

Fall 2009
AMC/BMS Core Curriculum
(AMC 505 / BMS 500)
MOLECULAR CELL BIOLOGY



9:00 - 11:00 AM
Tuesday and Thursday
MS-316
(4 credits)

Drs. Bill Wolfgang (BMS) and Susan LaFlamme (AMC)
Course Directors

Course Policies

The faculty from the Albany Medical College and from the Department of Biomedical Sciences of the SUNY School of Public Health welcomes you to AMC505/BMS500: Molecular Cell Biology. This course is part of the core curriculum requirements for all first year graduate students at Albany Medical College and in the BMS Department of the SUNY School of Public Health.

Lectures

This course covers subject areas that are essential knowledge for more advanced courses in all tracks and training programs at both institutions. Topics covered include Genetics/Genomics, Information Flow, Cytoskeleton, Cell Signaling, and Cell Division. Each set of lectures is designed to provide both a basic understanding of the subject and an appreciation of current experimental research in the field. This is intended to provide the student with essential knowledge to make significant and innovative contributions in the future.

Instructors will utilize classical and current research literature including use of primary data to define, analyze and complement their lectures. As a rule, the literature requirement will be one research paper and one review per four hours of lecture. The review articles will be taken from journals such as *Current Opinions*, *The Journal of Biological Chemistry*, *Cell*, *Molecular Cell* and the *Trends* journals. Materials from the assigned readings will be used within the lectures to impart general information, illustrate experimental design and methods, design homework problems, summarize and interpret the data in an exam, and stimulate discussion.

Suggested Textbook

The faculty of Molecular Cell Biology recommends that each student have *Molecular Biology of the Cell*, 5th edition (Alberts, et al). Instructors will also use material from other textbooks, especially *Molecular Cell Biology*, 5th Ed. (Lodish, et. al.). All textbooks used by instructors will be on reserve at both the AMC and Wadsworth (ESP) libraries. Both textbooks can also be accessed at the NCBI PubMed site on the internet at: www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=books. Instructors will identify reading material from these textbooks to provide additional foundation material essential for fully understanding each lecture. Textbook readings will be particularly important for students with limited background in cell or molecular biology. *Molecular Biology of the Cell* is available for purchase at the Albany Medical College Bookstore.

Syllabus and Lecture Material

In this packet are outlines for each lecture within the course. Each instructor will provide additional material that will include a more detailed outline (if necessary), copies of figures used in their lectures and required readings. Course and lecture material will be made available on the Course Web Site (see below). Students will be responsible for printing out lecture materials prior to class. If lecture materials are not available online at least 48 hours prior to the lecture, hard copies will be provided at the beginning of the lecture.

Course Web Site

The web site is at SUNY-Albany's electronic reserves <http://eres.ulib.albany.edu>. Click on 'Electronic Reserves and Reserves Pages'. Choose 'Course Reserves Pages by Department' and select Biomedical Sciences. Click on HBMS500 Molecular Cell Biology. The course password is mcb09. Files will be PDF, Word or PowerPoint files.

Homework, Help and Review Sessions

Faculty may provide students with homework problems that will be representative of their questions on the exams. The faculty, at their option, may discuss these homework problems during class time or at a review session that will precede the exam. There will be a scheduled review session preceding each exam. In addition, faculty will make themselves available for student questions either by appointment or during defined office hours. If you are experiencing any problems in understanding a lecture or group of lectures, you are encouraged to meet individually with the relevant instructor for additional help.

Examinations and Grading

There will be four 100 point exams given during the course of the semester as indicated in the attached schedule. Each exam will contribute equally to the final grade. Each exam will be given from 9:00 AM to 12:00 noon at Albany Medical College on the following dates:

September 24, 2009	EXAM 1 in J-305/306
October 13, 2009	EXAM 2 in J-305/306
November 10, 2009	EXAM 3 in J-305/306
December 11, 2009	EXAM 4 in J-305/306

Each student is expected to abide by the Honor Code regarding examinations. Students caught cheating will face disciplinary action. Exam questions will principally be short answer and essay style and may require problem solving. Each instructor will indicate the length of an acceptable answer by the space provided on the exam. For each instructor, specific questions will be asked that relate to the required research literature reading. If needed, these papers will be made available as part of the exam packet. Following each exam, students will be provided access to an answer key and will have the opportunity to discuss specific questions and answers with the instructors. All students will be provided with a range and point breakdown following each exam.

Final letter grades for the course will be determined by a consensus of the teaching faculty based on each student's average numerical score for the four exams. All students will be provided with the grade ranges and point breakdown for the entire course. Letter grades will be assigned relative to the class-average score and considering the distribution of scores around the mean. While there are no fixed rules for how grades are assigned, historically the class mean is typically a B to B+.

Evaluation by Students

This course will be formally evaluated by the Graduate Student Organizations at Albany Medical College and in the Department of Biomedical Sciences of the SUNY School of Public Health. The results of these evaluations will be provided to [1] the AMC and BMS curriculum committees; [2] the course directors; [3] the department chairs; [4] the graduate program directors; and [5] each faculty member involved. Included in this evaluation will be content, flow, lectures, instructors, and exams.

Molecular Cell Biology Teaching Faculty

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Lecture 1, September 1 - Human Genome; Chromosome and Gene Mutations

Dr. Robert Glaser

The Structure and Function of the Human Genome; Sequence Complexity in Genomes; Chromosome and Gene Mutations

READING: Relevant sections of Molecular Biology of the Cell (Alberts) 5th edition, Chapters 1 and 4. For mutations, relevant chapters in any general genetics text book. An Introduction to Genetic Analysis by Griffiths et. al. is on reserve at ESP and AMC, and is available on NCBI-Books. Chapters on gene mutations, chromosome structure mutations, chromosome number mutations.

LEARNING OBJECTIVES:

1. Know the amounts and types of sequences present in the human genome.
2. Know why the number of genes in an organism's genome is not correlated with the biological complexity of the organism.
3. Know how biological complexity can be encoded in the genome without changing gene number.
4. Appreciate the complexity of the transcriptome and its implications for understanding gene regulation
5. Understand how functional elements contained within the human genome are being identified by the ENCODE project.
6. Know the types and frequencies of genetic variation in the human genome.
7. Understand how mutations in chromosome number and structure arise, their incidence in human disease, and their mechanism of mutagenesis.
8. Be able to describe gene mutations at the level of the DNA, biological function, and phenotype.

OUTLINE:

I. The Human Genome

A. Genome sizes

B. Sequence components

1. unique - genes and regulatory sequences
2. middle repetitive - transposons
3. highly repetitive - satellite sequences and heterochromatin

C. Gene number

1. lower than expected and not correlated with biological complexity
2. gene functions and evolution
 - a) why so few essential genes?

D. Origins of genetic innovation that drive increases in biological complexity

1. the proteome
 - a) new protein domains
 - b) new domain architectures and domain accretion
 - c) post-translational modifications
2. the transcriptome
 - a) differential splicing, 5' and 3' UTR
 - b) increased regulatory complexity
 - c) non-coding and non-polyA transcripts

E. The ENCODE project - functional annotation of the human genome

1. most of the genome is transcribed
2. lots of unexpected transcriptional regulation

F. Genome variation - what makes us unique?

1. types of variation: SNPs and structural variations
2. the genomes of two individuals compared: the beginning of personal genomics
3. genomic variation and disease: what can my genome tell me?

II. Mutations

A. Chromosome mutations

1. changes in chromosome number: nondisjunction and aneuploidy
 - a. mechanism of somatic and germline nondisjunction
2. changes in chromosome structure: duplications, deficiencies, inversions, translocations
 - a. relation to sterility syndromes

B. Gene mutations

1. mutations at the level of the DNA
2. mutations at the level of phenotype
3. suppressor and enhancer mutations

Lecture 2, September 3 - Structure of DNA and Chromatin

Dr. Robert Glaser

DNA Structure and Topology; Topoisomerases; Chromatin Structure

READING: Relevant sections of Molecular Biology of the Cell (Alberts) 5th Ed, Chapters 1, 4 and 5. Supplemental: Biochemistry (Zubay) 4th Ed, Chapter 30 (library reserve)

LEARNING OBJECTIVES:

1. Know the biochemical structure of DNA and understand the forces responsible for stabilizing B-DNA.
2. Know non-B form DNA structures and the evidence they occur in vivo.
3. Understand DNA topology and how it is used to describe supercoiling of DNA.
4. Understand how topoisomerases alter DNA topology, what functions topoisomerases serve in vivo, and their relevance to treatments for cancer.
5. Know how DNA is packaged into nucleosomes, and the structure of the 11 nm and 30 nm chromatin fiber.
6. Know the different ways chromatin structure can be modified by cells to facilitate DNA-mediated processes, such as transcription, replication, and DNA repair.

OUTLINE:

I. DNA Structure

- A. Biochemical components of DNA
- B. Forces that stabilize B-DNA structure: base pairing, base stacking, electrostatic
- C. Non-B form DNA: A, Z, H, G-quadruplex
 1. evidence for G-quadruplex structures in vivo

II. DNA Topology

- A. DNA supercoiling in vivo: packaging and replication/transcription
- B. Definitions and relationships between parameters of topology
 1. linking number, supercoiling and superhelical density
 2. supercoiling is distributed between twist and writhe ($L=T+W$)
 3. toroidal and plectonemic supercoils and their relevance in vivo
- C. Topoisomerases
 1. basic properties of Type I and Type II topoisomerases
 2. functions in vivo
 3. mechanisms: nick & swivel versus strand passage
 4. source of negative supercoiling in eukaryotes
 5. topoisomerase inhibitors are important antitumor drugs
 - mechanism of fork collapse caused by topo inhibitors

III. Chromatin Structure

- A. Nucleosomes
 1. basic structure
 2. assembly of histones: the H3:H4 tetramer core + two H2A:H2B dimers
 3. 11 nm "beads-on-a-string" fiber
- B. The 30 nm fiber
 1. stabilization by histone H1 and histone tail interactions
 2. 11 nm - 30 nm transition is an important target of regulation

- C. Higher order structures
 - 1. looped domains
 - 2. SMC proteins and control of higher order domains
 - mitotic chromosome condensation, SMC proteins and condensin
- E. Chromatin modification
 - 1. post-translational modification of histone tails
 - a) types
 - b) functions: “histone code” versus changes in biophysical properties
 - 2. chromatin remodeling complexes
 - a) classes and their modes of action
 - b) interrelatedness with histone tail modifications
 - 3. variant histones
- F. Functions of chromatin modifications
 - 1. regulating transcription of specific genes
 - 2. controlling large domains of DNA
 - a) propagation by “reader-writer” complexes
 - b) heterochromatin formation in mammals and yeast
 - c) barrier proteins

Lecture 3, Sept. 8 - Mechanism of DNA Replication**Dr. Bill Wolfgang****Mechanism of replication in bacteria and eukaryotes; initiation and termination in bacteria**

READING: Molecular Biology of the Cell (Alberts) 5th edition, Chapter 5. Supplemental: Molecular Cell Biology (Lodish) 4th Ed, Chapter 12 (library reserve and NCBI-Books)

LEARNING OBJECTIVES:

1. Know the activities of DNA polymerases and the associated proteins that replicate chromosomal DNA.
2. Know how replication forks are extended, particularly as relates to differences between leading and lagging strand synthesis.
3. Know the differences between bacterial and eukaryotic DNA replication.
4. Know how replication is initiated and terminated in bacteria.

OUTLINE:**I. General characteristics**

- A. Semi-conservative
- B. Specific origins
- C. Bidirectional

II. Bacterial Replication**A. Polymerization**

1. Pol I, Pol II, Pol III
2. Pol III-associated proteins - clamp, clamp loader and τ
3. other replication proteins at the fork - SSB and helicase

B. Lagging strand synthesis

1. priming by the primasome
2. polymerization by Pol III
3. completion by Pol I and ligase
4. coupling of leading and lagging strand synthesis

C. Sources of replication fidelity

1. base-pair geometry
2. 3'-5' exonucleolytic proofreading
3. strand-directed mis-match repair

D. Initiation by DnaB at oriC**E. Termination**

1. ter and tus
2. topoisomerases

II. Eukaryotic Replication**A. Polymerization**

1. Polymerases $\alpha, \delta, \epsilon, \beta$ and γ
2. the clamp and clamp loader
3. the MCM/GNS complex and helicase activity

B. Lagging strand synthesis

1. priming by Pol α : primase
2. polymerization by Pol α and then Pol δ
3. completion by RNase/Fen1 and ligase

Lecture 4, Sept. 10 - Regulation of DNA replication**Dr. Bill Wolfgang****Replicating Chromatin; Telomeres; Initiation in Eukaryotes**

READING: Molecular Biology of the Cell (Alberts) 5th edition, Chapter 5 and pages 1067-1069
Chapter 17. Supplemental: Molecular Cell Biology (Lodish) 4th Ed, Chapter 12 (library reserve and NCBI-Books)

1. Know how DNA replication is initiated in eukaryotes, and how initiation is coupled to control of the cell cycle.
2. Know how eukaryotes replicate chromatin and why it's important for epigenetic control of development.
3. Know how eukaryotes replicate telomeres and the relationship of telomere replication to disease.

OUTLINE**I. Initiation in Eukaryotes**

- A. Complex developmental regulation in space and time
- B. Pre-RC formation and licensing
 1. the ORC complex
 2. Cdc6p and Cdt1p
 2. the Mcm complex
- C. Initiation - Mcm10, Cdc45p, GINS, and pol α : primase
 1. connecting initiation to M phase of the cell cycle
- D. Once-per-cell cycle regulation
 1. inhibiting formation of the pre-RC in S, M, and G2
 2. Orc1, Cdc6, Cdt1, Mcm

II. Replication of chromatin

- A. Retention of old nucleosome and assembly of new
 1. conservative or semiconservative replication of nucleosome?
- B. Retention of epigenetic "marks" and developmental memory

III. Telomeres

- A. Telomere structure
 1. 3' ssDNA overhang - origin and why a problem
 2. T-loop formation and heterochromatinization
- B. Telomerase
 1. TERC + TERT
 2. mechanism of maintenance
- C. Telomere shortening and disease
 1. telomerase in the germline, stem cells, and soma
 2. dyskeratosis congenita and stem cell disease
 3. replicative senescence, genomic instability and cancer in somatic cells

Lecture 5, Sept. 11 – DNA Repair and Disease**Dr. Thomas Begley****DNA Damage; DNA Damage Responses, DNA Repair; Cancer Onset**

READING: Molecular Biology of the Cell (Alberts) 5th edition, Chapter 5, pages 263-265, 295-304. Supplemental: See associated review articles in lecture notes

LEARNING OBJECTIVES:

1. Understand that DNA can be damaged by environmental and endogenous agents
2. Know how eukaryotes sense and respond to DNA damage
3. Know how direct repair works and understand the mechanism of Mgmt and AlkB like activities
4. Understand the difference between direct repair and excision based repair
5. Connect failures in the DNA damage response to human disease

OUTLINE**I. DNA damage**

- A. Environmental
- B. Endogenous
- C. Unifying features

II. Sensing DNA damage

- A. Signaling cascades associated with the DNA damage response
- B. ATM protein and associated kinase cascade
- C. Ataxia telangiectasia

III. DNA Repair

- A. One step repair activities
- B. Mgmt repair of O⁶-methylguanine lesions
- C. AlkB repair of 1-methyladenine lesions
- D. Preventing carcinogenesis by direct repair
- E. Direct versus excision based repair pathways

IV. Diseases associated with defects in DNA repair

- A. Long term carcinogenesis studies in mice
- B. Other diseases identified in mice deficient in DNA repair
- C. Xeroderma Pigmentosum in humans
- D. Cockayne's Syndrome in humans

Lecture 6, Sept. 15 – DNA Recombination and Disease
Dr. Thomas Begley

Double strand break; homologues recombination, non-homologues end joining; Nejmegen Brakeage Syndrome; Fanconi anemia

READING: Molecular Biology of the Cell (Alberts) 5th edition, Chapter 5 pages 302- 316.
Supplemental: See associated review articles in lecture notes

LEARNING OBJECTIVES:

1. Understand how double strand breaks are generated and how they can be repaired
2. Understand how homologues recombination works and the roles of proteins involved with homologues recombination
3. Understand how non-homologues end joining works and the roles of proteins involved with non-homologues end joining
4. Understand how defects in recombination lead to the phenotypes associated with Nejmegen Brakeage Syndrome
5. Understand how inter-strand cross links are repaired by Fanconi Anemia (FA) proteins and how FA proteins work in conjunction with homologous recombination to coordinate repair.

OUTLINE

I. Background information important for recombination

- A. DNA
- B. Cell cycle
- C. Mitosis and meiosis

II. Repair of double strand breaks

- A. Formation of double strand breaks
- B. Repair by homologous recombination
- C. Repair by single strand annealing, a variation of homologous recombination
- D. Repair by non-homologous end joining

III. Recombination proteins and mechanism of action

- A. Homologous recombination from *E. coli* to humans
- B. Non-homologous end joining in budding yeast and humans

IV. Diseases associated with defects in recombination

- A. Nejmegen Breakage Syndrome (NBS)
 1. Phenotypes
 2. experimental approaches to study defects in homologous recombination and NBS
- B. Fanconi anemia (FA), a defect in inter-strand crosslink repair
 1. FA proteins, complexes, and activities
 2. Coordination of homologous recombination with other pathways

Lecture 7, Sept 17, 2009
Dr. Jing-Ren Zhang
Prokaryotic Transcription

READING: Pages 379-385, 395-398, 414-415, and 418-419 in Chapter 7 of the text *Molecular Biology of the Cell*.

Supplemental Reading: Regulation at Complex Bacterial Promoters: How Bacteria Use Different Promoter Organizations to Produce Different Regulatory Outcomes, *Curr. Opin. Microbiol.*, vol. 7, pp. 102-108, 2004.

Bacterial Transcription Elongation Factors: New Insights into Molecular Mechanism of Action, *Mol. Microbiol.*, vol. 55, pp. 1315-1324, 2005.

OBJECTIVES:

The objectives of these lectures are to 1) Understand the structure of bacterial RNA polymerase and appreciate how its complexity allows for a maximal range of transcriptional regulation, 2) Understand the basis for RNA polymerase recognizing promoter sequences, 3) Understand the mechanisms of transcriptional initiation, elongation, and termination, 4) Provide an overview of the signal transduction mechanisms that bridge environmental signals with gene expression, and 5) Examine current research into bacterial transcriptional control using the regulation of the *fru* operon in *Lactococcus lactis* as a model.

OUTLINE:

I. Definitions

- A. Transcription
- B. Transcriptional unit
- C. Key considerations
 1. how does RNA polymerase find a promoter?
 2. how do regulatory elements interact with RNA polymerase to influence the rate of transcription?

II. RNA Polymerase

- A. Subunits
- B. Transcription overview
- C. Promoters
 1. consensus sequence
 2. strength
- D. Initiation
 1. various steps
 2. sigma factors
- E. Termination
 1. intrinsic terminators
 2. Rho-dependent terminators
 3. antitermination

III. Regulatory Elements

- A. Cis-acting elements versus trans-acting factors
- B. Negative control
- C. Positive control
- D. Small molecule inducers and corepressors

- IV. *Trp* Operon: Application of principles of trans-acting regulatory factors and introduction of attenuation
 - A. Negative control
 - B. Tryptophan as a small molecule corepressor
 - C. Attenuation
- V. Bridging the Environment and Gene Expression
 - A. Two-component histidine kinase systems
 - B. Other mechanisms
 - C. Global influences
 - 1. quorum sensing
 - 2. stringent response

Lectures 9 and 10, September 29, Oct 1
Dr. Dorina Avram
Eukaryotic Transcription and Its control

READING

- Alberts, et al, Molecular Biology of the Cell, 5th Edition, 2002 –chapter six: P329-345; chapter 7: p411-453; 459-477
- Margueron R, Trojer P, Reinberg D., The key to development: interpreting the histone code? *Curr Opin Genet Dev.* 2005 Apr;15(2):163-76.
- Roeder RG. Transcriptional regulation and the role of diverse coactivators in animal cells. *FEBS Lett.* 2005 Feb 7;579(4):909-15.
- Research paper (to be announced before the class)

OBJECTIVES

- Knowledge about transcription and its regulation in eukaryotes
- Knowledge about the eukaryotic RNA polymerases with emphasize on the Polymerase II and general transcription factors
- Knowledge about transcriptional regulators
- Understanding the role of chromatin modifying and remodeling in the context of transcriptional regulation
- Learning how to understand a research paper in the field of transcriptional control

OUTLINE

- Basic concepts in transcription and its control
- Eukaryotic promoters
- Eukaryotic RNA polymerases and genes they transcribe
- Comparison between eukaryotic and prokaryotic RNA polymerases (subunits)
- General transcription factors, preinitiation complex (PIC), Pol II
- Initiation, elongation, termination
- Transcriptional regulators (characteristics; classes of DNA-binding domains)
- Nuclear receptors:
 - type I, type II, orphans
 - structure, DNA-binding domain, helix 12
 - co-activator and co-repressor complexes associated with nuclear receptors
- Regulation of transcription:
 - Chromatin, nucleosomes and transcriptional regulation
 - Histone tail modifications
 - Histone methylation and DNA methylation
 - Transcriptional corepressor and coactivator complexes
 - Chromatin remodeling complexes

Lectures 11 & 12, October 6 & 8
Dr. Susan Madison-Antenucci
RNA Processing I & II

READING:

The relevant sections of either Molecular Cell Biology, Lodish et al 4th edition Chapter 11 or Molecular Biology of the Cell, Alberts et al 4th edition Chapter 6

OBJECTIVES:

1. Understand the basic mechanisms of mRNA processing, 5' cap formation, 3' cleavage and polyadenylation, and RNA splicing
2. Understand how mRNA processing is regulated to control gene expression.
3. Know how RNA processing and nuclear export are coordinated.
4. Understand how rRNA and tRNA genes are arranged and how the RNAs are processed.
5. Understand how RNAi, RNA turnover and RNA editing affect gene expression.

OVERVIEW:

Processing of eukaryotic mRNA

5' Cap formation

3' End formation, cleavage and polyadenylation

Splicing

Spliceosomes

SnRNPs

Transesterification

Splicing cycle and rearrangements

Nuclear localization and export

Role of hnRNPs

Nuclear export of mature mRNAs

Self-splicing

Group I and Group II introns

Regulation of mRNA processing

Processing of rRNA and tRNA

Other Mechanisms of Processing

RNA editing

RNA modification

Trans splicing

Post-transcriptional control of gene expression

RNA interference

Antisense RNA

mRNA stability and half-life

Lectures 13 - 14, Oct 15 and 20, 2009**TRANSLATION I, II****Scott A. Tenenbaum, Ph.D.**

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Literature:

1. Getting the message across: cytoplasmic ribonucleoprotein complexes. Bailey-Serres J, Sorenson R, Juntawong P. Trends Plant Sci. 2009 Jul 17.
2. Genome-wide identification of alternative splice forms down-regulated by nonsense mediated mRNA decay in Drosophila. Hansen KD, Lareau LF, Blanchette M, Green RE, Meng Q, Rehwinkel J, Gallusser FL, Izaurralde E, Rio DC, Dudoit S, Brenner SE. PLoS Genet. 2009 Jun;5(6):e1000525. Epub 2009 Jun 19.

Text Book References:

Molecular Biology of the Cell, by Alberts *et al*, 5th Edition.

Pages: 366-387	general protein synthesis
Pages: 488-495	prokaryotic and eukaryotic translation regulation

Translation I. The structure and function of the basic components that participate in protein synthesis will be introduced, including. Aspects of how translation is regulated will be discussed. Impact of recent advancements in RNA-binding proteins and our understanding of how they influence mRNA translation will be discussed. The main steps in protein synthesis will be introduced. While doing so, the main differences between prokaryotic and eukaryotic protein synthesis machinery will be outlined.

OBJECTIVES

1. Know the various components of protein synthesis machinery.
2. Understand the role RNA-binding proteins can play in influencing translation regulation.
3. Appreciate the complexity of ribosome structure.
4. Know how the genetic code works.
5. Know the mechanism of initiation of protein synthesis, and to understand the fundamental differences between the mechanisms of initiation in prokaryotic and eukaryotic systems.

OVERVIEW

1. Components of protein synthesis machinery:

tRNA:

- Review of structure and function
- Triplet nature of genetic code

- Overall structure of genetic code; universality and rare exceptions

Ribosome:

- Composition of rRNAs and proteins; similarities and differences between prokaryotic and eukaryotic ribosomes.
- Catalytic role of rRNA: role of small subunit rRNA in mRNA decoding

2. The role of RNA-binding proteins in Translation:

- The 5'-UTR
- The 3'UTR
- Interactions with the cap and the tail of the mRNA
- Associations with the ribosome

3. Initiation of protein synthesis:

Prokaryotic Initiation:

- Shine-Dalgarno sequences and influences
- fmet-tRNA_f^{Met}; prokaryotic initiation factors and roles

Eukaryotic Initiation:

- ribosome scanning rules; role of met-tRNA_i^{Met} in scanning
- modifications and exceptions to ribosome scanning: non-AUG start codons; leaky scanning; internal ribosome entry sites (IRESs)
- eukaryotic initiation factors: multiplicity and complexity

4. Elongation:

- Three primary tRNA-binding sites on the ribosome, recent insights and models
- Elongation factors: EF-Tu, EF-G, and tRNA molecular mimicry

5. Termination and ribosome recycling:

- nonsense codons; release factors (RFs); more of tRNA molecular mimicry
- ribosome-recycling factor (RRF) and its mode of action

6. Nonsense Mediated Decay

- The mechanism of NMD

Lecture 15, OCTOBER 22, 2009**Dr. Bruce McEwen****ELECTRON MICROSCOPY, CYTOSKELTON, & MOTORS****I. Principles of cellular electron microscopy***Text: Chapter 9, 604-613; Figs 9-1, 9-2.*

Teaching objectives: Impart a basic knowledge of the principles of modern biological electron microscopy and its application to cell biology. Enable students to recognize different types of specimen preparations from an EM image.

- A. Transmission electron microscopy (TEM)
 - 1. Fixation and dehydration
 - 2. Plastic embedding, positive staining
 - 3. Serial section ultramicrotomy
 - 4. Immuno-TEM
- B. Surface imaging
 - 1. Scanning Electron Microscopy (SEM)
 - 2. Metal Shadowing
- C. High resolution methods and 3D reconstruction
 - 1. Negative stain & Frozen-hydrated
 - 2. Averaging for higher resolution
 - 3. Electron tomography

II. Cytoskeleton*Text: Chapter 16, 966-1010.*

Teaching objectives: Impart an understanding of the structure, polarity, and dynamics of the three major components of the cytoskeleton, and an appreciation of how these basic characteristics of the cytoskeleton are utilized and regulated in the cell; Appreciation for the experimental basis for this knowledge

- A. Structure, assembly, and dynamics of cytoskeletal filaments
 - 1. Construction of F-actin and microtubules
 - 2. Assembly and polarity of F-actin and microtubules
 - 3. Nucleotide hydrolysis and dynamic instability
 - 4. Construction and properties of intermediate filaments
- B. Regulation of filament assembly and dynamic instability
 - 1. Pharmacology
 - 2. Cellular regulation
 - a. Control of assembly
 - b. Control of dynamic instability
- C. Formation of higher ordered arrays
 - 1. Actin arrays and cross-linking proteins
 - 2. Control and remodeling by severing proteins

III. Molecular motors

Text: Chapter 16, 1010-1025.

Teaching objectives: Impart knowledge of the basic structure and function of the three families of cytoskeleton motors and the variations within each motor family; Facilitate understanding how the energy of ATP hydrolysis is converted into mechanical force.

- A. Myosin superfamily:
 - 1. Common head domain that contains the motor activity
 - 2. Light chains; stalk
- B. Kinesin superfamily
 - 1. Common head domain; homology to myosin head domain
- C. Dyneins
 - 1. Bouquet structure
- D. Force generation during the mechanochemical cycle
 - 1. ATP hydrolysis is coupled to a conformational change.
 - 2. Contrast myosin and kinesin
 - 3. Processivity and directional of travel
- E. Regulation of motor activity
 - 1. Melanosome movement

Lecture 16, OCTOBER 27, 2009**Dr. Bruce McEwen****CELL MOTILITY & MITOSIS****IV. Cell Motility: Functional cytoskeletal-motor arrays***Text: Chapter 16, 1025-1030, 1036-1039*

Teaching objectives: Using two well-studied examples, impart a basic understanding of how cytoskeletal fibers, motors, and regulatory elements interact to create movements at the cellular and organism level in eukaryotes.

- A. Muscle.
- B. Cell migration

IV. Mitosis**Text: Chapter 17, 1071-1097; Chapter 16, 1034-1035**

Teaching Objectives: Impart a basic understanding of the modern view of genomic segregation during mitosis. Emphasis will be on the role of the cytoskeleton, motor proteins, spindle poles, and the kinetochore.

- A. Overview of M phase and genomic segregation
 - 1. Replication and segregation are separated in time
 - a. For both the genome and centrosomes
 - 2. Stages of Mitosis
- B. Setting the stage: prophase, early prometaphase
 - 1. Chromosome replication, condensation, cohesion.
 - 2. Centrosome duplication.
 - 3. Microtubule dynamics.
- C. Spindle Assembly
 - 1. Arrangement of Microtubules
 - 2. Role of motors in spindle assembly
 - 3. Two mechanisms contribute
- E. Chromosome alignment: prometaphase and metaphase
 - 1. Function and molecular construction of the kinetochore
 - 2. The kinetochore attachment to microtubules
 - 3. Force generation
 - 4. Chromosome alignment at the spindle equator
- D. Chromatid segregation: anaphase
 - 1. Spindle-attachment checkpoint
 - 2. Anaphase A - chromatid separation
 - 3. Anaphase B – spindle elongation
- E. Partitioning the cytoplasm: cytokinesis
 - 1. Determining the site of cleavage
 - 2. Contractile ring – role actin and myosin
 - 3. Organelle segregation

Lecture 17, October 29**Dr. Jim Drake****Intracellular Compartments & Protein Sorting****READING:**

1. All of Chapter 12 in Alberts, Johnson, Lewis, Raff, Roberts and Walter, 2002, *Molecular Biology of the Cell*, 5th Edition, Garland Science. New York
2. Bolender et al., 2008, Multiple pathways for sorting mitochondrial precursor proteins, *EMBO reports* 9:42.
3. Caramelo and Parodi, 2008, Getting In and Out from Calnexin/Calretuculin Cycles, *J. Biol. Chem.*, 283:10221.

LEARNING OBJECTIVES:

1. To gain a basic understanding of the spatial and topological organization of the various membrane-bound intracellular compartments and the problems this creates for the movement of proteins between these compartments.
2. To gain a basic understanding of the various mechanisms for translocating proteins across intracellular membranes which evolution has developed to dealing with the problems of moving proteins between intracellular compartments.

OVERVIEW:

- I. The basics of protein transport.
 - A. Topological considerations.
 - B. Transport mechanisms.
 - C. Signal sequences.
- II. Nuclear transport.
 - A. The nuclear pore complex.
 - B. Nuclear localization signals and nuclear import receptors.
- III. Mitochondria.
 - A. Organelle structure and topology.
 - B. Multiple transport complexes.
 - C. Mechanisms of transport into distinct intra-organelle spaces.
- IV. The endoplasmic reticulum and Golgi apparatus.
 - A. Glycosylation and quality control.
 - B. Chaperone proteins.
- V. Golgi to lysosome transport.
 - A. The mannose-6-phosphate receptor.
 - B. The hydrolyase signal patch.

Lecture 18, November 3**Dr. Jim Drake****Vesicular Transport****READING:**

1. All of Chapter 13 in Alberts, Johnson, Lewis, Raff, Roberts and Walter, 2002, *Molecular Biology of the Cell*, 5th Edition, Garland Science. New York.
2. Jahn and Grubmüller, 2002, Membrane fusion, *Current Opinion in Cell Biology*, 14:488

LEARNING OBJECTIVES:

1. To gain a basic understand of the mechanism by which transport vesicles bud from one intracellular compartment and then selectively deliver their contents to another intracellular compartment.
2. To gain a basic understanding the mechanisms controlling the vesicle-based movement of cellular proteins.

OVERVIEW:

- I. The basic mechanism of vesicular transport.
 - A. The nucleation and budding of transport vesicles from donor membranes.
 - B. The docking and fusion of transport vesicles with acceptor membranes.
- II. Coat proteins and vesicle formation.
 - A. Clathrin – endocytosis and Golgi transport
 - B. COPII vesicles and anterograde movement.
 - C. COPI vesicles, retrograde movement and the KDEL receptor.
 - D. Caveolin - endocytosis
 - E. Adaptor proteins
- III. The SNARE hypothesis.
 - A. SNARE structure.
 - B. v-SNAREs and t-SNAREs.
 - C. Regulatory proteins.
- IV. The endocytic pathway
 - A. Early and recycling endosomes
 - B. Late endosomes, multi-vesicular bodies and lysosomes
- V. Clathrin-mediated endocytosis
 - A. The dynamics of clathrin coated pit formation.
 - B. Receptor interaction with coated pits.

Lectures 19 & 20, November 12 & 13
Dr. Susan LaFlamme
Cell Signaling I & II

Reading: Alberts (2008) *Molecular Biology of the Cell* pp. 879-884; pp. 891-899; pp. 904-941; pp. 948-950; pp. 1115-1129

Objectives:

- I. To understand the general principles of cell signaling
- II. To know the major classes of signaling proteins and understand their mechanism of action

Outline

- I. Mechanisms of Intercellular Communication
 - A. Endocrine
 - B. Paracrine
 - C. Autocrine
- II. Types of Signaling Molecules and Receptors
- III. Signal Transduction Mechanisms
 - A. Kinases
 - B. Small GTP-Binding Proteins
 - C. Signaling Complexes
- IV. Cell Surface Receptors
 - A. G protein-coupled Receptors
 1. Activation of PLC and Ca²⁺ and IP₃ and PKC signaling
 2. Activation of Adenylyl Cyclase and cAMP signaling
 - B. Receptor Tyrosine Kinases
 1. Activation of the Ras-MAP kinase Pathway
 2. Activation of PI-3Kinase and PLC signaling
 - C. Receptor Serine-Threonine ---TGF- β receptor family
 1. SMAD signaling
 2. SMAD-independent signaling
 3. Tumor suppression and activation
- V. Classical Wg/Wnt Signaling Pathway
 - A. Mechanism of Activation
 - B. Role in Development and Tumorigenesis
- VI. Apoptosis
 - A. Role in Development and Tumor Suppression
 - B. Caspases-Mediators of Apoptosis
 - C. Intrinsic and Extrinsic pathways
- VII. Discussion of a recent paper involving one of more of these signaling pathways.

Lecture 21, November 17
Dr. Susan LaFlamme
Cell Adhesion

READING: Alberts (2008) *Molecular Biology of the Cell* pp.1131-1193

OBJECTIVES:

- I. To understand the contribution of extracellular matrix ECM and cell surface receptors for ECM in promoting tissue structure and regulating cell behavior.
- II. To understand the different types of cell-cell adhesion and how they contribute to tissue structure/function and cell behavior.

OUTLINE:

- I. Cell-ECM Adhesion
 - A. The ECM
 1. Components
 - Glycosaminoglycans (GAGs)
 - Proteoglycans
 - ECM Proteins
 2. Structure of ECM
 - Connective Tissues
 - Basement Membranes
 - B. Cell-ECM Adhesion
 1. Integrin Family
 2. Specialized Cell-ECM Adhesions
 - Focal Adhesions
 - Hemidesmosomes
 3. Integrin Signaling
 - Inside-out
 - Outside-in
 - C. ECM and Integrins in Normal and Pathophysiological Processes.
- II. Cell-Cell Adhesion
 - A. Adherens Junctions
 1. Structural Components
 2. Functions
 - B. Desmosomes
 1. Structural components
 2. Functions
 - C. Tight Junctions
 1. Structural components
 2. Functions
 - D. GAP Junctions
 1. Structural components
 2. Functions
 - E. Cell-Cell adhesion in normal and pathophysiological processes

Lectures 22 & 23, November 19 & 24**Dr. Tom Friedrich****Cell Cycle I & II**

READING Molecular Biology of the Cell (Alberts), 5th Edition, Chapter 17 or Molecular Cell Biology (Lodish) 5th Edition, Chapter 21

OBJECTIVES:

Students should be able to:

1. Describe events specific to each cell cycle phase in eukaryotes.
2. Explain the basic molecular mechanisms that control cell cycle transitions from G1 to S phase and from G2 to M phase.
3. Explain the molecular mechanisms of DNA damage checkpoints and their roles in cell cycle regulation.
4. Describe how disruption of cell cycle regulatory pathways leads to cancer.
5. Apply information from the lecture and readings to analysis of a recent scientific publication.

OVERVIEW:

I. Viewing cell proliferation in context of the life cycle of cells

II. The Cell Cycle

A. The G2/M transition

1. evidence for a factor that initiates mitosis
2. mitosis promoting factor (MPF)
3. cycling in a cell-free system
4. how does MPF trigger mitosis?
5. how is MPF regulated?
6. the G2 phase DNA damage checkpoint

B. M phase

1. the metaphase-anaphase transition
2. the spindle checkpoint
3. proteasome-dependent protein degradation and mitotic exit

C. The G1/S phase transition

1. G0 arrest
2. the restriction point
3. cyclins, cdks and inhibitors
4. the molecular basis of the restriction point
5. the G1 phase DNA damage checkpoint
6. G1/S control and cancer
7. update on the roles of cyclins and cdks

D. Discussion of a recent paper in cell cycle regulation and/or cancer biology

Lecture 24, December 1, 2009
Livingston Van De Water
Cells and Tissue Organization

Reading:

Molecular Biology of the Cell (5th ed.).

Chapter 19: pp. 1131-1195. (Cell junctions, cell adhesion and ECM)

Chapter 23: pp. 1417-1428; 1445-1450; 1467-1476. (Epithelia, connective tissue)

Chapter 22: pp. 1205-1223; 1240-1256. (Cancer cell behavior & microevolution)

Research paper: none

Learning objectives: The overall objective of this lecture is to integrate what you have learned about cell function into the context of tissues. We will emphasize how tissue compartments (especially epithelia and connective tissue) function cooperatively in wound repair and aberrantly in pathologies.

More specifically you will:

- Understand the classification and organization of tissues and the structural elements that hold tissues together.
- Know the types of cells found in epithelia and connective tissues and what they do.
- Learn about cooperative mechanisms used by tissues and cells to maintain/restore homeostasis (i.e., wound healing).
- Be introduced to mechanisms of pathogenesis.

Outline of lecture:

Organization of tissues:

1. Major tissue types.
2. Organization of epithelia and connective tissue.
3. Role of cell-cell and cell-matrix interactions
4. Signaling: paracrine, autocrine, mechanical, extracellular matrix

Skin as a model system:

1. Histology of skin
2. Cellular components in skin
3. Why study diseases?
4. Wound healing
5. Pathogenesis of cancer

Lecture 25, December 3**Dr. Sally Temple****Stem Cells & Early Vertebrate Development.****OBJECTIVES**

1. To understand basic concepts of developmental biology:
 - pattern formation
 - generation of cell diversity
2. To learn how the basic vertebrate body plan is established, using examples of systems pertinent to our major areas of study at AMC

OVERVIEW

1. Development as an initial blueprint inside the fertilized egg that is revealed by cell division, cell specialization, cell interactions and cell movement.
2. Three different ways to make cells diverse: morphogen gradients, cell induction, asymmetric cell division.
3. Early development: Egg Cleavage, Blastula, Gastrula and then Neurula: in *Xenopus* and Mammals
4. Embryonic stem cells and Adult stem cells
5. Formation of the 3 germ layers: ectoderm, endoderm and mesoderm
6. Examples of induction mechanisms underlying these early processes: A) patterning in the egg and early embryo, B) neural induction.
7. Basic body regional patterning: Example: anterior-posterior via Hox genes and the retinoic acid morphogen gradient
8. Adult stem/progenitor cells build tissues: Example blood stem cells
9. Placement of progenitor cells in space: Example Lateral inhibition
10. Stem cells self-renew via asymmetric cell division: SOP as example of asymmetric division
Patterning progenitors in time: Example, somite formation