MOLECULAR BIOLOGY OF EUKARYOTES 2019

03-442 Lectures: MWF 9:30-10:20 a.m. Wean Hall 5421
03-742 Advanced Discussion Section: Mon 4:30-6:00p.m. CNAST Conference room MI 622

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<th>Instructor</th>
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<tr>
<td>John Woolford</td>
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Preferred office hours for Dan: Mon-Fri 8a.m.-4p.m., and for John: anytime you can arrange

THE PRIMARY GOALS OF THIS COURSE AND, THEREFORE, MY EXPECTATIONS FOR YOU ARE:

(1) Learn to identify and solve important problems in a creative fashion, while learning how to think like a molecular biologist:

How do molecular biologists identify questions that are important to answer?
How does one develop a testable hypothesis?
How does one design experiments to test one's hypothesis?
If new tools are necessary to answer a question, how does one develop them?
How does one properly and critically evaluate results?

(2) I want to help you to learn more about: What is creativity? How can one become creative?

(3) I want to help you to learn how to read scientific papers more critically and more efficiently.

(4) I want to help you to improve your science communications skills: to speak and write more critically, persuasively, succinctly, and creatively.

(5) More specifically, I want to help you learn the basic principles of the molecular mechanisms underpinning the structure and expression of genomes, and the tools to study these. How do these steps work and how does one figure out how the molecules participating in these steps function together?
SYLLABUS

I. Structure and expression of eukaryotic genomes...what are the emerging principles of gene expression?

- What exactly are all of the steps required for a gene to be expressed?
- What are the 3-4 “BIGGEST QUESTIONS” in molecular biology?
- What are the “model” eukaryotic organisms used by biologists, and why are they model organisms?
- B.C. ...Before cloning: Different populations of genes are expressed in different cell types at different times. How was this initially discovered, and why was it of interest?

II. B.S. Before sequencing: Recombinant DNA techniques: how to clone your favorite gene (Weaver, Chapters 4&5) What motivated the development of cloning tools? From where did these tools come?

- Construction and use of recombinant DNA molecules: cDNA and genomic DNA clones

III. A.S. After sequencing: Genomics, Transcriptomics, Proteomics and other ‘omics – High throughput analysis once your favorite genome is sequenced (Weaver, Ch. 24). What motivated development of these approaches? How were they developed?

- Genomics: What have we learned from sequencing genomes of model organisms?
- The transcriptome: assaying amounts of all transcripts under different conditions using gene chips, microarrays, and now, high throughput RNA sequencing
- Proteomics: study of all proteins present under different conditions, using mass spectrometry
- Functional genomics and proteomics: (1) investigating gene function by constructing gene knockouts, knockdowns (RNAi) and knock-ins; CRISPR technology; (2) constructing conditionally expressed genes or mutant alleles by PCR, transformation and homologous recombination; (3) epitope-tagging, purification of multi-molecular complexes, and identification of constituents by mass spectrometry.
- How does gene expression vary among individual cells in a sample, e.g., one organ or tissue ...the emergence of single cell ‘omics
• What does the future hold?

➢ FIRST HOUR EXAM (~Friday, September 27)

IV. Translation of mRNA (Weaver, Chs. 17-19)

• Role of 5' caps and 3' poly(A): revisiting an old hypothesis
• Cis-regulation of translation by upstream open reading frames or IRES elements
• Trans-acting factors
• Specialized ribosomes
• Localized translation

V. Transcription of eukaryotic genes (Weaver, Chapters 10-13)

• Overview of transcription
• Temporal and spatial-specific gene expression
• Cis-acting elements (Weaver, Chapter 10)
  o Promoters, enhancers and silencers
  o Defining the eukaryotic promoter by mutating it in vitro, introducing it into living cells, and assaying expression in vivo
• Trans-acting factors (Weaver, Chs. 10-12)
  o Basal transcriptional machinery: identification by genetic and biochemical methods
  o Regulatory proteins: enhancer binding proteins and adaptor proteins
  o DNA-protein as well as protein-protein interactions
• Role of chromatin/chromosome structure in transcription: histones, nucleosomes, structural and posttranslational modification of nucleosomes, and the histone code (Weaver, Ch. 13)
• Unraveling the three-dimensional genome ...how is the genome organized in 3D space within the nucleus, and how does this affect gene expression?
• Regulation of transcription initiation
• Polymerase pausing and regulation of transcription elongation

SHOW AND TELL DAY: THE ART PROJECT (~Wednesday, October 16)

MID SEMESTER BREAK (Friday, October 18)

COMMUNITY ENGAGEMENT DAY NO CLASS FRIDAY, OCTOBER 25

➢ SECOND HOUR EXAM (~Friday, November 1)
VI. Pre-mRNA processing (Weaver, Chs. 14 and 15)

- Comparing and contrasting the structure of RNA vs. DNA; High throughput assays of RNA folding
- There are several different classes of processed RNAs and RNA processing reactions.
- Mechanism of pre-messenger RNA splicing in vivo and in vitro: essential cis-elements
- Trans-acting factors: Splicing factors and the splicing complexes (spliceosome)
- What have we learned from recent near-atomic resolution cryo-EM structures of splicing complexes?
- Regulation of splicing and alternative splicing -- another means to generate diversity in gene expression. Example: Regulation of sex determination in Drosophila
- Coupling of transcription and splicing - one large machine?

VII. mRNA localization: export of RNA from the nucleus to the cytoplasm and intracellular transport

Methods to detect intracellular localization of RNA molecules
Molecular machines that enable intracellular transport of RNA
Coupling of nuclear export of mRNA with its transcription and splicing

VIII. Storage and turnover of nuclear and cytoplasmic RNA
- Nobodies, P-bodies, Cajal bodies, stress granules, and others

RESEARCH PROPOSAL OUTLINE DUE (Friday, November 22)

- THIRD HOUR EXAM (~Friday, December 6)
- RESEARCH PROPOSAL DUE (Friday, December 13)
Your grade in this course will be determined by the following:

Oral and written summaries of journal articles: 20 points each 200 points

First hour exam: ~Friday, September 27 100 points

Art Project ~Wednesday, October 16 50 points

Second hour exam ~Friday, November 1 100 points

Research proposal outline ~Friday, November 22 50 points

Third hour exam ~Friday, December 6 100 points

Research proposal ~Friday, December 13 200 points 800 points

Research articles from scientific journals are the focus of this course. These are required reading, will be available on the class Canvas online, and each will be discussed in subsequent lectures. The exams will be based on the lectures, including information discussed from the articles. The textbook is Molecular Biology, Fifth Edition, (2011) by Robert Weaver, can be made available in the bookstore. Also, you can borrow a copy from me for a day.
Approximately once per week during the course, you will be asked to write a half page summary of one of the journal articles uploaded to Canvas. Ordinarily, the summary will be due in class at least one week after each assignment is announced. Generally, I will discuss this article in class soon after the summary is due. Thus, you will have read, thought, and written about the paper before we discuss it in class. However, during the first few weeks of the course, I will try to discuss the article before your summary is due to help you become acquainted with reading and understanding journal articles.

The summary should be approximately one-half page (some may need to be longer, although a page at the maximum), single-spaced, typed or handwritten legibly. In your own words (not those from the article) briefly describe:

1. What previously unanswered questions or issues were addressed by the authors' research described in the paper, i.e., what was the goal of the work described in the paper?
2. What experiments were done, i.e., how was the work accomplished, and importantly, why were THESE experiments chosen? i.e., what was their approach?
3. What did they observe in their experiments?
4. What was concluded by the authors, and how well supported were their conclusions?
5. What was the most interesting or exciting finding and why?

Later in the course I will also ask you to describe what additional questions should be addressed next and by what means (i.e. what OTHER experiments SHOULD be done).

6. Describe briefly any sections that were confusing or unclear to you and why in a few sentences, if any. This latter point is important in that it will alert me to clarify in class issues that are confusing to you.

To break up the tedium of the semester, and to develop alternative brain cells for conveying concepts of molecular biology, we will have a “SHOW AND TELL” day. Here, you will present to the class your art project, to illustrate a pathway in gene expression, for example changes in RNA-RNA interactions during spliceosome assembly and splicing, or how molecules are transported into and out of the nucleus through nuclear pores.

If you cannot bring your homework to class, please do not email it to me. Bring me a hard copy as soon thereafter as possible.
MOLECULAR BIOLOGY OF EUKARYOTES 2019

RESEARCH PROPOSAL

Outline due Friday November 22, 2019

Research proposal 7-typed pages, double-spaced -- due Friday, December 13, 2019

The term paper is the most critical "test" in this course. It is designed to assess your ability by the end of this course to critically evaluate the literature, to provide original suggestions for appropriate questions to be asked in eukaryotic molecular biology at this time, and to design appropriate experiments to try to answer these questions.

1. **Specific Aims** (approximately 1 page)

   State concisely and realistically what the research described in your proposal is intended to accomplish and/or what hypotheses are to be tested. Write one or two general paragraphs introducing the subject and its relevance to biology, and then simply list three or four specific questions to be addressed. This section is critical because it provides a framework for the reader to appreciate your proposal and the "connections" between sections of the proposal. Please make certain that you choose a problem of reasonable complexity, e.g. something comparable to a Ph.D. thesis project, performed by one human being.

2. **Significance** (approximately 2-3 pages)

   Briefly sketch the background to your proposal, critically evaluate existing knowledge, and specifically identify the gaps that the project is intended to fill. i.e., summarize the general knowledge of the field, and identify where your questions "fit in". This is an important section in that you display your knowledge and understanding of the field and its shortcomings at present. **What are the unanswered questions?**

3. **Experimental Design and Methods** (approximately 3-4 pages)

   Discuss in detail the experimental design and the procedures to be used to accomplish the specific aims of the project. Include control experiments, and potential difficulties and limitations of the proposed procedures and alternative approaches to achieve the aims. This section is where you will display the extent to which you have benefited from this course to learn contemporary methods to experimentally test hypotheses in molecular biology. Spend most of your intellectual energy here!

   Note that this proposal is analogous to the research proposal at the end of the first year of our graduate program. I will provide you with examples of such term papers from previous years.
MOLECULAR BIOLOGY OF EUKARYOTES 2019

READING A SCIENTIFIC PAPER

We will rely almost entirely on papers from the literature to provide us with both classical and newly developing approaches and ideas in molecular biology. It is essential to your development as a professional scientist that you learn how to read research papers critically and efficiently. We will go over the first few papers in greater detail in class to help you learn how to read journal articles.

A typical outline of a research paper is summarized below:

A. **Title and Abstract:** (200 words) The title and abstract are meant to attract your attention and summarize what the authors consider the most important points of the paper. Little if any background information is provided here, mostly new results.

B. **Introduction:** This is where the authors (try to) provide you with sufficient background information to understand where their work fits into the "big picture". This should not be a comprehensive review -- only salient points that allow you to get directly to the issue at hand. However, some writers often use (abuse) this space to write a comprehensive review article.

C. **Results:** This contains a description of experiments done - how and what was found, usually with some, but minimal interpretations of the significance of the results. It is here that Tables and Figures of the data are presented and explained. Look at the figures. An experienced reader of a well-written paper can determine and assess the results merely from the Figures. A key element to your success in reading papers is the ability to evaluate the credibility of the results including the possible significance of aberrant or unexpected results and sources of revolutionary changes in the field.

D. **Discussion:** Contains interpretations of what the authors find. Are strong conclusions justified? Are alternative interpretations considered? Are shortcomings or limitations of methods and approaches explained? Usually the authors' results are related to others' results -- do they agree or disagree and why?

E. **Methods:** Contains recipes and protocols for what they did. Are they appropriate and adequate? Could you repeat the work with the information provided? Methods may follow Introduction or Discussion, depending on the journal.
YOUR WELL BEING

Take care of yourself. Do your best to maintain a healthy lifestyle this semester by eating well, exercising, avoiding drugs and alcohol, getting enough sleep and taking some time to relax. This will help you achieve your goals and cope with stress.

All of us benefit from support during times of struggle. There are many helpful resources available on campus and an important part of the college experience is learning how to ask for help. Asking for support sooner rather than later is almost always helpful.

If you or anyone you know experiences any academic stress, difficult life events, or feelings like anxiety or depression, we strongly encourage you to seek support. Counseling and Psychological Services (CaPS) is here to help: call 412-268-2922 and visit their website at http://www.cmu.edu/counseling/. Consider reaching out to a friend, faculty or family member you trust for help getting connected to the support that can help.

If at any time this course creates undue stress or confusion for you, please feel free to come see me. We can try to organize your efforts to learn what you need most efficiently and with minimal stress. I am always happy to work with you individually to try to optimize your experience and your learning.

ACCOMODATIONS TO STUDENTS WITH DISABILITIES

If you have a disability, and are registered with the Office of Disability Resources, I encourage you to use their online system to notify me of your accommodations and discuss your needs with me as early as possible. I will work with you to ensure that accommodations are provided as appropriate. If you suspect that you may have a disability and would benefit from accommodations but are not yet registered with the Office of Disability Resources, I encourage you to contact them at access@andrew.cmu.edu.