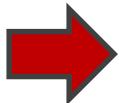


# Cours Biochimie I

## Leçon 6: Synthèse des protéines / expression recombinant



Leçon	Subject	Page en Stryer
1	Introduction en biochimie / classes de biomolécules / aminoacides	4-17 (1.2, 1.3), 25-40 (2.1, 2.2)
2	Composition et structure des protéines	40-59 (2.3-2.6)
3	Exploration des protéines et des protéomes	65-90 (3.1-3.3), 93-101 (3.5, 3.6)
4	Structure de la DNA et RNA	107-119 (4.1-4.3)
5	Explorer les gènes et les génomes	134-144 (5.1, 5.2 premières 3 pages)
6	Synthèse des protéines / expression recombinante	117-119 (4.3), 119-127 (4.4), 142-148 (5.2)

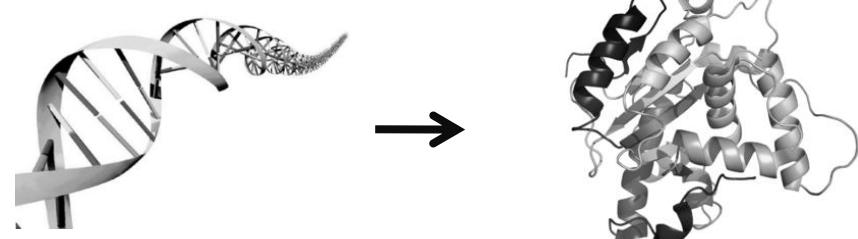
Christian Heinis

christian.heinis@epfl.ch, BCH 5305

# Leçon 6

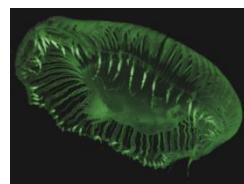
- Synthèse des protéines

- RNA
- Ribosome



- Expression de protéines recombinantes

- DNA isolé d'un donneur
- Plasmide (vecteur) de DNA
- Expression dans un hôte différent



une méduse

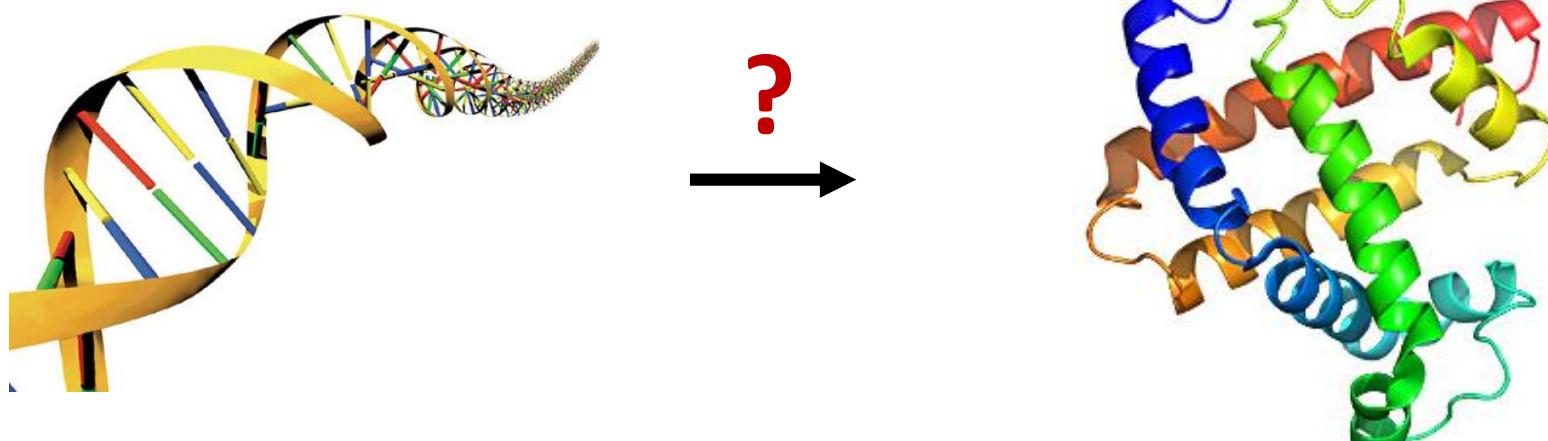


DNA d'une protéine  
fluorescente

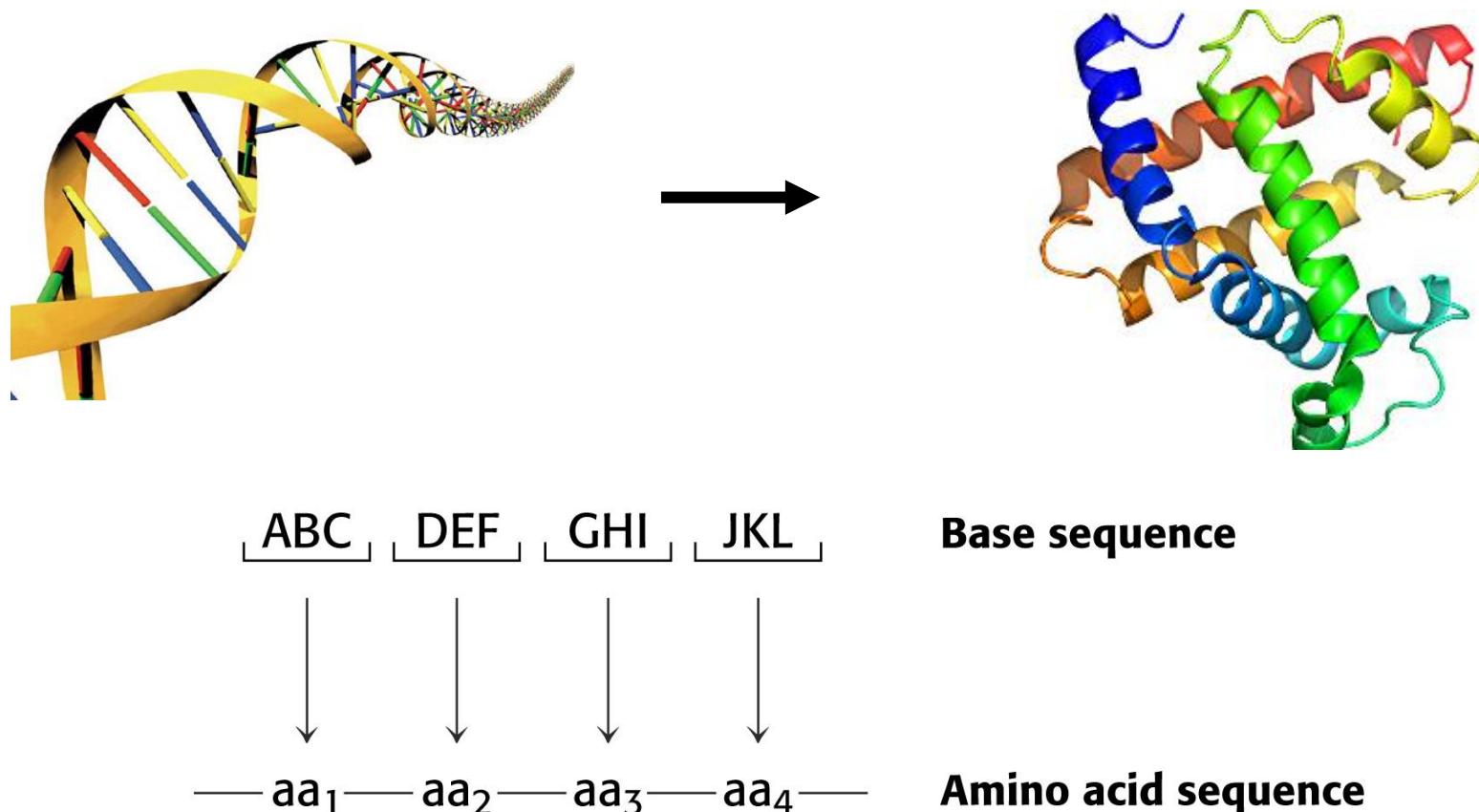


souris fluorescentes

# Synthèse des protéines



# Synthèse des protéines



**TABLE 5.4** The genetic code**64 codons**

First position (5' end)	Second position				Third position (3' end)
	T	C	A	G	
T	Phe	Ser	Tyr	Cys	T
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	T
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	T
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	T
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Note: This table identifies the amino acid encoded by each triplet. For example, the codon 5' AUG 3' on mRNA specifies methionine, whereas CAU specifies histidine. UAA, UAG, and UGA are termination signals. AUG is part of the initiation signal, in addition to coding for internal methionine residues.

**TABLE 5.4** The genetic code

First position (5' end)	Second position				Third position (3' end)
	T	C	A	G	
T	T	Ser	Tyr	Cys	T
	Phe	Ser	Tyr	Cys	C
	Phe	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	T
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	T
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	T
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Note: This table identifies the amino acid encoded by each triplet. For example, the codon 5' AUG 3' on mRNA specifies methionine, whereas CAU specifies histidine. UAA, UAG, and UGA are termination signals. AUG is part of the initiation signal, in addition to coding for internal methionine residues.

**Exemple:****TTC → Phe**

**TABLE 5.4** The genetic code

First position (5' end)	Second position				Third position (3' end)
	T	C	A	G	
T	Phe	Ser	Tyr	Cys	T
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	T
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	T
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	T
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Quelques amino acids ont plusieurs codes:

TCT → Ser

TCC → Ser

TCA → Ser

TCG → Ser

AGT → Ser

AGC → Ser

Note: This table identifies the amino acid encoded by each triplet. For example, the codon 5' AUG 3' on mRNA specifies methionine, whereas CAU specifies histidine. UAA, UAG, and UGA are termination signals. AUG is part of the initiation signal, in addition to coding for internal methionine residues.

**TABLE 5.4** The genetic code

First position (5' end)	Second position				Third position (3' end)
	T	C	A	G	
T	Phe	Ser	Tyr	Cys	T
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	T
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	T
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	T
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

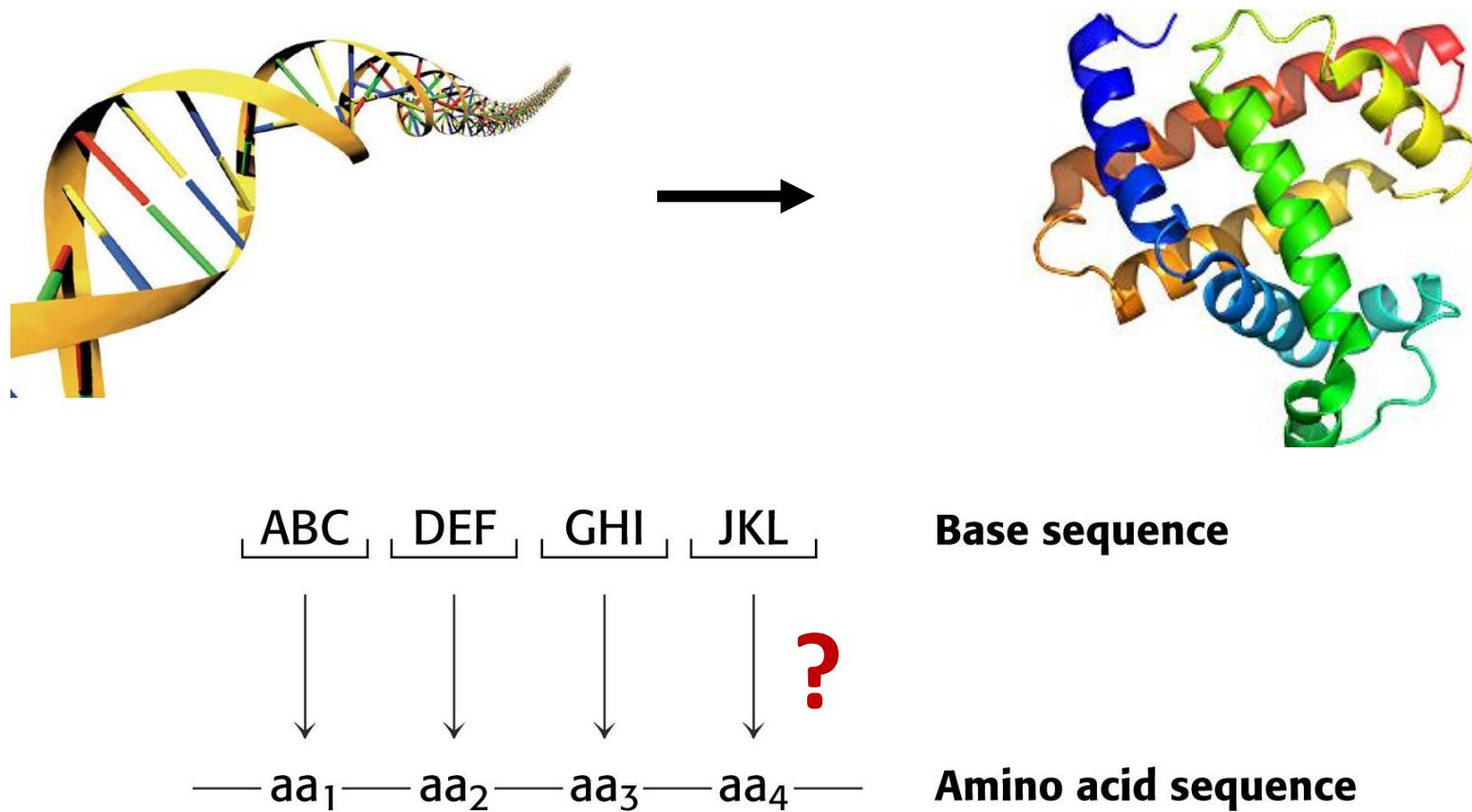
Quelques aminoacids ont seulement un code:

ATG → Met

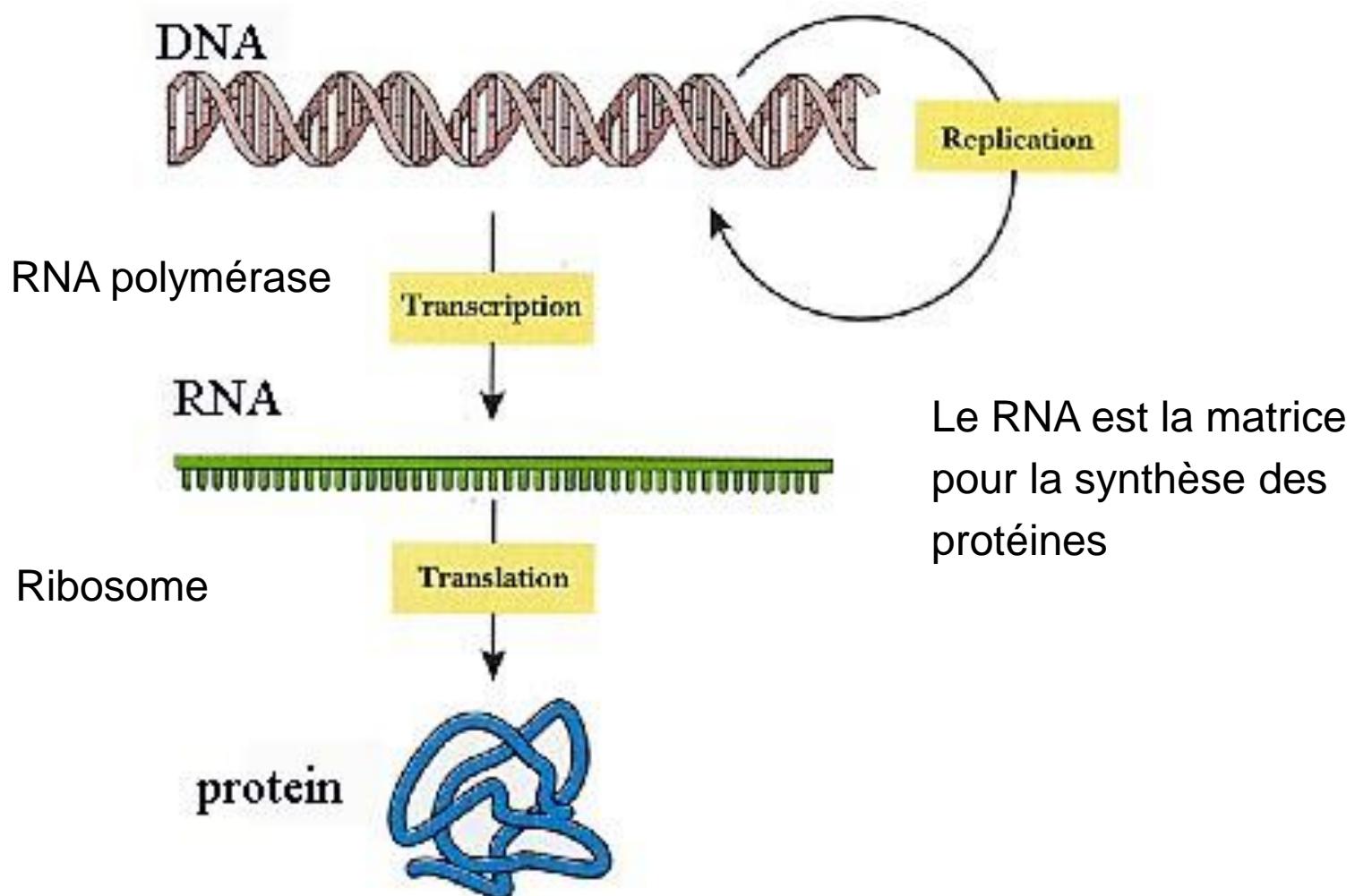
TGG → Trp

Note: This table identifies the amino acid encoded by each triplet. For example, the codon 5' AUG 3' on mRNA specifies methionine, whereas CAU specifies histidine. UAA, UAG, and UGA are termination signals. AUG is part of the initiation signal, in addition to coding for internal methionine residues.

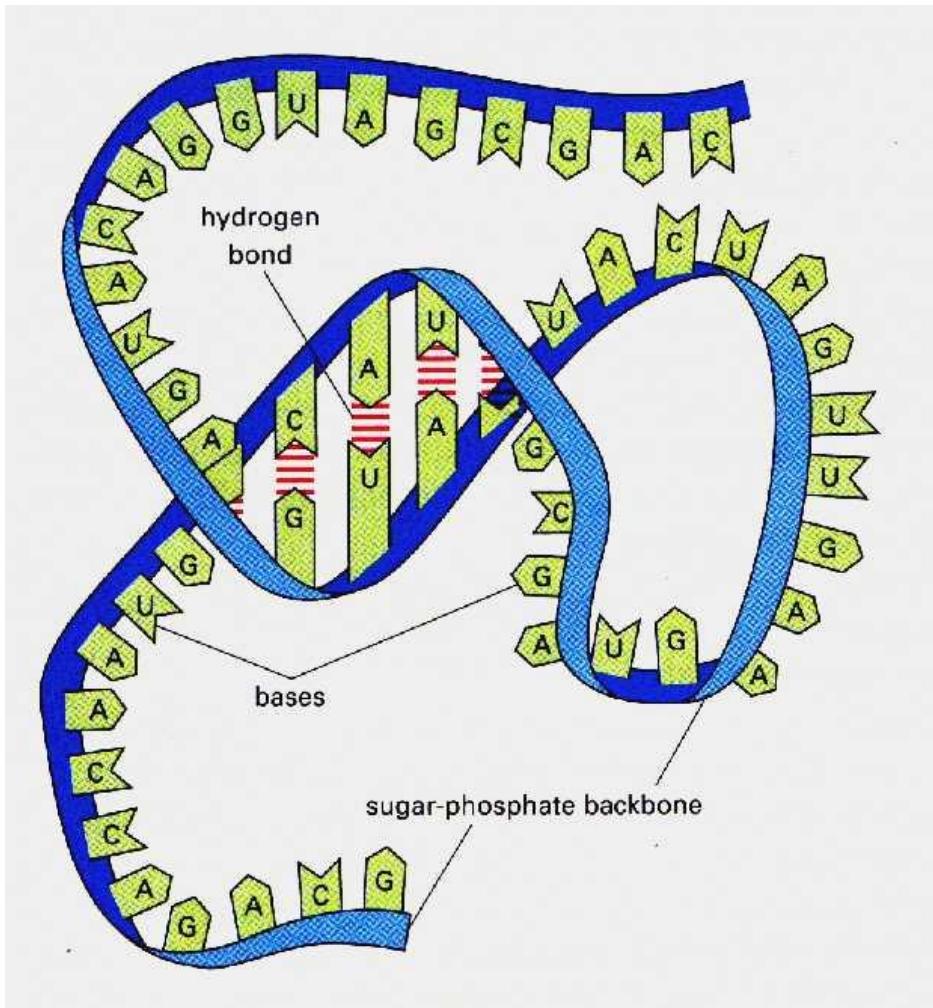
# Synthèse des protéines



# DNA → RNA → protéine

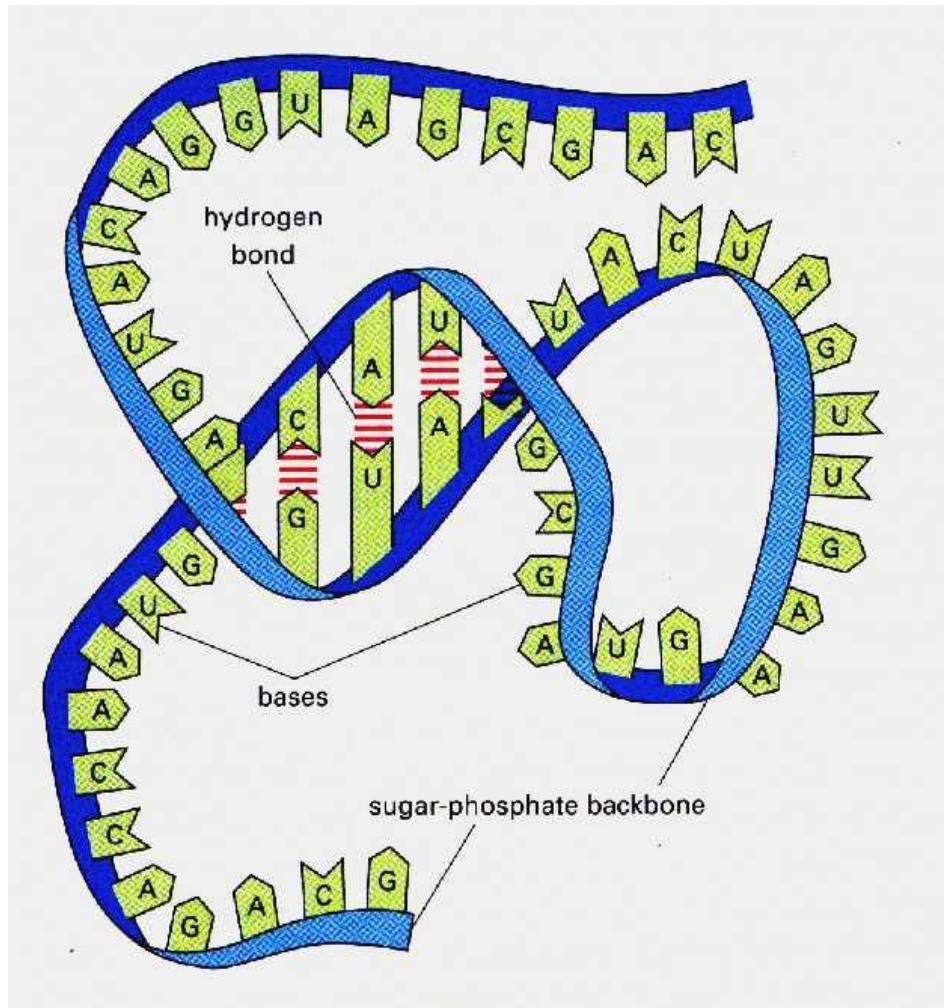


# RNA



- Le RNA est un polymère linéaire composé de nucléotides comme le DNA
- Le RNA possède un seul brin (ne forme pas de double hélices comme le DNA)

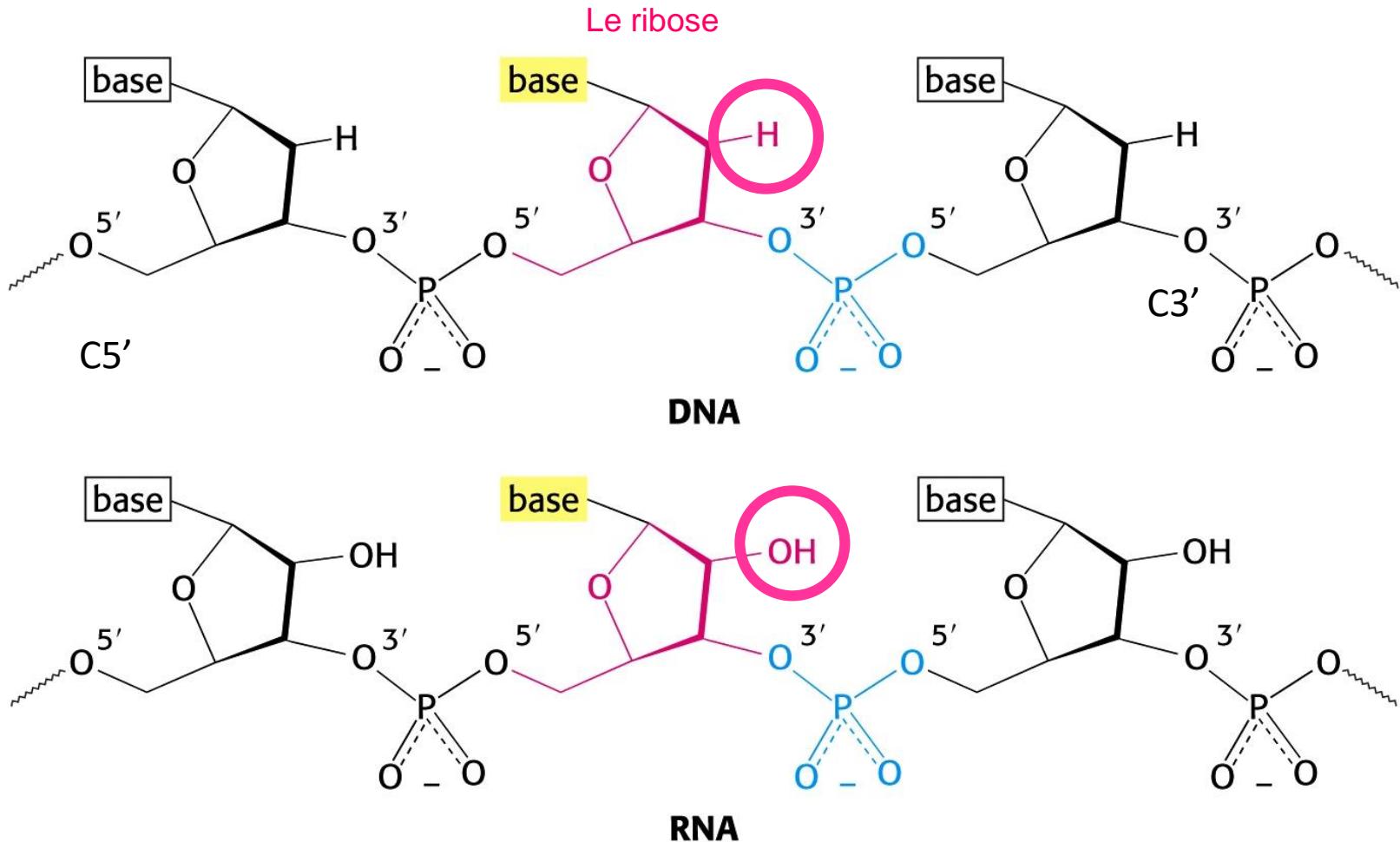
# RNA



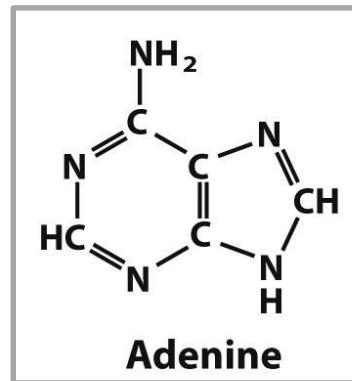
- Le RNA est un polymère linéaire composé de nucléotides comme le DNA
- Le RNA possède un seul brin (ne forme pas de double hélices comme le DNA)

*Quelles sont les différences entre la structure chimique du DNA et du RNA?*

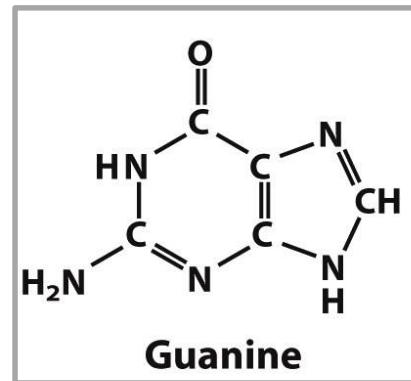
# Structure chimique de RNA



# Les bases de DNA et RNA

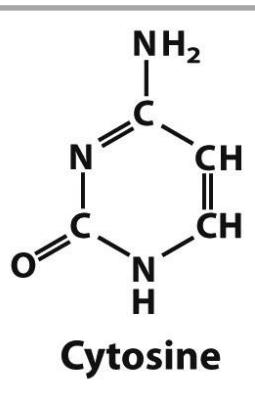


Adenine

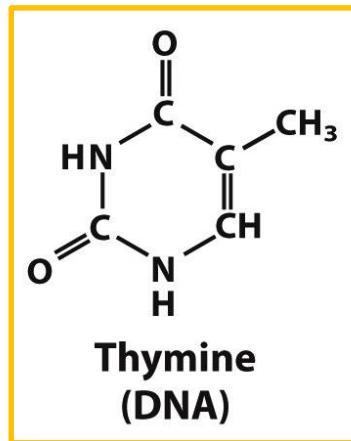


Guanine

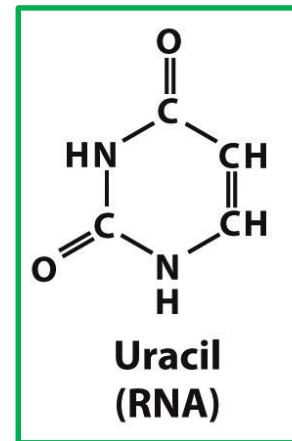
## Purines



Cytosine



Thymine  
(DNA)



Uracil  
(RNA)

## Pyrimidines

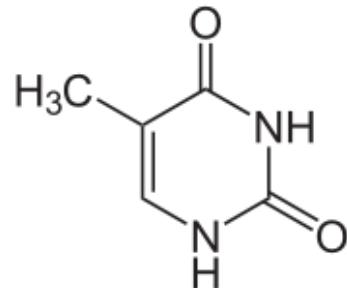
Figure 8-2

*Lehninger Principles of Biochemistry, Fifth Edition*

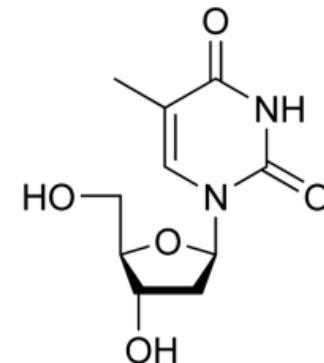
© 2008 W.H. Freeman and Company

# Les bases et nucléosides de DNA et RNA

DNA

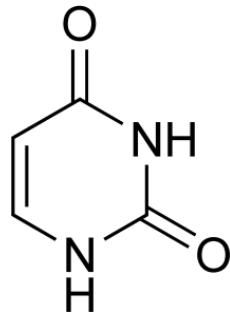


thymine

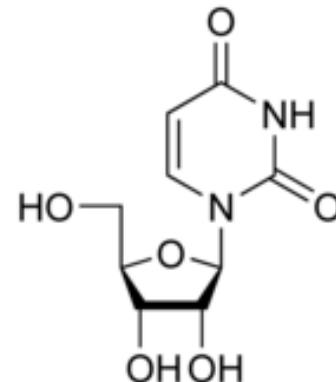


thymidine

RNA

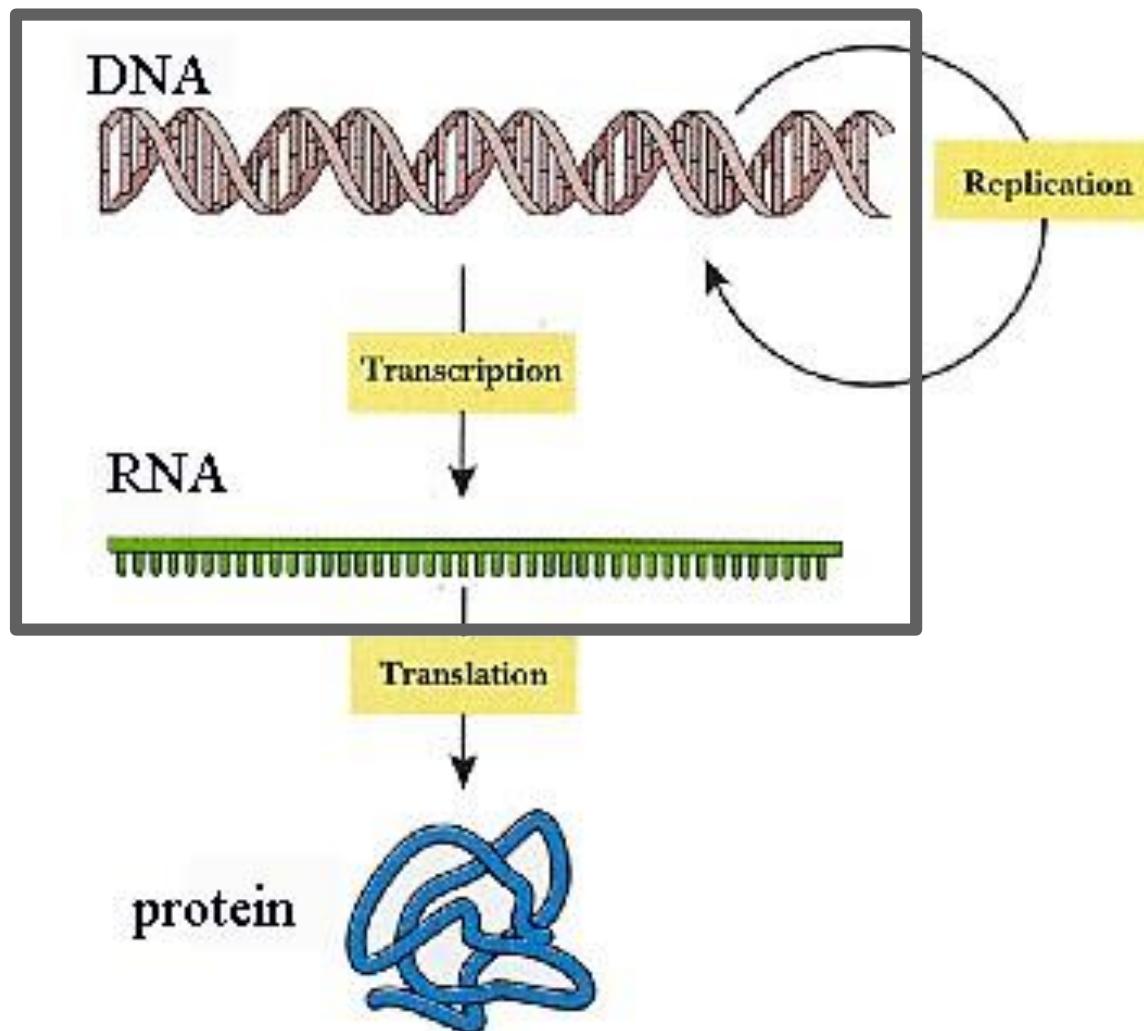


uracil

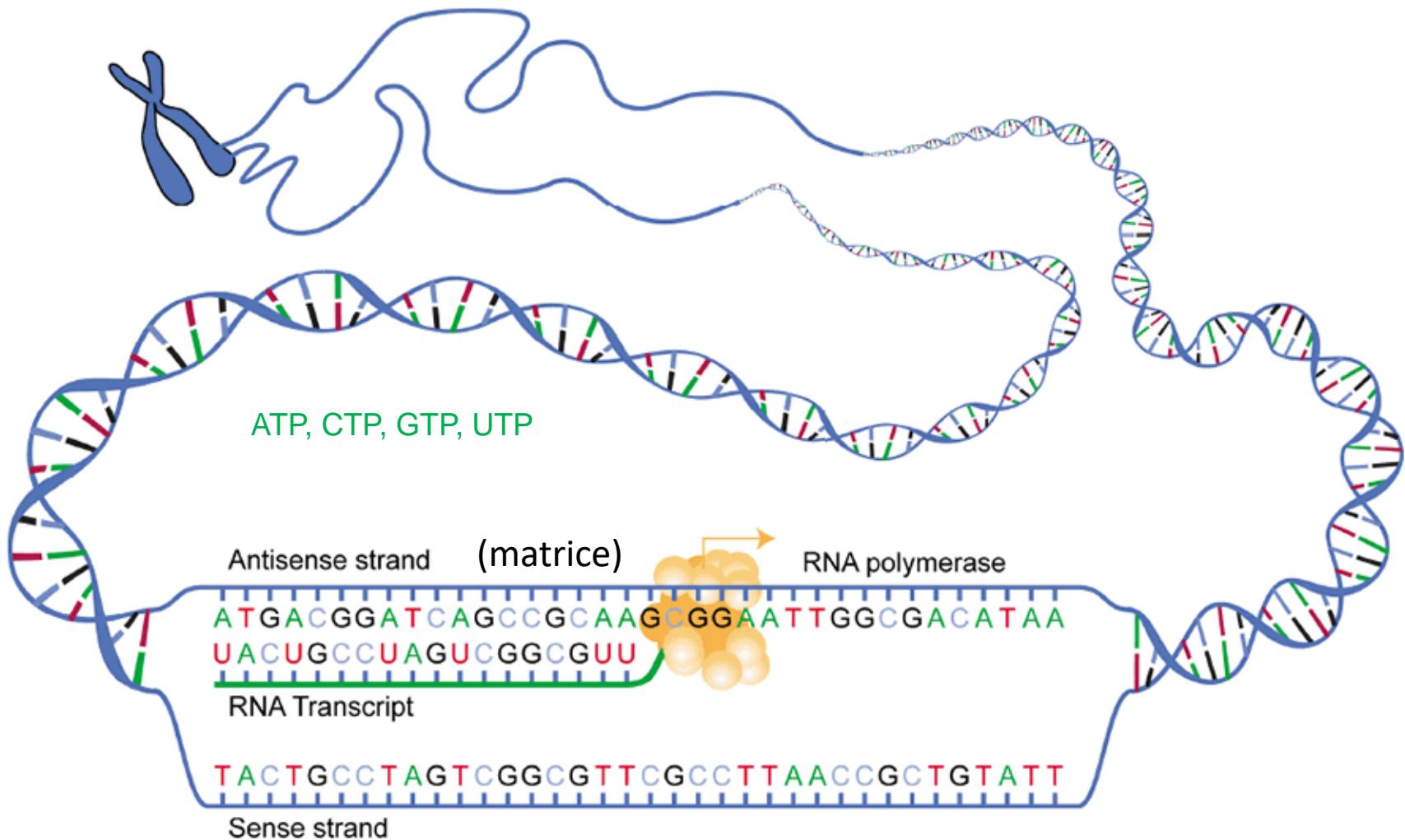


uridine

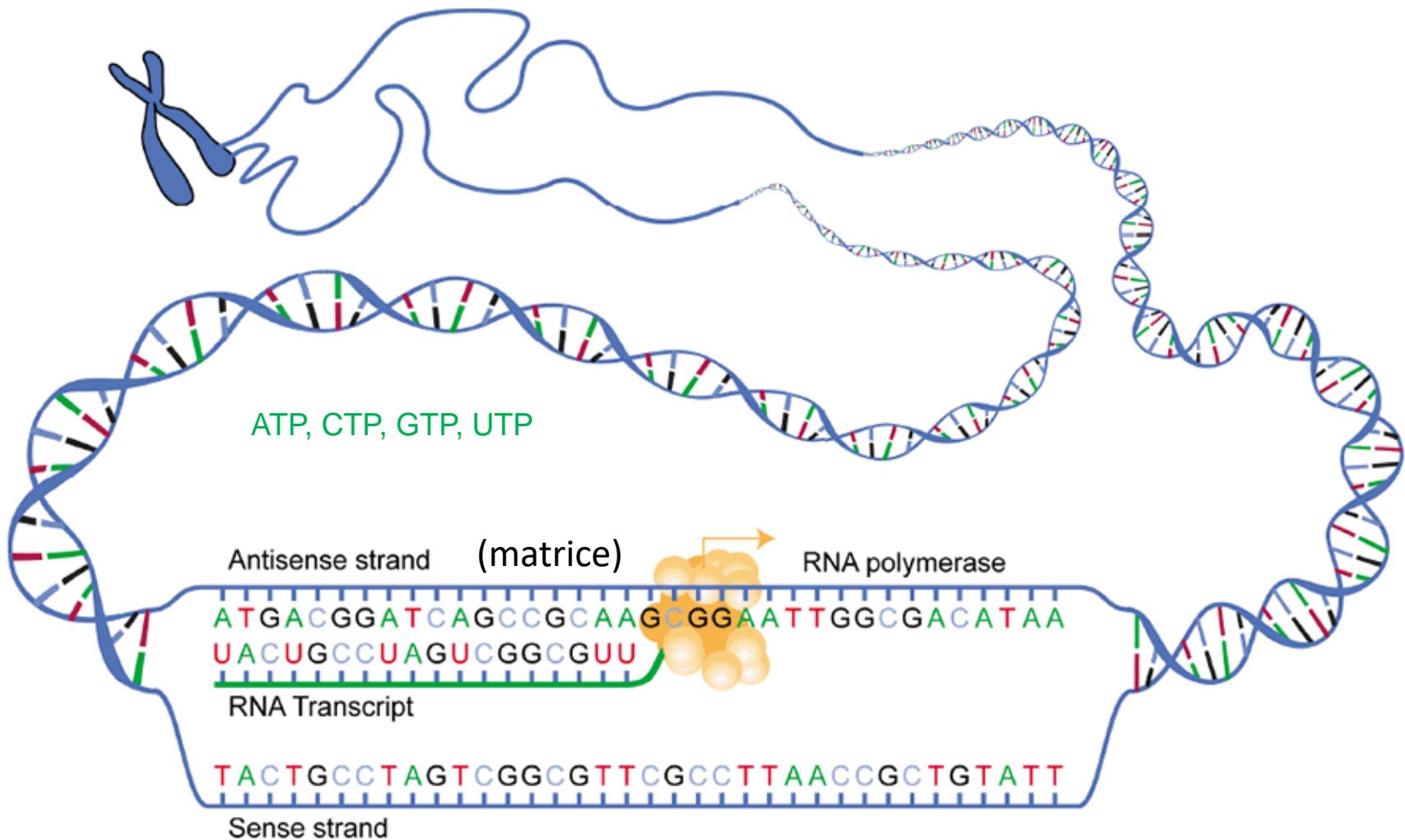
# Transcription



# Transcription



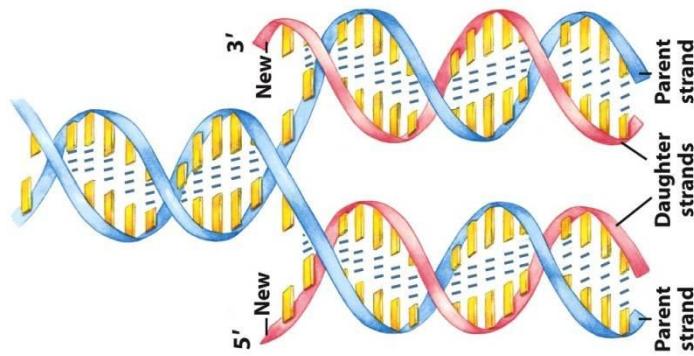
# Transcription



*Quel processus ressemble à la transcription ?*

# La synthèse du RNA est semblable à celle du DNA

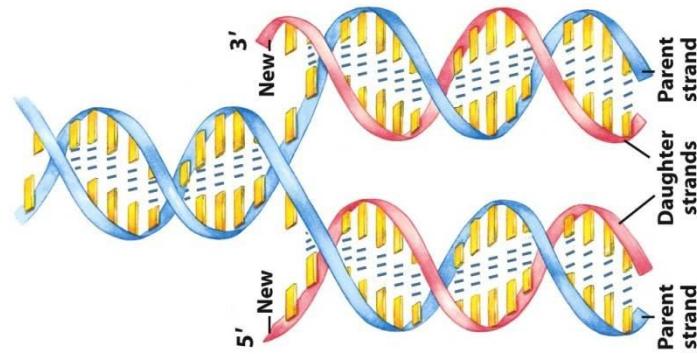
## Synthèse du DNA



processus pour doubler le DNA

# La synthèse du RNA est semblable à celle du DNA

## Synthèse du DNA



**Enzyme**

DNA polymérase

**Direction**

$5' \rightarrow 3'$

**Matrice**

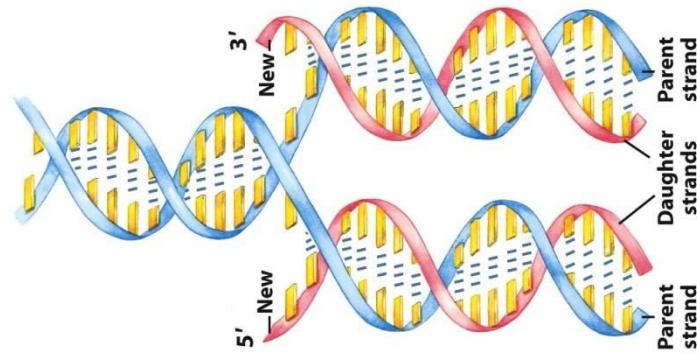
DNA

**Nucléotides**

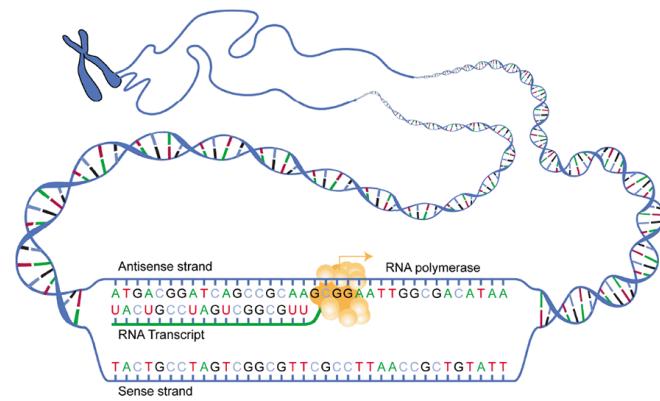
dATP, dCTP, dGTP, dTTP

# La synthèse du RNA est semblable à celle du DNA

## Synthèse du DNA



## Synthèse du RNA



**Enzyme**

DNA polymérase

**Direction**

5' → 3'

**Matrice**

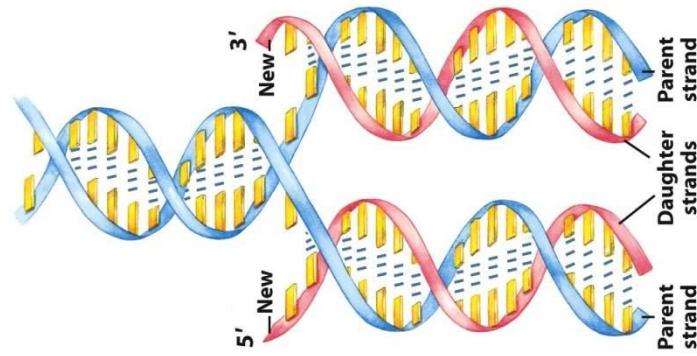
DNA

**Nucléotides**

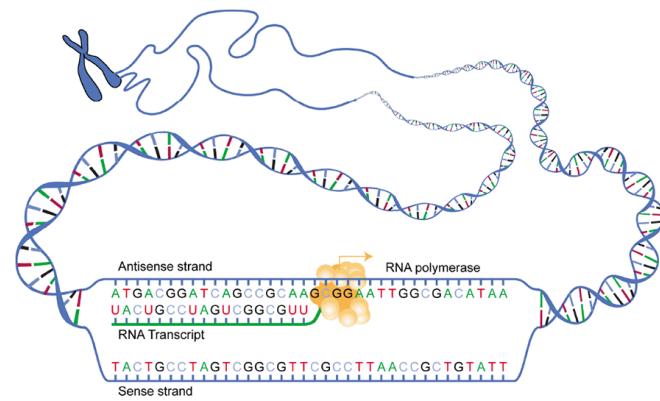
dATP, dCTP, dGTP, dTTP

# La synthèse du RNA est semblable à celle du DNA

## Synthèse du DNA



## Synthèse du RNA



Enzyme

DNA polymérase

Direction

5' → 3'

Matrice

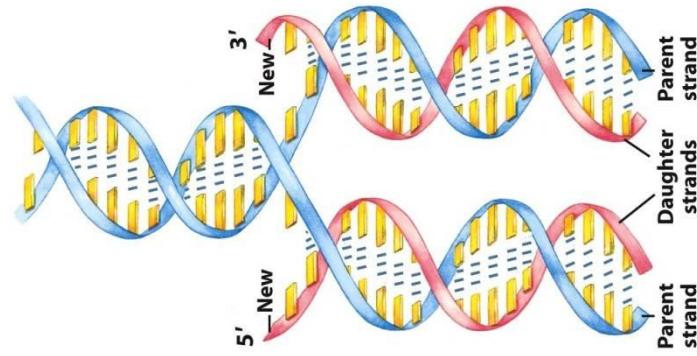
DNA

Nucléotides

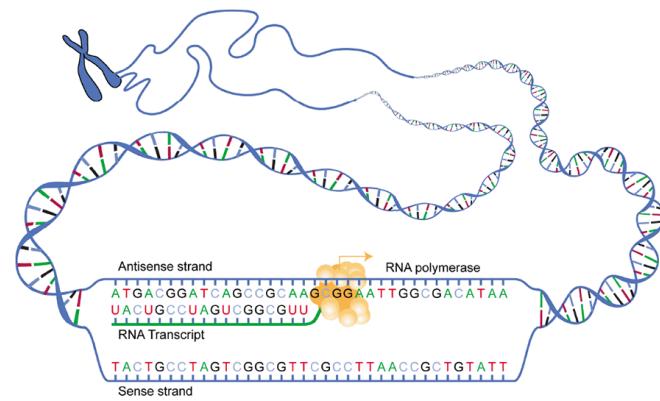
dATP, dCTP, dGTP, dTTP

# La synthèse du RNA est semblable à celle du DNA

## Synthèse de DNA



## Synthèse de RNA



Enzyme

DNA polymérase

Direction

5' → 3'

Matrice

DNA

Nucléotides

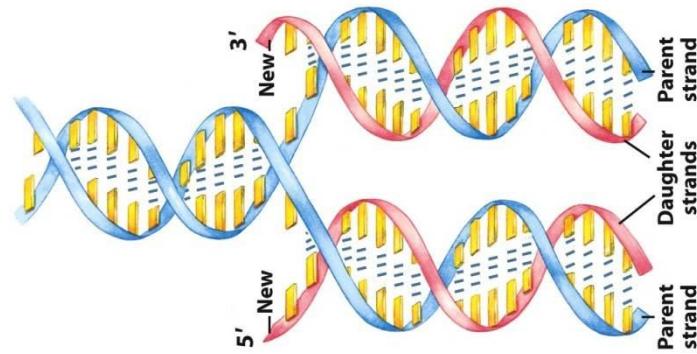
dATP, dCTP, dGTP, dTTP

RNA polymérase

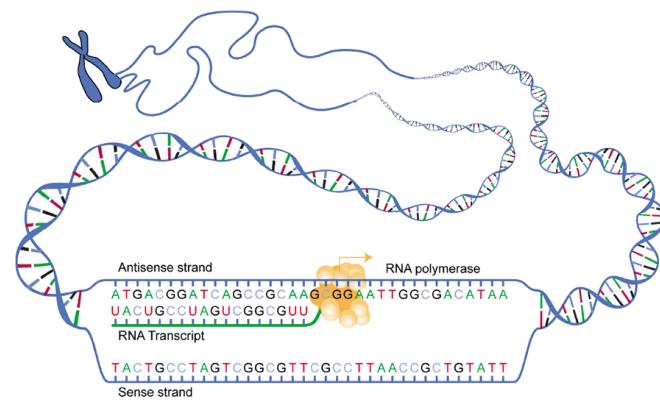
5' → 3'

# La synthèse du RNA est semblable à celle du DNA

## Synthèse de DNA



## Synthèse de RNA



Enzyme

DNA polymérase

Direction

5' → 3'

Matrice

DNA

RNA polymérase

5' → 3'

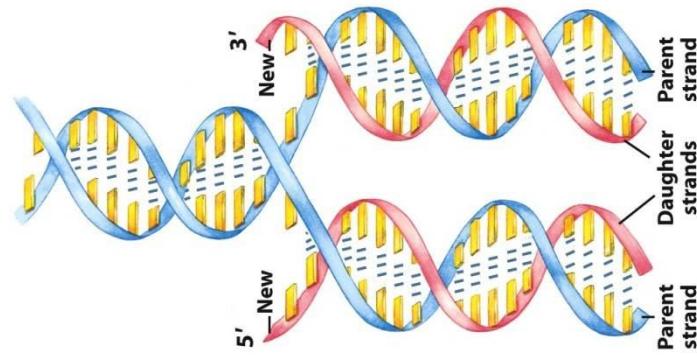
Nucléotides

dATP, dCTP, dGTP, dTTP

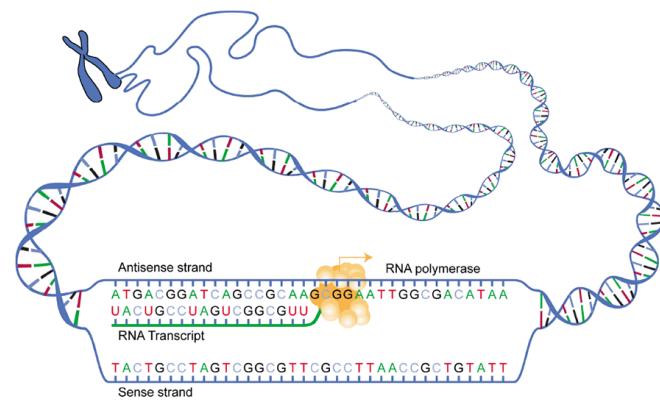
DNA

# La synthèse du RNA est semblable à celle du DNA

Synthèse de DNA



Synthèse de RNA



Enzyme

DNA polymérase

Direction

5' → 3'

Matrice

DNA

Nucléotides

dATP, dCTP, dGTP, dTTP

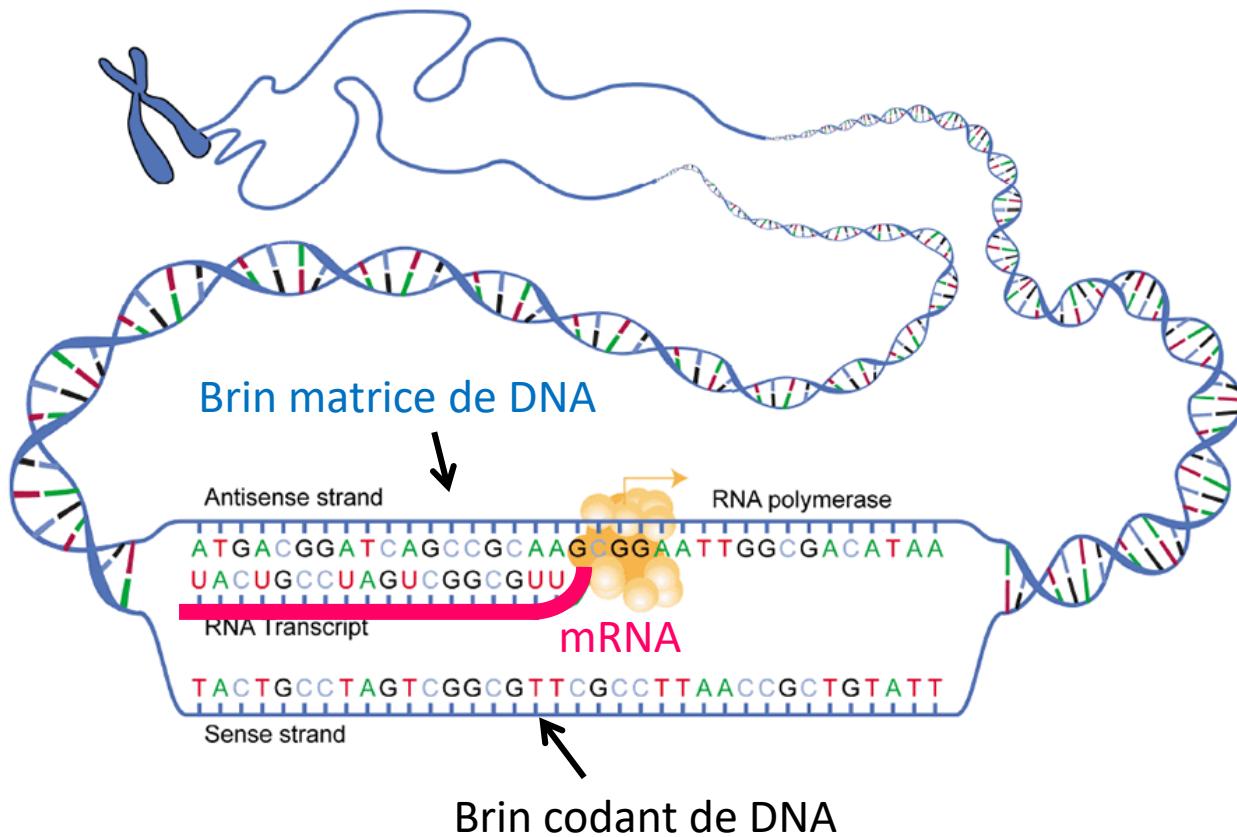
RNA polymérase

5' → 3'

DNA

ATP, CTP, GTP, UTP

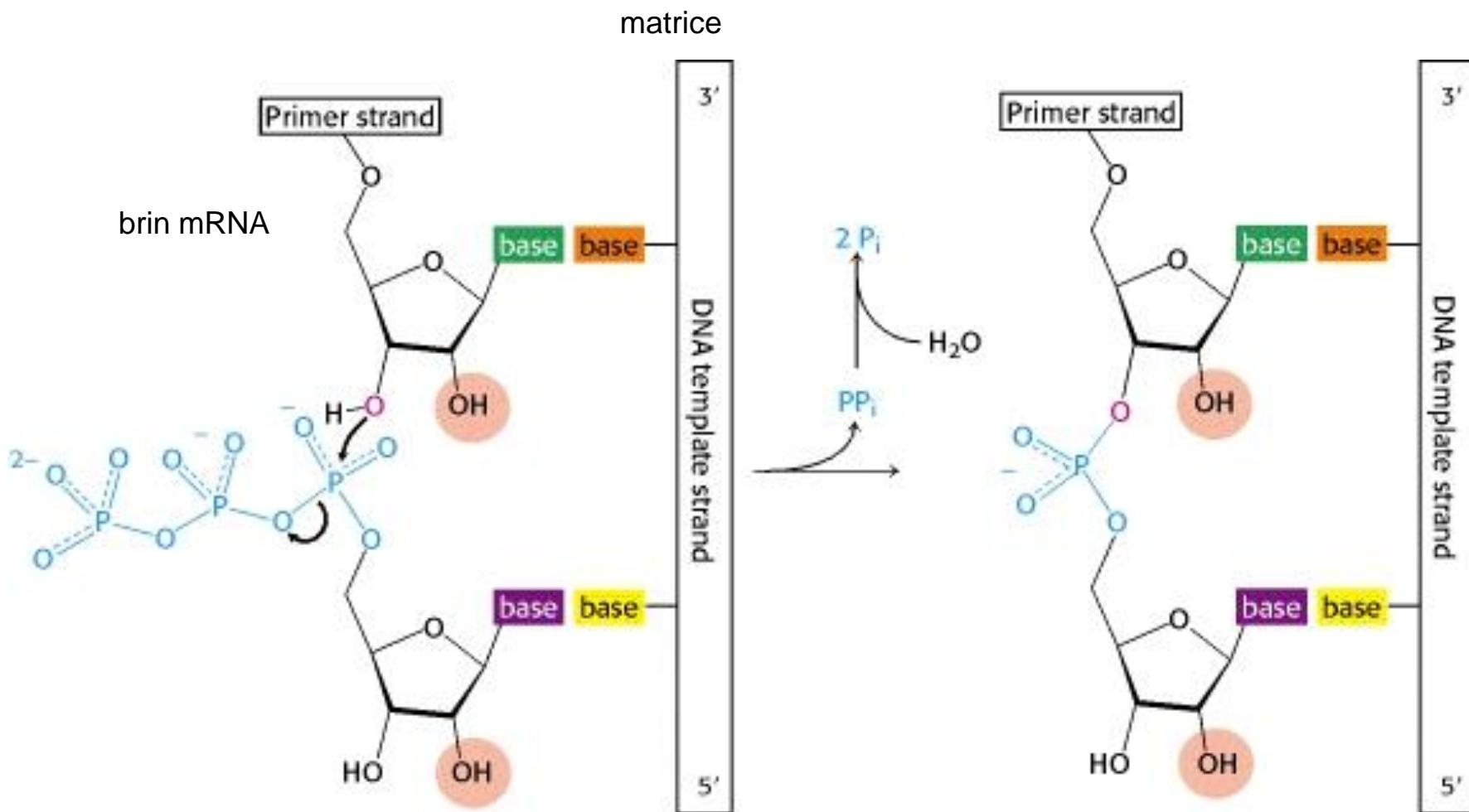
# Transcription du RNA



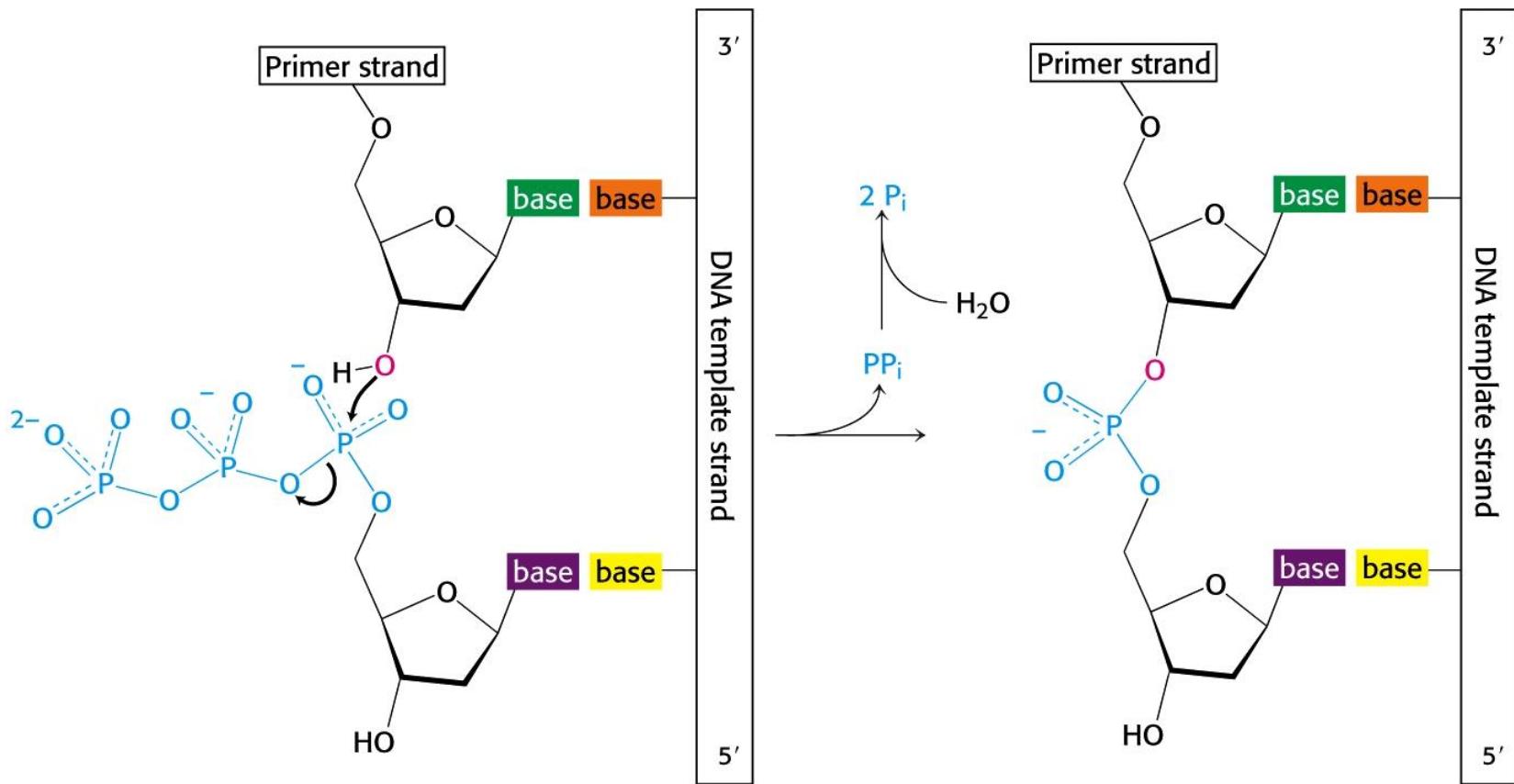
5'—GCGGCGACGCGCAGUUAAUCCCACAGCCGCCAGUUCCGUUGCGGCCAUUUU—3'  
3'—CGCCGCTGCGCGTCAATTAGGGTGTGGCGGTCAAGGCGACCCCGTAAAAA—5'  
5'—GCGGCGACGCGCAGTTAACCCACAGCCGCCAGTTCCGCTGGCGGCCATT—3'

mRNA (=messenger RNA)  
Brin matrice de DNA  
Brin codant de DNA

# Mécanisme de transcription

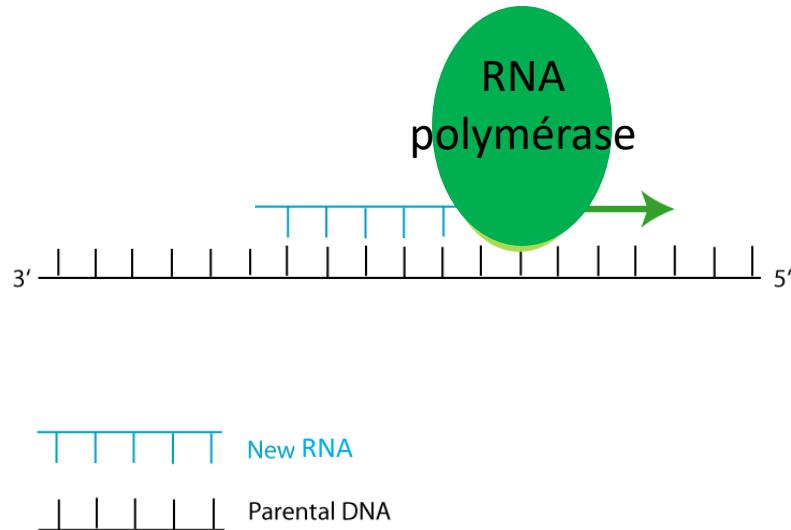


# Comparaison avec la DNA polymérisation

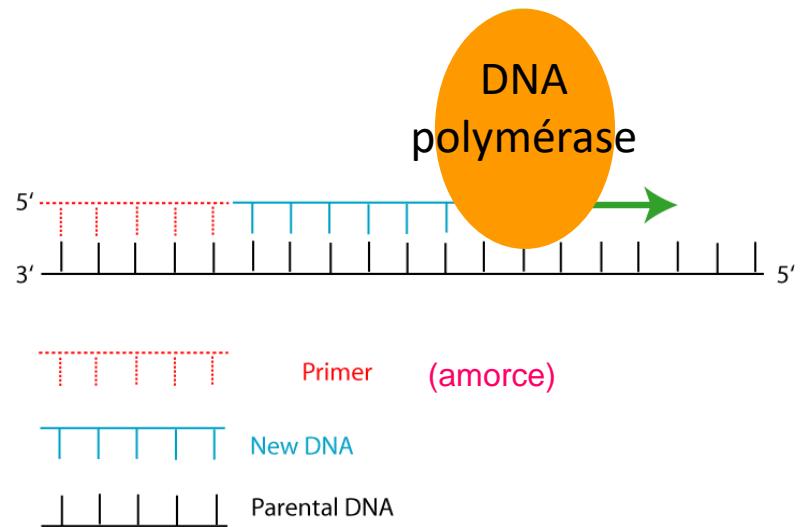


# Comparaison de la RNA et DNA polymérisation

Synthèse de RNA

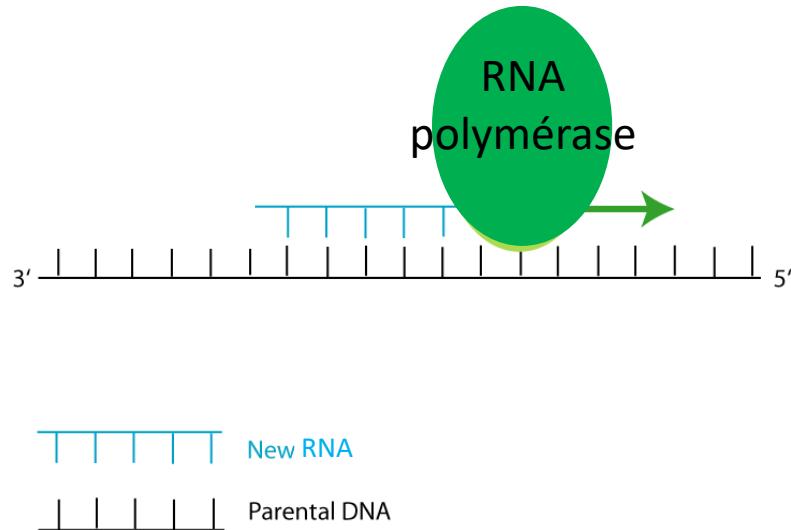


Synthèse de DNA

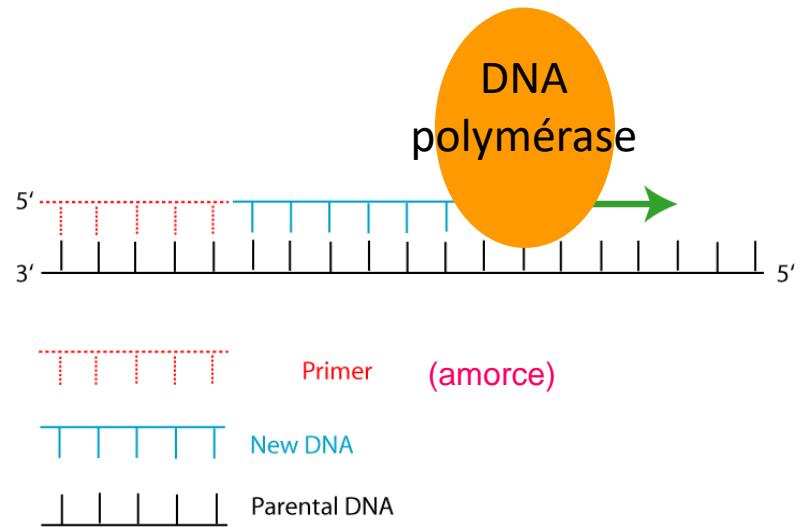


# Comparaison de la RNA et DNA polymérisation

Synthèse de RNA



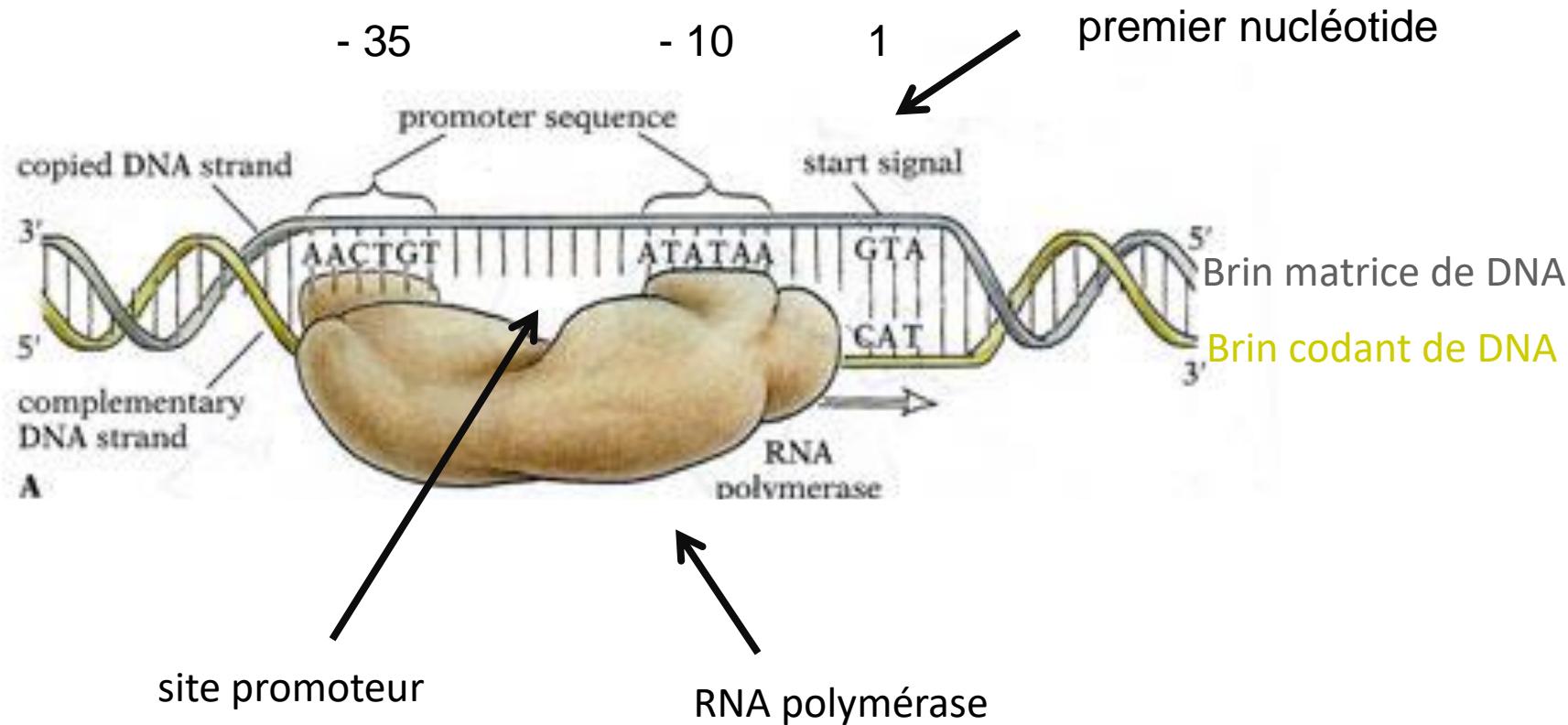
Synthèse de DNA



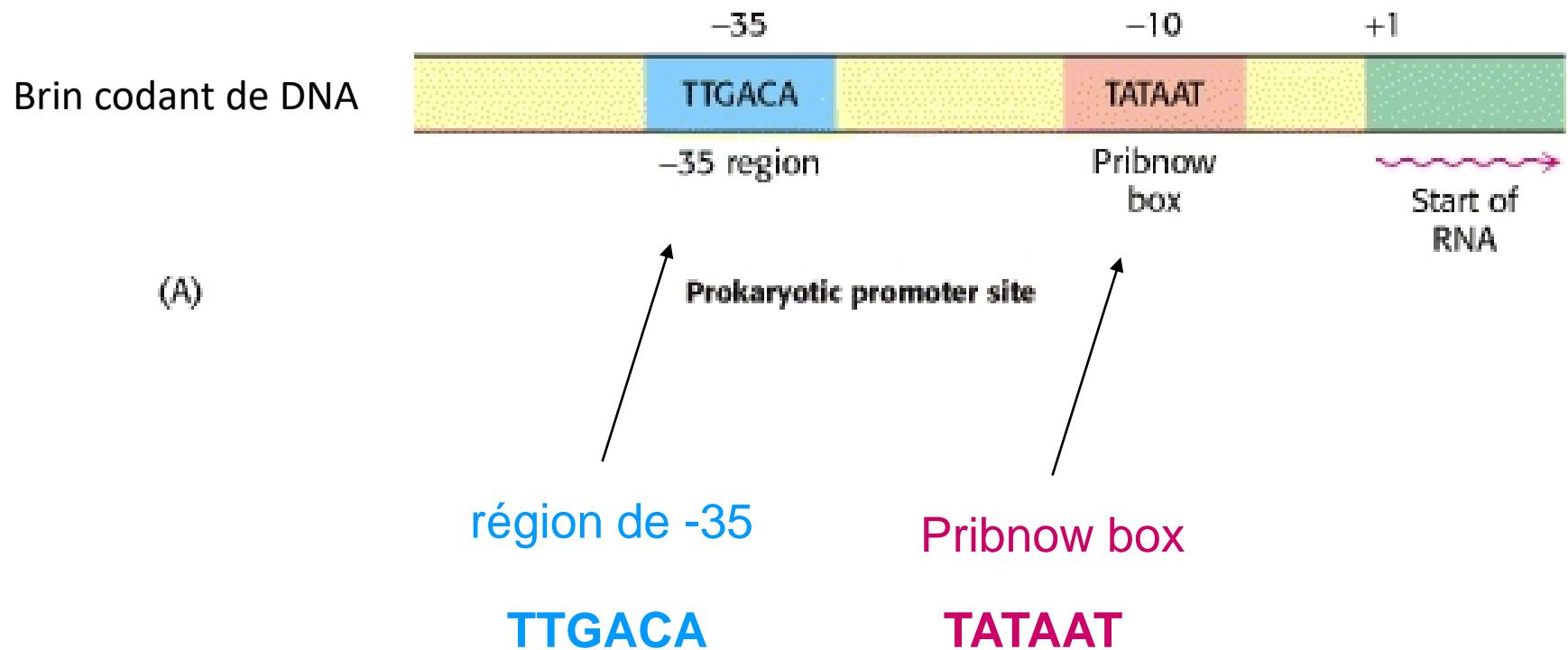
*Comment est-ce que la RNA polymérase sait-elle à quelle position dans le gène elle doit commencer?*

# La transcription commence près des sites promoteurs

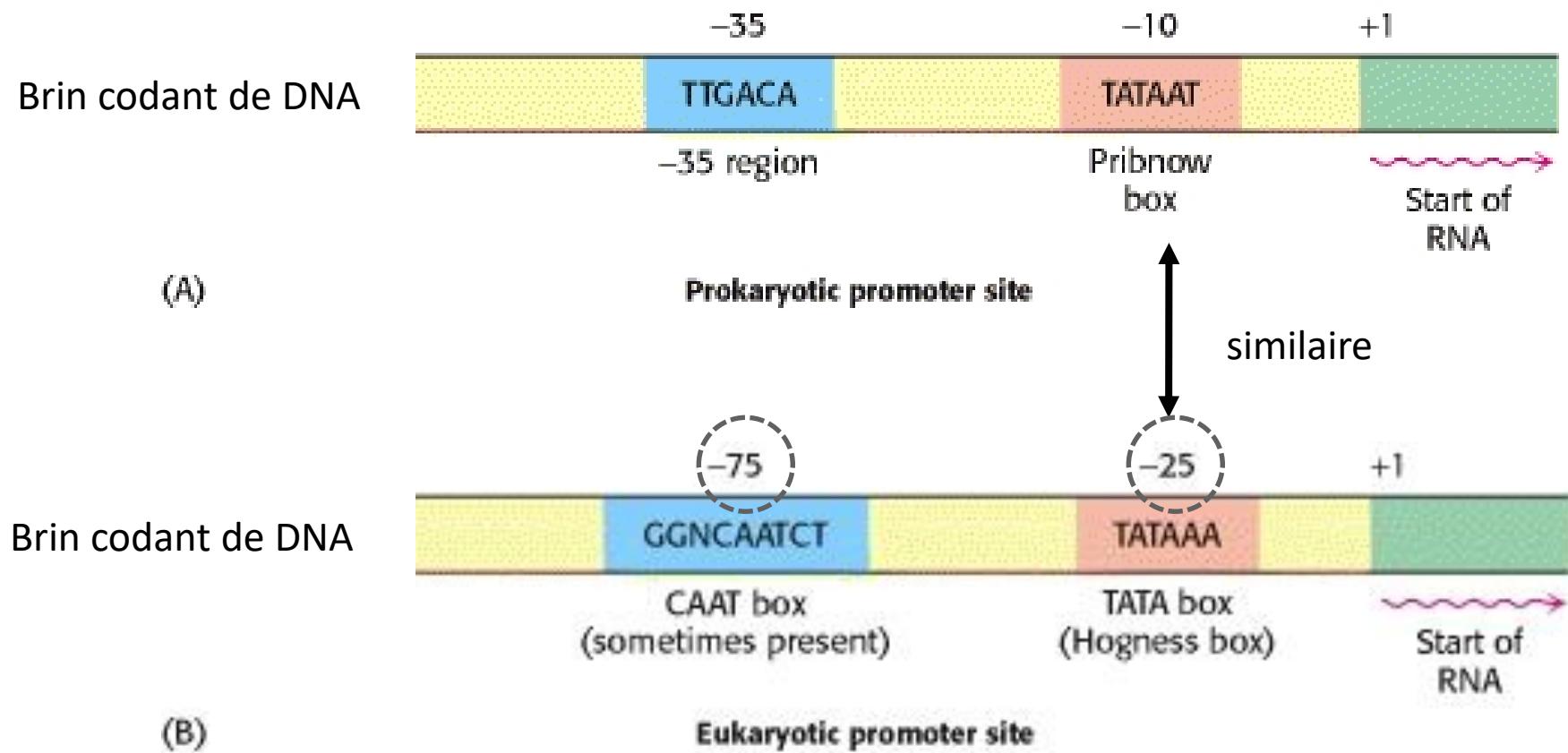
position relative au 5' terminus du mRNA



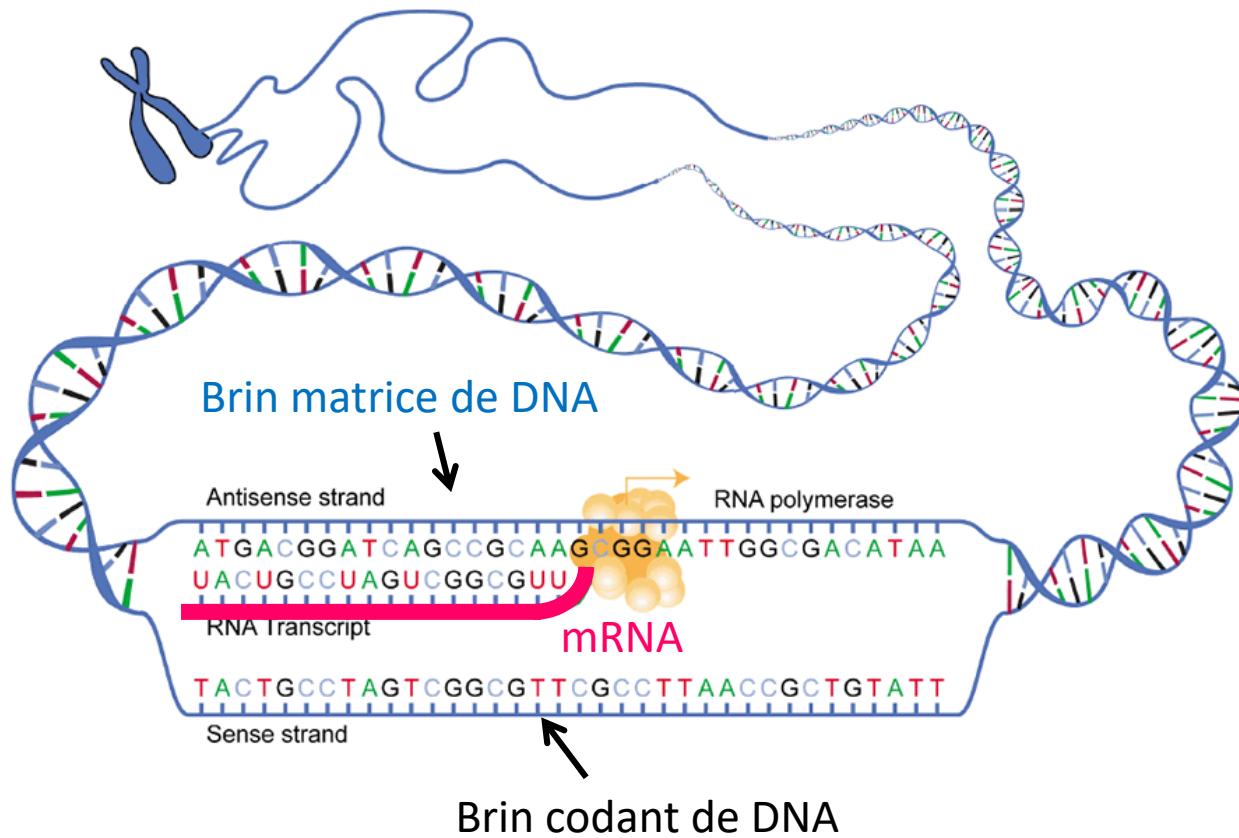
# La transcription commence près des sites promoteurs



# La transcription commence près des sites promoteurs

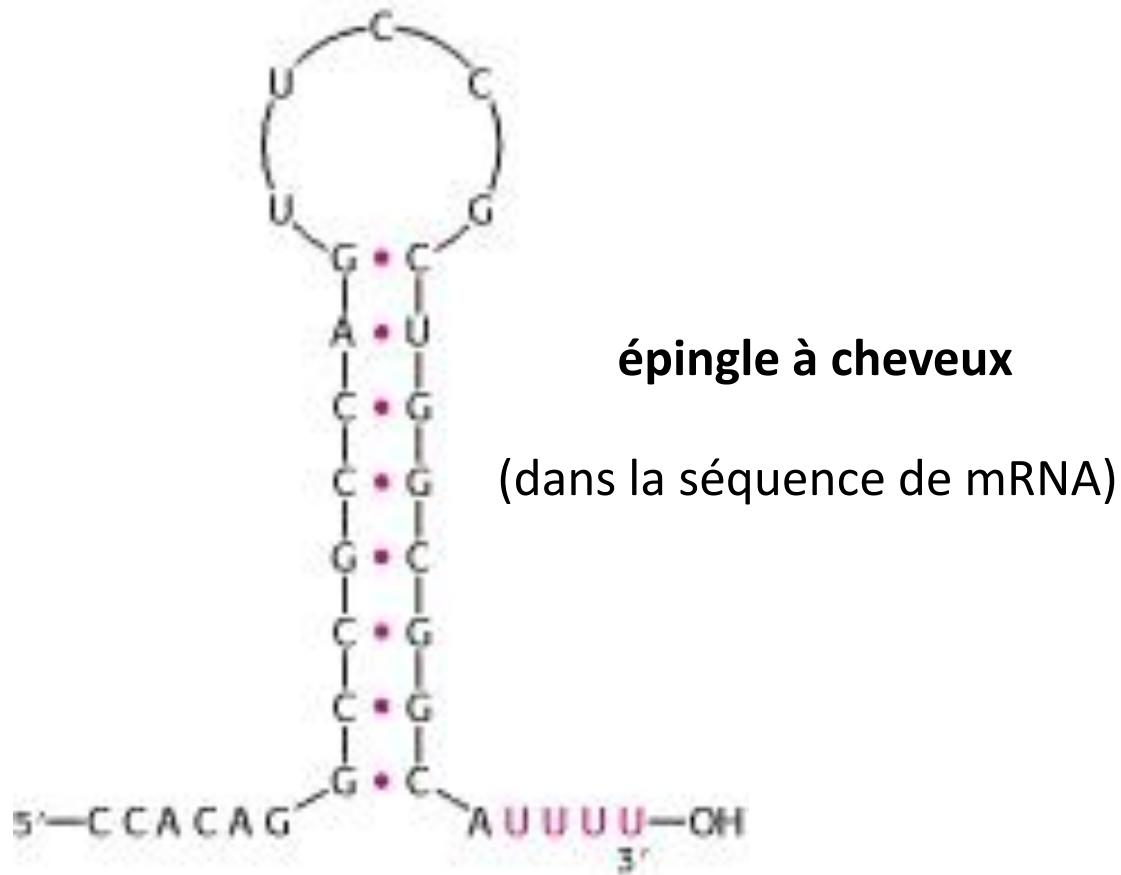


# Transcription du RNA

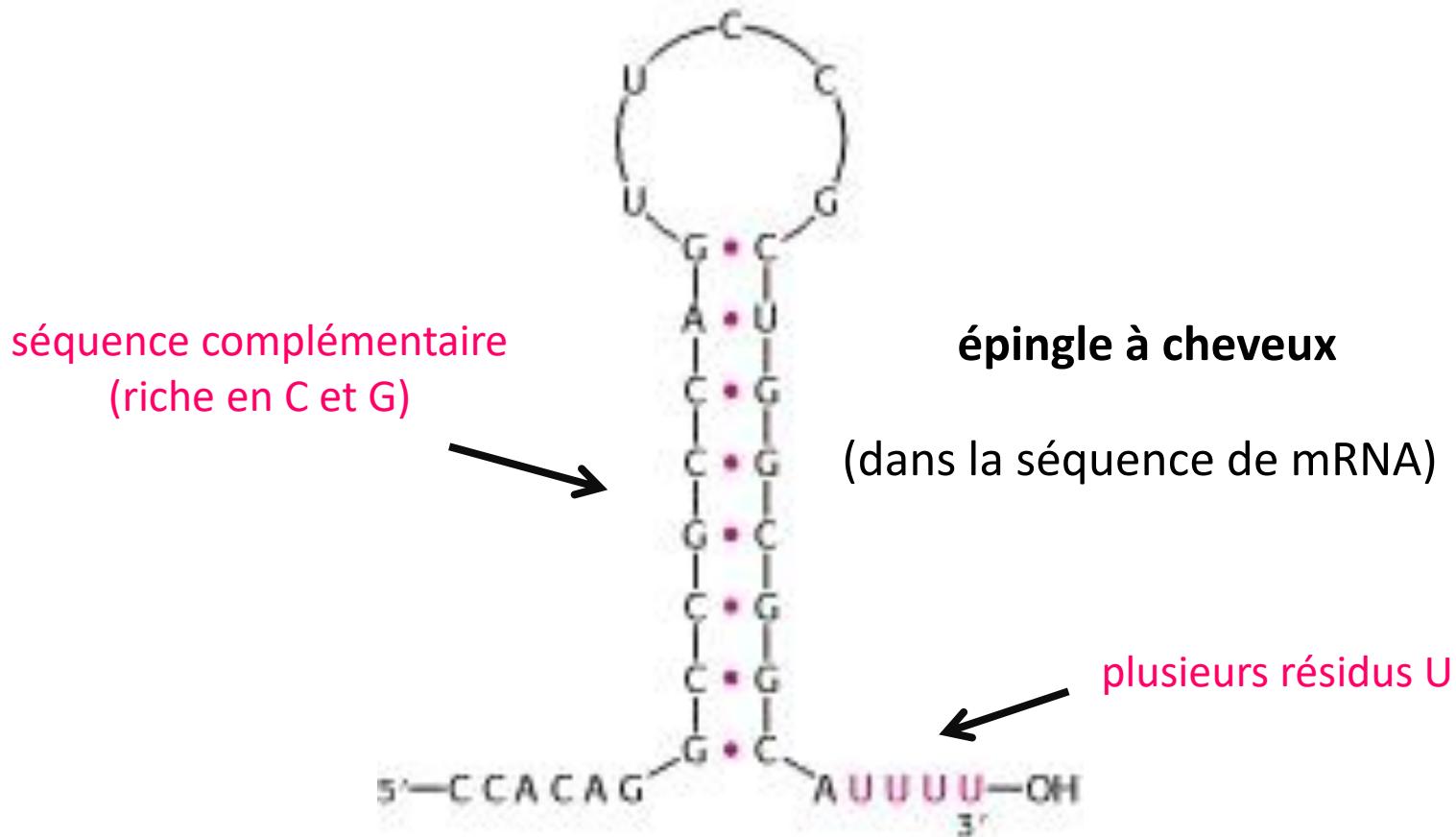


*Quand est-ce que la polymérase du RNA termine la synthèse?*

# Séquence de sites de terminaison

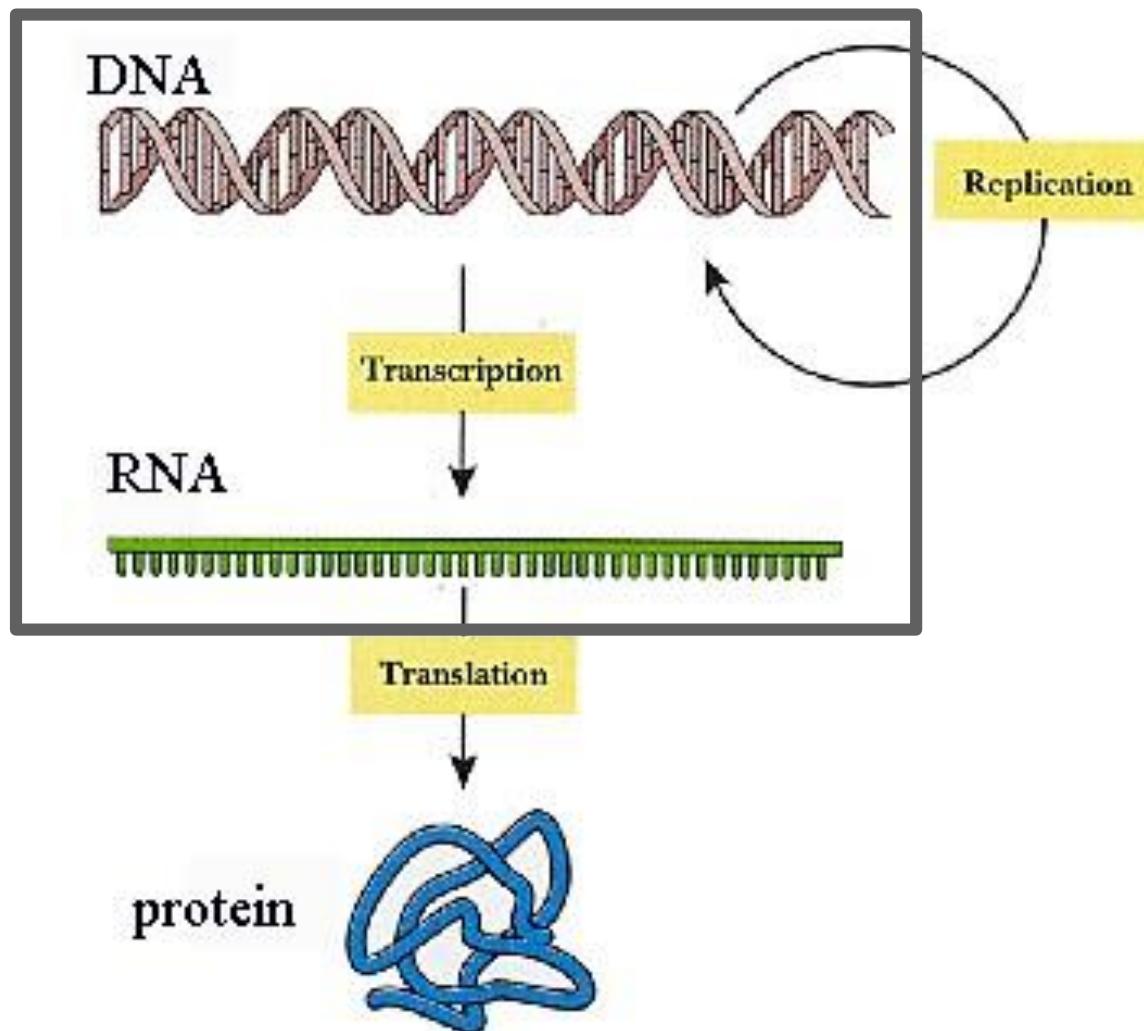


# Séquence de sites de terminaison

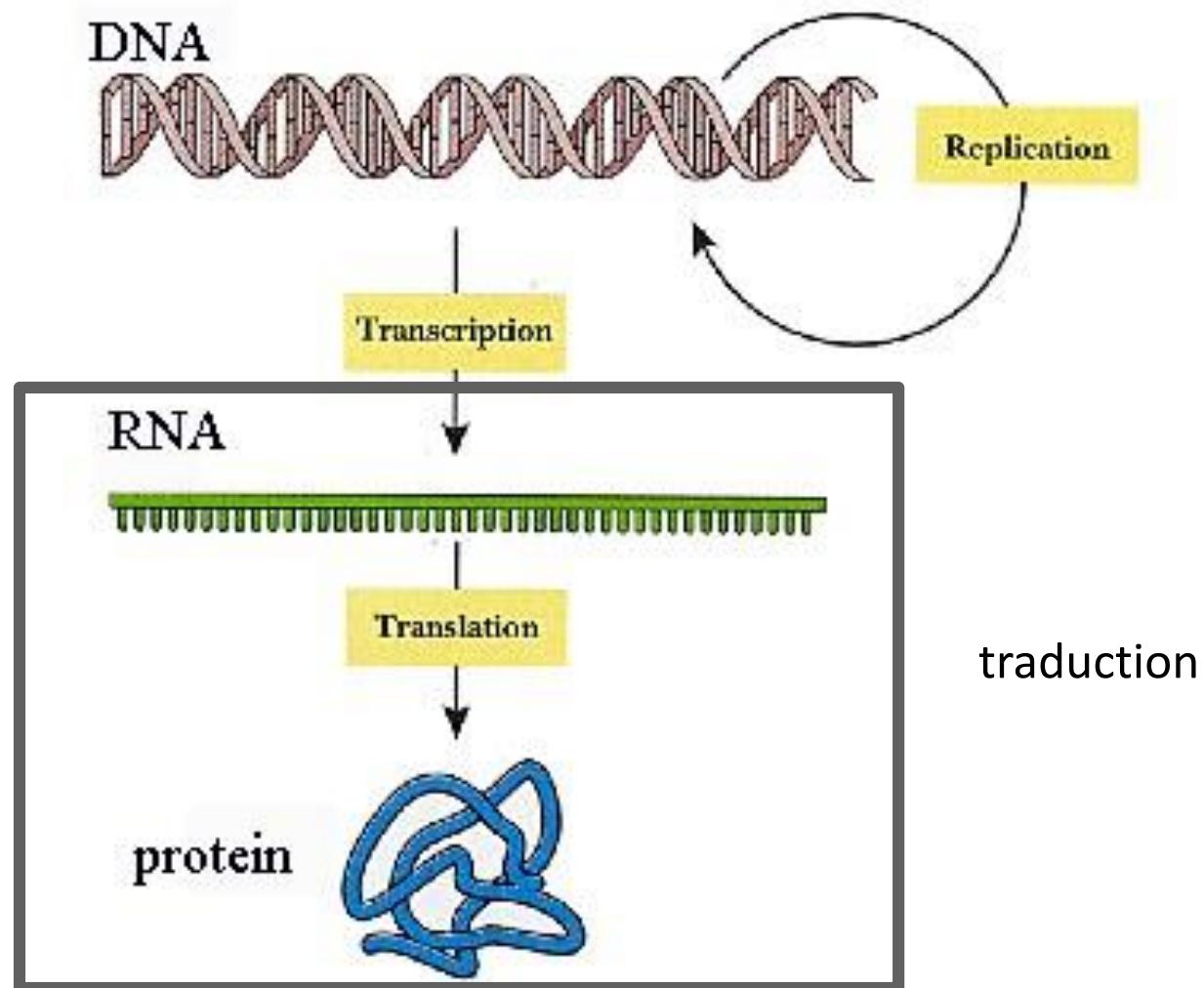


Dans les cellules eucaryotes: séquence d'adénulates (queue poly A)

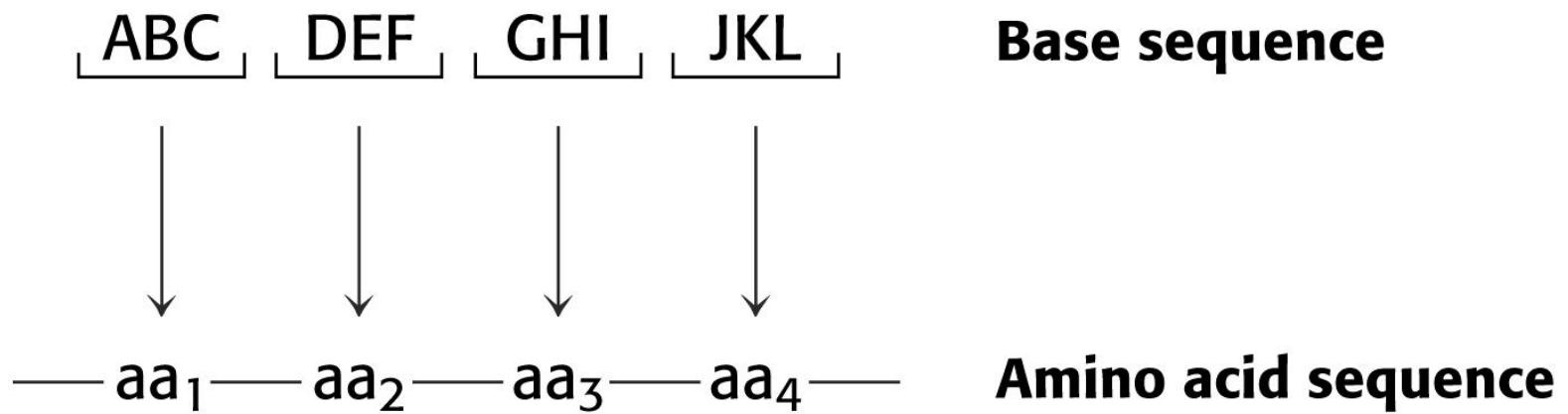
# Transcription



# Traduction

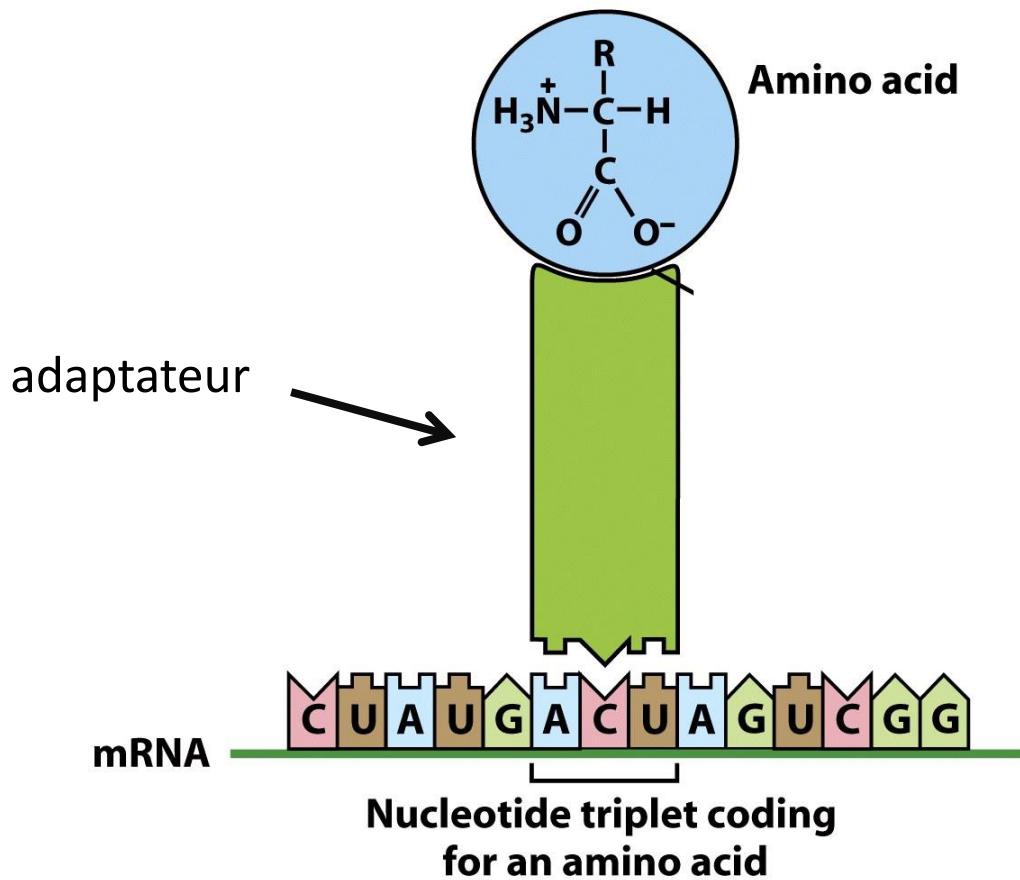


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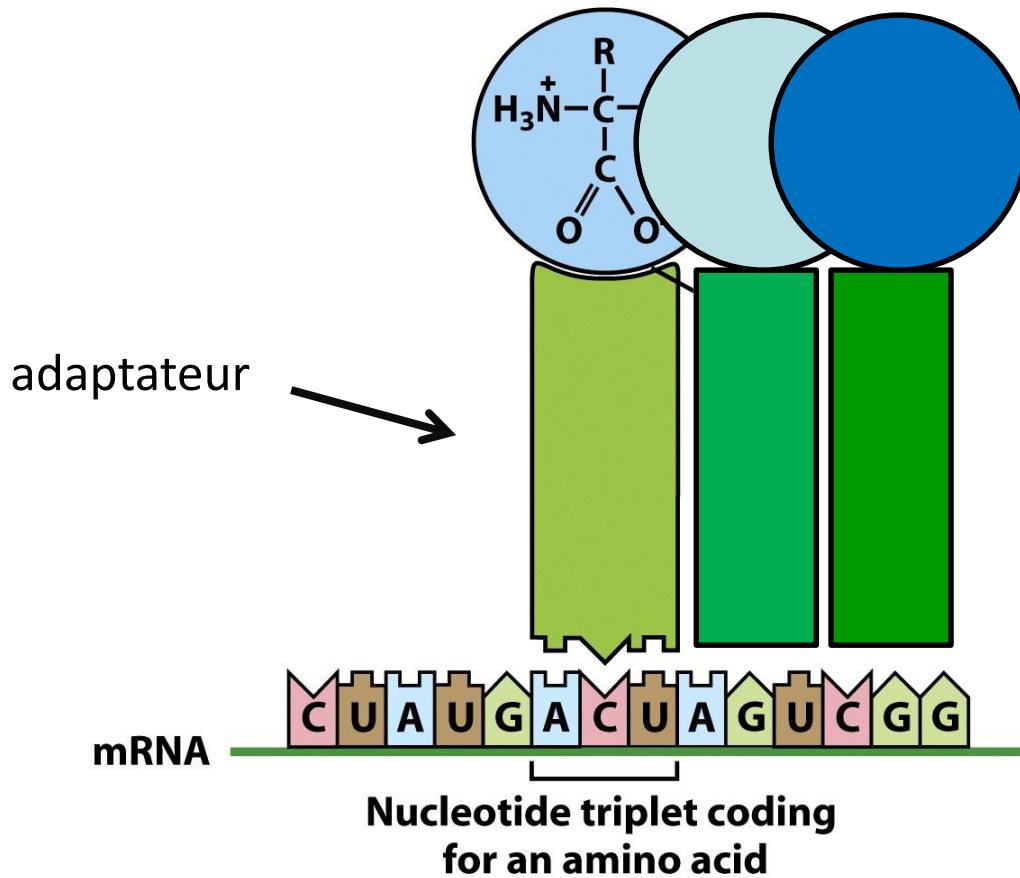
*Comment le RNA peut-il être traduit en protéines?*

# Translation de RNA



**Figure 27-2**  
*Lehninger Principles of Biochemistry, Fifth Edition*  
© 2008 W.H. Freeman and Company

# Translation de RNA



**Figure 27-2**  
*Lehninger Principles of Biochemistry, Fifth Edition*  
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# Translation de RNA

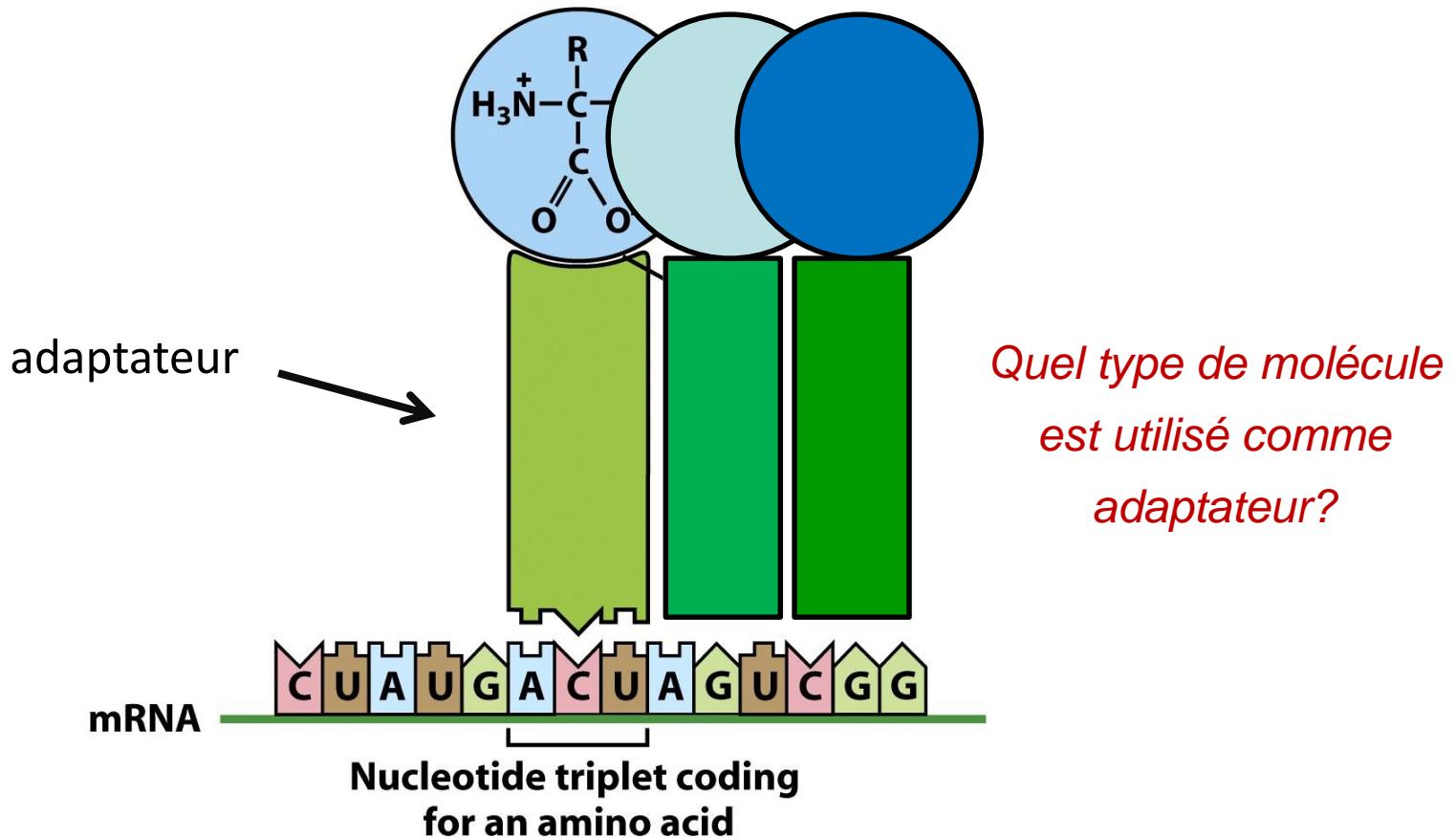
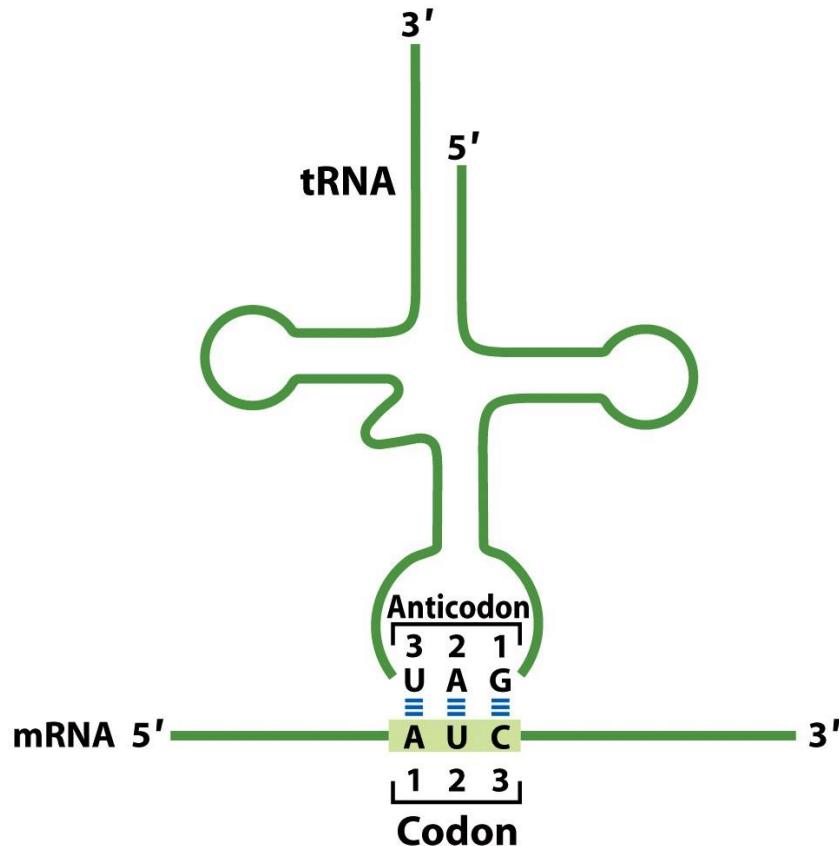


Figure 27-2  
*Lehninger Principles of Biochemistry, Fifth Edition*  
© 2008 W.H. Freeman and Company

# tRNA (RNA de transfert)



**Figure 27-8a**  
*Lehninger Principles of Biochemistry, Fifth Edition*  
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# tRNA (RNA de transfert)

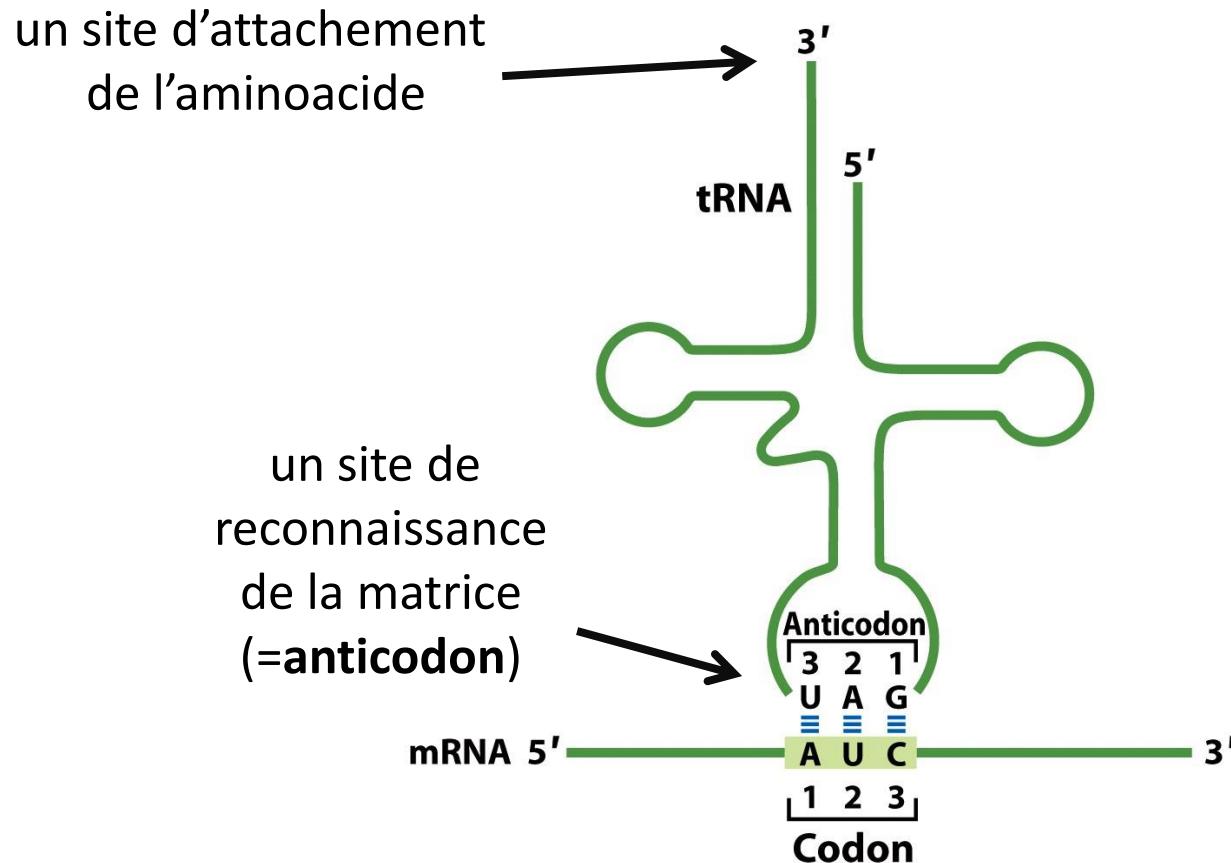


Figure 27-8a  
Lehninger Principles of Biochemistry, Fifth Edition  
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# tRNA (RNA de transfert)

un site d'attachement  
de l'acide aminé

un site de  
reconnaissance  
de la matrice  
(=anticodon)

*Comment*

*l'acide aminé peut-il  
être attaché?*

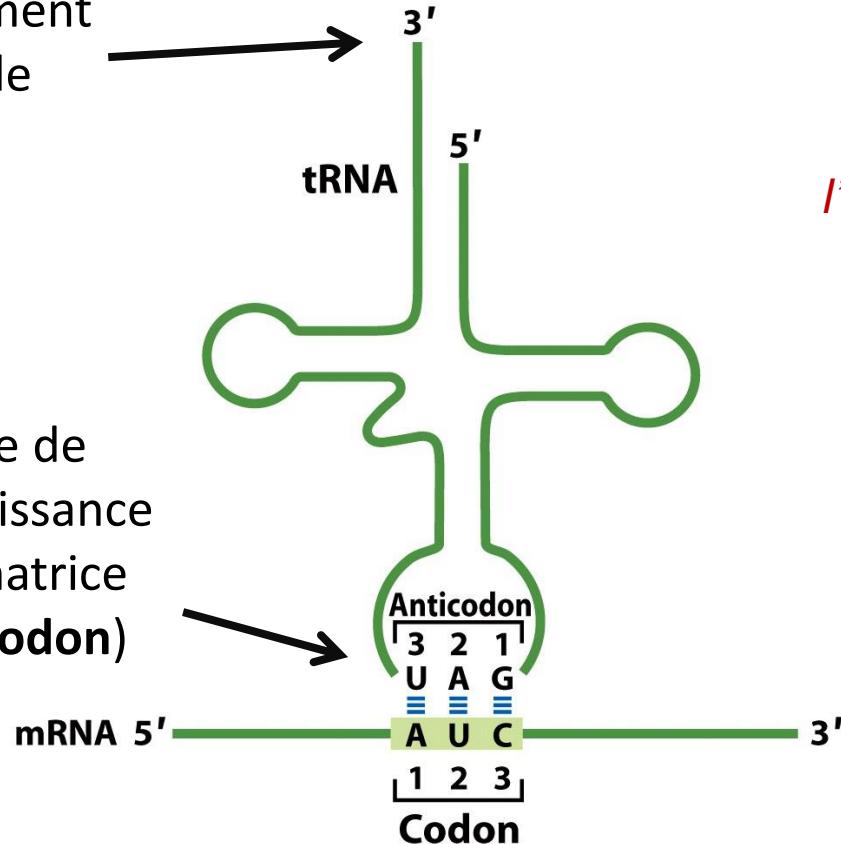
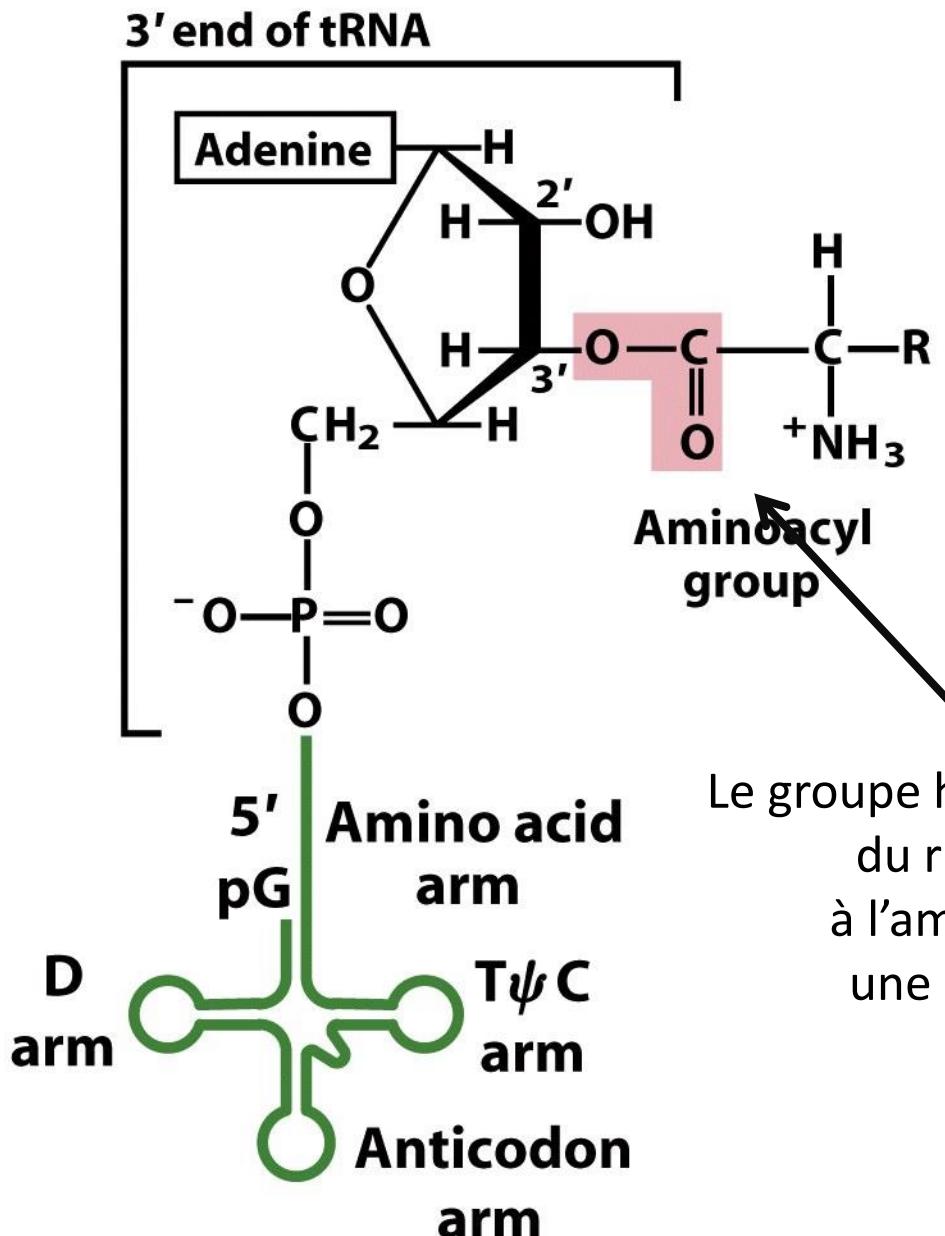


Figure 27-8a  
Lehninger Principles of Biochemistry, Fifth Edition  
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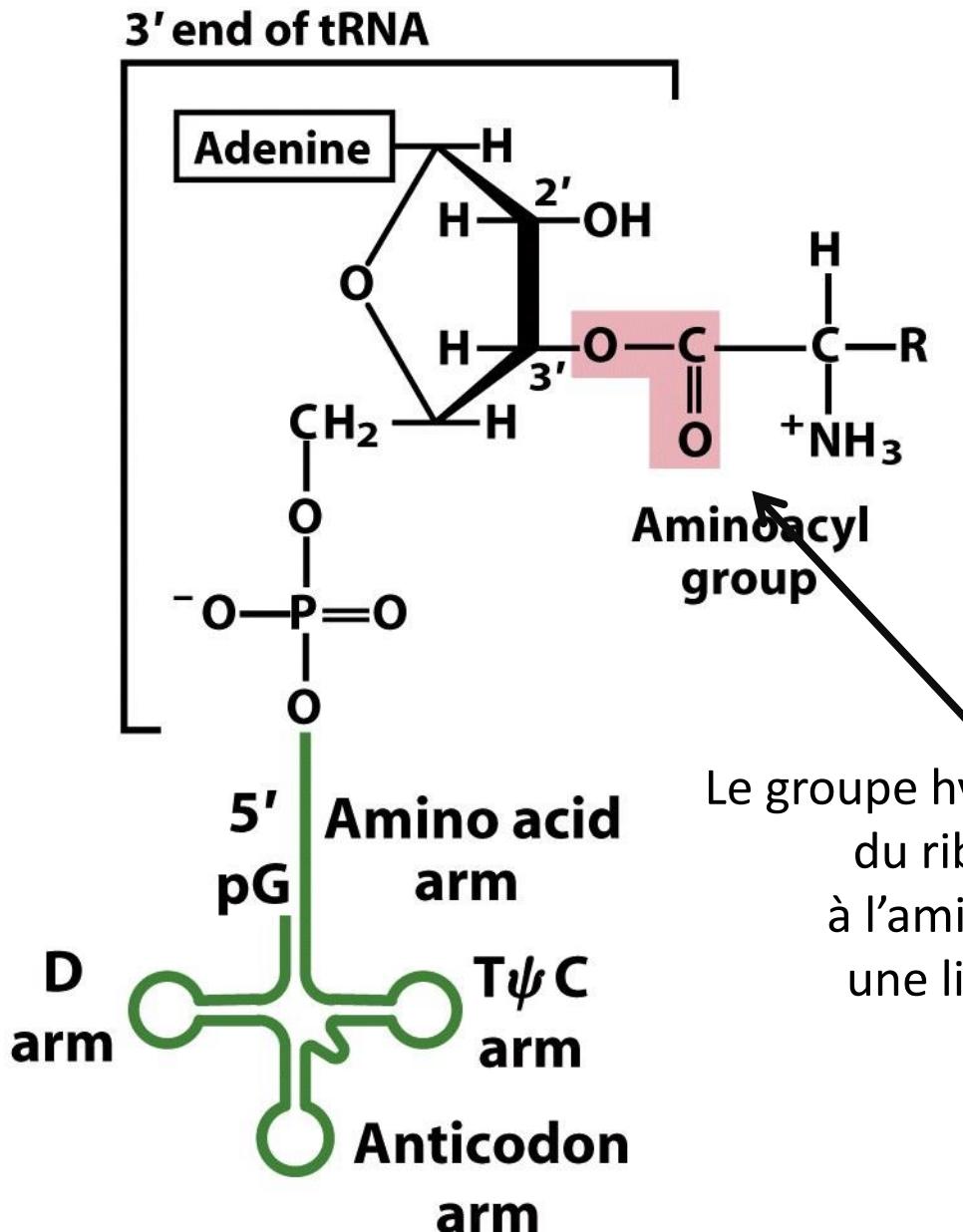


**Figure 27-20**

*Lehninger Principles of Biochemistry, Fifth Edition*

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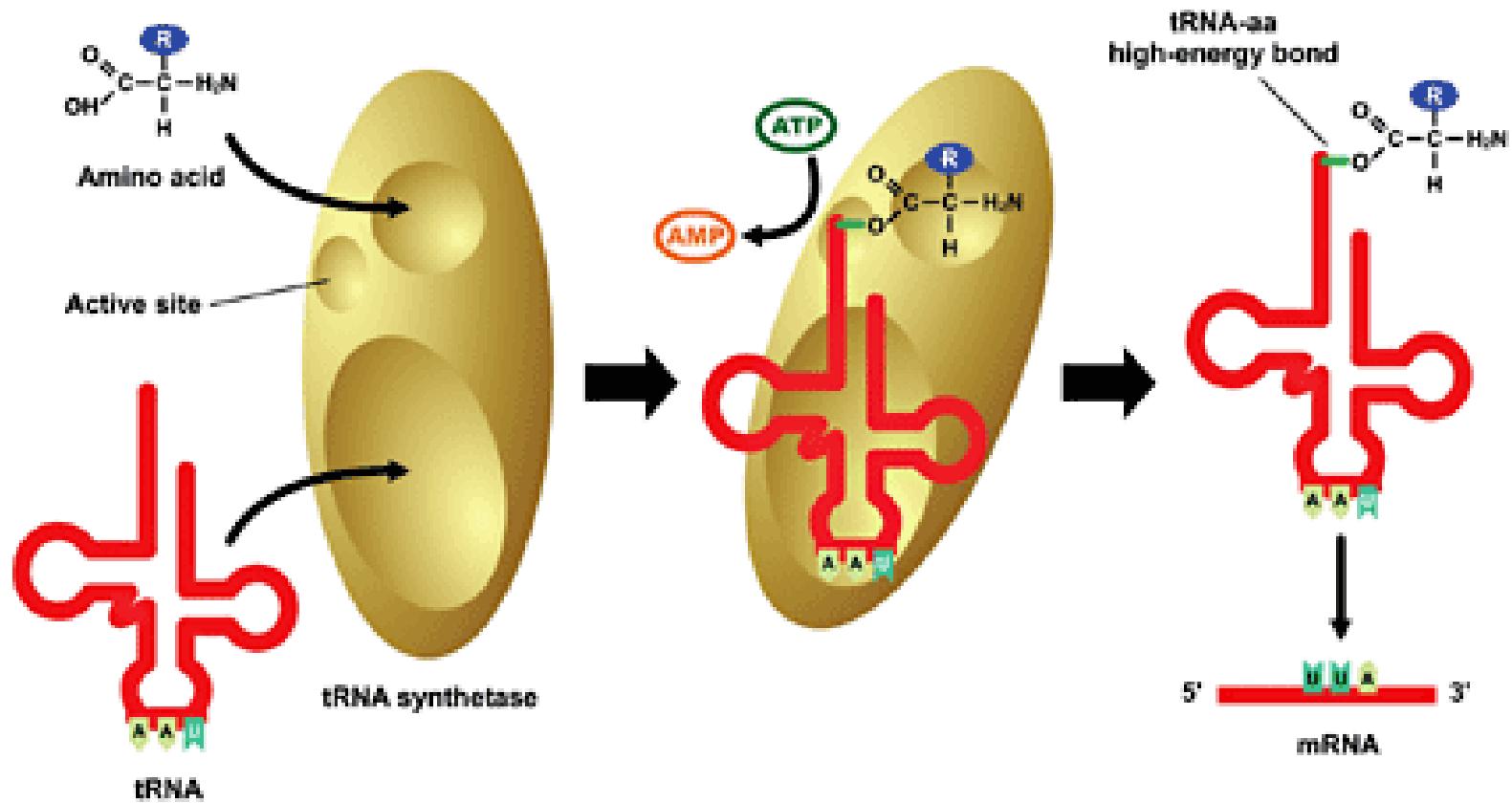
*Comment le tRNA peut-il être chargé avec un aminoacide?*



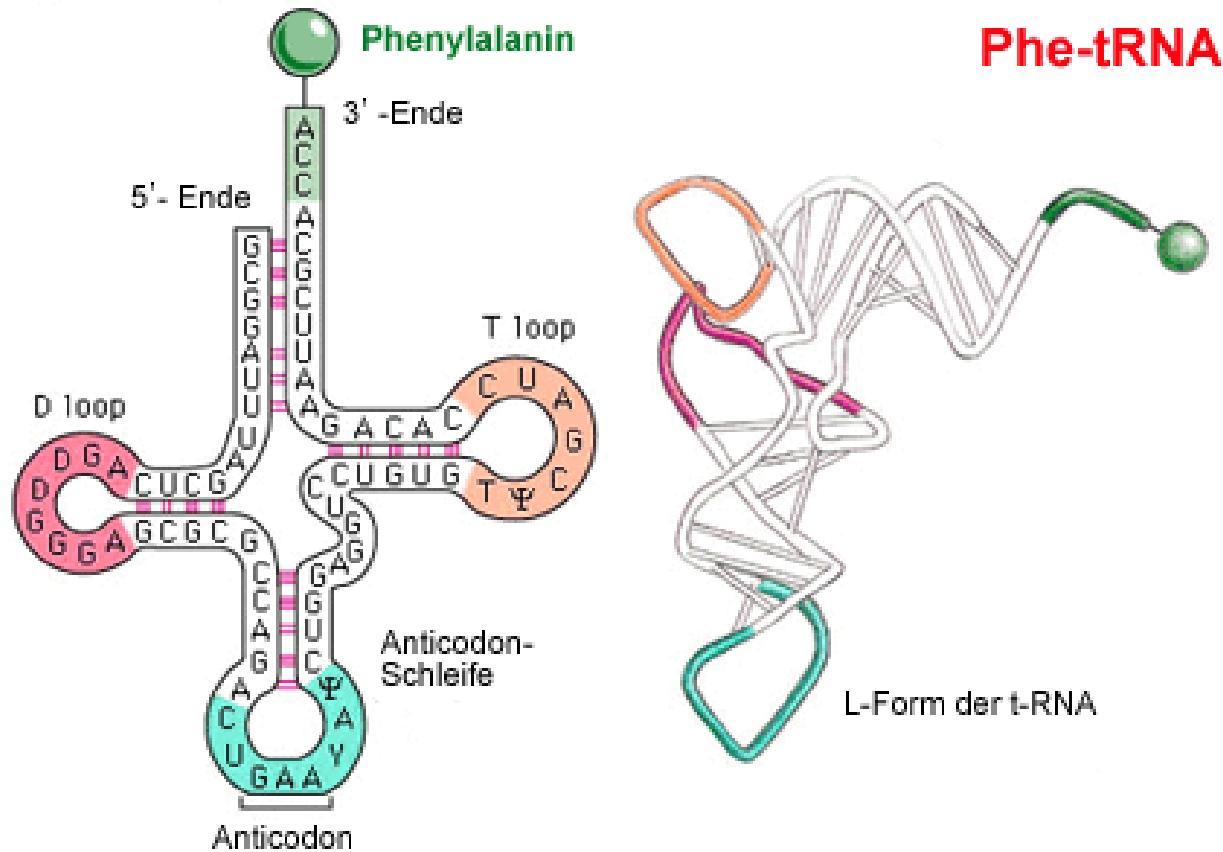
Le groupe hydroxyle (3' ou 2') du ribose est lié à l'aminoacide par une liaison **ester**

**Figure 27-20**  
*Lehninger Principles of Biochemistry, Fifth Edition*  
© 2008 W.H. Freeman and Company

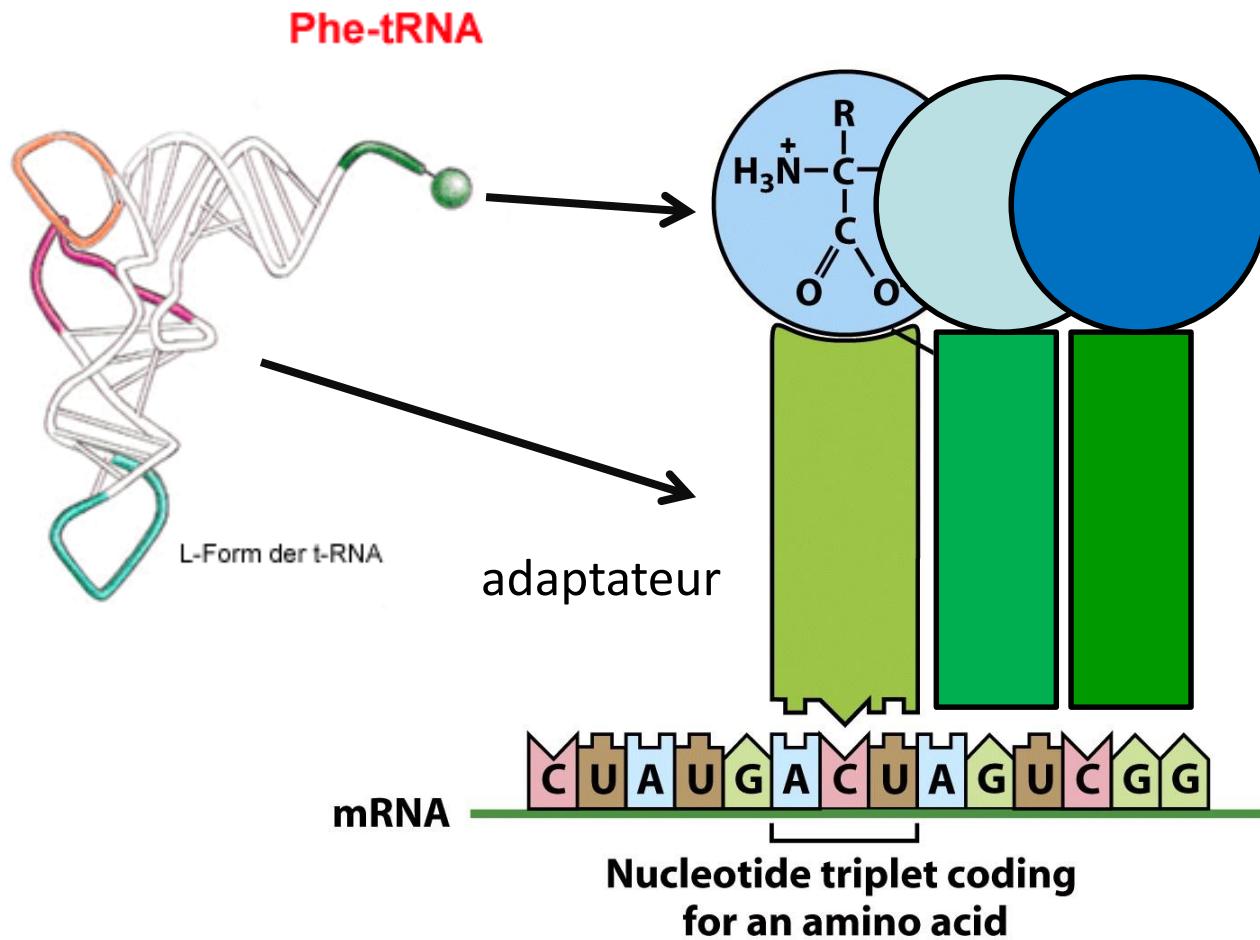
# Aminoacyl-tRNA synthétase



# tRNA (RNA de transfert)



# Translation de RNA



**Figure 27-2**  
*Lehninger Principles of Biochemistry, Fifth Edition*  
© 2008 W.H. Freeman and Company

# Translation de RNA

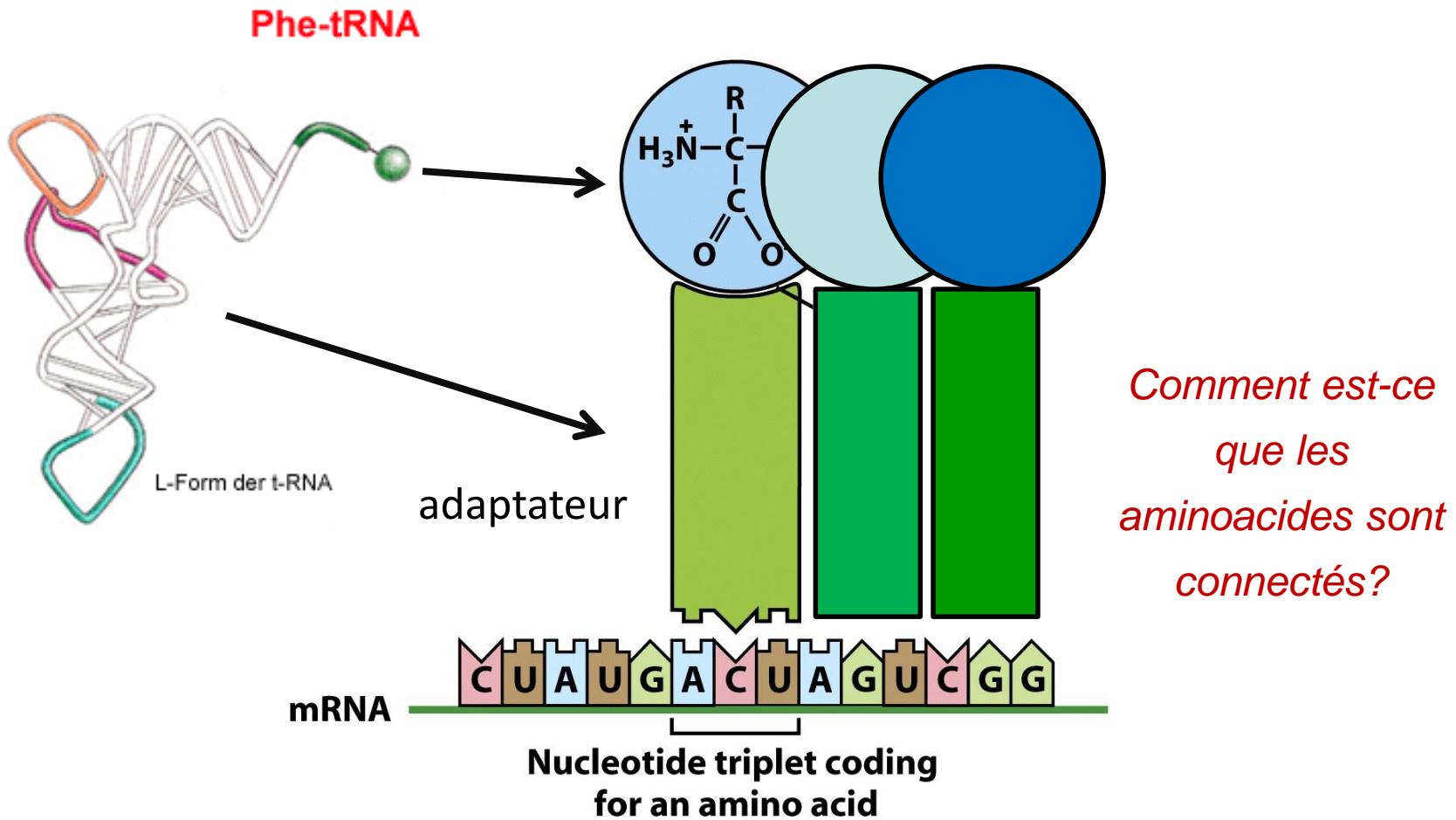
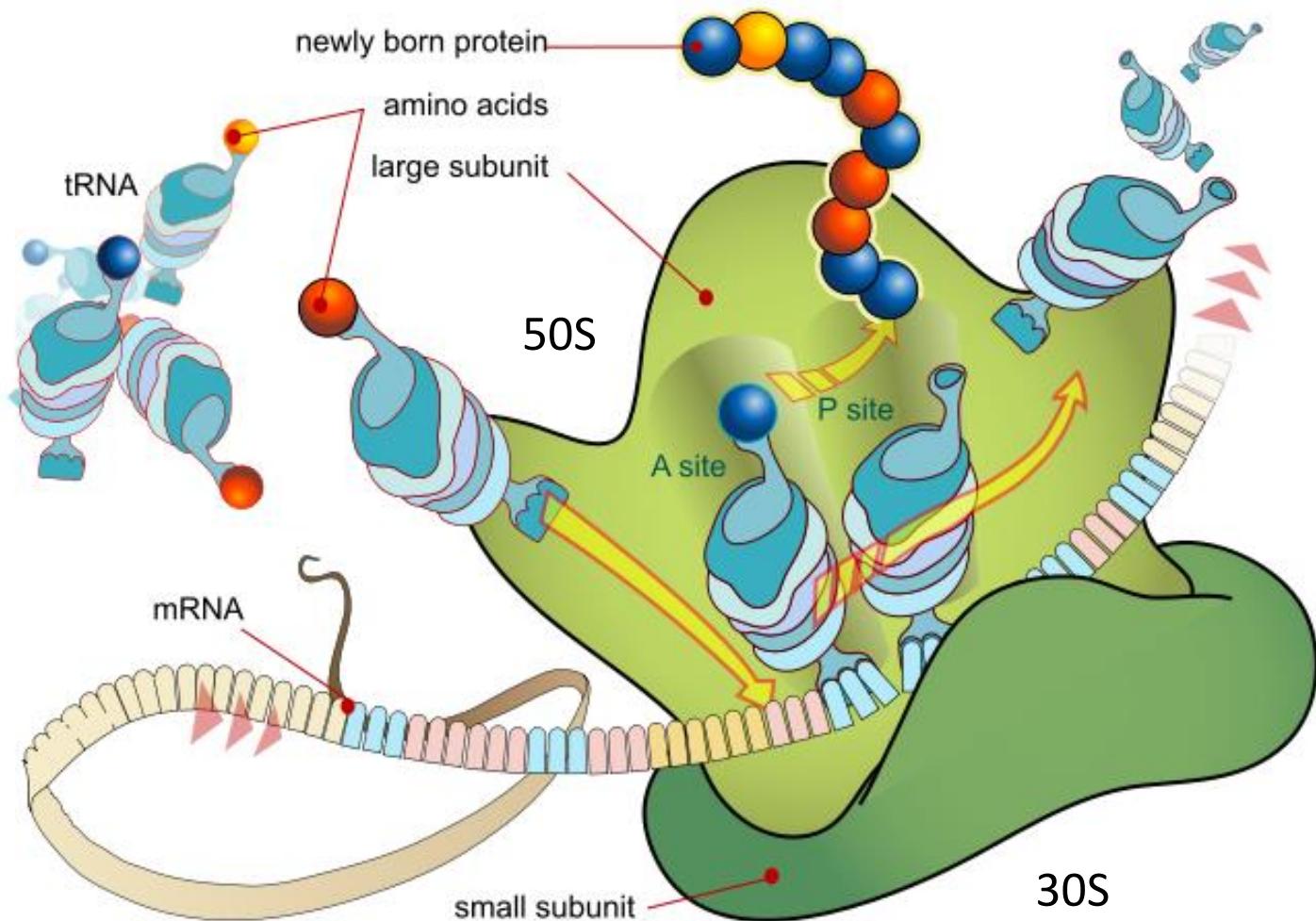
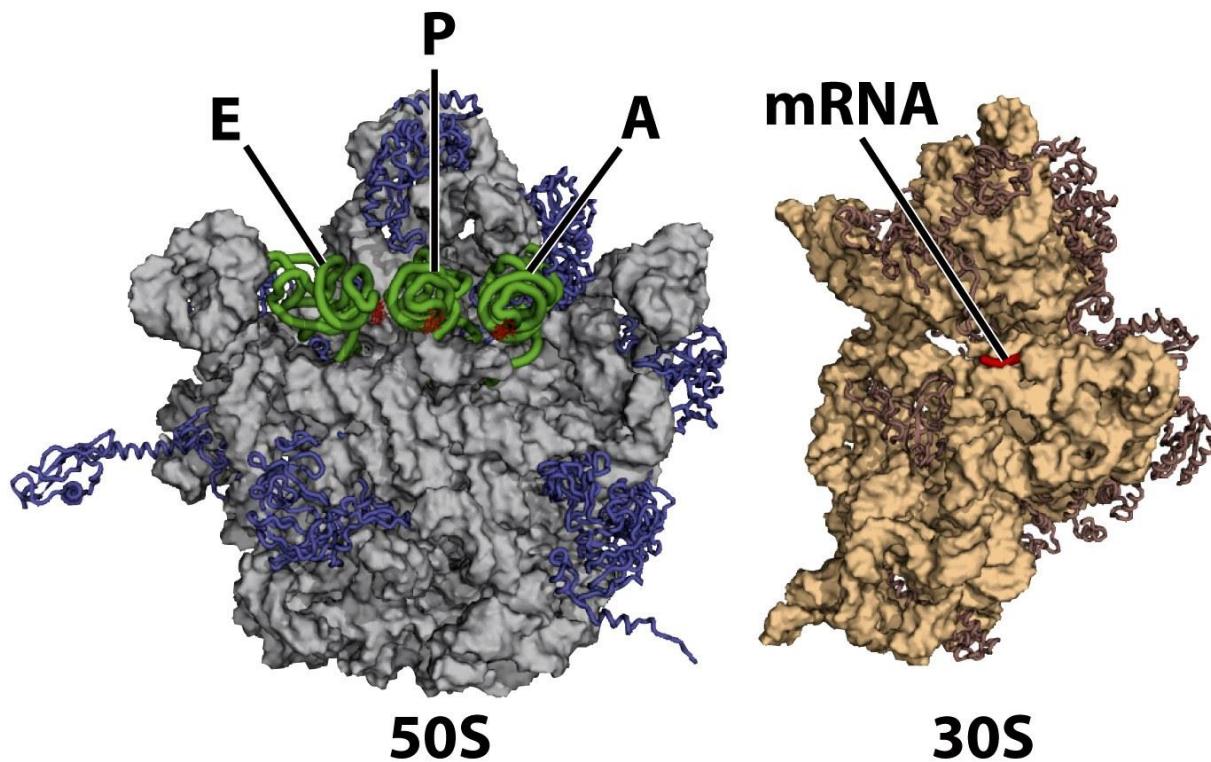


Figure 27-2  
Lehninger Principles of Biochemistry, Fifth Edition  
© 2008 W.H. Freeman and Company

# Ribosome

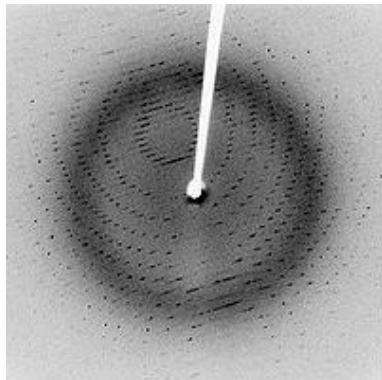


# Ribosome



- 2 sous-unités (50S et 30S)
- Constituants du ribosome: protéines et RNA (rRNA)

# Ada Yonath



# Traduction par le ribosome

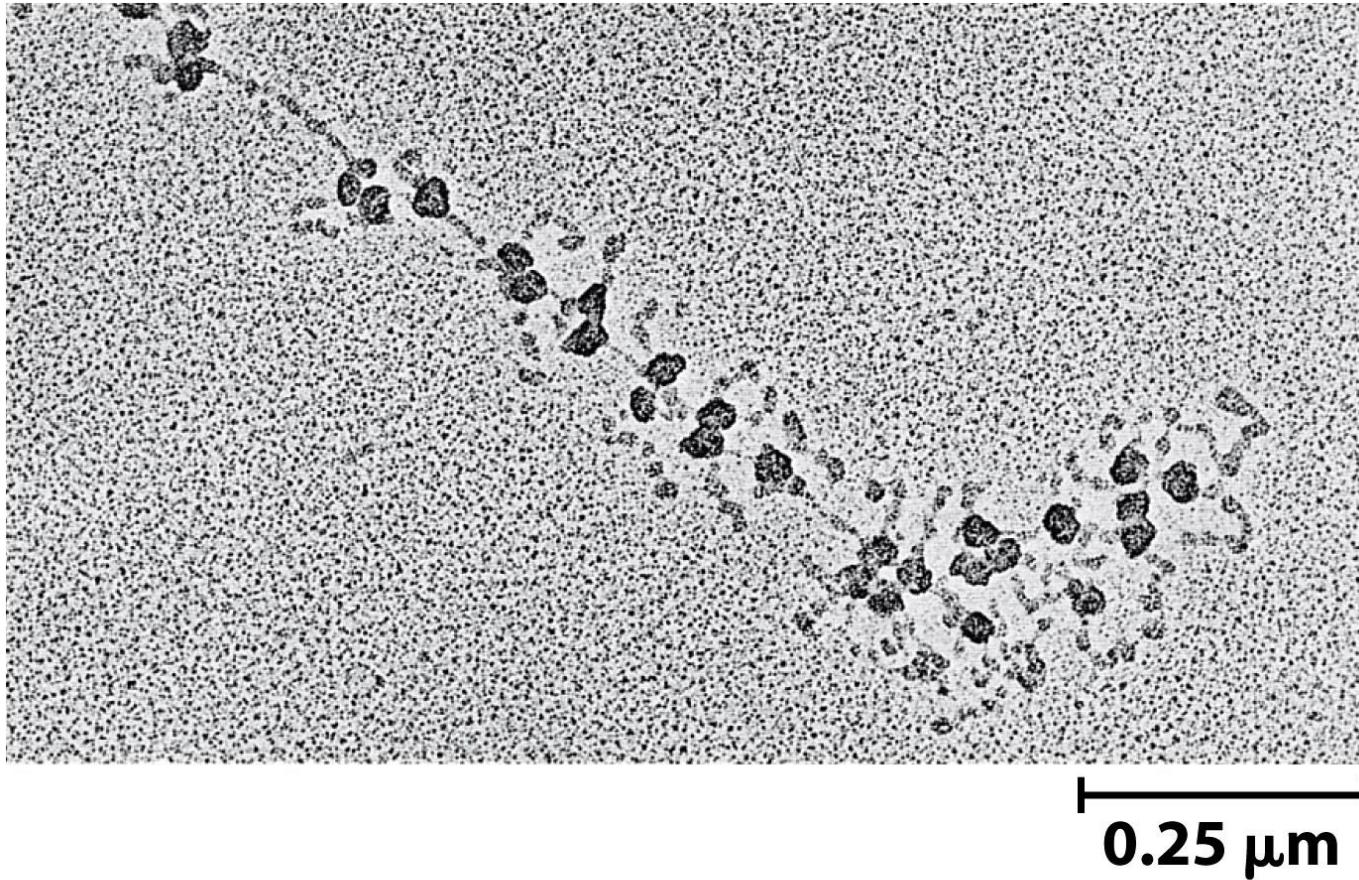
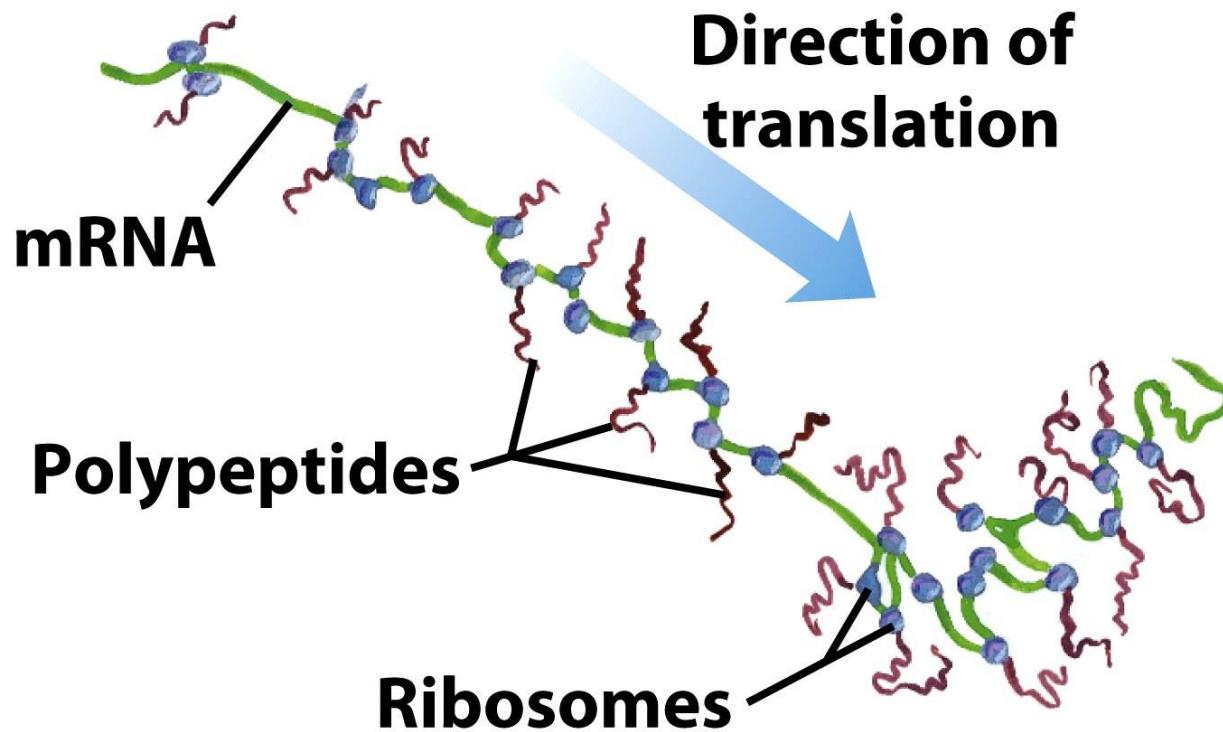


Image d'un mRNA et des ribosomes par microscopie électronique

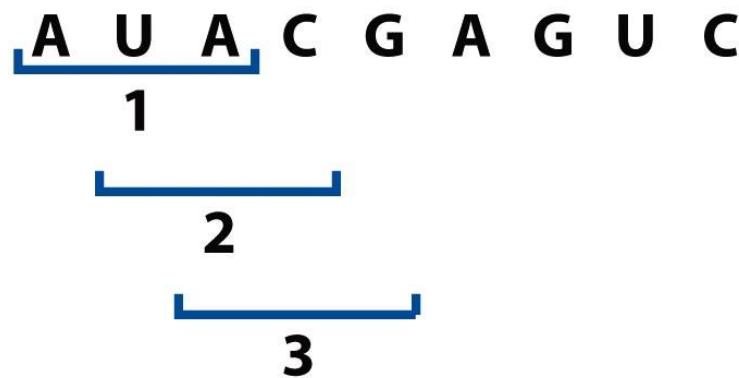
# Traduction par le ribosome



*Comment le ribosome sait-il à quelle position sur le mRNA il doit commencer la traduction?*

# Reading frame: 3 possibilités

**Overlapping  
code**



# Reading frame: 3 possibilités

Reading frame 1 5'---**U U C U C G G A C C U G G A G A U U C A C A G U**--- 3'

Reading frame 2 ---**U U C U C G G A C C U G G A G A U U C A C A G U**---

Reading frame 3 ---**U U C U C G G A C C U G G A G A U U C A C A G U**---

Phe – Ser – Asp – Leu - Glu - ...

Ser – Arg – Thr – Trp – Arg - ...

Leu – Gly – Pro – Gly - Asp - ...

# Reading frame: 3 possibilités

Reading frame 1 5'---[U U C] [U C G] [G A C] [C C U] [G G A] [G A U] [U U C] [A C A] [G U]--- 3'

Reading frame 2 ---[U U C] [U C G] [G A C] [C C U] [G G A] [G A U] [U U C] [A C A] [G U]---

Reading frame 3 ---[U U C] [U C G] [G G A] [C C U] [G G A] [G A U] [U U C] [A C A] [G U]---

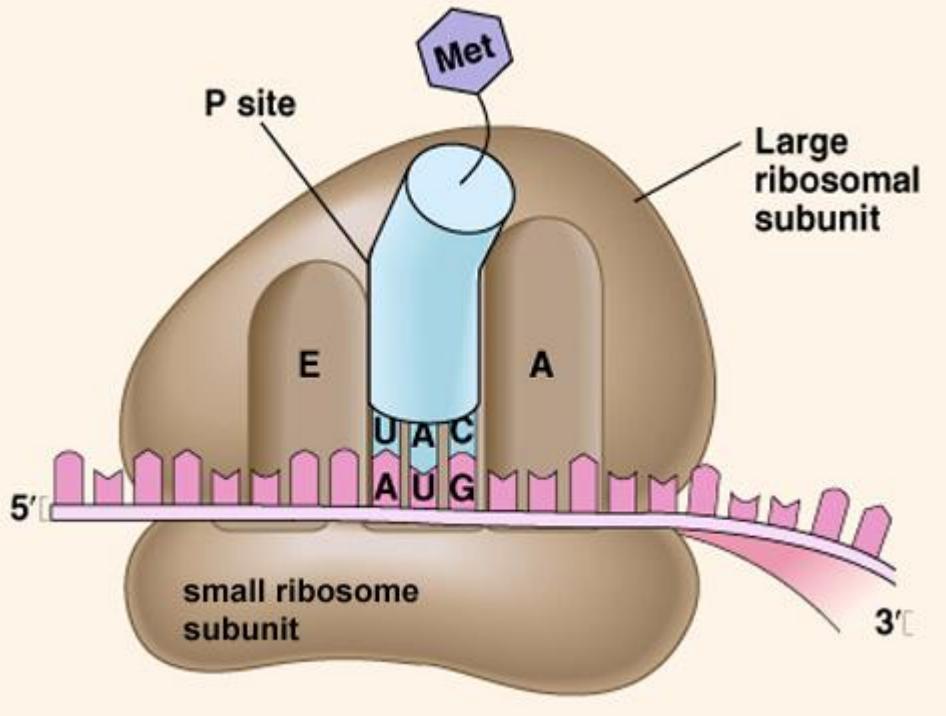
Phe – Ser – Asp – Leu - Glu - ...

Ser – Arg – Thr – Trp – Arg - ...

Leu – Gly – Pro – Gly - Asp - ...

*Comment le ribosome sait-il à quelle position  
sur le mRNA il doit commencer la traduction?*

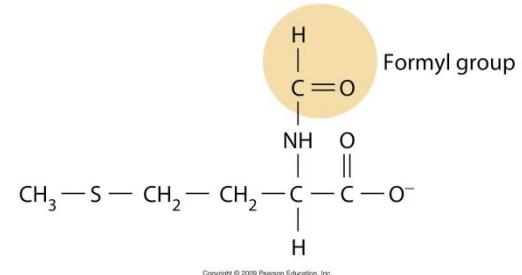
# La traduction commence toujours avec une méthionine



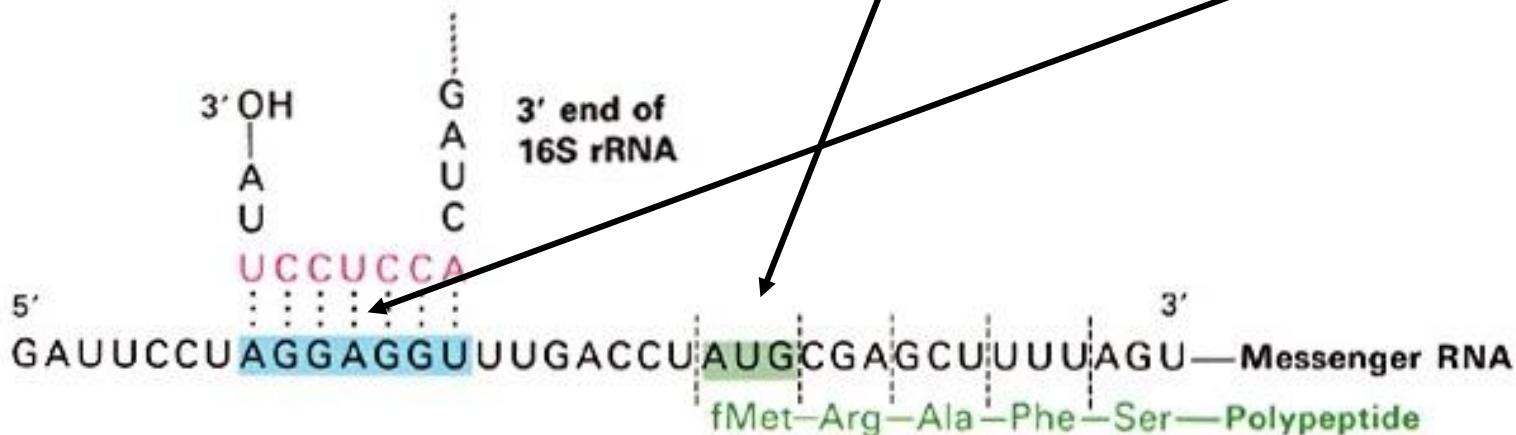
		Second Base					
		U	C	A	G		
First Base	U	UUU Phe UUC UUA Leu UUG	UCU Ser UCC UCA UCG	UAU Tyr UAC UAA Stop UAG Stop	UGU Cys UGC UGA Stop UGG Trp	U C A G	
	C	CUU CUC CUA Leu CUG	CCU CCC CCA Pro CCG	CAU His CAC CAA Gln CAG	CGU CGC Arg CGA CGG	U C A G	
A	A	AUU AUC Ile AUA AUG Met / Start	ACU ACC ACA Thr ACG	AAU Asn AAC AAA Lys AAG	AGU Ser AGC AGA Arg AGG	U C A G	
	G	GUU GUC GUA Val GUG	GCU GCC Ala GCA GCG	CAU Asp GAC GAA Glu GAG	GGU GGC Gly GGA GGG	U C A G	

Signal de départ (start codon): AUG

# Séquence de Shine-Dalgarno



- Premier aminoacide chez les procaryotes: **fMet**
- Signal d'initiation: région avec plusieurs bases de purine (**Shine-Dalgarno**)



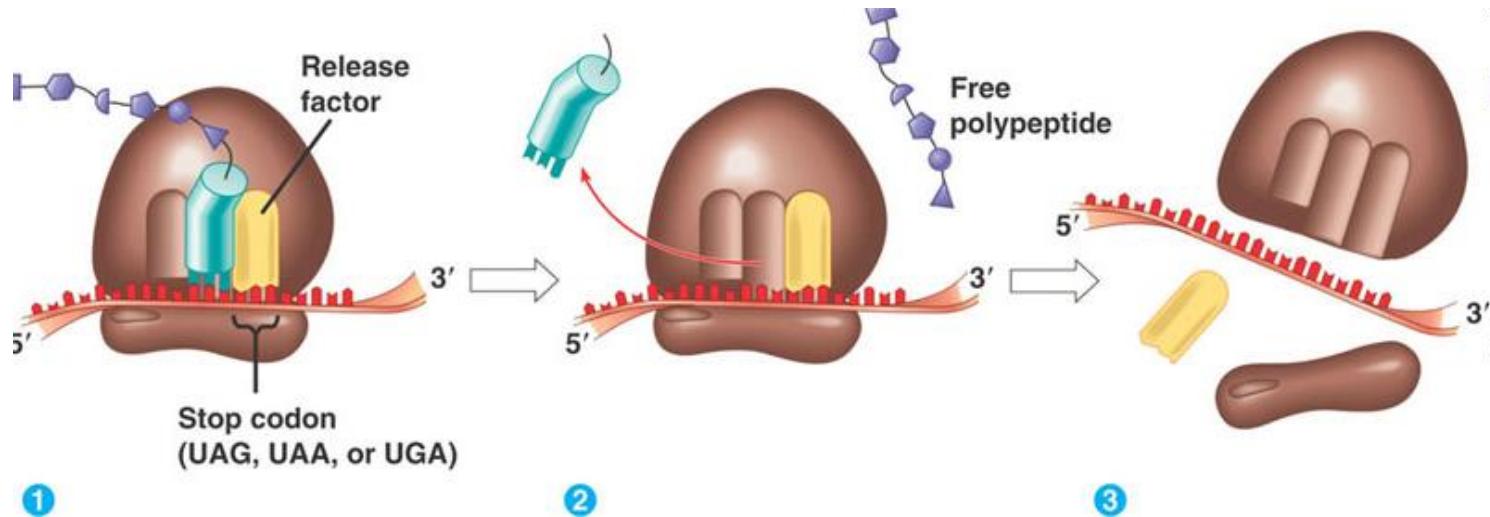
# Terminaison de la chaîne

*Comment le ribosome sait-il à quelle position  
sur le mRNA il doit **terminer** la traduction?*

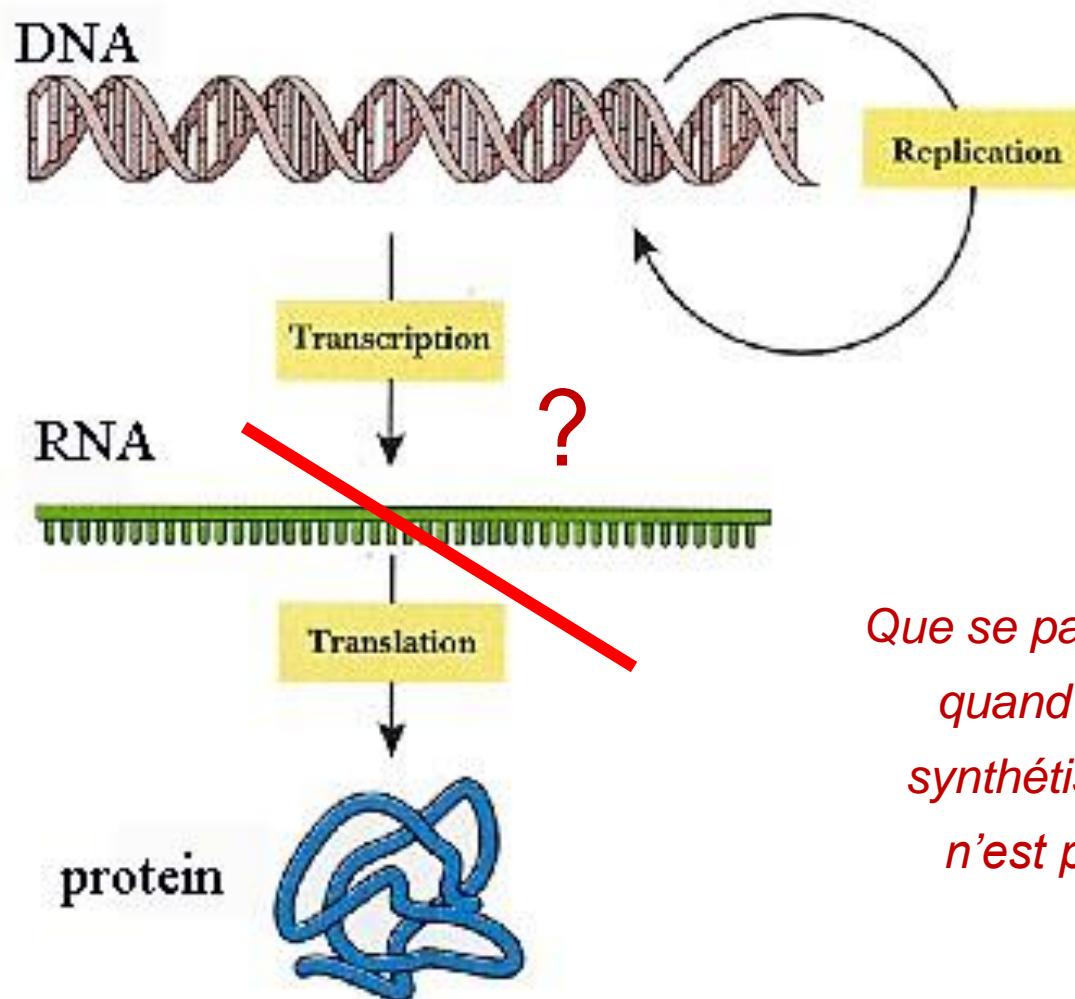
# Codons et facteurs de terminaison

		Second Base				
		U	C	A	G	
First Base	U	UUU Phe UUC UUA Leu UUG	UCU Ser UCC UCA UCG	UAU Tyr UAC UAA Stop UAG Stop	UGU Cys UGC UGA Stop UGG Trp	U C A G
	C	CUU CUC Leu CUA CUG	CCU CCC Pro CCA CCG	CAU His CAC CAA Gin CAG	CGU CGC Arg CGA CGG	U C A G
A	A	AUU AUC Ile AUA AUG Met / Start	ACU ACC Thr ACA ACG	AAU Asn AAC AAA Lys AAG	AGU Ser AGC AGA Arg AGG	U C A G
	G	GUU GUC Val GUA GUG	GCU GCC Ala GCA GCG	CAU Asp GAC GAA Glu GAG	GGU GGC Gly GGA GGG	U C A G

- Codons de terminaison: des tRNA n'existent pas pour quelques codons: **UAA, UAG, UGA**
- Facteurs de terminaison: des protéines spécifiques

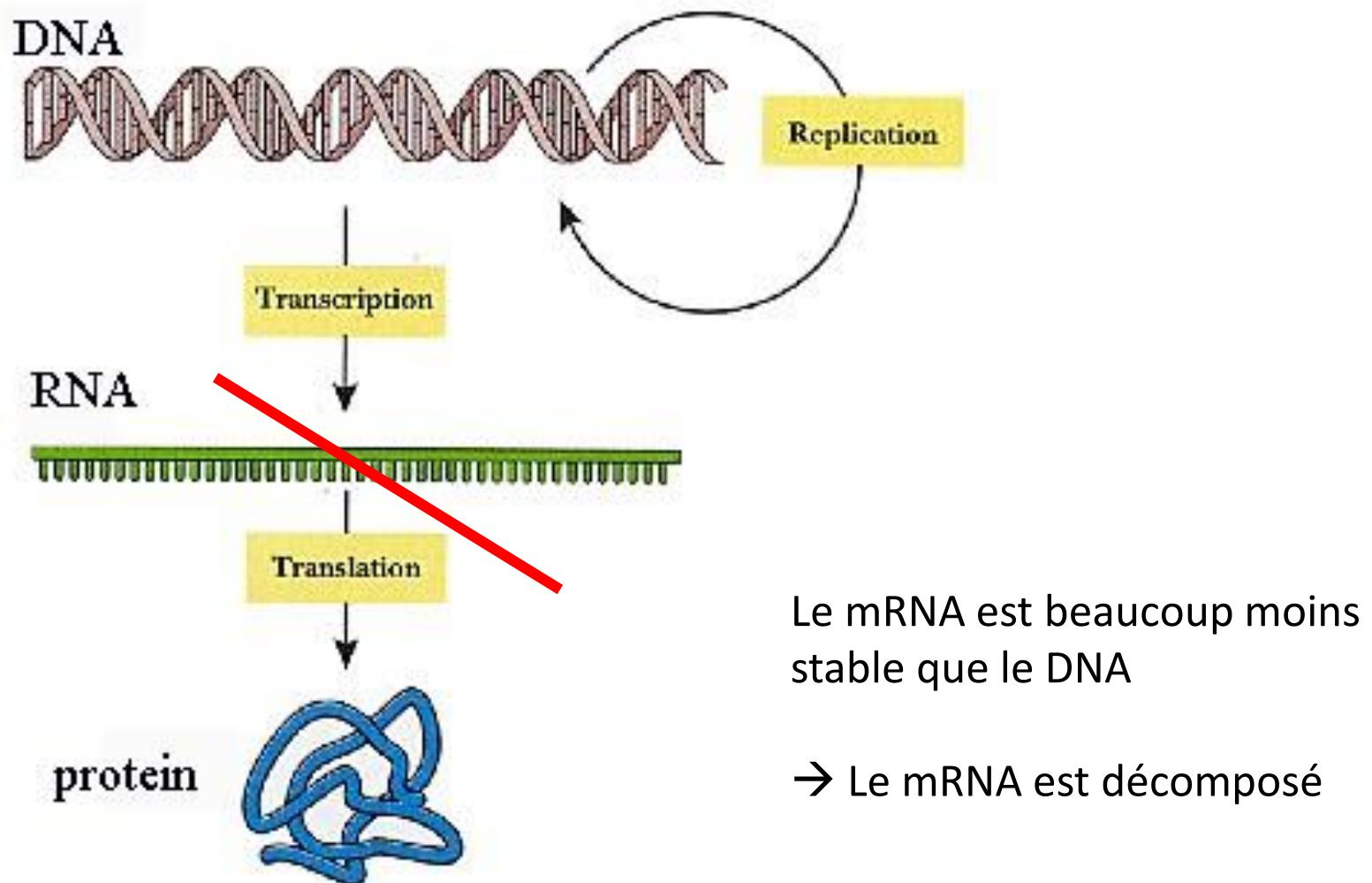


# Transcription et traduction

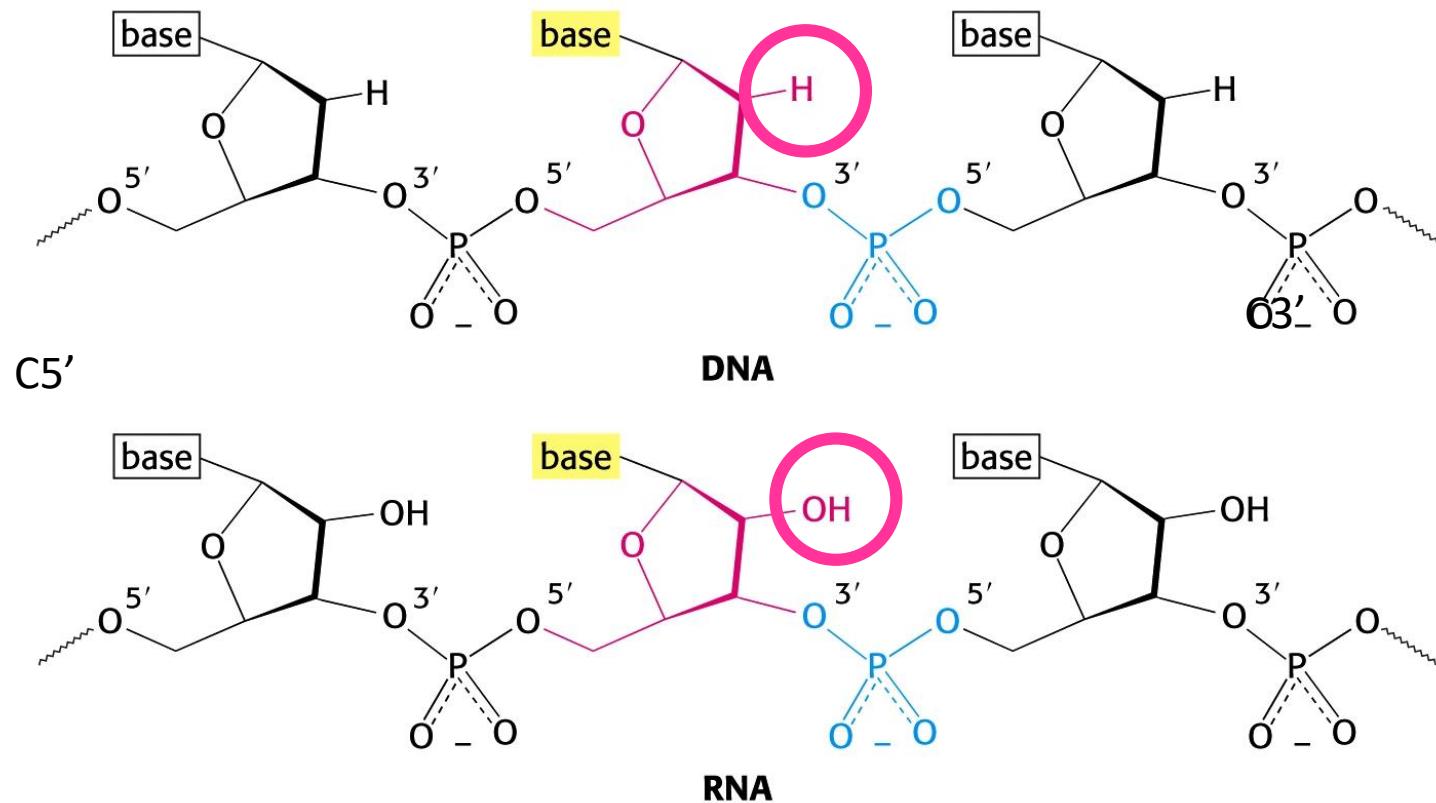


*Que se passe-t-il avec le RNA  
quand la protéine a été  
synthétisée et que le RNA  
n'est plus nécessaire?*

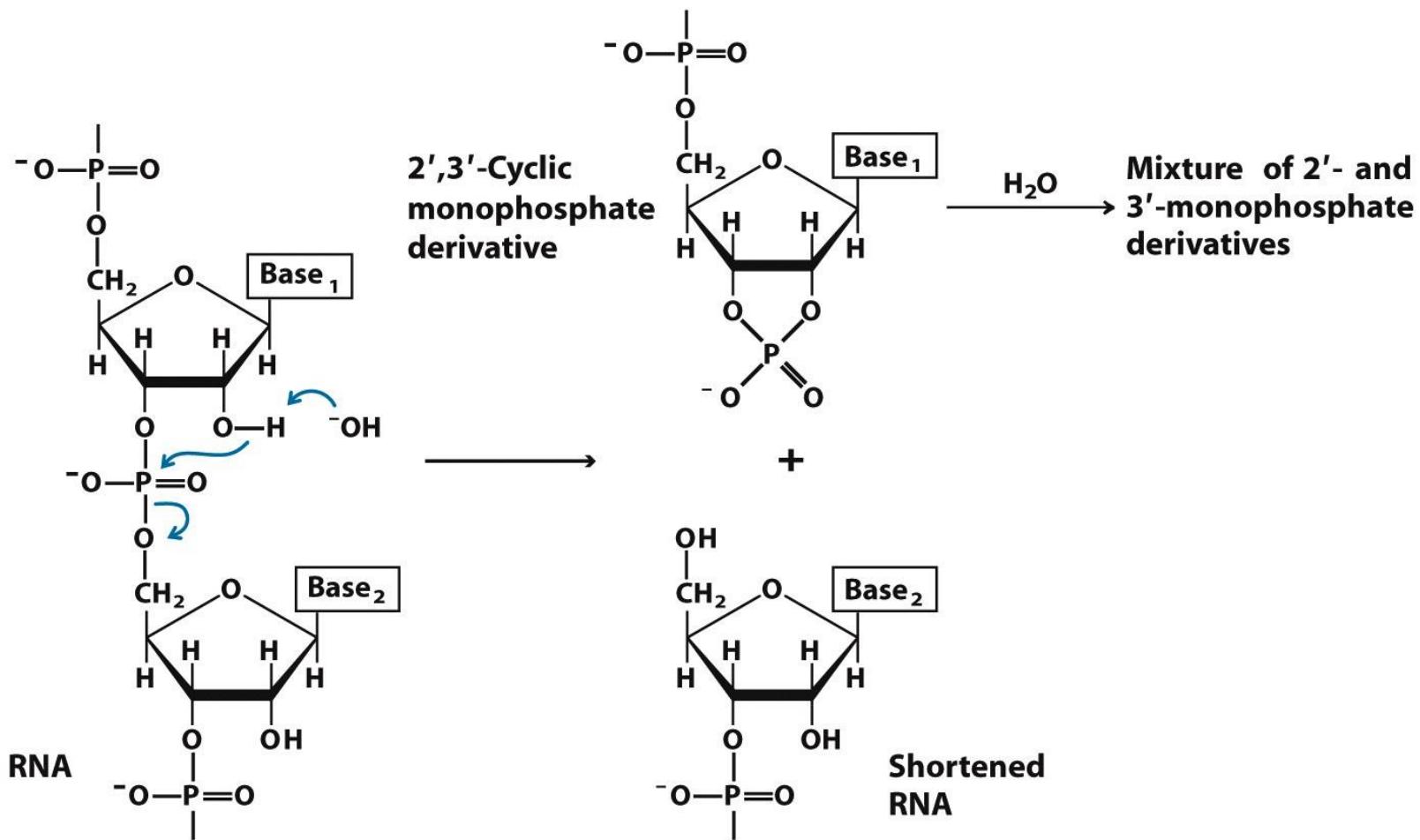
# Transcription et traduction



# Stabilité du DNA et RNA



*Pourquoi est-ce que le RNA est chimiquement moins stable que le DNA?*



**Figure 8-8**

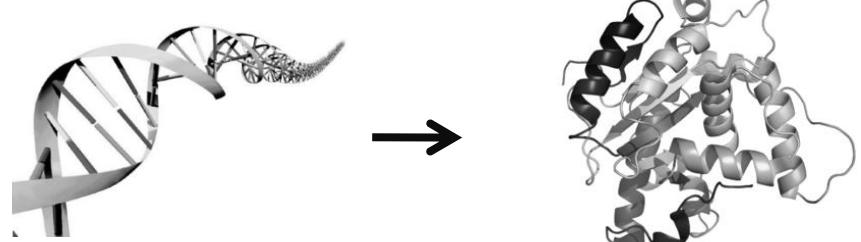
*Lehninger Principles of Biochemistry, Fifth Edition*

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# Leçon 6

- Synthèse des protéines

- RNA
- Ribosome



- Expression de protéines recombinantes

- DNA isolé d'un donneur
- Plasmide (vecteur) de DNA
- Expression dans un hôte différent



une méduse

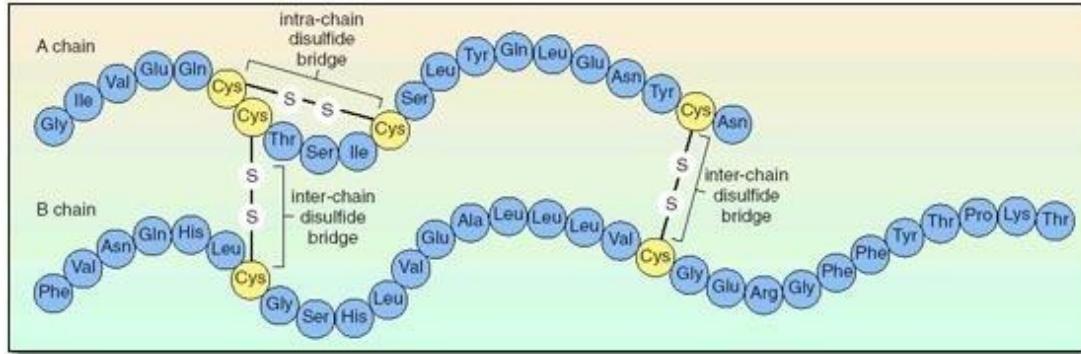


DNA d'une protéine  
fluorescente

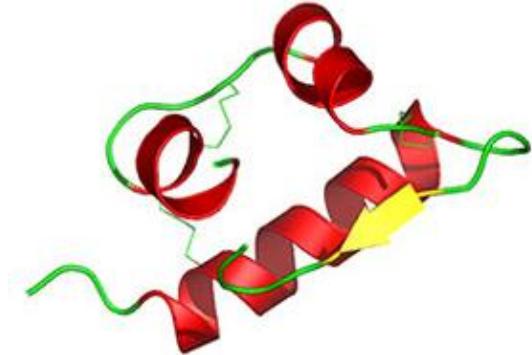


souris fluorescentes

# Insuline



structure primaire



structure tertiaire

- 51 aminoacides (5808 Da)
- Protéine importante pour la régulation de la concentration en glucose
- Une des premières protéines produites par la technologie recombinante

# Découverte de l'insuline

- 1921: Les injections d'extrait d'insuline d'un pancréas de chien diminuèrent la concentration en glucose
- L'insuline des animaux ( cochon, vache) est utilisée pour les personnes diabétiques

*Quelles sont les limitations de l'insuline porcine ou bovine?*



Banting and Best

# Découverte de l'insuline

- 1921: Les injections d'extrait d'insuline d'un pancréas de chien diminuèrent la concentration en glucose
- L'insuline des animaux ( cochon, vache) est utilisée pour les personnes diabétiques

*Quelles sont les limitations de l'insuline porcine ou bovine?*

- insuline de porc: 1 mutation d'aminoacide
- insuline bovine: 3 mutations d'aminoacide



Banting and Best

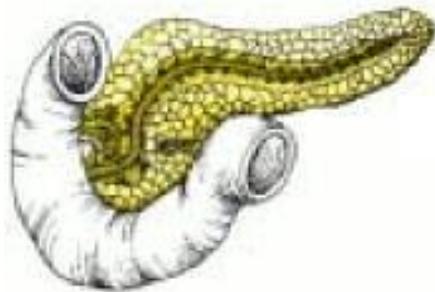
# Insuline humaine recombinante

- 1978 Genentech produit de l'insuline 'humaine' dans des bactéries de *Escherichia coli* par la technologie de DNA recombinant

**Genentech**

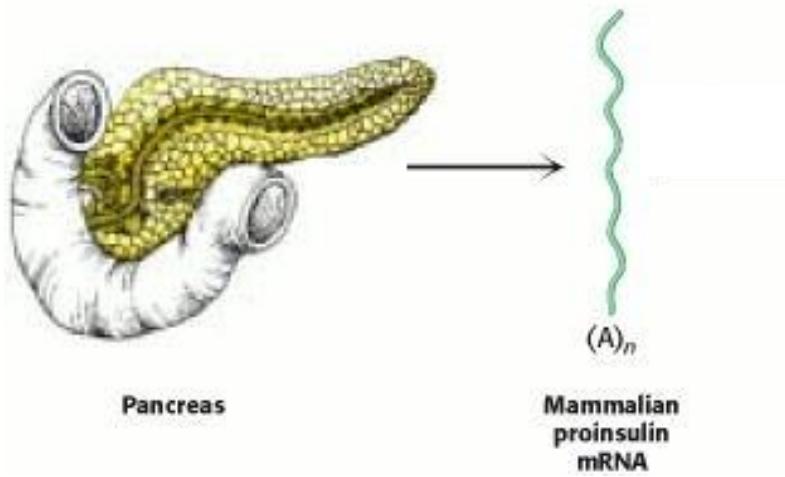


# Expression recombinante de insuline



Pancreas

# Expression recombinante de l'insuline

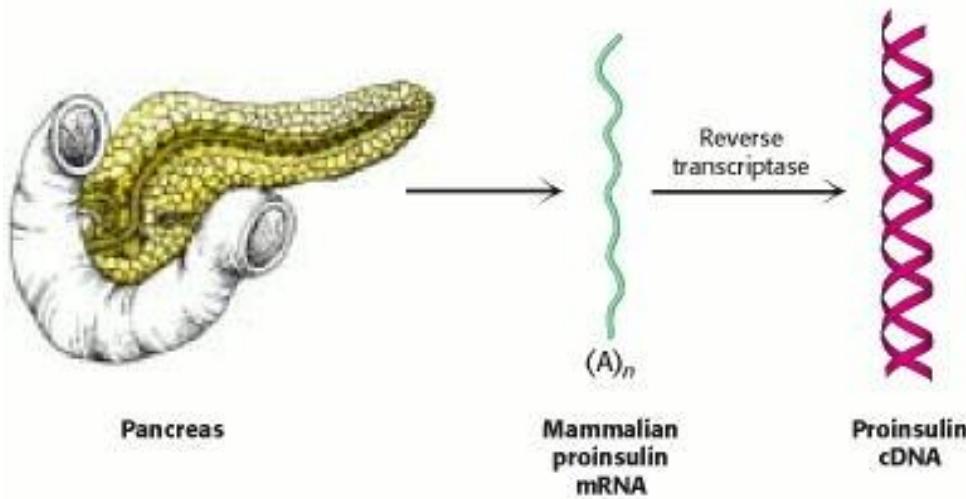


## Isolation du matériel

### génétique:

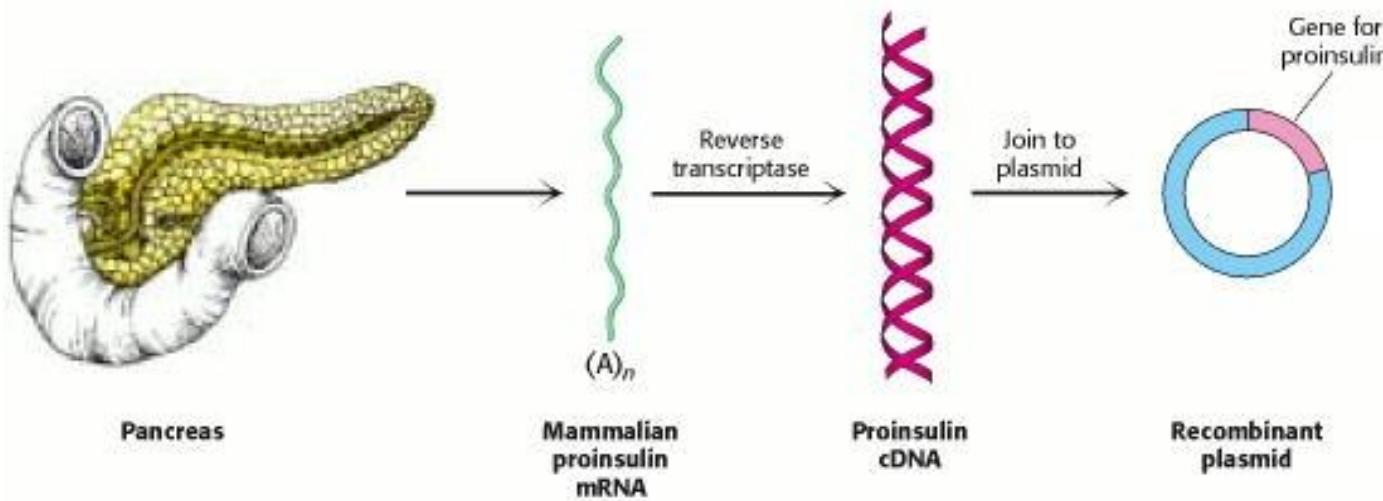
- DNA génomique
- **mRNA**

# Expression recombinante de l'insuline



- |   |                         |
|---|-------------------------|
| <b>Isolation du matériel génétique:</b> | <b>Synthèse de DNA:</b> |
| - DNA génomique                         | - <b>cDNA</b>           |
| - <b>mRNA</b>                           |                         |

# Expression recombinante de insuline



**Isolation du matériel génétique:**

- DNA génomique
- **mRNA**

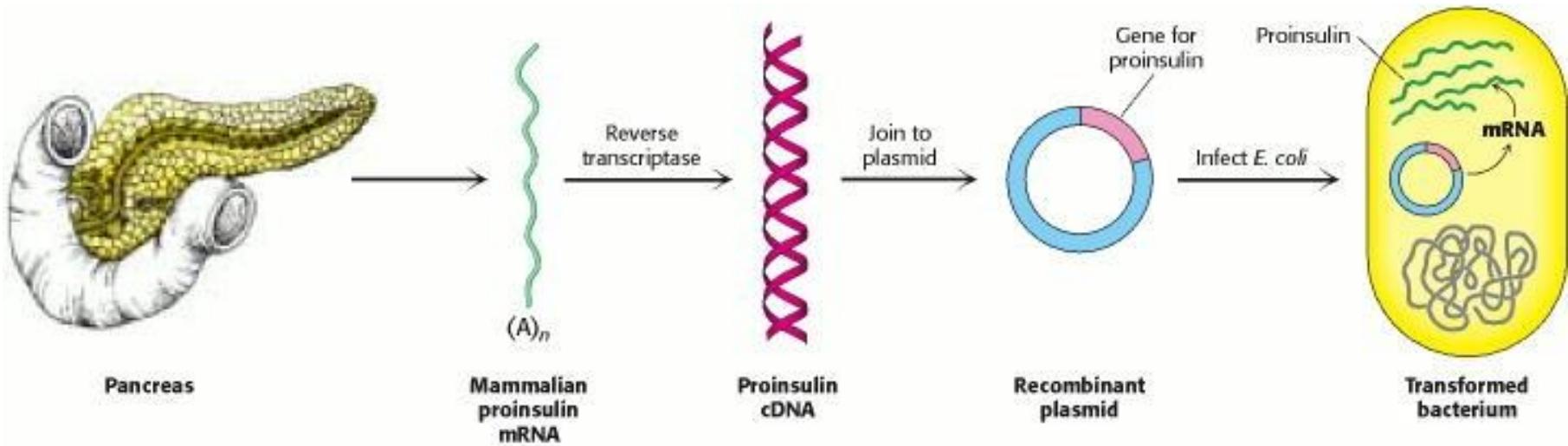
**Synthèse de DNA:**

- **cDNA**

**Insertion dans un vecteur:**

- **plasmide**

# Expression recombinante de insuline



**Isolation du matériel génétique:**

- DNA génomique
- **mRNA**

**Synthèse de DNA:**

- **cDNA**

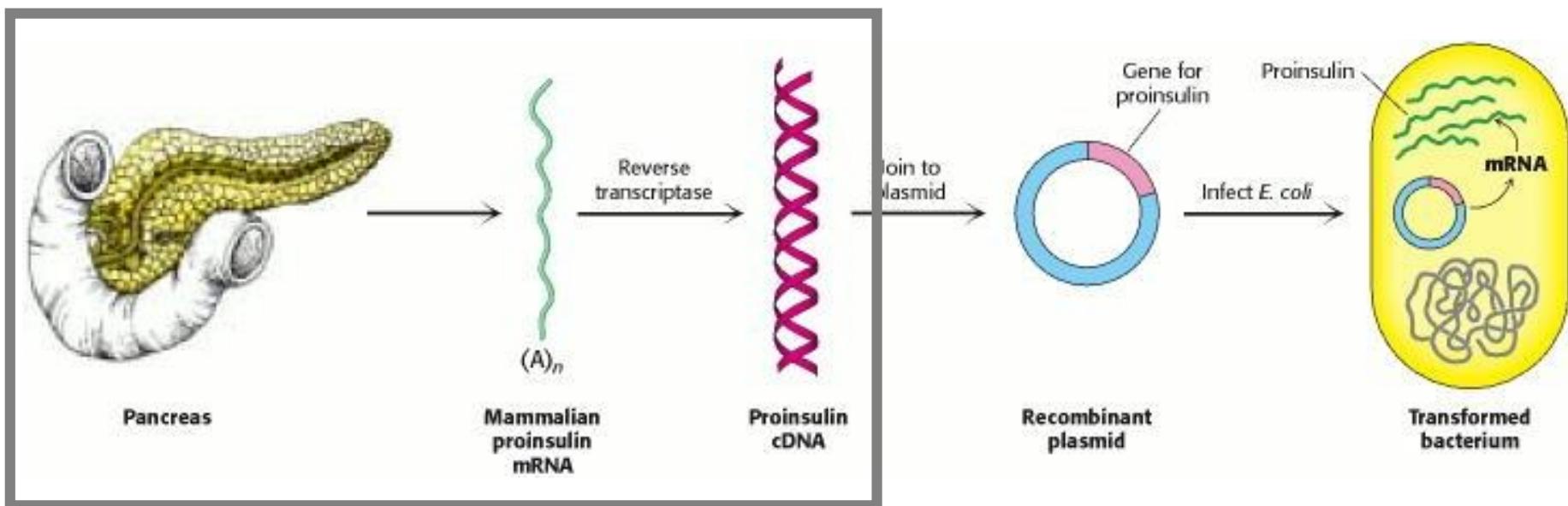
**Insertion dans un vecteur:**

- **plasmide**

**Transfert dans l'hôte:**

- **bactérie**

# Expression recombinante de insuline



**Isolation du matériel génétique:**

- DNA génomique
- **mRNA**

**Synthèse de DNA:**

- **cDNA**

**Insertion dans un vecteur:**

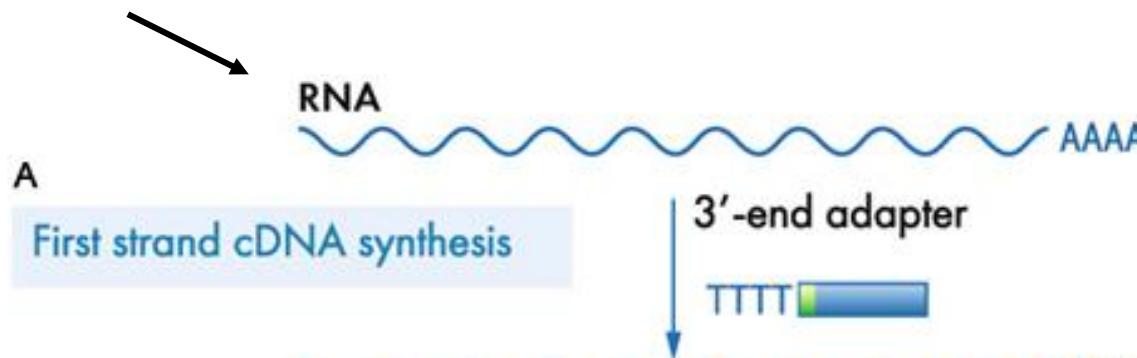
- **plasmide**

**Transfert dans l'hôte:**

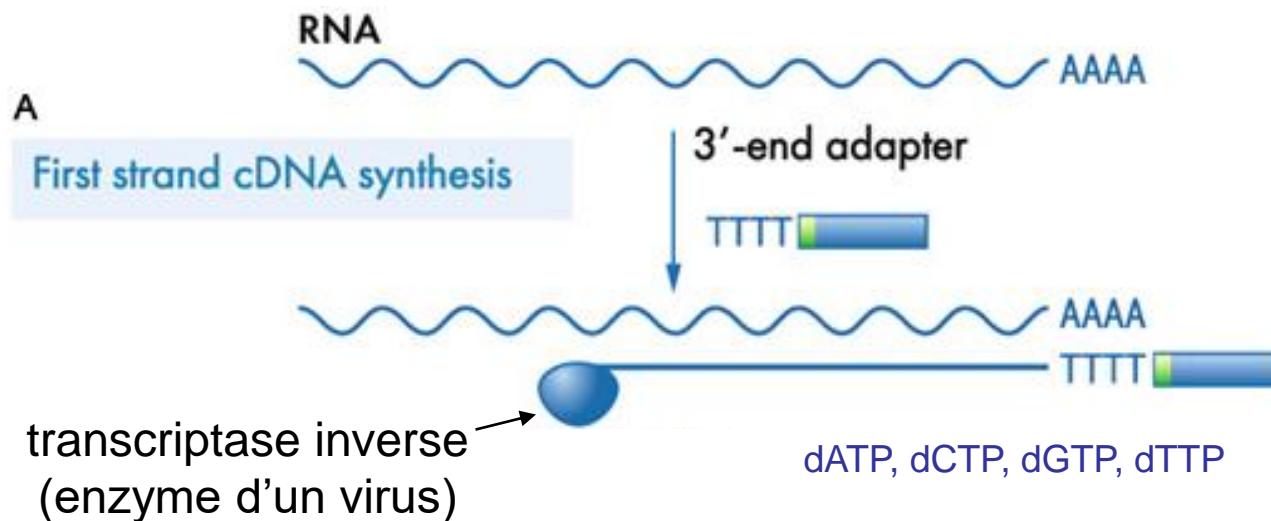
- **bactérie**

mRNA de  
plusieurs gènes

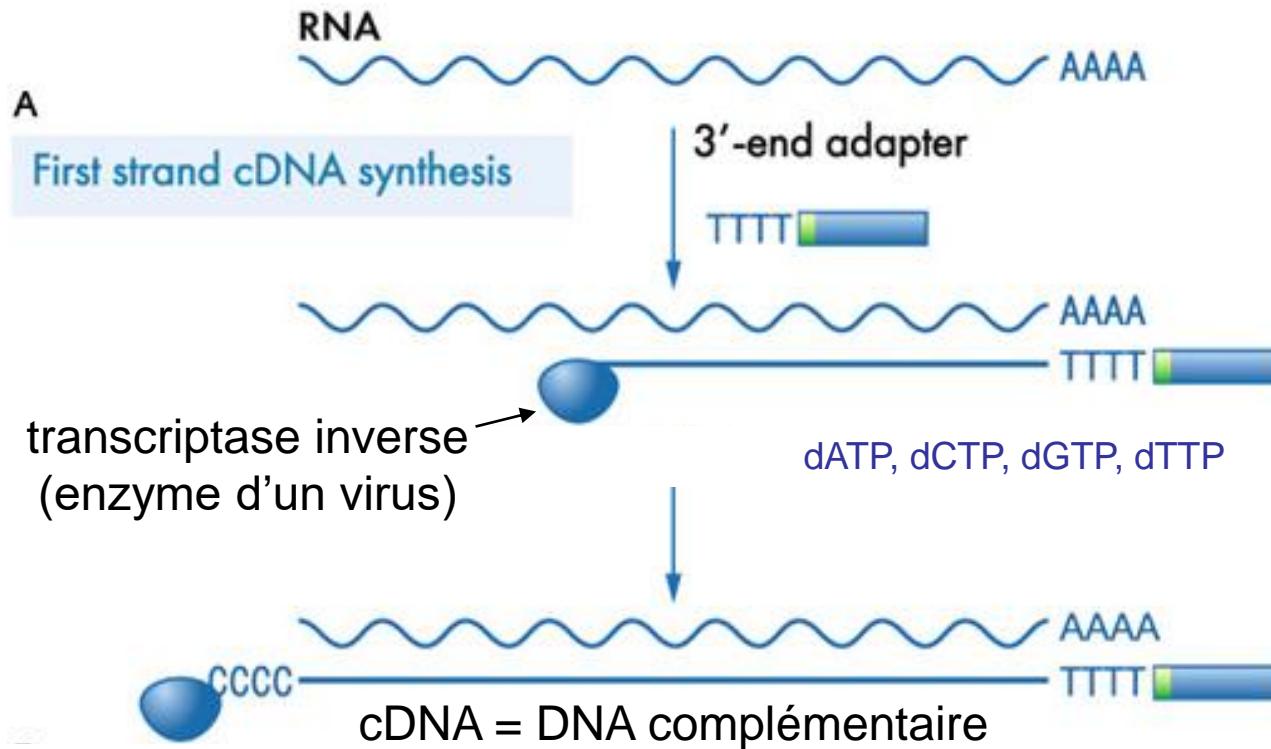
## Transcription inverse



# Transcription inverse

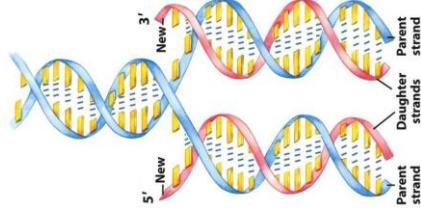


# Transcription inverse

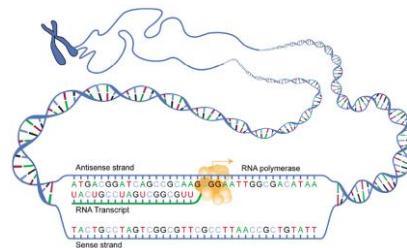


# Polymérases

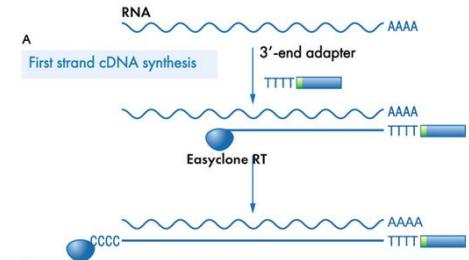
## DNA polymérase



## RNA polymérase



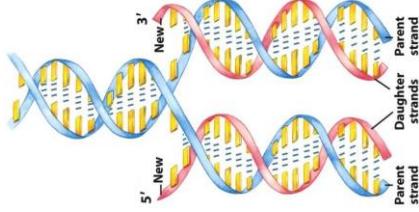
## transcriptase inverse



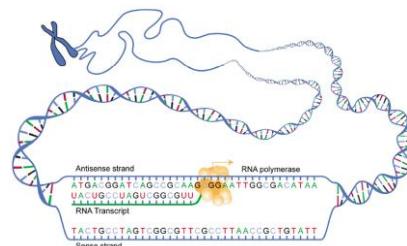
*Quelles sont les fonctions de ces trois polymérases?*

# Polymérases

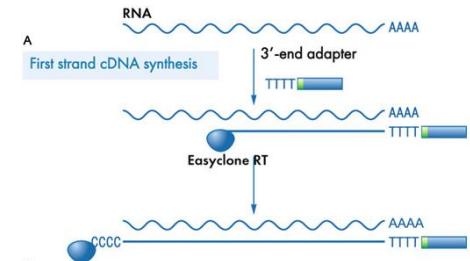
## DNA polymérase



## RNA polymérase



## transcriptase inverse



Fonction

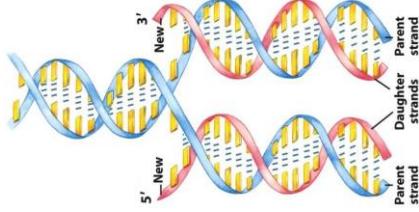
duplication de DNA

synthèse mRNA

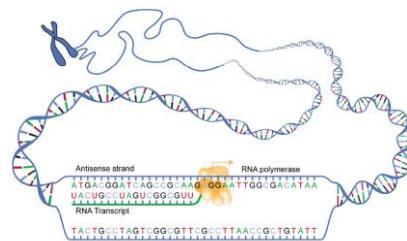
synthèse DNA

# Polymérases

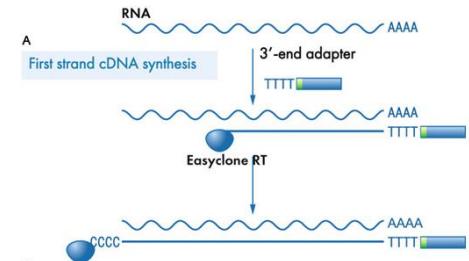
## DNA polymérase



## RNA polymérase



## transcriptase inverse



Fonction

duplication de DNA

synthèse mRNA

synthèse DNA

Matrice

DNA

DNA

RNA

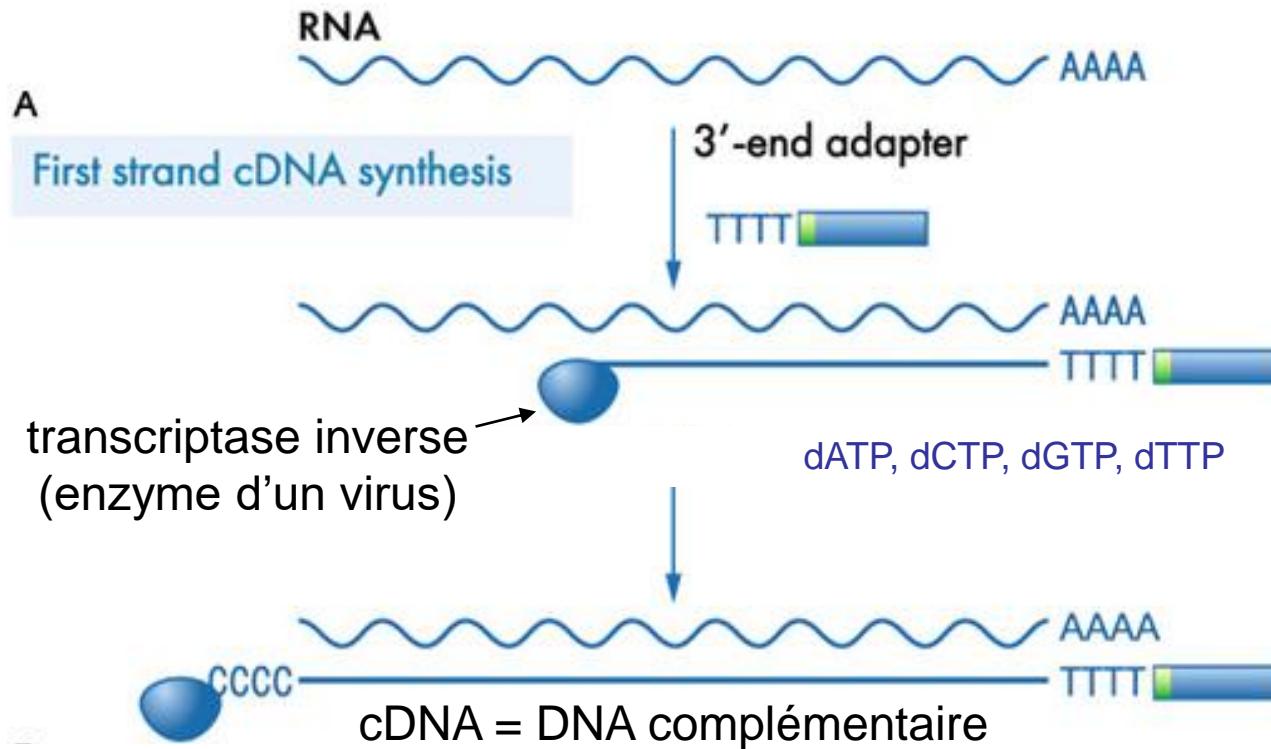
Nucléotides

dATP, dCTP, dGTP, dTTP

ATP, CTP, GTP, UTP

dATP, dCTP, dGTP, dTTP

# Transcription inverse



# Synthèse de DNA du gène d'insuline



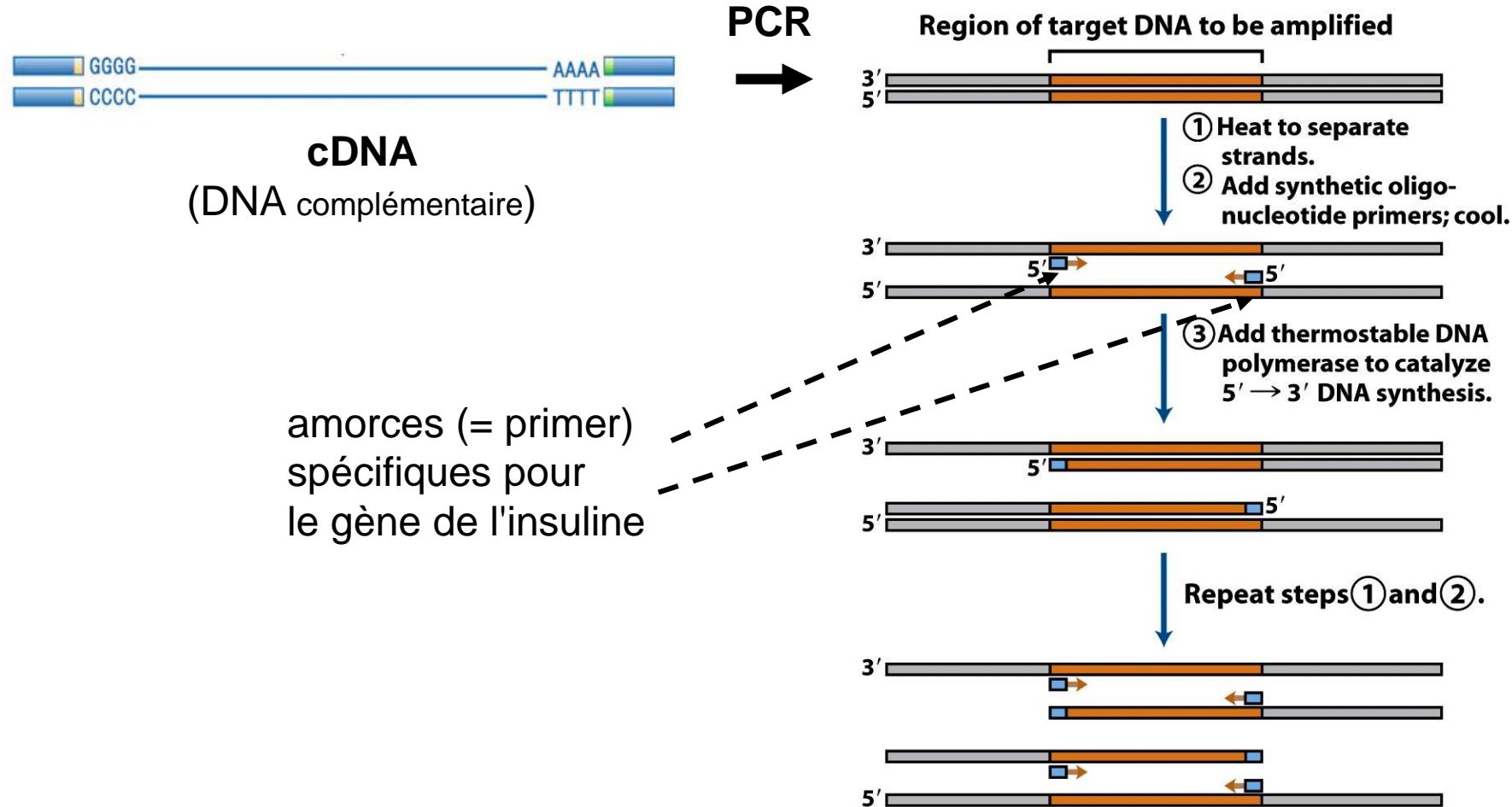
**cDNA**  
(DNA complémentaire)



cDNA de plusieurs gènes  
(pas seulement d'insuline!)

*Comment pourrait-on synthétiser de grandes quantités de  
DNA du **gène de l'insuline**?*

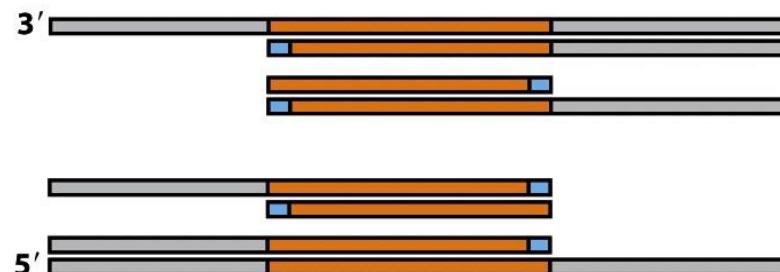
# Synthèse de DNA dans une réaction PCR



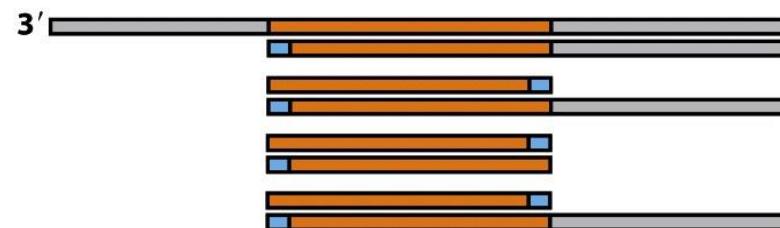
cDNA  
(DNA complémentaire)

amorces (= primer)  
spécifiques pour  
le gène de l'insuline

Figure 9-16a part 1  
*Lehninger Principles of Biochemistry, Fifth Edition*  
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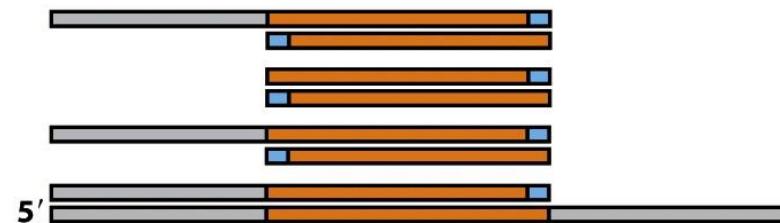
**Repeat steps ① through ③.**



25 cycles



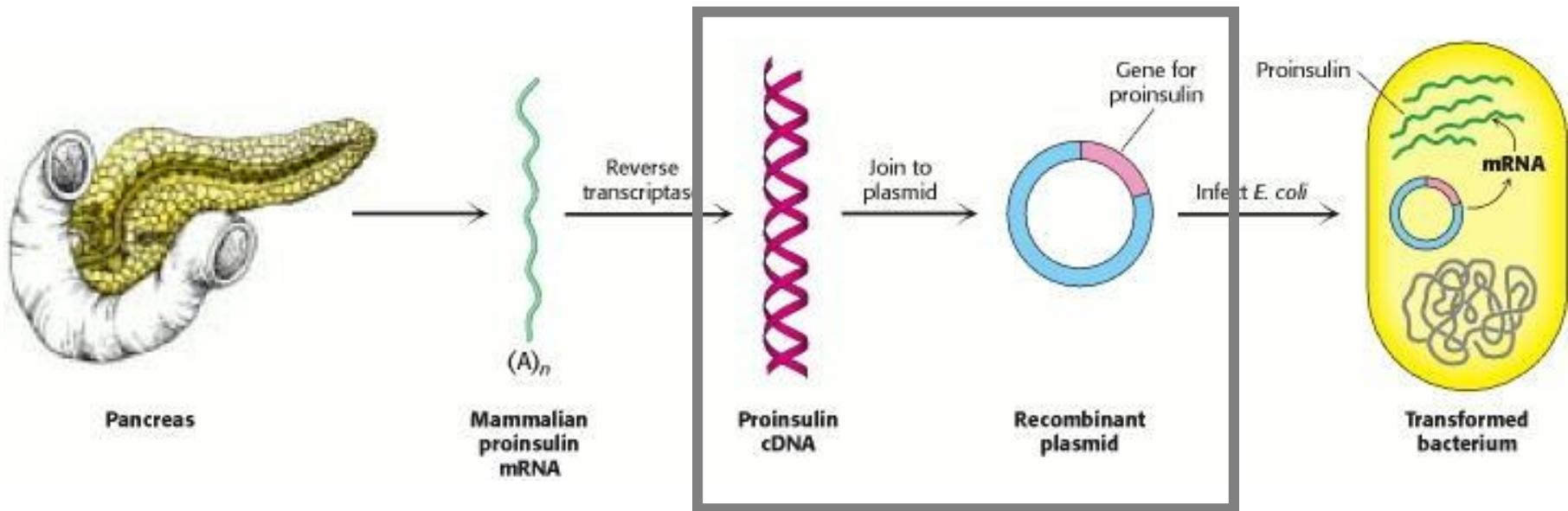
environ  $10^6$  copies  
du gène de l'insuline



**After 25 cycles, the target sequence has  
been amplified about  $10^6$ -fold.**

**Figure 9-16a part 3**  
*Lehninger Principles of Biochemistry, Fifth Edition*  
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# Expression recombinante de l'insuline



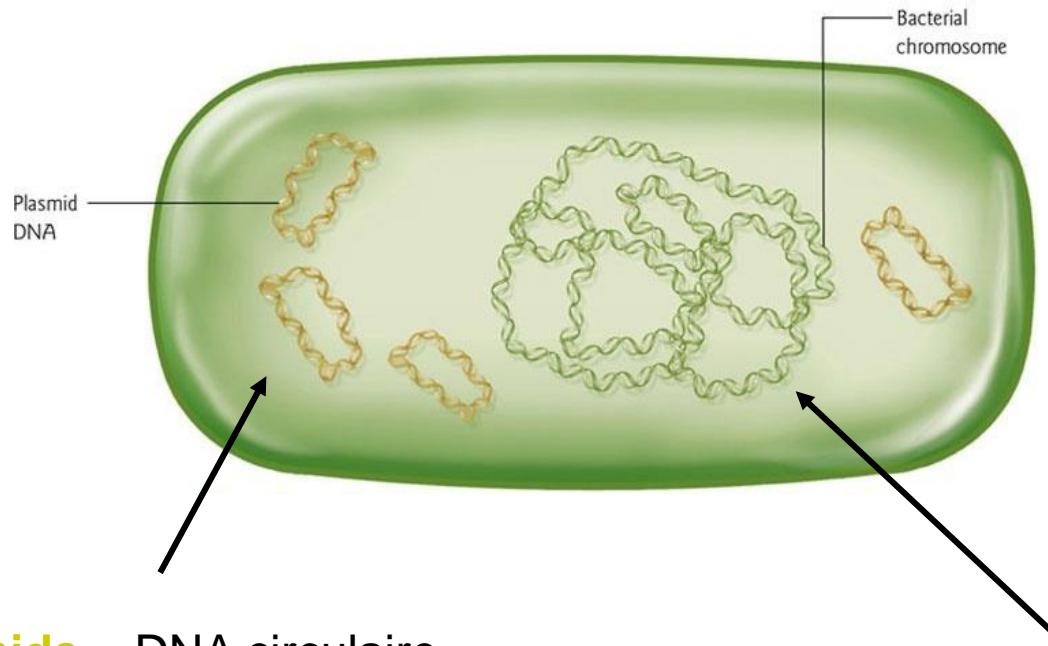
**Isolation du matériel génétique:**  
- DNA génomique  
- mRNA

**Synthèse de DNA:**  
- cDNA

**Insertion dans un vecteur:**  
- plasmide

**Transfert dans l'hôte:**  
- bactéries

# Plasmide de DNA



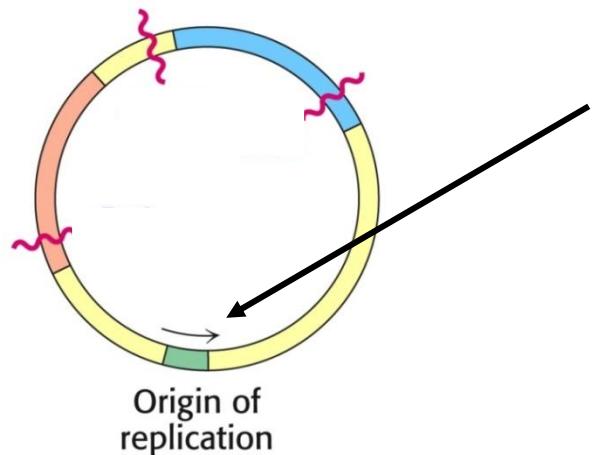
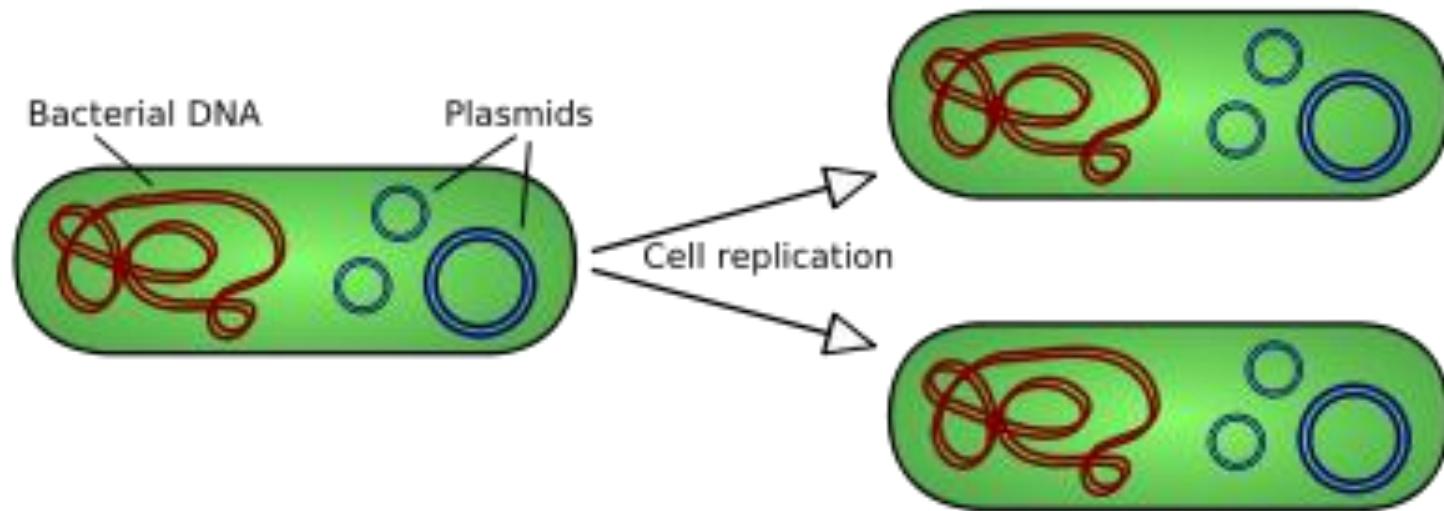
**Plasmide** = DNA circulaire

(environ 4000 nucléotides, peut se répliquer de façon autonome dans la bactérie)

**DNA génomique** de bactérie

(Dans *E.coli*, 4.6 million de nucléotides, contient tous les gènes de la bactérie)

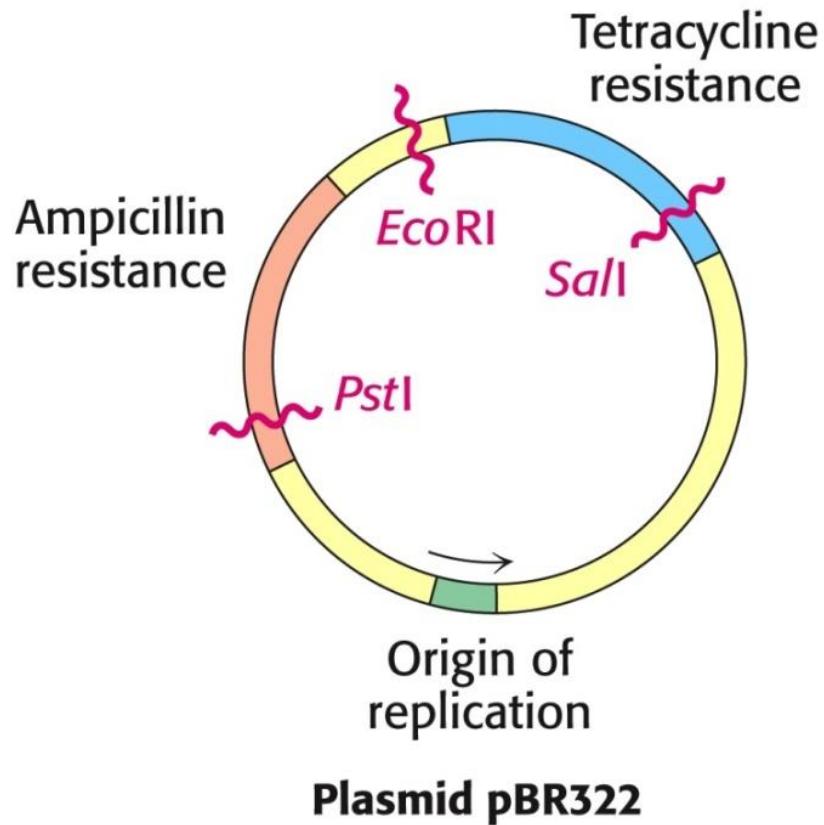
# Origine de réPLICATION



L'origine de réPLICATION permet au plasmide de se répliquer

Plasmid pBR322

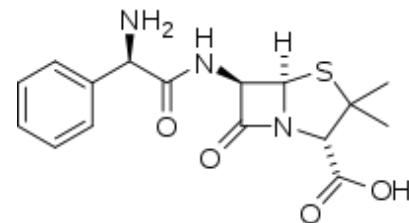
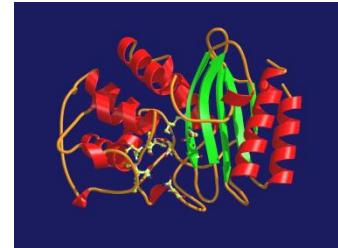
# Résistance d'antibiotique



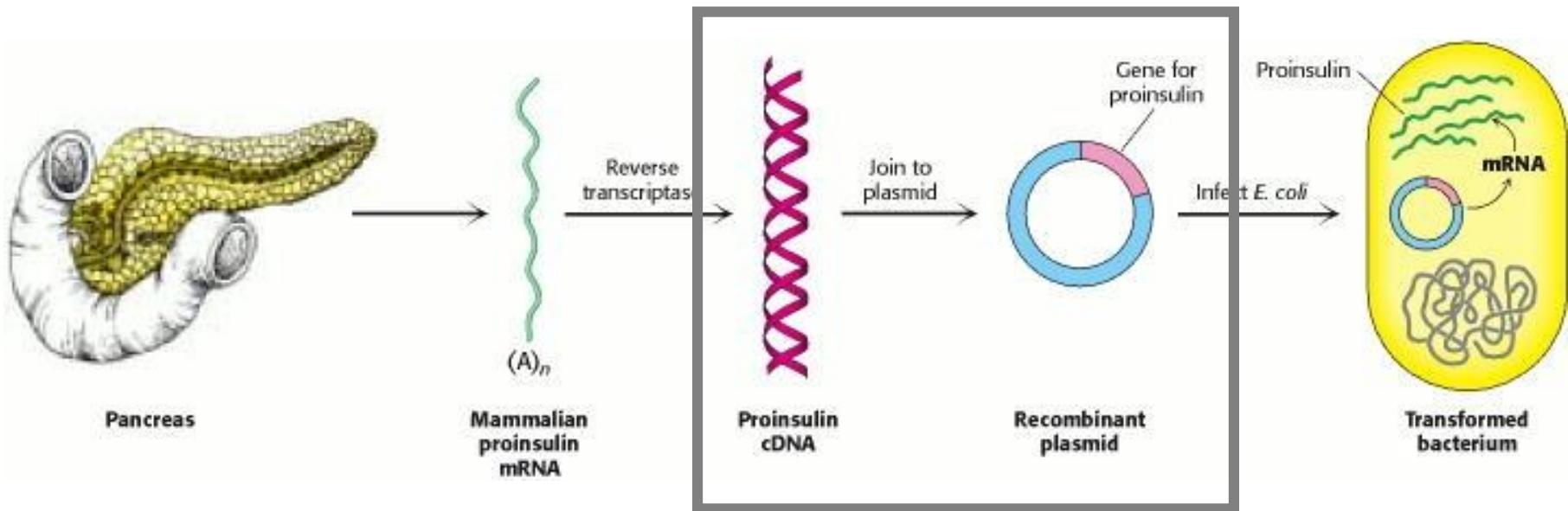
*E. coli* plasmid pBR322

**Example: résistance ampicillin**

→ expression de la protéine  
β-lactamase



# Expression recombinante de l'insuline



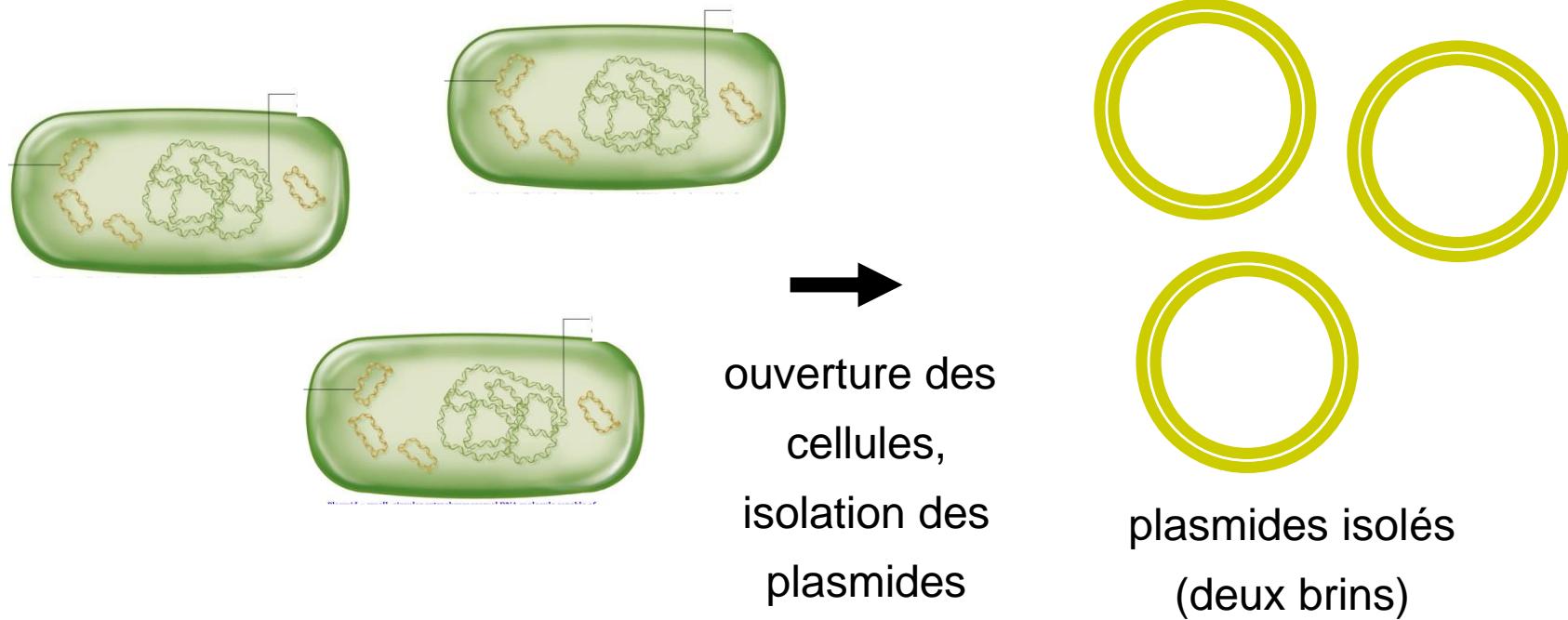
**Isolation du matériel génétique:**  
- DNA génomique  
- mRNA

**Synthèse de DNA:**  
- cDNA

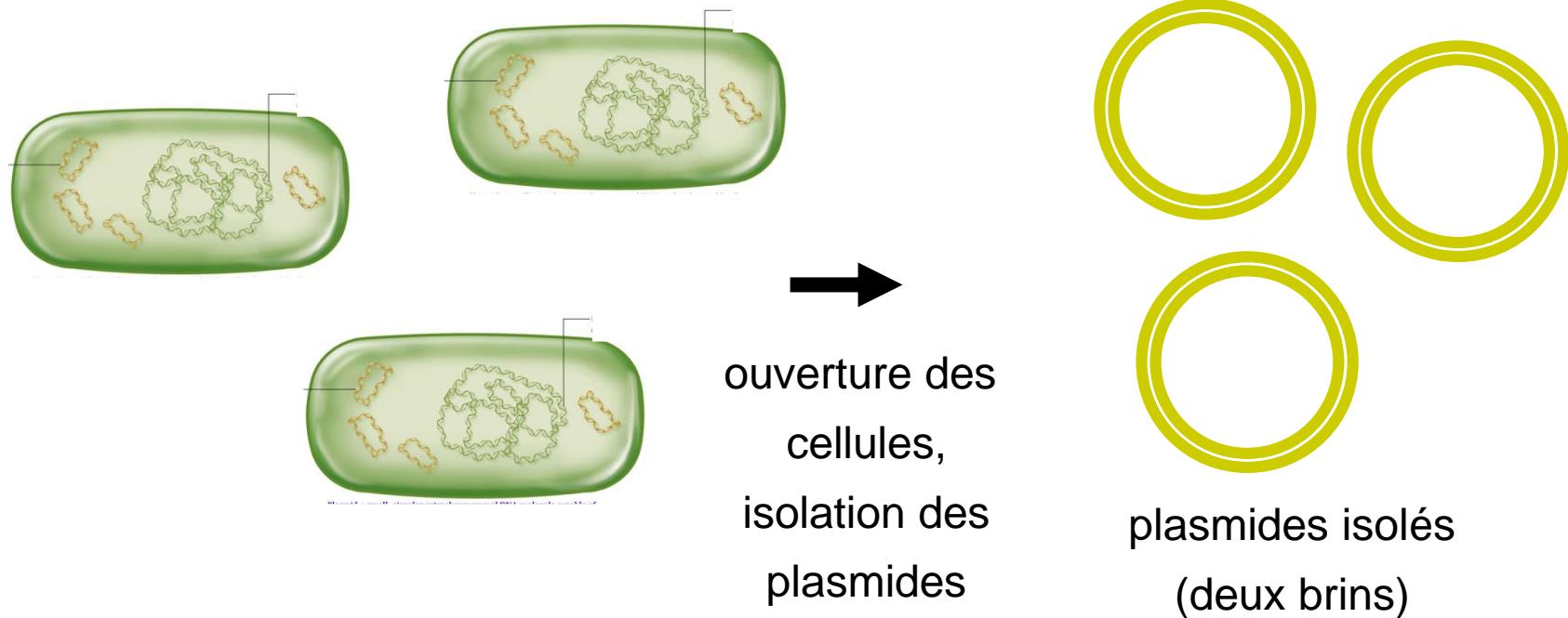
**Insertion dans un vecteur:**  
- plasmide

**Transfert dans l'hôte:**  
- bactéries

# Plasmide de DNA



# Plasmide de DNA



*Comment pourrait-on insérer le DNA de l'insuline dans un plasmide?*

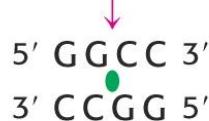
# Enzymes de restriction



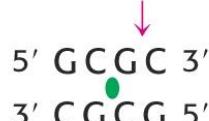
*BamHI*



*EcoRI*



*HaeIII*



*HhaI*



*XbaI*

circular  
double-stranded  
plasmid DNA  
(cloning vector)

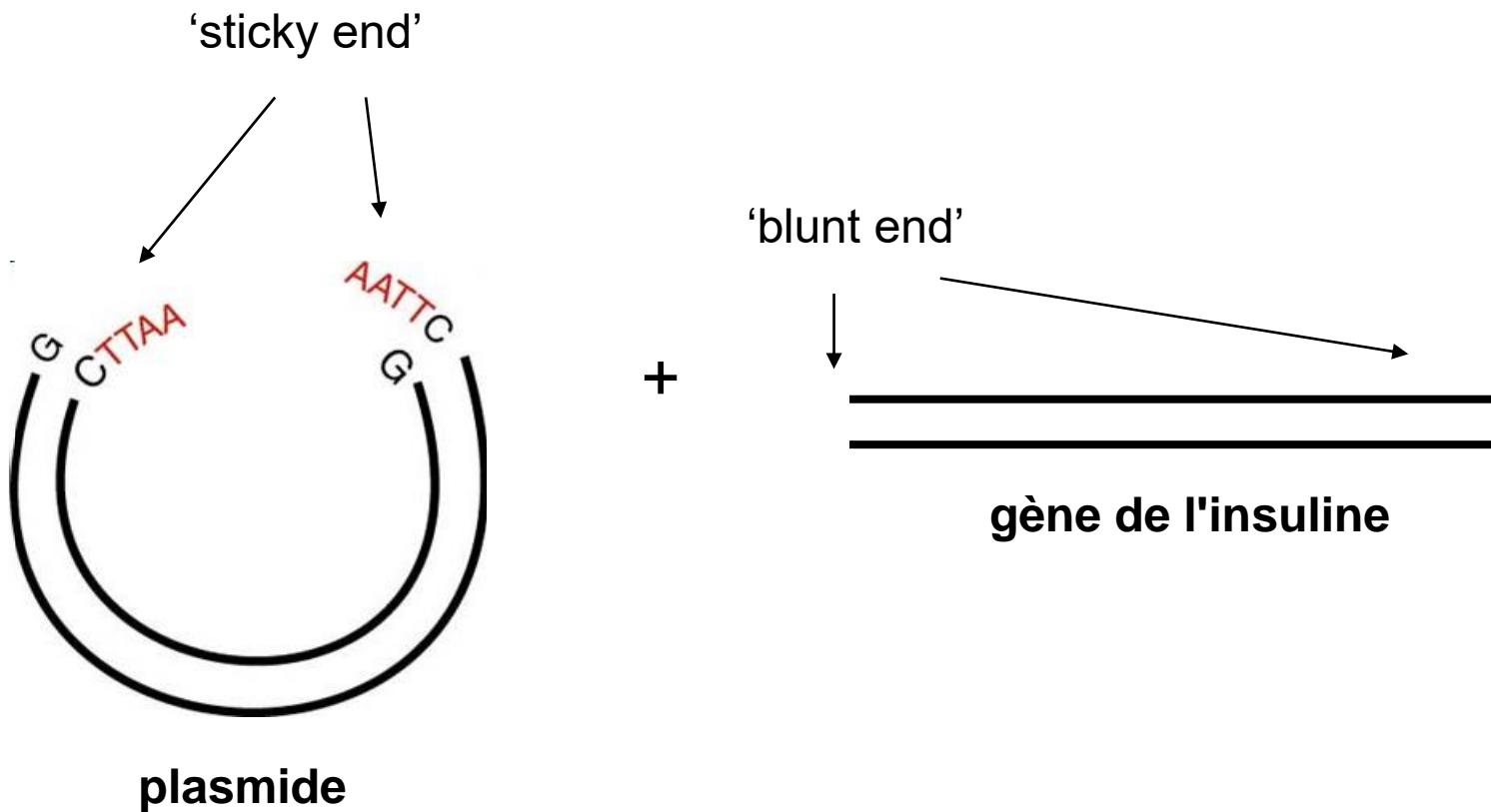
*EcoRI*

CLEAVAGE WITH  
RESTRICTION  
NUCLEASE

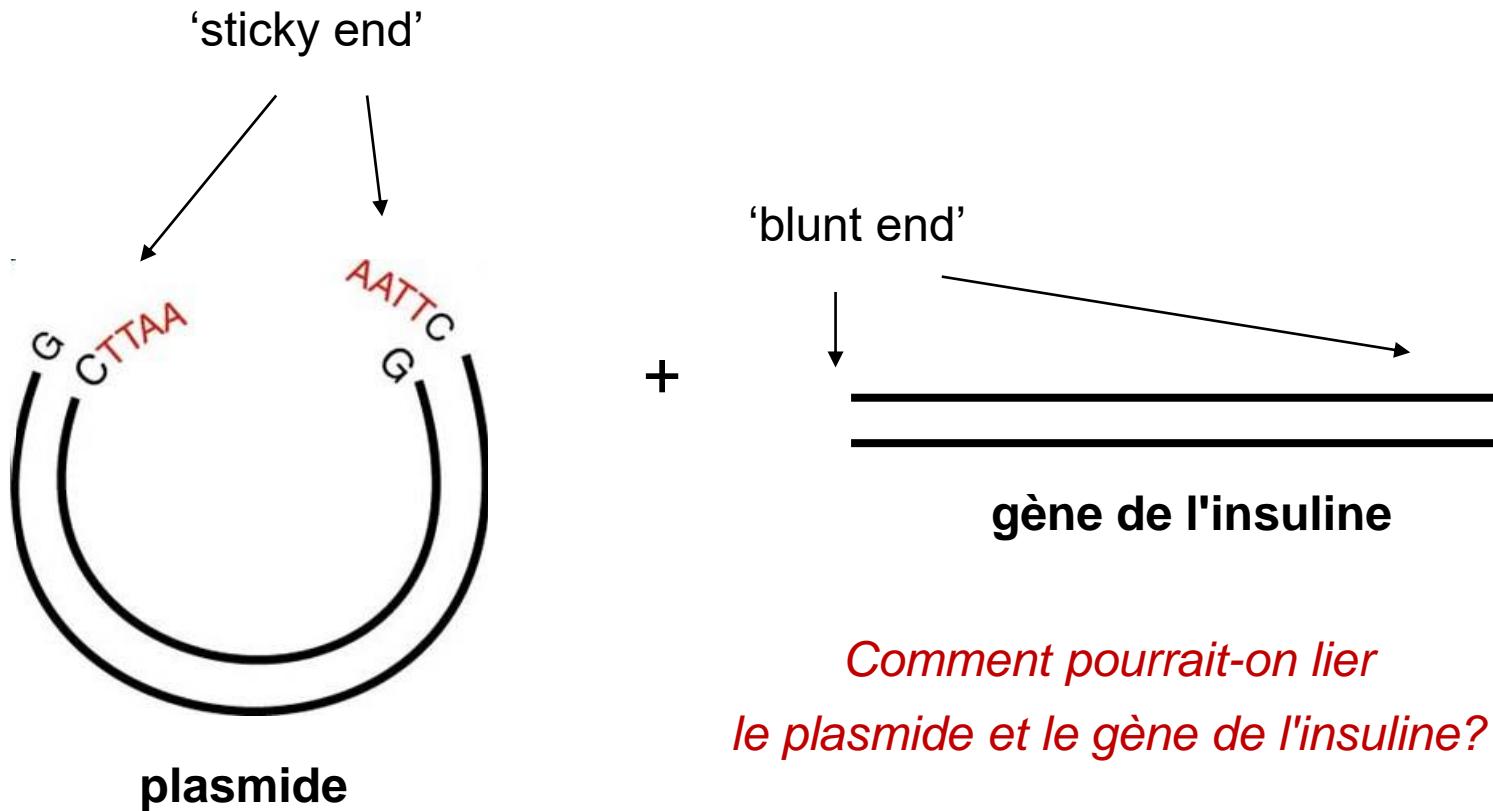


extrémités  
cohésives

# Ligation du gène de l'insuline dans un plasmide



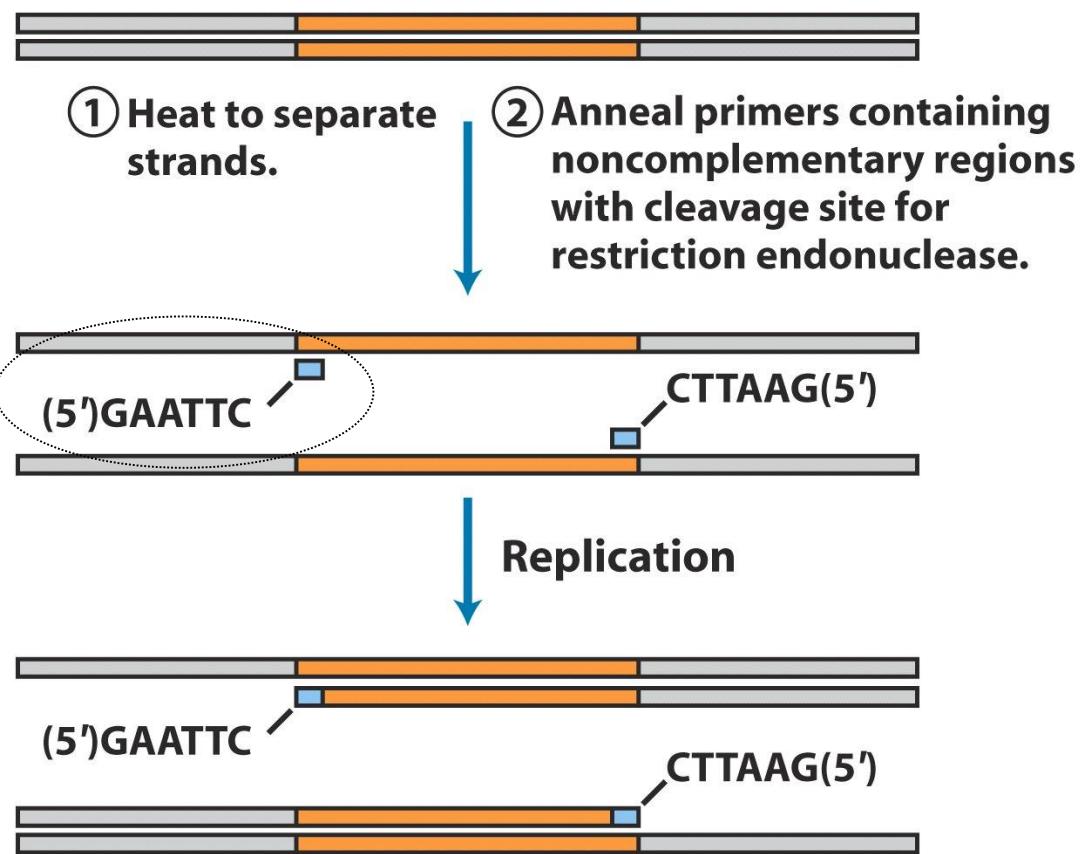
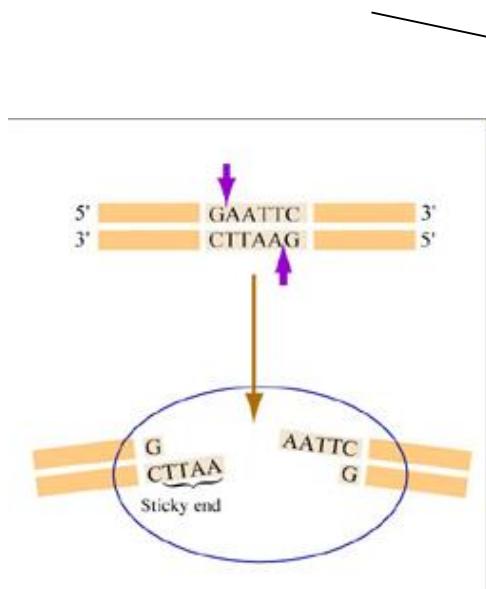
# Ligation du gène de l'insuline dans un plasmide

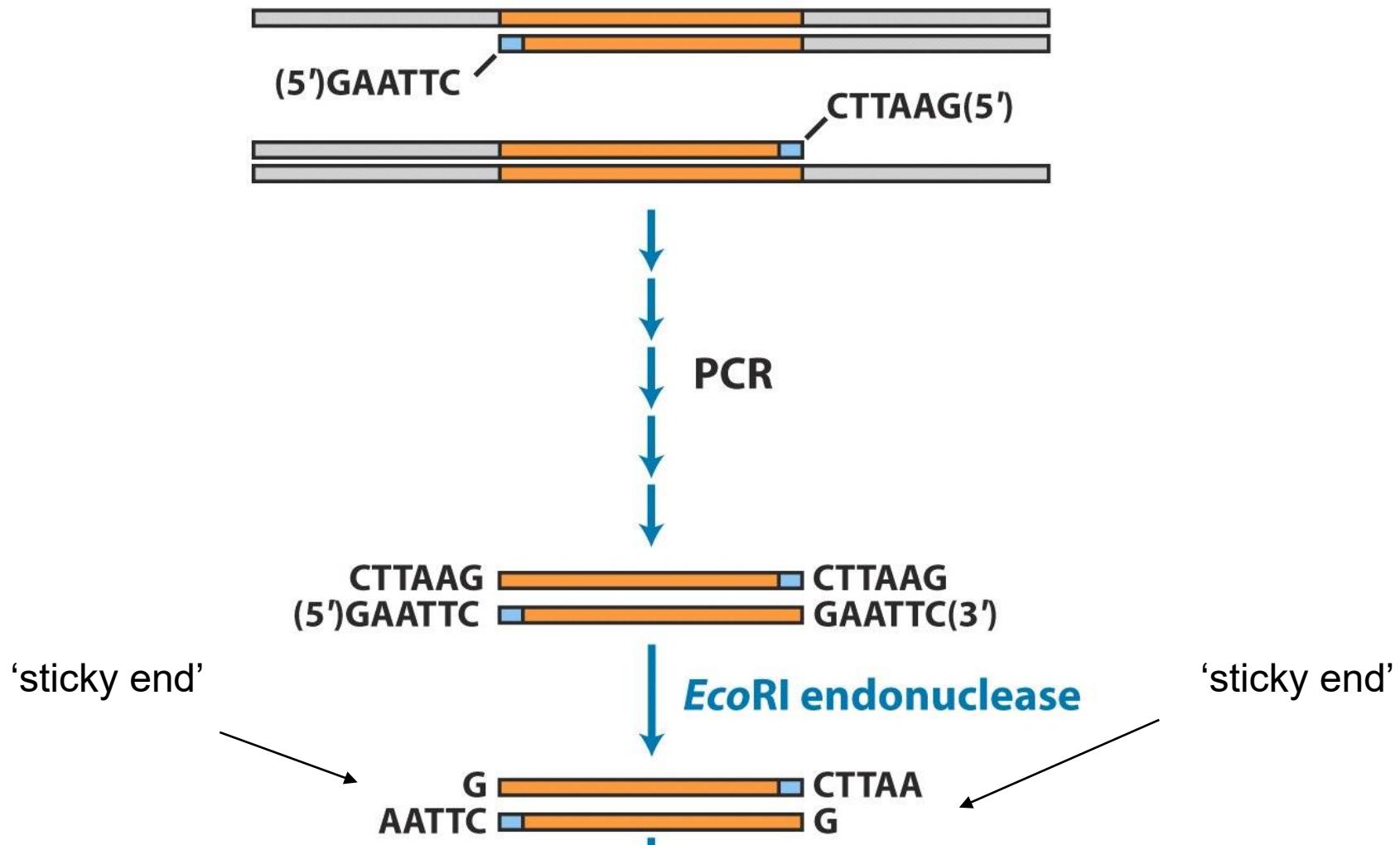


# Introduire des sites de restriction dans le gène de l'insuline par PCR

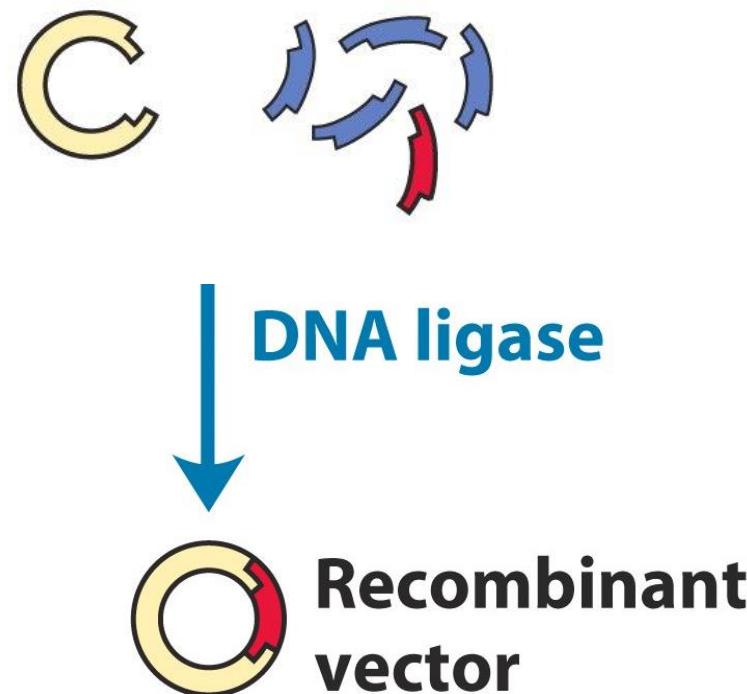
Exemple d'une amorce:

5'-GAATTC**GTACCGTTAGTG**-3'

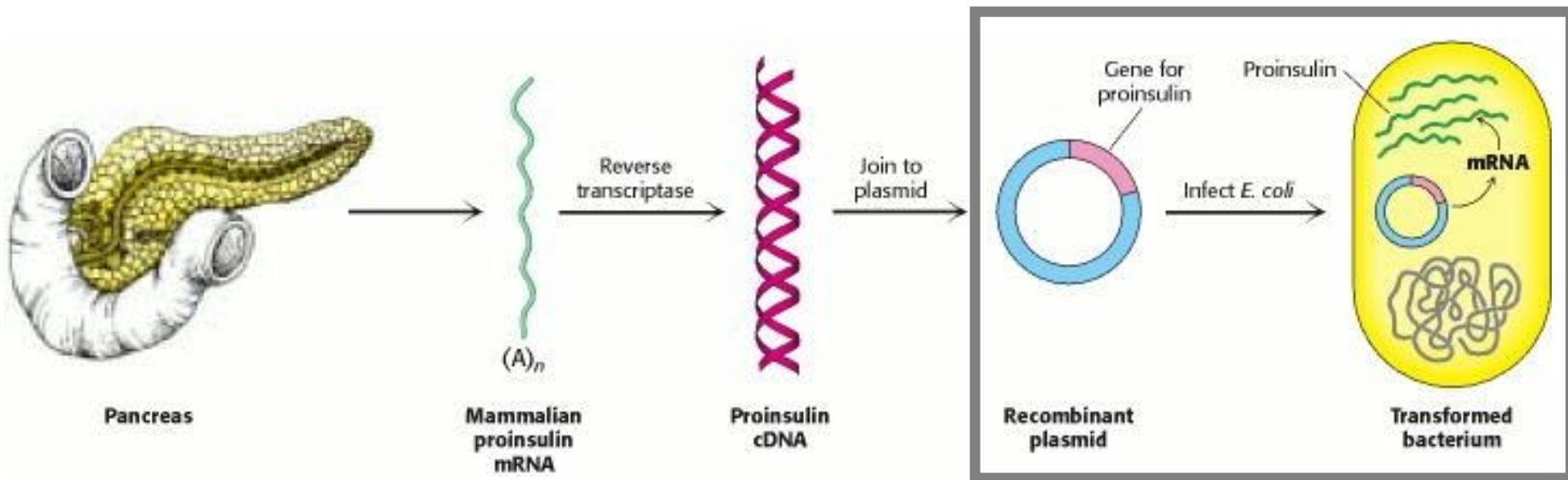




# Insertion d'un gène dans un plasmide



# Expression recombinante de l'insuline



**Isolation du matériel génétique:**  
- DNA génomique  
- mRNA

**Synthèse de DNA:**  
- cDNA

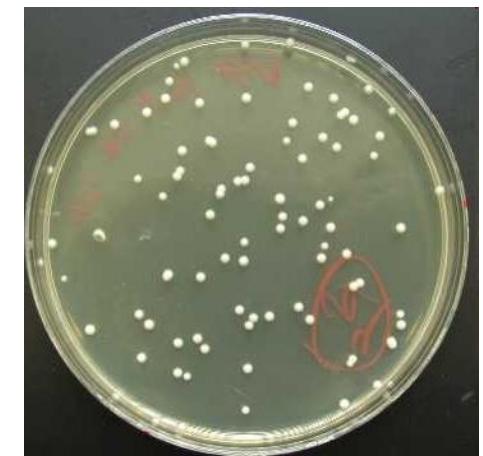
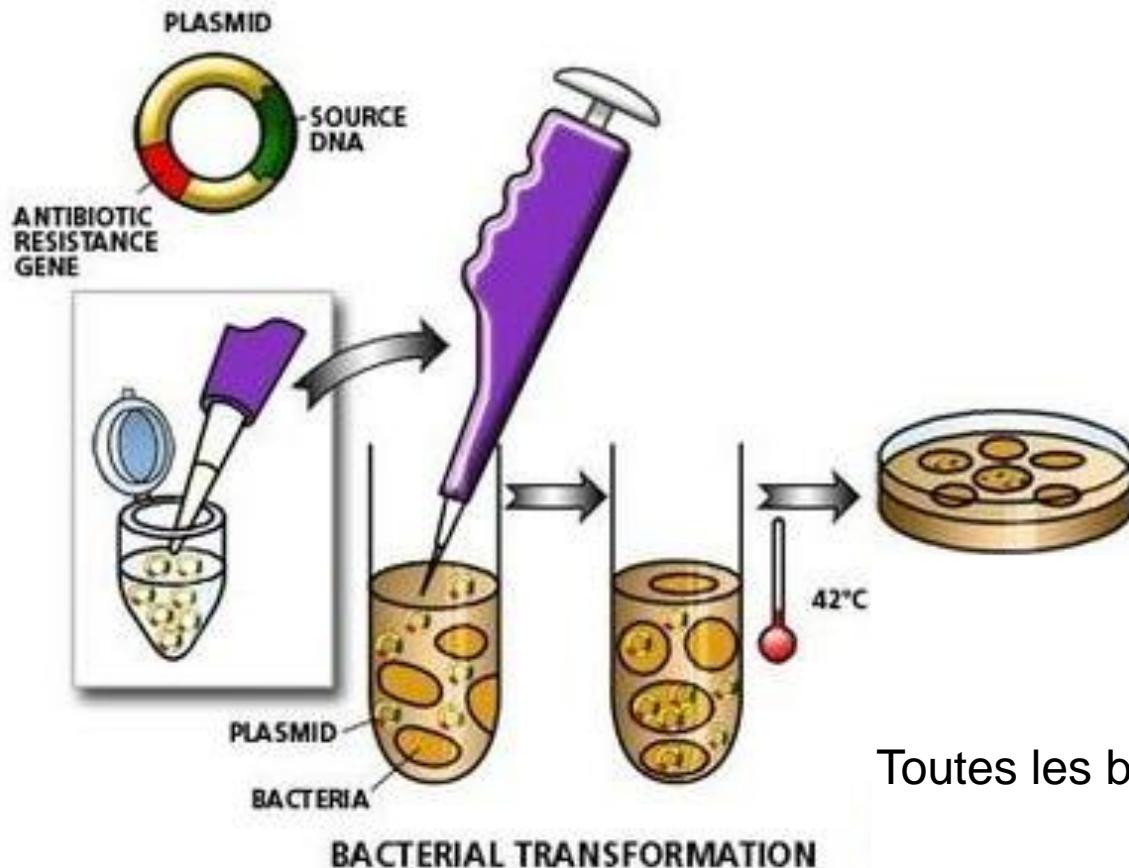
**Insertion dans un vecteur:**  
- plasmide

**Transfert dans l'hôte:**  
- bactéries

# Transformation du plasmide dans les bactéries

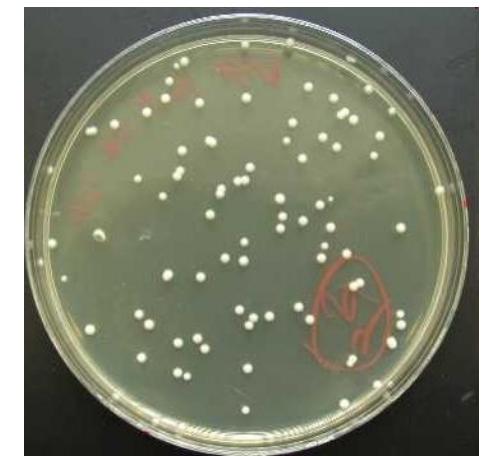
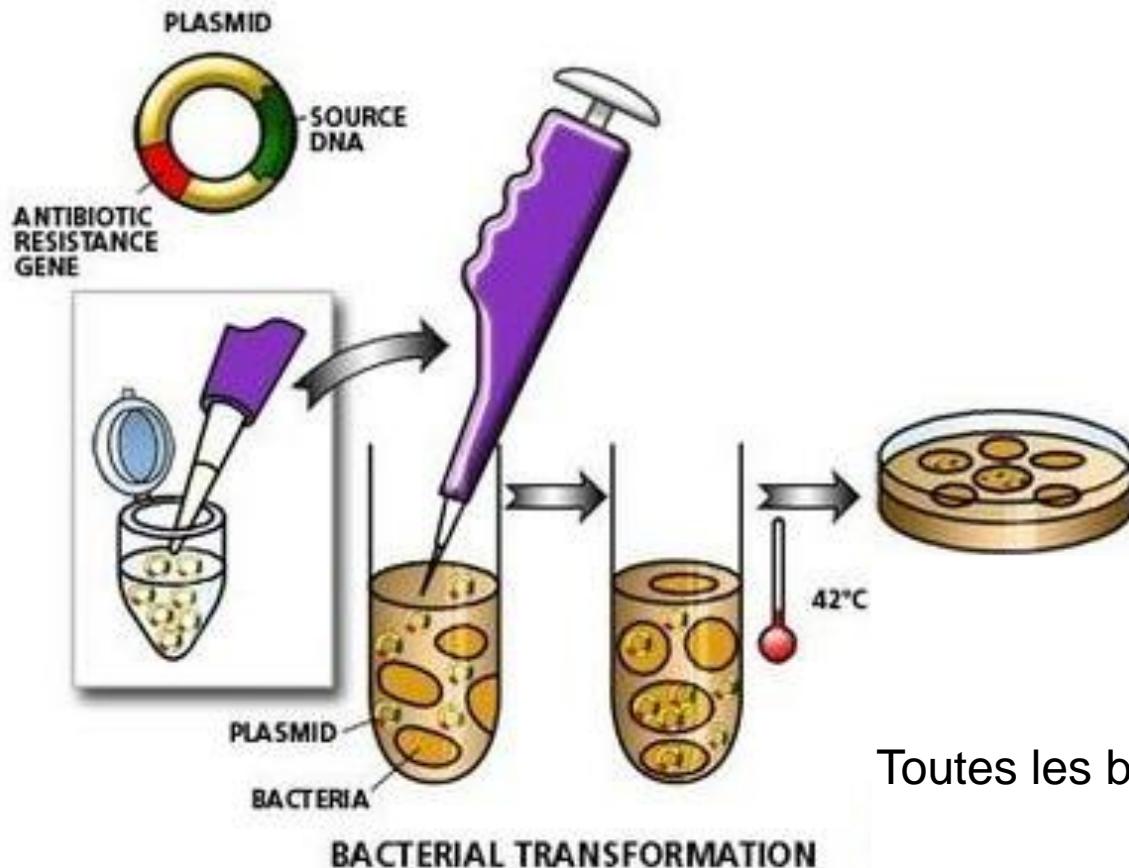
*Comment pourrait-on ‘injecter’ le plasmide dans les bactéries?*

# Transformation du plasmide dans les bactéries



Toutes les bactéries poussent sur la plaque

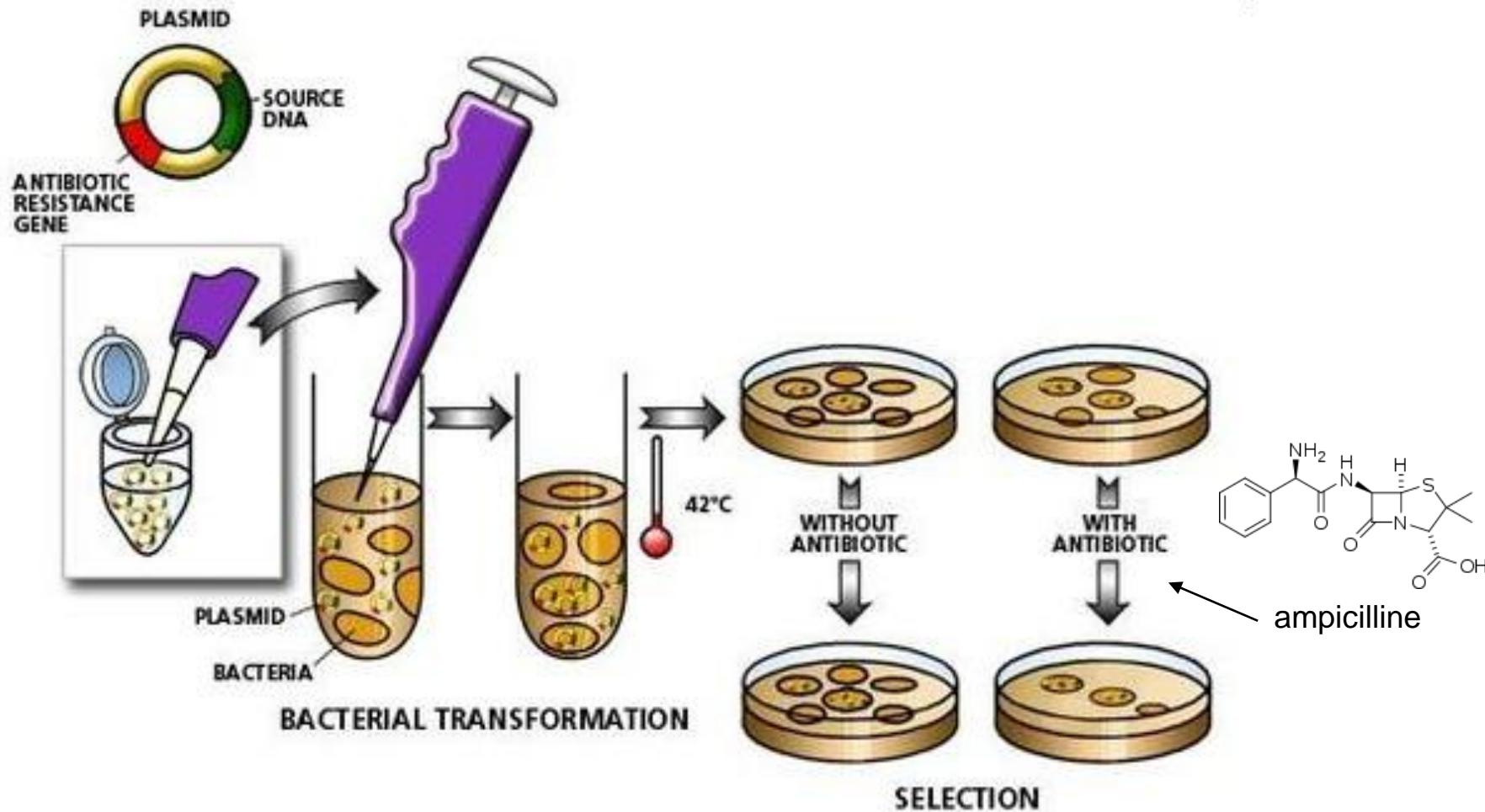
# Transformation du plasmide dans les bactéries



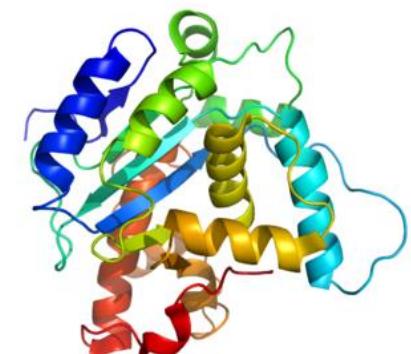
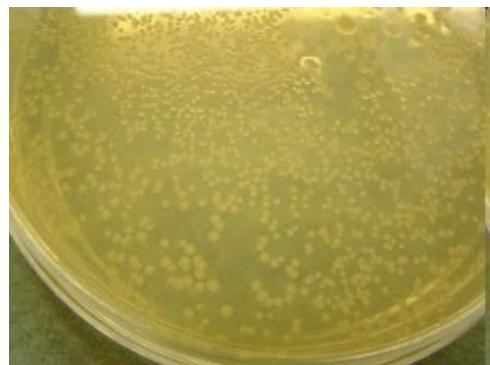
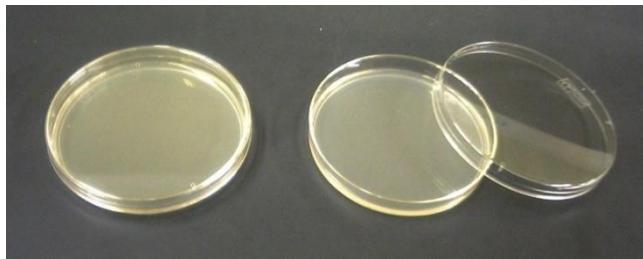
Toutes les bactéries poussent sur la plaque

*Comment pourrait-on identifier  
les bactéries qui contiennent le plasmide?*

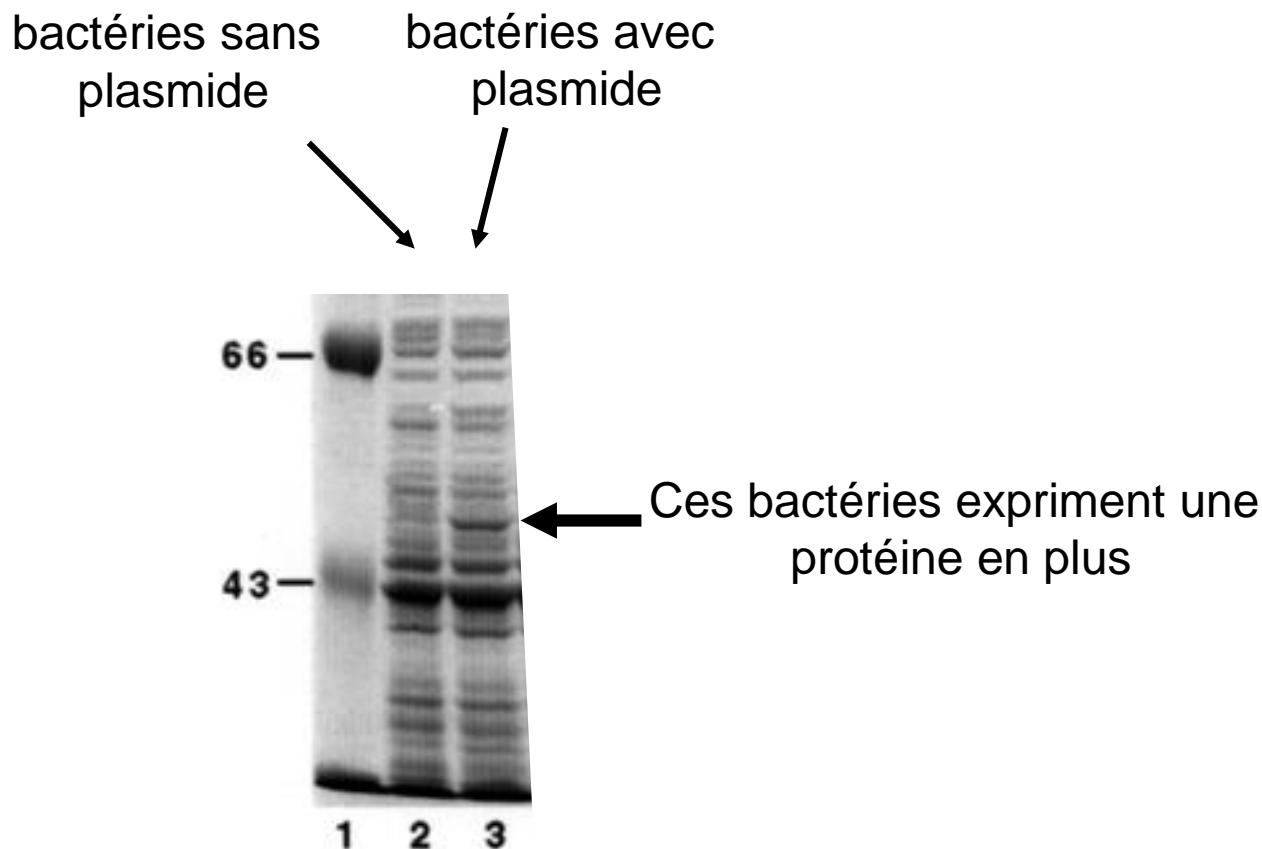
# Transformation du plasmide dans les bactéries



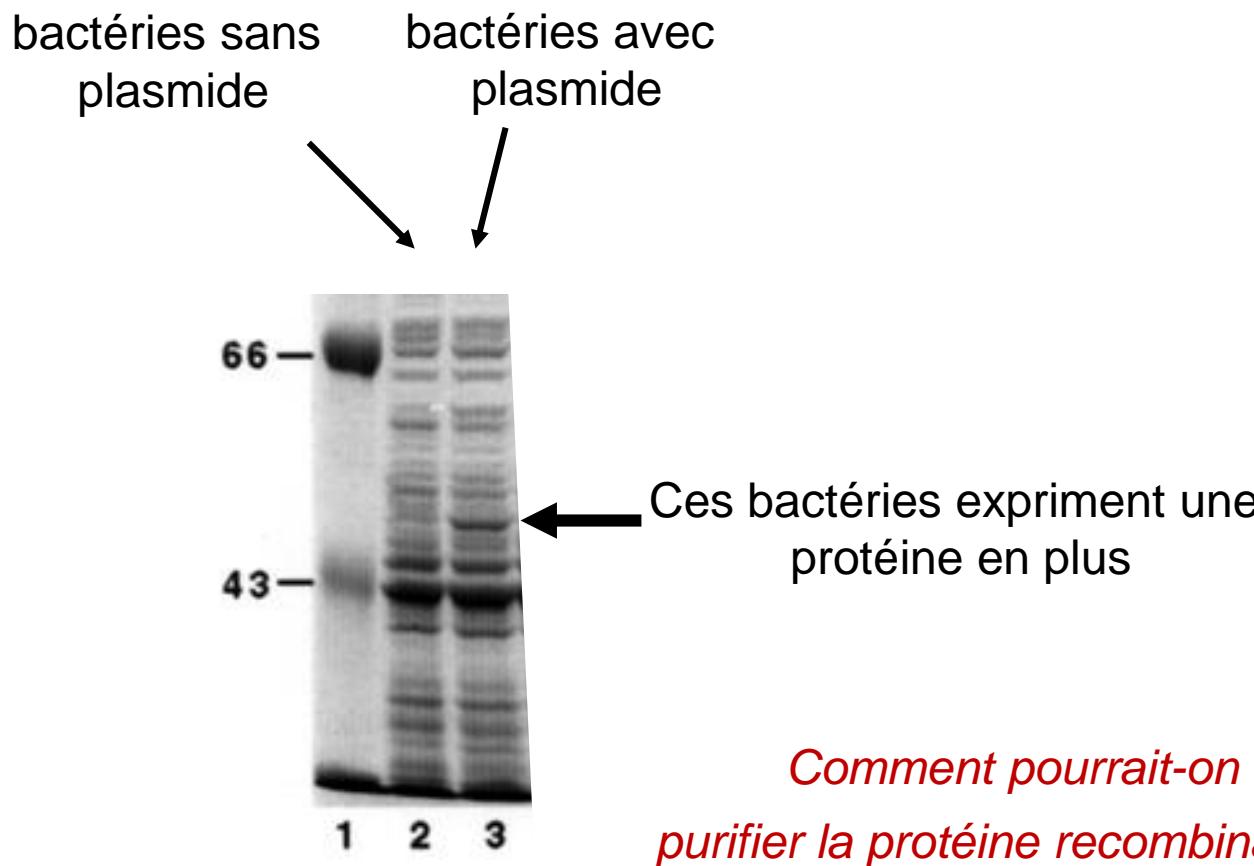
# Transformation du vecteur dans les bactéries et expression de la protéine



# Purification des protéines recombinantes



# Purification des protéines recombinantes



# **Méthodes pour la purification des protéines**

## **Méthode:**

Chromatographie par gel-filtration

Chromatographie par échange d'ions

Chromatographie d'affinité

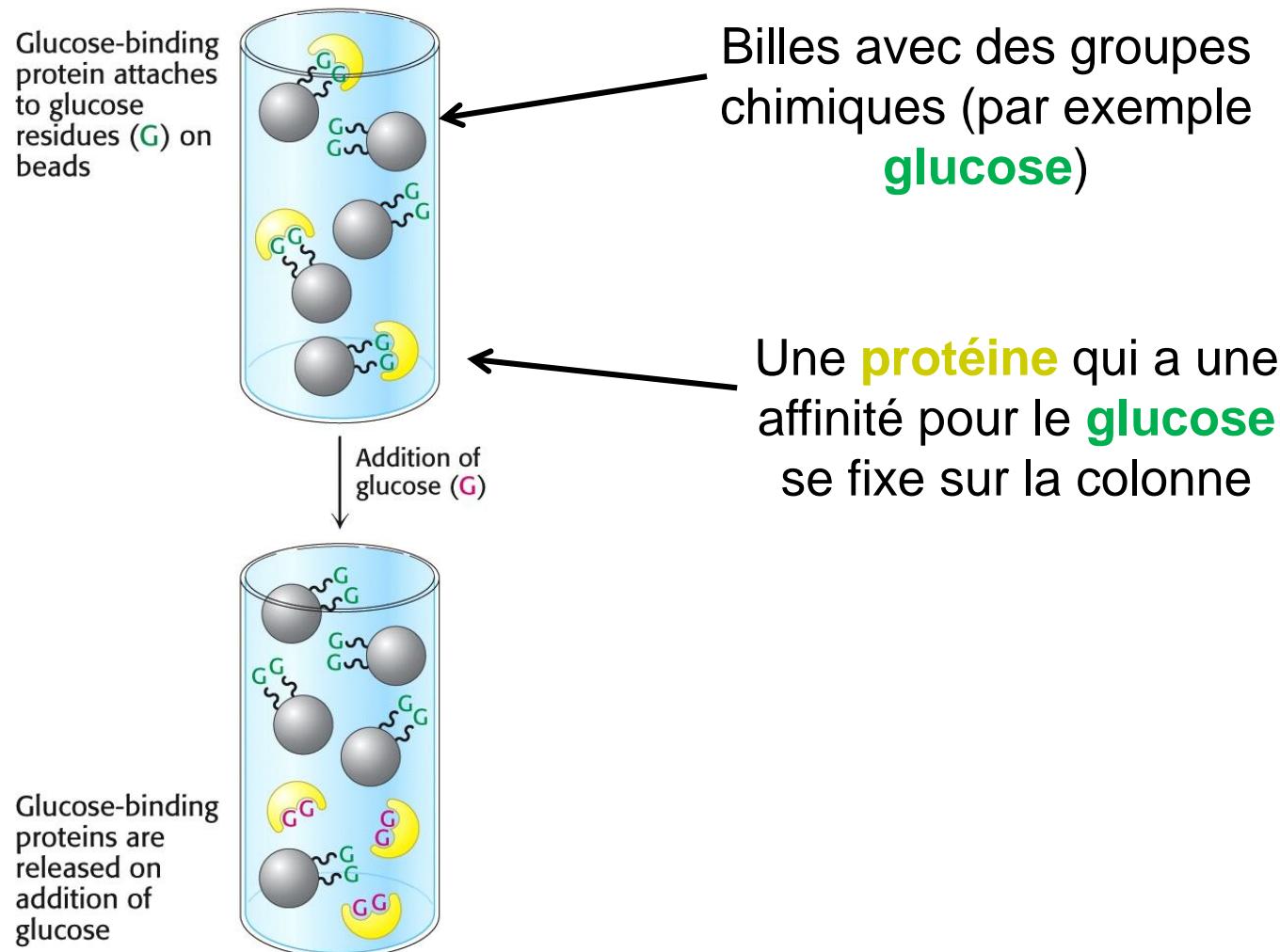
## **Principe de séparation:**

Taille

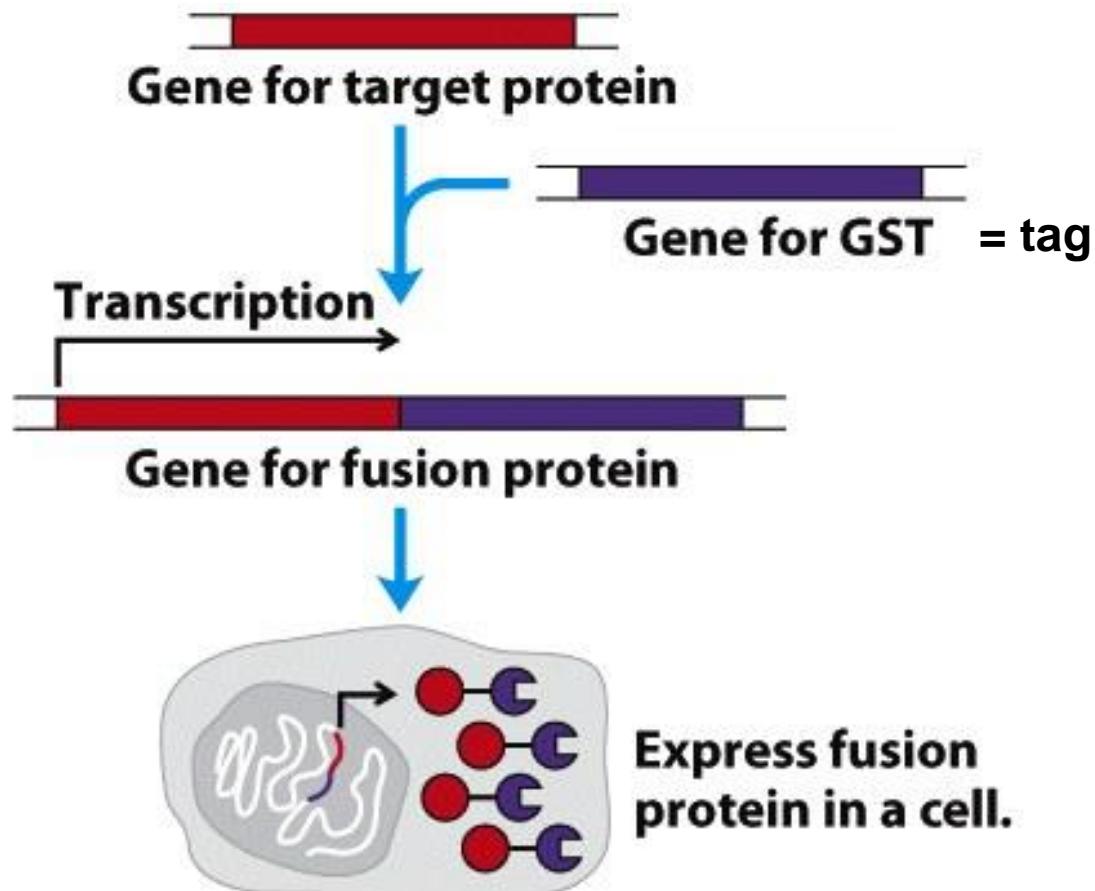
Charge

Affinité spécifique

# Chromatographie d'affinité

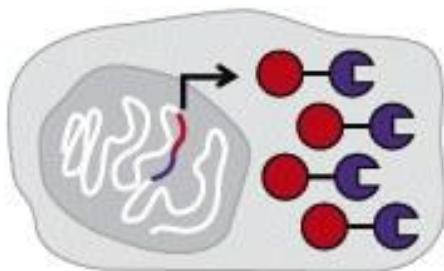
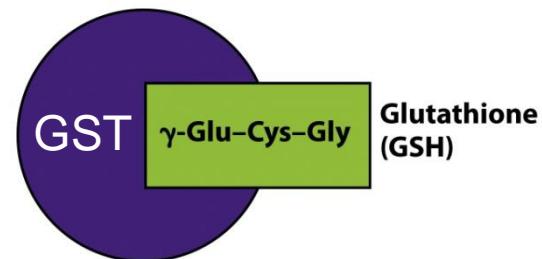


# Expression de protéines recombinantes avec un ‘tag’



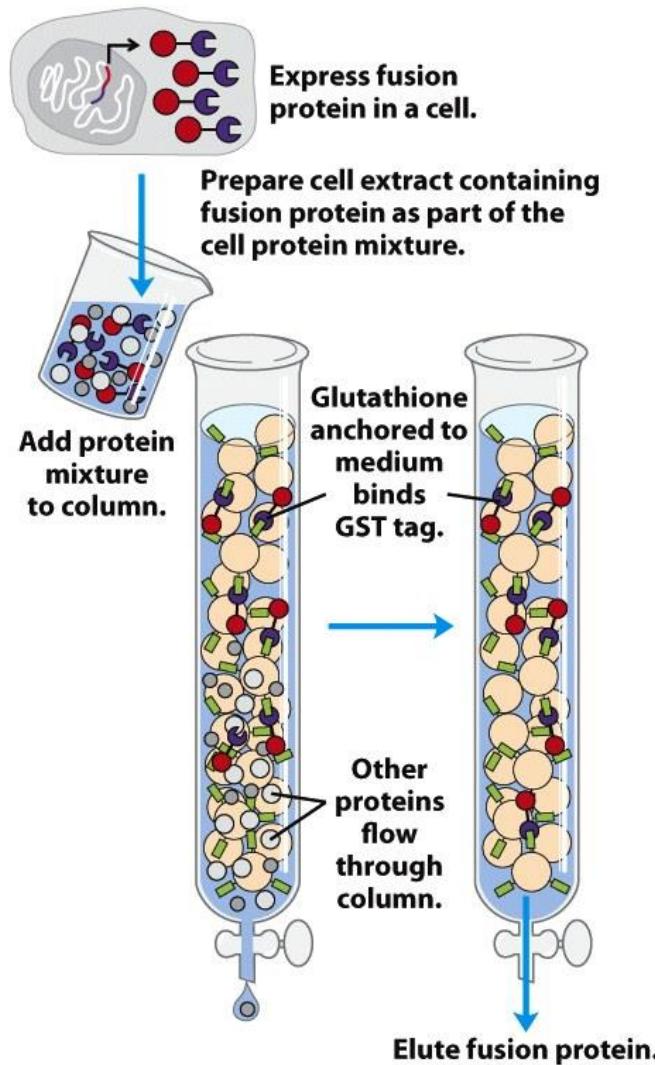
# Expression de protéines recombinantes avec un ‘tag’

La protéine ‘GST’ peut se lier  
au tripeptide ‘glutathione’



**Express fusion  
protein in a cell.**

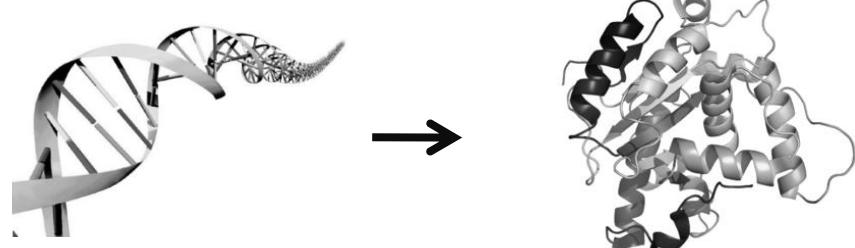
# Purification par chromatographie d'affinité



# Leçon 6

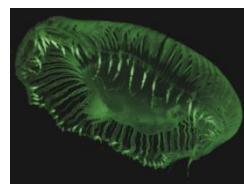
- Synthèse des protéines

- RNA
- Ribosome



- Expression de protéines recombinantes

- DNA isolé d'un donneur
- Plasmide (vecteur) de DNA
- Expression dans un hôte différent



une méduse



DNA d'une protéine  
fluorescente



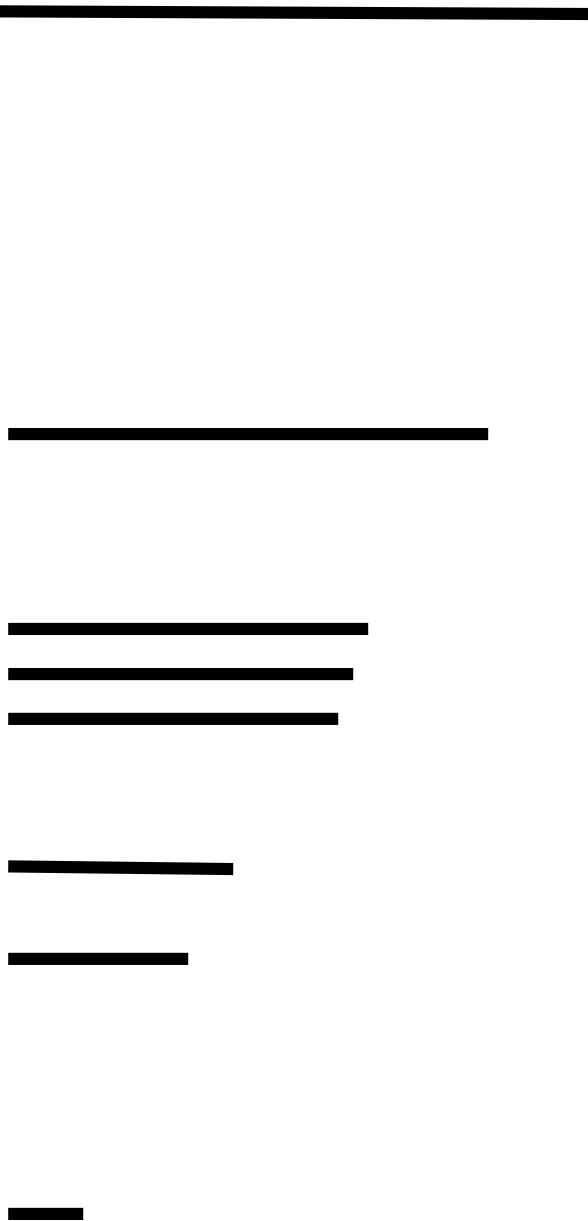
souris fluorescentes

# Série 5

## Question 1



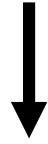
# Exon 3



3' — **GAATTXXXXXXXXXXXXXXXXXXXXXX** — 5'

5' — **CTTAA**

↑  
amorce de DNA  
(‘primer’)



DNA polymérase

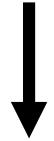
dATP  
dCTP  
dGTP  
dTTP  
  
ddGTP

5'-CTTAA**G**-3'

3' — **GAATTXXXXXXXXXXXXXXXXXXXX** — 5'

5' — **CTTAA**

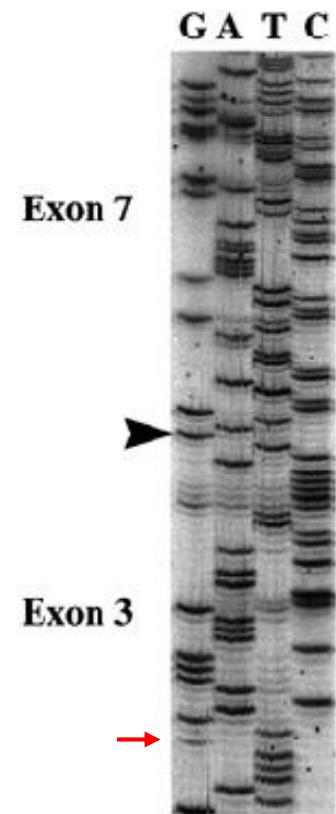
↑  
amorce de DNA  
(‘primer’)



DNA polymérase

5'-CTTAA**G**-3'

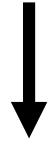
5'-CTTAAXXXXXX**G**-3'



3' — **GAATTXXXXXXXXXXXXXXXXXXXX** — 5'

5' — **CTTAA**

↑  
amorce de DNA  
(‘primer’)

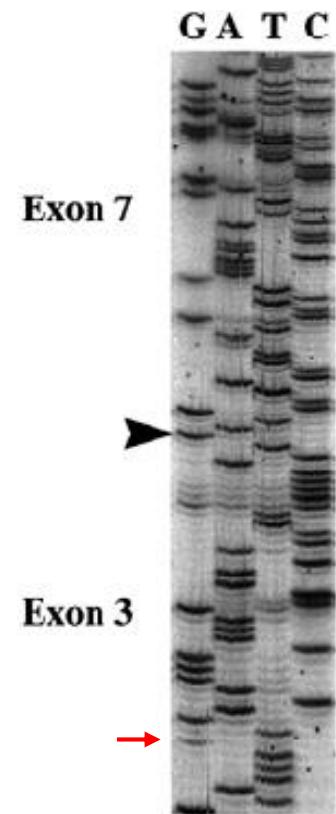


DNA polymérase

5'-CTTAA**G**-3'

5'-CTTAAXXXXXX**G**-3'

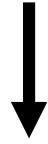
5'-CTTAA**GXXXXGXGXXXGGGXXXXG**-3'



3' — **GAATT**XXXXXXXXXXXXXXXXXXXXXX-5'

5' — **CTTAA**

↑  
amorce de DNA  
(‘primer’)

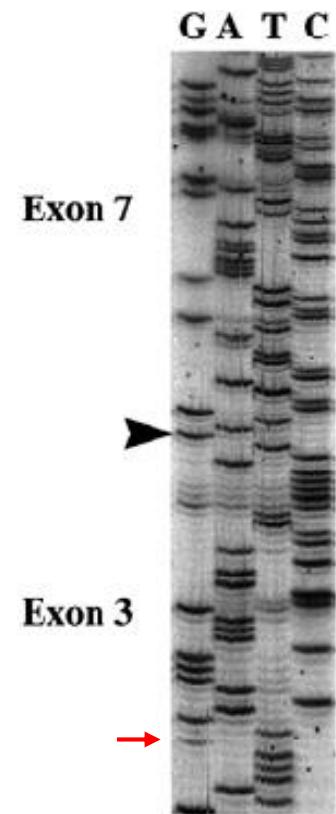


DNA polymérase

5'-CTTAA**G**-3'

5'-CTTAAXXXXXX**G**-3'

3'-GAATT**C**XXXXX**C****X****C**XXX**CCC**XXXX**C**-5'  
5'-CTTAA**G**XXXXX**G****X****G**XXX**GGG**XXXX**G**-3'



# Série 5

## Question 2

$$(10'000-5)/4^6 = 2.44 \rightarrow 2\text{-}3 \text{ fois}$$

5'-XXXXXXXXXX-3'

GAATTC

GAATTC

GAATTC

GAATTC

GAATTC

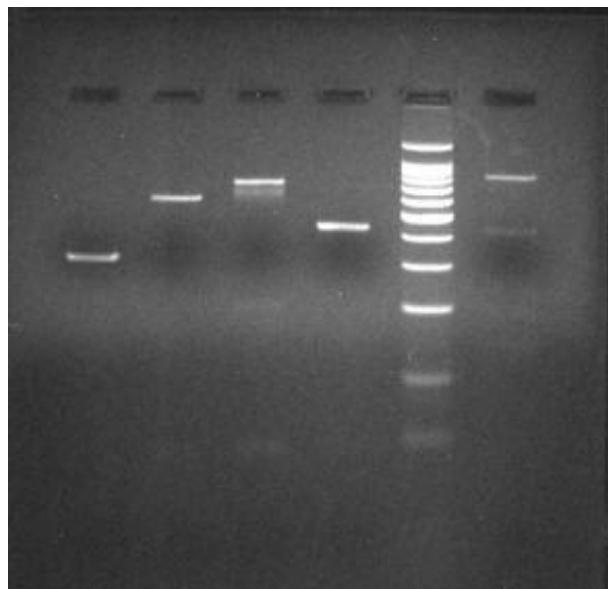
# Série 5

## Question 3

$$2^{20} = 1'048'576 \times 0.1 \text{ ng} \rightarrow 105 \mu\text{g}$$

# Série 5

## Question 4



Ligne 1 à 6 : 500 pdb, 2500 pdb, 4000 pdb, 1200 pdb, marker, 5000 pdb