Evolutionary significance of anaerobic energy metabolism in eukaryotes

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Endosymbiotic theory is traditionally founded in comparative physiology and biochemistry. That is also where it works best. Meserschkwsky’s first formulation of the endosymbiotic origin of plastids was based on the comparison of physiological attributes of plastids and cyanobacteria (Meserschkwsky 1905). The revival of endosymbiotic theory for the origin of mitochondria and chloroplasts by the late Lynn Margulis (Sagan 1967; Margulis 1970) was also very much based on comparative physiology, although her suggestion for an additional endosymbiont for the origin of flagella from spirochaetes was based on morphological similarity. When hydrogenosomes were discovered by Müller in 1973 (Lindmark and Müller 1973), they looked much more like chlorotidia than mitochondria from the standpoint of comparative biochemistry, but that is mainly because in 1973 the concept of mitochondria was built around investigations on rat liver mitochondria, a strictly aerobically functioning organelle, and in addition very little was known about the mitochondria of anaerobic eukaryotes. As advances have accrued in the understanding of mitochondria of anaerobic eukaryotes, the smaller have become the biochemical differences between mitochondria and hydrogenosomes (Müller et al. 2012). Today it is clear that they share a common ancestry and that they represent related biochemical manifestations of one and the same endosymbiotically derived organelle.

Introduction

The role of the Euglena gracilis mitochondrion as a biochemical missing link between typical mitochondria and hydrogenosomes, has been studied in recent years using the example of wax ester fermentation. While wax ester fermentation in the facultatively anaerobic mitochondrion of Euglena gracilis harbours undiminished general biochemi-
working hypothesis that these enzymes were present in the eukaryote common ancestor.

An aerobic energy metabolism of Euglena

Under aerobic conditions, Euglena expresses PDH in mito-

chondria (Hoffmeister et al. 2004) and respires O₂, but

using a slightly modified Krebs cycle that involves replace-

ment of α-ketoglutarate dehydrogenase by α-ketoglutarate
decarboxylase and succinate semialdehyde dehydrogenase
(Buetow 1989), as is also found among some α-

proteobacteria (Green et al. 2000) and cyanobacteria
(Zhang and Bryant 2011). Under anaerobic conditions,
Euglena uses acetyl-CoA as the terminal electron acceptor
and produces waxes as end products of metabolism
(Inui et al. 1982; Buetow 1989; Tucci et al. 2010), some-
times at levels up to 57% of dry weight (Tucci et al. 2010).

In general principle, Euglena’s wax-ester fermentation
is similar to the synthesis of branched short-chain fatty
acids in Ascaris or butyrate in Dasystricha (Müller et al.
2012), where fatty acids are synthesized from acetyl-CoA
condensation with an acyl-CoA (starting with acetyl-CoA or
propionyl-CoA), reduction of the resulting 3-oxoacid to the
3-hydroxy acid, dehydration thereof and reduction of the
resulting trans-2-enoyl-CoA to the elongated acyl-CoA (Müller
et al. 2012). The acetyl-CoA dependent Euglena route al-

ows net fermentative ATP synthesis from glucose, because
acetyl-CoA is condensed without prior ATP-dependent
carboxylation to malonyl-CoA (Inui et al. 1982; Schneider
and Betz 1985). The step catalyzed by trans-2-enoyl-CoA
reductase (NADPH-dependent) circumvents the reversal of
an O₂-dependent step in β-oxidation (Hoffmeister et al.
2005). A portion of the fatty acids is reduced to alcohols,
esterified with another fatty acid and deposited in the cyto-
sol as wax (wax ester fermentation). The fatty acyl-CoA
reductase and the wax synthase involved in the synthesis of
Euglena’s medium-chain wax aliphatic side chains were
recently characterized (Teerawanichpan and Qui 2010). When
oxygen again becomes available, the stored waxes are degrada-
ted via aerobic dissimilation in the mitochondri-
on (Inui et al. 1982).

Euglena’s wax ester fermentation involves mitochon-
drial fumarate reduction, and thus utilizes RQ (Hoffmeister
et al. 2004) for the synthesis of propionyl-CoA, similar to anaerobic mitochondria of metazoa (Müller et al. 2012),
although reduced RQ can also donate electrons to other components of the Euglena mitochondrial respiratory chain
(Castro-Guerrero et al. 2005), including the cytochrome bc₁
complex and the alternative oxidase (Castro-Guerrero et al.
2005). Propionyl-CoA is the starter for the synthesis of fatty
acids of odd-number chain length, 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 methyl-
malonyl-CoA route as is found in animal mitochondria
(Schneider and Betz 1985) and homologues for the under-
lying enzymes, methylmalonyl-CoA mutase and propionyl-
CoA carboxylase (that provides ATP via substrate-level
phosphorylation) are abundantly expressed in euglenid EST
data (Ahmadinejad et al. 2007). Methylmalonyl-CoA
mutase from Euglena was recently characterized (Miya-
 moto et al. 2010); the enzyme is also present in humans
where it is one of our only two vitamin B₁₂- (cobalamín-)
dependent enzymes (Roth et al. 1996), whereby Euglena
was once the standard assay for serum B₁₂ levels, because
of its B₁₂-dependent ribonucleotide reductase (Torrents et
al. 2006).

Under anaerobic conditions Euglena expresses py-
ruvate:NAD⁺ oxidoreductase (PNO) (Inui et al. 1985, 1987,
1991), which performs oxidative decarboxylation of py-
ruvate and is a fusion protein, with an N-terminal PFO
domain fused to a C-terminal flavoprotein domain with NAD⁺,
FMN, and P450-binding modules (Rotte et al. 2001;
Nakazawa et al. 2003). The flavoprotein domain is found in
many other proteins, sometimes alone as in the case of
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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 in Euglena and was
long thought to be unique to the Euglena lineage, but the
same PNO (PFO fusion) is found in the apicomplexan Crypt-
osporidium (Rotte et al. 2001) as well as in Blastocystis
(Lantsman et al. 2008). More recently, PNO homologues are
turning up abundantly in EST sequencing projects from
many disparate eukaryotic lineages (Hug et al. 2010; Atteia
et al. 2012). The accumulation of wax esters with even-
numbered chains in flufenacet-treated Euglena cultures and
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Trichomonas hydrogenosomes

Euglena is a thankful system from the standpoint of protein
biochemistry, because one can isolate proteins from kilo-
grams of cell material (Hoffmeister et al. 2005). But Euge-

Extracellular hydrogenosomes possess several compo-
ments of the mitochondrial protein targeting machinery and import across the two hydrogenosomal membranes is thought to entail components homologous to TOMs and TIMs (translocases of the outer and inner mitochondrial membrane, respectively) of mitochondrial membranes (Carlton et al. 2007; Dolezal et al. 2006; Shiflett and Johnson 2010; Rada et al. 2011). But there are clear differ-
ences and clues that the import machinery in T. vaginalis is
reduced relative to yeast, because Tom70, Tom20 and
Tom22 as well as Tim54, Tim50 and Tim21 appear to be
lacking (Shiflett and Johnson 2010; Rada et al. 2011).
Trichomonas hydrogenosomes lack cytochromes (Müller
1993) but seem to generate an electrochemical gradient
across the inner membrane (Vilela et al. 2010), which is
instrumental in protein import into yeast mitochondria
(Dolezal et al. 2005; Neupert and Herrmann 2007). Protein
import into hydrogenosomes is ATP-dependent (Bradley
et al. 1997) and it is possible, but has not been demonstrated,
that the generation of the membrane potential is as well.

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(Aurrecoechea et al. 2009). Hydrogenosome import on the order of 250 (Henze 2008) to 500 proteins, about half of which possess a short N-terminal extension (Carlton et al. 2007; Schneider et al. 2011; Burstein et al. 2012; Dyall and Johnson 2000). This extension is cleaved by the hydrogenosomal processing peptidase, a reduced homolog of the yeast mitochondrial processing peptidase (Brown et al. 2007; Smid et al. 2008).

Mentel et al. (2008) found that protein import into Trichomonas hydrogenosomes is not strictly dependent upon cleavable N-terminal extensions, but that internal signals contained within the import substrates, sufficient to direct the protein to the organelle. Two hydrogenosomal proteins with known cleavage sites for their N-terminal presequences, the α-subunit of succinyl-CoA synthase (SCSα) and pyruvate-ferredoxin oxidoreductase (PFOR), were investigated with respect to the ability of their presequences to target thioredoxin reductase to hydrogenosomes in transformed Trichomonas. Neither presequence directed TrxRh1 to hydrogenosomes, indicating that neither extension is, by itself, sufficient for hydrogenosomal targeting. Moreover, SCSα lacking its N-terminal extension was efficiently imported into hydrogenosomes, indicating that this extension is not required for import of this major hydrogenosomal protein. The finding that some hydrogenosomal enzymes require N-terminal signals for import, but that in others the N-terminal extension is not necessary for targeting, indicates the presence of (additional) targeting signals within the mature subunits of several hydrogenosome-localized proteins (Mentel et al. 2008).

The homologous succinyl-CoA synthase promoter works well in Trichomonas as does the dihemagglutinin tag (HA tag), which can be added in frame to constructs prepared for electroporation of T. vaginalis T1 cells, transformants of which can be selected on standard media containing 100 µg/ml of G418 (Mentel et al. 2008). This technique has permitted study of localization for several hydrogenosomal proteins to date. Three examples include the protein HydG and HydF, involved in [Fe]-hydrogenase maturation (Pütz et al. 2006), ruberythrin (Pütz et al. 2005), and thioredoxin reductase (Mentel et al. 2008). Good positive controls are also available using antibodies against native acetate:succinate CoA transferase (ASCT), which was recently characterized as the last remaining major protein of core carbon and energy metabolism in trichomonal hydrogenosomes (van Grinsven et al. 2008).

**Strain differences**

One important aspect that has recently emerged from investigations of eukaryotic anaerobes is that there is significant variability among strains of the same species with regard to the type and amounts of the metabolic end products accumulated. This has been shown for Euglena (Tucci et al. 2010) and for Trichomonas vaginalis (Rasoloson et al. 2002). The variability among different Euglena strains with respect to anaerobic growth and wax ester fermentation is substantial. Eight new strains of Euglena gracilis from the Göttingen collection in addition to two bleached mutants generated with standard ofloxacin and streptomycin treatment were examined for their capacity to grow anaerobically. These two mutants generated with standard ofloxacin and streptomycin treatment were examined for their capacity to grow anaerobically. The effect of phenacetin (Trenkamp et al. 2004) on wax ester accumulation, and the partitioning of carbon species (length and saturation) within the wax ester fraction using GC-MS (Tucci et al. 2010). Substantial differences even with respect to the ability to grow anaerobically were observed, as three of the Göttingen strains (1224-5/10, 1224-/15, and 1224-/27) did not undergo either increases in cell number or increases in biomass over 11 days of culturing, while other strains, such as 1224-/13, produced threefold the amounts of wax esters in comparison to “Z” (1224-5/25), amounting to a wax ester content of >30% dry weight in some cases (note: the dry cell mass of Euglena gracilis 1224-5/13 burns very nicely indeed, but emitting the scent of burned protein (mainly pellicula), of course. The specific composition of the fatty alcohol and fatty acid (methyl esters) constituents was determined for all of the wax ester fractions, phenacetin inhibited the accumulation of odd numbered chains (methylmalonyl-CoA derived propionyl-CoA starter for mitochondrial synthesis).

**The broader significance**

Some anaerobic eukaryotic microorganisms inhabit environments that are also sulfidic. Mitochondrial sulfide oxidation in animals that inhabit anaerobic and sulfidic environments has also been studied (Theissen and Martin 2008a, 2008b). The evolutionary analysis of the Chlamydomonas mitochondrial proteome suggested that the ancestor of mitochondria had a collection of genes that was more similar to that possessed by generalist α-proteobacteria such as Rhodobacter than to specialists such as the rickettsias (Atelea et al. 2008). In the larger context of things, the widespread occurrence of anaerobic mitochondria and the enzymes of aerobic energy metabolism in eukaryotes makes sense in the context of the new model of Prorozoezoic ocean chemistry that has emerged over the last 10 years in the field of geochemistry (Mentel and Martin 2008; Müller et al. 2012). This is an interesting development, because it is rare that major and independent conceptual revolutions in two separate scientific fields converge at the same answers. But in recent years have witnessed major upheavals in views about early eukaryotic evolution and the role of anaerobes therein, developments that have been paralleled by a similar upheaval in the Earth sciences regarding views about the prevalence of oxygen in the oceans during the Proterozoic eon (the time from about 2.5 to 0.57 billion years ago). New views of the Proterozoic ocean chemistry indicate that the oceans were anoxic and sulfidic during most of the Proterozoic eon ("Canfield eon") and only became oxic about 580 million years ago, at the time when the first macroscopic animal forms appear in the fossil record. New views of eukaryote evolution have it that anaerobes are not re-
stricted to primitive or early-branching lineages, but are distributed throughout the eukaryote tree of life. Because the widespread occurrence of hydrogenosomes and mitosomes among eukaryotic lineages and their evolutionary identity with mitochondria, the possession of mitochondria is no longer synonymous with an oxygen-dependent lifestyle. Eukaryotic anaerobes have mitochondria and many use them for ATP synthesis in the absence of oxygen. Once a puzzle, this is now readily understood in the new geochemical light of our comparatively recent anaerobic past (Mentel and Martin 2008; Müller et al. 2012). Taken together, these independent but mutually compatible developments enrich views of eukaryote ecology and evolution in the context of Earth history.

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References


