BARRY M. GOLDWATER SCHOLARSHIP
AND EXCELLENCE IN EDUCATION PROGRAM

Nominee's Essay

Discuss a significant issue or problem in your field of study that is of particular interest to you.

Your essay must include a description of the issue or problem, discuss an idea for research that could have significant impact on the issue or problem, describe an aspect of the research in which you would be involved, and explain the relevance of the issue or problem to you as a mathematician, scientist, or engineer. Assume that your reader is knowledgeable in mathematics, science, and engineering. The content and style of your essay will be important to the success of your scholarship application.

If your essay involves research in which you are or were involved, please indicate if you are or were the sole researcher or if you collaborated with another individual.

Include a bibliography, references, or illustrations, when appropriate, as part of the essay. Font size may not be smaller than 12 characters per inch, or 11 point. Your essay **must** be typed and confined to two pages (one side only). **Be sure to include your signature at the end of your essay.**

Title: The identification, characterization, and potential applications of RNA Diels-Alderases

The advent of antibiotics has often been hailed as one of the greatest medical breakthroughs of the twentieth century. However, the use and misuse of these "miracle drugs" have evoked an evolutionary response in bacteria, leading to the rise of microorganisms that are resistant to even the most potent drugs. On average, drug-resistant strains are identified within one to four years after the introduction of a new medication (1). Clearly, the need for novel and effective treatments is great. A major impediment to drug discovery is the inability of researchers to access and quickly explore the full spectrum of potential drug candidates. Medicinal chemists typically synthesize combinatorial libraries of 1,000 to 100,000 compounds based off of a known molecule that shows antimicrobial activity. However, the number of potential compounds consistent with one of these lead molecules is several orders of magnitude greater than the size of these synthetic pools. In addition, the size of combinatorial libraries prohibits the individual screening of each candidate (2). In order to quickly discover new pharmaceuticals, researchers must find ways first to generate libraries more fully representative of all of the chemical possibilities that are useful for a given target (3) and then to rapidly screen these enormous pools for antimicrobial activity. However, though this task seems daunting, researchers may be able to make progress towards these goals by harnessing the same force that led to the emergence of drug-resistant bacteria: evolution.

When exposed to drugs, a few bacteria that are able to survive this selective pressure soon come to comprise a substantial portion of the population. The process of in vitro selection employs the same principle of selection to identify RNA catalysts in the laboratory. In the in vitro selection process, a starting library of RNA molecules is sent through repeated selection cycles. In each round, those molecules that participated in a certain chemical event are isolated, amplified, and taken into the next round of selection. Over repeated rounds, catalytic sequences begin to dominate the pool of RNA (4). Using the in vitro scheme shown in Figure 1, Eaton and coworkers have isolated RNA catalysts for the Diels-Alder reaction (a cycloaddition between a diene and a dienophile) capable of accelerating the reaction by a factor of up to 10,000 (5). Initially, a pool of 100 trillion unique, chemically generated DNA sequences are transcribed to the corresponding RNA sequences with the enzyme T7 RNA polymerase. Each RNA sequence is then enzymatically tethered to a diene through a polyethylene glycol linker and a short DNA bridge, and the RNA-diene constructs are incubated with a biotinylated maleimide dienophile. Therefore, any Diels-Alder product that forms will be linked to both

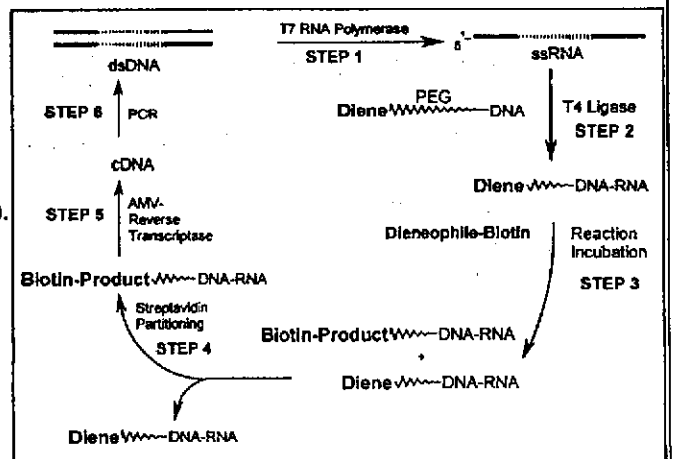

Figure1: In vitro selection of RNA Diels-Alderases

biotin and an RNA molecule. The RNA attached to the desired product can then be separated from RNA bound to unreacted material based upon the binding of biotin to streptavidin, reverse transcribed to the complementary DNA sequence, amplified by the polymerase chain reaction, and taken into the next round of selection (6). Variations on this selection process have revealed other potential applications of in vitro selection. By using an isolation procedure that relies on the affinity of a compound for the active site of an enzyme, rather than streptavidin-biotin binding, RNA molecules can be selected that use the most potent stereoisomer of a chiral enzyme inhibitor in preference to other stereoisomers (7). These results suggest that it might eventually be possible to use in vitro selection techniques to identify the most potent enzyme inhibitors, which are potential drug candidates, from mixtures of compounds.

In order to demonstrate that RNA catalysts isolated through in vitro selection are true catalysts that exhibit turnover, the Eaton group is currently working to characterize the kinetic behavior of their previously isolated Diels-Alderases under multiple turnover conditions. All previous kinetic studies of these Diels-Alderases were conducted under single turnover conditions (Figure 2a). Under these conditions, 32-P labeled RNA was tethered to the diene substrate, and therefore to the biotinylated Diels-Alder product. The product could be isolated and accurately quantitated using phosphorimaging. However, under multiple turnover conditions, the RNA is no longer tethered to the substrate or the product, and a method that can detect and measure the amount of the actual product, not the RNA, must be employed. Fluorimetry is one of the few techniques that is accurate and sensitive at the scale at which these assays are conducted. Members of the group have already shown that certain Diels-Alderases exhibit turnover (5), so I am currently working with the Eaton group to synthesize a diene labeled with fluorescein to use for RNA catalysts that use slightly different substrates than those studied thus far. The fluorescent-labeled diene will be incubated with a biotinylated maleimide while the RNA is free in solution. The Diels-Alder product can then be isolated by streptavidin-biotin binding and quantitated by fluorimetry, providing the data needed to develop a kinetic model (Figure 2b). Future work will include the synthesis of other Diels-Alderase substrates with fluorophores. Different fluorophores will be used to see if the identity of the fluorophore impacts the results. The fluorescent label will also be placed on the maleimide instead of the diene to investigate whether or not the kinetics are affected by which substrate bears the tag. Different dienes and dienophiles will be employed to probe the substrate specificity of the Diels-Alderases. The combined data from these assays will help the group to obtain a better understanding of the catalytic mechanism of these RNA catalysts and to provide evidence that in vitro selection is an effective method to rapidly screen enormous libraries of molecules.

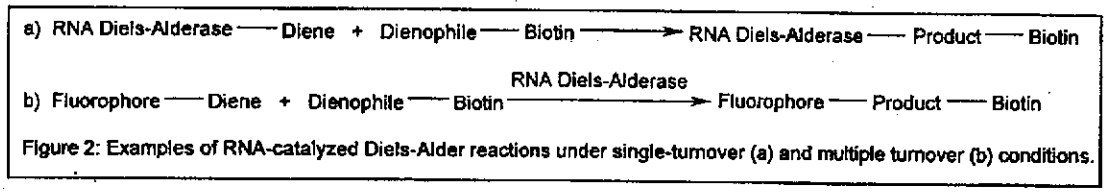

Figure 2: Examples of RNA-catalyzed Diels-Alder reactions under single-turnover (a) and multiple turnover (b) conditions.

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(2) Ghose, Arup K., and Vellarkad N. Viswanadhan, eds. Combinatorial Library Design and Evaluation. New York: M. Dekker, 2001.

(3) Feher, Miklos, and Jonathan M. Schmidt. "Property Distributions: Differences between Drugs, Natural Products, and Molecules from Combinatorial Chemistry," Journal of Chemical Information and Computer Sciences 43 (January 2003): 218-227.

(4) Roberts, Richard W, and William W. Ja. "In Vitro Selection of Nucleic Acids and Proteins: What are we Learning?," Current Opinion in Structural Biology 9 (August 1999): 521-529.

(5) Eaton, B. E., unpublished results.

(6) Tarasow, Theodore M., Sandra L. Tarasow, and Bruce E. Eaton, "RNA-catalysed Carbon-carbon Bond Formation," Nature 389 (September 1997): 54-57.

(7) Nieuwlandt, Dan, Madeline West, Xiaoqin Cheng, Gary Kirshenheuter, and Bruce E. Eaton. "The First Example of an RNA Urea Synthase: Selection through the Enzyme Active Site of Human Neutrophile

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