BI~~3ee~~ 362 Eukaryotic Chromatin Structure, Maintenance and Function

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Course Resources

Required Text: Molecular Biology of the Cell, 4th Edition (2002), Alberts, et al. Garland Publishing

Primary scientific research articles will be distributed in class

Course Overview

This course builds upon the fundamental aspects of eukaryotic gene expression you were exposed to in introductory biology courses (BI105/106) and in the core molecular biology course, BI2aa. In this course we will utilize information derived from the research of molecular biologists from many disciplines to come to a greater understanding of the regulation of chromatin, one of the most fundamental macromolecules shared by all of life. We will explore how chromatin is assembled, how it is faithfully maintained, and how its molecular architecture controls gene expression and the various the scientific methodologies used to investigate these questions. In addition we will examine how defects in many of these pathways contribute to heritable and spontaneous human disease states.

Course Goals and Objectives

1. To understand i) how DNA is packaged into chromatin and how chromatin is dynamically modified to form euchromatin or heterochromatin; ii) the mechanism by which DNA is faithfully replicated and repaired to ensure its integrity for subsequent generations; and iii) the mechanisms by which the expression of individual genes is regulated at the level of transcriptional control.

2. To understand experimental techniques used to assay and investigate chromatin structure, maintenance and function and to develop the ability to design experimental protocols using these techniques to address specific problems.

3. To gain experience in the critical evaluation of scientific research articles.

4. To strengthen written and oral skills in communicating scientific ideas.

Other Important Information Regarding This Course

1. This is a discussion-based course. As such, attendance is REQUIRED and multiple absences will influence your final grade. For example, three or more absences and you are vulnerable to receive a failing grade.

2. Examinations: There are four exams scheduled throughout the term. The final is cumulative. Exams must be taken on the scheduled day unless you have an exceptionally urgent and dire situation. There are NO retakes of exams.

3. The occasional unscheduled "pop" quiz should be anticipated.

4. The practice of pleading for "just one more point" is strongly discouraged. Requests for reassessment of a specific question will result in the re-grading of the entire exam.

5. Each student is responsible for the selection, study and presentation of a current scientific research article related to a topic we cover in class. Article must be current and published in one of the following peer reviewed journals found in the library: Cell, Molecular Cell; Genes and Development; or the EMBO Journal. A copy of the article must be submitted to me and approved by me at least two weeks prior to the scheduled date of presentation.

6. Grading: Exams 50%; Homework and Quizzes 10%; Research Article Oral Presentation 10%; Research Article Written Review 10%; Attendance and Classroom Participation 10%;

7. It is expected that your participation within this course will reflect the Skidmore Honor Code as stated in the Skidmore student handbook. Your signature on homework, quizzes, and exams is your pledge to uphold this code of conduct.

Bl 3ee Lecture Syllabus

Week #1

Lecture #1: Introductions, Course Outline, Expectations, and Goals, Entrance Survey

Section 1: Review of DNA Chemistry, Composition and Structure

Week #2

Lecture #1: The Molecular Arena: Atoms, Electronegativity, Chemical Bonding and Molecular Wind ()
Handouts-Three 1953 Nature Papers Describing the Structure of DNA

Lecture #2: DNA: Nucleotide Composition, Hydrogen Bonding, Structural Features (Major/Minor Grooves) and Implication for Function

Flex Hour: Journal Discussion: Three 1953 Nature Papers Describing the Structure of DNA

Week #3

Lecture #1: DNA-Protein Interactions I: Amino Acid Structures, Hydrogen Bond Donors and Acceptors, Helix-Loop-Helix Protein Interaction with the Major Groove of DNA

Lecture #2: Experimental Strategies to Investigate DNA-Protein Interactions.

a. Radioactive labeling of the 5' end and/or 3' ends of DNA molecules
b. Column Chromatography to separate the radioactively labeled DNA molecule from the remaining unreacted labeled nucleotides.
c. DNAseI footprinting to probe protein interaction sites within DNA.
d. Filter binding assays to determine the kinetics of binding.

Flex Hour: Exam #1

Section II: Chromatin Structure and Composition

Week #4

Lecture #1: Histone Composition and DNA Association
a. Biochemical purification and characterization of histone proteins
b. Protein-Protein Interactions that form the histone octamer
2. Nucleosome interactions with DNA: Assaying nucleosome formation and DNA packaging by DNAseI I probing.

Lecture #2: The influence of chromatin on gene activity.
a. In vitro reconstitution of chromatin to demonstrate repression of Class II genes: Assaying DNA transcription in vitro
b. Differential nucleosome formation controls 5s rRNA gene activity in germ line versus somatic cells
c. Assaying chromatin structure/function relationships.

Flex Hour: Study, presentation and discussion of a scientific article on the organization of DNA in eukaryotic nuclei (model for DNA packaging).

Week #5

Lecture 1: Nucleosome Positioning-The Influence of Transcription Factors
a. Nucleosome-free zones in SV40 chromatin: Probing the architecture of chromatin with restriction enzymes.
b. DNAseI hypersensitivity sites: Nucleosome free zones are an indicator of actively transcribed gene. Nucleosome free zones are roughly 100 fold more sensitive to DNAseI digestion than bulk chromatin.
c. Transcription factors can dictate where nucleosomes get positioned on an active gene.

Lecture 2: Modification of Chromatin Structure
a. Controlling Transcription via the acetylation or deaceytlation of lysine in histone tails by histone acetyl transferases and deacetylasese

b. Nuclear Hormone Receptors that mediate histone acetylation or histone deaceytlation depending upon the presence or absence of their ligand

Flex Hour: Study, presentation and discussion of scientific article on the post-translational modification of histone proteins during gene activation/gene silencing.

Week #6

Lecture 1: Heterochromatin
a. Centromeres-incorporation of a special, centromere specific histone H2A variant
b. Telomeres-association of special silencing proteins at the ends of chromatin that interact with histones
c. X-chromosome Inactivation-Transcription of the Xist gene

Lecture 2: Exam #2
Flex Hour: Journal article on X chromosome inactivation.

Section III: Chromatin Maintenance

Week #7

Lecture #1: Three Models for DNA Replication: The Meselsohn-Stahl Experiment
a. Use of heavy isotopes
b. Equilibrium density centrifugation

Lecture #2: DNA Replication is Discontinuous and Bidiirectional
a. Pulse Chase Analysis of the Kinetics of Replication

Flex Hour: Study, presentation and discussion of scientific article on hereditary disease associated with defects in DNA replication (tri-nucleotide expansion diseases)

Week #8

Lecture #1: Origins of Replication-defining a start site of a replicating unit
- a. Yeast genomic libraries
- b. Microarray technology

Lecture #2: DNA Polymerase-Mechanism and Components of the Machine.
- a. Mechanism of action
- b. Associated replication activities (Primase, RNAseH, DNA Ligase, 5'-3' and 3'-5' Exonuclease)

Flex Hour: Article on hereditary disease associated with defects in DNA replication continued

Week #9

Lecture #1: Initiation of DNA Replication
- a. Initiation of Replication in Prokaryotes
- b. Initiation of Replication in Eukaryotes

Lecture #2: Coordinating Eukaryotic DNA Replication with the Cell Cycle
- a. Phases of the cell cycle
- b. Regulators (cyclins and cyclin dependant kinases and others)

Flex Hour: Study, presentation and discussion of scientific article on yeast cell cycle mutants

Week #10

Lecture #1: Cell cycle control of DNA Replication: Ensuring that eukaryotic origins fire once and only once per cell cycle.
- a. The origin recognition complex-ORC
- b. Cdc-ORC complex recruits MCM complex
- c. Regulation of Cdcp stability by S-phase Cyclins (S-CDKs)

Lecture #2: Telomeres: Special ends on linear chromosomes.
- a. Telomerase-a Ribonucleoprotein particle (protein and RNA).
- b. Mechanism of expansion by Telomerase
- c. Detecting Telomerase Activity in cell extracts
- d. T-loops: Special structure at the end of the telomeres

Flex Hour: Study, presentation and discussion of scientific article on telomerase and cancer

Week #11

Lecture #1: DNA Damage: Surveying the DNA structure for Damage
- a. Mistakes in replication
- a. Spontaneous changes-Depurination and Deamination
- b. Induced changes-Thymine Dimers (UV light)

Lecture #2: DNA Repair Systems: MSH2 in Human hereditary colon cancer

Flex Hour: Exam #3

Section III: Chromatin Function

Week #12
Lecture #1: Eukaryotic RNA Polymerase II -Structure/Function of Subunits
Lecture #2: Eukaryotic RNA Polymerase II-Initiation, Elongation and Termination

Flex Hour: Signaling cascades to alter gene expression patterns

Week #13
Lecture #1: Oral presentations
Lecture #2: Oral presentations
Flex Hour: Oral presentations

Week #14: Study Week

Week #15: Final Exam