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Inducing Coexistence of Incompatible Single-Copy Plasmids with Selective Pressure

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Background and Significance

Plasmids, which are circular molecules of extrachromosomal DNA, are often found in bacterial cells. Traditionally plasmids have been considered simply accessories to chromosomal DNA. However, recent publications have demonstrated that plasmids may be more than simply an appendage to the bacterial chromosome. One such paper showed that plasmids may actually compete to exclude one another from a cell (1). This research shows that the current view of plasmids may be too limited, as plasmids may actually act as individuals in an environment. If that is the case, then plasmids should also be susceptible to selection just as other organisms in an ecosystem.

One necessary component of all plasmids is the origin of replication. Single-copy plasmids that contain the same origin of replication are said to be *incompatible*, because the plasmids will separate into two different daughter cells upon cell division. Copy number refers to the number of plasmid molecules within a cell. If plasmids are to be considered individuals in the ecosystem of a cell, then some sort of selective pressure could cause the incompatible plasmids to cooperate and coexist in the same cell. A mutational event of some sort would be the expected cause of such a cooperative transition.

The finding that plasmids adapt to and interact with their environment as individuals could have great impact on fields ranging from agriculture to medicine. Problems such as antibiotic resistance could be explained and perhaps controlled in a whole new way. The field of plasmid ecology is very new and, until recently, few studies have been performed to explore such an idea.

Results

My hypothesis was that two incompatible single-copy plasmids can be forced to coexist in a single cell by applying the appropriate selective pressure. I proposed to complete construction of 2 single-copy plasmids using the mini-f origin of replication, making them inherently incompatible. Each plasmid would have a different portion of the lac pathway (LacZ or LacY), both of which are essential for lactose metabolism, and a different fluorescence protein (green or yellow). Upon completion of the plasmids they were to be transformed into bacteria and then grown under various selective pressures.

In the short period of time that transpired between the awarding of the scholarship and the time of this report I was only able to partially complete the construction of the plasmids. I ran into a number of obstacles including finding the appropriate single-copy plasmid and getting viable constructions. Through a lot of reading and searching I was able to find the best single-copy plasmid for my project. Many of my initial attempts to isolate various antibiotic resistance genes failed but I was eventually able to find the appropriate targets and I was successful in isolating them.

This project has great potential for learning more about plasmid ecology and how they interact in the environment of the cell. More time is all that is needed to see this project to completion and the outcome will bring forth exciting new insight to a rapidly expanding area of research.

Through this experience I was able to see that research takes time and perserverance and that it is difficult to put a deadline on discovery.