School of Medicine M 504: Structure, Synthesis and Degradation of Macromolecules

Course Director

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Credit Hours

Graduate students take this course for 4 credit hours. Students are required to attend all the lectures, problem-solving conferences, workshops, and other activities scheduled. This course is identical to Biochemistry 534.

Disability Statement

Any student who has need for accommodation based on the impact of a disability should contact the course director privately to discuss the specific situation as soon as possible. Contact Disability Resources and Services at 215-204-1280 in 100 Ritter annex to coordinate reasonable accommodations for students with documented disabilities.

Course Objectives

The goal of Structure & Degradation of Macromolecules is to provide students with an in-depth understanding of the synthesis and regulation of molecules involved in gene expression.

Textbook

The textbook is Watson, J.D., Baker, T.A., Bell, S.B., Gann, A., Levine, M., and Losick, R. (2004) *Molecular Biology of the Gene*, 5th. Edition, Benjamin Cummings, San Francisco. ISBN 0-8053-4635-X

Other recent textbooks that are not required, but may prove helpful, are:

Alberts, B., Johnson, J., Lewis, J., Raff, M., Roberts, K., and Walter, P. (2002) Molecular Biology of the Cell, 4th. Edition, Garland Publishing, Inc,. New York ISBN 0-8153-3218-1

Lewin, B. (2004) *Genes VIII*, Person, Upper Saddle River, NJ ISBN 0-13-145140-5

Homework

Faculty will assign homework problems from time to time. **THESE PROBLEMS ARE AN IMPORTANT PART OF THE COURSE !** The homework problems will be discussed during the discussion/review sessions scheduled throughout the course. **Students are expected to have worked the problems BEFORE these discussion sessions and to turn in their written answers at the beginning of the discussion session.** Students will also be required to read and comprehend papers from the recent research literature. Questions about these papers will be assigned and each student is expected to provide written answers before the paper is discussed in class. Working together in groups on homework assignments is encouraged, but each student must turn in their own written answers to the assignment. If you want to keep a copy of your homework to assist your studying please make this copy BEFORE the discussion session begins. Written homework papers will not be accepted after the discussion sessions begin. The problem sets will be graded and used to compute 20% of the final grade.

Grades

Grades will be based upon exams as follows:

Exam I	25 %
Exam II	25 %
Final	30 %
Homework	20%

THE FINAL EXAM WILL BE COMPREHENSIVE AND WILL COVER THE ENTIRE COURSE. The final will, however, emphasize material covered since Exam II.

Student's participation in class will be used to make a final adjustment in grade.

Syllabus

1. Introduction, The Central Dogma, Basic Techniques of Molecular Biology
Overview: Transmutation of information. DNA, RNA, Protein. Gel electrophoresis (agarose and acrylamide), hybridization, restriction endonucleases, basics of cloning, simple plasmid and phage vectors, insertional inactivation as a means of identifying successful cloning events, Southern, Northern, Western Blots, RNAse protection.

2. Structure of DNA. Nucleosides and nucleotides, structure of DNA, A, B and Z forms of DNA, linking numbers, methods of analyzing DNA: zone sedimentation, isopycnic sedimentation, chromatin, nucleosomes.

3. Enzymes and Proteins of DNA Replication.Recombination and Repair/ Techniques used to study DNA Metabolism. Properties of DNA polymerases, exonucleases, endonucleases, helicases, Reverse transcriptase. Polymerase chain reaction (PCR), RTPCR, dideoxy DNA sequencing, hybridization, complementation, .Reverse genetics, site specific mutagenesis

4. Discussion/ Problem Solving Session. Solutions to assigned problems on molecular biology techniques will be discussed.

5. DNA Replication I. Basics of DNA synthesis, Okazaki fragments, leading and lagging strands, DNA polymerases from prokaryotes and eukaryotes, fidelity, proofreading, structure of DNA polymerases, exo- and endo-nucleases, helicases, topoisomerases, primase, single strand binding protein.

6. DNA Replication II. DNA replication, cycling, Initiation and regulation of DNA replication, origin of replication, eukaryotic origin recognition complex, licensing, disruption of DNA replication forks, re-initiation via recombination, the primosome, termination of DNA replication, telomerases, replication of RNA viruses, reverse transcriptase.

7. Homologous Recombination. Basic mechanisms of homologous recombination. The Holliday junction, RecA, the RecBCD enzyme, resolvase, the Radding-Meselson model, the single strand annealing model, double strand break repair and recombination. Enzymatic pathways of recombination in prokaryotes.

8. Genetic Rearrangement . Site specific recombination, illegitimate recombination, transposition, regulation of transposition, retrotransposons, deletion, gene duplication, trinucleotide repeat expansions.

9. Discussion / Problem Solving Session. Solutions to assigned problems on DNA replication and recombination will be discussed.

EXAM I.

10. The gene, genomic structure. Prokaryotes (operons) vs. eukaryotes (introns); Highly repetitive, middle repetitive, unique sequences; Tm analysis; genomics and bioinformatics, organization of the genome, microarrays.

11. Properties and types of RNA. RNA structure; ribonucleoproteins, Stability and degradation; hnRNA and mRNA; capping; polyadenylation; introduction to splicing; rRNA transcription and processing, small nucleolar RNAs (snoRNAs); tRNA structure, base modification.

12. RNA Splicing. pre-mRNA; mRNA; introns, Type I, II and III splicing mechanisms; snRNPs; lariat formation; branchpoint; splice acceptor; splice donor; hammerhead, hairpin, and axe-head ribozymes; RNA editing.

13. RNA Synthesis. Overview; RNA polymerases: bacteria, archaea, eukarya; Prokaryotes: promoter structure, bent DNA, initiation, sigma factors; Eukaryotes (emphasizing pol II basal transcription): promoter structure, initiation, transcription factors, CTD phosphorylation; chromatin.

14. Regulation of Transcription in Prokaryotes. The operon, positive and negative regulation, repressors, activators, regulation of the lac operon, catabolite repression, regulation of the arabinose operon.

15. First Hour: Discussion/Problem Session. Solutions to assigned problems on genome structure, RNA, and RNA synthesis.
Second Hour: Overview of Transcription in Eukaryotes. Overview of gene expression in eukaryotes. Levels of gene expression. Gene structure and promoter.

16. Regulation of Transcription in Eukaryotes II. Consensus DNA sequences (i.e. TATA, enhancer DNA sequences). Functional analysis of cis-acting DNA sequences in the promoter. Reporter assays (CAT, Luc, ,-gal). Trans-acting transcription factors which bind to cis-acting DNA sequences. Electrophoretic mobility shift assays (EMSA). Properties and structure of transcription factors. Regulation of transcription factors. Two hybrid screening techniques and GST pull down.

17. Regulation of Transcription in Eukaryotes III. Coactivators and corepressors, Overall anatomy of the transcription apparatus. Histone acetylases and deactylases. Chromatin remodeling. Other transcriptional mechanisms of gene regulation (alternative promoter usage and methylation of DNA). Posttranscriptional mechanisms of gene regulation (alternative splicing, RNA editing, RNA transport and mRNA half-life). Translational mechanisms of gene expression (rate of translation).

18. Hormonal Regulation of Gene Expression. Steroid hormone vs peptide hormones, Steroid hormone, Steroids, Nuclear receptors - structure, Cis-acting DNA sequences (hormone response elements), Ligand binding domain of nuclear receptors, Coactivators and corepressors.

19. Elongation and termination of transcription. RNA polymerase pausing; sliding backwards; TFIIS, Gre factors; Termination in prokaryotes: intrinsic, Rho-dependent; RNA 3' end formation in eukaryotes; attenuation; antitermination.

20. Discussion / Problem Solving Session. Solutions to assigned problems on gene expression will be discussed.

EXAM II. This exam will cover all topics covered between 10/12 up to and including 11/16. Knowledge of the material covered on the first exam is assumed. Thus, material in the early part of the course may be included implicitly in questions that emphasize the genome, RNA metabolism, transcription and gene expression.

21. The genetic code. Terms and features; wobble; kinds of mutations; nonsense suppressors, tRNA synthetases; ribosome structure.

22. Protein synthesis I. Prokaryotic protein synthesis; antibiotics; eukaryotic protein initiation.

23. Protein synthesis II. Accuracy & proofreading; regulation; expense; protein localization, esp. secretion; glycosylation; chaperones; protein degradation.

24. Mutagenesis. Types of mutations, transitions, transversions, the effect of DNA damage on mutagenesis, replication slippage, the mismatch repair system, adaptive mutagenesis, Oxy-guanine as a premutational lesion.

25. DNA Repair. Types of DNA damage, base excision repair, nucleotide excision repair, preferential repair of replicating genes, roles for transcription factors in DNA repair, adaptive response, SOS response, cell cycle check points, apoptosis, double strand break repair.

26. Discussion / Problem Solving session . Solutions to assigned problems on protein synthesis will be discussed

FINAL EXAM. The final exam is **COMPREHENSIVE**. It will cover all material in the course. There will be some emphasis on the topics covered between 11/23 and 12/14.