A welcome diversion from photorespiration

Richard C. Leegood

Suppressing conventional photorespiration in *Arabidopsis thaliana* increases biomass.

All life depends on photosynthetic carbon fixation, yet for most plants, this is a fundamentally inefficient process. Photosynthetic rates in C_3 plants are reduced by photorespiration, a competing pathway that fixes oxygen and releases as CO_2 around a quarter of the carbon fixed by photosynthesis[1]. In this issue, Kebeish *et al.*[2] show that photorespiratory losses in *Arabidopsis thaliana* can be alleviated by introducing into chloroplasts a bacterial pathway for the catabolism of the photorespiratory substrate, glycolate. The associated increases in photosynthesis and productivity observed in this model system suggest a promising strategy to increase productivity of C_3 crops such as wheat and rice.

The inefficiency of C_3 photosynthesis can be attributed largely to one enzyme. Rubisco—the primary carboxylase in most photo- and chemoautotrophs—catalyzes two competing reactions, carboxylation and oxygenation, the extent of which depends on the relative concentrations of CO_2 and O_2, as well as on temperature. Carboxylation leads to net CO_2 fixation, whereas oxygenation generates glycolate, which can be metabolized only outside chloroplasts by photorespiratory events in peroxisomes and mitochondria (**Fig. 1**).

Kebeish *et al.*'s diversionary tactic takes advantage of a fundamental difference between bacterial and plant enzymes capable of glycolate catabolism: whereas glycolate dehydrogenase (GDH) from *Escherichia coli* uses NAD^+ as an electron acceptor to oxidize glycolate to glyoxylate, plant glycolate oxidases use molecular oxygen and must be contained in peroxisomes to avoid the disastrous consequences of releasing hydrogen peroxide into metabolically active cell compartments. The authors first target the three subunits of GDH to *A. thaliana* chloroplasts and then introduce glyoxylate carboligase (GCL) and tartronic semialdehyde reductase (TSR) to complete the pathway that converts glycolate to glycerate in parallel with the endogenous photorespiratory pathway (**Fig. 1**).

The results of this manipulation, involving expression of five transgenes, are striking. The transformants have enhanced rates of CO_2 fixation, growth and biomass production, and these differences are eliminated by growth in elevated CO_2 concentrations that suppress photorespiration. Promotion of growth is particularly evident when plants are shifted to stress conditions, such as high temperature or strong light, which increase photorespiratory flux. The effects are apparent in the plants expressing GDH and are enhanced in plants that also express GCL and TSR.

What mechanisms might underlie this increase in plant productivity? Introducing the bacterial pathway for glycolate catabolism into plastids likely shifts at least some mitochondrial CO_2 release to chloroplasts, which could raise CO_2 concentrations in the vicinity of rubisco and enhance carboxylation relative to oxygenation. Although the ability of chloroplasts to accumulate CO_2 has been questioned because they are rather leaky to gases[3], the transgenic lines have a lower CO_2 compensation point—the CO_2 concentration at which the rate of CO_2 uptake equals the rate of CO_2 efflux from photorespiration. Along with the decreased inhibition of photosynthesis by O_2 that was observed, this is consistent with reduced release of photorespiratory CO_2 in the transformants. A smaller post-illumination CO_2 burst in transformants is also consistent with smaller pools of photorespiratory intermediates in the light.

Interestingly, a glycolate pathway similar to the one engineered by Kebeish *et al.* already exists in nature, in *Synechocystis sp.* strain PCC 6803 and possibly other cyanobacteria[4]. How this pathway interacts with normal photorespiration in these organisms is not well understood. In *A. thaliana*, the authors' findings, including assessment of CO_2 release from glycolate by isolated chloroplasts, suggest that flux through the engineered pathway is appreciable compared with that through the endogenous photorespiratory pathway.

Although formally, the engineered glycolate shunt is a photorespiratory pathway because it requires rubisco's oxygenase activity and releases

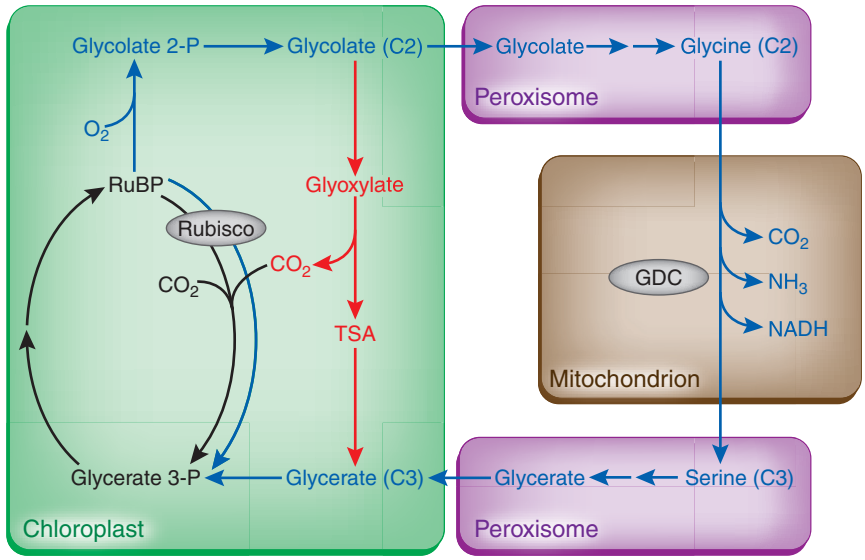

Figure 1 A simplified, nonstoichiometric representation of the normal photorespiratory pathway of C_3 plants in relation to a chloroplastic bypass for catabolism of glycolate to glycerate. Carboxylation of RuBP by rubisco generates glycerate 3-P for use within the Benson-Calvin cycle, whereas oxygenation of RuBP forms glycolate 2-P. Black arrows and metabolites indicate rubisco carboxylation and the Benson-Calvin cycle within the chloroplast, whereas blue arrows and metabolites depict the normal photorespiratory pathway using glycolate, which ultimately converts two C2 molecules into one C3 molecule (glycerate 3-P) with the loss of CO_2 and ammonia. Red arrows and metabolites indicate the glycolate catabolic pathway from *E. coli* engineered into the *A. thaliana* chloroplast. GDC, glycine decarboxylase; RuBP, ribulose 1,5-bisphosphate; rubisco, ribulose 1,5-bisphosphate carboxylase-oxygenase; TSA, tartronic semialdehyde.

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CO_2, its ability to generate energy distinguishes it from the endogenous photorespiratory pathway that consumes reductant and ATP[5]. Sidestepping the mitochondrial and peroxisomal photorespiratory reactions generates NADH while eliminating the expenditure of ATP and reductant normally required to refix ammonia by the glutamine synthetase/glutamate synthase cycle[6] (**Fig. 1**). However, as high fluxes of the engineered shunt might lead to redox imbalance within the chloroplast, a trade-off for the superior nitrogen-use efficiency and reduced energy consumption might be that it diminishes the role that photorespiration could play in the dissipation of excess energy as a stress response to adverse environmental conditions[5].

The work of Kebeish *et al.* is the latest of several efforts to diminish the impact of photorespiration. These include attempting to improve the efficiency of rubisco by rational engineering or by prospecting for more efficient natural variants[7] and concentrating CO_2 around rubisco by emulating C_4 plants or expressing a cyanobacterial bicarbonate transporter[8]. However, the engineering of C_4 photosynthesis into C_3 plants, such as rice[9,10], is inherently more ambitious than introducing the glycolate shunt as it requires the engineering of a photosynthetic pathway in two cell types, as well as structural modifications to reduce CO_2 leakage from the vicintity of rubisco, a problem that must always be considered in engineering CO_2-concentrating mechanisms.

The potential rewards of taming photorespiration are enormous. Enabling photosynthesis to operate at lower leaf CO_2 concentrations would increase the efficiency of using water (through reduced stomatal transpiration) and nitrogen (through more efficient use of rubisco, the most abundant protein in leaves). Improved CO_2 fixation could, therefore, not only increase the productivity of crops to meet the world's ever-increasing demands for food and fuel, but also simultaneously decrease consumption of water, fertilizer and land[9,10].

COMPETING INTERESTS STATEMENT
The author declares no competing financial interests.

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Metagenomic sorcery and the expanding protein universe

Eugene V Koonin

A metagenomic study of the Atlantic and Pacific oceans reveals dominant bacterial species, remarkable microdiversity and a wealth of new proteins.

The magisterial series of three articles published recently by J. Craig Venter and colleagues[1–3] in *PLOS Biology* establishes a new milestone for metagenomics. The previous groundbreaking work from this[4] and other groups[5,6] notwithstanding, the scale of the new study is such that for the first time we can begin to contemplate the planet-wide diversity of prokaryotic life in an important habitat.

The *Sorcerer II* expedition traveled from Nova Scotia in the Northwestern Atlantic to Hawaii in the tropical Pacific, producing an unprecedented 6.25 gigabases of prokaryotic DNA sequence from surface water samples collected at 41 locations. Fittingly, Venter and colleagues note that they drew their inspiration from the famous *HMS Challenger* survey of 1872–1876, which explored the worldwide diversity of macroscopic marine life. As 10 of their 41 samples were collected off the Galapagos Islands, one cannot help but think also of the voyage of the *HMS Beagle*. It took Darwin 20 years to transform the observations made during this voyage, particularly in the Galapagos Islands, into his sweeping vision of evolution by natural selection. Similarly, working out the full implications of the *Sorcerer II* study will take time. Nevertheless, the wealth of information and analytic results in the three papers already convey the general significance of these studies.

The overarching theme is diversity—within bacteria, viruses and protein families. The first paper[1] addresses genomic diversity. At the level of bacterial ribotypes (that is, distinct classes of prokaryotic sequences defined by 16S rRNA comparison and corresponding roughly to species) and, especially, higher taxa, the extent of diversity found was, in fact, relatively low. Altogether, 811 ribotypes were observed, about half of which are novel. However, only 60 ribotypes were abundant (together accounting for >70% of the 16S RNA sequences), and all but one of these had been described previously. Furthermore, ~70% of the sequence reads aligned to one or more of the 584 prokaryotic genomes available from public databases at the time of the analysis.

Considering the relatively low sensitivity of the assignment method (BLAST with nucleotide sequences[7]), it therefore seems that, at the level of the ribotype, most of the ocean's microbial diversity is already represented in genomics databases. That unknown prokaryotes, at least near the ocean's surface, largely fall within known groups might come as a surprise to many microbiologists given that most bacteria cannot currently be cultivated.

The *Sorcerer II* results suggest that the distribution of identifiable bacteria is strikingly nonuniform. The entire genomic landscape is dominated by only five bacterial genera, which together account for ~97% of the sequences that can be aligned to available genomes. More than 60% of these sequences are from *Pelagibacter* alone—a striking case of domination of the microbial communities by a select few (**Fig. 1a**).

The relative lack of diversity at the higher taxonomic levels contrasts with the extensive diversity within each ribotype. Quite strikingly, even though sequences aligning to many parts of the reference genomes of the abundant bacteria were sequenced hundreds of times over, very few identical sequences were found, suggesting that no two cells that contribute to the metagenome have identical genome sequences. Nevertheless, the diversity within ribotypes was shown by phylogenetic analysis to have a clear structure of distinct subtypes. Furthermore, there seems to be considerable variability in the gene composition of the abundant genomes, and each includes gaps (regions to which no or few environmental sequences are assigned) that are likely to be hotspots of horizontal gene transfer. Taking their cue from the *Beagle* naturalist and in accord with the theoretical predictions of intense selection acting on large populations[8], Venter and colleagues suggest that the diversity is largely adaptive: the different subtypes have distinct roles in the ecosystem and their combined presence provides the system with stability and robustness.

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