Biology 376 – Animal Development – Fall 2019

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Lecture: Mon/Wed/Fri, 9:05 – 10:00, MRH131 Lab: Wednesday, 2:30 - 6:30, SCST 330

Office Hours: Mon 10–11 AM, 1:15-2:15 PM, Tues 8:30-10:30 AM, Thurs 11-12 AM, or by appointment. Email is typically an good way to get a quick response to a question – although it is no substitute for one-on-one help with discussion in my office.

Course Home Page: **home.sandiego.edu/~cloer/bio376.html**
This is a good place to check for announcements and handouts. All lab handouts, lecture outlines and slides (in note-taking format), and non-text readings can be found here.

Main text (required) - *Developmental Biology,* 11th Edition. Authors – Gilbert & Barresi. Sinauer Associates, ©2016. (Textbook Home Page: www.devbio.com)

Books for laboratory (recommended) -
Atlas of Descriptive Embryology, 8th Ed., Mathews & Schoenwolf. Macmillan/Collier, 2006.
Student Handbook for Writing in Biology, 3rd Ed., Kinsely. W. H. Freeman Co., 2009 - this is the official writing manual for the Biology Department (so you should have a copy).
See the laboratory syllabus for information on additional lab supplies.

Goals of the Course

Students will learn patterns and mechanisms of animal development, with an emphasis on model organisms such as *C. elegans*, *Drosophila*, *Xenopus*, chick and mouse. A central theme will be development as a phenomenon of differential gene regulation. Stages of embryogenesis, morphogenesis, pattern formation and differentiation of developing organisms will be examined. Developmental mechanisms, especially at a molecular level, will be examined for differences and commonality among organisms, with a special focus on two specific signaling pathways: Wnt and RTK signaling. The relationships between developmental mechanisms and the molecular-genetic basis of human disease will also be examined and discussed.

At the end of Biology 376, a student should be able to:
* List and explain the principle features of animal development; compare and contrast these developmental stages in various organisms.
* Identify the three classical germ layers, understand how they arise and contribute to embryonic organization; explain conserved molecular mechanisms for their generation.
* Visualize embryonic development of selected organisms in four dimensions, from fertilization through early organogenesis; relate embryonic structure to cell/tissue interactions and mechanisms of cell/tissue specification.
* Explain how key historical experiments in developmental biology shape our current understanding of developmental events, mechanisms and evolution.
* Design an experiment to evaluate the role of a molecular component in a specific developmental decision, and critically analyze the results of such an experiment.
* Explain the components and functions of key molecular genetic and signaling pathways (especially RTK and Wnt), how they work to determine cell fate in selected organisms, and how their dysfunction causes human disease and malformation; use these understandings to hypothesize the function of conserved components in other organisms.
* Apply developmental principles to analyze and present current primary literature in the field, and integrate this new knowledge into the broader field.
* Consider the role of developmental biology in biomedical ethics and policies.

Course Mechanics

Attendance at all lectures is **strongly recommended**, but not required, except as noted below. If attendance appears to be lagging, however, I may begin taking attendance. Students who miss more than a few lectures often do poorly in class; such students will find little

sympathy for their plight. For any missed lecture, a student should consult a fellow student for notes; all lecture slides and outlines will be available online. Please note that attendance at class presentations /discussions and 'active learning' activities is **required**. There will be two group presentations earlier in the semester. Later in the semester, four sessions for individual literature presentations are scheduled during the Wednesday lecture time beginning in November. Attendance at any guest lectures during the semester is also **required**.

Missed quizzes, tests, presentations, laboratories, and other major class activities may be made up only for excused absences (e.g., serious illness). Students must inform the instructor of the reason for their absence as soon as possible by email or telephone; ideally, immediately prior to an anticipated absence (in the case of illness). Except in extraordinary circumstances, request for an excused absence must be made within 1-2 days after the absence (again, as soon as possible). Note also that grades on any assignments turned in late may be severely reduced except for excused absences.

Attendance at all laboratory sessions is **required**. If you miss a lab for an excused absence, you may have an opportunity to make it up later; however, if that lab session uses living material or requires a substantial lab preparation, this may be impossible or impractical. Some other form of makeup may be arranged.

Tests and Grading

There will be two hourly tests during the semester covering the material in lectures preceding them. The final exam will cover mainly previously untested material (essentially a third hourly test, although some material is cumulative by nature). The lab portion of the class will be 25% of your final grade (see the lab syllabus for more specifics). Class presentations and discussion participation on primary literature will count for a total of 14%. Work done for the class home page will count for 1% of your grade. More details will follow on class presentations and home page work. **Note that adjustments to grading percentages may be required if assignments are altered.** For breakdown on lab grade, see the syllabus in the lab manual.

Tests will emphasize lecture material. Assigned readings contain more material than will be covered in lecture. The quality of your writing on exams is important. Your answer to a question must be clear (and legible) to be correct. Spelling must also be correct, especially of new words you are adding to your biological vocabulary.

Grading Summary:

<table>
<tr><td>1st hourly test</td><td>20%</td><td>Class presentations</td><td>12%</td></tr>
<tr><td>2nd hourly test</td><td>20%</td><td rowspan="2">Discussion participation/reviewing, attendance at required events</td><td rowspan="2">2%</td></tr>
<tr><td>Final (3rd hourly)</td><td>20%</td></tr>
<tr><td>Lab</td><td>25%</td><td>'Read More About It' web page, etc.</td><td>1%</td></tr>
</table>

Academic Integrity

Copying of any material from current or former Biology 376 students is plagiarism and will not be tolerated. Do your own work and demand that others do theirs. Take similar care in your use of material from the class lab handouts and the textbook. All portions of lab reports and papers are expected to reflect ONLY your own work and your own writing. When working in groups, each member of the group is expected to synthesize the information, analyze data and prepare an individual report. If you use literature in your report, cite it appropriately. [For example, copying sentences from the book, followed by a parenthetical citation (Gilbert, 2016), is plagiarism.] **Be sure you understand what constitutes plagiarism.** If you have any questions about this, or any other item related to academic integrity, please ask. USD Academic Integrity policy will be strictly enforced. Please see the lab syllabus for further discussion of plagiarism.

Lecture Schedule Readings from Gilbert & Barresi, *Developmental Biology*, 11th ed., © 2016; or as indicated otherwise.

Sept. 4 (W)	Introduction to animal development. Review of syllabus; course mechanics. 'Principle features of development.' Questions & approaches of developmental biology. Reading: pp. 1–28 (Chapter 1), pp. 45-47 (diff. gene expr./central dogma).
Sept. 6 (F)	Cell signaling, signal transduction Reading: pp. 108-113, 116-120 (RTK signaling), 125-128, 131 (Wnt signaling), esp. Figs. 4.24, 4.26, 4.34, 4.37 (in Chapter 4).
Sept. 9 (M)	Types of cell specification. Induction at single cell resolution: *C. elegans* vulva. Reading: pp. 29-41 (Chapter 2), 138-139 [Most *C. elegans* info not in text.]
Sept. 11 (W)	Differential cell adhesion & cadherins, extracellular matrix and ECM receptors (integrins). pp. 97-107 (plus Fig 4.14 on p. 108), in Chapter 4.
Sept. 13 (F) Add/Drop Deadline	Evidence for genomic equivalence, animal cloning; in-class exercise on gene regulation (attendance required) – review what you know about gene structure, promoters, enhancers, eukaryotic transcription, transcription factors, chromatin structure, etc. Reading: all of Chapter 3, but primarily pp. 45-72.
Sept. 16 (M)	Fertilization I: gamete structure, echinoderm fertilization, acrosome reaction, blocks to polyspermy, cortical reaction, egg activation. Reading: pp. 217–239 (Chapter 7).
Sept. 18 (W)	Sea urchin early development. Types of cell movement (esp. in gastrulation). Reading: p. 14, Table 1.1; pp. 311-326 (in Ch. 10). VIDEO: Sea urchin development.
Sept. 20 (F)	Fertilization II: Mammalian fertilization. Reading: pp. 239–248 (Chapter 7). Patterns of metazoan cleavage, cytoskeletal mechanisms of karyo- & cytokinesis. Reading: pp. 11-13 (Fig. 1.5); Web topic 1.2 (http://11e.devbio.com/wt0102.html)
Sept. 23 (M)	Cleavage and developmental regulation gone awry: Introduction to cancer. Reading: pp. 754-761; 'The Hallmarks of Cancer,' Hanahan & Weinberg, 2000.
Sept. 25 (W)	Amphibian development: cleavage, gastrulation, neurulation; intro to ectodermal, mesodermal, endodermal derivatives. Reading: pp. 333-343, 413-429, 538-543 (see also Figs 18.1, 18.11, 18.18A), 653-661 (emphasis on phenomenon vs. mechanism). VIDEO: *Xenopus* (frog)
Sept. 27 (F)	Amniote early development: birds & mammals. Reading: pp. 379-399. VIDEO: chick development, mammalian early development.
Sept. 30 (M)	Mesodermal derivatives, heart formation. Endodermal derivatives. Extraembryonic membranes. Reading: 538-543, 653-661.
Oct. 2 (W)	*C. elegans* early development and cell specification. Reading: pp. 265-273.
Oct. 4 (F)	*Drosophila* development I. Early development and the maternal genes: determination of anterior-posterior polarity. Reading: pp. 38-41 (syncitial specification), 115-116 (morphogen gradients), 277-293. VIDEO: *Drosophila* embryogenesis.
Oct. 7 (M)	*Drosophila* II. Maternal genes continued, Zygotic genes: gap, pair-rule and segment polarity genes in anterior-posterior patterning. Homeotic selector genes. Reading: pp 284-303.
Oct. 9 (W)	Lecture catch-up / review
Oct. 11 (F)	**FIRST HOURLY EXAM**
Oct. 14 (M)	*Drosophila* III. Zygotic genes (cont.): gap, pair-rule and segment polarity genes in anterior-posterior patterning. Homeotic selector genes. Reading: same as above.
Oct. 16 (W)	The Homeotic Complex/Hox genes: Conservation of anterior/posterior pattern formation, evolution via changes Hox gene number & expression. Reading: pp. 301-303, 402-404, 789-792.

Oct. 18 (F)	**FALL HOLIDAY**
Oct. 21 (M)	Guest lecture (attendance required)
Oct. 23 (W)	Pattern formation in tetrapod limb. Reading: pp. 613-644 (most of Chapter 19).
Oct. 25 (F)	**PRIMARY LITERATURE PRESENTATION / DISCUSSION 1** (attendance required) Reed et al., 2008 article [see online page for assignments & details].
Oct. 28 (M)	Cell-cell interactions in vertebrate development: Spemann & Mangold and the 'organizer,' primary embryonic induction. Reading: pp. 343-348
Oct. 30 (W)	Molecular mechanisms of vertebrate axis formation. Reading: pp. 348-364.
Nov. 1 (F)	**PRIMARY LITERATURE PRESENTATION / DISCUSSION 2** (attendance required) Misale et al., 2012 article [see online page for assignments & details].
Nov. 4 (M)	Developmental Neurobiology I. Patterning of vertebrate CNS. Neural crest cell migration and specification. Reading: pp. 430-433, 463-486.
Nov. 6 (W)	Developmental Neurobiology II. Axonal outgrowth and guidance. Reading: pp. 488-509. 'Mol Bio of Axon Guidance' [pp. 1123–1124, 1130–1131]; .
Nov. 8 (F)	**SECOND HOURLY EXAM**
Nov. 11 (M)	Developmental Neurobiology III. Neuron-target interactions. Neurotrophic substances. Reading: pp. 511–513 (differential survival).
Nov. 13 (W)	Programmed cell death/apoptosis I. Roles of PCD in normal development, genetics of PCD in *C. elegans*. Reading: pp. 645-646, 509–511.
Nov. 15 (F)	Programmed cell death/apoptosis II. Molecular mechanisms. Same as above.
Nov. 18 (M)	**INDIVIDUAL LITERATURE PRESENTATIONS** (attendance required)
Nov. 20 (W)	Cancer and developmental biology II. More cancer molecular mechanisms, cell cycle regulation. Reading: Gilbert website, supplement(s).
Nov. 22 (F)	Cancer and developmental biology III.
Nov. 25 (M)	**INDIVIDUAL LITERATURE PRESENTATIONS** (attendance required)
Nov. 27-29	**THANKSGIVING HOLIDAY**
Dec. 2 (M)	**EVOLUTION AND DEVELOPMENT (EVO-DEVO) I. [OR OTHER TOPIC(S) TBD]**
Dec. 4 (W)	**INDIVIDUAL LITERATURE PRESENTATIONS** (attendance required)
Dec. 6 (F)	Evolution and development (Evo-devo) II. [or other topic(s) TBD]
Dec. 9 (M)	**INDIVIDUAL LITERATURE PRESENTATIONS** (attendance required)
Dec. 11 (W)	Evolution and development (Evo-devo) III. [or other topic(s) TBD]
Dec. 13 (F)	Lecture catch-up/review [last day of classes]
Dec. 16 - 20	**FINALS**
Dec. 18 (W)	**FINAL EXAM - 8:00 - 10:00 AM**

Bio 376 - Animal Development Lab Syllabus - Fall 2019

Wednesday, 2:30 - 6:30 PM, SCST 330
Office Hours: Mon 10-11 AM, 1:15-2:15 PM, Tues 8:30-10:30 AM, Thurs 11-12 AM

Texts:

Atlas of Descriptive Embryology, 8th Ed., Mathews & Schoenwolf. Macmillan/Collier, 2006 (recommended). [Note, nearly any edition of this atlas will be adequate.]

Animal Development Laboratory Manual. C. Loer, 2019 (individual labs will be available on the course website or occasionally by email).

Student Handbook for Writing in Biology, 3rd Ed., Kinsely. W. H. Freeman Co., 2009

Introduction - Because of the lab format – one 4 hour section per week – in order to perform experiments, you will sometimes need to return to lab outside of regularly scheduled hours to make observations, check embryos or other organisms, or change a solution. Some labs (particularly prior to the first practical exam) are oriented partly toward traditional embryology, mainly of vertebrates. Thus, these labs will be somewhat independent of lecture material, although we will attempt wherever possible to link the two. Finally, because availability of living organisms, their gametes and embryos is not absolutely predictable, a scheduled lab may be postponed or changed, even at the last minute. Be sure to check your email regularly, especially the day before lab.

Labs - Lab periods may include any of the following: short lecture by instructor, pop quiz, demonstration by instructor, observation of and experimentation with living material, examination of prepared slides, or viewing videos. (Yes, I know you've heard this before, but...) It is *essential* that you review the next lab's procedures before coming to lab since time may be short in the lab period, and in the case of living material, a developing organism waits for no one. Be prepared and be on time. To provide additional motivation for reading lab material ahead of time, a short test ('pop quiz') may be given at the beginning of some lab periods.

Whenever possible, given available materials, equipment and organisms, you will be able to do each exercise on your own, rather than having to share with a partner. This should allow you to work at your own pace, your own depth of interest, and with independence. This is not to say that you should not feel free to help one another, discuss one's findings, and, especially, to share viewing of any particularly clear, extraordinary or spectacular sights that appear under your microscope. This is true not only of living organisms, but also of prepared slides in which a given structure is especially obvious. We suggest that whenever possible (time and materials allowing) you make observations more than once. More often than not, you will see things you didn't see the first time around because you are now more familiar with the subject .

Some of the lab experiments we will attempt take some manual dexterity, which most people can learn with instruction, care and some practice. Please don't become frustrated if you can't do something right away. For example, moving a living 1 mm worm from a plate to a slide without killing it takes a little practice. Having accomplished the feat in question, however, allows you to do make interesting observations and in some cases to perform fascinating experiments.

Attendance

Attendance at all lab sessions is **required**. If you have a legitimate conflict (e.g., college athletics, religious holiday) with a given lab session **please let the instructor know as soon as possible**. If you miss a lab unexpectedly for a legitimate reason (e.g., sickness) you may have the opportunity to make it up later; however, if that lab session uses living material, this may be impractical or impossible. Ideally you should contact the instructor prior to the lab; otherwise as soon as possible (i.e., within 24 hrs). Some other form of makeup may be assigned. Missing any lab for an unexcused reason will result in complete loss of lab 'citizenship' points as well as any specific points for that lab.

Work outside of the regular lab period

Experiments in developmental lab cannot easily be restricted to one four-hour lab per week. It is unavoidable that students will be required on occasions to come to the lab outside the regular lab time to set up or complete parts of an experiment or project.

Lab Citizenship: Safety and Courtesy

Strictly following all safety rules is basic to good lab technique.

- ☞ No eating, drinking, smoking or application of cosmetics in the laboratory. Please do not bring any food or drink containers into the lab.
- ☞ Close-toed shoes that fully cover the feet must be worn at all times (i.e., no sandals or flip-flops).
- ☞ Lab coats, protective eyewear (goggles) and/or gloves must be worn at all appropriate times (as instructed).
- ☞ Contact lenses must not be worn in lab.
- ☞ No pipetting by mouth.
- ☞ No unauthorized experiments are to be performed.
- ☞ No unauthorized use of equipment.
- ☞ Follow carefully instructions for disposal of glass, living tissue, etc.
- ☞ Wash hands during lab as necessary, and thoroughly following lab.

Deviation from safety policies will be taken seriously.

In addition, please note that we share the room with other biology laboratory sections. As a courtesy to others, we must diligently clean up after ourselves and put away our equipment at the end of the lab period. Put away your microscope, slides and other equipment, and clean up as necessary. Don't leave it to someone else. Take care of your own mess! Furthermore, please do not disturb ongoing experiments of your classmates or those of other sections that may be in the room. Be a good lab citizen.

When you come to the lab outside of regular lab times, ask permission of the instructor if there is another lab class in session. Try to avoid the first hour of lab classes when the instructor may be lecturing or demonstrating.

Lab Citizenship: Electronic devices and communications

Your focus while in lab must be on studying development, monitoring procedures and experiments, and listening to instruction about the lab. Students are not to use cell phones, smart phones, tablets, laptops, etc. in lab for *any purpose other than class-related activities*, including texting, web-surfing, etc. This is distracting to you and your classmates. If you must communicate during lab time, do so **outside** the room, and only when you are working on your own time (usually only after the first 1-2 hours of lab).

Lab Supplies

You are required to have for use in lab the following:

Sharpie extra fine permanent marker (a few different colors may be useful or fun), used for marking slides and plates (they can write on glass or plastic).

Colored pencils or pens (Drawings of embryos should use standard embryological code: red = mesoderm, blue = ectoderm, yellow = endoderm; green is sometimes used for neural crest).

Lab notebook ~8.5 x 11 in.; I prefer a flat notebook to loose-leaf. Example: Bienfang 8.5x11 horizontal "NoteSketch" book (R239102) - typically available for purchase at the bookstore, or online.

Lab coat (You should bring this to lab and wear it when so instructed.)

Recommended: Flash drive for transferring and backing up computer projects or images taken with the microscope camera.

Lab Notebooks, Lab Reports

You are required to keep a lab notebook in which you will keep an accurate record of your observations and experiments. Keeping a good notebook will particularly helpful when it comes time to do a lab report. Your notebooks may be collected and evaluated periodically. Thus, they should be interpretable to someone other than just yourself. We understand, however, that a lab notebook is a working document, so we don't expect them to perfectly neat, just legible. They will be evaluated for thoroughness of recorded observations and usefulness for write-ups. Much of what you will be doing during the "embryology" part of the course will be sketching what you observe through the microscope. If you desire, you may make sketches on loose, unruled sheets, but these must be taped securely into your lab notebook immediately upon completion. Otherwise, taking data on loose sheets is strongly discouraged.

For labs that require a report, you will be given specific instructions on the format. See below for general guidelines. Since the kinds of labs vary through the course, the formats may be somewhat different for each report. The section of the lab manual for labs requiring a report should direct you to collect all the information you need for your lab report. Feel free to ask the instructor and TAs about these issues if you're not sure. Reports or other assignments turned in must be printed out (not hand-written) and neat. Lab reports that have excessive typographical errors or are intelligible will be returned immediately for correction. The quality of your writing is an important part of your grade in lab reports – correct grammar and spelling, logical organization, clear presentation, etc.

Each student must prepare a separate report, whether projects are individual, with a partner or a group. If you must use others' data for your report, be sure to make this clear in your report. (See also Academic Integrity section below.)

Grading Summary

Quizzes/Notebook checks: 24%	Practical/Exams: 35%
Lab Reports/Presentations: 36%	Citizenship/Participation: 5%

Living Organisms

We will be examining and experimenting with some living, developing animals. Students should treat these creatures with appropriate respect. Procedures should be followed as described and no unauthorized experiments or procedures performed. No organisms are allowed to leave the laboratory and all will be humanely destroyed

following their prescribed uses. (The South African clawed frog, *Xenopus*, for example, is not native to North America. Since its careless introduction to the wild, it has become a pest in some parts of the country, destroying native species.) Any deviation from these policies by students will be viewed with the utmost seriousness.

Slide Collections

During the semester, you will using microscope slides of prepared organisms and tissues. Although some of these slides may appear old, they are expensive to replace. You will be held fully responsible for any slides broken or lost. Specific slides will be available to you again at the beginning of any lab period when we will use them. Additional slides will be available during specific lab sessions; these are also to be returned to the same location you took them from at the end of lab periods.

Lab Equipment

The most expensive equipment you will use regularly in lab are the dissecting and compound microscopes. All lab equipment, and especially the microscopes, must be treated with appropriate care. The research grade compound microscope and camera at the back of the room is worth more than $50,000, and many seemingly insignificant parts worth hundreds or thousands of dollars. Reckless disregard for lab equipment could result in an expensive bill for you. We will discuss microscope use and care in the first lab session.

The only dumb question is the one you need an answer to, that you don't ask.

-- a Biology Professor

Some important notes on academic integrity in lab reports and presentations:

1. Although in some cases students may be using the same primary data to prepare reports, *each student must write a separate report, and prepare separate figures, tables, graphs and data analysis.* Students working together *must not turn in identical figures, graphs or tables* (except perhaps for some primary data), as this calls into question whether one of the students has done the work assigned.

On a related note, it is inappropriate and unwise to provide a copy of your lab report, complete or in draft form, to another student – including a lab partner. If the student with whom you share your text copies a portion of your report, then you also will be implicated for having provided prohibited assistance with an assignment, and will also be sanctioned for having violated academic integrity. If you are using the same data, you may of course share copies of data – but (as noted above) each student must perform analyses, and prepare figures and tables separately.

2. It is not appropriate to use direct quotations from a source without placing the material in quotation marks and attributing the source, *even if the source is cited*. This is plagiarism -- representing to the reader that another's writing is your own. Furthermore, in the context of a lab report, quotations should be used sparingly or not at all, and should never be more than a phrase or sentence long. A paragraph-long quotation means that you are *not writing*. Paraphrasing can a bit trickier -- a little harder to draw the line on what is plagiarism -- but if the sentence or sentences is almost identical to the source with a few changed words, or slightly rearranged, then that is likely to be plagiarism as well. A good way to avoid paraphrasing or unambiguous plagiarism from sources is to write *without having the source(s) in front of you*. This way the writing can only be in your own words, synthesized from your reading.

3. Copying of any material from former or students in this class is plagiarism and will not be tolerated. Do your own work and demand that others do theirs. Take similar care in your use of material from the lab manuals and the textbook. All portions of lab reports and papers are expected to reflect ONLY your own work and your own writing. When working in groups, each member of the group is expected to synthesize the information, analyze data and prepare an individual report. If you use literature in your report, cite it appropriately. [For example, copying sentences from the book, followed by a parenthetical citation (Weaver, 2002), is plagiarism.] **Be sure you understand what constitutes plagiarism.** If you have any questions about this, or any other item related to academic integrity, please ask. USD Academic Integrity policy will be strictly enforced.

4. Powerpoint presentations must also demonstrate *your work*. This can particularly be an problem when reviewing/presenting the scientific literature. It is NOT generally appropriate (and can be plagiarism) to copy sentences or significant phrases, etc. from an article you are presenting into your slides – you should present and summarize material in your own words. Rework/interpret in a way that demonstrates *your understanding* of the material rather than repeating those of the authors.

An example of plagiarism by paraphrasing

Each of the two paragraphs in the lab report below takes its organization and logical progression from one of two paragraphs in the indicated source, which is cited. Some of the individual phrases are not remarkable in themselves, and could theoretically have been independently coined. (Although some phrases are fairly particular – e.g., 'phenotype-based functional map.') Note that the progression of ideas in each paragraph, however, exactly matches that in each of the paragraphs in the source. This makes it clear that these phrases are taken from the source, despite some re-wording and re-ordering. It is interesting to note that the less common defining of siRNA as 'short interfering' (the abbreviation stands for '*small* interfering') found in the source is copied in the plagiarized passage.

Simply citing sources does not excuse the wholesale borrowing of ideas and phrases found there.

From plagiarized lab report introduction:

RNA interference (RNAi) is a method that uses double stranded RNA (dsRNA) to inhibit a gene's function. This is done in a sequence-dependent manner. Double stranded RNA is able to mimic the loss-of-function phenotypes of a particular gene when the dsRNA related to that particular gene is introduced to *C. elegans*. This dsRNA is referred to as dsRNA trigger. RNAi has been utilized to assess loss-of-function phenotypic data to construct a functional map of the *C. elegans* genome that is phenotype-based (Piano and Gunsalus, 2002).

RNA interference is used to regulate transcription, translation, as well as mRNA stability. The mechanism of RNAi is comprised of three steps. To begin, a dsRNA trigger is diced into small fragments in order to form short interfering dsRNAs (siRNAs). Then, these siRNAs are used to locate similar mRNAs and guide the cleavage of mRNA. This mRNA is then cleaved and degraded (Piano and Gunsalus, 2002).

From the Source (Piano & Gunsalus, 2002):

RNAi is a method that can specifically inhibit a gene's function in a sequence dependent manner using double-stranded RNA (dsRNA). When dsRNA corresponding to a particular gene (referred to as the dsRNA trigger) is introduced in *C. elegans*, it can mimic loss-of-function phenotypes of that gene. RNAi has been applied as a "functional genomics" approach to obtain loss-of-function phenotypic data associated with about one third of the currently predicted genes (Fig. **1a**), allowing an initial assessment of the prospects for using RNAi to build a phenotype-based functional map of the *C. elegans* genome.

Recent work has shown that RNAi is probably only one facet of several related and ancient phenomena that use small RNAs to regulate transcription, translation and mRNA stability (reviewed in [5, 13-15]; see also [16]). RNAi is thought to have evolved to protect cells from viruses and transposable elements. The RNAi pathway is comprised of at least three separable steps (Fig. **1b**). In the first step a dsRNA trigger is cut into small 21-23 nucleotide (nt) fragments to form "short interfering" dsRNAs (siRNAs). In the second step the siRNAs are used to find cognate mRNAs and guide cleavage of the mRNA. Finally, the cleaved mRNA is degraded.

Provisional Lab Schedule – Fall 2019 – Animal Development

Preparation for lab always includes reading the appropriate sections of the class lab manual, reviewing the embryology atlas, and (if indicated) viewing sections of *Vade mecum*[3] online (access code comes with the main text).

Sept. 4	Introduction to lab, safety, microscopy and measurements, Intro to *C. elegans*. Reading: 'Visualizing Cells,' Alberts et al., 2002 *Molecular Biology of the Cell* online (http://www.ncbi.nlm.nih.gov/books/NBK26880/). *Vade Mecum* online, sections on microscopy and lab safety. **Quiz on microscopy during lab.**
Sept. 11	*C. elegans* developmental genetics and reporter gene fusions. Discuss initial *C. elegans* project, form research groups, learn basic culture techniques for RNAi expt. ***C. elegans* quiz at beginning of lab. Digital pics of *lacZ* and FP reporter worms due by Mon. Sept 16, 5 PM.**
Sept. 18	Discuss and set up for first *C. elegans* RNAi / genetics experiment. **Finalize group genes selected for RNAi – individual proposals/literature reviews with first project plans due in class Fri. Sept 20.**
Sept. 25	Score phenotypes from first *C. elegans* RNAi / genetics experiment (or outside of regular class time). **First RNAi project reports due Wed., Oct. 2.**
Oct. 2	Echinoderm Gametes, Fertilization and Cleavage. Prepared starfish slide. Review *vade mecum*3, section on sea urchin. **Starfish and sand dollar digital pics due by Mon. Oct. 7, 5 PM.**
Oct. 9	Frog Embryogenesis (prepared slides & whole embryos). Review *vade mecum*3 section on amphibian and chick early development. Begin chick slides (wholemount). **Frog embryo & chick wm pics due Mon. Oct. 14, 5 PM.**
Oct. 16	Complete chick slide work, examine and dissect live chick embryos, start chick embryo histology. Review *vade mecum*3, section on histotechniques. Prepare for lab practical. **Chick section pics due Mon. Oct. 21, 5 PM.**
Oct. 23	**Lab Practical/Exam, turn in lab notebook and chick wholemount slide.**
Oct. 30	Begin Chick Teratogenesis experiments – Experimental design, using ANOVA; Inject embryos with teratogens. **ANOVA practice report due Wed. Nov. 6 in lab**
Nov. 6	Open eggs (Teratogenesis expt), begin evaluation and staining.
Nov. 13	Complete chick embryo histology and evaluation. Discuss / set up for next *C. elegans* research project lab. **Chick teratogenesis lab report due no later than Wed., Nov. 20, 5 PM.**
Nov. 20	*C. elegans* research project lab. (Note: work outside regular lab time.)
Nov. 29	THANKSGIVING HOLIDAY – No Lab
Dec. 4	*C. elegans* research project lab (continued).
Dec. 11	**Presentations** on *C. elegans* research project lab results. **Reports & project lab notebooks due by Fri., Dec. 13, 5 PM (last class day)**

Lab Report General Guidelines

When in doubt, refer to your official departmental writing guide: *A Student Handbook for Writing in Biology,* Knisely, 3rd Edition, © 2009. Lab write-ups for this class should generally take the form below (& see other instructions provided for a specific lab report):

Title: for example, "Fertilization in the sand dollar *Dendraster excentricus*," on a title page with your name. (If you're keen on saving paper, you may put the title on the same page as the introduction.)

Introduction: Introduce the topic, define key terms and explain the significance to study of development: give something about "the big picture." Please do not begin with "In this lab, we..." or "The purpose of this lab was to..." Try to make it interesting! For example: "Fertilization is the joining of male and female gametes to create a new life. Studying fertilization in a simple organism like the sea urchin can help us understand this process in more complex organisms, like humans..." Cite appropriate references. (Typically 1-2 pages in length)

Materials & Methods: a summary of important materials and organisms used. This should include the species and stages of embryos (the experimental subjects), materials used, and any experimental procedures performed. Your handout should be helpful in preparing this, but please don't make this section a repeat of the entire protocol, just a brief summary (e.g., "Worms were washed four times with M9 buffer to remove bacteria. The worms were then pipetted to a clean glass slide and desiccated in a vacuum chamber.") It's good to state the reason for a given procedure. You don't need to list basic supplies, such as Kimwipes, pipets and slides unless they are key to a specific result or new technique. You don't need to indicate trivial actions, e.g., that you 'labeled the tubes.' (Typically 1/2 - 1 page)

Results and Discussion:

(Note: For some reports, these sections will be written separately; for others they can be combined. Where separate, the results section is a "just-the-facts" section. The discussion is where conclusions and conjecture take place, as well as relating the results to other studies in the literature. The discussion should again end with "the big picture.")

The Results section should always constitute the bulk of your report. Include your observations and supplement, as appropriate, with labeled sketches you have made. Where possible, give numbers. What was the length of the slug or fruiting body; how many did you count? How long did it take for the changes you observed to occur? How many times (or in how many embryos) did you observe the phenomenon? You should be sure to report what you saw and not what you expected to see or what your fellow student reports that she saw. If you have no results to report and must use the observations or results of your fellow student, be sure to attribute the results appropriately. Also report what you tried, how many times and a guess as to why your observations or experiments failed. In "discussion" of your results, you should relate what you saw with what is already known, therefore, you should draw on information from lecture and/or reading. The lab report should include **all items explicitly requested in handout** - if something is missing due to failure of experiment, give

possible reason(s) for failure. (Length: as many pages as necessary, however, try to be succinct.)

The results section should always be in a *narrative form*, referring to data in figures and/or tables which are located *at the end of the report*. Long lists of all data should *never* be presented. Data should be processed / digested for the reader. Summarize data in tables, figures, charts, graphs, etc. Don't repeat details or numbers extensively in the text when they are presented in a figure, table or graph. Refer the reader to the item containing the data.

References

Works cited in your text should be listed on a separate page at the end of the report and use the following format described at the end of this section. Most references should be published literature (journal articles or books) and *not* websites. ***Wikipedia*** *or similar community-edited sites are not appropriate because information can be unreliable.*

Lab write-ups don't have to be epics. Try to keep them succinct. Length does not equal quality or a good grade; you shouldn't feel compelled to pad your reports. On the other hand, be sure to address points marked by a question in your lab handout. Feel free to ask the instructor about these issues if you're not sure.

Miscellaneous notes on preparing lab reports:

1. **Proofread** your report to avoid silly typographical errors or awful sentences like "Observations of the sea urchin embryos ... were observed." Spell-check can help, but will still miss many errors (including in spelling).

2. Make your prose **reader-directed** not **writer-directed.** That is, write so that what you say is clear to the reader, who cannot read your mind and may not have all the information you have at his fingertips. The report should be understandable to a reader independent of outside information. Would another biology major (not taking Animal Development) or one of your lab mates be able to understand what you have written? Imagine that you are reporting new findings to other scientists/biology students - without prior knowledge of the expected results.

3. Use the **past tense** when referring to something that you did in lab. You may use the **present tense** when referring to known facts relevant to your results. For example: "We added concentrated sperm to a slide of eggs to induce polyspermy. Sea urchin eggs are not normally exposed to high concentrations of sperm since the gametes are released into the sea and rapidly diluted." Often, such statements should be backed up with a citation. Also, whenever reasonable, use **active voice** versus **passive voice**. See the discussion of this issue on pp. 96-97 in the

4. Some common usage/spelling errors to avoid:
   a. Using the words **affect** and **effect** correctly -- You are usually safe if you use **affect** only as a verb, and **effect** only as a noun. Examples: “Alcohol had a detrimental effect on development of the embryo" (effect as noun) or "Alcohol affected the development of the embryo" (affect as verb).
   b. Less or fewer -- use **less** when modifying a mass amount; use **fewer** when modifying something countable. Examples: Less tissue, but fewer cells; less DNA, but fewer nucleotides.

5. **Avoid slang** and conversational English expressions. These have no place in formal writing. Samples from the previous lab reports: "a lot," "only so much could be seen," "In the part where..."

6. Avoid random abbreviations in your text. For example: "conc." for concentration; "difft" for different; "w/" for with. When using an abbreviation (such as for a long word used repeatedly in the text), spell the word out completely the first time it appears, followed by the abbreviation in parentheses. You may then use the abbreviation for the rest of the report. A list of approved abbreviations appears at the end of this section.

7. Be sure to underline or *italicize* the scientific names of organisms we work with, for example, *E. coli* or *C. elegans*. Do not capitalize the species portion of the name (e.g., do not write *E. Coli*) – note, this is a common problem caused by automatic capitalization after periods done by word processing programs – this can be caught by *proofreading*.

8. Draw and report on only what you actually saw in lab. If you cannot see a nucleus and acrosomal vesicle in a sperm head under the microscope, don't add it to your drawing.

9. Be sure to put **units** with your measurements, and make sure that your measurements make sense. Be sure you state what **dimension** you are measuring: length, diameter, etc.

10. Presenting numbers: If you give averages or percentages, show how numbers of items measured. How many measurements are averaged, how many were counted to obtain the reported percentage?

Example: All of the sperm tails measured 15 μm in length (n = 5).

Also provide some measure of the variability of the data with a statistical measure (standard deviation, SD, or standard error of the mean, SEM) or a range whenever appropriate.

For example, "The average diameter of the cells was 127 ± 15 μm (mean ± SD, n = 4)."
Or, "The average diameter of the eggs was 151 μm (Range 125 - 162 μm, n = 6)."

11. When writing decimal figures that are less than one, use a zero before the decimal point; i.e., write "0.4 g" and "0.01 ng" instead of ".4 g" and ".001 ng."

12. Use the following abbreviations in your lab reports. Do not put periods on the end or an 's' to pluralize. (E.g. use **"min"** not "min." or "mins" or "min's.")

Orders of magnitude:

milli	10^{-3}	kilo	10^{3}
micro	10^{-6}	mega	10^{6}
nano	10^{-9}	giga	10^{9}
pico	10^{-12}	tera	10^{12}
femto	10^{-15}	peta	10^{15}
atto	10^{-18}		

Mass:		**Volume:**		**Mol Wt/ Size/Amt:**	
gram	g	liter	l	dalton	Da

milligram	mg	milliliter	ml	kilodalton	kD
microgram	μg or ug	microliter	μl or ul	basepairs	bp
nanogram	ng			kilobases	kb
picogram	pg	**Time:**		megabases	Mb
kilogram	kg	second	sec	Molar	M
		minute	min	moles	mol
Activity:		hour	hr		
Unit	U	week	wk	**Other:**	
				° Celsius	C

Reference Format

(from the *Journal of Neuroscience*; the same or similar to many major science journals)

Within the body of the text, cite the author(s) and year of publication:

> The neurotransmitter serotonin has been detected in nematodes by various methods; it has been suggested to be involved in locomotion and reproductive behaviors (Croll, 1975; Horvitz et al., 1982; Avery and Horvitz, 1990).

Articles with more than two authors should be cited using the term **et al.**, which is an abbreviation for the Latin *et alia*, meaning "and others." Because **et** means "and," there should be no comma before it. Although such Latin expressions have traditionally been italicized, many journals no longer do this.

At the end of the paper, append a section entitled "References," with articles in the following format:

Journal articles:

> Croll N (1975) Indolealkylamines in the coordination of nematode behavioral activities. Can J Zool 53: 894-903.
>
> Horvitz HR, Chalfie M, Trent C, Sulston J, Evans P (1982) Serotonin and octopamine in the nematode *C. elegans*. Science 216: 1012-1014.

Book

> Gilbert S (2014) Developmental Biology. 10th ed., pp 123-124. Sunderland, MA: Sinauer.

Abbreviations of journal names in this reference form usually use only the first syllable or two, and only "J" for "Journal of." For example "Can J Zool " above is the Canadian Journal of Zoology. Single word journal titles (e.g., Cell, Science, Nature, etc.) are

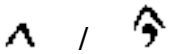
typically not abbreviated. (Note that there are no periods in journal abbreviations or author initials.)

Examples:

Full name of journal	Abbreviation
Annual Review of Genetics	Ann Rev Genet
Cell	Cell
Comparative & Biochemical Physiology	Comp Biochem Physiol
Development	Development
Developmental Genetics	Devel Genet
Genes and Development	Genes Devel
Journal of the American Medical Association	J Amer Med Assoc
Journal of Molecular Biology	J Mol Biol
New England Journal of Medicine	New Eng J Med
Proceedings of the National Academy of Science	Proc Nat Acad Sci
Journal of Teratology	J Teratol

Interpreting the instructor's markings on lab reports

Some of the following marks are standard proofreader's marks -- others are my particular notations.

In the margin	Meaning
sp	spelling
tr	transpose
	delete/take out
#	insert space
	close up / remove space
U or U?	usage incorrect or questionable
cap	capital
lc or l.c.	lower case
ital	italics
	Insert / e.g., insert comma
¶	make new paragraph
no ¶	no new paragraph, run in
ab	abbreviation non-standard or inappropriate
RO (cs)	run-on sentence (comma splice)
frag	sentence fragment
SVA	subject-verb agreement problem

Digital Embryo Photos for Bio 376 Lab

Turn in by email, preferably as a single file (unless prohibitively large). All images are due by 5 PM on the indicated due date. Images are done and turned in individually, not as a part of your research group. Note, these digital pics do *not* substitute for labeled drawings in your lab notebook.

For all images, **include both an unlabeled and labeled version of the image**. Images should be **centered**, **in focus**, and the subject should fill a good portion of the image (in other words, not too small). The field should be **evenly illuminated**, with a **good exposure** (not too dark or light) and **contrast**. The image should be of fairly high resolution, but try to keep the size of the document to a few MB for ease of emailing. To orient your image, note that the camera and to some degree the stage can be rotated to take photos. [**See the Rubric.**] By convention, organisms are usually oriented with anterior to the left (or sometimes up) and dorsal up (if possible). Ideally, images also have a labeled scale to show the size of the objects of interest.

Image names/titles should include something informative, no spaces, **plus your last name, or first 4 letters of your last name. If some else has the same last name, include a first initial.**

Examples of good image titles (with *my* name):
frog_neurula_LoerC.jpg / chick-48hr-xs-heart-LoerC.jpg / starfish_gastrula_LoerC.jpg

To label your images, use whatever image manipulation program you are comfortable with. (If you are not fully satisfied with your original image, you may also alter the brightness & contrast to enhance visibility of features prior to labeling). Image manipulation and labeling can be done, for example, in a Powerpoint slide. If you import your image into Powerpoint, you can present the unlabeled and labeled versions on successive slides. Also, expand the image *evenly* (vertical/horizontal) as needed to fill most of the slide. Be sure to complete any image manipulations *before* labeling so that you are using the same image for both versions. Make sure the scale is added *before* performing any size manipulations.

Labels on images should include anything noted for labeling in the lab handout (labeled like your drawings). This is not necessarily the same as found in the atlas - usually the atlas has a lot more structures identified. But, don't label something if can't be seen in the image (…so, if you can't identify much, perhaps you should try a different embryo/slide/section). If anatomical designations of animal/vegetal, dorsal/ventral, or anterior/posterior are possible, include them in your labeled image.

Due Mon Sept 16:
C. elegans lacZ strain – at least one photo showing stained worm(s)
C. elegans flourescent protein expression strain – at least one photo showing stained worm(s)

Due Mon Oct. 7:
Starfish (prepared slide) – at least one embryo of your choice (more than one cell).
Sand Dollar (live) – at least one photo with eggs & sperm / fertilized zygote / cleaved embryo(s), etc. [Note, I can show you how to set up DIC for a more beautiful image.]

Due Mon Oct. 14:
1. Frog – a. two embryo section pics of 2 different stages – cleavage/blastula, gastrula or neurula – your choice.
   b. one pic of a 4 mm frog cross section (xs) – your choice.
2. Chick wholemount – at least one embryo of your choice (24, 33 or 48 hr).
   [whole mounts may require more than one image that is montaged – joined together]

Due Mon Oct. 21:
1. Chick wholemount – another embryo of your choice (24, 33 or 48 hr), not same as last time.
2. Chick serial sections – at least one representative cross section from each 24 and 48 hr.

Rubric for Digital Embryo Photos (Labeled and unlabeled) – Bio 376

Area↓ Score→	Outstanding (10-8)	Good - Satisfactory (7 - 4)	Marginal (3 - 1)
Subject / Specimen	Subject is an excellent representative example of the type (e.g., correct stage of development, good example) Specimen is undamaged. Specimen shows clearly most or all of the desired structures.	Subject is a good to fair example of the desired type. Specimen may have some slight damage that does not interfere with identifying almost all or all of the desired structures.	Subject poorly represents the type desired, or is incorrect. Specimen may be damaged and/or many desired structures are not visible.
Lighting / Image quality	Brightness and contrast in specimen are excellent - neither too dim nor too bright; neither too little nor too much contrast. Microscopic field of view is evenly illuminated. Image resolution is high.	Brightness and contrast are good to fair. Field of view is well illuminated but may be less than perfect.	Image is dim or too bright, or low contrast or too high contrast, so that many structures are not visible. Image is unevenly lighted; portions are cropped; image is pixelated or distorted by uneven sizing.
Focus	Subject is in sharp focus. In a thicker specimen with multiple focal planes, the selected focus is consistent with desired structures to be seen, or a good compromise.	Subject is in good to fair focus, and most desired structures can be seen fairly well.	Subject is somewhat to very out of focus; many structures cannot be seen clearly.
Orientation	Subject is logically oriented (ideal – anterior to left, dorsal up or animal pole up).	Subject is fairly well oriented, and correctly identified.	Subject has a random orientation that is unclear, or incorrectly identified.
Labeling (labeled version of image) – note, max 40 pts (4 x score)	All labels are correct, including spelling of structure names. Labeling is complete, but without excess labels. Arrows or lines do not obscure other portions of the image that should be visible.	Most labels and spelling are correct, and mostly complete. Some may be missing or many extra, non-essential labels are present. Some labels, arrows etc. may slightly obscure structures.	Many labels are missing and/or incorrect; spelling is frequently incorrect. Arrows, etc. are missing and/or frequently obscure other structures. [0 – no labeled version]
Aesthetics (somewhat subjective)	Overall image is pleasing and beautiful. Subject is centered, and not clipped. Coloration is natural – similar or identical to what one sees by eye. Pleasing sizes, fonts, colors, and types of labels (labeled version).	Image is reasonably attractive or at least not distracting. Subject is pretty well centered and not clipped. Coloration may be a bit off. Choice of labels may be slightly distracting to viewer.	Overall image is displeasing or ugly. Subject is uncentered and cropped. Colors of image deviate greatly from that seen by eye. Poor choice of fonts, colors, and sizes/shapes of labels in labeled version may be odd, distracting or bizarre.
Other - scale	Labeled figure has a labeled scale in the lower right or left corner of the image that is attractive.	Labeled figure has a labeled scale that is satisfactory but may be too large or too small.	There is no scale, or the scale present is incorrect.

Adding a scale to your images:

1. Photograph a stage micrometer at the same magnification and resolution as your embryo image. (Note, once you've done this at each objective magnifications you should be able to reuse the images.)
2. Before performing any image size manipulations, copy a portion of the stage micrometer image into a lower corner of your embryo image.
3. For aesthetic reasons, you may wish to replace the actual stage micrometer image with another shape such as a rectangle of a matching a known size portion of the micrometer.