A New Light on Photosynthesis

A pioneer in photosynthesis research proposes a new mechanism of photophosphorylation. His peers greet it with skepticism.

New research findings that appear to upset a key tenet of the currently accepted mechanism for photosynthesis have led Daniel I. Arnon of the University of California at Berkeley to propose a radical new mechanism. Arnon, one of the pioneers in photosynthesis research, may have observed what T. H. Huxley has called "the great tragedy of science—the slaying of a beautiful hypothesis by an ugly fact." Other scientists in the field argue that Arnon's new observations are not as important as he makes them out to be and that the formulation of a new mechanism is premature, but Arnon has a long track record of introducing controversial theories that have subsequently been proved correct.

The thylakoid membranes of plant chloroplasts contain two different reaction centers, known as photosystems I and II (PSI and PSII). These use light at slightly different wavelengths to convert solar energy into two forms of chemical energy—reducing power and adenosine triphosphate (ATP), the primary energy carrier of the cell. These photosystems carry out two separate types of energy conversion—cyclic and noncyclic (linear) photophosphorylation. In the cyclic process, discovered by Arnon and his co-workers in 1954, the sole product of energy conversion is ATP. When quanta of light interact with PSI, they induce an electron flow that gives rise to a proton gradient. Protons from outside the chloroplast are carried to the inside of the thylakoid membrane in which the photosystem resides, establishing an electrochemical gradient of protons across the membrane.

In 1978, Peter Mitchell of the Glynn Research Laboratories in Bodmin, England, received the Nobel Prize in Chemistry for demonstrating that such an electrochemical gradient is the driving force for the production of ATP during photophosphorylation and respiration. Arnon named the process carried out by PSI cyclic photophosphorylation to fit his concept that light induces a cyclic electron flow. Mitchell's concept made it clear that protons also cycle in the process.

In 1957, Arnon and his colleagues discovered linear photophosphorylation, which produces not only ATP, but also free oxygen and a strong reductant, reduced nicotinamide adenine dinucleotide phosphate (NADPH). In the 1960's, Arnon's group found that the photoreduction of ferredoxin precedes the formation of NADPH and is coupled to ATP formation. They also identified ferredoxin as a catalyst of cyclic photophosphorylation.

In 1960, Robin Hill and Fay Bendall of Cambridge University proposed the essence of a mechanism for linear photophosphorylation in which both PSI and PSII participate. A refined version of this mechanism, known as the Z scheme because of the shape of a graph of the oxidation-reduction potentials involved, is now the accepted mechanism for linear photophosphorylation.

The Z scheme proposes two distinct steps, each requiring a quantum of light. In the first step, a quantum of light interacts with PSII to liberate an energized electron from a water molecule; four such interactions produce one molecule of oxygen. The energized electron is then transferred from PSII to PSI by a series of electron carriers, the most important of which is known as plastoquinone. In this process the electron gives up some of its energy and ATP is formed. Protons are assumed to diffuse from the thylakoid membrane to the chloroplast interior. In the second step, another quantum of light interacts with PSI and imparts enough additional energy to the electron to reduce ferredoxin. Through a subsequent series of reactions that take place in the dark, ATP and the reducing potential of ferredoxin (through NADPH) are used to reduce carbon dioxide and convert it into sugars.

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The assumptions implicit in the Z scheme may be incorrect.

Arnon postulates that linear photophosphorylation is carried out entirely by "photosystem II" and is activated by two quanta of light.

Implicit in the Z scheme are three assumptions, Arnon says. One is that PSI and PSII should exist in the chloroplast in more or less equal quantities, since one of each is required for linear photophosphorylation. The two systems should also be close together spatially, since an energetic electron must be transferred between them. And finally, the Z scheme says that ferredoxin cannot be reduced unless an energized electron is transferred from PSII to PSI by a series of carriers that includes plastoquinone. It now appears that none of these assumptions is correct.

Last year, Anastasios Melis and Jeanette S. Brown of the Carnegie Institution of Washington's Department of Plant Biology at Stanford University demonstrated that the ratio of PSII to PSI in chloroplasts varies widely. There can be as much as 3.5 times as much PSII as PSI. About the same time, Jan M. Anderson of the Division of Plant Industry of CSIRO in Canberra, Australia, and Bertil Andersson of the University of

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Lund in Sweden found that the two photosystems are located relatively far apart within the chloroplast—far enough that physical transport of an energetic electron between them would be quite difficult.

Skeptics think that Arnon's interpretation of his data goes too far.

The most critical evidence, however, has been provided in a series of reports during the past year by Arnon, Harry Y. Tsujimoto, and George M.-S. Tang. The early papers in this series demonstrated that inhibition of electron transport by plastoquinone with 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) does not stop the reduction of ferredoxin. According to the Z scheme, reduction of ferredoxin should not occur if electrons cannot be transferred between the photosystems.

In their most recent paper in the May issue of *Proceedings of the National Academy of Sciences** the three investigators report that complete inhibition of plastoquinone function does halt the reduction of ferredoxin. But this reducing activity can be restored by any of "four chemically diverse uncouplers, similar only in their ability to facilitate proton transport across membranes." It thus appears that the most important role of plastoquinone in linear photophosphorylation is the transport of protons originating from water.

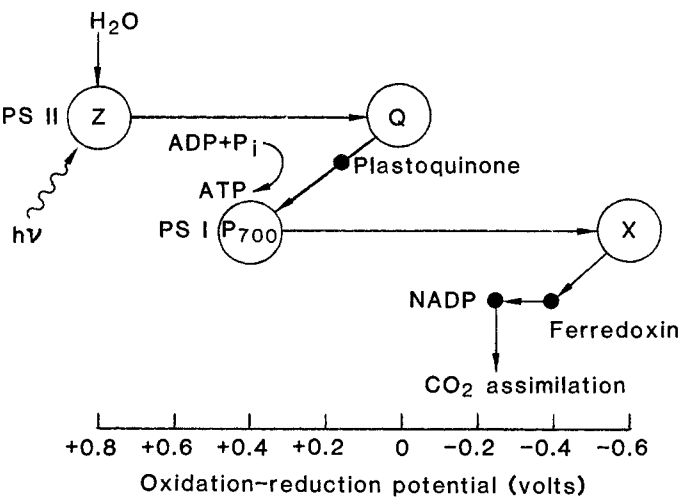
On the basis of this evidence, Arnon postulates a new mechanism in which linear photophosphorlyation is carried out entirely by PSII and activated by two quanta of light. His basic premise is that the flow of electrons from water is coupled with a removal from the membranes of the simultaneously generated protons. As one quantum is used to transfer an electron from water to ferredoxin on the outside, a second quantum is used to reduce plastoquinone and enable it to transport protons to the inside of the membrane.

The transport of protons to the inside of the membrane produces an electrochemical gradient that is the driving force in the production of ATP. Thus, two quanta of light are used to transfer one electron from water to ferredoxin. Four such events require eight quanta of light and produce one molecule of oxygen and two of ATP. The crucial difference between Arnon's new hypothesis and the Z scheme is the role of PSI. In the Z scheme, the noncyclic reduction of ferredoxin by electrons originating from water is carried out by PSI, but in Arnon's scheme by PSII. In his scheme, PSI drives only cyclic electron flow.

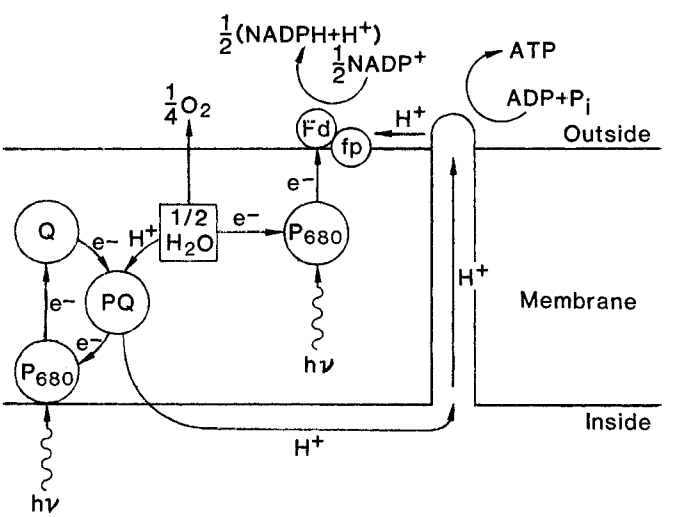
To avoid confusion with the Z scheme, Arnon terms this new mechanism for noncyclic photophosphorylation oxygenic photophosphorylation, since oxygen is liberated during the reaction. By analogy, he terms the cyclic mechanism that occurs in PSI anoxygenic photophosphorylation. Ferredoxin and plastoquinone serve as regulatory links between the two systems, he says, but the systems operate basically independently and simultaneously. Further evidence from his laboratory that has not yet been published supports this hypothesis, Arnon adds.

The reception of Arnon's proposal has not been very enthusiastic, to put it mildly. He was allowed only a short time to discuss the hypothesis at a recent Gordon Conference on photosynthesis, and the mood of the group, according to one participant who prefers to remain anonymous, "was that they didn't want to take time to discuss it." No one disputes that Arnon's observations are correct. "I have to respect his findings," says Bacon Ke of the Kettering Laboratories, but they nonetheless "need to be confirmed by other people." "He has found an anomaly," says the Gordon participant, "and I believe it is true. It certainly deserves further exploration and explanation. But it could be anomalous for trivial reasons."

"There is a tremendous amount of data on which the Z scheme is based," says Richard A. Dilley of Purdue University, but Arnon has taken "one piece of data, that is not very strong," and has constructed an alternative hypothesis that "doesn't explain a lot of the existing data." There are many other discrepancies in the Z scheme, adds Norman Good of Michigan State University, but Arnon's mechanism is nonetheless "somewhat contrived" and doesn't explain



The Z scheme of photosynthesis. Z is the primary electron donor of PSII; Q is the electron acceptor of PSII. P_{700} is a form of chlorophyll that is the electron donor of PSI, and X is the electron acceptor.



Oxygenic photophosphorylation, as proposed by Arnon. Q is the primary acceptor. PQ is plastoquinone. P_{680} is a chromophore that accepts light energy and transfers it to an electron. Fd is ferredoxin. [Illustration courtesy of Proceedings of the National Academy of Sciences of the United States of America]

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many other observations of the system. Furthermore, he adds, the uncouplers that Arnon says only transport protons across the membrane "have a lot of funny effects. It is more likely that they are inducing some kind of a bypass of plastoquinone." Dilley cites several other potential problems with Arnon's scheme. No one, he says, has ever been able to demonstrate reduction of ferredoxin by PSII in the absence of PSI—not even Arnon. Such a demonstration would be a convincing proof of Arnon's hypothesis. Furthermore, it has been shown by several investigators that tetramethylphenylene diamine can reverse inhibition of plastoquinone by DBMIB; that chemical, however, is a carrier of electrons rather than protons. And finally, DBMIB inhibition of plastoquinone blocks reduction of ferredoxin even when iodide ion is the source of electrons rather than water; when iodide ion is the electron source, no protons are generated, and Arnon's scheme suggests that ferredoxin reduction should not be affected. Were Arnon's data his, Good concludes, "I would say, 'Look boys, this observation doesn't fit the dogma. What do you make of it?' and leave it there."

Arnon is unfazed by the criticism: "Based on my past experience, I am not surprised by the skepticism with which the hypothesis has been received. It took years before we were able to get cyclic photophosphorylation accepted. The obstacles were formidable: the concept of cyclic electron transport flew in the face of theoretical arguments against the conversion of light energy into phosphate bond energy, and also challenged the then-entrenched view that mitochondria were the only organelles capable of ATP synthesis. There was even published evidence that chloroplasts could not form ATP unless mitochondria were present. When we discovered noncyclic photophosphorylation it was received with skepticism even by those who then accepted cyclic photophosphorylation. Likewise, our findings and concepts pertaining to ferredoxin have had tough sledding. In short, we have a long history of unpopular findings and theories that went counter to established doctrine. They've all met with great resistance, but have now become textbook facts." But despite Arnon's track record, says Dilley, "I would place my bet on the Z scheme at this time." Clearly, the last word on this subject has yet to be heard.—THOMAS H. MAUGH II

More Progress on Gene Transfer

Researchers appear to be on the verge of developing strains of animals that have functional foreign genes in their germ cells

The transfer of foreign genes into cultured cells, and their expression there, is becoming a common event (*Science*, 19 December 1980, p. 1334). In parallel with these in vitro experiments, investigators are also trying to introduce functional genes into the living animal, where the factors that control gene activities can be explored under more natural conditions. Ultimately they would like to produce strains of animals that carry foreign genes in their germ cells, the eggs and sperm. This would permit researchers to monitor the fate of an individual gene as a multicellular organism develops from a single fertilized egg, to see how a gene is turned on (or off) in the appropriate tissues during differentiation. Recent successes with egg injection experiments suggest that this goal may soon be achieved.

Recombinant DNA technology has made it possible to prepare purified genes in large enough quantities for injection into recently fertilized eggs (mouse eggs are generally used). As Beatrice Mintz of the Institute for Cancer Research in Philadelphia points out, "If you introduce recombinant DNA into the egg, you can get the DNA into all the the cells at once." Breeding the mice that develop from injected eggs would then provide large numbers of animals for use in studying the control of gene expression.

Mintz and her colleagues Erwin Wagner and Timothy Stewart now report* that a foreign gene, which was injected into fertilized mouse eggs, has produced a functional product in at least one of the embryos that subsequently developed. They injected a recombinant molecule (originally constructed by Tom Maniatis of Harvard University) consisting of bacterial plasmid DNA into which the thymidine kinase (*tk*) gene of herpes simplex virus (HSV) and the human β-globin gene had been inserted.

> According to Mintz, "One of the five animals was producing the herpes enzyme as well as its own . . . ."

Mintz, Wagner, and Stewart then implanted the injected eggs that survived without apparent damage in the oviducts of female mice which had been prepared for pregnancy by mating with vasectomized males. The investigators killed some of the females near the end of the gestation period and removed the individual fetuses. Each fetus and its placenta were separately analyzed for the two foreign genes and for the enzyme thymidine kinase, the product of the *tk* gene. There was not enough material to analyze for human β-globin.

"What we found was really very interesting," Mintz says. "An extremely high percentage—15 percent—of the developing mice had donor recombinant DNA. We found it in 5 animals out of 33, which is a very high take. Everyone of the five had both genes of interest in them. What is more important is that they had intact copies."

In similar experiments, Frank Ruddle and Jon Gordon of Yale University had previously shown that genetic material injected into mouse eggs would be retained in some of the animals that subsequently developed, but in rearranged form. The Mintz group also found evidence for rearranged viral *tk* and human β-globin genes in some of their animals.

Gordon and Ruddle have been continuing their egg injection work, but Gordon says, "We don't see intact genes yet." However, in a symposium last

*E. F. Wagner, T. A. Stewart, B. Mintz, *Proc. Natl. Acad. Sci. U.S.A.* (August 1981).

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