Among the more widely discussed biotech possibilities is altering the stomata, the porelike openings that stipple a plant's epidermis and control the in- and outtake of oxygen, carbon dioxide, and water. In most plants, the stomata are edged by two cells that resemble a pair of parentheses. When the plant takes in water, the stomatal cells swell open, allowing water to escape and permitting gas exchange; when the surroundings become drier or hotter, the stomata close. Because the stomata stay open longer than needed, most of the water that wheat and rice take in ends up in the atmosphere rather than being used in photosynthesis. "If you're irrigating, you might put up with the water loss in the name of getting the greatest biomass possible," says UC Davis's Loomis. "But if you're dry-land farming in Kansas, it might not be a good deal—you're using up water too fast."

To allow dry-land crops to use water more efficiently, stomata might be bioengineered to close more readily; in water-rich areas, they might be modified to stay open even longer. "That would give you better ventilation in the leaf, decreasing the canopy temperature and giving you better transport of CO_2, both of which could boost the rate of photosynthesis," says Fischer of the Australian Centre for International Agricultural Research.

Researchers have their eyes on two molecular targets that play a role in regulating the stomata: the plant hormone abscisic acid, which triggers closing, and an enzymatic process called farnesylation, which seems to impede ABA (*Science*, 9 October 1998, pp. 252, 287). By altering farnesylation, researchers may, in theory, be able to adjust plants' sensitivity to ABA and thus the tendency of the stomata to close. That task is daunting enough, but other researchers would like to go even further and tinker with the mechanisms of photosynthesis itself (see next story).

Many economists are confident that such efforts will eventually pay off and drive up crop yields again. But agronomists tend to view biotech as a long shot. Controlling such basic multigene traits, Fischer warns, is a "complex, unpredictable" task. Photosynthesis, notes Sinclair, is a process that evolution hasn't changed fundamentally "in a couple billion years." And even if the work is a technical success, the payoff may be minor, as traditional plant breeding has already pushed up crops' harvest index and ability to capture sunlight about as high as they can go. As Sinclair put it at the Irvine meeting, "Some of the hope for biotechnology seems analogous to the dreams of mechanical perpetual motion devices over a century ago: No matter how finely tuned the machine, reality does not allow output to exceed input."

Still, altering photosynthesis is "the great white hope" of the future of agriculture, as agricultural consultant Austin puts it. "All the relatively obvious steps have been taken. Photosynthesis is what's left."

Money woes

Re-engineering photosynthesis—or fundamentally improving crops in some other way—will require years of costly basic research, in Cassman's view. But a crucial source of support for agricultural science is eroding. For more than a century, according to Phil Pardey, an economist at IFPRI, government funding has supported long-term agricultural research. Although the biotech boom has spearheaded a recent massive increase in private-sector spending on agricultural R&D, notes Duvick, a former research director of agribusiness giant Pioneer Seeds, "even the big companies don't do a lot of long-term research."

But despite opposition from both the academic and corporate community, IRRI's budget in constant 1994 dollars has dropped from a high of $46.5 million in 1990 to $32.7 million in 1997, according to CGIAR figures. Similarly, CIMMYT's funding fell from $40.2 million in 1988 to $28.4 million in 1997. "We're taking away funding with the assumption that we've made it," says Dennis A. Ahlburg, a demographer at the London School of Hygiene and Tropical Medicine's Centre for Population Studies. "But if we don't continue to support [agricultural research], we'll slide backward."

"The scientific challenge [of feeding the world] has been grossly understated," Cassman says. "But even if I'm wrong, and we somehow can do it without special effort, I think you'd like to have a margin of security. … We are talking about the prospects for producing enough food to feed people in the next century, and a margin of security seems justified."

–CHARLES C. MANN

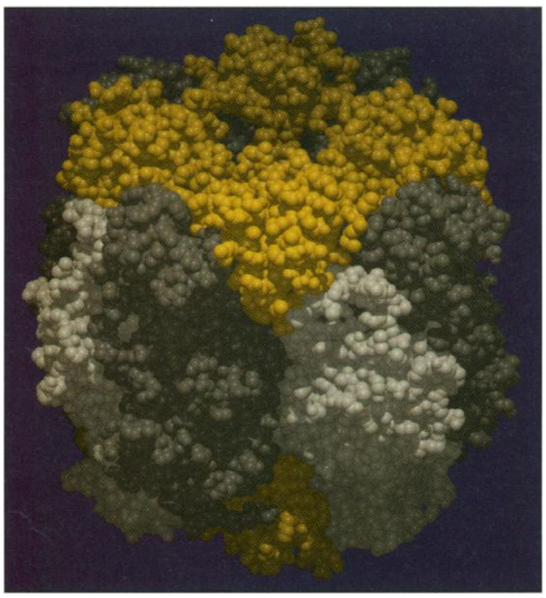
FUTURE FOOD

▶BIOENGINEERING

Genetic Engineers Aim to Soup Up Crop Photosynthesis

To improve crops' ability to turn atmospheric carbon into food, researchers hope to alter the principal enzyme or supercharge it with CO_2

Few nonbiologists may have heard of ribulose-1,5-bisphosphate carboxylase-oxygenase, the enzyme known as RuBisCO, but its importance is hard to overstate. The principal catalyst for photosynthesis, it is the basic means by which living creatures acquire the carbon necessary for life. By interacting with atmospheric carbon dioxide, RuBisCO—the world's most abundant protein—initiates the chain of biochemical reactions that creates the carbohydrates, proteins, and fats that sustain plants and other living things, ourselves included. But the enzyme also has another distinction, according to T. John Andrews, a plant physiologist at The Australian National University in Canberra: "RuBisCO is nearly the world's worst, most incompetent enzyme—it's almost certainly the most inefficient enzyme in primary metabolism that there is."



The enzyme that feeds the world. RuBisCO, which captures carbon dioxide and helps turn it into starches, sugars, and other compounds, is a target for genetic engineers.

RuBisCO's ineffectiveness has been a spur to scientists since it became fully apparent in the 1970s. Indeed, the quest for a better RuBisCO is "a Holy Grail in plant biology," says George Lorimer, a biochemist at the University of Maryland, College Park, who worked with the Swedish team that mapped the enzyme's structure in 1984. "Everyone always goes in with the hope of changing the face of agriculture." Despite more than 20 years of effort, the hopes have not yet paid

CREDIT: JOURNAL OF MOLECULAR BIOLOGY 259, 160 (1996)

off. But recent advances in molecular biology—and the unexpected discovery of more efficient RuBisCO in red algae—have given new impetus to the long struggle to modify the enzyme. In what may be the most ambitious genetic-engineering project ever tried, laboratories across the world are trying to improve the RuBisCO in food crops by either replacing the existing enzyme with the red algae form or bolting on what could be thought of as molecular superchargers.

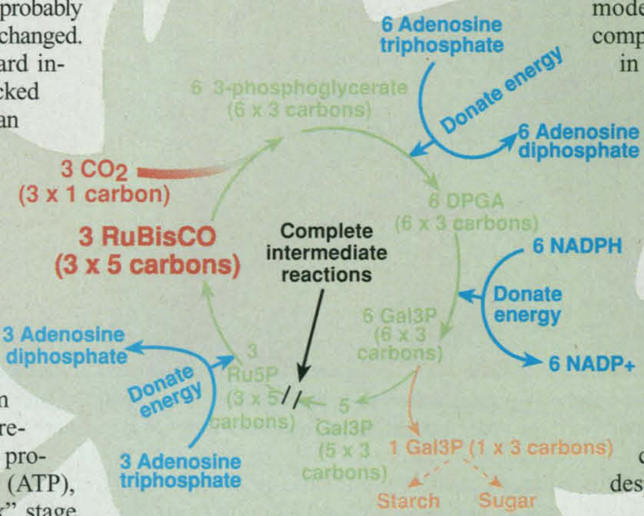
No one expects quick results—"I'm not for a second trying to minimize the task," says Andrews. The current state of the art in genetic engineering permits altering or splicing in single genes to improve a plant's resistance to pests, say, or to allow a crop to survive applications of weed-killing herbicide. To alter RuBisCO, by contrast, scientists must work with a 16-part molecule that is encoded by many genes in both the cell nucleus and the chloroplasts, where photosynthesis takes place. The enzyme also depends on a supporting cast of other enzymes, some of which will probably need to be revamped if RuBisCO is changed. But with many other avenues toward increasing crop yields seemingly blocked (see p. 310), RuBisCO has become an increasingly tempting target.

RuBisCO is just one actor in photosynthesis, which is a complex symphony of photochemical and enzymatic reactions. During the first, "light" stage of photosynthesis, chlorophyll—a green pigment in the chloroplasts—absorbs enough energy from sunlight to split off electrons from water molecules, simultaneously releasing oxygen gas and driving the production of adenosine triphosphate (ATP), which is used in the second, "dark" stage. This step begins when RuBisCO combines with carbon dioxide to produce 3-phosphoglycerate, or PGA (see diagram). Powered by energy from the ATP, a series of reactions transforms PGA into a host of starches, sugars, and other organic compounds.

Photosynthesis as a whole is not particularly efficient; a crop plant that stores as much as 1% of the total received solar energy is exceptional. As a result, the process offers many targets for bioengineers. But RuBisCO, far and away the biggest drag on the process, is the most appealing of them. First, it is torpid in the extreme—"perhaps the slowest known enzyme," William Ogren, a now-retired RuBisCO researcher from the University of Illinois, Urbana-Champaign, says with only slight exaggeration. Enzymatic rates are often on the order of 25,000 reactions per second; RuBisCO turnover in higher plants can be as little as two or three reactions per second. "Not one of evolution's finest efforts," says Ogren.

Second, RuBisCO triggers an additional reaction that interferes with the first. In 1971 Ogren and two other researchers discovered to their amazement that besides capturing and "fixing" carbon dioxide, RuBisCO catalyzes a second, opposing reaction. In what is called photorespiration, the enzyme combines with oxygen, rather than carbon dioxide, to create a compound that is subsequently converted partly into carbon dioxide. In other words, RuBisCO catalyzes one reaction that incorporates carbon into plants and another that ultimately strips them of carbon.

Typically, the RuBisCO in higher plants like rice and wheat is 100 times more likely to pick up CO_2 than O_2. But because the concentration of atmospheric O_2 is many times greater than that of CO_2, the greater affinity for CO_2 is largely canceled. As a result, 20% to 50% of the carbon fixed by photosynthesis is lost to photorespiration. "The oxygenation reaction is—as far as we can tell, and a lot of research over decades has gone into it—just a complete waste," says Andrews. "It doesn't do anything for the plant."



Virtuous cycle. The energy-carrying compounds ATP and NADPH, produced by photosynthesis, power a chain of reactions that begin when RuBisCO combines with carbon dioxide and ultimately produce starches and sugars.

SOURCE: HELENA CURTIS, *BIOLOGY* (WORTH PUBLISHERS, 1983)

This striking inefficiency was no handicap when photosynthesis first evolved 3 billion years ago, because the atmosphere was almost devoid of oxygen. After photosynthesis filled the air with oxygen and RuBisCO's weakness was revealed, it may have been too late for evolution to fix the problem, says Murray Badger, a RuBisCO specialist at The Australian National University. "It's a somewhat general correlation that the more specific and discriminatory a reaction becomes, the slower it gets," he says. As a result, mutations that made RuBisCO target carbon dioxide better might also have made it even slower.

If genetic engineers could find a way around RuBisCO's slowness and inefficiency, they might reap a double benefit. A faster, more efficient enzyme could help crops grow and increase their biomass, letting them produce more grain at a faster rate. In addition, explains Martin Parry of the Institute of Arable Crops Research-Rothamsted in Hertfordshire, Britain, RuBisCO's lethargy means that "plants need to invest incredibly heavily in it" to fix sufficient carbon. "A very large proportion of the plant's nitrogen requirements come from the need to produce the enzyme," which makes up as much as half the soluble protein in plant leaves. More efficient RuBisCO could thus lower crops' need for nitrogen, now mainly supplied by fertilizer in many countries.

A better RuBisCO. The discovery of photorespiration launched the effort to remodel RuBisCO. Researchers began by comparing the form of the enzyme found in higher plants with that in cyanobacteria—blue-green algae—which is even less efficient than the higher-plant version. To find out why, Lorimer's group, then based at DuPont, collaborated with Carl Branden's x-ray crystallography group in Sweden to determine the molecular structures of both forms, hoping to find telling differences. "We spent years creating high-resolution structures of spinach [a model plant in RuBisCO research] and cyanobacteria," says Lorimer. But despite the finely detailed results, "the sobering reality was that you can lay down [structural maps of] these two enzymes on top of each other and you're very hard pressed to see the difference." Even if the differences could be identified, Lorimer believes, they would be so numerous and subtle "that you could not rationally reason your way to what it was that you would need to improve the enzyme."

The failure of the structures to provide a path for modifying RuBisCO dismayed many researchers; Lorimer's group disbanded. Hopes reawakened in 1992, when F. Robert Tabita and B. R. Read of Ohio State University in Columbus discovered that some diatoms and red algae have more-specific RuBisCO than that in higher plants. In 1997, a team led by Akiho Yokota, a plant molecular physiologist at the Research Institute for Innovative Technology for the Earth, in the Keihanna Science City near Osaka, Japan, found red algae with RuBisCO that is about three times more efficient. "We've looked at a lot of the red algae," Tabita says, "and the trend is always the same, 2 1/2 to threefold higher than normal plants." No one yet knows why.

To try to exploit this advantage, Andrews's group is one of several that are attempting to insert RuBisCO genes from red-algal chloroplasts into the chloroplasts of higher plants, using techniques for manipulating chloroplastic DNA developed by Rutgers University biochemist Pal Maliga. "If it can be done, it would be really amazing," says Yokota, who also works at the Nara Institute of Science and Technology, in Kansai Science City, near Nara, Japan. Other groups are working on related approaches at Rothamsted, Ohio State, and the University of Nebraska, Lincoln.

"This is a little bit like transferring a V-8 engine from a big automobile into a small car," says Andrews. "It may not work." Even if the enzyme functions in its new, transgenic home, he cautions, "it's not enough simply to get the RuBisCO in there; it has to be assembled and produced in the right form, and also be connected to the regulation system that the chloroplast keeps control of RuBisCO with." Andrews hopes to see results in "about 10 years."

Fired-up photosynthesis. Red algae like this one have a RuBisCO that is as much as three times more efficient than the enzyme in green plants.

Supercharging photosynthesis. While most researchers trying to modify the genetic basis of photosynthesis are focusing on RuBisCO, a few are trying another, perhaps even more ambitious, strategy. Just as small engines can go faster if they are equipped with a supercharger, which force-feeds them with fuel, some plants have their own photosynthetic supercharger, known technically as the C_4 cycle. In C_4 plants, the bundle cells where photosynthesis takes place are surrounded by specialized "mesophyll" cells, which temporarily fix carbon dioxide and jam it into the bundle cells at such high concentrations that the oxygen reaction is effectively blocked. The C_4 cycle requires so much energy that C_4 plants cannot grow in dim light, but in the right, well-illuminated conditions, C_4 crops like sugarcane photosynthesize more efficiently than any others. About 5% of all terrestrial higher-plant species use the C_4 cycle; maize is economically the most important.

A joint team at Japan's National Institute of Agrobiological Resources and Nagoya University led by Nagoya microbiologist Makoto Matsuoka is now attempting to reproduce the C_4 cycle in rice. For the transformation to succeed, a host of altered enzymes would have to work together properly, and the plant's structure may have to be changed to create the equivalent of mesophyll cells. As a result, the project may well be the most fundamental genetic alteration that humankind has ever tried in any organism. "Don't hold your breath," Lorimer says.

Indeed, Matsuoka cautions, "I don't think we can really make a true C_4 [rice] plant." Rather than transferring the whole genetic structure for the C_4 cycle from, say, maize into rice, his team is trying to identify nonfunctioning equivalents of C_4-type genes in rice and selectively replace them with their active counterparts from maize.

In a paper in press at *Nature Biotechnology*, Matsuoka's group reports taking a first step by replacing three silent rice genes with their more lively equivalents in maize, including the important enzyme phosphoenolpyruvate carboxylase (PEPC), which catalyzes the beginning of the C_4 cycle. "We succeeded in getting [PEPC] highly expressed in a rice plant," Matsuoka says. "This is a world first." After transferring each gene to a different rice plant, the group is now crossing the results to obtain rice that produces all three enzymes.

That may not be enough to replicate C_4-like photosynthesis in rice, says Matsuoka. Rice has mesophyll-like cells that are not photosynthetically active, and these may have to be activated. And some of the changes may actually be deleterious—the transgenic rice with only the C_4 enzyme NADP-malic tends to die quickly, for example. Still, Matsuoka says, preliminary evidence suggests that active PEPC in rice cuts the destructive oxygen reaction by about a third.

Even as the work to alter photosynthesis begins to gain momentum, some critics question whether it will benefit agriculture. Since at least 1970, research has shown little correlation between crops' photosynthesis rates and their yields, suggesting that improvements in RuBisCO won't automatically translate into better harvests. But according to Steven P. Long, a plant physiologist at the University of Essex in the U.K., the correlation may simply be hidden by the propensity of higher yielding cultivars to have bigger leaves, which increases the amount of self-shading and thus lowers the mean photosynthetic rate. When he and his colleagues temporarily boosted photosynthesis rates in wheat by flooding open fields with enough CO_2 to increase local atmospheric levels by 50%, grain yield went up 10% to 12% in two consecutive growing seasons.

Long is more skeptical about the value of importing the C_4 cycle into crops like wheat and rice. Because the C_4 cycle imposes a high energy cost on the plant's metabolism, it only pays off at higher temperatures—that's why there is no winter maize crop. "You can model it fairly easily," Long says. "Below 28°C, the [standard photosynthesis] is more effective, and above 28°C the C_4 is more efficient." The payoff threshold will rise even higher as the atmosphere's CO_2 concentration increases because of human activity. And if scientists like Andrews succeed in increasing the specificity of RuBisCO, the threshold for adding the C_4 cycle will go still higher—perhaps to 40°C, he says. "There'd be no point in going to C_4 then."

Nonetheless, Long favors working on both approaches. "These are such major steps that we don't even know how many unknowns there are in doing this. Pursuing all options is well worthwhile."

"We've now reached the limits of what we can do [with conventional breeding]," Rothamsted's Parry says. "So therefore we have to solve the next problem, which is putting in a bigger engine." It's a challenge, he observes, "with considerable practical interest." **–CHARLES C. MANN**

With reporting by Dennis Normile in Tokyo.

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