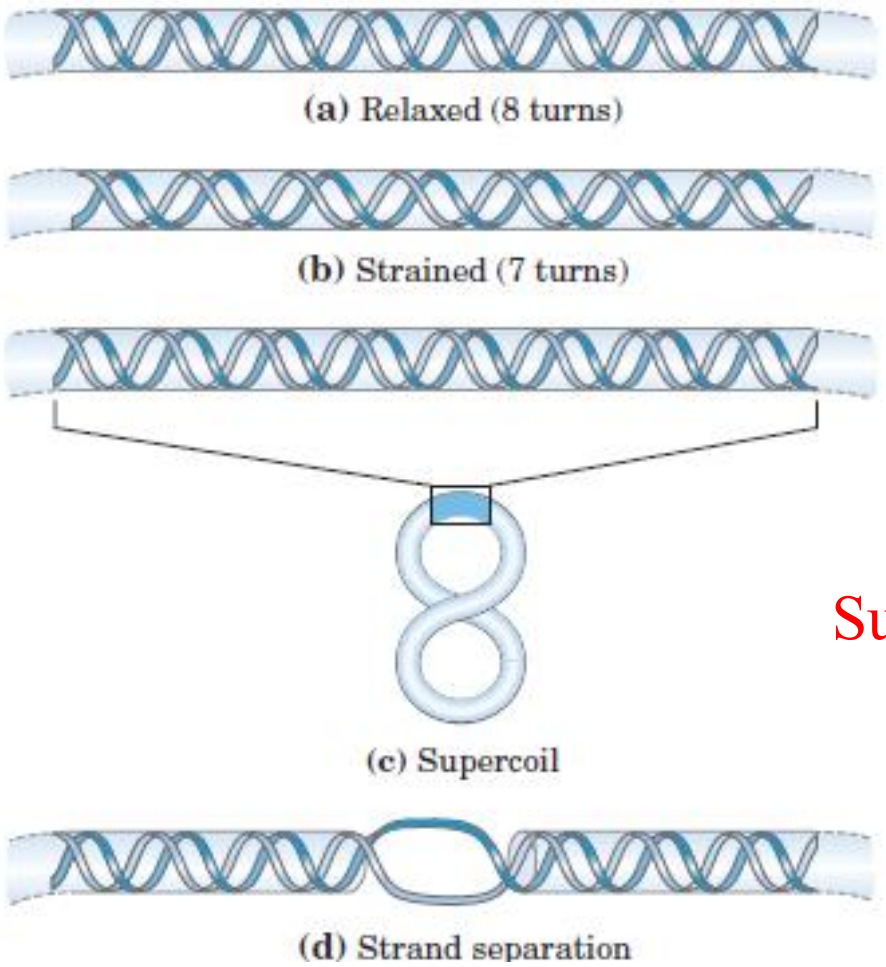
Topoisomerases

Dr. Suheir Ereqat 2017/2018

supercoiling



84/10.5=8

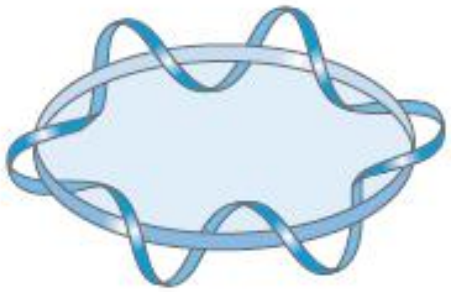
Unstable (12bp/turm)

Supercoiling

Linking Number

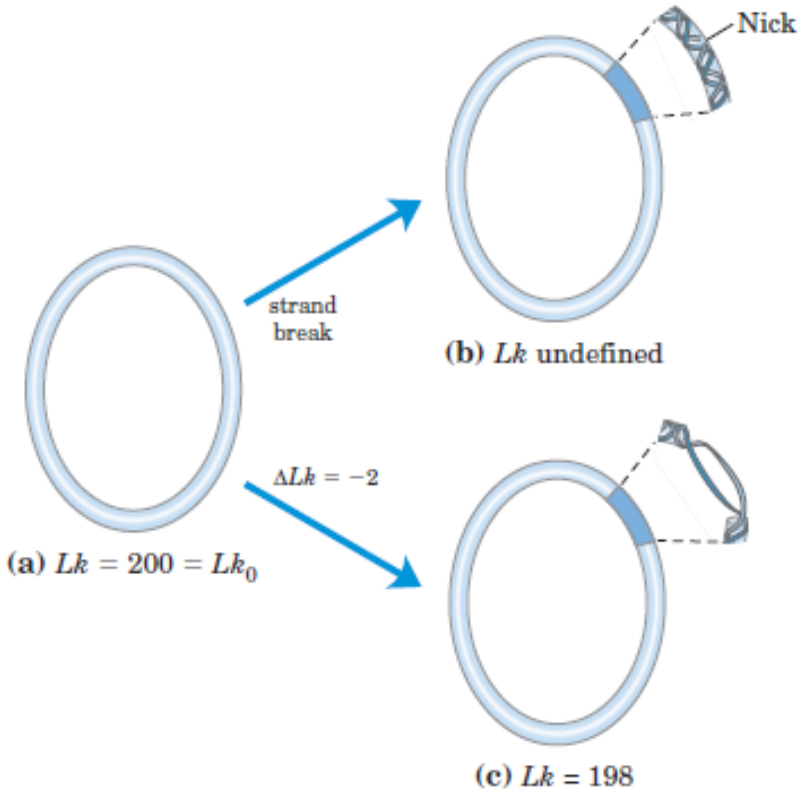
The linking number defines the number of times a strand of DNA winds in the right-handed direction around the helix axis

(a) $Lk = 1$



(b) $Lk = 6$

DNA of most organisms is negatively supercoiled.



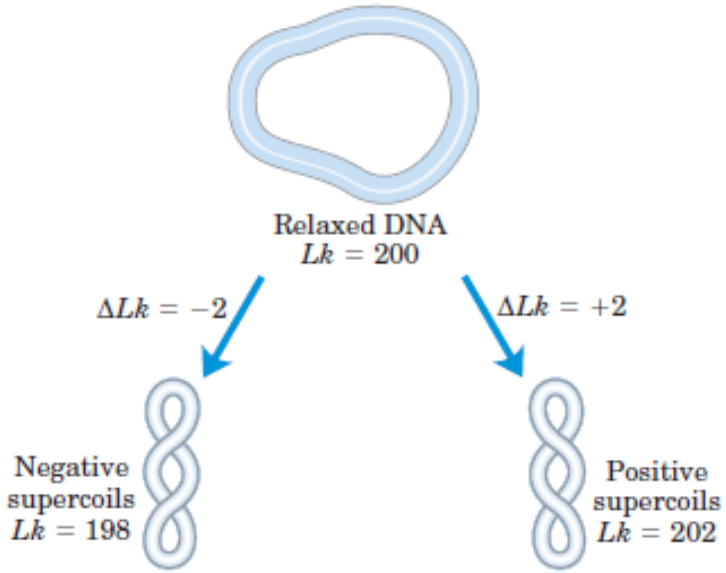
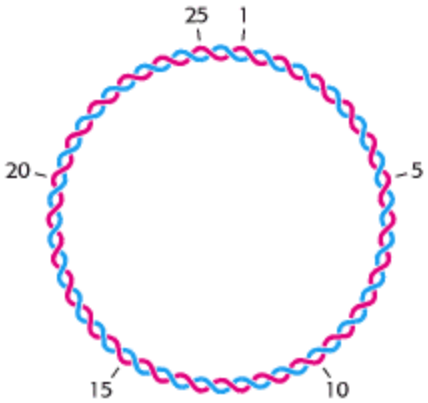


FIGURE 24–17 Negative and positive supercoils. For the relaxed DNA molecule of Figure 24–16a, underwinding or overwinding by two helical turns (Lk = 198 or 202) will produce negative or positive supercoiling, respectively. Note that the DNA axis twists in opposite directions in the two cases.

(A)



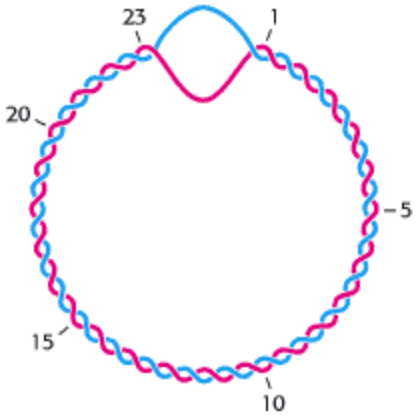
(B) $Lk = 25, Tw = 25, Wr = 0$
Relaxed Circle

Twist-no. of helical turn=LK(relaxed) writhe-= is the number of times the double helix crosses over on itself

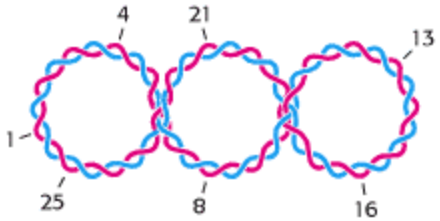
$$L=T+W$$

Linear DNA unwound by two right-hand turns

(C)



(D) $Lk = 23, Tw \sim 23, Wr \sim 0$
Unwound Circle



(E) $Lk = 23, Tw \sim 25, Wr \sim -2$
Negative superhelix
(right-handed)

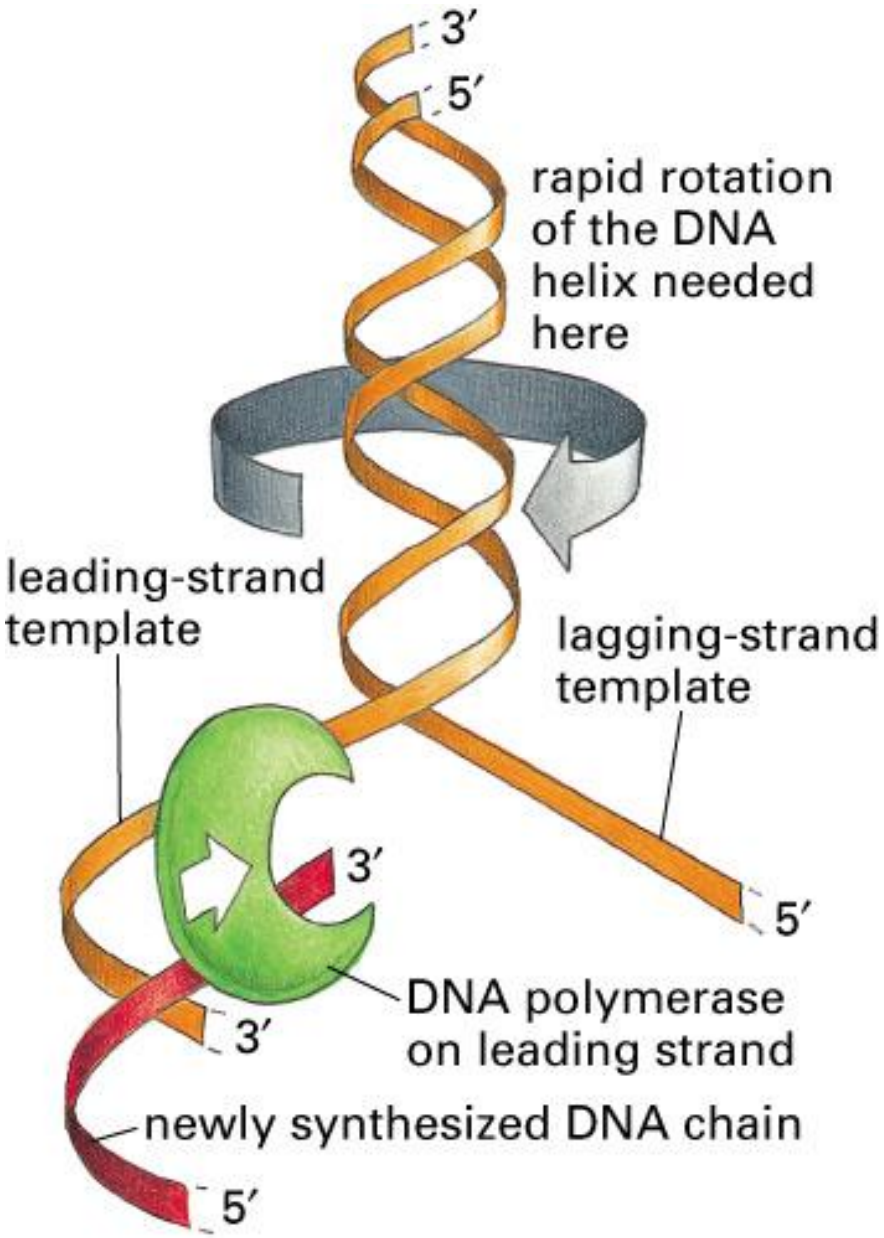
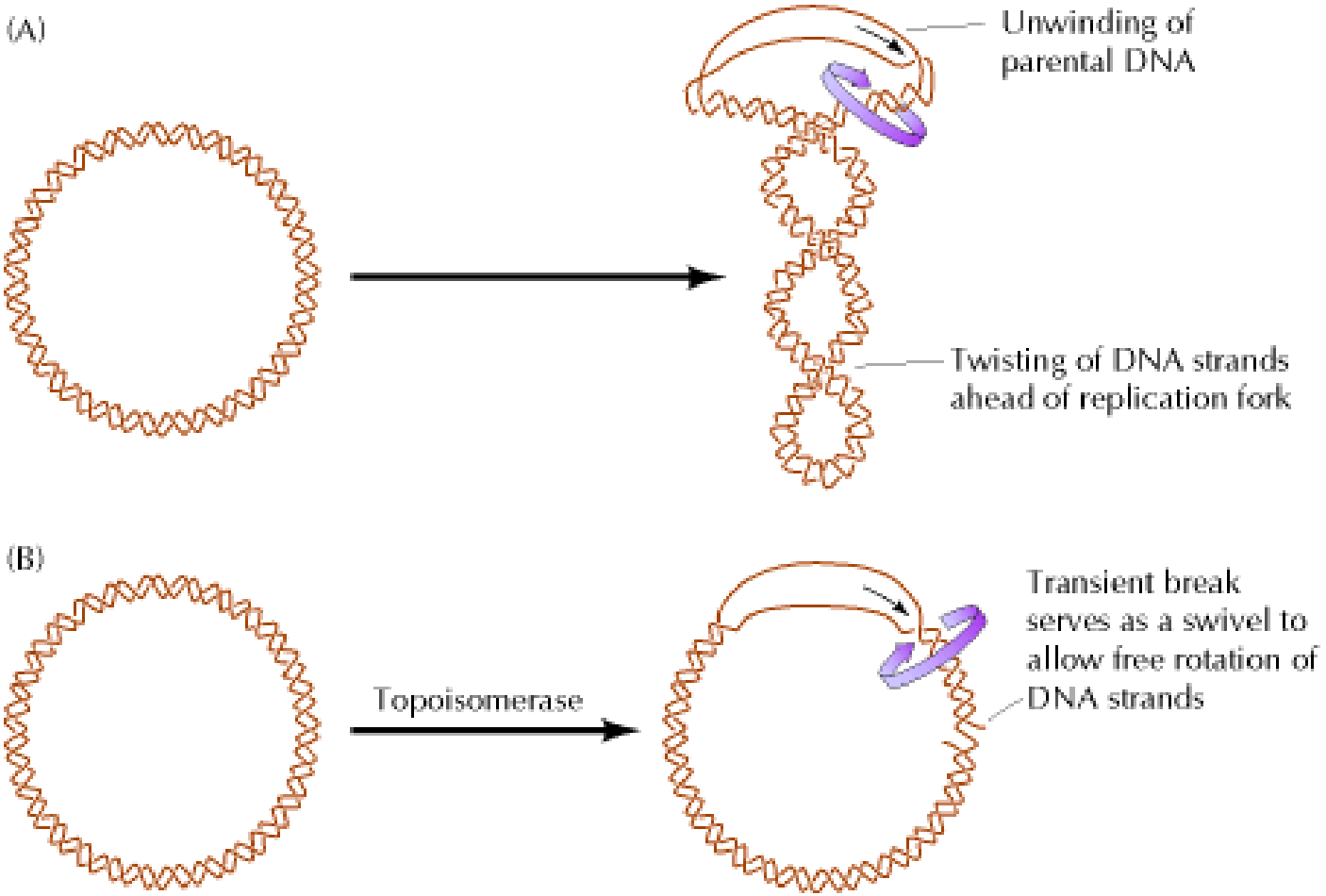
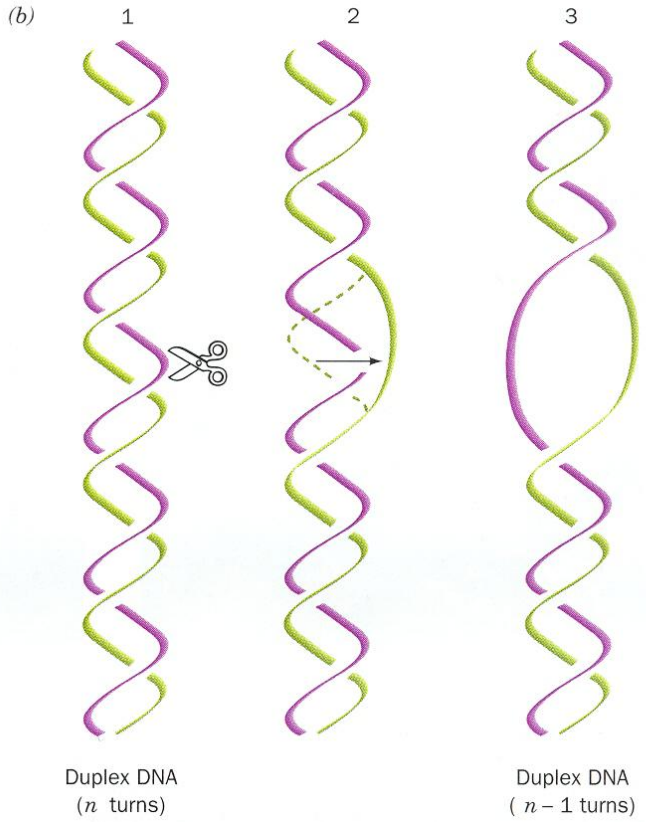


Figure 5–24. Molecular Biology of the Cell, 4th Edition.



Action of topoisomerases during DNA replication (A) As the two strands of template DNA unwind, the DNA ahead of the replication fork is forced to rotate in the opposite direction, causing circular molecules to become twisted around themselves. (B) This problem is solved by topoisomerases, which catalyze the reversible breakage and joining of DNA strands. The transient breaks introduced by these enzymes serve as swivels that allow the two strands of DNA to rotate freely around each other.

What can Topoisomerase I do to the DNA?



DNA topoisomerase I

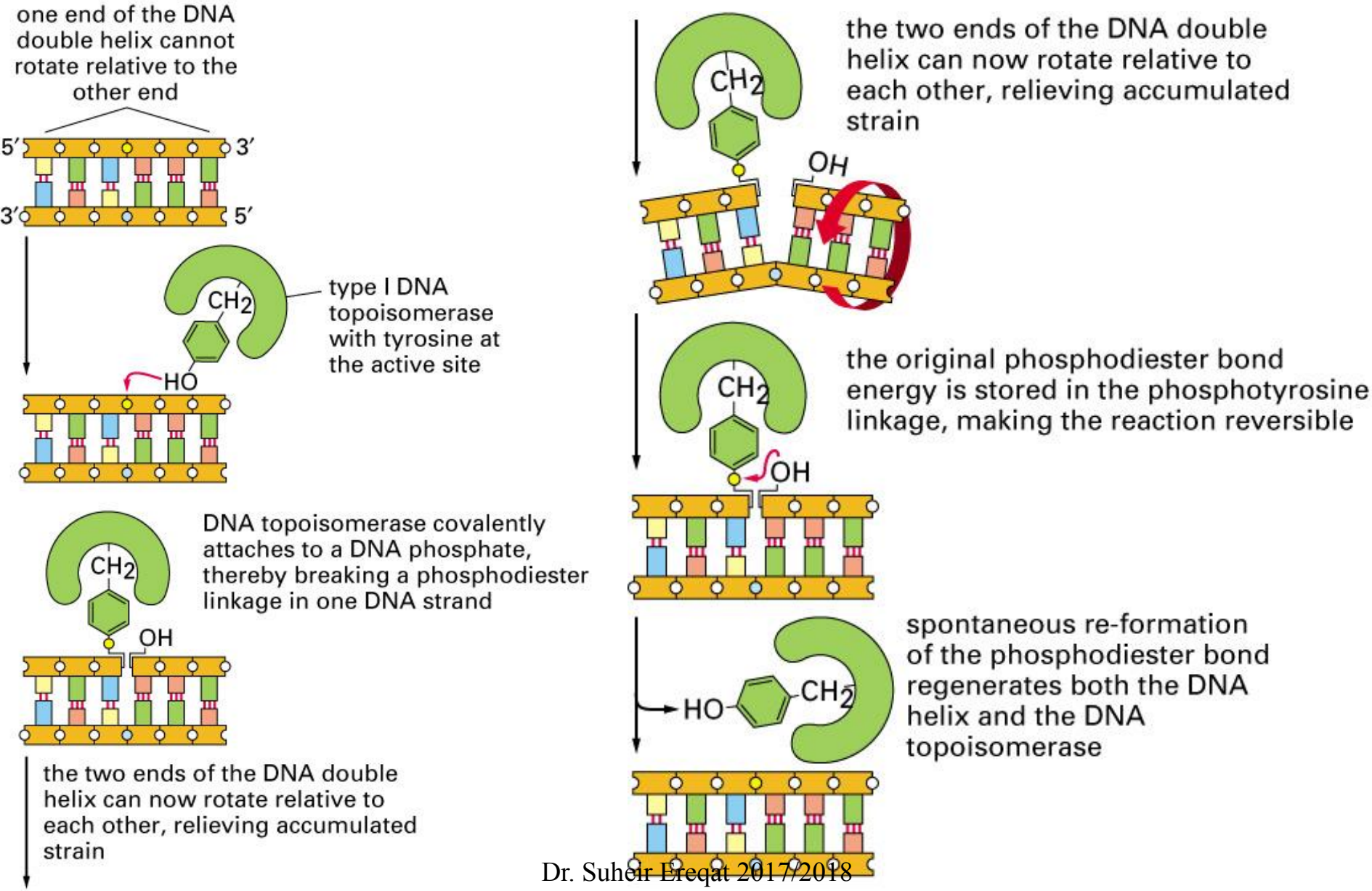


Figure 5–25 part 1 of 2. Molecular Biology of the Cell, 4th Editic

Figure 5–25 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

DNA topoisomerase II

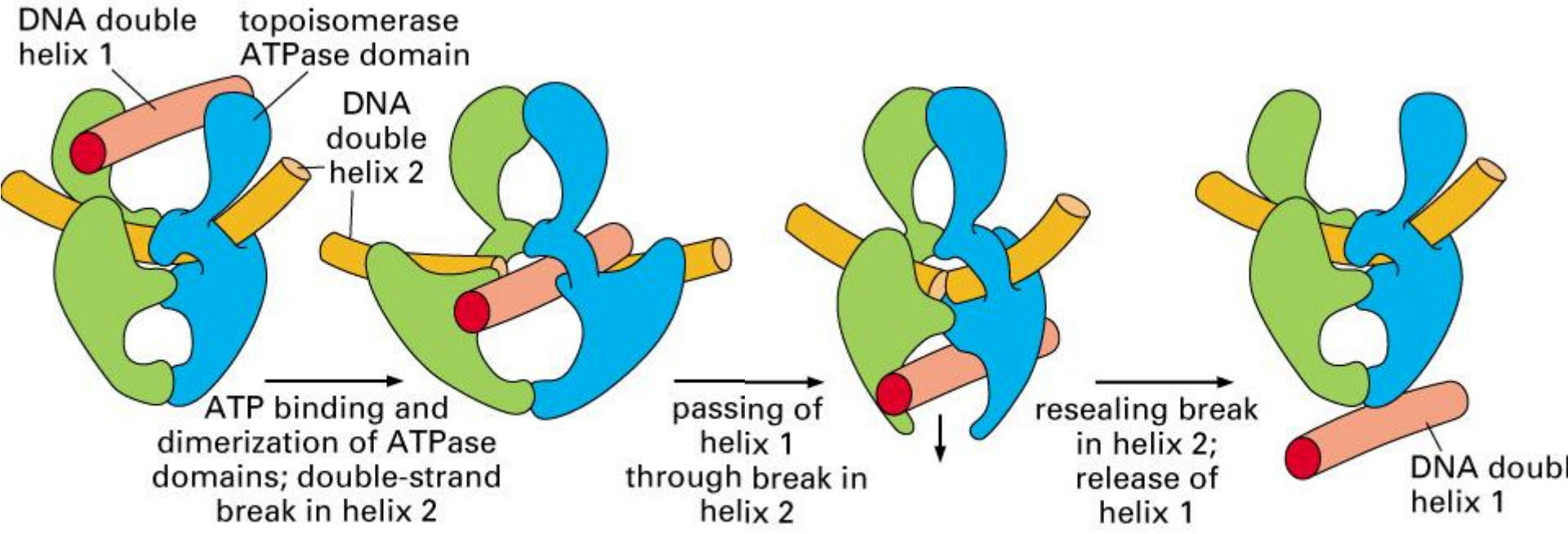


Figure 5–26 part 1 of 2. Molecular Biology of the Cell, 4th26 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

DNA topoisomerase II

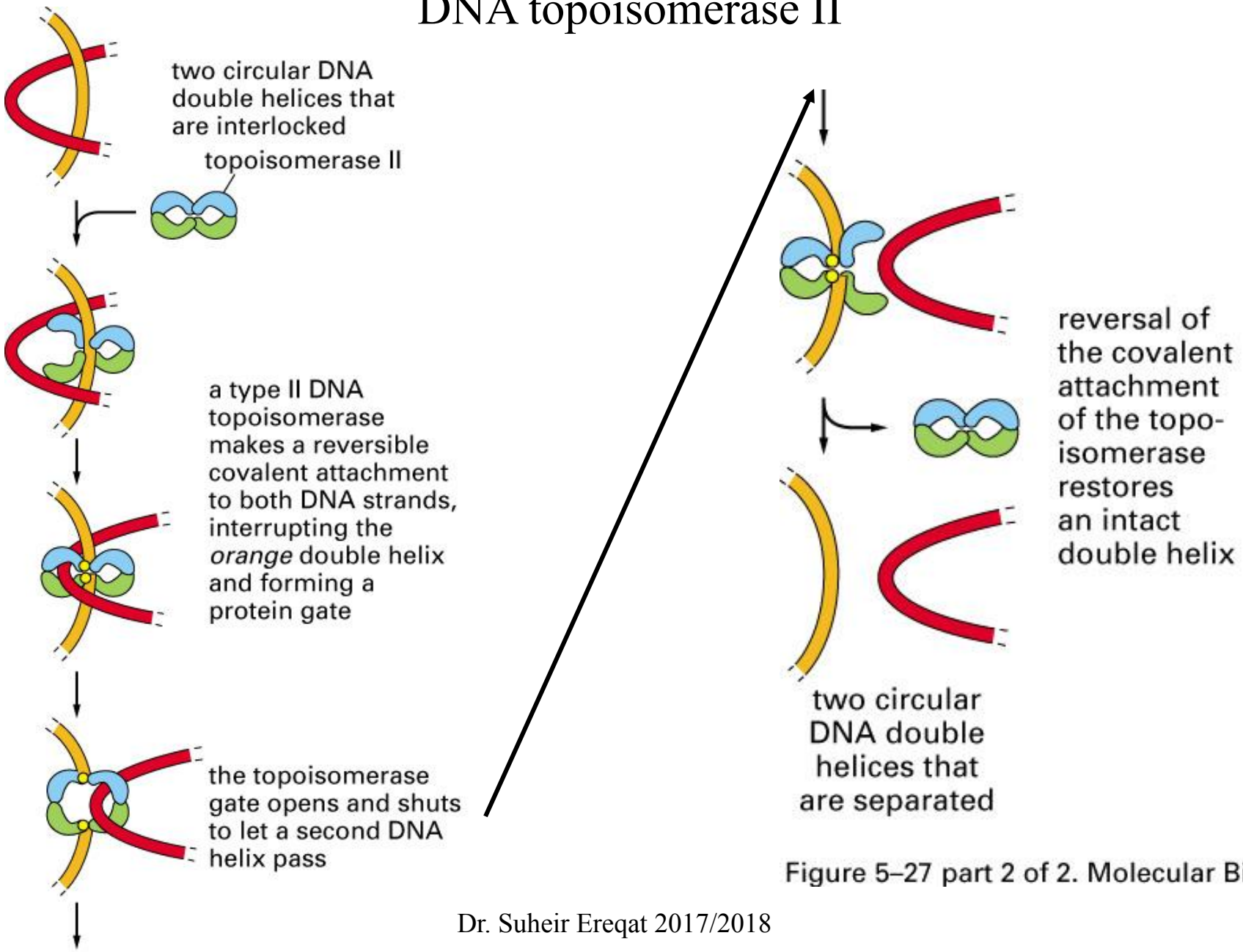
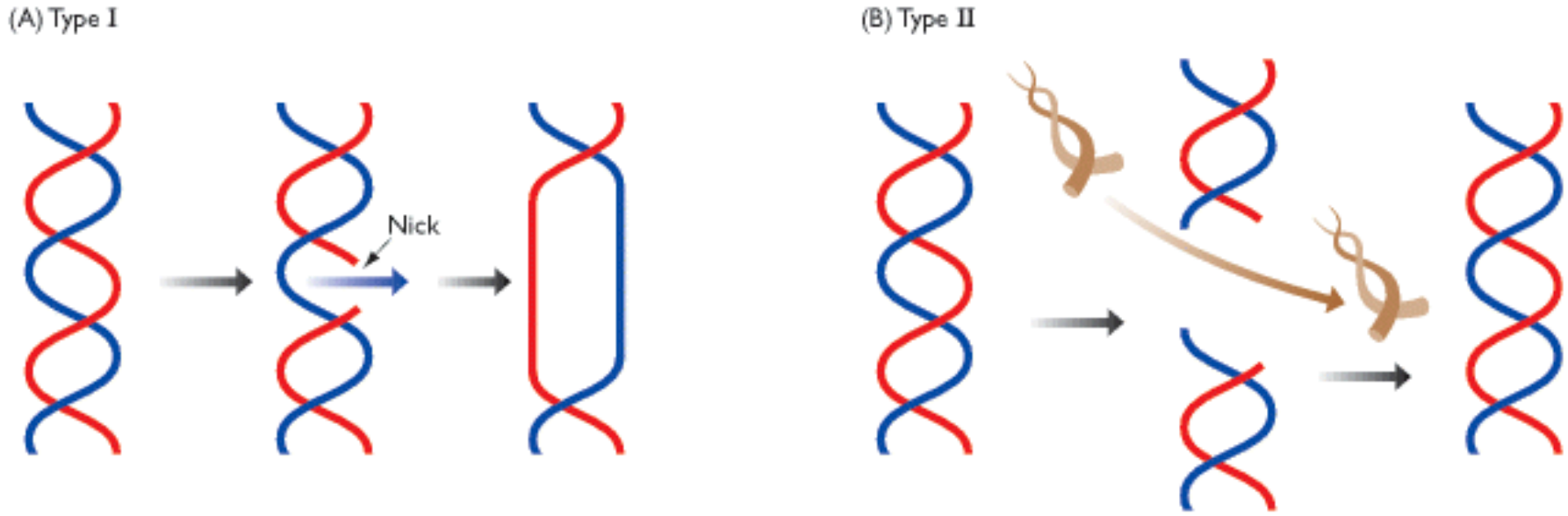


Figure 5–27 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Figure 5–27 part 2 of 2. Molecular Bi

Summary



The mode of action of Type I and Type II DNA topoisomerases. (A) A Type I topoisomerase makes a nick in one strand of a DNA molecule, passes the intact strand through the nick, and reseals the gap. (B) A Type II topoisomerase makes a double-stranded break in the double helix, creating a gate through which a second segment of the helix is passed.

DNA Repair

Dr. Suheir Ereqat 2017/2018

Sources of damage

endogenous damage: such as attack by ROS and replication errors

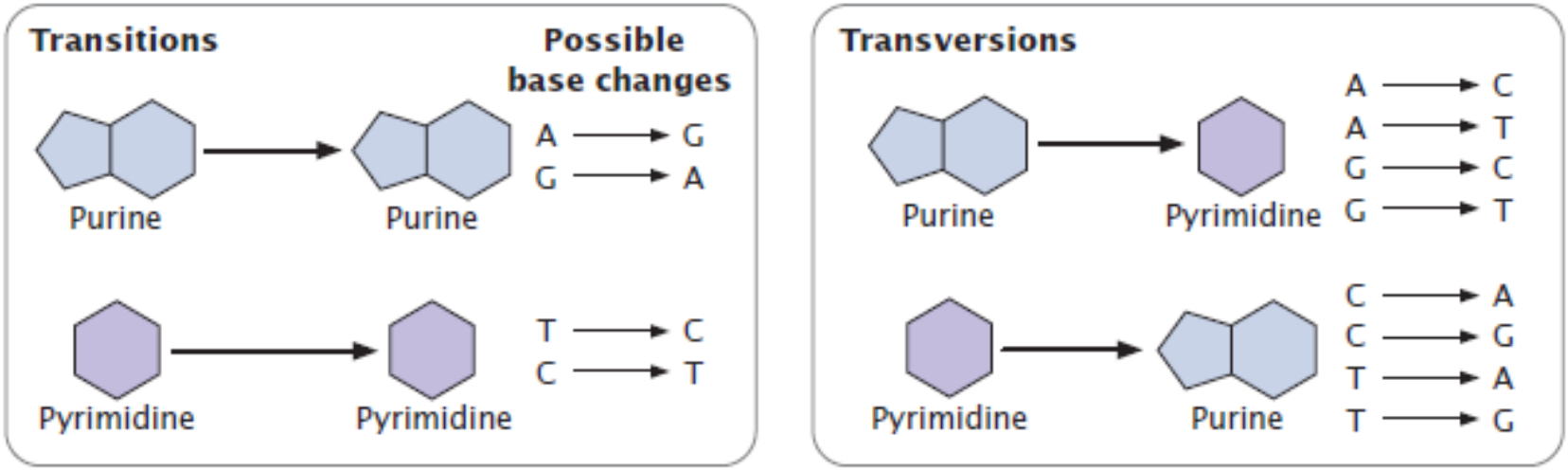
exogenous damage caused by external agents:
UV light, x-rays and gamma rays

plant toxins

human-made mutagenic chemicals,
DNA intercalating agents

viruses

Base substitution:Transitions Vs transversions



18.3 A transition is the substitution of a purine for a purine or of a pyrimidine for a pyrimidine; a transversion is the substitution of a pyrimidine for a purine or of a purine for a pyrimidine.

Mutation:

A permanent change in the nucleotide sequence.

Categorized by the A) nature of bases

- Substitution mutation: transition, transversion

- Insertion or deletion mutation:

B) effect on coding sequence

- Silent mutation do not alter the amino acid encoded

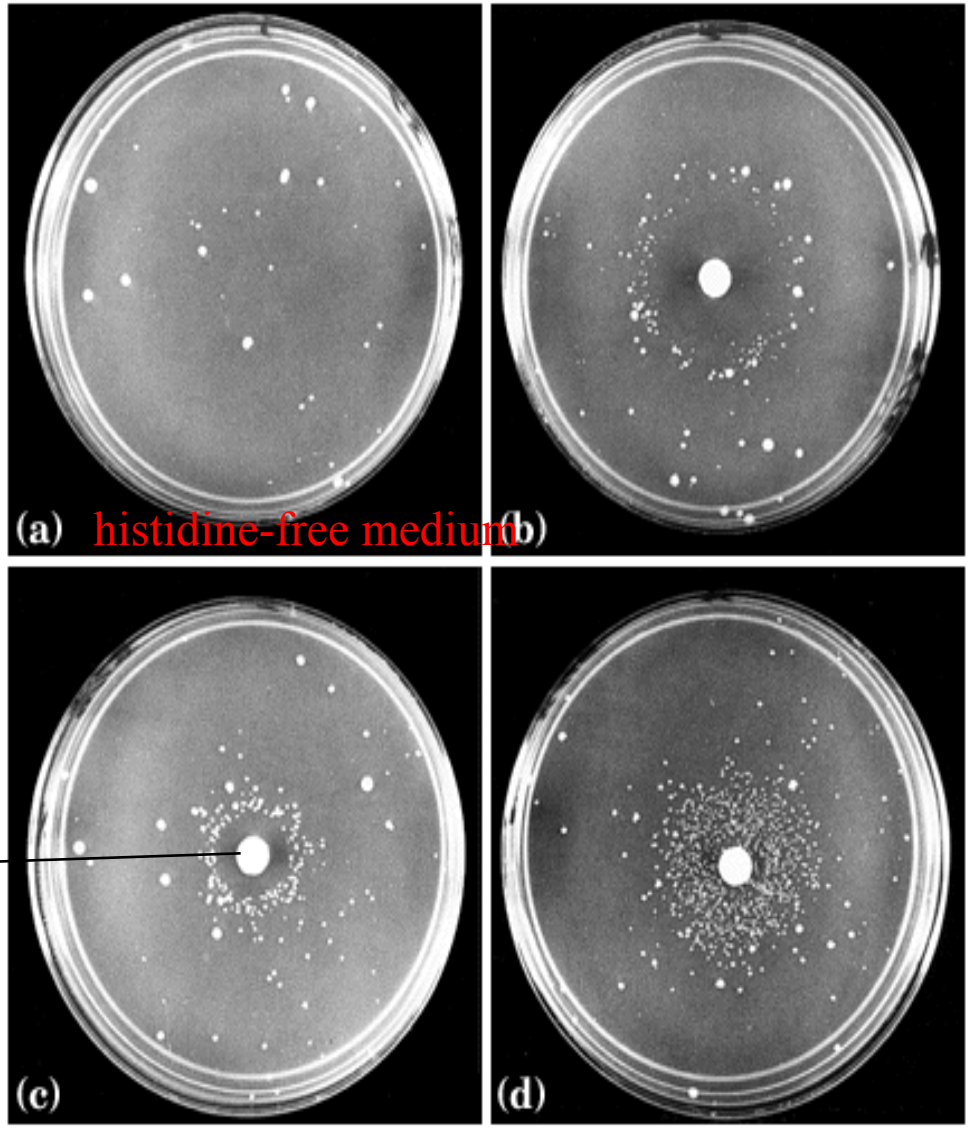
- Missense mutation: change amino acid encoded
- Nonsense mutation: stop codon

Mutations and Cancer

Ames Test for Carcinogens:

Measure the potential of a chemical to induce mutations in bacteria(may act as a carcinogen)

Salmonella typhimurium having a mutation
Cant synthesize histidine



Filter disc: mutagen increases the rate of back-mutation and hence the number of colonies

table 25–5

Types of DNA Repair Systems in *E. coli*

Enzymes/proteins	Type of damage
Mismatch repair	
Dam methylase	Mismatches
MutH, MutL, MutS proteins	
DNA helicase II	
SSB	
DNA polymerase III	
Exonuclease I	
Exonuclease VII	
RecJ nuclease	
Exonuclease X	
DNA ligase	
Base-excision repair	
DNA glycosylases	Abnormal bases (uracil, hypoxanthine, xanthine); alkylated bases; pyrimidine dimers in some other organisms
AP endonucleases	
DNA polymerase I	
DNA ligase	
Nucleotide-excision repair	
ABC excinuclease	DNA lesions that cause large structural changes (e.g., pyrimidine dimers)
DNA polymerase I	
DNA ligase	
Direct repair	
DNA photolyases	Pyrimidine dimers
O^6-Methylguanine-DNA methyltransferase	O^6-Methylguanine

Deamination of G

Deamination of C

Deamination of A

Methyl A/G

*

*

*

Single-strand damage

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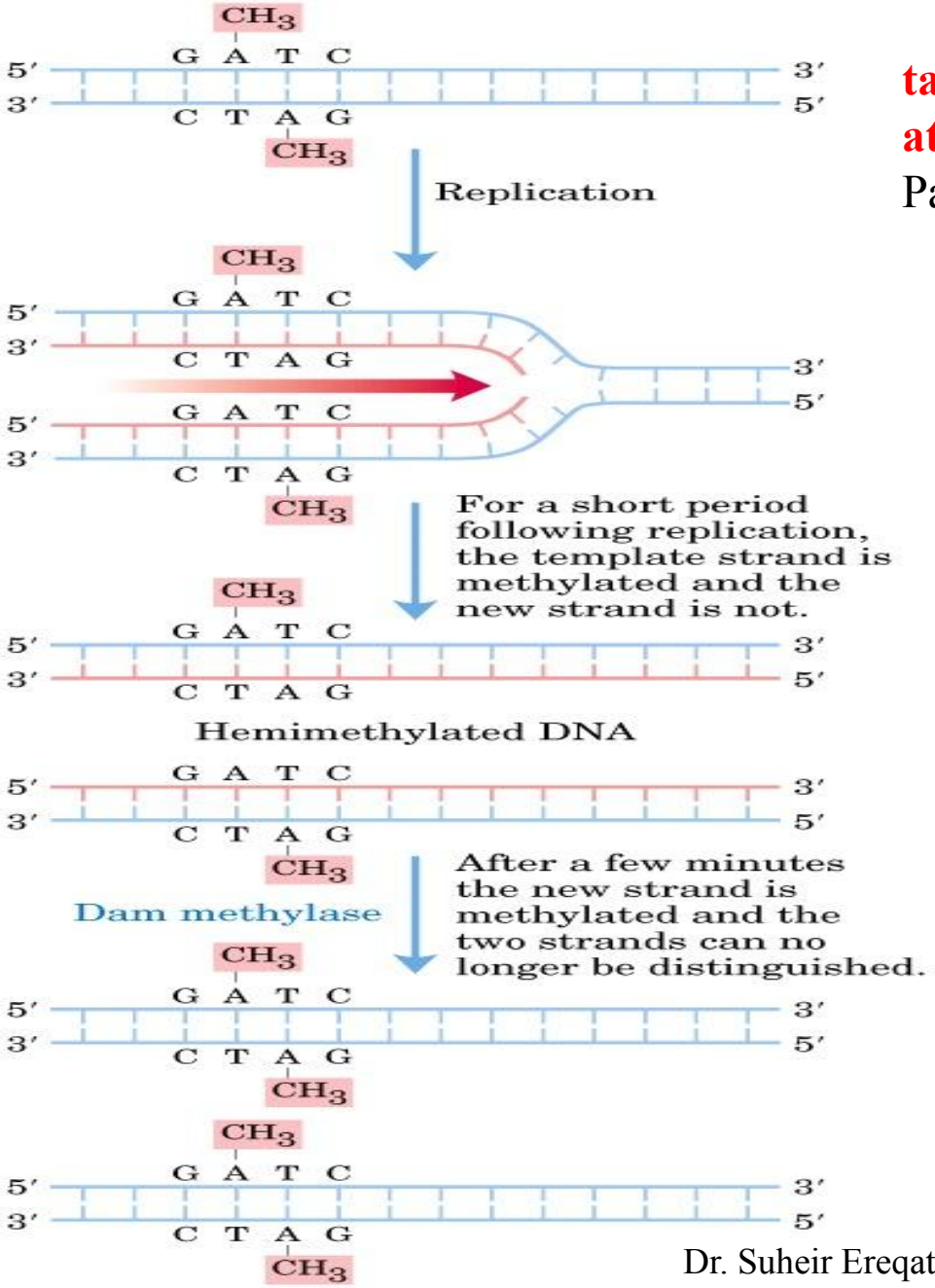
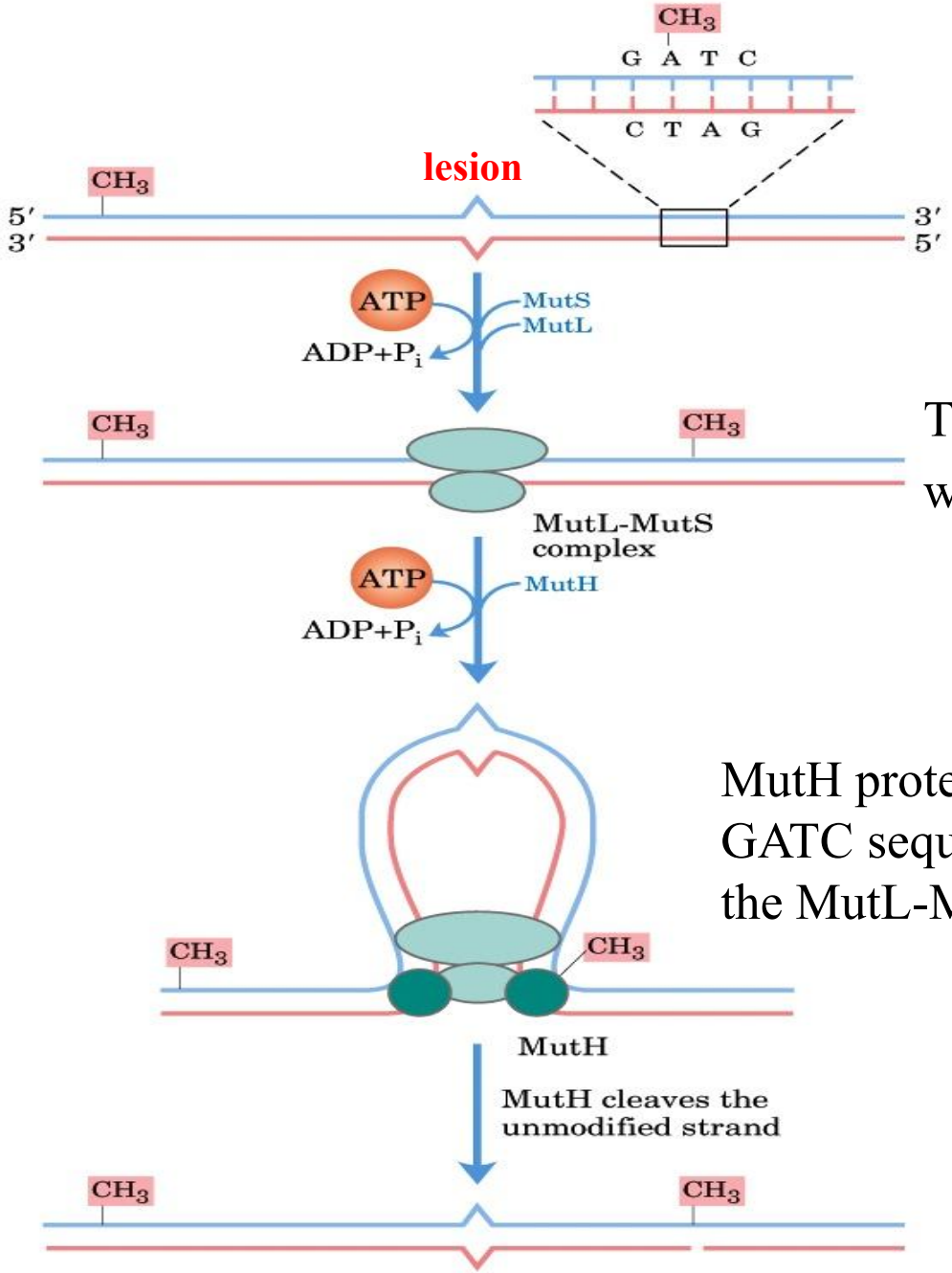
1) Methyl- directed Mismatch Repair:



tagging by Dam methylase at(5)GATC

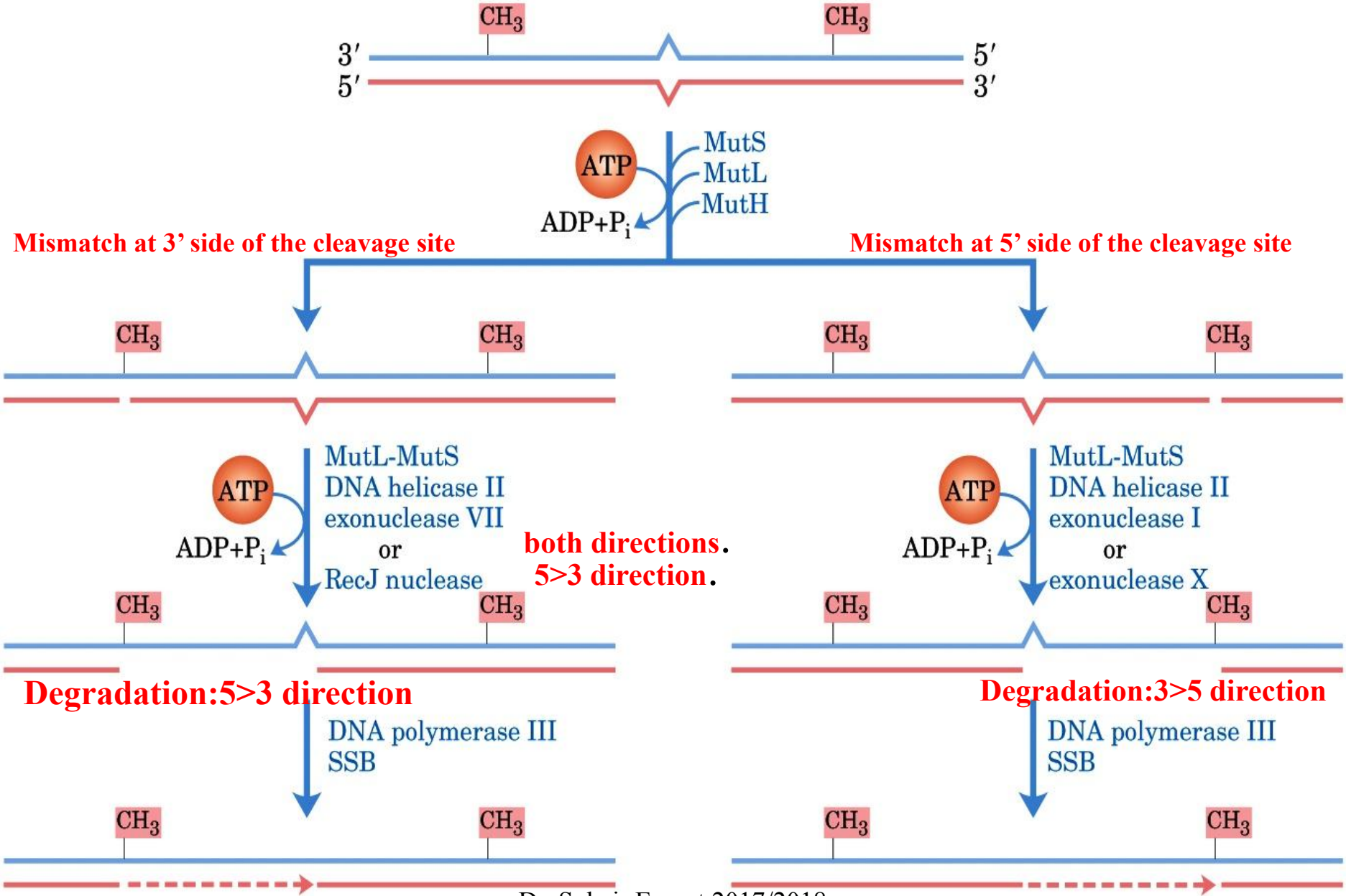
Palindromic sequence

Methyl- directed mismatch repair:



The MutL protein forms a complex with MutS at the mismatch.

MutH protein binds to MutL and to GATC sequences encountered by the MutL-MutS complex= DNA loop


CH3
CH3
3′
5′
5′
3′
ATP
ADP+Pi
MutS
MutL
MutH
Mismatch at 3’ side of the cleavage site
Mismatch at 5’ side of the cleavage site
MutL-MutS
DNA helicase II
exonuclease VII
or
RecJ nuclease
MutL-MutS
DNA helicase II
exonuclease I
or
exonuclease X
both directions.
5>3 direction.
Degradation:5>3 direction
Degradation:3>5 direction
DNA polymerase III
SSB

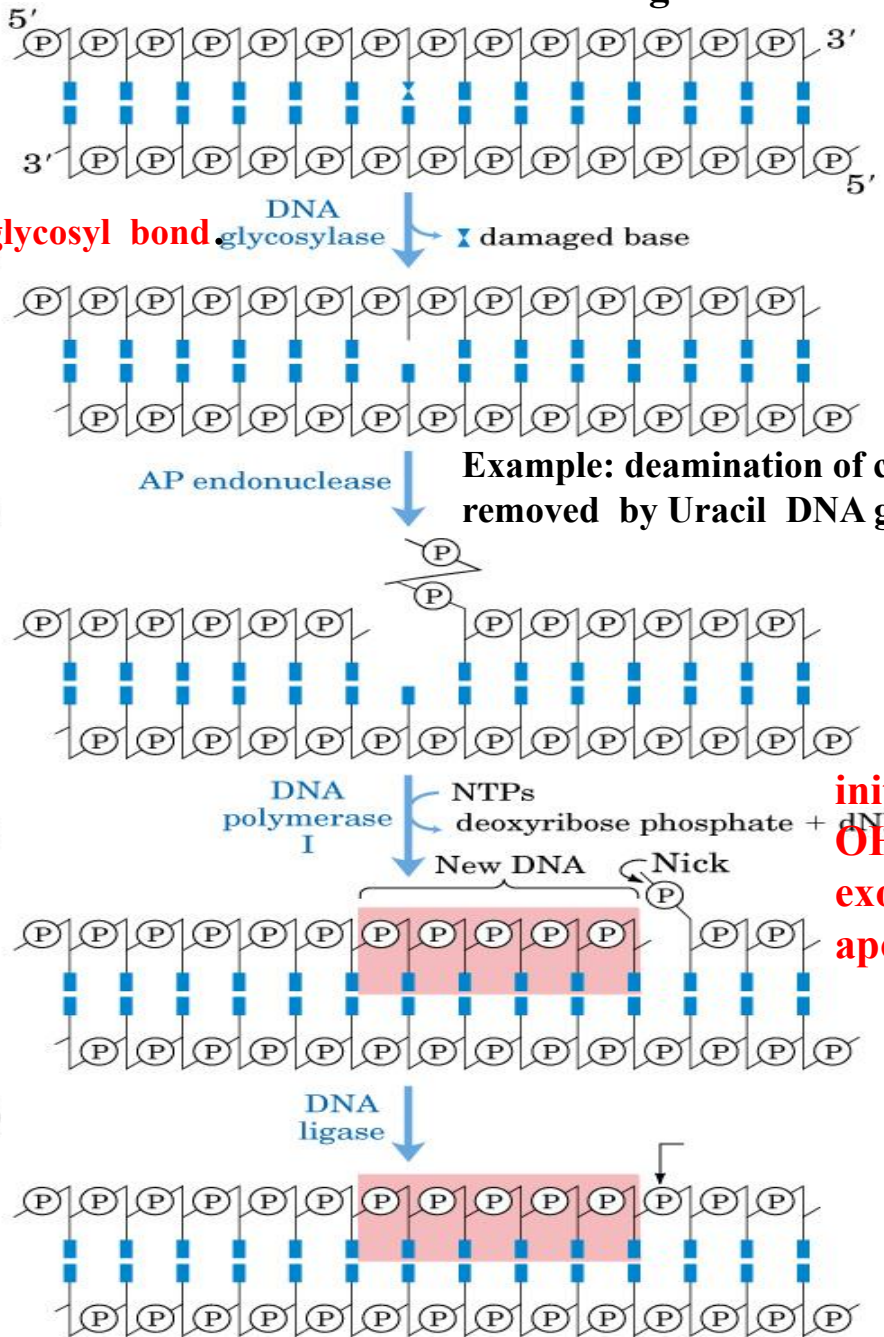
Eukaryotes:

- Similar to MutS and MutL.
- MutS homologus for eukaryotes from yeast to humans.

  MSH2 (Muts homolog 2) MSH3, MSH6.

- Mutated in CANCER=$\uparrow$ mutation rate

2) Base- Excision Repair: recognize and repair damage caused by environmental agents



cleaving the N-glycosyl bond.

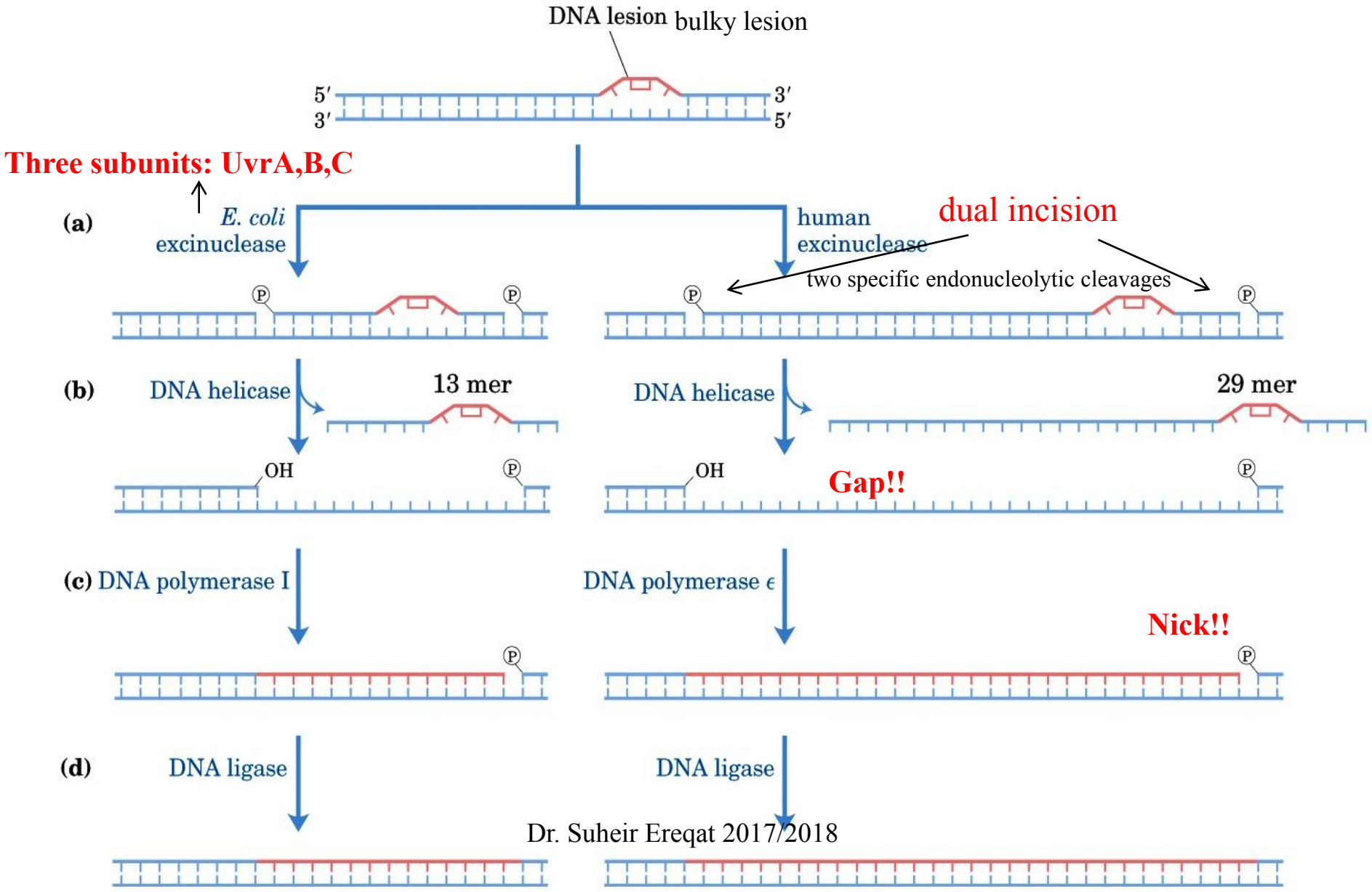
abasic site (AP site)

Example: deamination of cytosine=Uracil removed by Uracil DNA glycosylases, human=4 UNG,

initiates repair synthesis from the free 3 OH at the nick, removing (with its 5>3 exonuclease activity) and replacing aportion of the damaged strand.

3) Nucleotide Excision Repair:

recognize and remove bulky lesions and pyrimidine dimers.



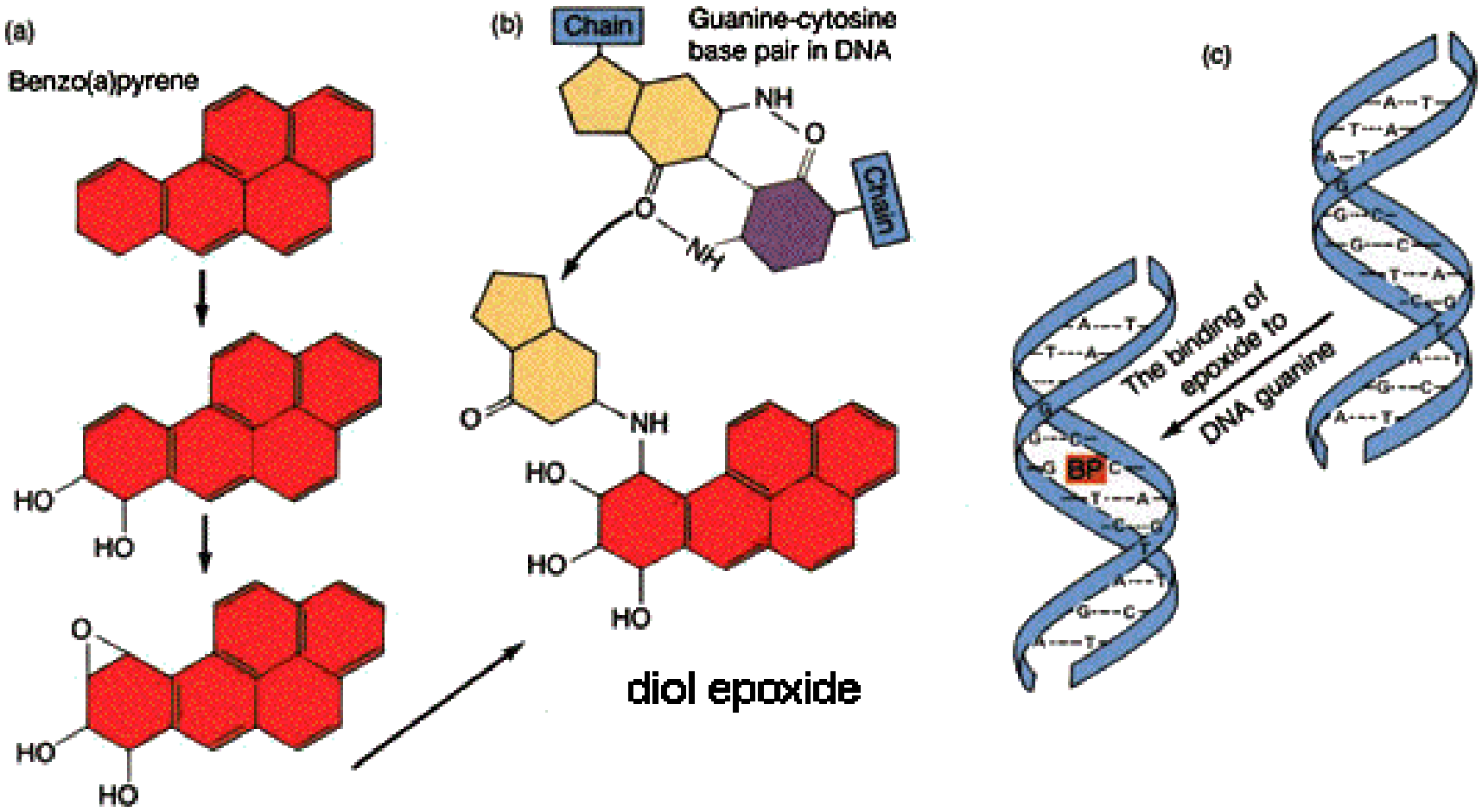
This pathway the primary route for many lesion types :

- pyrimidine dimers (T dimer).
- base adducts: benzo pyrene-guanine (formed in DNA by **cigarette smoke**).

Xeroderma pigmentosum (XP):

rare inherited disease (pigmented lesions on skin + skin cancer+ also have neurological abnormalities) due to mutations(XPA-XPG) in Nucleotide Excision Repair system (the sole repair pathway for pyrimidine dimers in humans).

HNPCC (hereditary non-polyposis colorectal cancer)
= defect miss match repair

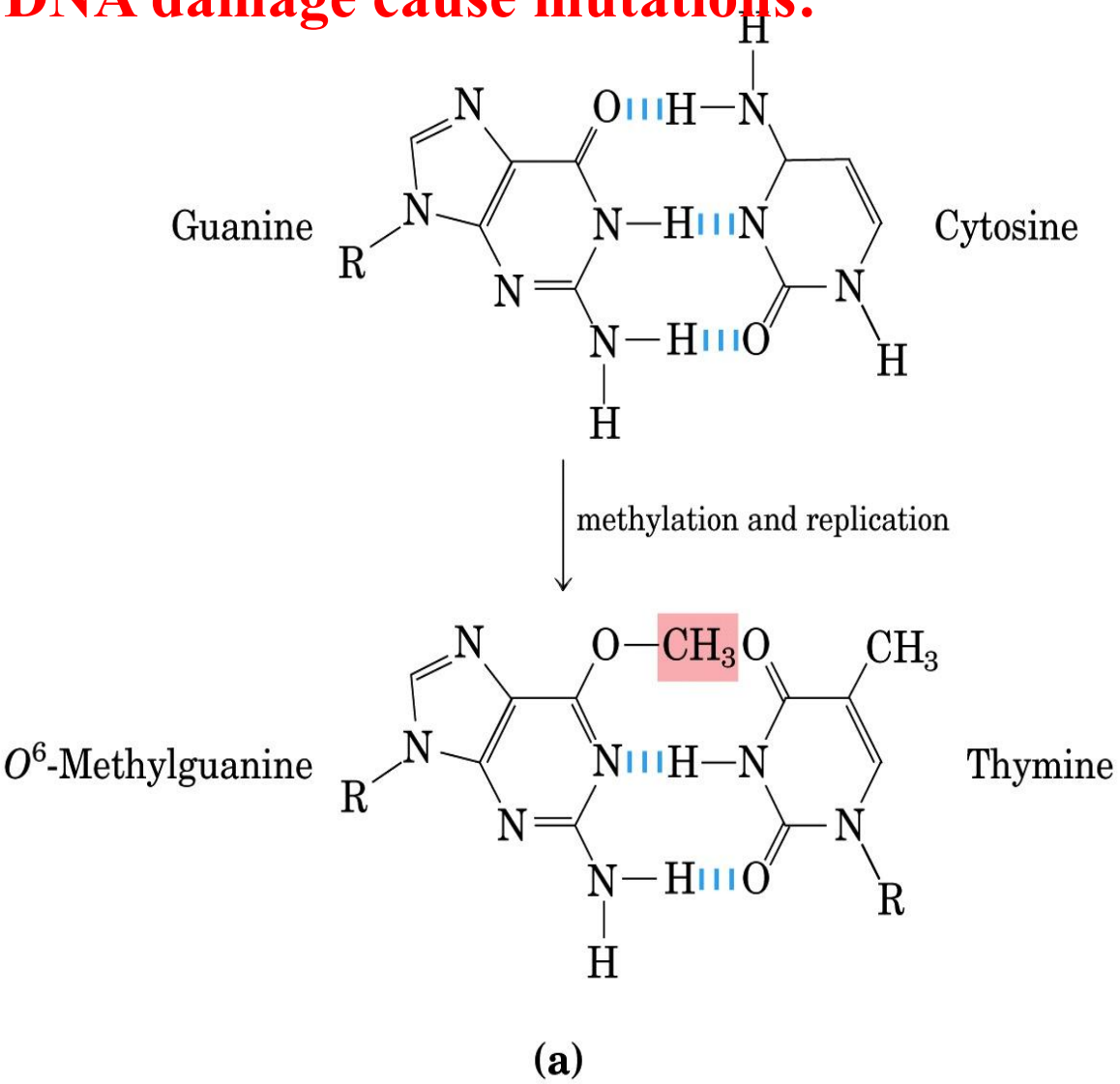

(a)
Benzo(a)pyrene
HO
HO
O
HO
HO
(b)
Chain
Guanine-cytosine
base pair in DNA
NH
O
O
NH
Chain
O
NH
HO
HO
HO
diol epoxide
(c)
The binding of
epoxide to
DNA guanine
BP

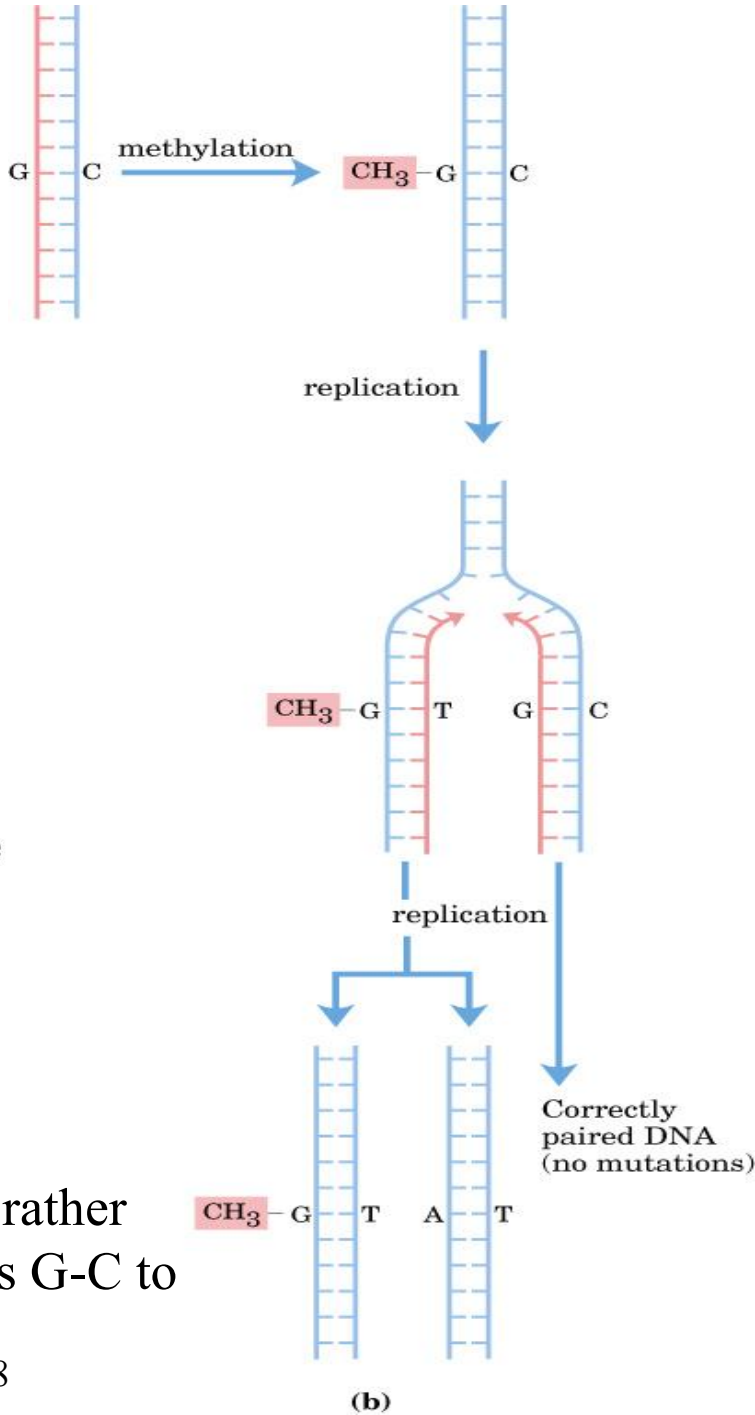
HNPCC:

It can present with rectal bleeding, stomach pain and cancer-related symptoms like unexplained weight loss and fatigue.

The most prevalent are defects in the *hMLH1* (human MutL homolog 1) and *hMSH2 (human MutS* homolog 2)

DNA damage cause mutations:

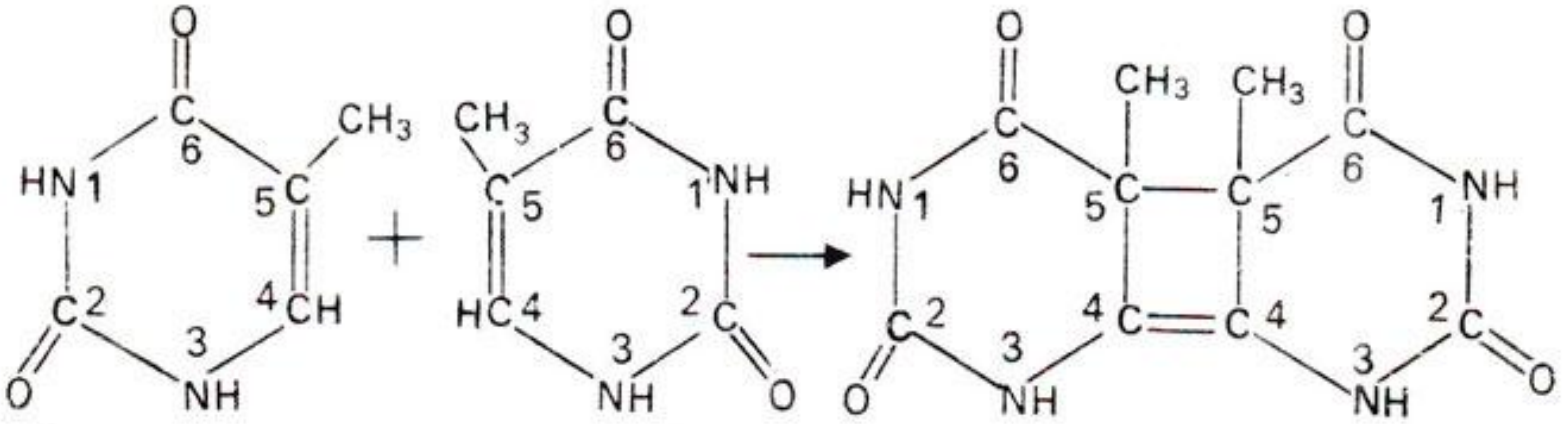




O6-methylguanine forms tends to pair with thymine rather than cytosine during replication, and therefore causes G-C to A-T mutations

4) **Direct Repair:** Repair with no excision / removal of a base or a nucleotide.

A) Photoreactivation: Pyrimidine dimers result from a UV-induced reaction, and **photolyases** use energy derived from absorbed light to reverse the damage

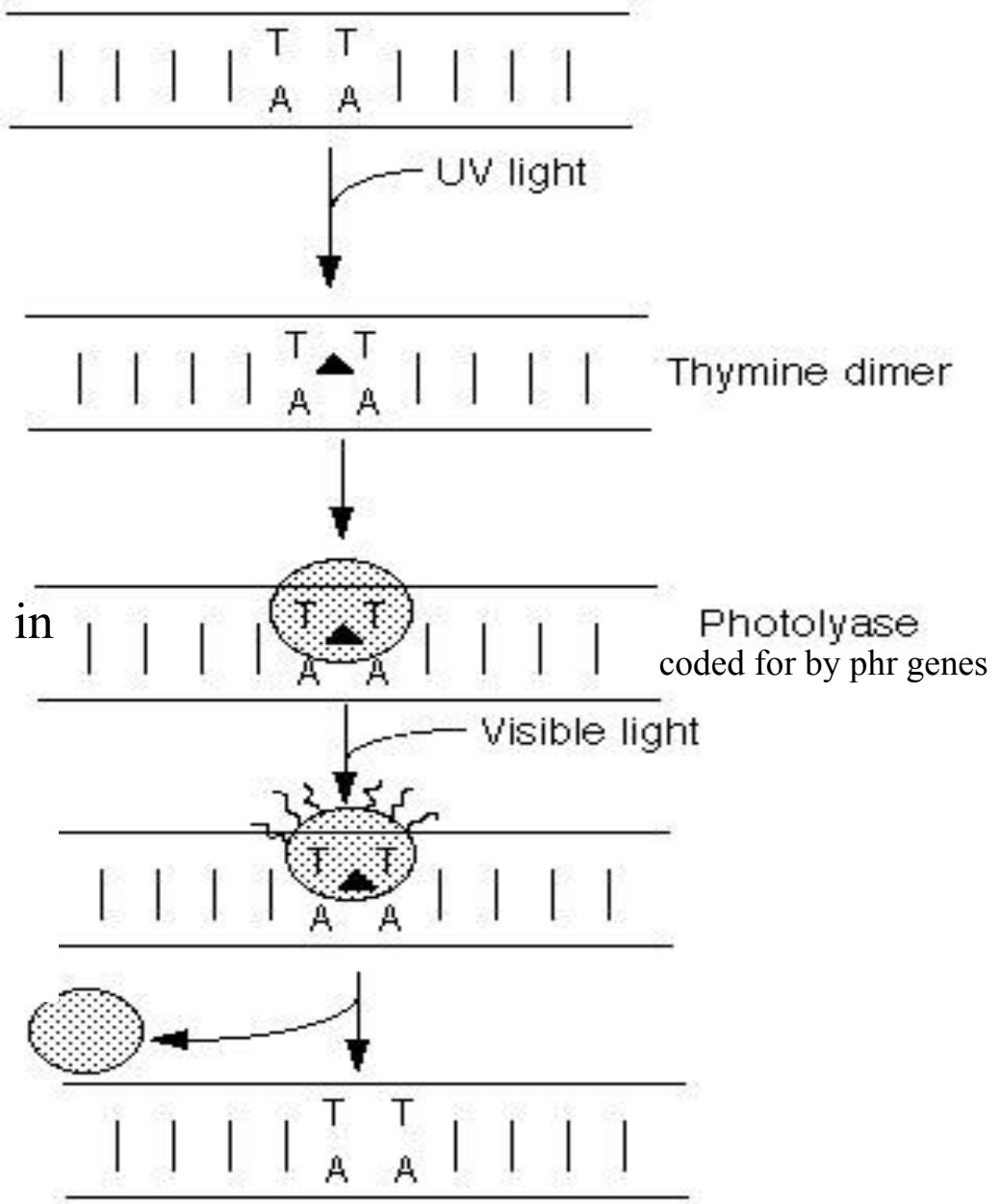


Photoreactivation Repair

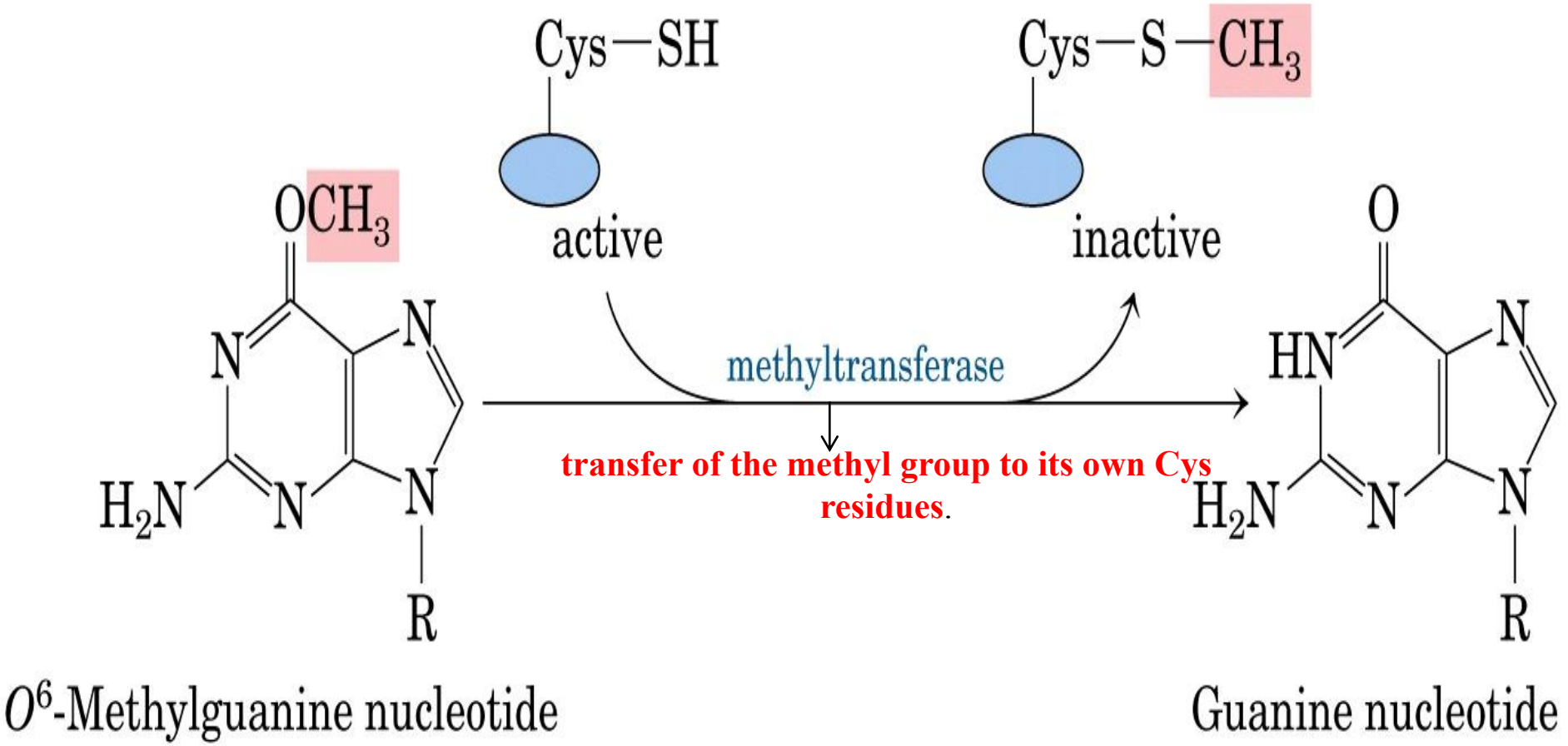
UV light strikes one of the adjacent thymines, creating a **thymine dimer**.

DNA photolyases recognize the "kink" in the DNA, and bind to the site

When excited by blue light (350-500 nm wavelength), the photolyases change conformation, breaking apart the dimer.



B)-repair of nucleotides with alkylation damage



c- Direct Repair: oxidative demethylation of alkylated nucleotides by the AlkB protein,

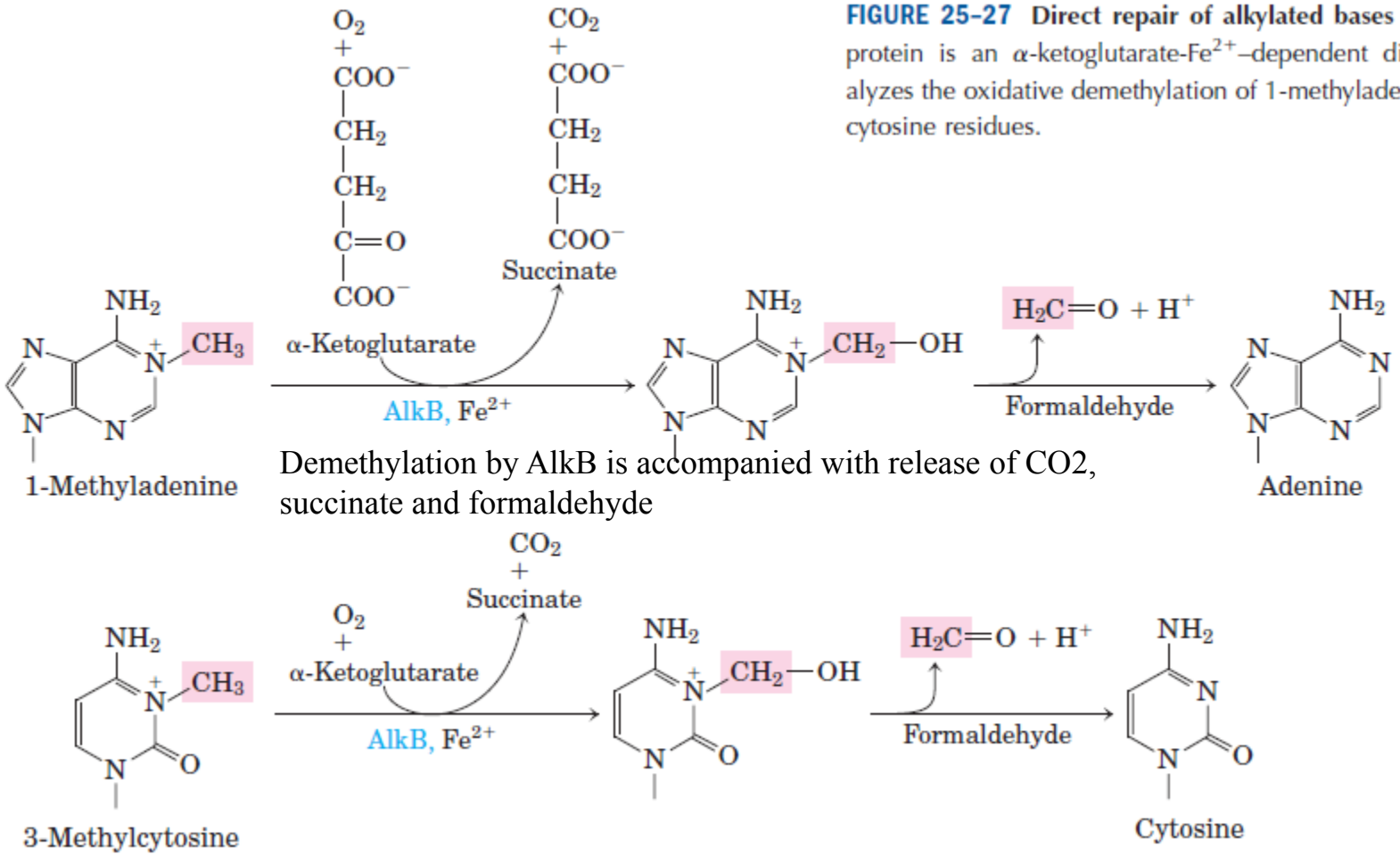


FIGURE 25-27 Direct repair of alkylated bases by AlkB. The AlkB protein is an α-ketoglutarate-Fe^{2+}–dependent dioxygenase. It catalyzes the oxidative demethylation of 1-methyladenine and 3-methylcytosine residues.

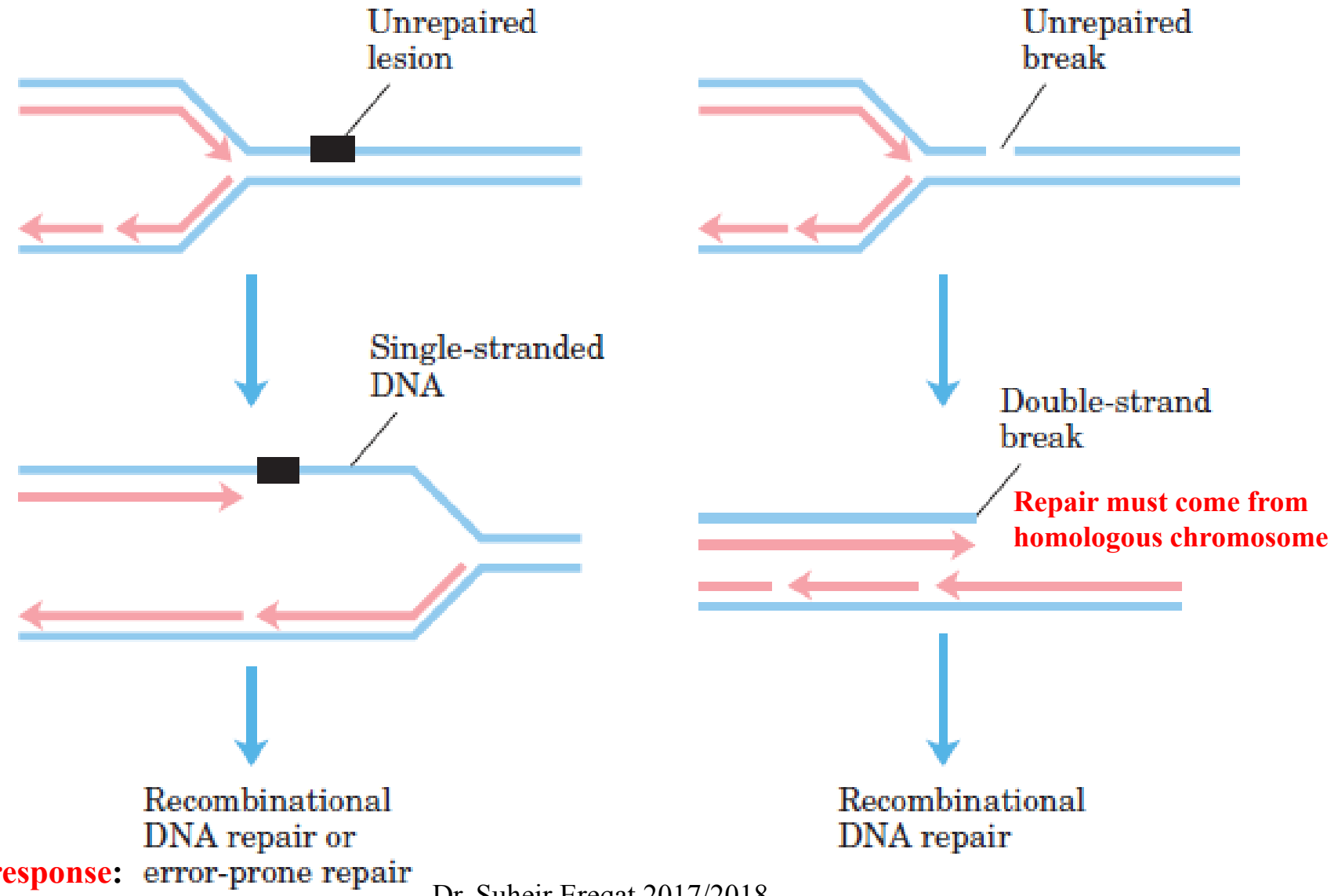
Demethylation by AlkB is accompanied with release of CO2, succinate and formaldehyde

The AlkB enzyme couples oxidative decarboxylation of -ketoglutarate to the hydroxylation of the methylated bases in DNA, resulting in direct reversion to the unmodified base and the release of formaldehyde.

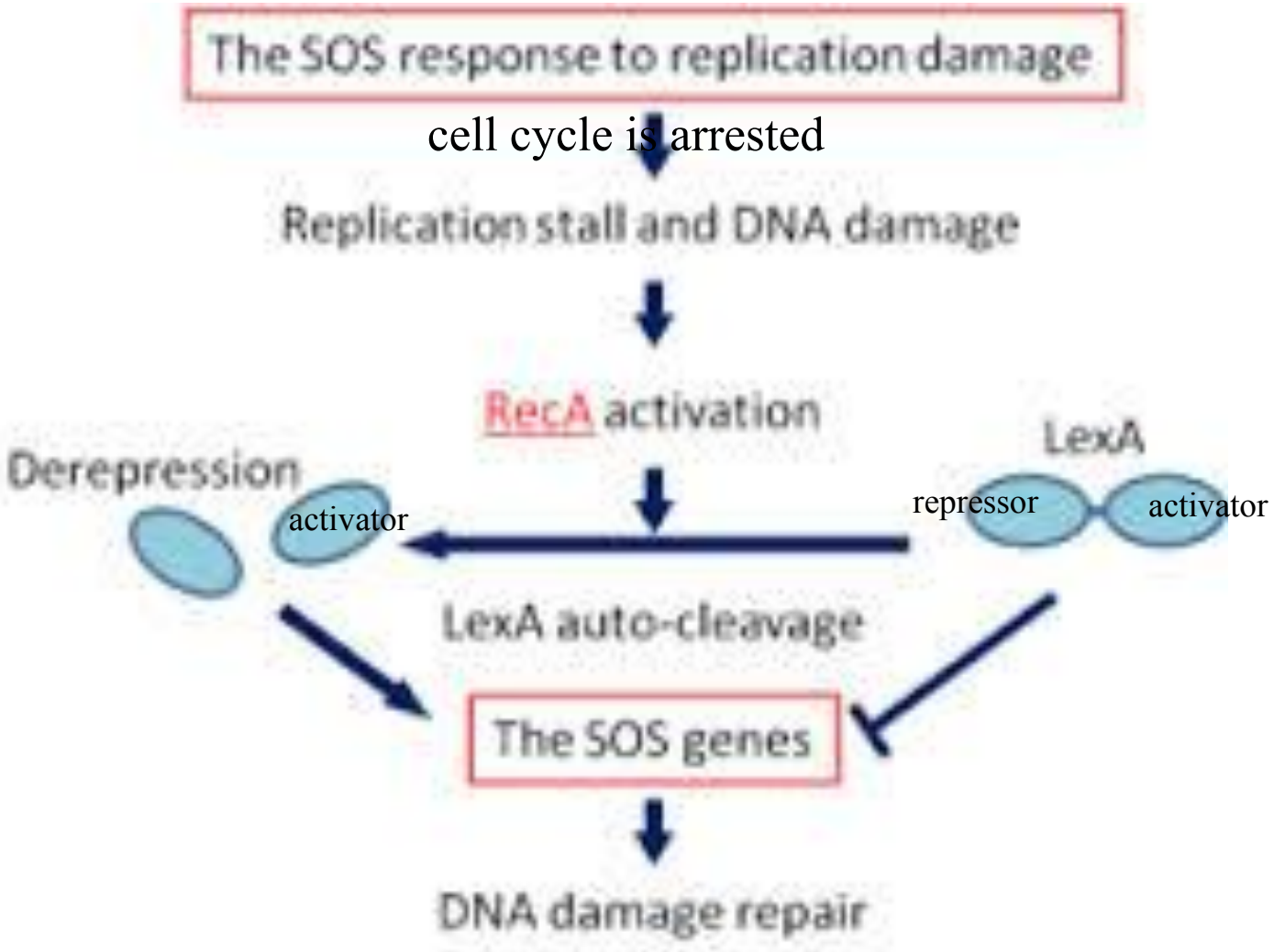
There are nine human homologs of AlkB.

- ALKBH1, ALKBH2, ALKBH3, ALKBH4, ALKBH5, ALKBH6, ALKBH7, ALKBH8, FTO

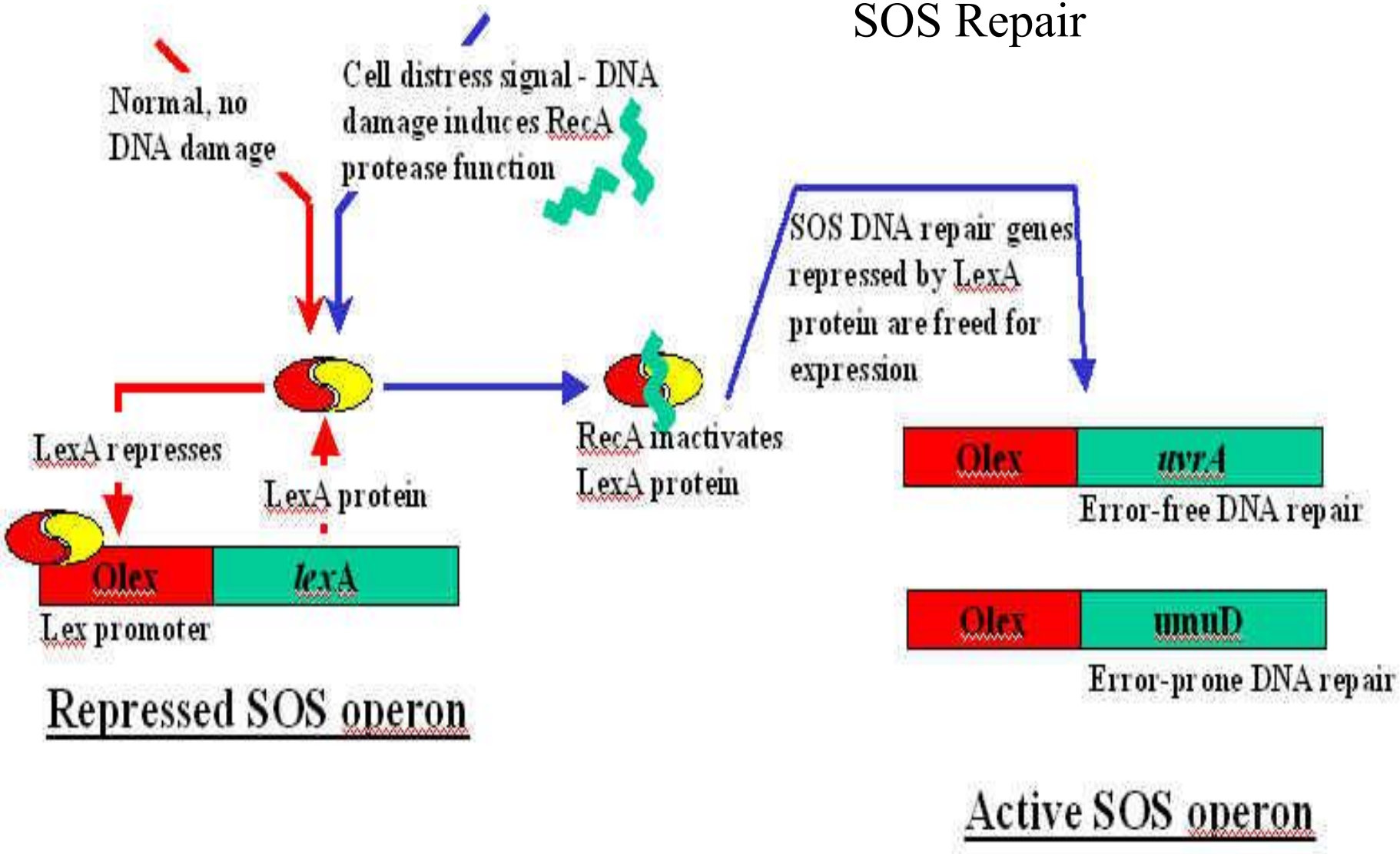
The Interaction of Replication Forks with DNA Damage



SOS response:


The SOS response to replication damage
cell cycle is arrested
Replication stall and DNA damage
RecA activation
Derepression
activator
LexA
repressor
activator
LexA auto-cleavage
The SOS genes
DNA damage repair

SOS Repair


Normal, no DNA damage
Cell distress signal - DNA damage induces RecA protease function
SOS DNA repair genes repressed by LexA protein are freed for expression
LexA represses
LexA protein
RecA inactivates LexA protein
Olex
lexA
Lex promoter
Repressed SOS operon
Olex
uvrA
Error-free DNA repair
Olex
umuD
Error-prone DNA repair
Active SOS operon

1-**Recombinational DNA repair**.

2-**Error prone translesion DNA synthesis (TLS).**

UmuD' complex with **UmuC→ DNA pol V** replicate many lesions that normally would block replication. **Pol IV**, induced under SOS response which is also highly error-prone..

- Proper base pairing is nearly impossible→ **inaccurate repair + high mutation rate.**
- SOS activated UmuD' +UmuC only when all replication forks blocked { result of extensive DNA damage}.
- The bacterial DNA polymerases IV and V are part of a family of TLS polymerases found in all organisms. These enzymes lack a proofreading exonuclease activity thus have low fidelity.
- Other polymerases in eukaryotes: DNA polymerase **eta, iota.**

- Rec A
- Lex A

table 25–6

Genes Induced as Part of the SOS Response in *E. coli*

Gene name	Protein encoded and/or role in DNA repair
Genes of known function	
*pol*B (*din*A)	Encodes polymerization subunit of DNA polymerase II, required for replication restart in recombinational DNA repair
*uvr*A, *uvr*B	Encode ABC excinuclease subunits UvrA and UvrB
*umu*C, *umu*D	Encode DNA polymerase V
*sul*A	Encodes protein that inhibits cell division, possibly to allow time for DNA repair
*rec*A	Encodes RecA protein required for error-prone repair and recombinational repair
*din*B	Encodes DNA polymerase IV
Genes involved in DNA metabolism, but role in DNA repair unknown	
ssb	Encodes single-stranded DNA-binding protein (SSB)
*uvr*D	Encodes DNA helicase II (DNA-unwinding protein)
*him*A	Encodes subunit of integration host factor, involved in site-specific recombination, replication, transposition, regulation of gene expression
*rec*N	Required for recombinational repair
Genes of unknown function	
*din*D	
*din*F	

Note: Some of these genes and their functions are further discussed in Chapter 28.