Teaching the Central Dogma through an Inquiry-Based Project Using GFP

Cynthia Bujanda, Nadja Anderson

ABSTRACT

The Central Dogma is a crucial concept needed to understand biotechnology and molecular biology. High school students often struggle with a meaningful understanding of this abstract concept. This paper presents an inquiry-based approach to increase critical thinking and understanding of the Central Dogma. Commonly used in high school classrooms is Bio-Rad pGLO plasmid containing green fluorescent protein (GFP), because of its accessibility and the fluorescence it emits when exposed to ultraviolet light. We use the expression of GFP in a high school hands-on class project so that students can visualize and understand the abstract concepts of the Central Dogma. Students will also explore protein structure and its importance for a functional protein. During the entire project, students will be guided by the instructor to build hypotheses and design experiments to test those hypotheses, exercising the scientific method.

Key Words: central dogma; GFP; inquiry; transformation.

Introduction

Green fluorescent protein (GFP), a protein isolated from a bioluminescent jellyfish, Aequorea victoria, is of interest to researchers because of its bright fluorescence when exposed to ultraviolet (UV) light (Prasher et al., 1992). Historically, GFP has been used in high school classrooms since the mid-1990s; this was made possible after GFP was cloned and expressed in E. coli in 1994 (Ward et al., 2000), making it accessible for biotechnology education. Commonly used in the classroom is the transformation of E. coli using the Bio-Rad pGLO plasmid, which contains GFP as well as a gene for ampicillin resistance (bla) and a regulatory protein that binds to the promoter side pBAD (AraC) (Crameri et al., 1995; Deutch, 2019). In the presence of arabinose, the bound regulator on the promoter (AraC) changes shape, allowing RNA polymerase to bind to the promoter. This facilitates the gene expression of GFP by the production of mRNA, followed by the translation of mRNA into protein.

We can use the expression of GFP to explain the Central Dogma, which was first described by Francis Crick in 1970 to explain the transfer of information within cells (Figure 1). The Central Dogma states that the information in DNA is transferred to RNA and that in turn is transferred to a specific sequence of amino acids to produce a polypeptide. This concept is crucial in the understanding of biotechnology and molecular biology. However, biology education research has shown that, when taught under conventional modes of instruction, students have a difficult time understanding the abstract concepts of gene expression and protein translation that are core to the Central Dogma (Lewis & Kattmann, 2004; Newman et al., 2016; Reinagel & Bray Speth, 2016).

Part of the teachers’ challenge to maximize students’ learning of abstract concepts is that students are being asked to understand concepts, objects, and processes that are not visible (Hinze et al., 2013). Additionally, using technical vocabulary to convey genetic concepts proves burdensome for students (Reinagel & Bray Speth, 2016). Student understanding of abstract concepts is significantly increased with hands-on, kinesthetic learning (Fyfe et al., 2014; Hayes & Kraemer, 2017); this is even more apparent for ESL students (Llosa et al., 2016; National Academies of Sciences, Engineering, and Medicine, 2018). By broadening students’ knowledge on genetic concepts and molecular biology, we educate students in scientific literacy. In today’s society this becomes increasingly important because of the relevance of DNA techniques used in medicine, laboratory diagnostics, and criminology (Duncan & Reiser, 2007).

To efficiently teach the Central Dogma, it is important that we present lessons that mirror scientific research by inquiry-based exploration and meaningful reflection on results (Burnette &
We report a lesson in which students will develop their own hypothesis and conduct experiments with the guidance of the instructor in order to reach conclusions and maximize their learning. The main objective of this lesson is to teach the abstract concepts of one of the most important lessons in molecular biology, the Central Dogma, targeting transcription and translation in a way that students experience the concepts rather than merely reading and hearing them.

We further expand our lesson by exploring protein structure and examining how the information flow from DNA will eventually be correctly expressed in proteins. Proteins are complex molecules, their structure being crucial to their function and regulation of cells, and are essential in all biological processes. Their function is dependent on their properly folded three-dimensional structure, which is dictated by the translated sequence of amino acids in the protein. The importance of protein structure can be seen by comparing denatured proteins and native proteins, which is accomplished in this lesson as a secondary objective.

These activities are accessible to schools with polymerase chain reaction (PCR) and electrophoresis materials and flexible to adjust to high school schedules. Teachers can use the pBADgfpuv plasmid, which is readily available through Bio-Rad Laboratories (as pGLO).

**Objective**
Engage students in an inquiry-based hands-on project that will broaden their knowledge of the Central Dogma in molecular biology.

**Secondary Objectives**
1. Students will hypothesize and experimentally test their hypotheses.
2. Students will be introduced to transformation, sterile technique, DNA extraction, PCR, electrophoresis, DNA sequencing, BLAST, and SDS-PAGE protein gels.
3. Students will observe the functional differences between denatured and native GFP.

**Methods, Materials & Results**
This set of lab activities takes approximately 10 days of class time. It can be broken up into different segments to fit the class schedule and curriculum. The details of the protocols and student guides are available on the BIOTECH Project website: http://biotech.bio5.org/publications. Overview of the entire class project can be seen in Figure 2.

**Transformation of pBADgfpuv Plasmid**
Teachers will provide students with an agar plate previously transformed with pBADgfpuv. Students begin the transformation process by extracting the plasmid DNA from glowing *E. coli* (Figure 3A). A single colony of glowing *E. coli* is grown in a liquid LB-plus-ampicillin culture to grow enough cells to extract plasmid DNA. Any plasmid DNA extraction kit can be used; we used Omega BIO-TEK EZDNA Plasmid DNA Mini Kit and followed the manufacturer’s instructions. The students can visualize the characteristics of the gene that is being expressed in the initial colonies and show that DNA is the genetic material involved in the transformation process. They should be able to hypothesis that if DNA is the genetic material seen in the transformation, the glowing characteristic will be transferred to the transformed *E. coli*. As an extension, students can further confirm that DNA is the genetic material by adding protease or DNAase to their transformation, much as Oswald Avery did to confirm Fred Griffith’s results (Avery et al., 1944; Griffith, 1928). Between 20 and 50 ng of the extracted plasmid DNA can be used for bacterial transformation. Most plasmid DNA extraction kits will yield between 50 and 100 ng/µl.

Students introduce the transformed *E. coli* onto LB and LB-plus-ampicillin plates using a standard chemically competent cells transformation protocol (Green & Rogers, 2013). The following day, students analyze the results and observe transformed colonies (based on ampicillin selection, ampicillin resistance being another gene on the plasmid). At this point, students should wonder why their colonies are not glowing (Figure 3B), since they used plates without arabinose. This being the big question, students will need to find the answer by testing different hypotheses.

The instructor should remind students that their hypothesis of DNA being the genetic material seen in transformation was supported due to the selection of colonies on ampicillin. They should encourage the students to hypothesize as to why their colonies are not glowing, as was seen in the original colonies they used to extract DNA (Figure 3A), and provide guidance as to how they can test each hypothesis. This is a crucial step.
that builds the next set of experiments conducted by the students. Table 1 lists potential hypotheses students may propose. Hypothesis 5 requires students to have more background information, but the others are generally proposed by students (Table 1). It is essential that the instructor guide the students into formulating their thoughts into hypotheses.

Table 1. Student hypothesis—five common hypotheses proposed by students.

| Hypothesis | How to Test Hypothesis (Teacher Guided) |
|------------|----------------------------------------|
| GFP gene is mutated | PCR/DNA sequence |
| GFP gene is not in plasmid | PCR/gel electrophoresis |
| Plasmid/DNA is not present | Can be eliminated—ampicillin selection was seen |
| GFP gene is not expressed | Look for presence of mRNA (northern blot, Q-PCR) |
| Protein is not produced or is improperly folded | Protein extraction/SDS-PAGE |

○ DNA Analysis: PCR & Gel Electrophoresis

To test Hypotheses 1 and 2 in Table 1, the instructor guides students to utilize PCR to look for the presence of GFP. The PCR product can then be sequenced and analyzed for mutations. If the students do not have any previous PCR experience, the instructor should dedicate time to give an overview of the technique. There are many animations to help students understand the process, including the DNA Learning Center PCR Animation on YouTube (https://www.youtube.com/watch?v=JRAA4C2OPwQ).

To set up the PCR reactions, students can use any scientific supply company's master mix; we used Promega GoTaq, which contains the Taq polymerase, dNTPs, Mg²⁺, and buffers to simulate the cellular conditions. Each reaction will need forward and reverse primers (GFP Forward: 5’TCCCATACCCGTTTTTTTG3’ and GFP Reverse: 5’CGTTTTTATCAGACCGCCTTC3’) with nuclease-free water making up the remaining volume. A small dab of an E. coli colony will be used for the students' template DNA. PCR cycles for amplification of GFP are as follows:

One cycle of:
- 94°C for 5 minutes (initial denaturation and E. coli cell disruption)

30 cycles of:
- 94°C for 30 seconds (denaturation of DNA)
- 55°C for 45 seconds (primer annealing)
- 72°C for 1.5 minutes (DNA extension)

One cycle of:
- 72°C for 7 minutes (final extension)

Students will also need to run positive and negative controls to compare their PCR product. A small volume (0.5 μl) of plasmid DNA will be added for the positive control, and either 0.5 μl of water or nothing will be added as the negative control. We have found that a typical class time best allows student groups to set up two reactions; all the groups run a PCR of their nonglowing colony, one group can amplify a positive control, another can amplify the negative control, and two groups can view their results together on an electrophoresis gel. Alternatively, with longer class periods, teachers can choose to have each group run both positive and negative controls. An example of PCR results can be seen in Figure 4.

PCR products are analyzed by gel electrophoresis and compared to a molecular weight (MW) marker (Invitrogen 1 Kb Plus). If there is a band corresponding to the MW of GFP (~800 bp), then GFP was amplified and can be sequenced (Figure 4). At the end of the DNA analysis, students will have experience in molecular techniques, pipetting, DNA extraction, transformation, PCR, and gel electrophoresis. While students are waiting for the sequencing results, another hypothesis can be investigated. Most high schools do not have the ability to conduct a northern blot or have access to a Q-PCR, potentially making it difficult to test Hypothesis 4, though gene expression will be revisited at the end of the lesson. As an alternative, students can focus on testing for the presence and structure of the protein.

Figure 4. Gel electrophoresis of PCR of GFP, 0.8% agarose in TAE, stained with methylene blue.
Protein Analysis: Protein Extraction, Bradford Assay, & SDS-PAGE

To analyze GFP, students can extract cellular proteins from both the original glowing and nonglowing *E. coli* (Figure 1). We will refer to the glowing sample as green and the nonglowing as white. Students should collect half the colonies on each plate with a 10μl loop and resuspend the bacteria colonies in a microcentrifuge tube containing 500μl of LB broth by twirling the loop in the broth. Centrifuge the samples, discard the supernatant, and resuspend the bacteria pellet in 400μl of Camilo buffer. Aliquot half of each sample to a new tube and heat at 95°C for 5 minutes to ensure complete denaturation of the proteins. At this point, each group should have four samples, green heated (G+), green unheated (G-), white heated (W+), and white unheated (W-). An introduction to protein folding and protein structure can be used to illustrate the denaturation process expected with high temperatures (https://youtu.be/8k6D8ajTRlc). Using a Bradford assay, students will determine the protein concentration of each sample. They will make the appropriate dilutions with Laemml buffer for a final concentration of 0.5 mg/ml of protein in a final volume of 0.1 ml.

Protein analysis is conducted with SDS-PAGE, which separates proteins by size; this is based on an adaptation of “Biotechnology Explorer Protein Electrophoresis of GFP” from Bio-Rad Laboratories. Once the samples have migrated into the gel and the dye front is at the bottom, observation of the gel cassette with UV light will allow students to see one green, structurally functional GFP band glowing at a size of approximately 50 kD (Figure 5B). We recommend using a fluorescent marker, such as BioRad's Precision Plus Protein Kaleidoscope (product 1610375), to assess the size of the glowing band. Students should see the difference between green heated and unheated samples. The instructor can help students interpret that in order to have the functional protein it needs to be folded properly. Students will also see that properly folded, functional, glowing GFP proteins are not visible in the white colony samples.

In order to address whether nonfunctional GFP is present in the students’ white colonies, stain the gel with Coomassie blue and visualize the protein profiles of these samples. An example of the Coomassie stained gel can be seen in Figure 5A. Comparing G+ and G-, we can see the contrast in that G+ has abundant protein at ~27 kD (marked with a white arrow), which is interpreted to be the denatured variant of GFP. Folded and denatured proteins can migrate at vastly different “sizes” in the gel, hence the size difference. The predicted size of GFP monomer is 27 kD. Additionally, the functional glowing protein may be migrating as a dimer. The denatured variant of GFP is absent in the white colony samples (W+ and W-). The G-lane shows abundant protein (marked with the red arrow) at the same size as the fluorescent band, which is also absent in the W- or W+. Students may notice that other proteins are expressed in both white colony samples that are not seen in the GFP expressing samples. This is not uncommon, since the expression of GFP is taking up much of the cells resources and therefore they are not able to express their normal abundance of other proteins. These, however, are seen in the white colonies where resources are not being taxed by the overexpression of a nonessential protein. After finishing the protein analysis, students should conclude that the GFP wasn’t being produced by their nonglowing, transformed colonies.

Sequence Analysis of GFP: NCBI/BLAST

Instructors can find the document with DNA sequence information on the BIOTECH Project website. We recommend doing the protein analysis before the sequence analysis. Returning to the hypothesis of whether the GFP gene was mutated, students will analyze the sequence of the PCR product. Students are introduced to the NCBI website, specifically the BLAST tool. In this program, the sequence query (PCR product) will be compared to sequences in the database to find similarities. In the BLAST results, an option of “cloning vector pBAD-GFPuv, complete sequence” will display. The query alignment to the subject (pBAD-GFPuv Accession U62637) will show no apparent mutations, thus refuting the first hypothesis (Table 1). Looking at the sequence entry of the entire plasmid sequence, three genes are features in this plasmid: araC, gfpuv, and bla. Further investigation of the genes allows students to determine that bla provides ampicillin resistance and that araC encodes for the araC protein. At this point, students should question the purpose of araC. Students can Google araC, leading them to the AraC Wikipedia page (https://en.wikipedia.org/wiki/AraC). The information on this page indicates that araC is a component of the L-arabinose operon in *E. coli*. Further investigation of the L-arabinose link will allow the students to elucidate that arabinose is necessary to activate the genes of the L-arabinose operon. The instructor helps the students identify that AraC acts as a repressor on the promoter of these genes by blocking the DNA binding site for RNA polymerase, thus blocking the expression of the BAD genes (genes on the L-arabinose operon). In the presence of arabinose, AraC protein is altered, allowing the binding of RNA polymerase and expression of the BAD genes. AP biology classes will be able to relate this to Lac-operon gene expression.

The students should hypothesize that pBAD-GFPuv is using this regulated promoter for GFP expression and, if so, that the addition of arabinose to these cells will allow GFP to be expressed and the colonies to glow. By discovering the function of AraC on BAD promoter expression, they are tying together the concepts of the Central Dogma. The instructor provides arabinose to be added to their transformed nonglowing plates (which have been stored at 4°C to avoid overgrowth of colonies). After incubation at 37°C overnight, students can see glowing colonies. This reinforces that DNA is the
instruction manual that has the information. To produce a protein, DNA must transcribe its information in RNA so that the translation to amino acids (the language of proteins) can occur.

**Conclusion**

This lab project aims to explain gene expression and how information transfers within cells. The most important takeaway from this project is the Central Dogma of molecular biology, in a way that students can experience through an inquiry-based project rather than lecture-based instruction. Additionally, protein folding/denaturation and protein function were addressed. Using heat to alter the shape of the protein, rendering it nonfunctional, students can learn how important protein structure is for proper function.

The role of the instructor is to ignite curiosity and engagement in the students, to guide in students' development of hypotheses and apply the scientific method. This inquiry-based project will promote critical thinking to an abstract concept—the Central Dogma.

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CYNTHIA BUJANDA is a designated campus colleague in the Department of Molecular and Cellular Biology at the University of Arizona and a high school teacher at Sunnyside High School; e-mail: cindybjd@email.arizona.edu. NADJA ANDERSON is the director of the Biotech Project in the Department of Molecular and Cellular Biology at the University of Arizona; e-mail: nadja@bio5.org.
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