MCB316 Genetics and Disease
Topics in 2019:

Introduction to course and basic cell structure (compare and contrast bacteria, plant cells, and animal cells; internal structures including genomes)

General discussions on meiosis and mitosis, reproduction in relation to the life cycles of mammals, yeast (budding and fission), plants (*Arabidopsis*, *Zea*) and flies (*Drosophila*)

Mendelian genetics (monohybrid/dihybrid crosses, inheritance patterns in model organisms with varying numbers of genes determining a trait, pedigree analysis in humans)

Non-Mendelian inheritance patterns (incomplete dominance, codominance, incomplete penetrance, epistatic interactions, lethal mutations, multiple alleles, gene complementation tests, variable expressivity)

Effects of mutations on protein expression (dominant vs. recessive, gain-of-function vs. loss-of-function, dominant negatives, multigene biochemical pathways)

Sex-linked inheritance patterns, disease examples (hemophilia, color-blindness, Fragile X syndrome) and methods of detection (karyotyping, PCR-based genetic testing of disease))

Molecular basis of sex-determination in mammals (*SRY* and *TFM* loci) and *Drosophila* (X/A ratios and *Sxl* splicing cascade)

Dosage compensation in *Drosophila* (X-chromosome hyperactivation) and mammals (X-chromosome inactivation)

Cytological analysis, detection of chromosome abnormalities, polyploid chromosomes and genes in humans, plants and *Drosophila*

Chromosome rearrangements (inversions, translocations, chromosome disjunction, chromosome fusions)

Genetic strategies for localizing genes in model organisms (recombination frequencies in plants and *Drosophila*, cytogenetic mapping in *Drosophila* deletion and duplication stocks) and humans (pedigree mapping, RFLP analysis, somatic-cell hybrids) and disease examples (Duchenne muscular dystrophy, hemophilia, color blindness)

Linkage analysis in *Saccharomyces* and *Neurospora*

DNA and chromatin organization (general vs. specialized at centromeres and telomeres, nucleosome positioning), analysis of genome complexity with $C_o t$ curves, organization of repeat sequences in genomes

DNA replication (leading and lagging strand synthesis, methods for measuring nucleic acid synthesis (pulse vs. pulse-chase), methods for sizing nucleic acids and separating cellular

components (density gradients, velocity gradients, differential centrifugation), DNA polymerases and error rates, DNA mismatch repair, replication complications caused by nucleosomes and telomeres, disease effects (telomerase deficiencies)

Translation (comparison of ribosomal components in bacterial vs. eukaryotes, disease effects (ribosomopathies), consequences of nucleotide mutations on protein structure, tRNA suppressors)

Mutations (silent, missense, nonsense, somatic, germinal, isoalleles, null alleles, transitions, transversions, conditional alleles) and disease examples caused by mutations in protein structure (sickle cell, thalessemias, Tay-Sachs, phenylketonuria, albinism, etc.), mechanisms causing natural and induced mutations (tautomeric shifts, chemicals, radiation, transposons)

RNA synthesis (methods measuring RNA synthesis and half-life (pulse vs. pulse-chase), methods locating introns (heteroduplex analysis), four methods mapping transcription start sites, specificities of eukaryotic polymerases), RNA maturation (pre-mRNA capping, polyadenylation, and splicing, alternative splicing, RNA editing) and disease examples caused by mutations in RNA structures (thalessemias, myosin and tropomyosin defects)

DNA transposons (inverted repeat maize *Ac/Ds*-elements, *Drosophila P*-elements, *mariner* elements) and retrotransposons (LTR (endogenous retroviruses), non-LTR (LINEs), processed pseudogenes, SINEs), proportions of transposons in genomes, effects on genome evolution and transcript expression, disease examples caused by transposon insertions (L1, Alu, SVA insertions in embryonic cells, in neuronal cells, in cancer cells), effects of pseudogenes on RNA and protein expression patterns

Molecular genetic techniques for cloning genes (vectors (plasmid, phage, phagemid, bacterial artificial chromosomes, yeast artificial chromosomes), construction of cDNA and genomic DNA libraries, screening techniques (plaque hybridization, protein expression, complementation screening), copy number determination (Southern analysis, *in situ* hybridization), sequencing (dideoxy)

Molecular genetic techniques for transcript profiling (Northern analysis, RT-PCR and qRT-PCR analyses, EST libraries, oligonucleotide arrays, microarrays, cluster analysis)

Methods for locating genes (genetic maps, cytological maps, physical maps, RFLP and VTNR/STR variations, chromosome walking, chromosome jumping, chromosome synteny) and examples of diseases mapped by these techniques (Huntington's disease and > 40 trinucleotide repeat disorders, cystic fibrosis, Fragile X syndrome, sickle cell anemia)

Molecular genetic strategies for correcting genetic defects (gene-product, gene-addition, gene-replacement, gene-knockout), vectors for expression in mammalian cells (retrovirus, lentivirus, adenovirus-associated virus, helper-dependent adenovirus, liposome, BACs), diseases initially tackled (ADA$^-$SCID, other single-gene candidates including X-linked and

autosomal recessive deficiencies), problems encountered with integrative vs. transient vectors

Techniques for transforming embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, for improving gene targeting (zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), clustered regularly spaced short palindromic repeats (CRISPR/Cas9), for elimination of transcripts (RNAi/dsRNA), for production of proteins in bacteria and transgenic plants, diseases tackled with these corrective strategies (older examples of retroviral vectors for ADA$^-$SCID and X-linked SCID, newer examples using ZFN, TALEN and CRISPR systems for mutagenesis of promoters and coding sequences, insertion of amino acid tags), diseases tackled with gene-product deliveries (growth hormone, insulin, clotting factors, etc.), advantages and disadvantages of producing proteins in *E. coli*, mammalian and plant cells

Genetic basis of cancers (cell cycle regulation, checkpoint control, viral and cellular oncogenes, tumor suppressors), examples of oncogenes affected by point mutations (*c-H-Ras*) and translocations (*bcr/abl*, *myc*/*IGH*, promoter fusions to oncogenes (*ETS*, *ERG*) etc), examples of tumor suppressor genes affected by point mutations and translocations (*RB*, *TP53*, *BRCA1*, *BRCA2*, *ALK*), gene amplifications (*ALK*, *MYCN*), genetic changes in core promoters, super-enhancers and 3' UTRs, unusual translation of short 5' ORFs occurring in cancer drivers, cancer examples caused by oncogene rearrangements (chronic and acute myeloid leukemias, Burkitt's lymphoma, prostate cancers) and point mutations (neuroblastomas, carcinomas) vs. tumor suppressor mutations (retinoblastomas, many p53-deficient cancers, breast cancers, etc.), techniques for validating importance of gene rearrangements (CRISPR/Cas9-mediated chromosome swaps)

Drug-based therapies for cancers (small molecules targeted to constitutively active kinase catalytic sites and allosteric sites, to transcription factor interactions with super-enhancers and RNA polymerase, to DNA repair pathways and to bystander gene), immunotherapies, RNA-based therapies for eliminating oncogene transcripts and correcting genetic defects (RNAi, siRNA, CRISPR/Cas9 deletions, CRISPR/Cas9 insertions of suicide genes), cancers tackled with these strategies (leukemias, neuroblastomas, prostate cancers, breast cancers, colorectal cancers, squamous cell carcinomas, melanomas), mechanisms of drug resistance (amino acid changes in kinase target protein, amplification of resistance gene, activation of bypass pathways, decreased expression of drug import pumps, increased expression of drug efflux pumps)

Diseases tackled with these (and other) therapeutic strategies (Duchenne muscular dystrophy (many types of mutations in *dystrophin* gene), cystic fibrosis (point mutations and ΔF508 in *CFTR* gene), spinal muscle atrophy (mutations in *SMN1* and *SMN2* genes), myotonic dystrophy (CTG repeat expansions in *DMPK* gene), hemophilia A (mutations in *F8* and *F9* genes), Huntington's disease (CUG repeat expansions in *HT* gene), breast cancer (mutations in *BRCA1* and *BRCA2* genes)

Scientific articles and essay topics in 2019:

(1) X-chromosome inactivation:

Carrel and Willard (2005) X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature 434, 400-404.

Liu, Wang and Zheng (2010) X-linked tumor suppressors: perplexing inheritance, a unique therapeutic opportunity. Trends Genetics 26, 260-265.

Engreitz et al. (2013) The Xist lncRNA exploits three-dimensional genome architecture to spread across the X chromosome. Science 341, 1237973.

(2) Medical genetics, RNA and disease:

Everts (2015) Uncovering the splicesome's secrets. Chem Engin News, 93, 10-14.

Cooper, Wan and Dreyfuss (2009) RNA and disease. Cell 136, 777-793.

Hsu, Simon, Neill, Marcotte, et al. (2015) The spliceosome is a therapeutic vulnerability in MYC-driven cancer. Nature 525, 384-388.

choice of:

Vigevani and Valcarcel (2014) A splicing magic bullet. Science 345, 2.

Naryshkin, Weetal, Dakka, Narasimhan and Metzger (2014) SMN2 splicing modifiers improve motor function and longevity in mice with spinal muscular atrophy. Science 345, 6.

Bonnal, Vigevani and Valcarcel (2012) The spliceosome as a target of novel antitumour drugs. Nat Rev Drug Discov 11, 847-859.

(3) CRISPR/Cas9 in cancer and disease therapeutics:

Doudna and Charpentier (2014) Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 346, 1258096.

Choi and Meyerson (2014) Targeted genomic rearrangements using CRISPR/Cas technology. Nat Commun 5, 3728.

choice of:

Young et al. (2016) A single CRISPR-Cas9 deletion strategy that targets the majority of DMD patients restores dystrophin function in hiPSC-derived muscle cells. Cell Stem Cell 18, 533-540.

Wu, Liang, Wang, Bai et al. (2013). Correction of a genetic disease in mouse via use of CRISPR-Cas9. Cell Stem Cell 13. 659-662.

Schwank, Koo, Sasselli, Dekkers et al. (2013) Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell 13, 653-658.

Options for student presentation topics for 2019:

1. **Do mutations in enhancers have a role in disease?**
   Lead-in articles: Sur and Taipale. The role of enhancers in cancer. Nature Reviews 16, 483-493 (2016)
2. **What types of genetic mutations and copy number defects have a role in the development of Parkinson's disease?**
   Lead-in articles: Nuytemans et al. Genetic etiology of Parkinson's disease associated with mutations in the SNCA, PARK2, PINK1, PARK7 and LRRK2 genes: a mutation update. Human Mut 31, 763-780 (2010)
   Genetic variability in SNCA and Parkinson's disease. Neurogenetics 12, 283-293 (2011)
3. **At what levels can defects in trinucleotide repeat lengths lead to problems in gene expression and protein function?**
   Lead-in article: A human huntingtin SNP alters post-translational modification and pathologic proteolysis of the protein causing Huntington disease. Martin et al. Scientific Reports 8: 8096 (2018)
4. **How can deregulation of the protein kinase mTOR lead to cancer defects?**
   Lead-in article: Murugan. mTOR: role in cancer, metastasis and drug resistance. Semin. Cancer Biol. pii: S1044-579X(18)30135-4. doi: 10.1016/j.semcancer.2019.07.003 (2019)
5. **How does the transcription factor MYC control normal cell growth and how does its misregulation lead to cancer?**
   Lead-in article: Dang. MYC on the path to cancer. Cell 1349, 22-35 (2012)
6. **How active are retrotransposons in cancer genomes?**
   Lead-in article: Kemp et al. Crossing the LINE toward genomic instability: LINE-1 retrotransposition in cancer. Front. Chem. doi:10.3389/fchem.2015.00068 (2015)
7. **What role do mutations in ALK and other genes have in the development of neuroblastomas?**
   Lead-in article: Schleiemacher et al. Recent insights into the biology of neuroblastoma. Inter J Cancer 135, 2249-2261 (2014)
8. **How do defects in the *BRCA* genes impact DNA repair systems and cancer?**
   Lead-in articles: Venkitaraman. Cancer suppression by the chromosome custodians, BRCA1 and BRCA2. Science 343, 1470-1475 (2014)
   King Nature The race to clone BRCA1. Science 343, 1462-1465 (2014)
9. **What new database methodologies are being used to evaluate the effects of genetic variations?**
   Lead-in article: Rivas et al. Effect of predicted protein-truncating genetic variants on the human transcriptome. Science 348, 666-669 (2015)
   Cummings et al. Improving genetic diagnosis in Mendelian disease with transcriptome sequencing. Sci Transl Med 9, 386 (2017)
10. **How have synthetic lethal therapeutic approaches been developed and impacted treatment of some diseases?**
    Lead-in articles: Heitz et al. Poly(ADP-ribosyl)ation polymerases: mechanism and new target of anticancer therapy. Expert Rev Anticancer Ther 10, 1125-1136 (2010)

Leung et al. Synthetic lethality in lung cancer and translation to clinical therapies. Mol Cancer 15, 61 (2016)

11. **What genetic mechanisms lead to drug resistance?**
    Lead-in articles: Golberg et al. Outwitting evolution: fighting drug-resistant TB, malaria and HIV. Cell 148, 1271-1283 (2012)
12. **What strategies are being used to overcome drug resistance in cancers?**
    Lead-in article: Workman et al. Genome-based cancer therapeutics: targets, kinase drug resistance and future strategies for precision oncology. Curr Opinion Pharmacology13, 486-496 (2013)