Replication

Objectives

Steps of DNA Synthesis

DNA polymerases in prokaryotes and Eukaryotes

Difference in function of topoisomerase and ligase

Replication inhibitors

DNA repair

Action of Telomerase

Case Report

- An 8-year-old female, born out of the second degree consanguineous marriage presented with complaints of hypo- and hyper-pigmented spots since the age of 1 year all over the body which gradually increased in size and number associated with small warty growths over face for 5 years. She had a history of acute sunburn all over the body

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- except armpits and perineum after sun exposure. The patient also had photophobia with redness, watering of eyes. On examination, she had multiple hyperpigmented warty growths over face [Figure 1]. The patient was advised biopsy and photoprotection, but parents denied biopsy. After 1 year, patient came with an increase in number, size of the growths. All the growths were, warty and firm in consistency [Figure 1b].

- A nontender nodular growth measuring about 2 cm × 3 cm was present over the right mandibular region from which biopsy was taken which was suggestive of nodular melanoma [Figure 2]. Another biopsy from growth over right temporal region showed features of hypertrophic type of actinic keratosis . Routine investigations were within normal limits.

- The patient was referred to oncology department, but relatives refused for any further management. The patient visited our department again after 2 years of the first visit with small fungating growths over face, scalp (4 cm × 4 cm) and large foul smelling fungating growth over nape of the neck extending over to upper back (10 cm × 8 cm) [Figure 3].

- Biopsy was taken from the growth over the nape of the neck which showed features of squamous cell carcinoma [Figure 3]. There was a history of injury to the right eye, in between the two visits for which evisceration was done. The left eye was congested; right orbital socket was empty

Fig 1:Photograph of the patient at first visit

Fig 2: A well circumscribed nodular lesion with excess melanin pigment deposition and irregular arrangement of neoplastic cells

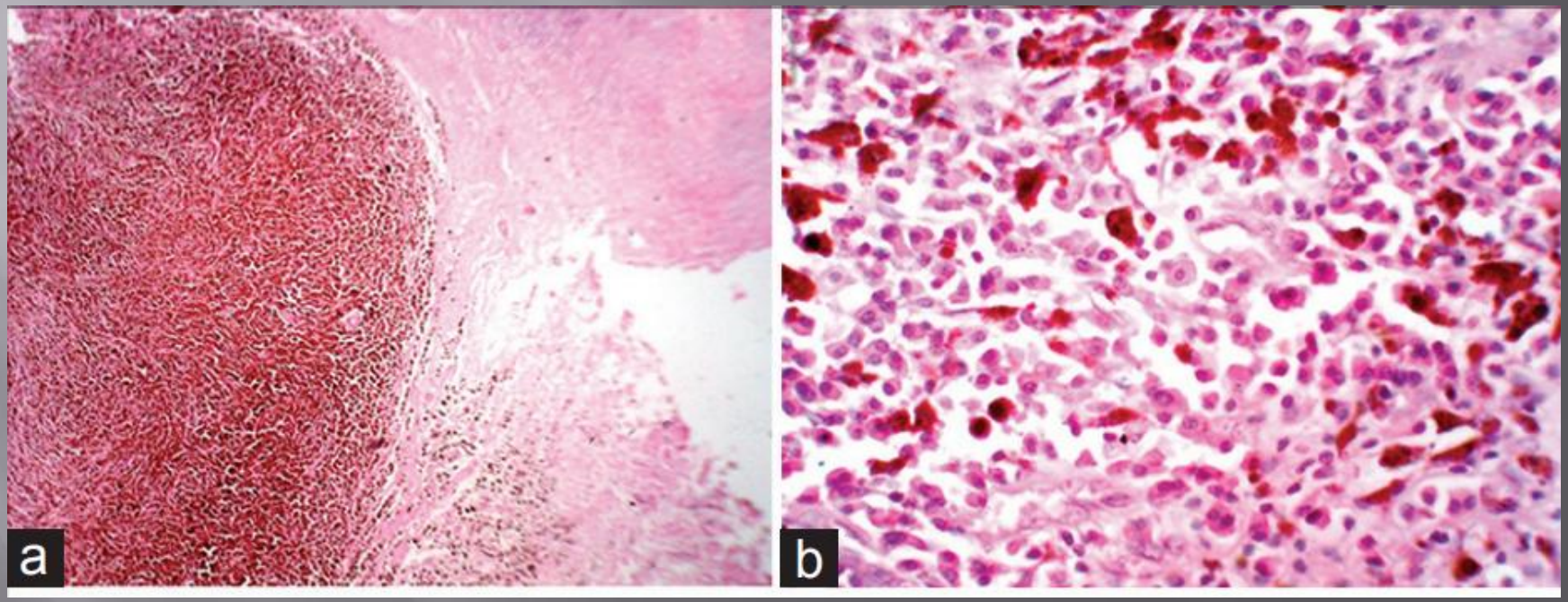
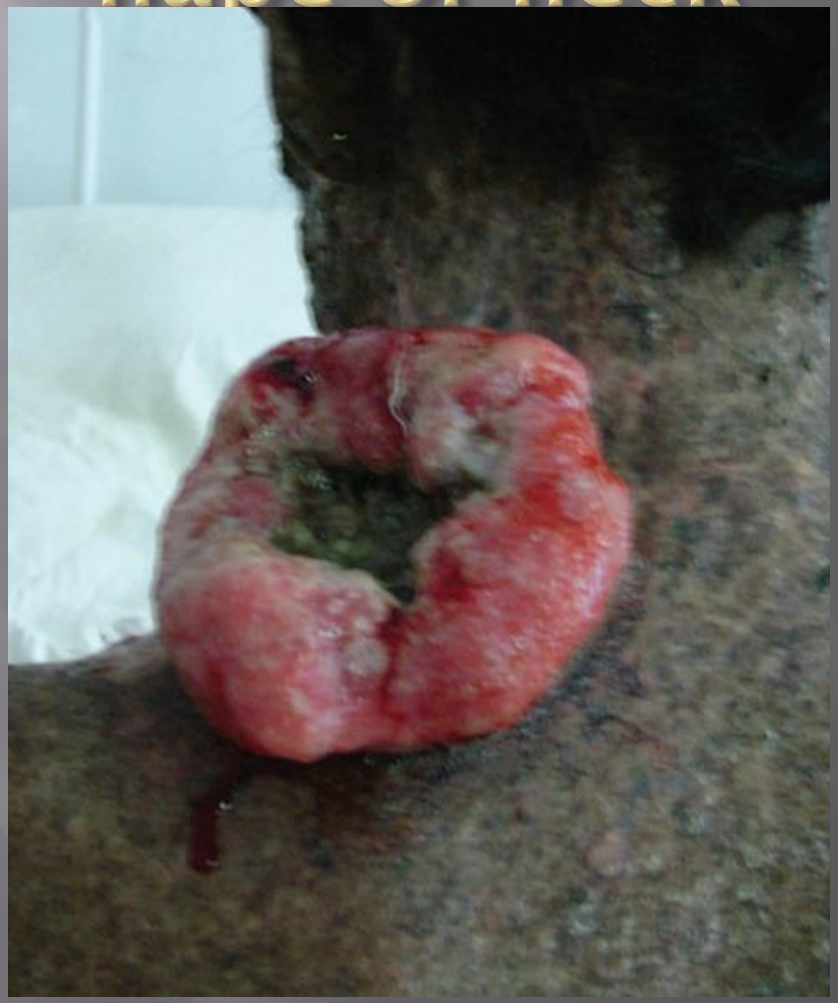


Fig 3:Large fungating growth with bleeding over left side of nape of neck



- What is the likely diagnosis?
- How would you make a definite diagnosis?
- What is the pathophysiology of this disorder?

- What is replication?

  Biologic process of producing 2 identical replicas of DNA from one original DNA molecule.

- Purpose :

  Provision of progeny with genetic information

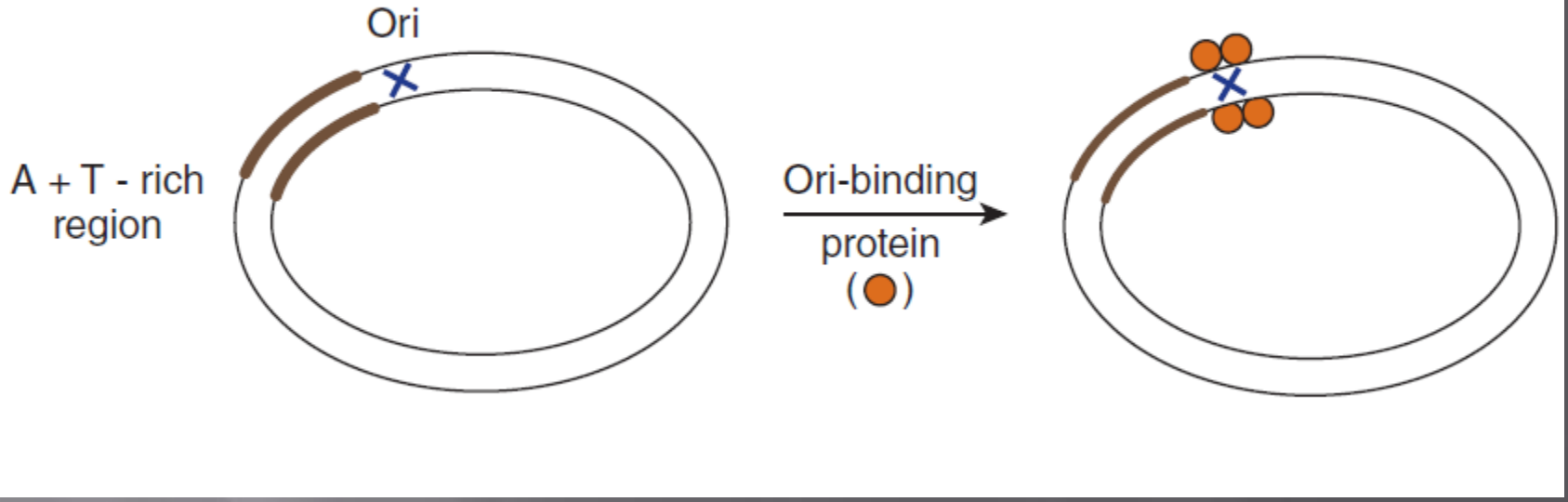
History

- First enzymologic observations on DNA replication were made by
  - Arthur Kornberg
  - Existence of replication enzyme
    - DNA polymerase I

Steps Involved in DNA Replication in Eukaryotes

1. Identification of the origins of replication
2. ATP hydrolysis-driven unwinding of dsDNA to provide an ssDNA template
3. Formation of the replication fork; synthesis of RNA primer
4. Initiation of DNA synthesis and elongation
5. Formation of replication bubbles with ligation of the newly synthesized DNA segments
6. Reconstitution of chromatin structure

Origin of DNA replication



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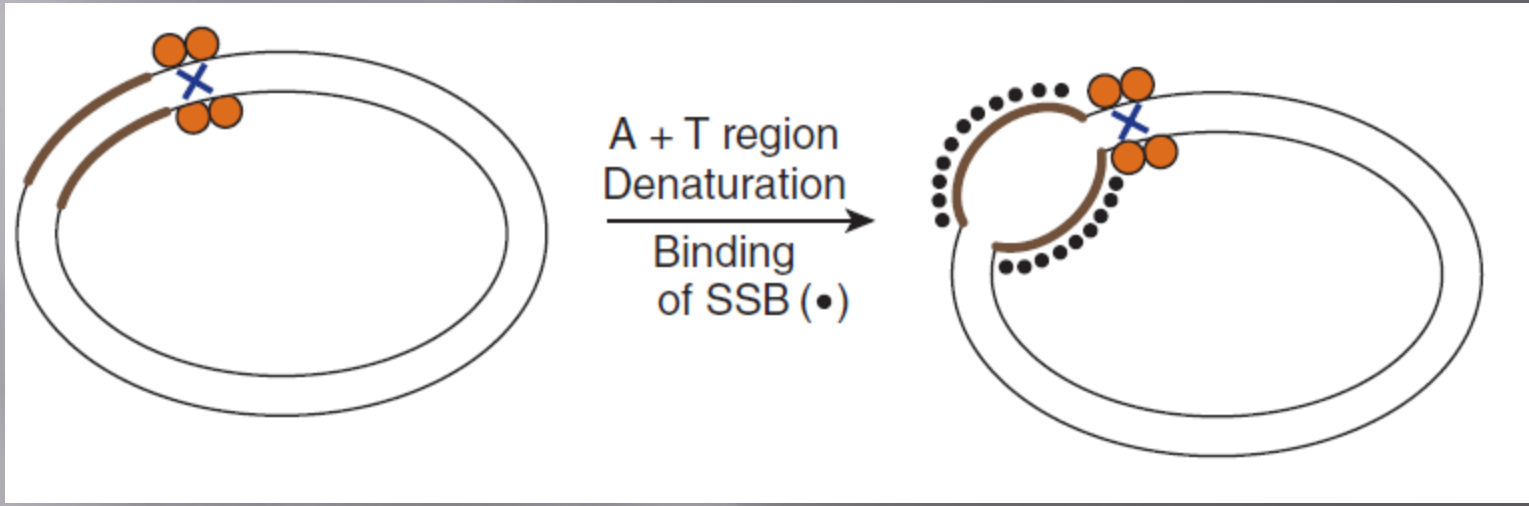

Unwinding of DNA

Binding of factors, formation of replication fork, initiation of replication

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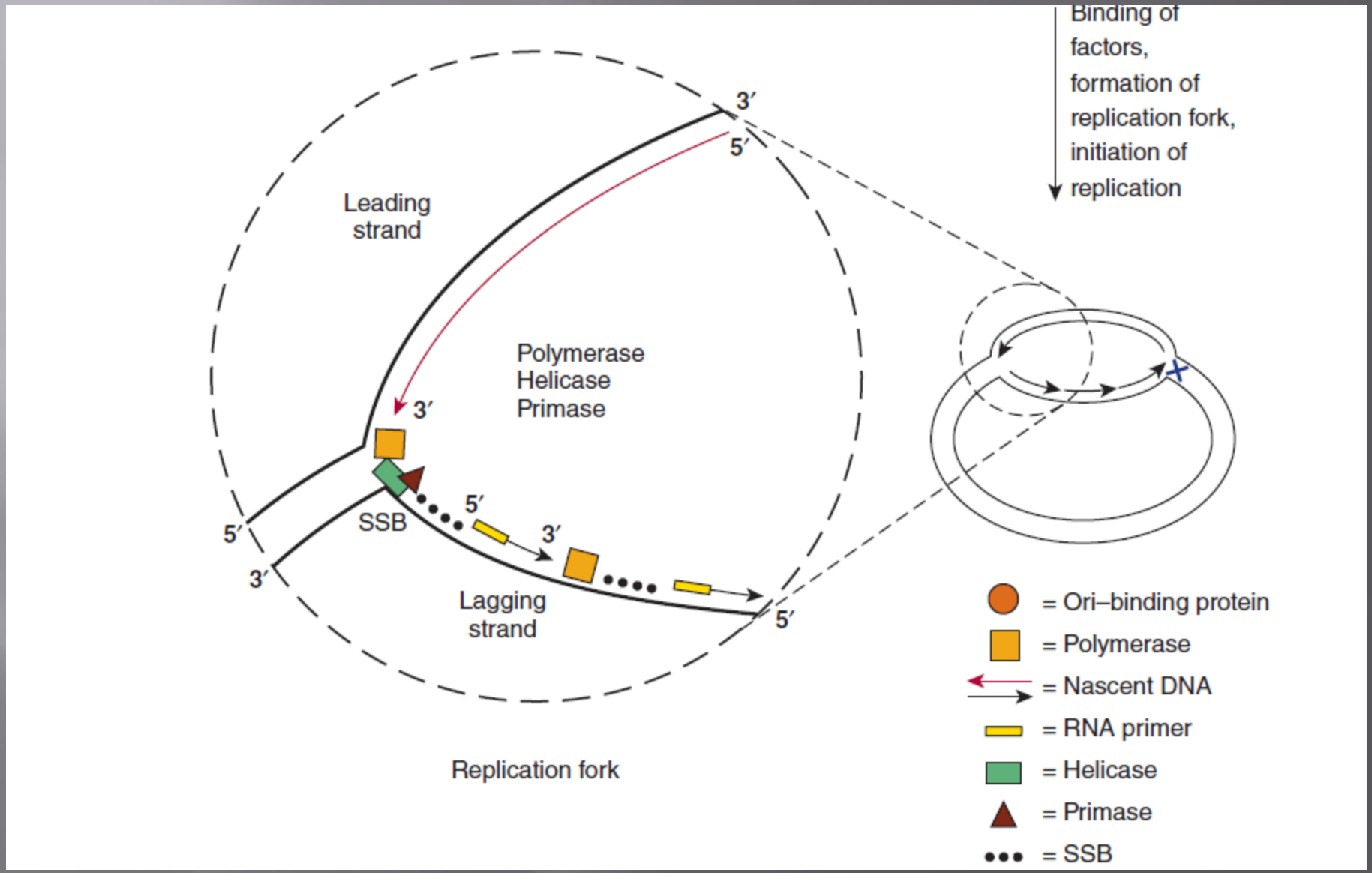
Formation of Replication fork: 4 components



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Classes of Proteins Involved in Replication

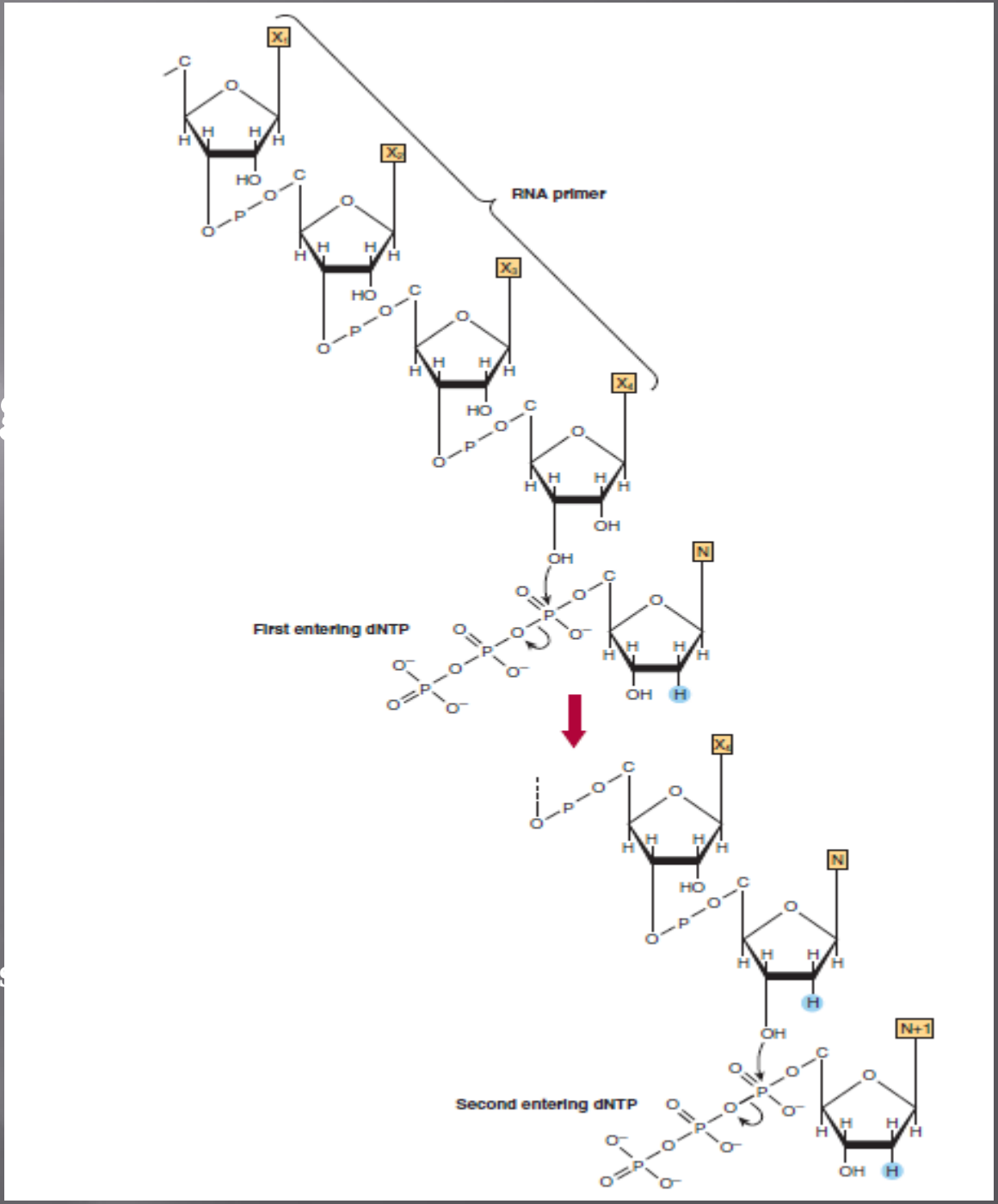
Protein	Function
DNA polymerases	Deoxynucleotide polymerization
Helicases	ATP -driven processive unwinding of DNA
Topoisomerases	Relieve torsional strain that results from helicase-induced unwinding
DNA primase	Initiates synthesis of RNA primers
Single-strand binding proteins (SSBs)	Prevent premature reannealing of dsDNA
DNA ligase	Seals the single strand nick between the nascent chain and Okazaki fragments on lagging strand

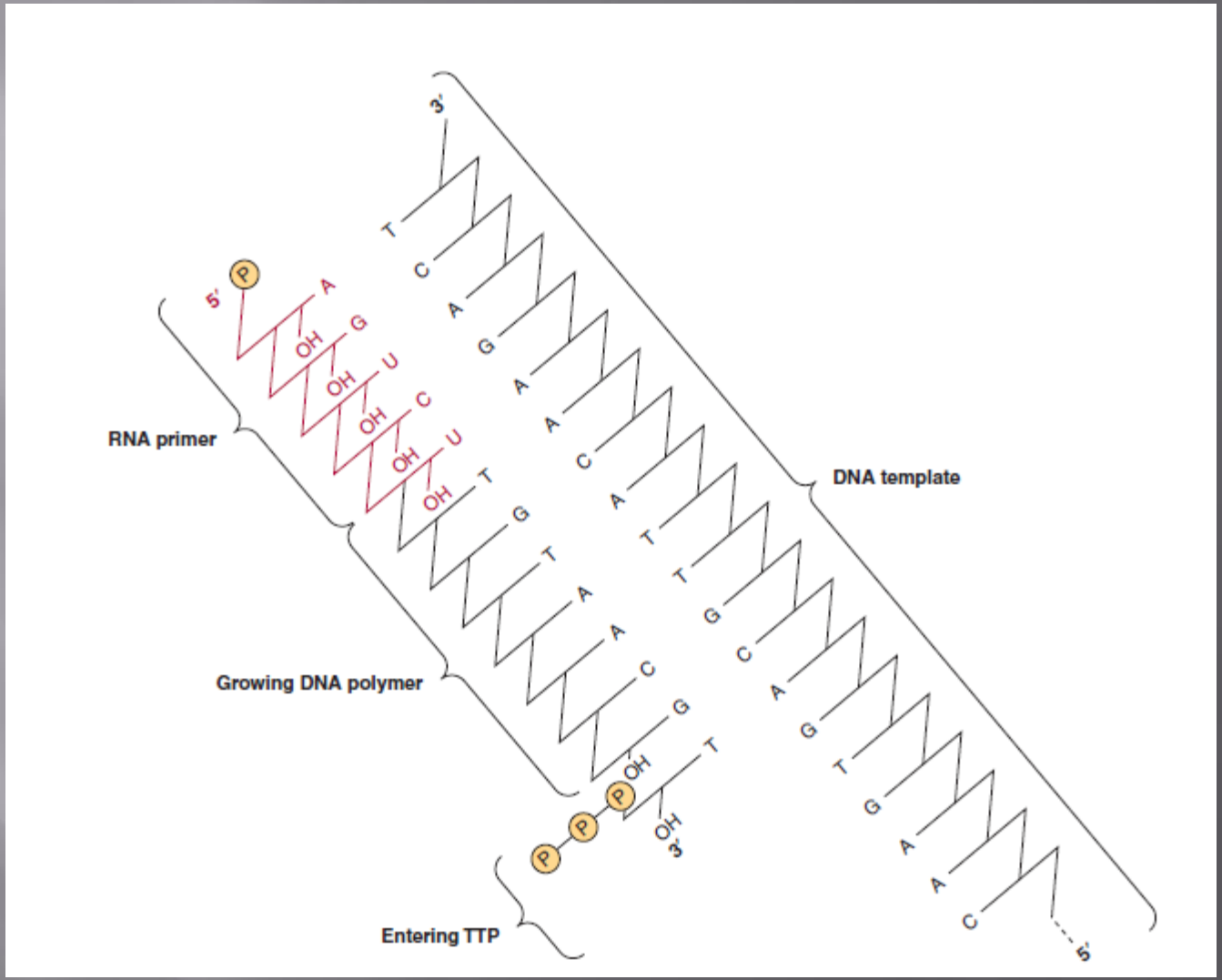
DNA Polymerase

- Chain elongation
  - Rate at which polymerisation
- Processivity
  - Number of nucleotides added to the nacsent chainbefore the polymerase disengages from template
- Proof reading
  - Identify copying errors

Initiation and Elong of DNA synthesis

The initiation of DNA synthes upon a primer of RNA





The RNA-primed synthesis of DNA demonstrating the template function of the complementary strand of parental DNA

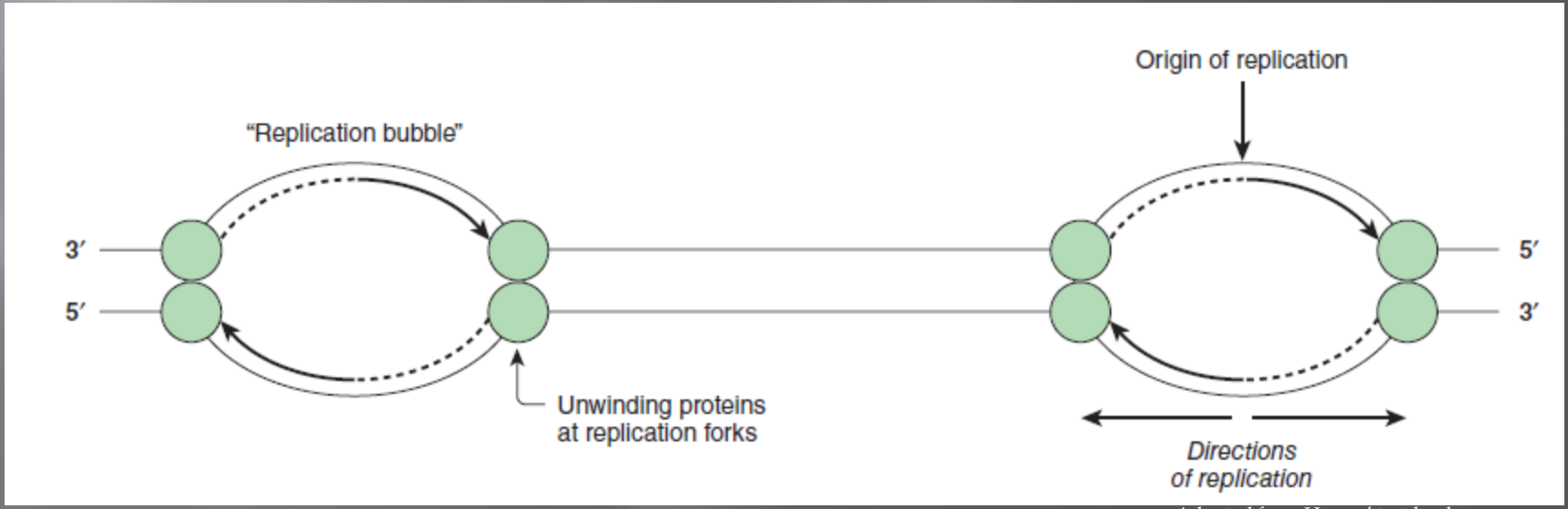
The generation of "replication bubbles" during the process of DNA synthesis

E coli genome: 5X10^6 bp
Replication rate: 3X10^5 bp/min
How long = 30 min

Mammalian genome: 3X10^9 bp

How to solve?

1. Bideirectional
2. Multiple origins



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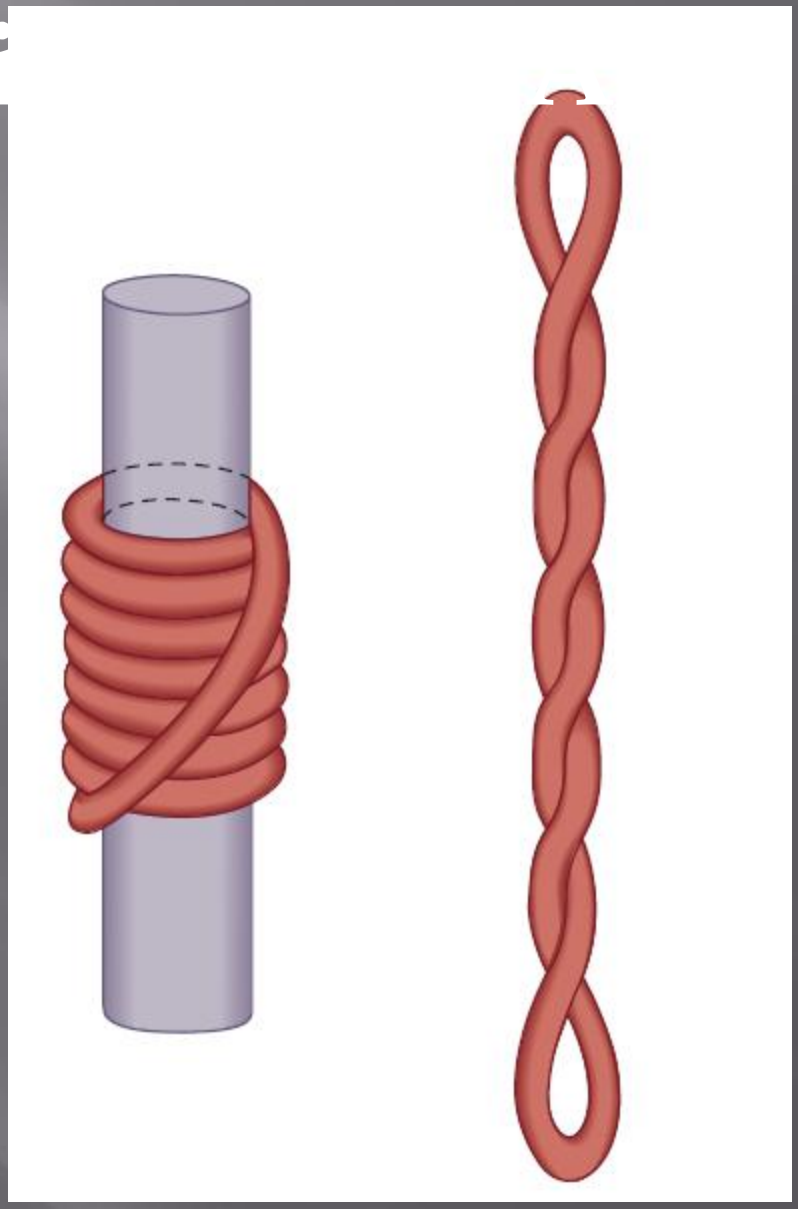
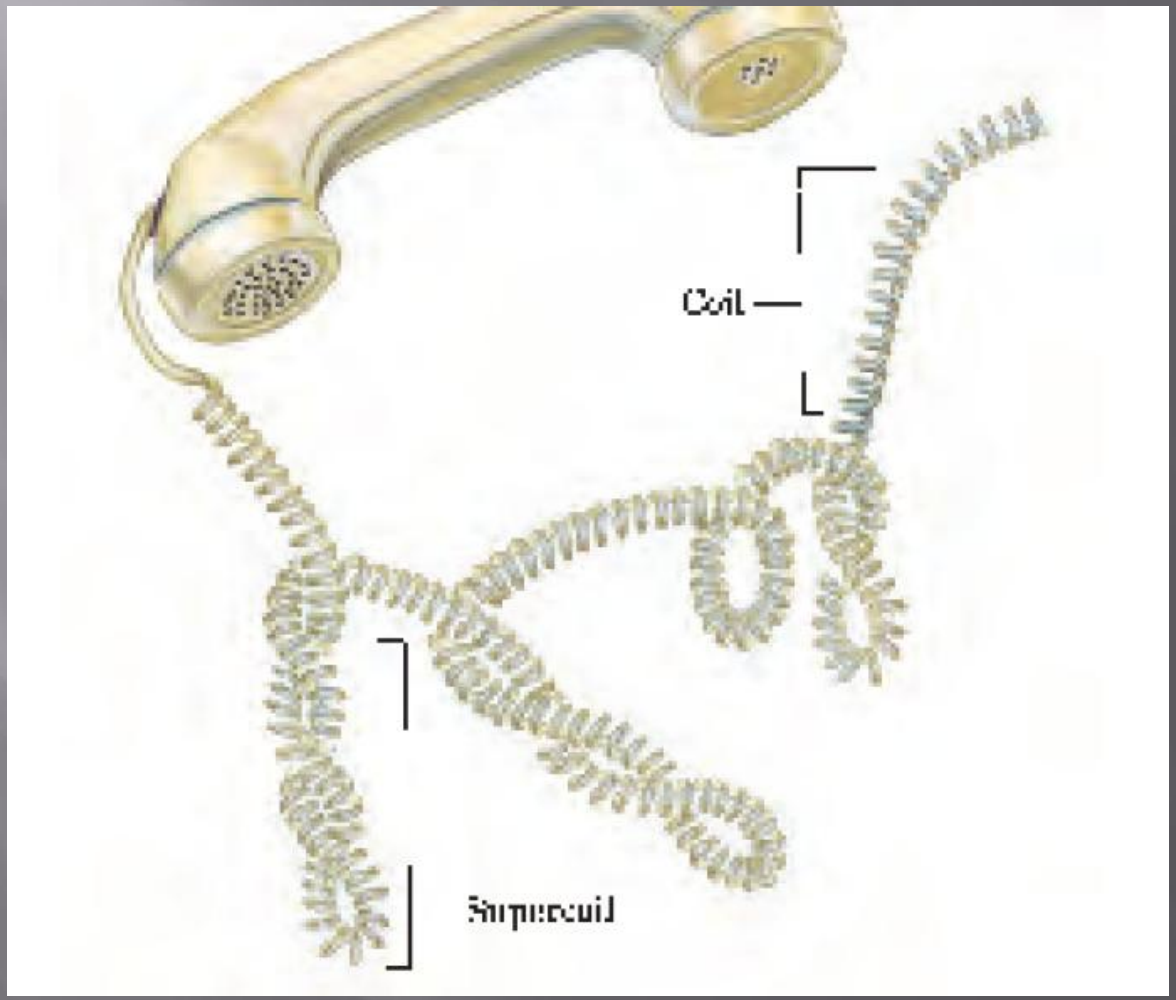
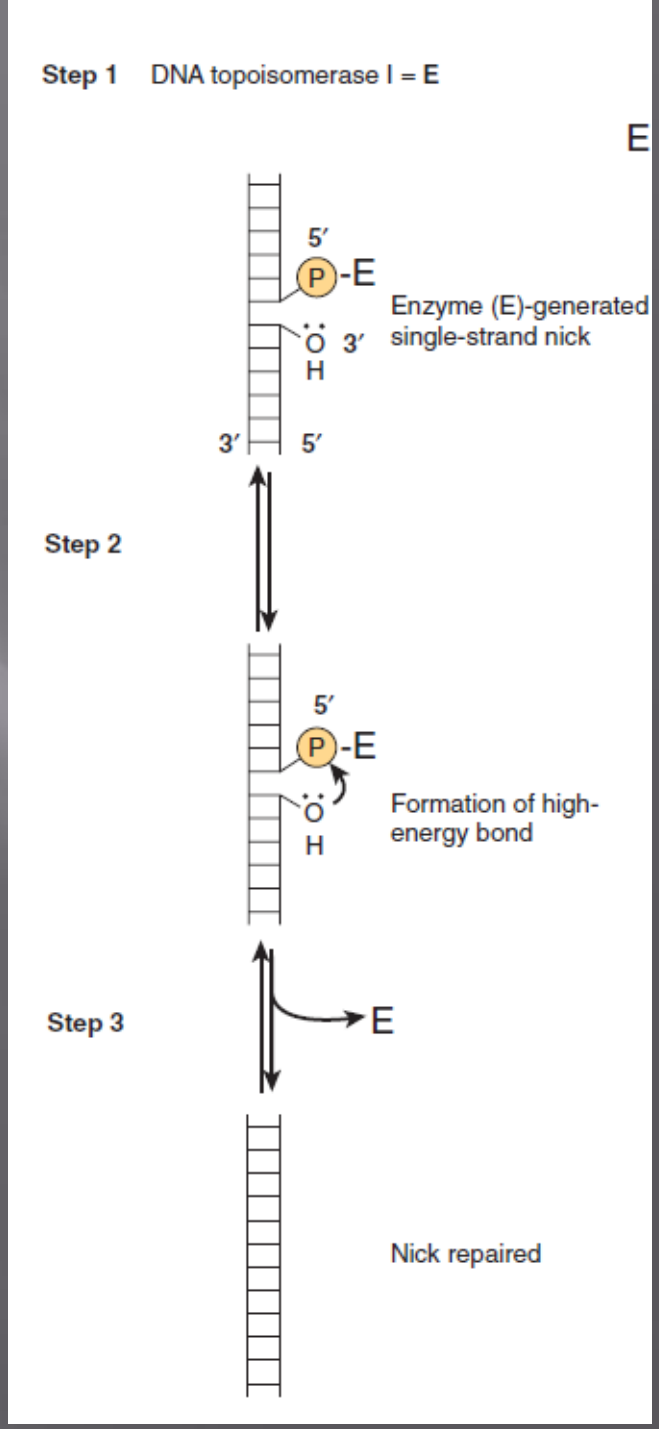
Supercoili




Coil
Supercoil

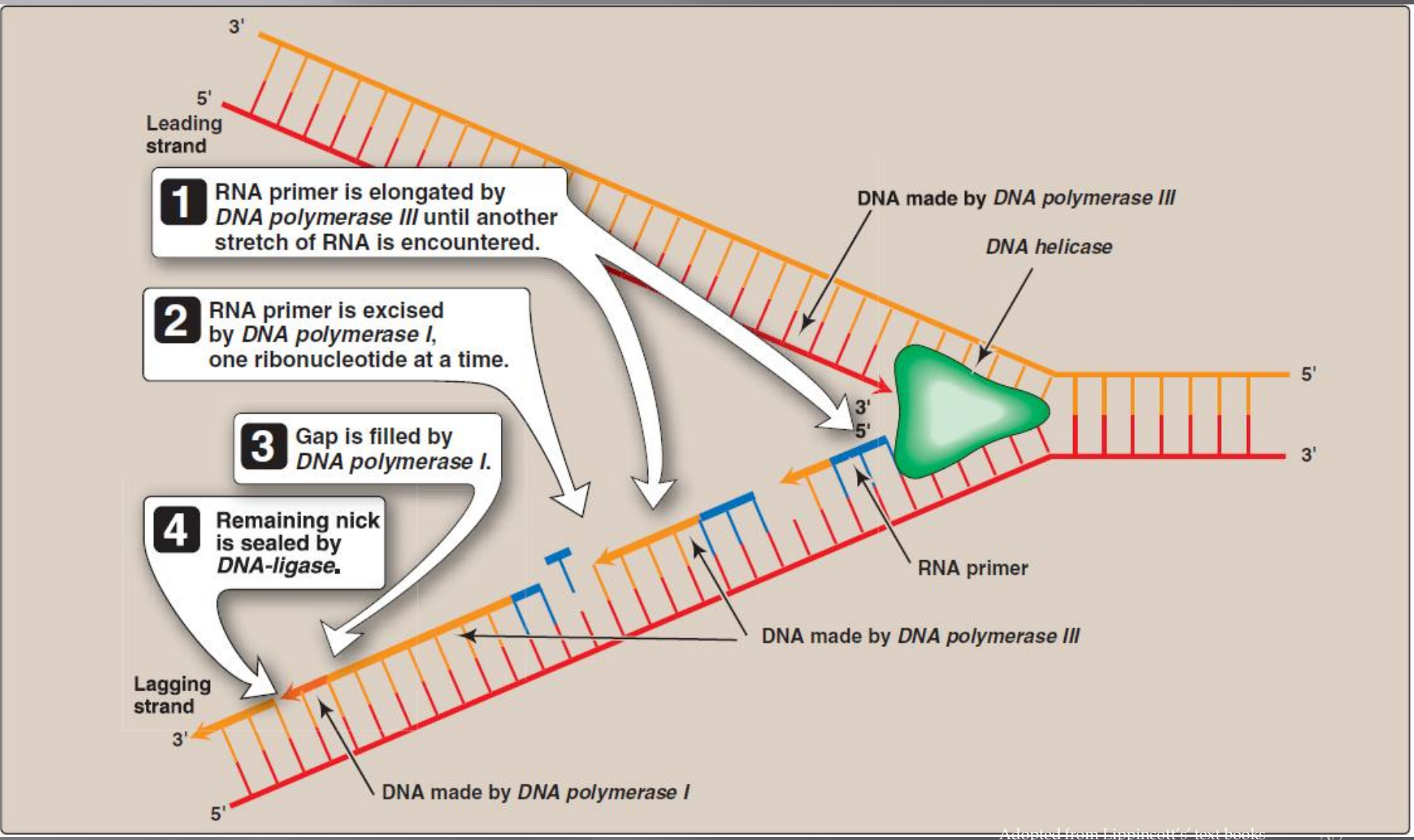
Reactions catalyzed by DNA topoisomerase I



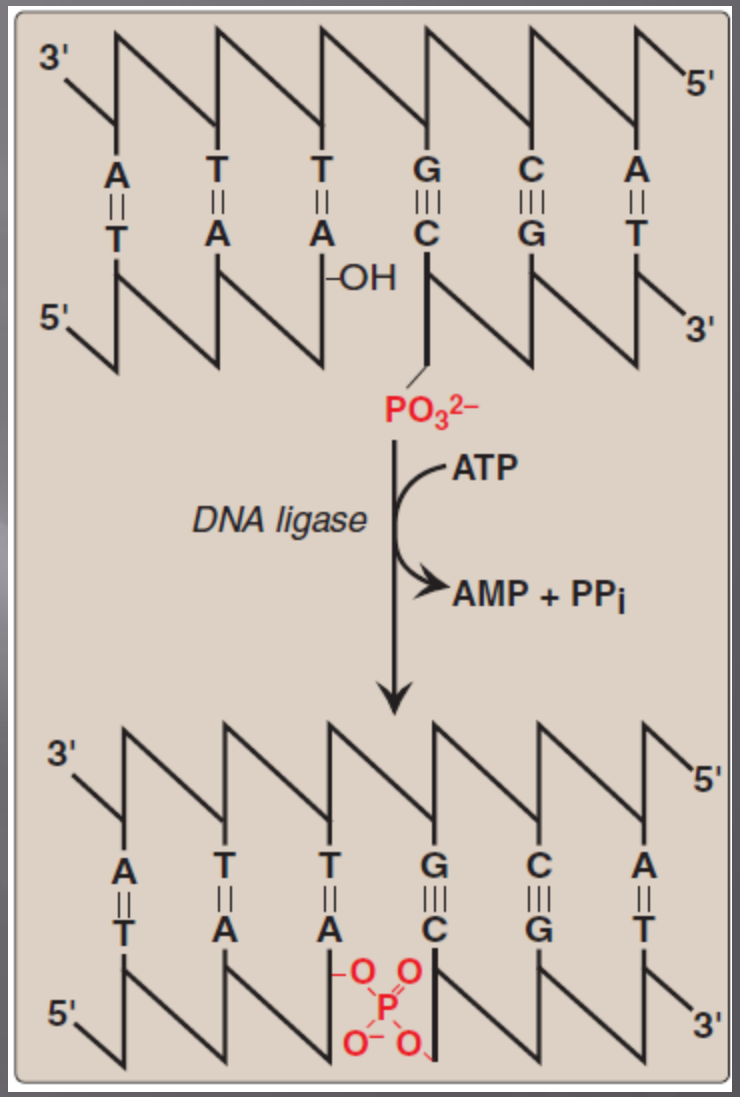
Proof reading of newly synthesized DNA

- Should be as few error as possible
- 3′ to 5′ exonuclease activity

Removal of RNA primer and filling of the resulting "gaps" by *DNA polymerase I*



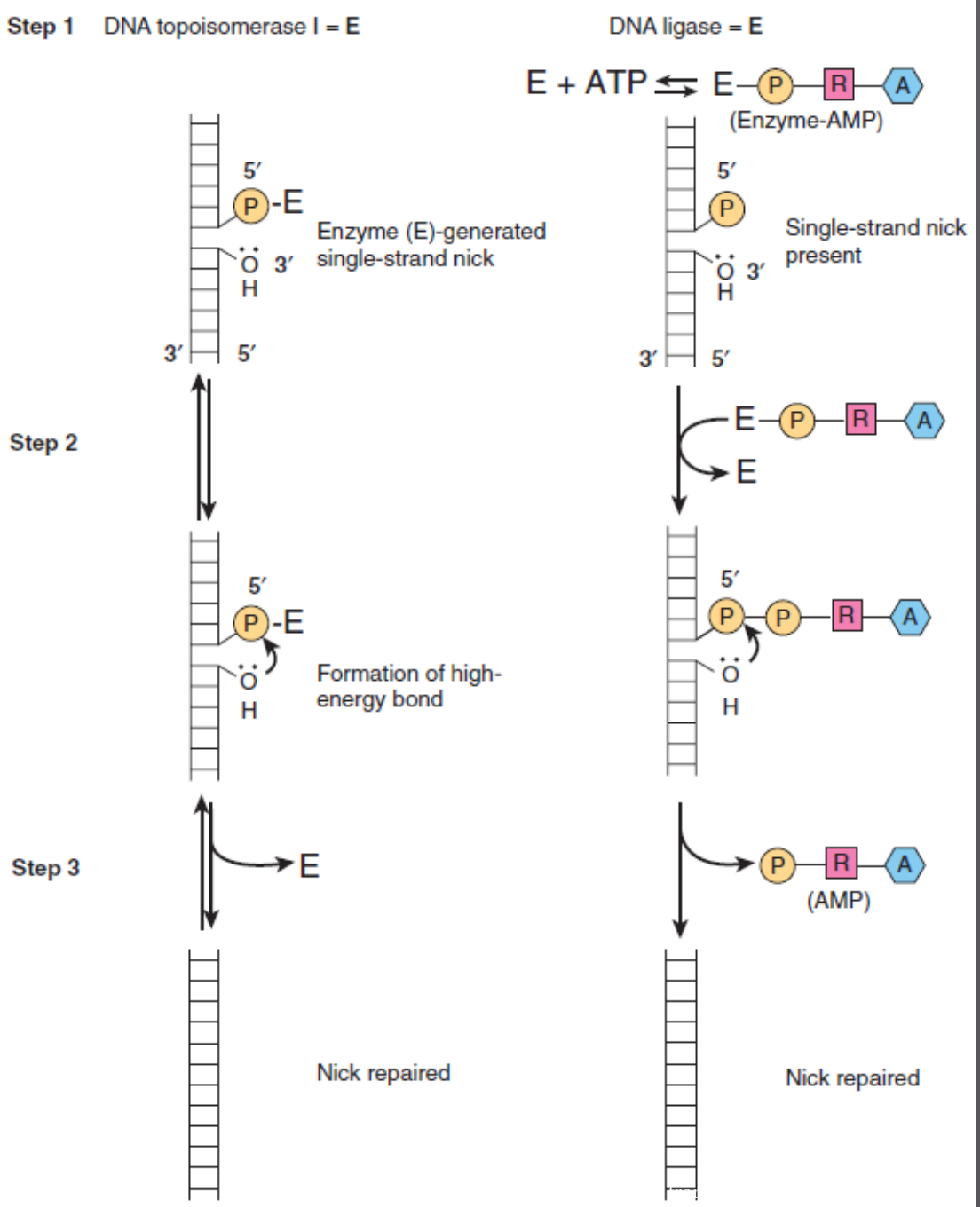
Action of DNA ligase: Formation of a phosphodiester bond



Reconstitution of chromatin structure

- Rate of polymerization in eukaryotic cells compared to prokaryotes…..?
- Nuclear organization and chromatin structure determines the regulation and initiation of DNA synthesis
- Replicated DNA assembled into nucleosome and histone octamers distributed to each arm of the replication fork
- Facilitated by histone chaperone proteins working in concert with chromatin remodelling complexes

Comparison of two types of nick-sealing reactions on DNA



Functionally similar proteins

Prokaryote	Eukaryote	function
Hexameric Dnaβ complex	Hexameric minichromosomal maintenance (MCM) complex	Unwinds the DNA
SSB	Replication protein A	Prevent reannealing
DnaG	Polα	Primase synthesizes RNA primer
Pol III	Pol ε (leading) and Pol δ (Lagging)	Processive, leading and lagging strand synthesis
Pol I	Rnase H and FEN1 (flap of endonuclease)	RNA primers removed
Pol II	β	DNA repair
Pol I		Gap filling following DNA replication, repair, and recombination
	γ	Mitochondrial DNA synthesis
β subunit of Pol III	PCNA (Proliferating cell nuclear antigen)	Sliding clamp for high procesivity

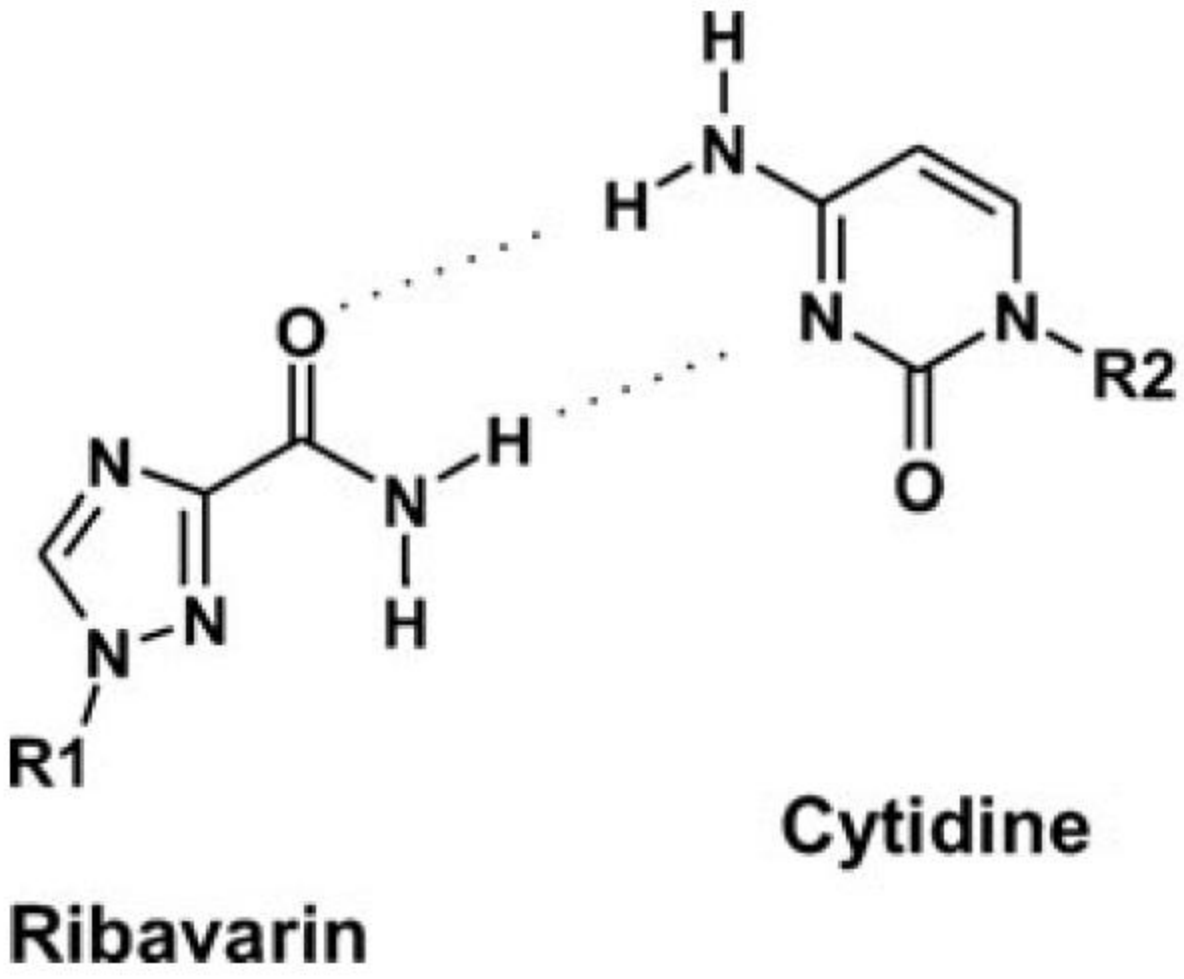
Case 2

- A 21-year-old college student presents to the clinic complaining of a sudden onset of chills and fever, muscle aches, headache, fatigue, sore throat, and painful nonproductive cough 3 days prior to fall final exams. Numerous friends of the patient in the dormitory reported similar symptoms and were given the diagnosis of influenza. He said that some of them were given a prescription for ribavirin. On examination, he appears ill with temperature 39.4°C (103°F). His skin is warm to the touch, but no rashes are appreciated. The patient has mild cervical lymph node enlargement but otherwise has a normal examination.

- ◆ **What is the most likely diagnosis?**

- ◆ **What is the biochemical mechanism of action of ribavirin?**

Ribavarin



Inhibitors of DNA Replication

Inhibitors of DNA Replication

Antibacterial agents	Action
Ciprofloxacin	Bacterial DNA gyrase
Nalidixic acid	Bacterial DNA gyrase
Anticancer agents	
Adriamycin	Human topoisomerase
Etoposide	Human topoisomerase
6-mercaptopurine	Human DNA polymerase
5-fluoro uraci	Thymidylate synthase
Cytosine arabinosidede (Cytarabine)	Nucleoside analog
Anti viral agent	
adenine arabinoside (Vidarabine)	Nucleoside analog

Summary

- Each strand of the double helix serves as a **template (semiconservative replication).**
- occurs in the **S phase of the cell cycle and begins at the origin of replication.**
- strands are separated locally, forming **replication forks.**
- Replication of dsDNA is **bidirectional**
- **Helicase unwinds** the double helix.
- As the two strands of the double helix are separated, **supercoils are produced**

DNA topoisomerases Types I and II remove supercoils

Summary (contd)

- The primer for de novo DNA synthesis is a short stretch of RNA synthesized by **primase**
- **DNA polymerases synthesize new DNA strands only in the 5'→3'** direction.
- one of the newly synthesized stretches of

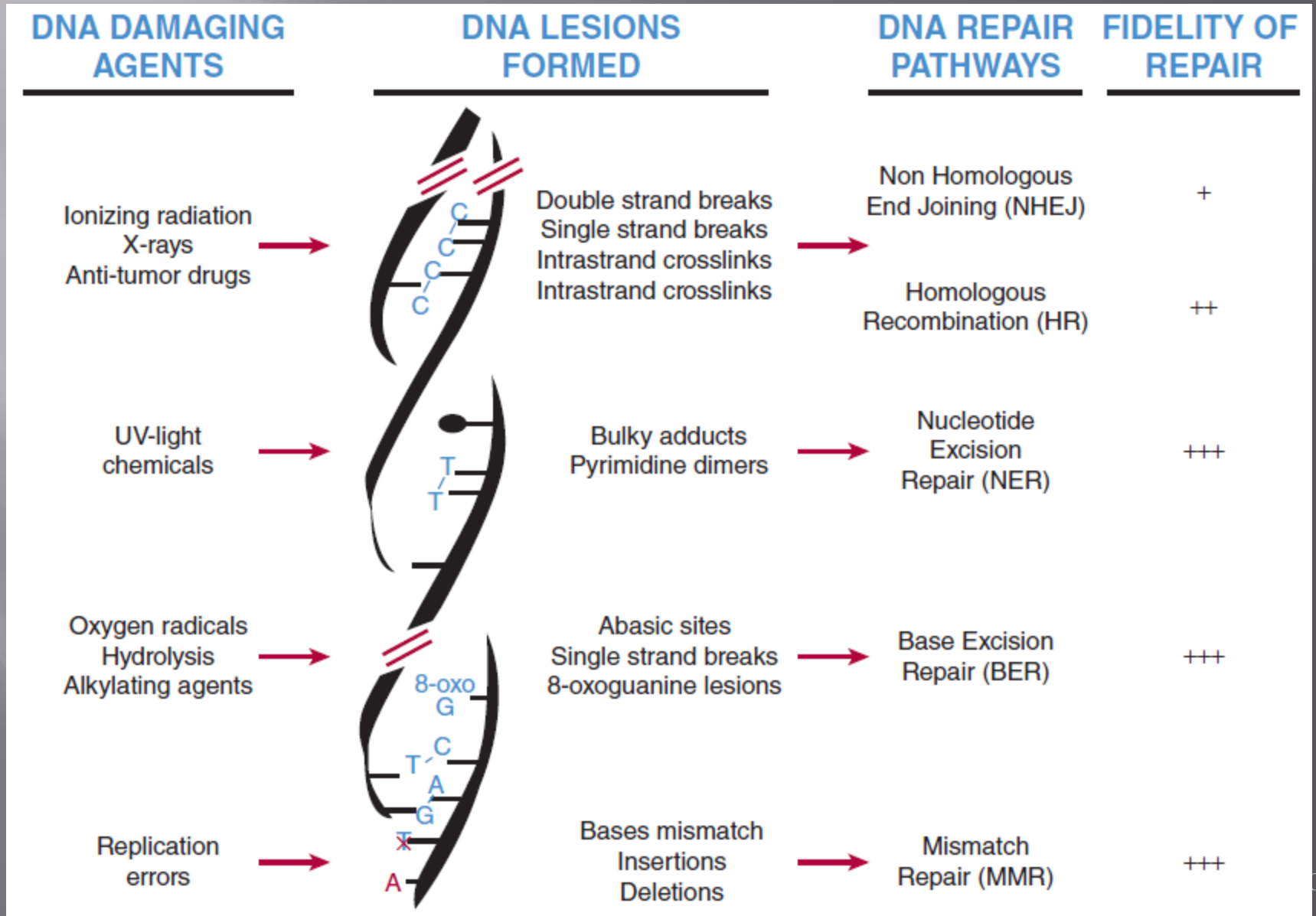
nucleotide chains must grow in the 5'→3' direction toward the replication fork (**leading strand)**

- In E. coli DNA chain elongation is catalyzed by **DNA polymerase III**
- The enzyme **"proofreads" the newly synthesized** DNA with its **3'→5' exonuclease activity.**
- RNA primers are removed by **DNA polymerase I, using its 5'→3' exonuclease activity.**
- The final phosphodi-ester linkage is catalyzed by **DNA ligases**
- at least five **eukaryotic DNA polymerase**
- **Nucleoside analogs containing modified sugars can be used to** block DNA chain growth.

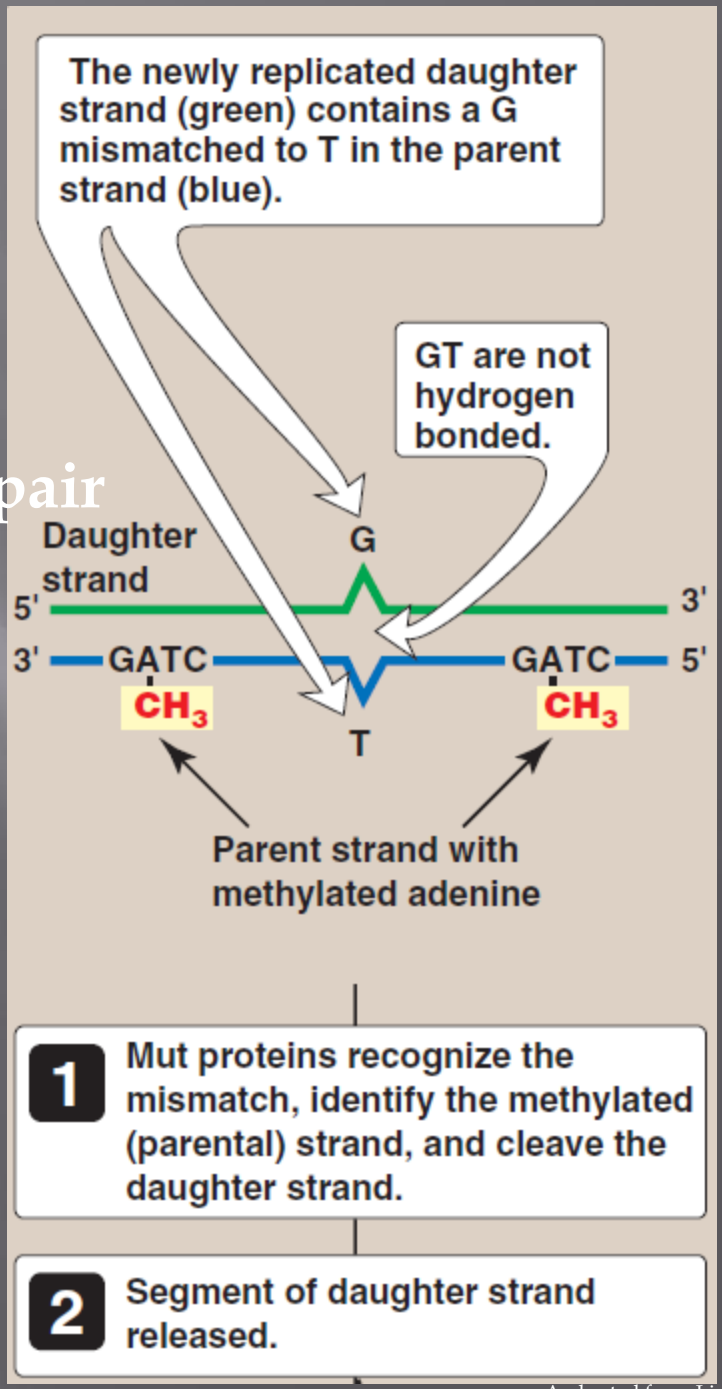
- Video on DNA Replication

DNA Repair

Multiple DNA repair pathways of variable accuracy



1. Methyl-directed mismatch repair



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Methyl-directed mismatch repair

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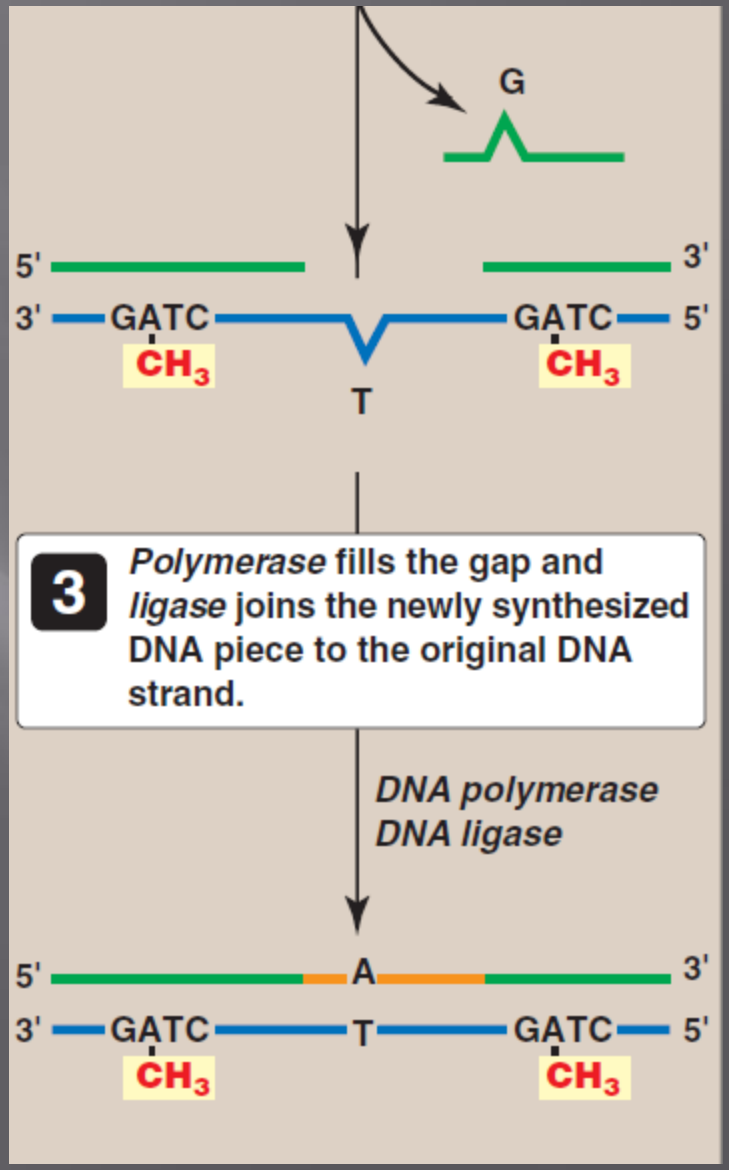

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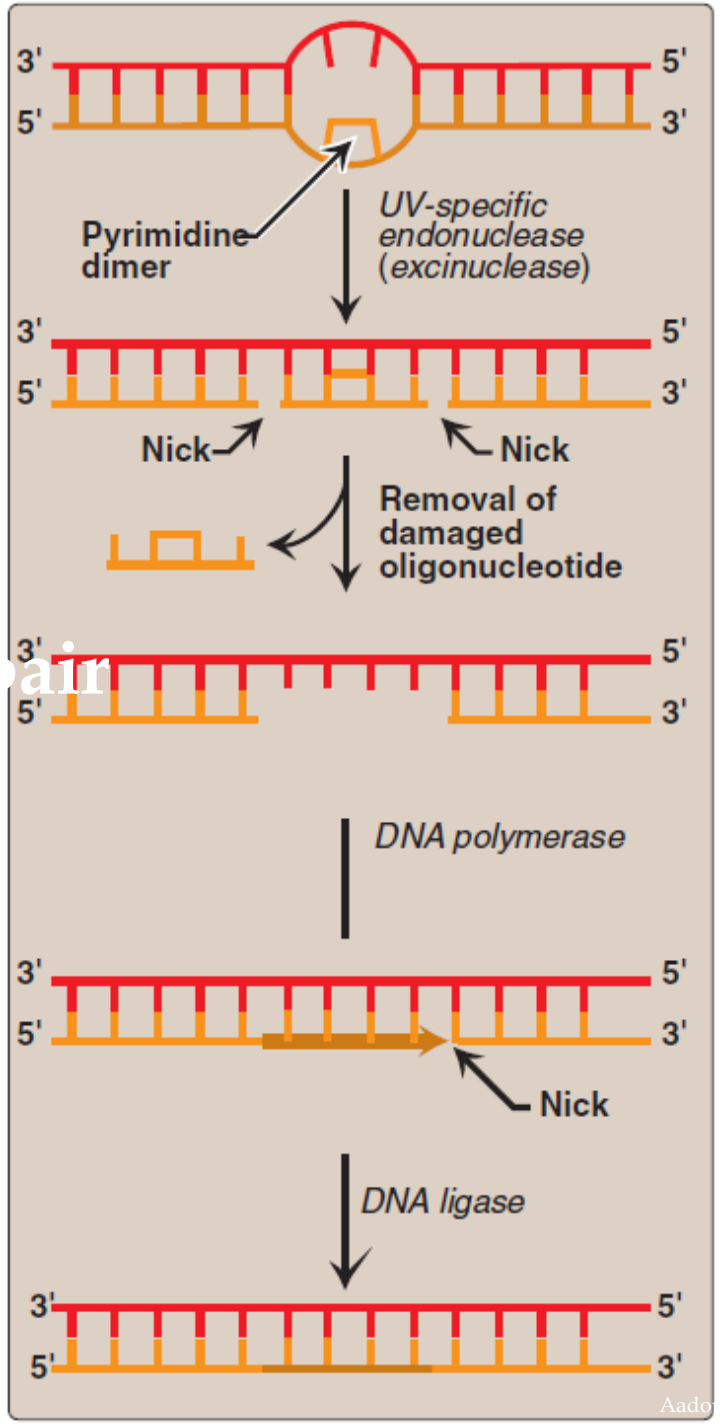
2. Nucleotide excision repair



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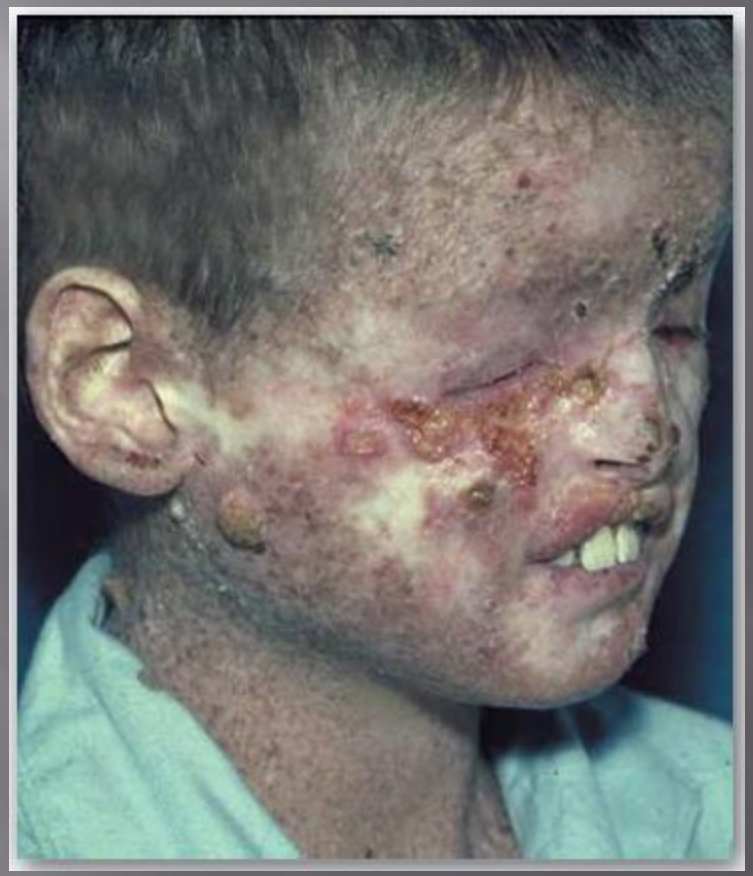
Patient with xeroderma pigmentosum



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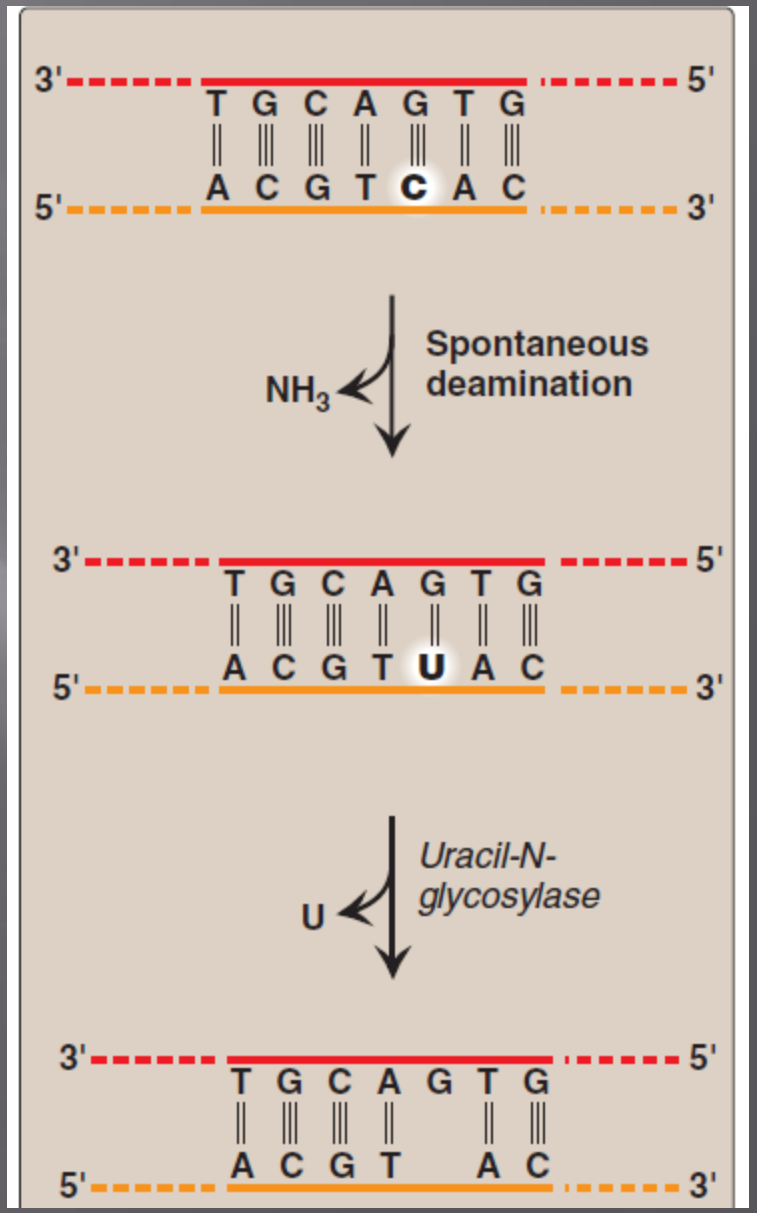
Xeroderma pigmentosum (XP)

- inherited as an autosomal recessive trait
- characterized
  by photosensitivity, pigmentary changes, premature skin ageing and malignant tumour development
- defect in DNA repair (NER)

3. Base excision repair



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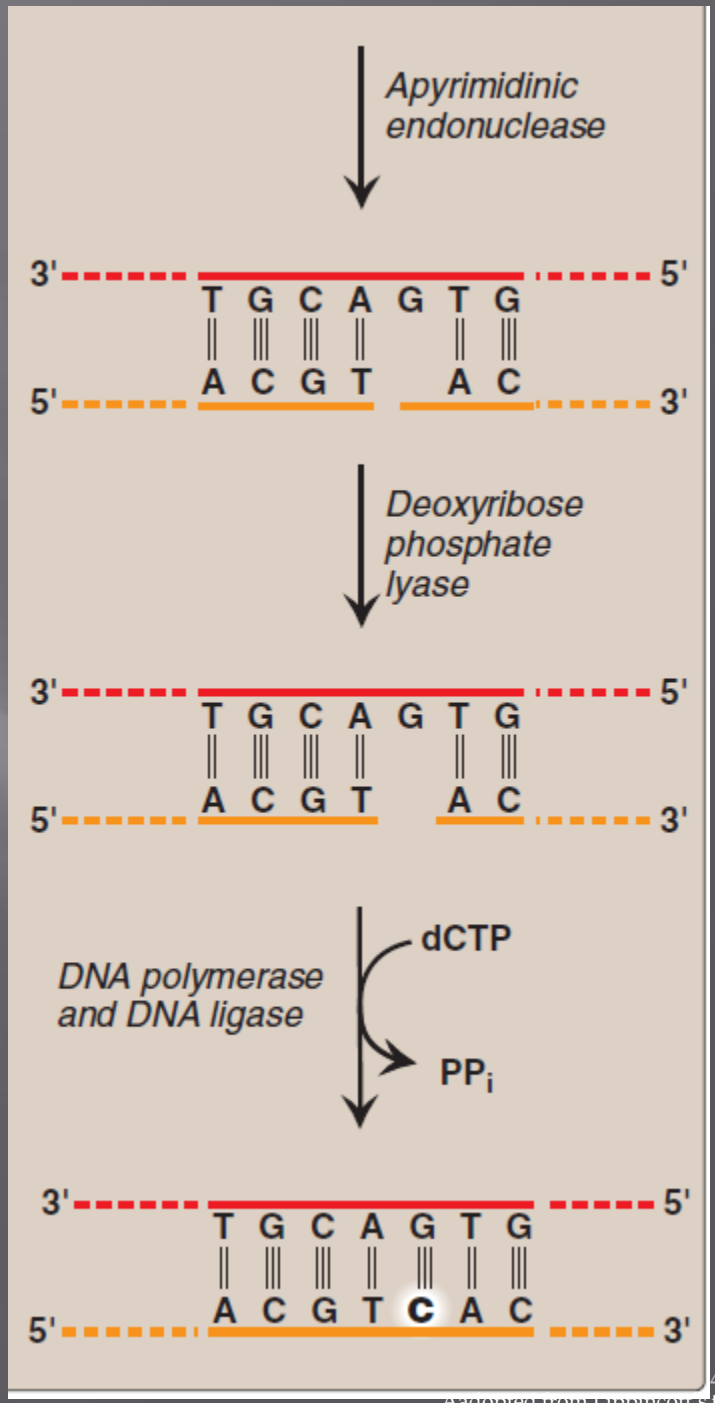
Correction of base alterations by base excision repair



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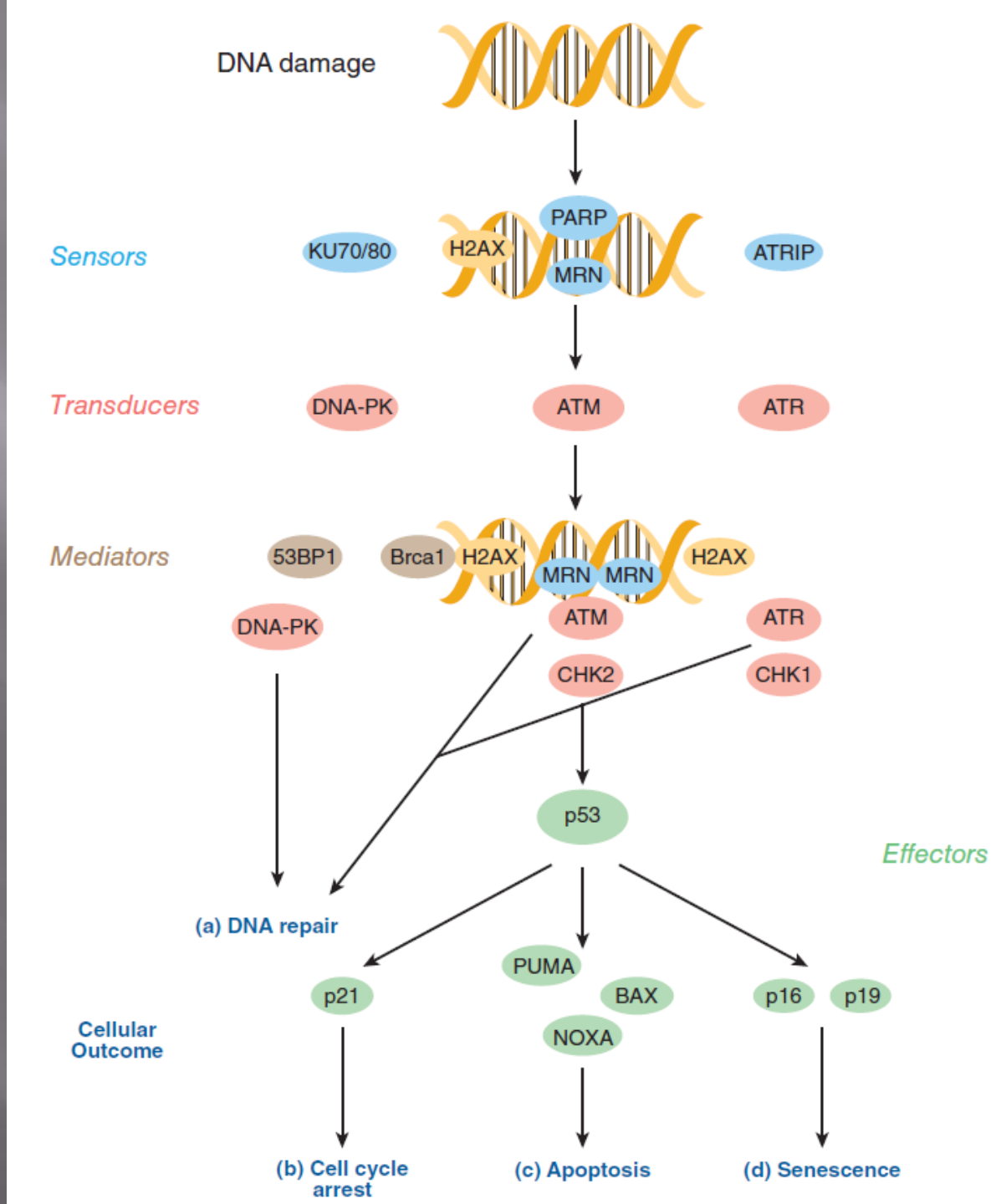
4. The multistep mechanism of DNA double-strand break repair



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- Clinical implications

Human Diseases of DNA Damage Repair

Defective Nonhomologous End Joining Repair (NHEJ)

- Severe combined immunodeficiency disease (SCID)
- Radiation sensitive severe combined immunodeficiency disease (RS-SCID)

Defective Homologous Repair (HR)

- Bloom syndrome (BS)
- Werner syndrome (WS)
- Breast cancer suspectibility 1 and 2 (BRCA1, BRCA2
- AT -like disorder (AT LD)

Human Diseases of DNA Damage Repair

Defective DNA Nucleotide Exicision Repair (NER)
Xeroderma pigmentosum (XP)
Cockayne syndrome (CS)
Trichothiodystrophy (TT D)

Defective DNA Base Excision Repair (BER)
MUTYH-associated polyposis (MAP)

Defective DNA Mismatch Repair (MMR)
Hereditary nonpolyposis colorectal cancer (HNPCC)

MCQ 1

- If a double-stranded DNA molecule undergoes two rounds of replication in an in vitro system that contains all of the necessary enzymes and
nucleoside triphosphates that have been labeled with 32P, which of the
following best describes the distribution of radioactivity in the four
resulting DNA molecules?
-
A. Exactly one of the molecules contains no radioactivity.
B. Exactly one of the molecules contains radioactivity in only one strand.
C. Two of the molecules contain radioactivity in both strands.
D. Three of the molecules contain radioactivity in both strands.
E. All four molecules contain radioactivity in only one strand.

MCQ 2

- A 48-year-old man has had a lengthy history of skin cancer. In the past 6 years he has had over 30 neoplasms removed from sun-exposed areas and has been diagnosed with xeroderma pigmentosum. Which of the following best describes the enzymatic defect in patients with xeroderma pigmentosum?
-

A. DNA polymerase α
B. DNA polymerase γ
C. DNA ligase
D. Excision repair enzymes
E. RNA polymerase III

MCQ 3

- A 10-year-old girl is brought to the dermatologist by her parents. She has many freckles on her face, neck, arms, and hands, and the parents report that she is unusually sensitive to sunlight. Two basal cell carcinomas are identified on her face. Which of the following processes is most likely to be defective in this patient?
- A. Repair of double-strand breaks.
  B. Removal of mismatched bases from the 3'-end of Okazaki fragments.
  C. Removal of pyrimidine dimers from DNA.
  D. Removal of uracil from DNA.

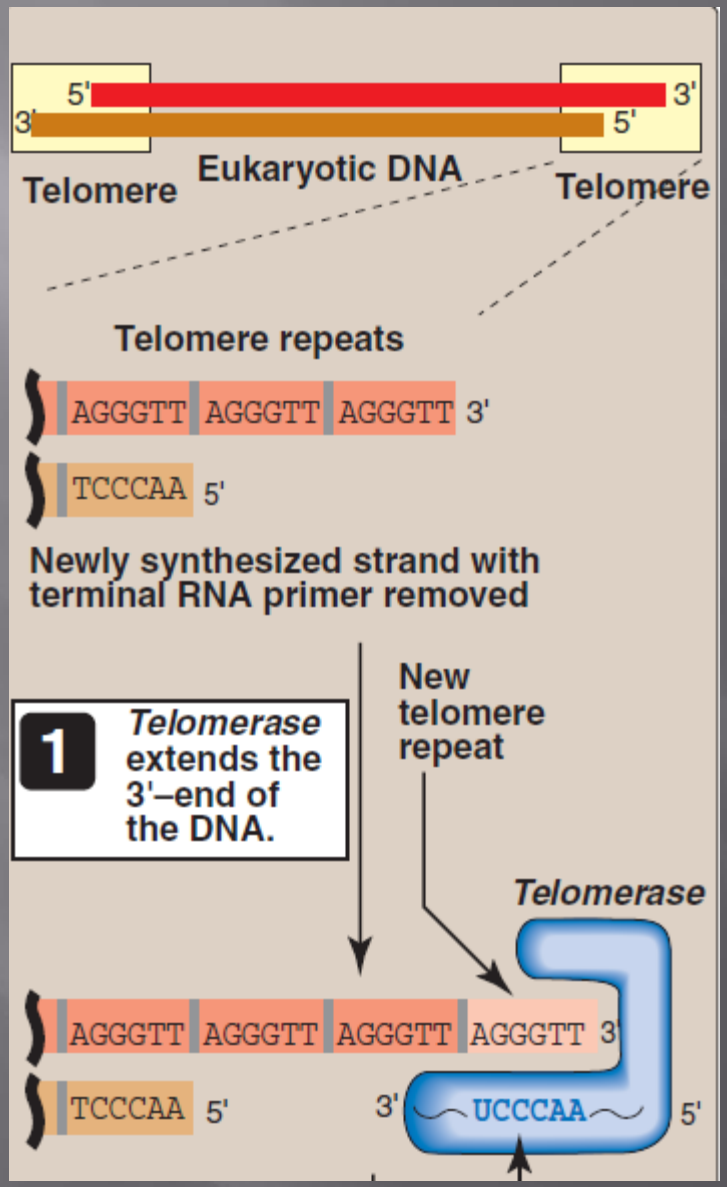
Molecular count down clock?

What is telomere

- Several thousand tandem repeats of GT rich hexamer (AGGGTT) that cap the ends of eukaryotic chromosomes that help stabilize the chromosome., preventing attack by nucleases
- **Telomeres (Greek *telos, "end") : complexes of DNA plus proteins (Shelterin)***
- To accommodate the wastage that occurs during replication
- Allow repair system to distinguish

- Organisms are able to generate progeny that contain full length telomeres .
- How is it possible?
- Telomerase
  - Ribonucleoprotein expressed in germ cells, stem cells , most cancer cells
  - NOT in SOMATIC cells
- Replicative senescence

Mechanism of action of *telomerase*



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Mechanism of action of *telomerase*

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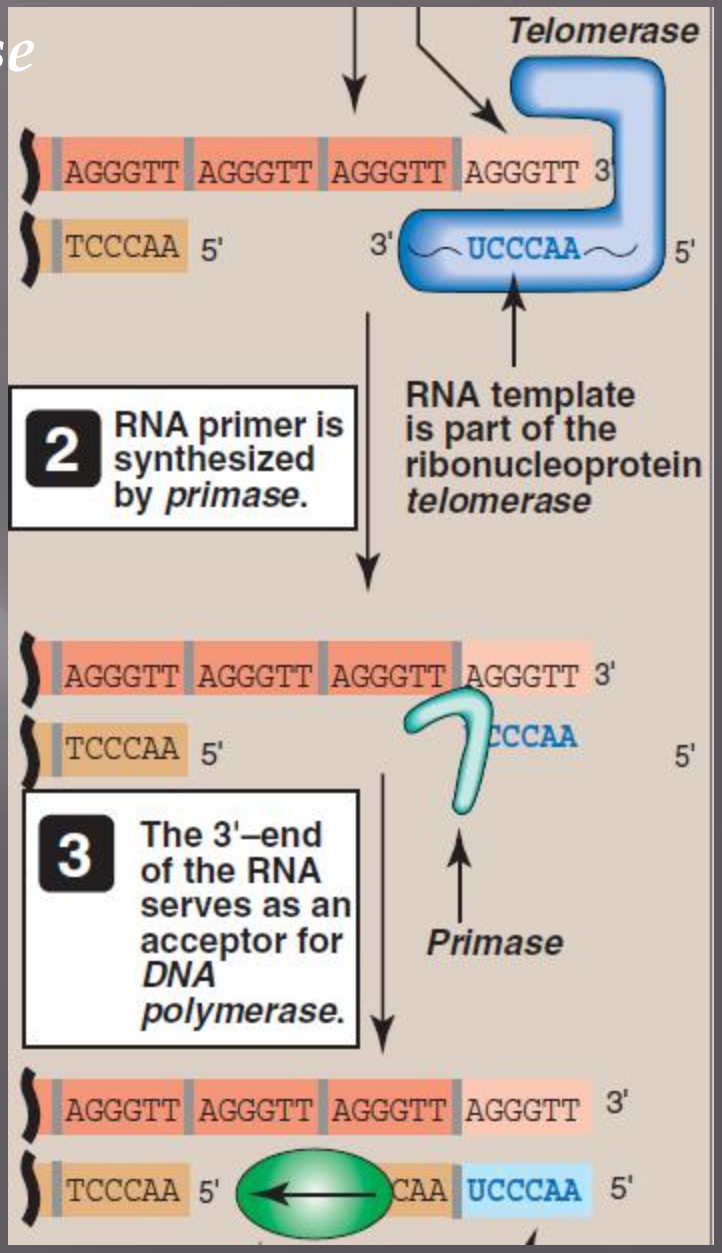

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Mechanism of action of *telomerase*

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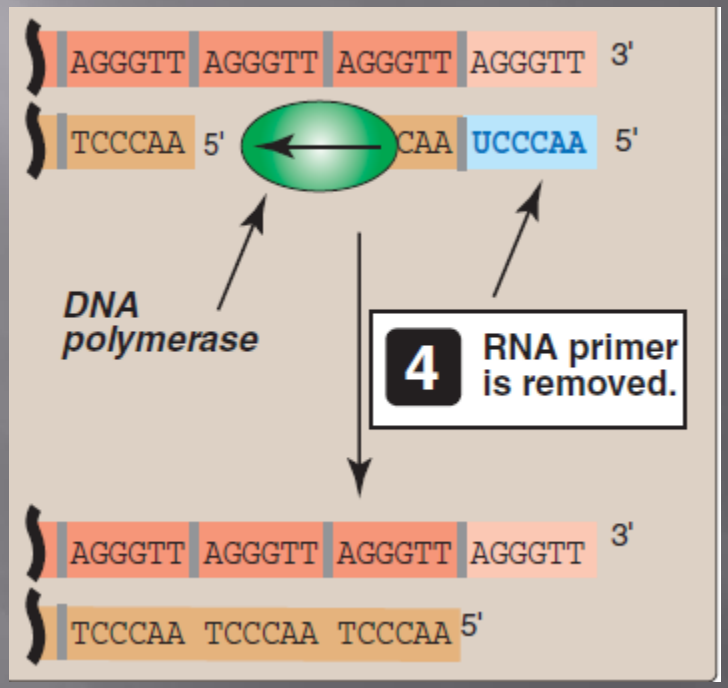

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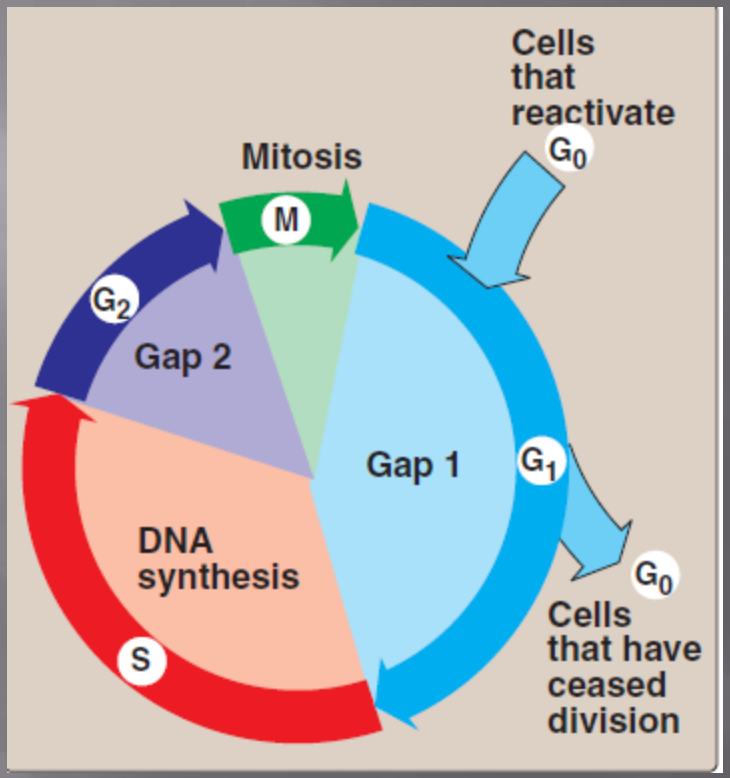
Reverse transcriptase activity in eukaryotes

- Telomerase
- Transposons
  - Most transposons transcribed into RNA which is used as a template for DNA synthesis by a reverses transcriptase encoded by transposons and the DNA is randomly inserted into the genome
  - Called retrotransposon or retroposon

MCQ

- Telomeres are complexes of DNA and protein that protect the ends of linear chromosomes. In most normal human somatic cells, telomeres shorten with each division. In stem cells and in cancer cells, however, telomeric length is maintained. In the synthesis of telomeres:

- A. telomerase, a ribonucleoprotein, provides both the RNA and the polymerase needed for synthesis.
  B. the RNA of telomerase serves as a primer.
  C. the polymerase of telomerase is a DNA-directed DNA polymerase.
  D. the shorter, 3'→5' strand gets extended.
  E. the direction of synthesis is 3'→5'.



The eukaryotic cell cycle

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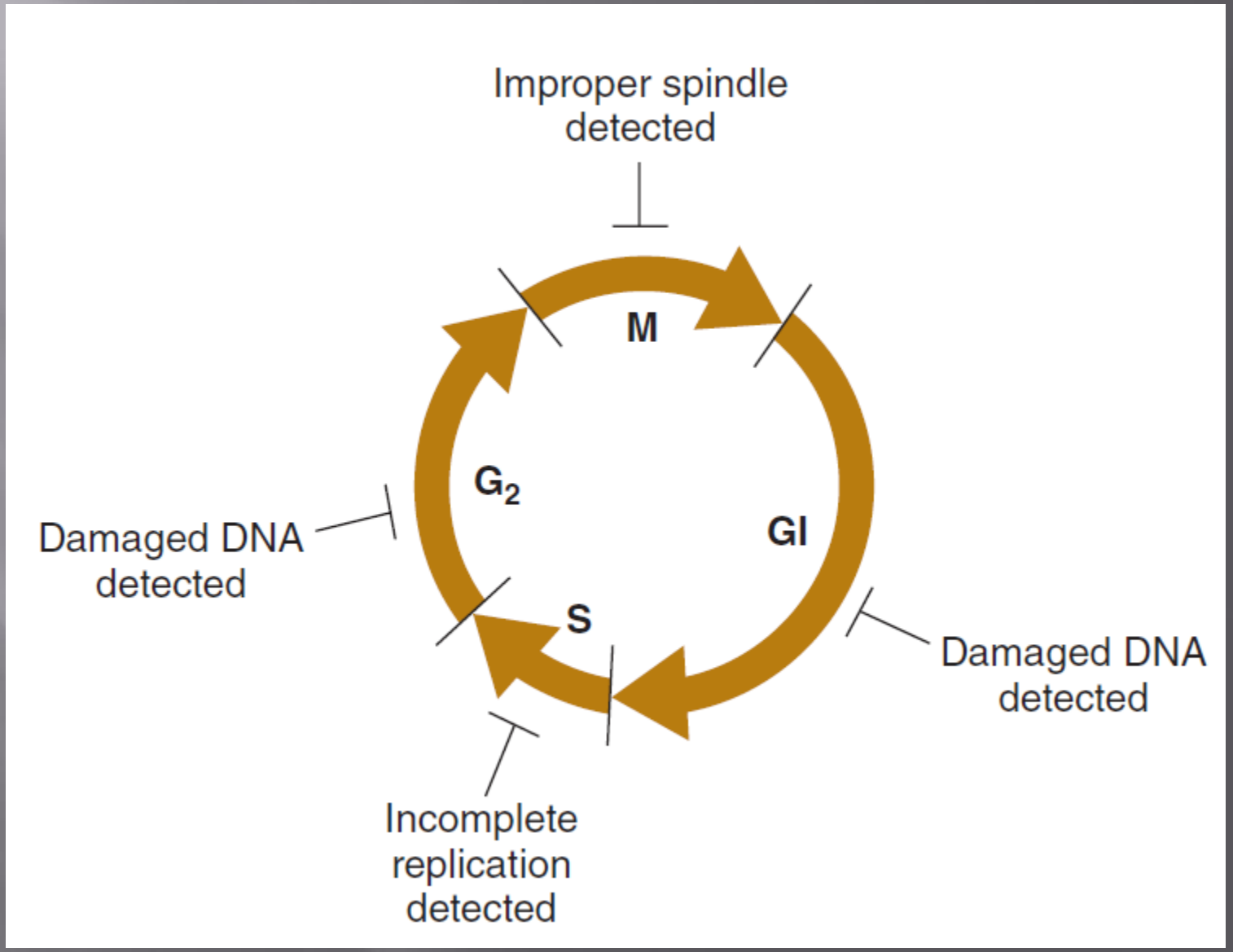
Replication licensing

- During the S phase, the **nuclear DNA is completely replicated once and only once.**
- Once chromatin has been replicated, it is marked so as to prevent its further replication

  until it again passes through mitosis.
- molecular mechanisms
  - dissociation and/or cyclin-CDK phosphorylation
  - and subsequent degradation of several origin binding proteins

- origins fire only once per cell cycle.

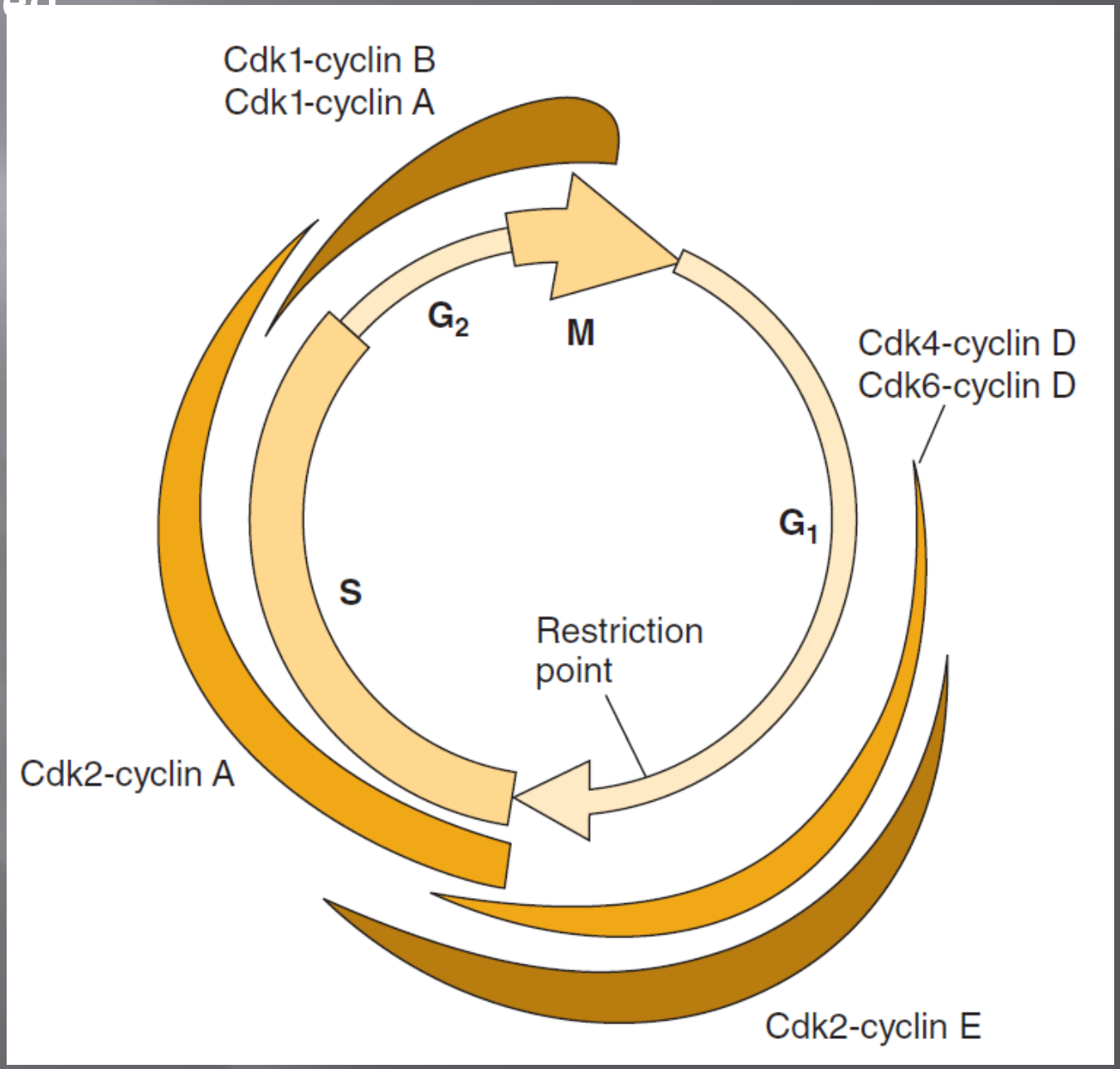
Progress through the mammalian cell cycle is continuously monitored via multiple cell-cycle checkpoints



Cyclins and Cyclin-Dependent Kinases Involved in Cell-Cycle Progression

Cyclin	Kinase	Function
D	CDK4, CDK6	Progression past restriction point at G1/S boundary
E, A	CDK2	Initiation of DNA synthesis in early S phase
B	CDK1	Transition from G2 to M

Points during which the indicated cyclins and cyclin-dependent kinases are activated



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