Syllabus: MOLECULAR BIOLOGY
Course code: 275
Semester: 3rd (Fall)
Type: Obligatory course

Course Overview

1: Genes are DNA and encode RNAs and polypeptides
Contents

1. DNA Is the genetic material of bacteria and viruses
2. DNA Is the genetic material of eukaryotic cells
3. Polynucleotide chains have nitrogenous bases linked to a sugar—phosphate backbone
4. DNA is a double helix
5. DNA replication is semiconservative
6. Polymerases act on separated DNA strands at the replication fork
7. Nucleic acids hybridize by base pairing
8. Mutations change the sequence of DNA
9. Mutations can affect single base pairs or longer sequences
10. The effects of mutations can be reversed
11. Most genes encode polypeptides
12. Mutations in the same gene cannot complement
13. Mutations may cause loss of function or gain of function
14. A locus can have many different mutant alleles
15. A locus can have more than one wild-type allele
16. The genetic code is triplet
17. Every coding sequence has three possible reading frames
18. Proteins are trans-acting but sites on DNA are cis- acting

Learning outcomes
Upon successfully completing this course, students should be able to:

- Describe the two experiments that led molecular biologists to conclude that genes are made of DNA, and state the limitations of each experiment
- Discuss the evidence that Watson and Crick used to deduce the double helix structure of DNA and
- List the key features of DNA structure
- Explain how RNA differs from DNA
- Distinguish between a missense and a nonsense mutation.
- Discuss if an insertion or deletion more likely to be deleterious than a substitution?

2: The interrupted Gene

Contents

1. An interrupted gene has exons and introns
2. Exon and intron base compositions differ
3. Organization of interrupted genes can be conserved
4. Exon sequences under negative selection are conserved but introns vary
5. Exon sequences under positive selection vary but introns are conserved
6. Genes show a wide distribution of sizes due primarily to intron size and number variation
7. Some DNA sequences encode more than one polypeptide
8. Some exons correspond to protein functional domains
9. Members of a gene family have a common organization
10. There are many forms of information in DNA

Learning outcomes

Upon successfully completing this course, students should be able to:

- Describe the structure of a eukaryotic gene
- Explain the diversity of introns sequences and positions
- Discuss the functional roles of introns

3: The messenger RNA

Contents

1. mRNA is produced by transcription and is translated
2. Transfer RNA forms a cloverleaf
3. The acceptor stem and anticodon are at ends of the tertiary structure
4. Messenger RNA is translated by ribosomes
5. Many ribosomes bind to one mRNA
6. The life cycle of bacterial messenger RNA
7. Eukaryotic mRNA is modified during or after its transcription
8. The 5 ′ end of eukaryotic mRNA is capped
9. The 3 ′ terminus is polyadenylated
10. Bacterial mRNA degradation involves multiple enzymes
11. mRNA stability depends on its structure and sequence
12. mRNA degradation involves multiple activities
13. Eukaryotic RNAs are transported
14. mRNA can be specifically localized

Learning outcomes

Upon successfully completing this course, students should be able to:

- Describe the factors that influence the lifespan of mRNA in the cytoplasm.
- Compare the longevity of mRNA in prokaryotes and eukaryotes.
- Explain the factors that are involved in mRNA degradation

4: Translation

Contents

1. Translation occurs by initiation, elongation, and termination
2. Special mechanisms control the accuracy of translation
3. Initiation in bacteria needs 30s subunits and accessory factors
4. A special initiator tRNA starts the polypeptide chain
5. Use of fMet-tRNAf is controlled by IF-2 and the ribosome
6. Initiation involves base pairing between mRNA and rRNA
7. Small subunits scan for initiation sites on eukaryotic mRNA
8. Eukaryotes use a complex of many Initiation Factors
9. Elongation factor Tu Loads aminoacyl-tRNA into the A site
10. The polypeptide chain is transferred to aminoacyl- tRNA
11. Translocation moves the ribosome
12. Elongation factors bind alternately to the ribosome
13. Three codons terminate translation
14. Termination codons are recognized by protein factors
15. Ribosomal RNA is found throughout both ribosomal subunits
16. Ribosomes have several active centers
17. 16S rRNA plays an active role in translation
18. 23S rRNA has peptidyl transferase activity

Learning outcomes

Upon successfully completing this course, students should be able to:

- Describe the structure and function of tRNAs
- Describe the process by which tRNA is joined to the appropriate amino acid
- Explain the structure and functions of ribosomes
- Describe the process of translation (including initiation, elongation and termination)
- Explain which enzymes, protein factors, and energy sources are needed for each stage
- Define the significance of polyribosomes

5: The Genetic Code

Contents

1. Related Codons Represent Chemically Similar Amino Acids
2. Codon—Anticodon Recognition Involves Wobbling
3. tRNAs Are Processed from Longer Precursors
4. tRNA Contains Modified Bases
5. Modified Bases Affect Anticodon—Codon Pairing
6. The Universal Code Has Experienced Sporadic Alterations
7. Novel Amino Acids Can Be Inserted at Certain Stop Codons

8. tRNAs Are Charged with Amino Acids by Aminoacyl- tRNA Synthetases
9. Aminoacyl-tRNA Synthetases Fall into Two Classes
10. Synthetases Use Proofreading to Improve Accuracy
11. The Ribosome Influences the Accuracy of Translation
12. Frameshifting Occurs at Slippery Sequences

Learning outcomes

Upon successfully completing this course, students should be able to:

- Define “codon” and explain the relationship between codons on mRNA and the amino acids in a polypeptide
- Explain the significance of the “reading frame” during translation
- Describe the properties of the genetic code – how many codons code for amino
- acids, stop codons, redundancy, universality
- Explain the evolutionary significance of a nearly universal genetic code
- Discuss the significance of the wobble hypothesis

6: Transcription

Contents

1. Transcription occurs by base pairing in a “bubble” of unpaired DNA
2. The transcription reaction has three stages
3. Bacterial RNA polymerase consists of multiple subunits
4. RNA polymerase holoenzyme consists of the core enzyme and sigma factor
5. How does RNA polymerase find promoter sequences?
6. The holoenzyme goes through transitions in the process of recognizing and escaping from promoters
7. Sigma factor controls binding to DNA by recognizing specific sequences in promoters
8. Promoter efficiencies can be increased or decreased by mutation
9. Multiple regions in RNA polymerase directly contact promoter DNA
10. RNA polymerase—promoter and DNA—protein interactions are the same for promoter recognition and DNA melting
11. Interactions between sigma factor and core RNA polymerase change during promoter escape
12. A model for enzyme movement is suggested by the crystal structure
13. A stalled RNA polymerase can restart
14. Bacterial RNA polymerase terminates at discrete sites
15. How does Rho factor work?
16. Competition for sigma factors can regulate initiation
17. Sigma factors can be organized into cascades
18. Antitermination can be a regulatory event

Learning outcomes

Upon successfully completing this course, students should be able to:

- Explain how information flows from gene to protein.
- Discuss why every cell does not express all of its genes
- Define differential gene expression levels
- List the steps in prokaryotic transcription
- Discuss the role of RNA polymerase in prokaryotic transcription
- Describe how and when transcription is terminated
- Explain the role of promoters in prokaryotic transcription
- Explain why the sigma factors are required for accurate initiation of transcription

7: The operon

Contents

1. Structural gene clusters are coordinately controlled
2. The lac operon is negative inducible
3. The lac repressor is controlled by a small-molecule inducer
4. *cis*-acting constitutive mutations identify the operator
5. *trans*-acting mutations identify the regulator gene
6. The lac repressor is a tetramer made of two dimers
7. lac Repressor binding to the operator is regulated by an allosteric change in conformation
8. The lac repressor binds to three operators and interacts with RNA polymerase
9. The operator competes with low-affinity sites to bind repressor
10. The lac operon has a second layer of control: catabolite repression
11. The trp operon is a repressible operon with three transcription units
12. The trp operon is also controlled by attenuation
13. Attenuation can be controlled by translation
14. Stringent control by stable RNA transcription

Learning outcomes

Upon successfully completing this course, students should be able to:

- Explain the adaptive advantage of bacterial genes grouped into an operon
- Using the trp operon as an example, explain the concept of an operon and the function of the operator, repressor, and corepressor
- Explain how repressible and inducible operons differ and how those differences reflect differences in the pathways they control
- Describe how the lac operon functions and explain the role of the inducer, allolactose
- Distinguish between positive and negative control. Give examples of each from the lac operon
- Explain how cyclic AMP and catabolite activator protein are affected by glucose concentration
- Describe two main strategies that cells use to control metabolism

8: Regulatory Circuits

Contents

1. Antisense RNA can be used to inactivate gene expression
2. Small RNA molecules can regulate translation
3. Bacteria contain regulator RNAs
4. MicroRNAs are regulators in many eukaryotes
5. RNA interference is related to gene silencing

Learning outcomes

Upon successfully completing this course, students should be able to:

- Describe the formation of microRNAs (miRNAs).
- Distinguish between small interfering RNAs (siRNAs) and miRNAs.
- Explain the evolutionary significance of cellular RNA interference (RNAi) pathways

9: The replicon

Contents

1. Replicons can be linear or circular
2. An Origin Usually Initiates Bidirectional Replication
3. Origins can be mapped by autoradiography and electrophoresis
4. The Bacterial Genome Is (Usually) a Single Circular Replicon
5. Each Eukaryotic Chromosome Contains Many Replicons
6. Replication Origins Can Be Isolated in Yeast
7. The ends of linear DNA are a problem for replication
8. Rolling circles produce multimers of a replicon
9. Rolling circles are used to replicate phage genomes
10. Replication is connected to the cell cycle
11. Plasmid incompatibility is determined by the replicon
12. The ColE1 compatibility system is controlled by an RNA regulator

Learning outcomes

Upon successfully completing this course, students should be able to:

- Understand the concept of replicon, origin of replication, and types of replication control.
- Provide examples that illustrate single and multiple copy replication control
- Basic definitions associated with the mechanism of replication such as replication fork and replication eye
- Be able to differentiate between the unidirectional and bidirectional replication forks.
- Be familiar with structural organization and processes occurring at the prokaryotic origin of replication.
- The core consensus sequence of the bacterial oriC origin of replication

10: DNA replication

Contents

1. DNA Polymerases are the enzymes that make DNA
2. DNA Polymerases have various nuclease activities
3. DNA Polymerases control the fidelity of replication
4. DNA Polymerases have a common structure
5. The two new DNA strands have different modes of synthesis
6. Replication requires a helicase and a single-stranded binding protein
7. Priming is required to start DNA synthesis
8. Coordinating synthesis of the lagging and leading strands
9. DNA Polymerase holoenzyme consists of subcomplexes
10. The clamp controls association of core enzyme with DNA
11. Okazaki fragments are linked by ligase
12. Separate eukaryotic DNA polymerases undertake initiation and elongation
13. Creating the replication forks at an origin
14. Common events in priming replication at the origin
15. The primosome is needed to restart replication
16. Does methylation at the origin regulate initiation?
17. Lesion bypass requires polymerase replacement
18. Termination of replication

Learning outcomes

Upon successfully completing this course, students should be able to:

- Describe the relationship between the structure of a DNA molecule and the means by which DNA is replicated.
- Outline the basic steps involved in DNA replication: strand separation, pairing of complementary nucleotides, polymerization, and termination.
- understand replication terms helicase, polymerase, primase, Okazaki fragments, and ligase
- Review the major differences of replication between eukaryotes and bacteria.
- Explain how eukaryotes overcome the difficulty of replicating the ends of linear chromosomes

11: The chromosomes

Contents

1. Viral genomes are packaged into their coats
2. The bacterial genome is a nucleoid
3. The bacterial genome is supercoiled
4. Eukaryotic DNA has loops and domains attached to a scaffold
5. Specific sequences attach DNA to an interphase matrix
6. Chromatin is divided into euchromatin and heterochromatin
7. Chromosomes have banding patterns
8. Polytene chromosomes form bands

9. Polytene chromosomes expand at sites of gene expression
10. Centromeres have short DNA sequences in S. cerevisiae
11. The centromere binds a protein complex
12. Centromeres may contain repetitious DNA
13. Telomeres have simple repeating sequences
14. Telomeres seal the chromosome ends
15. Telomeres are synthesized by a ribonucleoprotein enzyme
16. Telomeres are essential for survival

Learning outcomes

Upon successfully completing this course, students should be able to:

- Define the term 'chromosome'
- Recognize and name the physical features of a chromosome
- Distinguish between the terms ‘constitutive heterochromatin’, ‘facultative heterochromatin’ and ‘euchromatin’
- Understand the use of a cytological map
- Describe key concepts for telomere length regulation and structure of telomeric chromatin.
- Illustrate how packaging is achieved in various prokaryotic organisms

12: The nucleosomes

Contents

1. The nucleosome is the subunit of all chromatin
2. DNA is coiled in arrays of nucleosomes
3. Nucleosomes have a common structure
4. DNA structure varies on the nucleosomal surface
5. The periodicity of DNA changes on the nucleosome
6. The path of nucleosomes in the chromatin fiber
7. Organization of the histone octamer
8. The N-terminal tails of histones are modified
9. Reproduction of chromatin requires assembly of nucleosomes
10. Are transcribed genes organized in nucleosomes?
11. Histone octamers are displaced by transcription

Learning outcomes

Upon successfully completing this course, students should be able to:

- Understand the various strategies employed by eukaryotes to compact their genomes into a nucleus.
- Explain the significance of histone proteins, including their charge and amino-terminal tails.
- Explain the interactions between the DNA double helix and the nucleosome

13: Promoters and enhancers

Contents

1. Eukaryotic RNA polymerases consist of many subunits
2. Promoter elements are defined by mutations and footprinting
3. RNA polymerase I has a bipartite promoter
4. RNA polymerase III uses both downstream and upstream promoters
5. The startpoint for RNA polymerase II
6. TBP is a universal factor
7. TBP binds DNA in an unusual way
8. The basal apparatus assembles at the promoter
9. Initiation is followed by promoter clearance
10. A connection between transcription and repair
11. Short sequence elements bind activators
12. Promoter construction is flexible but context can be important
13. Enhancers contain bidirectional elements that assist initiation
14. Enhancers contain the same elements that are found at promoters
15. Enhancers work by increasing the concentration of activators near the promoter
16. Gene expression is associated with demethylation
17. CpG islands are regulatory targets
18. Insulators block the actions of enhancers and heterochromatin

Learning outcomes

Upon successfully completing this course, students should be able to:

- List the steps in eukaryotic transcription
- Compare and contrast the three RNA polymerases
- Discuss the role of RNA polymerases in transcription
- Describe the role of promoters in RNA transcription
- Distinguish between general and specific transcription factors
- Describe the role of the transcription initiation complex.
- Explain the significance of transcription factors
- Explain the role of promoters, enhancers, activators, and repressors in transcriptional control

14: RNA Splicing and Processing

Contents

1. Nuclear splice junctions are short sequences
2. Splice junctions are read in pairs
3. pre-mRNA splicing proceeds through a lariat
4. snRNAs are required for splicing
5. U1 snRNP initiates splicing
6. The E complex can be formed by intron definition or exon definition
7. 5 snRNPs form the spliceosome
8. An alternative splicing apparatus uses different snRNPs

9. Splicing is connected to export of mRNA
10. Alternative splicing involves differential use of splice junctions
11. The 3 ′ ends of mRNAs are generated by cleavage and polyadenylation
12. Cleavage of the 3 ′ end of histone mRNA may require a small RNA
13. Cleavage of the 3 ′ end of histone mRNA may require a small RNA

Learning outcomes

Upon successfully completing this course, students should be able to:

- Outline the steps of pre-mRNA processing in eukaryotes.
- Understand the significance of exons, introns, and splicing
- Describe the process and significance of alternative RNA splicing.
- Describe the processing of pre-mRNA in eukaryotes
- Describe the general mechanism of the spliceosome doing splicing of mRNA precursors
- Explain how CTD of Pol II coordinates splicing