**17BTU302 MOLECULAR BIOLOGY 4H - 4C**

**Total hours/week: L:4 T:0 P:0 Marks: Internal: 40 External: 60 Total: 100**

**Scope:** Molecular biology deals with the molecular characterization and structures and functions of cells.

**Objective:** This paper will enable the students to learn about the cells at molecular level.

**UNIT-I**

**DNA structure and organization:** DNA as genetic material, Structure of DNA, Types of DNA, Organization of DNA in prokaryote and eukaryotic cells, Chromosome biology – histone and non-histone proteins, organization.

**UNIT-II**

**DNA replication:** Replication of DNA in prokaryotes and eukaryotes: Semi-conservative nature of DNA replication, Bi-directional replication, DNA polymerases, Replication complex: Pre-priming proteins, primosome, replisome, Rolling circle replication, Unique aspects of eukaryotic chromosome replication, Fidelity of replication.

**UNIT-III**

**Transcription and RNA processing:** RNA structure and types of RNA, Transcription in prokaryotes: Prokaryotic RNA polymerase, role of sigma factor, promoter, Initiation, elongation and termination of RNA chains Transcription in eukaryotes: Eukaryotic RNA polymerases, transcription factors, promoters, enhancers, mechanism of transcription initiation, promoter clearance and elongation RNA splicing and processing: processing of pre-mRNA: 5' cap formation, polyadenylation, splicing, rRNA and tRNA splicing.

**UNIT-IV**

**Regulation of gene expression and translation:** Regulation of gene expression in prokaryotes: Operon concept (inducible and repressible system), Genetic code and its characteristics, Prokaryotic and eukaryotic translation: ribosome structure and assembly, Charging of tRNA, aminoacyl tRNA synthetases, Mechanism of initiation, elongation and termination of polypeptides, Fidelity of translation, Inhibitors of translation, Posttranslational modifications of proteins.

**UNIT-V**

**DNA damage, repair and homologous recombination:** DNA damage and repair: causes and types of DNA damage, mechanism of DNA repair: Photoreactivation, base excision repair, nucleotide excision repair, mismatch repair, trans-lesion synthesis, recombinational repair, nonhomologous end joining. Homologous recombination: models and mechanism.

**References**

1. Karp, G. (2013). *Cell and Molecular Biology: Concepts and Experiments* (7th ed.). Hoboken, US: John Wiley & Sons. Inc.
2. Watson, J. D., Baker T.A., Bell, S. P., Gann, A., Levine, M., & Losick, R. (2008). *Molecular Biology of the Gene* (6th ed.). Cold Spring Harbour Lab. Press, Pearson Pub.
3. De Robertis, E.D.P., & De Robertis, E.M.F. (2006). *Cell and Molecular Biology* (8th ed.). Lippincott Williams and Wilkins, Philadelphia.
4. Becker, W.M., Kleinsmith, L.J., Hardin. J., & Bertoni, G. P. (2009). *The World of the Cell* (7th ed.). San Francisco: Pearson Benjamin Cummings Publishing.

# KARPAGAM ACADEMY OF HIGHER EDUCATION

DEPARTMENT OF BIOTECHNOLOGY
II B.Sc., BIOTECHNOLOGY – SEMESTER III
LECTURE PLAN – MOLECULAR BIOLOGY (17BTU302)

| S.No | Lecture Duration (hr) | Topics | Support materials |
|---|---|---|---|
| 1. | 1 | DNA as genetic material – Basic information about DNA | T1: 85 |
| 2. | 1 | Griffith Experiment | T1: 86 |
| 3. | 1 | Avery and Hershey - Chase Experiment | T1: 87 |
| 4. | 1 | Different types of DNA – A DNA, B-DNA, Z-DNA, Chargaff's Rule. | R1: 281<br>T1: 88 |
| 5. | 1 | Watson and Crick model – Double helix structure of DNA, Parallel and Antiparallel | T1: 88-90 R2: 287 |
| 6. | 1 | Organization of DNA in prokaryotes<br>- Chromatin, Chromosome, Nucleosome | R1: 962, 963 |
| 7. | 1 | Organization of DNA in Eukaryotes<br>- Histone Proteins, Non-Histone proteins | R1: 964, 965 |
| 8. | 1 | Centromere, Telomere, C- Value paradox | T1- 109-110 |
| 9. | 1 | Revision | - |
| 10. | 1 | Revision | - |
| 11. | 1 | Replication – Introduction, Replication in Prokaryotes – Initiation, Elongation , Termination | T1: 1195; T1: 1188; T1: 1208 |
| 12. | 1 | Rolling circle method – Plasmid DNA model, Bacteriophage Model | T1: 1190-1193 |
| 13. | 1 | DNA polymerases – DNA polymerase I, II, III | T1: 1176-1181; T1: 1126-1127; T1: 1181-1187 |
| 14. | 1 | Eukaryotic replication – Leading strand formation, | T1: 1201-1213; |
| 15. | 1 | Lagging strand formation, Okazaki fragments | T1-1174-1175 |

| | | | |
|---|---|---|---|
| 16. | 1 | Role of Topoisomerase I and II | T1:1209-1213 |
| 17. | 1 | RNA primer – DNA polymerase I as DNA repair | T1: 1176 |
| 18. | 1 | Fidelity of DNA replication | T1: 1200 - 1202 |
| 19. | 1 | Revision | - |
| 20. | 1 | Revision | - |
| 21. | 1 | DNA damage and repair - Types of DNA damage, Mechanism of DNA repair: | T1: 1214-1216 |
| 22. | 1 | Photoreactivation DNA damage | T1: 1214-1215 |
| 23. | 1 | Base excision repair mechanism | T1: 1218 |
| 24. | 1 | Nucleotide excision repair | T1: 1216-1217 |
| 25. | 1 | Mismatch repair | T1: 1220 |
| 26. | 1 | Trans-lesion synthesis | T1: 1221 |
| 27. | 1 | Recombinational repair | T1: 1225 |
| 28. | 1 | Non-homologous end joining | T1: 1225 |
| 29. | 1 | Homologous recombination: models and mechanism | T1 -1225-1226 |
| 30. | 1 | Revision | - |
| 31. | 1 | Revision | - |
| 32. | 1 | RNA structure and types of RNA | T1: 1264 |
| 33. | 1 | Transcription in Prokaryotes - RNA polymerase, Role of sigma factor, Promoter, Initiation, Elongation and Termination of RNA chains | T1: 1265; T1:1266; T1: 1267-69; T1: 1275-1277 |
| 34. | 1 | Transcription in Eukaryotes - Mechanism of Initiation | T1: 1277-1279; |
| 35. | 1 | Promoter clearance and Elongation | T1:1284-1286: T1: 1292-1293 |
| 36. | 1 | RNA splicing and Processing | T1: 1307 |
| 37. | 1 | 5’ cap formation | T1:1286-1302 |
| 38. | 1 | Polyadenylation (poly A tail) | T1: 1302-1304 |
| 39. | 1 | r RNA and t RNA splicing | T1: 1305-1306 |
| 40. | 1 | Processing of Pre m RNA | T1: 1324-1332 |

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|---|---|---|---|
| 41. | 1 | Revision | - |
| 42. | 1 | Revision | - |
| 43. | 1 | Regulation of gene expression in prokaryotes | T1:1308-1325 |
| 44. | 1 | Operon concept – Lac Operon | T1:1262-1266 |
| 45. | 1 | Trp Operon | T1:1296-1298 |
| 46. | 1 | Genetic Code and its Charcteristics | T1 – 1338-1342 |
| 47. | 1 | Translation – Structure and assembly | T1:1349-1342 |
| 48. | 1 | Charging of t-RNA | T1: 1363-1365 |
| 49. | 1 | Mechanism of Initiation, Elongation, Termination of polypeptides | T1: 1373-1378; T1: 1379-1390; T1: 1391-1394 |
| 50. | 1 | Fidelity of Translation | T1:1395-1396 |
| 51. | 1 | Post translational modification of proteins | T1:1403-1407 |
| 52. | 1 | Inhibitors in translation | T1:1408-1421 |
| 53. | 1 | Regulation of gene expression in prokaryotes | T1:1308-1325 |
| 54. | 1 | Revision | - |
| 55. | 1 | Revision | - |

**References**

T1: Donald Voet and Judith Voet; 2012, Biochemistry, 4th Edition, John Wiley and Sons. Inc.

R1: Daniel L. Nelson and Michael M. Cox; 2005 Biochemistry – Lehininger 5th Edition, W. H. Freeman and Company, Newyork.

R2: Kenneth A. Mason, Jonathan B. Losos and Susan R. Singer; 2017, Biology, MC. GrawHill, Education, Newyork.

## MOLECULAR BIOLOGY 17BTU302

### UNIT I

**DNA structure and organization:** DNA as genetic material, Structure of DNA, Types of DNA, Organization of DNA in prokaryote and eukaryotic cells, Chromosome biology – histone and non-histone proteins, organization.

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References

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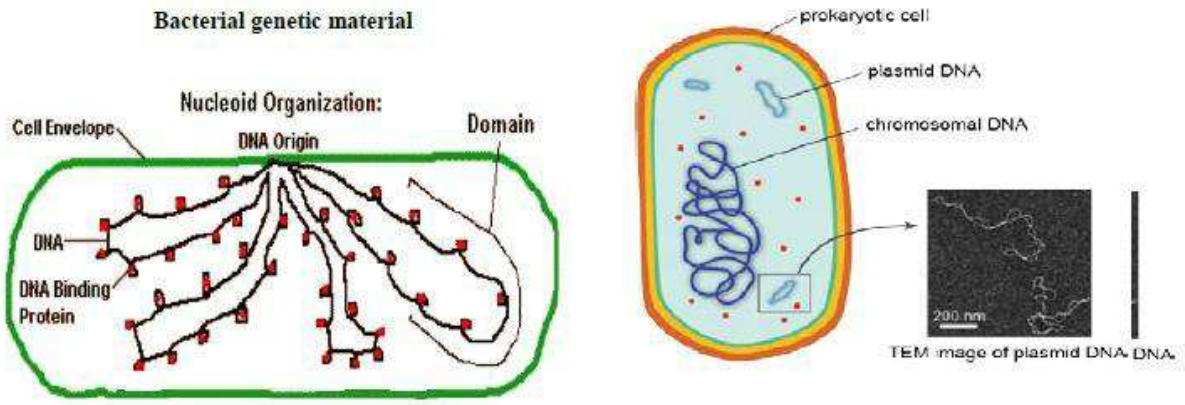
# DNA as Genetic Material

- All cells have the capability to give rise to new cells and the encoded information in a living cell is passed from one generation to another. The information encoding material is the genetic or hereditary material of the cell.
- The research at the end of the 19th century had verified Mendelian inheritance and it was also believed that the genetic material is in the chromosome.
- However, scientists still didn't know the true features of the genetic material. In the early twentieth century, biologists believed that proteins carried genetic information.
- But the Griffith experiment with *Streptococcus pneumoniae* (1928), Avery, MacLeod and McCarty experiment (1944) on transforming principle and Hershey-Chase experiment (1952) on bacteriophage T2, confirms that DNA is genetic material.
- Genetic material is the material that determines the inherited characteristics of a functional organism. It has the following properties:
  - It must be stable
  - It must be capable of being expressed when needed
  - It must be capable of accurate replication
  - It must be transmitted from parent to progeny without change

## Prokaryotic genetic material

1. The prokaryotic (bacterial) genetic material is usually concentrated in a specific clear region of the cytoplasm called nucleiod.
2. The bacterial chromosome is a single, circular, double stranded DNA molecule mostly attached to the plasma membrane at one point. It does not contain any histone protein. *Escherichia coli* DNA is circular molecule 4.6 million base pairs in length, containing 4288 annotated protein-coding genes (organized into 2584 operons), seven ribosomal RNA (rRNA) operons, and 86 transfer RNA (tRNA) genes. Certain bacteria like the *Borrelia burgdorferi* possess array of linear chromosome like eukaryotes.
3. Besides the chromosomal DNA many bacteria may also carry extra chromosomal genetic elements in the form of small, circular and closed DNA molecules, called plasmids. They

generally remain floated in the cytoplasm and bear different genes based on which they have been studied. Some of the different types of plasmids are F plasmids, R plasmids, virulent plasmids, metabolic plasmids etc. Below Figure depicts a bacterial chromosome and plasmid.




**Virus genetic material**

1. The chromosomal material of viruses is DNA or RNA which adopts different structures.
2. It is circular when packaged inside the virus particle.

**Eukaryotic genetic material**

1. A Eukaryotic cell has genetic material in the form of genomic DNA enclosed within the nucleus.
2. Genes or the hereditary units are located on the chromosomes which exist as chromatin network in the non-dividing cell/interphase. This will be discussed in detail in the coming sections.

## The Griffith Experiment - Hereditary Information Can Pass between Organisms

- The identification of the nucleus as the repository of hereditary information focused attention on the chromosomes, which were already suspected to be the vehicles of Mendelian inheritance.
- Specifically, biologists wondered how the **genes,** the units of hereditary information studied by Mendel, were actually arranged in the chromosomes.
- They knew that chromosomes contained both protein and deoxyribonucleic acid (DNA). Which of these held the genes? Starting in the late 1920s and continuing for about 30 years, a series of investigations addressed this question.

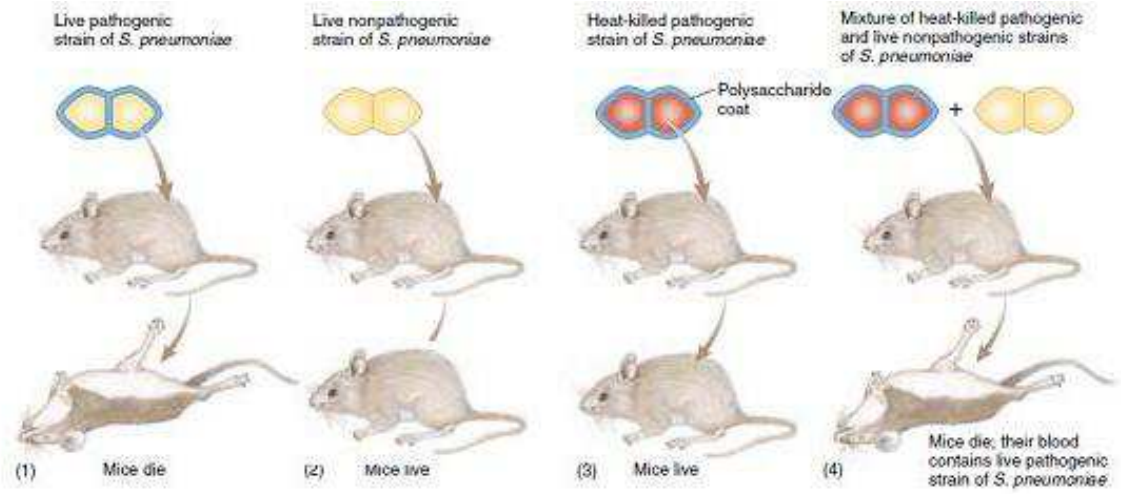
- In 1928, British microbiologist Frederick Griffith made a series of unexpected observations while experimenting with pathogenic (disease-causing) bacteria. When he infected mice with a virulent strain of *Streptococcus pneumonia* bacteria (then known as *Pneumococcus*), the mice died of blood poisoning.

- However, when he infected similar mice with a mutant strain of *S. pneumoniae* that lacked the virulent strain's polysaccharide coat, the mice showed no ill effects. The coat was apparently necessary for virulence. The normal pathogenic form of this bacterium is referred to as the S form because it forms smooth colonies on a culture dish. The mutant form, which lacks an enzyme needed to manufacture the polysaccharide capsule, is called the R form because it forms rough colonies.

- To determine whether the polysaccharide coat itself had a toxic effect, Griffith injected dead bacteria of the virulent S strain into mice; the mice remained perfectly healthy.

- As a control, he injected mice with a mixture containing dead S bacteria of the virulent strain and live coatless R bacteria, each of which by itself did not harm the mice. Unexpectedly, the mice developed disease symptoms and many of them died. The blood of the dead mice was found to contain high levels of live, virulent *Streptococcus* type S bacteria, which had surface proteins characteristic of the live (previously R) strain.

- Somehow, the information specifying the polysaccharide coat had passed from the dead, virulent S bacteria to the live, coatless R bacteria in the mixture, permanently transforming the coatless R bacteria into the virulent S variety. **Transformation** is the transfer of genetic material from one cell to another and can alter the genetic makeup of the recipient cell.



**Griffith's discovery of transformation -**

(*1*) The pathogenic of the bacterium *Streptococcus pneumoniae* kills many of the mice it is injected into. The bacterial cells are covered with a polysaccharide coat, which the bacteria themselves synthesize.

(*2*) Interestingly, an injection of live, coatless bacteria produced no ill effects. However, the coat itself is not the agent of disease.

(*3*) When Griffith injected mice with dead bacteria that possessed polysaccharide coats, the mice were unharmed. (*4*) But when Griffith injected a mixture of dead bacteria with polysaccharide coats and live bacteria without such coats, many of the mice died, and virulent bacteria with coats were recovered. Griffith concluded that the live cells had been ―transformed‖ by the dead ones; that is, genetic information specifying the polysaccharide coat had passed from the dead cells to the living ones.

## The Avery Experiments

- The agent responsible for transforming *Streptococcus* went undiscovered until 1944. In a classic series of experiments, Oswald Avery and his coworkers Colin MacLeod and Maclyn McCarty characterized what they referred to as the ―transforming principle.‖
- They first prepared the mixture of dead S *Streptococcus* and live R *Streptococcus* that Griffith had used. Then Avery and his colleagues removed as much of the protein as they could from their preparation, eventually achieving 99.98% purity. Despite the removal of nearly all protein, the transforming activity was not reduced.

Moreover, the properties of the transforming principle resembled those of DNA in several ways:

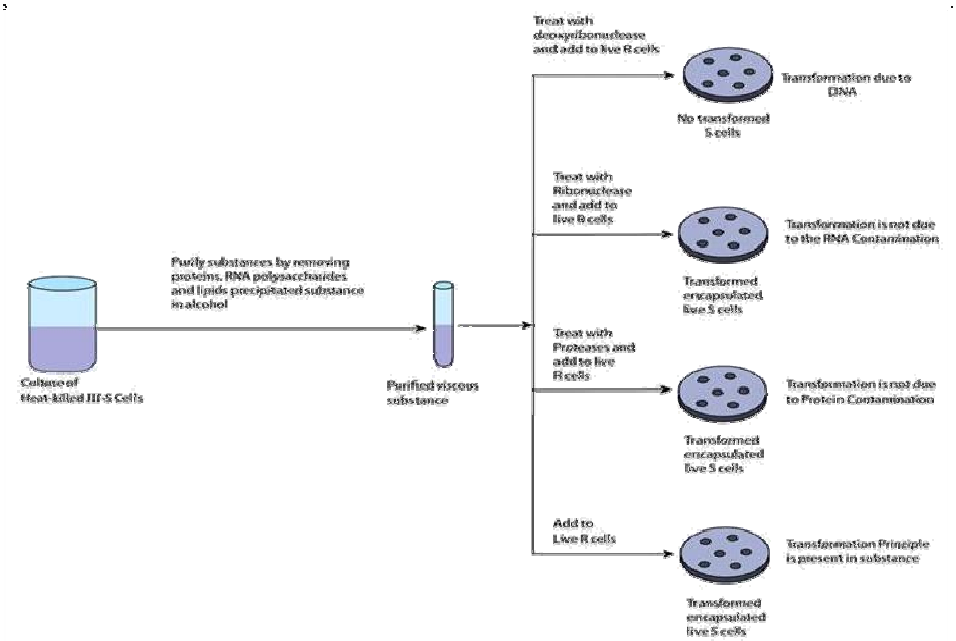
**1.** When the purified principle was analyzed chemically, the array of elements agreed closely with DNA.

**2.** When spun at high speeds in an ultracentrifuge, the transforming principle migrated to the same level (density) as DNA.

**3.** Extracting the lipid and protein from the purified transforming principle did not reduce its activity.

**4.** Protein-digesting enzymes did not affect the principle's activity; nor did RNA-digesting enzymes.

**5.** The DNA-digesting enzyme DNase destroyed all transforming activity. The evidence was overwhelming. They concluded that ―a nucleic acid of the deoxyribose type is the fundamental unit of the transforming principle of *Pneumococcus* Type III‖—in essence, that DNA is the hereditary material.




Avery, Macleod and McCarty's experiment demonstrating the Griffith's transformation

## The Hershey–Chase Experiment

- Avery‘s results were not widely accepted at first, as many biologists preferred to believe that proteins were the repository of hereditary information. Additional evidence supporting

  Avery‘s conclusion was provided in 1952 by Alfred Hershey and Martha Chase, who experimented with **bacteriophages,** viruses that attack bacteria.

- Viruses, consist of either DNA or RNA (ribonucleic acid) surrounded by a protein coat. When a *lytic* (potentially cell-rupturing) bacteriophage infects a bacterial cell, it first binds to

  the cell‘s outer surface and then injects its hereditary information into the cell. There, the hereditary information directs the production of thousands of new viruses within the bacterium. The bacterial cell eventually ruptures, or lyses, releasing the newly made viruses.

- To identify the hereditary material injected into bacterial cells at the start of an infection, Hershey and Chase used the bacteriophage T2, which contains DNA rather than RNA. They

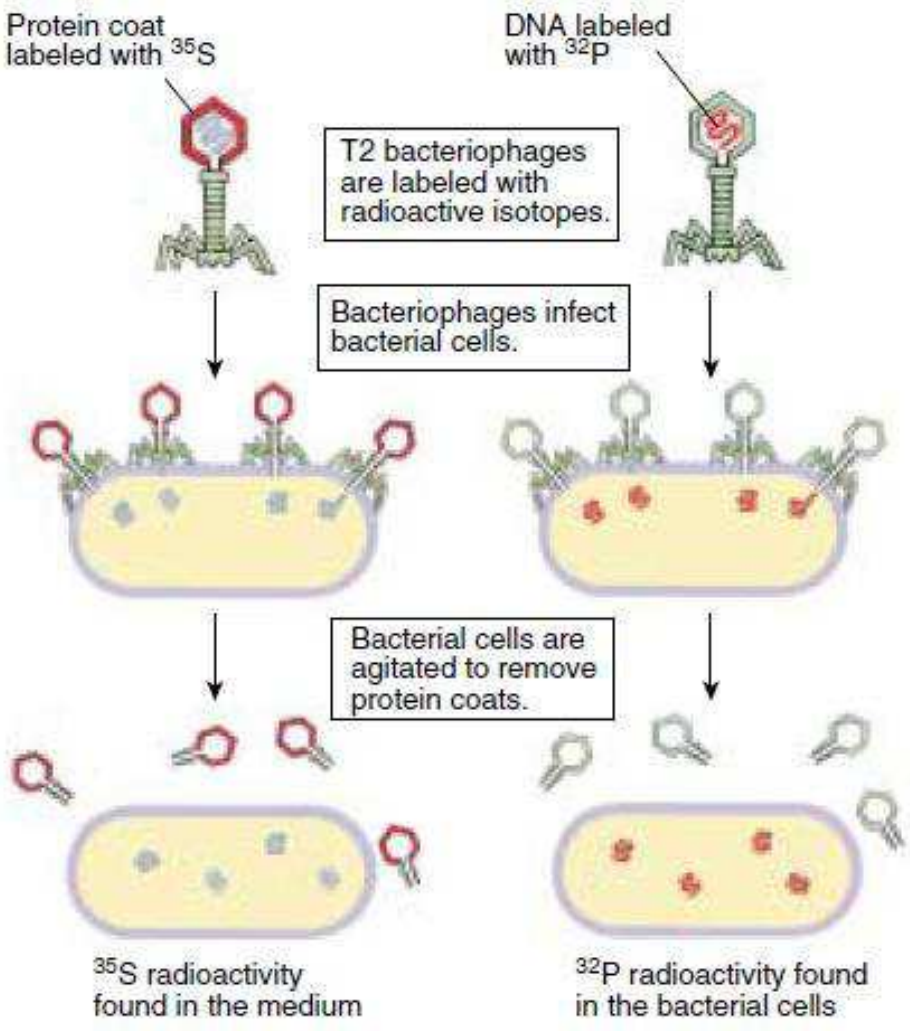
  labeled the two parts of the viruses, the DNA and the protein coat, with different radioactive isotopes that would serve as tracers.

- In some experiments, the viruses were grown on a medium containing an isotope of phosphorus, 32P, and the isotope was incorporated into the phosphate groups of newly

  synthesized DNA molecules. In other experiments, the viruses were grown on a medium

containing 35S, an isotope of sulfur, which is incorporated into the amino acids of newly synthesized protein coats. The 32P and 35S isotopes are easily distinguished from eachother because they emit particles with different energies when they decay.

- After the labeled viruses were permitted to infect bacteria, the bacterial cells were agitated violently to remove the protein coats of the infecting viruses from the surfaces of the bacteria. This procedure removed nearly all of the 35S label (and thus nearly all of the viral protein) from the bacteria. However, the 32P label (and thus the viral DNA) had transferred to the interior of the bacteria and was found in viruses subsequently released from the infected bacteria. Hence, the hereditary information injected into the bacteria that specified the new generation of viruses was DNA and not protein.




**Hershey and Chase experiment -** and that 35S radioactivity did not infected cells and radioactivity did. concluded that DNA, not was for the of new .

## Different forms of DNA

- The Watson-Crick structure is also referred to as B-form DNA, or B-DNA. The B form is the most stable structure for a random-sequence DNA molecule under physiological conditions and is therefore the standard point of reference in any study of the properties of DNA. Two structural variants that have been well characterized in crystal structures are the A and Z forms.

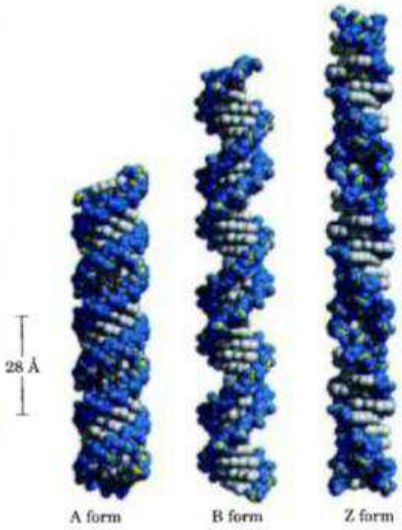
- These three DNA conformations are shown, with a summary of their properties. The A form is favored in many solutions that are relatively devoid of water. The DNA is still arranged in a right-handed double helix, but the helix is wider and the number of base pairs per helical turn is 11, rather than 10.5 as in B DNA.

- The plane of the base pairs in A-DNA is tilted about 20° with respect to the helix axis. These structural changes deepen the major groove while making the minor grooves hallower. The reagents used to promote crystallization of DNA tend to dehydrate it, and thus most short DNA molecules tend to crystallize in the A form.

- Z-form DNA is a more radical departure from the B structure; the most obvious distinction is the left handed helical rotation. There are 12 base pairs per helical turn, and the structure appears more slender and elongated. The DNA backbone takes on a zigzag appearance. Certain nucleotide sequences fold into left-handed Z helices much more readily than others. Prominent examples are sequences in which pyrimidines alternate with purines, especially alternating C and G or 5-methyl-C and G residues. To form the left handed helix in Z-DNA, the purine residues flip to the syn conformation, alternating with pyrimidines in the anti-conformation. The major groove is barely apparent in Z-DNA, and the minor groove is narrow and deep.

- Whether A-DNA occurs in cells is uncertain, but there is evidence for some short stretches (tracts) of Z-DNA in both bacteria and eukaryotes' These ZDNA tracts may play a role (as yet undefined) in regulating the expression of some genes or in genetic recombination.

| | A form | B form | Z form |
|---|---|---|---|
| Helical sense | Right handed | Right handed | Left handed |
| Diameter | ~26 Å | ~20 Å | ~18 Å |
| Base pairs per helical turn | 11 | 10.5 | 12 |
| Helix rise per base pair | 2.6 Å | 3.4 Å | 3.7 Å |
| Base tilt normal to the helix axis | 20° | 6° | 7° |
| Sugar pucker conformation | C-3′ endo | C-2′ endo | C-2′ endo for pyrimidines; C-3′ endo for purines |
| Glycosyl bond conformation | Anti | Anti | Anti for pyrimidines; syn for purines |




Comparison of A, B, and Z forms of DNA. Each structure shown here has 36 base pairs. The bases are shown in gray, the phosphate atoms in yellow, and the ribose and phosphate oxygens in Blue. Blue is the color used to represent DNA strand is in later chapters.The table summarizes some properties of the three forms of DNA.

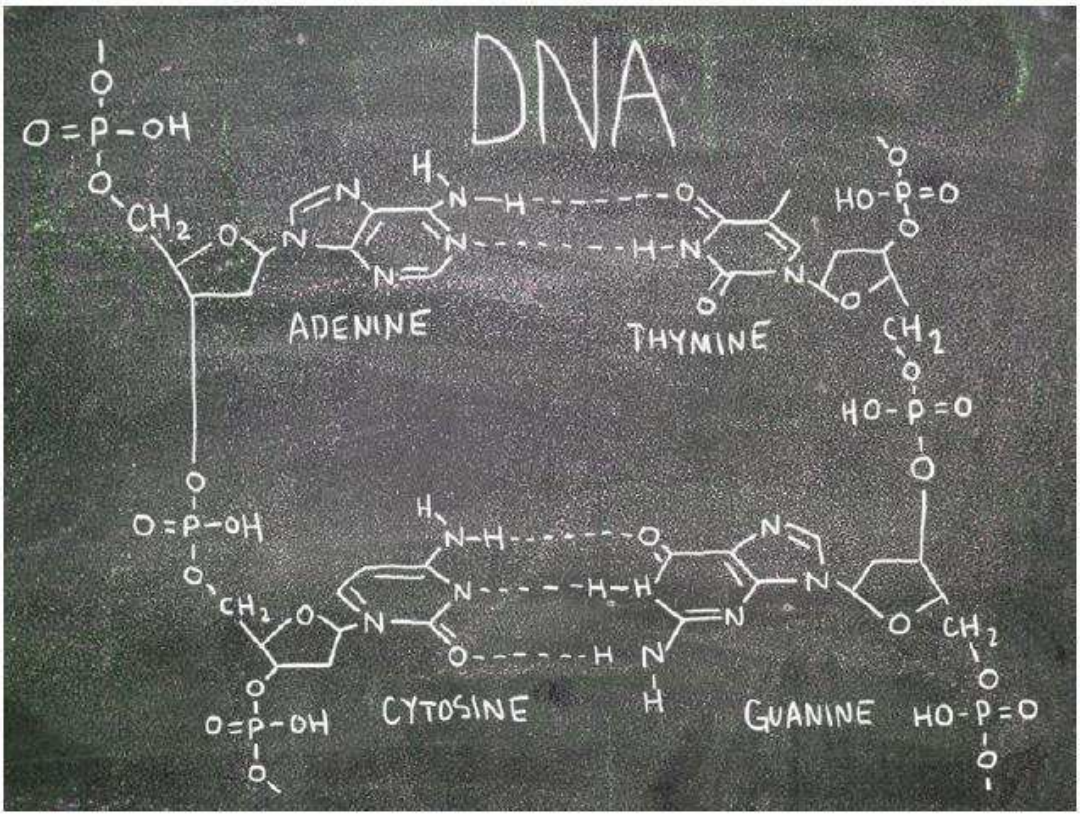
## Chargaff's Rules

It was known that DNA is composed of nucleotides, each of which contains a nitrogen-containing base, a five carbon sugar (deoxyribose), and a phosphate group. In these nucleotides, there is one of the four possible bases: adenine (A), guanine (G), cytosine (C), or thymine (T). Adenine and guanine are purine bases, and cytosine and thymine are pyrimidine bases. Erwin Chargaff (1905-2002), an Austrian-American biochemist from Columbia University, analyzed the base composition of the DNA of various species. This led him to propose two main rules that have been appropriately named Chargaff's rules.

**Rule 1** - Chargaff determined that in DNA, the amount of one base, a purine, always approximately equals the amount of a particular second base, a pyrimidine. Specifically, that in any double-stranded DNA the number of guanine unit sequals approximately the the number of cytosine units and the number of adenine units equals approximately the number of thymine units. Human DNA is 30.9% A and 29.4% T, 19.9% G and 19.8% C. The rule constitutes the basis of base pairs in the DNA double helix: A always pairs with T, and G always pairs with C.

He also demonstrated that the number of purines (A+G) always approximates the number of pyrimidines (T+C), an obvious consequence of the base-pairing nature of the DNA double helix.

**Rule 2** - In 1947 Chargaff showed that the composition of DNA, in terms of the relative amounts of the A, C, G and T bases, varied from one species to another. This molecular diversity added evidence that DNA could be the genetic material.




## Watson and Crick model

- The determination of the structure of DNA by Watson and Crick in 1953 is often said to mark the birth of modern molecular biology. The **Watson–Crick structure** of DNA is of such importance because, in addition to providing the structure of what is arguably the central molecule of life, it suggested the molecular mechanism of heredity. Watson and Crick‘s accomplishment, which is ranked as one of science‘s major intellectual achievements, tied together the less than universally accepted results of several diverse studies.

- Information that DNA is a helical molecule. This was provided by an X-ray diffraction photograph of a DNA fiber taken by Rosalind Franklin (DNA, being a threadlike molecule, does not crystallize but, rather, can be drawn out in fibers consisting of parallel bundles of molecules).
- This photograph enabled Crick, an X-ray crystallographer by training who had earlier derived the equations describing diffraction by helical molecules, to deduce (a) that DNA is a helical molecule and (b) that its planar aromatic bases form a stack of parallel rings which is parallel to the fiber axis.
- This information only provided a few crude landmarks that guided the elucidation of the DNA structure. It mostly sprang from Watson and Crick‘s imaginations through model building studies. Once the Watson–Crick model had been published, however, its basic simplicity combined with its obvious biological relevance led to its rapid acceptance. Later investigations have confirmed the essential correctness of the Watson–Crick model,although its details have been modified.

**The Watson–Crick Structure: B-DNA**

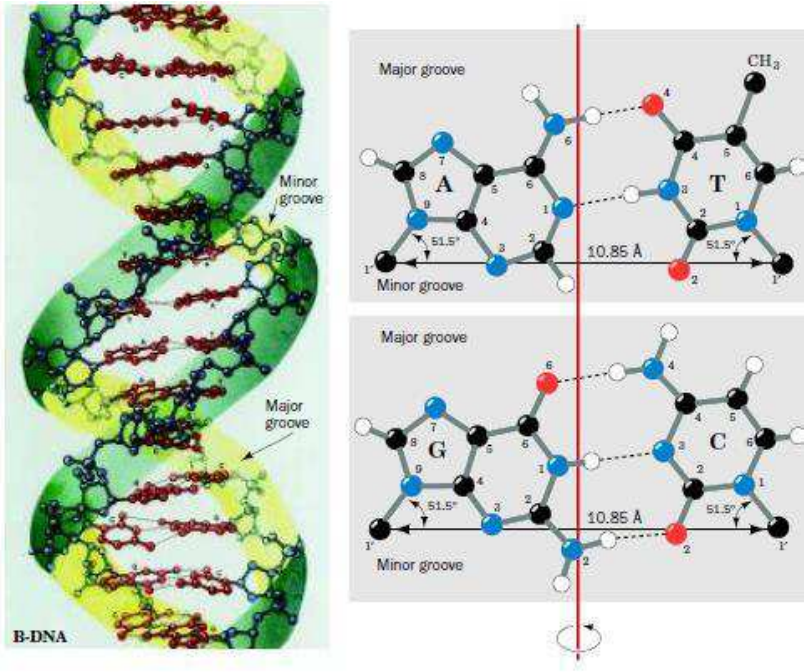
Fibers of DNA assume the so-called B conformation, as indicated by their X-ray diffraction patterns, when the counterion is an alkali metal such as Na1 and the relative humidity is .92%. **B-DNA** is regarded as the **native** (biologically functional) form of DNA because, for example, its X-ray pattern resembles that of the DNA in intact sperm heads. The Watson–Crick structure of B-DNA has the following major features:

**1.** It consists of two polynucleotide strands that wind about a common axis with a right-handed twist to form an ,20-Å-diameter double helix. The two strands are antiparallel (run in opposite directions) and wrap around each other such that they cannot be separated without unwinding the helix. The bases occupy the core of the helix and the sugar–phosphate chains are coiled about its periphery, thereby minimizing the repulsions between charged phosphate groups.

**2.** The planes of the bases are nearly perpendicular to the helix axis. Each base is hydrogen bonded to a base on the opposite strand to form a planar base pair. It is these hydrogen bonding interactions, a phenomenon known as **complementary base pairing,** that result in the specific association of the two chains of the double helix.

**3.** The ―ideal‖ B-DNA helix has 10 base pairs (**bp**) per turn (a helical twist of 36° per bp) and, since the aromatic bases have van der Waals thicknesses of 3.4 Å and are partially stacked on

each other (**base stacking**), the helix has a **pitch** (rise per turn) of 34 Å. The most remarkable feature of the Watson–Crick structure is that it can accommodate only two types of base pairs: Each adenine residue must pair with a thymine residue and vice versa, and each guanine residue must pair with a cytosine residue and vice versa. The geometries of these A ? T and G ? C base pairs, the so-called **Watson–Crick base pairs**. It can be seen that both of these base pairs are interchangeable in that they can replace each other in the double helix without altering the positions of the sugar–phosphate backbone‘s C1¿ atoms. Likewise, the double helix is undisturbed by exchanging the partners of a Watson–Crick base pair, that is, by changing a G ? C to a C ? G or an A ? T to a T ? A. In contrast, any other combination of bases (e.g., A ? G or A ? C) would significantly distort the double helix since the formation of a non-Watson–Crick base pair would require considerable reorientation of the sugar–phosphate chain. B-DNA has two deep exterior grooves that wind between its sugar–phosphate chains as a consequence of the helix axis passing through the approximate center of each base pair. However, the grooves are of unequal size because (1) the top edge of each base pair, is structurally distinct from the bottom edge; and (2) the deoxyribose residues are asymmetric. The **minor groove** exposes that edge of a base pair from which its C1atoms extend whereas the **major groove** exposes the opposite edge of each base pair. Although B-DNA is, by far, the most prevalent form of DNA in the cell, double helical DNAs and RNAs can assume several distinct structures.

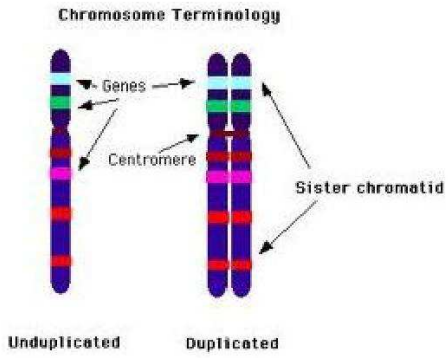



**Watson–Crick base pairs.** The line joining the C1atoms is the same length in both base pairs and makes equal angles with the glycosidic bonds to the bases.This gives DNA a seriesof pseudo-twofold symmetry axes (often referred to as **dyad axes**) that pass through the center of each base pair (*red line*)and are perpendicular to the helix axis. Note that A ? T base pairsassociate via two hydrogen bonds, whereas C ? G base pairs are joined by three hydrogen bonds.

**Chromosome:**

- German biologist Walter Flemming in the early 1880s revealed that during cell division the nuclear material organize themselves into visible thread like structures which were named as chromosomes which stains deep with basic dyes. The term chromosome was coined by W. Waldeyer in 1888. Chrome is coloured and soma is body, hence they mean ―colored bodies‖ and can be defined as higher order organized arrangement of DNA and proteins. It contains many genes or the hereditary units, regulatory elements and other nucleotide sequences. Chromosomes also contain DNA-bound proteins, which serve in packaging the DNA and control its functions. Chromosomes vary both in number and structure among organisms (Table 1) and the number of chromosomes is characteristic of every species. Benden and Bovery in 1887 reported that the number of chromosomes in each species is constant. W.S. Sutton and T. Boveri in 1902 suggested that chromosomes are the physical structures which acted as messengers of heredity.

- Chromosomes are tightly coiled DNA around basic histone proteins, which help in the tight packing of DNA. During interphase, the DNA is not tightly coiled into chromosomes, but exists as chromatin. The structure of a chromosome is given in Figure 2. In eukaryotes to fit the entire length of DNA in the nucleus it undergoes condensation and the degree to which DNA is condensed is expressed as its packing ratio which is the length of DNA divided by the length into which it is packaged into chromatin along with proteins. The shortest human chromosome contains 4.6 x 107 bp of DNA. This is equivalent to 14,000 µm of extended DNA. In its most condensed state during mitosis, the chromosome is about 2 µm long. This gives a packing ratio of 7000 (14,000/2). The DNA is packaged stepwise into the higher order chromatin structure and this is known as ―hierarchies of chromosomal organization‖.



**Chromosome number**

- There are normally two copies of each chromosome present in every somatic cell. The number of unique chromosomes (N) in such a cell is known as its haploid number, and the total number of chromosomes (2N) is its diploid number. The suffix _ploid' refers to chromosome _sets'. The haploid set of the chromosome is also known as the genome.

  Structurally, eukaryotes possess large linear chromosomes unlike prokaryotes which have circular chromosomes. In Eukaryotes other than the nucleus chromosomes are present in mitochondria and chloroplast too. The number of chromosomes in each somatic cell is same for all members of a given species. The organism with lowest number of chromosome is the nematode, *Ascaris megalocephalusunivalens* which has only two chromosomes in the somatic cells (2n=2).

**Autosomes and sex chromosomes**

- In a diploid cell, there are two of each kind of chromosome (termed homologus chromosomes) except the sex chromosomes. In humans one of the sex has two of the same kind of sex chromosomes and the other has one of each kind. In humans there are 23 pairs of homologous chromosomes (2n=46). The human female has 44 non sex chromosomes, termed autosomes and one pair of homomorphic sex chromosomes given the designation XX. The human male has 44 autosomes and one pair of heteromorphic sex chromosomes, one X and one Y chromosome.

**Morphology:**

**Size:** The size of chromosome is normally measured at mitotic metaphase and may be as short as 0.25µm in fungi and birds to as long as 30 µm in some plants such as Trillium. However, most mitotic chromosome falls in the range of 3µm in Drosophila to 5µm in man and 8-12µm in maize. The monocots contain large sized chromosomes as compared to dicots. Organisms with less number of chromosomes contain comparatively large sized chromosomes. The chromosomes in set vary in size.

**Shape:** The shape of the chromosome changes from phase to phase in the continuous process of cell growth and cell division. During the resting/interphase stage of the cell, the chromosomes occur in the form of thin, coiled, elastic and contractile, thread like stainable structures, the chromatin threads. In the metaphase and the anaphase, the chromosome becomes thick and filamentous. Each chromosome contains a clear zone, known as centromere or kinetochore,

along their length. The centromere divides the chromosome into two parts and each part is called chromosome arm. The position of centromere varies from chromosome to chromosome providing it a different shape. They could be telocentric (centromere on the proximal end of the chromosome), acrocentric (centromere at one end giving it a very short and another long arm), submetacentric (J or L shaped chromosome with the centromere near the centre), metacentric (v shaped with centromere at the centre).

**Structure of Chromosome**

- A chromosome at mitotic metaphase consists of two symmetrical structures called chromatids. Each chromatid contains a single DNA molecule and both chromatids are attached to each other by centromere and become separated at the beginning of anaphase. The chromomeres are bead like accumulations of chromatin material that are sometimes visible along interphase chromosomes. The chromomere bearing chromatin has an appearance of a necklace in which several beads occur on a string. Chromomeres are regions of tightly folded DNA and become especially prominent in polytene chromosomes.

- Centromere in a chromosome contains specific DNA sequences with special proteins bound to them, forming a disc shaped structure, called kinetochore. In electron microscope the kinetochore appears as a plate or cup like disc, 0.20-0.25 nm, in diameter situated upon the primary constriction or centromere. The chromosomes of most organisms contain only one centromere and are known as monocentric chromosomes. Some species have diffused centromeres, with microtubules attached along the length of the chromosomes and are termed holocentric chromosomes. Chromosomes of *Ascaris megalocephala* are examples of diffused centromeric chromosomes. Telomere is the chromosomal ends which prevents other chromosomal segments to be fused with it. Besides the primary constrictions or centromeres, chromosomes also possess secondary constriction at any point of the chromosome and are constant in their position and extent. These constrictions are helpful in identifying particular chromosomes in a set. Chromosomes also contain nucleolar organizers which are certain secondary constrictions that contain the genes coding for 5.8S, 18S and 28S ribosomal RNA and induce the formation of nucleoli. Sometimes the chromosomes bear round, elongated or knob like appendages known as satellites. The satellite remains connected with the rest of the chromosomes by a thin chromatin filament

## Chromatin

### Chemical composition of chromatin

Chromatin consists of DNA, RNA and protein. The protein of chromatin could be of two types: histones and non-histones.

**DNA**

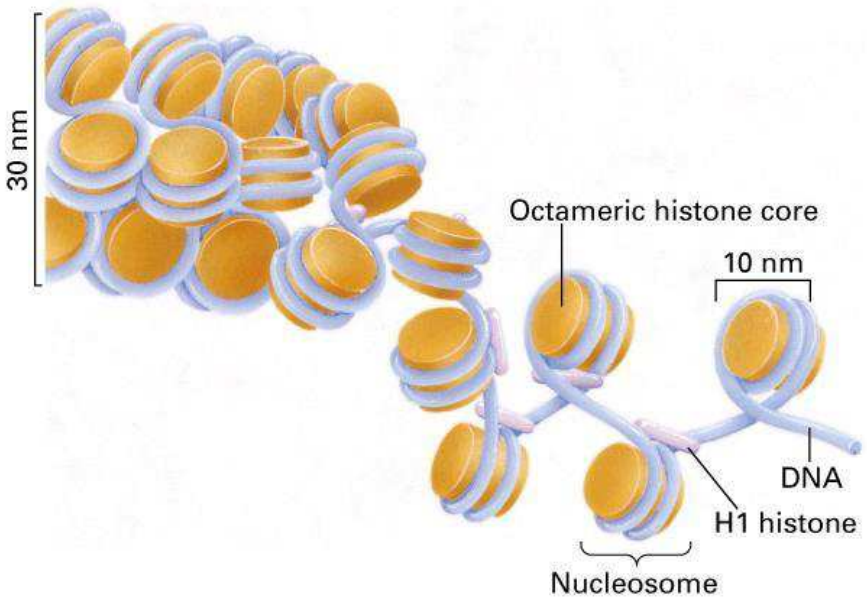
- DNA is the most important chemical component of chromatin, since it plays central role of controlling heredity and is most conveniently measured in picograms.
- In addition to describing the genome of an organism by its number of chromosomes, it is also described by the amount of DNA in a haploid cell. This is usually expressed as the amount of DNA per haploid cell (usually expressed as picograms) or the number of kilobases per haploid cell and is called the C value. This is constant for all cells of a species. For diploid cells it is 2C. Extending the C value we reach the C-value paradox. One immediate feature of eukaryotic organisms highlights a specific anomaly that was detected early in molecular research. Even though eukaryotic organisms appear to have 2-10 times as many genes as prokaryotes, they have many orders of magnitude more DNA in the cell.
- Furthermore, the amount of DNA per genome is correlated not with the presumed evolutionary complexity of a species. This is stated as the **C value paradox**: the amount of DNA in the haploid cell of an organism is not related to its evolutionary complexity. Lower eukaryotes in general have less DNA, such as nematode *Caenorhabditis elegans* which has 20 times more DNA than *E. coli*. Vertebrates have greater DNA content about 3pg, in general about 700 times more than *E. coli*. Salamander *Amphiuma* has a very high DNA content of about 84pg. Man has about 3pg of DNA per haploid genome.

**Histones**

- Histones are basic proteins as they are enriched with basic proteins arginine and lysine. At physiological pH they are cationic and can interact with anionic nucleic acids. They form a highly condensed structure. The histones are of five types called H1, H2A H2B, H3, and H4- which are very similar among different species of eukaryotes and have been highly conserved during evolution. H1 is the least conserved among all and is also loosely bound with DNA. H1 histone is absent in *Sacharomyces cerevisiae*.

**Non-histones**

- In addition to histones the chromatin comprise of many different types of non-histone proteins, which are involved in a range of activities, including DNA replication and gene expression. They display more diversity or are not conserved. They may also differ between different tissues of same organism. Roger Kornberg in 1974 described the basic structural unit of chromatin which is called the nucleosome.




**Euchromatin**

- The lightly-stained regions in chromosome when stained with basic dyes are called euchromatin and contain single-copy of genetically-active DNA. The extent of chromatin condensation varies during the life cycle of the cell and plays an important role in regulating gene expression. In the interphase of cell cycle the chromatin are decondensed and known as euchromatin leading to gene transcription and DNA replication.

**Heterochromatin**

- The word heterochromatin was coined by Emil Heitz based on cytological observations. They are highly condensed and ordered areas in nucleosomal arrays. About 10% of interphase chromatin is called heterochromatin and is in a very highly condensed state that resembles the chromatin of cells undergoing mitosis. They contain a high density of repetitive DNA found at centromeres and telomeres form heterochromatin.

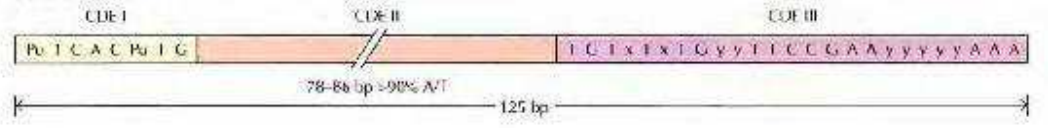
- Heterochromatin are of two types, the constitutive and facultative heterochromatin. The regions that remain condensed throughout the cell cycle are called constitutive heterochromatin whereas the regions where heterochromatin condensation state can change are known as facultative.

Constitutive heterochromatin is found in the region that flanks the telomeres and centromere of each chromosome and in the distal arm of the Y chromosome in mammals. Constitutive heterochromatin possesses very few genes and they also lead to transcriptional inactivation of nearby genes. This phenomenon of gene silencing is known as ―position effect‖. Constitutive heterochromatin also inhibits genetic recombination between homologous repetitive sequences circumventing DNA duplications and deletion. Whereas facultative heterochromatin is chromatin that has been specifically inactivated during certain phases of an organism's life or in certain types of differentiated cells. Dosage compensation of X-chromosome or X-chromosome inactivation in mammals is an example of such heterochromatin (Karp 2010). Heterochromatin spreads from a specific nucleation site, causing silencing of most of the X chromosome, thereby regulating gene dosage.

**Centromeres**

- Centromeres are those condensed regions within the chromosome that are responsible for the accurate segregation of the replicated chromosome during mitosis and meiosis. When chromosomes are stained they typically show a dark-stained region that is the centromere. The actual location where the attachments of spindle fibres occur is called the kinetochore and is composed of both DNA and protein. The DNA sequence within these regions is called *CEN* DNA. Because *CEN* DNA can be moved from one chromosome to another and still provide the chromosome with the ability to segregate, these sequences must not provide any other function. Typically CEN DNA is about 120 base pairs long and consists of several sub-domains, CDE-I, CDE-II and CDE-III. Mutations in the first two sub-domains have no effect upon segregation, but a point mutation in the CDE-III sub-domain completely eliminates the ability of the centromere to function during chromosome segregation. Therefore CDE-III must be actively involved in the binding of the spindle fibers to the centromere. The protein component of the kinetochore is only now being characterized. A complex of three proteins called Cbf-III binds to normal CDE-III regions but cannot bind to a CDE-III region with a point mutation that prevents mitotic segregation. Furthermore, mutants of the genes encoding the Cbf-III proteins also eliminates the ability for chromosomes to segregate during mitosis. Additional analyses of the DNA and protein

components of the centromere are necessary to fully understand the mechanics of chromosome segregation.




The *S. cerevisiae* centrosome. The *S. cerevisae* centromere (CEN) sequences consist of two short conserved sequences (CDE I and CDE Ill) separated by 78 to 86 base pairs (bp) of ATrich DNA (CDE II). The sequences shown are consensus sequences derived from analysis of the centromere sequences of individual yeast chromosomes. Pu = A or G; x =A or T; y = any base. The figure has been adapted from ―The Cell, A Molecular Approachl by Geoffrey M. Cooper, 4th Ed. 2007.

**Telomeres**

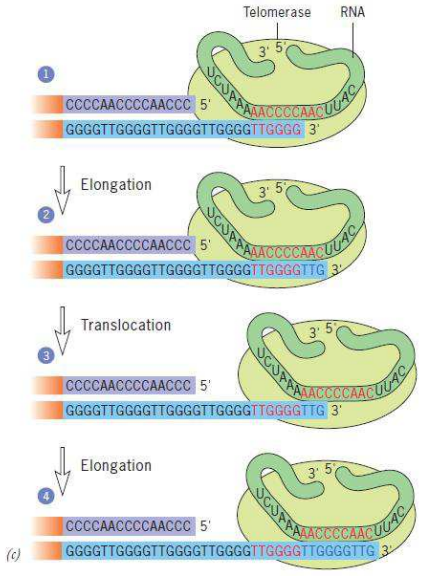
- Telomeres are the region of DNA at the end of the linear eukaryotic chromosome that are required for the replication and stability of the chromosome. McClintock recognized their special features when she noticed, that if two chromosomes were broken in a cell, the ends were sticky and end of one could attach to the other and vice versa. However she never observed the attachment of the broken end to the end of an unbroken chromosome suggesting that the end of chromosomes have unique features.

- Telomere sequences remain conserved throughout vertebrates and they form caps that protect the chromosomes from nucleases and other destabilizing influences; and they prevent the ends of chromosomes from fusing with one another.The telomeric DNA contains direct tandemly repeated sequences of the form $(T/A)_xG_y$ where x is between 1 and 4 and y is greater than 1. Human telomeres contain the sequence TTAGGG repeated from about 500 to 5000 times. Certain bacteria possess telomeres in their linear genetic material which are of two types; one of the types is called a hairpin telomere. As its name implies, the telomeres bend around from the end of one DNA strand to the end of the complimentary strand. The other type of telomere is known as an invertron telomere. This type acts to allow an overlap between the ends of the complimentary DNA strands.

**Telomere replication**

- Telomere replication is an important aspect in DNA replication. The primary difficulty with telomeres is the replication of the lagging strand. Because DNA synthesis requires a RNA template (that provides the free 3'-OH group) to prime DNA replication, and this template is eventually degraded, a short single-stranded region would be left at the end of the chromosome.

This region would be susceptible to enzymes that degrade single-stranded DNA. The result would be that the length of the chromosome would be shortened after each division. This is known as the end replication problem which is not observed. The action of the telomerase enzymes ensure that the ends of the lagging strands are replicated correctly. Telomerase was discovered in 1984 by Elizabeth Blackburn and Carol Greider of the University of California, Berkeley. It is a reverse transcriptase that synthesizes DNA using an RNA template. Unlike most reverse transcriptases, the enzyme itself contains the RNA that serves as its template, i.e., telomerase can add new repeat units to the 3‘ end of the overhanging strand. A well-studied system involves the *Tetrahymena* protozoa organism. The telomeres of this organism end in the sequence 5'-TTGGGG-3'. The telomerase adds a series of 5'-TTGGGG-3' repeats to the ends of the lagging strand. A hairpin occurs when unusual base pairs between guanine residues in the repeat form. Next the RNA primer is removed, and the 5' end of the lagging strand can be used for DNA synthesis. Ligation occurs between the finished lagging strand and the hairpin. Finally, the hairpin is removed at the 5'-TTGGGG-3' repeat. The replication of telomere has been presented in Figure 4. Telomerase activity is retained in germ cells and zygote and somatic cells after few cell division cycles do not show such activities because otherwise they would divide indefinitely and lead to cancer. Thus telomeres shrink causing chromosome shortening to a critical point when the cell ceases to grow and divide. An inherited disease called the Werner‘s syndrome that causes patients to age much more rapidly abnormal telomere maintenance.




**Telomerase replication** - Telomerase contains an RNA primer that is complementary to the end of the G-rich strand, which extends past the C-rich strand. The telomerase RNA binds to the protruding end of the G-rich strand in step 1 and then serves as a template for the addition of nucleotides onto the 3‘ terminus of the strand in step 2. After a segment of DNA is synthesized, the telomerase RNA slides to the new end of the strand being elongated in step 3 and serves as the template for the incorporation of additional nucleotides in step 4. The gap in the complementary strand is filled by the replication enzymes polymerase α-primase. This figure has been adapted from Cell and Molecular Biology Concepts and Experiments by Karp, 2010.

## MOLECULAR BIOLOGY 17BTU302

### UNIT II

**DNA structure and replication:** Replication of DNA in prokaryotes and eukaryotes: Semi-conservative nature of DNA replication, Bi-directional replication, DNA polymerases, Replication complex: Pre-priming proteins, primosome, replisome, Rolling circle replication, Unique aspects of eukaryotic chromosome replication, Fidelity of replication.

| S.No | Lecture Duration (hr) | Topics | Support materials |
|---|---|---|---|
| 1 | 1 | Replication – Introduction, Replication in Prokaryotes – Initiation, Elongation , Termination | T1: 1195; T1: 1188; T1: 1208 |
| 2 | 1 | Rolling circle method – Plasmid DNA model, Bacteriophage Model | T1: 1190-1193 |
| 3 | 1 | DNA polymerases – DNA polymerase I, II, III | T1: 1176-1181; T1: 1126-1127; T1: 1181-1187 |
| 4 | 1 | Eukaryotic replication – Leading strand formation, | T1: 1201-1213; |
| 5 | 1 | Lagging strand formation, Okazaki fragments | T1-1174-1175 |
| 6 | 1 | Role of Topoisomerase I and II | T1:1209-1213 |
| 7 | 1 | RNA primer – DNA polymerase I as DNA repair | T1: 1176 |
| 8 | 1 | Fidelity of DNA replication | T1: 1200 - 1202 |

Reference

T1: Donald Voet and Judith Voet; 2012, Biochemistry, 4th Edition, John Wiley and Sons. Inc.

## DNA replication

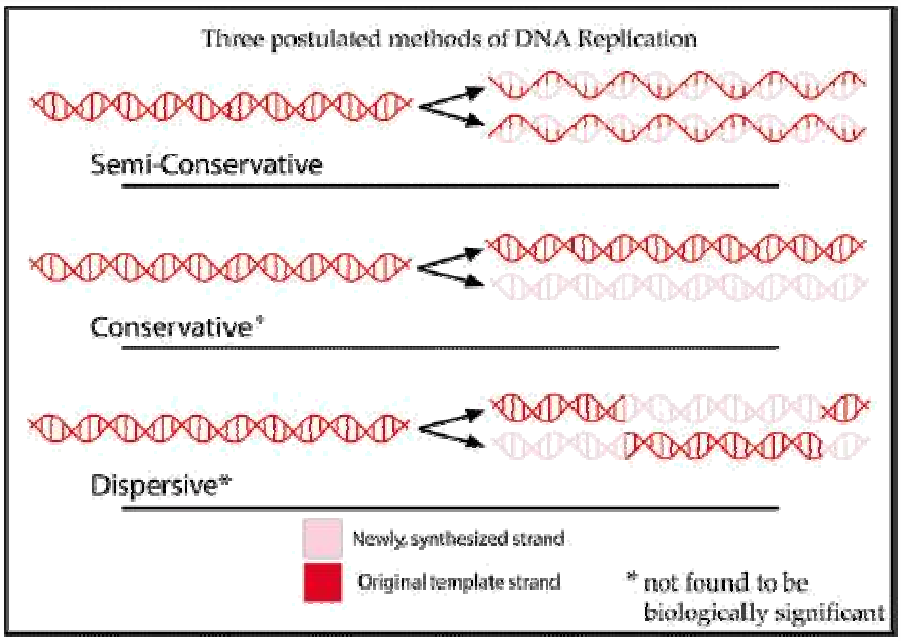
- In molecular biology, DNA replication is the biological process of producing two identical replicas of DNA from one original DNA molecule. This process occurs in all living organisms and is the basis for biological inheritance. DNA is made up of a double helix of two strands, and each strand of the original DNA molecule serves as a template for the production of the complementary strand, a process referred to as semiconservative replication. Cellular proofreading and error-checking mechanisms ensure near perfect fidelity for DNA replication.

- In a cell, DNA replication begins at specific locations, or origins of replication, in the genome. Unwinding of DNA at the origin and synthesis of new strands results inreplication forks growing bidirectional from the origin. A number of proteins are associated with the replication fork which helps in terms of the initiation and continuation of DNA synthesis. Most prominently, DNA polymerase synthesizes the new DNA by adding complementary nucleotides to the template strand.

## Templated replication

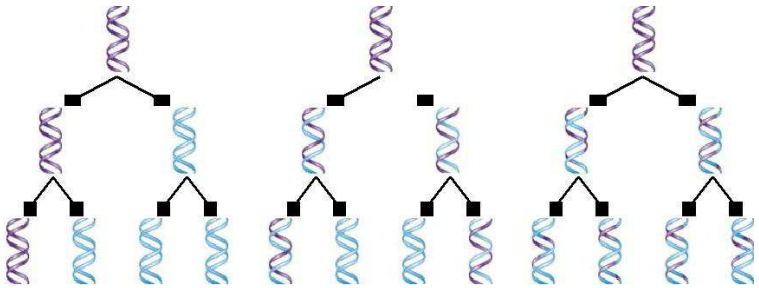
- **Sense and antisense strands:** The sense strand of a double-stranded DNA molecule has a base sequence similar to that of the RNA that is transcribed from the DNA. The antisense strand (also known as template strand) has a sequence that is the reverse complement of the sense strand (base paired to it with an antiparallel 5' to 3' polarity).

- **Semiconservative replication:** Discovery of the double helical structure of DNA immediately suggested a mechanism for precise replication -- namely separation of the two strands and template synthesis of a new copy of each of the missing complementary strands. This model predicted that each new double helix should have one strand of parental DNA and one strand of newly synthesized DNA.

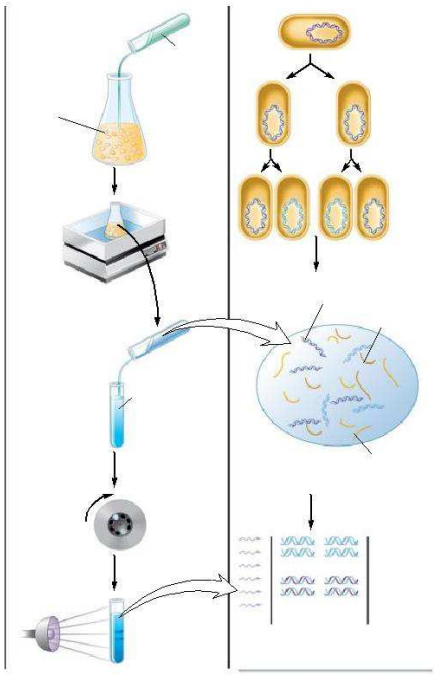



Three postulated meth ods of replication: 1. Semiconservative, 2. Conservative, 3. Dispersive

**Models of replication**



- **Messelson-Stahl experiment:** Semiconservative replication was demonstrated by starting with bacteria whose DNA was labeled with heavy nitrogen (15N) and then allowing various amounts of growth to occur in media labeled with normal nitrogen. After one round of replication, the entire DNA had an intermediate density. After two rounds, one half was light and the other half still had an intermediate density. There was no conservation of heavy DNA. However, the data suggested that each of the labeled strands from the original DNA remained intact and separated from its partner in each replication cycle.



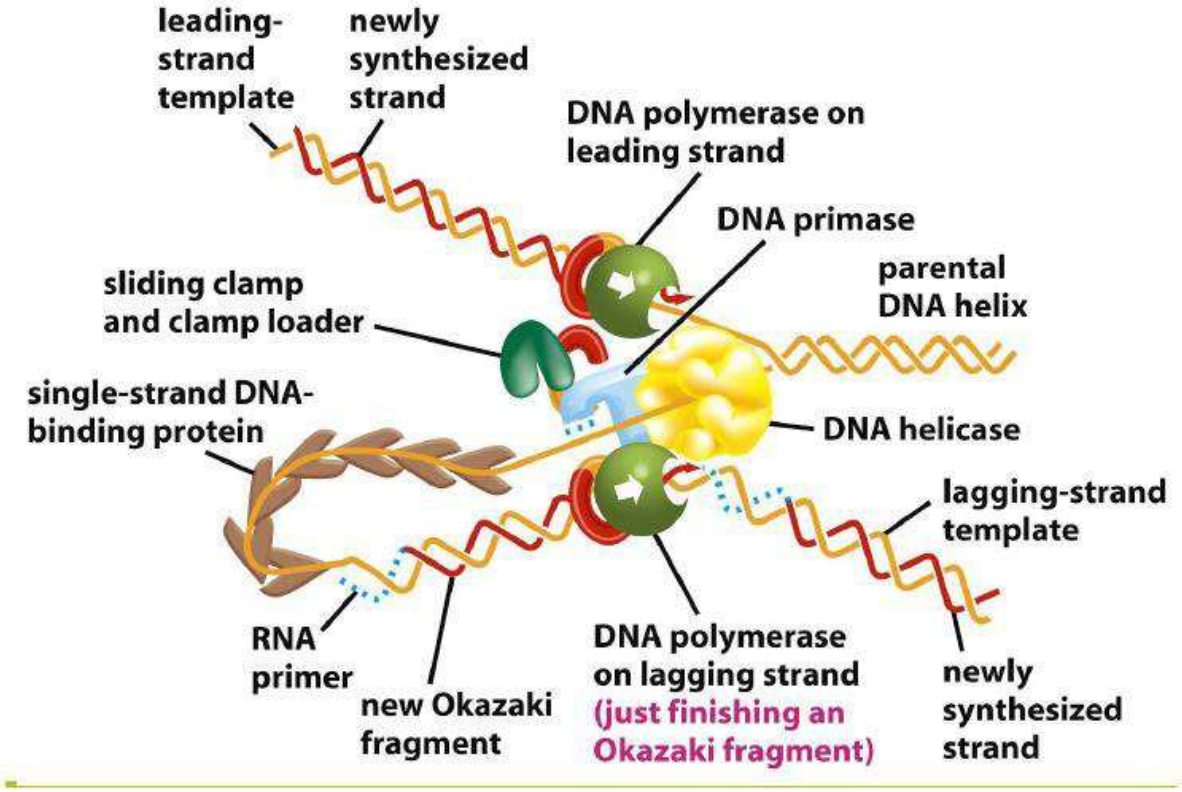
**Initiation of replication**

• **Origin of Replication:** The DNA of a bacterial chromosome is a closed circular structure. DNA replication begins at a specific site (origin of replication) characterized by the presence of repeated 9 base and 13 base nucleotide sequences. The repeating units are referred to as 9-mers and 13-mers. The 13 mers are AT rich, making easier to separate the two strands of the double-stranded DNA.

• **DNAa protein:** The protein coded by the DNA a gene binds to the repeated 9mers. This forms a tight loop and generates a strain that causes strand separation in the region containing the AT-rich 13-mers • Helicase: Enzymes called helicases use energy derived from ATP to further separate the two strands of the DNA double helix.• Topoisomerase I: Separation of the two strands of the DNA double helix requires substantial unwinding of the helix. An enzyme known as topoisomerase I(gyrase) relieves twisting strain that is generated by unwinding the double helix. It is believed to act by cutting one of the strands such that the other strand can rotate freely to relieve the strain, and then resealing the strand that has been temporarily cut.

• **Keeping the helix open:** Single stranded DNA-binding proteins (SSBPs) attach to the single stranded DNA generated by unwinding the double helix and temporarily keep it from reforming double helical structures.

**Patterns of replication**




• **Replication forks:** When the two strands of double helical DNA separate and replication of both strands begins, a forked or Y-shaped structure is formed.

• **Bidirectional replication:** In bacterial cells, replication starts at a specific origin of replication within the circular DNA molecule and proceeds in both directions away from the origin. This results in the formation of a replication "bubble", which continues to elongate as replication proceeds. A similar pattern is seen in eukaryotic chromosomes, except that multiple origins are involved.

• **Theta structure in bacteria:** As replication proceeds in both directions around the circular chromosomes of bacteria, a structure reminiscent of the Greek letter theta is formed.

• **Replicons:** The very long DNA double helices found in eukaryotic chromosomes contain multiple origins of replication, which often initiate bidirectional replication more or less synchronously. Each unit of replication is called a replicon. Replication continues until the replication bubbles fuse to yield fullyreplicated DNA strands.

• **Other patterns** There are also two alternative strategies for replication of circular DNA that are more complex than the simple bidirectional model. These are the rolling circle mode (sigma mode) used by some types of viruses, and the D loop mode, used for replication of mitochondrial and chloroplast DNAs.

**5'-to-3' synthesis**

• **Unidirectional addition of nucleotides:** Polymerization of DNA, and also of RNA, occurs by condensing a 5'-nucleoside triphosphate (dNTP or NTP) onto the 3' hydroxyl group of another nucleotide, or onto the 3'-end of a growing polynucleotide chain. No mechanism exists for extending the 5'-end of a nucleotide chain.

• **Energy for synthesis:** Hydrolysis of the dNTP or NTP that is being added provides the energy needed to form a covalent bond. The outer two phosphates of the triphosphate are split off and the innermost phosphate forms an ester linkage with the 3'-hydroxyl group at the end of the pre-existing chain. This results in a phosphodiester bond

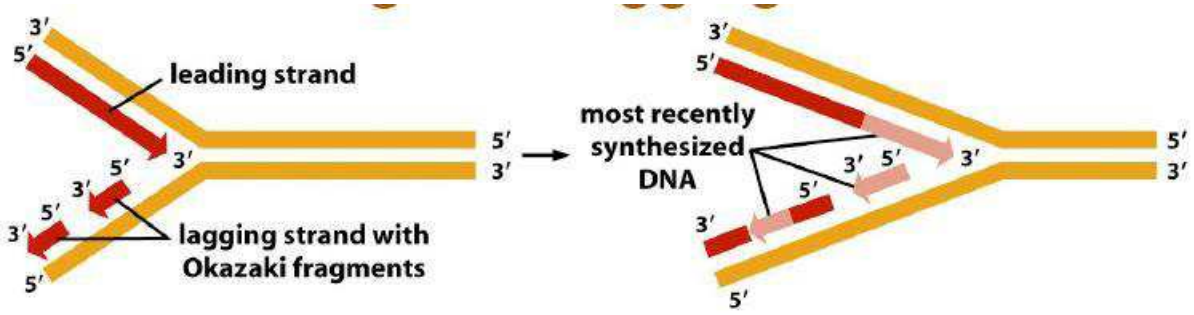
**RNA priming**

• **No new DNA starts**: Deoxynucleotides can only be added to 3'-end of a preexisting strand of DNA (or RNA). There are no enzymes capable of initiating the synthesis of a DNA-templated DNA molecule at the level of a single nucleotide. This makes necessary to use an indirect priming procedure.

• **RNA primers:** A variety of enzymes are capable of initiating new DNAtemplated RNA synthesis (as in transcription). New DNA synthesis is primed with a short segment of RNA that is later removed

• **Primase:** A separate enzyme in the initiation complex called primase synthesizes a short RNA primer each time that new DNA synthesis begins, including all new starts in the discontinuous pattern of synthesis described below.

**Leading and lagging strands**




• **Unidirectional synthesis of antiparallel DNA:** The inability to synthesize new chains in a 3' to 5' direction adds a major complication to the replication of antiparallel double-stranded DNA. At any replication fork, one of the template strands has a 3' to 5' orientation, which is what is needed for synthesis of a new complementary strand in a 5' to 3' direction. Synthesis on that template is primed and starts very quickly. However, the other template strand has a 5' to 3' orientation and is thus unable to support synthesis beginning at the origin and moving away from it in a 3' to 5' direction. Because of this, the 5' to 3' template strand accumulates in a single stranded configuration until there is a sufficient length so that synthesis of its complementary antiparallel strand can be primed and initiated in a 5'-to-3' direction ("backward" toward the origin of replication). The strand whose synthesis begins immediately is called the "leading" strand, and the one whose synthesis is delayed is called the "lagging" strand.

• **Discontinuous synthesis -- Okazaki fragments:** As synthesis of the leading strand continues, more and more of the single stranded DNA of the lagging strand is unwound. Each time that a sufficient length is reached, synthesis of a new segment of the complementary strand begins. If the replicating DNA is denatured (separated into individual strands) before the newly synthesized pieces of the lagging strand have been ligated together, a number of relatively small fragments of newly synthesized DNA will be recovered, together with the much longer strands

produced by continuous synthesis in the leading strand. The small fragments are called "Okazaki fragments", named for the person who first discovered them.

**DNA polymerase III**

**Template DNA synthesis:** After the RNA primer has reached an adequate length, DNA polymerase III begins synthesis of DNA, which proceeds to completion in the leading strand, and proceeds until the 5'-end of the previous primer is encountered in the lagging strand.

• **DNA polymerase III holoenzyme:** DNA polymerase III is a highly complex dimeric aggregate, consisting of 20 or more protein subunits. The alpha subunits perform the actual DNA synthesis, but operate in conjunction with multiple accessory proteins

• **Simultaneous synthesis of leading and lagging strands:** There is yet another complicating factor in DNA synthesis. It is now generally believed that the leading and lagging strands are synthesized simultaneously by a single dimeric DNA polymerase III complex. This requires formation of a looped structure with the leading and lagging strands so positioned that their synthesis can occur side by side in the same orientation, despite the fact that the newly synthesized chains are growing in opposite directions relative to the overall DNA that is being replicated. A similar looping also appears to occur in eukaryotic DNA replication.

• **Clamping function of beta subunits:** The beta subunits appear to have a clamping function that keeps the leading and lagging strands appropriately aligned with the catalytically active alpha subunits.

• **Replisome complex:** The entire DNA-synthesizing complex at each replication fork, which also includes topoisomerase, helicase, and primase, is sometimes referred to as a replisome.

**DNA polymerase I**

• Not the primary enzyme for DNA synthesis: DNA polymerase I is so named because it was the first of the DNA polymerase enzymes to be isolated and characterized. Although it is capable of template-directed DNA synthesis, it is now known not to be the enzyme primarily responsible for new DNA synthesis, but it does have other important roles, as described below.

• **5' to 3' exonuclease activity:** DNA polymerase I has an unusual 5' to 3' exonuclease activity. This gives it the ability to start at a single-stranded break and progressively remove nucleotides and replace them in a 5' to 3' direction.

• **Removal of RNA primer:** In the lagging strand, when DNA polymerase III runs into the 5'-end of the previous RNA primer, it is unable to proceed further. Although the new DNA butts up against the primer, it is not covalently joined to it. DNA polymerase III dissociates, leaving a "nick" (a single stranded gap) between the new DNA and the primer. Starting at the nick, DNA polymerase I removes the primer ribonucleotides one at a time, using its 5' to 3' exonuclease activity, and replaces them with deoxyribonucleotides, using its DNA polymerase activity..

• **DNA repair:** DNA polymerase I is also used to fill in short gaps in DNA, often as a part of a repair process that excises part of a damaged strand and replaces it with new DNA templated from the remaining strand. Proofreading and DNA repair

• Removal of mismatched bases: DNA polymerases III and I both have 3' to 5' exonuclease activity. This allows them to remove a mismatched nucleotide that has just been added to a growing DNA chain and make another attempt to insert the correct nucleotide. This "proofreading" function helps to reduce the number of mistakes in DNA synthesis that would otherwise result in mutation

•**DNA polymerase II**: There is yet another prokaryotic DNA polymerase, designated DNA polymerase II, whose main function appears to be to synthesize replacement DNA during DNA repair.

**Final steps in DNA synthesis**

• DNA Ligase: After the last ribonucleotide is removed from the primer and replaced with a deoxynucleotide, there is still a nick in the newly synthesized lagging strand. This nick is closed by DNA ligase, which forms a covalent phosphodiester bond between the Okazaki fragments, joining them into a continuous strand of DNA. Note that Okazaki fragments accumulate when ligase function is impaired.

• Topoisomerase II: Its role is to cut and reseal the newly synthesized DNAs as needed so that they can separate from each other (it is easy to visualize two circular genomes linked through each other at the end of replication).

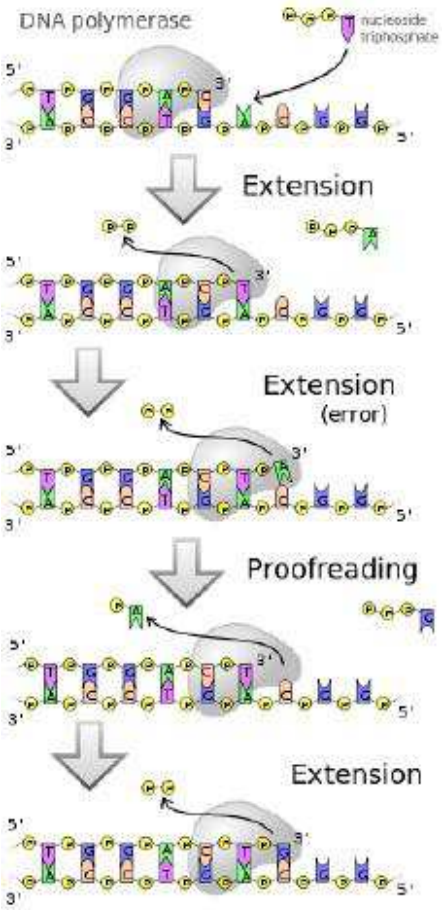


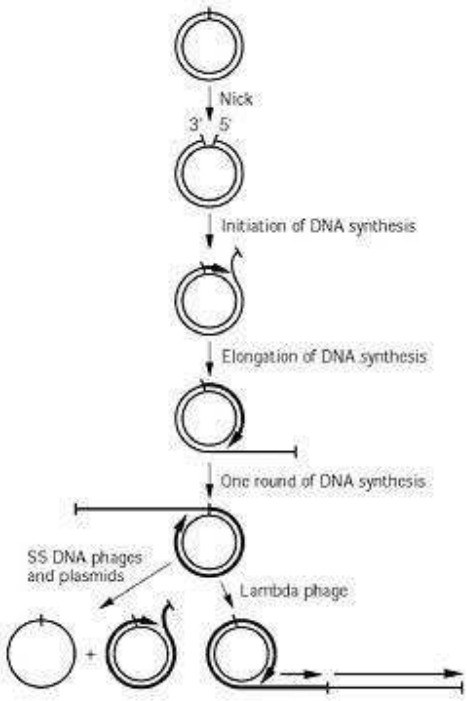

**Diagram of DNA polymerase extending a DNA strand and proof-reading**

## Rolling cycle method of Plasmid DNA

➢ In DNA replication, the DNA polymerase cannot initiate the synthesis of a new DNA strand and must rely on a priming device. In general, an RNA primer is synthesized at or near a replication origin to start synthesis of the leading strand. However, a DNA primer terminus can be generated by a nuclease-generated nick at a specific place in some circular duplex DNA, and replication will then proceed unidirectionally. This mode of replication is called rolling circle replication and is found for replication of the replicative form (RF) form of

bacteriophage single-stranded genomes of Gram-negative bacteria and of the multicopy plasmids of Gram-positive bacteria (see Single-Stranded DNA Replication).

- Rolling circle replication is also observed in the late stage of the replication of the lambda phage genome and in the process of the conjugative transfer of bacterial plasmids.




- DNA synthesis initiates using the free 3′ -OH end at the nick as a primer, and a replication fork proceeds around the template. In the process, the newly synthesized strand displaces the old strand from the template.
- In the case of replication of the RF form of single-stranded phage genomes and of plasmids of Gram-positive bacteria, the displaced old strand is cleaved off after one round of replication and is converted into the circular, double-stranded form. In contrast, in phage lambda replication, the replication fork proceeds a number of revolutions around the template without cleavage of the displaced strand, and the displaced strand becomes double-stranded as it is peeled off.
- The linear concatemer thus created is cleaved into one unit length and packaged into the phage particles. In the conjugation process of plasmids, the displaced strand is transferred into the new cell.

- The initial nick of rolling circle replication is introduced by an endonuclease specific for each system. The similarity of the amino acid sequences of the initiator endonucleases and of the proteins (known as relaxases) involved in the initiation and termination of conjugative DNA strand transfer clearly indicate that they are evolved from a common ancestor.

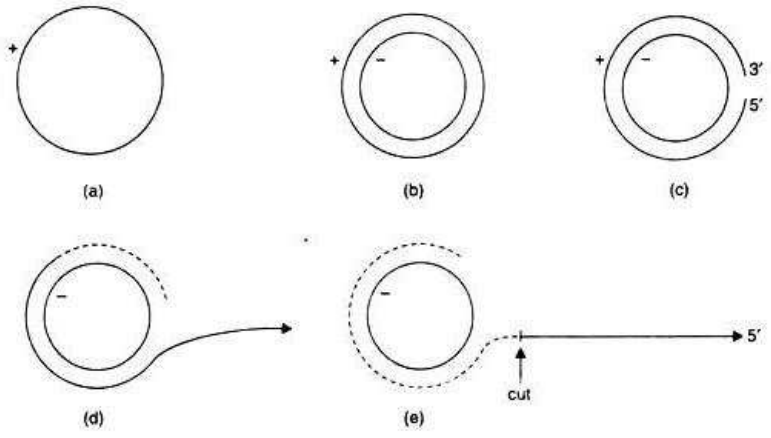
## Rolling cycle method of DNA replication in φ X 174

- All circular genomes do not follow the same pattern of replication described in *E. coli*. In some bacteriophages (phi, lambda and phi X 174), in mitochondrial chromosomes and during bacterial mating an alternative method known as the rolling circle has been demonstrated. This method as seen in the small bacteriophage φ X 174 will be described here.

- φ X174 has a single stranded DNA molecule only about 6,000 nucleotides in length. Its newly formed DNA is linear, not circular. The process takes place in following way. Soon after φ X174 enters the host E. coli cell its single stranded parent DNA (designated + strand) synthesizes a complementary minus strand (with the help of host cell enzymes) to form duplex DNA (+ -). This is the replicative form (RF) of φXI74.

- An endonuclease enzyme produces a nick in the + strand, exposing a free 3′ end and a free 5′ end. DNA synthesis begins by addition of deoxyribonucleotides by the enzyme DNA polymerase to the free 3′ end of the + strand using the strand as template.

- The minus strand revolves while serving as template hence the name rolling circle. The addition of nucleotides displaces the free 5′ end outwards in the form of a free tail. The rolling circle revolves a number of times thus increasing the length of the + strand by a corresponding number of + complements.




The rolling circle method of DNA replication in ϕX174.

It appears that the tail is finally cut into the correct genome lengths by an endonuclease to provide as many copies of free, linear DNA molecules. The free ends joined by the newly formed linear DNA are joined by the enzyme DNA ligase to form closed, circular progeny chromosomes.

**Fidelity of Replication**

- Since a single polypeptide as small as the Pol I Klenow fragment can replicate DNA by itself, why does *E. coli* maintain a battery of >20 intricately coordinated proteins to replicate its chromosome? The answer apparently *is to ensure the nearly perfect fidelity of DNA replication required to preserve the genetic message's integrity from generation to generation.* The rates of reversion of mutant *E. coli* or T4 phage to the wild type indicates that only one mispairing occurs per $10^8$ to $10^{10}$ base pairs replicated. This corresponds to _1error per 1000 bacteria per generation. Such high replication accuracy arises from four sources:

**1.** Cells maintain balanced levels of dNTPs through the mechanism discussed in Section 28-3Ad. This is an important aspect of replication fidelity because a dNTP present at aberrantly high levels is more likely to be misincorporated and, conversely, one present at low levels is more likely to be replaced by the dNTPs present at higher levels.

**2.** The polymerase reaction itself has extraordinary fidelity. This is because, as we have seen (Section 30-2Ae), the polymerase reaction occurs in two stages: (1) a binding step in which the incoming dNTP base-pairs with the template while the enzyme is in an open conformation that cannot catalyze the polymerase reaction; and (2) a catalysis step in which the polymerase forms a closed conformation about the newly formed base pair, which properly positions its catalytic residues (induced fit). Since the formation of the closed conformation requires that the incoming dNTP form a Watson–Crick-shaped base pair with the template, the conformation change constitutes a double check for correct base pairing.

**3.** The 3¿ S 5¿ exonuclease functions of Pol I and Pol III detect and eliminate the occasional errors made by their polymerase functions. In fact, mutations that increase a DNA polymerase's proofreading exonuclease activity decrease the rates of mutation of other genes.

**4.** A remarkable battery of enzyme systems, contained in all cells, function to repair residual errors in the newly synthesized DNA as well as any damage that it may incur after its synthesis through chemical and/or physical insults.

- In addition, *the inability of a DNA polymerase to initiate chain elongation without a primer is a feature that increases DNA replication fidelity.* The first few nucleotides of a chain to be coupled together are those most likely to be mispaired because of the cooperative nature of base pairing interactions. The editing of a short duplex oligonucleotide is similarly an error-prone process.

- The use of RNA primers eliminates this source RNA is eventually replaced by DNA under conditions that permit accurate base pairing to be achieved. One might wonder why cells have evolved the complex system of discontinuous lagging strand synthesis rather than a DNA polymerase that could simply extend DNA chains in their 3'- 5' direction. Consideration of the chemistry of DNA chain extension also leads to the conclusion that this system promotes high-fidelity replication.The linking of 5'-deoxynucleotide triphosphates in the 3'- 5' direction would require the retention of the growing chain's 5'-terminal triphosphate group to drive the next coupling step.

- On editing a mispaired 5'-terminal nucleotide, this putative polymerase would—in analogy with Pol I, for example excise the offending nucleotide, leaving either a 5'-OH or a 5'-phosphate group. Neither of these terminal groups is capable of energizing further chain extension. A proofreading 3'- 5' DNA polymerase would therefore have to be capable of reactivating its edited product. The inherent complexity of such a system has presumably selected against its evolution.

## MOLECULAR BIOLOGY 17BTU302

### UNIT III

**DNA damage, repair and homologous recombination:** DNA damage and repair: causes and types of DNA damage, mechanism of DNA repair: Photoreactivation, base excision repair, nucleotide excision repair, mismatch repair, trans-lesion synthesis, recombinational repair, nonhomologous end joining. Homologous recombination: models and mechanism.

| S.No | Lecture Duration (hr) | Topics | Support materials |
|---|---|---|---|
| 1 | 1 | DNA damage and repair - Types of DNA damage, Mechanism of DNA repair: | T1: 1214-1216 |
| 2 | 1 | Photoreactivation DNA damage | T1: 1214-1215 |
| 3 | 1 | Base excision repair mechanism | T1: 1218 |
| 4 | 1 | Nucleotide excision repair | T1: 1216-1217 |
| 5 | 1 | Mismatch repair | T1: 1220 |
| 6 | 1 | Trans-lesion synthesis | T1: 1221 |
| 7 | 1 | Recombinational repair | T1: 1225 |
| 8 | 1 | Non-homologous end joining | T1: 1225 |
| 9 | 1 | Homologous recombination: models and mechanism | T1 -1225-1226 |

Reference

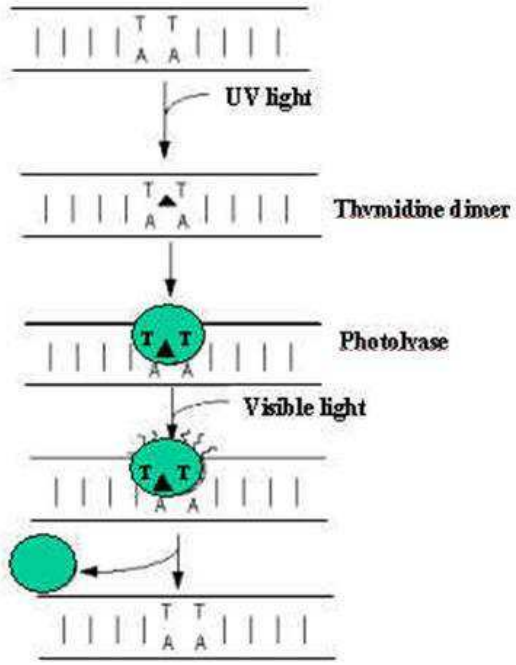
T1: Donald Voet and Judith Voet; 2012, Biochemistry, 4th Edition, John Wiley and Sons. Inc.

## DNA REPAIR

It can be defined as **"any one of the cellular process that attempts to correct errors in cellular DNA which occurred during cell division or by external environment".** There are many cellular proteins and enzymes which help in repairing most of the errors by means of different mechanisms.

### Photoreactivation

- Catalyzed by DNA-Photolyase (DPL)
- Reverses cyclobutyl pyrimidine dimers resulting from UV irradiation.
- It has low abundance (10 to 20 molecules per cell) in E. coli.
- DPL binds irradiated DNA 100x better than non-irradiated DNA.
- DPL contains a non-covalently associated chromophore (FADH or FADH2).
- The photochemical mechanism of photorepair has been proposed to be light-dependent redox reaction between the singlet excited state of FADH2 and the pyrimidine dimer.
- Photolyase has not yet been identified in placental mammals.




**Photoreactivation**

**Aberrant Methylation**

- Catalyzed by 6-O-methylguanine methyltransferase (6-O-MGM)
- 6-O-MGM recognizes 6-O-methylguanine in DNA and removes the methyl group, transferring the group to an amino acid on itself in a "suicide" mechanism.
- 6-O-MGM is encoded by the ada gene in E. coli and the MGMT gene in eukaryotes.
- Mice knocked out for MGMT are cancer-prone and sensitive to methylation agents

**Common Features of Excision-Type Repair Pathways**

- Recognition: Altered DNA is recognized and bound by a specific damage-recognition protein. This first step recruits other components required for the repair reaction.
- Excision: Damaged base(s), and in some cases adjacent nucleotides, are excised from the strand by exonucleases, resulting in a gapped DNA.
- Resynthesis: The gap is refilled by a DNA polymerase using the complementary strand as a template.

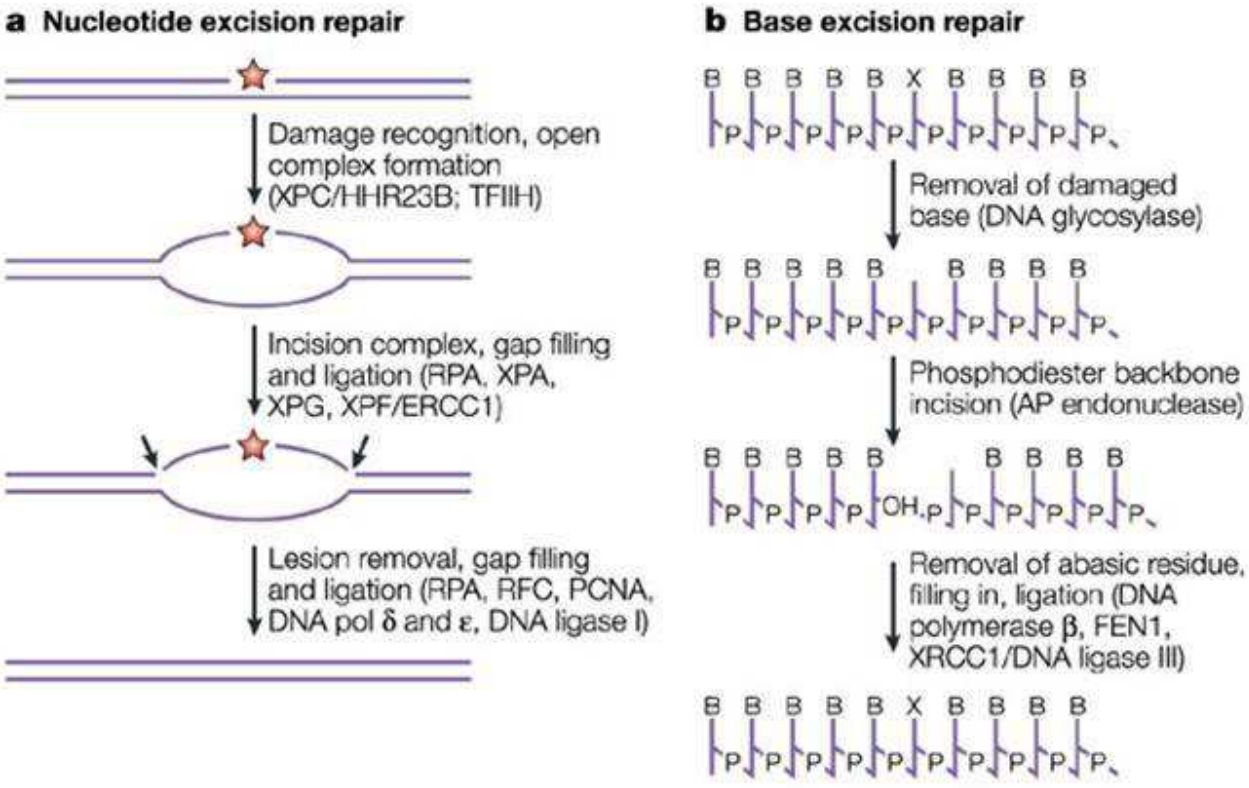
**Base-Excision Repair (BER)**

- Damaged bases are removed as free bases.
- BER primarily handles oxidative and alkylative damage.
- BER is thought to have an important role in aging.
- BER recognizes base deamination, oxidative damage, and other minor base modifications.
- Five gene products are required for BER: glycosylase, AP endonuclease, phosphodiesterase, DNA polymerase, and DNA ligase.
- DNA glycosylase recognizes the damaged base and removes it, generating an AP (apurinic, apyramidinic) site.
- AP endonuclease cleaves the phosphodiester bond, generating a single-strand break with a 5'-terminal deoxyribophosphate moiety.
- The 5'-deoxyribophosphate is excised by action of a DNA phosphodiesterase.
- The resulting single-nucleotide gap is repaired by DNA polymerase β (beta).
- The resulting nick is sealed by DNA ligase.

- BER is relatively inefficient, due to the large number of peptides needed to recognize each damage type.

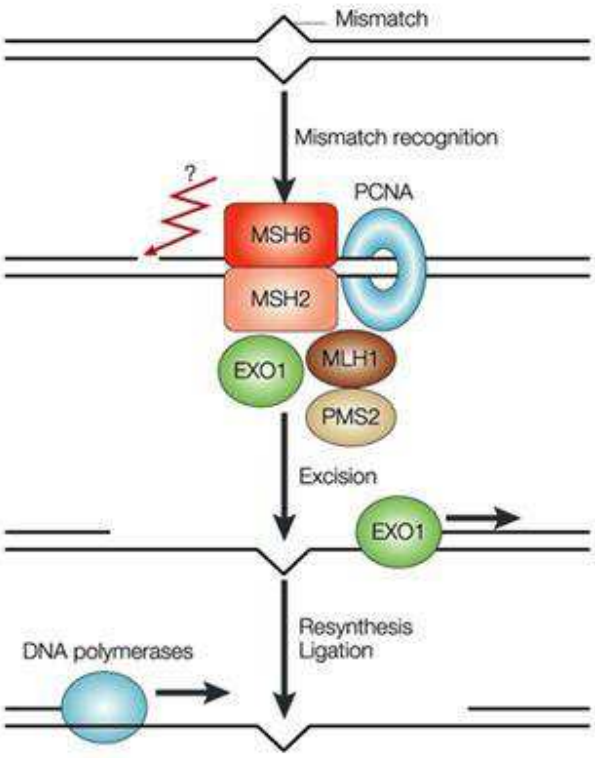
**Nucleotide-Excision Repair (NER)**

- Damaged bases are removed as oligonucleotides.
- NER is primarily responsible for removal of UV-induced damage and bulky adducts, but also removes ~20% of oxidative damage.
- Deficiencies cause many human disorders.
- Xeroderma Pigmentosum (both classical and variant) and Cockayne's syndrome are caused by defects in NER, resulting in various detrimental effects upon exposure to UV light.



**DNA Mismatch Repair (MMR)**

- Mismatches are removed as long oligonucleotides.
- MMR is primarily responsible for removal of replicative errors.
- Also prevents recombination of non-homologous sequences
- Deficient in many human cancers.
- MMR recognizes base-base mismatch and small insertion/deletion loops.




**DNA mismatch repair**

**Error prone translation DNAsynthesis**

When this pathway is active, DNA repair becomes significantly less accurate and a high mutation rate occurs. It is a part of cellular stress response to extensive DNA damage, also known as SOS response. The cell, at all cases had to survive, so DNA gets repaired, although it contains a lot of errors.

**Types of Mutations**

**NOTE**: For all others examples cited below, the below given DNA sequence shall be considered as wild type:

- ATG CCG TGT CAG ATG TTC ---------- DNA
- AUG CCG UGU CAG AUG UUC ---------- mRNA

Met Pro Cys Gln met phe ------ Amino acid sequence

**1. Synonymous / Silent Mutations**

1. No alteration in polypeptide product of the gene
2. Single base pair substitution
3. Occur in the third position of a codon
4. Codes for the same amino acid
5. No alteration of the protein

Example: ***Samesense Mutation*:** a codon is changed to a different codon that specifies the same amino acid.

ATG CCG TGC CAG ATG TTC -----MUTATED DNA (compare with wild type)
AUG CCG UGC CAG AUG UUC ----- mRNA
Met Pro Cys Gln Met Phe -------- amino acid sequence

**2. Non-Synonymous Mutations**

1. Occur less frequently than synonymous mutations
2. Leads to alteration in the encoded polypeptide
3. Result in abnormal function® disease

Occur in one of three main ways:

1. Missense
2. Nonsense
3. Frameshift

**Missence mutation:** a codon is changed to a different codon that specifies a different amino acid.

ATG CCG TGG CAG ATG TTC -----MUTATED DNA

AUG CCG UGG CAG AUG UUC ----- mRNA

Met Pro Trp Gln Met Phe -------- amino acid sequence

**Nonsense Mutation***:* A codon that specifies an amino acid is changed to a stop codon. (termination codon), this mutation usually destroys the function of the gene product.

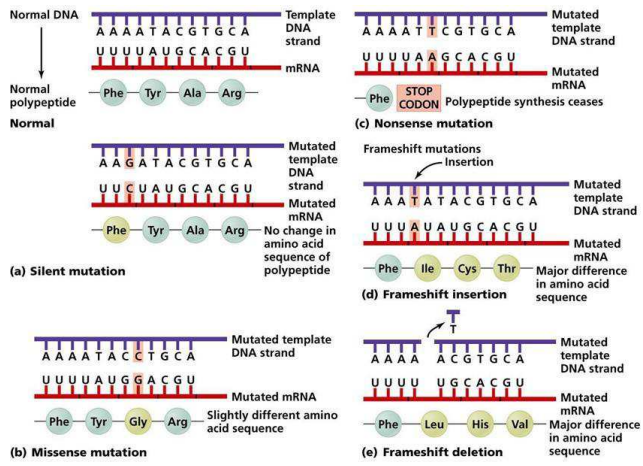
ATG CCG TGA CAG ATG TTC ----- MUTATED DNA

AUG CCG UGA CAG AUG UUC ----- mRNA

Met Pro STOP -------- amino acid sequence

**Frameshift Mutations**:

One or 2 nucleotide pairs are inserted into or deleted from the molecule, causing an alteration of the *reading frame.* As the result of this shift, codons downstream of the insertion or deletion site specify an entirely new sequence of amino acids. Depending on where the insertion or deletion occurs in the gene, different effects can be generated. In addition to producing an entirely new polypeptide sequence immediately after the change, frameshift mutations usually produce a stop or termination codon within a short distance of the mutation. This codon terminates the already altered polypepetide chain. A frame shift in a gene specifying an enzyme usually result in a loss of enzyme activity. If the enzyme is an essential one, the effect on the organism can be disastrous.




**Types of mutations**

**Mutation in non-coding DNA**

They will have a phenotyping effect if it occurs in Regulatory elements (e.g. TATA box) → affect level of gene expression

1. Splicing of introns: highly conserved GT & AG at the end of introns

Either:

a. coding sequences being lost
b. intronic sequences being added to the mRNA

**Functional effects of Mutation on the Protein**

1. Loss of function
2. Gain of Function

Loss of Function

- Reduce in activity

In heterzygous state → half normal levels of the protein product

- Or complete loss of the gene product

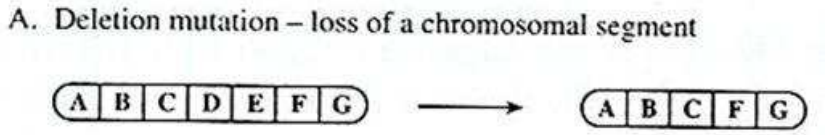
Gain of Function:

- Increased levels of gene expression
- Development of a new function(s) of the gene product

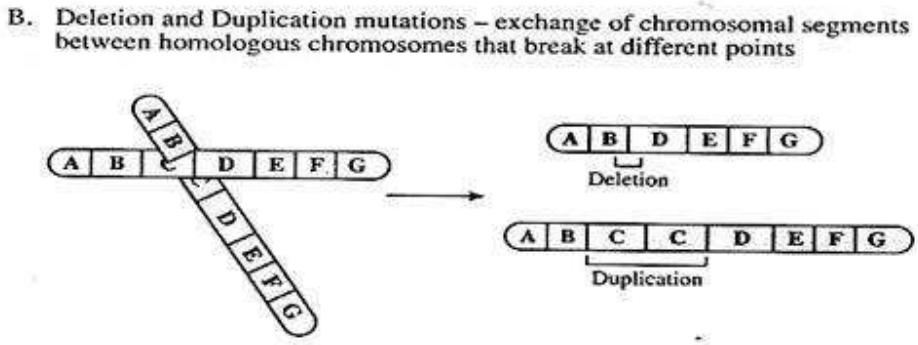
**Chromosomal Mutations:**

Chromosomal mutations are grouped into four broad types:

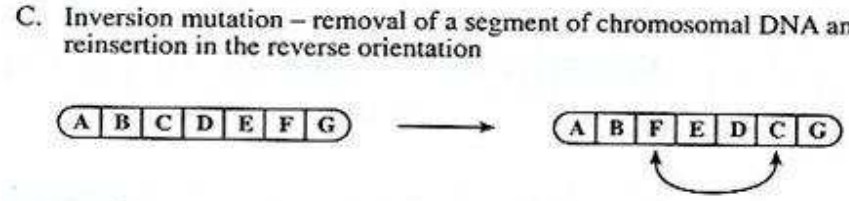
1. **Deletion**: are chromosomal changes in which one or more genes or segments of chromosomal DNA are lost.



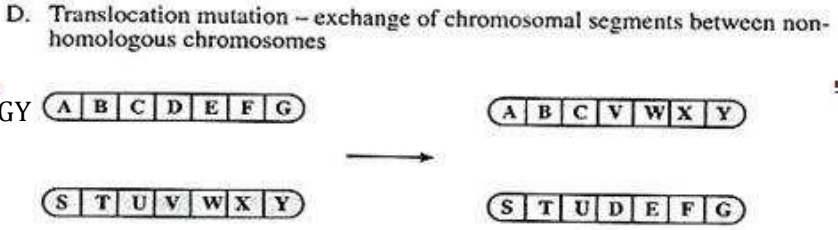

2. Duplication: are chromosomal changes in which one copy or more copies of a gene are present on the same chromosome




3. Inversion: in which a segment of DNA is released and rotated 180 degrees before being reinserted into the DNA. If the inverted DNA segment carries part of the protein-coding sequence, the resulting protein would be drastically altered and most likely nonfunctional.




4. Translocation: occur when a segment of DNA moves from one chromosome and inserted into a different nonhomologous chromosome. Translocation can also be reciprocal, that is two nonhomologous chromosome may break and trade pieces of DNA. Translocation mutations frequently cause



problems in meiosis and sometimes lead to aneuploidy (the gain or loss of chromosome)

**DNA recombination**

DNA recombination involves the exchange of genetic material either between multiple chromosomes or between different regions of the same chromosome. This process is generally mediated by homology ; that is, homologous regions of chromosomes line up in preparation for exchange, and some degree of sequence identity is required.

- Homologous recombination occurs between two homologous chromosomes.
- Nonhomologous, or illegitimate recombination occurs between two different chromosomes, though the segment at recombination sites may be related.
- Site-specific recombination can result in integration of viral, bacterial, or plasmid DNA into a chromosome at a specific location (such as att).
- Replicative recombination results in sequence transposition, and is mediated by transposase enzymes.

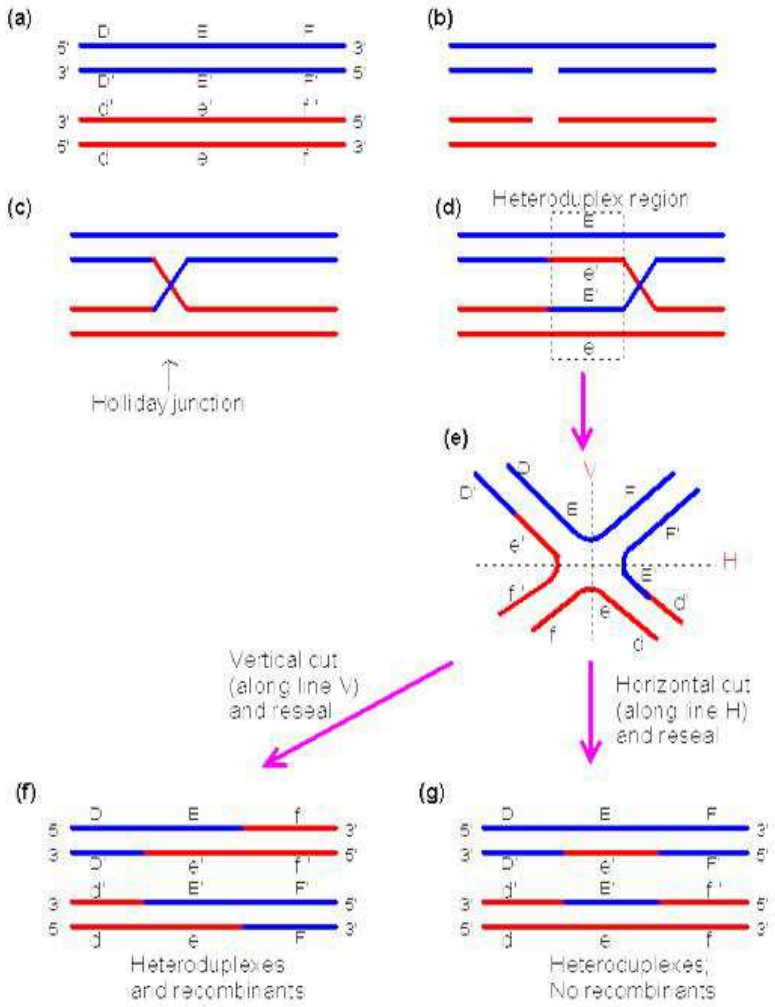
**Types of Homologous Recombination**

- Reciprocal recombination results from two chromosomes exchanging the same amount of DNA.
- Non-reciprocal recombination results from two chromosomes exchanging different amounts of DNA.
- Intramolecular recombination results from recombination in chromosomal loops.
  - Direct repeats cause plasmid formation from the intervening loop.
  - Inverted repeats cause the intervening loop to change direction.
- Double crossover results in exchanging a short segment of DNA, rather than a large segment of chromosome from the crossover point to the terminus.

**Holliday Model for Homologous Recombination**

- Two DNA molecules with nicks induced by an endonuclease in strands of the same polarity can invade each other with free single strands.

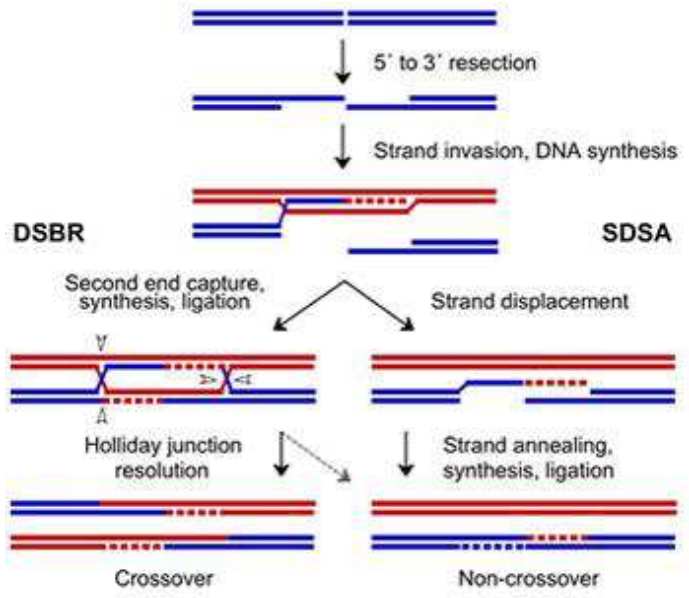
- A Holliday junction, or Chi structure, is formed as a recombination intermediate, and is solidified by ligase sealing the nicks, uniting the two homologues.
- Branch migration allows exchange of material, since the loose area around the junction allows unzipping/rezipping.
- New nicks allow separation of recombined DNA and resolution of the recombination intermediate.
- The Holliday model assumes reciprocal and equal exchange of genetic material between DNA molecules.




**Holliday model**

**Double-Strand Break Repair Model (Szostak Model) for Recombination**

- Double-strand breaks are introduced into one of the homologues.
- Broken ends are recessed by specific exonucleases which produce longer 3' single strands.
- The single strands can invade a region of homologous DNA.
- The invading end serves as a primer for a polymerase which extends it, unwidnding the template DNA and generating a D-loop. A Holliday junction is formed, and DNA synthesis proceeds until it reaches the opposing end of the recombining chromosome.
- The displaced DNA of the opposing end of the invading chromosome anneals to the template (invaded chromosome), forming a second Holliday junction by ligation of the strands. Holliday junctions can migrate prior to resolution with resolvase endonucleases.
- Resolution can cut the invading strands, resulting in a non-crossover event, since each chromosome has only a tiny fragment of DNA from the other chromosome.
- Crossover results from cutting the strands not participating in the recombination, generating a crossover by leaving a large segment of each chromosome attached to the other.
- The Szostak model accounts for non-reciprocal recombination.




**Double and single strand break repair model**

**Factors Involved in Each Recombination Step**

- The Szostak model is an error-free pathway (since it can only occur in homologues), and is of major importance in yeasts and mammals.
- A 5' to 3' recession reaction is mediated by endonucleases.
- Strand invasion requires multiple Rad proteins, including DNA helicases and DNA end-binding proteins.
- New DNA synthesis requires DNA Pol δ and ε (delta and epsilon).

**Repair of DSBs by Non-Homologous End Joining (NHEJ)**

- NHEJ is an error-prone pathway, and is the major pathway in mammals for repair of DSBs.
- DNA-PKcs (DNA protein kinase, catalytic subunit) binds ends of the DSB (double-strand break).
- Endonucleases create blunt (no overhang) ends.
- Synapsis is achieved through microhomologies.
- Ends are ligated, and, due to end-blunting and other factors, small insertions and deletions are introduced. The errors are often of little consequence, since most DNA is non-coding.

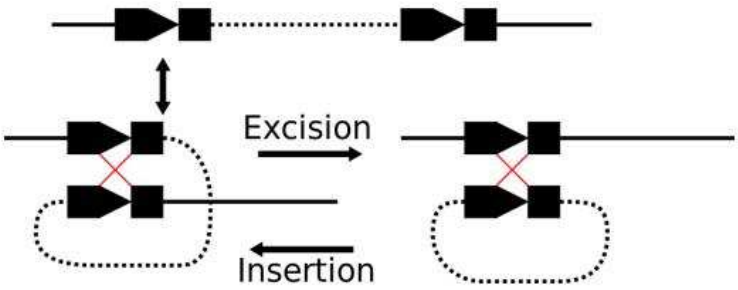
**Site specific recombination**

**Site-specific recombination** , also known as **conservative site-specific recombination** , is a type of genetic recombination in which DNA strand exchange takes place between segments possessing only a limited degree of sequence homology. Site-specific recombinases perform rearrangements of DNA segments by recognizing and binding to short DNA sequences (sites), at which they cleave the DNA backbone, exchange the two DNA helices involved and rejoin the DNA strands. While in some site-specific recombination systems just a recombinase enzyme and the recombination sites is enough to perform all these reactions, in other systems a number of accessory proteins and/or accessory sites are also needed.

Site-specific recombination systems are highly specific, fast and efficient, even when faced with complex eukaryotic genomes. They are employed in a variety of cellular processes, including

bacterial genome replication, differentiation and pathogenesis, and movement of mobile genetic elements. For the same reasons, they present a potential basis for the development of genetic engineering tools.

Recombination sites are typically between 30 and 200 nucleotides in length and consist of two motifs with a partial inverted-repeat symmetry, to which the recombinase binds, and which flank a central crossover sequence at which the recombination takes place. The pairs of sites between which the recombination occurs are usually identical, but there are exceptions (e.g. attP and attB of λintegrase,)




Insertion and excision mediated by aligned Lox sites and the cre recombinase. Red X designates recombination.

**MOLECULAR BIOLOGY 17BTU302**

**UNIT IV**

**Transcription and RNA processing:** RNA structure and types of RNA, Transcription in prokaryotes: Prokaryotic RNA polymerase, role of sigma factor, promoter, Initiation, elongation and termination of RNA chains Transcription in eukaryotes: Eukaryotic RNA polymerases, transcription factors, promoters, enhancers, mechanism of transcription initiation, promoter clearance and elongation RNA splicing and processing: processing of pre-mRNA: 5' cap formation, polyadenylation, splicing, rRNA and tRNA splicing.

| S.No | Lecture Duration (hr) | Topics | Support materials |
|---|---|---|---|
| 1 | 1 | RNA structure and types of RNA | T1: 1264 |
| 2 | 1 | Transcription in Prokaryotes - RNA polymerase, Role of sigma factor, Promoter, Initiation, Elongation and Termination of RNA chains | T1: 1265; T1:1266; T1: 1267-69; T1: 1275-1277 |
| 3 | 1 | Transcription in Eukaryotes - Mechanism of Initiation | T1: 1277-1279; |
| 4 | 1 | Promoter clearance and Elongation | T1:1284-1286: T1: 1292-1293 |
| 5 | 1 | RNA splicing and Processing | T1: 1307 |
| 6 | 1 | 5' cap formation | T1:1286-1302 |
| 7 | 1 | Polyadenylation (poly A tail) | T1: 1302-1304 |
| 8 | 1 | r RNA and t RNA splicing | T1: 1305-1306 |
| 9 | 1 | Processing of Pre m RNA | T1: 1324-1332 |

Refernce

T1: Donald Voet and Judith Voet; 2012, Biochemistry, 4th Edition, John Wiley and Sons. Inc.

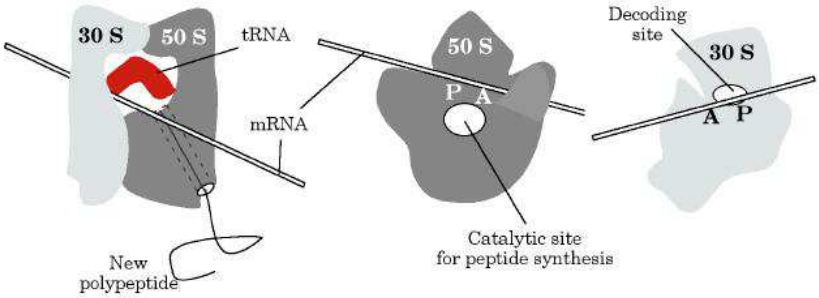
## RNA

- RNA differs from DNA in both structural and functional respects. RNA has two major structural differences: each of the ribose rings contains a 2´-hydroxyl, and RNA uses uracil in place of thymine.
- RNA molecules are capable of base pairing, but generally will not form large regions of stable RNA-RNA double helix.
- RNA can act as a genetic material (although this role, at least for current organisms, seems to be restricted to viruses). Unlike DNA, RNA can form complex three-dimensional structures. As a result, RNA can also exhibit catalytic activity.
- The combination of the ability to store genetic information with the ability to catalyze reactions has resulted in a proposal for the origin of life: the "RNA World".
- The RNA world hypothesis proposes that RNA molecules once filled all of the roles of protein and nucleic acid macromolecules, and acted in both an information storage capacity and as the source of the enzymatic activity required for metabolic reactions. In general, RNA is less suited to acting as genetic material than DNA, and is less suited to forming efficient catalysts than proteins.
- Assuming that the RNA world once existed, nearly all of its functions have been taken over by other biological molecules. However, some vestiges of the RNA world may still exist. The vast majority of RNA functions are concerned with protein synthesis.

**The major types of RNA**

**Ribosomal RNA (rRNA)**

- Ribosomal RNA molecules comprise 65 to 70% of the mass of the ribosome (the machinery responsible for protein synthesis). Ribosomes are very large objects; prokaryotic ribosomes have molecular weights of about 2.5 million, while eukaryotic ribosomes have molecular weights of about 4 million.



- The original studies on ribosomes used relatively crude techniques that were unable to measure size in terms of molecular weight. Instead the size of the ribosomal particles and their components were measured by their rate of sedimentation (movement driven by gravitational acceleration or centrifugal acceleration).

- Sedimentation is a function of size, shape, and density, with larger objects tending to sediment faster than smaller ones. Object sizes are measured in Svedberg units. Prokaryotic ribosomes are 70 S particles, with each comprised of a large (50 S) and a small (30 S) subunit. Eukaryotic ribosomes are 80 S particles, comprised of a large (60 S) and a small (40 S) subunit. You will notice that the Svedberg units are not additive for the particles sizes; this is due to the effects of shape on sedimentation.

- The eukaryotic 40S Ribosome contains 1 rRNA (18 S rRNA = 1900 bases) and about 35 different proteins. The 60S ribosome contains 3 rRNA (5 S = 120 bases, 5.8 S = 160 bases, and 28 S = 4700 bases), and about 50 proteins. The 5 S rRNA has its own gene; the others are synthesized as a single transcript that is then cleaved to release the mature RNA molecules that become part of the ribosome. Until relatively recently, it was assumed that the ribosomal RNA performed a largely structural function.

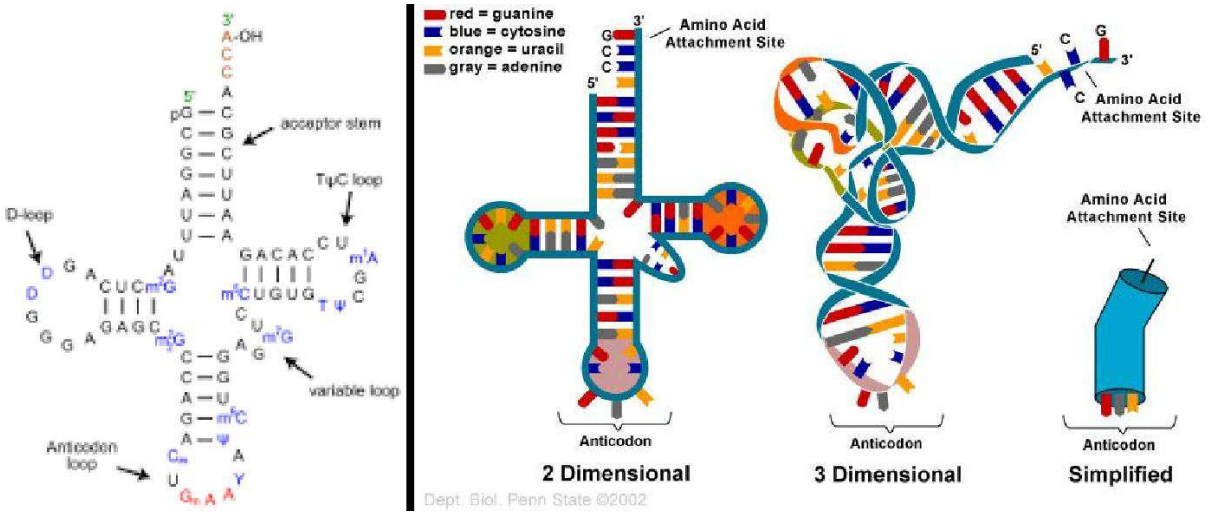
- However, more recent data strongly suggests that the rRNA acts as the enzyme, with the protein acting as the structural scaffolding. These data include results from the recent high-resolution (2.4 Å) X-ray diffraction structure of the large subunit and low-resolution (5 Å) structure of the complete ribosome from the bacterium *Haloarcula marismortui*. Examination of the high-resolution structure of the large subunit, and of the lower resolution structures of the entire particle (used to generate the cartoons above) strongly suggests that only RNA is present at the catalytic site.

- In the structure below, proteins are shown in black, and the orientation of the large subunit is similar to the center cartoon above. Examination of this structure suggests that no protein is present in the catalytic site.

**Transfer RNA (tRNA)**

- tRNA is a ~75 base molecule that carries the amino acids, and transfers them to the growing protein. tRNAs are thought to have a common tertiary structure structure based on X-ray diffraction analysis is shown below. Analysis of the tRNA sequence suggests a

cloverleaf secondary structure formed by regions of base pairing between the sections of the RNA strand, with this cloverleaf folding into the three-dimensional structure.




**Messenger RNA (mRNA)**

- mRNA molecules contain the coding sequence for proteins. The mRNA molecules can vary considerably in size, with eukaryotic transcripts including the largest known ribonucleic acids. This is most obvious before splicing of introns, because many transcripts exceed 100 kb in length.

**snRNA small nuclear ribonucleic acid (snRNA),**

- It is also commonly referred to as **U-RNA**, is a class of small RNA molecules that are found within the splicing speckles and Cajal bodies of the cell nucleus in eukaryotic cells. The length of an average snRNA is approximately 150 nucleotides. They are transcribed by either RNA polymerase II or RNA polymerase III, and studies have shown that their primary function is in the processing of pre- messenger RNA (hnRNA) in the nucleus. They have also been shown to aid in the regulation of transcription factors (7SK RNA) or RNA polymerase II (B2 RNA), and maintaining thetelomeres.

- snRNA are always associated with a set of specific proteins, and the complexes are referred to as small nuclear ribonucleoproteins (snRNP, often pronounced "snurps"). Each snRNP particle is composed of several Sm proteins, the snRNA component, and snRNP-specific proteins. The most common snRNA components of these complexes are known, respectively, as: U1 spliceosomal RNA, U2 spliceosomal RNA, U4 spliceosomal RNA, U5 spliceosomal

RNA, and U6 spliceosomal RNA. Their nomenclature derives from their high uridine content.

- snRNAs were discovered by accident during a gel electrophoresis experiment in 1966. An unexpected type of RNA was found in the gel and investigated. Later analysis has shown that these RNA were high in uridylate and were established in the nucleus.
- A large group of snRNAs are known as small nucleolar RNAs (snoRNAs). These are small RNA molecules that play an essential role in RNA biogenesis and guide chemical modifications of ribosomal RNAs (rRNAs) and other RNA genes (tRNA and snRNAs). They are located in the nucleolus and the Cajal bodies of eukaryotic cells (the major sites of RNA synthesis), where they are called scaRNAs (small Cajal body-specific RNAs).

**Classes of sn RNA**

- snRNA are often divided into two classes based upon both common sequence features as well as associated protein factors such as the RNA-binding LSm proteins.
- The first class, known as **Sm-class snRNA**, is more widely studied and consists of U1, U2, U4, U4atac, U5, U7, U11, and U12. Sm-class snRNA are transcribed by RNA polymerase II.

  The pre-snRNA are transcribed and receive the usual 7-methylguanosine five-prime cap in the nucleus. They are then exported to the cytoplasm through nuclear pores for further processing. In the cytoplasm, the snRNA receive 3' trimming to form a 3' stem-loop structure, as well as hypermethylation of the 5' cap to form trimethylguanosine. The 3' stem structure is necessary for recognition by the survival of motor neuron(SMN) protein. This complex assembles the snRNA into stable ribonucleoproteins (RNPs). The modified 5' cap is then required to import the snRNP back into the nucleus. All of these uridine-rich snRNA, with the exception of U7, form the core of the spliceosome. Splicing, or the removal of introns, is a major aspect of post-transcriptional modification, and takes place only in the nucleus of eukaryotes. U7 snRNA has been found to function in histone pre-mRNA processing.
- The second class, known as **Lsm-class snRNA**, consists of U6 and U6atac. Lsm-class snRNAs are transcribed by RNA polymerase III and never leave the nucleus, in contrast to Sm-class snRNA. Lsm-class snRNAs contain a 5'-γ-monomethylphosphate cap and a 3'

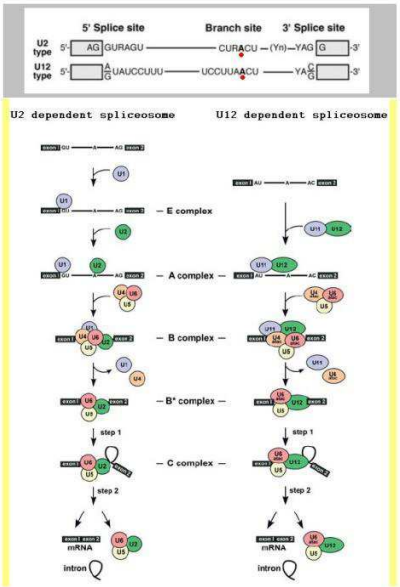
stem–loop, terminating in a stretch of uridines that form the binding site for a distinct heteroheptameric ring of Lsm proteins.

**SnRNA in the splicosome**

- Spliceosomes are a major component of an integral step in eukaryotic precursor messenger RNA maturation. A mistake in even a single nucleotide can be devastating to the cell, and a reliable, repeatable method of RNA processing is necessary to ensure cell survival. The spliceosome is a large, protein-RNA complex that consists of five small nuclear RNAs (U1, U2, U4, U5, and U6) and over 150 proteins.

- The snRNAs, along with their associated proteins, form ribonucleoprotein complexes (snRNPs), which bind to specific sequences on the pre-mRNA substrate. This intricate process results in two sequential transesterification reactions. These reactions will produce a free lariat intron and ligate two exons to form a mature mRNA. There are two separate classes of spliceosomes. The major class, which is far more abundant in eukaryotic cells, splices primarily U2-type introns.

- The initial step of splicing is the bonding of the U1 snRNP and its associated proteins to the 5' splice end to the hnRNA. This creates the commitment complex which will constrain the hnRNA to the splicing pathway. Then, U2 snRNP is recruited to the spliceosome binding site and forms complex A. U2 snRNP changes the conformation of the hnRNA-snRNP complex, exposing the nucleotide favorably for splicing. Following the conformation change, the U4/U5/U6 tri-snRNP complex binds to complex A to form the structure known as complex B. After rearrangement, complex C is formed, and the spliceosome is active for catalysis.




*U5 is believed to be the only common component between major and minor spliceosomes.*
*Reference: Will CL, Luhrmann R.Biol Chem. 2005 Aug;386(8):713-24.*

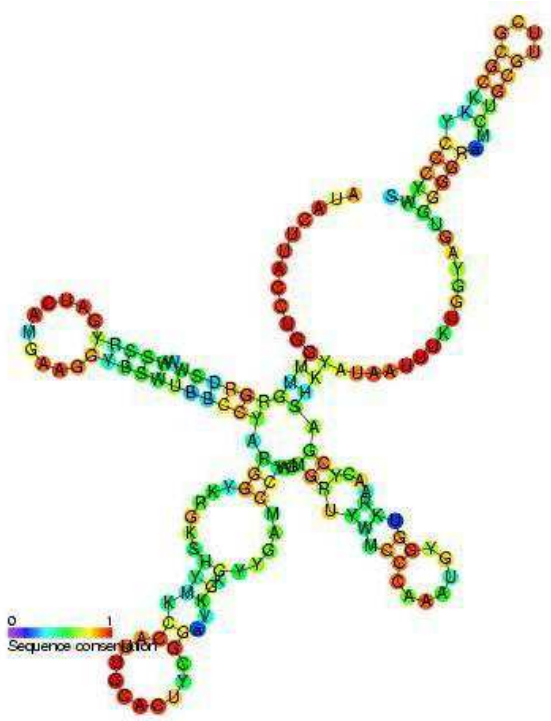
- In addition to this main spliceosome complex, there exists a much less common (~1%) minor spliceosome. This complex comprises U11, U12, U4atac, U6atac and U5 snRNPs. These snRNPs are functional analogs of the snRNPs used in the major spliceosome. The minor spliceosome splices U-12 type introns. The two types of introns mainly differ in their

splicing sites: U2-type introns have GT-AG 5' and 3' splice sites while U12-type introns have AT-AC at their 5' and 3' ends. The minor spliceosome carries out its function through a different pathway from the major spliceosome.

**U1 snRNA**

- U1 snRNP is the initiator of spliceosomal activity in the cell by base pairing with the hnRNA. In the major spliceosome, experimental data has shown that the U1 snRNP is

present in equal stoichiometry with U2, U4, U5, and U6 snRNP. However, U1 snRNP's abundance in human cells is far greater than that of the other snRNPs. Through U1 snRNA gene knockdown in HeLacells, studies have shown the U1 snRNA holds great importance for cellular function. When U1 snRNA genes were knocked out, genomic microarrays showed an increased accumulation of unspliced pre-mRNA. In addition, the knockout was shown to cause premature cleavage andpolyadenylation primarily in introns located near the beginning of the transcript. When other uridine based snRNAs were knocked out, this effect was not seen. Thus, U1 snRNA–pre-mRNA base pairing was shown to protect pre-mRNA from polyadenylation as well as premature cleavage. This special protection may explain the overabundance of U1 snRNA in the cell.



**Transcription**

• Transcription is a fundamental cellular process: RNA polymerases "transcribe" the genetic information on DNA into RNA strands. All cells have RNA polymerases (RNAP).

• The RNA polymerases increase in complexity as you go from viruses (example, T7 RNA polymerase is made up of a single protein), to bacterial systems (one RNA polymerase made up of the proteins - beta, beta', 2 x alpha, omega and the sigma factor), and finally to eukaryotic systems (Three RNA polymerases - Pol I, Pol II, and Pol III, each with ten or more subunits).

• While the RNA polymerases have become increasing complex as life evolved, their overall structure (as evidenced by crystallographic structures of bacterial RNA polymerase and Pol II) show remarkable similarity. There is also sequence similarity between the bacterial polymerase protein subunits and the proteins that make up the eukaryotic polymerases.

**Transcription of any gene usually involves three distinct stages:**

1. First, the RNA polymerase has to find the start site of a gene. The "holoenzyme" form of the polymerase does this by looking for the "promoter" site that exists just upstream of the gene start site. This process is termed "transcription initiation". This is followed by opening up (melting) of the duplex DNA to form an "open complex".

2. This is followed by a rapid change into the "elongation" phase of transcription where the "core polymerase" part of the RNA polymerase rapidly transcribes an RNA strand that is complementary to the "template" strand of the DNA. The change into the elongation phase usually occurs after a few bases of RNA have been transcribed (typically about 8-9 bases of RNA in bacterial system which form a RNA-DNA hybrid with the template strand), and involves a "clamping down" on the DNA to prevent the polymerase from falling off the DNA.

3. The final stage of the transcription of a gene is "termination", after the stop codon of the gene. The process of termination usually involves sequences where the polymerase slows down or stalls, and the polymerase-RNA-DNA complex (often proteins such as rho and NusA are involved in bacterial systems).

• Genes have to be transcribed to mRNAs before they can be translated into proteins; more or less mRNA from a particular gene equals more or less of the protein encoded by the gene.

Transcription is, thus, an important point in the control of gene "expression". Most genes are controlled transcriptionally, usually by regulation of the level of transcriptional initiation. For example, if a gene has a strong promoter, it will be more highly expressed when compared to another gene with a weak promoter site. Similarly if a regulatory protein can bind the promoter site of a gene (and prevent transcription initiation), then it can turn off the expression of that gene.

• Transcriptional control of genetic expression is vital for cellular functions, and many diseases and cancers are results of defects in the transcriptional control of essentials genes.

**Transcription initiation**

• Transcription initiation in bacteria (prokaryotes) involves sigma factors. The sigma factor combine with the core RNA polymerase to form a holoenzyme that is competent for promoter binding. The core RNA polymerase, by itself, cannot bind the promoter site.

• The sigma factor can be thought of as the specificity factor in the RNA polymerase.

Each bacteria has several different sigma factors that recognize slightly different promoter sequences. Predominant among these is the sigma70 (70 kDa in E. coli; also called rpoD), which initiates the transcription of most genes in exponentially growing cells. There are two general classes of sigma factors - sigma 70 class and sigma 54 class:

• The sigma 70 class of sigma factors share extensive sequence homology, and bind to two conserved sequences upstream of the gene start site (the -35 box and the -10 box). Each of these sigma factors recognizes slight variations in these conserved sequence boxes. Sigma70 binds promoter DNA as a part of the holoenzyme, and binds DNA very poorly in absence of the core enzyme.

• The sigma54 class of genes (54 kDa in E. coli; also called rpoN) controls a much smaller set of genes than sigma70. It recognizes different conserved sequences (the -12 and -24 boxes). Unlike Sigma70, sigma54 can bind DNA even in the absence of the core RNA polymerase. It however lacks the ability to melt promoter DNA on its own - for this it needs to interact with other activator proteins that bind further upstream of the promoter site, as well as the core RNAP.

• In eukaryotic systems, transcription initiation is very different. There are no sigma factors. Instead, the central protein in forming the "pre-initiation complex " (PIC) is the TATA binding protein (TBP), that binds to the TATA box, a conserved sequence just upstream of the

initiation region. A large number of other general transcription factors such as TFIIB, TFIIE, TFIIF, TFIIH (TF stands for transcription factor; II stands for Pol II; there are similar factors for Pol I and Pol II) and others assemble to form the multisubunit TFIID complex. This PIC then recruits the RNA polymerase (Pol I, II, or III in eukaryotic cells) to initiate transcription. The PIC often remains at the promoter site, and is then available to initiate another round of transcription.

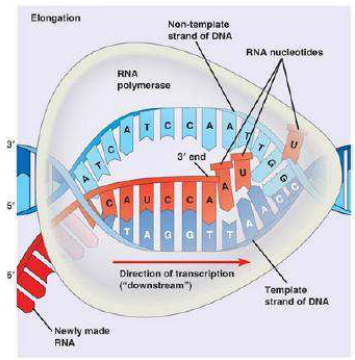
• TBP is a universal transcription factor, and is seen in all eukaryotes and archaea. It sharply bends DNA at the TATA box.

**Transcription elongation**

• Once initiation is complete and the open complex forms, the RNA polymerase begins to read the template strand and add corresponding RNA nucleotides. This process is not always efficient, and the polymerase may make several passes at this. After a long enough RNA-DNA hybrid is made, the polymerase clears the promoter region and moves rapidly downstream. This is preceeded by a large conformational change in the polymerase core enzyme, as it clamps down on the DNA and becomes quite processive.

• While transcription elongation is quite rapid, the polymerase does not transcribe all sequences with equal efficieny. The elongation rate is not uniform, and there can be pausing or stalling. Elongation factors (GreA/GreB in bacterial systems; TFIIS in eukaryotes) act to help the polymerase along by stimulating backtracking and cleavage of the newly formed RNA (from the 3' end).

• Other factors are involved in the elongation cycle - for example, in eukaryotic Pol II there are the elongins and ELL proteins that increase the elongation rate, as well as factors to remodel chromatin.




**Transcription elongation**

**Transcription activation & repression**

• There are multiple modes of transcription activation. All these usually involve different protein factors (activators) that bind DNA sequences (enhancer sequences) in and around the promoter site. All these act to increase the affinity of the initiation complex or RNA holoenzyme at the promoter (thus enhancing the chance that a productive open complex will form and transcription will initiate).

• When enhancer elements combine with poor promoter sequences, activators can modulate the activity of a gene by several hundred or thousand fold. For example, if a gene has a poor -35 or -10 box in its promoter site, it will be poorly expressed - an activator protein can, in such a case, enhance the activity of the gene by several orders of magnitude, by recruiting the RNA polymerase (or other initiation factors) to the promoter site.

• Repression, on the other hand, works by having protein (repressors) that sit on or close to the promoter regions of the DNA, preventing RNA polymerase or initiator/activator proteins from starting transcription initiation (thereby "turning-off" the gene).

• Activators and repressors are usually DNA binding proteins. These proteins have common DNA binding motifs such as Zn-fingers, helix-turn-helix, etc. Activators also have regions that interact with different domains of the RNA polymerase (parts of alpha, sigma, etc.).

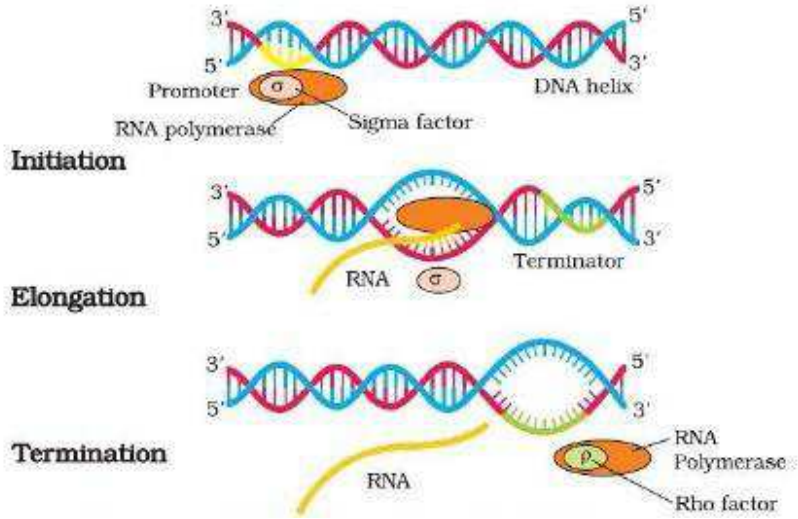
• In addition to protein activators, there are also DNA sequences that can directly interact with RNA polymerase components. For example, the alpha subunit in the bacterial polymerase has two domains - the alpha NTD (n-terminal domain) and the alpha CTD (c-terminal domain), linked by a flexible linker region. The alpha CTD can bind DNA sequences (UP elements) upstream of the promoter region, enhancing the affinity of the polymerase on the promoter.

**Transcription termination**

• Termination in bacterial system can be broadly classified as "rho independent", and "rho dependent". o In rho independent termination, there is formation of a stable GC rich stem-loop in the newly synthesized RNA followed by a string of U's (A's in the template strand) spaced about 20 bases downstream (these sites are often called intrinsic terminators). The stem loop "snares" the polymerase, slowing or stalling it. This pause, coupled with the low stability of the RNA-DNA hybrid at the active site (run of A=U basepairs) allows the RNA polymerase to fall off the template DNA and terminates the RNA transcription for that gene.

o In rho dependent termination, rho binds the newly formed RNA (as hexamers), and stalls the RNA polymerase by interacting with it. In some models, the Rho hexamer translocates on the RNA. Rho termination activity is stimulated by ATP hydrolysis. This activity is greatly enhanced by Nus factors.

• In eukaryotic cells, transcription termination involves cleavage of the elongating RNA chain by specific endonucleases which recognize particular sequences (AAUAAA) in the newly formed RNA. Once this happens, the RNA elongation complex is destabilized, and falls off the DNA. It is then available to attach to another nearby PIC, and start transcribing again.




**Process of transcription in bacteria**

**RNA processing (for eukaryotes)**

Eukaryotic genes contain Exons (which encode information that will end up being translated into protein) separated by Introns (sequences that will not encode proteins). At the 5' end of a gene we find a promoter and often a CpG island. Both of these elements regulate the transcription of a gene. At the 3' end of a gene we find a stop sequence, and a signal for polyadenylation (AAUAAA). In Eukaryotes this primary RNA transcript is then processed into a messenger RNA (mRNA) if the gene is to be translated into protein. This involves removing introns, which do not encode for protein, and splicing the remaining exons together. At the 5´ end of the mRNA a 7-Methyguanosine residue is added to provide a protective cap. At the 3´ end of the mRNA, 50-100 adenosine residues are added, generating a poly A tail.

**REVERSE TRANSCRIPTION**

Some viruses (such as HIV, the cause of AIDS), have the ability to transcribe RNA into DNA. HIV has an RNA genome that is duplicated into DNA. The resulting DNA can be merged with the DNA genome of the host cell. The main enzyme responsible for synthesis of DNA from an RNA template is called reverse transcriptase. In the case of HIV, reverse transcriptase is responsible for synthesizing a complementary DNA strand (cDNA) to the viral RNA genome. An associated enzyme, ribonuclease H, digests the RNA strand, and reverse transcriptase synthesizes a complementary strand of DNA to form a double helix DNA structure. This cDNA is integrated into the host cell's genome via another enzyme (integrase) causing the host cell to generate viral proteins that reassemble into new viral particles. In HIV, subsequent to this, the host cell undergoes programmed cell death, apoptosis of T cells. However, in other retroviruses, the host cell remains intact as the virus buds out of the cell.

Some eukaryotic cells contain an enzyme with reverse transcription activity called telomerase. Telomerase is a reverse transcriptase that lengthens the ends of linear chromosomes. Telomerase carries an RNA template from which it synthesizes DNA repeating sequence, or "junk" DNA. This repeated sequence of DNA is important because, every time a linear chromosome is duplicated, it is shortened in length. With "junk" DNA at the ends of chromosomes, the shortening eliminates some of the non essential, repeated sequence rather than the protein-encoding DNA sequence farther away from the chromosome end. Telomerase is often activated in cancer cells to enable cancer cells to duplicate their genomes indefinitely without losing important protein-coding DNA sequence. Activation of telomerase could be part of the process that allows cancer cells to become *immortal*.

**Promoters**

A promoter is a region of DNA that initiates transcription of a particular gene. Promoters are located near the transcription start sites of genes, on the same strand and upstream on the DNA (towards the 5' region of the antisense strand). Promoters can be about 100–1000 base pairs long.

For transcription to take place, the enzyme that synthesizes RNA, known as RNA polymerase, must attach to the DNA near a gene. Promoters contain specific DNA sequences such as response elements that provide a secure initial binding site for RNA polymerase and for

proteins called transcription factors that recruit RNA polymerase. These transcription factors have specific activator or repressor sequences of corresponding nucleotides that attach to specific promoters and regulate gene expression.

**In bacteria**

The promoter is recognized by RNA polymerase and an associated sigma factor, which in turn are often brought to the promoter DNA by an activator protein's binding to its own DNA binding site nearby.

**In eukaryotes**

The process is more complicated, and at least seven different factors are necessary for the binding of an RNA polymerase II to the promoter.

Promoters represent critical elements that can work in concert with other regulatory regions (enhancers, silencers, boundary elements/insulators) to direct the level of transcription of a given gene.

**Promoter elements**

Core promoter – the minimal portion of the promoter required to properly initiate transcription [3]

- Includes the transcription start site (TSS) and elements directly upstream
- A binding site for RNA polymerase
- RNA polymerase I: transcribes genes encoding ribosomal RNA
- RNA polymerase II: transcribes genes encoding messenger RNA and certain small nuclear RNAs and microRNA
- RNA polymerase III: transcribes genes encoding transfer RNAs and other small RNAs
- General transcription factor binding sites, e.g. TATA box
- Proximal promoter – the proximal sequence upstream of the gene that tends to contain primary regulatory elements
- Approximately 250 base pairs upstream of the start site
- Specific transcription factor binding sites
- Distal promoter – the distal sequence upstream of the gene that may contain additional regulatory elements, often with a weaker influence than the proximal promoter

- Anything further upstream (but not an enhancer or other regulatory region whose influence is positional/orientation independent)
- Specific transcription factor binding sites

**Operator**

- an **operator** is a segment of DNA to which a transcription factorbinds to regulate gene expression. The transcription factor is a repressor, which can bind to the operator to prevent transcription.

- The main operator (O2) in the classically defined *lac* operon is located slightly downstream of the promoter. Two additional operators, O1 and O3 are located at -82 and +412, respectively.

**Mechanism**

- The repressor protein physically obstructs the RNA polymerase fromtranscribing the genes.
- An inducer (small molecule) can displace a repressor (protein) from the operator site (DNA), resulting in an uninhibited operon.
- Alternatively, a corepressor can bind to the repressor to allow its binding to the operator site. A good example of this type of regulation is seen for the trp operon.

**Terminator**

**transcription terminator** is a section of nucleic acid sequence that marks the end of a gene or operon in genomic DNA during transcription. This sequence mediates transcriptional termination by providing signals in the newly synthesized mRNA that trigger processes which release the mRNA from the transcriptional complex. These processes include the direct interaction of the mRNA secondary structure with the complex and/or the indirect activities of recruitedtermination factors. Release of the transcriptional complex frees RNA polymerase and related transcriptional machinery to begin transcription of new mRNAs.

Two classes of transcription terminators, Rho-dependent and Rho-independent, have been identified throughout prokaryotic genomes. These widely distributed sequences are responsible for triggering the end of transcription upon normal completion of gene or operon transcription, mediating early termination of transcripts as a means of regulation such as that observed in transcriptional attenuation, and to ensure the termination of runaway transcriptional complexes

that manage to escape earlier terminators by chance, which prevents unnecessary energy expenditure for the cell.

**Rho-dependent terminators**

Rho-dependent transcription terminators require a protein called Rho factor, which exhibits RNA helicase activity, to disrupt the mRNA-DNA-RNA polymerase transcriptional complex. Rho-dependent terminators are found in bacteria andphage. The Rho-dependent terminator occurs downstream of translational stop codons and consists of an unstructured, cytosine-rich sequence on the mRNA known as a Rho utilization site (*rut*) for which a consensus sequence has not been identified, and a downstream transcription stop point (*tsp*). The *rut* serves as a mRNA loading site and as an activator for Rho; activation enables Rho to efficiently hydrolyze ATP and translocate down the mRNA while it maintains contact with the rut site. Rho is able to catch up with the RNA polymerase, which is stalled at the downstream *tsp* sites. Contact between Rho and the RNA polymerase complex stimulates dissociation of the transcriptional complex through a mechanism involvingallosteric effects of Rho on RNA polymerase.

**Rho-independent terminators**

Intrinsic transcription terminators or Rho-independent terminators require the formation of a self-annealing hairpin structure on the elongating transcript, which results in the disruption of the mRNA-DNA-RNA polymerase ternary complex. The terminator sequence contains a 20 basepair GC-rich region of dyad symmetry followed by a short poly-T tract or "T stretch" which is transcribed to RNA to form the terminating hairpin and a 7-9 nucleotide "U tract" respectively. The mechanism of termination is hypothesized to occur through a combination of direct promotion of dissociation through allosteric effects of hairpin binding interactions with the RNA polymerase and "competitive kinetics". The hairpin formation causes RNA polymerase stalling and destabilization, leading to a greater likelihood that dissociation of the complex will occur at that location due to an increased time spent paused at that site and reduced stability of the complex. Additionally, the elongation protein factor NusA interacts with the RNA polymerase and the hairpin structure to stimulate transcriptional termination.

**Termination in eukaryotes**

In eukaryotic transcription of mRNAs, terminator signals are recognized by protein factors that are associated with the RNA polymerase II and which trigger the termination process. Once the poly-A signals are transcribed into the mRNA, the proteinscleavage and polyadenylation specificity factor (CPSF) and cleavage stimulation factor (CstF) transfer from the carboxyl terminal domain of RNA polymerase II to the poly-A signal. These two factors then recruit other proteins to the site to cleave the transcript, freeing the mRNA from the transcription complex, and add a string of about 200 A-repeats to the 3' end of the mRNA in a process known as polyadenylation. During these processing steps, the RNA polymerase continues to transcribe for several kilobases and eventually dissociates from the DNA and downstream transcript through an unclear mechanism; there are two basic models for this event known as the torpedo and allosteric models.

**Torpedo model**

After the mRNA is completed, the residual RNA strand remains in association with the DNA template and the RNA polymerase II, continuing to be transcribed. XRN2 (5'-3' Exoribonuclease 2), a RNase, attaches to the carboxyl terminal domain of RNA polymerase II and proceeds to degrade the uncapped residual RNA from 5’ to 3’ until it reaches the RNA pol II. 5' cap refers to a modified guanine added to the front of mRNA for protection from RNase. 3' poly(A) tail is added to the end of a mRNA strand for protection from exonucleases. Similar to Rho-dependent termination, XRN2 triggers dissociation of RNA polymerase II by either pushing the polymerase off of the DNA template or pulling the template out of the RNA polymerase. The entire mechanism remains unclear.

**Allosteric model**

RNA polymerase normally is capable of transcribing DNA into single-stranded mRNA efficiently. However, upon transcribing over the poly-A signals on the DNA template, a conformational shift is induced in the RNA polymerase from the proposed loss of associated proteins from its carboxyl terminal domain. This change of conformation reduces RNA polymerase'sprocessivity making the enzyme more prone to dissociating from its DNA-RNA substrate. In this case, termination is not completed by degradation of mRNA but instead is mediated by limiting the elongation efficiency of RNA polymerase and thus increasing the likelihood that the polymerase will dissociate and end its current cycle of transcription.

**Enhancer**

An enhancer is a short region of DNA that can be bound with proteins (namely, the trans-acting factors, much like a set of transcription factors) to enhance transcription levels of genes (hence the name) in a gene cluster. While enhancers are usually cis-acting, an enhancer does not need to be particularly close to the genes it acts on, and need not be located on the same chromosome.

In eukaryotic cells the structure of the chromatin complex of DNA is folded in a way that functionally mimics the supercoiled state characteristic of prokaryotic DNA, so that although the enhancer DNA is far from the gene in regard to the number of nucleotides, it is geometrically close to the promoter and gene. This allows it to interact with the general transcription factors and RNA polymerase II. An enhancer may be located upstream or downstream of the gene that it regulates.

Furthermore, an enhancer does not need to be located near to the transcription initiation site to affect the transcription of a gene, as some have been found to bind several hundred thousand base pairs upstream or downstream of the start site.**Enhancers do not act on the promoter region itself, but are bound by activator proteins**. These activator proteins interact with the mediator complex, which recruits polymerase II and the general transcription factors which then begin transcribing the genes. Enhancers can also be found within introns. An enhancer's orientation may even be reversed without affecting its function. Additionally, an enhancer may be excised and inserted elsewhere in the chromosome, and still affect gene transcription. That is the reason that intron polymorphisms are checked though they are not translated.

**Silencers**

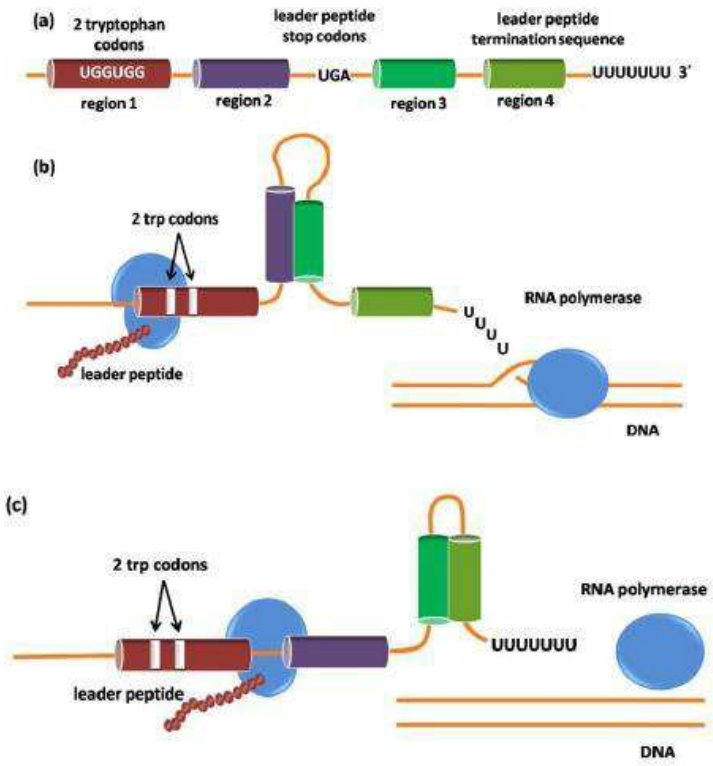
In genetics, a **silencer** is a DNA sequence capable of binding transcription regulation factors, called repressors. DNA contains genes and provides the template to produce messenger RNA (mRNA). That mRNA is then translated into proteins that activate or inactivate gene expression in cells. When a repressor protein binds to the silencer region of DNA, RNA polymerase is prevented from transcribing the DNA sequence into RNA. With transcription blocked, the translation of RNA into proteins is impossible. Thus, silencers prevent genes from being expressed as proteins.

RNA polymerase, a DNA-dependent enzyme, transcribes the DNA sequences, called nucleotides, in the 3' to 5' direction while the complementary RNA is synthesized in the 5' to 3' direction. RNA is similar to DNA, except that RNA contains uracil, instead of thymine, which forms a base pair with adenine. An important region for the activity of gene repression and expression found in RNA is the 3' untranslated region. This is a region on the 3' terminus of RNA that will not be translated to protein but includes many regulatory regions.

Not much is yet known about silencers but scientists continue to study in hopes to classify more types, locations in the genome, and diseases associated with silencers.

**Attenuation**

Premature termination of primary transcript in the leader region i.e. before the first structural genes is called attenuation. Attenuation is carried out by attenuator, a sequence within leader region of the tryptophan operon, (Fig 2). At this site, choice is made by RNA polymerase either to terminate or continue transcription. Mutants with small deletions in this region produce tryptophan synthesizing enzymes even in the presence of tryptophan.




**Termination of Transcription regulated by attenuation (a) Stem-loop structures of the trp operon in the mRNA; (b) Low level of trp full length mRNA made; (c) High level transcription of the trp operon is prematurely halted**

**Expression of genes**

• DNA serves two major functions:

1. Encoding structural information that can be converted into RNA and (usually), hence into protein sequence.

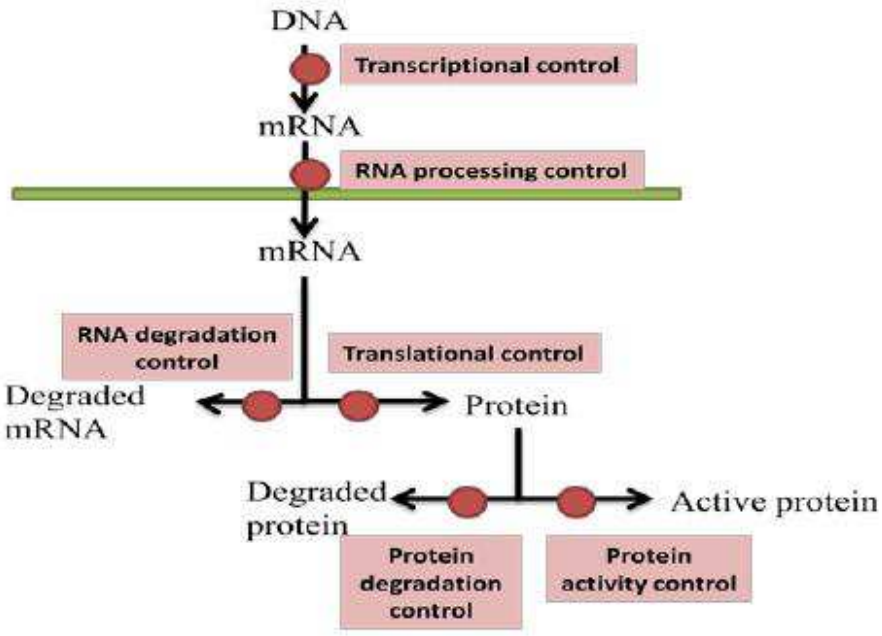
2. Encoding regulatory signals that allow certain proteins to decide where to begin or terminate reading DNA

• The "***central dogma***" of molecular biology: information flows from nucleic acids to proteins, not the reverse:

**DNA ----> RNA ----> polypeptides (proteins) Transcription** = the process of making RNA from DNA templates

• **Translation** = the process of making polypeptides

**Control of gene expression:**




**Points of control of gene expression The eukaryotic gene expressions in a cell are controlled at about six stages**

• Transcriptional control decides when and how frequently a given gene be transcribed. It enables the cell to check any discrepancy in the quantity of transcriptomes generated.

• RNA processing regulates how the RNA transcript is spliced or otherwise processed (applicable only to eukaryotes), which have split genes consisting of exons and introns.

• Translational control decides which mRNAs in the cytoplasm are to be translated by ribosomes.

• mRNA degradation control selectively destabilize some mRNA molecules in the cytoplasm.

• Protein activity control selectively activates, inactivates, degrades, or compartmentalizes specific protein molecules after they have been synthesized. (applicable only to eukaryotes)

**Regulatory elements:**

Gene expression is a complex multi-step process. Amongst various steps involved, transcription initiation is a vital key point in controlling gene expression.

**Fundamental elements that regulate the process of transcription are:**

◦ cis-acting elements comprising of special DNA sequences.

◦ trans-acting elements comprising of regulatory proteins.

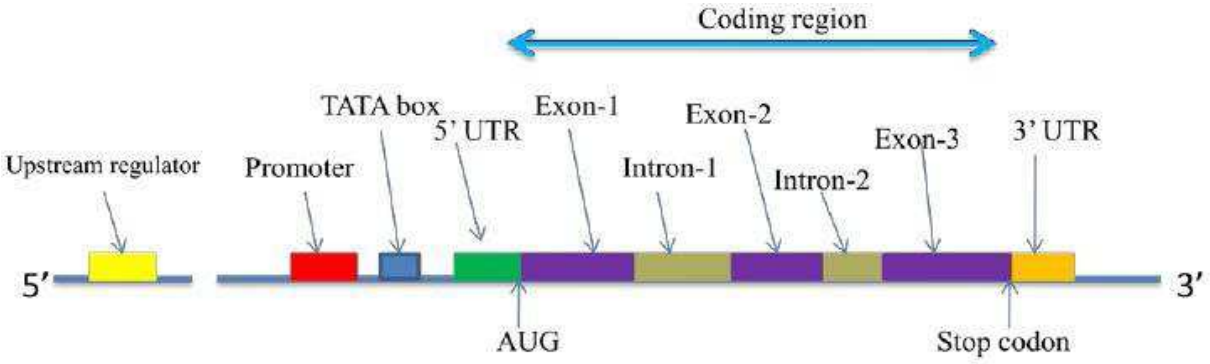
◦ DNA –protein interaction.

◦ Protein-protein interaction

◦ RNA polymerase

**cis-acting elements:**

In Latin “cis”, means "same side as". Cis acting elements are thus a region of DNA or RNA that regulates the expression of genes located on the same molecule. The cis-regulatory elements are often binding sites for one or more trans-acting factors. Cis-element may be located upstream to the coding sequence of the gene it controls (in the promoter region or further upstream 5'), in an intron, or 3' to the gene's coding sequence, either in the untranslated or untranscribed region.




**Structure of eukaryotic gene with upstream regulatory elements**

**Examples of cis-acting elements:**

**Prokaryotic systems:**

◦ Promoter is a DNA sequence where RNA polymerase binds to initiate transcription. There are two promoter sequences in prokaryotic systems known as -10 (Pribnow box or Pribnow-Schaller box) and -35 sequences.

◦ Operator is regulatory sequence of DNA located immediately upstream of the structural gene that controls transcription of an operon.

◦ Inducers are located upstream of promoter region.

◦ Downstream regulatory sequences comprise of GC-rich inverted repeats followed by four adenine (A) residues signal the termination of transcription.

**Trans-acting elements:**

The protein factors which regulate the expression of gene by binding to cis acting DNA sequence are termed as trans-acting elements. Trans-acting molecules generally have two domains: DNA binding domain (which binds to cis elements) and protein binding domain (required for activation or suppression of transcription). Transcription initiation is a tightly regulated process controlled by trans-acting elements both in prokaryotes as well as eukaryotes.

**DNA – Protein interactions:** Gene regulatory proteins and the transcription factors are capable of binding to the DNA based on the interaction of amino acids of the protein with the nucleotides of the DNA. The regulation is implemented through various interactions between cis-acting elements and trans-acting factors. Examples of some DNA binding proteins are:

◦ Eukaryotic TATA-binding protein

◦ σ subunit of bacterial RNA polymerase etc.

**There are four types of structures of DNA binding proteins,**

**1.** Zinc finger proteins

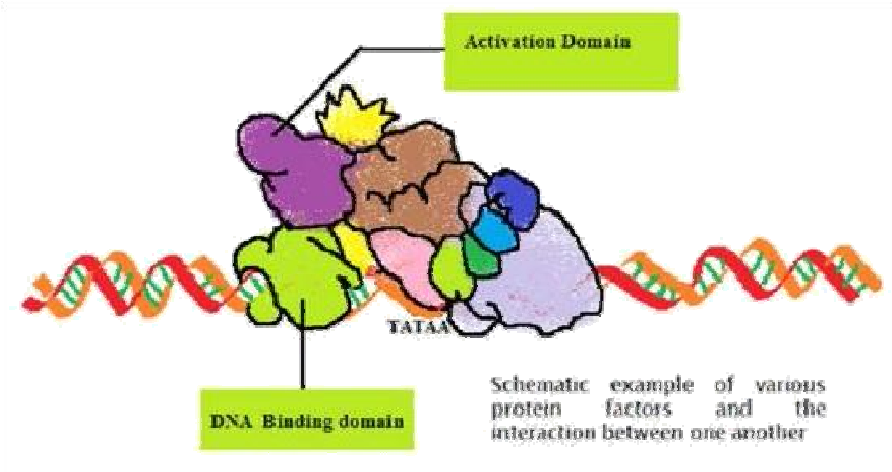
2. Helix loop Helix protein

3. Leucine zipper proteins

4. Homeodomain proteins

**Protein – Protein interactions:**

Protein-protein interaction is present in both prokaryotes and eukaryotes. The external signals affect the gene expression with the help of such interaction. Proteins interact with each other to form a homo or hetero-dimers before binding to DNA molecules. Eg. transcription initiation factors interacts with TATA box binding proteins (TBP) to activate transcription of a gene.




**Interaction between various protein factors**

**RNA Polymerase:**

• A single RNA polymerase is responsible for transcribing all types of RNA in prokaryotic system.

• However, eukaryotes have three different RNA polymerases, which have been found to specialize in the synthesis of various types ofRNA:

• RNA polymerase I (Pol I) -transcribes rRNA (ribosomal RNA) genes.

• RNA polymerase II (Pol II) - transcribes protein-coding genes or mRNA (messenger RNA).

• RNA polymerase III (Pol III) - transcribes other functional RNA genes (e.g., tRNA).

• In eukaryotes, transcription occurs inside the nucleus. All the enzymes responsible for translation are present in the cytosol therefore the transcripts formed then move out of the nucleus through nuclear pores into the cytosol (the liquid phase of the cytoplasm), where translation occurs. Organelle genomes (like the mitochondrial genome) are transcribed within the organelle (the mitochondria) and translation is also within the organelle (the mitochondria).

• Since prokaryotes have no nucleus, the step involving the movement of transcripts from nucleus to cytoplasm does not take place, and translation can take place immediately in the cytoplasm, directly on the growing transcript.

## MOLECULAR BIOLOGY 17BTU302

### UNIT V

**Regulation of gene expression and translation:** Regulation of gene expression in prokaryotes: Operon concept (inducible and repressible system), Genetic code and its characteristics, Prokaryotic and eukaryotic translation: ribosome structure and assembly, Charging of tRNA, aminoacyl tRNA synthetases, Mechanism of initiation, elongation and termination of polypeptides, Fidelity of translation, Inhibitors of translation, Posttranslational modifications of proteins.

| S.No | Lecture Duration (hr) | Topics | Support materials |
|---|---|---|---|
| 1 | 1 | Regulation of gene expression in prokaryotes | T1:1308-1325 |
| 2 | 1 | Operon concept – Lac Operon | T1:1262-1266 |
| 3 | 1 | Trp Operon | T1:1296-1298 |
| 4 | 1 | Genetic Code and its Charcteristics | T1 – 1338-1342 |
| 5 | 1 | Translation – Structure and assembly | T1:1349-1342 |
| 6 | 1 | Charging of t-RNA | T1: 1363-1365 |
| 7 | 1 | Mechanism of Initiation, Elongation, Termination of polypeptides | T1: 1373-1378; T1: 1379-1390; T1: 1391-1394 |
| 8 | 1 | Fidelity of Translation | T1:1395-1396 |
| 9 | 1 | Post translational modification of proteins | T1:1403-1407 |
| 10 | 1 | Inhibitors in translation | T1:1408-1421 |

Reference

T1: Donald Voet and Judith Voet; 2012, Biochemistry, 4th Edition, John Wiley and Sons. Inc.

# GENE REGULATION

- Each cell expresses, or turns on, only a fraction of its genes. The rest of the genes are repressed, or turned off. The process of turning genes on and off is known as gene regulation. Gene regulation also allows cells to react quickly to changes in their environments. Gene regulation can occur at any point during gene expression, but most commonly occurs at the level of transcription (when the information in a gene's DNA is transferred to mRNA). Signals from the environment or from other cells activate proteins called transcription factors. These proteins bind to regulatory regions of a gene and increase or decrease the level of transcription. By controlling the level of transcription, this process can determine the amount of protein product that is made by a gene at any given time.

**Introduction**

- *E. coli* lives in colon. It has a metabolic pathway that allows for the synthesis of the amino acid tryptophan (Trp). o This pathway starts with a precurser molecule and proceeds through five enzyme catalyzed steps before reaching the final Trp product.
- It is important that *E. coli* be able to control the rate of Trp synthesis because the amount of Trp available from the environment varies considerably.
- If we eat a meal with little or no Trp, the *E. coli* in our gut must compensate by making more.
- If we eat a meal rich in Trp, *E. coli* doesn't want to waste valuable resources or energy to produce the amino acid because it is readily available for use.
- Therefore, *E. coli* uses the amount of Trp present to regulate the pathway. o If levels are not adequate, the rate of Trp synthesis is increased.
- If levels are adequate, the rate of Trp synthesis is inhibited.
- The first method works to decrease the synthesis of Trp by inhibiting the first enzyme in the pathway, preventing the rest of the pathway from proceeding.
- What inhibits the first enzyme? Trp does!  The more Trp in the cell, the more that can bind to the first enzyme and prevent it from catalyzing the first step.
- This method of regulation is feedback inhibition in which the end product of a pathway acts as an inhibitor of an enzyme in that pathway.

- The other method of control stops the production of the enzymes in the pathway at the transcription level. o Remember that enzymes are proteins that must be transcribed and translated from the genetic code.
- If the genes for the enzymes are not transcribed to mRNA, then translation to the enzymes cannot occur.
- Without enzymes, there is no Trp synthesis.
- This method of control is called regulation of gene expression because control is taking place at the genetic level. This method of control will now be examined in detail.

**Trp Operon**

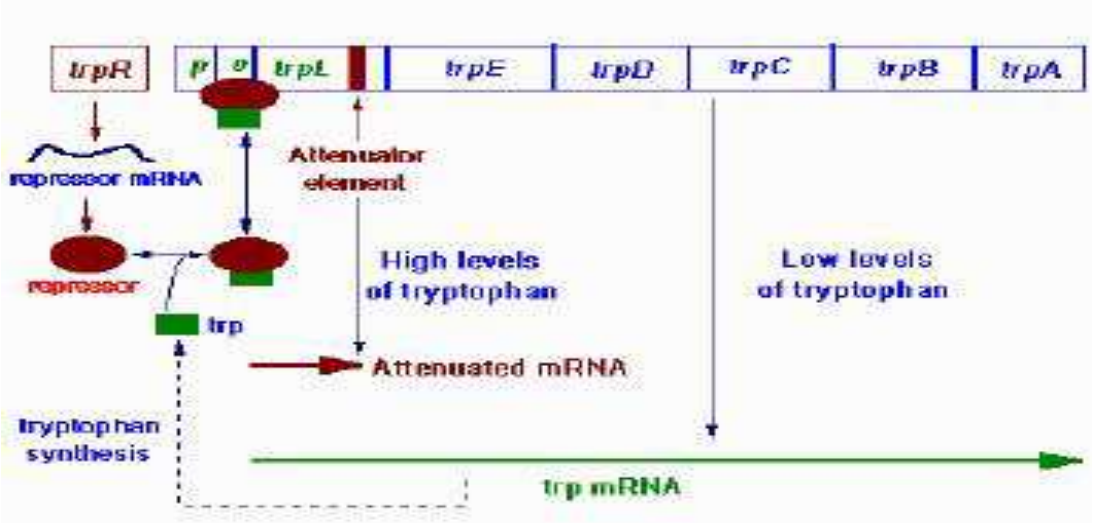
The genes for the five enzymes in the Trp synthesis pathway are clustered on the same chromosome in what is called the Trp operon.

• The Trp operon has three components: o Five Structural Genes:  These genes contain the genetic code for the five enzymes in the Trp synthesis pathway

o One Promoter: DNA segment where RNA polymerase binds and starts transcription

o One Operator: DNA segment found between the promoter and structural genes. It determines if transcription will take place. If the operator is turned "on", transcription will occur.

• When nothing is bonded to the operator, the operon is "on". o RNA polymerase binds to the promoter and transcription is initiated.




**Structure of trp operon**

o The five structural genes are transcribed to one mRNA strand.

o The mRNA will then be translated into the enzymes that control the Trp synthesis pathway.

• The operon is turned "off" by a specific protein called the repressor. o The repressor is a product of the regulator gene which is found some distance from the operon.  Transcription of the regulator produces mRNA which is translated into the repressor.

The repressor is inactive in this form and cannot bind properly to the operator with this conformation.

o To become active and bind properly to the operator, a co-repressor must associate with the repressor. The co-repressor for this system is Trp.  This means it is available from the environment

*E. coli* does not want to synthesize Trp if it is available

 The more Trp available, the more that can associate with repressor molecules.

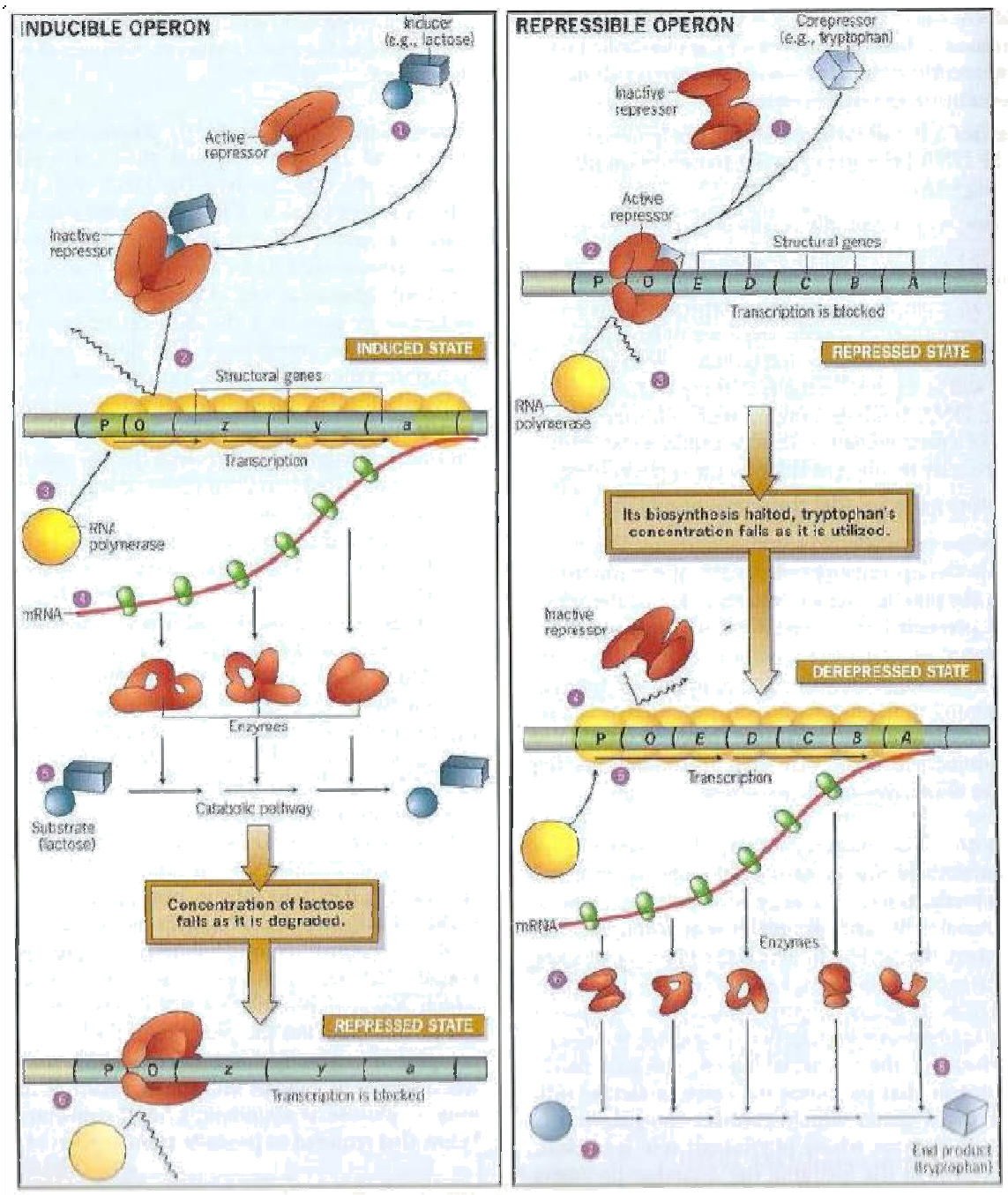
o An active repressor binds to the operator blocking the attachment of RNA polymerase to the promoter.

o Without RNA polymerase, transcription and translation of the structural genes can't occur and the enzymes needed for Trp synthesis are not made.

**Repressible vs Inducible Systems**

• The Trp pathway is anabolic as Trp is being synthesized. The Trp and other regulated anabolic pathways are usually repressible because the system can be repressed by an overabundance of the end product. o The end product, Trp, in this case, decreases or stops the transcription of the enzymes necessary for its production.

• Regulated catabolic pathways, on the other hand, are usually inducible because the pathway is stimulated rather than inhibited by a specific molecule. An example of an inducible system is lactose metabolism.




**Inducible and repressible operons**

**The lac Operon**

• The genes that code for the enzymes needed for lactose catabolism are clustered on the same chromosome in what is called the *lac* operon.

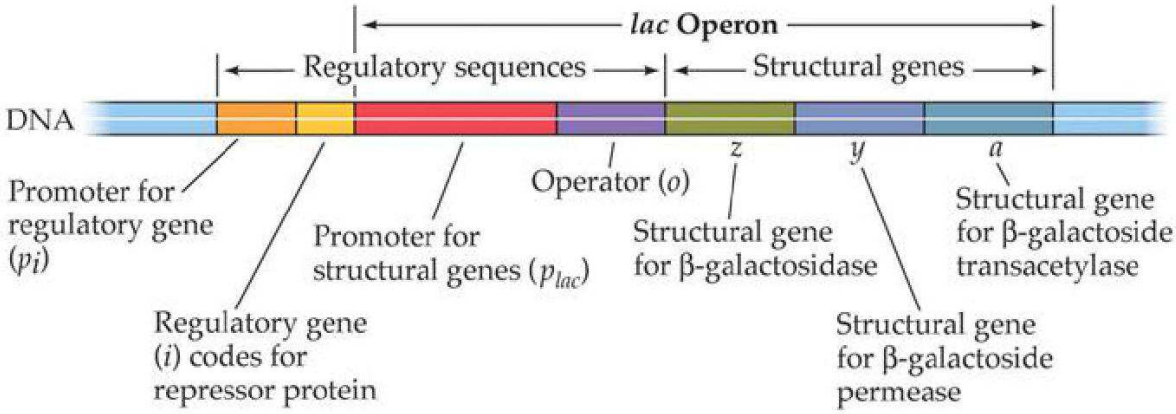
• The Lac operon has three components: o Three Structural Genes:  These contain the genetic code for the three enzymes in the lac catabolic pathway

o One Promoter:  DNA segment where RNA polymerase binds and starts transcription

o One Operator:  DNA segment found between the promoter and structural genes.

 It determines if transcription will take place.

 If the operator in turned "on", transcription will occur.




**The *lac* operon**

• As in the Trp operon, the Lac operon is turned "off" by a specific protein called the repressor. o The repressor is the product of the regulator gene which is found outside the operon.

 Transcription of the regulator produces mRNA which is translated into the repressor.

o But unlike the Trp operon, the repressor is active in this form and does not require a co-repressor.

o The active repressor binds to the operator blocking the advancement of RNA polymerase to the structural genes.

o Without RNA polymerase, transcription and translation of the genes can't occur and the enzymes needed for Lac metabolism are not made.

• What turns the Lac operon "on"? Lactose does! o This makes sense because the cell only needs to make enzymes to catabolize lactose if lactose is present.

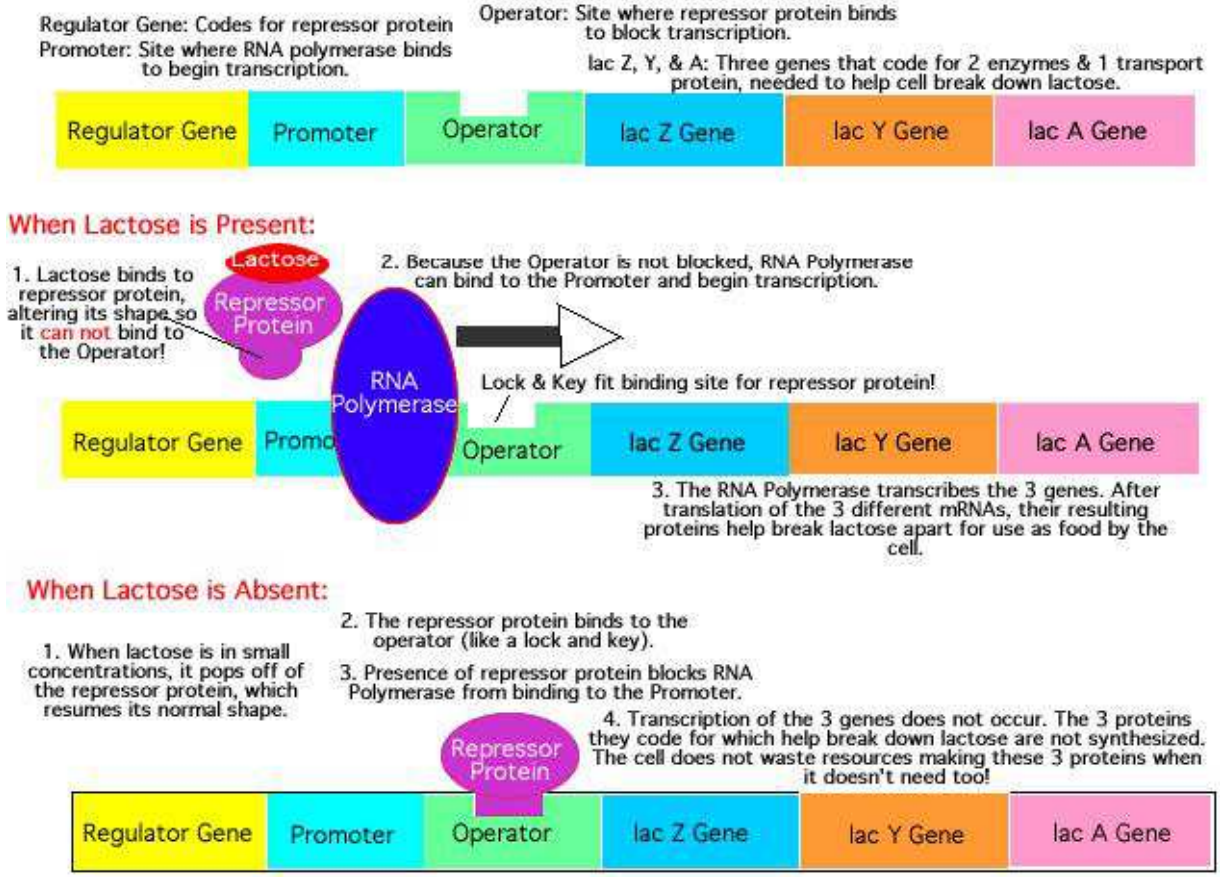
• When lactose enters the cell, allolactose, an isomer of lactose is formed.

• Allolactose binds to the repressor and alters its conformation so that it can't bind to the operator.

• RNA polymerase can now start transcription. o The three structural genes are transcribed to one mRNA strand.

o The mRNA will then be translated into the enzymes that control lactose catabolism.

• In this sense, allolactose is an inducer.




**The *lac* operon: a model of gene regulation in prokaryotic cells Negative vs Positive Control**

• While the Trp operon is an example of repressible gene regulation and the Lac operon is an example of inducible gene regulation, both are examples of negative control of genes because both operons are shut "off" by an active repressor.

• Gene regulation would be positive; on the other hand, if an activator molecule turned the operon "on".

• The Lac operon is also an example of a positive control system and is turned on by the cAMP-CAP complex, as described below:

• *E. coli* can be described as a fussy eater. o Its first choice at every meal is glucose because glucose supplies maximum energy for growth.

o Therefore, *E. coli* will only metabolize lactose if concentrations of glucose are low.

• For this to work, there must be a signal to tell the Lac operon that glucose is not available and to start transcribing the genes to metabolize lactose. o This signal is a small molecule called cyclic AMP (cAMP). □ The amount of cAMP present in a cell is inversely proportional to the amount of glucose present.

□ As a result, the absence of glucose results in an increase in cAMP in the cell.

• The following describes the situation where there is lactose but no glucose available to the cell: o No glucose means high levels of cAMP.

o cAMP binds to a molecule known as CAP.

o CAP, when in association with cAMP, can bind to the promoter at the CAP binding site.

o Here, the cAMP-CAP complex stimulates transcription by helping RNA polymerase bind to the promoter. □ RNA polymerase has a weak affinity for the Lac promoter and will not bind without this help.

o Remember with lactose present so is allolactose. □ Allolactose binds to the repressor and prevents it from binding to the operator.

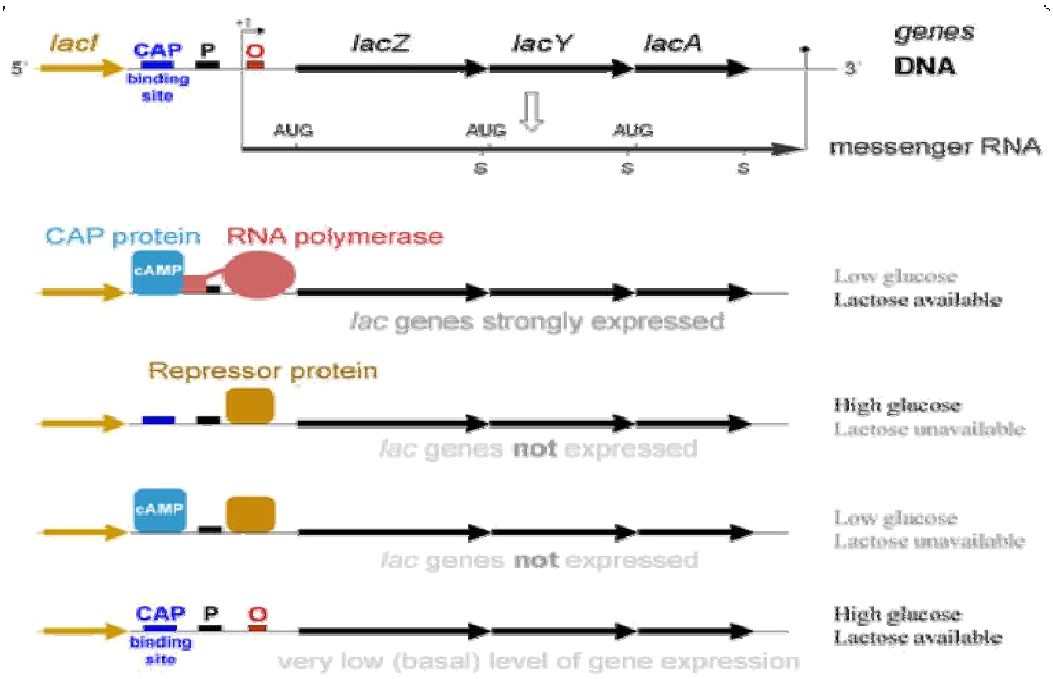
□ Therefore, transcription and translation of the genes can occur.

o The following depicts what happens when glucose and lactose are both present for *E. coli* to metabolize: □ With glucose present, there is very little or no cAMP.

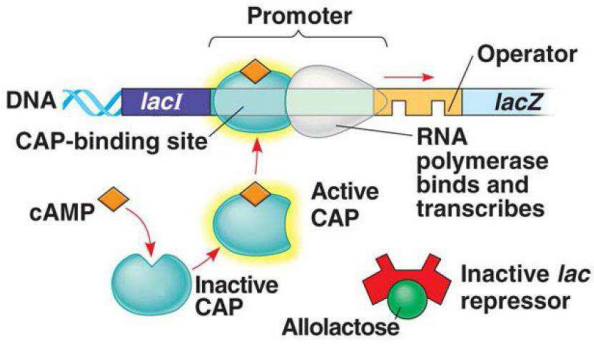
□ It cannot bind to the CAP binding site.

□ Without this complex, RNA polymerase cannot bind to the promoter and transcription cannot occur.

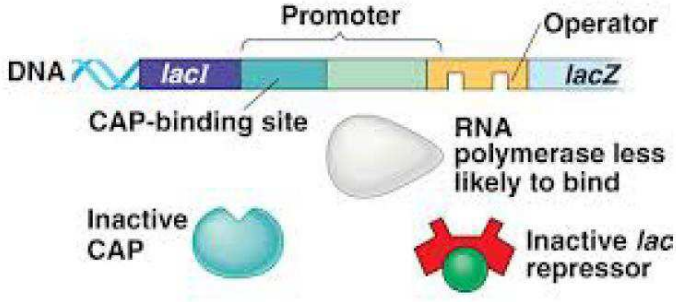
□ Even though allolactose is present and blocks the action of the repressor, there is no transcription of the lac genes because glucose is present.




**Overall structural elements of Lac Operon**




**Lactose present, glucose scarce (cAMP level high): abundant lac mRNA synthesized**




**Lactose present, glucose present (cAMO level low): little lac mRNA synthesized**

## The Genetic code

- Since there are 20 different kinds of amino acids in proteins and only four kinds of nucleotides in DNA, the relationship between the gene and its most elementary functional product, i.e., between DNA and protein, can hardly be interpreted through a code of one nucleotide = one amino acid.

- A coding sequence of two nucleotides for one amino acid, or a doublet code, would produce only 16 possible coding combinations, or codons. Codons are group of nucleotides that specifies one amino acid. By the **genetic code** (George Gamov, 1954), we mean, a collection of base sequences (codons) that correspond to each amino acid and to translation signals.

- A codon size of three nucleotides for one amino acid are triplet codon seems more likely, since it produces 64 possible codons, however, only 20 amino acids need to be coded, 44 codons in a triplet code seem to be superfluous. To account for the excess of codons beyond the necessary 20, we can suppose that more than one codon can code for a particular amino acid. For example, if each kind of amino acid were coded by three different possible codons, 60 possible codons would be accounted for.

- A code in which there is more than one codon for the same amino acid, is called degenerate. It is also possible that some or all of the codons in excess of 20 do not code for any amino acid and are therefore nonsense codons.

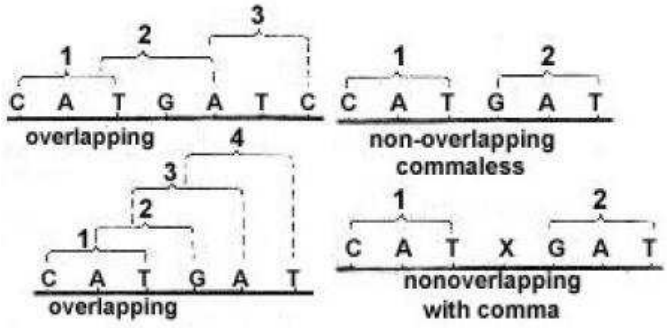
**Second letter**

| First letter | U | C | A | G | Third letter |
|---|---|---|---|---|---|
| U | UUU, UUC: Phe<br>UUA, UUG: Leu | UCU, UCC, UCA, UCG: Ser | UAU, UAC: Tyr<br>**UAA Stop**<br>**UAG Stop** | UGU, UGC: Cys<br>**UGA Stop**<br>UGG Trp | U C A G |
| C | CUU, CUC, CUA, CUG: Leu | CCU, CCC, CCA, CCG: Pro | CAU, CAC: His<br>CAA, CAG: Gln | CGU, CGC, CGA, CGG: Arg | U C A G |
| A | AUU, AUC, AUA: Ile<br>AUG: Met | ACU, ACC, ACA, ACG: Thr | AAU, AAC: Asn<br>AAA, AAG: Lys | AGU, AGC: Ser<br>AGA, AGG: Arg | U C A G |
| G | GUU, GUC, GUA, GUG: Val | GCU, GCC, GCA, GCG: Ala | GAU, GAC: Asp<br>GAA, GAG: Glu | GGU, GGC, GGA, GGG: Gly | U C A G |

The genetic code has following general properties, mostly applicable to the genes of all the organisms:

- **Genetic code is triplet**

  As discussed earlier, singlet and doublet codons cannot form 20 combinations, which is the minimum requirement; therefore triplet codon is a necessity, so that all the amino acids must be coded. Genetic code is non-overlapping. During translation, the codons are read one after another, in a sequence. One base of a codon is not used by the other codons. Therefore, if there are six bases, they will code for two amino acids only. e.g.; in case of non-overlapping, a gene sequence of UUUCCC only two amino acids will be coded, phenylalanine (UUU) and proline (CCC), whereas for an overlapping code, more than two amino acids could be coded, phenylalanine (UUU), serine (UCC) and proline (CCC).




**Overlapping and non-overlapping genetic code**

- **Genetic code is commaless**

  The bases are read one after the other in the codons, i.e., no bases or codons are reserved for punctuation or comma. When the first amino acid is coded, the second will be coded by the next three bases immediately, and no base will be wasted to serve as a comma. Once the translation begins, the codons are read one after the other with no break or demarcating signals in between them.

- **Genetic code is non-ambiguous**

  Each codon has a particular amino acid for coding, and it will code for that amino acid only. There is one to one relationship between codon to amino acid. However, there is an exception, AUG codes for methionine and GUG codes for valine, but if AUG is absent, then GUG codes for methionine, as starting codon for protein synthesis. In an ambiguous code, one codon can code for more than one amino acid. In certain rare cases, the genetic code is found to be ambiguous i.e, some codons codes for different amino acids under different

conditions, e.g. in streptomycin sensitive strain of E.coli the codon UUU normally codes for phenylalanine, but it may also code for isoleucine, serine when treated with streptomycin. This ambiguity is enhanced at high Mg ion concentration, low temperature and the presence of ethyl alcohol.

- **Genetic code is degenerate.** Since there are more codons than the amino acids, more than one codon may specify the same amino acid. Such different codons that specify the same amino acid are called as synonymous codons. e.g., UUU = UUC = phenylalanine.

- **Genetic code has start/stop signals.** Some codons are specially meant for initiation and termination of protein synthesis. e.g.: AUG codes for methionine, serves as initiation codon in eukaryotes and GUG in case of prokaryotes. Three codons UAG (**amber**), UAA (**ochre**), UGA (**opal**) are called as termination codons, because they terminate protein synthesis. Earlier, they were called as nonsense codons, because they do not code for any amino acid, but, since they are involved in termination of protein synthesis, they are called as termination codons. The initiation and termination codons are known as **signals** and this phenomenon is known as **punctuation**.

- **Genetic code is polar.** It means that the genetic code has a fixed start and termination ends, and is always read in a fixed direction, i.e. in 5'→ 3' direction and the polypeptide chain is synthesized in N→C direction i.e., from amino group (NH2) to carboxylic group (COOH).

- **Genetic code is universal.** The same genetic code is applicable to all organisms, from bacteria to man, i.e.; the codons have the same meaning in all the organisms. e.g., UUU = phenylalanine in bacteria, mouse, man and tobacco. The universality of the genetic code, however, does not mean that DNA base ratios must be similar in different species for genes specifying similar proteins. The fact that the code is degenerate enables many bases to be changed by mutation in a sequence of mRNA, but this mRNA could still produce the same amino acid sequence. In 1979 investigators began started DNA sequencing of mitochondrial DNA in humans, cattle and mice. During their experiments, they were surprised to learn that the genetic code used by the mitochondrial DNA was not the same as the universal genetic code. e.g. UGA, which is a non-sense codon, but it codes for tryptophan in mtDNA, AGG which codes for arginine is a non-sense codon in mtDNA. So, extra chromosomal DNA such as mtDNA and ctDNA do not come under the universality of the genetic code.

- **Wobble Hypothesis.** Out of the 64 codons, three are involved in termination process. So, there are only 61 codons specifying the amino acids, and the cell should have 61 different types of tRNAs, each having a different anticodon for the recognition of codons. However, the actual number of tRNA is found to be much less than 61. This means that the anticodons of tRNA read more than one codon on the mRNA. **Crick** (1966) proposed a hypothesis to explain the degeneracy of the genetic code; the hypothesis is known as Wobble hypothesis.

- According to this hypothesis, the major degeneracy occurs at the third position, i.e., the third codon is not important in base pairing, and the actual pairing occurs only in the first two codon-anticodon pairs. The base at the 5′ end of the anticodon and the base at the 3′ end of the codon form hydrogen bonds without any specificity. The third base is called as the **wobble base**. This wobble base of codon lacks specificity and the base in the first position of the anticodon is usually abnormal e.g., inosine, tyrosine, etc. These abnormal bases are able to pair up with more than one nitrogen base at the same position e.g., inosine (I) can pair up with A, C and U. The pairing between unusual base of tRNA and wobble base of mRNA is called **wobble pairing**.

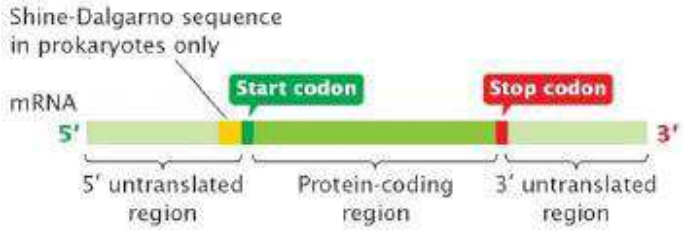
## Translation: the Synthesis of Proteins

**Role of mRNA**

• carries **codons** (3-nucleotide sequences) arranged in linear fashion that code for amino acids

• also carries signals needed to tell how to recognize ribosomes, start and stop signals for decoding protein

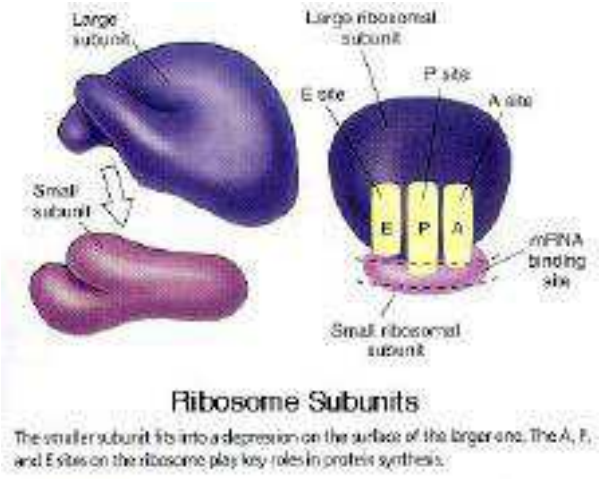
• **leader sequence** on small ribosome subunit binds to complementary sequence on mRNA, allows initial formation of RNA-ribosome complex.




**Role of mRNA**

**Role of ribosome**

- two parts: a **small subunit** and a **large subunit**. These are separated except when attached to m-RNA
- ribosomes contain a set of ribosomal proteins and several types of ribosomal RNA (r-RNA)
- ribosomes catalyze the formation of peptide bonds; intially thought to be due to protein activity (enzyme), but now known to be due to RNA catalytic activity (**ribozyme**).

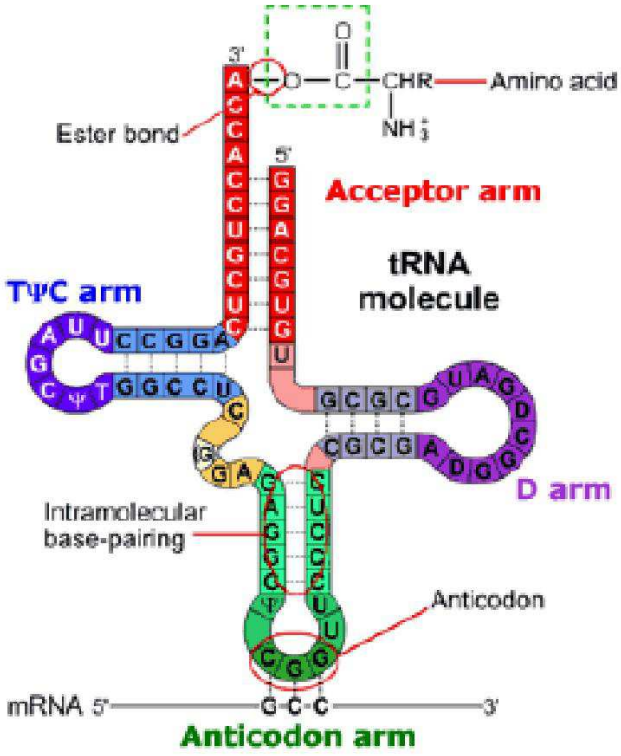



**Ribosome**

**Role of tRNA**

- structure: 4 loops, anticodon, AA binding site
- ~ 60 types in bacteria (>100 in mammals)
- only 73-93 nucleotides long
- some bases modified after transcription; like pseudouridine.
- extensive hairpin loops
- **anticodon** site: recognizes codon on mRNA
    - Activation of tRNA: adding amino acids o requires special enzyme: AA-tRNA activating enzymes

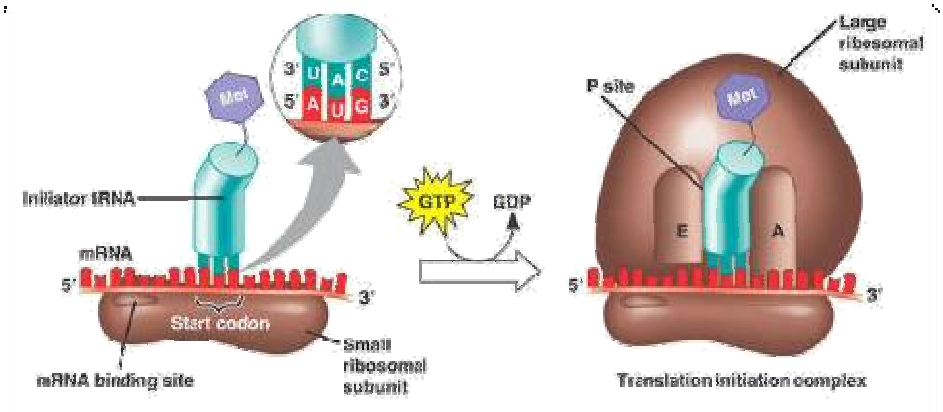
    o ATP required, forms AA-AMP + PP, then AA-tRNA + AMP




**tRNA strcuture**

**Initiation of Translation**

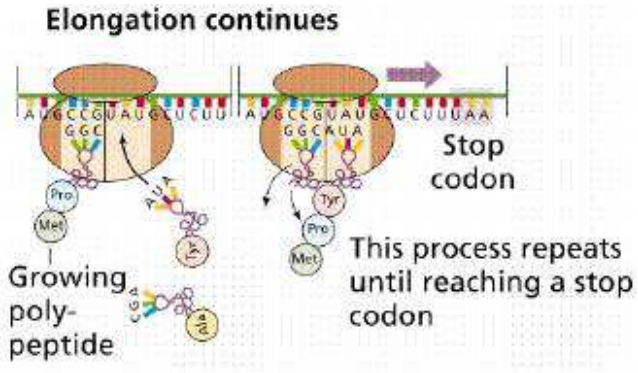
- small ribosomal subunit initiates binding to mRNA
- locates 5' end of mRNA
- small subunit ribosome finds first **AUG codon** = start codon
- large ribosome binds
- tRNA carries the amino acid **methionine** to first position




**Initiation of translation**

**Elongation of Translation**

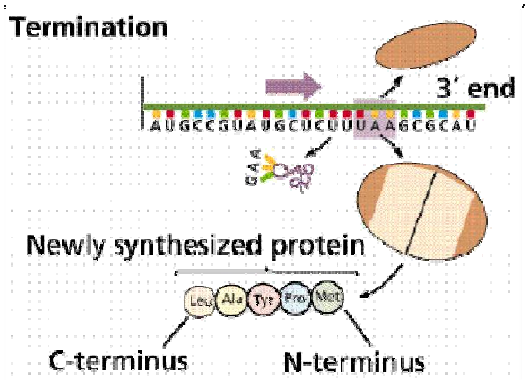
- 2 adjacent sites on ribosome: P (Peptide) and A (Amino Acid) site
- A site accepts a new tRNA-AA
- P site holds existing chain
- peptide transferred from P site tRNA to A-site AA
- enzyme activity is in ribosomal RNA (ribozyme)
- also required: Energy (GTP) and elongation factors




**Elongation of translation**

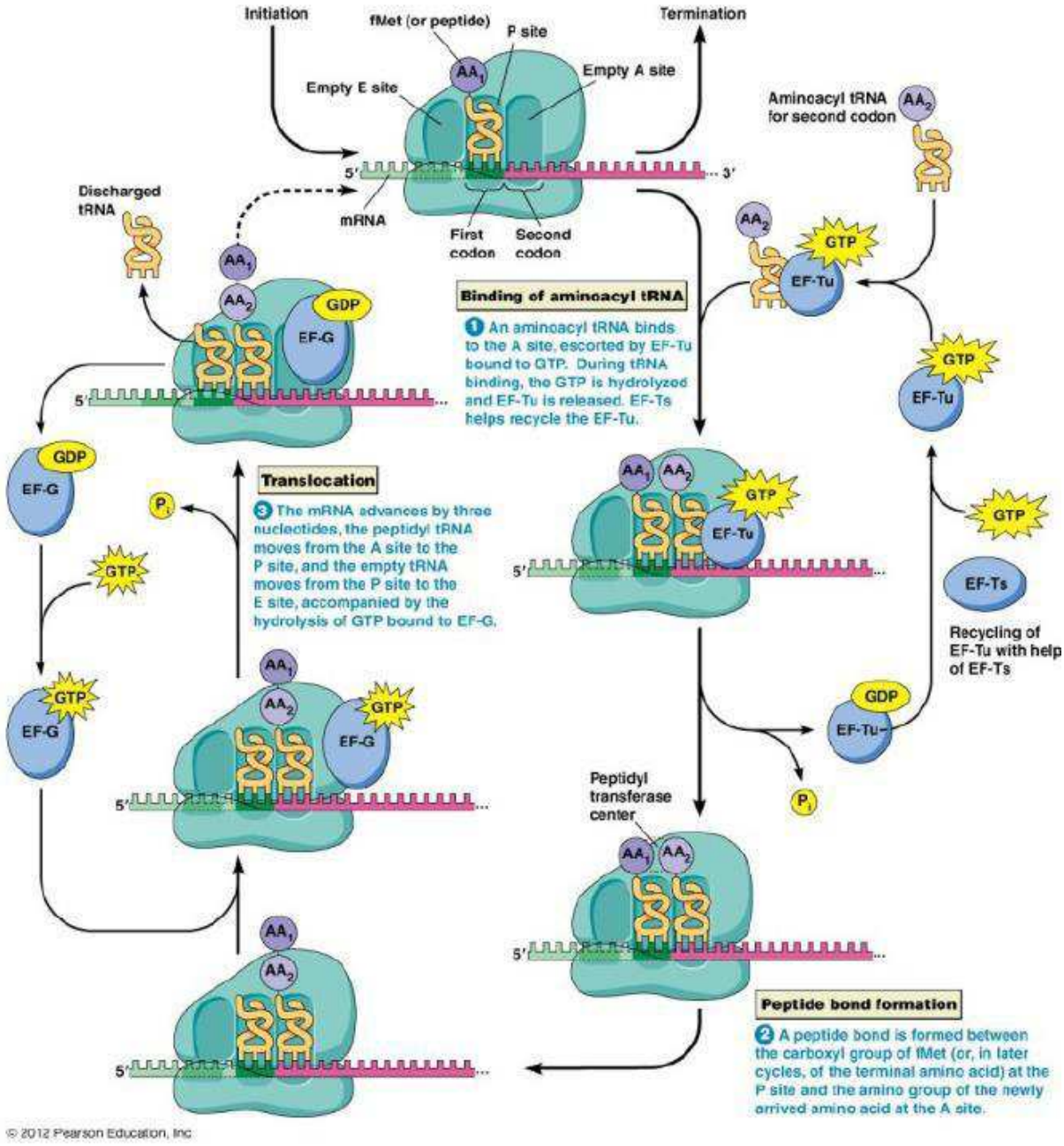
**Termination of Translation**

- reach a "stop codon" UAG, UAA, or UGA
- no t-RNAs bind
- instead, specific **release factors** required
- Net cost of translation: 4 phosphate bonds/amino acid added.




**Termination of translation**

**NOTE**: Typical RNA polymerization rate (~ 40 nts/second at 37 °C in bacteria - close to translation rate of about ~15 aa/second).




**The complete process of translation**

**Two marks**

1. Write about the structural difference between B-form (Watson-Crick) DNA and Z-form DNA.
2. Write about Histone proteins.
3. What is Euchromatin and Heterochromatin?
4. Write short note on chromosomes.
5. Write short note on Chargaffs rule..
6. Write short note on Okazaki fragments.
7. What are primosome?
8. Write about the importance of SSB's in DNA replication.
9. Define telomeres.
10. Write in detail about the topoisomerase enzyme.
11. What are Uvr proteins?
12. Write short note on Nonsense mutation.
13. Write short note on photoreactivation.
14. Write the function of photolyase.
15. Discuss on Depurination.
16. Give two examples for housekeeping gene.
17. What are RNA polymerases?
18. Discuss on the role of promtors in transcription.
19. What is 5' capping?
20. What are enhancers?
21. Write the three stop codons with their names.
22. Define the term translation.
23. What are Rho proteins?
24. Write about Transposable elements.
25. Define the term repressors.

**Six Marks**

1. Describe the experiment of Hershey and Chase on bacteriophages.
2. Write in detail about Watson - Crick model of DNA.
3. Explain in detail about the different types of DNA.
4. Write briefly about the Avery experiment on DNA as genetic material.
5. Explain in detail about the packing of DNA in histone proteins.
6. Write an experiment to prove DNA as genetic material.
7. Explain in detail about the Griffith's experiment on Transformation.
8. Explain about C- value paradox.
9. Describe about the super coiling of DNA.
10. Write about the phosphodiester bond formation in DNA structure.
11. Explain in detail on semiconservative nature of DNA replication
12. Elaborate on various steps involved in DNA replication in eukaryotes.
13. Explain about the bidirectional property of DNA replication.
14. Write in detail about the role of different polymerases enzyme in replication.
15. Explain the fidelity of replication
16. Describe the experiment of Meselson and stahl on replication.

17. Write briefly on Rolling circle replication.
18. Describe the various steps involved in eukaryotic replication.
19. Explain in detail about the semi-discontinuous property of DNA.
20. Describe the formation of replication fork.
21. Explain about homologous recombination.
22. Write a short note on proteins involved in base excision repair.
23. Briefly explain about the different types of DNA damages.
24. Define Mutation. Explain in detail about different types of Mutations.
25. Write about the Nucleotide excision repair.
26. Describe about non homologues recombination.
27. Explain the detail process of DNA repair mechanism.
28. Write about the formation of Holliday model.
29. Write about the Mismatch repair occurring during DNA damage.
30. Explain in detail on the formation of thymine – thymine and its repair mechanism.
31. Write in detail on t RNA splicing.
32. Elaborate on the mechanism of transcription in eukaryotes.
33. Describe about the mechanism of transcription in prokaryotes
34. Explain the different types of RNA, its structure and role.
35. Explain the various elongation factors involved in transcription?
36. Write about the role of tRNA and mRNA in transcription.
37. Explain about the transcription process in prokaryotes.
38. Write in detail on RNA splicing.
39. Write about the role of transcription factors.
40. Give a detailed study on RNA processing in Transcription.
41. Write about post-translational modification of proteins
42. Describe in detail about the tryptophan operon
43. Explain the detail process of regulation of gene expression in prokaryotes.
44. Explain in detail about the process for protein synthesis.
45. Write about the ribosome structure and assembly.
46. Elaborate on various steps involved in DNA replication in prokaryotes.
47. Explain: (i) the features of genetic code, (ii). Poly-adenylation.
48. Explain in detail about *Lac* operon
49. Explain about Operon concept with an example.
50. Elaborate on fidelity of translation.

| Questions | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
|---|---|---|---|---|---|
| **Unit I** | | | | | |
| The backbone of nucleic acid structure is | Hydrogen bond | Phosphodiester bond | Ionic bond | Hydrophobic bond | Hydrogen bond |
| The functional unit of DNA is | Genome | Gene | Nucleotide | Chromosome | Nucleotide |
| Base pairing of DNA is based on _____ | Arther kornberg | Charhoff | Meselson | Mc clintock | Charhoff |
| In Hershey and Chase experiment P32 is incorporated in formation of---------- | Protein | RNA | DNA | Gene | DNA |
| RNA contain uracil in place of | Adenine | Guanine | Cytosine | Thymine | Adenine |
| The proteins associated with eukaryotic | Trypsin | Histones | Collagen | Elastin | Histones |
| DNA double helix does not have | Antiparallel configuration | Complementary base pairing | Major and minor grooves | Uracil | Uracil |
| The total genetic information contained in a DNA is referred to as | Gene | Genome | Ribosome | Chromosome | Genome |
| of_____ | Rewinding of | Polymerize of | Ligase of DNA | Unwinding of | Unwinding of DNA |
| Histones are rich in | Arg & Lys | Lys & Gly | Arg & Glu | Arg & Gly | Arg & Lys |
| Fundamental unit of DNA organization is | Replisome | Nucleosome | Primosome | Chromosome | Nucleosome |
| B-DNA form was discovered by | Watson and Crick | Hoogsteen | Chargaft | Wang and Rick | Watson and Crick |
| A nucleotide in DNA is composed of _____. | Deoxyribose sugar, phosphate & nitrogen base | Deoxyribose sugar & nitrogen base | Deoxyribose sugar & phosphate | Deoxyribose sugar | Deoxyribose sugar, phosphate & nitrogen base |
| In a nucleotide, the nitrogen base is joined to the sugar molecule by | Phosphodiester bond | Glycosidic bond | Hydrogen bond | Hydrophobic bond | Glycosidic bond |
| Change in nucleotide sequence of a short region of genome is called | replication | recombination | repair | mutation | mutation |
| form | nucleotides | nucleosides | nucleosomes | nucleofomes | nucleosomes |
| In which direction does DNA replication take place? | 3'-5' | 5'-3' | 5' | 3' | 5'-3' |
| The ends of the linear chromosomes are maintained by | helicase | primase | gyrase | telomerase | telomerase |

| | | | | | |
|---|---|---|---|---|---|
| The experiments by Hershey and Chase helped confirm that DNA was the hereditary material on the basis of | radioactive phage were found in the pellet | radioactive cells were found in the supernatant | radioactive sulfur was found inside the cell | radioactive phosphorus was found in the cell | radioactive phosphorus was found in the cell |
| Which histone protein is not part of a core particle of nucleosome? | H1 | H2a | H2b | H4 | H1 |
| Heterochromatin is DNA in which form? | Naked DNA form | extended nucleosome form | solenoid form | looped solenoid form | Naked DNA form |
| Double strrand nature of DNA proposed by | Watson & crick | Arther kornberg | Stahl & | Mc clintock | Watson & crick |
| An enzyme that catalyses phosphodiester linkages in DNA strands is called | DNA polymerase | DNA ligase | RNA polymerase | Topoisomerase | DNA ligase |
| In DNA, the genetic information resides in_____ bases | purine bases | pymidine bases | purine & pyrimidine bases | Sugar | purine & pyrimidine bases |
| Z-form of DNA is | Left handed | Right handed | Both right and left handed | Non directional | Left handed |
| DNA synthesis takes place during | G1 Phase | S-Phase | G 2 Phase | Interphase | S-Phase |
| with | RNA | Ribosome | Protein | DNA | Ribosome |
| Hershey and Chase experiment on---------- | T2-Phages | Salmonella | Amoebae | Bacterium | T2-Phages |
| RNA has the following bases | ATG | AUG | APG | ATT | AUG |
| Chromatin composed of | Nucleic acids and proteins | Nucleic acids only | Proteins only | RNA only | Nucleic acids and proteins |
| Nucleosome assembly is | initiated by nucleoplasmin | spontaneous | inhibited by topoisomerase | induced | spontaneous |
| Polytene chromosomes are produced by | gene inversion | Gene conversion | DNA amplification | gene deletion | DNA amplification |
| In DNA double helix, the two DNA chains are held together by _____ bonds between the base pairs | Covalent | Hydrogen | Ionic | Hydrophobic | Hydrogen |
| Smaller blocks occur at the end of chromosome arm is called _____ | centromere | Telomere | blastomeres | blastocyst | Telomere |

| | | | | | |
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| Proteins that can unwind a DNA helix are | Helix destabilizing proteins | Melting proteins | Helix destabilizing proteins and | Single strand binding proteins | Helix destabilizing proteins and Melting proteins |
| The length of the DNA segment present in the nucleosome core particle is | 140 bp | 200bp | 166 bp | 114 bp | 140 bp |
| The molecular chaperone which causes the nucleosome assembly is | nucleoplasmin | histone | Hu protein | Ubiquitin | histone |
| The base pair between adenine and thymine forms __ hydrogen bonds | **2** | 1 | 3 | 4 | 2 |
| Bacterial transformation is ---------- | Griffith effect | Averys effect | Hershey effect | Joule effect | Griffith effect |
| The process DNA duplication | Replication | Transcription | Translation | Reverse | Replication |
| The process of making copy of RNA from DNA is _____ | Replication | Transcription | Translation | Reverse transcription | Transcription |
| The process of synthesizing protein from | Replication | Transcription | Translation | Reverse | Translation |
| The process of making copy of DNA from RNA is | Replication | Transcription | Translation | Reverse transcription | Reverse transcription |
| Satellite DNA is rich in _____base | AT | GC | GCAT | GT | AT |
| Important chaecteristic of satellite DNA is, they contain | repetitive base sequence | GC rich | Unique sequence | solitary sequence | repetitive base sequence |
| Chicken lysozyme gene is a good example of | Single copy DNA | Moderatively Repetitive DNA | Simple sequence DNA d. | highly Repetitive DNA | Single copy DNA |
| In linker DNA, which histone is involved | H1 | H2 | H3 | H4 | H1 |
| The sister chromatids will be attached by | a centromere | a telomere | a centriole | golgi bodies | a centromere |
| ---------- is the division of the cytoplasm, separating the organelles and other cellular components. | Mitosis | Meiosis | Cytokinesis | Proface | Cytokinesis |
| Chromatin condenses into chromosomes, the nucleolus dissolves, nuclear membrane is disassembled, and the spindle apparatus | Prophase | Metaphase | Anaphase | Telophase | Prophase |
| Simple non-nucleated cells are --------- | Prokaryotic cells | Eukaryotic cells | Stem cells | Blood cells | Prokaryotic cells |
| Chromosomes are seen in the equatorial | Prophase | Metaphase | Anaphase | Telophase | Metaphase |

| | | | | | |
|---|---|---|---|---|---|
| An interphase between nucleus and | Nuclear envelop | Nuclear | Nuclear pores | Perinuclear space | Perinuclear space |
| The cell cycle consists of ---------- | two distinct phases | three distinct | four distinct | five distinct | four distinct phases |
| Sucide bags are otherwise called | Ribosomes | Lysosomes | Golgi apparatus | Endoplasmic reticulum | Lysosomes |
| The Golgi apparatus important for | Protein synthesis | DNA synthesis | RNA synthesis | Packaging and secretion of | Packaging and secretion of proteins |
| Rough endoplasmic reticulum is embedded | RNA | Ribosome | Protein | DNA | Ribosome |
| In rolling circle replication single stranded viral DNA otherwise called as | Positive strand | Negative strand | Replicative strand | Rolling strand | Positive strand |
| Which of the following catalize the formation of negative coils during DNA | helicases | gyrases | primosome | polymerase | gyrases |
| In Hershey and Chase experiment $S^{35}$ is incorporated in formation of---------- | DNA | Proteins | Cytoplasm | RNA | Proteins |
| **Unit II** | | | | | |
| The DNA replication is semi conservative was demonstrated by | Meselson &Stahl | Okazaki | Lehniger | Lorunberg | Meselson &Stahl |
| The process DNA duplication is | Replication | Transcription | Translation | Transversion | Replication |
| The seperation of two DNA strands for replication is brought by | DNA polymerase III | DNA helicase | DNA polymerase I | DNA ligase | DNA helicase |
| deoxyoligonucleotides will hybridize with a DNA containing the sequence (5')AGACTGGTC(3')? | (5')TCTGACCAG(3') | (5')CTCATTGAG(3') | (5')GAGTCAACT(3') | (5')GACCAGTCT(3') | (5')GACCAGTCT(3') |
| Negative supercoils are removed by | Topoisomerase I | gyrase | helicase | rep protein | Topoisomerase I |
| The term Okazaki fragments refers to the | discontinuously synthesized DNA pieces on the lagging strand | discontinuously synthesized DNA pieces on the leading | DNA fragments formed by the action of DNA ligase | DNA fragment from pol I | discontinuously synthesized DNA pieces on the lagging strand |
| The synthesis of new DNA (replication) in prokaryotes is catalysed by | DNATopoisomerase | DNA polymerase I | DNA polymerase II | DNA polymerase III | DNA polymerase III |
| Okazaki fragements are initiated with | DNA primer | RNA primer | DNA template | RNA template | RNA primer |

| | | | | | |
|---|---|---|---|---|---|
| Semiconservative mechnism of replication was proposed by | Crick | Watson | Watson & Crick | Benjamin &Franklin | Watson & Crick |
| Eukaryotic cells have _____ kinds of RNA polymerase | 1 | 2 | 3 | 4 | 3 |
| The lagging strand is characterized by except | a constant supply of RNA primers | discontinuous synthesis of new strand | replication of DNA in 5'-3' direction | continuous synthesis of new strand | continuous synthesis of new strand |
| Replication is characterized by the following except it is | semiconservative | bi-directional | semidiscontinuou | dependent on DNA primer | dependent on DNA primer |
| The process linking two circular DNA molecules to form a chain is called | decatenation | catenation | annealing | nick translation | catenation |
| The rep protein moves along the | leading strand template in 3'-5' direction | lagging strand template in 3'-5' direction | leading strand template in 5'-3' direction | lagging strand template in 5'-3' direction | lagging strand template in 3'-5' direction |
| Ori C is rich in _____sequence | GC | AT | ATGC | GATC | AT |
| The coding strand of DNA is | the plus strand | the minus strand | the antisense | template strand | the plus strand |
| A replicon is | region with DNA origin | regulatory gene | Inverted repeats | tandom repeats | region with DNA origin |
| The DNA replication is discontinuous was proved by | Messelson-Stahl | Reigi Okazaki | Albert Lehninger | Arthur Kornberg | Reigi Okazaki |
| Watson and Crick elucidated ds DNA structure by using | NMR spectroscopy | X-ray diffraction | Circular dichroism | IR and Raman spectroscopy | X-ray diffraction |
| . The enzyme primarily involved in DNA repair in bacteria is | DNA pol I | DNA pol II | DNA pol III | DNA pol a | DNA pol I |
| In bacteria, which enzyme unwinds, binds ss DNA, denatures ds DNA and matches the ss DNA with complementary denatured | Rec A | Uvr ABC | Uvr D | Dna B | Dna B |
| The melting temperature of DNA is | Directly propotional to A-T | Directly propotional to G- | Not related to base composition | Directly propotional to | Directly propotional to G-C content |
| Genetic information is perpetuated by | repair | transcription | translation | replication | replication |
| The catenated circules in E. Coli are | Topoisomerase I | Topoisomerase | Topoisomerase | Topoisomerase | Topoisomerase IV |

| | | | | | |
|---|---|---|---|---|---|
| E. Coli DNA ligase utilizes | NAD+ | ATP | GTP | NADH | NAD+ |
| . ---------- is an E.Coli protein that melts duplex DNA | DNa A | DNa B | DNa C | DNa D | DNa A |
| The Klenow fragment exhibits the activity of | 5'-3' exonuclease | polymerase and 3'-5' exo | polymerase and 5'-3' exo | an endo nuclease | polymerase and 3' -5' exo nuclease |
| For the polymerase I activity | primer is not required | primer with either free 3' OH or 5' – P is | Primer with free 3'-OH is required | Primer with free 5 '- OH is required | Primer with free 3'-OH is required |
| DNA polymerase I has all the activities except | 3'- 5' exonnclease activity | 5' – 3' exonnclease | endonuclease activity | Helicase | Helicase |
| Among the following which is a substrate for DNA polymerase I? | 5' monophosphate | 5' diphosphate | 5'triphosphate | deoxy ribose | 5'triphosphate |
| In DNA replication helix unwinding is accomplished by enzymes called | helicases | gyrases | topoisomerases | polymerases | helicases |
| The term processivity with reference to DNA polymerase refers to | the rate at which polymerization occurs | the number of nucleotides added before the enzyme dissociates from | the rate at which the enzyme dissociates from the template | fidelity of DNA replication | the number of nucleotides added before the enzyme dissociates from the template |
| The initiation of a new round of replication in E.coli is regulated by the gene | dna C | dna B | dna A | pol A | dna C |
| RNA primers are removed by | DNA polymerase I | DNA polymerase II | DNA polymerase III | topoisomearse | DNA polymerase I |
| E.coli DNA replicase is | Pol III | Pol II | pol I | ligase | Pol III |
| The two replicating forks travel in opposite directions until they reach either end of that unit, the two end points are called | termini | replication fork | intron | exon | termini |
| dna B code for the enzyme | helicase | primse | polymerase | ligase | helicase |
| The presence of following four deoxy ribonucleoside triphosphate is essential for replication | dATP, dUTP, dCTP, dTTP | dATP, dGTP, dUTP, dTTP | dATP, dGTP, dCTP, dTTP | dATP, dGTP, dCTP, dUTP | dATP, dGTP, dCTP, dTTP |

| | | | | | |
|---|---|---|---|---|---|
| The synthesis of new DNA (replication) in prokaryotes is catalysed by | DNA polymerase I | DNA polymerase II | DNA polymerase III | DNA topo isomerase | DNA polymerase III |
| Single strand binding protein binds to single strand DNA | to prevent replication | . to repair base pairs | to initiate transcription | to prevent reformation of duplex state. | to prevent reformation of duplex state. |
| Which of the following possesses both 5′-3′ and 3′-5′ exonuclease activity? | Kornberg enzyme | DNA polymerase III | Taq DNA polymerase | DNA polymerase I | Kornberg enzyme |
| In E.coli, which enzyme synthesizes the RNA primer for Okazaki fragments? | DnaA | DnaB | DnaC | DnaG | DnaG |
| DNA gyrase is inhibited by | tetracycline | Nalidixic acid | Streptomycin | cephalosporin | Nalidixic acid |
| DNA replication rates in prokaryotes are approximately of the order of | 10 bases per second | 100 bases per second | 1000 bases per second | 10,000 bases per second | 1000 bases per second |
| new copy of a DNA molecule is precisely synthesized? | Trasformation | Transcription | Translation | Replication | Replication |
| Which of the following repairs nicked DNA by forming a phosphodiester bond between adjacent nucleotides? | Helicase | DNA gyrase | Topoisomerases | DNA ligase | DNA ligase |
| The scientist involved in discovery of DNA as chemical basis of heredity were. | Hershey and Chase | Griffith and Avery | Avery, MacLeod and McCarty | Waston and Crick | Avery, MacLeod and McCarty |
| DNA strands apart while they are being replicated? | primase | ligase | DNA polymerase | single strand binding proteins | single strand binding proteins |
| What is the role of DNA ligase in the elongation of the lagging strand during DNA replication? | synthesize RNA nucleotides to make a primer | catalyze the lengthenin g of telomeres | join Okazaki fragments together | unwind the parental double helix | join Okazaki fragments together |
| Which of the following covalently connects segments of DNA? | helicase | DNA polymerase III | ligase | DNA polymerase I | ligase |
| What is the function of helicase? | It forms bonds between DNA nucleotides | It adds new nucleotides to the DNA helix. | It forms the DNA helix | It separates DNA strands | It separates DNA strands |
| Nucleosome assembly is | initiated by nucleoplasmin | spontaneous | inhibited by topoisomerase | induced | spontaneous |

| | | | | | |
|---|---|---|---|---|---|
| The coiling that cannot be separated except by unwinding is called | supercoiling | negative super coiling | plectonemic coiling | anti parellel coiling | plectonemic coiling |
| If replication was completely conservative then | one heavy and one light strand would be seen | both heavy strands would be seen | both light strands would be seen | Mixed strands | one heavy and one light strand would be seen |
| Specific chromosomal locations from where replication begins are called | locus | origin of replication | loci | chromosomal arms | origin of replication |
| the | 3' end to 5' end | 5' end to 3' end | both A and B | 5' end to 5' end | 5' end to 3' end |
| with | pyrimidine | pyrol | purine | phenazine | purine |
| Change in nucleotide sequence of a short region of genome is called | replication | recombination | repair | mutation | mutation |
| When DNA opens up y shaped structures are called | replication forks | single stands | parent strand | double strand | replication forks |
| All newly synthesized polynucleotide strands must be initiated by | RNA polymerase | DNA polymerase | gyrase | helicase | RNA polymerase |
| **Unit III** | | | | | |
| enzymes cut out a damaged section of DNA and other enzymes then repair the resulting break is known as | excision repair | recombination repair | SOS repair | Photo reactivation | excision repair |
| Removal of thymine dimer after replication process is done by | recombination repair | excision repair | light repair | photo reactivation | recombination repair |
| Excision repair is carried by | Uvr ABC endonuclese | AP endonuclease | Methyl transferase | Glycosylase | Glycosylase |
| In the following statement all are related to photoreactivation repair except | an enzymatic cleavage of thiamine dimers | activated by visible light | uses light energy to cleave C-C bonds of | removal of short segment of DNA | removal of short segment of DNA |
| The enzyme responsible for the replication of mitochondrial DNA | DNA polymerase $\alpha$ | DNA polymerase $\beta$ | DNA polymerase $\gamma$ | DNA polymerase $\delta$ | DNA polymerase $\gamma$ |
| 5-bromo uracil damages DNA. It is an | Uracil | Thymine | Cytosin | Guanine | Thymine |

| | | | | | |
|---|---|---|---|---|---|
| The type of point mutation which could be detected only after nucleic acid sequencing is known as | silent mutation | missense mutation | nonsense mutation | . Transversion mutation | silent mutation |
| Which of the following enzyme selectively degrades single stranded nucleic acid | DNA ligase | . DNA polymerase | Ribonuclease | S1 nuclease | S1 nuclease |
| Pyrimidine dimers are mended byall except | excision repair | photo lyase | recombination repair | Mismatch repair | Mismatch repair |
| Altered bases in DNA are removed by | Photolyase | Glycosylase | Apendo nuclease | UvrABC endonulease | Glycosylase |
| Hot spots are sites containing | MeC | MeG | MeA | MeA | MeC |
| Which of the following have proof reading | Rep protein | polymerase I | polymerase II | polymerase III | polymerase I |
| Which of the following thymine dimer repair pathway is dependent on light? | excision repair | recombination repair | SOS repair | Photo reactivation | Photo reactivation |
| Daughter strand repair is the other name for | SOS repair | excision repair | recombination | photo reactivation | recombination |
| Dna A protein recognize and binds to | 49 bp repeats in ter C | 13 bp AT rich segment | 49 bp repeats in Ori C | RNA polymerase | 4 9 bp repeats in Ori C |
| sequence of parent and daughter strand is observed in | mismatch repair | excision repair | recombination repair | photo reactivation | mismatch repair |
| Rec A protein can bind to | ssDNA | ds DNA | RNA | protein | ssDNA |
| Chemical adducts are DNA region | having chemically modified bases | having unusal bases | rich in AT | rich in GC | having chemically modified bases |
| The proof reading activity of the newly synthesized DNA is present on the enzyme | DNA helicase | DNA polymerase I | DNA polymerase II | DNA polymerase III | DNA polymerase I |
| The type of point mutation which could be detected only after nucleic acid sequencing is known as | silent mutation | missense mutation | nonsense mutation | . Transversion mutation | silent mutation |
| Common lesions found in DNA after exposure to ultraviolet light | Pyrimidine dimers | Single strand breaks | Base deletion | Purine dimers. | Pyrimidine dimers |
| Genetic recombination in bacteria discovered by ----- | Lederberg and tautum | Hershey and chase | Avery and Mccarthy | Griffith | Lederberg and tautum |

| | | | | | |
|---|---|---|---|---|---|
| Exchange of a single nucleotide (A ↔ G) or (C ↔ T).is ---------- | transition | transversion | recombination | translation | transition |
| Point mutation which code for the differnt amino acid is | missense mutation | nonsense mutation | silent mutation | inseration | missense mutation |
| Point mutation which code for a stop and truncate the protein is | missense mutation | nonsense mutation | Silent mutation | inseration | nonsense mutation |
| Mutation occur in non coding region is ----- | missense mutation | nonsense | Silent mutation | inseration | Silent mutation |
| The biochemical mechanism tolerance of the damage is | SOS repair | Excision repair | Recombination | reverse mutation | SOS repair |
| -------- Includes a bypass system that allows DNA chain growth across damaged segment at the cost of fidelity of replication | SOS repair | Excision repair | Recombination Excision repair | Incision repair | SOS repair |
| The drug chloramphenical blocks | cellwall formation | transcription | translation termination release factors | polypeptide chain elongation. | polypeptide chain elongation. |
| AT repeats are the examples of | insertion | deletion | transversion | translocation | insertion |
| A base is changed by the repositioning of a hydrogen atom. | tautomerism | depurination | deamination | transition | transition |
| Loss of a purine base (A or G) | tautomerism | depurination | deamination | transition | depurination |
| A purine changes to another purine, or a pyrimidine to a pyrimidine | tautomerism | depurination | deamination | transition | transition |
| which of the following is an alkylating | NTG | BU | NH2OH | HA | NTG |
| Common lesions found in DNA after exposure to ultraviolet light | Pyrimidine dimers | Single strand breaks | Base deletion | Purine dimers. | Pyrimidine dimers |
| During mutation substitution of Adenine by Guanine is known as | Transition | Transversion | Deletion | Transpositions | Transition |
| ---------- Mutation leads to the death of | Lethal | Point | Frame shift | Missense | Lethal |
| Which of the following leads to frame shift | base analog | ionization | uv radiation | 5 BU | uv radiation |
| Which one is an mutagen | EMS | EES | NTG | HA | EMS |
| Which of the following is not require for plasmid to select recombination? | ori | marker site | restriction enzyme site | recombination site | recombination site |

| | | | | | |
|---|---|---|---|---|---|
| Transposons are _____ | Regulatory | Jumping genes | Enzymes | restriction | Jumping genes |
| Transposable elements were discovered by _____ | Mendel | Darwin | McClintock | Tomizerwa | McClintock |
| One of the following is not a kind of mobile genetic elements? | Operon | Plasmid | Transposon | Bacteriophage. | Operon |
| The insertion sites used repeatedly are called _____ | Cols spots | Hot spots | Random spots | Unique spots | Hot spots |
| The termini of each Is elements have _____ | Inverted repeat sequence | Initiation repeat sequence | Contrast repeat sequence | Plasmid repeat sequence | Inverted repeat sequence |
| LTR strands for | Long terminal repeats | Left terminal repeats | Large terminal repeats | L terminal repeats | Long terminal repeats |
| The genetic organization of the Ty elements resembles that of the eukaryotic | Retro viruses | Mosaic viruses | Sendai viruses | Animal viruses | Retro viruses |
| The genes present in a provirus's is | Gap gene | Pol gene | Env gene | Tus gene | Gap gene |
| The yeast Ty elements are sometimes called as | Simple transposons | Complex transposons | Retro transposons | Co- transposons | Simple transposons |
| The Holliday intermediate is also known as | α-form | β form | μ- form | א- form | א- form |
| Holliday model of recombination is observed in which stage of cell cycle | DNA Synthesis | G1 | G2 | Meiosis | Meiosis |
| Repressor binding site on DNA is | Activator | Operator | enhancer | stimulators. | Operator |
| House keeping gene is the gene that | rarely | constantly | over expressed | under expressed | constantly |
| Lac repressor is a | Monomer | Dimer | Trimer | Tetramer | Tetramer |
| Antisnse RNA is related | PCR | RNAi | Southern blot | Western blot | RNAi |
| tRNA acts as _____ molcule | intergrator | Adaptor | regulator | promoter | Adaptor |
| Double strand RNA is degraded by | endouclease | protease | RNase | Dnase | RNase |
| Site specific recombination require the recognition of unique nucleotide sequences in both DNA molecules by enzymes | recombinase | Helicase | Isomerase | ligase | recombinase |
| Recombination in Meselson-Radding model is initiated by | ss nicks | ds nicks | exchange process | mispairing | ss nicks |
| RNA polymerase binds to DNA at | activator | Operator | Promoter | repressor | Promoter |

| **Unit IV** | | | | | |
|---|---|---|---|---|---|
| 5' cap in mRNA is done by | ATP | GTP | CTP | UTP | GTP |
| Large protein complex that involved in intron removal is | replisome | primosome | spliceosome | Chromosome | spliceosome |
| One of the following is distinctive Structure of eukarytic mRNA | poly A tail | poly T tail | polu U tail | poly G tail | poly A tail |
| Eukaryotic mRNA was synthesised by | DNA Pol I | RNA Pol I | RNA Pol II | RNA Pol III | RNA Pol II |
| The strand of DNA that directs the synthesis of mRNA is | sense strand | leading strand | antisense strand | lagging strand | sense strand |
| Primase initiates all excxept | leading strand synthesis | replication | Okazaki fragments | translation | translation |
| Positive superhelicity generated during DNA replication is removed by | DNA polymerase | DNA isomerase | DNA gyrase | DNA helicase | DNA gyrase |
| The problem of supercoils during DNA replication is overcome by a group of | DNA topoisomerases | DNA ligases | DNA polymerases | DNA helicases | DNA topoisomerases |
| The DNA element enhancer _____ | can act only in cis | . can act only in trans | can act in cis or in trans | can act at medial | can act only in cis |
| Telomerase is a ____ | primase | polymerase | reverse | helicase | reverse transcriptase |
| For initiation ___subunit of RNA pol | alpha | beta | gamma | sigma | sigma |
| Pribnow box consists of the following 6 nucleotide bases _____ | TATAAT | TATATA | TTAAAT | TAATAT | TATAAT |
| Rho protein is involved in _____ | transcription initiation | transcription elongation | transcription termination at some terminators | . transcription termination at all terminators | transcription termination at some terminators |
| 5SrRNA are synthesized by RNA polymerase ____ | I | II | III | IV | III |
| Transfer RNA has all except _____ | Clover leaf structure | Anticodon arm | Minor bases in addition to normal bases A, U, G and C | proteins | proteins |

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| snRNAs are involved in the eukaryotic _____ | Transcription | Splicing of mRNA | transfer of amino acids | splicing of tRNA | Splicing of mRNA |
| Rho factor is a ____ | Dimer | Trimer | hexamer | Tetramer | hexamer |
| RNA self splicing was discovered by | Watson & Crick | McClintock | Sanger | Thomas Cech | Thomas Cech |
| Transcription charecterised by all except _____ | one strand of DNA serve as template | synthesis of RNA | Synthesize proteins | Participation of RNA polymerase | Synthesize proteins |
| Termination of transcription is brought about by _____ | sigma factor | RNA polymerase | rho factor | TATA box | rho factor |
| In eukaryotic cells, the synthesis of mRNA is carried out by _____ | RNA polymerase I | RNA polymerase II | RNA polymerase III | DNA polymerase | RNA polymerase II |
| Rho factor is ____ | an enzyme | accessory protein | rich in U residues | rich in G-C with a palindromic sequence. | accessory protein |
| The protein that bind to the TATA box in the promoter region are called _____ | coregulators | coactivators | . enhancers | Transcriptional factors | Transcriptional factors |
| Who demonstrated that poly U stimulate the synthesis of poly phenylalanine? | Nirenberg and Leder | . Nirenberg and Ochoa | Nirenberg and Mathai | H. Gobind Khorana | Nirenberg and Mathai |
| Shine dalgarno sequence is rich in _____ | purines | . pyrimidines | . only A | only T | purines |
| The enzyme responsible for processing the rRNAs is _____ | Rnase III | Rnase H | Rnase P | Rnase F | Rnase III |
| Hirpin loop like structure in transcription is related to _____ | Initiation | Elongation | termination | Initiation&elongation | termination |
| Proceesing of tRNA involves adding _____ | CCA to the 3' end | CCA to the 5' end | 7-methyl G to the 3' end | poly A to the 3' end | CCA to the 3' end |
| A tRNA molecule must be able to _____ | recognize a codon | recognize an anticodon | distinguish one amino acid from another | recognize DNA molecules | recognize a codon |
| The following are associated with transcription except _____ | -35 sequence | pribnow box | promoter | spacer | spacer |
| What is the characteristic form introns have after being cut from a pre-mRNA? | Linear structure | circular form | lariat shaped | theta structure | lariat shaped |

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| During the course of transcriptuon or replication for the addition each nucleotide to the growing chain the following moiety is | phosphate | pyrophosphate | nitrogenous base | nucleotide | pyrophosphate |
| The RNA polymerase of E.coli complex holoenzyme composed of 5 polypeptide | 2a,2b and one sigma factor | 2a 2b and one rho factor | 2a 1b,1b and one sigma factor | 2a,1b,1b and one rho factor | 2a 1b,1b and one sigma factor |
| RNA polymerase utilizes the following RNA triphosphates for the formation of | ATP, GTP, CTP and TTP | ATP, GTP, CTP and UTP | ATP, CTP, UTP and UTP | CTP, UTP | ATP, GTP, CTP and UTP |
| tRNA specifically reacts with | mRNA | nuclear RNA | amino acyl adenylates | ribosomal RNA | amino acyl adenylates |
| Pseudo uridine is found in _____ | mRNA | snRNA | rRNA | tRNA | tRNA |
| mRNAs are synthysized by RNA | . I | II | III | I & II | II |
| Mature mRNAs has the following properties except _____ | 5'Cap | Poly A tail | Exon | Intron | Intron |
| Rho factor catalyses the unwinding of RNA-DNA double helices and _____ | Hydrolysis of ATP | RNA-RNA double helices | Migrates along the RNA in 3'-5' direction | Migrates along the RNA in 5'-3' direction | Hydrolysis of ATP |
| Prokaryotic transcription initiation is inhibited by _____ | . Rifamycin | Rifampicin | ricin | abrin | Rifampicin |
| Non template strand is otherwise called as _____ | template strand | coding strand | non coding strand | sense strand | coding strand |
| ____activates transcription | CRP | GMP | RNase | ribozyme | CRP |
| The region on t RNA recognizes the appropriate base sequence m RNA is called | codon loop | anti-codon loop | . P site | . A site | anti-codon loop |
| Enhancers are entry point on DNA for RNA polymerase | I | II | III | I and III | II |
| Promoters are recognized by _____ | alpha sub unit | gamma subunit | beta subunit | sigma subunit | sigma subunit |
| Eukaryotic mRNA that specifies proteins is modified before translation by all except | Addition of 5' cap | removal of exons | addition of 3' poly A tail | removal of introns | removal of introns |
| The 5' terminus of a eukaryotic mRNA molecule is _____ | 2-amino purine | 5 bromo uracil | 7 methyl guanosine | formyl methionine | 7 methyl guanosine |

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| . When a number of genes are transcribed as one mRNA, the mRNA is said to be ___ | multimeric | . polymeric | polycistronic | polyclonal | polycistronic |
| Transposase is encoded by _____ | IS element | bacterial trnasposon | . rRNA | . tRNA | bacterial trnasposon |
| Premature chain termination in prokaryotes and eukaryotes is caused by | ricin | abrin | erythromycin | . puromycin | ricin |
| Rho dependant termination sites are rich in | only U | . Only GC | Both U and GC | . AT | Both U and GC |
| Internal promoters are present in the gene sequence of | 18SrRNA gene | 28SrRNA genes | mRNA genes | t RNA | t RNA |
| The hexa nucleotide AAU AAA provides the signal for | Capping | Generation of eukaryotic 3'-poly A tail | Initiation of translation | Peptide bond formation | Generation of eukaryotic 3'- poly A tail |
| RNA polymerases present in eukaryotes | RNA pol I | RNA pol II | RNA pol III | RNA pol I,II and | RNA pol I,II and III |
| The mature 5'end of tRNA in E.coli formed as a result of endonucleolytic cleavage by | RNase P | RNAse D | RNase M5 | RNase M13 | RNase P |
| The enzyme responsible for reverse transcription | DNA dependent DNA polymerase | DNA dependent RNA polymerase | RNA dependent DNA polymerase | RNA dependent RNA polymerase | RNA dependent DNA polymerase |
| DNA Polymerase I has | 5'-3' Exonuclease activity | Proof reading activity | Polymerising activity | 5'-3' Endonuclease | Polymerising activity |
| An E.Coli strain lacking DNA polymerase I would be deficient in DNA | Repair | Splicing | Methylation | Transcripition | Repair |
| Single standered circular DNA is present in | j/174 | all viruses | Animal viruses | Plant viruses | bacteriophages |
| In the classical model of transcriptional control described by Jacob and Monod, a repressor protein binds to | Enhancer | AUG sequence | Operator | Ribosome binding site | Operator |
| **Unit V** | | | | | |
| Translation refers to | DNA from RNA | RNA from | protein from | protein from | protein from RNA |
| The chain initiating amino acid in protein biosynthesis in E.Coli is | Methionine | . Cysteine | N-formyl methionine | . Arginine | N-formyl methionine |
| _____are the factories for protein | mitochondria | nucleus | ribosomes | cytoplasm | ribosomes |
| The initiating codon in protein synthesis | UAA | . UAG | UGA | AUG | AUG |

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| Genetic code is the dictionary of the following nucleotide bases | A, G, C and T | A, G, C and U | . A, G, T and U | . A, G, T and T | A, G, C and U |
| The Wobble hypothesis accounts for the | triple nature of codon | degeneracy of codon | universality of the codon | doublet nature of the codon | degeneracy of codon |
| Which of the following amino acids is specified by only a single codon? | . Glutamine | . tryptophan | asparagine | isoleucine | isoleucine |
| The enzyme responsible the cleavage of the bond connecting amino acid and tRNA | amino acyl-tRNA synthetase | peptidyl transferase | aminoacyl transferase | tRNA deacylase | tRNA deacylase |
| Catalytic RNA is | Abzyme | ribozyme | enzyme | protein | ribozyme |
| _____are proteins that block the synthesis of RNA at gene level | Initiators | Repressors | stimulators | retarders | Repressors |
| tRNA, 5S RNA, Special RNA s are synthesised by RNA Pol_____ | I | II | III | IV | III |
| Which of the following antibiotics is an inhibitor of protein synthesis | Chloramphenicol | Penicillin | Bacitracin | Cephalosporin | Chloramphenicol |
| Coupled transcription and translation occurs in _____ | only in eucaryoptes | only in prokaryotes | both in prokaryotes and in eukaryotes | neither in prokaryotes nor in eukaryotes | only in prokaryotes |
| Which of the following are termination | UAG, UAA, UGA | UUA, AUC, | . UAG, | . UAG, GAA, | UAG, UAA, UGA |
| Nucleic acid concerned with the protein synthesis is | DNA | mRNA | . ribosomal RNA | tRNA | . ribosomal RNA |
| One of the following compound competitively inhibits the enzyme peptidyl transferase and interfere with elongation of | Streptomycin | Puromycin | Chloramphenicol | Terracycline | Chloramphenicol |
| The stop codon UAA is otherwise called as | Amber | Ochre | opal | none | Ochre |
| The binding of $tRNA^{fmet}$ to the P site is inhibited by | Streptomycin | . Chloramphenicol | Erythromycin | Lincomycin | Streptomycin |
| Translocase is an enzyme required in the process of | .DNA replication | RNA synthesis | Initiation of protein synthesis | Elongation of peptides | Elongation of peptides |

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| Which one of the following bases if present in the third position of an anticodon cannot form wobbl base pairing. | G | C | U | A | A |
| How many polypeptide chains can be formed simultaneously by a given | one | about a dozen | up to 30 | variable depending on | one |
| Diphtheria toxin inhibits elongation step in translation through | Glycosylation | Methylation | ADP-ribosylation | Ubiquitinylation | ADP- ribosylation |
| Selection of translational initiation site is carried out by | Base pairing between mRNA | initiation codon | termination codon | promoter | Base pairing between mRNA and |
| Principle function of RNA pol II in Eukaryote is synthesis of | mRNA | tRNA | rRNA | tRNA | mRNA |
| Which of the following inhibits the peptidyl transferase activity of the eukaryotic 60S ribosomal subunit | Chloroamphenical | Cycloheximide | Erythromycin | Puromycin | Cycloheximide |
| Large subunit of ribosome is present in _____eukaryotes | 60s | . 40s | 30s | 80s | 60s |
| In protein synthesis aminoacids are joined by _____ bond | peptide | phosphodiester | disulfide | phosphodiester and disulfide | peptide |
| Which of the following Binds to 50S subunit and inhibits translocation | Chloroamphenical | Cycloheximide | Erythromycin | Puromycin | Erythromycin |
| The codon that terminates protein synthesis | UGG | UGA | UGC | UGU | UGA |
| Shine Dalgarno sequence is | rich in pyrimindine | rich in purine | centered 100 nucleotide upstream from the start codon | centered 100 nucleotide downstream from the start codon | rich in purine |
| The following are the termination or non sense codons except | AUG | UAA | UAG | UGA | AUG |
| The specific information required for the sequence amino acids in protein is located | rRNA | mRNA | tRNA | hnRNA | mRNA |
| The largest subunit of ribosomal RNA in prokaryotes | 30S | 50S | 60S | 40S | 50S |

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| Which one of the following post translational modifications occurs in | hydroxylation | methylation | carboxylation | phosphorylation | carboxylation |
| The antibiotic cycloheximide which _____ | blocks chain elongation during protein synthesis | blocks glycosylation of proteins | resembles aminoacyl tRNA | inhibits the formation of peptide bond. | inhibits the formation of peptide bond. |
| Post dimer initiation is responsible for | recombination | SOS repair | excision repair | photo reactivation | recombination |
| The association of 50S and 30Ssubunit is prevented by_____ | IF 1 | IF 2 | IF 3 | EF2 | IF 3 |
| Wobble hypothesis explained by one of the following characteristics of the genetic code | Universality | Specificity | non overlapping | degenerate | degenerate |
| The codon (of mRNA) and anticodon (of tRNA) recognize each other by pairing | 5'-3' of mRNA with 5'-3' of tRNA | 3'-5' of mRNA with 3'-5' of | 5'-3' of mRNA with 3'-5' of | 3'-5' of mRNA with 5'-3' of | 5'-3' of mRNA with 3'-5' of tRNA |
| Proteins are synthesized in _____ direction | Amino to carboxyl | Carboxyl to amino | Both amino and carboxyl | other groups like phosphoric acid | Amino to carboxyl direction |
| Post translational modifications involve the following covalent changes | Synthesis mRNA | hydroxylation | carboxylation | Phospho diester linkage formation | Phospho diester linkage formation |
| Wobble hypothesis is characterized by recognition of'_____ | a single codon by a single tRNA | more than one codon by asingle tRNA | a single codon by more than one tRNA | more than one codon by more tRNA | more than one codon by asingle tRNA |
| Rifampicin inhibit Protein synthesis by binding to | beta subunit of RNA pol | delta subunit of RNA pol | gamma subunit of RNA pol | alpha subunit of RNA pol | beta subunit of RNA pol |
| In prokaryotes protein synthesis is carried out by ribosomes of | 80s,30s, and 50s | 80s,40s, and 50s | 70s,30s, and 50s | 70s,40s, and 60s | 70s,30s, and 50s |
| Premature termination of poly peptide is caused by | Erythromycin | Puromycin | Streptomycin | Ricin | Puromycin |
| Which of the following is not a necessary component of translation? | Anticodon | mRNA | Ligase | Amino acid | Ligase |
| Eukaryotic mRNA binding to the ribosomes is facilitated by _____ | the Shine Dalgarno sequence | the 7-methyl guanosine cap | tRNA | poly A tail | the 7-methyl guanosine cap |
| The structure in a bacterium that indicates an active site for protein synthesis is | a chromosome. | a cell membrane, | a flagellum. | a polysome. | a polysome. |

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|---|---|---|---|---|---|
| In the presence of high levels of tryptophan | attenuator allows transcription of trp structural genes | attenuator propogates transcription | attenuator terminates transcription | attenuator terminates translation | attenuator terminates transcription |
| Which of the following occur in the presence of glucose? | *lac* Z gene expression is increased | cAMP increases | Binding of CAP-cAMP complex to the promoter area decreases | cAMP decreases | Binding of CAP-cAMP complex to the promoter area decreases |
| The lac operon is translated into _____ proteins. | 1 | 2 | 3 | 4 | 3 |
| In the presence of tryptophan in the cell, the repressor bound to | tryptophan | DNA | DNA and tryptophan | neither tryptophan nor DNA | DNA and tryptophan |
| _____ control on transcription of the lac operon genes. | positive | negative | may be positive or negative | positron | positive |
| DNA is methylated at _____ | AC sequences | sequences | CAAT sequences | CG sequences | CG sequences |
| Which type of ribonucleic acid combines with proteins to form a ribosome? | mRNA | tRNA | sRNA | rRNA | rRNA |
| How many bases are needed to code for two amino acids? | 2 | 3 | 6 | 12 | 6 |
| What does the operon model attempt to explain? | the coordinated control of gene expression in bacteria | bacterial resistance to antibiotics | how genes move between homologous regions of DNA | the mechanism of viral attachment to a host cell | the coordinated control of gene expression in bacteria |
| result in the inability of the cell to ″turn off″ genes: | inducer | promoter | repressor | corepressor | corepressor |
| The lactose operon is likely to be transcribed when | there is more glucose in the cell than lactose. | the cyclic AMP levels are low. | there is glucose but no lactose in the cell. | the cyclic AMP and lactose levels are both high within the cell. | the cyclic AMP and lactose levels are both high within the cell. |
| During translation, chain elongation continues until what happens? | All tRNAs are empty | The polypeptide is long enough | A stop codon is encountered. | The ribosomes run off the end of mRNA. | A stop codon is encountered. |