DNA replication

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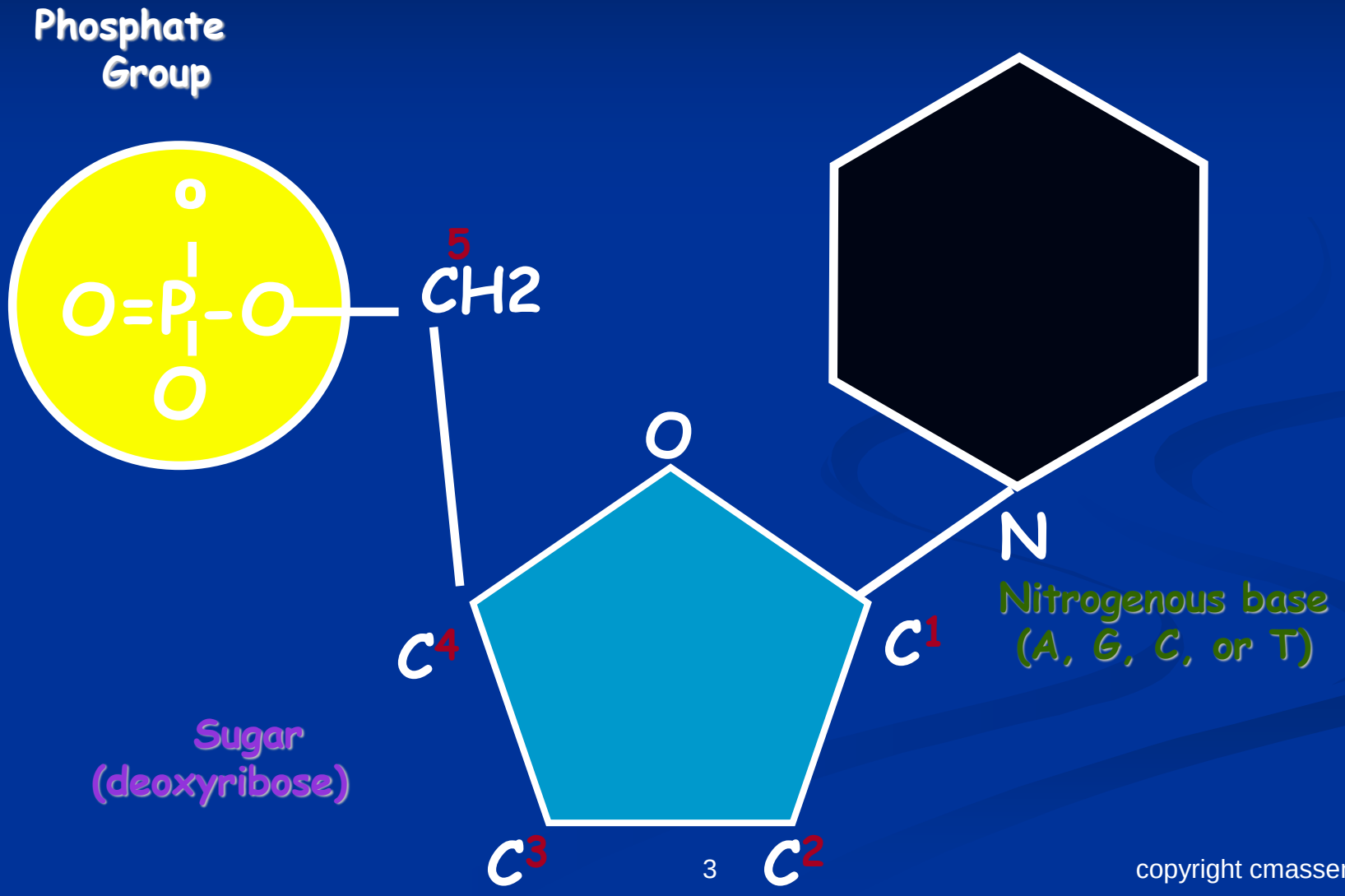
DNA

- Stands for Deoxyribonucleic acid
- Made up of subunits called nucleotides
- Nucleotide made of:
    1. Phosphate group
    2. 5-carbon sugar
    3. Nitrogenous base

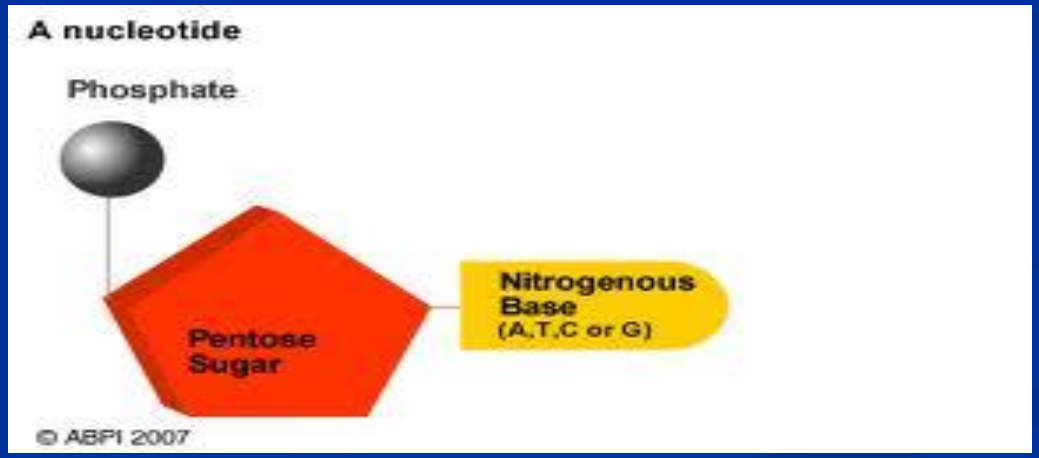
DNA Nucleotide



Sugar +Base = nucleoside

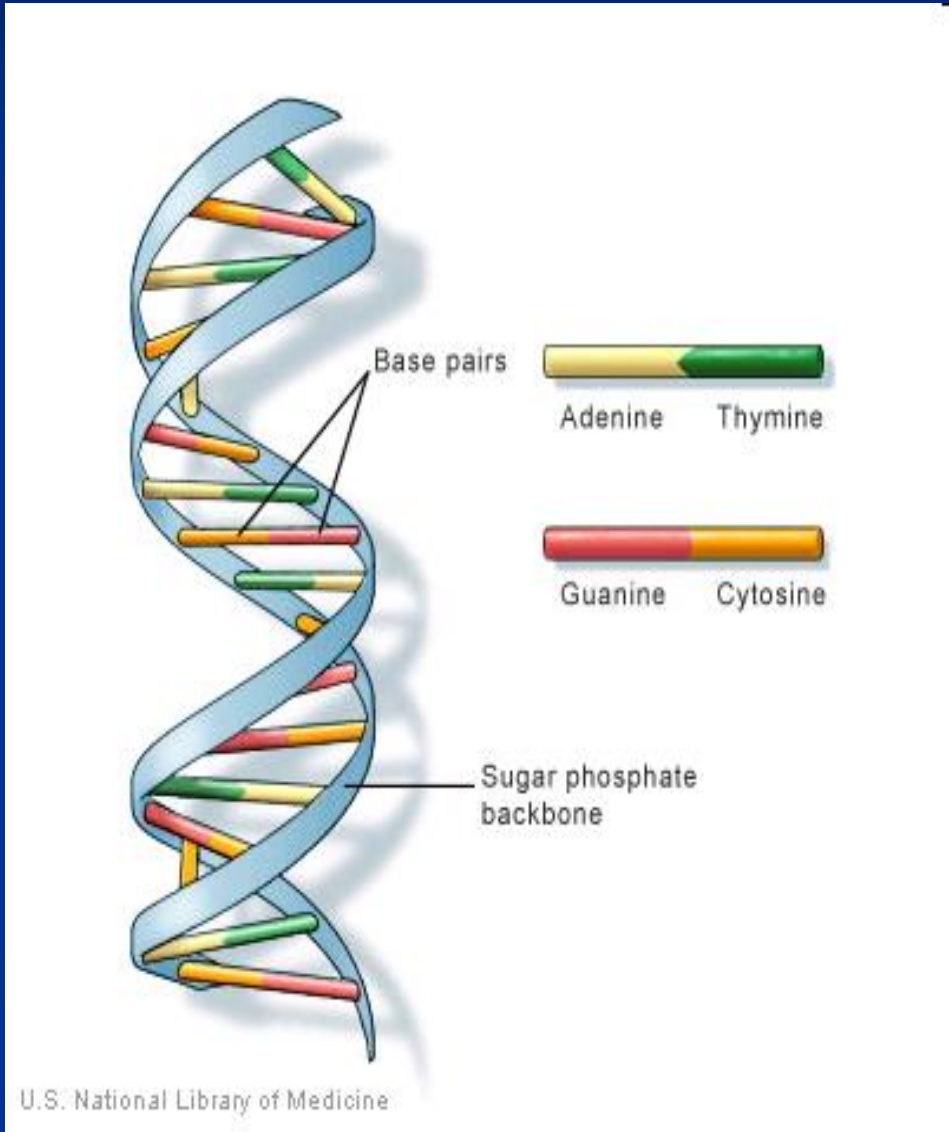
nucleoside

Phosphate+ sugar + Base = nucleotide

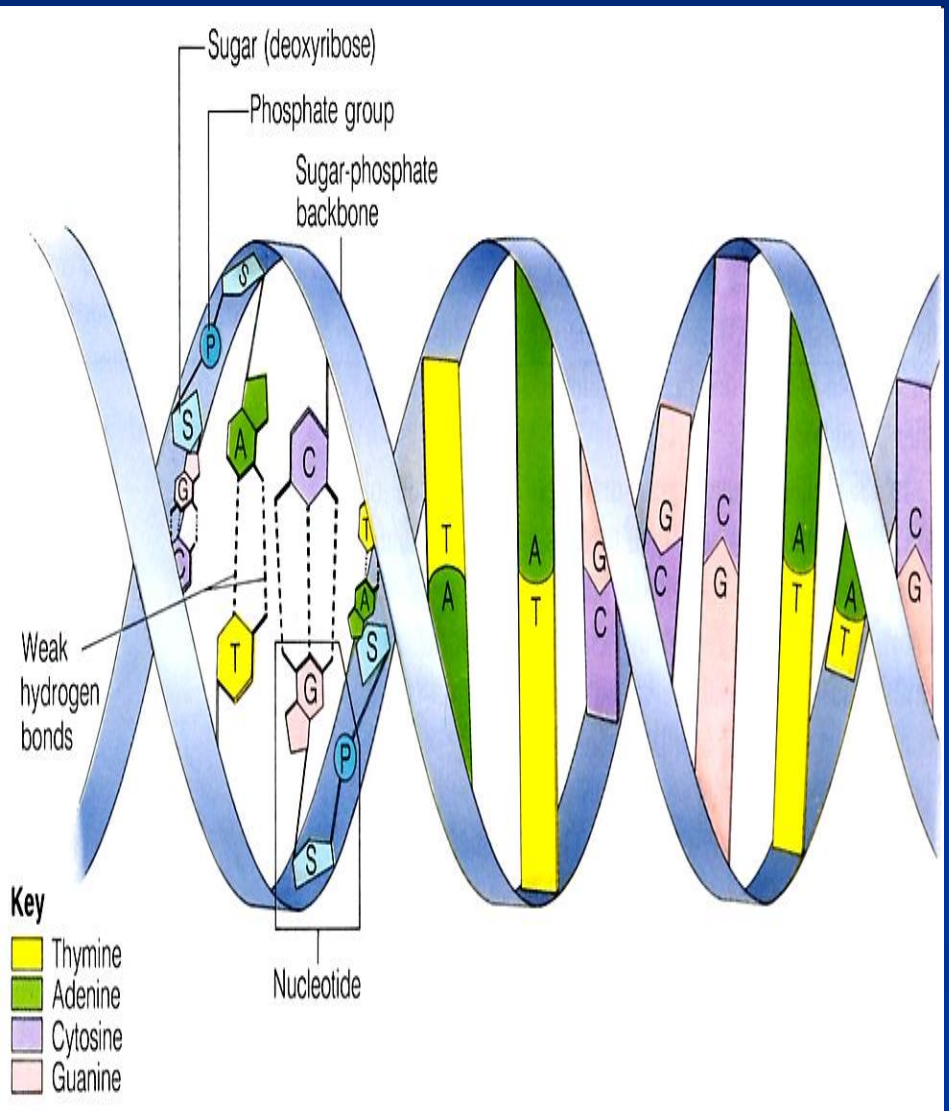


DNA - Structure

- The nucleotide remains as the fundamental unit (monomer) of the nucleic acid polymer. There are four nucleotides: those with cytosine (C), those with guanine (G), those with adenine (A), and those with thymine (T).



DNA

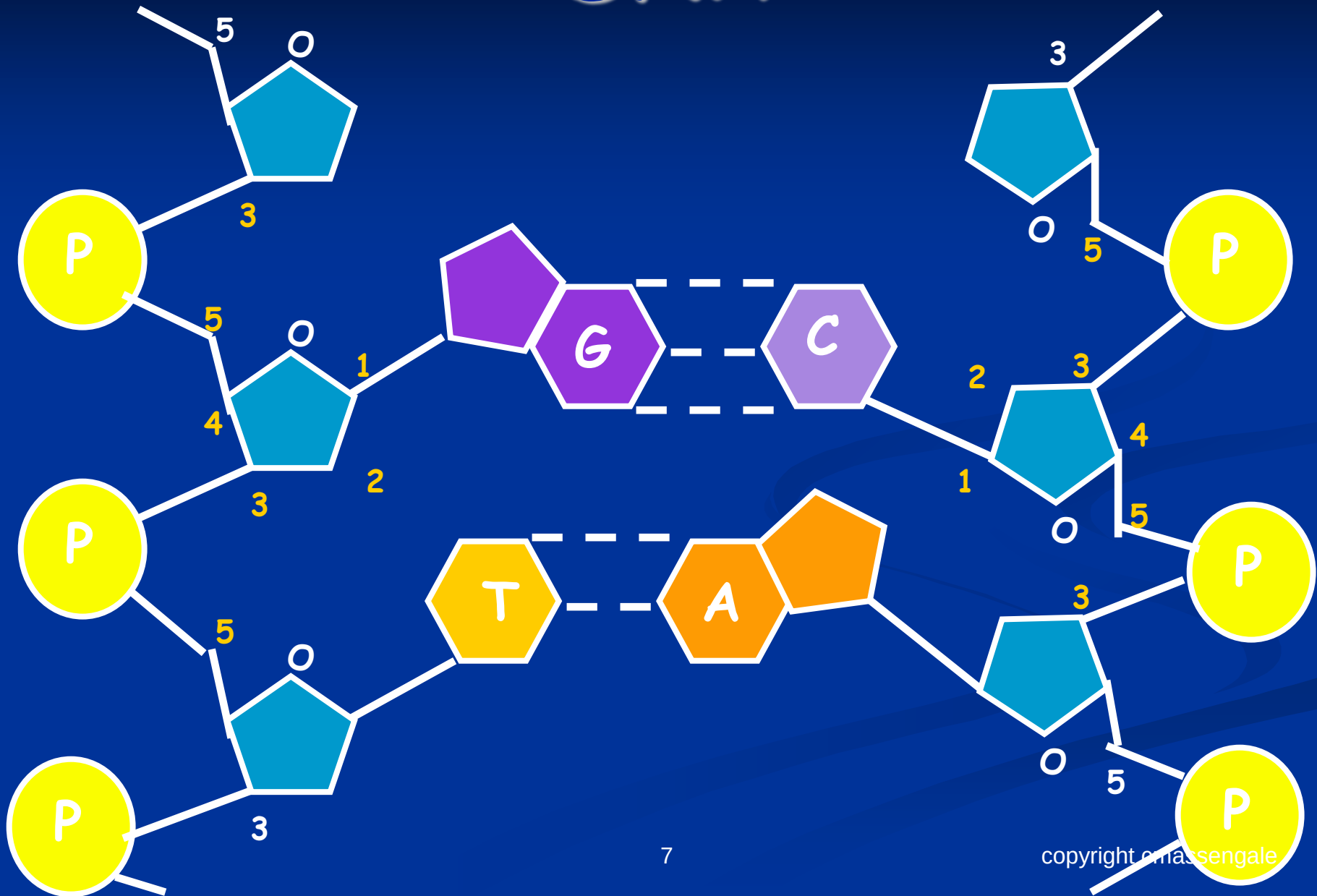


A purine always links with a pyrimidine base to maintain the structure of DNA.

Adenine (A) binds to Thymine (T), with two hydrogen bonds between them.

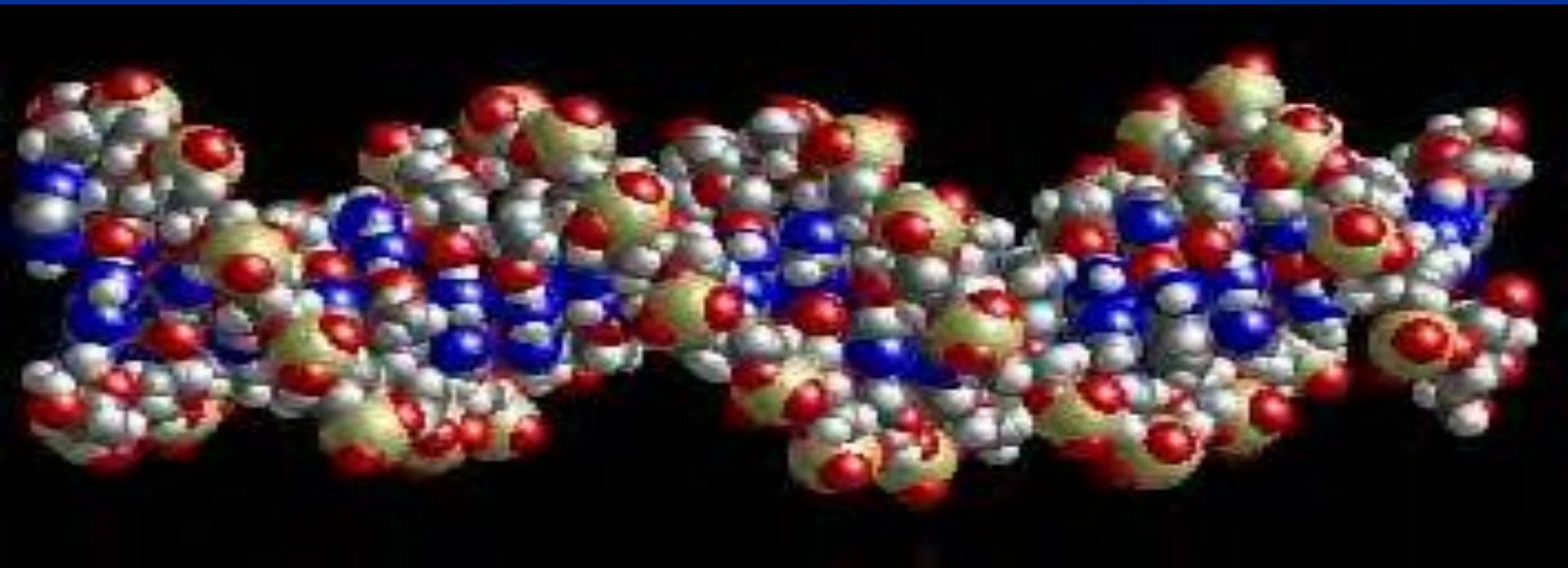
Guanine (G) binds to Cytosine (C), with **three hydrogen bonds** between them.

DNA



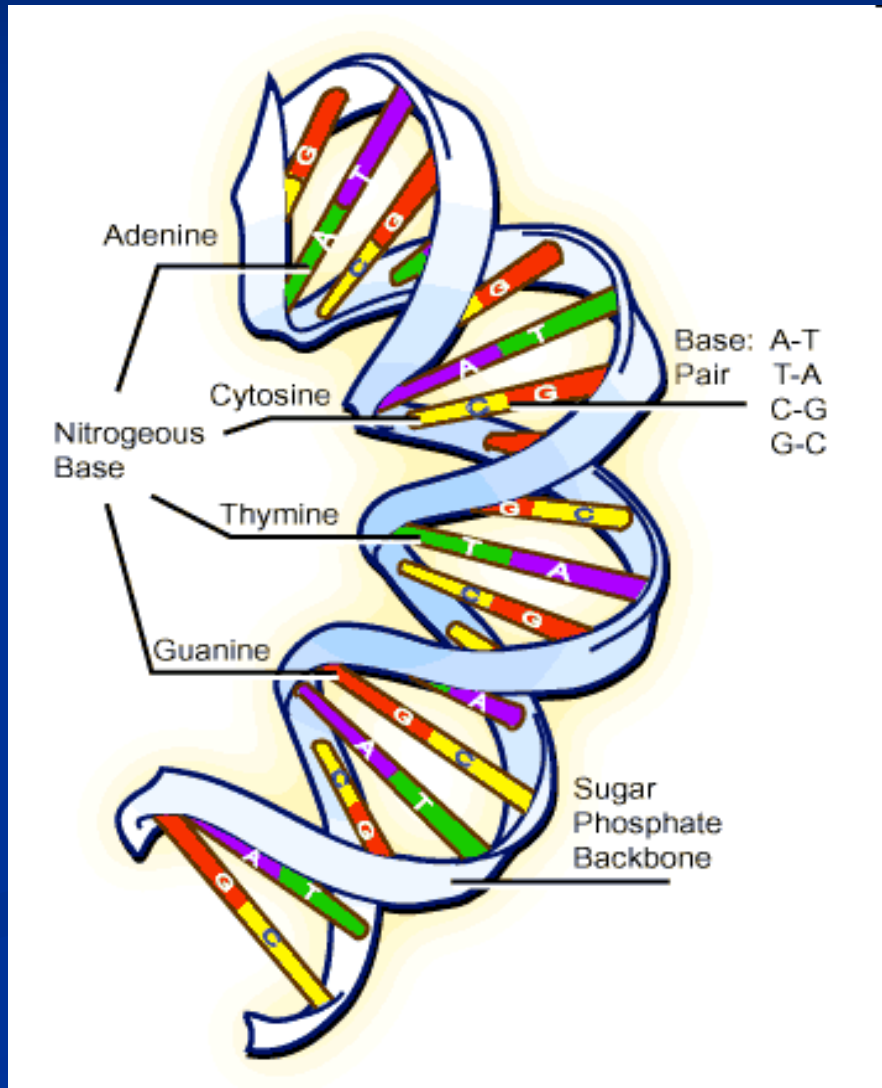
DNA

- Example
- First strand **GGGTTTAAACCC**
- Second strand **CCCAAATTTGGG**



DNA is Endless structure

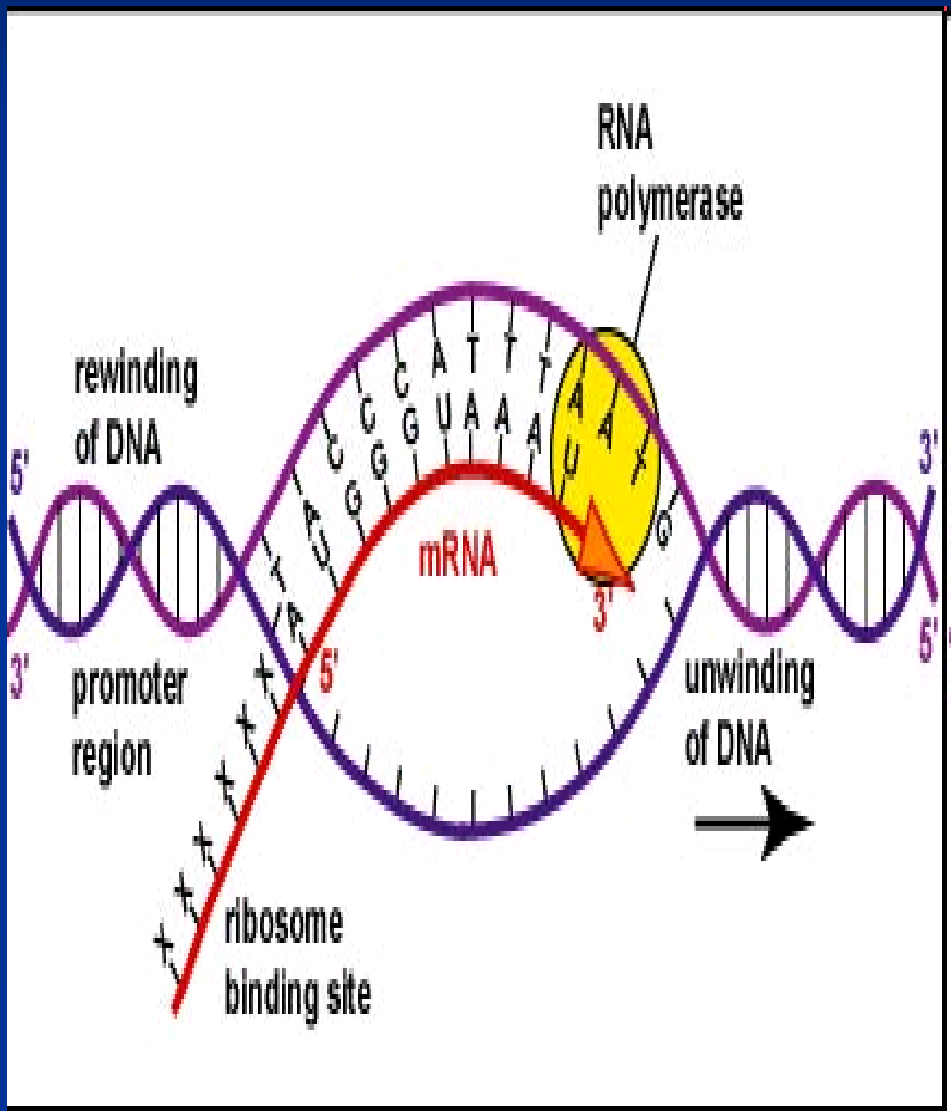
- The rungs of the ladder can occur in any order (as long as the base-pair rule is followed)
- Those 4 bases have endless combinations just like the letters of the alphabet can combine to make different words.



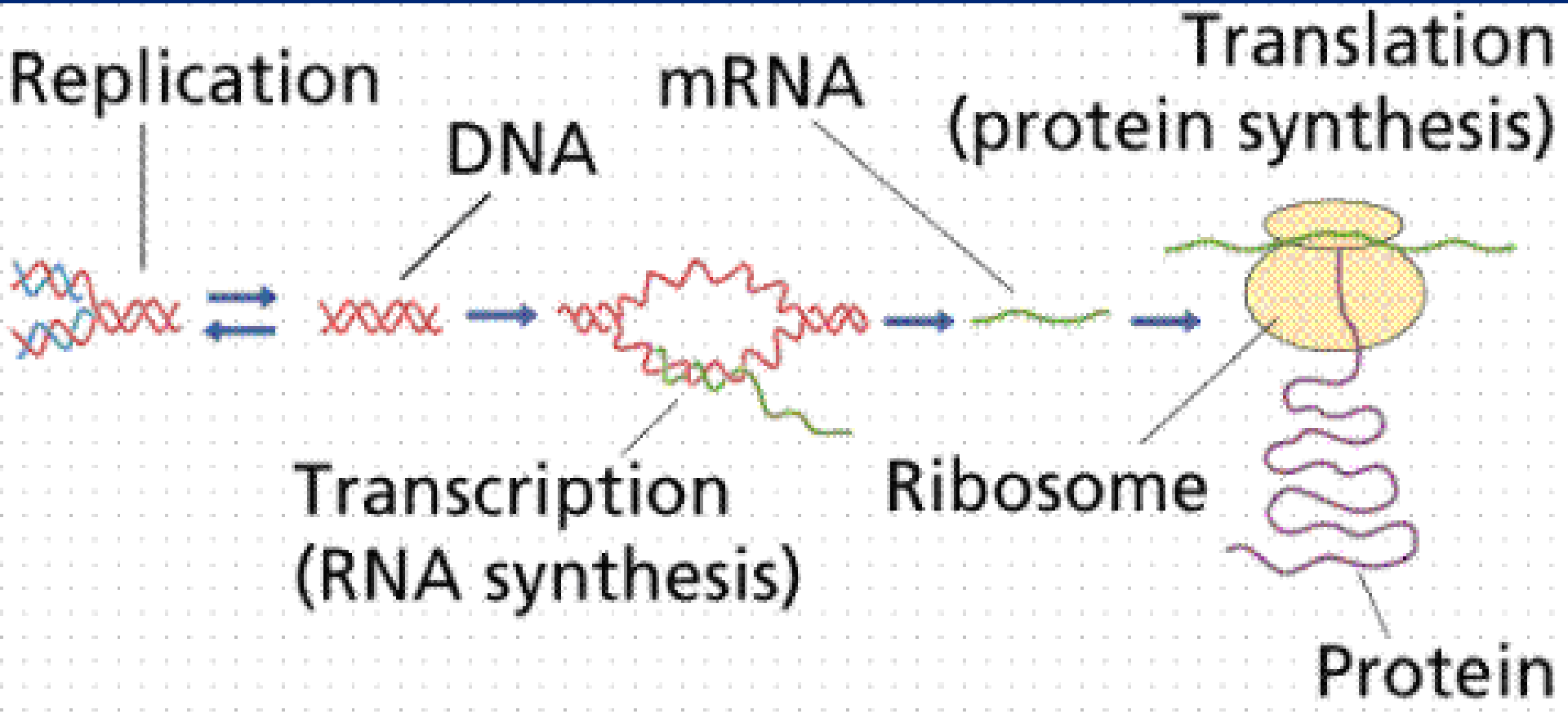
DNA to RNA

creates functional translations

- DNA remains in the nucleus, but in order for it to get its instructions translated into proteins, it must send its message to the ribosomes, where proteins are made. This message is taken by the **Messenger RNA**



Central Dogma of Molecular Biology



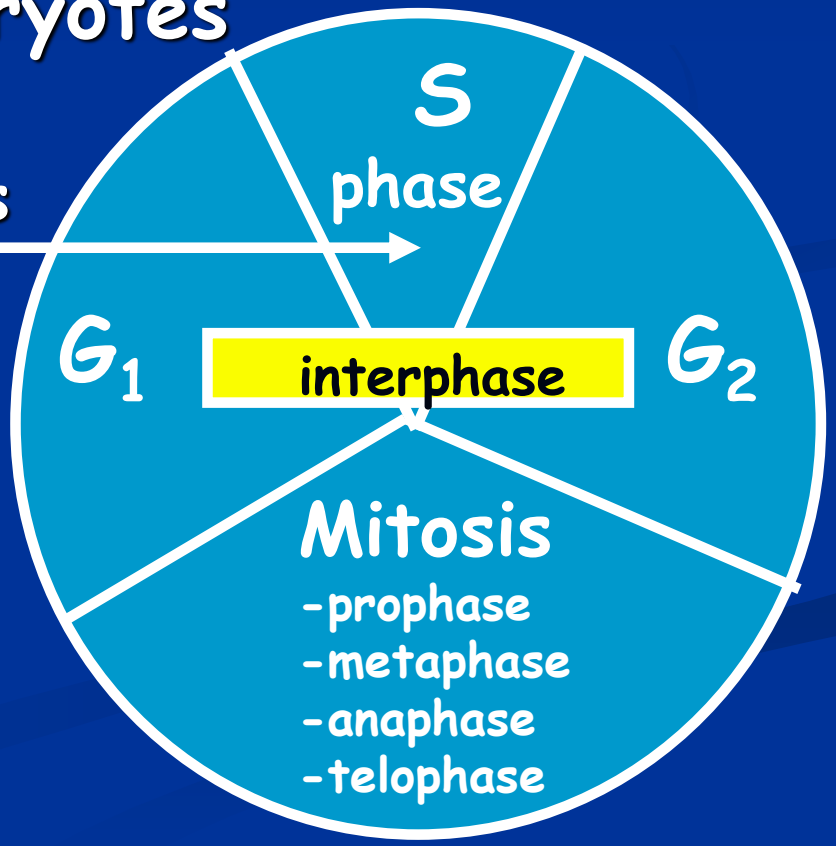
Replication

- Replication is a process in which DNA copies itself to produce identical daughter molecules of DNA

Synthesis Phase (S phase)

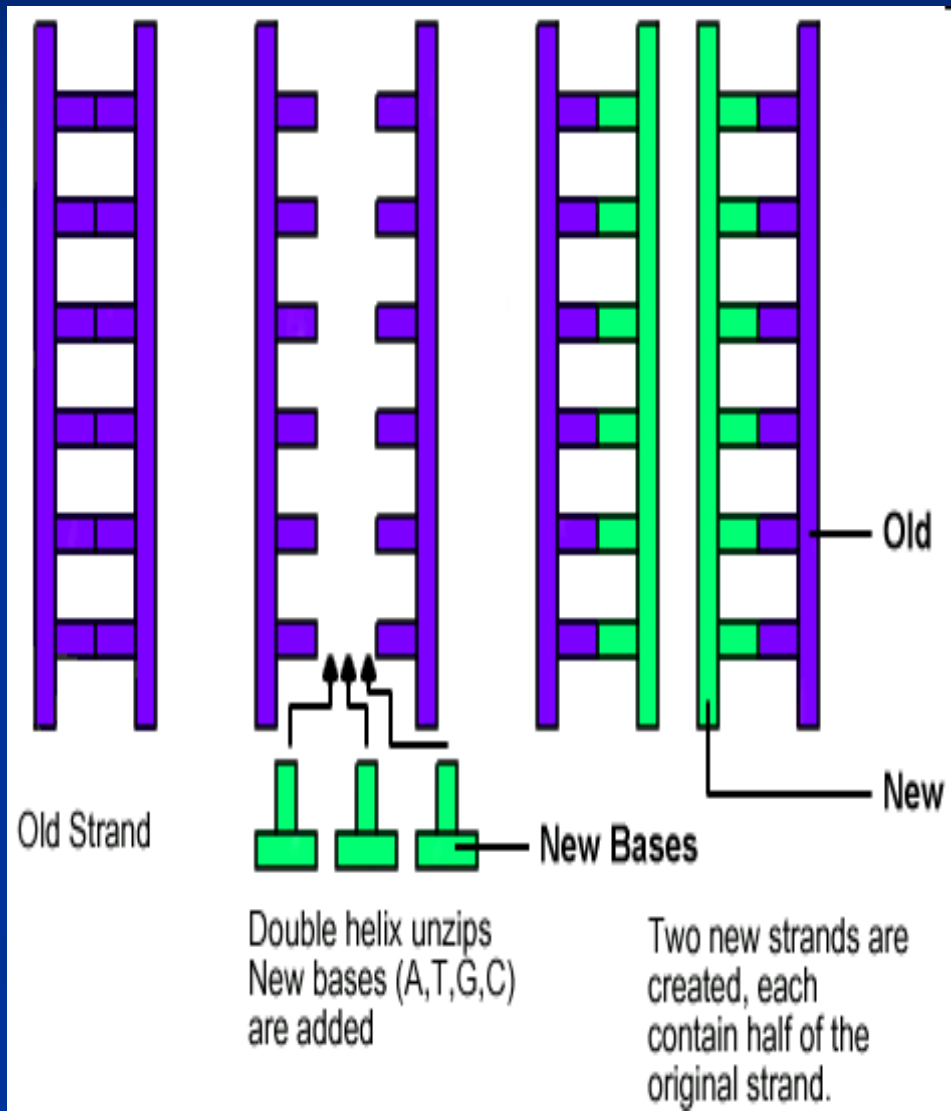
- S phase during interphase of the cell cycle
- Nucleus of eukaryotes

DNA replication takes place in the S phase.



DNA Replication

- DNA replication is **semi-conservative.** That means that when it makes a copy, one half of the old strand is always kept in the new strand. This helps reduce the number of copy errors.



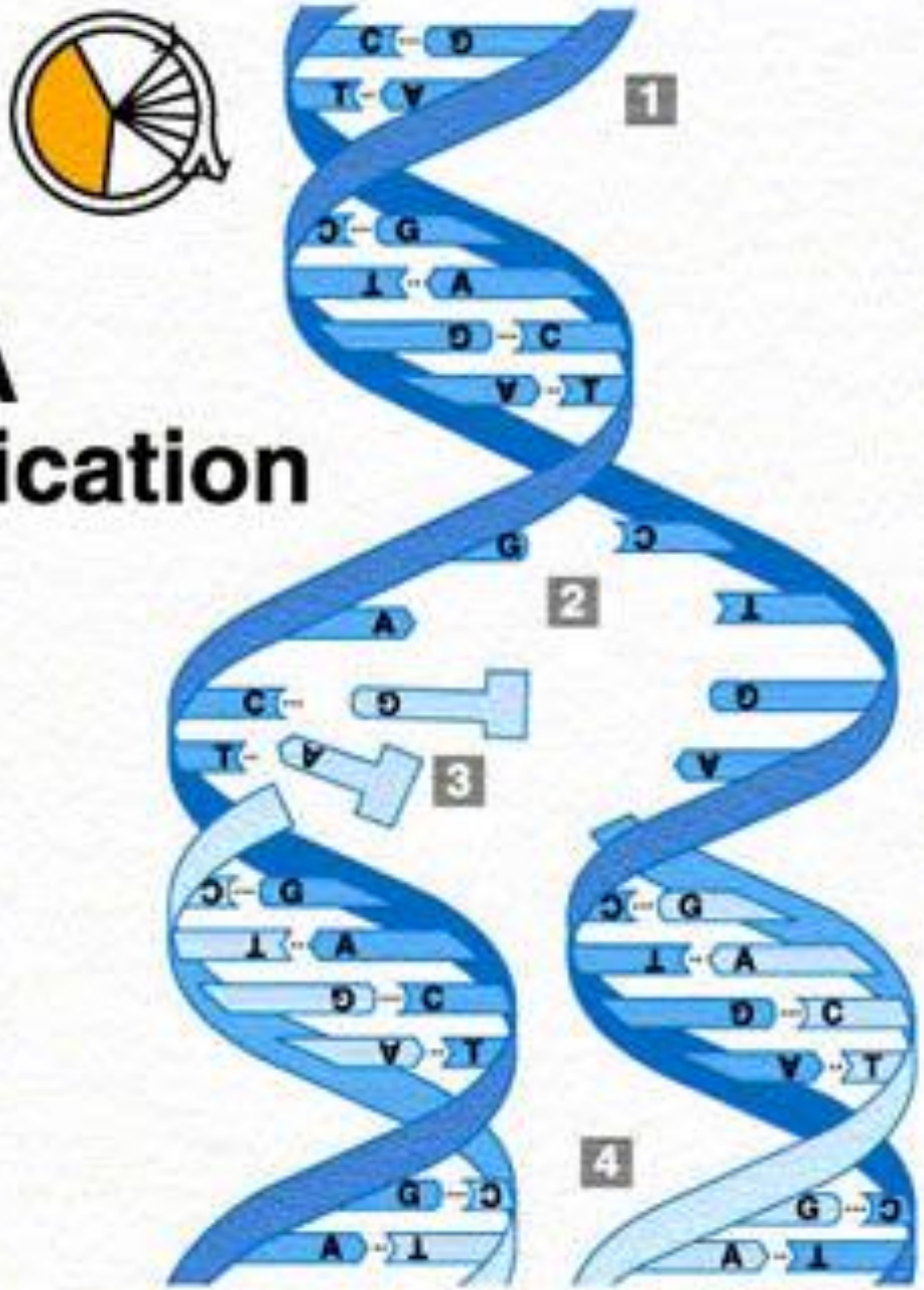
DNA replication

- DNA replication, the basis for biological inheritance, is a fundamental process occurring in all living organisms to copy their DNA.

- In the process of "replication" each strand of the original double-stranded DNA molecule serves as template for the reproduction of the complementary strand.

- Two identical DNA molecules have been produced from a double-stranded DNA molecule.

DNA replication



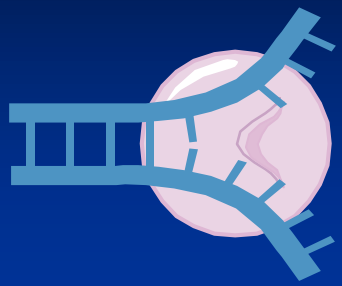
1 Parent DNA molecule; two complementary strands of base-paired nucleotides.

2 Parental strands unwind and separate at several points along the DNA molecule, forming replication forks.

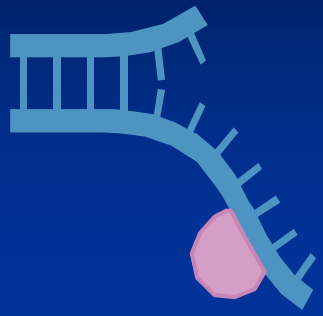
3 Each parental strand provides a template that attracts and binds complementary bases, A with T and G with C.

4 Sugar-phosphate back-bone of daughter strands closed. Each new DNA molecule consists of one parental and one daughter strand, as a result of semiconser-vative replication.

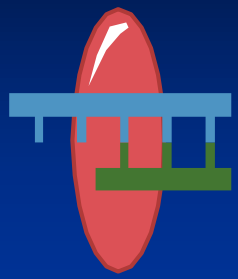
Enzymes in DNA replication



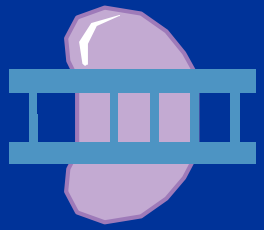
Helicase unwinds parental double helix



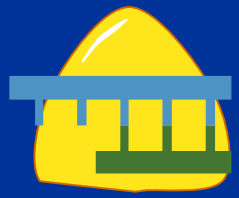
Binding proteins stabilize separate strands



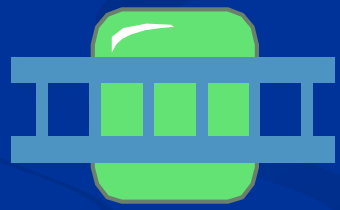
Primase adds short primer to template strand



DNA polymerase III binds nucleotides to form new strands



DNA polymerase I (Exonuclease) removes RNA primer and inserts the correct bases



Ligase joins Okazaki fragments and seals other nicks in sugar-phosphate backbone

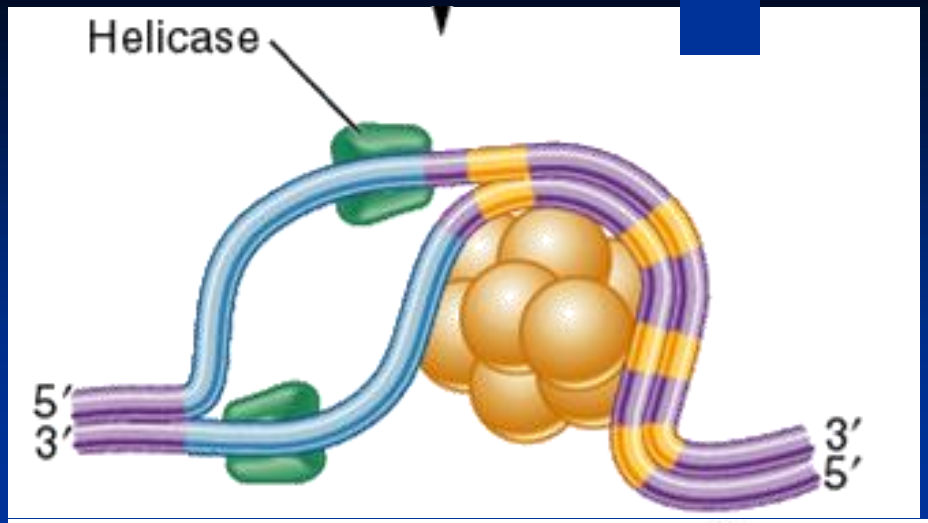
Initiation of Replication

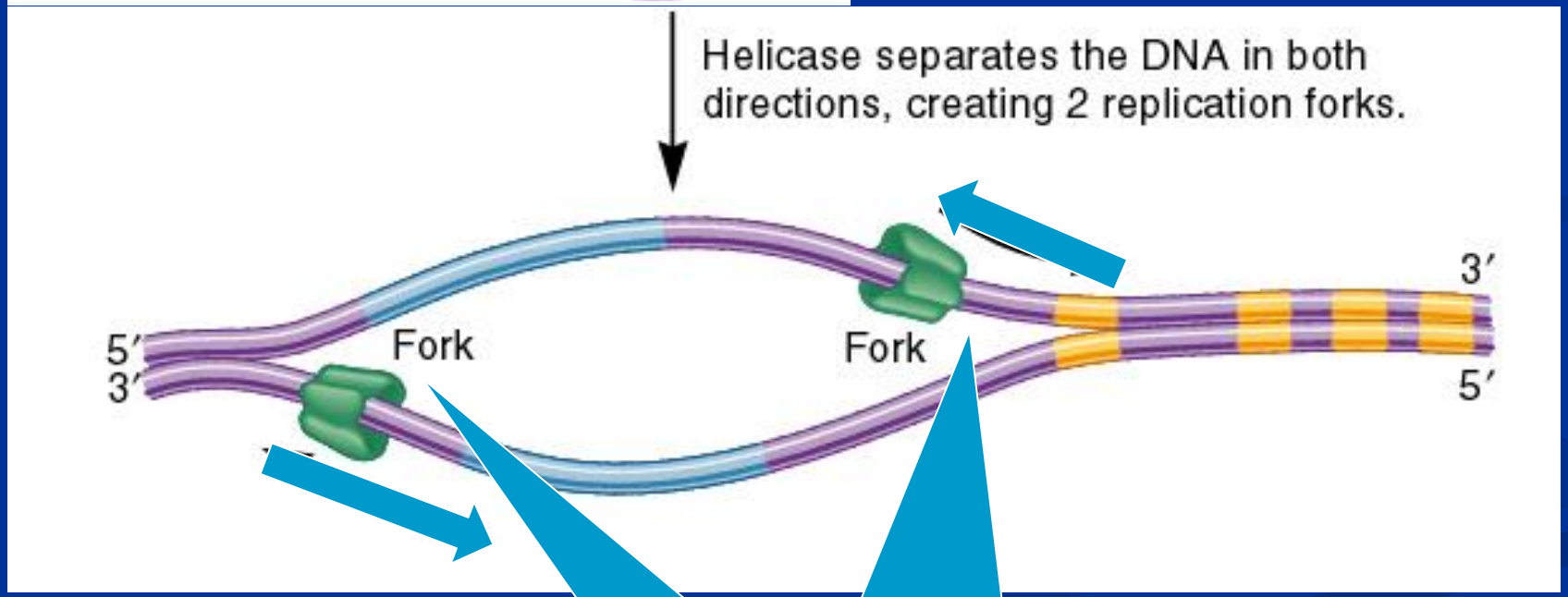
- In order for the two strands of the parental double helical DNA to be replicated, they must first separate (or "melt"), at least in a small region, because the polymerases use only single-stranded DNA as a template.
- In prokaryotic organisms, DNA replication begins at a single, unique nucleotide site called the **origin of replication**
- In Eukaryotes replication begins at multiple sites along the DNA helix. These sites include a short sequence composed almost exclusively of AT base pairs.

Formation of Replication Fork

- As the two strands unwind and separate they form a "V" where active synthesis occurs. This region is called the **replication fork or replication bubble.**
- It moves along the DNA molecule as synthesis occurs.
- Replication of double-stranded DNA is bidirectional-that is, the replication forks move in both directions away from the origin .

Replication Initiation





Bidirectional replication

Direction of DNA Replication

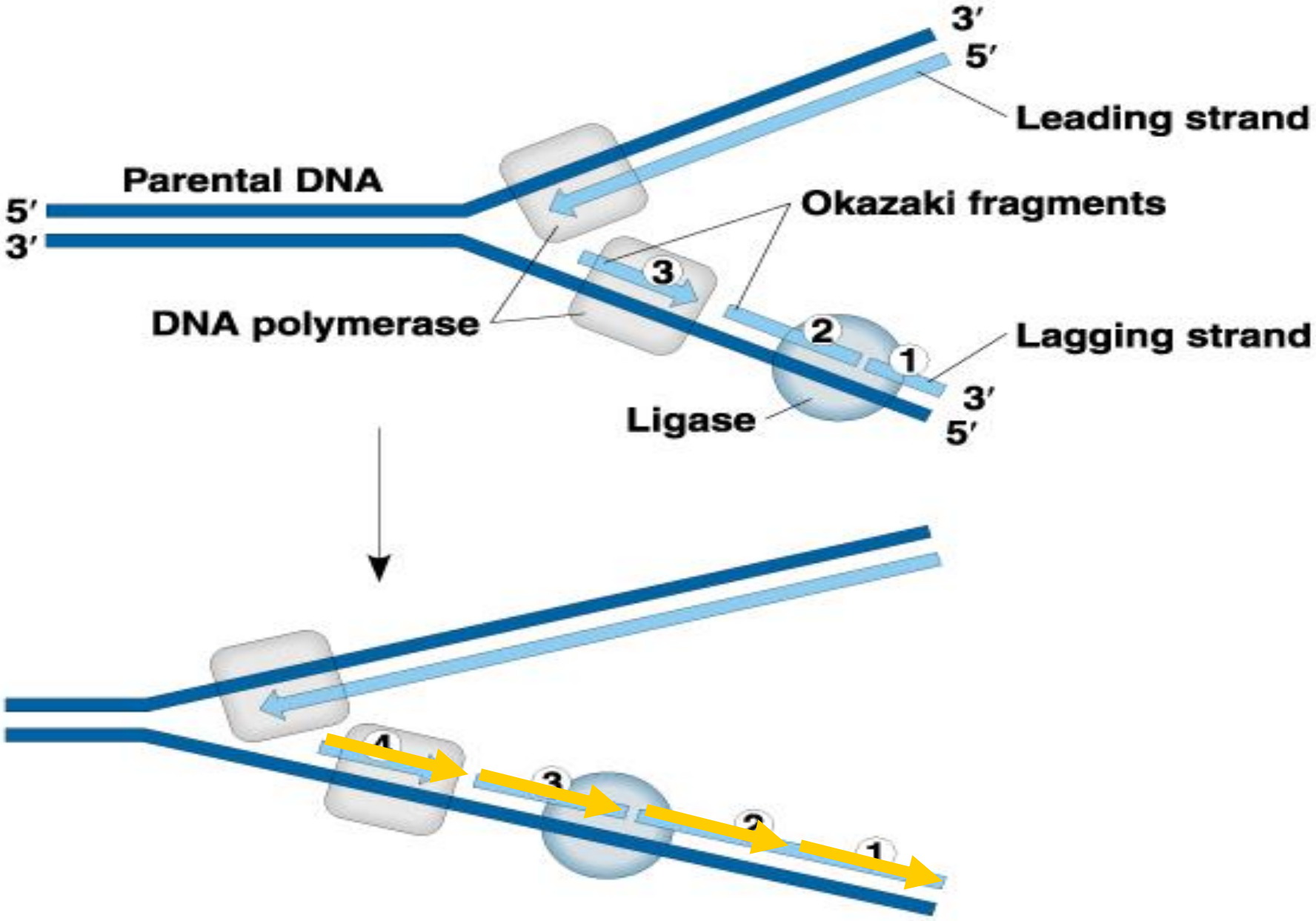
- The DNA polymerases responsible for copying the DNA templates are only able to "read" the parental nucleotide sequences in the 3'-»5' direction, and they synthesize the new DNA strands in the 5'-»3' (anti parallel) direction.
- Therefore, beginning with one parental double helix, the two newly synthesized stretches of nucleotide chains must grow in opposite directions—both in 5'-»3' direction .

Leading strand

- The strand that is being copied in the direction of the advancing replication fork is called the leading strand and is **synthesized almost continuously.**

Lagging Strand

- The strand that is being copied in the direction away from the replication fork is synthesized dis continuously, with small fragments of DNA being copied near the replication fork. These short stretches of discontinuous DNA, termed **Okazaki fragments**, are eventually joined to become a single, continuous strand. The new strand of DNA produced by this mechanism is termed the **lagging strand**.


3′
5′
Leading strand
Parental DNA
5′
3′
Okazaki fragments
3
DNA polymerase
2
1
Lagging strand
3′
5′
Ligase
4
3
2
1

DNA Helicases

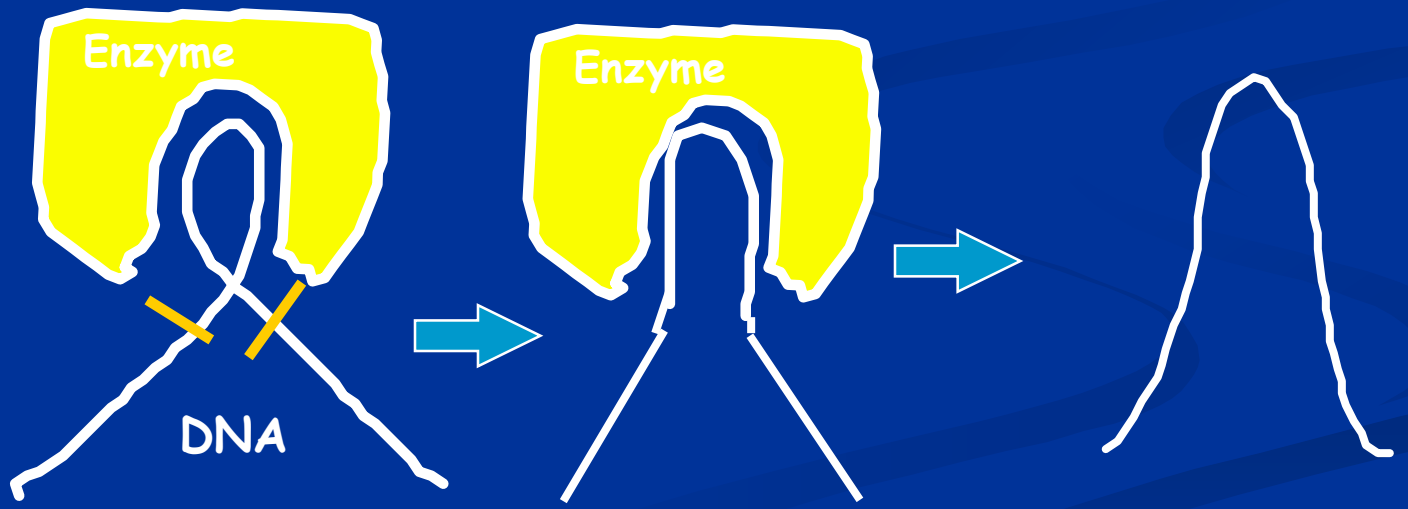
- These enzymes bind to single-stranded DNA near the replication fork, and then move into the neighboring double-stranded region, forcing the strands apart - in effect, unwinding the double helix.
- Helicase require energy provided by ATP.
- When the strands separate, SSB proteins bind, preventing reformation of the double helix

SSBP

- These proteins bind to the ss DNA generated by helicase
- These proteins not only keep the two strands of DNA separated in the area of the replication origin, thus providing the single-stranded template required by polymerases, but also protect DNA from nucleases that cleave single-stranded DNA.

Topoisomerases

- Enzyme **Topoisomerase** attaches to the 2 forks of the bubble to **relieve stress** on the **DNA molecule** as it separates



Super coil and DNA Topoisomerase

- The problem of super coil in the way of DNA replication is solved by DNA Topoisomerases
- **DNA Type I topoisomerases** cuts the single DNA strand to overcome the problem of super coil and then reseal the strand
- **DNAType II topoisomerase** (DNA gyrase) cuts both the strand

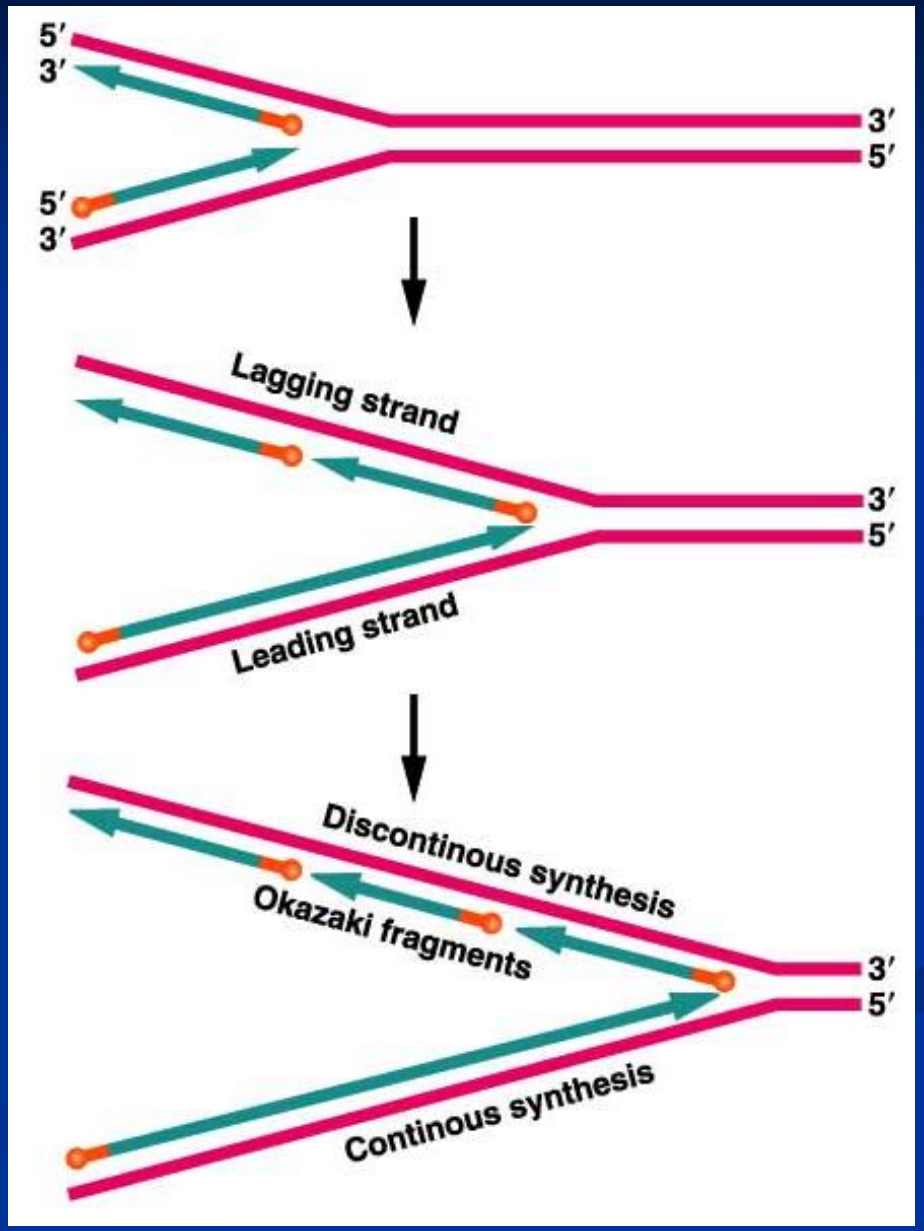
RNA Primer

- DNA polymerases cannot initiate synthesis of a complementary strand of DNA on a totally single-stranded template.
- Rather, they require an RNA primer which is a short region consisting of RNA base-paired to the DNA template, with a free group on the 3'-end of the RNA strand.
- This group serves as the first acceptor of a nucleotide by action of DNA polymerase.

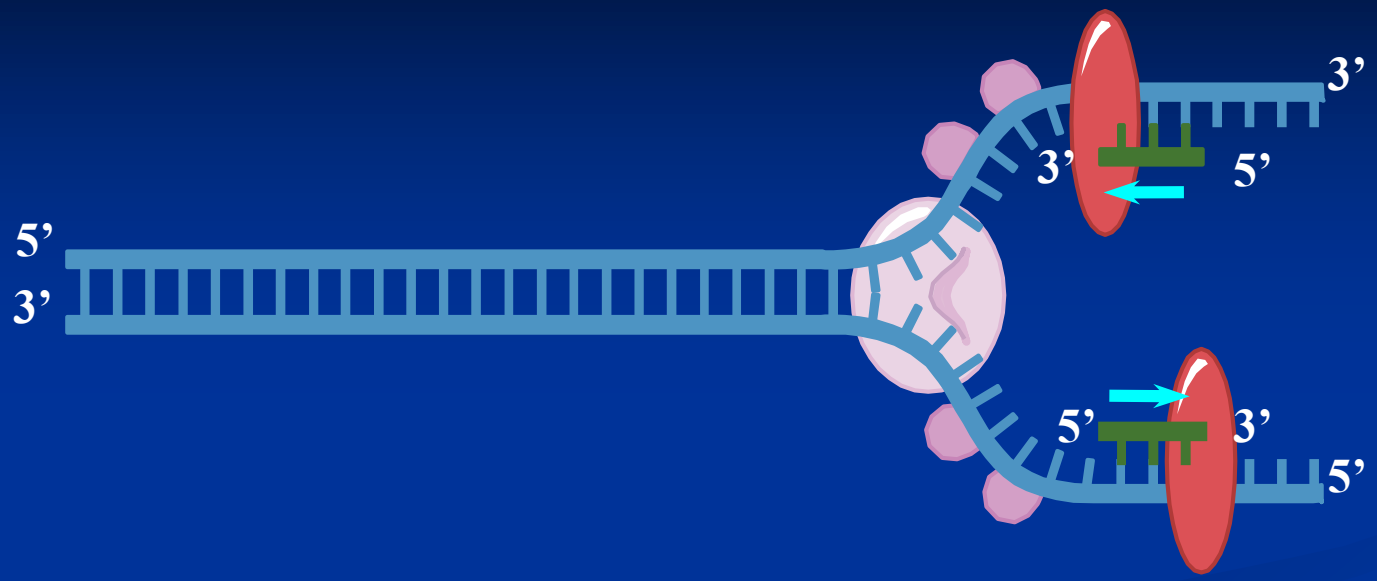
- A specific RNA polymerase, called primase synthesizes the short stretches of RNA (approximately ten nucleotides long) that are complementary and antiparallel to the DNA template.
- The resulting hybrid duplex, the U in RNA pairs with A in DNA.
- These short RNA sequences are constantly being synthesized at the replication fork on the lagging strand, but only one RNA sequence at the origin of replication required on the leading strand.

Okazaki Fragments

- If model is correct, should be able to find lagging strand fragments
- 1000-2000 long DNA fragments
- Discovered by Reiji & Tuneko Okazaki



Replication

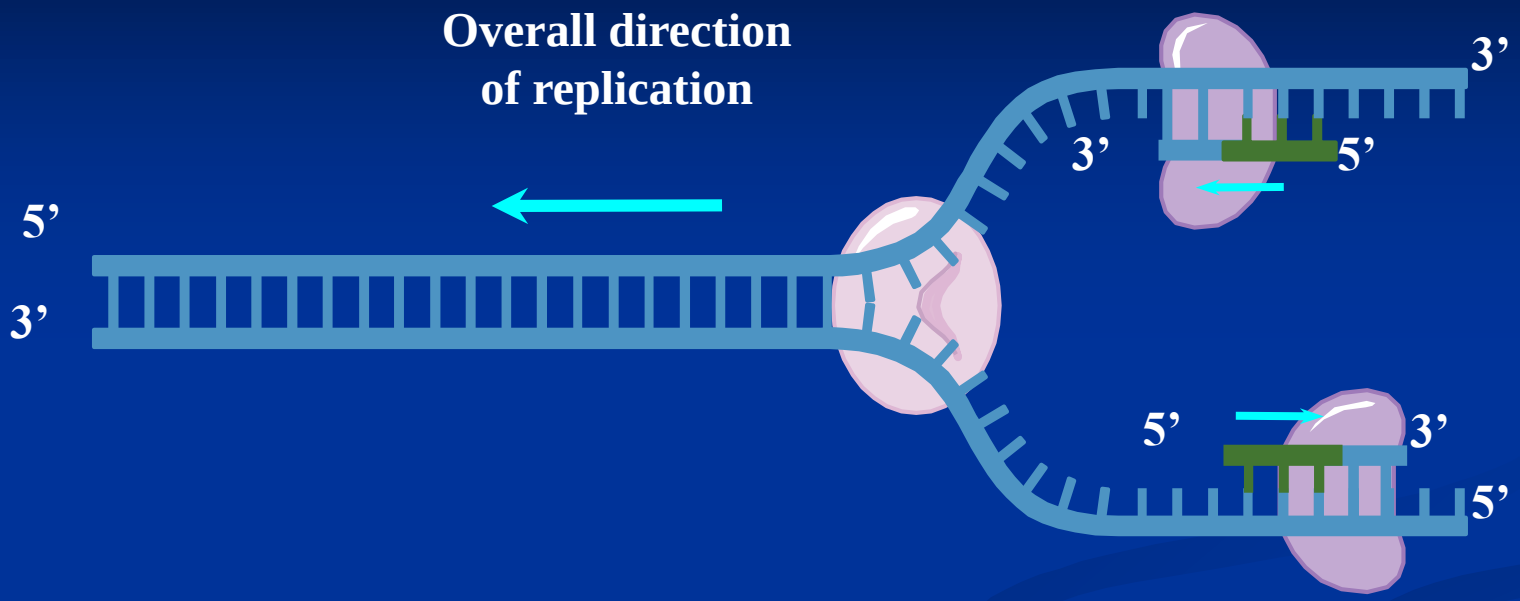


Helicase protein binds to DNA sequences called origins and unwinds DNA strands.

Binding proteins prevent single strands from rewinding.

Primase protein makes a short segment of RNA complementary to the DNA, a primer.

Replication



DNA polymerase enzyme adds DNA nucleotides to the RNA primer.

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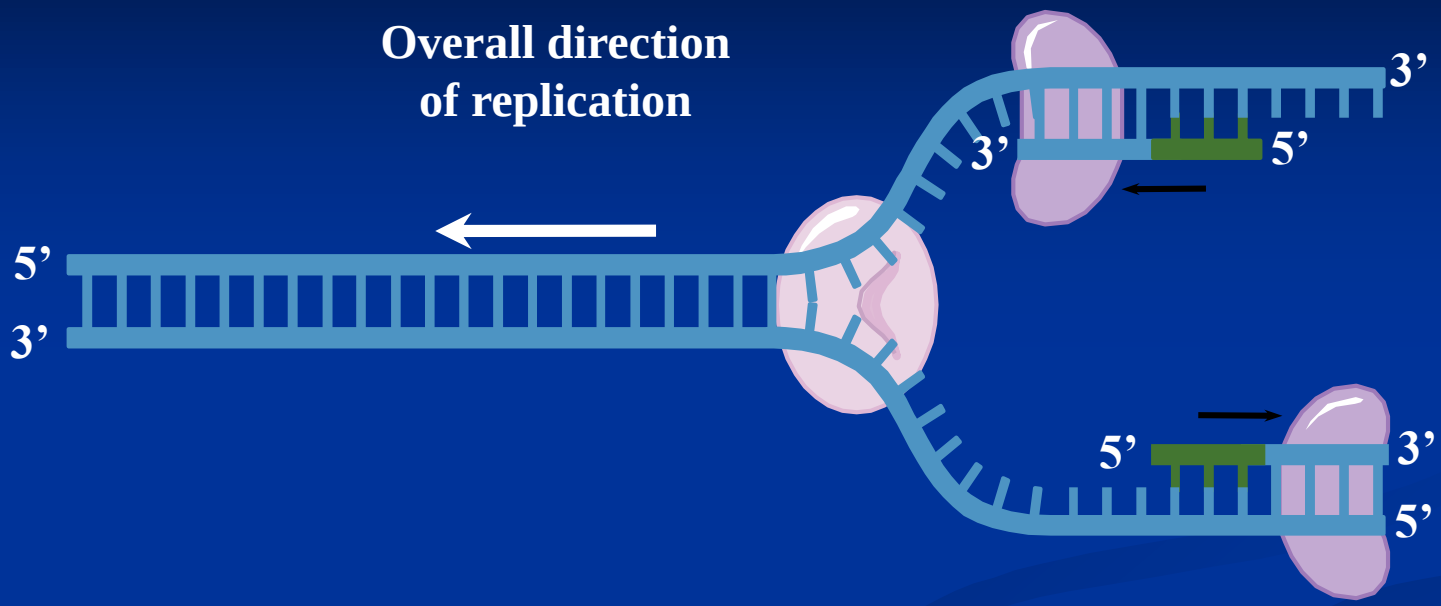
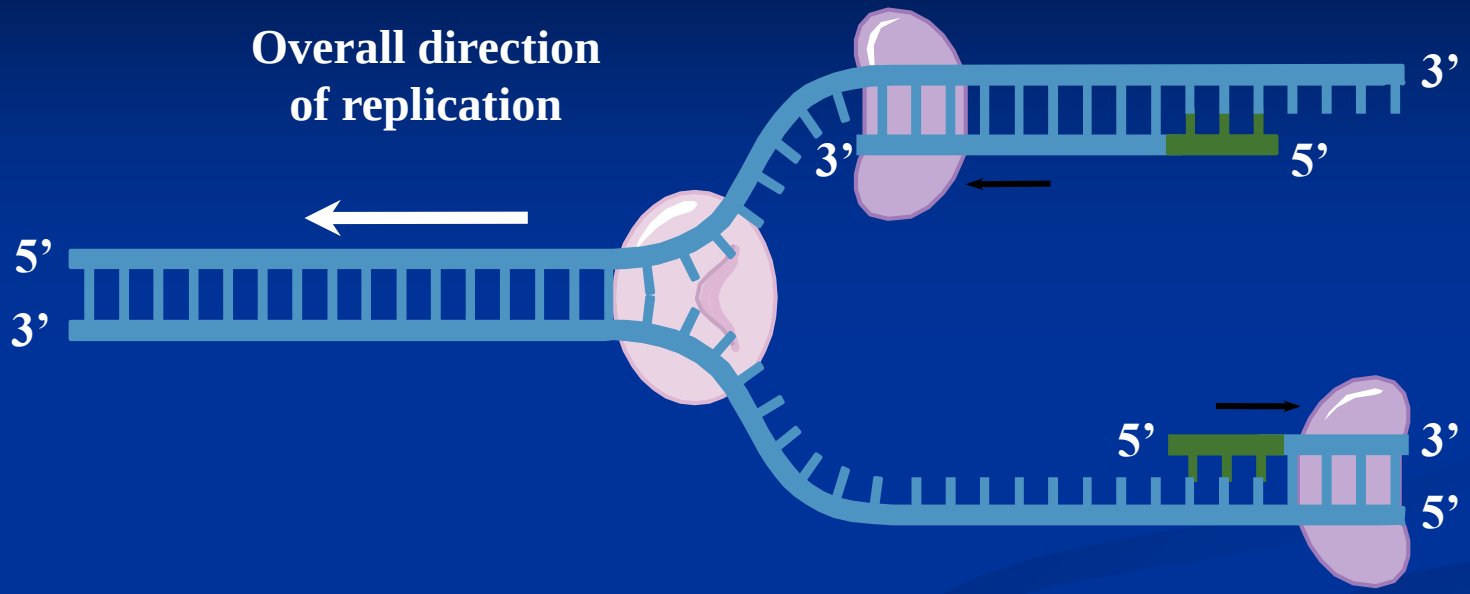


DNA polymerase enzyme adds DNA nucleotides to the RNA primer.

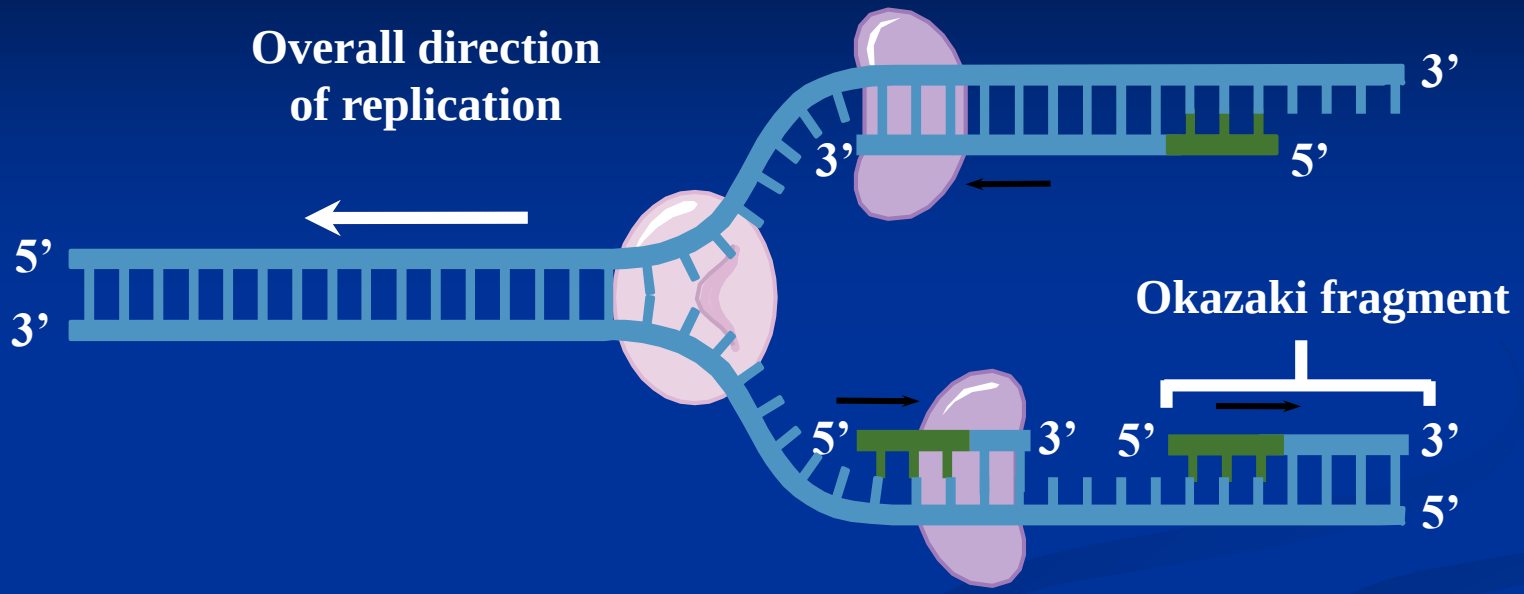
DNA polymerase proofreads bases added and replaces incorrect nucleotides.

Replication



Leading strand synthesis continues in a 5’ to 3’ direction.

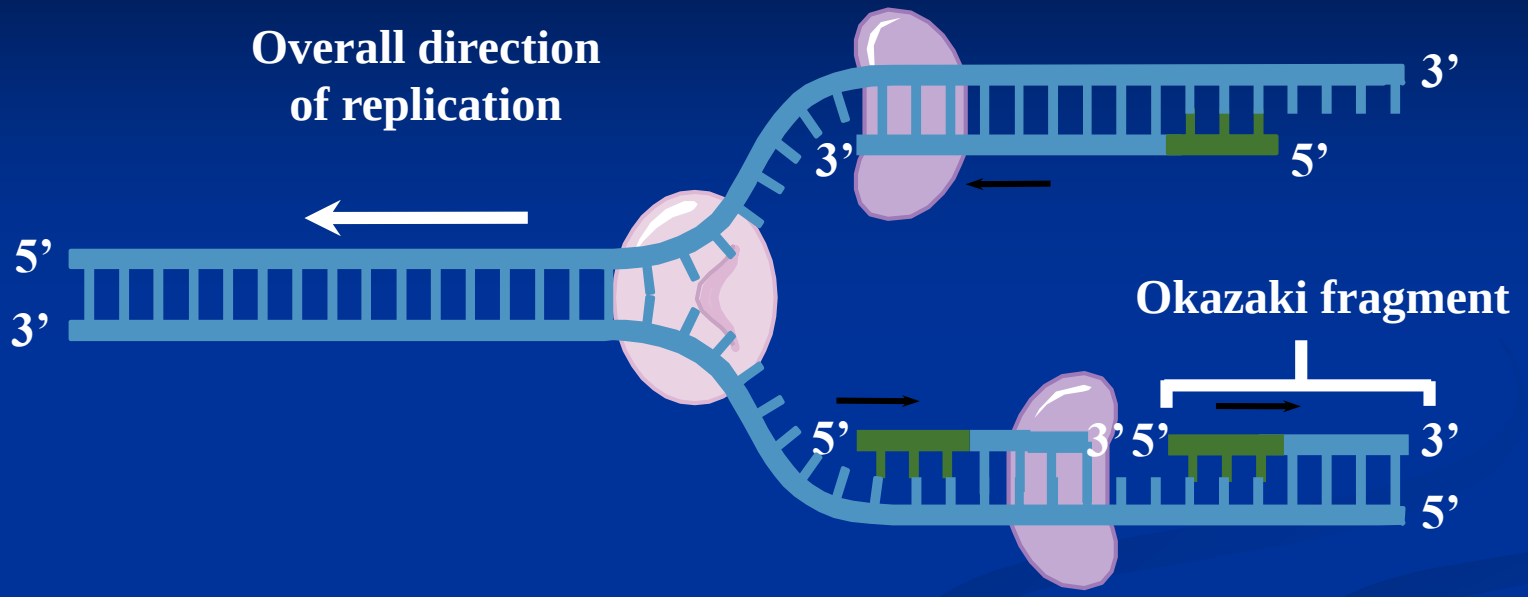
Replication



Leading strand synthesis continues in a 5’ to 3’ direction.

Discontinuous synthesis produces 5’ to 3’ DNA segments called Okazaki fragments.

Replication



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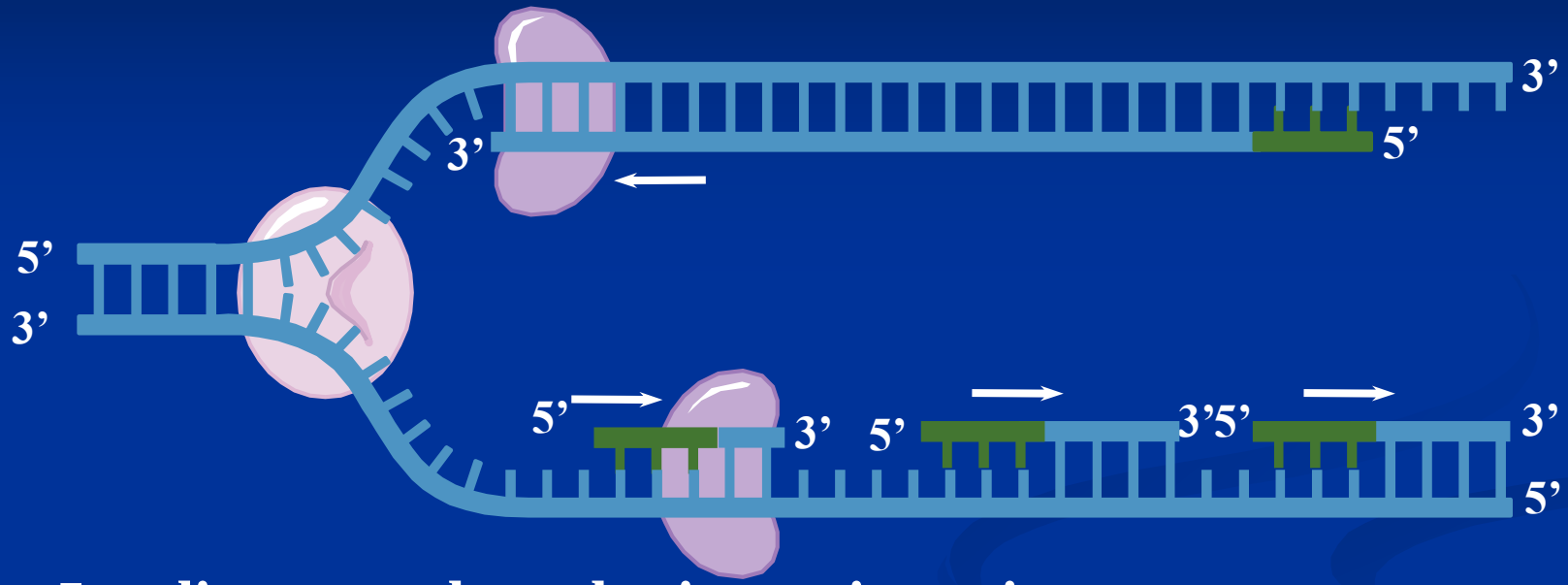

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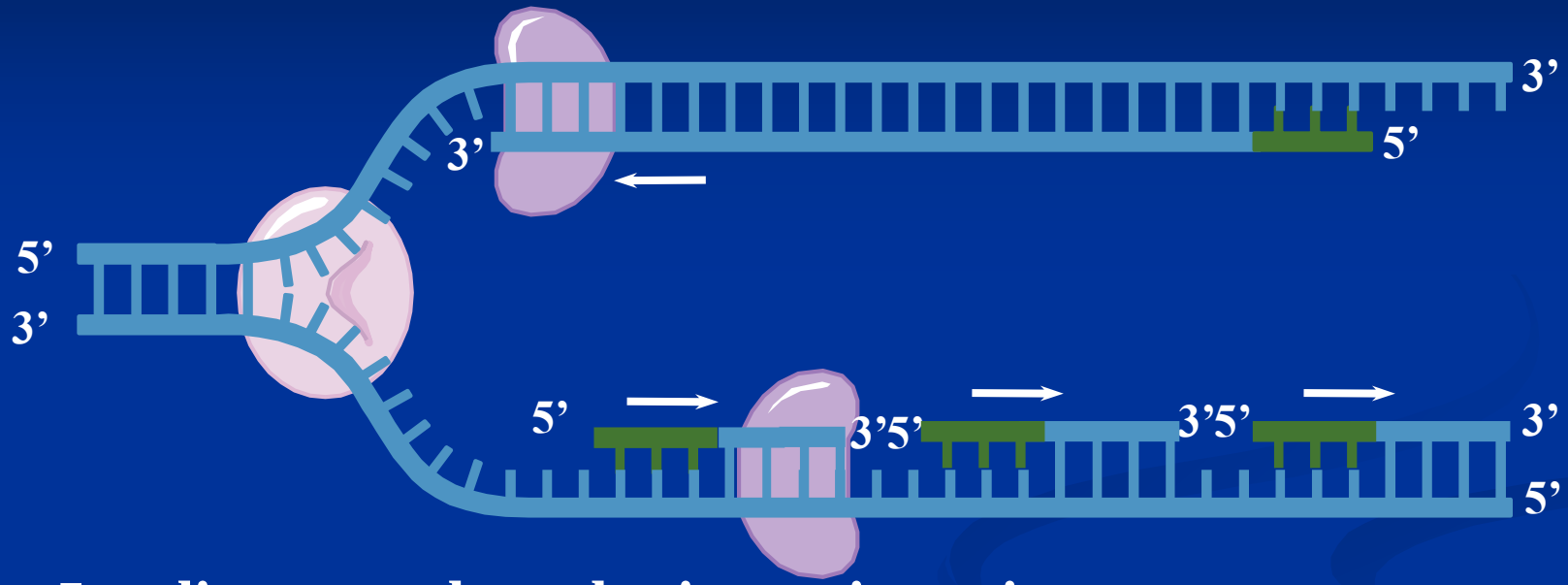

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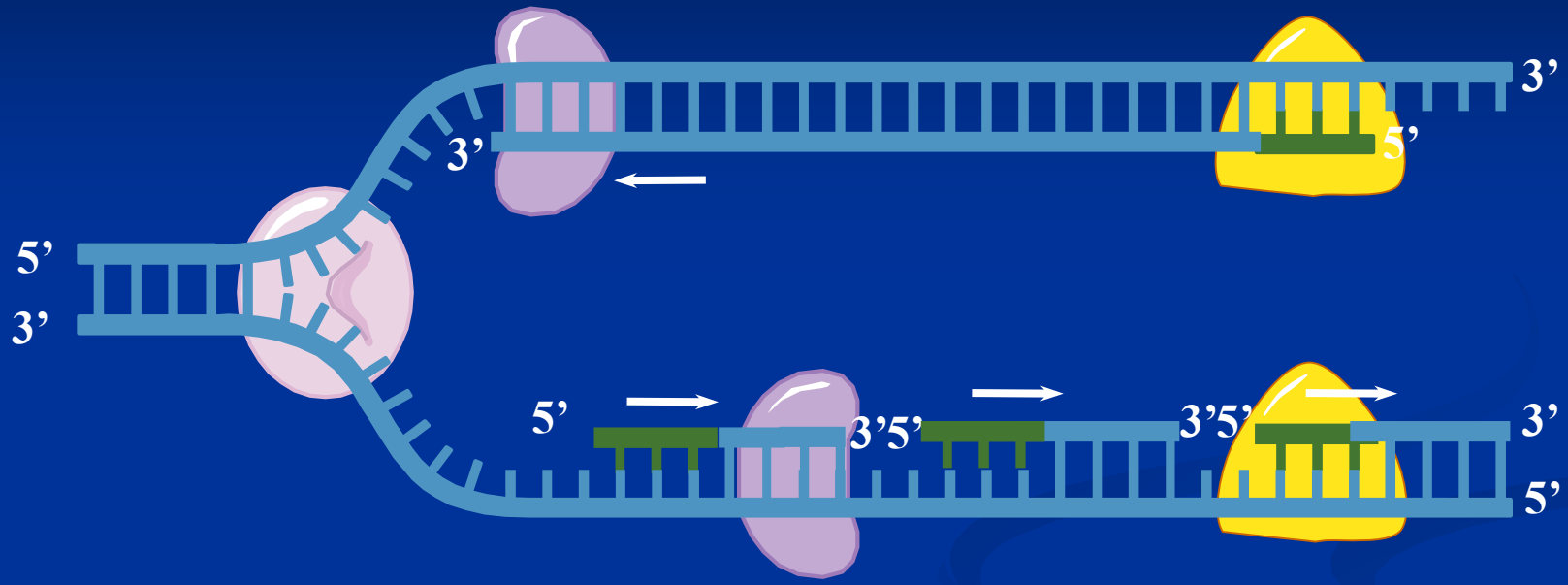
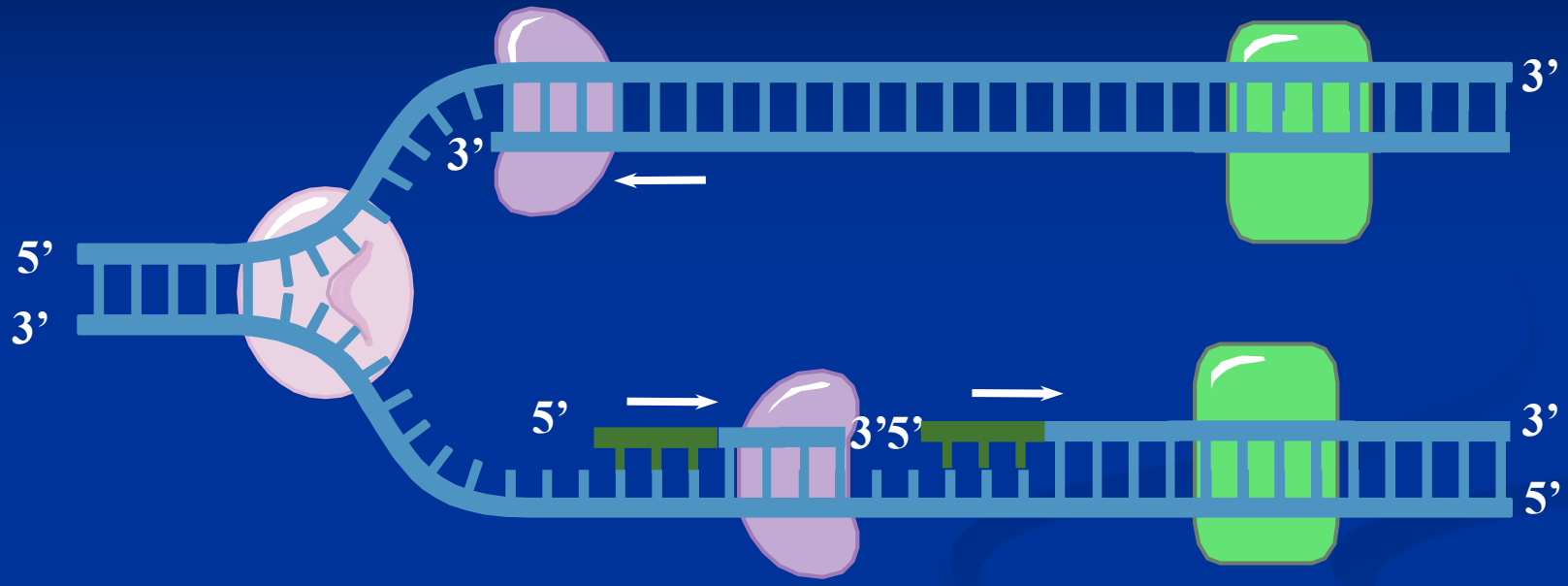


Exonuclease activity of DNA polymerase I removes RNA primers.

Replication



Polymerase activity of DNA polymerase I fills the gaps.

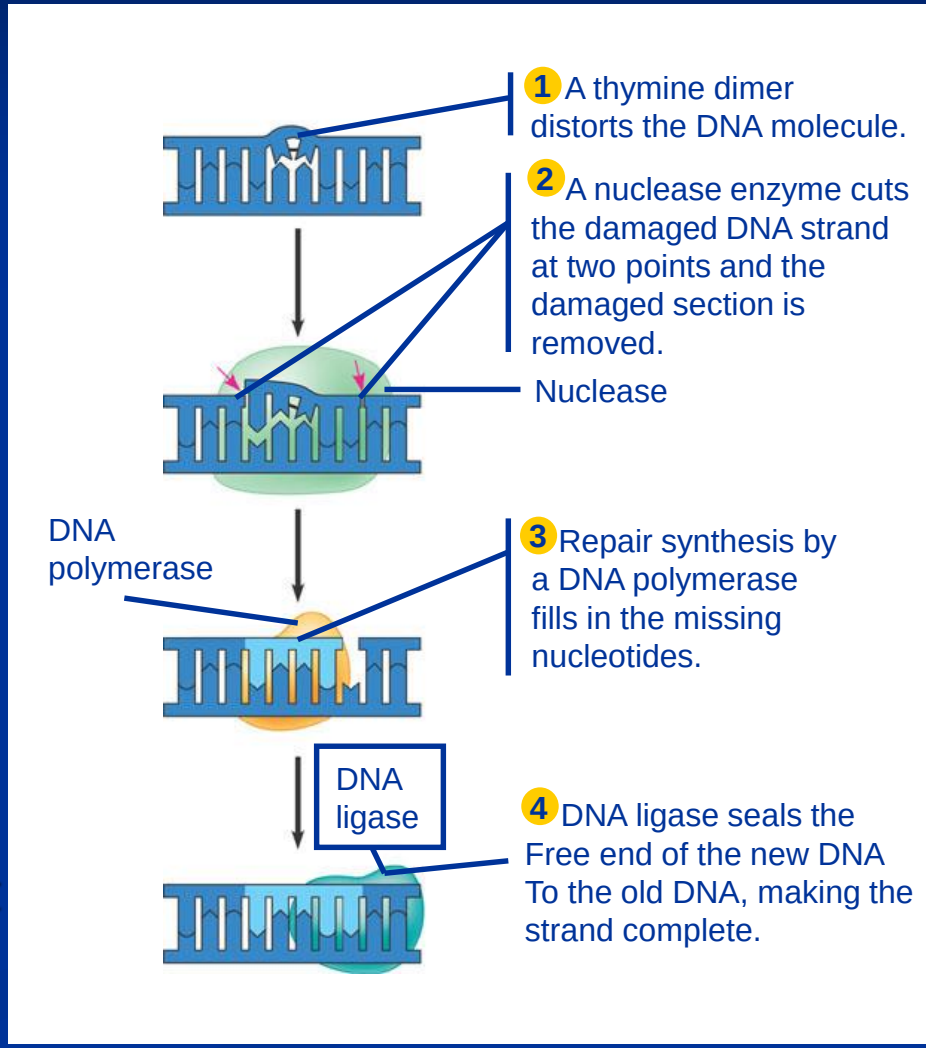
Ligase forms bonds between sugar-phosphate backbone.

Mutations

- A mismatching of base pairs, can occur at a rate of 1 per 10,000 bases.
- DNA polymerase proofreads and repairs accidental mismatched pairs.
- Chances of a mutation occurring at any one gene is over 1 in 100,000
- Because the human genome is so large, even at this rate, mutations add up. Each of us probably inherited 3-4 mutations!

Proofreading and Repairing DNA

- DNA polymerases proofread newly made DNA, replacing any incorrect nucleotides
- In mismatch repair of DNA, repair enzymes correct errors in base pairing
- In nucleotide excision DNA repair nucleases cut out and replace damaged stretches of DNA



Base Excision Repair (BER)

Variety of DNA glycosylases, for different types of damaged bases.

AP endonuclease recognizes sites with a missing base; cleaves sugar-phosphate backbone.

Deoxyribose phosphodiesterase removes the sugar-phosphate lacking the base.

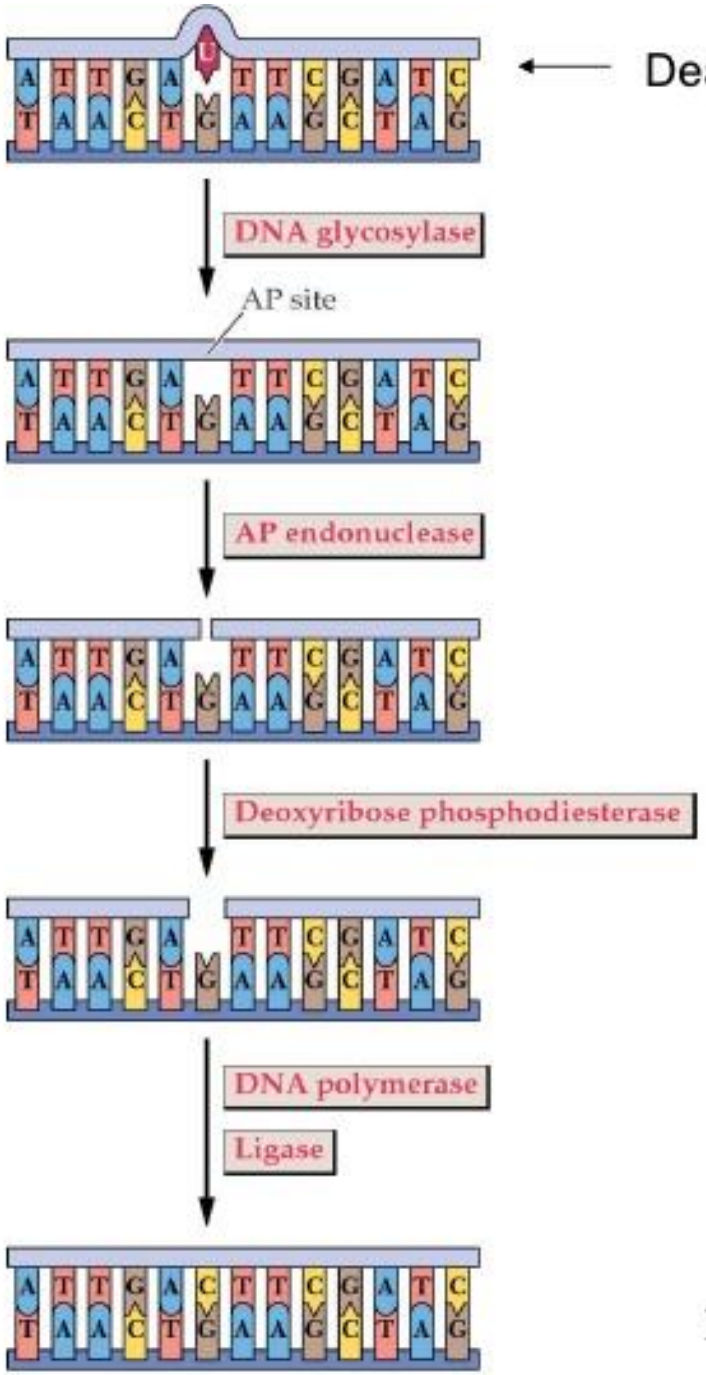


Fig. 6.15

The Genetic code

- The genetic code is a dictionary that identifies the correspondence between a sequence of nucleotide bases and a sequence of amino acids
- Each individual word in the code is composed of three nucleotides bases
- These genetic words are called codons.

- Codons are presented in the mRNA language of adenine (A), Guanine (G), Cytosine (C) and Uracil (U)
- The four nucleotide bases produce 64 different combinations of three base codons
- The nucleotide sequence of codon on mRNA is written from 5’ to 3’ end
- 61 codons code for 20 amino acids found in protein.

Genetic Code- Table

1st letter	Second Letter: U	Second Letter: C	Second Letter: A	Second Letter: G	3rd letter
U	UUU Phe UUC UUA Leu UUG	UCU UCC Ser UCA UCG	UAU Tyr UAC UAA Stop UAG Stop	UGU Cys UGC UGA Stop UGG Trp	U C A G
C	CUU CUC Leu CUA CUG	CCU CCC Pro CCA CCG	CAU His CAC CAA Gln CAG	CGU CGC Arg CGA CGG	U C A G
A	AUU AUC Ile AUA AUG Met	ACU ACC Thr ACA ACG	AAU Asn AAC AAA Lys AAG	AGU Ser AGC AGA Arg AGG	U C A G
G	GUU GUC Val GUA GUG	GCU GCC Ala GCA GCG	GAU Asp GAC GAA Glu GAG	GGU GGC Gly GGA GGG	U C A G

Non Sense Codon

- Three of the codons, UAG, UGA and UAA do not code for amino acids and are called termination codon
- When one of these codons appear in mRNA sequence, it signals synthesis of the protein coded for by that mRNA is completed
- AUG codes for methionine which is the first amino acid in the poly peptide ,known as the start codon.

Characteristics of Genetic Code

- Specificity (unambiguity)
- Universality
- Degeneracy
- Non overlapping
- Comma less ness

Specificity

- A particular codon always codes for the same amino acid
- For example: UGG is a code specified for Tryptophan

Universality

- The same codons are used to code for the same amino acids in all living organisms.
- Thus genetic code has been conserved during the course of evolution.
- Exception to universality is found in mitochondrial genome where ‘AUA’ codes for methionine instead of isoleucine & UGA for tryptophan instead of stop codon.

Degeneracy

- Degeneracy means having multiple domain elements corresponding to one element.
- Most of the amino acids have more than one codon
- The codon is degenerate since there is 61 codons for 20 amino acids
- The codons that designate the same amino acids are called **synonyms.**

Degeneracy

- Often the base of the third position is insignificant meaning the codons representing the same amino acid differ in the third base ,e.g; UCU,UCC, UCA, UCG all code for serine.
- The reduced specificity of the last position is known as **“third base degeneracy or wobbling phenomenon”.**
- This feature minimizes the effects of mutations.

Non-overlapping

- All codons are independent set of 3 bases, read from a fixed point as a continuous base sequence.
- There is non-overlapping, i.e.no base functions as a common member of two consecutive codons.
- For example: UUUCUUAGA is read as UUU/CUU/AGA/
- Addition or deletion of one or two bases will change the message sequence in mRNA

Comma less ness

- Codons are arranged in a continuous structure.
- There is not one or more nucleotides between consecutive codons.
- The last nucleotide of preceding codon is immediately followed by the first nucleotide of the succeeding nucleotide.