Syllabus and Instructions for Carey & Berk Gene Regulation M254A – Biochemistry/Genomics Section (2020)

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Office Hours: By appointment.

Focus: Covers papers on gene regulation mechanisms with an emphasis on the transcriptional machinery and chromatin structure. The course combines biochemistry of the RNA Pol II machinery and its major co-activators, chromatin remodeling, and genome wide approaches that employ next generation sequencing, and single molecule imaging, to explore mechanisms for how gene regulation is achieved. This information is helpful when rotating in epigenomics labs utilizing RNA-Seq, ChIP-Seq and other Next Gen Sequencing analyses in their research.

Faculty/Student Tues, Fri Online 10 AM-12 PM
Student only Mon, Thurs Online 1:30-3:30 PM

Final Exam: November 1, online 10 AM-12 PM

Course Web Site: https://ccle.ucla.edu/course/view/20F-MOLBIO254A-3
We will try to use the website for announcements and to provide additional class materials. But download the papers yourselves from the journal websites.

If you are using an off campus computer you will need to set up the BOL (Bruin On-Line) proxy server - follow instructions on the BOL site.

Recent papers from Elsevier (Cell, Molecular Cell) must be ordered from the library well ahead of your need for them due to the dispute between UC and the publisher. Go to the following Link https://ucelinks.cdlib.org/sfx_local/cgi/core/citation-linker.cgi (see full instructions below).

Learning Objectives: Students will learn to 1. analyze the content of primary research articles on regulation of transcription by RNA Pol II including the research problem, hypothesis (if one is posed), methods and results; 2. improve skills for researching background material on a topic of interest; 3. develop oral presentation skills for scientific communication; 4. critique experimental design/procedure by identifying limitations, challenges, and experimental alternatives. At the end of course, the students should have a strong grasp of the mechanics of Pol II initiation/elongation in vitro, and how it is regulated in vivo during development and differentiation. They should understand major genomics techniques. and how higher order chromatin organization impacts gene regulation. Students will learn how to apply rigor to scientific questions, and the importance of reproducibility in scientific research.
Rigor and Reproducibility (from NIH): Two of the cornerstones of science advancement are rigor in designing and performing scientific research and the ability to reproduce biomedical research findings. The application of rigor ensures robust and unbiased experimental design, methodology, analysis, interpretation, and reporting of results. When a result can be reproduced by multiple scientists, it validates the original results and readiness to progress to the next phase of research." Scientific rigor is the strict application of the scientific method to ensure unbiased and well-controlled experimental design, methodology, analysis, interpretation and reporting of results.

Grading Rubric: Letter Grade. 50% based on in class presentations and participation; 50% based on grade from a written final exam. In class grading, 10 points max per session graded in real time by instructors. Presenters: 2 points (max) each for understanding of specific problem, understanding of data, clarity/quality of presentation (according to attached guidelines), critical analysis of data, and ability to expand beyond figure and tie into previous and following figures. Leaders: 2.5 points each for organization of presentation, introduction of subject, proper coordination of figures/speakers, final wrap-up.

Class Meeting: Guidelines for presentation are attached at the end. Read these instructions carefully and follow them. Each student in the class will present a figure when prompted by the leader during the course of the session. At the end of the class, there will be a discussion section where students break up into groups of 3 to discuss their answers to the discussion questions. They will present their answer to the class as a group using Zoom.

UCLA policies that support tolerance: All students are asked to treat one another with kindness and respect. Harassment and discrimination based on: race, ethnicity, ancestry, color; sex, gender, gender identity, gender expression, sexual orientation; national origin, citizenship status; religion; disability, pregnancy, medical condition, genetic predisposition; domestic partnership/marital status; age; or veteran status may violate UCLA regulations and lead to serious consequences. Information on how to obtain redress or counseling if you are subjected to such harassment or discrimination can be found at https://equity.ucla.edu/report-an-incident/.

UCLA is bound by Title IX, a federal law that applies to any education program receiving federal assistance. Title IX prohibits gender discrimination, including sexual harassment, domestic and dating violence, sexual assault, and stalking. Students who have experienced sexual harassment or sexual violence can receive confidential support and advocacy at the CARE Advocacy Office for Sexual and Gender-Based Violence, 1st Floor Wooden Center West, CAREadvocate@caps.ucla.edu, (310) 206-2465. You can also report sexual violence or sexual harassment directly to the University’s Title IX Coordinator, 2241 Murphy Hall, titleix@conet.ucla.edu, (310) 206-3417.

Student Resources:
It is normal for students to feel stress about assignments, exams and life in general and there are many resources on campus for students in need of various types of counseling. These include:

UCLA Behavioral Wellness Center For Graduate Students
A student mental health center primarily for GPB graduate students, medical students, and medical residents.

**The Bruin Resource Center** (http://www.brc.ucla.edu/).

**Support for Undocumented Students:** UCLA provides many resources to support undocumented students and links to many of them can be found on the following web site: https://equity.ucla.edu/know/immigration/.

https://medschool.ucla.edu/bwc

**Class Sessions:**

**Session 1. Oct. 6. Introduction to Eukaryotic Transcription. Lecture and Discussion. Lecture will be loaded onto CCLE.**

**Session 2. Oct. 9. The Pol II Pre-initiation Complex (PIC) and transcription initiation (Carey).**

The PIC comprises the general transcription factors (GTFs), Pol II. Understanding the role of the GTFs in initiation has been a goal of the field for over 25 years. This paper reports a high-resolution structure of the PIC and explains the role of various GTFs, Mediator and their interactions with each other and Pol II.


**Useful general background references:**


There should be two leaders: One should describe background on promoters, GTFs, and Pol II. The other leader should describe Cryo-EM using Figures from the Nogales Reviews. Maybe 6 or 7 slides max. No need to show all figures from reviews. Each student gets two slides max for their figure of the paper and 5 minutes max to present it. Follow the instructions provided below for presenting figures and make sure to touch on all 5 aspects mentioned in the grading Rubric above.

Although the paper focuses mainly on Mediator and TFIIH, you should discuss the core PIC and roles of GTFs, some of which is covered in the second Nogales review. The main question that each student should consider during their figure presentation is "what is the specific role of each factor in PIC assembly and function?" For example if you are covering TFIIIE, don't simply describe where it binds but also what its purpose is in the PIC.
Roundtable questions:
1. What would be necessary to allow PICs to form on chromatin?
2. What step is regulated when considering a gene that is activated by activators bound at promoters and enhancers?
3. What step is regulated by heat shock transcription factor and HIV Tat?


PICs can be studied in vivo using genomewide approaches. Because the PIC is a major target of gene regulation, an understanding of how it assembles in cells is important.


The leader should present a brief introduction to the PIC and the techniques of ChIP, ChIP-Exo and next generation sequencing: (https://www.youtube.com/watch?v=fCd6B5HRaZ8). The students should present the figures in the paper with an emphasis on explaining how the various subunits of the PIC are localized and quantitated. Be able to clearly present the axes on the graphs, heatmap quantitation and so on. Be very specific about what is being shown and explain it clearly. The key questions to focus on include: how similar are PICs in vitro and in vivo; what are the limitations of using ChIP to study PICs in vivo in terms of PIC composition; the Nogales review suggest that PICs assemble in a stepwise manner via protein-protein and protein-DNA interactions so how might you analyze the protein-protein interactions in the PIC in vivo (note there are many ways of rapidly removing a GTF in vivo using anchor away or degrons); where is mediator (has it been ChIPed and what is the current view)?

Group Questions:
Group 1: In yeast, promoters are controlled by UASs. What are UASs and what do they bind? Give an example of a well characterized UAS.
Group 2: How would one study whether PIC assembly is regulated by an inducible activator like GAL4 or HSF in vivo?
Group 3: Because all of the GTFs and Pol II are encoded by essential genes, what in vivo techniques could one use to study how depletion of a GTF affects PIC assembly in vivo? Note there are many techniques for transiently depleting factors from yeast: inducible degrons, anchor away and others. Be aware of these and use them in your answer.

Session 4. Oct. 16. Bacterial Adaptive immunity and Genome Engineering in Eukaryotes by the CRISPR/CAS system. (Berk)
The CRSPR-Cas system has enabled rapid revolutionary advances in genome editing that can be used to study genes involved in human disease, understand how DNA elements like enhancers and promoters function and possibly be used to create genetically modified humans to correct both somatic and germline defects.


The leader should present a review of the key elements of the CRSPR-CAS system using recent reviews. What is the biology and what is known of the structure, i.e., key features such as the protein domains, catalytic residues, nucleic acid strands. The leader should require that students presenting various figures from main paper address how the PAM sequence is recognized and the structural basis for target DNA switching from duplex to a sgRNA-DNA hybrid at the PAM boundary.

**Group Questions:**
1. How is CRSPR being used to create enhancer and promoter deletions?
2. How is CRSPR being used to target proteins to genetic loci like promoters/enhancers to activate genes?
3. How is CRSPR being used to repress transcription of specific genes or IncRNAs?

**Session 5. Oct. 20. Chromatin Remodeling (Carey)**

In order for PICs to be assembled, chromatin remodeling enzymes have to remove nucleosomes around promoters. The mechanisms controlling this removal are not well known. This paper by Shore and colleagues examines the mechanism using some new approaches.


The leader should present an overview of chromatin remodeling and transcription using figures from the review. Use the model provided by the authors to set up the various figures. The students should present the figures in paper. As you go along address mechanistically the following questions: how RSC is recruited to the promoter; how RSC works to move or remove nucleosomes; what are fuzzy nucleosomes; what are histone
variants; what are pioneer factors and why are they important in higher eukaryotes. There is a nice commentary by Steve Henikoff accompanying the paper.

**Group Questions:**
1. What is known about the role of activators in recruitment of remodeling enzymes?
2. What is known about the role of histone modifications in recruitment of remodeling enzymes?
3. Activators interact with many different co-activators, chromatin modifying factors and so on. How would you determine whether these interactions were an ordered progression of events or occurred simultaneously?

**Session 6. Oct. 23. Evidence against regular higher order chromatin structure beyond the nucleosome. (Berk)**


**Session 7. Oct. 27. Super-resolution microscopy of chromatin (Berk)**


**Session 8. Oct. 30. Phase-Separated Nuclear Condensates are Critical to Enhancer Function (Berk)**

*Main papers:*


Dr. Berk will provide instructions, questions and additional information before session.

**Session 9. Nov 3. Transcriptional Bursting (Berk)**

Dr. Berk will provide instructions, questions and additional information before session.

Exam. Nov. 6.
Guidelines for Student Presentations

This section outlines the style, organization and general considerations for a typical 1 hour or so presentation. It will have to be tailored to both the paper and each presenter’s style. Remember, since each student will present part of the paper, it is important to work out transitions and such in the Student meeting. The overall presentation should be a coherent product created by the concerted effort of all students, and not a series of unconnected mini-talks.

I. Introduction (5-10 min):

1. The leader should begin presentations by very briefly stating the main problem(s) or hypotheses being addressed (2-5 min). Then introduce the authors, the paper and point out why the paper is important (e.g., "One of the unanswered questions in the field of eukaryotic transcription is what is the structure of the Pol II initiation complex and what specific roles do the GTFs play during initiation by Pol II? This paper by the famous scientists Dr. Smith et al., entitled "The answer to FAQs about the PIC" is exciting/great science and describes a structure of the initiating PIC that reveals the functions of the GTFs and how the TSS is melted to allow Pol II initiation...."). This need only be a brief overview. You will cover the rationale and background in more detail below.

2. The leader should give a short review of the necessary background information (5-10 min or so). The leader should read a review article or a previous paper from the authors and use figures from the review in the background to provide an intellectual framework for the problem or the area being covered in the paper. Never present some random web figure, wikipedia figure or undergrad textbook figure as background. Use recent review articles. Don't make the introduction overly broad or you'll lose focus. Usually three or four schematic illustrations of the process being discussed and a description of any key proteins/systems covered in the paper will provide the necessary framework. These diagrams should be referred to periodically throughout the talk to organize the presentation as it becomes more sophisticated and begins to focus on data. An organizational slide outlining the talk is recommended and should be inserted by the leader at key transition points with a few conclusions to date (i.e., every couple of figures have a brief wrap-up). The transitions can be done by the leader or the figure presenter.

3. At the end of the Introduction, restate the specific problem being covered in a paper and how this paper addresses it. Present the main conclusions of paper up front. This helps the audience to understand the logic behind the experiments the group will be describing next (1 min) (e.g., "This paper shows that the translocation of DNA by TFIIH is a key step in promoter opening and that the transition is accompanied by re-arrangement of the GTFs...etc."). If there is a concluding model also show that up front. This strategy helps the class to arrange a logical progression of the data.

4. Briefly outline the experimental design for the paper, (e.g., "The authors used Cryo-EM, a melted DNA template along with superimposed crystal structures to understand...." (2-3 min). No need to dwell on this too long as the other students will have to describe the specifics when showing the figures.
Presenting Data/Figure Slides

5. Each student except the leader must then explain, using key figures from the paper, how the data support the conclusions and fit into the model. Do not try to cover every single figure or nuance in the paper or the seminar will drag out. But be careful not to make your treatment of the paper superficial. The correct balance is essential! Remember to critique the data, i.e., control experiments that should have been done or alternate explanations for the results. Use clearly labeled Powerpoint slides when summarizing the key figures. Each student except the leader should present some data. If the figures haven’t been properly labeled by the authors then you do it, i.e., adding a circle or arrows to the area you will concentrate on helps focus the audience. Also, when showing figures from a paper never use the pdf figures -- they are low resolution! Would you give a talk at a major meeting and show low res figures of your research? Please say no. Ok then, go directly to the journal/paper website and copy the HTML high resolution JPEG files onto the slide. Often this entails resizing the figure, cropping it so as to show only the relevant panels and then labeling it with a title and any comments you wish to make. The only caveat is that we do not currently have web access to Elsevier articles and you will need to use pdf figures for these. Make sure they are as high a resolution as possible.

6. When each student describes an individual figure, the student should first state the reason for this particular experiment or structure analysis and its conclusion (e.g., "The authors wished to determine the structure of TFIIB in the PIC while Pol II was initiating transcription. They show that the key interactions are with Pol II and the consequences of those interactions are..."). Then briefly mention how this particular experiment was done and describe what you are showing (e.g., "The Cryo-EM was solved using this... methodology. This is a rendering of the TFIIB-Pol II interface...") and be sure it is well labeled, even if the authors failed to do so. Point out landmarks (e.g., "The structure reveals this specific interaction between this domain of TFIIB and this feature of Pol II is ... etc."). Organize panels thematically (e.g., "The panel shows this aspect of the interaction while these panels show a different aspect... etc."). Remember that it is not necessary to explain every aspect or panel. Pick out the crucial ones and always contrast a result with the control when showing blots or biochemical figures, e.g., "Figure 1A shows TFIIB in the closed complex and Figure 1B shows how this changes after TFIIB translocates the DNA." Or "Lane 3 shows that transcription is increased when adding the activator as compared to the control in Lane 1." Sometimes it is necessary to describe in more detail how an experiment was performed; be prepared to explain the methodology if the audience or instructor feels it has not been explained well enough. Finally, end the description of each figure with a brief conclusion and transition to the next slide or next presenter. The transition should never be "Next the authors wanted to show..." Instead, provide a logical segue to the next slide (e.g., "The authors showed TFIIE interacts across the cleft with the Rpb1 and 2 subunits of Pol II in the closed complex. This next slide addresses what happens during promoter melting"). The group should use the thematic repeating slide generated by the leader to organize different sections of the presentation.

III. Summary (5 min):

7. After the group has covered the figures, the leader should finish with a summary of the
conclusions and a summary figure (usually a schematic), describe how the data support (or not) the original hypotheses, and present any general comments you might have on the paper. Don't be shy! It is very important to have an opinion. Be critical if you feel the data do not support the conclusions or if you feel there are caveats. Finally, the leader should state what the group believes the future problems or directions in the field should be (maybe even add these into a slide). You may think you've said something before but restating the rationale and conclusions at different stages of the talk often helps the audience follow the paper and encourages critical discussion.

IV. Ending. The leader should conclude the presentation with wrap-up summary followed by a simple "Thank you for your attention" or "Thank you. Are there any questions?" This tidy little ending will eliminate the awkward pause that sometimes occurs when the audience doesn't realize the speaker has finished.

V. Group Questions: Then transition to the group roundtable questions. Each member of each group should present part of the roundtable answer. Keep it tight.

Points to enhance the presentations:

• All presentations should be in Powerpoint format and displayed from an LCD projector attached to your computer or iPad. Bring the correct computer-LCD projector adapters with you. Use the online JPEG versions of the figures. Do not copy and paste figs from the pdf!
• It is essential to **rehearse your blurbs extensively**. The presentation should only be 1 to 1.5 hour max so use the student sessions to discuss, develop and practice your descriptions of figures. We need the extra time for discussion and roundtable. Verbalizing a blurb helps one to work out the bugs and avoid hidden tongue twisters or logistical issues. Also, rehearsing in front of the class allows them to inform you as to what's clear and good or what's unclear and well, bad.
• Don’t be defensive. Acknowledge a good idea, a piece of advice or so on.
• Speak to the audience not to the screen. Do not stand directly in front of the screen or walk around nervously. Keep your hands out of your pockets and avoid odd habits like scratching your head, grabbing your hair or saying “um” or “like” all the time. Try to suppress giggles and no profanities or you will be asked to leave. Show respect for your colleagues.
• Use the pointer to illustrate what part of the figure you are referring to as you speak.
• Speak loudly enough for those in the back of the room to hear you. Do not speak in monotone. Add some emotion and cadence. Smile and be happy and enthusiastic. This is graduate school -- the first step in a long but exciting journey!!!!
• Figures must be large and readable from the back of room. A single slide should show one piece of data or at most two. If a figure in a paper has too many panels, break it up and present the pieces separately. This is quite easy to do using the cropping tools in Powerpoint.
• Each data slide should have a title with the conclusion of the slide.
• Don't overdo the text on a slide. Long sentences and paragraphs often distract the audience because rather than listen to you they try to read the whole paragraph. Keep the text elements of a slide very brief and use bullets or numbers to delineate separate points or ideas. Don't simply read the text. Have it be a short summary of what you are
• Avoid superficiality: Know your paper, know how experiments were carried out and know why they are important. Read the Materials and Methods and Supplementary Materials! The instructors will expect that the students understand each aspect of the figures they present and sometimes refer to Supplemental figures when they address something not directly shown in the paper. Minimize these as they are often pdfs.
• You will be interrupted and questioned by the instructors to clarify. Welcome these breaks and, if appropriate, use them as springboards for side discussions. Always be prepared to deviate from your game plan if necessary. Good presentations incite and invite discussion. It is the instructors’ roles to ensure everyone gets the take home point and to expand on ideas based on their experience in the field.
• Leave your phones in your backpacks purses or pockets. Anyone texting in class will be asked to leave.
• No side conversations unrelated to the talk and definitely no mocking your fellow students (or the instructors). You are in a professional environment.
• Good luck and don't be nervous or defensive. We’re all friends and this is meant to be an educational training experience!

Student Meeting Responsibilities

Group Leader(s):
1. Assign figures and any additional tasks (i.e., additional background information) deemed necessary at end of previous class session or by email so that they can be discussed in the student meeting. The leader should introduce the paper, why it is important and then present several slides of background on the paper. Each student has a max of 2 slides to present data figures. No more.
3. Meet briefly with Instructor after the student meeting to go over the paper and powerpoint slides of paper.
4. Leader leads the Student-only meetings on either the Monday or Thursday prior to the Tuesday and Friday Faculty-Student meetings.
5. Leader prepares the final powerpoint presentation of slides from paper including paper title and abstract. Use the JPEG online versions of the figures for powerpoint, not the pdf! Did I say this already? The leader is responsible for other students using JPEGs.
6. Will assign questions and figures to members of the class.
7. Will incorporate any background slides provided by class members for addressing their figures or answering general questions posed in the syllabus.
8. Will begin discussion of class sessions by stating the paper, what it is about, present background and then lead session by picking the assigned students to come up to front of room to present figures.
9. Will conclude formal part of Faculty-Student meeting and initiate post-paper roundtable discussion.

All Students:
1. All students must have read paper, review article(s) and answered questions or prepared figure presentations assigned by the Group leader prior to the student meetings on Mondays and Thursdays. There are two purposes for these meetings. One is to discuss the paper, its importance and to help each other understand. The other is to go
over your figure blurbs, discuss them with other students, and to put the final powerpoint presentation together.
2. Attendance at all sessions is required.
3. Prepare answers to questions or provide background information as directed by group leader.
4. Provide group leader with any necessary slides associated with your question.
5. Participation in class beyond presenting your own slides.

**Second half: Round Table Questions** The students should break into three groups (these groups should stay constant throughout the course) and craft their answers to the discussion questions. In most cases this will require different members of the group doing a little background research on different ideas. In class, each member of each group should discuss one aspect of the group answer. No more than 8 minutes per group.

**Before you leave:** Spend 10 minutes on question/figure assignments for next class. Who does what question? Groups should assign tasks for each member to address group questions. By the next student session everyone should be ready.

**Your other responsibilities:**

- A central aspect of graduate school is self-learning. This includes performing literature searches to answer your questions, using pymol to render, learning techniques on your own and so on. Asking the instructors to explain figures defeats the training objective.
Instructions for Accessing 2019 Elsevier (i.e., Cell, Molecular Cell ...etc.) Articles:

Step 1: Go to the following Link  
https://ucelinks.cdlib.org/sfx_local/cgi/core/citation-linker.cgi  
Fill out required info and click "Continue"

Locate the article from your citation


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Journal title and date are required.

Author

Last name
First name
Initials

Article Title* Features that Drive Formation of Transcriptional Condensates

Journal Title* Molecular Cell

For best results use full title, not abbreviations, i.e., use Journal of popular music studies, not J pop music stud

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Step 4. You must specify a library to "Deliver my request to" although this is for UC record keeping purposes. Make sure your email address is added. I'm not sure if you need to use a UCLA address or not. The article will be emailed to you. Check the box to Send a confirmation email.
Step 5: Check the info and then click "Request it!"
Step 6: You will receive the confirmation below. It should take less than 4 days. Then log out.