Unit 3

Fundamental Process

Unit Map

3.A DNA replication, repair and recombination

DNA replication

The Central Dogma of Molecular Biology

The central dogma of molecular biology describes the flow of genetic information from DNA to RNA and RNA to protein in a biological system. It was firstly given by Francis Crick in 1958 and published in nature in 1970. The double stranded DNA molecule serves as a template for the synthesis of new DNA molecule. DNA replication is the process of making an identical copy of double-stranded DNA, using existing DNA as a template for the synthesis of new DNA strands.

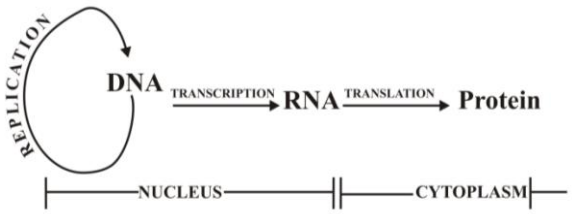

Figure 3.A.1-1
Central Dogma of Molecular Biology
It proposed that information flows in a one-way direction, from DNA to RNA to protein in a biological system and provided a molecular basis for the connection between genotype and phenotype.

3.A.1 Important rules for DNA replication

- **Semi conservative replication**

Semi conservative replication means each strand of DNA serves as a template for the synthesis of a new strand. Watson and Crick proposed the hypothesis of semi conservative replication and the hypothesis was proved by experiments carried out by Matthew Meselson and Franklin Stahl in 1957. In their experiment the *E.coli* cells were grown 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 so that all the nitrogen in their DNA was ^{15}N. The *E. coli* cells grown in the ^{15}N medium were transferred to a fresh medium containing only the ^{14}N isotope, where they were allowed to grow until the cell population just doubled. The DNA isolated from the first-generation cells formed a single band in the CsCl density gradient at a position indicating that the double helical DNA molecules of the daughter cells were hybrids containing one new ^{14}N strand and one parent ^{15}N strand.

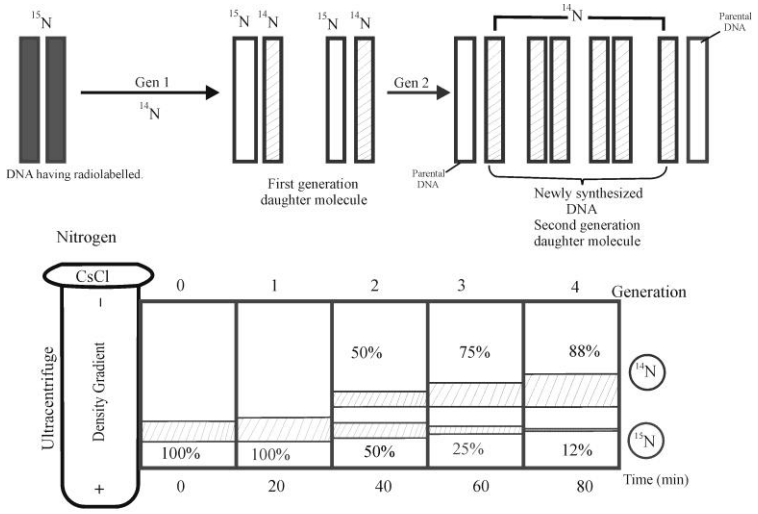

Figure 3.A.1-2
Semi-conservative Replication
In this the two nucleotide strands unwind and each serves as a template for a new DNA molecule. Meselson and Stahl demonstrated that DNA replication is semi-conservative means one strand comes from parent and one is synthesized new.

- **Replication at origin and replication fork**

Ross Inman and colleagues developed a technique denaturation mapping, which is used to determine whether the replication loops originate at a unique point. Inman showed that DNA could be selectively denatured at sequences unusually rich in AT base pairs as AT base pair requires less energy as comparison to GC base pair, generating a reproducible pattern of single-strand bubbles. Isolated DNA containing replication loops can be partially denatured in the same way. For circular DNA molecules, the two-replication forks meet at a point on the side of the circle opposite to the origin. Inman and Schnos by using the technique denaturation mapping demonstrated the bidirectional replication in lambda phage the circular DNA from θ shaped structure and the linear DNA produced eye shaped structure.

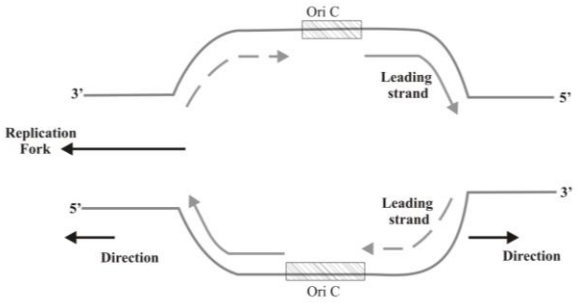

Figure 3.A.1-3
Replication Begins at an Origin and Usually Proceeds Bidirectional
The technique of denaturation mapping revealed that replication loops always initiate at a unique point, which was termed as origin and that replication is usually bidirectional. DNA replication at a single replication fork begins when a double-stranded DNA molecule unwinds to provide two single-strand templates.

DNA replication is semi-discontinuous and precedes in 5' to 3' direction the polymerization in DNA follows SN^2rx i.e. the free 3'OH end attacks the γ- phosphoryl group of following NTP's. As the two strands are antiparallel so the synthesis of leading strand is continuous along the direction of replication fork & the lagging strand is synthesized discontinuously as a single polymerase is functional over both the strands. The lagging strand is being synthesized over a 3'→5' strand by forming small Okazaki fragments.
Reiji Okazaki found that one of the new DNA strands is synthesized in short pieces, called ***Okazaki fragments***. One DNA strand is synthesized continuously and the other discontinuously. The continuous strand, or leading strand, is the one in which 5'to 3' synthesis proceeds in the same direction as replication fork movement. The discontinuous strand, or lagging strand, is the one in which 5'to 3' synthesis proceeds in the direction opposite to the direction of fork movement. A new strand of DNA is always synthesized in the 5' to 3' direction, with the free 3' OH as the point at which the DNA is elongated. Because the two DNA strands are anti-parallel, the strand serving as the template is read from its 3' end toward its 5' end.

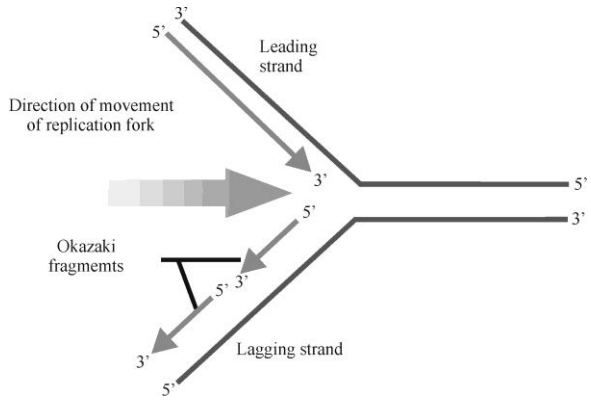

Figure 3.A.1-4
DNA synthesis is continuous on one template strand of DNA and discontinuous on the other
- The synthesis of the new DNA strand occurs in the 5'→3' direction and the template are read in the opposite direction, i.e. 3'→5'.
- The leading strand is continuously synthesized in the direction taken by the replication fork.
- The other strand, the lagging strand, is synthesized discontinuously in short pieces (Okazaki fragments) in a direction opposite to that in which the replication fork moves.
- The Okazaki fragments are joined together by DNA ligase.

➢ **Fidelity of DNA replication**

Fidelity of DNA replication is a key determinant of genome stability and is central to the evolution of species and to the origins of human diseases. The error rate per base pair per round of replication is about 10^{-9} to 10^{-10}. Two cellular systems aid the fidelity of replication. These include the following

i. The 3'-5' exonuclease activity of DNA polymerases acts as a "proofreading" system. It slows down the rate at which incorrectly paired nucleotides are extended by the DNA polymerase. Corrected nucleotides subsequently put on to them very fast. Nucleotides which are incorporated incorrectly are not readily extended by the polymerase very efficiently.

ii. The mismatch repair system makes an additional contribution to accuracy of about 100-fold. It works by scanning the newly replicated DNA, excising any residues that are not properly base-paired and replacing them with the correct nucleotides.

The fidelity of DNA replication can be measured easily and has been for many different types of organisms. It can be expressed in a number of ways. For instance, errors per nucleotide incorporated. It varies substantially between prokaryotes, eukaryotes, unicellular and multicellular organisms. A typical value given by some genetics textbooks is 1 error/genome/1000 bacterial

➢ **Accurate replication**

Replication proceeds with an extraordinary degree of fidelity. In *E.coli*, in vivo base substitution error rate is in the range of 10^{-9}-10^{-11}. The differences between correct and incorrect nucleotides relies on the hydrogen bonds which specify the

correct pairing between complementary bases and also on the geometry of the standard A=T and G=C base pairs. The active site of DNA polymerase I accommodates only base pairs with this geometry. An incorrect nucleotide which is able to hydrogen-bond with a base in the template is generally unable fit into the active site.

➢ **DNA damage**

DNA damage is due to environmental factors and normal metabolic processes inside the cell, occurs at a rate of 1,000 to 1,000,000 molecular lesions per cell per day. While this constitutes only 0.000165% of the human genome's approximately 6 bases, unrepaired lesions in critical genes can impede a cell's ability to carry out its function and appreciably increase the chances of tumor formation. DNA damage can be subdivided into two main types

i. Endogenuos damage such as attack by reactive oxygen species produced from normal metabolic byproducts, especially the process of oxidative deamination; also includes replication errors.

ii. Exogenous damage caused by external agents such as

- Ultraviolet [UV 200-300nm] radiation from the sun
- Other radiation frequencies, including x-rays and gamma rays
- Hydrolysis or thermal disruption
- Certain plant toxins
- Human-made mutagenic chemical, especially aromatic compounds that act as DNA intercalating agents
- Cancer chemotherapy and radiotherapy
- Viruses

The replication of damaged DNA before cell division can lead to the incorporation of wrong bases opposite damaged ones. Daughter cells that inherit these wrong bases carry mutations from which the original DNA sequence is unrecoverable (except in the rare case of a back mutation, for example, through gene conversion).

Damages are physical abnormalities in the DNA. These may include single and double strand breaks, 8-hydroxydeoxyguanosine residues and polycyclic aromatic hydrocarbon adducts. These damages can be recognized by the enzymes and thus they can be correctly repaired if specific information, such as the undamaged sequence in the complementary DNA strand or in a homologous chromosome, is available for copying. If a cell retains DNA damage, transcription of a gene can be prevented and thus translation into a protein will also be blocked. Replication may also be blocked.

3.A.2 Enzymes and proteins involved in DNA replication

➢ **DNA polymerases**

DNA polymerase was purified by Arthur Kornberg in 1955 from *E.coli* cells now known as DNA polymerase I and in 1959; Kornberg was awarded with Noble Prize in physiology and medicine. *E.coli* has five distinct DNA polymerases.

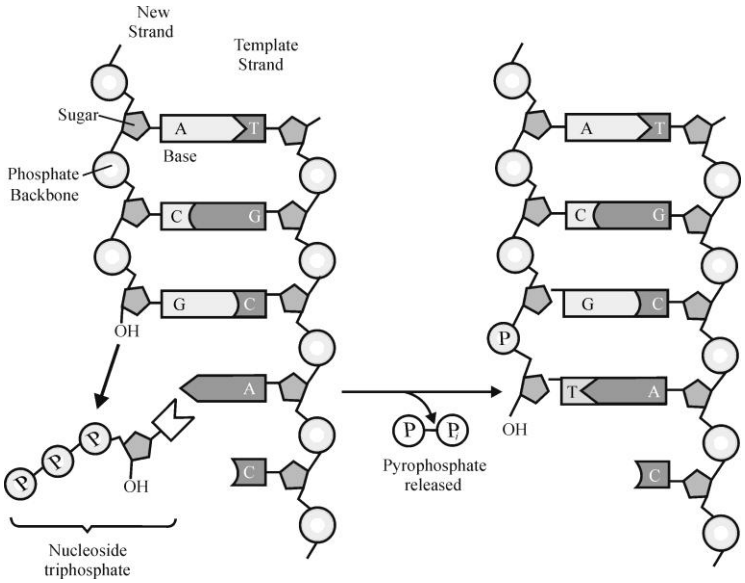

Figure 3.A.2.1
DNA is synthesized by DNA polymerases

- DNA polymerase activity requires a single unpaired strand to act as template and a primer strand to provide a free hydroxyl group at the 3' end, to which a new nucleotide unit is added.
- Each incoming nucleotide is selected in part by base pairing to the appropriate nucleotide in the template strand.
- The reaction product has a new free 3' hydroxyl, allowing the addition of another nucleotide.

DNA synthesis occurs by phosphoryl group transfer. 3′-OH group is nucleophile and attacks α phosphorous of the incoming deoxynucleoside 5′-triphosphate. The reaction is

$$(dNMP)n + \alpha NTP \rightarrow (dNMP)n+1+PPi$$
DNA Lengthened DNA

Important points about DNA polymerase

- DNA polymerases require a template
- Polymerases require a primer
- A primer is a strand segment with a free 3′-OH to which a nucleoside can be added
- Primer terminus is the free 3′-OH group. Most primers are RNA oligonucleotides rather than DNA
- DNA polymerase either dissociates or moves along the template and adds nucleotide after adding nucleotides to the growing DNA strand.

- ***E.coli* has 5 DNA polymerases**
  DNA Pol I (Kornberg enzyme)It possesses four enzymatic activities
  - 5′-3′ (forward) DNA-dependent polymerase activity requiring 3′ primer site and a templates strand
  - 3′-5′ (reverse) exo-nuclease activity that mediates to proof reading
  - 5′-3′ (forward) exo-nuclease activity mediating nick translation during DNA repair
  - 5′-3′ (forward) RNA dependent DNA polymerase activity

  DNA Pol II It helps in DNA repair
  DNA Pol III It is a primary enzyme known as Holoenzyme involved in DNA replication in ***E.coli.*** It consists of three assemblies i.e. the Pol III core, the beta sliding clamp processivity factor and the clamp loading complex.
  Core subunits α-polymerase activity hub, ξ-exonucleolytic proof reader, θ-stabilizer for ξ
  DNA Pol IV and V Both are γ family DNA polymerase, have no proofreading ability.

- **Eukaryotic DNA Polymerases**
  DNA Pol III It is main enzyme responsible for replication, it is λ, β, γ, δ, δ', ε, θ, Ƭ, ϰ, Ψ in eukryotes. Where γ δ complex (γ, ϰ, δ, δ', Ψ) is sliding clamp loader, β subunit is sliding clamp. The λ-subunit have 5′→3′ polymerase activity, Ƭ subunit is basically dimerization unit, ε subunit have 3′→5′exo-nuclease activity so perform proofreading. The subunits λ, ε, θ are core enzyme.

The function of each subunit of DNA polymerase is summarized in the table below

Subunit	Function of Subunit	
α	Polymerization activity	
ε	3′→5′ Proofreading exonculease	
θ	Core polymerase	
τ	Stable template binding; core enzyme dimerization	
γ	Clamp load	Clamp-loading (λ) complex thatloads β subunits on lagging strand at each Okazaki fragment
δ	Clamp opener	
δ′	Clamp loader	
χ	Interaction with SSB	
Ψ	Interaction with γ and χ	
β	DNA clamp required for optimal processivity	

Comparison between 3 polymerases

DNA polymerase	I	II	II
Structural gene	polA	polB	polC (dnaE)
Subunits (number of different types)	1	7	>10
3′→5′Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/s)	16-20	40	250-1,000
Processivity (nucleotides added before polymerase dissociates	3-200	1,500	>500,000