Biology 4141 – Current Topics and Methods in Cell Biology  
(Integrated with Biology 5064)  
Fall 2009 Course Outline

**Calendar Description:** Selected topics in cell biology, such as membrane dynamics, cell cycle control, apoptosis, signal transduction, and cellular rhythmicity. Presentation and critical discussion of recent research papers, emphasizing current methods and experimental design. Three lecture hours. One term. Three credits.

**Prerequisites:** SC/BIOL 2020 4.0; SC/BIOL 2021 4.0; or equivalent.

**WARNING:** Students without pre-requisites will be de-enrolled unless they have advanced standing or permission of the instructor.

**Time and location:** Tues-Thurs 10:00-11:30, TEL 1004

**Course Director:** Dr. Pat Lakin-Thomas, 005 Farquharson, x33461
Office hours: Tues & Thurs 11:30 – 1:00
e-mail: clocklab@yorku.ca I will try to respond within one working day, or answer your question at the next class meeting if appropriate.
Listserv: BIOL4141@yorku.ca; you can use this to set up study groups, or find someone to swap presentation dates with

**Objectives**
1. Learn about recent developments in a selected set of topics at the frontiers of cell biology research
2. Learn about methodologies for investigating cell structure and function
3. Learn about experimental design
4. Practice skills in critically reading original research papers
5. Practice skills in presentation

**Structure**
Five current topics will be covered. Each will be introduced by a recent review followed by several original papers relevant to the topic. Students will present the papers and the class will discuss the papers in detail.

**Assessment**

**Undergraduates:** Presentation 30%, one midterm 30%, final exam 40%

If there are more students than presentation slots, the remaining students will submit a written critical analysis of a paper (5 pages maximum, due on the day of the presentation) using the list of discussion topics as a guide. Oral/written presentation topics and dates will be assigned at random by the instructor.

**Graduates:** Presentation 30%, one midterm 15%, final exam 25%, essay 30%

Essay will be an in-depth analysis of a paper from the student’s own research topic, using the list of discussion topics. Essay will be due on Dec. 8.

**NOTE:** Final course grades may be adjusted to conform to Program or Faculty grade distribution profiles.

**Exams**
Midterm date: Oct. 27, in class.
Exam format: open-note, short answers and paragraph-length essay answers. You may bring the papers and your notes to the exam. Final exam may include questions about a paper to be assigned at the last class meeting, to test your ability to critically read and understand the literature. You are encouraged to work together on reading and analyzing the assigned paper before the exam.

Sample exam questions:
1. What is the experimental evidence to support a particular conclusion?
2. Given a particular experimental approach, suggest some controls that should be included in the experimental design and explain why they are useful.
3. What techniques would be appropriate to investigate a particular question, and what information would these techniques provide?
4. For a particular figure from a paper, explain the experimental design and explain what information was gained from the results.

Late policy
Presentations and essays will not be accepted after the assigned date unless you have a well-documented excuse, in which case the presentation or essay will be given at the first opportunity. If the midterm is missed due to a documented excuse, the weight will be assigned to the final exam. If the final exam is missed, the Biology Department’s deferred exam policy will apply. A doctor’s note is not sufficient; you must fill out an Attending Physician’s Statement and have it signed by your doctor:
http://www.registrar.yorku.ca/services/petitions/forms.htm#6

Academic Integrity
Students are expected to be familiar with and follow York University’s policies regarding academic integrity. Please consult the website below for more details:
http://www.yorku.ca/academicintegrity/students/index.htm

Accommodation Statement
Students who feel that there are extenuating circumstances that may interfere with their ability to successfully complete the course requirements are encouraged to discuss the matter with the Course Director as soon as possible. Students with physical, learning or psychiatric disabilities who require reasonable accommodation in teaching style or evaluation methods should discuss this with the Course Director early in the term so that appropriate arrangements can be made. The religious accommodation policy can be found at this website:
https://w2prod.sis.yorku.ca/Apps/WebObjects/cdm.woa/wa/regobs

Required Text
Gillen, C.M. (2007) Reading Primary Literature, Pearson Benjamin Cummings.
This short pamphlet is an excellent introduction to critical reading and experimental design (and how science works). Copies are for sale in the bookstore and also on reserve at Steacie.

Finding papers
All papers for this course are available to download from the journals’ own websites. You do not need to photocopy paper journals, and there are no copies of the papers on reserve. Please do not copy from the original journals, to save wear-and-tear on the bound volumes. Go first to the York Library eResources and find by title, then find the citation by volume and page number. (You might need to be connected via a York computer, or know how to get access from your own computer using Passport York.) If not
there, then go directly to the journal’s own website for access. (Google the name of the journal in quotes.)

Be sure to download the pdf version (not full text/html) for printing. Download the html version for high-resolution figures (to use in your presentation). Also be sure to check for and download any supplemental files: many papers have additional information that is not included in the printed version and is only available to download. Many papers require color for some of the figures; if you don’t print in color, you may need to look at the figures in the electronic version to understand them.

Finding information on methods
(Include your sources in your presentation, at the bottom of the slide.)

1) Go first to your cell biology textbook (Alberts), and the index. Try other textbooks such as molecular biology or genetics texts.
2) Look in Sambrook for molecular methods.
3) Try Current Protocols Online: Select “Current Protocols in Cell Biology” (or another topic) and use the search function with a keyword.
4) Try websites of companies named in the paper that manufacture kits or supply reagents. This is a good source for proprietary (patented) kits and methods.
5) Wikipedia is surprisingly good for background information but don’t use it as a primary reference—look at the bibliography at the bottom of the Wikipedia page and find those references. There is a Wiki project for molecular and cell biology: http://en.wikipedia.org/wiki/Portal:MCB
6) You can Google the name of the method but this is inefficient and sometimes unreliable. Check the reliability of the websites: often they will not be primary (reliable) sources, but rather someone’s lecture notes.

General References
Very useful for background information and basic methods. CD has good figures to use in presentations.

Current Protocols Online:  
http://www3.interscience.wiley.com/browse/?type=CURRENT_PROTOCOL  
Online resource that describes methods in cell biology, cytometry, molecular biology, and other titles.

The standard reference for molecular biology methods.

Methods In…  
Stacie holds several series of publications with this title, including Enzymology (covering far more than enzymes), Cell Biology, Molecular Biology, etc. Each volume is on a specific topic and is cataloged under its specific title. They are best located by doing a Library Catalogue search > search by keyword > series “methods”, subject “whatever” (e.g. “cell cycle”, “electrophysiology”).

Model Organisms:  
www.ceolas.org/VL/mo/  
Online catalog of websites with information about various model organisms.
www.nih.gov/science/models/  
Information on model organisms for biomedical research from the NIH
Presentation of Papers

**Time limit:** Approx. 20 minutes (15-25 min) plus questions. Practice and time your talk.

**Evaluation** will be based on the criteria listed on the evaluation form. Students will provide evaluations of their peers to be taken into consideration by the professor, but the final evaluation will be the professor’s subjective judgment.

**Three types of presentations** will be assigned, either “review” or “methods” or “results”. For most papers, two students will present the methods and results from the paper. There may also be a third student writing an essay on the same paper; you are welcome to get together as a group to work on the paper, but grades will be assigned individually. There will also be a few presentations of the review papers at the beginning of each section.

**Methods presentation**
Research the methods used: start with textbooks, then read references cited in the methods section of the paper if necessary. Your presentation should begin by briefly summarizing the introduction to the paper to put the methods into context. Explain the background to the experiments: Why is this an interesting question to try to answer? Then present a detailed description of methods, with appropriate diagrams to illustrate techniques. Concentrate on the methods unique to your paper but mention other important methods used in the paper. Most of these are listed for each paper on the course outline, but don’t limit yourself to that list if you believe other methods should also be discussed. Describe what information the methods provide, and what controls are necessary.

**Results presentation**
Present and explain the most significant results from the paper (using figures from the paper). You don’t need to cover all the figures if there are too many. Summarize the conclusions and explain how the results lead to those conclusions. Briefly summarize the major points in the discussion section of the paper. Don’t critique the paper- leave that for class discussion.

**Review presentation**
Summarize the important points and explain any figures. Use background information from textbooks if appropriate to introduce the subject. Explain how the selected papers we will be reading fit into the review topic.

**Questions:** Be prepared to answer questions about the subject of your presentation.

**Visual Aids:** Use document camera for hard copy, and/or chalkboard, and/or PowerPoint. I will provide the paper and supplements on CD and/or a colour printout of the paper for you to use during your presentation if you want it. Supplemental movies will be available on a CD. **WARNING:** If you use PowerPoint, bring your presentation on a CD or a USB drive and come early to class to get it loaded onto the computer. If your presentation fails to run for technical reasons you will NOT be allowed to postpone it, so test it beforehand AND bring a backup (hard copy of your slides or a plan to write on the document camera or chalkboard in case the computer fails). Email your presentation to yourself so you can download it if your CD or USB drive fails. I will edit presentations and post them on WebCT.

**Handouts:** Handouts can sometimes be useful, but are not required. A handout could be an outline of your talk, a summary of the main points in the paper, a diagram or additional data/figures not in the paper. Please limit the length to one page. Do not bring copies of all of your overheads to distribute.
Topics for Critical Discussion of Papers

(Read Gillen for further explanations of these questions.)

1. Do the title and abstract accurately reflect the contents of the paper?

2. Does the introduction provide adequate background and historical perspective to explain why the work was undertaken and why it’s important? Have the authors referenced the work of others or do they cite only their own work?

3. Are the methods described or referenced in sufficient detail to be able to reproduce the experiments?

4. What are the strengths and limitations of the methods? What are the advantages/disadvantages of the choice of organism/cell type?

5. Are the results presented in a clear and meaningful way? Are figures and tables appropriate and understandable? Are figure legends clear and complete?

6. What evidence is presented that the results are reproducible? (Were appropriate statistical tests used?)

7. What controls were run, and why?

8. Are the experiments causative studies or merely correlational?

9. Are the conclusions supported by the data?

10. Does the discussion adequately put the results in the context of other work?

11. Have the authors discussed the limitations of the methods? Have the authors considered alternative explanations for the results?

12. Are the results physiologically relevant? (Do they tell us how real live cells behave?)

13. What does the paper tell us that is genuinely novel?

14. What questions are left unanswered? What new questions have been raised? Have the authors suggested further experiments?

15. Do the authors have any conflicts of interest that might have influenced their work?
### Biology 4141- Fall 2009  Presentation Evaluation

**Speaker:**

**Date:**

**Paper:**

<table>
<thead>
<tr>
<th>Weight (%)</th>
<th>Mark (0-100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>Did the presentation accurately summarize the important points from the paper, including background information from additional sources if necessary?</td>
</tr>
<tr>
<td>25%</td>
<td>Was the material presented clearly and in a well-organized way?</td>
</tr>
<tr>
<td>10%</td>
<td>Were the visual aids clear and readable and appropriate?</td>
</tr>
<tr>
<td>5%</td>
<td>Was the level of explanation appropriate, not too difficult and not too simple?</td>
</tr>
<tr>
<td>5%</td>
<td>Were the timing and pacing right, and was the speaker loud enough and understandable?</td>
</tr>
<tr>
<td>5%</td>
<td>Did the speaker project enthusiasm and make the subject interesting?</td>
</tr>
</tbody>
</table>


Does the speaker have any mannerisms they should try to avoid?

**Other comments:**
Tips on Presentations

• Make an outline first of the points you want to make and the pictures/graphics you will need to illustrate those points. Try to have an illustration for every important point. A picture is worth a thousand words.

• Start with the title of your presentation and YOUR NAME.

• Acknowledge your sources. Give credit for pictures you download from a website or copy from a published source. Put the reference at the bottom of the slide.

• Practice and time your presentation. You should try for 20 min, no less than 15 and no more than 25. You might get cut off if you run on too long.

• Speak up and make eye contact with the audience. Don’t talk to the computer or screen.

• Don’t read a script. Use notes if you need them but put only a few words on the notes to remind you what to say.

Tips on visual aids and PowerPoint

• Choose a simple design with high contrast between text and background. Black text on a pale background or white or yellow text on dark blue work well.

• Use large font sizes, at least 24 point. Use 28 or 32 for text and 36 to 44 for titles.

• Choose a clean, standard font like Arial or Times New Roman. Do not use many different fonts, or unusual fonts that might not be found in all standard computer installations.

• Don’t put too much on one slide. Slides don’t cost anything so you don’t have to be frugal with them. One point per slide is a good rule.

• Don’t write whole sentences and then read them out word-for-word. Use a few words or a phrase to communicate the key words and ideas.

• Don’t use fancy transitions and moving text. It is not helpful, just distracting. There are rare times when a simple animation can help to make a point, but usually it is a waste of your time and the audience’s patience.

• Put a black slide after your last slide so you don’t drop out of the presentation if you advance after the last slide. Add some extra slides after your last slide with information you might use to answer questions from the audience.

• Check your presentation on a computer similar to the one you will be presenting on. (This is a warning to Mac users like me: Sometimes Windows versions of PowerPoint don’t display your slides the way you see them on your Mac. Movies are a particular problem.)
A. Cell culture
    1. Primary cultures vs. cell lines
    2. Growth media

B. Electron microscopy
    1. Transmission EM (TEM)
       Thin section, Shadowing, Freeze-fracture
    2. Scanning EM (SEM)

C. Fluorescence
    1. Fluorescence microscopy
    2. Confocal microscopy
    3. Fluorescence-activated cell sorting (FACS) (flow cytometry)

D. Antibodies
    1. Types
       Polyclonal vs. Monoclonal
    2. Uses of antibodies
       a. Unconjugated
          Immunoprecipitation, Co-immunoprecipitation
       b. Conjugated
          Immunofluorescence, Immunogold, Enzyme-linked
       c. Secondary antibodies

E. Molecular methods
    1. DNA cloning
    2. Polymerase chain reaction (PCR)
    3. Blotting techniques
       a. Southern (DNA blot)
       b. Northern (RNA blot)
       c. Western (Protein blot, Immunoblot)
    4. Microarrays
    5. in situ methods
       a. in situ hybridization
       b. Immunohistochemistry (Immunolocalization)
    6. Transgenic methods
       a. Transfection methods
          Biochemical, Physical, Viral
       b. Transfection results
          Transient vs. Stable
       b. Gene manipulation
          i. in vitro mutagenesis
          ii. Ectopic promoters
          iii. Reporter genes
             LacZ, GFP, luciferase
BIOL 4141/5064 Schedule, Fall 2009

Note: There will be two or three student presentations per day, except where noted. For each data paper there will be a methods and a results presentation. Additional presenters will cover reviews.

Th Sept 10
First class meeting, introduction
Dr. Pat: general methods, part 1

Sept 15, 17, 22, 24, 29: Cell Size

T Sept 15 (three presenters)
Two presenters: general methods, parts 2 and 3

Third presenter: Review

Th Sept 17

Methods: Drosophila as a model system, induction of mitotic clones, Gal4/UAS system for gene expression from tissue-specific promoters, FACS, apoptosis assays, “minute” technique

T Sept 22

Methods: S. cerevisiae as a model system, ORF deletion strains, synthetic genetic array, epistasis analysis, coulter counter for cell size assays, elutriation, alpha-factor resistance

Th Sept 24


Methods: Arabidopsis as a model organism, trichomes as a model single-cell system, transgenic plants, vital dyes, GUS assay, DAPI for DNA content, double-mutant analysis for protein/protein interactions in vivo
T Sept 29
Methods: Rat Schwann cells and fibroblasts as model systems, immunopanning, cell culture and serum-free medium, cell volume by confocal sectioning and Coulter counter, BrDU for cell cycle timing, Phalloidin for actin staining, mathematical model

Oct 1, 6, 8 and 20: Apoptosis

Th Oct 1 (three presenters)
Methods: *C. elegans* as a model organism, nematode genetics, gene cloning strategy, in vitro protein binding assays, yeast 2-hybrid analysis

T Oct 6
Methods: human colon cancer cells, overexpression and knockout cell lines, apoptosis assays, GFP fusion proteins, immunostaining, immunoprecipitation, subcellular fractionation, caspase inhibitors

Th Oct 8
Methods: HeLa cells, human glioblastoma cells, synthetic chemistry, fluorescence polarization assay for molecular binding, GST-tagged proteins and biotin tags for protein-protein interaction assays, native PAGE for protein complex formation, caspase assays

(Oct 10 - 16: Reading Week)

T Oct 20
Methods: Jurkat T leukemic cells, overexpression cell lines, flow cytometry, Annexin V and propidium iodide for apoptosis assay, MTS cell survival assay, Westerns for caspase-3, cytochrome c and BAK activation, mitochondrial isolation
Th Oct 22: Free day, midterm review

T Oct 27: Midterm

Oct 29, Nov 3 and 5: Golgi and protein trafficking

Th Oct 29 (three presenters)
Review

Methods: COS7 cells, rat thyroid cells, transfection, in vitro mutagenesis, GFP, Golgi and ER marker proteins, immunofluorescence, photobleaching and FRAP

T Nov 3
Methods: NRK cells, GFP-tagged Golgi proteins, transient transfection, immunofluorescence, immunogold labelling, photobleaching and FRAP, Brefeldin A, nocodazole, Sar1 and Arf1 mutants

Th Nov 5
Methods: yeast, 4-D confocal microscopy, immunofluorescence, Golgi marker proteins, radioactive pulse-chase, immunoprecipitation, alpha-factor

(Friday Nov 6: Drop deadline)

Nov 10, 12, 17 and 19: Cellular rhythms and cell signalling

T Nov 10 (three presenters)
Review.

Methods: *Drosophila* locomotor rhythms, GAL4/UAS and cell-specific drivers, Ca\textsuperscript{2+} imaging, Ca\textsuperscript{2+} buffer protein, calmodulin and CaMKII mutants

**Th Nov 12**

Methods: *Arabidopsis*, microarrays, cADPR assays, Ca\textsuperscript{2+} assay with aequorin, clock gene mutants, Ca\textsuperscript{2+} pharmacology, mathematical model, inducible transgenes

**T Nov 17**

Methods: rat fibroblasts, transient transfection, cell-permeant proteins, His tag for protein purification, luciferase reporter, knockout mouse fibroblasts, Ca\textsuperscript{2+} assay with fura-2

**Th Nov 19**

Methods: mouse brain slices *in vitro*, NIH3T3 cells, luciferase reporter for gene expression rhythms, cAMP assays, cAMP pharmacology (agonists and antagonists)

**Nov 24, 26, Dec 1 and 3: Stem Cells**

**T Nov 24 (three presenters)**

Review.


Methods: Rag knockout mice, nuclear transfer and isolation of ES cells, tetraploid embryo complementation, HoxB4 expression, B-and T-cell markers, FACS, ELISA

**Th Nov 26**

Methods: primary fibroblast cell culture, karyotype, flow cytometry, FISH, oocyte preparation and nuclear transfer, parthenogenetic activation, DNA fingerprinting, mitochondrial DNA sequencing
T Dec 1
Methods: retroviral transduction of mouse fibroblasts, embryonic cell culture on feeder cells, assays for pluripotency, chromatin immunoprecipitation assays, karyotyping, promoter methylation assay, teratomas and embryoid bodies

Th Dec 3
Methods: comparison of viral transfection methods, *piggyBac* transposition, doxycycline-inducible promoter, Southern blots for transposition events, ES cell pluripotency markers

T Dec 8: Last class, free day, final review
Due date for grad student essays (5064 students)