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A "Choose-Your-Own" Classroom-Based Activity That Promotes Scientific Inquiry about RNA Interference †

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RNA interference (RNAi), the process that results in the degradation of a target gene's mRNA, is a fundamental part of eukaryotic gene regulation and is also an important molecular technique that allows for experimental manipulation of gene expression without altering DNA sequences. Despite the importance of RNAi, there have been relatively few lecture-based activities designed to teach about the consequences of this process and counter common misconceptions. I present here an inquiry-based activity that is centered around a "choose your own experiment" design where students generate hypotheses and critically evaluate their ideas by choosing several simulated experiments. The activity presents students with one of the original puzzling observations, the discovery that triggering overexpression of a given gene in a flower resulted in an opposite change in phenotype than expected, and the subsequent discovery that there was a dramatic decrease of that gene's mRNA, that sparked the discovery of RNAi. Students then propose a molecular mechanism for these results before using a limited budget of funding to simulate their choice of experiments. Simulated results are provided for these experiments, and students must work together to interpret and discuss these results before deciding on the next experiment. I provide a guide for instructors on how to implement this activity, with suggestions on how to vary the activity to fit different class sizes as well as an abbreviated version for instructors who are short on time. Finally, I include an aligned assessment so that instructors may check student learning about the impacts of RNAi.

INTRODUCTION

The process of RNA interference (RNAi), which degrades target mRNA and leads to a downregulation or silencing of gene expression, is critical in eukaryotic gene regulation. The discovery of RNAi resulted in a Nobel Prize and has enabled the insertion of exogenous double-stranded RNA to trigger RNAi and knock down gene expression without altering the DNA sequence (1–3). Similarly, there has been an increasing emphasis in teaching RNAi, including incorporating RNAi into lab courses (4–6). However, there remains a paucity of lecture-based activities to teach about RNAi. Here, I present an inquiry-based activity designed to model the scientific process, where students choose different experiments, receive simulated results, and make decisions about next steps in order to differentiate between competing hypotheses. A follow-up assessment is used to counter common misconceptions about RNAi. This activity is geared toward mid-level college courses on molecular genetics, though it can easily be adapted for introductory or high school courses.

LEARNING OBJECTIVES, PREREQUISITE KNOWLEDGE, AND TARGET AUDIENCE

At the end of these activities, which are designed to fit within an 80-minute class (or across two 50-minute classes), students will be able to 1) describe the impact (or lack thereof) of RNAi on transcription, translation, and the amount of mRNA and protein expected, 2) explain when an organism might naturally induce RNAi, and 3) evaluate and predict the impact of various experimental techniques that study gene expression. This activity is designed to occur after instruction on chromatin remodeling, transcriptional control, and RNA processing. The module works best in small classes but can be adapted for larger classes. This module focuses on the consequences of RNAi and is not designed to teach about the molecular mechanisms of how RNAi works; instructors may choose to introduce the RNAi mechanism between the activity and assessment.

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PROCEDURE

Part I of the activity (see Appendix 1 for instructor guide and Appendix 2 for student handout; see Fig. 1 for flowchart of activity components) begins by introducing the main results from work published by Napoli and colleagues in 1990 prior to the discovery of RNAi (7). Napoli *et al.* attempted to overexpress the gene *chalcone synthase* (*CHS*) in petunias. They found that, instead of leading to an increase in the amount of *CHS* mRNA, the procedure resulted in a dramatic decrease in *CHS* mRNA while producing no decrease in *CHS* transcription. The authors could not provide a mechanistic molecular explanation for these results (which we now know are due to RNAi), but their results triggered the eventual discovery of this pathway. The activity—which should be done in class prior to any discussion of RNAi—begins with a brief summary of these puzzling results (excluding the finding of lowered mRNA levels) and then challenges students (working in groups) to develop possible explanations of the puzzling results. Following this, students are asked to outline an experiment to test one of their hypotheses. No prior knowledge of experimental techniques is required; instead, the activity encourages students to focus on what they would want to manipulate and measure in an experiment in order to test their hypothesis. Students are then asked to make predictions of the results if their hypothesis is either correct or incorrect. Part I of the activity thus provides opportunities for students to engage scientifically by challenging them to critically think through the actual results that sparked the discovery of RNAi. At the end of Part I, instructors should lead a class-wide discussion on the hypotheses, experiments, and predictions generated. They can then introduce Part II by highlighting a few hypotheses to continue testing. If the students have not generated a diverse set of hypotheses, the instructor may wish to introduce some, such as the (correct) hypothesis that there is a mechanism to degrade mRNA if the organism detects an overabundance of those transcripts.

The second part (See Appendix 1 for instructor guide, Appendix 2 for student handout, and Appendix 3 for corresponding experimental results) continues the scientific inquiry by providing students with possible experiments to evaluate the different hypotheses. Inspired by other "choose-your-own-experiment" case studies (e.g., [8]), I generated 13 possible experiments based on content I had previously covered in class. Instructors are encouraged to modify this list of experiments or add new experiments tailored to class content. Each experiment provides a realistic (albeit simplified) experimental manipulation or collaboration. Each group is given a limited budget of "grant funding," and each possible experiment comes with an associated cost. Instructors may also remove the grant funding aspect and instead limit students to two to three experiments if they wish to streamline the activity. Students must choose which experiment to run first; they will then receive a de-

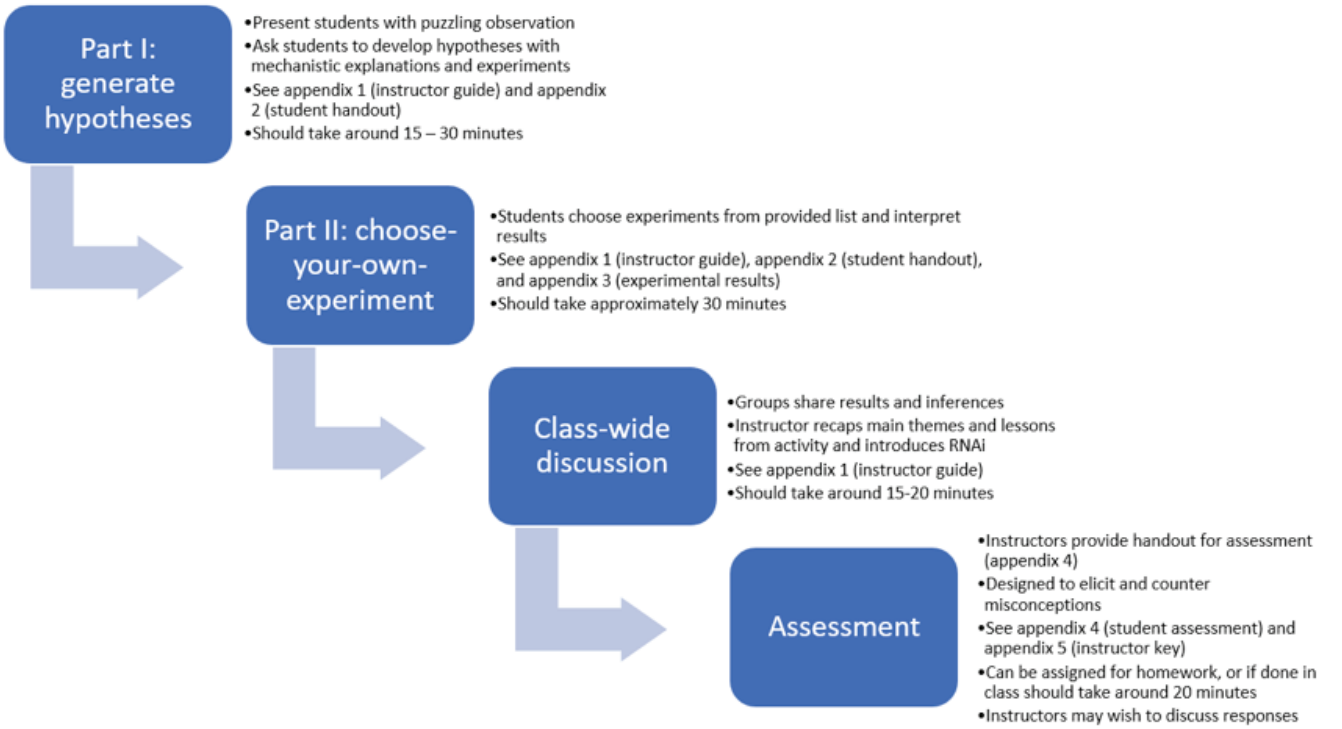

FIGURE 1. A flowchart showing the different parts of the activity, along with the estimated time required and the relevant corresponding sections of the supplement.

scription or diagram of the results. Each group must then discuss these results and decide which experiment to run next with the remaining funds. They continue doing so until they are out of funds. The instructors can then allow groups to collaborate and share results or ask groups to share any conclusions they have drawn about their hypotheses. After this discussion, the instructor can introduce the mechanisms of RNAi and highlight how the students have just critically synthesized key results based on a real paper.

I also provide an assessment (Appendices 4 and 5) that I recommend giving after teaching about the mechanisms of RNAi. The assessment is designed to measure student learning on the consequences of RNAi and counter a common student misconception, namely, that RNAi would directly impact the rate of transcription. In the assessment, students make predictions and compare the transcription rate, translation rate, and amount of mRNA of three genes. No information is provided about two of the genes (and thus no inferences can be made about their expression levels), but endogenous RNAi has been triggered for the third gene, providing insight that the transcription and mRNA levels of this gene are putatively high, thus necessitating RNAi. The activity also includes additional questions assessing understanding of the mechanisms of RNAi.

For instructors short on time, I have also provided an alternate condensed version. In this abbreviated version, Parts I and II are compressed into a think-pair-share activity based on the same scenario as outlined above, guided by instructor discussion. The assessment can still follow the abbreviated version.

CONCLUSION

I present an inquiry-based activity that simulates discovery of a mechanism to degrade mRNA. Students are challenged to think critically about the original puzzling observations, where attempted overexpression of a gene leads to decreased mRNA levels, and are then presented with a "choose-your-own-experiment" case study in which they work together to decide which experiment to run with a limited budget, interpret the results, and iterate until reaching a conclusion. I also present suggestions for how this activity—and the accompanying assessment—can be modified to fit different curricula and class sizes. This activity provides a novel and creative approach to teaching RNA interference in lecture courses.

SUPPLEMENTAL MATERIALS

Appendix 1. Instructor guide
Appendix 2. Student handout for activity
Appendix 3. Experimental results
Appendix 4. Assessment for activity
Appendix 5. Assessment key

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