Spring 2-1-2018

BIOB 425.01: Advanced Cell & Molecular Biology

Mark L. Grimes
University of Montana - Missoula, Mark.Grimes@mso.umt.edu

Advanced Cell and Molecular Biology/Cell Organization and Mechanisms
BIOB 425/BCH 600 Spring 2018

Instructor: Mark Grimes
243-4977; HS 306
mark.Grimes@mso.umt.edu

TA: Lauren Foltz
HS 301
lauren.foltz@umconnect.umt.edu

Class Meeting Time and Place:
1:00 pm - 1:50 pm MWF Health Science 411

G 600 Cell Organization and Mechanisms. Offered every other spring. Prereq., BCH 480 or consent of instr. Same as BMED 600. Primary literature exploration of the regulation of structure, function, and dynamics of eukaryotic cells. Topics include membranes, cytoskeleton, transcription, translation, signal transduction, cell motility, cell proliferation, and programmed cell death.

BIOB 425 - Adv Cell & Molecular Biology. 3 Credits. Offered spring. Prereq., BIOB 260 and 272; BCH 380 strongly recommended. Cell structure and function, cell cycle, cellular signaling, molecular basis of cancer, regulated cell death, membrane transport, organelle dynamics, cytoskeleton, cell adhesion, and the molecular basis of learning and memory.

Overview

Cell Biology is vast and dense and encompasses biochemistry, biophysics, molecular biology, microscopy, genetics, physiology, bioinformatics, and developmental biology. We will explore the topics listed below by reading reviews and papers from the primary literature. Papers will be chosen, where possible, that are at the interface between two fields, so a large amount of background reading will be necessary to understand the paper and put it in context. The main learning goals are 1) to learn about a number of topics in cell biology; 2) understand a number of laboratory techniques, their purpose, and how to interpret and evaluate data derived from them; and 3) to gain the confidence and skills to attack any scientific paper even if it is in an unfamiliar area; in other words to learn how to learn new things. Alberts, et al., *Molecular Biology of the Cell,* 6h ed. (Garland Science) is recommended as excellent textbook that frames the background as we explore the primary literature. (The 4th ed. is available online at http://www.ncbi.nlm.nih.gov/books/NBK21054/. Lodish et al., *Molecular Cell Biology,* 7th ed. may be substituted if you have already purchased this book. Lecture figures will come from Alberts, et al. and reviews.)

Format

Presentations will be made by the instructor and graduate students. Instructor will typically introduce a topic with a lecture, and a student presentation of a paper with data will follow in the next session. All students will have a one page paper summary assignment due the day of the paper presentation.

The student's presentation should set the stage for the paper being presented with a brief introduction that draws on recent reviews. Keep in mind the following questions when presenting a paper. What is the hypothesis? What is the key question being addressed by the experiments? What are the key experiments that address the question? Do you believe their interpretation, and did they do the proper controls? Many of the methods used to study cell biology and biochemistry are evolving, and far from perfect, so it is important to look with a critical eye at the data, the methods used to obtain the data, and how the data are interpreted.

When tackling any research paper in an unfamiliar area, the best way to start is by reading one or more textbooks (use the index and table of contents) and reviews, looking up unknown concepts mentioned in the paper's introduction (often reviews are cited there too). Then look at the data in the figures. If you don't

understand the methods, look them up. Then read the results and discussion, and decide whether the author's interpretation of the data is the same or different than yours.

Student's presentation papers will be assigned in advance to allow time for preparation. Students are strongly encouraged to ask questions by email, phone, or by stopping by any time. We will schedule a "study jam" session with the TA. We intend a relaxed atmosphere where we all ask questions, and no questions are dumb questions. All students will be required to read all papers and ask questions of the presenter. It is expected that the student presenting the paper will be well informed on the topic, which will require extra work. While errors and misunderstandings are forgivable, we expect you to make an effort to understand the paper being presented, especially if you are the one presenting it!

Mentorship Activity and Presentations

The purpose of having graduate students pair up with undergrads for paper presentations is to enhance communication skills and ensure that undergraduates are not left behind as we explore the scientific literature.

We recognize that students have diverse learning styles, personal styles, ethnicity, gender, nationality, and experience. Science is about learning new things, and communicating these things to other people, regardless of this diversity among people.

On Day 1: Partition into two groups: graduate students and undergraduates. Further partition by the amount of experience working in a research laboratory: line up so that the graduate students with the most laboratory experience are across from the undergraduates with the least research experience. Pick a "lab partner" from the opposite group who is most different from yourself. Take a few minutes to introduce yourselves, then introduce your partner to the class.

Graduate students will be assigned one paper from the list below to present to the rest of the class. Before the student presentation to the class, there are two mandatory meetings. First, the student will prepare a draft presentation and go over it with either the TA (Lauren) or instructor (Mark). Second, do a practice presentation with the undergraduate partner. The presenter should make sure that the undergraduate understands the background, motivation, hypothesis, question, experimental techniques, data from key experiments, and conclusions in the paper. The expectations for the undergraduate are 1) to ask questions - no questions are dumb questions - to make sure the presentation is clear; and 2) identify a laboratory technique that is used in one of the key experiments to present to the class (and has not been discussed already). The presentation to the class will be a team effort, with the graduate student doing the bulk of the talking. The undergraduate will step in to describe the key experimental technique and how to interpret the data just before the key figure is presented, then hand the floor back to the graduate student to finish presenting the paper.

Writing Assignments

Paper summaries. For all students except for the presenters: one for each paper from the primary literature. Due on the day of the paper presentation. One page maximum, double-spaced, 10 point font min. In your own words (do *not* cut and paste from the paper), in a cohesive summary paragraph, write one-two sentence(s) to answer each question:

1. What is the research topic/question and why is it important/interesting? Include at least one statement of hypothesis, as in, "The authors hypothesize that cortactin binds to a protein at the plasma membrane."
2. What are the main results of the paper? (This may take three-four sentences, but should not include details.)
3. What method/approaches are used? When describing experiments, motivate them as a question. For example, "The investigators asked whether cortactin and protein X were co-localized at the plasma membrane using two experimental approaches."
4. What are the conclusions from the experiments, and their significance?
5. What would you like to know more about/understand better? Write down at least one question that you plan to ask the presenter.

Revisions: After the presentation we will hand back your assignment with comments. You will revise the page and resubmit the revised version. **Graduate Students**: Identify a question that emerges from the paper that

represents a next step towards understanding the biological mechanism under study. What technique(s) would you use to answer the question with an experiment? Keep this brief, but think about it carefully.

The typical timing is expected to be: student presentations on Fridays; summaries handed back the next Monday; revisions due Wednesday.

Bioinformatics assignment

1. See http://www.ncbi.nlm.nih.gov/guide/ and http://www.genenames.org/. Pick a gene, preferably a gene from the paper you will be presenting (you may find this exercise useful as background information for your presentation), and find its HUGO gene name, nucleic acid and protein sequence, and domain structure. If there is a structure for the protein, find the structure. Use http://www.hprd.org and http://www.phosphosite.org to find the domain structure and post-translational modifications of the protein.
2. Use PSICQUIC (http://www.ebi.ac.uk/Tools/webservices/psicquic/view/main.xhtml), or GeneMANIA (genemania.org) **and** String (string-db.org) to retrieve interacting partners based only on physical interactions, pathways (knowledge), genetic interactions, and predictions from interactions known to occur in other species or due to the domain structure (*not* text-mining and co-expression). (If there are no known interacting partners, pick another gene.) Use PubMed (https://www.ncbi.nlm.nih.gov/pubmed) to find evidence (published papers) showing that your gene and one of the interacting partners retrieved by GeneMANIA or String actually interact with each other.
3. Report the gene name, synonyms, sequences, structure(s), modifications, network graphs, and one owe two references for an interaction. Optional: use Cytoscape (http://www.cytoscape.org) to graph the networks, otherwise copying and pasting diagrams and graphs from the websites is sufficient. Write a short paragraph summarizing the results and conclusions. Save the report as a pdf file and email it to the instructor.

You may work with your "lab partner" on this assignment, but each student must pick their own gene and submit their own report. Due by the 5th week of class (February 23 or sooner).

Assessment

The course grade will be assigned based on oral presentations, written assignments, and exams. There will be two exams in which interpretation of data will be emphasized. The instructor will identify key figures to focus on from papers that have been presented. Expect broad questions about the motivation for the experiments (*e.g.,* "What signaling pathway is under investigation?"), and specific questions about the data (*e.g.,* "Which lane in Figure 4 shows that cortactin is bound to a plasma membrane protein?"). Written questions from all students (except presenting students) will be required for student presentations, and students will be expected to ask those questions (or other questions) during class.

Point values:
Bioinformatics assignment: 20 points.
Paper review summaries: 10 points each (5 each for first drafts and revisions). One lowest score will be dropped.
Exams: 100 points each.
Presentation: Graduate students 100 points; undergraduates 20 points.

The Provost's Official Fine Print
All students must practice academic honesty. Academic misconduct is subject to an academic penalty by the course instructor and/or a disciplinary sanction by the University.

All students need to be familiar with the Student Conduct Code. The Code is available for review online at http://www.umt.edu/safety/policies/. Treat each other with respect!

Topics

Review and Overview of Cells and Biochemistry (Alberts, Chapters 1 and 2)

Oral questions will be used to assess how well students are prepared for this class.

Review and Overview of Methods (Alberts, Chapters 8 and 9)

Students will be asked in class to explain different methods used to manipulate nucleic acids and proteins, and to visualize cells. These chapters are a good first review or source for methods.

Cell structure, lipids and membrane traffic (Alberts, Chapters 10-13)

Reviews

Schöneberg, J., Lee, I.-H., Iwasa, J. H., & Hurley, J. H. (2017). Reverse-topology membrane scission by the ESCRT proteins. Nature Reviews Molecular Cell Biology, 18(1), 5–17. http://doi.org/10.1038/nrm.2016.121

van Niel, G., D'Angelo, G., & Raposo, G. (2018). Shedding light on the cell biology of extracellular vesicles. Nature Reviews Molecular Cell Biology, 1–16. http://doi.org/10.1038/nrm.2017.125

Primary papers

Instructor presentation: Matsuo, H., Chevallier, J., Mayran, N., Le Blanc, I., Ferguson, C., Faure, J., Blanc, N.S., Matile, S., Dubochet, J., Sadoul, R., Parton, R.G., Vilbois, F., and Gruenberg, J. (2004). Role of LBPA and Alix in multivesicular liposome formation and endosome organization. Science *303*, 531-534.

Two papers together: Wollert, T., and J. H. Hurley. 2010. Molecular mechanism of multivesicular body biogenesis by ESCRT complexes. Nature 464:864-869.

Wollert, T., C. Wunder, J. Lippincott-Schwartz, and J. H. Hurley. 2009. Membrane scission by the ESCRT-III complex. Nature 458:172-177.

Signal transduction and intracellular localization (Alberts, Chapters 15 as well as 12, 13, and 16)

Reviews

McCrea, P. D., and Gottardi, C. J. (2015). Beyond β-catenin: prospects for a larger catenin network in the nucleus. Nat Rev Mol Cell Biol 17, 55–64.Tomas, A., Futter, C. E., & Eden, E. R. (2014).

Manning, B. D., & Toker, A. (2017). AKT/PKB Signaling: Navigating the Network. Cell, 169(3), 381–405. http://doi.org/10.1016/j.cell.2017.04.001

Mayer, B. J. (2015). The discovery of modular binding domains: building blocks of cell signalling. Nat Rev Mol Cell Biol 16, 691–698.

Primary papers

Taelman, V. F., R. Dobrowolski, J. L. Plouhinec, L. C. Fuentealba, P. P. Vorwald, I. Gumper, D. D. Sabatini, and E. M. De Robertis. 2010. Wnt signaling requires sequestration of glycogen synthase kinase 3 inside multivesicular endosomes. Cell 143:1136-1148.

Vasudevan, K. M., D. A. Barbie, M. A. Davies, R. Rabinovsky, C. J. McNear, J. J. Kim, B. T. Hennessy, H. Tseng, P. Pochanard, S. Y. Kim, I. F. Dunn, A. C. Schinzel, P. Sandy, S. Hoersch, Q. Sheng, P. B. Gupta, J. S. Boehm, J. H. Reiling, S. Silver, Y. Lu, K. Stemke-Hale, B. Dutta, C. Joy, A. A. Sahin, A. M. Gonzalez-Angulo, A. Lluch, L. E. Rameh, T. Jacks, D. E. Root, E. S. Lander, G. B. Mills, W. C. Hahn, W. R. Sellers, and L. A. Garraway. 2009. AKT-independent signaling downstream of oncogenic PIK3CA mutations in human cancer. Cancer Cell 16:21-32.

Tassew, N. G., Charish, J., Shabanzadeh, A. P., Luga, V., Harada, H., Farhani, N., et al. (2017). Exosomes Mediate Mobilization of Autocrine Wnt10b to Promote Axonal Regeneration in the Injured CNS. Cell Reports, 20(1), 99–111. http://doi.org/10.1016/j.celrep.2017.06.009

Instructor presentation: Zheng, Y. et al. (2013). Temporal regulation of EGF signalling networks by the scaffold protein Shc1. Nature 499, 166–171.

The Cell Cycle (Alberts Chapters 17 and 20)

Reviews

Craney, A., & Rape, M. (2013). Dynamic regulation of ubiquitin-dependent cell cycle control. Current Opinion in Cell Biology, 25(6), 704–710. doi:10.1016/j.ceb.2013.07.004

Primary papers

Bashir, T., Dorrello, N.V., Amador, V., Guardavaccaro, D., and Pagano, M. (2004). Control of the SCF(Skp2-Cks1) ubiquitin ligase by the APC/C(Cdh1) ubiquitin ligase. Nature *428*, 190-193.

Wei, W., Ayad, N.G., Wan, Y., Zhang, G.J., Kirschner, M.W., and Kaelin, W.G., Jr. (2004). Degradation of the SCF component Skp2 in cell-cycle phase G1 by the anaphase-promoting complex. Nature *428*, 194-198.

Programmed cell death (Alberts Chapter 18)

Reviews

Fuchs, Y., & Steller, H. (2011). Programmed cell death in animal development and disease. Cell, 147(4), 742–758. http://doi.org/10.1016/j.cell.2011.10.033

Primary papers

Liu, X., Kim, C. N., Yang, J., Jemmerson, R., & Wang, X. (1996). Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell, 86(1), 147–157.

The cytoskeleton and adhesion (Alberts, Chapters 16 and 19)

Reviews

Leterrier, C., Dubey, P., & Roy, S. (2017). The nano-architecture of the axonal cytoskeleton. Nature Reviews Neuroscience, 18(12), 713–726. http://doi.org/10.1038/nrn.2017.129

Primary paper

Cai, X., D. Lietha, D. F. Ceccarelli, A. V. Karginov, Z. Rajfur, K. Jacobson, K. M. Hahn, M. J. Eck, and M. D. Schaller. 2008. Spatial and temporal regulation of focal adhesion kinase activity in living cells. Mol Cell Biol 28:201-214.

Xu, Z., Schaedel, L., Portran, D., Aguilar, A., Gaillard, J., Marinkovich, M. P., et al. (2017). Microtubules acquire resistance from mechanical breakage through intralumenal acetylation. Science (New York, NY), 356(6335), 328–332. http://doi.org/10.1126/science.aai8764

Asymmetric Cell Division (Alberts Chapters 17 and 21)

Review

Knoblich, J. A. 2010. Asymmetric cell division: recent developments and their implications for tumour biology. Nat Rev Mol Cell Biol 11:849-860.

Primary paper

Cicalese, A., G. Bonizzi, C. E. Pasi, M. Faretta, S. Ronzoni, B. Giulini, C. Brisken, S. Minucci, P. P. Di Fiore, and P. G. Pelicci. 2009. The tumor suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells. Cell 138:1083-1095.

Regulation of gene expression (Alberts Chapters 4, 6 and 7)

Reviews

Cech, T. R., & Steitz, J. A. (2014). The noncoding RNA revolution-trashing old rules to forge new ones. Cell, 157(1), 77–94. http://doi.org/10.1016/j.cell.2014.03.008

Bergmann, J. H., & Spector, D. L. (2014). Long non-coding RNAs: modulators of nuclear structure and function. Current Opinion in Cell Biology, 26, 10–18. doi:10.1016/j.ceb.2013.08.005

Scadden, D. 2009. A NEAT Way of Regulating Nuclear Export of mRNAs. Mol Cell 35:395.

Primary paper

Chen, L. L., and G. G. Carmichael. 2009. Altered nuclear retention of mRNAs containing inverted repeats in human embryonic stem cells: functional role of a nuclear noncoding RNA. Molecular cell 35:467-478.

Stem cells and chromatin modifications (Alberts Chapters 7 and 22)

Reviews

Dang-Nguyen, T. Q., and Torres-Padilla, M.-E. (2015). How cells build totipotency and pluripotency: nuclear, chromatin and transcriptional architecture. Current Opinion in Cell Biology 34, 9–15.

Hajkova, P. 2010. Epigenetic reprogramming--taking a lesson from the embryo. Current Opinion in Cell Biology 22:342-350.

Primary paper

Hajkova, P., S. J. Jeffries, C. Lee, N. Miller, S. P. Jackson, and M. A. Surani. 2010. Genome-wide reprogramming in the mouse germ line entails the base excision repair pathway. Science 329:78-82.

Stem Cells and Organoids (Alberts Chapter 22)

Reviews

Giandomenico, S. L., & Lancaster, M. A. (2017). Probing human brain evolution and development in organoids. Current Opinion in Cell Biology, 44, 36–43. http://doi.org/10.1016/j.ceb.2017.01.001

Quadrato, G., & Arlotta, P. (2017). Present and future of modeling human brain development in 3D organoids. Current Opinion in Cell Biology, 49, 47–52. http://doi.org/10.1016/j.ceb.2017.11.010

Primary Paper

Bershteyn M, Nowakowski TJ, Pollen AA, Di Lullo E, Nene A, Wynshaw-Boris A, Kriegstein AR: Human iPSC-derived cerebral organoids model cellular features of lissencephaly and reveal prolonged mitosis of outer radial glia. Cell Stem Cell 2017, 20:435-449 e434.

CRISPR

Reviews

Dominguez, A. A., Lim, W. A., and Qi, L. S. (2016). Beyond editing: repurposing CRISPR-Cas9 for precision genome regulation and interrogation. Nat Rev Mol Cell Biol 17, 5–15.

Johnson, J. Z., and Hockemeyer, D. (2015). Human stem cell-based disease modeling: prospects and challenges. Current Opinion in Cell Biology 37, 84–90.

Zeltner, N., and Studer, L. (2015). Pluripotent stem cell-based disease modeling: current hurdles and future promise. Current Opinion in Cell Biology 37, 102–110.

Primary Paper

Kleinstiver, B. P., Pattanayak, V., Prew, M. S., Tsai, S. Q., Nguyen, N. T., Zheng, Z., and Joung, J. K. (2016). High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects. Nature, 1–17.

Endoplasmic Reticulum: The Unfolded Protein Response (Alberts Chapter 12)

Reviews

Wang, M., and Kaufman, R. J. (2016). Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature 529, 326–335. Hetz, C.

Volmer, R., and Ron, D. (2015). Lipid-dependent regulation of the unfolded protein response. Current Opinion in Cell Biology 33, 67–73.

Hetz, C. (2012) The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nature Reviews Molecular Cell Biology, 13(2), 89–102. . http://doi.org/10.1038/nrm3270

Primary paper

Volmer, R., van der Ploeg, K., and Ron, D. (2013). Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains. Proc Natl Acad Sci USA 110, 4628–4633.