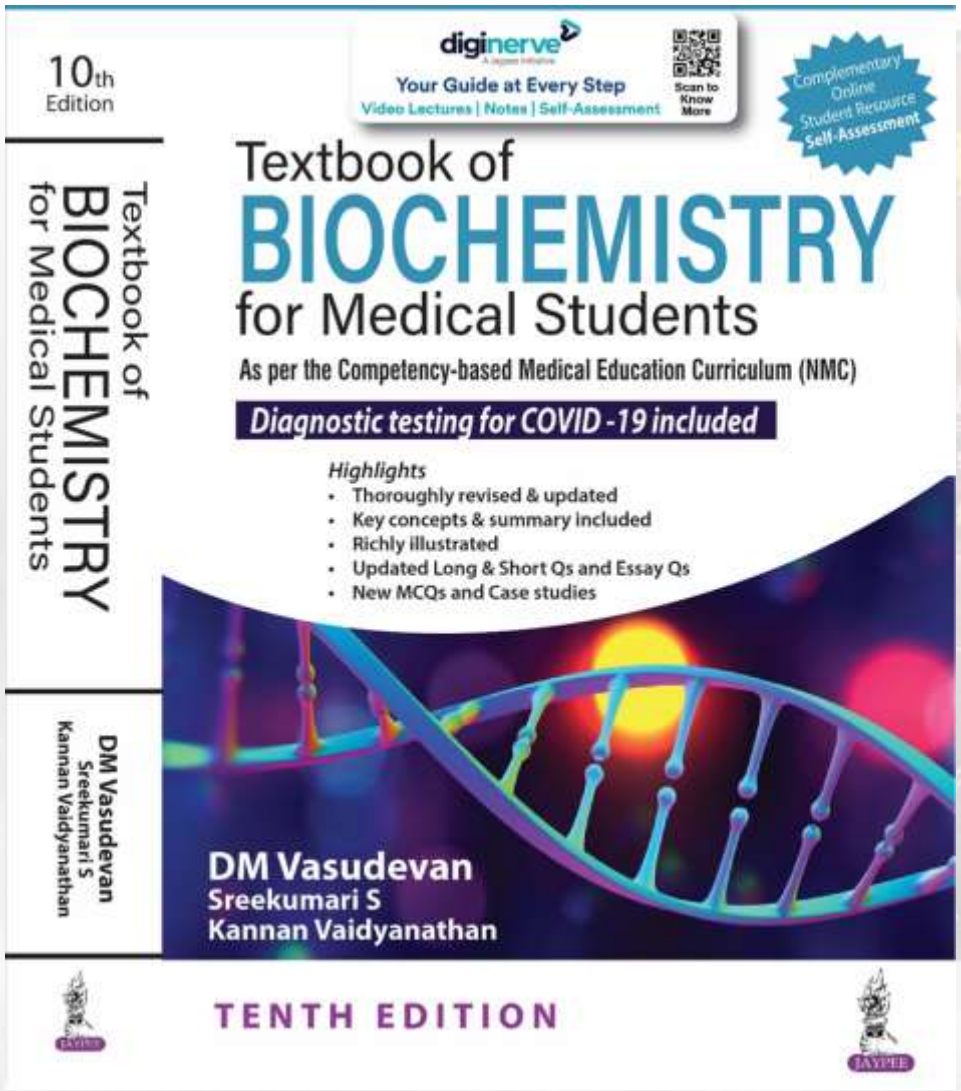


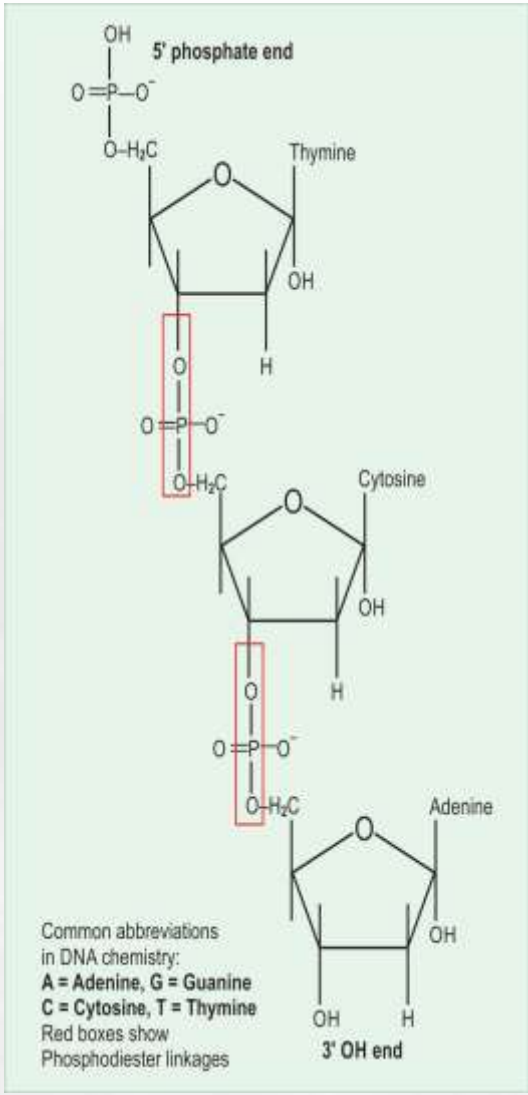
Chapter 34:

Deoxyribonucleic Acid: Structure, Organization, and Replication

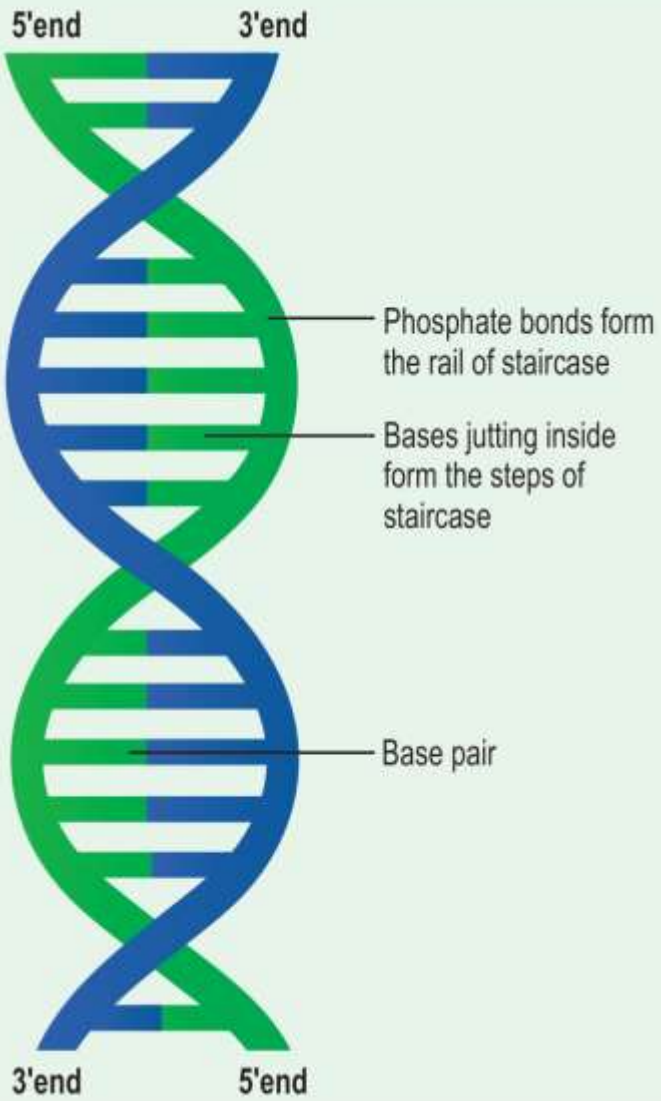
Textbook of
BIOCHEMISTRY
for Medical Students

By DM Vasudevan, *et al.*

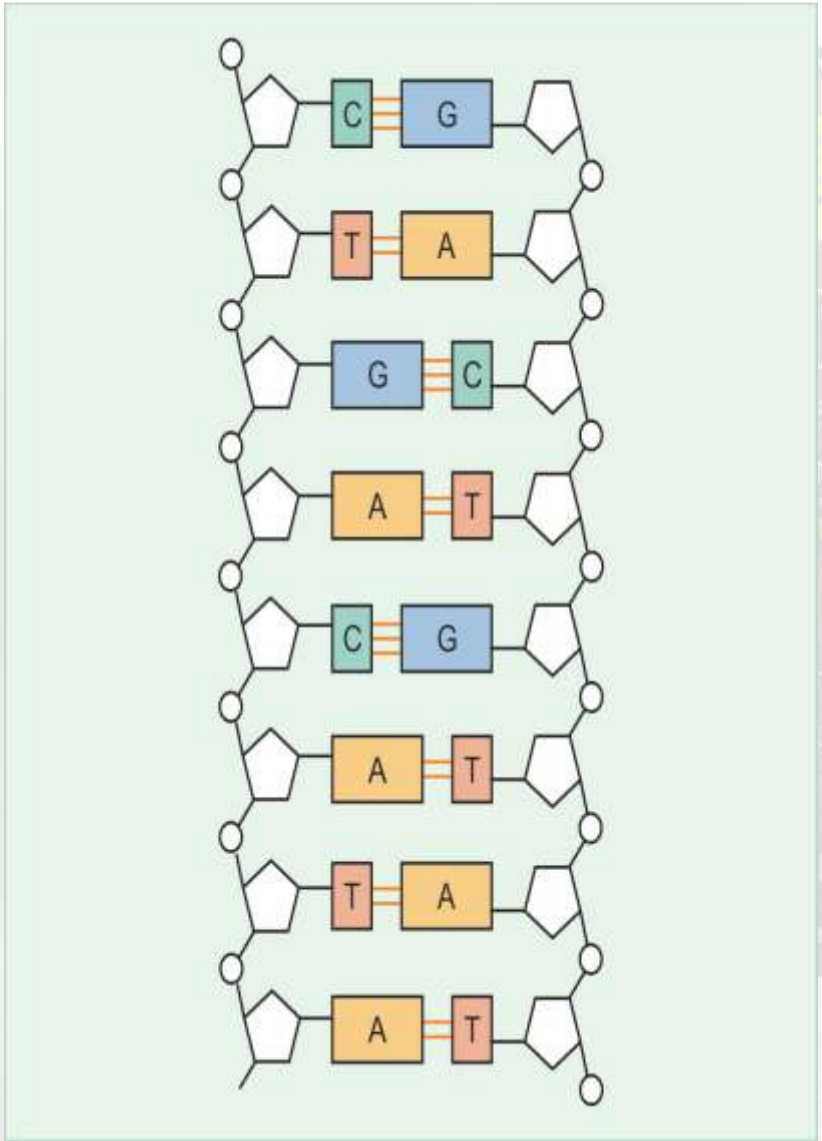
TENTH EDITION



Polynucleotide.



Watson–Crick model of double helical structure of DNA. Adjacent bases are separated by 0.34 nm. The diameter or width of the helix is 2 nanometers.



Base pairing rule. Base pairing of A with T and G with C. Hydrogen bonds keep the bases in position.

Watson Crick Model of DNA Structure

- Right handed double helix

  2 polydeoxy ribonuleotide chains twisted around one another in a right handed double helix.

  Backbone or handrail constituted by sugar and phosphate, bases are perpendicular to helix axis. Sugars nearly at right angles. Backbone has a net negative charge.

- Base pairing rule (Chargaff's rule)

  Adenine always pairs with thymine (double bond) and thymine always pairs with guanine (triple bond).

  This maintains complementarities of the two strands, and A always equals T, C always equals G.

- Hydrogen bonding

  DNA strands held together by hydrogen bonds.

  2 hydrogen bonds between A and T, three between C and G. CG bond stronger than AT.

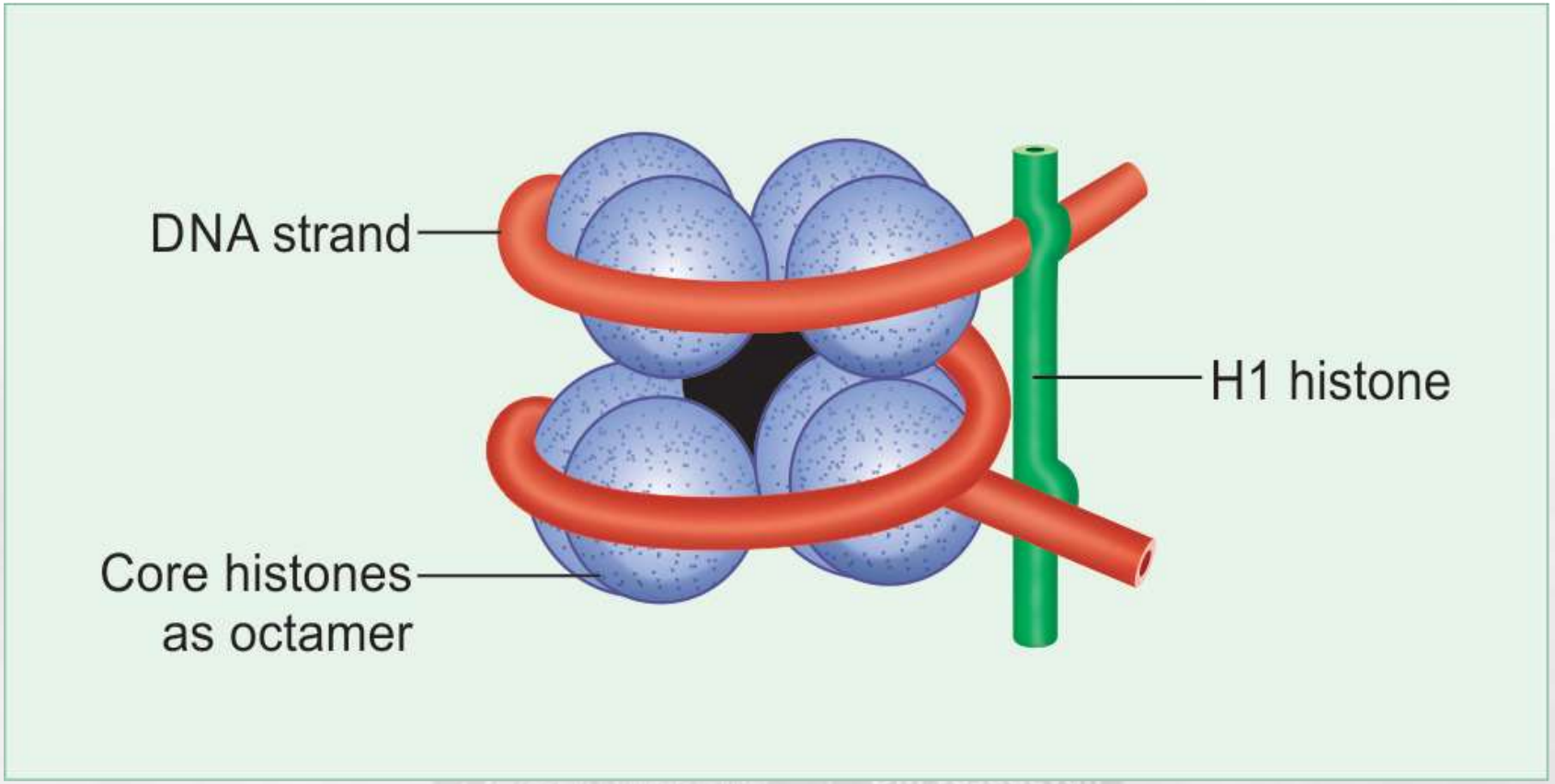
  DNA bases are planar.

  Base stacking, hydrophobic interactions between adjacent base pairs, also contributes significantly to DNA stability.

- Antiparallel

  2 strands run in the antiparallel direction, one strand in the 5’ – 3’ direction, while the other runs in 3’ – 5’ direction.

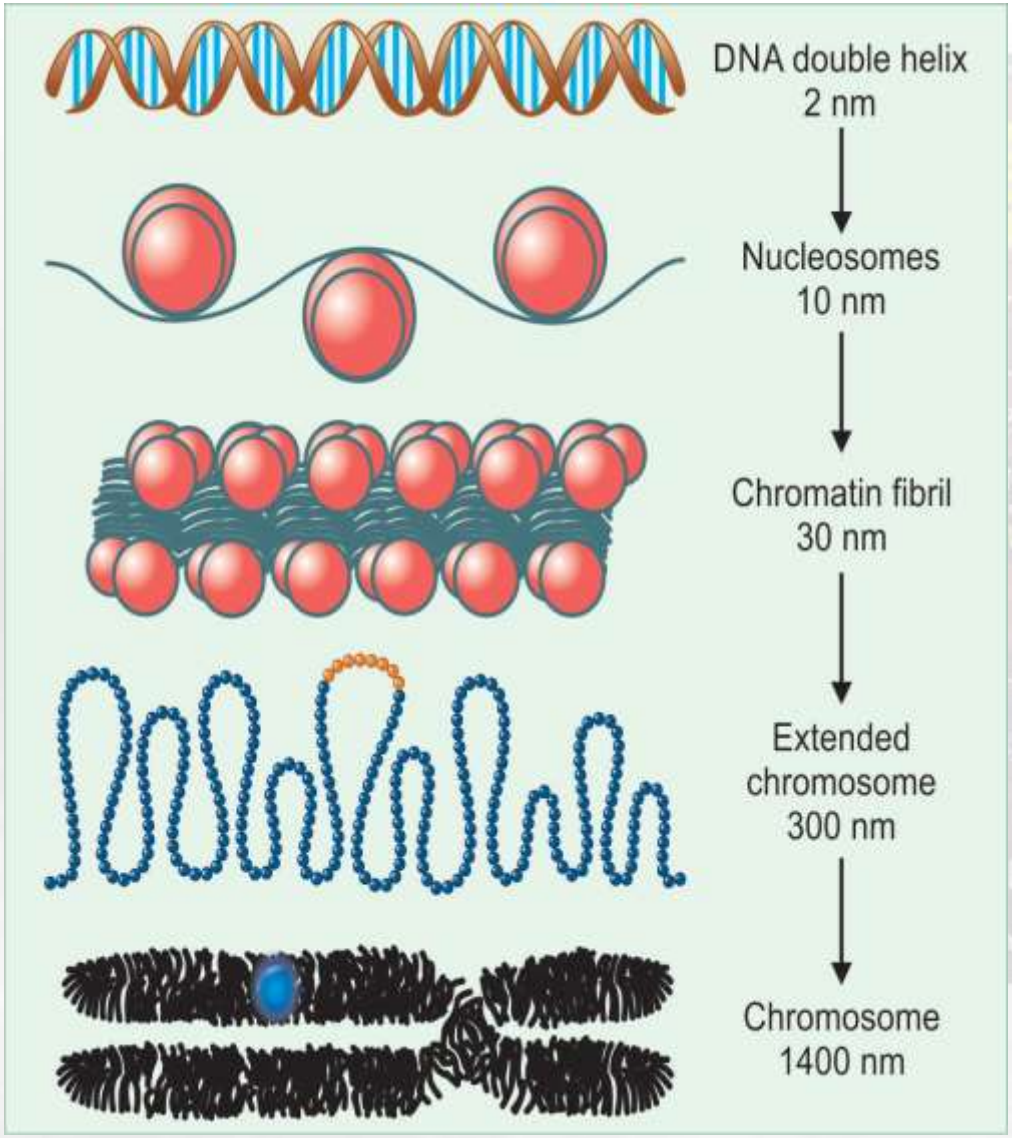
- Each strand acts as a template for the other strand during DNA replication (semi conservative replication).
- Pitch of the spiral is 3.4nm per turn.
- Diameter (width) of the axis is 1.9-2.0 nm.
- Proteins interact with DNA at the exposed bases present in two grooves, major groove (1.2nm) and minor groove (0.6nm). These are parallel to the phosphodiester backbone and wind along the molecule.



DNA wraps twice around histone octamer to form one nucleosome

Histones

- Proteins containing unusually higher concentrations of basic amino acids.
- 5 classes – H1, H2A, H2B, H3 & H4.
- H1 is loosely associated with DNA.
- Core histones – Other histones. H2A and H2B are lysine rich histones. H3 and H4 are arginine rich histones.
- All histones, except H1 are present in equimolar concentrations. H1 is half concentration.
- Histone synthesis stops when DNA synthesis stops.
- Histone synthesized in the cytoplasm migrate to the nucleus.
- Histones are modified by acetylation, methylation and phosphorylation.
- Phosphorylation of serine and threonine residues of H1 occurs prior to mitosis.
- Phosphorylation of H2B is also associated with S phase of cell cycle.



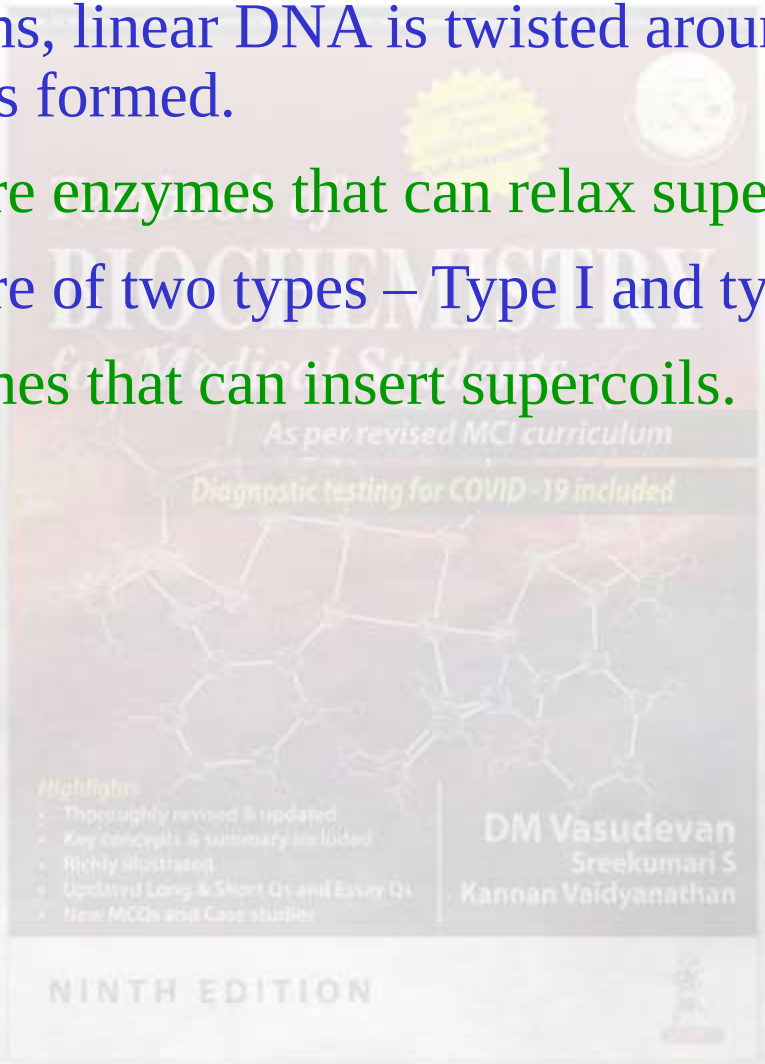
DNA condenses repeatedly to form chromosome

DNA Denaturation

- Melting of DNA is denaturation and separation by heat.
- Optical absorbance of DNA is increased in this process – Hyperchromacity of denaturation
- Melting temperature (Tm) – Temperature when half of DNA is denatured.
- GC rich DNA melts at a higher temperature.
- Melted strands are re-associated at lower temperature – Annealing
- Formamide is a commonly used agent in DNA hybridisation studies, it lowers Tm by disrupting hydrogen bonding between bases.
- Phosphodiester bonds are not broken in the process.

Supercoiling of DNA

- In higher organisms, linear DNA is twisted around its own axis, when a supercoil is formed.
- Topoisomerases are enzymes that can relax supercoils.
- Topoisomerases are of two types – Type I and type II
- Gyrases are enzymes that can insert supercoils.

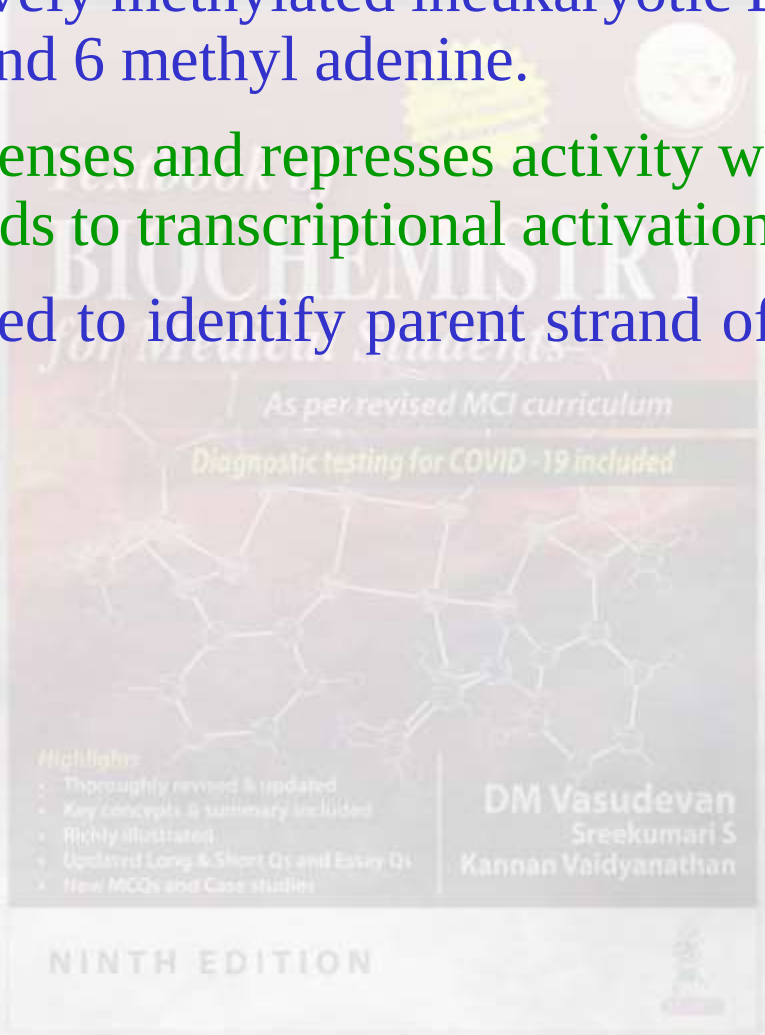


Higher Organization of DNA

- In higher organisms, DNA is organized inside the nucleus.
- Double stranded DNA is first wound over histones, it is called nucleosomes.
- Chromatin – Long stretch of DNA in association with histones.
- Chromosome – Condensed chromatin.
- Extra – chromosomal DNA include plasmids which are found in most bacteria and other organisms.

Base Modifications

- ✓Bases are extensively methylated ineukaryotic DNA to form 6 methyl cytosine and 6 methyl adenine.
- ✓Methylation condenses and represses activity whereas demethylation leads to transcriptional activation.
- ✓Methylation is used to identify parent strand of DNA, during cell replication.



Types of Genes

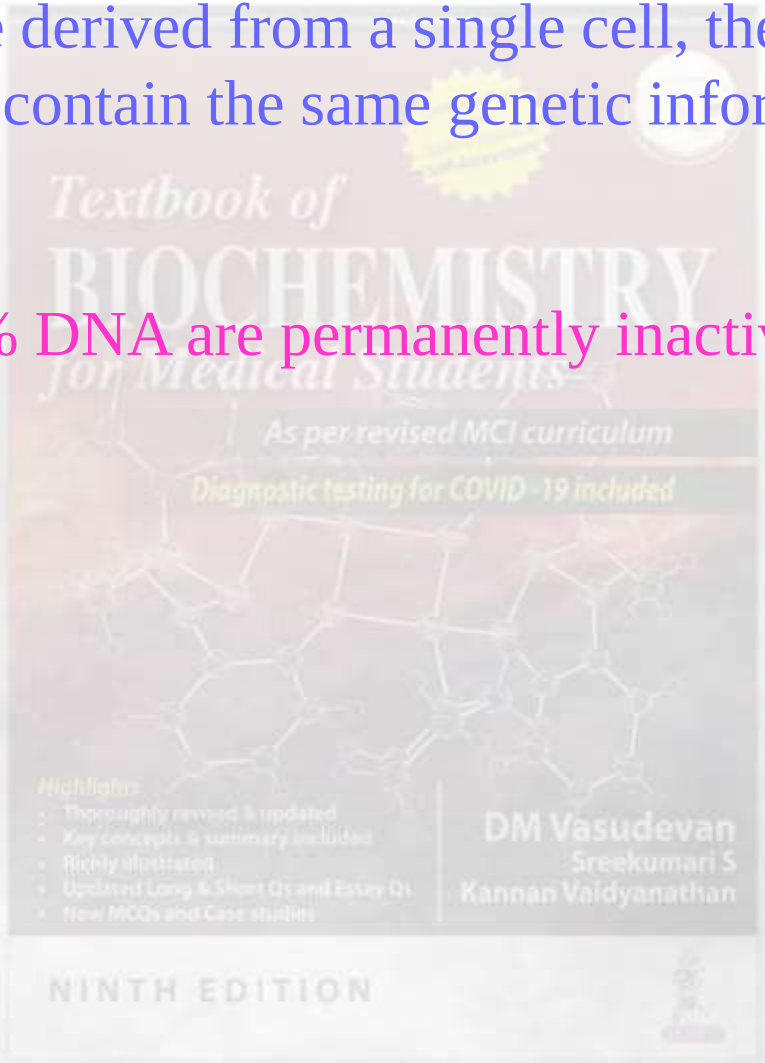
- ✓Homeotic
- ✓Mobile
- ✓Pseudo
- ✓Structural
- ✓Regulator
- ✓Oncogenes
- ✓Tumor suppressor
- ✓Designer
- ✓Morphogenesis
- ✓Transposons
- ✓Not expressed
- ✓Specify protein synthesis
- ✓Produce repressors
- ✓Malignancy
- ✓Malignancy suppression
- ✓Synthetic genes

Inactivation of DNA During Differentiation

All human cells are derived from a single cell, the zygote. Therefore, all cells contain the same genetic information.

In a cell, about 90% DNA are permanently inactive.

Differentiation.



Introns, Exons, Cistrons

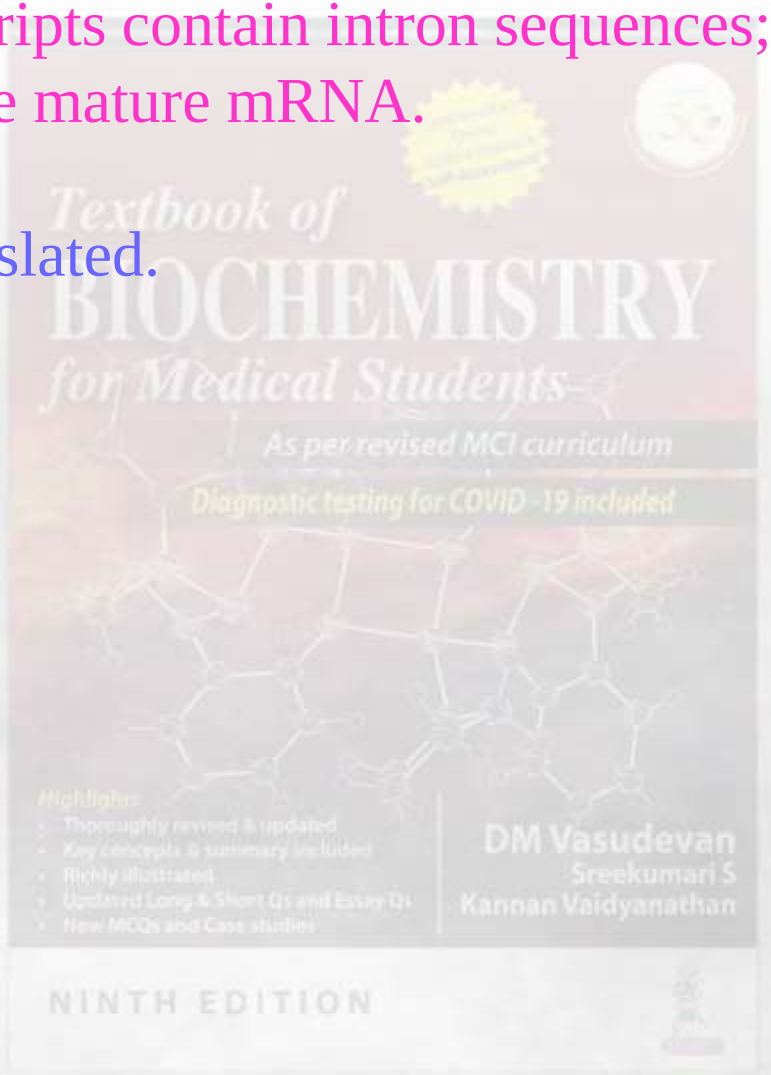
Only about 10% of the human DNA contain genes; the rest are silent areas.

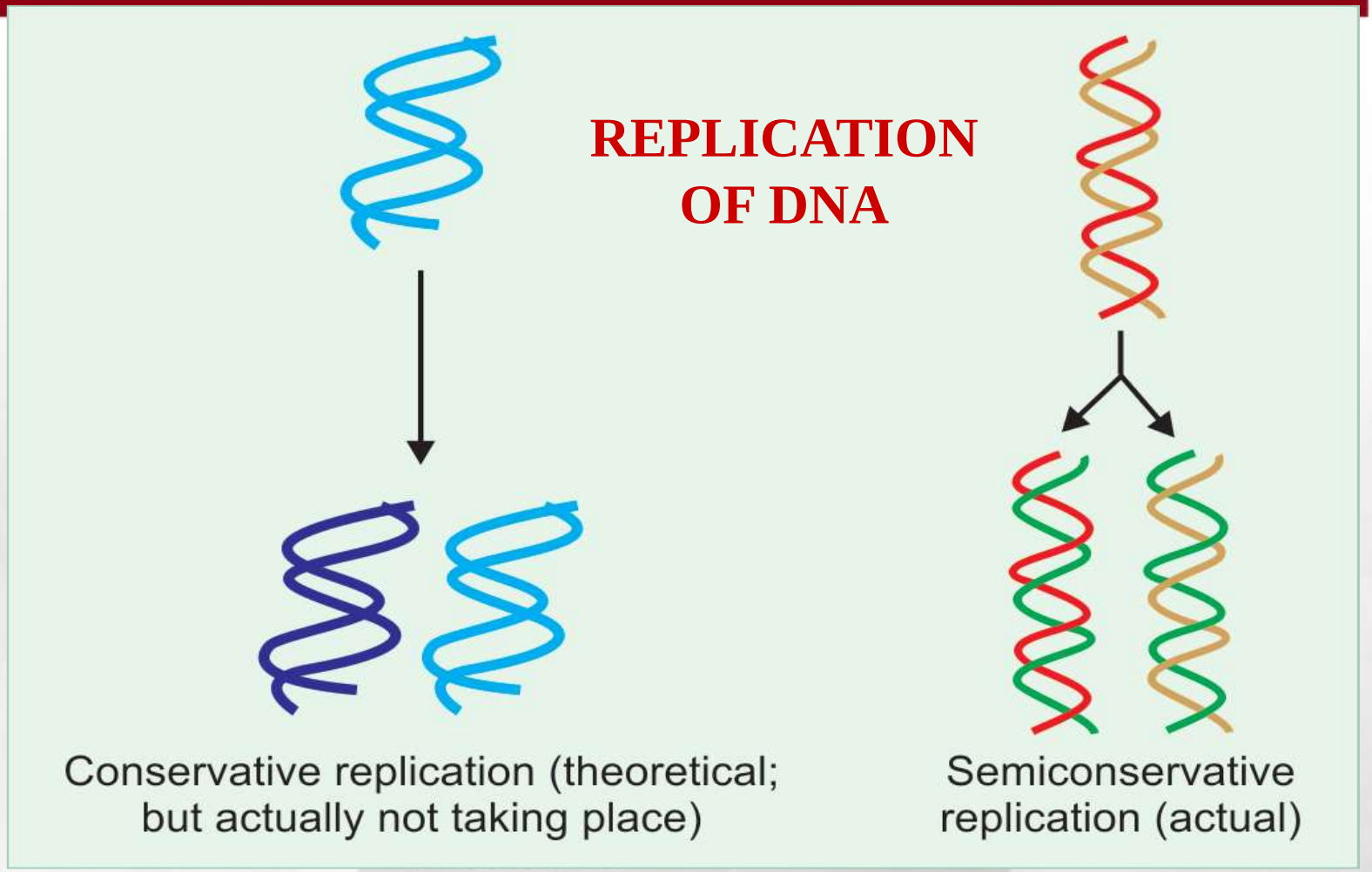
The segments of the gene coding for proteins are called **exons** (expressed regions).

They are interspaced in the DNA with stretches of silent areas, called **introns** (intervening areas).

The primary transcripts contain intron sequences; which are later removed to produce mature mRNA.

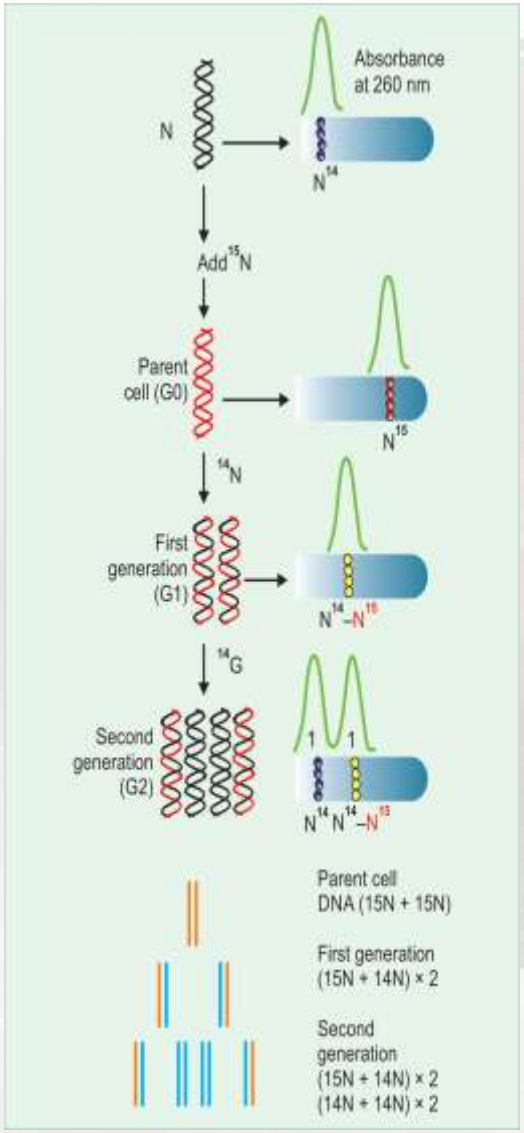
Introns are not translated.





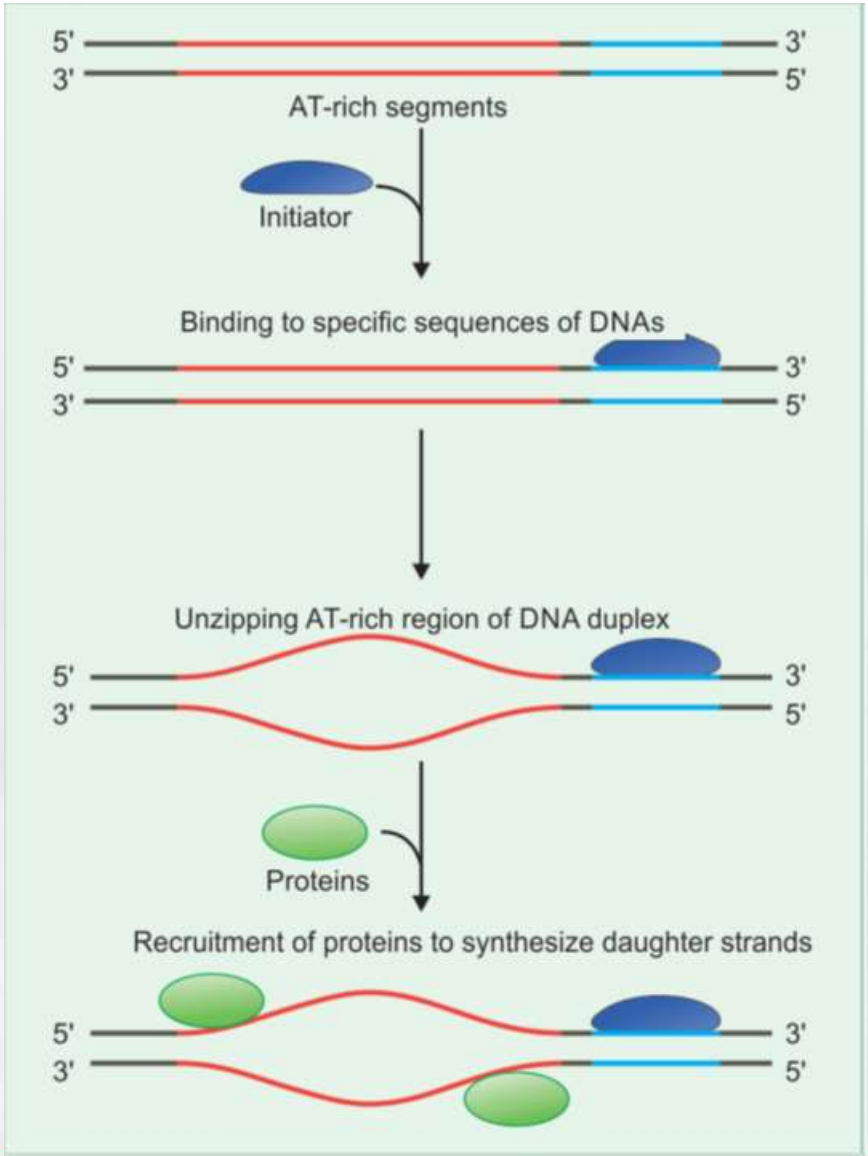
Semiconservative replication. A new complementary strand is synthesized over the old template.

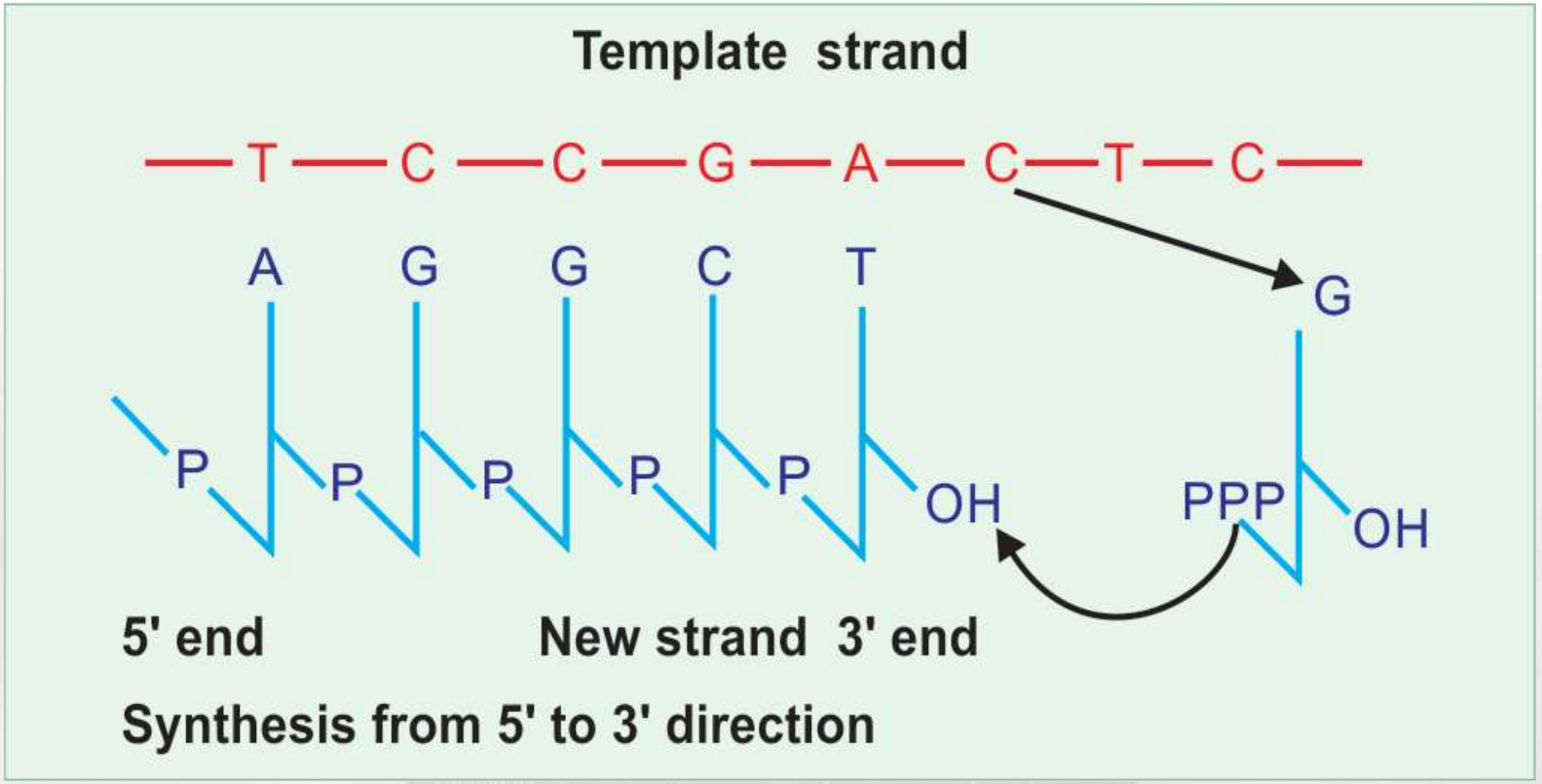
Meselson-Stahl Experiment



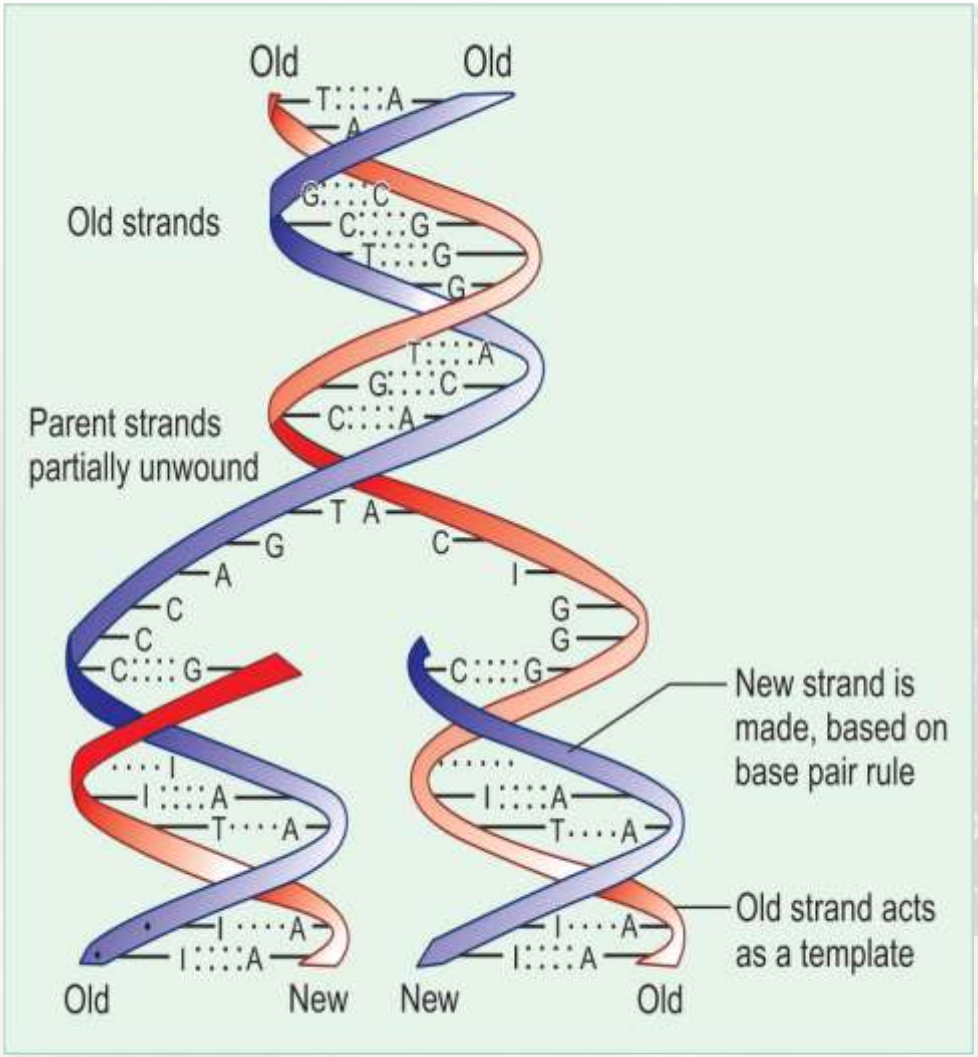
Bacteria were grown in a medium containing the heavy isotope of nitrogen (15N), when all the DNA was labeled with heavy nitrogen. These cells were allowed to divide in a medium containing normal nitrogen, (14N). In the first generation, all DNA molecules were half labeled. In the second generation half labelled And completely unlabeled molecules were present in equal numbers. From this experiment it was proved that *DNA replication is semiconservative in vivo*. The **base pairing** rule is always maintained. The new strand is joined to the old strand by hydrogen bonds between base pairs (A with T and G with C).

Origin of replication





New strand is synthesized from 5' to 3' direction. Base pairing rule is always maintained.

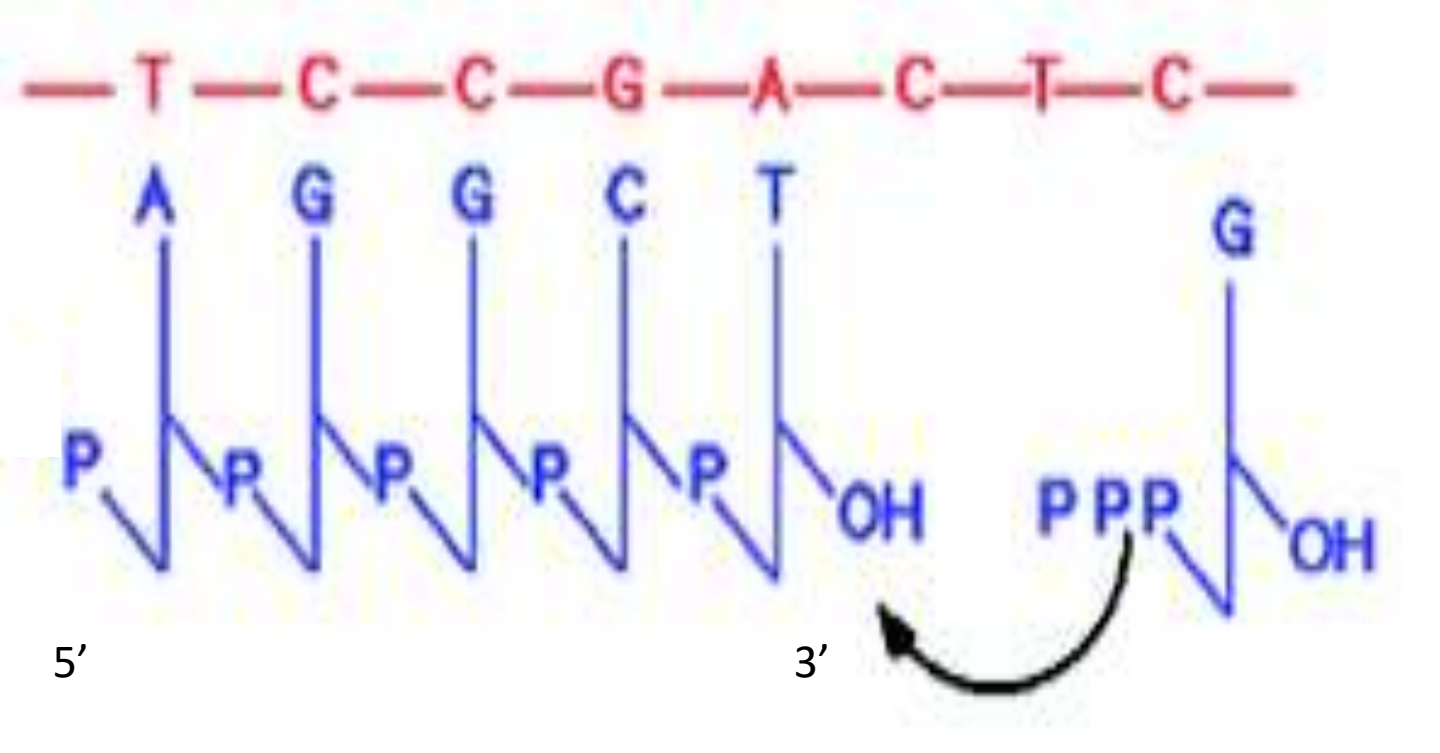


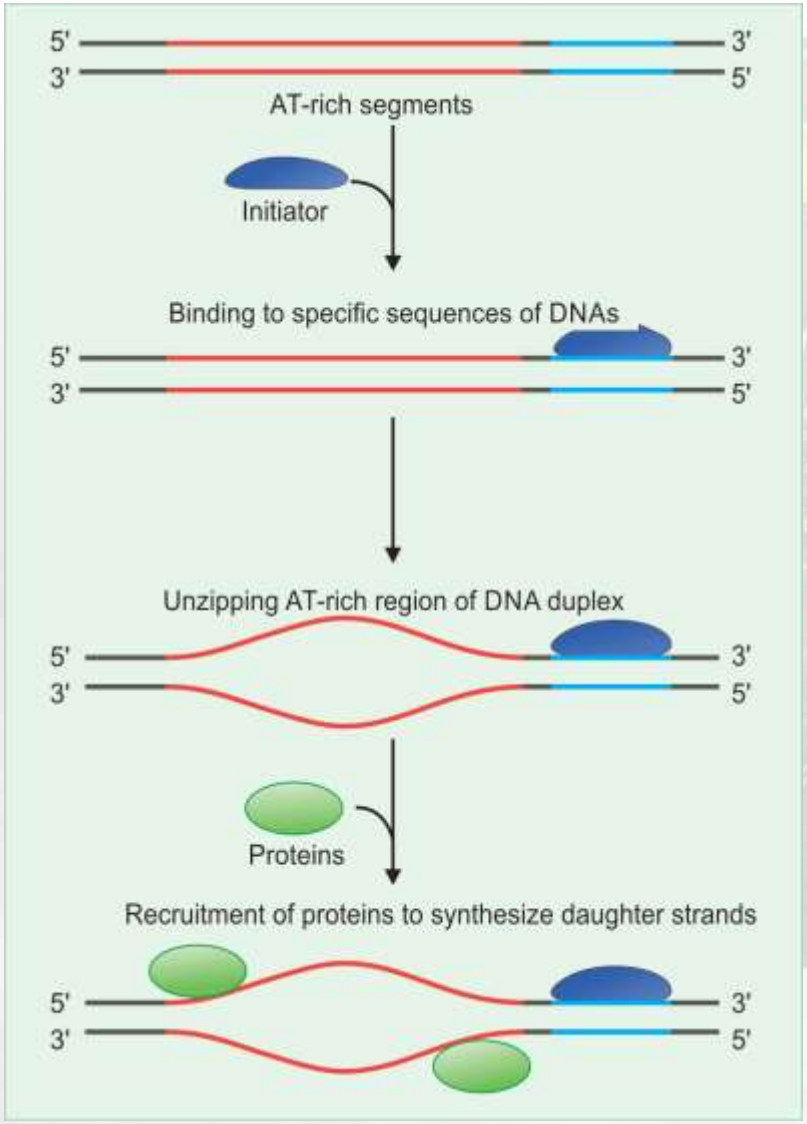
Both strands are replicated.

Salient Features of Replication

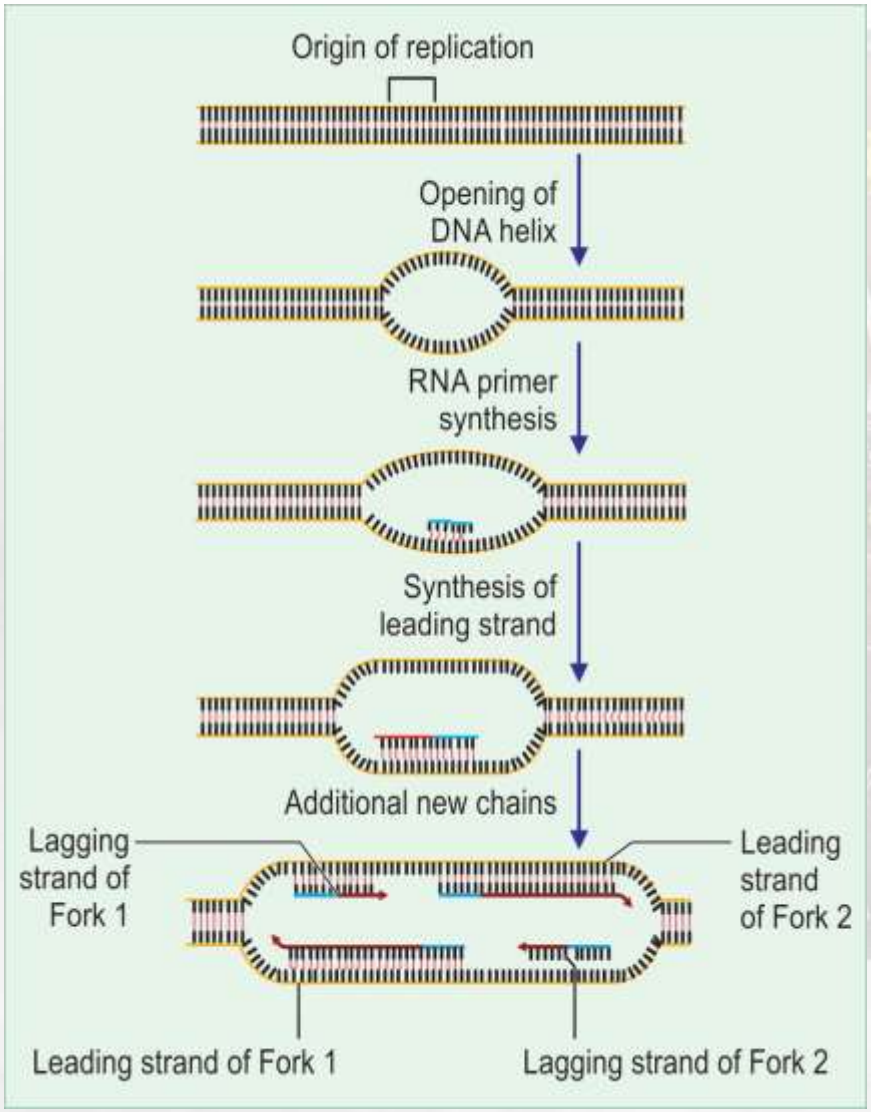
1. Each strand serves as a TEMPLATE over which new COMPLEMENTARY strand is synthesised
2. Base parining rule, A with T; G to C
3. Polymerisation of the new strand is taking place from 5’ to 3’ direction

DNA Polymerase Action

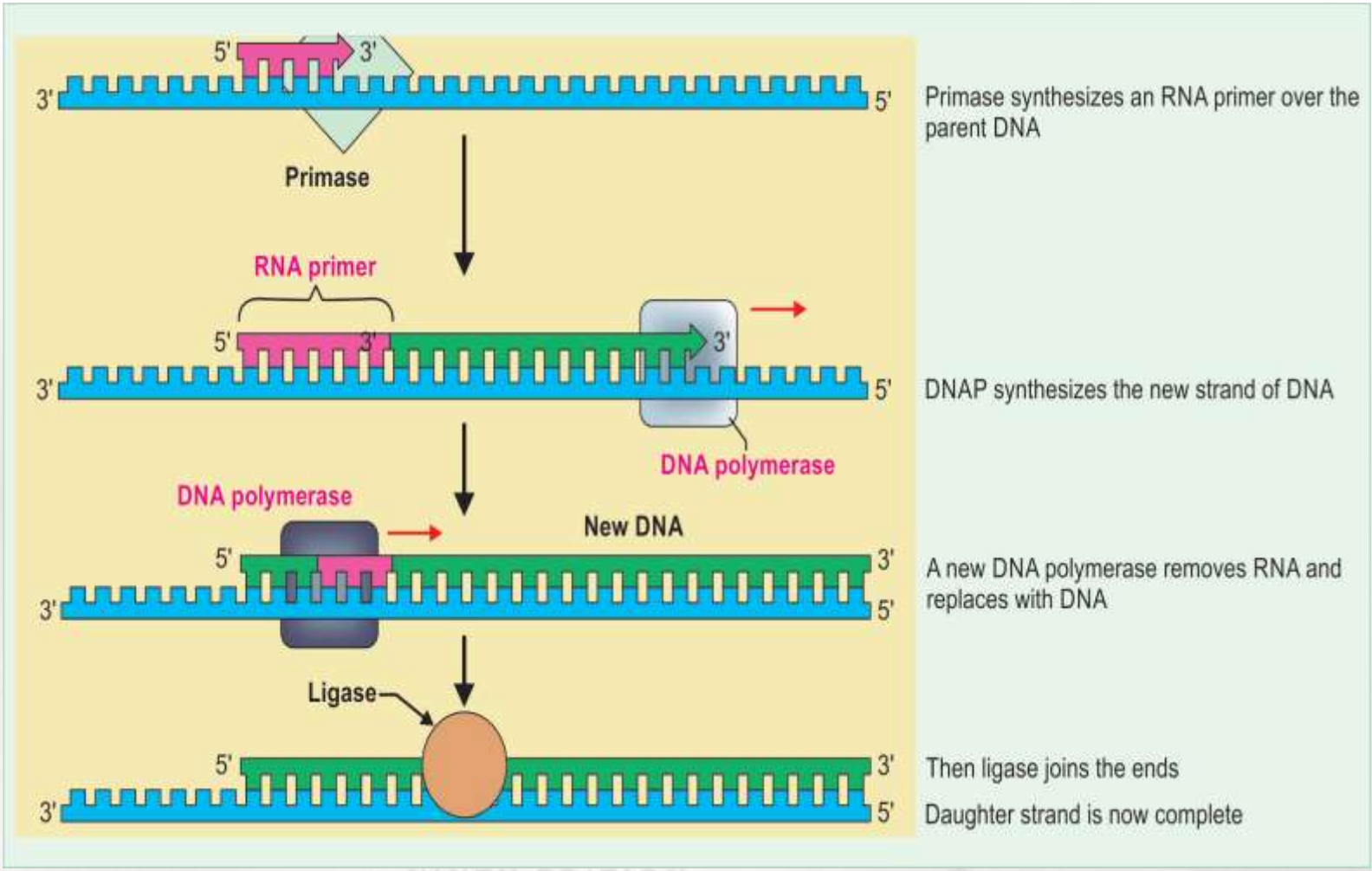




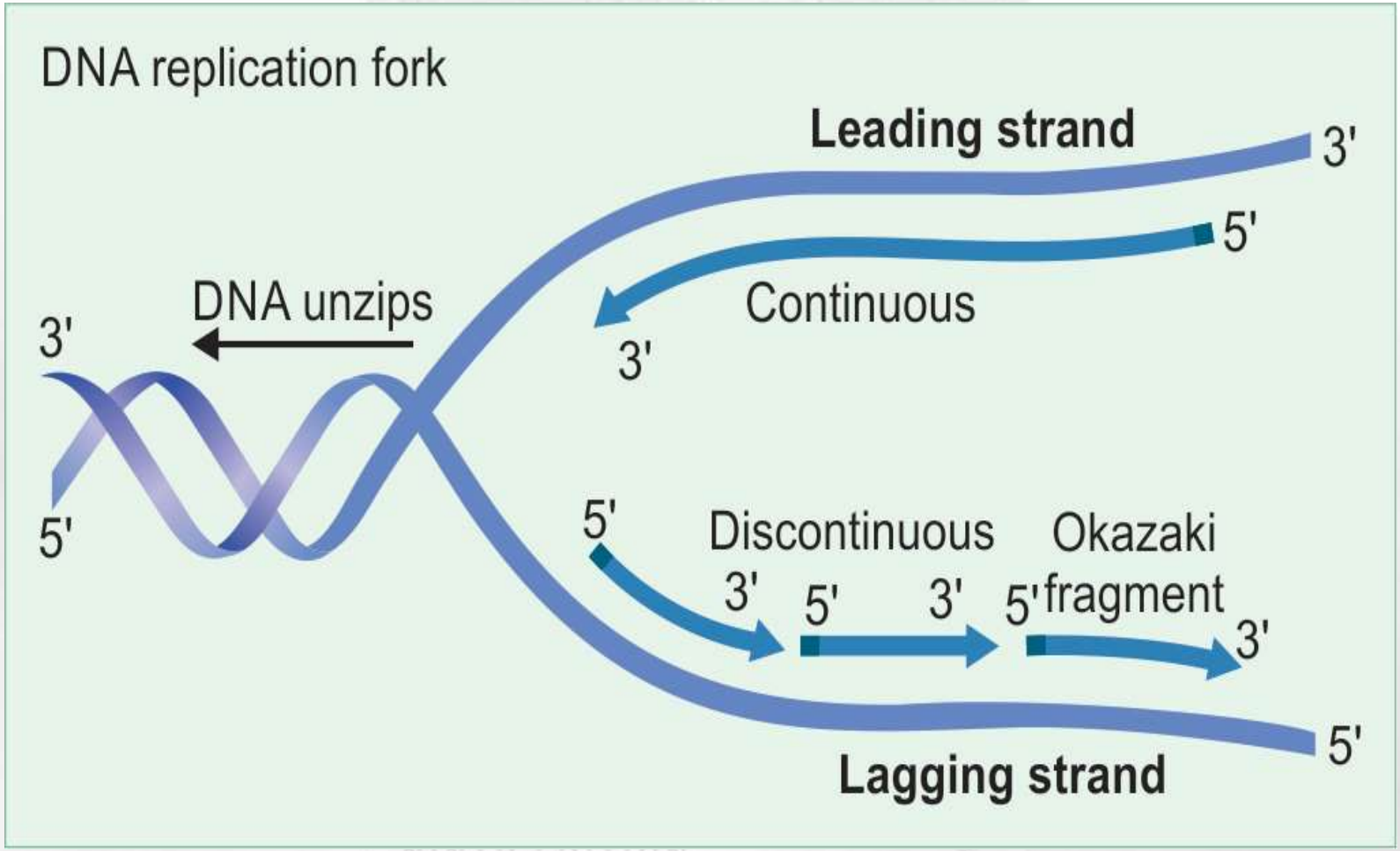
Origin of replication.



Replication bubble (replication fork).



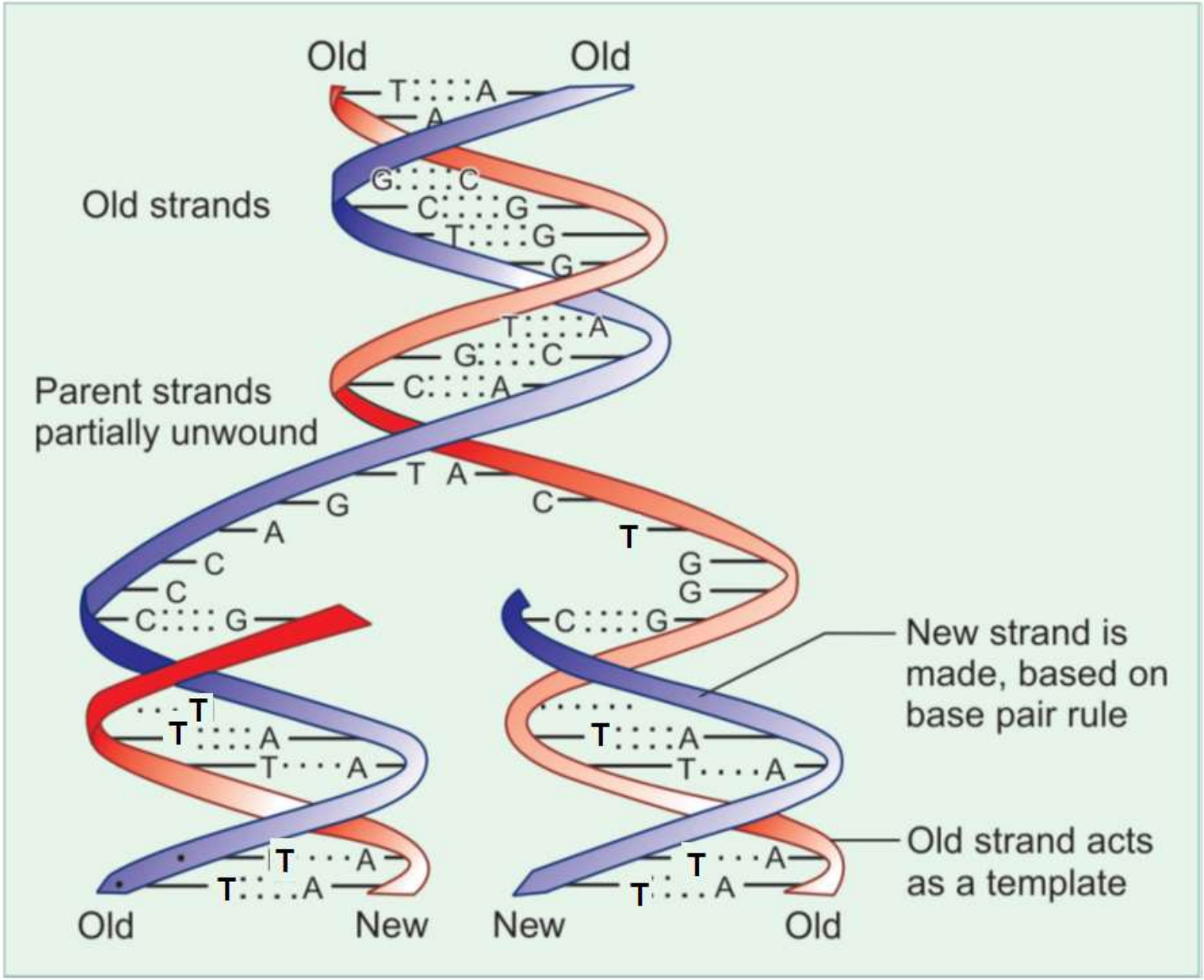
RNA primer is needed for the DNA synthesis.


DNA replication fork
Leading strand
3'
5'
DNA unzips
Continuous
3'
3'
5'
5'
Discontinuous
Okazaki
3'
5'
3'
5'
fragment
3'
5'
Lagging strand

Summary of DNA Replication

- Origin of replication is identified. Then unwinding of parental DNA to form a replication fork.
- RNA primer complementary to the DNA template is synthesized by RNA primase.
- DNA synthesis is continuous in the leading strand (toward replication fork) by DNA polymerase.
- DNA synthesis is discontinuous in the lagging strand (away from the fork), as Okazaki fragments.
- Elongation: In both strands, the synthesis is from 5' to 3‘ direction.
- Then the RNA pieces are removed; the gaps are filled by deoxynucleotides by DNAP and the pieces are ligated by DNA ligase.
- Proofreading is done by the DNA polymerase.
- Finally organized into chromatin.
- Main enzymes involved in replication are: DNA polymerases, helicases, topoisomerases, DNA primase, single-strand binding proteins, and DNA ligase.

Summary of DNA Replication



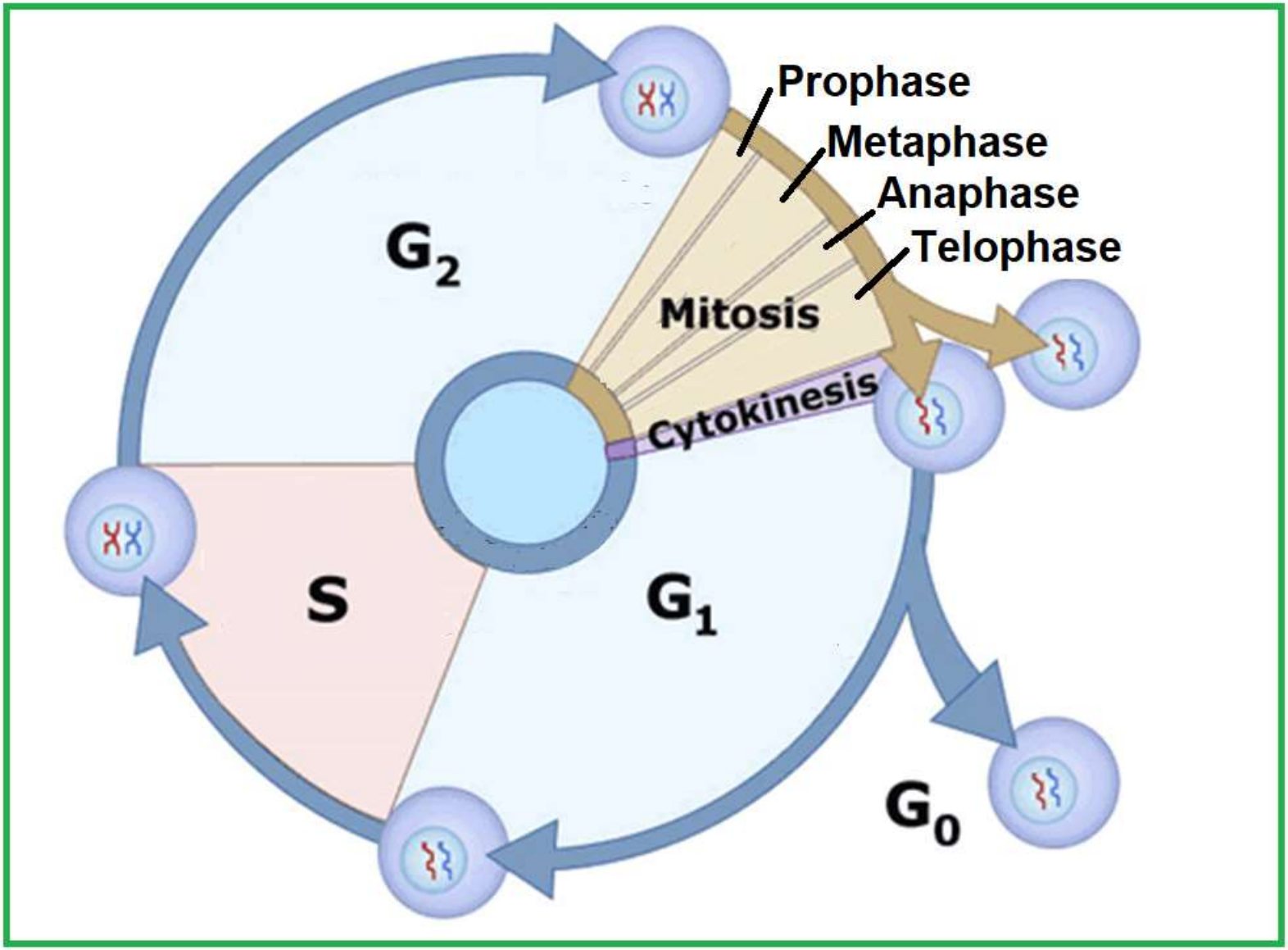
Comparison of features of Replication in Prokaryotic and Eukaryotic Cells

	Prokaryotes	Eurokaryotes
DNA	Circular DNA	Linear
Origin	Origin of replication	Replication at multiple sites
Single strand binding proteins	Co-operative binding to the SS DNA	SS DNA binding proteins bind at the replication fork.
RNA primer	Required for synthesis of both strands	DNAP alpha has primase activity and initiates synthesis of both lagging and leading strands
DNA polymerrases	Major polymerizing enzyme is DNAP–III	Major polymerizing enzymes are DNAP delta and DNAP epsilon.

Comparison of features of Replication in Prokaryotic and Eukaryotic Cells

	Prokaryotes	Eurokaryotes
Proof-reading	DNAP III also has 3' to 5' exonuclease activity so that any wrong base is removed and the correct one added	DNAP delta and epsilon both have 3'→ 5' exonuclease activity and therefore serve the proofreading function
Gap filling	The RNA primer is removed and gap filled by DNAP	RNA primer is removed by RNAse H and FEN1. Polymerase beta is involved in gap filling and DNA repair.
Inhibitors	Ciprofloxacin and Novobiocin inhibit topoisomerase	Etoposide, Adriamycin and Camptothecin inhibit Topoisomerase

Cell Cycle


Prophase
Metaphase
Anaphase
Telophase
Mitosis
Cytokinesis
G_2
S
G_1
G_0

Cell Cycle

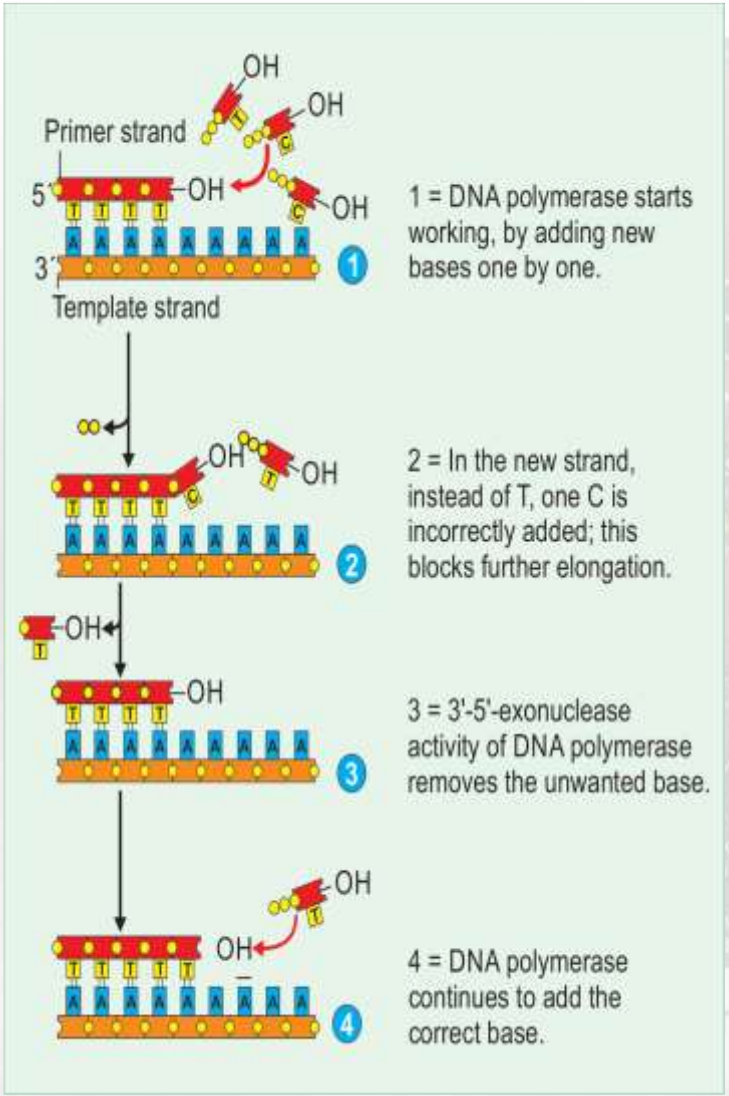
The four phases of the cell cycle are G1, G2, S, and M. In G1 (gap 1) phase, the cell prepares for DNA synthesis. DNA synthesis occurs during the S (synthesis) phase of the cell cycle (Fig. 34.30). During the S phase, DNA is completely replicated, but only once. Cell prepares for mitosis in G2 (gap 2) phase, when proteins necessary for daughter cells are synthesized. Then the cell enters into the M (mitotic) phase, when the chromosomes are visible under the microscope. The whole cycle lasts about 24 hours; out of which M phase is only 1–2 hours. Those cells which are not in division are said to be in G0 phase or resting phase.

Types of DNA Damage

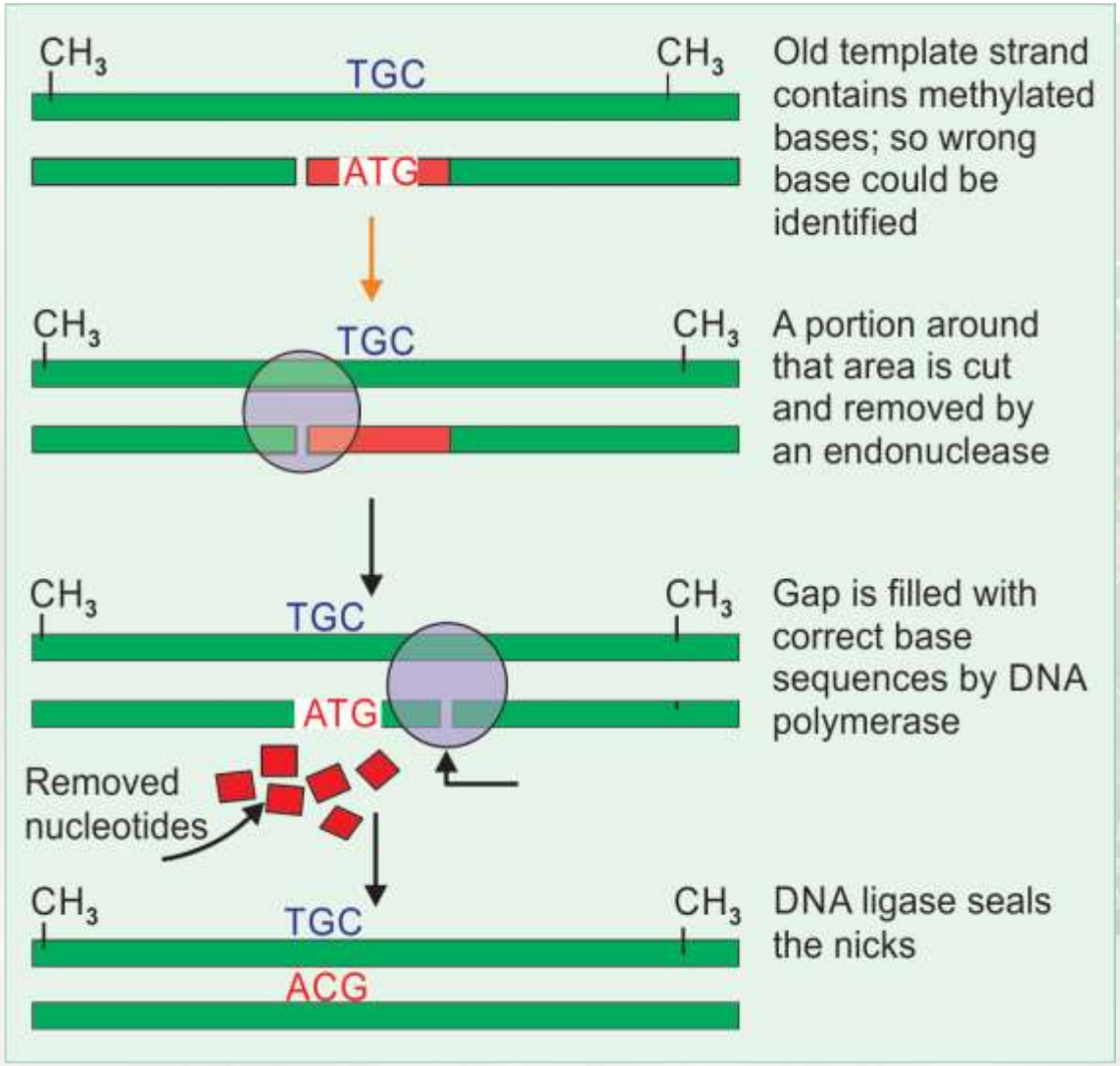
- Oxidation of bases (e.g. 8-oxo-7,8-dihydroguanine).
- Alkylation (methylation) of bases, such as 7-methyl guanosine, 1-methyl adenine, 6-methyl guanine.
- Hydrolysis of bases, such as deamination, depurination, and depyrimidination.
- Adduct formation, e.g. benzo[a]pyrene diol epoxide causes dG adduct.
- Mismatch of bases, due to errors in DNA replication.
- Monoadduct damage cause by change in single nitrogenous base of DNA.
- Ultraviolet-B (UV-B) light causes cross-linking between adjacent cytosine and thymine bases creating pyrimidine dimers. This is a direct damage.
- Ultraviolet-A light creates mostly free radicals, causing indirect DNA damages.
- Ionizing radiation such as gamma-rays may induce irreparable DNA damage.
- Elevated temperature causes depurination (loss of purine bases from the DNA backbone) and single strand breaks.
- Chemicals such as aromatic hydrocarbons cause DNA adducts.

DNA Repair Mechanisms

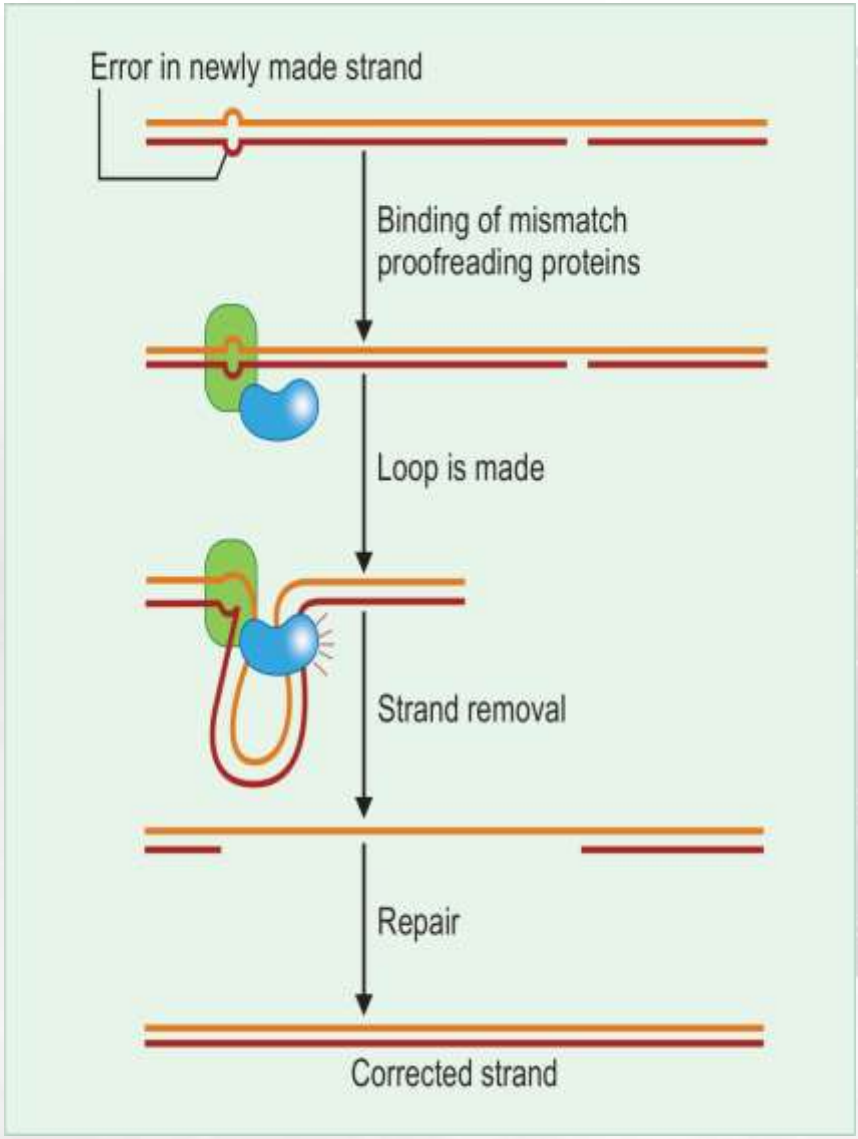
Mechanism	Defect	Repair
Mismatch repair	Copying error 1-5 bases unpaired	Strand cutting, exonuclease digestion
Nucleotide exicision repair (NER)	Chemical damage to a segment	30 bases removed; then correct bases added
Base excision repair	Chemical damage to single base	Base removed by N-glycosylase; new base added
Double strand break	Free radicals and radiation	Unwinding, alignment, ligation



Exonucleolytic proofreading of DNA polymerase.



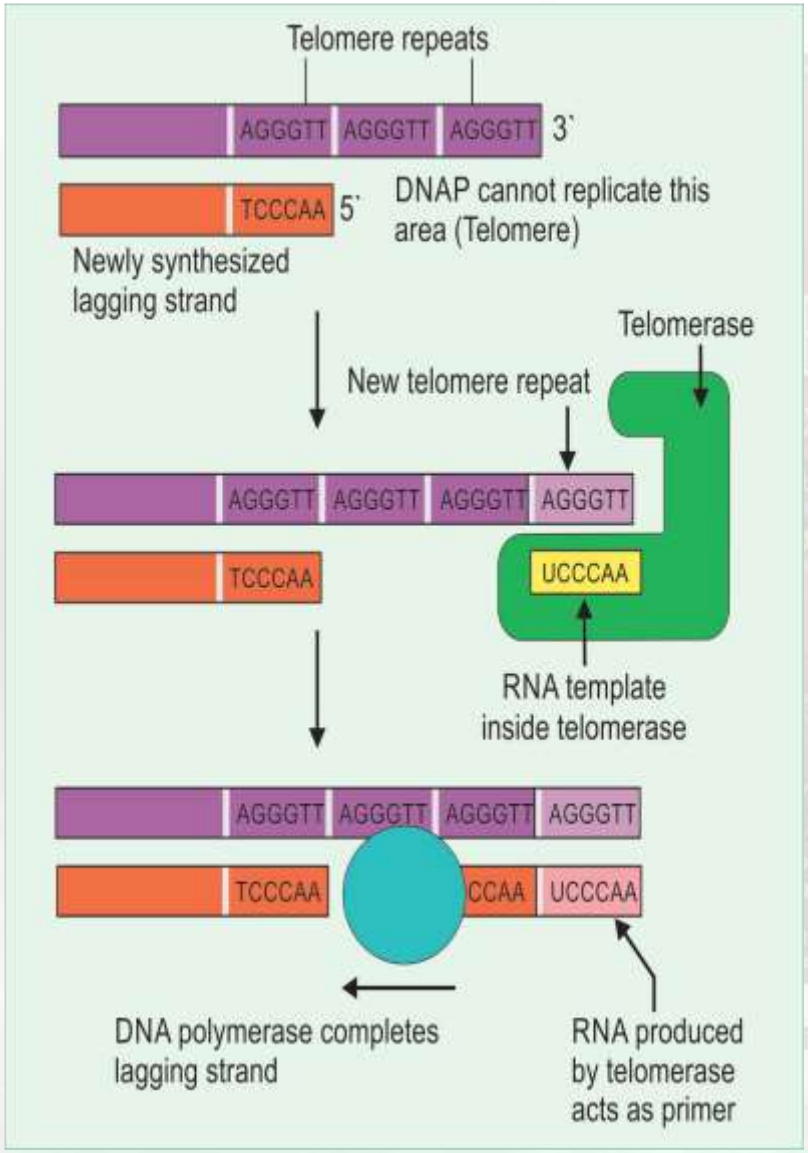
Nucleotide excision repair (NER).



Strand directed mismatch repair.

Diseases Associated with DNA Repair Mechanisms

- *Xeroderma pigmentosum (XP):* Defective nucleotide excision repair (NER) mechanism; sensitivity to UV light; skin cancers.
- *Ataxia telangiectasia (AT):* Defective ATM gene; sensitivity to UV light; lymphoreticular neoplasms.
- *Fanconi anemia*: Defective genes are in chromosomes 20q and 9q. Defect in DNA cross-link repair; increased occurrence of cancer.
- *Bloom's syndrome*: Gene is in 15q. Defect is in DNA ligase or helicase; lymphoreticular malignancies.
- *Cockayne syndrome*: Defect in NER mechanism; transcription factor II H is defective; stunted growth and mental retardation.
- *Hereditary polyposis colon cancer (Lynch syndrome):* Defective gene in chromosome 2. Defect in *hMSH* 1 and 2 genes; mismatch repair is defective.



Telomere and Telomerase

The replication always takes place from 5' to 3' direction in the new strand. The DNA polymerase enzyme is not able to synthesise the new strand at the 5' end of the new strand. Thus, a small portion (about 300 nucleotides) in the *3' ends of the parent strands could not be replicated*. This end piece of the chromosome is called telomere. Therefore, another enzyme, *telomere terminal transferase* or telomerase takes up this job of replication of the end piece of chromosomes.

Unless there is some mechanism to replicate telomeres, the length of the chromosomes will go on reducing at each cell division (gene loss). The stability of the chromosome is thus lost. The shortening of telomere end is prevented by an enzyme telomerase. It contains an essential RNA component, which provides the template for telomerase repeat synthesis. Telomerase acts like a reverse transcriptase. Telomerase recognises 3' end of telomere, and then a small DNA strand is synthesised.

Inhibitors of DNA Replication

Drug	Action (inhibition of)
Antibacterial agents	
Ciprofloxacin	Bacterial DNA gyrase
Nalidixic acid	do
Novobiocin	do
Anticancer agents	
Etoposide	Human topoisomerase
Adriamycin	do
Doxorubicin	do
6-mercaptopurine	Human DNA polymerase
5-fluorouracil	Thymidylate synthase

Clinical uses of DNA analysis

- DNA analysis has been widely used in the diagnosis of viral and bacterial pathogens with high accuracy and sensitivity.
- DNA based vaccines have been widely used in the prevention of viral infections including Covid-19.
- Analysis of cell free DNA (cfDNA) in maternal circulation is useful in non-invasive prenatal testing (NIPT).
- Study of cell free tumour DNA (cftDNA) is useful in the assessment of progression and recurrence of cancer