IOWA STATE UNIVERSITY
Veterinary Microbiology and Preventive Medicine

University Honors Program
December 7, 2020

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Developing a chromogenic protein model adapted to high school STEM research and education

INTRODUCTION

The characteristic purple color of the coral species *Acropora millepora* is resultant of the expression of chromogenic protein (CP), single polypeptide chains that can exhibit an array of colors. To employ these chromogenic properties for a variety of applications, we synthesized CP for expression in *Escherichia coli*. We reasoned that, thanks to its striking phenotype, this system would be an effective tool for teaching the principles of the central dogma (Fig. 1). To reinforce the steps of information flow, we designed experiments demonstrating transcription (through regulated expression of CP), replication (through site-directed mutagenesis), and translation (through informational suppression). These systems have been implemented in undergraduate genetics curricula, and also hold potential for high school students as a visually engaging system for introducing students to molecular biology and sparking interest in STEM, research, and biotechnology, as well as a research tool to differentiate between bacterial strains in synthetic communities.

FIGURE 1. The central dogma of biology.

METHODS

Basic methods of bacteriology and molecular biology were used throughout the course of this study and associated curriculum design. Inverse PCR was used to alter the DNA sequence corresponding to amino acid 62 of wild-type CP placed under a rhamnose-inducible promoter (Fig. 2). The linear PCR product was self-ligated and transformed into competent K12 *E. coli* and plated onto chloramphenicol selective media with L-rhamnose, yielding a variety of colored variants, depending on the amino acid substitution. Plasmid DNA was isolated from the resultant uniquely colored colonies and the 62-position amino acid was confirmed via nucleotide sequencing. This method was incorporated into undergraduate teaching curriculum as an illustration of gene regulation and informational suppression using the CP_{Amber} synthetic variant, and adapted to the high-school level with additional instruction on core concepts and technical skills.

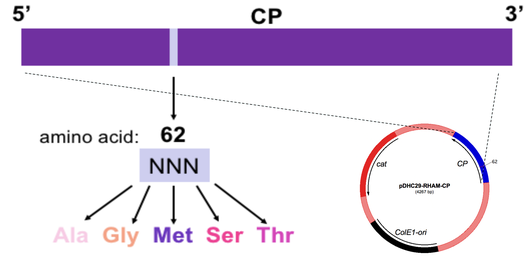

FIGURE 2. Mutagenesis method for the nucleotides encoding the 62-position amino acid of CP. Also shown in the map of the CP expressing recombinant plasmid.

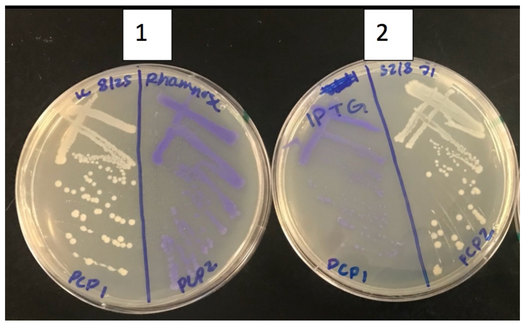

FIGURE 3. CP induced by rhamnose (left) and IPTG (right).

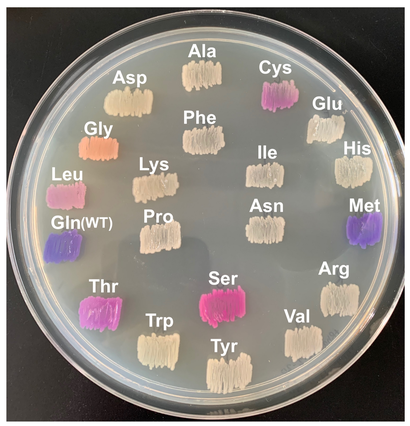

FIGURE 4. Substituting for the wild-type glutamine in the chromophore yields a colorful array of variants. We hypothesize that large, bulky, or charged amino acids do not produce color.

RESULTS

Transcription. CP was placed under rhamnose (*rha*) and lactose (*lac*) inducible promoters to demonstrate principles of transcriptional regulation (Fig 3).

Replication and Mutagenesis. Site-directed mutagenesis of wild-type CP with inverse PCR reinforced the processes of DNA replication and mutagenesis (Fig. 2).

Translation. Amino acid 62's location at the interior of the β-barrel structure of CP's chromophore makes this model a useful tool for teaching students about informational suppression, because it plays a critical role in the colored phenotype displayed (Fig. 5).

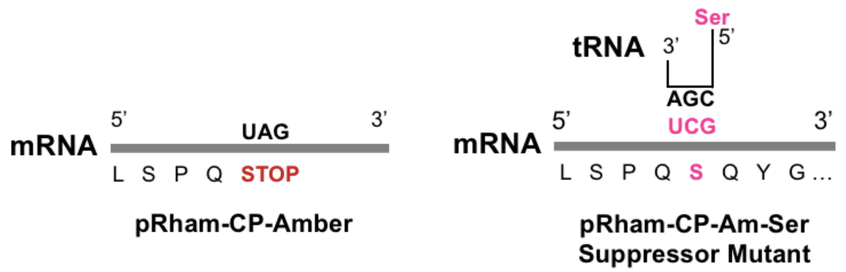

FIGURE 5. tRNA with a modified anticodon loop recognizes the Amber stop codon as a sense codon, and incorporates an amino acid that imparts a distinct color.

CONCLUSIONS

The use of CP is visually engaging, and for this reason we believe it is well-suited not only to undergraduates but also to high school students who may not have had much education in the biological sciences. Clearly identifiable phenotypes corresponding to modified genotypes (Fig. 4) offers students a solid grasp on the central dogma, and the colorful array of variants is likely to spark interest in STEM—particularly research in the biological sciences, where this model may serve as a differential tool quantifying strains' fitness in microbial communities.

REFERENCES

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Langan PS, Close DW, Coates L, Rocha RC, Ghosh K, Kiss C, Waldo G, Freyer J, Kovalevsky A, Bradbury ARM. 2016. Evolution and characterization of a new reversibly photoswitching chromogenic protein, Dathail. J. Mol. Bio. 428.

Acknowledgements: Megan Jones