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College Of Education For Pure Sciences
Chemistry department

Transfer of genetic information by DNA, replication of DNA, damage and repair of DNA

DNA copying

- Each cell division cell must copy its entire DNA
- So each daughter cell gets a complete copy
- Rate of synthesis
  - Bacteria = 1000 bases per second
  - Mammals = 100 bases per second
- Problem - with a single replication origin in DNA
  - Bacteria genome is 4 x 10E6. Takes 20 minutes to copy.
  - Human is 3.2 x 10E9. Would take 10,000 times longer.

DNA copying solutions

- Double helix has to be copied
- Semi-conservation solution exists
  - Each daughter cells gets one of the original copies

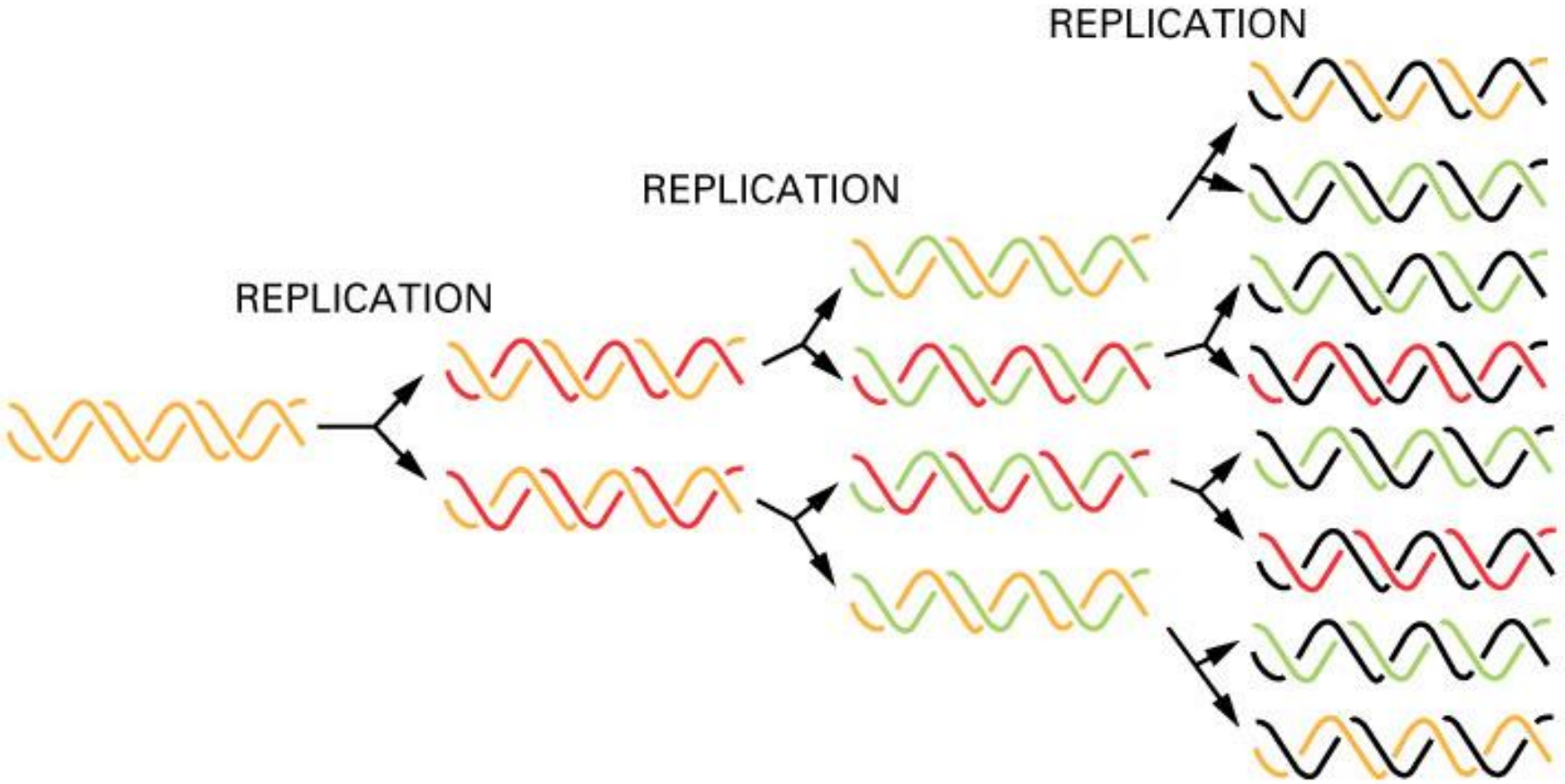


Figure 6-4 Essential Cell Biology, 2/e. (© 2004 Garland Science)

DNA copying solutions

- Double helix has to be copied
- Semi-conservation solution exists
  - Each daughter cells gets one of the original copies
- Unwind at one point and use that as the origin of replication

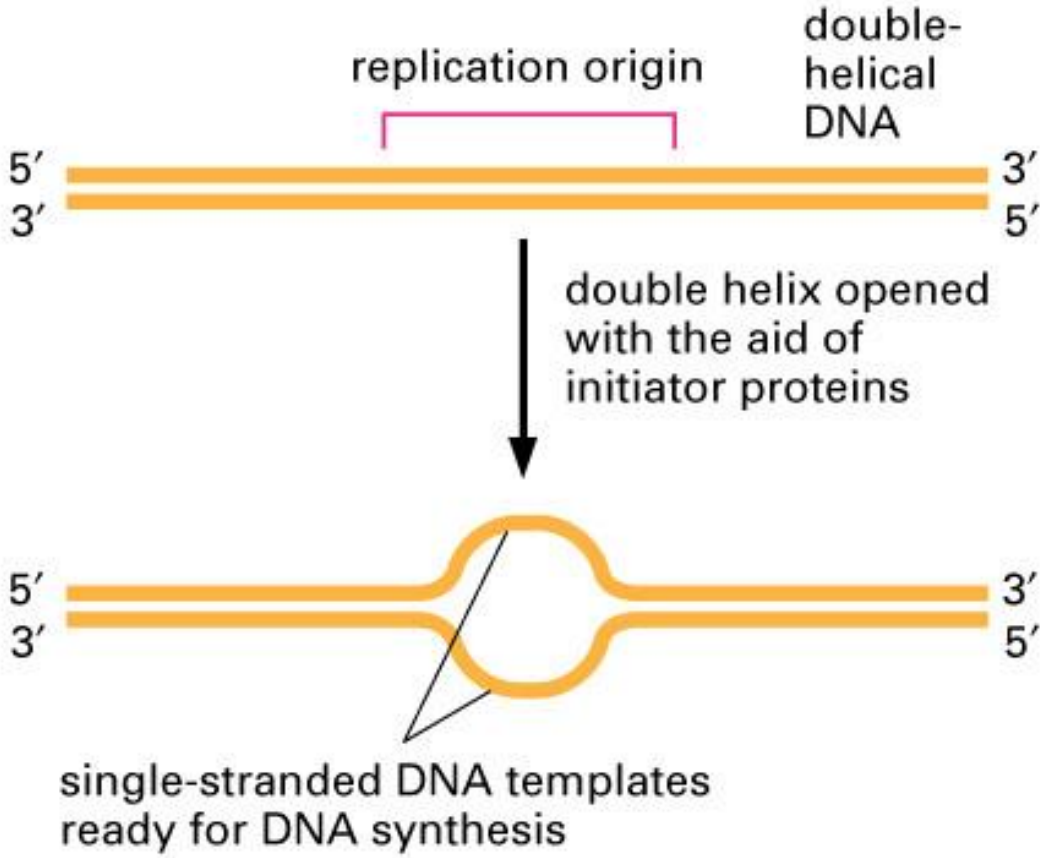


Figure 6-5 Essential Cell Biology, 2/e. (© 2004 Garland Science)

DNA copying solutions

- Double helix has to be copied
- Semi-conservation solution exists
  - Each daughter cells gets one of the original copies
- Unwind at one point and use that as the origin of replication
- Region is AT rich to allow easy separation
- Eukaryotes have multiple replication origins (Humans = 10,000)

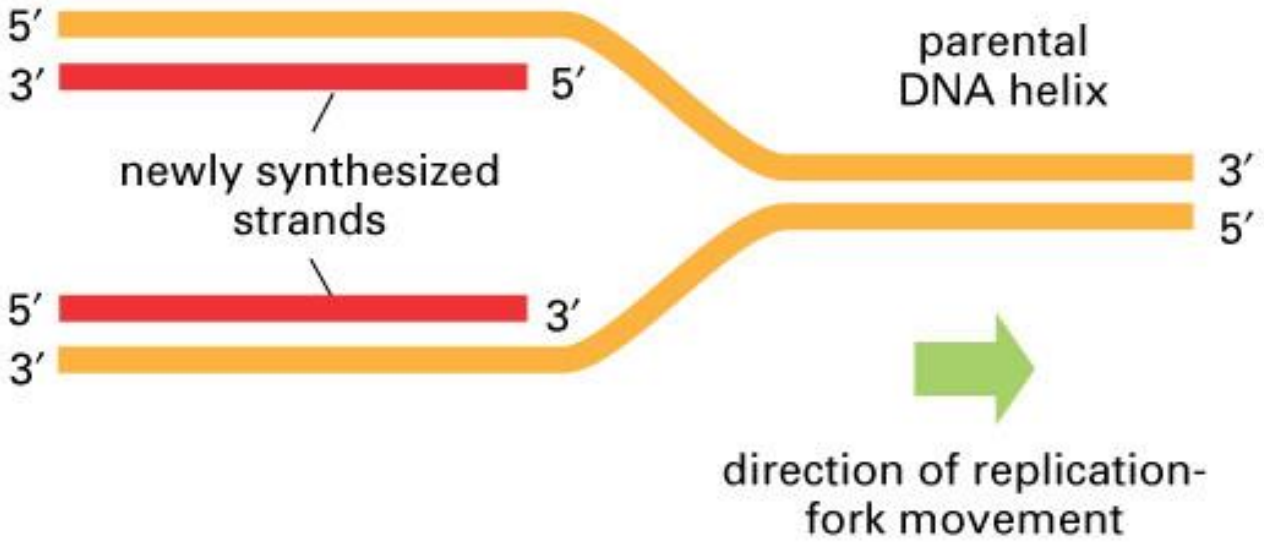


Figure 6-11 Essential Cell Biology, 2/e. (© 2004 Garland Science)

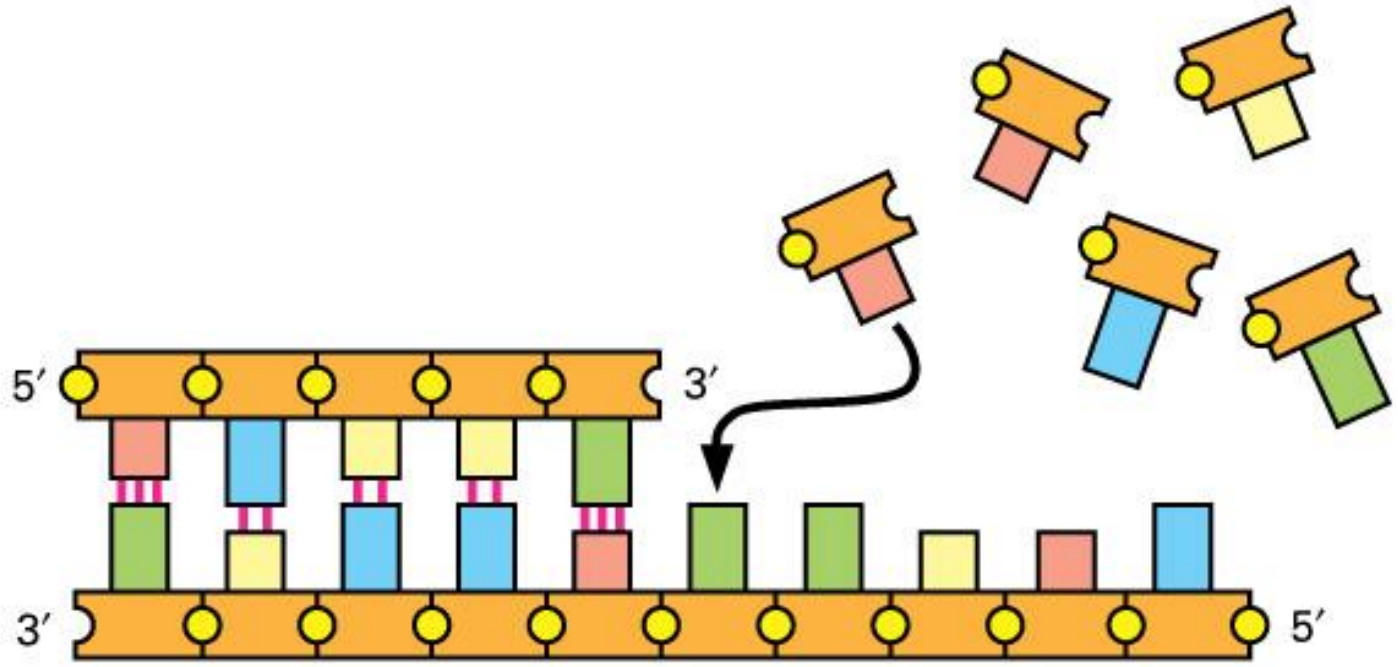
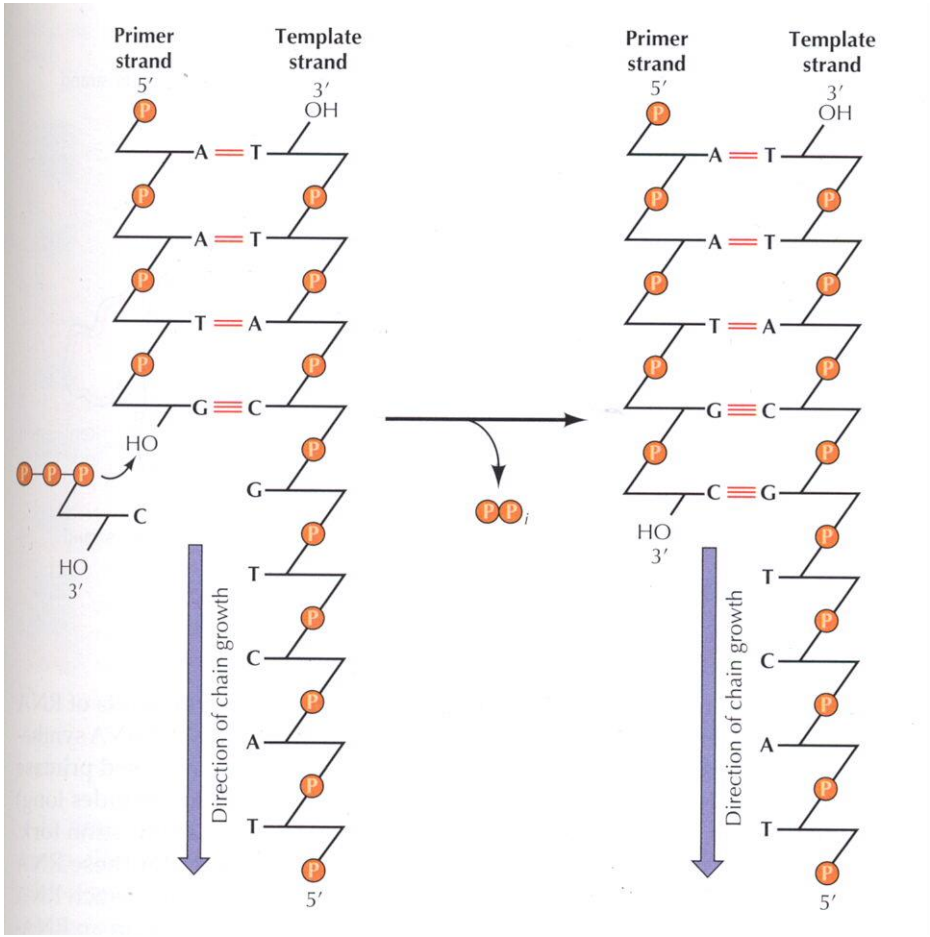


Figure 6-2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Schematic 2

The addition of dNTPs to the growing DNA strand



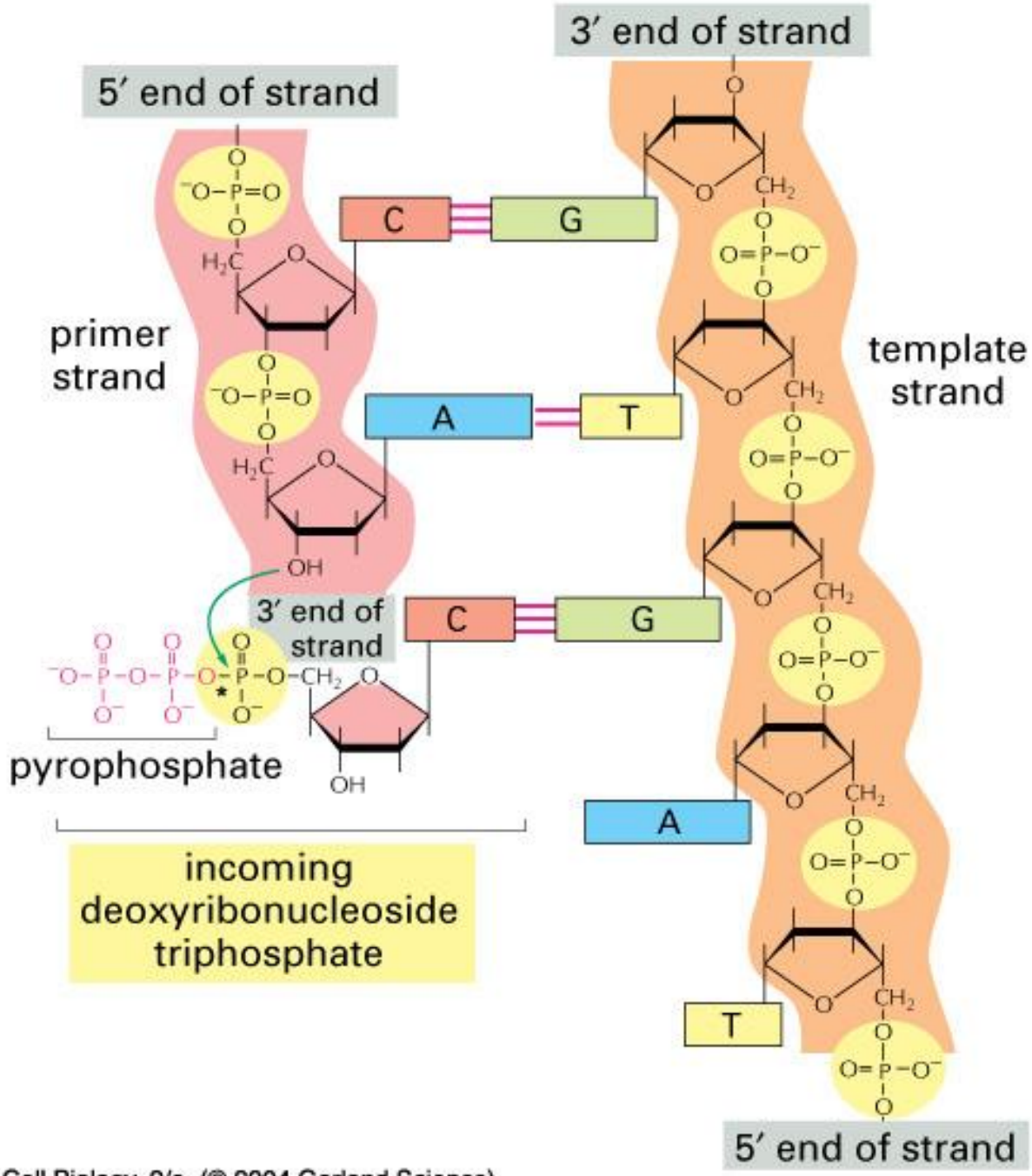


Figure 6-10 Essential Cell Biology, 2/e. (© 2004 Garland Science)

DNA Replication

- The DNA is copied by a replication machine that travels along each replication fork.
- One of the most important members of this complex is DNA POLYMERASE
- It is able to add DNA subunits, making new DNA

DNA Polymerase

- First discovered in 1956 by Kornberg
- Bacteria E.coli
- Bacteria have 3 types
  - DNA Pol I, II, and III
    - DNA Pol III involved in replication of DNA
    - DNA Pol I involved in repair
- Humans have 4 types (you need to know, now)
    - DNA Pol alpha, beta, delta - nuclear DNA
    - DNA Pol gamma - mitochondrial DNA

DNA Polymerase…

- ALL DNA Pol's have 2 properties
  - Only synthesize DNA in one direction 5' to 3'
  - Only add to the end of existing double stranded DNA

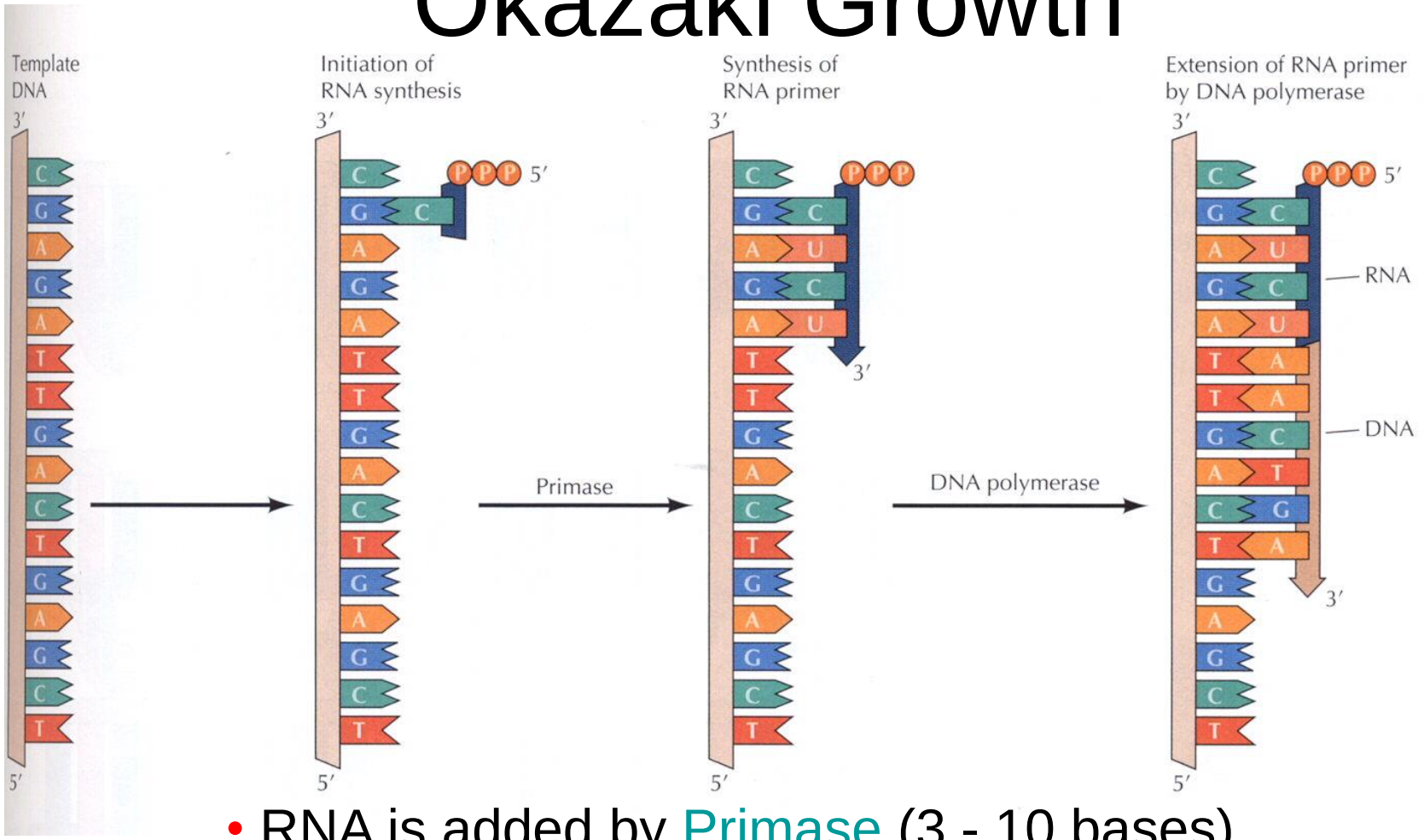
Therefore they CANNOT start synthesis of DNA from scratch.

RNA polymerases can, but not DNA polymerases

What happens at the other strand?

- 3’ to 5’ strand replication
- Solved by Reiji Okazaki
- He saw - one strand made in a continuous manner (leading strand) and the other from short discontinuous pieces (lagging strand)
- The discontinuous pieces are known as Okazaki fragments

Okazaki Growth



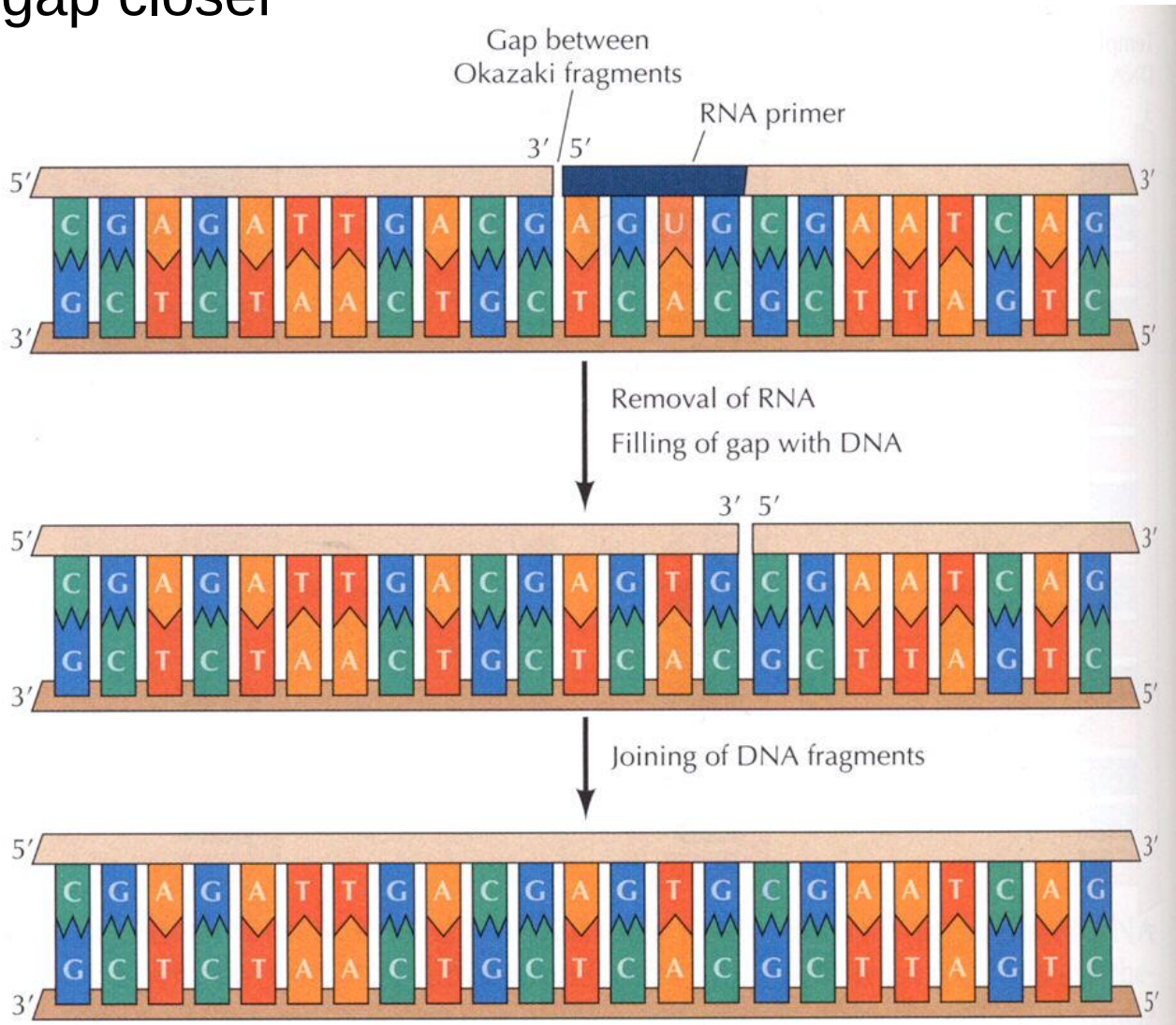
- RNA is added by Primase (3 - 10 bases)
- DNA is added by DNA Pol alpha

Primase can start RNA synthesis de novo

We have a RNA/DNA joint, so RNA is involved in eukaryote DNA replication

Closing the gap between Okazaki fragments

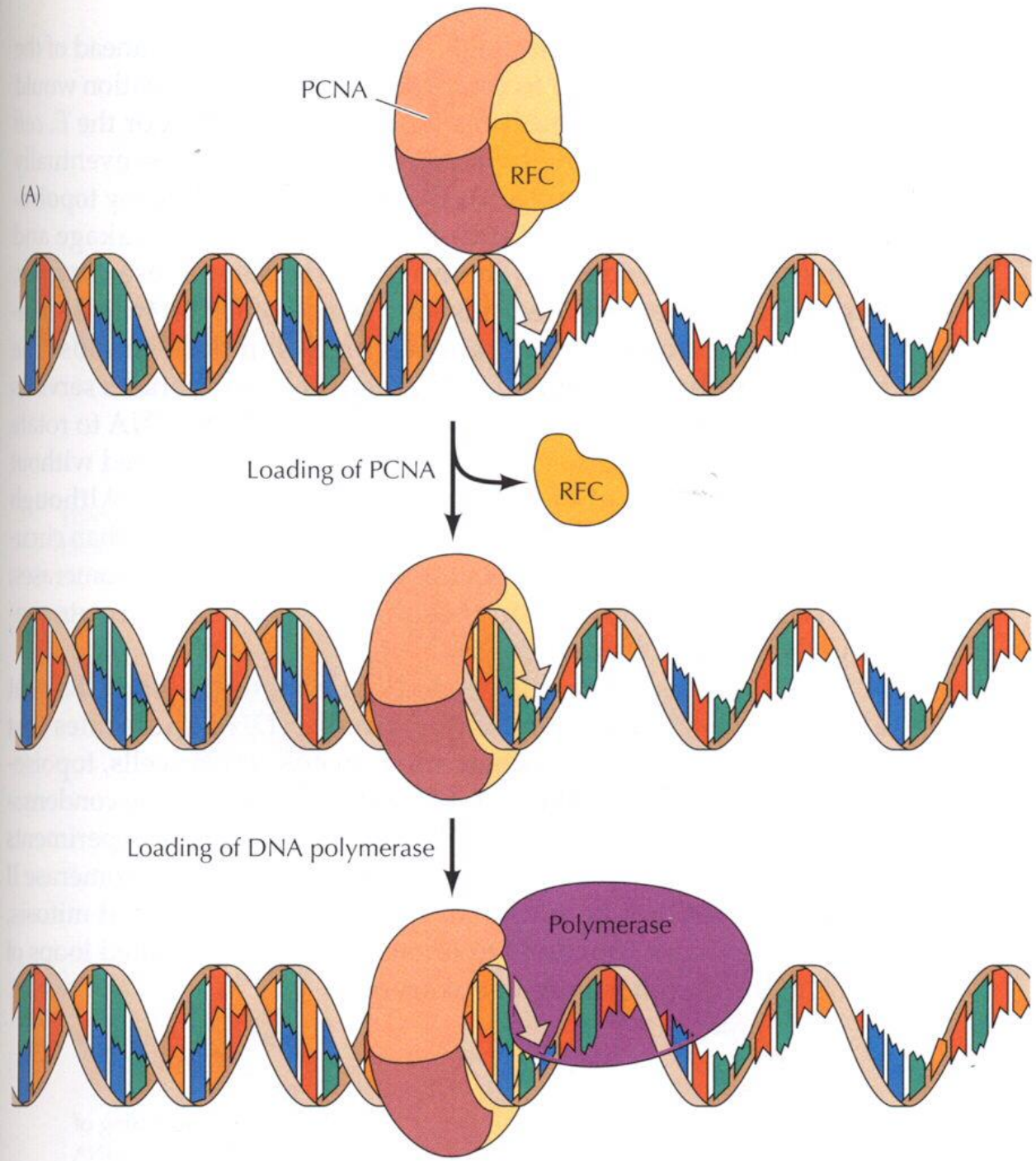
- RNA primer removed by RNase H
- DNA Pol delta fills the gap
- DNA Ligase - the gap closer

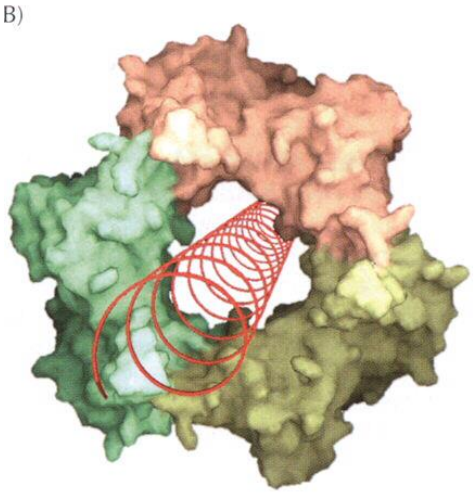


Stabilization of the replication machine

- DNA Pol delta and epsilon are attached to the DNA and held in place by other proteins
  - Sliding-clamp proteins (proliferating cell nuclear antigen - PCNA), allow the stable binding of DNA Pol and strand synthesis
  - Clamp-loading proteins (replication factor C - RFC), aid in attaching the sliding-clamp proteins.

DNA Pol loading





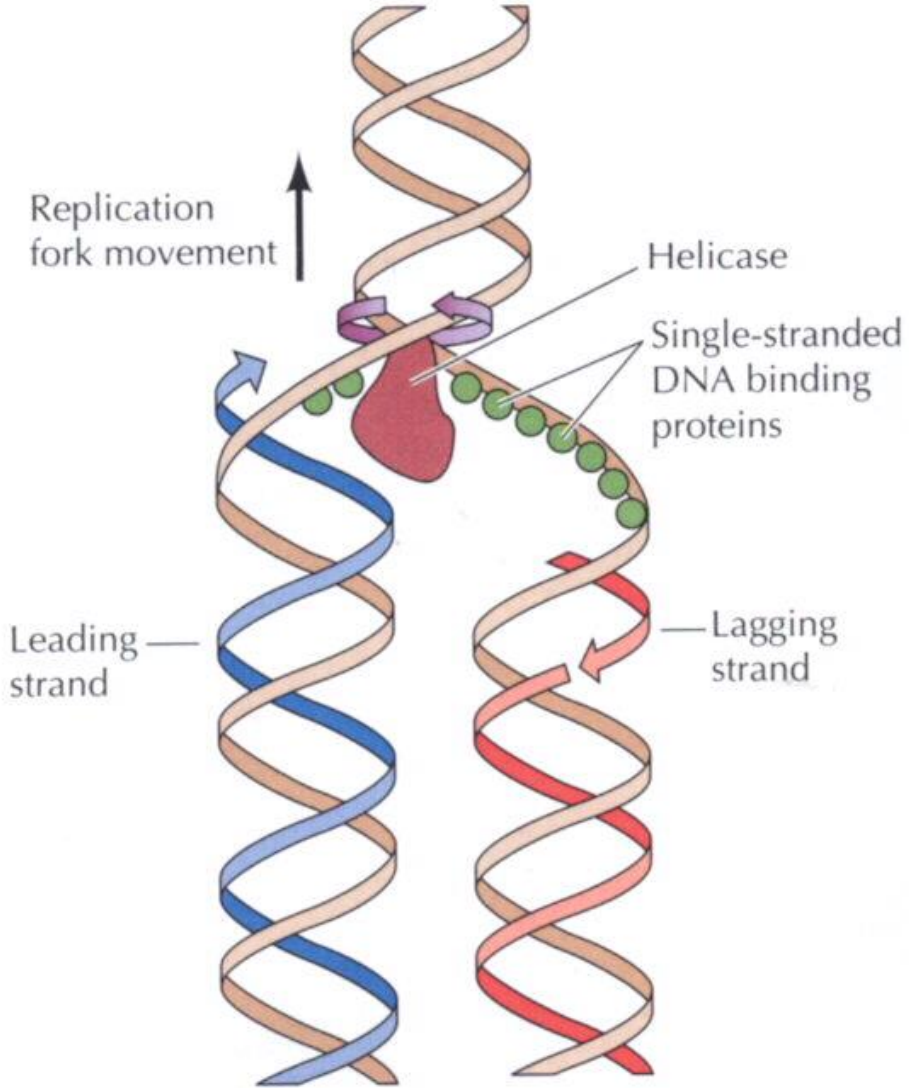
DNA Pol… …more facts

- Has a proofreading mechanism built in
- Checks for base matching
- Removes mismatched bases by going backwards
- Reason why it is not able to build DNA in the 3’ to 5’ direction. No energy from ATP hydrolysis.
- Makes just one error in 10E8 or 10E9 (billion) bases added

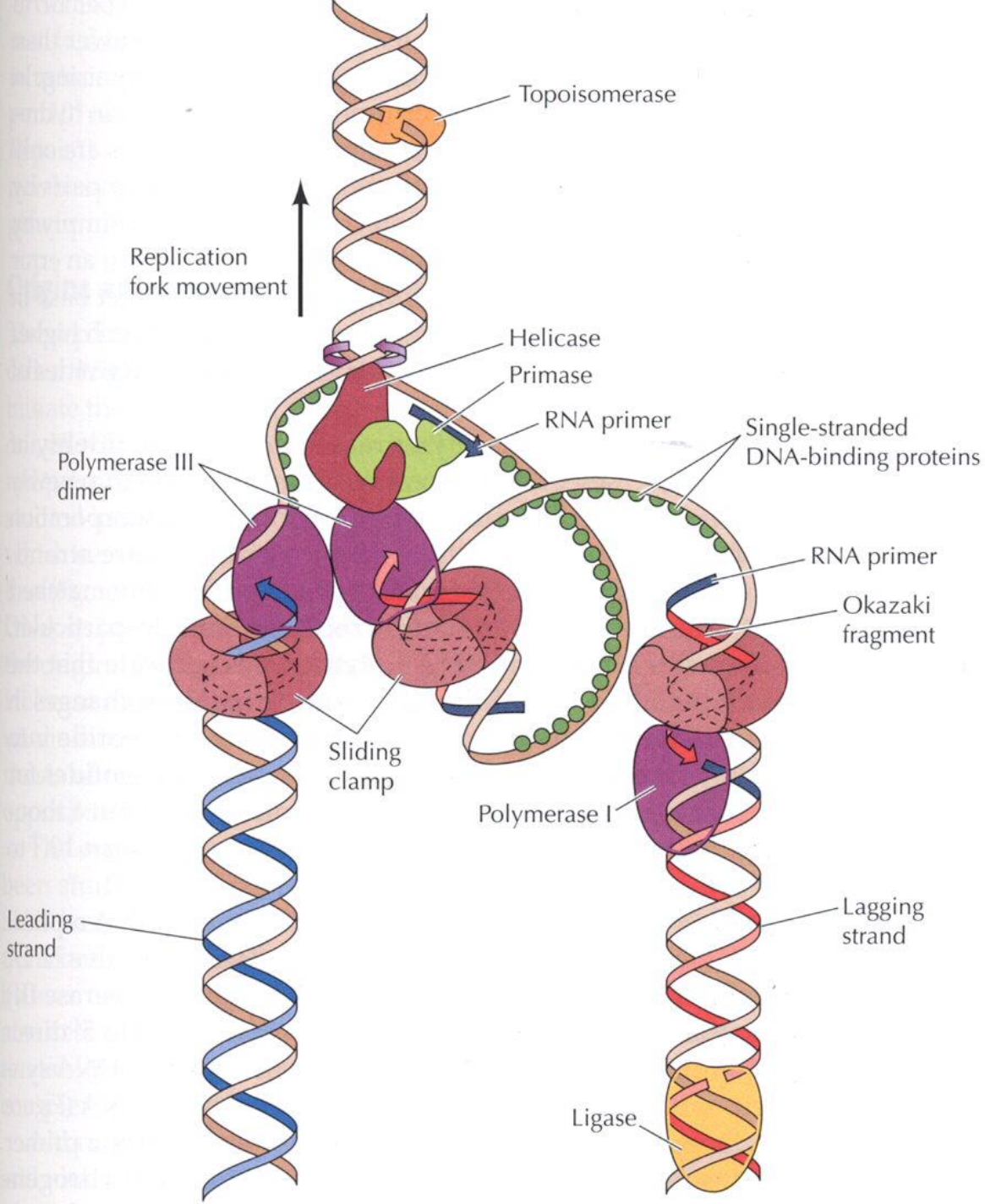
Other helpers

- Unwinding of DNA strands by Helicases
  - Require ATP
- Single-stranded DNA-binding proteins
  - Bind to single stranded DNA to stabilize structure
  - RPA (replication protein A - in eukaryotes)
- Topoisomerase - helps with prevention of DNA strand twisting - ‘swivels’
  - Two types
    - Type I - Break one strand only and then rejoin
    - Type II - Break both strands and then rejoin

Protein aids



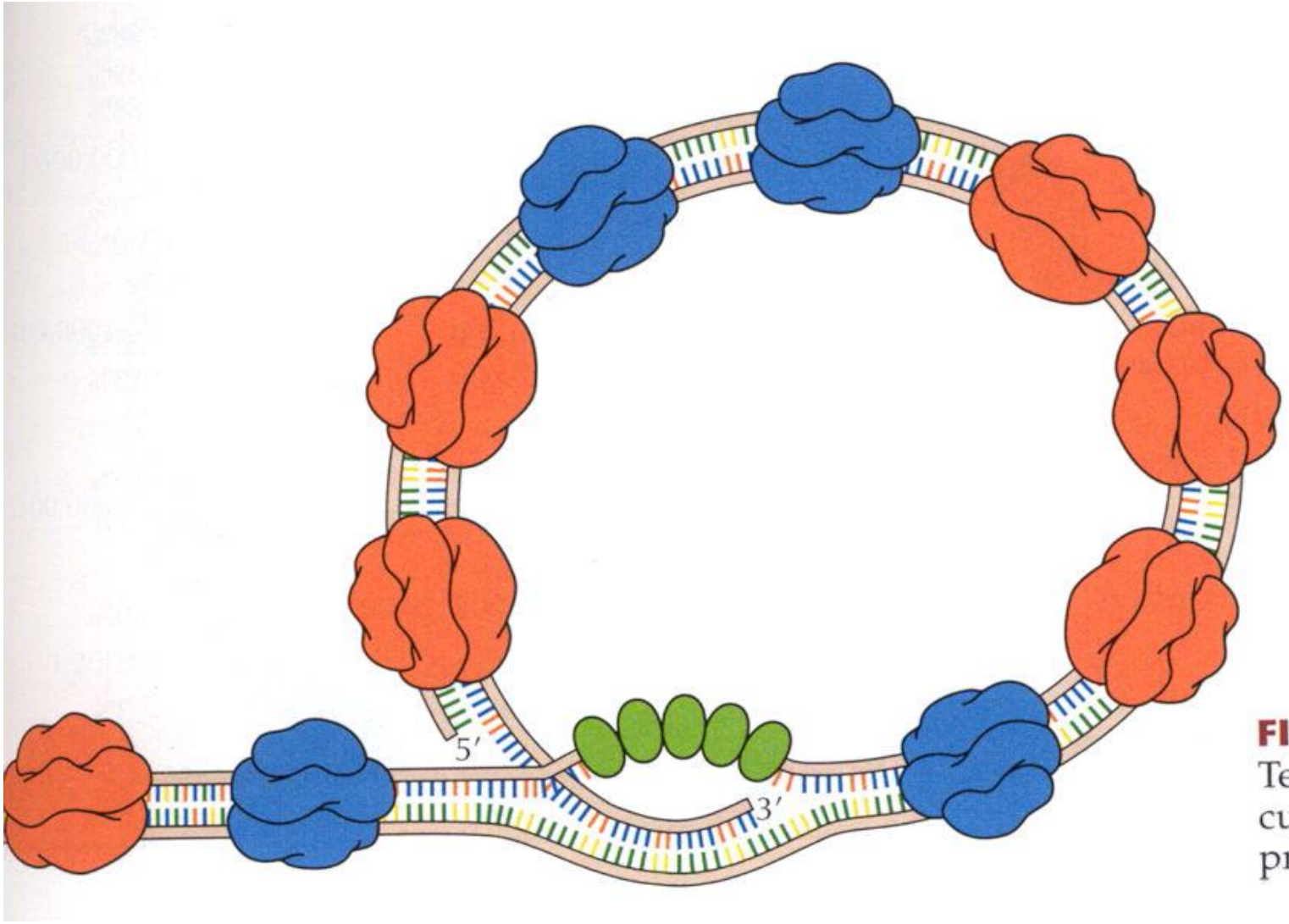
Overview


Topoisomerase
Replication fork movement
Helicase
Primase
RNA primer
Single-stranded DNA-binding proteins
Polymerase III dimer
RNA primer
Okazaki fragment
Sliding clamp
Polymerase I
Leading strand
Lagging strand
Ligase

What happens to the histones

- Nucleosomes are disrupted during replication
- Each ds strand gets half
- New histones added by chromatin assembly factors

Telomers - present challenge



FIGU
Telon
cular
prote

Telomerase

- Enzyme
- Reverse transcriptase
- Acts without DNA template, as it has its own RNA template stored inside

- RESULT = DNA is extended each time it is copied, however other mechanisms restore length in germ cells. Somatic cells do not have enough telomerase to keep this process going so they lose telomere length and this is thought to result in cell death.

Accuracy and Fidelity

Maintenance of DNA Sequences

Maintenance of DNA Sequences

DNA Polymerase as Self Correcting Enzyme

- Correct nucleotide has greater affinity for moving polymerase than incorrect nucleotide
- Exonucleolytic proofreading of DNA polymerase
  - DNA molecules w/ mismatched 3’ OH end are not effective templates; polymerase cannot extend when 3’ OH is not base paired
  - DNA polymerase has separate catalytic site that removes unpaired residues at terminus

Maintenance of DNA Sequences

DNA Polymerase as Self Correcting Enzyme

Two catalytic sites

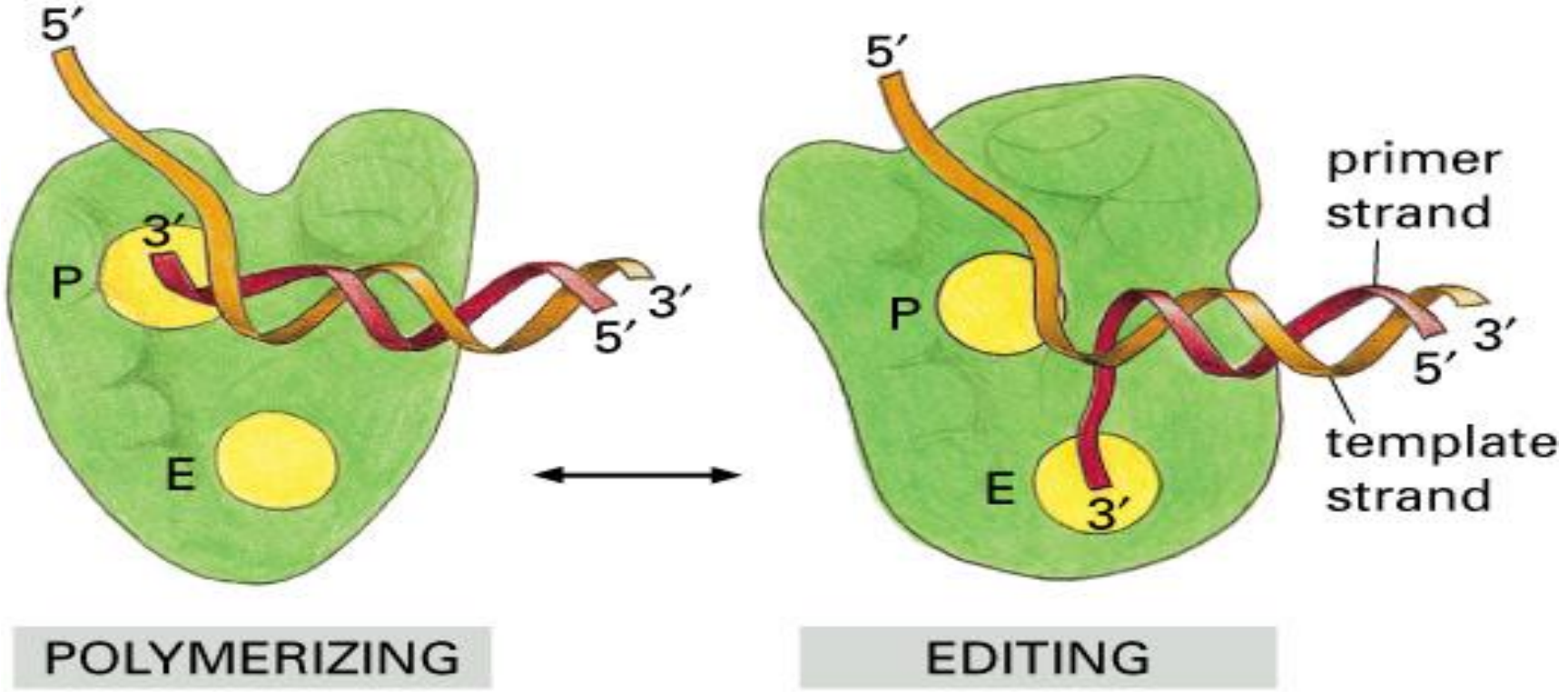


Figure 5–10. Molecular Biology of the Cell, 4th Edition.

Strand Directed Mismatch Repair in Mammals

- Newly synthesized strand is preferentially nicked and can be distinguish in this manner from parental strand
- Defective copy of mismatch repair gene predisposed to cancer

Strand Directed Mismatch Repair System

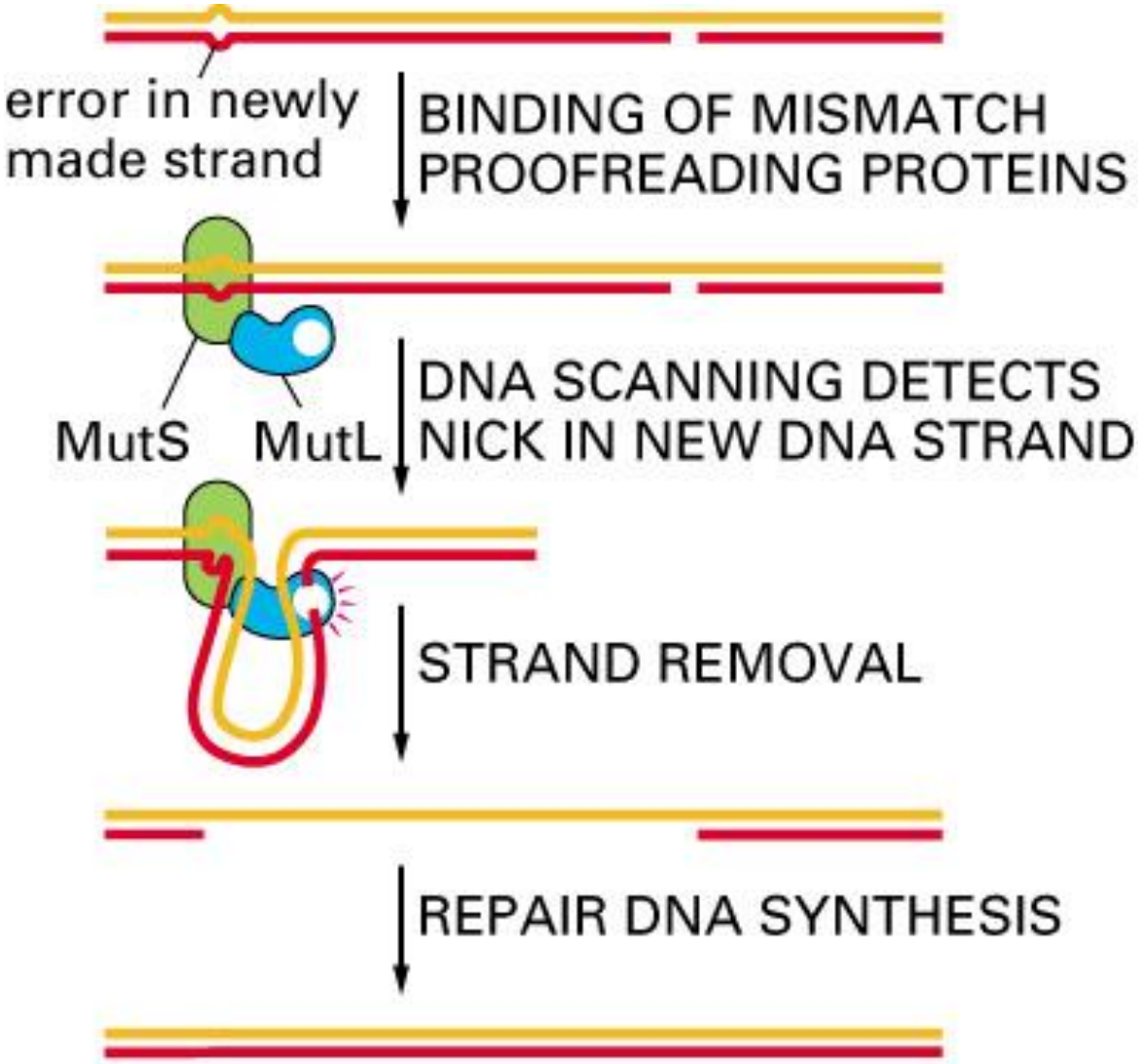
- Removes replication errors not recognized by replication machine
- Detects distortion in DNA helix
- Distinguishes newly replicated strand from parental strand by methylation of A residues in GATC in bacteria
- Methylation occurs shortly after replication occurs
- Reduces error rate 100X
- 3 Step Process

  recognition of mismatch

  excision of segment of DNA containing mismatch

  resynthesis of excised fragment

Strand Directed Mismatch Repair System



Causes of DNA Damage

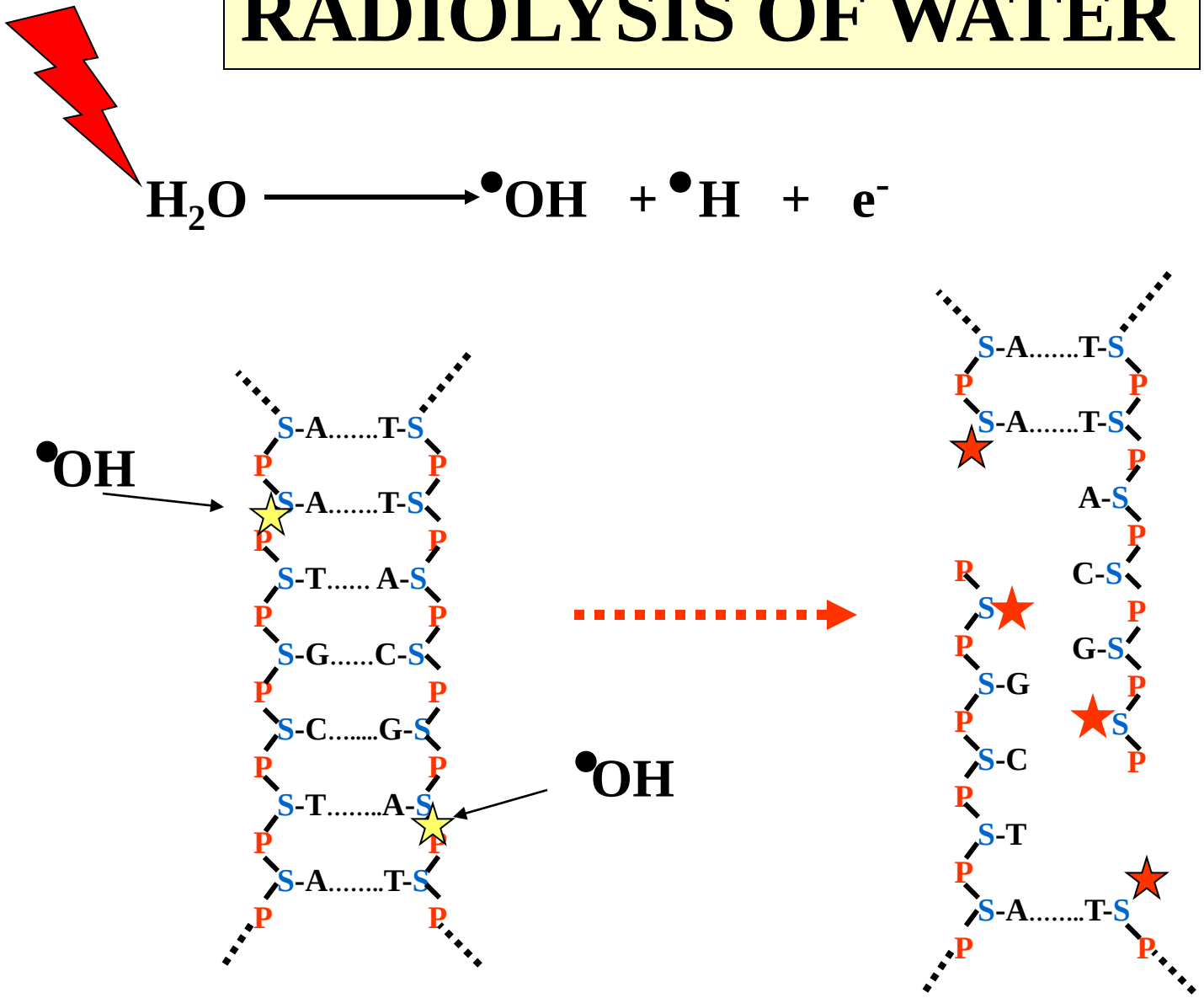
- Chemical mutagens
- Radiation
- Free radicals

RADIATION = ENERGY

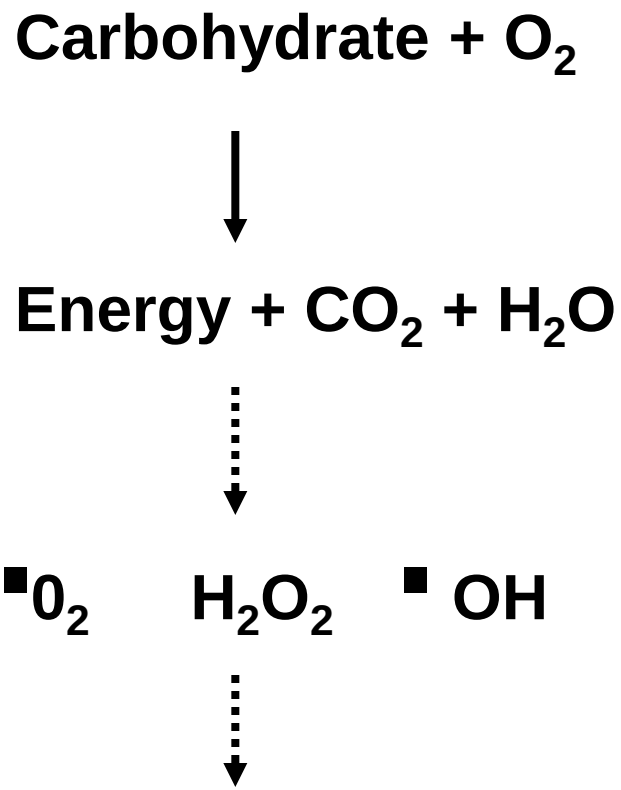
ENERGY DEPOSITION IN DNA

DNA DAMAGE

RADIOLYSIS OF WATER

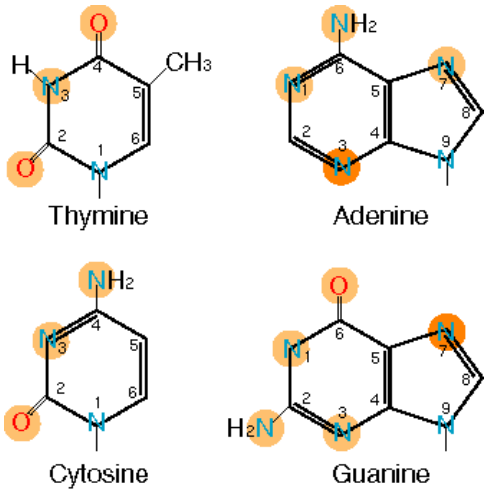


RESPIRATION AND AGEING

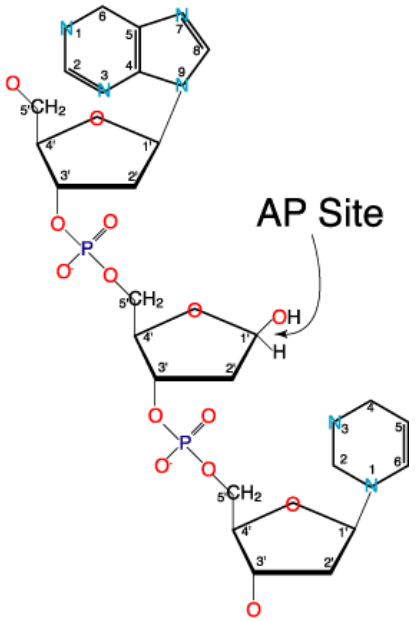


DNA Repair

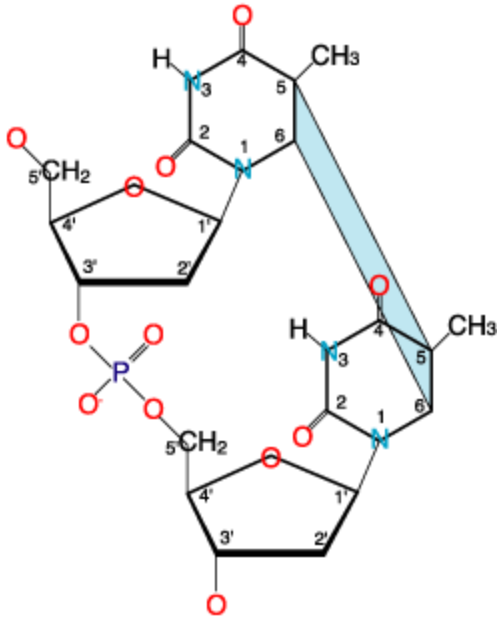
Types of DNA Damage: Base Loss and Base Modification



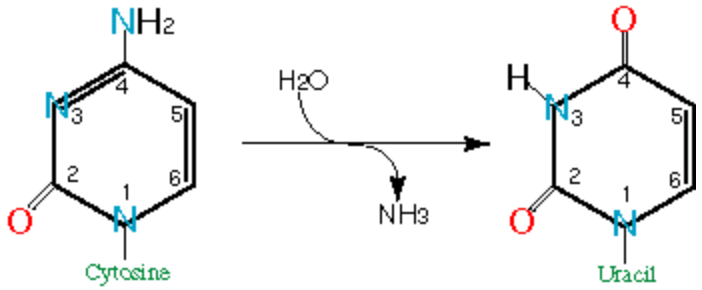
Chemical Modification



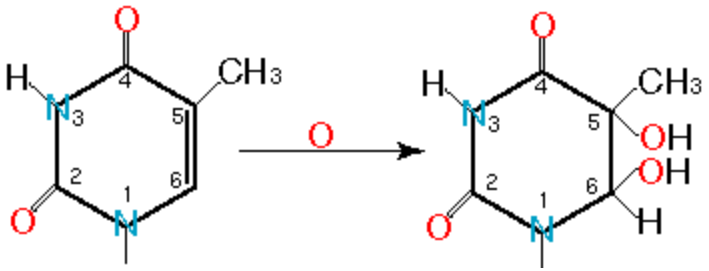
Depurination



Photodamage thymine dimer



Deamination



Chemical Modification by O2 free radicals

DNA Repair

- Despite 1000's of alterations that occur in DNA each day, few are retained as mutations
- Efficient repair mechanisms
- Importance of DNA repair highlighted by:

  Number of genes devoted to DNA repair

  ↑ mutation rates with inactivation or loss of DNA repair gene
- Defects in DNA repair associated with several disease states

DNA replication and repair disorders

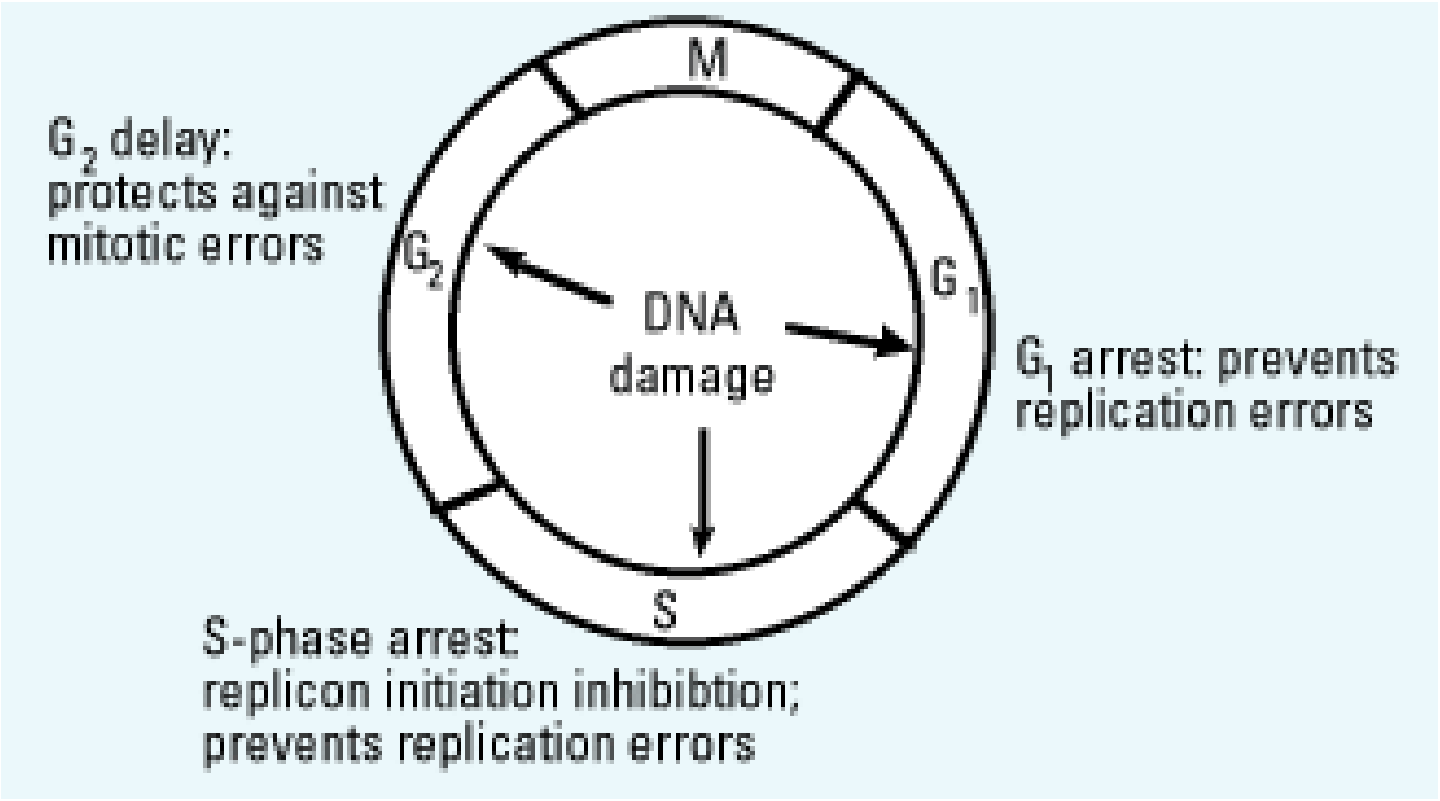
Disorder	Frequency	Defect
Fanconi's anaemia	1/22,000 in some popns.	Deficient excision repair
Hereditary nonpolyposis colon cance	1/200	Deficient mismatch repair
Werner's syndrome	3/1,000,000	Deficient helicase
Xeroderma pigmentosum	1/250,000	Deficient excision repair

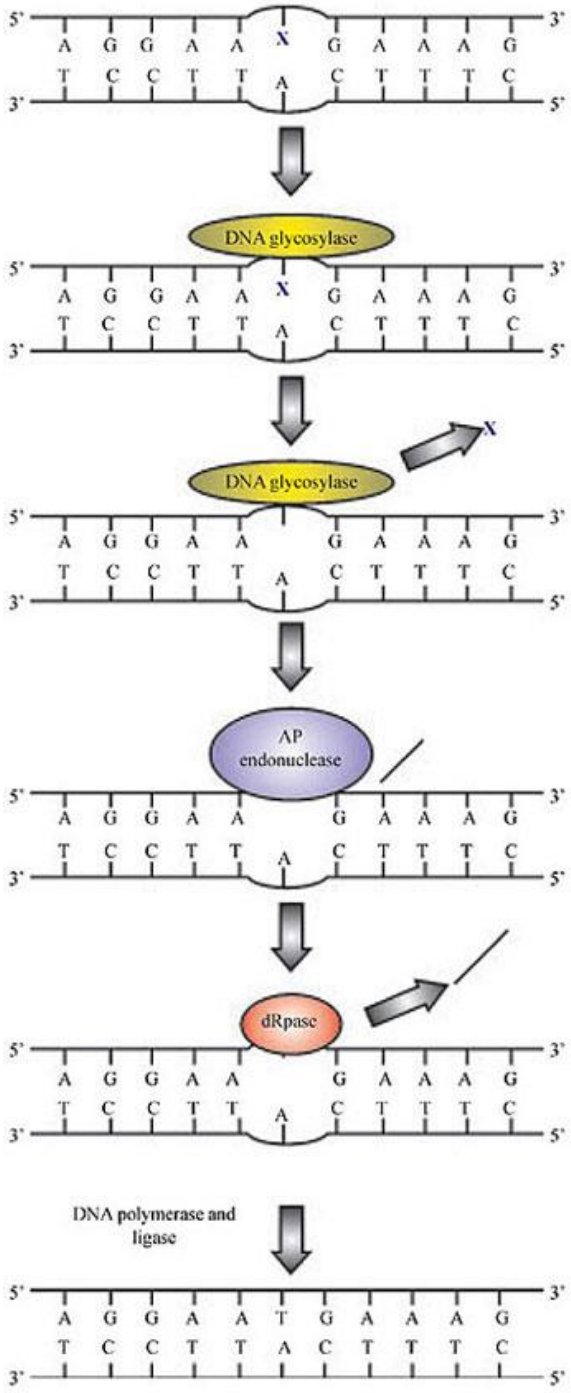
DNA Repair

DNA Damage Can Activate Expression of Whole Sets of Genes

- Heat Shock Response
- SOS Response

DNA Repair


M
G2 delay:
protects against
mitotic errors
G2
G1
DNA
damage
G1 arrest: prevents
replication errors
S
S-phase arrest:
replicon initiation inhibibtion;
prevents replication errors



DNA Repair

Base Excision Repair

a. DNA glycosylase recognizes damaged base

b. Removes base leaving deoxyribose sugar

c. AP endonuclease cuts phosphodiester backbone

d. DNA polymerase replaces missing nucleotide

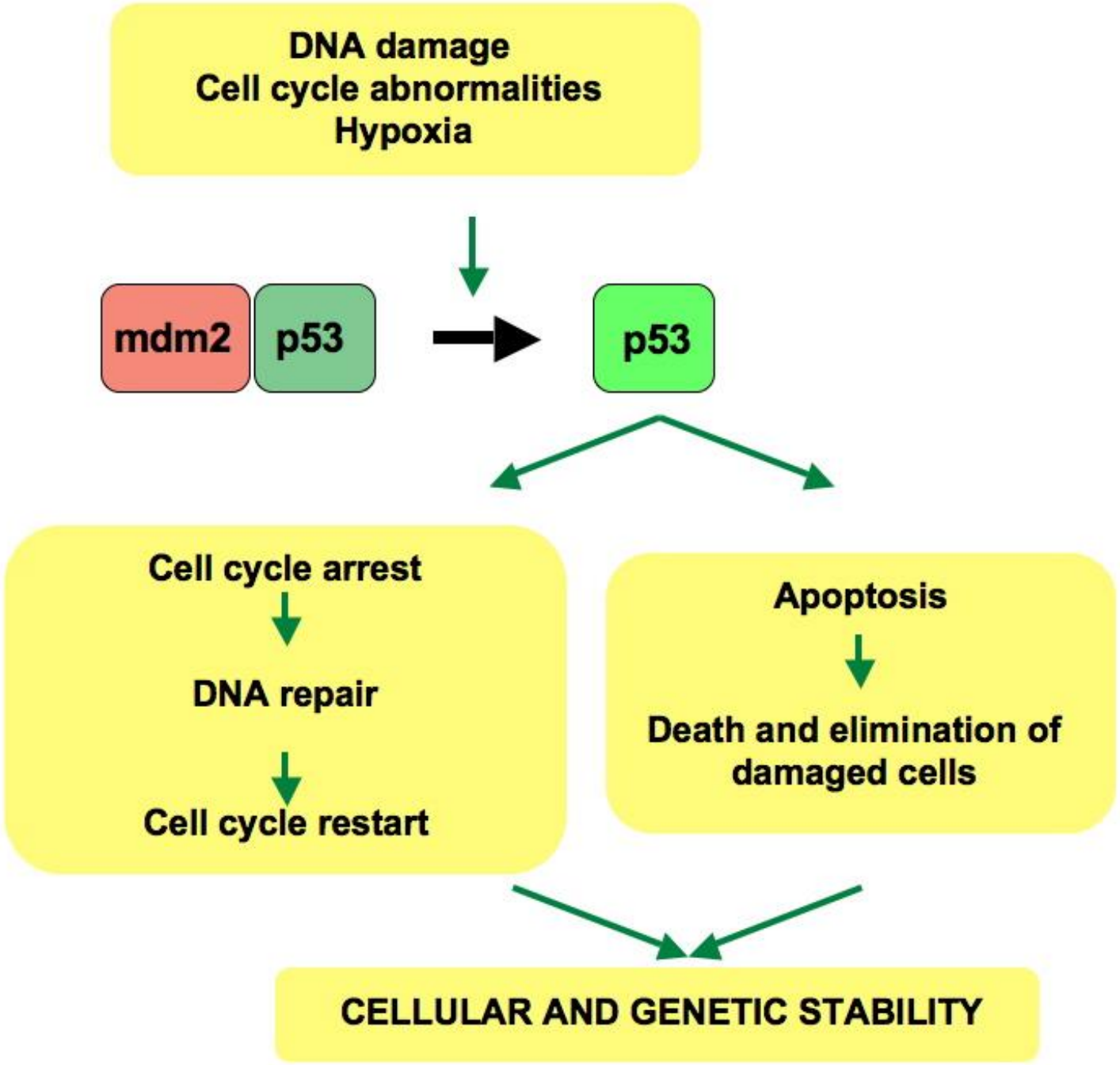
e. DNA ligase seals nick

Failure of DNA repair

- When DNA repair fails, fewer mutations corrected → increase in number of mutations in the genome.

- The protein p53 monitors repair of damaged DNA.
- If damage too severe, p53 protein promotes programmed cell death (apoptosis)

- Mutations in genes encoding DNA repair proteins can be inherited → overall increase in mutations as errors or damage to DNA no longer repaired efficiently.


DNA damage
Cell cycle abnormalities
Hypoxia
mdm2
p53
p53
Cell cycle arrest
DNA repair
Cell cycle restart
Apoptosis
Death and elimination of damaged cells
CELLULAR AND GENETIC STABILITY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