

# Biosynthesis of Arachidonic Acid in the Oleaginous Microalga *Parietochloris incisa* (Chlorophyceae): Radiolabeling Studies

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**ABSTRACT:** The fresh-water green alga *Parietochloris incisa* is the richest plant source of the polyunsaturated fatty acid (PUFA) arachidonic acid (20:4n-6, AA). To elucidate the biosynthesis of AA in this alga we labeled cultures of *P. incisa* with radioactive precursors. Pulse chase labeling with acetate resulted in its incorporation via the *de novo* biosynthesis pathway of fatty acids. However, labeled acetate was also utilized for the elongation of C<sub>16</sub> and C<sub>18</sub> PUFA. Labeling with [1-<sup>14</sup>C]oleic acid has shown that the first steps of the lipid-linked fatty acid desaturations utilize cytoplasmic lipids. Phosphatidylcholine (PC) and diacylglyceryltrimethylhomoserine are the major lipids involved as acyl carriers for the Δ12 and Δ6 desaturations of oleic acid, leading sequentially to linoleic and γ-linolenic acid. The latter is released from its lipid carrier and elongated to 20:3n-6, which is reincorporated primarily into phosphatidylethanolamine and PC and finally desaturated to AA. Galactolipids, mostly monogalactosyldiacylglycerol (MGDG), serve as substrates for the chloroplastic Δ12 desaturase and, apparently, the ω3 desaturation, common to higher plants and many green algae. The predominant sequence desaturates the 18:1/16:0 molecular species of MGDG stepwise to the 18:3n-3/16:3n-3 molecular species similar to the prokaryotic pathway of higher plants and green algae.

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Arachidonic acid (20:4n-6, AA) and docosahexaenoic acid (22:6n-3, DHA), which are the major polyunsaturated fatty acids (PUFA) of brain membrane phospholipids, aid development of infants (1,2). DHA and AA are transferred directly from mother to infant during the last intrauterine trimester and after birth by breast-feeding (3,4). Therefore, formula-fed preterm infants require an external supply of AA and DHA (5). Indeed, various health authorities recommend the incorporation of both AA and DHA into baby formula (6), and the U.S. Food and Drug Administration has recently approved its use.

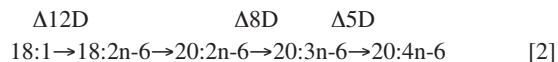
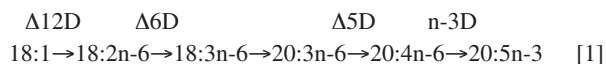
In a search for PUFA-rich algal strains, we found the fresh-water green microalga *Parietochloris incisa* comb. nov. (7)

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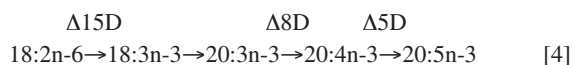
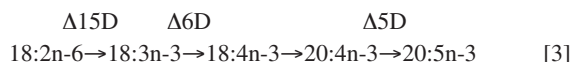
Abbreviations: AA, arachidonic acid (20:4(6)); DGDG, digalactosyldiacylglycerol; DGTS, diacylglyceryltrimethylhomoserine; DHA, docosahexaenoic acid (22:6ω3); EPA, eicosapentaenoic acid (20:5ω3); MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PUFA, polyunsaturated fatty acid; TAG, triacylglycerols. Pairs of numbers representing the fatty acids, when separated by a slash, designate the components in the *sn*-1 and *sn*-2 positions, respectively, of the molecular species.

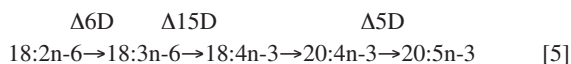
to be the only AA-rich vegetal organism (8). Under conditions conducive to oil accumulation, the AA content of this alga exceeds 20% of dry weight, and the proportion of AA reaches 50% of total fatty acids (9). Over 90% of cell AA is deposited in triacylglycerols (TAG).

Whereas the biosynthesis of C<sub>18</sub> PUFA, which are present in the entire vegetal kingdom, has been studied in depth (10,11), very little is known about that of C<sub>20</sub> PUFA in algae. The order of the elongation and desaturation steps varies in the different microorganisms, showing a multiplicity of biosynthetic pathways. Recently, Shiran *et al.* (12) and Khozin *et al.* (13) have described the biosynthesis of AA and eicosapentaenoic acid (20:5n-3, EPA) in the red microalga *Porphyridium cruentum*. Oleate is stepwise desaturated to 18:3n-6, which is then elongated to 20:3n-6 and further desaturated to AA (Sequence 1). However, it was claimed that in Euglenophyceae, the elongation of 18:2n-6 to 20:2n-6 precedes the desaturations to 20:3n-6 and to AA (Sequence 2) (14).



In the diatom, *Phaeodactylum tricorutum*, EPA is synthesized through four different routes, starting from 18:1n-9, utilizing phosphatidylcholine (PC) as the lipid carrier for the desaturations (15,16). In one pathway (Sequence 3), 18:1 is successively desaturated via 18:2n-6 and 18:3n-3, to 18:4n-3, elongated to 20:4n-3, and subsequently desaturated to 20:5n-3. In the second pathway (Sequence 4) 18:3(3 is elongated to 20:3n-3 before being desaturated to 20:4n-3 and 20:5n-3. In a mixed n-6/n-3 pathway, 18:2n-6 is desaturated to 18:3n-6, which is n-3 desaturated to 18:4n-3 and elongated to 20:4n-3 before the final desaturation to 20:5n-3 (Sequence 5). The fourth n-6 pathway is similar to the one described for *P. cruentum* (Sequence 1).





The lipids to which the fatty acyl groups are attached prior to undergoing these desaturations seem to be species-specific. In the green alga *Chlamydomonas reinhardtii*, the desaturation of saturated and monounsaturated fatty acids involves these fatty acids esterified in diacylglyceryltrimethylhomoserine (DGTS) (17). In the cryptomonad *Chroomonas salina*, C<sub>18</sub> PUFA are located predominantly in galactolipids, whereas EPA and DHA are found almost exclusively in phospholipids (18). Schneider and Roessler (19) suggested that in the eustigmatophyte *Nannochloropsis*, PC and phosphatidylethanolamine (PE) were the lipid carriers for the desaturations of C<sub>18</sub> and C<sub>20</sub> fatty acids, respectively.

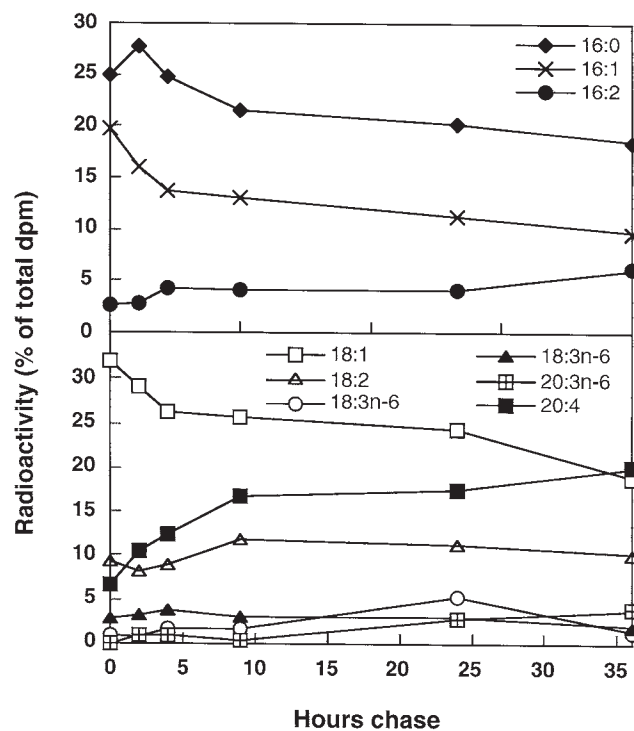
In this study, we attempted to elucidate the biosynthesis of AA in *P. incisa* at the fatty acid and lipid levels by pulse labeling with various radioactive fatty acid precursors. Our data indicate that PC and DGTS are involved in the  $\Delta 12$  and, subsequently, the  $\Delta 6$  desaturations of oleic acid, whereas PE and PC are the major substrates for the  $\Delta 5$  desaturation of 20:3n-6 to AA. The elucidation of the biosynthetic pathways leading to the production of AA and its accumulation in TAG will provide the basis for genetic engineering approaches aimed at the development of PUFA-rich algal strains.

## MATERIALS AND METHODS

**Growth condition.** Cultures of *P. incisa* in the logarithmic phase were cultivated on BG11 medium (20) in 150-mL Erlenmeyer flasks under an air/CO<sub>2</sub> (99:1, vol/vol) atmosphere. The flasks were shaken at 25°C and illuminated from above at a light intensity of 115 mmol quanta m<sup>-2</sup> s<sup>-1</sup>. Cultures were diluted daily for at least 4 d prior to the onset of the experiment.

**Radiolabeling experiments.** [1-<sup>14</sup>C]Oleic acid (sp. act. 55 mCi/mmol) and sodium [2-<sup>14</sup>C]acetate (sp. act. 58 mCi/mmol) were obtained from Amersham (Little Chalfont, United Kingdom). Cultures were labeled with 250  $\mu$ Ci sodium [2-<sup>14</sup>C]acetate (corresponding to 4.3  $\mu$ moles of acetate) or 20  $\mu$ Ci [1-<sup>14</sup>C]oleic acid. Labeled oleic acid was added as its ammonium salt, which was obtained by neutralization of the free fatty acid with an equimolar amount of 2 M NH<sub>4</sub>OH. Exponentially growing cells were concentrated to half of the original volume by centrifugation and pulse-labeled for 30 min (oleate) or 1 h (acetate) in the remaining medium. Cells were centrifuged and washed repeatedly with the growth medium until it was label-free, resuspended to the original volume (100 mL) with growth medium (oleate labeling) or 5 mM (500  $\mu$ mol acetate) acetate-containing growth medium (acetate labeling), and cultivated as described above. Experiments were repeated two or three times. Figures 1–9 depict representative experiments.

**Lipid extraction.** Lyophilized biomass was extracted with methanol containing 10% (vol/vol) DMSO by heating for 5



**FIG. 1.** Redistributive of radioactivity in the fatty acids (*p*-bromophenacyl esters) of *Parietochloris incisa* after 1 h of pulse labeling with [2-<sup>14</sup>C]acetate ( $19.3 \times 10^6$  dpm incorporated) and chase with 5 mM sodium acetate.

min at 40°C min and stirring at 4°C for another 1 h. The mixture was centrifuged, the supernatant removed, and the pellet re-extracted with hexane/ether (1:1, vol/vol). Diethyl ether, hexane, and water were added to the supernatant so as to form a ratio of 1:1:1:1 (by vol). The mixture was shaken and then centrifuged for 5 min at 1000  $\times$  g and the upper phase was collected; the water phase was re-extracted with a mixture of diethyl ether/hexane (1:1, vol/vol). The organic phases were combined and evaporated to dryness. The diethyl ether used for the extractions was peroxide-free and contained 0.01% butylated hydroxytoluene.

**Fatty acid and lipid analysis.** Fatty acid methyl esters of total and individual lipids were obtained by transmethylation with 0.36 M sulfuric acid in methanol (13). In the experiments with sodium [2-<sup>14</sup>C]acetate, lipids were hydrolyzed with 5% (wt/vol) KOH in 95% methanol at 60°C. *p*-Bromophenacyl esters of fatty acids were prepared from the free fatty acids according to Borch (21). Methyl esters and *p*-bromophenacyl esters were separated by reversed-phase HPLC (RP-18 column, 5  $\mu$ m, 250 mm, Lichrospher 100; Merck, Darmstadt, Germany) using a solvent system of methanol/acetonitrile/water (76:12:12, by vol) detected at 205 and 242 nm, respectively, and identified using authentic standards. Radioactivity of individual peaks was determined by a Flo-One/Beta series A-100 detector (Radiomatic Instruments and Chemical Co. Inc., Tampa, FL). Distribution of radioactivity among individual lipids was assessed by TLC on 10  $\times$  10 cm plates (Silica Gel 60, 0.25 mm thickness; Macherey-

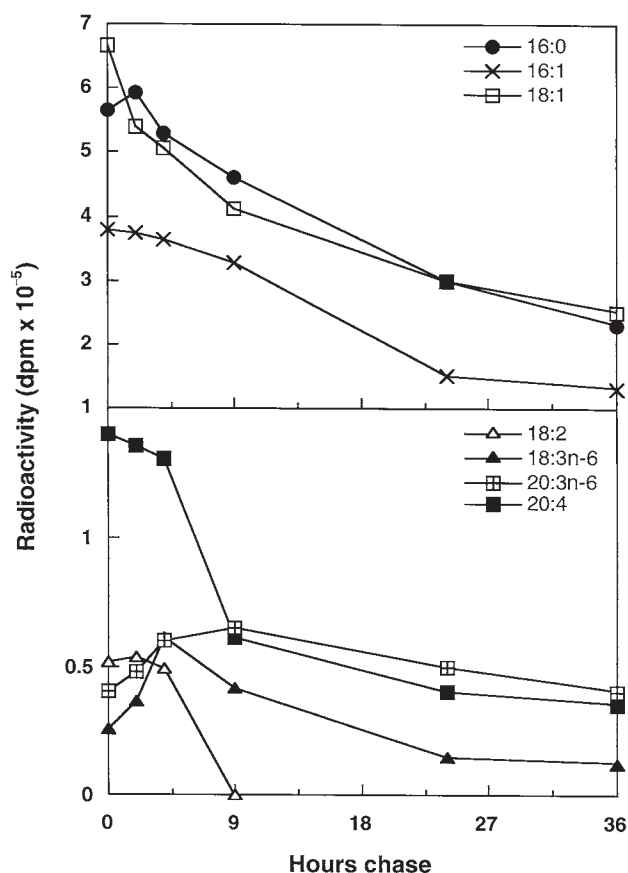


FIG. 2. Redistribuition of radioactivity in the fatty acids (*p*-bromophenacyl esters) of phosphatidylethanolamine of *Parietochloris incisa* after pulse-chase labeling with [2-<sup>14</sup>C]acetate (details as in Fig. 1). The two isomers (*n*-9 and *n*-7) of 18:1 were not resolved.

Nagel, Düren, Germany). Polar lipids were separated by two-dimensional TLC using a solvent system of chloroform/methanol/water (65:25:4, by vol) for the first direction and chloroform/methanol/1-ethylpropylamine/conc. ammonia (25%) (65:35:0.5:5, by vol) for the second direction. Neutral lipids were resolved with petroleum ether/diethyl ether/acetic acid (80:20:1, by vol). Radioactivity was detected by autoradiography with X-ray films (X-OMAT AR; Kodak, Rochester, NY) exposed to the TLC plates for 24 h. Lipid spots were scraped directly into scintillation vials containing 1 mL of methanol and 1 mL of scintillation cocktail (Ultima Gold, Packard), and radioactivity was measured in a liquid scintillation counter (Rackbeta LKB, model 1217; LKB, Wallac Oy, Finland).

## RESULTS AND DISCUSSION

**Incorporation of [2-<sup>14</sup>C]sodium acetate.** Cells of *P. incisa* were pulse labeled with [2-<sup>14</sup>C]acetate and chased after 1 h with 0.5 mM acetate. The most labeled fatty acids immediately after the pulse were 16:0, 16:1, and 18:1 (Fig. 1). How-

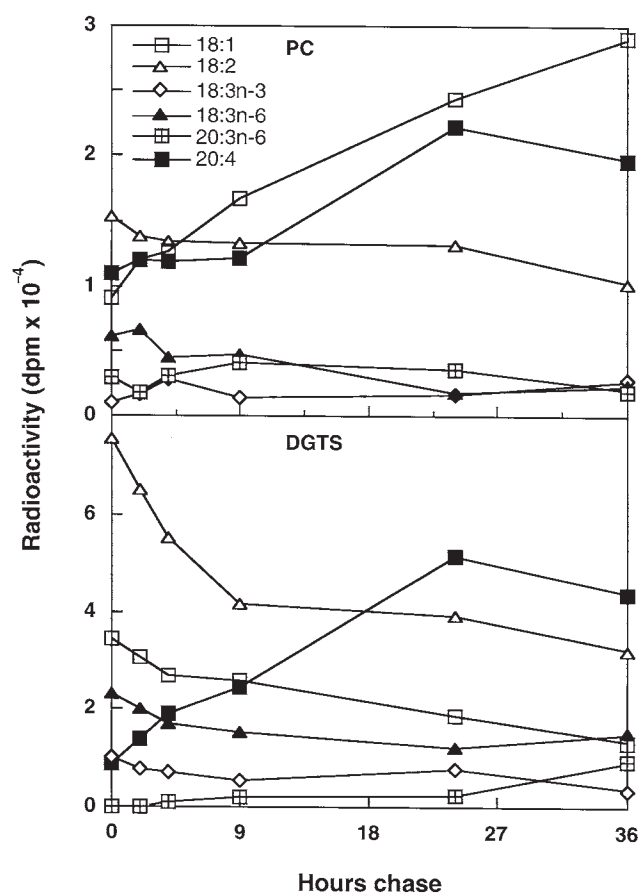


FIG. 3. Redistribuition of radioactivity in the fatty acids (*p*-bromophenacyl esters) of phosphatidylcholine (PC) and diacylglyceroltrimerethylhomoserine (DGTS) after pulse-chase labeling of *Parietochloris incisa* cultures with [2-<sup>14</sup>C]acetate (details as in Fig. 1). Palmitic acid was also highly labeled (not shown).

ever, all other C<sub>18</sub> as well as C<sub>20</sub> fatty acids were already labeled, even after a shorter pulse (0.5 h, data not shown). This finding clearly suggested that labeled acetate participated not only in the *de novo* synthesis but also in the elongation of C<sub>18</sub> to C<sub>20</sub> fatty acids. A similar phenomenon was reported to take place in *Pavlova lutheri* (22). During the chase, AA became the second-most labeled fatty acid after 16:0. The presence of labeled 18:1, 18:2, 18:3n-6 and 20:3n-6 and the lack of labeling in 20:2n-6 indicated that, at the fatty acid level, the biosynthetic pathway leading to AA is the same as that of *Porphyridium cruentum* (Sequence 1).

In PE, 16:0, 16:1, 18:1, and AA were immediately labeled and lost most of their label during the chase (Fig. 2). Labeling of 18:2, 18:3n-6, and 20:3n-6 peaked after 2, 5, and 9 h, respectively. In PC, the label of 18:1 increased throughout the time course, whereas that of 18:2, 18:3n-6, 20:3n-6, and AA peaked after 0, 1, 9, and 24 h (Fig. 3). In DGTS, all C<sub>18</sub> fatty acids were immediately labeled (Fig. 3), and lost labels with time. However, the labeling of 20:3n-6 was rather low and increased only slightly toward the end of the chase. As with PC, labeling of AA peaked after 24 h.

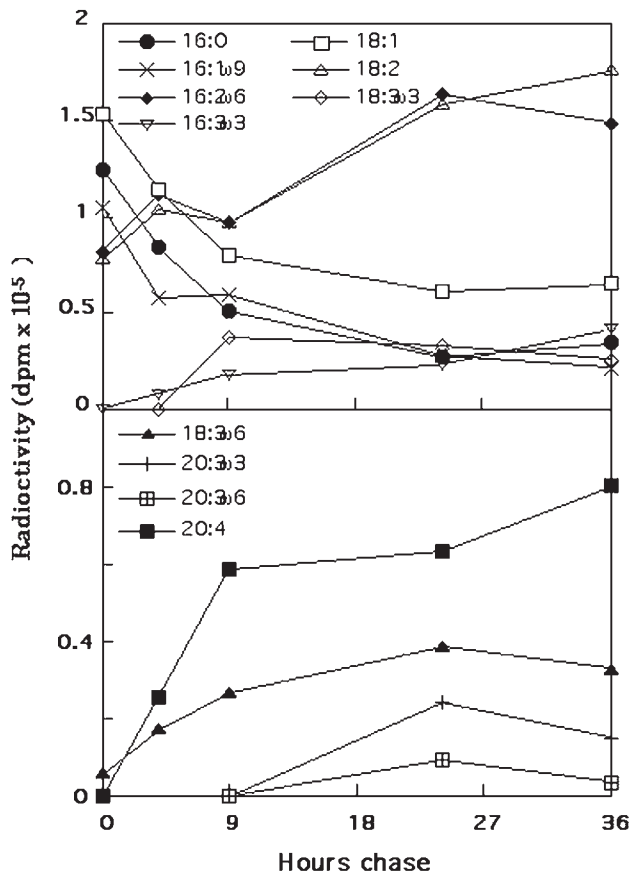


FIG. 4. Redistributiion of radioactivity in the fatty acids (p-bromophenacyl esters) of monogalactosyldiacylglycerol after pulse-chase labeling with  $[2-^{14}\text{C}]$ acetate (details as in Fig. 1).

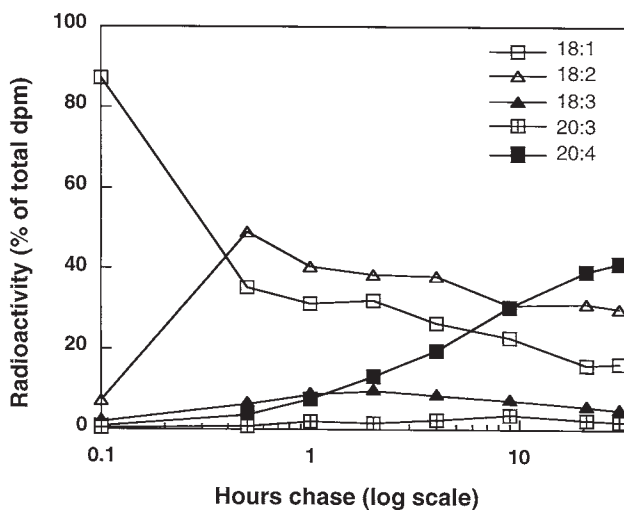


FIG. 5. Redistributiion of radioactivity in the fatty acids of *Parietochloris incisa* after 30 min of pulse labeling with  $[1-^{14}\text{C}]18:1n-9$  ( $19.8 \times 10^6$  dpm incorporated). Isomers of 18:3 were not resolved.

The labeling kinetics of the fatty acids of monogalactosyldiacylglycerol (MGDG) (Fig. 4) show a high initial labeling of 18:1 that gradually turns over in favor of 18:2n-6 and

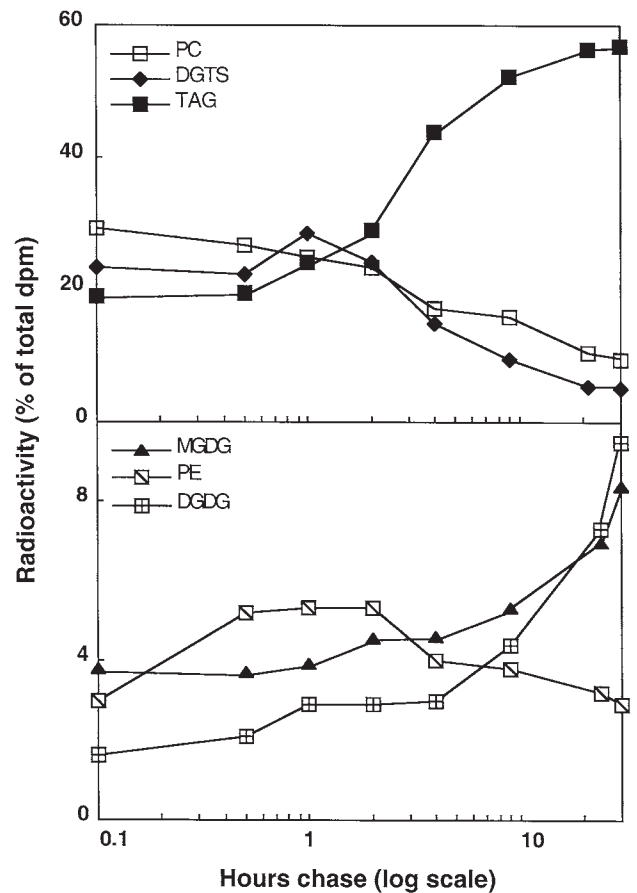
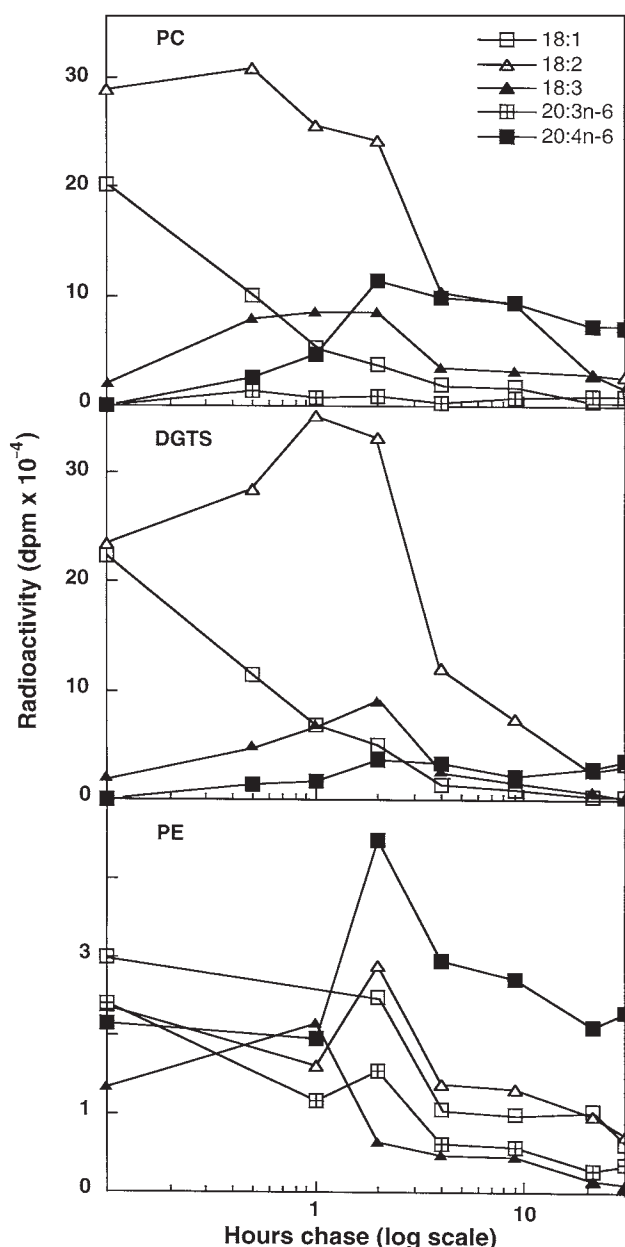


FIG. 6. Redistributiion of radioactivity in the major lipids of *Parietochloris incisa* after 30 min of pulse labeling with  $[1-^{14}\text{C}]18:1n-9$ . TAG, triacylglycerol; DGDG, digalactosyldiacylglycerol; for other abbreviations see Figures 2-4.

18:3n-3. Similarly, 16:0, which was also highly labeled, lost its label to 16:1, 16:2n-6, and 16:3n-3. This pattern suggests a pathway by which the 18:1/16:0 molecular species are stepwise desaturated to produce the prokaryotic molecular species, 18:3n-3/16:3n-3, in keeping with the classical prokaryotic pathway described for *Arabidopsis thaliana* (23) and for *Chlorella vulgaris* (24). Indeed, molecular species analysis showed the presence of both 18:2/16:2 and 18:3n-3/16:3n-3 in MGDG of *P. incisa* (9). Labeled AA and 18:3n-6 gradually increased; however, 20:3n-6 was not detected until much later, indicating that AA is probably being imported from extrachloroplastic lipids.

**Incorporation of  $[1-^{14}\text{C}]$ oleic acid.** The apparent participation of acetate at both the *de novo* synthesis and in the extrachloroplastic elongation of  $\text{C}_{18}$  to  $\text{C}_{20}$  fatty acids did not allow the elucidation of the role of each of the individual lipids involved in the biosynthesis. We have thus chosen to label the culture with long-chain fatty acids, although we were aware that the results might not completely reflect the *de novo* pathway. Attempts to label the culture with  $[1-^{14}\text{C}]$ lauric or  $[1-^{14}\text{C}]$ palmitic acid failed, as the labels were incorporated into lipids but were not further metabolized

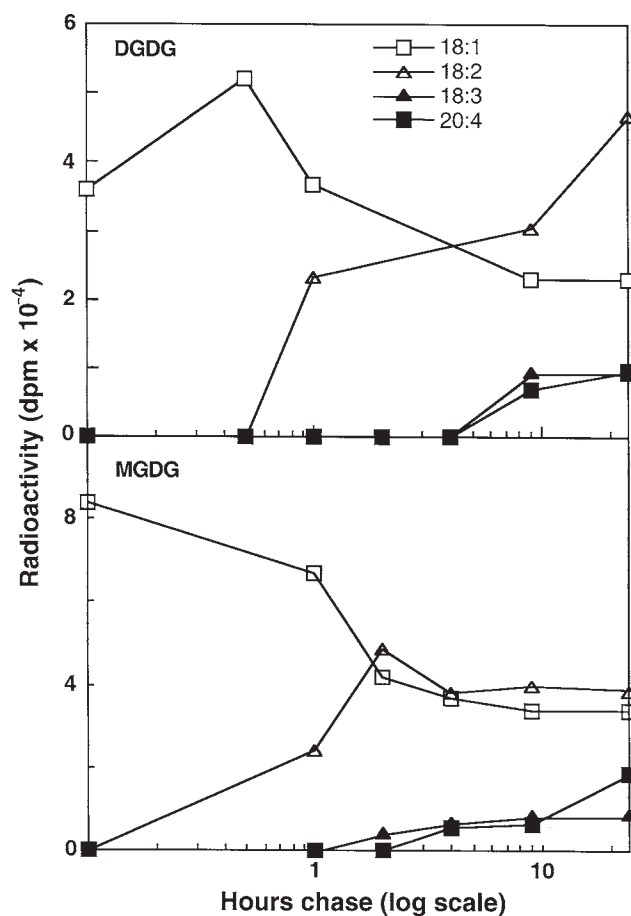


**FIG. 7.** Redistribution of radioactivity in the fatty acids of PC, DGTS and PE after labeling with  $[1-^{14}\text{C}]18:1n-9$ . Isomers of 18:3 were not resolved. For abbreviations see Figures 2 and 3.

(data not shown). We have thus used  $[1-^{14}\text{C}]$ oleic acid in subsequent labeling experiments.

The labeling kinetics following the incorporation of labeled oleic acid (Fig. 5), as well as that obtained following the label with acetate (Figs. 1–3), suggested a rapid turnover of 18:1 to 18:2, 18:3, and finally to 20:3n-6 and AA, according to the n-6 pathway shown in Sequence 1. Fatty acids shorter than 18:1 were not labeled, indicating that breakdown of labeled oleate and re-incorporation of the label through the *de novo* synthesis did not occur.

Initial label from oleate was mainly incorporated into PC, DGTS, and TAG (Fig. 6). During the time course, PC and



**FIG. 8.** Redistribution of radioactivity in the fatty acids of DGDG and MGDG after labeling with  $[1-^{14}\text{C}]18:1n-9$ . Isomers of 18:3 were not resolved. For abbreviations see Figures 4 and 6.

DGTS turned over their label slightly to the galactolipids but mostly to TAG, which eventually accounted for almost 60% of total label. The labeling within PE increased briefly but decreased to its original level after 3 h. Labeling of MGDG and digalactosyldiacylglycerol (DGDG) did not significantly change for the first 4 h but doubled thereafter.

We also followed the label of fatty acids within each of these lipids. Although we did not separate 18:3 into its isomers, we assume that 18:3n-6 was the major isomer, since there were not even traces of either 18:4n-3 or 20:3n-3. The initial high level of labeled 18:1 in PC and in DGTS was succeeded by the movement of the label into 18:2 and 18:3 (Fig. 7).  $C_{18}$  fatty acyl groups of PE were much less labeled. In PE, however, the initial label of 20:3n-6 was relatively more significant, especially if the lower molar concentration of PE is taken into consideration (Table 1). The label of 20:3n-6 was high already after the pulse and was turned over in favor of AA, the latter becoming the most labeled fatty acid after 2 h. In contrast, PC and later also DGTS lost most of the label of  $C_{18}$  fatty acids but very little of that label was reintroduced into  $C_{20}$  fatty acids. In PC, labeling of 20:3n-6 was rather low and in DGTS it was not detected at all. Following the decline

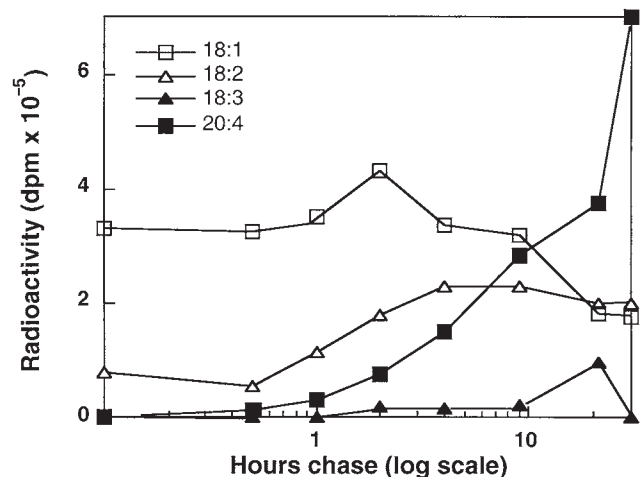


FIG. 9. Redistribution of radioactivity in the fatty acids of TAG after labeling with  $[1-^{14}\text{C}]$  18:1n-9. Isomers of 18:3 were not resolved. For abbreviation see Figure 6.

in the label of  $\text{C}_{18}$  fatty acids, AA constituted much of the residual label of PC and later also in DGTS.

The labeling kinetics of this experiment as well as the labeling with acetate (Fig. 2) indicated PC and DGTS as the most likely substrates for the  $\Delta 12$  and  $\Delta 6$  desaturations of 18:1 to 18:3n-6 (Sequence 1). PE is apparently much less involved in these desaturations. Treatment with the inhibitor salicylhydroxamic acid produced further evidence implicating PC and DGTS as the lipid carriers for the  $\Delta 12$  and  $\Delta 6$  desaturases (9). As elongation of 18:3 takes place at its carboxylic end, the fatty acyl groups of 18:3n-6 must be detached from its phospholipid carrier prior to its elongation to 20:3n-6. Since labeled 20:3n-6 was detected first in PE and then in PC but not in DGTS, we deduce that the former lipids, especially PE, are the most likely substrates for the  $\Delta 5$  desaturation of 20:3n-6 to AA. However, much more labeled AA appeared in PC than in PE. This finding could possibly be ex-

plained by a rapid methylation of 20:4n-6-PE to 20:4n-6-PC. The presence of molecular species common to both PC and PE, e.g., AA/AA, 18:2/AA, 18:1/AA, and 16:0/AA, further supports this suggestion (9). However, direct transfer of AA from PE to PC is also possible.

Labeled 18:1 was incorporated into both MGDG and DGDG and was subsequently desaturated to 18:2 and 18:3 (presumably 18:3n-3, since only this isomer is found in the galactolipids). Labeled 20:3n-6 was conspicuously absent in these lipids (Fig. 8). Labeled AA, however, was detected after 4 and 9 h, respectively, concomitant with its decrease in PE and PC. These findings indicate that the galactolipids can serve as substrates for the chloroplastic  $\Delta 9$  and  $\Delta 12$  desaturations and apparently also the  $\Delta 15$  desaturation of  $\text{C}_{18}$  fatty acids that are common to higher plants and many green algae, whereas the  $\Delta 6$  and  $\Delta 5$  desaturations take place only in the cytoplasm. Similar findings were reported for the biosynthesis of 20:5n-3 in *Phaeodactylum tricorutum* (15,16), *Nannochloropsis* sp (19), and *Monodus subterraneus* (25). However, in *Porphyridium cruentum* a  $\Delta 17$  chloroplastic desaturase is capable of desaturating 20:4n-6 to 20:5n-3.

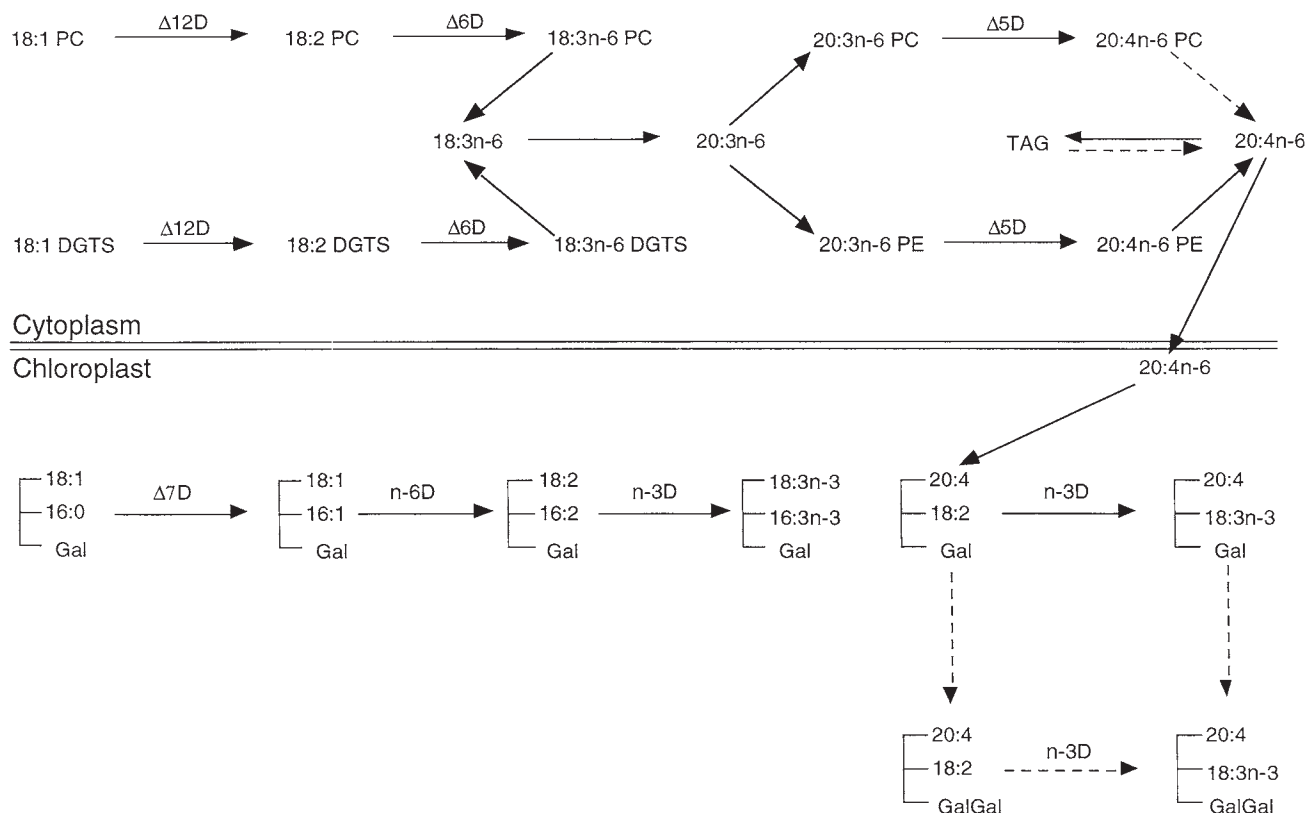
The biosynthesis of the prokaryotic molecular species in MGDG of *P. incisa*, is apparently similar to that described for *Chlorella vulgaris* (24) and *A. thaliana* (23). However, the occurrence of AA-containing eukaryotic molecular species of MGDG and DGDG (9) suggests that AA is imported from extrachloroplastic lipids. A similar import of AA was demonstrated in *Porphyridium cruentum* (13). Translocation of single fatty acids from the cytoplasm was also reported to occur in *Pavlova lutheri* (22).

Most of the initial label within the TAG lipids consisted of 18:1 and 18:2 acyl groups, with the temporary appearance of 18:3 (9). The label of AA increased continuously and after 3 h was responsible for the entire gain of label in TAG. The decrease of labeled AA in PC and PE and its subsequent increase in TAG indicate PC and PE as the most likely contributors of AA to TAG. Once AA is synthesized in phospho-

TABLE 1  
Fatty Acid Composition of the Lipids of *Parietochloris incisa*<sup>a</sup>

Lipid class	Lipid distribution (mol%)	Fatty acid composition (mole %)													
		16:0	16:1 n-11	16:1 n-7	16:2 n-6	16:3 n-3	18:0	18:1 n-9	18:1 n-7	18:2 n-6	18:3 n-6	18:3 n-3	20:3 n-6	20:4 n-6	20:5 n-3
Biomass		16.4	4.9	Tr	1.4	5.8	1.0	14.8	Tr	14.9	1.6	12.2	0.8	23.8	1.6
TAG	35.4	11.1	0.2	0.2	0.2	0.7	2.4	24.6	3.6	12.5	1.2	2.3	1.0	36.3	2.3
MGDG	17.2	3.3	1.6	≠	8.3	27.4	0.4	4.7	0.8	12.2	0.8	31.8	≠	7.5	0.6
DGDG	10.6	35.6	1.1	0.4	2.8	3.5	0.9	8.5	2.1	19.2	0.9	18.5	≠	5.5	1.4
SQDG	11.0	66.6	0.3	0.2	≠	≠	0.7	5.6	5.5	11.4	0.2	9.2	≠	0.4	≠
PG	2.9	44.7	7.3	0.3	0.6	Tr	1.0	10.2	3.7	17.0	1.3	3.1	0.2	3.6	≠
PC	6.8	18.9	0.3	1.0	0.3	0.2	3.7	8.0	7.1	18.5	7.5	4.7	2.9	23.8	1.0
DGTS	5.4	35.1	0.4	1.3	0.0	0.3	2.6	6.0	2.8	20.8	8.7	4.6	1.0	13.6	0.8
PE	2.0	7.5	0.0	1.2	0.5	≠	3.3	3.6	19.4	6.0	3.1	1.4	10.9	40.1	1.7
PA	1.7	50.7	1.1	0.2	0.8	1.4	4.2	18.2	3.0	12.9	0.3	5.6	≠	1.6	≠
PI	1.7	56.1	0.1	0.8	0.5	0.1	3.5	14.6	4.3	15.0	1.0	1.3	Tr	2.0	Tr

<sup>a</sup>The culture was diluted daily to a chlorophyll concentration of 5–6  $\mu\text{g}/\text{mL}$ . PA, phosphatidic acid; PG, phosphatidylglycerol; PI, phosphatidylinositol; SQDG, sulfoquinovosyldiacylglycerol; TAG, triacylglycerols; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PC, phosphatidylcholine; DGTS, diacylglyceryltrimethylhomoserine; PE, phosphatidylethanolamine. The fatty acids 18:4n-3, 20:0, 20:1, and 20:2 were present at less than 0.5 mol%. In PG, 16:1 $\Delta 3t$  constituted 6.9 mol%.



**FIG. 10.** Suggested pathways for the biosynthesis of polyunsaturated fatty acids in *P. incisa*.  $\Delta nD$ , a fatty acid desaturase that introduces a double bond at the  $n$ th carbon atom, counting from the carboxyl group; Gal, galactosyl; for other abbreviations see Figures 2, 3, and 6.

lipids, it is exported predominantly to TAG but also to the galactolipids and, apparently, also to DGTS. Recently, we found that the substituted pyridazinone, SAN 9785, inhibited the assembly of TAG but not the production of AA (9). A similar effect of this herbicide on *Pavlova lutheri* was reported by Siljegovic-Hänggi and Eichenberger (26). Owing to this inhibition, AA was mainly accumulated in PC, further supporting its role as a possible donor of AA to TAG. In contrast to higher plants, TAG of algae generally contain saturated and monounsaturated fatty acids (27,28). *Parietochloris incisa* is thus one of the very few reported cases where PUFA accumulate within TAG lipids.

The desaturases that produce PUFA in algae use PC, DGTS, and PE as acyl carriers for their substrates. In *Chlamydomonas reinhardtii*, which does not contain PC, 18:1 is stepwise desaturated up to 18:4n-3 while linked to the *sn*-2 position of DGTS or PE (18). Similarly, *Ochromonas danica*, which does contain PC, utilizes only DGTS and PE for these desaturations (17). Schneider and Roessler (19) have shown that the  $\Delta 12$  and  $\Delta 6$  desaturases of *Nannochloropsis* operate on  $C_{18}$  fatty acids attached to PC whereas the  $\Delta 5$  and the  $\Delta 17$  desaturases utilize  $C_{20}$  fatty acids esterified to PE. In *Porphyridium cruentum*, the desaturations of both  $C_{18}$  and  $C_{20}$  fatty acids occur mostly while being constituents of PC (13). The pathways we suggest for *P. incisa*, in which PC and DGTS are the major substrates for the  $\Delta 12$  and  $\Delta 6$  desaturases

and PC and PE for the  $\Delta 5$  desaturase, are thus different from any of the previously described models. Figure 10 summarizes these observations and suggests likely pathways for the biosynthesis of AA in the cytoplasm and n-3 PUFA in the chloroplast.

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Notes to Authors

Re: L8885

1. In the Acknowledgments, please provide the Contribution number.
2. Reference 9. My impression is that Ben Gurion University has several campuses. At which campus did this author earn the Ph.D.?

Thank you for your contribution to Lipids.

Sincerely,

Meg Griffin  
Copy Editor