



Cours de Génie Génétique

Licence biologie moléculaire

Chapitre I

Concepts de base et introduction au génie génétique

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Faculté des Sciences de la nature et de la vie

Département de biologie

Université Ziane Achour Djelfa

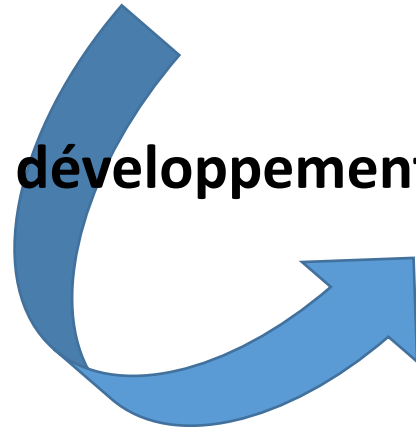
2016/2017

L'homme et la recherche



Survivre

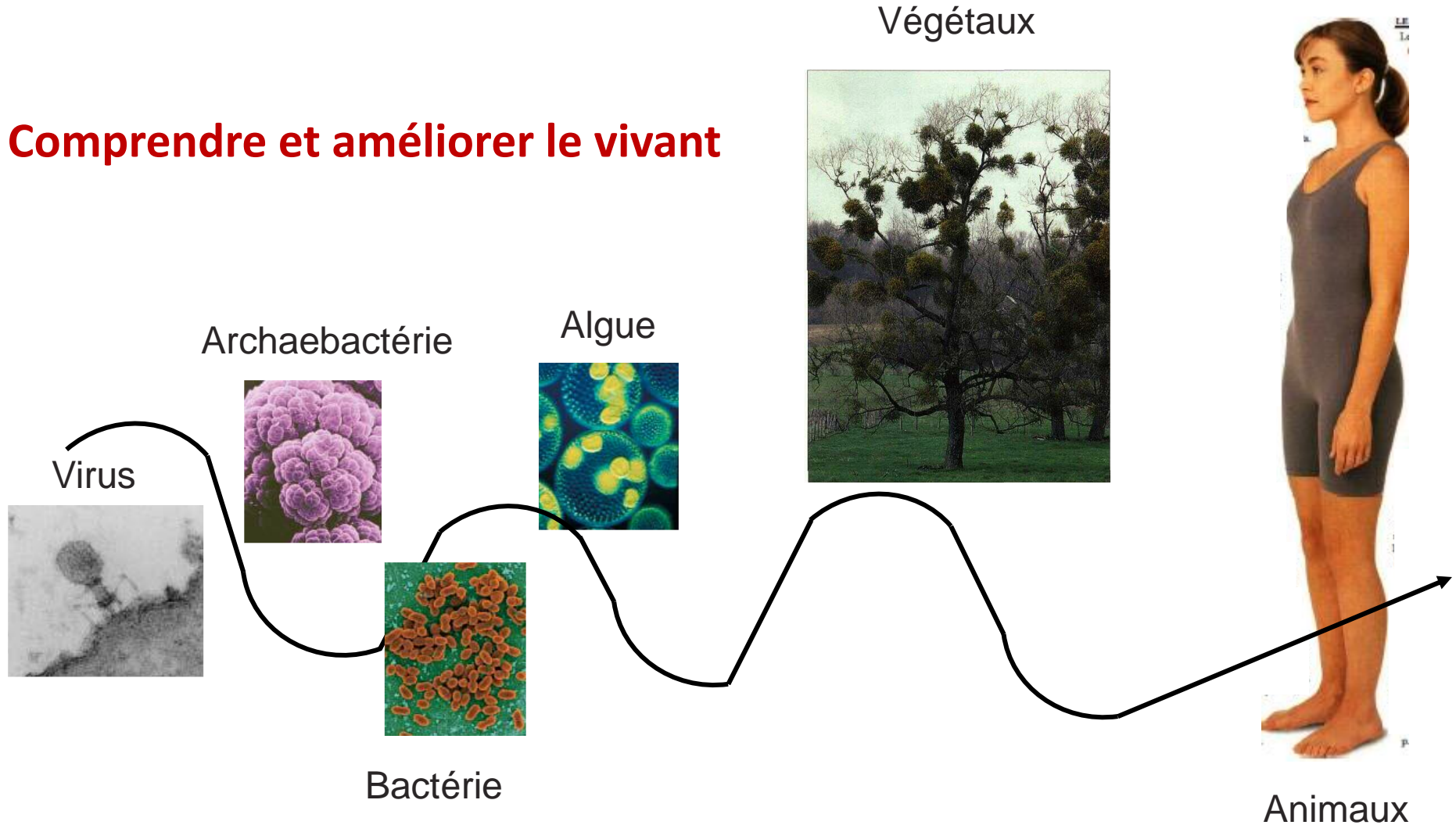
Recherche et le développement



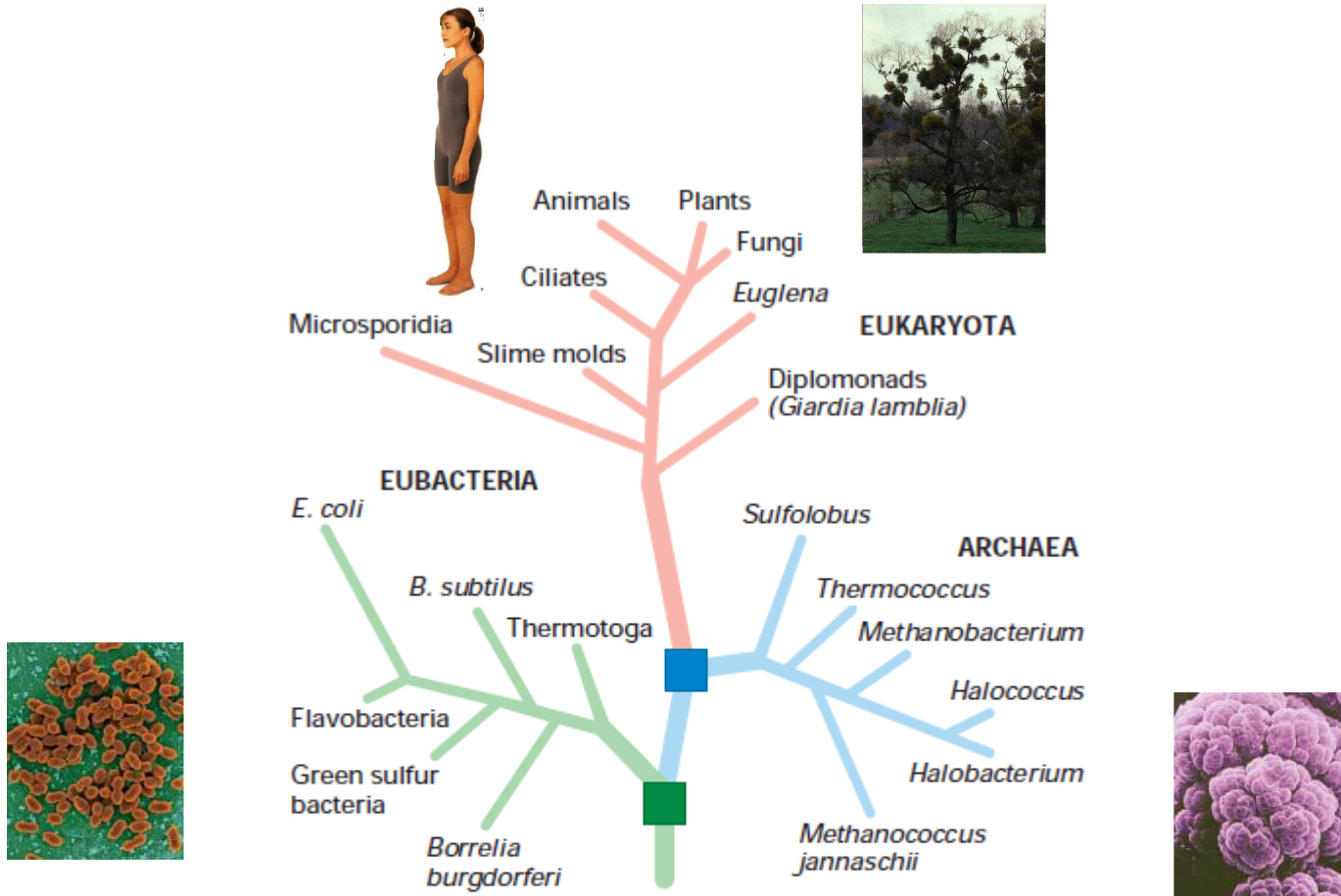
Vivre

Objectif du génie génétique

Comprendre et améliorer le vivant



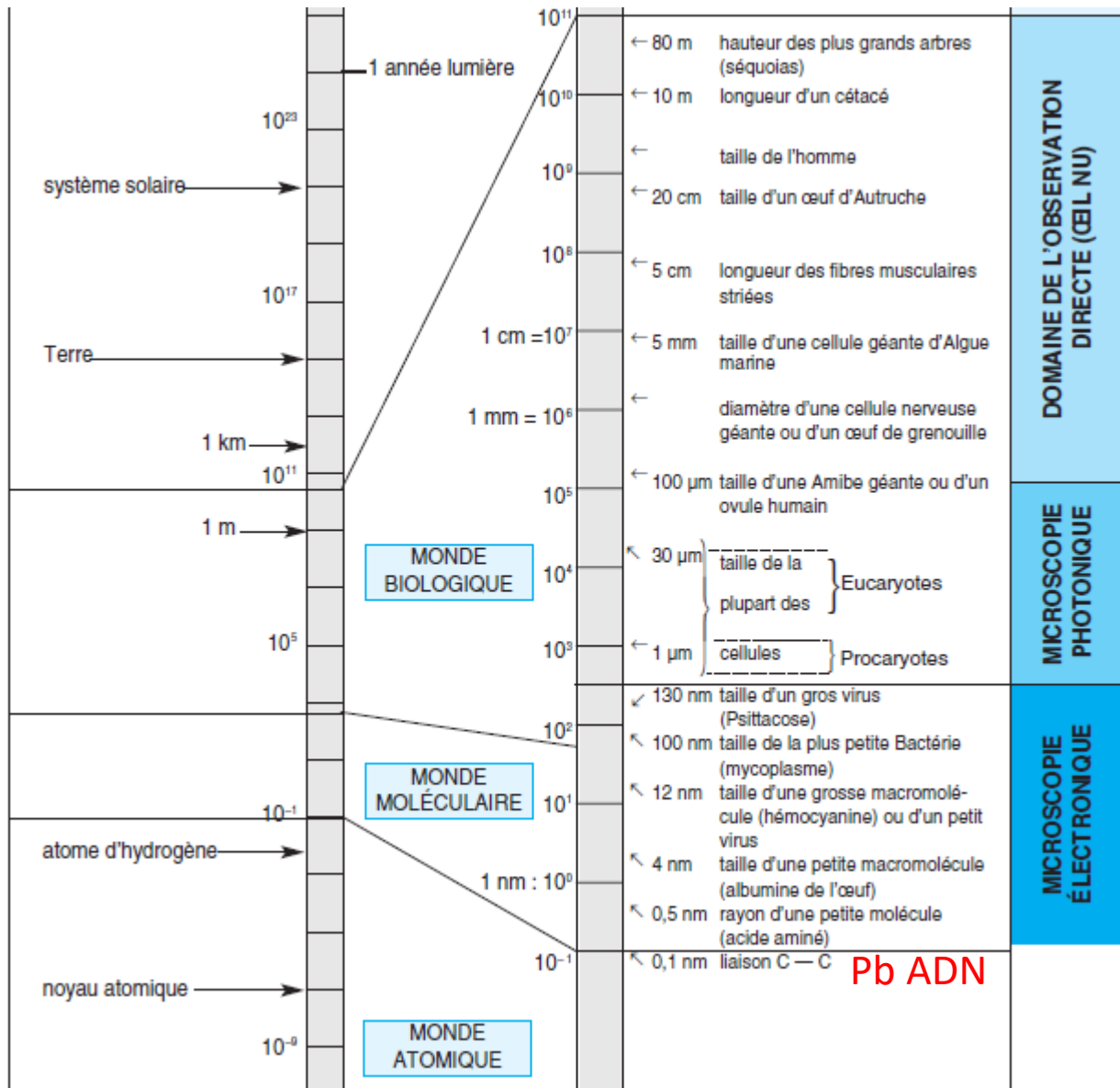
Organisation du vivant



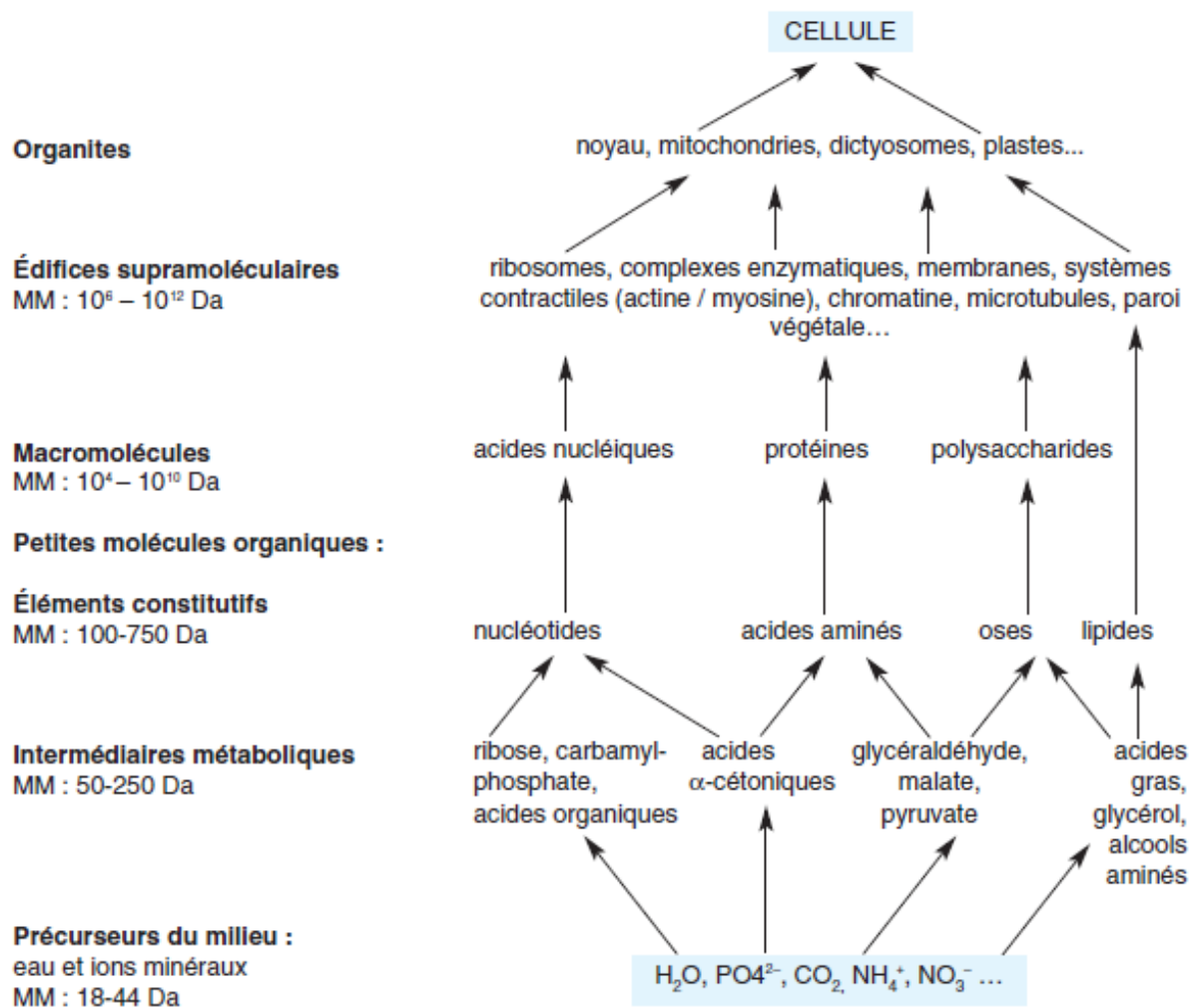
■ Presumed common progenitor of all extant organisms

■ Presumed common progenitor of archaeobacteria and eukaryotes

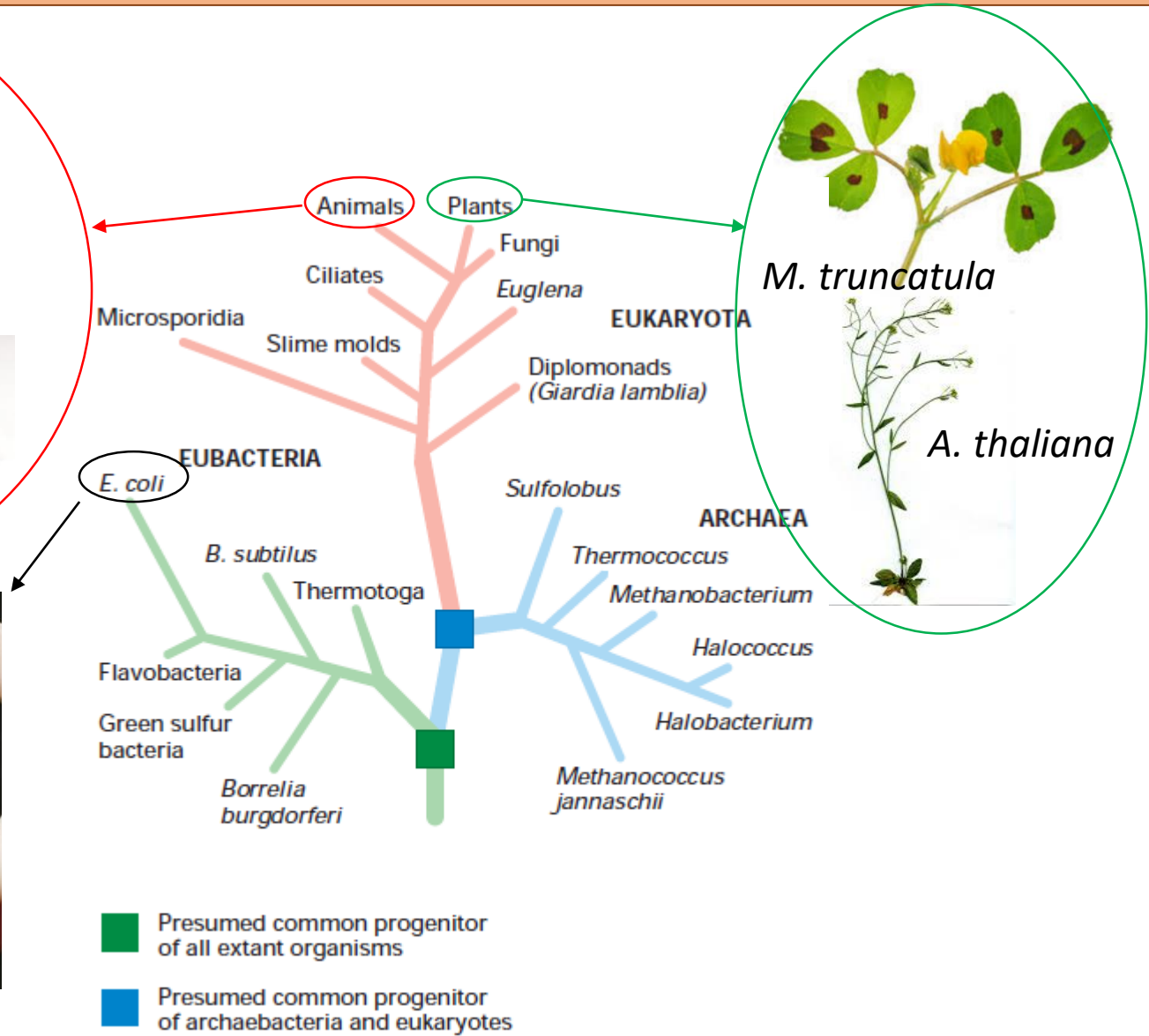
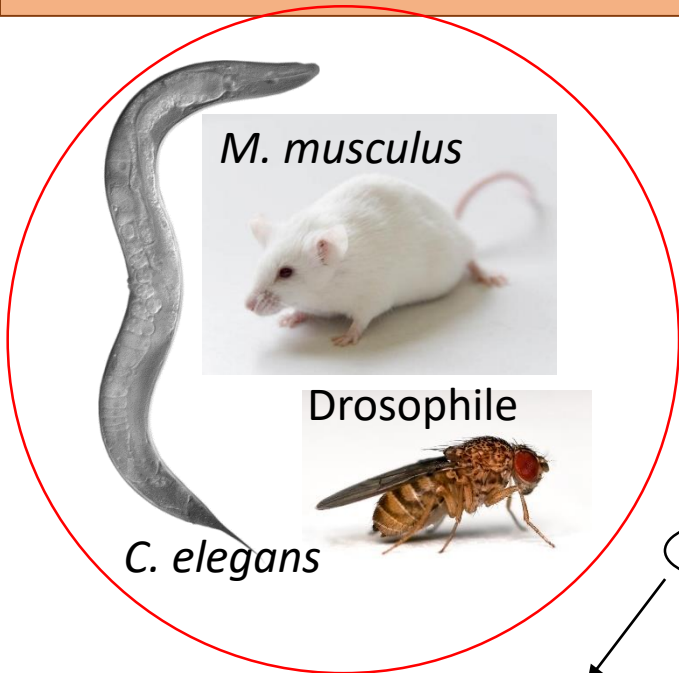
Les dimensions du vivant



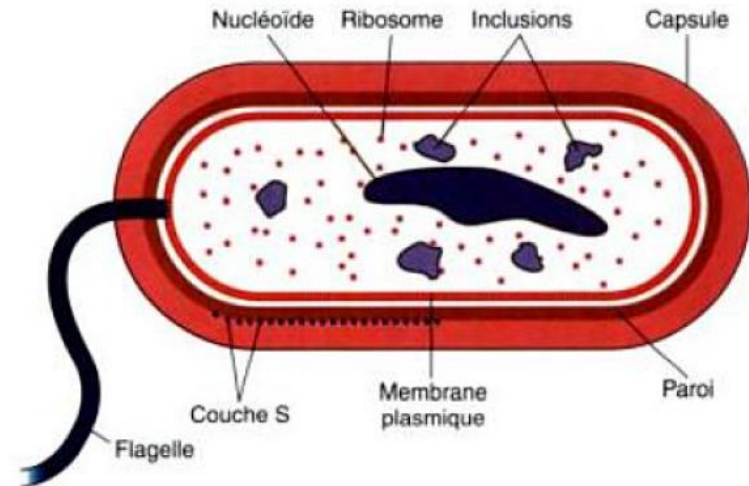
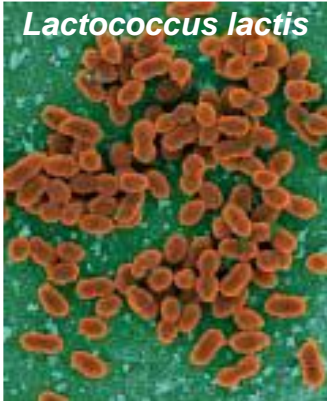
De la molécule au vivant



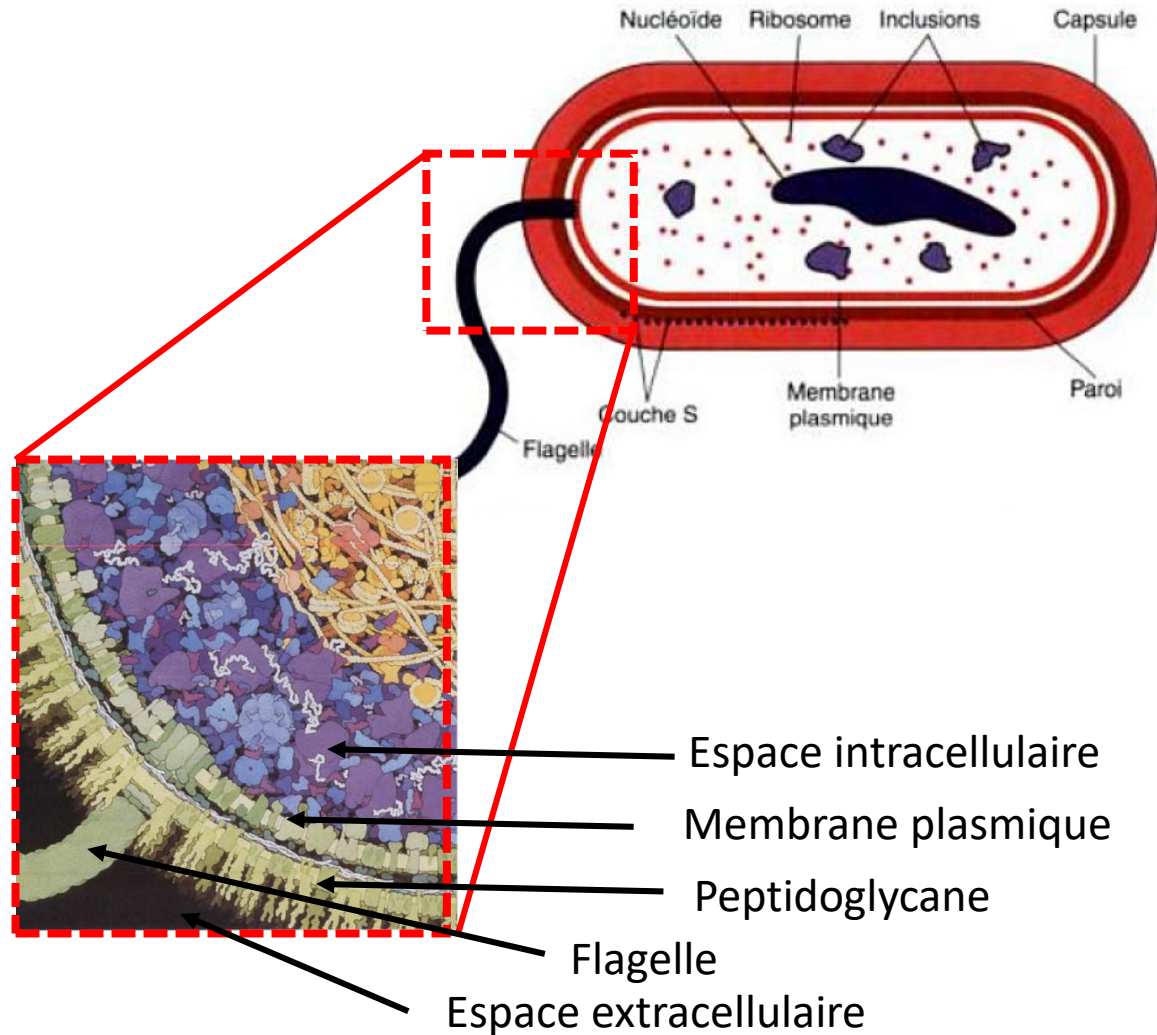
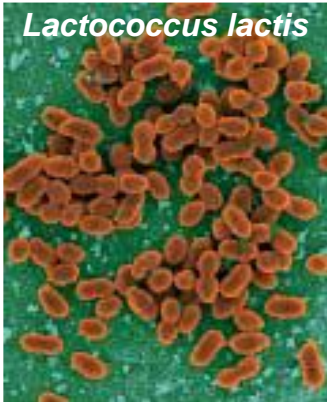
Notion d'espèce modèle



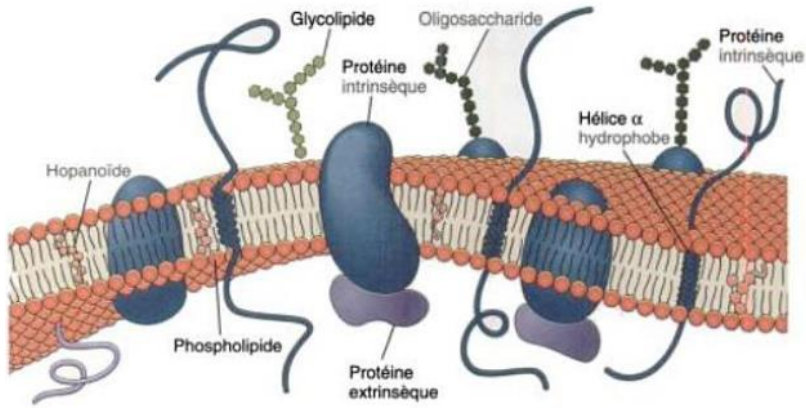
Exemple de procaryote ; les bactéries



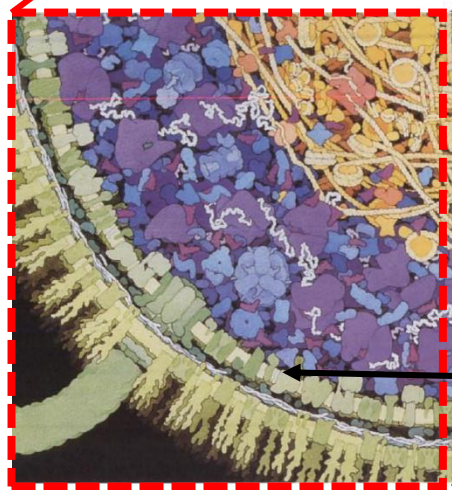
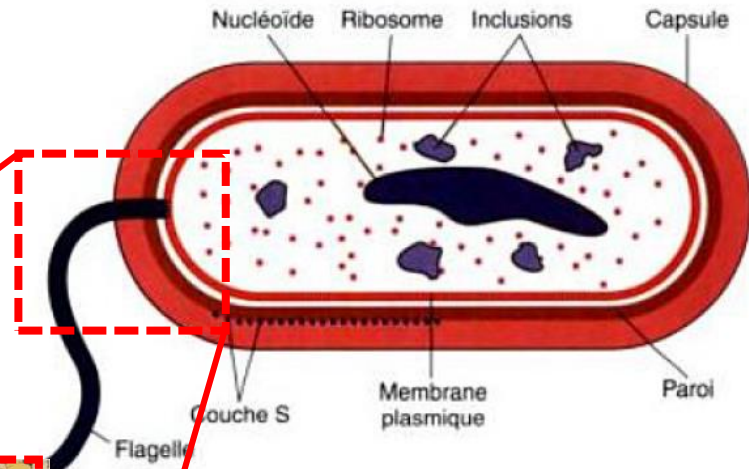
Exemple de procaryote ; les bactéries



Exemple de procaryote ; les bactéries

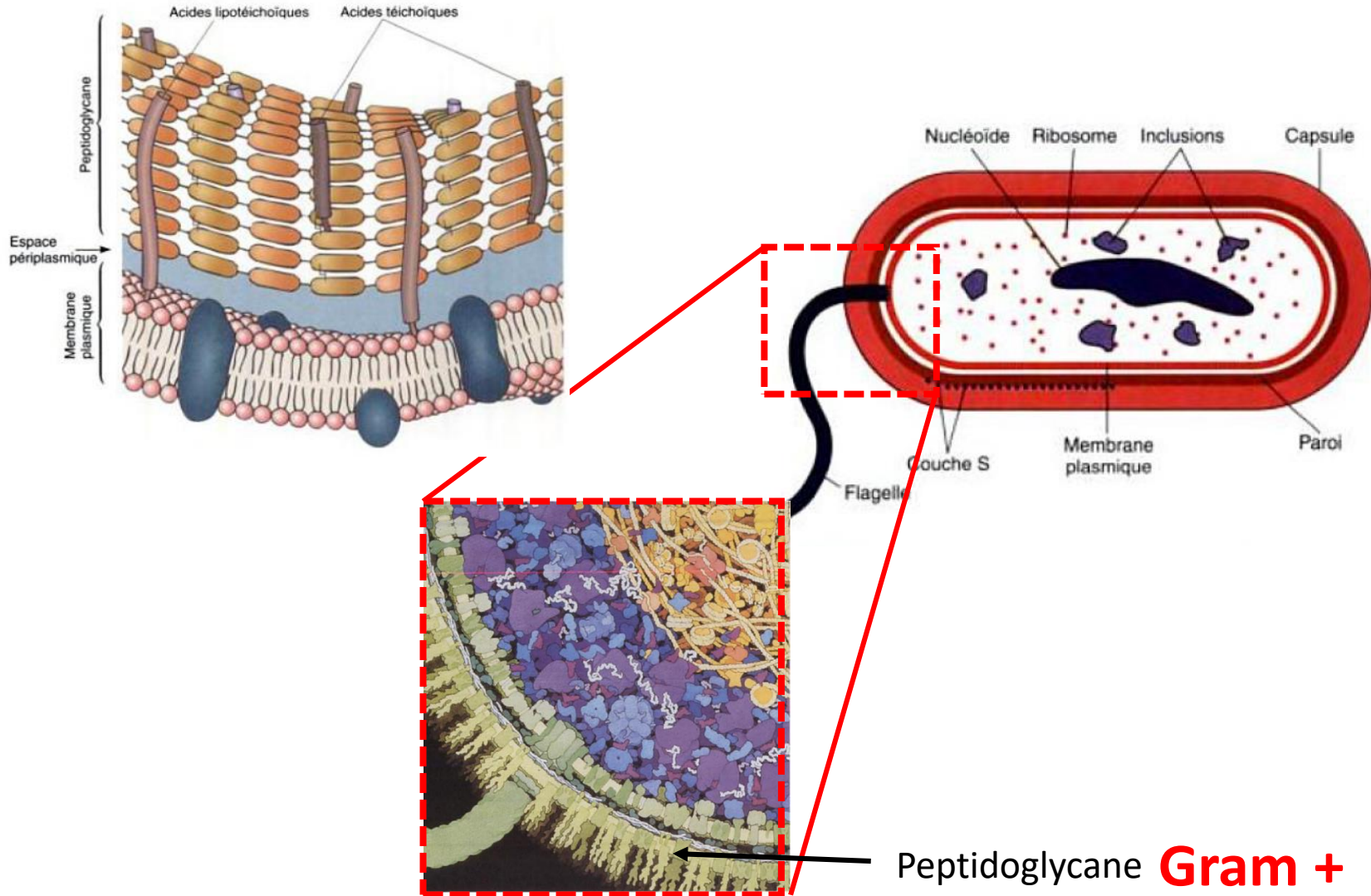


Membrane plasmique

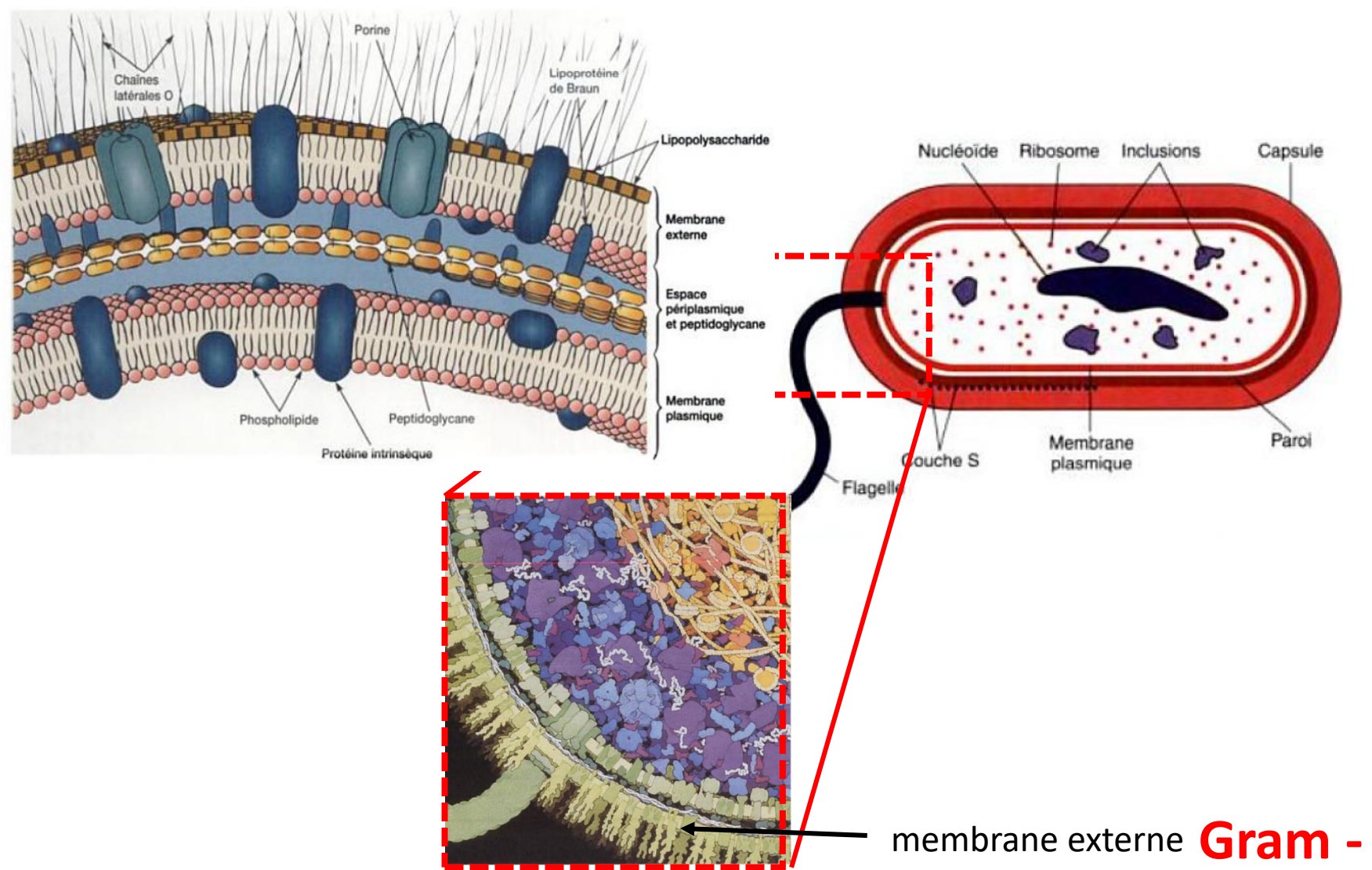


Membrane plasmique

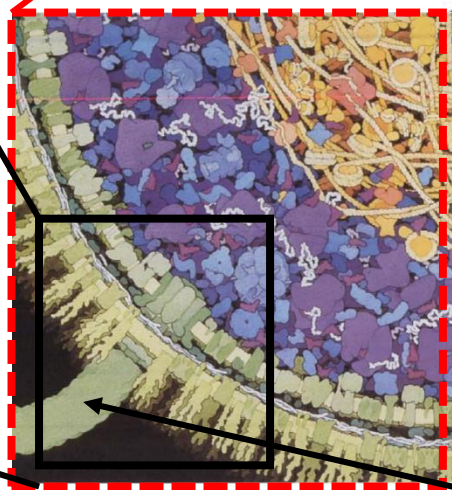
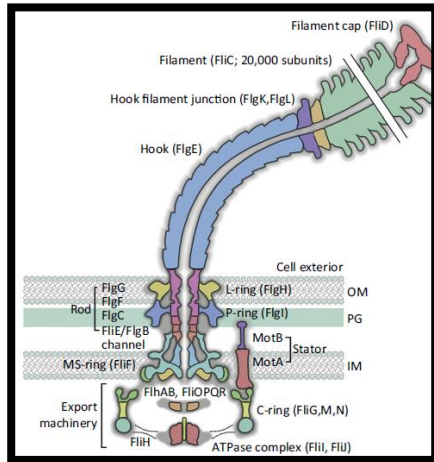
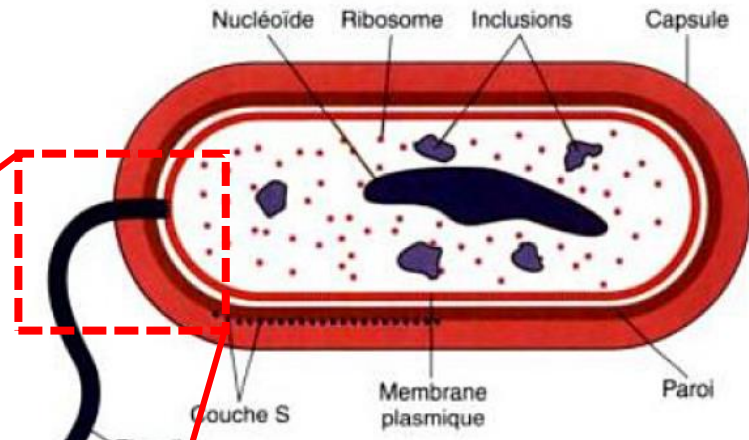
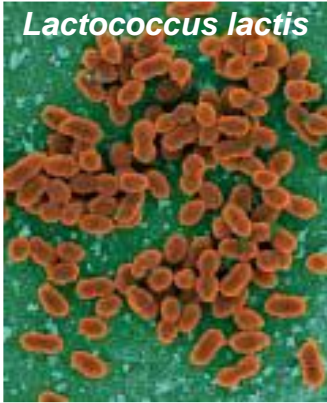
Exemple de procaryote ; les bactéries



Exemple de procaryote ; les bactéries

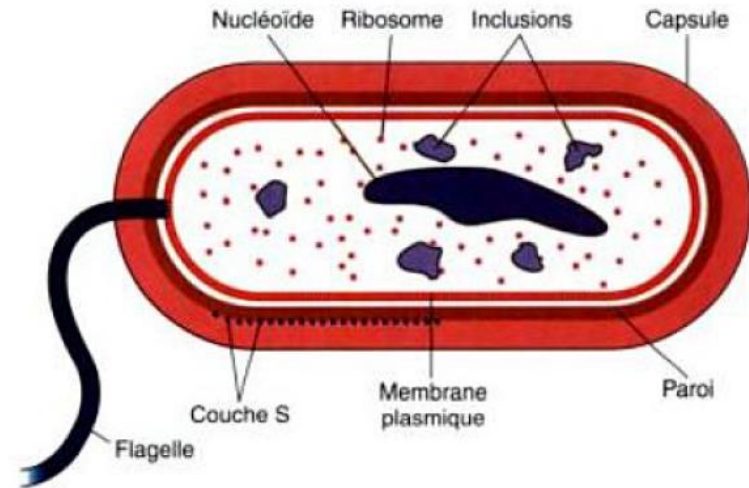
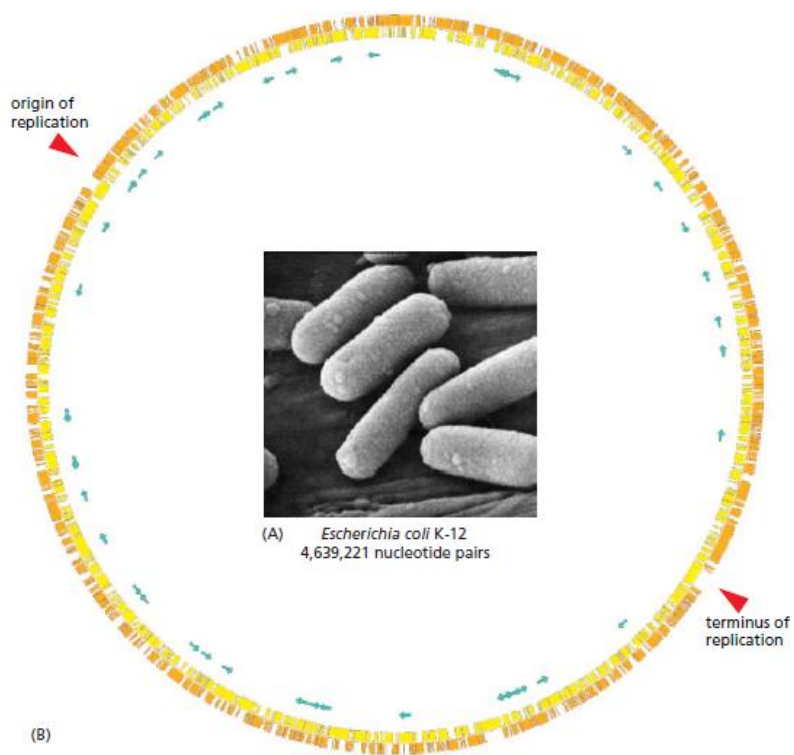


Exemple de procaryote ; les bactéries



Flagelle

Exemple de procaryote ; les bactéries



Plasmide

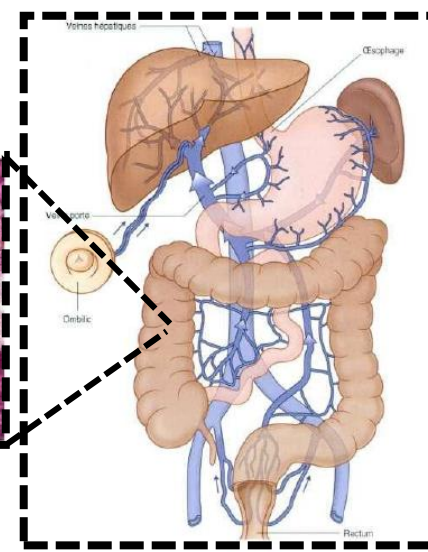
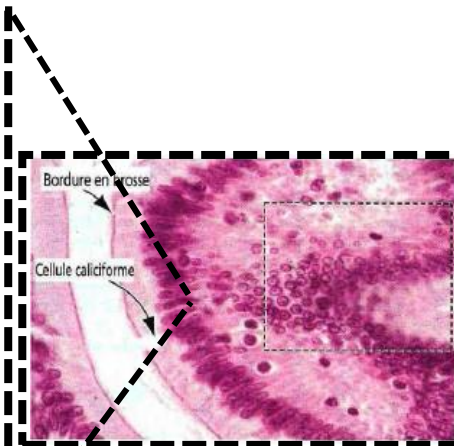
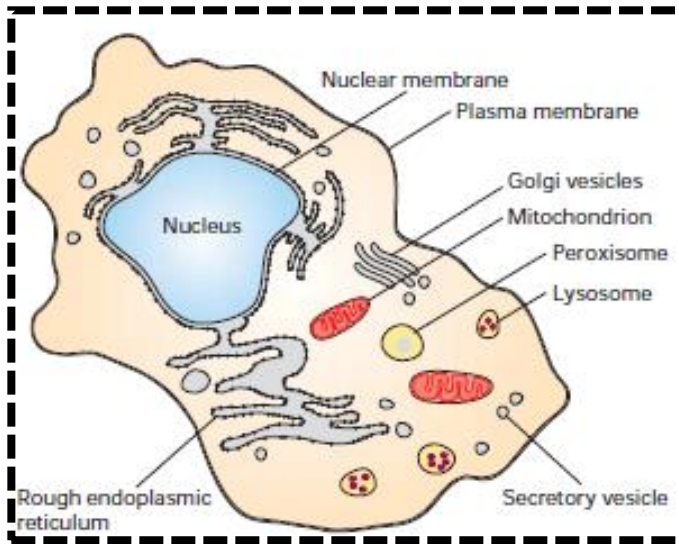
Les organismes supérieurs

Cellules

Tissus

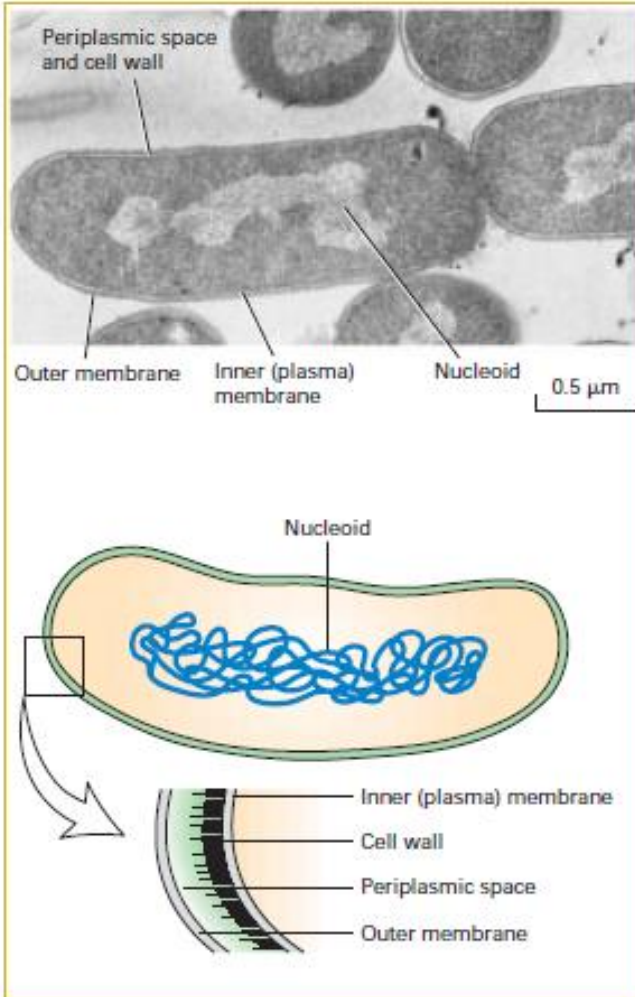
Organes

Organisme

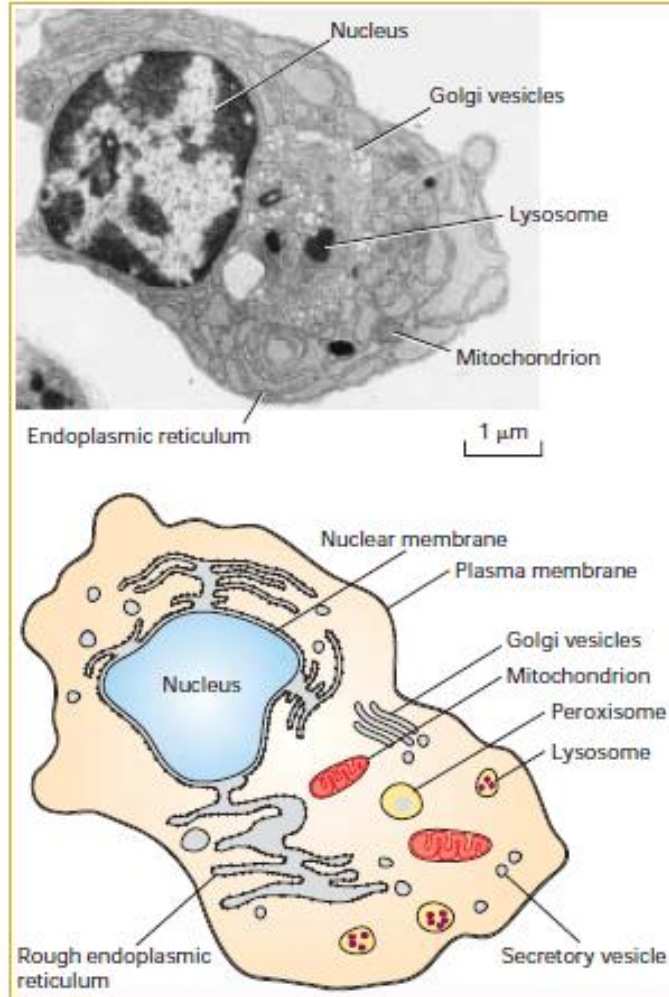


Pro vs Eucaryotes

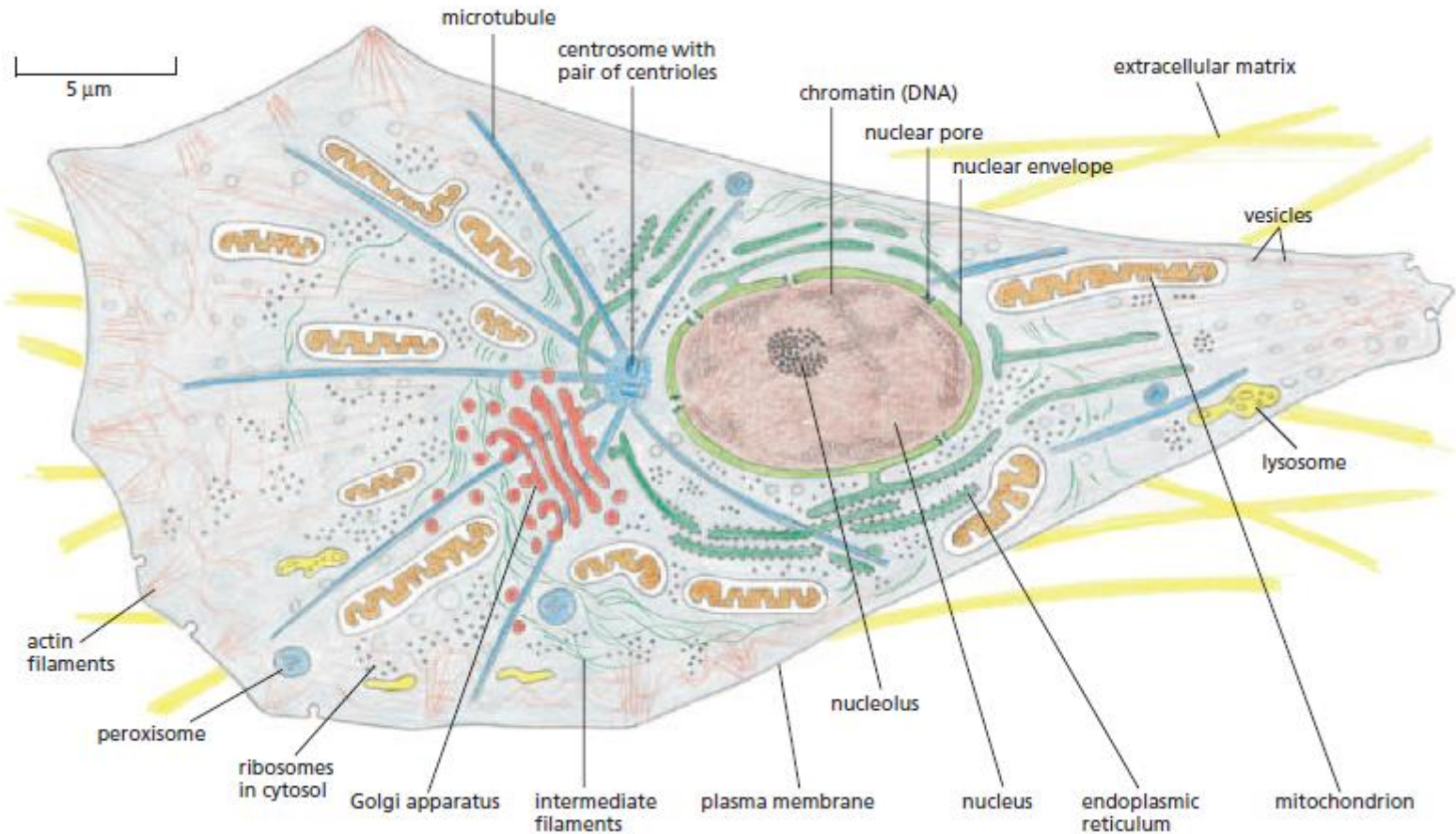
(a) Prokaryotic cell



(b) Eukaryotic cell



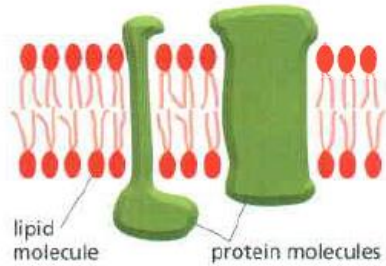
Organisation d'une cellule eucaryote



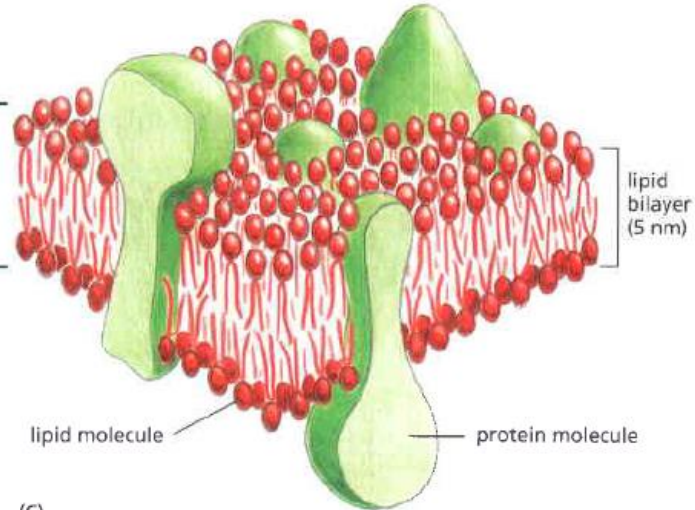
Organisation de la membrane plasmique



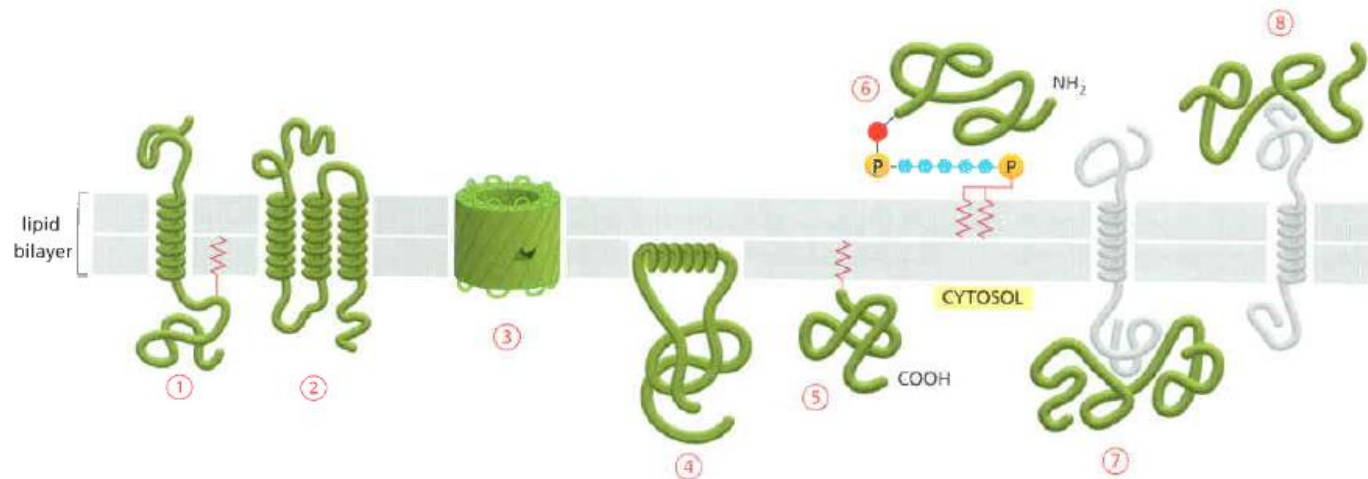
(A)



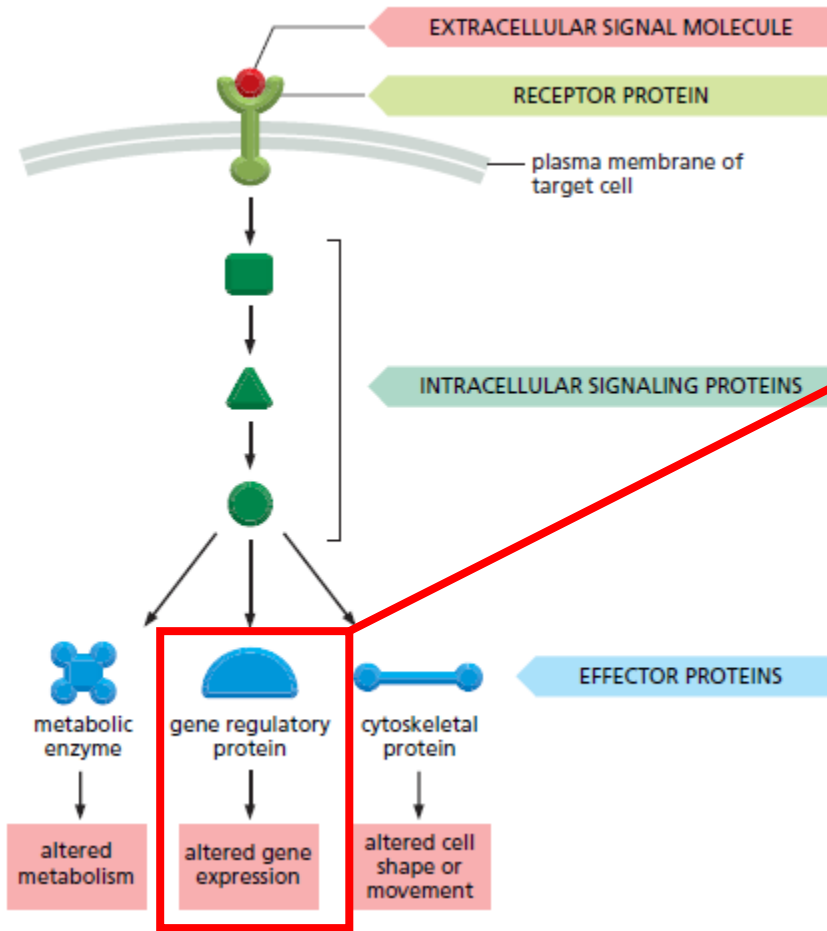
(B)



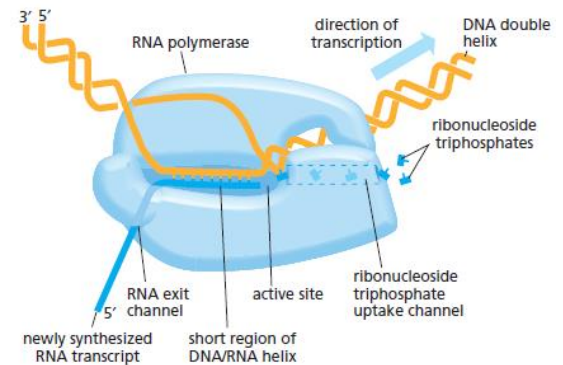
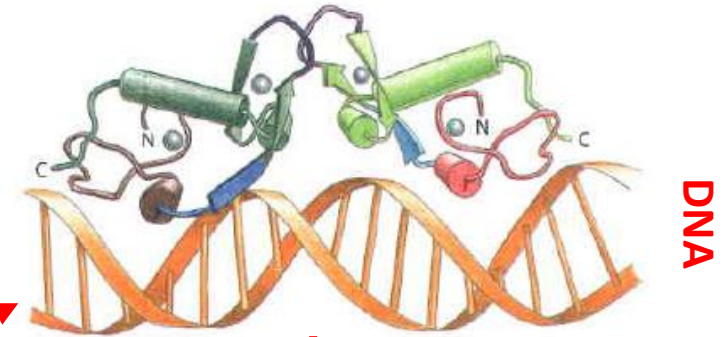
(C)



La signalisation cellulaire



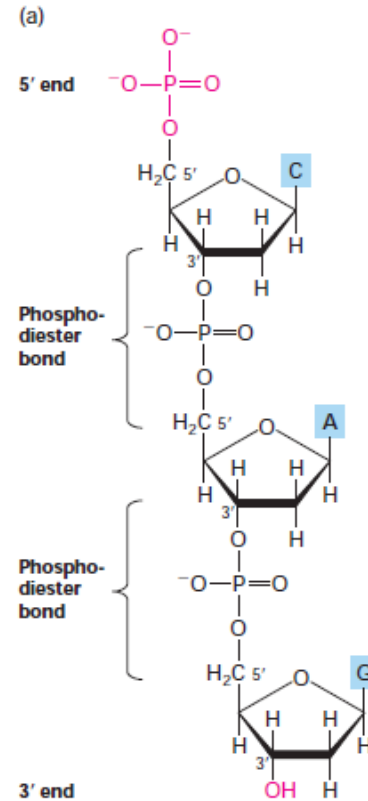
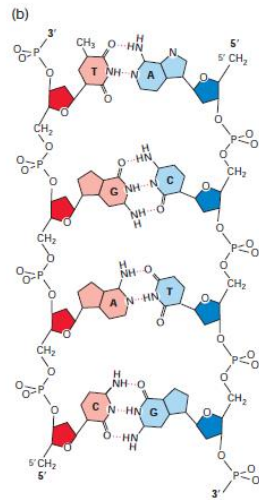
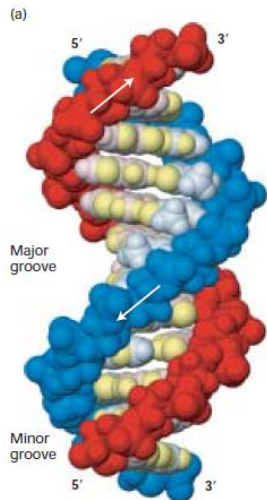
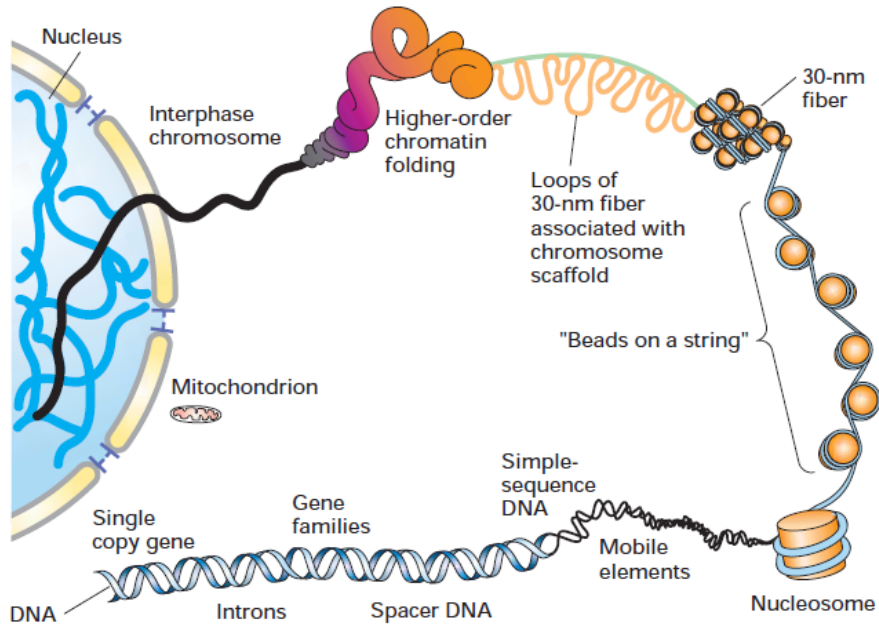
Transcription factor



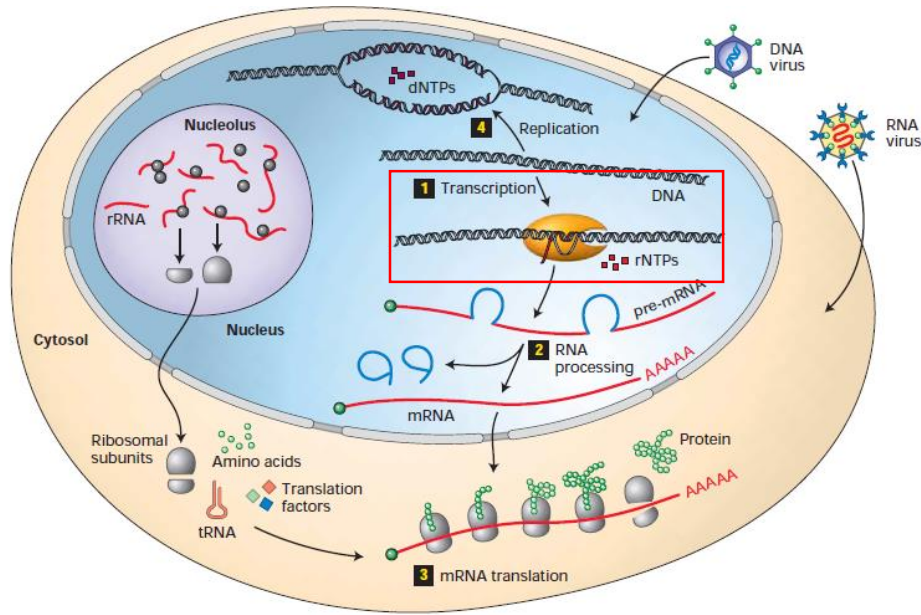
Gene expression

Gene Repression

L'ADN, base de l'information génétique

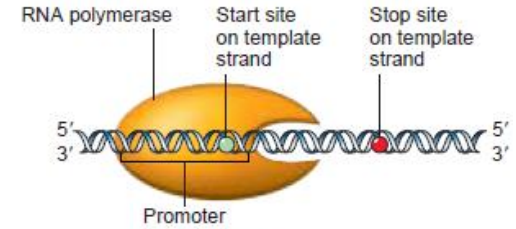


La transcription et la traduction

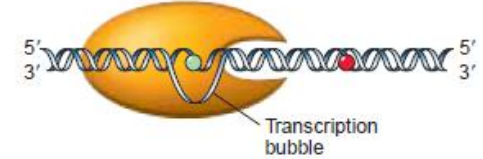


INITIATION

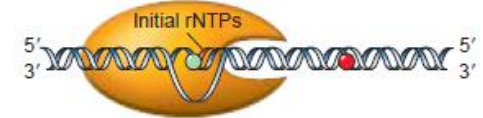
1 Polymerase binds to promoter sequence in duplex DNA. "Closed complex"



2 Polymerase melts duplex DNA near transcription start site, forming a transcription bubble. "Open complex"

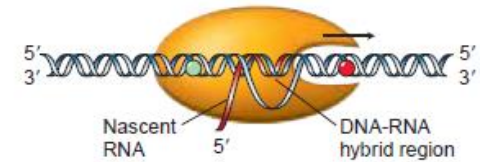


3 Polymerase catalyzes phosphodiester linkage of two initial rNTPs.



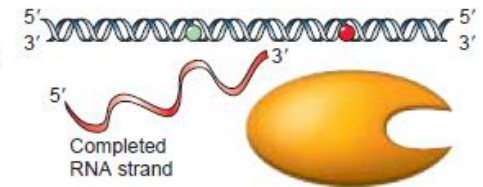
ELONGATION

4 Polymerase advances 3' → 5' down template strand, melting duplex DNA and adding rNTPs to growing RNA.

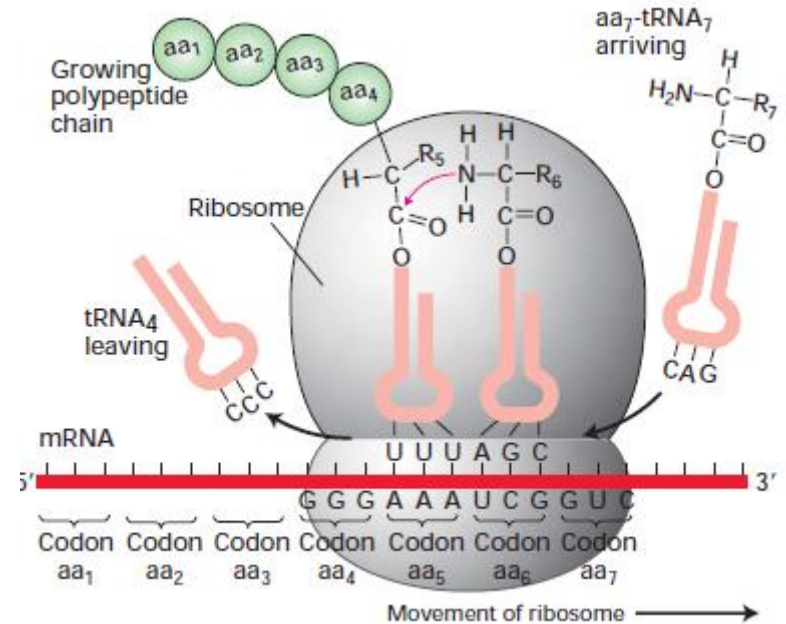
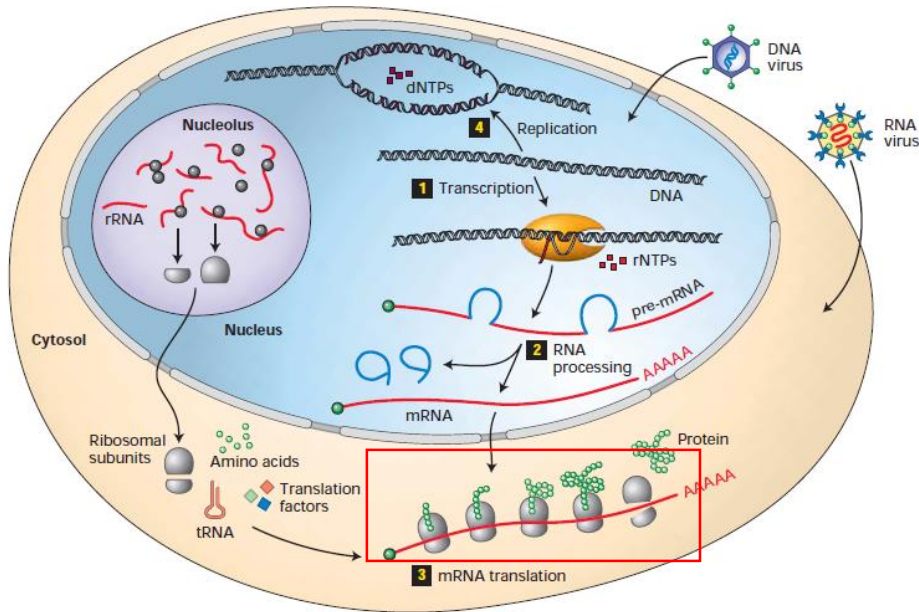


TERMINATION

5 At transcription stop site, polymerase releases completed RNA and dissociates from DNA.

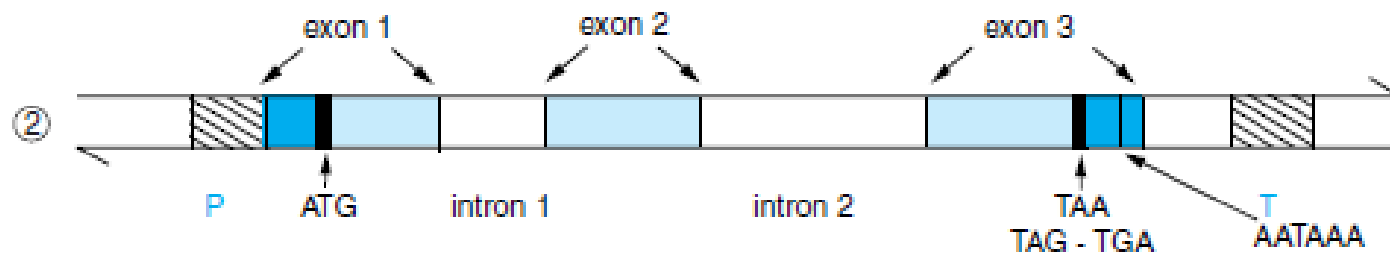
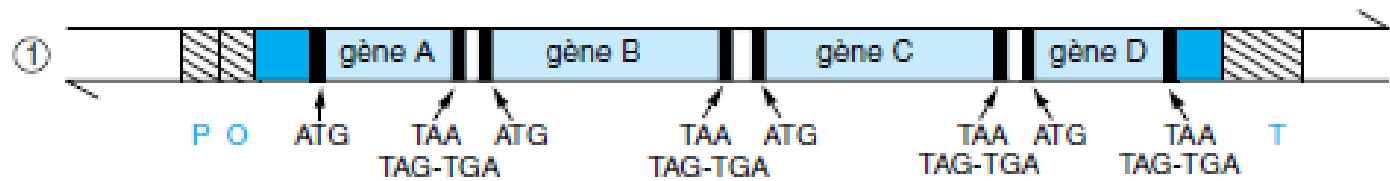


La transcription et la traduction



Structure d'un gène

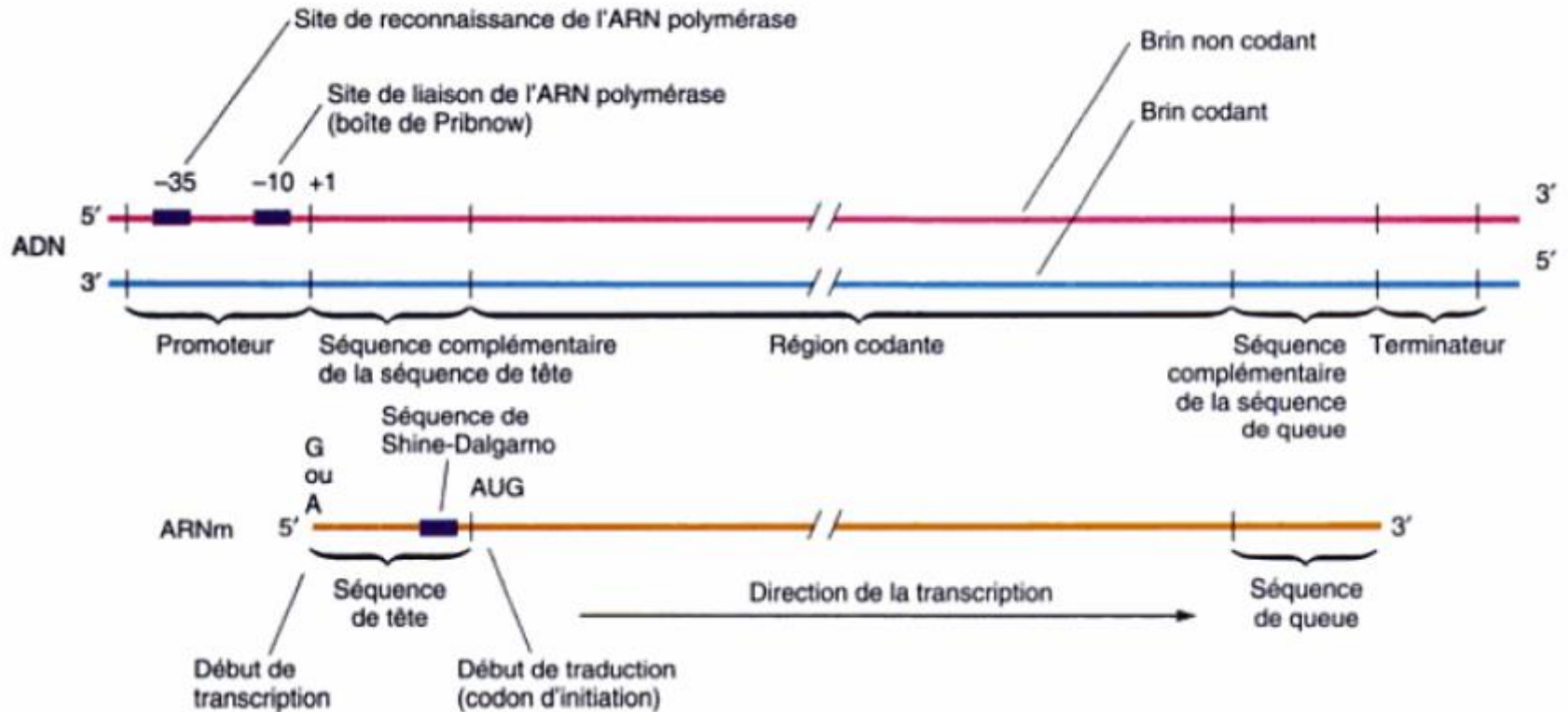
Procaryote



Eucaryote

ORF

Structure d'un gène



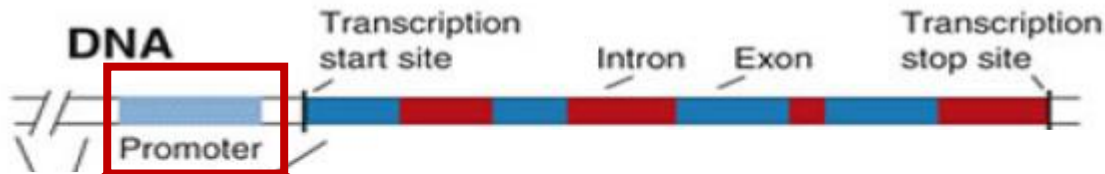
Quesqu'un promoteur ?



Gene	-35 region	Pribnow box (-10 region)	Initiation site (+1)
<i>araB-AD</i>	GGATCGTACCTGACGGCTTTTATCGCAA	CTCTACTGTTTCTCCAT	AGCCC
<i>araC</i>	GCCGTGATTATAGACACTTTTGTACCGCTTTT	TGTCATGGCTTT	CGTCCC
<i>bisA</i>	TTCCAAAACGGTGTTTTTTGTGTTAAATTCGGTGTAGACTTGTAA	ACCTAAA	AT
<i>bisB</i>	GATAATCGACTTGTAAAGCAAATTGAAAAGATT	TAGCTTTACAAGTC	TACAC
<i>galP2</i>	ATTTATTTCATGTCACACTTTTGGCATCTTTGTTATGCTATGGTT	ATTTGAT	ATTTGAT
<i>lac</i>	AGCCCAAGCTTTACACTTTATGCTTCGGCTCGTATCTTGTGTGCA	ATTGTC	
<i>lacI</i>	CCATCGAATGGGCGAAAACCTTTCCGGGTATGGCATGATAGCCGCCCGAAGA		
<i>rnaI</i>	AAAAATAAATGCTTGACTCTGTACGGCGAAGGGCTATTATCAACCC	CCGGCC	
<i>rnaII</i>	CAAAAAAATACTTGTGCAAAAATTTGGGATCCCTATAATGCGCTCCGTTG		
<i>rnaI7</i>	CAATTTTTCTATTGCGGGCTGCGGAGAACTCCCTATAATGCGCTCCATCG		
<i>rRNA^{Tr}</i>	CAACGTAAACACTTTACAGCGGCGCGTCATTTIGATATGATGCGCCCCGCTTCC		
<i>trp</i>	AAATGAGCTGTTGACAATTAATCATCGA	ACTAGTTAACTAGTACGCA	AGTTC

Consensus sequences:	-35 region	Pribnow box	Initiation site
	T C T T G A C A T ... [11-15 bp] ...	T A T A A T ... [5-8 bp] ...	A
	42 38 82 84 79 64 53 45 41	79 95 44 59 51 96	51 C T 50 G 2

La régulation de l'expression des gènes



**Interaction ADN-
Facteur de
transcription**

NNNNNNNNN **TGGGGAATTCCC** NNNNNNNNN



**Motif protéique
A**

NNNNNNN **CTGGGGAATTTCCCAAG** NNNNNNN



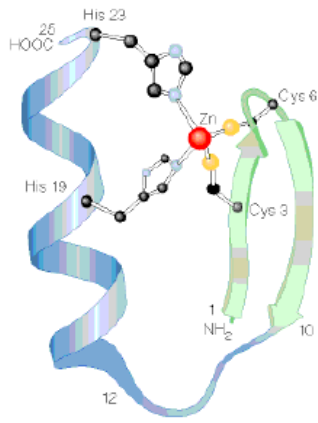
**Motif protéique
B**

NNNNNNNNNNN **GAAATCCC** NNNNNNNNNNNN

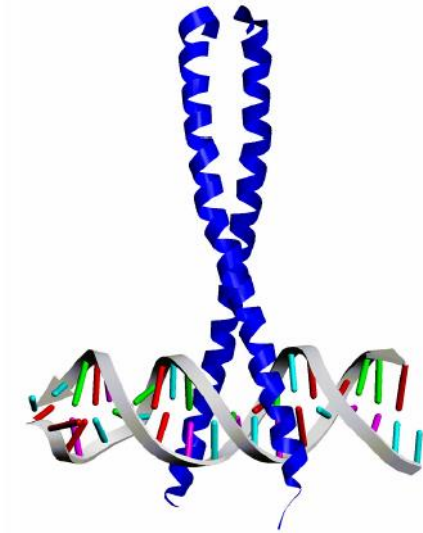


**Motif protéique
C**

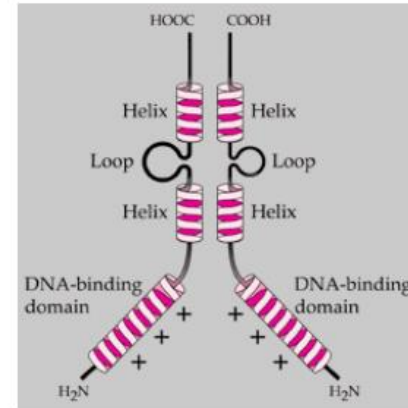
Exemple de motifs protéiques



Zinc Finger

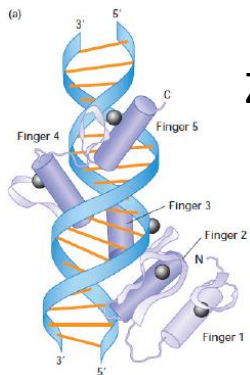
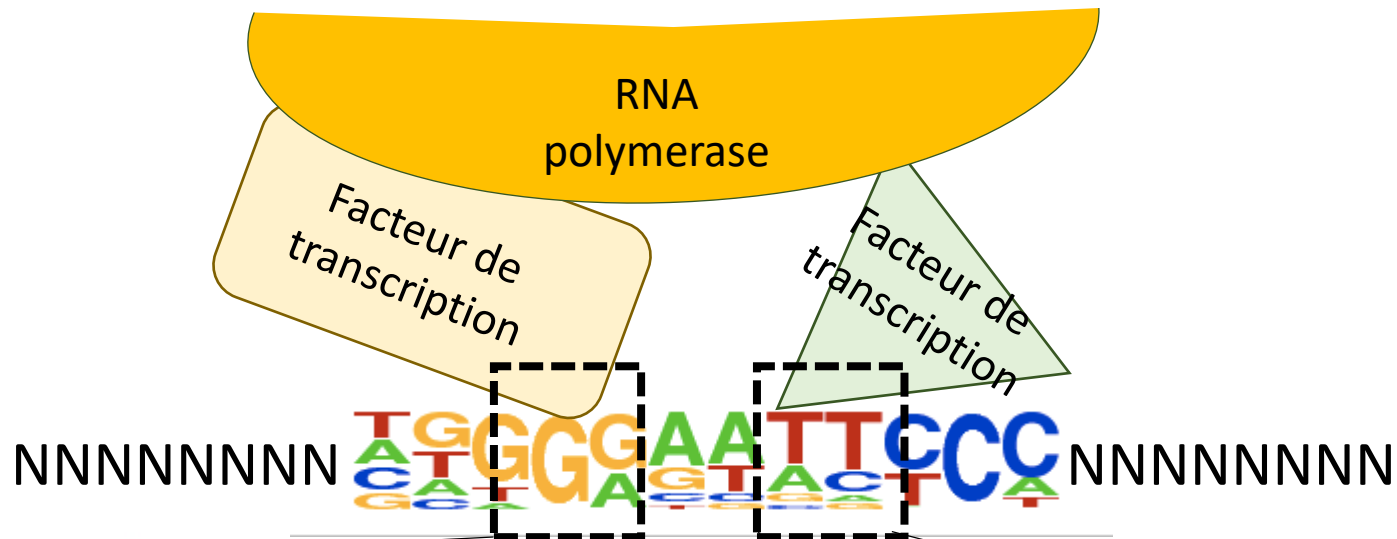
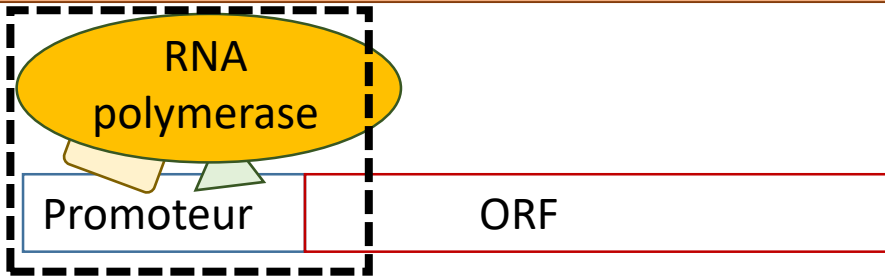


Leucine zipper



bHLH

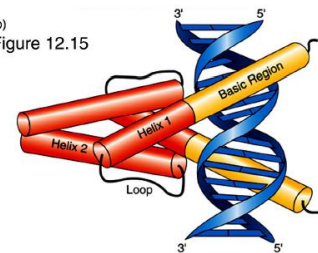
Eléments Cis-Trans dans la régulation



Zinc finger

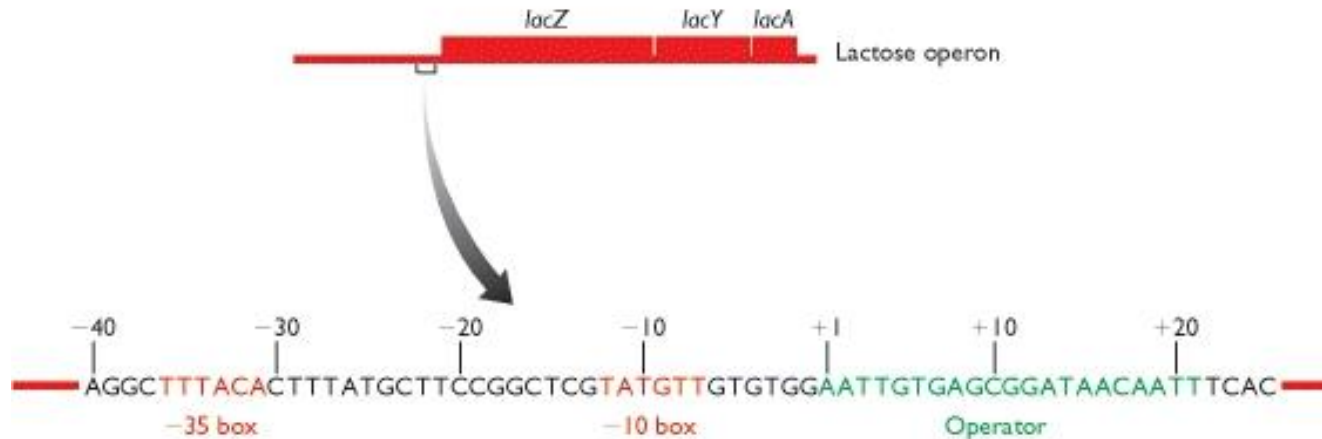
bHLH

(b)
Figure 12.15

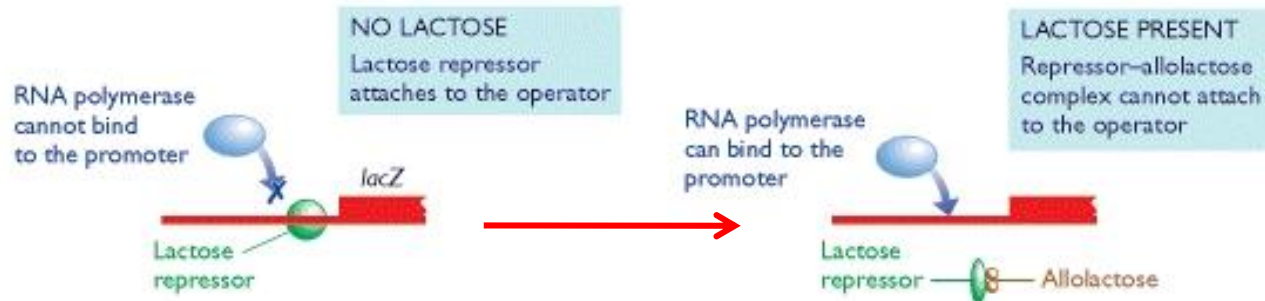


Contrôle négatif d'un gène inductible (éteint)

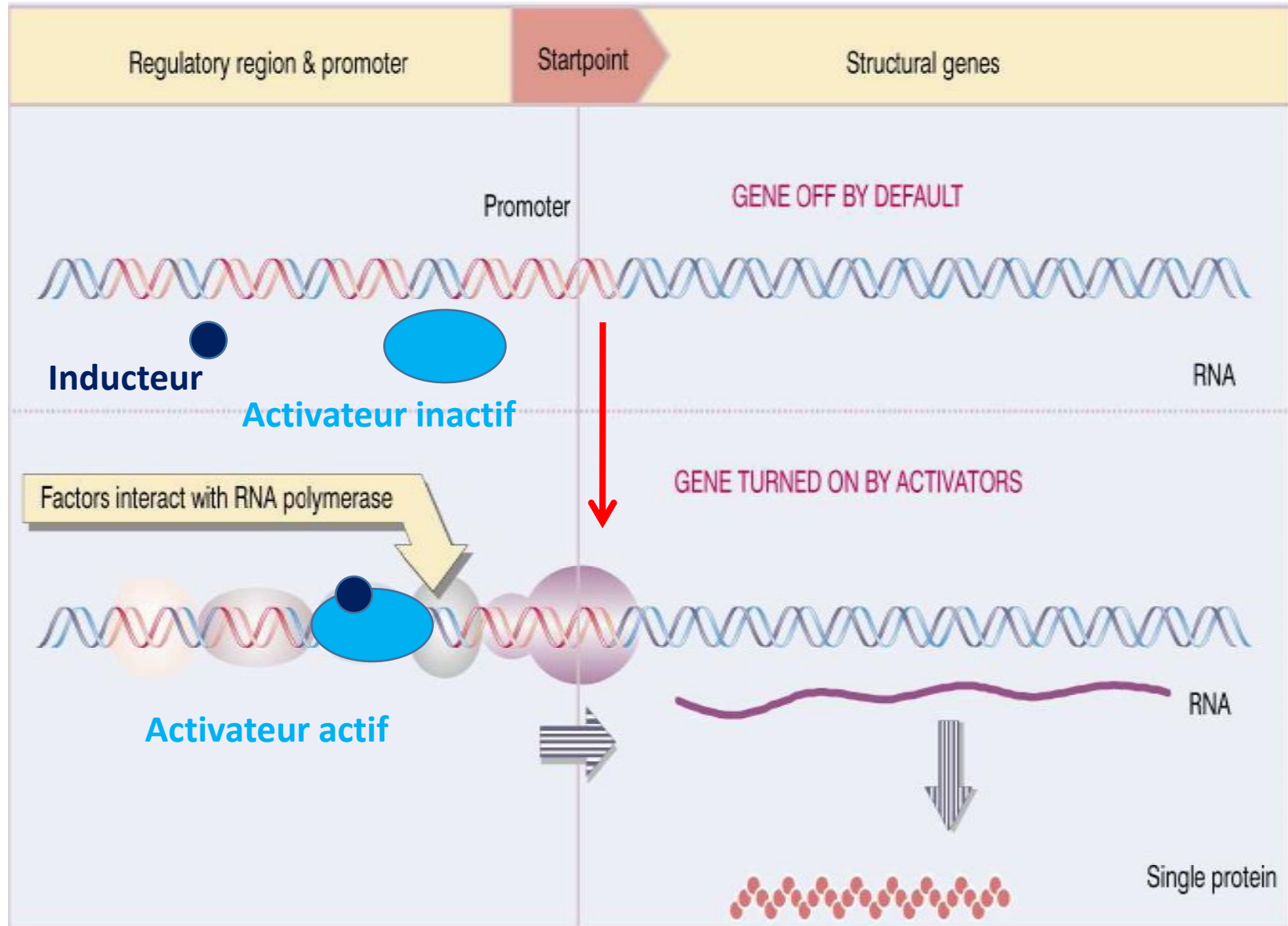
(A) The lactose operator



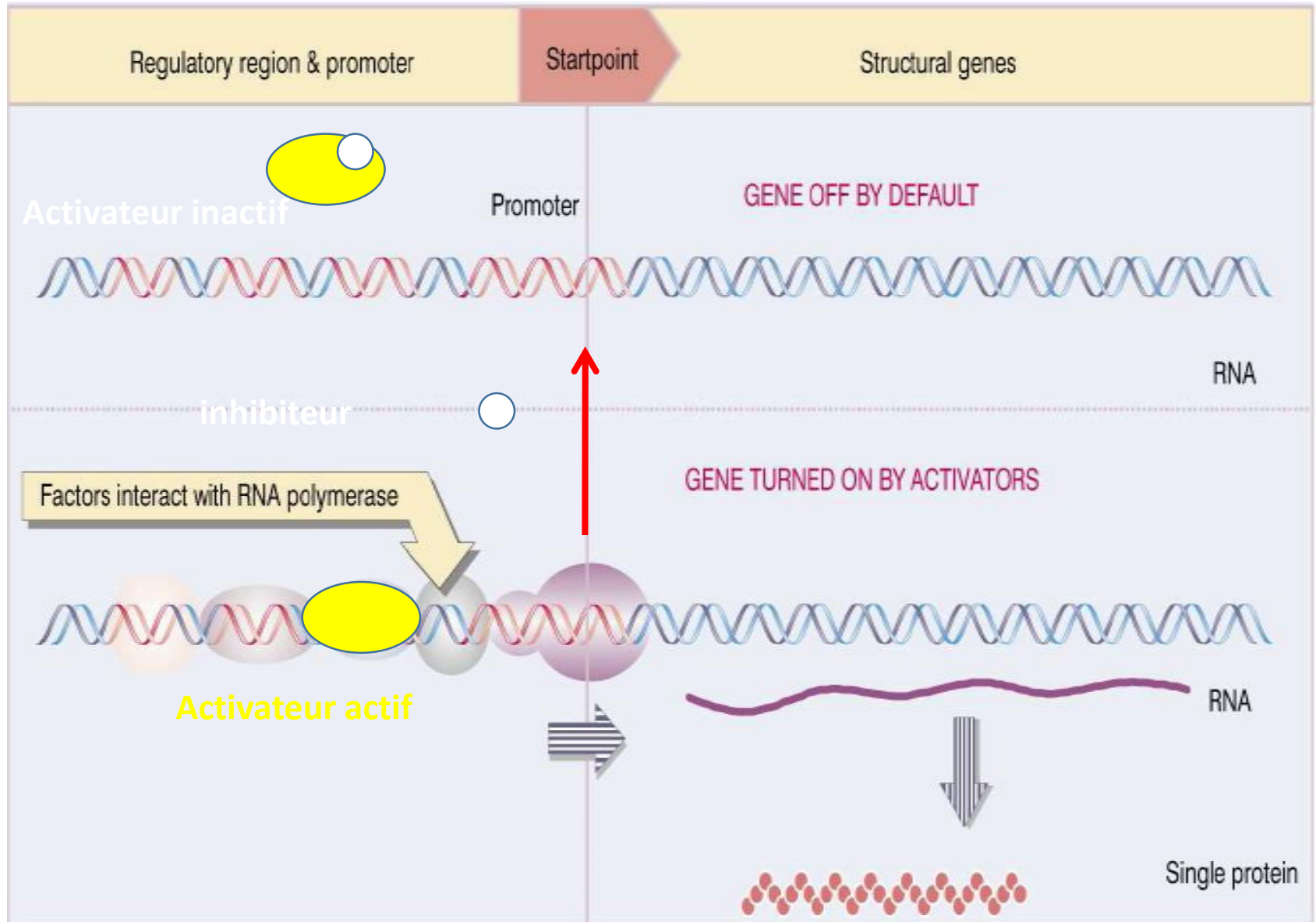
(B) The original model



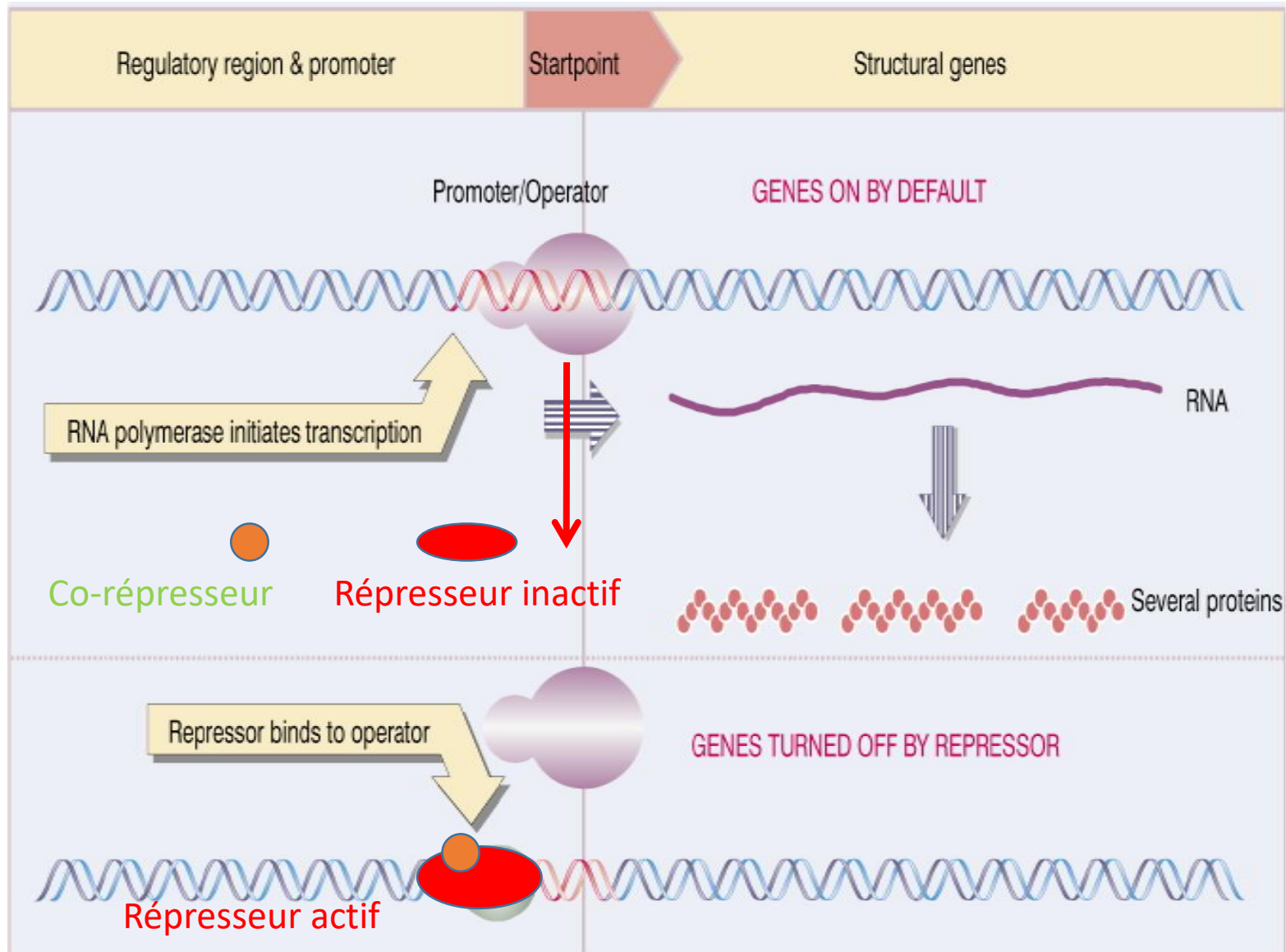
Contrôle positif d'un gène inductible (éteint)



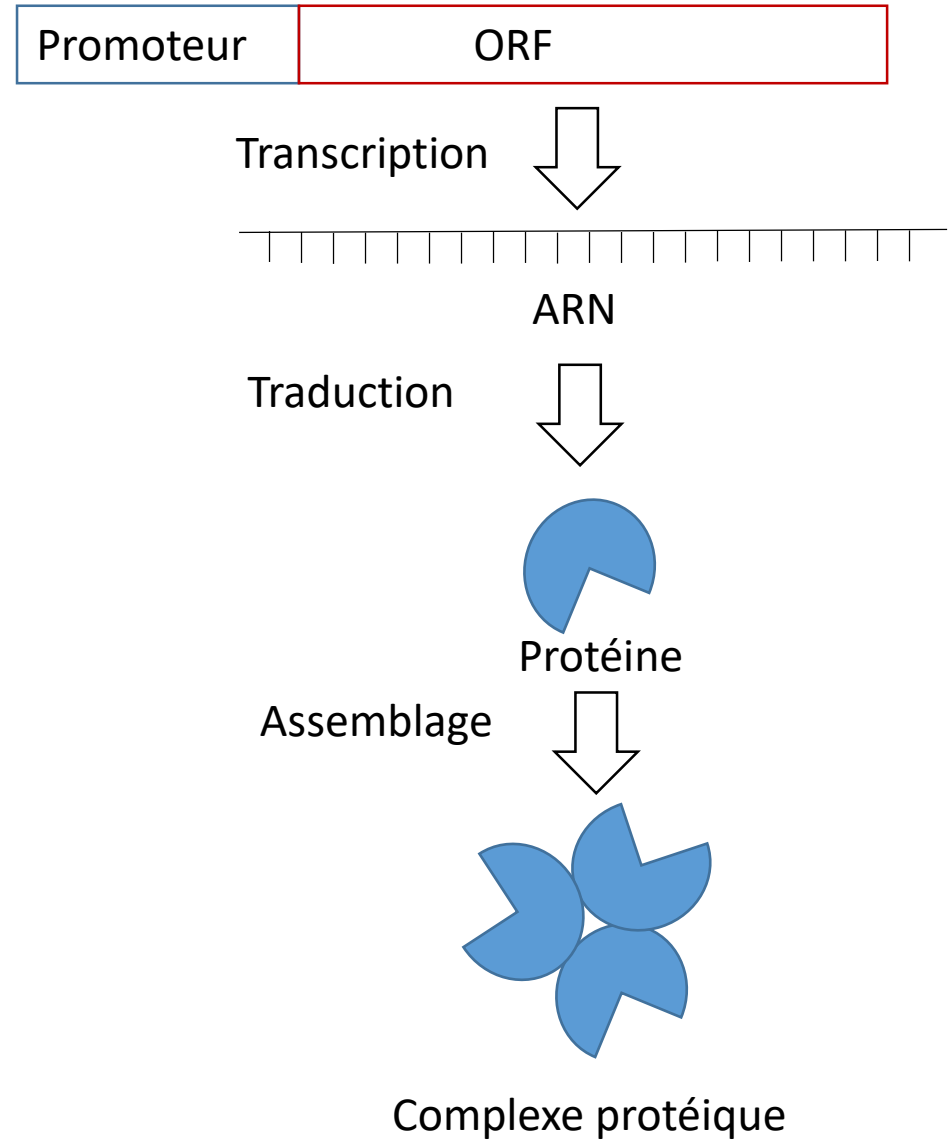
Contrôle positif d'un gène répressible (allumé)



Contrôle négatif d'un gène répressible (allumé)



La régulation de l'expression



La régulation de l'expression

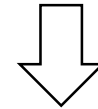
Régulation cis-trans



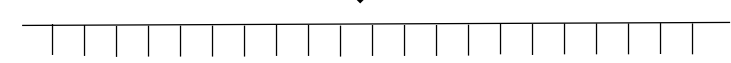
Avortement de la transcription



Transcription



Régulation de la durée de vie

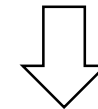


ARN

Avortement de la traduction



Traduction



Régulation de la fonction
/dégradation/Inhibition

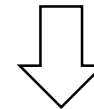


Protéine

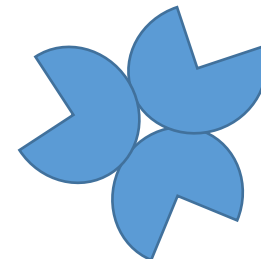
Régulation de l'assemblage



Assemblage

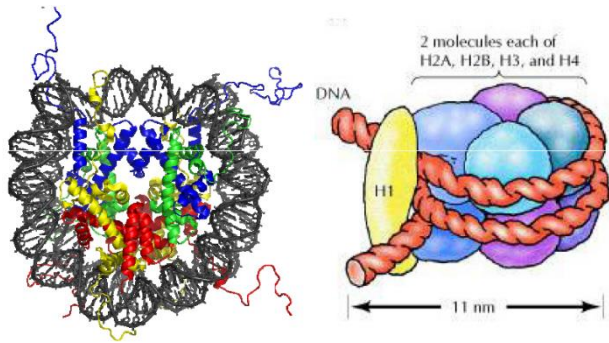


Désassemblage
/dégradation/
inhibition



Complexe protéique

La superstructure de l'ADN dans la régulation



courte région de la double hélice d'ADN

2 nm

chromatine en forme de « perles sur un fil »

11 nm

fibre de chromatine de 30 nm, avec des nucléosomes empilés

30 nm

partie de chromosome sous une forme allongée

300 nm

partie condensée du chromosome

700 nm

chromosome mitotique entier

1400 nm

centromère

RÉSULTAT NET : CHAQUE MOLÉCULE D'ADN A ÉTÉ EMPAQUETÉE DANS UN CHROMOSOME MITOTIQUE 50 000 FOIS PLUS COURT QUE LA MOLÉCULE DÉROULÉE

SAR: protéine associée à l'armature.

HMG: protège l'ADN.

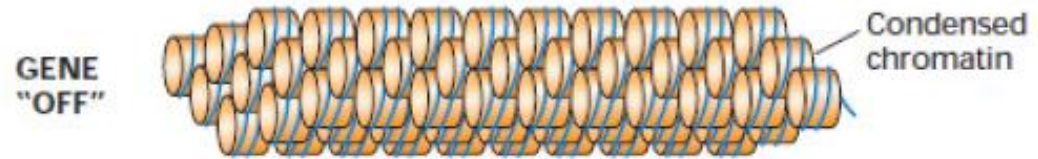
Topoisomérase.

HAT et DHAC.....

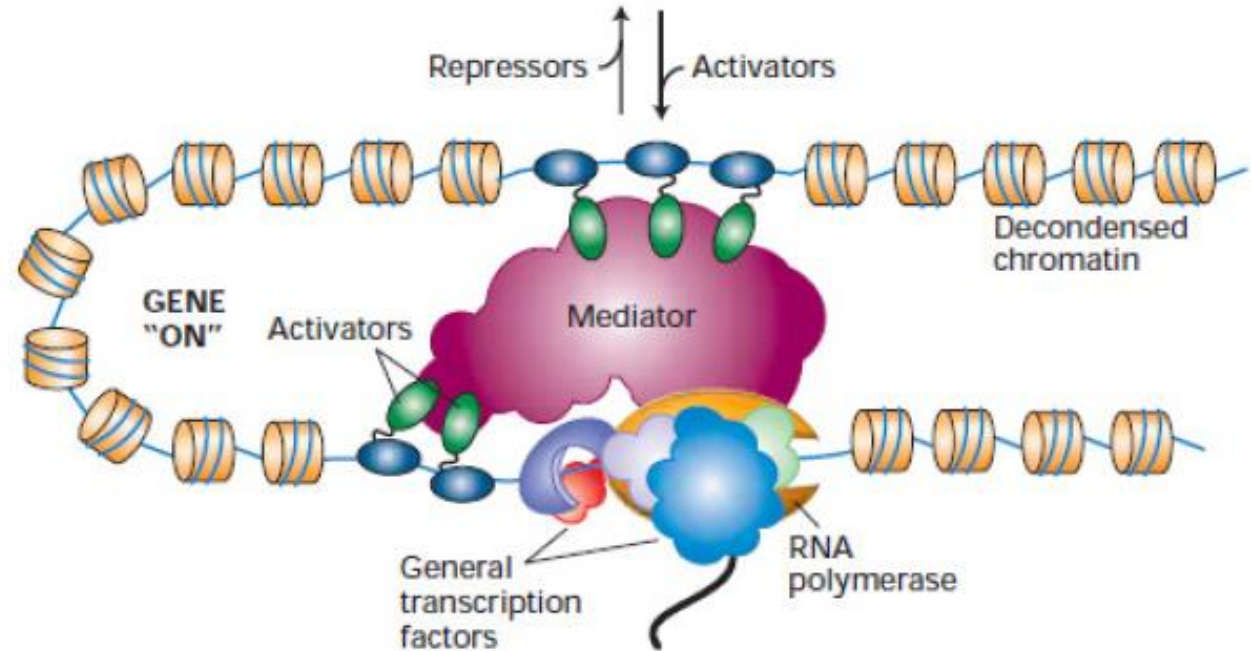
En plus des facteurs de transcriptions et co-transcriptions.

Réguler l'expression en régulant l'accessibilité des zones à transcrire

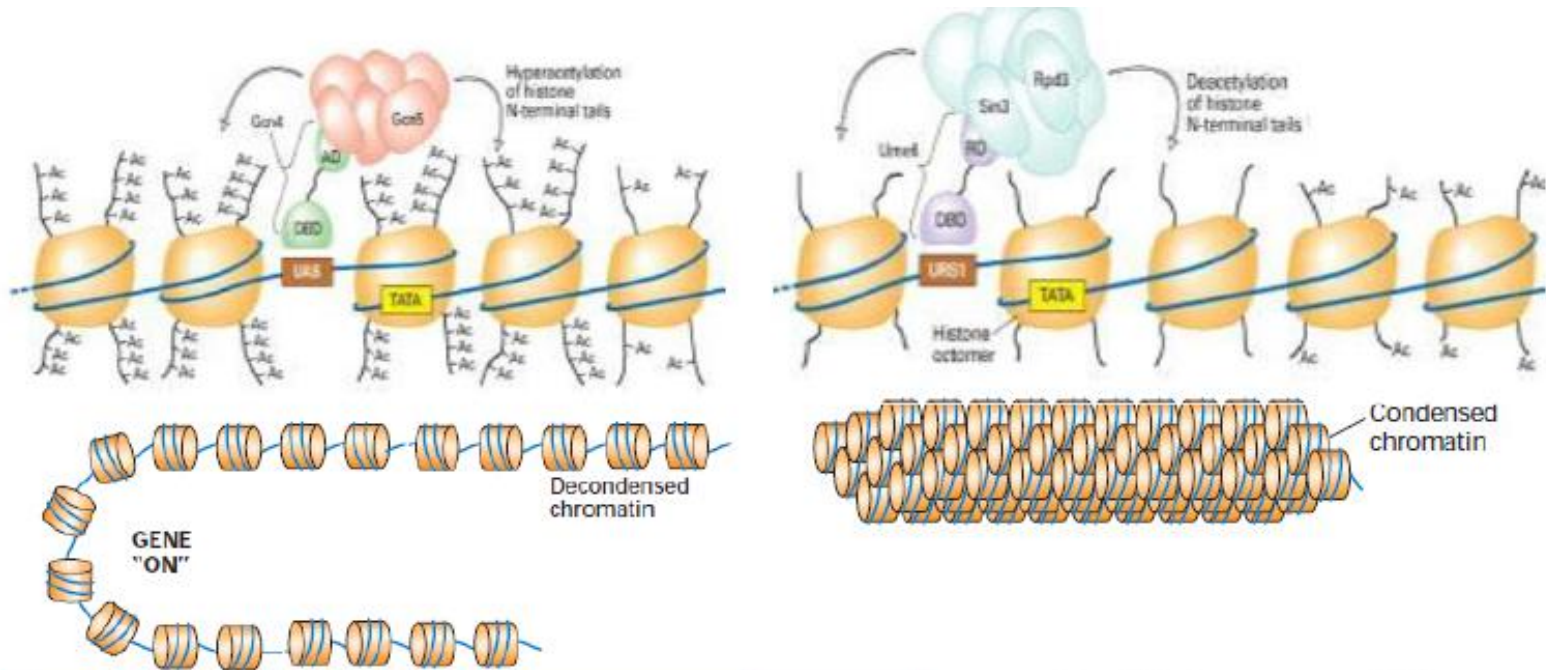
Hétérochromatine



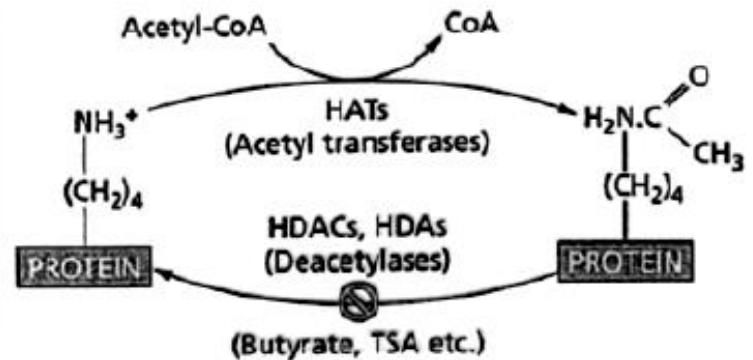
Euchromatine



L'acétylation des histones

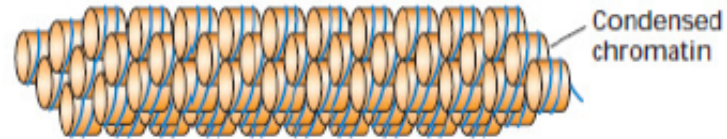


- Acetylation histone (HAT) = activation
- Déacétylation histone (HDAC) = inhibition (complexe SIN)

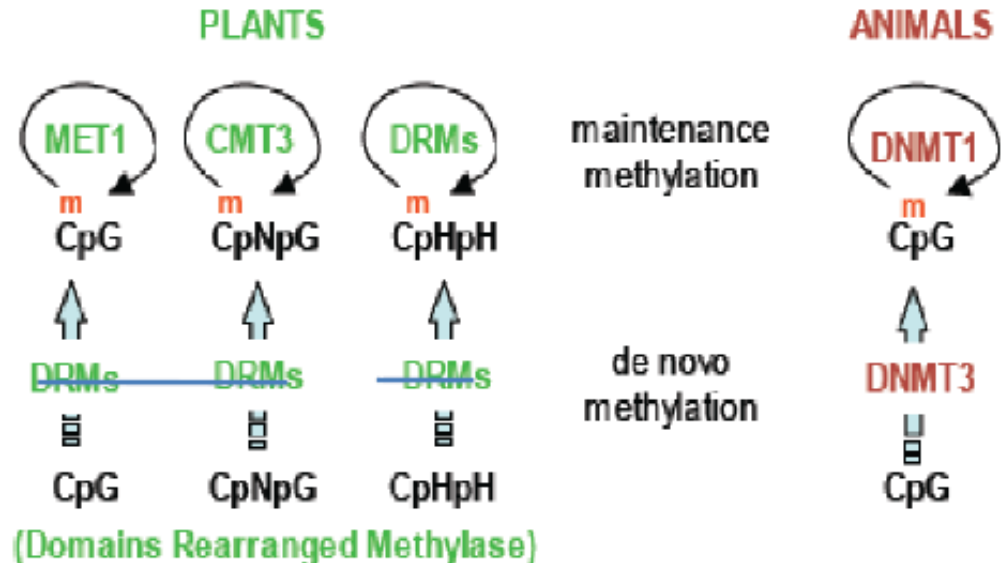


La méthylation de l'ADN

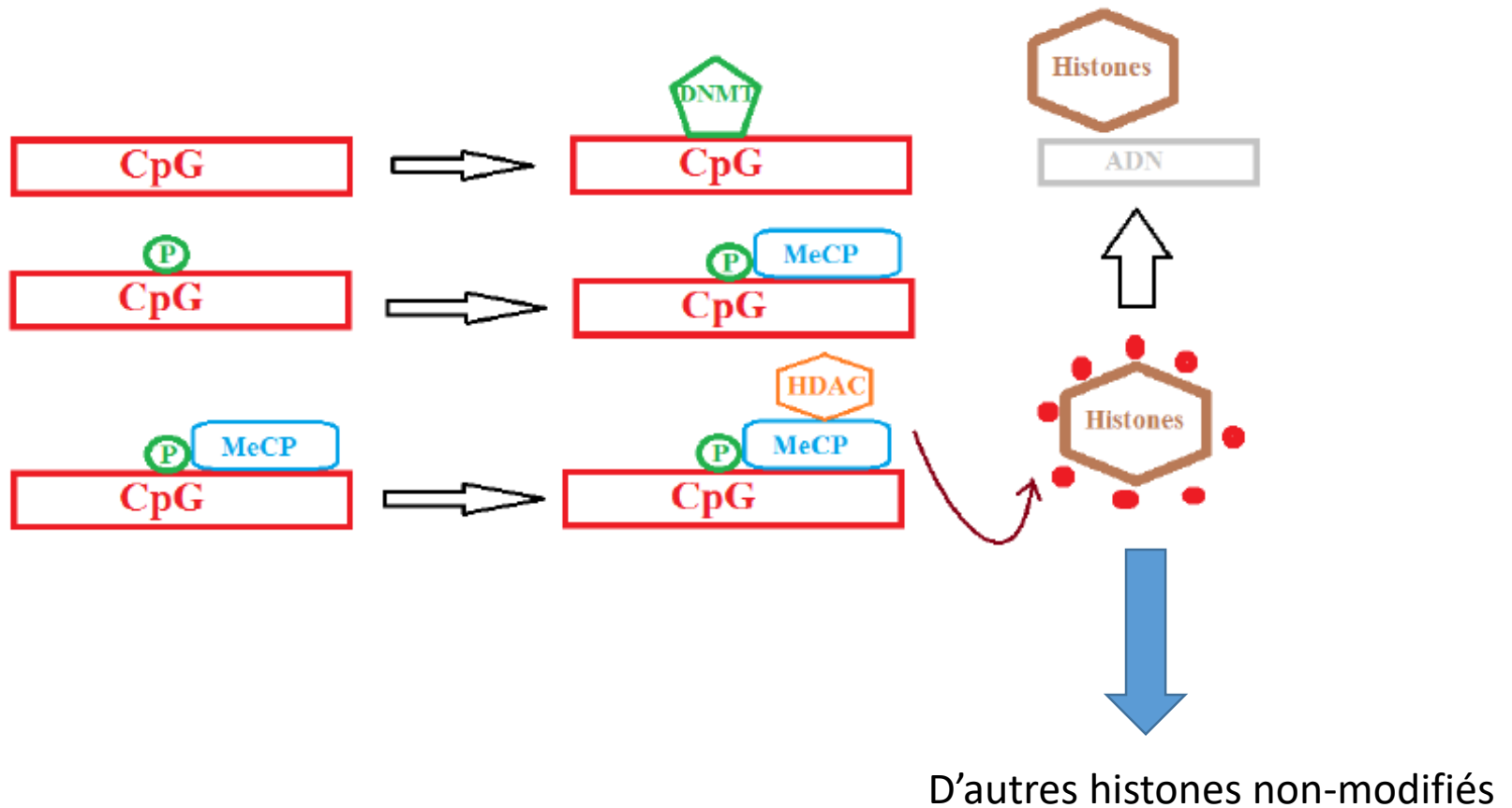
- * Méthylation constitutive ou inductible.
- * Affecte les cystéines.
- * Effet répressif: formation d'hétérochromatine



Symétrique	Asymétrique
5'-CG-	-CNG-
3'-GC-	-GN'C-
	-CNN-
	-GN'N' -



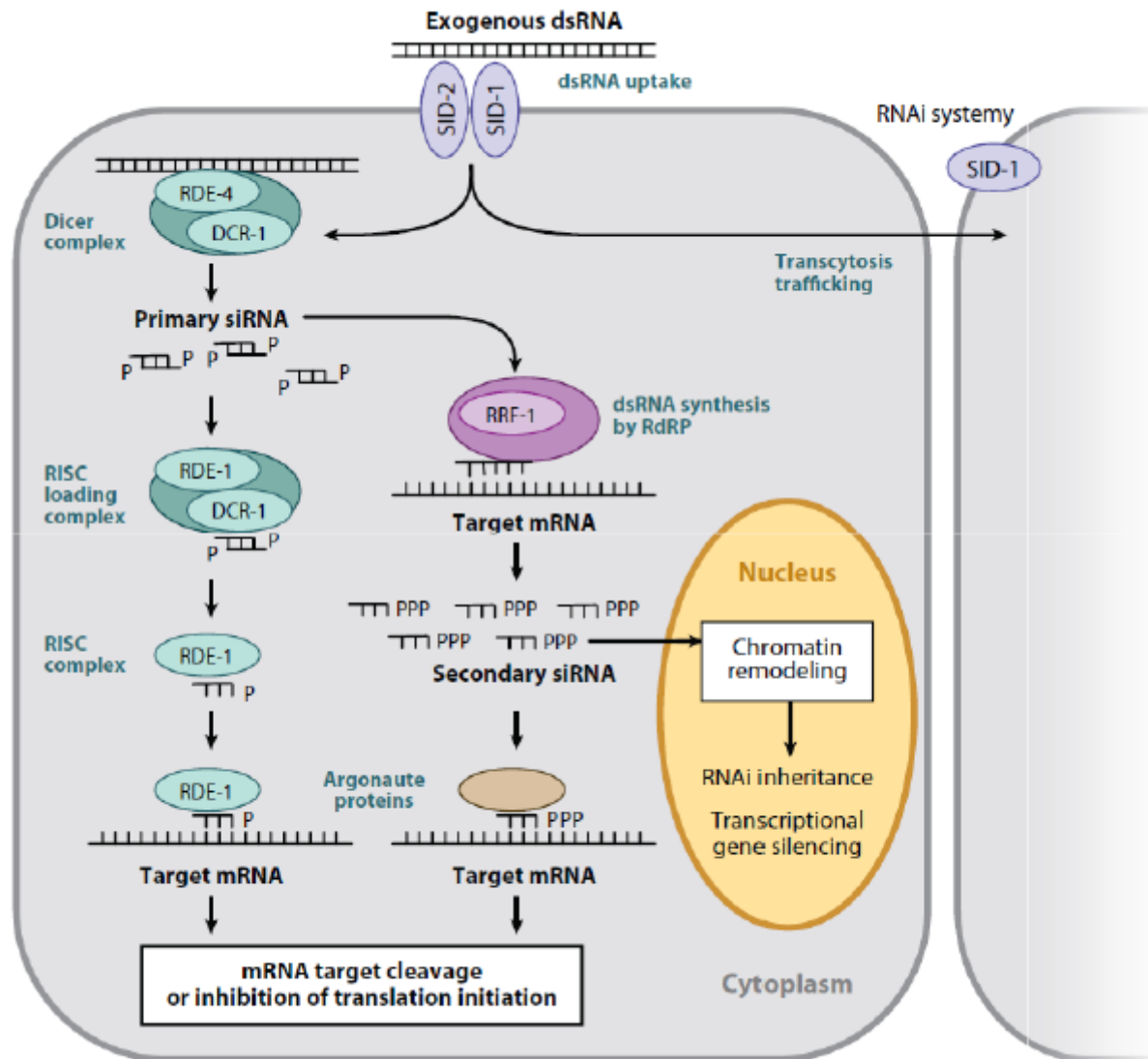
Les effets croisés méthy/acetylation, histone/ADN



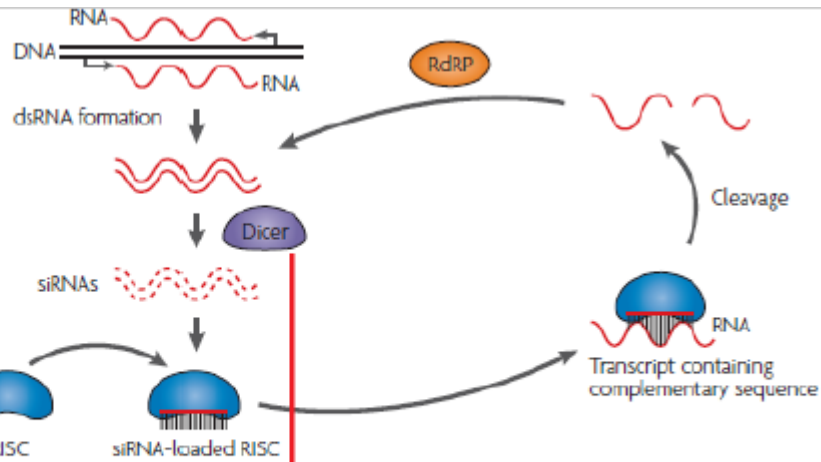
L'ubiquitination dans la régulation de l'expression

- Ajout de résidus ubiquitine sur les K des protéines induits:
 - dégradation via protéasome
 - inactivation par modification structurel, modification des interactions et compétition avec d'autre modification post-traductionnelle.
- Effet dépend de la position et du nombre de résidus d'ubiquitine fixés.
- Effet sur co/facteurs de transcriptions mais aussi sur histones

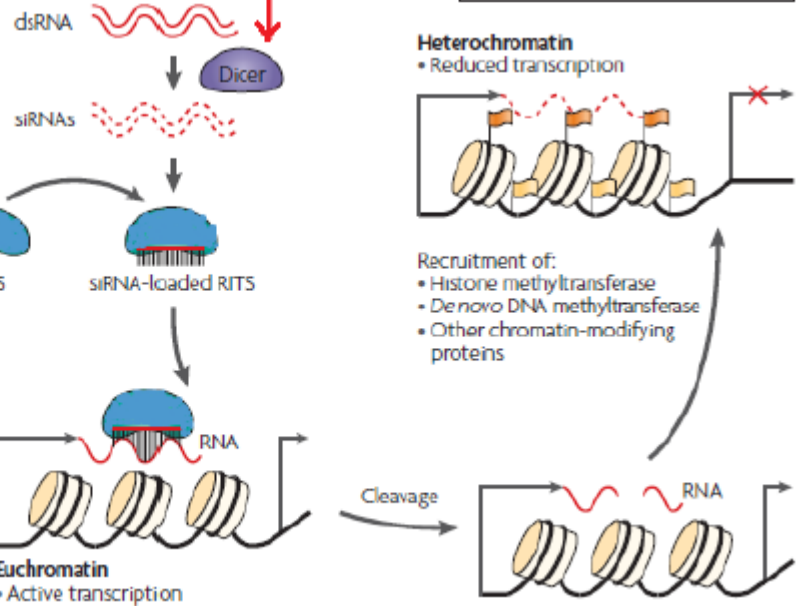
Les petits ARNs dans la régulation d'expression



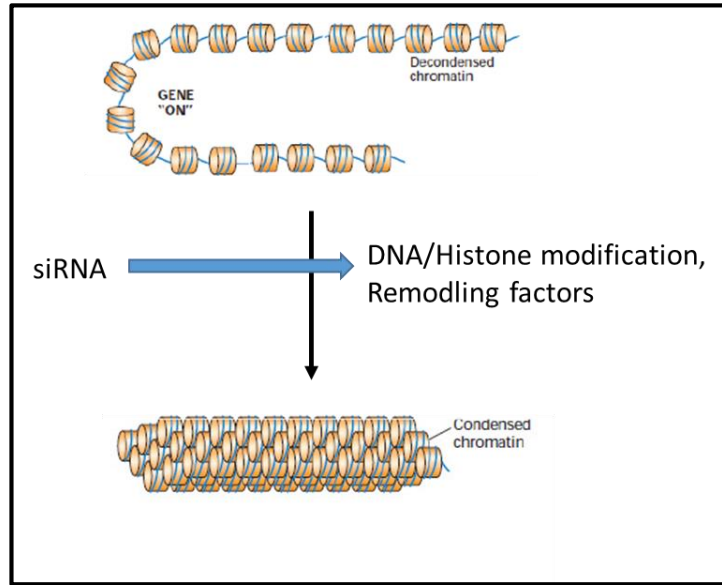
Les petits ARNs dans la régulation d'expression



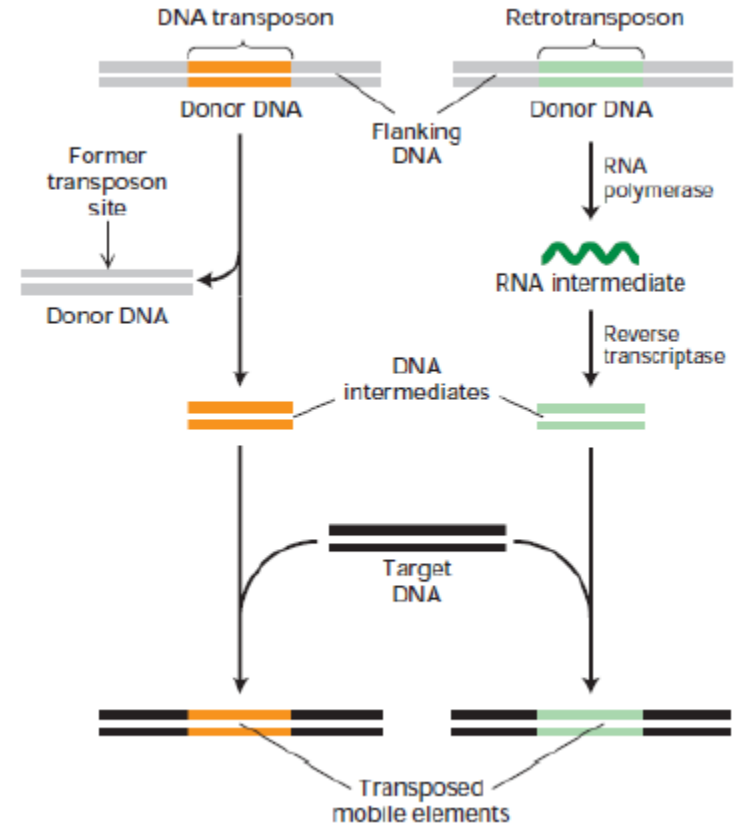
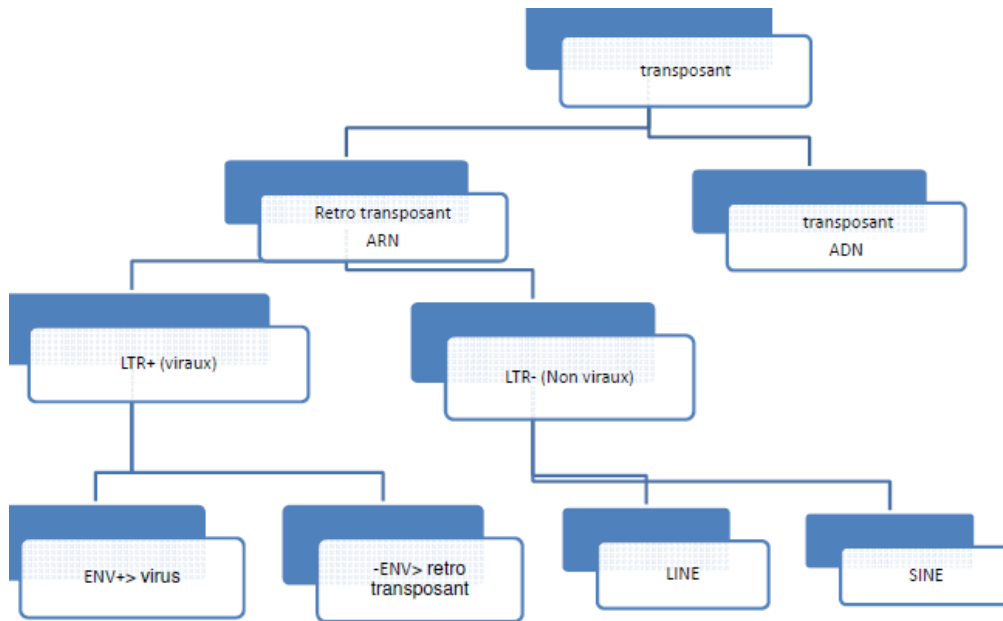
Methylation at histone H3K9
 CpG DNA methylation



RITS= RISC (levure)

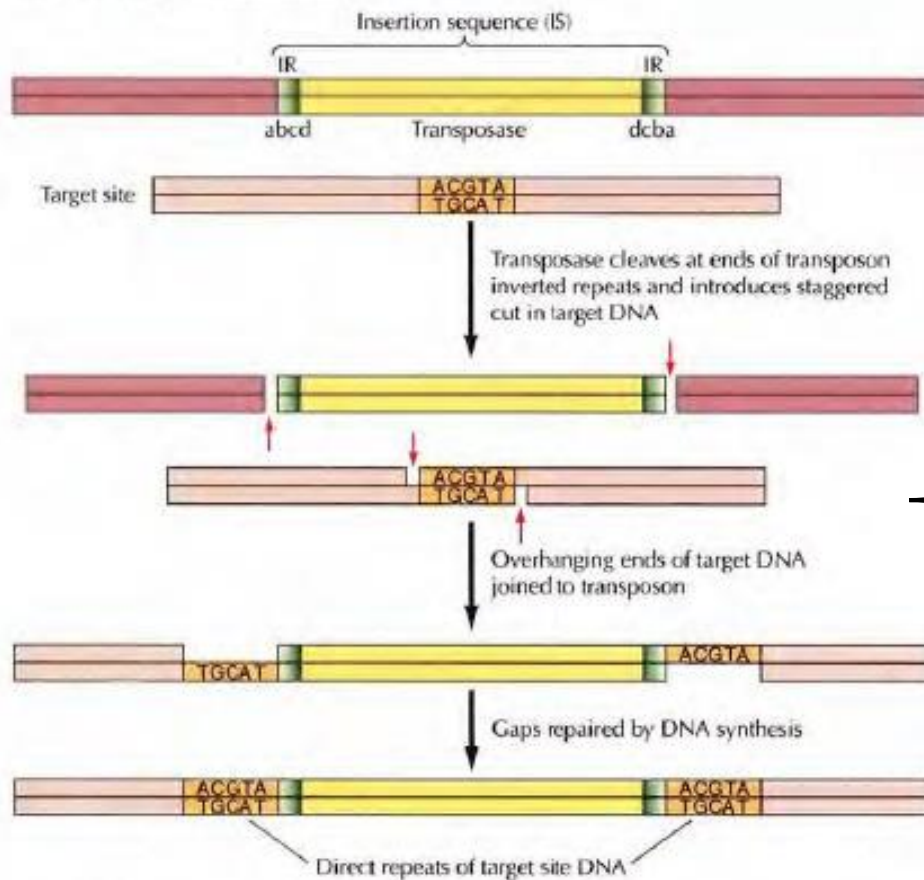


Les transposants

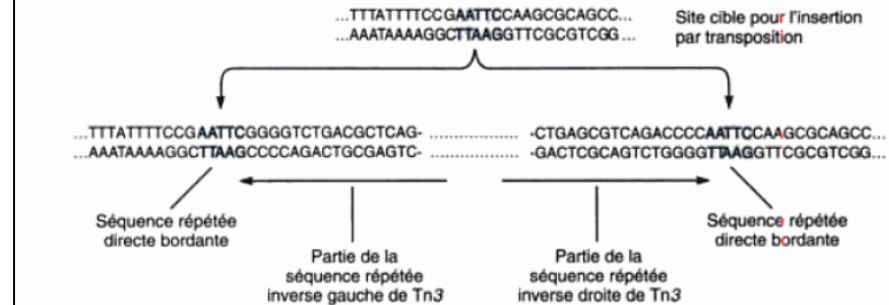


Les éléments d'insertion chez les bactéries

Les IS bactériens sont l'équivalent des transposant ADN chez les eucaryotes



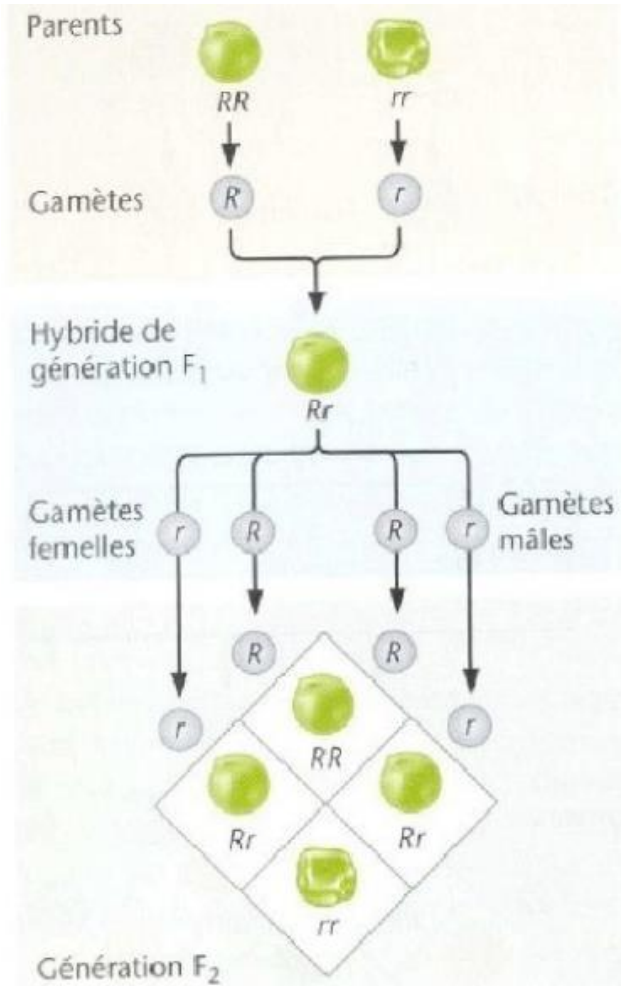
Site cible du transposon Tn3



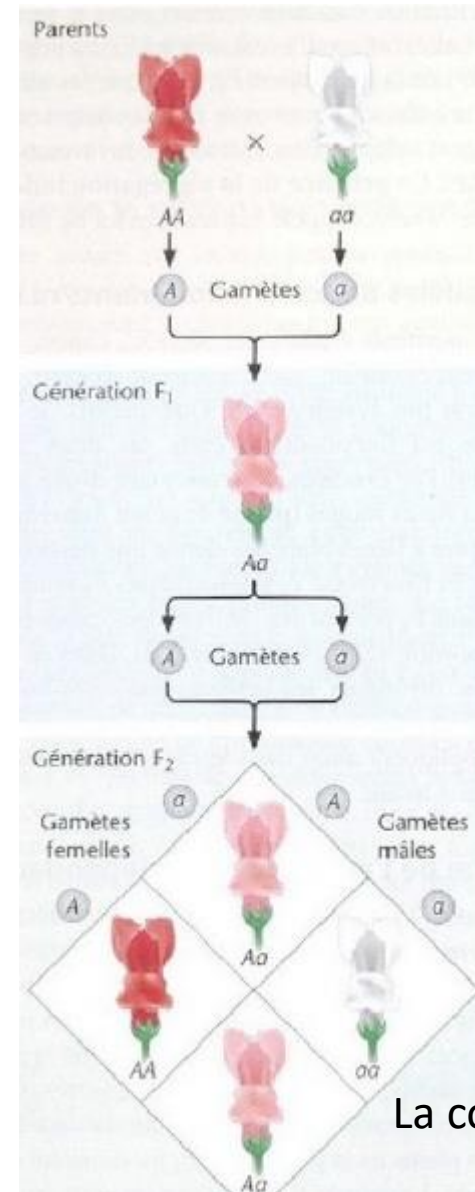
Effets des transposants sur la régulation de l'expression

- Les transposants sont généralement associées à l'hétérochromatine et au région pericentromérique/subtélomérique.
- Ils forment des insulateurs.
- L'insertion des transposants peut modifier l'organisation de la séquence d'ADN et donc perturber les interactions entre éléments CIS et Trans.
- Certains transposants peuvent piéger des CIS éléments de régulation.
- Indispensabilité de maintien de répression lors de la divisions.
- Présence d'une spécialisation régionale en terme de transposant et d'induction de transposition.

La génétique classique

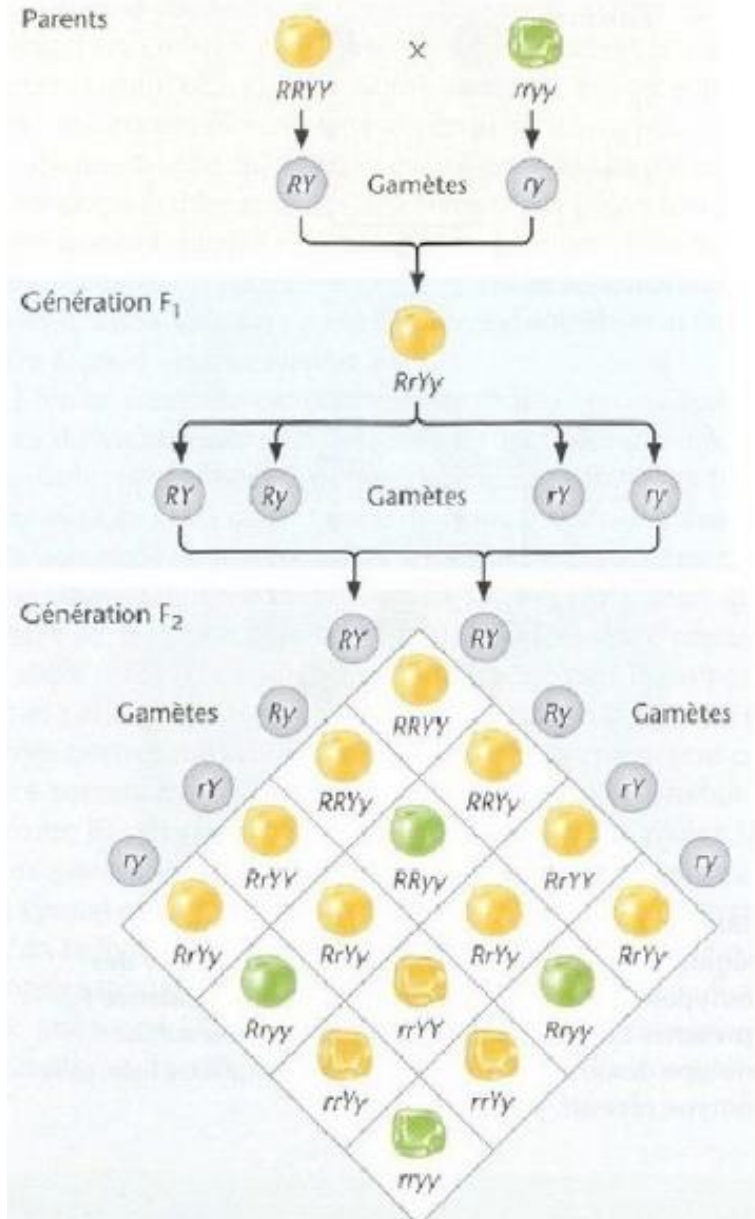


Transmission d'un caractère simple 3:1



La codominance 1:2:1

La génétique classique



Transmission de deux caractères

	RY	Ry	rY	ry
RY	RYRY	RYRy	rYRY	Ryry
Ry	RyRY	RyRy	RyrY	Ryry
rY	rYRY	rYRy	rYrY	ryry
ry	ryRY	ryRy	ryrY	ryry

Phénotype Parental:

Jaune lisse: 9

Vert rugueux: 1

Phénotype hybride:

Jaune rugueux: 3

Vert lisse: 3

Les distances génétiques : the crossing over

La distance génétique désigne la distance virtuelle entre deux locus situés sur un même chromosome. Elle s'exprime le plus souvent en centimorgans (Wikipedia)

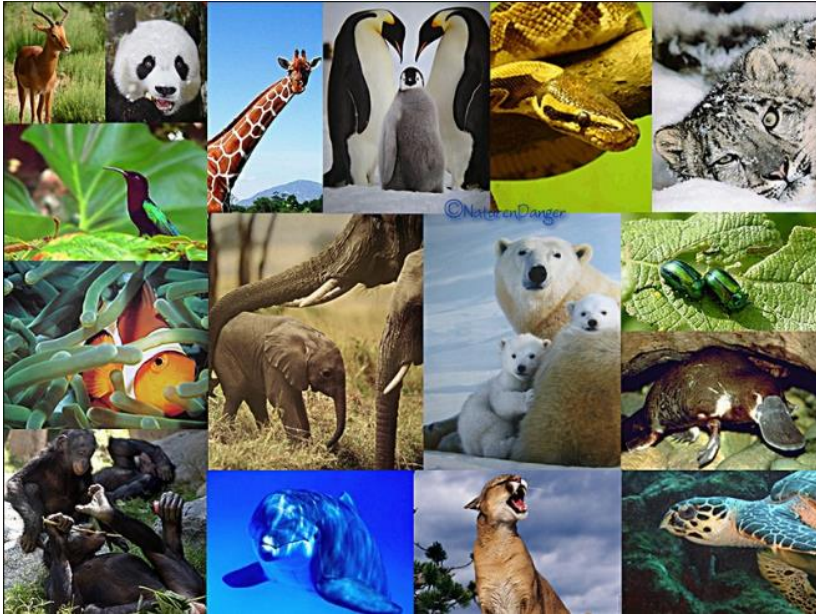


La distance génétique désigne se calcule par : nombre de recombinant/effectif totale

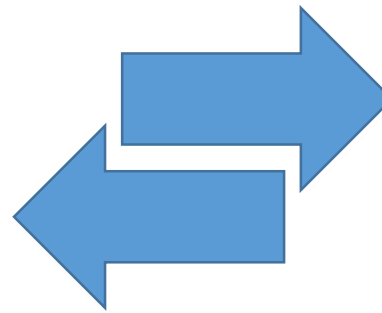
234 Rrbb ---> Parental
246 rrBb ---> Parental
54 RrBb ---> recombiné
66 rrbB ---> recombiné

Distance génétique entre R et B : $(54+66)/600 = 0,2$ soit 20cM

Objectifs du génie génétique



Comprendre le vivant



Améliorer le vivant



Fin du chapitre I