Gene Mutation and DNA Repair.

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.

Mutations Are Classified in Various Ways

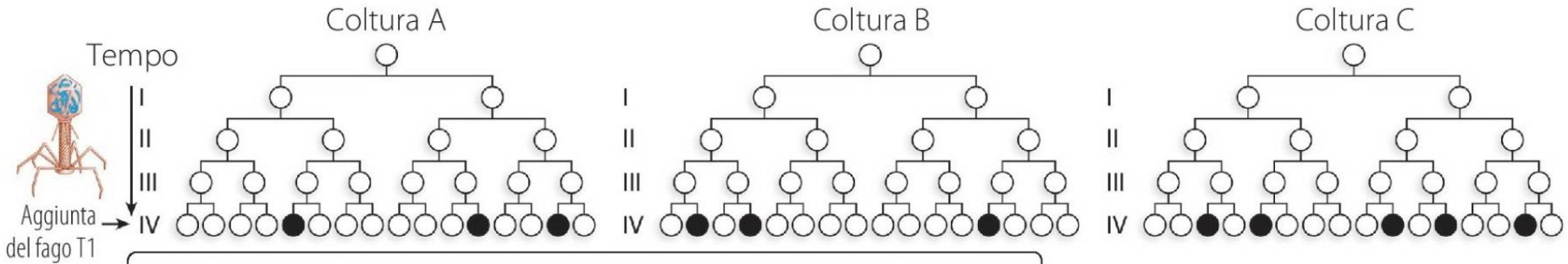
- The Luria-Delbrück fluctuation test demonstrated that mutations are not adaptive but occur spontaneously.

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

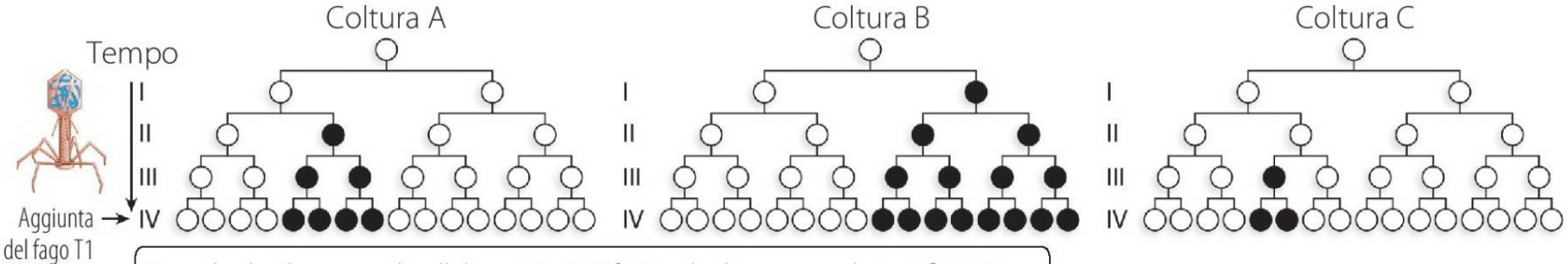
Source: After Luria and Delbrück (1943).

(**a**) Ipotesi della mutazione adattativa



Prevede che tutte le popolazioni siano portatrici approssimativamente della stessa proporzione di cellule resistenti al fago.

(**b**) Ipotesi della mutazione casuale



Prevede che il numero di cellule resistenti ai fagi tra le diverse popolazioni fluttui in misura sostanziale in conseguenza della comparsa casuale della mutazione.

Mutations Are Classified in Various Ways

- **Somatic mutations** occur in any cell except germ cells and are not heritable. **Germ-line mutations** occur in gametes and are inherited.
- **Autosomal mutations** occur within genes located on the autosomes, whereas **X-linked mutations** occur within genes located on the X chromosome.

Mutations Are Classified in Various Ways

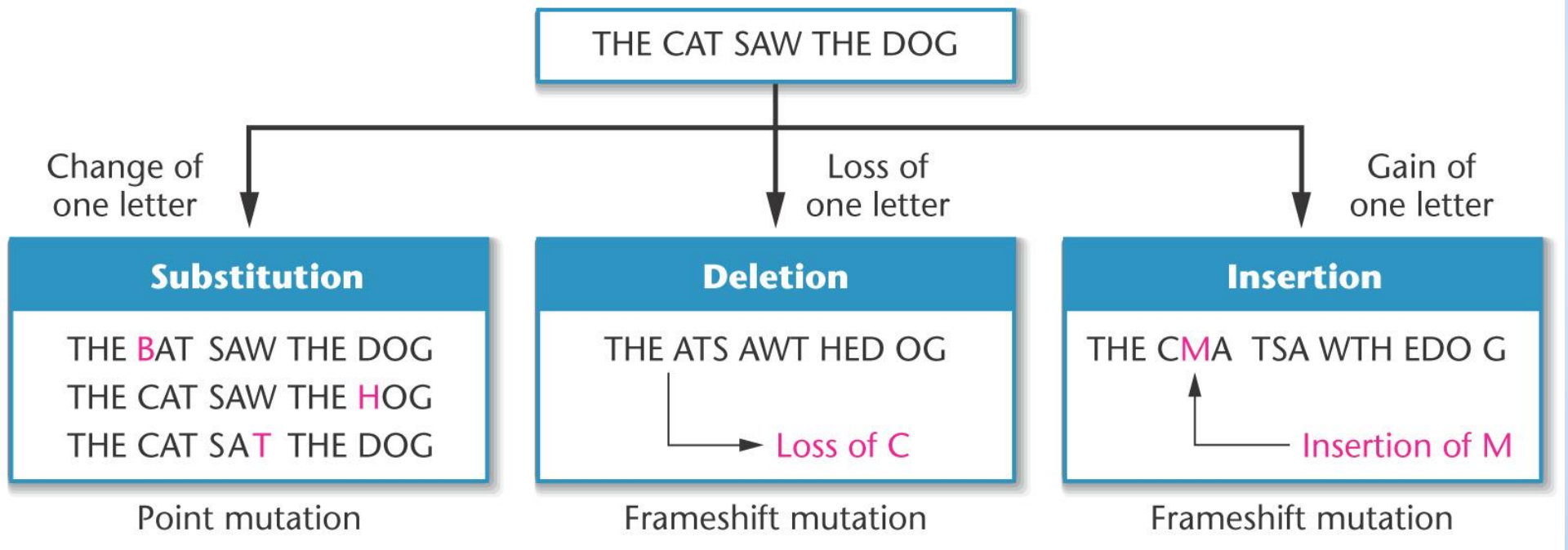
- When a **recessive autosomal mutation** occurs in a somatic cell of a diploid organism, it is unlikely to result in a detectable phenotype.

Mutations Are Classified in Various Ways

- **Inherited dominant autosomal mutations** will be expressed phenotypically in the first generation.
- **X-linked recessive mutations** arising in the gametes of a homogametic female may be expressed in hemizygous male offspring.

Mutations Are Classified in Various Ways

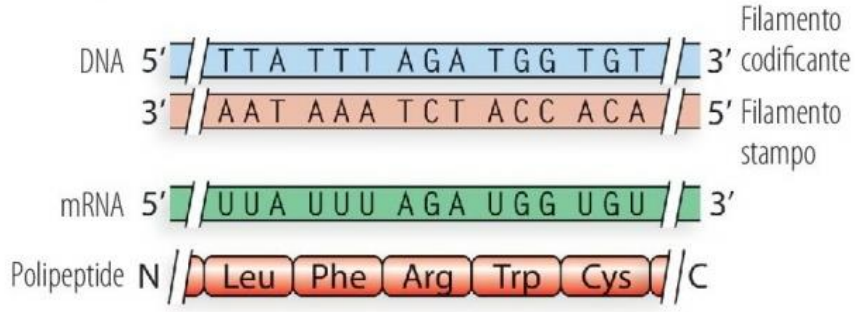
- Mutations can be classified based on type of molecular change. **Point mutations** are base substitutions in which one base pair is altered.



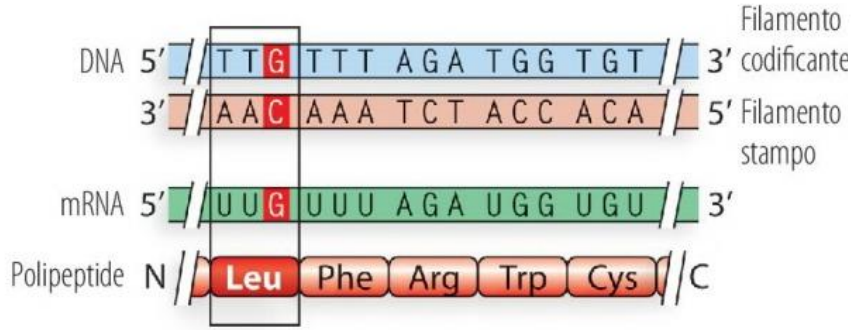
Mutations Are Classified in Various Ways

- **Missense mutations** change a codon within a protein-coding portion of a gene.
- A **nonsense mutation** changes a codon into a stop codon and results in premature termination of translation.
- A **silent mutation** alters a codon but does not result in a change in the amino acid at that position of the protein.

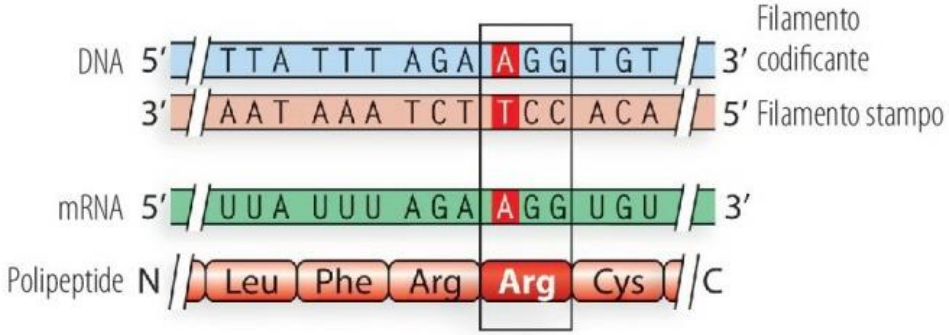
(**a**) Sequenza selvatica



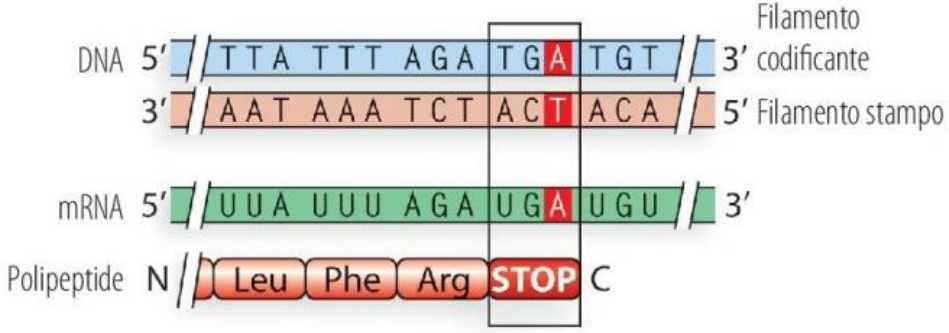
(**b**) Mutazione silente



(**c**) Mutazione missenso



(**d**) Mutazione nonsenso



Mutations Are Classified in Various Ways

- If a pyrimidine replaces a pyrimidine or a purine replaces a purine, a **transition** has occurred.
- If a purine and a pyrimidine are interchanged, a **transversion** has occurred.
- A **frameshift mutation** occurs when any number of bases are added or deleted, except multiples of three, which would reestablish the initial frame of reading.

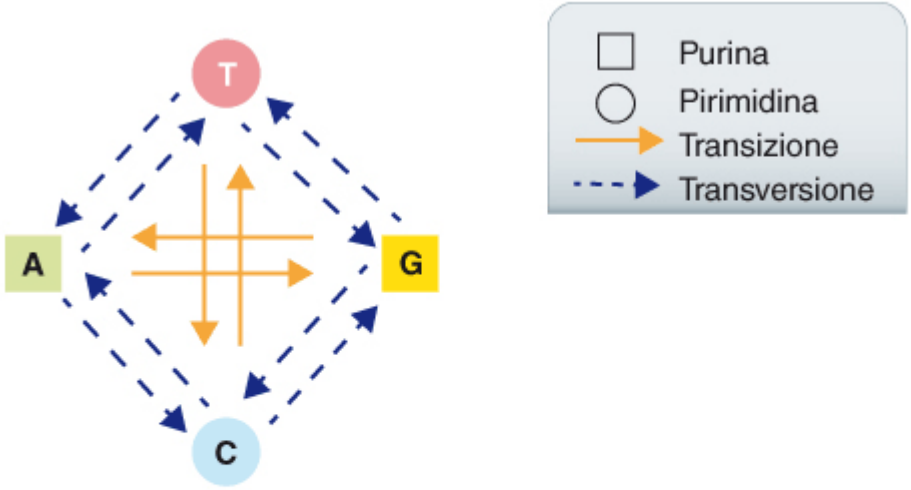
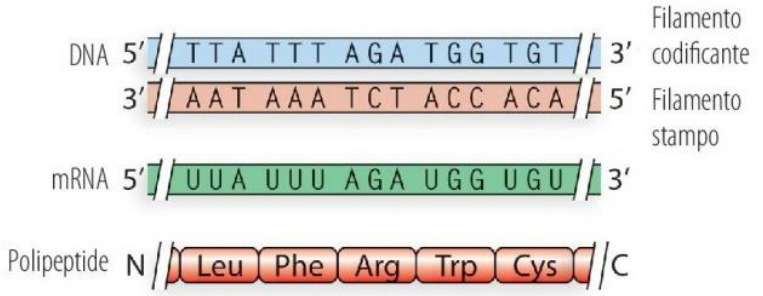
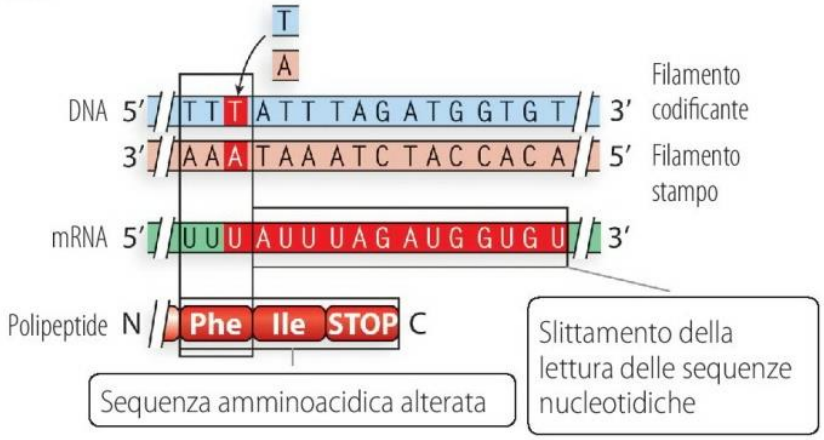


FIGURA 12.1 ► Sostituzioni nucleotidiche possibili. Nel DNA si possono verificare dodici differenti sostituzioni di basi, suddivise in transizioni (quando una purina sostituisce un'altra purina o una pirimidina sostituisce un'altra pirimidina) e transversioni (quando una purina sostituisce una pirimidina, e viceversa).

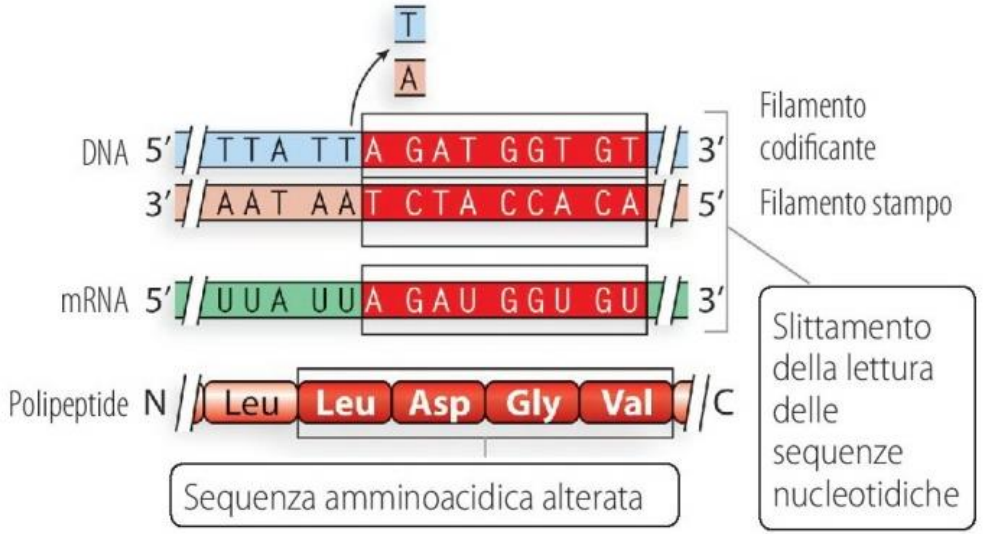
(**a**) Sequenza selvatica



(**b**) Mutazione frameshift: inserzione di una singola coppia di basi



(**c**) Mutazione frameshift: delezione di una singola coppia di basi



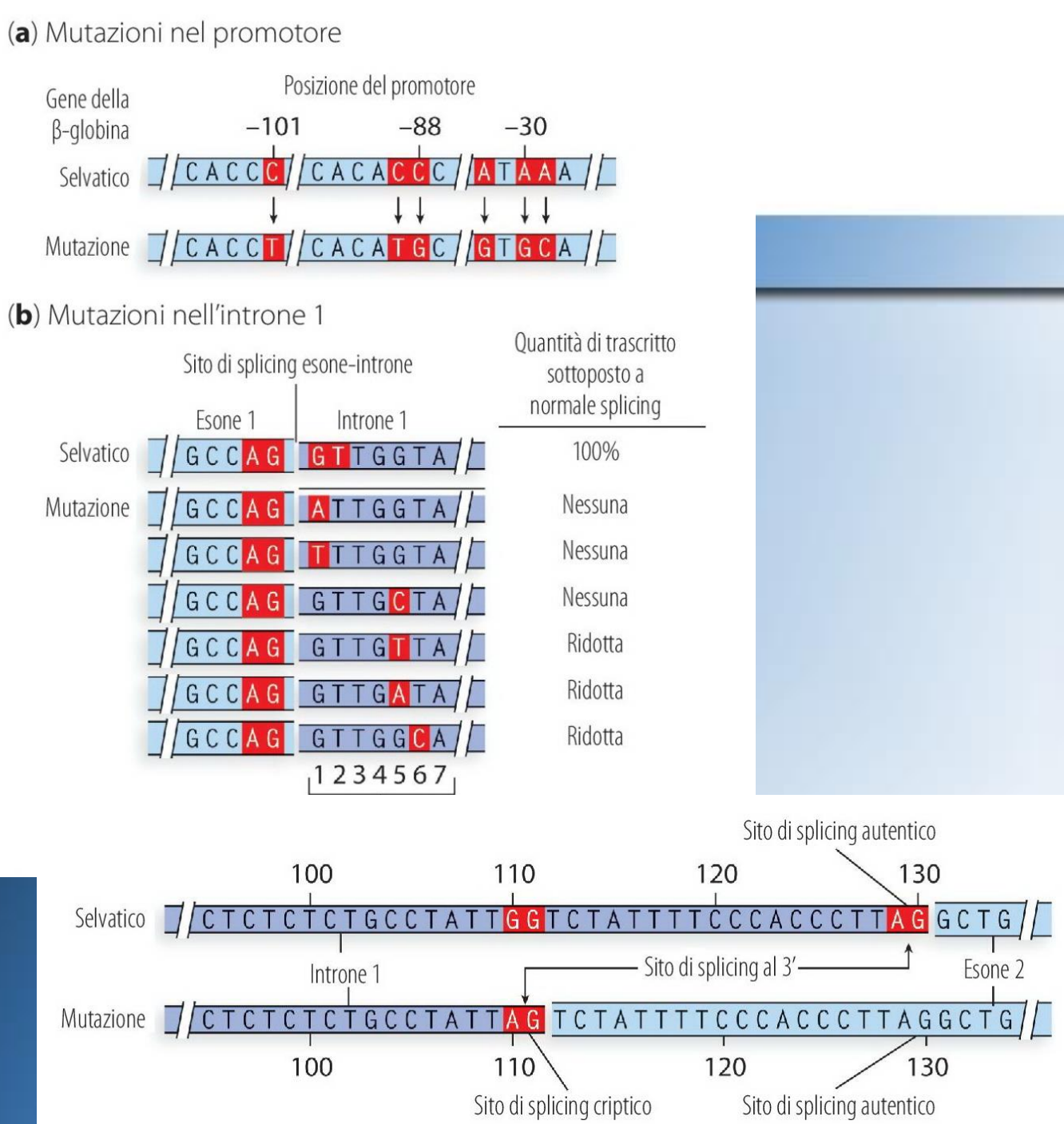


Figura 11.5 Splicing criptico. La sostituzione della coppia di basi G–C in A–T nella posizione 110 dell'introne 1 del gene della β-globina umana crea un sito di splicing criptico al 3′.

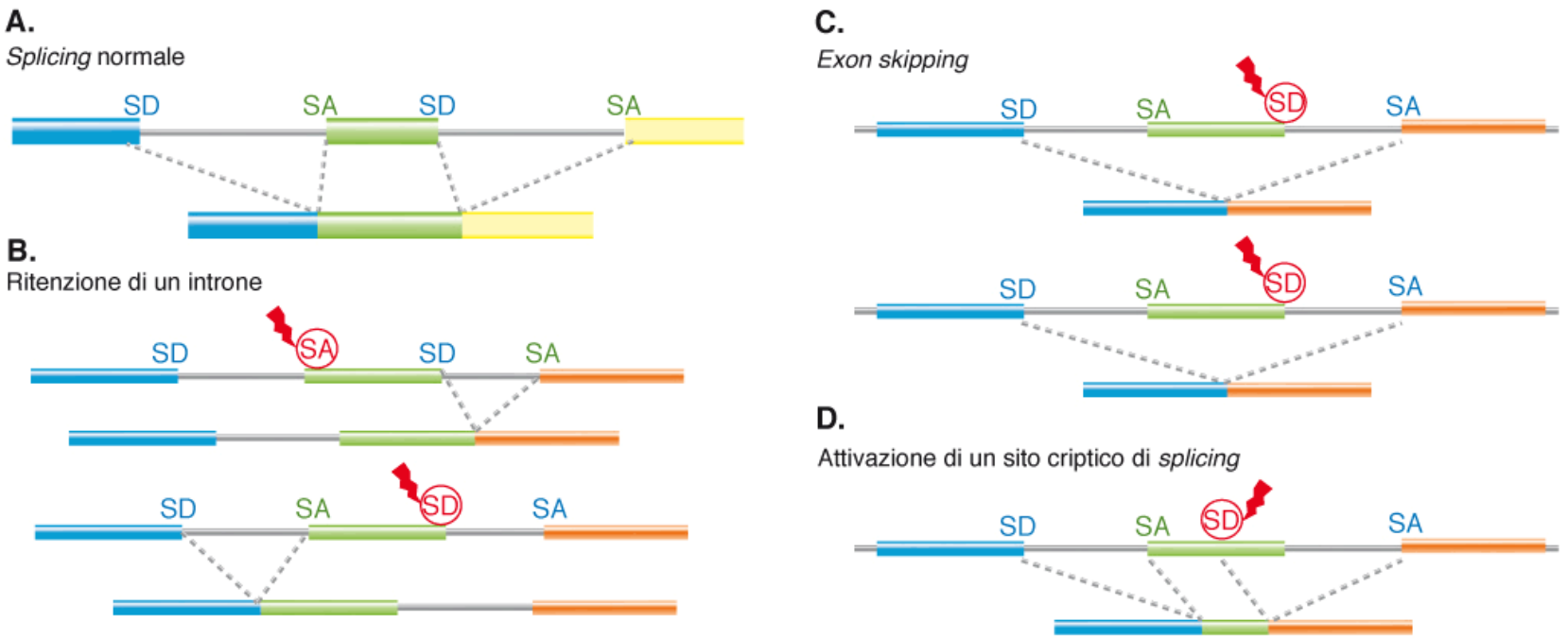


FIGURA 12.10 ► Diverse conseguenze di mutazioni nei siti di *splicing*. Gli esoni sono indicati con box di diversi colori, gli introni con linee. Sono indicati i siti donatori (SD) e quelli accettori (SA) di *splicing*. Le linee tratteggiate indicano la rimozione della regione intronica. Le frecce indicano gli eventi mutazionali. **(A)** *Splicing* normale. **(B)** Ritenzione di un introne. La mutazione di un sito accettore determina la ritenzione dell'introne a monte, quella di un sito donatore la ritenzione dell'introne a valle. **(C)** *Exon skipping* (perdita di un esone). L'esone localizzato a monte di un sito donatore mutato, o a valle di un sito accettore mutato, viene eliminato. **(D)** Attivazione di un sito di *splicing* criptico. La mutazione, creando un sito di *splicing* criptico all'interno di un esone, altera la normale posizione dello *splicing*, causando l'eliminazione di parte di un esone dalla regione codificante.

Mutations Are Classified in Various Ways

- Mutations can be classified according to their phenotypic effects as:

  - loss-of-function,
  - gain-of-function,
  - morphological,
  - nutritional (biochemical),
  - behavioral, or
  - regulatory.

Mutations Are Classified in Various Ways

- **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.

The Spontaneous Mutation Rate Varies Greatly among Organisms

- **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.

Retromutazioni, reversione e soppressione

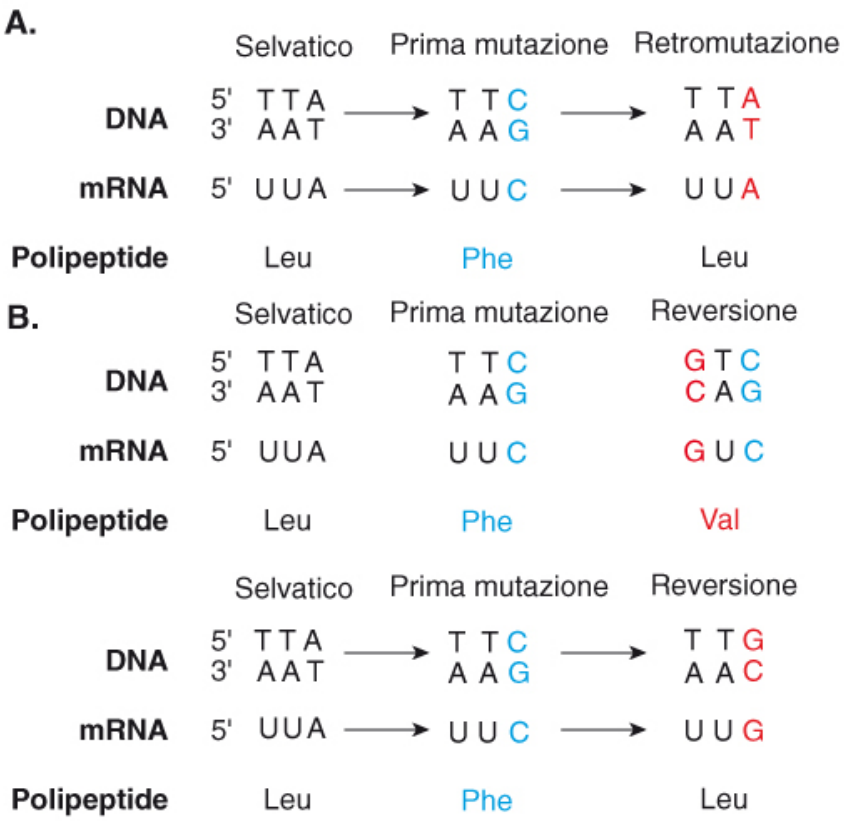
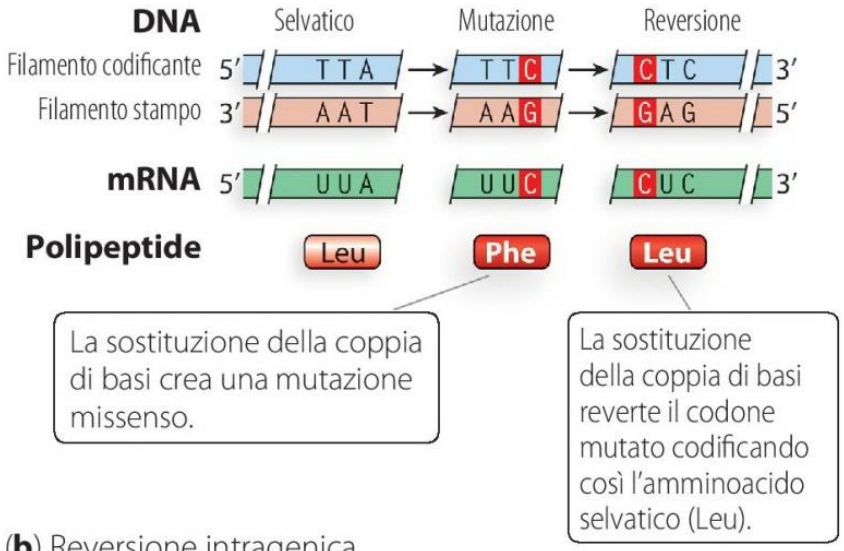
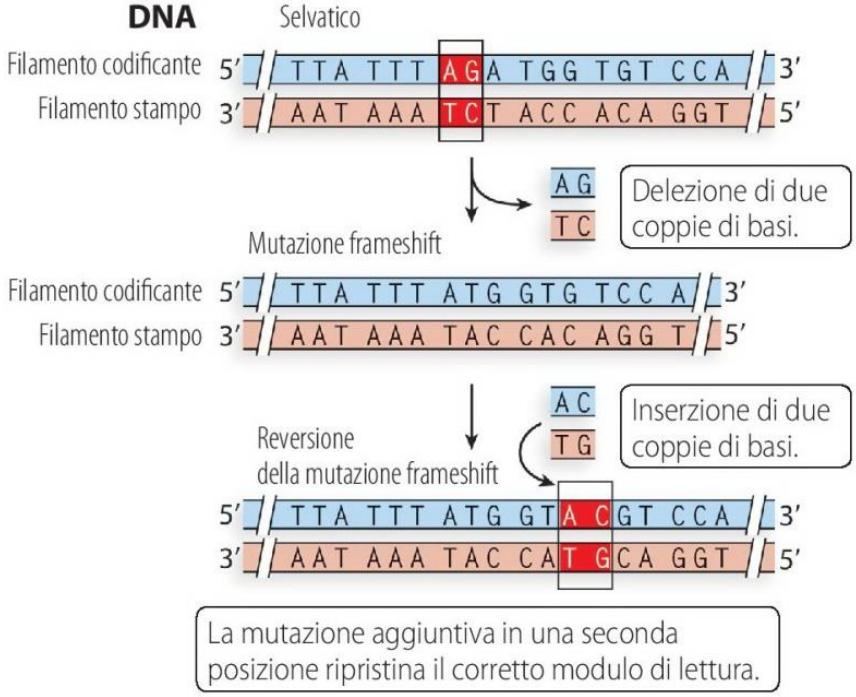


FIGURA 12.11 ► Mutazioni di andata e di ritorno. (A) Retromutazione: una mutazione viene cancellata da un secondo evento (in rosso) che reintroduce la sequenza nucleotidica originale. **(B)** Reversione: una seconda mutazione avviene nello stesso codone. La seconda mutazione può determinare una sostituzione aminoacidica compatibile con la funzionalità della proteina (sopra) oppure può, grazie alla degenerazione del codice, riportare allo stesso aminoacido pur non ripristinando la sequenza nucleotidica originale (sotto).

(**a**) Reversione vera



(**b**) Reversione intragenica



(**c**) Reversione al secondo sito

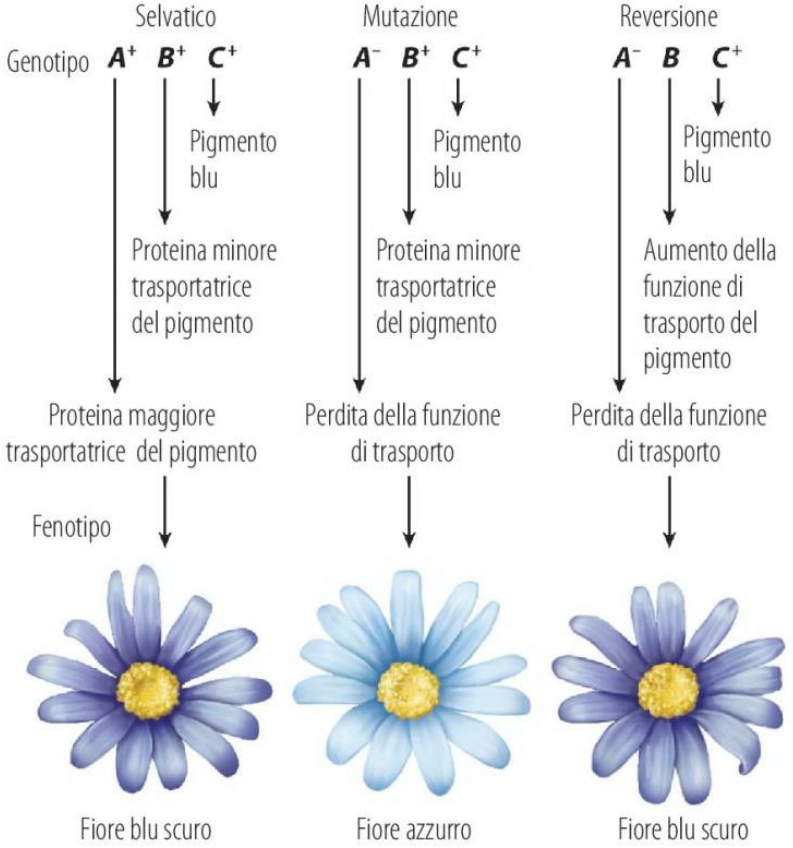


Figura 11.6 Mutazioni per reversione. (**a**) Questa reversione vera ripristina la sequenza amminoacidica selvatica del polipeptide. (**b**) Questa reversione intragenica inverte un mutazione frameshift causata da una delezione di 2 bp in seguito all'inserzione di 2 bp in un sito vicino nel gene. (**c**) La reversione al secondo sito ripristina un fenotipo quasi selvatico mediante una mutazione compensatoria di un secondo gene.

The Spontaneous Mutation Rate Varies Greatly among Organisms

- The rate of spontaneous mutation varies by organism but is exceedingly low.

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

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

The Spontaneous Mutation Rate Varies Greatly among Organisms

- Recent molecular techniques indicate that the rate of deleterious mutation in humans is at least 1.6 per individual per generation.

Spontaneous Mutations Arise from Replication Errors and Base Modifications

- DNA polymerase occasionally inserts incorrect nucleotides, generally due to **mispairing**. These types of errors predominantly lead to point mutations.
- **Slippage** during replication can lead to **small insertions or deletions**. (indels)

Espansioni per Slippage

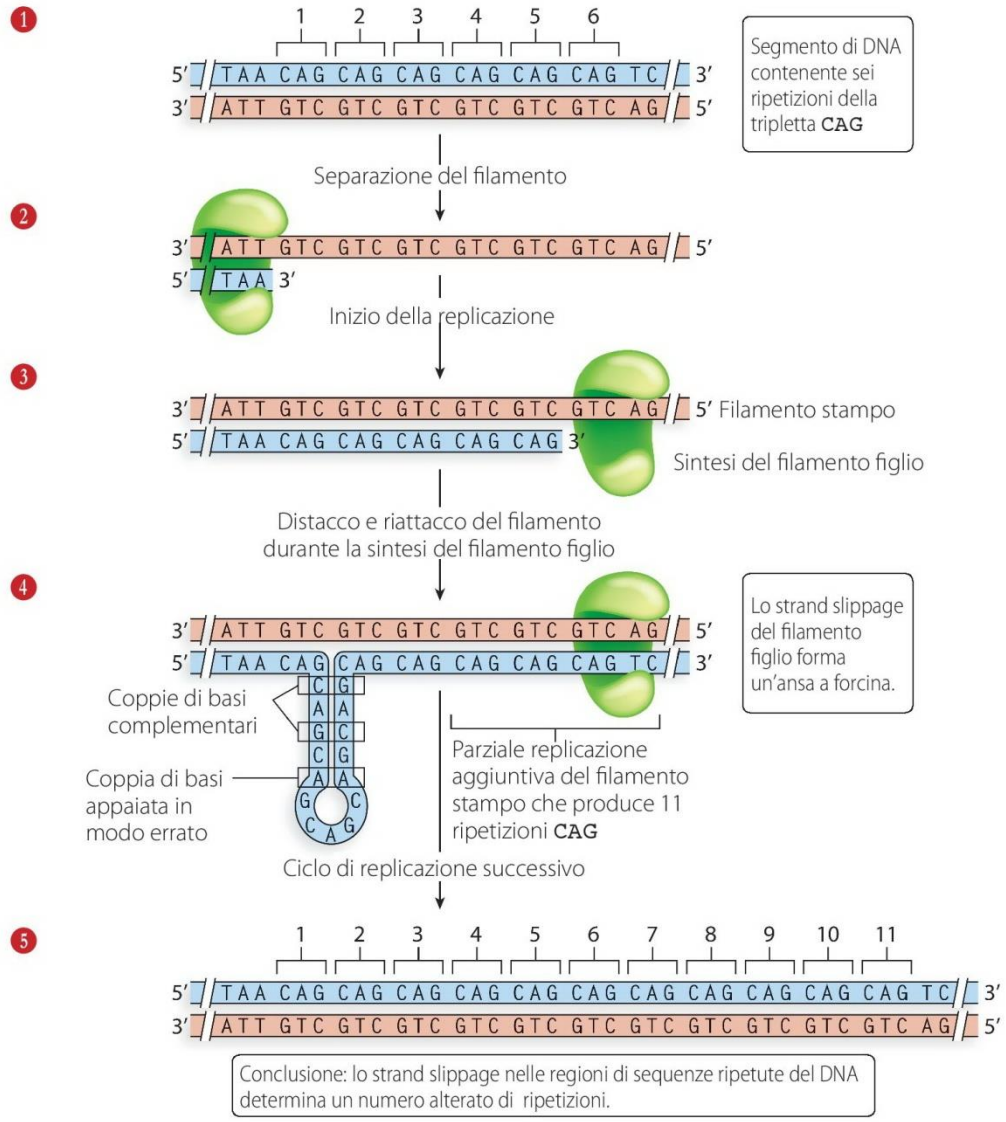
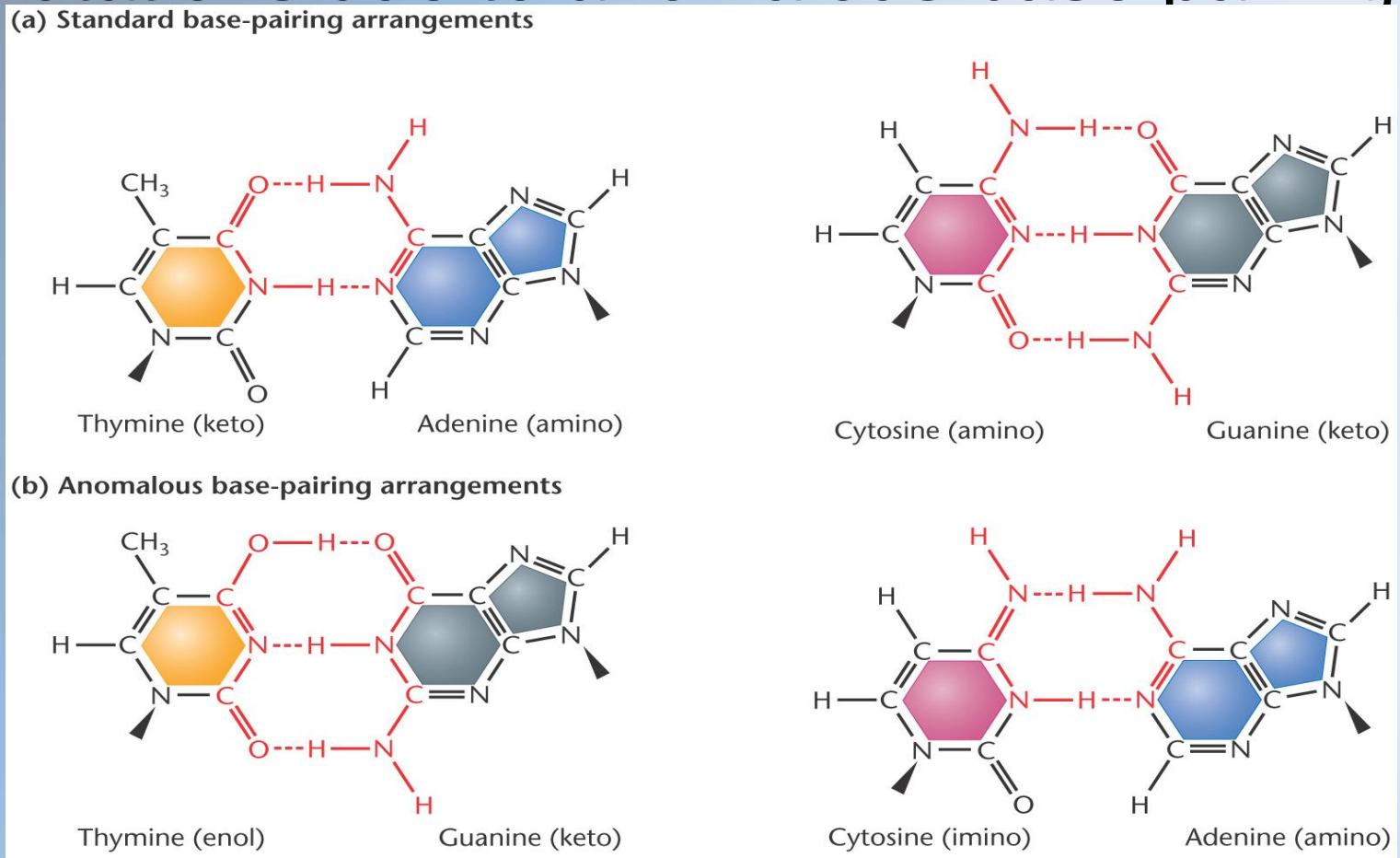
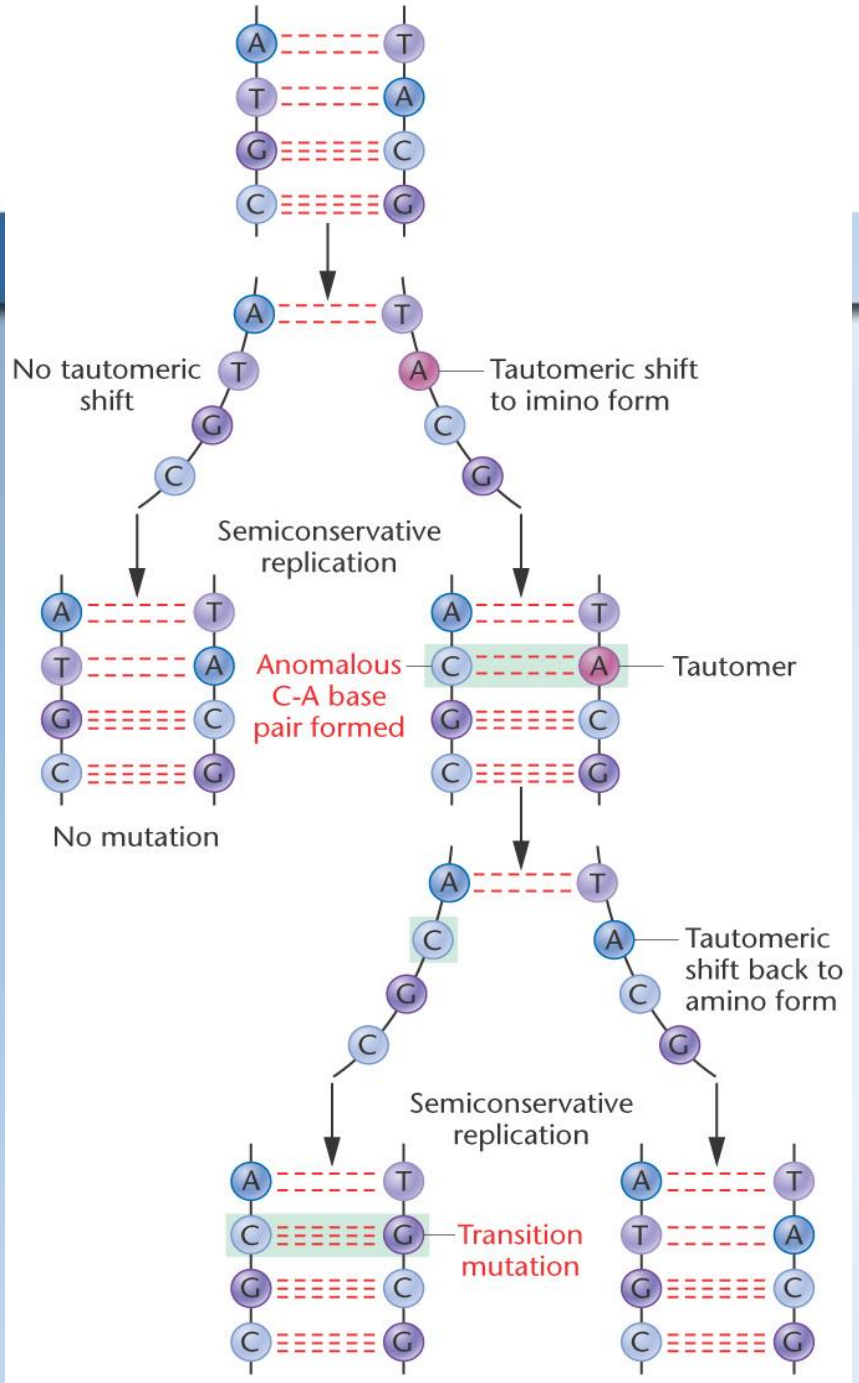


Figura 11.7 Strand *slippage* durante la replicazione del DNA.

Spontaneous Mutations Arise from Replication Errors and Base Modifications

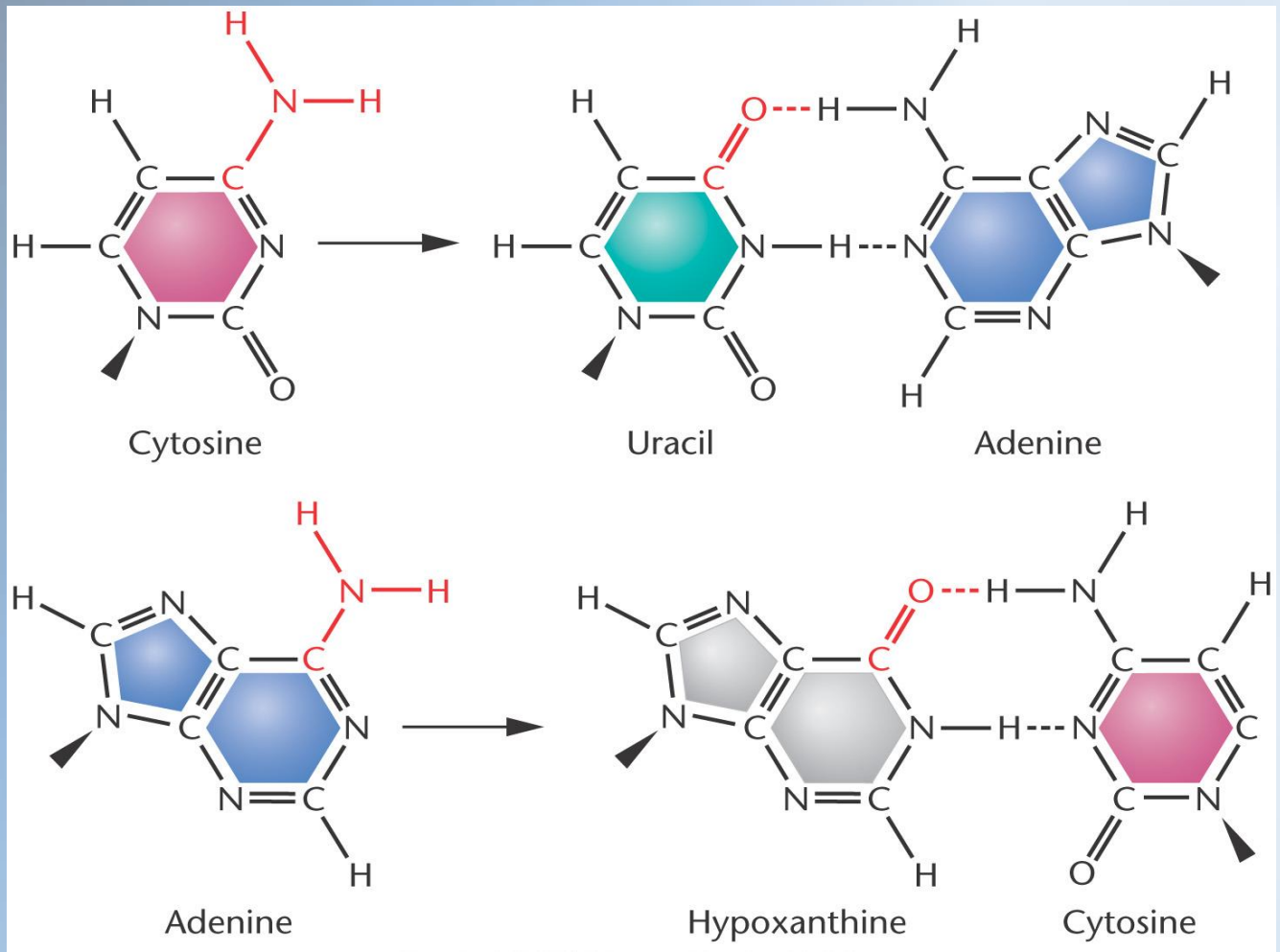
- Tautomeric shifts in nucleotides can result in mutations due to anomalous base pairing.



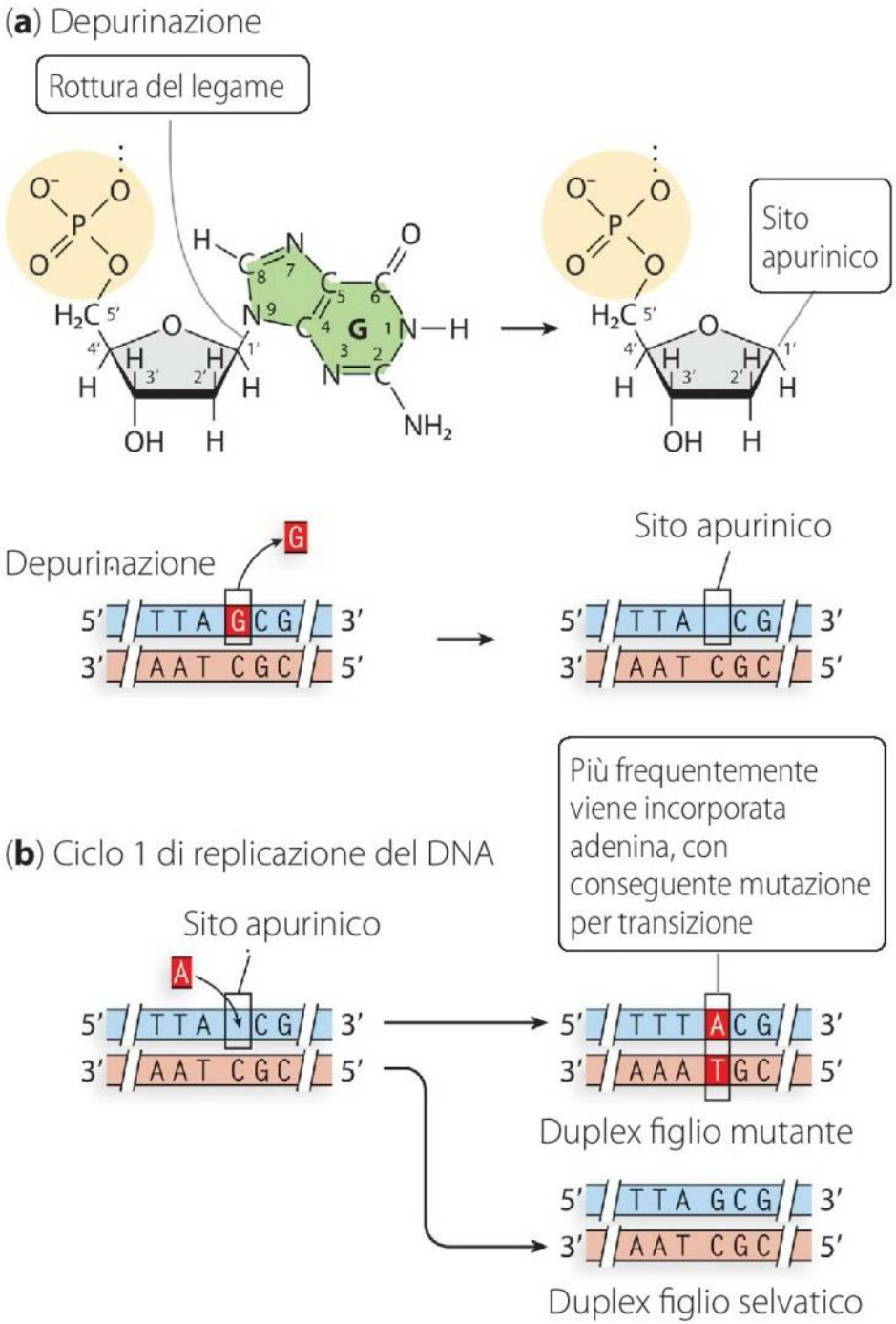

No tautomeric shift
Tautomeric shift to imino form
Semiconservative replication
Anomalous C-A base pair formed
Tautomer
No mutation
Tautomeric shift back to amino form
Semiconservative replication
Transition mutation

Spontaneous Mutations Arise from Replication Errors and Base Modifications

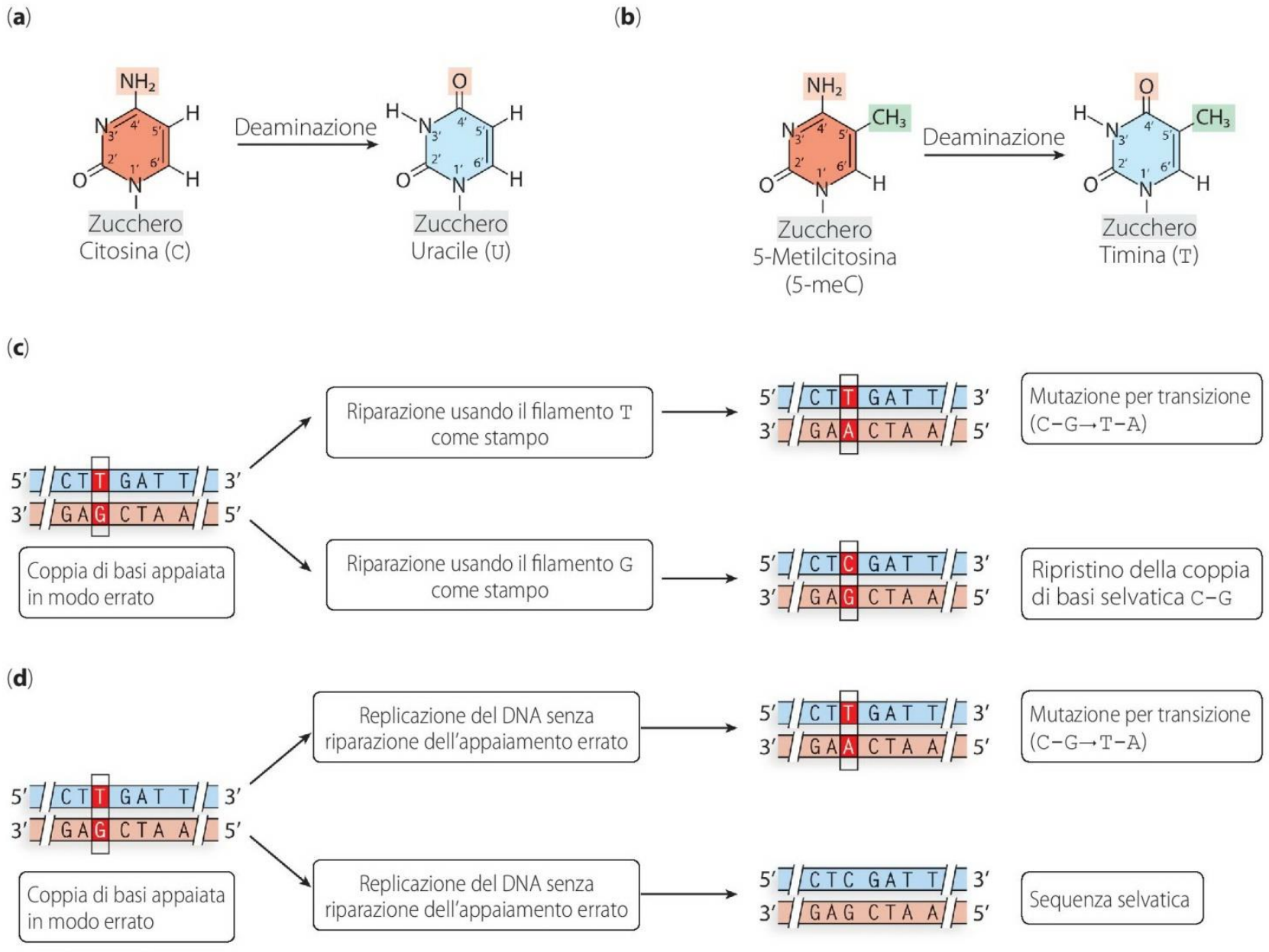
- DNA base damage by **depurination** and **deamination** is the most common cause of spontaneous mutation.



Depurination



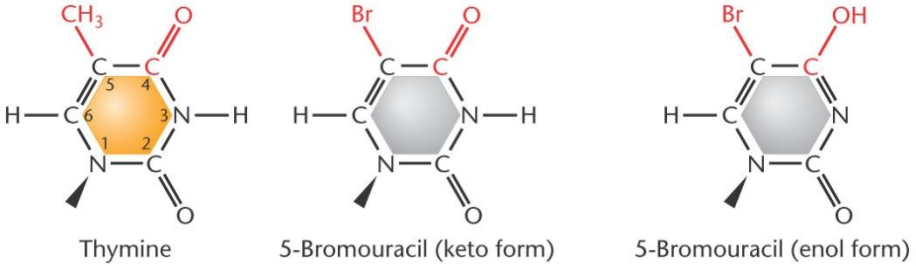
Deamination

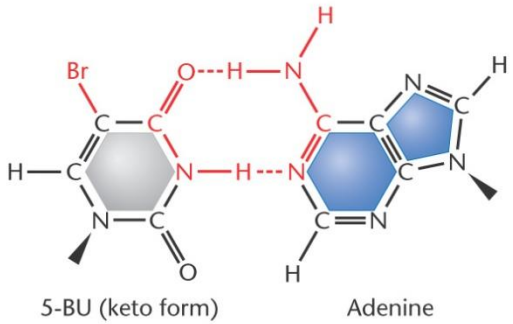


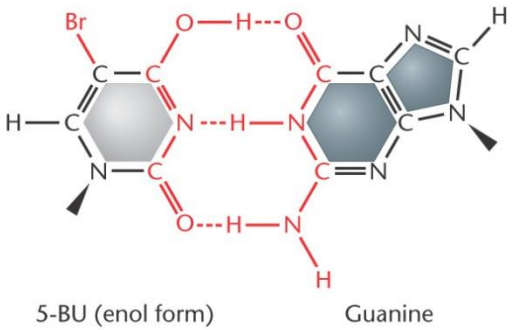
Induced Mutations Arise from DNA Damage Caused by Chemicals and Radiation

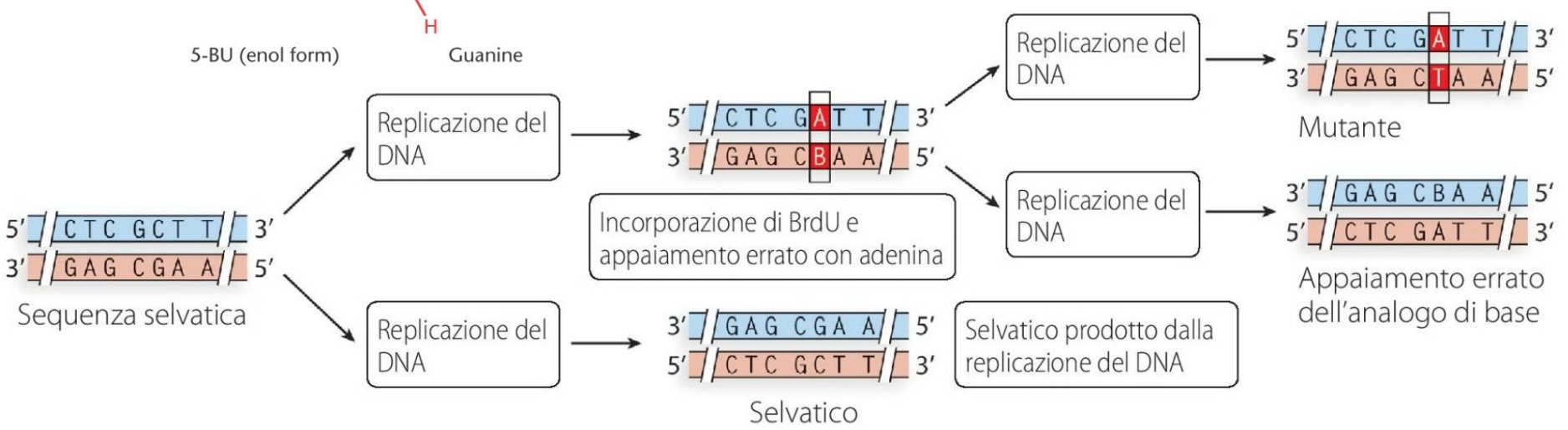
- **Mutagens** are natural or artificial agents that induce mutations.
- **Base analogs** can substitute for purines and pyrimidines during nucleic acid replication.

Base analogs



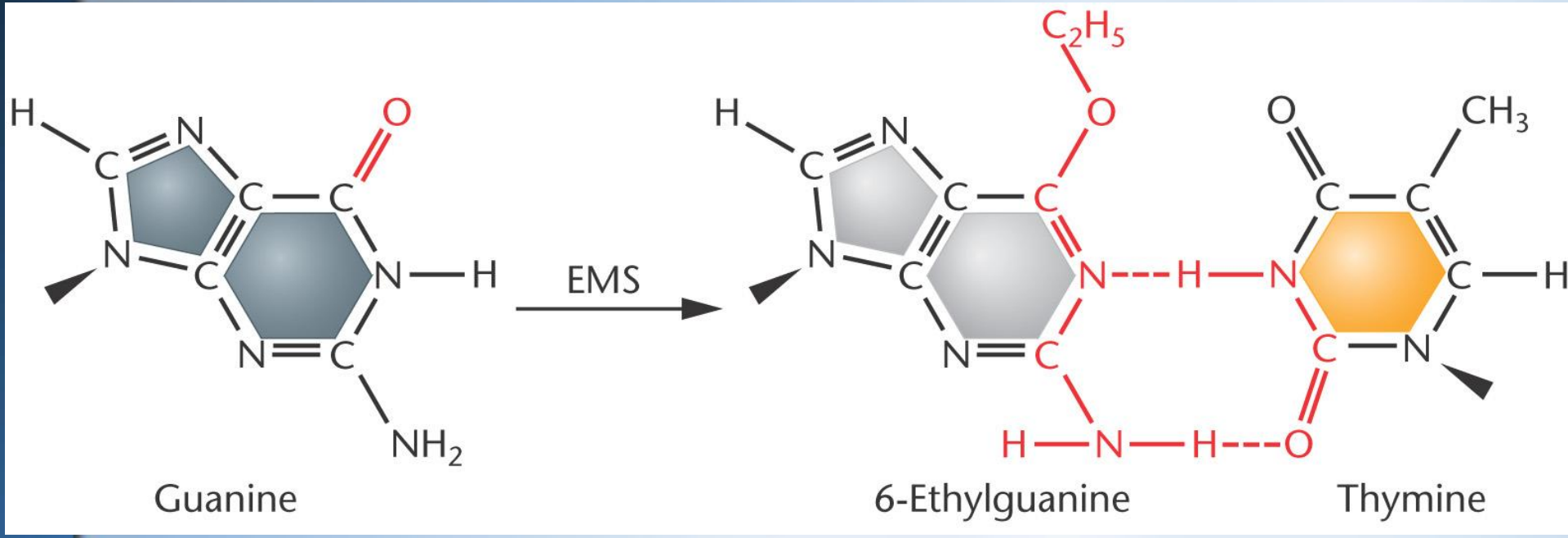






Induced Mutations Arise from DNA Damage Caused by Chemicals and Radiation

- Alkylating agents donate an alkyl group to amino or keto groups in nucleotides to alter base-pairing affinity.



Induced Mutations Arise from DNA Damage Caused by Chemicals and Radiation

TABLE 15.3 ALKYLATING AGENTS

Common Name or Symbol	Chemical Name	Chemical Structure
Mustard gas (sulfur)	Di-(2-chloroethyl) sulfide	$Cl-CH_2-CH_2-S-CH_2-CH_2-Cl$
EMS	Ethylmethane sulfonate	$CH_3-CH_2-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-CH_3$
EES	Ethylethane sulfonate	$CH_3-CH_2-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-CH_2-CH_3$

Chemical mutagens

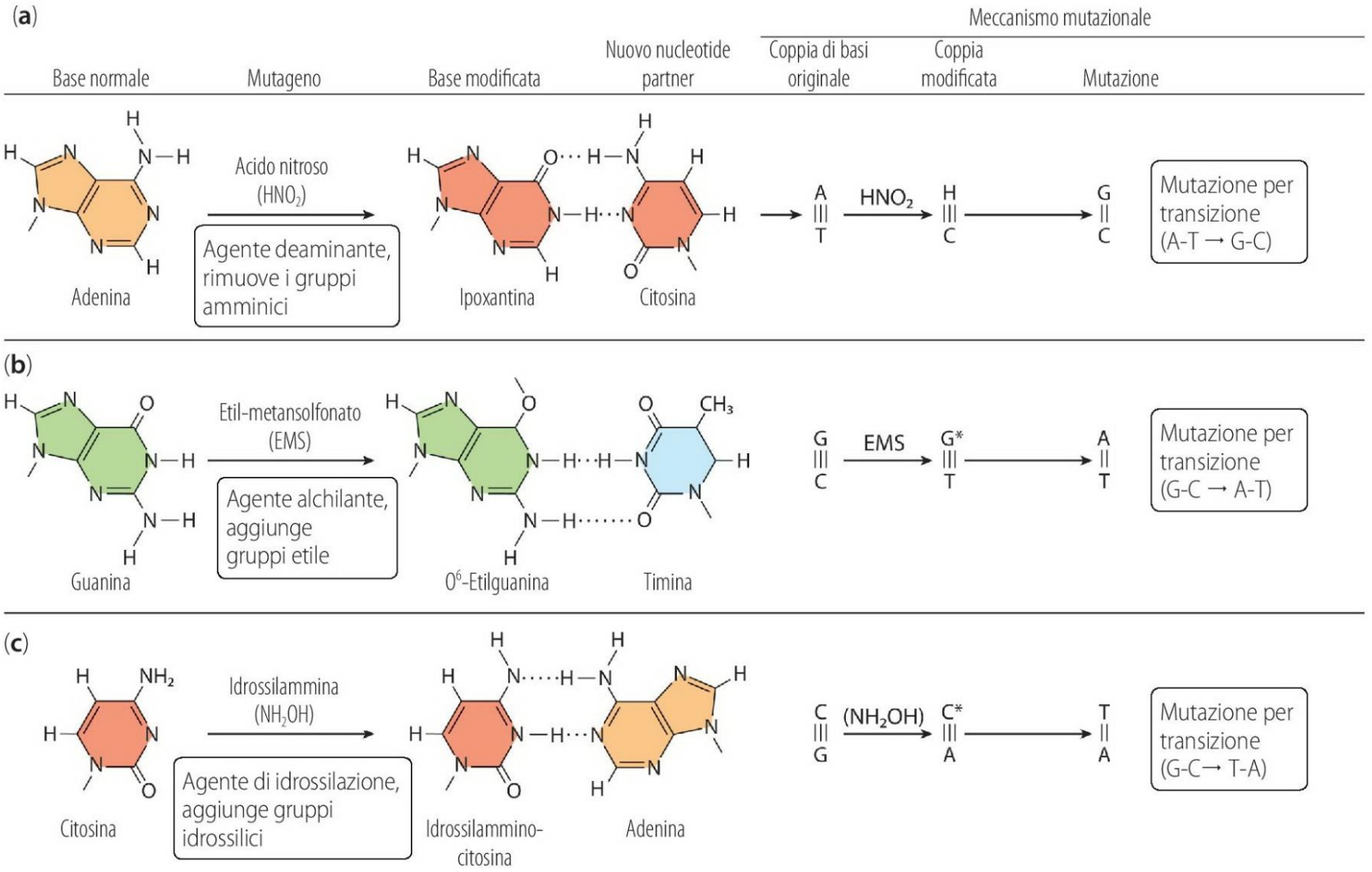
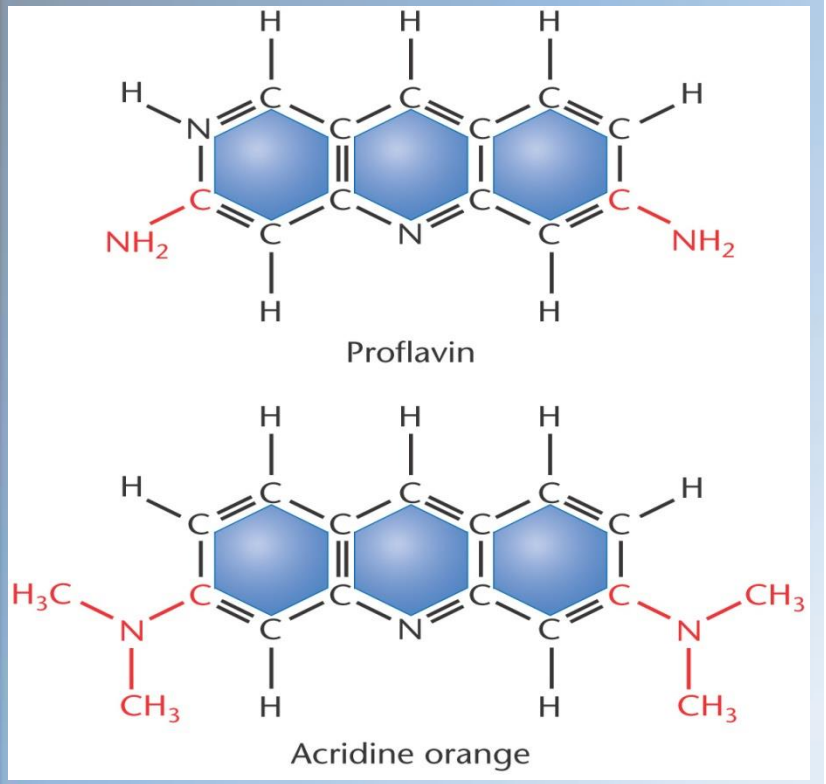
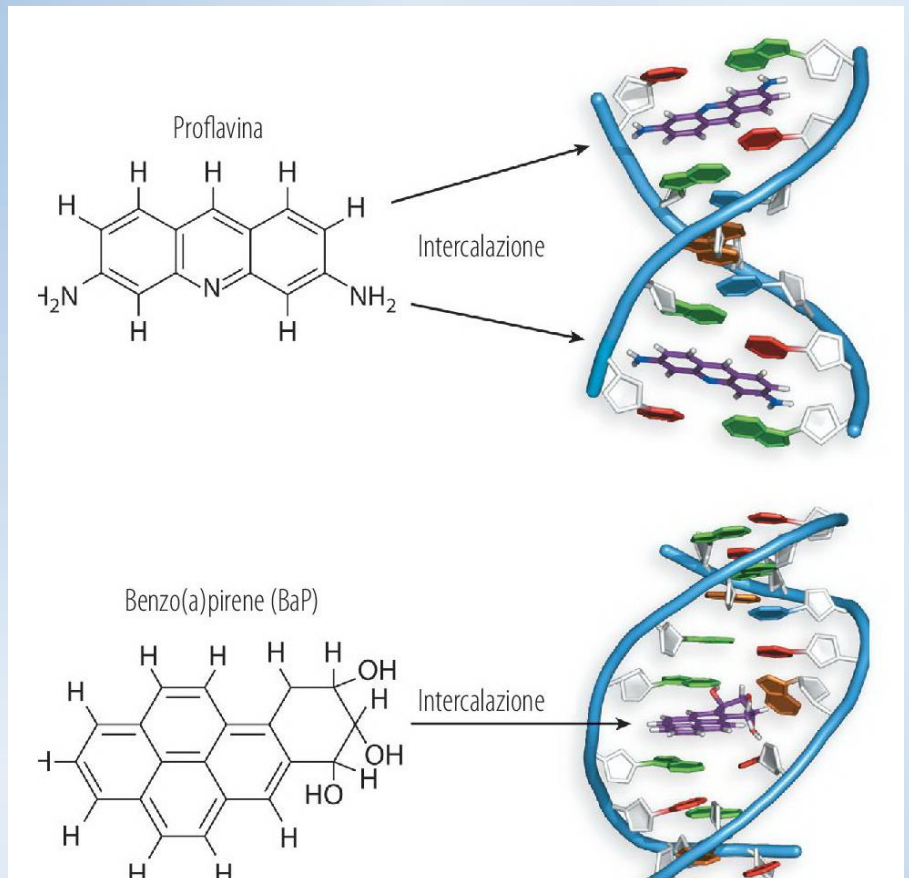


Figura 11.13 Esempi dell'azione esercitata dai mutageni chimici. In (**a**), H è ipoxantina. In (**b**) e (**c**), gli asterischi (*) indicano i nucleotidi modificati.

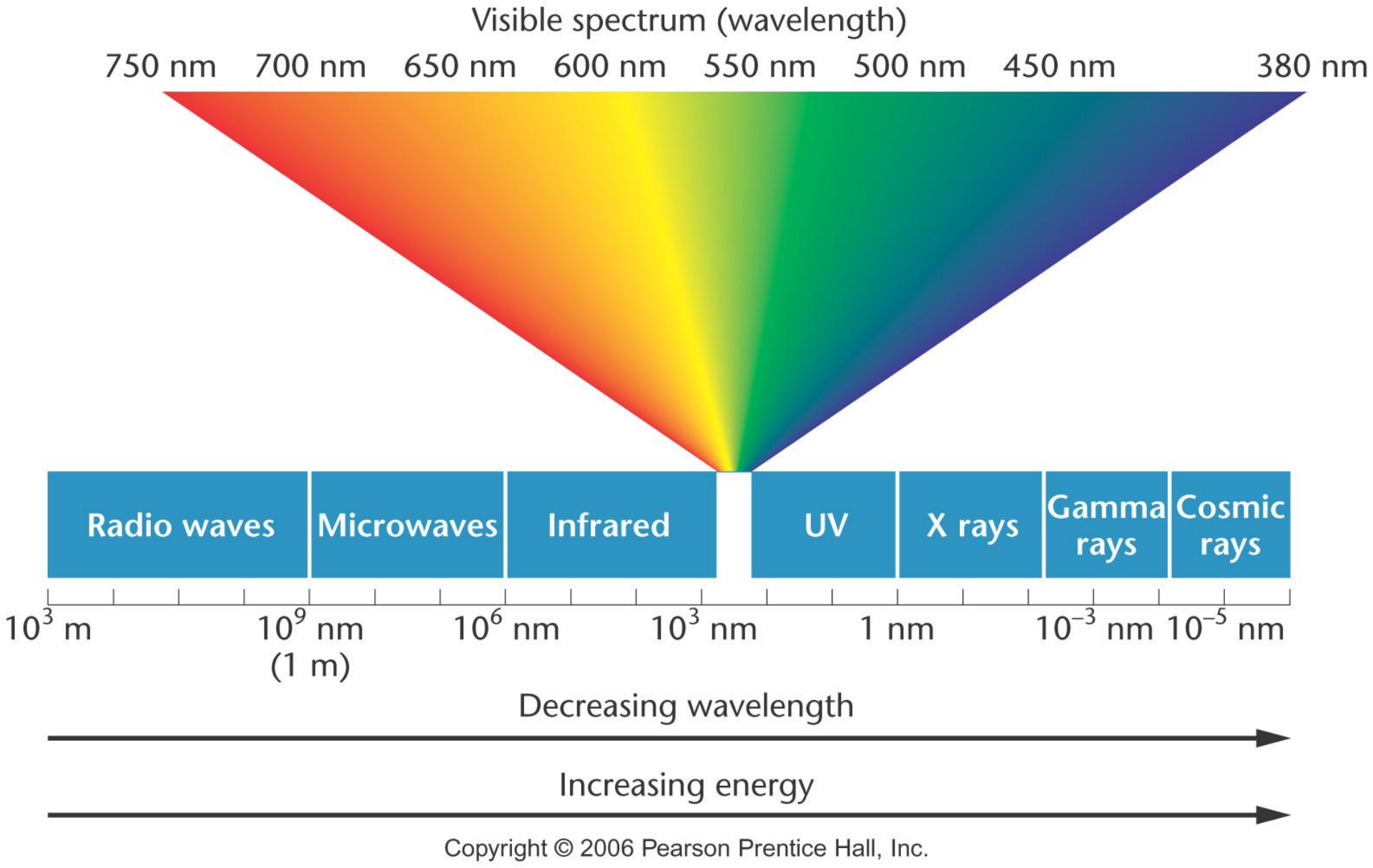
Induced Mutations Arise from DNA Damage Caused by Chemicals and Radiation

- Acridine dyes cause **frameshift mutations** by intercalating between purines and pyrimidines.



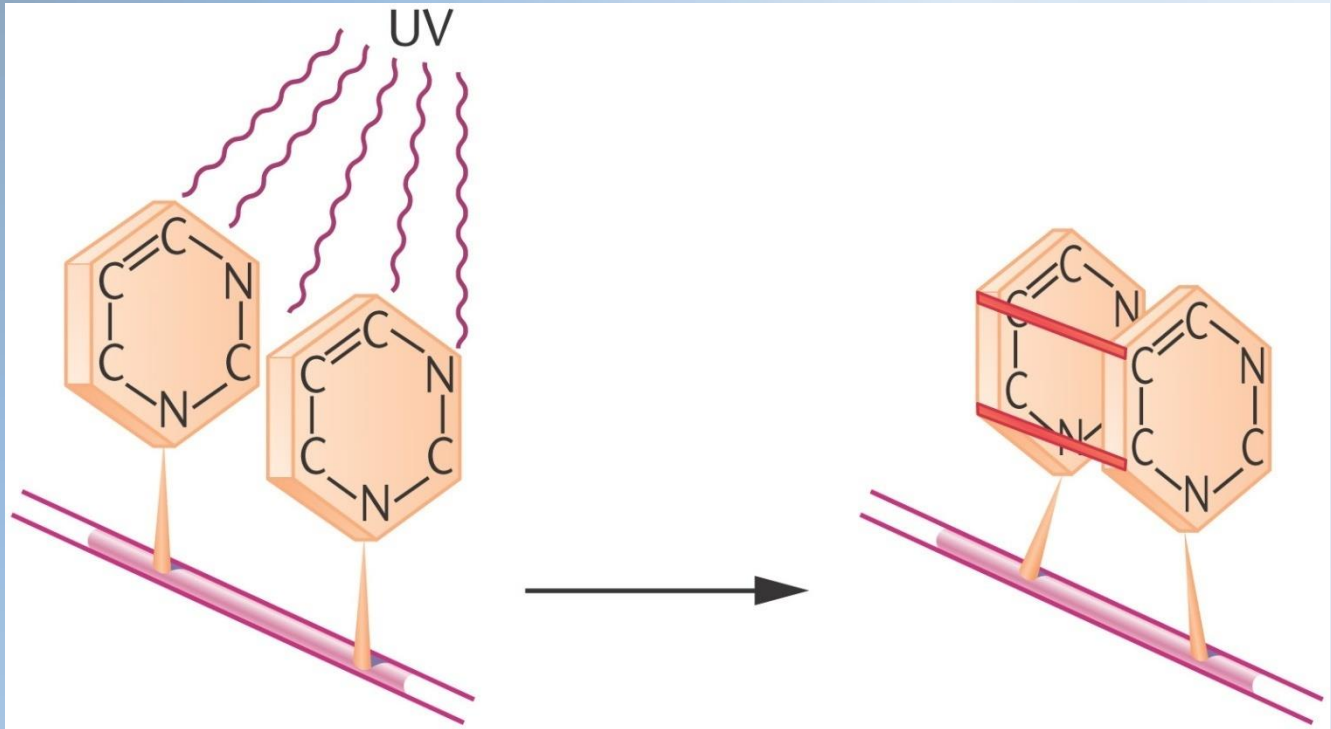


Induced Mutations Arise from DNA Damage Caused by Chemicals and Radiation



Induced Mutations Arise from DNA Damage Caused by Chemicals and Radiation

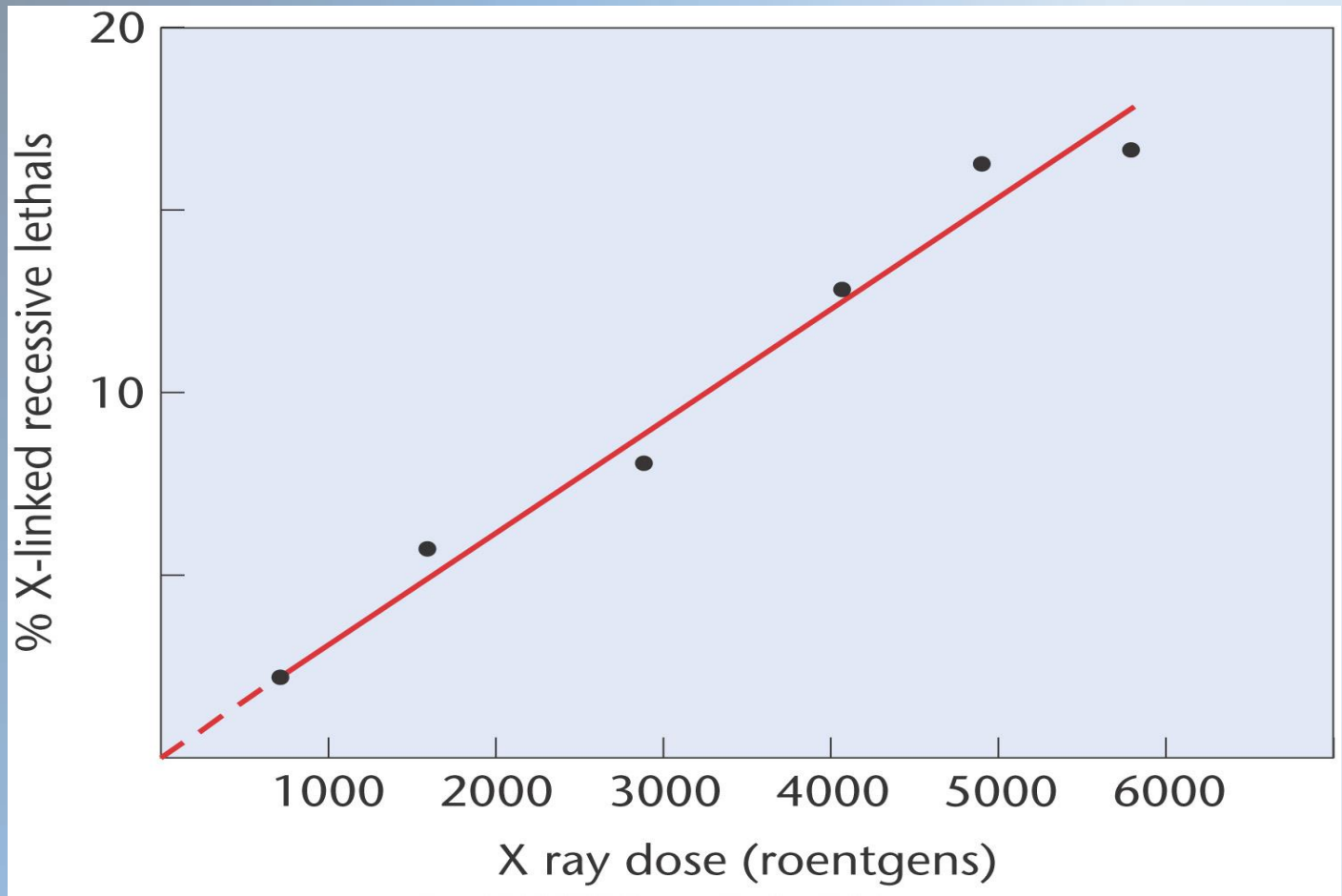
- UV radiation creates **pyrimidine dimers** that distort the DNA conformation in such a way that errors tend to be introduced during DNA replication.



Dimer formed between adjacent thymidine residues along a DNA strand

Induced Mutations Arise from DNA Damage Caused by Chemicals and Radiation

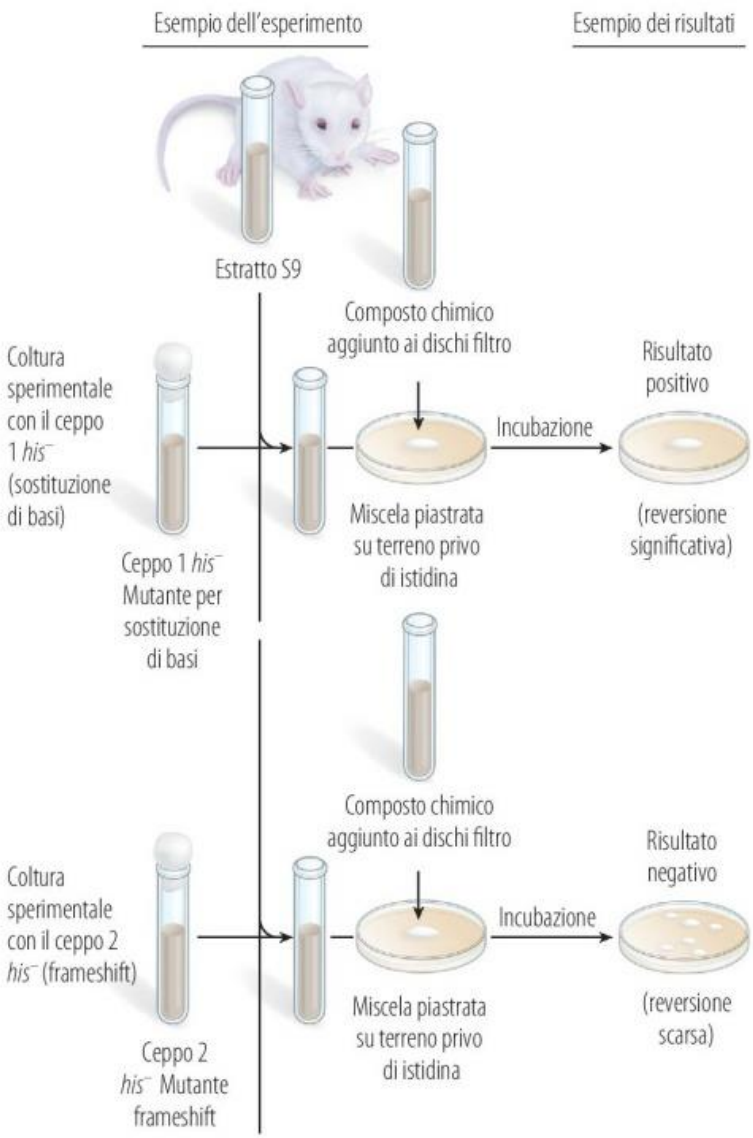
- Ionizing radiation in the form of X fays, gamma rays, and cosmic rays are mutagenic.



The Ames Test Is Used to Assess the Mutagenicity of Compounds

- The **Ames test** uses four strains of *Salmonella typhimurium* selected for their sensitivity to specific types of mutagenesis to screen compounds for potential mutagenicity.
- Strains *his*$^{-}$
- Many carcinogens have been shown by the Ames test to be strong mutagens.

Test di ames



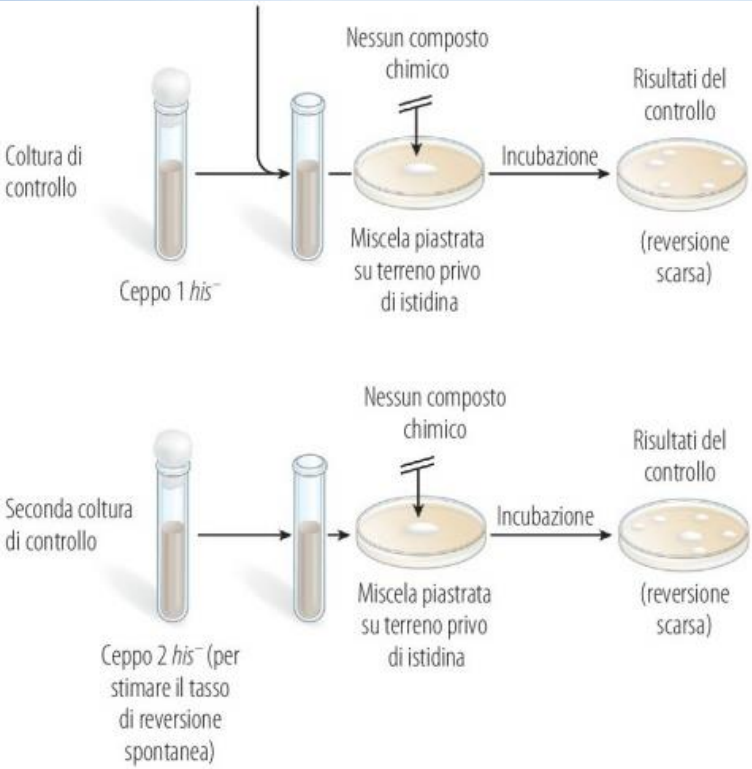
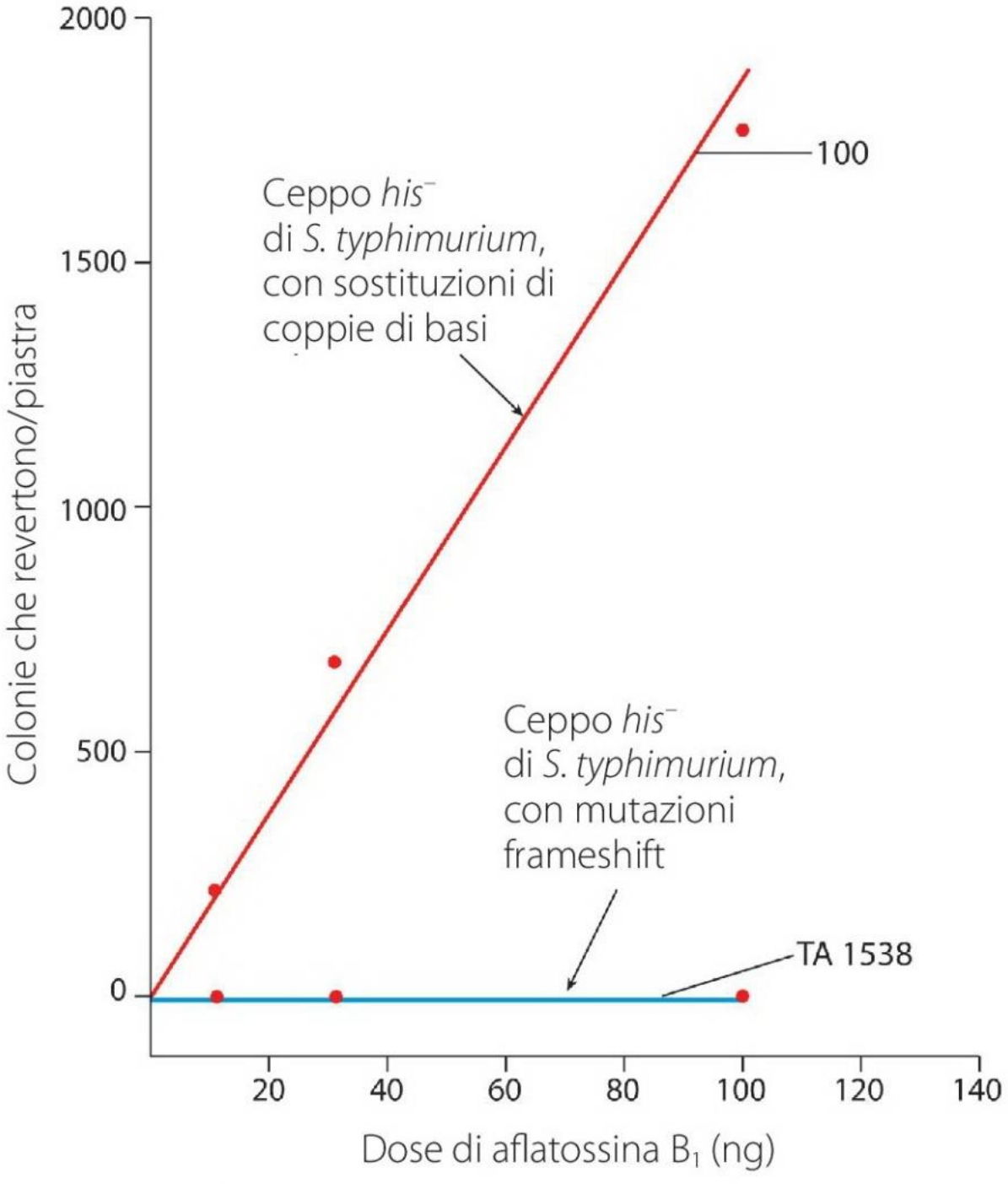
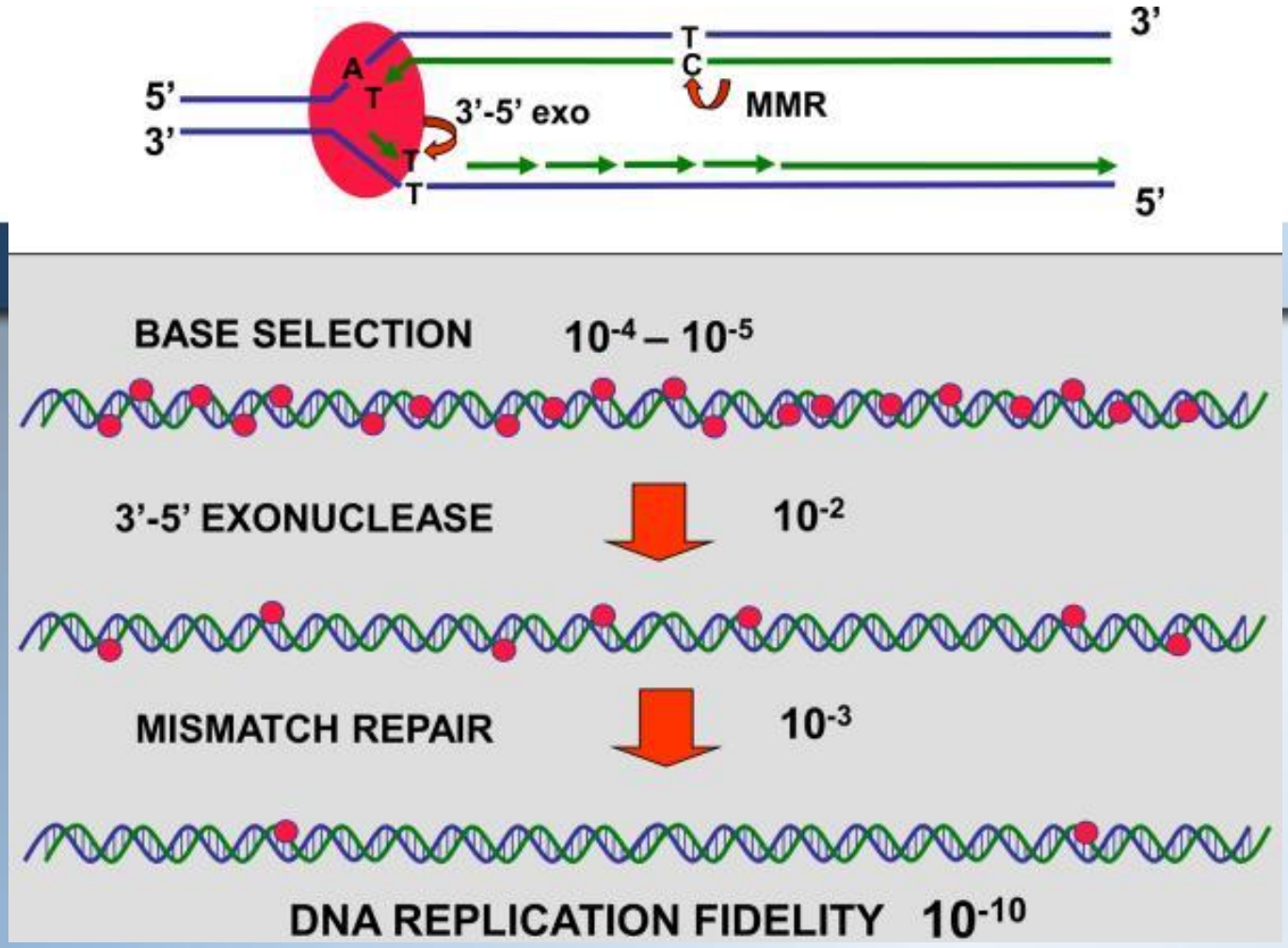


Figura 11.16 Test di Ames per la valutazione della potenziale mutagenicità dei composti chimici.


2000
1500
1000
500
0
Colonie che revertono/piastra
Ceppo *his*⁻
di *S. typhimurium*,
con sostituzioni di
coppie di basi
100
Ceppo *his*⁻
di *S. typhimurium*,
con mutazioni
frameshift
TA 1538
20
40
60
80
100
120
140
Dose di aflatossina B_1 (ng)

Organisms Use DNA Repair Systems to Counteract Mutations

- Bacterial **DNA polymerase III** is able to recognize and correct errors in replication, a process called **proofreading**.



General scheme indicating how three serial fidelity steps during chromosomal replication can produce the low error rate of ~10^{-10} errors per base per round of replication. The steps are: (a) discrimination by the polymerase against inserting an incorrect base, (error rate ~10^{-5}); (b) proofreading (editing) of misinserted bases (T·T, example) by the 3'→5' exonuclease associated with the polymerase (escape rate ~10^{-2}); and (c) removal of remaining mismatches (G·G, example) by postreplicative DNA Mismatch Repair (MMR) (escape rate ~10^{-3}).

Tabella 13.1 Alcune lesioni al DNA generate da agenti endogeni e loro frequenza

Danno endogeno al DNA	*Lesione generata*	*Numero di lesioni/cellula/giorno*
Depurinazione	Sito AP	18.000
Deaminazione della citosina	Transizione di base	100-500
Metilazione dovuta alla *S*-adenosilmetionina	3meA 7meG O^6meG	600 4000 10-30
Ossidazione	8oxoG	400-1500

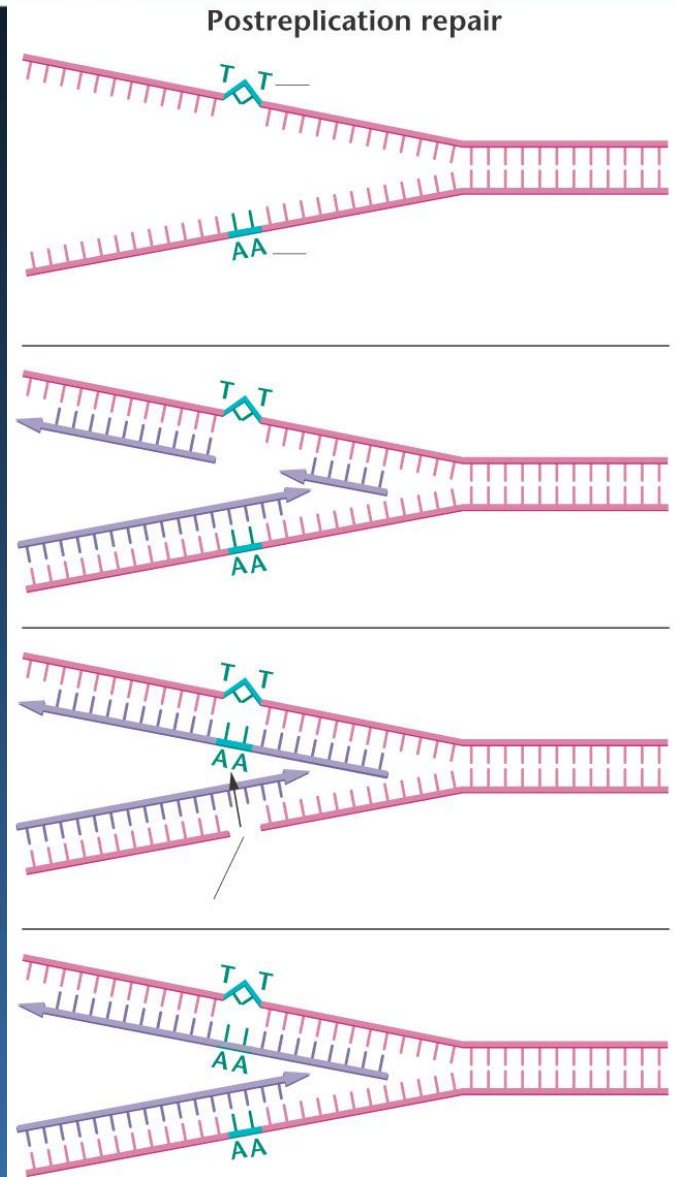
Abbreviazioni: AP, apurinico; 3meA, 3-metiladenosina; 7meG, 7-metilguanina; O^6meG, O^6-metilguanina; 8oxoG, 7,8-diidro-8-oxoguanina.
Da "Mutagenesi e Riparazione del DNA", Friedberg, E. C., Walker, G. C., Siede, W., Wood, R. D., Schultz, R. A., & Ellenberger, T. *DNA repair and mutagenesis* (ASM Press).

Organisms Use DNA Repair Systems to Counteract Mutations

- **Mismatch repair** corrects errors that remain after proofreading. The correct DNA strand is *recognized* based on DNA methylation of the parental strand.

- **Postreplication repair** occurs when DNA replication skips over a lesion and requires homologous recombination mediated by the **RecA protein.**

Post replication repair



. Through the process of recombination, the correct complementary sequence is recruited from the parental strand and inserted into the gap opposite the lesion. The new gap is filled by DNA polymerase and DNA ligase.

Organisms Use DNA Repair Systems to Counteract Mutations

- The **SOS repair system** allows DNA synthesis to become error-prone. Although SOS repair is itself mutagenic, it may allow the cell to survive DNA damage that might otherwise kill it.

Organisms Use DNA Repair Systems to Counteract Mutations

- **Photoreactivation** repair removes thymine dimers caused by UV light. The process depends on the activity of a protein called the photoreactivation enzyme (PRE).
- DNA photolyase

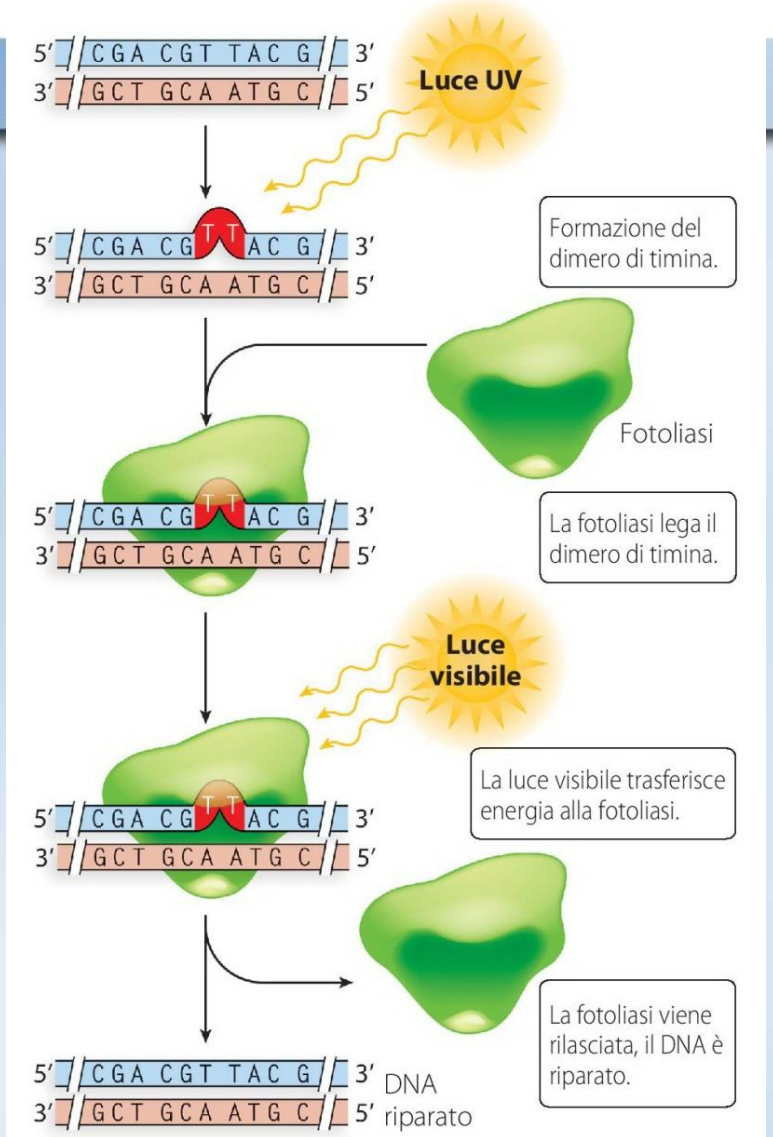


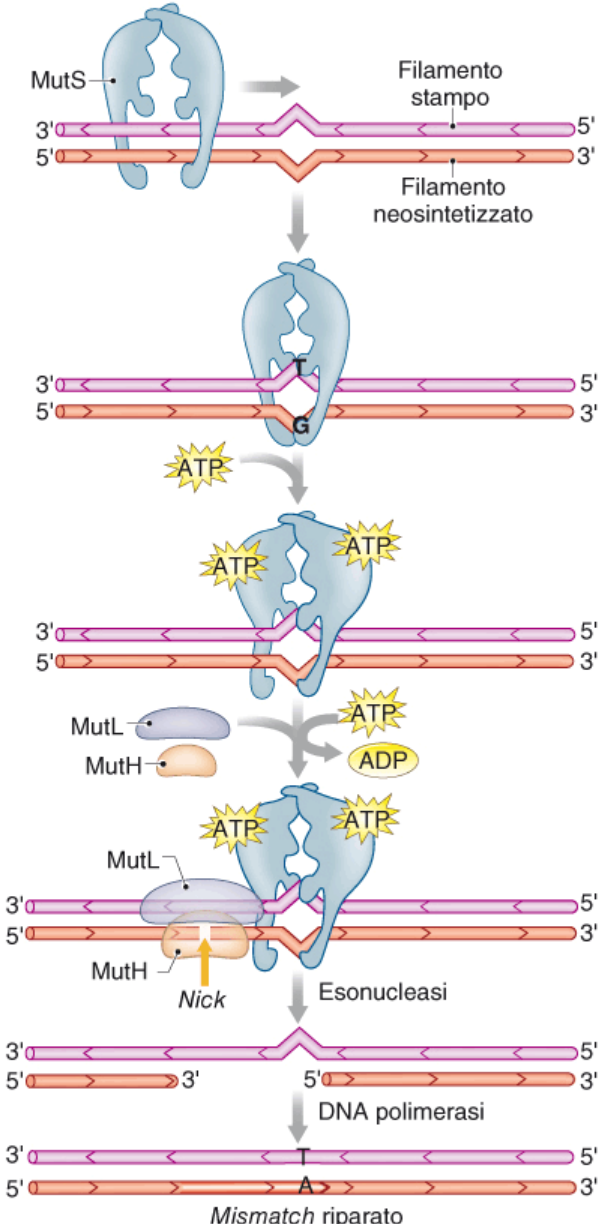
Figura 11.18 **Riparazione fotoreattiva.**

Organisms Use DNA Repair Systems to Counteract Mutations

- **Excision repair** involves three steps:

  - removal of the mutation by a nuclease,
  - gap filling by DNA polymerase, and
  - sealing of the nick by DNA ligase.

Mismatch repair (MMR)



G. Binelli, D. Ghisotti
Genetica
EdiSES

FIGURA 13.5 ► MMR in *Escherichia coli*. L'omodimero MutS scorre lungo il DNA, piegandolo leggermente. Questa sua attività gli permette di riconoscere un *mismatch*, in quanto in quel punto il DNA si piega maggiormente a causa dello scorretto appaiamento tra le basi. MutS recluta quindi MutL e MutH. Il ruolo esatto dell'attività ATPasica di MutS nel MMR non è chiaro. MutH è un'endonucleasi che produce un taglio in prossimità del *mismatch*. Quindi un'esonucleasi digerisce il filamento interrotto, raggiungendo e superando la regione male appaiata. Infine, la DNA polimerasi riempie la zona a singolo filamento.

BOX 13.1 MMR negli eucarioti

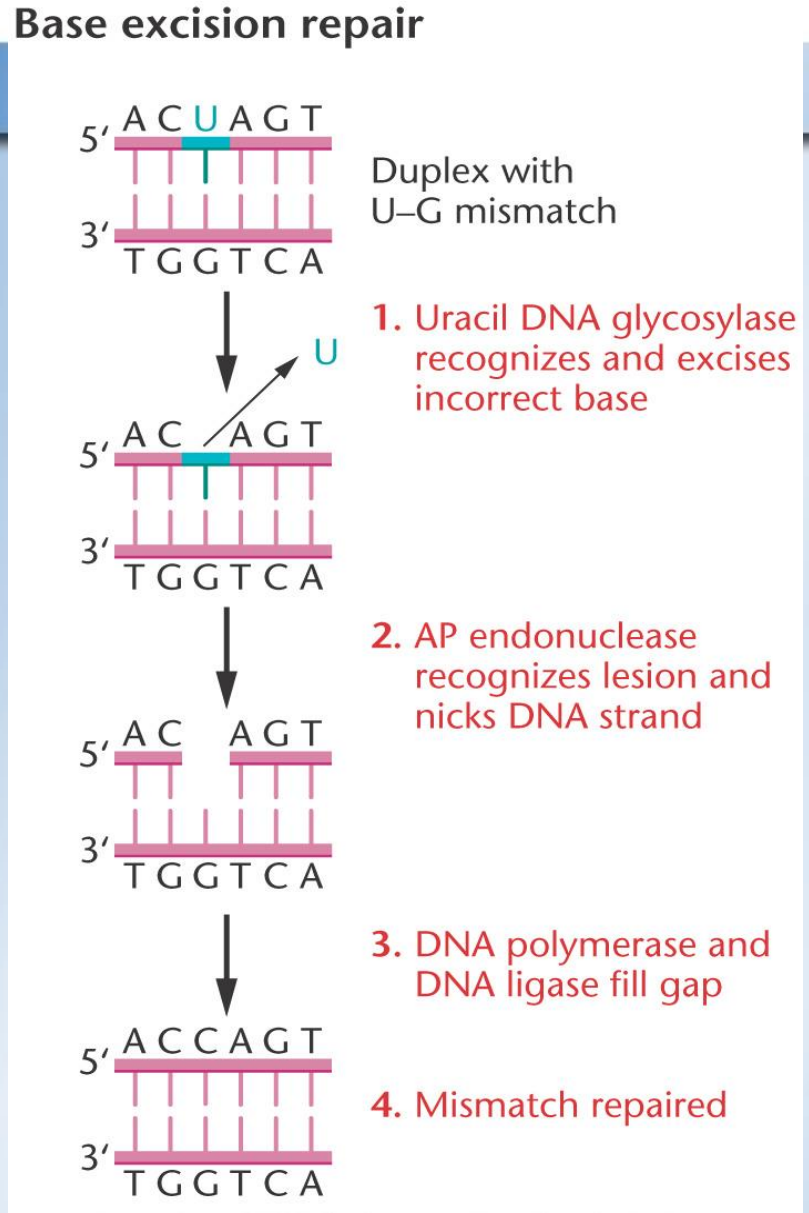
Il MMR è uno dei *pathway* di riparazione dei danni al DNA più conservato dall'evoluzione. Mentre in *E. coli MutS* è un omodimero, negli eucarioti cinque diversi geni MSH codificano proteine omologhe a MutS che formano eterodimeri: MutSα, formata da MSH2 e MSH6, riconosce i *mismatch* e inserzioni o delezioni di una singola base rispetto al DNA stampo; MutSβ, formata da MSH2 e MSH3, riconosce inserzioni o delezioni di 2 o 4 basi rispetto allo stampo; MSH4 e MSH5 svolgono il loro ruolo durante la ricombinazione meiotica. Analogamente, anche l'omodimero MutL è un eterodimero negli eucarioti, che può essere costituito da quattro diverse proteine.

Negli eucarioti è differente anche la discriminazione tra filamento parentale e filamento di neosintesi. La metilazione, in questo caso, non sembra avere un ruolo importante. Dati recenti suggeriscono che probabili interazioni proteina-proteina tra fattori di replicazione e proteine del MMR possano svolgere un ruolo chiave nella scelta del filamento che deve essere riparato. Inoltre, il meccanismo di discriminazione sembra essere diverso per il filamento *leading* e per quello *lagging*.

È importante sottolineare come difetti nel MMR portino a un accumulo di mutazioni in tutti gli organismi. Nell'uomo è stato scoperto che nel 60% dei pazienti che soffrono di tumore ereditario al colon (*Hereditary Non-Polyposis Colon Cancer*) sono presenti mutazioni in geni che codificano proteine coinvolte nel MMR. Il ruolo del MMR nel processo di tumorigenesi è stato anche evidenziato da studi sui topi: l'inattivazione di geni del MMR è compatibile con la vita, ma i topolini mutati sono soggetti all'insorgenza di tumori.

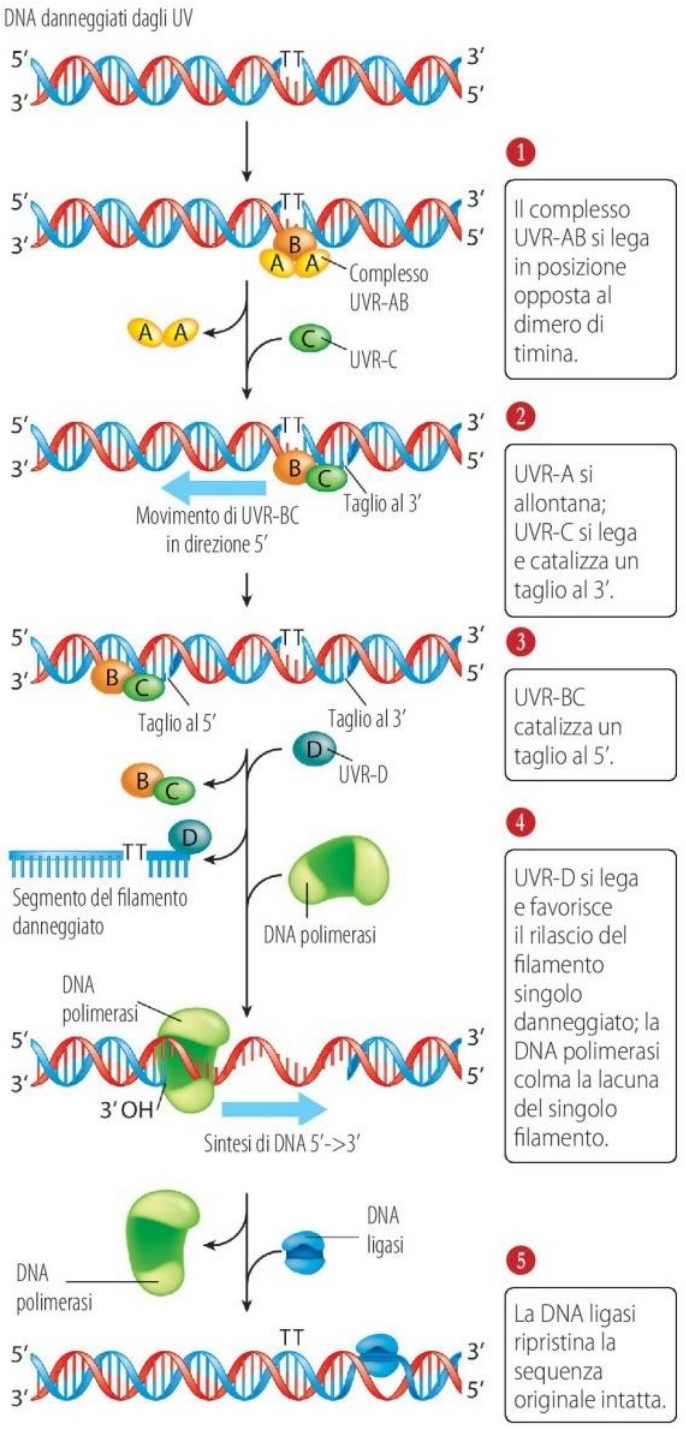
Organisms Use DNA Repair Systems to Counteract Mutations

- **Base excision repair (BER)** involves recognition of the erroneous base by **DNA glycosylase** and cutting of the DNA backbone by endonuclease.



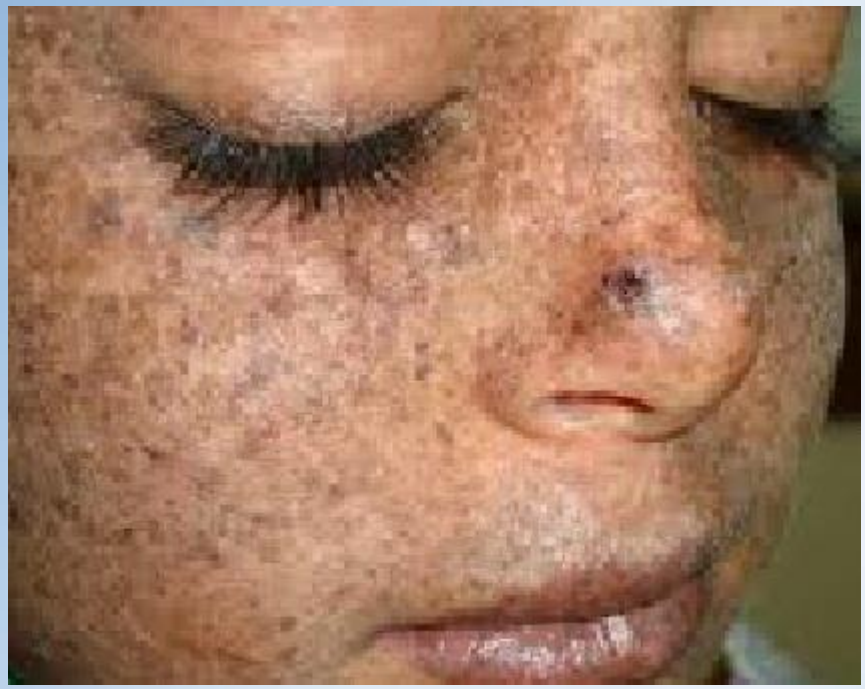
Organisms Use DNA Repair Systems to Counteract Mutations

- **Nucleotide excision repair (NER)** repairs bulky lesions and involves the *uvr* genes.



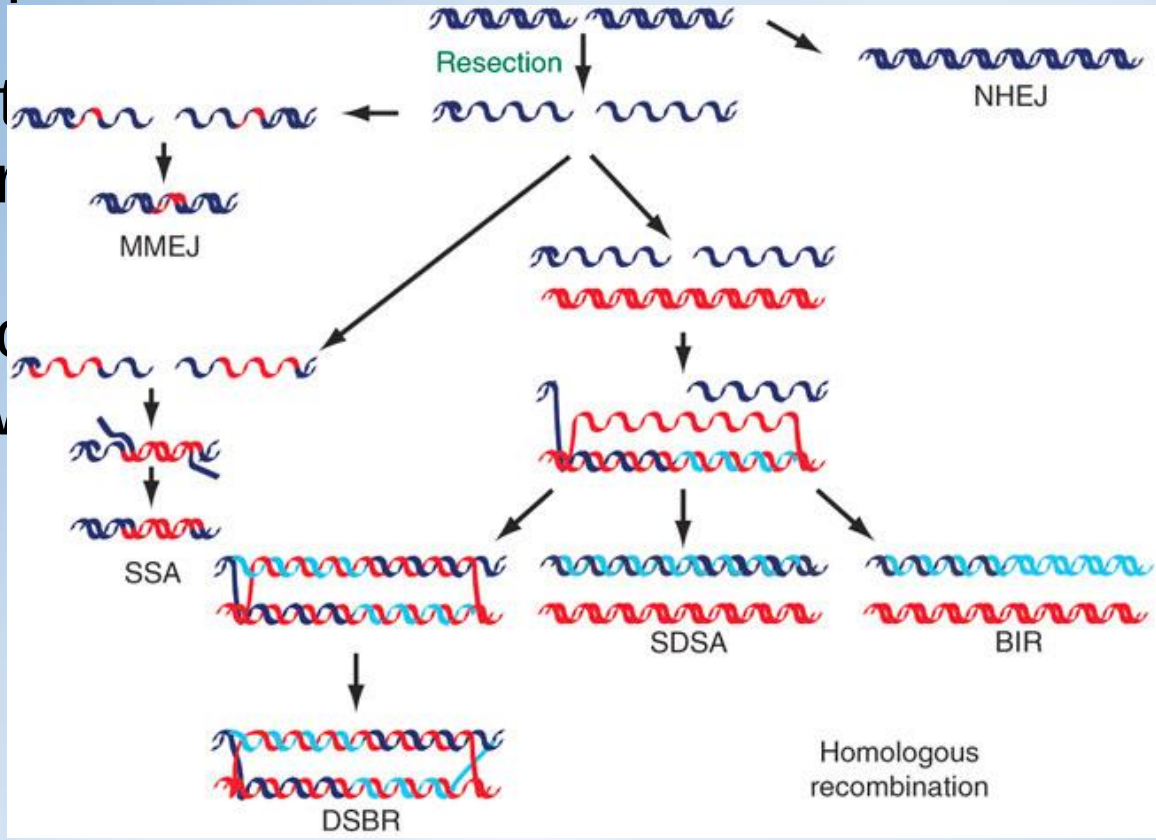
Organisms Use DNA Repair Systems to Counteract Mutations

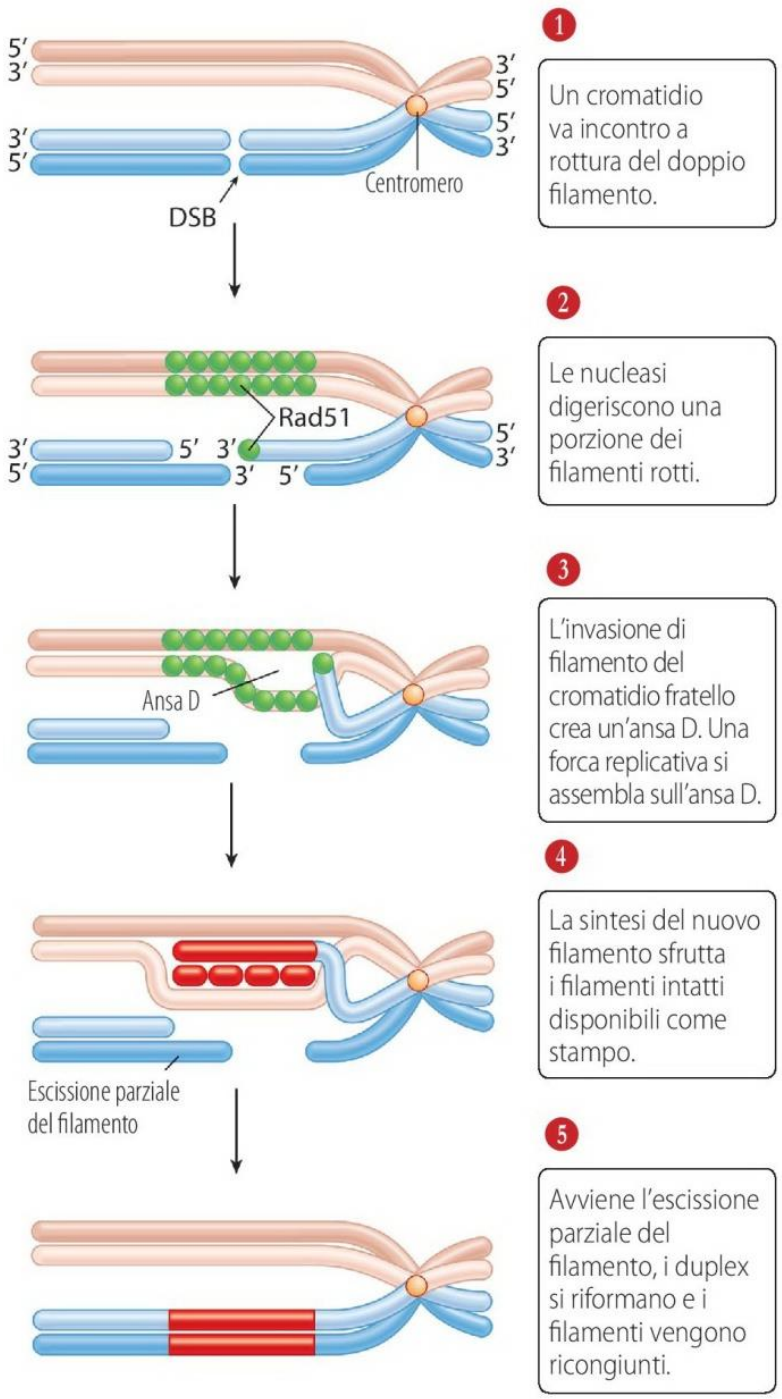
- Individuals with **xeroderma pigmentosum** have lost the ability to undergo nucleotide excision repair.



Organisms Use DNA Repair Systems to Counteract Mutations

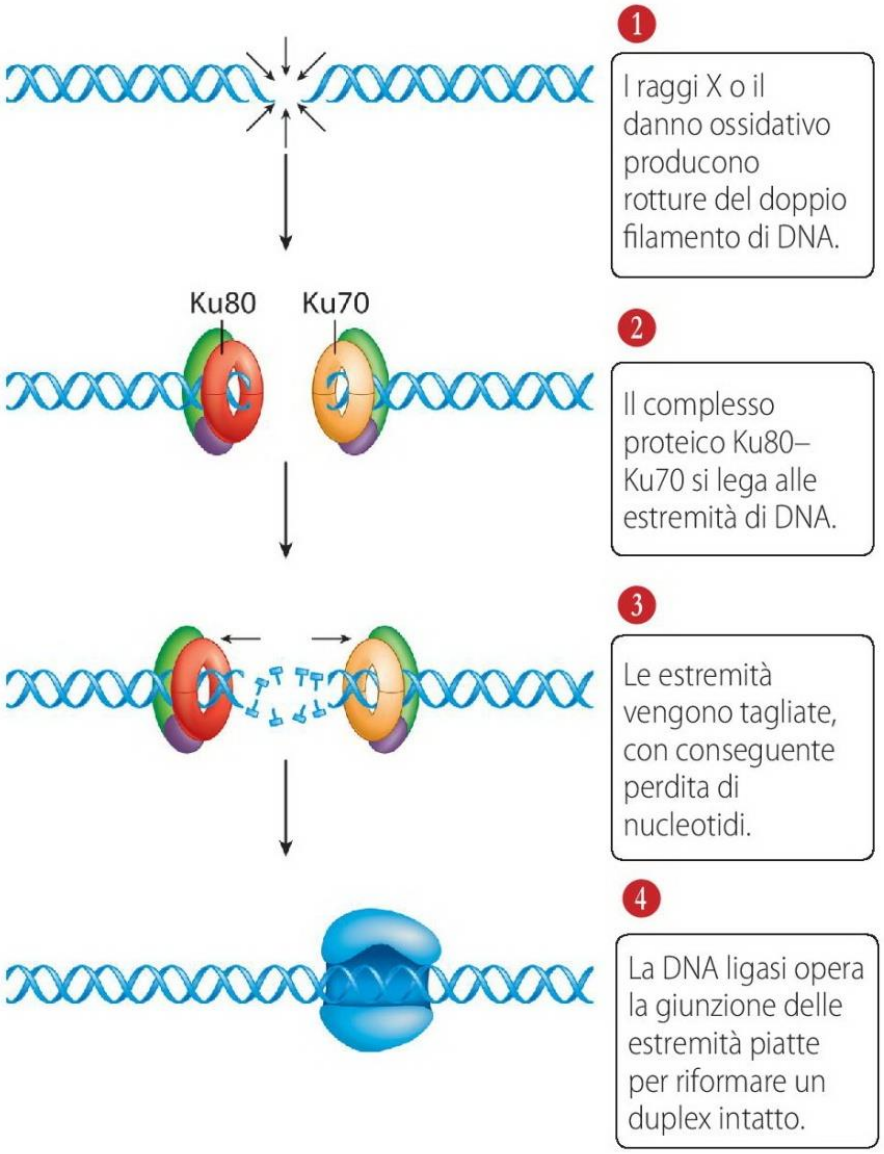
- **DNA double-strand break (DSB)** repair i
  activated when bot
  DNA strands ar
  cleaved and
  responsible fo
  reannealing the tw
  strands.





Double strand break

Organisms Use DNA Repair Systems to Counteract Mutations



- **End joining repairs double-stranded breaks** but does not require a homologous region of DNA during repair.
- Three proteins link to the ends of the broken DNA
- Error prone repair system