PERSPECTIVES: PLANT BIOLOGY

The Green Revolution Strikes Gold

Mary Lou Guerinot

For millennia, breeders have concentrated on modifying the traits of plants to influence their growth performance in the field. The late 20th-century version of this effort is the production of transgenic plants. Crops such as Roundup Ready soybeans developed by Monsanto and corn expressing *Bacillus thuringiensis* (Bt) toxin reduce costs to the farmer by minimizing the application of herbicides and insecticides. Other genetically engineered traits increase the cash value of a crop providing us, for example, with canola plants that produce oils high in unsaturated fatty acids. However, the crops that would make the biggest difference for the largest number of people in the world are those that would serve as better sources of essential nutrients. Because extreme poverty continues to limit access of much of the world's population to food, it is important that affordable food be as nutritious as possible. The report on page 303 of this issue by Ye *et al.* (*1*), who engineered rice grains to produce provitamin A (β-carotene), exemplifies the best that agricultural biotechnology has to offer a world whose population is predicted to reach 7 billion by 2013.

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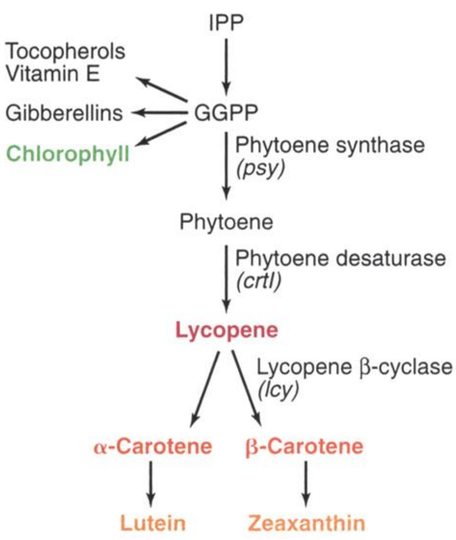
Although half of the world's population eat rice daily and depend on it as their staple food, rice is a poor source of many essential micronutrients and vitamins. In Southeast Asia, 70% of children under the age of five suffer from vitamin A deficiency, leading to vision impairment and increased susceptibility to disease (*2*). UNICEF (United Nations Children's Fund) predicts that improved vitamin A nutrition could prevent 1 to 2 million deaths each year among children aged 1 to 4 years (*3*).

How, then, can one improve the provitamin A content of rice? Mammals make vitamin A from β-carotene, which is one of the most abundant carotenoids found in plants. Carotenoids are yellow, orange, and red pigments that are essential components of the photosynthetic membranes of all plants. They serve as accessory light-harvesting pigments and as antioxidants that quench tissue-damaging free radicals such as singlet oxygen species. Rice in its milled form contains neither β-carotene nor any of its immediate precursors. In their successful bid to engineer rice to produce β-carotene, Ye and colleagues have been greatly aided by recent progress in dissecting the carotenoid biosynthetic pathway (*4*, *5*).

Carotenoids, along with a variety of other compounds including gibberellins, sterols, chlorophylls, and tocopherols, are derived from the general isoprenoid biosynthetic pathway (see the figure, below). Immature rice endosperm synthesizes the carotenoid precursor geranyl geranyl diphosphate (GGPP) (*6*). To convert GGPP to β-carotene, Ye *et al.* programmed the endosperm to carry out the necessary additional enzymatic steps. Two molecules of GGPP must first be condensed to form phytoene, which is then desaturated to lycopene and finally cyclized to form β-carotene. The successful introduction of three genes that encode the additional enzymes is a technical tour de force. Most traits engineered to date have only required the introduction of a single gene. The authors also needed to ensure that the introduced genes would only be expressed in the endosperm as this is the part of the rice grain that remains after polishing.

Most remarkably, the investigators successfully introduced two of the required genes (encoding phytoene synthase and phytoene desaturase) on a construct that did not have a selectable marker. They achieved this by simultaneously introducing another construct, which carried the third gene of interest (lycopene β-cyclase) as well as a selectable antibiotic resistance gene. This cotransformation strategy should enable Ye *et al.* to segregate the antibiotic resistance gene away from the phytoene synthase and phytoene desaturase genes, thereby addressing one of the major concerns of opponents of genetically engineered crops. Such plants should still be able to produce β-carotene because the authors have also shown that plants engineered with standard transformation procedures to express only the phytoene synthase and phytoene desaturase genes do not accumulate lycopene as predicted (see the figure, below). Instead these plants produce essentially the same end products (β-carotene, lutein, and zeaxanthin) as plants engineered to express all three carotenoid genes. The authors speculate that the enzymes necessary to convert lycopene into β-carotene, lutein and zeaxanthin are constitutively expressed in normal rice endosperm or are induced when lycopene is formed. In addition, the fact that rice plants normally do make carotenoids should go a long way toward calming fears about "Frankenfoods." Perhaps the only objection to golden rice, in the end, will be its color.

There is plenty of work still to do. Initial calculations suggest that these engineered plants can provide enough provitamin A to satisfy the recommended dietary allowance with a daily ration of rice. But only when true-breeding lines are available will it be possible to accurately determine levels of each type of



Turning carotene into gold. The carotenoid biosynthetic pathway of plants. Carotenoids are synthesized in the central isoprenoid pathway within plastids. All isoprenoids (more than 20,000 different compounds exist in plants) are built from the common precursor isopentenyl diphosphate (IPP). IPP is thought to be synthesized in plastids from pyruvate and glyceraldehyde-3-phosphate. The first committed step in the carotenoid pathway is the head-to-head condensation of two molecules of geranyl geranyl diphosphate (GGPP) to produce phytoene. Phytoene synthase is encoded by *psy*, phytoene desaturase by *crtI*, and lycopene β-cyclase by *lcy*. Golden rice engineered to produce β-carotene accumulates lutein and zeaxanthin as well.

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Home with the harvest. Young girl carrying rice in eastern Bhutan.

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carotenoid. The production of various carotenoids other than β-carotene could provide additional health benefits as carotenoids have been implicated in reducing the risk of certain types of cancers, cardiovascular disease, and age-related macular degeneration. Fortunately, excess dietary β-carotene, in contrast to excess vitamin A, has no harmful effects, so plants with enhanced β-carotene content should be a safe and effective means of vitamin delivery.

Field-testing will tell us whether production of carotenoids in rice endosperm will entail any metabolic trade-offs. Shunting more of the common precursor GGPP into carotenoid production might result in a decrease in other compounds whose synthesis is dependent on GGPP. For example, tomatoes engineered to produce more phytoene exhibit signs of dwarfism, attributed to a 30-fold reduction in the plant hormone gibberellic acid, which shares the precursor GGPP with phytoene (*7*). However, unlike tomato plants that express phytoene synthase in all their tissues, the rice plants engineered by Ye *et al.* express the introduced phytoene synthase only in the endosperm, which reduces the potential for metabolic disruption throughout the plant.

Presumably, it should be possible to engineer the pathways for many of the 13 essential vitamins into plants, once the pathways are known and the corresponding genes have been cloned (*8*). Indeed, the model plant *Arabidopsis* has already been successfully engineered to synthesize vitamin E (*9*). Improving the mineral content of plants so that they can serve as sources of the 14 minerals required in the human diet presents researchers with a different set of challenges (*8*). Unlike vitamins, which are synthesized by the plants themselves, plants must take up essential minerals from the soil. Iron deficiency is the leading nutritional disorder in the world today, affecting over 2 billion people. As with vitamins, many of the world's staple foods are not good sources of iron. Current efforts are centered on understanding how plants take up and store iron (*10*, *11*). Rice has been engineered to have higher levels of the iron storage protein ferritin in the grain (*12*), but the question remains as to whether these engineered rice plants will be a good source of dietary iron.

The road to better nutrition is not paved with gold and, hence, agribusiness has not centered its efforts on the nutritional value of food. The work that culminated in the production of golden rice was funded by grants from the Rockefeller Foundation, the Swiss Federal Institute of Technology and the European Community Biotech Program. Like the plant varieties that made the green revolution so successful, the rice engineered to produce provitamin A will be freely available to the farmers who need it most. One can only hope that this application of plant genetic engineering to ameliorate human misery without regard to short-term profit will restore this technology to political acceptability.

References and Notes

1. X. Ye *et al. Science* **287**, 303 (2000).
2. www.who.int/nut/
3. www.unicef.org/vitamina/
4. F. X. Cunningham and E. Gantt, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **49**, 557 (1998).
5. J. Hirschberg, *Curr. Opin. Biotechnol.* **10**, 186 (1999).
6. P. K. Burkhardt *et al., Plant J.* **11**, 1071 (1997).
7. R. G. Fray, A. Wallace, P. D. Fraser, D. Valero, P. Hedden, *Plant J.* **8**, 693 (1995).
8. M. A. Grusak and D. DellaPenna, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50**, 133 (1999); D. DellaPenna, *Science* **285**, 375 (1999).
9. D. Shintani and D. DellaPenna, *Science* **282**, 2098 (1998).
10. D. Eide, M. Broderius, J. Fett, M. L. Guerinot, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 5624 (1996).
11. N. J. Robinson, C. M. Proctor, E. L. Connolly, M. L. Guerinot, *Nature* **397**, 694 (1999).
12. F. Goto, T. Yoshihara, N. Shigemoto, S. Toki, F. Takaiwa, *Nature Biotechnol.* **17**, 282 (1999).

13. I thank R. McClung and J. Hirschberg for assistance in preparing this perspective.

PERSPECTIVES: PLUTONIUM CHEMISTRY

Toward the End of PuO_2's Supremacy?

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Owing to the ubiquitous presence of oxygen in the terrestrial environment, oxides occupy a central position in the chemistry of many elements. This is true not only for natural elements but also for artificial elements, particularly for the most famous one: plutonium. On page 285 of this issue, Haschke *et al.* (*1*) demonstrate convincingly that the supremacy of plutonium dioxide (PuO_2), long thought to be the most stable plutonium oxide under oxidizing conditions, is over. The results have implications for both military and civilian applications and for the long-term storage of plutonium.

The element plutonium was first created in December 1940 at the University of Berkeley, California, by a team of American scientists headed by Glenn T. Seaborg (*2*). During the summer of 1942, Cunningham and Werner (*3*) prepared a weighable amount of a solid plutonium compound, PuO_2 (2.77 μg). Thus, for the first time in human history, an artificial element was made visible to human eyes. This historical sample of PuO_2 is still kept at the Lawrence Hall of Science in Berkeley, California (*4*). Humanity became inescapably aware of the implications of these discoveries at the end of World War II: The atomic bomb that destroyed Nagasaki, Japan, on 9 August 1945 was made of plutonium prepared as part of the Manhattan Project.

It was soon recognized that plutonium chemistry is dominated by the existence of numerous oxidation states, from +III to +VI. In 1967, Russian scientists discovered that Pu(VII) can also exist (*5*). Despite the fact that plutonium thus possesses five oxidation states (III to VII), its oxide chemistry is far simpler. According to most textbooks (*4*, *6–8*), the plutonium-oxygen phase diagram contains the following crystalline solid oxides: $PuO_{1.50}$, $PuO_{1.52}$, $PuO_{1.61}$, PuO_{2-x}, and $PuO_{2.00}$, all of which involve only plutonium oxidation states III and IV. No plutonium oxide with an O/Pu stoichiometry higher than 2 was observed, despite numerous attempts to prepare PuO_3, the oxide corresponding to Pu(VI) (*4*).

It has therefore been assumed for

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