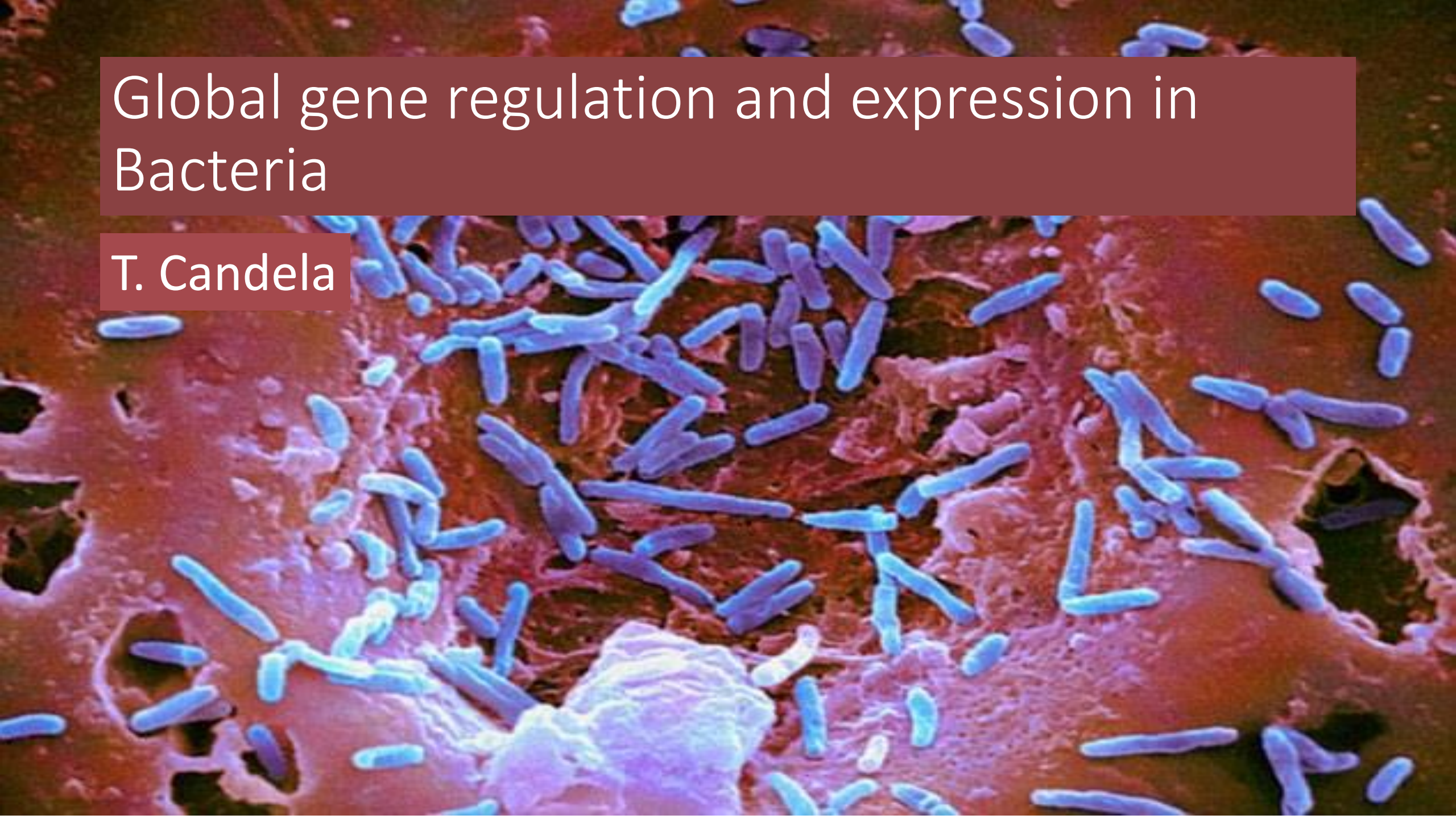
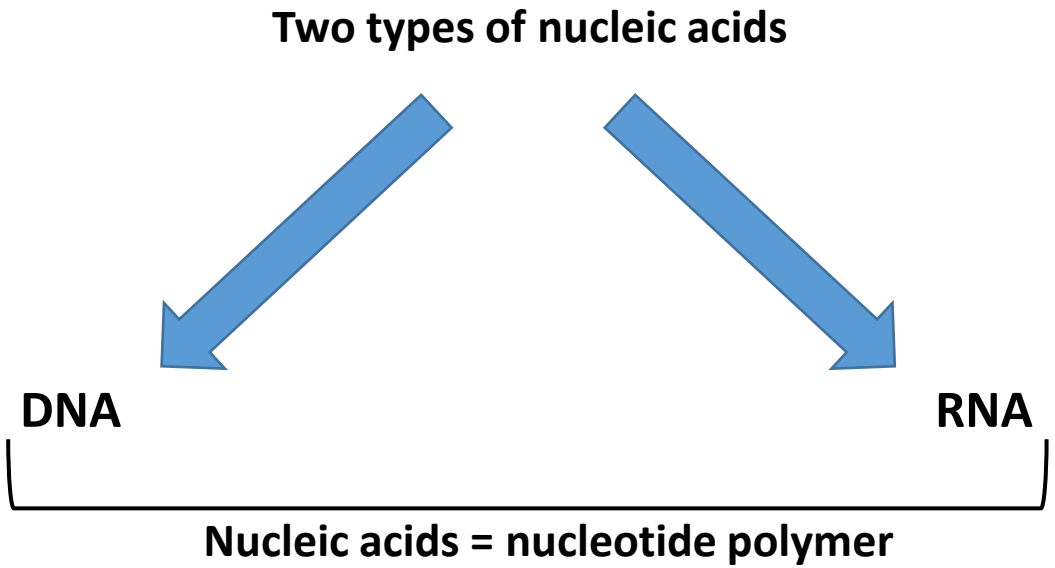
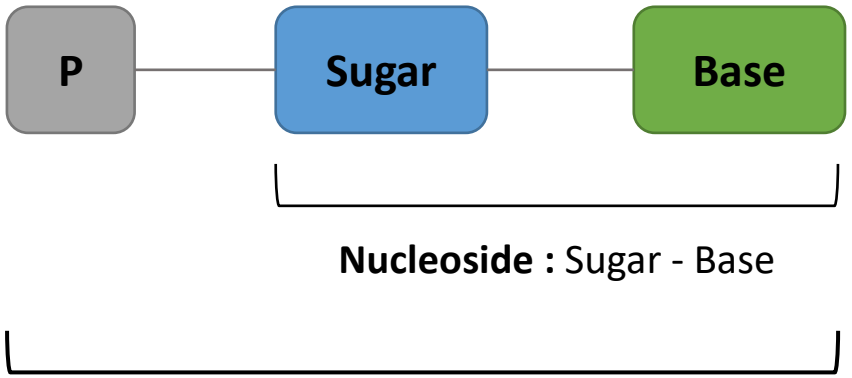
Global gene regulation and expression in Bacteria

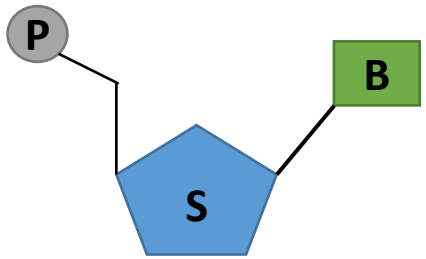
T. Candela



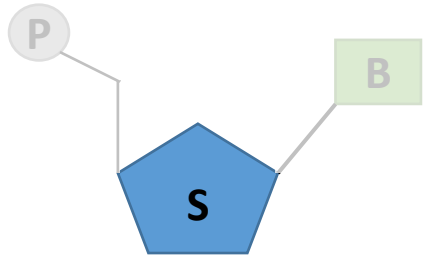
Reminder on DNA and RNA



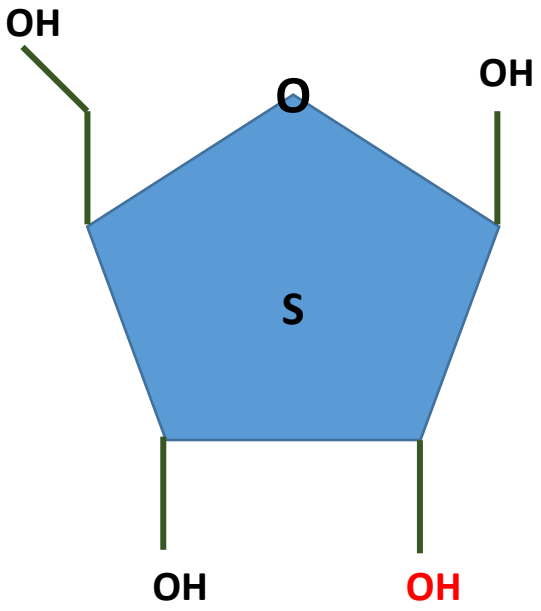




Reminder on DNA and RNA

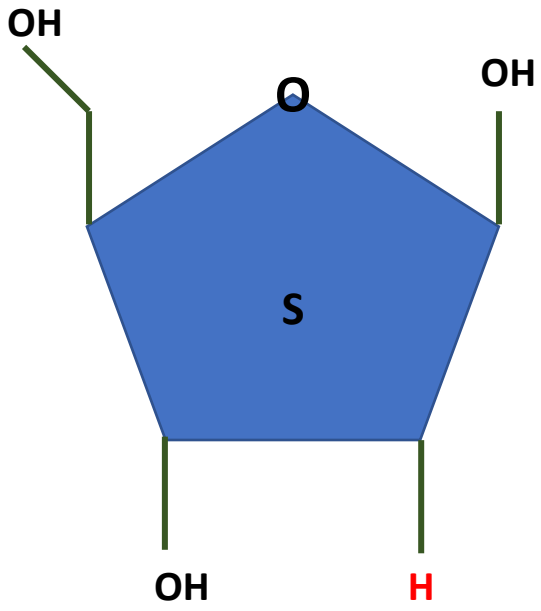


Sugars : Ribose and Desoxyribose



Ribose

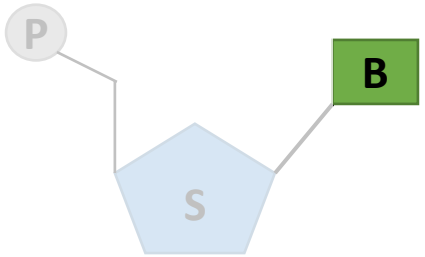
RNA



DesoxyRibose

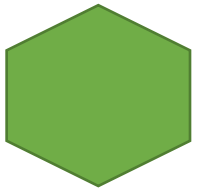
DNA

Reminder on DNA and RNA



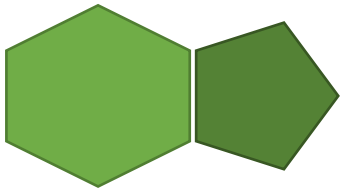
Purine and pyrimidine bases :

Pyrimidine base



Cytosine (C) **Thymine (T)** **Uracile (U)**

Purine base



Adenine (A) **Guanine (G)**

Repartition : ADN : A/**T**, G/C

ARN : A/**U**, G/C

Reminder on DNA and RNA

Nucleic acids = nucleotide polymer

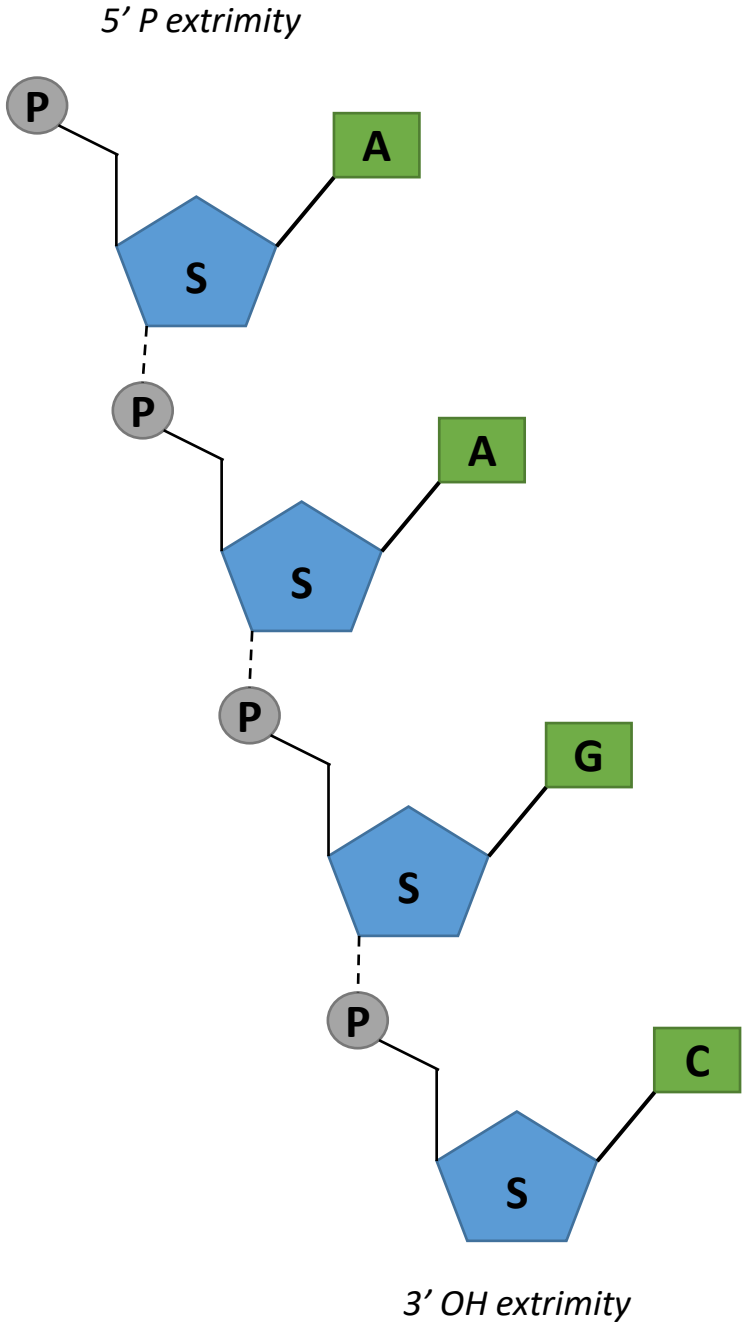
Nucleic acid chains are characterized:

- sugar nature : r*ibose/desoxyribose*

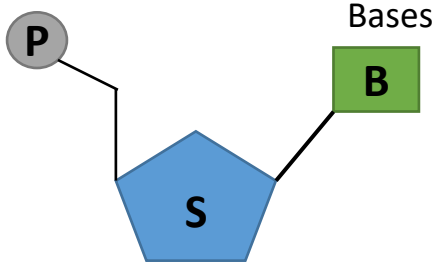
- number, nature and nucleotide sequences

<u>Writting Convention :</u>

From left to right (5’ phosphate to 3’OH)
(5’P) A-A-G-C (3’OH)



DNA structure



Bases : A, T, G, C

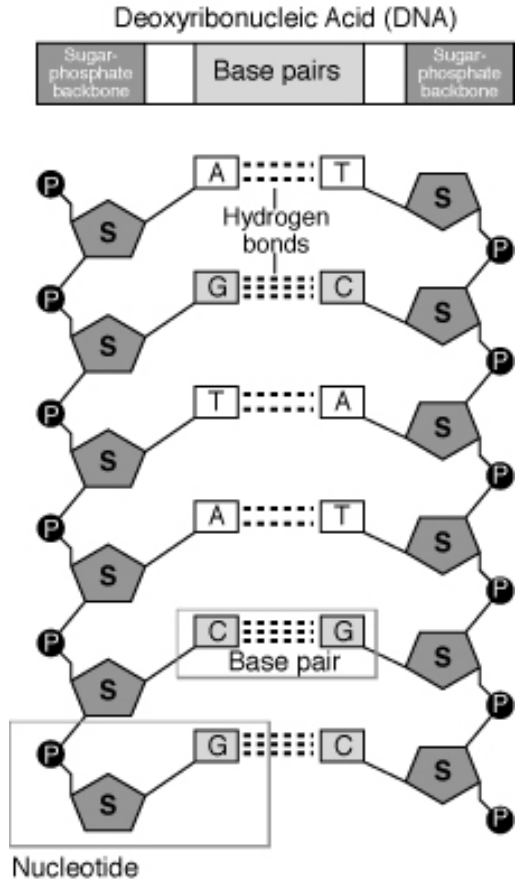
Sucre : Desoxyribose

Nucleotide

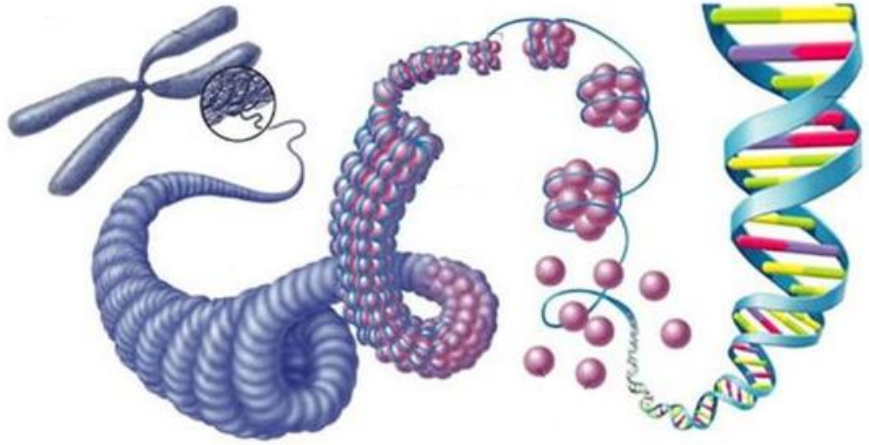
Nucleic chain

Two complementary nucleic chains = **Double Helix**

A <-> T
G <-> C

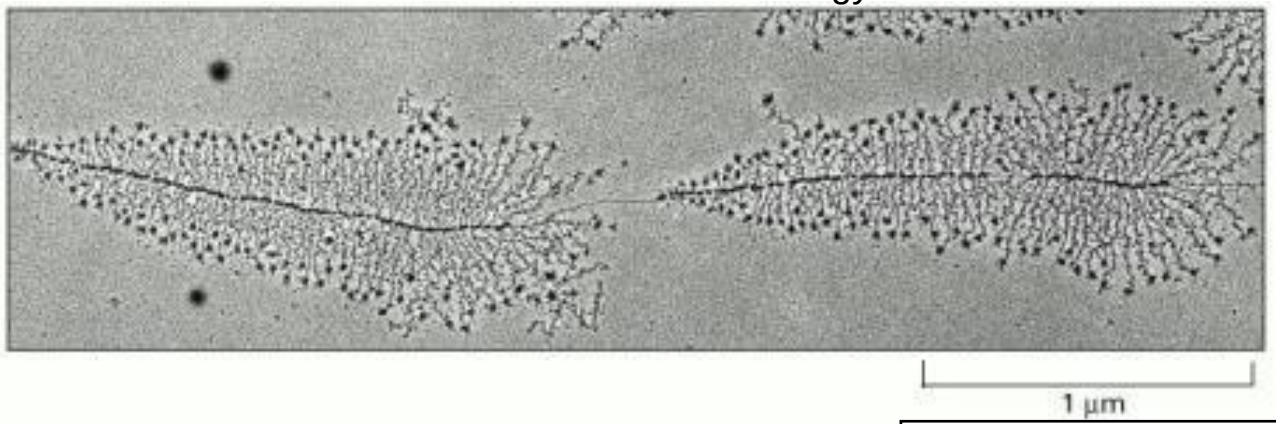


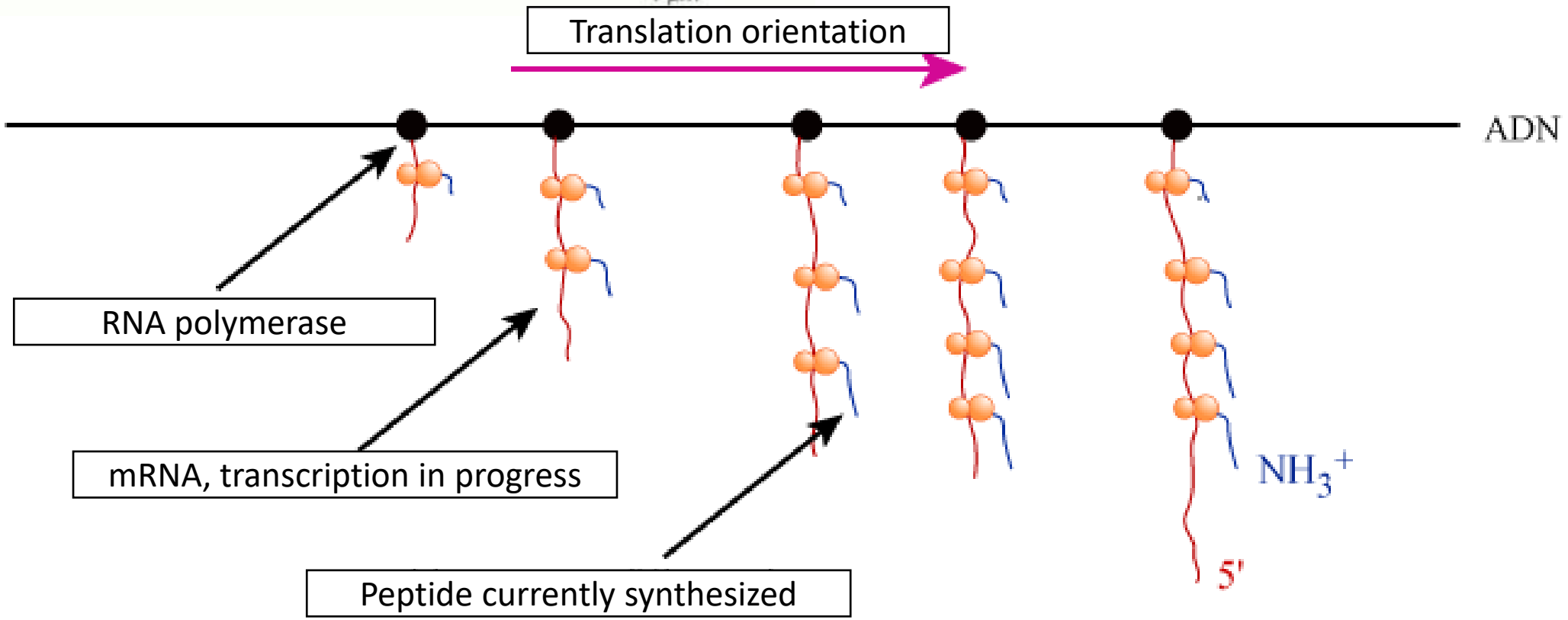
Chromosomes



From DNA to proteins in bacteria

Molecular Biology of the Cell. 4th edition.





Open Reading Frame (ORF)

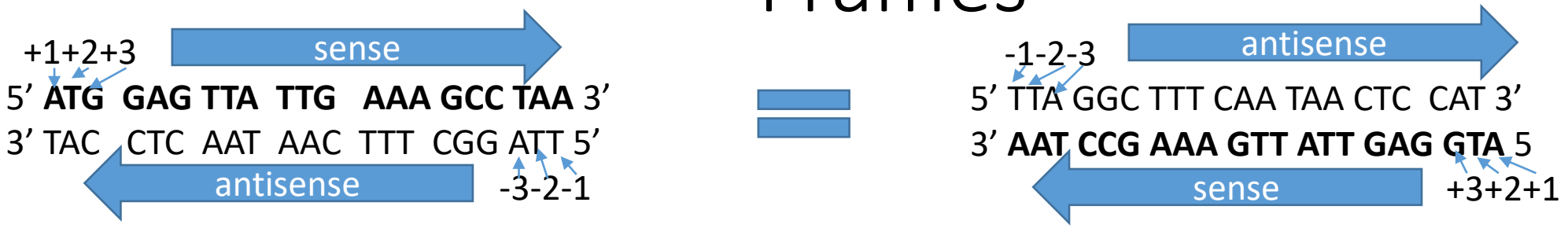
Open reading frame (ORF) allows to encode a protein:
One codon= 3 bases
One codon encodes one amino acid

Example:
DNA: ATG GAG TTA TTG AAA GCC TAA
RNA: AUG GAG UUA UUG AAA GCC UAA
Protein: Met-Glu- Leu- Leu- Lys- Ala-STOP

First nucleotide	Second nucleotide: U		Second nucleotide: C		Second nucleotide: A		Second nucleotide: G		Third nucleotide
U	UUU, UUC	phényl-alanine	UCU, UCC, UCA, UCG	sérine	UAU, UAC	tyrosine	UGU, UGC	cystéine	U C A G
	UUA, UUG	leucine			UAA, UAG	STOP	UGA	STOP	
							UGG	tryptophane	
C	CUU, CUC, CUA, CUG	leucine	CCU, CCC, CCA, CCG	proline	CAU, CAC	histidine	CGU, CGC, CGA, CGG	arginine	U C A G
					CAA, CAG	glutamine			
A	AUU, AUC, AUA	isoleucine	ACU, ACC, ACA, ACG	thréonine	AAU, AAC	asparagine	AGU, AGC	sérine	U C
	AUG	méthionine			AAA, AAG	lysine	AGA, AGG	arginine	A G
G	GUU, GUC, GUA, GUG	valine	GCU, GCC, GCA, GCG	alanine	GAU, GAC	acide aspartique	GGU, GGC, GGA, GGG	glycine	U C A G
					GAA, GAG	acide glutamique			

In bacteria, the usual codons to start are most often **ATG**
and sometimes **TTG**
The **stops codon are : TAA, TGA, TAG**

Frames



Frame +1

DNA
5’ **ATG GAG TTA TTG AAA GCC TAA** 3’
3’ TAC CTC AAT AAC TTT CGG ATT 5’

RNA: AUG GAG UUA UUG AAA GCC UAA

Protein: Met-Glu- Leu- Leu- Lys- Ala-STOP

Frame -1

DNA
5’ TTA GGC TTT CAA TAA CTC CAT 3’
3’ **AAT CCG AAA GTT ATT GAG GTA** 5’

RNA: UUA GGC UUU CAA UAA CUC CAU

Protein: Leu- Gly- Phe- Gln-STOP

Frame +2

DNA
5’ **TGG AGT TAT TGA AAG CCT AA** 3’
3’ ACC TCA ATA ACT TTC GGA TT 5’

RNA: UGG AGU UAU UGA AAG CCU AA

Protein: Trp- Arg- Tyr- STOP

Frame -2

DNA
5’ TAG GCT TTC AAT AAC TCC AT 3’
3’ **ATC CGA AAG TTA TTG AGG TA** 5’

RNA: UAG GCU UUC AAU AAC UCC AU

Protein: STOP

Frame +3

DNA
5’ **GGA GTT ATT GAA AGC CTA A** 3’
3’ CCT CAA TAA CTT TCG GAT T 5’

RNA: GGA GUU AUU GAA AGC CUA A

Protein: Gly- Val- Ile- Glu- Ser- Leu- …

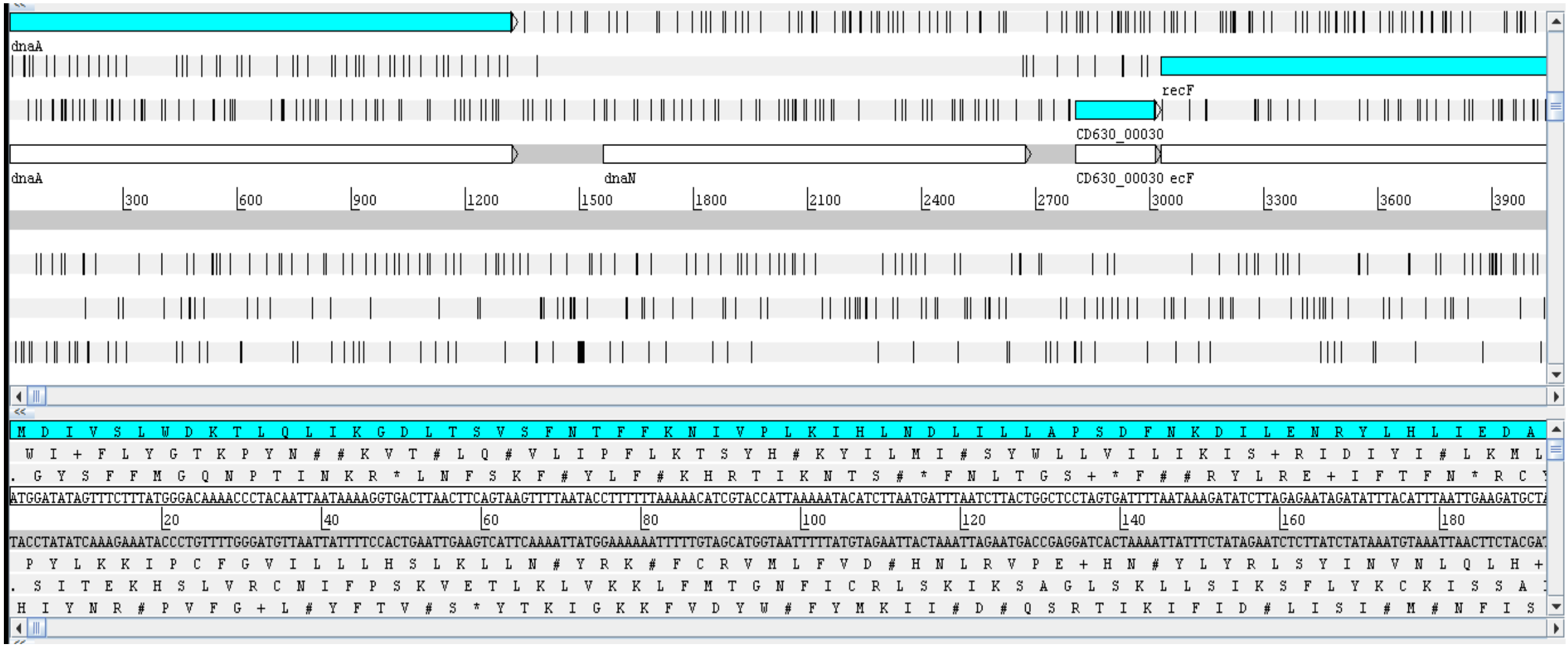
Frame -3

DNA
5’ AGG CTT TCA ATA ACT CCA T 3’
3’ **TCC GAA AGT TAT TGA GGT A** 5’

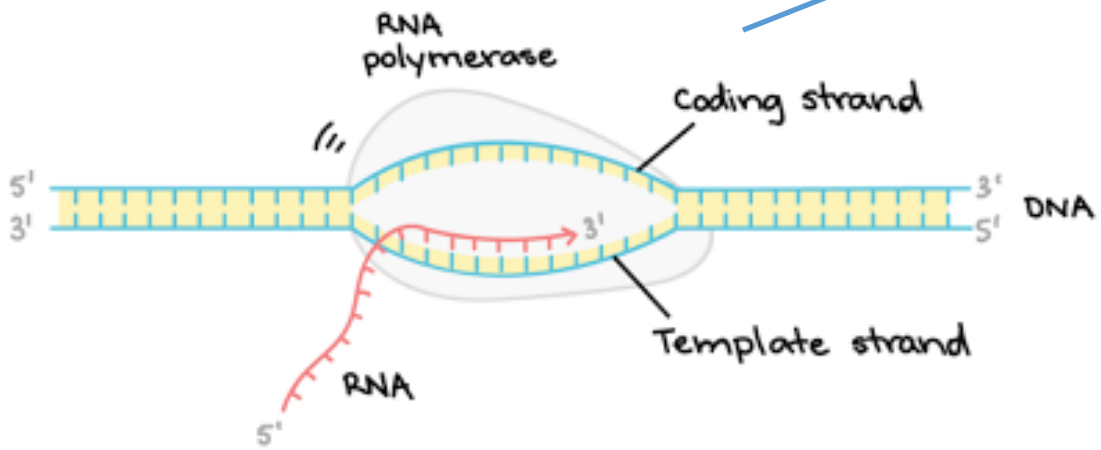
RNA: AGG CUU UGA AUA ACU CCA U

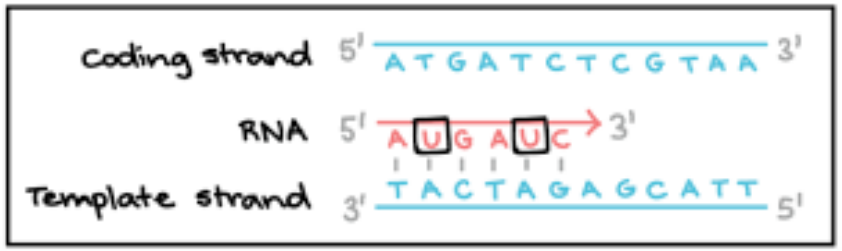
Protein: Arg- Leu- STOP

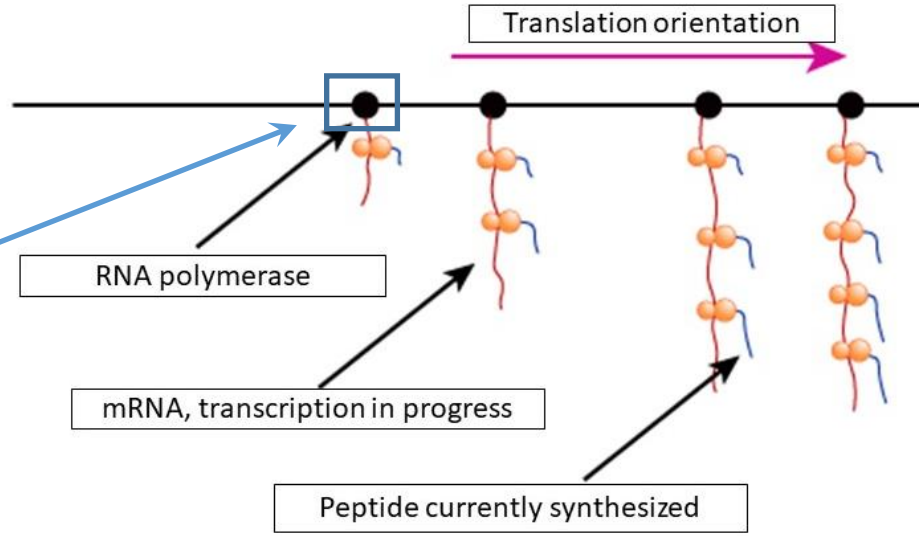
DNA, what is an ORF



Transcription







Transcription

Initiation

Elongation

Termination

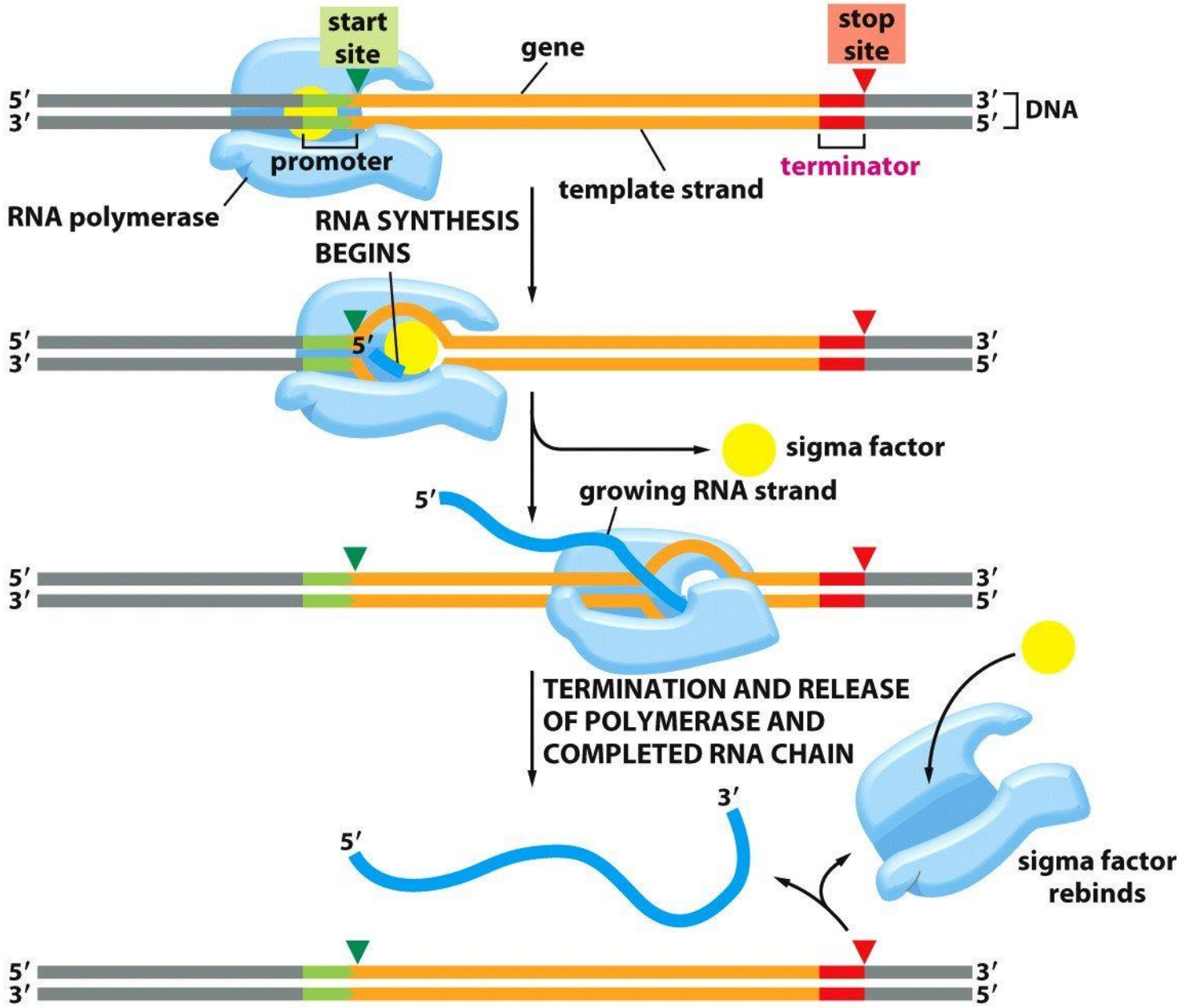
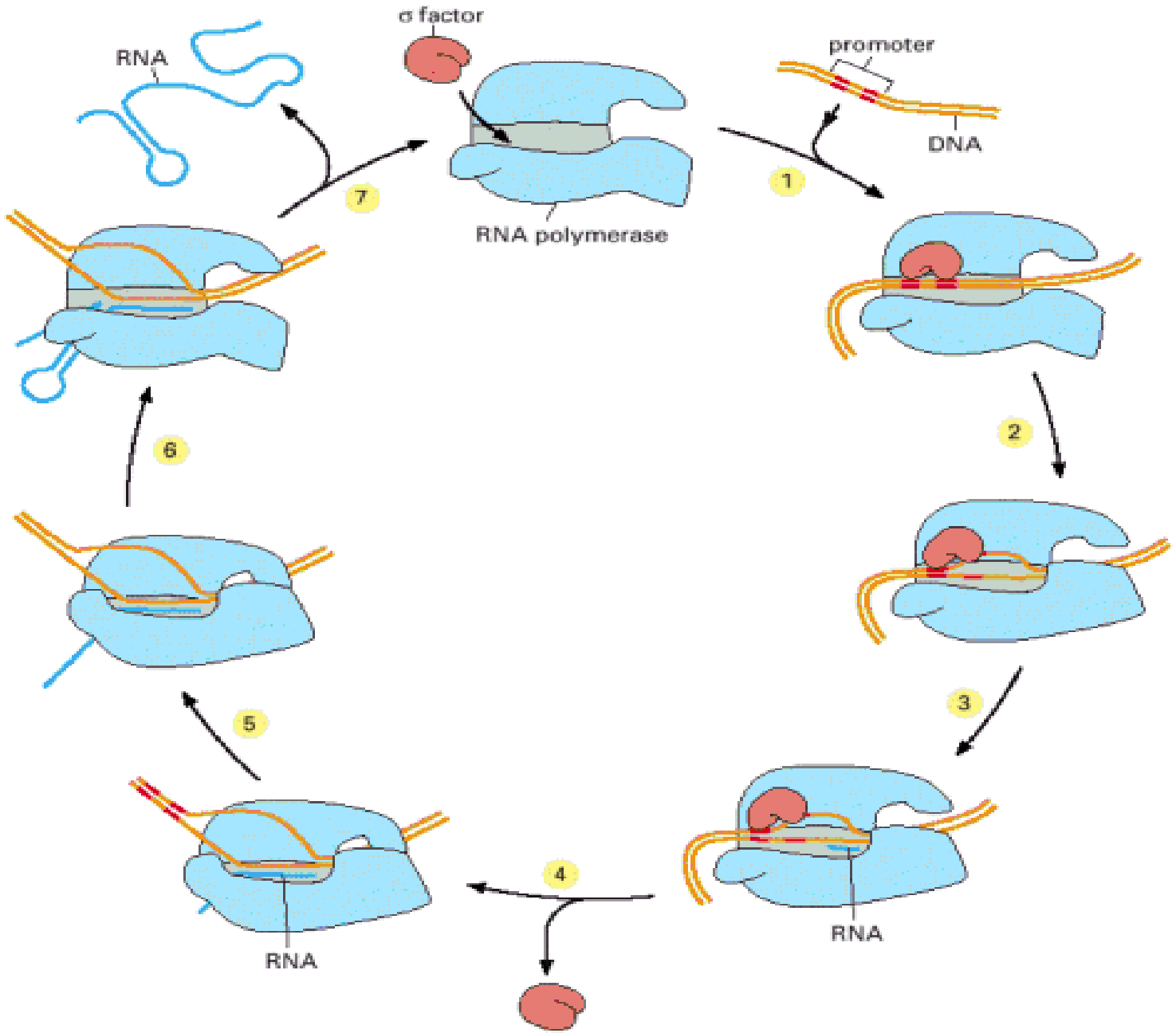
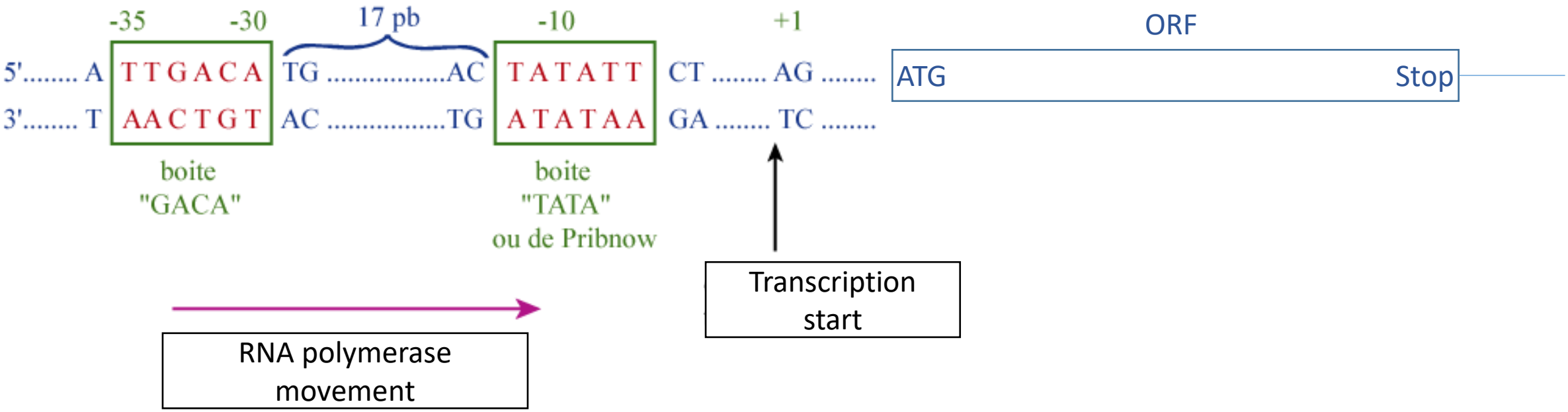


Figure 7-9 Essential Cell Biology 3/e (© Garland Science 2010)

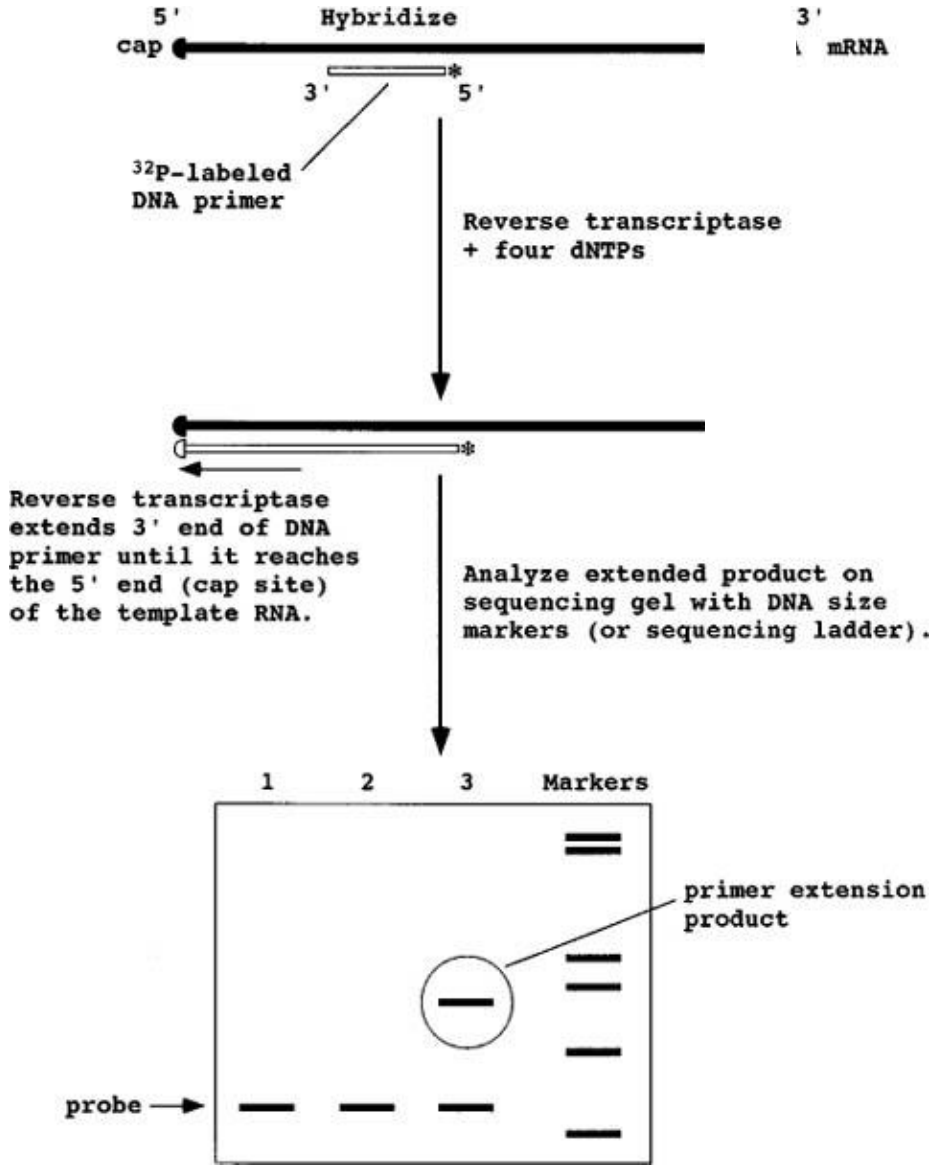
Sigma factors



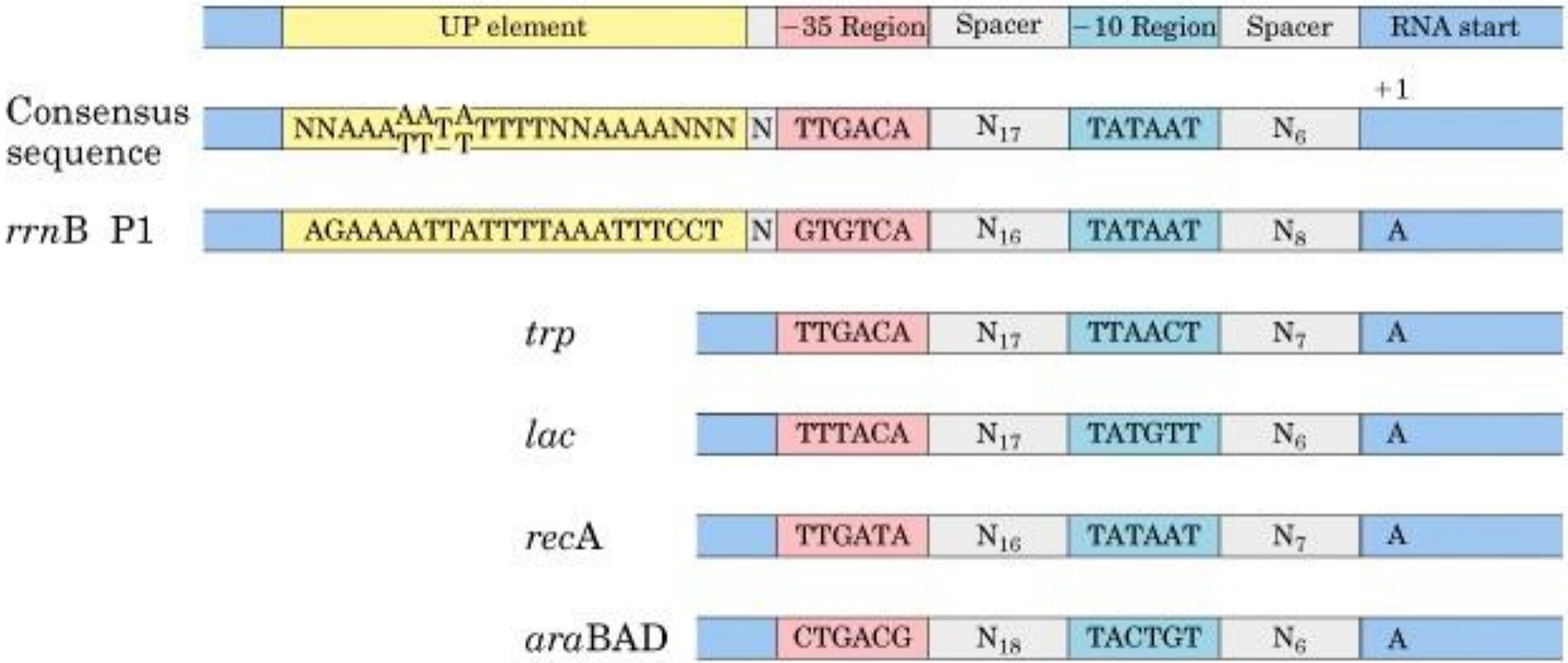
Promoter


-35
-30
17 pb
-10
+1
ORF
5'..... A T T G A C A TG .....AC T A T A T T CT ..... AG .....
3'..... T A A C T G T AC .....TG A T A T A A GA ..... TC .....
ATG
Stop
boite "GACA"
boite "TATA" ou de Pribnow
Transcription start
RNA polymerase movement

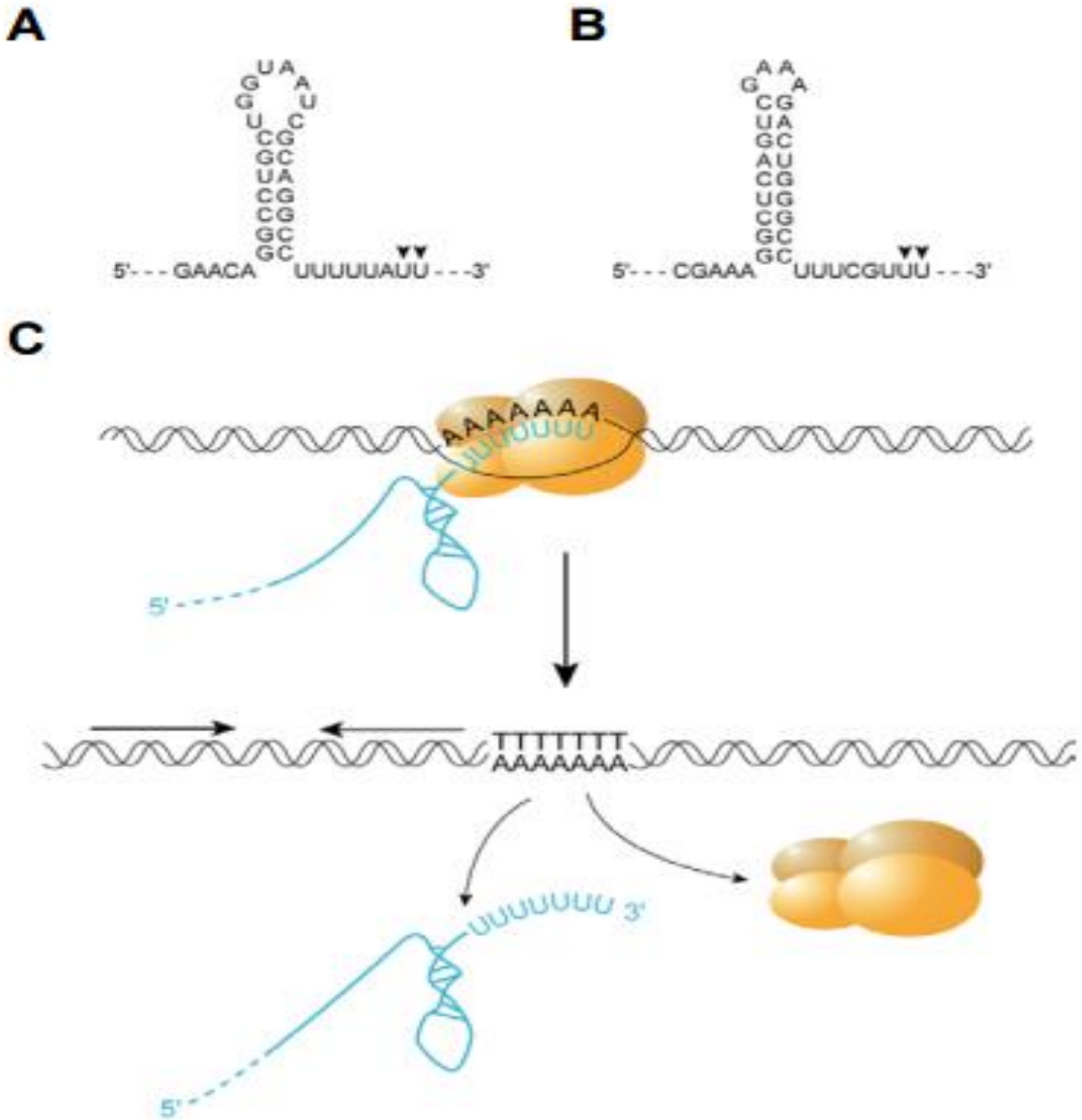
Amorce Extension to look for the “+1” of transcription



Promoters


UP element
−35 Region
Spacer
−10 Region
Spacer
RNA start
+1
Consensus sequence
NNAAA(AA/TT)(A/T)TTTTNNAAAANNN N TTGACA N17 TATAAT N6
rrnB P1
AGAAAATTATTTTAAATTTCCT N GTGTCA N16 TATAAT N8 A
trp
TTGACA N17 TTAACT N7 A
lac
TTTACA N17 TATGTT N6 A
recA
TTGATA N16 TATAAT N7 A
araBAD
CTGACG N18 TACTGT N6 A

Transcription termination

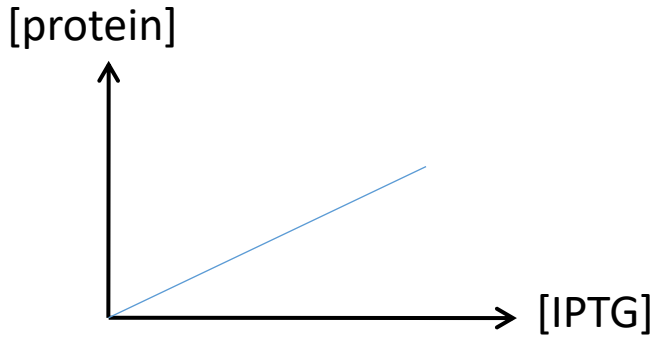


Gene expression : promoter types

<u>Inducible Promoters</u> :

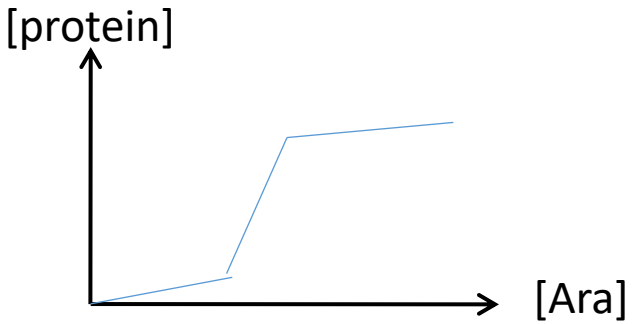
* IPTG or

Xylose (used in *Bacillus*) :



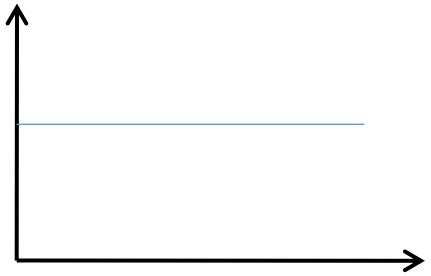
Linear induction

* Arabinose



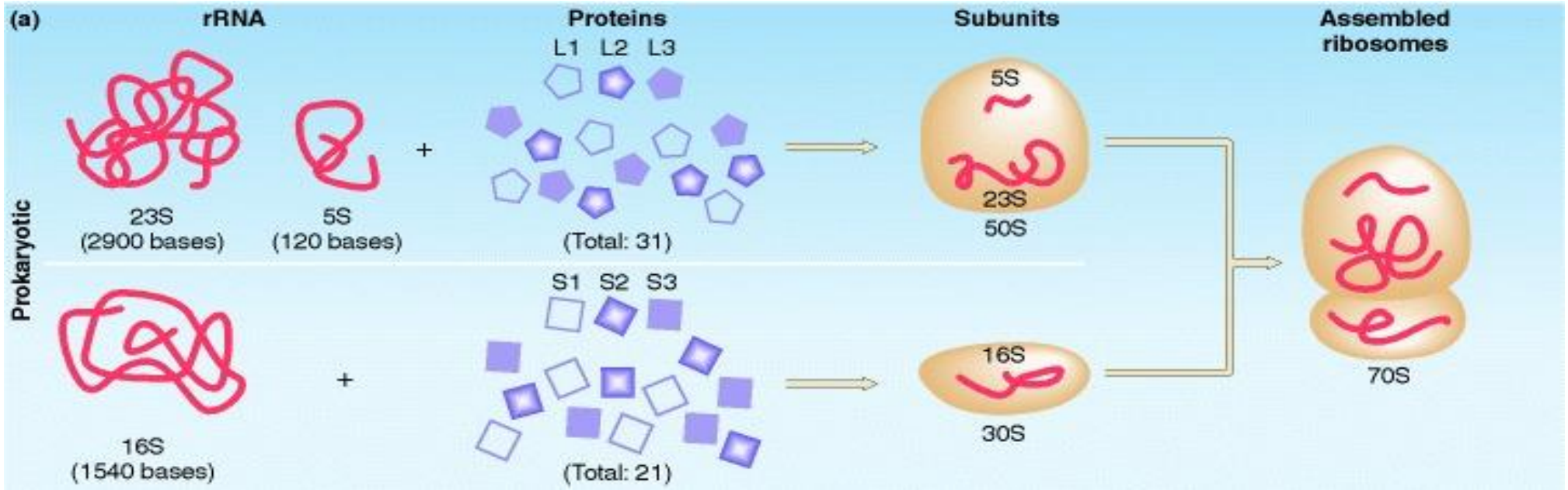
« ON/OFF » induction

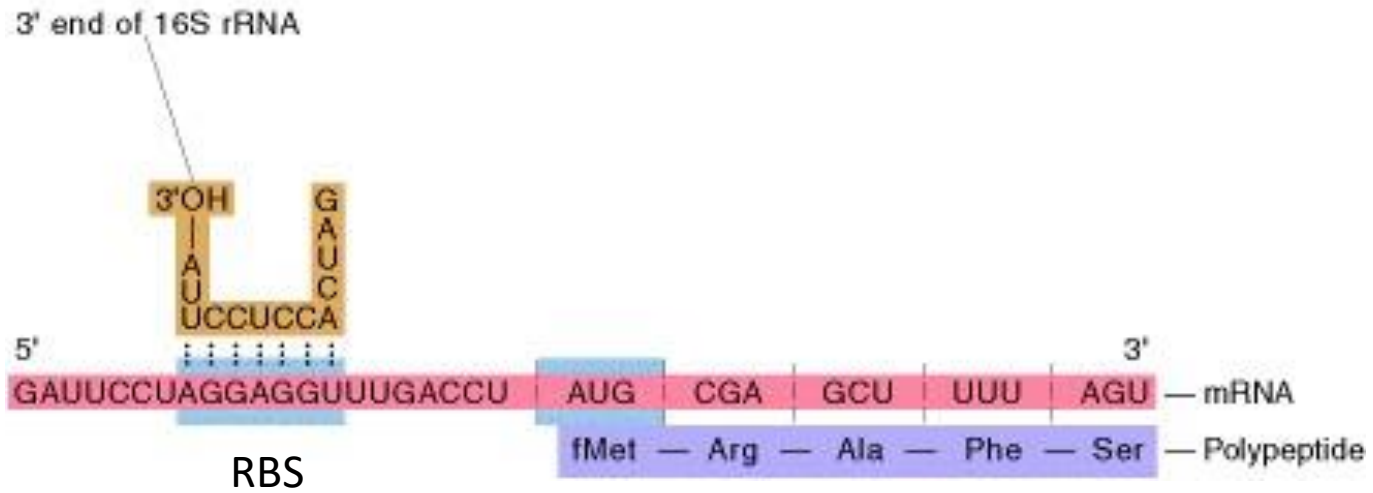
<u>Constitutive Promoters</u>



Translation

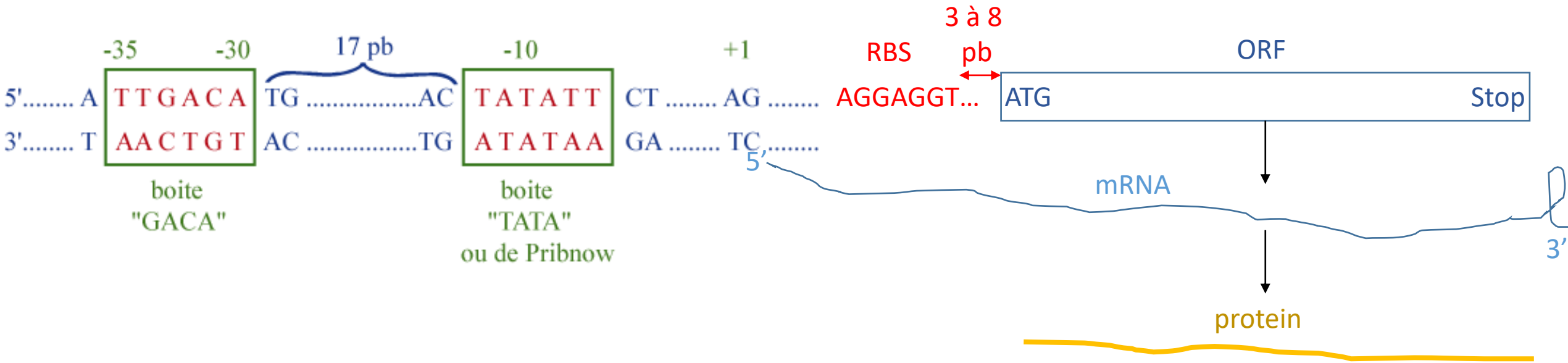
An Introduction to Genetic Analysis. 7th edition.



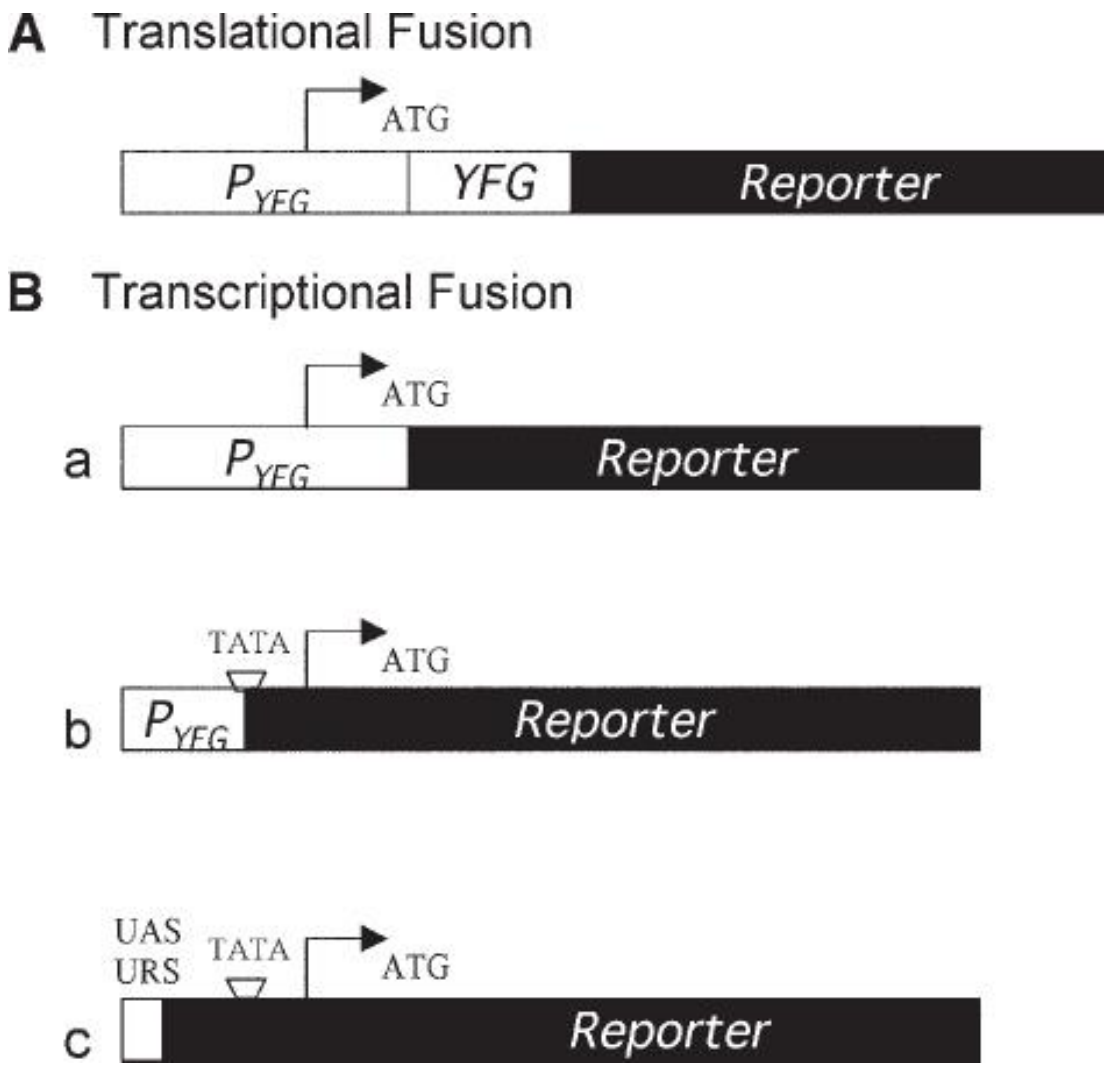


RBS: Ribosome Binding site
Also caled Shine Dalgarno

Necessary sequence to express one gene:

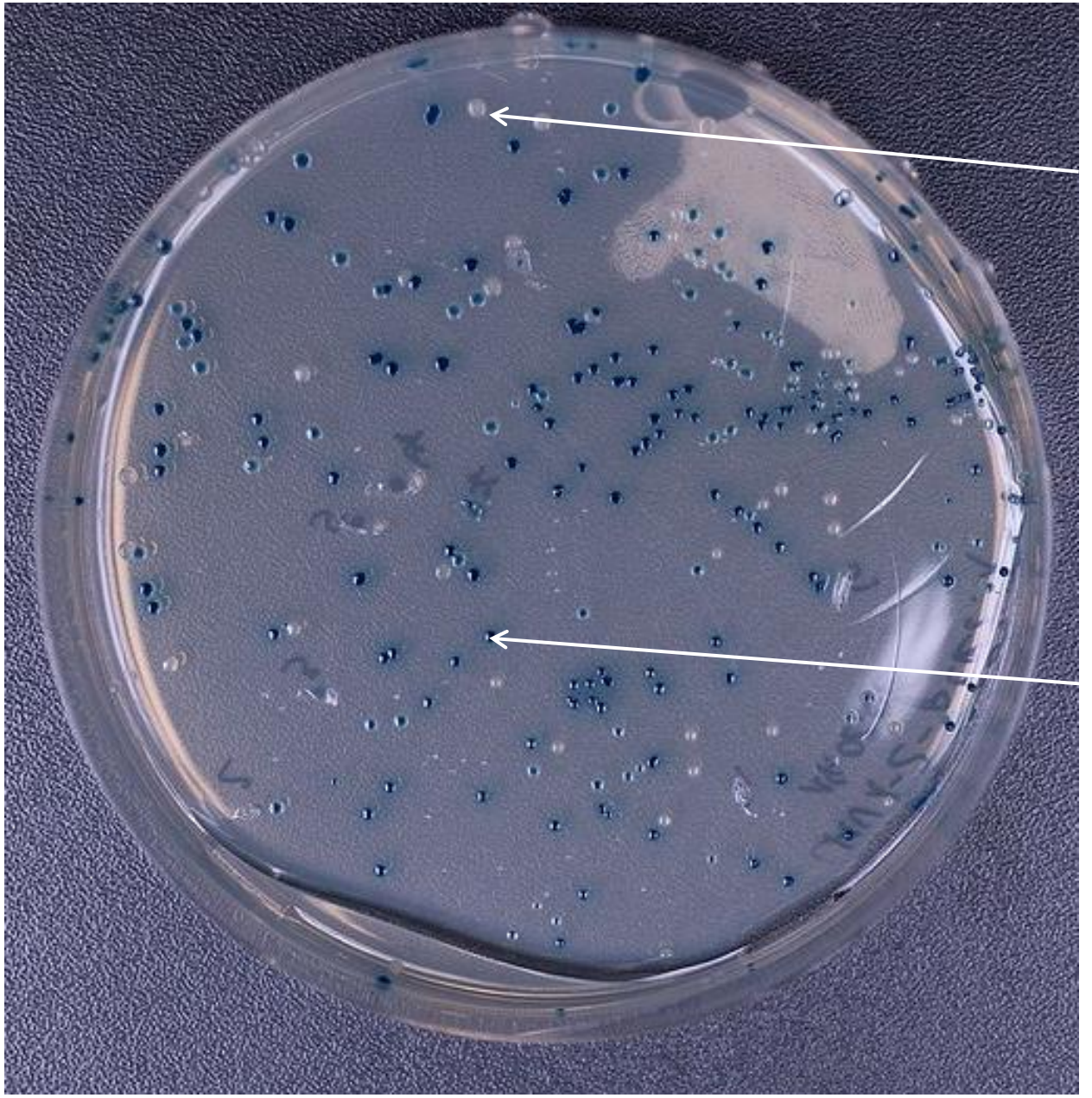


Use of a reporter

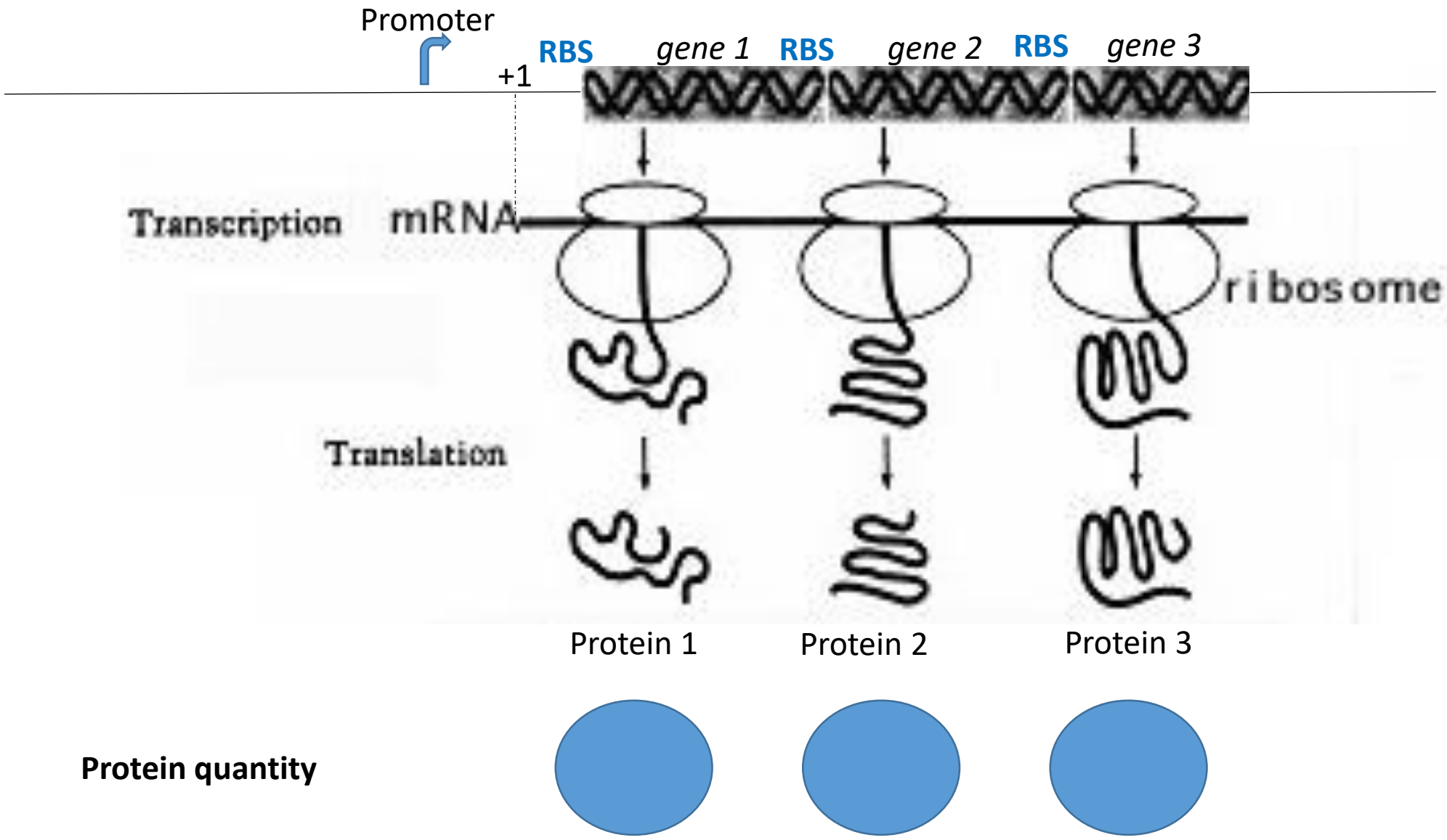


Example : LacZ reporter

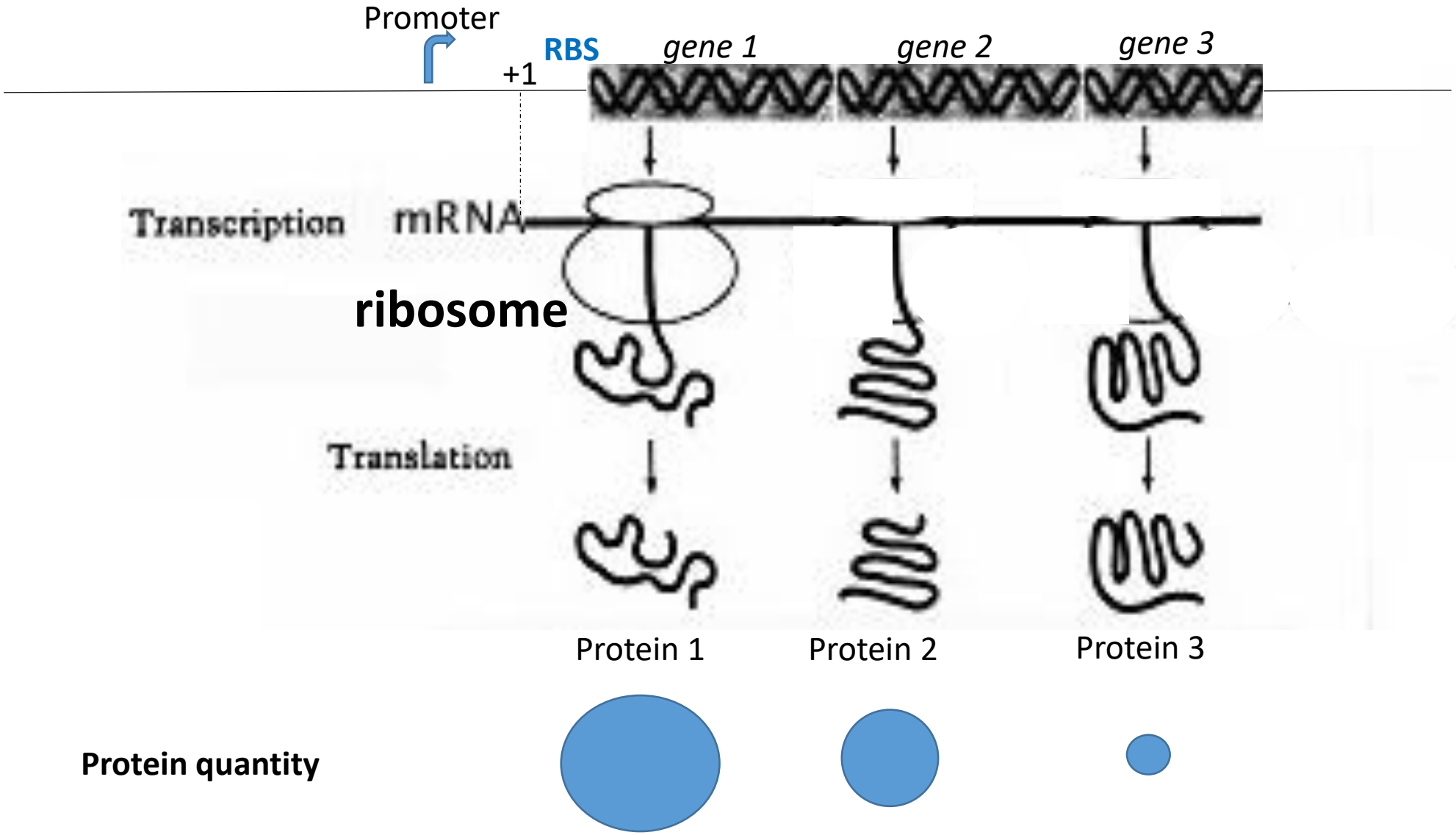
Rich medium containing Xgal



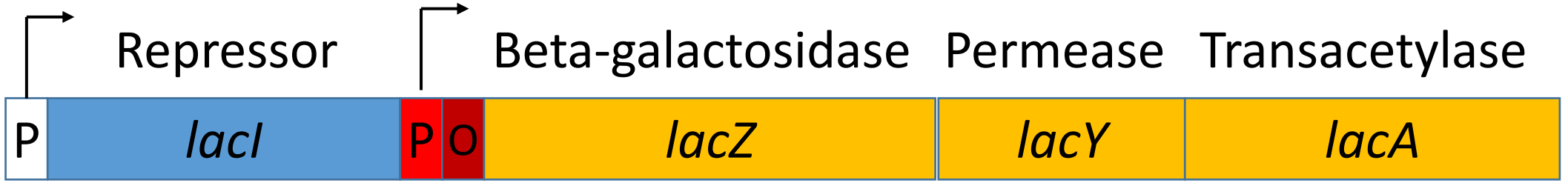
Operon


Promoter
+1
RBS
gene 1
RBS
gene 2
RBS
gene 3
Transcription
mRNA
ribosome
Translation
Protein 1
Protein 2
Protein 3
Protein quantity

Operon, one RBS

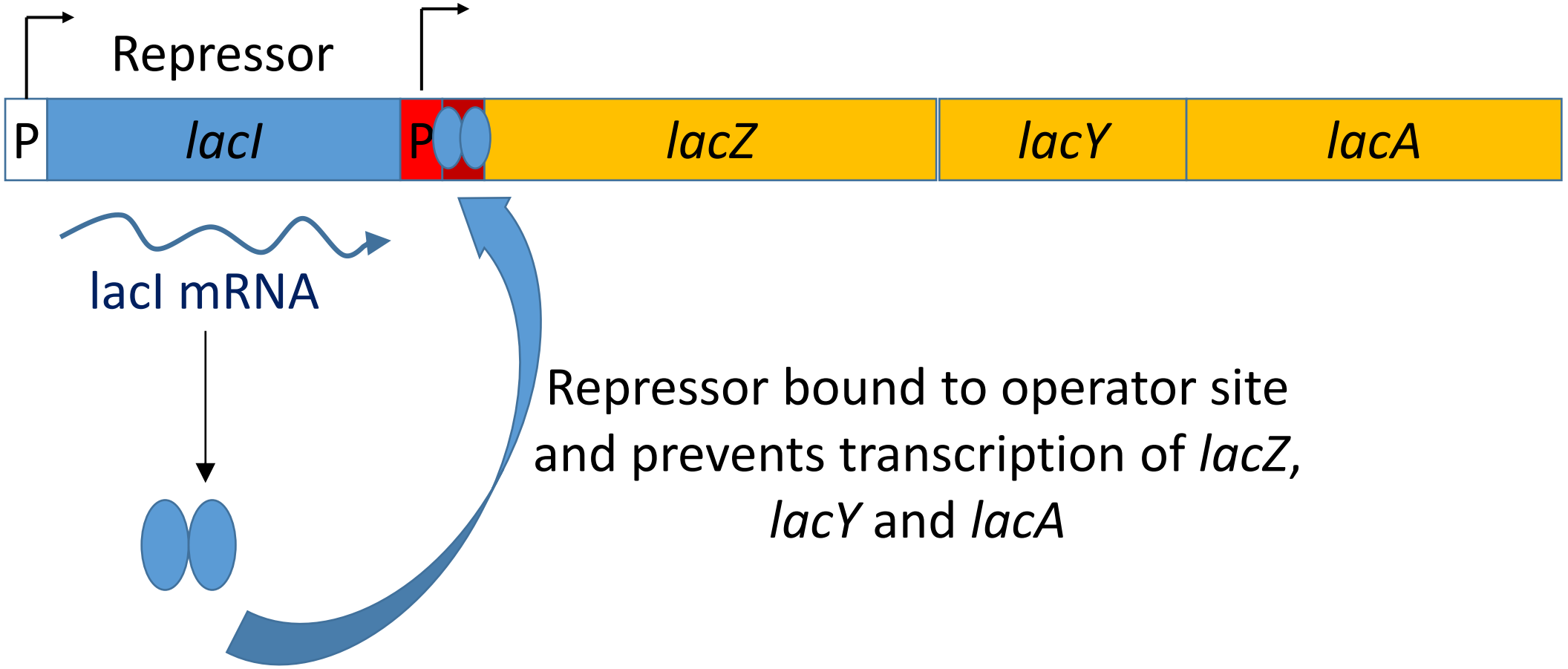


Regulations at the transcription level: example of thelactose operon



Lactose operon:

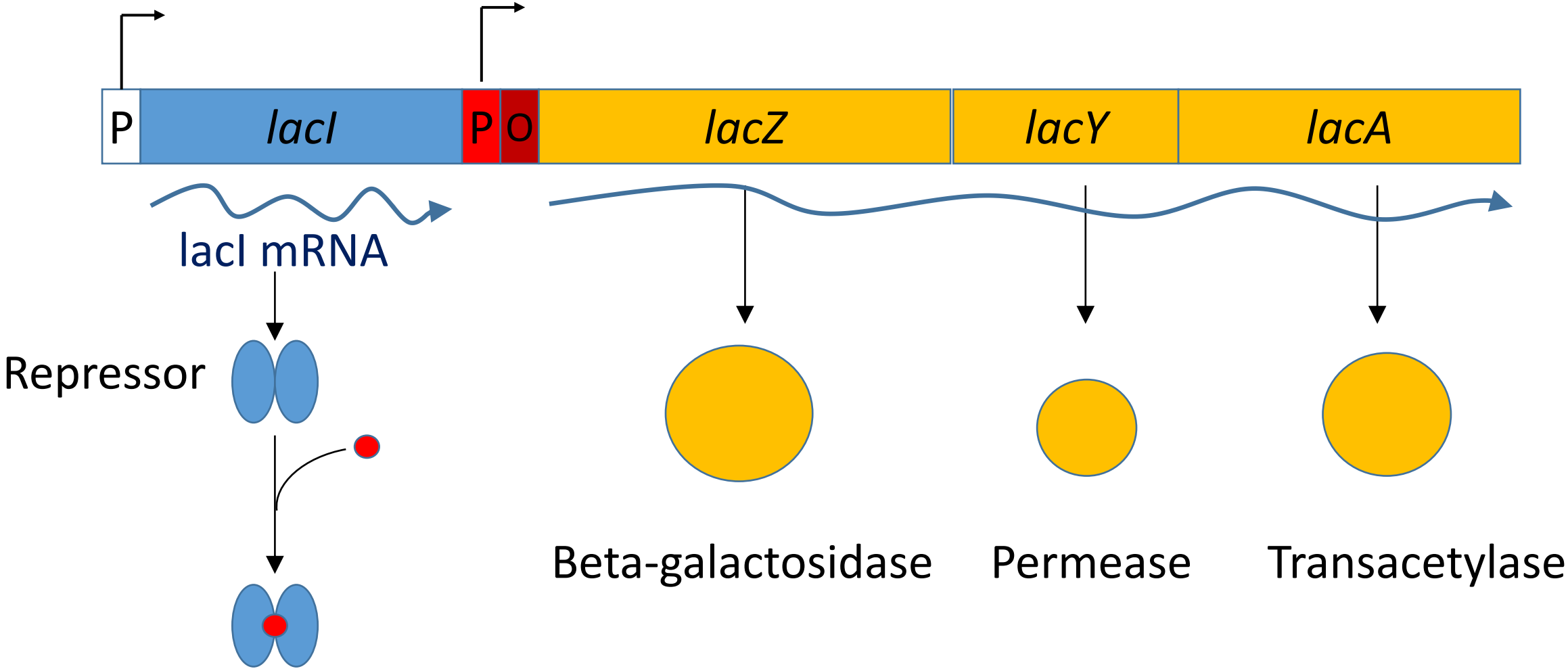
Lactose absence



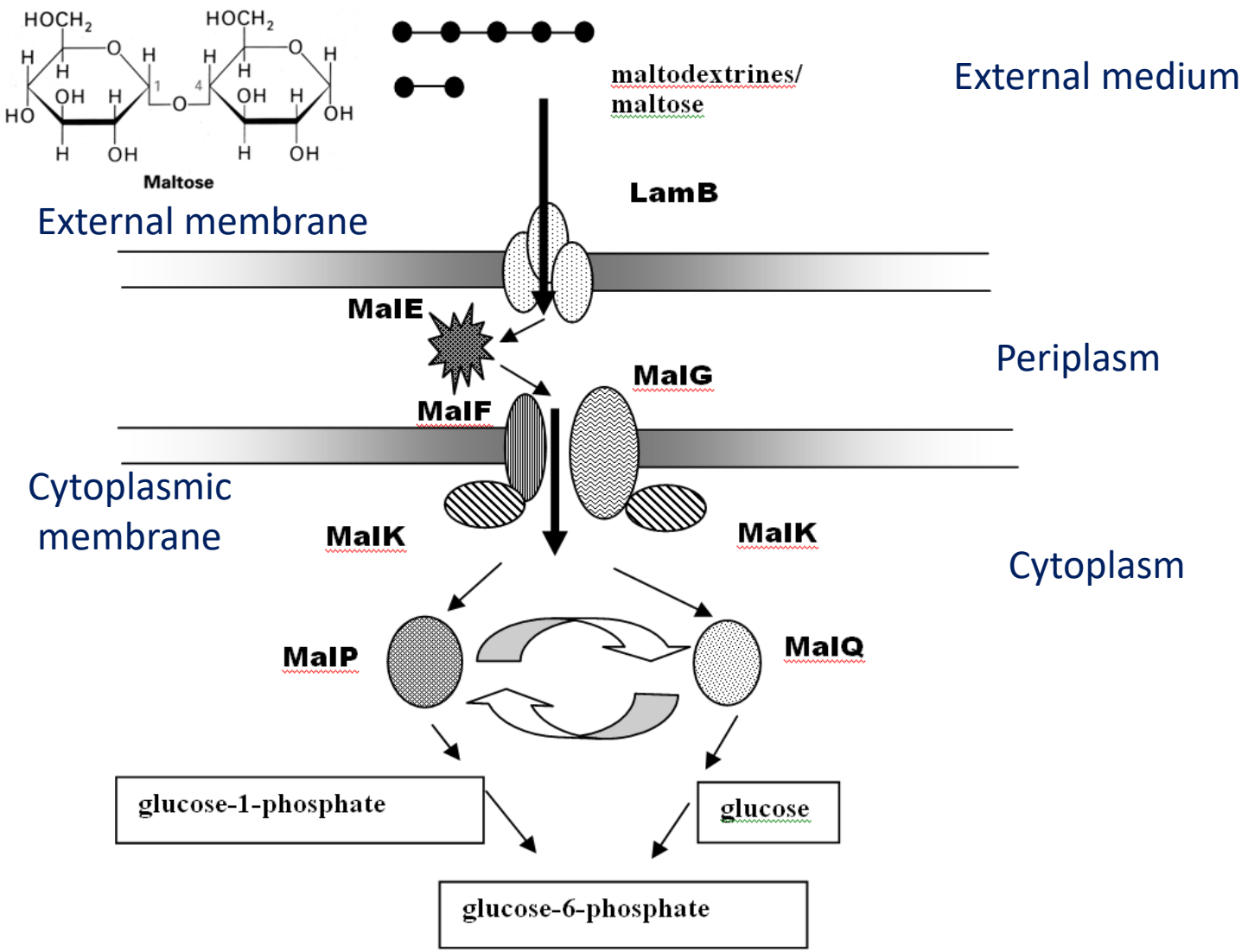
Repressor bound to operator site and prevents transcription of *lacZ*, *lacY* and *lacA*

Lactose operon:

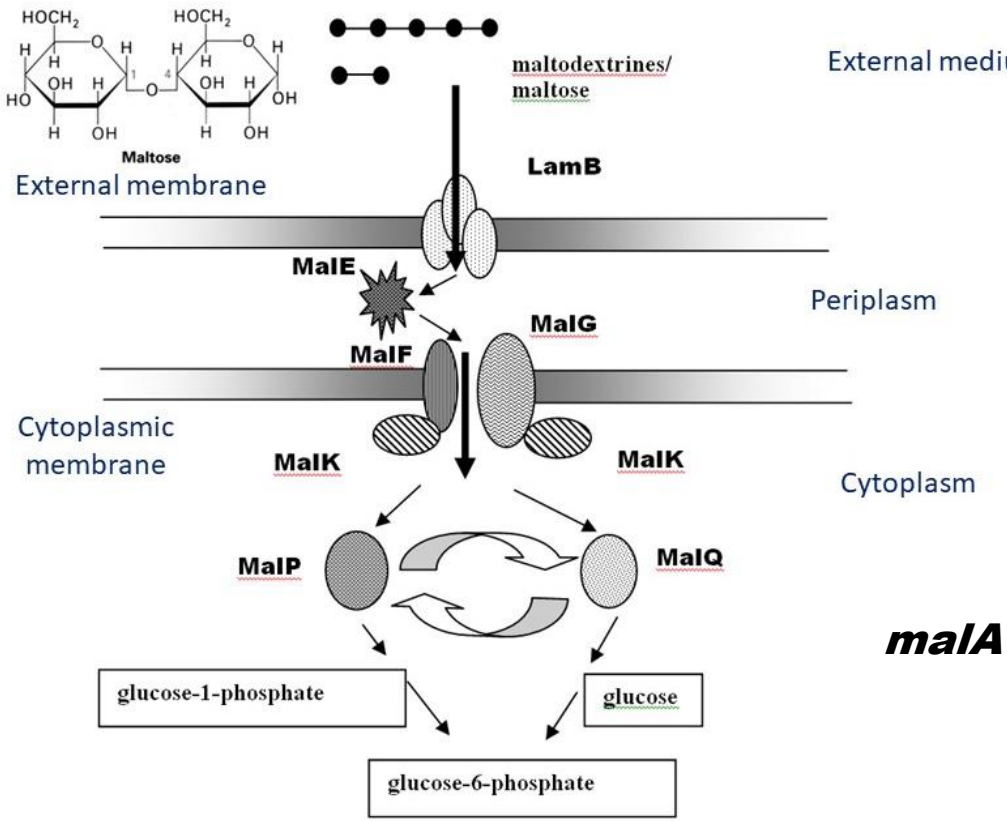
Lactose presence

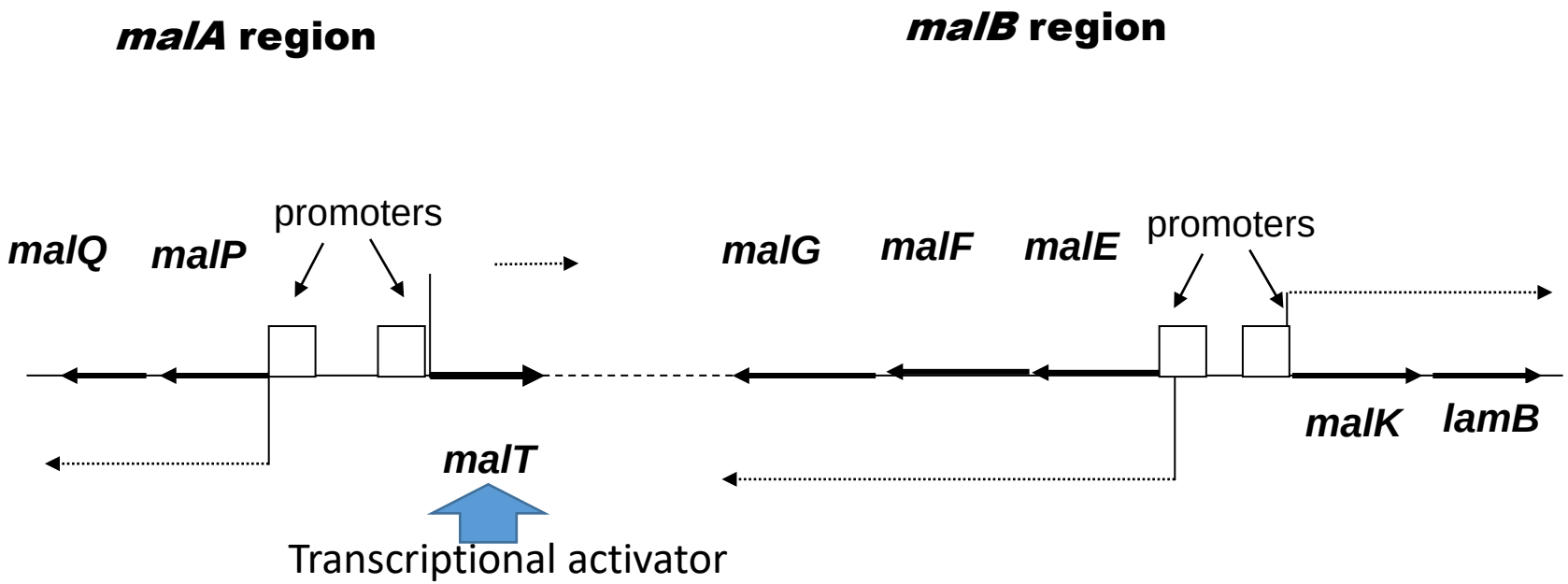


Regulon: example of maltose

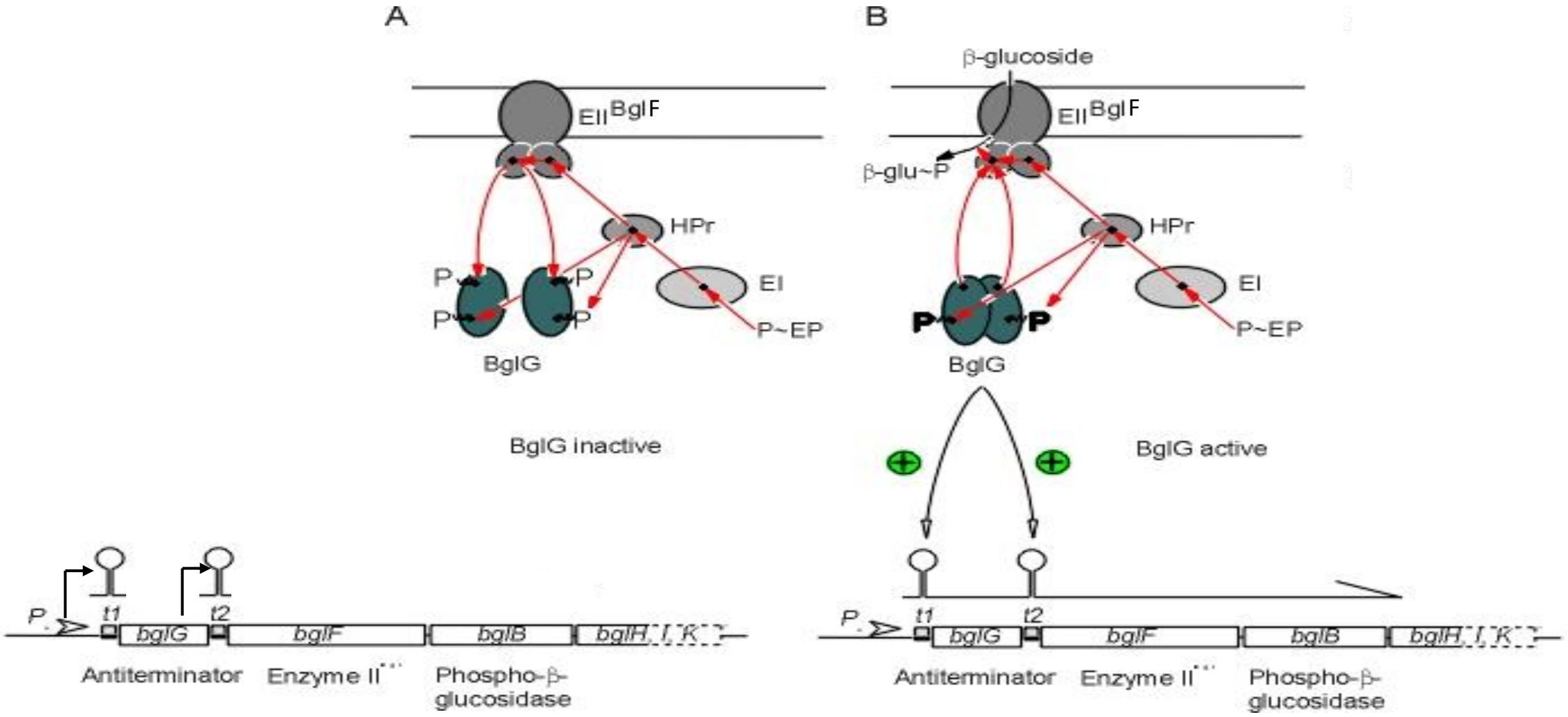


Regulon: example of maltose

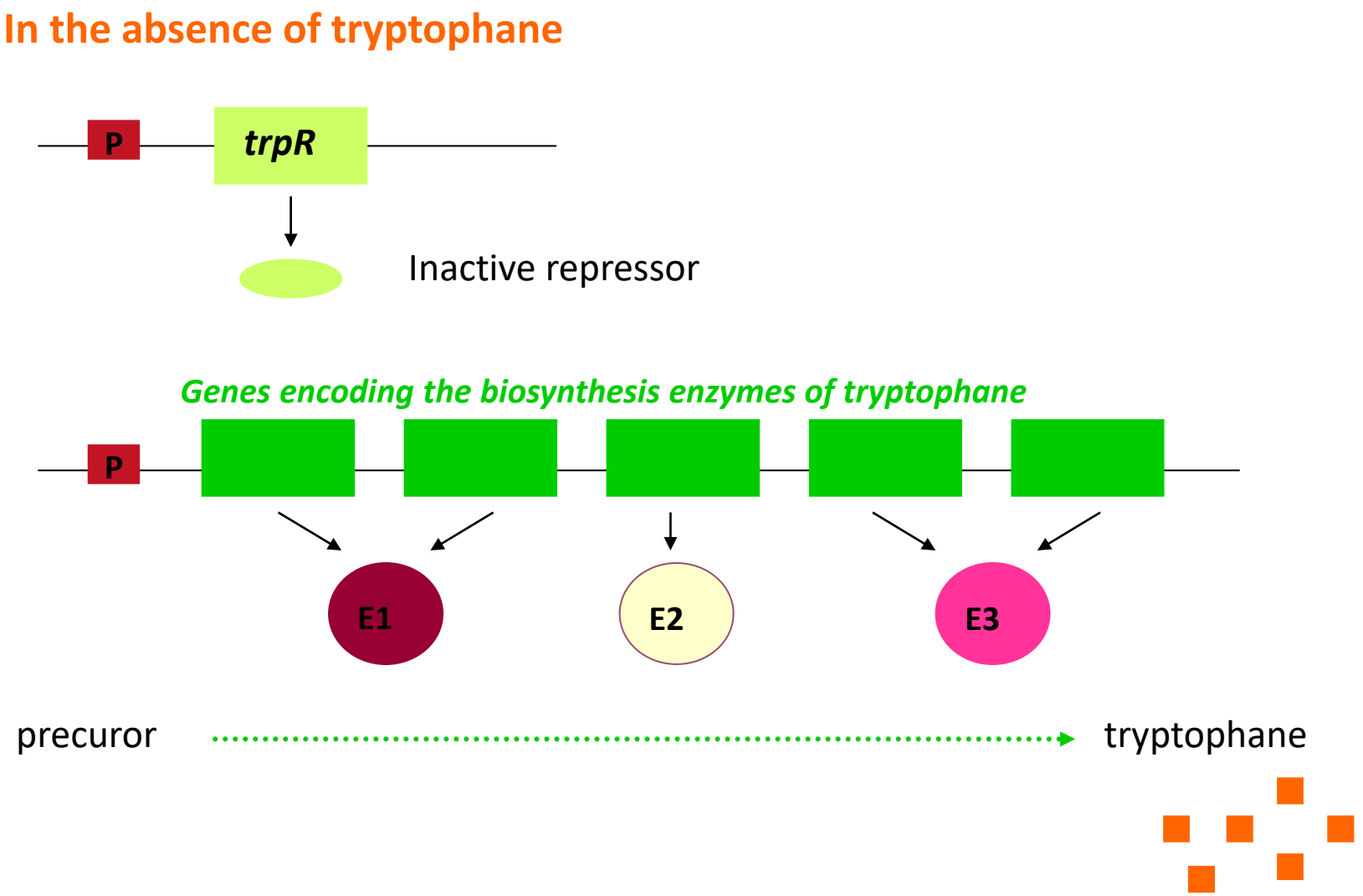




Antitermination regulation : example of the *bgl operon*

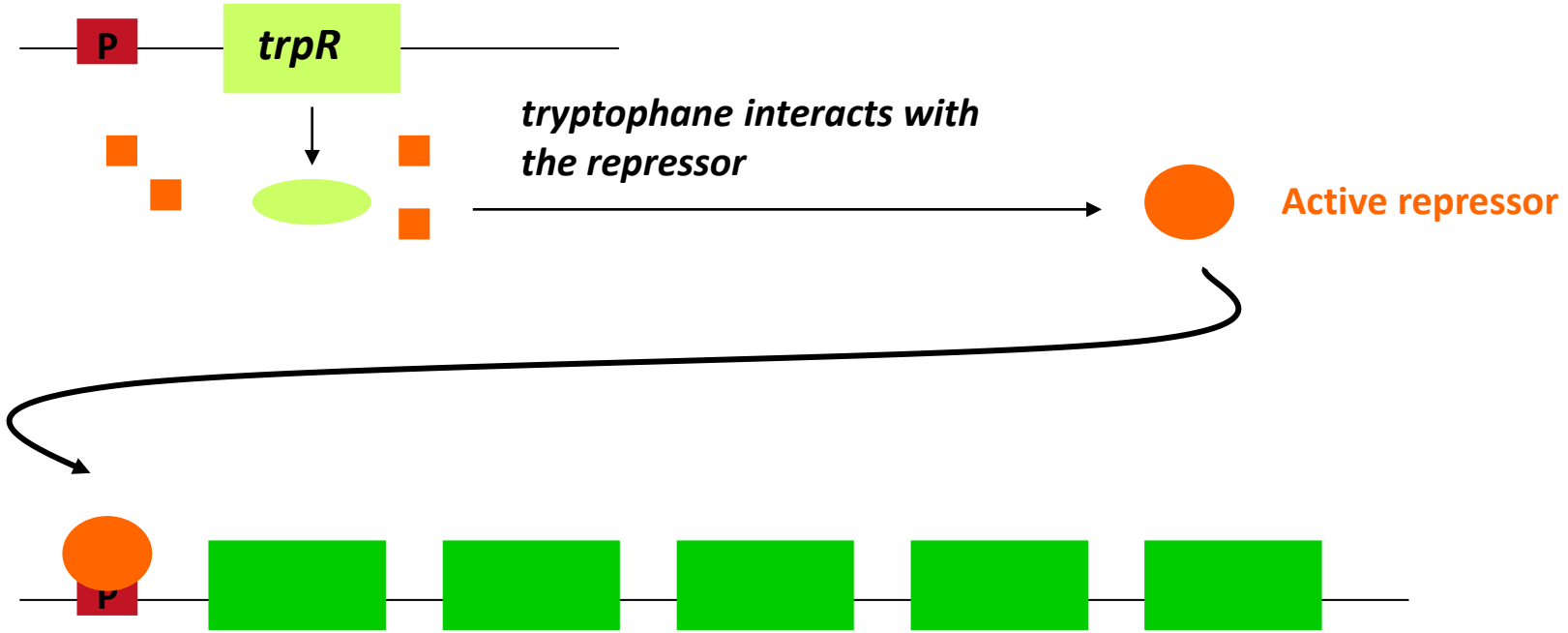


Transcriptional Attenuation: example of the tryptophane operon



Transcriptional Attenuation: example of the tryptophane operon

In the presence of tryptophane : regulation by negative control (repression)



Promoter is repressed No synthesis of the tryptophane biosynthesis enzymes

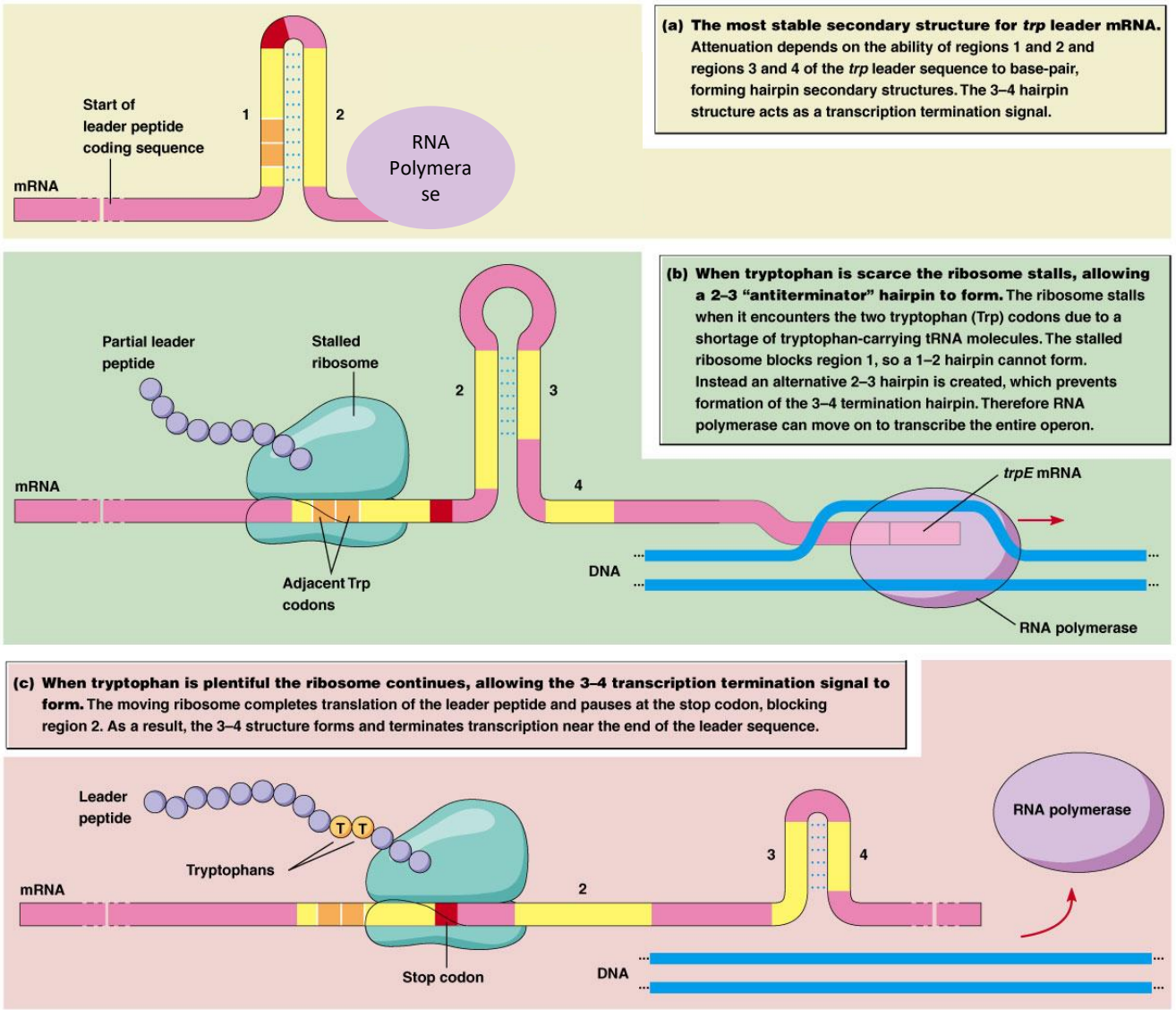
→ No tryptophane synthesis

Transcriptional Attenuation: example of the tryptophane operon

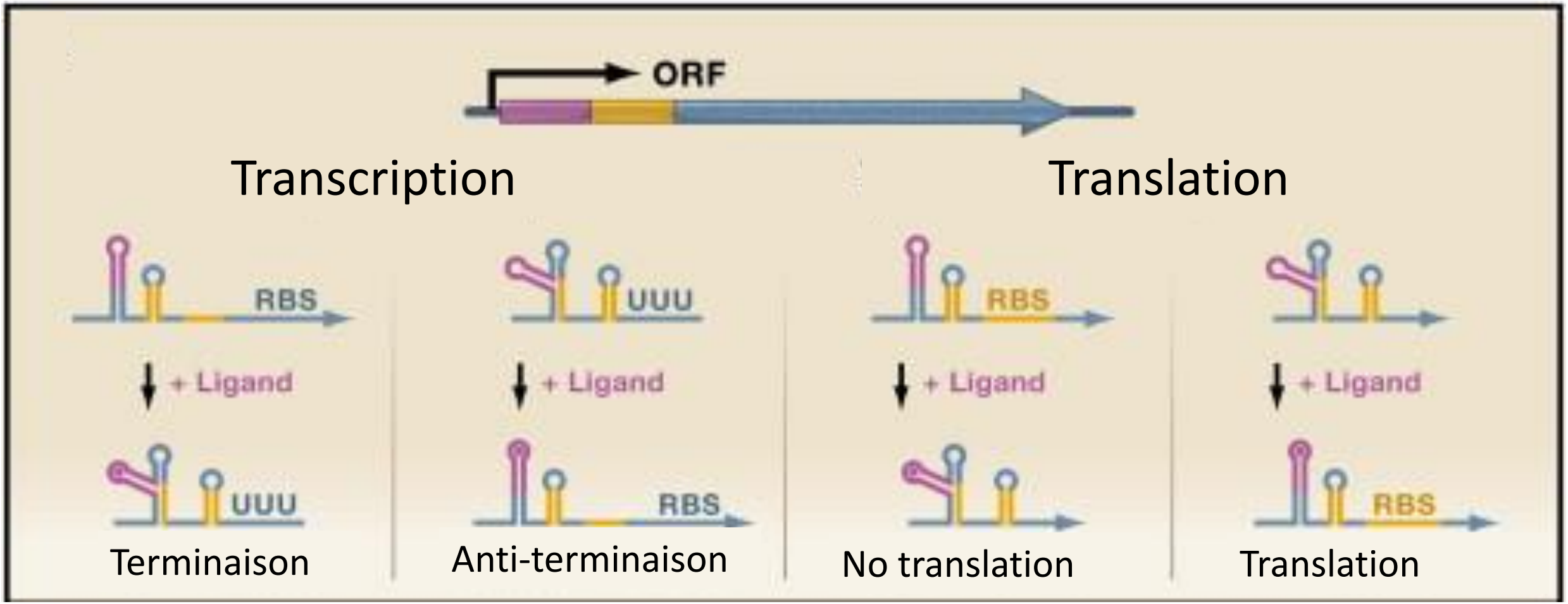
Tryptophane absence

Tryptophane absence

Tryptophane presence

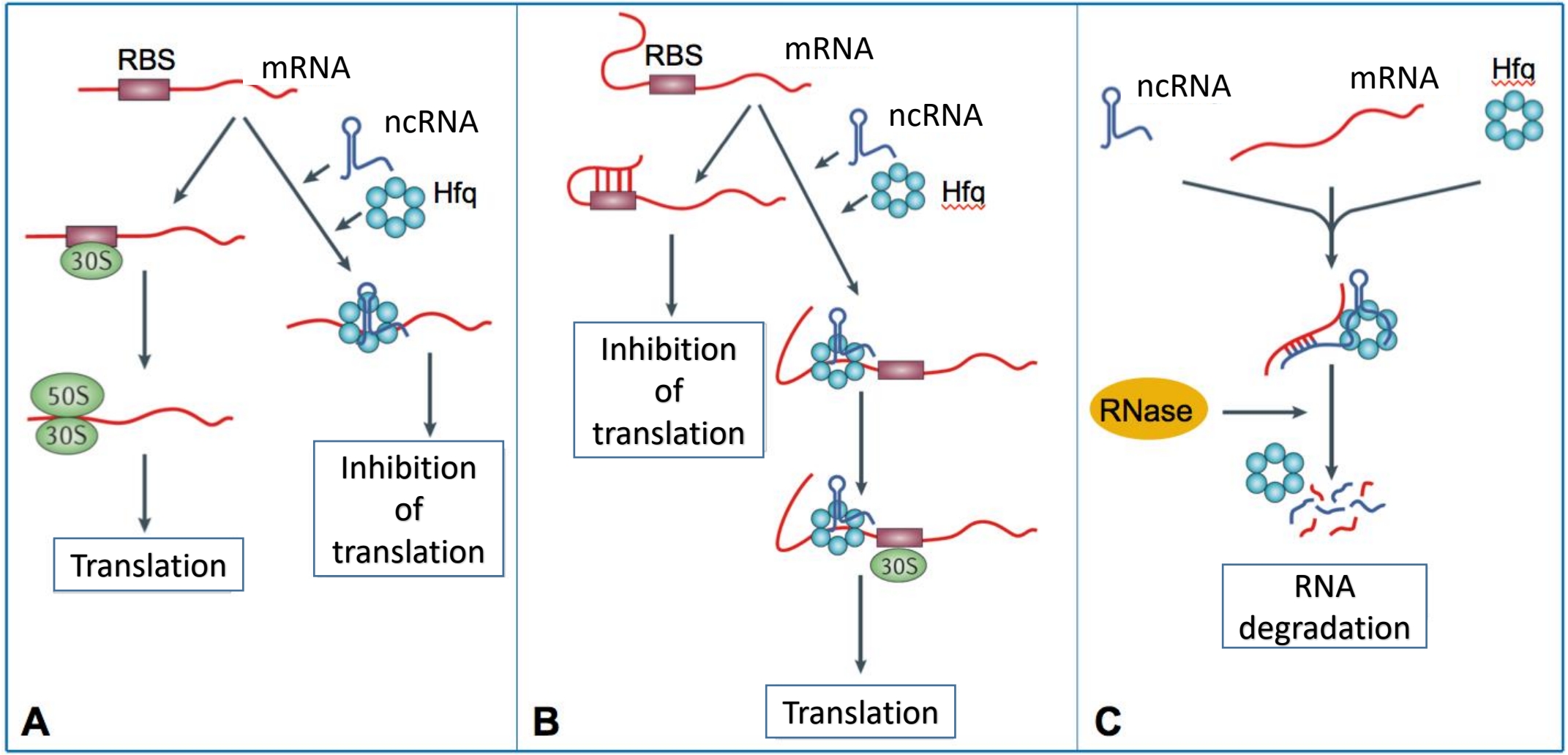


Riboswitch regulations



Cell Volume 136, Issue 4, 20 February 2009, Pages 615–628

Regulations trough a pleiotropic factor: example of Hfq



mRNA Messenger RNA
ncRNA Non coding RNA

From Vogel et Luisi, 2011

Small RNAs

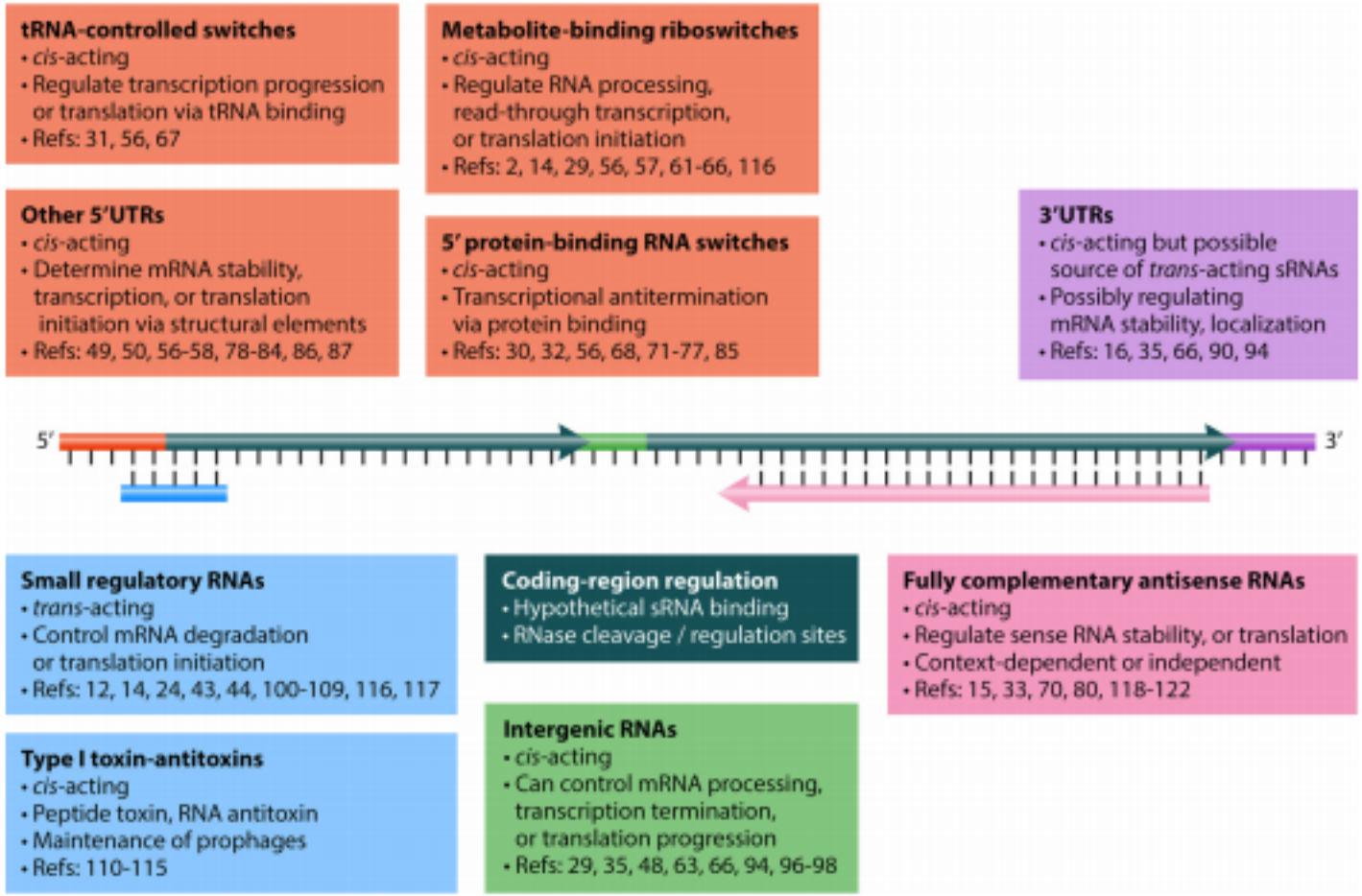
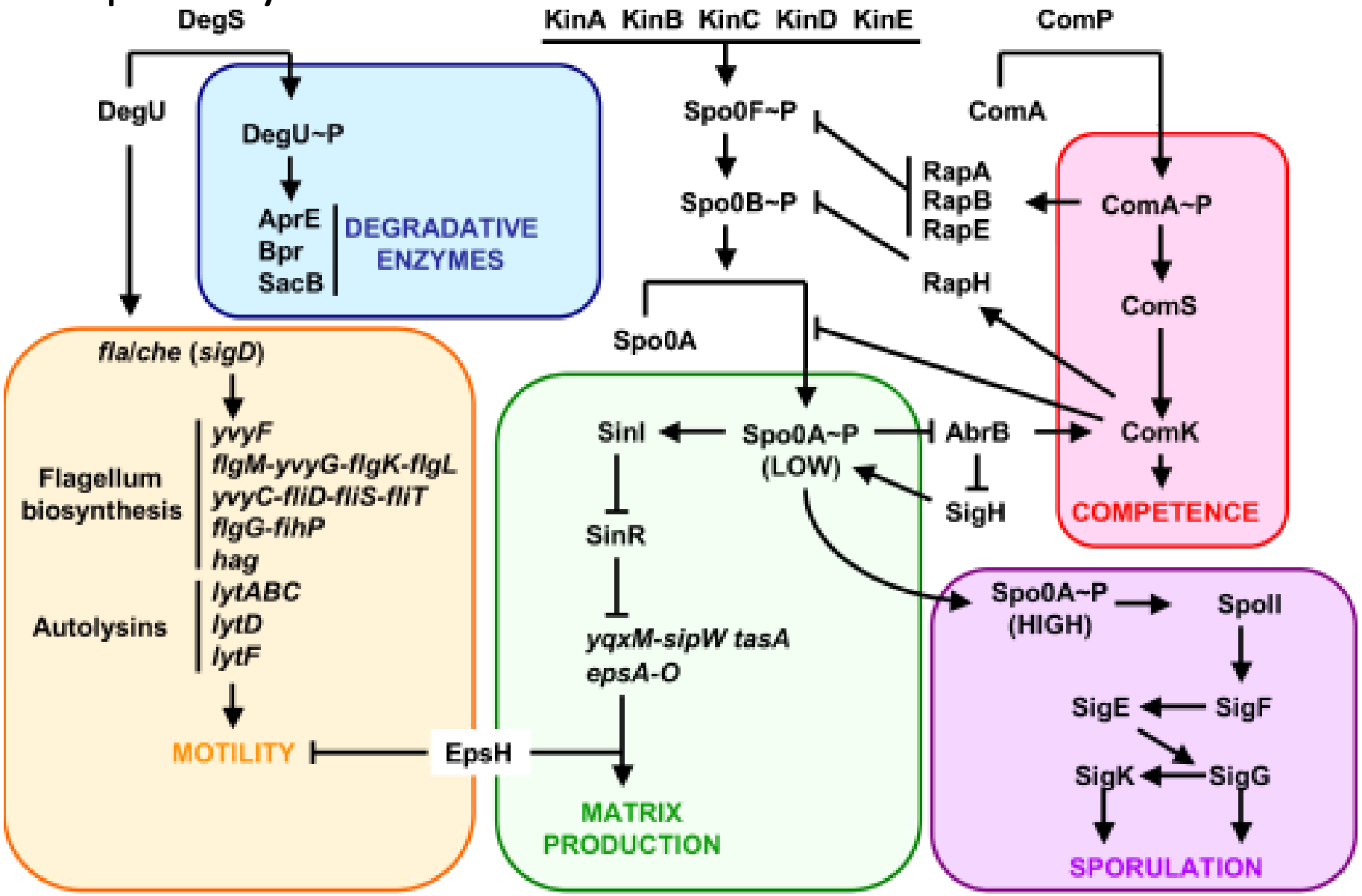


FIG 1 Graphic summary of possible regulatory RNA functions.

Ruben A. T. Mars, MMBR, oct 2016

Post-translational regulations: example of phosphorylation

Generation of multiple cell types in *Bacillus subtilis* Daniel Lopez, Hera Vlamakis & Roberto Kolter
Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA, USA

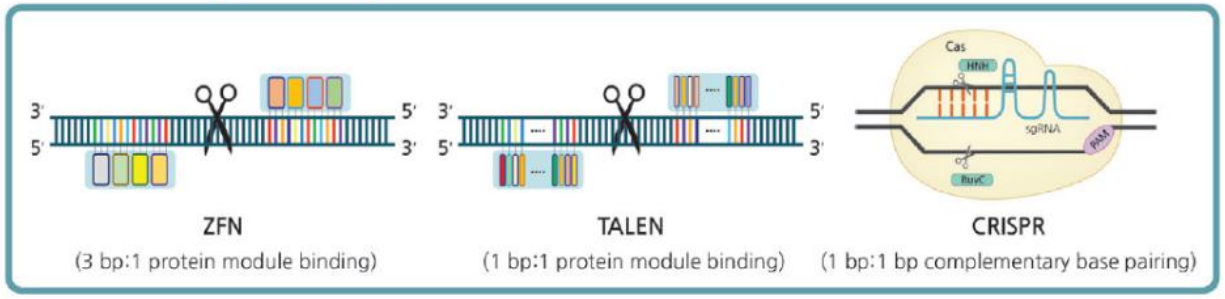


Bacterial Regulation

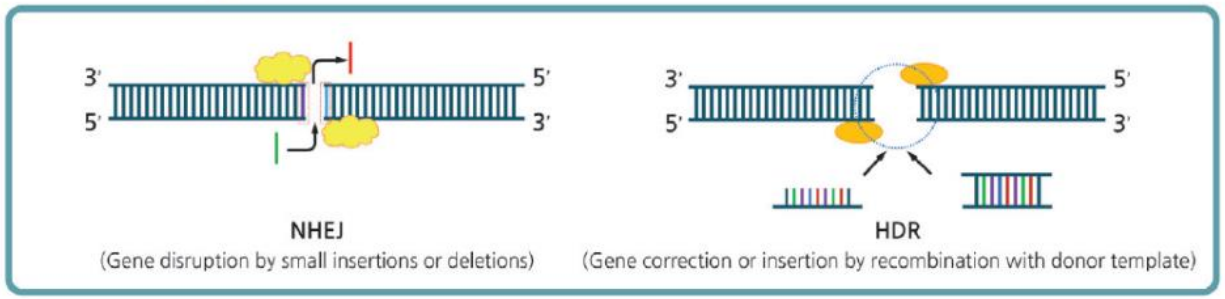
- Transcription
- Translation
- Post-translation
- Gene location on the chromosome

Gene editing in eukaryotic cells

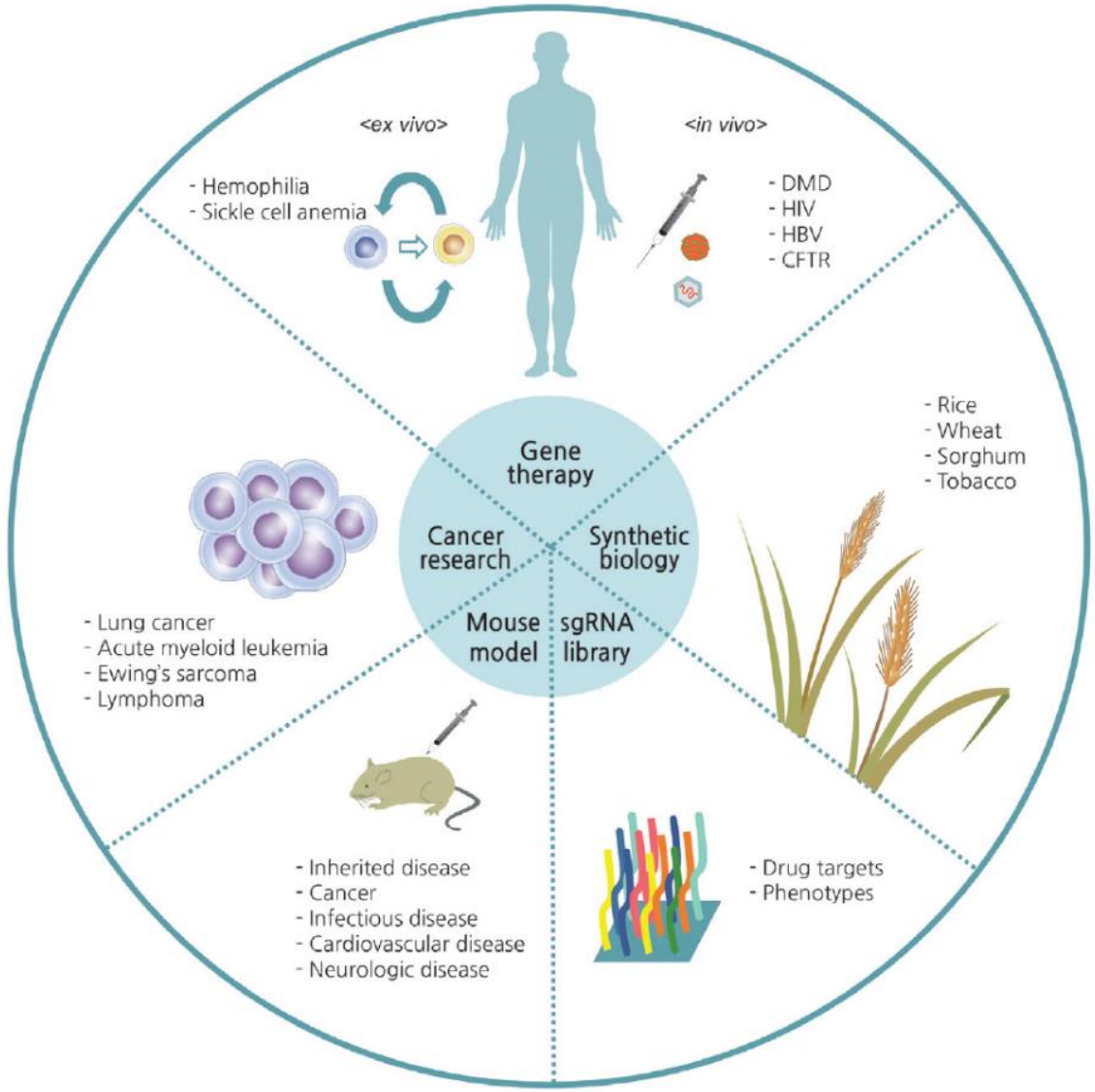
Gene editing technique



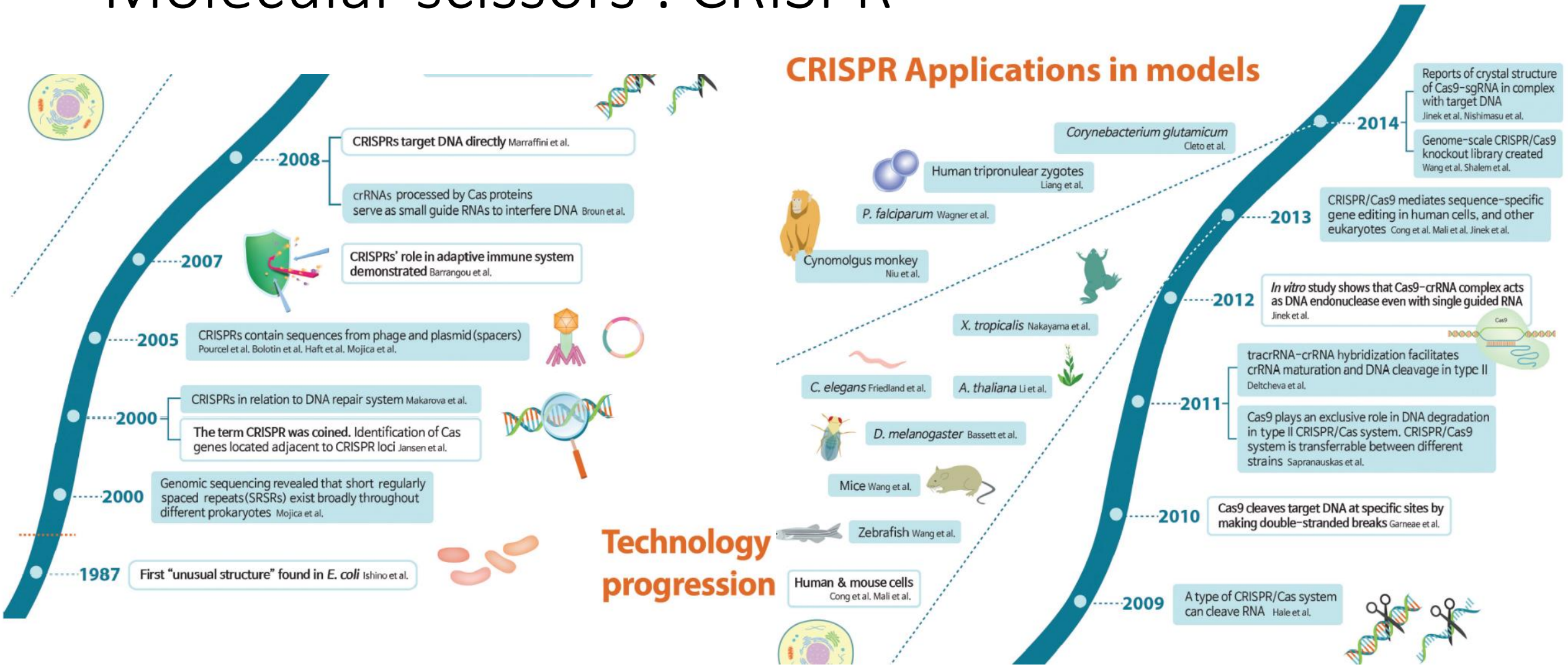
Gene editing mechanism



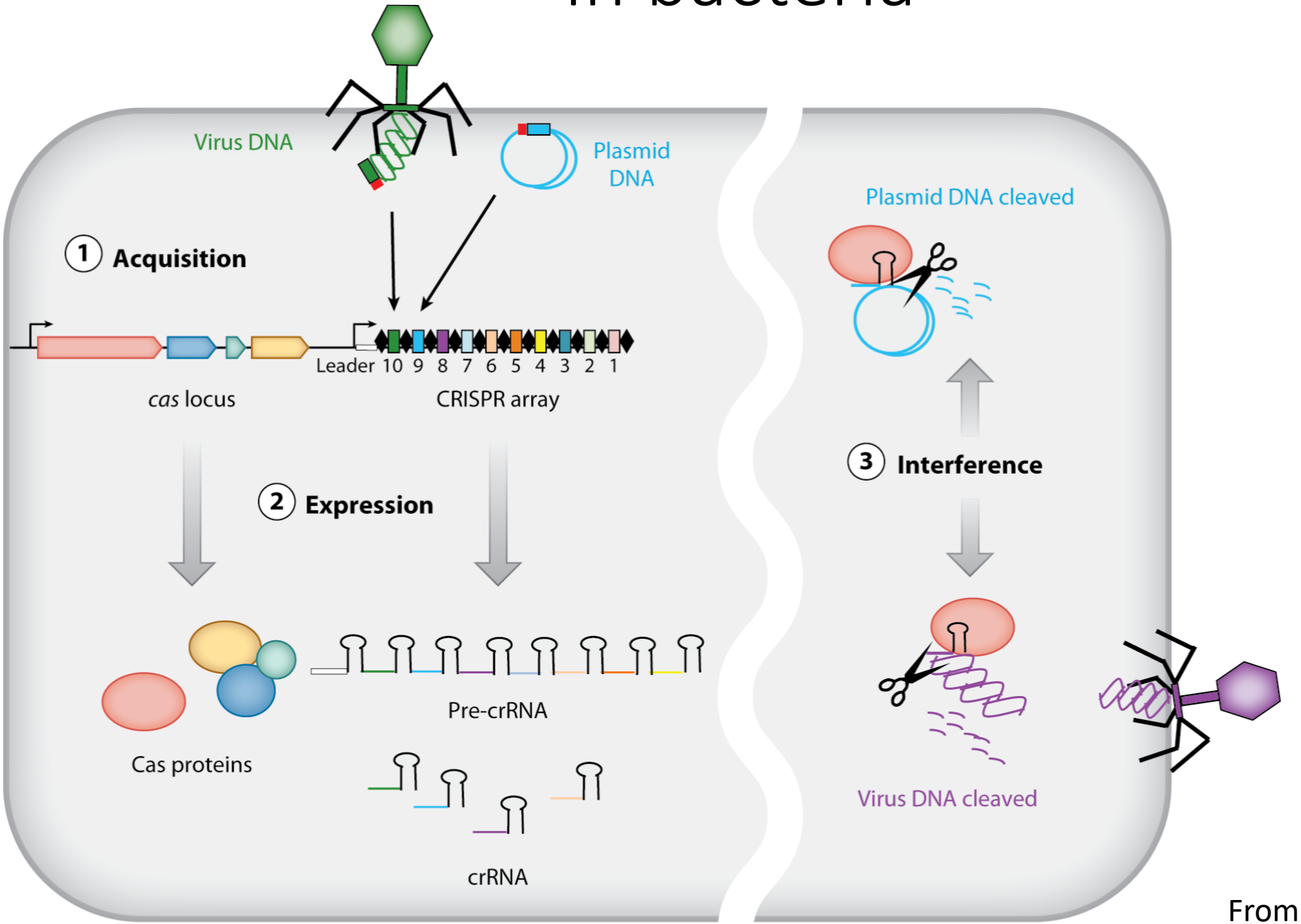
Gene editing applications



Molecular scissors : CRISPR

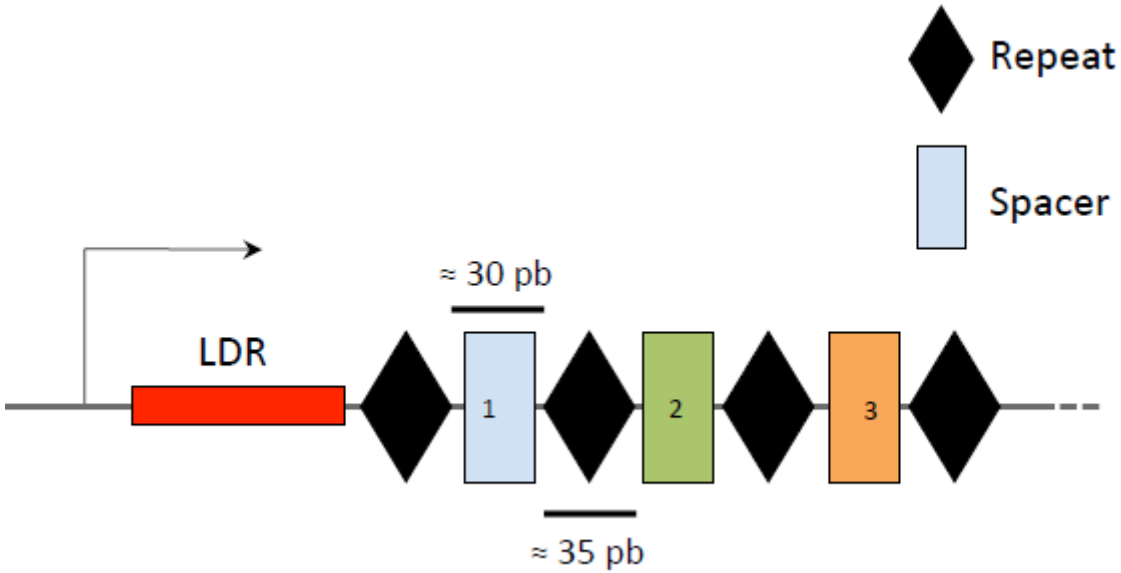


In bacteria



From Bhaya *et al.*, 2011

Genetic element

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                Leader motif          DR1                Spacer 1                DR2
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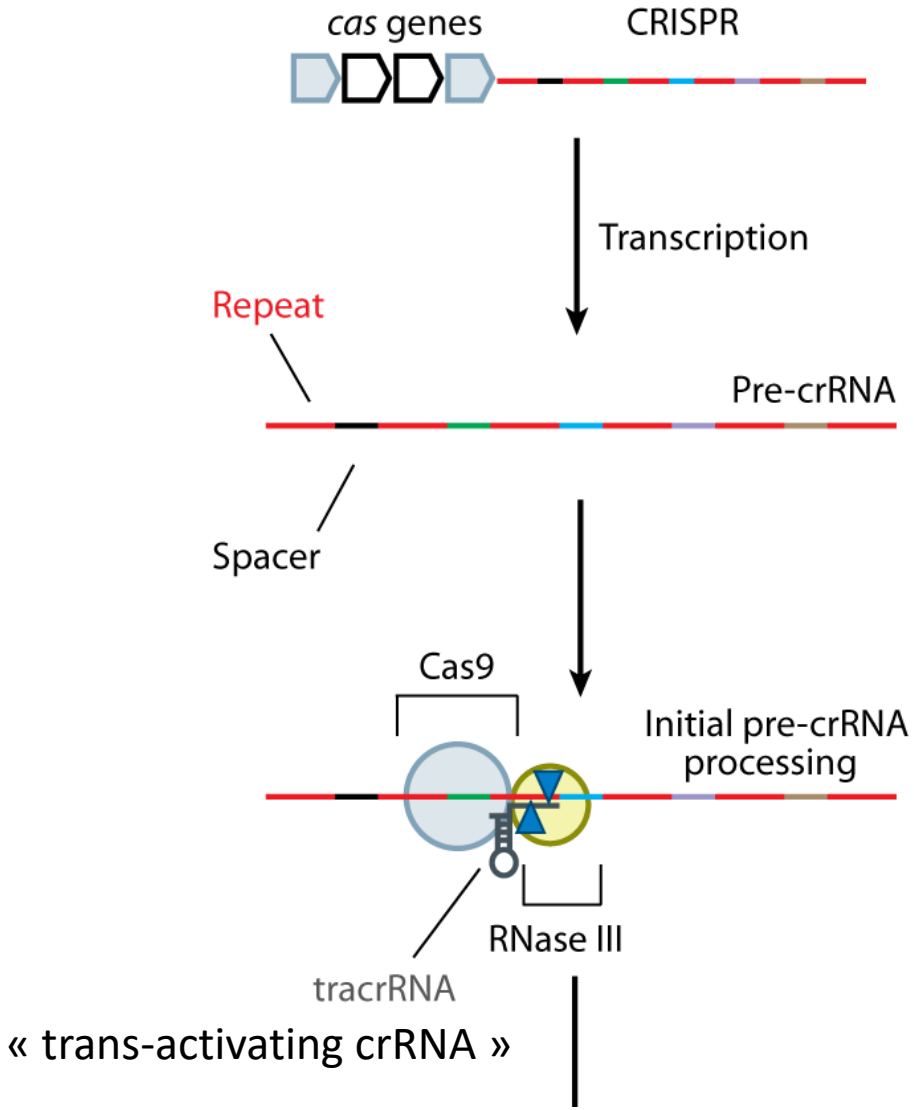
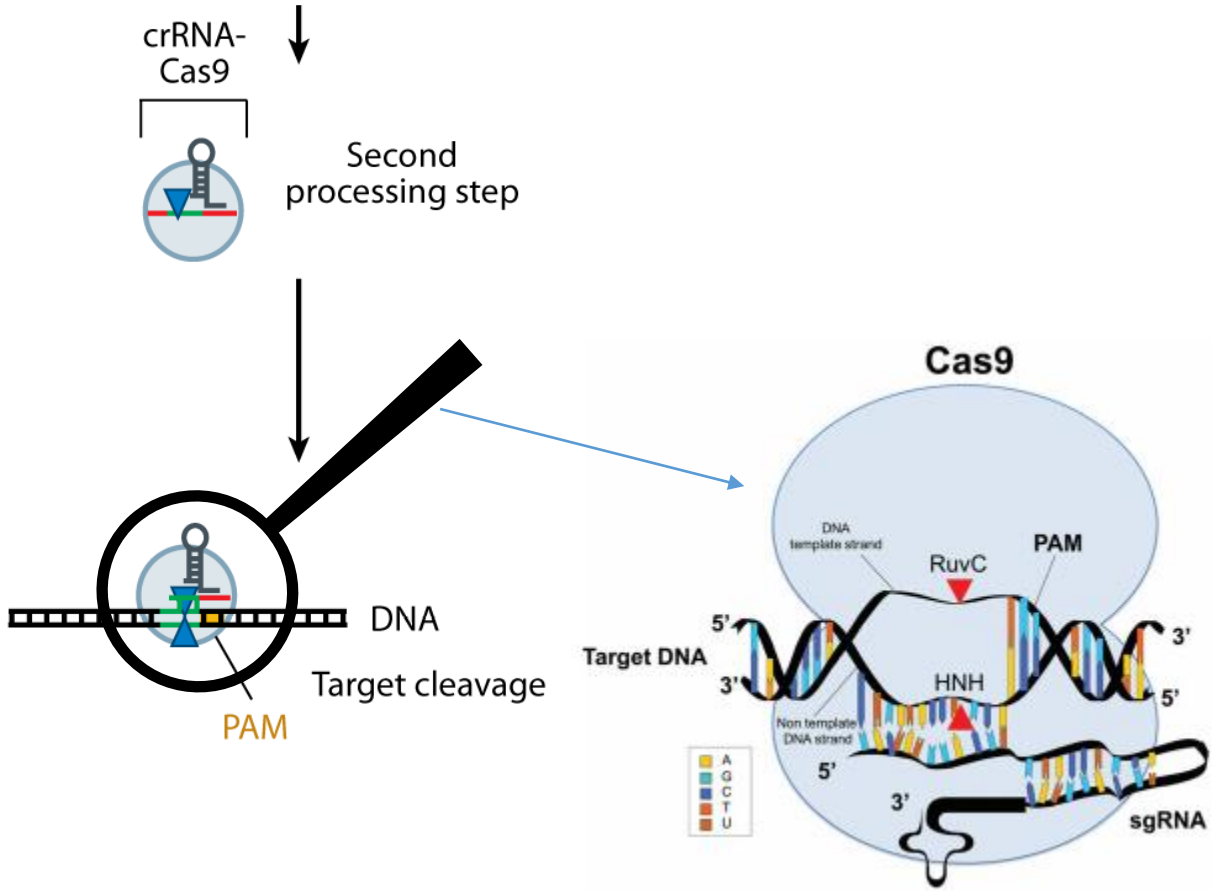
P. Boudry Thesis

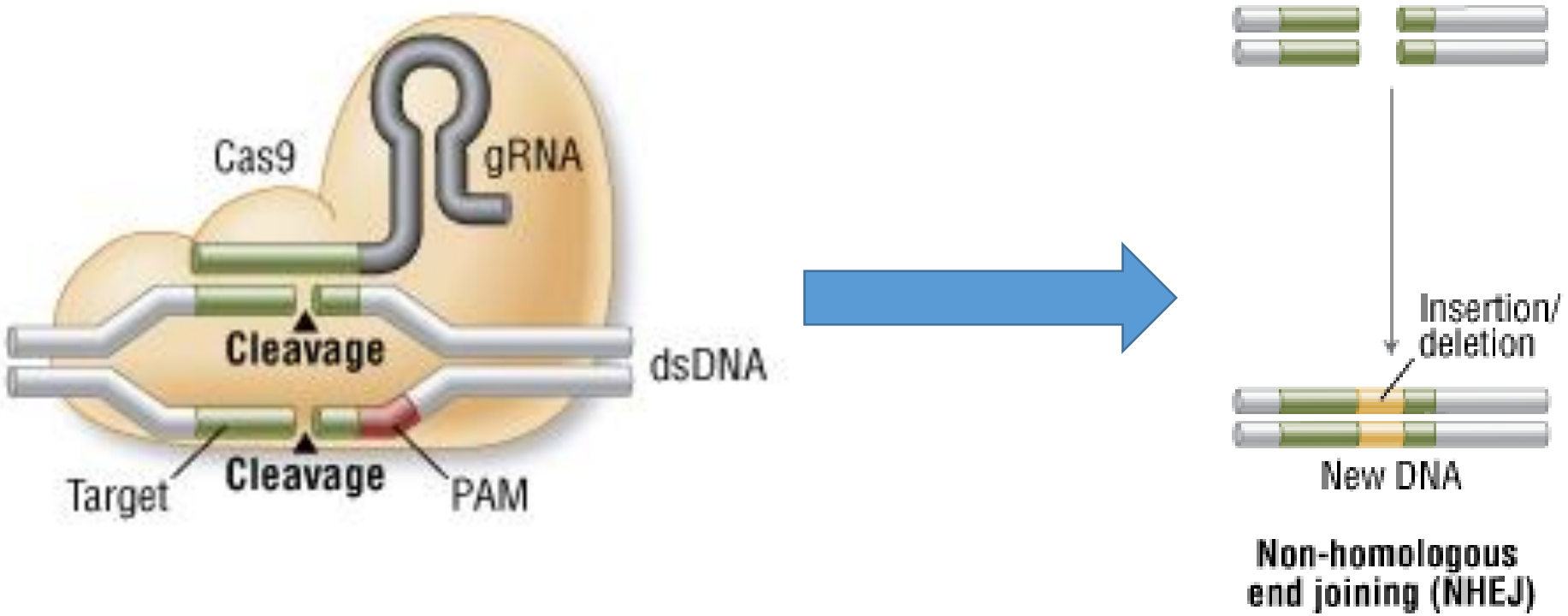
Cas9 protein: action mode





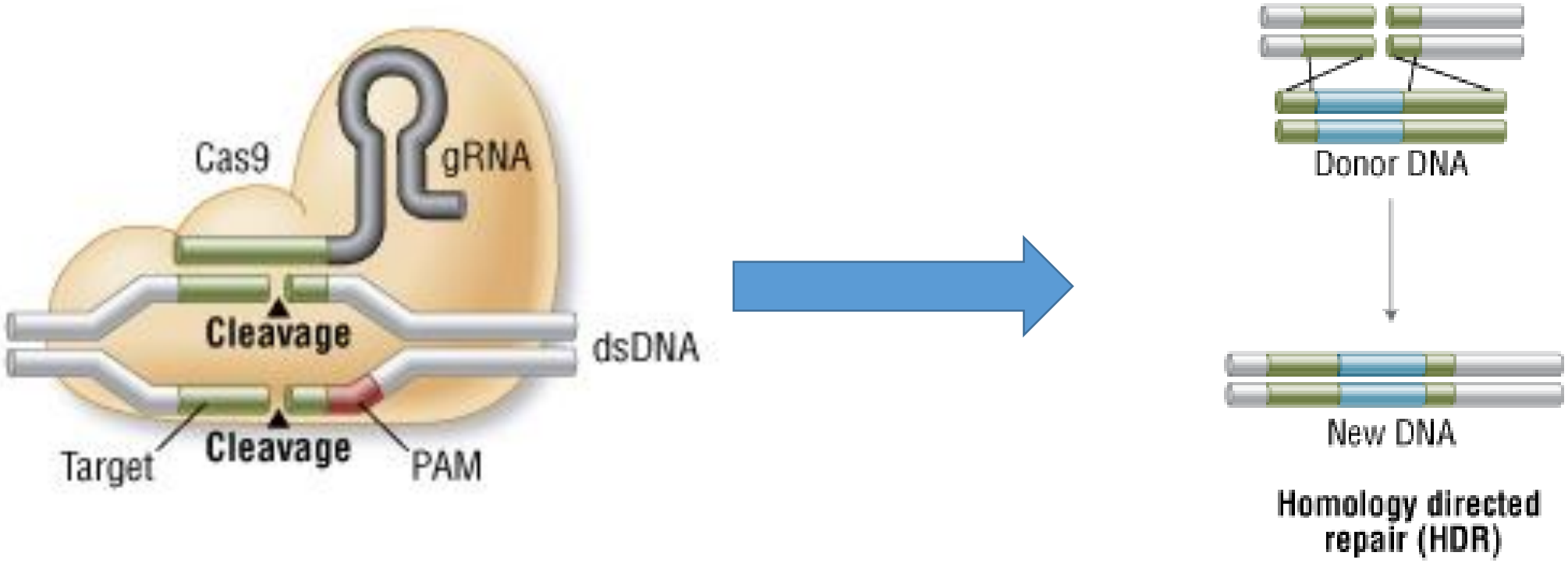
P. Boudry Thesis

Use in Eukariotic cells: mutant creation



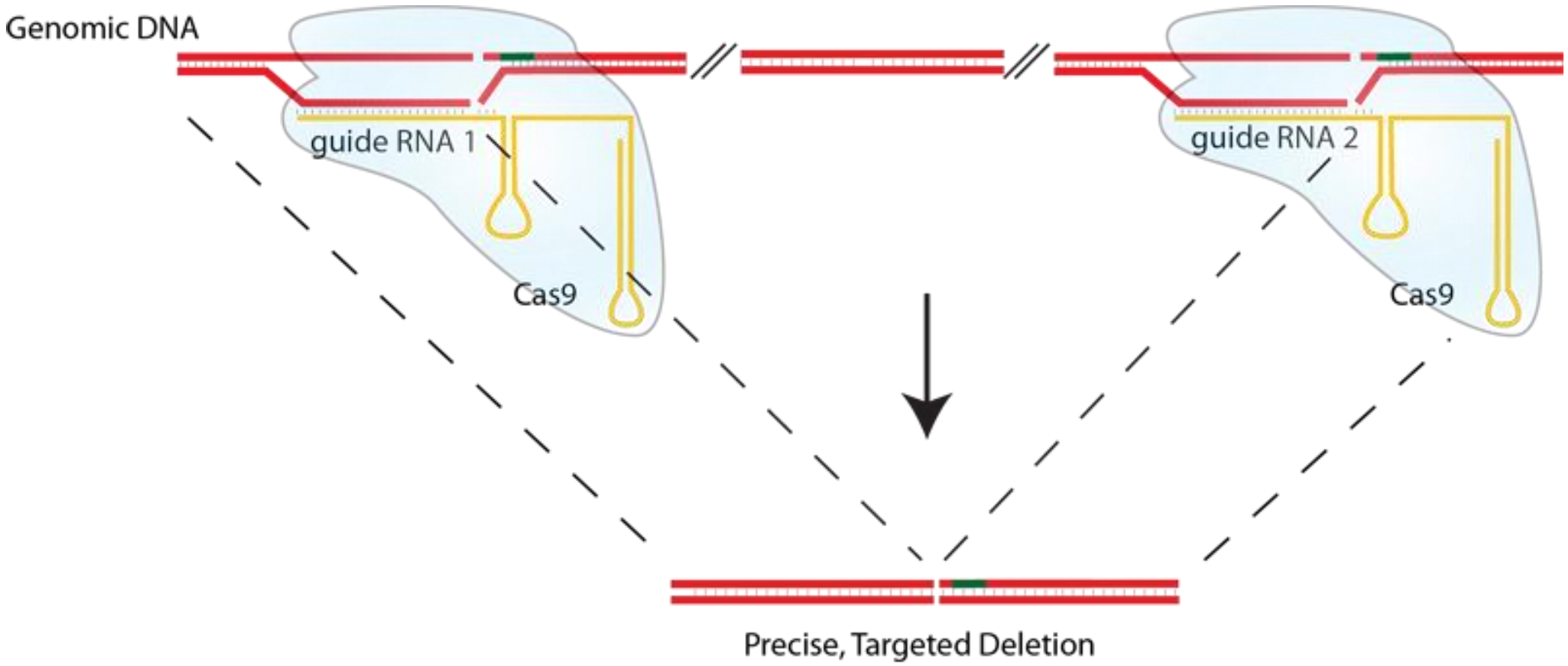
Neb biolabs

Use in Eukariotic cells: insertion



Neb biolabs

Use in Eukariotic cells: deletion



http://ko.cwru.edu/services/crispr_cas.shtml