INQUIRY & INVESTIGATION

The Use of Inquiry-Based Activities with *Caenorhabditis elegans* to Enable Students to Learn about UV Radiation

RECOMMENDED FOR *AP Biology*

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ABSTRACT

In traditional microbiology laboratory activities, different strains of bacteria are exposed to UV radiation for varying lengths of time. This article provides information that expands on these activities (or uses them alone) with Caenorhabditis elegans *as a model organism with which to study the effects of UV radiation. These activities might be used in an introductory class to teach students the basics of working with* C. elegans. *In cell biology and microbiology classes, students might learn about how UV radiation can damage cells and cause cancer. These activities can also be used to teach the students about how genetic background can affect the sensitivity to UV radiation. In the laboratory, the students design their own activities by altering the parameters of the basic UV radiation experiment. By performing these laboratory activities, students will learn about UV radiation and about all parts of the scientific process.*

Key Words: *guided inquiry; UV radiation;* C. elegans.

Introduction

Faculty members have long thought about ways to improve undergraduate biology education. One common approach involves engaging the students in the process rather than just teaching the concepts. The students become active learners through a hands-on approach to learning. This may include designing classes that provide inquiry-based experiences in which students design and develop their own independent research projects instead of performing experiments where the results are already known. At times, the students might also be able to attend a conference and present their inquiry-based research. All of these strategies support vision and change in Next Generation Science Standards (NGSS, http://www.nextgenscience.org/).

Various studies have shown that incorporating inquiry-based activities into the classroom helps engage students with learning.

Various studies have shown that incorporating inquiry-based activities into the classroom helps engage students with learning. It is important for students to perform original research to develop new ideas rather than just learn the basic facts relating to a topic. In so doing, students can ask their own questions and learn all stages of the research process. In these types of inquiry-based learning experiences, the answer to the questions and experiments are unknown. Groups of students can be involved in a research project, which can be beneficial to all students. Studies have shown that inquiry-based experiences help students in their careers. It is not always possible for all students to perform independent research with a faculty member, whether because not enough faculty exist who can mentor all of the students, or the schedules of the students (particularly with non-traditional students) will not allow for it. Therefore, by embedding inquiry-based learning into the classes, all students will have some exposure to original research. (Auchincloss et al., 2014; Beach & Alvarez, 2015; Gray et al., 2015; Shapiro et al., 2015; Symes et al., 2015). Not all students will be able to work one-on-one with a faculty member in a research internship but when faculty use course-based undergraduate research experiences, students will still be exposed to inquiry-based learning (Auchincloss et al., 2014).

It is also important that students take these inquiry-based classes early in their careers. However, there may be additional obstacles, such as the background of the students and the fact that introductory classes are usually larger than more advanced classes (Bugarcic et al., 2012; Rodenbusch et al., 2016). A large study of 4,898 students at the University of Texas–Austin, performed from 2006 to 2014, showed that students participating in these inquiry-based courses for three semesters starting as freshman were more likely to graduate from a STEM discipline within a six-year period. To gain the maximum effects of the research experience, it is

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important for the students to complete all three research-based courses (Rodenbusch et al., 2016).

Caenorhabditis elegans (*C. elegans*) has a number of attributes that classify it as a model organism for use in inquiry-based activities. Some of these features are similar to other model organisms, such as their ease of use, the limited time required for the experiments (rapid reproduction of the organism), a measurable phenotype, and the cost in maintaining the organisms (Dassarma et al., 2016; Layton & Belden, 2016). In addition, *C. elegans* was the first organism to have its genome sequenced. It is also small in size and has a transparent body, allowing visualization of different processes. *C. elegans* hermaphrodites can self-fertilize so that maintaining the different lines is not time-consuming. The lifespan of *C. elegans* is relatively constant, which is important for aging and survival studies. *C. elegans* has also been used for RNAi interference studies, which can easily be adapted to undergraduate laboratories (Andersen et al., 2008; Tejeda-Benitez & Olivero-Verbel, 2016; Tissenbaum, 2015). There are also a number of online resources that can help students and faculty who are new to this field; see Appendix Table 1.

The main goal of this article is to provide foundation materials and strategies to faculty so that they can develop inquiry-based laboratory activities in their own classes that use *C. elegans*. The main concept centers on how UV radiation can harm the *C. elegans*. Besides performing the laboratory activities and discussing the effects of radiation on the *C. elegans*, faculty might also want to use case studies about cancer to tie this laboratory more directly to concepts being taught in the class. Links for case studies on different types of cancer are provided in the Appendix.

The rest of this article gives information and details on how to set up these activities. Suggestions are presented for ideas on how to make these experiments into an inquiry-based laboratory for students. This handout should be modified by different faculty depending on the time that is available. The initial supplies can be prepared for the students, including making up agar plates and adding OP-50 bacteria and *C. elegans* to the plates. The objectives of this project may also vary based on the level and content of the class being taught.

○ Student Handout

C. elegans is a model system that can be used to study various processes in microbiology, cell biology, genetics, and introductory biology laboratories. This article describes activities that use UV radiation as a concept to introduce students to inquiry-based activities with *C. elegans*.

Since UV radiation causes thymine dimers and, in doing so, inhibits the process of DNA replication, investigators must use the *C. elegans* at a stage when they are actively replicating, which is when they are very young. The lifecycle of *C. elegans* consists of the eggs, stages L1–L4, and then the adult stage (Altun et al., 2009). UV radiation activities should be set up when the *C. elegans* are at the egg or L1 stages.

For faculty and students who are new to the *C. elegans* field, Appendix Table 1 lists online resources for obtaining background information and protocols for working with *C. elegans*. It is advised that teachers explore these resources so that they can provide adequate background to the students before they begin their activities with *C. elegans*.

Prior to starting these activities, faculty must order OP-50 bacteria and the *C. elegans* strains from the *Caenorhabditis* Genetics Center and expand them. These items are inexpensive, but it is worthwhile to invest in a few dissecting scopes that have more than the minimal magnification to view the worms. The *C. elegans* can be grown at room temperature or at 20–22°C but not at 37°C. The OP-50 bacteria are grown at 37°C.

In preparation for these activities, faculty will first need to make NGM agar plates and add the OP-50 bacteria to them. Then, the *C. elegans* strains will be grown on the plates. The protocols for maintaining *C. elegans* have been previously described (Stiernagle, 2006). The Appendix also includes other resources for working with *C. elegans*. Other videos on using *C. elegans* can be found on the website for the Journal of Video Experiments (JOVE, search "*C. elegans* maintenance").

One radiation-sensitive strain of *C. elegans* is called SP483. To get information about this strain and to order them, investigators should go to the website for the *Caenorhabditis* Genetics Center. Additional details can also be found on the Wormbase website. A published study has characterized the phenotype of this strain (Dittrich, 2012). Other radiation-sensitive *C. elegans* or related strains (for example, DNA repair mutants) can also be found by searching in Wormbase. See O'Neil and Rose (2006), for a list of strains that are deficient in DNA repair genes.

Faculty will need to have these strains on hand before the students start the laboratory activities. However, it is a good exercise to teach the students how to find information on them.

The first step of the laboratory activity introduces the students to the inquiry-based activities. The students will design an activity examining the effects of UV radiation on *C. elegans*. Students should be given an explanation of the project and then be given time to get with their group and decide how they will set up their activities. This planning is important because a number of plates with *C. elegans* growing on them should be prepared before the laboratory activities begin. It is best if the faculty member or laboratory prep person makes these plates (unless the teacher has a lot of class time for these activities).

Objectives for These Laboratory Activities

After completing these activities, the student should be able to:

- Understand how the steps of the scientific method apply to an inquiry-based laboratory activity.
- Design an independent research project with appropriate control and experimental groups.
- Understand that these activities should be set up with more than one sample.
- Analyze data of the UV radiation activities, including graphing data and possible use of statistics.
- Understand how genetic background can affect the results (the relationship between genotype and phenotype).
- Discuss the effects of UV radiation, including how mutations in DNA can lead to cancer and death.
- Discuss the relationship between repair genes and cancer.
- Understand that the effects of UV radiation and repair genes can be studied in different model systems.

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Student-Designed Activities

The teacher should guide the students based on some of the learning objectives for the course. For example, these laboratory activities could be used in a lower-level biology class to introduce the students to the use of *C.elegans* and how to set up experiments using the scientific method. In more advanced classes such as genetics or cell biology, the teacher may want the students to focus more on different strains of *C. elegans* or on how UV radiation causes cancer and can kill the organism. This concept can be further expanded to also test compounds that can block the effects of UV radiation. Some teachers might want to alter the objectives listed above. Described below are some suggestions and details for inquiry activities that students might pursue, but they should be given latitude to design other, independent inquiries. It is recommended that at least two plates be used for each group.

Activity 1. In this activity, wild-type N2 *C. elegans* will be exposed to UV radiation for different time periods. Group A should be an unirradiated control. Group B should be irradiated for 15 seconds, group C for 30 seconds, group D for 1 minute, and group E for 2 minutes.

Activity 2. In this activity, wild-type N2 will be compared to radiation-sensitive *C. elegans* for the different time periods. It is recommended that the wild-type *C. elegans* be compared to only one radiation-sensitive strain since a number of plates will be needed for these activities. Groups A and B should be the unirradiated *C. elegans* from each strain. The other groups should consist of each line irradiated according to the indicated times given in Activity 1, above.

Activity 3. In this activity, sunscreen will be used to block the effects of the UV radiation. Although this activity tests for the ability of sunscreen to block the lethal effects of radiation, there are a number of variables that can be tested. First, one time point for the irradiation should be selected. The most basic experiment would consist of four different groups. For group A, the *C. elegans* eggs would not be irradiated. Group B would be *C. elegans* irradiated for the selected time. Group C would also contain the irradiated *C. elegans*, but plastic wrap should be placed over the plates. The results from groups B and C will let the students determine if UV radiation can pass through the plastic wrap. Group D should have sunscreen placed on top of the plastic wrap.

This activity can be modified by varying the strength of the sunscreen (SPF value). This would entail adding groups beyond D for the different strengths. Students can also use sunscreens produced by different companies, and again, by adding groups beyond D. The sunscreen could be applied in different manners (for example spraying vs. spreading on plastic wrap). Students could also vary the thickness of the sunscreen applied or the distance from the plate when spraying. The faculty should emphasize that the inquiry experiments should have appropriate controls, and that it is difficult to test for multiple variables in one activity.

Activity 4. In this activity, the age of the *C. elegans* could be varied—for example, eggs vs. older *C. elegans.* It is also somewhat straight forward (provided that relatively high-power dissecting microscopes are available) to start with a synchronized population of L4 *C. elegans* (Montserrat et al., 2012). This activity should be set up in a similar manner to Activity 1, except there should be twice as many plates. Half of the plates would use the eggs irradiated for the different times, and the other half would use the L4 *C. elegans*.

Activity 5. In this activity, other compounds could be tested, besides sunscreen, that may block the effects of UV radiation. These activities should model inquiry Activity 3. Students would first need to research these compounds.

Activity 6. In this activity, the effects of non-ionizing radiation could be compared to ionizing forms of radiation. This activity may require additional equipment. Faculty will need to provide details of this activity, based on the equipment in their teaching laboratories.

Activity 7. In this activity, students could investigate the mechanism whereby UV radiation exerts its effects and repair mechanisms. Additional research will need to be performed to set up these activities.

Activity 8. This activity might vary the distance from the lamp to the plates when examining the effects of radiation alone. The distance will have to be determined based on the actually lamp and how it bends. In this activity, a set time for radiation should be selected, then the different groups used should vary in the distance from the UV lamp to the plates.

The students should be reminded that the experimental group should differ from the control group in only one variable. There may be more than one control group if the activity is testing for more than one variable. For example, in an activity testing for the optimal dose of UV radiation needed to kill the *C. elegans*, the control group needed would be a plate without radiation. In an activity testing the effects of sunscreen on the ability to block the UV radiation, the sunscreen could be applied on top of a piece of plastic wrap and, the control group needed would have plastic wrap but no sunscreen.

○ Protocols and Notes for Instructors

Isolating *C. elegans* Eggs and Removing Them from the Bacterial Food Source Prior to Performing UV Radiation Activities

This procedure has two purposes. First, by isolating eggs, an investigator is creating a synchronized population of *C. elegans* that are all of the same age. Secondly, when performing the UV radiation studies, the *C. elegans* need to be separated from the bacteria.

Care must be taken to isolate the *C. elegans* eggs since they are so small. If extra time is available, it might be helpful to have the students practice this technique.

Presented below is the method to obtain the synchronized *C. elegans* population that is free of bacteria (Stiernagle, 2006). This method has been tested with undergraduate research students and with a microbiology laboratory class, and it was found that the students are capable of isolating the *C. elegans* eggs.

Egg Isolation Protocol

1. Wash the plate with sterile H_2O. Pipet the H_2O across the plate several times to loosen worms and eggs that are stuck in bacteria.
2. Collect the liquid in a sterile 5 ml conical centrifuge tube with cap, and add H_2O to a total of 3.5 ml.
3. Mix 0.5 ml of 5N NaOH with 1 ml of bleach (make a fresh solution). Add to the centrifuge tube with 3.5 ml of worms.

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4. Shake well or vortex the tube for a few seconds. Repeat the shaking/vortex every 2 minutes for a total of 10 minutes.
5. Spin the tube in a tabletop centrifuge for 30 seconds at 1300 g force to pellet the eggs.
6. Aspirate to 0.1 ml.
7. Add 5 ml sterile H_2O to 0.1 ml of centrifuge tube. Shake well or vortex for a few seconds.
8. Repeat centrifuge (at same settings) and aspirate to 0.05 ml for each plate used. The number of groups needed for the experiments will determine how many plates the students will need in the activity. At this step, all tubes should be combined into one.
9. Use a pipet to transfer the eggs in the remaining 0.05 ml of liquid to the edge of a clean NGM plate. It is best to make a small circle on the plate to indicate where the eggs were placed. If a larger volume than 0.05 ml is used, the size of the circle will need to be large. If these eggs will be used for the radiation experiments, the NGM plates should not have bacteria on them. In a subsequent step, after the UV radiation exposure, the *C. elegans* should be transferred to plates with food. A spatula should be used to gather a chunk from the area where the eggs were placed. This chunk should then be transferred to a plate with food.

It is important that all plates start with the same number of eggs. In the last step of the isolation procedure, all eggs should be combined into one tube. Then, 50 µL of this suspension should be added to each NGM plate that will be used in the activity. It is best to use the eggs isolated from one large petri dish (100 mm) with a near confluent population of *C. elegans* to set up a laboratory activity that will be plated on two petri dishes. For instance, if six plates are needed for the activity, the students should start with three 100-mm petri dishes with *C. elegans*. In the last step of the egg isolation procedure, the *C. elegans* should be aspirated to a final volume of 300 µL (6 plates × 50 µL). Then, 50 µL of eggs should be added to each plate without food to start the activity.

Notes on Exposure to UV Radiation

Immediately after the eggs are placed on the plates, they should be moved to a laminar flow hood, the lid of the plates removed, and the eggs exposed to UV radiation for the time(s) selected. For some activities, plastic wrap will also be used. The radiation source should be adjusted so that the plates are directly under the UV source. The lamp used in this author's laboratory is 8 watts and 57 volts, and emits light at a wavelength of 254 nm. These numbers may vary with different equipment, but the distance from the lamp to the plates should be measured and kept consistent. It might also be possible to use a handheld UV source, but fewer plates could be treated at one time.

After isolating the eggs, the plates should be exposed to UV radiation, and then the eggs transferred to plates with food to continue their growth. This is done by chunking. The investigator could vary the starting age of the *C. elegans*, but the younger worms are more susceptible to the effects of radiation (Kan et al., 2010).

Notes on Application of Sunscreen

When sunscreen is applied, it should be sprayed on a piece of plastic wrap that covers the plates instead of the petri dish lid. Previous studies have shown that plastic wrap transmits UV radiation (Slieman & Nicholson, 2000; Xue & Nicholson, 1996). After drying briefly, these plates should then be exposed to UV radiation for the selected amount of time.

Notes on Counting the Surviving *C. elegans*

To determine the number of *C. elegans* surviving the experiments, the bottom half of the Petri dishes should be divided into quarters with a marking pen. All of the *C. elegans* in each quarter of the plate should be counted. The *C.elegans* will need to be observed over a week after setting up the UV laboratory activities. After 2 days, a small number of *C. elegans* should be visible around the edges of the chunk. At this point, the students can get an idea of the differences between groups, but there may not be enough *C. elegans* to perform statistical analysis.

Students should next observe the plates 7 days after setting them up, but at this time it will probably be difficult to count individual *C. elegans*. The students will then score the plates as no growth (–), low growth (+), middle growth (++) and maximum growth (+++). If students are available to come and check their plates outside of class time, then it would be best for them to observe the plates more than two times during the week. However, the results should be dramatic, with the UV-irradiated groups showing almost complete death while the unirradiated controls showing *C. elegans* growing on the plates.

Based on the results from these activities, students should then be instructed to use Excel or other computer program to graph their data or make a table. Students should average the data for plates treated in the same manner. Some teachers may also want the students to use statistics to determine if differences exist between groups.

Other possible Activities

Depending on the size of the class, the availability of teaching assistants, and the level of the students, many other types of laboratory activities can be performed. In addition to the other possible activities suggested, students should perform their own research and determine their own methods for their work with *C. elegans* to study some aspect of radiation. The teachers who will use these laboratory activities may want to help guide their students in their selections based on what type of class is being taught and what concepts they want their students to learn. For instance, genetics classes might want to concentrate on the different strains of *C.elegans* by comparing wild types to the UV radiation strains, and by determining if each of these strains results in similar or different results. These laboratory activities would teach the students how the genetic background of the strains makes them more or less resistant to the effects of radiation. Exercises in microbiology classes might concentrate more on how the radiation affects the *C. elegans*.

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APPENDIX

If faculty want to use case studies on cancer to supplement these laboratory activities, they could find information at the links below:

- Cancer Network: www.cancernetwork.com/case-studies
- NCCSTS, National Center for Case Study Teaching in Science: http://sciencecases.lib.buffalo.edu/cs/results.asp?search=cancer&Submit.x=0&Submit.y=0
- Annenberg Learner: https://www.learner.org/courses/biology/casestudy/cancer.html
- American Association of Immunologists: http://www.aai.org/education/Summer_Teachers/Docs/Archive/2014_Aguilar_Robert_Final.pdf

Faculty who are new to working with *C. elegans* may want to consult the links in Table 1 to provide students with background information and needed protocols. *Wormbook* contains all of the needed protocols for setting up laboratory experiments with *C. elegans*. The *Journal of Video Experiments* contains many videos for working with *C. elegans*.

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Table 1. Online resources available for obtaining background information and protocols for working with *C. elegans*.

Name	Uses	Website
Caenorhabditis Genetics Center (CGC)	Maintains stocks of wildtype and mutant strains of *C. elegans*, as well as stocks of OP-50 *E. coli*	cbs.umn.edu/cgc/home
Journal of Video Experiments (JOVE)	Provides videos on basic maintenance of *C. elegans*	www.jove.com/science-education/5104/c-elegans-maintenance
WormAtlas	Database showing different behavioral phenotypes and structural anatomy of *C. elegans*	www.wormatlas.org
WormBase	Variety of resources: gene names, meetings, discussion forum, etc.	www.wormbase.org
WormBook	Good resource for different techniques for working with *C. elegans*	www.wormbook.org
Worm Breeder's Gazette	Publishes brief articles on *C. elegans*	wbg.wormbook.org/
WormClassroom	Provides numerous educational resources	wormclassroom.org

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