HHMI Course-based Research Experiences (CRE) Course Development Grant Program

Cover Sheet

Name of Course being created/revised:
Check the appropriate development type: [ ] Module [x] Full Course
Name of PI: Benjamin Kopek
Name(s) of collaborators: 
Total Budget Requested: $ 10,000

<table>
<thead>
<tr>
<th>Anticipated Long-term costs associated with this course:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurring equipment/supplies costs ($)</td>
</tr>
<tr>
<td>~ $1500</td>
</tr>
<tr>
<td>Recurring student assistant costs (hrs/semester)</td>
</tr>
<tr>
<td>$ 6 hrs/week x 15 weeks = 120 hr (TA)</td>
</tr>
<tr>
<td>Recurring staff costs (contact hours/semester)</td>
</tr>
<tr>
<td>6 hr</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Approvals</th>
<th>Signature</th>
<th>Date</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>Benjamin Kopek</td>
<td>2/16/15</td>
<td></td>
</tr>
<tr>
<td>Chairperson</td>
<td>OM Donahue</td>
<td>2/16/15</td>
<td></td>
</tr>
<tr>
<td>Dean</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Abstract
In this proposal, I detail plans to fully integrate course-based research into the recently offered Virology course (BIOL 395). In Fall 2014, 22 mostly upperclassmen took Virology and its associated lab. Virology will be offered again in Fall 2015 and expanded to 36 students with two laboratory sections of 18 students each. The course lab in 2014 involved performing basic molecular virology techniques with no research component. For Fall 2015, I am proposing to integrate the learning of molecular virology skills within an authentic research experience. The research will focus on elucidating the role of host-factors involved in virus replication using the model system Flock House virus (FHV). Viruses are obligate parasites and thus rely on host cell factors for many steps in their replication. Understanding the role of various host factors in aiding or inhibiting viral replication may assist development of novel antiviral approaches. The course-based research will give students the opportunity to perform cutting-edge research while learning basic molecular virology concepts and techniques.

Research Description

Introduction
Positive-strand RNA [(+)+RNA] viruses are significant threats to human health and include pathogens that are the leading cause of liver cancer and foodborne illnesses in the U.S. Despite the threat (+)RNA viruses pose to human health, few antivirals exist to combat virus infection and replication. As obligate parasites, viruses modulate host functions to create a favorable environment for replication (Ahlquist et al., 2003). In turn, host cells modulate their own functions (i.e., immune systems) in a cat-and-mouse game to halt virus replication. Elucidating virus-host interactions may provide novel routes for controlling viruses.

The model (+)RNA virus students will use is Flock House virus (FHV). FHV is a simple (+)RNA virus with a 4.5 kb bipartite genome (Ball & Johnson, 1998). Insect cells (Drosophila melanogaster) are a natural host for FHV, but FHV will also replicate in a wide-variety of organisms including mammalian, plant, and yeast (Ball & Johnson, 1998). The diversity of organisms that support FHV replication suggests that host factors affecting FHV replication are widely conserved. FHV is an ideal system for an undergraduate laboratory since it replicates robustly in insect cells and is non-pathogenic to humans.

Multiple large scale screens have been performed to identify host factors involved in FHV replication (Hao et al., 2014; Kemp et al., 2013). Hao et al. took advantage of a system whereby FHV is capable of replicating in Saccharomyces cerevisiae (Price, Rueckert, & Ahlquist, 1996) to perform a genetic screen for factors affecting FHV replication using a yeast knockout strain library. This genetic screen identified 65 gene products implicated in affecting FHV replication. Using a transcriptomic approach, Kemp et al. identified 279 host genes whose expression was modulated upon FHV infection of Drosophila cells (Kemp et al., 2013). Changes in gene expression in response to infection could result from several factors including an immune response or the altering of the cellular environment by the virus to assist replication. The roles of very few host factors involved in viral replication have been studied in depth, likely due to the sheer number identified in screens. Herein lies an
opportunity for students to contribute to an important field, as understanding how host factors are involved in virus replication will help to illuminate important pathways in virus-host interactions.

**Approach**

Students will begin their research by examining the lists of host genes identified in screens as having roles in FHV replication. Working in groups of four, students will identify four host genes of interest and validate the previously published results.

Validation and extension of the yeast knockout data will be done by identifying *Drosophila* homologs of the yeast genes followed by genetic knockdown of the genes using RNA interference (RNAi). Templates to generate dsRNA for RNAi assays will be purchased from the *Drosophila* RNAi Screening Center at Harvard Medical School. Students will evaluate FHV replication and gene specific knockdown using reverse-transcription quantitative PCR (RT-qPCR). Proper controls such as cell viability assays, control gene knockouts, and off-target effects will be discussed and performed.

Validation of the transcriptomic screen will be done by examining the expression levels of those genes using qPCR in response to FHV infection. The role of the gene will be further evaluated by knocking down the gene using RNAi. If a gene is knocked down and viral replication increases, the gene is possibly involved in a host defense response to limit infection and replication. This may lead to insights into the molecular mechanisms of innate immunity. Alternatively, if a gene is knocked down and viral replication decreases, the gene is possibly contributing, either directly or indirectly, to viral replication.

Once students validate prior results, each group will select one host factor to examine in more detail. Students will perform an extensive literature search of their selected host factor and generate a hypothesis as to its role in viral replication. Once students generate a testable hypothesis, they will design experiments. At this point, groups will write and submit a “grant” with their preliminary data, background, specific aims, experimental design, and expected outcomes. A panel (the instructor, TA, and another group of students) will review the grant and decide whether to “fund” the study or ask for revisions before funding. This is an important aspect of the process, not only because it gives students experience with grant writing and review, but also ensures students are proposing experiments that are feasible within the real constraints of time and money. Once each group’s proposal is “funded”, it becomes a truly independent project for the students and each group will likely take a unique strategy to determine the role of the host factor in FHV replication.

Figure 1 shows a simplified view of the major steps in (+)RNA virus genome replication. Data exists for host factor involvement in most of the steps shown including virus entry, translation of viral replication proteins, replicase protein targeting, RNA recruitment, virus assembly, and virus egress. Based on their literature review, students will generate a hypothesis as to what step in the viral replication cycle the host gene may be influencing.
One example of a student project could be to examine one of the genes identified in the yeast knockout screen that functions in protein trafficking and vesicle mediated transport between sub-cellular organelles. Students may hypothesize that this particular gene interrupts the transport of the viral replication protein to mitochondria. Students could then test this thru a variety of biochemical and molecular biology techniques including fractionation assays, fluorescence microscopy, and antibody detection assays.

Another example could be an examination of the gene perilipin whose expression is induced by FHV infection. Perilipin is involved in the mobilization and metabolism of lipids (Grahn et al., 2013). Like all (+)RNA viruses, FHV replicates in association with host intracellular membranes, thus students may hypothesis that upregulation of perilipin may be a cellular response to the interaction of FHV proteins with cellular membranes. A literature review would reveal that the replication proteins of several (+)RNA viruses are involved in remodeling the cellular lipid environment to aid virus replication. Students may then propose experiments such as the expression of viral proteins alone, in the absence of full virus infection, to determine if perilipin expression is still increased. More in depth experiments could also examine the changes in lipid composition of FHV-infected cells if perilipin expression is limited by RNAi.

Some students will have the opportunity to continue the research project during the spring semester in my laboratory and present their results through posters at the Hope College Annual Celebration of Undergraduate Research and Creative Performance. Since the identification of host factors involved in viral replication is
critical to the development of new anti-viral strategies, this work will provide a foundation for student work in my own laboratory.

**Education**

Few Colleges and Universities have a virology course as part of their curriculum (at both the graduate and undergraduate levels) and fewer still have associated labs. Thus, there are no standardized curricular guidelines for general virology courses. Guidelines exist for clinical virology programs, but working with most human infectious agents is outside of the scope of this course and the biohazard capabilities of this institution. In general, the types of assays we will perform (PCR, antibody detection) are all assays commonly used in clinical virology labs to detect pathogens (e.g., HIV, Ebola, Influenza). Therefore, students will be learning methodologies that could be extended to more pathogenic specimens. However, most important is that students will be learning the process of science and scientific thinking.

**Anticipated Learning Gains**

By the end of the semester, students will be able to:

- Perform a literature review and critically analyze and interpret scientific literature.
- Formulate testable hypotheses based on literature and prior results.
- Design experiments to test hypotheses.
- Write a hypothesis-driven grant proposal based on preliminary results and review of the literature.
- Analyze and interpret results from experiments.
- Write a summary of their project that is intelligible and logical.
- Orally communicate scientific results to their peers in a coherent fashion.
- Learn effective means of collaboration during all aspects of the scientific process from hypothesis generation to presentation of results.

By the end of the semester, students will be able to perform the following laboratory skills in a safe and technically sound manner:

- Animal cell culture and sterile technique
- Proper biological safety methods with appropriate protective equipment and procedures.
- Reverse-transcription quantitative PCR
- Viral infection at a proper multiplicity of infection
- Lipid-based transfection
- Antibody detection methods
- General and fluorescence microscopy

Students will gain these skills through the course-based research project proposed. Evaluation of student learning will be based on a grant proposal, a final paper, and oral presentation of the results. Much like the grant proposal, the paper will be peer-evaluated in a style of a manuscript submitted to a peer-reviewed journal. Students will “submit” their results to a journal. The instructor will serve as a “managing editor” and send the manuscript out for review. Another group will act as anonymous reviewers to evaluate if the manuscript is technically sound, if the data support the conclusions, if the
statistical analysis has been performed appropriately and rigorously, and if the manuscript is presented and written in an intelligible fashion. Students will then have a chance to respond to any criticism from the reviewers and revise the manuscript. Lastly, students will present their work in the form of a 10-minute oral presentation.

Resources

The schedule for Virology will include 3 laboratory hours per week during the fall semester. There will be two laboratory sections of 18 students each. The laboratories have been scheduled back-to-back to create a 6-hour block of dedicated virology laboratory time when the instructor and a teaching assistant will be available to assist students. Since this is project based, students will be encouraged to come in before and stay after their assigned laboratory section. Literature review, hypothesis generation, manuscript and oral presentation preparation will all occur outside of the dedicated lab time. Students will also be required to come in on days when their lab does not meet in order to perform certain exercises but longer assays that require instructor or TA assistance will initially be encouraged to be performed during the dedicated lab period.

A major cost is the acquisition of templates for dsRNA production that will be used in RNAi screens. I will initially purchase templates for all the ~350 genes identified in the screens from the Drosophila RNAi Screening Center at Harvard Medical School. Each template costs $8 for a total of ~$2800 but can then be reused and regenerated indefinitely. Other costs will include RT-qPCR reagents (~$800), oligonucleotide primers (~$400), in vitro transcription kits ($300), transfection reagents ($300). Some reagents and kits can be used over multiple years. Continuing yearly costs include cell culture supplies (media, flasks, plates, etc.) and other consumables (pipettes, pipette tips, etc.). After the initial purchase of the RNAi screening templates, recurring costs would be the normal amount typical for an advanced cell and molecular biology course laboratory.

Sustainability of the course also depends on the number of host factors available for screening. On average, 10 host factors will be examined in-depth per year. With over 300 host genes identified in the literature to play roles in FHV replication there is conservatively 30 years of laboratories that could be run.

Implementation Timeline

Summer 2015 – Development of a laboratory manual, including standard protocols to be used by students during independent projects. Acquisition of reagents and other materials. Preparation of pre- and post- assessments of learning gains.

Fall 2015 – Implementation of laboratory.

Spring/Summer 2016 – Evaluate and revise the course based on student learning gains, feedback from students and the teaching assistant, and project data.


