cannot rule out the possibility that TBR serum may have precipitated insulin receptors indirectly—for example, by virtue of pp60^{src} binding of insulin receptors and consequent bridging of the receptors to antibodies to pp60^{src}. However, this seems less likely since antibodies to the insulin receptor do not precipitate ^{35}S-labeled pp60^{src} (that is, a protein with an apparent molecular weight of 60,000 daltons) in association with insulin receptors labeled biosynthetically with [^{35}S]methionine (*17*).

NICOLA PERROTTI
SIMEON I. TAYLOR*
Diabetes Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Bethesda, Maryland 20205
NANCY D. RICHERT
Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Maryland 20205
ULF R. RAPP
Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland 21701
IRA H. PASTAN
Laboratory of Molecular Biology, National Cancer Institute
JESSE ROTH
Diabetes Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases

References and Notes

1. J. Downward *et al.*, *Nature (London)* **307**, 521 (1984); A. Ullrich *et al.*, *ibid.* **309**, 418 (1984); Y. Xu *et al.*, *ibid.*, p. 806; T. Yamamoto *et al.*, *Cell* **35**, 71 (1983).
2. J. M. Bishop, *Annu. Rev. Biochem.* **52**, 301 (1983). It has not been possible to show in vitro activity as tyrosine-specific protein kinases for all of the members of the *src* family. Moreover, certain members of the *src* family may be capable of phosphorylating substrates other than tyrosine residues on proteins (for example, phosphoinositides and serine or threonine residues) under certain circumstances.
3. L. E. King, Jr., G. Carpenter, S. Cohen, *Biochemistry* **19**, 1524 (1980); S. Cohen, G. Carpenter, L. E. King, Jr., *J. Biol. Chem.* **255**, 4834, 1980; M. Kasuga, F. A. Karlsson, C. R. Kahn, *Science* **215**, 185 (1982); Y. Zick, M. Kasuga, C. R. Kahn, J. Roth, *J. Biol. Chem.* **258**, 75 (1983); B. Ek, B. Westermark, A. Wasteson, C. H. Heldin, *Nature (London)* **295**, 419 (1982); B. Ek and C. H. Heldin, *J. Biol. Chem.* **257**, 10486 (1982); S. Jacobs *et al.*, *ibid.* **258**, 9581 (1983); J. B. Rubin, M. A. Shia, P. F. Pilch, *Nature (London)* **305**, 438 (1983); Y. Zick *et al.*, *Biochem. Biophys. Res. Commun.* **119**, 6 (1984).
4. P. F. Pilch and M. P. Czech, *J. Biol. Chem.* **255**, 1722 (1980); S. I. Taylor *et al.*, *Diabetes* **33**, 778 (1984).
5. Insulin, but not IGF I, competitively inhibited the affinity labeling of the peptide precipitated by TBR serum. This supports the view that the insulin receptor rather than the IGF I receptor is precipitated by TBR serum.
6. J. S. Brugge and R. L. Erikson, *Nature (London)* **269**, 346 (1977).
7. A. E Purchio, E. Erikson, J. A. Brugge, R. L. Erikson, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1567 (1978); J. S. Brugge, E. Erikson, R. L. Erikson, *Virology* **84**, 429 (1978).
8. In these studies, we used two different lots of TBR serum: lot C-5, which appeared to be monospecific for pp60^{src} and did not precipitate other virally encoded proteins, and lot C-8, which had antibodies to several viral proteins in addition to pp60^{src} (*18*). Also, we used a polyclonal antiserum that was raised by immunization of rabbits with purified pp60^{src} produced in *E. coli* by means of genetic engineering techniques (*9*).
9. When the supernatant from the TBR serum immunoprecipitation was subjected to a second precipitation with TBR serum, it was possible to precipitate a similar fraction (10 to 20 percent) of the affinity-labeled insulin receptor. Thus, the precipitation of only a small amount (10 to 20 percent) of the insulin receptors by TBR serum probably results from the relatively low affinity of that antiserum for the insulin receptor. Moreover, these observations rule out the alternative possibility that the receptors precipitated by TBR serum make up a subpopulation that is different from the receptors not precipitated during the first immunoprecipitation.
10. D. L. Blithe, N. D. Richert, I. H. Pastan, *J. Biol. Chem.* **257**, 7135 (1982); N. D. Richert, D. L. Blithe, I. H. Pastan, *ibid.*, p. 7143. We have shown that four separate preparations of p54 were capable of inhibiting the ability of TBR serum to immunoprecipitate affinity-labeled insulin receptors. Moreover, when p54 was boiled or precipitated with trichloroacetic acid, this destroyed the ability of p54 to act as a protein kinase and to inhibit immunoprecipitation of insulin receptors.
11. G. E. Mark and U. R. Rapp, *Science* **224**, 285 (1984).
12. The various synthetic peptides used for Fig. 2 have amino acid sequences corresponding to dodeca- and tridecapeptides derived from the predicted sequence of the *raf* gene product (*11*).
13. R. L. Erikson *et al.*, *J. Virol.* **45**, 462 (1983).
14. N. Perrotti, U. Rapp, S. I. Taylor, unpublished observations.
15. M. Kasuga, Y. Fujita-Yamaguchi, D. L. Blithe, M. F. Withei, C. R. Kahn, *J. Biol. Chem.* **258**, 10973 (1983).
16. In this regard, Kasuga *et al.* (*15*) studied the ^{32}P-labeled insulin receptor whereas we studied the affinity-labeled insulin receptor. In our experiments, we showed that the precipitation of the insulin receptor occurs only when it is covalently cross-linked to insulin. The results of initial studies suggest that phosphorylation of the receptor with adenosine triphosphate substantially inhibits (by about 50 percent) the ability of TBR serum to precipitate the affinity-labeled insulin receptor. Cross-linking of insulin to its receptor may expose cryptic epitopes that enable TBR serum to bind to the receptor. Similarly, phosphorylation of the receptor may render those same epitopes less available for antibody binding.
17. E. Van Obbergen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1052 (1981).
18. N. Richert and I. H. Pastan, unpublished observations.
19. We thank R. L. Erikson for the polyclonal antiserum used in these studies.

6 August 1984; accepted 11 October 1984

A Mutant of *Arabidopsis* Lacking a Chloroplast-Specific Lipid

Abstract. *In order to investigate the functional significance of membrane lipid unsaturation, we have isolated a series of mutants of* Arabidopsis thaliana *which are deficient in particular membrane fatty acids. The first of these mutants completely lacks* Δ*3-trans-hexadecenoate, an acyl group that until now has been thought to play an important role in the structure or function of thylakoid membranes in photosynthetic eukaryotes. The apparent absence of any marked physiological effect of the mutation illustrates the potential of this approach to the analysis of membrane structure and function.*

In most eukaryotes each lipid class has a characteristic fatty acyl composition defined by chain length and degree, position, and stereochemistry of unsaturation. However, with a few exceptions the functional significance of lipid acyl unsaturation remains uncertain in both animals (*1*) and plants (*2*). Much of the work on plants has been dominated by a hypothesis that suggested a causal relation between membrane lipid unsaturation and chilling sensitivity (*3*) and has frequently involved an attempt to correlate species differences in membrane composition with thermal tolerance. In addition, it is widely proposed that the unusual fatty acid composition that characterizes chloroplast membranes is critical to the maintenance of photosynthetic function (*4*). We have taken a novel approach to these questions by isolating a series of mutants of the crucifer *Arabidopsis thaliana* (L.) Heynh. with specific

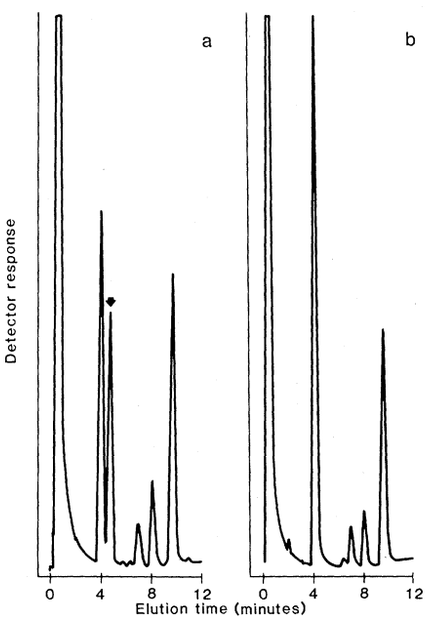

Fig. 1. Fatty acid composition of PG from wild-type (a) and mutant (b) *Arabidopsis*. PG was purified by preparative thin-layer chromatography of a chloroform-methanol extract of leaves (*20*). Lipid-containing zones were visualized with iodine vapor, the lipids were extracted from the silica gel, and fatty acid methyl esters were prepared as described in the legend to Table 1. Individual lipids were identified by comparison of Rf (relative mobility) values with those of known lipid standards. The major peaks (from left to right) and the percentages of total fatty acid in the wild-type sample were solvent, $C_{16:0}$ (34 percent); *trans*-$C_{16:1}$ (20 percent); $C_{18:1}$ (4 percent); $C_{18:2}$ (10 percent); and $C_{18:3}$ (32 percent).

alterations in leaf membrane fatty acyl composition. Here we describe the methods by which the mutants were isolated and the properties of one of them, a mutant that lacks a chloroplast-specific lipid species.

The mutants were isolated without selection by direct analysis of the leaf fatty acid composition of individual M_2 plants. The M_2 population was created by mutagenizing approximately 20,000 wild-type seeds (the M_1 generation) with 0.3 percent aqueous ethyl methanesulfonate for 16 hours and permitting the resulting plants to self-fertilize (*5*). To reduce spurious variability in fatty acid composition, plants were grown in continuous illumination (200 $\mu E/m^2$-sec) and constant temperature (23°C) and relative humidity (70 percent). A leaf was removed from individual M_2 plants and the fatty acid composition was determined by gas-liquid chromatography, as described in the legend to Table 1. From among approximately 2000 plants examined in this way, 89 were retained for subsequent analysis because of anomalies in fatty acyl composition. These plants were self-fertilized, four to ten individual M_3 progeny from each of the 89 lines were analyzed for inheritance of the altered fatty acid composition, and putative mutants were advanced by single-seed descent. By repeating this process for several generations, at least seven of the original 89 lines were found to have stably inherited changes in fatty acid composition.

One of these lines, designated JB60, lacked detectable levels of the unusual fatty acid Δ3-*trans*-hexadecenoic acid (*trans*-$C_{16:1}$) and had a corresponding increase in palmitic acid ($C_{16:0}$), but was otherwise indistinguishable from the wild type in fatty acid composition (Table 1) or morphology. The other mutants contained altered levels of polyunsaturated fatty acids, and most were also similar to the wild type in growth rate and morphology. However, in at least one case elevated temperatures (>25°C) were essential for normal growth. Although the specific enzymatic lesions in these other mutants are still under investigation, we can distinguish at least five classes of mutants by genetic complementation tests and gross differences in fatty acyl composition. The recovery of seven mutants with lesions at five loci from among only 2000 M_2 plants is very similar to the frequencies with which mutants have been recovered after chemical mutagenesis in other diploid plant species, such as barley (*6*) and maize (*7*).

The genetic basis for the alteration in membrane composition in the mutant lacking *trans*-$C_{16:1}$ was determined by measuring the fatty acid composition of the F_1 and F_2 progeny from a cross between mutant and wild type. The F_1 progeny had approximately 50 percent as much *trans*-$C_{16:1}$ as the wild type (0.89 ± 0.25 percent in the F_1 progeny versus 1.74 ± 0.21 percent in the wild type), suggesting a simply inherited nuclear mutation. The frequency of the homozygous mutant phenotype in the F_2 generation was determined by measuring the fatty acid composition of 57 F_2 plants from the JB60 × wild-type cross. Of these, 13 completely lacked *trans*-$C_{16:1}$. This very good fit to the 3:1 hypothesis [$\chi^2(1) = 0.14$; $P > 0.9$] indicates that the deficiency is due to a single nuclear mutation at a locus that we have designated *fadA* (fatty acid desaturation).

The fatty acid *trans*-$C_{16:1}$, which is atypical both in the occurrence of the *trans* unsaturation and in the position of the double bond near the carboxyl end of the molecule, is esterified only to position two of phosphatidyl glycerol (PG) (*8*). Thus it was of interest to compare the fatty acid composition of PG from the mutant and the wild type. The results of these measurements (Fig. 1), which substantially increased the limit of detection, confirmed that the mutant completely lacked *trans*-$C_{16:1}$. The decrease in *trans*-$C_{16:1}$ was compensated by a proportional increase in the $C_{16:0}$ content of PG. These observations suggest that the mutant is deficient in activity for a proposed desaturase that specifically converts $C_{16:0}$ at position two of PG to *trans*-$C_{16:1}$ (*9*). If this interpretation is correct, the presence of intermediate levels of *trans*-$C_{16:1}$ in the heterozygote would suggest that the amount of this

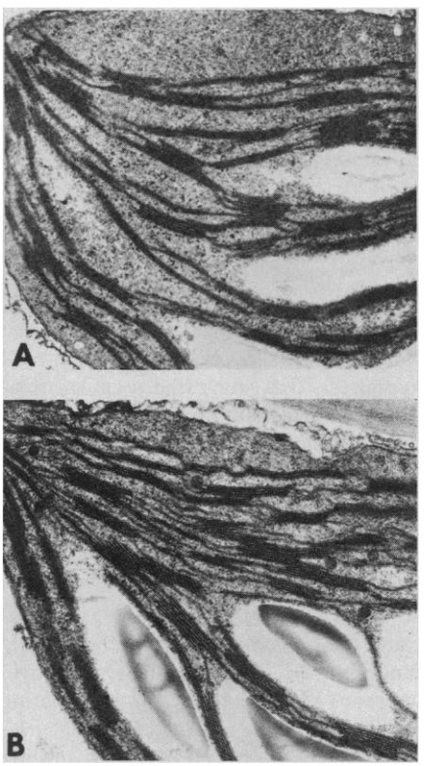

Fig. 2. Transmission electron micrographs of ultrathin (80 to 100 nm) sections of whole leaves from wild-type (a) and mutant (b) *Arabidopsis* at ×22,500 magnification. Leaves from 2-week-old plants grown under constant light and temperature (*5*) were fixed in 4 percent glutaraldehyde, washed in 0.1*M* phosphate (*p*H 7.2), and stained with 1 percent OsO_4 in the same buffer for 1.5 hours at 23°C. The samples were washed, infiltrated with a graded series of ethanol (25 to 100 percent), placed in epoxy resin (Epon-Araldite), and thin-sectioned with a Porter-Blum MT-2 microtome. Sections were poststained in 2 percent aqueous uranyl acetate Reynold lead citrate solution. Electron microscopy was carried out with a Philips 201 transmission electron microscope.

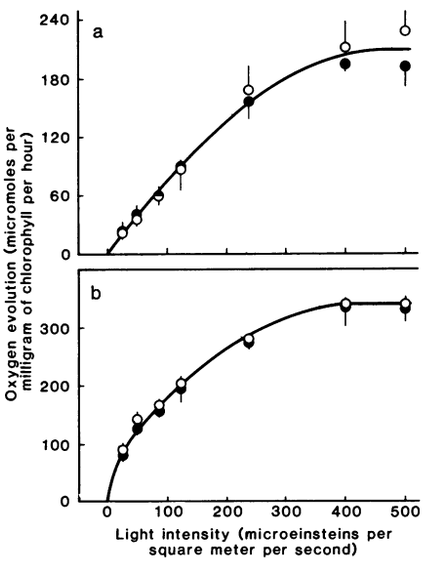

Fig. 3. Effects of light intensity on photosystem II (a) and photosystem I (b) activity of thylakoids from wild-type and mutant *Arabidopsis*. Thylakoids were isolated by grinding leaves in 50 m*M* Tricine (*p*H 7.8), 10 m*M* NaCl, 10 m*M* EDTA, and 400 m*M* sorbitol. The homogenate was passed through cheesecloth and centrifuged at 300*g* for 5 minutes. The pellet was washed in 10 m*M* Tricine (*p*H 7.8), 10 m*M* NaCl, and 5 m*M* EDTA and resuspended in 10 m*M* Tricine (*p*H 7.8), 10 m*M* NaCl, 5 m*M* $MgCl_2$, and 100 m*M* sorbitol. Electron transport was measured in a Rank oxygen electrode at 25°C. Photosystem II activity (a) was measured by the rate of O_2 evolution with ferricyanide as the electron acceptor. Photosystem I activity (b) was measured after differential inhibition of photosystem II by 3-(3,4-dichlorophenyl)-1,1-dimethylurea. Ascorbate and 2,6-dichlorophenol-indophenol were used to supply electrons directly to photosystem I, and removal of O_2 by methyl viologen was used as an indicator of electron transport rates. The responses of the mutant JB60 and the wild type are indicated by open and closed circles, respectively.

fatty acid is regulated directly by the amount of enzyme activity rather than by some mechanism that senses and responds to the absolute concentration of this fatty acid in the membrane. Since this desaturase activity has not, as yet, been demonstrated by in vitro assay of cellular extracts or by tracer studies with intact chloroplasts (*10*), the precise enzymatic lesion in the mutant cannot be evaluated by these means at present.

The absence of *trans*-$C_{16:1}$ in a barley mutant deficient in chloroplast ribosomes (*11*) and the inhibition of *trans*-$C_{16:1}$ synthesis by chloroplast protein synthesis inhibitors (*12*) have been interpreted as possible evidence that a gene for *trans*-$C_{16:1}$ synthesis is chloroplast-encoded. The Mendelian segregation of the *fadA* mutation does not support this concept; however, our results do not exclude the possibility that one or more proteins encoded by the chloroplast genome are also required for *trans*-$C_{16:1}$ synthesis.

The ubiquitous presence of the unusual fatty acid *trans*-$C_{16:1}$ in the thylakoid membranes of all higher plants and green algae (*13*) has invited considerable speculation about a possible role in membrane organization or function (*8*). Because *trans*-$C_{16:1}$–PG is not present in etiolated tissue but accumulates during light-induced chloroplast development (*14, 15*) and occurs only in chloroplast membranes, it has been inferred that this lipid has a specific role associated with the light reactions of photosynthesis. Recently, attention has been focused on an apparent association of this lipid with the light-harvesting chlorophyll a/b protein complex (LHCP) (*16, 17*), which also accumulates in thylakoid membranes during light-induced chloroplast development and is thought to have an important role in the formation of the appressed membranes of the grana (*18*). To evaluate the possibility that *trans*-$C_{16:1}$ is also involved in this process, we analyzed the thylakoid ultrastructure of mutant and wild-type chloroplasts by electron microscopy of thin sections of whole leaves. The micrographs (Fig. 2) showed no obvious differences in the size or extent of grana development or in any other major ultrastructural feature of the chloroplast—compelling evidence against obligatory involvement of *trans*-$C_{16:1}$ in the development of thylakoid structure.

Recent models for the native structure of LHCP based on Fourier analysis of high-resolution electron micrographs of two-dimensional LHCP crystals have indicated that LHCP is a trimer of three structurally equivalent subunits (*19*). It is believed that this oligomeric structure corresponds to a high molecular weight form of LHCP observed after electrophoresis of detergent-solubilized thylakoid proteins in acrylamide gels containing small amounts of ionic detergents (*16, 17*). The reported presence of approximately one molecule of bound *trans*-$C_{16:1}$–PG per LHCP monomer in such oligomeric complexes (*18, 17*) is suggestive of a specific role for the lipid. Since the function of LHCP is to enhance the capture of light energy by photosystem II, we examined the effect of the *fadA* mutation on the irradiance response curve for the light reactions catalyzed by isolated thylakoids (Fig. 3). A major role for *trans*-$C_{16:1}$ in LHCP function would be expected to result in a reduced rate of electron transport in the mutant lines at low irradiance. However, no difference was observed between the photosynthetic activities of mutant and wild type. We have also performed extensive analysis of energy transfer from LHCP to photosystem II by sensitive fluorescence techniques at a wide range of temperature and salt conditions without finding any major functional difference between the mutants and the wild type.

The apparently universal occurrence of *trans*-$C_{16:1}$–PG in eukaryotic photosynthetic membranes suggests that the lipid confers some selective advantage. Also, although we cannot preclude the possible effect of functionally unrelated secondary mutations, we have observed that the mutant grows slightly more slowly than the wild type. However, the results presented here clearly demonstrate that *trans*-$C_{16:1}$–PG does not play any major functional or structural role related to the light reactions of photosynthesis in normal environmental conditions. We must, therefore, conclude that the role of *trans*-$C_{16:1}$–PG is more subtle than previously suggested (*8*) or is manifest only during particular developmental stages or environmental conditions. It is expected that the *fadA* mutant, which is available on request, will be particularly useful in formulating and testing new hypotheses concerning the functional significance of this lipid species. More generally, our results demonstrate that mutational analysis can be a powerful approach to the study of the relations between fatty acyl composition and membrane function in a higher eukaryote.

Table 1. Fatty acid composition of leaves from mutant and wild-type *Arabidopsis*. To prepare fatty acid methyl esters, a leaf was sealed under nitrogen in a tube containing 1 ml of 1.0*M* methanolic HCl and refluxed for 1 hour at 80°C. One milliliter of 0.9 percent NaCl was added and the fatty acid methyl esters were extracted into 0.3 ml of hexane. Gas-liquid chromatography analysis was performed at 170°C on a 2-m column containing 10 percent diethylene glycol succinate and equipped with a flame ionization detector. Peaks were identified by comparing the elution times with known standards of fatty acid methyl esters. Each value is the mean ± standard error of independent measurements made on ten leaves of each line.

Fatty acid	Percentage of total	
	Wild type	Mutant JB60
$C_{16:0}$	15.8 ± 0.4	18.3 ± 0.3
trans-$C_{16:1}$	1.8 ± 0.2	0
$C_{16:2}$	0.7 ± 0.1	0.8 ± 0.1
$C_{16:3}$ + $C_{18:0}$	12.6 ± 0.8	11.6 ± 0.6
$C_{18:1}$	2.7 ± 0.4	2.7 ± 0.5
$C_{18:2}$	18.9 ± 0.8	18.9 ± 0.5
$C_{18:3}$	47.4 ± 1.5	47.2 ± 0.9

J. BROWSE
Plant Physiology Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand
P. MCCOURT
C. R. SOMERVILLE*
DOE Plant Research Laboratory, Michigan State University, East Lansing 48824

References and Notes

1. C. D. Stubbs and A. D. Smith, *Biochim. Biophys. Acta* **779**, 89 (1984).
2. P. J. Quinn and W. P. Williams, *ibid.* **737**, 223 (1983).
3. J. B. Lyons and J. K. Raison, *Plant Physiol.* **45**, 386 (1970).
4. K. Gounaris and J. Barber, *Trends Biochem. Sci.* **8**, 378 (1983).
5. C. R. Somerville and W. L. Ogren, in *Methods in Chloroplast Molecular Biology*, M. Edelman, R. Hallick, N. H. Chua, Eds. (Elsevier, Amsterdam, 1982), pp. 129–138.
6. R. L. Warner, C. J. Lin, A. Kleinhofs, *Nature (London)* **269**, 406 (1977).
7. M. G. Neuffer and W. G. Sheridan, *Genetics* **95**, 929 (1980).
8. J. P. Dubacq and A. Tremolieres, *Physiol. Veg.* **21**, 293 (1983).
9. B. W. Nichols, P. Harris, A. T. James, *Biochem. Biophys. Res. Commun.* **21**, 473 (1965).
10. S. A. Sparace and J. B. Mudd, *Plant Physiol.* **70**, 1260 (1982).
11. A. J. Dorne, J. P. Carde, J. Joyard, T. Borner, R. Douce, *ibid.* **69**, 1467 (1982).
12. K. Strazalka and E. Machowicz, in *Advances in Photosynthetic Research*, C. Sybesma, Ed. (Nijhoff, Amsterdam, 1984), vol. 4, pp. 653–656.
13. A. T. James and B. W. Nichols, *Nature (London)* **210**, 372 (1966).
14. R. O. Mackender, *Plant Sci. Lett.* **16**, 101 (1974).
15. T. Guillot-Salomon, C. Tuquet, M. F. Hallais, M. Signol, *Biol. Cell.*. **28**, 168 (1977).
16. R. Remy *et al.*, *FEBS Lett.* **137**, 271 (1982).
17. A. Tremolieres, J. P. Dubacq, F. Ambard-Bretteville, R. Remy, *ibid.* **130**, 27 (1981).
18. J. Bennett, *Biochem. J.* **212**, 1 (1983).
19. W. Kuhlbrandt, *Nature (London)* **307**, 478 (1984).
20. P. G. Roughan, C. R. Slack, R. Holland, *Lipids* **13**, 497 (1978).
21. We thank K. Baker, J. Watson, and T. Shimei for technical assistance. Supported in part by grants from Department of Energy (AC02-76ER01338), the National Science Foundation (PCM8351595), the McKnight Foundation, and the U.S.-N.Z. Agreement for Scientific and Technological Cooperation.
* To whom correspondence should be addressed.

1 October 1984; accepted 26 November 1984