The Mitochondrion of *Euglena gracilis*

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Abstract

In the presence of oxygen, *Euglena gracilis* mitochondria function much like mammalian mitochondria. Under anaerobiosis, *E. gracilis* mitochondria perform a malonyl-CoA independent synthesis of fatty acids leading to accumulation of wax esters, which serve as the sink for electrons stemming from glycolytic ATP synthesis and pyruvate oxidation. Some components (enzymes and cofactors) of *Euglena's* anaerobic energy metabolism are found among the anaerobic mitochondria of invertebrates, others are found among hydrogenosomes, the H_2-producing anaerobic mitochondria of protists.

Keywords

Euglena gracilis • Mitochondrion • Anaerobiosis • Wax ester fermentation • Mitochondrial genome

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Abbreviations

ADH	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
AOX	Alternative oxidase
APX	Ascorbate peroxidase
ATP	Adenosine triphosphate
CCR	Crotonyl-CoA reductase
CoA	Coenzyme A
FAD	Flavin adenine dinucleotide
FAS	Fatty acid synthase
FMN	Flavin mononucleotide
FRD	Fumarate reductase

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ICL	Isocitrate lyase
MS	Malate synthase
NAD^+	Nicotinamide adenine dinucleotide (oxidized form)
NADH	Nicotinamide adenine dinucleotide (reduced form)
$NADP^+$	Nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
PDH	Pyruvate dehydrogenase
PFO	Pyruvate:ferredoxin oxidoreductase
PKS	Polyketide synthase
PNO	Pyruvate:$NADP^+$ oxidoreductase
Prx	Peroxiredoxin
ROS	Reactive oxygen species
RQ	Rhodoquinone
TER	*trans*-2-enoyl-CoA reductase

2.1 Introduction

Mitochondria are essential components of eukaryotic cells. In eukaryotes that spend all of their life cycle in oxic environments, the inner mitochondrial membrane is the site of chemiosmotic ATP synthesis. Eukaryotes that spend all or part of their life cycle in anoxic environments harbor anaerobic forms of mitochondria. Some forms of anaerobically functioning mitochondria possess cytochromes and quinones, generating proton gradients through an electron transport chain using terminal acceptors other than oxygen, while others generate ATP through fermentative substrate level phosphorylation, and still others have no role in ATP synthesis at all (Müller et al. 2012). The mitochondrion of *Euglena gracilis* generates ATP with and without the help of oxygen as a terminal acceptor. The organelle was last reviewed in the classical chapter by Buetow (1989). The purpose of the current chapter is to cover more recent progress in understanding the *Euglena* mitochondrion.

Euglenids are a broad and diverse group of unicellular eukaryotes that typically inhabit shallow aquatic environments. In the region around the northern Rhine river, springtime rains can lead to small puddles alongside bicycle paths and forest trails. If the puddles remain for a couple of weeks or so, they tend to turn green, as a rule due to growth of euglenids. Though typically encountered in oxic freshwater systems, euglenids also occur in anoxic freshwater ecosystems (Simpson et al. 1997; Klaveness and Løvhøiden 2007) and in anoxic marine sediments (Bernhard et al. 2000; Edgcomb et al. 2011). Some euglenids possess organelles ultrastructurally similar to hydrogenosomes (Simpson et al. 1997; Edgcomb et al. 2011), the hydrogen-producing forms of mitochondria found in diverse anaerobic protists (Müller et al. 2012). From the standpoint of core carbon and energy metabolism, only one member of the euglenids has been extensively studied: the photosynthetic flagellate *Euglena gracilis*.

2.2 The Morphology of Mitochondria

The mitochondrion of *Euglena* is a single, large and reticulated organelle, a trait that is by no means unique among algae (Leedale et al. 1965; Wolken 1967; Buetow 1989; Hayashi and Ueda 1989). As with many other protists (Bereiter-Hahn and Vöth 2005), the mitochondria of *Euglena* have been reported to change shape in response to different conditions of growth, in response to different modes of nutrition (Brönstrup and Hachtel 1986) or when exposed to a variety of inhibitors (Calvayrac and Butow 1971; Calvayrac et al. 1971; Nass and Ben-Shaul 1972; Michaels and Gibor 1973; Neumann and Parthier 1973). In dark-adapted *Euglena*, the network of mitochondria is located close to the cell surface and is adjacent to other organelles (Pellegrini 1980; Hayashi and Ueda 1989). In light-adapted cells, the mitochondrial reticulum is more randomly distributed throughout the cytoplasm, but still tends to localize adjacent to the plastids, which might relate to local oxygen production of the latter (Fig. 2.1) (Wolken 1967). Like mitochondria of higher animals and plants, *Euglena* mitochondria have flat cristae (Stewart and Mattox 1980). The main contents of *Euglena* mitochondria (apart from water and protein) are lipids, amino acids (with a

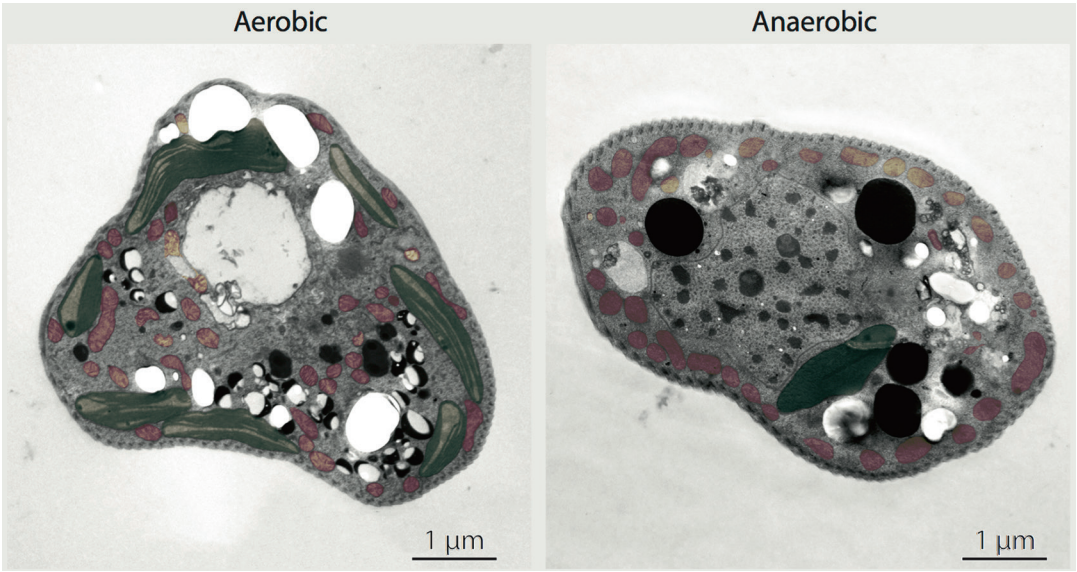


Fig. 2.1 Transmission electron microscopy (TEM) images taken from aerobic and anaerobic grown *E. gracilis* cultures. Mitochondria are highlighted by false colouring in *red*, plastids are shaded in *green*. In anaerobic cultures of *E. gracilis*, the network of mitochondria is located closer to the cell surface and adjacent to other organelles. In comparison to the aerobic cultures the mitochondria are more randomly distributed throughout the cell, but still tend to localize adjacent to the plastids. *White* parts (*holes*) are preparation artefacts

large amount of proline), thiamine, cobalamins, quinones, tocopherols (vitamin E) and cytochromes, which *Euglena* loses during prolonged anoxia (Buetow 1989).

2.3 Aerobic and Anaerobic Metabolic Pathways

Organisms that can grow either with or without oxygen are called facultative anaerobes. Many eukaryotes can use their mitochondria for ATP synthesis and growth under aerobic or anaerobic conditions (Tielens et al. 2002; Tielens and van Hellemond 2007; Müller et al. 2012). Such organisms can be said to possess facultatively anaerobic mitochondria, which is the case for *E. gracilis*. It can grow autotrophically in the light, producing oxygen through photosynthesis and consuming oxygen via mitochondrial respiration, but many strains can also grow as anaerobic heterotrophs in the dark. Importantly, there are distinct differences among *E. gracilis* strains when it comes to the issue of being able to simply survive anoxic conditions versus being able to actively grow under anoxic conditions. Most *E. gracilis* strains have no problem surviving anoxia, but only some strains have the ability to actively grow heterotrophically under anoxia in the dark (Tucci et al. 2010). In our experience each new strain has to be tested, and recent work shows that anoxic cultivation on plates tends to deliver faster growth rates than anoxic liquid culture (C. Rauch, unpublished). Because *Euglena*'s anaerobic energy metabolism entails many components, as we will elaborate in subsequent sections, it is safe to assume that those strains that do not grow anaerobically have lost the ability to do so during the prolonged laboratory culture.

Some eukaryotes with oxygen respiring mitochondria can survive anoxia for prolonged periods using cytosolic fermentations. Goldfish (*Carassius auratus*) and crucian carp (*Carassius carassius*) provide such an example, being able to survive complete anoxia for several weeks via cytosolic ethanol fermentation (van Waarde et al. 1993). *Euglena* belongs to the eukaryotes with facultatively anaerobic mitochondria, that is, it

synthesizes ATP anaerobically in mitochondria. The biochemistry and physiology of anaerobic mitochondria and facultatively anaerobic mitochondria has been studied in only a few model systems (Tielens et al. 2002; Mentel and Martin 2008; Müller et al. 2012).

2.3.1 Aerobic Metabolic Pathway

In *Euglena*, pyruvate from the glycolysis pathway enters the mitochondrion and in the presence of oxygen undergoes oxidative decarboxylation via pyruvate dehydrogenase (PDH) (Tielens et al. 2002; Hoffmeister et al. 2004; Müller et al. 2012). *Euglena* PDH was shown to be active under aerobic conditions (Krnáčová et al. 2015), but the question remains open whether the *Euglena* mitochondrion imports pyruvate or malate, which in many eukaryotes with anaerobic mitochondria is converted into pyruvate in the organelle by malic enzyme (Tielens et al. 2002; Müller et al. 2012). Oxidative decarboxylation yields acetyl-CoA that enters a modified Krebs cycle in which alpha-ketoglutarate decarboxylase and succinate semialdehyde dehydrogenase (Buetow 1989) replace the alpha-ketoglutarate dehydrogenase of the classical Krebs cycle. This modification of the Krebs cycle is also found among some alphaproteobacteria, like *Bradyrhizobium* (Green et al. 2000), and among cyanobacteria (Zhang and Bryant 2011).

During aerobic ATP synthesis, electrons from glucose breakdown are transferred via a branched respiratory chain to oxygen as the terminal electron acceptor (Fig. 2.2) (Buetow 1989; Kitaoka et al. 1989). The *E. gracilis* respiratory chain consists of the multi-subunit complexes I, II, III and IV of canonical mitochondrial electron-transport chains, plus a plant-like alternative oxidase, as explained later in this section. The respiratory chain complexes of *E. gracilis* harbor in total at least 48 subunits that *Euglena* shares with various eukaryotes and 41 subunits that they share exclusively with trypanosomatids (Duarte and Tomas 2014; Perez et al. 2014). Characterization of enzymes involved in *E. gracilis* oxidative phosphorylation revealed the presence of two catalytically active forms of complex I, but only a single active form of complex II (Krnáčová et al. 2015). Isolated complex III (cytochrome c reductase) from *E. gracilis* contained cytochrome c_1 and b. It contained 10 subunits, showed only a low activity, was not sensitive to antimycin A and the cytochrome c_1 had an atypical heme-binding structure (Mukai et al. 1989; Braun and Schmitz 1995). The presence of two complexes III in *E. gracilis* was suggested by Moreno-Sánchez et al. (2000); one sensitive to antimycin A and resistant to myxothiazol and *vice versa* for the other one, while complexes III from other organisms are sensitive to both inhibitors. Complex IV (cytochrome *c* oxidase) was also purified from *E. gracilis* mitochondria. It contains 15 subunits and showed a high activity in the presence of '*Euglena* cytochrome c', however it had a very low activity in the presence of horse cytochrome c (Brönstrup and Hachtel 1989; Mukai et al. 1989). For the ATP synthase, two main active forms were detected in *Euglena* (Krnáčová et al. 2015).

The activity of enzymes involved in *Euglena* oxidative phosphorylation did not show any effects in comparisons of light vs. dark or functionality vs. nonfunctionality of plastids (Krnáčová et al. 2015). Isolated mitochondria of *E. gracilis* used NADH as an external substrate for oxygen consumption (Uribe et al. 1994; Jasso-Chávez and Moreno-Sánchez 2003; Krnáčová et al. 2015). This seems surprising at first sight, because NADH is generally thought not to be transported across the mitochondrial inner membrane, and the enzyme or enzymes responsible for the use of NADH as an exogenous substrate during oxygen consumption in *E. gracilis* mitochondria have not been identified (Krnáčová et al. 2015).

There is evidence for alternative respiratory pathways in *E. gracilis*: an alternative oxidase (AOX) transfers electrons directly from ubiquinol to oxygen (Devars et al. 1992). The presence of AOX was confirmed *in silico* (Perez et al. 2014) and *in vivo* (Krnáčová et al. 2015). Another pathway includes lactate dehydrogenase, which transfers electrons from lactate directly to ubiquinone (Moreno-Sánchez et al. 2000) and is part of the lactate/pyruvate shuttle (Jasso-Chávez et al.

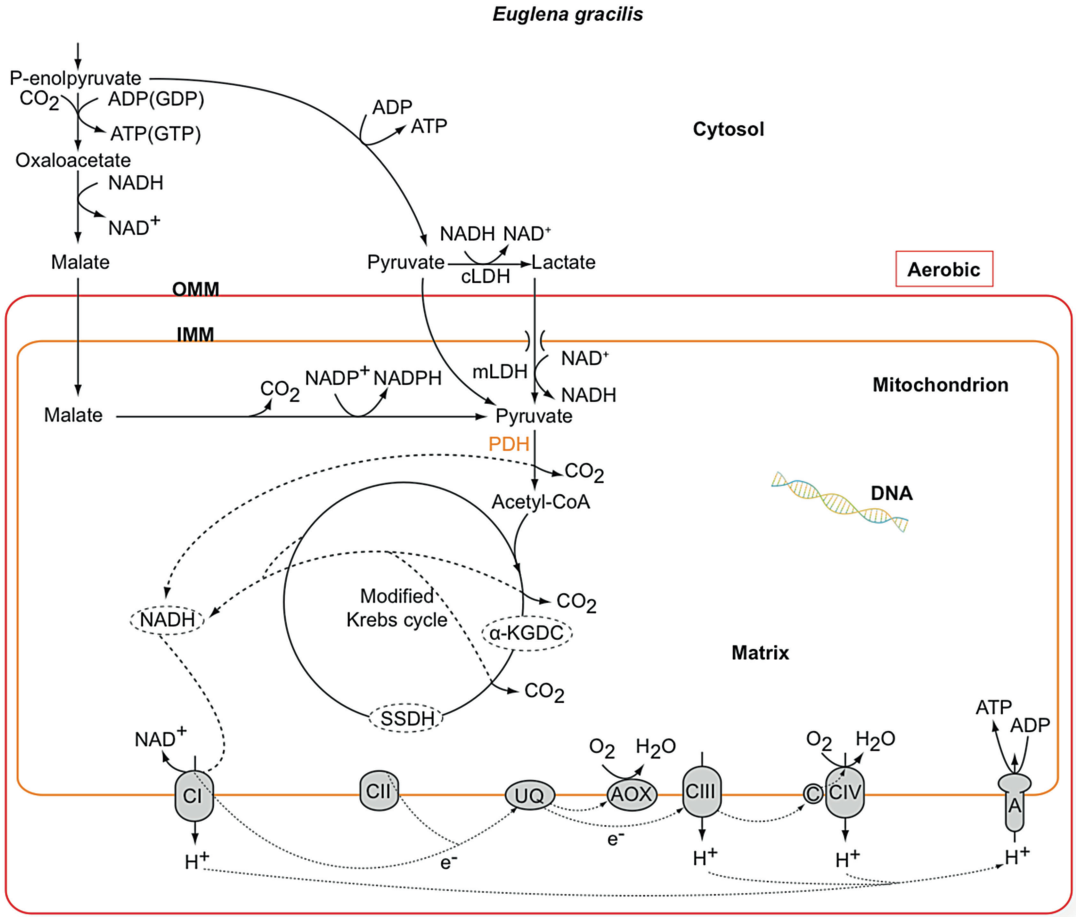


Fig. 2.2 Metabolic pathways in mitochondria of the facultatively anaerobic photosynthetic flagellate *Euglena gracilis*. The map is redrawn based on Müller et al. (2012). Under aerobic conditions (shown in the upper part in *red*) either pyruvate from the glycolytic pathway enters the mitochondrion or malate or lactate via the lactate shuttle enter the mitochondrion, where they are converted to pyruvate. Pyruvate undergoes oxidative decarboxylation via pyruvate dehydrogenase (PDH) yielding acetyl-CoA that enters a modified Krebs cycle in which alpha-ketoglutarate decarboxylase and succinate semialdehyde dehydrogenase replace the alpha-ketoglutarate dehydrogenase and succinate thiokinase (STK) of the classical Krebs cycle. Electrons are transferred via a branched respiratory chain to oxygen as the terminal electron acceptor generating the proton gradient for ATP synthesis. *A* ATPase, *AOX* alternative oxidase, *C* cytochrome c, *CI to CIV* respiratory complexes I to IV, *FRD* fumarate reductase, *IMM* inner mitochondrial membrane, *LDH* lactate dehydrogenase, *OMM* outer mitochondrial membrane, *RQ* rhodoquinone

2001, 2005; Jasso-Chávez and Moreno-Sánchez 2003). Alternative respiratory pathways are likely involved in cellular responses to oxidative stress (Castro-Guerrero et al. 2004, 2008), since *E. gracilis* lacks catalase, but contains ascorbate peroxidase (APX), which localizes exclusively to the cytosol (Shigeoka et al. 2002). Other enzymes involved in detoxification of reactive oxygen species (ROS) have not been found yet, thus little is known about ROS metabolism in *Euglena* (Tamaki et al. 2014). *Euglena* was shown to contain 2-CysPrxs in the mitochondria (Castro and Tomás 2008). Tamaki et al. (2014) examined four peroxiredoxins (Prx), two localized in the cytosol and the others in the plastids and the mitochondria, respectively. All could reduce both H_2O_2 and alkyl hydroperoxide and might contribute to ROS metabolism – also in mitochondria

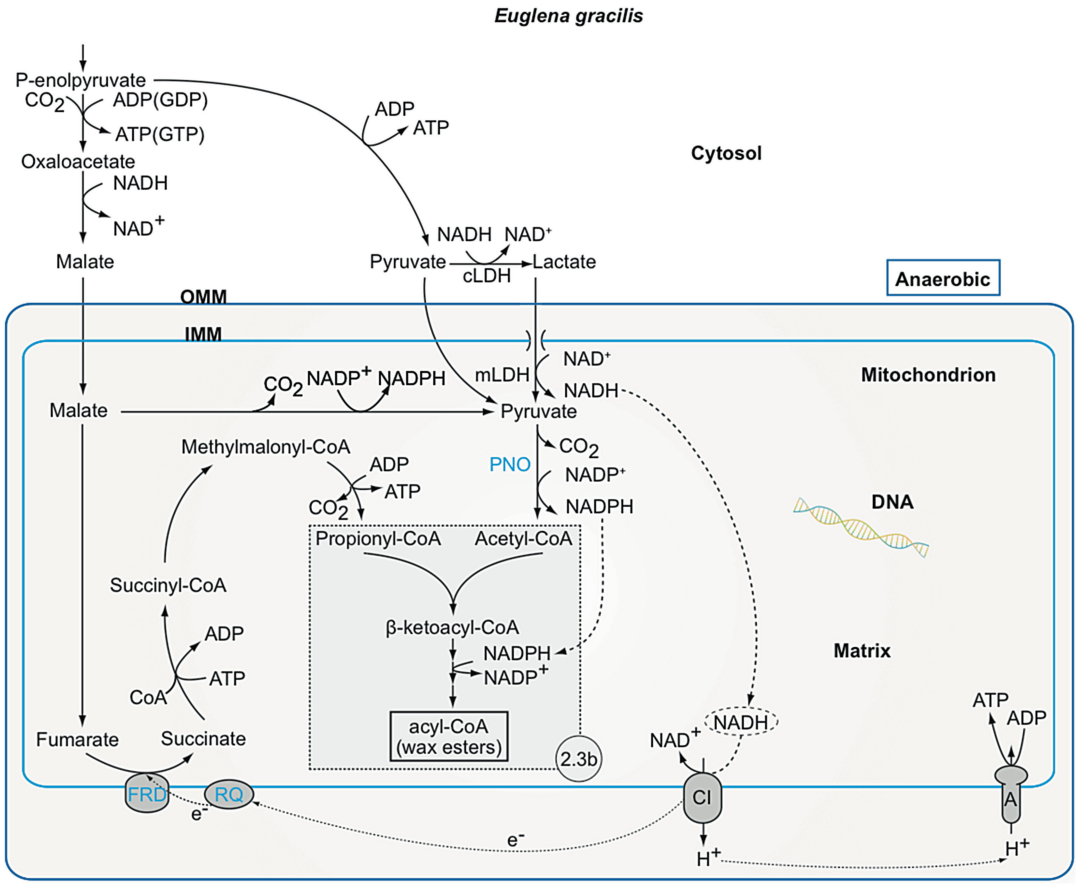


Fig. 2.3 Metabolic pathways in mitochondria of the facultatively anaerobic photosynthetic flagellate *Euglena gracilis*. The map is redrawn based on Müller et al. (2012). Under anaerobic conditions (shown at the bottom in blue) *E. gracilis* uses acetyl-CoA produced by pyruvate:NADP oxidoreductase as the terminal electron acceptor, leading to the formation of an unusual end product among eukaryotes: wax esters. Mitochondrial wax ester fermentation includes anaerobic fumarate respiration and the same propionyl-CoA formation pathway as the one found in mitochondria of facultative anaerobic animals excreting propionate. Note however that succinate thiokinase participates in the propionyl-CoA generating pathway under anaerobic conditions and that STK homologues are present in *Euglena* EST libraries (Müller et al. 2012). *A* ATPase, *AOX* alternative oxidase, *C* cytochrome c, *CI to CIV* respiratory complexes I to IV, *FRD* fumarate reductase, *IMM* inner mitochondrial membrane, *LDH* lactate dehydrogenase, *OMM* outer mitochondrial membrane, *RQ* rhodoquinone

(Tamaki et al. 2014). But H_2O_2 can also diffuse from the mitochondria into the cytosol, where it is then detoxified by APX (Ishikawa et al. 1993).

2.3.2 Anaerobic Metabolic Pathway

When oxygen is absent, *E. gracilis* uses acetyl-CoA as the terminal electron acceptor of glucose oxidation and produces wax esters as end products of its metabolism (Figs. 2.3 and 2.4) (Buetow 1989; Inui et al. 1982, 1983, 1984; Tucci et al. 2010; Ogawa et al. 2015). When shifted from aerobic to anaerobic conditions, PDH protein levels decrease in *Euglena* (Hoffmeister et al. 2004). The shift to anaerobic conditions leads to the malonyl-CoA independent synthesis of wax esters, levels of which can reach up to 40 μg per 10^6 *Euglena* cells (Inui et al. 1982). The wax esters are not excreted but accumulate in the cytosol instead (Tucci et al. 2010; Ogawa et al. 2015). Some *E. gracilis* strains accumulate wax

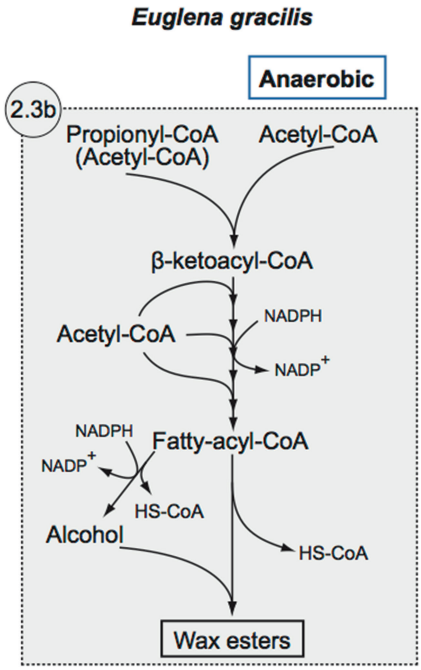


Fig. 2.4 Expanded view of the pathway leading to formation of wax-esters shown in Fig. 2.3a. The fatty acid CoA-ester is elongated by starting again at ß-ketoacyl-CoA. A part of the final fatty acid product is reduced to the alcohol which is then esterified with other fatty acids and deposited in the cytosol as wax-esters. Fatty acid synthesis is mitochondrial, wax ester synthesis is cytosolic. Odd chain fatty acids (roughly 40% of the total, see text) start from propionyl-CoA

esters at levels of up to 65% total dry weight (Tucci et al. 2010) and acid mine drainage biofilms mainly constituted by *Euglena mutabilis* contain large amounts of wax esters (Dasgupta et al. 2012). Because of this high wax ester production there is a general interest in selective breeding of *E. gracilis* for biofuel, nutritional foods and cosmetic purposes (Yamada et al. 2016) (see Chaps. 13 and 14 of this volume). Under aerobic conditions, the wax esters can be converted back to acetyl-CoA, which can be oxidized to CO_2 in the mitochondria or used to form paramylon (β-1,3-glucan) reserves (Inui et al. 1992; Ono et al. 1995b; Atteia et al. 2013). This metabolism probably involves β-oxidation, the glyoxylate cycle and conversion of acetyl-CoA, which either enters the glyoxylate cycle to be converted to succinate which can be used for gluconeogenesis, or the acetyl-CoA enters the modified Krebs cycle to be oxidized to CO_2 (Ono et al. 2003; Hoffmeister et al. 2005).

2.3.3 Five Different Systems for Fatty Acid Synthesis

Five different systems of fatty acid synthesis have been reported for *E. gracilis* (Kitaoka et al. 1989). The first two systems, localized in the chloroplasts, are acyl-carrier protein dependent systems (FAS II), which synthesize products with chain lengths of C16 and C18 that can be further elongated to C20–C24 (Ernst-Fonberg and Bloch 1971; Hendren and Bloch 1980). The third, a multifunctional fatty acid synthase (FAS I) located in the cytosol of *Euglena*, has as main products C16 fatty acids with C14 and as minor products C18 chain lengths (Goldberg and Bloch 1972; Walker et al. 1981). The fourth is a microsomal fatty acid synthase activity (Khan and Kolattukudy 1973). The fifth is involved in anaerobic wax ester fermentation and part of the mitochondrial system. It uses acetyl-CoA as a primer and C2-donor, a portion of the fatty acids is reduced to alcohols, esterified with another fatty acid and deposited in the cytosol as waxes (Inui et al. 1982, 1984; Buetow 1989). The mitochondrial system synthesizes products of chain length C8–18 with a majority of C14 (mainly myristyl myristate) (Inui et al. 1983, 1984). Synthesis of odd numbered fatty acids starts from propionyl-CoA, which is synthesized via the methylmalonyl-CoA pathway (Nagai et al. 1971; Schneider and Betz 1985; Pönsgen-Schmidt et al. 1988) and requires the participation of rhodoquinones (Hoffmeister et al. 2004; Castro-Guerrero et al. 2005). Labeling experiments indicate the involvement of Krebs cycle intermediates leading to propionyl-CoA in the synthesis of odd numbered chains during anaerobic wax ester fermentation (Padermshoke et al. 2016).

The mitochondrial wax ester fermentation by *Euglena* is similar in general principle to the synthesis of branched short-chain fatty acids in *Ascaris* or butyrate in *Dasytricha*. The adult parasitic nematode *Ascaris suum* is known to be

adapted to anaerobic conditions in that they possess anaerobically functioning mitochondria (Kita et al. 2002). Early larval stages of *Ascaris* are aerobic and their mitochondria possess a functional Krebs cycle and cytochrome *c* oxidase (Komuniecki and Vanover 1987; Takamiya et al. 1993) whereas adults use organic acids instead of oxygen as terminal electron acceptors (Komuniecki et al. 1981; Mei et al. 1997). Main end products of the anaerobic energy metabolism of *Ascaris* mitochondria are the methyl-branched fatty acids 2-methylbutyrate and 2-methylvalerate (Komuniecki and Tielens 2003). These reactions of anaerobic mitochondrial metabolism resemble *Euglena* mitochondrial fatty acid synthesis in that acetyl-CoA and propionyl-CoA condensations are involved and the final reduction is catalyzed by a 2-methyl branched chain enoyl-CoA reductase (Komuniecki and Tielens 2003; Komuniecki et al. 1987). However, the *Ascaris* 2-methyl branched chain enoyl-CoA reductase does not accept NADH as an electron donor, in contrast to *trans*-2-enoyl-CoA reductase (TER) in *Euglena* (Hoffmeister et al. 2005), but accepts electrons from an electron transporting flavoprotein and shows sequence similarities to acyl-CoA dehydrogenases (Komuniecki et al. 1985; Duran et al. 1993, 1998). This indicates that *Euglena* TER is clearly distinct from the *Ascaris* enzyme.

In *Euglena*, fatty acids for wax esters are synthesized via condensation of acetyl-CoA with an acyl-CoA (starting with acetyl-CoA or propionyl-CoA), a reduction of the resulting 3-oxoacid to 3-hydroxy acid, dehydration thereof, and a reduction of the resulting trans-enoyl-CoA to the elongated acyl-CoA. The acetyl-CoA stems from pyruvate via the oxygen-sensitive enzyme; pyruvate:$NADP^+$ oxidoreductase (PNO) (Inui et al. 1987; Nakazawa et al. 2000), the core catalytic component of which is pyruvate:ferredoxin oxidoreductase, a typical enzyme of hydrogenosomes (Rotte et al. 2001). In contrast to malonyl-CoA-dependent fatty acid synthesis, the acetyl-CoA-dependent *Euglena* route allows net fermentative ATP synthesis from glucose, because acetyl-CoA is condensed without prior ATP-dependent carboxylation to malonyl-CoA (Inui et al. 1982, 1984; Schneider and Betz 1985).

The condensing enzyme 3-ketoacyl-CoA thiolase (EC 2.3.1.9 and 2.3.1.16) catalyzes the condensation of propionyl-CoA and acetyl-CoA in the mitochondrial fatty acid synthesis in *E. gracilis*. A recent study showed that gene silencing of this enzyme altered both the wax ester content and the wax ester composition in *E. gracilis*, inducing a significant compositional shift to shorter carbon chain lengths in wax esters (Nakazawa et al. 2015). Six putative 3-ketoacyl-CoA thiolase isoenzymes were found in an *E. gracilis* EST database (O'Brien et al. 2007), but only the gene silencing of three of them had an effect on wax esters. For two isoforms (3 and 1) the total wax ester production was lowered to approximately 25% and 85% of the control level, when cells were exposed to hypoxic conditions for 48 h. Gene silencing of isoforms 1 and 2 also induced different acyl chain length distributions of wax esters compared to those in control cells. C28 wax esters were dominant in the control cells and in thiolase-silenced cells C26 (isoform 1) and C27 (isoform 2). Gene silencing of these two isoforms significantly increased the amount and the ratio of wax esters with an acyl chain length shorter than C26 (Nakazawa et al. 2015). The study suggests different roles for each isoenzyme in hypoxic wax ester production (Nakazawa et al. 2015). Isoenzyme 3 might be a short- and medium-chain-specific enzyme important for the hypoxic synthesis of wax esters from paramylon, while isoforms 1 and 2 might be medium- and long-chain-specific in the acyl-CoA production in mitochondrial fatty acid synthesis (Nakazawa et al. 2015).

Euglena's mitochondrial fatty acid synthesis has been formally described as a reversal of β-oxidation (Inui et al. 1984; Buetow 1989) because it proceeds via CoA rather than acyl carrier protein (ACP) bound intermediates. However, the key difference relative to β-oxidation is that *trans*-2-enoyl-CoA reductase (TER; EC 1.3.1.44) reduces the double bond in enoyl-CoA to produce acyl-CoA (Inui et al. 1984). In β-oxidation this step is oxidative and irreversible under physiological conditions because acyl-CoA dehydrogenase (mitochondrial β-oxidation) and acyl-CoA oxidase (peroxisomal β-oxidation) are both linked to

O_2-reduction (Kunau et al. 1995; Graham and Eastmond 2002). A portion of the fatty acids is reduced to alcohols, esterified with another fatty acid, and deposited into the cytosol as wax (wax ester fermentation). The fatty acyl-CoA reductase and the wax synthase involved in the synthesis of the medium-chain wax aliphatic side chains of *Euglena* were recently characterized (Teerawanichpan and Qiu 2010); the gene is ubiquitous among eukaryotes. Upon a return to an oxic environment, the stored waxes are degraded via aerobic dissimilation in the mitochondrion (Inui et al. 1982).

An NADPH-specific crotonyl-CoA reductase (CCR) from *Streptomyces collinus* catalyzes the synthesis of butyryl-CoA from crotonyl-CoA (Wallace et al. 1995), a reaction very similar to that catalyzed by *Euglena* TER. However, no detectable sequence similarity exists between TER and CCR (Hoffmeister et al. 2005). Furthermore, *S. collinus* CCR shows no activity with NADH in contrast to *Euglena* TER (Wallace et al. 1995; Liu and Reynolds 1999). CRR provides butyryl-CoA units for synthesis of polyketides (Reynolds et al. 1992), but is again distinct from *Euglena* TER.

A variety of enoyl reductases (ER) and ER-modules are known among polyketide synthases (Metz et al. 2001; Wallis et al. 2002), but none characterized so far has sequence similarity with *Euglena* TER. TER is expressed both under aerobic and anaerobic conditions, in line with findings from other anaerobic mitochondria, which seem to have adopted a strategy of being prepared for anaerobiosis without the need for specific enzyme induction (Hoffmeister et al. 2004; Tielens 1994). *Euglena* TER has strong sequence similarity to proteins annotated as hypothetical reading frames in several dozen prokaryotes, most conspicuously among gamma-proteobacteria. The operon context of TER homologues from sequenced genomes revealed that TER homologues (annotated so far as hypothetical reading frames) most commonly occur next to other hypothetical reading frames, but sometimes occur downstream of enzymes associated with fatty acid biosynthetic pathways and in one case downstream of a polyketide synthase in the *S. avermitilis* genome (Hoffmeister et al. 2005). This raises the possibility that TER homologues (and *Euglena* TER itself) could operate in fatty acid biosynthesis, polyketide biosynthesis, or conceivably both, since both pathways involve thioester-bound enoyl reduction. TER is active as a monomer, suggesting that catalysis of the NADH- and NADPH-dependent reduction of the double bond in crotonyl-CoA does not require interactions with other proteins (Hoffmeister et al. 2005).

2.4 The Biosynthetic Route to Wax Esters in *Euglena*

Under anaerobic conditions *Euglena gracilis* produces wax esters as end products of its metabolism (Fig. 2.4) (Buetow 1989; Inui et al. 1982, 1983, 1984; Tucci et al. 2010; Ogawa et al. 2015). The key enzyme is pyruvate:$NADP^+$ oxidoreductase, which decarboxylates pyruvate to acetyl-CoA. Acetyl-CoA serves as the terminal electron acceptor from glucose oxidation whereby fatty acids are synthesized. The fatty acids contain 8 to 18 carbon atoms and consist mainly of myristic acid and myristic alcohol (C14) (Inui et al. 1983). Sixty percent of the produced fatty acids are even number carbon chains (Tucci et al. 2010). Acetyl-CoA is used both as a primer and a C2 donor during even-numbered fatty acid synthesis in mitochondria. In addition to these even-numbered wax esters, also odd-numbered wax esters are synthesized. The synthesis of odd numbered wax esters start from propionyl-CoA instead of acetyl-CoA, stemming from the methyl-malonyl-CoA pathway (Nagai et al. 1971; Schneider and Betz 1985; Pönsgen-Schmidt et al. 1988).

The fatty acids are subsequently transported into the cytosol, where a portion of the fatty acids is reduced to alcohol, esterified with another fatty acid in the microsomes (Kolattukudy 1970; Khan and Kolattukudy 1973) and accumulate in the cytosol in an insoluble, crystalline form: waxes, hence this biochemical pathway is called wax ester fermentation. An unusual phenomenon, because fermentations usually generate soluble substances like alcohols or organic acids as end products. *Euglena* cells are able to produce 40 μg

of wax per 10^6 cells in the first 24 h of anaerobic incubation (Tucci et al. 2010).

Fatty acid biosynthesis catalyzed by fatty acid synthase (FAS) complexes and polyketide biosynthesis catalyzed by polyketide synthases (PKS) involve four fundamentally similar steps: (i) the condensation of an acetyl moiety onto an acyl thioester to form a β-ketoacyl thioester, (ii) reduction of the keto group to a β-hydroxyacyl thioester, (iii) dehydration to form the double bond in the resulting enoyl thioester, and (iv) reduction of the double bond to form an acyl thioester two carbons longer than the first one. The thiol moiety of the thioester is pantothenate, which is covalently bound to acyl carrier protein (ACP) in PKS and FAS complexes (Hopwood and Sherman 1990; Bentley and Bennett 1999; Hoffmeister et al. 2005). Fatty acid elongases, which extend C18 fatty acids to C20 and longer, generally operate with CoA esters (Trenkamp et al. 2004). In mitochondria and peroxisomes, β-oxidation operates through steps (iv) to (i) with the CoA esters, whereby the oxidative step (iv) is physiologically irreversible, since it is finally linked to oxygen reduction (Reddy and Hashimoto 2001).

TER catalyzes the step corresponding to (iv) above with the CoA ester, reducing crotonyl-CoA to butyryl-CoA (Hoffmeister et al. 2005; Tucci et al. 2010). No TER activity was found in the reverse reaction using butyryl-CoA as substrate with either NAD^+ or $NADP^+$ (Hoffmeister et al. 2005). The recombinant TER enzyme showed highest activity with crotonyl-CoA but also reduces C6 enoyl-CoAs and thus relates in terms of substrate preference to enoyl-CoA reductase previously described by Inui et al. (1984). Under anaerobiosis, active photosynthesis and consumption of external carbon sources, activities of glutamate dehydrogenase and $NADP^+$-dependent malic enzyme were enhanced, and malate dehydrogenase activity was reduced (Santiago-Martínez et al. 2015).

The synthesis of fatty acids, in particular long-chain polyunsaturated fatty acids (PUFAs), via polyketide synthesis has been shown both for the prokaryote *Shewanella* and the eukaryote *Schizochytrium* (Metz et al. 2001; Napier 2002). TER homologues are found within many sequenced prokaryotic genomes, however no true homologues for TER have so far been identified from any eukaryote other than *Euglena.* We must however qualify the foregoing statement as being only conditionally true, because several sequences in the 2016 release of GenBank have highly significant sequence identity to *Euglena* TER and are currently annotated as existing in eukaryotic genomes (*Nematostella* for example), but these are prokaryotic contaminations in the eukaryote assembly; such cases are fairly common and are often mistaken for lateral gene transfer (Ku and Martin 2016). Comprehensive analyses indicate that eukaryotes have rarely, if ever, acquired genes outside the context of the endosymbiotic origins of chloroplast and mitochondria (Ku et al. 2015; Ku and Martin 2016).

The apicomplexan protist *Cryptosporidium parvum*, which possesses the same unusual pyruvate:$NADP^+$-oxidoreductase as *Euglena* (Rotte et al. 2001), was found to encode a polyketide synthase (Zhu et al. 2002), and various groups of dinoflagellates synthesize large and complex polyketides (Rein and Borrone 1999). However, polyketides are not specific to eukaryotic groups that possess plastids (or, in the case of *Cryptospridium*, have secondarily lost plastids), since fungi also synthesize polyketides and possess PKS genes (Bohnert et al. 2004).

2.5 Rhodoquinone

Similar to the situation for anaerobic mitochondria of metazoa (Tielens et al. 2002; van Hellemond et al. 2003), wax ester fermentation of *Euglena* involves malate dismutation and mitochondrial fumarate reduction, and thus entails rhodoquinone (RQ) (Hoffmeister et al. 2004) for the synthesis of propionyl-CoA. In its physiological role, RQ is reduced by complex I and oxidized by fumarate reductase (FRD) (van Hellemond et al. 2003), but it can also donate electrons to other components of the *Euglena* mitochondrial respiratory chain (Castro-Guerrero et al. 2005), including the cytochrome bc_1 complex and the alternative oxidase (Castro-Guerrero et al. 2004).

Propionyl-CoA is used as the starter for the synthesis of fatty acids with odd-number chain lengths, which comprise about 50% (by weight) of hydrocarbon chains in accumulated wax esters under various conditions (Kawabata and Kaneyama 1989) and in different *Euglena* strains (Tucci et al. 2010). Propionyl-CoA is produced via the same short methylmalonyl-CoA-dependent route as that found in animal mitochondria (Schneider and Betz 1985), and homologs of the underlying enzymes, methylmalonyl-CoA mutase and propionyl-CoA carboxylase (which provides ATP via substrate-level phosphorylation), are abundantly expressed in euglenoid EST data (Ahmadinejad et al. 2007). Methylmalonyl-CoA mutase from *Euglena* has been characterized (Miyamoto et al. 2010); the enzyme is also present in humans, where it is one of our only two vitamin B_{12} (cobalamin)-dependent enzymes (Roth et al. 1996). *Euglena* growth was once the standard assay for serum B_{12} levels, because of its B_{12}-dependent ribonucleotide reductase (Torrents et al. 2006).

When grown under anaerobic conditions, *Euglena* expresses PNO (Inui et al. 1985, 1987, 1991), which performs the oxidative decarboxylation of pyruvate and is a fusion protein, with an N-terminal PFO (pyruvate:ferredoxin oxidoreductase) domain fused to a C-terminal flavoprotein domain with NAD-, FMN-, and FAD-binding modules (Nakazawa et al. 2003; Rotte et al. 2001). The flavoprotein domain is found in many other proteins, sometimes alone as NADPH:cytochrome P450 reductase and sometimes fused to other domains, such as in nitric oxide synthases of metazoans. The flavoprotein domain is best understood as a transducer of one-electron transport (from the FeS clusters of the PFO domain) to two-electron transport (NADPH). PNO supplies acetyl-CoA and NADPH for the production of wax esters. PNO was first described for *Euglena* and was long considered an enzyme unique to the *Euglena* lineage, but the same PNO (PFO fusion) is found in the apicomplexan *Cryptosporidium* (Rotte et al. 2001) as well as in *Blastocystis* (Lantsman et al. 2008). PNO homologs from many disparate eukaryotic lineages have been turning up in EST sequencing projects (Hug et al. 2010), indicating that the enzyme is far more widespread among eukaryotes than previously thought. Sequences clearly homologous to PFO occur in "typical" eukaryotes as well but in the guise of sulfite oxidases among fungi (Horner et al. 1999; Rotte et al. 2001), which exhibit fusions similar to those of *Euglena* mitochondrial PNO.

2.6 The Mitochondrial Genome

It is known that mitochondrial genomes of excavates are complicated to sequence, however in 2015 the mitochondrial genome and transcriptome of *E. gracilis* was described by Dobáková et al. (NCBI BioProject PRJNA294935). The mitochondrial DNA of *E. gracilis* appears to be combined with proteins to form nucleoids in the organelles. Most nucleoids in the mitochondria are spherical or ovoid and are, with a diameter of 70–130 nm, smaller than those in plastids (Hayashi-Isimaru et al. 1993). Many attempts to analyze the mitochondrial genome of *E. gracilis* failed and led only to the detection of a single cox1-bearing fragment (Tessier et al. 1997; Yasuhira and Simpson 1997), so mtDNA structure and size remained elusive (Roy et al. 2007), until Spencer and Gray (2011) established the general architecture of *E. gracilis* mtDNA. They isolated mtDNA comprising a heterodisperse collection of short linear fragments of heterogeneous size (modal size 4 kbp) bearing terminal repeats and many small, likely nonfunctional protein and rRNA gene fragments and showed that the mitochondrial large and small subunit rRNA are each split into two pieces (Spencer and Gray 2011). The mitochondrial genome of *E. gracilis* is fragmented, like Flegontov et al. (2011) suggested. Dobáková et al. (2015) analysed the mitochondrial genome in detail and reported that it encodes only a very limited set of seven protein-coding genes (three subunits of respiratory complex I, one subunit of complex III, and three subunits of complex IV) and a single SSU rRNA gene fragment. Surprisingly not a single subunit of the ATP synthase is encoded in the mitochondrial genome. The transcriptomic data

revealed that the transcripts of the mitochondrial genes are not post-translationally modified. RNA editing seems not to operate in the mitochondrion of *E. gracilis* as it does in diplonemids and kinetoplastids, the sister groups of euglenids. These findings suggest that mitochondrial panediting and processing machineries in diplonemids and kinetoplastids evolved late in evolution from the genome and transcriptome of the common ancestor of the Euglenozoa (Dobáková et al. 2015).

2.7 Mitochondrial Targeting

The majority of mitochondrial proteins in *E. gracilis* (and all eukaryotes) is encoded in the nucleus and imported into the organelles after translation in the cytosol. The mitochondrial import apparatus of Euglenozoa is supposed to be primitive; most components of the canonical mitochondrial import machinery could not be identified in euglenozoan sequences – including Tom40 (Schneider et al. 2008). Recently a protein assumed to have the same function as Tom40 in eukaryotes (Zársky et al. 2012) was found in trypanosomes: ATOM (Pusnik et al. 2011), but it was not found in the transcriptomes of euglenids (Krnáčová et al. 2012).

The mitochondrial proteins of *Euglena* possess presequences with a common (M/L)RR motif at the N-terminus or within the presequence for the organellar targeting (Krnáčová et al. 2012), as in some trypanosomatid mitochondrial proteins (Häusler et al. 1997). The presequences seem to be highly variable in sequence length (5–118 amino acids) with an average size of 31–41 amino acids, but apparently share statistically significant similarities. In most presequences the (M/L)RR motif is followed by a positively charged, hydrophobic region rich in alanine, leucine, and valine (Krnáčová et al. 2012). The presequences in *E. gracilis* can be divided into two types: i) short (up to 10 amino acids long) and ii) long (10–30 amino acids) – although some presequences can be longer (up to 118 amino acids). It was not possible to find predicted cleaved presequences in all analyzed mitochondrial proteins of *E. gracilis*, although they were predicted to be targeted to mitochondria (Krnáčová et al. 2012), similar to hydrogenosomes of *Trichomonas vaginalis*, where the majority of hydrogenosomal proteins do not harbor an N-terminal targeting sequence (Garg et al. 2015).

2.8 Alcohol Metabolizing Enzymes

An investigation of alcohol metabolizing enzymes in *E. gracilis* revealed their presence in the cytosol and the mitochondria (Yoval-Sánchez et al. 2011). Several alcohol dehydrogenases (ADHs) have been reported to localize to the cytosol (Munir et al. 2002; Palma-Gutiérrez et al. 2008), whereas prior work had identified ADHs only in *Euglena* mitochondria (Ono et al. 1995a). Furthermore, one aldehyde dehydrogenase (ALDH) (Ono et al. 1995b; Rodríguez-Zavala et al. 2006) and one acetyl-CoA synthase (Ono et al. 1995a) were found in the mitochondria. Ethanol is converted in the mitochondria to acetaldehyde by alcohol dehydrogenase and the produced acetaldehyde is converted to acetate by aldehyde dehydrogenase. Acetate is converted to acetyl-CoA by acetyl-CoA synthetase and can enter the modified Krebs cycle and after two reactions isocitrate is formed which can then either be further oxidized to carbon dioxide, or be converted by the bifunctional glyoxylate cycle enzyme to malate and succinate. Although significant fractions of ADH activity (26%), and ALDH activity (24%) were found in the cytosol, 68% of the ADH and 67% of the ALDH activities were measured in the mitochondria (Yoval-Sánchez et al. 2011). Most of the mitochondrial ADH activity was found in the mitochondrial matrix (60%), while 27% was detected in the inner membrane fraction. For ALDH activity it was *vice versa*: 20% in the mitochondrial matrix and 60% in the mitochondrial membrane fraction. Nevertheless, the active sites of both enzymes were not exposed to the cytosolic compartment. Furthermore, both enzymes were able to use aliphatic substrates of different chain

length, so they might be active in the metabolism of fatty alcohols released from wax esters (Yoval-Sánchez et al. 2011).

2.9 Glyoxylate Cycle

In 2003, a glyoxylate cycle to synthesize four-carbon compounds from two-carbon compounds, such as acetate and ethanol, was shown to be present only in mitochondria of *E. gracilis* (Ono et al. 2003). The glyoxylate cycle is important in gluconeogenesis and connects the conversion of fat to carbohydrate with some enzymes for β-oxidation of fatty acids. In those eukaryotes that possess a glyoxylate cycle, it is usually present in specialized peroxisomes known as glyoxysomes. Isocitrate lyase (ICL) and malate synthase (MS) are the main enzymes of the glyoxylate cycle. Isocitrate is reversibly cleaved by isocitrate lyase to succinate and glyoxylate, and the latter condenses with acetyl-CoA to malate catalyzed by malate synthase (Nakazawa et al. 2011). The bifunctional protein, called glyoxylate cycle enzyme (also known as the bifunctional glyoxylate cycle enzyme, malate synthase/isocitrate lyase) (Liu et al. 1995, 1997), is a fusion protein with ICL and MS domains. The N-terminal domain (amino acids 1–519) of *Euglena* glyoxylate cycle enzyme has high sequence similarity to other MS enzymes and the C-terminal domain (amino acids 597–1165) is related to ICL family proteins (Nakazawa et al. 2005). Despite the resemblance of the C-terminal domain to ICLs it was not possible to yield ICL activity by expression of the domain in *E. coli*. Instead the N-terminal domain was sufficient to provide MS and ICL activities. The ICL activity might be regulated by acetyl-CoA, binding to a site other than the catalytic center, for the MS reaction (Nakazawa et al. 2011).

The detection of the *Euglena* glyoxylate cycle enzyme in the mitochondria shows that these organelles play an important role in providing carbon sources for gluconeogenesis during wax ester and ethanol utilization. The isocitrate is metabolized in the mitochondria via two cycles, the glyoxylate cycle and the Krebs cycle (Ono et al. 2003).

2.10 A multifunctional β-oxidation Enzyme

Winkler et al. purified a novel multifunctional β-oxidation complex composed of 3-hydroxyacyl-CoA dehydrogenase, 2-enoyl-CoA hydratase, thiolase, and epimerase activities from *E. gracilis* mitochondria (Winkler et al. 2003). The novel β-oxidation complex resembles the composition of monofunctional β-oxidation enzymes and consists of 45.5-, 44.5-, 34- and 32-kD subunits. On the large subunits hydratase and thiolase functions were located and on the two smaller subunits two different 3-hydroxyacyl-CoA dehydrogenase functions were found, whereas epimerase activity was found in the complete enzyme. The 3-hydroxyacyl-CoA dehydrogenases were specific to L-isomers, the 2-enoyl-CoA hydratase catalyzed the formation of 1-hydroxyacyl-CoA isomers. This indicates that the multifunctional *Euglena* enzyme might belong to the family of β-oxidation enzymes that degrade acyl-CoAs via L-isomers (Winkler et al. 2003).

2.11 Evolutionary Considerations

Metabolic maps summarizing the main contours of energy metabolism in the mitochondria of *Euglena* grown under aerobic (Fig. 2.2) and anaerobic conditions (Figs. 2.3 and 2.4) are presented. Currently, the main interest in *Euglena* mitochondria focuses on the application of the enzymes of wax ester fermentation to biofuel production. The energetic finesse behind wax ester fermentation in *Euglena* resides in the condensing enzyme of mitochondrial fatty acid biosynthesis; it can condense acetyl-CoA to 2-oxoacyl-CoA moieties (Inui et al. 1984), in contrast to standard fatty acid biosynthesis, which condenses malonyl-CoA units. The malonyl-CoA independence means that the one ATP per pyruvate obtained from glycolysis need not be spent on synthesis of malonyl-CoA for fatty acid synthesis (conversion of acetyl-CoA to malonyl-CoA requires one ATP), allowing net gain of one ATP per pyruvate during wax ester accumulation.

The ability of *Euglena* mitochondria to function in ATP synthesis with or without oxygen raises a number of interesting evolutionary questions, and there has been considerable interest in the evolutionary origin of anaerobic energy metabolism in *Euglena* mitochondria (Rotte et al. 2001; Hoffmeister et al. 2005; Müller et al. 2012). When the cells are grown aerobically, *Euglena* mitochondria appear to function much like a normal textbook rat liver mitochondrion, except for the modified Krebs cycle. When the cells are cultured anaerobically, *Euglena* mitochondria share properties both with hydrogenosomes and with the anaerobic mitochondria characterized from several metazoans. Shared with hydrogenosomes is, for example, the use of PFO in the PNO fusion protein for oxidative decarboxylation of pyruvate. Shared with anaerobic mitochondria of animals is, for example, the use of RQ and the propionate cycle involving methylmalonyl-CoA mutase, a cobalamin dependent enzyme. With one exception, none of the proteins involved in anaerobic energy metabolism of eukaryotes investigated so far are lineage specific, they all trace to the eukaryote common ancestor (Müller et al. 2012). The single exception is currently the TER enzyme of *Euglena*, which is still unique among eukaryotes studied to date.

2.12 Future Directions

Recent studies have investigated transcriptomic comparisons of anaerobically versus aerobically treated *E. gracilis* cultures (Yoshida et al. 2016). But the anaerobic treatments were restricted to 24 h and no data were reported about growth of the cultures under anaerobic conditions. Additional transcriptomic studies comparing suitable *Euglena* strains that grow and divide under a variety of aerobic and anaerobic conditions are needed in order to better understand the corresponding differences in mitochondrial energy metabolism. An earlier proteomic study of short-term treated aerobic and anaerobic cultures revealed that the transition to anaerobic conditions did not result in an induction of enzymes specific for anaerobic function, but that enzymes and cofactors (RQ) required for anaerobiosis were already present before anaerobic treatment, with proteins for aerobic ATP synthesis being lost during anaerobic adaptation (Hoffmeister et al. 2004). This indicates that *Euglena* is prepared for anaerobic conditions before they are encountered, a strategy often encountered among eukaryotes. The biosynthesis of RQ has still not been elucidated in any eukaryote, this is an important topic for future work on *Euglena*.

References

Ahmadinejad N, Dagan T, Martin W (2007) Genome history in the symbiotic hybrid *Euglena gracilis*. Gene 402:35–39

Atteia A, van Lis R, Tielens AG, Martin WF (2013) Anaerobic energy metabolism in unicellular photosynthetic eukaryotes. Biochim Biophys Acta 1827:210–223

Bentley R, Bennett JW (1999) Constructing polyketides: from collie to combinatorial biosynthesis. Annu Rev Microbiol 53:411–446

Bereiter-Hahn J, Vöth M (2005) Dynamics of mitochondria in living cells: shape changes, dislocations, fusion and fission of mitochondria. Microsc Res Tech 27:198–219

Bernhard JM, Buck KR, Farmer MA, Bowser SS (2000) The Santa Barbara Basin is a symbiosis oasis. Nature 403:77–80

Bohnert HU, Fudal I, Dioh W, Tharreau D, Notteghem JL, Lebrun MH (2004) A putative polyketide synthase/peptide synthetase from *Magnaporthe grisea* signals pathogen attack to resistant rice. Plant Cell 16:2499–2513

Braun HP, Schmitz UK (1995) Molecular structure of the 8.0 kDa subunit of cytochrome *c* reductase from potato and its $\Delta\Psi$-dependent import into isolated-mitochondria. Biochim Biophys Acta 1229:181–186

Brönstrup U, Hachtel W (1986) Protein synthesis in isolated mitochondria from a streptomycin-bleached mutant of *Euglena gracilis* Klebs. J Plant Physiol 124:45–53

Brönstrup U, Hachtel W (1989) Cytochrome *c* oxidase of *Euglena gracilis*: purification, characterization, and identification of mitochondrially synthesized subunits. J Bioenerg Biomembr 21:359–373

Buetow DE (1989) The mitochondrion. In: Buetow DE (ed) The biology of *Euglena*, vol 4. Academic Press, San Diego, pp 247–314

Calvayrac R, Butow RA (1971) Action de l'antimycine A sur la respiration et la structure des mitochondries d'*Euglena gracilis*. Arch Microbiol 80:62–69

Calvayrac R, van Lente F, Butow RA (1971) *Euglena gracilis*: formation of giant mitochondria. Science 173:252–254

Castro H, Tomás AM (2008) Peroxidases of trypanosomatids. Antioxid Redox Signal 10:1593–1606

Castro-Guerrero NA, Krab K, Moreno-Sanchez R (2004) The alternative respiratory pathway of *Euglena* mitochondria. J Bioenerg Biomembr 36:459–469

Castro-Guerrero NA, Jasso-Chavez R, Moreno-Sanchez R (2005) Physiological role of rhodoquinone in *Euglena gracilis* mitochondria. Biochim Biophys Acta 1710:113–121

Castro-Guerrero NA, Rodríguez-Zavala JS, Marín-Hernández A, Rodríguez-Enríquez S, Moreno-Sánchez R (2008) Enhanced alternative oxidase and antioxidant enzymes under Cd(2+) stress in *Euglena*. J Bioenerg Biomembr 40:227–235

Dasgupta S, Fang J, Brake SS, Hasiotis ST, Zhang L (2012) Biosynthesis of sterols and wax esters by *Euglena* of acid mine drainage biofilms: implications for eukaryotic evolution and the early Earth. Chem Geol 306:139–145

Devars S, Torres-Márquez ME, González-Halphen D, Uribe A, Moreno-Sánchez R (1992) Cyanide-sensitive and cyanide-resistant respiration of dark-grown *Euglena gracilis*. Plant Sci 82:37–46

Dobáková E, Flegontov P, Skalicky T, Lukeš J (2015) Unexpectedly streamlined mitochondrial genome of the euglenozoan *Euglena gracilis*. Genome Biol Evol 7:3358–3367

Duarte M, Tomas AMJ (2014) The mitochondrial complex I of trypanosomatids – an overview of current knowledge. Bioenerg Biomembr 46:299–311

Duran E, Komuniecki RW, Komuniecki PR, Wheelock MJ, Klingbeil MM, Ma YC, Johnson KR (1993) Characterization of cDNA clones for the 2-methyl branched-chain enoyl-CoA reductase. An enzyme involved in branched-chain fatty acid synthesis in anaerobic mitochondria of the parasitic nematode Ascaris suum. J Biol Chem 268:22391–22396

Duran E, Walker DJ, Johnson KR, Komuniecki PR, Komuniecki RW (1998) Developmental and tissue-specific expression of 2-methyl branched-chain enoyl CoA reductase isoforms in the parasitic nematode, *Ascaris suum*. Mol Biochem Parasitol 91:307–318

Edgcomb VP, Breglia SA, Yubuki N, Beaudoin D, Patterson DJ, Leander BS, Bernhard JM (2011) Identity of epibiotic bacteria on symbiontid euglenozoans in O_2-depleted marine sediments: evidence for symbiont and host co-evolution. ISME J 5:231–243

Ernst-Fonberg ML, Bloch K (1971) A chloroplast-associated fatty acid synthetase system in *Euglena*. Arch Biochem Biophys 143:392–400

Flegontov P, Gray MW, Burger G, Lukeš J (2011) Gene fragmentation: a key to mitochondrial genome evolution in Euglenozoa? Curr Genet 57:225–232

Garg S, Stölting J, Zimorski V, Rada P, Tachezy J, Martin WF, Gould SB (2015) Conservation of transit peptide-independent protein import into the mitochondrial and hydrogenosomal matrix. Genome Biol Evol 7:2716–2726

Goldberg I, Bloch K (1972) Fatty acid synthetases in *Euglena gracilis*. J Biol Chem 247:7349–7357

Graham IA, Eastmond PJ (2002) Pathways of straight and branched chain fatty acid catabolism in higher plants. Prog Lipid Res 41:156–181

Green LS, Li Y, Emerich DW, Bergersen FJ, Day DA (2000) Catabolism of α-ketoglutarate by a *sucA* mutant of *Bradyrhizobium japonicum*: evidence for an alternative tricarboxylic acid cycle. J Bacteriol 182:2838–2844

Häusler T, Stierhof YD, Blattner J, Clayton C (1997) Conservation of mitochondrial targeting sequence function in mitochondrial and hydrogenosomal proteins from the early-branching eukaryotes *Crithidia*, *Trypanosoma* and *Trichomonas*. Eur J Cell Biol 73:240–251

Hayashi Y, Ueda K (1989) The shape of mitochondria and the number of mitochondrial nucleoids during the cell cycle of *Euglena gracilis*. J Cell Sci 93:565–570

Hayashi-Isimaru Y, Ueda K, Nonaka M (1993) Detection of DNA in the nucleoids of chloroplasts and mitochondria in *Euglena gracilis* by immunoelectron microscopy. J Cell Sci 105:1159–1164

Hendren RW, Bloch K (1980) Fatty acid synthetases from *Euglena gracilis*. Separation of component activities of the ACP-dependent fatty acid synthetase and partial purification of the beta-ketoacyl-ACP synthetase. J Biol Chem 255:1504–1508

Hoffmeister M, van der Klei A, Rotte C, van Grinsven KW, van Hellemond JJ, Henze K, Tielens AG, Martin W (2004) *Euglena gracilis* rhodoquinone:ubiquinone ratio and mitochondrial proteome differ under aerobic and anaerobic conditions. J Biol Chem 279: 22422–22429

Hoffmeister M, Piotrowski M, Nowitzki U, Martin W (2005) Mitochondrial trans-2-enoyl-CoA reductase of wax ester fermentation from *Euglena gracilis* defines a new family of enzymes involved in lipid synthesis. J Biol Chem 280:4329–4338

Hopwood DA, Sherman DH (1990) Molecular genetics of polyketides and its comparison to fatty acid biosynthesis. Annu Rev Genet 24:37–66

Horner DS, Hirt RP, Embley TM (1999) A single eubacterial origin of eukaryotic pyruvate:ferredoxin oxidoreductase genes: implications for the evolution of anaerobic eukaryotes. Mol Biol Evol 16:1280–1291

Hug LA, Stechmann A, Roger AJ (2010) Phylogenetic distributions and histories of proteins involved in anaerobic pyruvate metabolism in eukaryotes. Mol Biol Evol 27:311–324

Inui H, Miyatake K, Nakano Y, Kitaoka S (1982) Wax ester fermentation in *Euglena gracilis*. FEBS Lett 150:89–93

Inui H, Miyatake K, Nakano Y, Kitaoka S (1983) Production and composition of wax esters by fermentation of *Euglena gracilis*. Agric Biol Chem 47:2669–2671

Inui H, Miyatake K, Nakano Y, Kitaoka S (1984) Fatty acid synthesis in mitochondria of *Euglena gracilis*. Eur J Biochem 142:121–126

Inui H, Miyatake K, Nakano Y, Kitaoka S (1985) The physiological role of oxygen-sensitive pyruvate

dehydrogenase in mitochondrial fatty acid synthesis in *Euglena gracilis*. Arch Biochem Biophys 237:423–429

Inui H, Ono K, Miyatake K, Nakano Y, Kitaoka S (1987) Purification and characterization of pyruvate:NADP+ oxidoreductase in *Euglena gracilis*. J Biol Chem 262:9130–9135

Inui H, Yamaji R, Saidoh H, Miyatake K, Kitaoka S (1991) Pyruvate:$NADP^+$ oxidoreductase from *Euglena gracilis*: limited proteolysis of the enzyme with trypsin. Arch Biochem Biophys 286:270–276

Inui H, Miyatake K, Nakano Y, Kitaoka S (1992) Synthesis of reserved polysaccharide from wax esters accumulated as the result of anaerobic energy generation in *Euglena gracilis* returned from anaerobic to aerobic conditions. Int J Biochem 24:799–803

Ishikawa T, Takeda T, Shigeoka S, Hirayama O, Mitsunaga T (1993) Hydrogen peroxide generation in organelles of *Euglena gracilis*. Phytochemistry 33:1297–1299

Jasso-Chávez R, Moreno-Sánchez R (2003) Cytosol-mitochondria transfer of reducing equivalents by a lactate shuttle in heterotrophic *Euglena*. Eur J Biochem 270:4942–4951

Jasso-Chávez R, Torres-Márquez ME, Moreno-Sánchez R (2001) The membrane-bound L- and D-lactate dehydrogenase activities in mitochondria from *Euglena gracilis*. Arch Biochem Biophys 390:295–303

Jasso-Chávez R, García-Cano I, Marín-Hernández A, Mendoza-Cózatl D, Rendón JL, Moreno-Sánchez R (2005) The bacterial-like lactate shuttle components from the heterotrophic *Euglena gracilis*. Biochim Biophys Acta 1709:181–190

Kawabata A, Kaneyama M (1989) The effect of growth temperature on wax ester content and composition of *Euglena gracilis*. J Gen Microbiol 135:1461–1467

Khan AA, Kolattukudy PE (1973) A microsomal fatty acid synthtase coupled to acyl-CoA reductase in *Euglena gracilis*. Arch Biochem Biophys 158:411–420

Kita K, Hirawake H, Miyadera H, Amino H, Takeo S (2002) Role of complex II in anaerobic respiration of the parasite mitochondria from *Ascaris suum* and *Plasmodium falciparum*. Biochim Biophys Acta 1553:123–139

Kitaoka S, Nakano Y, Miyatake K, Yokota A (1989) Enzymes and their functional localization. In: Buetow DE (ed) The biology of Euglena, vol 4. Academic Press, San Diego, pp 2–135

Klaveness D, Løvhøiden F (2007) Meromictic lakes as habitats for protists: life in the chemocline and below? In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Dordrecht, pp 59–78

Kolattukudy PE (1970) Reduction of fatty acids to alcohols by cell-free preparations of *Euglena gracilis*. Biochemist 9:1095–1102

Komuniecki R, Tielens AGM (2003) Carbohydrate and energy metabolism in parasitic helminths. In: Marr JJ, Nilsen TW, Komuniecki RW (eds) Molecular medical parasitology. Academic press, San Diego, pp 339–358

Komuniecki PR, Vanover L (1987) Biochemical changes during the aerobic-anaerobic transition in Ascaris suum larvae. Mol Biochem Parasitol 22:241–248

Komuniecki R, Komuniecki PR, Saz HJ (1981) Relationships between pyruvate decarboxylation and branched-chain volatile acid synthesis in *Ascaris* mitochondria. J Parasitol 67:601–608

Komuniecki R, Fekee S, Thissen-Parra J (1985) Purification and characterization of the 2-methyl branched-chain Acyl-CoA dehydrogenase, an enzyme involved in NADH-dependent enoyl-CoA reduction in anaerobic mitochondria of the nematode, *Ascaris suum*. J Biol Chem 260:4770–4777

Komuniecki R, Campbell T, Rubin N (1987) Anaerobic metabolism in *Ascaris suum*: Acyl CoA intermediates in isolated mitochondria synthesizing 2-methyl branched-chain fatty acids. Mol Biochem Parasitol 24:147–154

Krnáčová K, Vesteg M, Hampl V, Vlček Č, Horváth A (2012) *Euglena gracilis* and Trypanosomatids possess common patterns in predicted mitochondrial targeting presequences. J Mol Evol 75:119–129

Krnáčová K, Rýdlová I, Vinarčíková M, Krajčovič J, Vesteg M, Horváth A (2015) Characterization of oxidative phosphorylation enzymes in *Euglena gracilis* and its white mutant strain W(gm)ZOflL. FEBS Lett 589:687–694

Ku C, Martin WF (2016) A natural barrier to lateral gene transfer from prokaryotes to eukaryotes revealed from genomes: the 70% rule. BMC Biol 14:89

Ku C, Nelson-Sathi S, Roettger M, Sousa FL, Lockhart PJ, Bryant D, Hazkani-Covo E, McInerney JO, Landan G, Martin WF (2015) Endosymbiotic origin and differential loss of eukaryotic genes. Nature 524:427–432

Kunau WH, Dommes V, Schulz H (1995) β-oxidation of fatty acids in mitochondria, peroxisomes, and bacteria: a century of continued progress. Prog Lipid Res 34:264–342

Lantsman Y, Tan KSW, Morada M, Yarlett N (2008) Biochemical characterization of a mitochondrial-like organelle from *Blastocystis* sp. subtype 7. Microbiology 154:2757–2766

Leedale GF, Meeuse BJD, Pringsheim EG (1965) Structure and physiology of *Euglena spirogyra* I and II. Arch Microbiol 50:68–102

Liu H, Reynolds KA (1999) Role of crotonyl coenzyme A reductase in determining the ratio of polyketides monensin A and monensin B produced by *Streptomyces cinnamonensis*. J Bacteriol 181:6806–6813

Liu F, Thatcher JD, Barral JM, Epstein HF (1995) Bifunctional glyoxylate cycle protein of *Caenorhabditis elegans*: a developmentally regulated protein of intestine and muscle. Dev Biol 169:399–414

Liu F, Thatcher JD, Epstein HF (1997) Induction of glyoxylate cycle expression in *Caenorhabditis elegans*: a fasting response throughout larval development. Biochemistry 36:255–260

Mei B, Komuniecki R, Komuniecki PR (1997) Localization of cytochrome oxidase and the 2-methyl branched-chain enoyl CoA reductase in muscle and hypodermis of *Ascaris suum* larvae and adults. J Parasitol 83:760–763

Mentel M, Martin W (2008) Energy metabolism among eukaryotic anaerobes in light of Proterozoic ocean

chemistry. Philos Trans R Soc Lond B 363: 2717–2729

Metz JG, Roessler P, Facciotti D, Levering C, Dittrich F, Lassner M, Valentine R, Lardizabal K, Domergue F, Yamada A, Yazawa K, Knauf V, Browse J (2001) Production of polyunsaturated fatty acids by polyketide synthases in both prokaryotes and eukaryotes. Science 293:290–293

Michaels A, Gibor A (1973) Ultrastructural changes in *Euglena* after ultraviolet irradiation. J Cell Sci 13:779–809

Miyamoto E, Tanioka Y, Nishizawa-Yokoi A, Yabuta Y, Ohnishi K, Misono H, Shigeika S, Nakano Y, Watanabe F (2010) Characterization of methylmalonyl-CoA mutase involved in the propionate photoassimilation of *Euglena gracilis* Z. Arch Microbiol 192:437–446

Moreno-Sánchez R, Covián R, Jasso-Chávez R, Rodríguez-Enríquez S, Pacheco-Moisés E, Torres-Márquez ME (2000) Oxidative phosphorylation supported by an alternative respiratory pathway in mitochondria from *Euglena*. Biochim Biophys Acta 1457:200–210

Mukai K, Yoshida M, Toyosaki H, Yao Y, Wakabayashi S, Matsubara H (1989) An atypical heme-binding structure of cytochrome *c*1 of *Euglena gracilis* mitochondrial complex III. Eur J Biochem 178:649–656

Müller M, Mentel M, van Hellemond JJ, Henze K, Woehle C, Gould SB, Yu R-Y, van der Giezen M, Tielens AGM, Martin WF (2012) Biochemistry and evolution of anaerobic energy metabolism in eukaryotes. Microbiol Mol Biol Rev 76:444–495

Munir I, Nakazawa M, Harano K, Yamaji R, Inui H, Miyatake K, Nakano Y (2002) Occurrence of a novel $NADP^+$ –linked alcohol dehydrogenase in *Euglena gracilis*. Comp Biochem Physiol B Biochem Mol Biol 132:535–540

Nagai J, Otha T, Saito E (1971) Incorporation of propionate into wax esters by etiolated *Euglena*. Biochem Biophys Res Commun 42:523–529

Nakazawa M, Inui H, Yamaji R, Yamamoto T, Takenaka S, Ueda M, Nakano Y, Miyatake K (2000) The origin of pyruvate:$NADP^+$ oxidoreductase in mitochondria of *Euglena gracilis*. FEBS Lett 479:155–156

Nakazawa M, Takenaka S, Ueda M, Inui H, Nakano Y, Miyatake K (2003) Pyruvate:$NADP^+$ oxidoreductase is stabilized by its cofactor, thiamin pyrophosphate, in mitochondria of *Euglena gracilis*. Arch Biochem Biophys 411:183–188

Nakazawa M, Minami T, Teramura K, Kumamoto S, Hanato S, Takenaka S, Ueda M, Inui H, Nakano Y, Miyatake K (2005) Molecular characterization of a bifunctional glyoxylate cycle enzyme, malate synthase/isocitrate lyase, in *Euglena gracilis*. Comp Biochem Physiol B Biochem Mol Biol 141:445–452

Nakazawa M, Nishimura M, Inoue K, Ueda M, Inui H, Nakano Y, Miyatake K (2011) Characterization of a bifunctional glyoxylate cycle enzyme, malate synthase/isocitrate lyase, of *Euglena gracilis*. J Eukaryot Microbiol 58:128–133

Nakazawa M, Andoh H, Koyama K, Watanabe Y, Nakai T, Ueda M, Sakamoto T, Inui H, Nakano Y, Miyatake K (2015) Alteration of wax ester content and composition in *Euglena gracilis* with gene silencing of 3-ketoacyl-CoA thiolase isozymes. Lipids 50:483–492

Napier JA (2002) Plumbing the depths of PUFA biosynthesis: a novel polyketide synthase-like pathway from marine organisms. Trends Plant Sci 7:51–54

Nass MMK, Ben-Shaul Y (1972) A novel closed circular duplex DNA in bleached mutant and green strains of *Euglena gracilis*. Biochim Biophys Acta 272:130–136

Neumann D, Parthier B (1973) Effects of nalidixic acid, chloramphenicol, cycloheximide, and anisomycin on structure and development of plastids and mitochondria in greening *Euglena gracilis*. Exp Cell Res 81:255–268

O'Brien EA, Koski LB, Zhang Y, Yang L, Wang E, Gray MW, Burger G, Lang BF (2007) TBestDB: a taxonomically broad database of expressed sequence tags (ESTs). Nucleic Acids Res 35:D445–D451

Ogawa T, Kimura A, Sakuyama H, Tamoi M, Ishikawa T, Shigeoka S (2015) Identification and characterization of cytosolic fructose-1,6-bisphosphatase in *Euglena gracilis*. Biosci Biotechnol Biochem 79:1957–1964

Ono K, Kawanaka Y, Izumi Y, Inui H, Miyatake K, Kitaoka S, Nakano Y (1995a) Mitochondrial alcohol dehydrogenase from ethanol-grown *Euglena gracilis*. J Biochem 117:1178–1182

Ono K, Miyatake K, Inui H, Kitaoka S, Nakano Y (1995b) Wax ester production by anaerobic *Euglena gracilis*. J Mar Biotechnol 2:157–161

Ono K, Kondo M, Osafune T, Miyatake K, Inui H, Kitaoka S, Nishimura M, Nakano Y (2003) Presence of glyoxylate cycle enzymes in the mitochondria of *Euglena gracilis*. J Eukaryot Microbiol 50:92–96

Padermshoke A, Ogawa T, Nishio K, Nakazawa M, Nakamoto M, Okazawa A, Kanaya S, Arita M, Ohta D (2016) Critical involvement of environmental carbon dioxide fixation to drive wax ester fermentation in *Euglena*. PLoS One 11:e0162827

Palma-Gutiérrez HN, Rodríguez-Zavala JS, Jasso-Chávez R, Moreno-Sánchez R, Saavedra E (2008) Gene cloning and biochemical characterization of an alcohol dehydrogenase from *Euglena gracilis*. J Eukaryot Microbiol 55:554–561

Pellegrini M (1980) Three-dimensional reconstruction of organelles in *Euglena gracilis* Z. II. Qualitative and quantitative changes of chloroplasts and mitochondrial reticulum in synchronous cultures during bleaching. J Cell Sci 46:313–340

Perez E, Lapaille M, Degand H, Cilibrasi L, Villavicencio-Queijeiro A, Morsomme P, González-Halphen D, Field MC, Remacle C, Baurain D, Cardol P (2014) The mitochondrial respiratory chain of the secondary green alga *Euglena gracilis* shares many additional subunits with parasitic Trypanosomatidae. Mitochondrion 19:338–349

Pönsgen-Schmidt E, Schneider T, Hammer U, Betz A (1988) Comparison of phosphoenolpyruvate-carboxykinase from autotrophically and heterotrophi-

cally grown *Euglena* and its role during dark anaerobiosis. Plant Physiol 86:456–462

Pusnik M, Schmidt O, Perry AJ, Oeljeklaus S, Niemann M, Warcheid B, Lithgow T, Meisinger C, Schneider A (2011) Mitochondrial preprotein translocase of trypanosomatids has a bacterial origin. Curr Biol 21:1738–1743

Reddy JK, Hashimoto T (2001) Peroxisomal beta-oxidation and peroxisome proliferator-activated receptor alpha: an adaptive metabolic system. Annu Rev Nutr 21:193–230

Rein KS, Borrone J (1999) Polyketides from dinoflagellates: origins, pharmacology and biosynthesis. Comp Biochem Physiol B Biochem Mol Biol 124:117–131

Reynolds KA, Wang P, Fox KM, Speedie MK, Lam Y, Floss HG (1992) Purification and characterization of a novel enoyl coenzyme A reductase from *Streptomyces collinus*. J Bacteriol 174:3850–3854

Rodríguez-Zavala JS, Ortiz-Cruz MA, Moreno-Sánchez R (2006) Characterization of an aldehyde dehydrogenase from *Euglena gracilis*. J Eukaryot Microbiol 53:36–42

Roth JR, Lawrence JG, Bobik TA (1996) Cobalamin (coenzyme B_{12}): synthesis and biological significance. Annu Rev Microbiol 50:137–181

Rotte C, Stejskal F, Zhu G, Keithly JS, Martin W (2001) Pyruvate:$NADP^+$ oxidoreductase from the mitochondrion of *Euglena gracilis* and from the apicomplexan *Cryptosporidium parvum*: a biochemical relic linking pyruvate metabolism in mitochondriate and amitochondriate protists. Mol Biol Evol 18:710–720

Roy J, Faktorov D, Lukeš J, Burger G (2007) Unusual mitochondrial genome structures throughout the Euglenozoa. Protist 158:385–396

Santiago-Martínez MG, Lira-Silva E, Encalada R, Pineda E, Gallardo-Pérez JC, Zepeda-Rodriguez A, Moreno-Sánchez R, Saavedra E, Jasso-Chávez R (2015) Cadmium removal by *Euglena gracilis* is enhanced under anaerobic growth conditions. J Hazard Mater 288:104–112

Schneider T, Betz A (1985) Wax ester fermentation in *Euglena gracilis* T. Factors favouring the synthesis of odd-numbered fatty acids and alcohols. Planta 166:67–73

Schneider A, Bursa D, Lithgow T (2008) The direct route: a simplified pathway for protein import into the mitochondrion of trypanosomes. Trends Cell Biol 18:12–18

Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y, Yoshimura K (2002) Regulation and function of ascorbate peroxidase isoenzymes. J Exp Bot 53:1305–1319

Simpson AGB, van den Hoff J, Bernard C, Burton HR, Patterson DJ (1997) The ultrastructure and systematic position of the Euglenozoon *Postgaardi mariagerensis*, Fenchel et al. Arch Protistenkd 147:213–225

Spencer DF, Gray MW (2011) Ribosomal RNA genes in *Euglena gracilis* mitochondrial DNA: fragmented genes in a seemingly fragmented genome. Mol Gen Genomics 285:19–31

Stewart KD, Mattox K (1980) Phylogeny of phytoflagellates. In: Cox ER (ed) Phytoflagellates. Elsevier, North Holland, pp 433–462

Takamiya S, Kita K, Wang PP, Weinstein A, Hiraishi H, Oya H, Aoki T (1993) Developmental changes in the respiratory chain of *Ascaris* mitochondria. Biochim Biophys Acta 1141:65–74

Tamaki S, Maruta T, Sawa Y, Shigeoka S, Ishikawa T (2014) Identification and functional analysis of peroxiredoxin isoforms in *Euglena gracilis*. Biosci Biotechnol Biochem 78:593–601

Teerawanichpan P, Qiu X (2010) Fatty acyl-CoA reductase and wax synthase from *Euglena gracilis* in the biosynthesis of medium-chain wax esters. Lipids 45:263–273

Tessier LH, van der Speck H, Gualberto JM, Grienenberger JM (1997) The *cox1* gene from *Euglena gracilis*: a protist mitochondrial gene without introns and genetic code modifications. Curr Genet 31:208–213

Tielens AGM (1994) Energy generation in parasitic helminths. Parasitol Today 10:346–352

Tielens AGM, van Hellemond JJ (2007) Anaerobic mitochondria: properties and origin. In: Martin W, Müller M (eds) Origin of mitochondria and hydrogenosomes. Springer, Berlin, Heidelberg, pp 85–103

Tielens AGM, Rotte C, van Hellemond JJ, Martin W (2002) Mitochondria as we don't know them. Trends Biochem Sci 27:564–572

Torrents E, Trevisiol C, Rotte C, Hellman U, Martin W, Reichard P (2006) *Euglena gracilis* ribonucleotide reductase: the eukaryotic class II enzyme and the antiquity of eukaryotic B_{12}-dependence. J Biol Chem 281:5604–5611

Trenkamp S, Martin W, Tietjen K (2004) Specific and differential inhibition of very-long-chain fatty acid elongases from *Arabidopsis thaliana* by different herbicides. Proc Natl Acad Sci U S A 101:11903–11908

Tucci S, Vacula R, Krajcovic J, Proksch P, Martin W (2010) Variability of wax ester fermentation in natural and bleached *Euglena gracilis* strains in response to oxygen and the elongase inhibitor flufenacet. J Eukaryot Microbiol 57:63–69

Uribe A, Cávez E, Jiménez M, Zazueta C, Moreno-Sánchez R (1994) Characterization of Ca^{2+}-transport in *Euglena gracilis* mitochondria. Biochim Biophys Acta 1186:107–116

Van Hellemond JJ, van der Klei A, van Weelden SWH, Tielens AGM (2003) Biochemical and evolutionary aspects of anaerobically functioning mitochondria. Philos Trans R Soc Lond Ser B Biol Sci 358:205–213

van Waarde A, Van den Thillart G, Verhagen M (1993) Ethanol formation and pH regulation in fish. In: Hochachka PW et al (eds) Surviving hypoxia. CRC Press, Boca Raton, pp 157–170

Walker TA, Jonak ZL, Worsham LM, Ernst-Fonberg ML (1981) Kinetic studies oft he fatty acid synthetase multienzyme complex from *Euglena gracilis* variety *bacillaris*. Biochem J 199:383–392

Wallace KK, Bao ZY, Dai H, Digate R, Schuler G, Speedie MK, Reynolds KA (1995) Purification of crotonyl-CoA

reductase from *Streptomyces collinus* and cloning, sequencing and expression of the corresponding gene in *Escherichia coli*. Eur J Biochem 233:954–962

Wallis JG, Watts JL, Browse J (2002) Polyunsaturated fatty acid synthesis: what will they think of next? Trends Biochem Sci 27:467–473

Winkler U, Säftel W, Stabenau H (2003) A new type of a multifunctional β-oxidation enzyme in *Euglena*. Plant Physiol 131:753–762

Wolken JJ (1967) *Euglena*—an experimental organism for biochemical and biophysical studies, 2nd edn. Springer, New York

Yamada K, Suzuki H, Takeuchi T, Kazama Y, Mitra S, Abe T, Goda K, Suzuki K, Iwata O (2016) Efficient selective breeding of live oil-rich *Euglena gracilis* with fluorescence-activated cell sorting. Sci Rep 6:26327

Yasuhira S, Simpson L (1997) Phylogenetic affinity of mitochondria of *Euglena gracilis* and kinetoplastids using cytochrome oxidase I and hsp60. J Mol Evol 44:341–347

Yoshida Y, Tomiyama T, Maruta T, Tomita M, Ishikawa T, Arakawa K (2016) De novo assembly and comparative transcriptome analysis of *Euglena gracilis* in response to anaerobic conditions. BMC Genomics 17:182

Yoval-Sánchez B, Jasso-Chávez R, Lira-Silva E, Moreno-Sánchez R, Rodríguez-Zavala JS (2011) Novel mitochondrial alcohol metabolizing enzymes of *Euglena gracilis*. J Bioenerg Biomembr 43:519–530

Zársky V, Tachezy J, Dolezal P (2012) Tom40 is likely common to all mitochondria. Curr Biol 22:R479–R481

Zhang SY, Bryant D (2011) The tricarboxylic acid cycle in cyanobacteria. Science 334:1551–1553

Zhu G, LaGier MJ, Stejskal F, Millership JJ, Cai X, Keithly JS (2002) *Cryptosporidium parvum*: the first protist known to encode a putative polyketide synthase. Gene 298:79–89

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