We live in the “post-genomic” era, in which the availability of complete genome sequences from a host of organisms offers exciting opportunities for undergraduate research. In this course, we will use some of the strain and clone resources generated by the *Saccharomyces* genome project to investigate the evolution of genes involved in the synthesis of methionine and cysteine, essential sulfur-containing amino acids.

**Investigations in Molecular Cell Biology**

- Course design and learning goals
- Pathways over Time: Our research project
- Course overview
- References
- Acknowledgments
Chapter 1

Welcome to Investigations in Molecular Cell Biology, a new kind of introductory lab course that incorporates an authentic research project in functional genomics. It has been said that we live in a “post-genomic” era. Large-scale genome projects have generated tremendous amounts of sequence data, and complete genome sequences are available for thousands of organisms. In a typical genome project, genes are identified largely by their sequence similarity to known genes from other organisms (Goffeau et al., 1996), with the assumption that the proteins encoded by the genes perform similar functions. These “loose ends” connecting sequence and function provide exciting opportunities for undergraduate students to participate in functional genomics research. In this course, students will study the functional conservation of the genes involved in methionine (Met) and cysteine (Cys) biosynthesis. Met and Cys are essential amino acids in all living cells. These two amino acids contain sulfur in their side chains, which allows Met and Cys to play unique roles in proteins.

We expect that students will make novel findings in their projects each semester and that students will be able to build upon the results obtained in preceding semesters. We hope that you enjoy the research experience and we look forward to your experimental results!

Course design and learning goals

Biology education at the undergraduate level is undergoing a transformation. For decades, many have viewed biology as an encyclopedic subject, because of the vast amount of content matter included in the undergraduate curriculum. A recent reevaluation of undergraduate biology education, however, is guiding biology curricula in a new direction, stressing the importance of involving students in the process of scientific investigation in their coursework (Bauerle et al., 2011). This reevaluation process has also challenged educators to sort through the vast amount of content in introductory biology to identify the core concepts that students should learn and the key competencies that students should acquire during their undergraduate education. This course has been designed in line with these recommendations.

Our course research project is designed to illustrate the core concepts of biology:

- **Evolution**: The proteins involved in Met and Cys synthesis show varied patterns of conservation during evolution.
- **Structure and function**: The structures of the proteins involved in Met and Cys synthesis are adapted to their catalytic roles.
- **Information transfer**: Met and Cys synthesis requires enzymes encoded by multiple genes.
- **Pathways and energy transformation**: The enzymes involved in Met and Cys synthesis are parts of intersecting energy-consuming pathways.
- **Systems biology**: The reactions involved in sulfur amino acid synthesis intersect with many other metabolic pathways in cells.
Introduction

During the semester, students will develop proficiency in some key competencies of professional biologists. Working in teams, students will:

- propose hypotheses and design experiments to test their hypotheses.
- learn basic skills of molecular cell biology.
- collect, organize and interpret experimental data
- find and use information from the primary scientific literature and online databases.
- communicate scientific results in a series of short oral presentations and written reports.
- use feedback from their peers and the teaching staff to compile data from the short interim reports into a final poster and a final report written in the format of a scientific publication.

Pathways over Time: our research project

In the 2015-2016 academic year, we will explore the conservation of Met and Cys biosynthetic enzymes between the budding yeast, *Saccharomyces cerevisiae*, and the fission yeast, *Schizosaccharomyces pombe*. As their names imply, *S. cerevisiae* and *S. pombe* are sugar-loving fungi that were originally isolated from beer. *S. pombe* and *S. cerevisiae* are members of the phylum Ascomycota that can be propagated in both diploid and haploid forms. In response to various stresses, haploid strains of opposite mating types are induced to mate and undergo meiosis. The four spores generated from meiosis are contained within a resistant structure known as the ascus, from which the phylum derives its name. The two species are thought to have diverged from a common ancestor about 1 billion years ago (Hedges, 2002). Since their divergence, the *S. cerevisiae* lineage has undergone a whole genome duplication, followed by rounds of gene elimination and diversification (Mortimer, 2000). Today, the size of the *S. cerevisiae* genome (Kellis *et al.*, 2004), ~12.5 Mbp, is similar to that of *S. pombe*. Because it has undergone less genome diversification, *S. pombe* is considered to be much closer to ancestral members of the phylum.

Diversification of selected yeast species within the Phylum Ascomycota

Different mechanisms of cell replication in *S. cerevisiae* and *S. pombe* are apparent in electron micrographs. (*S. cerevisiae* image reproduced with permission of Christopher Buser. *S. pombe* image from Hochstenbach *et al.*, Copyright National Academy of Sciences, U.S.A (1998), is reproduced with permission.)
Chapter 1

Of the two yeasts, *S. cerevisiae* is far and away the more thoroughly studied. Scientists have worked with genetically pure strains of *S. cerevisiae* for over a century, and it is widely used as a model organism (Botstein and Fink, 2011). *S. cerevisiae* has many of the same biochemical pathways as higher eukaryotes, but its genome is significantly smaller than vertebrate genomes and powerful genetic techniques are available for manipulating gene expression. For these reasons, the *S. cerevisiae* genome was the first eukaryotic genome to be sequenced in its entirety. Completion of the yeast genome sequence (Goffeau et al., 1996) allowed researchers to prepare genome-wide collections of mutant strains (Winzeler et al., 1999) and plasmids (Gelperin et al., 2005) that are available to the yeast community. This semester, we will use *S. cerevisiae* strains with defined defects in Met and Cys biosynthesis as the hosts for homologous genes from *S. pombe*. If the *S. pombe* gene restores the ability of the *S. cerevisiae* mutant to synthesize Met, in a process known as complementation, we will know that gene function has been conserved over the evolutionary time that separates the two species.

Course overview

The course can be viewed as a series of six related units (see opposite page).

1. **Boot camp**: Students will become acquainted with basic laboratory equipment and techniques for handling and viewing yeast. Students will also be introduced to some of the many online databases, which are important sources of gene and protein information.

2. **Yeast deletion strains**: Students will use selective growth media and the polymerase chain reaction (PCR) to identify three *S. cerevisiae* mutants, each of which is missing a single gene involved in Met or Cys synthesis (Winzeler et al., 1999). Students will use one of the deletion strains for the remaining experiments of the semester.

3. **Plasmid identification**: Students will isolate yeast overexpression plasmids from bacterial stocks and use restriction endonucleases to identify the plasmids. One plasmid contains the *S. cerevisiae MET* gene that is missing in their yeast strain (Gelperin et al., 2005). A second plasmid carries the *S. pombe* homolog for the *S. cerevisiae MET* gene, and the third plasmid carries an unrelated bacterial gene.

4. **Transformation and complementation**: Students will transform their yeast Δmet strain with the three plasmids. Students will use selective plates to determine if the plasmid-encoded genes complement the met deletions in transformed strains.

5. **Analysis of protein expression**: Students will use western blots to analyze expression of plasmid-encoded genes. The plasmid-encoded proteins are fusion proteins with epitopes at their C-termini that can be recognized by antibodies.

6. **Team-designed experiment**: Teams will design and conduct their own experiments, based on questions that have arisen during the previous experiments.
Overview of the semester’s experiments

1. Introduction to the lab (boot camp)
   Micropipettes and measurement (Ch. 2)
   Meet the microbes (Ch. 3)
   Yeast culture techniques (Ch. 4)
   Databases (Ch. 5)

2. Identify yeast deletion strains
   Identify missing genes in 3 Δmet strains by: their ability to grow on sulfur sources (Ch. 6) and yeast colony PCR (Chs. 7-8)

3. Characterize \textit{MET} expression plasmids
   Identify \textit{S. pombe} homolog (Ch. 9)
   Isolate plasmids (Ch. 10)
   Restriction mapping (Ch. 11)

4. Transform strain with expression plasmids
   Has complementation occurred? (Ch. 12)

5. Analyze Metp expression with western blots
   Prepare cell extracts (Ch. 13)
   Separate proteins by SDS-PAGE (Ch. 14)
   Analyze expression on western blots (Ch. 15)

6. Team-designed follow-up experiment
Chapter 1

Acknowledgments

It has been a pleasure to work with many Boston College colleagues and students on this fifth edition Investigations in Molecular Cell Biology. In particular, Dr. Douglas Warner has helped to design the experiments and has contributed many revisions to the text. Many thanks to Holli Rowedder for her careful editing and her help with the experiments.

Over the past five years, many teaching assistants have provided able leadership for their sections and offered valuable feedback about the effectiveness of this manual and suggestions for improving both the manual and the course. Hundreds of undergraduate students have also contributed very constructive comments and suggestions. Special thanks are due to David Chou, Class of 2012, who designed the cover and layout of this manual.

Finally, I would like to acknowledge support provided by the National Science Foundation for the Pathways over Time project through grant NSF114028.

References


