

SAMPLE

2005-06 UROP STUDENT APPLICATION FORM

University of Colorado at Denver and Health Sciences Center

NOTE: Submit file as an attachment to roxanne.vidal@storm.cudenver.edu by 5:00 PM on 11 March 2005.

A. Student Principal Investigator:

1. Name Xyuuu Crow _____
(Principal Investigator or PI)
NOTE: If team grant, please identify PI and list co-investigator(s) below; only the PI has budgetary authority.

Major Biology _____ Student No. 111-11-1111 _____

Address 1055 E. Sett Ave. _____

City Denver _____ State CO _____ Zip 8888 _____

Telephone 720 555 5555 _____ E-mail xxxx@xxxx.com _____

Year: Fr ___ So ___ Jr ___ Sr X_ Graduation Date SPR 2006 _____ GPA 3.6 _____

Project Title "YOUR TITLE HERE" _____

Duration of Project (Dates): From April 2005 To April 2006 _____

Total Funds Requested: Stipend 500_ Expenses 1800_ Total 2300_

Project involves use of: animal subjects human subjects ___ neither ___
(check all that apply)

B. Team Grant Co-Investigators (modify to accommodate more investigators):

2. Name Axxxx Mxxxxx _____
(Co-Investigator)

Major Biology _____ Student No. 111 11 1111_

Address 11111 East Vin St _____

City Aurora _____ State CO _____ Zip 8888 _____

Telephone 303 555 5555 _____ E-mail xxxx@xxxx.com

Year: Fr ___ So ___ Jr ___ Sr X_ Graduation Date Spr 2006 GPA 3.5 _____

2005-06 UROP STUDENT Budget FORM
University of Colorado at Denver and Health Sciences Center

Principal Investigator: Xyuuu Crow
 Title here _____
 Project Title: _____

	Amount
Stipend	500
Animal cost	300
Equipment	
Supplies	1500
Computer Services	
Library fees	
Lab fees	
Copying Costs	
Travel Expenses	
Total Request	\$2300

- (maximum: \$1150 per student; \$2300 per team of two; \$3000 per team of three or more)

Budget Justification: Explain what you will do and how much time it will take, including the number of hours for the stipend. Justify the need for each of the other expenses.

Costs include obtaining animals (\$25 for each male or female frog; additional costs for food and care of the animals). The use of these animals has been approved by the University animal care committee. Supplies include chemicals for the centrifugation procedure to obtain the rafts (about \$200 as we have some small supply already: protease inhibitors, calcium chelators: EDTA and EGTA, molecular biology grade sucrose, and the detergent - molecular biology grade Triton X-100- is obtained under nitrogen). Other supplies include two ELISA kits for recording Src activity (already perfected in Dr. CCCC's lab; about \$650) and PLC activity (about \$650). The use of preassembled "kits" facilitates completion of the experiments by undergraduate researchers yet still provides an excellent learning experience. Explain stipend here.

2005-06 UROP FACULTY SPONSOR FORM
University of Colorado at Denver and Health Sciences Center

NOTE: Sponsor should submit this file on the web by the deadline. The attachment should clearly identify the PI and major as part of "name" for the attachment, such as UROPSmithChemistry.doc. The subject line of the e-mail should also identify UROP Sponsor, as well as the name and major of the PI.

To be completed and submitted by Faculty Sponsor:

Name Prof name here
(Faculty Sponsor)

Department Biology Campus Box 171

E-mail xxxx@cudenver.edu Telephone 6 0000

Name and Axxxx Mxxxxx
(Student Principal Investigator)

Comment on the ability of the student(s) to carry out the proposed project.

Axxxx Mxxxxx received a B and Xyuuu Crow received an A from my difficult General Cell Biology class and Axxxx Mxxxxx is earning an A in my developmental biology class (he got the second highest grade just last week on exam one). Since last fall, these students have expressed an interest in working in the lab and this project provides an excellent opportunity for them to learn basic lab skills and procedures associated with fertilization. Axxxx Mxxxxx has already worked in my lab for a few months and has learned much (she is very dedicated as she has worked without payment, or even credit hours). I am impressed with not only their intelligence and their interest in working in the lab (begun well before this proposal was initiated), but also with their strong work ethic (as noted, in part, by the many drafts that we went through to write this UROP grant proposal).

Comment on the role that this project will play in your scholarly activities.

As we have measured whole cell fertilization events with PA, the experiments described here with the raft preparation **represent a totally new direction**. By removing most of the cell, we can center in on and more carefully define those early fertilization event that take place at the plasma membrane- when the sperm first contacts the egg. Dr. Sato has worked out all details of this new and valuable preparation (as evidenced by his many publications in excellent journals). This is the **central project in the lab** over the next year.

Comment on the merit of this project and its potential impact.

This is the next logical step in our work to understand how the sperm activates fertilization events (such as Src and PLC). Knowledge of how the sperm acts may help those infertile couples hoping to have a child.

I have reviewed the UROP Proposal and Budget Request for the aforementioned principal investigator and, by submitting this form, agree to serve as faculty sponsor to the student(s) and evaluate their accomplishments at the end of the semester/funding period. Further-more, I understand that the student principal investigator is the only one authorized for budget transactions and proposal revisions.

Introduction: The objective of this UROP project is to examine the mechanism of fertilization. Our model is that a lipid found in sperm (phosphatidic acid or PA), or this lipid PA produced in the egg after fertilization, stimulate Src and PLC to induce fertilization events (Fig. 1). As sperm and egg bind at “membrane rafts” on the egg surface, we will explore this model by isolating these membrane rafts.

Figure 1:

Sperm PA? Or Egg PA? | → stimulates Src in the egg → stimulates egg Phospholipase C (PLC) → **FERTILIZATION**

((NOTE THAT FIGURES HAVE TO BE SENT AS AN ATTACHMENT—MAX OF TWO FIGURES)))

First, we will learn Dr. Ken-Ichi Sato’s technique of isolating membrane rafts from *Xenopus* (frog) eggs. Second, as Dr. Sato has done, we will learn how to add sperm to the isolated egg membrane rafts and record the induction of fertilization events. Finally, we will remove the enzyme that makes lipid PA in the egg and see if this blocks fertilization events. If the removal of this enzyme does not block fertilization events, then we will say that sperm PA is sufficient for fertilization. Dr. CCCCC has already shown that PA is found in sperm in unusually high quantities, that PA in the egg (or zygote) increases immediately after fertilization and that PA can stimulate Src and PLC.

This work is truly significant to the scientific community for a variety of reasons. First, an exact pathway of conception will be obtained (Figure 1). Note in this figure that activation of Src and PLC are central to fertilization, but currently no one knows the mechanism by which sperm activates Src. Finally, the unique nature of this work is that we will be lowering the “background noise” of the egg by isolating egg membrane rafts, thus removing the extraneous cell parts. Furthermore, this cell free preparation will allow us to remove the enzyme that produces PA in the egg raft to determine whether sperm PA, or PA produced in the egg, is required for fertilization events.

Background: Dr. CCCCC, of CU-Denver, in a collaborative effort with Dr. Ken-Ichi Sato of Kobe University in Japan, is attempting to determine the events that occur at the moment of creation- when the

sperm first interacts with the egg. Sato has isolated membrane rafts so that ninety-nine percent of the *Xenopus* egg can be discarded and analysis of the initial fertilization events occurring between the sperm and egg membrane raft can be emphasized --without interference from subsequent events.

Membrane rafts are microdomains of the plasma membrane that contain high concentrations of cholesterol, sphingomyelin and glycosphingolipids. These lipid rafts are specialized areas on the surface of the egg plasma membrane where a sperm will bind to an egg. In addition, many studies in other systems have found that membrane rafts are integral in cell regulation. Through this egg raft assay, Sato¹ has shown that sperm can activate a tyrosine kinase called Src, and that the Src kinase then activates an enzyme called Phospholipase C (PLC). The activation of PLC then initiates the release of calcium (Ca²⁺), which leads to all of the major fertilization events. However, the mechanism by which sperm activate Src kinase is still unknown. Dr. CCCC has suggested that a lipid called PA resides in sperm and acts as a catalyst for the activation of both the Src kinase and later PLC.

Methodology

The membrane raft preparation of Dr. Ken-Ichi Sato is an *in vitro* assay system. It is simple to obtain by: 1. homogenization of the egg using detergent Triton X 100 to solubilize membranes; 2. separation of egg rafts from other cell parts by density gradient centrifugation (separates cell parts based on density and size). The final centrifugation will occur at 150,000 X g for twenty minutes and utilize the Biology Department ultracentrifuge. Homogenization of eggs takes place in protease inhibitors (to prevent degradation of Src and PLC) and various concentrations of salt and sucrose.

After isolating the membrane rafts by centrifugation, we will add sperm and record fertilization events. Specifically, we will learn how to record Src and PLC activity (Dr. CCCC has found that PA can activate Src and PLC). Both of these assays have been perfected by Dr. CCCC and have been performed by undergraduates. We will then remove the egg enzyme that makes PA and see if this makes any difference in the ability of sperm to induce fertilization events in the egg rafts. That is, we will use an

¹ Sato, Dr. Ken-Ichi, et al. "Reconstitution of Src-dependent Phospholipase c Phosphorylation and Transient Calcium Release by Using Membrane Rafts and Cell-free Extracts from *Xenopus* Eggs." *The Journal of Biological Chemistry*, vol. 278, no. 40, October 2003, pp. 38413-38420.

antibody to the enzyme that makes PA to immunoprecipitate or remove the enzyme from the egg membrane raft (something that cannot be done in an intact cell but can be done in the raft preparation). Then we will see if sperm addition can stimulate the two fertilization events (Src and PLC activation). If PA from sperm is all that is needed for fertilization (not the PA increase that occurs in the egg raft after fertilization), then this removal of the egg enzyme should not prevent sperm activation of Src and PLC. If PA produced in the egg raft is required, then we will block induction of fertilization events by PA since we removed the enzyme that produces the PA in the membrane raft. We already have the antibody and have shown that it “immunoprecipitates” or removes the enzyme that makes PA. In addition, Sato has already used this immunoprecipitation technique with egg rafts (he removed Src, showing that it is required) therefore all steps have already been worked out.

Timeline

Due to the fact that the ultracentrifuge equipment is already located in the Biology Department at UCD, and since the analyses of Src and PLC have already been developed by Dr. CCCC’s lab, we anticipate rapid progress. We will begin working this summer since we have already had the course background. We have both taken Dr. CCCC’s Cell Biology class where many topics related to this project were introduced. Currently, as an undergraduate student, Xuuu is enrolled in Dr. CCCC’s graduate level Developmental Biology class, and one of the main aspects of this class is an understanding of fertilization. We have already begun visiting his lab and observing fertilization procedures.

Collaboration

We will work very closely with Dr. CCCC to learn the use of the ultracentrifuge to isolate membrane rafts; this equipment is an expensive but rather easy to use. We will also work with experienced graduate students in his lab. After developing many basic skills, we will begin to work more independently, at this time, we will meet with Dr. CCCC for less time but we will talk each day that we work. Starting even last fall, Dr. CCCC, xUUU and xAAA spent extensive time reviewing fertilization and Sato’s technique in preparation for the initiation of our project.