Evolution of peroxisomes illustrates symbiogenesis

Dave Speijer

Recently, the group of McBride reported a stunning observation regarding peroxisome biogenesis: newly born peroxisomes are hybrids of mitochondrial and ER-derived pre-peroxisomes. What was stunning? Studies performed with the yeast \textit{Saccharomyces cerevisiae} had convincingly shown that peroxisomes are ER-derived, without indications for mitochondrial involvement. However, the recent finding using fibroblasts dovetails nicely with a mechanism inferred to be driving the eukaryotic invention of peroxisomes: reduction of mitochondrial reactive oxygen species (ROS) generation associated with fatty acid (FA) oxidation. This not only explains the mitochondrial involvement, but also its apparent absence in yeast. The latest results allow a reconstruction of the evolution of the yeast's highly derived metabolism and its limitations as a model organism in this instance. As I review here, peroxisomes are eukaryotic inventions reflecting mutual host endosymbiont adaptations: this is predicted by symbiogenetic theory, which states that the defining eukaryotic characteristics evolved as a result of mutual adaptations of two merging prokaryotes.

See also the video abstract here: https://youtu.be/HtyKhQ3DSxg

Keywords: beta-oxidation; FADH$_2$/NADH ratio; peroxisome evolution; reverse electron transport (RET); symbiogenesis

Introduction

When one contemplates biological processes that seem hard to understand, sometimes superficially unrelated biological questions turn out to be related at a deeper level. An example of two such questions might be found in the following: why do our mitochondria break down small, medium and long chain fatty acids (FAs), but are very long chain FAs (VLCFAs; >22C atoms) exclusively catabolized by peroxisomes, universal eukaryotic organelles with diverse metabolic functions of which FA (beta) oxidation seems the most ancient [1]? Why do neurons not use beta oxidation for energy generation, even though they are major energy consuming cells and FAs contain more than twice the amount of energy per gram as compared to lactate/glucose, which are used instead? The available answers (VLCFAs are just too long for mitochondria; FAs cannot pass the blood–brain barrier, neurons have very low activities of essential beta oxidation enzymes, the 3-ketoacyl-CoA thiolase (KAT) [2] and carnitine acyltransferases, with KAT activities being rate-limiting [3]) turn out to be untrue or unconvincing. Mitochondria have separate chain-length specific (overall: long, medium, and small) acyl-CoA dehydrogenases (ACADs), enoyl-CoA hydratases (EHs), 3-hydroxyacyl-CoA dehydrogenases (3HADs), and KATs for sequential, cyclic, FA breakdown ending with acetyl-CoA. Nobody ever gives a physical reason why, suddenly, VLCFAs have to be broken down somewhere else. Interestingly, the peroxisomal system needs only one type of each enzyme to go from >C22 to ~C8, but now peroxisomes cannot degrade FAs to completion, releasing them either as small FAs or as carnitine esters, which can be directly broken down further in mitochondria. Again, no physical explanation for the strange cellular distribution of essentially the same process is given (see e.g. [4]). Efficient transport of FAs over the blood–brain barrier was already demonstrated with $^{13}$C labelled FAs in 1988 [5], and has been consistently observed ever since, though the mechanism(s) involved are still debated (see e.g. [6] and [7]). Lastly, the neuronal KAT indeed has very low activity, but this regulation has been established during evolution and might easily be changed, leaving the question: “what is the selective advantage of not using FA oxidation for energy generation in neurons?” unanswered.
However, one can make sense of the superficially disparate observations mentioned above if one thinks about the kinetics of the mitochondrial respiratory chain during the oxidation of different substrates. During normal breakdown of glucose or lactate the respiratory chain oxidizes 1 FADH2 for 5 NADH molecules (a FADH2/NADH or F/N ratio of 0.2), while catabolism of FAs tends toward 1 FADH2 for 2 NADH molecules (a ratio of 0.5; the longer the FA, the higher the ratio). This would give rise to a situation in which, in the absence of further adaptations, complex I (NADH:ubiquinone oxidoreductase) would encounter a much higher level of ubiquinol (QH2), the reduced form of its acceptor ubiquinone (Q) during FA (beta) oxidation than e.g. during glucose use (reflected in a high QH2/Q ratio). Hence, reverse electron transport (RET; which depends on concomitant high membrane potentials (Δp) and QH2/Q ratios) would occur much more frequently during beta oxidation. The resulting ROS formation by complex I would lead to severe oxidative stress inside the newly merged pre-eukaryote [8]. This model (schematically depicted in Fig. 1) explains the two “unrelated” biological phenomena. Outsourcing part of beta oxidation to a new cellular entity, generating NADH, in the “absence” of FADH2 would thus be selected for. This is exactly what occurs in peroxisomes. Peroxisomes thus evolved to lessen the amount of beta oxidation in the mitochondria, reducing the overall F/N ratio [8–10]. The recent finding that new peroxisomes can be hybrids of mitochondrial and ER-derived pre-peroxisomes is the last in a series of outcomes that seems to show this part of the model to be correct [11, 12]; see also below.

Neurons cannot use a “high-ROS” substrate like FA because neuronal networks contain the irreplaceable information of memory (the plethora of highly specific interconnections between neurons) and should last a lifetime. This is a crucial difference with other major energy consuming cells, such as muscle cells, which can (to a certain extent) be functionally replaced upon dying from oxidative damage, because they do not contain positional information that is lost upon death. A good example can be found in cardiomyocytes, which preferentially indeed use long-chain FAs as their energy source and contain large amounts of mitochondria [13]. The large majority of these cells is not able to regenerate, but a small population of “hypoxic” cardiomyocytes (those that have stabilized the hypoxia-inducible factor 1 alpha subunit) can contribute to new cardiomyocyte formation for ongoing repair of the adult heart [14, 15]. There is a parallel here with the elusive neural stem cell that allows neuronal regeneration [16]. All this is of little use after massive damage upon sudden large-scale hypoxia and this holds true for both brain and heart. One crucial difference remains, however, while the low level repair of the heart can be complete, the death of neurons will always lead to “informational” loss.

**Figure 1.** ROS formation in Complex I due to high QH2 levels during beta oxidation or use of the glycerol-3-phosphate shuttle. A: Glucose oxidation (low F/N ratio) with adequate electron-acceptor (Q) for Complex I. B: High F/N ratios with insufficient electron-acceptor (Q) for Complex I; ROS formation at Complex I via RET (•). RET depends on high QH2 levels and a high Δp (indicated). C: ROS reduction by either less FA oxidation (e.g. use of peroxisomes) or enhanced QH2 oxidation (e.g. more uncoupling or ATP formation). IMS = intermembrane space; Complex I (not to scale; based on [72]) purple, Complex II light green, ETF-complex dark green, glycerol-3-phosphate dehydrogenase pink, ubiquinone (Q) red; electron flow black arrows. The specific ROS generating site of complex I upon RET is still debated: The flavin containing site (ln) [73] or the Q binding site (lQ) [74]. F = FADH2 oxidizing complex, N = NADH oxidizing complex. For details see text. Extended and adapted from [10].

**Did peroxisomes indeed evolve to suppress mitochondrial ROS formation?**

Since the proposal that peroxisomes evolved as a method to reduce mitochondrial F/N ratios was made, many new findings (in the fields of evolutionary genomics, eukaryotic metabolism, peroxisome formation, and the evolution of the eukaryotic endomembrane system) have not only supported the model and its relevance for peroxisomal evolution, but allow us to reconstruct the birth of peroxisomes in the context of the mutual adaptation of archaeal host and bacterial endosymbiont. Here, I will discuss these new discoveries and show that the detailed reconstruction of the path to peroxisomes they allow, fully support symbiogenesis: the model in which most, if not all, eukaryotic inventions can be understood in the light of necessary mutual prokaryotic adaptations. Peroxisomes, which were once even seen as remnants of a very old pre-mitochondrial endosymbiosis, now seem to make up one of the strongest cases for the symbiogenetic model.

The claim that peroxisomes evolved to suppress the overall F/N ratio might, at first sight, seem a bit hasty. Why not just allow all FA oxidation to migrate to peroxisomes if this process leads to an increase in endogenous mitochondrial ROS formation? One should not forget that the FADH2 formed during peroxisomal FA oxidation (by the universal “eukaryotic” acyl-CoA oxidase, ACOX, replacing an ACAD enzyme) does not contribute to the Δp of the mitochondrial inner membrane anymore – the electrons ending up in H2O instead of H2O2 – and thus ATP is lost, while catalase is needed to get...
The specific peroxisomal breakdown of VLCFAs makes perfect sense: using only these (which have the highest F/N ratios) the cell obtains a relatively strong reduction of the overall F/N ratio at minimal ATP loss [8]. When this kinetic theory of mitochondrial ROS formation was published, it was already known that two of the four recurring steps of peroxisomal FA oxidation were catalyzed by a single bi-functional enzyme (Pox2p; yeast nomenclature), of alpha-proteobacterial (mitochondrial) origin, making the conclusion almost inescapable that peroxisomes indeed evolved after uptake of the endosymbiont [1]. Also the many links between peroxisomes and mitochondria were already becoming ever more apparent, see e.g. [17]. Above, I mentioned that beta oxidation seems to be the oldest metabolic function of peroxisomes. Recent genomes becoming available have strengthened this perception, with many more specialized, mostly oxidative, functions coming in later, as illustrated by box 1 of a recent review [18], which shows only beta oxidation and the associated catalase function to be universally present. However, in addition to the enzymes involved in beta oxidation, e.g. peroxisomal (as based on the presence of targeting signals) urate oxidase has been detected in many metazoans, plants and fungi (though protozoan associations are somewhat less easily found). In general, H2O2 generating enzymes, such as D-amino acid oxidase, have been localized to the peroxisome. The question how many of these already belonged to the LECA peroxisomal proteome, apart from the beta oxidation enzymes, awaits further phylogenetic analyses.

Reconstructing steps leading to peroxisomes

Thus, peroxisomes evolved after (and most likely in response to) alpha-proteobacterial uptake. This does not mean we can reconstruct how this occurred in great detail. In the original formulation of the hypothesis I speculated about the combination of catalase containing secretory vesicles of the host with positive selection of low levels of omnipresent mislocalization at the start of peroxisome evolution [8]. However, the surprising (universal?) presence of the ACAD-11 protein of unknown function in peroxisomes [19–21] might indicate a different scenario. The protein is of alpha-bacterial origin and most likely came along with the pre-mitochondrial bacterium. The different scenario is partly based on the exciting recent proposal that the complete eukaryotic endomembrane system is derived from the outer membrane vesicles (OMVs) that the pre-mitochondrion started shedding into the cell, nicely explaining the total replacement of the archaeal isoprene-based membrane lipids by the bacterial FA-based ones as well [22]. Such vesicles might on occasion contain functional proteins, especially if they associate with membranes. Most proteins that form part of the same metabolic pathway have physical interactions (they are part of modules, as e.g. shown in yeast [23]). Thus OMV’s containing all the (few) enzymes necessary for FA oxidation (though they of course are normally present in the matrix) might appear. However, they would be both highly inefficient, as there is no regular electron-acceptor for the ACAD reaction present, and dangerous, as ROS would easily be produced in the absence of such an acceptor. Merging such vesicles with the secretory vesicles of the host containing catalase would partially mend the second problem, but an efficient reaction would only be achieved upon the arrival of ACOX. Possibly, the peroxisomal ACAD-11 protein retained a modulating function in the process (perhaps enhanced by the amino glycoside phosphotransferase domain it gained early on), as speculated upon in [21]. OMV’s containing metabolic modules could also have been at the base of lysosome and ER evolution. Of note, this does not mean that metabolic pathways are easily duplicated or relocated in cells during evolution (see [24] and references therein). In this hypothetical scenario, gene migration to the nucleus, which occurs much more easily, and organelle targeting would stabilize the situation in the evolving eukaryote.

How do other recent findings stack up?

Since the original proposal of the kinetic theory of mitochondrial ROS formation it has been both strengthened by new findings and found to have bigger explanatory powers [9]. Internal ROS formation could help explain several eukaryotic inventions, such as uncoupling proteins, the carnitine shuttle for mitochondrial FA import, mitochondrial genome reduction and meiotic sex [25, 26]. Uncoupling lowers the Δp, which is required to be high for RET and subsequent ROS formation by complex I to occur (see Fig. 1). Let us look at the regulation of UCP2 protein as an example. UCP2 is regulated at transcrip- tional and (post)translational levels. It is positively regulated by FAs and by PPAR transcription factors (which signal the presence of FAs), QH2, ROS (at multiple levels, including direct activation); see [10] and references therein. All this makes perfect sense if beta oxidation indeed leads to enhanced internal ROS formation. Interestingly, UCP2 and UCP3 transcription is also triggered in target cells upon activation by the thyroid T3 hormone [27]. Here the link does not seem to be in increased FA oxidation, but in activation of the Glycerol-3-Phosphate Dehydrogenase by T3 [28]. Thus, the link between uncoupling and high F/N (QH2/Q) ratios is retained independent of the specific FADH2 utilizing complex (see Fig. 1C).

A rash of recent experimental results have not only demonstrated ROS production by complex I upon RET due to high F/N (QH2/Q) ratios, but also its physiological importance in metabolic adaptations and in the choice of cellular developmental programs. To name but a few: Enriquez and colleagues show that Q oxidation capacity cannot keep up during a switch to FA oxidation, resulting in RET to complex I in their mice fibroblast cell lines. They next demonstrate that the ensuing ROS oxidizes specific complex I subunits, which are degraded. This leads to disintegration of complex I and reconfiguration of supercomplexes (freeing up complex III), a process that increases electron flux via FADH2. All in all, Q redox status acts as a metabolic sensor to adjust the electron transport chain to the high F/N ratio associated with beta oxidation [29]. Mills et al. describe the involvement of F/N (QH2/Q) ratios in macrophage activation [30]. Upon lipopoly-saccharide stimulation, macrophages shift to glycolysis but also increase succinate levels. Increased complex II activity in
combination with elevated Δp then leads to ROS production, in accordance with the model. Controls include: inhibiting succinate oxidation with dimethyl malonate, blocking ROS production with rotenone (a complex I inhibitor), or expressing an alternative oxidase to oxidize QH₂. As expected, they all abolish the pro-inflammatory phenotype [30]. Surprisingly, in a Drosophila brain study, RET induced ROS formation by Complex I could delay ageing [31], by activating a range of eukaryotic anti-oxidant measures. Naïve interpretations of such observations have led the mitochondrial ROS theory of ageing to be criticized, but it seems to be basically correct [32]. So this specific mitochondrial ROS formation mechanism is indeed active, highly important, and often repurposed as a signalling mechanism. Are there further findings in accord with its predicted role in the evolution of peroxisomes?

**The evolution of peroxisomes only makes sense in the presence of mitochondria**

Recent discoveries with regard to the involvement of a mitochondrial F/N reduction mechanism in the evolution of peroxisomes have been supportive of the model. First of all of course absolutely predicts that peroxisomes evolved later than, and in response to, the entry of the pre-mitochondrion. Bolte et al. performed phylogenetic analyses of the peroxisomal beta-oxidation enzymes and found strong support for mitochondria-first models [33]. Only one of the enzymes involved in peroxisomal FA oxidation does not seem to be directly derived from the endosymbiont (not surprisingly, the H₂O₂ producing ACOX), the provenance of which remains unclear. It is best understood as a eukaryotic invention driven by mitochondrial F/N reduction and the replacement of the archaeal membrane by the alphaproteobacterial one, a development that increased cellular levels of FAs [22, 33]. Tabak et al. had already shown that, in yeast, peroxisomes are derived from the ER (see e.g. [34, 35]), making it highly unlikely that they are endosymbiont remnants. Direct contact between peroxisomes and mitochondria was detected in HeLa cells, with cargo-selected transport from mitochondria to peroxisomes [36]. The peroxisomal protein import machinery was shown to be related to the ER-associated degradation (ERAD) system [1, 37–39], and, more recently, a related mitochondrion-associated degradation system (MAD) has been shown to exist [40]. Also a protein (Vms1) was identified, linking mitochondrial quality control to Cdc48/VCP/p97, a ubiquitin/proteasome system component of ERAD [41]. It was known that inhibition of the hsp90/proteasome quality control system has direct effects on mitochondria as well [42]. Work by Schrader et al. shows that mitochondria and peroxisomes also share many fission machinery components [43, 44]. Contacts between mitochondrial derived vesicles and peroxisomes are getting more and more attention [36, 45]. However, though such contacts are proof for metabolic cooperation, they are only consistent with the model to explain peroxisomal evolution presented in [8], that is discussed here. Peroxisomes are of course completely wired into the metabolism of present-day eukaryotes: their most abundant contacts (~80% in HepG2 cells [46]) are with the ER and even lysosome contacts, important for intracellular cholesterol transport, have been observed [47].

Can these observations linking growth and maintenance of mitochondria, peroxisomes and ER be understood? Actually they make sense in the light of symbiogenesis [48, 49] and the new proposal that the eukaryotic endomembrane system is derived from OMVs that the pre-mitochondrion secretes into the cell [22], referred to above. Using and modifying available archaenal secretion/translocation systems to target this evolving endomembrane system, illustrates the evolutionary parsimony principle (recycling mechanisms and components present over and over again to meet new physiological challenges). The shared characteristics of the peroxisomal protein import machinery and the ERAD machinery, just mentioned, constitutes an example. Interestingly, the original selective force proposed in [8], managing ROS formation associated with beta-oxidation by removing (part of) it to peroxisomes, seems to have blossomed into a full scale homeostatic mechanism in which a large part of the cellular ROS load is dealt with by a shared contribution of peroxisomes and mitochondria; see [50] and references therein.

**Recent findings: Mitochondrial membranes are involved in biogenesis of peroxisomes**

Last, but certainly not least, the group of McBride just published their surprising finding: In human fibroblasts without peroxisomes, peroxisomal import receptors Pex3 and Pex14 end up in mitochondria to be released in pre-peroxisomal vesicles next. These vesicles fuse with Pex16 containing ER-derived vesicles to give rise to de novo peroxisomes (as defined by import of peroxisomal proteins) [11]. In the “News & Views” accompanying the article, Hettema and Gould pinpoint differences between de novo peroxisome formation in mammalian cells and yeast [12]. While the mammalian mechanism needs PEX16, the yeast S. cerevisiae lacks this gene, which can be seen as another instance of the substantial genome reduction of this model organism. More importantly, during pre-peroxisome formation in yeast, Pex3 seems to transit through the ER, and not through mitochondria, as now shown for human fibroblasts. So why did this hybrid origin of peroxisomes not show up in yeast experiments; see [37] and references therein? The highly specific, and often forgotten, derived metabolic nature of several yeasts might contain the answer. S. cerevisiae has discarded complex I and relocated all FA oxidation to its peroxisomes, thus severing the ancient functional link to mitochondria that, I argue, gave rise to peroxisomes in the first place; see [10] and references therein. For a more detailed explanation of the model possibly explaining the difference between yeast cells and fibroblasts, see Box 1.
**Box 1**

**Yeast as a model system: How about studying peroxisomes?**

**Yeast as a model system**

*S. cerevisiae* (Baker’s yeast) has been used as a eukaryotic model system because of its ease of cultivation, relatively small eukaryotic genome, easy and cheap genetic manipulation options and because of a crucial chance fact: its economic importance meant it was studied ahead of other single celled eukaryotes. Overall it has turned out to be an inspired choice. Yeast seems to be a good model system for animals in general [63]. It was important for five Nobel prizes up till now: cell cycle study/regulation (2001), eukaryotic transcription (2006), telomerase action (2009), vesicular transport (2013), and autophagy (2016). It allowed the demonstration of protein modularity in the cell machinery [23]. It has also been used to study aging, metabolism, apoptosis and even neurodegenerative diseases; see [63] and references therein. It was instrumental in showing that peroxisomes can be formed de novo from ER membranes [34, 35, 37]. However, the indications for a mitochondrial connection recently demonstrated in human fibroblasts by the group of McBride [11] have not been observed in yeast. How can this be? To try to understand this apparent contradiction, we might focus on the (highly derived) metabolism of this organism.

**Yeast metabolism: Idiosyncrasies**

Let us first note important differences between yeast and a “generic” animal cell. Yeast prefers anaerobic glycolysis to aerobic processes. It has replaced the normal proton pumping complex I by a non-pumping single subunit enzyme and relocated all FA oxidation to its peroxisomes; see [10] and references therein. Yeast ATPase has 10 c subunits, while its “generic” animal counterpart has 8. All of these changes mean less energy from oxidative processes. Complex I does not contribute to the proton motive force (PMF) anymore and FA oxidation in peroxisomes allows the energy of its first oxidative step to end up in H$_2$O$_2$, instead of contributing to the PMF [8, 10]. Last but not least, the yeast ATPase must use 10 protons to allow a full (360°) rotary movement resulting in the formation of 3 ATP, instead of 8 protons, which constitutes a 20% reduced efficiency. However, the enzyme is predicted to be able to synthesize ATP at a lower PMF. Real-time ATP synthesis experiments in proteoliposomes comparing a bacterial enzyme with either 11 or 12 c subunits showed that the larger c(12) ATPase is indeed functional at lower PMF [64].

**Using other “yeasts”?!**

The methylotrophic yeasts Hansenula polymorpha and Pichia pastoris both lack mitochondrial beta oxidation and their Pex3 homologs are also sorted to the peroxisome via the endoplasmic reticulum [69–71]. A yeast that has retained the complementing FA oxidation pathways in peroxisomes and mitochondria is Ustilago maydis. Studies with a pexisome-deficient Δpex3 mutant have been performed [21], but no studies have been done reintroducing the pex3 protein so far. Will it visit mitochondria first?
A non-phagocytotic merger at the basis of symbiogenesis

Upon uptake of the bacterium by a non-phagocytotic mechanism, the new cellular entity entered a phase characterized by enhanced internal ROS formation. Hence, migration of bacterial genes to the host genome and retargeting of encoded products to the bacterium was selected for; see [51] and references therein. Relocating part of FA oxidation to the OMVs was clearly selected for as well. Thus, both driving force and physical incarnation of the peroxisomes seem to stem from bacterial entry. A possible reconstruction of the eukaryotic invention of peroxisomes in the light of these driving forces is shown in Fig. 2.

I should explain why I favor a non-phagocytotic mechanism for the original uptake event. Recent studies with reconstituted archaeal genomes [52, 53] have allowed the discovery of new phyla of Archaea. These are more closely related to the host in the merger of archaeon and bacterium at the basis of the eukaryotic tree than ever found before. The composite genomes contain new “eukaryote-specific” genes, some of which resembling ones involved in membrane-trafficking and vesicle biogenesis (with implications for the possibility of phagocytosis). Indeed in [53] it is speculated that “...these findings may suggest the ability of simple phagocytic capabilities in the archaeal host...”. I originally thought such phagocytic capabilities not unlikely [54], but actually there are many arguments against it, see e.g. [22, 26]. Though the archaeal host thus already seems to have had some important components for later phagocytosis, the process is still missing many key pieces that have to come from the bacterial endosymbiont and others that we have to classify as “eukaryotic inventions”. There are no indications that the archaea in question do not have an ATP generating membrane potential anymore, which seems unlikely to coexist with a phagocytic capability [24]. Then there is the question of size and cost: a cell that, to all intents and purposes, is still an archaeon taking up a bacterium with a machinery that is beyond its energetic means in operation and maintenance (the advent of mitochondrial ATP generation will allow this to evolve later on). Only the original two bacterial membranes are present (as is the case for chloroplasts), while later (secondary) eukaryotic acquisitions often show membrane traces of phagocytotic uptake [55–57], implying that only the “uptake membrane” would have disappeared in this instance. Such membranes were most likely never there: phagocytic uptake of the alpha-proteobacterium destined to become the mitochondrion is a classic example of putting the cart before the horse. Just recently, a very thorough analysis of all the problems associated with “pre-mitochondrial phagocytosis” appeared, which seems to settle the issue [58].

How strong are alternative models for the evolution of peroxisomes?

The last gasp of the old theories describing peroxisomes as derived from another endosymbiont with a more ancient, and less efficient, form of FA oxidation, probably came with the idea that peroxisomes are of actinobacterial origin [59]. Many findings described earlier showed them to be real eukaryotic inventions instead, while beta oxidation – their oldest pathway – came from the endosymbiont, meaning peroxisomes had to appear after alpha-proteobacterial uptake. Clearly there has been less agreement with regard to driving forces in the meantime. A popular idea is that VLCFAs are “just too long” for the mitochondrial oxidation system. But mitochondria have several strongly homologous enzymes specializing on breakdown of different classes of (small, medium, and long chain) FAs and there is no hint of an explanation why this class would pose specific problems (see above).

After contributing to the important insight that a significant fraction (~20%), of the peroxisomal proteome is of alpha-proteobacterial origin [1], Gabaldón also published an alternative hypothesis for the driving forces involved in peroxisome evolution in 2014. Here ROS formation in the ER (!)
during FA metabolism (stressing anabolism, such as the synthesis of poly-unsaturated FAs) is considered to be the main force [60]. In his model, peroxisomes can be present before endosymbiont entry, a possibility even explicitly mentioned. Gabaldón is also rather vague regarding the precise mechanics of ROS formation in the ER. In contrast, the model he wants to improve upon has a specific mitochondrial mechanism (RET) and unequivocally predicts that peroxi-
somes evolved in response to entry of the pre-mitochon-
drion [8, 61]. In a more recent contribution Gabaldón and co-
workers try to accommodate some of the new findings. This
gave rise to e.g. the following observation: “...the bacterial-
derived component of peroxisomes was diminished in more
ancestral stages...”, which is still not easily reconciled with
peroxisomes evolving in response to pre-mitochondrial endosymbiont entry. On the whole, their reasoning seems
somewhat muddled and unconvincing; see [62].

Conclusions and outlook
Recent discoveries allow a logical reconstruction of peroxi-
somal evolution completely in line with symbiogenetic
predictions: peroxisomes are indeed eukaryotic inventions
that can only be understood as the result of complex, mutual,
prokaryotic adaptations. The symbiogenetic model looks
stronger than ever.

Acknowledgments
I thank Fred Meijer for directing me towards the “glycerol-3-
phosphate UCP” connection. I would also like to thank Bill
Martin and Sven Gould for some nice pointers to improve an
earlier version of the article. An unknown reviewer performed the
same function for a later version. As usual, the help of Andrew
Moore in improving the manuscript was much appreciated.

The author has declared no conflict of interest.

References
1. Gabaldon T, Snel B, van ZF, Hemrika W, et al., 2006. Origin and
evolution of the peroxisomal proteome. Biol Direct 1: 8.
2. Yang SY, He XY, Schulz H. 1987. Fatty acid oxidation in rat brain is
limited by the low activity of 3-ketoacyl-coenzyme A thiolase. J Biol Chem
262: 13027–32.
3. Bird MI, Munday LA, Saggerson ED, Clark JB. 1985. Carnitine
acyltransferase activities in rat brain mitochondria. Bimodal distribution,
kinetic constants, regulation by malonyl-CoA and developmental pattern.
Biochem J 226: 323–30.
4. Wangers RJ, Ruiter JP, IJlst L, Waterham HR, et al., 2010. The
enzymology of mitochondrial fatty acid beta-oxidation and its application
to follow-up analysis of positive neonatal screening results. J Inherit
Metab Dis 33: 473–94.
5. Spector R. 1988. Fatty acid transport through the blood-brain barrier.
J Neurochem 50: 639–43.
6. Hamilton JA, Brunaldi K. 2007. A model for fatty acid transport into
the brain. J Mol Neurosci 33: 12–7.
7. Dally S, Hieu Nguyen NO, Pogorzelc M, Miller DW, et al., 2014. The
blood-brain barrier – regulation of fatty acid and drug transport. In:
Heinbockel T, ed: Neurochemistry. Rijeka: InTech.
8. Speijer D. 2011. Oxygen radicals shaping evolution: why fatty acid
catabolism leads to peroxisomes while neurons do without it. BioEssays
33: 88–94.
9. Speijer D. 2014. How the mitochondrion was shaped by radical
differences in substrates: what carnitine shuttles and uncoupling tell
us about mitochondrial evolution in response to ROS. BioEssays 36: 632–33.
10. Speijer D. 2016. Being right on Q: shaping eukaryotic evolution. Biochem J
473: 4103–27.
11. Sugiuara A, Mattie S, Prudent J, McBride HM. 2017. Newly born
peroxisomes are a hybrid of mitochondrial and ER-derived pre-
peroxisomes. Nature 542: 251–4.
12. Hettema EH, Gould SJ. 2017. Cell biology: organelle formation from
scratch. Nature 542: 174–5.
13. Heier C, Haemmerle G. 2016. Fat in the heart; the enzymatic machinery
regulating cardiac triacylglycerol metabolism. Biochim Biophys Acta
1860: 1500–12.
14. Kimura W, Xiao F, Cansuco DC, Muralidhar S, et al., 2015. Hypoxia fate
mapping identifies cycling cardiomyocytes in the adult heart. Nature
523: 226–30.
15. Nakada Y, Cansuco DC, Ther S, Abdissaam S, et al., 2017. Hypoxia
induced heart regeneration in adult mice. Nature 541: 227–32.
16. Gage FH, Temple S. 2013. Neural stem cells: generating and
regenerating the brain. Neuron 80: 588–601.
17. Plomer S, Gronborg S, Gartner J. 2009. Organellar interplay in
peroxisomal disorders. Trends Mol Med 15: 293–302.
18. Speijer D. 2015. Peroxisomes take shape. Nat Rev Mol Cell Biol
14: 803–17.
19. Shen YQ, Lang BF, Burger G. 2009. Diversity and dispersal of a
ubiquitous protein family: acyl-CoA dehydrogenases. Nucleic Acids Res
37: 5619–31.
20. Sivigonova Z, Mohsen AW, Vockley J. 2009. Acyl-CoA dehydrogen-
as: dynamic history of protein family evolution. J Mol Evol 69:
176–93.
21. Cameos F, Islinger M, Guiamae SC, Kilaru S, et al., 2015. New
insights into the peroxisomal protein inventory: acyl-CoA oxides and
-dehydrogenases are an ancient feature of peroxisomes. Biochim
Biophys Acta 1853: 111–25.
22. Gould SB, Garg SG, Martin W. 2016. Bacterial vesicle secretion and the
evolutionary origin of the eukaryotic endomembrane system. Trends
Microbiol 24: 525–34.
23. Gavin AC, Aloy P, Grandi P, Krause R, et al., 2006. Proteome survey
reveals modularity of the yeast cell machinery. Nature 440: 631–6.
24. Speijer D. 2017. Alternating terminal electron-acceptors at the basis of
symbiogenesis: how oxygen ignited eukaryotic evolution. BioEssays
39: 133–47.
25. Speijer D, Lukses J, Elias M. 2015. Sex is a ubiquitous, ancient, and
inherent attribute of eukaryotic life. Proc Natl Acad Sci USA 112:
8827–34.
26. Speijer D. 2016. What can we infer about the origin of sex in early
eukaryotes? Philos Trans R Soc Lond B Biol Sci 371: 20150530.
27. Barbe P, Larrouy D, Boulanger C, Chevillotte E, et al., 2001.
Eukaryotic cells: phylogenetic analyses of evolutionary origin of the
endomembrane system. DNA Cell Biol 17: 301–9.
28. Vanhoutte M, Songsasen N, Barbe P, Tintignac JL, et al., 2004.
Triiodothyronine-mediated up-regulation of UCP2 and UCP3 mRNA
expression in human skeletal muscle without coordinated induction of
mitochondrial respiratory chain genes. FASEB J 18: 13–5.
29. Cong DW, Si, Weintraub BD, Neidman M. 1998. Rat mitochondrial
glycerol–3-phosphate dehydrogenase gene: multiple promoters, high
levels in brown adipose tissue, and tissue-specific regulation by thyroid
hormone. DNA Cell Biol 17: 301–9.
30. Guaras A, Perales-Clemente E, Calvo E, Acin-Perez R, et al., 2016. The
CoQH2/CoQ ratio serves as a sensor of respiratory chain efficiency. Cell
Rep 15: 197–209.
31. Mills EL, Kelly B, Logan A, Costa AS, et al., 2016. Succinate
dehydrogenase supports metabolic repurposing of mitochondria to drive
inflammatory macrophages. Cell 167: 457–70 e13.
32. Scioli F, Sirrim A, Fernandez-Ayala D, Gubina N, et al., 2016.
Mitochondrial ROS produced via reverse electron transport extend
animal lifespan. Cell Metab 23: 725–34.
33. Barja G. 2013. Updating the mitochondrial free radical theory of aging: an
integrated view, key aspects, and confounding concepts. Antioxid Redox
Signal 19: 1420–45.
34. Bolte K, Rensing SA, Maier UG. 2015. The evolution of eukaryotic cells
from the perspective of peroxisomes: phylogenetic analyses of
peroxisomal beta-oxidation enzymes support mitochondria-first models
of eukaryotic cell evolution. BioEssays 37: 195–203.
35. Tabak HF, van der Zand A, Braakman I. 2008. Peroxisomes: minted by
the ER. Curr Opin Cell Biol 20: 393–400.
36. van der Zand A, Gent J, Braakman I, Habak HF. 2012. Biochemically
distinct vesicles from the endoplasmic reticulum fuse to form
peroxisomes. Cell 149: 397–409.
Problems and Paradigms

36. Neuspiel M, Schauss AC, Braschi E, Zunino R, et al., 2008. Cargo-selected transport from the mitochondria to peroxisomes is mediated by vesicular carriers. Curr Biol 18: 102–8.
37. Tabak HF, Braakman I, van der Zand A. 2013. Peroxisome formation and maintenance are dependent on the endoplasmic reticulum. Annu Rev Biochem 82: 723–44.
38. Bolte K, Gruenheit N, Felsner G, Sommer MS, et al., 2011. Making new out of old: recycling and modification of an ancient protein translocation system during eukaryotic evolution. Mechanistic comparison and phylogenetic analysis of ERAD, SELMA and the peroxisomal importer. BioEssays 33: 368–76.
39. Schlesw B, Girzalsky W, Erdmann R. 2010. Peroxosomal protein import and ERAD: variations on a common theme. Nat Rev Mol Cell Biol 11: 885–90.
40. Taylor EB, Rutter J. 2011. Mitochondrial quality control by the ubiquitin-proteasome system. Nature 473: 173–9.
41. Zimorski V, Martin WF. 2003. Beta' and the 'little sister' closer than assumed? Biochem Biophys Acta 169: 2259–66.
42. Murphy MP. 2009. How mitochondria produce reactive oxygen species. Biochem J 417: 1–3.
43. Formentini L, Sanchez-Arago M, Sanchez-Cenizo L, Cuezva JM. 2012. The mitochondrial ATPase inhibitory factor 1 triggers a ROS-mediated retrograde prosurvival and proliferative response. Mol Cell 45: 41–42.
44. Kramer GF, Ames BN. 1987. Oxidative mechanisms of toxicity of low-intensity near-UV light in Salmonella typhimurium. J Bacteriol 169: 2259–66.
45. Potts M. 1994. Desiccation tolerance of prokaryotes. Microbiol Rev 58: 755–805.
46. Baerends RJ, Rasmussen SW, Hilbrands RE, van der Heide M, et al., 1996. The Hansenula polymorpha PER9 gene encodes a peroxisomal membrane protein essential for peroxisome assembly and integrity. J Biol Chem 271: 887–94.
47. Zimorski V, Martin WF. 2014. Subcellular targeting of proteins and pathways during evolution. New Phytol 201: 1–2.
48. Peng G, Fritsch G, Zickermann V, Schagger H, et al., 2003. Isolation, characterization and electron microscopic single particle analysis of the NADH:ubiquinone oxidoreductase (complex I) from the hyperthermophilic bacterium Aquifex aeolicus. Biochemistry 42: 3032–9.
49. Chouchani ET, Pell VR, James AM, Work LM, et al., 2016. A unifying mechanism for mitochondrial superoxide production during ischemia-reperfusion injury. Cell Metab 23: 254–63.
50. Treberg JR, Quinlan CL, Brand MD. 2011. Evidence for two sites of superoxide production by mitochondrial NADH-ubiquinone oxidoreductase (complex I). J Biol Chem 286: 27103–10.