ONLINE INQUIRY & INVESTIGATION

Cookie-ases:

Interactive Models for Teaching Genotype-Phenotype Relationships

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The ideas of genotype and phenotype and their intricate relationship are often initially presented simply as terms to be memorized. Many times the volume of material to be taught leaves little time to revisit their true meaning, and how an organism's genetic makeup (genotype) generates its phenotype. The idea that a gene encodes a protein establishes the fundamental relationship, but it is often presented as an idea that the student memorizes. The student may also memorize that a mutant gene can generate a mutant phenotype. Further, the student may memorize that changing the sequence of the gene changes the amino acid sequence of the protein. The extra step of melding these memorized passages into a coherent model that incorporates the connection of protein activity and phenotype is rarely achieved without additional assistance. It is truly important that students grasp these abstract concepts, because they are core ideas that are important in a number of biological disciplines, including genetics, biochemistry, molecular and cellular biology, and physiology. It is because these concepts are so abstract that an active learning approach that is visual, concrete, and includes role-playing and modeling is most likely to be effective (Anderson, 1996; Rutledge, 2001).

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Several hands-on and wet laboratory activities have been proposed to model similar genetic concepts (Lanza & Cress, 2001; Heim, 1991; Guilfoile & Plum, 2000; Pigage, 1991; Bio-Rad Laboratories pGLO Bacterial Transformation Kit). The exercise presented here is a novel, time effective, student-centered, role-playing activity in which students learn about the intricate connection between genotype and phenotype by exploring the fundamental effect of mutation on protein function beginning with a very real and human phenotype, albinism. This exercise is based on a long established role-playing model of enzyme kinetics (author unknown) by allowing students to act out the role of enzyme (Oreo-ase). However, in this model exercise, instead of learning only about the enzyme, students learn about the genes and mutations, bringing this model to its full genetic extension.

Classroom Logistics

This exercise can be done within a 50-minute class period with students in groups of four or five around small tables or with chairs arranged in circles. It is recommended for students in grades nine and above and has been performed in situations where class size ranges from seven to 24.

The Activity

Students learn about the gene and enzyme (see Figure 1 and the Albinism Background Section) responsible for

the albinism phenotype (Figure 2) before proceeding to the role-playing model where one of the students in each group "becomes" the enzyme. The instructor may wish to open the exercise with an "Invitation to Inquiry" (see p. 11) or show examples of individuals with albinism. Figure 2 shows one example of the albinism phenotype from a particularly nice Web site with many images by photographer Charles Eisenmann titled "Nineteenth Century Images of Albinism." The Web site was compiled and is maintained by photographic historian Marcel Safir and can be accessed at http://members.optusnet.com.au/~msafier/albinism/c19albinos.html.

Once the students appear appropriately grounded in the background material and its connection to the exercise, they are permitted to begin the role-playing game. The function (job) of the enzyme (student) is to generate (break open) as many substrate molecules (sandwich cookies, Figure 3a) to form product molecules (sandwich cookie halves, Figure 3b) as possible in 15 seconds. Each of the other students in the group plays one of several crucial roles. One key role is the "mutation chooser." This individual draws and reads a mutation card that states what mutation has occurred to the gene encoding the enzyme, how that mutation will affect the enzyme's function, and how the enzyme is to behave. One key point is that mutations are made to the enzyme such that the enzyme's activity is altered. For example, one mutation generates an enzyme that must open cookies with only little fingers (Figure 4a). Another mutation generates an enzyme that must open cookies while standing on one foot (Figure 4b). Other student jobs include timekeeper, grapher, and recorder. If there are fewer than five students in one group, all students except the enzyme can have more than one job. Each mutation generates a different problem for the enzyme, which will be played out by the student and be observable in the number of cookies that the student can open. Following the exercise, you can choose to have the students graph the data over time to give them practice graphing and to provide a visual picture of the effects of various mutations on substrate turnover rate. Depending on the time permitted, the instructor may choose to give some direction to their graphing. An example of the data generated by one group is shown in Figure 5. The accompanying figure legend describes the data generated by this group in greater detail. Follow-up questions are given to help the students interpret, reach conclusions, and explore the meaning of the exercise more fully. Experimental outcomes are more readily interpreted if students focus on the 15-second timepoints for each allele. It is important to note that the student "enzyme" represents the protein activity derived from only a single allele and that humans, being diploid, will have two alleles.

Figure 1. Metabolic pathway illustrating melanin production, highlighting the points at which tyrosinase acts. (Mendelian Inheritance in Man, 2003)

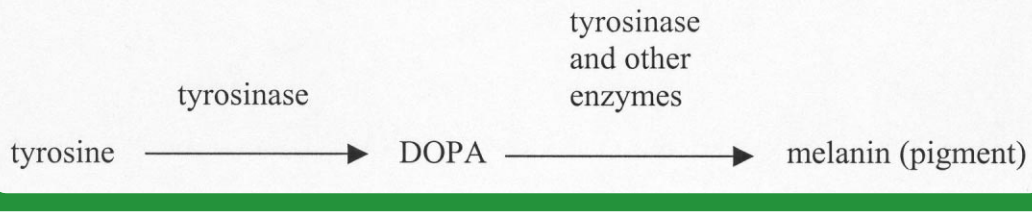


Figure 2. Examples of Human Oculocutaneous Albinism Type IA Phenotype. Mary and Florence Martin were a pair of sisters with albinism who lived in the nineteenth century. Permission to reproduce the photograph was kindly provided by M. Safir. (Safir, 2003)

Figure 3a. Closed cookie representing tyrosine and open cookie representing DOPA. **Figure 3b.** Cookie-ase, Elishea Nolan, opens substrate (tyrosine, cookies) to form product (DOPA, open cookies) while recorder, Rodney Kincaid, records the data.

3a.

3b.

Albinism Background

Albinism is one of the first human genetic metabolic disorders described by the father of human biochemical genetics, Archibald Garrod, as an "inborn error of metabolism"(Garrod, 1923). One form of albinism, oculocutaneous albinism Type 1, is a recessively inherited genetic disorder that is caused by mutations in the gene encoding tyrosinase (Online Mendelian Inheritance in Man: MIM #203100, MIM #606952). Tyrosinase acts on the substrate tyrosine in a hydroxylation reaction to generate dihydrohyphenlalanine (DOPA; see Figure 1; Michal, 1999). DOPA is then further converted in subsequent biochemical reactions that cyclize and condense the products into melanin, the pigment found in the hair, skin, and eyes. Tyrosinase is known to act in at least three distinct steps in the biochemical pathway to melanin production. The phenotype for this metabolic disorder is a lack of pigment in the skin, eyes, and hair (Figure 2) and may include visual problems such as limited visual acuity (Online Mendelian Inheritance in Man: MIM #203100, MIM #606952).

Optional Invitation to Inquiry

Alberta and Raymond both exhibit a lack of pigment in their skin, hair, and eyes that is caused by a mutation in one gene. This gene normally produces a protein that generates the pigment, as well as performing other functions in the body. Raymond exhibits a slight yellowing of the hair, eyes, and skin, but he is still very pale. However, Alberta's lack of pigment is much more severe. She shows no coloration in her skin, hair, or eyes. How might the differences between Alberta and Raymond be explained?

Student Objectives

Learn how different genetic alterations (mutations) affect enzyme activity.

Figure 4a. Cookie-ase, Keith Beckman, demonstrates the functional effect of a mutant allele that is destructive to the enzyme's active site; the enzyme uses only the little fingers. **Figure 4b.** Cookie-ase, Emily Wolfe, demonstrates the functional effect of a mutant allele that does not affect the active part of the enzyme; the enzyme stands on one foot. Recorder, Wesley Skelton; grapher, Will Isom; and time keeper/mutation chooser, Leanna Brownfield, form the remainder of the group from left to right.

Determine that enzyme activity correlates to phenotype.

Observe that mutations in the same gene can result in a variation in protein activity and variability of the resulting phenotype (expressivity).

Materials

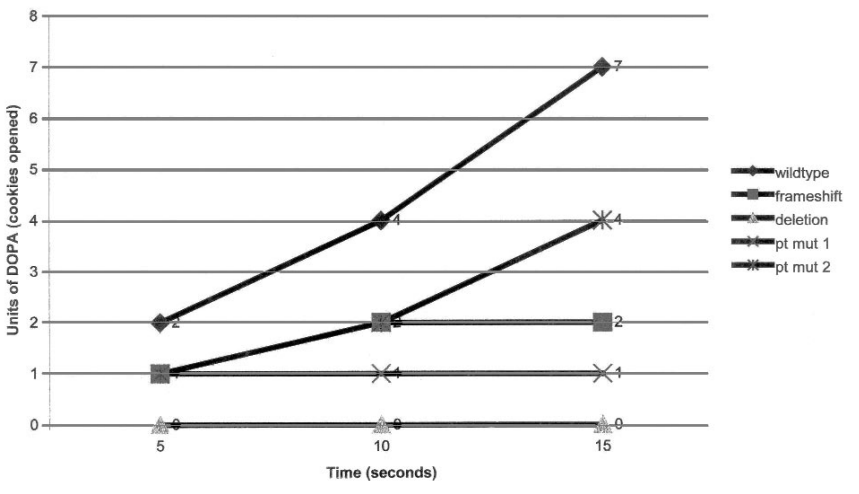
- index cards noting jobs for students (Figure 6)
- index cards noting mutations and effects on enzyme (Figure 7)
- graph paper
- one watch with a second hand per group
- three-pound-package of sandwich cookies per group (cost is usually $1.50-$2.50 per package, if a store brand is used)*

*Note: Store brand (Walmart and Bi-Lo) vanilla and/or chocolate sandwich cookies yield excellent results. A single three-pound package of cookies is more than sufficient for one group performing all five allele simulations.

Student Directions

In this exercise one of you will be the enzyme tyrosinase

Figure 5. Example of results generated from an undergraduate sophomore genetics group.



that converts tyrosine (represented by a "closed" sandwich cookie) to DOPA (which is represented by an "open" sandwich cookie). DOPA is a precursor of melanin, the pigment in the skin, hair, and eyes. The two halves of the sandwich cookie must be completely separated to count. Other students in the group will select mutations, and time and graph the results when mutations are introduced into the gene encoding the enzyme.

1. Divide yourselves into groups so that 4 or 5 students are in each group.
2. Acquire a stack of job cards, a stack of mutation cards, and a three-pound package of cookies.
3. Select a single job card. All jobs should be taken. Any member of the group can have more than one job, except for the enzyme.
4. The mutation chooser gets to select cards from the mutation card stack and tell the enzyme what to do.
5. The enzyme should be allowed at least one practice round of opening as many cookies as he/she can in 15 seconds.
6. The timekeeper starts and stops the enzyme at the correct time.
7. The recorder records the numbers of cookies opened at 5, 10, and 15 seconds for each mutation.
8. The grapher is responsible for helping the recorder and also graphing the results for the group.

Student Follow-up Questions

Following the activity, you will need to answer the following questions.

How many units of DOPA (opened cookies) per 15 seconds did your "wildtype" enzyme produce? Remembering that humans are diploid (having two alleles), how many units of DOPA would a homozygous wild-type individual produce?

The minimum number of DOPA units required for a "normal" pigmented phenotype is the number you noted in the first question for a single allele. Rank the mutations in order of most severe albino phenotype to least severe phenotype. Which mutation results in the most severe mutant phenotype? Why?

A person having two different mutant alleles is called a compound heterozygote. How could this affect the severity of mutant phenotype?

How would your answers above change if 15 units of DOPA per 15 seconds were required for a normal phenotype?

Did any of the mutations result in an enzyme with increased activity?

Figure 6. Job cards to be randomly drawn by students.

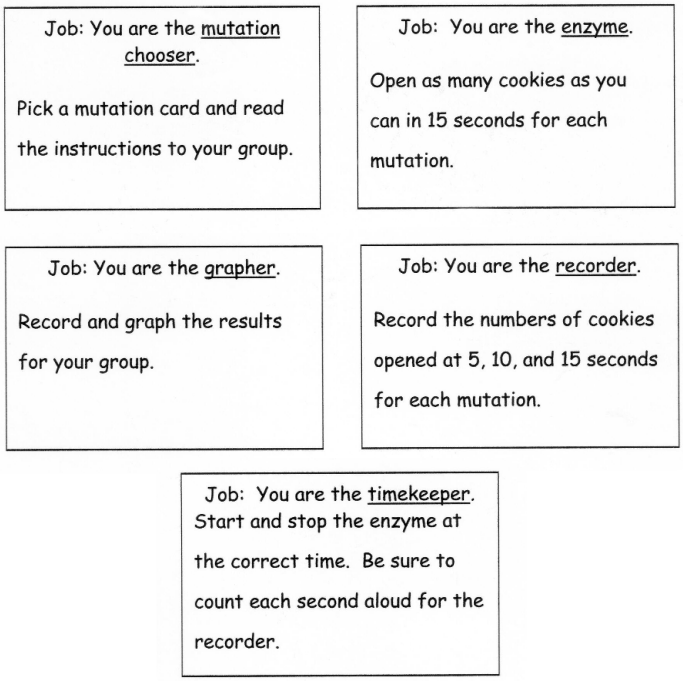
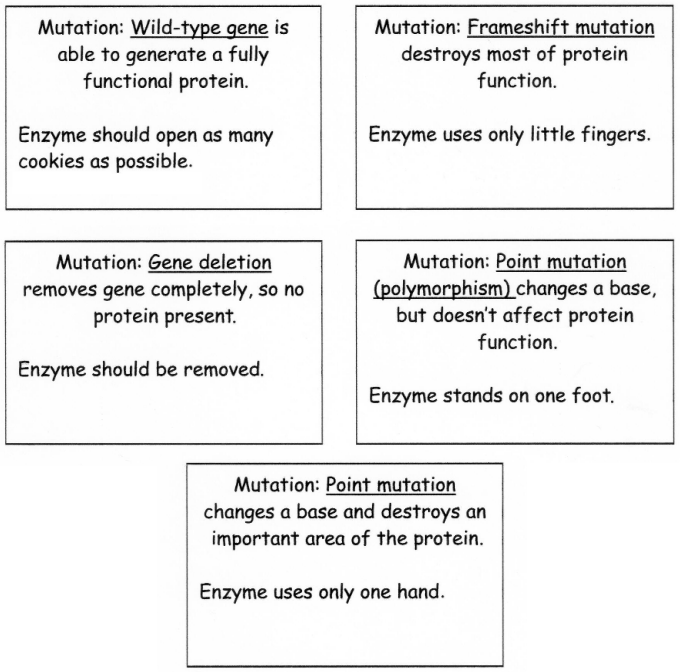


Figure 7. Mutation cards to be given to the "mutation chooser" and randomly drawn and read by "mutation chooser."



Classroom Considerations & Extensions

This exercise can be adapted easily to students of various grade levels. I have used variations in general Genetics, a sophomore level undergraduate genetics course, and Human Genetics, an upper level undergraduate genetics course. Students are extremely animated and interactive during the exercise and always appear eager to learn the effect on the enzyme and outcome of the next mutation. Several pre-service teachers/students I have taught have expressed interest in using the exercise in their own classrooms. I also have shared it with high school teachers and teachers of undergraduate general biology, who used it successfully in their classrooms. One general biology teacher wrote, "Students love it and they really get an understanding of why some alleles are considered dominant or recessive" (Teresa Fulcher, personal communication).

To approach this exercise at the most basic level, the instructor may consider downplaying the idea of a more or less severe phenotype, which is known as expressivity. Should the instructor choose, though, this model can be used as a springboard to discuss expressivity in greater detail. Alternatively, by choosing different "normal" phenotype limits and focusing on different follow-up questions, the model could be used to enhance and develop a better student understanding of allele dominance and recessiveness. In addition, this model can also be used to begin discussions of the existence of multiple alleles in nature, beyond blood type alleles. One study conducted in 1992 (Tripathi et al., 1992) noted 60 different alleles for tyrosinase-related albinism, while another study found a number of those exhibiting albinism were compound heterozygotes (Oetting & King, 1993). For those seeking a very advanced extension, this exercise could be used as a springboard to investigate the variation in human cytochrome genes, their importance in drug metabolism, and their future in individualized medicine (Johnson, 2003; Human Genetic Variation NIH Curriculum Supplement, 1999). For a review of other human genetic disorders that may be of interest in forming similar models, see Steward et al. (2003).

Laboratory Extensions

As noted earlier, a few laboratory exercises have been proposed to illustrate how concepts of genotype and phenotype relate to one another. Examples include the pGLO bacterial transformation kit available from Bio-Rad Laboratories (Hercules, CA) and an activity described by Guilfoile and Plum (2000). These activities will be excellent options for many educators, but are time-intensive, somewhat costly, and can be intimidating or overwhelming to students (and teachers) who are unfamiliar with biotechnology laboratory skills. This role-playing exercise is offered as a complement to these laboratory exercises for advanced students or as an introduction for the less advanced student of genotype-phenotype relationships.

College-level teachers who have prior recombinant DNA and biochemistry laboratory expertise that are seeking a truly advanced laboratory complement to this exercise may wish to consider generating mutant alleles of a gene encoding an enzyme whose activity can be readily assayed. One example of this type of laboratory investigation focused on the comparative kinetics of isozymes of the puromycin-sensitive aminopeptidase gene (Thompson et al., 2003). In this example, wildtype and mutant alleles were expressed in insect cells and biochemically purified prior to performing each assay. In this biochemical reaction, the puromycin-sensitive aminopeptidase (wildtype or mutant) catalyzes the hydrolysis of alanine-p-nitroanilide to alanine and p-nitroanilide, which is detectible at 405 nm in a standard spectrophotometer. Therefore, this complement of role-playing and laboratory exercises could also be used to teach the principles of basic enzyme kinetics.

Acknowledgments

The author is especially indebted to Dr. Philip M. Mathis for helpful discussions and comments, and for reviewing the manuscript. In addition, the author wishes to thank Drs. Michael W. Thompson, Michael L. Rutledge, and Kim C. Sadler for reviewing the manuscript and for their helpful comments.

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