

Analyzing Function by Classical Genetic Approaches

Classical genetic analysis is a powerful approach to dissect complex biological processes. Selective removal, addition, or alteration of specific proteins creates mutant phenotypes that give insight into the normal roles of those genes. Analysis of double mutants lets one deduce the order of events and infer interactions between proteins. These methods let one propose mechanisms for processes that may be too complex to study effectively with biochemical or molecular approaches. Genetic studies can also provide definitive functional assignments for predicted genes, regardless of whether those genes exhibit sequence homology or have orthologues in other systems.

We will employ genetic analysis to identify and characterize genes governing mating of yeast and patterning of the *Drosophila* body and the *Arabidopsis* flower. We will discuss methods for disrupting gene function randomly, through large-scale mutagenesis screens, and specifically, through homologous recombination or construction of chimeric genes. This latter approach will let us investigate structure/function relationships within proteins. We will evaluate the nature of mutant alleles, considering the effects of dominant vs. recessive mutations and the impact each has on our interpretation of a process. We will use epistasis tests to establish the order of genes in a pathway. Finally, we will discuss the tissue and temporal requirement for gene function and consider how these insights from model organisms inform our approaches to understand phenotypes in other organisms.

Instructor

Christine Queitsch queitsch@u.washington.edu 685-8935 Foegen S410A

Meeting Time and Place

Tuesday / Thursday 9:00 am – 10:20 am Foegen S110

Course Materials**SLN 13854**

- 1) Web page with link to Course syllabus:
<http://www.gs.washington.edu/academics/courses/>
- 2) Electronic copies (pdf format) of each assigned article are on reserve in the UW Catalogue under electronic "Course Reserves". Log into the Course Reserves page using your MyUW id and look up the course by title (GENOME 553) or instructor (Queitsch).
- 3) Download "Questions for Thought" in pdf format by linking to the QfT on the syllabus page.

Grading

25% assigned homework for each reading
25% participation in class discussion (ASK QUESTIONS!)
50% final exam

Syllabus

Part One: Genetic Analysis in a Single-cell Eukaryote

Tues Jan 5 Introduction: When and why should one use genetic analysis? What is genetic analysis? To understand the major concepts in genetic analysis, we will begin by studying the work carried out by Lee Hartwell and colleagues to analyze cell-cycle regulation in yeast, emphasizing the control of cell-cycle progression by the mating pathway. The first meeting will consist of a lecture format introducing students to the yeast system, cell cycle, and nomenclature, and ending with a synopsis of Lee Hartwell's initial screen for temperature-sensitive mutants in yeast. Future meetings will consist of discussions based on assigned readings and "Questions for Thought".

Assigned: Sullivan, W. 1993. The Salvation of Doug: A tale of two retired scientists and some rope. *GENERations* vol. 1 #3. <http://bio.research.ucsc.edu/people/sullivan/savedoug.html>

Optional Background Reference: Hartwell, L. H., Hood, L., Goldberg, M. L., Reynolds, A. E., Silver, L. M., and Veres, R. C. 2004. **Reference A:** *Saccharomyces cerevisiae*: genetic portrait of a yeast. In: *Genetics from Genes to Genomes* 2nd edition. pp. 739–754. This chapter summarizes the useful features and outstanding genetic and molecular tools available in yeast. The Hartwell text also provides a good introduction to classical genetics and modern genomics.

Optional Review: Konopka J., and Fields, S. 1992. The pheromone signal pathway in *Saccharomyces cerevisiae*. *Antonie Van Leeuwenhoek*. **62**: 95–108.

General Background References for Yeast:

Yeast Data Base (SacchDB): <http://www.yeastgenome.org/>

Guthrie, C. and Fink, G.R., Editors. *Guide to Yeast Genetics and Molecular Biology*. Methods in Enzymology, Volume 194, Academic Press, New York, 1991.

Broach, J.R., Pringle, J.R. and Jones, E.W., Editors. *The Molecular and Cellular Biology of the Yeast Saccharomyces: Cell Cycle and Cell Biology*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1997.

Thurs Jan 7 How mutant phenotypes give insight into a biological process.

Assigned: Hartwell, L. 1980. Mutants of *Saccharomyces cerevisiae* unresponsive to cell division control by polypeptide mating hormone. *J. Cell Biol.* **85**: 811–822.

Tues Jan 12 Ordering genes in a pathway by double mutant analysis.

Assigned: Hereford, L. and Hartwell, L. 1974. Sequential gene function in the initiation of *Saccharomyces cerevisiae* DNA synthesis. *J. Mol. Biol.* **84**: 445–461.

Thurs Jan 14 How to interpret function based on differences in alleles.

Assigned: Whiteway, M., et al. 1989. The *STE4* and *STE18* genes of yeast encode potential β and γ subunits of the mating factor receptor-coupled G protein. *Cell* **56**: 467–477.

Tues Jan 19 Using genetics to characterize the structure and function of a protein.

Assigned: Mody, A, Weiner, J and Ramanathan, S. 2009 Modularity of MAP kinases allows deformation of their signaling pathways *Nat Cell Biol.* Apr;11(4):484-91.

Part Two: Genetic Analysis in a Metazoan System

Thurs Jan 21 The genetic principles used to characterize biological phenomena in single-celled organisms also apply to multi-cellular organisms. In these more complex systems, the methods and logic emphasize the analysis of tissue-specific vs. pleiotropic functions, and redundant genes or pathways. We will examine genetic control of morphogenesis in flies, beetles, and flowers as a tool for understanding metazoan genetics. The first lecture will introduce students to fly and Arabidopsis genetics, nomenclature, and genomics.

Assigned: Greenspan, R. 1997. *Fly Pushing* **Chapter 1:** 3–17.

Jones, A., et al. 2008. The impact of Arabidopsis on human health: diversifying our portfolio. *Cell* **133:** 939-943

Optional Background References:

Hartwell, L. H., Hood, L., Goldberg, M. L., Reynolds, A. E., Silver, L. M., and Veres, R. C. 2004.

Reference D: *Drosophila melanogaster*: genetic portrait of the fruit fly. In: *Genetics from Genes to Genomes* 2nd edition. pp. 813–838. This chapter summarizes the useful features and outstanding genetic and molecular tools available in flies.

Hartwell, L. H., Hood, L., Goldberg, M. L., Reynolds, A. E., M., and Veres, R. C. 2004.

Reference B: *Arabidopsis thaliana*: genetic portrait of a model plant. In: *Genetics from Genes to Genomes* 2nd edition. pp. 759–784. This chapter summarizes the useful features and outstanding genetic and molecular tools available in Arabidopsis.

Theissen, G. and Melzer, R., 2007. Molecular Mechanisms underlying origin and diversification of the angiosperm flower. *Annals of Botany* **100:** 603-619

General Background References:

FlyBase <http://flybase.bio.indiana.edu/>

Ashburner, M., Golic, K., Hawley, R. S. *Drosophila* A Laboratory Handbook. 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2005.

Arabidopsis thaliana data base: TAIR <http://www.arabidopsis.org/>

Weigel, D. and Glazebrook, J., *Arabidopsis*. A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2002

Tues Jan 26 Defining the pathway: Isolation of mutants; phenotypic characterization; mosaic analysis.

Assigned: Sanchez-Herrero, E., et al. 1985. Genetic organization of the *Drosophila* bithorax complex. *Nature* **313:** 108–113.

Thurs Jan 28 Exploiting evolution: understanding developmental pathways through comparative genetics.

Assigned: Shippy, T.D., et al. 2008. Analysis of the *Tribolium* homeotic complex: insights into mechanisms constraining insect Hox clusters. *Dev. Genes Evol.* **218:** 127–139.

Tues Feb 2 Arriving at pathway models: dissecting complex phenotypes with allelic series.

Assigned: Bowman, J. L., et al. 1991. Genetic interactions among floral homeotic genes of Arabidopsis. *Development* **112:** 1–20.

Thurs Feb 4 Necessary and sufficient: reverse genetics solves complications of redundancy.

Assigned: Pelaz, S., et al. 2000. B and C floral organ identity functions require SEPALLATA MADS-box genes. *Nature* **405:** 200–203.

Wed Feb 18 Take-home Exam Due, **5 pm.** NO EXCEPTIONS.