Replication

Replication

- **DNA replication** is the biological process of producing two identical replicas of DNA from one original DNA molecule.

1. DNA replication occurs during the S-stage of interphase

2. DNA replication begins at specific locations, or origins of replication, in the genome

3. During replication, these strands are separated. Each strand of the original DNA molecule then serves as a template for the production of its counterpart, a process referred to as semiconservative replication

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4. In general, DNA polymerases are highly accurate, with an intrinsic error rate of less than one mistake for every 10^7nucleotides added

5. The mutation rate per base pair per replication during phage T4 DNA synthesis is 1.7 per 10^8

6. 1000 to 100000 replication each cycle

7. 3.4 minute embryonic cell while in 10 h Drosophilla

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Replication in Prokaryotes

Initiation

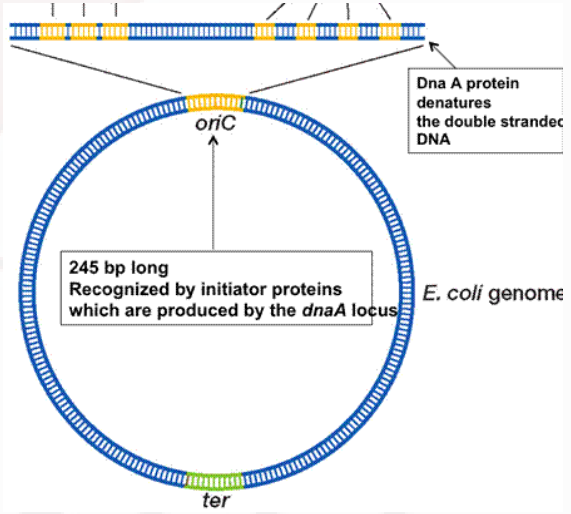
Origin C: **DnaA-ATP** complex bind inverted repeat region of origin C (9x4)

GATCTNTTNTTTT
(13MER x 3)

TTATCCACA (9MER)

- Promote opening of DNA from 13 bp sequence region(13x3)

- HU protein required for this step



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Initiation

- This process is initiated at particular points in the DNA, known as "origins", which are targeted by initiator proteins.

- In *E. coli* this protein is

1. DnaA: recognize origin

2. DnaC protein + DnaB approach

   DnaC protein" recognice DnaA

   Dna B/helicase break H bonds form Replication fork

3. SSB protein bind prevent recoiling

4. Topoisomerase II DNA gyrase – reduce torsion

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DNA Helicase enzyme

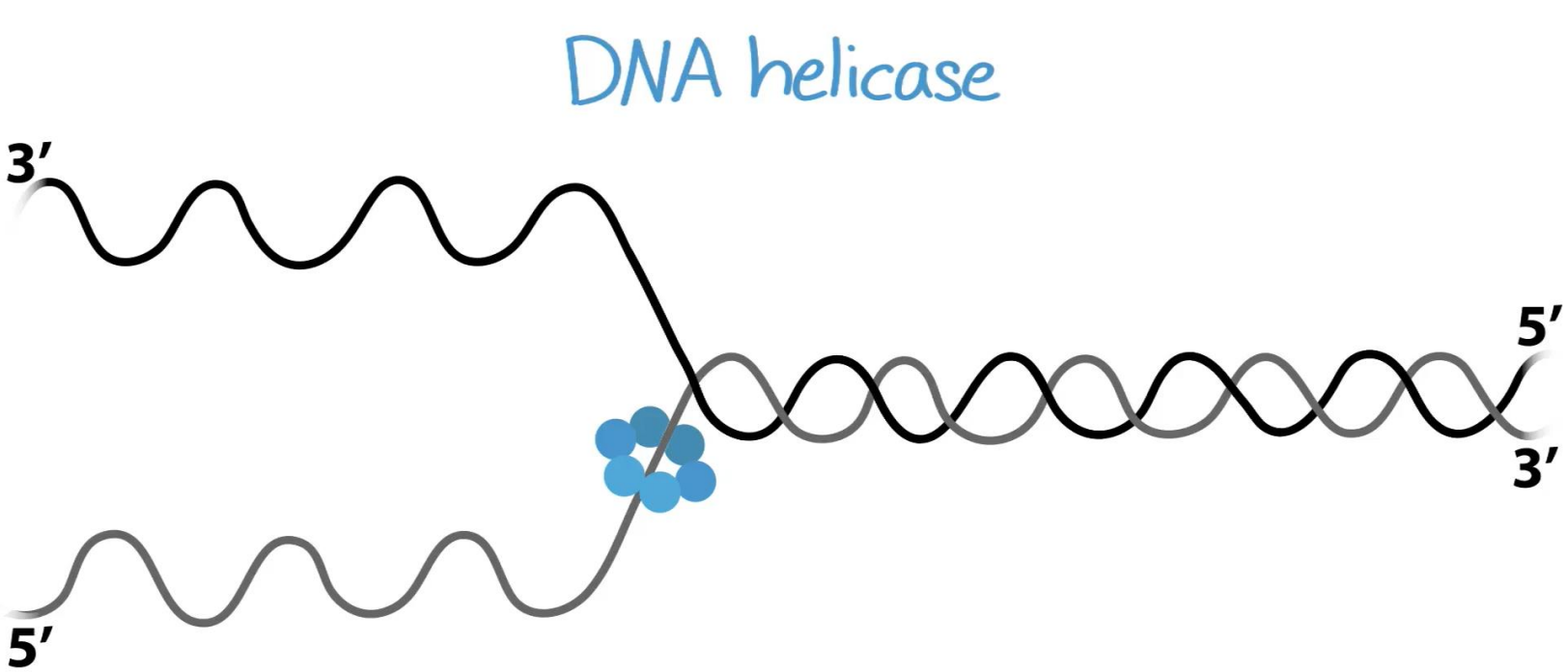
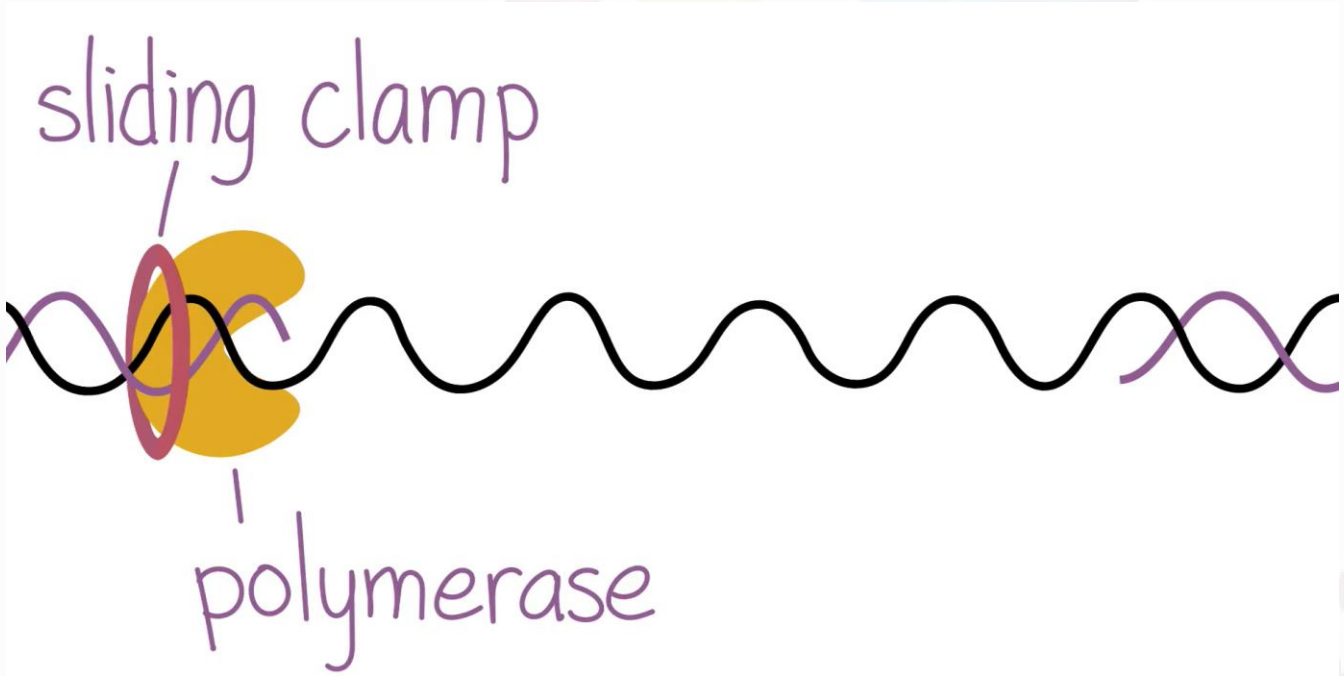


Diagram was redrawn from E.dx. Replication course (Massachusetts Institute of Technology (MIT)

DNA Polymerase enzyme bound with sliding clamp



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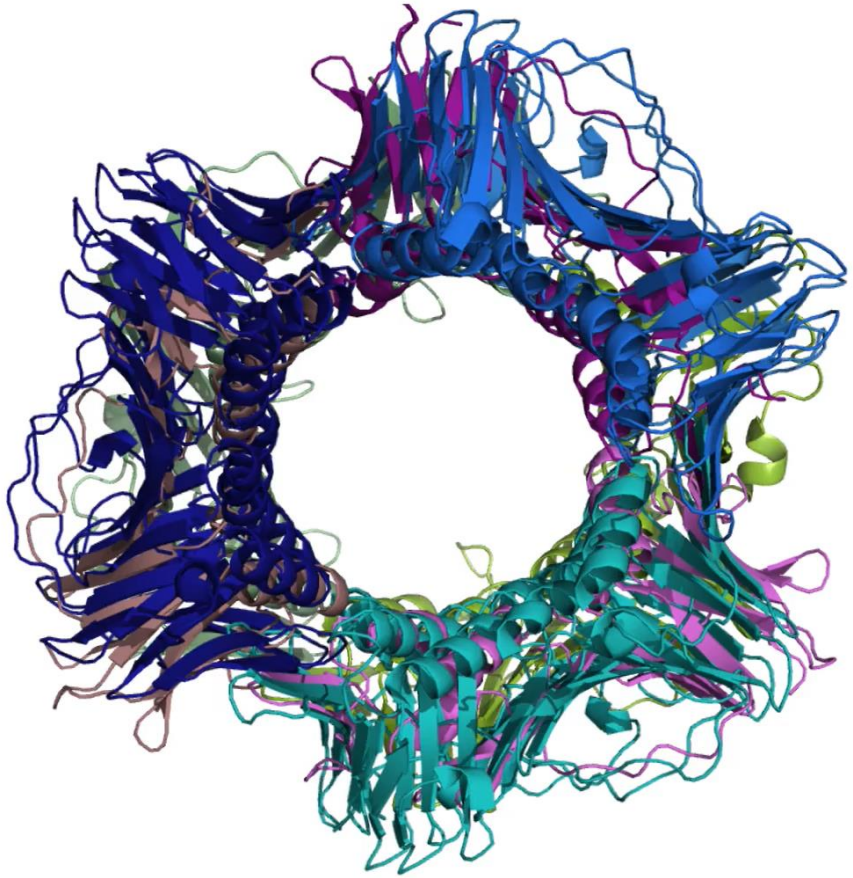
Subunit polymerization of sliding clamp

Diagram was redrawn from E.dx. Replication course (Massachusetts Institute of Technology (MIT)



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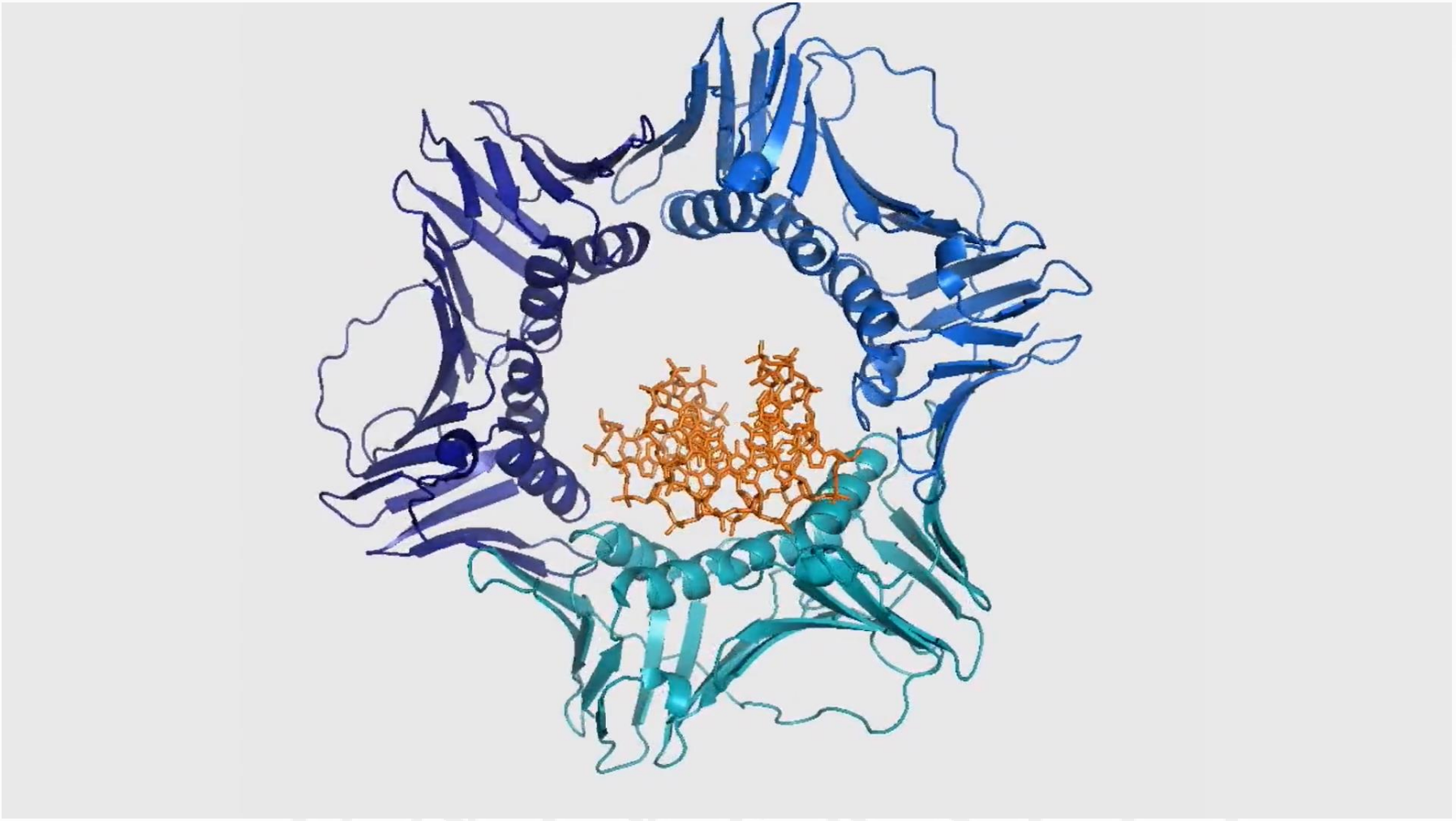

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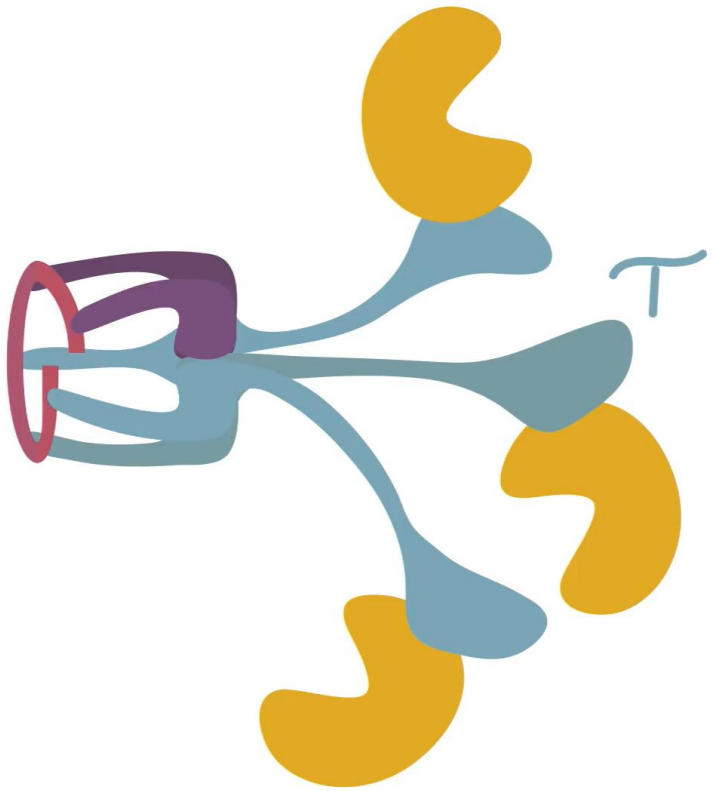
Sliding Clamp Bounded DNA Polymerase Enzyme



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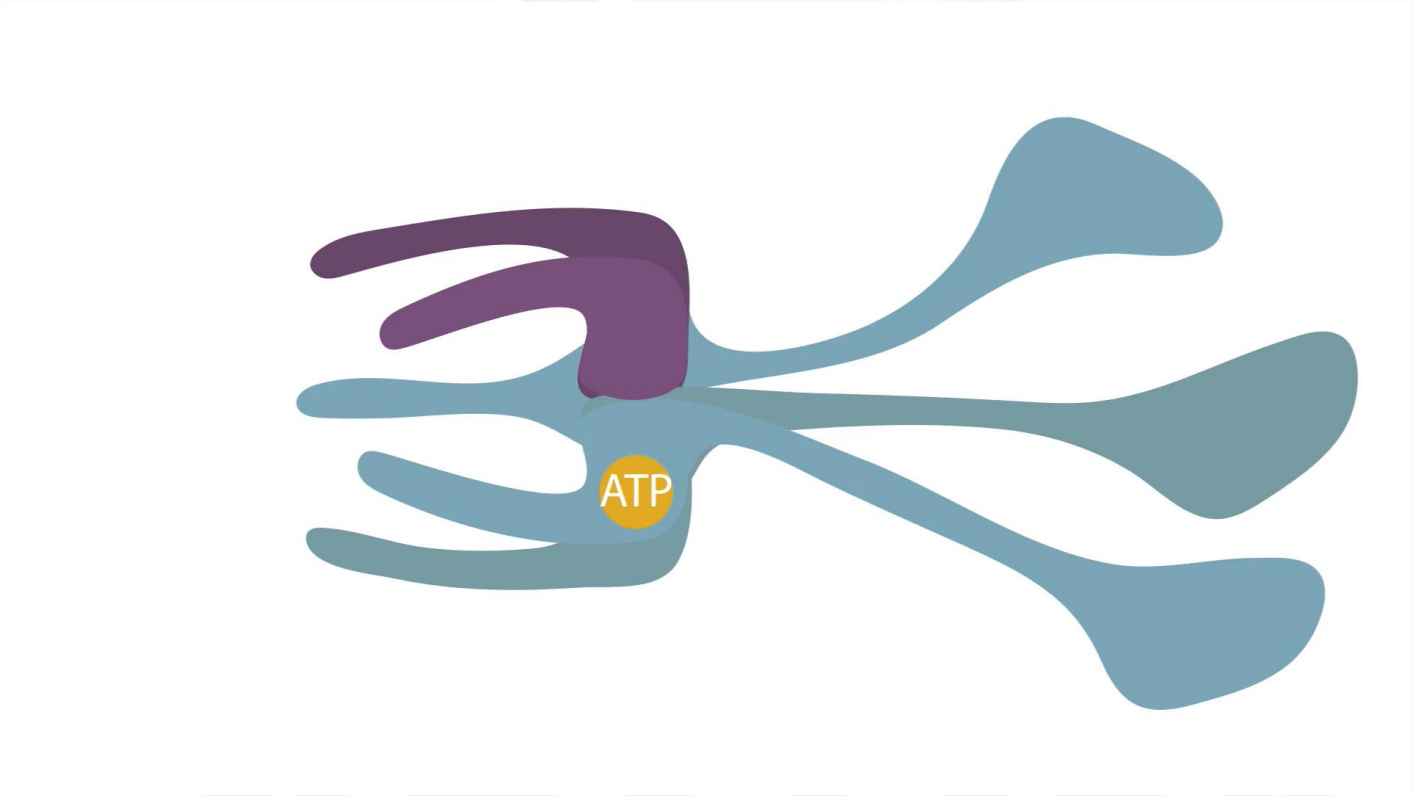
ATP Bind with DNA Polymerase enzyme



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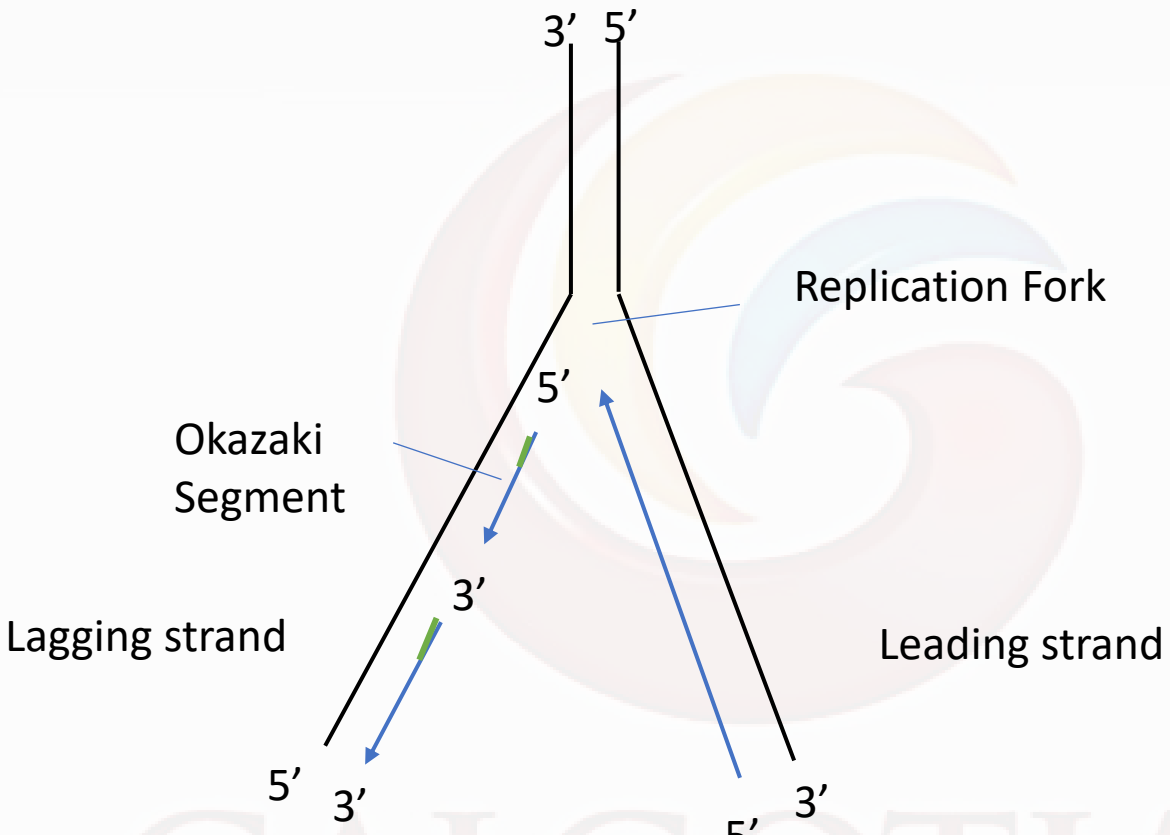

Ray diagram showing Mechanism of Replication

- **Okazaki fragments**, named for their discovery in the 1960s by Reiji and Tsuneko Okazaki

- They are short, newly synthesized DNA fragments that are formed on the lagging template strand during DNA replication.

- Okazaki fragments are between 1000 and 2000 nucleotides long in prokaryotes (e.g. *Escherichia coli*) and are roughly 100 to 200 nucleotides long in eukaryotes.

- They are separated by ~12-nucleotide RNA primers

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- DNA Pol I is the enzyme responsible for replacing RNA primers with DNA

- Primosome primase + helicase

- Repliosome helicase primase polymerase

- Bidirectional replication

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Elongation

- RNA Primer add
- DNA Polymerase III: add deoxyribonucleotide
- DNA Polymerase I: proofreading
- DNA Ligase: seal nick by forming phosphodiester bond

Termination: Ter sites where termination occur.

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History of molecular discoveries

1. **1978** – Discovery of the first eukaryotic DNA helicases, isolated from the lily plant.
2. **1982** – "T4 gene 41 protein" is the first reported bacteriophage DNA helicase
3. **1985** – First mammalian DNA helicases isolated from calf thymus
4. **1986** – SV40 large tumor antigen reported as a viral helicase (1st reported viral protein that was determined to serve as a DNA helicase)
5. **1986** – ATPaseIII, a yeast protein, determined to be a DNA helicase
6. **1988** – Discovery of seven conserved amino acid domains determined to be helicase motifs
7. **1989** – Designation of DNA helicase Superfamily I and Superfamily II
8. **1989** - Identification of the DEAD box helicase family
9. **1990** - Isolation of a human DNA helicase
10. **1992** – Isolation of the first reported mitochondrial DNA helicase (from bovine brain)
11. **1996** – Report of the discovery of the first purified chloroplast DNA helicase from the pea

Replication in Eukaryotes

Origin in Replication Eukaryotes

- Origin contain 2 part
- 1. core component
- Auxiliary component

- Core component: It has 3 region 1. ORE, 2. DUE, 3. A/T
- ORE (origin recognition complex)
- DUE: DNA unbinding element

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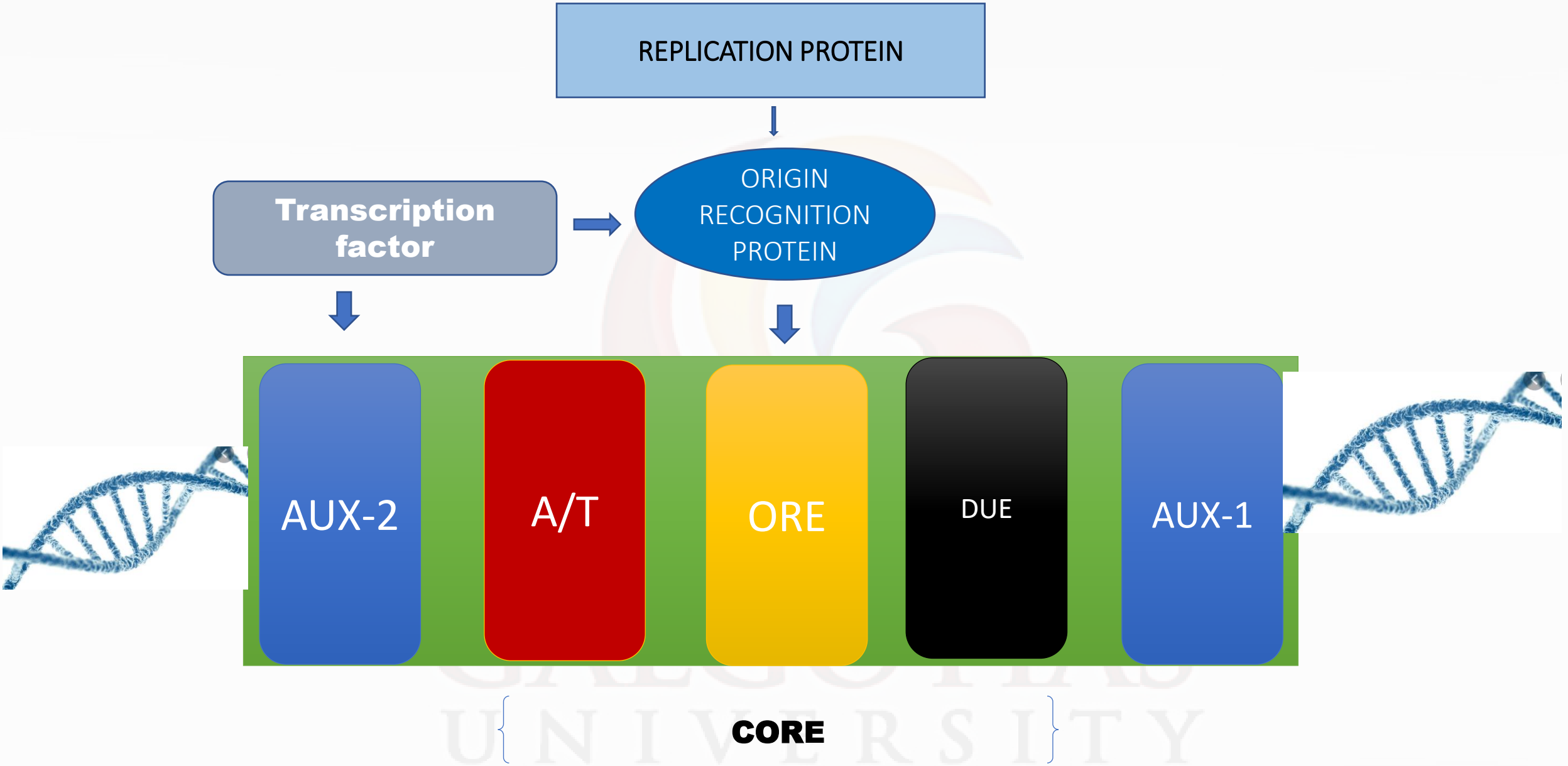

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- Auxiliary component provide the site of transcription.
- Core component is analogous to transcription promotor

- Transcription factor have 2 domain DNA binding domain and activation domain, involve activation of origin recognition protein by protein -protein interaction.

- In eukaryotes (ORE) origin recognition element have ARS (autonomic replicating sequence)

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Replicon

- The unit of DNA in which individual act of replication occur called replicon
- Replicon is usually applied to eukaryotic chromosome
- Eukaryotic replicon is relatively small
- The rate of replication is much slower in than the rate of bacterial replication fork movement

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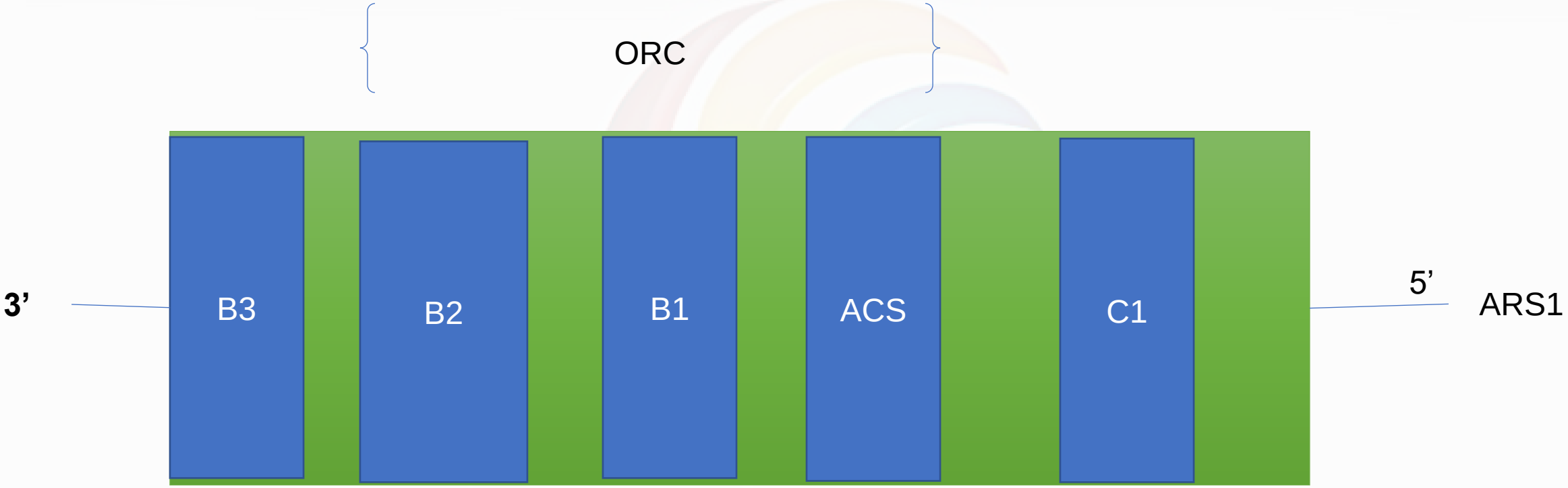

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ORC is act as loading pad for many protein like MCM

Eukaryotic initiator

- Initiator protein in eukaryotes are MCM and initiation factors
- MCM is minimicrosome or plasmid maintenance protein
- **MCM**: The MCM proteins are essential for the maintenance of some ARS contains plasmid and its origin site of phosphorylation. Where many kinase cyclin B-CDK bind and initiate replication

Eukaryotic DNA polymerase

DNA pol alpha: Its equivalent to DNA pol I in prokaryotes

- cytoplasmic polymerase called
- Large polymerase
- Found in nucleus and cytoplasm
- Act on lagging strand

DNA pol Beta:

- small polymerase
- Found in nucleus
- Found in vertebrate
- Absent in yeast

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DNA pol gamma: chloroplast

- Mitochondrial polymerase found in nucleus of mitochondria

DNA pol Delta: Having PCNA dependent DNA synthetic processivity

- Its equivalent to DNA pol III in prokaryotes
- Act on leading strand

DNA pol epsilon: repair and proofreading activity

Helicase: hexamer of identical subunits require ATP for hydrolysis posses ATPase activity

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Thanks