Botany

Manipulation of photosynthesis

from J. Barber

BEFORE the goal of creating improved cultivars of crop plants by *in vitro* genetic engineering can be reached, it will be necessary to establish appropriate molecular biological techniques for precise gene transfer and to identify the key physiological processes and biochemical reactions that must be changed to produce the new varieties. In response to these challenges Britain's Agricultural and Food Research Council (AFRC) sponsor two priority programmes. One is concerned with developing techniques for the genetic manipulation of plant cells (see *Nature* **304**, 498; 1984). The groups that contribute to the other programme, which aims to elucidate the processes that limit photosynthesis and plant growth, met recently to consider the outcome of its first five years. A clear message from the meeting* was that there has been a substantial increase in knowledge which is now starting to form a firm basis for closer interaction with the molecular geneticists.

Our knowledge of the organization and functioning of the light-harvesting system and associated electron-flow processes has advanced to such a level that it is now possible to give rational explanations to the mechanisms by which some, but not all, plants can adjust to growth at different temperatures and under different lighting conditions. Both of these environmental factors are commercially important to the agricultural and horticultural industries. As emphasized by C. Pollock (Welsh Plant Breeding Station, Aberystwyth), the control of photosynthesis and plant growth is not just at the level of electron transport but also involves optimization of carbon metabolism and the adoption of various strategies for partitioning assimilates.

It is seldom clear which specific proteins should be the (indirect) targets of genetic manipulation in order to create new crop plants but there are already some examples where molecular biology is having considerable impact. For example, there is the 32,000-molecular weight protein which is a structural component of the photosystem II complex and is known to bind a wide range of commercially important herbicides, including the triazines, ureas and nitrophenols. This hydrophobic protein is coded for in the chloroplast genome and its amino acid sequence has been determined from the gene sequence (Zurawski, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7699; 1982). J. Hirschberg and L. McIntosh have sequenced the same gene, but isolated from the atrazine-resistant biotype of the weed *Amaranthus hybridus*, and conclude that the resistance can be accounted for by a single amino acid change (*Science* **222**, 1346; 1983). According to Alison Telfer (Imperial College, London) there is little difference in the photosynthetic capacity of atrazine-resistant and atrazine-susceptible plants of four different species, suggesting it would not be detrimental to 'engineer' atrazine-resistance into crop plants.

Techniques for specifically manipulating the chloroplast genome have not so far been developed, but site-directed mutagenesis is proving to be a valuable approach when altering plant proteins synthesized by gene expression in bacteria, in particular the enzyme ribulose bisphosphate (RUBP) carboxylase. It is this enzyme which fixes CO_2 by using the reducing power and ATP generated by light-induced electron transport. In higher plants the enzyme is a complex consisting of eight large and eight small subunits while in some photosynthetic bacteria the enzyme structure is simpler. As G.H. Lorimer (Du Pont de Nemours Co., Wilmington) outlined in an overview lecture, the carboxylase activity of this enzyme requires the initial formation of a chemical complex of CO_2 and a divalent cation. Its activation and subsequent catalysis are affected by a range of metabolites, the significance of which is not yet fully understood for the *in vivo* system. Perhaps the most striking and important feature of the enzyme is its ability to catalyse an alternative reaction involving the oxygenation of sugar bisphosphate. Both the carboxylase and oxygenase reactions occur at the same site and compete with each other. The oxygenase reaction produces phosphoglycollate and the metabolism of the resulting hydroxy acid leads to release of CO_2 in a process known as photorespiration.

Because this alternative pathway is energetically wasteful, especially at limiting light intensities, it is a goal of the AFRC programme to manipulate the enzyme genetically so as to increase its carboxylase activity and decrease its oxygenase activity. Site-directed mutagenesis is the obvious approach, and forms the basis of some encouraging work by S. Gutteridge (Rothamsted Experimental Station) in collaboration with G. Lorimer, on the *in vivo* site-directed mutagenesis of the RUBP carboxylase of the photosynthetic bacterium *Rhodospirillum rubrum*. Although this enzyme consists of only two identical subunits, its amino acid sequence is remarkably similar to that of RUBP carboxylase from higher plants, particularly in a region of acidic amino acids involved in activation of the enzyme. At residue 198 there is a highly conserved aspartic acid which Gutteridge and Lorimer chose to replace by glutamic acid. The modified enzyme, synthesized in *Escherichia coli*, was found to have lower carboxylase and oxygenase activities as well as a modification in its divalent cation-binding properties. This result not only provides further evidence for the common catalytic site of carboxylase–oxygenase activity but also represents the starting point for a series of experiments to manipulate the enzyme and elucidate the molecular mechanism by which it functions.

Precisely this approach is also needed to study the RUBP carboxylase–oxygenase activities of higher plants, although a complication arises because the small subunits of the enzyme are encoded in the nuclear DNA while the gene for the large subunit is located in the chloroplast where final assembly takes place. Even so, A. Gatenby (Plant Breeding Institute, Cambridge) could report that genes for the small and large subunits have been cloned and separately expressed in *E. coli*. Unfortunately the products did not assemble into an active enzyme. It may be necessary to introduce both genes into the same *E. coli* cell to overcome this problem. One approach would be to isolate appropriate genes from cyanobacteria which have RUBP carboxylase similar to higher plants but do not have the complication of differentiation into chloroplast and nuclear DNA. In any event it seems highly likely that *in vitro* methods will soon produce active RUBP carboxylase so that the powerful technique of site-directed mutagenesis can be exploited. To aid these developments detailed structural studies of the crystallized enzyme by X-ray diffraction will be useful and progress in this direction was reported by Professor Sir David Phillips' group from Oxford.

I left the meeting feeling optimistic that we would eventually be able simultaneously to reduce oxygenase activity of RUBP carboxylase and to increase the efficiency of the carboxylation step. It remains to be seen, however, whether the overall efficiency of photosynthesis in the intact plant will thereby be increased, as hoped, or whether photorespiration turns out to be obligatory for effective carbon assimilation by the C3 pathway. □

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*Agricultural and Food Research Council's Meeting on Photosynthesis, 15–17 April, University of Sheffield, where Professor David Walker has recently established a Research Institute of Photosynthesis.

100 years ago

THE first mail from Kadiak Island received this season has arrived at San Francisco, bringing dates to May 2. According to the correspondent of the *Bulletin*, the account of the eruption of the volcano on Augustine Island, Cook's Inlet, sent by the last advices of 1883, was much exaggerated. The island "was not split in two, and no new island was formed but the west side of the summit has fallen in, forming a new crater, while the whole island has arisen to such an extent as to fill up the only bay or boat harbour." No tidal waves were observed on the west shore of Cooke's Inlet or on Kadiak Island.

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