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DNA Replication

DNA is the carrier of genetic information. Before a cell divides, DNA must be precisely copied, or "replicated," so that each of the two daughter cells can inherit a complete genome, the full set of genes present in the organism. In eukaryotes, the DNA molecules that make up the genome are packaged with proteins into chromosomes, each of which contains a single linear DNA molecule. Eukaryotic chromosomes are found in a special compartment called the cell nucleus. The genomes of bacterial cells which lack a nucleus are typically circular DNA molecules that associate with special structures in the cell membrane. Despite the hundreds of millions of years of evolutionary history separating eukaryotes and prokaryotes, the features of the replication process have been highly conserved between them.

Replication can be broadly defined as genome duplication, essential process for the propagation of cellular genomes and those of 'molecular parasites' - viruses, plasmids and transposable elements. The genome to be duplicated is the parental genome, and the copies are daughter genomes.

At the biochemical level, replication is defined as a template-directed nucleic acid synthesis reaction where the template and nascent (growing) strand are the same type of nucleic acid. Replication is a polymerization reaction and can be divided into stages of initiation, elongation and termination. The elongating replication center requires the coordination of many different enzyme activities, collectively described as the replisome.

Models of Replication

The Watson–Crick model while describing their structure of DNA molecule suggested that the basis for copying the genetic information is complementarity. One chain of the DNA molecule may have any conceivable base sequence, but this sequence completely determines the sequence of its partner in the duplex. For example, if the sequence of one chain is 5'- ATTGCAT-3', the sequence of its partner must be 3'-TAACGTA-5'.

Thus, each chain in the duplex is a complement of the other. The complementarity of the DNA duplex provides a ready means of accurately duplicating the molecule. If one were to "unzip" the molecule, one would need only to assemble the appropriate complementary nucleotides on the exposed single strands to form two daughter duplexes with the same sequence. This form of

DNA replication is called semiconservative, because while the sequence of the original duplex is conserved after one round of replication, the duplex itself is not. Instead, each strand of the duplex becomes part of another duplex.

Two other hypotheses of gene replication were also proposed.

The conservative model stated that the parental double helix would remain intact and generate DNA copies consisting of entirely new molecules.

The dispersive model predicted that parental DNA would become dispersed throughout the new copy so that each strand of all the daughter molecules would be a mixture of old and new DNA.

Meselson and Stahl's Experiment

1. The three hypotheses of DNA replication were evaluated in 1958 by Matthew Meselson and Franklin Stahl of the California Institute of Technology.

2. They grew bacteria in a medium containing the heavy isotope of nitrogen, 15N, which became incorporated into the bases of the bacterial DNA. After several generations, the

DNA of these bacteria was denser than that of bacteria grown in a medium containing the lighter isotope of nitrogen, 14N.

3. They then transferred the bacteria from the 15N medium to the 14N medium and collected the DNA at various intervals. By dissolving the DNA they had collected in a heavy salt, cesium chloride ($CsCl_2$) and then spinning the solution at very high speeds in an ultracentrifuge, they were able to separate DNA strands of different densities. The enormous centrifugal forces generated by the ultracentrifuge caused the cesium ions to migrate toward the bottom of the centrifuge tube, creating a gradient of cesium concentration, and thus of density.

4. Each DNA strand floats or sinks in the gradient until it reaches the position where its density exactly matches the density of the cesium there. Because 15N strands are denser than 14Nstrands, they migrate farther down the tube to a denser region of the cesium gradient.

5. The DNA collected immediately after the transfer was all dense. However, after the bacteria completed their first round of DNA replication in the 14N medium, the density of their DNA had decreased to a value intermediate between 14N-DNA and 15N-DNA.

6. After the second round of replication, two density classes of DNA were observed, one intermediate and one equal to that of 14N-DNA

7. Meselson and Stahl interpreted their results as follows: after the first round of replication, each daughter DNA duplex was a hybrid possessing one of the heavy strands of the parent

molecule and one light strand; when this hybrid duplex replicated, it contributed one heavy strand to form another hybrid duplex and one light strand to form a light duplex.

8. Thus, this experiment clearly confirmed the prediction of the Watson - Crick Model that DNA replicates in a semi conservative manner.

Semidiscontinuous Replication.

Watson and Crick's semi conservative model of DNA replication predicted the existence of a replication fork, a dynamic Y-shaped structure with a barrel composed of parental duplex DNA and arms composed of daughter duplex DNA, each daughter duplex consisting of one parental and one daughter strand. At the center of the fork, the parental duplex would be unwound and nucleotides would be added to the growing daughter strands. The existence of replication forks has been confirmed directly by incorporating radioactive nucleotides into replicating bacterial DNA and observing the intermediate structures by electron microscopy. However, this model reveals a paradox which can be summarized as follows:

(1) cellular DNA replication is semi conservative;

(2) both daughter strands are extended simultaneously;

(3) the strands of the parental duplex are antiparallel;

(4) DNA polymerases extend DNA only in the 5'→3' direction.

How can simultaneous 5"→ 3' elongation of both daughter strands occur at a replication fork when the parental templates have opposite polarity?

This can be achieved by semi discontinuous replication, where one strand is extended continuously and the other is synthesized discontinuously as a collection of short fragments. The mechanism of semi discontinuous DNA replication can be formally expressed as the leading strand - lagging strand model.

The leading strand is the nascent strand which is synthesized continuously in the direction of fork movement because its 3' end is exposed to the DNA polymerase. The leading strand template is thus the forward template.

The lagging strand is the nascent strand which is synthesized discontinuously in the opposite direction to fork movement because its 5' end, the end which cannot be extended, is exposed to the DNA polymerase. The lagging-strand template is thus the retrograde template.

The mechanism can be summarized as follows: -as the replication fork moves forward and the leading strand is extended, a portion of retrograde template is exposed. DNA polymerase can

then synthesize a small fragment of DNA, an Okazaki fragment, by moving backwards over the template in relation to the fork progression.

The lagging strand is so called because the leading strand must be synthesized first to uncover the corresponding portion of lagging strand template. The enzyme dissociates from the template when it reaches the previously synthesized Okazaki fragment, by which time a further portion of retrograde template has been exposed. The enzyme can then reinitiate and synthesize a new Okazaki fragment. By repeating this back-stitching process over and over, the lagging strand would appear to grow in the 3'→5' direction.

Because DNA polymerase cannot initiate de novo strand synthesis, each Okazaki fragment needs to be individually primed. The dissociation- reassociation cycle therefore requires a priming step.

Note: Evidence supporting the leading strand-lagging strand model includes the presence of DNA primase at the replication fork in both bacterial and eukaryotic replisomes. Also, both replisomes are asymmetrical, reflecting the presence of one highly processive DNA polymerase for leading-strand synthesis and one distributive DNA polymerase for lagging-strand synthesis. Pulse chase experiment confirm that 50% of nascent DNA in cellular replication occurs as low molecular weight fragments

The Replication Process-at a glance

The replication of the DNA double helix is a complex process that has taken decades of research to understand. It takes place in five interlocking steps:

Opening up the DNA double helix. The very stable DNA double helix must be opened up and its strands separated from each other for semiconservative replication to occur. *Stage one: Initiating replication.* The binding of initiator proteins to the replication origin starts an intricate series of interactions that opens the helix.

Stage two: Unwinding the duplex. After initiation, "unwinding" enzymes called helicases bind to and move along one strand, shouldering aside the other strand as they go.

Stage three: Stabilizing the single strands. The unwound portion of the DNA double helix is stabilized by single-strand binding protein, which binds to the exposed single strands, protecting them from cleavage and preventing them from rewinding.

Stage four: Relieving the torque generated by unwinding. For replication to proceed at 1000 nucleotides per second, the parental helix ahead of the replication fork must rotate 100 revolutions per second! To relieve the resulting twisting, called torque, enzymes known as

topisomerases—or, more informally, gyrases— cleave a strand of the helix, allow it to swivel around the intact strand, and then reseal the broken strand.

2. Building a primer. New DNA cannot be synthesized on the exposed templates until a primer is constructed, as DNA polymerases require 3' primers to initiate replication. The necessary primer is a short stretch of RNA, added by a specialized RNA polymerase called *primase* in a multisubunit complex informally called a *primosome.* Why an RNA primer, rather than DNA? Starting chains on exposed templates introduces many errors; RNA marks this initial stretch as "temporary," making this error-prone stretch easy to excise later. 3. Assembling complementary strands. Next, the dimeric DNA polymerase III then binds to the replication fork. While the leading strand complexes with one half of the polymerase dimer, the lagging strand is thought to loop around and complex with the other half of the polymerase dimer (figure 14.17). Moving in concert down the parental double helix, DNA polymerase III catalyzes the formation of complementary sequences on each of the two single strands at the same time. 4. Removing the primer. The enzyme DNA polymerase I now removes the RNA primer and fills in the gap, as well as any gaps between Okazaki fragments. 5. Joining the Okazaki fragments. After any gaps between Okazaki fragments are filled in, the enzyme DNA ligase joins the fragments to the lagging strand.

During the process of DNA replication, the strands are unwound by an enzyme called DNA helicase, and a new strand of DNA is synthesized on each of the old (template) strands by an enzyme called DNA polymerase, which joins incoming nucleotides together in a sequence that is determined by the sequence of nucleotides present in the template strand. Prokaryotic replication can take as little as twenty minutes, while replication in eukaryotes takes considerably longer, approximately eight hours in mammals.

Initiation of DNA Replication

DNA replication begins (initiates) at special sites called origins of DNA replication. Eukaryotic DNA contains multiple replication origins, Replication spaced at intervals of approximately 100 kb along the length of the DNA. Prokaryotic chromosomes typically have a single replication origin.

Replication origins are composed of special sequences of DNA that are recognized by replication initiator proteins, which bind to the origin sequences and then help to assemble other proteins required for DNA replication at these sites. The eukaryotic replication initiator protein is a

complex containing six different subunits called the *origin recognition complex* (ORC). The bacterial replication initiator protein is called the *dnaA* protein.

The timing of DNA replication is regulated by controlling the assembly of complexes at replication origins. The distinct steps in the initiation of replication are understood better in bacteria than in eukaryotes, but several key steps are common to both.

The first step is a change in the conformation of the initiator protein, which causes limited "melting" (*i.e*, the separation of the two strands) of the double stranded DNA next to the initiator binding site, thus exposing single-stranded regions of the template . Two more proteins, *DNA helicase* and *DNA primase*, then join the complex. Replication initiation is triggered by the activation of the helicase and primase, and the subsequent recruitment of *DNA polymerase*. In prokaryotes, the particular form of the enzyme is called DNA polymerase III. Other proteins are also recruited, each of whose functions are discussed below.

The Replication Fork

The separation of the two template strands and the synthesis of new daughter DNA molecules creates a moving "replication fork", in which double-stranded DNA is continually unwound and copied. The unwinding of DNA poses special problems, which can be visualized by imagining pulling apart two pieces of string that are tightly wound around each other. The pulling apart requires energy; the strands tend to rewind if not held apart; and the region ahead of the separated strands becomes even more tightly twisted.

Proteins at the replication fork address each of these problems. DNA polymerases are not able to unwind double-stranded DNA, which requires energy to break the hydrogen bonds between the bases that hold the strands together. This task is accomplished by the enzyme DNA helicase, which uses the energy in ATP to unwind the template DNA at the replication fork.

The single strands are then bound by a *single-strand binding protein (called SSB in bacteria and RPA in eukaryotes)*, which prevents the strands from re-associating to form double-stranded DNA. Unwinding the DNA at the replication fork causes the DNA ahead of the fork to rotate and become twisted on itself. To prevent this from happening, an enzyme called *DNA gyrase (in bacteria) or topoisomerase (in eukaryotes)* moves ahead of the replication fork, breaking, swiveling, and rejoining the double helix to relieve the strain.

Leading Strands and Lagging Strands

The coordinated synthesis of the two daughter strands posed an important problem in DNA replication. The two parental strands of DNA run in opposite directions, one from the 5'→3'end, and the other from the 3'→5'end.

However, all known DNA polymerases catalyze DNA synthesis in only one direction, from the 5'→3'end, adding nucleotides only to the 3' end of the growing chain. The daughter strands, if they were both synthesized continuously would have to be synthesized in opposite directions, but this is known not to occur. How, then, can the other strand be synthesized?

The resolution of the problem was provided by the demonstration that only one of the two daughter strands, called the leading strand, is synthesized continuously in the overall direction of fork movement, from the 5'→3 end. The second daughter strand, called the lagging strand, is made discontinuously in small segments, called Okazaki fragments.

Each Okazaki fragment is made in the 5'→3' direction, by a DNA polymerase whose direction of synthesis is backwards compared to the overall direction of fork movement. These fragments are then joined together by an enzyme called DNA ligase.

The Need for Primers

DNA polymerases are unable to initiate synthesis of a new DNA strand from scratch; they can only add nucleotides to the 3' end of an existing strand, which can be either DNA or RNA. Thus, the synthesis of each strand must be started (primed) by some other enzyme.

The priming problem is solved by a specialized RNA polymerase, called *DNA primase*, which synthesizes a short (3-10 nucleotides) RNA primer strand that DNA polymerase extends. On the leading strand, only one small primer is required at the very beginning. On the lagging strand, however, each Okazaki fragment requires a separate primer.

Before Okazaki fragments can be linked together to form a continuous lagging strand, the RNA primers must be removed and replaced with DNA. In bacteria, this processing is accomplished by the combined action of RNase H and DNA polymerase I. RNase H is a ribonuclease that degrades RNA molecules in RNA/DNA double helices. In addition to its polymerase activity, DNA polymerase I is a 5'→3' nuclease, so it too can degrade RNA primers. After the RNA primer is removed and the gap is filled in with the correct DNA, DNA ligase seals the nick between the two Okazaki fragments, making a continuous lagging strand.

The Replisome.

The replisome (replication complex, center, machine etc.) is the dynamic complex of enzymes and other proteins found at the replication fork during elongation. Despite the elegant simplicity of the underlying mechanism of replication, the logistics of the operation require many different enzyme activities to be coordinated for continued, accurate DNA synthesis. There is a formidable energy requirement to unwind the supercoiled DNA**,** and in eukaryotes, DNA is organized into chromatin which must be negotiated by the replisome. The components of the cellular replisome and their functions are listed in the following table-

Replisome components	**Function during replication**
DNA helicase	Unwinds DNA strand of replication fork
DNA ligase	Joins fragments of repaired lagging strand
DNA polymerase	DNA synthesis, repair of gaps in lagging strand. The replisome may contain several distinct forms of DNA polymerase, one for leading-strand synthesis, one for lagging-strand repair.
DNA primase	Primes Okazaki fragment synthesis
DNA topoisomerases	Releases tortional strain caused by helicase activity, decatinates linked circles following replication.
RNaseH	Removes RNA primers from lagging strand
Single stranded binding protein	Stabilizes single-stranded regions of replication fork. May interact with other replisome components to stimulate their activity

The replisome assembles from its components during initiation and is only found at the replication fork - it does not exist as a separate entity in the cell. Simpler replicons may require fewer replisome components -the minimal requirement being a single polymerase enzyme.

Termination

In some DNA molecules, replication is terminated whenever two replication forks meet. In others, specific termination sequences block further replication. A termination protein, called *Tus* in *E. coli,* binds to these sequences. *Tus* blocks the movement of helicase, thus stalling the replication fork and preventing further DNA replication

DNA Polymerase

Enzymes catalyzing DNA synthesis on a DNA template are DNA polymerases. They perform two primary functions in the cell: the synthesis of DNA during genome replication, and the

resynthesis of missing DNA following damage or recombination, and following primer excision from the lagging strand. All DNA polymerases possess a 5'→3' polymerase activity and a pyrophosphorylysis activity, which together facilitate DNA synthesis. Unlike RNA polymerases, DNA polymerases are unable to initiate *de novo* strand synthesis and therefore require a primer. Most DNA polymerases also possess further intrinsic activities). The most important is a 3'→5' exonuclease activity, which is the basis of proofreading

The DNA polymerases of *E. coli*.

The *E. coli* genome encodes three DNA polymerases (DNA polymerases I, II and III, or pol I, pol II and pol III). Pol I and Pol II are single polypeptides whose primary role appears to be DNA repair.

Pol I (also known as Kornberg polymerase) is the predominant polymerase activity in the cell and possesses a unique 5'→3' exonuclease activity which facilitates primer excision from the lagging strand during repair synthesis *in vivo.* Although all the enzymatic activities of pol I lie on a single polypeptide of 109 kDa, each arises from a specific domain and proteolytic cleavage can generate a large C-terminal fragment *(Klenow fragment, Klenow polymerase)* which lacks the 5'→3' exonuclease activity and is useful for *in vitro* applications where excision would be undesirable (e.g.. *random priming, in vitro mutagenesis).* Klenow polymerase is produced commercially by expressing a truncated polA gene**.**

Pol II is a minor component of the cell during normal growth but is inducible by the SOS *response*. It appears that this enzyme allows nucleotide incorporation opposite AP *sites (apurinic site)* i.e. lesions which stall pol I and pol III - it thus facilitates *translesion synthesis*

The principle replicative DNA polymerase of *E. coli* is Pol III which, unlike the other enzymes, is a multisubunit complex, the Pol III holoenzyme. The holoenzyme functions as a heterodimer of complexes at the replication fork, with each monomer seeing to the synthesis of one daughter strand.

In vitro, the *α,* ε and θ subunits associate to form the core enzyme, which contains the essential enzyme activities. Addition of the other subunits promotes dimerization and increases the processivity of the enzyme.

The assembly of the holoenzyme *in vivo* occurs as follows: the βsubunit functions as a dimer and forms a ring or clamp which can slide along single-stranded DNA. This is a processivity factor which keeps the core enzyme attached to the template. The β subunit is loaded onto the template-primer by the γ complex, an ATP-dependent process, to formt he pre-initiation complex. The loading of the β subunit allows the core enzyme to bind, and addition of the τ

subunit facilitates dimerization. The holoenzyme is symmetrical except for the γ complex, which is associated with only one of the monomers. The γ complex is required for both loading and unloading the β subunit from DNA, and hence controls the processivity of the enzyme. The presence of the γ complex allows the β subunit to disassociate from the template primer when the polymerase encounters the 5' end of a previously synthesized Okazaki fragment on the retrograde template. The lagging-strand core enzyme will thus be released from the holoenzyme when it has completed the Okazaki fragment and can reassociate with a pre-initiation complex which has loaded at the next available template primer, provided by DNA primase.

*Note:-*The DNA polymerases of other bacteria are similar to those of *E. coli,* and in fact all DNA polymerases can be grouped and classified according to the structure of *six* conserved domains. The most remarkable polymerases are those of thermophilic and hypothermophilic prokarytoes, which can catalyze DNA synthesis at temperatures above 100°C, greatly in excess of the T_m, of DNA. In such organisms, the melting of DNA is likely to occur passively, but the polymerases must demonstrate an extraordinary capability to clamp the primer on the template in order to extend it. *Taq* DNA polymerase, originally isolated from *Thermus aquaticus,* is widely used in the *polymerase chain reaction* It is homologous to *E. coli* pol I, although it lacks 3'→5' exonuclease activity and therefore does not carry out proofreading. Other thermostable DNA polymerases do possess proofreading activity (e.g. *Pfu* polymerase from *Pyrococcus jilriosus).*

Features of Replication in Eukaryotic Cells

The steps in DNA replication in eukaryotic cells are very much the same as the steps in bacterial replication discussed above. The differences in bacterial and eukaryotic replication relate to the details of the proteins that function in each step. Although amino acid sequences of eukaryotic and prokaryotic replication proteins have diverged through evolution, their structures and functions are highly conserved. However, the eukaryotic systems are often somewhat more complicated. Replication continues until two approaching forks meet. The tips of linear eukaryotic chromosomes, called telomeres, require special replication events. Bacterial chromosomes, which contain circular DNA molecules, do not require these special events.

Eukaryotic DNA polymerases.

Eukaryotic cells contain four nuclear DNA polymerases and a fifth which is responsible for organelle genome replication The nuclear enzymes are DNA polymerases α, β, δ and ε. DNA polymerases α and δ are responsible for chromosomal replication. DNA polymerase α has an

associated primase activity but no 3'→5' exonuclease activity, whereas DNA polymerase δ has a proofreading capability. DNA polymerase δ binds an accessory factor called proliferating cell nuclear antigen (PCNA), analogous to the *E. coli* pol III β subunit in that it acts as a sliding ring to increase enzyme processivity. DNA polymerase δ synthesizes both the leading and lagging strands. The function of DNA polymerase α is to extend the RNA primer on the lagging strand and provide the template for an accessory factor, replication factor C (RF-C) whose role may be analogous to that of the *E. coli* γ-complex, *i.e*. to load DNA polymerase and control the processivity of replication on the lagging strand. The role of DNA polymerase ε is unclear. It is structurally very similar to DNA polymerase δ but does not associate with PCNA. It may have a role in the replication fork, or it may be involved in DNA repair, like DNA polymerase β. β is the smallest of the five enzymes and the one with the lowest fidelity and processivity. DNA polymerase γ (similar to *E. coli* pol I) is responsible for the replication of mitochondrial DNA, and a similar enzyme has been isolated from plant chloroplasts.

The two molecules of DNA polymerase used for the synthesis of both leading and lagging strands in bacteria are both DNA polymerase III. They are actually tethered together at the fork by one of the subunits of the protein, keeping their progress tightly coordinated. Many of the other players involved are also linked, so that the entire complex functions as a large molecular replicating machine.

DNA polymerase III has several special properties that make it suitable for its job. Replication of the leading strand of a bacterial chromosome requires the synthesis of a DNA strand several million bases in length. To prevent the DNA polymerase from “falling off” the template strand during this process, the polymerase has a ring-shaped clamp that encircles and slides along the DNA strand that is being replicated, holding the polymerase in place. This sliding clamp has to be opened like a bracelet in order to be loaded onto the DNA, and the polymerase also contains a special clamp loader that does this job.

A second important property of DNA polymerase III is that it is highly accurate. Any mistakes made in incorporating individual nucleotides cause **mutations**, which are changes in the DNA sequence. These mutations can be harmful to the organism. The accuracy of the DNA polymerase results both from its ability to select the correct nucleotide to incorporate, and from its ability to “proofread” its work. Appropriate nucleotide selection depends on base-pairing of the incoming nucleotide with the template strand. At this step, the polymerase makes about one mistake per 1,000 to 10,000 incorporations. Following incorporation, the DNA polymerase

has a way of checking to see that the nucleotide pairs with the template strand appropriately (that is, A only pairs with T, C only pairs with G). In the event that it does not, the DNA polymerase has a second enzymatic activity, called a proofreading exonuclease, or a 3'→5'_ exonuclease, that allows it to back up and remove the incorrectly incorporated nucleotide. This ability to proofread reduces the overall error rate to about one error in a million nucleotides incorporated. Other mechanisms detect and remove mismatched base pairs that remain after proofreading and reduce the overall error rate to about one error in a billion.

Telomere replication

A fundamental difference between eukaryotic and bacterial replication arises because eukaryotic chromosomes are linear and thus have ends. As already stated, the 3'-OH group needed for replication by DNA polymerases is provided at the initiation of replication by RNA primers that are synthesized by primase. This solution is temporary, because eventually the primers must be removed and replaced by DNA nucleotides.

In a circular DNA molecule, elongation around the circle eventually provides a 3'-OH group immediately in front of the primer. After the primer has been removed, the replacement DNA nucleotides can be added to this 3'-OH group. In linear chromosomes with multiple origins, the elongation of DNA in adjacent replicons also provides a 3'-OH group preceding each primer.

At the very end of a linear chromosome, however, there is no adjacent stretch of replicated DNA to provide this crucial 3'-OH group. Once the primer at the end of the chromosome has been removed, it cannot be replaced with DNA nucleotides, which produces a gap at the end of the chromosome, suggesting that the chromosome should become progressively shorter with each round of replication. The chromosome would be shortened each generation, leading to the eventual elimination of the entire telomere, destabilization of the chromosome, and cell death.

But chromosomes don't become shorter each generation and destabilize; the ends of chromosomes—the telomeres—possess several unique features, one of which is the presence of many copies of a short repeated sequence. In the protozoan *Tetrahymena,* this telomeric repeat is CCCCAA), with the G-rich strand typically protruding beyond the C-rich strand): The single-stranded protruding end of the telomere can be extended by telomerase, an enzyme with both a protein and an RNA component (ribonucleoprotein). The RNA part of the enzyme contains from 15 to 22 nucleotides that are complementary to the sequence on the G-rich strand. This sequence pairs with the overhanging 3' end of the DNA and provides a template for the synthesis of additional DNA copies of the repeats. DNA nucleotides are added to the 3' end

of the strand one at a time and, after several nucleotides have been added, the RNA template moves down the DNA and more nucleotides are added to the 3’ end. Usually, from 14-16 nucleotides are added to the 3’ end of the G-rich strand. In this way, the telomerase can extend the 3’ end of the chromosome without the use of a complementary DNA template.

How the complementary C-rich strand is synthesized is not yet clear. It may be synthesized by conventional replication, with primase synthesizing an RNA primer on the 5’ end of the extended (G-rich) template. The removal of this primer once again leaves a gap at the 5’ end of the chromosome, but this gap does not matter, because the end of the chromosome is extended at each replication by telomerase; no genetic information is lost, and the chromosome does not become shorter overall. The extended single-strand end may fold back on itself, forming a terminal loop by nonconventional pairing of bases. This loop could provide a 3’-OH group for the attachment of DNA nucleotides along the C-rich strand**.**

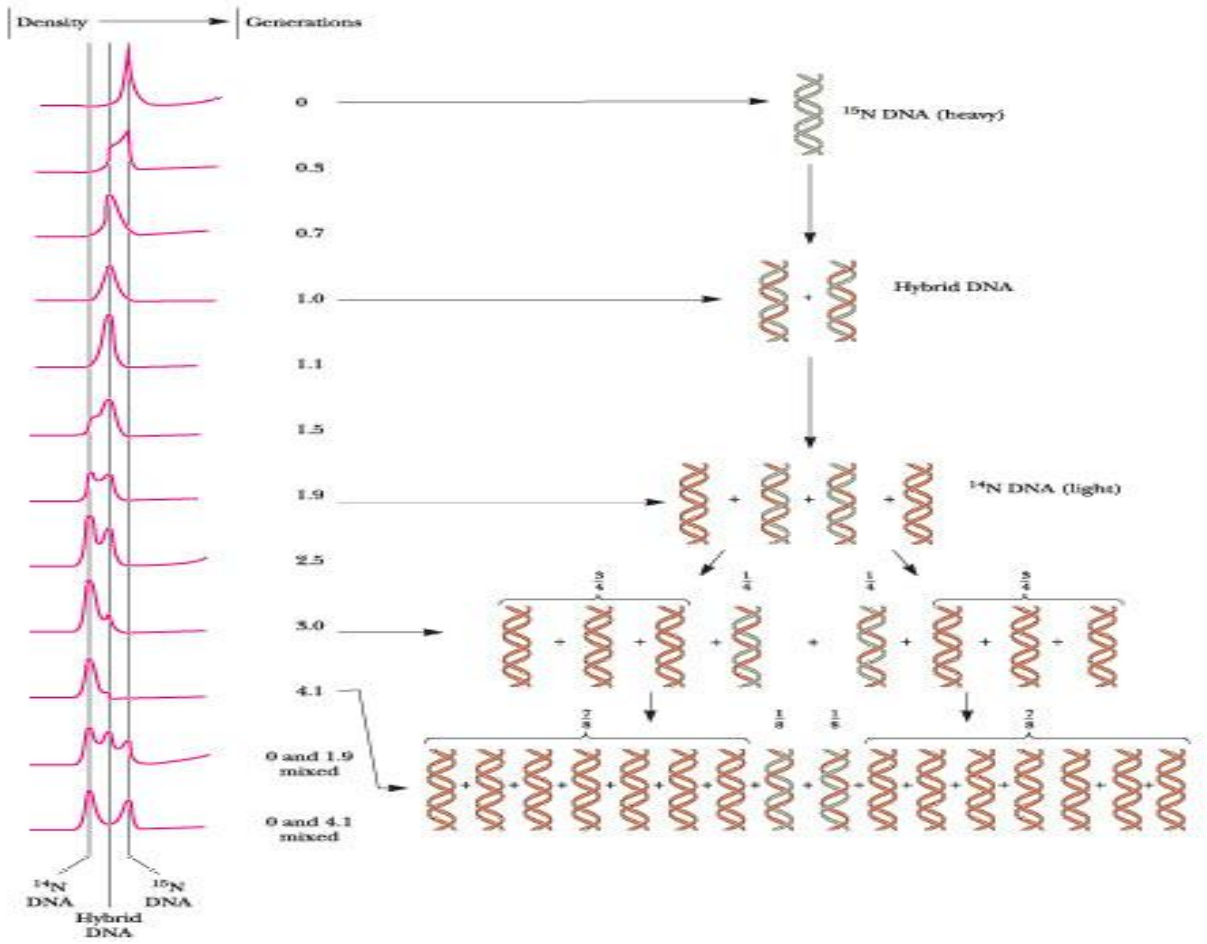

FIG 1-The Meselson and Stahl experiment demonstrating that DNA replication is semiconservative. On the left are shown densitometric traces made of UV absorption photographs taken of the ultracentrifugation cells containing DNA isolated from E. *coli* grown for various generation times after ^{15}N-labeling. The photographs were taken once the migration of the DNA in the density gradient had reached equilibrium. Density increases from left to right. The peaks reveal the positions of the banded DNA with respect to the density of the solution. The number of generations that the E. *coli* cells were grown (following 14 generations of ^{15}N density-labeling) is shown down the middle of the figure. A schematic representation interpreting the pattern expected of semiconservative replication is shown on the right side of this figure.

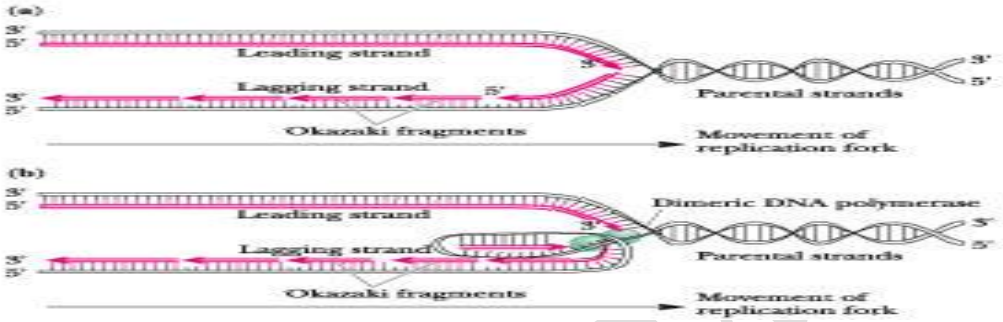

FIG 2-The semidiscontinuous model for DNA replication

FIG3-The chain elongation reaction catalyzed by DNA polymerase.

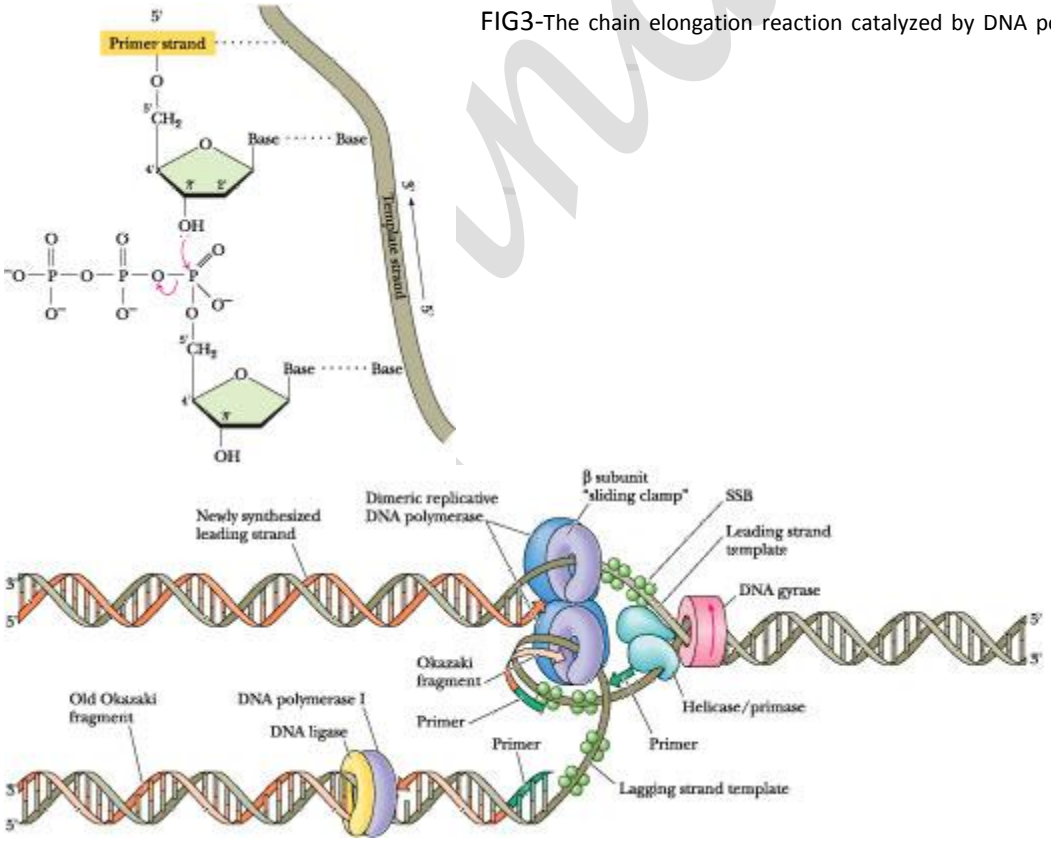

FIG 4-General features of a replication fork. The DNA duplex is unwound by the action of DNA gyrase and helicase, and the single strands are coated with SSB (ssDNA-binding protein). Primase periodically primes synthesis on the

lagging strand. Each half of the dimeric replicative polymerase is a “core” polymerase bound to its template strand by a b-subunit sliding clamp. DNA polymerase I and DNA ligase act downstream on the lagging strand to remove RNA primers, replace them with DNA, and ligate the Okazaki fragments.

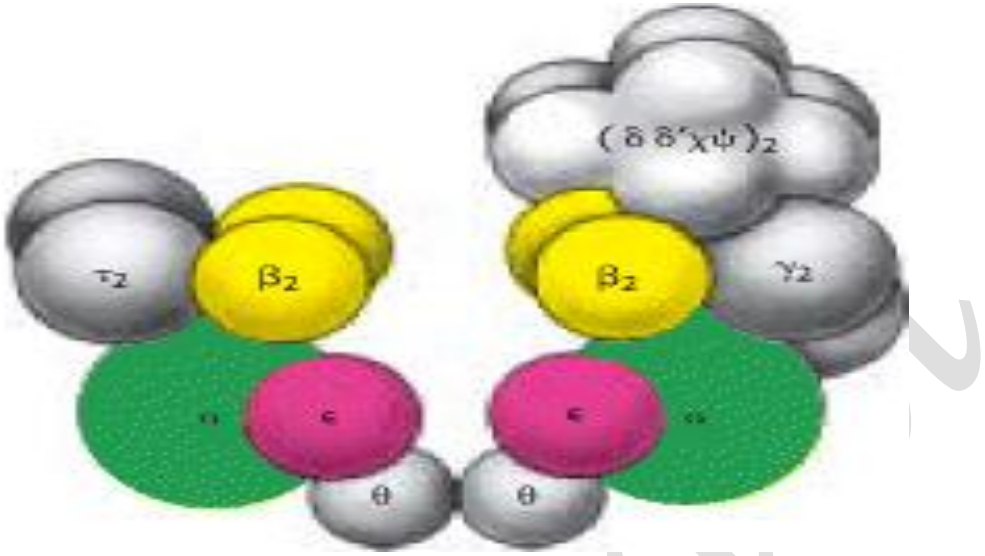

FIG5-Proposed Architecture of DNA Polymerase III Holoenzyme.

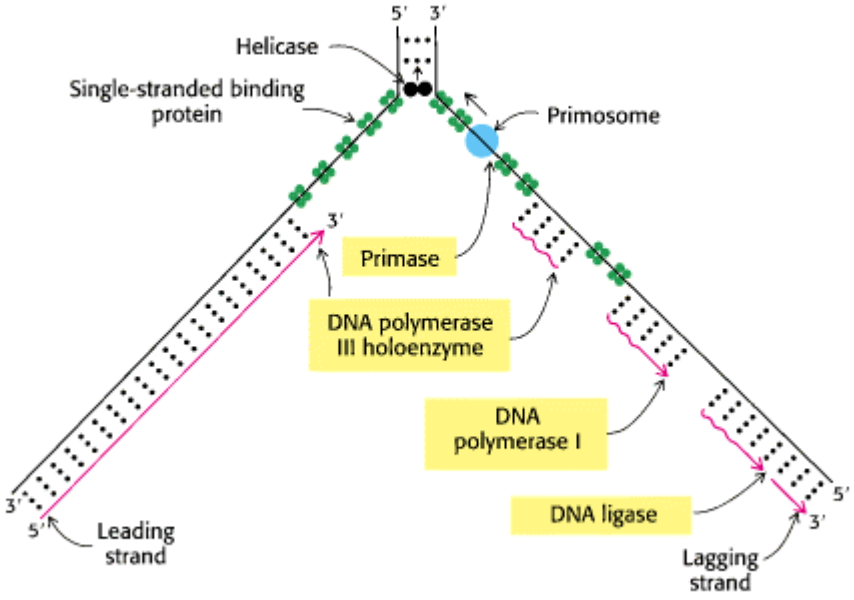

FIG6-Replication Fork

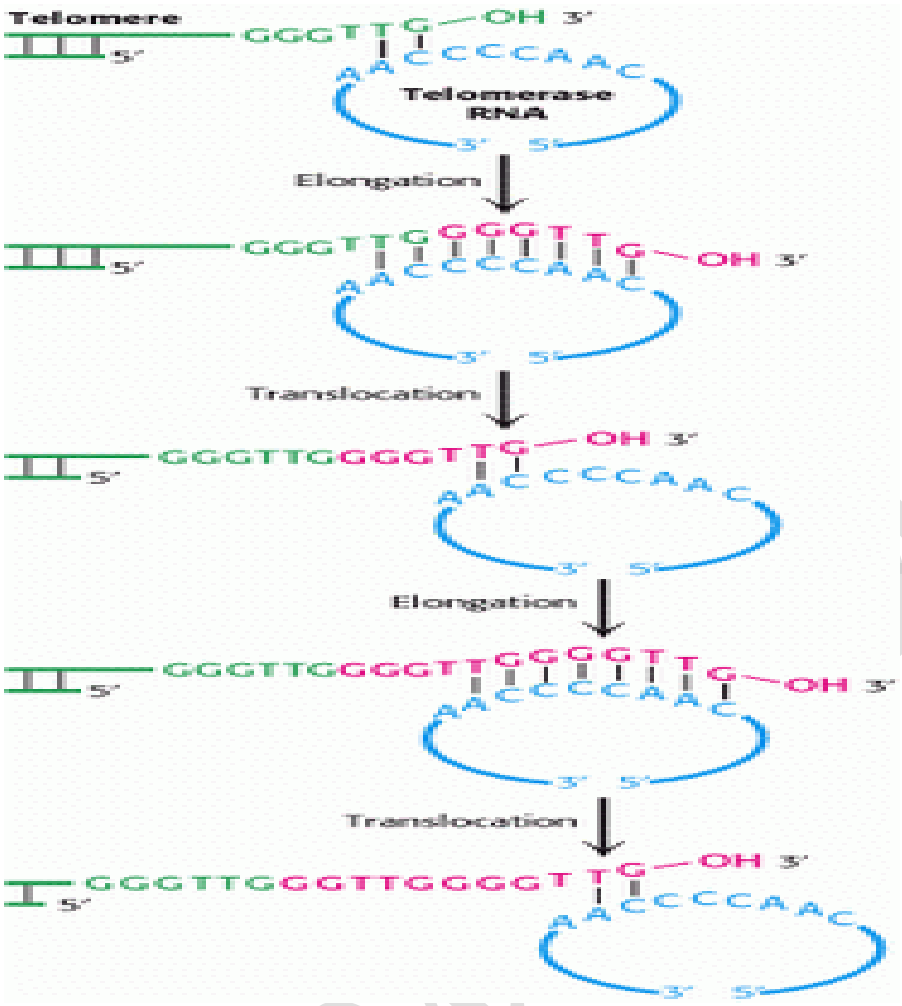

FIG7-Telomere Formation. Mechanism of synthesis of the G-rich strand of telomeric DNA.