DEPARTMENT OF BIOLOGICAL SCIENCES
MCDB CORE COURSE SYLLABUS

FALL 2007 – SPRING 2008

MTHF 2:30-3:50

Crawford Hall, Room 343

Course Objectives

This is the primary course taken by graduate students during their first year in the Molecular, Cellular and Developmental Biology Graduate Program in the Department of Biological Sciences. It is an intensive course designed to provide instruction and stimulate discussion on all the important topics in molecular, cellular and developmental biology, with emphasis placed on the experimental approaches being taken to understand these topics. It is loosely divided into six modules: DNA structure and function, RNA structure and function, Protein structure and function, Genetics, Cell Biology and Developmental Biology. However, compartmentalization in Biological Sciences is becoming increasingly blurred so that an integrative approach will be emphasized.

Instructors

The course is taught by most of the faculty in the MCDB program, with an occasional guest lecture from researchers outside the Department. Instructors are usually experts in the fields being taught. The office location and e-mail address of each instructor is provided at the beginning of each module listed below. The Module Leader is the instructor denoted in each module with an asterisk (*) and should be consulted regarding any general issues for a particular module. If students are having difficulties with specific subjects being taught they are encouraged to contact the instructor directly responsible for teaching that subject.

Classes, Exams and Grades

A list of classes is provided for each of the modules. There are four classes per week which last ~1 hr 20 min. These will consist, in part, of standard lectures but questions and discussion on the subjects being taught are encouraged. Where appropriate, standard lectures will be replaced by discussions on relevant research papers that will be assigned prior to class. An exam will be set after each module and will only include material covered during that module (this includes any of the research papers discussed during class or assigned by the instructors). Unless informed otherwise, exams will take place in the same room and at the same time of day as the lectures. If a student feels a mistake has been made in grading their exam, they should return the relevant section to the appropriate instructor along with a written explanation.

MCDB students should be familiar with University of Pittsburgh policies related to Graduate Studies found in the FAS Grad-Guide (http://www.umc.pitt.edu/bulletins/graduate/FASinfo.htm), where it states that a minimum QPA of 3.0 is required of all graduate students (but is not a guarantee of promotion to the second year). For students in the MCDB program, even if a QPA of 3.0 is attained, any modules for which a grade of C+ or lower was attained must be repeated in the second year.

Reading materials

Required Text (for background reading): Molecular Biology of the Cell, Alberts *et al*., Fourth

Edition, Garland Science. Although this is an excellent textbook, it will only cover many subjects superficially so that additional background reading will be assigned.

Evaluations

Students will be requested to complete a questionnaire after completing each module. This will be done at the beginning of the next module and will remain anonymous. These surveys are invaluable for the faculty to improve the course in future years. Any suggestions from students on how the course could be improved are welcome at any time.

Module 1: DNA Structure and Function

Instructors

Name	Office Address	e-mail address	Phone	Lectures
Jeff Hildebrand	103 LSA	jeffh@pitt.edu	4-6987	1
Lew Jacobson	304 Langley	ljac@pitt.edu	4-4647	2-3
James Pipas	559 Crawford	pipas@pitt.edu	4-4691	4
Richard Wood	Hillman Cancer Center	rdwood@pitt.edu	3-7766	5-6
Graham Hatfull	A234 Langley	gfh@pitt.edu	4-4350	7
Anthony Schwacha*	560 Crawford	schwacha@pitt.edu	4-4307	8-10
Joseph Martens	247 Crawford	martens@pitt.edu	4-2649	11
Karen Arndt	269 Crawford	arndt@pitt.edu	4-6963	12-16

* Module leader

Introduction

Students will become acquainted with DNA, and how this material is physically and functionally integrated into the nucleus of a cell in both prokaryotes and eukaryotes. Beginning with a structural investigation of DNA and protein-DNA interactions and common techniques in molecular biology, this module will explore the 3 “Rs” of DNA (replication, recombination, and repair) with emphasis on the biological implications of these processes. The mechanistic aspects of transcription and its regulation will be presented with special emphasis on the important role that chromatin plays in this process. A lecture on the structure and function of the nucleus will highlight the dynamic properties that DNA plays within the organelle.

Lectures

Aug 27 **Hildebrand**

Lecture 1 Introduction

- Course expectations
- Library resources
- CourseInfo website
- What to do/who to go to if you are having problems

Aug 28 **Jacobson**

Lecture 2 Nucleic acid structure

- Physical parameters of nucleic acids
- Forces stabilizing secondary structure
- DNA bending
- Topology, supercoiling, topoisomerases

Aug. 30 **Jacobson**

Lecture 3 DNA/protein interactions

- Forces/structures that favor protein/nucleic acid interactions
- specificity of protein/DNA binding
- protein/DNA bending
- introduction to the nucleosome

Aug. 31: Note: Dept. Kick-Off meeting will be held at 3:30–CLASS BEGINS AT 2 PM

Lecture 4 Structure and function of the nucleus **Pipas**

- nuclear membrane, pores, lamins, transport

- chromosome cohesion
- chromosome condensation
- structure, organization and dynamics of chromosomes
- spatial organization of nuclear processes

Sept 3 — Labor Day

Sept 4 — **Wood**
Lecture 5 **DNA repair 1**
- DNA damage/mutagenesis
- DNA damage checkpoint
- Mismatch repair

Sept 6
Lecture 6 **DNA repair 2** — **Wood**
- translesion DNA polymerases
- base excision repair
- nucleotide excision repair

Sept 7 — **Hatfull**
Lecture 7 **Recombination 1 - Site specific recombination**
- Transposition phenomena and molecular details
- Immunoglobulin switching

Sept 10 — **Schwacha**
Lecture 8 **Mechanisms of prokaryotic/eukaryotic DNA replication**
- DNA polymerase – mechanism and limitations
- DNA replication accessory factors
- Initiation
- Elongation and function of the replication fork
- Termination and telomerase

Sept 11 — **Schwacha**
Lecture 9 **Regulation of DNA replication in prokaryotes/eukaryotes**
- Replication checkpoint and genomic instability
- Cell cycle control of DNA replication
- SOS response

Sept 13
Lecture 10 **Recombination 2 – General recombination** — **Schwacha**
- RecA, homologous recombination and meiosis
- Non-homologous end joining

Sept 14 **Department 30th Birthday Celebration** — **No Class**

Sept 17 — **Martens**
Lecture 11 **Replication of Chromatin – issues involved in copying the epigenetic state**
- Nucleosome addition
- Nucleosome modification
- DNA methylation

Sept 18 **Arndt**

Lecture 12 Prokaryotic transcription mechanism and control

- Bacterial RNA polymerase structure
- Sigma Factors
- Mechanism of initiation, elongation, and termination
- The impact of translation on transcriptional control

Sept 20 **Arndt**

Lecture 13 Eukaryotic RNA pol II and general transcription factors

- Promoter architecture and recognition
- RNA polymerase II structure
- Properties and functions of the RNA pol II carboxy terminal domain
- The general transcription factors

Sept 21 **Arndt**

Lecture 14 Regulation of RNA pol II transcription

- Activator and repressor proteins
- Coactivators and corepressors
- Mechanisms of activation and repression

Sept 24 **Arndt**

Lecture 15 Chromatin remodeling and modification

- Chromatin structure and its effects on transcription
- ATP dependent chromatin remodeling
- Histone modification
- Histone variants

Sept 25 **Arndt**

Lecture 16 Transcription elongation

- The role of the RNA pol II CTD during elongation
- The impact of chromatin on elongation
- Elongation factors and their mechanisms of action
- Coupling elongation with transcription termination and RNA processing

Sept 27 Reading Day

Sept 28 Exam I

<u>Techniques to be covered</u>

- Basic DNA cloning methodology
- DNA sequencing
- Polymerase chain reaction, RT-PCR
- Nucleic acid hybridization – Southerns and Northerns
- Protein/DNA footprinting
- EMSA
- Velocity sedimentation
- Antibodies and immunopreciptation
- Chromatin immunoprecipitation
- Microarray basics
- Chromatin technology – micrococcal nuclease ladders, indirect end labelling, TAU gels
- Primer extension analysis and S1 nuclease protection analysis

Module 2: RNA Structure and Function/Translation

Instructors

Name	Office Address	e-mail address	Phone	Lectures
Paula Grabowski	A502 Langley	pag4@pitt.edu	4-6983	2, 3, 6-10
Craig Peebles	357A Crawford Hall	cpeebles+@pitt.edu	4-4648	1, 5, 8
Roger Hendrix	A340 Langley	RHX@pitt.edu	4-4674	11-13
Jeff Brodsky *	274 Crawford	jbrodsky@pitt.edu	4-4831	14, 15

* Module leader

Introduction

This module will begin by examining the fate and functions of diverse RNA molecules during post-transcriptional processing pathways with an emphasis on pre-tRNA, pre-mRNA and pre-rRNAs. The roles of small RNAs and proteins in these processes will be examined in view of mechanism, regulation and quality control. We will then turn to ribosome assembly and the mechanism and regulation of protein synthesis. Finally we will examine polypeptide maturation, secretion and trafficking. Some problems to be discussed include: How do RNA molecules with only four types of nucleotide building blocks fold into functional and highly specific catalysts? Why are ribonucleoprotein machineries like the spliceosome and the ribosome modular, how are they regulated, and how is accuracy maintained? And, how are newly synthesized polypeptides “handled” by cytoplasmic “machines” during and after translation?

Lectures

Oct 1 — Peebles

Lecture 1 **Introduction** (evaluations for Module 1 will be conducted first)

- Diverse RNA Functions in Living Cells: Problems and Prospects
- Chemistry and conformations of RNA components; RNA structural motifs

Oct 2 — Grabowski

Lecture 2 **RNA Structure, Folding and Catalysis**

- Secondary/tertiary structure dynamics and role of metal ions
- Experimental methods to examine the inside and outside of functional RNAs (case study)

Oct 4 — Grabowski

Lecture 3 **Ribozyme Mechanisms and Self-splicing Introns**

- Group I and Group II introns
- Case study of the Tetrahymena P4-P6 catalytic core: conformational changes, tetraloop receptors and structured water molecules

Oct 5 — Peebles

Lecture 4 **Ribonucleoprotein ribozymes: RNase P and the ribosome**

- Role of RNAse P in pre-tRNA processing
- Evidence for ribozyme activities in RNase P and the ribosome
- Role of protein components

Oct 8 — Peebles

Lecture 5 **Protein machineries: CCA-adding Enzyme and Poly-A Polymerase**

- Problem: how to polymerize a specific nucleotide sequence without a nucleic acid template (case study)

Oct 9 Grabowski
Lecture 6 Pre-mRNA Processing, snRNPs and Spliceosomes
- 5´ capping, splicing and 3´ end formation
- Role of snRNPs in splicesome assembly, exon definition
- Proteomic analysis of the spliceosome; evidence for coupling (case study)
- RNA helicases and structural transitions
- Exon junction complex and nonsense mediated mRNA decay

Oct 11 Grabowski
Lecture 7 Alternative Pre-mRNA Splicing and Combinatorial Control
- Role of RNA binding proteins in splicing enhancement and silencing (case study)
- Computational and large-scale approaches for analysis; exon-junction microarrays

Oct 12 Grabowski
Lecture 8 Pre-rRNA Processing and snoRNAs
- How snoRNAs guide cleavages and chemical modifications in ribosome assembly pathways
- Ro and RNA folding quality control
- Protein composition of ribosome assembly intermediates (case study)

Oct 15 Grabowski
Lecture 9 Biochemistry of RNAi
- Phenomenon of mRNA interference mediated by small interfering RNAs
- Protein machineries involved in conversion of double-stranded RNA into siRNAs
- How is the guide strand of siRNAs delivered to the target mRNA? (case study)

Oct 16 Grabowski
Lecture 10 MicroRNAs and miRNPs
- Association of miRNAs in the SMN complex
- Role of miRNPs in snRNP biogenesis

Oct 18 Hendrix
Lecture 11 Protein synthesis I
- Overview & introduction: tRNA, rRNA, mRNA, genetic code, wobble
- Normal initiation and elongaton cycles, Prok & Euk
- Release factors and normal termination, Prok & Euk

Oct 19 Hendrix
Lecture 12 Structure and function of the ribosome
- Functions of the ribosome, antibiotic sites, R'some structure
- R'some sites (A, P, E), their functions & partial reactions,
- R'some sites (A, P, E), their functions & partial reactions, (decoding center, peptidyl transferase center, exit tunnel)

Oct 22 Hendrix

Lecture 13	**Nonstandard interpretations of mRNA reading frames** • Frameshifting (+1, -1, -2) • Hopping, skipping, sliding, etc • Trans-translation • IRESs and gene regulation (virus? Fe?)	
Oct 23		Brodsky
Lecture 14	**Secretion & maturation of polypeptides** • Signal sequences, SRP and entry to the secretory pathway • Spontaneous vs. Chaperone-mediated protein folding	
Oct 25		Brodsky
Lecture 15	**Protein trafficking and maturation** • ERAD and UPR response systems • Golgi maturation & modifications	
Oct 26		Reading Day
Oct. 29		Exam II

<u>Techniques to be covered</u>

UV absorption spectroscopy of helix-coil transitions involved in RNA folding
Computational tools for RNA structure prediction
Phylogenetic analysis of functional RNA structures
RNA footprinting with Fe(II) EDTA and other chemical reagents
Nucleotide analog interference analysis
SELEX (Systematic evolution of RNA ligands with exponential enrichment)
Affinity chromatography with maltose binding protein and tobramycin affinity tags
Proteomic analysis of spliceosomes using 2D-PAGE and mass spectrometry
Psoralen crosslinking and Northern blot analysis of mRNA/snRNA interactions
Filter binding and gel mobility gel shift analysis of RNA-protein complexes
Large-scale methods for analysis of alternative splicing; exon junction microarrays

Module 3: Protein Structure and Function

Instructors

Name	Office Address	e-mail address	Phone	Lectures
Roger Hendrix	A340 Langley	RHX@pitt.edu	4-4674	2, 3, 9
Linda Jen-Jacobson*	321 Clapp	ljen@pitt.edu	4-4969	10-13
Michael Grabe	242 Crawford Hall	mdgrabe@pitt.edu	4-4266	1, 8
Andy VanDemark	518 Langley Hall	andyv@pitt. edu	8-0110	4,5,7
Gordon Rule (CMU)	755 Mellon Institute	rule@andrew.cmu.edu	412-268-1839	6
John Hemple *	A356 Langley Hall	hempel+@pitt.edu	4-016	14-16

* Module leader

Introduction

This module will begin by reviewing the basic structural elements in proteins and will then introduce the techniques used to determine protein structure. We will also discuss the thermodynamics underlying protein structure and the forces involved in maintaining protein-DNA complexes. Finally, students will gain a basic appreciation of chaperone-assisted protein folding, enzyme kinetics, and techniques used in proteomics.

Lectures

Oct 30 — Grabe

Lecture 1 **Protein Structure: Introduction to forces at the molecular level** (Module 2 evaluations will be conducted first)

- vanderWalls / Ionic
- Hydrophobic effect
  - Hydrogen bonding, the peptide bond

Nov 1 — Hendrix

Lecture 2 **Protein Structure: Building blocks**

- Helices
- Strands
- Turns
- Loops & Disordered structure

Nov 2 — Hendrix

Lecture 3 **Protein Structure: Motifs & Domains; patterns of structures**

- All á structures
- All â structures
- á/â structures
- Other

Nov 5 — Van Demark

Lecture 4 **Protein Xtallography: properties of crystals, gathering data**

- The crystal state and its biological relevance

- Process of diffraction
- Image production

Nov 6 — Van Demark

Lecture 5 Protein Structure Determination: X-ray crystallography

- Why not all structures are created equal - validation
- Agreement with the diffraction data and stereochemistry
- Intro to computer graphics

Nov 7 — Rotation Talks (I)

Nov 8 — Rule

Lecture 6 Protein Structure Determination: 2D NMR

- Fundamentals of NMR Spectroscopy
- Multi-dimensional NMR
- Determination of protein structure by NMR
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Nov 9 — Van Demark

Lecture 7 Protein Structure: Working with structures, modeling techniques

- Modeling techniques and in silico mutagenesis
- Homology modeling
- State of the art: CASP and CAPRI

Nov 12 — Grabe

Lecture 8 Protein Structure: Folding – Thermodynamics & kinetics, prediction

- Interplay of Forces in Protein Folding
- Thermodynamic Signatures in Protein Folding
- Energy landscapes in Protein Folding

Nov 13 — Hendrix

Lecture 9 Protein Structure: Folding requiring chaperonins

- Mechanism of GroEL/ES function
- Post-translational folding trigger factor
- Chaperones and protein degradation
-

Nov 15 — Jen-Jacobson

Lecture 10 Protein-DNA Interactions:

- **DNA structural parameters**
- Sequence dependent DNA conformation and flexibility
- Sequence dependent recognition of DNA conformation

Nov 16 — Jen-Jacobson

Lecture 11 Protein-DNA Complexes: Structure and Energetics

- Structural view of Protein-DNA Interfaces
- Mechanisms for discrimination between closely related sites
- Favorable and Unfavorable Energetic Contributions to Specificity

Nov 19 — Jen-Jacobson

Lecture 12 Proteins-DNA interactions: Quantification

- Quantifying binding affinity

- Quantifying specificity
- Role of water and ions

Nov 20 Jen-Jacobson

Lecture 13 Protein-DNA Interactions: A case study

- Cooperative networks at protein-DNA interfaces and their energetic consequences
- Experimental evaluation of structural and energetic factors that contribute to specificity
- Integration of Structural and Energetic Perspectives

Nov 22, 23 **Thanksgiving** **No classes**

Nov 26 Hemple

Lecture 14 Introduction to Enzyme Chemical Mechanisms

- Pyridoxal-phosphate-dependent enzymes
- Serine and thiol proteases
- Aspartyl proteases
- Aldehyde dehydrogenase

Nov 27 Hemple

Lecture 15 Mass Spectrometry

- MALDI
- Electrospray Tandem mass spectrometry (MS/MS); de novo sequence determination

Nov 29 Hemple

Lecture 16 Proteomics

- The databases
- 2D gels, combinatorial approach to searches
- Virtual Mass Spec Lab

Dec 30 Reading Day

Dec 3 Exam III

<u>Techniques to be covered</u>

UV/Vis spectroscopy
Mass spectrometry
X-ray diffraction
2D NMR

Module 4: Genetics

Instructors

Name	Office Address	e-mail address	Phone	Lectures
Valerie Oke	527 Langley	voke@pitt.edu	4-4635	1-7
Jeff Hildebrand	103A LSA	jeffh@pitt.edu	4-6987	8-10
Jeffrey Lawrence*	346 Crawford	jlawrenc@pitt.edu	4-4204	11-15

Introduction

Genetics and genomics are approaches to address questions in many fields of biology. Rather than studying biochemical or physiological functions directly, inferences are made based on the effects of changes in the DNA. Therefore, genetics is a way of thinking rather than a body of knowledge. In this module you will learn approaches to examining biological problems based on analyzing small numbers of genes or whole genomes.

Lectures

Jan 7 Oke

Lecture 1 **Introduction** (evaluations for Module 3 will be conducted first)

- What is a gene?
- Types of crosses (monohybrid, dihybrid, backcross, testcross, reciprocal cross)
- Genetic concepts: genotype, phenotype, dominance, pleiotropy, penetrance, complementation, cis-trans position effect)

Jan 8 Oke

Lecture 2 **Genetics: a How-To guide**

- Choosing an organism—what do you need for genetics
- Overview of model organisms
- Screens vs. selections
- Controls and pilot experiments
- You get what you ask for, not necessarily what you want

Jan 10 Oke

Lecture 3 **Genetics: Tools of the trade**

- Selectable markers
- Insertion mutagenesis
- Mutagens
- Targeted mutations
- Markers and mapping
- Protein inactivation (temperature sensitive alleles)
- Inducible promoters
- Gene fusions
- Protein localization

Jan 11 Oke

Lecture 4 **Genetics: What you get**

- Types of mutations (gain of function, loss of function, lethal, conditional, etc)

- Penetrance and expressivity
- Pleiotropy
- Mutations that separate functions
- Suppression/reversion
- Gene interactions
- Ways of ordering pathways

Jan 14 — Oke

Lecture 5 Using genetics to dissect complex systems I

- Use papers to illustrate concepts introduced in the first four days. In depth analysis of two papers describing the genetics used to identify and order genes involved in vulval development in *C. elegans*.

Jan 15 — Oke

Lecture 6 Using genetics to dissect complex systems II

Jan 17 — Oke

Lecture 7 Inter-organismal genetics

- Use papers to illustrate concepts introduced in the first four days. In depth analysis of two papers describing genetic screens used to uncover interacting genes in both partners of a plant-pathogen interaction (Arabidopsis-*Pseudomonas*).

Jan 18 — Hildebrand

Lecture 8 Transgenes

- The production of genetically modified animals.
- Using transgenic animals to answer specific biological questions.

Jan 21 — Martin Luther King Day

Jan 22 — Hildebrand

Lecture 9 Epigenetics:

- Imprinting and specialize modes of gene regulation in mammals.

Jan 24 — Hildebrand

Lecture 10 Applications of the above methods and an introduction to interactions screens.

- Paper discussions of transgenics and epigenetics.
- 1-, 2-, 3-hybrid screens, small and genome-wide scales.

Jan 25 — Lawrence

Lecture 11 Tools and techniques of genomics

Jan 28 — Lawrence

Lecture 12 Similarity and difference: why they are not reciprocal

Jan 29 — Lawrence

Lecture 13 Doing genetics using genomics

Jan 31 — Lawrence

Lecture 14 New insights from genomics

Feb 1	Lawrence
Lecture 15 Metagenomics	
Feb 4	Reading Day
Feb 5	Exam IV
Feb 6	**Rotation Talks (II)**

Module 5: Cell Biology

Instructors

Name	Office Address	e-mail address	Phone	Lectures
Jeff Brodsky	274 Crawford	jbrodsky@pitt.edu	4-4831	1, 2
Kirill Kiselyov	523 Langley	kiselyov@pitt.edu	4-4317	3, 4, 14
Bill Saunders	264 Crawford	wsaund@pitt.edu	4-4320	5-7
Jeff Hildebrand	103A LSA	jeffh@pitt.edu	4-6987	8-10, 15
Beth Stronach *	202A LSA	stronach@pitt.edu	8-7658	11-13

* Module leader

Introduction

This module will focus on understanding some of the basic biological processes that modulate cellular behaviors. Of particular interest is understanding the molecular and biochemical nature of how cells regulate and carry out the complex processes that are needed for normal homeostasis as well as specialized cellular responses induced by extracellular signals. Emphasis will be placed on understanding how specific problems in cell biology are being addressed experimentally and how basic paradigms of cell biology are conserved in divergent organisms. Finally, where applicable, the cellular basis of human diseases and therapeutics will be discussed.

Lectures

Feb 8 — Brodsky

Lecture 1 **Introduction to the cell (evaluations for Module 4 will be conducted first)**
- Organelles
- Cytoskeleton
- imaging techniques

Feb 11 — Brodsky

Lecture 2 **The proteasome and protein degradation.**
- Ubiquitin ligases
- The proteasome

Feb 12 — Kiselyov

Lecture 3 **Protein and membrane trafficking I**
- membrane structure
- ER function
- Protein folding
- snares, COPI, rabs, etc

Feb 14

Lecture 4 **Protein and membrane trafficking II** — Kiselyov
- Exocytosis and endocytosis
- Mechanisms of protein sorting
- directional transport and secretion
- Lysosome function
- storage diseases

Feb 15 — Saunders

Lecture 5 **Basic of Cytoskeletal Elements:**
- Actin, microtubule, and intermediate filaments
- Motor proteins
- regulatory proteins

Feb 18 Saunders
Lecture 6 **Cell division cycle and checkpoint control**
- Mechanics of cell cycle progression
- Error signaling
- Mechanisms of cell cycle arrest
- Cell cycle defects in human diseases

Feb 19 Saunders
Lecture 7 **Cell division**
- Spindles
- Kinetochores
- Cytokinesis
- Divisional defects in human disease

Feb 21 Hildebrand
Lecture 8 **Cytoskeletal dynamics II:**
- Rho-family GTPases
- Cytoskeleton in cell migration and other processes.

Feb 22 Hildebrand
Lecture 9 **Cell-cell and cell-ECM interactions**
- Adherens junctions
- Integrins
- Tight junctions
- Regulation

Feb 25 Hildebrand
Lecture 10 **Cellular polarity**
- apical-basal polarity
- embryonic polarity
- planar polarity

Feb 26 Stronach
Lecture 11 **Cell signaling I:**
- RTK and Map kinase
- protein scaffolds
- signal integration

Feb 28 Stronach
Lecture 12 **Cell signaling II:**
- Wnts, SHH, BMPs

Feb 29 Stronach
Lecture 13 **Apoptosis**
- Death Receptors
- Caspases
- Pro and anti-apoptotic proteins

- AKT

Mar. 3 — Kiselyov
Lecture 14 Cell signaling III
- Calcium
- Lipids
- GPCR
- Rafts

March 4 — Hildebrand
Lecture 15 Intracellular pathogens: bacterial and viral
- Probes for cellular processes
- Subversion of growth regulation
- Subversion of the cytoskeleton

March 6 — Reading Day

March 7 — Exam V

<u>Techniques to be covered</u>

- imaging (fixed and real time)
- FRET
- siRNA
- Fractionation
- yeast screens mutations in specific transport pathways
- biochemical assays (kinase, phosph-specific antibodies, protein-protein interactions)
- 3-D culture system
- electrophysiology
- migration assays
- EM

Module 6: Developmental Biology

Instructors

Name	Office Address	e-mail address	Phone	Lectures
Deborah Chapman*	219 Clapp Hall	dlc7@pitt.edu	4-0774	1, 3-6, 14
Gerard Campbell	215 Clapp Hall	camp@pitt.edu	4-6812	7-9, 12-15
Lew Jacobson	304 Langley	ljac@pitt.edu	4-4647	10
Beth Roman	201A	romanb@pitt.edu	3-5297	2, 11

* Module leader

Introduction

This module will integrate the knowledge you have gained on nucleic acids, proteins, genetics and cell biology into understanding the mechanisms used to transform a single cell, the fertilized egg, into a multicellular animal. A review of different model systems will be backed-up by an in-depth analysis of the molecular mechanisms operating to control pattern formation, cell differentiation, cell movement and cell proliferation during embryogenesis. This will include discussions of the role of cell-cell signaling via different signaling molecules, localized determinants in eggs, and how developmental paradigms have been modified over the course of evolution.

Lectures

March 10-14 Spring Break, No classes

March 17 — Chapman

Lecture 1 **Introduction** (evaluations for Module 5 will be conducted first)

- Outline of module
- Basic principles; differentiation, pattern formation, induction
- Definitions/ terminology and processes
- Brief introduction to model systems

March 18 — Roman

Lecture 2 **Model systems I: Zebrafish**

- Introduction to the system; specific techniques, transplantation, genetics; advantages/disadvantages
- Basic embryogenesis; cleavage; Fate mapping
- Signaling centers/organizers

March 20 — Chapman

Lecture 3 **Model systems II: Chick and Mouse**

- Introduction to the system; specific techniques, transplantation, genetics; advantages/disadvantages
- Basic embryogenesis; cleavage; Fate mapping
- Signaling centers/organizers

March 21 — Chapman

Lecture 4 **Axis formation in vertebrate embryos I**

- A/P patterning
- D/V patterning
- Signaling pathways

- Cell-cell interactions
- Cell movements/morphogenesis
- Molecular analysis of embryogenesis

March 24 Chapman
Lecture 5 Axis formation in vertebrate embryos II

March 25 Chapman
Lecture 6 Axis formation in vertebrate embryos III

March 27 Campbell
Lecture 7 Model systems III: *Drosophila*

- Introduction to the system, techniques; advantages/disadvantages
- Description of embryogenesis
- Genetic screens for patterning mutants
- Localized determinants in eggs and egg shells
- A/P patterning/segmentation: from gradients of transcription factors to boundaries
- D/V and terminal patterning: graded extracellular gradients to intracellular gradients
- The chicken or the egg – generation of localized determinants in eggs during oogenesis

March 28 Campbell
Lecture 8 Axis formation in *Drosophila* embryos II

March 31 Campbell
Lecture 9 Axis formation in *Drosophila* embryos III

April 1 Jacobson
Lecture 10 Model systems IV. Axis formation in *C. elegans*

- Introduction to the system, techniques; advantages/disadvantages
- Description of embryogenesis
- Cell lineage
- Blastomere specification

April 3 Roman
Lecture 11 Organogenesis I: The vascular system

- Introduction to the vascular system
- Determinants of vascular organization
- Cellular interaction in vascular development
- Molecular basis of angiogenesis/vasculogensis
- Developmental defects and disease

April 4 Campbell
Lecture 12 Organogenesis II: Limb development

- Limb development in *Drosophila*
- Limb development in vertebrates
- Signaling centers
- Signaling and gene expression

April 7 Campbell

Lecture 13 Morphogens and developmental gradients

- Secreted signaling polypeptides as morphogens
- Theory and tests for morphogens
- Examples of morphogens
- Establishing and regulating signaling gradients

April 8 Chapman

Lecture 14 Germ cells and stem cells

- What are stem cells and where can they be found
- Niches
- Pluripotency
- Cloning
- Germ cells: what are they and where do they come from?

April 10 Campbell

Lecture 15 Transcriptional regulation of development

- Hox genes: master control genes
- Signal modulated transcription factors and tissue specific transcription factors
- Identification of cis-regulatory elements
- Characteristics of cis-regulatory elements
- Identifying targets of transcription factors

April 11 Campbell

Lecture 16 Developmental mechanisms of evolutionary change

- Universality of developmental mechanisms
- Changes in body plan: acquisition and loss of structures
- Gene duplication and divergence

April 14 Reading Day

April 15 Exam VI

April 16 Rotation Talks (III)

Techniques to be covered

- Manipulation of embryos
- Genetic screens to identify developmental mutants
- Reverse genetics in model systems, transgenic techniques
- Creating genetic mosaics
- Misexpression of genes in different systems
- Analyzing gene expression: in situ hybridization, antibody staining, reporter genes