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Endosymbiosis and the design of eukaryotic electron transport

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Abstract

The bioenergetic organelles of eukaryotic cells, mitochondria and chloroplasts, are derived from endosymbiotic bacteria. Their electron transport chains (ETCs) resemble those of free-living bacteria, but were tailored for energy transformation within the host cell. Parallel evolutionary processes in mitochondria and chloroplasts include reductive as well as expansive events: On one hand, bacterial complexes were lost in eukaryotes with a concomitant loss of metabolic flexibility. On the other hand, new subunits have been added to the remaining bacterial complexes, new complexes have been introduced, and elaborate folding patterns of the thylakoid and mitochondrial inner membranes have emerged. Some bacterial pathways were reinvented independently by eukaryotes, such as parallel routes for quinol oxidation or the use of various anaerobic electron acceptors. Multicellular organization and ontogenetic cycles in eukaryotes gave rise to further modifications of the bioenergetic organelles. Besides mitochondria and chloroplasts, eukaryotes have ETCs in other membranes, such as the plasma membrane (PM) redox system, or the cytochrome P450 (CYP) system. These systems have fewer complexes and simpler branching patterns than those in energy-transforming organelles, and they are often adapted to non-bioenergetic functions such as detoxification or cellular defense.

Keywords: Archaea; Bacterium; Chloroplast; Mitochondrion; Photosynthesis; Respiration

1. Introduction

Bioenergetics played a central role in the emergence of the eukaryotes, which was closely related to the acquisition of the mitochondrion, an organelle specialized in producing ATP by aerobic respiration. According to the endosymbiosis theory, the ancestors of mitochondria were free-living bacteria that were engulfed by a host cell. In particular, members of the α-proteobacterial order Rickettsiales, such as *Rickettsia prowazekii* (the agent of epidemic typhus), appear to be good models for the ancestor of mitochondria, which are most likely of monophyletic origin [1–4].

Some anaerobic eukaryotes lack mitochondria, and in the past this condition was regarded as primitive (see, e.g. Ref. [5]). Thus amitochondriate eukaryotes seemed to be a kind of "living fossil", branching off from the eukaryote tree before the appearance of mitochondria. However, during the last decade a large amount of evidence has accumulated, indicating that the amitochondriate eukaryotes are polyphyletic and initially had mitochondria, which then were either transformed into another organelle (the hydrogenosome; see Section 4), or completely lost [1,6–11]. Therefore, apparently no bona fide model of a eukaryotic cell before the acquisition of the α-proteobacterial endosymbiont exists.

The chloroplast and derived plastids of phototrophic eukaryotes evolved from endosymbiotic cyanobacteria in a eukaryotic host. Like mitochondria, plastids harbor their own genome and are probably monophyletic [12–14]. Certain cyanobacteria, the prochlorophytes, utilize chlorophylls *b* and *c*, which are also found in eukaryotes, and therefore they were initially considered good models for the origin of chloroplasts. However, subsequently it became evident that the prochlorophytes are no monophyletic subgroup within the cyanobacteria, indicating that chlorophyll *b* or *c* emerged several times independently [15,16]. The use of chlorophyll *b* or *c* can be therefore no diagnostic criterion for the ancestor of plastids. Recently, it was shown that the closest known relative of plastids in terms of genetic

Abbreviations: AOX, alternative oxidase; CYP, cytochrome P450; cyt, cytochrome; ETC, electron transport chain; NDH, NAD(P)H dehydrogenase; PM, plasma membrane; PQ, plastoquinone; PS, photosystem; ROS, reactive oxygen species; SDH, succinate dehydrogenase; UQ, ubiquinone

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similarity is the cyanobacterium *Nostoc punctiforme* [17]. The genus *Nostoc* has generally a high potential to form symbioses with various eukaryotes [13,18,19], which provides further support for an origin of plastids in this genus. A number of phototrophic eukaryotes (e.g., dinoflagellates, euglenoids, cryptomonads) have plastids surrounded by three or four membranes; they are derived from secondary endosymbioses of green or red algae in heterotrophic eukaryotic hosts [12,14,20,21].

In contrast to the excellent bacterial models for the ancestors of mitochondria and chloroplasts, less is known about the prokaryotic ancestor(s) of the proto-eukaryotic host cell. Eukaryotic proteins for information storage and processing and for cell cycle control indicate a contribution from archaea; this may imply that an archaeon was the host that engulfed an α-proteobacterium to become the first eukaryotic cell [9,22] (Fig. 1a). Other authors suggested that only the nucleus is derived from an archaeal endosymbiont, living within a bacterial host [23,24] (Fig. 1b). The lack of a consensus is reflected by the widely divergent proposals for the archaeal-bacterial merger, including anaerobic and aerobic members from both major subdivisions of the archaea (Euryarchaeota and Crenarchaeota) [9,22,24–27].The model proposing an anaerobic, hydrogen-dependent archaeon, e.g. a methanogen, as host for an α-proteobacterium [9,22] has made several predictions that were confirmed since it was initially published in 1998. In particular, the observation of structural or molecular vestiges of mitochondria in virtually all amitochondriate eukaryotes (see above) fits the notion that acquisition of the mitochondrion and emergence of the eukaryote cell were a single event.

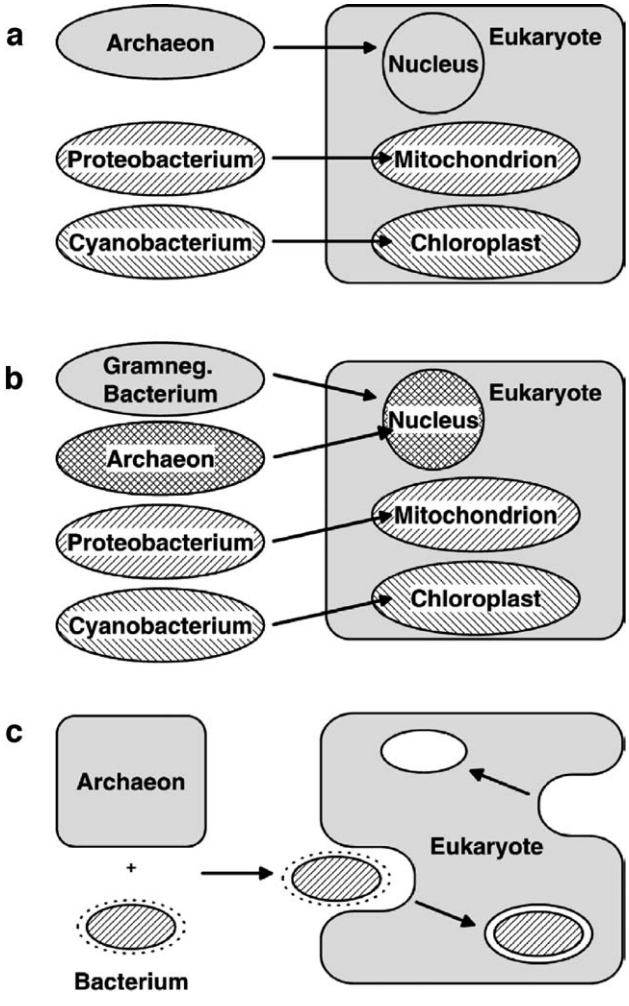

Fig. 1. Schematic representation of endosymbiosis events. (a, b) Different scenarios for the origin of the eukaryotic cell, and (c) resulting membrane topology of eukaryotes.

The difficulties to reconstruct the emergence of eukaryotes are also reflected by the controversies on the root of the eukaryote tree (see Refs. [28–30] for discussion). A novel area of research, being still in its infancy, is the investigation of ultra-small eukaryotes. This may have profound consequences for the reconstruction of early eukaryote phylogeny, as these small, newly detected eukaryotes have cell sizes and probably also genome sizes comparable to prokaryotic ranges [30].

Fig. 1c shows in a schematic fashion the different membranes of eukaryotic cells. Due to their endosymbiotic origin, mitochondria and chloroplasts are surrounded by two membranes, the outer of which being probably derived from the plasma membrane (PM) of the host cell. Both proteobacteria and cyanobacteria have, in addition to their PM, also a periplasmic membrane, but the latter is not a barrier in the chemiosmotic sense (accordingly shown as a dotted line in Fig. 1c) and was apparently lost in mitochondria and chloroplasts, respectively. (According to an alternative proposal, the bacterial membranes have been retained completely in both mitochondria and plastids, so that the outer mitochondrial membrane and the outer chloroplast envelope membrane would be derived from the respective periplasmic membranes [20]). In addition, the eukaryotic cell has several organelles, such as the endoplasmic reticulum, the Golgi apparatus, and microbodies. In the main part of this paper, I will review the effects of endosymbiosis on the bioenergetic functions of the different membranes, with a focus on convergent processes and common trends of bioenergetic evolution [31]. In particular, two aspects are interesting for such a comparative approach: First, the parallel processes during the transitions α-proteobacteria–mitochondria and cyanobacteria–chloroplasts, respectively. Second, the reinvention of some bacterial pathways that apparently were initially lost in eukaryotes.

2. When less is more: the streamlining of bacterial pathways in eukaryotes

Compared to other bacteria, *R. prowazekii* has a rather small genome (~ 1.1 Mbp, 834 protein-encoding genes) reflecting its intracellular mode of life [3,4]. Genome reduction in mitochondria has gone even further, and their genomes are about one order of magnitude smaller [1,2]. A

similar reduction occurred in chloroplast genomes, which harbor about 60–200 genes [12,13,17], as opposed to several-thousand genes in cyanobacteria.

Two processes have caused this organelle genome reduction. First, many genes have been transferred to the nucleus [2,12,32–36]; one major driving force is probably the higher rate of mutation in the organelle. This effect would be directly related to bioenergetic reactions: The electron transport chains (ETCs) in both mitochondria and chloroplasts appear to be sources of DNA-damaging reactive oxygen species (ROS), in particular superoxide anions. For plant and animal mitochondria, ROS formation is discussed in the context of ubisemquinone oxidation at various sites of the ETC [37,38], but also for oxidation of cyt *c* released from the respiratory chain [39]. The major source of ROS in chloroplasts is the Mehler reaction, i.e. the reduction of oxygen to superoxide by photosystem I [40,41]. So the question arises why not all genes were transferred to the nucleus. Again, the answer seems related to electron transport in the organelles: the remaining genes in the organelle genomes may enable an efficient control of protein synthesis in dependence on the local redox state, which may differ between individual mitochondria or chloroplasts, respectively, within a cell [42,43].

The second process of organelle genome reduction is the complete loss of many genes. In this respect mitochondria and chloroplasts follow a general trend in endocellular symbionts and parasites [2–4,13,20,32,44]. In particular, a loss of bioenergetic complexity and versatility is a frequent feature of intracellular organisms [45]. *Paracoccus denitrificans*, an α-proteobacterium with a well-characterized ETC, serves here as an example for bacterial respiration (Fig. 2a). The electron transport system shows an apparently redundant branching pattern that enables the use of various electron donors and acceptors; the terminal oxidases have different oxygen affinities and are expressed differentially in dependence on the oxygen concentration [46–48]. *E. coli*, a universal "model organism" of biology, is atypical insofar as it has not the cyt *bc*-type complex, which occupies a central position, for instance, in *P. denitrificans*. However, bacterial respiration in general uses branched electron transport systems centered around soluble carriers (quinones and *c*-type cytochromes), with multiple points for entry and exit of electrons to utilize a range of donors and acceptors under fully aerobic, microaerobic, and anaerobic conditions [47,49]. For comparison, the canonical complexes of mitochondrial respiration are highlighted in gray in Fig. 2a. The decreased metabolic flexibility in comparison to free-living bacteria is obvious. [Some yeasts such as *S. cerevisiae* have additionally lost the NADH dehydrogenase (Complex I).] Mitochondria operate within the homeostatic environment of a host cell, so they are optimized for efficient ATP synthesis fuelled by a small set of substrates being present within a predictable concentration range. In contrast, free-living bacteria are subject to environmental fluctuations, and they must be able to thrive upon a number of different

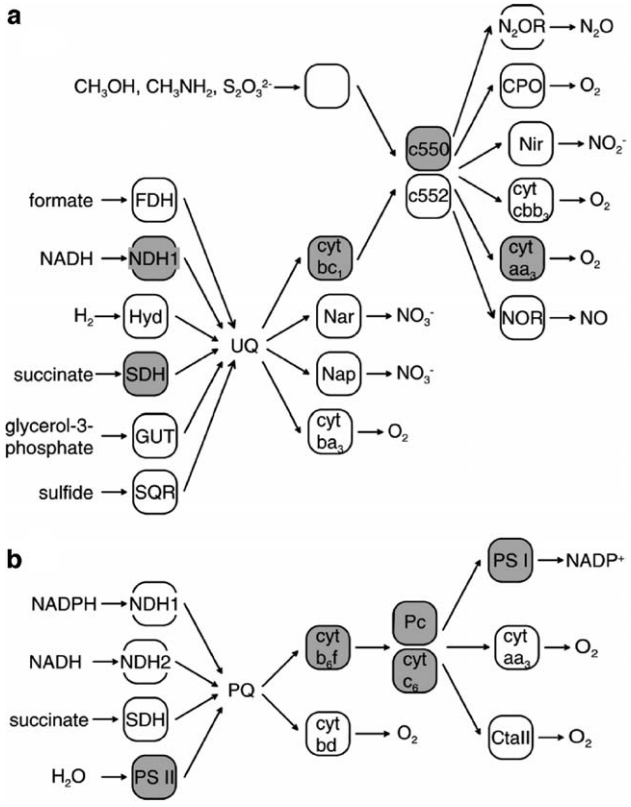

Fig. 2. Electron transport systems of *Paracoccus denitrificans* (a) and *Synechocystis* sp. strain PCC 6803 (b). Standard electron transport components of mitochondria and chloroplasts, respectively, are highlighted in gray. Note that *Synechocystis* 6803 has two membrane systems with differing composition: the photosystems operate only in thylakoids, while the cyt *c* oxidase CtaII operates in the plasma membrane. Other abbreviations: CPO, cyt *c* peroxidase; FDH, formate dehydrogenase; GUT, glycerol-3-phosphate:ubiquinone oxidoreductase; Hyd, hydrogenase; N_2OR, N_2O reductase; Nap, periplasmic nitrate reductase; Nar, membrane-bound nitrate reductase; Nir, nitrite reductase; NOR, NO reductase; Pc, plastocyanin; SQR, sulfite:ubiquinone oxidoreductase.

substrates of changing availability. However, "textbook mitochondria" are essentially mammalian mitochondria, and mammals are extreme cases in two respects: they have a particularly low number of mitochondrial electron transport complexes, and they (together with birds) have the tightest homeostatic control of the internal milieu that maintains temperature, glucose level, and oxygen concentration within narrow limits.

A similar reduction of the ETC took place in photosynthesis. *Synechocystis* sp. PCC 6803, probably the cyanobacterium with the best known ETC at present [50–55], has a branched electron transport system with the soluble electron carriers plastoquinone and plastocyanin/cyt c_6 acting as central crossroads (Fig. 2b), resembling the picture in other heterotrophic and phototrophic bacteria (e.g., in Fig. 2a). In addition, there are several parallel routes for cyclic electron transport around PS I [55] (not shown in Fig. 2b for clarity), which generate a pH gradient at the thylakoid membrane for ATP synthesis without net NADP reduction.

The canonical complexes of eukaryotic photosynthetic electron transport [56] are again highlighted in gray, and it is evident that a streamlining of the ETC has occurred, like the parallel trend in mitochondria. The ETCs of algae and higher plants are generally similar, but reductive evolution in plants has gone one step further, and they use only plastocyanin as soluble electron carrier, while cyt c_6 is still present in algae [57,58].

The photosynthetic apparatus of phototrophic eukaryotes originating from secondary endosymbioses (meta-algae) [20,21] has not yet been characterized in detail, compared to the wealth of information on higher plants and green algae, and it would be difficult to make generalizations on specific modifications resulting from secondary endosymbiosis. Photosynthetic reactions in thylakoids from the diatoms *Cylindrotheca fusiformis* and *Phaeodyctylum tricornutum* resemble those in higher plants [59,60]. On the other hand, several meta-algae have lost photosynthesis (see below), such as the malaria parasite, and sometimes even the plastid is lost [20,61]. General features of all secondary endosymbioses seem to be the loss of the mitochondria of the endosymbiont and the strong reduction of both its nuclear and plastid genomes [20].

3. Novel eukaryotic proteins and pathways

3.1. New subunits for old complexes

While the number of ETC components has decreased in mitochondria, there is an opposite trend with respect to the subunit composition of the individual complexes: The eukaryotic cyt bc_1 complex has 11 subunits, while a three-subunit minimum version is found in *P. denitrificans* [62–64]. Likewise, eukaryotic cyt *c* oxidase has 13 subunits, while *P. denitrificans* utilizes a minimum version of only four subunits [65,66]. NDH-1 (NADH:ubiquinone oxidoreductase) is quite large already in proteobacteria, where it has 14 subunits; but again a large number of additional subunits occur in mitochondria, the total number of proteins in eukaryotic NDH-1 being up to 46 [67–69]. Only succinate dehydrogenase (SDH) does not fit into this picture, having only three to four subunits in bacteria and eukaryotes alike [70–72]. The new mitochondrial proteins, which are specific eukaryotic and have no α-proteobacterial homologues, are generally nuclear encoded [2]. It is often difficult to assign a function for them, but many of these "supernumerary" subunits seem to be involved in biogenesis and stabilization of the complexes [64,68]. The integration of a mitochondrial processing peptidase into the cyt bc_1 complex is an example of a functional assignment [73–75]. At least five additional subunits occur already in the cyt bc_1 complex of the jakobid flagellate *Seculamonas ecuadoriensis* [76], indicating an early emergence of the novel eukaryotic subunits, because jakobids have the most ancient, bacteria-like mitochondrial genomes of all eukaryotes [1,2].

A comparable trend towards increased size of electron transport complexes is not obvious for chloroplasts, and both PS I and PS II, respectively, have rather similar subunit compositions in cyanobacteria and higher plants. PS I has acquired three additional small subunits (PsaH, PsaG, and PsaN) [77–79], but in general new peripheral subunits have been added mainly as replacements for older ones. Plant PS II has lost two small cyanobacterial membrane-extrinsic proteins on the lumenal side (a 12-kDa protein and cyt c_{550}), and acquired instead two new small subunits (the 17-kDa PsbQ and the 23-kDa PsbO) [80,81]. Likewise, the cyt b_6f complex has an identical set of the four core subunits (cyt b_6, cyt *f*, Subunit IV, and Rieske protein) in both cyanobacteria and eukaryotes, and minor differences occur with respect to the peripheral small subunits without cofactors [82,83]. Replacement dominated also in the evolution of the light-harvesting pigment-binding proteins: The cyanobacterial phycobilisomes [84,85] and the PS I antenna IsiA [86,87] have been deleted in phototrophic eukaryotes (except for Rhodophyta, which still have phycobilisomes). They were replaced by eukaryotic LHC proteins, which contain chlorophyll $a+b$ (as in green algae, higher plants, and euglenoids) or chlorophyll $a+c$ (as in red algae) [88,89].

So the following resume can be drawn: Bacterial core subunits, being essential for electron transport, have been retained in mitochondria and chloroplasts without major changes, and they are preferentially encoded by the organelle genomes. For peripheral subunits a difference exists between the two organelles, as mitochondrial complexes acquired numerous additional subunits (frequently of unknown function), while in the chloroplast there was rather a replacement without altering the net number of subunits or the overall size.

3.2. Novel complexes and the branching of eukaryotic ETCs

In addition to the canonical complexes of mitochondrial respiration shown in Fig. 2a, many eukaryotes have additional components in their ETCs (Fig. 3a), so that sometimes a branching pattern similar to the ancestral bacterial one is restored. Alternative NAD(P)H dehydrogenases of the soluble NDH-2 type are found frequently in plants and fungi, where they are associated with both faces of the mitochondrial inner membrane [90–93]. Some yeasts have respiratory dehydrogenases for further substrates, such as glycerol-3-phosphate, D- and L-lactate [92]; membrane-bound D- and L-lactate dehydrogenases occur also in mitochondria of the photosynthetic protist *Euglena gracilis* [94]. The function of these complexes is still under investigation; they may be involved in fermentative metabolism and in the regulation of the energetic yield (H^+/e^- ratio) of respiratory electron transport because the H^+/e^- ratio is different, for instance, for electron transport via NDH-1 versus NDH-2.

In mitochondria from algae, plants, fungi, and protozoa, a quinol oxidase has been found, which is a non-heme iron protein with a di-iron center [95–99]. This alternative oxidase (AOX) catalyzes the oxidation of ubiquinol and

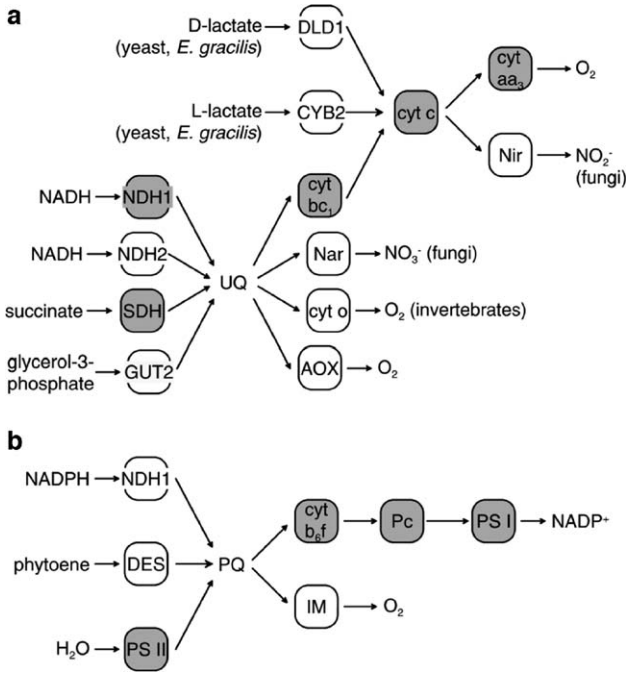

Fig. 3. Nonstandard electron transport pathways in mitochondria (a) and chloroplasts (b); standard components are highlighted in gray. Note that (a) is a composite picture that is not necessarily realized in any single eukaryotic species. Abbreviations (see also Fig. 2): DLD1 and CYB2, D- and L-lactate:cytochrome *c* oxidoreductase, respectively; DES, phytoene desaturase.

the reduction of molecular oxygen to water, but does not pump protons; it is cyanide-insensitive and can occur in monomeric or dimeric forms, depending on the species. AOX is subject to different types of regulation; in plants it is stimulated by organic acids, while in fungi and protozoa activation occurs by purine nucleotides. AOX has not been found in animals, but several unrelated invertebrates have another ubiquinol oxidase instead, serving as adaptation to low or fluctuating oxygen levels. This so-called cyt *o* complex is a *b*-type cytochrome [100–102]. (The use of nitrate and nitrite as electron acceptors in fungi is discussed in Section 4.)

In the last decade it became evident that chloroplasts also have ‘‘non-standard’’ complexes (Fig. 3b) that transcend the simple picture of photosynthetic electron transport shown in textbooks (Fig. 2b). Chloroplasts have inherited from cyanobacteria a homologue of mitochondrial Complex I [103,104]; the participation of this NDH-1 in cyclic electron transport around PS I was shown for cyanobacteria [53,55] and higher plants [104,105]. Chlororespiration, a respiratory electron transport in chloroplasts of algae and higher plants in the dark, has been discussed controversially, and some early reports on the phenomenon may have been confounded by interaction of thylakoid reactions with mitochondrial electron transport, or by plastoquinol autoxidation [106]. However, meanwhile there is sufficient evidence to demonstrate the existence of genuine chlororespiration beyond doubt [107,108]. Plastoquinone reduction in the dark can occur via the chloroplast NDH-1, and the so-called IM (or IMMUTANS) protein is the chlororespiratory quinol oxidase of eukaryotic thylakoids. The identification of IM as a thylakoid-located quinol oxidase is crucial because it confirms the existence of a real respiratory chain in this membrane. IM operates as an electron sink in carotenoid biosynthesis by transferring electrons from phytoene desaturase via plastoquinol to oxygen, and it is homologous to AOX from plant mitochondria, i.e., it is a eukaryotic protein not inherited from cyanobacteria. A novel eukaryotic complex is also the hydrogenase, utilized by green algae as an anaerobic electron sink that reduces protons to molecular hydrogen [109,110]; it is of the Fe-type and unrelated to the cyanobacterial Ni-Fe-type hydrogenase.

Based on the principle of parsimony, the following course of evolution can thus be proposed for eukaryotic terminal oxidases (Fig. 4): a widespread bacterial quinol oxidase is the cyt *bd* complex [111], and genes for both subunits are also present in the genome of *R. prowazekii* [4]. (*P. denitrificans* shown in Fig. 2a has no cyt *bd* complex, but uses a cyt ba_3 quinol oxidase instead.) Therefore, it is likely that a cyt *bd* complex was present in the ancestor of mitochondria. Given the absence of cyt *bd* in all eukaryotes and the wide phylogenetic distribution of AOX, it is furthermore likely that cyt *bd* was lost early and replaced by AOX as novel eukaryotic quinol oxidase. A secondary loss of AOX apparently occurred in the animal line, and here instead the cyt *o* complex emerged. Again, this happened perhaps shortly after the origin of the metazoa, as cyt *o* is found in unrelated invertebrate lines such as polychaetes and bivalves. A loss of AOX occurred also in the mitochondria of *Polytomella* sp., a colorless, non-photosynthetic chlorophyte [112]. The ancestor of chloroplasts is likely to have possessed both a cyt aa_3 and a cyt *bd* oxidase, as it is typical for cyanobacteria [51,54], but both these terminal oxidases were lost in chloroplasts. Instead, chloroplasts acquired another complex, the IM alternative oxidase, which arose by gene duplication from the homologous mitochondrial AOX.

Irrespective of their diverse biochemical nature, resulting from multiple episodes of loss and reinvention, the terminal quinol oxidases seem to have similar roles across bacteria and eukaryotes. A salient similarity of cyt *bd* and AOX (and probably also IM and cyt *o*) is the absence of vectorial proton pumping. The short-circuited electron transport via these proteins is inefficient in terms of proton pumping, and results in an uncoupling of electron transport from phosphorylation and the dissipation of free energy. Accordingly, one function of AOX in certain plants is thermogenesis in floral tissues; some more generally applicable functions of terminal quinol oxidases discussed in the literature are [90,93,95–99,102,107,108,113]:

- Electron sink for catabolic and anabolic reactions
- Electron valve to prevent over-reduction of the quinone pool

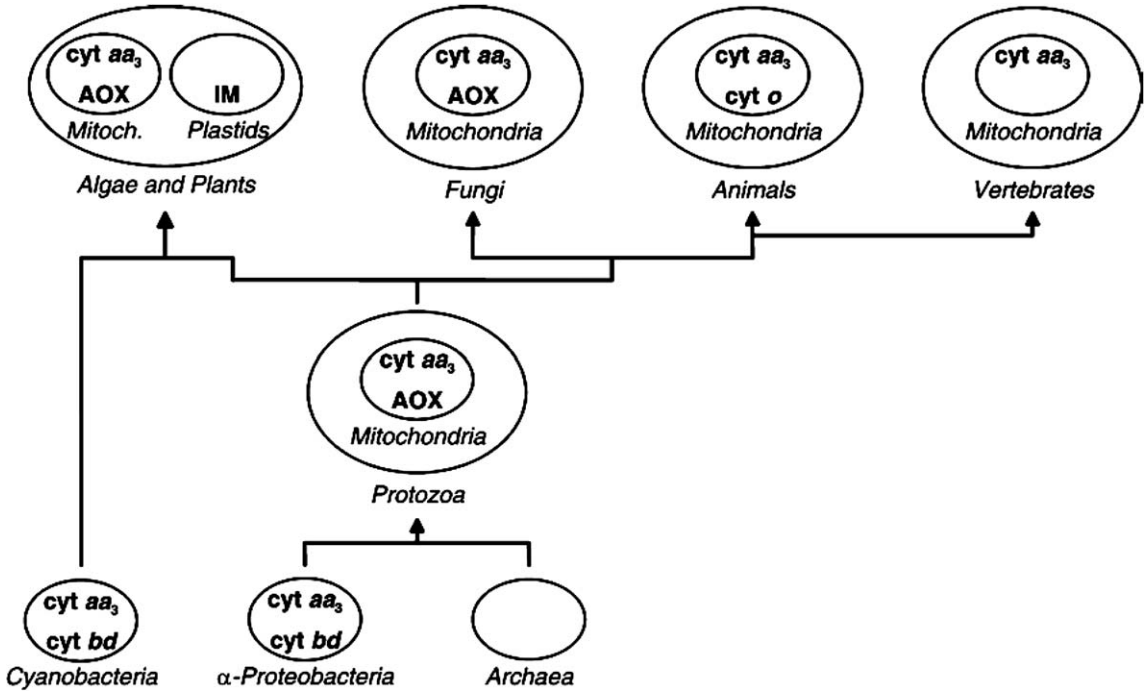

Fig. 4. Hypothetical reconstruction of the utilization of different terminal respiratory oxidases in eukaryotes, see text for details.

- Adaptation to different oxygen levels
- Scavenger of oxygen to prevent formation of ROS
- Decoupling of electron transport and phosphorylation for regulatory purposes
- Backup system in case of failure in the main pathway due to toxins or mutations.

The aspects in this list are analogously discussed for bacterial ETCs [46–49].

3.3. Changes in membrane architecture

A parallel trend in mitochondria and chloroplasts is the emergence of elaborate folding patterns of the bioenergetic membranes. This means an increase of membrane surface and, thus, of net biosynthetic capacity per organelle volume in comparison to α-proteobacteria and cyanobacteria, respectively. In addition, the intricate membrane topology has functional implications: in chloroplasts, there emerged a spatial separation of the two photosystems and a differentiation between different regions of the thylakoid membrane [114,115]. The stacked grana membranes contain PS II and cyt b_6f; they support linear photosynthetic electron transport. In contrast, the exposed membranes of the stroma lamellae contain PS I, cyt b_6f, and the CF_0CF_1 ATP synthase. This part of the thylakoid membrane functions in both linear electron transport and the PS I cycle, which generates ATP without net NADPH production.

For mitochondria, the textbooks usually present a simple internal structure, where the cristae are depicted as mere dents of the inner membrane. However, it has become evident that mitochondrial ultrastructure is far more complex [116–119], with a three-dimensional network of membranes reminiscent of the folding pattern of the thylakoid membrane. And like in chloroplasts, there is evidence for restricted diffusion and functional differentiation between different regions of the mitochondrial inner membrane. In particular, the F_0F_1 ATP synthase and supplementary carriers for P_i and ADP/ATP seem to be located only in the cristae, a situation which strikingly resembles the restriction of the chloroplast ATP synthase to particular regions of the thylakoid membrane.

4. Anaerobiosis and parasitism

Aerobic respiration is typical for eukaryotes, but several groups have secondarily adapted to anaerobiosis to cope with anoxic or hypoxic environments and with fluctuating oxygen concentrations. Many of these anaerobic eukaryotes are parasites, in particular endocellular ones. The bioenergetic reactions of anaerobic eukaryotes can be grouped into anaerobic respiration and fermentation.

Several lines, in particular invertebrates, can use fumarate as an anaerobic electron acceptor, which is reduced by Complex II, i.e., an SDH operating in reverse direction [120,121]. This fumarate reductase arose by gene duplication from the normal eukaryotic SDH. Fumarate respiration is known from bacteria [122,123] and was obviously rediscovered independently in eukaryotes, but with a characteristic difference: fumarate, being a low-potential acceptor, requires also a low-potential donor, and bacteria therefore

use menaquinol for fumarate reduction. Eukaryotes are unable to synthesize menaquinol, probably because the ancestral mitochondrion was functioning obligatorily aerobic and used only ubiquinol, and so they have invented their own low-potential quinone, rhodoquinone, which is an amino-substituted ubiquinone [121,124]. In parasitic helminths there are also special isoforms of subunit 1 of Complex I, which operates as an NADH:rhodoquinone oxidoreductase under anaerobic conditions [125].

Another type of anaerobic metabolism known from bacteria and reinvented by eukaryotes is denitrification, observed in mitochondria of fungi such as *Fusarium oxysporum* and *Cylindrocarpon tonkinense* [126–128] (Fig. 3a). These fungi possess a NO reductase of the cyt P450-type, which is a eukaryotic invention unrelated to bacterial NO reductases of the cyt *bc*-type [127,129]. Another difference between fungal and bacterial pathways is the oxygen requirement in fungi, where a minimal amount of oxygen is needed for induction of denitrification [128]. On the other hand, the fungal nitrite and nitrate reductases (Nir and Nar, respectively) resemble their bacterial counterparts; one possible explanation would be that these proteins were inherited from the ancestor of mitochondria [126]. (Note the presence of the same proteins in proteobacteria such as *P. denitrificans*, Fig. 2a.)

Many parasites have life cycles where different ontogenetic stages switch between mitochondrial aerobic respiration and anaerobic metabolism [120], such as mitochondrial fumarate respiration in helminths (see above). Trypanosomatids like *Trypanosoma brucei*, the causative agent of sleeping sickness, have realized this aerobic/anaerobic switch in a different (and apparently independently evolved) way [130–133]: they utilize normal mitochondrial respiration in the procyclic form that lives, for instance, in the midgut of the tsetse fly, and glycolysis in the bloodstream form in the vertebrate host. Glycolysis occurs in a specialized, peroxisome-like organelle, the glycosome, where a novel soluble NADH:fumarate oxidoreductase serves for maintaining the NAD^+/NADH balance [131]. Several glycosome proteins were reported to be homologous to plant and algal proteins, and it was proposed that trypanosomatids are derived from meta-algae, which lost their chloroplasts completely [133].

Various eukaryotes have gone one step further and lost mitochondrial respiration and oxidative phosphorylation altogether. These fermenting amitochondriate species can be classified into Type I, which has no specialized compartments for energy metabolism, and Type II, which has hydrogenosomes. A consensus has emerged that the absence of mitochondria is a secondary loss, rather than representing an early, pre-mitochondrial stage of eukaryote evolution [1,6–11]. Accordingly, the hydrogenosome is most likely a transformed mitochondrion. The parabasalia, such as *Trichomonas vaginalis*, belong to Type II and represent an early branch of the eukaryote tree [10,134]. Another early branch is probably the diplomonads such as *Giardia lamblia*, which have Type I-metabolism [6,8]. No hydrogenosomes have been found also in microsporidia, but they are derived from fungi, thus stressing the polyphyletic character of the amitochondriate condition [11,135].

A large group of parasites are the apicomplexa, which encompass a big number of human and veterinary pathogens, such as *Plasmodium falciparum*, the cause of malaria, or *Toxoplasma gondii* [7,136,137]. The apicomplexa probably arose from a secondary endosymbiosis of an alga within a heterotrophic eukaryote host, and have retained a residual plastid, the apicoplast. However, apicomplexa are non-photosynthetic, in accordance with their parasitic life style, and all photosynthetic functions have been deleted in the apicoplast. The remaining physiological roles of this organelle are biosynthesis of fatty acids, isoprenoids, and perhaps also heme [136,137]. Similarly, the loss of photosynthetic and chlororespiratory electron transport has been reported for the vestigial plastid of *Epifagus virginiana*, a non-photosynthetic, parasitic plant [138].

5. Consequences of multicellular organization

Bacteria are able to form cellular associations, such as filaments, and in some filamentous cyanobacteria there is even a functional differentiation between cells. Nevertheless, multicellular organization with differentiation into tissues and organs is observed only in eukaryotes. The energetic performance of mitochondrial respiration may be a key feature to enable complex body plans. On the other hand, the mitochondria themselves (and chloroplasts, too) have undergone major changes and acquired new functions as a consequence of multicellular organization. In particular, three aspects affect the bioenergetic functions of mitochondria and chloroplasts: the differentiation into different tissues, the existence of distinct ontogenetic stages, and the large variation of body mass giving rise to allometric scaling of metabolic rates.

5.1. Tissue-specific modifications

Chloroplasts have diversified into a range of other plastids in non-photosynthetic tissues, which have lost the photosynthetic function and which are adapted for a variety of tasks, such as biosynthesis and storage of starch, fatty acids, amino acids, secondary metabolites, and pigments [139–141]. The rule "delete photosynthesis, but keep the plastid" thus seems to be common for non-photosynthetic tissues of green plants, the leaves of parasitic plants, and apicomplexa alike. In a certain sense, this pattern can be traced back already to the cyanobacteria because some species form N_2-fixing heterocysts and other specialized cells, which have lost all photosynthetic functions. Such cellular plasticity is especially pronounced in the genus *Nostoc* [18,19], which appears to be closely related to the ancestor of plastids.

Compared to the drastic variation of morphology and function in plastids, mitochondria from different tissues of the same organism seem to show a lower degree of flexibility. Nevertheless, modifications of mitochondria in dependence on tissue type or ontogenetic stage are observed in animals [118,142,143] and plants [144]. The particularly strong genome reduction observed in animal mitochondria may be related to the emergence of multicellular body plans in the metazoa, as their closest relatives among the protozoa have significantly larger mitochondrial genomes [145]. Mitochondrial plasticity occurs also in unicellular and multicellular fungi, where structure, density per cell, and biochemical properties of the organelle vary in dependence on metabolic state, developmental stage, and substrate supply [90,93,146,147]. In particular, the composition of the ETC and the balance between phosphorylating and alternative electron transport pathways in fungi are subject to such modifications.

The structural variation of mitochondria is not yet fully understood. In a naive view, the total surface of the inner membrane per mitochondrion should be directly correlated to the organelle's phosphorylation capacity, as the crucial protein complexes for electron transport and ATP synthesis are membrane-bound. However, the observed intricate differences in cristae morphology may have functional implications beyond such a simple proportionality [116,117], and an inverse relation between mitochondrial size and bioenergetic capacity was shown for the rat cerebellum [148]. Tissue-specific modifications of localization, metabolism, and structure of mitochondria occur in cells with high energy demand such as neurons [119,149] and muscle cells [150–152]. The mitochondria of pancreas β-cells have acquired a specific role as fuel sensors and are involved in the regulation of insulin secretion [153]. Another tissue-specific function of mitochondria is thermogenesis in brown adipose tissue (BAT) of mammals, which results in structural and biochemical modifications [154]. A crucial biochemical adaptation of BAT mitochondria is the expression of UCP1, a proton-conducting protein in the inner mitochondrial membrane, which uncouples respiratory electron transport from ATP synthesis and induces the dissipation of free energy as heat. The UCP proteins form a large family and their possible roles as metabolic regulators, in addition to thermogenesis by UCP1, are discussed intensively [155–159]. UCP homologues occur furthermore in ectothermic vertebrates, invertebrates, fungi, and plants, where in most cases a function in thermogenesis seems to be excluded [160–162]. (However, as mentioned above, certain plants have thermogenic floral tissue, where also a dissipative electron transport via AOX is found [95,96].) A tissue-specific modification at the level of individual ETC proteins is observed in cyt *c* oxidase, where different isoforms of subunit VI give rise to different H^+/e^- ratios and, thus, different energetic efficiency [163,164]; energy dissipation by cyt *c* oxidase especially in birds may be related to their elevated metabolic rate and thermogenesis. Subunit VIII of cyt *c* oxidase seems to have been specifically adapted in anthropoid primates, including our own species, and accelerated rates of nonsynonymous substitutions occur also in other COX subunits of anthropoids [165]. The authors of Ref. [165] suggest that these cyt *c* oxidase subunits have been optimized for energetic efficiency, concomitant with the emergence of large and energy-demanding brains in this lineage.

5.2. Mitochondria and ontogenesis

Mitochondria have been integrated into ontogenetic processes in animals and plants in several ways; a major mechanism is the release of cyt *c* during apoptosis, giving rise to cytotoxic superoxide [39,117,166–170]. A second link between the mitochondrial ETC and apoptosis is provided by the identification of the cell death regulator GRIM-19 as a subunit of bovine Complex I [171]. The connection between apoptosis and redox reactions—including both mitochondrial and PM electron transport—is conspicuous, and it has been proposed that apoptotic cell death has evolved together with redox signaling as a means to prevent the selfish reproduction of individual cells in early multicellular associations [172]. In fact, proteins with homologies to metazoan cell death factors occur in sponges [173] and in the single-celled *Dictyostelium discoideum* [174]. Programmed death occurs also in bacteria, but no clear evidence seems to link these processes in bacteria and mitochondria [175].

A further connection between mitochondrial electron transport and development is the implication of ubiquinone [176–178]. Mutants of *Caenorhabditis elegans* and mouse with deletions in the last step of ubiquinone biosynthesis show almost normal mitochondrial electron transport because the accumulating precursor demethoxyubiquinone can substitute for UQ as electron carrier. Nevertheless, these mutants have severe defects in their development, indicating a hormone-like function specifically of ubiquinone. Likewise, the cattle parasite *Setaria digitata* uses ubiquinone-6 for mitochondrial electron transport in both larval and adult stages, but UQ-8 occurs additionally in the larvae as an antioxidant, partially replacing other compounds such as tocopherol [179].

5.3. Scaling of metabolic rates

Another aspect of multicellularity with important physiological consequences is the enormous span of body mass in eukaryotes, giving rise to allometric laws of the type $R \sim M^a$ where R is the metabolic rate, M the body mass, and a the scaling exponent [180–183]. Scaling exponents $a = 3/4$ seem to apply to many groups of organisms (but see the discussion in Ref. [180] with respect to endotherms). The observed allometries partially result from effects at higher morphological levels, such as transport processes in the circulation system or the plant vascular system, but

metabolic scaling can be also related to the organelle level: the mitochondrial volume per cell is subject to allometric scaling [184], and additionally the basal proton permeability of the inner mitochondrial membrane and thus the rate of non-phosphorylating oxygen consumption decrease with increasing body mass [185,186]. At the same time, the energetic efficiency of oxidative phosphorylation in the mammalian heart decreases with increasing body mass [187], which is unexpected given the lower contribution of H^+ leakage at high body mass. Therefore, there must be another parameter involved; one explanation could be a higher H^+/e^- coupling stoichiometry of the ETC in small animals (see Refs. [56,188] for a discussion of variable versus fixed stoichiometry in respiratory and photosynthetic electron transport).

6. Other eukaryotic membranes

Prokaryotes usually have ETCs in their PM, and in principle one might expect to find vestiges of these ETCs in all eukaryotic membranes that are derived from prokaryotic PMs (Fig. 1c). In addition to the eukaryotic PM itself and the inner membrane of mitochondria, which is homologous to the α-proteobacterial PM, this concerns the outer membrane of mitochondria (derived from the PM of the host cell), and the inner chloroplast envelope membrane (derived from the PM of the endosymbiotic cyanobacterium). In contrast, the outer chloroplast envelope membrane is already derived from a eukaryotic PM, as it arose from the engulfment of a cyanobacterium in a eukaryotic host. Finally, there is a number of other organelles such as the ER and microbodies, which, unlike mitochondria and chloroplasts, have only one membrane and do not harbor their own genome; they may have originated from invaginations of the (proto-) eukaryotic PM as shown in Fig. 1c. Another proposal is the evolution of these endocellular membranes from vesicles that originated when lipid biosynthesis of the bacterial endosymbiont occurred within the host cell [9].

The eukaryotic PM and the various organellar membranes do in fact harbor electron transport components (Fig. 5). Redox reactions in PMs are involved in bioenergetic functions, such as control of membrane potential and cytoplasmic pH, nutrient uptake, and sodium cycling, but a range of additional functions can be attributed to these ETCs [189,190], like cell-cycle control and signaling. Superoxide anions are generated in an NADPH-dependent pathway as a defense against pathogens (Fig. 5a), for instance in the ‘‘oxidative burst’’ of neutrophil cells, but also in plants [191,192], and similar reactions are involved in metazoan apoptosis [193,194] and plant development [195]. Ascorbate, serving as extracellular antioxidant, is regenerated at animal and plant PMs by NADH via ubiquinol [196,197] (Fig. 5b).

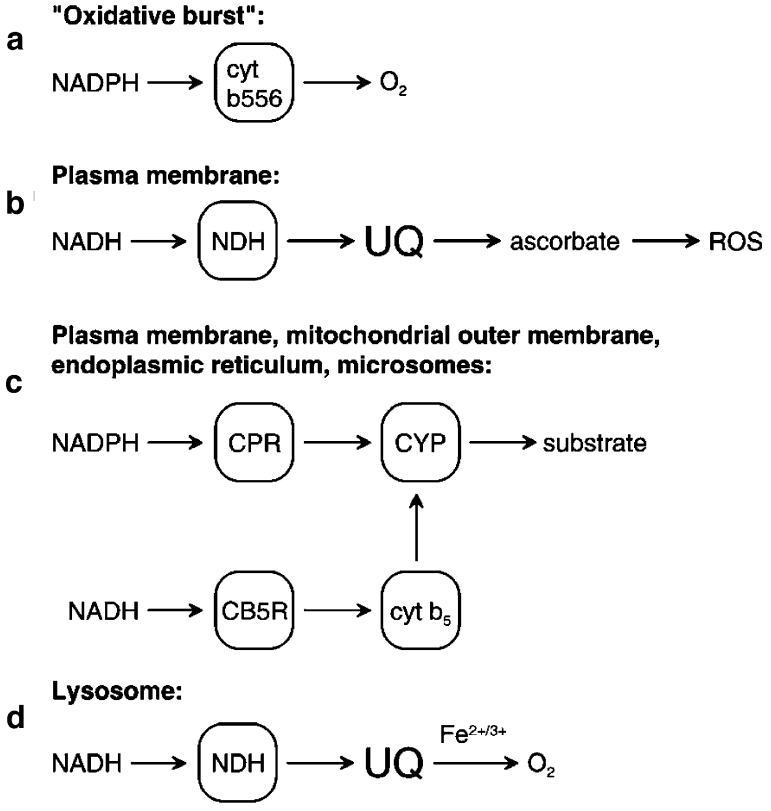

Fig. 5. Electron transport chains in eukaryotic membranes with non-bioenergetic functions; see text for details. Abbreviations: CB5R, cyt b_5 reductase; CPR, cyt P450 reductase.

Molecular trafficking, rather than energy conversion, is the central task of those membranes which form the interface between the bioenergetic organelles and the cytoplasm. Because most mitochondrial and plastidal proteins are nuclear-encoded, they must be translocated into the organelle; several pathways exist with homologies to bacterial protein export mechanisms [198]. Equally important is the transport of small molecules between organelle and cytoplasm, which are the substrates and end products of the bioenergetic and biosynthetic reactions in mitochondria and plastids [139,199,200]. However, the mitochondrial outer membrane is also involved in electron transport activities, in particular those of the cytochrome P450 (CYP) system [201–203], which in mitochondria participates in the biosynthesis of steroids [204,205]. Members of the CYP family oxidize a broad range of hydrocarbons in the monooxygenase reaction $RH + O_2 + 2H^+ + 2e^- \rightarrow ROH + H_2O$, which requires the transfer of two electrons. The details of the reaction have been controversial, but the branched scheme of Fig. 5c has emerged as a consensus [201,206,207]. Components of the mitochondrial outer membrane electron transport may also interact with the ETC of the inner membrane, through cyt *c* diffusing across the intermembrane space [208,209].

Gloeobacter violaceus is the only known cyanobacterium without thylakoids and has instead a green PM harboring both photosynthetic and respiratory electron transport [210]. *G. violaceus* thus may represent an ancient condition before the invention of the internal thylakoid membrane. All other cyanobacteria perform photosynthesis exclusively in the thylakoids and photosystems are absent from the PM. However, the PM of these "advanced" species is still involved in the biogenesis of the photosystems [211]. This evolutionary relic was also forwarded to the chloroplasts but with a further modification: eukaryotes assemble the photosystems directly in the thylakoid membranes but crucial steps of chlorophyll metabolism are still located in the chloroplast envelope [139,212]. Like in the outer mitochondrial membrane, there are electron transport components in the chloroplast envelope: quinone species, flavins, iron–sulfur centers, and a member of the cyt P450 family (CYP86B1) have been detected [213,214], and at least some of these compounds were shown to constitute a working ETC [215]. Its function may be related to the biosynthetic reactions located in the plastid envelopes, such as formation of lipids, plastoquinone, carotenoids or chlorophyll [139,212].

The CYP system plays a major role in the membranes of ER and microsomes [201–204]. It participates in many biosyntheses and metabolic reactions, in particular detoxification reactions. The isoform CYP3A4 is a major constituent in liver ER and is responsible for the biotransformation of many drugs. A different type of ETC exists in lysosomes (Fig. 5d): Electrons are transferred from NADH to ubiquinone; reoxidation at the lumenal side of the lysosome membrane is catalyzed by iron species and generates superoxide anions and an acidic pH in the lumen [216,217].

So virtually all eukaryotic membranes harbor ETCs, but, compared to the ETCs of core energy metabolism in mitochondria and chloroplasts, they are shorter and mostly unbranched. The ETCs of core energy metabolism in bacteria and eukaryotes have a similar overall structure (Figs. 2 and 3), which is probably shaped by two opposite criteria: on one hand, the chain should be short to minimize dissipative side reactions; in particular the number of diffusive electron carriers should be small [218]. On the other hand, a high energy yield in terms of pumped protons per electron requires a high number of coupling sites, i.e., high number of different proteins, and this was probably a driving force towards the evolution of more elaborate chains [219]. Obviously, the second criterion was irrelevant for the ETCs of the various eukaryotic membranes discussed in this section, and they were not optimized for chemiosmotic efficiency, in accordance with the fact that they do not drive ATP synthesis. Nevertheless, ubiquinone is found in virtually all eukaryotic membranes [176–178,196,216,217], and ubiquinol oxidation with concomitant proton deposition occurs at the P-side of the membrane [31], as in phosphorylating ETCs. This may indicate that eukaryotic non-bioenergetic ETCs are generally derived from systems that were initially involved in energy metabolism, as proposed for components of the CYP system [205].

Depending on the preferred scenario for the origin of the eukaryotic cell, Fig. 1a (archaeal host) or Fig. 1b (bacterial host), one might expect a homology of eukaryotic PMs with archaeal or bacterial ones, respectively. Metabolic pathways in eukaryotes appear to be more similar to bacterial ones than to archaeal ones, and this also holds for the electron transport systems of the diverse cellular membranes: eukaryotic members of the cyt P450 family have bacterial homologues [201, 204], while certain components with a unique archaeal signature [220] are absent in eukaryotic membranes, such as the isoprenoid ether lipids forming the archaeal PM, or the S-heterocyclic quinones used by some species. Taken at face value, such evidence seems to favor the scenario of Fig. 1b. An alternative explanation is a metabolic "takeover", where archaeal membrane lipids and proteins were replaced by eubacterial biochemistry, after the endosymbiosis according Fig. 1a had been established [9,22]. Evidence for an archaeal contribution to eukaryotic membranes is, for instance, provided by the V-type ATPase of eukaryotic PMs and various organelle membranes, which resembles more the archaeal A-type complex than the bacterial F-type ATPase that occurs in mitochondria and chloroplasts [221,222].

Recently, in the α-proteobacterium *Agrobacterium tumefaciens* the presence of organelles similar to acidocalcisomes was reported [223]. Acidocalcisomes are membrane-enclosed organelles known from unicellular eukaryotes; they are acidified by a H^+-pyrophosphatase and accumulate ions such as calcium and magnesium. The same features have been detected now for the bacterial vesicles, and this is the first report of an organelle that seems to be homologous

in the cytoplasm of bacteria and eukaryotes. It may have developed before the emergence of the eukaryotes [223], and if this finding is corroborated, in particular by demonstration of acidocalcisomes in other bacteria, then it may have also profound implications for the reconstruction of the eukaryotic origin.

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