New method of DNA editing allows synthetic biologists to unlock secrets of a bacterial genome

December 5 2013

A group of University of Illinois researchers, led by Centennial Chair Professor of the Department of Chemical and Biomolecular Engineering Huimin Zhao, has demonstrated the use of an innovative DNA engineering technique to discover potentially valuable functions hidden within bacterial genomes. Their work was reported in a *Nature Communications* article on December 5, 2013.

The genome of every bacterial species contains genes that can synthesize a diverse arsenal of compounds. These include natural antibiotics, antifungals, and other biochemicals that help the bacteria fight off unfriendly fellow microbes; such compounds are of potentially great medical importance. The genes encoding the enzymes a bacterium needs to create these compounds are often arranged in clusters. Each gene corresponds to one of a set of proteins that work together in a biochemical pathway to create one or a few products.

If a colony of bacteria is producing a biologically active compound, sometimes referred to as a natural product, scientists can isolate it, study its structure and function, and discover its potential uses. Many natural products have already been discovered by screening the compounds produced by different bacterial and other microbial species.

The compounds discovered so far, however, represent a small fraction of those that bacteria are capable of producing.

Bacteria are masters at survival; their genomes represent a set of contingency plans for a wide array of environmental situations. Like a painter laying out a palette with only the colors needed that day, a bacterium will only express the genes and synthesize the compounds that will help it thrive in its current setting. Constant expression of the gene clusters that aren't useful in a given situation would be energetically wasteful.

This conservation of energy is good for bacteria, but bad for researchers hoping to discover new natural products. This was the challenge that Zhao and colleagues hoped to address when they began their project. "Sequence analysis of bacterial genomes indicates that there are many cryptic or silent pathways that have not been discovered," Zhao said. " . . .they need the right signal to turn on expression of the whole gene cluster."

Several strategies have been employed to trick cells into activating their little-used, "cryptic" gene clusters, such as culturing bacteria in a variety of harsh conditions or inserting sets of genes from one species of bacteria into the genome of another species. These techniques involve labor-intensive trial and error, with no guarantee of success.

Zhao's group, rather than attempting to manipulate the environment, focused on reprogramming the control of gene expression within the cell. They used a genetic engineering method previously developed by Zhao's laboratory, called DNA assembler, to insert small sections of DNA between each gene in a cryptic gene cluster. The sections of DNA added were promoters, specialized regions that help control when and how much nearby genes are expressed. By adding the right promoters, Zhao and colleagues forced the cell to increase expression of every gene in the cluster.

What makes Zhao's strategy possible is the ability of the DNA assembler

method to join many different fragments of DNA in a single step. Previous methods for DNA editing limited researchers to making a series of sequential changes; the number of experimental steps required to add a promoter to each gene in even a small cluster would have been prohibitive. In contrast, Zhao said, "we can actually build the whole cluster, so that gives us ultimate flexibility, because we can add different promoters," ensuring that every gene within the cluster is consistently activated.

For the study published in *Nature Communications*, Zhao and his coauthors modified a cryptic cluster of six genes from Streptomyces griseus, a species of soil bacterium. They added a promoter before each gene in the cluster to increase expression, and inserted the cluster into a related bacterial species, Streptomyces lividans, that is easier to grow in a laboratory setting.

The resulting bacterial strain expressed all the genes in the previously silent cluster, and produced several previously unknown compounds. These compounds belonged to a class of natural products called polycyclic tetramate macrolactams or PTMs, many of which have useful biomedical applications. By examining the compounds produced by strains missing one of the six genes in the cluster, the researchers were able to discover the function of each gene's encoded protein, leading to a better understanding of how bacteria synthesize PTMs.

Zhao sees the work as an important step toward a larger goal: to create a generalized, automated high-throughput method to reconstruct any biochemical pathway in a target experimental organism. Zhao is the leader of the recently formed Biosystems Design Research Theme at the Institute for Genomic Biology, University of Illinois, and development of this type of method is a major goal of the Theme.

"We want the technology platform established, then we can actually

work on mammalian systems, on plant systems, on microorganisms," said Zhao. Yet his ultimate motivation is the discovery of potentially useful biochemicals: "It's very likely some of the compounds will turn into new drugs, and that's very exciting."

More information: DOI: 10.1038/ncomms3894

Provided by University of Illinois at Urbana-Champaign