Opinion

Something old, something new, something borrowed, something blue: the anaerobic microbial ancestry of aerobic respiration

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Aerobic respiration evolved by bricolage, with modules cobbled together as microbial biochemistry coevolved with Earth's geochemistry. The mitochondrial electron transport chain represents a patchwork of respiratory modules inherited from microbial methanogenesis, iron oxidation, anoxygenic photosynthesis, and denitrification pathways, and preserves a biochemical record of Earth's redox environment over its four-billion-year history. Imprints of the anoxic early Earth are recognizable in Complex I's numerous iron-sulfur cofactors and vestigial binding sites for ferredoxin, nickel-iron, and molybdopterin, whereas the more recent advent of oxygen as a terminal electron acceptor necessitated use of heme and copper cofactors by Complex IV. Bricolage of respiratory complexes resulted in supercomplexes for improved electron transfer efficiency in some bacteria and archaea, and in many eukaryotes. Accessory subunits evolved to wrap mitochondrial supercomplexes for improved assembly and stability. Environmental microbes with 'fossil' proteins that are similar to ancestral forms of the respiratory complexes deserve further scrutiny and may reveal new insights on the evolution of aerobic respiration.

Deep breaths: the evolution of aerobic respiration

Broadly defined, respiration is the transfer of electrons coupled to the pumping of ions (protons or sodium ions) across the membrane with formation of a transmembrane gradient of protons or sodium ions. This gradient then discharges through ATP synthase to make ATP. Aerobic respiration in mitochondria and many free-living bacteria starts with Complexes I and II, which transfer electrons to ubiquinone (UQ) from NADH and succinate, respectively. Reduced UQ carries electrons through the inner membrane to Complex III, which transfers them to cytochrome c and then on to Complex IV, where the terminal reduction of molecular oxygen (O_2) to water occurs. In the process, Complexes I, III, and IV pump a total of ten protons across the membrane, generating a proton gradient that drives ATP synthesis.

The evolution of aerobic respiration is an example of **bricolage** (see Glossary) [1]. Respiratory complexes in our mitochondria did not originate with the same substrates and **cofactors** that today shepherd electrons from the food we eat to the oxygen (O_2) we breathe; rather, aerobic respiration emerged from bricolages of anaerobic respiratory modules that transport electrons to a terminal oxidase. Over billions of years of evolution, prokaryotic respiratory complexes evolved with the rising oxidation state of the Earth's surface, from the hydrogen (H_2) redox potential to the O_2 redox potential, from iron-sulfur proteins to heme proteins and blue-copper proteins [2–5]. Individual proteins merged into modules, which assembled into multimodular complexes [6], which coalesced into electron transport chains. Each of these complexes has

Highlights

The mitochondrial electron transport chain is derived from modular enzyme complexes assembled from microbial pathways, including methanogenesis, iron oxidation, anoxygenic photosynthesis, and denitrification.

The vestiges of ancient anaerobic respiratory complexes are especially evident in Complex I, which represents a bricolage of modules, each of which originated with a function different from that which they have today.

Ancestral Complex II likely ran in the opposite direction for anaerobic respiration and was later co-opted to provide additional reducing power for aerobic respiration.

Complex III likely originated in anaerobic anoxygenic photosynthetic bacteria.

Ancestral Complex IV was likely involved in denitrification, and iron-oxidizing bacteria may have contributed to adaptation of Complex IV to higher oxygen levels.

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its own multi-billion-year history. Just as unpeeling the onion of the ribosome can transport us back to the origin of translation, unraveling respiratory molecular machines can teleport us back to the very beginning of microbial metabolism.

Something old: the Q module at the core of Complex I

In many bacteria and most eukaryotes, Complex I (NADH:Q oxidoreductase) contains three modules [7], each of which performs a key function: the N module accepts electrons from NADH (Figure 1A), the Q module passes the electrons to the quinone Q (Figure 1B), and the P module translocates protons across the inner membrane (Figure 1C). The original ancestor of the Q module likely arose prior to the divergence of archaea and bacteria [8], and probably functioned as a hydrogenase [9–11]. Phylogenetic analyses suggest that Complex I's Q module evolved from nickel-iron membrane-bound hydrogenases most like those found in methanogenic archaea [2,11]. As the oxidation state of the Earth rose, the Q module lost its hydrogenase activity and began passing electrons to quinone-based **coenzymes** with higher midpoint redox potential [2,12], first probably to the low-potential menaquinone (MQ), and eventually to the high-potential UQ in aerobic bacteria (Figure 1D) and plastoquinone (PQ) in cyanobacteria (and, later, in plants). Electron transport via lipid-soluble quinone/quinol coenzymes enabled life to harvest more energy per electron by expanding beyond a single complex into a chain of complexes along the inner membrane.

Something new: growth of Complex I

Over billions of years, Complex I continued growing by bricolage. Merger of the Q module with a transmembrane monovalent **antiporter** (the P module; Figure 1C) enabled coupling of transport of electrons and antiport of monovalent ions [13–15]. Acquisition of the P module was a multistep event, with antiporter subunits likely acquired one at a time, as reflected in the fact that different prokaryotic relatives of mitochondrial complex I have different numbers of antiporter subunits [2]. This bricolage necessitated synchronization of electron transfer with slower rates of ion translocation [16]. These ions were primarily sodium in more ancient respiratory pathways, while protons became widespread in more recently evolved respiratory pathways [17,18]. After the acquisition of the N module, the Q module acquired numerous iron-sulfur clusters from agglomeration of iron-sulfur enzymes in order to establish a connection with the remote NADH-binding site [16]. The presence of vestigial binding sites for ferredoxin and molybdopterin in the N module suggests that ancestral forms used lower redox potential coenzymes, which were later replaced by higher redox-potential NADH and flavin mononucleotide (Figure 1E) [2,16,19,20].

Something borrowed: Complexes II and III

Complex II (succinate dehydrogenase), composed of heme b, three iron-sulfur clusters, and flavin adenine dinucleotide (FAD) (Figure 1G), is shared with the citric acid cycle and provides another source of reduced UQ (ubiquinol) to the respiratory chain. Succinate dehydrogenase is thought to have evolved from membrane-bound fumarate reductase in anaerobic fumarate respiration, which is essentially the same enzyme run in reverse (Figure 1G), with electrons from a lower redox-potential quinol, likely menaquinol [21]. As atmospheric O_2 levels rose and anaerobic terminal electron acceptors like fumarate were replaced by O_2 (see discussion of Complex IV later), fumarate reductase was refashioned to serve as a source of reduced UQ with electrons from succinate.

Complex III (cytochrome bc_1 complex) is an example of 'borrowed' machinery that is used for the same purpose in a different metabolism. Modern Complex III accepts two electrons from reduced UQ and shuttles the electrons through the Rieske iron-sulfur protein to cytochrome c, the high-potential redox carrier that can reduce O_2 to water in Complex IV (Figure 1H). The other electron is recycled back to the UQ pool via cytochrome b in a so-called Q-cycle [22]. Complex III

Glossary

Antiporter: a membrane protein that transports two molecules at the same time in opposite directions.
Bricolage: from the French word meaning construction (as of a sculpture or a structure of ideas) achieved by using whatever comes to hand; used here to mean the appearance of new molecular structures by combining and alteration of pre-existing ones.
Chemolithotroph: an organism that uses inorganic reduced compounds as a source of energy.
Coenzyme: an organic compound that binds to an enzyme to catalyze a reaction.
Cofactor: a non-protein chemical compound or metal ion required for an enzyme's role as a catalyst.
**Great Oxidation Event:** the period ~2.4 billion years ago when molecular oxygen (O_2) produced by cyanobacteria began to accumulate in the Earth's atmosphere and shallow oceans.
Magnetotactic bacteria: bacteria that produce magnetic minerals to align with the Earth's magnetic field and access redox gradients for respiration.
Respirasome: macromolecular assemblies of the respiratory chain complexes I, III, and IV in the inner mitochondrial membrane.
Respiratory supercomplex: membrane-bound clusters of respiratory complexes.
Supercomplex: a stable association of two or more complexes of biological macromolecules that occur separately elsewhere.
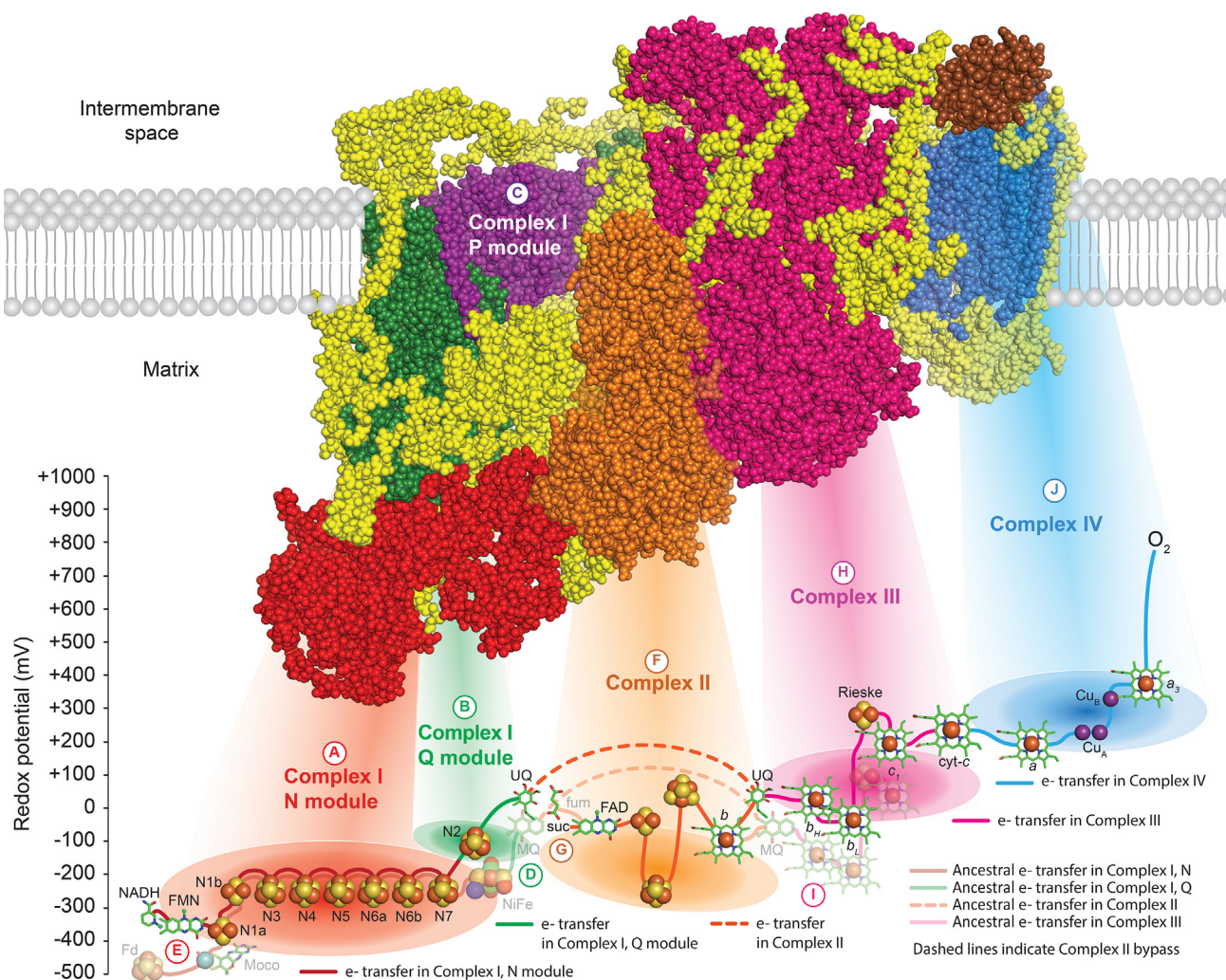
Figure 1. The respirasome evolved via bricolage of subunits, with modern cofactors at higher redox potential than original cofactors. Top: Mammalian respirasome (PDB 5J4Z; CI:CIII$_2$:CIV), CII (PDB 1NEK), and cytochrome *c* (PDB 1HRC). Bottom: Cofactors and coenzymes in aerobic electron transport chain, plotted by redox potential, in millivolts, from electron donor (NADH at –320 mV) to electron acceptor (O$_2$ at +815 mV). Inorganic cofactors in mitochondrial respiration include iron-sulfur clusters (labeled with CI annotation), heme molecules, and copper atoms, where red spheres are iron atoms, yellow spheres are sulfur atoms, and purple spheres are copper atoms. Only redox-active functional groups are shown for organic cofactors (NADH, FMN, Moco, UQ, MQ, FAD), with each small green sphere representing a ribonucleotide substituent. Ancestral coenzymes and cofactors (Fd, Moco, NiFe, MQ, and Complex III cofactors) have lower redox potentials than their modern replacements and are shown as partially transparent, with the teal sphere representing a molybdenum atom and the blue sphere representing a nickel atom. Lines represent electron transfer through the modules, with ancestral pathways shown as partially transparent. Broken lines indicate flow from quinones to Complex III, bypassing Complex II. Each 'spotlight' color represents a respiratory module: red/green/purple, N/Q/P-modules (Complex I); orange, succinate dehydrogenase (Complex II); pink, cytochrome *bc$_1$* complex (Complex III); blue, cytochrome *c* oxidase (Complex IV); brown, cytochrome *c*; yellow, additional subunits in mammalian respirasome. Circled labels A–J are described in the main text.

Abbreviations: CI–IV, Complex I–IV; cyt, cytochrome; FAD, flavin adenine dinucleotide; Fd, ferredoxin; FMN, flavin mononucleotide; fum, fumarate; Moco, molybdopterin; MQ, menaquinone/menaquinol; NiFe, nickel-iron cofactor; O$_2$, molecular oxygen; ox, oxidized; red, reduced; suc, succinate; UQ, ubiquinone/ubiquinol.

translocates four protons across the membrane per two-electron cycle. The cytochrome *b*-Rieske protein core of Complex III appears to have originated in anaerobic anoxygenic photosynthetic bacteria [23–26], and then spread through lateral gene transfer. This evolutionary scenario is consistent with the requirement of the Q-cycle for high-potential electron acceptors, which were limited to photosynthetic charge separation on the anoxic early Earth [25]. Greater availability of O$_2$ after

the **Great Oxidation Event** resulted in more widespread use of higher redox potential quinones (e.g., UQ instead of MQ) and a corresponding rise in the redox potential of cofactors in Complex III [27] (Figure 1I).

Something blue: Complex IV

Complex IV (cytochrome c oxidase) couples proton translocation to reduction of O_2. In mitochondria and their bacterial relatives, Complex IV contains two copper (Cu) cofactors (Figure 1J): a binuclear Cu_A that passes electrons from cytochrome c to the catalytic site and is thought to have evolved from a combination of two blue copper-type cofactors [28], and a mononuclear Cu_B that forms the catalytic site with a high-spin heme and a crosslinked tyrosine [29]. Mitochondrial heme-copper oxidase (HCO) belongs to type A1 of the HCO superfamily. A-type HCOs have lower affinity for O_2, and a higher proton-pumping efficiency (four protons per catalytic cycle) than C-type HCOs in bacteria adapted to low O_2, which pump two protons per catalytic cycle [29]. The HCO superfamily also includes nitric oxide reductase, which contains an Fe atom in place of Cu_B and performs the second to last step in the anaerobic respiratory pathway of complete denitrification [30].

The evolutionary history of Complex IV has long been hazy. High conservation of histidine residues serving as metal ligands and 12 transmembrane helices forming a catalytic subunit structure led to a consensus that all cytochrome c oxidases in the HCO family share a common ancestor [31–33]. Yet the lack of an obvious root to the HCO phylogenetic tree has frustrated efforts to resolve that ancestor [30,34]. The similarity of the Cu_A site in cytochrome c oxidase subunit II and the Cu_A site in the last enzyme in denitrification, nitrous oxide reductase, as well as the homology between nitric oxide reductases and cytochrome c oxidase, led to the hypothesis that aerobic respiration evolved from a bricolage of the last two enzymes in denitrification [35,36], with nitric oxide reductase predating cytochrome c oxidase [29,37]. While A-type HCOs are inhibited by nitric oxide [38–40], C-type HCOs reduce nitric oxide to nitrous oxide [26] using the same mechanism as O_2 reduction [41], suggesting that the ancestral HCO might have reduced nitric oxide as well as O_2. Ancestral HCOs might also have served primarily as electron sinks, with proton pumping for energy conservation as a later addition [42]. The role of ancestral HCOs in denitrification is also supported by the role of HCO homologs in denitrification and detoxification of reactive nitrogen species [31] and the geochemical likelihood that nitric oxide would likely have been more bioavailable than O_2 before the Great Oxidation Event ~2.4 billion years ago (Ga) [37,43], especially given evidence of the onset of nitrification and denitrification by ~2.5 Ga [44–46]. An alternative theory posits that the widespread nature of A-type enzymes suggests antiquity, while the more patchy distribution of C-type enzymes and nitric oxide reductases implies their more recent origin [47,48].

Newer phylogenies offer another explanation: that all the sequences from laboratory cultures were relatively young forms of the enzyme, and a C-type enzyme from environmental metagenomes of *Nitrospirae* is more similar to the originator of all extant HCO types [49,50]. Intriguingly, ancestral features (two transmembrane helices at the N terminus and four protonable residues lining the K/K^c channel) are shared between most A- and C-type oxidases [46]. Further, one of the deepest branching C-type enzymes belongs to *Leptospirillum*, an acidophilic, iron-oxidizing bacterium that lives in low-O_2, low-pH environments [50,51]. These environments also host **chemolithotrophic** basal proteobacteria, such as *Acidithiobacillus*, which use the *rus* pathway to oxidize ferrous iron and sulfide, and contain a deep-branching form of heme A synthase [51]. Likewise, **magnetotactic** alphaproteobacteria may be a 'living fossil' in the evolutionary history between C-type and A-type cytochrome c oxidase [33]. Ancestors of iron-oxidizing bacteria are implicated with the origins of crucial genes for the eventual emergence of mitochondrial HCOs, and bioleaching of copper sulfides by these organisms may have liberated copper for use in HCOs [51].

Wrapping it all together: respiratory supercomplexes and accessory subunits

Bricolage of bricolages emerged through the evolution of **respiratory supercomplexes**, which are especially prevalent in mitochondrial respiratory chains. **Supercomplexes** are beneficial because they increase electron transport efficiency by decreasing the distance required for diffusion of electron carriers [52] while also minimizing production of harmful reactive oxygen species [53]. Complexes III+IV supercomplexes are well known in yeast mitochondria [54] and also occur in some bacteria and archaea [55,56]. Complex I+II supercomplexes are common in plant mitochondria [57]. The most abundant respiratory supercomplex in mammals, also known as the mitochondrial '**respirasome**', contains Complexes I+III+IV in a ratio of 1:2:1 [58]. The same three complexes form a 1:4:4 supercomplex in *Paracoccus denitrificans* [59].

The last mitochondrial common ancestor, which lived ~1.55 Ga [60], already possessed Complexes I–IV, as well as additional bioenergetic and detoxification systems that were later lost in animal lineages, but retained in some basal eukaryotes, especially those that regularly encounter low-oxygen environments [61,62]. The widespread occurrence and diverse composition of respiratory supercomplexes across the tree of life suggests that supercomplexes independently evolved multiple times in different lineages, before and after the endosymbiotic event that created the mitochondrion.

After the endosymbiotic event, bricolage continued in eukaryotic mitochondria with the buildup of additional subunits involved in assembly, stability, and regulation around each respiratory complex or supercomplex (Figure 1, yellow). Over two dozen additional subunits were added to mitochondrial Complex I, on top of the 14 core subunits shared with bacteria [63]. Four additional accessory subunits were added to mitochondrial Complex II in plants [64]. Mammalian Complex III and IV were appended by six and 11 subunits, respectively, in addition to three core subunits in each. Intricate coordination is required to produce and assemble respiratory complexes from combinations of products from mitochondrial and nuclear genomes [65]. While most respiratory complex genes are encoded by the nucleus, a core group of respiratory complex genes are universally retained in mitochondrial genomes. Retention of these core genes is generally believed to be due to the high hydrophobicity of the membrane proteins they encode, which complicates mitochondrial import [66].

Concluding remarks and future perspectives

The mitochondrial electron transport chain evolved over billions of years from bricolage of anaerobic respiratory proteins. But much remains to be resolved (see Outstanding questions). To rewind the clock on the evolution of respiratory complexes, ancestral sequence reconstruction (ASR) of respiratory enzymes offers a powerful technique to study the characteristics and biochemistry of ancient proteins [67,68] and could inform about the nature of key evolutionary intermediates in aerobic respiration. Estimates of the timing of these deep evolutionary events rely on molecular clock studies, which are challenging due to the absence of body fossils for Precambrian microbial lineages. While mitochondrial genes have been used extensively to identify and date the divergence of animal lineages [69], extending these dates to the microbial origins of the HCO superfamily is notoriously challenging. Geobiological studies that merge environmental constraints from the rock record with protein substrate requirements, such as the O_2 levels required for heme A synthesis [51], hold promise for finally filling in the timeline on the history of respiratory enzymes.

Although textbooks often present mitochondrial Complexes I–IV and ATP synthase as the sole example of respiration, there are in fact multitudes of multicomplex respiratory chains in bacteria and archaea. Each of these respiratory chains evolved to harvest redox energy to generate a

Outstanding questions

When did the last common ancestor of each mitochondrial respiratory complex emerge?

What are the biochemical characteristics of uncharacterized deep-branching clades (e.g., 'clade G' in cytochrome b_6f/bc complexes and heme-copper oxidase homologs)?

How can ancestral sequence reconstruction inform the evolution of respiration?

What is the evolutionary connection, if any, of the Cu_A site in heme-copper oxidase and the Cu_A site in nitrous oxide reductase?

What was the impact of the evolution of respiratory complexes on Earth's atmospheric composition (e.g., methane, nitrous oxide, oxygen) over its four-billion-year history?

gradient of monovalent ions across the membrane to drive ATP synthesis [70–73]. These diverse bacterial and archaeal complexes deserve scrutiny as they may hold clues to missing links in the evolution of aerobic respiration prior to the emergence of eukaryotes. Studies are inherently limited by biochemical knowledge of little-studied protein 'fossils' of respiratory evolution, such as the uncharacterized clade G of cytochrome b_6f/bc complexes [24]. These genes are often present in lesser-studied environmental microbes from far afield locales, as opposed to the better characterized isolates from soil or the human microbiome. Ongoing metagenomic sequencing efforts of diverse environments will continue to be valuable for fleshing out deep-branching lineages, as recently demonstrated by phylogenetic placement of cytochrome c oxidases from metagenomic assembled bins [28–33,74]. Additional crystal structures from protein homologs of complexes on the mitochondrial electron transport chain, such as the hydrogen gas-evolving membrane-bound hydrogenase [75,76], will be insightful for deconvolving the structural foundations of bricolage over the eons. Such investigations will help to fill in missing pieces of the puzzle to reveal the full picture of the anaerobic origins of aerobic respiration.

Declaration of interests

No interests are declared.

References

1. Jacob, F. (1977) Evolution and tinkering. *Science* 196, 1161–1166
2. Schut, G.J. *et al.* (2016) The role of geochemistry and energetics in the evolution of modern respiratory complexes from a proton-reducing ancestor. *Biochim. Biophys. Acta Bioenerg.* 1857, 958–970
3. Baltscheffsky, H. (1974) A new hypothesis for the evolution of biological electron transport. In *Cosmochemical Evolution and the Origins of Life* (Oró, J. *et al.*, eds), pp. 387–395, Springer
4. Barnabas, J. *et al.* (1982) Evolution of major metabolic innovations in the Precambrian. *Orig. Life* 12, 81–91
5. Raanan, H. *et al.* (2020) Small protein folds at the root of an ancient metabolic network. *Proc. Natl. Acad. Sci. U. S. A.* 117, 7193–7199
6. Calisto, F. and Pereira, M.M. (2021) Modularity of membrane-bound charge-translocating protein complexes. *Biochem. Soc. Trans.* 49, 2669–2685
7. Friedrich, T. and Böttcher, B. (2004) The gross structure of the respiratory complex I: a Lego System. *Biochim. Biophys. Acta Bioenerg.* 1608, 1–9
8. Baymann, F. *et al.* (2003) The redox protein construction kit: pre-last universal common ancestor evolution of energy-conserving enzymes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 358, 267–274
9. Friedrich, T. and Scheide, D. (2000) The respiratory complex I of bacteria, archaea and eukarya and its module common with membrane-bound multisubunit hydrogenases. *FEBS Lett.* 479, 1–5
10. Albracht, S.P. and Hedderich, R. (2000) Learning from hydrogenases: location of a proton pump and of a second FMN in bovine NADH–ubiquinone oxidoreductase (Complex I). *FEBS Lett.* 485, 1–6
11. Boyd, E.S. *et al.* (2014) Hydrogen metabolism and the evolution of biological respiration. *Microbe* 9, 361–367
12. Degli Esposti, M. (2016) Genome analysis of structure–function relationships in respiratory complex I, an ancient bioenergetic enzyme. *Genome Biolog. Evol.* 8, 126–147
13. Mathiesen, C. and Hägerhäll, C. (2002) Transmembrane topology of the NuoL, M and N subunits of NADH: quinone oxidoreductase and their homologues among membrane-bound hydrogenases and bona fide antiporters. *Biochim. Biophys. Acta Bioenerg.* 1556, 121–132
14. Moparthi, V.K. *et al.* (2014) Functional role of the MrpA-and MrpD-homologous protein subunits in enzyme complexes evolutionary related to respiratory chain complex I. *Biochim. Biophys. Acta Bioenerg.* 1837, 178–185
15. Moparthi, V.K. and Hägerhäll, C. (2011) The evolution of respiratory chain complex I from a smaller last common ancestor consisting of 11 protein subunits. *J. Mol. Evol.* 72, 484–497
16. Gnandt, E. *et al.* (2016) The multitude of iron–sulfur clusters in respiratory complex I. *Biochim. Biophys. Acta Bioenerg.* 1857, 1068–1072
17. Mulkidjanian, A.Y. *et al.* (2008) Evolutionary primacy of sodium bioenergetics. *Biol. Direct* 3, 1–19
18. Mulkidjanian, A.Y. *et al.* (2008) The past and present of sodium energetics: may the sodium-motive force be with you. *Biochim. Biophys. Acta Bioenerg.* 1777, 985–992
19. Finel, M. (1998) Organization and evolution of structural elements within complex I. *Biochim. Biophys. Acta Bioenerg.* 1364, 112–121
20. Rothery, R.A. *et al.* (2008) The prokaryotic complex iron–sulfur molybdoenzyme family. *Biochim. Biophys. Acta Bioenerg.* 1778, 1897–1929
21. Jardim-Messeder, D. *et al.* (2017) Fumarate reductase superfamily: a diverse group of enzymes whose evolution is correlated to the establishment of different metabolic pathways. *Mitochondrion* 34, 56–66
22. Mitchell, P. (1975) The protonmotive Q cycle: a general formulation. *FEBS Lett.* 59, 137–139
23. Schütz, M. *et al.* (2000) Early evolution of cytochrome bc complexes. *J. Mol. Biol.* 300, 663–675
24. Dibrova, D.V. *et al.* (2013) Evolution of cytochrome bc complexes: from membrane-anchored dehydrogenases of ancient bacteria to triggers of apoptosis in vertebrates. *Biochim. Biophys. Acta Bioenerg.* 1827, 1407–1427
25. Dibrova, D.V. *et al.* (2017) Emergence of cytochrome bc complexes in the context of photosynthesis. *Physiol. Plant.* 161, 150–170
26. Lebrun, E. *et al.* (2006) The Rieske protein: a case study on the pitfalls of multiple sequence alignments and phylogenetic reconstruction. *Mol. Biol. Evol.* 23, 1180–1191

27. Bergdoll, L. *et al.* (2016) From low-to high-potential bioenergetic chains: thermodynamic constraints of Q-cycle function. *Biochim. Biophys. Acta Bioenerg.* 1857, 1569–1579

28. Rydén, L.G. and Hunt, L.T. (1993) Evolution of protein complexity: the blue copper-containing oxidases and related proteins. *J. Mol. Evol.* 36, 41–66

29. Sharma, V. and Wikström, M. (2014) A structural and functional perspective on the evolution of the heme–copper oxidases. *FEBS Lett.* 588, 3787–3792

30. Sousa, F.L. *et al.* (2012) The superfamily of heme–copper oxygen reductases: types and evolutionary considerations. *Biochim. Biophys. Acta Bioenerg.* 1817, 629–637

31. Pei, J. *et al.* (2014) Conserved evolutionary units in the heme-copper oxidase superfamily revealed by novel homologous protein families. *Protein Sci.* 23, 1220–1234

32. Pereira, M.M. *et al.* (2001) A novel scenario for the evolution of haem–copper oxygen reductases. *Biochim. Biophys. Acta Bioenerg.* 1505, 185–208

33. Degli Esposti, M. *et al.* (2019) Oxygen reductases in alphaproteobacterial genomes: physiological evolution from low to high oxygen environments. *Front. Microbiol.* 10, 499

34. Ducluzeau, A.-L. *et al.* (2014) The evolution of respiratory O_2/NO reductases: an out-of-the-phylogenetic-box perspective. *J. R. Soc. Interface* 11, 20140196

35. Viebrock, A. and Zumft, W. (1988) Molecular cloning, heterologous expression, and primary structure of the structural gene for the copper enzyme nitrous oxide reductase from denitrifying *Pseudomonas stutzeri. J. Bacteriol.* 170, 4658–4668

36. Saraste, M. and Castresana, J. (1994) Cytochrome oxidase evolved by tinkering with denitrification enzymes. *FEBS Lett.* 341, 1–4

37. Ducluzeau, A.-L. *et al.* (2009) Was nitric oxide the first deep electron sink? *Trends Biochem. Sci.* 34, 9–15

38. Cooper, C.E. (2002) Nitric oxide and cytochrome oxidase: substrate, inhibitor or effector? *Trends Biochem. Sci.* 27, 33–39

39. Poderoso, J.J. *et al.* (2019) The effect of nitric oxide on mitochondrial respiration. *Nitric Oxide* 88, 61–72

40. Blomberg, M.R. and Ädelroth, P. (2018) Mechanisms for enzymatic reduction of nitric oxide to nitrous oxide – a comparison between nitric oxide reductase and cytochrome c oxidase. *Biochim. Biophys. Acta Bioenerg.* 1859, 1223–1234

41. Blomberg, M.R. (2020) Role of the two metals in the active sites of heme copper oxidases – a study of NO reduction in cbb_3 cytochrome c oxidase. *Inorg. Chem.* 59, 11542–11553

42. Chen, J. and Strous, M. (2013) Denitrification and aerobic respiration, hybrid electron transport chains and co-evolution. *Biochim. Biophys. Acta Bioenerg.* 1827, 136–144

43. Stanton, C.L. *et al.* (2018) Nitrous oxide from chemodenitrification: a possible missing link in the Proterozoic greenhouse and the evolution of aerobic respiration. *Geobiology* 16, 597–609

44. Stüeken, E.E. *et al.* (2016) The evolution of Earth's biogeochemical nitrogen cycle. *Earth Sci. Rev.* 160, 220–239

45. Zerkle, A. and Mikhail, S. (2017) The geobiological nitrogen cycle: from microbes to the mantle. *Geobiology* 15, 343–352

46. Garvin, J. *et al.* (2009) Isotopic evidence for an aerobic nitrogen cycle in the latest Archean. *Science* 323, 1045–1048

47. Brochier-Armanet, C. *et al.* (2009) The multiple evolutionary histories of dioxygen reductases: implications for the origin and evolution of aerobic respiration. *Mol. Biol. Evol.* 26, 285–297

48. Gribaldo, S. *et al.* (2009) Evolution of the haem copper oxidases superfamily: a rooting tale. *Trends Biochem. Sci.* 34, 375–381

49. Murali, R. *et al.* (2021) Diversity and evolution of nitric oxide reduction. *bioRxiv* Published online November 10, 2021. https://doi.org/10.1101/2021.10.15.464467

50. Degli Esposti, M. (2020) On the evolution of cytochrome oxidases consuming oxygen. *Biochim. Biophys. Acta Bioenerg.* 1861, 148304

51. Degli Esposti, M. *et al.* (2021) Respiratory heme A-containing oxidases originated in the ancestors of iron-oxidizing bacteria. *Front. Microbiol.* 12, 1465

52. Berndtsson, J. *et al.* (2020) Respiratory supercomplexes enhance electron transport by decreasing cytochrome c diffusion distance. *EMBO Rep.* 21, e51015

53. Diaz, F. *et al.* (2012) Cells lacking Rieske iron-sulfur protein have a reactive oxygen species-associated decrease in respiratory complexes I and IV. *Mol. Cell. Biol.* 32, 415–429

54. Brzezinski, P. *et al.* (2021) Structure and mechanism of respiratory III–IV supercomplexes in bioenergetic membranes. *Chem. Rev.* 121, 9644–9673

55. Magalon, A. *et al.* (2012) Supramolecular organization in prokaryotic respiratory systems. *Adv. Microb. Physiol.* 61, 217–266

56. Melo, A.M. and Teixeira, M. (2016) Supramolecular organization of bacterial aerobic respiratory chains: from cells and back. *Biochim. Biophys. Acta Bioenerg.* 1857, 190–197

57. Dudkina, N.V. *et al.* (2005) Structure of a mitochondrial supercomplex formed by respiratory-chain complexes I and III. *Proc. Natl. Acad. Sci. U. S. A.* 102, 3225–3229

58. Gu, J. *et al.* (2016) The architecture of the mammalian respirasome. *Nature* 537, 639–643

59. Stroh, A. *et al.* (2004) Assembly of respiratory complexes I, III, and IV into NADH oxidase supercomplexes stabilizes complex I in *Paracoccus denitrificans. J. Biol. Chem.* 279, 5000–5007

60. Wang, S. and Luo, H. (2021) Dating Alphaproteobacteria evolution with eukaryotic fossils. *Nat. Commun.* 12, 1–9

61. Degli Esposti, M. *et al.* (2014) Evolution of mitochondria reconstructed from the energy metabolism of living bacteria. *PLoS One* 9, e96566

62. McDonald, A.E. and Gospodaryov, D.V. (2019) Alternative NAD(P)H dehydrogenase and alternative oxidase: proposed physiological roles in animals. *Mitochondrion* 45, 7–17

63. Gabaldón, T. *et al.* (2005) Tracing the evolution of a large protein complex in the eukaryotes, NADH: ubiquinone oxidoreductase (Complex I). *J. Mol. Biol.* 348, 857–870

64. Huang, S. *et al.* (2019) Mitochondrial complex II of plants: subunit composition, assembly, and function in respiration and signaling. *Plant J.* 98, 405–417

65. Isaac, R.S. *et al.* (2018) The multiple levels of mitonuclear coregulation. *Annu. Rev. Genet.* 52, 511–533

66. Björkholm, P. *et al.* (2015) Mitochondrial genomes are retained by selective constraints on protein targeting. *Proc. Natl. Acad. Sci. U. S. A.* 112, 10154–10161

67. Garcia, A.K. and Kaçar, B. (2019) How to resurrect ancestral proteins as proxies for ancient biogeochemistry. *Free Radic. Biol. Med.* 140, 260–269

68. Kacar, B. *et al.* (2017) Resurrecting ancestral genes in bacteria to interpret ancient biosignatures. *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* 375, 20160352

69. Hebert, P.D. *et al.* (2003) Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 270, 313–321

70. Simon, J. *et al.* (2008) The organisation of proton motive and non-proton motive redox loops in prokaryotic respiratory systems. *Biochim. Biophys. Acta Bioenerg.* 1777, 1480–1490

71. Schoepp-Cothenet, B. *et al.* (2013) On the universal core of bioenergetics. *Biochim. Biophys. Acta Bioenerg.* 1827, 79–93

72. Spero, M.A. *et al.* (2015) Phylogenomic analysis and predicted physiological role of the proton-translocating NADH: quinone oxidoreductase (complex I) across bacteria. *mBio* 6 e00389-00315

73. Richardson, D.J. (2000) Bacterial respiration: a flexible process for a changing environment. *Microbiology* 146, 551–571

74. Soo, R.M. *et al.* (2017) On the origins of oxygenic photosynthesis and aerobic respiration in Cyanobacteria. *Science* 355, 1436–1440

75. Yu, H. *et al.* (2018) Structure of an ancient respiratory system. *Cell* 173, 1636–1649

76. Yu, H. *et al.* (2020) Structure of the respiratory MBS complex reveals iron-sulfur cluster catalyzed sulfane sulfur reduction in ancient life. *Nat. Commun.* 11, 5953