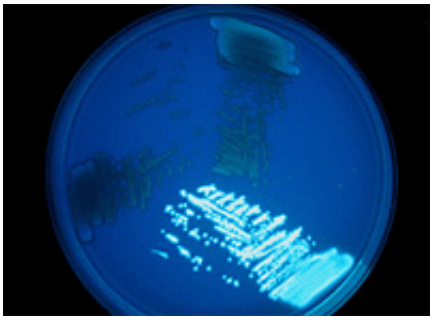
Science class experiment reveals vitamin B12 secret

February 22 2006



Under ultraviolet light in a Petri dish containing laundry whitener, symbiotic bacteria with a mutant bluB gene (lower right) fluoresce brightly, while the same bacteria with no mutation only glow slightly (top right), and bacteria with another mutation (in the exoY gene) are completely dark. Image: Michiko Taga

For decades, scientists have wondered how living organisms manufacture the essential vitamin B12. Now, using laundry whitener and dirt-dwelling bacteria--the everyday ingredients of an undergraduate science experiment--researchers may have found the major clue they need to solve the mystery.

Researchers led by Graham Walker, a Howard Hughes Medical Institute (HHMI) professor and American Cancer Society research professor at the Massachusetts Institute of Technology, have discovered the first known mutant bacteria with a specific defect in a gene involved in the least-understood part of B12 synthesis. They report their findings in the

early online edition of the Proceedings of the National Academy of Sciences, published February 20, 2006. HHMI professors are leading research scientists who received $1 million grants from the Institute to find ways to bring the excitement of the research lab into undergraduate science classrooms.

In the ancient world, B12 was probably catalyzing reactions before cells even existed. Now, all animals need B12 to help make the building blocks of DNA, and children need enough of the vitamin to help their brain develop normally. Most people consume enough B12 through animal products or fortified foods in their diet. On the other hand, animals that do not eat other animal products acquire the nutrient from bacteria in their guts or from bacteria-infected dirt on their plant food. An estimated one-quarter of people older than 60 in this country have trouble absorbing B12. B12 deficiency can lead to nerve damage, anemia, and forgetfulness.

Walker's team's genetic discovery was made possible by a gimmick Walker designed to capture the attention of undergraduate biology students in the early 1980s. When he added a laundry whitener to a lab dish, the symbiotic bacteria he studied glowed in ultraviolet light, just as the additive makes clothes look brighter in the sun.

The teaching trick soon became a popular tool in Walker's lab for research that had nothing to do with vitamin B12. There, researchers have been focusing on how symbiotic bacteria form and invade the nodules in alfalfa roots that provide the plant with nitrogen and the bacteria with food. The scientists noticed that some of the bacteria on the glowing lab dishes did not light up. These stubborn dark spots revealed bacteria missing key genes needed to construct and enter the nodules in plant roots, they discovered. By analyzing various mutations, the researchers were able to track the molecular details of how the bacteria provide the plant with the nutrients it needs to grow.

Several years ago, Walker's graduate student Gordon Campbell decided to look for symbiosis defects in bacterial mutants that, instead of being dark spots on the glowing lab dish, were even brighter than their normal counterparts. His findings enabled Campbell and his co-authors to answer a question being asked by many researchers studying B12 synthesis.

"That is what is so great about basic research," Walker said. "It finds answers to things you cannot get at in a direct way."

Campbell isolated the brightest mutants and put them onto the roots of alfalfa seedlings. Healthy symbiotic partnerships show up on the plant roots as long pink nodules stuffed with bacteria. In contrast, seedlings sharing a dish with the most obviously defective bacteria were stunted and their roots had small white nubs with barely any bacteria inside. For one of these bright mutants, it turned out that the root of the problem was the mutant bacteria's inability to produce B12.

Adopting nomenclature traditional in their field, the researchers named the mutated gene bluB, after a similar gene found in another kind of bacteria.

"The important clue came when we noticed bluB was grouped with other genes important for making vitamin B12 in the other bacterium," Walker said. "That's not something we are expert in." So the researchers contacted co-author John Roth, a professor of molecular biology at University of California, Davis, who has studied in detail the intricate series of steps required to assemble B12, the largest known natural compound that is not made out of repeating units.

"Out of our conversations came the idea that bluB might be required for an unknown part of the pathway," Walker said. "B12 is a big, complicated molecule. Researchers have been unable to crack the

problem of how to make the lower ligand," a segment of the molecule known as DMB.

It was a simple experiment, said Michiko Taga, a postdoctoral fellow and co-first author of the paper. Taga took over the research when Campbell graduated. "If the mutant was broken because it could not make DMB," she said, "then if we added DMB back it should be okay. So we added DMB, and the bacteria went back to acting like ordinary [symbiotic] bacteria. That was the defining experiment."

When the researchers provided DMB so that the bacteria did not have to manufacture it themselves, the bacteria's extraordinary brilliance subsided to a more uniform fluorescence on the lab dish with the laundry whitener. And in the lab dish with the seedlings, the restored bacteria produced a bigger, healthier plant. Chemist and co-author Kavita Mistry followed up with biochemical experiments to prove that the bluB mutant could not make B12 without added DMB.

"Our findings just mean bluB is necessary for the reaction," Taga said. "We are currently doing experiments to show that it directly catalyzes the reaction."

But Roth said the discovery gives him hope for finding all the steps in the pathway for synthesizing B12. "This is the part that has resisted genetics and chemistry," he explained. "We've tried it. Others have tried it. This appears to be the first enzyme dedicated to synthesizing the part."

Other bacteria, such as the Salmonella that Roth studies, appear to substitute other molecules in place of DMB, stymieing genetic approaches. But the form of B12 that people need contains DMB.

The discovery of the bluB mutant may overturn a theory that DMB

spontaneously forms without enzymes to speed up the reaction, Roth said. Before the bluB mutant was identified, that theory made sense because the reactions that make B12 do not require energy, in contrast to most biosynthetic reactions.

Taga and Walker are following up to figure out how the bluB mutation affects the symbiotic relationship between the bacteria and the plant.

Source: Howard Hughes Medical Institute