AMERICAN SOCIETY FOR MICROBIOLOGY | JMBE Journal of Microbiology & Biology Education

Curriculum

Changing colors and understanding: the use of mutant chromogenic protein and informational suppressor strains of *Escherichia coli* to explore the central dogma of molecular biology

Sarah DeWolf,[1] Maartje Van den Bogaard,[2] Rachael Brady Hart,[3] Sparrow Hartman,[4] Nancy Boury,[2] Gregory J. Phillips[1]

AUTHOR AFFILIATIONS See affiliation list on p. 9.

ABSTRACT The central dogma of molecular biology is a key concept for undergraduate students in the life sciences as it describes the flow of information in living systems from gene-to-gene product. However, despite often being covered in many introductory life science courses, students may still have misconceptions surrounding the central dogma even as they move on to advanced courses. Active learning strategies such as laboratory activities can be useful in addressing such misconceptions. In the laboratory exercise presented here, senior undergraduate students explore the intricacies of nonsense suppressor mutations to challenge their understanding of the central dogma. The students introduce a plasmid carrying a nonfunctional chromogenic protein gene due to a nonsense mutation in a codon encoding the chromophore to various nonsense suppressor strains of *Escherichia coli*. Students then observe distinct chromogenic phenotypes, depending on the suppressor strain. Students showed a moderate increase in understanding of the central dogma. While the central dogma remains a challenging concept, active learning strategies like the one presented here can help reduce conceptual errors.

KEYWORDS genetics instruction, mutations, central dogma, chromogenic bacteria

Fundamental to the field of molecular genetics is the central dogma (CD) of molecular biology: DNA stores and transmits information, which is transiently copied into RNA intermediates and in turn used to the direct synthesis of proteins that carry out essential cellular functions (1). The ability to understand and apply the CD is a core concept of Vision and Change (2, 3). It is also central to the American Society for Microbiology (ASM) curriculum guidelines pertaining to information flow (ASM, 2014). Unfortunately, despite being a threshold concept of molecular biology, the CD proves to be a very challenging subject for many students in biology (1, 4–6). Duncan and Reiser observed that the CD is a multilevel phenomenon and cite studies that identify that the CD requires "*the need to reason across multiple organization levels from the molecular to the macro scale (page 951)*." Duncan and Reiser (4) add another difficulty: genetic phenomena are a hybrid hierarchical system that contains both the information and the physical level. Students often miss or cannot apply the knowledge of the mechanisms that connect the information level with the physical one and vice versa. Briggs et al. (7) found in a study into misconceptions in microbiology that the CD is both common and persistently difficult for students to fully comprehend. For example, they found that confusion about the role of RNA in DNA replication and transcription is one of the most common misconceptions in students taking introductory microbiology courses. Newman et al. (1) created and validated the central dogma concept inventory (CDCI) that contains

Editor David R. Wessner, Davidson College, Davidson, North Carolina, USA

Address correspondence to Nancy Boury, nan1@iastate.edu, or Gregory J. Phillips, Greg.Phillips@uga.edu.

Sarah DeWolf and Maartje Van den Bogaard contributed equally to this article. Author order was determined by time spent teaching this lesson.

The authors declare no conflict of interest.

Received 13 July 2023
Accepted 22 September 2023
Published 25 October 2023

questions on the levels of the hybrid system and the mechanisms that link them. To build the assessment, they tested the instrument with early and advanced biology students and found that the early students consistently scored low, yet in a pre-/post-test setup in an advanced molecular biology class that had a particular focus on the CD, students scored significantly higher after the class than before.

Coley and Tanner (8) and Stern and Kampourakis (9) assert that humans use intuitive conceptual systems to reason about the world around them. These preconceptions start developing at an early age and continue to exist in adults, even after years of scientific training (8–11). Not all intuitive preconceptions are wrong, yet when they are wrong or scientifically illegitimate, they are called misconceptions (9). As they progress through their program of study, students in biology become less receptive to intuitive reasoning (12). Giving students the correct, factual information on biological processes is not helpful in addressing these misconceptions (13, 14). Exercises designed to help students identify and destabilize the intuitive thinking that supports their misconceptions, for instance, through having students verbalize their reasoning and assess it for elements of intuitive reasoning, are effective in some classrooms (15–17). Duncan and Reiser (4) suggest addressing the mechanisms that connect the different levels of the CD hierarchical system specifically.

There have been many efforts to create stimulating learning environments and materials to support students in learning the CD to date (6, 18–22). One of the most powerful active learning strategies is the experimental module, where students actively develop hypotheses, generate data, and draw conclusions informed by evidence (23). The laboratory has always been a popular environment to teach scientific concepts, yet not all lab experiences are effective. For example, traditional "cookbook" labs are highly structured and focus on the verification of concepts. They leave little space for student agency. Solutions are right or wrong with nothing in between, and these labs lack the authenticity of real lab settings, which are ill-structured and where many experiments fail (24). Holmes argues that effective labs do not need infinite student agency or need always to be as ill-structured as possible; however, there needs to be space for students to be engaged in authentic activities that grant them some form of agency and are not focused on right or wrong outcomes. Instead, the labs should focus on the process of inquiry (25–28). Bachhawat et al. (29) experimented with organizing biology labs "the wrong way." In their setup, students worked in groups to complete variations on the same experiment to understand the impact of different variables on the experimental design and results. They concluded that this approach is highly effective in terms of the depth of student understanding but at the expense of the breadth of topics discussed in a given lab course.

This laboratory exercise focuses on the role of translation in the CD. Students completing this exercise used amber nonsense suppressor strains to change the visual phenotype of chromogenic protein (CP)-expressing bacteria. We use a gene encoding CP from coral *Acropora millepora* (Genebank accession # AY646075) that when expressed in *Escherichia coli* imparts a purple color to the colonies (30, 31). To better demonstrate the role of translation in gene expression, we constructed a variant of the CP gene by introducing an amber nonsense mutation (TAG) into the CP gene (CP_{am}) at the position encoding the chromophore (glutamine at amino acid 62) of the CP protein. When expressed from a recombinant plasmid in wild-type (WT) *E. coli,* i.e., lacking suppressor mutations, CP_{am} yields a nonfunctional CP due to the early termination of translation at the amber codon. *E. coli* expressing the loss of function CP_{am} are readily identified by the loss of purple colony color. The plasmid carrying the wild-type CP is named pRCP (Addgene 195862), and the plasmid with the amber mutation is called pRCPam (Addgene 195861).

In this experiment, students transform both WT *E. coli* and nonsense suppressor strains with pRCPam. The nonsense suppressor strains each carry a mutation in tRNA genes corresponding to the anticodon region of different tRNAs, making the anticodon complementary to the amber stop codon and allowing translation to continue (Fig. 1)

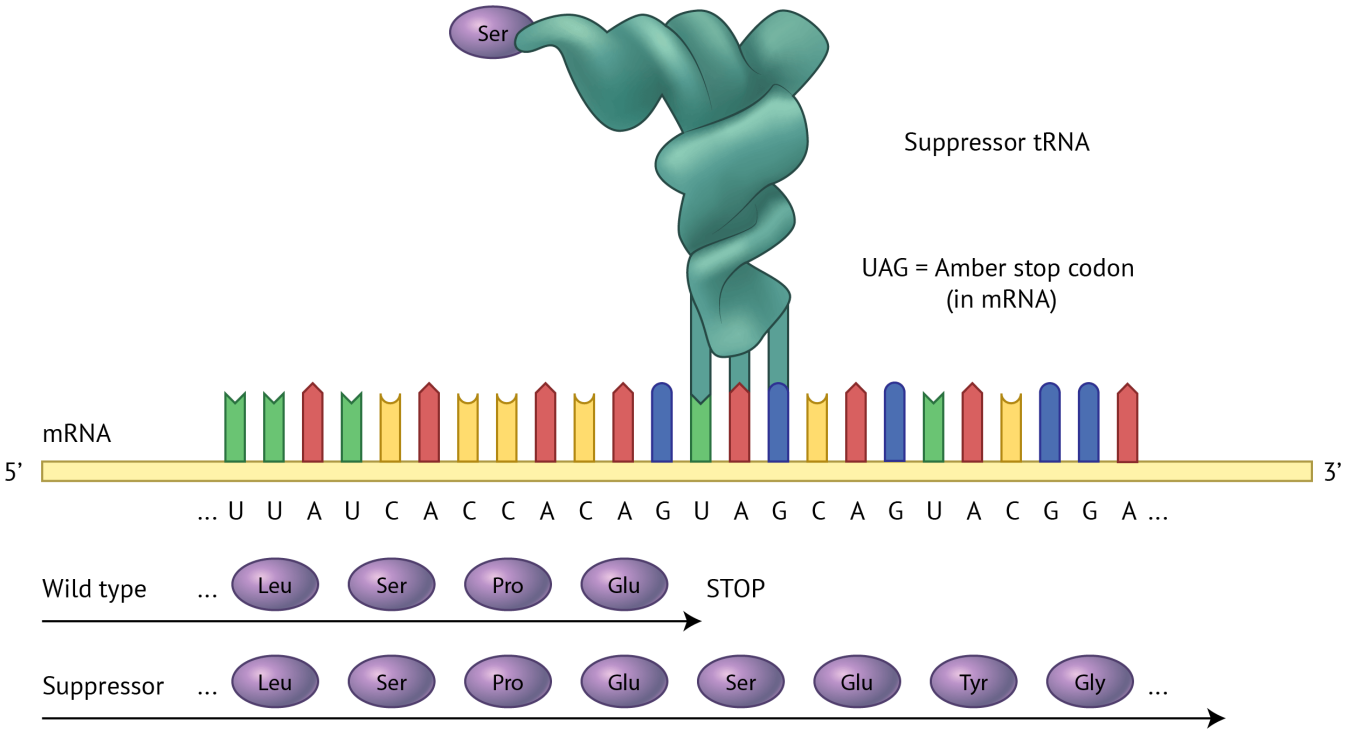

FIG 1 Suppressor tRNA strains alter the phenotype without altering the genotype of the chromogenic protein amber mutant gene. When plasmids carrying the gene encoding chromogenic protein (CP) with an amber mutation are transformed into wild-type *E. coli* strains, no change in colony color is observed. The nonsense mutation causes translation to stop prematurely, leading to the production of a truncated and nonfunctional protein. When the same plasmids are transformed into amber suppressor strains, the amber stop codon (UAG) is read as a sense codon allowing translation to continue. Different suppressor strains insert different amino acids at the location specified by the amber mutation. A serine suppressor tRNA is shown.

(32, 33). Since each strain has a mutation in a different tRNA gene, a distinct amino acid is inserted into the CP chromophore in each suppressor strain. As a result, different chromogenic phenotypes can be observed in the different transformants (Fig. 2). The resulting changes in *phenotype* without a change in CP gene *genotype* challenge students' understanding of information flow in living systems as they make predictions as to the results of their transformation experiment. The resolution of this cognitive dissonance requires a deeper understanding of transcription and translation, which helps students clarify their thinking about the central dogma to address misconceptions they still might have in their senior year. The guiding questions (Appendix 1) that students use to complete the lab report for this project require students to create a model of how transcription and translation work in coordination in WT strains and to evaluate their data with the suppressor strains against the results in the WT strain. Sample results (Appendix 5) illustrate how students are able to understand and communicate this complex phenomenon.

Intended audience

The lab we present in this contribution is part of a capstone lab course in microbiology focused on the CD and is intended for senior students in the life sciences and veterinary science. Students in general genetics or cell biology labs could also benefit from this exercise.

Learning time

The experiment is set up for students to be in the lab for three lab periods. Before the lab, students read through the instruction worksheet and complete the modified prelab CD concept inventory (1) and a prelab quiz (Appendix 2) that is intended to verify

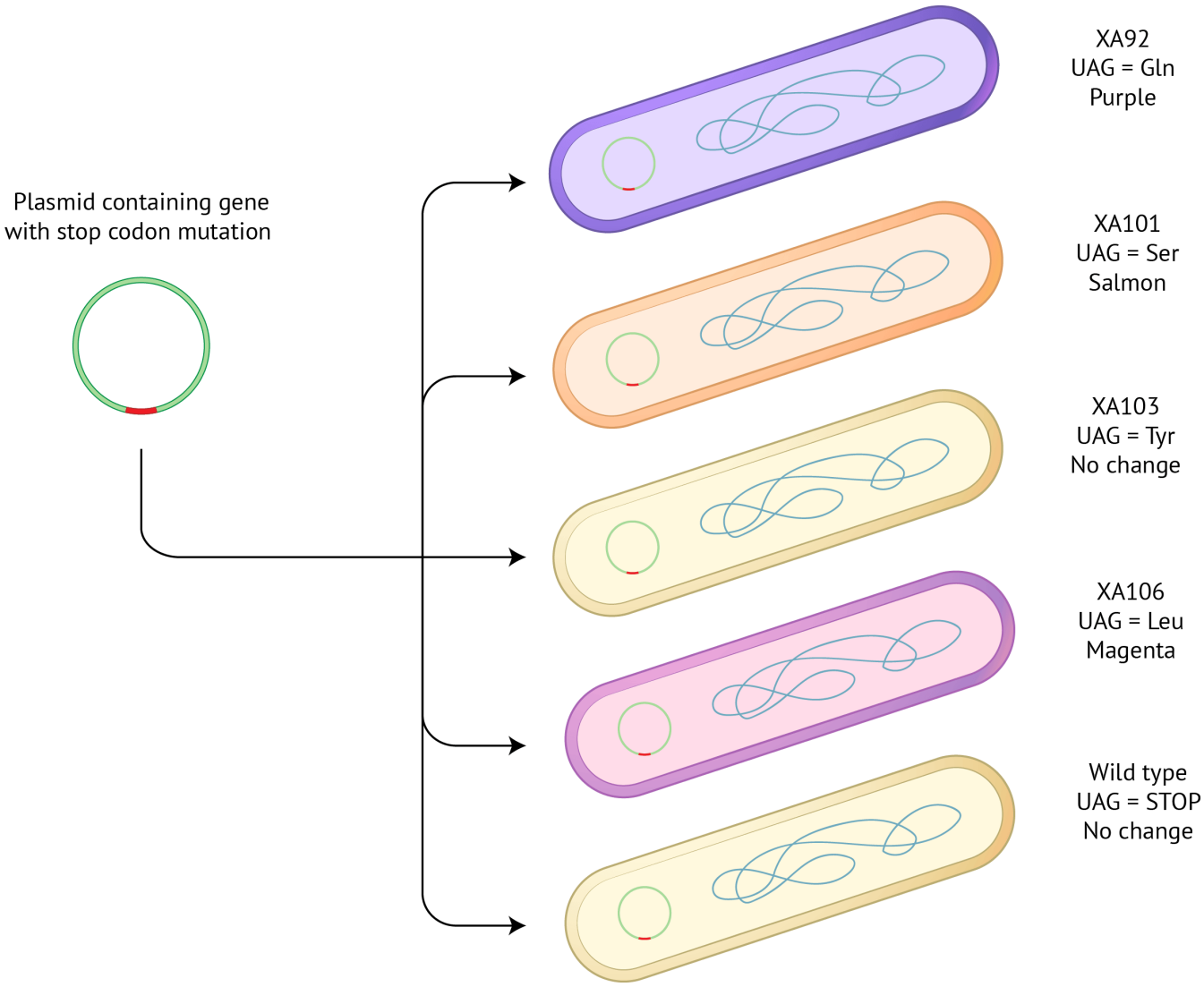

FIG 2 Different tRNA informational suppressor strains (XA92, XA101, XA103, XA106) confer different phenotypes without a change of genotype. Each of the different strains will read the UAG (stop) codon and insert a different amino acid because each of the informational suppressor strains has a different tRNA gene mutation.

that students have familiarized themselves with the goals and setup of the experiment. This way, students will be more focused on the lab activities while in the lab (34, 35). On the first day of the experiment, students spend about 2 h in the lab working to transform the WT and suppressor *E. coli* strains with the CP amber mutation plasmid. On days 2 and 3, students spend about half an hour in the lab following up on the construction of the strains and observing the phenotypic results of transformation into different strains and effects of the inducer, rhamnose, on CP expression. Students are expected to record their observations carefully and write a lab report, reflecting on their experiences and observations, and contemplate the reflective questions on the process of construction and the process of scientific inquiry that are included in the student worksheet (Appendix 1). After the lab, the students complete a modified postlab CD concept inventory.

Prerequisite student knowledge

This lab is intended for senior undergraduate students in microbiology, so students should have previously completed classes in introductory chemistry and microbiology or molecular biology, which would provide them with background on gene expression (replication, transcription, and translation). Students should be familiar with sterile techniques and basic bacteriology techniques such as streak plating.

Student learning objectives for the lab

1. Students will be able to identify the unique features and cellular components of translation that differentiate it from transcription and replication.
2. Students will be able to differentiate between informational, intragenic, and intergenic suppression mechanisms.
3. Students will be able to evaluate their data and use it to explain how informational suppression changes the phenotype a mutated CP gene confers without changing its genotype.

These learning objectives are aligned with the ASM core concept of Information Flow and Genetics (36) and the Genetics Society of America (GSA)'s education framework (37).

PROCEDURE

Materials

Student worksheet (Appendix 1): The student worksheet contains a description of how the CD works on the level that we address in this lab. It contains all lab instructions and reflective questions that aim to support students in reflecting on the process of bacterial growth and gene expression and the process of scientific inquiry. Students use these questions as a scaffold for the lab report associated with this assignment.

Modified pre-/postlab CD concept inventory: This assessment is based on the CDCI and modified by eliminating questions focused on eukaryotic processes and adding questions probing student understanding of mutations.

Pre-lab quiz with answer key (Appendix 2): This quiz is designed to help students focus on the core concepts that underlie the lab.

Instructions and assessment rubric for lab report (Appendix 3)

Instructions for lab setup (Appendix 4): This document contains the instructions for the technical staff that prepares the labs and the equipment for the students.

Samples of student lab reports (Appendix 5): This appendix summarizes the student work documenting the results of their experiments and the students' discussion of their findings.

Student instructions

Before beginning this lab, students are responsible for reading the worksheet, taking a prelab quiz, drawing a flowchart of the procedure, and writing a brief introduction in their laboratory notebooks about the purpose of this activity. The students also completed a modified prelab CD concept inventory (1). On the first day of lab, students transform four nonsense suppressor mutant strains and one WT control strain with a plasmid carrying a CP gene with an amber nonsense mutation that alters the mRNA codon specifying the amino acid glutamine at position 62 of the chromogenic protein (pRCPam, Addgene 195861). They then plate the bacteria on LB containing chloramphenicol (Cam) to select for transformants. In the following lab period, students observe their transformation plates and pick single colonies from each plate. Bacteria from these isolated colonies are streaked onto fresh LB agar plates containing Cam with rhamnose added to induce expression of the CP gene. On the third day of lab, students examine their plates and document the new chromogenic phenotypes observed with

the different nonsense suppressor strains. Throughout the activity, students use iPads to take pictures of results and record observations in Microsoft OneNote, their digital lab notebook. After, the lab students write a lab report that is assessed with a rubric (Appendix 3). They also completed the modified postlab CD concept inventory.

Faculty instructions

Prior to the first day of this lab activity, each *E. coli* strain will need to be made competent as described in Appendix 4. Faculty or lab staff should prepare enough competent cells of each suppressor strain for each student to do a single transformation of each strain, including the wild-type (control) strain. Alternatively, students can prepare their own competent cells of each strain using standard methods. Suppressor strains are available through the Coli Genetic Stock Center (CGSC) (Table 1). Additionally, pRCPam will need to be mini or midi prepped prior to the first day of lab; 1 µL of ~1–100 ng/µL plasmid will be needed per transformation, so if students are working individually, 5 µL will be needed per student to transform the four suppressor strains and one WT control strain. The plasmid sequence is available on Addgene (195861). Finally, instructors should make the lab instructions (Appendix 1) available to students, either as a handout or available online. The prelab quiz (Appendix 2) that students complete before the first day of lab can be used to encourage students to read the instructions before the activity begins.

While the student handout includes a thorough explanation of nonsense suppressors, we find that giving a short prelab talk to review the background and the lab procedure can be helpful. Depending on how much time is available, this lecture could take place on the first or second day of the lab activity. If lecturing on the second day, this discussion should include the instructor showing students the clean negative control plates, i.e., no growth on the LB +Cam plates. This control represents competent cells where no plasmid DNA was added to the transformation reactions and can be prepared either with the students on day 1 or outside of class time. Faculty should arrange for any students who had unsuccessful transformations to take colonies from other students' plates, as they only need one colony per transformation. Additionally, on the third day, faculty can show students a plate displaying the different chromogenic phenotypes. This could include showing the class an additional control, the WT CSH108 strain transformed with a plasmid carrying the WT CP gene without an amber mutation, pRCP (Addgene 195862). The instructor then asks questions to guide a discussion about how the different amino acids inserted into the CP protein at position 62 by way of the nonsense suppressor mutations are affecting what they see on their plates.

We use this lab lesson with students in the context of a larger module focused on genetics, and as such, our students have already been exposed to techniques such as transformation and streak plating. If students are unfamiliar with these techniques, instructors should demonstrate these and other sterile techniques to the students.

If possible, instructors should allow for several days' time between the second and third days of this lab, and they should transfer the students' plates from 37°C to room temperature after about 24 h, as the color of the colonies will grow more striking and distinct the longer the plates are stored at room temperature. It can also be helpful for

TABLE 1 List of bacterial strains[a]

Strain	CGSC#	Suppressor gene mutation
XA92	7895	*glnX*
XA101	7898	*serU*
XA103	7899	*tyrT*
XA106	7896	*leuX*
CSH108	8081	None

[a]Bacterial strains used in suppressor experiment with Yale University Coli Genetic Stock Center (CGSC) ID numbers. Note that in our experiments, we used CSH130 (Miller, 1992) as our wild-type control strain rather than CSH108. CSH130 is not available through the CGSC, and we recommend CSH108 as an alternative. The nonsense suppressor strains as well as CSH130 are also available pretransformed with pRCPam through Addgene (195855-195859).

students to seal their plates with parafilm on the second day to prevent the media from drying out during extended incubation.

Suggestions for determining student learning

Data from the prelab quiz (Appendix 2) can be used to adjust the discussion questions during each lab session. Instructors can add discussion questions and think–pair–share activities to elaborate on the details of those topics that students did not fully understand by reading over the lab instructions (Appendix 1).

The reflection questions at the end of the lab instructions (Appendix 1) are designed to guide students as they complete their lab reports. These reports are formatted as a results and discussion section of a primary paper, written in ASM format. We provide a detailed rubric (Appendix 3) with the lab report instructions. The writing assignment is a summative assessment of students' ability to analyze and interpret their data in the context of current literature. The rubric also emphasizes the communication of data in tables and figures and following ASM format guidelines.

For quantitative data that measures student understanding of the central dogma of molecular biology, we used a modified version of the Central Dogma Concept inventory (1).

Sample data

The lab reports that the students produced after completing this activity required that the students create a results and discussion section in ASM format. The papers often included results sections that described the different phenotypes and linked these phenotypes to the different amino acid substitutions and the induction of the colored phenotypes with rhamnose. For the discussion, the students used the questions at the end of their lab protocol (Appendix 1) to describe the exact mutation (amino acid 62), diagram the effect of nonsense suppressor tRNAs on the CP protein, and differentiate between suppression (intergenic and intragenic) and reversion. In short, most lab reports connected the molecular events during translation to the alterations in phenotype (Appendix 5).

Safety issues

All bacterial strains used are derivatives of K-12 *E. coli* and can be handled under BSL1 biosafety conditions. During the course of the experiment, the use of aseptic technique with proper PPE is reinforced. The use of Bunsen burners presents a fire hazard, so many labs have moved to the use of bench-top incinerators. Iowa State University's Institutional Biosafety Committee has reviewed and approved these lab procedures in terms of the creation of recombinant organisms and the safe use of the bacterial strains in a teaching lab (IBC #18–181)

DISCUSSION

Field testing

This experiment was completed by senior undergraduate students majoring in microbiology across four semesters at an R1 public university in laboratories of about 16 students each semester.

Evidence of student learning from field testing

Prior to the lab and after finishing the lab, students were asked to do a pre- and post-test with 16 questions based on the CD concept inventory (1). The data collection process was reviewed by Iowa State University's Institutional Review Board and was determined to be classroom data, therefore exempt (IRB # 17–460). In the CDCI, students are given three to five statements, where multiple statements can be correct. We removed the questions focused on eukaryotic genetic processes and added three questions on

information suppressors specifically probing student understanding of the lab exercise. The maximum score a student could obtain by selecting the correct statements and not selecting any distractors was 67. Concept inventories may not be the ideal instrument to assess learning in a single course, as the concept inventories tend to have a high level of abstraction, and this tends to lead to low student scores (38). However, concept inventories are used to assess student learning with new or novel pedagogies, as they are standardized instruments that have been tested for reliability and validity (39). We collected data on the cohorts of Fall 2019, Spring 2020, Spring 2021, Fall 2021, and Spring 2022. The data for Spring 2021 was incomplete and could not be included in the analysis. The Kolmogorov–Smirnov test showed that the data were not normally distributed; hence, we ran a nonparametric two-tailed Wilcoxon signed-rank test to test for differences between the pre- and post-test scores. In Table 2, we show the results.

The results show an overall significant increase in scores on the posttest, with the median score increasing by three more correct answers on the post-test. We also calculated the normalized learning gain (NLG), which is a rough measure of learning often used in the sciences as it is a standardized measure that compares how much was learned with how much students could have learned (40). It is a percentage measure of learning that can range from –1 to 1 (41). The NLG is 0.14 or 14%. Nissen et al. report that the NLG tends to have a bias toward populations that have higher pretest scores, is predisposed to not having any losses in the post-test, and tends to get distorted by the distribution of the data. McKagan et al. (41) argue that effect size accounts for the size of the class and for the variation in students within the class, while the NLG does not. This makes effect size a more sensitive measure, especially in small or diverse classes such as ours. The effect size of 0.33 is indicative of a small to medium effect (42) and a medium-sized effect for educational outcomes (43). We see some loss on questions 9 and 13, yet we see increases on scores of all other questions and a significant difference overall between the pre- and post-test. During the lab, most students were able to explain the concepts relevant to the topic. This may indicate that students mix up details, yet overall, they may have a good understanding of these topics. This underlines the importance of continuing to focus on these topics and having students verbalize their understanding of the topics during the labs. These findings show that the central dogma continues to be a difficult topic for students, yet learning activities such as the one presented in this work make an important contribution to students' understanding of this fundamental concept of molecular biology.

Possible adjustments

If time is short, instructors can also do the transformations into the different informational suppressor strains as part of lab preparation, or they can obtain the WT strain and four suppressor strains pretransformed with pRCPam through Addgene (195855–195859). In this case, instructors should make it clear to students that the strains are all transformed with the same plasmid; an advantage of having the students perform the transformations themselves is for them to see that the same plasmid was used but each strain produces different phenotypes.

There are several additional genetics concepts that could also be illustrated using this system. If the class is investigating the regulation of gene expression, instructors could provide students with wild-type *E. coli* pretransformed with pRCP (Addgene 195862), a WT CP plasmid that does not carry the amber mutation, for students to culture on agar plates with and without rhamnose. Students could then design an experiment

TABLE 2 Results of Wilcoxon signed-rank test for the Fall 2019, Spring 2020, Fall 2021, and Spring 2022 cohorts on the pre- and post test[a]

	Mean	SD	Median	*z*-value	*P*	*r*	NLG
Total pre	50.0	6.6	50	-3.67	0.000	0.33	0.14
Total post	52.2	7.2	53				

[a]*N*=–55, the maximum score on the concept inventory is 67. *r* is the effect size, which was calculated as $r = Z/(n_x + n_y)$ (Field, 2013).

to determine the concentration of rhamnose needed to induce CP gene expression or investigate the ability of other sugars to induce CP gene expression.

Instructors can also have students construct foldable paper models of green fluorescent protein (GFP) to emphasize the connection between structure and function in these molecules, as GFP is largely homologous to CP (31). These paper models are available through the Research Collaboratory for Structural Bioinformatics Protein Data Bank at pdb101.rcsb.org. Students can take pictures of their completed models to refer to in their final reports.

ACKNOWLEDGMENTS

We would like to thank the students from Micro 440 for providing constructive feedback about this exercise and the teaching and learning assistants who have helped streamline the lab prep.

This material is based upon work supported by the National Institute of Food and Agriculture, USDA, Hatch project 242IOW04208, #14647, and a Miller Faculty Fellowship Award.

AUTHOR AFFILIATIONS

[1]Department of Veterinary Microbiology and Preventative Medicine, Iowa State University, Ames, Iowa, USA
[2]Department of Plant Pathology, Entomology, and Microbiology, Iowa State University, Ames, Iowa, USA
[3]Undergraduate Program in Genetics, Iowa State University, Ames, Iowa, USA
[4]Undergraduate Program in Biological and Premedical Illustration, Iowa State University, Ames, Iowa, USA

AUTHOR ORCIDs

Nancy Boury http://orcid.org/0000-0003-1438-8299
Gregory J. Phillips http://orcid.org/0000-0002-0048-512X

AUTHOR CONTRIBUTIONS

Sarah DeWolf, Data curation, Project administration, Visualization, Writing – original draft | Maartje Van den Bogaard, Formal analysis, Methodology, Validation, Writing – original draft | Rachael Brady Hart, Investigation, Methodology, Project administration, Supervision | Sparrow Hartman, Resources, Software, Visualization | Nancy Boury, Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review and editing | Gregory J. Phillips, Conceptualization, Methodology, Project administration, Supervision, Writing – review and editing

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Appendices 1 through 5 (jmbe00094-23-s0001.pdf). Appendix 1 (student instructions), Appendix 2 (prelab quiz with answer key), Appendix 3 (assessment rubric for lab reports), Appendix 4 (instructions for lab set up), and Appendix 5 (summary of student lab reports).

REFERENCES

1. Newman DL, Snyder CW, Fisk JN, Wright LK. 2016. Development of the central dogma concept inventory (CDCI) assessment tool. CBE Life Sci Educ 15:ar9. https://doi.org/10.1187/cbe.15-06-0124
2. Honey M, Alberts B, Bass H, Castillo C, Lee O, Strutches M 2020. STEM education for the future: a visioning report. Tech Rep:36. https://www.

nsf.gov/edu/Materials/STEM%20Education%20for%20the%20Future%20-%202020%20Visioning%20Report.pdf
3. McLaughlin J, Metz A. 2016. Vision & change: why it matters. Am Biol Teach 78:456–462. https://doi.org/10.1525/abt.2016.78.6.456
4. Duncan RG, Reiser BJ. 2007. Reasoning across ontologically distinct levels: students' understandings of molecular Genetics. J Res Sci Teach 44:938–959. https://doi.org/10.1002/tea.20186
5. Champagne Queloz A, Klymkowsky MW, Stern E, Hafen E, Köhler K, Hermes-Lima M. 2017. Diagnostic of students' misconceptions using the biological concepts instrument (BCI): a method for conducting an educational needs assessment. PLoS ONE 12:e0176906. https://doi.org/10.1371/journal.pone.0176906
6. Reinagel A, Bray Speth E. 2016. Beyond the central dogma: model-based learning of how genes determine phenotypes. CBE Life Sci Educ 15:ar4. https://doi.org/10.1187/cbe.15-04-0105
7. Briggs AG, Hughes LE, Brennan RE, Buchner J, Horak REA, Amburn DSK, McDonald AH, Primm TP, Smith AC, Stevens AM, Yung SB, Paustian TD. 2017. Concept inventory development reveals common student misconceptions about microbiology. J Microbiol Biol Educ 18:30. https://doi.org/10.1128/jmbe.v18i3.1319
8. Coley JD, Tanner KD. 2012. Common origins of diverse misconceptions: cognitive principles and the development of biology thinking. CBE Life Sci Educ 11:209–215. https://doi.org/10.1187/cbe.12-06-0074
9. Stern F, Kampourakis K. 2017. Teaching for genetics literacy in the post-genomic era. Stud Sci Educ 53:193–225. https://doi.org/10.1080/03057267.2017.1392731
10. Kelemen D, Rosset E. 2009. The human function compunction: teleological explanation in adults. Cognition 111:138–143. https://doi.org/10.1016/j.cognition.2009.01.001
11. Kelemen D. 2019. The magic of mechanism: explanation-based instruction on counterintuitive concepts in early childhood. Perspect Psychol Sci 14:510–522. https://doi.org/10.1177/1745691619827011
12. Richard M, Coley JD, Tanner KD, Sevian H. 2017. Investigating undergraduate students' use of intuitive reasoning and evolutionary knowledge in explanations of antibiotic resistance. CBE Life Sci Educ 16:ar55. https://doi.org/10.1187/cbe.16-11-0317
13. Pickett SB, Nielson C, Marshall H, Tanner KD, Coley JD. 2022. Effects of reading interventions on student understanding of and misconceptions about antibiotic resistance. J Microbiol Biol Educ 23:e00220–21. https://doi.org/10.1128/jmbe.00220-21
14. Hartelt T, Martens H, Minkley N. 2022. Teachers' ability to diagnose and deal with alternative student conceptions of evolution. Sci Educ 106:706–738. https://doi.org/10.1002/sce.21705
15. Wingert JR, Bassett GM, Terry CE, Lee J. 2022. The impact of direct challenges to student endorsement of teleological reasoning on understanding and acceptance of natural selection: an exploratory study. Evo Edu Outreach 15:4. https://doi.org/10.1186/s12052-022-00162-6
16. Ginnobili S, González Galli L, Ariza Y. 2022. Do what Darwin did: how to deal with teleological misconceptions in the classroom. Sci Educ 31:597–617. https://doi.org/10.1007/s11191-020-00186-8
17. Nehm RH, Finch SJ, Sbeglia GC. 2022. Is active learning enough? the contributions of misconception-focused instruction and active-learning dosage on student learning of evolution. BioScience 72:1105–1117. https://doi.org/10.1093/biosci/biac073
18. Tripepi M, Clear M, Bondi JL, Brunelli E, Berardi A. 2020. From gene to function: a safe laboratory exercise promoting the learning of the central dogma of biology for the undergraduate biology curriculum. J Microbiol Biol Educ 21:30. https://doi.org/10.1128/jmbe.v21i2.2131
19. Silveira LF, Xavier CS, Perez MA, Bozza DA, Furtado-Alle L, Hass I, Tureck LV. 2023. Unravelling the central dogma of biology in an active way: a case study. J Biol Educ 57:101–114. https://doi.org/10.1080/00219266.2021.1877780
20. Bujanda C, Anderson N. 2022. Teaching the central dogma through an inquiry-based project using GFP. Am Biol Teach 84:33–37. https://doi.org/10.1525/abt.2022.84.1.33
21. Prevost LB, Smith MK, Knight JK, Momsen J. 2016. Using student writing and lexical analysis to reveal student thinking about the role of stop codons in the central dogma. CBE Life Sci Educ 15:ar65. https://doi.org/10.1187/cbe.15-12-0267
22. Takemura M, Kurabayashi M. 2014. Using analogy role - play activity in an undergraduate biology classroom to show central dogma revision. Biochem Mol Biol Educ 42:351–356. https://doi.org/10.1002/bmb.20803
23. Olimpo JT, Esparza D. 2020. Active learning and conceptual understanding in biology, p 43–57. In Mintzes JJ, EM Walter (ed), Active learning in college science: the case for evidence-based practice. Springer, Cham. https://doi.org/10.1007/978-3-030-33600-4
24. Holmes NG. 2020. Why traditional labs fail… and what we can do about it, p 271–290. In Mintzes JJ, EM Walter (ed), Active learning in college science: the case for evidence-based practice. Springer, Cham. https://doi.org/10.1007/978-3-030-33600-4
25. Schussler EE, Bautista NU, Link-Pérez MA, Solomon NG, Steinly BA. 2013. Instruction matters for nature of science understanding in college biology laboratories. BioScience 63:380–389. https://doi.org/10.1525/bio.2013.63.5.11
26. Röllke K, Sellmann-Risse D, Wenzel A, Grotjohann N. 2021. Impact of inquiry-based learning in a molecular biology class on the dimensions of students' situational interest. Int J Sci Educ 43:2843–2865. https://doi.org/10.1080/09500693.2021.1993377
27. Deacon C, Hajek A. 2011. Student perceptions of the value of physics laboratories. Int J Sci Educ 33:943–977. https://doi.org/10.1080/09500693.2010.481682
28. Brownell SE, Kloser MJ, Fukami T, Shavelson R. 2012. Undergraduate biology lab courses: comparing the impact of traditionally based "cookbook" and authentic research-based courses on student lab experiences. J Coll Sci Teach 41:36–45.
29. Bachhawat AK, Pandit SB, Banerjee I, Anand S, Sarkar R, Mrigwani A, Mishra SK. 2020. An inquiry - based approach in large undergraduate LABS: learning, by doing it the "wrong" way. Biochem Mol Biol Educ 48:227–235. https://doi.org/10.1002/bmb.21331
30. Liljeruhm J, Funk SK, Tietscher S, Edlund AD, Jamal S, Wistrand-Yuen P, Dyrhage K, Gynnå A, Ivermark K, Lövgren J, Törnblom V, Virtanen A, Lundin ER, Wistrand-Yuen E, Forster AC. 2018. Engineering a palette of eukaryotic chromoproteins for bacterial synthetic biology. J Biol Eng 12:8. https://doi.org/10.1186/s13036-018-0100-0
31. Alieva NO, Konzen KA, Field SF, Meleshkevitch EA, Hunt ME, Beltran-Ramirez V, Miller DJ, Wiedenmann J, Salih A, Matz MV, El-Shemy HA. 2008. Diversity and evolution of coral fluorescent proteins. PLoS ONE 3:e2680. https://doi.org/10.1371/journal.pone.0002680
32. Cupples CG, Miller JH. 1988. Effects of amino acid substitutions at the active site in *Escherichia coli* beta-galactosidase. Genetics 120:637–644. https://doi.org/10.1093/genetics/120.3.637
33. Porter JJ, Heil CS, Lueck JD. 2021. Therapeutic promise of engineered nonsense suppressor tRNAs. Wiley Interdiscip Rev RNA 12:e1641. https://doi.org/10.1002/wrna.1641
34. Mshayisa VV, Basitere M. 2021. Flipped laboratory classes: student performance and perceptions in undergraduate food science and technology. J Food Sci Educ 20:208–220. https://doi.org/10.1111/1541-4329.12235
35. Cann AJ. 2016. Increasing student engagement with practical classes through online pre-lab quizzes. J Biol Educ 50:101–112. https://doi.org/10.1080/00219266.2014.986182
36. Merkel S, Reynolds J, Hung K, Smith H, Siegesmund A, Smith A, Baker N, Chang A 2012. Recommended curriculum guidelines for undergraduate microbiology education. American Society for Microbiology. https://asm.org/Guideline/ASM-Curriculum-Guidelines-for-Undergraduate-Microb
37. Committee GSoAE. 2015. Genetics learning framework, on genetics society of America. Available from: http://www.genetics-gsa.org/education/GSAPREP_CoreConcepts_CoreCompetencies.shtml. Retrieved 15 Oct 2016.
38. Nissen JM, Talbot RM, Thompson AN, Dusen B. 2018. Comparison of normalized gain and Cohen's *d* for analyzing gains on concept inventories. Phys Rev Phys Educ Res 14:010115. https://doi.org/10.1103/PhysRevPhysEducRes.14.010115
39. Klymkowsky MW, Garvin-Doxas K. 2020. Concept inventories: design, application, uses, limitations, and next steps, p 775–790. In Mintzes JJ, EM Walter (ed), Active learning in college science. Springer, Cham. https://doi.org/10.1007/978-3-030-33600-4
40. Hake RR. 1998. Interactive-engagement versus traditional methods: a six-thousand-student survey of mechanics test data for introductory physics courses. Am J Phys 66:64–74. https://doi.org/10.1119/1.18809

41. McKagan S, Sayre E, Madsen A. 2016. Normalized gain: what is it and when and how should i use it. https://www.physport.org/recommendations/Entry.cfm?ID=93334.
42. Cohen J. 2013. Statistical power analysis for the behavioral sciences. Academic press. https://doi.org/10.1016/C2013-0-10517-X
43. Arnold I. 2011. John Hattie: Visible learning: a synthesis of over 800 meta-analyses relating to achievement. Int Rev Educ 57:219–221. https://doi.org/10.1007/s11159-011-9198-8