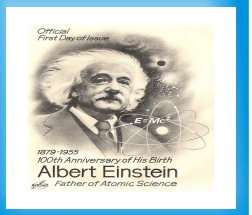

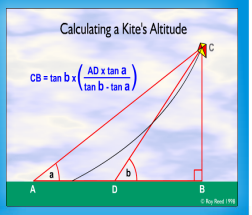

GATE SCIENCE - LIFE SCIENCE

SAMPLE THEORY

DNA REPLICATION

- *INTRODUCTION*
- *BIOCHEM ISTRY OF DNA REPLICATION*
- *PROKARYOTIC DNA REPLICATION*
- *EUKARYOTIC DNA REPLICATION*
- *SOLVED QUESTIONS*

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INTRODUCTION

- **DNA replication is a semi conservative process** in which each parental strand serves as a template for the synthesis of a new complementary daughter strand.
- Central enzyme involved is DNA polymerase, which catalyzes the joining of deoxyribonucleoside 5' - triphosphates (dNTPs) to form the growing DNA chain.
- **Meselson and Stahl** grew *E. coli* cells for many generations in a medium in which the sole nitrogen source (NH_4Cl) contained ^{15}N, the "heavy" isotope of nitrogen, instead of the normal, more abundant "light" isotope, ^{14}N.

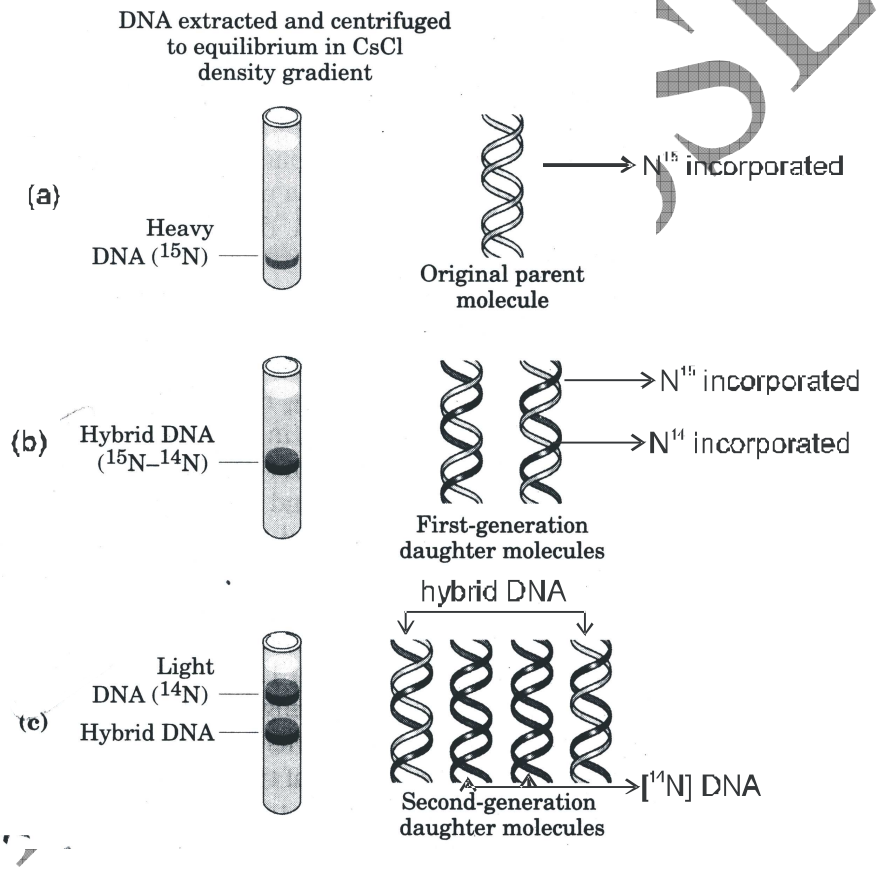


Fig: Meselson and Stahl's experiment demonstrating that DNA replicated by a semiconservative mechanism in *E.coli*. The diagram shows that the result of their experiments is those expected if the *E.coli* chromosome replicates semi conservatively.

REPLICATION FORK

- In each replicon, replication is continuous from the origin to the terminus and is accompanied by the movement of the replicating point, called the replication fork.

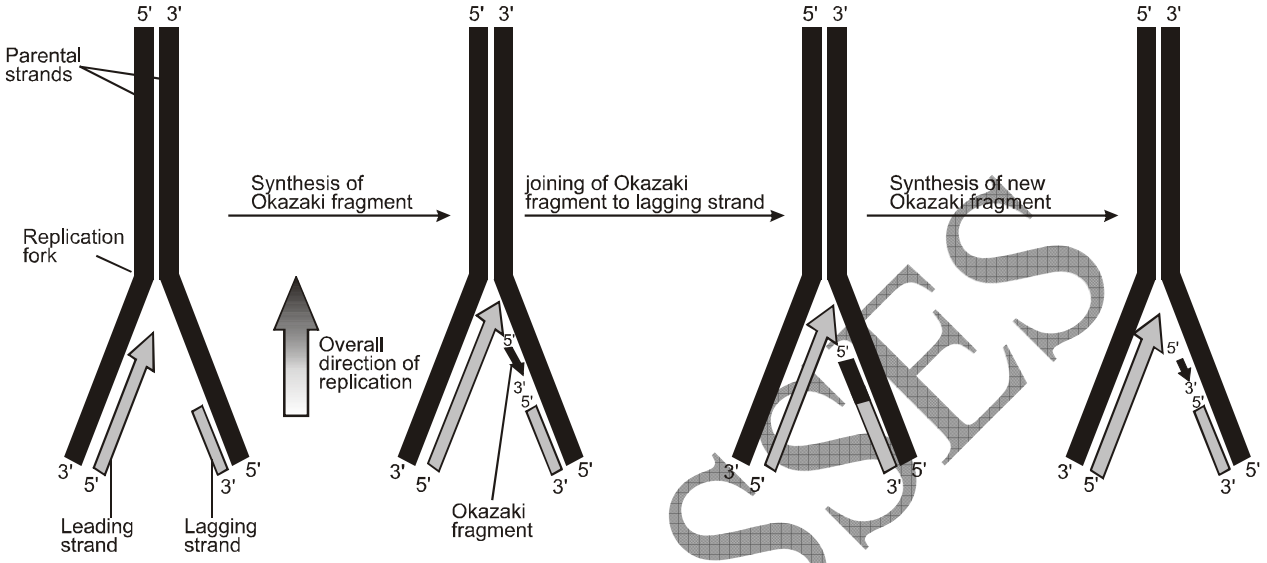


Figure : Synthesis of leading and lagging strands of DNA The leading strand is synthesized continuously in the direction of replication fork movement. The lagging strand is synthesized in small pieces (Okazaki fragments) backward from the overall direction of replication. The Okazaki fragments are then joined by the action of DNA ligase.

REPLICON & ORIGIN OF REPLICATION

- DNA replication does not start at random locations but at particular sites, called the origins of DNA replication. A strand whose replication starts from an origin and proceeds bidirectional or unidirectional to terminus site is called a replicon, a unit of DNA replication.
- In bacterial cells, the circular chromosome contains a unique origin and DNA replication proceeds bidirectional from the origin to the terminus. Therefore, the whole bacterial genome (~4.6 Mbp for *E. coli*) is a single replicon (monorepliconic).
- On the other hand, eukaryotic cells contain multiple replication origins on each chromosome and hence many replicons (multirepliconic).
- The *E.coli* origin of replication is referred to as oriC. It spans approximately 245 bp of DNA. It contains two short repeat motifs, one of nine nucleotides and the other of 13 nucleotides.
- The nine-nucleotide repeat, five copies of which are dispersed throughout oriC, is the binding site for a protein called **DnaA**.
- The result of DnaA binding is that the double helix opens up ('melts') within the tandem array of three AT-rich, 13-nucleotide repeats located at one end of the oriC sequence.

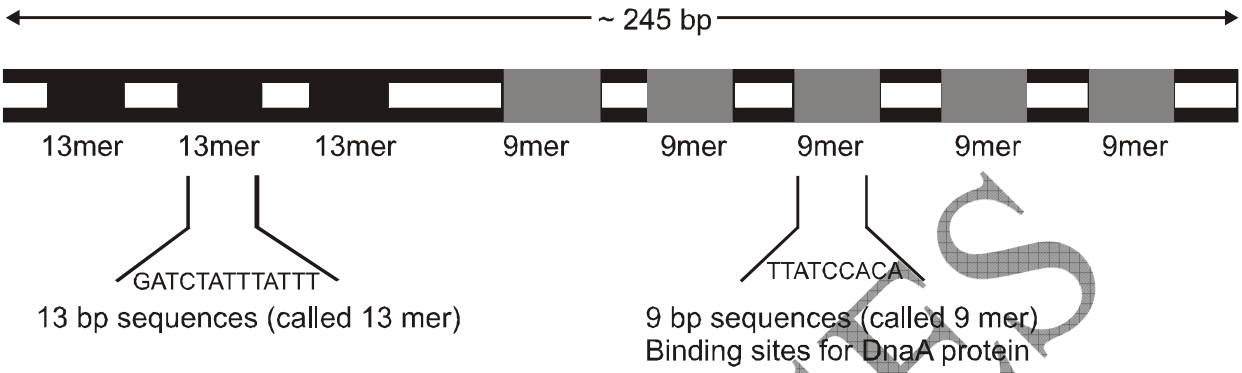


Fig. : E. coli origin of replication, oriC, oriC contains repetitive 6 -bp and A.T rich 13-bp sequences, referred to as 9-mers and 13-mers, respectively.

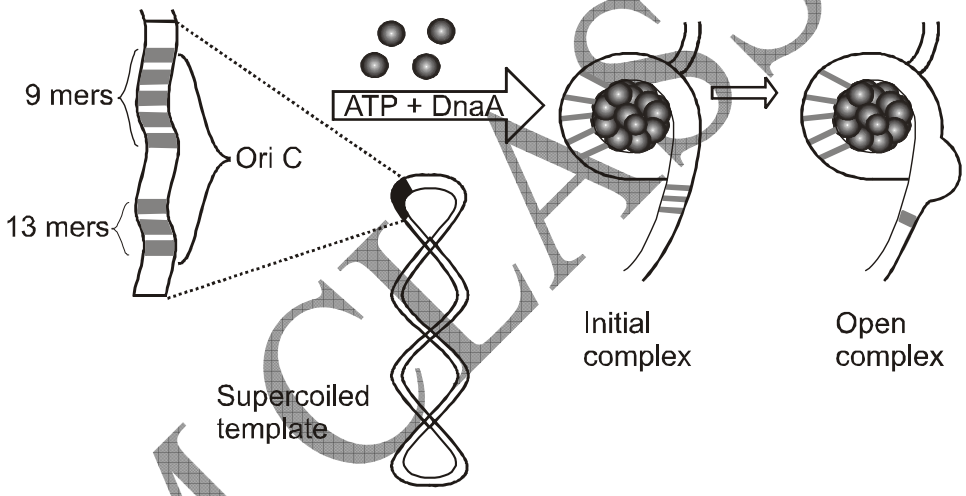


Fig. Initiation at oriC occurs after DnaA protein binds the five 9 mers. The 13 mer region is then denatured, and this open complex serves as a replication start site.

- oriC contains 11 5'-GATC-3' repeats that are methylated on adenine on both strands. Only fully methylated origins can initiate replication; hemimethylated daughter origins cannot be used again until they have been restored to the fully methylated state.
- **Common features of replication origins:**
  1. Replication origins are unique DNA segments that contain multiple short repeated sequences.
  2. These short repeat units are recognized by multimeric origin-binding proteins. These proteins play a key role in assembling DNA polymerases and other replication enzymes at the sites where replication begins.

3. Origin regions usually contain an AT-rich stretch. This property facilitates unwinding of duplex DNA because less energy is required to melt A=T base pairs than GC base pairs.

BIOCHEMISTRY OF DNA REPLICATION

Enzymes Involved in DNA Replication

- **Helicases** are enzymes that catalyze the unwinding of parental DNA, coupled to the hydrolysis of ATP, ahead of the replication fork.
- **Single-stranded DNA-binding proteins** (e.g., eukaryotic replication protein A [RPA]) then stabilize the unwound template DNA, keeping it in an extended single-stranded state so that it can be copied by the polymerase.
- As the strands of parental DNA unwind, the DNA ahead of the replication fork is forced to rotate. Unchecked, this rotation would cause circular DNA molecules (such as SV40 DNA or the *E. coli* chromosome) to become twisted around themselves, eventually blocking replication.
- This problem is solved by **topoisomerases enzymes** that catalyze the reversible breakage and rejoining of DNA strands.
- **Type I topoisomerases** break just one strand of DNA; type I and II topisomerases serve as "swivels" that allow the two strands of template DNA to rotate freely around each other.
- In ***E. coli*** there is an enzyme called DNA gyrase which is able to produce negative superhelicity.
- Positive superhelicity is removed by gyrase introducing negative twists by binding ahead of the advancing replication fork.
- The synthesis of RNA primer can initiated de novo, and an enzyme called **primase** synthesizes short fragments of RNA (e.g., three to ten nucleotides long) complementary to the lagging strand template at the replication fork.
- **RNA primers** must eventually be removed from the Okazaki fragments and replaced with DNA.
- In prokaryotes, RNA Primers are removed by the action of polymerase I.
- In addition to its DNA polymerase activity, **polymerase I** acts as an exonuclease that can hydrolyze DNA (or RNA) in either the 3' to 5' or 5' to 3' direction.
- The action of polymerase I as a 5' to 3' exonuclease removes ribonucleotides from the 5' ends of Okazaki fragments.
- In eukaryotic cells, RNA primers are removed by the combined action of **R Nase H**, an enzyme that degrades the RNA strand of RNA-DNA hybrids, and 5' to 3' exonuclease.

PROKARYOTIC DNA POLYMERASE

3 different types of prokaryotic DNA polymerase are known:

1) DNA POLYMERASE-I

- It was first of all isolated by **Arthur Kornberg** in 1960.
- It is now considered to be a DNA repair enzyme rather than a replication enzyme.
- The exonuclease activity of DNA polymerase-I also helps in the removal of mismatch base pairs and this process of making correction is known as **"proofreading".**
- DNA polymerase-I consist of 2 types of fragments, larger **Klenow fragment** → 3'- 5' exonuclease activity and 5'-3' polymerizing activity.
- Smaller fragment → 5'-3' exonuclease activity.

2) DNA POLYMERASE-II

It resembles DNA polymerase-I in bringing up the growth in 5'-->3' using 3'-OH group.

- Although it has 3'-5' exonuclease activity, it lacks 5'-->3' exonuclease activity.
- It is not a replicating enzyme.It is involved in the process of DNA repair.

3) DNA POLYMERASE-III

• It plays an important role in DNA replication, it is also called hetero multimeric enzyme with 10 units.

• This replication complex is an asymmetric dimer. The catalytic core of DNA pol III composed of α^{-}, ε, θ-subunits, contains the polymerase activity and a 3' → 5' exonuclease for proofreading.

Table: Activities and Functions of DNA polymerases

Prokaryotic (E.coli)	Number of subunits	Function
Pol I	1	RNA primer removal, DNA repair
Pol II (Din A)	1	DNA repair
Pol III holoenzyme	9	Chromosome replication
Pol IV (Din B)	1	DNA repair, Translesion Synthesis (TLS)
Pol V (Umuc, $UmuD'_2$ C)	3	TLS
Eukaryotic	**Number of subunits**	**Function**
Pol α	4	Primer synthesis during DNA replication
Pol β	1	Base excision repair
Pol γ	3	Mitochondrial DNA replication and repair
Pol δ	2 - 3	DNA replication; nucleotide and base excision repair
Poll ε	4	DNA replication; nucleotide and base excision repair

- In eukaryotic cells, nuclear DNA replication requires three polymerase, **Pol α, Pol δ, and Pol ε**. Pol γ is a sole polymerase participating in mitochondrial DNA replication.
- DNA Pol α is unusual because it has the ability to initiate a new strand. It is used to initiate both the leading and lagging strands. Among the replicative polymerases, Pol α is unique in possessing a primase activity, the only such activity so far identified in eukaryotic cells, suggesting that Pol α may play a role in the priming of DNA synthesis.
- DNA synthesis by Pol α, lacking the 3'-5' exonuclease activity, is inaccurate and shows a low processivity. These enzymatic characteristics make Pol α poor candidate for the major replicative polymerase.
- The Pol α binds to the initiation complex at the origin and synthesize a short strand consisting of ~10 bases of RNA followed by 20-30 bases of DNA.
- Then it is replaced by an enzyme that will extend the chain. On the leading strand, this is DNA pol δ. This event is called the **polymerase switching**.
- DNA pol δ, Pol ε both posses a 3'-5' exonuclease activity and are able to carry out highly processive DNA synthesis with the aid of a sliding clamp and a clamp loader.
- DNA pol δ elongates the leading strand and pol ε may be involved in lagging strand synthesis.

PROKARYOTIC DNA REPLICATION

(1) Initiation of Replication in a Double Strand of DNA

- For the initiation of replication from ori-c, DnaA protein which is a product of dnaA gene form complex with the ATP molecule (DnaA-ATP complex).
- Now this complex (DnaA-ATP complex) combines at ori-c region in order to promote the unwinding of DNA in a region of 13 base pair sequence.
- During the unwinding the excessive super coiling of DNA also occurs ahead of the replication fork.
- In case of circular DNA molecules the problem of super coiling is overcome by topoisomerase enzyme.

RNA PRIMING: Unwinding is followed by the synthesis of RNA primers by DNA-primase through it's interaction with DnaB and the primers are further elongated by DNA polymerase-III holoenzyme.

(2) CHAIN ELONGATION

- The elongation of the chain involves loading and activation of Dna-B helicase enzyme because this enzyme will then activate a series of other enzyme such as DNA polymerase-III holoenzyme to promote chain elongation.
- DNA polymerase-III holoenzyme is a large multiprotein complex made up of two components i.e. core and accessory component.
- When both the components are linked together, enzyme becomes functional.

- As a result of this process the two types of strands are synthesized out of which one is leading strand and the other is lagging strand.

(3) CHAIN TERMINATION

- The prokaryotic DNA strand contains large termination zone opposite to Ori-C region which can block the progress of the replication.
- Replication of genome terminates at terminus region containing multiple copies of about 23 bp sequences called **Ter (for terminus) sequences**.
- Seven of these have been identified in the *E.coli* genome, each one acting as the recognition site for a sequence-specific DNA-binding protein called **Tus** (terminus utilization substance).
- When approached from one direction, Thus blocks the passage of the Dna B helicase, which is responsible for progression of the replication fork. But when approaching from other direction, Dna B is able to cross the Tus protein.

EUKARYOTIC DNA REPLICATION

- DNA replication in eukaryotes involves the following steps:

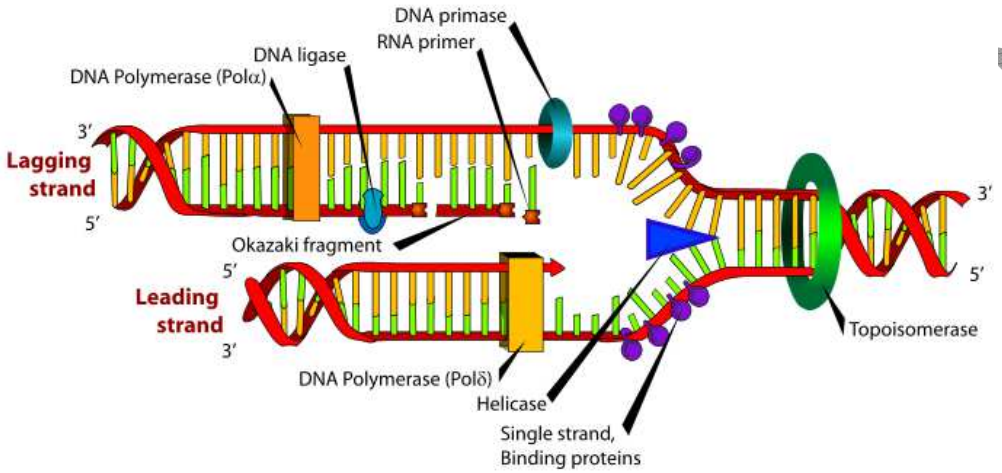
Chain initiation

- In the eukaryotic genome the origin of replication is also known as **autonomously replicating sequence (ARS).**
- ARS contains 11 base pairs A=T rich consensus sequences or ACS domain [Autonomous replication consensus sequences).
- Unwinding occurs from the domain in order to form a replication fork. The replication fork in this case of eukaryotes has directional progress i.e. it is bidirectional in nature.
- The protein involved in the unwinding is **T-Ag protein** which requires the presence of **ATP.**
- The same protein is also associated with the **replication factor A** (RF-A) and the topoisomerase enzyme which performs the function of helicase & brings about the local unwinding of the DNA molecule.
- Just as soon as the replication fork is formed S.S.B binds so as to unwound single stranded DNA.
- Next step of eukaryotic replication is the RNA priming.

Chain Elongation

- DNA polymerase α synthesize lagging strands or the Okazaki fragments on 5'-3' DNA template.
- **RF-C** [Replicating Factor-C] & **PCNA** [Proliferating Cell Nuclear Antigen] or **cycline** helps in switching off DNA polymerase, so that poly-α is replaced by poly δ which will then continuously synthesize DNA as a leading strand in 3'-->5' direction

- Another Okazaki fragment is then synthesized from the replication fork on the lagging strand by poly α primase complex.
- This step is repeated again and again till the entire DNA molecule is covered.
- The RNA primers are later on removed and the gaps are filled as in case of prokaryotic DNA replication. Thus, the chain elongation involves the switching between ploy α and poly δ.
- Aphidicolin, a tetracyclic diterpenoid, is a potent inhibitor of mammalian nuclear DNA polymerases. It does not affect mitochondrial DNA polymerase.



POINTS TO REMEMBER

- DNA replication is semiconservative in nature..
- Replication could be either unidirectional or bidirectional and these can be distinguished by autoradiography:
- DNA replication does not start at random locations but at particular sites, called the origins of DNA replication.
- Origins of replication in yeast in known as *autonomously replicating sequences* or **ARSs**.
- The *E.coli* origin of replication is referred to as oriC.
- Origin regions usually contain an AT-rich stretch.

SOLVED EXAMPLES

1. Eukaryotic DNA synthesis is inhibited by

(A) Aphidicoline

(B) Cycloheximide

(C) Chloramphenicol

(D) Ampicillin

2. What would be the consequence of mutation in the 3' to 5' exonuclease activity of DNA polymerase III on the fidelity of replication of *E.coli* DNA?

(A) DNA polymerases are unable to initiate DNA synthesis

(B) Unable to remove RNA primers

(C) Would have a high frequency of mutation each time the DNA is replicated

(D) Would not synthesize RNA primers

3. The function of the 3' to 5' exonuclease activity of a DNA polymerase is to

(A) Remove the 5' end of the polynucleotide from the template strand that is being copied

(B) Remove damaged nucleotides from the template strand during DNA synthesis

(C) Remove nucleotides from the ends of DNA molecules to ensure the generation of blunt ends.

(D) Remove incorrect nucleotides from the synthesized strand of DNA

4. All of the following statements about Type I topoisomerase (Topo I) are true except:

(A) topo I removes DNA supercoils in an ATP-dependent reaction

(B) topo I from eukaryotic cells removes both positive and negative supercoils

(C) topo I from E. coli can remove only negative supercoils

(D) topo I is essential for viability in E. coli

5. DNA replication in prokaryotes requires

(A) Primase to make small primers containing deoxyribo nucleotides

(B) DNA polymerase III to join Okazaki fragments

(C) DNA polymerase alpha to synthesize DNA

(D) DNA polymerase I to cut out primers and fill in gaps in the lagging strand

Answer key: 1. (A) , 2. (C) , 3.(D), 4 (A) , 5 (D)

1.(A) Aphidicolin, a tetracyclic diterpenoid, is a potent inhibitor of mammalian nuclear DNA polymerases. It does not affect mitochondrial DNA polymerase.

2.(C) 3'to 5' exonuclease activity is required for the excision of mismatched bases in newly synthesized DNA during proofreading. A mutant *E.coli* with a DNA pol.III lacking this activity would have a high frequency of mutations each time the DNA is replicated.

3.(D) 3' - 5' exonuclease activity involves mainly proof reading or editing function i.e. correcting unpaired or mismatch bases in the DNA.

4.(A) Topoisomerase I removes supercoiling but not require ATP for this activity.

5.(D) DNA replication in prokaryotes requires DNA polymerase DNA to cut out primers and fill in gaps in the lagging strand.