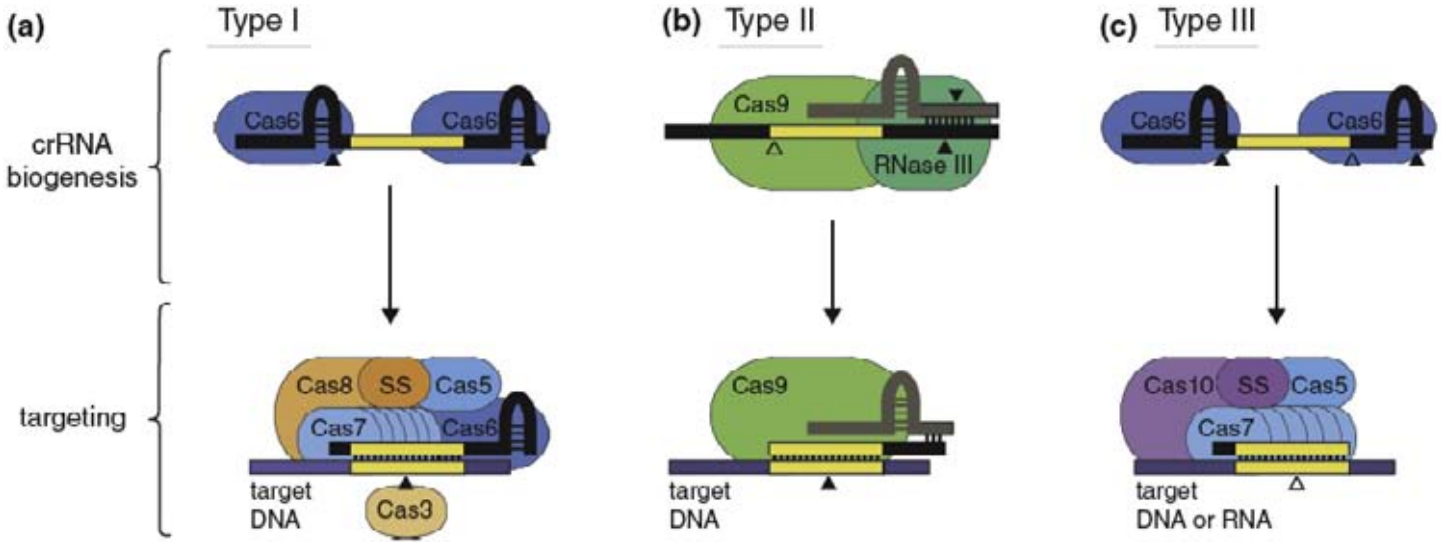
CRISPR/Cas adaptive bacterial immunity



Wiedenheft et al, Nature, 2012

crRNA biogenesis and function





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

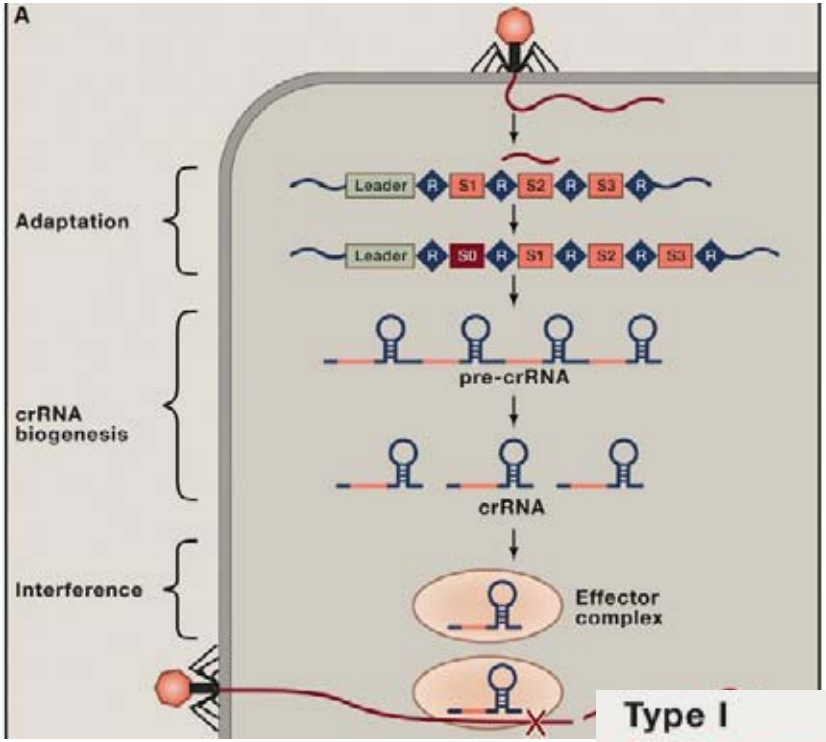
CRISPR/Cas types

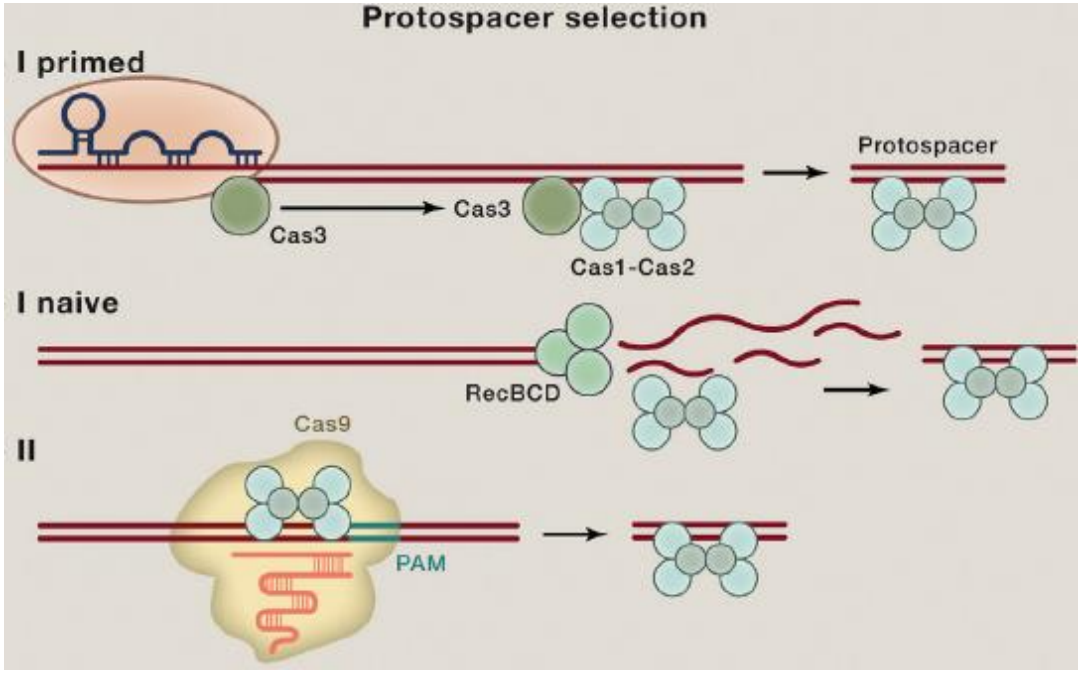
Table 1. Classification and Examples of CRISPR Systems

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

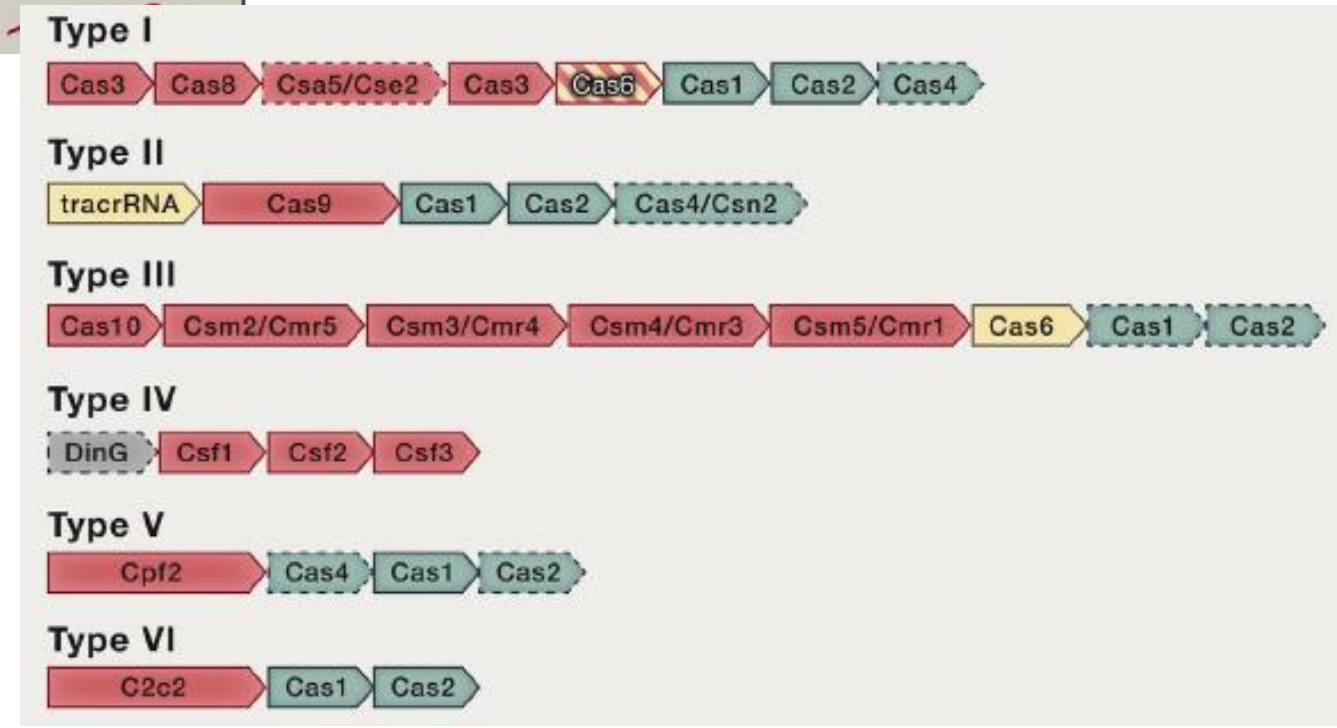
CRISPR systems are currently organized into two overarching classes: Class 1, which contain multi-subunit effectors, and Class 2, which contain single protein effectors. These classes are subdivided into five types (Makarova et al., 2015), with type IV remaining a putative type within Class 1. Although only Class 2 systems have been adapted for genome engineering, the results described in this review emerged from studying a diversity of CRISPR-Cas systems. (Type III-B systems are not discussed but represent an unusual system that targets RNA rather than DNA [Hale et al., 2009].)

Lander, Cell, 2016





CRISPR/Cas types

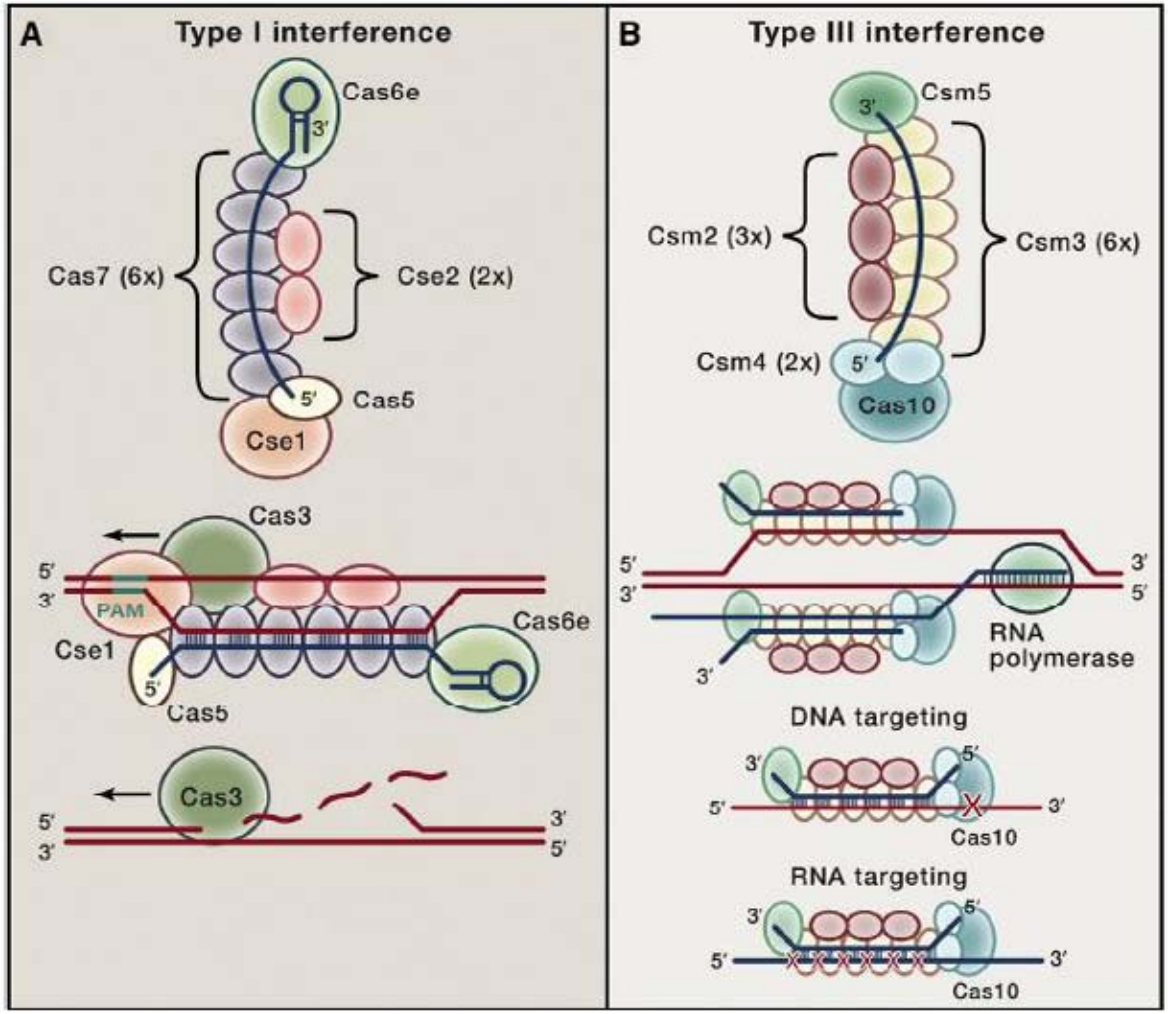


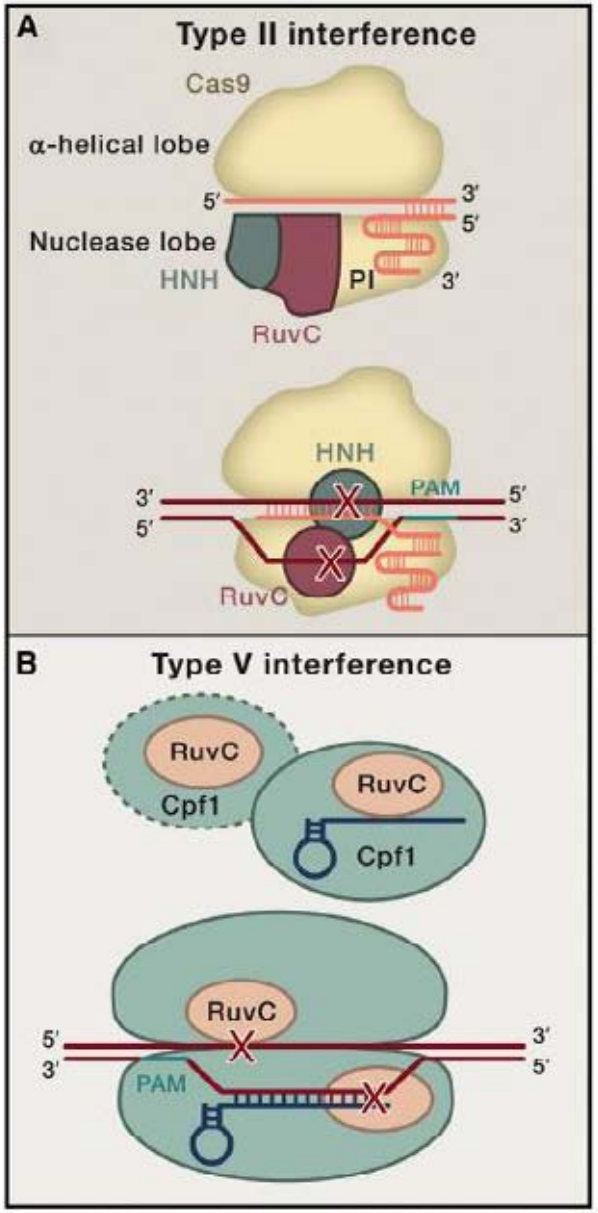
Wright et al, Cell, 2016

CRISPR/Cas types

targets DNA
(via PAM)

targets RNA and actively
transcribed DNA





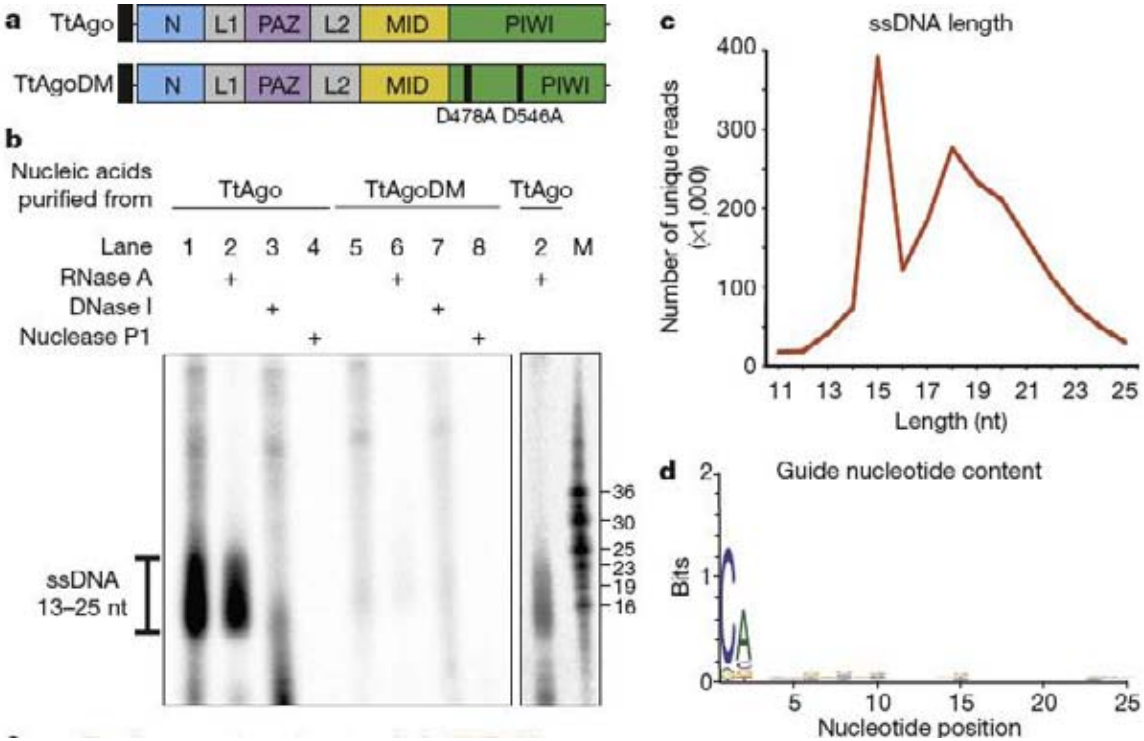
Wright et al, Cell, 2016

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

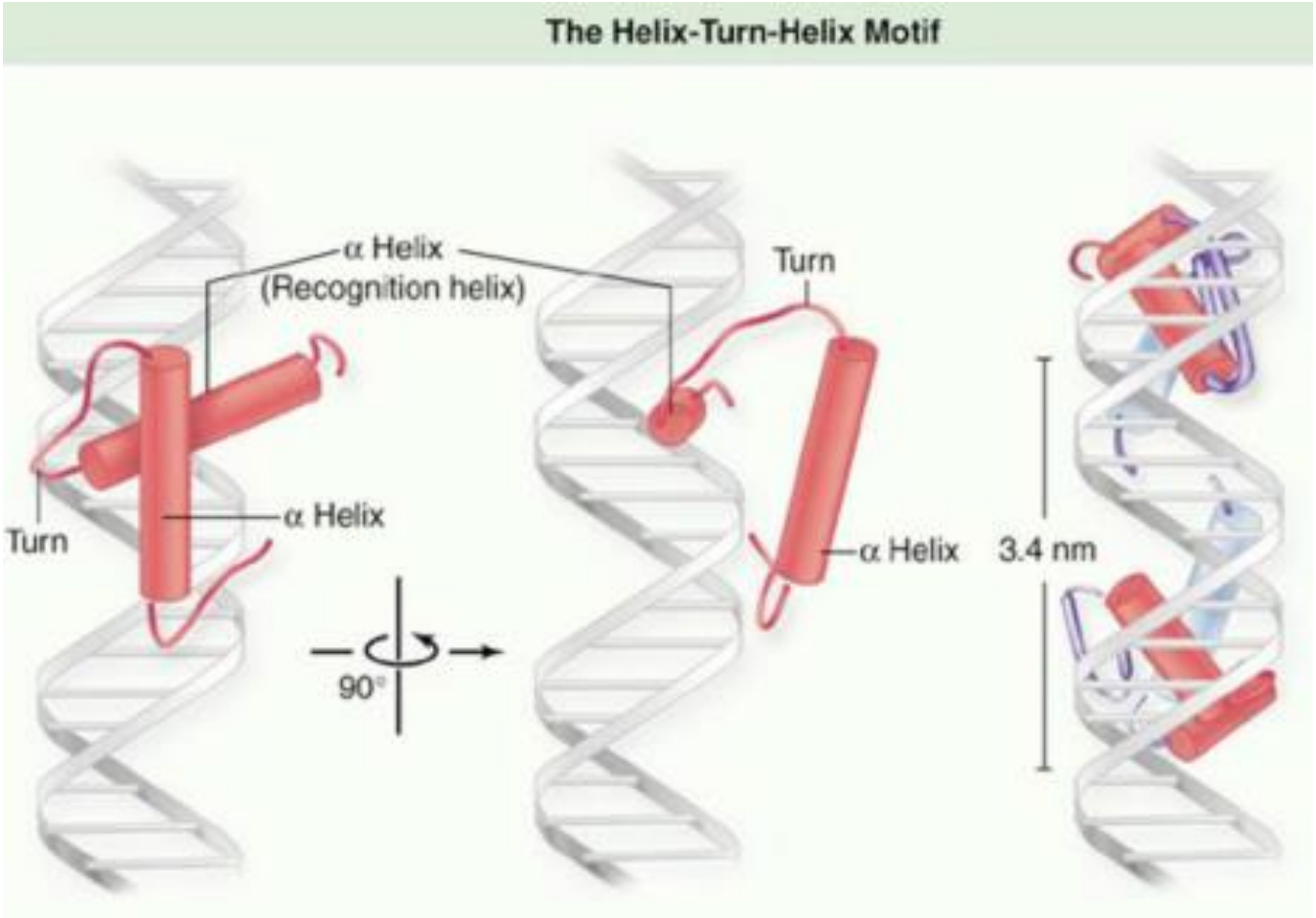
- polyadenylation-assisted RNA degradation

(occurs also in Eukaryotes)

- no cap-dependent translation or ribosome scanning

- tmRNA tagging for protein degradation

REGULATORY PROTEINS



REGULATORY PROTEINS