Mutation → Change in DNA sequence

Spontaneous mutations → occurred spontaneously

Induced mutations → treating an organism with a mutagenizing agent

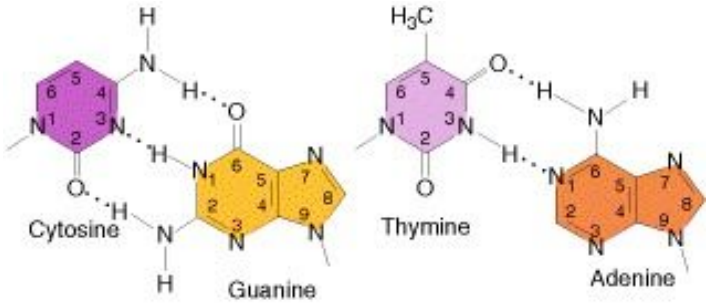
The spontaneous mutation rate varies ranging from 2 x 10^{-6} to 40 x 10^{-6} mutations per gamete per gene and usually involves one or few base pairs.

Transition: G-C in A-T

Transversion: purine in pyrimidine (A-T in T-A o C-G)

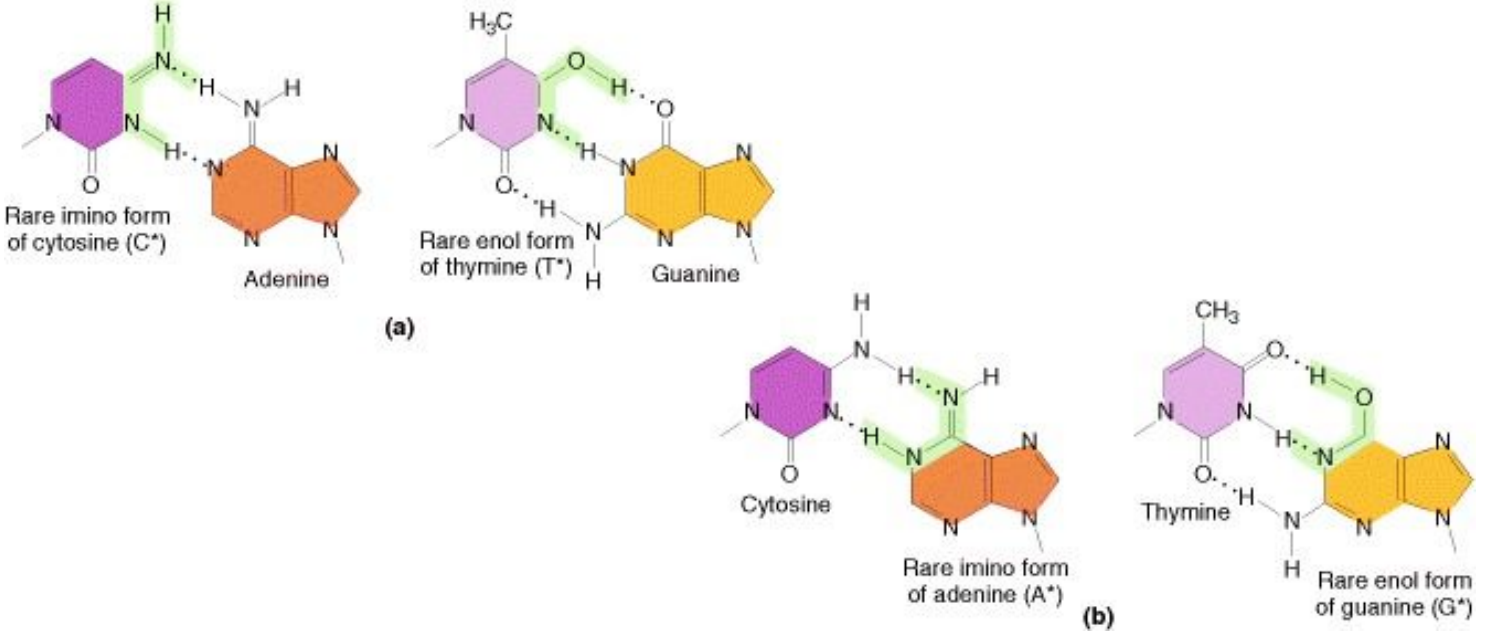
Spontaneus Mutations

An error in DNA replication can occur when an illegitimate nucleotide pair (say, A–C) forms in DNA synthesis, leading to a base substitution.



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.



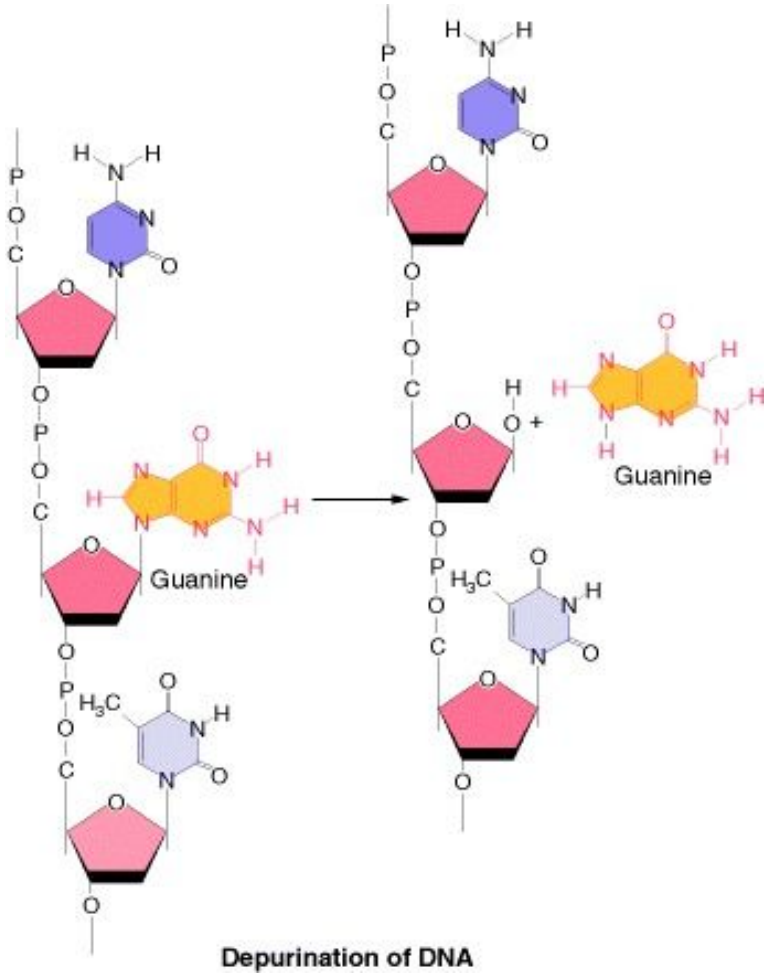
HHMI

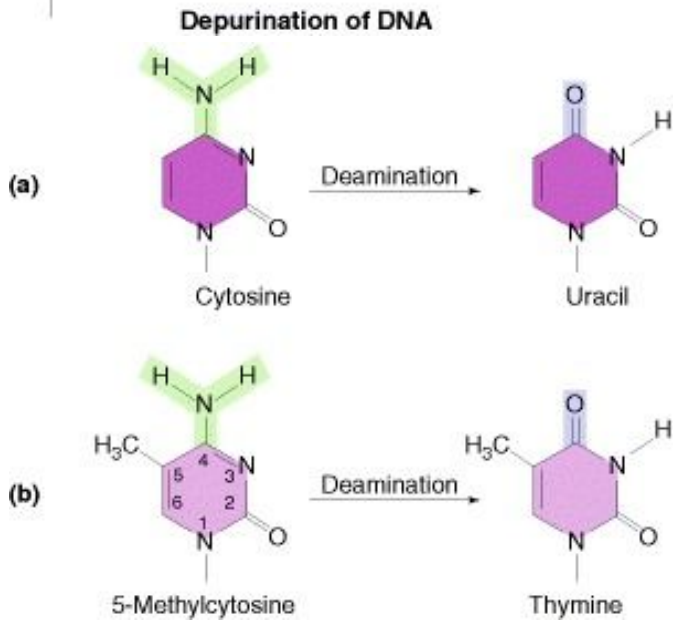
Depurination: the loss of a purine residue (guanine) from a single strand of DNA. The sugar-phosphate backbone is left intact.

Deamination : the loss of an amine group.

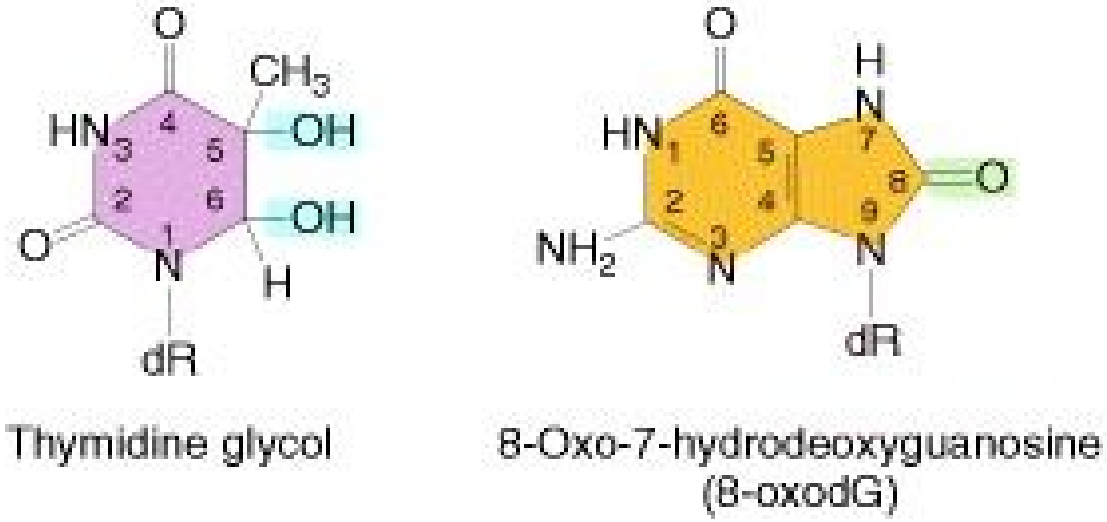
a) Repaired by the Uracil-DNA glycosylase.

b) HOT SPOTS not repaired by the Uracil-DNA glycosylase.





Oxidatively damage: Active oxygen species, such as superoxide radicals ($O_2 \cdot$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH \cdot$), are produced as by-products of normal aerobic metabolism.



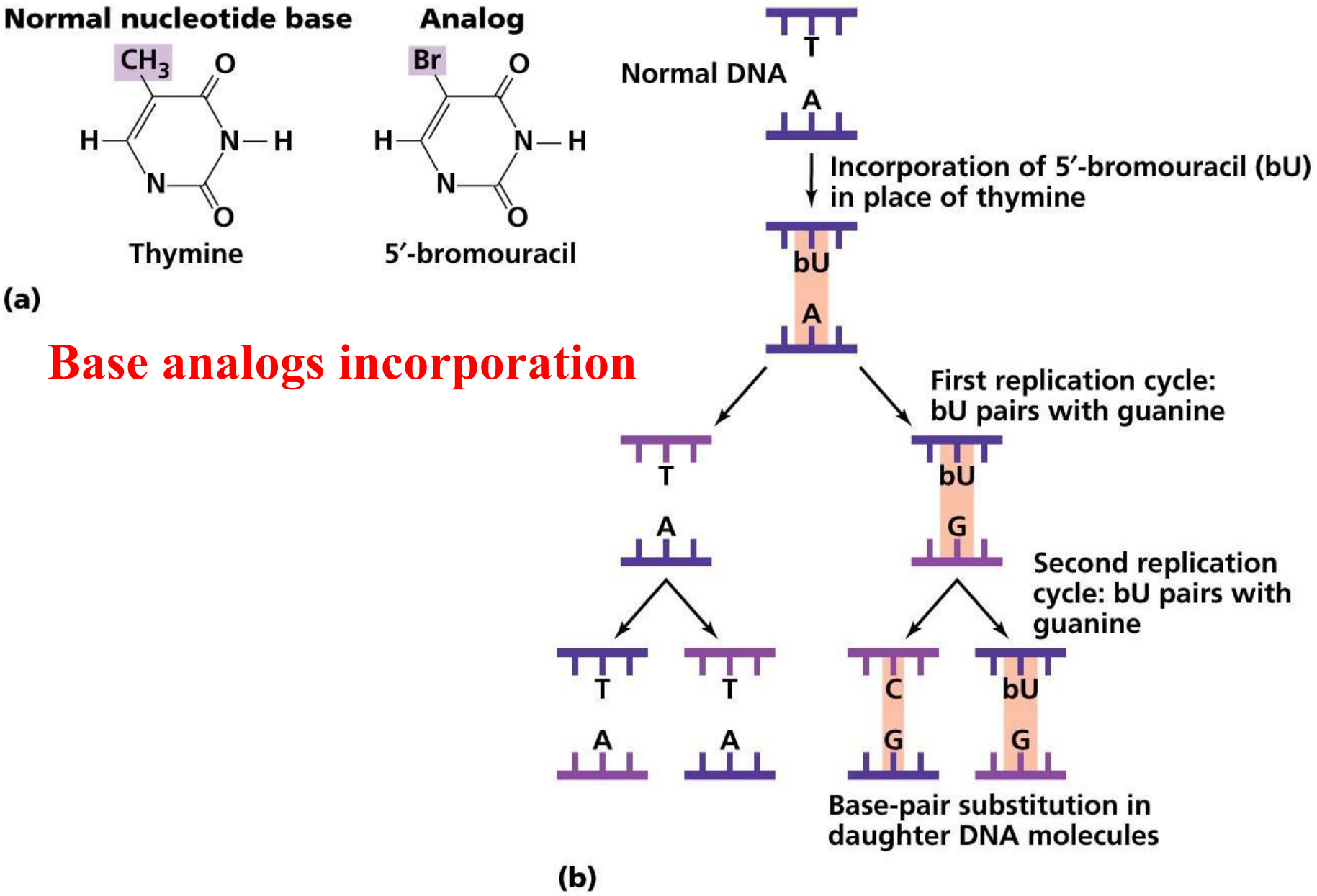
Thymidine glycol

8-Oxo-7-hydrodeoxyguanosine
(8-oxodG)

DNA damage products formed after attack by oxygen radicals.
dR = deoxyribose.
Mispairs with A, resulting in a high level of G → T transversions.

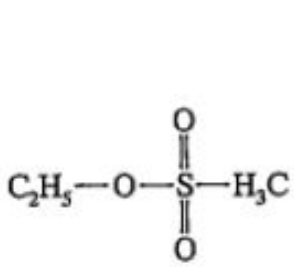
Induced mutations

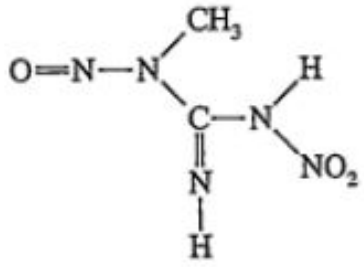
Mutagens → chemicals, radiations

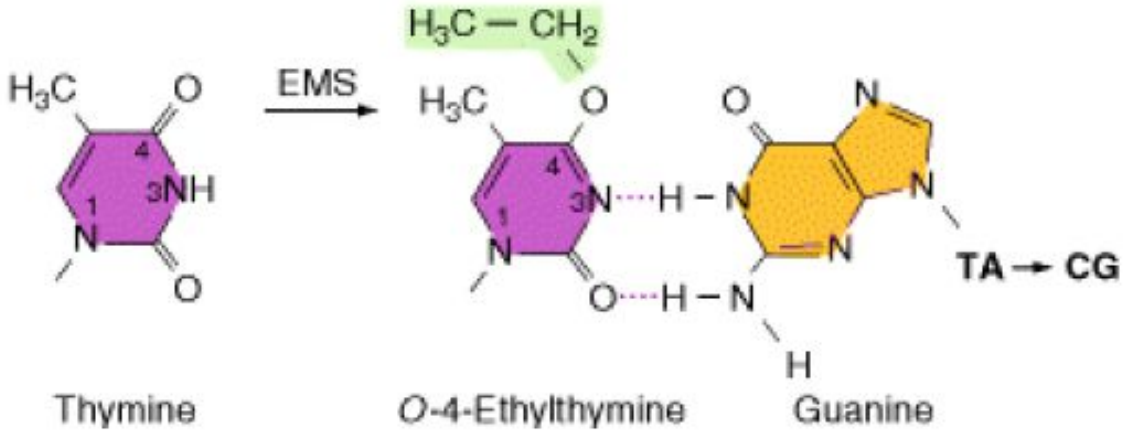


Alkylating agents

EMS (Ethyl methanesulfonate)
NG (nitrosoguanidine)



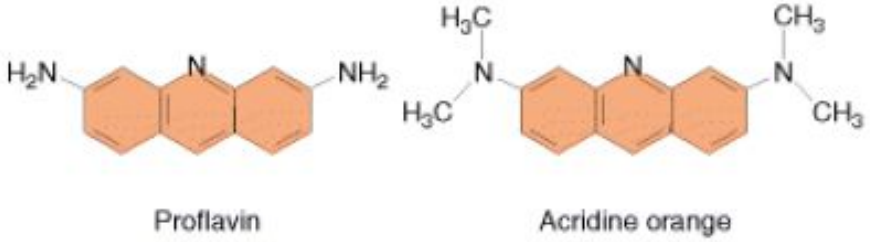




Intercalating agents

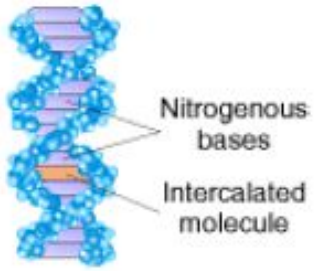
Proflavin
Acridine orange
Ethidium bromide

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.



(a)

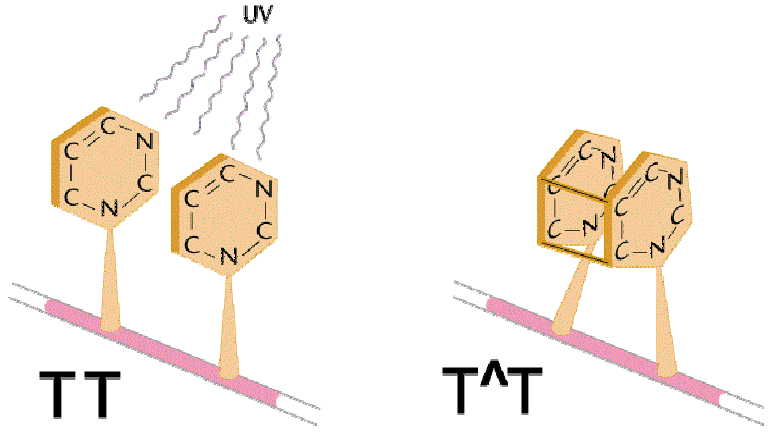
Ethidium bromide

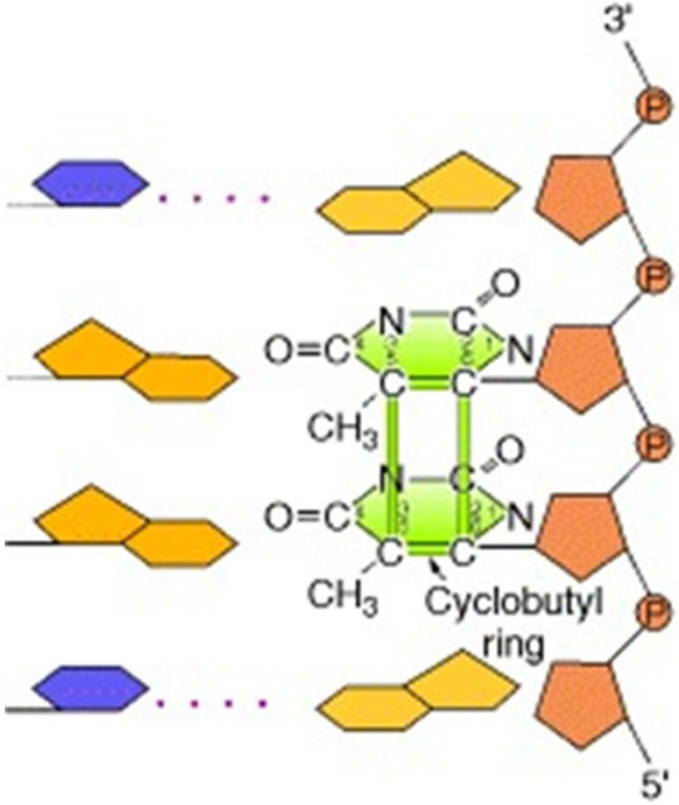


(b)

UV radiation

Generates a number of photoproducts in DNA





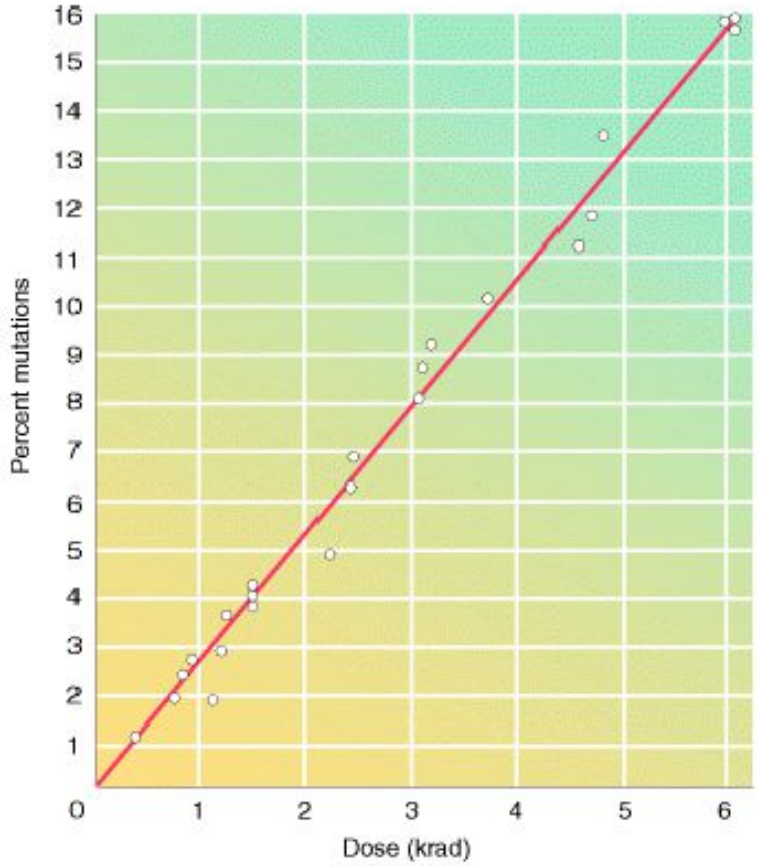
Ultraviolet light stimulates the formation of a four-membered cyclobutane ring (green) between two adjacent pyrimidines on the same DNA strand by acting on the 5,6 double bonds.

Ionizing radiation (X-Rays)

Results in the formation of ionized and excited molecules that can cause damage to cellular components and to DNA

Type of radiation	Percentage of male X chromosomes bearing recessive lethal mutations after a dose of 1000 roentgens*
Visible light (spontaneous)	0.15
X rays (25 Mev)	1.70
β rays, γ rays, hard X rays	2.90
Soft X rays	2.50
Neutrons	1.90
α rays	0.84

* The roentgen (r) is a unit of radiation energy.



DNA repair Systems

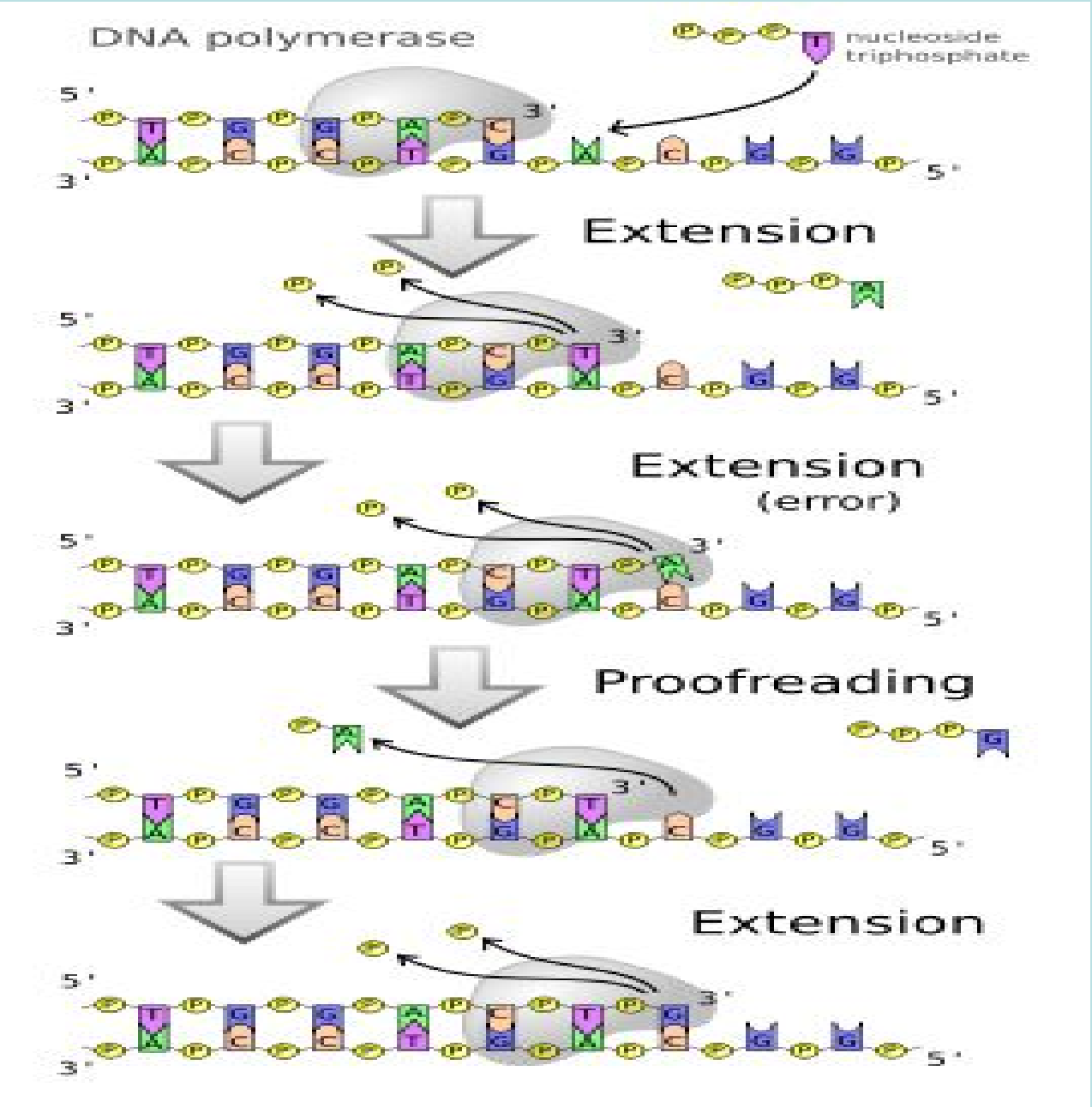
DNA damage, due to environmental factors and normal metabolic processes inside the cell, occurs at a rate of 1,000 to 1,000,000 molecular lesions per cell per day. (Lodish et al., 2004). While this constitutes only 0.000165% of the human genome's approximately 6 billion bases (3 billion base pairs) unrepaired lesions in critical (such as tumor suppressor genes) can impede a cell's ability to carry out its function and appreciably increase the likelihood of tumor formation.

Cells cannot function if DNA damage corrupts the integrity and accessibility of essential information in the genome (but cells remain superficially functional when so called "non-essential" genes are missing or damaged). Depending on the type of damage inflicted on the DNA's double helical structure, a variety of repair strategies have evolved to restore lost information. If possible, cells use the unmodified complementary strand of the DNA or the sister chromatid as a template to losslessly recover the original information. Without access to a template, cells use an error-prone recovery mechanism known as translesion synthesis as a last resort. (this can be also a standard mechanism: many double strand breaks in mammalian cells, for example, are repaired without any template to be read).

The types of molecules involved and the mechanism of repair that is mobilized depend on:

1. **The type of damage that has occurred to DNA**
2. **If the cell is in a senescence status**
3. **The phase of the cell cycle that the cell is in.**

Proofreading by DNA Polymerase corrects copying errors



Single strand damage

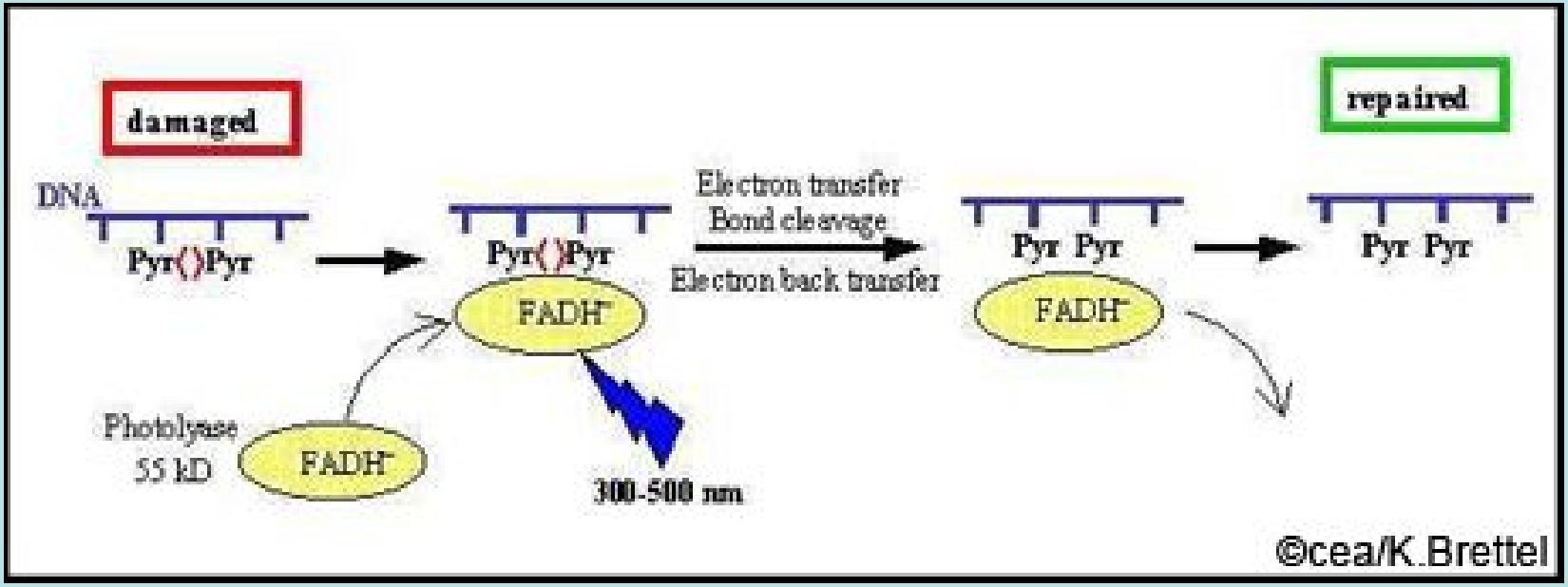
When only one of the two strands of a double helix has a defect, the other strand can be used as a template to guide the correction of the damaged strand.

1. Single step reactions, a direct reversal by a single enzyme through different specific mechanisms. Examples are the methyl guanine methyl transferase (MGMT) that cuts off methyl groups specifically from guanine, and the bacterial photolyase, that breaks chemical bonds formed from UV radiation between neighboring thymine bases.
For this kind of processes no template strand is needed.

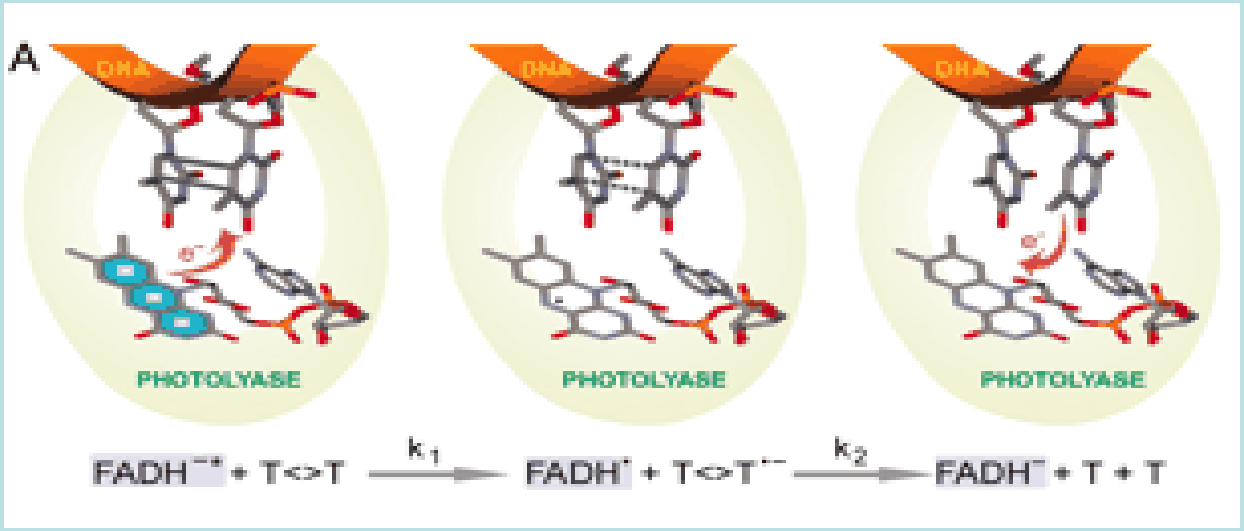
2. Excision repair mechanisms, remove the damaged nucleotide and replace it with an undamaged nucleotide complementary to that found in the undamaged DNA strand (Watson., 2004). Different types of repair mechanisms are found in mammalian system:

a. **Base Excision Repair, (BER) which repairs damage due to a single nucleotide caused by oxidation, alkylation, hydrolysis, or deamination;**
b. **Nucleotide Excision Repair, (NER), which repairs damage affecting longer strands of 2-30 bases. This process recognizes bulky, helix-distorting changes such as thymine dimers as well as single-strand breaks (repaired with enzymes such UvrABC endonuclease). A specialized form of NER known as Transcription-Coupled Repair (TCR) deploys high-priority NER repair enzymes to genes that are being actively transcribed;**
c. **Mismatch Repair (MMR), which corrects errors of DNA replication and recombination that result in mispaired nucleotides following DNA replication..**

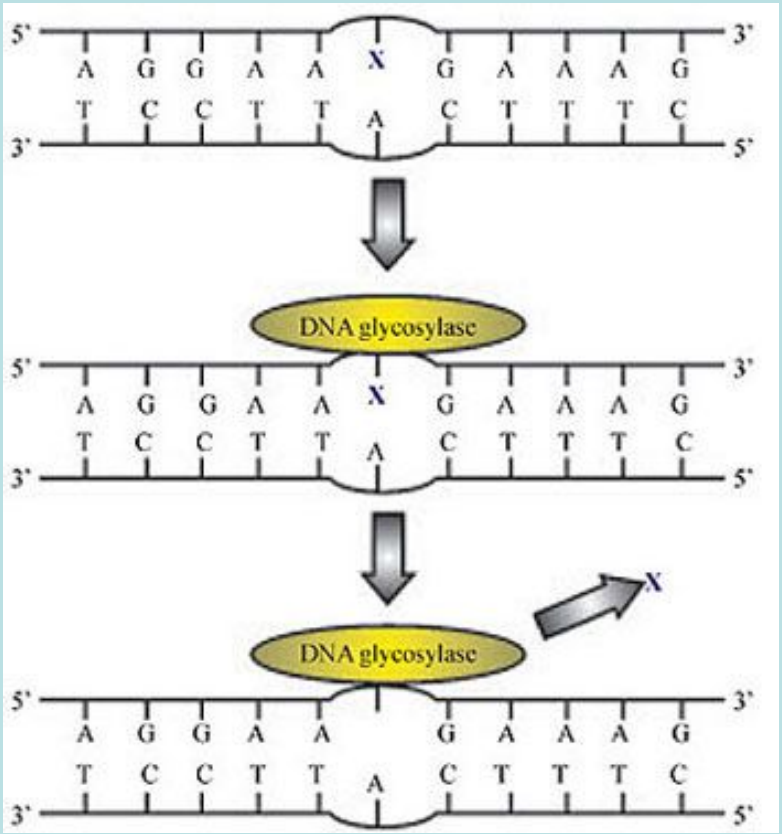
Damage Direct Reverse System

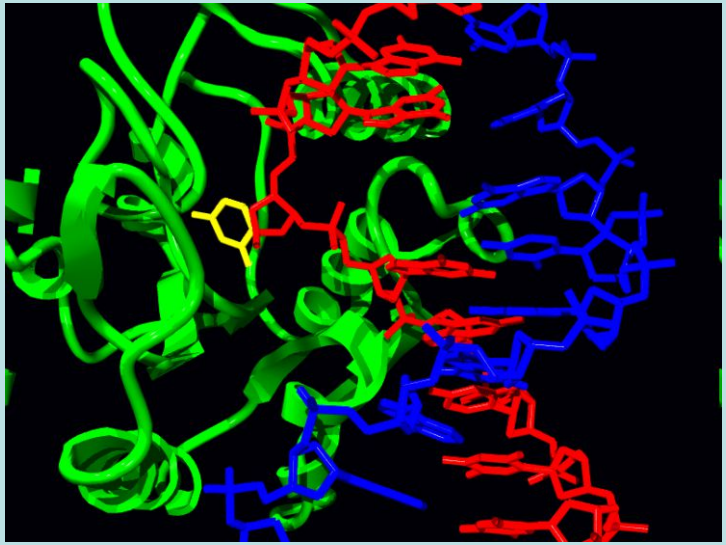


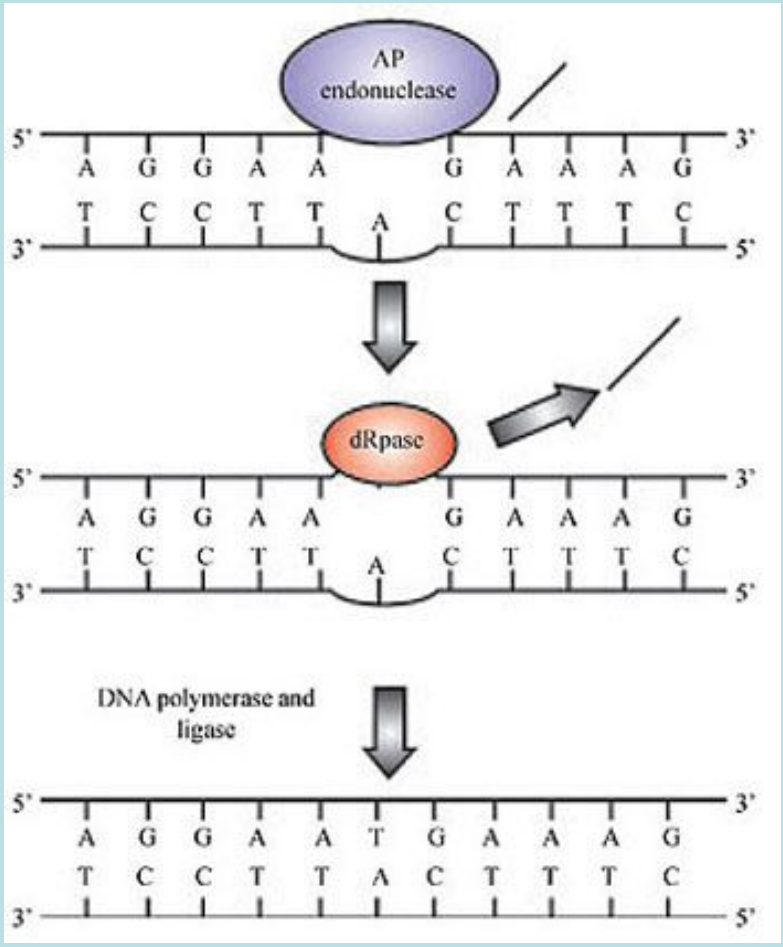
Photolyase binds specifically to damaged DNA. The repair reaction starts most likely with an electron transfer from photoexcited $FADH^-$ to the pyrimidine dimer.



Base Excision Repair System

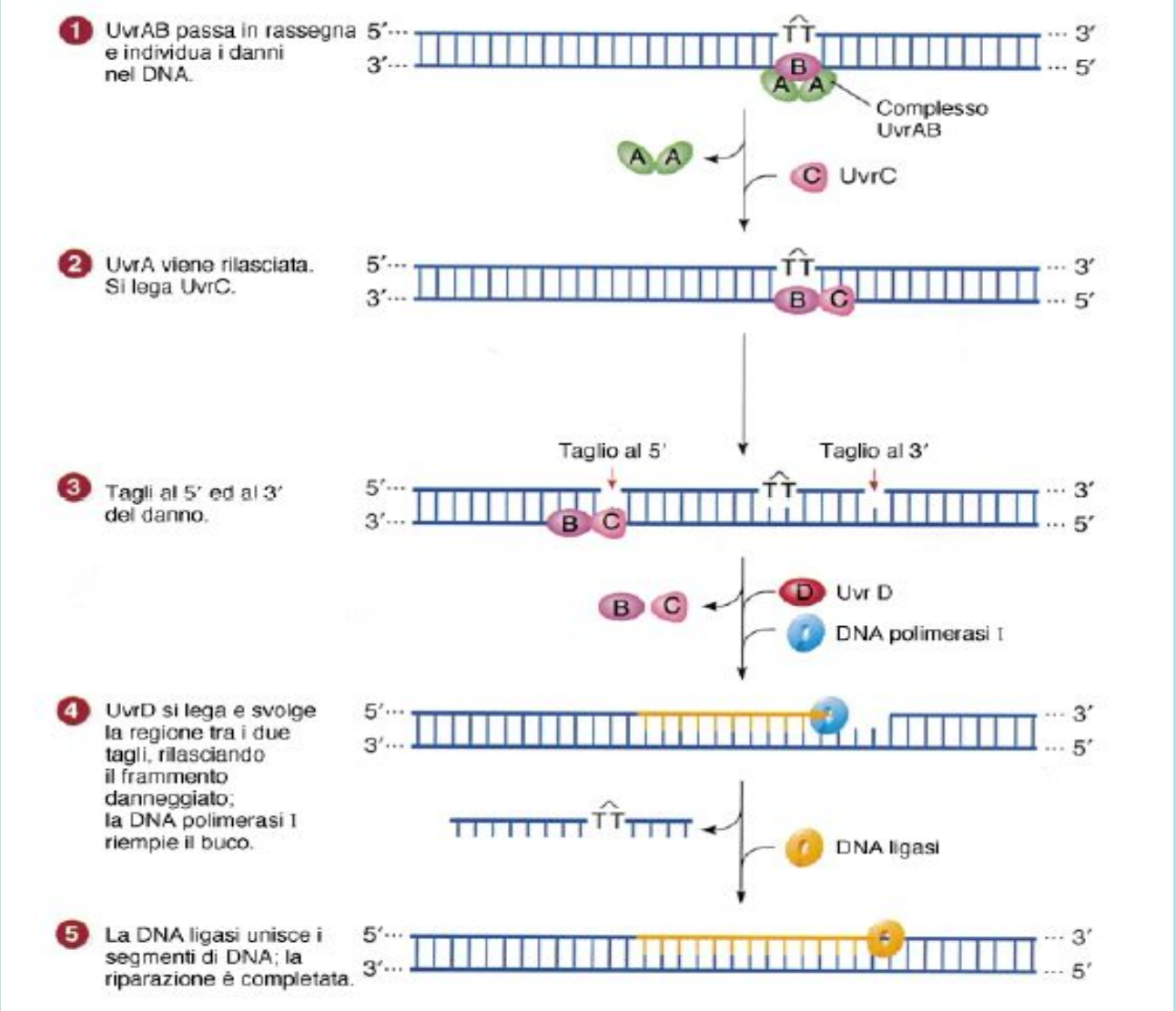






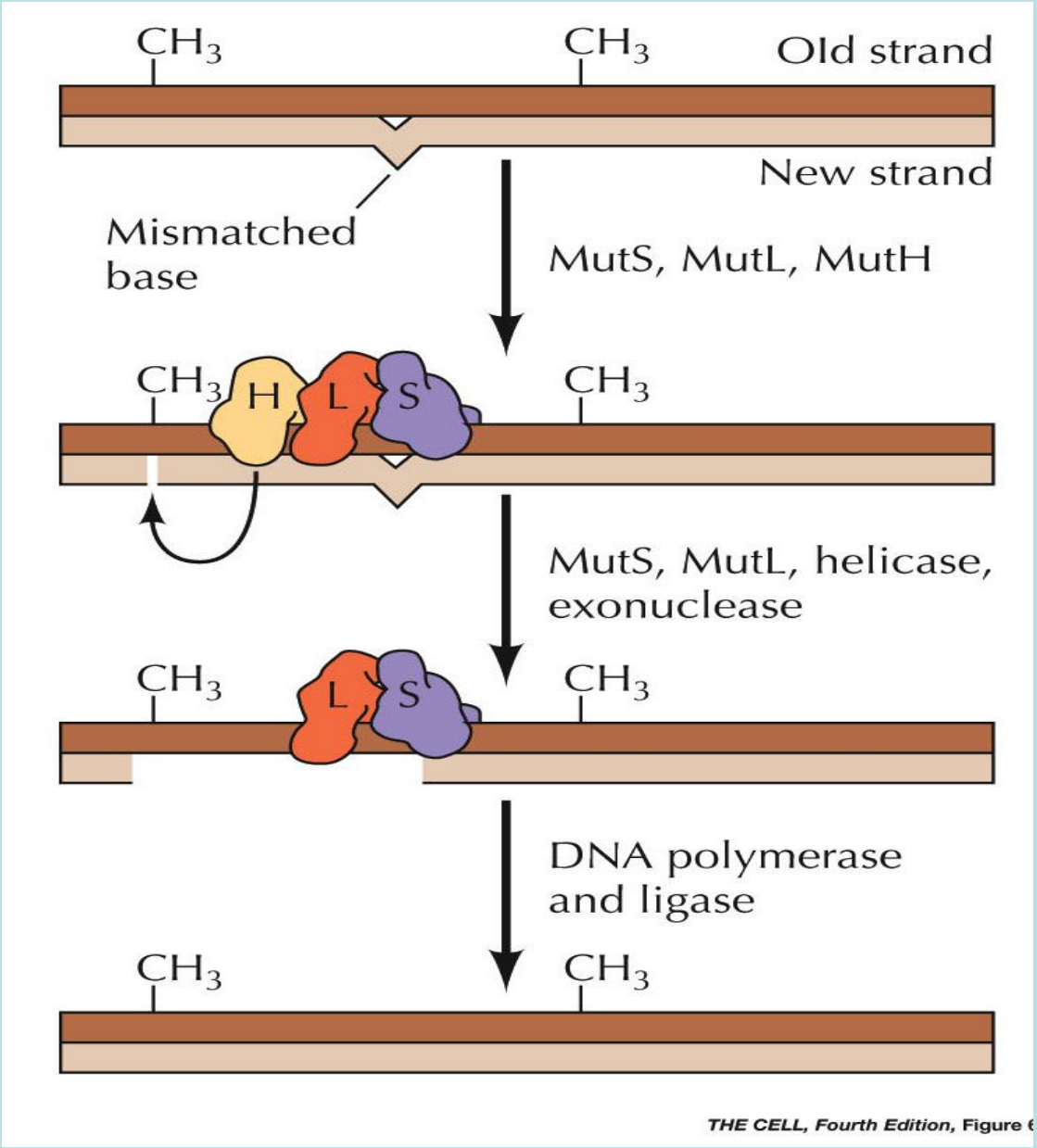
Uracil DNA glycosylases remove uracil from DNA, which can arise either by spontaneous deamination of cytosine or by the misincorporation of dU opposite dA during DNA replication.

Nucleotide Excision Repair System
(UvrACB mechanism of *E.coli*)



In eukaryotes exist a similar mechanism using proteins homologues to UvrABC

Mismatch Repair System



In *E. coli*, immediately after replication only the original parental strand carries methyl groups (it is methylated after a period at particular sequences (E.G. 6-Met adenine in GATC sequence)) → the hemimethylated state is used to distinguish replicated origins from nonreplicated. It is not known how other prokaryotes and eukaryotes recognize the daughter strand during mismatch repair.

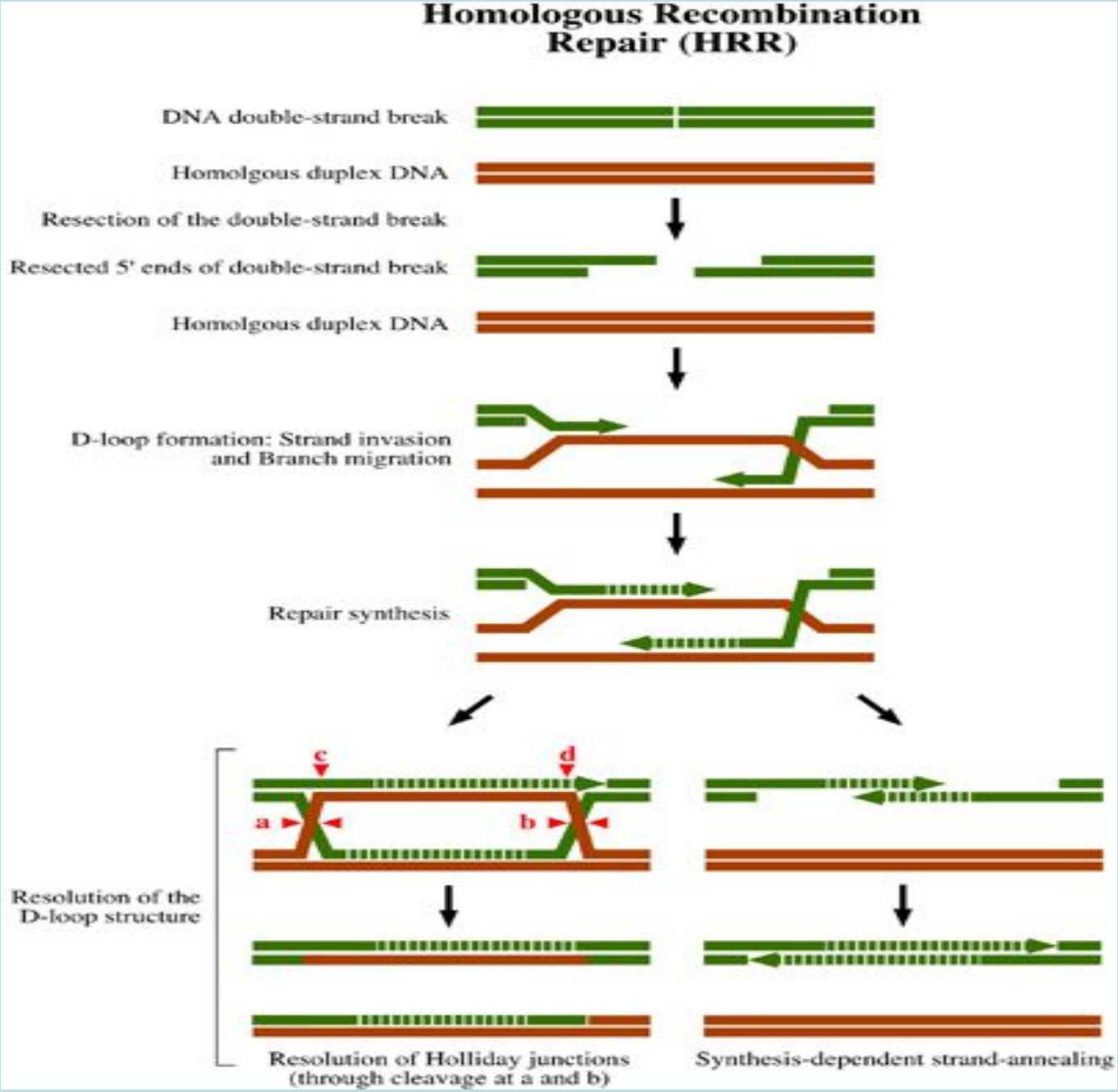
Also in this case a similar mechanism exist in eukaryotes

Double strand break

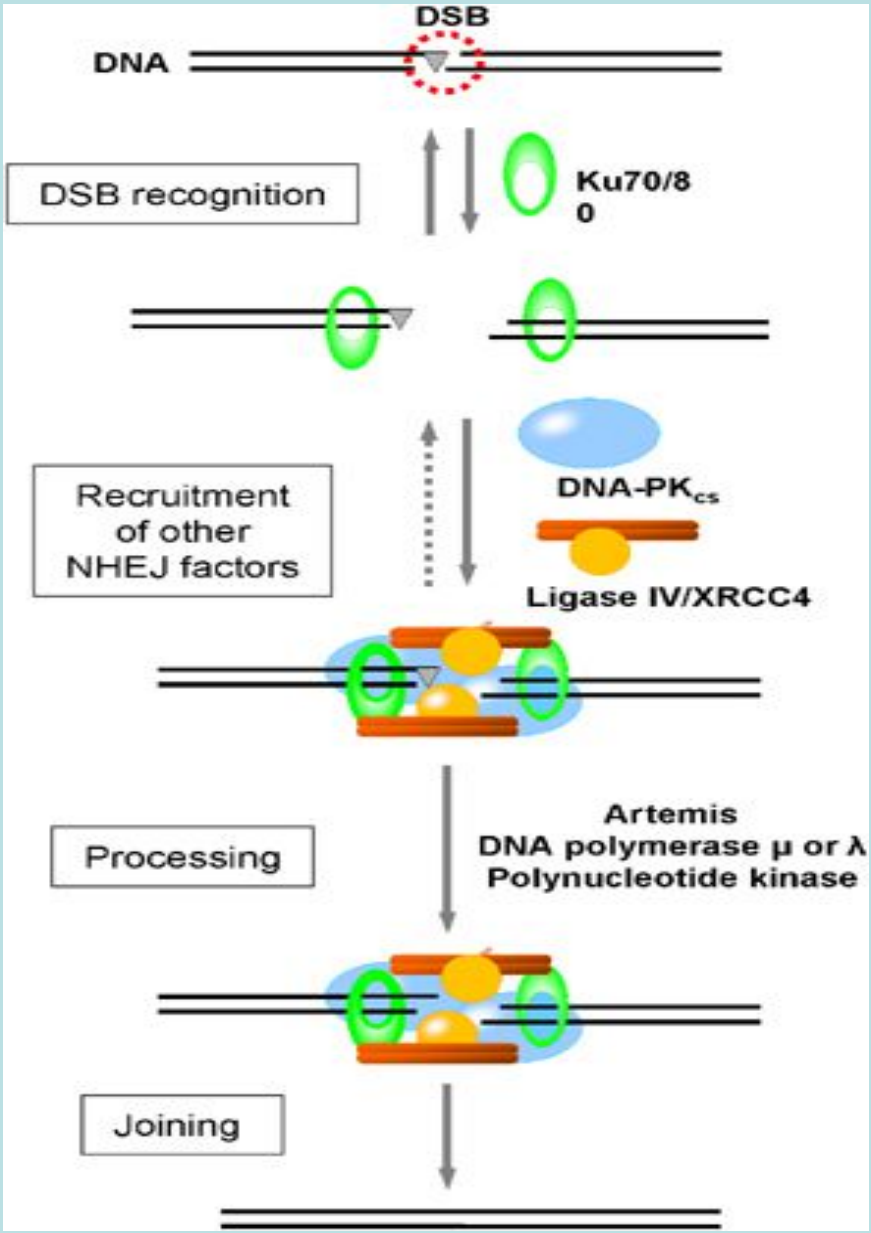
During DNA replication, certain sequences termed fragile sites are particularly susceptible to double-strand break formation, a very dangerous damage for cells under replication.
There are two main mechanisms used to solve this kind of problems, usually known as:

1. **Recombination-Repair System, when the single strand of another duplex is used to replace the gap.**
2. **Non-Homologous End-Joining, that repairs the double-strand breaks when homologous sequence is not available**

Recombination-Repair System



Non-Homologous End-Joining (NHEJ)



NHEJ is the repair system mainly used to resolve double-strand breaks. If the ends are homologous the end-joining will not loose information, otherwise <u>mutations are generated</u> through nucleotide deletion and insertion that occurs during the processing steps prior to ligation. Moreover the inability to repair double-strand breaks in DNA is particularly severe and leads to chromosomal instability, resulting in a genome alteration.

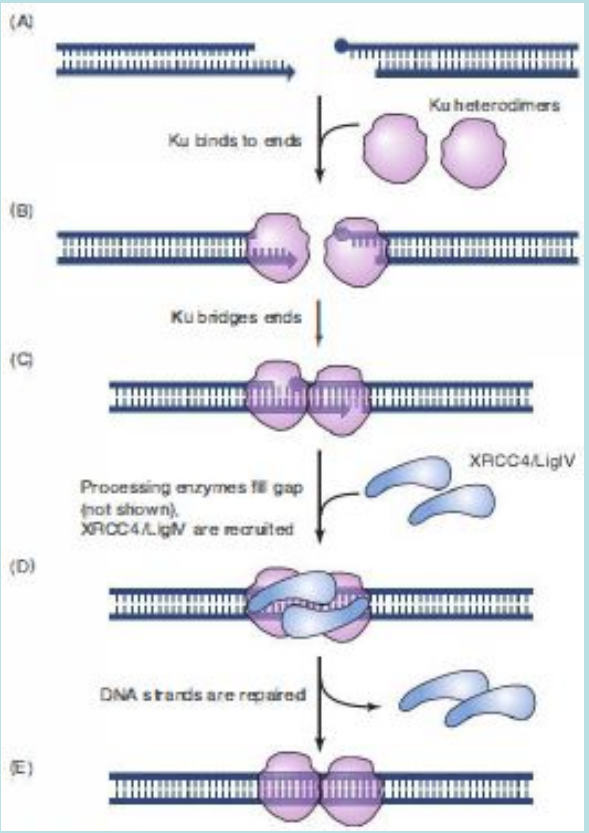


FIGURE 16.25 Nonhomologous end-joining. The blue dot on one of the two DSB ends signifies a nonligatable end (A). The double-strand break ends are bound by the Ku heterodimer (B). The Ku:DNA complexes are juxtaposed (C) to bridge the ends and the gap is filled in by processing enzymes and Pol lambda or Pol mu. The ends are ligated by the specialized DNA ligase LigIV with its partner XRCC4 (D) to repair the double-strand break (E).

The Immune System

The immune system of vertebrates mounts a protective response that distinguishes foreign (nonself) soluble molecules or molecules on microorganisms from molecules or cells of the organism itself (self).

Responses to antigens on viruses and bacteria, are highly specific and are the expression of adaptive (acquired) immunity.

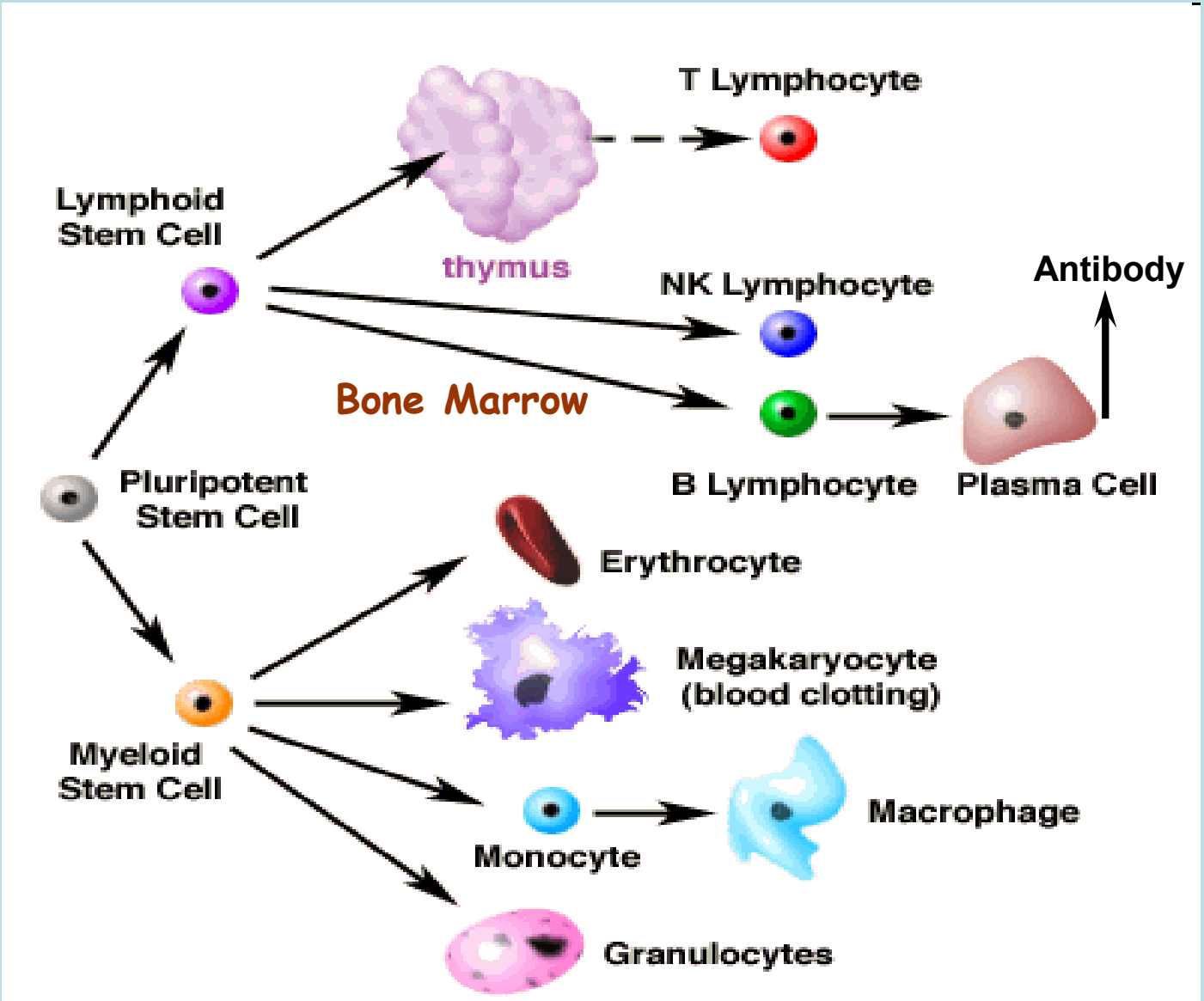
In contrast to adaptive immunity, innate immunity provides an immediate (without latency) first line of defense against invading microorganisms. Invertebrates have an innate immune system but no adaptive system.

• **Innate immunity**: it depends on receptors encoded in the gennline to recognize shared and conserved structural patterns, as occurring on microbial pathogens. The innate response is nonspecific for any given pathogen and does not generate memory. It is triggered in different ways and to different degrees, as determined by the nature of the foreign microbial antigen inducing it.

• **Adaptive immunity**: The adaptive immune response is characterized by a latency period-generally lasting a few days-required for the clonal selection and expansion oft he B cells and/or T cells specific for the antigen. Clonal selection of B cells or T cells relies on binding of antigen to the surface B cell receptors (BCR) and T cell receptors (TCR). The structural basis for this selection process is provided by the generation of a very large number of BCRs/TCRs to create a high probability of recognizing any foreign molecule. The specific (adaptive) immune response is defined according to whether it is effected mainly by B cells (antibodies) or T cells and can be divided into:

- The antibody response
- The cell-mediated response

The immune system cells



Innate immunity

Microbe-Associated Molecular Patterns

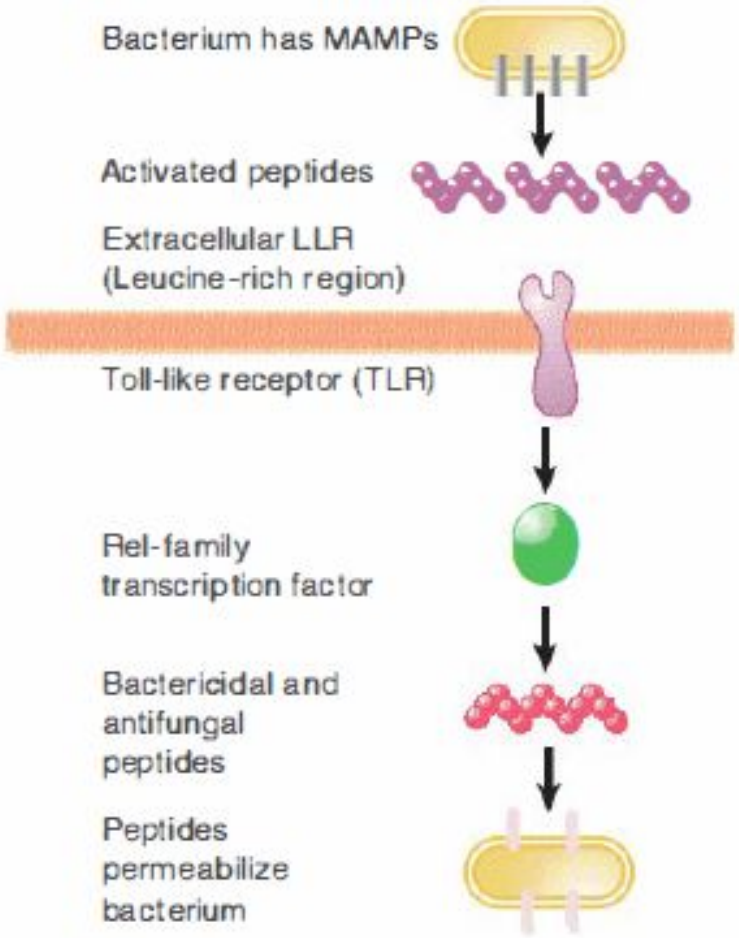


FIGURE 18.2 Innate immunity is triggered by MAMPs. In flies, MAMPs cause the production of peptides that activate Toll-like receptors. The receptors lead to a pathway that activates a transcription factor for the Rel family. Target genes for this factor include bactericidal and antifungal peptides. The peptides act by permeabilizing the membrane of the pathogenic organism.

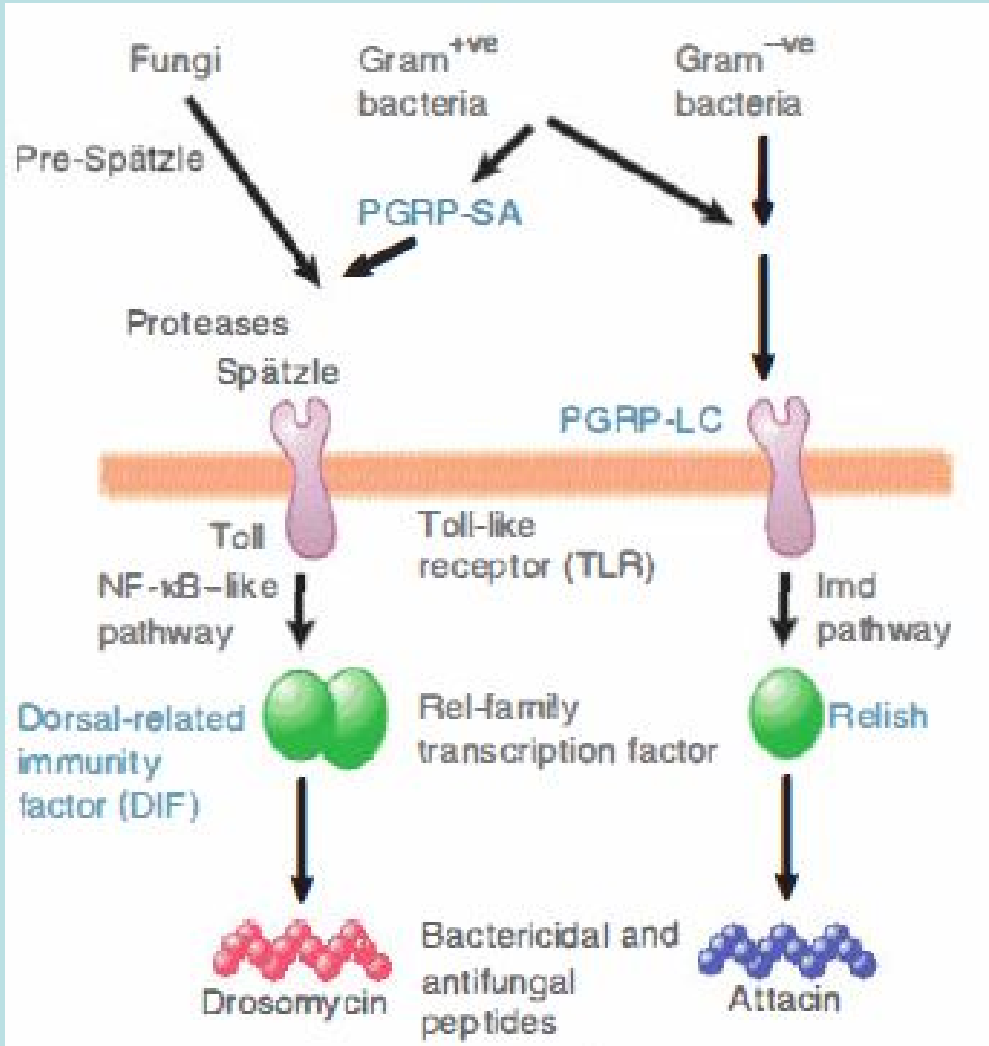


FIGURE 18.3 One of *Drosophila*'s innate immunity pathways is closely related to the mammalian pathway for activating NF-κB; the other has components related to those of apoptosis pathways.

The antibody response

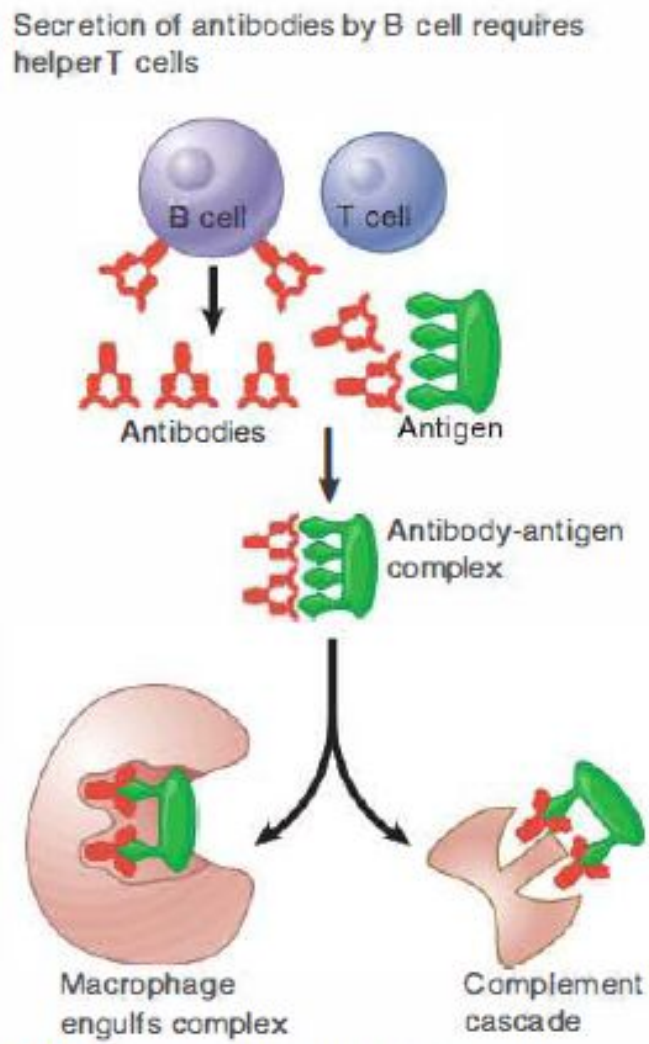


FIGURE 18.4 Free antibodies bind to antigens to form antigen–antibody complexes that are removed from the bloodstream by macrophages or that are attacked directly by the activated complement cascade.

The cell-mediated response

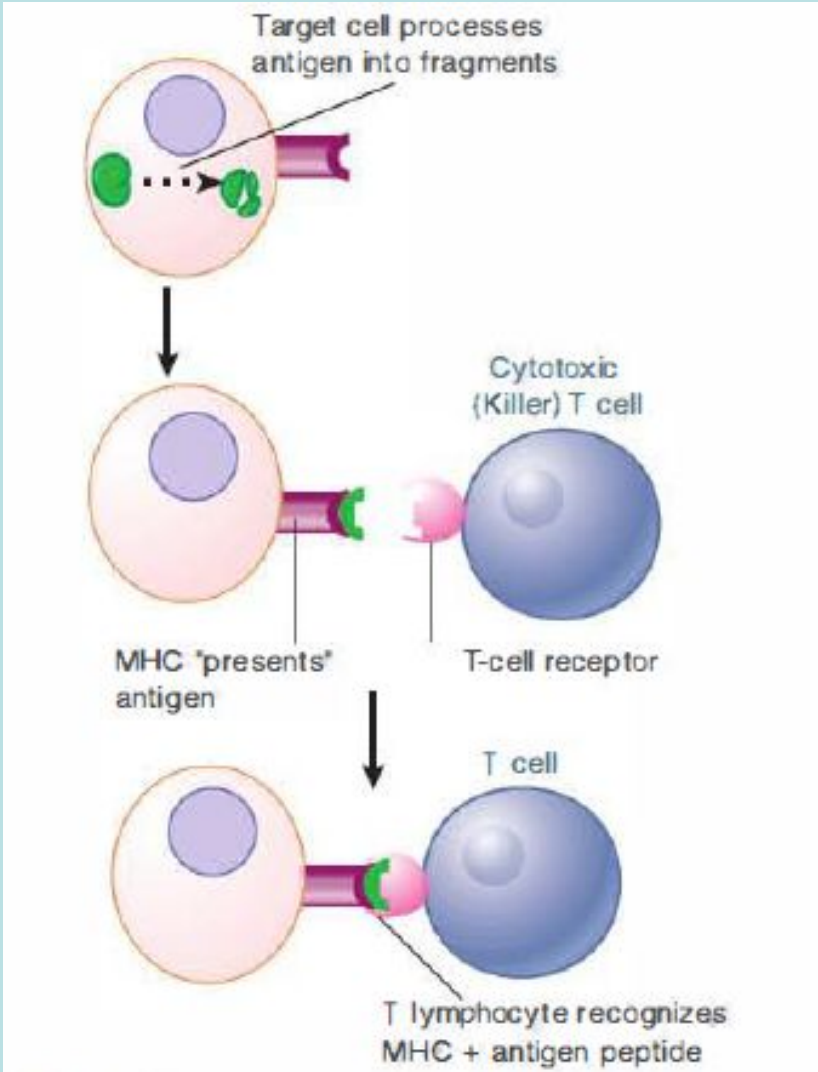
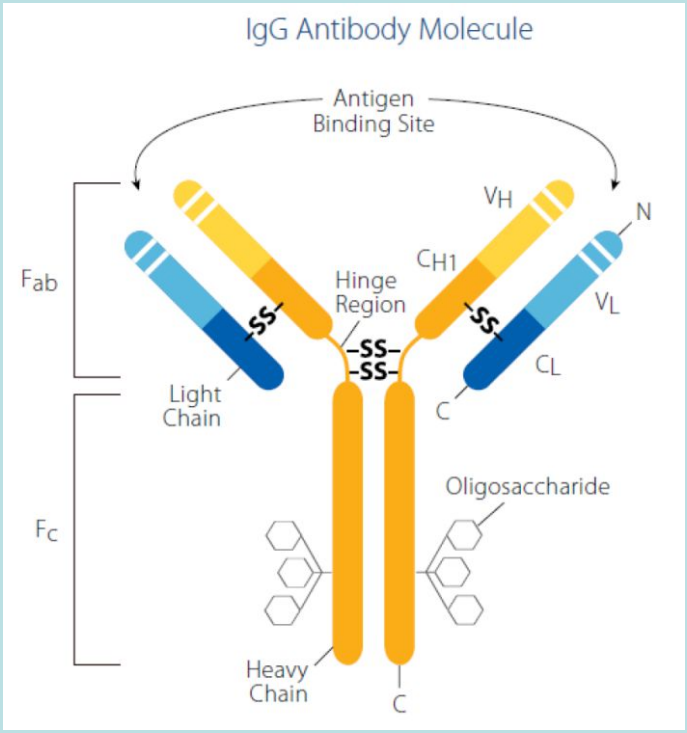
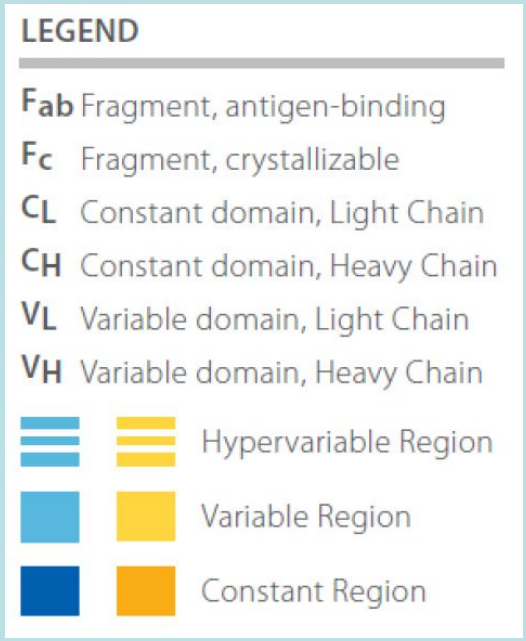
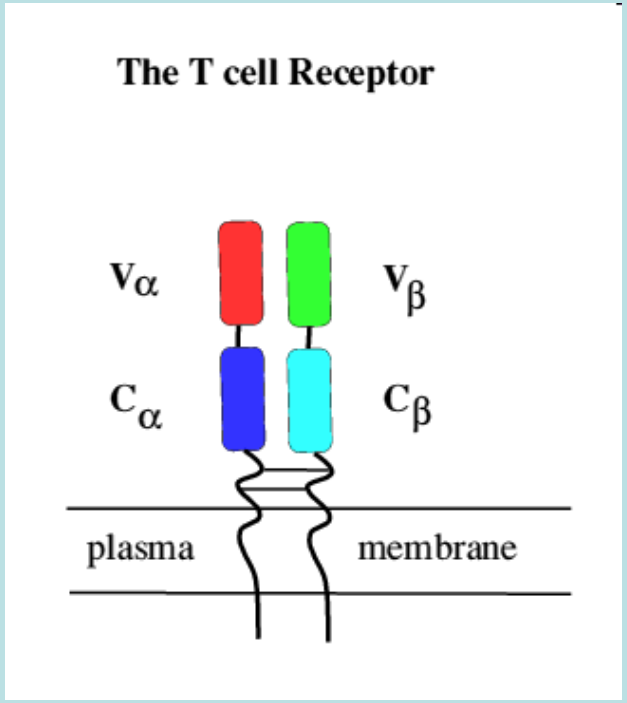


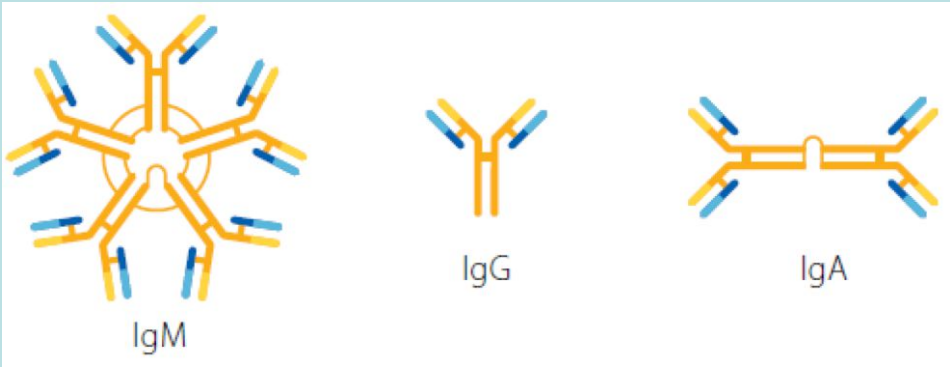
FIGURE 18.5 In cell-mediated immunity, cytotoxic T cells use the T cell receptor (TCR) to recognize a peptide fragment of the antigen that is presented on the surface of the target cell by the MHC molecule.

Antibody (Immunoglobulins) and T cell receptors









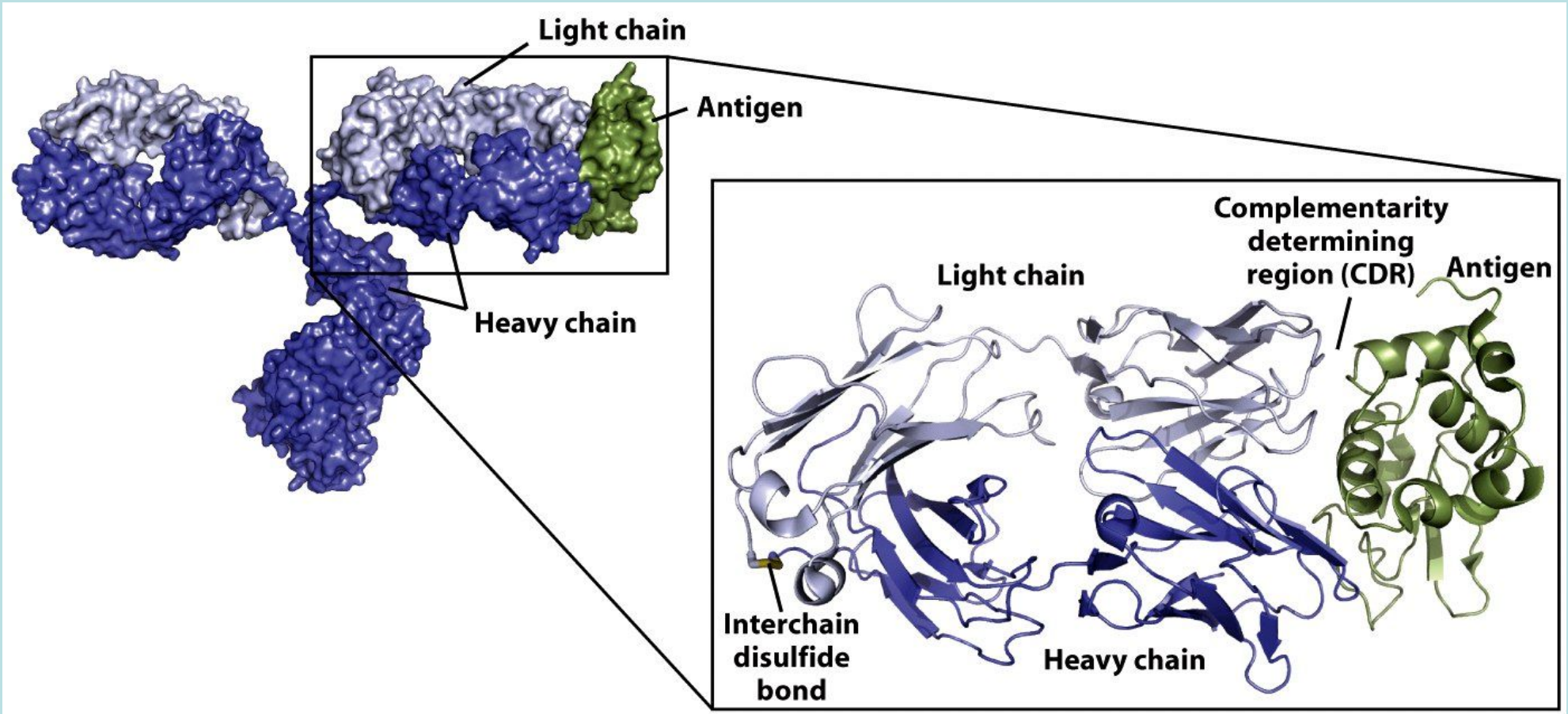
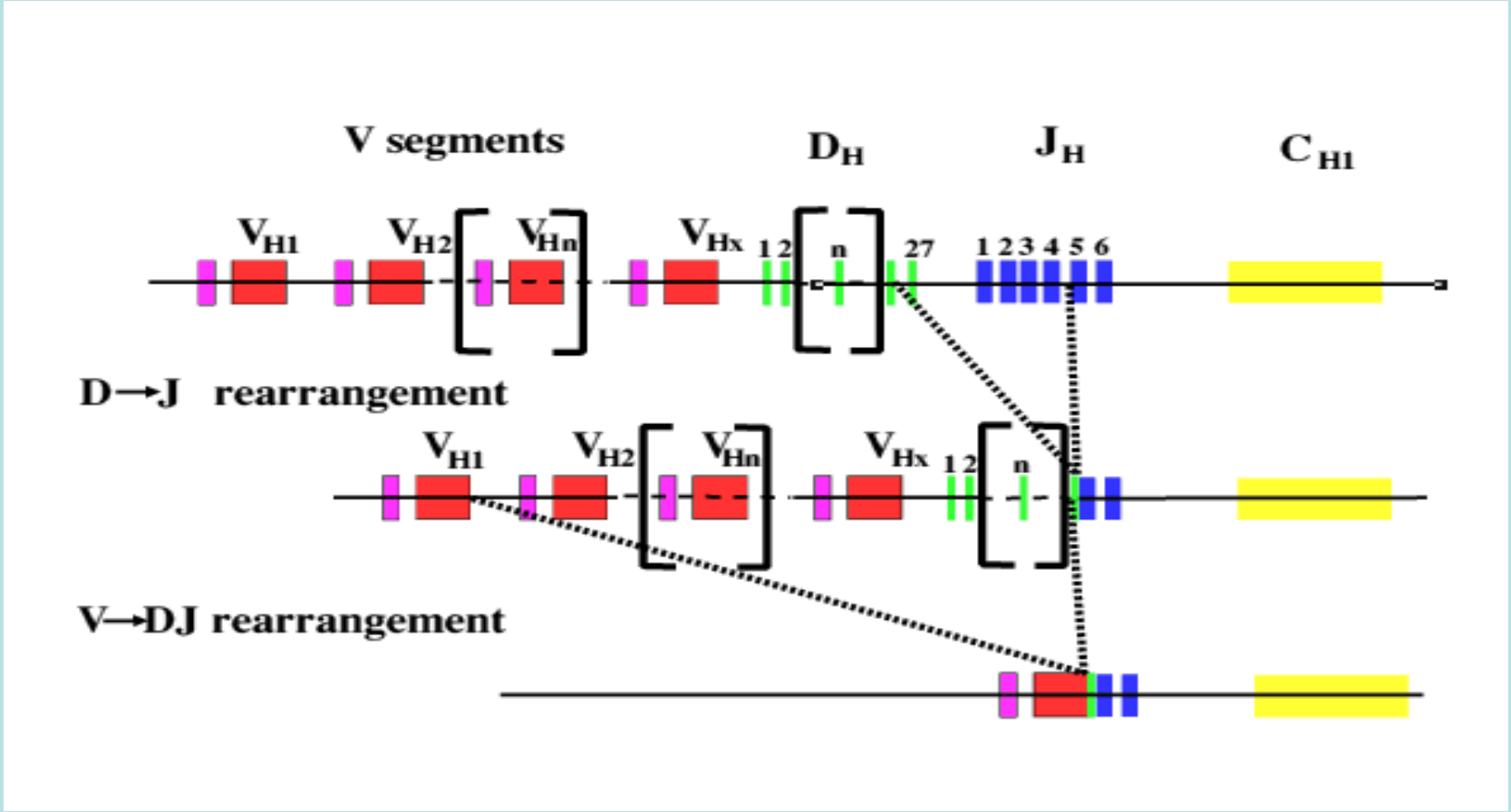


Figure 24-13
Molecular Cell Biology, Sixth Edition

VJ (or VDJ) recombination in immune system cells

V(D)J recombination is a mechanism of genetic recombination that occurs in vertebrates, which randomly selects and assembles segments of genes encoding specific proteins with important roles in the immune system. This site-specific recombination reaction generates a diverse repertoire of T cell receptor (TCR) and immunoglobulin (Ig) molecules that are necessary for the recognition of diverse antigens from bacterial, viral, and parasitic invaders, and from dysfunctional cells such as tumor cells.



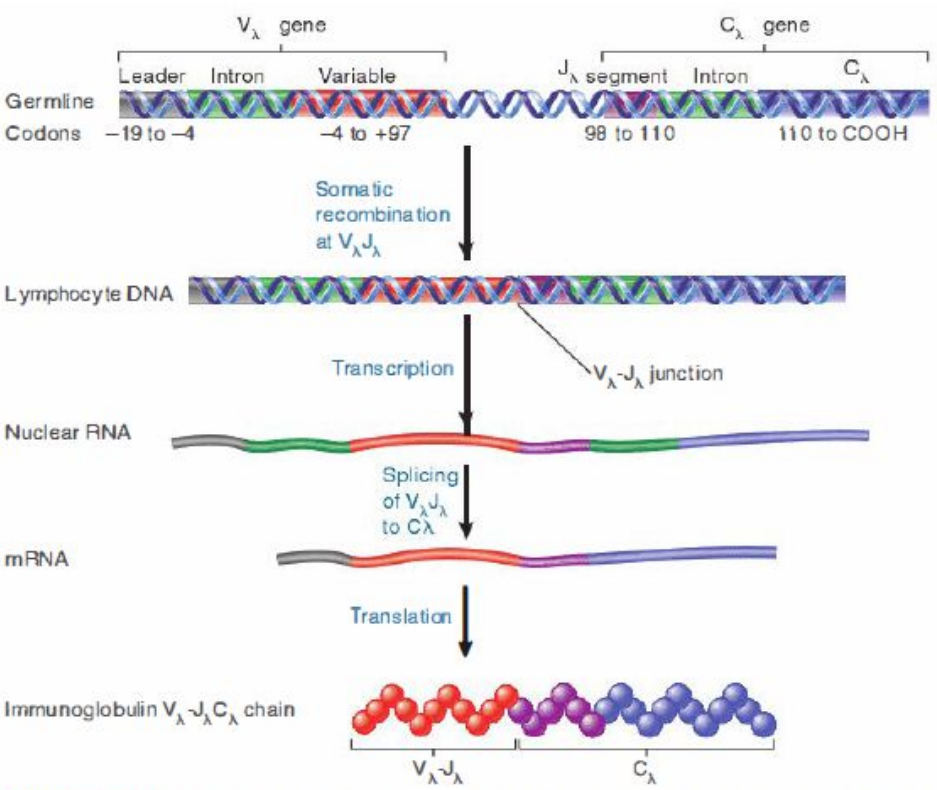


FIGURE 18.8 The C_λ gene segment is preceded by a J_λ segment, so that V_λ-J_λ recombination generates a productive V_λ-$J_\lambda C_\lambda$.

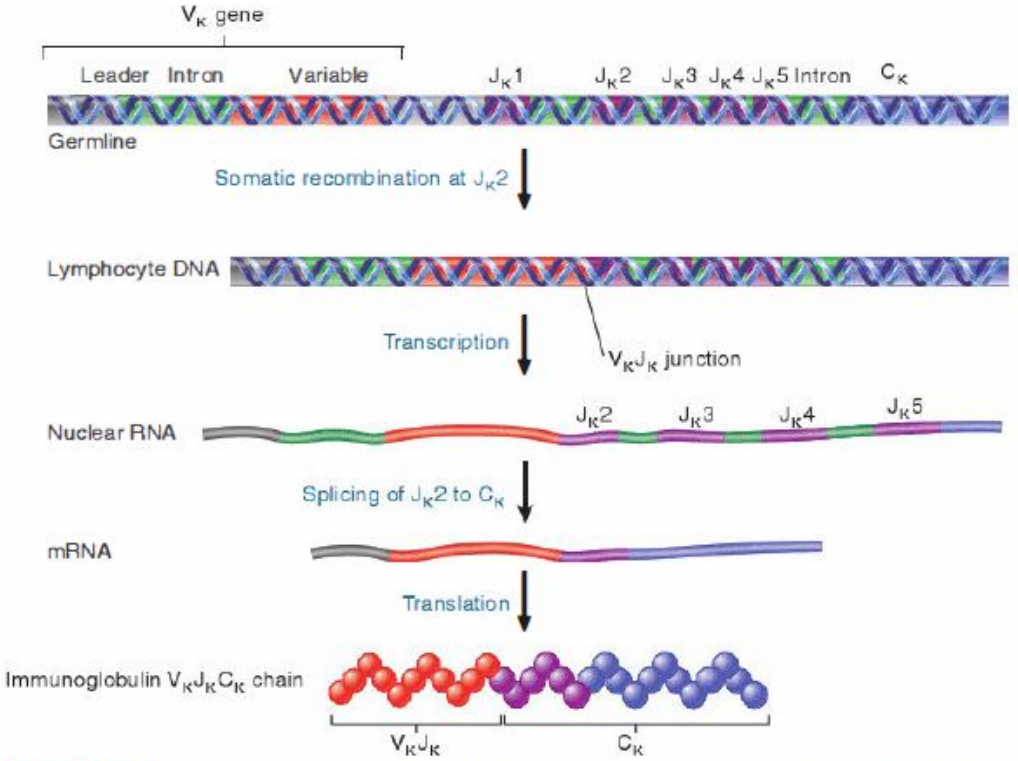


FIGURE 18.9 The C_κ gene segment is preceded by multiple J_κ segments in the germline. V_κ-J_κ joining may recognize any one of the J segments, which is then spliced to the C gene segment during RNA processing.

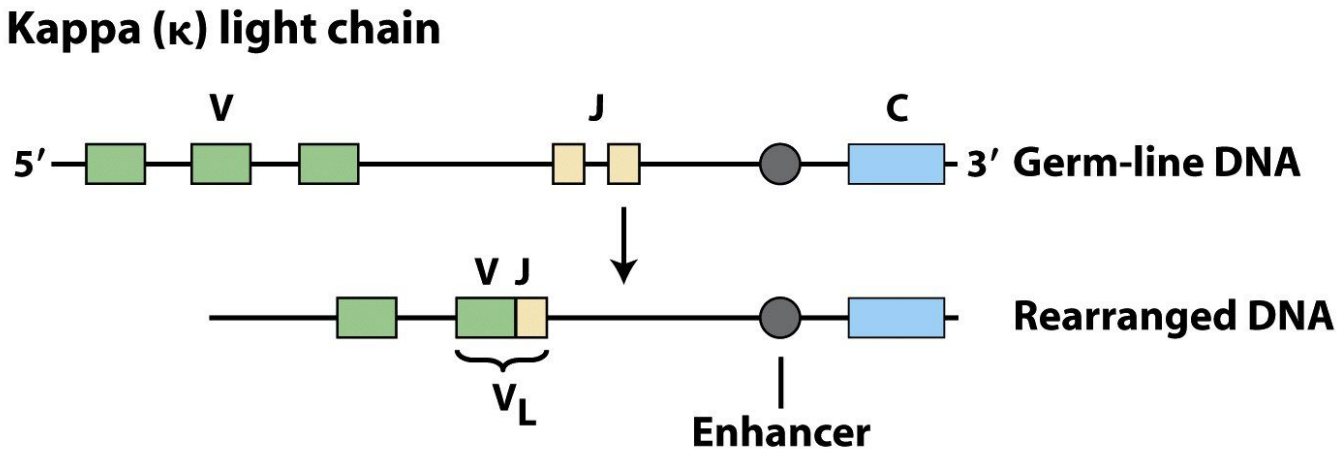


Figure 24-14a
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In the developing B cell, the first recombination event to occur is between one D and one J gene segment of the heavy chain locus. Any DNA between these two genes is deleted. This **D-J recombination** is followed by the joining of one V gene, from a region upstream of the newly formed DJ complex, forming a rearranged **VDJ gene**. All other genes between V and D segments of the new VDJ gene are now deleted from the cell's genome.

A single mature B cell possesses a peculiar VDJ combination thus encoding for a specific antibody.

All other cells do not undergo to V(D)J recombination in their genome and do not express antibodies.

Light locus: 300 V and 4-5 C genes => 1000 different light chains
Heavy locus: 300 V, 20 D and 4 J genes => 4000 different heavy chains

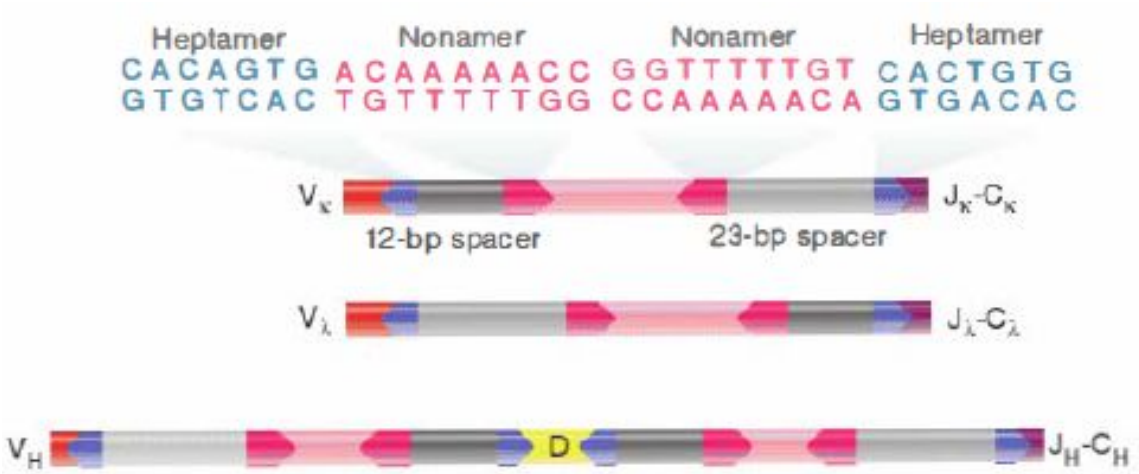
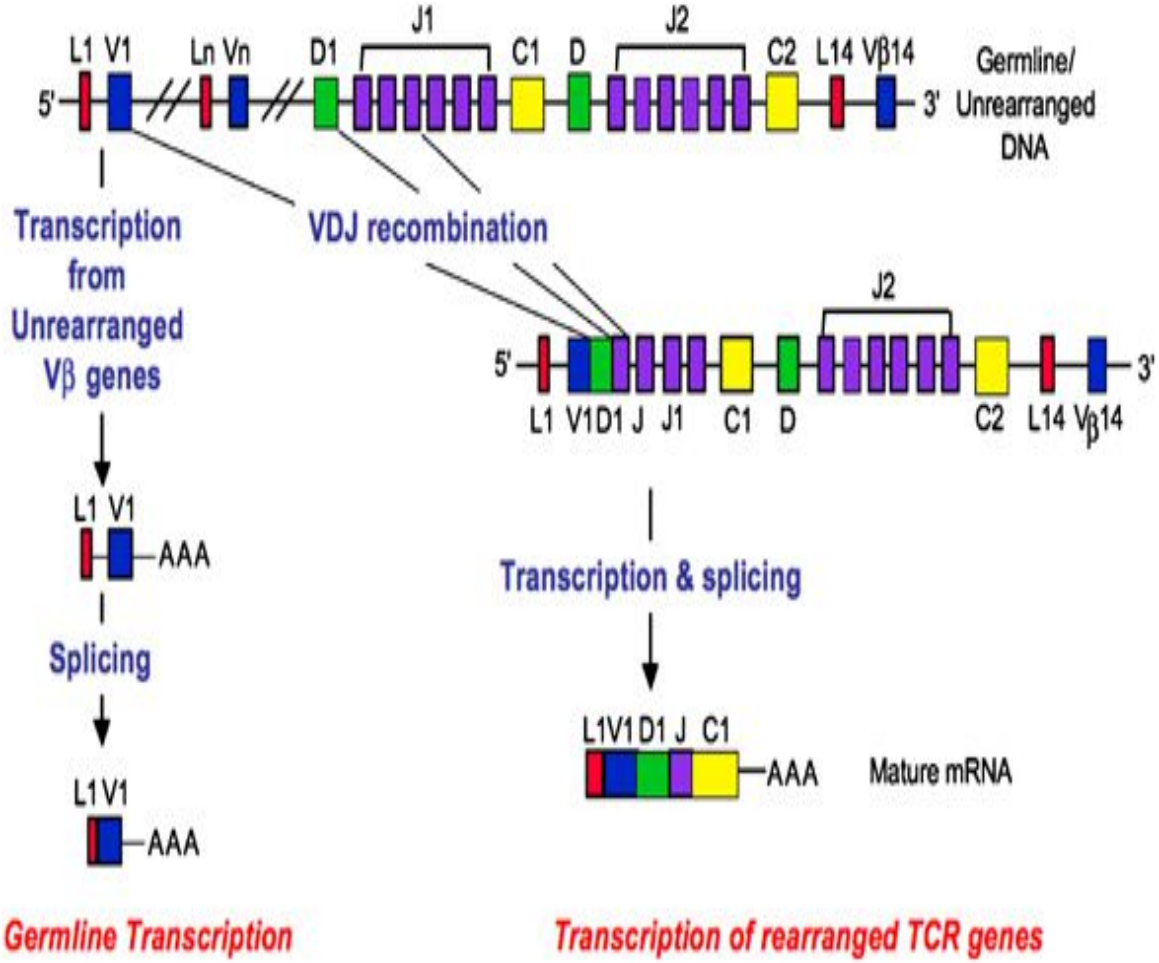
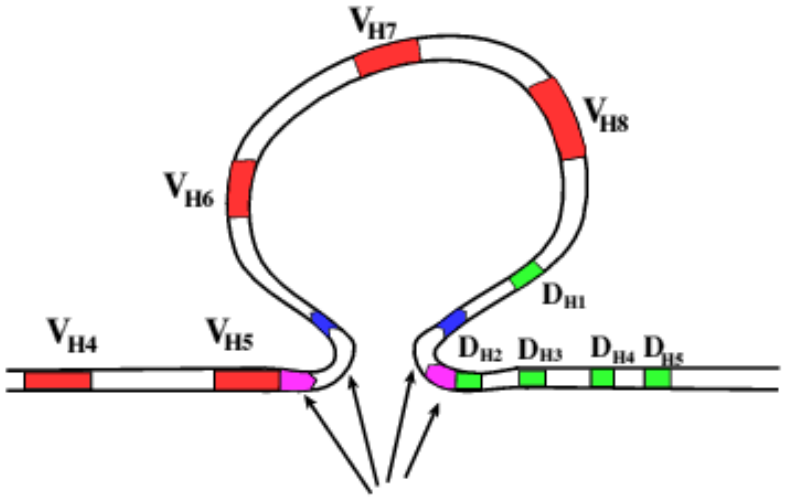


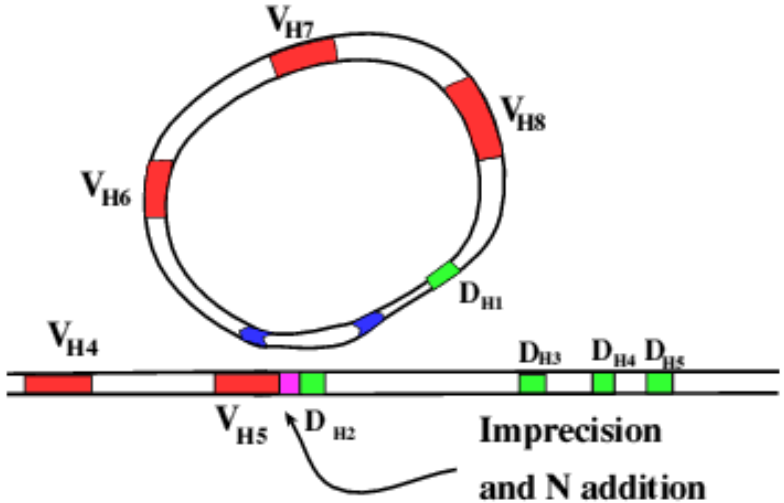
FIGURE 18.14 RSS sequences are present in inverted orientation at each pair of recombining sites. One member of each pair has a 12-bp spacer between its components; the other has a 23-bp spacer.



VDJ joining occurs by DNA recombination



Heptamer and nonamer signal sequences



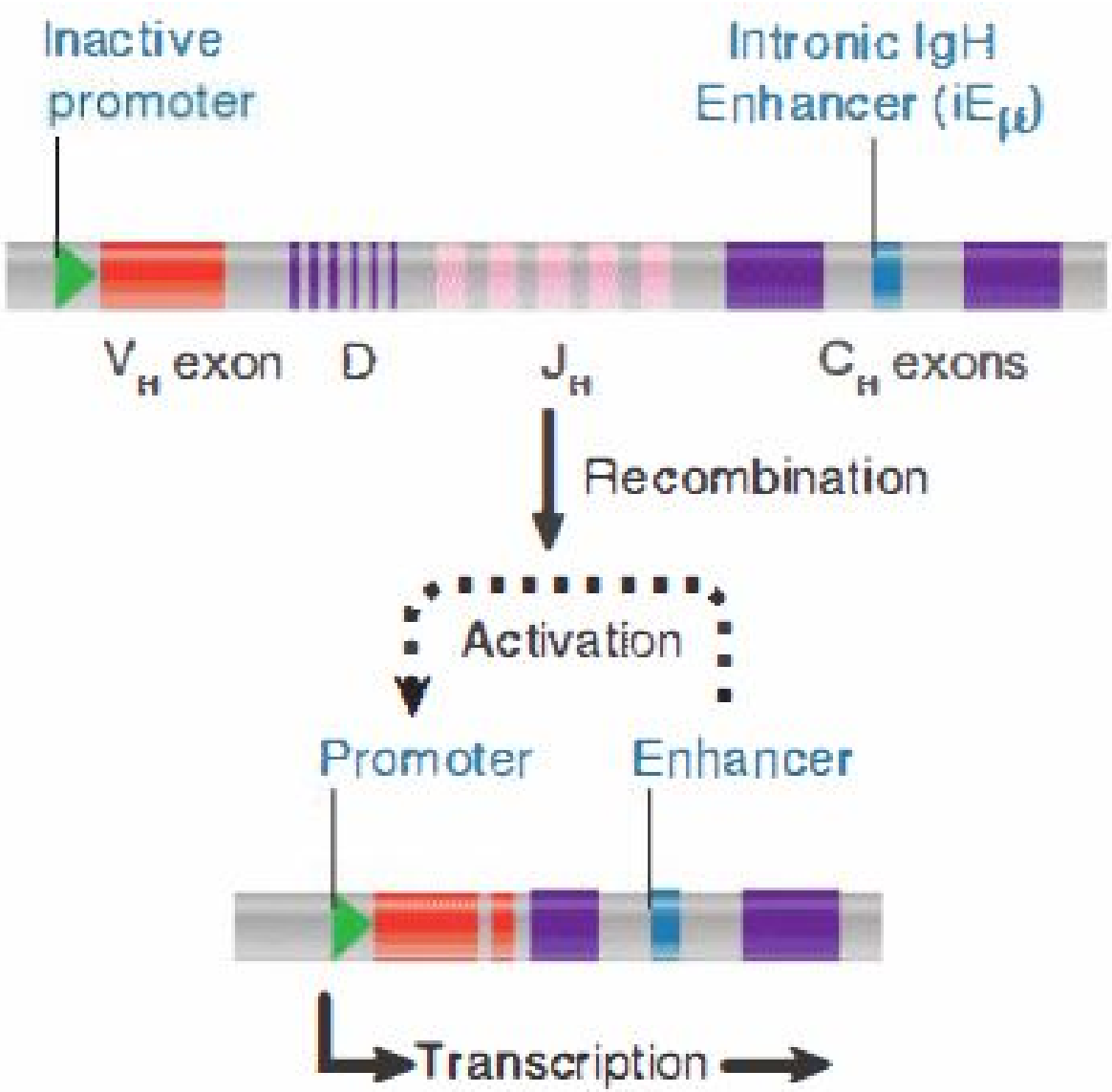


FIGURE 18.18 A V gene promoter is inactive until recombination brings it into the proximity (and therefore under the influence) of the iEμ enhancer that lies downstream of the Sμ region and upstream of the Cμ exon cluster. The enhancer is active only in B lymphocytes.

VDJ recombinase refers to a collection of enzymes some of which are lymphocyte specific, and some that are expressed in many cell types. The initial steps of VDJ recombination are carried out by critical *lymphocyte specific* enzymes, called recombination activating gene-1 and -2 (RAG1 and RAG2).

VDJ recombination occurs
with a mechanism similar to **NHEJ**.

The loss or the addition of nucleotides at the joining points greatly **increases the variability** in the variable regions of expressed antibodies.