School of Biological Sciences
Core Course on Scientific Reasoning and Logic, Fall 2022
Course Outline

Module 1: Gene Expression

Course Faculty

Instructor: Alex Gann
Invited Experts: Adrian Krainer
Christopher Vakoc

Tutor: Jonathan Cahn

Lecture 1: Gann
- Early ideas and bacterial models

Lecture 2: Gann
- Understanding a regulatory network

Lecture 3: Gann
- Eukaryotic networks and development

Lecture 4: Krainer
- Splicing mechanisms and regulation

Lecture 5: Vakoc
- Emerging concepts and mechanisms of transcriptional control

Student Evaluation:
- Problem Set 40%, Discussions 40%, Class Participation 20%

Learning Objectives
- Transcriptional activation and repression
- Cooperativity and specificity in gene regulation
- Signal integration and combinatorial control
- Regulation by RNAs
- Splicing and processing of RNAs
- Histone and DNA Modification

Learning Outcomes
- Explain how genes are turned on and off
- Explain how specificity of gene regulation is achieved
- Discuss establishment vs. maintenance of gene expression

Reference Material

Textbooks:
- Watson, J.D. *et al., Molecular Biology of the Gene,* 2013, Chapters 13, 14, 18, 19, 20
- Ptashne, M. *A Genetic Switch,* 3rd Edition, 2004

Reviews:
- Ptashne, M. 2014. The chemistry of gene regulation. *J. Biol Chem* **289:** 5417-5435
- Gann, A. 2010. Jacob and Monod: From Operons to EvoDevo. *Curr Biol*. **20**: R718-R723.
- Deneris, E. and Hobert, O. 2014. Maintenance of postmitotic neuronal cell identity. *Nat Neurosci*. **17:** 899-907.
- Zabidi, M. and Stark, A. 2016. Regulatory Enhancer-Core-Promoter communication via transcription factors and cofactors. Trends Genet. **32**: 801-814.

Problem Set Papers:
- Zeitlinger, J., Stark, A, Kellis, M., Hong, J-W., Nechaev, S., Adelman, K., Levine, M., and Young, R.A. 2007. RNA polymerase stalling at developmental control genes in the *Drosophila melanogaster* embryo. *Nat Genet*. **39**: 1512-1516.
- Ruskin, B., Krainer, A.R., Maniatis, T., and Green, M.R. 1984. Excision of an intact intron as a novel lariat structure during pre-mRNA splicing in vitro. *Cell* **38**: 317-331.

Discussion 1: Gann

- Atsumi, S., and Little, J.W. 2006. A synthetic phage lambda regulatory circuit. *Proc Natl Acad Sci U S A*. **103**: 19045-19050.
- Cui, L., Murchland, I., Shearwin, K.E., and Dodd IB. 2013. Enhancer-like long-range transcriptional activation by λ CI-mediated DNA looping. Proc Natl Acad Sci U S A. **110**: 2922-2927.

Discussion 2: Krainer

- Smith, D.J., Query, C.C., and Konarska, M.M. 2007. trans-splicing to spliceosomal U2 snRNA suggests disruption of branch site-U2 pairing during pre-mRNA splicing. *Mol Cell* **26**: 883-890.
- Zhang, Z., Pinto, A.M., Wan, L., Wang, W., Berg, M.G., Oliva, I., Singh, L.N., Dengler, C., Wei, Z., Dreyfuss, G. 2013. Dysregulation of synaptogenesis genes antecedes motor neuron pathology in spinal muscular atrophy. Proc Natl Acad Sci U S A. **110**: 19348-19353.

CSHL School of Biological Sciences
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Module 2: Gene Regulatory Logic and the Construction of Multicellular Organisms: Insights from humans, flies, and worms

Course Faculty
Lead Instructor: Christopher Hammell
Tutor: Peipei Wu

Lecture 1: Hammell
- Cell fate specification and the construction of a basic organ
- Overview of how intracellular and extracellular signaling define an array of distinct cell fates using the C. elegans vulva as a model.
- Integrate these general principles of intra- and extra-cellular signaling in the context of disease.

Lecture 2: Hammell
- Control of temporal gene expression
- Overview of *C. elegans* development and the utility of having a hard-wired developmental program verses the spatially-defined one discussed in last lecture.
- *C. elegans* heterochronic pathway and the emergent themes that are common to all metazoans.
- Comparison of temporal gene expression strategies: developmental timers, circadian timers, and biological oscillators that construct repeated, spatial elements in development.

Lecture 3: Hammell
- Control to size and death during development
- Overview of forms of animal/plant growth.
- Insights from single cells and model organism.
- Cell autonomous and non-autonomous regulatory networks and how to find them genetically.
- Cell death and its regulation.

Lecture 4: Hammell
- Germline formation
- Overview of forms of animal/plant growth.
- Examples from model organisms and the genetic analysis of the problem.
- Cell autonomous and non-autonomous regulatory networks and how to find them genetically.

Student Evaluation:
- 50% participation in daily discussions during lectures
- 50% based on paper discussions

Learning Objectives
- To understand the fundamentals of recurrent Gene Regulatory Networks (GRN) that orchestrate various types of cell fate specification.
- To understand what makes a good "model system" for developmental biology.
- To define what a stem cell is and how they operate in embryogenesis, post-embryonic development, tissue regeneration and the germ line.
- To understand what the limitations of studying developmental biology from a genetic perspective and to determine what are the solutions to this problem.
- Integrate large-scale gene expression studies to understand the coordination of gene expression during development.
- Gain a practical understanding of how cell death, developmental timing, cell and organ growth control, germline

development and tissue regeneration contribute to normal developmental processes.

Learning Outcomes

- Elaborate on an understanding of a functional model system for a particular developmental problem
- Design tractable methods to investigate developmental problems.
- Critically access modern literature focused on developmental biology.
- Gain a fundamental understanding of how high-volume genomic approaches contribute to our understanding of gene expression trajectories and progression of developmental processes.

Reference Material

Textbooks:

- Gilbert, S.F. 2003. *Developmental Biology*, 7th ed. Sinauer Associates, Inc.
- Wolpert, L., R. Beddington, J. Brockes, T. Jessell, P. Lawrence, and E. Meyerowitz. 2002. *Principles of Development*. 2nd ed. Oxford University Press.
- Stern, C. 2004. Gastrulation: From Cells to Embryo. CSHL Press.
- Wilt F. H., Hake, S.C. 2004. *Principles of Development*. W.W. Norton & Company, Inc.
- Alon, Uri. 2006. *An Introduction to Systems Biology: Design Principles of Biological Circuits*. Chapman and Hall. CRC Pr.

Reviews:

- Raj, A. and van Oudenaarden, A. 2009. Single-molecule approaches to stochastic gene expression. *Annu Rev Biophys.* **38**: 255–270.
- Roth, S. and Lynch, J. 2013. Does the bicoid gradient matter? *Cell* **149**: 511–512.
- Tumaneng, K., Russell, R. C. and Guan, K.-L. 2013. Organ size control by Hippo and TOR pathways. *Curr Biol* **22**: R368–79.
- Zhao, B., Tumaneng, K. and Guan, K.-L. 2011. The Hippo pathway in organ size control, tissue regeneration and stem cell self-renewal. *Nat. Cell Biol.* **13**: 877–883.

Problem Set Papers:

- Raj, A., Rifkin, S. A., Andersen, E. and van Oudenaarden, A. 2010. Variability in gene expression underlies incomplete penetrance. *Nature* **463**: 913–918.
- Tursun, B., Patel, T., Kratsios, P., and Hobert, O. 2011. Direct conversion of C. elegans germ cells into specific neuron types. *Science* **331**: 304-308.

Discussion 1 Papers:

- la Cova, de, C., Abril, M., Bellosta, P., Gallant, P. and Johnston, L. A. 2004. *Drosophila* myc regulates organ size by inducing cell competition. *Cell* **117**: 107–116.
- Böhni, R., Riesgo-Escovar, J., Oldham, S., Brogiolo, W., Stocker, H., Andruss, B.F., Beckingham, K., and Hafen, E. 1999. Autonomous control of cell and organ size by CHICO, a *Drosophila* homolog of vertebrate IRS1-4. *Cell* **97**: 865–875.

Discussion 2 Papers:

- Raj, A., Rifkin, S. A., Andersen, E. and van Oudenaarden, A. 2010. Variability in gene expression underlies incomplete penetrance. *Nature* **463**: 913–918.
- Tursun, B., Patel, T., Kratsios, P., and Hobert, O. 2011. Direct conversion of *C. elegans* germ cells into specific neuron types. *Science* **331**: 304-308.

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Module 3: The Brain: genes, circuits, and behavior

Course Faculty
Organizer: Jessica Tollkuhn

Lecture 1: Tollkuhn
Behavior
• Neuroethology, innate behavior
• Sex-differences in the brain and behavior: hard-wired or plastic?
• Assessing validity of rodent models of psychiatric disease
• Rodent behavior assays

Lecture 2: Tollkuhn
Circuits
• Overview of neural circuit approaches:
Circuit mapping, genetic and viral methods, imaging neural activity, optogenetics, chemogenetics.
• In-class paper discussion: Hashikawa K, Hashikawa Y, et al. 2017. Esr1+ cells in the ventromedial hypothalamus control female aggression. *Nat Neurosci*. **20**:1580-1590

Lecture 3: Tollkuhn
Genes
• Activity-dependent gene expression
• Methodologies for studying gene regulation
• DNA methylation, MeCp2, Rett Syndrome
• Neuroepigenetics: searching for causality
• Transgenerational inheritence: facts and controversies

In-class paper reading: Chen MB, Jiang X, Quake SR, Südhof TC. 2020. Persistent transcriptional programmes are associated with remote memory. *Nature* **587**:437-442.

Lecture 4: Tollkuhn
Neurodevelopment and Cell Identity
• Cortical patterning
• Neuronal migration, "inside-out" corticogenesis
• Neural Cell Types, insights from single-cell sequencing
• Critical periods, development of the visual system

Discussion 1: Tollkuhn "Validity of rodent disease models"
- Bosch OJ, Nair HP, Ahern TH, Neumann ID, Young LJ. 2009. The CRF system mediates increased passive stress-coping behavior following the loss of a bonded partner in a monogamous rodent. *Neuropsychopharmacology* **34**:1406-15.
- Peça J, Feliciano C, Ting JT, Wang W, Wells MF, Venkatraman TN, Lascola CD, Fu Z, Feng G. 2011. Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature* **472**:437-42.
- Wright, EC, Culkin, HI, Sekar S, Kapoor A, Corbett C, Trainor, BC. 2020. Pubertal Androgens Reduce the Effects of Social Stress on Anxiety-related Behaviors in California Mice *bioRxiv* 2020.12.02.408526

Discussion 2: Tollkuhn "Discussion of mystery paper"

Problem Set Paper
Mystery paper! Students will write the title and abstract for the preprint.

<u>Student Evaluation:</u>
- Problem Set 40%, Discussions 40%, Class Participation 20%

Learning Objectives

Gain proficiency in the following:

- Activity-dependent gene expression
- DNA methylation and chromatin organization in neurons
- Epigenetic gene regulation in neurons
- Ocular dominance columns and ocular dominance shift
- Critical periods for visual system and brain sexual differentiation
- Innate behaviors: fear, aggression, parenting, pair-bonding
- Sex differences in the brain and behavior
- Common rodent behavior paradigms and what they measure
- Rodent models of autism, schizophrenia, depression, anxiety
- Morphogens and transcription factor gradients in neurodevelopment
- Corticogenesis: excitatory and inhibitory neurons
- The importance of single-cell sequencing for neuroscience

Learning Outcomes

- Students will gain proficiency in reading and discussing papers outside of their expertise
- Discuss common pitfalls in experimental design and interpretation of neuroepigenetic studies
- Demonstrate an understanding of critical periods in brain development
- Be familiar with common rodent models of human psychiatric conditions
- Discuss how neural activity leads to changes in gene expression
- Discuss how hormones organize and activate innate behavior circuitry
- Demonstrate an understanding of epigenomic and single-cell approaches in neuroscience

Lecture #1 Reference Material

- Gray JM, Kim TK, West AE, Nord AS, Markenscoff-Papadimitriou E, Lomvardas S 2015. Genomic Views of Transcriptional Enhancers: Essential Determinants of Cellular Identity and Activity-Dependent Responses in the CNS *J Neurosci*. **35**:13819-26
- Yap EL, Greenberg ME. 2018 Activity-Regulated Transcription: Bridging the Gap Between Neural Activity and Behavior. *Neuron* **100**:330-348

Lecture #2 Reference Material

- Wei D, Talwar V, Lin D 2022 Neural circuits of social behaviors: innate yet flexible *Neuron* **109**:1610-20
- Nestler EJ, Hyman SE. 2010. Animal models of neuropsychiatric disorders. *Nat Neurosci* **13**: 1161-1169
- Jones CA, Watson DJ, Fone KC. 2011. Animal models of schizophrenia. Br J Pharmacol. **164**:1162-1194

Lecture #3 Reference Material

- Lister R, Mukamel E, et al. 2013. Global Epigenomic Reconfiguration During Mammalian Brain Development. *Science*. **341**:629-643
- Tillotson R et al. 2021. Neuronal non-CG methylation is an essential target for MeCP2 function. *Mol Cell* **81**:1260-1275
- Heard E, Martienssen RA 2014 Transgenerational epigenetic inheritance: myths and mechanisms *Cell* **157**:95-109

Lecture #4 Reference Material

- Greig LC, Woodworth MB, Galazo MJ, Padmanabhan H, Macklis JD. 2013. Molecular logic of neocortical projection neuron specification, development and diversity. *Nat Rev Neurosci* **14**: 755-69.
- Bartolini G, Ciceri G, Marin O. 2013. Integration of GABAergic interneurons into cortical assemblies: lessons from embryos and adults. *Neuron* **79**: 849-64
- Tasic B, et al. 2018 Shared and distinct transcriptomic cell types across neocortical areas. *Nature* **563**: 72-78

- BRAIN Initiative Cell Census Network (BICCN) 2021. A multimodal cell census and atlas of the mammalian primary motor cortex. *Nature* **598:** 86–102

Core Course on Scientific Reasoning and Logic
Module 4: Macromolecular Structure and Function
Course Syllabus

Course Faculty
Organizer: Leemor Joshua-Tor
Module Tutor: Ankur Garg (garg@cshl.edu)

Lecture 1: Joshua-Tor
- Basic principles

Lecture 2: Garg
- Pymol Tutorial

Lecture 3: Joshua-Tor
- A structural perspective of RNA interference

Lecture 4: Joshua-Tor
- X-ray crystallography

Lecture 5: Joshua-Tor
- CryoEM and other methods in structural biology

Student Evaluation:
Presentation and written portion of protein tales: 60%
Lecture participation: 20%
Problem Set: 20%

Learning Objectives
- Elements of macromolecular structure
- Hydrophobic vs. ionic interactions
- Protein-nucleic acid and protein-protein interactions
- RNA folding/recognition
- Crystallography in a nutshell – what you need to know in reading structure papers critically
- Single particle negative stain and cryoEM
- Principles of CD, SAXS, NMR spectroscopy

Learning Outcomes
- Understand the principles of RNA interference pathways
- Demonstrate understanding of protein and nucleic acid structure and their utility in understanding biology
- Have the ability to download structures, visualize and interrogate them.
- Demonstrate understanding of protein-nucleic acid and protein-protein interactions
- Design methods to distinguish between direct and indirect protein interactions
- Discuss strategies for obtaining macromolecular structure and learn to decide which approach to use and what information one can obtain from each method
- Learn how to read structural biology papers and critically assess them

Reference Material
Textbooks:
- Watson, J.D. *et al., Molecular Biology of the Gene,* 2013
- Liljas, et al., Textbook on Structural Biology
  Alberts, B. *et al., Molecular Biology of the Cell,* 2008, pp. 329-400 and 411-454.
- Rupp, *Biomolecular Crystallography*
- McPherson, *Introduction to Macromolecular Crystallography*

Reviews:
- Ipsaro, J.J. and Joshua-Tor, L. 2015. From guide to target: molecular insights into eukaryotic RNA-interference machinery. *Nat Struc Mol Biol*. **22**: 20-28.

- Ozata, DM et al and Zamore, PD. 2019. PIWI-interacting RNAs: small RNAs with big functions. *Nat Rev Genet*, **20**, 89-108.
- Gutbrod, MJ and Martienssen, RA, 2020. Conserved chromosomal functions of RNA interference. *Nat Rev Genet*, **21**, 311-331.
- Crowther, R.A. 2016. *Methods in Enzymology* **579**: 2-445.

Discussion 1: Protein Tales

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Module 5: Study Section

Course Faculty
Instructor: Linda Van Aelst

Session 1: Van Alest
- Module Overview
- The NIH Grant System
- Grants Distribution

Session 2: Van Aelst
- Study Section I

Session 3: Van Aelst
- Study Section II

Session 4: Van Alest
- Study Section III

Student Evaluation:
- Primary reviewer presentation 40%, Written review 30%, Secondary reviewer presentation 20%, Class Participation 10%

In the Study Section Module, you will read and critique grants, much as an NIH Study Section reviewing applications would do (although we will have much more time per grant for presentation and discussion). We have pre-selected real grants for review. Every student is expected to read every grant and participate in the discussion of every grant. In addition to this, each student will be assigned as PRIMARY on one grant as SECONDARY on another grant and as a READER on a third grant.

PRIMARY reviewers will: (A) Prepare a 30 minute-presentation to serve as the basis of discussion of the grant. 15 minutes will be on the scientific Background and to introduce Specific Aims (Background presentation). 15 minutes will be devoted to summarize Preliminary Results and to evaluate the Experimental Design (Grant presentation) and 5 minutes will be devoted to the PI, environment, etc. (B) Each primary reviewer will also prepare a written critique of the grant, along the lines of the NIH Center for Scientific Review guide (http://www.csr.nih.gov/guidelines/R01.htm).
SECONDARY reviewers will read the grant in detail and in advance of the meeting. They should prepare specific commentary and questions and will have ~ 10 minutes to present them before the general discussion period.
READERS will read the grant in detail and in advance of the meeting. They should prepare specific questions for the general discussion period.

Written Critiques
The written critiques do not have to provide introductory information. They should begin by summarizing the overall goal of the proposed research in context with the importance of the questions to be addressed within the field of proposed study. The strengths and weaknesses of each approach should be outlined, with an aim-by-aim critique being generally the easiest to present and understand. Alternative and perhaps better ways to approach each question should be presented if they exist. In exemplary cases, it would be good to postulate why such approaches might not have been proposed. The review should finish with a brief discussion of the P.I. and their qualifications, the environment in which the research is to be performed and the appropriateness of the available facilities and budget/personnel. Any concerns about

regulatory issues with vertebrate animals, human subjects, data sharing and human embryonic stem cell research should be noted. An overall score should then be suggested based on the standard NIH priority score rating scale.

The oral presentations for the primary referee should proceed similarly except that they should include an introduction to the field and the work proposed. This should be sufficient to bring a non-expert up to speed with the topic of the grant. Secondary referees should follow the primary referee with comments and concerns on the science proposed and other areas (P.I. etc.) outlined above. The reader will not be responsible for a formal presentation but is expected to support the discussion.

Learning Objectives

- Gain an understanding of the structure and practices of an
- NIH study section
- Gain an understanding of the scored criteria of an NIH R01 grant
- Learn to write a written grant review
- Learn to present a grant as the Primary and Secondary Reviewer

Learning Outcomes

- Demonstrate an understanding of the structure and practices of an NIH study section
- Provide an oral and written critique of an NIH R01 grant
- Contribute to the critical analysis of an NIH R01 grant as a Group

Reference Material

- There are a number of sources to assist you in this assignment. The NIH has produced a video of a mock study section. "Inside the NIH Grant Review Process", is a 39-minute video developed by the NIH Center for Scientific Review. The video includes excerpts from the reviews of 3 types of NIH applications: R01 - Research project grant, K08 - Mentored clinical scientist career development grant and R03 - Small research grant.

- This video (or transcript of the video) can be viewed at http://www.csr.nih.gov/Video/Video.asp. Companion materials to the study section include the grant applications and summary statements used in the discussions. The summary statements, often referred to as "pink-sheets" because they used to be sent on pink copy paper, are the written critiques prepared by the study section, similar to this assignment.

- An annotated research grant is available from the NIH's National Institute of Allergy and Infectious Disease http://www.niaid.nih.gov/ncn/grants/app/default.htm.
- You might also find the list of guidelines for a study section chair useful. These can be found at http://www.csr.nih.gov/events/guidelineschairs.htm.

- Some terms you may come across (from the Center for Scientific Review):
  Percentile: represents the relative position or rank of each priority score (along a 100.0 percentile band) among the scores assigned by a particular study section.
  Priority score: A numerical rating that reflects the scientific merit of the proposed research relative to the "state of the science."
  Study section: panel of experts established according to scientific disciplines or current research areas for the primary purpose of evaluating the scientific and technical merit of grant applications. Also called scientific review groups (SRGs).
  Summary statement: a combination of the reviewers' written comments and the SRA's summary of the members' discussion during the study section meeting. It includes the recommendations of the study section, a recommended budget, and administrative notes of special consideration.