**MOLECULAR BIOLOGY OF EUKARYOTES**

**2024**

Lectures for 03-442, 03-642, and 03-742 sections: MWF 9:00-9:50a.m. Wean Hall 5403

03-742 Advanced Discussion section: Mon 4:00-5:30p.m. CNAST Room Mellon Institute 624

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***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:
   1. How do molecular biologists identify questions that are *important* to answer?
   2. How can you figure out the *most impactful* directions in which your field is going? In other words, where will the waves be breaking (in the future)? Which of these waves should you catch, and how? What interests you the most? What might you be best equipped to investigate?
   3. To do so, it helps to study previous wave patterns. What can we learn from studying work previously published?

* 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?

1. I also want to help you to learn more about *creativity*. What does this mean in terms of predicting the behavior of biomolecules, and the structures and pathways in which they function? How can you become creative?
2. I want to help you to learn how to read scientific papers more critically and more efficiently.
3. I want to help you to improve your science communications skills: to speak and write more critically, persuasively, succinctly, and creatively.
4. 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?
   1. classic and emerging techniques to study genome organization and expression...from cloning genes to 'omics
   2. transcription of RNA from DNA
   3. removal of introns from pre-mRNA by splicing
   4. RNA transport and localization within cells
   5. translation of mRNA to synthesize proteins
   6. quality control in cells: turnover of RNA and proteins

**HOW TO SUCCEED IN THIS COURSE**

**As implied above, our goal is not to emphasize memorizing facts; Siri has many of the answers that we need. Rather, we want to help you learn how to think. Therefore, please pay attention to the general principles that drive molecular biology and the ways of thinking that lead one to discover these principles.**

**STRONG RECOMMENDATION: TODAY, RIGHT NOW, take a quick look**

**on the Canvas site at the exams from previous years. See examples of what we expect you to learn.**

1. **Class attendance**: **It is critical to try your very best to attend every class.** During the 44 years that I have taught this course, over and over and over, by far, the best learning occurs and the best grades are earned by those who attend class. Most of what we will teach you in this course is not found in succinct form in textbooks. Homework often will be based on published review articles and research papers. Yet the most important take- home messages will be emphasized, and when necessary, clarified, in lectures in class.
2. **Class participation: Questions during class are highly encouraged.** If lectures or discussions are not clear, then it is critical for me to stop and try to do a better job to explain the material to you. Do not be shy about asking questions. If the material is not clear to you, or if there is another aspect of the material about which you are curious, chances are very high that all of us will learn from your questions!!
3. **More questions after class:** I will stay after each class to answer questions, until everyone has been helped to their satisfaction.
4. **Collaboration:** Unless otherwise specified, all work should be your own…homework, quizzes, exams, term paper. However, I might ask you to break into groups in class to brainstorm on a problem, and report back to the rest of us. Science is collaborative, but alas! individual grades must be assigned. One exception: the art project can be done in teams of 2-4 people.
5. **Homework reading assignments:** there will be ~ one HW assignment each week requiring that you read a paper and answer questions about it. Please note that there is a section below advising you how most efficiently and effectively to read scientific research and review articles. Often you will be asked to read a paper before it is discussed in the lecture, to better prepare you for the lecture. Do not get bogged down in details…focus on the primary take-homes. These take-homes will be made clear in the lectures.
6. **Grading:** see section below on page 7.

# SYLLABUS

1. 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 five of the “BIGGEST QUESTIONS” in molecular biology?
   * What are four “general trends “ in patterns of gene expression to keep in mind?
   * What are the “model” eukaryotic organisms used by biologists, and *why* are they model organisms?
   * B.C. …Before cloning: Different and large populations of genes are expressed in different cell types at different times. WHY was this phenomenon interesting and important to investigate? How was this *temporal and spatial-specific* gene expression initially discovered? How was gene expression investigated before cloned genes were available, i.e., without nucleic acid probes for specific mRNAs?
2. 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? **IMPORTANT!!!!!**
3. 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
* HOW MIGHT THESE SINGLE CELL DATA BE VERY DIFFERENT FROM BULK DATA? Is every cell in a tissue identical? What does “stochastic” gene expression mean? Does this make it seem like gene expression is “NOT carefully programmed”? What is really going on at the molecular level?
* Spatial ‘omics

**SHOW AND TELL DAY 1: ART PROJECT #1 (~Friday, September 27)**

* **FIRST HOUR EXAM (~Wednesday, October 2)**

1. 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
   * Colliding ribosomes

* **MID SEMESTER BREAK (Monday, October 14- Friday, October 18)**
* **SHOW AND TELL DAY 2: ART PROJECT #2 (~Wednesday, October 30)**
* **SECOND HOUR EXAM (~Friday, November 1)**

1. Transcription of eukaryotic genes (Weaver, Chapters 10-13)
   * Overview of transcription
   * Temporal and spatial-specific gene expression
   * *Cis*-acting elements (Weaver, Chapter 10)
   * Promoters, enhancers and silencers
   * 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)
   * Basal transcriptional machinery: identification by genetic and biochemical methods
   * Regulatory proteins: enhancer binding proteins and adaptor proteins
   * 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
2. Pre-mRNA splicing (Weaver, Chapters 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.
   * Coupling of transcription and splicing - one large machine?
3. 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
4. Storage and turnover of nuclear and cytoplasmic RNA
   * Nobodies, P-bodies, Cajal bodies, stress granules, and others

**SHOW AND TELL DAY 3: ART PROJECT #3 (~Wednesday December 4)**

* **THIRD HOUR EXAM (~Friday, December 6)**
* **RESEARCH PROPOSAL (TERM PAPER)**
  + **Outline due Friday, November 22**
  + **Final proposal due Friday, December 13**

**MOLECULAR BIOLOGY OF EUKARYOTES 2024 – GRADING**

**Your grade in this course** will be determined by the following:

Oral and written summaries of journal articles (usually40 points each) 400 points

Art Project 1\* **~Friday, September 27** 50points\*

First hour exam **~ Wednesday, October 2** 100 points

Art Project 2\* **~Wednesday, October 30** 50points\*

Second hour exam **~Friday, November 1** 100 points

Research proposal outline **~ Friday, November 22** 50 points

Art Project 3\* **~Wednesday, December 4** 50 points\*

Third hour exam **~Friday, December 6** 100 points

Research proposal **~ Friday, December 13** 200 points **1000** points

\*Note: each person will do only one of the three art projects

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) by Robert Weaver, can be made available in the bookstore. Also, you can borrow a copy from me for a day.

**MOLECULAR BIOLOGY OF EUKARYOTES 2024**

**SUMMARIES OF JOURNAL ARTICLES**

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. You will hand in your HW on the course Canvas site.

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).

1. 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.

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**RESEARCH PROPOSAL**

**Outline due Friday, November 22**

**Research proposal 7-typed pages, double-spaced -- due Friday, December 13, 2024**

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.

1. **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?**

1. **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.

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**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:

1. **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.** Often, little if any background information is provided here, mostly new results.
2. **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.
3. **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.
4. **Discussion:** Contains interpretations of what the authors discovered.

* 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?

1. **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**

This semester is unlike any other. We are all emerging from a period of stress and uncertainty into normalcy. This can itself be stressful. 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. Reach out to your support system if you need to do so, but feel free to contact me (jw17@andrew.cmu.edu). We can all benefit from support in times of stress, and this semester is no exception.

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/.](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 very 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 have an accommodations letter from the Disability Resources office, I encourage you to discuss your accommodations and needs with me as early in the semester 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](mailto:access@andrew.cmu.edu).