Trends in Microbiology

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pinion

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

Jennifer B. Glass [1,*] Claire E. Elbon [1] and Loren Dean Williams [2]

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 denitri cation 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 ef ciency 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.

Highlights

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

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 denitri cation, and iron-oxidizing bacteria may have contributed to adaptation of Complex IV to higher oxygen levels.

Deep breaths: the evolution of aerobic respiration

Broadly de ned, 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

[1]School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA, USA
[2]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA, USA

*Correspondence: Jennifer.Glass@eas.gatech.edu J.B. Glass).

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], rst 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 eld 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.

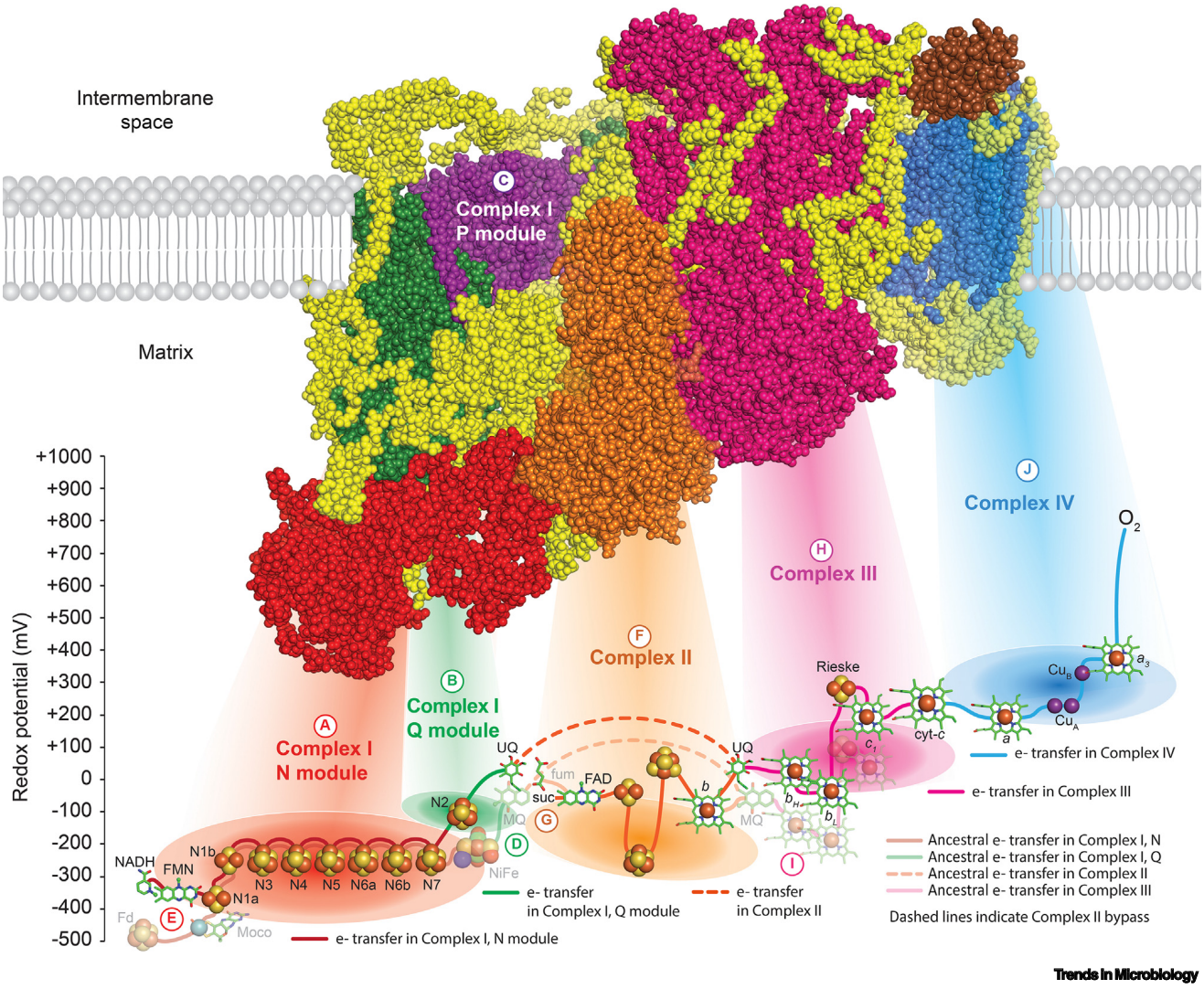

Figure 1. The respirasome evolved via bricolage of subunits, with modern cofactors at higher redox potential than original cofactors. Top: Mammalian respirasome (PDB 5J4Z; CI:$CIII_2$:CIV), CII (PDB 1NEK), and cytochrome *c* (PDB 1HRC). Bottom: Cofactors and coenzymes in aerobic electron transport chain, 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 af nity for O_2, and a higher proton-pumping ef ciency 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 denitri cation [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 denitri cation, 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 denitri cation [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 denitri cation is also supported by the role of HCO homologs in denitri cation and detoxi cation 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 nitri cation and denitri cation 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 sul de, 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 sul des 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 bene cial because they increase electron transport ef ciency 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 denitri cans* [59].

The last mitochondrial common ancestor, which lived ~1.55 Ga [60], already possessed Complexes I–IV, as well as additional bioenergetic and detoxi cation 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 nally lling 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 a eld 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 ll in missing pieces of the puzzle to reveal the full picture of the anaerobic origins of aerobic respiration.

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Declaration of interests

No interests are declared.

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