

Fatty acid synthesis system in Glaucophyta: Further evidence for the lack of chloroplast fatty acid synthesis in glaucophyte algae

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Abstract

We previously showed that the chloroplast of the glaucophyte *Cyanophora paradoxa* does not have fatty acid synthesis activity, based on both experimental and bioinformatic analyses. The present study was intended to extend the bioinformatic analysis to a wider range of Glaucophyta, using published genomic data. The results confirmed that the four species of Glaucophyta analyzed do not have prokaryotic acetyl-CoA carboxylase, which is found in the chloroplasts of Rhodophyta and Viridiplantae. Only the eukaryotic type of acetyl-CoA carboxylase was identified. Some components of prokaryotic fatty acid synthase (FAS II), such as acyl carrier protein (ACP), 2-oxoacyl ACP synthase (KAS I/II), and FabD, were detected in the glaucophytes, but other components especially KAS III were not detected. In contrast, the eukaryotic fatty acid synthase (FAS I) was found in Gloeochaete as well as *Cyanophora*. Although the genome data is still to be amended, the available evidence suggests that the lack of fatty acid synthesis in the chloroplast is a trait of not just *Cyanophora* but also shared by the phylum Glaucophyta. The relationship between photosynthesis and fatty acid synthesis is discussed with special attention to the origin of chloroplasts.

Introduction

Fatty acids are major components of membrane lipids in all bacteria and eukaryotes, except Archaea. Chloroplasts (or plastids) are known to be the major site of fatty acid biosynthesis in plants and algae (Guan et al 2020; Misra et al. 2012; Ohlrogge and Jaworski 1997). Mitochondria also have minor activity in fatty acid synthesis in all eukaryotes, possibly for the synthesis of lipoate (Wada et al. 1997; Wada et al. 2002; Wedan et al. 2023). In contrast, the cytosol is the site of fatty acid synthesis in fungi and animals (Maier et al. 2010). Fatty acid synthesis consists of two major steps: the activation of acetyl-CoA to malonyl-CoA, and the main cycle of condensation of C2 units. The first process is catalyzed by acetyl-CoA carboxylase, and the second process by fatty acid synthase. Two different forms are known for both enzymes and are found in different organisms and compartments.

Bacterial acetyl-CoA carboxylase consists of four subunits (Cronan 2021; Polyak et al. 2012): biotin bound to biotin-carboxyl carrier protein (BCCP or AccB) is carboxylated by biotin carboxylase (AccC), and then the carboxyl group is transferred to acetyl-CoA by carboxyltransferase (AccA and AccD). Non-photosynthetic eukaryotes such as fungi and animals possess a large, multifunctional acetyl-CoA carboxylase (ACC I), which catalyzes all steps and is located in the cytosol (Supplementary Fig. S1) (Tong 2005). Plants and many algae studied so far possess both types of acetyl-CoA carboxylases: the multi-subunit type in the chloroplast and the multifunctional type in the cytosol. Malonyl-CoA is synthesized from malonate and CoA by malonyl-CoA synthetase in plant mitochondria (Guan and Nicolau 2016). The chloroplast genome encodes AccD in many plants and algae, while AccA and AccB are also encoded by the chloroplast genome in red algae (Sato 2020a). Exceptionally, some *Poaceae* chloroplasts lack the multi-subunit type, which is replaced by a multifunctional type (Sasaki et al. 1995). Recently, we found that the glaucophyte *Cyanophora paradoxa* also lacks the multi-subunit type acetyl-CoA carboxylase (Sato et al. 2025).

Fatty acid synthases (FAS) are classified into two types: Type II FAS (FAS II) consists of individual, monofunctional enzymes, such as 2-oxoacyl ACP synthase (called KAS I and II), 2-oxoacyl dehydrogenase, dehydratase, and enoyl reductase, each catalyzing a reaction in the main condensation cycle (Rock and Jackowski 2002). In addition, KAS III (FabH) is known to catalyze the synthesis of the initial C4 unit in the chloroplast, and FabD transfers malonyl-CoA to acyl-carrier protein (ACP). This type of FAS is present in chloroplasts and mitochondria (excluding KAS III) as well as bacteria. In plants, many of the FAS II components are known to be targeted to both chloroplasts and mitochondria (Guan et al. 2020). Type I FAS (or FAS I) consists of a high-molecular weight enzyme complex consisting of multifunctional polypeptides. This type of enzyme exists in the cytosol of fungi and animals, as well as in some actinobacteria (Bukhari et al. 2014; Jenke-Kodama et al. 2005; Maier et al. 2010; Smith and Tsai 2007). Fungal and animal enzymes are structurally distinct and are called fFAS and mFAS, respectively. Polyketide synthase and fatty acid synthase are related (Jenke-Kodama et al. 2005; Smith and Tsai 2007), and the two types of FAS I enzymes originated from bacterial polyketide synthases of different clades. We found that the glaucophyte *Cyanophora paradoxa* lacks many components of FAS II, while it has several copies of FAS I (Sato et al. 2025).

Currently, the fatty acid synthesis system is well documented in *Rhodophyta* (Mori et al. 2019; Sato and Moriyama 2007) and *Viridiplantae* (Ohlrogge and Jaworski 1997; Sato 2020a), whereas *Cyanophora paradoxa* is the only species that has been studied for fatty acid synthesis among *Glaucophyta* (Sato et al. 2025), another of the three main lineages of *Archaeplastida* (Adl et al. 2005; Jackson et al. 2015). To extend knowledge of the fatty acid synthesis system in *Glaucophyta*, we used the comparative genomics database Gclust (Sato 2009) to identify homologs of components of acetyl-CoA carboxylase and fatty acid synthase. Phylogenetic analysis was also used to confirm the identification. The results suggest that the *Glaucophyta* lack a fatty acid synthesis system in the chloroplast, as reported in *Cyanophora*. This finding contradicts the simplistic idea that both photosynthesis and fatty acid synthesis were implemented by the cyanobacterial endosymbiosis in the common ancestor of *Archaeplastida*. The advantages and disadvantages of fatty acid synthesis with different allocation of enzyme components are discussed.

Materials and methods

Sequence data

To identify clusters of proteins related to fatty acid synthesis enzymes, we used the preformed homolog clusters in the Gclust database (Sato 2009) at <https://gclust.jp/>. For this analysis, we employed two datasets, Gclust2024 and APclust2024, as described previously (Sato and Takano 2026). Briefly, Gclust2024 is a general-purpose dataset encompassing a variety of organisms, while APclust2024 includes data from 6 *Rhodophyta*, 4 *Glaucophyta*, 19 *Streptophyta* (including 4 land plants), and 31 *Chlorophyta*. The four species of *Glaucophyta* in this dataset are *Cyanophora paradoxa* NIES-547 (CPA, from the Cyanophora Genome Project of Rutgers University), *Cyanoptyche glaucocystis* (CyGL, from the entry JKHA of the 1000 Plants Project or 1KP in CNGBdb), *Glaucocystis cf. nostochinearum* (GINO, from

the entry POOW of 1KP in CNGBdb), and *Gloeochaete witrockiana* SAG46-84 (GIWI, from the entry HBOJ01.1 of NCBI). The amino acid sequence data from 1KP resulted from rather small transcriptome sizes and lacked data on large proteins. We had to combine partial sequences to reconstruct a complete sequence in the case of acetyl-CoA carboxylase. This approach was not successful in FAS I. The lack of an amino terminus in these data often made it difficult to infer intracellular targeting.

We identified ortholog clusters of enzymes related to fatty acid synthesis. The homolog sequences found in APclust2024 and Gclust2024 were combined. Sequences from our previous report (Sato 2020a) were also integrated where necessary. After removing redundancies and outdated versions, the homolog groups underwent phylogenetic analysis.

Phylogenetic analysis

Phylogenetic analysis was conducted following the methods described in previous studies (Sato 2020a; Sato and Takano 2026). Briefly, a multiple alignment was prepared by MAFFT version 7.490 with the option “--auto” (Katoh and Standley, 2013). Sites containing a gap in more than 20% (or 25%) of sequences were removed. The ML tree was constructed using IQ-tree version 3.0.1 for macOS (Minh et al., 2020) by finding the best model (options: -m MFP -madd LG4X, LG4M -B 1000 -T 4) each time. The Q.PFAM model was selected as the best in most cases, with the LG model as the second best. In some cases, however, the LG model was the best. The +I, +F, or +R5 (or +R6) options were also selected depending on the data. The Bayesian Inference (BI) tree was constructed using MrBayes version 3.2.7 (Ronquist et al., 2012) (rates=invgamma, aamodelpr=fixed(lg), ratepr=variable). The LG model was the best model available in MrBayes software. The BI calculations were performed on the Supercomputer at the Human Genome Center, the University of Tokyo. The final graphical representation of the trees was created using FigTree version 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>) and further decorated using Adobe Illustrator version CS6.

Estimation of intracellular localization

The amino acid sequences were analyzed using the DeepLoc-2.1 software (Ødum et al. 2024), which can be accessed at <https://services.healthtech.dtu.dk/services/DeepLoc-2.1/>.

Results

Analysis of acetyl-CoA carboxylases in Glaucophyta

None of the subunits of the multi-subunit type of acetyl-CoA carboxylase was detected in the genomic (or transcriptome) data of the four *Glaucophyta* analyzed (Fig. 1). The multifunctional type of acetyl-CoA carboxylase (Cluster 126 in APclust2024) was detected in *Cyanophora paradoxa* (CPA_99385_g24767.t1) and *Gloeochaete witrockiana* (GIWI_0185350418). However, further examination of the sequences with partial homology (“Related groups” in the Gclust database) detected partial sequences in all four species

of *Glaucophyta*. Using *Arabidopsis* ACC1 as a template, the partial sequences were assembled into a single large multifunctional enzyme (Fig. 1, Supplementary Fig. S1). This assembly is justified by the fact that we can clearly distinguish between multi-subunit and multifunctional types by sequence homology (Fig. 2a), namely, the multifunctional ACC is not just an assembly of the four subunits of multi-subunit-type enzymes. Therefore, the partial sequences are part of a multifunctional enzyme but not a multi-subunit type. Phylogenetic analysis of the multifunctional ACC enzymes showed that the *Glaucophyta* enzymes (single polypeptides and assembled sequences) formed a single clade except for an assembled sequence of *Gloeochaete* (Fig. 2b). This justified the identification and assembly of the ACC sequences in the four species of *Glaucophyta*. The only outlier, *Gloeochaete*, could reflect minor misassembly, which will be resolved with more refined genome data in the future.

Analysis of FAS II components in *Glaucophyta*

Among the FAS II components, only acyl carrier protein (ACP), FabB/F, and FabD were detected by the Gclust database in the four *Glaucophyta* species analyzed (Fig. 1). All these components are known to reside in both chloroplasts and mitochondria in *Arabidopsis*. The plastid genome encodes one ACP in *Cyanophora*; therefore, it is a plastid protein (Reyes-Prieto and Russell 2018). This ACP is not related to cyanobacterial ACP, but branched from within the clade of actinobacterial ACP in a previously published phylogenetic tree (Sato 2020a). Therefore, this ACP is not included in the phylogenetic analysis of the present study.

Another, nuclear-encoded ACP (CPA_01107_g7100.t1) is predicted to be targeted to mitochondria (probability $P = 0.95$). No other plastid genomes of *Glaucophyta* encode ACP. *Cyanoptyche* has two mitochondrial ACP copies ($P = 0.82$ and 0.94), but no plastid copy. *Glaucocystis* has a single ACP, which is targeted to the plastid ($P = 0.83$). *Gloeochaete* has a plastid ACP ($P = 0.96$) and three mitochondrial ACPs ($P = 0.89, 0.95, 0.95$). All these targeting predictions were made by DeepLoc-2.1 software, which works even with unidentified *N*-terminus sequences. Because of the short sequence lengths, we were able to construct only a low-quality phylogenetic tree of ACPs (Supplementary Fig. S2). However, mitochondrial and plastid ACPs were roughly classified by the phylogenetic tree.

The FabB/F was detected in all four species of *Glaucophyta*. In *Cyanophora*, one copy is targeted to plastids ($P = 0.99$), whereas another is targeted to mitochondria ($P = 0.68$) (Fig. 1). In the three other species, the FabB/F proteins are predicted to be targeted to neither plastids nor mitochondria. However, phylogenetic analysis clearly classified them into two groups, one sister to plant mitochondrial FabF and the other (including the *Cyanophora* FabF estimated to be targeted to plastids) sister to a clade consisting of plant/algal FabF and *Chlamydia* FabF (Fig. 3). *Cyanoptyche* has another short copy (CyGL_2031013), possibly a member of polyketide synthase but not plastid or mitochondrial FabF. The phylogenetic relationship was further confirmed by the detailed phylogenetic trees of the respective groups (Supplementary Figs. S3 and S4). These results indicate that each of the four glaucophyte species possesses a mitochondrial FabF. The *Gloeochaete* protein (GIWI_0184373254) is likely a plastid

protein like the *Cyanophora* ortholog (CPA_00342_g24242.t1), but these two could be distinct from the FabF (KAS I/II) of *Viridiplantae* and *Rhodophyta*.

A single copy of the FabD protein is detected in each glaucophyte (Fig. 1). The FabD in *Cyanophora* and *Gloeochaete* was predicted to be targeted to the mitochondria. In contrast, cytoplasmic localization was predicted for the FabD in the other two species. The phylogenetic tree (Supplementary Fig. S5) of FabD proteins showed two *Viridiplantae* clades: one presumably chloroplastic clade of green plants and algae, and the other a cytosolic or mitochondrial clade of green algae in close association with animal mitochondrial FabD. Rhodophyte FabD (presumably with both plastid and mitochondrial localization, with comparable values of *P*) formed a distinct clade. The four FabD proteins of *Glaucophyta* were spread over clades with fungal, protist, and chlamydial homologs. We therefore assume that the FabD of *Cyanoptycha* and *Glaucocystis* are also mitochondrial proteins.

Putative FabG was found in three glaucophytes except *Cyanoptycha* (Fig. 1). *Gloeochaete* FabG was predicted to be targeted to mitochondria (*P* = 0.84). The other enzymes were cytoplasmic proteins. A putative FabI was detected in *Cyanophora*, but there is no ortholog in other glaucophytes.

These results indicate that some FAS II components, such as ACP, FabF, FabD, and FabG, are present. The mitochondria might have a single copy of each of these, whereas each copy of ACP and FabF could be present in the plastid. FabH, the initiator synthesizing component in the plastid system in *Viridiplantae* and *Rhodophyta*, is not detected in any of the four species of *Glaucophyta* analyzed.

Analysis of FAS I in Glaucophyta

In a previous publication (Sato et al. 2025), we described three copies of FAS I in *Cyanophora*. All are fungal-type FAS I. A full-length FAS I was also detected in *Gloeochaete* (GIWI_0184342518). This FAS I was found to be related to the three copies of *Cyanophora* FAS I (Fig. 3). However, FAS I was not detected in the transcriptome data of *Cyanoptycha* and *Glaucocystis* obtained from CNGBdb (JKHA and POOW, respectively), maybe because the transcriptome data were incomplete for detecting a large protein like FAS I. Nevertheless, a scrutiny of the normalized transcriptome data in EMBL detected a short fragment of *Glaucocystis* transcript (ENA|EC122136) encoding the C-terminus of FAS I (Supplementary Fig. S6). Despite its very short length, this sequence suggests the presence of a FAS I protein in *Glaucocystis*, because the C-terminus corresponds to the acyl-carrier-protein synthase (or 4'phosphopantetheinyl transferase) domain of fFAS, which is distinct from the monofunctional AcpS of bacteria or plants. The results suggest that fatty acid synthesis is performed by the fungal-type FAS I in at least three species of *Glaucophyta*. We also expect to find a FAS I in *Cyanoptycha* in the future.

Discussion

Lack of fatty acid synthesis system in the plastids in Glaucophyta

As an extension of our previous study on *Cyanophora*, we surveyed the presence or absence of acetyl-CoA carboxylase and fatty acid synthase in four species of *Glaucophyta*, based on published genomic or transcriptomic data. Multi-subunit type and multifunctional type are known for both enzymes. The multifunctional types of acetyl-CoA carboxylase and fatty acid synthase are both cytosolic enzymes, whereas the multi-subunit types are localized to the chloroplast. Mitochondria also have a multi-subunit type of fatty acid synthase for limited lipoate synthesis in all eukaryotes.

Glaucophytes possess only the multifunctional type of acetyl-CoA carboxylase, which is localized to the cytosol. Some subunits of the multi-subunit type of fatty acid synthase, such as ACP, FabF, FabD, and FabG, are detected in *Glaucophyta* and are localized to either chloroplasts or mitochondria. However, the key component of chloroplast fatty acid synthesis, FabH, is not detected in any *Glaucophyta* species. This suggests that the multi-subunit type of fatty acid synthase may be functional in mitochondria but not in chloroplasts. The multifunctional type of fatty acid synthase was found in *Gloeochaete* (full-length) and *Glaucocystis* (fragment), as well as in *Cyanophora* (full-length). Although further studies are needed in *Cyanoptycha*, we can conclude that fatty acid synthesis is performed in the cytosol (and mitochondria) but not in the chloroplasts in *Glaucophyta*.

Photosynthesis and fatty acid synthesis

The localization of the fatty acid synthesis system in the chloroplast in *Viridiplantae* and *Rhodophyta* can be understood in terms of the efficiency of coupling photosynthesis and fatty acid synthesis (Fig. 4a). Here, the metabolic pathway is expressed using glyceraldehyde 3-phosphate (GAP) as the unit. In this representation, three carbon atoms are fixed by the photosynthetic carbon fixation cycle to produce a GAP molecule, which is subsequently decarboxylated to give an acetyl-CoA molecule through several steps, the building block of a fatty acyl chain ($-C_2H_4-$). During this process, 12 electrons reduce two CO_2 molecules to $-C_2H_4-$. The CO_2 released by pyruvate reductase is readily refixed in the chloroplast. The ATP and 2 NAD(P)H produced during the conversion of GAP to acetyl-CoA are used for the reduction steps during the fatty acid synthesis cycle. This scheme does not use 1 ATP, but it may be used during the conversion of starch to GAP. This integrated pathway of photosynthesis and fatty acid synthesis suggests that fatty acids are also products of photosynthesis. The activation of acetyl-CoA carboxylase through light-induced reduction of thioredoxin supports the idea of the coupling between photosynthesis and fatty acid synthesis (Sasaki et al. 1997).

In heterotrophs such as yeasts and mammals (Fig. 4c), glucose is used for the synthesis of fatty acids. This process does not involve net oxidation or reduction of carbons, because one carbon is oxidized to CO_2 and two others are reduced to $-C_2H_4-$. Heterotrophic organisms can therefore synthesize fatty acids by releasing CO_2 as much as 50% of the fatty acid products. In this case, no net production or consumption of ATP and NAD(P)H occurs.

Glaucophytes are intermediate between the two (Fig. 4b). Fatty acid synthesis and starch synthesis are localized in the cytosol. Three carbons are fixed in the chloroplast to produce GAP, which is subsequently decarboxylated in the cytosol to give acetyl-CoA. In this case, the released CO₂ could be transported to the chloroplasts and efficiently re-fixed. If starch is used to synthesize fatty acids, the situation is similar to that in heterotrophs. Although the coupling of photosynthesis and fatty acid synthesis, as in *Viridiplantae* and *Rhodophyta*, is functionally meaningful, the spatial separation of the two processes could also be functional if heterotrophic metabolism involving starch is considered. In this sense, the chloroplast is specialized for photosynthesis in *Glaucophyta*.

The origin of fatty acid synthesis in photosynthetic organisms

In current views of *Archaeplastida* evolution, *Rhodophyta* diverged first, followed by *Viridiplantae* and *Glaucophyta* (Adl 2024; Bowles et al. 2023; Strassert et al. 2021). In the conventional theory of endosymbiotic origin of chloroplasts (Adl et al. 2019, see also Sato 2020b, 2021), the endosymbiotic cyanobacterium introduced both photosynthesis and fatty acid synthesis, as well as many other metabolic pathways. However, the lack of fatty acid synthesis in the glaucophyte chloroplasts is hard to explain. If the initial chloroplast gained FAS II from the endosymbiotic cyanobacterium, many components of FAS II must be lost when the *Glaucophyta* diverged. At the same time, *Glaucophyta* must acquire FAS I. FAS I could have been acquired at the root of *Archaeplastida*, because *Rhodospirillum rubrum*, a non-photosynthetic sister of *Rhodophyta* (see explanation in Adl 2024), also has a FAS I (Fig. 3). Still, in this scenario, FAS I must be lost in *Rhodophyta* and *Viridiplantae* independently. The fact that the glaucophyte FabF is not monophyletic with FabF (KAS I/II) of *Viridiplantae* and *Rhodophyta* is not consistent with the endosymbiotic acquisition hypothesis.

Currently, we can imagine no simple, plausible hypothesis for the absence of fatty acid synthesis in chloroplasts in *Glaucophyta*. The phylogenetic complexity is also documented for the peptidoglycan synthesis enzymes, which are found in *Viridiplantae* and *Glaucophyta* but not in *Rhodophyta* (Sato and Takano 2026). Only four of the enzymes originated from cyanobacteria, while the origin of many other enzymes differs between *Viridiplantae* and *Glaucophyta*. The origin of *Glaucophyta* might not be as simple as it was considered in the original *Archaeplastida* hypothesis (Adl et al. 2005).

Declarations

Competing interests

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Figures

Pathway	Enzyme	ID	APclust2024 Cluster #	Viridiplantae	Glaucophyta			
				Arabidopsis thaliana	Cyanophora paradoxa	Cyanoptycye gloeocystis	Glaucocystis cf nostochinearum	Gloeochaete wirockiana
Acetyl-CoA carboxylase	Prokaryotic acetyl-CoA carboxylase	AccA	2564	AT2G38040=CAC3	none	none	none	none
		AccB	1112	AT5G16390=BCCP1 (CAC1)	none	none	none	none
		AccC	126	AT5G35360=CAC2	none	none	none	none
		AccD	8563	ATHc_accD	none	none	none	none
	Eukaryotic multifunctional acetyl-CoA carboxylase	ACC	126 11113 34654 229743 279745 280465	AT1G36160=ACC1	00350_g24319.t1 +00350_g24318.t1 00385_g24767.t1	CyGL_2005686 + CyGL_2008724 + CyGL_2008727	GINO_2058125 + GINO_2058049	GIWI_0184350418 GIWI_0184346346 + GIWI_0184370582 + GIWI_0184345692
Fatty acid synthesis	Acyl carrier protein	ACP (Cp)	286	AT3G05020=ACP1 (+4 ACP)	CypaCp009=acpP	none	GINO_2009443	GIWI_0184333576
		ACP (Mt)		AT2G44620=mtACP1 (+ 2 mtACP)	01107_g7100.t1	CyGL_2008671 CyGL_2008673	(none?)	GIWI_0184334654 GIWI_0184332184 GIWI_0184349794
	Condensing enzyme	FabBF (CpKAS)	197	AT5G46290	00342_g24242.t1	CyGL_2031013 (short)	none	GIWI_0184373254
		MtKAS	9454	AT2G04540	00053_g23493.t1	CyGL_2027372	GINO_2011364	GIWI_0184362418
	Malonyl-ACP synthesis enzyme	FabD (Cp)	1550	AT2G30200	none	none	none	none
		FabD (Mt)	4833 20495		21127_g18828.t1	CyGL_2120112 (short)	GINO_2011442	GIWI_0184340708
	FAS subunits (reductase/dehydra tase/reductase)	FabG	7931 77469	AT1G24360	00944_g5923.t1	none	GINO_2055076	GIWI_0184333078
		FabI	1954	AT2G05990 AT3G45770	20572_g11549.t1 (short)	none	none	none
		FabZ	1584	AT2G22230 AT5G10160	00403_g302.t1	none	none	none
	Condensing enzyme	FabH (KAS III)	3951 7231 13717 15517	AT1G62640	none	none	none	none
	Multifunctional FAS	FAS I	Large proteins	none	01335_g8213.t1 00912_g5450.t1 00025_g7952.t1	(none?)	[EC122136 ?]	GIWI_0184342518

Localization

Chloroplast

Mitochondrion

Cytosol or ER

Figure 1

Summary of glaucophyte enzymes for fatty acid synthesis.

The components of fatty acid synthesis were searched in the Gclust database using the APclust2024 dataset. The Arabidopsis enzymes are shown as a *Viridiplantae* reference. The localization of each enzyme was estimated using the DeepLoc-2.1 software, and the results are shown as background color: green, chloroplast; orange, mitochondrion; white, cytosol or endoplasmic reticulum (ER). The gray background indicates the absence of the enzyme. The dual colors within a cell show the dually targeted enzymes of *Arabidopsis*. For *Cyanophora* sequences, "CPA_" is omitted to shorten the name.

a. Cross homology of Eukaryotic and chloroplast ACCs (BLAST E-values)

Query	Subject											
	ATH_ACC1	CPA_00385	Mv06825	GIWI_0184350418	ATH_CAC3	CME_AccA	ATH_BCCP2	CME_AccB	ATH_CAC2	CME_AccC	ATH_AccD	CME_AccD
ATH_ACC1	0.0E+00	0.0E+00	0.0E+00	0.0E+00			8.8E-01		6.0E-62	3.0E-74	8.0E-03	4.3E-02
CPA_00385	0.0E+00	0.0E+00	0.0E+00	0.0E+00				0.0E+00	3.0E-67	1.0E-76		
Mv06825	0.0E+00	0.0E+00	0.0E+00	0.0E+00			1.1E-01		6.0E-61	9.0E-68		6.2E-01
GIWI_0184350418	0.0E+00	0.0E+00	0.0E+00	0.0E+00			7.0E-01		2.0E-64	8.0E-70		
ATH_CAC3	3.8E-01		8.6E-01		0.0E+00	1.0E-96						
CME_AccA					6.0E-97	0.0E+00						
ATH_BCCP2	1.0E-01	2.3E-01	1.1E-02	7.6E-02			0.0E+00	6.0E-21				
CME_AccB			8.9E-02	2.9E-01	2.7E-01		4.0E-21	2.0E-97				
ATH_CAC2	2.0E-62	8.0E-68	1.0E-61	4.0E-65					0.0E+00	1.0E-175		
CME_AccC	1.0E-67	2.0E-76	2.0E-68	2.0E-70	5.0E-01				1.0E-175	0.0E+00		
ATH_AccD	2.0E-03	2.3E-01	8.8E-01								0.0E+00	1.0E-81
CME_AccD	5.0E-03	6.3E-02	1.9E-01								6.0E-62	0.0E+00

b. ML tree of Eukaryotic ACCs (ML/BI)

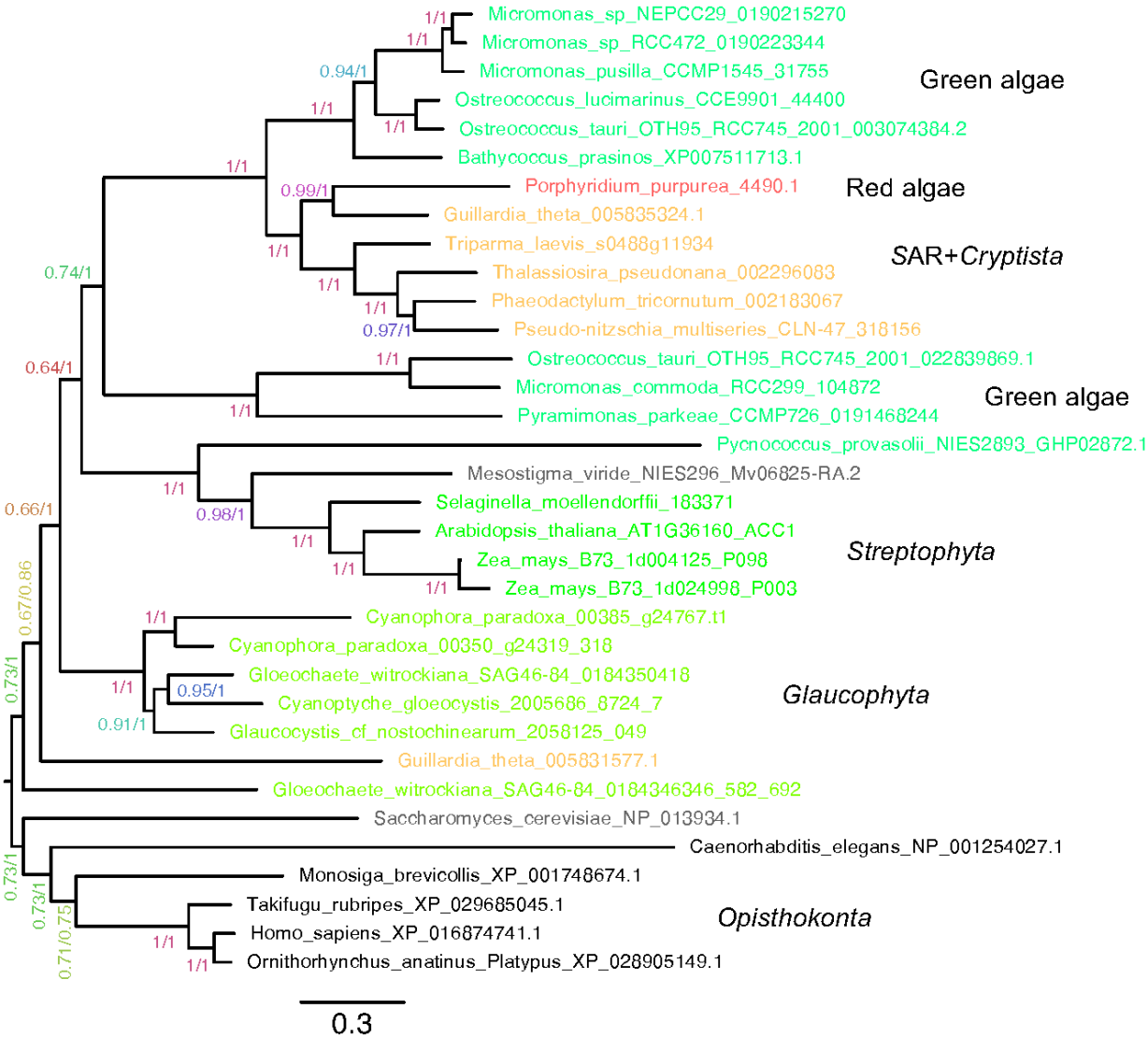


Figure 2

Bioinformatic analysis of acetyl-CoA carboxylases.

a. Limited similarity of prokaryotic and eukaryotic acetyl-CoA carboxylases. BLAST E-values lower than 10 are shown as a measure of similarity. The similarity of orthologs are colored with pink, whereas the cross similarity ($E < 1.0E-60$) of eukaryotic ACC I and prokaryotic Acc subunits are colored with light

blue. ATH, *Arabidopsis thaliana* (land plant, *Viridiplantae*); CME, *Cyanidioschyzon merolae* (*Rhodophyta*); CPA, *Cyanophora paradoxa* (*Glaucophyta*); Mv, *Mesostigma viride* (streptophyte alga, *Viridiplantae*); GIWI, *Gloeochaete witrockiana* (*Glaucophyta*). The upper four enzymes are eukaryotic acetyl-CoA carboxylases. Below them are shown two entries each of prokaryotic acetyl-CoA carboxylase subunits, AccA, AccB, AccC, and AccD, some of which are encoded by the chloroplast genome (see text).

b. Phylogenetic tree of eukaryotic acetyl-CoA carboxylases. The ML tree is presented. The values on each branch indicate confidence levels for the ML and BI trees. For simplicity, 1.00 is shown as "1". Confidence values are shown only on large branches. The *Opisthokonta*(animals) clade is taken as the outgroup.

Phyla are color-coded (according to the color scheme in the FigTree software. RGB intensities are shown in brackets. All color codes used in the present study are listed here: Green (or Spring) [0,255,0], *Streptophyta*; Sea Foam [0,255,128], Green algae; Lime [0,128,255], *Glaucophyta*; Cyan (or Turquoise) [0,255,255], cyanobacteria; Red (or Maraschino) [255,0,0], α -proteobacteria; Tangerine [255,128,0], β -proteobacteria; Grape [128,0,255], γ -proteobacteria; Clover [0,128,0], *Chlorobi* and *Chloroflexi*; Midnight [0,0,128], Firmicutes; Mocha [128,64,0], actinobacteria; Salmon [255,128,0], *Rhodophyta*; Cantaloupe [255, 204, 102], *Stramenopiles* and *Cryptista*; Black [0,0,0], other bacteria.

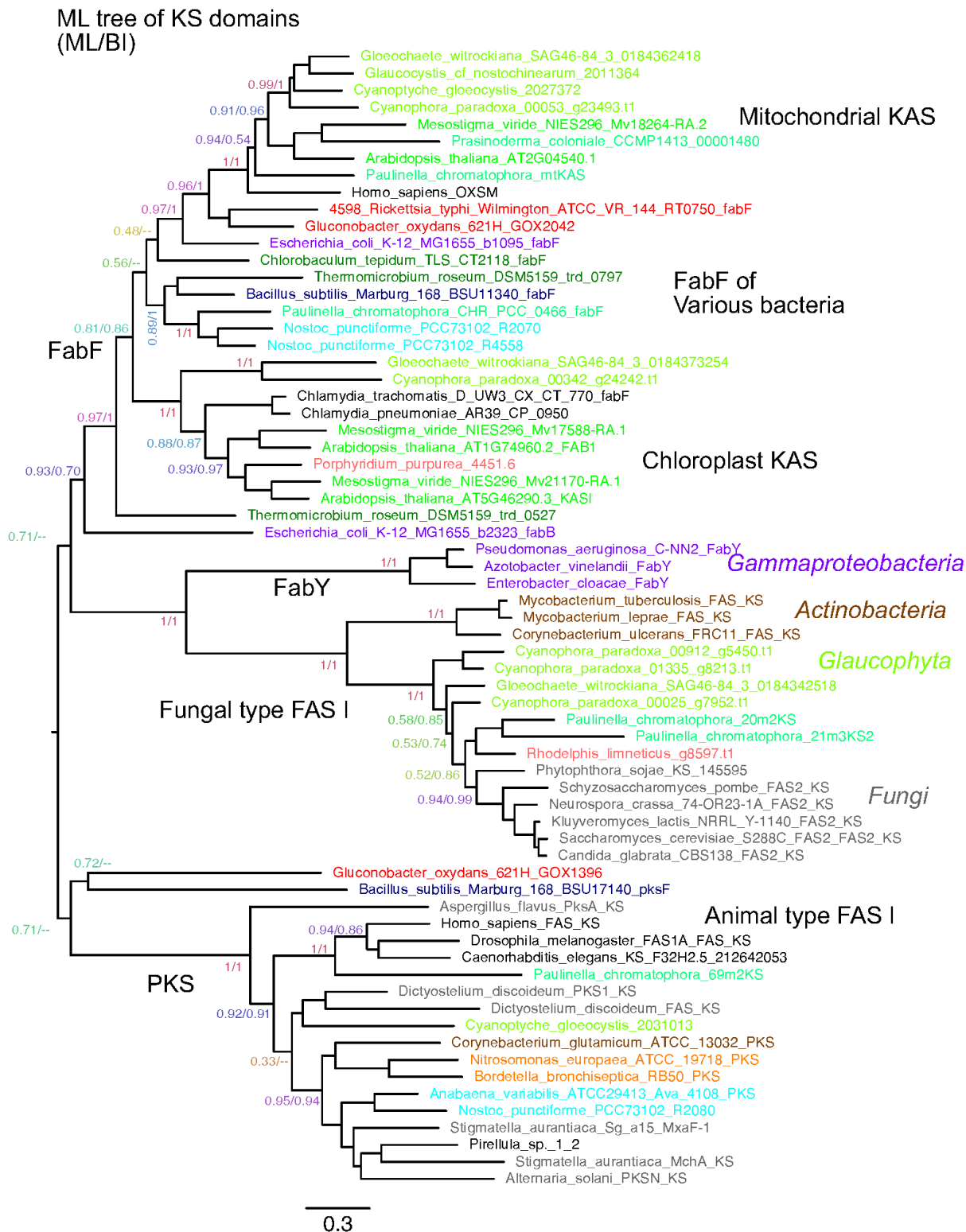


Figure 3

Phylogenetic tree of prokaryotic KAS/PKS and KS domains of eukaryotic FAS I.

The sequence data were mainly obtained from the previous tree data (Sato et al. 2025). Glaucophyte data and some bacterial data were added from the Gclust database (dataset APclust2024 and Gclust2024). The ML tree is presented. Branch values indicate branching confidence levels for the ML

and BI trees. For simplicity, 1.00 is shown as "1". Confidence values are shown only on large branches. The root obtained by midpoint rooting coincided with a split between the broad PKS clade (PKS + animal FAS) and the FAS clade. For color codes, see the legend for Fig. 2.

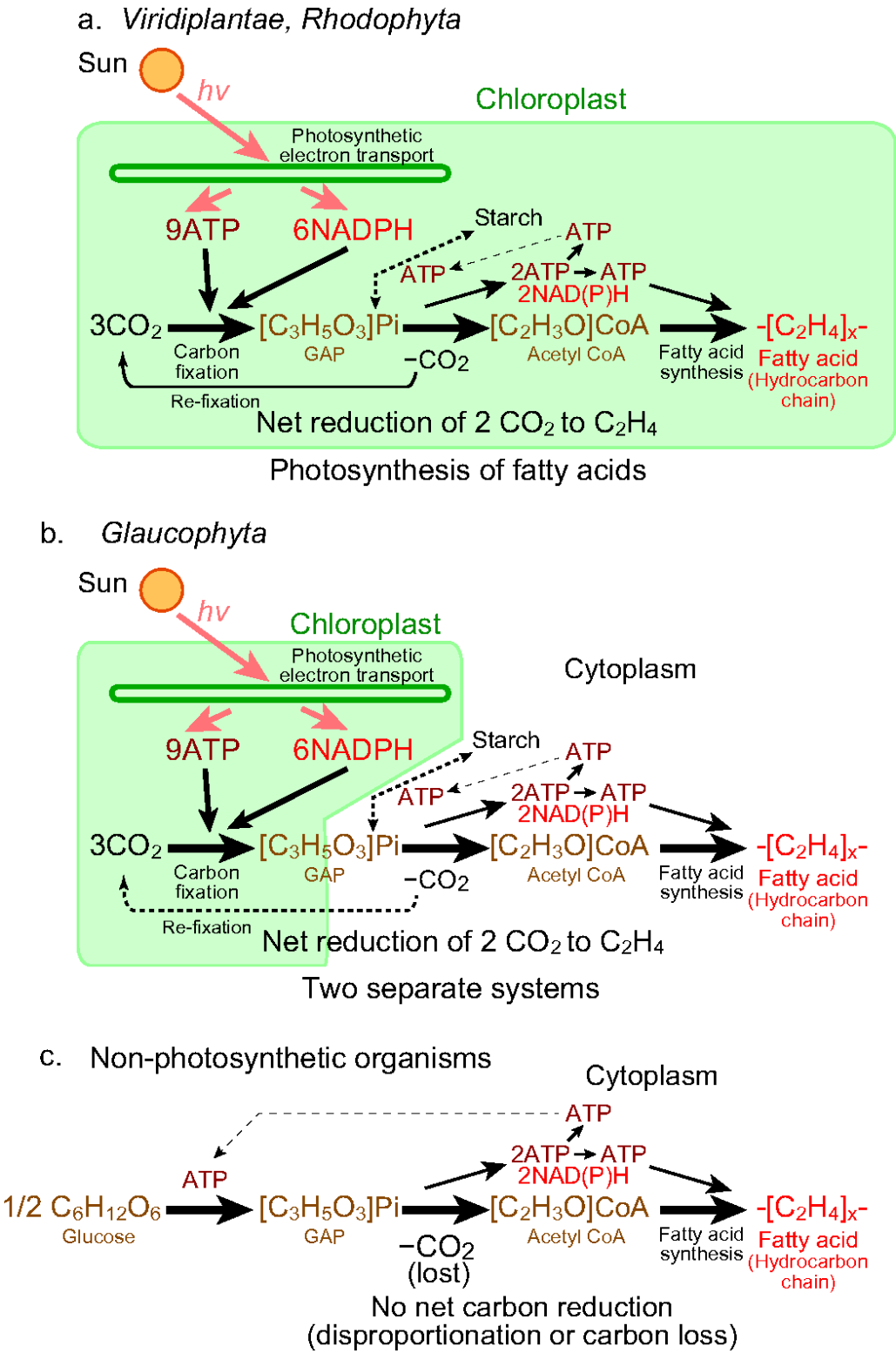


Figure 4

Metabolic coupling of photosynthesis and fatty acid synthesis.

The flow of photosynthetically fixed carbon to fatty acid synthesis is shown in a simplified form for *Viridiplantae* and *Rhodophyta*(a) and *Glaucophyta* (b). Panel (c) is the carbon flow from glucose to fatty acid in heterotrophs. All numbers of molecules are based on a glyceraldehyde 3-phosphate (GAP) molecule.

Supplementary Files

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