

enzyme activity behaved differently: it increased in accordance with the treatment time of BA, and the increase after 12 h of BA treatment was especially significant (Y. Yamaryo, Y. Yamamoto, D. Kanai, K. Awai, T. Masuda, H. Shimada, K.-i. Takamiya and H. Ohta, unpublished work).

In the same manner, the effects of illumination on mRNA level of MGDG synthase and enzyme activity were determined. mRNA showed a transient increase at 3 h of illumination (Figure 1). In the case of the enzyme activity, a noteworthy increase after 24 h of illumination was observed (Y. Yamaryo, Y. Yamamoto, D. Kanai, K. Awai, T. Masuda, H. Shimada, K.-i. Takamiya and H. Ohta, unpublished work). Here again, the existence of the time lag between an increase in the mRNA level and enzyme activity was found.

To clarify the mechanism for the induction of gene expression, promoter assaying was performed as a detailed analysis. A plasmid construct, containing the 5'-upstream region of a *MGD/LUC* gene fusion, was utilized for the assay. The chimaeric gene was introduced into etiolated cucumber cotyledons by particle bombardment. The promoter activities were analysed in terms of the transient expression of the reporter gene in response to BA and illumination. The transient assays clearly demonstrated the elevated levels of LUC expression in response to BA and light (Y. Yamaryo, Y. Yamamoto, D. Kanai, K. Awai,

T. Masuda, H. Shimada, K.-i. Takamiya and H. Ohta, unpublished work).

From these results, it was revealed that the mRNA level of MGDG synthase showed a transient increase in response to both BA treatment and illumination. Moreover, there was a time lag between the peak of the mRNA level and the enzyme activation. Promoter assay with chimaeric genes showed the existence of light- and cytokinin-responsive elements in the 5'-upstream region. Consequently, it may be inferred that the activation of MGDG synthase is regulated, at least in part, at the post-transcription level, in addition to regulation at the transcription level.

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## The role of triacylglycerol as a reservoir of polyunsaturated fatty acids for the rapid production of chloroplastic lipids in certain microalgae

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### Abstract

Many microalgae are known to accumulate triacylglycerols (TAGs), especially under nitrate starvation. Generally, these TAGs are predominantly constructed of saturated and mono-unsaturated fatty acids. In contrast, the TAGs of the red microalga *Porphyridium cruentum* are rich in arachidonic acid (AA, 20:4  $\omega$ 6) and eicosapenta-

enoic acid (EPA, 20:5  $\omega$ 3). A mutant of this alga, impaired of growth at suboptimal temperatures, was shown to have reduced levels of EPA and of the eukaryotic molecular species of monogalactosyldiacylglycerol (MGDG) and an elevated level of TAG. Labelling experiments have shown that labelling of wild-type TAGs decreased, whereas that of the mutant remained high. Contemporarily, eukaryotic MGDG of the mutant was less labelled. Similarly, TAGs of the green alga T12, which can grow at a low temperature, are extremely rich in AA. We have labelled exponentially growing cultures of T12 kept at 25 °C with radioactive AA and cultivated the cultures for a further 12 h at 25 °C, 12 °C or 4 °C. At low

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Key words: arachidonic acid, LC PUFA, long-chain PUFA, *Porphyridium cruentum*.

Abbreviations used: TAG, triacylglycerol; PUFA, polyunsaturated fatty acid; AA, arachidonic acid.

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temperatures, labelled AA was transferred from TAGs to polar lipids. These findings may indicate that polyunsaturated fatty acids that can be incorporated into the membranes, enabling the organism to quickly respond to low-temperature-induced stress.

## Introduction

Cultivation of algae under growth-limiting conditions was shown to result in accumulation of storage lipids such as triacylglycerols (TAGs) [1]. However, even in algae that are rich in polyunsaturated fatty acids (PUFAs), TAGs are mostly constituted by saturated and mono-unsaturated fatty acids [2]. Relatively high proportions of PUFAs are found only in TAGs of a few algae. We have recently found a freshwater alga (code name T12) that is unique in its ability to accumulate TAGs that are rich in arachidonic acid (AA, 20:4  $\omega$ 6). Under nitrogen-starvation conditions, TAGs accounted for over 90% of total acyl lipids and AA constituted up to 60% of the fatty acids of TAGs [3]. In the red microalga *Porphyridium cruentum*, N starvation resulted in an enhanced accumulation of TAGs [4]. Concomitantly, the proportion of the PUFA AA in TAGs increased sharply [5]. Similar findings were obtained for other red algae [6]. Recently, we have described a mutant of *P. cruentum*, impaired of growth at sub-optimal temperatures, that had an elevated level of TAGs and reduced level of the 20:5/20:5 eukaryotic molecular species of MGDG [7]. Labelling experiments revealed that mutant TAGs accumulated a higher percentage of the initial label and were severely limited in turning over the label in comparison to the wild type. Subsequently, eukaryotic MGDG was much less labelled. In this work, we have studied growth resumption of *P. cruentum* following nitrogen starvation and changes in the lipid composition of T12 following temperature reduction. Based on our findings we hypothesize that PUFA-rich TAGs may have a role as a depot of PUFAs that can be mobilized for the construction of chloroplastic membranes under certain environmental conditions.

## Materials and methods

*P. cruentum* strain 1380.1d was obtained from the Göttingen Algal Culture Collection and was grown on Jones' medium as described previously [8]. Cultures of T12 were cultivated on BG11 medium [9]. Cultures of *P. cruentum* were resuspended in nitrate-free medium. After 2 days of

nitrogen starvation, the cultures were labelled for 24 h with ammonium salts of 5  $\mu$ Ci of [1- $^{14}$ C]AA (specific activity 58 mCi/mmol; Amersham) and resuspended in fresh medium afterwards. *P. cruentum* lipids were extracted using the procedure of Bligh and Dyer [10], and lipids of T12 were extracted with 10% DMSO in methanol. Lipid analysis was carried out as described previously [11].

## Results

### *P. cruentum*

Cultures of *P. cruentum* were starved of nitrogen for 48 h, labelled with AA for another 24 h and resuspended in full medium. Following the pulse, the wild type and the mutant were similarly labelled and the label was similarly distributed between cytoplasmic and chloroplastic lipids (Figure 1, left-hand panel). There was no degradation of labelled AA. Phosphatidylcholine of both cultures lost about 70% of their label, whereas wild-type TAGs lost 95% of its original label in comparison with only 59% in the mutant (Figure 1, right-hand panel). The prokaryotic species of MGDG (20:4/16:0 and 20:5/16:0) were similarly labelled, while labelling of the eukaryotic species (20:5/20:5, 20:4/20:4, 20:4/20:5 and 20:5/18:2) of the mutant was about half of that of the wild type (Figure 1, right-hand panel).

### T12

In an attempt to assess the effect of a sudden temperature shift on the mobility of acyl groups from TAGs, exponential cultures of T12 kept at 25 °C were labelled with [1- $^{14}$ C]AA and cultivated for another 12 h at 25 °C, 12 °C or 4 °C. Total radioactivity did not decline significantly and AA was still the only labelled fatty acid at the end of the experiment, indicating that no breakdown and reassembly of fatty acids had occurred. At 25 °C, the level of radioactivity remained relatively stable in both TAGs and polar lipids, indicating that any transfer of acyl groups, between polar lipids and TAGs, was already in equilibrium. However, at 12 °C and even more so at 4 °C, a rapid transfer of label from TAGs to polar lipids took place throughout the time course of the experiment (Figure 2).

## Discussion

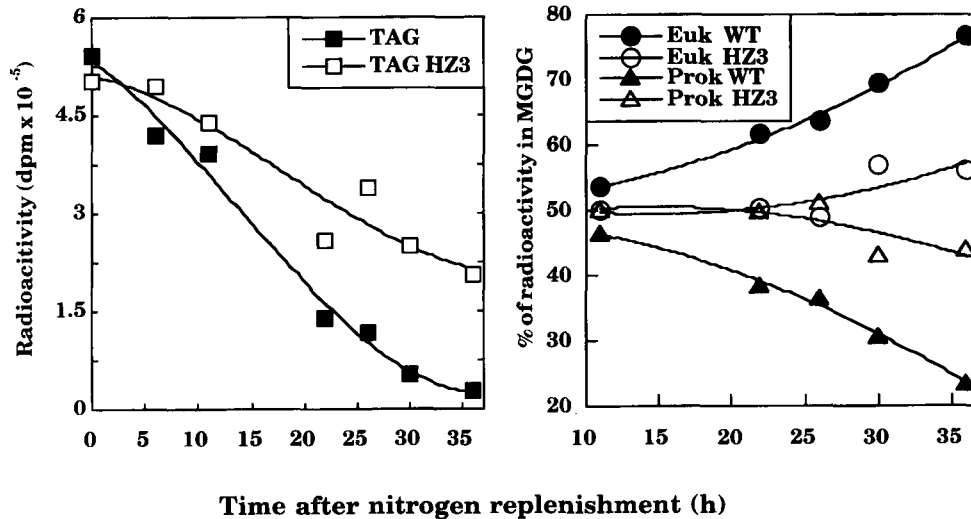
The increase in the degree of unsaturation of membranal fatty acids upon reduction of the temperature is a universal phenomenon [12].

Therefore, adaptation to sudden decreases in temperature must include a mechanism for fast enhancement of the PUFA content of the membranes. We thus suggest that in algae that contain long-chain PUFAs and whose natural habitat is characterized by rapid changes in environmental conditions, particularly sudden drops in temperature, PUFA-rich TAGs might be involved as a buffer capacity for PUFAs. The transfer of label from TAGs to eukaryotic MGDG following nitrogen replenishment in *P. cruentum* or to polar

lipids subsequent to temperature reduction in T12 could possibly indicate that under conditions that require rapid, enhanced desaturation and perhaps production of new chloroplastic membranes, cells of *P. cruentum* and T12 can utilize TAGs as a reservoir of AA, for the construction of their membranes. Indeed, Wanner and Kost [13] demonstrated that the degradation of the cellular membrane pool in starved cells of *P. cruentum* is reversible and occurs in correlation with the formation of lipid bodies. Regeneration of plastids

**Figure 1**

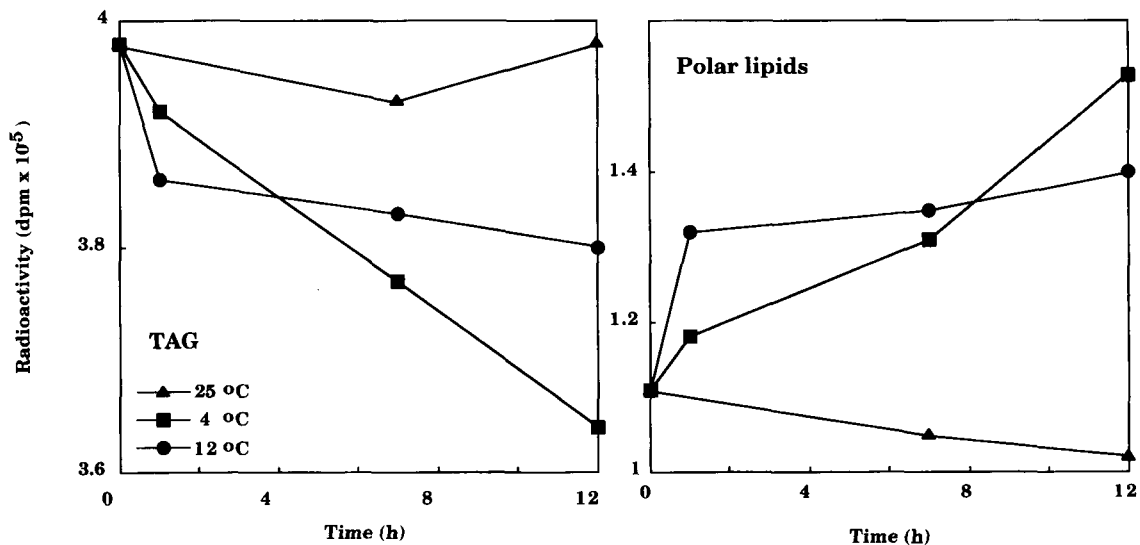
Changes in the label of TAG (left-hand panel) and in the proportion of labelled prokaryotic (prok) and eukaryotic (euk) molecular species of MGDG (right-hand panel) in wild-type (WT) and HZ3 mutant *P. cruentum* following recovery from nitrogen starvation and labelling with 10  $\mu$ Ci of [ $1-^{14}$ C]20:4  $\omega$ 6



**Figure 2**

Effect of temperature downshift on the composition of labelled lipids of T12

Exponential cultures were pulse-labelled with [ $1-^{14}$ C]20:4  $\omega$ 6 for 12 h at 25 °C and cultivated for another 12 h at 25 °C, 12 °C or 4 °C.



is accompanied with the disappearance of the lipid bodies and occurs even when fatty acid synthesis is inhibited by cerulenin, indicating that the lipid bodies can be utilized for the construction of membrane lipids. Likewise, Makewicz et al. [14] suggested that TAGs of the phaeophyte *Ectocarpus fasciculatus* are involved in the transfer of fatty acids to MGDG and other polar lipids. Furthermore, Garces et al. [15] and Sarmiento et al. [16] have shown that following a temperature drop in sunflowers, 18:1 of TAG was transferred to PC where it was desaturated to 18:2. We hypothesize, therefore, that one of the roles of TAG in long-chain-PUFA-rich algae is to serve as a reservoir of AA or eicosapentaenoic acid which enables the organism to quickly respond to low temperature-induced stress.

This may indeed be the case for T12. This alga was isolated from a snow patch [3] and was shown to be relatively resistant to low temperatures. Similarly, *P. cruentum* is mainly found in shallow marshes where temperature fluctuations are rapid and more pronounced than in deeper bodies of water. The increase in the proportion of EPA in MGDG at low temperatures and especially in that of the eukaryotic component of MGDG, 20:5/20:5, could possibly be attributed to the organism's attempt to cope with the stress inflicted by sudden drops in temperature.

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