Alternating terminal electron-acceptors at the basis of symbiogenesis: How oxygen ignited eukaryotic evolution

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What kind of symbiosis between archaeon and bacterium gave rise to their eventual merger at the origin of the eukaryotes? I hypothesize that conditions favouring bacterial uptake were based on exchange of intermediate carbohydrate metabolites required by recurring changes in availability and use of the two different terminal electron chain acceptors, the bacterial one being oxygen. Oxygen won, and definitive loss of the archaeal membrane potential allowed permanent establishment of the bacterial partner as the proto-mitochondrion, further metabolic integration and highly efficient ATP production. This represents initial symbiogenesis, when crucial eukaryotic traits arose in response to the archaeon-bacterium merger. The attendant generation of internal reactive oxygen species (ROS) gave rise to a myriad of further eukaryotic adaptations, such as extreme mitochondrial genome reduction, nuclei, peroxisomes and meiotic sex. Eukaryotic origins could have started with shuffling intermediate metabolites as is still essential today.

Keywords:
- eukaryotic evolution; FADH₂/NADH ratio; genome reduction; LECA; mitochondria; oxygen radicals; symbiogenesis

Introducing symbiogenetic models

In 1998, a highly important Nature paper by Martin and Muller proposed that the eukaryotes started out from a symbiosis between a hydrogen-producing and a hydrogen-consuming archaeal methanogen [1]. However, this hydrogen hypothesis invokes a metabolic distribution (i.e. a fermenting organelle with a respiring host) that is never found in the eukaryotes. Eukaryotes are characterized by fermenting cytosols (the compartment representing the original host) and (almost) universally respiring organelles. I will introduce an alternative symbiotic mechanism more in line with the distribution of tasks observed. Before elaborating the model, I have to stress the following: Even more important than the specific incarnation of the hydrogen-hypothesis was the revolutionary underlying framework: The idea that most, if not all, of the defining eukaryotic characteristics can be explained as resulting from the merger of two prokaryotic cells. The merger extended a symbiotic relation between the cells that existed prior to uptake of one of the partners. This conceptual scheme, christened the symbiogenetic hypothesis for eukaryotic evolution, turned out to be extremely fruitful, and subsequent analyses — as well as (phylo) genomic studies — have convinced more and more researchers that it is a broadly correct description of how eukaryotic evolution occurred [2, 3]. Further work, almost exclusively by Martin and coworkers, filled in more and more of the details of a possible developmental path starting out with an archaeon-bacterium symbiosis and ending with the fully-fledged last eukaryotic common ancestor (LECA). The relevant phylogenomic data include the following: First of all, group-wise gene acquisitions of complete functional metabolic units from bacteria, possibly reflecting a consorted uptake, turned out to be run of the mill for archaea. Such uptakes resulted in 13 sets of innovations defining higher archaeal taxa [4]. Thus, extensive bacterial gene pool uptake accompanied by subsequent smaller-scale loss is a recurrent theme in archaeal evolution [5]. In perfect agreement with symbiogenesis, the latest findings place core eukaryotic genes within the Archaea and,
Figure 1. **A:** A schematic history of live events. The timeline gives their relative positions. Eukaryotic development was most likely an extremely rapid process of adaptations to specific pressures arising from endosymbiotic engulfment (see main text). Timing of life’s origin and the arrival of eukaryotes are uncertain: It occurred before 3.8, and between 2.1 and 1.6 billion years ago, respectively (arrows). Of note, eukaryotes arrived late AND after the great oxygenation event (GOE). **B:** Symbiogenesis. A (brown) — “Lokiarchaeon”; B (blue) — “alpha-Proteobacterium”; C — LECA with “bacterial” membranes, a nucleus formed in response to endosymbiont entry (red) and assorted new compartments; D (green) — Cyanobacterium. An example of a further symbiosis after development of LECA: E — Archaeoplastida (plants and algae); Adapted and extended from [3].

**How did the merger of two prokaryotes lead to LECA?**

We can tentatively reconstruct the sequence of events leading to LECA as (mostly) defined by Martin and co-workers. Starting out with the presumed symbiosis of hydrogen producing bacteria and archaeal methanogens [1], we get uptake of the bacterium either by a primitive phagocytic process available to the Lokiarchaeota, or, more likely (see below), by an uncharacterized non-phagocytic process such as we find in yeasts today. Examples of bacteria taking up other bacteria are found in the tripartite Mealybug system, in which the insect cells contain beta-Proteobacteria, which themselves harbor gamma-Proteobacteria [8, 9]. Of course, this occurs in the context of a very elaborate symbiosis within a multicellular eukaryote. Of note, the engulfing beta-Proteobacterium does not generate its own ATP anymore, and thus lacks a membrane potential-generating machinery [10].

Upon uptake, internal membrane blebbing from the mitochondrion-to-be gave rise to cytoplasmic vesicle formation and furnished building blocks for the complete set of eukaryotic vesicle structures such as nucleus, endoplasmic reticulum (ER), Golgi, autophagosomes, lysosomes, and peroxisomes [11]. Thus, outer membrane vesicles (OMVs) resulting from the endosymbiont’s bacterial secretion system are crucial for the development of a large part of eukaryotic complexity. Interestingly, this model also gets rid of somewhat awkward ad hoc proposals to explain the complete replacement of archaeal host membranes by bacterial ones [3], because OMVs would accumulate in the cytosol, where they fuse with both plasma membrane and each other, as bacterial sn-G3P lipids and archaeal sn-G1P lipids can be stably mixed [12].

Because the host encoded an archaeal secretory pathway with cotranslational protein insertion, this could be combined with outward bound endosymbiont-derived vesicles, allowing a primordial, secretory ER to develop [11]. Previously, the driving force behind the formation of the nucleus was hypothesized to be that it allowed slow mRNA splicing (a necessity because intron-filled genes migrated to the unprepared host from the endosymbiont) to be completed after transcription in a compartment separated from the cytoplasm where translation occurs. Strictly cytosolic translation would then occur using fully-spliced mRNAs only, preventing widespread aberrant translation [13]. A (necessary?) intermediate stage in the evolution towards LECA could possibly have consisted of a so-called coenocytic/syncytial eukaryote in the making form [14].

As depicted in Fig. 1A the eukaryotes made a late appearance in life’s history. Not only that, they appeared quickly and as highly complex fully formed cells with all the characteristics mentioned so far (see Fig. 1B). Intermediate stages have never been found, implying a rapid, evolutionarily unstable, bottleneck in their development [3, 15, 16]. A syncytial stage, such as the one described [14], might help to partly explain this. Garg and Martin propose that eukaryotic chromosome division and full meiotic sex arose in a filamentous, syncytial, multinucleated ancestor, allowing nuclei with insufficient chromosome numbers to complement one another using shared cytosolic mRNA while trying out new chromosome combinations via the development of karyogamy (fusion of nuclei). Such a sequence of events is in line with the observation that the specifics of eukaryotic chromosome separation overall seem to be more universally conserved [17, 18] than those of eukaryotic cell division (e.g. budding, fission, etc.). The syncytial ancestor, using the host’s vesicle secretion machinery could release vesicular feelers (containing different genetic combinations and endosymbiont content) into the environment [14]. This occurs against the background of abundant ATP supply from the endosymbiont being streamlined and metabolically integrated (see below) [3, 14, 19], effectively...
paying for the experiments. There are two important aspects combined in the single Coeca concept: (i) It represents a mixed experimentation chamber in which the gene content available allows for error-prone processes because the total entity (up to a certain limit) remains viable; (ii) It also embodies a testing ground in the form of a fertile field of secreted vesicles, with only those (originally probably extremely rare) vesicles that can make it on their own being selected. Thus, the model has evolution speeding up, first allowing essential relaxed selection, to be followed by strong selection later. Whether such a chamber ever existed or whether the primary symbiosis only gave rise to normal cellular intermediate stages, it is certain that highly efficient ATP generation (see below) by the endosymbiont was accompanied by intensive internal ROS production: Driving evolution of most (all?) of the eukaryotic inventions [15, 20]. One salient example is the universal eukaryotic peroxisome, which is best understood as an adaptation that allowed a decrease in mitochondrial ROS formation by partly rerouting fatty acid (β) oxidation to a separate location in the cell. Oxidation of substrate uses temporary electron carriers such as FADH$_2$ and NADH, which donate their electrons at different sites of the mitochondrial electron transport chain (ETC). Fatty acids (FAs) have a much higher FADH$_2$/NADH ratio than carbohydrates. This means that upon total oxidation of an FA a maximum of one pair of electrons will enter the ETC via FADH$_2$ for every two pairs coming from NADH, while the ratio for a sugar might be as low as 1:5 [21, 22]. The evolution of peroxisomes allowed an overall lowering of the ratio during beta oxidation, especially of longer FAs [23], hence suppressing ROS formation at the cost of slightly less efficient ATP generation; for details see [21, 24, 25]. Along these lines we end up with LECA in the form of a highly active, bi-flagellated textbook-eukaryote capable of meiotic sex (see section “Eukaryotic features that arose as a result of enhanced internal ROS formation”). We can safely conclude that we are getting closer to a viable reconstruction of the path to the eukaryote. However, as already mentioned, I think there is room for improvement: I will introduce an alternative origin scenario and try to fill in some further details.

### Problems with assuming hydrogen exchange at the basis of the primordial symbiosis leading to eukaryotes

As mentioned in the introduction, the conceptual impact of the hydrogen hypothesis for eukaryogenesis should not be underestimated. However, does it describe our best bet for the primeval symbiosis giving rise to eukaryotes? Possibly not. The hypothesis seems to have some problems. Let us first consider the timing of the event (see Fig. 1A). The symbiosis described could not have occurred much earlier, because a minimum level of oxygen was needed. This is due to the need for oxygen as the bacterial electron acceptor, because high abundance of ATP – now considered a sine qua non of eukaryotic development – depends heavily on O$_2$ as endpoint of a respiratory chain. Thus, the bacterium needs oxygen, but the symbiosis based on hydrogen exchange has to occur under anaerobic conditions. As mentioned in the introduction, the mitochondrial precursor). This precursor could respire, but also generate hydrogen as waste during anaerobic metabolism. Hydrogen production thus only goes up whenever (O$_2$) becomes limiting. The affinity of present day terminal mitochondrial oxidase (cytochrome c oxidase) for O$_2$ is very high (K_d for O$_2 < 1$ μM; [27]). As O$_2$ concentrations were lower at the time of eukaryotic origins, it is not unreasonable to claim that such a high affinity as has been measured had already been attained. Thus the host is presumed to have become dependent upon the H$_2$ waste under anoxic conditions. But how could the future endosymbiont have retained its complex multi-subunit aerobic respiratory chain under prolonged anoxic conditions? Do not prokaryotes rapidly lose what they do not use? On the other hand, as soon as O$_2$ is present, the ETC of the archaeon would be damaged (in particular, unadjusted iron-sulphur clusters would be vulnerable to oxidation [28]). Indeed, upon prolonged exposure to O$_2$, the ETC of the archaeal ETC did completely disappear (it is absent in LECA).

Clearly, most viable theories explaining eukaryogenesis have to solve this problem of an anoxic host teaming up with an aerobic endosymbiont, but for making it thus points out an exciting alternative. For this symbiosis to work the environment has to have both oxygen – though still at lower levels than currently available – and (relative) anoxia. The electron transfer chain (ETC) of the host is (strongly) hampered by O$_2$, but not all ETC complexes in the host cells will be irretrievably damaged by low, fluctuating, O$_2$ levels. This points to microenvironments characterized by fluctuating O$_2$ levels with more or less steep oxygen gradients. Let us model the crucial interactions between archaeon and proto-mitochondrial bacterium under such conditions.

### Introducing an alternative: Temporary electron-acceptor shifts at the basis of symbiogenesis

The new symbiosis scenario (see Box 1) gives O$_2$ levels a prominent role from the start. As mentioned, the (host) archaeon and a bacterium use different terminal acceptors for their ETC’s. In many microenvironments natural (O$_2$) gradients occur. They often result from physical barriers, where the gradient is sharpened as a result of efficient organismal (mostly prokaryotic) oxygen scavenging. For example, sediments as well as wetland soils can be characterized by oxygenated surfaces with redox stratification into an
Hypotheses

In water, (O₂) declines from

dients can then exist over tiny distan-

try inactivity of one of the two

otic (O₂) has indeed been around in

rium at the start of eukaryotic evolution.

the initial association of host and bacte-

scavenging might have been the basis for

fixing cyanobacteria [30]. Such efficient

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important inactivity of one of the two

features of both partners. For the present purposes the specific group-

ing of the proto-mitochondrion in the alpha-Proteobacteria is not essential,

and the discussion regarding its possible relation to the SAR11 group of

marine alpha-Proteobacteria and/or the Rickettsiales [33, 34], I will not
deal with (see [35]). The future endosymbiont clearly encoded (aero-

bic) catabolic pathways burning carbo-

hydrates (including glycolysis), lipids, glycerol, and several amino

 acids, with (a few of the) citric acid cycle enzymes, and a FA (β) oxidation

pathway linked to an oxidative respira-

tory chain. This is consistent with a

reconstruction of ancestral proto-mi-

tochondrial metabolism indicative of

respiratory oxidation of (at least)

lipids, glycerol, and amino acids

obtained from the host [36]. With

the assembly of a Lokiarchaeota

geno me we obtain more insight in

the metabolic pathways that the host

probably brought into the merger

(see e.g. http://www.genome.jp/
kegg-bin/show_pathway?category =
Lokiarchaeota; [7]). Surprisingly, the

assembly hints at a hydrogen-depen-

dent metabolism [37, 38]. If this turns

out to be the case, I should stress that

this observation is compatible with

both the hydrogen hypothesis and the

alternative model presented here. But

we also find both a glycolytic pathway

and a lactate dehydrogenase enzyme

(oxidizing NADH), allowing glycoly-

sis to proceed in the absence of NAD+

regeneration by a functional ETC.

Perhaps the occurrence of a hexose

pathway ending in pyruvate/lactate

in the host raises some eyebrows,

because the current eukaryotic path-

way seems completely bacterial in

origin [2]. However, clearly archaea

contain pathways of central carbohy-

drate metabolism such as the classi-

cal Embden–Meyerhof (mostly

referred to as glycolysis) and Ent-

ner–Doudoroff routes; though con-
taining some twists and, on the whole, less extensive allosteric regu-

lation of individual enzyme (com-

plexes) [39, 40]. The much higher

level of allosteric control that came

along with replacement by bacterial
counterparts probably was the driv-

ing force behind the takeover in
eukaryotes. Thus, eukaryotes are less

Box 1
Essentials of the alternating terminal electron-acceptor model for symbiogenesis at the basis of eukaryotic evolution

Model:

The preexisting symbiosis leading to a merger between the archaeal host and the bacterial pre-mitochondrion is hypothesized to have started with the exchange of intermediate carbohydrate metabolites. This was driven by the alternation between the two different electron chains (ETCs) as a result of changes in (O₂), because the bacterial acceptor is O₂, while the host chain is oxygen-sensitive.

Important aspects:

The model does not have to be explicit regarding the nature of the terminal electron-acceptor of the archaeon. Its ETC only has to be inhibited by oxygen, which is known to occur for different acceptors, for the symbiosis to work. In essence, electron-rich compounds are directed to the cell with an active ETC, and this redistribution is based on known characteristics of membrane transporters (see main text).

Empirical support:

Molecular oxygen and reactive oxygen species have had a crucial impact on eukaryogenesis from the start. This makes an initial anaerobic symbiosis less likely, though not impossible. The dominant specific complementation of the initial symbiosis that shaped the organization of the eukaryotic cell is still characteristic of almost all eukaryotes today: Fermenting hosts (cytosol) and respiring oxidizing mitochondria, exchanging intermediate carbohydrate metabolites.

oxygen-depleted zone. Steep (O₂) gradients can then exist over tiny dis-
cances [29]. In water, (O₂) declines from 100% to ~0% local saturation in less than a millimetre from the surface into the interior of aggregates of detritus or N₂-fixing cyanobacteria [30]. Such efficient scavenging might have been the basis for the initial association of host and bacte-
rium at the start of eukaryotic evolution.

Protection of archaeal respiration against toxic (O₂) has indeed been around in quite a few symbiogenetic theories (e.g. see [31, 32]). However, details of most of these theories have proven false, and it is quite hard to understand how the protective factory ends up on the inside of the cell. On top of this, the border and contacts described can hardly be consid-
ered stable. Martin and Muller were correct in surmising a more substantial bond in the form of metabolite exchange. This is where temporary electron-acceptor shifts enter the picture. Both external (O₂) fluctuations and, even more likely, border ruptures would lead to temporary inactivity of one of the two final acceptors. Thus, both cell types could be alternatively robbed of a final acceptor, the bacterial one by low (O₂), the archaeal one for instance by inhibition due to increased (O₂) [28]. If substrate-level ATP generation prior to further energy extraction in ETC’s was available for both prokaryotes, we get the conditions conducive to a mutually beneficial (!) symbiosis (see Fig. 2). Temporary molecular oxygen shifts will establish a symbiosis based on intermediate carbohydrate metabolite (y in Fig. 2) exchange; these metabolites being fur-
ther processed by the cell with the ETC that still functions. Only afterwards, upon prolonged O₂ abundance, would the archaeal ETC and its associated membrane potential disappear, allowing bacterial uptake and the first steps of eukaryotic metabolic differentiation (see Fig. 2).

Can we find evidence that this scheme might be correct? To recon-
struct the sequence of events leading to primeval endosymbiont uptake, we first have to describe basic metabolic
versatile in their choice of electron donors and acceptors (in their case, in the end it practically always has to be O₂) than prokaryotes, but more versatile in fine-tuning and adapting the intermediate metabolism to nutrient availability and demand. Another line of reasoning also predicts the presence of a glycolytic pathway in the host. Though it is quite easy for gene products to start functioning in a new eukaryotic cell compartment [41], the complete relocation of a pathway, having higher interconnectivity, is harder to accomplish. This is sometimes referred to as the complexity hypothesis [42, 43]. It is nicely illustrated by the fact that most of the genetic information for the ETC complexes in eukaryotes is encoded by the nuclear genome (in the case of complex II, succinatidehydrogenase, even completely) while the pathway itself of course has not moved. This also holds true for non-membrane bound pathways. In principle a path only functions as a whole, and cannot easily be moved in a piecemeal fashion. The few exceptions illustrate this clearly. Bacterial (mitochondrial) FA (β) oxidation was duplicated (so, not replaced) in peroxisomes. This new organelle even seems to have come into being under selection to allow lowering of FADH₂/NADH ratios during fat(ty acid) breakdown (see section “How did the merger of two prokaryotes lead to LECA?”), hence minimizing mitochondrial ROS formation [21, 44]. Complete β-oxidation is a recurrent concise 4-step process. In the case of peroxisomal duplication, the first step is the only really new one (i.e. the one that allows oxidation without generating FADH₂ for the mitochondrial ETC), leaving only the three last steps to be derived from duplicated mitochondrial genes (steps 2 and 3 even as a single bifunctional enzyme) [23, 45]. These aspects nicely clarify this exception with regard to retargeting complex pathways. Peroxisomes are also responsible for another exceptional, much later, example of retargeting complex pathways, in their guise of kinetoplastid glycosomes. Especially in bloodstream forms of some kinetoplastid parasites relying heavily on glycolysis, retargeting of the first part of this pathway to glycosomes seems essential for its regulation [46].

**How did the association of two prokaryotes with ETC’s using different final acceptors give rise to stable symbiosis?**

Let us discuss how the two prokaryotes with (partly) duplicated carbohydrate metabolisms but ETC’s evolved with different final acceptors formed a stable symbiosis. As soon as one of the two ETC’s is severely hampered (too little or too much O₂), the products of the intermediate metabolism will build up in the afflicted cell. Let us take lactate as the y of Fig. 2 as an example. Transporters for carbohydrates either work by facilitated passive diffusion or (in)direct active transport. When only powered by differences in concentrations, such build-up could immediately lead to spontaneous efflux, especially because the lactate is effectively removed by uptake (again, passively or actively) and oxidation by the alternative ETC of the other cell. This is reminiscent of overflow metabolism, which occurs under substrate (e.g. glucose) excess conditions in bacteria. It is characterized by secretion of metabolites: e.g. acetate or lactate [47]. Surprisingly, the metabolite exchange needs no new mechanisms, using only metabolite transporters already present and pathways allowing temporary fermentation in either cell. This would allow survival on substrate-level ATP generation in the afflicted cell. As always: Fermentation depends on respiration [16]. One could even envisage: (i) more efficient substrate uptake by the fermenting partner in a form of feed forward; and/or (ii) short- or long-range product inhibition, lessening substrate uptake by the respiring cell via allosteric regulation. This would further stabilize the symbiosis (not shown in Fig. 2). Next, upon the replacement of a fluctuating environment by a constant

![Figure 2. A highly schematic reconstruction of the sequence of events leading to primeval endosymbiotic uptake and metabolic specialization. Archaea (brown) and bacteria (blue) could define a sharp oxygen gradient (due to bacterial oxygen scavenging at the basis of their association). Both donate substrate electrons to their ETC’s (depicted as strings of spheres), the bacterial one using oxygen as its final acceptor. Low-level substrate-linked ATP generation prior to further energy extraction in the ETC’s can occur in both prokaryotes. Temporary molecular oxygen fluctuations will establish a symbiosis based on the exchange of intermediate metabolites (y), y being further processed by the cell with a functioning ETC. Upon O₂ ascendance, the archaeal ETC and its membrane potential disappear, allowing bacterial uptake and eukaryotic metabolic differentiation. For further details see main text. Of note: Substrate, x and y can differ between the two prokaryotes. Metabolic paths are depicted in red. The black lines indicate a border. PMF – proton-motive force.](image-url)
Hypotheses

made by the proto-mitochondrion and contributions to overall ATP generation could have been based on fluctuating bacterium. allowing the host to engulf the oxidizing ETC would deteriorate and disappear, substrate) even prefer fermentation and growth under conditions of plentiful dormant states with spectacular out- yeasts (eukaryotes that alternate specific cellular adaptations. Many aspects of the eukaryotic lifestyles and mitochondrial involvement can have spec-

Eukaryotic features that arose as a result of enhanced internal ROS formation

The uptake of the bacterium created a charged situation full of promise and difficulties. Interestingly, varying O2 levels can enhance ROS formation, which possibly already led to ROS pre-adaptations in the merging partners. However, the crucial factor in eukaryotic evolution probably was the enhanced production of internal ROS by mitochondria, see [3, 22] and references therein. A far from complete list of eukaryotic features most likely (strongly) influenced by enhanced internal ROS formation could include: superior protein quality control [51] and chromosomal DNA repair (see [52] and references therein), dynamic mitochondrial super-complex formation, mitochondrial uncoupling (proteins), carnitine shuttles, multiple antioxidant mechanisms, mitochondrial fusion/fission cycles and autophagy (to allow specific removal of parts damaged by ROS). Further adaptations include peroxisome formation (see section “How did the merger of two prokaryotes lead to LECA?”), internal, e.g. nuclear, membrane formation, as well as meiotic sex; for further discussion see [3] and references therein. The influence of ROS is far-ranging, as exemplified by replacing isoleucine with antioxidant methionine via AUA codon reassignment in mitochondrial DNA [53]. Thus, ROS has even rewritten the mitochondrial genetic code. Another surprising and elegant mechanism was demonstrated in yeast, in this case via translational control by reprogramming of ribosomes in response to ROS [54]. ROS leads to a Trm4 methyltransferase-dependent increase of tRNA (Leu [CAA]) containing m(5)C at the wobble position, improving selective translation of TTG enriched mRNAs, such as the one encoding ribosomal protein RPL22A. As expected, loss of TRM4 and/or RPL22A makes yeast more susceptible to ROS. The ROS impact is further reflected in the plethora of metabolic pathways that eukaryotes influence via ROS signalling, often using reversible modulation of reactive Cys residues in target proteins. For an overview, see [55]. This led to the concept of mitohormesis, referring to a coordinated response upon low-level mitochondrial stress and ROS production, allowing cells to more easily cope with subsequent challenges [56, 57]. This observation helps to explain the mixed blessings of anti-oxidant supplements.

Extreme mitochondrial genome re-
duction can be understood in the context of endogenous ROS formation [58] as well. Among the ideas to explain large scale early migration of most bacterial genes, a molecular ratchet model for gene migration as described in [59] nicely dovetails with the driving force(s) probably responsible. At the beginning of endosymbiosis some dividing prokar-
yotes will lyse, and fragments of their DNA will end up in the host genome. This mostly seems to occur via the straightforward incorporation of large DNA seg-

ments via recombination (see [60, 61] and references therein). The host genome cannot return the favour, as its fragmentation means the end. Currently, yeast DNA seems to make the journey from nucleus to mitochondrion at least 100,000 fold less efficiently than the other way around [62]. However, though clearly part of the explanation, there are caveats. First of all, especially in the beginning, retargeting of gene products to organelles is no small or cheap feat, which seems to invite a driving force (e.g. ROS mutating the bacterial DNA bound to the inner membrane [63]). Secondly, the extended and improved archaeal DNA repair mechanisms, up to meiotic sex [64], are most likely the result of ROS-associated selection pressures to begin with. Thirdly, if the syncytial, multinucleated ancestor [16], described above, indeed existed, the ratchet is (at least temporarily) weakened, as the host DNA can now be fragmented as well. Of note, such a long-lived, steady state ancestor must have been even more susceptible to the cumulative effects of endogenous ROS formation. Other models stress the superior repair and translational control in the host genome, but, as already mentioned, these can be seen as resulting from the primary driving force
(ROS). The combination of higher mutation rates due to ROS in ETC-containing organelles (see e.g. [65]) and possible mutational meltdown in the absence of meiotic recombination [66] would allow Muller’s ratchet to overcome the organelle in the absence of gene migration. In conclusion, a combination of differential mutation and biased transfer rates seems to explain the observed genome reduction [67]. So, organelar genes hide out in the nucleus. But where did the nucleus itself come from?

Was the nucleus shaped by internal ROS as well?

Part of the question regarding nuclear origins seems to have been answered recently. As mentioned earlier, the membranes allowing the nucleus’ formation very likely came from internal membrane vesicle secretion from the bacterial endosymbiont [11]. Thus, the outer membrane vesicles (OMVs) are envisaged stacking around the host’s DNA (a configuration that temporarily appears in metazoans during karyokinesis [68]). But why was this configuration (resembling a defence) selected? As described above, nucleus formation might have allowed completion of mRNA splicing – of intron-riddled genes derived from the endosymbiont – before export. Restricting translation to fully-spliced mRNAs in the cytoplasm would prevent translation errors [13]. One might object that this did not pose a problem to the endosymbiont itself. However, while the bacterial genome had been constantly purged by the energy cost of introns, the host genome energetic costs were steadily becoming less of a problem, because of the energy revolution of eukaryotic endosymbiosis [19]. More intronic passengers could be supported, so they were. Thus the problem is real, but could it have been the first driving force for the host DNA sequestration? When a nuclear membrane is in place this scenario adds a further driving force for specialization and further adaptations. However, stating that even slight retention of large mRNAs by a nuclear membrane would be positively selected sounds less convincing: would not the large ribosomes be retained as well? Protection of host DNA from ROS damage seems a plausible candidate as the primary driving force behind cellular enlargement as well as nucleus formation. This framework possibly also explains the appearance of polyunsaturated lipids in eukaryotic membranes. Bacterial mono-unsaturated lipids are relatively unreactive [69], while the eukaryotic variants could function as both defence and signalling molecules.

Why do some mitochondrial genes stay put?

All mitochondria retain the genes for a tiny fraction of the proteins required for their function. The same gene set encoding core proteins is thus almost always present on mtDNA. These are central, very hydrophobic, components of complex I, III, and IV of the ETC, and subunit(s) of the ATP synthase. This is e.g. the case in the human mitochondrion genome; a typical instance [70]. Some components of the translational machinery are preserved as well (the 2 rRNA and 22 tRNA genes [70]), mitoribosomes having to be built locally as long as the mitochondrion retains its own genome. There are deviations on both sides of the norm [71]: e.g. (much) further reduction in eukaryotic parasites, but also Jakobid organisms (an order of protists), having retained ~100 mitochondrial genes [72, 73]. The extreme reduction allowed outlandish systems to arise, exemplified by gene fragmentation and RNA editing [74, 75], and it can be seen as part of a mostly dominating evolutionary trend toward streamlining and simplification [76]. For our current discussion however, the reasons for more extensive retention are more interesting: How did Jakobid organisms hang on to more of their mitochondrial genomes? The most simple explanation: Jakobid mitochondria kept the ancestral bacterial RNA polymerase [72], which was replaced by a viral-type polymerase in all other eukaryotes. Possibly, the absence of this polymerase made relocation of specific mitochondrial genes somewhat more difficult to accomplish. Yet, further analysis of Jakobid metabolism and nuclear genomes might turn up indications for connections to ROS-related mechanisms.

How about the generally retained set of core mitochondrial genes enumerated above? Why are they still there? Three, not mutually exclusive, answers have been given (see [77] and references therein). Two important obstacles to transfer were quickly invoked. First of all, many mitochondrial genomes have genetic code differences (small genomes being able to implement deviations from the standard code), such as the AUA codon reassignment (isoleucine to methionine) mentioned earlier [53]. A transferred gene would thus encode an incorrect peptide. Secondly, proteins still encoded in mitochondria are overwhelmingly highly hydrophobic. This could not only interfere with efficient import upon cytosolic synthesis, but also with refolding. Often overlooked, the efficient biogenesis of the large complexes, in which the mitochondrialy encoded subunits form part of the hydrophobic central core modules, is a highly complex orchestrated sequence that might not be amenable to import of the central players (e.g. because of lacking membrane interactions). For a beautiful overview of such a finely orchestrated biogenetic dance in the case of complex I, see [78]. A more recent idea is the CoRR, co-location for redox regulation, hypothesis [16, 79, 80]. It posits that expression of some genes encoding parts of ETC’s has to respond directly to changes in the redox state of the local environment (e.g. signaled by ROS) and for this, local genomes are necessary. Thus, to retain function, a complete redox regulatory system must remain functional within the original membrane-bound compartment [80]. Paradoxically, the highly evolved eukaryotic antioxidant measures might make a local sensing mechanism more and more important. There are indeed tantalizing indications for such a system: The mitochondrial topoisomerase I is ROS sensitive and influences mitochondrial transcription [81]. However, though I find the idea of direct ROS signalling as the reason behind mitochondrial DNA retention enticing, the jury is still out regarding the hypothesis. As stated in [77]: “There is no evidence for obstacles as severe as code disparity or hydrophobicity.” The longtime independent evolution of both mitochondrial DNA and ETC biogenesis, starting out
with the highly hydrophobic core, might preclude further genome reduction and transfer. Without manipulation, that is. If we artificially transfer the remaining sequences (after getting rid of code disparity and adding a mitochondrial localization signal) to the nuclear genome, CoRR could be tested. Reducing hydrophobicity levels (while retaining function!) as well, might enable the ultimate test. If such artificial cells, when able to form functional ETCs in the absence of mitochondrial DNA, produce more ROS, this would indicate CoRR to be more than just an exciting idea.

The CoRR hypothesis also predicted the presence of organellar DNA in a specific class of hydrogenosomes (those that have a membrane potential; see below), which turned out to be true with the discovery of the anaerobic ciliate Nyctotherus ovalis, inhabiting the anoxic hindgut of a cockroach [82]. This constituted the first example of an organelle containing mitochondrial DNA producing H₂ [83]. Another, convergent, example of an organelle blurring the line between mitochondria (again having its own DNA) and hydrogenosomes is found in Blastocystis spec [84, 85]. These observations do seem in line with the theory, but of course do not demonstrate the truth of the CoRR hypothesis. However, they constitute important findings when discussing Martin and Muller’s hydrogen hypothesis [1, 83].

**What do eukaryotic hydrogenosomes tell us about the hydrogen hypothesis?**

The hydrogen hypothesis posited a facultative aerobic bacterium as the ancestor of our current-day mitochondrion. Not only that, upon anaerobic fermentation it would produce hydrogen [1]. Hydrogenosomes are found in many unicellular eukaryotes living in hypoxic or anoxic environments. Although the precise pathways differ quite extensively they all lack (at least) the terminal oxidase, cannot use oxygen anymore, and make varying amounts of hydrogen. Apart from the two examples already mentioned, amongst others, the parbasalid Trichomonas vaginalis, the heterolobosean Psalteriomonas lanternae [86] and some chytrid fungi contain hydrogenosomes (though organellar DNA is absent in all these cases); information to be found in excellent overviews by Yarlett and Hackstein, and Stairs et al. [87, 88]. The hydrogen hypothesis was partially inspired by the presence of hydrogenosomes, instead of normal mitochondria, in many, evolutionarily diverse, eukaryotic lineages. It was speculated that these represented examples of the bacterium-derived organelle holding on to its hydrogen producing capacity because of the anoxic environments they found themselves in. Do any of them represent the genuine article? Most current analysis does not make that likely [87, 88]. The best way to understand these organelles is probably as steps in a general pathway to adapt to hypoxic, and later on, anoxic conditions, encountered by free-living and parasitic protists; some generalities of this progression are described in [88].

The genes for the enzymes needed seem to be laterally acquired because the enzymes involved (e.g. pyruvate formate lyases, hydrogenases, pyruvate:ferredoxin oxidoreductases) are not found in aerobic eukaryotes. To put it another way: The endosymbiont-derived genes for *aerobic* metabolism clearly are derived from an alpha-Proteobacterium-like organism, while this cannot be said for those involved in *anaerobic* metabolism. Is this a blow to the hydrogen hypothesis? I do not think so. The concentrated period of metabolically expensive adaptations, needing O₂, that gave rise to LECA (see Fig. 1) always made retention of bacterial anaerobic enzymes, if present to begin with, rather unlikely. Of note, the anaerobic archaeal ETC has completely disappeared. These kinds of analyses cannot decide whether bacterial hydrogen generation was available, let alone important, during the primordial symbiosis.

Interesting from the standpoint of the hydrogen hypothesis is the interaction observed between many of the hydrogenosome-containing eukaryotes and methanogenic archaea. However, none of the hydrogenosomes have hung on to their ETCs for oxidation. The question then becomes: Do such interactions emulate the symbiosis at the origin of the eukaryotes or, instead, illustrate their lack of viability as far as models for symbiogenesis are concerned?

**Conclusions and outlook**

The hydrogen hypothesis envisages a fermenting organelle and a respiring host at the basis of eukaryotic evolution. However, such a configuration is not observed in eukaryotes. They are characterized by fermenting cytosols (representing the original host) and respiring organelles, the logical outcome of the alternating terminal electron-acceptor model. What kind of findings would shed further light on whether this model could be correct? The discovery of an organelle/endo-symbiont capable of producing hydrogen while having retained a functional oxidative ETC would be a boost to the alternative hydrogen hypothesis. In an experimental setting, models of prokaryotic symbiosis might be manipulated with regard to ETC functions. This would possibly allow the study of engulfment mechanisms available upon loss of membrane potential – subsequently evolving into eukaryotic endocytosis? – and of the exchange of intermediate carbohydrate metabolites under such conditions.

Fluctuating oxygen levels could thus have been the crucial determinant allowing the original symbiosis at the basis of eukaryotic evolution. The alternating electron-acceptor model depicts eukaryogenesis in a sequence of logical steps, starting out with interactions still enshrined in the day-to-day function of all eukaryotic cells. Oxygen, as the perfect final electron acceptor, also gave rise to unlimited ATP, allowing the myriad of inventions that now define the gulf between prokaryotic and eukaryotic cells. Finally, oxygen, in the form of ROS, presented a number of selection pressures that drove many of these eukaryotic inventions, in a process of creative destruction. Building on initial differences in host and endosymbiont efficiencies, the eukaryote ended up with archival information processing hardware and bacterial, allosterically regulated, metabolic enzymes. It definitely got the best of both worlds as well as abundant
energy to make the most of its new possibilities.

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