An inquiry-based lab course to study casein kinase 1 structure-function relationships in *Saccharomyces*

Lucy C. Robinson  
LSUHSC, Shreveport, LA  
Cynthia J. Brame  
Centenary College, Shreveport, LA
Biology 313 (Genetics) Lab
Centenary College

- Paired with intermediate level Genetics lecture course
- Required for biology majors and elective for biochemistry, biophysics and neuroscience majors
- 40-50 students in three lab sections, ~equal sophomore, junior and senior students
- Requires general chemistry and introduction to cell biology
Biology 313 (Genetics) Lab

Skills and ideas to be learned
  : Hypothesis generation and testing
  : Introduction to lab research
  : Inheritance and genetic analysis
    - Complementation testing
  : Molecular genetics techniques
    - Cloning
    - Site-directed mutagenesis
  : Analysis and communication of project results
Biology 313 Lab- Genetics

System:

- *S. cerevisiae YCK2* gene
- *YCK2* + *YCK1* are redundant and essential genes encoding casein kinase 1 (CK1) isoforms
- Much known about protein kinases in general, some about CK1 proteins; catalytic domains conserved among subfamilies; differs in some respects from those of other protein kinases
- Yck2 C-terminus modified, and modification is essential for localization and function
Biology 313 Lab - Genetics

• GFP fusion to Yck2 functional as kinase and visible with clear localization patterns in growing cells

• Have thermosensitive \textit{yck} mutant for testing complementation; also can use plasmid shuffle with double \textit{yck}1 \textit{yck}2 deletion mutant
Biology 313 Lab I, 2006

Original conception:

- Instructor-chosen mutations and primers
- Testing effects of deletions in C-terminus on localization and function
- Outcomes known to instructors
- 54 students (3 days)
- Weekly handouts describing goals, approach, experimental details
- Final lab report covered semester project
Biology 313 Lab II, 2008

• Addition of bioinformatics (database searching, Clustal W alignment) and structural biology (Cn3D use) components as well as original research
• 54 students, again 3 lab sections
• Students identified CK1-specific conserved sequences in kinase domain and proposed possible functions for sequences based on structure
• Students chose mutations and primers, carried out mutagenesis, cloned mutant alleles into expression vectors, and tested biological function and localization
• Outcomes not known to students OR instructors
ClustalW alignment of CK1 sequences, and identification of conserved segments
Students found several CK1-specific sequences; proposed function for three of these in ATP or substrate binding

Groups from each lab day chose one sequence to explore

Each group chose a mutation to generate (deletion, substitution)

Primer choices left to students BUT instructors chose differently for several- in those cases, both sets made and tested
Biology 313 Lab II, 2008

- Two substantial lab reports covering semester project, as well as lab notebook and exam results, allowed assessment of progress

  Results and assessment published:

- Three students chose to continue working on their mutation of interest as summer projects

- All three students presented their work at Centenary and a FASEB meeting
Biology 313 Lab III, 2009

- Focus on conserved structural features
  - Hypothesis that activation loop in CK1 phosphorylated, and that interaction of the loop with the RD pocket residues INHIBITS activity

- Weekly mini lab report replaced larger reports

- Second mini lab report included outline and flow chart of semester project
CK1 Structural Features

Activation loop - potential phosphorylation sites

RD pocket

Y225  S243  R194, K223, K240
Semester Project Overview

Ahmad Azzawe and Josh Phillips, Mini lab report 2
Biology 313 Lab III, 2009

- Final lab report was in the form of a poster section. Posters were presented at Centenary Research Forum—students in all biology lab courses (or working in labs at LSUSHC-S) can present projects.

- Three posters from Biology 313 Lab
Investigation of the Conserved RD Pocket in the CK1 protein kinase family

Authors: Dallas Krentzel*, Noor Azzawe, Michaela Beng, Caitlin Cavarra, John Cefalu, Jordan Day, Rob Grand, Sarah Kurniavila, Ruth Latinaoiz, Colin McCue, Marco Raje, Kelly Reed, Tyler Smith, Garrett Vick, Heather Wender

ADVISORS: Cynthia J. Brune and Lucy C. Robinson

ABSTRACT. Members of the CK1 subfamily of protein kinases regulate cell differentiation and proliferation, chromosome segregation, and circadian rhythm. Like all protein kinases, CK1s catalyze the transfer of phosphate from ATP to a protein substrate. One of the domain-specific features of CK1 kinases is a late activation loop and an RD pocket, an area of positively charged residues on the surface of the enzyme. In some kinase families, phosphorylation within the activation loop may activate the enzyme via binding of the phosphorylated residues to the RD pocket. We hypothesize that the RD pocket is involved in inhibiting CK1 kinases by binding phosphorylated residues from the activation loop. Here we have used a model CK1, YCK2, from the yeast Saccharomyces cerevisiae. Each of the three positively charged residues comprising the RD pocket was mutated to a negatively charged glutamate and a neutral glutamine via site-directed mutagenesis. We predicted that substitution of neutral residues would slightly decrease YCK2 activity while substitution of negative residues would significantly decrease YCK2 activity. Plasmids containing the mutant alleles were cloned, isolated, and sequenced to confirm introduction of the mutations. The mutant YCK2 alleles were then expressed in yeast. Effect on function was assessed by complementation tests and microscopy.

Introduction
Protein kinases are a diverse set of enzymes that are responsible for modifying proteins through phosphorylation. Most, but not all, regulatory protein kinases are found to be dimerized based on bioactivity detection, wherein both contain specific conserved residues that are essential for the function of the individual protein kinases (Belevc et al., 2018). The RD pocket is a conserved region in the carboxy terminus of protein kinases. All protein kinases consist of a similar basic fold structure which includes a larger linear-templates fold that is involved in ATP binding, a smaller linear-templates fold that is involved in protein binding, and a smaller core enzyme structure that is responsible for catalysis and catalytic nucleotide binding (Black and Salkoff, 1995). Additionally, the structure of CK1 includes a dimeric form that is involved in ATP binding (Black and Salkoff, 1995). It is known that the RD pocket can be an active site, which contributes to the activity of protein kinases. The RD pocket is a basic residue that is involved in binding to ATP and phosphorylating other proteins. The RD pocket has been shown to be important in the activity of protein kinases, but that it is not essential for catalysis (Black and Salkoff, 1995). Previous CK1 phosphorylation data has been shown in other studies, however, we hypothesize that the RD pocket is involved in phosphorylation within the activation loop (Black and Salkoff, 1995). We further hypothesize that the phosphorylated site would be blocked by the negatively charged glutamate. We further hypothesize that the phosphorylated site would be blocked by the negatively charged glutamate.

In our hypothesis, we determined the RD pocket to be a potential site for inhibiting glutamate (Glu) residues within each of the three isoforms comprising the RD pocket (see Figure 1). Our hypothesis is that the positive charge residues of the negatively charged glutamate will interact with the negatively charged glutamate within the RD pocket, then decreasing the activity of the enzyme (see Figure 1). The RD pocket consists of a basic residue that is involved in binding to ATP and phosphorylating other proteins. The RD pocket has been shown in other studies, however, we hypothesize that the RD pocket is involved in phosphorylation within the activation loop (Black and Salkoff, 1995). We further hypothesize that the phosphorylated site would be blocked by the negatively charged glutamate.

To test our hypothesis, we determined the RD pocket to be the potential site for inhibiting glutamate (Glu) residues within each of the three isoforms comprising the RD pocket (see Figure 1). Our hypothesis is that the positive charge residues of the negatively charged glutamate will interact with the negatively charged glutamate within the RD pocket, then decreasing the activity of the enzyme (see Figure 1). The RD pocket consists of a basic residue that is involved in binding to ATP and phosphorylating other proteins. The RD pocket has been shown in other studies, however, we hypothesize that the RD pocket is involved in phosphorylation within the activation loop (Black and Salkoff, 1995). We further hypothesize that the phosphorylated site would be blocked by the negatively charged glutamate.

In our hypothesis, we determined the RD pocket to be a potential site for inhibiting glutamate (Glu) residues within each of the three isoforms comprising the RD pocket (see Figure 1). Our hypothesis is that the positive charge residues of the negatively charged glutamate will interact with the negatively charged glutamate within the RD pocket, then decreasing the activity of the enzyme (see Figure 1). The RD pocket consists of a basic residue that is involved in binding to ATP and phosphorylating other proteins. The RD pocket has been shown in other studies, however, we hypothesize that the RD pocket is involved in phosphorylation within the activation loop (Black and Salkoff, 1995). We further hypothesize that the phosphorylated site would be blocked by the negatively charged glutamate.

In our hypothesis, we determined the RD pocket to be a potential site for inhibiting glutamate (Glu) residues within each of the three isoforms comprising the RD pocket (see Figure 1). Our hypothesis is that the positive charge residues of the negatively charged glutamate will interact with the negatively charged glutamate within the RD pocket, then decreasing the activity of the enzyme (see Figure 1). The RD pocket consists of a basic residue that is involved in binding to ATP and phosphorylating other proteins. The RD pocket has been shown in other studies, however, we hypothesize that the RD pocket is involved in phosphorylation within the activation loop (Black and Salkoff, 1995). We further hypothesize that the phosphorylated site would be blocked by the negatively charged glutamate.
Biology 313 Lab III, 2009

• Two students working on projects from 2010 iteration in LCR lab; a third student to begin Fall 2010
**Materials Needed**

- Plasmids
- Yeast strains
- Competent E. coli
- Culture media- yeast and bacterial- and incubators
- Reagents and equipment for transformation, plasmid prep, restriction digestion, gel electrophoresis
- QuikChange components
- Primers
- Thermocycler
- Sequencing service