THE MOLECULAR BASIS OF GENE

MUTATION

Major types of mutations and their distinguishing features

Basis of classification	Major types of mutations	Major features
1- Origin	Spontaneous	Occurs in absence of known mutagen
	Induced	Occurs in presence of known mutagen
2- Cell type	Somatic	Occurs in nonreproductive cells
	Germ-line	Occurs in reproductive cells
3- Expression	Conditional	Expressed only under restrictive conditions (such as high temperature)
	Unconditional	Expressed under permissive conditions as well as restrictive conditions
4- Effect on function	Loss-of-function (knockout, null)	Eliminates normal function
	Hypomorphic (leaky)	Reduces normal function
	Hypermorphic	Increases normal function
	Gain-of-function (ectopic expression)	Expressed at incorrect time or in inappropriate cell types
5- Molecular change	Base substitution	One base pair in duplex DNA replaced with a different base pair
	Transition	Pyrimidine (T or C) to pyrimidine, or purine (A or G) to purine
	Transversion	Pyrimidine (T or C) to purine, or purine (A or G) to pyrimidine
	Insertion	One or more extra nucleotides present
	Deletion	One or more missing nucleotides
6- Effect on translation	Synonymous (silent)	No change in amino acid encoded
	Missense (nonsynonymous)	Change in amino acid encoded
	Nonsense (termination)	Creates translational termination codon (UAA, UAG, or UGA)
	Frameshift	Shifts triplet reading of codons out of correct phase

Germline Mutation - affecting tissues that produces **eggs & sperm**
(**heritable** meiotically between generations)
Somatic Mutation- affecting other body tissues (heritable mitotically, *e.g.* cancer)

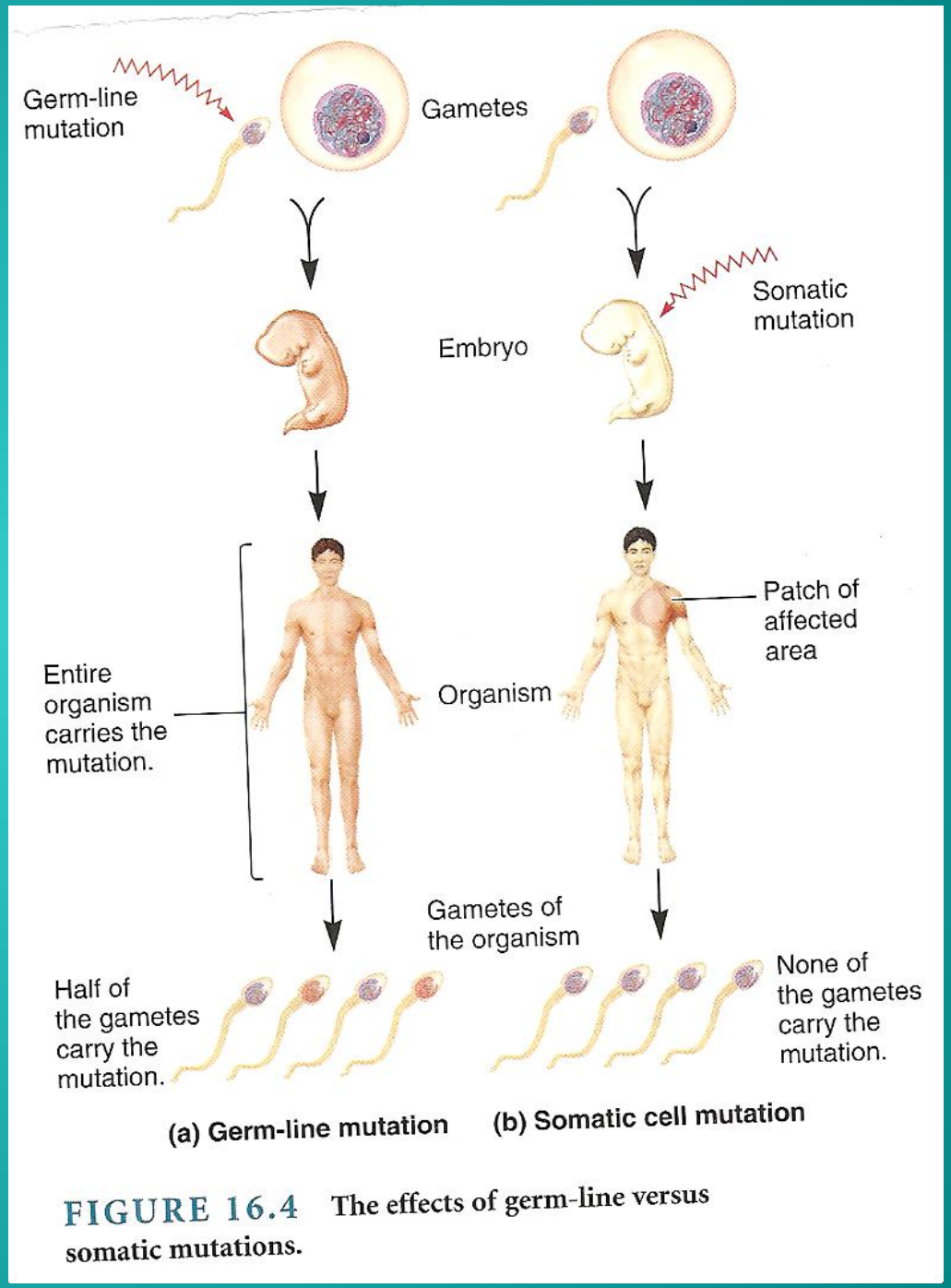


FIGURE 16.4 The effects of germ-line versus somatic mutations.

- Mutations are categorized as *spontaneous* or *induced*
- **Spontaneous mutations** are those that arise in the absence of known mutagen treatment. They account for the "background rate" of mutation and are presumably the ultimate source of natural genetic variation that is seen in populations.
- **Induced mutations** are defined as those that arise after purposeful treatment with **mutagens,** environmental agents that are known to increase the rate of mutations

Mechanism of Spontaneous Mutation

➢Spontaneous mutations are naturally occurring mutations and arise in all cells.

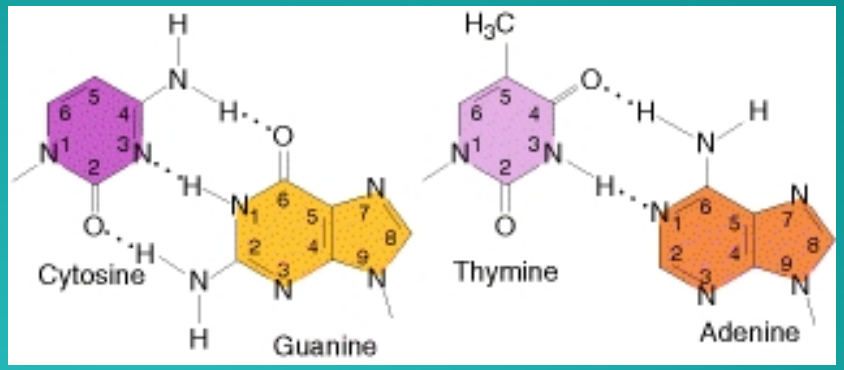
➢Spontaneous mutations arise from a variety of sources, including errors in DNA replication, spontaneous lesions, and transposable genetic elements

➢Each of the bases in DNA can appear in one of several forms, called tautomers

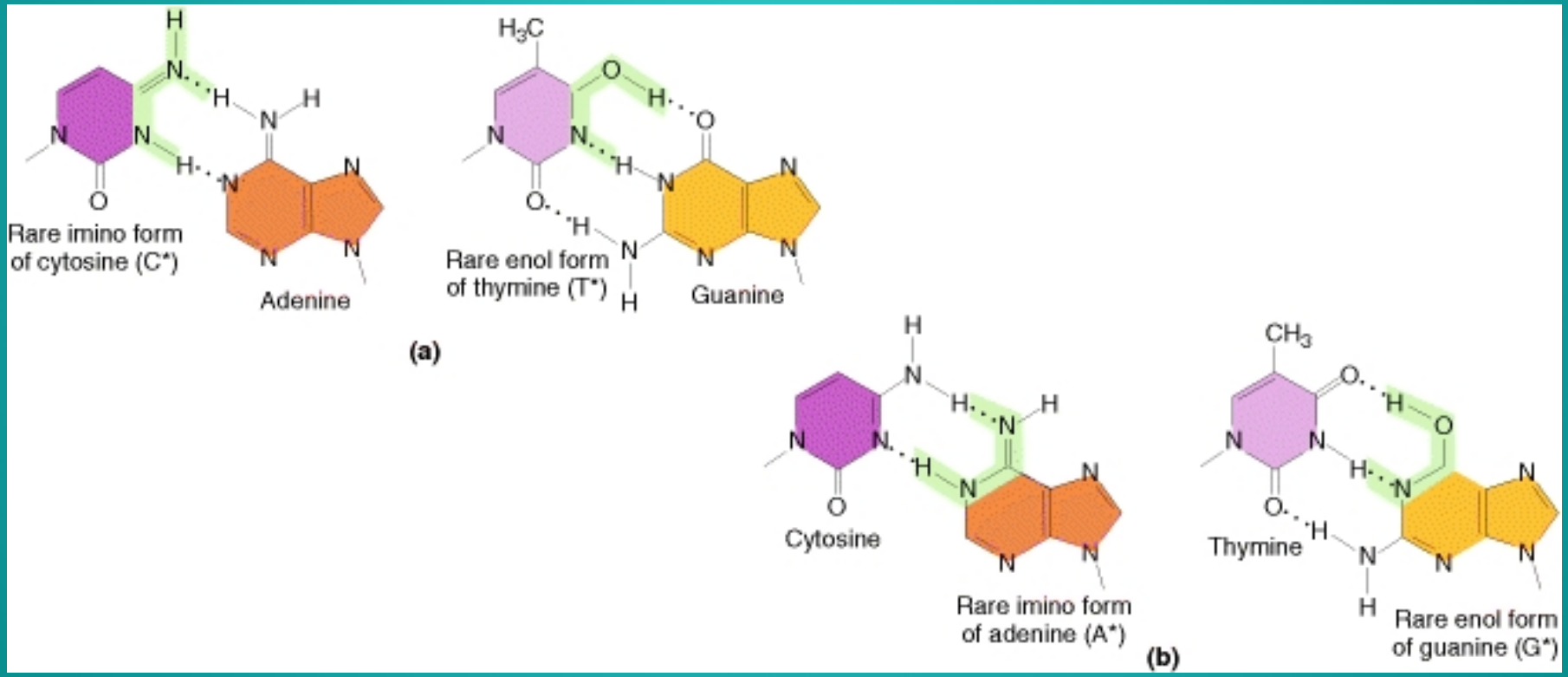
➢These are Isomers that differ in the positions of their atoms and in the bonds between the atoms. The forms are in equilibrium.

➢The keto form of each base is normally present in DNA, whereas the imino and enol forms of the bases are rare.

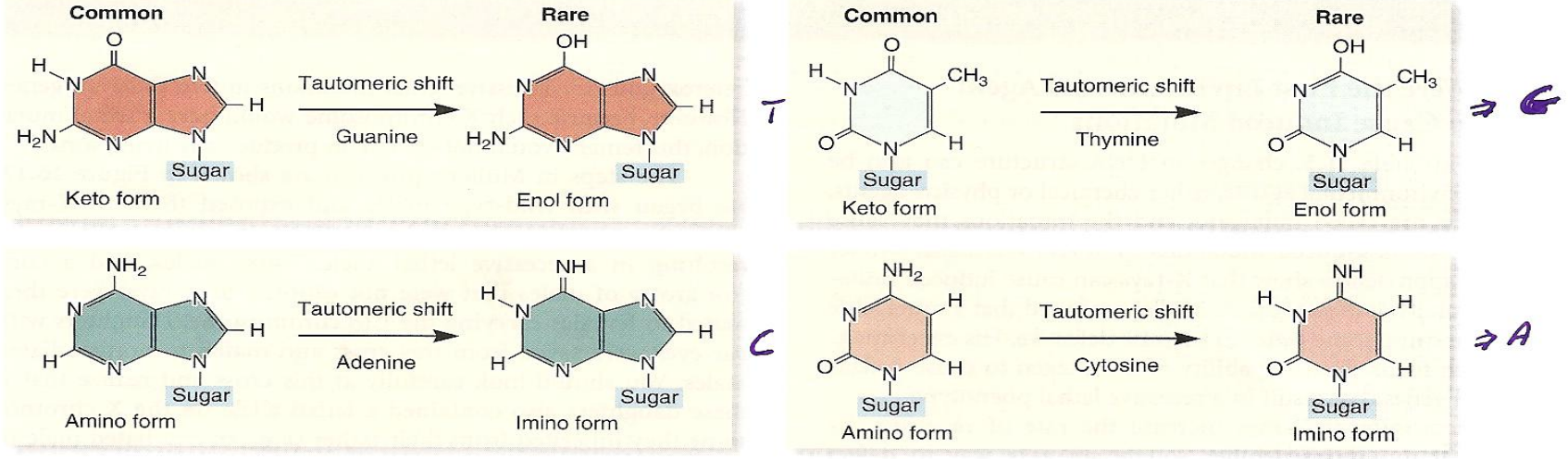
Errors in DNA Replication



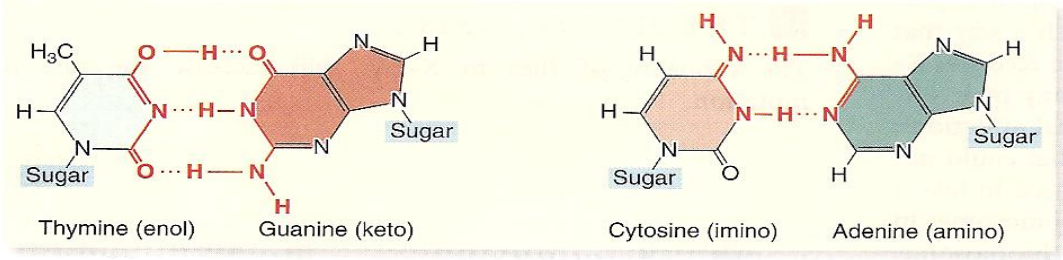
Pairing between the normal (keto) forms of the bases



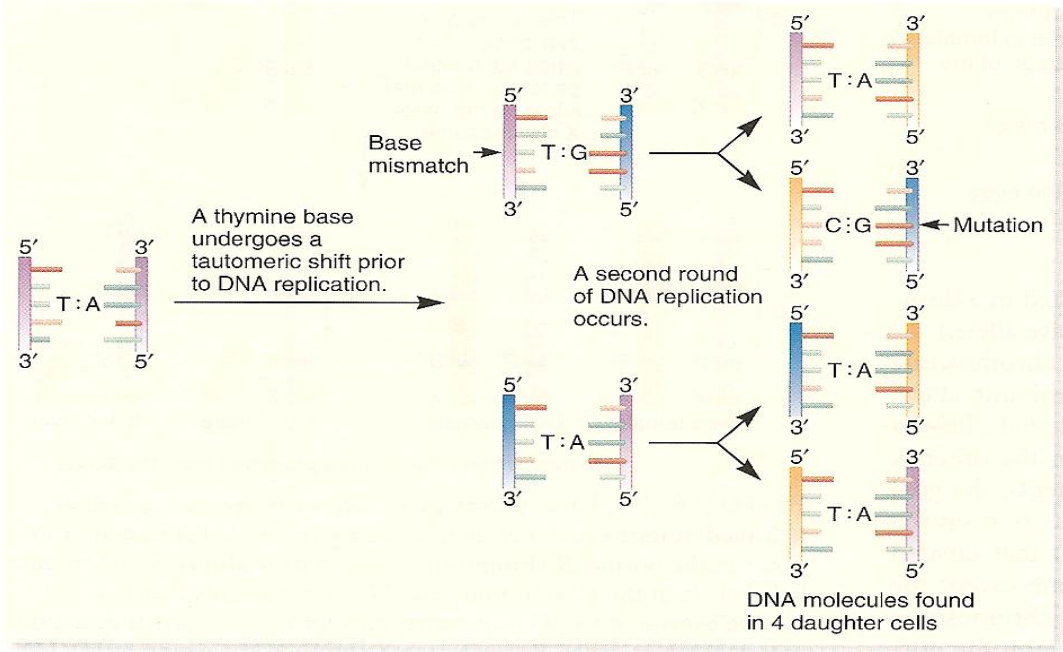
Mismatched bases. (a) Mispairs resulting from rare tautomeric forms of the pyrimidines; (b) mispairs resulting from rare tautomeric forms of the purines



(a) Tautomeric shifts that occur in the 4 bases found in DNA



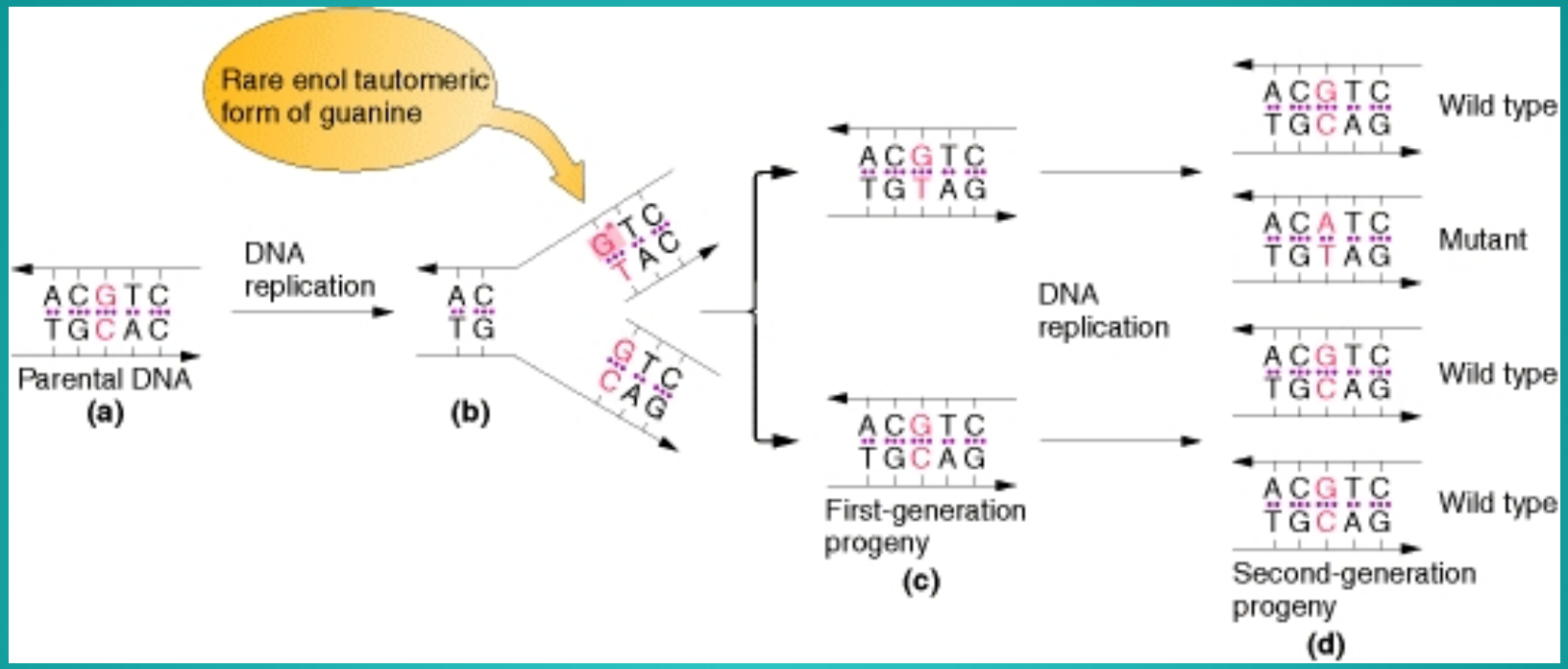
(b) Mis–base pairing due to tautomeric shifts



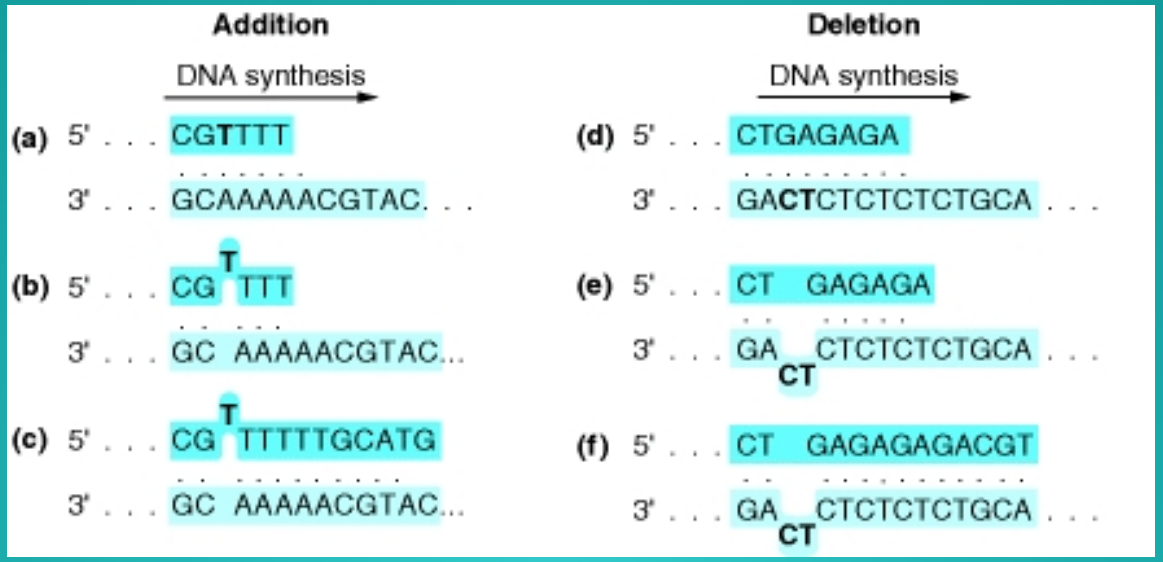
(c) Tautomeric shifts and DNA replication can cause mutation

FIGURE 16.10 Tautomeric shifts and their ability to cause mutation. (**a**) The common forms of the bases are shown on the left, and the rare forms produced by a tautomeric shift are shown on the right. (**b**) On the left, the rare enol form of thymine pairs with the common keto form of guanine (instead of adenine); on the right, the rare imino form of cytosine pairs with the common amino form of adenine (instead of guanine). (**c**) A tautomeric shift occurred in a thymine base just prior to replication, causing the formation of a TG base pair. If not repaired, a second round of replication will lead to the formation of a permanent CG mutation. Note: A tautomeric shift is a very temporary situation. During the second round of replication, the thymine base that shifted prior to the first round of DNA replication is likely to have shifted back to its normal form. Therefore, during the second round of replication, an adenine base will be found opposite this thymine.

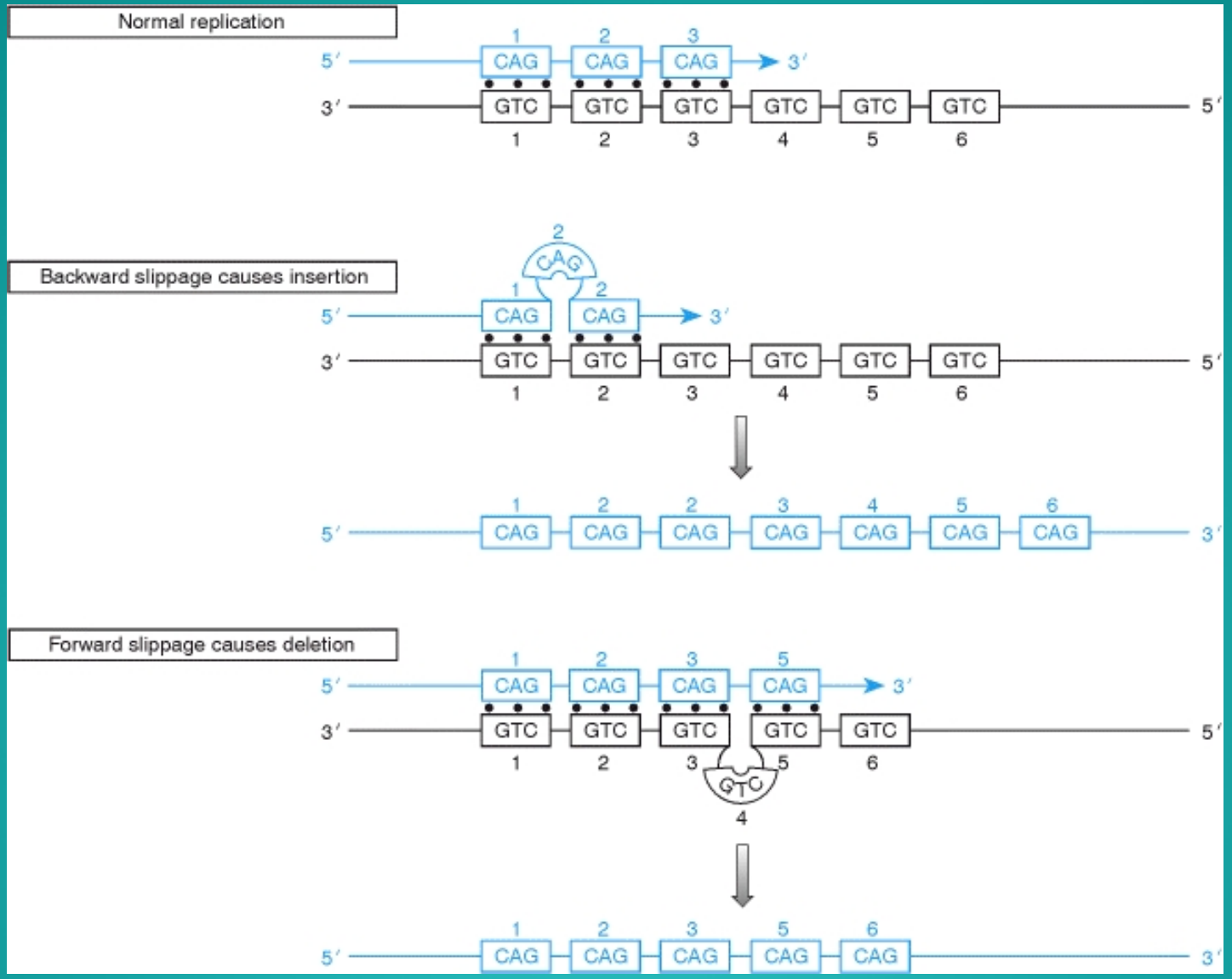
Transitions



Mutation by tautomeric shifts in the bases of DNA. (a) In the example diagrammed, a guanine undergoes a tautomeric shift to its rare enol form (G*) at the time of replication. (b) In its enol form, it pairs with thymine. (c and d) In the next replication, the guanine shifts back to its more stable keto form. The thymine incorporated opposite the enol form of guanine, seen in part b, directs the incorporation of adenine in the subsequent replication, shown in parts c and d. The net result is a GC → AT mutation. If a guanine undergoes a tautomeric shift from the common keto form to the rare enol form at the time of incorporation (as a nucleoside triphosphate, rather than in the template strand diagrammed here), it will be incorporated opposite thymine in the template strand and cause an AT → GC mutation. (From E. J. Gardner and D. P. Snustad, *Principles of Genetics,* 5th ed. Copyright © 1984 by John Wiley & Sons, New York.)



A simplified version of the Streisinger model for frameshift formation. (a–c) In DNA synthesis, the newly synthesized strand slips, looping out one or several bases. This loop is stabilized by the pairing afforded by the repetitive-sequence unit (the A bases in this case). An addition of one base pair, A–T, will result at the next round of replication in this example. (d–f) If, instead of the newly synthesized strand, the template strand slips, then a deletion results. Here the repeating unit is a CT dinucleotide. After slippage, a deletion of two base pairs (C–G and T–A) would result at the next round of replication.



Slipped strand mispairing during DNA replication can cause insertions or deletions

Short tandem repeats are thought to be particularly prone to slipped strand mispairing, i.e. mispairing of the complementary DNA strands of a single DNA double helix. The examples show how slipped strand mispairing can occur during replication, with the lower strand representing a parental DNA strand and the upper blue strand representing the newly synthesized complementary strand. In such cases, slippage involves a region of nonpairing (shown as a bubble) containing one or more repeats of the newly synthesized strand (backward slippage) or of the parental strand (forward slippage), causing, respectively, an insertion or a deletion on the newly synthesized strand. *Note* that it is conceivable that slipped strand mispairing can also cause insertions/deletions in nonreplicating DNA. In such cases, two regions of nonpairing are required, one containing repeats from one DNA strand and the other containing repeats from the complementary strand

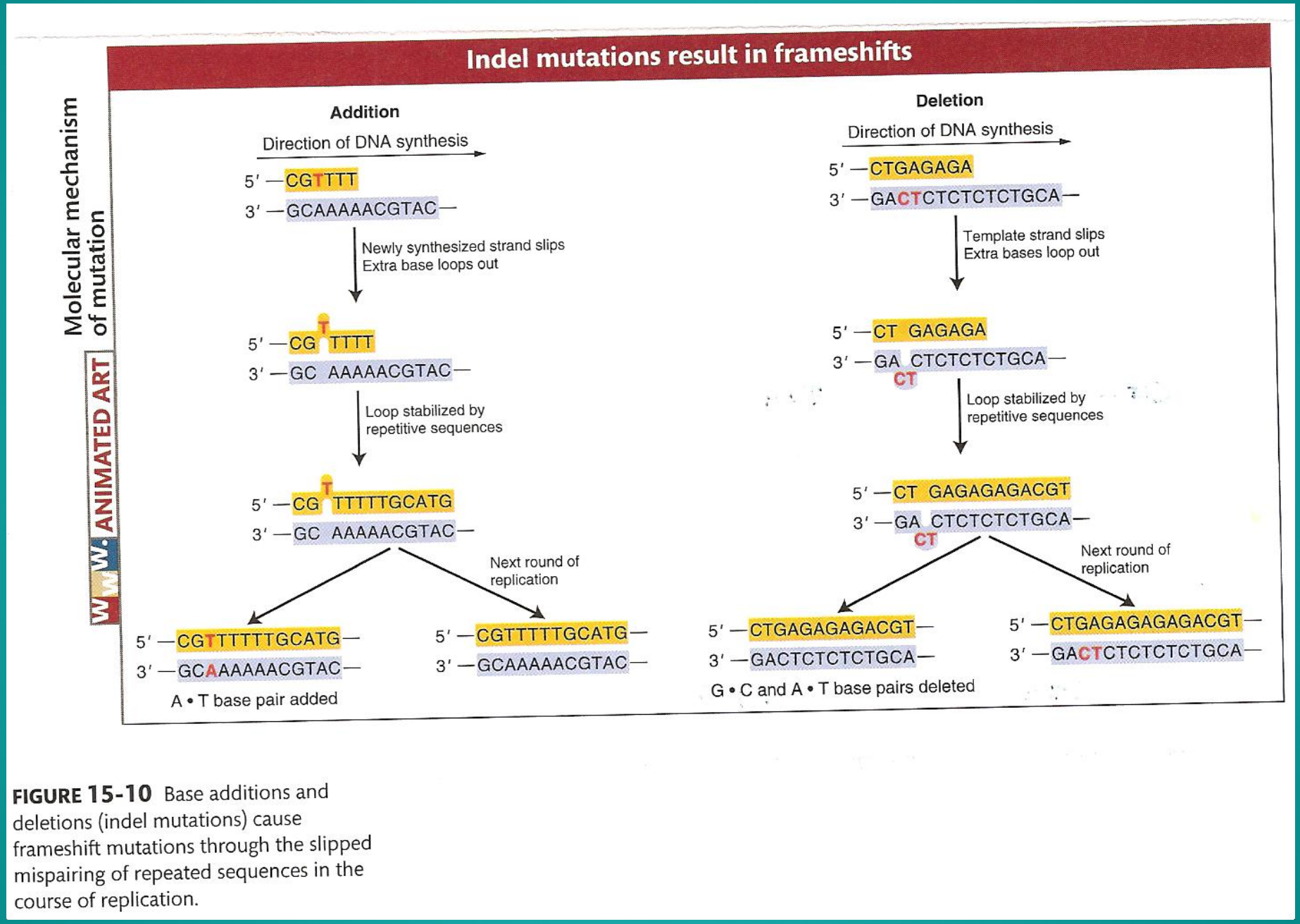
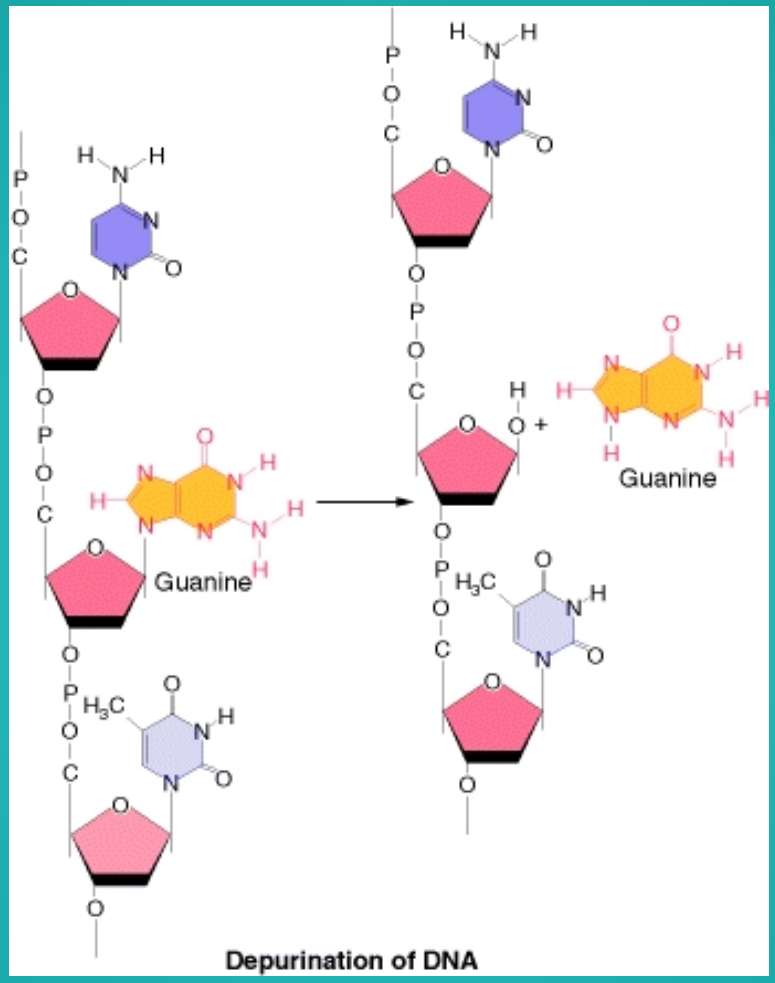


FIGURE 15-10 Base additions and deletions (indel mutations) cause frameshift mutations through the slipped mispairing of repeated sequences in the course of replication.

Spontaneous Lesions



The loss of a purine residue (guanine) from a single strand of DNA. The sugar-phosphate backbone is left intact

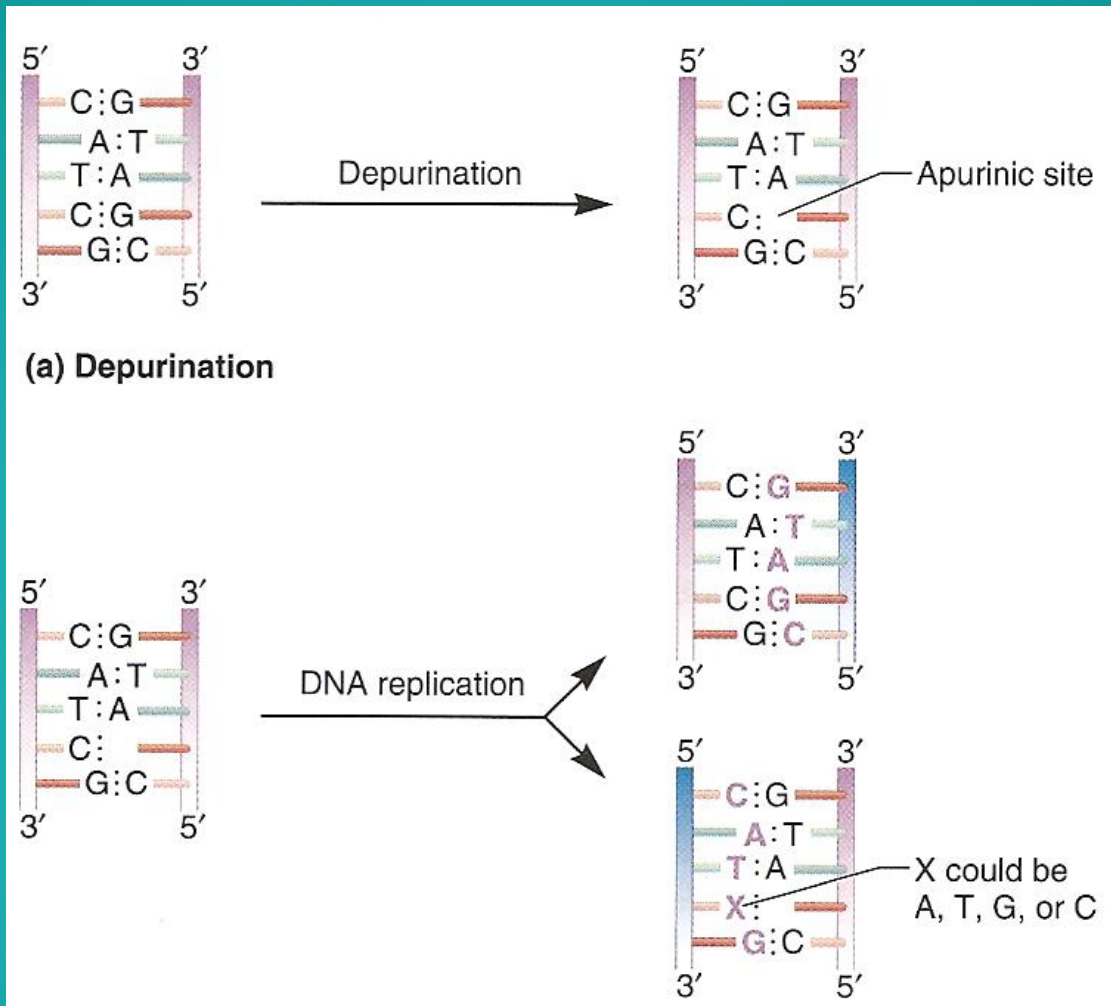
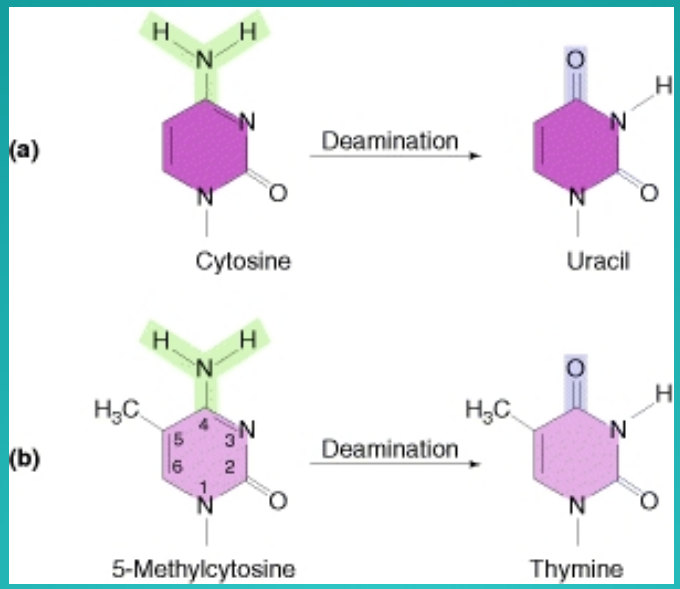


FIGURE 16.8 **Spontaneous depurination.** (a) The bond between guanine and deoxyribose is broken, thereby releasing the base. This leaves an apurinic site in the DNA. (**b**) If an apurinic site remains in the DNA as it is being replicated, any of the four nucleotides can be added to the newly made strand. Because three out of four (A, T, and G) are the incorrect base, the chance of causing a mutation is 75%.

Depurination, the more common of the two, consists of the interruption of the glycosidic bond between the base and deoxyribose and the subsequent loss of a guanine or an adenine residue from the DNA.

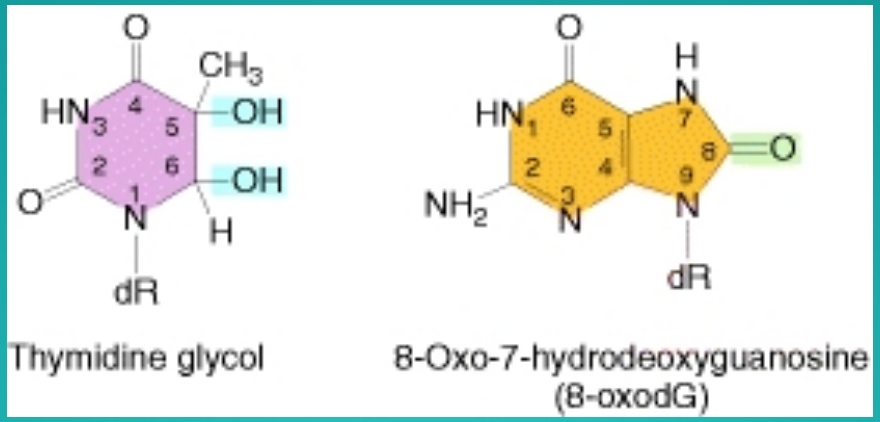
A mammalian cell spontaneously loses about 10,000 purines from its DNA in a 20-hour cell-generation period at 37°C. If these lesions were to persist, they would result in significant genetic damage because, in replication, the resulting **apurinic sites** cannot specify a base complementary to the original purine.

However, efficient repair systems remove apurinic sites. Under certain conditions, a base can be inserted across from an apurinic site; this insertion will frequently result in a mutation.



Deamination of (a) cytosine and (b) 5-methylcytosine.

The **deamination** of cytosine yields Uracil. Unrepaired uracil residues will pair with adenine in replication, resulting in the conversion of a G–C pair into an A–T pair (a **GC → AT transition**). In 1978, deaminations at certain cytosine residues were found to be the cause of one type of mutational hot spot. DNA sequence analysis of GC → AT transition hot spots in the *lacI* gene showed that 5-methylcytosine residues are present at each hot spot. (Certain bases in prokaryotes and eukaryotes are methylated.)



DNA damage products formed after attack by oxygen radicals. dR = deoxyribose.

Oxidatively damaged bases represent a third type of spontaneous lesion implicated in mutagenesis.

Active oxygen species, such as superoxide radicals (O2·), hydrogen peroxide (H2O2), and hydroxyl radicals (OH·), are produced as by-products of normal aerobic metabolism.

They can cause oxidative damage to DNA, as well as to precursors of DNA (such as GTP), which results in mutation and which has been implicated in a number of human diseases.

The above figure shows two products of oxidative damage. The 8-oxo-7-hydrodeoxyguanosine (8-oxodG, or GO) product frequently mispairs with A, resulting in a high level of G → T transversions.

Thymidine glycol blocks DNA replication if unrepaired but has not yet been implicated in mutagenesis.

Mechanism of Induced Mutation

Mutagens induce mutations by at least three different mechanisms.

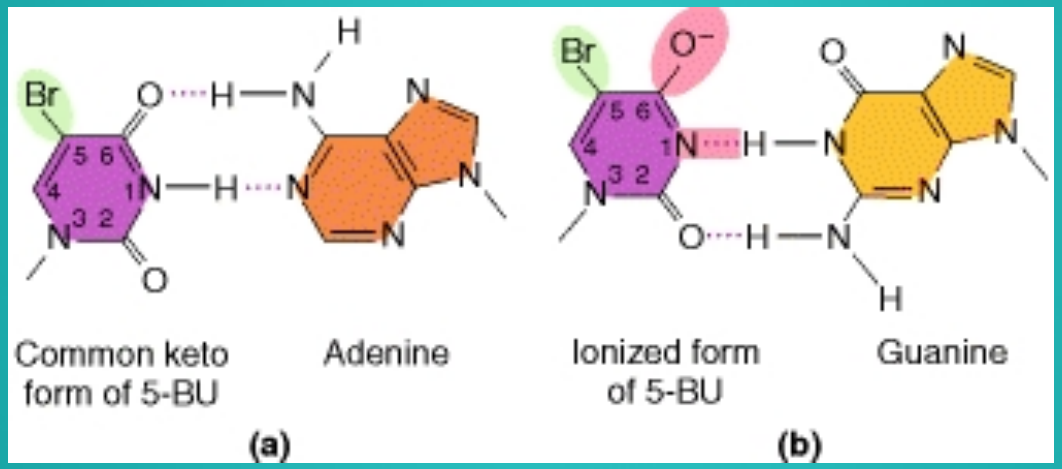
➢They can replace a base in the DNA

➢Alter a base so that it specifically mispairs with another base,

➢Or damage a base so that it can no longer pair with any base under normal conditions.

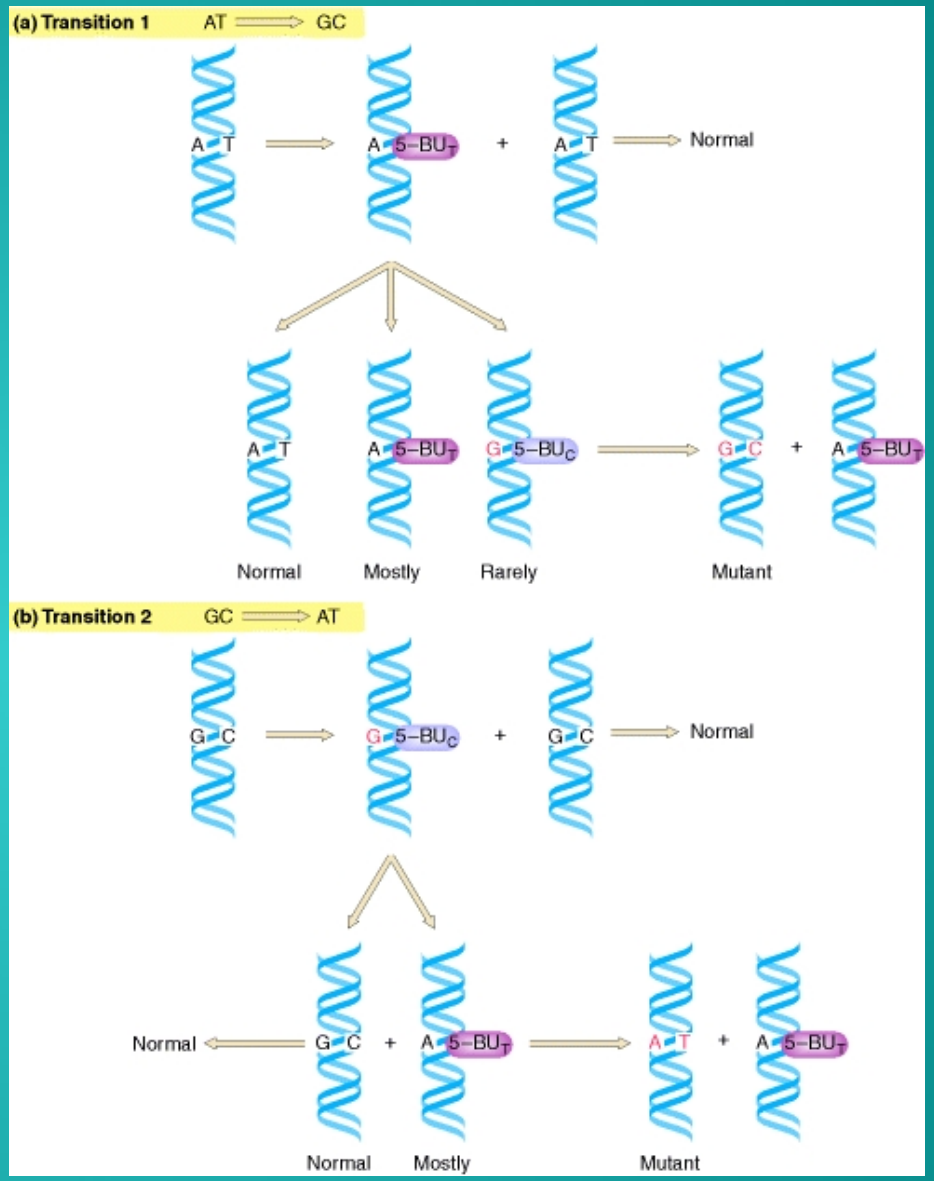
Incorporation of base analogs

Some chemical compounds are sufficiently similar to the normal nitrogen bases of DNA that they occasionally are incorporated into DNA in place of normal bases; such compounds are called base analogs



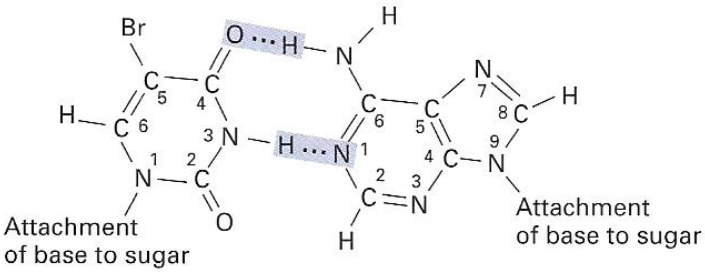
Alternative pairing possibilities for 5-bromouracil (5-BU). 5-BU is an analog of thymine that can be mistakenly incorporated into DNA as a base. It has a bromine atom in place of the methyl group. (a) In its normal keto state, 5-BU mimics the pairing behavior of the thymine that it replaces, pairing with adenine. (b) The presence of the bromine atom, however, causes a relatively frequent redistribution of electrons, so that 5-BU can spend part of its existence in the rare ionized form. In this state, it pairs with guanine, mimicking the behavior of cytosine and thus inducing mutations in replication.

The mechanism of 5-BU mutagenesis. 5-BU causes mutations when it is incorporated in one form and then shifts to another form. (a) In its normal keto state, 5-BU pairs like thymine (5-BUT). Thus, 5-BU is incorporated across from adenine and subsequently mispairs with guanine, resulting in AT → GC transitions. (b) In its ionized form, 5-BU pairs like cytosine (5-BUC). Thus, 5-BU is misincorporated across from guanine and subsequently pairs with adenine, resulting in GC → AT transitions.



Mutagenic effects of the base analog 5-bromouracil (5BU). (a) In its normal state 5BU pairs with adenine. (b) In its rare state, 5BU (indicated by white letters on magenta) pairs with guanine. (c) The two possible mutation mechanisms. 5BU induces transition mutations when it incorporates into DNA in one state, then shifts to its alternate state during the next round of DNA replication.

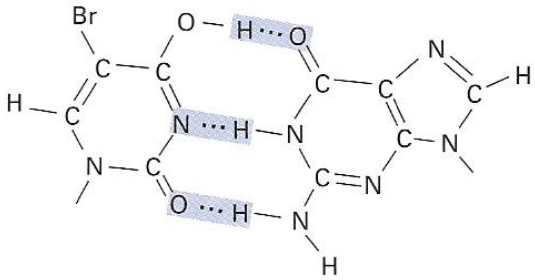
a) Base-pairing of 5-bromouracil in its normal state



5-bromouracil (behaves like thymine; normal state)

Adenine (normal state)

b) Base-pairing of 5-bromouracil in its rare state

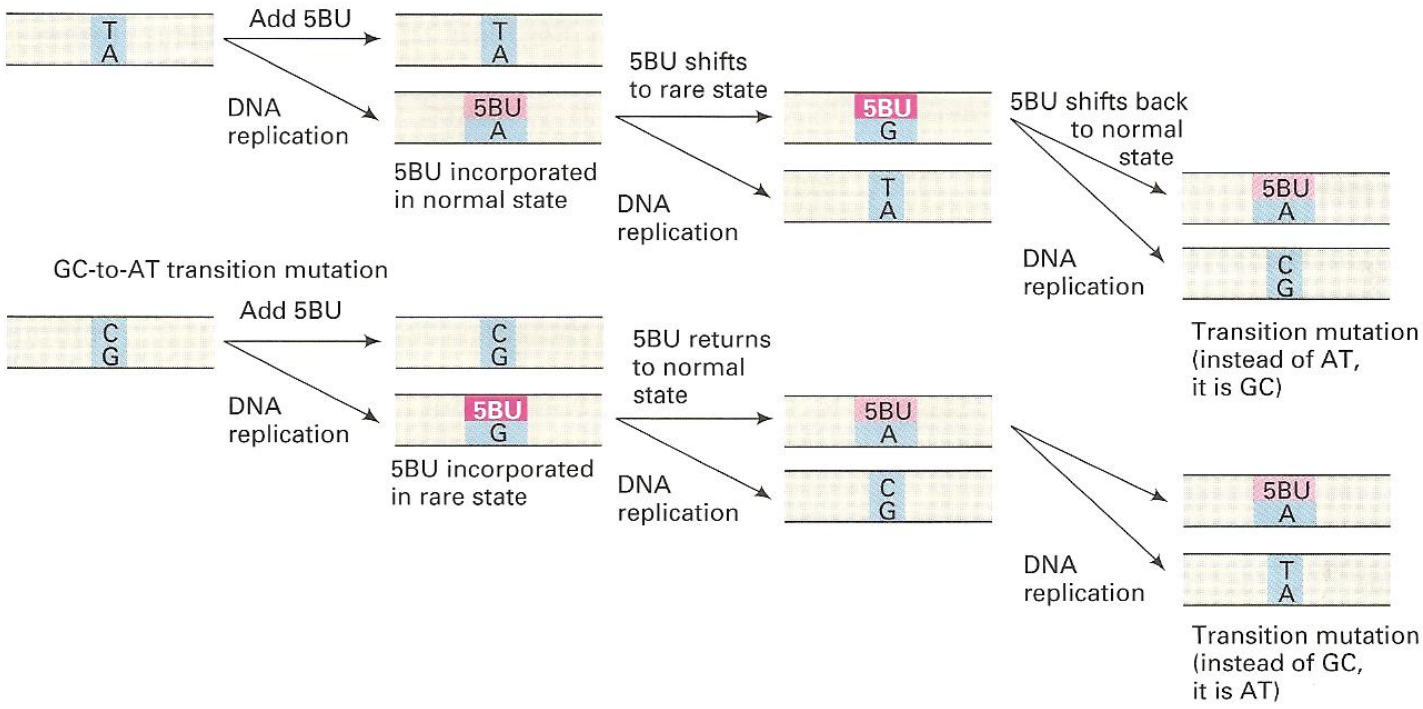


5-bromouracil (behaves like cytosine; rare state)

Guanine (normal state)

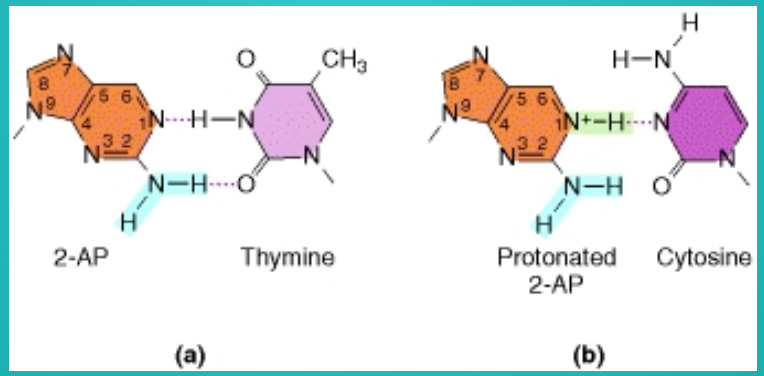
c) Mutagenic action of 5BU

AT–to–GC transition mutation



Another analog widely used in research is **2-amino-purine (2-AP),** which is an analog of adenine that can pair with thymine but can also mispair with cytosine when protonated

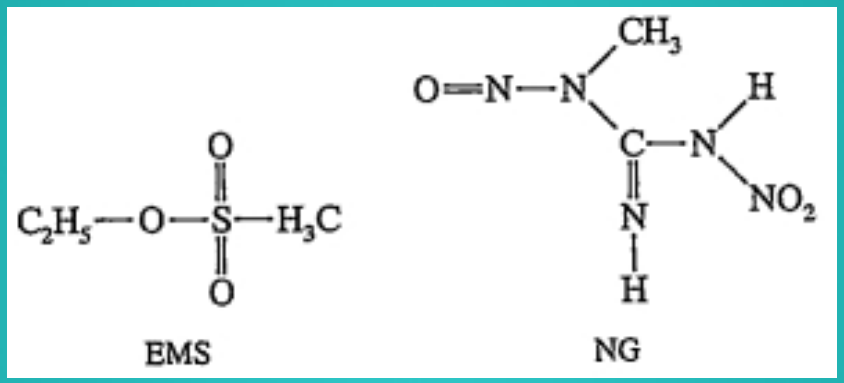
Therefore, when 2-AP is incorporated into DNA by pairing with thymine, it can generate AT → GC transitions by mispairing with cytosine in subsequent replications. Or, if 2-AP is incorporated by mispairing with cytosine, then GC → AT transitions will result when it pairs with thymine.



Alternative pairing possibilities for 2-aminopurine (2-AP), an analog of adenine. Normally, 2-AP pairs with thymine (a), but in its protonated state it can pair with cytosine (b).

Specific Mispairing

Some mutagens are not incorporated into the DNA but instead alter a base, causing specific mispairing. Certain **Alkylating agents**, such as **ethylmethanesulfonate (EMS)** and the widely used **nitrosoguanidine (NG),** operate by this pathway:

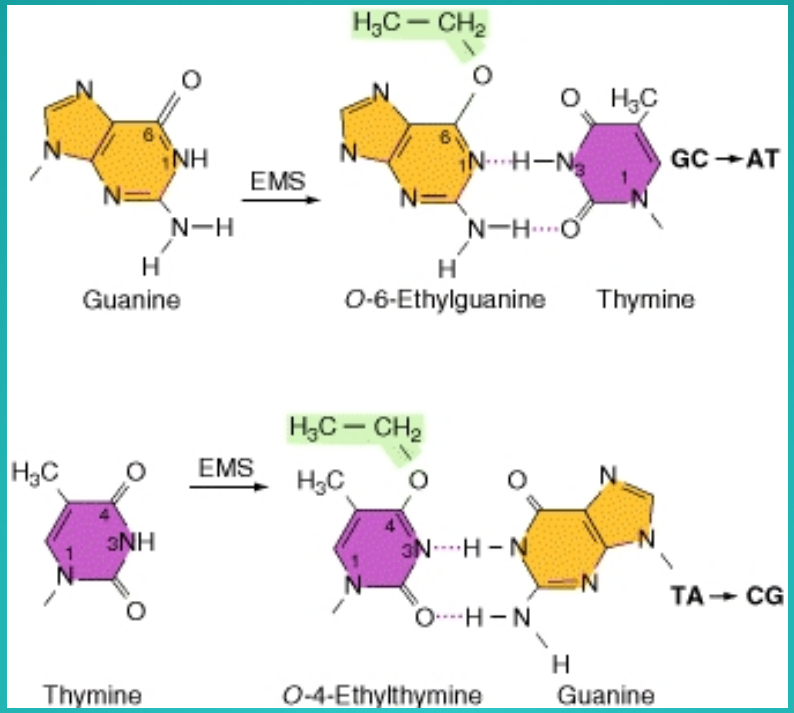


Alkylating agents add alkyl groups (an ethyl group in EMS and a methyl group in NG) to many positions on all four bases

Mutagenicity is best correlated with an addition to the oxygen at the 6 position of Guanine to create an *O*-6-alkylguanine. This addition leads to direct mispairing with thymine,

determinations of mutagenic specificity for EMS and NG show a strong preference for GC → AT transitions

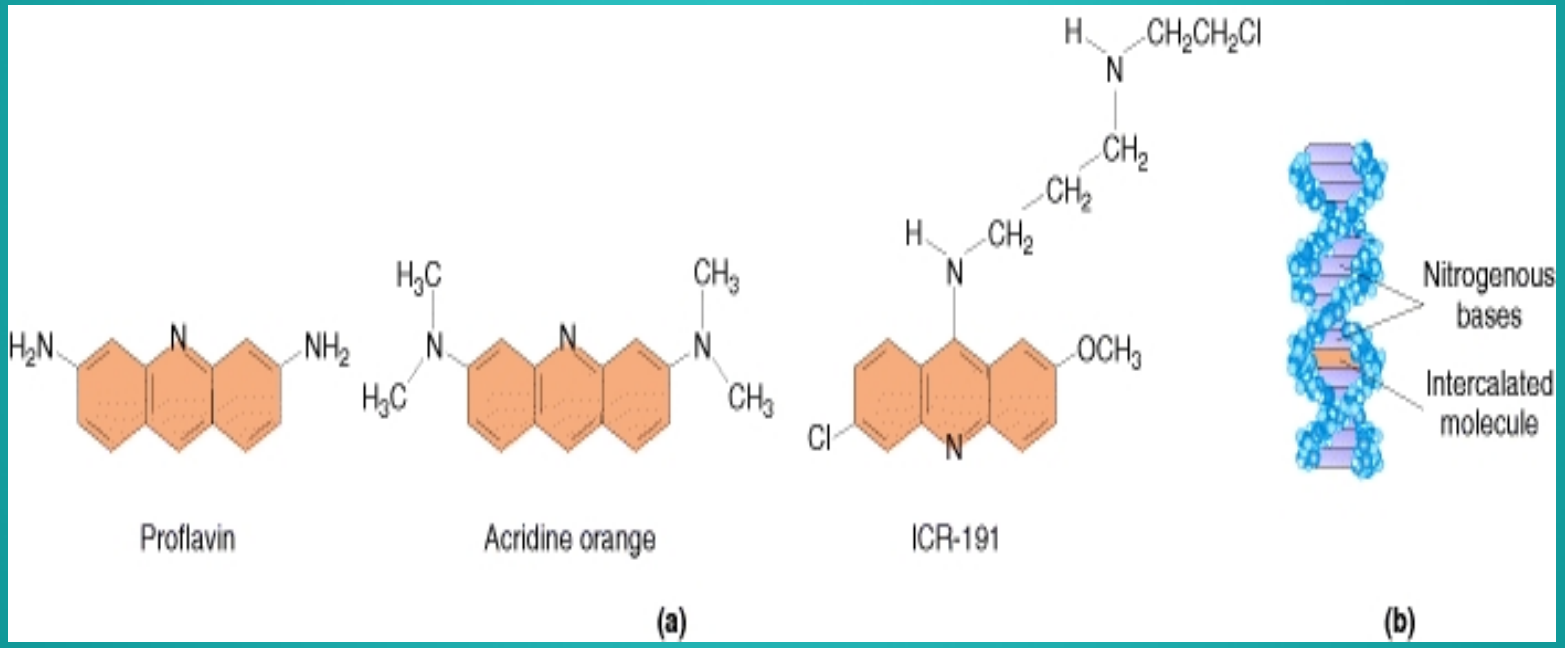
Alkylating agents can also modify the bases in dNTPs (where N is any base), which are precursors in DNA synthesis



Alkylation-induced specific mispairing. The alkylation (in this case, EMS-generated ethylation) of the O-6 position of guanine and the O-4 position of thymine can lead to direct mispairing with thymine and guanine, respectively, as shown here. In bacteria, where mutations have been analyzed in great detail, the principal mutations detected are GC → AT transitions, indicating that the O-6 alkylation of guanine is most relevant to mutagenesis

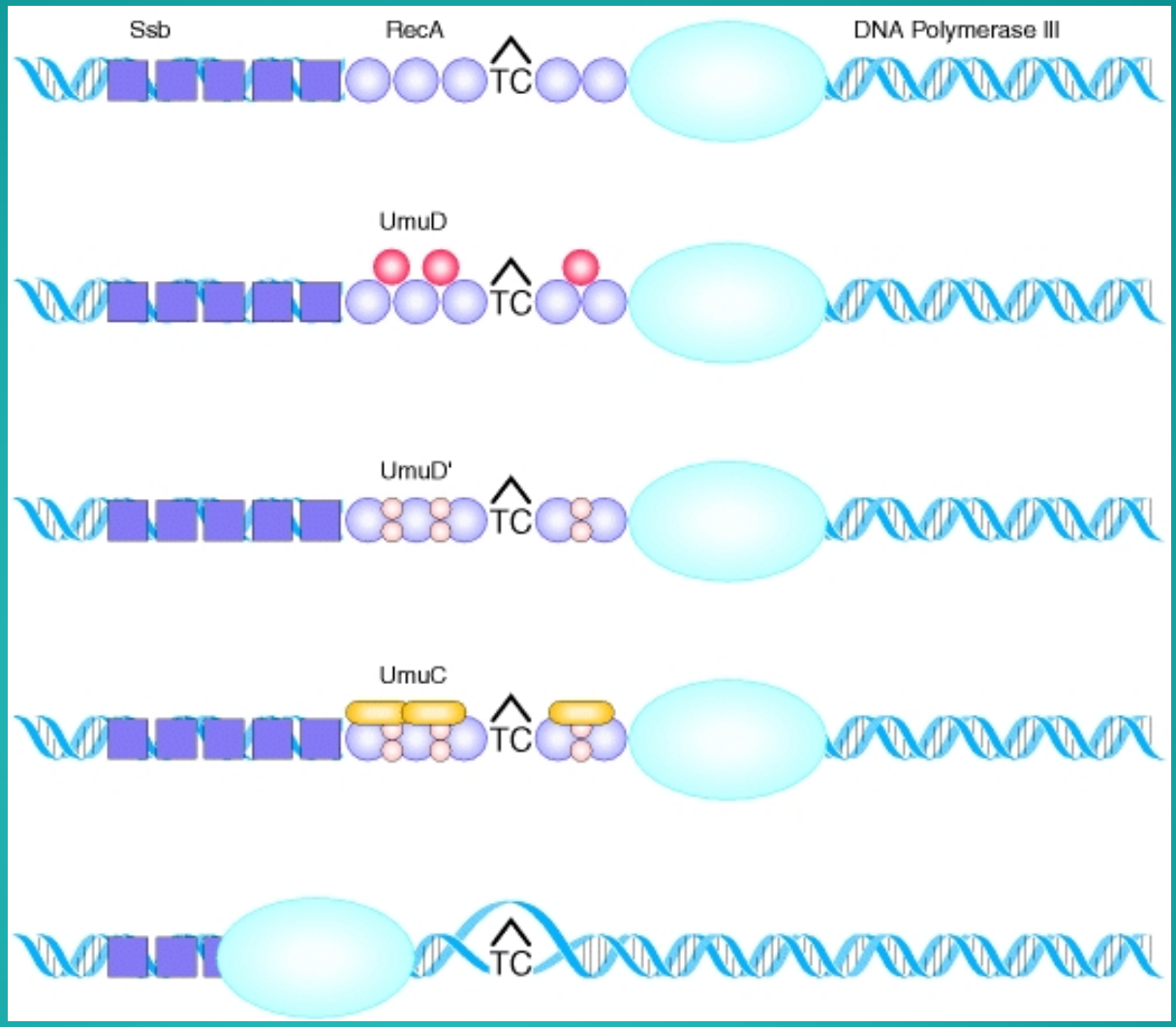
The **intercalating agents** form another important class of DNA modifiers.

These agents are planar molecules, which mimic base pairs and are able to slip themselves in **(intercalate)** between the stacked nitrogen bases at the core of the DNA double helix



Intercalating agents. (a) Structures of the common agents proflavin, acridine orange, and ICR-191. (b) An intercalating agent slips between the nitrogenous bases stacked at the center of the DNA molecule. This occurrence can lead to single-nucleotide-pair insertions and deletions

Base Damage



The SOS system. DNA polymerase III, shown in blue, stops at a noncoding lesion, such as the T–C photodimer shown here, generating single-stranded regions that attract the Ssb protein (dark purple) and RecA (light purple), which forms filaments. The presence of RecA filaments helps to signal the cell to synthesize UmuD (red circles), which is cleaved by RecA to yield UmuD′ (pink circles) and UmuC (yellow ovals). The UmuC is recruited to form a complex with UmuD′ that permits DNA polymerization to proceed past the blocking lesion

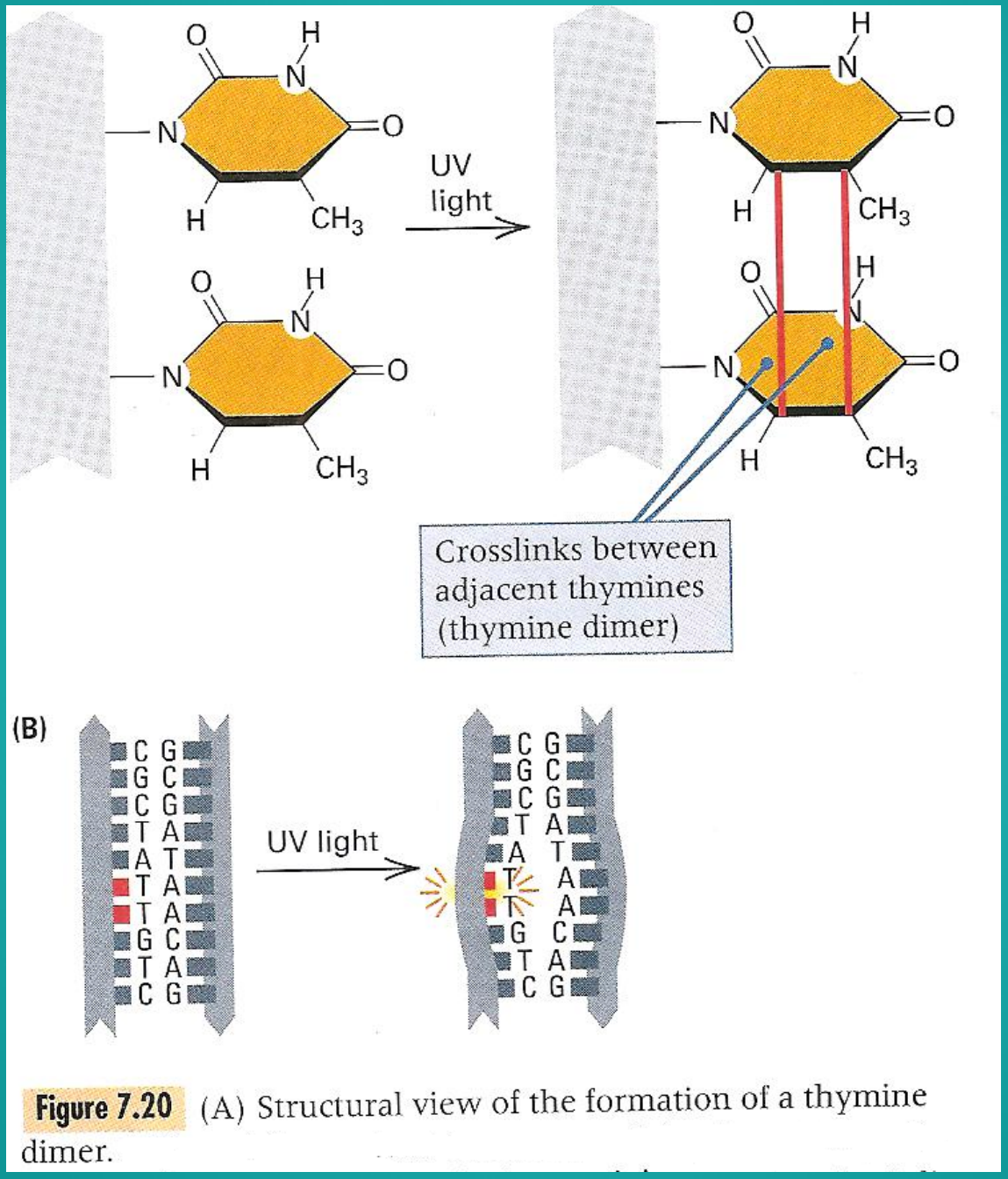
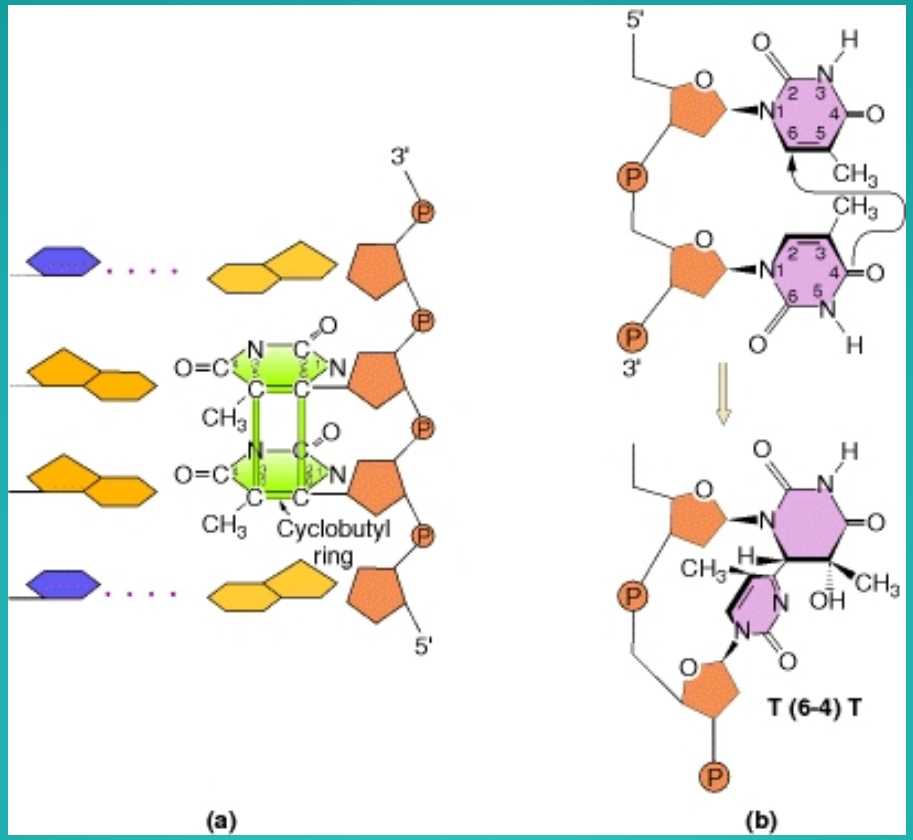


Figure 7.20 (A) Structural view of the formation of a thymine dimer.



Structure of a cyclobutane Pyrimidine dimer. Ultraviolet (UV) light stimulates the formation of a four-membered cyclobutane ring (green) between two adjacent Pyrimidine on the same DNA strand by acting on the 5,6 double bonds. (b) Structure of the 6-4 photoproduct. The structure forms most prevalently with 5′-CC-3′ and 5′-TC-3′, between the C-6 and the C-4 positions of two adjacent pyrimidines, causing a significant perturbation in local structure of the double helix

Ionizing Radiations

- Ionizing radiation results in the formation of ionized and excited molecules that can cause damage to cellular components and to DNA.
- Because of the aqueous nature of biological systems, the molecules generated by the effects of ionizing radiation on water produce the most damage.
- Many different types of reactive oxygen species are produced, including superoxide radicals, such as ·OH.
- The most biologically relevant reaction products are ·OH, O_2^-, and H_2O_2. These species can damage bases and cause different adducts and degradation products. Among the most prevalent, which result in mutations, are thymine glycol and 8-oxodG
- Ionizing radiation can cause breakage of the N-glycosydic bond, leading to the formation of AP sites, and can cause strand breaks that are responsible for most of the lethal effects of such radiation.

Aflatoxin B1 is a powerful carcinogen.

It generates **apurinic sites** following the formation of an addition product at the N-7 position of guanine.

Studies with apurinic sites generated in vitro demonstrated a requirement for the SOS system and showed that the SOS bypass of these sites leads to the preferential insertion of an adenine across from an apurinic site.

Thus agents that cause depurination at guanine residues should preferentially induce GC → TA transversions.

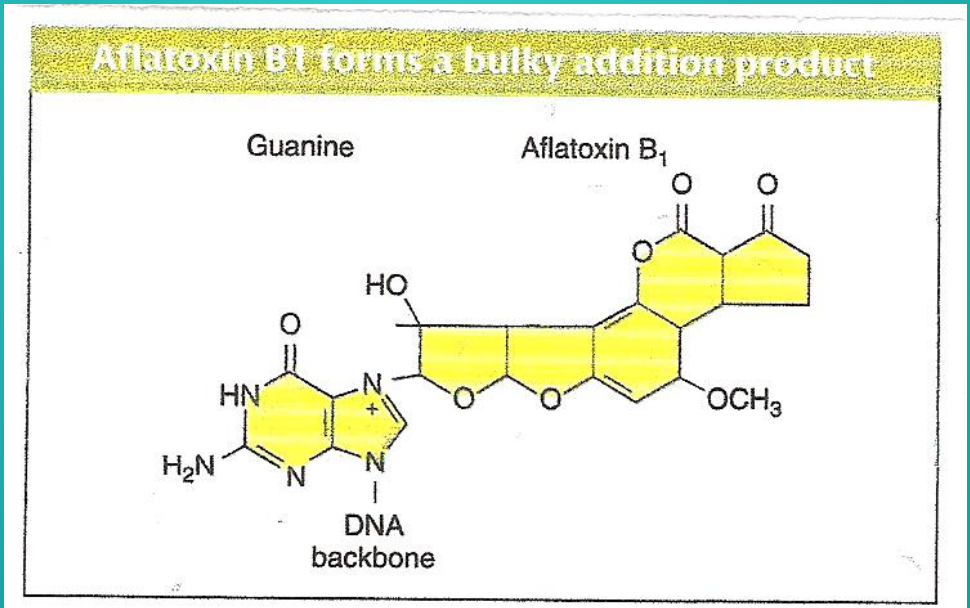


FIGURE 15-19 Metabolically activated aflatoxin B1 binds to DNA.

Table 9.1 Incidence of mutation classes in the human genome

Mutation class	Type of mutation	Incidence
Base substitutions	All types	Comparatively common type of mutation in coding DNA but also common in noncoding DNA
	Transitions and transversions	Unexpectedly, transitions are commoner than transversions, especially in mitochondrial DNA
	Synonymous and nonsynonymous substitutions	Synonymous substitutions are considerably more common than nonsynonymous substitutions in coding DNA; conservative substitutions are more common than nonconservative
	Gene conversion-like events (multiple base substitution)	Rare except at certain tandemly repeated loci or clustered repeats
Insertions	Of one or a few nucleotides	Very common in noncoding DNA but rare in coding DNA where they produce frameshifts
	Triplet repeat expansions	Rare but can contribute to several disorders, especially neurological disorders (see Box 16.7)
	Other large insertions	Rare; can occasionally get large-scale tandem duplications, and also insertions of transposable elements (Section 9.5.6)
Deletions	Of one or a few nucleotides	Very common in noncoding DNA but rare in coding DNA where they produce frameshifts
	Larger deletions	Rare, but often occur at regions containing tandem repeats (Section 9.5.3) or between interspersed repeats (see Section 9.5.4 and Figure 9.9)
Chromosomal abnormalities	Numerical and structural	Rare as constitutional mutations, but can often be pathogenic (see Section 2.6). Much more common as somatic mutations and often found in tumor cells

Table 9.5 Effect of location and class of mutation on gene function

Location and nature of mutation	Effect on gene function	Comments
Extragenic mutation	Normally none	Rare mutations may result in inactivation of distant regulatory elements required for normal gene expression (see Figure 8.23)
Multigene deletion	Abolition	Associated with contiguous gene syndromes (see Figure 16.9)
Whole gene deletion	Abolition	
Whole gene duplication	Can have effect due to altered gene dosage	Large duplications including the peripheral myelin protein 22 gene can cause Charcot-Marie-Tooth syndrome (see Figure 16.7)
Whole exon deletion	Abolition or modification	May cause shift in reading frame; protein often unstable
Within exon	Abolition	If loss/change of key amino acids, shift of the reading frame or introduction of premature stop codon
	Modification	If nonconservative substitutions, small in-frame insertions or other mutations at some locations
	None	If conservative/silent substitutions or mutation at nonessential sites
Whole intron deletion	None	
Splice site mutation	Abolition or modulation of expression	Conserved GT and AG signals are critically important for normal gene expression. Mutations may induce exon skipping or intron retention
Promoter mutation	Abolition or modulation of expression	Deletion, insertion or substitution of nucleotides within promoter may alter expression. Complete deletion abolishes function
Mutation of termination codon	Modification	Additional amino acids are included at the end of the protein until another stop codon is reached
Mutation of poly(A) signal	Abolition or modulation of expression	Deletion, insertion or substitution of nucleotides within poly(A) site may alter expression. Complete deletion abolishes function
Elsewhere in introns/UTS	Usually none	

Further Readings/ References

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Principles of Genetics

Gardner, Simmons, Snustad

THANK YOU