

studies have produced unexpected results. Only a decade ago, for example, many geologists assumed that as the climate grows warmer and wetter, erosion and chemical weathering (the chemical breakdown of soil and rocks) accelerate. But this notion has itself been slowly eroding. Clifford Riebe, a geomorphologist at UC Berkeley, has used cosmogenics to produce some of the first quantitative evidence that challenges the old assumption. When he compared weathering rates with long-term erosion rates at seven sites that span wide climate ranges in California's Sierra Nevada mountains, Riebe found that erosion and weathering did indeed go hand in hand but that climate had

little effect on either. "Given that chemical weathering gets its name from weather," Riebe says, his results "came as a surprise."

Instead, Riebe discovered, erosion was swiftest on steep slopes near geologic faults or river canyons—evidence that tectonic activity eclipsed climate in driving erosion and weathering. Extrapolated to a global scale, that conclusion bolsters a 13-year-old theory that the uplift and erosion of the Himalayas—and the ensuing consumption of atmospheric CO₂ by chemical weathering reactions—triggered a global cooling that began about 40 million years ago. Riebe is now roaming from the rainforests of New Zealand to the deserts of Mexico to see whether tectonics

dominates weathering rates even in those extreme climates.

As cosmogenic studies of erosion move out of the hands of specialists and into more widespread usage, new applications are blossoming. Researchers like Vermont's Bierman readily rattle off long lists of their plans and predictions for future research. "Overall, what keeps this exciting for me is [applying the techniques to] problems that we otherwise haven't been able to solve," Bierman says. "It's not an incremental learning experience. It's a major learning experience."

—LIESE GREENSFELDER

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BIOENGINEERING

Plant Scientists See Big Potential in Tiny Plastids

Tinkering with plant cells' second genome could boost photosynthesis or turn plants into drug factories

When it comes to genetic engineering, the genes inside the nucleus get all the attention. But plants have an unassuming second genome inside tiny organelles called plastids. And although this small, circular genome carries far fewer genes than its nuclear counterpart, researchers say its potential for genetic engineering far outstrips its size.

The plastid genome arose some 1.5 billion years ago, when the ancient ancestors of modern plants are thought to have engulfed

this secondary genome. The most famous member of the plastid family is the chloroplast, the photosynthesis factory. Others include the amyloplasts, which store starch, and the oil-producing elaioplasts.

Because plastids present an easy way to produce proteins in high concentrations and offer unique access to the photosynthetic machinery, some plant scientists believe they offer some of the best opportunities to make transgenic crops that grow more efficiently,

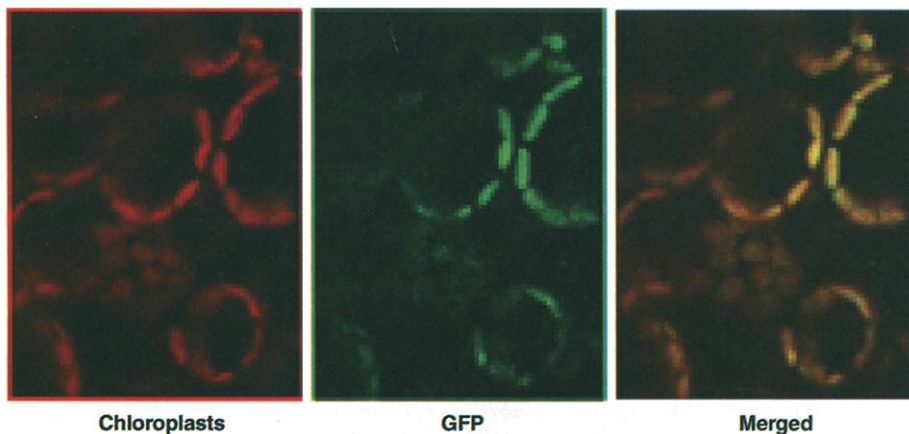
compartment," Bogorad says. But transforming plastids is technically tricky, and the field, although growing, remains small.

The promise of plastids

For years, researchers have dreamed of tinkering with the genes in plants to turn them into living, photosynthesizing drug factories. If plants could be engineered to pump out lots of therapeutic proteins, these could be isolated and made into medicines. But, although creating transgenic plants by altering their nuclear DNA has become routine, it remains extremely difficult to get these plants to produce the desired protein—say, antibodies against herpesviruses or enzymes for diagnostic kits—in large quantities. In most such plants, the new protein accounts for a paltry 1% of the plant's total protein output, although levels as high as 25% have been reported in a few exceptional cases.

Transgenic plants made with altered plastids are much more productive than nuclear-engineered plants. Last year, geneticist Henry Daniell of the University of Central Florida in Orlando inserted a gene cluster for an insecticidal *Bacillus thuringiensis* toxin into the chloroplasts of tobacco plants; the chloroplasts churned out vast amounts of the crystallized protein—45% of the cell's total protein output. Levels routinely reach 5% to 15% in the latest studies, says geneticist Pal Maliga of Rutgers University, New Brunswick, New Jersey.

Engineering the plastid genome has additional advantages over nuclear transformation. For example, the risk that foreign genes introduced into plastids will spread to other plants is much lower than the risk that nuclear genes will make such a leap. This is because plastid DNA in most crop species is transmitted only from generation to generation through the ovules, the plant "egg," not through pollen, the plant "sperm"—just as animals' mitochondrial DNA is passed down only through the egg. Plants produce



Neon plastids. When inserted into the plastid genome, a gene for a fluorescent marker protein (GFP) signals a successful transformation.

photosynthetic bacteria and put them to work manufacturing food. Over time, many of the original plastid genes slipped into the nucleus, but a small genome with about 100 genes remains. Plants now contain undifferentiated organelles that can diversify into a number of specialized plastids, each of which carries

for instance, or that manufacture medicines. "You get high yields" of proteins produced by plastids, says emeritus Harvard molecular biologist Lawrence Bogorad. And when it comes to boosting photosynthesis, "you can probably do things in the chloroplast compartment that you can't do in the nuclear

thousands of tiny pollen grains that can be spread uncontrollably by wind or insects over wide distances, but seeds developing from ovules stay with the plant.

What's more, the rules of molecular biology are different in the plastid than in the nucleus. In the nuclear genome, each gene is turned on and off by its own control sequence. That makes it difficult to engineer complex traits controlled by many genes. But in the plastid, multiple genes are controlled by the same genetic switch, as is the case in bacteria. "It's like a bacterial fermenter in a plant cell," says research director Peter Heifetz of the Torrey Mesa Research Institute in San Diego. With plastids, he sums up, "you solve a lot of problems in one shot."

Technical difficulties

But plastid genomes have often defied the best efforts to modify them. Since Maliga modified the plastid genome of tobacco more than a decade ago, scientists have been able to transform plastid genomes in just a handful of plant species. The technical knowledge required to rework plastids is spreading, but slowly.

Modifying the plastid genome is tough for the same reason that it's promising: Tens of thousands of copies of the genome may be present in any given cell. A single plastid can have hundreds of copies of the genome, and a plant cell can have hundreds of plastids. For successful transformation, the new gene must be present in each copy of the plastid genome within each cell.

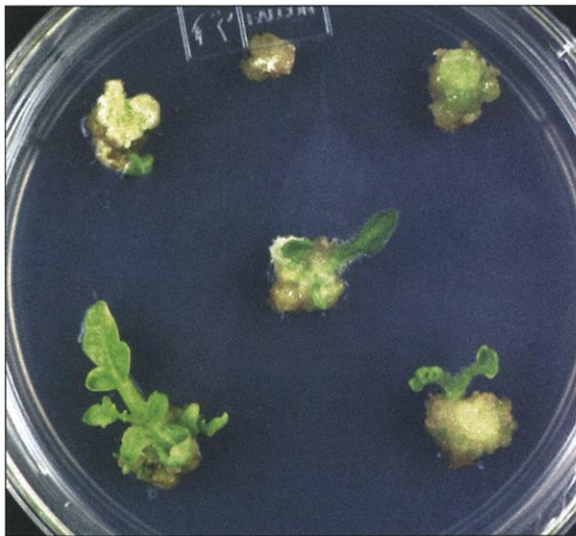
To achieve that, scientists first insert their gene of choice into a single plastid and then allow the cell to divide many times in culture. Then they apply a delicate balance of chemicals that allows cells with more copies of the gene to prosper. After months of selection—if all goes well—the culture will contain only transformed cells.

At this point, scientists are left with a plate of undifferentiated cells that they have to turn back into a plant. Doused with the proper cocktail of plant hormones, tobacco, potato, tomato, and other plants in the nightshade family are easy to regenerate—and plastid engineering has been relatively successful in them. But many of these techniques are species-specific, and it's been difficult to apply them to other plants.

Healthful tobacco?

So far, tobacco has yielded the most plastid engineering successes. In 2000, plant geneticist Jeffrey Staub and his colleagues at Monsanto in St. Louis genetically altered tobacco chloroplasts so that they produced a correctly folded human protein called

somatotropin, which is used to treat dwarfism in children. Maliga calls the findings a "milestone," because they convinced skeptics that the bacteriumlike genetic machinery in plastids was capable of correctly folding mammalian proteins. The study also showed that the plastid-engineered plants don't modify proteins after synthesizing them—a major drawback when making human proteins in a plant's nucleus. Monsanto does not plan to commercialize the plants, which produce the protein at levels 300-fold higher than do their nuclear transgenic counterparts. The researchers chose to work with somatotropin, which has a well-studied structure, only as proof of principle; however, the company is now rumored to be working on expressing other human proteins in tobacco. "We may actually find something very



Shooting up. After researchers weed out nontransformed tomato cells, they sprout the cells with souped-up plastids.

useful to do with tobacco," says retired Duke geneticist Nicholas Gillham.

Tobacco isn't an ideal host, however. For one, it grows in a restricted geographical area. But, more important, it would be difficult to extract proteins of interest from the plants, which produce other troublesome compounds such as nicotine.

Making a fruit or tuber with genetically transformed plastids would get around many of tobacco's limitations. The edible result would be big enough to contain large quantities of the compounds of interest. In 1999, Staub and his Monsanto colleagues produced potato plants with genetically modified plastids. But the tubers expressed the foreign genes at a concentration 100 times lower than that in the leaves, equivalent to levels achievable by nuclear transformation.

Engineered tomatoes have met with more success. Ralph Bock and his colleagues at

the University of Freiburg, Germany, spent more than 2 years developing transformation and regeneration conditions for a tomato. In the September 2001 issue of *Nature Biotechnology* they report proof of principle that the tomato fruit can be modified. The 1% expression levels the researchers achieved were fairly low, but on the bright side, the fruits produced fully half as much protein as the plants' green leaves. This suggests that the same tricks that help increase expression levels in tobacco leaves might lead to fruits that express engineered plastid proteins in large quantities. "These are first-generation expression levels," says Heifetz, who expects that the researchers will be able to improve the fruits' output.

Future harvests

Photosynthesis is very inefficient, turning less than 1% of the incoming solar radiation into food. If it could be slightly improved, the face of agriculture would change dramatically, as plants could grow bigger with less sunlight.

Until the invention of plastid transformation, scientists could not tinker with RuBisCO, the key carbon-fixing enzyme of photosynthesis, because half of its subunits are encoded in the chloroplast genome. Dozens of research groups have since tried to alter the molecule to improve the efficiency of photosynthesis, but none so far have succeeded (*Science*, 15 January 1999, p. 314).

Now, however, plant physiologists Spencer Whitney and T. John Andrews at Australian National University in Canberra have taken a key step toward this goal, creating the first viable plants with an altered RuBisCO. They report in the 4 December 2001

issue of the *Proceedings of the National Academy of Sciences* that photosynthetic efficiency drops predictably when the RuBisCO of tobacco plants is replaced with the less efficient RuBisCO from the red alga *Rhodospirillum rubrum*. Even though the plants are less efficient, not more, the advance is exciting, according to Maliga, because it is "the first time that [researchers have] actually changed the properties of the photosynthetic machinery in a predicted fashion."

Andrews ties the success of the study to the spread of the technical skills needed to conduct plastid transformation. "I think it hasn't been done before because the technology for plastid transformation is not that widely dispersed," says Andrews. As that knowledge becomes more widespread, the humble plastid may acquire mightier powers.

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