General goals of BIOL 331-Part 1:

- To convey the message cells are **dynamic**.
- To expose students to cells of a wide range of organisms (prokaryotic and eukaryotic model organisms).
- To conceptualize cellular strategies- why do eukaryotic cells have compartments? How did these compartments arise during evolution? Discuss evidence and controversies of current theories.
- To familiarize students with the basic properties of cellular components, organelles and trafficking processes.

**Independent learning goals:**

- To allow students to look for answers to problems which were solved by scientific discoveries, and contrast them with the most current accepted answers.
- To motivate and guide students to read not only the chapter from Karp, but review the literature as well (at least reviews on a topic). This should allow students to identify what kind of questions researchers are currently asking.
- To stimulate students to formulate questions related to lecture topics and to address selected questions in lectures and tutorials.
- To direct students to explore the WWW for images and movies resources in the field of cell and molecular biology.
- To provide students with basic knowledge of tools and techniques used in cell and molecular biology (tutorials) in order for them to interpret evidence and to relate it to the material learned (lectures).

**CONTENT**

**General**

- basic properties of all cells
- differences between *prokaryotic* and *eukaryotic* cells.
- dimensions important to Cell Biology

**Membranes**

- Membranes organize cells into functionally distinct compartments.
- Fluid mosaic model of cell membranes (Singer and Nicolson, 1972), what has changed 40 years later?
- Chemical composition of membranes
- Role of membranes- Membrane fluidity
- Membrane proteins
- Membrane transport -selective permeability Active and passive transport across membranes
- Membrane potential
- Electrochemical gradient
• Non-excitable and excitable cells- ion channels
• Nerve impulse-action potential, depolarization, refractory period

**Endomembrane system**
• Biosynthetic –secretory and endocytic pathways
• Ribosome assembly and protein synthesis
• Endoplasmic reticulum
• Protein synthesis in the ER -Synthesis of Proteins on Membrane-Bound versus Free Ribosomes. What determines where a protein is synthesized?
• Post-translational modifications, mechanisms of quality control
• Golgi complex
• Vesicular traffic: specificity of the fusion reaction- directionality of anterograde and retrograde pathways- signalling lipids (phosphoinositides), coats, G-proteins, SNAREs
• sorting at TGN
• lysosomes
• endocytosis
• Receptor-mediated endocytosis of LDL (example of Nobel prize 1985)

**Other organelles**
• Location, location, location, how does a protein get to the correct cellular location?
• Mitochondrion
• Chloroplast
• Peroxisomes
• Lipid droplets

**Cytoskeleton**
• Microfilaments
• Microtubules
• Intermediate Filaments
Lecture plans for three plant cell biology lectures in Biology 331 – Doug Muench

These three lectures are typically offered midway through this course, and follow lectures that contain material related to membrane/organelle biogenesis and function. These membrane/organelle lectures gives the students with a strong knowledge base that provides a nice segue into the plant lectures.

The material presented in these three plant lectures focuses on aspects of cell biology that are unique to plant cells, and is divided into 6 parts. The material that is presented in the first lecture is quite broad in scope, discussing plant cell evolution, basic structural features of plant cells, intracellular movement and the cytoskeleton. The second and third lectures are more focused, and emphasize cell wall biogenesis, cell division, plasmodesmata and vacuoles.

Lecture 1 - An Introduction to Plant Cell Biology (Part I) and the Cytoskeleton (Part II)

- General characteristics of plant cells – plant organelles, cell wall, cell types, totipotency, dynamic movement of organelles
- Cytoskeleton – cytoskeletal components, motors, microtubule organizing centres, the four microtubule arrays

Lecture 2 – Cell Wall (Part III) and Cell Division (cytokinesis, Part IV)

- Cell wall - structure and components, cell wall synthesis and organization, cell wall involvement in cell growth
- Cell division – cell plate formation, pre-prophase band of microtubules, phragmoplast, deposition of Golgi-derived vesicles

Lecture 3 – Plasmodesmata (Part V) and Vacuoles (Part VI)

- Plasmodesmata – structure and biogenesis, selective movement of macromolecules, role in non-autonomous cell development
- Vacuole – structure, roles, two types of vacuoles
In the second half of Biol 331 we cover 4 main modules for which the lectures come full circle and are interconnected. Tying the material together is very important for higher concept level learning.

1. Interactions between cells and environment
2. Cell communication
3. Control of cellular differentiation and epigenetic reprogramming

LECTURE 1 Cell-cell interactions

Tie lecture into Tumor suppressor lecture at the end, come full circle with E-cadherin

Objective: How do ‘junctionless’ proteins orchestrate cell to cell interactions?

Learning Objectives:
- What are some adhesive proteins and how do they contribute to selective cell interactions?
- ...During embryogenesis?
- ...During inflammation?
- What are the steps in the process of transendothelial migration (during inflammation)?

Principles of cell to cell interactions
- Cells can selectively interact with each other to organize organs or to carry out a specific function
- Cells interact using very specific protein-protein interactions

Topics covered
- Selectins
- Immunoglobulin super family
- Members of the Integrin family
- Cadherins
- Cell selective interactions during embryogenesis
- Cell interactions during inflammation – invasion and extravasation

Textbook readings
Karp 6th edition p245-250
Sections 7.3 – cadherins (inclusive)

Additional web sites:
http://astro.temple.edu/~jbs/courses/204lectures/neutrophil-js.html
http://multimedia.mcb.harvard.edu/media.html
LECTURE 2  Cell Junctions
Tie into apoptosis lecture, substrates of caspases and with cancer biology lectures, tumor suppressors

**Objective:** How do proteins of epithelial cell junctions contribute to tissue permeability, cell polarity, tissue strength, cell-cell communication and cell motility?

**Learning Objectives:**
- What is the function of each cellular junction?
- Where are they located with respect to an epithelial cell attached to the extracellular matrix?
- Which cellular junction(s) binds the actin cytoskeleton and which one(s) binds the intermediate filament cytoskeleton?

**Principles of cell junctions**
- Cells can contact each other through specialized contacts
- The proteins in these contacts interact specifically to others on the neighboring cell or substratum...
- ...and to other proteins inside the cell
- The properties of these proteins and their interactions dictates their function

**Topics covered:**
- The junctional complex
- Tight junctions
- Adherens junctions
- Desmosomes
- Gap junctions
- Focal adhesions
- Hemidesmosomes

**Textbook readings**
Karp p250-258
7.3 Adherens Junctions... To end of Gap Junctions (not plasmodesmata)

**Additional web sites:**
http://jcb.rupress.org/content/suppl/2001/12/20/jcb.200107107.DC1/1

LECTURE 3 Extracellular matrix - (ECM)  
Tie this lecture in with cell-interaction lecture – integrins and back into protein secretion in first half of course.

**Objective:** How do proteins of the ECM direct tissue properties, cell behaviour, survival and motility?
Learning Objectives:
- What is the extracellular matrix?
- Name and describe some component proteins of the ECM, detailing if and how they bind to proteins of the same family, to other ECM components or the cell surface.
- What is an RGD sequence? Which proteins carry this sequence and to which proteins can they bind and why?

Principles of cell-substrate interactions
- Solid tissues can only be formed if cells interact in specific ways with other cells and the extracellular material.
- Cells interact with the extracellular material using specific protein-protein interactions.
- The extracellular material can in turn influence cell behaviour.

Topics covered:
- Glycocalyx
- Extracellular matrix (ECM)
- Focal adhesions connect to the ECM
- Integrin signaling – inside-out and outside-in
- RGD peptides and RGD binding site
- ECM and cellular differentiation
- ECM proteins – collagen, proteoglycans, fibronectin, laminin

Textbook readings
Karp p230-245
Sections 7.0-7.2 inclusive

Additional web sites:

LECTURE 4 Signal Transduction I Communication between cells - Introduction

Objective: How do external signals make a change inside the cytoplasm and nucleus (instruct gene expression)?

Learning Objectives:
- What are the different mechanisms that cells can send and receive signals?
- Why are phosphorylation and dephosphorylation important?
- What are the steps during prolactin signal transduction?

Principles of signal transduction
- Most signals are extracellular and must bind a receptor to be functional.
- Signals that can’t pass the membrane must transmit their signal by a change in the receptor’s shape (conformation).
• The signal is amplified in the cell

Topics covered:
- Cell signaling mechanisms
- Formation and action of cell stimuli
- Classes of receptors
- Extracellular messengers
- Basic elements of cell signaling systems
- Second messengers
- Phosphorylation cascades
- Reversible phosphorylation
- Control of signal transduction
- Prolactin-Jak2-Stat signal transduction

Textbook readings
Karp p605-609
Section 15.0-15.2 inclusive

Additional web sites:

LECTURE 5 Cell Signaling II - G protein coupled receptors and cAMP

Objective: to understand how G-protein coupled receptors function, using an example of glucagon signaling

Learning Objectives:
• How do G proteins act as a switch for signal transduction?
• How are heterotrimeric G proteins regulated?
• Define the term ‘second messenger’ and how does it function in the glucagon pathway of glucose mobilization?

Principles of
• The receptor undergoes a conformational change during activation or deactivation
• G-proteins help activate specific effector molecules
• Signal amplification takes place at key steps in cascade

Topics covered:
• G-proteins are a common regulatory switch
• G-protein coupled receptors
• Heterotrimeric G-protein activation and termination of response
• Cyclic AMP as a 2nd messenger and the example of glucose mobilization with adenylyl cyclase as the effector
Textbook readings
Karp 609-612; 614; 619-622
Section 15.3 (G-protein coupled receptors and termination of the response); Second messengers cAMP; Glucose mobilization including cAMP signal transduction pathways

Additional web sites:
http://highered.mcgraw-hill.com/sites/0072507470/student_view0/chapter17/animation__membrane-bound_receptors_that_activate_g_proteins.html

LECTURE 6 Cell Signaling III: IP3/Ca^{2+}/PKC pathways

Objective: to understand how lipid based second messengers are generated using an example of acetylcholine regulation of smooth muscle contraction

Learning Objectives:
- How are second messengers generated from phospholipids (e.g. phosphatidylinositol)?
- Identify the effector in the generation of lipid second messengers and their signal amplification.
- Describe a method to measure the concentration of free calcium ions in a living cell.
- How do Ca^{2+} ions act as second messengers? How is their concentration carefully regulated?

Principles of IP3/Ca^{2+}/PKC pathways
- Some 2nd messengers are generated from lipids
- Calcium can act as a 2nd messenger
- 2 messengers amplify the signal transduction cascade

Topics covered:
- phospholipids and phospholipase generation of second messengers
- PH domain
- generation of second messengers from breakdown of phosphoinositides
- Activation of a G protein coupled receptor by acetylcholine – for smooth muscle contraction
- IP3 receptor is a ligand gated calcium channel
- How can we visualize calcium in a cell?

Textbook readings
Karp 614-617; 634-638
Sections 15.3, 15.5
Additional web sites:

**LECTURE 7 Cell Signaling IV: Receptor Protein-Tyrosine Kinases**
*Tie this lecture into G-protein lecture*

**Objective:** understand how intrinsic protein tyrosine kinases propagate signal transduction eg. Ras-MAP kinase pathway

**Learning Objectives:**
- How does ligand binding lead to receptor activation? Compare and contrast ligand-induced receptor dimerization versus receptor-mediated dimerization.
- What is an SH2 domain? Provide examples of proteins with this domain.
- How are RTKs linked to Ras proteins, and what is the function of the components?
- Name three modulators of monomeric G proteins (accessory proteins) and discuss how they regulate G protein activity.
- What is convergence, divergence and cross-talk?

**Principles of Receptor Protein-Tyrosine Kinases (RTKs)**
- RTKs possess tyrosine kinase activity within a domain of the receptor amino-acid chain
- A conformational change induced by ligand binding activates the kinase activity
- The kinase activity enhances its own activity through auto-phosphorylation of the receptor chain and phosphorylation of the receptor also creates docking sites for signaling molecules

**Topics covered:**
- Steps in the activation of RTKs
- Different RTK ligand binding domains
- Docking site partners
- Diversity of signaling proteins and examples of – adaptor proteins, docking proteins, transcription factors, signaling enzymes
- Ras small monomeric G-protein – compare with heterotrimeric G-protein in earlier lecture
- The cycle of small monomeric G-proteins
- The AMPK pathway – illustration of an RTK, Ras G-protein activation, phosphorylation cascade and cell function
- Introduce cross-talk – convergence, divergence – use examples from the past Cell Signaling lectures to review

**Textbook readings**
Karp P623-632;639-640
Sections 15.4 (up to glucose transport), 15.6

**Additional web sites:**

**LECTURE 8 Steroid hormones and gene regulation**

**Objective:** to understand how transcription factors and chromatin can contribute to gene regulation (epigenetics).

**Learning Objectives:**
- Understand the zinc finger motif of how transcription factors can interact with DNA
- Describe how chromatin modifiers can also regulate transcription both positively and negatively

**Principles of Epigenetics**
- The DNA and protein that makes up chromatin can be modified chemically and this contributes to gene regulation

**Topics covered:**
- Nuclear hormone receptors, eg. Estrogen receptor, glucocorticoid receptor
- DNA methylation, histone acetylation, histone methylation
- A model for transcriptional activation at the promoter
- A model for transcriptional repression at the promoter

**Textbook readings**
Karp p508-521
Section 12.4 (The role of TF... To end of DNA methylation)

**Additional web sites:**
http://www.youtube.com/watch?v=GRL_rdB30GY

**LECTURE 9 Cell proliferation, tissue renewal and stem cells**

Tie this lecture into epigenetic control of gene expression

**Objective:** to understand the difference between reproductive and therapeutic cloning and the reprogramming events involved in cloning

**Learning Objectives:**
- Define therapeutic versus reproductive cloning
- Define self-renewal versus commitment
- Define potential for differentiation and know what controls it
• Discuss the concept of plasticity

**Principles of Stem Cells**
• Every tissue likely has a tissue specific stem cell for repair and maintenance
• Only the embryo is totipotent, the embryonic stem cell is pluripotent, the tissue specific stem cell is multi-potent

**Topics covered:**
• Cloning for therapeutic purposes or reproduction
• Stem cell capacity, totipotent, pluripotent, multipotent
• Cellular differentiation involves epigenetic changes
• Developmental potential and epigenetic states of cells at different stages of development
• Epigenetic changes are reprogrammed during cloning in the egg

**Textbook readings**
Karp 19-21; 503-504
Human Perspective Prospect of cell replacement therapy, Section 12.3

**Additional web sites:**

**LECTURES 10 and 11 Cell cycle**
Tie these lectures to growth factor initiation of the cell cycle and to DNA damage checkpoints

**Objective:** understand how the cell regulates the precision of cell division events

**Learning objectives:**
• Describe each phase of the cell cycle.
• How are cdks and cyclins regulated?
• What do cell fusions tell us about control over the cell cycle?
• Describe the roles of CAK and Wee1 in the regulation of the cdk-cyclin complex.
• How is the cell cycle regulated by proteolysis?
• What are cdk inhibitors and how do they function?

**Topics covered:**
• Phases of the cell cycle
• Mitotic cyclins
• Cdk inhibitors
• Cell phase transitions
• pRetinoblastoma
Textbook readings
• Karp 560-567; 570; 599-602
• Section 14.1

LECTURE 12 DNA damage and Cell cycle checkpoints
Tie this lecture into Cell Cycle lectures (Wee1, CDC25, p21, cdk4, cdc2) and be prepared to tie together with tumor suppressor and apoptosis lecture materials

Objective: understand how the cell responds to different forms of DNA damage that allows time for repair, repair or death

Learning Objectives:
• Distinguish the major pathways of the DNA repair response, involving ATM and ATR
• Understand the function of the sensors, signal transducers and effectors in these pathways, and
• Understand the consequences of their activation during G1/S and G2/M phases of the cell cycle.

Principles of DNA damage and cell cycle checkpoints
• Normal cells can not proceed through the cell cycle if their DNA is damaged
• If cells override the cell cycle checkpoints with existing DNA damage, this could lead to cancerous mutations

Topics covered:
• Sources of DNA damage
• ATM and double strand DNA breaks
• Review G2/M and G1/S checkpoints
• Models for the mechanism of action of two DNA damage checkpoints
• ATM, ATR, BRCA1(highlight), CHK1, CHK2, p53, p21
• DNA damage and the G1/S checkpoint – wee1 and cdc25
• DNA damage and the G2/M checkpoint

Textbook readings
Karp 567-569
Section 14.1 Checkpoints, Kinase Inhibitors, and Cellular Responses

Additional web sites:

LECTURE 13 Apoptosis - Programmed Cell Death
Tie into cancer biology and DNA damage lectures

Objective: to understand how a cell can initiate a suicide program or respond to external death signals
Learning Objectives:
• Compare apoptosis versus necrosis.
• Describe the normal roles that apoptosis fulfills.
• Discuss the role of caspases.
• Compare the internal and external signals of apoptosis and the order and nature of the molecular events for each pathway.

Principles of apoptosis
• Programmed cell death (apoptosis) avoids an immune response to the cellular contents
• Can be initiated from an internal (intrinsic) or an external (extrinsic) stimulus

Topics covered:
• Failure of DNA damage repair leads to programmed cell death
• Necrosis vs apoptosis
• Examples of apoptosis in the body
• Stages of apoptosis
• Caspases and their substrates
• Ladder assay
• The extrinsic, receptor-mediated pathway of apoptosis – eg. Tumor Necrosis Factor
• The intrinsic, mitochondria-mediated pathway of apoptosis

Textbook readings
Karp 4th 642-646
Section 15.8

Additional web sites:

LECTURE 14 Cancer Biology
Tie back to first lecture – E-cadherin, cell interaction lectures, cell cycle

Objective: to understand the causes, properties and progression of cancer

Learning Objectives:
• List and define the properties of cancer cells
• Understand which viruses are involved in transformation
• Describe the steps and proteins involved in metastasis (spread of cancer)

Principles of Cancer Biology
• List and define the properties of cancer cells
• Understand which viruses are involved in transformation
• Describe the steps and proteins involved in metastasis (spread of cancer)

Topics covered:
• Sarcomas, leukemias and lymphomas, carcinomas
• Incidence of cancer
• Properties of cancer cells
• Abnormal karyotype – major genetic aberrations
• Density dependent inhibition of growth – loss of
• Contact inhibition – loss of
• Serum induced growth – loss of dependence
• Progression of cancer, colon carcinoma as an example
• Viruses and cancer

Textbook readings
Karp 4th 650-656; 669-671
Sections 16-16.3, up to Tumor-suppressor Genes... But including Gene Expression
Analysis

Additional web sites:

LECTURE 15/16 Tumor suppressors, Proto-oncogenes and Oncogenes (newly condensed from 3 to 2 lectures)

Tie these lectures into cell-cell interaction lecture, cell junction lecture, cell cycle lecture, DNA repair lecture, RTK lecture, apoptosis lecture...

Objective: to understand how oncogene activation and tumor-suppressor loss contribute to cancerous progression

Learning Objectives:
• How does Knudson’s two hit hypothesis apply to tumour suppressors?
• Describe the tumour suppressive function of pRB and p53
• distinguish between proto-oncogenes and oncogenes
• describe different mechanisms to activate oncogenes
• know the names and functions of common oncogenes

Principles of tumor suppressors, proto-oncogenes and oncogenes
• Tumor-suppressors are the brakes on the cell and will be lost during cancerous progression
• Protooncogenes are the accelerators on the cell and will be activated to oncogenes during cancerous progression

Topics covered:
• Knudson’s 2 hit hypothesis
• Tumor suppressors – RB, retinoblastoma – tie into cell cycle lecture
• Tumor suppressor – p53, BRCA1/2 - tie into DNA repair lecture
• Oncogenes – at the focal adhesion – tie into lecture on cell interactions, c-myc, cyclin D1, Ras – tie into RTK lecture, BCl2 – apoptosis lecture
• Protoconcogene activation

Textbook readings
Karp 4th p677-697
  Karp 5th p670-681

Additional web sites: