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Nature's conductors: what can microbial multi-heme cytochromes teach us about electron transport and biological energy conversion?

Grace W Chong[1], Amruta A Karbelkar[2] and Mohamed Y El-Naggar[1,2,3]

Abstract

Microorganisms can acquire energy from the environment by extending their electron transport chains to external solid electron donors or acceptors. This process, known as extracellular electron transfer (EET), is now being heavily pursued for wiring microbes to electrodes in bioelectrochemical renewable energy technologies. Recent studies highlight the crucial role of multi-heme cytochromes in facilitating biotic–abiotic EET both for cellular electron export and uptake. Here we explore progress in understanding the range and function of these biological electron conduits in the context of fuel-to-electricity and electricity-to-bioproduct conversion. We also highlight emerging topics, including the role of multi-heme cytochromes in inter-species electron transfer and in inspiring the design and synthesis of a new generation of protein-based bioelectronic components.

Addresses
[1] Molecular and Computational Biology Section, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-2910, USA
[2] Department of Chemistry, University of Southern California, Los Angeles, CA 90089-1062, USA
[3] Department of Physics and Astronomy, University of Southern California, Los Angeles, CA 90089-0484, USA

Corresponding author: El-Naggar, Mohamed Y (mnaggar@usc.edu)

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Introduction — wiring microbes to the electrodes of energy technologies

Electron transfer, when coordinated with proton transfer in respiration, charges biological membranes for the synthesis of energy-storing ATP molecules. For many respiratory organisms, maintaining this electron flow depends on the availability of soluble electron donors (food) and acceptors (e.g. O_2) that can enter cells to interact with bioenergetic machinery. However, some microorganisms are equipped with *extracellular* electron transfer (EET) conduits that bridge intracellular reactions to insoluble oxidants or reductants, including electrodes, outside the cells. Microbes evolved this strategy to access abundant redox-active respiratory electron acceptors and donors (e.g. S, Fe, Mn) found in minerals [1].

The two most well-studied EET-capable microbes are the metal-reducing bacteria *Shewanella* and *Geobacter*, first discovered three decades ago [2,3]. Since then, extensive studies of *Shewanella oneidensis* and *Geobacter sulfurreducens* have revealed the central role of multi-heme cytochromes (MHCs) in moving electrons between cell interior and exterior [1,4,5]. With a redox potential range that spans nearly 1 V and stability against chemical modifications relative to other metalloproteins [6], it is perhaps not surprising that cytochromes have been selected for use as electron conductors in extracellular environments and at biotic–abiotic interfaces. A fundamental understanding of microbial EET has many promising applications, especially in the development of bioelectrochemical systems (BES) [7,8]. As depicted in Figure 1, BES generally rely on microbes catalyzing activity on an anode, cathode, or both, to achieve a desired outcome, such as: (1) electricity generation via cell-to-electrode EET in microbial fuel cells [9]; (2) wastewater treatment powered by microbial oxidation of organic wastes [10]; (3) bioremediation, where toxic contaminants are transformed into less soluble, less toxic forms [11]; and (4) production of biofuels or other value-added products, as done by electrode-to-cell EET in microbial electrosynthesis cells [12].

Here, we highlight recent studies that describe how MHCs contribute to both outward and inward EET. We also discuss novel discoveries of direct interspecies electron transfer that are thought to involve MHCs, and recent work on the synthesis of EET-inspired bioelectronic components.

Multi-heme cytochromes in outward extracellular electron transfer

The genomes of *S. oneidensis* and *G. sulfurreducens* encode for 73 and 42 MHCs, respectively [13,14], including a combination of inner membrane, periplasmic, and outer membrane-associated MHCs with overlapping redox

Figure 1

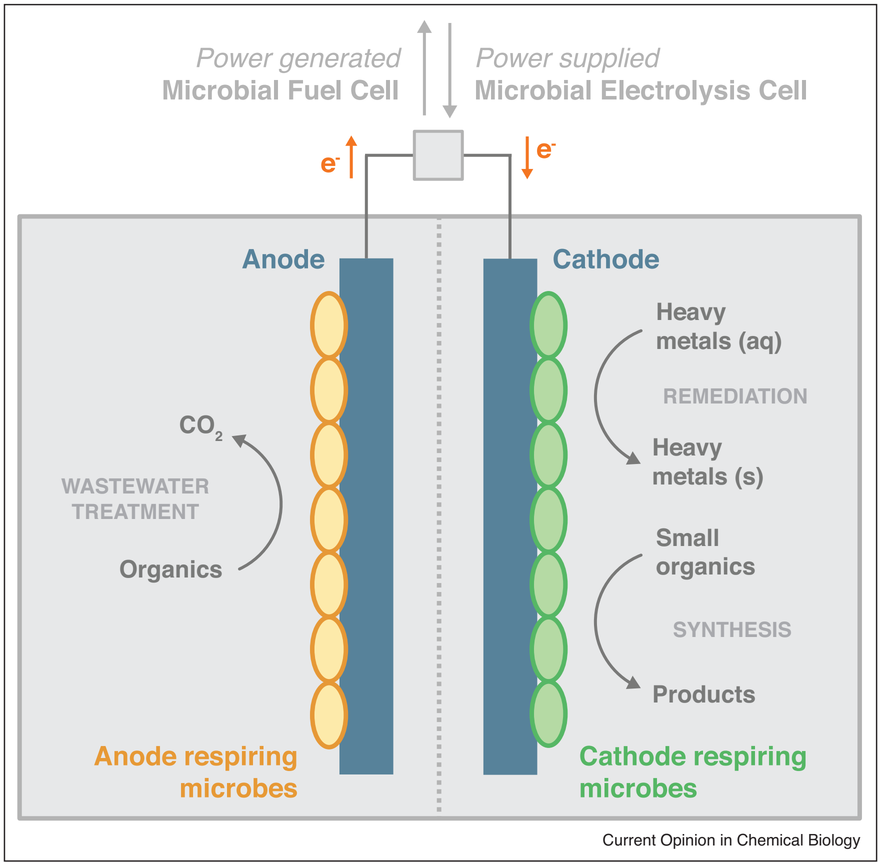

Diagram of a bioelectrochemical system (BES). Microbes can catalyze environmentally or industrially relevant processes at electrodes, including the production of electricity, wastewater treatment, bioremediation of toxic compounds, and the synthesis of biofuels or other useful products.

potentials that relay electrons towards the cell surface [5,15]. *Geobacter*'s EET network is particularly intricate, as multiple homologues and parallel pathways exist for EET across the cell envelope [[16•]]. In the *Shewanella* EET network, electrons proceed from the inner-membrane-associated tetraheme menaquinol dehydrogenase CymA to periplasmic MHCs (STC and FccA) that in turn transfer electrons to the MtrABC porin–cytochrome conduit. The latter allows two decaheme cytochromes, the periplasmic MtrA and outer-membrane-associated MtrC, to interact through the MtrB porin. MtrC, and a partnering decaheme cytochrome OmcA, function as terminals for reduction of external surfaces in a variety of proposed ways: (1) directly contacting electron acceptors at the cell surface through solvent–exposed hemes [4]; (2) incorporating into outer-membrane extensions that may function as nanowires for long-distance EET to distant acceptors [16•]; and (3) reducing soluble redox-active molecules, such as secreted flavins, which may shuttle electrons from cell-surface MHCs to distant acceptors [17]. A simplified model of these EET mechanisms, as described in *S. oneidensis*, is illustrated in Figure 2.

The role of flavins has recently attracted significant interest [18]. Flavins enhance EET from *Shewanella* to electrodes [17], and can be reduced by outer membrane-associated MHCs [19] that contain flavin-binding sites [20–22]. In addition, a bacterial flavin exporter was recently identified in *S. oneidensis* [17]. These observations support the role of flavins as soluble electron carriers in EET. Recent studies also highlight an alternative model where flavins function as MHC-bound cofactors rather than free soluble shuttles, perhaps by promoting rate-limiting proton transport [22–25]. Edwards *et al.* proposed that MHCs form stable flavocytochrome complexes in anaerobic conditions that dissociate in the presence of oxygen [22]. This was investigated electrochemically by Xu *et al.*, who simultaneously captured the redox signatures of free and cytochrome-bound flavins, suggesting that the latter play the primary role while stressing the extent to which different electrode materials and their properties (surface area, flavin affinity) impact different pathways [24]. Flavins also enhanced interfacial electron transfer in cell-free systems where MHCs were embedded in liposomes [26]. Recent molecular dynamics