quinone pool and only then does the protonmotive force drive them into $NADP^+$. Both conflicting hypotheses can be discriminated by applying uncouplers and plastoquinone antagonists. Until the necessary tests have been performed, the question of an efficient, physiologically relevant, direct reduction of $NADP^+$ by PSII, and hence the challenge to the general validity of the Z-scheme of Hill and Bendall[13], remains unsettled.

Armen Y. Mulkidjanian, Wolfgang Junge
Division of Biophysics,
Faculty of Biology/Chemistry,
University of Osnabrück,
D-49069 Osnabrück, Germany

GREENBAUM *ET AL.* REPLY — In our Letter we reported a new phenomenon, CO_2 fixation and hydrogen and oxygen evolution, at wild-type rates, in a mutant alga that lacked the photosystem I reaction centre. We did not, however, attempt to elucidate the mechanism or the pathway of this discovery, or claim evidence for "the direct reduction of $NADP^+$ by pheophytin..."

Mulkidjanian and Junge speculate that the mechanism of 'PSII photosynthesis' is reversed electron flow through the NAD(P)H:plastoquinone (PQ) oxidoreductase in the thylakoid membrane, as previously advanced by Peltier and Thibault[14] to explain electron transport in isotopic oxygen exchange experiments in F18, another PSI-deficient mutant of *Chlamydomonas reinhardtii*. We considered this mechanism, but we are convinced that this explanation is not correct. Antimycin A is known to inhibit chlororespiration by blocking electron transport between NAD(P)H and the PQ pool which is mediated by the thylakoid membrane-bound NAD(P)H–PQ oxidoreductase[15,16]. Our recent experiments (J.W.L. and E.G., manuscript in preparation) indicate that antimycin A has no effect on PSII photosynthesis.

In addition, we have found that 5 μM FCCP (carbonyl cyanide trifluoromethoxyphenyl hydrazone) completely inhibits CO_2 photoassimilation but increases the sustained simultaneous photoevolution of molecular hydrogen and oxygen. FCCP is a protonophore that dissipates proton gradients across the photosynthetic membrane and thus inhibits synthesis of ATP, which is essential for CO_2 assimilation by the Calvin cycle but not essential for hydrogen production by the ferredoxin/hydrogenase pathway. It is known that the reverse-operating NAD(P)H:PQ oxidoreductase is driven by a proton gradient across the thylakoid membrane. If such a reverse-operated mechanism were responsible for electron transfer from PQH_2 to ferredoxin, FCCP would inhibit not only CO_2 assimilation but also H_2 photoevolution, because both are dependent on electron transfer from PQH_2 to ferredoxin. FCCP's effect cannot be explained by the reverse-operating NAD(P)H:PQ oxidoreductase mechanism.

Moreover, we have demonstrated that PSI-deficient green algae can grow photoautotrophically using CO_2 as the sole source of carbon, light as the sole source of energy, and water as the sole source of electrons under both aerobic and anaerobic conditions in a minimal medium (water plus mineral elements but without organic nutrients; J.W.L., C.V.T., L.J.M., T.G.O. and E.G., manuscript submitted). Photoautotrophic growth and the quantum requirement of photosynthesis in PSI-deficient mutants (E.G., J.W.L. and C.V.T., manuscript in preparation) preclude the reverse-operating NAD(P)H:PQ oxidoreductase from being responsible for PSII photosynthesis.

Mulkidjanian and Junge state that their proposed mechanism uses the scalar reducing potential of the ubiquinone/ubiquinol redox pair plus the electrochemical proton potential difference to drive the reduction of $NADP^+$. Even if the operation of that mechanism can generate NADPH, it will leave little or no proton-gradient energy for synthesis of ATP, which is required for CO_2 fixation by the Calvin cycle as well as for cell growth. This mechanism cannot, therefore, explain our newly discovered PSII photosynthesis and its support of photoautotrophic growth. We believe that the mechanism of PSII photosynthesis involves electron flow from PSII to ferredoxin/$NADP^+$ reduction through the plastoquinone pool and cytochrome *b/f* complex (J.W.L. and E.G., manuscript in preparation).

E. Greenbaum, J. W. Lee
C. V. Tevault, S. L. Blankinship
Chemical Technology Division,
Oak Ridge National Laboratory,
Oak Ridge, Tennessee 37831, USA
L. J. Mets
Department of Molecular Genetics and Cell Biology,
University of Chicago,
Chicago, Illinois 60637, USA

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Lateral proton diffusion

Sir — The lateral diffusion of protons along membranes could provide a direct link between sources and sinks involved in chemiosmotic coupling[1]. Recently, a long-distance migration of protons along membranes has been observed in purple membranes and reconstituted bacteriorhodopsin[2–5]. This was suggested to be due either to protonation/deprotonation reactions of amino groups or polar headgroups of lipids, or to a movement along interfacial water molecules[2–5]. Scherrer has suggested that evidence for a lateral movement of protons along a surface could be obtained by modulation of the chemical character of the lipid headgroups[5]. The dwell time of protons depends on the lipid headgroups[6], and so this is expected to control any lateral proton movement. Studies on dissociation rate constants concluded that surface-to-bulk proton transfer was not retarded and so there was no lateral proton movement[7,8].

The experiments proposed by Scherrer have already been performed in lipid monolayers, by comparing long-range movements of protons from a source to detectors either at the membrane level or in the bulk medium (as reviewed in ref. 9). Lateral migration of protons is observed with many phospholipids as long as the molecular assembly is in the fluid state, and is therefore not controlled by the chemical character of the polar heads. Rather, the migration of protons along membranes may be supported by a hydrogen-bond network involving polar headgroups and interfacial water molecules. A 'hop and turn' mechanism would be involved and controlled by the correlation time of the lipid headgroups, as experimentally observed, and not their chemical character. As soon as the continuity of the network is broken (for physical or chemical reasons), the lateral migration of protons is prevented.

Migration was critically controlled by the composition of lipid/detergent mixed

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