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MINI-REVIEW

Membrane Acclimation by Unicellular Organisms in Response to Temperature Change

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Abstract

Unicellular organisms possess a wide variety of molecular mechanisms for altering the lipid composition (and thereby the physical properties) of their membranes in response to changes in environmental temperature. These are discussed with a view to establish which of the mechanisms are of more importance to bacteria, algae, and protozoa in coping with extremes of temperature.

Key Words: Temperature acclimation; membranes; lipid metabolism; unicellular organisms.

Introduction

Free-living unicellular organisms are very susceptible to environmental stress because of their small size and bulk. They often inhabit moist soil, which may be subject to rapid changes in temperature. Many aquatic species exist in bodies of water too small to buffer the effects of changing air temperature. Therefore, these organisms must acclimate to their environmental temperature in order to survive.

Needless to say, a great variety of unicellular species have successfully evolved mechanisms for surviving extremes of temperature. One very important key to survival involves maintaining adequate membrane function. It is generally acknowledged that membrane performance is to a large extent dependent on membrane fluidity or, in other words, the physical state of the membrane (Shinitzky, 1984). Leakiness of membranes and dysfunction of

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membrane-embedded proteins have frequently been observed at temperatures approaching 0°C. This is currently thought to result from phase separation and other organizational derangements of the various lipid classes, upon which the physical state of the membrane depends.

On the other hand, exposure of these cells to high temperatures, while generally increasing membrane fluidity, is not thought to damage membrane functions any more seriously than it damages other cellular processes. Accordingly, the majority of studies into membrane responses to temperature stress have centered on low-temperature conditions, where membrane dysfunction may be the primary cause of injury.

The present discussion emphasizes the response of membranes following the exposure of unicellular organisms to low, but above freezing, temperatures. As freezing occurs, a number of complications arise, such as osmotic effects accompanying ice crystal formation (Steponkus, 1984). These are not considered here, even though many of the membrane alterations that provide a degree of protection against chilling damage also engender resistance to freezing injury.

When one thinks of membrane adaptation to low-temperature stress, lipid modifications immediately come to mind. These changes appear to far overshadow any alterations to proteins in reshaping the physical state of the membrane. Many different mechanisms have evolved to effect the stress-induced lipid changes. Several of these are shared by a wide variety of microorganisms, while others are unique to a limited range of species. Because it would lead to considerable duplication, I have opted not to follow taxonomic lines in discussing the major strategies by which different microorganisms alter their membrane lipids during low-temperature acclimation. Instead, each biochemical mechanism is summarized, using examples often chosen from work with organisms used in my own laboratory. For a more extensive review of the pathways discussed, a recent chapter (Lynch and Thompson, 1988) covering regulatory mechanisms is recommended.

The Role of Fatty Acid Biosynthesis in Temperature Acclimation

In *Tetrahymena* (Martin *et al.*, 1976) and many other cells, fatty acid biosynthesis is sharply curtailed by sudden chilling. This reduced supply of saturated fatty acids contributes indirectly to the increasing membrane fluidity since the desaturation of those previously formed saturated fatty acids that are available continues at only a slightly decreased rate. In the anaerobic bacteria, however, low temperature exerts a much more direct effect on fatty acid biosynthesis. A typical response is exhibited by *Escherichia coli*. Unlike aerobic cells, both prokaryotic and eukaryotic, which

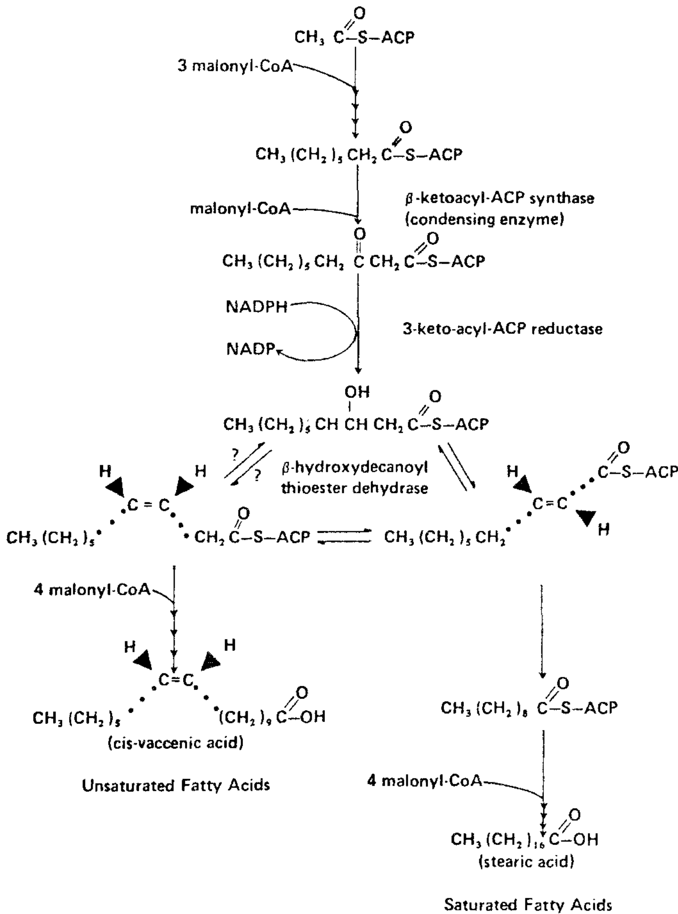

Fig. 1. The pathway of fatty acid biosynthesis in *Escherichia coli*, showing the bifurcation leading to the formation of unsaturated fatty acids (left) and saturated fatty acids (right). Reprinted, with permission, from Thompson and Martin (1984).

place double bonds into saturated long-chain fatty acids by an oxygen-requiring desaturation reaction, *E. coli* inserts a double bond only during the biosynthetic process *per se*. As illustrated in Fig. 1, the chief unsaturated fatty acid, *cis*-vaccenate, is made by one branch of the biosynthetic pathway while palmitic acid arises through a separate branch. At low temperatures, the proportion of *cis*-vaccenate increases, leading to an increased membrane fluidity.

Cronan and coworkers recognized the mechanism underlying this control step as being the existence of two distinct forms of the enzyme β-ketoacyl-ACP synthase (De Mendoza and Cronan, 1983). Synthase II has

a much greater preference for elongating palmitoleoyl-ACP than for elongating palmitoyl-ACP. This specificity, coupled with the higher activity of synthase II at low temperature, ensures that cells transferred to low temperature will increase their content of *cis*-vaccenate relative to palmitate.

Modification of Fatty Acids During Temperature Acclimation

Membrane fluidity can be increased by enzymatically modifying saturated fatty acids in a number of ways. The addition of methyl groups or cyclopropane, cyclopentane, or cycohexyl rings to straight-chain fatty acids by bacteria (Russell, 1984) imparts a fluidizing effect on the membrane. It is not clear, however, that this is a bacterial strategy specifically associated with acclimation to temperature stress.

An unusual strategy for fatty acid modification has evolved in the psychrophilic bacterium *Micrococcus cryophilus* (Russell, 1984). This aerobe, when chilled from 20°C to 0°C, increases its membrane fluidity not through desaturating fatty acids, but rather by decreasing the C_{18}/C_{16} ratio of its fatty acids from 3 to 1. This is achieved by a removal of C_2 units from stearate and using these selectively to elongate a C_{14} intermediate to palmitate. The membrane-bound elongase is apparently regulated by the physical properties of its host membrane.

The best known stress-induced lipid modification is the insertion of *cis*-double bonds into saturated fatty acids. This response is widespread in aerobic bacteria as well as in plants and animals (note, however, that anaerobic bacteria insert double bonds during the course of fatty acid synthesis). It is widely, but not universally, assumed that the ability of a cell to increase fatty acid unsaturation in its membrane lipids at low temperature evolved because of its survival value.

The mechanism utilized by aerobic bacteria to regulate the degree of fatty acid unsaturation has been thoroughly investigated by Fulco and associates (reviewed by Fulco, 1984), using *Bacillus megaterium* and *B. licheniformis*. *Bacillus megaterium* contains only saturated fatty acids if grown at $\geqslant 35$°C. If the culture temperature is decreased to 30°C, however, unsaturated fatty acids rapidly appear. This is explained by the finding that fatty acid desaturase mRNA is synthesized only at relatively low temperatures. Interestingly, the initial burst of unsaturated fatty acid formation soon tapers off to an optimal level under the growing influence of a modulator protein whose synthesis is also triggered by low temperatures, but only after a brief delay.

Certain cyanobacteria (blue-green algae) respond to low temperature in a rather similar fashion. Thus, *Anacystis nidulans* produces substantial

amounts of mono-unsaturated fatty acids (Sato *et al.*, 1979) and *Anabaena variabilis* generates polyunsaturated fatty acids (Sato *et al.*, 1979; Sato and Murata, 1981) only when chilled to low temperatures. As in the bacilli, fatty acid desaturation in *A. variabilis* depends on induced protein synthesis.

The low-temperature-induced increase in unsaturated fatty acid content has been extensively studied in the ciliate *Tetrahymena pyriformis* (Thompson and Nozawa, 1984). Considerable evidence is available to indicate two complementary and sometimes simultaneous modes of regulation, namely, via the persistent action of existing desaturase molecules and via the induced synthesis of additional enzyme. There has been a tendency to consider the former mode as the short-term response and the latter as the preferred mechanism when a more extended response is needed. This interpretation may be oversimplified when one considers that, not one, but a family of desaturases is involved in the desaturation of fatty acids. The initial reaction, insertion of a double bond at the $\omega 9$ position of 16:0 or the $\omega 9$ position of 18:0, is carried out by a desaturase acting on the CoA form of the substrate (Nagao *et al.*, 1978). An induced synthesis of this enzyme has been shown to occur when *Tetrahymena* cells are chilled (Umeki *et al.*, 1982).

Additional double bonds, if added, are usually inserted after the fatty acid is incorporated into a glycerolipid. In *Tetrahymena*, as many as three double bonds may be added following a pattern of introduction typical of animal cells, but different from the sequence followed in plants (Fig. 2). There

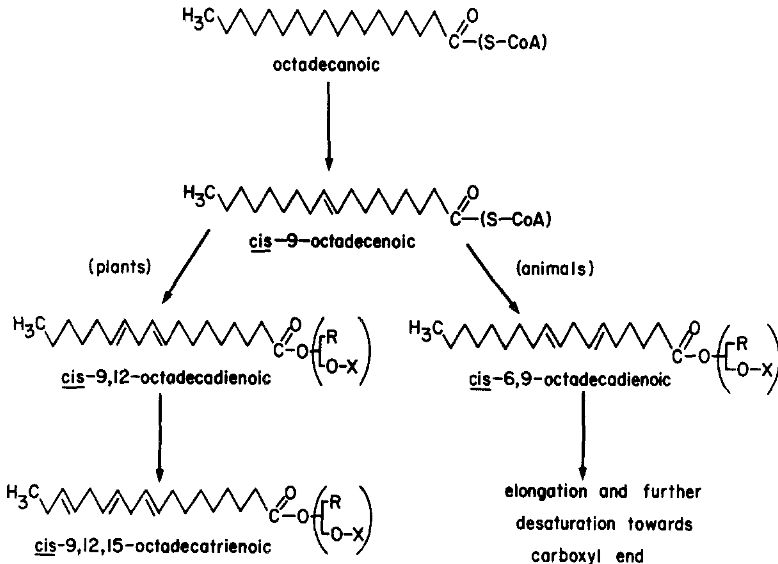

Fig. 2. The common sequence of fatty acid desaturations in plants (left) and animals (right).

is apparently a different desaturase responsible for desaturation at each position. Action by one or another of these becomes more prominent at low temperature, not because of an absolute increase in its enzymatic activity, but rather because activity declines only slightly against a background of sharply declining fatty acid biosynthesis (Martin *et al.*, 1976).

Although a variety of indirect evidence supports the concept of lowered membrane fluidity somehow maintaining desaturases embedded in the endoplasmic reticulum in an unusually active state at low temperature, it has so far been impossible to establish unequivocally that the changing physical state of the membrane is the factor directly regulating enzyme activity. The recent advent of a nontoxic homogeneous hydrogenation catalyst capable of hydrogenating the double bonds of membrane lipids *in vivo* (Vigh *et al.*, 1988) may permit a straightforward correlation between membrane fluidity and desaturase activity. Using this approach, it should be feasible to reduce the fluidity of the endoplasmic reticulum without incurring the many metabolic complications associated with lowering the growth temperature. Preliminary experiments with *Tetrahymena* have been promising (Y. B. Pak, and G. A. Thompson, Jr., unpublished data). Relatively few unicellular systems have been studied for their responses to chilling as extensively as has *Tetrahymena*, but it appears likely that the dual system of control is widespread, at least among eukaryotic animal cells.

Fatty acid unsaturation also increases markedly in *Neurospora crassa* shifted from high (37°C) to low (15°C) temperature (Martin *et al.*, 1981). The most remarkable change is in the tenfold rise in the level of phospholipid-bound α-linolenate (18 : 3) in certain strains.

Regulation of fatty acid desaturation in algal cells is complicated by certain spatial considerations not applicable in animals. Plants synthesize all of their fatty acids within the chloroplast or other forms of plastids. The products, mainly palmitic and oleic acids, have two destinations. Some are used within the chloroplast for the formation of phosphatidylglycerol, sulfolipid, and, in some plants, a large percentage of the abundant galactolipids. The remaining fatty acids are exported from the chloroplast and utilized by enzymes in the endoplasmic reticulum for the formation of phospholipids needed in the growth of nonchloroplast membranes. In many types of plants, e.g., grasses, phosphatidylcholine, the major lipid formed in the endoplasmic reticulum also serves as the prime source of diacylglycerol trans-shipped back into the chloroplast for the synthesis of galactolipids (Fig. 3).

The chloroplast and the endoplasmic reticulum each possesses its own system of fatty acid desaturases. Both appear to act on glycerolipid-bound acyl chains. The temporal response of these two systems to chilling has been compared in *Dunaliella salina* cells shifted from a growth temperature of 30 to 12°C. The chloroplast desaturases were relatively unresponsive to

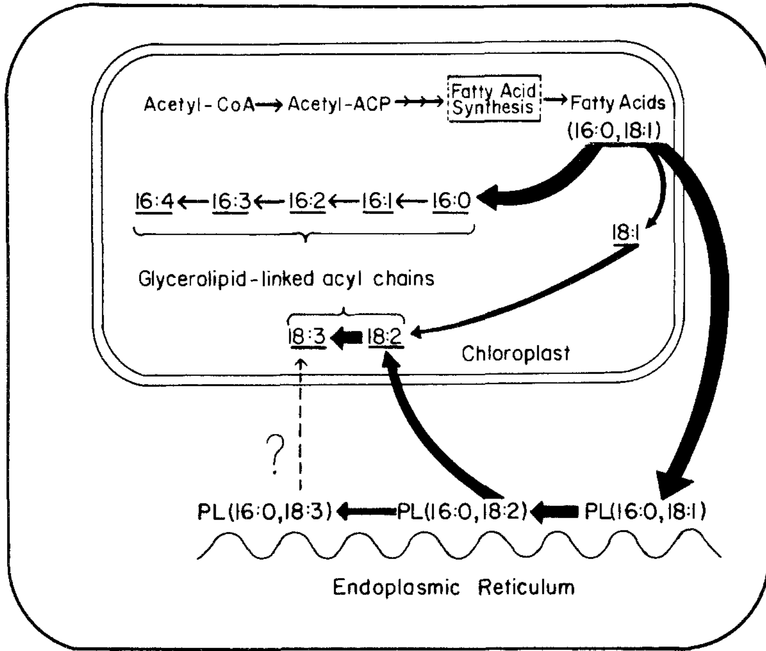

Fig. 3. Typical membrane lipid trafficking in plant cells. Palmitate (16 : 0) and oleate (18 : 1) synthesized in the chloroplast are either utilized for galactolipid, sulfolipid, or phosphatidylglycerol synthesis in the chloroplast or exported to the endoplasmic reticulum for use in the formation of phosphatidylcholine and other phospholipids. A different system of fatty acid desaturases operates in each compartment. Fatty acids desaturated in the endoplasmic reticulum may be returned to the chloroplast for incorporation into galactolipids.

chilling as compared with equivalent enzymes of the microsomes. Thus, major changes in microsomal lipid composition were detectable within 12 h whereas the principal changes in chloroplast lipids were made only after 36 h (Lynch and Thompson, 1984a, c). In studies with the cyanobacterium *Anacystis nidulans*, Gombos and Vigh (1986) also concluded that the cytoplasmic membrane plays a determinative role in thermal acclimation.

One may therefore surmise that, in chilled plant cells, it is more important to restore an optimal fluidity to nonchloroplast membranes, especially the plasma membrane, than it is to regain function in the chloroplast. In fact, it may work to the cell's advantage to shut down many of the chloroplast functions partially while the temperature remains depressed in order to preclude photochemical damage (Martin and Ort, 1985).

In some systems, the level of dissolved O_2 has been implicated as a factor in controlling fatty acid desaturase activity. The yeast *Candida lipolytica* increases the degree of its microsomal fatty acid unsaturation under the

influence of increased aeration as well as lowered temperature (Ferrante *et al.*, 1983). The fatty acid unsaturation level decreases as the cell density of the cultures rises, perhaps reflecting a limitation reached by one or more fatty acid desaturase for O_2.

The Effect of Temperature on the Molecular Species Composition of Membrane Lipids

In addition to the well-known membrane-fluidizing effect brought on by the increasing fatty acid unsaturation that usually accompanies low-temperature acclimation, other more subtle changes in fatty acid metabolism can also help to restore an optimal fluidity. The most prominent of these is chilling-induced retailoring of phospholipid molecular species (Lynch and Thompson, 1984b).

Retailoring refers to the process of swapping fatty acyl chains among the individual phospholipid molecules so as to make different combinations, each of which which has its own phase transition temperature (T_m). The T_m can vary dramatically depending upon the chain length and degree of unsaturation in the two fatty acids (Fig. 4). A simple example would involve reorganization of an equimolar mixture of distearoylphosphatidylcholine and dioleoylphosphatidylcholine in such a way that each resulting phosphatidylcholine molecule contained stearic acid at the *sn*-1 position and oleic acid at the *sn*-2 position. While the overall fatty acid composition would remain constant following this change, the physical properties of the lipids would be dramatically altered. The complex thermotropic behavior of the original mixture (phase transitions at $-22°$ and 30–53°C) would be transformed into a single sharp phase transition at 3°C. The molecular species composition can also significantly influence the temperature at which lipids change from the lamellar to the hexagonal phase ($L_\alpha \rightarrow H_{II}$) (Tate and Gruner, 1987).

In natural membranes, which routinely contain nearly 100 different phospholipid molecular species, low-temperature-induced phase transitions of phase separations are much harder to discern than in the above example, yet the physical state of the lipid bilayer may be significantly influenced by enzymatic retailoring. Three enzymes are typically involved in the process. Action by a phospholipase A_1 or A_2 is needed to remove the acyl chain from the *sn*-1 or *sn*-2 position, respectively. Replacement of the detached fatty acid by a different or an identical fatty acid requires that the fatty acid first be converted to its CoA derivative by acyl–CoA synthetase and then attached to the lysophospholipid acceptor by an acyltransferase (Fig. 5).

It seems that deacylation–reacylation reactions occur continuously in unicellular animal cells and in at least the nonchloroplast membranes of algal

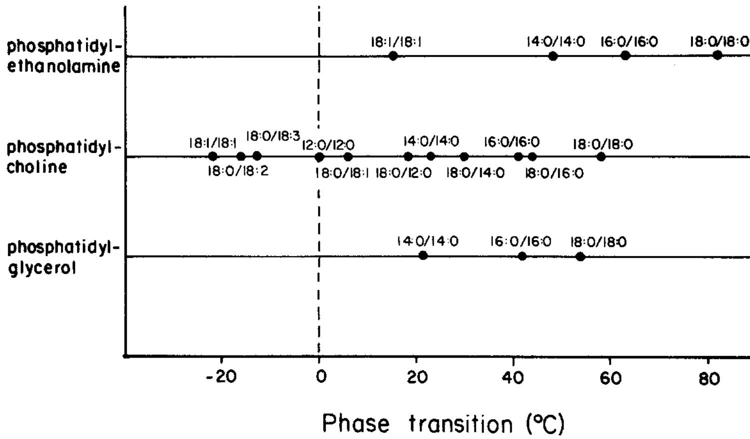

Fig. 4. Phase transition temperatures (T_m) of selected phospholipid molecular species. From Coolbear *et al.* (1983).

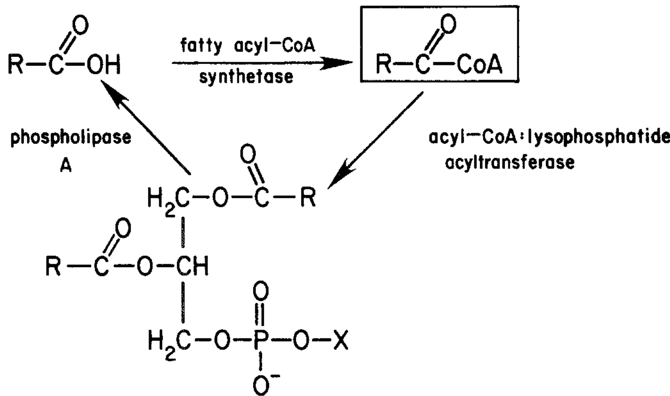

Fig. 5. The enzymes involved in phospholipid molecular species retailoring via deacylation and reacylation. The acyl chain located at the *sn*-1 and/or the *sn*-2 position of the phospholipid may be involved.

cells as a normal part of metabolic turnover. Fatty acyl chain turnover is less apparent in bacteria, but recent findings suggest that it does occur (Cooper *et al.*, 1987). Under stable environmental conditions, the deacylation–reacylation process does not lead to lipid compositional differences because the newly made lipids are identical to those being replaced.

Only recently has it been recognized that rapid and sizable phospholipid molecular species changes occur in low-temperature-stressed cells. A primary factor contributing to this realization has been the development of sensitive gas chromatographic and high-performance liquid chromatographic

techniques for phospholipid molecular species analysis (Lynch and Thompson, 1986; Smith and Thompson, 1987). Using these procedures, phospholipid molecular species retailoring has been shown to be an important part of the short-term response of *Tetrahymena* (Ramesha and Thompson, 1983, 1984; Kameyama *et al.*, 1984; Yoshioka *et al.*, 1984) and *Dunaliella* (Lynch and Thompson, 1984a, b) to chilling. The initial retailoring of phospholipid molecular species seems to precede other lipid acclimation responses, such as fatty acyl chain desaturation. The compositional changes occuring through the deacylation–reacylation reactions quickly cause measurable changes in physical properties of the membrane lipids (Dickens and Thompson, 1982).

How does temperature change bring about a retailoring of phospholipid-catalyzed molecular species? In *Tetrahymena* cilia, phospholipase-A hydrolysis is believed to be the rate-limiting step (Ramesha and Thompson, 1984). The lysophospholipids produced by hydrolysis may be more important in determining the pattern of retailoring than are the other products, fatty acids, since the latter compounds are always available to the cell in small, but presumably adequate, quantities (Ryals and Thompson, 1987).

Although considerable information is available regarding the specificity of acyltransferases (reviewed in Lynch and Thompson, 1988), it is still not known how production of the phospholipid molecular species unique to the low-temperature-acclimated cells is controlled. For example, phosphatidylcholine and phosphatidylethanolamine of *Tetrahymena* chilled from 39°C to 15°C accumulated abnormally large amounts of 18:3 in the *sn*-1 position (Watanabe *et al.*, 1981). Such a pattern, in theory, could be achieved either through manipulating the pool of lysophospholipid acceptors, altering the specificity of the acyltransferase(s), or controlling the availability of specific acyl-CoAs. Because the participating enzymes are sensitive to small fluctuations in the concentration of Ca^{2+} and other ligands, establishing the enzymatic preferences through *in vitro* studies may prove very difficult.

Changes in Membrane Lipid Classes

Large changes in the relative proportions of different phospholipid and glycosphingolipid classes and in sterol/phospholipid ratios have been observed in unicellular organisms grown at different temperatures. Typical examples are reported for *Tetrahymena pyriformis* (Thompson and Nozawa, 1984), *Neurospora crassa* (Aaronson and Martin, 1983), and *Dunaliella salina* (Lynch and Thompson, 1982). In those cases where such changes are recorded during the course of adaptation, especially in the more detailed study of *Tetrahymena*, the polar head group alterations occurred after other acclimation processes, such as phospholipid molecular species retailoring

and fatty acid desaturation, had been nearly completed. The effect on physical properties of the complex phospholipid polar head group changes that came later are not presently interpretable by following any single straightforward principle. However, one might speculate that the quantitative changes in lipid class proportions constitute a final adjustment of lipid molecular dimensions so that a balance of conical, inverted conical, and cylindrical lipid shapes is reached that confers stability to the membrane (Israelachvili *et al.*, 1980).

Particularly interesting from this latter point of view are changes in the molar ratio of monogalactosyldiacylglycerol (MGDG) to digalactosyldiacylglycerol (DGDG) that are associated with changes in growth temperature. Since MGDG is a lipid prone to form nonbilayer conformations and DGDG is not (Williams and Quinn, 1987), these ratio changes could have far-reaching consequences with regard to membrane thermotropic behavior, especially at high-temperature extremes, i.e., 45–55°C. In *Dunaliella*, the MGDG/DGDG ratio of 12°-grown cells was reduced by almost 50% from the value found in 30°-grown cells. *Acholeplasma laidlawii*, too, developed a much lower MGDG/DGDG ratio when shifted from a growth temperature of 37°C to 17°C (Wieslander *et al.*, 1980). On the other hand, in the higher plant *Phaseolus vulgaris* (Süss and Yordanov, 1986), the MGDG/DGDG ratio decreased from 1.3 to 0.9 as the growth temperature was elevated from 25°C to 50°C.

There is a natural tendency to assume that lipid compositional changes such as those discussed above are experienced uniformly throughout the membrane. This may be especially dangerous in the case of thylakoid membranes. It is known that the relative proportions of different thylakoid protein complexes can vary with such environmental factors as temperature, e.g., in rye (Krupa *et al.*, 1987) and *Dunaliella* (Horváth, unpublished data) and light intensity, e.g., in *Dunaliella* (Pick *et al.*, 1987). It has also been determined that the MGDG/DGDG molar ratio is quite nonuniform within different domains of the thylakoid, being, for example, 2.9 in free lipid, 2.0 in oligomeric light-harvesting chlorophyl–protein complex, and 0.9 in photosystem-I (CPI_a) of *Dunaliella* (Cho and Thompson, submitted for publication). Significant MGDG/DGDG ratio changes might be effected simply by altering the proportions of protein complexes within the thylakoid. Such changes would not necessarily entail any localized change in lipid composition around a given protein type.

Spatial and Temporal Effects

Temperature extremes influence not only the biochemical reactions capable of modifying lipids, but also the mechanism by which lipids are disseminated throughout the cell following their modification. Studies with

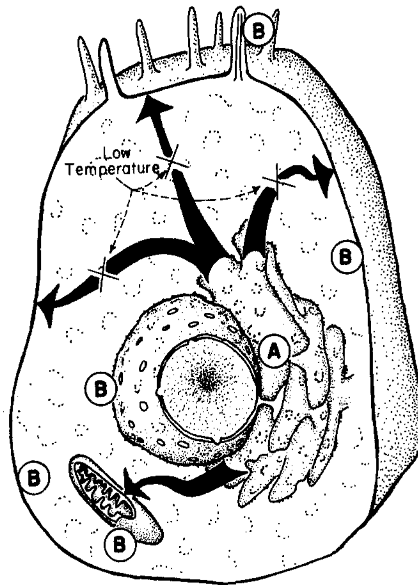

Fig. 6. Lipids synthesized or modified in the endoplasmic reticulum (A) must be translocated to other cellular membranes (B) in order to affect the physical state of those membranes. The translocation process is retarded at low temperatures.

Tetrahymena, e.g., by Ramesha and Thompson (1982), have shown that low temperature markedly reduces the intracellular movement of phospholipid-associated fatty acids desaturated by desaturases of the endoplasmic reticulation (Fig. 6). Such movements probably occur via a combination of bulk membrane flow and the dissemination of individual lipid molecules mediated by phospholipid or sterol transfer proteins. Phospholipid transfer proteins have been identified in several unicellular organisms (Tai and Kaplan, 1985), including *Saccharomyces cerevisiae* and *Rhodopseudomonas sphaeroides*. The cessation of membrane vesicle flux in temperature-sensitive *S. cerevisiae* mutants did not affect phospholipid transfer to the plasma membrane, indicating that transfer-protein-mediated exchange is the predominant mechanism involved. Whereas *S. cerevisiae* phosphatidylcholine-specific phospholipid transfer protein activity was reported to be rather constant over time and presumaby is active even under nongrowing conditions, *R. sphaeroides* phospholipid transfer activity rose to its highest rate at the time of cell division (Tai and Kaplan, 1985). The process is believed to play a large role in lipid transfer from the cytoplasmic membrane to the cell's outer membrane.

The tendency for intracellular lipid movement to slow at low temperature enhances the importance of metabolic responses that act independently

throughout many compartments of the cell. Chief among these is phospholipid molecular species retailoring. The ciliary membrane of chilled *Tetrahymena* cells was estimated to receive radiolabeled phospholipids from the endoplasmic reticulation only following a 12-hr transit time (Ramesha and Thompson, 1982). Fortunately, an active retailoring of phospholipids by enzymes resident in the cilia enabled a much more rapid modification of the lipid composition and physical state of the ciliary membranes (Ramesha and Thompson, 1982, 1984).

The Effects of Temperature on Lipid–Protein Interactions

As improved instrumentation for the detailed analysis of lipids becomes available, new opportunities arise for investigating the subtle ways in which membrane lipids participate in physiological temperature responses. Space does not permit elaboration of all such interactions presently under study. I shall summarize a single ongoing series of investigations that utilizes both higher plants and algae to illustrate some of the exciting, but still tentative, interpretations that have been made.

The experiments under consideration bear on the process of photosynthesis and its marked sensitivity to extremes of high and low temperature (Berry and Bjorkman, 1980; Martin and Ort, 1985). In 1982–83, it was observed by Murata and colleagues (Murata *et al.*, 1982; Murata, 1983) that phosphatidylglycerol isolated from many chilling-sensitive plants contains a considerably higher proportion of 16:0/16:0 plus 16:0/*trans*-16:1 molecular species than does phosphatidylglycerol from chilling-resistant plants. Because the *trans*-16:1 of plant phosphatidylglycerol is *trans*-Δ^3-hexadecenoate, which resembles in its physical properties saturated fatty acids more than the usual *cis*-unsaturated acids (Bishop and Kenrick, 1987), these two molecular species together constitute a sizable subpopulation of chloroplast membrane lipids that are capable of undergoing a thermotropic phase transition above 0°C (Murata and Yamaya, 1984). It was postulated that a low-temperature-induced phase separation of the high-melting-point phosphatidylglycerol species might well cause packing defects in the lipid bilayer, thereby allowing a leakage of critical ions.

Efforts have been made to determine why chilling-sensitive plants contain elevated levels of these high-melting-point phosphatidylglycerols. In one such higher plant, *Amaranthus lividus*, the *sn*-glycerol-3-phosphate acytransferase responsible for acylating the phosphatidylglycerol precursor was found to have a specificity for fatty acid donors that leads to a high proportion of saturated phosphatidylglycerol molecular species (Cronan and Roughan, 1987).

Recent efforts have been successful in characterizing three isomeric forms of acyl (acyl-carrier protein): glycerol-3-phosphate acyltransferase from another chilling-sensitive higher plant *Cucurbita mosehata* (squash) and showing that two of the isoforms favor the attachment of saturated acyl groups at the *sn*-1 position of phosphatidylglycerol (Frentzen *et al.*, 1987).

Fortuitously, phosphatidylglycerol is restricted to chloroplast membranes, making it feasible to correlate the content of high-melting-point phosphatidylglycerol molecular species and damage to photosynthetic membranes in a wide spectrum of plant types without the need for cell fractionation. When the survey was thus broadened, enthusiasm for this appealing hypothesis was somewhat dampened by the discovery that the postulated correlation does not always hold (Roughan, 1985; Bishop, 1986). It now seems likely that any key role for these molecular species of phosphatidylglycerol in determining chilling sensitivity is not due simply to their bulk content in chloroplast membranes. A more localized role within the chloroplast remains a distinct possibility, as illustrated below.

During the course of the investigations described above, a parallel series of studies in other laboratories was developing information on a different involvement of phosphatidylglycerol with low-temperature sensitivity. Experiments with green algae and higher plants detecting a strong association of *trans*-16 : 1-containing phosphatidylglycerol with the light-harvesting chlorophyll *a*/*b* protein (LHCP) of thylakoids (Dubacq and Trémolières, 1983). When LHCP was resolved by non-denaturing sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) into its oligomeric form (LHCP-1), which is thought to be physiologically active *in vivo*, and its inactive monomeric form (LHCP-3), *trans*-16 : 1 phosphatidylglycerol was found predominantly with LHPC-1. Mutants of *Chlamydomonas reinhardtii* lacking *trans*-16 : 1 also lacked LHCP-1 and, interestingly, were quite inefficient in regulating the transfer of energy from the main light-harvesting complexes to the photochemical centers (Maroc *et al.*, 1987).

Based on findings such as these, it has been suggested that *trans*-16 : 1 phosphatidylglycerol has a special function in stabilizing light-energy transfer in thylakoids. Its presence is clearly not an absolute requirement, as evidenced by the fact that a mutant of *Arabidopsis thaliana* lacking *trans*-16 : 1 appeared normal in appearance and growth rate despite having thylakoid LHCP that showed an abnormally pronounced tendency to dissociate into the monomeric form on electrophoretic gels (McCourt *et al.*, 1985). Clues for a possible physiological function for *trans*-16 : 1 arose through studies of changes in levels of this lipid during low-temperature acclimation. Winter rye seedlings grown at 5°C exhibited a 67% decrease in the *trans*-16 : 1 level of phosphatidylglycerol over that found in 20°C-grown plants (Huner *et al.*,

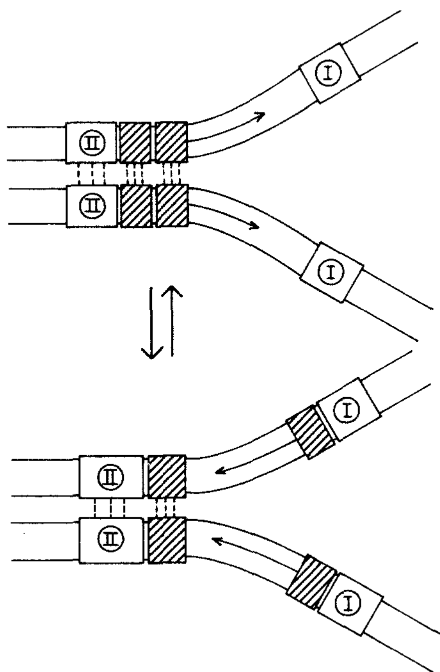

Fig. 7. A simplified representation of reversible movement of the light-harvesting complex (LHCP) (hatched rectangles) from a photosystem-II-associated oligomeric form, above, to a monomeric form, below, which can associate with photosystem I. LHCP dissociation may be modulated by the presence of *trans*-Δ^3-hexadecenoic acid-containing phosphatidylglycerol as well as by phosphorylation of LHCP.

1987). The cold-hardened rye also revealed a 60% lower LHCP-1 content than did the nonhardened seedlings (Krupa *et al.*, 1987).

A similar though somewhat less pronounced decrease in the content of chloroplast *trans*-16:1 phosphatidylglycerol was found when comparing the green alga *Dunaliella salina* grown at 12°C with cells grown at 30°C (Lynch and Thompson, 1984a). SDS-PAGE electrophoresis of these thylakoids confirmed the pattern of sharply decreasing oligomeric LHCP–monomeric LHCP at low temperature (15°C) (Horváth, unpublished data). In this latter study, an effort was made to destabilize the *Dunaliella* LHCP oligomers by catalytically hydrogenating thylakoids from the 30°C-acclimated cells with palladium di (sodium alizarine monosulfonate). Although this treatment successfully reduced the *trans*-16:1 content to a level slightly below that in the cold-acclimated cells, LHCP oligomer dissociation was not enhanced, raising the unexpected possibility that additional factors besides the *trans*-double bond are responsible for oligomer stability.

While no clear benefits of the fluctuating *trans*-16 : 1 phosphatidylglycerol levels have been authenticated, a possible advantage of modulating LHCP oligomer stability through its *trans*-16 : 1 phosphatidylglycerol content can be visualized. Chilling-sensitive plants grown at relatively high temperatures and then chilled are much more severely damaged if they are maintained at the lower temperature in bright light as opposed to darkness (Martin and Ort, 1985). This may reflect an inability of such plants to control adequately the reversible dissociation of LHCP from photosystem II—the so-called state 1 to state 2 transformation, which appears designed to prevent a damaging superabundance of electrons from entering the electron transport system of photosystem II. In fact, the phosphorylation of LHCP, which normally regulates the state 1 to state 2 transition, is known to be inhibited at low temperature (Moll *et al.*, 1987). One might postulate that a second mechanism involving *trans*-16 : 1 phosphatidylglycerol complements the previously discovered phosphorylation-regulated state 1 to state 2 conversion, fine tuning it for more sensitive response to varying temperature. Thus, the gradual reduction of the *trans*-16 : 1 phosphatidylglycerol content during hardening would constitute a backup system facilitating dissociation of oligomeric LHCP when decreased electron flow into photosystem II is needed.

Only time and the further accumulation of data from increasingly sophisticated research will clarify the precise nature of lipid involvement in cells acclimating to low temperature. Meanwhile, we may feel confident that the refined nature of many hypotheses currently being tested will greatly accelerate progress toward truly appreciating lipid function at the molecular level.

Acknowledgments

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