PERSPECTIVES: PLANT BIOLOGY

Some Like It Hot

Thomas D. Sharkey

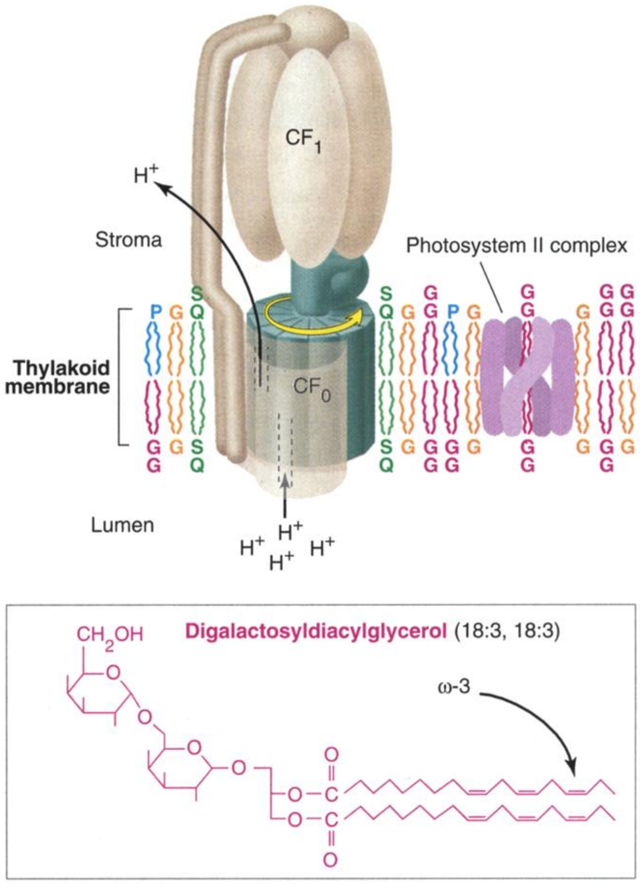
Photosynthesis—the process through which green plants harvest light energy to make organic compounds from water and carbon dioxide (CO_2)—is inhibited by moderate to high temperatures, but the causes of this inhibition are not clear. An increase in temperature is thought to be detrimental to the membranes of chloroplasts—the organelles, akin to mitochondria, where photosynthesis takes place—but this has been difficult to prove at moderate temperatures (about 35°C) known to inhibit photosynthesis in many plants. The decreased photosynthesis at moderate temperatures could also be due to a corresponding increase in respiration or photorespiration, or a decline in the activation state of the CO_2-fixing enzyme rubisco (*1*). On page 476 of this issue, Murakami *et al.* (*2*) now provide the best evidence yet that the number of unsaturated lipids (that is, fatty acids with double bonds) in the thylakoid membrane of chloroplasts—which contain the light-absorbing system, electron transport chain, and ATP synthase—is important in determining a plant's ability for growth and photosynthesis at temperatures of 35°C or more.

The investigators show that it is possible to improve the thermotolerance of plants by genetic modification. They silenced the gene encoding the chloroplast version of the ω-3 fatty acid desaturase enzyme (which synthesizes lipids containing three double bonds). This resulted in a decrease in unsaturated lipids with three double bonds and a corresponding increase in lipids with two double bonds in thylakoid membranes. The reduced level of lipid unsaturation improved the rate of photosynthesis at 40°C and markedly improved plant growth at 36°C. This provides an unambiguous demonstration of how reducing the unsaturation of thylakoid membrane lipids improves photosynthesis and growth at moderately high temperatures.

Membranes are made up of a bilayer of amphipathic lipids composed of hydrophobic fatty acids (which constitute the interior of the membrane) and hydrophilic polar head groups (which face out into the aqueous environment). For example, the chloroplast's thylakoid membrane separates the aqueous stromal compartment (where CO_2 is fixed) from the aqueous lumen (where protons for photophosphorylation accumulate). Thylakoid membranes have a high density of unsaturated fatty acids, which makes them very fluid (just as polyunsaturated corn oil is more fluid than monounsaturated or saturated corn oil that forms margarine). They must be sufficiently fluid to allow the "spinning" of ATP synthase (*3*) yet solid enough to produce the proton-motive force needed to propel this spinning (see the figure). The thylakoid membrane is heterogeneous, and the lipids must be kept properly dispersed to prevent them from concentrating and forming nonbilayer structures (*4*). Heating followed by cooling disrupts the intricate organization of the thylakoid membrane (*5*).

Unsaturated lipids in membranes have been shown to protect plants against damage during cold spells (*6*). Light-induced damage (photoinhibition) at low temperatures is repaired more slowly when plants or photosynthetic bacteria have been modified to have fewer unsaturated lipids in their photosynthetic membranes. These studies found that reducing unsaturation did not translate into a gain in thermotolerance (as measured by analyzing photosynthetic electron transport with a parabenzoquinone reduction assay) (*6*). The finding that the carbon metabolism enzyme rubisco activase was inhibited by moderately high temperatures (*1*) seemed to confirm that the effect of temperature on photosynthesis at 35°C or below did not depend on the unsaturated state of the membrane lipids.

Others have reported that thylakoid membranes become leaky to protons at moderate temperatures before any reduction in photosynthesis is seen (*7*). This would uncouple photosynthetic electron transport from photophosphorylation (light-dependent ATP synthesis). Thylakoid leakiness to protons would not be detected by parabenzoquinone reduction measurements. This shifts the focus of high-temperature studies back to membranes. When membranes become permeable to protons, the ability to make ATP is compromised but other measures of electron transport may be unaffected. This may explain why Moon *et al.* (*6*) found no effect of lipid composition on the response of photosynthetic electron transport to temperature. The parabenzoquinone reduction assay they used to assess photosynthetic electron transport would not be affected by membrane leakiness. Because rubisco activase requires the



Fat, a temperature-sensitive issue. (Top) The thylakoid membrane of chloroplasts is composed of 50% protein and 50% lipid (principally galactolipid). Monogalactosyldiacylglycerol does not form bilayers spontaneously and may be associated with the periphery of the photosystem II photosynthetic complex. Digalactosyldiacylglycerol may be intrinsic to the photosystem II complex. Sulfolipids are associated with the CF_0 ATP synthase complex. The CF_0 spins in the membrane at a frequency of between 100 and 200 Hz. P, phosphatidylglycerol; G, monogalactosyldiacylglycerol; GG, digalactosyldiacylglycerol; SQ, sulfoquinovosyldiacylglycerol. **(Bottom)** Structure of the digalactosyldiacylglycerol head group and its two linolenic acid (18:3) unsaturated fatty acid chains.

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production of ATP, membrane leakiness may lead to subtle changes in ATP availability, causing reduced activity of this enzyme at lower temperatures than would be required for the inhibition of other photosynthetic reactions. The increased thylakoid leakiness at or below 35°C is rapidly reversible and at moderate temperatures can be partly compensated for by increased cyclic photophosphorylation (which uses photosystem I to boost ATP synthesis) (*8*). Very high temperatures (45°C and above) may irreversibly damage the photosynthetic machinery by causing the disintegration of the protein complex responsible for oxygen production during photosynthesis (*9*).

The substantial effects found by Murakami and colleagues after they reduced the level of membrane lipid unsaturation may reflect the specific double bonds they eliminated. They silenced the *FAD7* gene, which encodes a chloroplast-localized ω-3 desaturase. This enzyme converts 16:2 fatty acids (16 carbons long with two double bonds) to 16:3 molecules, or 18:2 fatty acids to 18:3 molecules, by desaturating the third to last carbon-carbon bond (see the figure). Other studies have used chemical hydrogenation (*10*) (which randomly saturates double bonds) or mutation to bring about fatty acid desaturation at other depths within the membrane (*11*). Some plants with decreased lipid unsaturation exhibit variation in chloroplast structure (*12*)—this makes it more difficult to demonstrate specific effects on thermotolerance.

Murakami *et al.* showed that their transgenic plants grew much better than controls at higher temperatures. Differences in growth rate were noted at 36°C, and transgenic plants survived for 2 hours at 47°C, a treatment that killed their wild-type counterparts. This demonstrates that thermotolerance is related to membrane properties, and that the growth and survival of plants can be determined by the thermotolerance capabilities of photosynthesis. With increasing concentrations of greenhouse gases in the atmosphere, the effect of high temperature on plants is an important area of study. The Murakami *et al.* report may provide valuable information about the best approach to engineering plants that can carry out photosynthesis in the face of heat stress.

References

1. R. Law and S. J. Crafts-Brandner, *Plant Physiol.* **120**, 173 (1999).
2. Y. Murakami, M. Tsuyama, Y. Kobayashi, H. Kodama, K. Iba, *Science* **287**, 476 (2000).
3. T. Elston, H. Y. Wang, G. Oster, *Nature* **391**, 510 (1998).
4. W. P. Williams, *Lipids in Photosynthesis: Structure, Function and Genetics*, P. A. Siegenthaler and N. Murata, Eds. (Kluwer Academic, Dordrecht, Netherlands, 1998), p. 103.
5. K. Gounaris, A. P. R. Brain, P. J. Quinn, W. P. Williams, *Biochim. Biophys. Acta* **766**, 198 (1984).
6. B. Y. Moon, S.-I. Higashi, Z. Gombos, N. Murata, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 6219 (1995).
7. N. G. Bukhov, C. Wiese, S. Neimanis, U. Heber, *Photosynth. Res.* **59**, 81 (1999).
8. C. Pastenes and P. Horton, *Plant Physiol.* **112**, 1253 (1996).
9. D. Nash, M. Miyao, N. Murata, *Biochim. Biophys. Acta* **807**, 127 (1985).
10. P. G. Thomas *et al.*, *Biochim. Biophys. Acta* **849**, 131 (1986).
11. L. Kunst, J. Browse, C. Somerville, *Plant Physiol.* **91**, 401 (1989).
12. P. McCourt, L. Kunst, J. Browse, C. Somerville, *Plant Physiol.* **84**, 353 (1987).

PERSPECTIVES: POLYMER CHEMISTRY

Nickel Comes Full Cycle

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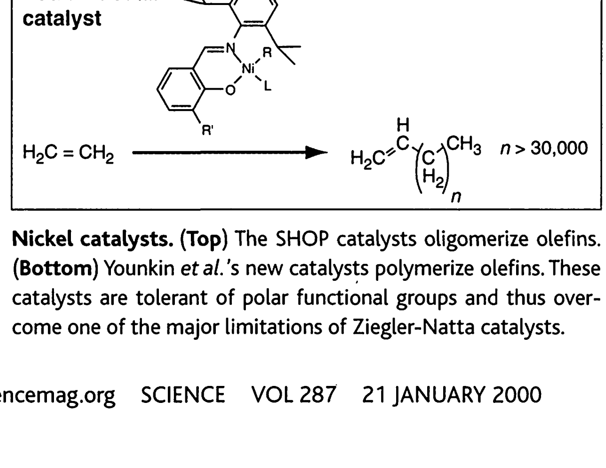
Some of the greatest scientific discoveries have resulted from what at first seemed to be failed experiments; famous examples include Fleming's discovery of penicillin or Penzias's and Wilson's discovery of the cosmic background radiation. The modern era of polyolefin chemistry, which has helped to make plastics an important presence in our everyday lives, also has its origins in such an experiment. In 1953, Erhard Holzkamp, a doctoral student working under Karl Ziegler at the Max-Planck-Institute for Coal Research in Mülheim, Germany, was struggling to reproduce a reaction discovered in Ziegler's lab a few years earlier. In this so-called "Aufbau" reaction, a molecule of ethylene is inserted into the carbon-metal bonds of triethylaluminum. Careful investigation revealed that nickel impurities, which were introduced by cleaning the stainless steel reaction vessel with acid, were inhibiting the Aufbau reaction in Holzkamp's experiments, a phenomenon that came to be known as the "nickel effect" (*1*). The observation of such a drastic change in reactivity imparted by small amounts of a transition metal salt led the Ziegler lab to carry out a systematic study of the effect of salts of other elements on the Aufbau reaction. To their surprise, they observed that the addition of early transition metal halides, such as $TiCl_3$ or $ZrCl_4$, to trimethylaluminum led to a completely different type of catalyst—one that affected the polymerization of ethylene at room temperature and ambient pressure, resulting in the formation of a polymer with unprecedented properties. Giulio Natta soon discovered that these new catalysts also catalyzed the stereoregular polymerization of monosubstituted olefins (α-olefins). The importance of these discoveries is reflected only in part by the fact that Ziegler and Natta were awarded the Nobel Prize for chemistry less than a decade after Holzkamp's initial struggles. More than 40 million tons of polyolefins are produced by Ziegler-Natta polymerization every year, and these "plastics" impact our daily lives in countless beneficial ways.



Nickel catalysts. (Top) The SHOP catalysts oligomerize olefins. **(Bottom)** Younkin *et al.*'s new catalysts polymerize olefins. These catalysts are tolerant of polar functional groups and thus overcome one of the major limitations of Ziegler-Natta catalysts.

The initial discoveries made by Ziegler and Natta inspired an extraordinary level of research activity that has been sustained for the past three and a half decades. This can be ascribed to the fact that, although the Ziegler-Natta polymerization answered a crucial practical concern through the introduction of low-cost, high-quality polyethylene and polypropylene polymers, it left many questions unanswered. One of the most intriguing of these is whether it might be possible to polymerize olefins

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