Unit 3: The replication of DNA (10 lectures)
Chemistry of DNA synthesis (Kornberg's discovery); general principles – bidirectional, semi conservative and semi discontinuous replication, RNA priming; various models of DNA replication, including rolling circle, θ (theta) mode of replication, replication of linear ds-DNA, replication of the 5'end of linear chromosome; Enzymes involved in DNA replication.

Semi-discontinuous DNA replication

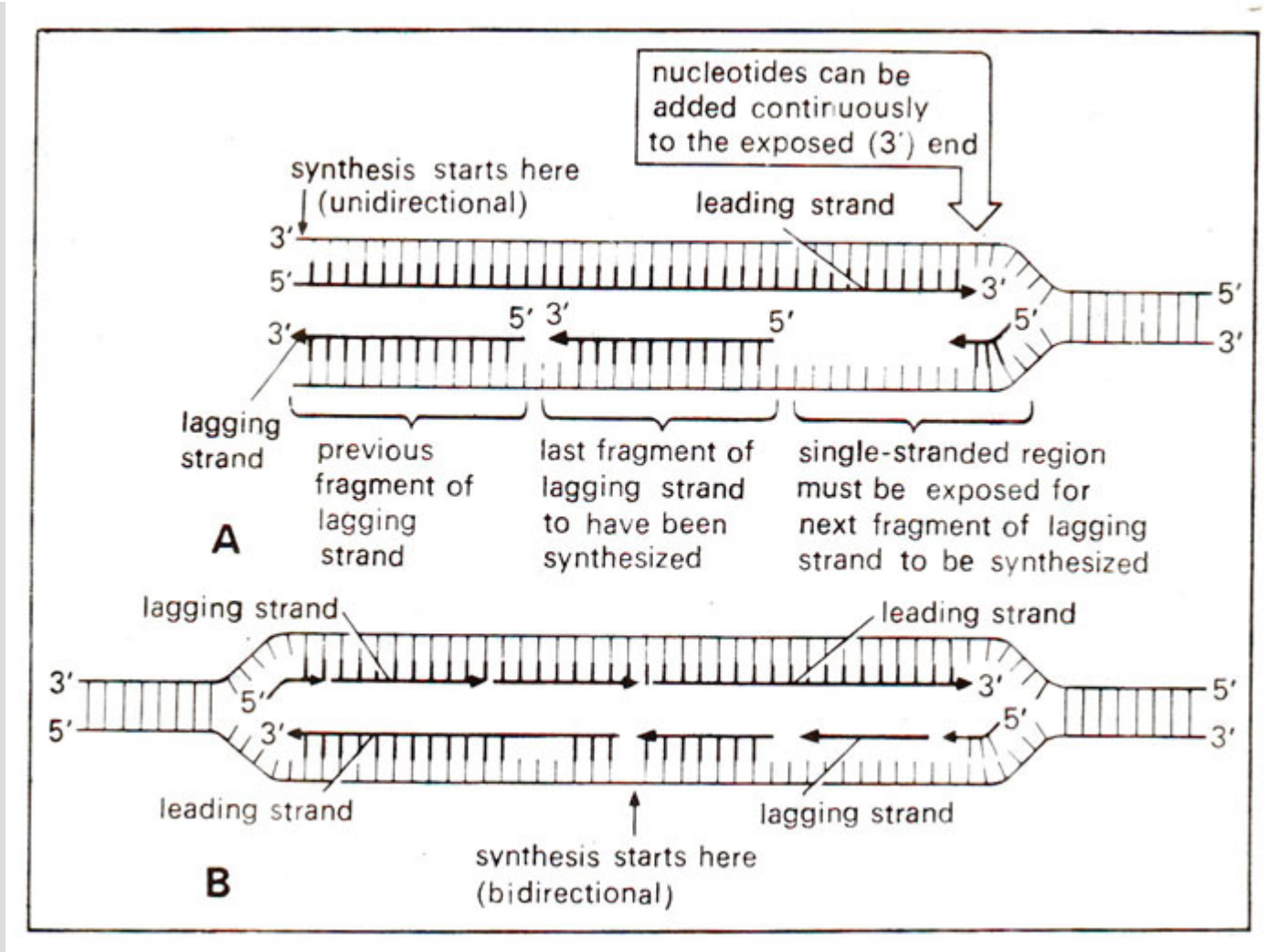


Fig. 26.6. Semi-discontinuous DNA replication under conditions of (A) unidirectional replication, (B) bidirectional replication.

A. Kornberg initially characterized an enzyme, now called **DNA polymerase I** and believed it to be responsible for DNA-replication. However, later it was shown that this enzyme is mainly involved in DNA repair and not in DNA replication. Another enzyme **DNA polymerase III** is now known to be responsible for DNA replication and synthesizes DNA in 5'→3' direction. Since the two strands of DNA have opposite polarities, DNA synthesis can not proceed on both strands, utilizing same enzyme, unless the synthesis proceeds in pieces. Such pieces called Okazaki **pieces** (after the name of discoverer) have actually been observed and it is established

At one time, it was felt that there was enough evidence to suggest that DNA synthesis is discontinuous on both the strands. However, now it is known that DNA synthesis is continuous on one strand and discontinuous on the other strand (Fig. 26.6). When the double helix of DNA unwinds, DNA replication on one of the two strands (3' to 5' stand) can easily proceed continuously in 5' to 3' direction. This is the **leading strand.** On the other strand (5'-3') if the synthesis has to take place in 5' to 3' direction, it has to be synthesized in a direction opposite to that on the leading strand. This strand is the **lagging strand** and on this strand, synthesis takes place in segments discontinuously and these segments are then fused to create an intact lagging strand. This behaviour where the leading strand is synthesized continuously and the lagging strand is synthesized discontinuously is called semi-discontinuous replication.

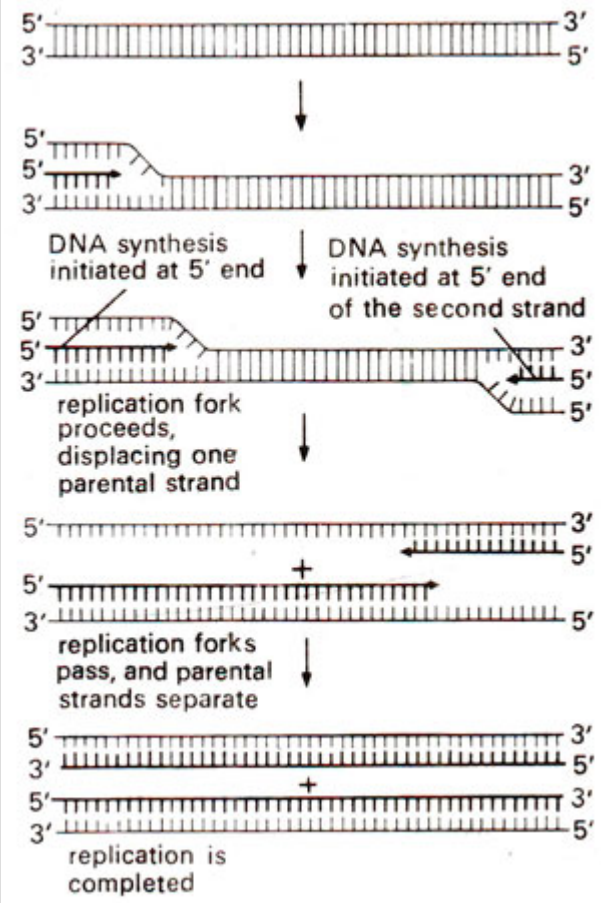
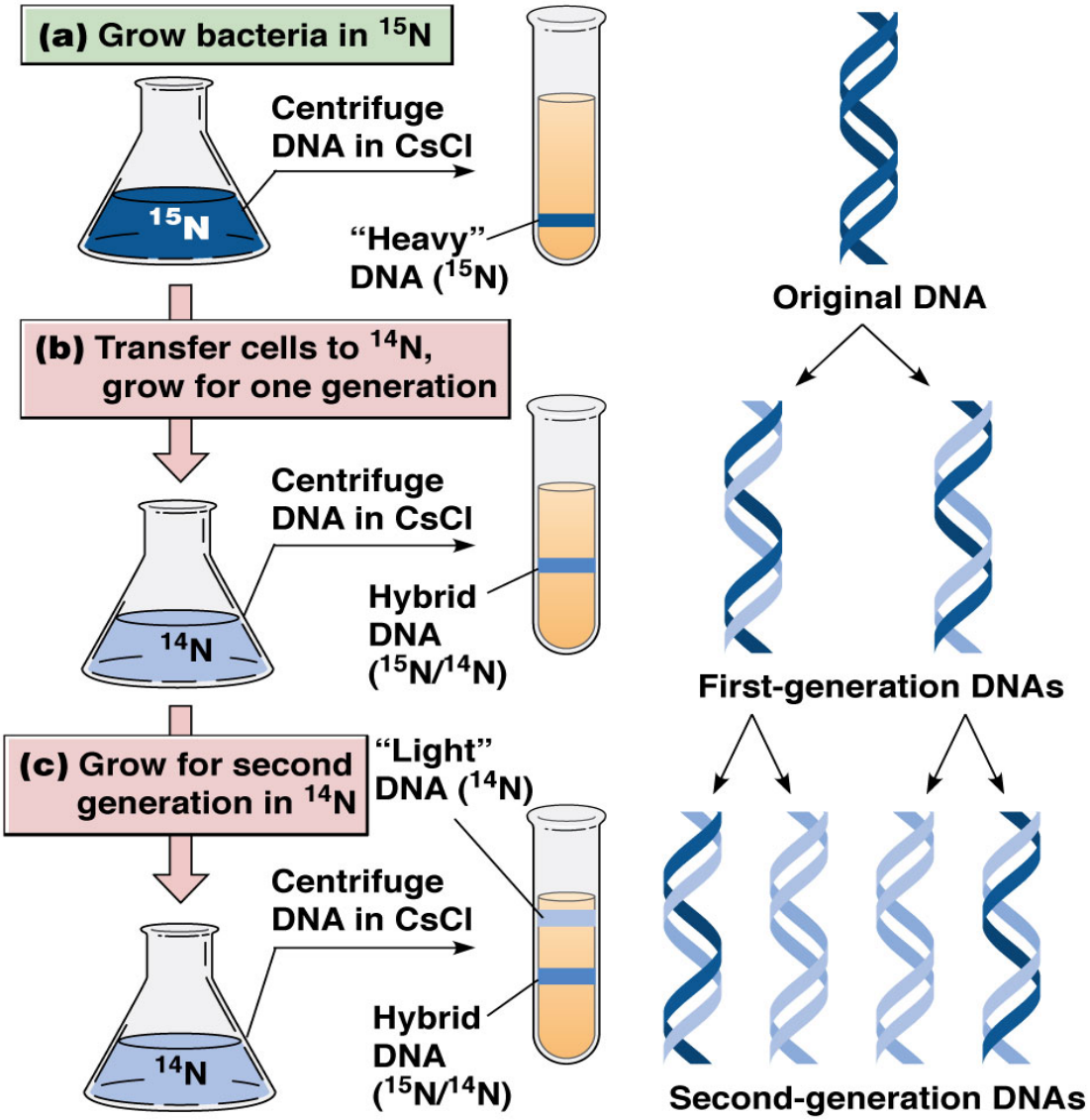


Fig. 26.7. Continuous DNA replication on both strands initiated independently at the two ends (as observed in adenovirus).

In certain viruses like **adenovirus** or Φ29, linear DNA replicates from the two ends by strand . displacement, so that both strands can be copied in 5' to 3' direction simultaneously without any need for discontinuous replication (Fig. 26.7). Therefore, it is obvious that semi-discontinuous replication is the result of a need to synthesize both strands simultaneously from the same origin.

DNA SYNTHESIS/REPLICATION



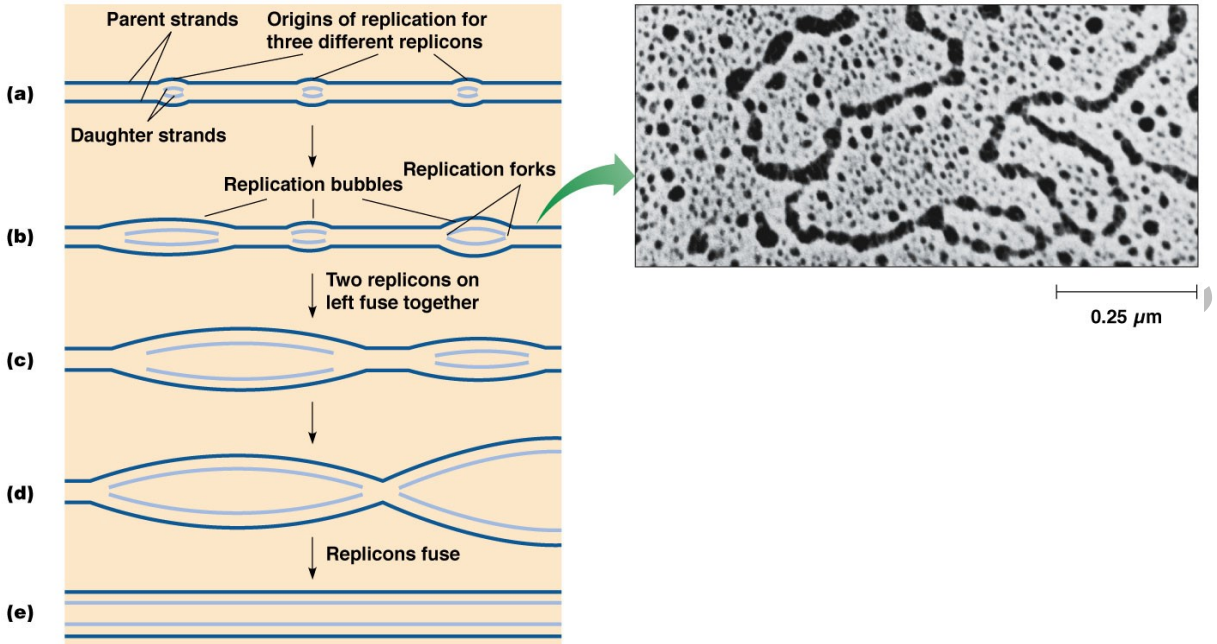
DNA Replication is a semiconservative process that results in a double-stranded molecule that synthesizes to produce two new double stranded molecules such that each original single strand is paired with one newly made single strand.

This was demonstrated by equilibrium density centrifugation (see chapter 19 for details).

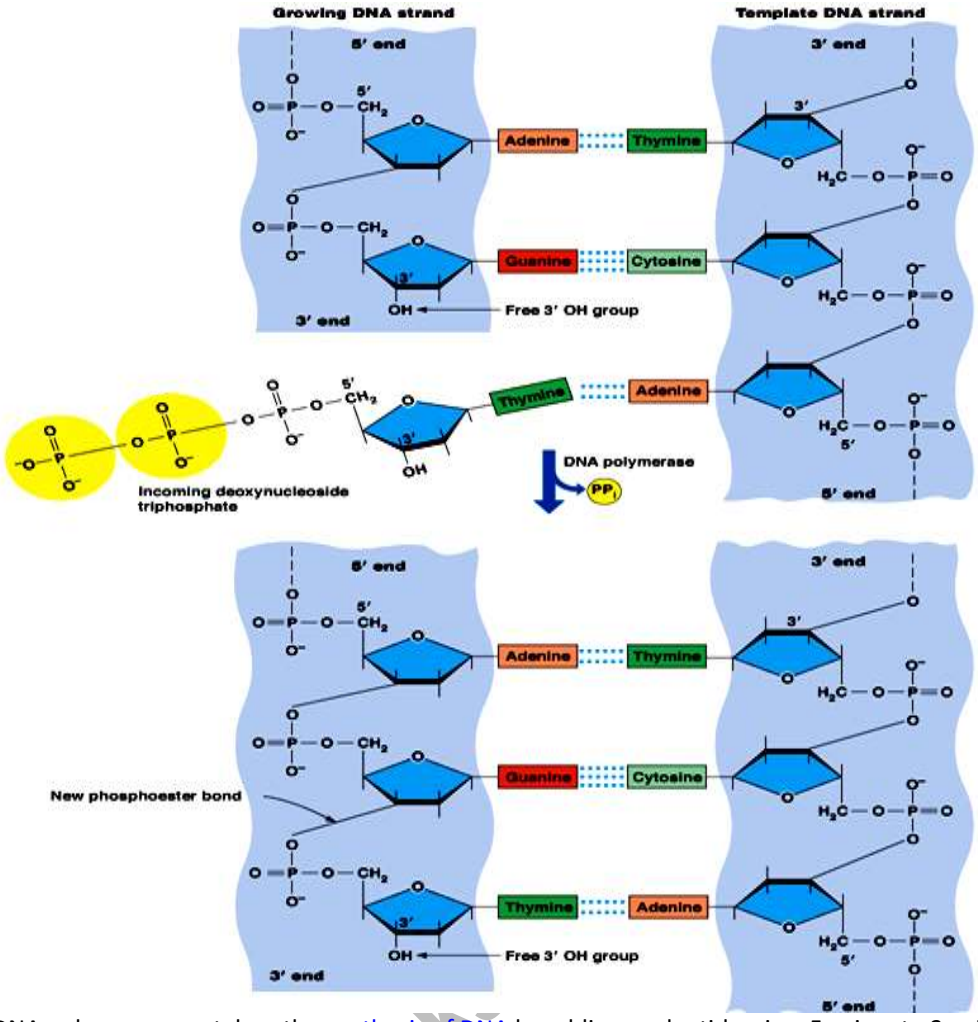
The replication of DNA most often occurs in a bidirectional manner from an origin of replication from which two replication forks move in opposite direction.

In prokaryotes, bidirectional DNA synthesis of the circular genome produces a theta structure (theta replication).

In eukaryotes, DNA replication is initiated in multiple sites along a chromosomes called replicons.



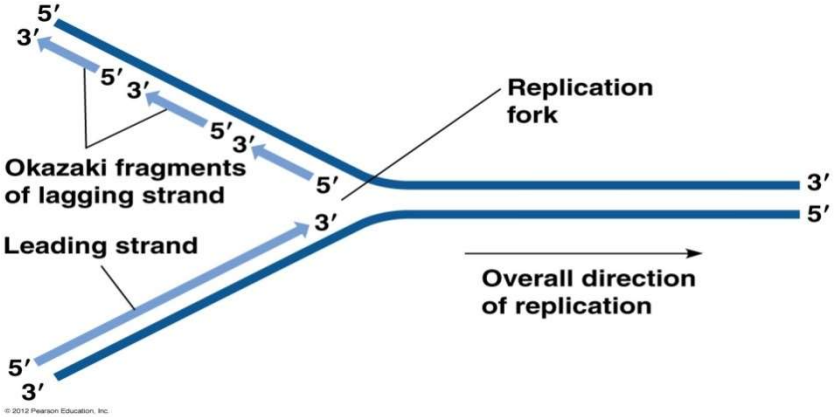
Thousands of replicons, each covering 50 to 300 kilobases, form replication bubbles that fuse to, in the end, make two daughter double stranded DNA molecules.
A number of proteins and protein complexes are involved in DNA synthesis.



DNA polymerases catalyze the synthesis of DNA by adding nucleotides, in a 5-prime to 3-prime direction, utilizing a single stranded region of DNA as a template.

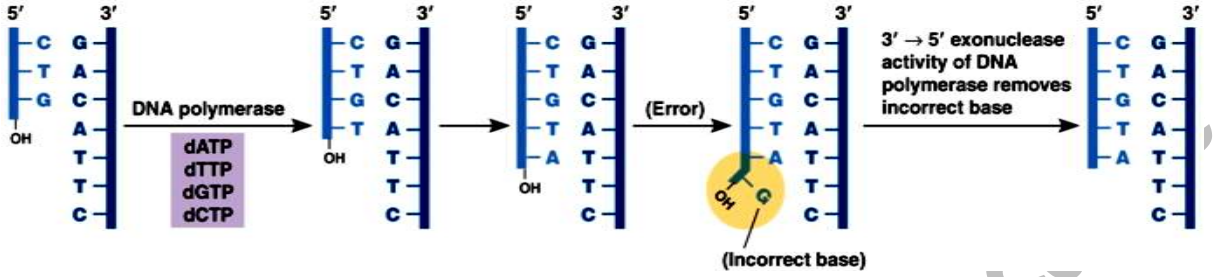
Four deoxynucleotide triphosphates (dATP, dCTP, dGTP,dTTP) only are incorporated into the growing chain by releasing the terminal two phosphate groups and covalently bonding the remaining phosphate to the 3-prime hydroxyl group of the previous nucleotide residue.

Since the 5-prime end does not get added to and the 3-prime end repeatedly does, the DNA strand is said to grow in a 5-prime to 3-prime manner.

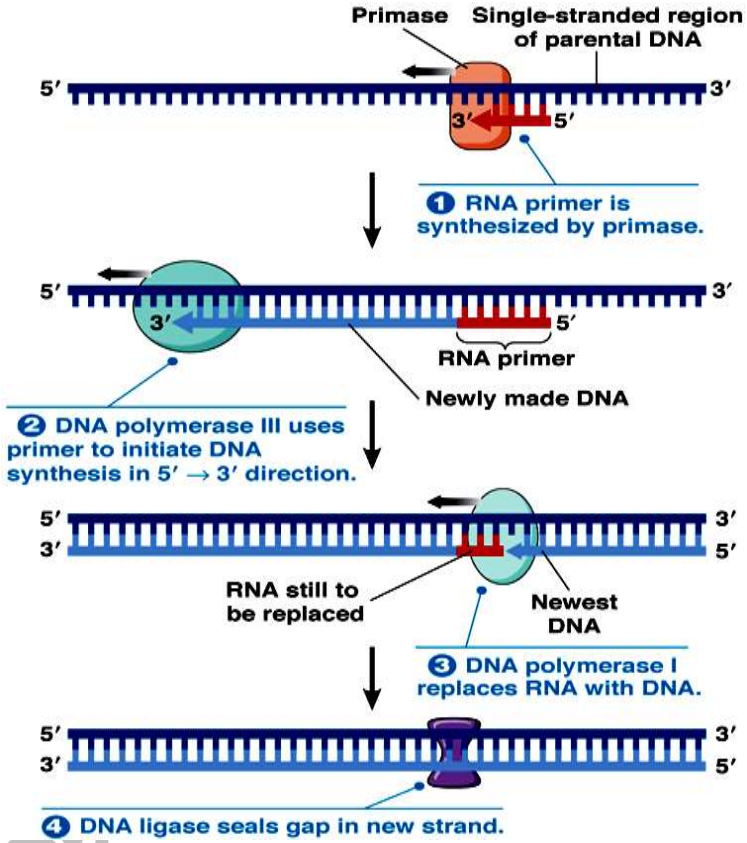


At a replicon, one strand of DNA is made in a continuous manner (the leading strand) and the other in a discontinuous manner (the lagging strand).

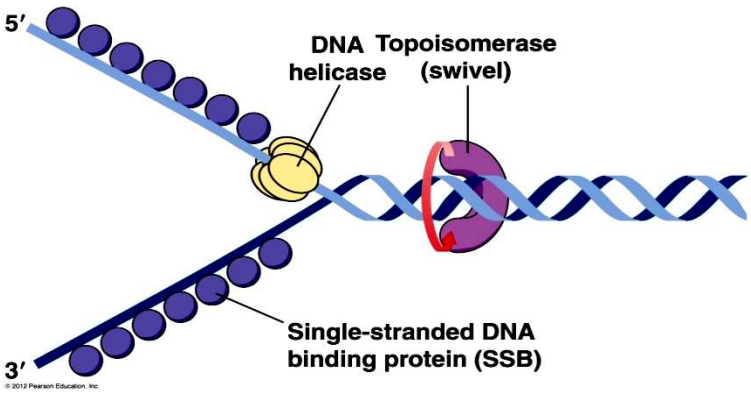
DNA is made in only the 5-prime to 3-prime direction and the replication bubble opens the original double stranded DNA to expose both a 3-prime to 5-prime template (Leading strand template) and it complement.
The lagging strand must be synthesized as a series of discontinuous segments of DNA.
These small fragments are called Okazaki fragments and they are joined together by an enzyme known as DNA ligase.



DNA synthesis is not perfect initially, so a proofreading mechanism is performed by a 3-prime to 5-prime exonuclease activity that is part of DNA polymerase enzyme itself.
Since DNA polyerase requires a template and a free 3-prime hydroxyl group to add nucleotides on to, RNA primers are required to initiate DNA polymerization.

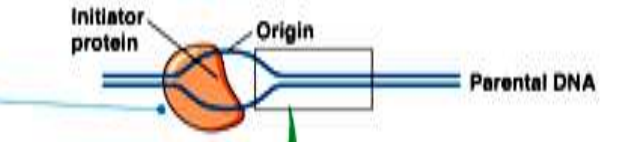


An enzyme, primase which is part of a large complex of proteins called the primosome, synthesizes a small stretch of RNA (the primer) of 3-10 nucleotide in length, which will act as a starting site for the DNA polymerase.
Okazaki fragments are initally made with RNA 5-prime ends which is digested away by the 3-prime to 5-prime exonuclease activity of the adjacent DNA polymerase enzyme just prior to the ligation of the two fragments.
To make the DNA single stranded in the first place to allow DNA synthesis, the DNA must be unwound.

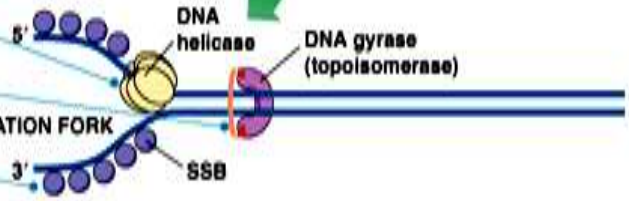


Unwinding double stranded DNA requires helicases, topoisomerases and single-strand binding proteins (SSBPs).

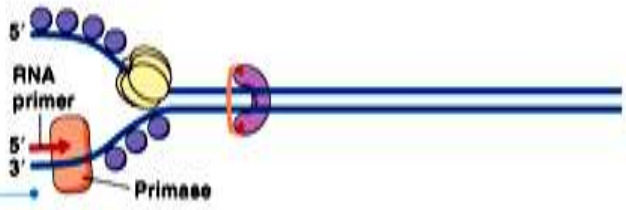
❶ Initiator protein binds to double-stranded DNA at the origin of replication and, using ATP energy, slightly unwinds the DNA.



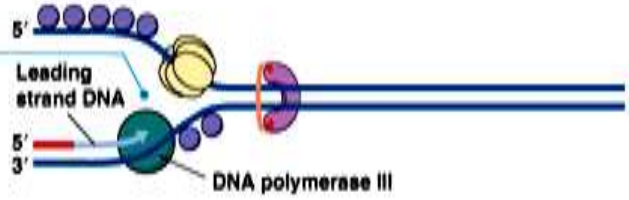
❷ DNA helicase binds to the unwound DNA and continues the unwinding. Meanwhile, DNA gyrase promotes strand separation by inducing negative supercoiling in front of the DNA helicase. Strand separation is maintained by molecules of single-stranded DNA binding protein (SSB), which stabilize the unwound region and allow the separated strands to serve as templates. A replication fork is now in evidence.



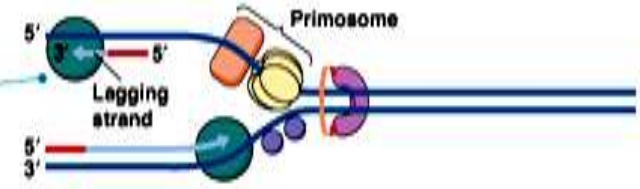
❸ Primase binds to the first initiaing sequence on the leading strand template and synthesizes a short RNA primer that is complementary to the DNA template.



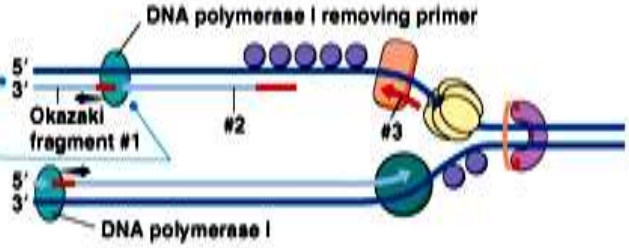
❹ DNA polymerase III (polymerase α followed by polymerase δ or ε in eukaryotes) uses the primer to initiate DNA synthesis by adding deoxyribonucleotides to its 3′ end. The leading strand requires only one priming event because DNA synthesis is continuous in the 5′ → 3′ direction.



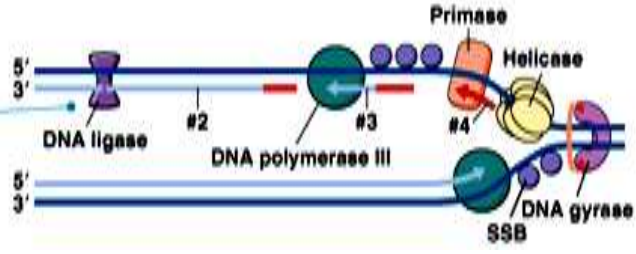
❺ An RNA primer is made for the lagging strand and DNA polymerase then extends the strand. In *E. coli*, the primase is part of the primosome, a protein complex that includes the DNA helicase.



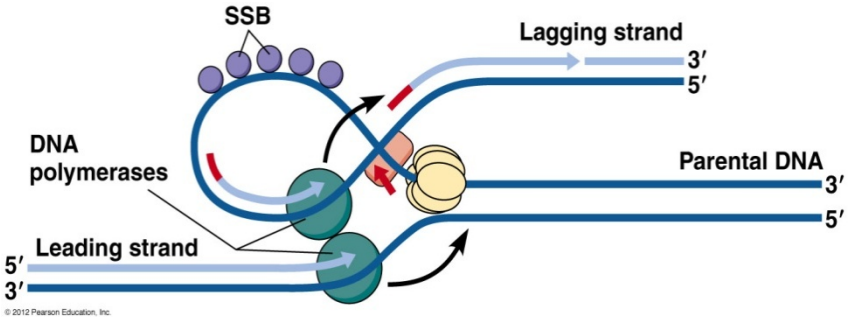
❻ For the lagging strand, DNA synthesis is discontinuous and requires a series of RNA primers (shown in red). DNA is synthesized at the 3′ end of each primer, generating an Okazaki fragment that grows until it meets the adjacent fragment. The RNA primer is then removed by the 5′ → 3′ exonuclease activity of DNA polymerase I and replaced with DNA by the polymerase activity of the same enzyme.



❼ DNA ligase links together adjacent Okazaki fragments. Hereafter, DNA ligase, polymerase I, polymerase III, primase, DNA helicase, and DNA gyrase will be working simultaneously in the vicinity of the replication fork.



The proteins discussed above form a ribosome-sized complex referred to as the replisome.



Arrangement of the replisome such that the lagging-strand is "looped around" allows the machinery to move in one direction while synthesizing DNA from strands in opposing polarities.

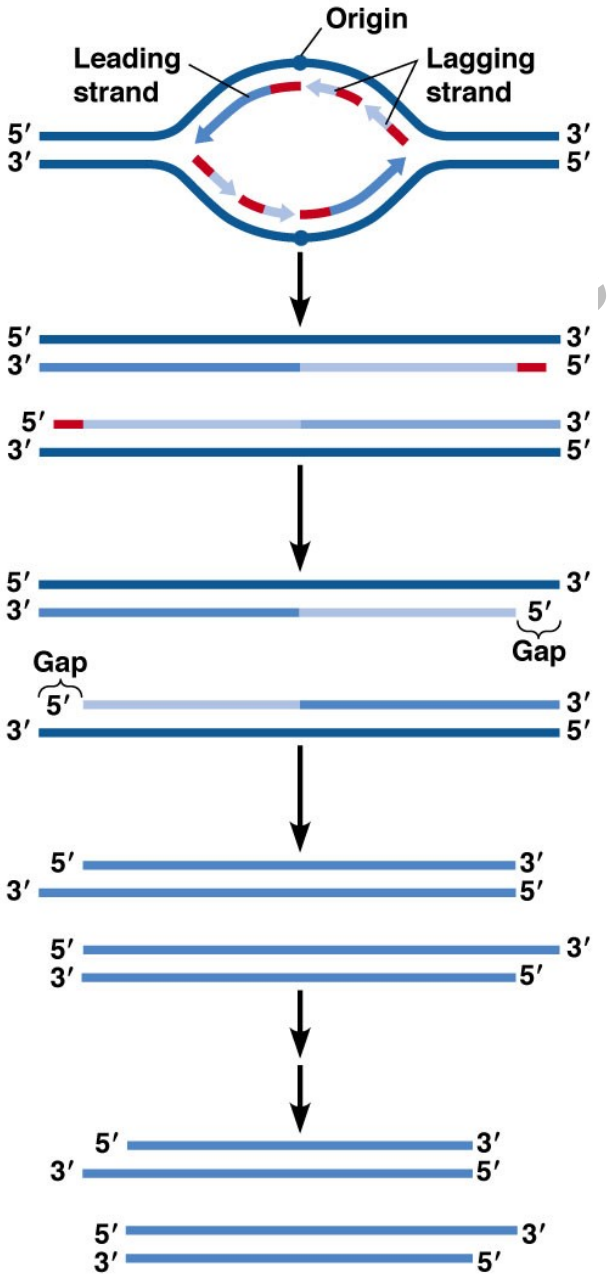
As DNA synthesis requires a RNA primer that will eventually be digested away, standard DNA replication would result in linear chromosmes that would shrink with every round of replication.

1. DNA replication is initiated at the origin; the replication bubble grows as the two replication forks move in opposite directions.

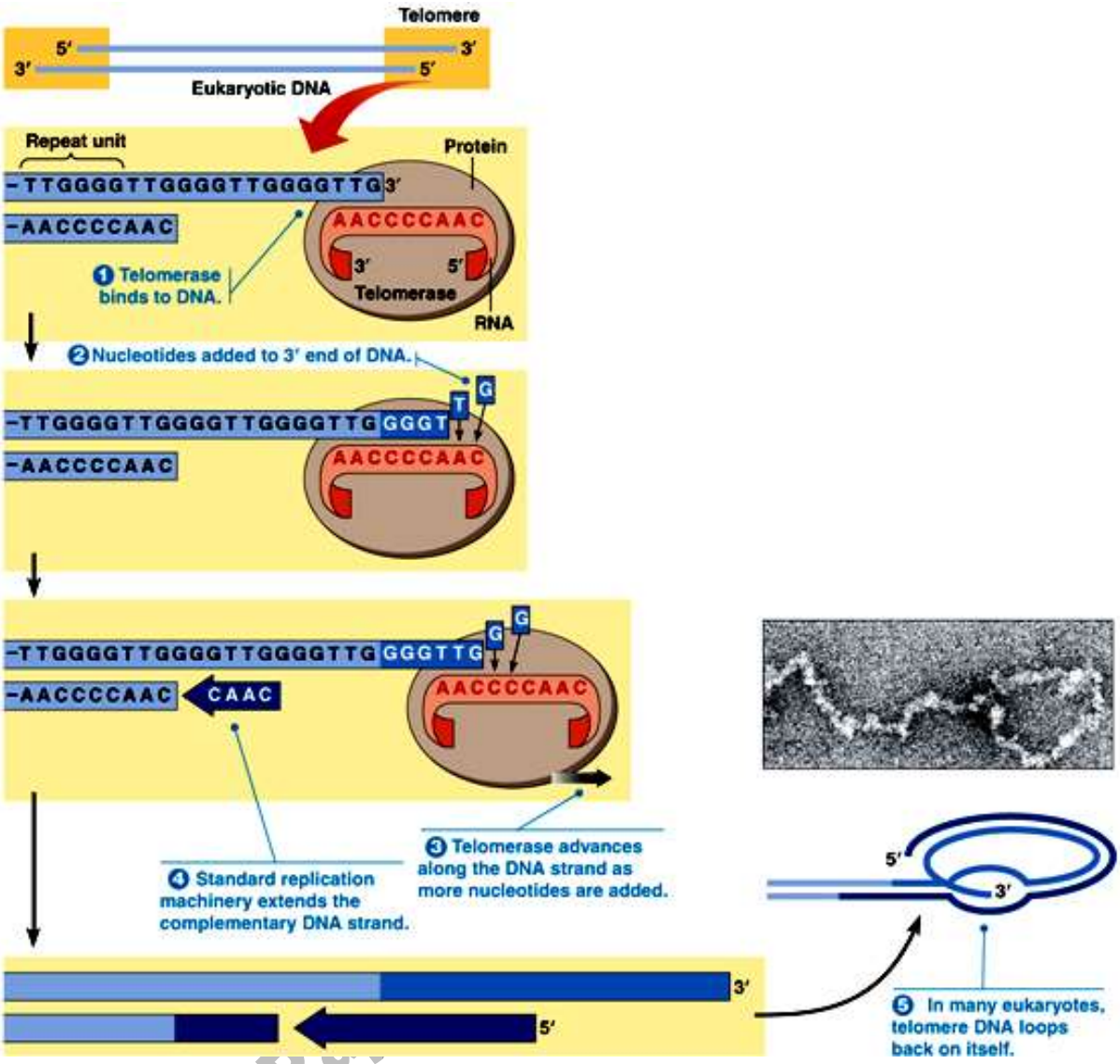
2. Finally only one primer (red) remains on each daughter DNA molecule.

3. The last primers are removed by a 5′→ 3′ exonuclease, but no DNA polymerase can fill the resulting gaps because there is no 3′ OH available to which a nucleotide can be added.

4. Each round of replication generates shorter and shorter DNA molecules.



This is resolved in bacteria by the circular genome which does not have an end.
In linear chromosomes, a specialized structure the telomere solves the end of DNA replication problem.



Telomeres have highly repeated DNA sequences 5'-TTAGGG-3'.
Human chromosomes have between 100 and 1500 copies of this sequence.
Telomerase, a special DNA polymerase, can add additional copies of the 5'-TTAGGG-3' to the end of a chromosome.
The telomerase enzyme is actually a complex containing protein and RNA (a "ribozyme").
The RNA portion has a 5'-CCCTAA-3' region that acts as a template for adding the DNA repeat to the chromosome ends.
The telomerase enzyme is found mostly in the germ cells of multicellular organisms.
In somatic cells, the absence of telomerase results in shorter chromosomal ends with each division and may be the limiting factor in an organism's life span.