

An Experimental Program for Introducing First and Second Year Biology Majors to Primary Literature

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The field of Cell Biology encompasses a vast array of subjects. Although it is important for instructors to cover a broad range of topics, it is also important to allow students to delve into specific subjects using discovery based learning (Wilke et al, 2001). A group of students were introduced to two hallmark papers through a series of lectures and assignments given intermittently during the course of their Cell Biology class. The course was designed to introduce a paradigm for sifting through and interpreting the data presented by scientific literature. For each paper students were given the necessary information needed to interpret a paper's results. Tables and figures were then discussed in class with an emphasis on student debate and participation. Groups of several students were then asked to perform an assignment which related to several or all of the figures and tables discussed by the class. Strong emphasis was placed on understanding each table or figure's role in the logical progression of the article. During the course there were also constant reminders of how each fact in their text books was at one time or another reported in a journal of some kind and scrutinized by scientific minds. This paper reports the successes and failures of such a class.

Introduction:

An undergraduate education in Biology, like most educational fields, strives to acquaint students with the current opinions, methods and knowledge of the subject. Given the depth and variety within this field such a task can be difficult. This may leave many introductory biology/lower level college biology classes with a large range of topics to cover in a short period of time. "Publishers produce ever larger and continuously heavier

textbooks, because these books are what faculties select. It is important to note that faculty, not students, choose textbooks. As long as faculty insist on broad coverage, continually adding to the volume without eliminating some subjects, there is no limit to the eventual size of textbooks” (Carter et al., 1990). Although the previous quote may seem somewhat ridiculous, it raises an interesting point. As the field of biology expands will introductory courses be forced to cover even more subject matter than they have previously?

Even though introducing the student to a wide variety of topics is integral to any education, it can also have its pitfalls. “New approaches to the teaching of biology at all levels must emphasize the conceptual framework of biology, reduce the excessive terminology that characterizes so many courses, consider the strengths and limitations of the scientific process, and deal explicitly with human problems for which biological data and methods can suggest solution” (NSF 1989). If introductory level courses attempt to cover a wider subject area then inevitably other areas of the course will suffer. More specifically, students may not be exposed to the practical aspects of biology. One way to alleviate this problem would be acquainting students with primary literature. “The value and appeal of using primary literature in the classroom are rooted in literature’s unique potential to instruct students on the nature of scientific reasoning and communication” (Muench, 2000).

The incorporation of scientific literature can help prime students for possible research careers. Such an approach has been tried, in several forms, at different schools. In a study at UCLA, a group of undergraduate students, in addition to doing research, were accepted into an 18 month program during their junior year during which time they participated in a weekly journal club, research presentations, and other biology related programs (Kozeracki, 2006). The students periodically presented journal articles as well as critiqued articles with their peers. Alumni of the program, most of whom obtained an M.D. or Ph.D. degree, attribute much of their current success to the journal club they took part in. More specifically many alumni felt that analyzing primary literature helped them improve their critical thinking skills. However, it should be noted the students who were selected for participation in the journal club were exceptional to begin with.

It has been shown that in-classroom educational programs employing primary source literature improve student confidence and reasoning skills. In a sophomore level physiology class at Earham College, students investigated protein structure and function using research articles which they presented to the class (Mulnix, 2003). The study indicated that the students were receptive to the approach and gained an increased confidence for reading scientific literature. An advanced genetics class at Ithaca College was centered entirely on discussion groups (Cameron, 2003). For a number of the discussions the students were required to read primary literature articles. The students were quizzed on the content of these articles. Survey data taken at the class's end showed that most students enjoyed this approach. Both cases suggest that the integration of primary literature into the classroom is possible.

Such a suggestion is in agreement with the analysis presented by *BIO2010: Transforming Undergraduate Education for Future Research Biologists* (National Research Council, 2003) which states "Students should be taught the way scientists think about the world, and how they analyze a scientific problem in particular." What better way to engage students in scientific thinking than to learn through scientific literature. The previously mentioned studies involved such learning. This paper highlights another study in which primary literature was incorporated into a sophomore level cell biology class at Worcester Polytechnic Institute.

The successful incorporation of primary literature into college classes relies on the selection and presentation of appropriate articles. Selection depends heavily on the goals the instructor has in mind (Muench, 2000). For example if the instructor wants to convey scientific reasoning then articles which contain methods and experiments already known to the students are most suitable. Whereas if the instructor wants only to teach a new method then obviously articles which employ methods previously unknown to the students would be selected. The goal of the course discussed in this paper was to impart to the students a new respect for the material read about in their text books. More specifically, the instructors wanted the students to understand how much it takes to make any scientific discovery, no matter how small. Lastly, we wanted the students to understand that all scientific facts, if they can be named as such, have a strong foundation in experimentation.

Another factor to consider when designing a primary literature course is the class's level of expertise. This primarily concerns how the primary sources material is presented to the students, along with performance expectations. It may be easier to ask advanced students to read and analyze primary source material independently, while novice and beginning biology students will most likely require carefully collected background information about the experiments employed and how to interpret the results.

There are many benefits to successfully integrating primary literature into first and second year biology classes. Programs incorporating hallmark literature could possibly instill a deeper respect in students for the material which they might otherwise take for granted, such as how we know that DNA is the genetic material or how Darwin formulated his theory of natural selection. Students will be encouraged to ask deeper, more scientific questions. However, for first and second year biology classes to successfully integrate scientific literature, the right approach must be found through assessing the successes and shortcomings of experimental programs.

The program discussed in this paper uses a novel method for educating freshman and sophomores who are considered to be relatively new to the field of biology. The novelty of this method resides in how the papers were presented. Rather than asking students to read the articles in their entirety, they were given information packets and lectures and asked to interpret the paper's data independently without the aid of the author's interpretation. After scouring the literature on pedagogy the author of this paper could not find a similar study, suggesting its novelty.

During the program, students in a cell biology class were introduced to two hall mark papers, Hershey and Chase's classic 1952 paper along with Mello and Fire's 1998 paper on RNAi, which were intended to supplement other pedagogical approaches in the course. During the program the students were not given either paper in its entirety and were discouraged from accessing them. Instead they were given a packet containing an introductory section explaining the paper's context, experiments used and relevant scientific terminology, along with selected tables and figures which had been copied and pasted out of the original papers. Then, using an informal lecture format, the isolated tables and figures were displayed during the class and the students were asked to explain them with the guidance of a peer instructor who had read and understood the paper in

advance filling in any gaps of knowledge. After the paper's tables and figures had been adequately discussed in class, groups of students were asked to answer homework questions designed to integrate the qualitative and quantitative information they had been given within the tables and figures.

The entire course centered on asking undergraduate students to begin to think like scientists and appreciate the experimental basis for the textbook information they may have taken for granted. Additionally, the course also intended to prime these students for data analysis tasks which they may encounter later on in their careers. The courses designers sought to create structure, yet also foster creativity. Structure was created by asking the students specific questions which were answerable if the student referred to introductory packet he or she was given. Yet creativity was also fostered when students were asked to judge or criticize the validity of the findings. The success of this course is gauged in this paper using student feedback collected through surveys along with instructor evaluated success. This study reports that student feedback and the instructor's personal evaluation suggest that this course was successful. However, aspects of the program will need to be modified for future use.

Materials and Methods:

Instruction: All lectures related to the primary literature project were designed and delivered by an undergraduate biology major at Worcester Polytechnic Institute who was in his junior year when the course was administered. Designing the project was part of a required student project, which WPI refers to as an Interdisciplinary Qualifying project. The student worked under the supervision of two faculty advisors one of whom was the instructor for the cell biology class which incorporated the experimental course on primary literature. The supporting rationale behind allowing an undergraduate student to design a course was that an undergraduate might have a better understanding of how to present the material to his peers.

General Class Information: A class of 51 students enrolled in Cell Biology class at Worcester Polytechnic Institute took part in the experimental program described in this paper. The Cell Biology course ran from January to March 1 during 2006-2007 academic year. Mondays, Tuesdays, Thursdays and Fridays were devoted to normal class activities, reviewing assigned reading, lectures, quizzes and tests. Wednesdays were devoted to discussions and assignments pertaining to the primary literature course module..

Class Composition: The class was composed of 51 students, 16 females and 35 males (Table 1). These gender ratios differ slightly from WPI's overall proportions: 26 percent women; 74 percent men. There were a variety of majors within the class: 8 biochemistry students, 11 biology students, 17 biomedical engineering students and 15 students who were either undeclared or majoring in a field not directly related to biology. The majority of students in the Cell Biology class were either sophomores or freshman who are exactly the students for whom the experimental course discussed in this paper was intended .

Table 1. Class Demographics

Total Number of Students	51
Students by Major	8 Biochemistry 11 Biology 17 Biomedical Engineering 15 Non Biology or Undeclared
Students by Gender	68.6% Male 31.4% Female
Student by Year	31.4% Freshmen 49% Sophomores 11% Juniors 7.8% Seniors

Lecture Content: Lectures were given every Wednesday as well as one Thursday over a period of four weeks. Each lecture was approximately 45 to 50 minutes in length. During the first lecture the students were given an overview of the experimental program. The students were then told they would be placed into groups of three to four and would be analyzing two high impact and historically important biology papers over the next several weeks. The peer instructor then presented background information for the first paper, *Independent Functions of Viral Protein and Nucleic Acid in Growth of Bacteriophage* (Hershey and Chase, 1952). At the end of class the students were told that given this information, they would now be expected to analyze, with their group members, assigned figures from the Hershey and Chase paper. Each group only had to analyze two figures or tables. The information presented by the peer instructor was also posted online in our electronic course management site for suggested perusal later on (Appendix A).

Prior to the second lecture the class was e-mailed in advance and told they would receive extra credit if they actively participated in an in class discussion of the paper. At the beginning of the second lecture the instructor gave a short introduction on the importance and context of the work done by Hershey and Chase. The instructor then went through each one of the assigned data tables and figures by displaying them on a projection screen. The class was designed to be entirely dependent on student participation. Before hand a series of questions had been written up by the instructor intended to lead the students towards the scientifically accepted conclusion.

The third lecture was given a day after the previous one because we found that 50 minutes was not enough time to analyze the paper in class. The instructor spent most of his time making sure that the students understood each one of the figures they were assigned.

The fourth lecture was spent going over all the background information required to understand the paper, *Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans* (Mello and Fire, 1998). The students were given information ranging from the use of markers such as GFP to the reasons for using *Caenorhabditis elegans* as a model organism. At the end of the lecture the class was told they would be working in the same groups and each group would now be expected to analyze every data table and figure from the paper.

In advance of the fifth lecture the class was e-mailed and told they would receive extra credit for any class participation. The fifth lecture format was highly interactive and used a preconceived set of questions designed to lead the students towards the scientifically acceptable conclusion. Because this paper was admittedly easier to analyze it took only a single lecture.

Assignments: The assignments were designed to focus students on the analysis of the data (appendices B and E) In order to do this, selected data tables and figures were cut from the articles and placed into a separate document. Each assignment was also supplemented with a general information packet which defined terms as well as procedures which might be unfamiliar to the students. (appendices A and D). A total of two assignments were given.

For the first assignment the groups were given only two out of six possible figures or tables from Hershey and Chase's paper. There are more than 6 data tables and figures in the actual paper however these additional tables and figures were omitted to make analysis of the data easier. These tables or graphs were omitted for two reasons. Some were not included because it was thought they were above the level of understanding for students in the class. Others were seen as only reinforcing points already illustrated by previous tables and graphs in the paper. The groups were given a series of questions for each data table or figure which they were expected to answer. No group was given all six figures or data tables. This was done in the interest of not overwhelming the students. Deciphering any one of these tables or figures was a daunting task in itself. Interpretation of the results involved becoming familiar with new concepts and experimental techniques. Also, these assignments were done in addition to the regular course reading assignments.

For the second assignment the groups were now expected to analyze the Mello and Fire paper. All the data tables and figures were cut and pasted out of the paper and placed in a separate document alongside pertinent information and questions which asked the students to analyze the data. The groups also received a general packet which clarified any unknown terminology or figures. The second assignment held a greater amount of relative credit (250% in comparison to assignment 1). It was expected that the students would be more capable when it came to answering questions about this paper because of the experience and skills they gained in the first half of the project

Criteria for Paper Selection: Hershey and Chase's "Independent Functions of Viral Protein and Nucleic Acid in Growth of Bacteriophage" as well as Mello and Fire's "Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*" were chosen for several reasons. Both of these papers can be considered to be the hallmarks papers of Nobel Prize laureates. These papers were also chosen because they were pertinent to the subject matter discussed in the cell biology class the program was embedded in. The Mello and Fire paper was also chosen because it was easy to interpret, the figures follow a logical progression and Craig Mello lives and conducts research very close to WPI. The Hershey and Chase paper was chosen because it is considered to be a classic experiment and is presented in many introductory texts. It also

forces students to understand and analyze an experiment considered key to one of modern biology's most important concepts, DNA as the genetic material.

Grading: Assignments were graded using an answer sheet written in advance (appendices C and F). Groups received full credit for their answers if they had come to a plausible conclusion based on the data and information they had been give. Points were awarded for proper use of the data as well as a demonstration of understanding. Groups were penalized for poor writing and for including information in their arguments which was not known during the year the paper was published.

Group Selection: Groups were put together by the peer instructor and were designed so that all the groups were multidisciplinary. Group sizes ranged from two to four students. There was also one group of five. The size of groups varied to such a significant extent because several students were no longer apart of the class after the groups had been conceived. Only one group was altered in between assignments one and two.

Survey Data: At the end of the course the students were asked to fill out an assessment form (appendix G). This data was then compiled and analyzed using Microsoft Excel. Students were asked to rate various portions of the course using a numerical system as well as provide written feedback. Before writing their assessments the class was informed that their input would shape future versions of this course and that they would all remain completely anonymous. Also because they did not place their names on this assessment they remained anonymous to the instructor as well.

Results:

The overall class performance on the two assignments is shown in Table 2. Assignment one was worth 20 points while assignment two was worth 50 points. Based on these results it can be shown that students were able to complete these assignments in a more than satisfactory manner, with average scores on both assignments in the high B range.-

Table 2. Overall class performance on each assignment

	Assignment 1	Assignment 2
Numerical Average	17.5/20	44.4/50
Numerical Standard Deviation	2.28	4.5
Percentage Average	87.50%	88.80%
Percentage Standard Deviation	11.40%	9%

At the end of the course a survey was given to gauge student satisfaction with the course (Appendix G). It should be noted that the survey was anonymous, so the students could be completely honest in their replies. The summary information from this survey can be found in Table 3. The data can be interpreted with assumption that, for questions one through three, the scale value five is a neutral value, as it would be on a Likert scale. However, for question four, zero is the neutral value on the scale. For each value, using Microsoft Excel, a 95% confidence interval was constructed. This was done by inputting the standard deviation, sample size, and α value 0.05 corresponding to a 95% confidence interval. Additionally, p values were calculated using the “One Sample T Test” function in maple.

The results in the first question indicate that all responses are above the neutral value of five, which corresponds to a response of indifference for course usefulness. Meaning, that on average students in all groups found the class to be useful. However only the values for the biomedical engineering, non biology and the overall class

response are statistically significant in relation to the neutral value of five. Additionally, although there is no statistical basis for the difference, the non biology majors found the course more useful than the biology and biochemistry majors.

Table 3. Class Survey data for Class and by Major

**Question 1: On a Scale of 1 to 10 how useful was this course?
(10 being very useful)**

Biochemistry Majors	5.3 ± 2.1
Biology Majors	6.0 ± 1.2
Biomedical Engineering Majors	7.1 ± 0.66 **
Non Biology Majors or Undeclared	7.0 ± 0.69 **
Overall Class response	6.3 ± 0.62 **

**Question 2: On a scale of 1 to 10 rate the quality of instruction.
(10 being very useful)**

Biochemistry Majors	6.7 ± 2.3
Biology Majors	7.4 ± 1.2 **
Biomedical Engineering Majors	7.1 ± 0.93 **
Non Biology Majors or Undeclared	6.8 ± 0.91 **
Overall Class response	6.9 ± 0.66 **

**Question 3: On a scale of 1 to 10 how difficult was the course?
(10 being most difficult)**

Biochemistry Majors	6.9 ± 1.3 *
Biology Majors	6.7 ± 0.72 **
Biomedical Engineering Majors	6.7 ± 0.83 **
Non Biology Majors or Undeclared	6.5 ± 0.91 *
Overall Class response	6.6 ± 0.49 **

**Question 4: If 5 is a positive change and -5 is a negative change rate how this course has changed your perspective of biology.
(-5 being a large negative shift in perspective while 5 is a large positive one)**

Biochemistry Majors	0.57 ± 2.5
Biology Majors	1.1 ± 1.3
Biomedical Engineering Majors	1.4 ± 0.89 **
Non Biology Majors or Undeclared	1.6 ± 0.84 **
Overall Class response	1.2 ± 0.64 **

To the right of each average there are 95% confidence intervals. Data in questions one to three which are statistically different from the neutral value five have a star next to them when $p < 0.05$ and two stars if $p < 0.01$. The labeling system is the same for question four except the neutral value is zero.

The second question which corresponds to the quality of instruction, like the first, also yields positive results. In this question results above the neutral value of five correspond to higher than average quality of instruction. All measured groups are above the neutral value of five ($p < 0.01$), except for the biochemistry majors. The average response for biology majors (7.4) was highest, however due to the small sample sizes used for these groups no differentiation could be made when comparing this value to the other groups. Like the previous question, the most important data point is the overall class response which was 6.9. This indicates that the instruction style used in this course was, in the eyes of the students, a success.

In the course difficulty question all values above five correspond to higher difficulty. While five would be considered average difficulty. This was the only question in which all of the data points could be statistically separated ($p < 0.05$) from the neutral value. These results suggest that the class as a whole, as well as all the subgroups considered this aspect of the course to be difficult. This is not necessarily a positive aspect of the course. If the course was too difficult it might have frustrated as well as discouraged the students in relation to their studies.

The last quantitative question on the survey asked the students if they felt the course had changed their perspective of biology. The scale for this question was different than for the preceding three questions. No change in perception would have resulted in a score of zero. A positive change was scored on a scale of 1-5. The average class response for this question was 1.2 ($p < 0.01$), meaning that with 99% confidence it can be said that on average the class's overall perspective of the biological sciences changed for the better.

Thus, the data indicates that the course was at least moderately successful. However, this survey may have been more useful if each individual number in the scale had been defined. For example, the value five should have been defined as a neutral response.

Discussion:

The design of the experimental course was intended to familiarize first and second year college students with primary literature, provide insight into pragmatic real world science and foster critical thinking skills. More specifically it was hoped that the students would understand, at the course's end, that everything we hold to be fact in the field of biology is based on a strong experimental foundation. Also, familiarizing freshman and sophomore students with primary literature would make them more capable data analysts later on in their careers. Each of the primary literature papers, with which these goals were accomplished, was selected for specific pedagogical reasons to accomplish these goals.

The Hershey and Chase paper was selected because it allowed the students to challenge the idea of DNA being the genetic material. It was also chosen due to its unique experimental design. In retrospect this may not have been the best paper to work with. The results were in some cases very convoluted and required large amounts of explanation. Additionally, the techniques used were antiquated and difficult to interpret out of their historic context. The Mello and Fire paper was chosen to show students that the science of biology is still changing and under revision. The paper had only been published in 1998 and already has helped elicit a Nobel prize as well as spark numerous studies and techniques. The paper was also chosen because the data were straightforward and allowed the students to easily follow the logical progression presented by the paper's figures and data tables. Lastly, Craig Mello, one of the paper's authors, was located only several miles away from WPI.

To ensure that limited knowledge, on the student's part was not problematic, information packets (appendices A & D) were posted online to serve as mental tools for interpreting the paper's data tables and figures. Various concepts such as centrifugation, the composition of virus particles and even more general topics such as analyzing data tables were also discussed in class. Students were also encouraged to ask for any additional information during class or through e-mail. It was hoped that since the student would have all the necessary information at his or her fingertips reaching conclusions and critically thinking about the figures and tables would not be impeded. However, many

students were still confused by some of the conclusions drawn by the Hershey and Chase paper. This may have occurred because each group was only responsible for two tables and/or figures. It was thought it might be better to only give the students a small amount of data to interpret at first, so they might not feel overwhelmed. However, a better approach may have been to give the students an easier paper, one which is more up to date, and has data more conducive to conclusive analysis. Still, a paper such as Hershey and Chase's may still have been suitable for such a course, after the students had substantial experience with data interpretation.

When data tables and figures were presented during class, lectures were kept informal. The instructor attempted to lead the students by asking a series of questions, encouraging students to reach their own conclusion. The instructor later reported that this was admittedly quite difficult. Due to the size of the class many students easily avoided participation. If the class had been smaller, then a greater proportion of students might have participated. Also, it would have been easier to keep track of such incentives as extra credit. Future versions of this type of course should attempt to minimize class sizes. Furthermore, students may have felt intimidated when considering whether to make a comment in front of such a large group of their peers.

After grading the assignments, several problems the students had, revealed themselves. Many students would not include the actual data in their answers even though it had been previously explained to the class that answers should include specific data. One of the experiments, described in a data table, involved a series of low and high speed centrifugations. Most of the class was confused by this experiment and it probably could have been avoided if the instructor had given the class a chart or table indicating what becomes supernatant or pellet during different types of centrifugation. Also several of the groups would often jump to conclusions in their answers without providing their rationale. In future versions of the course these problems can most likely be avoided if proper instruction is given in advance.

For assignment two, the Mello and Fire paper, the expectations were raised. Instead of giving group two tables or figures each group was responsible for interpreting every table and figure in the Mello and Fire paper (appendix E). As with the previous assignment they were also given a general information packet (appendix D). The class

seemed to do quite well on this assignment. The class average was 88.8%. Although this score does not appear to be very different from the scores on assignment one, the class as whole seemed to perform much better. The grading was simply more critical. From the instructor's point of view this paper was also much easier to teach and an excellent class example because the experiments followed a clear and logical flow.

Several students approached the instructor during the later stages of the course eager to discuss the current paper or talk about how they were starting to feel reading primary literature had been personally demystified for them. In the student survey several students actually mentioned they would have liked to look at more papers during the course. It was also suggested that there should have been more time spent on the actual experimental design. In order to be objective it should also be mentioned that there were several students who expressed a strong dislike for the course. Much of the students's frustration seemed to arise from the group format of the assignments. This might be corrected by allowing student selected groups. However, if the students picked their group members poorly then additional problems might arise. One way to remedy this would be to have group member's grade each other at the end of each project. The score which each student received from his or her group members could then be converted to a percentage and used as a multiplier for the grade which they received on the paper.

The survey data collected may have been misleading. Even though the students were told prior to the survey that it only applied to the primary literature course module, because it was not specifically written on the survey some students may have assumed that this survey was for the entire cell biology course. In the future such problems can easily be rectified. Still, considering that most students would have known that the survey only applied to the module, this experimental primary literature course seems to have been successful. Over all class responses for course usefulness and quality of instruction was positive. It was also reported that on average the entire class felt their perspective of the biological sciences had been changed for the better.

Taken together, the anecdotal and survey data seem to suggest that there is real and perceived value to introducing the primary literature, during freshman and sophomore level classes, using the aforementioned methods. If this project were to be repeated, some revisions are suggested. Further investigation is needed to determine what

types of scientific literature should be used to teach first and second year students. Is it best to use data with easy to read and interpret data? Or does such an approach betray the student only putting off what they will have to inevitably face, that being the occasional disorganized, convoluted or complex scientific paper. One strategy may be to first introduce students to primary literature using easy to follow papers and then gradually introducing more difficult material. In this regard, the Hershey Chase paper was probably not the best choice.

There are of course other methods for introducing students to primary literature. One which has been shown to be quite successful (Mulnix, 2003) involves giving groups of students their own paper which they are responsible for interpreting and then later presenting to the class as a whole. However, such a course does not offer what has been discussed in this paper. In the experimental course described above the students were asked to interpret tables and figures with little interpretive aid from the author's whose paper they were presented in. This stemmed from the fact that the tables and figures were presented to the students out of context. They were expected to read using only the quantitative or qualitative results, some background knowledge on the experimental techniques used and a few selected definitions. Such a unique approach certainly deserves a second look.

As the field of biology expands it will be important to remember what is truly vital to a science education. It cannot be denied that the learning of theories and facts allow students to build mental paradigms as well as enrich their understanding of the life sciences, are important. However, it is necessary to be reminded that biology majors, like all science majors, should also be able to analyze and interpret new data and experiments, as well as challenge what is held to be convention. The facts and theories we learn through our education may fade in time. However, once honed the ability to analyze and discovered will never waver.

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Appendix A:

Assignment 1(general packet):

The scientific community communicates through the papers they publish. Being able to analyze and understand these papers is an essential skill for any aspiring scientist. However these skills are not just essential for would be investigators. Learning to understand the language of science can lend itself to many walks of life aside from the sciences. It confers the ability to think logically, creatively and sometimes in an ingeniously indirect manner. That being said one should consider that any diligent effort to try and understand a classic scientific paper will not go unrewarded.

Many of those who read a scientific paper for the first time, to be quite honest, will be unpleasantly surprised. They can be very difficult to understand if one is not in the field the paper was written for. This is because the paper may use techniques or language you are not familiar with. So in order to work through a difficult paper you will have to try very hard to understand the jargon and the experiments being performed. Then with this understanding you must try and interpret the data which the writers have presented you with. If there are multiple data tables and figures then you are going to have to ask yourself how they all fit together.

The first time you really try to understand a paper you will find it is a lot of work. Do not despair; with practice you can become more proficient at understanding these papers and this course is going to help you do just that. In order to do this we are going to start small by only presenting you with small pieces of data. The entire class as you know has been divided into groups and each group has been given a small section of data from a classic paper which will remain unknown at this time. We will be presenting you with all the background you need to understand this data. It will then be your job to interpret the data. Then you will present what you have learned in class. If each group presents their interpretation we can begin piecing together the classic paper from which your data came.

Before you start interpreting the data you have been given some useful information:

Interpreting Imperfect Data: To understand the tables and figures you have been given you need a framework for interpreting the numerical data they present. To make things easier I have presented you with a few hard and fast rules.

Rule 1: Experiments and People are Imperfect so their data will be as well. When you look at the numbers from any experiment there is going to be a natural amount of variation due to variables beyond the researchers control and the data from the paper you are looking at is no exception. Because of this you need to keep an open mind.

Rule 2: If the numbers are Similar then perhaps they are the same, or that is to say they represent the same story. This may sound like mathematical fallacy but in terms of statistics it actually isn't. For example suppose I

really want to know how many people prefer chicken over beef. In order to answer this important question I recruit two trained statisticians sally and roger. I tell sally and roger that I want to minimize variation in the data so I give them identically protocols to follow for data collection and interpretation. Then on the morning of two weeks later sally tells me that 58 percent of the general population prefers chicken over beef. Later on during the afternoon of the same day roger tells me that 60 percent of the population prefers chicken over beef. Sally and mikes number's are different but does that make one or perhaps both of them wrong. The answer is of course no because in any study there is a certain amount of variation which cannot be controlled no matter how hard you try. What makes the most sense is that sally and mike are both right. Both 58 and 60 percent tell us that a little over half of the population prefers chicken over beef. So because both 58 and 60 percent tell the same story in a sense through their similarity they are the same.

Rule 3: The range which needs to exist between two or more pieces of data to make them different or the same will vary from experiment to experiment. The figures and tables you have been given are all from a paper which is notorious for large amounts of statistical variation. Many have said that if this paper were submitted for publishing today it would not make it and would have to be revised.

Rule 4: Every table or figure has something important to tell you which fits into the larger scope of the paper. This rule really only applies to the papers we will be giving you because we have reviewed each figure and table and would not give them to you if they didn't help to support the paper's thesis. So if you think that there is nothing you can take away from the figure you have been given then think again.

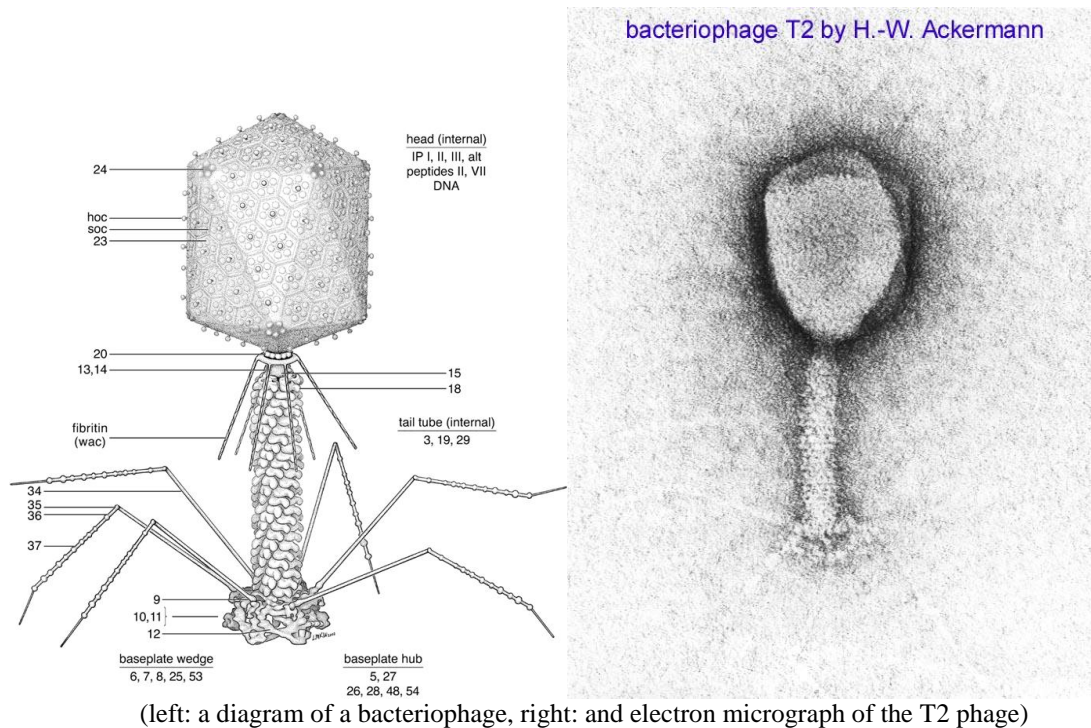
1 .Radioactive Isotopes: In nature there are radioactive isotopes. These isotopes are just atoms which are identical to the elements they are derived from except they have an excess of neutrons. As far as studies in biology are concerned it is not necessary to get into details about what happens to these isotopes as they decay. It is only necessary to know that we can track the location of these isotopes with a Geiger counter. You will soon find this knowledge useful because the researchers who produced the data you will be looking at exploit this knowledge.

2. The chemical Content of Proteins vs. DNA: When speaking of chemical differences between DNA and protein, phosphorus can be considered as exclusive to DNA and sulfur as being exclusive to protein. This fact will become very important when interpreting the data you will be presented with.

3. The Solubility of DNA: DNA is a negatively charged compound. When DNA is present in small enough fragments it is soluble in solution as long as that solution is

acidic. This happens because excessive amounts of hydrogen atoms will interact with water and the DNA. However this really only happens with small fragment of DNA and normally DNA exists as a relatively massive polymer. Unless of course you could somehow cut it into smaller more soluble fragments and as it turns out there is an enzyme used specifically for this purpose. This enzyme is called DNAase.

4. What is a T2 phage?: The T2 phage is a type of virus which has the ability to infect certain strains of *e. coli*. It is known to be composed of DNA and protein. When it infects its host it confers some of form information (through DNA or possibly protein) that allows for the production of other phages by the host which will cause the host cell to lyse(break open).



(left: a diagram of a bacteriophage, right: and electron micrograph of the T2 phage)

In the paper some of the tables will mention a process called plasmolysis. The specifics of the procedure are not important but what is important is that when you plasmolyze a phage you rip it open and loose “something” from the phages interior(note there is a good reason for my vagueness here).

5. Precipitation by Antiphage: An antiphage is an antibody that recognizes the phage particle. When you have a soluble virus particle you can place anti-phage in solution with that particle to precipitate it or make it insoluble. This happens because the anti-phage and phage form very large complexes that fall out of solution.

6. What is a Ghost Phage: A ghost phage for the purposes of the data you will be looking at is a phage that has a hollow appearance. In this paper ghost phages are created when normal phage are plasmolyzed. The phage retains its exterior structure but appears

to have lost something during plasmolysis. It also retains the ability to attach to sensitive bacteria.

7. Heat killed Bacteria: Applying heat to bacteria can cause damage. One effect is that the cell membrane loses its integrity and can no longer hold its intercellular constituents, such as the products of metabolism, mRNA, ribosomes, cytosolic proteins or possibly infectious factors(hint hint).

8. Centrifugation: This is possibly one of the most common tools of the molecular biologist. It involves spinning samples in a centrifuge to separate the components of a solution based on weight. In a centrifuge the strength of centripetal forces acting on a molecule are directly proportionally and positively correlated with that molecules weight. Or to put it another way; when you place a test tube holding a heterogeneous solution the heavier components will have a tendency to separate from the lighter ones by sinking to the bottom. As you increase the speed of centrifugation you decrease the threshold for how heavy something has to be to sediment.

Centrifugation Definitions:

Low speed Centrifugation: For the purposes of this paper this a centrifugation which will only force cells into the sediment.

High Speed Centrifugation: For the purposes of this paper this speed will cause cells as well as viruses and free DNA (as long as it is fully intact) to move into the sediment.

Fractionation: A step which splits a sample into multiple parts.

Supernatant: The liquid portion of centrifuged sample which will contain the less dense components of the sample.

Sediment: The solid and denser portion of the centrifuged sample which will form at the bottom of the test tube.

9. DNAase: This is an enzyme which cells manufacture to degrade DNA. When DNA is placed in a solution with DNAase under the right conditions the DNAase will cut the DNA into smaller pieces. If this were to occur in a highly acidic environment the negatively charged DNA might be made soluble because of its reduction in size.

10. The Waring Blender: The researchers used a blender to mechanically remove the components of the phage which adsorbed to the exterior of the cell. As one would expect as the cells spend more time in the Waring Blender more of the phages are removed from the bacterial cell surface.

Useful Definitions:

Adsorption: When a gas, liquid or a solute attaches to a solid or sometimes liquid phase. In the context of the data you have been presented with adsorption will mean the process by which a phage virus attaches to the surface of a bacterial cell in a reversible manner.

Elute: To extract (one material) from another, usually by means of a solvent (definition from freedictionary.com)

On a final note:

Between this packet and the assignment sheet you have been given enough information to answer the questions for assignment one. However it is going to be up to you to put the facts together. Be prepared to look at the data you are being presented and the additional information you have been given for about an hour. Also be prepared to discuss the data with your peers and see what they took away from it. Science is a team effort because it requires many modes of thinking that can never be provided by just one person. Sometimes you may not see what your peers are seeing and vice versa. By sharing interpretations of the data you may often find that the whole becomes greater than the sum of its parts.

Appendix B:

Assignment One(specific Assignments):

TABLE I
Composition of Ghosts and Solution of Plasmolyzed Phage

Per cent of isotope]	Whole phage labeled with		Plasmolyzed phage labeled with	
	P ³²	S ³⁵	P ³²	S ³⁵
Acid-soluble.....	—	—	1	—
Acid-soluble after treatment with DNase.....	1	1	80	1
Adsorbed to sensitive bacteria.....	85	90	2	90
Precipitated by antiphage.....	90	99	5	97

What do you need to know to interpret this table:

1. Before this data was collected the researchers knew about a phenomenon called plasmolysis. To plasmolyze a phage the phages are placed in a solution of high sodium chloride concentration. After that a large volume of water is added diluting the solution. When this happens the ions which were adhering to the phages exterior coat are pulled in all directions and this chaotic ionic dispersal creates a force strong enough to tear the phages open. This creates what is called a ghost phage. We call them ghost phages because when these phages are examined under the electron microscope they appear hollow.
2. You should also keep in mind that antiphages will interact with the exterior of the phage. This is important because if an antiphage attaches to a phage under conditions where the phage is normally soluble the phage may become insoluble and then precipitate.
3. DNAase is an enzyme that chops the DNA up into little pieces. Under many conditions large pieces of DNA are not soluble because the solution cannot support their size. However if the DNA is chopped up into small enough pices then it can be soluble.
4. Remember to consult your general packet for any other pieces of information.
5. The numbers given in these tables represent percentages of total isotope. The researchers were essentially monitoring where the isotope was traveling.

Experimental Design: The researcher’s plasmolyzed the phage by suspending them in three molar(a term for concentration in units of moles per liter) sodium chloride for 5 minutes at room temperature and then adding 40 volumes of distilled water. This leaves only two percent survivors(viable phages or phages which can still infect). They examined the behavior of phages labeled with phosphorus (only found on DNA) and

sulfur(only found on protein). The researchers “label” phages by growing them in the presence of the isotopes so when the phages are built they incorporate the radioactive isotopes into themselves. Each type of labeled phage was looked at under a variety of conditions.

Answer these Questions based on the information you have been presented with.

1. We already know that Plasmolyzed phage retains the ability to attach to sensitive bacteria, but what is the plasmolyzed phage/ghost phage composed of? If the plasmolyzed phage or ghost phage is made mostly of protein then we should expect to see the protein of plasmolyzed phage attaching to the cell surface. If the plasmolyzed phage or ghost phage is made mostly of DNA then we should expect to the DNA of the plasmolyzed phage attaching to the cell surface. You should look at the data specifically referring to percent isotope adsorbed to sensitive bacteria. It should give you enough information to answer the question. Also be to include the data in your answer. (1, 2 and 6)
2. What happens to the DNA when the bacteria are plasmolyzed? To answer this is will be useful to look at the data you used to support your last question. You will also need to consider under what conditions is DNA made acid soluble by the enzyme DNAase. It is important to remember that DNAase would not be able to access DNA if it were associated with the phage. It is also important to include the data in your answer. (1, 2, 3 and 9)
3. Based on your previous two answers tell whether you think protein, DNA, or both perform the function of attaching the phage to bacteria for subsequent infection? Your answer must be based on the previous answers and the data.(1, 2, 6 and 9)
4. Summarize the data table in one to two sentences?

TABLE II
Sensitization of Phage DNA to DNase by Adsorption to Bacteria

Phage adsorbed to	Phage labeled with	Non-sedimentable isotope, per cent	
		After DNase	No DNase
Live bacteria.....	S ³⁵	2	1
“ “	P ³²	8	7
Bacteria heated before infection.....	S ³⁵	15	11
“ “ “ “	P ³²	76	13
Bacteria heated after infection.....	S ³⁵	12	14
“ “ “ “	P ³²	66	23
Heated unadsorbed phage: acid-soluble P ³²	70°.....	P ³²	5
	80°.....	P ³²	13
	90°.....	P ³²	81
	100°.....	P ³²	88

What do you need to know to interpret this table:

1. DNAase is an enzyme which cuts DNA into smaller pieces. It can only gain access to DNA in this experiment if the DNA is unprotected.
2. Non-sedimentable isotopes are the isotopes which stay in the supernatant after centrifugation. It is important to keep in mind that anything which is attached to a cell is probably going to sediment during centrifugation.

Experimental Design: They examined the behavior of phages labeled with phosphorus (only found on DNA) and sulfur (only found on protein). The researchers “label” phages by growing them in the presence of the isotopes so when the phages are built they incorporate the radioactive isotopes into themselves. Each type of labeled phage was looked at under a variety of conditions.

The researchers are examining three different conditions. In the first they infect live bacteria with phages labeled with sulfur or phosphorus, in the second they heat the bacteria to eighty degrees Celsius before infection (this would cause the cells to break open) and in the third they heat the bacteria after the infection (damaging the cells once again). There is also a control experiment the researchers perform to show the temperature at which the structure of the phage begins to be compromised.

Answer these Questions based on the information you have been presented with.

1. An increase in non-sedimentable isotope after DNAase treatments tells us that the DNA has become more accessible to DNAase. That being said what conditions cause an increase in the sensitivity of DNA to DNAase? Be sure to support your claims using the data. (9, 7, 3, 2 and 1)

2. What would you theorize is happening to the DNA under the conditions in which it is sensitive? (9, 7, 3, 2 and 1)

3. The researchers heated unadsorbed phages and attempted to make their phosphorus contents acid soluble under different heating conditions. It is important to know that when the bacteria heated they were only heated to 80° Celsius. Why did they do this (hint this is a control)? Be sure to use the data in your answer.

4. Summarize the table in one or two sentences.

TABLE III
Sensitization of Intracellular Phage to DNase by Freezing, Thawing, and Fixation with Formaldehyde

	Unadsorbed phage frozen, thawed, fixed	Infected cells frozen, thawed, fixed	Infected cells fixed only
Low speed sediment fraction			
Total P ³²	—	71	86
Acid-soluble.....	—	0	0.5
Acid-soluble after DNase.....	—	59	28
Low speed supernatant fraction			
Total P ³²	—	29	14
Acid-soluble.....	1	0.8	0.4
Acid-soluble after DNase.....	11	21	5.5

The figures express per cent of total P³² in the original phage, or its adsorbed fraction.

What do you need to know to interpret this table:

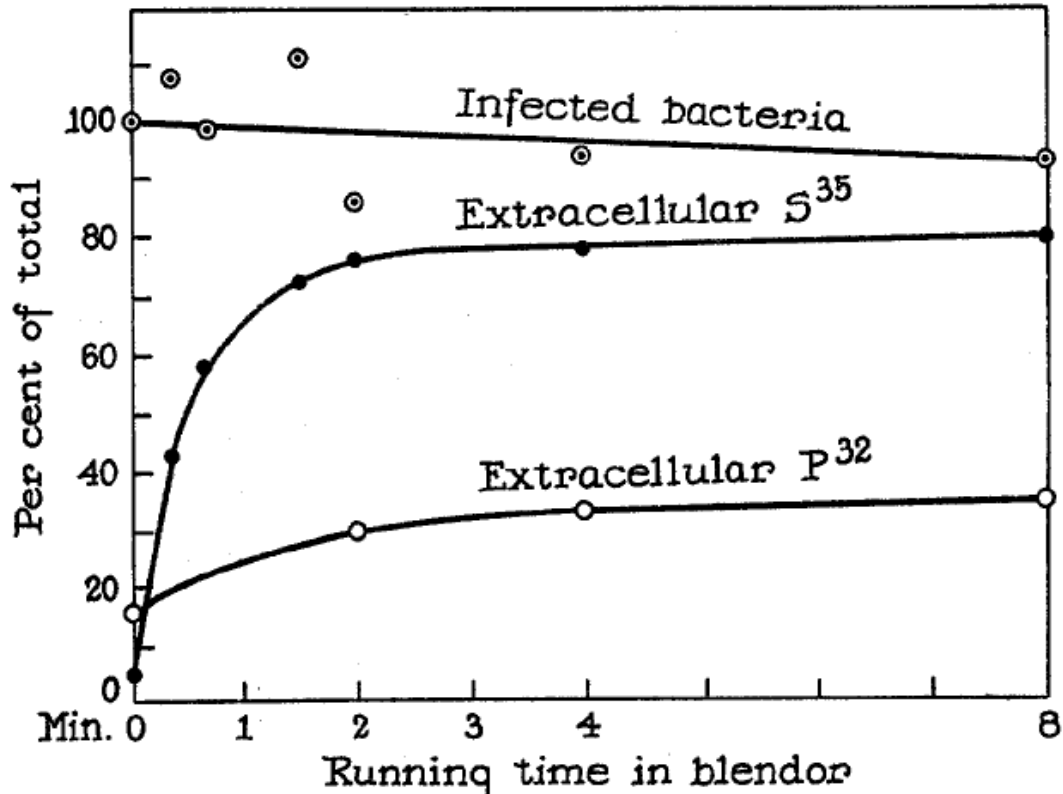
1. When bacteria are frozen, thawed and fixed with formaldehyde and then examined using microscopy the cells appear to be empty membranes many of which have the appearance of being broken open. For the purposes of this assignment assume that most of the bacteria will be broken open or lysed that undergo this process.
2. When cells are only fixed they generally remain in tact.
3. When performing a centrifugation you can perform the speed at which the centrifuge spins. These speeds can also be thought of as multiples of earth's gravity. It is important to keep in mind that in a low speed centrifugation or as they refer to it above a low speed fraction you only expect to see cells or anything attached to them in the sediment. Phages which have not been adsorbed should be in the supernatant.
4. Much of this data table hinges on the whether or not DNA is accessible to DNAase and why it is accessible. Previous information from this paper has hinted towards DNA being taken up by the bacteria during infection. When DNA is within the cell it should not be accessible to DNAase. However if conditions are altered then it the phage DNA can become sensitive to the DNAase.

Experimental Design: The researchers first grew up the bacteria then they centrifuged them and re-suspended the cells in adsorption media. Then the researchers infected the bacteria with P³² labeled phages. Then the bacteria were re-centrifuged and diluted with a new solution. The un-adsorbed phages would have presumably been separated because

whereas the cells would be in the sediment, and the phages adsorbed to them, the unadsorbed phages would be in the supernatant. The bacterial suspension was then frozen and thawed with “a minimum warming three times in succession”. After the third “warming” the cells were fixed using formaldehyde. Then after about thirty minutes the cells were dialyzed free of formaldehyde and centrifuged again. The researchers also brought T2 phages by themselves through the experiment so they could examine the effects of the experiment on the phage itself.

Answer these Questions based on the information you have been presented with.

1. Why is the phosphorus content only marginally affected by an increase in blending time?(1, 2, 4, 8 and 10)
2. Why is sulfur content greatly affected by an increase in blending time? Use the data to support you answer. (1, 2, 4, 8, and 10)
3. In terms of DNA or protein being the genetic material, what were the researchers trying to support with this table? (1, 2, 4, 8, and 10)
4. Create a more convincing figure that would support the above conclusion to a greater extent. The figure should be similar to the one you have just examined. The only difference should be the values of the data points.



What do you need to know to interpret this table:

1. According to Andersen in 1951 and his electron micrographs phage particles attach to the membranes of cells by their tails. The researchers built the bulk of their experiment on this theory. They hypothesized that if one were to apply a shearing force by way of a blender to bacterial cells adsorbing to phage particles then one could remove the phage particles. The researchers decided to do such a thing and they found they were able to remove the phage particles from the cell.
2. From previous data tables in the paper from which the figure has been extracted from we have already discovered and began to realize that when phages infect bacterial cells they transfer their DNA into the bacterial cell.
3. We are looking at two types of phages one labeled with phosphorus (only found on DNA) and the other labeled with sulfur (only found in protein). The researchers "label" phages by growing them in the presence of the isotopes so when the phages are built they incorporate the radioactive isotopes into themselves.
4. After centrifugation only cells and the particles which are associated with them internally or externally should be in the sediment.
5. The above figure shows the percent of total isotope found in the supernatant.

Experimental Design: Phages were allowed to adsorb to bacteria for a fixed amount of time after which they were subjected to mechanical shearing by means of a blender. They were then centrifuged and the supernatant was analyzed for sulfur or phosphorus content.

Answer these Questions based on the information you have been presented with.

1. Why is the phosphorus content only marginally affected by an increase in blending time?(1, 2, 4, 8 and 10)
2. Why is sulfur content greatly affected by an increase in blending time? Use the data to support you answer. (1, 2, 4, 8, and 10)
3. In terms of DNA or protein being the genetic material, what were the researchers trying to support with this table? (1, 2, 4, 8, and 10)
4. Create a more convincing figure that would support the above conclusion to a greater extent. The figure should be similar to the one you have just examined. The only difference should be the values of the data points.

TABLE V

Effect of Multiplicity of Infection on Elution of Phage Membranes from Infected Bacteria

Running time in blender	Multiplicity of infection	P^{32} -labeled phage		S^{35} -labeled phage	
		Isotope eluted	Infected bacteria surviving	Isotope eluted	Infected bacteria surviving
<i>min.</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0	0.6	10	120	16	101
2.5	0.6	21	82	81	78
0	6.0	13	89	46	90
2.5	6.0	24	86	82	85

The infected bacteria were suspended at 10^9 cells per ml. in water containing per liter 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 0.1 gm. gelatin. Samples were withdrawn for assay of extracellular isotope and infected bacteria before and after agitating the suspension. In either case the cells spent about 15 minutes at room temperature in the eluting fluid.

What do you need to know to interpret this table:

1. According to Andersen in 1951 and his electron micrographs phage particles attach to the membranes of cells by their tails. The researchers built the bulk of their experiment on this theory. They hypothesized that if one were to apply a shearing force by way of a blender to bacterial cells adsorbing to phage particles then one could remove the phage particles. The researchers decided to do such a thing and they found they were able to remove the phage particles from the cell.
2. From previous data tables in the paper from which the figure has been extracted from we have already discovered and began to realize that when phages infect bacterial cells they transfer their DNA to the cell.
3. We are looking at two types of phages one labeled with phosphorus (only found on DNA) and the other labeled with sulfur (only found in protein). The researchers "label" phages by growing them in the presence of the isotopes so when the phages are built they incorporate the radioactive isotopes into themselves.
4. After centrifugation only cells should be present in to sediment.
5. Multiplicity of infection refers to the concentration of phage particles that the bacteria were subjected to.

Experimental Design: The researchers grew up phages in the presence of phosphorus or sulfur. They then infected sensitive bacterial cultures using two different multiplicities of infection. They then measured the amount of isotope which they could elute in solution. It is important to know that when sulfur or phosphorus cannot be eluted from solution this means that it the isotopes are either attached to the surface of the bacteria or within the bacteria beneath the membrane.

Answer these Questions based on the information you have been presented with.

1. When a bacterium dies it can become degraded and self lyse because it is not longer able to maintain itself. As a result when bacteria dies they release a large amount of their intracellular contents. Given this fact why do you think the researchers thought it necessary to tell the percent of infected bacteria which were surviving?
2. What happens to the amount of isotopes eluted when the suspension is placed in a blender? Describe this for sulfur and phosphorus in conditions of both high and low multiplicity of infection.(hint: imagine you are conveying the data to someone over a telephone) Use the data to back up your answer. (1, 2 and 10)
3. As the multiplicity of infection increases is there a substantial increase in the amount of Phosphorus which is eluted after 2.5 minutes of blending? Why?(1, 2, and 10) Use the data to back up your answer.
4. Venture an educated guess and tell what you think this data tells us about the roles and action Protein? Your answer only needs to be one to two sentences.

TABLE VI
Per Cent Distributions of Phage and S³⁵ among Centrifugally Separated Fractions of Lysates after Infection with S³⁵-Labeled T2

Fraction	Lysis at <i>t</i> = 0 S ³⁵	Lysis at <i>t</i> = 10 S ³⁵	Maximal yield	
			S ³⁵	Phage
1st low speed sediment	79	81	82	19
2nd " " "	2.4	2.1	2.8	14
High speed "	8.6	6.9	7.1	61
" " supernatant	10	10	7.5	7.0
Recovery	100	100	96	100

Infection with S³⁵-labeled T2, 0.8 particles per bacterium. Lysing phage UV-killed *h* mutant of T2. Phage yields per infected bacterium: <0.1 after lysis at *t* = 0; 0.12 at *t* = 10; maximal yield 29. Recovery of S³⁵ means per cent of adsorbed input recovered in the four fractions; recovery of phage means per cent of total phage yield (by plaque count before fractionation) recovered by titration of fractions.

What do you need to know to interpret this table:

1. If you were to infect a bacteria with a T2 phage and then look at the phage content on and within that bacteria at different times you would see that initially(at *t*=0) there are only the original parental phage particles attached to the bacterium. However if you waited a little longer (*t*=10) allowing the phage to transmit its replicative information and allowed it to utilize the host cells protein synthesizing material you would find that not only are there the original parental phages attached to the cell but there are also many other progeny phages within the cell preparing to lyse the cell so they can go on to infect other bacteria.
2. We already know that phages are made up of protein and DNA.
3. We also know that sulfur is found exclusively in protein. In this table the researchers show us how they monitored viral proteins by tracking the sulfur.
4. Anything which is the genetic material would probably be transferred from the parent to progeny. So if labeled sulfur is transferred from the phage parents to progeny then we might suspect that protein is the genetic material.
5. The researchers "label" phages by growing them in the presence of the isotopes so when the phages are built they incorporate the radioactive isotopes into themselves.

Experimental Design: The researchers fractionated two different batches of bacteria which were infected with phage labeled with sulfur. Each batch was give cyanide(HCN), which stops phage growth, at two different times(*t*=0 and *t*=10). Then they were given a UV-killed lysing phage which caused the cells to lyse as well as blocked all sites of

attachment which progeny phages might attach to after leaving their host cells. The bacterial and phage solutions were first subjected to a low speed centrifugation, which should only drag down cells and what is attached to them into the sediment. Then they subjected the low speed supernatant from the first centrifugation to a high speed centrifugation, which should have dragged the unadsorbed phages and any progeny phages that have left the cell into the supernatant. Then the sediment was the high speed centrifugation was re-suspended and subjected to a low speed centrifugation. What is really important here is that if any sulfur is being transferred to the progeny phages then it there should be a large difference between sulfur content of the two batches in relation to the high speed centrifugation of the supernatant from the first centrifugation.

1. This table is trying to show us the variation or lack thereof in the distribution of the sulfur isotope across a series of centrifugations between $t=0$ (when the phages have just begun to infect the bacteria) and $t=10$ (when the phages have had sufficient time to infect and multiply within their host bacterium). Does this distribution change? Support your answer using the data.

2. Let suppose for a moment that if anything were going to be the genetic material then part of it should be transferred to its progeny. That this genetic material should act as a direct transforming factor. That being said do you think protein would fit the description of the genetic material? This may require some serious thought. (hint: the answer lies in how the distribution might or might not change because of an increase in infection time).

Appendix C:

Assignment 1 Answer Sheet:

Table 1:

1. We already know that Plasmolyzed phage retains the ability to attach to sensitive bacteria, but what is the plasmolyzed phage/ghost phage composed of? If the plasmolyzed phage or ghost phage is made mostly of protein then we should expect to see the protein of plasmolyzed phage attaching to the cell surface. If the plasmolyzed phage or ghost phage is made mostly of DNA then we should expect to the DNA of the plasmolyzed phage attaching to the cell surface. You should look at the data specifically referring to percent isotope adsorbed to sensitive bacteria. It should give you enough information to answer the question. Also be to include the data in your answer. (1, 2 and 6)

-When we look at percentage of isotope adsorbed to the bacteria we find that 85 percent of phosphorus and 90 percent of sulfur from whole phages will be adsorbed to bacteria. Then when we look at the percentage of isotope adhering to bacteria in plasmolyzed phage the sulfur percentage remains the same but the amount of phosphorus adsorbing to bacteria drops to 2 percent. Then because we already know that ghost phages can still adsorb to bacteria we must conclude that they are most likely composed of protein.

2. What happens to the DNA when the bacteria are plasmolyzed? To answer this is will be useful to look at the data you used to support your last question. You will also need to consider under what conditions is DNA made acid soluble by the enzyme DNAase. It is important to remember that DNAase would not be able to access DNA if it were associated with the phage. It is also important to include the data in your answer. (1, 2, 3 and 9)

-80 percent of the radiolabelled phosphorus which is used to label DNA was made acid soluble after treatment with DNAase in plasmolyzed phage. As opposed to the phosphorus from whole phage which was not made acid soluble. Also only 2 percent of the DNA in plasmolyzed phage will attach to bacteria. This leads us to the conclusion that during plasmolysis the phage DNA is separated from the ghost phage, which is composed of mostly protein, and becomes sensitive to DNAase.

3. Based on your previous two answers tell whether you think protein, DNA, or both perform the function of attaching the phage to bacteria for subsequent infection? Your answer must be based on the previous answers and the data.(1, 2, 6 and 9)

-Even after plasmolysis has occurred and protein separated from DNA it still retains the ability to adsorb to sensitive bacteria whereas DNA does not. Based on this I would have to say that one of protein's exclusive functions is helping the phage adsorb to sensitive bacteria.

4. Summarize the data table in one to two sentences?

-The outer coat of the phage particles is composed of protein and that coat reduces the sensitivity of the associated DNA to DNAase. The table tells us that protein is playing a major role in the adsorption whereas the function of the DNA still remains obscure.

Table 2:

1. An increase in non-sedimentable isotope after DNAase treatments tells us that the DNA has become more accessible to DNAase. That being said what conditions cause an increase in the sensitivity of DNA to DNAase? Be sure to support your claims using the data. (9, 7, 3, 2 and 1)

-There is a marked increase in the amount of non-sedimentable phosphorus isotopes after phages have been allowed to infect bacteria which were heated before or after infection. More specifically 76 percent of the isotope was non-sedimentable when applied to already heated bacteria and 66 percent of the isotope non-sedimentable when applied to bacteria which would be heated subsequently. Because phosphorus labeling is exclusive to DNA and DNA is only non-sedimentable when cut into smaller pieces then the phage DNA must only be sensitive when it has infected damaged or soon to be damaged bacteria.

2. What would you theorize is happening to the DNA under the conditions in which it is sensitive? (9, 7, 3, 2 and 1)

-Heating the bacterial cells kills them and leads to the cell membrane losing its integrity therefore allowing any unanchored cellular constituents within the cytoplasm to escape. The DNA is within or associated to the plasma membrane after infection. If the cells are damaged then the phage DNA is released and accessible to the DNAase.

3. The researchers heated unadsorbed phages and attempted to make their phosphorus contents acid soluble under different heating conditions. It is important to know that when the bacteria heated they were only heated to 80° Celsius. Why did they do this (hint this is a control)? Be sure to use the data in your answer.

-Large amounts of phosphorus isotope, 81 percent to be precise, does not become soluble until the phages are heated to 90 degrees, whereas at 80

degrees only 13 percent of the labeled phosphorus becomes soluble. This was done to support that the most of the DNA which becomes sensitive to DNAase comes DNA which has been transported to the sensitive bacteria and most of it is not coming directly from phage particles that are being damaged by heating.

4. Summarize the table in one or two sentences.

-Phage viruses will shuttle DNA from the virus to sensitive bacteria.

Table 3:

1. Compare the amount of soluble phosphorus which is made acid soluble after DNAase in infected cells which have been frozen thawed and fixed and those which have been just fixed. Under which conditions is the Phosphorus most soluble? Explain using the data.(1, 2, 3, 8 and 9)

-When cells are frozen thawed and fix there is an increase in the amount of phosphorus which becomes acid soluble after DNAase has been applied to it. More specifically 59 percent of the DNA is sensitive when the bacteria are frozen thawed and fixed whereas only 28 percent of the DNA is sensitive when the bacteria are only fixed. Based on the data it would be reasonable to assume that the DNA is becomes accessible to DNAase when the infected bacteria are opened and much it's intracellular contents are released or exposed to extra cellular enzymes.

2. The phage DNA should have been present in the cells before they underwent this process meaning they were retained within the cell even after much of the cytoplasm was released. Why might the phage DNA not be released when the cells are lysed? What evidence supports that the DNA is not being released? Be sure to use the data in your answer. (1, 2, 3, 8 and 9)

-There must be something holding the DNA back. There must be some sort of ordered structure which is attached directly to the bacterium. We can suspect this because 71 percent of the total isotope in bacteria which have been fixed, frozen and thawed travels with the bacteria into the sediment, whereas only 21 percent of the isotope remains in the supernatant. DNA by itself should not be pulled down into the sediment by a low speed centrifugation unless it is attached to something larger such as a cell.

3. In several sentences what does this table ultimately tell us?

-Upon infection phage DNA is transported inside the cell. Once inside it interacts with some sort of an intracellular structure, but if the cells integrity is compromised the DNA can become accessible to DNAase.

4. Draw a picture of what happens to phage DNA when the phage particle it is in attaches to a cell that has been frozen thawed and fixed.

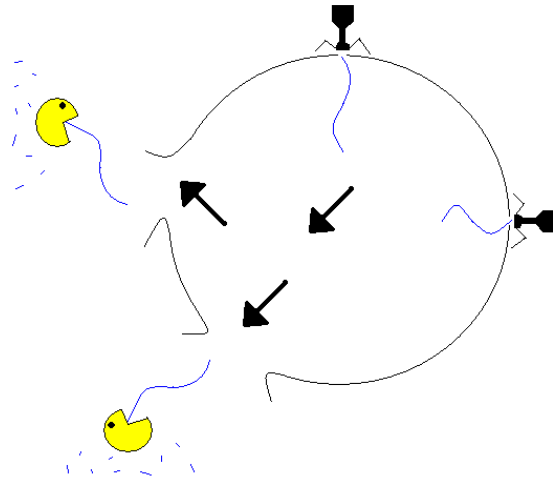


Figure 1:

1. Why is the phosphorus content only marginally affected by an increase in blending time?(1, 2, 4, 8 and 10)
 - Phosphorus is an isotope which exclusively labels DNA. The phosphorus content is only marginally affected because any DNA which is transferred into the cells sediments with the cells regardless of blending time.
2. Why is sulfur content greatly affected by an increase in blending time? Use the data to support you answer. (1, 2, 4, 8, and 10)
 - Sulfur is a component of protein and protein is what forms the outer coat of the phage . The protein facilitates attachment to the cells and is adsorbed to the cells during infection. When cells which have adsorbed the phages are placed in a blender the phage coat or proteins can be mechanically removed because they only interact weakly with the host cell's surface. The data show this because as the run time in the blender is increased substantially eighty percent of the sulfur will be found in the supernatant.
3. In terms of DNA or protein being the genetic material, what were the researchers trying to support with this table? (1, 2, 4, 8, and 10)
 - The researchers wanted to support that during infection DNA enters the cell but protein does not holding up the idea that DNA is the genetic material and not protein. Only DNA has the ability to pass the necessary information to the host cell telling how to construct its progeny.

4. Create a more convincing figure that would support the above conclusion to a greater extent. The figure should be similar to the one you have just examined. The only difference should be the values of the data points.

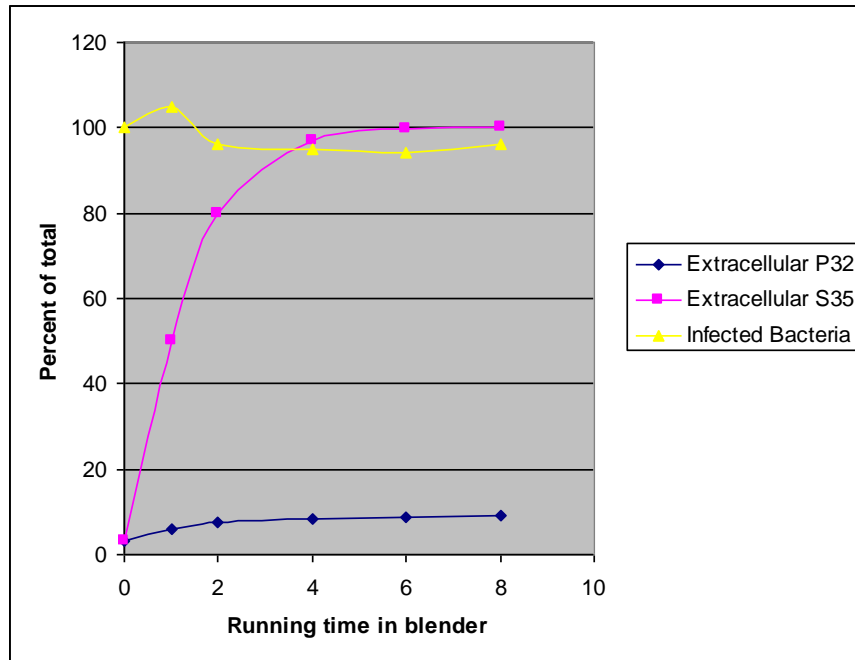


Table 5:

- When a bacterium dies it can become degraded and self lyse because it is not longer able to maintain itself. As a result when bacteria dies they release a large amount of their intracellular contents. Given this fact why do you think the researchers thought it necessary to tell the percent of infected bacteria which were surviving?

-If the percentage of surviving bacteria changed drastically from experiment to experiment then we would have knowing way of no what causes the changes in isotope elution.

- What happens to the amount of isotopes eluted when the suspension is placed in a blender? Describe this for sulfur and phosphorus in conditions of both high and low multiplicity of infection.(hint: imagine you are conveying the data to someone over a telephone) Use the data to back up your answer. (1, 2 and 10)

-The amount of sulfur eluted increases substantially when placed in a blender. Under conditions of low multiplicity of infection elution increased from 16 to 81 percent. Then under conditions of high multiplicity of infection it increased from 46 to 81 percent. Conversely there is very little effect on the amount of phosphorus eluted when it is placed in a blender. Under conditions of low multiplicity elution increased

from 10 to 21 percent. Then under conditions of high multiplicity of infection elution increased from 13 to 24 percent.

3. As the multiplicity of infection increases is there a substantial increase in the amount of Phosphorus which is eluted after 2.5 minutes of blending? Why?(1, 2, and 10) Use the data to back up your answer.

-When the multiplicity of infection is 0.6 the amount of phosphorus eluted is 21 percent. When the multiplicity of infection is increased to 6(ten fold) the amount of phosphorus eluted is 24 percent. So as multiplicity of infection increases there is only a marginal increase in the elution of phosphorus. This happens because in both low and high degrees of infection the DNA is transferred inside the cell regardless of changes in phage concentration.

4. Venture an educated guess and tell what you think this data tells us about the roles and action Protein? Your answer only needs to be one to two sentences.

-Protein only functions to transfer DNA inside the cell.

Table 6:

1. This table is trying to show us the variation or lack there of in the distribution of the sulfur isotope across a series of centrifugations between $t=0$ (when the phages have just begun to infect the bacteria) and $t=10$ (when the phages have had sufficient time to infect and multiply within there host bacterium). Does this distribution change? Support your answer using the data.

-79 and 81 percent in this context can be said to be similar and statistically the same. The same goes for comparisons of the other fraction percentages. They are all statistically identical. This leads me to believe that the overall distribution of the sulfur isotope does not change as the infection time increases.

2. Let suppose for a moment that if anything were going to be the genetic material then part of it should be transferred to its progeny. That this genetic material should act as a direct transforming factor. That being said do you think protein would fit the description of the genetic material? This may require some serious thought. (hint: the answer lies in how the distribution might or might not change because of an increase in infection time).

-If protein was the genetic material then there should have been a greater percentage of sulfur, a label for protein, in the high speed centrifugation of the batch of cells which underwent lysis at $t=10$. This would have indicated that a large amount of phage created within the cells had incorporated the labelled protein. However this was not the case and we are led to believe that protein is not the genetic material.

Appendix D:

General Packet:

The RNAi Paper

What is so wonderful about Biology is that even at the fundamental level it is constantly changing. Every year we learn about new life governing mechanisms. I am sure it could be argued that sciences such as physics and chemistry are changing as well. However it cannot be said that they are transforming at a rate even comparable to Biology. This is mainly because of two reasons. One being that modern Biology is a relatively new science. So there is still so much to be discovered. It is also because it is a very complex science. It encompasses every aspect of life. Stretching from reactions taking place on the nano-scale to the behavior of large multi-cellular organisms. It gives us an almost endless reservoir of phenomenon to examine and marvel at always causing us to change our viewpoint.

In this part of the course you will be looking at one of the most recent and largest change which has occurred in biology. It has to do with regulation of transcription. Within the cell there are many tiny chopped up pieces of messenger RNA floating around in the cytoplasm. You should not that when I say tiny I mean in the sense that these mRNAs are much smaller than the average mRNA, because as you may know all mRNA could be considered small. It was originally thought that these pieces of mRNA were just the result transcript degradation. We knew that mRNA was degraded by RNAases for a long time so we simply assumed that these small pieces of RNA were a byproduct of that. In a sense we took these pieces of RNA for granted and wrongly so as you will discover.

You have been given data from a paper which was investigating the role of RNA in the interference of gene expression. It was already known that somehow when strand which are complimentary to mRNA strands being expressed are placed in various cell types it creates an interference effect specific to that gene. The researchers in this paper wanted to ask what conditions allow this to happen. They accomplished this by injecting the nematode *C. elegans* with various types of RNA and then observing the resulting phenotype. They would also use reporter genes to monitor the expression of various genetic elements in the presence of injected RNA or lack there of. It very important to remember that all these experiments are performed on the wild type not mutants.

Your job will be to interpret the data you have been given based on the information you have been given. Whenever you answer a question you may only use the data and information on this sheet. You should also keep in mind that every conclusion you make must be supported.

Important Information

Sense RNA vs. Antisense RNA: Sense RNA is the mRNA which directly codes for a protein. If sense RNA is processed by a ribosome then it will produce a functional protein as long it has been expressed in the right cell and is not mutated. Antisense RNA is a negative version of the Sense RNA or it is complimentary to the Sense RNA. Also whenever the researchers refer to “sense + antisense” they are referring to double stranded RNA.

F₁ Phenotype: F₁ refers to the first filial generation or the first generation emanating from the parental mating set up by the researchers. Phenotype simply means a discernible property of the organism that results from a gene or combination of genes. The property can be physical or behavioral. It could be anything from the metabolic capabilities of a microorganism to the stability of beavers damn.

Exons vs. Introns: Exons are coding regions that direct the synthesis for part of or a whole polypeptide where as introns or intervening sequences are non-coding regions that are selectively spliced out of genes during post transcriptional processing within the nucleus. It should also be known that the concept of gene containing coding regions interrupted by non coding regions is almost purely eukaryotic.

Caenorhabditis elegans: The data you will analyze was collected from this organism. *C. elegans* is a model organism which has become quite popular especially for studying development as well as neurobiology. The nematode *C. elegans* is quite small, less than a millimeter in length and thinner than an eyelashes. It is composed of just under a thousand cells. Yet it possesses reproductive organs, a digestive tract as well as a nervous system. By understanding *C. elegans* researchers hope they can draw links to human development. One big advantage with *C. elegans* is that it is transparent. This allows researchers to express fluorescent proteins in the organism and use them as markers. A lot of the studies on this organism employs genetic principle in which phenotypes and genotypes are examined by looking at the progeny which result from genetic crosses.

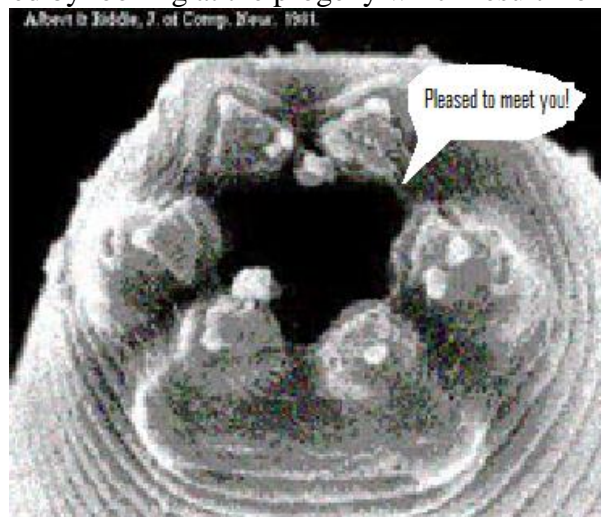


Image from www.cecs.cl/web/cecs_index.php?area=educacion&dep=main&idioma=es&pagina=mon_exp&id=21&tema=

Micro Injection RNA: This entire paper relies on the injection of various types of RNA into the gonad of *C. elegans* and then subsequent observation of the results. It is important to understand how the procedure works to understand the paper. The gonad is the portion of the animal which produces germ cells. By injecting the gonads of wild type adults with a transforming factor we can ensure that we will be able to affect most of if not all of their progeny. Just about all the data you will look at are collected from the progeny of these injected worms.

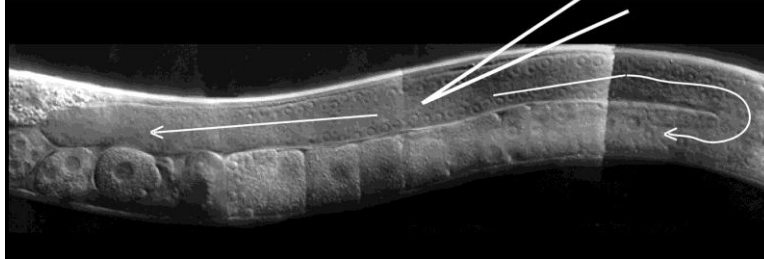


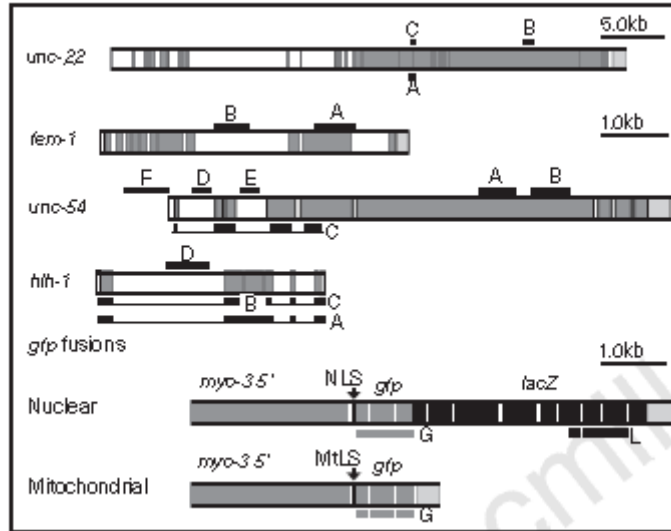
Image taken from wormbook.org

Wild type: This simply refers to worms that appear and behave relatively normal. They are the nematodes which you would be most likely to encounter outside the laboratory. They are also the type of nematode upon which these experiments were performed.

What does unc mean?: “unc” simply stands for uncoordinated. Whenever you see a gene called unc followed by some number then the researchers are trying to tell you that the gene they are referring to when mutated will alter the nematodes ability to maneuver itself. A great variety of genes can make an organism uncoordinated and because uncoordinated is such a broad term it can also apply to many phenotypes. For example, worms which have the unc-26 mutant gene might tend to move in clockwise circles where as worms that have the unc-57 mutant gene might move slower than wild type. It should also be noted that just like all other genes unc genes can be dominant or recessive.

Gene definitions:

-For any of the genes being studied you will find that the researchers used various regions of the genes such as specific introns or exons and in various form of sense, antisense or double strand.



1. *unc-22* : A gene which codes for a non essential myofilament protein which when mutated leads to uncoordinated movement.
2. *fem-1*: A gene which has been found to be a crucial secondary messenger in the sex-determination pathway.
3. *unc-54*: this gene encodes a crucial portion of muscle myosin and is needed for locomotion and egg laying.
4. *hth-1*: A transcription factor which is key in regulating the development of body wall muscle cells.
5. *gfpG*: A green fluorescent protein which the researchers fused to other proteins by means of genetic engineer so they could observe the expression of the gene to which *gfpG* would be attached to. We often refer to genes of these types as reporter genes.
6. *lacZ*: another reporter gene which encodes for the β -galactosidase protein, a protein which cleaves the disaccharide lactose therefore creating glucose and galactose.
7. *myo-3*: A gene which encodes for a myosin heavy chain and is important for filament formation, viability, movement and embryonic elongation.
8. *mex-3*: a gene which is abundantly transcribed in the early stages of worm development.

Information for gene definitions obtained from wormbase.org

Appendix E:

Assignment 2

Table 1A:

Gene	segment	Size (kilobases)	Injected RNA	F ₁ phenotype
<i>unc-22</i>				<i>unc-22</i> -null mutants: strong twitchers ^{1*}
<i>unc22A*</i>	Exon 21-22	742	Sense Antisense Sense + antisense	Wild type Wild type Strong twitchers (100%)
<i>unc22B</i>	Exon 27	1,033	Sense Antisense Sense + antisense	Wild type Wild type Strong twitchers (100%)
<i>unc22C</i>	Exon 21-22†	785	Sense + antisense	Strong twitchers (100%)

Background Information: The researchers injected *C. elegans* with various segments of the *unc-22* gene (If you want to know what each segment corresponds to then you can simply look at the information packet you have been given). What is important to know is that when *C. elegans* are “strong twitchers” then they are displaying a phenotype which is indicative of an *unc-22* mutation.

1. What happens when single stranded RNA (sense or antisense) from coding regions of *unc22* is injected into wild type worms? Explain using the data.
2. What happens when double stranded RNA (sense + antisense) from coding regions of *unc22* is injected into wild type worms? Use the data to back up your point.
3. In your own words what does this data table tell us?

Table 1B:

Gene	segment	Size (kilobases)	Injected RNA	F ₁ phenotype
<i>fem-1</i>				<i>fem-1</i> -null mutants: femal (no sperm) ⁶
<i>fem-1A</i>	Exon 10†	531	Sense Antisense Sense + antisense	Hermaphrodite (98%) Hermaphrodite (>98%) Female (72%)
<i>fem-1B</i>	Intron 8	556	Sense + antisense	Hermaphrodite (>98%)

Background Information: The researchers injected *C. elegans* with various segments of the *fem-1* gene. Normally about 98 percent of the *C. elegans* wild type population will be hermaphrodites and the remaining two percent will be males. So it is important to realize when *C. elegans* are mostly female then they are displaying an abnormal and mutant phenotype.

1. What happens when single stranded RNA (sense or antisense) from coding regions (an exon) of *fem-1* is injected into wild type worms? Explain using the data.
2. What happens when double stranded RNA (sense + antisense) from coding regions (an exon) of *fem-1* is injected into wild type worms? Use the data to back up your point.
3. What happens when double stranded RNA of a non coding region (an intron) from *fem-1* is injected into wild type worms? Use the data to back up your point.
4. In your own words what new piece of information does this table tell us? In other words what makes the conclusions from this table different from the previous one?

Table 1C:

Gene	segment	Size (kilobases)	Injected RNA	F ₁ phenotype
<i>unc-54</i>				<i>unc-54</i> -null mutants: paralysed ⁹¹
<i>unc54A</i>	Exon 6	576	Sense Antisense Sense + antisense	Wild type (100%) Wild type (100%) Paralysed (100%)
<i>unc54B</i>	Exon 6	651	Sense Antisense Sense + antisense	Wild type (100%) Wild type (100%) Paralysed (100%)
<i>unc54C</i>	Exon 1-5	1,015	Sense + antisense	Arrested embryos and larvae (100%)
<i>unc54D</i>	Promoter	567	Sense + antisense	Wild type (100%)
<i>unc54E</i>	Intron 1	369	Sense + antisense	Wild type (100%)
<i>unc54F</i>	Intron 3	386	Sense + antisense	Wild type (100%)

Background Information: It is important to keep in mind that genes can be multifunctional and that various regions of the genes can be involved with these various functions. In this case disruption in the expression of *unc-54* using different coding regions can lead to paralysis and arrested embryos larvae.

1. Do promoters or introns double stranded form produce interference? Support using the data.
2. If I wanted to “knockout” a gene using injected RNA what region of the gene would I need to use and in what form?
3. In your own words what do the above data tell us?

4. Table 1D:

Gene	segment	Size (kilobases)	Injected RNA	F ₁ phenotype
<i>hh-1</i>				<i>hh-1</i> -null mutants: lumpy-dumpy larvae ¹⁶
<i>hh1A</i>	Exons 1-6	1,033	Sense Antisense Sense + antisense	Wild type (<2% lpy-dpy) Wild type (<2% lpy-dpy) Lpy-dpy larvae (>90%)
<i>hh1B</i>	Exons 1-2	438	Sense + antisense	Lpy-dpy larvae (>80%)
<i>hh1C</i>	Exons 4-6	299	Sense + antisense	Lpy-dpy larvae (>80%)
<i>hh1D</i>	Intron 1	887	Sense + antisense	Wild type (<2% lpy-dpy)

Background Information: lpy-dpy refers to worms that have a fat and and stubby appearance. There are a variety of genes that when you mutated can produce phenotypes along these lines.

1. In your own words what do the above data tell us?
2. Give a good reason for the researchers to test their theory about RNA interference using multiple genes?

Table 1E:

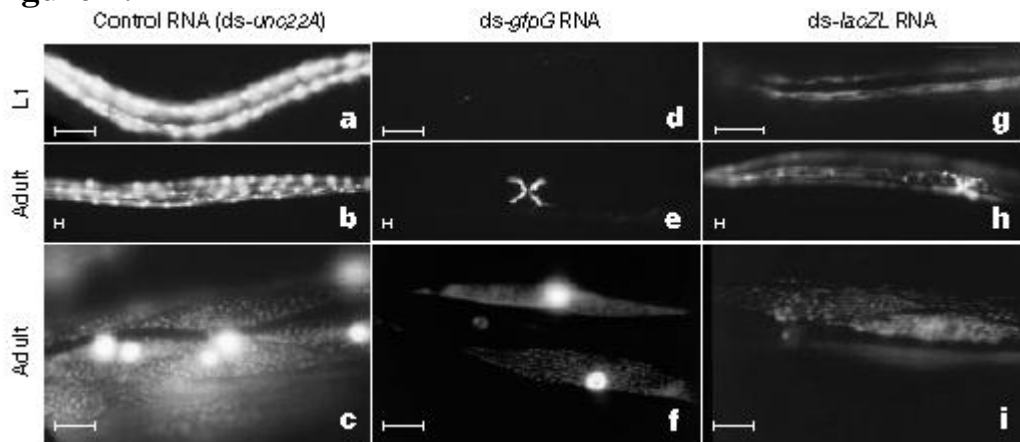
Gene	segment	Size (kilobases)	Injected RNA	F ₁ phenotype
<i>myo-3-driven GFP transgenes</i>				
<i>myo-3::NLS::gfp::lacZ</i>				Makes nuclear GFP in body muscle
<i>gfpG</i>	Exons 2-5	730	Sense Antisense Sense + antisense	Nuclear GFP-LacZ pattern of parent strain Nuclear GFP-LacZ pattern of parent strain Nuclear GFP-LacZ absent in 98% of cells
<i>lacZL</i>	Exon 12-14	830	Sense + antisense	Nuclear GFP-LacZ absent in >95% of cells
<i>myo-3::MtLS::gfp</i>				Makes mitochondrial GFP in body muscle
<i>gfpG</i>	Exons 2-5	730	Sense Antisense Sense + antisense	Mitochondria kGFP pattern of parent strain Mitochondria kGFP pattern of parent strain Mitochondria kGFP absent in 98% of cells
<i>lacZL</i>	Exon 12-14	830	Sense + antisense	Mitochondria kGFP pattern of parent strain

Background Information: To understand this figure you need to know that the researchers studied two different types engineered genes, each of which was attached to myo-3 gene and contained a gfp reporter gene as well. When the reporter gene is expressed GFP can be seen under a fluorescent scope. The two gene types were also expressed in two different locations. The myo-3::NLS::gfp::lacZ gene was expressed into the nucleus of body muscle and also contained the lacZ gene. The other gene, myo-3:MtLS::gfp, was expressed in the mitochondria and did not have a the lacZ attached to it. To see the layout of these genes just look at the figure given in your general information packet.

The researchers injected RNA from the gfpG gene and the LacL gene into the *C. elegans* which had one of these two genes and observed to expression patterns so they could have an idea of how the RNA was affecting the organism at the cellular level.

1. The worms with the myo-3:MtLS::gfp genotype are affected when injected with double stranded gfp-g coding region RNA but not by lacZL RNA. How does the data show this?
2. What story does the data tell us?

Figure 2:

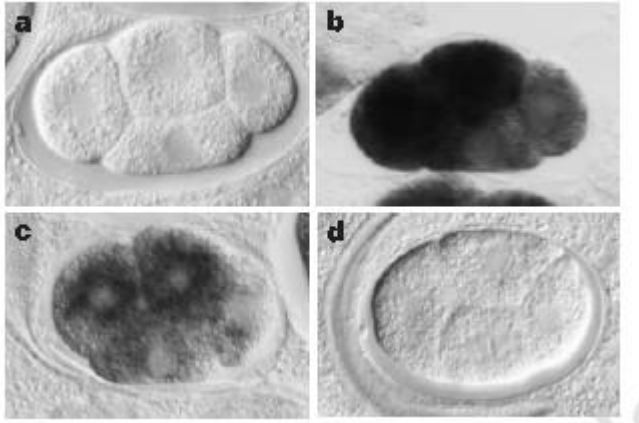


Background Information: These pictures are the data collected for an experiment in which the researchers injected three different types of double stranded RNA into adult and L1(very young) worms of a specific strain called PD4251. In order to understand the pictures above you need to know that PD4251 has been genetically modified to carry both the nuclear and the mitochondrial *gfp* fusion gene(as shown in the figure in your packet). Notice how these are practically identical except for the sequences that determine where they are expressed in the cell and the addition or lack of the *lacZ* gene.

The Researchers photographed the worms in three ways. They took photos of the L1 worms(very young), adult worms, and close-ups of the adult body wall. Under normal conditions PD4251 will express *gfp* throughout the worm's body because it is coupled to the expression of the *myo* gene. So when there is not interference you should see a lot of GFP(or glowing). In pictures "a" through "c" they inject these worms with double stranded *unc-22*. Then in d through f they injected the worms with double stranded *gfpG*. Finally in g through I they injected the worms with double *lacZL*.

1. Using the data why is there GFP expression in a-c but very little in d-f?
2. Explain with aid from the data why there are expression differences between d-f and g-i?
3. Were the researchers able to silence the *gfp-G* gene in every cell of the worms in d-f? Propose an explanation for your answer.
4. Summarize the story the data is trying to tell us.

Figure 3:



Background Information: In this experiment the researchers wanted to know why the genes corresponding to these double stranded RNAs being injected into the cell were no longer active. To probe this question they decided to be more specific and ask “what happens to the mRNAs of these silenced genes within the cell?”. The researchers used in situ hybridization track the mex-3 transcripts within the cell. All you need to know in order to understand this experiment is that when mex-3 mRNA is present the cells will appear dark and the intensity of that darkness gives us a good idea of how much endogenous mex-3 mRNA there is.

Picture A: A negative control in which no staining has taken place because there is no hybridization probe.

Picture B: An embryo from a parent which has not been injected with double stranded mex-3 RNA.

Picture C: An embryo from a parent which has been injected with antisense(single strand) mex-3 RNA.

Picture D: An embryo from a parent which has been injected with double stranded mex-3 RNA.

1. What does the fact that D shows no apparent staining tell us? Please rationalize your response using the data.
2. If the differences in endogenous mRNA concentrations between B and C are not due to normal statistical variation then what could cause this difference? Explain using the data. (hint: what type of RNA is being injected for picture C).

Table 2:

Table 2 Effect of site of injection on interference in injected animals and their progeny

dsRNA	Site of injection	Injected-animal phenotype	Progeny phenotype
None	Gonad or body cavity	No twitching	No twitching
None	Gonad or body cavity	Strong nuclear and mitochondrial GFP expression	Strong nuclear and mitochondrial GFP expression
<i>unc22B</i>	Gonad	Weak twitchers	Strong twitchers
<i>unc22B</i>	Body-cavity head	Weak twitchers	Strong twitchers
<i>unc22B</i>	Body-cavity tail	Weak twitchers	Strong twitchers
<i>gfpG</i>	Gonad	Lower nuclear and mitochondrial GFP expression	Rare or absent nuclear and mitochondrial GFP expression
<i>gfpG</i>	Body-cavity tail	Lower nuclear and mitochondrial GFP expression	Rare or absent nuclear and mitochondrial GFP expression
<i>lacZL</i>	Gonad	Lower nuclear GFP expression	Rare or absent nuclear-GFP expression
<i>lacZL</i>	Body-cavity tail	Lower nuclear GFP expression	Rare or absent nuclear-GFP expression

Background Information: In most of the data tables the researchers had injected RNA into the gonads of parents and then examined the phenotypes of their progeny. Now in this table the researchers try and see what happens when they inject the parents in the head or tail region and they observe the phenotype of the injected animal and their progeny.

1. Is there a qualitative difference between progeny which have been subjected to different methods of injection? Use the data to explain why.
2. What story does the data from this table tell us?

Appendix F:

Assignment 2 Answer Sheet

Table 1A:

1. What happens when single stranded RNA(sense or antisense) from coding regions of *unc22* is injected into wild type worms? Explain using the data.

For all instances the worms retain the wild type phenotype, indicating that the microinjection had not affect.

2. What happens when double stranded RNA(sense + antisense) from coding regions of *unc22* is injected into wild type worms?. Use the data to back up your point

For all instances of double stranded microinjection the F1 phenotype of all worms is a “strong twitchers”, which is indicative of an *unc22* mutant strain.

3. In your own words what does this data table tell us?

The table tells us that the mutant phenotype in the F1 progeny, for the respective gene, is only created when double stranded (sense and sense) is injected into the parent strain.

Table 1B:

1. What happens when single stranded RNA(sense or antisense) from coding regions(an exon) of *fem-1* is injected into wild type worms? Explain using the data.

The phenotypic gender ratio is the same as the wild type, 98% hermaphrodite.

2. What happens when double stranded RNA(sense + antisense) from coding regions(an exon) of *fem-1* is injected into wild type worms?. Use the data to back up your point

Contrary to sense or anti-sense injection, microinjections of double stranded RNA from the coding regions of the *fem1* gene produce an altered mutant phenotype, a 72% female population. This is an alteration of the normal gender ratio of the worms.

3. What happens when double stranded RNA of a non coding region(an intron) from *fem-1* is injected into wild type worms? Use the data to back up your point.

This produces the wild type phenotype, for the gender ratio, in the F1 generation, 98% hermaphrodite.

4. In your own words what new piece of information does this table tell us? In other words what makes the conclusions from this table different from the previous one?

We already knew that the injecting both sense and anti sense RNA from a gene could produce a mutant phenotype corresponding to that gene. Now, however, this paradigm is refined. We learn that, perhaps, only coding regions can produce the mutant phenotype.

Table1C:

1. Do promoters or introns double stranded form produce interference? Support using the data.

It appears that neither promoters or introns produce interference, at least for the unc-54 gene. This is evidenced by the fact that 100% of the F1 progeny for the respective microinjections produce a wild type phenotype. Also, this cannot be attributed to the gene itself, because, sense and antisense RNA from exons are injected they produce a mutant phenotype in the form of arrested embryos and larvae (100% of F1 progeny).

2. If I wanted to “knockout” a gene using injected RNA what region of the gene would I need to use and in what form?

You would have to inject both sense and antisense RNA from an exon (coding region) of the gene of interest.

3. In your own words what do the above data tell us?

Only sense and antisense RNA from a coding region will knockout a gene. However, these tables have only displayed a limited number of genes. If knocking out a gene is a global phenomenon then more genes would have to be tested.

Table 1D:

1. In your own words what do the above data tell us?

Varying populations can be created by injected different coding regions of a gene.

2. Give a good reason for the researchers to test their theory about RNA interference using multiple genes?

Once again, in order to determine if this is a global phenomenon (applying to all genes), many genes would have to be tested. At this point we have no way of knowing whether or not the researchers chose these genes for their susceptibility to this gene knockout phenomeon.

Table 1E:

1. The worms with the myo-3:MtLS::gfp genotype are affected when injected with double stranded gfp-g coding region RNA but not by lacZL RNA. How does the data show this?

These are fusion proteins which both contain GFP, however the lacZL coding region is attached to the gfpG gene but not to the myo-3 gene. This is shown by the fact that the lacZL, sense and antisense RNA, does not knock out GFP expression in the F1 generation.

2. What story does the data tell us?

The data hints at the fact that gene knockout is happening at the transcript level

Figure 2:

1. Using the data why is there GFP expression in a-c but very little in d-f?

In d through f double stranded gfpG RNA has been injected. So, we are seeing the visual manifestation of a gene knock out.

2. Explain with aid from the data why there are expression differences between d-f and g-i?

Although GFP expression in panels g to i is not as marked as a through c, the net reduction in GFP expression is not as large when compared to d through f.

3. Were the researchers able to slience the gfp-G gene in every cell of the worms in d-f? Propose an explanation for your answer.

No they were not able to. Perhaps, there are certain cells which have an inherent resistance to gene knockout via double stranded coding RNA.

Figure 3:

1. What does the fact that D shows no apparent staining tell us? Pleas rationalize your response using the data.

Injection of double stranded coding RNA completely removes the RNA sequence from these cells.

2. If the differences in endogenous mRNA concentrations between B and C are not due to normal statistical variation then what could cause this difference? Explain using the data. (hint: what type of RNA is being injected for picture C).

Sense RNA is already present in figure C.

Table 2:

1. Is there a qualitative difference between progeny which have been subjected to different methods of injection? Use the data to explain why.

There is no phenotypic difference between these two injection types. This suggests that even when RNA is injected away from the gonad, or germline, the RNA is still able to migrate towards the newly forming progeny.

Appendix G:

Introduction to Scientific Literature: Assessment

What is your major or if undecided what major(s) are you leaning towards?

As a result of this course have you began thinking about a specific career path, possibly changing major or simply reaffirming you interest in the biological sciences?

In terms of helping you better understand and read scientific literature rate the usefulness of this course (10 being very useful)

1 2 3 4 5 6 7 8 9 10

On a scale of one to ten please rate the quality of instruction (10 indicates that instruction was very useful and helped you understanding of the figures)

1 2 3 4 5 6 7 8 9 10

What did you take away from this portion of the course if anything?

Do you have any suggestions for how this portion of the course could be changed?

Rate the difficulty of this course (10 being most difficult)

1 2 3 4 5 6 7 8 9 10

Did this course change your perspective, in the positive or negative direction, in terms of what it means to be a scientist in the field of biology? (If you perspective change is most

negative then select -5. Conversely if your perspective change is most positive then select 5)

-5 -4 -3 -2 -1 0 1 2 3 4 5

Are there any additional comments you would like to make?