Biological electron transport goes the extra mile

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Microorganisms stand out by the creative ways in which they gain energy during respiration. Using versatile metabolic networks, respiratory microorganisms harvest electrons from organic or inorganic donor molecules and transfer them to specialized electron transport chains, which redirect their flow toward a terminal electron acceptor. Respiratory electrons move with a purpose: to generate a transmembrane ion gradient, most often of protons, that drives the synthesis of ATP by the ATP synthase complex. Thus, the electron carriers team up and transfer electrons in sequential steps to minimize energy losses to heat while simultaneously pumping protons, consuming them in the cytoplasm, and/or releasing them outside the membrane (Fig. 1). In PNAS, Bjerg et al. (1) report that the electron transport chain of some marine bacteria sequentially transfers electrons at several millimeters, something long believed to be unattainable in biological systems.

How Far Can Electrons Travel in Respiratory Chains?

The traditional view of microbial electron transport chains is that of a set of membrane-bound electron carriers organized from high to low redox potentials to permit the spontaneous flow of electrons to the terminal electron acceptor (Fig. 1). The membrane carriers are not structurally linked so they can diffuse laterally in the membrane and collide with one another to promote the rapid exchange of electrons (2). The model bacterium *Escherichia coli*, for example, uses lipophilic organic molecules called quinones to electronically link a dehydrogenase enzyme complex to a specific terminal reductase (3). If oxygen is available, ubiquinone links two proton pumps, the NADH dehydrogenase complex I (NDH I) and the cytochrome *bo*₃ (Fig. 1). In contrast, menaquinone links NDH I to a fumarate reductase complex during the reduction of fumarate to succinate, a reaction that consumes protons in the cytoplasm (Fig. 1).

This relatively simple electron transport chain lacks the reach needed to transport electrons to extracellular electron acceptors, such as iron oxide minerals.

Thus, iron oxide-reducing bacteria rely on complex and yet not fully understood electron transport chains to transfer electrons across the cell envelope (reviewed in ref. 4). In the model representative *Geobacter sulfur-reducens*, the menaquinone pool electronically links a proton-pumping NAD I to one of two putative quinol oxidases (IcmH and CbcL) of the inner membrane (Fig. 1). From here, electrons are transferred to periplasmic and outer membrane c-type cytochromes, including some that are organized on a porin scaffold (Pcc complexes B and C) (Fig. 1). The periplasmic cytochromes (PpcA–D) are particularly abundant and two of them (PpcA and its homolog PpcD) could also contribute to the proton motive force that drives ATP synthesis (5, 6). These cytochromes can also act as capacitors, storing electrons in the cell envelope (7). This maintains the proton motive force until the cells establish electronic contact with the iron oxide minerals, a step that requires the synthesis of conductive pili (8). These unique protein appendages are an assembly of a very short type IVa pilin peptide (8) and, without organic and inorganic cofactors, they can transport close to one billion electrons per second at the low potential difference (~100 mV) that exists between the inner membrane/periplasmic electron carriers and the extracellular iron oxide minerals (9). A conserved type IV pilus apparatus polymerizes and depolymerizes the pilin peptides on the inner membrane and exposes the base of the pilus to the periplasmic cytochromes to facilitate electron discharges. These antagonistic cycles of protrusion and retraction also recycle the pilins in the membrane and permit multiple rounds of mineral binding, reduction, and detachment (10).

The *Geobacter* pili extend the redox-active surface of the cell at micrometer distances and provide a competitive advantage to locate the minerals, which are often dispersed in soils and sediments and therefore difficult to reach. The pili also mediate the extracellular reduction of soluble toxic metals, such as the uranyl cation, which would otherwise traverse the outer membrane and mineralize in the periplasmic space (11). Furthermore, the pili promote cell–cell

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Fig. 1. Electron transport chains for the cytoplasmic reduction of O₂ and fumarate in *E. coli* (Left) and the extracellular reduction of Fe(III) oxide minerals in *G. sulfurreducens* (Right). Abbreviations: *bo*₃, cytochrome *bo*₃; Frd, fumarate reductase complex; IM, inner membrane; MQ, menaquinone; MQH₂, menaquinol; NDH I, NADH dehydrogenase complex I; OM, outer membrane; Omc, outer membrane cytochrome; PpcB/C, porin-cytochrome complex B or C; PpcA–E, periplasmic cytochromes A–E; PS, periplasmic space; Qox, one of two putative quinol oxidases (ImcH and CbcL); UQ, ubiquinone; UQH₂, ubiquinol.

aggregation and the formation of electroactive biofilms (12, 13), where cells are embedded in a matrix of *c*-type cytochromes and conductive pili that interact to promote the collective discharge of respiratory electrons across biofilms tens of micrometers thick (14). In PNAS, and defying all expectations, Bjerg et al. (1) describe a mechanism involving cytochromes that allows some marine filamentous bacteria to transport electrons at millimeter distances.

In Cable Bacteria, Electrons Just Keep Going... and Going

In marine sediments, the respiratory activities of microorganisms are vertically stratified from top to bottom following the hierarchical position of the terminal electron acceptors in a redox tower, from the highest to the lowest reduction potential (Fig. 2). The oxic layer, just below the water layer, provides the electron acceptor for aerobic respiratory microorganisms. Once oxygen is exhausted, nitrate becomes the preferred electron acceptor for respiration, then manganese and iron oxides. Sulfate reduction to sulfide gas takes place deeper in the sediment, sometimes 1–4 cm away from the water layer. Despite their spatial separation, the oxygen and sulfide layers are interconnected and expand and contract in response to the availability of sulfide and oxygen. The responses are so fast that they can only be explained if involving electric currents (15). In 2012, Pffefer et al. (16) linked the spatial coupling of sulfide oxidation and oxygen reduction in the sediments to the presence of a novel group of yet to be cultivated bacteria in the family *Desulfobulbaceae*. These bacteria, also known as “cable bacteria,” grow as multicellular filaments across the sediments and form dense networks that physically connect the sulfide and oxide layers (16). By consuming the sulfide deep in the sediment, cable bacteria contain the spread of the toxic gas and prevent its ascent to the oxic layer, where it would otherwise poison the electron transport chains of aerobic organisms (17).

Cable bacteria have intriguing morphological and electronic features that hinted early on at their ability to act as bacterial power cords (16). Their outer membrane is ridged with tubular channels that connect the periplasmic space of neighboring cells. Electrostatic force microscopy suggests that the content of the periplasmic conduits is electrically charged (16). To investigate the nature of the charged carriers, Bjerg et al. (1) constructed a microscope chamber with spatially separated sulfide and oxic layers that permitted the growth of cable bacteria and the probing of

their periplasmic content via Raman spectroscopy. The Raman spectra revealed the distinctive spectroscopic signatures of *c*-type cytochromes and peak intensities consistent with an accumulation of reduced cytochromes in the sulfide region. However, the ratio of reduced-to-oxidized cytochromes decreased progressively toward the oxic layer without any changes in the levels of cytochrome expression. Taken together, the results provide compelling evidence for long-range conduction via cytochromes in cable bacteria. The cytochromes serve as capacitors, storing electrons harvested from the oxidation of the electron donor (sulfide) deep in the

Fig. 2. Spatial coupling of sulfide oxidation and oxygen reduction by cable bacteria. The arrow at left shows the alignment of electron acceptors (O₂, nitrate [NO₄²⁻], Mn(IV), Fe(III), and sulfate [SO₄²⁻]) based on their standard reduction potential (E⁰, in volts). This hierarchical order of the electron acceptors mirrors the stratification of respiratory activities in the sediments. Cable bacteria harvest electrons from sulfide, the product of sulfate reduction, and use periplasmic conduits with cytochromes to transport the electrons to the upper cells, which use them to reduce O₂. The color of the cells in the filament represents the gradient of reduced (negatively charged; maroon) to oxidized (positively charged; blue) cytochromes.

sediments. Once the filaments grow and make the electronic connection with the electron acceptor (oxygen), they discharge the respiratory electrons. This generates a redox gradient of reduced to oxidized cytochromes along the filaments (Fig. 2). As noted by Bjerg et al. (1), an electron path of only cytochromes is unlikely to support transport rates high enough to match the electrical responses of the cable bacteria in the sediments. As in *Geobacter* biofilms (14), a second path, perhaps wired with conductive pili, could mechanistically stratify the electron transport chain and promote optimal rates of electron transfer.

Bjerg et al. (1) also consider a role for the cytochromes in loading and discharging electrons at each end of the filaments. The team estimates that the polarization of cable bacteria, like a battery, could result in an internal voltage drop of ~13 mV per millimeter of filament. Thus, a trade-off exists between filament length and energy losses during the aerobic oxidation of sulfide. Such energy cues could control the growth of the multicellular filaments and the electrogenic coupling of sulfide oxidation to alternative electron acceptors, such as nitrate, which is spatially closer to the sulfide layer and also reduced by cable bacteria (18). Syntrophic interactions with other bacteria may also coordinate the growth of the filaments and the electrical coupling of spatially

separated geochemical reactions. Movies of cable bacteria often show filaments surrounded by a swarm of bacteria. The identity and function of these bacteria and their potential contribution to long-range charge transport remain unknown. More studies are also needed to investigate if a wired pathway does indeed exist in cable bacteria and how it interacts with the periplasmic cytochromes. The type of cytochromes involved in long-range electron transfer through the periplasmic conduits also warrants consideration. Hampering these studies is the recalcitrance of cable bacteria to cultivation. However, even with partial enrichments of the filaments it may be possible to apply omics tools and gain molecular insights into their respiratory metabolism. Ultimately, it is important to understand how the electron carriers organize in the periplasmic conduits to transport electrons at distances 1,000-fold the length of individual cells. "Life," as the Nobel laureate Albert Szent-Györgyi once said, "is nothing but an electron looking for a place to rest." In cable bacteria, electrons just go the extra mile.

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