PHOTOSYNTHESIS

Clue from Mutant Alga

from our Photosynthesis Correspondent

THERE is every indication that mutant strains of algae may prove to be powerful tools for studying the reactions which occur on the water-splitting, oxidizing side of photosystem two (S2). These reactions are among the most poorly characterized of all the events that take place during photosynthesis by algae and higher plants. It is this part of the photosynthetic apparatus which extracts electrons from water resulting in the production of molecular oxygen and the release of protons. The electrons are then raised to a reducing potential capable of combining the protons with carbon dioxide to produce carbohydrate. The energy for this process is, of course, obtained from light which is probably absorbed by two photosystems S2 and S1 acting in series (see D. A. Walker, *Nature*, **226**, 1204; 1970).

Most workers in photosynthesis would now agree that manganese and chloride ions act as cofactors for the S2 oxidizing reactions, but their mode of action is far from certain. Extensive studies from the laboratories of Kok (B. Kok, B. Forbush and N. McGloin, *Photochem. Photobiol.*, **11**, 457; 1970) and Joliot (G. Barbieri, R. Delosme and P. Joliot, *Photochem. Photobiol.*, **12**, 197; 1970) have indicated that a multivalent component is oxidized by S2 light but its chemical nature is not known. Although information about this part of the electron transport chain is flimsy at present, an encouraging development which will undoubtedly extend what is known of the primary reactions involved in oxygen evolution is outlined in an article from Levine's laboratory (B. L. Epel and R. P. Levine, *Biochim. Biophys. Acta*, **226**, 154; 1971).

Epel and Levine have successfully isolated a mutant strain of the unicellular alga *Chlamydomonas reinhardi* that has lost the capacity to carry out reactions on the oxidizing side of S2. The mutant cells were unable to evolve oxygen although their chlorophyll content falls within the range generally found for the wild type. They also had a normal ratio of chlorophyll *a* to *b*. Although chloroplasts isolated from the mutant showed little or no Hill activity, they were able to carry out S1 reactions such as the photoreduction of NADP using a 2,6-dichlorophenolindophenol–ascorbate couple. As would be expected the mutant showed no time dependent changes in the yield of chlorophyll fluorescence. Of course, normal photosynthetic systems show changes in chlorophyll fluorescence as the number of open reaction centre traps varies with time. Epel and Levine did show, however, that a p-hydroquinone–ascorbate electron donor system could restore the fluorescence yield changes, confirming Yamashita and Butler's contention (*Plant Physiol.*, **44**, 435; 1969) that this donor system provides electrons to the oxidizing side of S2.

Perhaps the most surprising feature of this mutant was the weakness of its chlorophyll fluorescence as compared with wild-type cells. This was an unexpected observation because it is well known that blocking electron flow on the reducing side of the S2 traps usually results in an increase of fluorescence yield. This increase in fluorescence can be simply explained in terms of a closing of the S2 traps which would also be predicted for a similar blocking on the oxidizing side of S2. To explain the apparent contradiction between experimental findings and theoretical expectation, Epel and Levine suggest that a rapid back reaction occurs between the primary reductant of S2 and the oxidizing S2 reaction centre. In this case the chlorophyll fluorescence is kept low because the S2 traps are maintained in a state capable of accepting absorbed light energy in the form of an excited electron.

So far the preliminary studies on this mutant have not added much new information about the reactions associated with the photo-oxidation of water, but they do open up many possible lines of research.

Controlled Transcription *in vitro*

NEXT Wednesday's issue of *Nature New Biology* carries the story of one of those *tour de force* experiments with which molecular biologists periodically dazzle their expectant audiences. De Crombrugghe and six colleagues, a modestly sized group by the standards of the day and by the experiments, have reconstructed a cell free system which includes all the enzymes, regulator molecules and activators required for the regulated transcription of the genes of the lactose (lac) operon of *Escherichia coli*. Their experiments indicate that neither more nor less than seven components are necessary and sufficient for the initiation, elongation and termination of lac operon RNA molecules and the repression of this set of genes.

As a source of the lac operon DNA, de Crombrugghe *et al.* have used the DNA of a hybrid temperate bacteriophage which carries the entire *E. coli* lac operon. This phage DNA provides them not only with a suitable template but also allows them to exploit a neat hybridization method for measuring the amount of lac operon RNA transcribed. Apart from the DNA template, the system requires *E. coli* RNA polymerase, complete with its sigma factor, together with another specificity factor called cyclic AMP receptor protein (CRP) and cyclic AMP itself. The so-called rho factor is needed to accurately terminate RNA synthesis, and to switch the process on and off an appropriate inducer molecule and lac repressor protein have to be added to the system.

How do all these various components function in a coordinated manner? From the results of their own experiments and the vast assemblage of information about the lac operon which has been accumulated over the years, de Crombrugghe *et al.* paint the following picture. The lac repressor protein sits on the lac DNA blocking the initiation of transcription; in this state the genes are switched off. When, however, an inducer molecule such as a galactoside is added, it homes onto the repressor, binds to it and inactivates it. Once active repressor has been effectively removed from the system, the cyclic AMP binding protein associates with cyclic AMP and then tightly binds to some sequence at the beginning of the lac DNA. The formation of this complex between the lac DNA and the cyclic AMP protein is, they believe, a prerequisite for the attachment of *E. coli* RNA polymerase, complete with sigma factor, to the promoter site of the operon.

Once the polymerase has attached to the DNA and initiated the synthesis of lac RNA, the enzyme can proceed to transcribe the entire operon. When it reaches the end of the genetic message, the rho factor somehow causes the polymerase to terminate RNA synthesis and cast off from the DNA template releasing its RNA transcript. The only other molecule which may play a part in this process is the guanosine tetraphosphate ppGpp which significantly inhibits transcription by the purified, complete cell-free system. The mechanism of this inhibition is unknown.

The experiments of de Crombrugghe *et al.* define all the essential components required for the controlled transcription of this operon. Their system seems to be complete and it leaves no room for further, so far undiscovered, elements. It provides the most detailed explanation so far available of how the expression of a set of related genes can be controlled.