# GENETICS AND MOLECULAR BIOLOGY FOR ENVIRONMENTAL ANALYSIS

# MOLECULAR ECOLOGY
## LESSON 2

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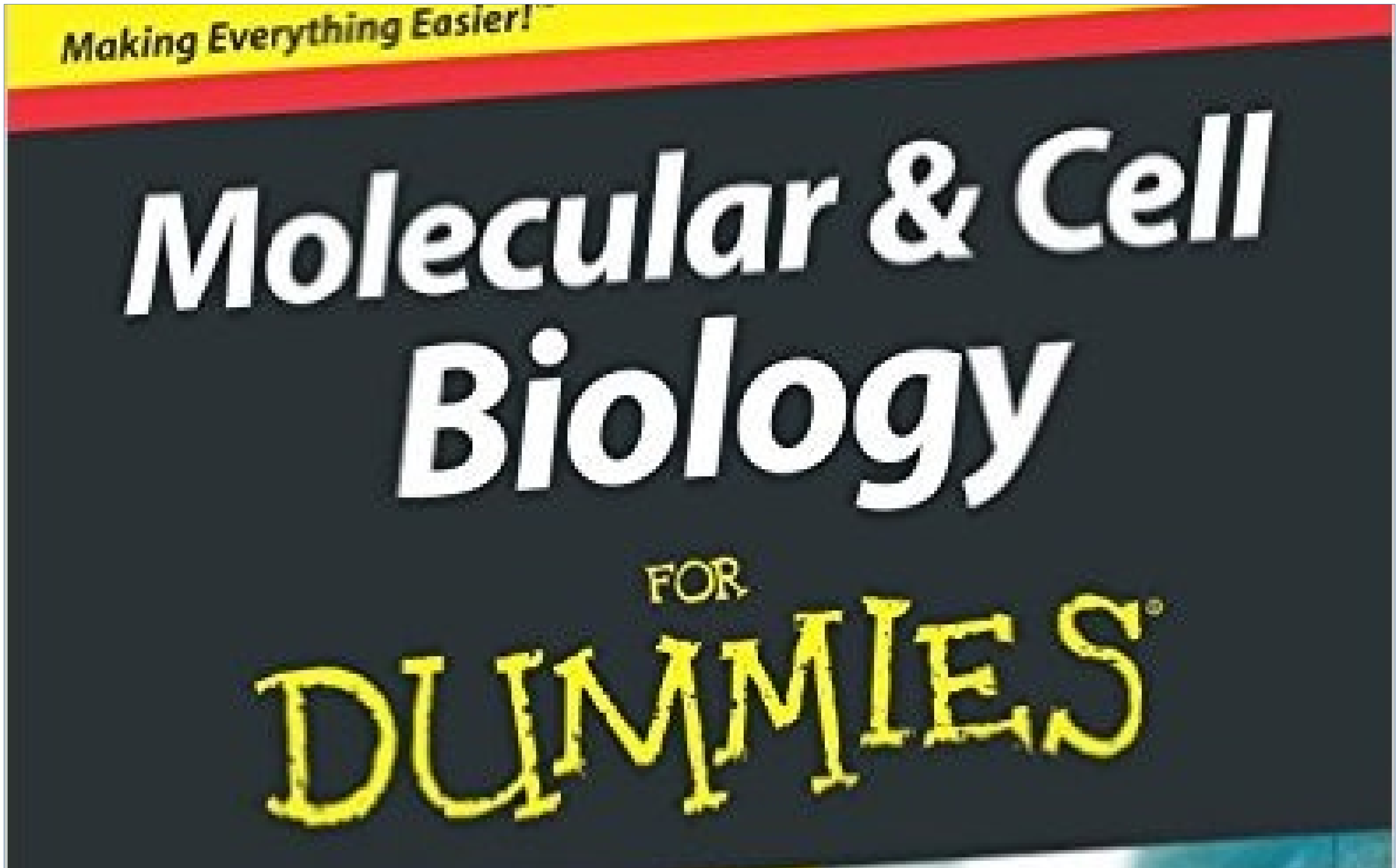
**pallavic@units.it**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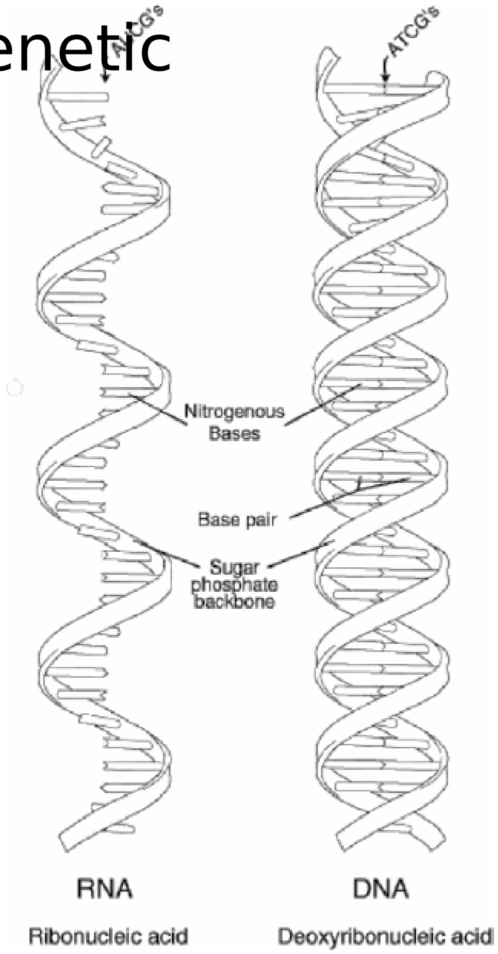
# NUCLEIC ACID CHEMISTRY

- DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) store and transfer genetic information in living organisms.
- • DNA:
  - – major constituent of the nucleus
  - – stable representation of an organism's complete genetic makeup
- • RNA:
  - – found in the nucleus and the cytoplasm
  - – key to information flow within a cell



# NUCLEIC ACID CHEMISTRY

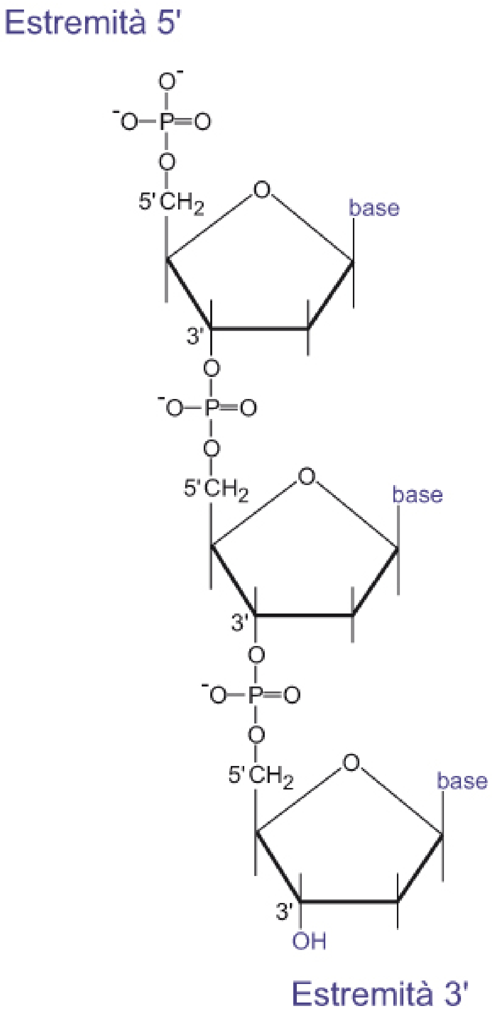



**Figura 1.1** Lo scheletro del DNA.

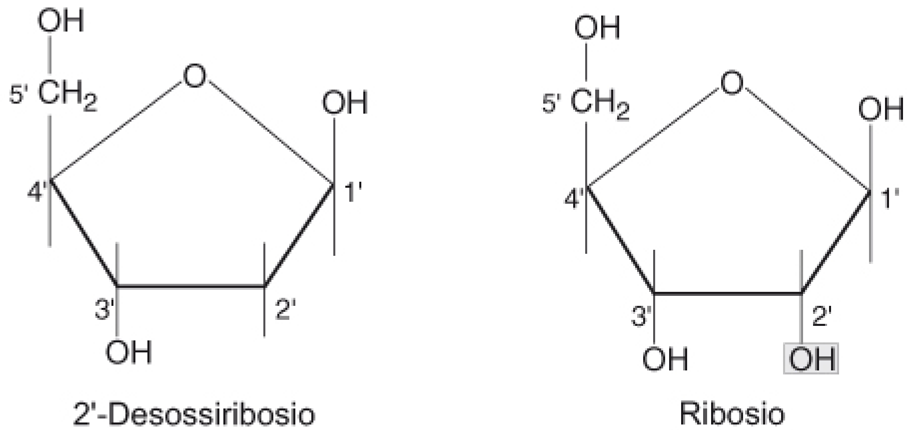



**Figura 1.2** Gli zuccheri degli acidi nucleici.

# NUCLEIC ACID CHEMISTRY

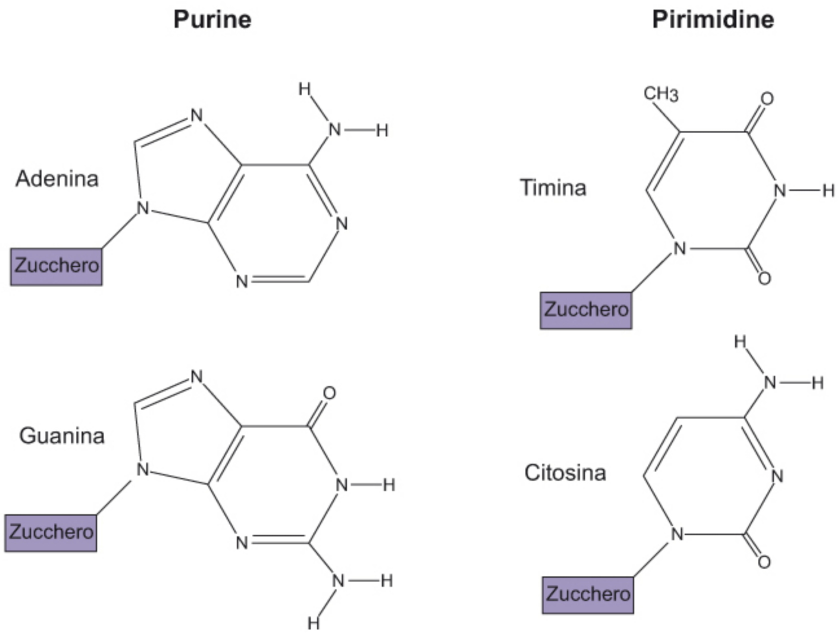



**Figura 1.3** Le basi degli acidi nucleici.

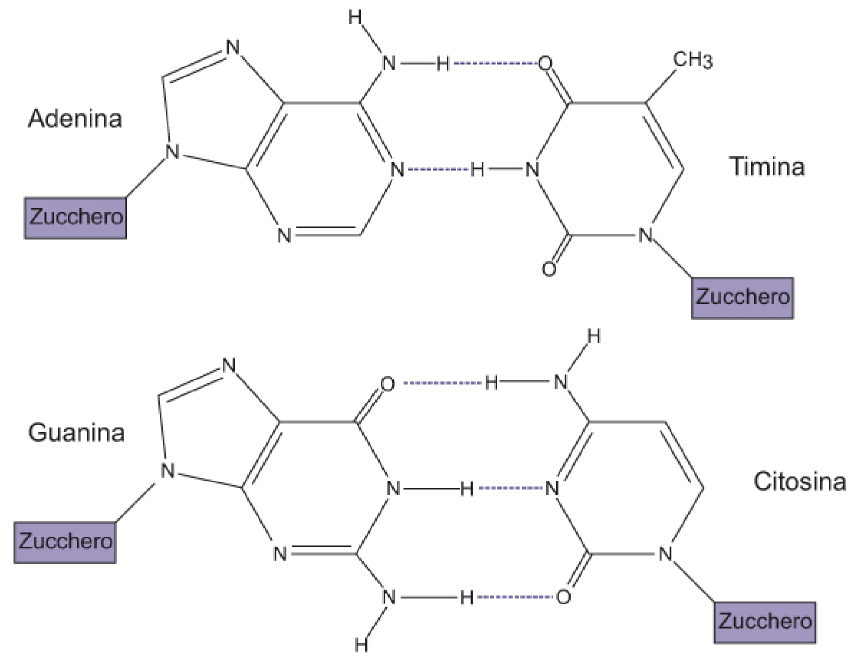



**Figura 1.4** Gli appaiamenti fra le basi del DNA.

# NUCLEIC ACID CHEMISTRY

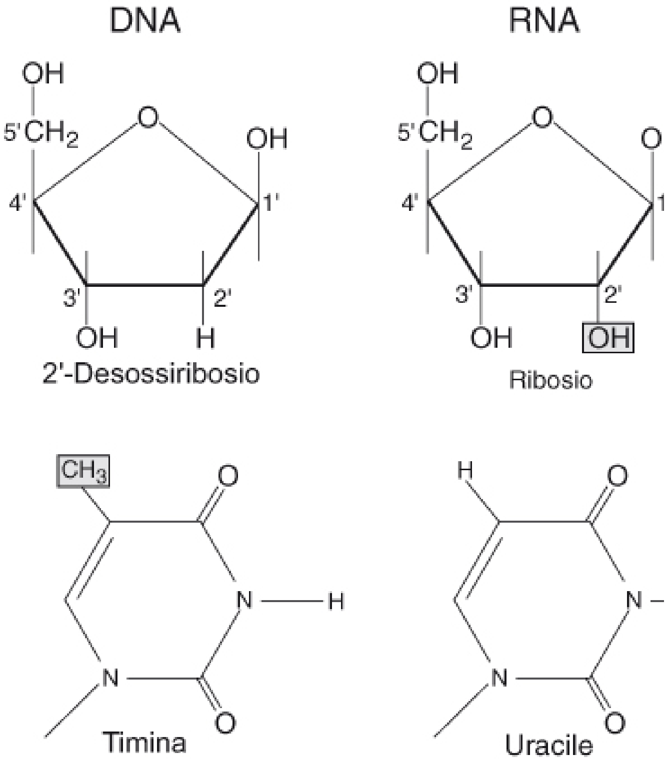



Figura 1.6 Differenze fra DNA ed RNA.

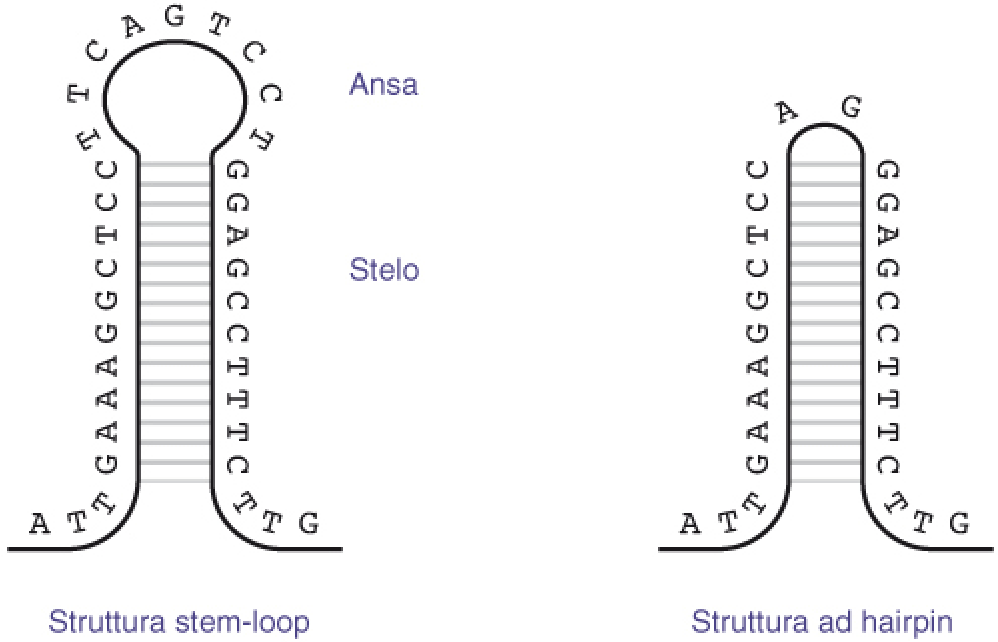



Figura 1.5 Struttura a stem-loop (stelo ed ansa) e ad hairpin (forcina

# NUCLEIC ACID CHEMISTRY

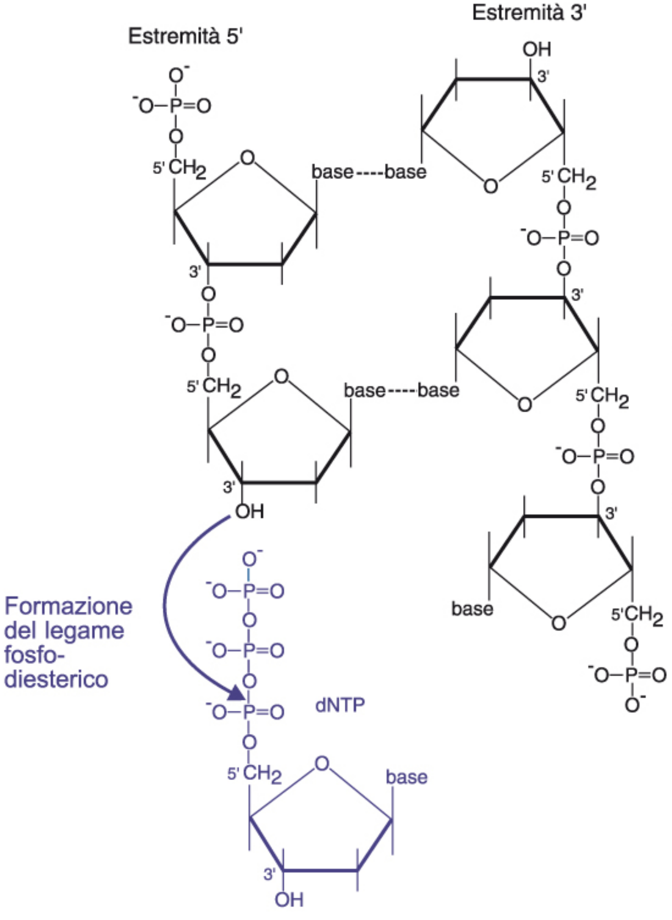
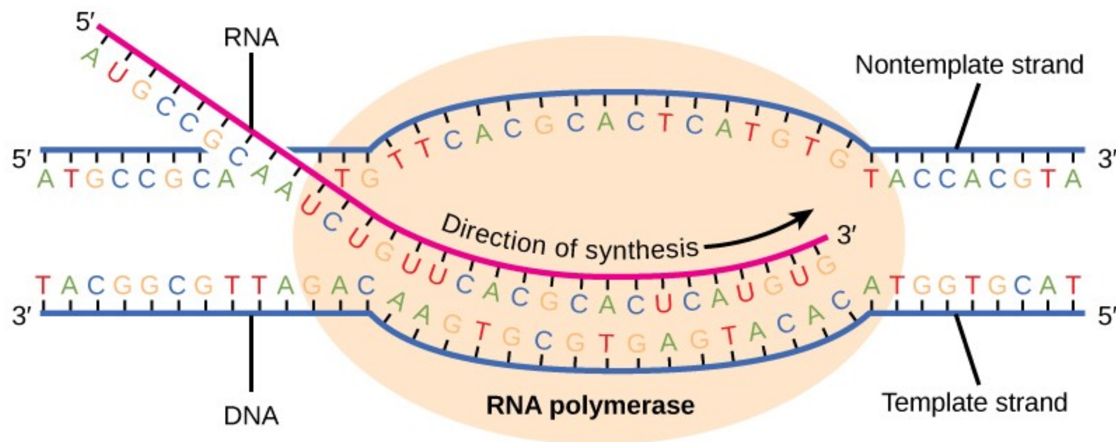
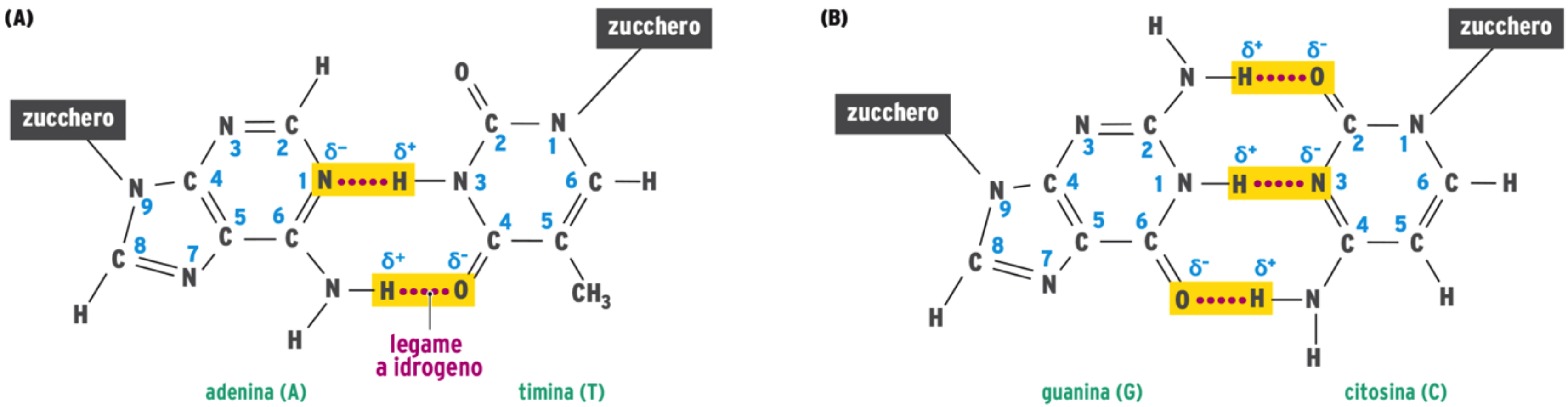
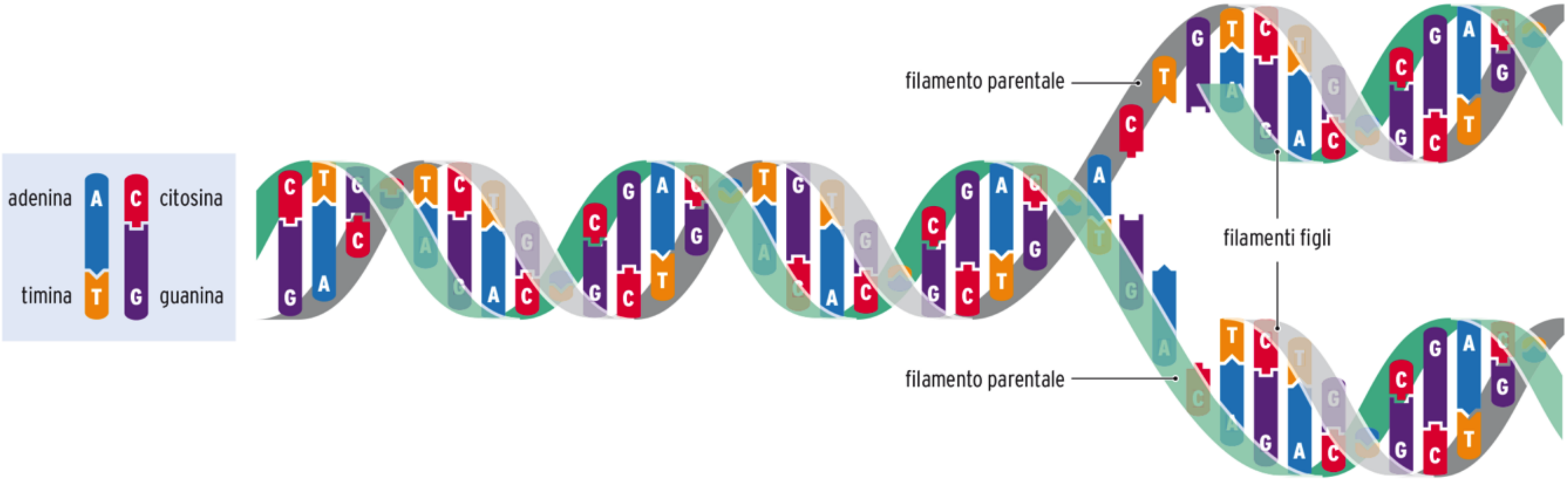
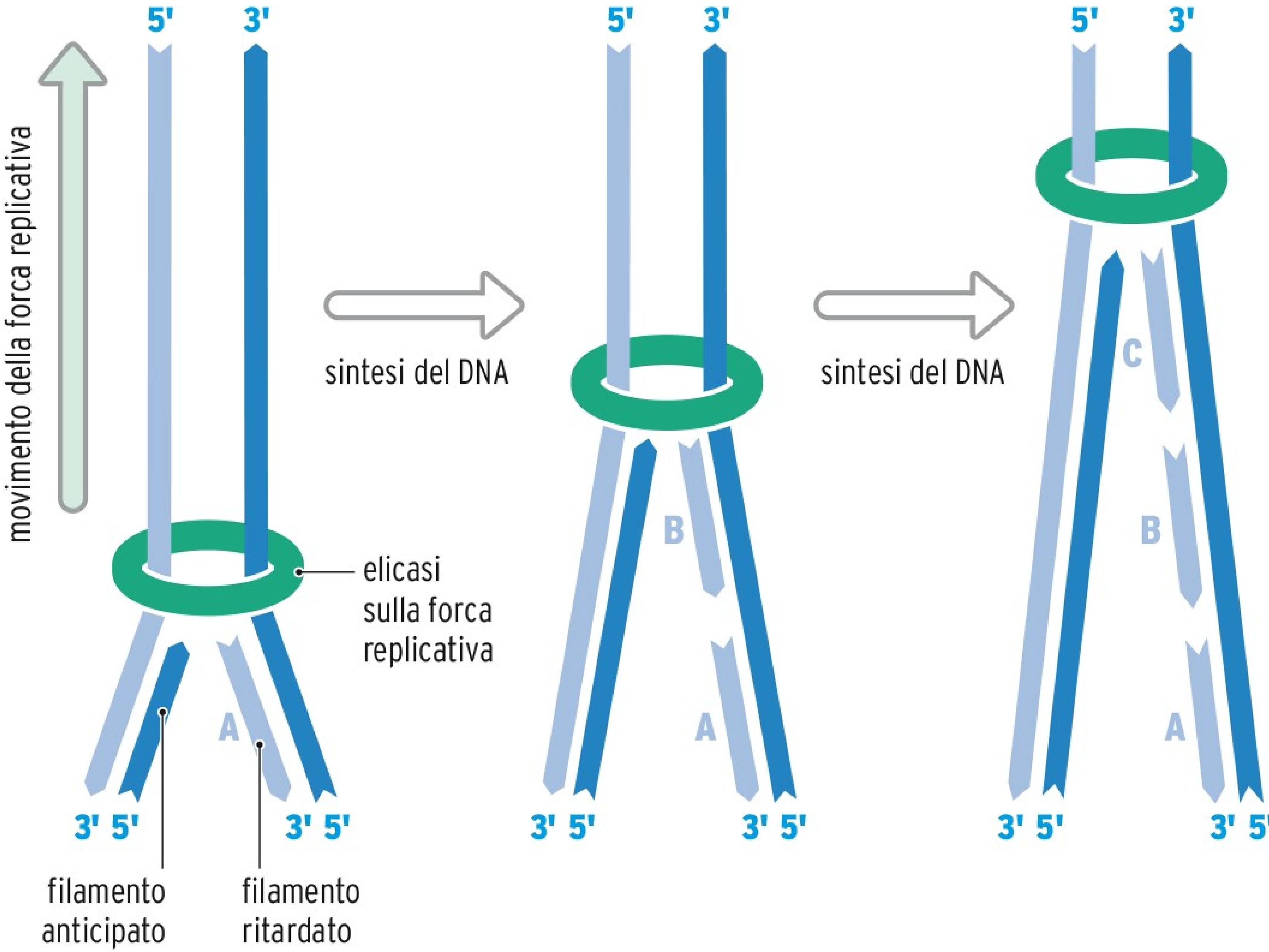



Figura 1.7 Sintesi del DNA.




(A)
zucchero
zucchero
legame
a idrogeno
adenina (A)
timina (T)
(B)
zucchero
zucchero
guanina (G)
citosina (C)


adenina
citosina
timina
guanina
filamento parentale
filamenti figli
filamento parentale



5'
3'
movimento della forca replicativa
elicasi
sulla forca
replicativa
A
3' 5'
3' 5'
filamento
anticipato
filamento
ritardato
sintesi del DNA
5'
3'
B
A
3' 5'
3' 5'
sintesi del DNA
5'
3'
C
B
A
3' 5'
3' 5'

# THE CENTRAL DOGMA

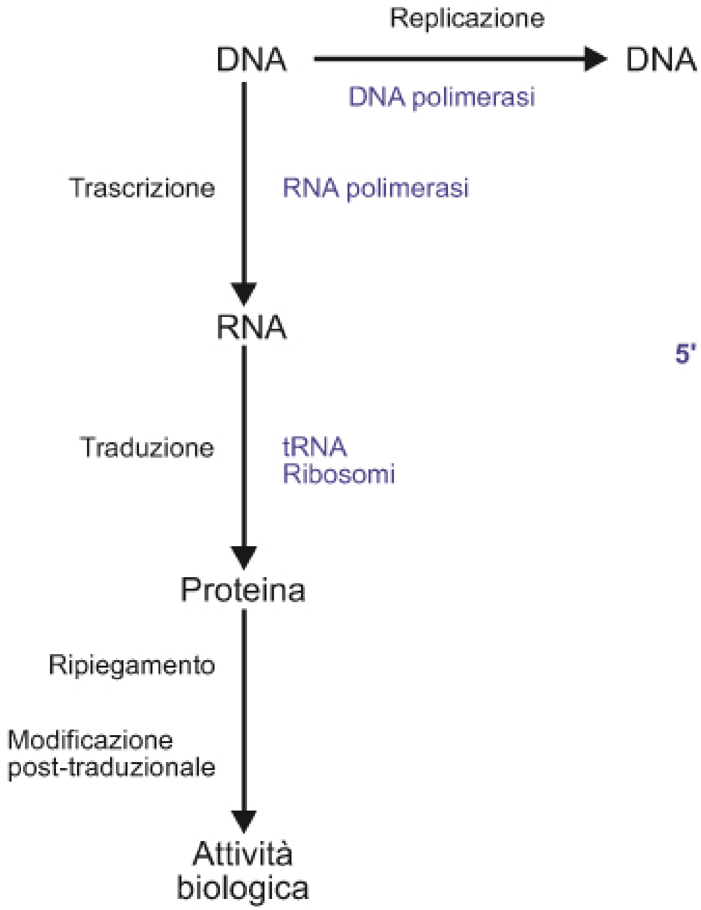



**Figura 1.8** Flusso dell'informazione

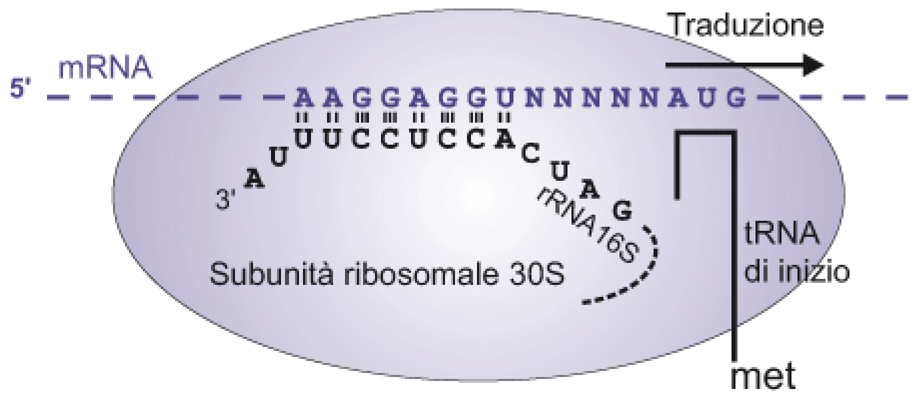
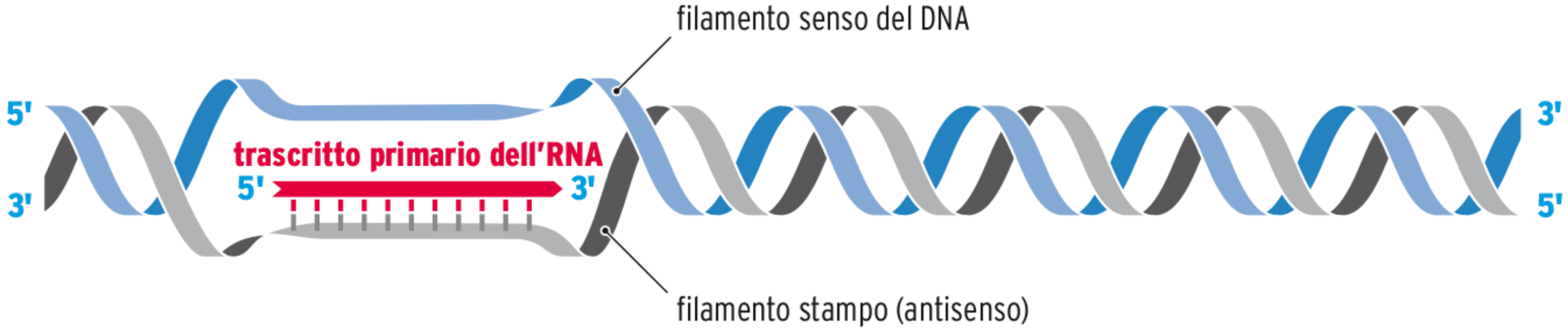
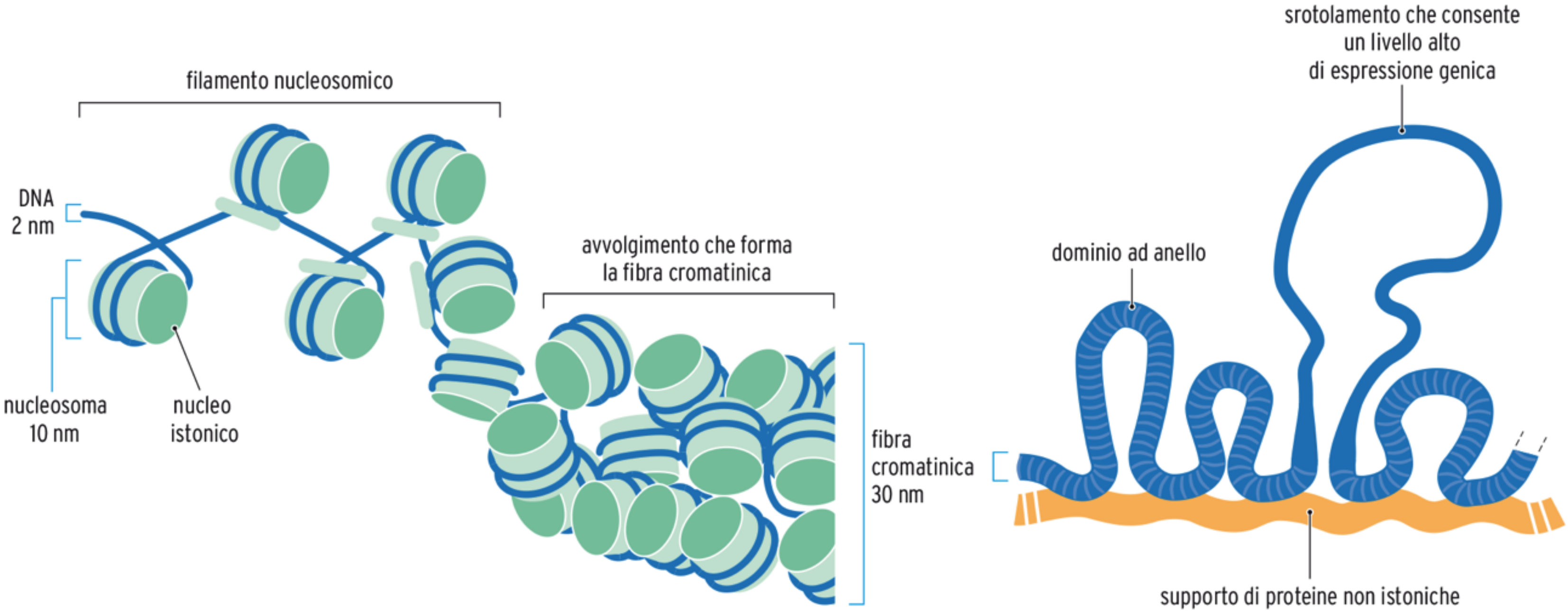
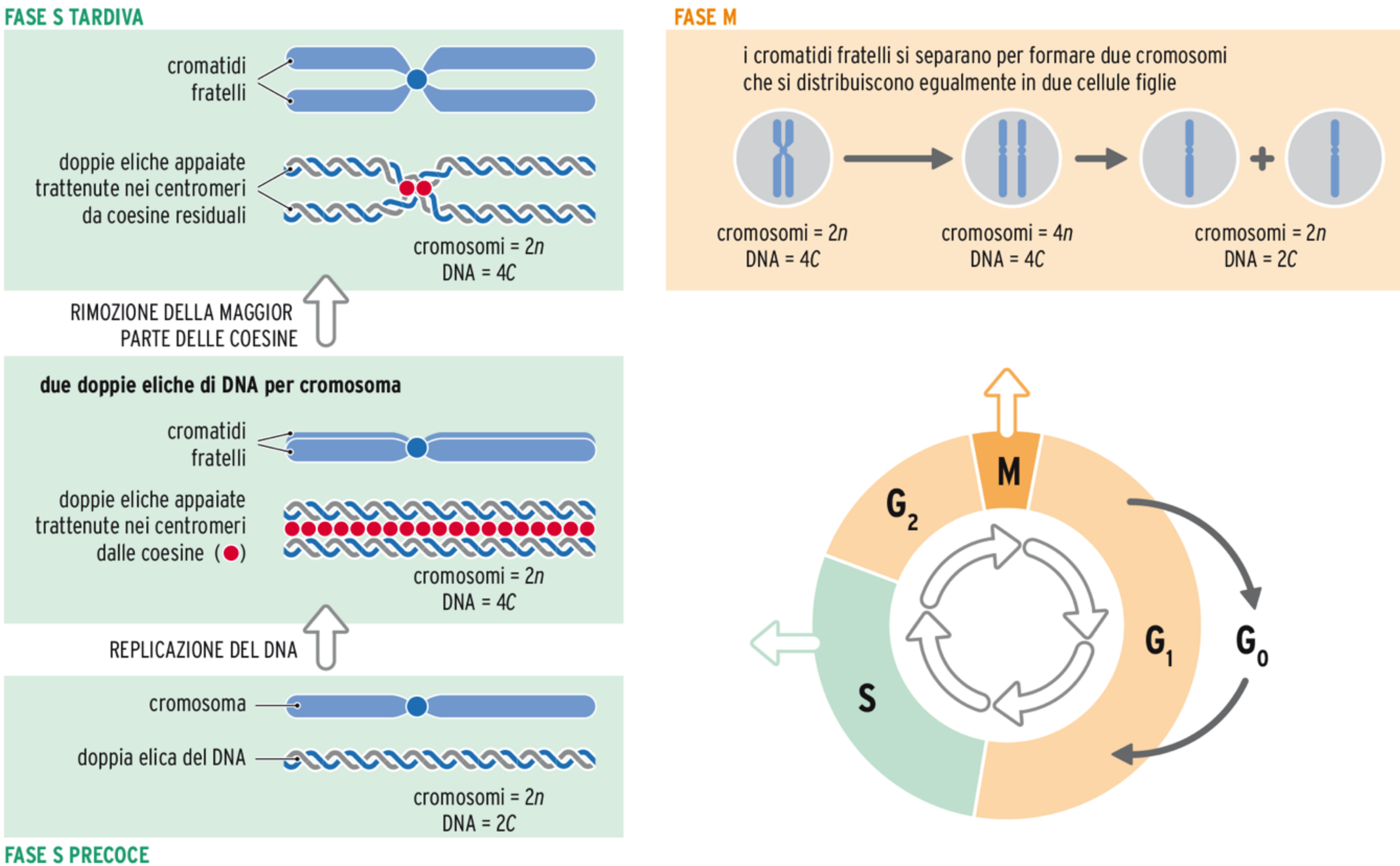
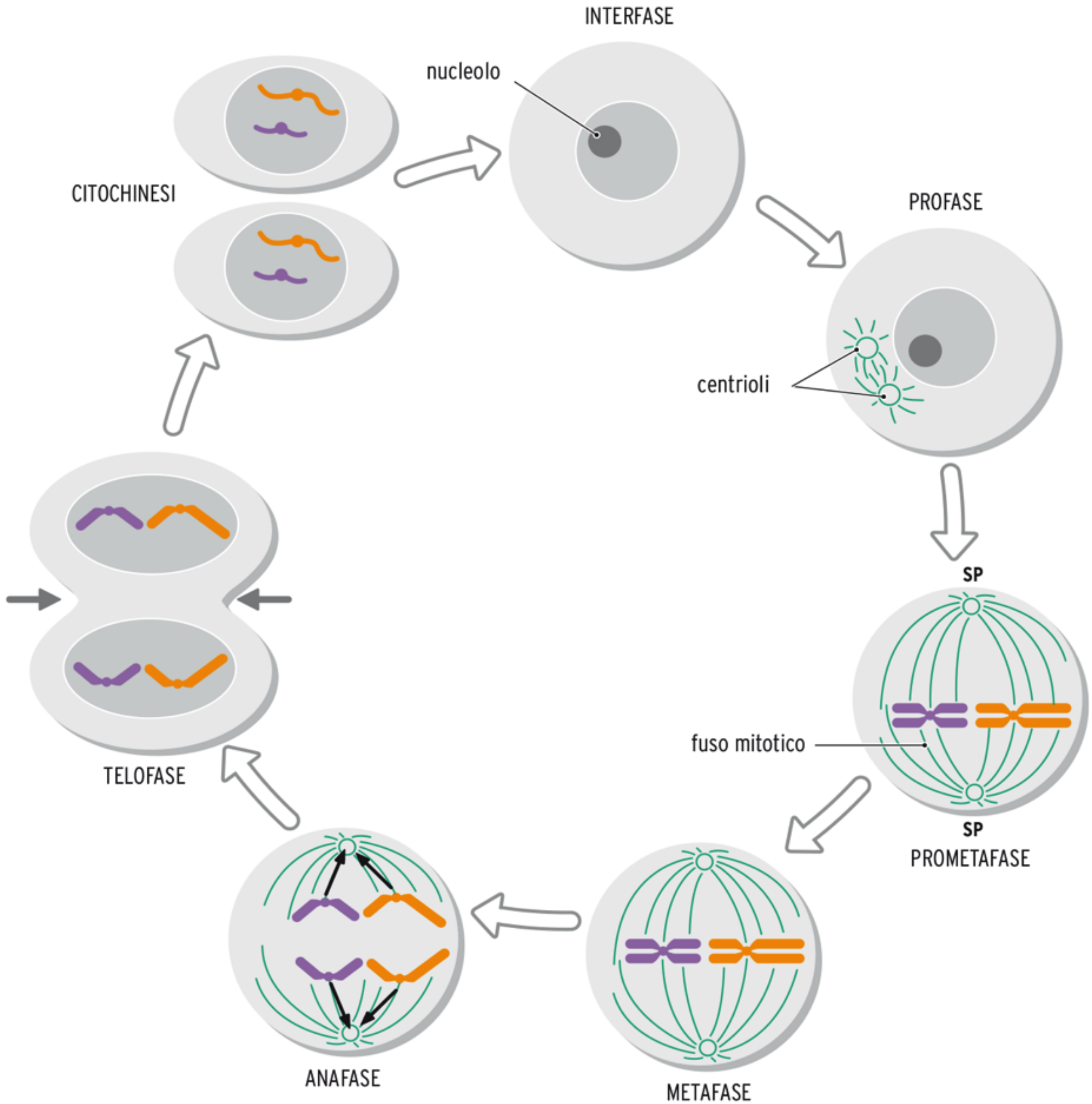



**Figura 1.10** Il sito di legame del ribosoma batterico.

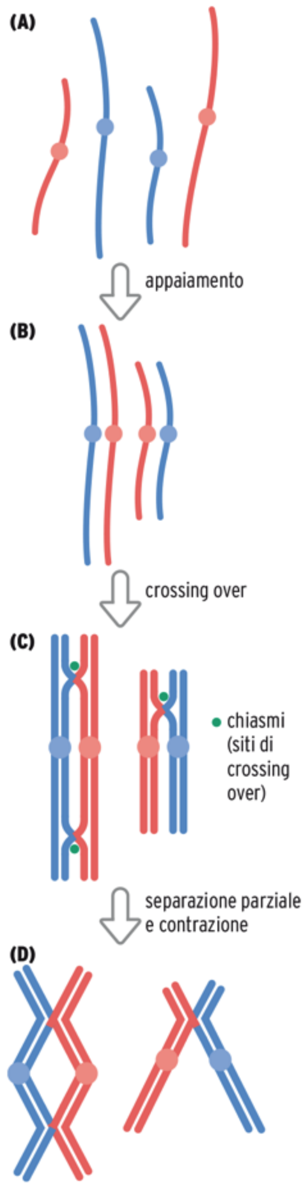

filamento senso del DNA
5'
3'
trascritto primario dell'RNA
5'
3'
3'
5'
filamento stampo (antisenso)

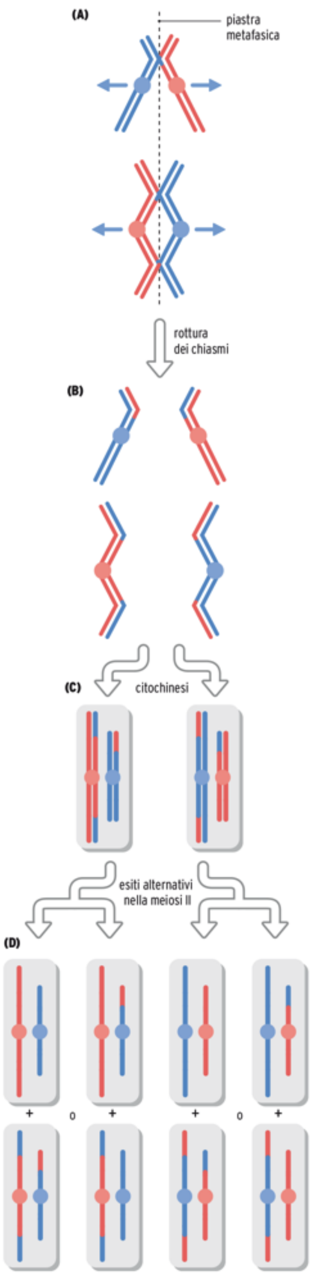

srotolamento che consente
un livello alto
di espressione genica
filamento nucleosomico
DNA
2 nm
avvolgimento che forma
la fibra cromatinica
dominio ad anello
nucleosoma
10 nm
nucleo
istonico
fibra
cromatinica
30 nm
supporto di proteine non istoniche


FASE S TARDIVA
cromatidi
fratelli
doppie eliche appaiate
trattenute nei centromeri
da coesine residuali
cromosomi = 2n
DNA = 4C
RIMOZIONE DELLA MAGGIOR
PARTE DELLE COESINE
due doppie eliche di DNA per cromosoma
cromatidi
fratelli
doppie eliche appaiate
trattenute nei centromeri
dalle coesine ( )
cromosomi = 2n
DNA = 4C
REPLICAZIONE DEL DNA
cromosoma
doppia elica del DNA
cromosomi = 2n
DNA = 2C
FASE S PRECOCE
FASE M
i cromatidi fratelli si separano per formare due cromosomi
che si distribuiscono egualmente in due cellule figlie
cromosomi = 2n
DNA = 4C
cromosomi = 4n
DNA = 4C
cromosomi = 2n
DNA = 2C
M
G2
G1
G0
S

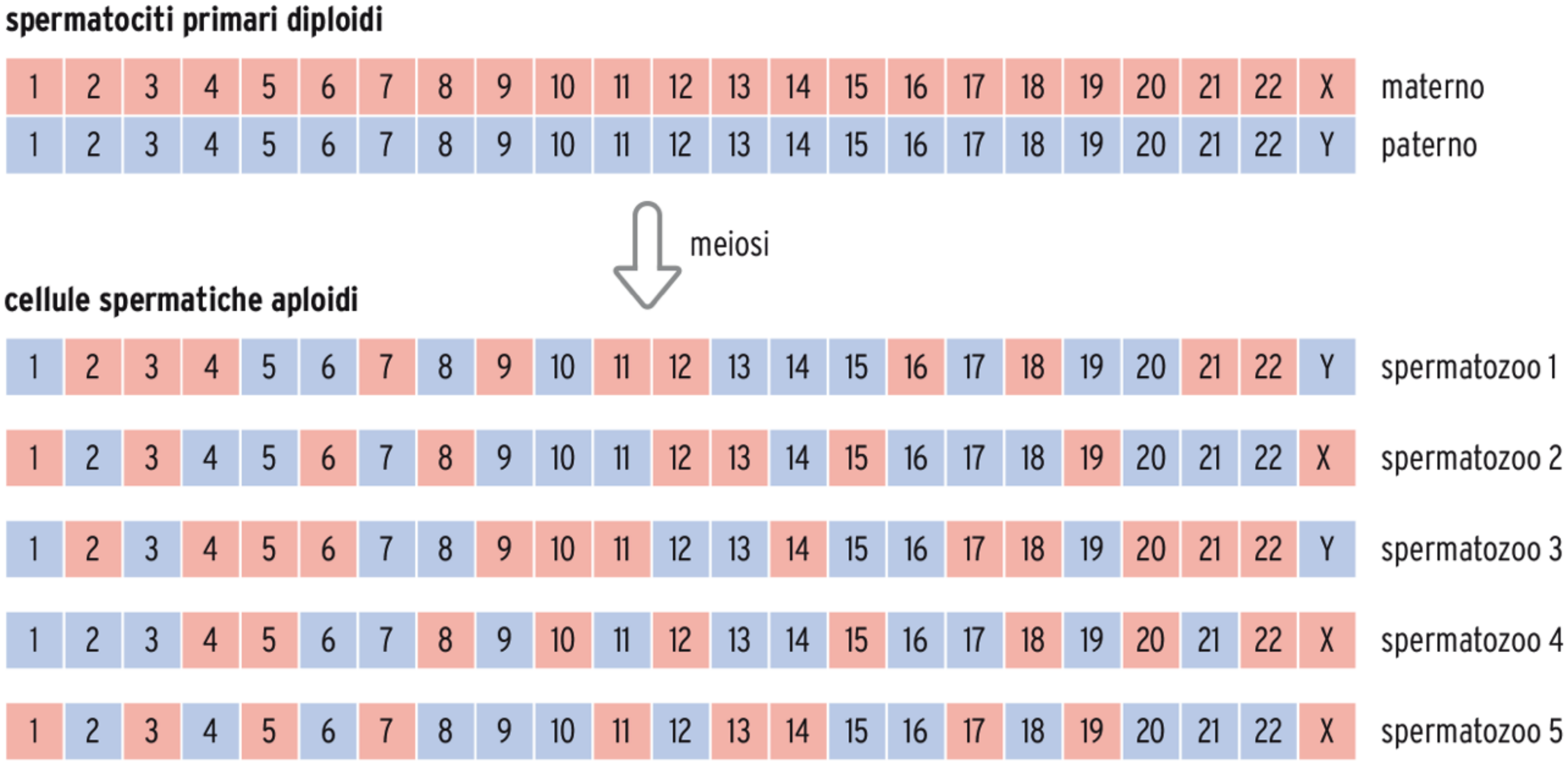


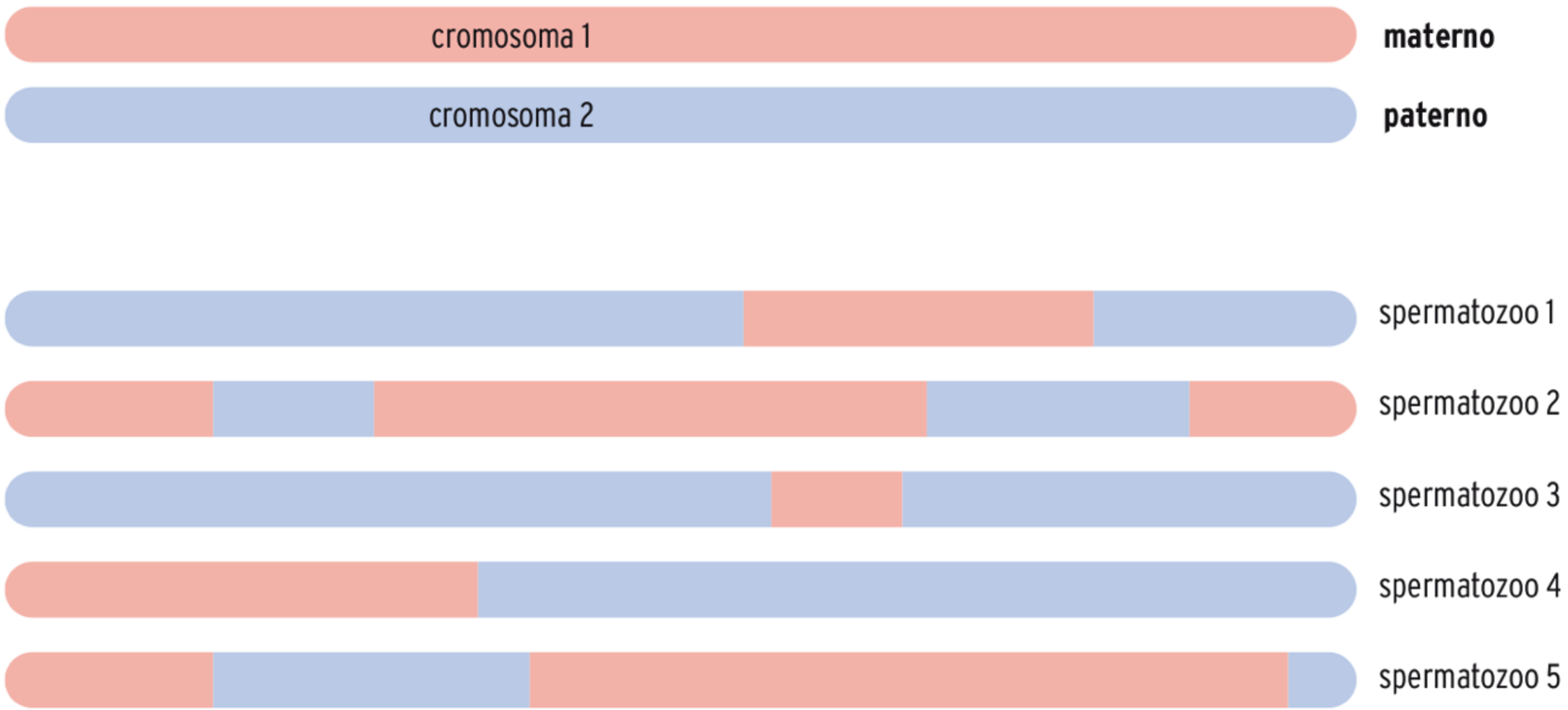
INTERFASE
nucleolo
PROFASE
centrioli
SP
fuso mitotico
SP
PROMETAFASE
METAFASE
ANAFASE
TELOFASE
CITOCHINESI


(A)
appaiamento
(B)
crossing over
(C)
chiasmi
(siti di
crossing
over)
separazione parziale
e contrazione
(D)



(A)
piastra
metafasica
rottura
dei chiasmi
(B)
(C)
citochinesi
esiti alternativi
nella meiosi II
(D)
+
o
+
+
o
+


spermatociti primari diploidi
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X
materno
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 Y
paterno
meiosi
cellule spermatiche aploidi
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 Y
spermatozoo 1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X
spermatozoo 2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 Y
spermatozoo 3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X
spermatozoo 4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X
spermatozoo 5


cromosoma 1
materno
cromosoma 2
paterno
spermatozoo 1
spermatozoo 2
spermatozoo 3
spermatozoo 4
spermatozoo 5

# GENE STRUCTURE: THE PROMOTER

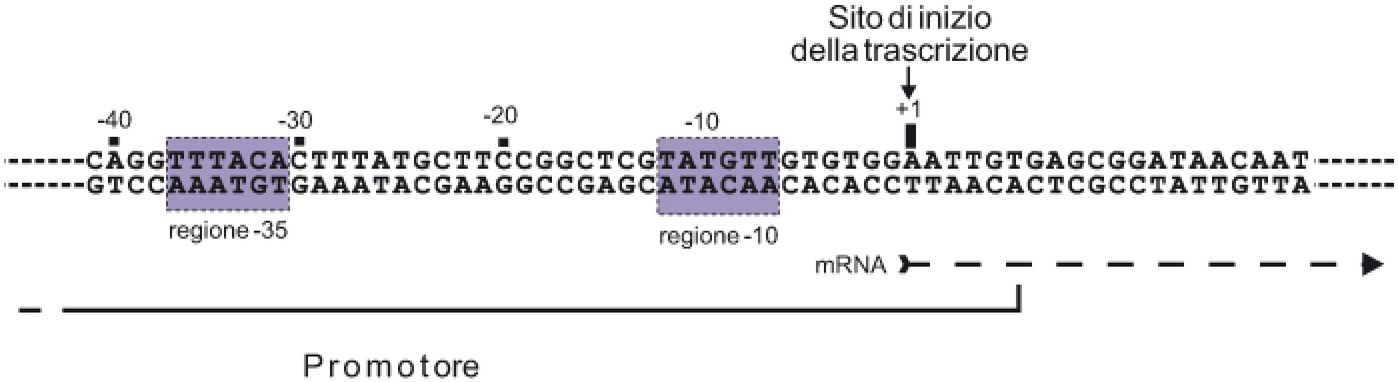



**Figura 1.9** Struttura della regione promotore dell'operone *lac*; notare che le regioni −35 e −10 del promotore *lac* non corrispondono esattamente alle sequenze consenso TTGACA e TATAAT, rispettivamente.

# GENE STRUCTURE: THE PROKARYOTIC OPERON

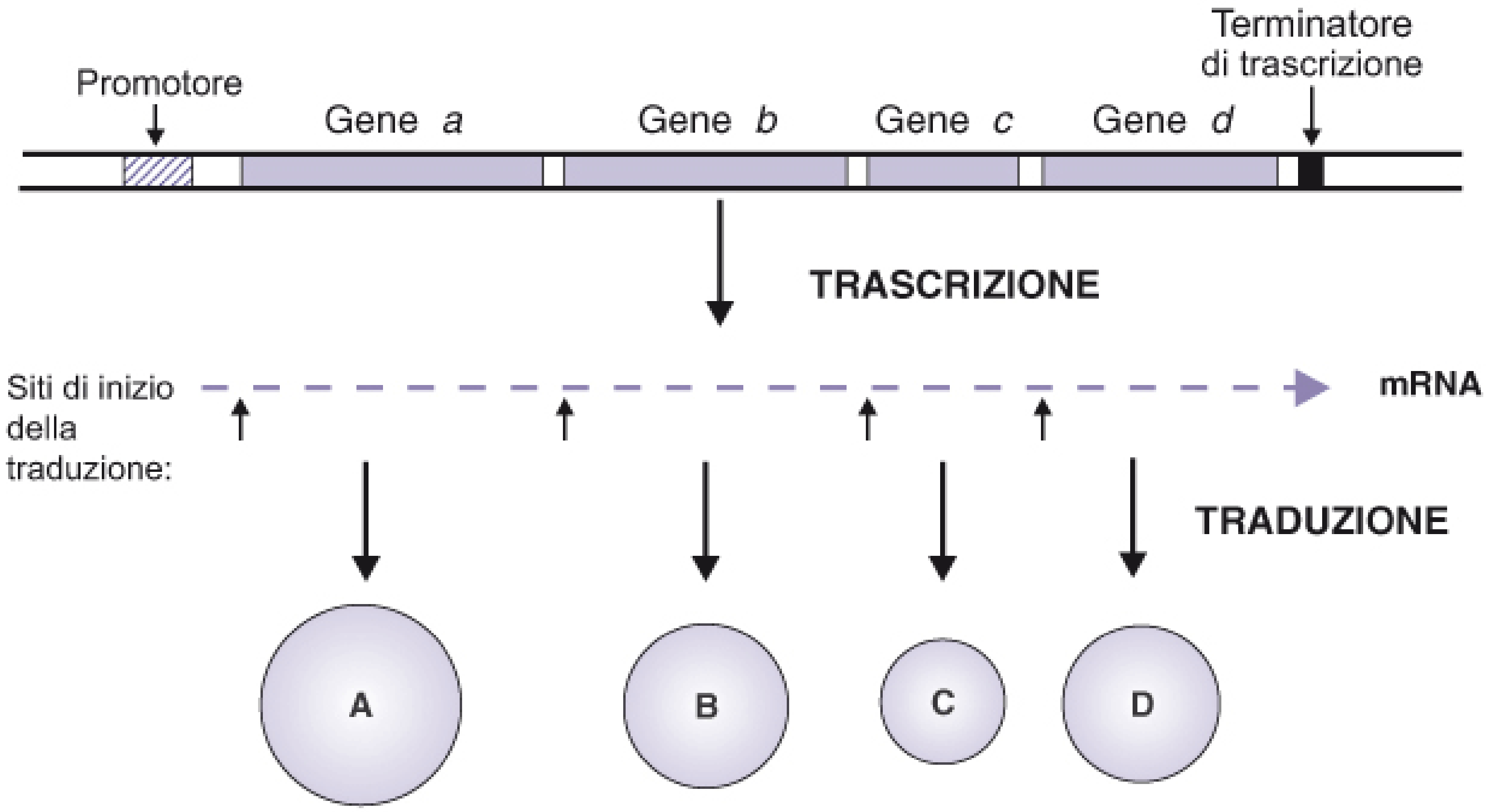
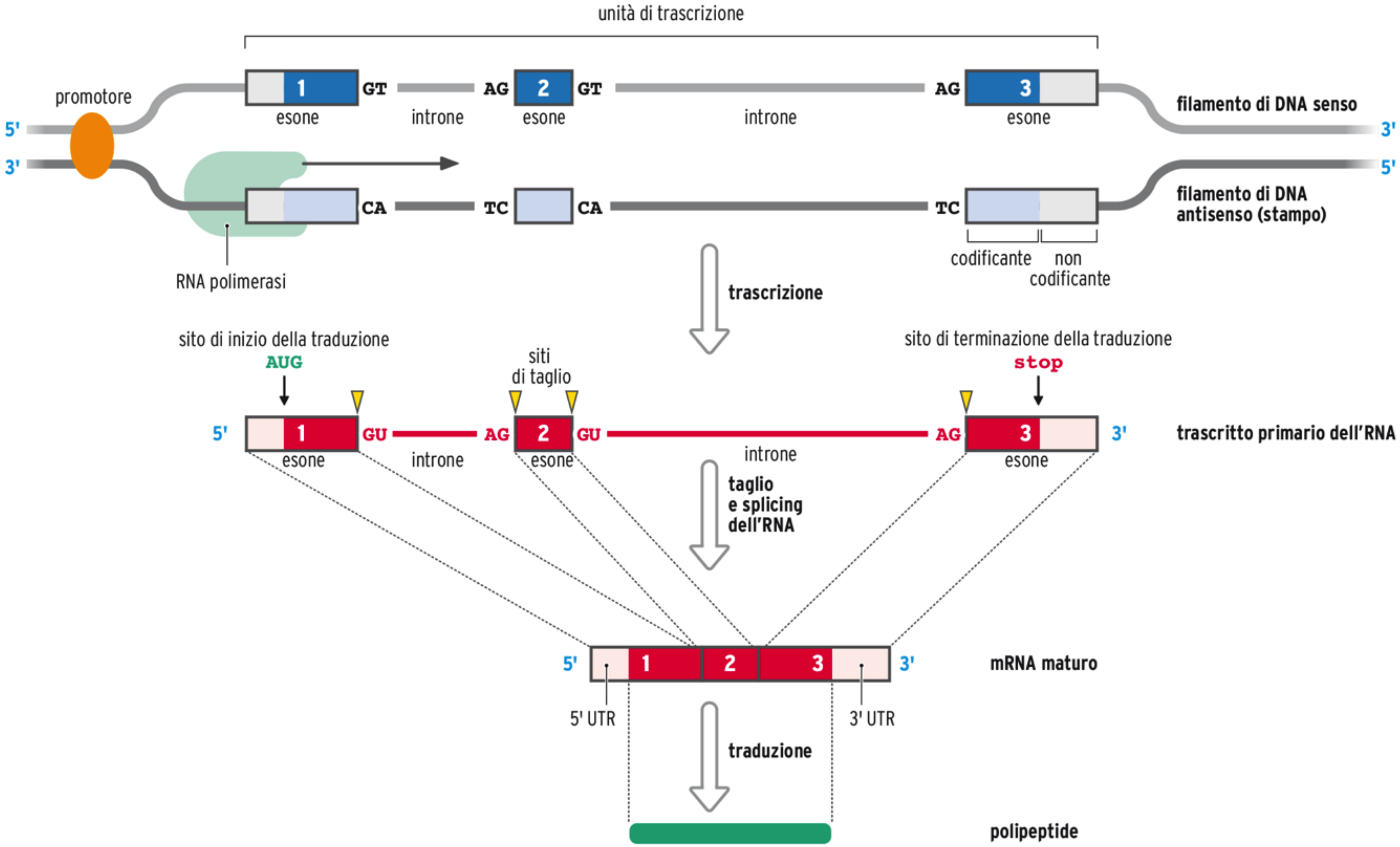



**Figura 1.11** Struttura di un operone.

# GENE STRUCTURE: THE EUKARYOTIC



# MUTATIONS ARE CLASSIFIED IN VARIOUS WAYS

- **Spontaneous mutations** happen naturally and randomly and are usually linked to normal biological or chemical processes in the organism.
- **Induced mutations** result from the influence of an extraneous factor, either natural or artificial.
- **Adaptative mutations** (from the Lamark hypothesys)

# MUTATION AND ADAPTATION:

- Jean-Baptiste Lamarck (1744-1829)

Lamarck

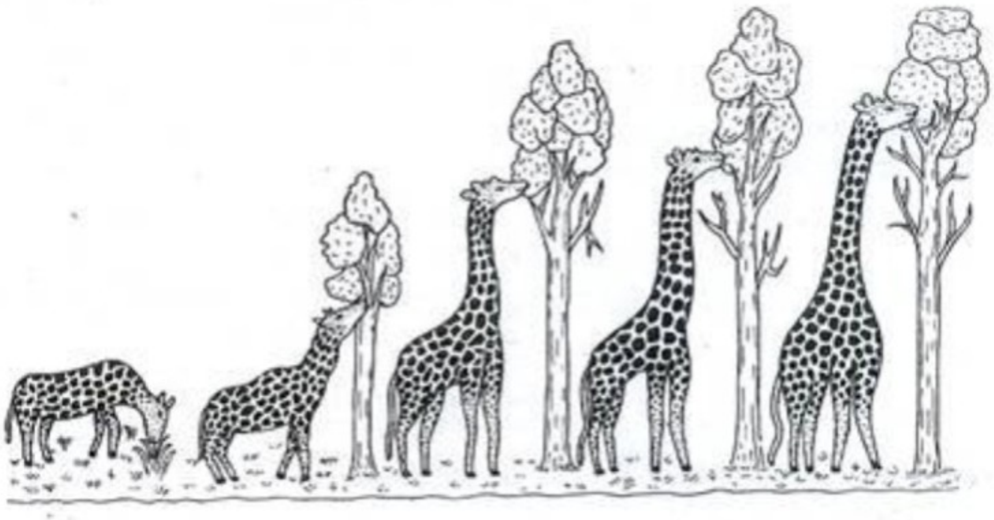
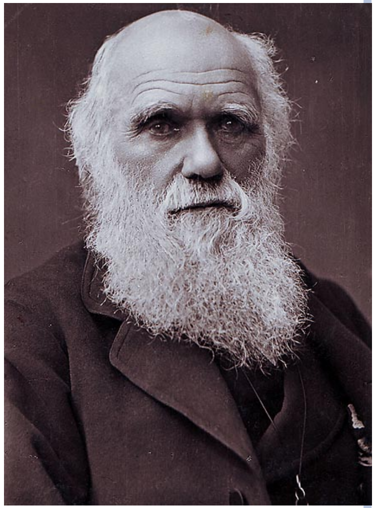


Diagram showing the elongation of a giraffe's neck according to Lamarck's theory of the heritability of acquired traits.

# MUTATION AND ADAPTATION:

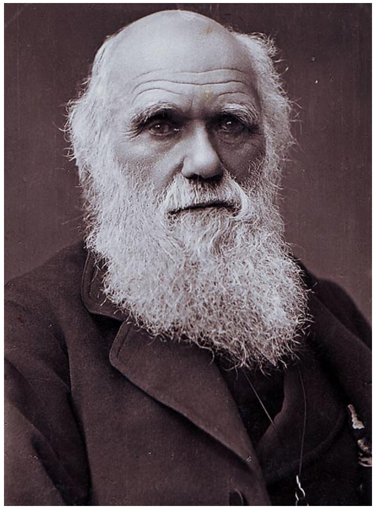
- Ideas largely ignored or attacked during his lifetime.
- Never won the acceptance and esteem of his colleagues and died in poverty and obscurity.
- Today Lamarck is mostly associated with a discredited theory of heredity (Lamarckism persisted until 1930s/1940s).

# MUTATION AND ADAPTATION: CHARLES DARWIN (1809-1882)



- Heritable adaptive variation results from random mutation and natural selection (1859, *The Origin of Species*).
- Contrary to Larmarck, inheritance of adaptive traits does not result from induction by environmental influences.
- But differential survival (selection) and heritable variation (arising from mutation in the DNA sequence).

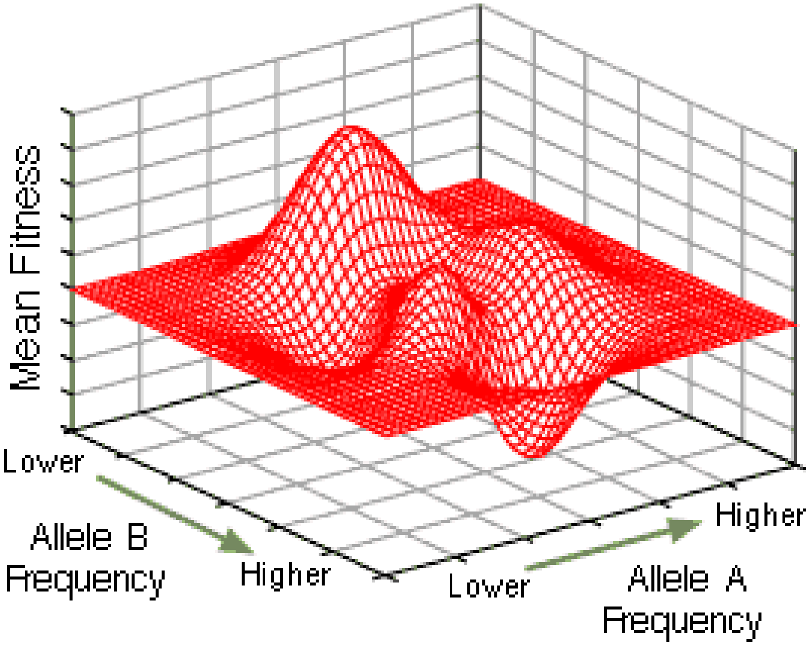
# MUTATION AND ADAPTATION: CHARLES DARWIN (1809-1882)



- Years following Darwin and rediscovery of Mendel resulted in controversy (until 1930s/1940s) about the relative importance of mutation and selection.
- Largely resolved by theoretical and empirical work of Fisher, Haldane, and Wright.

# Population genetics

Wright introduced the most compelling metaphor in population genetics, known as the "adaptive landscape"



# MUTATION AND ADAPTATION:

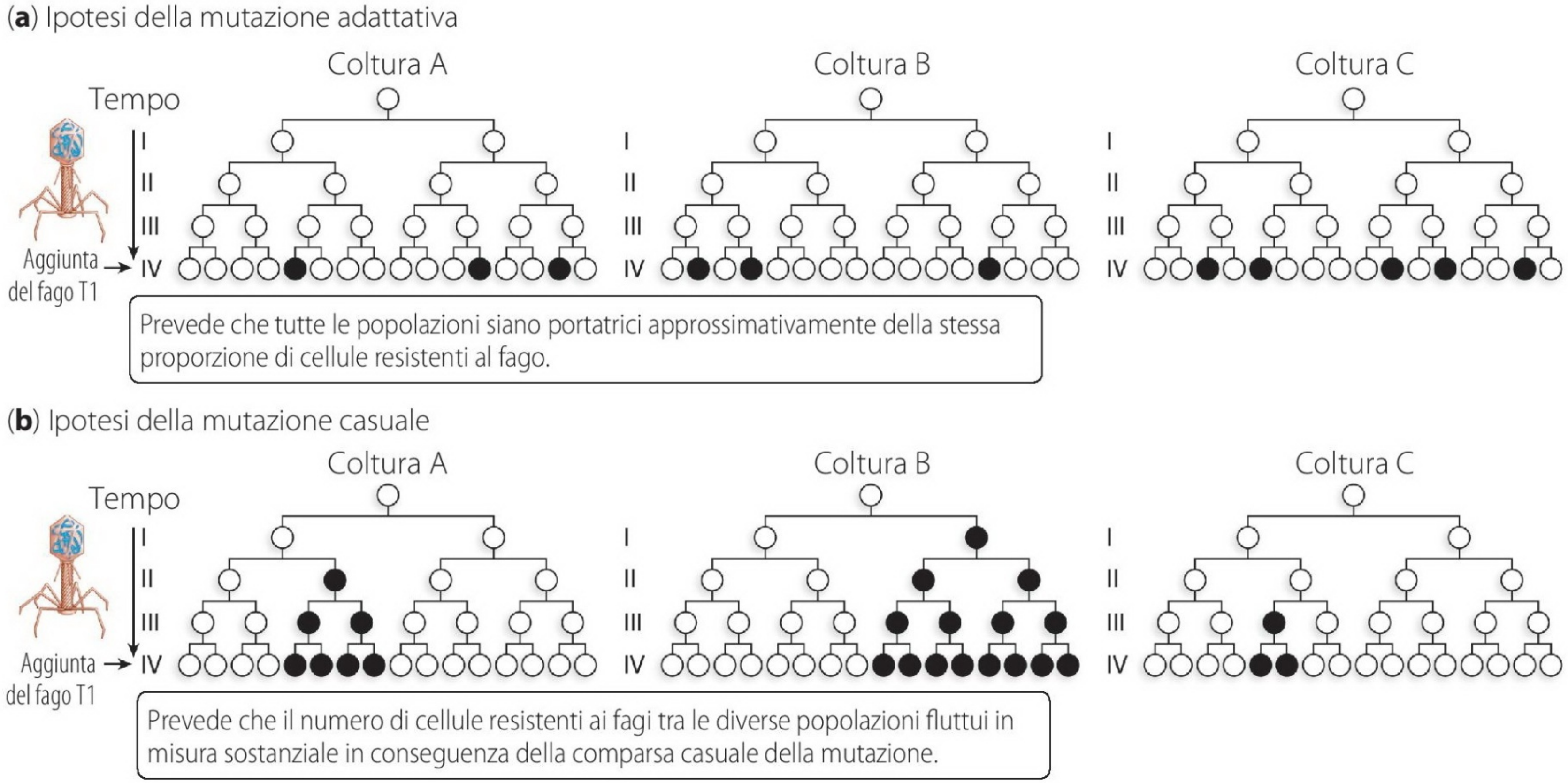
- The Luria-Delbrück **fluctuation test** demonstrated that mutations are not adaptive but occur spontaneously.

**TABLE 15.1** THE LURIA–DELBRÜCK EXPERIMENT DEMONSTRATING THAT SPONTANEOUS MUTATIONS ARE THE SOURCE OF PHAGE-RESISTANT BACTERIA

| | Number of T1-Resistant Bacteria | |
|---|---|---|
| Sample No. | Same Culture (Control) | Different Cultures |
| 1 | 14 | 6 |
| 2 | 15 | 5 |
| 3 | 13 | 10 |
| 4 | 21 | 8 |
| 5 | 15 | 24 |
| 6 | 14 | 13 |
| 7 | 26 | 165 |
| 8 | 16 | 15 |
| 9 | 20 | 6 |
| 10 | 13 | 10 |
| Mean | 16.7 | 26.2 |
| Variance | 15.0 | 2178.0 |

*Source*: After Luria and Delbrück (1943).

# MUTATION AND ADAPTATION:

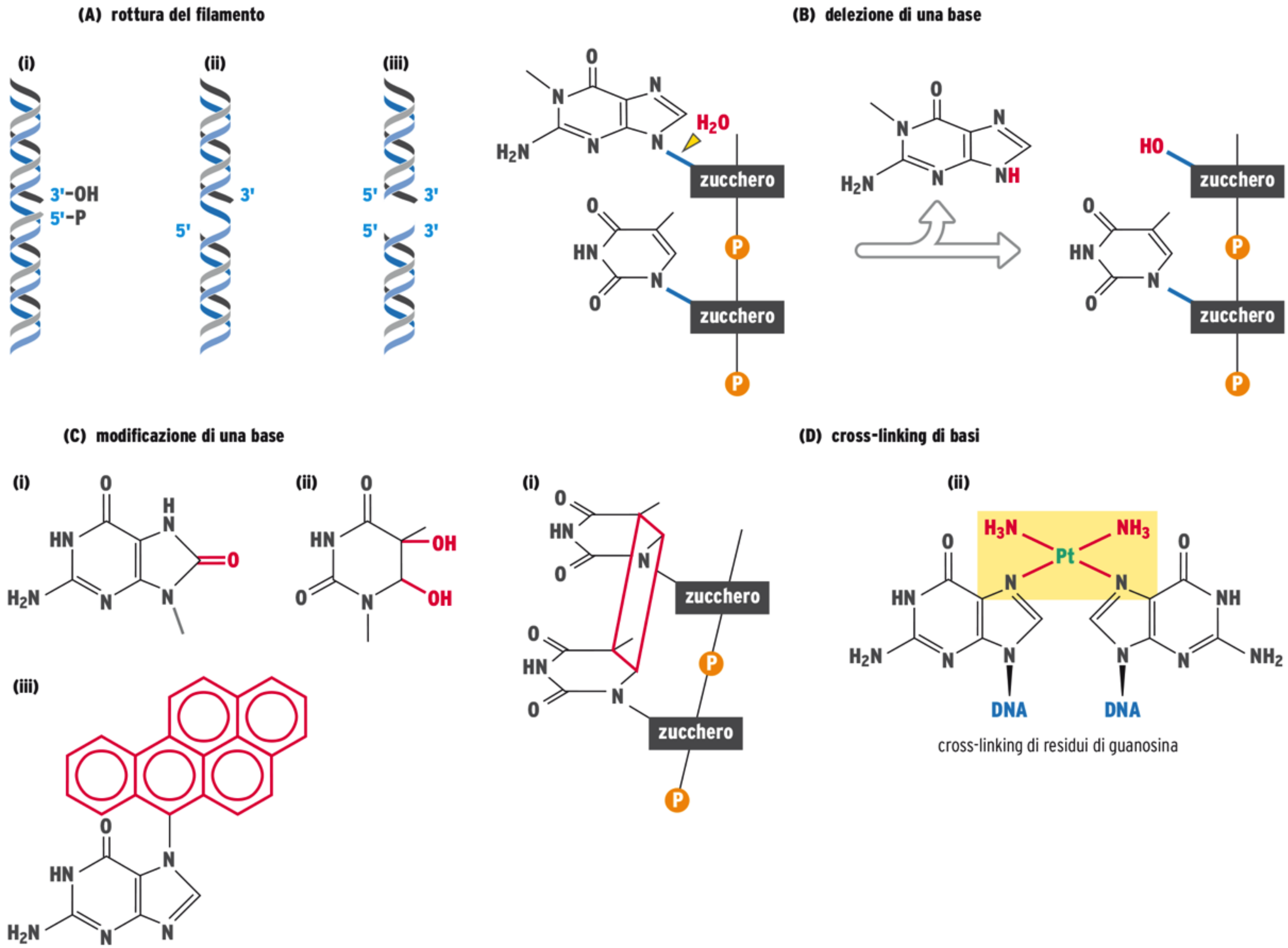


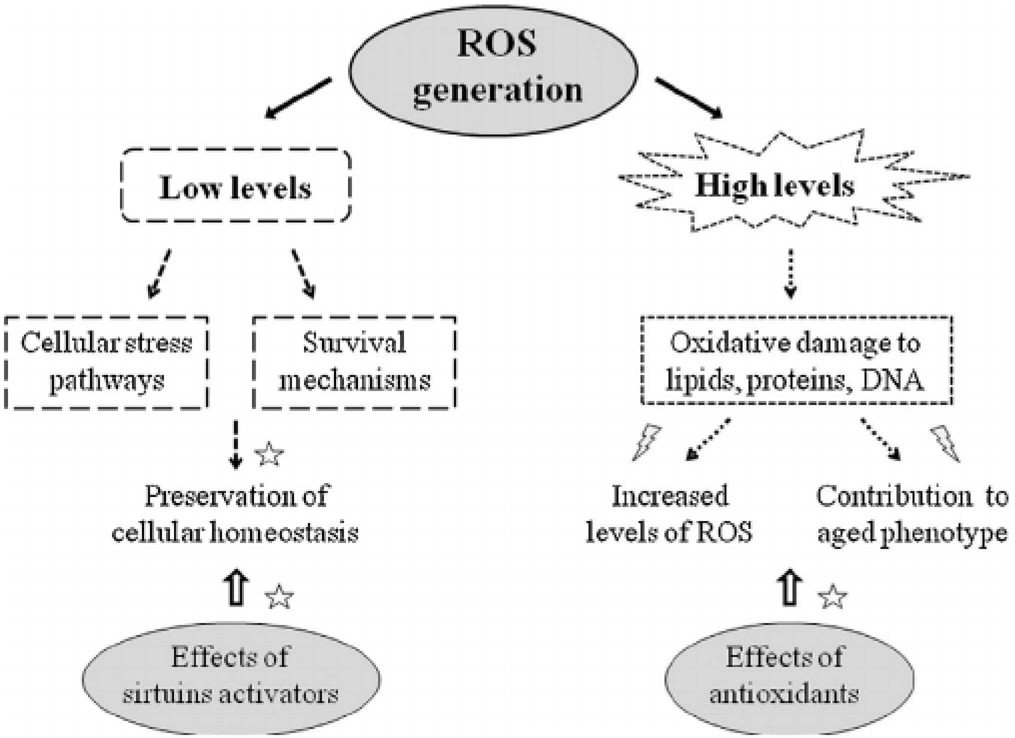
# MUTATION AND ADAPTATION:

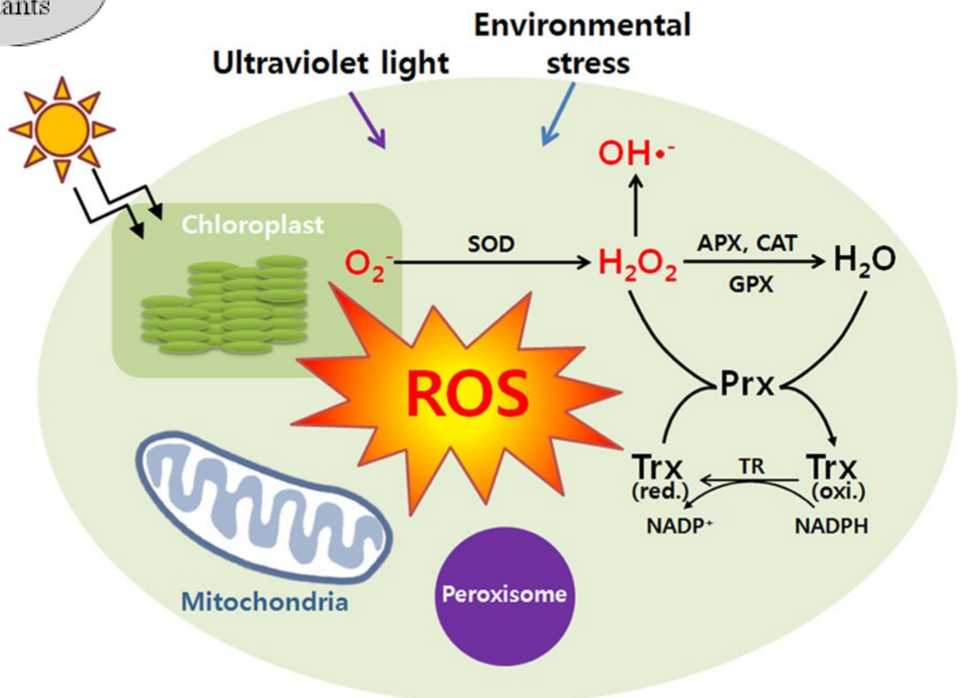
- What is a mutation?
  - Substitution, deletion, or insertion of a base pair.
  - Chromosomal deletion, insertion, or rearrangement.
- Somatic mutations occur in somatic cells and only affect the individual in which the mutation arises.
- Germ-line mutations alter gametes and passed to the next generation.

# MUTATION AND ADAPTATION:

- Mutations are quantified in two ways:
  1. Mutation rate = probability of a particular type of mutation per unit time (or generation).
  2. Mutation frequency = number of times a particular mutation occurs in a population of cells or individuals.


(A) rottura del filamento
(i)
(ii)
(iii)
3'-OH
5'-P
3'
5'
5'
3'
5'
3'
(B) delezione di una base
O
N
N
H2N
N
N
H2O
zucchero
O
HN
O
N
P
zucchero
P
O
N
N
H2N
N
NH
HO
zucchero
O
HN
O
N
P
zucchero
P
(C) modificazione di una base
(i)
O
H
N
HN
=O
H2N
N
N
(ii)
O
HN
OH
O
N
OH
(iii)
O
HN
N
H2N
N
N
(D) cross-linking di basi
(i)
O
HN
O
N
zucchero
O
HN
O
N
P
zucchero
P
(ii)
H3N
NH3
Pt
O
O
N
N
HN
NH
H2N
N
N
N
N
NH2
DNA
DNA
cross-linking di residui di guanosina


ROS generation
Low levels
High levels
Cellular stress pathways
Survival mechanisms
Oxidative damage to lipids, proteins, DNA
Preservation of cellular homeostasis
Increased levels of ROS
Contribution to aged phenotype
Effects of sirtuins activators
Effects of antioxidants


Ultraviolet light
Environmental stress
Chloroplast
$O_2^-$
SOD
$H_2O_2$
$OH\cdot^-$
APX, CAT
GPX
$H_2O$
Prx
Trx (red.)
Trx (oxi.)
TR
$NADP^+$
NADPH
ROS
Mitochondria
Peroxisome

**DANNO OSSIDATIVO**

- siti suscettibili

**mitocondriale**
(fosforilazione ossidativa)

$O_2$

↓

$O_2^-$

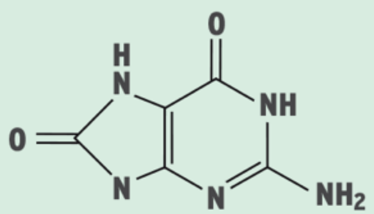
↓

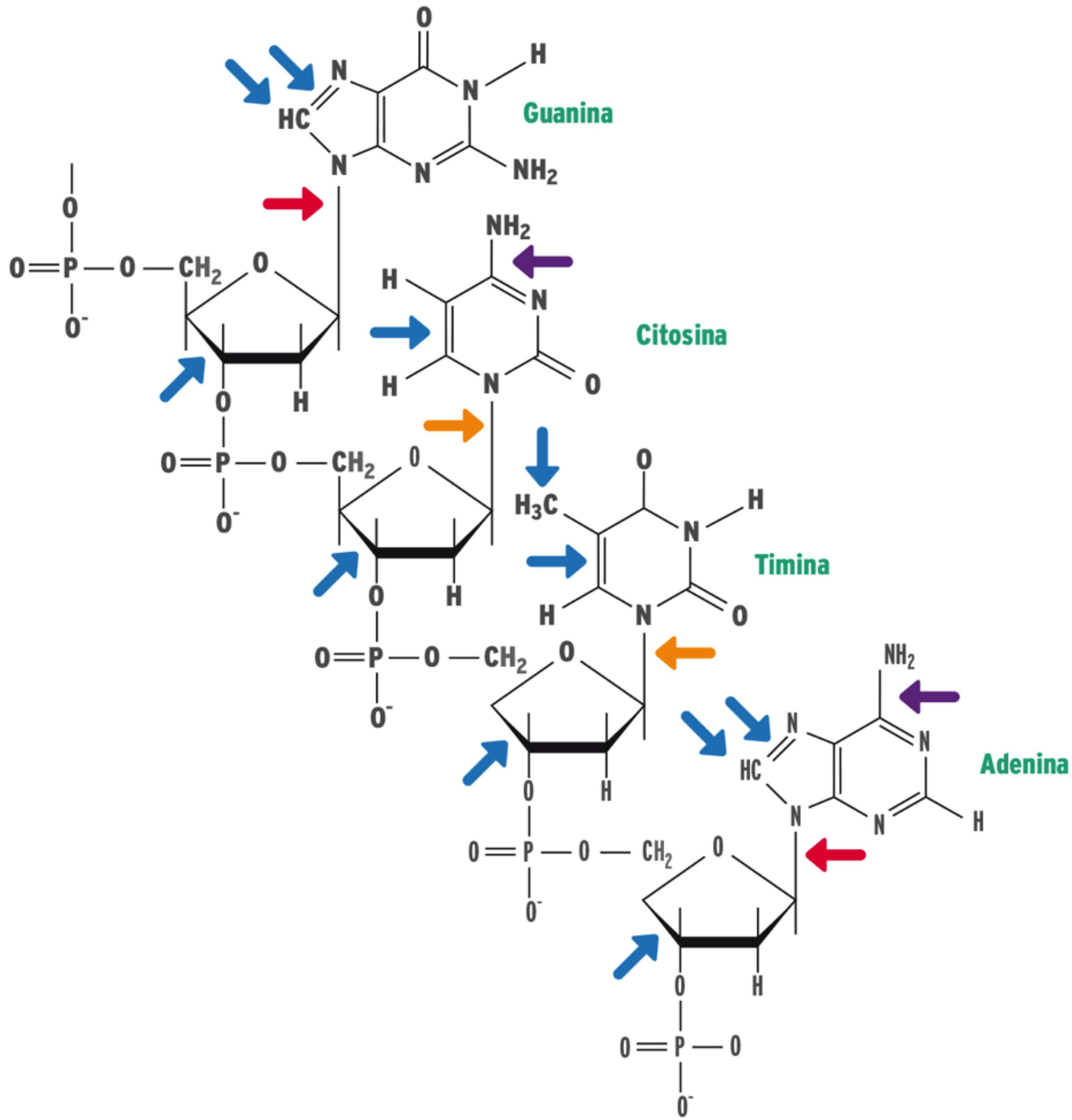
$H_2O_2$

**ATTACCO IDROLITICO**

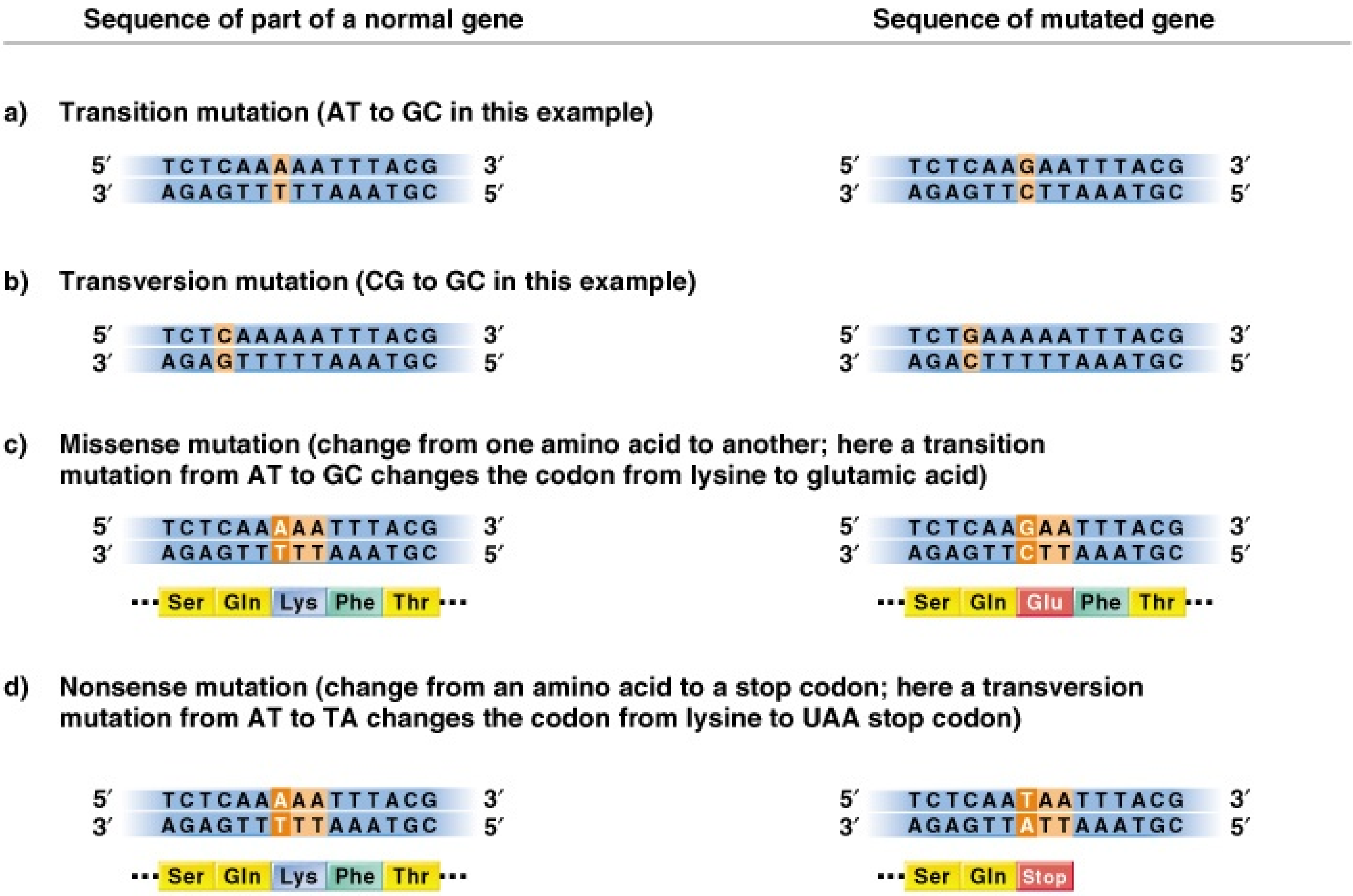
- depurinazione
- depirimidinazione
- deamminazione

**8-oxoguanina**

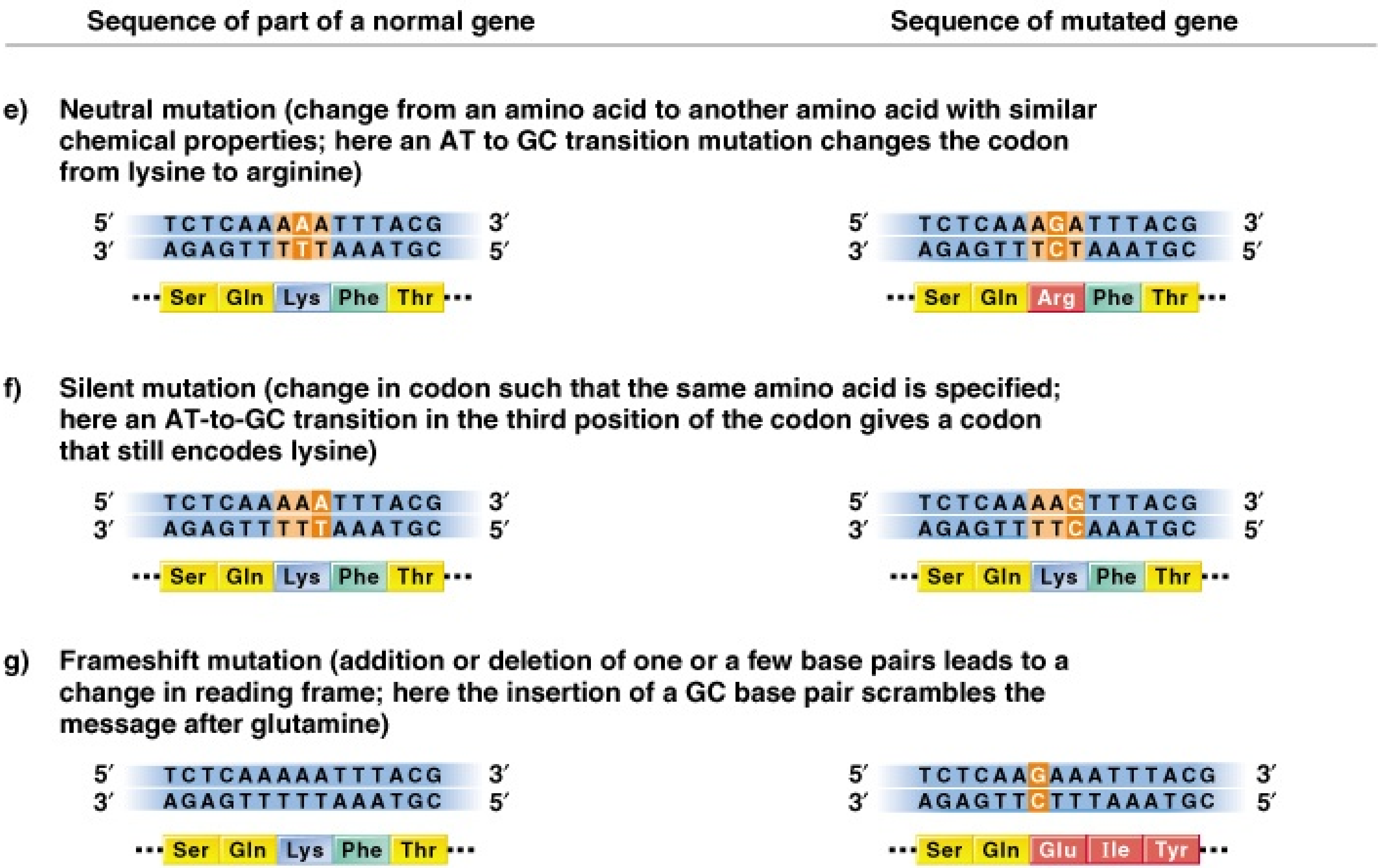




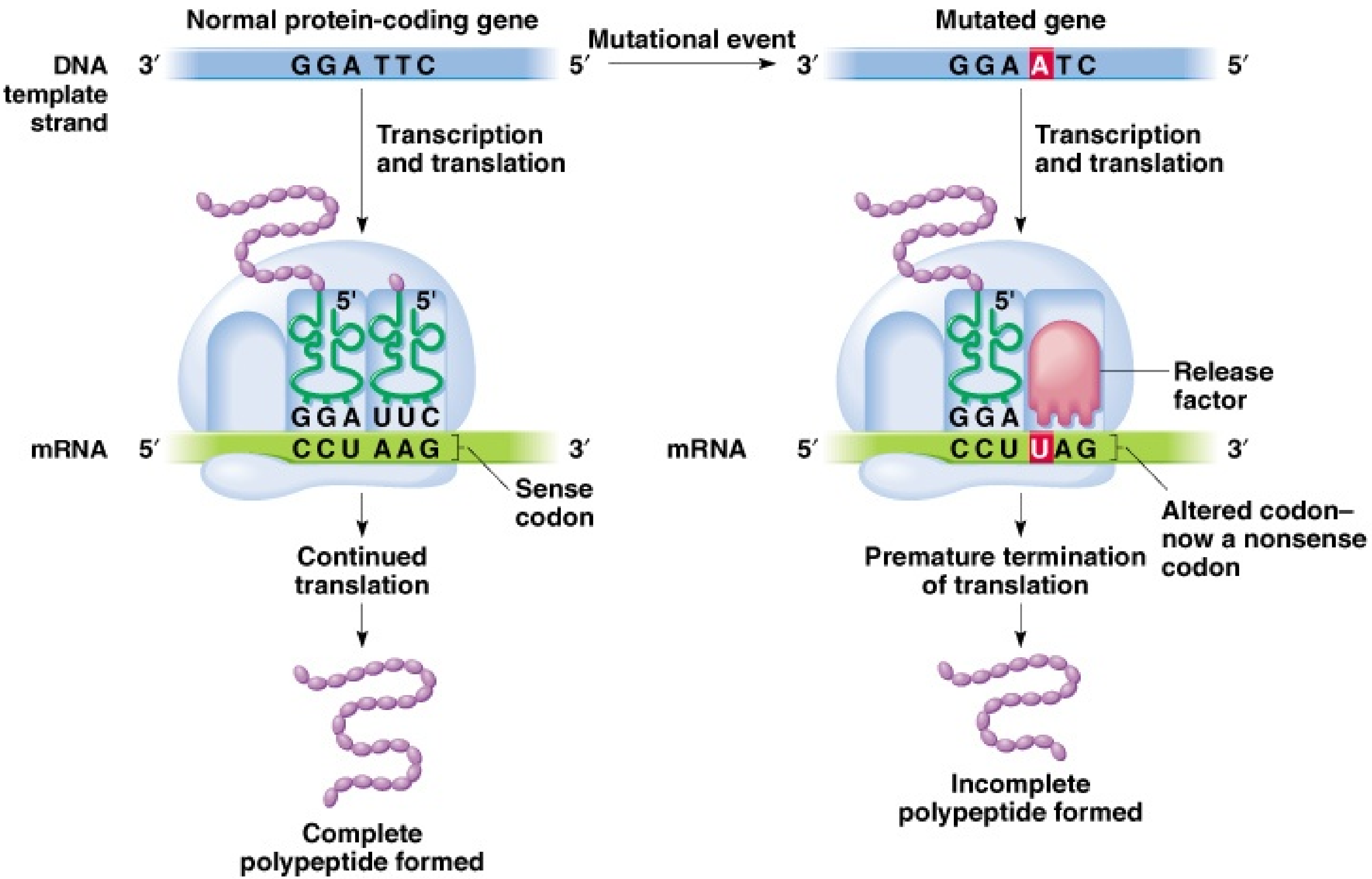
# MUTATION AND ADAPTATION:



# MUTATION AND ADAPTATION:



# MUTATION AND ADAPTATION: NON SENSE MUTATION



# MUTATION AND ADAPTATION

- **Lethal mutations** interrupt an essential process and result in death.
- The expression of **conditional mutations** depends on the environment in which the organism finds itself. A good example is a temperature-sensitive mutation.
- **Neutral mutations**, the vast majority of all mutations, occur in the large portions of the genome that do not contain genes and therefore have no effect on gene products.

# MUTATION AND ADAPTATION

**TABLE 15.2** RATES OF SPONTANEOUS MUTATIONS AT VARIOUS LOCI IN DIFFERENT ORGANISMS

| Organism | Character | Gene | Rate | Units |
|---|---|---|---|---|
| Bacteriophage T2 | Lysis inhibition | $r \rightarrow r^+$ | $1 \times 10^{-8}$ | Per gene replication |
| | Host range | $h^+ \rightarrow h$ | $3 \times 10^{-9}$ | |
| | Lactose fermentation | $lac^- \rightarrow lac^+$ | $2 \times 10^{-7}$ | |
| | Lactose fermentation | $lac^+ \rightarrow lac^-$ | $2 \times 10^{-6}$ | |
| | Phage T1 resistance | $Tl\text{-}s \rightarrow Tl\text{-}r$ | $2 \times 10^{-8}$ | |
| | Histidine requirement | $his^+ \rightarrow his^-$ | $2 \times 10^{-6}$ | |
| | Histidine independence | $his^- \rightarrow his^+$ | $4 \times 10^{-8}$ | |
| *E. coli* | Streptomycin dependence | $str\text{-}s \rightarrow str\text{-}d$ | $1 \times 10^{-9}$ | Per cell division |
| | Streptomycin sensitivity | $str\text{-}d \rightarrow str\text{-}s$ | $1 \times 10^{-8}$ | |
| | Radiation resistance | $rad\text{-}s \rightarrow rad\text{-}r$ | $1 \times 10^{-5}$ | |
| | Leucine independence | $leu^- \rightarrow leu^+$ | $7 \times 10^{-10}$ | |
| | Arginine independence | $arg^- \rightarrow arg^+$ | $4 \times 10^{-9}$ | |
| | Tryptophan independence | $trp^- \rightarrow trp^+$ | $6 \times 10^{-8}$ | |
| *Salmonella typhimurium* | Tryptophan independence | $trp^- \rightarrow trp^+$ | $5 \times 10^{-8}$ | Per cell division |
| *Diplococcus pneumoniae* | Penicillin resistance | $pen^s \rightarrow pen^r$ | $1 \times 10^{-7}$ | Per cell division |
| *Chlamydomonas reinhardi* | Streptomycin sensitivity | $str^r \rightarrow str^s$ | $1 \times 10^{-6}$ | Per cell division |
| *Neurospora crassa* | Inositol requirement | $inos^+ \rightarrow inos^-$ | $8 \times 10^{-8}$ | Mutant frequency among asexual spores |
| | Adenine independence | $ade^- \rightarrow ade^+$ | $2 \times 10^{-8}$ | |
| *Zea mays* | Shrunken seeds | $sh^+ \rightarrow sh^-$ | $1 \times 10^{-6}$ | Per gamete per generation |
| | Purple | $pr^+ \rightarrow pr^-$ | $1 \times 10^{-5}$ | |
| | Colorless | $c^+ \rightarrow c^-$ | $2 \times 10^{-6}$ | |
| | Sugary | $su^+ \rightarrow su^-$ | $2 \times 10^{-6}$ | |
| *Drosophila melanogaster* | Yellow body | $y^+ \rightarrow y$ | $1.2 \times 10^{-6}$ | Per gamete per generation |
| | White eye | $w^+ \rightarrow w$ | $4 \times 10^{-5}$ | |
| | Brown eye | $bw^+ \rightarrow bw$ | $3 \times 10^{-5}$ | |
| | Ebony body | $e^+ \rightarrow e$ | $2 \times 10^{-5}$ | |
| | Eyeless | $ey^+ \rightarrow ey$ | $6 \times 10^{-5}$ | |
| *Mus musculus* | Piebald coat | $s^+ \rightarrow s$ | $3 \times 10^{-5}$ | Per gamete per generation |
| | Dilute coat color | $d^+ \rightarrow d$ | $3 \times 10^{-5}$ | |
| | Brown coat | $b^+ \rightarrow b$ | $8.5 \times 10^{-4}$ | |
| | Pink eye | $p^+ \rightarrow p$ | $8.5 \times 10^{-4}$ | |
| *Homo sapiens* | Hemophilia | $h^+ \rightarrow h$ | $2 \times 10^{-5}$ | Per gamete per generation |
| | Huntington disease | $Hu^+ \rightarrow Hu$ | $5 \times 10^{-6}$ | |
| | Retinoblastoma | $R^+ \rightarrow R$ | $2 \times 10^{-5}$ | |
| | Epiloia | $Ep^+ \rightarrow Ep$ | $1 \times 10^{-5}$ | |
| | Aniridia | $An^+ \rightarrow An$ | $5 \times 10^{-6}$ | |
| | Achondroplasia | $A^+ \rightarrow A$ | $5 \times 10^{-5}$ | |

# MUTATION AND ADAPTATION

- Detecting environmental mutations: