PLANT SCIENCE

Towards turbocharged photosynthesis

The development of tobacco plants that are genetically engineered to produce a more efficient form of Rubisco, an enzyme involved in photosynthesis, marks a step towards increasing crop yields. SEE LETTER P.547

G. DEAN PRICE & SUSAN M. HOWITT

As the world's population increases, the spectre of severe food shortages is growing, with the United Nations predicting[1] that food production will need to double by 2050. It has been proposed that cyanobacteria — which obtain their energy from a highly efficient form of photosynthesis — might hold the key to increasing the yield of our most important crops and vegetables. On page 547 of this issue, Lin *et al.*[2] report a major step towards realizing this possibility, finding that cyanobacteria can be used to improve photosynthesis in the leaves of crops.

Photosynthesis harnesses sunlight to convert carbon dioxide into simple sugars. Rubisco, the key enzyme for CO_2 fixation into sugar, is inefficient because it cannot easily discriminate between oxygen and CO_2 and so wastes energy by fixing O_2. The enzyme evolved at a time when O_2 levels in the atmosphere were much lower than they are today, and there was therefore little evolutionary pressure to select for an ability to discriminate between the two molecules. Photosynthetic organisms have evolved to circumvent the problem of rising atmospheric O_2 levels in two ways: first, by making more of a slower-acting version of Rubisco with an improved ability to discriminate; or second, by using various 'add-ons', called CO_2-concentrating mechanisms (CCMs), to elevate CO_2 levels in the vicinity of the enzyme.

Most crops have adopted the first strategy, making Rubisco possibly the most abundant enzyme on Earth. This approach, however, results in a 30% reduction in photosynthetic efficiency through the associated O_2 fixation. That can be partly ameliorated

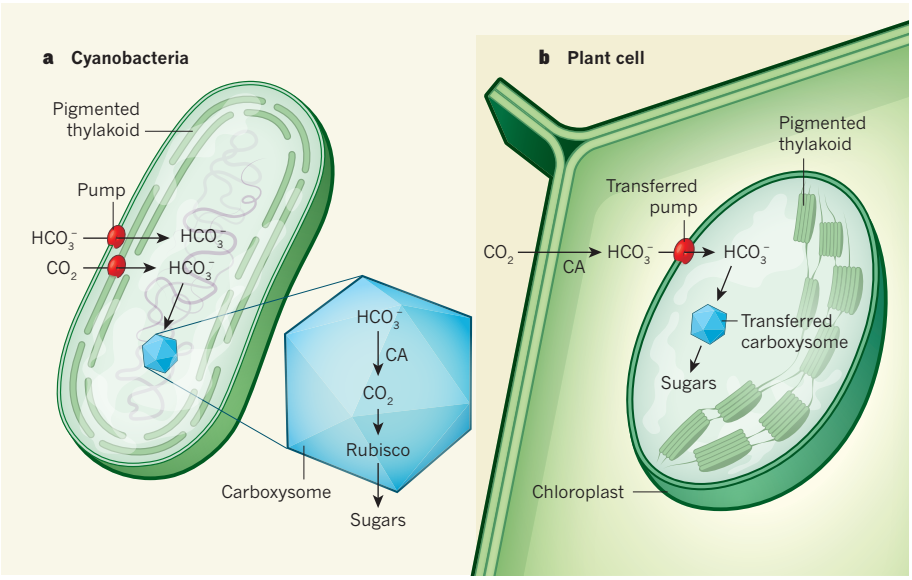

Figure 1 | A proposed method for improving photosynthesis in crops. **a**, In cyanobacteria, a carbon dioxide-concentrating mechanism (CCM) ensures that photosynthesis is effective (the energy for photosynthesis is provided through light harvesting by structures called pigmented thylakoids). The CCM includes pumps that take up CO_2 and bicarbonate (HCO_3^-) molecules; the CO_2 pump also converts CO_2 to HCO_3^-, which then enters microstructures called carboxysomes. Here, HCO_3^- is converted to CO_2 by the enzyme carbonic anhydrase (CA), elevating CO_2 concentrations around the Rubisco enzyme to increase the efficiency with which it converts CO_2 into sugars. **b**,Transfer of cyanobacterial carboxysomes or CCM pumps into plant cells has been posited as a way to improve crop yields. Lin *et al.*[2] took us a step closer to achieving this aim by replacing Rubisco in the chloroplasts of tobacco plants with a more efficient, cyanobacterial form of the enzyme. In the plant-cell cytoplasm, CA can also convert CO_2 to HCO_3^-.

50 Years Ago

The capacity exhibited by all organisms to develop tolerance for new environmental conditions has been an important factor in the continuing existence of life on Earth. It has also had adverse effects on some creatures. To man, one of the most disturbing of these effects is being manifested through the resistance certain microbes develop for the 'wonder' drugs he uses to counteract microbial diseases. The peculiar capacity of pathogenic micro-organisms to develop strains which are resistant to drugs which on initial application are lethal to most individuals — and eventually to thrive on those drugs — did not come into clear focus until Paul Ehrlich *et al.* made their famous chemotherapeutic researches during the first decade of this century.
From *Nature* 26 September 1964

100 Years Ago

The council of the Senate of the University of Cambridge has offered to professors, teachers, and students of the University of Louvain such facilities in the way of access to libraries, laboratories, and lectures, together with the use of lecture-rooms, as may secure the continuity of the work of that University during the present crisis. Hospitality in the way of living accommodation and so forth will probably be offered by the individual colleges and by private residents. The professors of the University of Oxford have offered a home for the winter to the young children of the professors of the University of Louvain; and the academic staff of University College (University of London) offers hospitality to about seventy members of French and Belgian universities, whether professors, teachers, or students, men or women, who may find it necessary to take refuge in this country.
From *Nature* 24 September 1914

by raising CO_2 levels around the leaf[3] in a manner conceptually similar to adding a CCM. There is currently increased focus on the second strategy — if a CCM could be introduced into crops, it might turbocharge photosynthetic CO_2 fixation. CCMs have evolved independently in cyanobacteria, microalgae and some plants (mostly those regarded by us as weeds). Although several types of CCM are being considered for introduction into crops, Lin and colleagues' work focuses on the cyanobacterial CCM.

This CCM involves a series of membrane-based pumps for CO_2 and bicarbonate (HCO_3^-), and special microcompartments called carboxysomes, which contain Rubisco[4]. HCO_3^- is pumped into the cell, then converted to CO_2 in the carboxysomes by the enzyme carbonic anhydrase (Fig. 1). The resulting high local CO_2 concentrations increase Rubisco efficiency, and so almost eliminate O_2 fixation[4]. Furthermore, thanks to the CCM, cyanobacteria have retained an ancient form of Rubisco that is almost three times as efficient as that found in most crops[5].

Lin *et al.* engineered tobacco plants to express a functional cyanobacterial form of Rubisco. This enzyme usually consists of a complex of eight large subunits and five to eight small subunits. The authors replaced DNA that encodes the large subunit of Rubisco in the tobacco plant with that encoding the cyanobacterial enzyme, ensuring that the photosynthesis and growth they observed occurred as a result of the introduced Rubisco, rather than the native version. This DNA is located in the cells' photosynthesizing factories, structures called chloroplasts.

Lin and colleagues' approach differs from those of earlier, unsuccessful efforts[6] in several ways; most notably, the authors co-expressed the cyanobacterial Rubisco with proteins that are involved in the enzyme's assembly. They found that co-expression of cyanobacterial Rubisco with either the RbcX chaperone protein (which helps protein folding) or a carboxysomal protein called CcmM35 (a Rubisco-organizing protein) were equally effective at forming functional Rubisco. However, the latter approach produced large complexes of Rubisco, which seemed to be related to those that form during the assembly of pre-carboxysomes in cyanobacteria. This is because CcmM35 mimics three of Rubisco's small subunits and so is incorporated into Rubisco. But the protein also crosslinks to other Rubisco complexes, producing enzyme aggregates[4].

The authors did not demonstrate whether the addition of CcmM35 or RbcX was the pivotal step in successfully expressing cyanobacterial Rubisco in tobacco, or whether other elements of the experimental design provided the crucial advantage. Earlier this year, the same group showed that co-expression of several carboxysomal shell proteins in chloroplasts can produce structures suggestive of carboxysome self-assembly[7]. Thus, prospects for building functional carboxysomes in tobacco-plant chloroplasts are now quite good. However, extending this to crops would be greatly aided by the development of technologies for altering the chloroplast genomes of key crop species.

In the past two years, the sequence of events required to build a cyanobacterial CCM in the chloroplast has been identified in detail[8,9]. Stand-alone addition of cyanobacterial Rubisco, or even of carboxysomes, to chloroplasts provides no obvious advantage. In fact, Lin *et al.* show that their modified plants survive only at high CO_2 concentrations. To provide an advantage, both CO_2 and HCO_3^- pumps are required, to elevate HCO_3^- levels in the chloroplast and so turbocharge CO_2 levels in the carboxysomes. And even when these remaining steps have been achieved in model plants such as tobacco, improved crops are still some way off. However, this work is a milestone on the road to boosting plant efficiency. The advance can be likened to having a new engine block in place in a high-performance car engine — now we just need the turbocharger fitted and tuned. ■

G. Dean Price *and* **Susan M. Howitt** *are in the Research School of Biology, Australian National University, Canberra, ACT 0200, Australia.*
e-mails: dean.price@anu.edu.au; susan.howitt@anu.edu.au

1. www.un.org/News/Press/docs/2009/gaef3242.doc.htm
2. Lin, M. T., Occhialini, A., Andralojc, P. J., Parry, M. A. J. & Hanson, M. R. *Nature* **513,** 547–550 (2014).
3. Long, S. P., Zhu, X.-G., Naidu, S. L. & Ort, D. R. *Plant Cell Environ.* **29,** 315–330 (2006).
4. Rae, B. D., Long, B. M., Badger, M. R. & Price, G. D. *Microbiol. Mol. Biol. Rev.* **77,** 357–379 (2013).
5. Tcherkez, G. G. B., Farquhar, G. D. & Andrews, T. J. *Proc. Natl Acad. Sci. USA* **103,** 7246–7251 (2006).
6. Kanevski, I., Maliga, P., Rhoades, D. F. & Gutteridge, S. *Plant Physiol.* **119,** 133–141 (1999).
7. Lin, M. T. *et al. Plant J.* **79,** 1–12 (2014).
8. Price, G. D. *et al. J. Exp. Bot.* **64,** 753–768 (2013).
9. McGrath, J. M. & Long, S. P. *Plant Physiol.* **164,** 2247–2261 (2014).

This article was published online on 17 September 2014.

NETWORK BIOLOGY

A compass for stem-cell differentiation

The development of CellNet — network-biology software that determines how cell types generated *in vitro* relate to their naturally occurring counterparts — could improve our ability to produce desirable cells in culture.

FRANZ-JOSEF MÜLLER & JEANNE F. LORING

Over the past few years, websites such as Facebook and Google have attained an uncanny ability to understand us and to predict our behaviour, even before we have consciously decided what to do. This predictive power is achieved through the systematic application of statistical 'inference algorithms' to the vast numbers of connections and links that users establish when browsing the Internet — making up a 'social graph' that can be exploited to characterize distinct groups of Internet users. It would be wonderful to have such a graph to characterize distinct groups of cells. This could then be used in regenerative medicine to overcome the challenge of coercing stem cells to become the cell type needed for a particular therapy. Writing in *Cell*, Cahan *et al.*[1] and Morris *et al.*[2] describe a network-biology platform, CellNet, that takes a first step in this direction.

The most popular representation of the differentiation of cells from immature precursors to mature cell types was, for many years, the 'epigenetic landscape' diagram conceived by the biologist Conrad Hal Waddington[3,4]. This diagram evokes a set of one-way paths down which immature cells roll along defined routes to more-differentiated cellular states. But over the past decade, this simple model has morphed into the concept of a multidirectional cell-identity transfer hub.

In 2007, Yamanaka and colleagues[5] reprogrammed ordinary human skin cells called dermal fibroblasts into induced pluripotent stem (iPS) cells using transcription factors that are highly expressed in embryonic stem (ES) cells, an equivalent cell type that is derived from early embryos. Both iPS cells and ES cells are pluripotent — they can, given the correct molecular cues, differentiate into almost any cell in the body, forming any one of hundreds of different cell types. Each of these mature cell types is characterized by distinct networks of highly expressed transcription factors, which regulate the expression of large sets of genes. Researchers have used transcription-factor cocktails specific to cell types of interest to try to directly convert one cell type, such as a