Curr Genet (1998) 33: 165–170

ORIGINAL PAPER

Ryuuichi Itoh · Kyoko Toda · Hidenori Takahashi
Hiroyoshi Takano · Tsuneyoshi Kuroiwa

Delta-9 fatty acid desaturase gene containing a carboxyl-terminal cytochrome b5 domain from the red alga *Cyanidioschyzon merolae*

Received: 6 October / 22 December 1997

Abstract A delta-9 fatty acid desaturase gene, homologous to animal and fungal acyl-coenzyme A (CoA) desaturases, was isolated from the red alga *Cyanidioschyzon merolae* using a degenerate PCR strategy. This gene, designated as *CmFAD9*, has no intron. The encoded delta-9 fatty acid desaturase (CmFad9p) consists of 476 amino acids and has an estimated molecular mass of 55.4 kDa. CmFad9p is a unique delta-9 fatty acid desaturase among plants, in that it is fused with the cytochrome b5 domain at its carboxyl terminus. This is characteristic of yeast acyl-CoA desaturase. Genomic Southern hybridization suggested that the *C. merolae* genome contains a single gene for delta-9 fatty acid desaturase of the animal and fungal type. Southern hybridization combined with pulsed-field gel electrophoresis revealed that *CmFAD9* is probably located on chromosome XI of the 17 *C. merolae* chromosomes. A 1.6-kb product of this gene was transcribed throughout a light/dark synchronization culture. The discovery of *CmFAD9* indicates the existence of a novel type of plant delta-9 fatty acid desaturase that may function in the endoplasmic reticulum, but not in the plastid.

Key words *Cyanidioschyzon merolae* · Cytochrome b5 · Delta-9 fatty acid desaturase · Red alga

Introduction

Fatty acids are an essential component of membrane lipids. The fatty acid composition of each membrane determines the characteristics of the membrane, such as its fluidity, permeability, and ultrastructure. Furthermore, a series of recent yeast genetic studies have shown that fatty acid synthesis is closely related to the structure, function, and inheritance of organelles (Schneiter and Kohlwein 1997). In the budding yeast *Saccharomyces cerevisiae* the *mdm2* (*m*itochondrial *d*istribution and *m*orphology *2*) allele of *OLE1*, encoding delta-9 acyl-coenzyme A (CoA) desaturase, interferes with the transfer of mitochondria into the bud and leads to the aggregation and fragmentation of those mitochondria. (McConnell et al. 1990; Stewart and Yaffe 1991). Mitochondrial movement and morphology are probably extremely sensitive to changes in the fluidity of mitochondrial membranes resulting from the absence of unsaturated fatty acids produced by the fatty acid desaturase (Stewart and Yaffe 1991).

Delta-9 acyl-CoA desaturase, a microsomal membrane intrinsic enzyme, synthesizes mono-unsaturated fatty acids from saturated fatty acids in animal and fungal cells. This enzyme introduces a double bond between the ninth and tenth carbons of both palmitoyl (16:0) and stearoyl (18:0) CoA substrates to make the monoenoic products palmitoleic (16:1) and oleic (18:1) acid respectively. To perform this reaction, the microsomal membrane enzyme cytochrome b5 must transfer electrons from its internal heme to the iron centers of the delta-9 fatty acid desaturase. Interestingly, the delta-9 fatty acid desaturases of *S. cerevisiae* (Ole1p) and other yeast species are chimeric proteins of these two enzymes. Cytochrome b5 is fused to the carboxyl terminus of the delta-9 fatty acid desaturase (Mitchell and Martin 1995).

Two other types of delta-9 fatty acid desaturases have been identified in cyanobacteria and plants (Fukuchi-Mizutani et al. 1995). Delta-9 acyl-lipid desaturases in cyanobacteria desaturate fatty acids bound to glycerolipids. Delta-9 acyl-acyl carrier protein (ACP) desaturases in the chloroplast stroma of higher plants desaturate fatty acids esterified to ACP. Delta-9 acyl-ACP desaturase is structurally unrelated to either delta-9 acyl-CoA desaturase or delta-9 acyl-lipid desaturase, although the last two desaturases have similar amino-acid sequences (Shanklin and Somerville 1991; Sakamoto et al. 1994).

R. Itoh (✉) · K. Toda · H. Takahashi · H. Takano · T. Kuroiwa
Department of Biological Sciences,
Graduate School of Science, University of Tokyo,
Hongo, Tokyo 113-0033, Japan
Fax: +81-3-3814 1408
e-mail: rgitoh@biol.s.u-tokyo.ac.jp

Communicated by J.-D. Rochaix

We isolated the delta-9 fatty acid desaturase gene from the unicellular red alga *Cyanidioschyzon merolae* as the first step toward determining which type of delta-9 fatty acid desaturase functions in algae, and in an attempt to understand the mechanism by which fatty acid desaturase regulates mitochondrial morphology and inheritance. We believe that *C. merolae* is a model organism for studying the morphology and inheritance of mitochondria for four reasons:

(1) It contains a single, simple-shaped mitochondrion per cell (Kuroiwa et al. 1994).
(2) This mitochondrion multiplies by binary division during cell division (Ohta et al. 1993; Suzuki et al. 1994).
(3) Mitochondrial division can be synchronized by the use of light-dark cycles and inhibitor treatment (Terui et al. 1995; Itoh et al. 1996, 1997).
(4) It is the only organism in which the apparatus of mitochondrial division, the mitochondrion-dividing ring, can be visualized by electron microscopy (Kuroiwa et al. 1993, 1995).

Therefore, by studying *C. merolae* we can increase our understanding of how the intracellular distribution and temporal expression of delta-9 fatty acid desaturase affects morphological changes and the division of mitochondria. In this paper we describe the isolation of a yeast-like delta-9 fatty acid desaturase gene, containing the carboxyl terminal cytochrome b5 domain, from *C. merolae*. This is the first report of the existence of a yeast-like delta-9 fatty acid desaturase in the plant kingdom.

Materials and methods

Material and culture conditions. The *C. merolae* cells were the generous gift of Prof. G. Pinto (University of Napoli, Italy). The culture and synchronization of *C. merolae* cells were performed according to Suzuki et al. (1994).

PCR amplification. Degenerate PCR was used to prepare a delta-9 fatty acid desaturase gene-specific probe. Nuclear DNA of *C. merolae*, the generous gift of Dr. N. Ohta (Waseda University, Japan), was used as a template. A pair of oligonucleotides [5′-CA(CT)(AC)GI(CT)TITGG(AT)(GC)ICA(CT)(AC)G-3′ and 5′-(AG)TG(AG)TG(AG)AA(AG)TT(AG)TG(AG)TAICC(CT)TCICC-3′] corresponding to the highly conserved amino-acid sequences HRLWSHR and GEGYHNFHH flanking the delta-9 fatty acid desaturase of *S. cerevisiae* (Ole1p) and rat liver stearoyl-CoA desaturase (Stukey et al. 1990), were synthesized and used as primers. PCR was performed for 40 cycles of 94°C for 1 min/56°C for 1 min/72°C for 1 min. Under these conditions, a single 525-bp fragment was amplified.

DNA cloning and sequencing. The genomic DNA library (Takahashi et al. 1995) was screened according to the method of Takahashi et al. (1995) using the 525-bp PCR fragment as a probe. One positive candidate was obtained after a second screening. Phage DNA was isolated from this candidate by the liquid lysate method (Sambrook et al. 1989). The phage DNA was digested with *Not*I and Southern hybridization (Takahashi et al. 1995) was performed using the degenerate PCR fragment as the probe. The 7.7-kbp fragment identified was cloned into pBluescript II SK+ (Stratagene, USA). This clone was digested with *Sal*I and the resulting 4.2- and 2.8-kb fragments, both of which hybridized with the probe, were subcloned. Deletion clones were obtained from these three clones, covering the entire region of the delta-9 fatty acid desaturase gene, using the pBluescript II Exo/Mung DNA Sequencing System (Stratagene, USA) according to the instruction manual supplied. Sequencing of the deletion clones was performed according to Matsunaga et al. (1996).

Southern hybridization. Genomic Southern hybridization was carried out using the degenerate PCR fragment as a probe under the following low-stringency conditions. The DNA probe was labelled with [α-^{32}P]-dCTP using a Megaprime DNA labelling system (Amersham LIFE SCIENCE, USA) according to the manufacturer's instructions. Pre-hybridization and hybridization were performed at 42°C in hybridization solution [6 × SSC (90 mM sodium citrate and 0.9 M sodium chloride), 5 × Denhardt's solution (0.1% (w/v) Ficoll 400, 0.1% (w/v) polyvinylpyrrolidone, and 0.1% (w/v) bovine serum albumin), 0.5% (w/v) sodium dodecyl sulfate (SDS), and 100 μg/ml denatured salmon testis DNA] for 1 h and 16 h respectively. After hybridization the membrane was washed with a series of buffers (2, 2, 1, 0.5, and 0.1 × SSC each containing 0.1% (w/v) SDS) at 42°C for 20 min per buffer. The chromosome location of the fatty acid desaturase gene was determined by pulsed-field gel electrophoresis (Takahashi et al. 1995) and Southern hybridization using Fluorescein Gene Images (Amersham LIFE SCIENCE, USA) according to the supplied instruction manual, using the 2.8-kb *Sal*I clone as the probe.

Northern hybridization. Total RNA of *C. merolae* was extracted by the method of Ohta et al. (1994). Northern hybridization was performed using the degenerate PCR fragment as a probe under the following high-stringency conditions. One microgram of total RNA was glyoxylated and electrophoresed in a 1.1% agarose gel in 10 mM sodium phosphate buffer (pH 7.0) for 2.5 h at 70 V with rapid circulation of the buffer. After electrophoresis, the samples were transferred to a nylon membrane filter, baked for 2 h at 80°C, and boiled in TE buffer [10 mM Tris-HCl (pH 7.5), 1 mM EDTA] at 100°C for 5 min. Pre-hybridization and hybridization were performed at 42°C in hybridization solution [5 × SSPE (0.75 M sodium chloride, 43.25 mM NaH_2PO_4, 6.25 mM EDTA), 50% (v/v) formamide, 5 × Denhardt's solution, 0.5% (w/v) SDS, and 20 μg/ml of denatured salmon testis DNA] for 1 h and 16 h respectively. After hybridization the membrane was washed in a series of buffers: 2 × SSPE, 0.1% (w/v) SDS at room temperature twice for 10 min, 1 × SSPE, 0.1% (w/v) SDS at 65°C twice for 20 min, and 0.1 × SSPE, 0.1% (w/v) SDS at 65°C for 20 min.

Results and discussion

Structure of the *C. merolae* delta-9 fatty acid desaturase gene

We designed oligonucleotide primers based on conserved histidine motifs in delta-9 fatty acid desaturases to isolate the gene from *C. merolae*. PCR with these primers amplified a single 525-bp fragment. The nucleotide sequence of this fragment showed significant DNA homology to animal and fungal delta-9 acyl CoA desaturases. Using this fragment as a probe, we screened the *C. merolae* genomic DNA library. Screening produced a single candidate. DNA sequence analysis indicated that this clone contained an open reading frame of 1431 bp, including the sequence identical to the probe (Fig. 1). It encoded a protein composed of 476 amino acids with an estimated molecular mass of 55.4 kDa. A search of genetic databases using the BLAST algorithm (Altschul et al. 1990) and the derived amino-acid sequence revealed significant homologies with fungal and animal delta-9 acyl-CoA desaturases and the delta-9 acyl-lipid desaturases of cyanobacteria. The se-

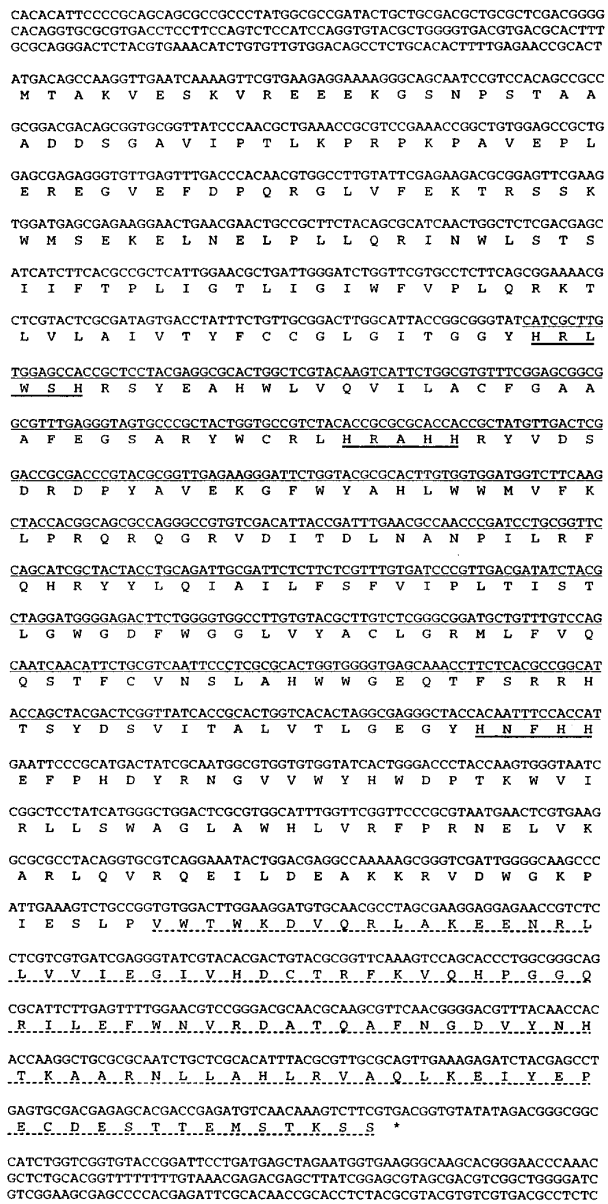

Fig. 1 The nucleotide sequence of the *C. merolae* delta-9 fatty acid desaturase gene (*CmFAD9*) and its 5′ and 3′ flanking regions. The derived amino-acid sequence is shown below the nucleotide sequence. Nucleotide and residue numbers are indicated to the left of each line. An *asterisk* under codon TGA indicates a stop codon. *Underlining* of a nucleotide sequence indicates the probe used to screen the genomic DNA library, as well as for genomic Southern hybridization and Northern hybridization. Three *bold lines* beneath the amino-acid sequence indicate the conserved histidine boxes. *Broken lines* beneath the amino-acid sequence indicate the cytochrome b5 domain of CmFad9p. This sequence data will appear in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases with the accession number AB006677

quence of the *C. merolae* desaturase gene was well conserved compared to those of other species, except at the amino- and carboxyl-termini. The histidine motifs, which are associated with the diiron-oxo moieties of desaturase [HRXXXH (residues 130–135 in Fig. 1), HRXHH (167–171), and HNXHH (304–308)], were highly conserved. The similarity of the *C. merolae* desaturase amino-acid sequence to that of other species suggests that the *C. merolae* desaturase gene has no intron. We designated this gene as *CmFAD9* (for *C. merolae* delta-9 fatty acid desaturase). Until now, a plant delta-9 fatty acid desaturase gene homologous to animal and fungal delta-9 acyl-CoA desaturase has only been reported in rose petals (Fukuchi-Mizutani et al. 1995). *CmFAD9* is the second gene reported in plants, and the first in algae, that encodes a fatty acid desaturase homologous to the delta-9 acyl-CoA desaturases of animals and fungi. We could not find any signal peptide sequences that indicated the localization of the protein.

The outstanding feature of *CmFAD9* is its carboxyl-terminal region, which resembles those of yeast. In the yeast delta-9 fatty acid desaturases, a cytochrome b5-like domain is linked to the carboxyl terminus. The cytochrome b5 domain of yeast delta-9 fatty acid desaturase plays a role in the desaturation of fatty acids, as revealed by truncation or disruption experiments of this domain, while cytochrome b5 protein itself is not required for fatty acid desaturation (Mitchell and Martin 1995). Alignment of the carboxyl termini of *C. merolae* (CmFad9p) and *S. cerevisiae* (Ole1p) desaturases with the entire sequence of cytochrome b5 of several species revealed that CmFad9p is also a chimeric protein of delta-9 fatty acid desaturase and cytochrome b5 (Fig. 2). The conserved amino acids that correspond to the heme-binding pockets common to cytochrome b5, (D/E)HPGG (residues 44–48 in Fig. 2), DAT (59–61), and H(S/T) (71–72), were also conserved in CmFad9p. The only difference was a Q at position 44 instead of a D or an E. The discovery of *CmFAD9* indicates the existence of a novel type of delta-9 fatty acid desaturase in the plant kingdom, a fusion enzyme combining delta-9 fatty acid desaturase and cytochrome b5.

Copy number, chromosome location, and expression of the *C. merolae* delta-9 fatty acid desaturase gene

The copy number of the delta-9 fatty acid desaturase gene in the *C. merolae* genome was studied by genomic Southern hybridization (Fig. 3). Under low-stringency conditions, a single obvious band appeared in the *Eco*RI and *Xho*I restriction digests. *Pst*I-digested genomic DNA produced two bands, perhaps because the *Pst*I site is located in the middle of the region that the probe hybridized to. From this we conclude that the *C. merolae* genome probably contains a single delta-9 fatty acid desaturase gene. The homology between the cytochrome b5 region of *CmFAD9* and the cytochrome b5 genes of other species is low at the nucleotide-sequence level. It therefore appears to be too difficult to detect the gene for the only cytochrome b5 protein of *C. merolae* by Southern hybridization using the *CmFAD9* cytochrome b5 region as a probe.

Southern hybridization combined with pulsed-field gel electrophoresis was used to determine the chromosomal location of *CmFAD9*. Electrophoretic karyotyping revealed that *C. merolae* has 17 chromosomes (Takahashi et al. 1995). The chromosomal DNA was separated using a

```
                10         20         30         40         50         60         70         80
Cm     V-WTW-KDV- ----QRLAK- -EENRL-LVV IEGIVHDCTR FKVQHPGGQR ILEFWNVRDA TQAFNGDVYN HTKAARNLLA
Sc     M---WDKQ-- --TF--LAKS KE-NKG-LVI ISGIVHDVSG YISEHPGGET LIKTALGKDA TKAFSGGVYR HSNAAQNVLA
Hs.b5  MAEQSDEAVK YYTLEEIQKH NH-SKSTWLI LHHKVYDLTK FLEEHPGGEE VLREQAGGDA TENF-EDV-G HSTDAREMSK
Os.b5  MSNDNKK-V- -YTLEEVAKH NS-KDDCWLI IGGKVYNVSK FLEDHPGGDD VLLSSTGKDA TDDF-EDV-G HTTTARAMMD
Sc.b5  MP----K-V- -YSYQEVAEH NG-PQNFWII IDDKVYDVSQ FKDEHPGGDE IIMDLGGQDA TESF-VDI-G HSDEALRLLK

                90         100        110        120        130        140        150
Cm     HLRVAQLK-- EIYEPECDE- --STTEM--- --STKSS... .......... .......... .......... ..........
Sc     DMRVAVIK-- ESKNSAIRM- ---------- ASKRGEIYET GKFF...... .......... .......... ..........
Hs.b5  TFIIGELH-- ----PDDRPK LNKPPETLIT TIDSSSSWWT NWVIPAISAV AVALMYRLYM AED.......
Os.b5  EYYVGDID-- TSTIP-ARTK YVPPKQPHYN QDKTPEFIIK ILQFLVPLAI LGLAVAIRIY TKSESA....
Sc.b5  GLYIGDVDKT SERVSVEK-- -VSTSE---N QSKGSGTLVV ILAIL----M LGVAY----Y LLNE......
```

Fig. 2 Comparison of the carboxyl-terminal sequence of CmFad9p (*Cm*) with the carboxyl terminus of *S. cerevisiae* delta-9 fatty acid desaturase Ole1p (*Sc*; accession number J05676) and the entire cytochrome b5 sequences of *Homo sapiens* (*Hs.b5*; L39945), *Oryza sativa* (*Os.b5*; X75670), and *S. cerevisiae* (*Sc.b5*; L22494). The positions of conserved amino acids that correspond to a heme-binding pocket common to cytochrome b5 are indicated by *double underlines*

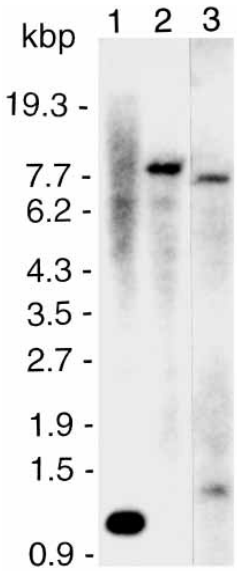

Fig. 3 Genomic Southern hybridization with the *CmFAD9* probe. In each case, 1 μg of nuclear DNA from *C. merolae* was digested with *Eco*RI (*1*), *Xho*I (*2*), and *Pst*I (*3*) and then Southern hybridization was performed on each restriction digest under low-stringency conditions. λDNA digested with *Sty*I was used for size markers

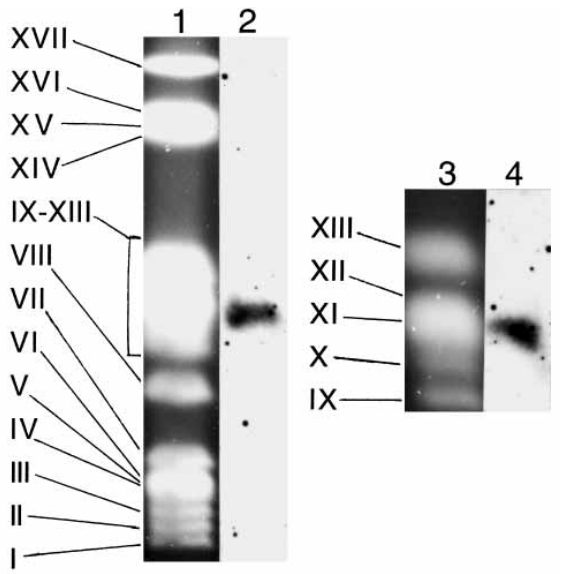

Fig. 4 Southern hybridization analysis of chromosomes separated by pulsed-field gel electrophoresis with the *CmFAD9* probe. Ethidium bromide-staining patterns (*1, 3*) and the corresponding autoradiograms (*2, 4*) are indicated. Separation of chromosomes was made using standard conditions for overall separation (*1, 2*) and then with specialized conditions to separate the five mid-sized chromosomes (auto-algorithm for separation of DNAs between 650 kb and 770 kb; *3, 4*)

modification of the standard conditions described by the supplier for separation of *S. cerevisiae* chromosomal DNA. With Southern blotting, the hybridized band was detected in the region of five stacked chromosomes (IX–XIII; Fig. 4-1, 2). To determine which chromosome hybridized with the *CmFAD9* probe, these five chromosomes were further separated. Re-hybridization provided a single band in the region of two chromosomes (XI and XII; Fig. 4-3, 4). These two chromosomes were difficult to separate completely, because their sizes are too similar. We concluded that *CmFAD9* is probably on chromosome XI, because the hybridized band on the autoradiogram was located in the lowest part of this region.

The cell and organelle division cycles of *C. merolae* were synchronized by subjecting cells to a 12-h light/12-h dark cycle. Most cells are in interphase during the light period. At the beginning of the succeeding dark period, the mitochondrion, chloroplast, and nucleus begin to divide, in that order. Once division of these three organelles is complete, cytokinesis occurs during the middle of the dark period. To assess the relationship between organelle division and expression of the delta-9 fatty acid desaturase gene, total RNA was extracted at various times during the light/dark cycle and was studied by Northern hybridization (Fig. 5). A 1.6-kb *CmFAD9* molecule is transcribed at all stages examined. No stage-specificity of *CmFAD9* expression was observed. This suggests that CmFad9p functions constitutively throughout the cell cycle. Western-blot analysis and immunolocalization of CmFad9p during the cell cycle will be the subject of future studies.

Evolutionary implications

Why does *C. merolae* possess a yeast-like chimeric fatty acid desaturase different from other plant fatty acid desa-

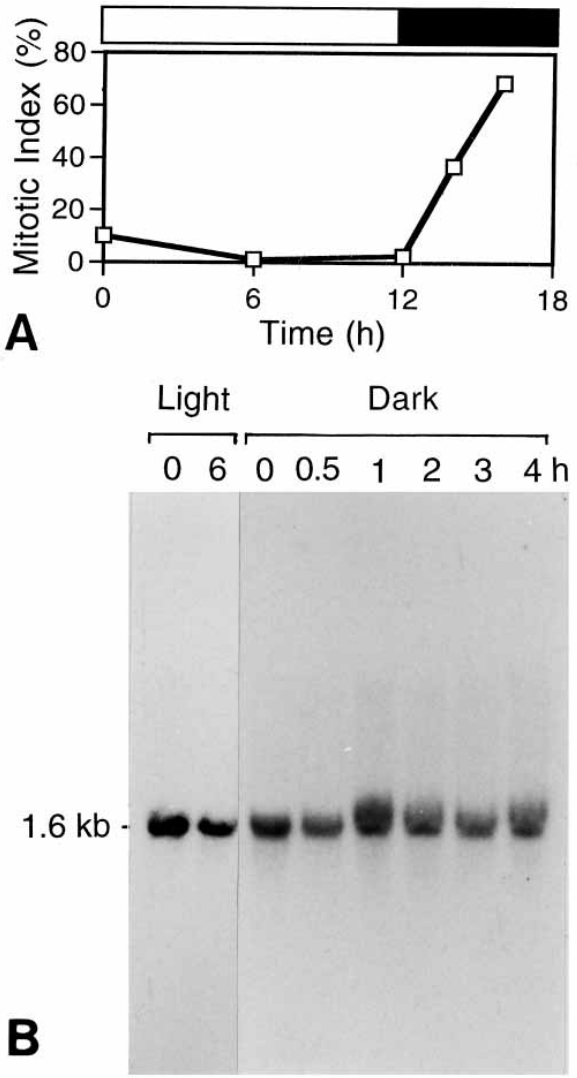

Fig. 5A, B *CmFAD9* expression during mitotic cycle. **A** synchronization of cell division of *C. merolae* in a 12-h light/dark culture. The x-axis indicates time after the beginning of the light period. The y-axis indicates the frequency of cell division. Five hundred cells were observed for each time. **B** Northern hybridization with the *CmFAD9* probe. In each case, 1 μg of total RNA from *C. merolae* at the different times indicated above was hybridized under high-stringency conditions. A 0.24–9.5-kb RNA Ladder (GIBCO BRL, USA) was used for size markers

turases, and how did this evolve? One hypothesis is that *CmFAD9* is derived from the inherent delta-9 fatty acid desaturase and cytochrome b5 of an ancestral *C. merolae* in a fusion event, independently of yeasts. Mitchell and Martin (1995) point out that there might be some selective advantage for this type of enzyme system. Tethering cytochrome b5 to the fatty acid desaturase could potentially speed up electron transfer, by positioning a correctly oriented heme group with respect to the dioxo-iron cluster. This orientation would eliminate the need for diffusion of NADH cytochrome b5 reductase, the electron donor, across the microsomal membrane to the fatty acid desaturase, and re-orientation of the reduced cytochrome b5. Other types of plant desaturases containing an amino-terminal cytochrome b5 domain have been discovered recently, including the acyl-lipid desaturase of sunflower (Sperling et al. 1995) and the delta-6 fatty acid desaturase of borage (Sayanova et al. 1997). These findings support the hypothesis that fusion of the fatty acid desaturase and cytochrome b5 occurred independently in *C. merolae* and yeasts.

An alternative hypothesis is that the fatty acid desaturase-cytochrome b5 fusion enzyme of *C. merolae* and yeasts is derived from a common ancestor. According to this hypothesis, the fusion event occurred once in either yeasts or *C. merolae*, and the other group obtained the fusion enzyme by horizontal transfer, although the mechanism of this process is unknown.

It is also possible that the chimeric enzyme of desaturase and cytochrome b5 was present in an ancient ancestor and split into two independent enzymes in the phylogenetic lines that led to today's higher organisms. This seems unreasonable, however, in view of the advantage conferred by the chimeric fatty acid desaturase discussed above. To determine which hypothesis is more appropriate, delta-9 fatty acid desaturase should be isolated, and its structure determined, for more taxa, including green and brown algae and many phyla of protozoa and fungi.

Acknowledgements R. I. was supported by a research fellowship (no. 3641) from the Japan Society for the Promotion of Science for Young Scientists. The work was also supported by a grant-in-aid for specially promoted research (no. 06101002) to T. K. from the Japanese Ministry of Education, Science and Culture.

References

Altschul SF, Gish W, Miller W, Meyers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Fukuchi-Mizutani M, Savin K, Cornish E, Tanaka Y, Ashikari T, Kusumi T, Murata N (1995) Senescence-induced expression of a homologue of Δ9 desaturase in rose petals. Plant Mol Biol 29: 627–635

Itoh R, Takahashi H, Toda K, Kuroiwa H, Kuroiwa T (1996) Aphidicolin uncouples the chloroplast division cycle from the mitotic cycle in the unicellular red alga *Cyanidioschyzon merolae*. Eur J Cell Biol 71:303–310

Itoh R, Takahashi H, Toda K, Kuroiwa H, Kuroiwa T (1997) Checkpoint control on mitochondrial division in *Cyanidioschyzon merolae*. Protoplasma 196:135–141

Kuroiwa T, Suzuki K, Kuroiwa H (1993) Mitochondrial division by an electron-dense ring in *Cyanidioschyzon merolae*. Protoplasma 175: 173–177

Kuroiwa T, Kawazu T, Takahashi H, Suzuki K, Ohta N, Kuroiwa H (1994) Comparison of ultrastructures between the ultra-small eukaryote *Cyanidioschyzon merolae* and *Cyanidium caldarium*. Cytologia 59:149–158

Kuroiwa T, Suzuki K, Itoh R, Toda K, O'Keefe TC, Kuroiwa H (1995) Mitochondria-dividing ring: ultrastructural basis for the mechanism of mitochondrial division in *Cyanidioschyzon merolae*. Protoplasma 186:12–23

Matsunaga S, Kawano S, Takano H, Uchida H, Sakai A, Kuroiwa T (1996) Isolation and developmental expression of male reproductive organ-specific genes in a dioecious campion, *Melandrium album* (*Silene latifolia*). Plant J 10:679–689

McConnell SJ, Stewart LC, Talin A, Yaffe MP (1990) Temperature-sensitive yeast mutants defective in mitochondrial inheritance. J Cell Biol 111:967–976

Mitchell AG, Martin CE (1995) A novel cytochrome b5-like domain is linked to the carboxyl terminus of the *Saccharomyces cerevisiae* Δ-9 fatty acid desaturase. J Biol Chem 270: 29766–29772

Ohta N, Suzuki K, Kawano S, Kuroiwa T (1993) Direct evidence of mitochondrial nuclear division in the ultra-micro alga *Cyanidioschyzon merolae*. Cytologia 58:471–476

Ohta N, Sato N, Kawano S, Kuroiwa T (1994) The *trpA* gene on the plastid genome of *Cyanidium caldarium* strain RK-1. Curr Genet 25:357–361

Sakamoto T, Wada H, Nishida I, Ohmori M, Murata N (1994) Delta-9 acyl-lipid desaturases of cyanobacteria: molecular cloning and substrate specificities in terms of fatty acids, *sn*-position, and polar head groups. J Biol Chem 269:25576–25580

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Sayanova O, Smith MA, Lapinskas P, Stobart AK, Dobson G, Christie WW, Shewry PR, Napier JA (1997) Expression of a borage desaturase cDNA containing an N-terminal cytochrome b^5 domain results in the accumulation of high levels of Δ^6-desaturated fatty acids in transgenic tobacco. Proc Natl Acad Sci USA 94: 4211–4216

Schneiter R, Kohlwein SD (1997) Organelle structure, function, and inheritance in yeast: a role for fatty acid synthesis? Cell 88: 431–434

Shanklin J, Somerville C (1991) Stearoyl-acyl-carrier-protein desaturase from higher plants is structurally unrelated to the animal and fungal homologs. Proc Natl Acad Sci USA 88: 2510–2514

Sperling P, Schmidt H, Heinz E (1995) A cytochrome b5-containing fusion protein similar to plant acyl lipid desaturases. Eur J Biochem 232:798–805

Stewart, LC, Yaffe MP (1991) A role for unsaturated fatty acids in mitochondrial movement and inheritance. J Cell Biol 115: 1249–1257

Stukey JE, McDonough VM, Martin CE (1990) The *OLE1* gene of *Saccharomyces cerevisiae* encodes the Δ9 fatty acid desaturase and can be functionally replaced by the rat stearoyl-CoA desaturase gene. J Biol Chem 265:20114–20149

Suzuki K, Ehara T, Osafune T, Kuroiwa H, Kawano S, Kuroiwa T (1994) Behavior of mitochondria, chloroplasts and their nuclei during the mitotic cycle in the ultramicroalga *Cyanidioschyzon merolae*. Eur J Cell Biol 63:280–288

Takahashi H, Takano H, Yokoyama A, Hara Y, Kawano S, Toh-e A, Kuroiwa T (1995) Isolation, characterization and chromosomal mapping of an actin gene from the primitive red alga *Cyanidioschyzon merolae*. Curr Genet 28: 484–490

Terui S, Suzuki K, Takahashi H, Itoh R, Kuroiwa T (1995) Synchronization of chloroplast division in the ultramicroalga *Cyanidioschyzon merolae* (Rhodophyta) by treatment with light and aphidicolin. J Phycol 31: 958–961