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Minireview

The roles of outer membrane cytochromes of *Shewanella* and *Geobacter* in extracellular electron transfer

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Summary

As key components of the electron transfer (ET) pathways used for dissimilatory reduction of solid iron [Fe(III)] (hydr)oxides, outer membrane multihaem *c*-type cytochromes MtrC and OmcA of *Shewanella oneidensis* MR-1 and OmcE and OmcS of *Geobacter sulfurreducens* mediate ET reactions extracellularly. Both MtrC and OmcA are at least partially exposed to the extracellular side of the outer membrane and their translocation across the outer membrane is mediated by bacterial type II secretion system. Purified MtrC and OmcA can bind Fe(III) oxides, such as haematite (α-Fe_2O_3), and directly transfer electrons to the haematite surface. Bindings of MtrC and OmcA to haematite are probably facilitated by their putative haematite-binding motifs whose conserved sequence is Thr–Pro–Ser/Thr. Purified MtrC and OmcA also exhibit broad operating potential ranges that make it thermodynamically feasible to transfer electrons directly not only to Fe(III) oxides but also to other extracellular substrates with different redox potentials. OmcE and OmcS are proposed to be located on the *Geobacter* cell surface where they are believed to function as intermediates to relay electrons to type IV pili, which are hypothesized to transfer electrons directly to the metal oxides. Cell surface-localized cytochromes thus are key components mediating extracellular ET reactions in both *Shewanella* and *Geobacter* for extracellular reduction of Fe(III) oxides.

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Introduction

In the absence of other terminal electron acceptors, some bacterial species can couple oxidation of organic matter to reduction of oxidized metals, such as iron [Fe(III)] and manganese [Mn(IV)] (hydr)oxides, via a biological process termed dissimilatory metal reduction (DMR) that can be coupled to energy conservation. Dissimilatory metal reduction is directly involved in biogeochemical cycles of carbon and various nutrient elements in addition to Fe, Mn, and a range of trace metals. Microorganisms that carry out DMR can also be used to remediate organic, radionuclide and metal contaminants and to generate electricity via microbial fuel cells (MFCs) (Lovley, 2006; Fredrickson and Zachara, 2008; Fredrickson *et al.*, 2008; Lovley, 2008a,b). Because Fe(III)/Mn(IV) oxides are poorly soluble in water at neutral pH and in the absence of strong complexing ligands, some of the dissimilatory metal-reducing bacteria (DMRB) have developed the ability to transfer electrons across the bacterial cell envelope to the surface of Fe(III)/Mn(IV) oxides external to the cells (for recent reviews, see Richardson, 2000; Schroder *et al.*, 2003; Lovley, 2006; 2008a; Weber *et al.*, 2006; Gralnick and Newman, 2007; Shi *et al.*, 2007; Fredrickson and Zachara, 2008; Fredrickson *et al.*, 2008). Multihaem *c*-type cytochromes (*c*-Cyts) are the major electron carrier proteins used by the Gram-negative bacteria *Shewanella oneidensis* MR-1 and *Geobacter sulfurreducens*, which are the two best-studied DMRB, for reducing metal oxides. These *c*-Cyts are strategically located in the bacterial cell envelope to facilitate the electron transfer **(**ET) from the quinone/quinol pool in the inner membrane to the periplasm and then to the outer membrane (OM) where they are positioned to transfer electrons to the metal oxides either directly, indirectly or both (Figs 1 and 2). This review focuses on the insights gained recently into the roles of OM *c*-Cyts (OMCs) of *S. oneidensis* MR-1

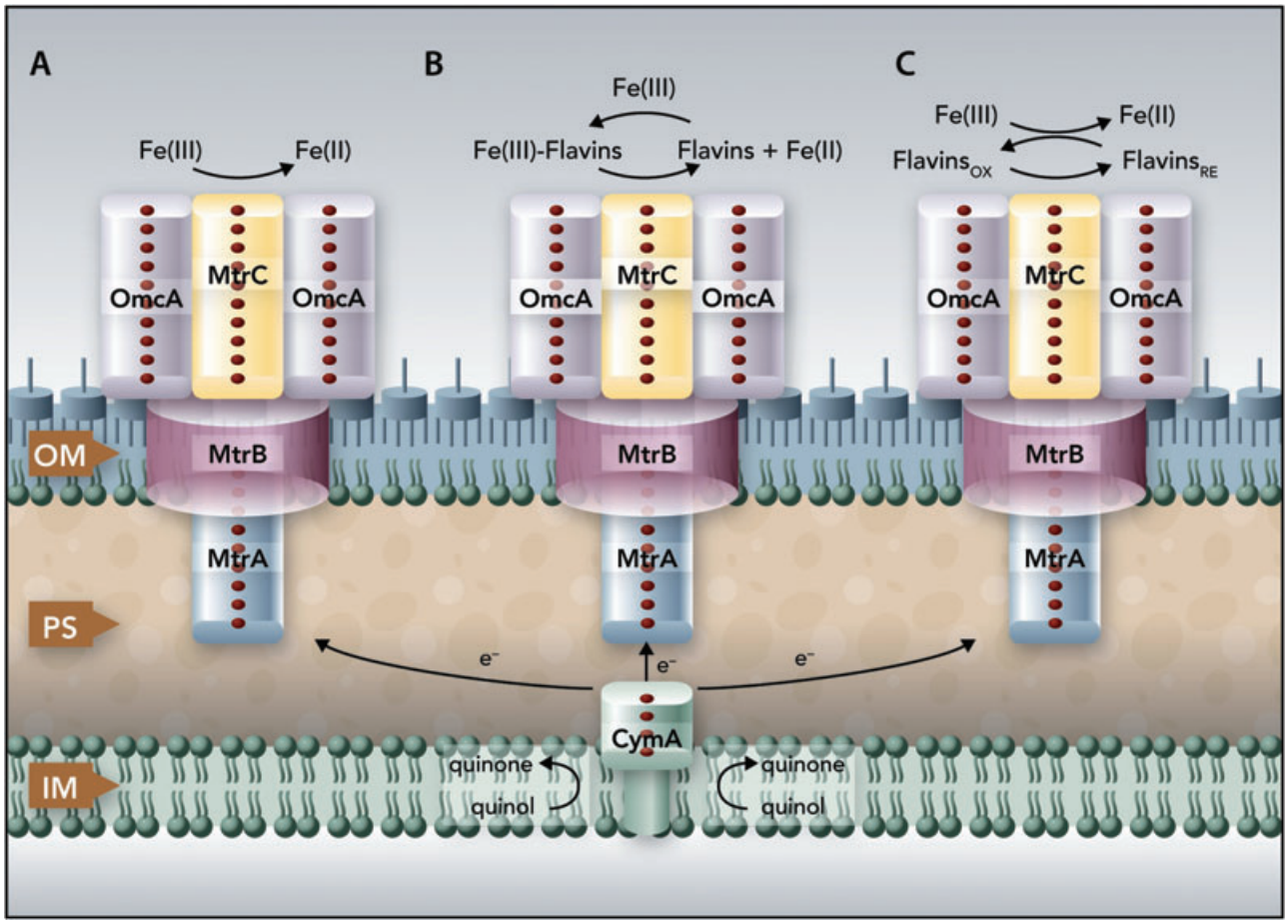

Fig. 1. The roles of MtrC and OmcA in *Shewanella oneidensis* MR-1-mediated extracellular reduction of Fe(III) oxides. Proteins that are known to be directly involved in the reduction include (i) the inner membrane (IM) tetrahaem *c*-Cyt CymA that is a homologue of NapC/NirT family of quinol dehydrogenases, (ii) the periplasmic decahaem *c*-Cyt MtrA, (iii) the outer membrane (OM) protein MtrB and (iv) the OM decahaem *c*-Cyts MtrC and OmcA. Together, they form a pathway for transferring electrons from quinone/quinol pool in the IM to the periplasm (PS) and then to the OM where MtrC and OmcA can transfer electrons directly to the surface of solid Fe(III) oxides (A). MtrC and OmcA might also reduce Fe(III) oxides indirectly by transferring electrons to either flavin-chelated Fe(III) (B) or oxidized flavins (C).

and *G. sulfurreducens* in mediating extracellular ET to Fe(III)/Mn(IV) oxides.

Shewanella OM cytochromes

Direct ET

The OMC MtrC (also known as OmcB) and OmcA have been directly implicated in *S. oneidensis* MR-1-mediated reduction of Fe(III) oxides. The genes encoding MtrC and OmcA are located in a metal-reductase-containing locus that also includes two additional genes, which encode a periplasmically exposed decahaem *c*-Cyt (MtrA) and an integral OM protein (MtrB) (Fig. 1). To date, all sequenced *Shewanella* genomes, with exception of the non-metal-reducing species *Shewanella denitrificans*, possess this metal-reductase-containing locus where *mtrABC* homologues are more conserved than *omcA* homologues (Fredrickson *et al.*, 2008; Wang *et al.*, 2008a). While deletions of the gene encoding MtrA or MtrB impair the bacterial ability to reduce Fe(III)/Mn(IV) oxides and to generate electrical current in *Shewanella* MFCs, the double mutant without either MtrC or OmcA only exhibits diminished ability for Fe(III) oxide reduction and for electricity production and can still reduce Mn(IV) oxide normally. In addition, the rates of Fe(III) oxide reduction and current production for the MtrC/OmcA double-deletion mutant are much higher than that for the mutants that lack MtrA or MtrB, suggesting that MtrC and OmcA (i) are not involved in Mn(IV) oxide reduction and (ii) are not the only OMCs that can serve as terminal reductases for Fe(III) oxides (Bretschger *et al.*, 2007). Both MtrC and OmcA are lipoproteins that are at least partially exposed on the extracellular side of the OM (Myers and Myers, 2003; 2004; Lower *et al.*, 2009), and their translocation across the OM to the cell surface is mediated by bacterial type II secretion system (Donald *et al.*, 2008; Shi *et al.*, 2008).

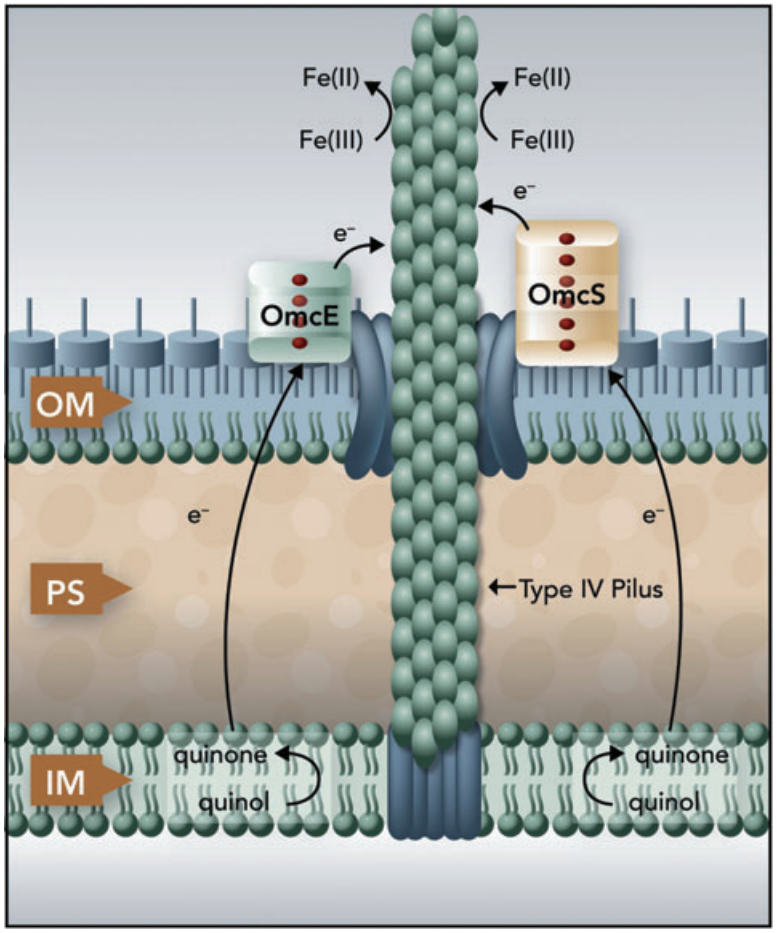

Fig. 2. The roles of OmcE and OmcS in *Geobacter sulfurreducens*-mediated extracellular reduction of Fe(III) oxides. The OM tetrahaem *c*-Cyt OmcE and hexahaem *c*-Cyt OmcS are believed to be located on the cell surface where they are suggested to transfer electrons to type IV pili. Type IV pili are hypothesized to relay electrons directly to Fe(III) oxides. OmcE and OmcS also receive the electrons originating from the quinone/quinol pool in the inner membrane (IM). Although other multihaem *c*-Cyts are believed to be the key components of the pathway that transfers electrons from the IM to OmcE and OmcS (Lovley, 2006; Weber *et al.*, 2006), their direct involvements in the related ET processes has yet to be established experimentally.

Protein purification and *in vivo* cross-linking results demonstrate that MtrABC and MtrC/OmcA can form a stable protein complex, respectively, indicating that together they form a molecular wire to transfer electrons across the bacterial OM (Shi *et al.*, 2006; Ross *et al.*, 2007; Tang *et al.*, 2007; Zhang *et al.*, 2008; 2009) (Fig. 1).

Analyses of purified MtrC and OmcA show that both OMCs can bind metal oxides, such as haematite (α-Fe_2O_3), and directly transfer electrons to the haematite and electrode surfaces. Results of the measurements with fluorescence correlation spectroscopy, optical waveguide lightmode spectroscopy and protein film voltammetry (PFV) show that OmcA binds haematite with an affinity of 1.2–2.6 nmol cm^{-2} (Xiong *et al.*, 2006; Eggleston *et al.*, 2008), and the binding is ionic-strength and pH dependent (Eggleston *et al.*, 2008). The same studies also indicate that OmcA conformation changes after binding to the haematite surface, which might be related to the changes of OmcA redox state (Xiong *et al.*, 2006; Eggleston *et al.*, 2008). By using atomic force microscopy, Lower and colleagues (2007) showed that OmcA and MtrC bind haematite as evidenced by distinct force characteristics. The strength of the OmcA-haematite attraction is about twice that of the MtrC-haematite attraction, but the binding frequency of MtrC to haematite is twice higher than that of OmcA to haematite. Furthermore, there is a very strong correlation between the force signatures observed for purified OMCs and those for intact cells (Lower *et al.*, 2001), emphasizing the direct ET role of MtrC and OmcA in Fe(III) oxide reduction (Lower *et al.*, 2007). By using phage-display technology, Lower and colleagues (2008) also identified a peptide with a haematite-binding motif whose conserved sequence is Ser/Thr-hydrophobic/aromatic-Ser/Thr–Pro–Ser/Thr. Molecular dynamics simulations of the peptide Ser–Pro–Ser indicate that (i) hydrogen bonding occurs between two serine amino acids and the hydroxylated haematite surface, and (ii) the proline induces a structure-binding motif by limiting the peptide flexibility. Both MtrC and OmcA possess the putative haematite-binding motif of Thr–Pro–Ser/Thr (Lower *et al.*, 2008). Spectroscopic and PFV measurements show that OmcA transfers electrons directly to haematite with an apparent rate constant of 0.11 s^{-1} (i.e. one OmcA molecule transfers 0.11 electron per second) (Xiong *et al.*, 2006; Eggleston *et al.*, 2008). Likewise, the ET rate from MtrC to haematite is 0.26 s^{-1}

(L. Shi, unpubl. data). Although it is impossible to directly compare these rates with those measured with intact cells, these low ET rates are in general agreement with the observations reported by other researchers that *Shewanella*-mediated reduction of haematite is relatively slow (Hansel *et al*., 2004) and that *S. oneidensis* MR-1 uses both direct and indirect mechanisms to transfer electrons to haematite (Bose *et al*., 2009). Protein film voltammetry measurements also show that MtrC and OmcA can transfer electrons directly to graphite electrodes and the interfacial ET rates between MtrC and the electrodes range from 100 to 276 s^{-1} (Hartshorne *et al*., 2007; Firer-Sherwood *et al*., 2008). It should be noted that binding of *c*-Cyts to the electrode surfaces is facilitated by neomycin (Firer-Sherwood *et al*., 2008) and the functional groups on the electrode surfaces (Leger *et al*., 2003), which make it possible for interfacial ET to occur between the *c*-Cyts and electrode surfaces as well as to greatly enhance the ET rates. Nevertheless, the observed interfacial ET between the *c*-Cyts and haematite or electrodes clearly indicates that at least one of the haem groups is exposed or near to the *c*-Cyt surfaces where it can mediate direct ET to the surfaces of haematite and the electrodes. Together, these results are consistent with MtrC and OmcA functioning as terminal reductases for transferring electrons extracellularly to Fe minerals via direct binding (Fig. 1A).

Indirect ET

Shewanella oneidensis MR-1 cells secrete water-soluble riboflavin and riboflavin-5'-phosphate (also known as flavin mononucleotide or FMN) to facilitate extracellular ET to Fe(III) oxides and glassy carbon electrodes (von Canstein *et al*., 2008; Marsili *et al*., 2008). The secreted flavins have been hypothesized to function as electron carriers that 'shuttle' electrons from cell-associated ET proteins to Fe(III) oxides or as chelators that solubilize Fe(III) oxides and increase their availability to the cell for reduction (von Canstein *et al*., 2008; Marsili *et al*., 2008). Although the exact mechanism by which flavins facilitate bacterial Fe(III) oxide reduction remains undetermined, cell surface localization would permit MtrC and OmcA to interact directly with flavin-chelated Fe(III) or oxidized flavins in the extracellular environment, bypassing the need for translocating the flavins across the OM.

The capacity of flavins for dissolving Fe(III) via complexation is unknown. By using cathodic squarewave voltammetry, Taillefert *et al.* observed the presence of organic–Fe(III) complexes during *Shewanella putrefaciens* strain 200-mediated reduction of Fe(III)-citrate and different Fe(III) oxides, including goethite (α-FeOOH). These Fe(III) complexes were not detected in abiotic and heat-killed biotic controls. The amount of organic–Fe(III) complexes formed depends on the numbers of initial bacterial cells used for reduction: the more cells used, the more organic–Fe(III) complexes formed. However, the identity of this organic compound that complexes Fe(III) still remains unknown (Taillefert *et al*., 2007). Nevertheless, given that (i) purified MtrC and OmcA exhibit strong reductase activity towards the Fe(III) chelated by citrate, ethylenediaminetetraacetic acid or nitrilotriacetic acid (NTA) (Borloo *et al*., 2007; Hartshorne *et al*., 2007; Wang *et al*., 2008b), and (ii) MtrC and OmcA are proposed to function as the terminal reductases for Fe(III)-NTA *in vivo* (Borloo *et al*., 2007), they could also potentially serve as the reductases for the Fe(III) that may be chelated by complexing ligands released by cells (Fig. 1B).

Although the ability of the OMCs to reduce oxidized flavins has not been experimentally demonstrated, MtrC and MtrB are required for extracellular reduction of anthraquinone-2,6-disulfonate (AQDS) (Lies *et al*., 2005). AQDS is routinely used as an artificial electron shuttle for mediating reduction of metal oxides and has a standard redox potential (E'_0) of −184 mV versus standard hydrogen electrode (SHE) (Rosso *et al*., 2004; Adachi *et al*., 2008). The involvement of MtrC in extracellular reduction of AQDS suggests that MtrC and probably OmcA could serve as AQDS reductases (Lies *et al*., 2005; Gralnick and Newman, 2007). In addition, both MtrC and OmcA have broad operating redox potential ranges (e.g. +100 to −500 mV versus SHE for MtrC) (Hartshorne *et al*., 2007; Firer-Sherwood *et al*., 2008), which make it thermodynamically feasible to transfer electrons directly from these OMCs to flavins whose E'_0 are about –200 mV versus SHE (Thauer *et al*., 1977). Thus, it is reasonable to suggest that MtrC and OmcA could also function as extracellular flavin reductases to reduce oxidized flavins with the reduced flavins ferrying electrons to Fe(III) oxides (Fig. 1C). Given that MtrC and OmcA directly bind Fe(III) oxides, the distance that flavins need to travel from the OMCs to Fe(III) oxide surface could be relatively short. If they do indeed function as flavin reductases, MtrC and OmcA would resemble functionally the bacterial assimilatory ferric reductases that can use flavins as diffusible cofactors to reduce ferric iron (Schroder *et al*., 2003).

Extracellular and electrically conductive materials termed nanowires are observed in *S. oneidensis* MR-1 cells where they are implicated in the bacteria-mediated reduction of metal oxides and electricity production in MFCs. MtrC and OmcA were proposed to be the key components of nanowires (Gorby *et al*., 2006). However, physical association of the OMCs with nanowires has yet to be established experimentally.

***Geobacter* OM cytochromes**

The OmcE, OmcS, and possibly OmcT, have also been implicated in extracellular ET by *G. sulfurreducens*.

Evidence for this is based on the observation that deletion of the genes encoding these OMCs impairs the ability to reduce Fe(III)/Mn(VI) oxides and to produce electricity in MFCs (Mehta *et al*., 2005; Holmes *et al*., 2006). The proposed role for OmcE and OmcS in metal oxide reductions differs significantly from their suggested role in electricity production in *Geobacter* MFCs. Because they are readily released into the solution phase by weak shearing forces, OmcE and OmcS are believed to be loosely associated with the cell surface where they are thought to serve as intermediates in the relay of electrons to electrically conductive type IV pili (T4P, also known as geopili). The charged T4P are then hypothesized to transfer electrons directly to the metal oxides (Fig. 2) (Mehta, *et al*., 2005; Reguera *et al*., 2005; Lovley, 2006; 2008a). However, to date there has been no experimental evidence demonstrating the inter-ET from the OMCs to T4P. The electrochemical mechanisms by which T4P transfer electrons are unknown as the pilin proteins are not known to contain any cofactors, such as haem group and Fe–S cluster, that are typically involved in ET reactions. In addition, the mechanisms by which electrons are transferred across the OM to the cell surface where the OMCs are located remain unclear (Fig. 2). In contrast to OmcE and OmcS, T4P are not required for the bacterial-mediated extracellular ET to MFC electrodes. Based on these observations, OmcS and OmcE are proposed to transfer electrons directly to the electrode surface (Holmes *et al*., 2006; Lovley, 2008b), similar to the role of MtrC and OmcA in *S. oneidensis* MR-1 (Bretschger *et al*., 2007) (Fig. 1A). Deletion of the gene encoding OmcF, a monohaem OMC, also impairs the bacterial ability to generate electricity in MFCs (Kim *et al*., 2008; Pokkuluri *et al*., 2009). However, OmcF is not involved in transferring electrons to the electrodes. Instead, it regulates indirectly surface localization of OmcE and OmcS because (i) compared with that of wild-type cells, OmcE and OmcS of the mutant without OmcF are not readily released into solutions by weak shearing forces, and (ii) deletion of *omcF* decreases the mRNA levels of the genes that might encode a bacterial type I secretion system (Kim *et al*., 2008). It will be interesting to learn whether the mutant without OmcF also exhibits impaired ability to reduce Fe(III) oxides.

In contrast to MtrC in MR-1 and other strains of dissimilatory metal-reducing *Shewanella*, neither OmcE nor OmcS is conserved among sequenced *Geobacter* species (Lovley, 2008a). In addition, no strain of *Geobacter* has been reported to secrete flavins or other water-soluble molecules that could serve as metal chelators or electron shuttles. Despite these differences, both *S. oneidensis* MR-1 and *G. sulfurreducens* use cell surface-localized OMCs to reduce metal oxides via mechanisms that are direct, indirect or both.

Other DMRB species might also use OMCs for extracellular reduction of metal oxides. Recent genomic sequencing results showed that the DMRB *Anaeromyxobacter dehalogenans* strain 2CP-C contained 68 putative *c*-Cyts (Thomas *et al*., 2008). Further research is needed to determine whether any of these putative *c*-Cyts is cell surface-exposed. Cell surface-exposed OMCs have also been implicated in extracellular ET reactions in bacteria that are physiologically distinct from DMRB. For example, the cell surface-localized OMCs MCA0338, MCA0421, MCA0423 and MCA2259 of the methanotroph *Methylococcus capsulatus* (Bath) are proposed to mediate extracellular ET to copper ions [Cu(II)], as Cu(II) regulates the OMC abundances. MCA2259 was the dominant OMC when the Cu(II) concentration was less than 0.8 μm in the growth medium. When the Cu(II) concentration was between 0.8 and 1.6 μM, the dominant OMCs were MCA0421 and MCA0423. MCA0338 became the most abundant OMC when the Cu(II) concentration in the medium was between 5 and 10 μM (Karlsen *et al*., 2008). These latest findings suggest that cell surface-localized OMCs as the key components for facilitating ET to substrates external to the cells may be more commonly distributed among Gram-negative bacteria than previously believed.

Conclusions and perspectives

Because of (i) their cell surface localization, (ii) their ability to transfer electrons to Fe(III) oxides via direct binding and (iii) their broad operating redox potential ranges, MtrC and OmcA of *S. oneidensis* MR-1 can serve as reductases for substrates external to the cells, such as Fe(III) oxides, soluble metal complexes and redox active organic compounds. The specific roles of MtrC and OmcA in extracellular ET are unclear although they do appear to be functionally redundant in regard to reduction of Fe(III) oxides. Because of the extensive amount of *in vivo* and *in vitro* characterization of MtrC and OmcA, they are attractive models for investigating different aspects of the OMC-mediated extracellular ET processes (e.g. direct and indirect ET) in reduction of metal oxides as well as in electricity production in MFCs. Meanwhile, ET intermediates OmcE and OmcS of *G. sulfurreducens* represent an extracellular ET paradigm that appears distinct from that mediated by MtrC and OmcA of *S. oneidensis* MR-1.

In order to provide a frame work for better understanding of the extracellular ET reactions mediated by surface-exposed OMCs, future research is needed to address the following important questions. First, how are electrons translocated across the OM and delivered to the surface-exposed OMCs? Surface-exposed OMCs receive the electrons originating from the inner membrane. In *S. oneidensis* MR-1, MtrA and MtrB are proposed to deliver

electrons to MtrC and probably OmcA (Fig. 1). Similar components might also exist in *Geobacter* (Lovley, 2006) but have yet to be discovered. Identification and functional characterization of these components will not only help to understand the extracellular ET pathways but also provide the mechanistic insights of electron translocation across the bacterial OM. Another key question is how OMCs mediate interfacial ET to the mineral surfaces? All available information clearly indicates that the OMCs of *Shewanella* and probably *Geobacter* are capable of transferring electrons directly to solid surfaces, such as those of minerals and electrodes. However, the molecular mechanisms by which the OMCs transfer electrons to the solid surfaces remain undetermined. Elucidation of how electrons are transferred via multiple haem groups to the oxide surfaces and how the proteins interface with and achieve appropriate orientation on the mineral surfaces will contribute to our understanding at the molecular level of ET reactions at the microbe–mineral interface. This understanding has important implications for biogeochemical cycling of metals, biotransformation of contaminants and electricity generation in MFCs. One final question is whether these OMCs have other functions in addition to reduction of Fe(III)-containing minerals. Although it is highly speculative, extracellular ET to other substrates, such as O_2, could avoid accumulation of O_2 and/or the harmful products of O_2 reduction, such as H_2O_2, in the periplasm of facultative anaerobic Gram-negative bacteria, where the redox proteins that are sensitive to the damage caused by O_2 or other reactive oxygen species might be abundant. Identification of these roles for surface-exposed OMCs will advance our understanding of bacterial physiology related to extracellular ET reactions.

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References

Adachi, M., Shimomura, T., Komatsu, M., Yakuwa, H., and Miya, A. (2008) A novel mediator-polymer-modified anode for microbial fuel cells. *Chem Commun (Camb)* **7:** 2055–2057.

Borloo, J., Vergauwen, B., De Smet, L., Brige, A., Motte, B., Devreese, B., and Van Beeumen, J. (2007) A kinetic approach to the dependence of dissimilatory metal reduction by *Shewanella oneidensis* MR-1 on the outer membrane cytochromes *c* OmcA and OmcB. *FEBS J* **274:** 3728–3738.

Bose, S., Hochella, M.F., Jr, Gorby, Y.A., Kennedy, D.W., McCready, D.E., Madden, A.S., and Lower, B.H. (2009) Bioreduction of hematite nanoparticles by the dissimilatory iron reducing bacterium *Shewanella oneidensis* MR-1. *Geochim Cosmochim Acta* **73:** 962–976.

Bretschger, O., Obraztsova, A., Sturm, C.A., Chang, I.S., Gorby, Y.A., Reed, S.B., *et al.* (2007) Current production and metal oxide reduction by *Shewanella oneidensis* MR-1 wild type and mutants. *Appl Environ Microbiol* **73:** 7003–7012.

von Canstein, H., Ogawa, J., Shimizu, S., and Lloyd, J.R. (2008) Secretion of flavins by *Shewanella* species and their role in extracellular electron transfer. *Appl Environ Microbiol* **74:** 615–623.

Donald, J.W., Hicks, M.G., Richardson, D.J., and Palmer, T. (2008) The *c*-type cytochrome OmcA localizes to the outer membrane upon heterologous expression in *Escherichia coli*. *J Bacteriol* **190:** 5127–5131.

Eggleston, C.M., Vörös, J., Shi, L., Lower, B.H., Droubay, T.C., and Colberg, P.J.S. (2008) Binding and direct electrochemistry of OmcA, an outer-membrane cytochrome from an iron reducing bacterium, with oxide electrodes: a candidate biofuel cell system. *Inorg Chim Acta* **361:** 769–777.

Firer-Sherwood, M., Pulcu, G.S., and Elliott, S.J. (2008) Electrochemical interrogations of the Mtr cytochromes from *Shewanella*: opening a potential window. *J Biol Inorg Chem* **13:** 849–854.

Fredrickson, J.K., and Zachara, J.M. (2008) Electron transfer at the microbe–mineral interface: a grand challenge in biogeochemistry. *Geobiology* **6:** 245–253.

Fredrickson, J.K., Romine, M.F., Beliaev, A.S., Auchtung, J.M., Driscoll, M.E., Gardner, T.S., *et al.* (2008) Towards environmental systems biology of *Shewanella*. *Nat Rev Microbiol* **6:** 592–603.

Gorby, Y.A., Yanina, S., McLean, J.S., Rosso, K.M., Moyles, D., Dohnalkova, A., *et al.* (2006) Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. *Proc Natl Acad Sci USA* **103:** 11358–11363.

Gralnick, J.A., and Newman, D.K. (2007) Extracellular respiration. *Mol Microbiol* **65:** 1–11.

Hansel, C.M., Benner, S.G., Nico, P., and Fendorf, S. (2004) Srtuctural constraints of ferric (hydr)oxides on dissimilatory iron reduction and fate of Fe(II). *Geochim Cosmochim Acta* **68:** 3217–3229.

Hartshorne, R.S., Jepson, B.N., Clarke, T.A., Field, S.J., Fredrickson, J., Zachara, J., *et al.* (2007) Characterization of *Shewanella oneidensis* MtrC: a cell-surface decaheme cytochrome involved in respiratory electron transport to extracellular electron acceptors. *J Biol Inorg Chem* **12:** 1083–1094.

Holmes, D.E., Chaudhuri, S.K., Nevin, K.P., Mehta, T., Methe, B.A., Liu, A., *et al.* (2006) Microarray and genetic analysis of electron transfer to electrodes in *Geobacter sulfurreducens*. *Environ Microbiol* **8:** 1805–1815.

Karlsen, O.A., Lillehaug, J.R., and Jensen, H.B. (2008) The

presence of multiple *c*-type cytochromes at the surface of the methanotrophic bacterium *Methylococcus capsulatus* (Bath) is regulated by copper. *Mol Microbiol* **70:** 15–26.

Kim, B.C., Postier, B.L., Didonato, R.J., Chaudhuri, S.K., Nevin, K.P., and Lovley, D.R. (2008) Insights into genes involved in electricity generation in *Geobacter sulfurreducens* via whole genome microarray analysis of the OmcF-deficient mutant. *Bioelectrochemistry* **73:** 70–75.

Leger, C., Elliott, S.J., Hoke, K.R., Jeuken, L.J., Jones, A.K., and Armstrong, F.A. (2003) Enzyme electrokinetics: using protein film voltammetry to investigate redox enzymes and their mechanisms. *Biochemistry* **42:** 8653–8662.

Lies, D.P., Hernandez, M.E., Kappler, A., Mielke, R.E., Gralnick, J.A., and Newman, D.K. (2005) *Shewanella oneidensis* MR-1 uses overlapping pathways for iron reduction at a distance and by direct contact under conditions relevant for biofilms. *Appl Environ Microbiol* **71:** 4414–4426.

Lovley, D.R. (2006) Bug juice: harvesting electricity with microorganisms. *Nat Rev Microbiol* **4:** 497–508.

Lovley, D.R. (2008a) Extracellular electron transfer: wires, capacitors, iron lungs, and more. *Geobiology* **6:** 225–231.

Lovley, D.R. (2008b) The microbe electric: conversion of organic matter to electricity. *Curr Opin Biotechnol* **19:** 564–571.

Lower, B.H., Shi, L., Yongsunthon, R., Droubay, T.C., McCready, D.E., and Lower, S.K. (2007) Specific bonds between an iron oxide surface and outer membrane cytochromes MtrC and OmcA from *Shewanella oneidensis* MR-1. *J Bacteriol* **189:** 4944–4952.

Lower, B.H., Lins, R.D., Oestreicher, Z., Straatsma, T.P., Hochella, M.F., Jr, Shi, L., and Lower, S.K. (2008) In vitro evolution of a peptide with a hematite binding motif that may constitute a natural metal-oxide binding archetype. *Environ Sci Technol* **42:** 3821–3827.

Lower, B.H., Yongsunthon, R., Shi, L., Wildling, L., Gruber, H.J., Wigginton, N.S., *et al.* (2009) Antibody-recognition force microscopy shows that outer membrane cytochromes OmcA and MtrC are expressed on the exterior surface of *Shewanella oneidensis* MR-1. *Appl Environ Microbiol* **75:** 2931–2935.

Lower, S.K., Hochella, M.F., Jr, and Beveridge, T.J. (2001) Bacterial recognition of mineral surfaces: nanoscale interactions between *Shewanella* and alpha-FeOOH. *Science* **292:** 1360–1363.

Marsili, E., Baron, D.B., Shikhare, I.D., Coursolle, D., Gralnick, J.A., and Bond, D.R. (2008) *Shewanella* secretes flavins that mediate extracellular electron transfer. *Proc Natl Acad Sci USA* **105:** 3968–3973.

Mehta, T., Coppi, M.V., Childers, S.E., and Lovley, D.R. (2005) Outer membrane *c*-type cytochromes required for Fe(III) and Mn(IV) oxide reduction in *Geobacter sulfurreducens*. *Appl Environ Microbiol* **71:** 8634–8641.

Myers, C.R., and Myers, J.M. (2003) Cell surface exposure of the outer membrane cytochromes of *Shewanella oneidensis* MR-1. *Lett Appl Microbiol* **37:** 254–258.

Myers, C.R., and Myers, J.M. (2004) The outer membrane cytochromes of *Shewanella oneidensis* MR-1 are lipoproteins. *Lett Appl Microbiol* **39:** 466–470.

Pokkuluri, P.R., Londer, Y.Y., Wood, S.J., Duke, N.E., Morgado, L., Salgueiro, C.A., and Schiffer, M. (2009) Outer membrane cytochrome *c*, OmcF, from *Geobacter sulfurreducens*: high structural similarity to an algal cytochrome *c*6. *Proteins* **74:** 266–270.

Reguera, G., McCarthy, K.D., Mehta, T., Nicoll, J.S., Tuominen, M.T., and Lovley, D.R. (2005) Extracellular electron transfer via microbial nanowires. *Nature* **435:** 1098–1101.

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

Ross, D.E., Ruebush, S.S., Brantley, S.L., Hartshorne, R.S., Clarke, T.A., Richardson, D.J., and Tien, M. (2007) Characterization of protein–protein interactions involved in iron reduction by *Shewanella oneidensis* MR-1. *Appl Environ Microbiol* **73:** 5797–5808.

Rosso, K.M., Smith, D.M.A., Wang, Z., Ainsworth, C.C., and Fredrickson, J.K. (2004) Self-exchange electron transfer kinetics and reduction potentials for anthraquinone disulfonate. *J Phys Chem A* **108:** 3292–3303.

Schroder, I., Johnson, E., and de Vries, S. (2003) Microbial ferric iron reductases. *FEMS Microbiol Rev* **27:** 427–447.

Shi, L., Chen, B., Wang, Z., Elias, D.A., Mayer, M.U., Gorby, Y.A., *et al.* (2006) Isolation of a high-affinity functional protein complex between OmcA and MtrC: two outer membrane decaheme *c*-type cytochromes of *Shewanella oneidensis* MR-1. *J Bacteriol* **188:** 4705–4714.

Shi, L., Squier, T.C., Zachara, J.M., and Fredrickson, J.K. (2007) Respiration of metal (hydr)oxides by *Shewanella* and *Geobacter*: a key role for multihaem *c*-type cytochromes. *Mol Microbiol* **65:** 12–20.

Shi, L., Deng, S., Marshall, M.J., Wang, Z., Kennedy, D.W., Dohnalkova, A.C., *et al.* (2008) Direct involvement of type II secretion system in extracellular translocation of *Shewanella oneidensis* outer membrane cytochromes MtrC and OmcA. *J Bacteriol* **190:** 5512–5516.

Taillefert, M., Beckler, J.S., Carey, E., Burns, J.L., Fennessey, C.M., and DiChristina, T.J. (2007) *Shewanella putrefaciens* produces an Fe(III)-solubilizing organic ligand during anaerobic respiration on insoluble Fe(III) oxides. *J Inorg Biochem* **101:** 1760–1767.

Tang, X., Yi, W., Munske, G.R., Adhikari, D.P., Zakharova, N.L., and Bruce, J.E. (2007) Profiling the membrane proteome of *Shewanella oneidensis* MR-1 with new affinity labeling probes. *J Proteome Res* **6:** 724–734.

Thauer, R.K., Jungermann, K., and Decker, K. (1977) Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol Rev* **41:** 100–180.

Thomas, S.H., Wagner, R.D., Arakaki, A.K., Skolnick, J., Kirby, J.R., Shimkets, L.J., *et al.* (2008) The mosaic genome of *Anaeromyxobacter dehalogenans* strain 2CP-C suggests an aerobic common ancestor to the delta-proteobacteria. *PLoS ONE* **3:** e2103.

Wang, F., Wang, J., Jian, H., Zhang, B., Li, S., Zeng, X., *et al.* (2008a) Environmental adaptation: genomic analysis of the piezotolerant and psychrotolerant deep-sea iron reducing bacterium *Shewanella piezotolerans* WP3. *PLoS ONE* **3:** e1937.

Wang, Z., Liu, C., Wang, X., Marshall, M.J., Zachara, J.M., Rosso, K.M., *et al.* (2008b) Reduction kinetics of Fe(III) complexes by outer membrane cytochromes MtrC and OmcA of *Shewanella oneidensis* MR-1. *Appl Environ Microbiol* **74:** 6746–6755.

Weber, K.A., Achenbach, L.A., and Coates, J.D. (2006) Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. *Nat Rev Microbiol* **4:** 752–764.

Xiong, Y., Shi, L., Chen, B., Mayer, M.U., Lower, B.H., Londer, Y., *et al.* (2006) High-affinity binding and direct electron transfer to solid metals by the *Shewanella oneidensis* MR-1 outer membrane *c*-type cytochrome OmcA. *J Am Chem Soc* **128:** 13978–13979.

Zhang, H., Tang, X., Munske, G.R., Zakharova, N., Yang, L., Zheng, C., *et al.* (2008) *In vivo* identification of the outer membrane protein OmcA–MtrC interaction network in *Shewanella oneidensis* MR-1 cells using novel hydrophobic chemical cross-linkers. *J Proteome Res* **7:** 1712–1720.

Zhang, H., Tang, X., Munske, G.R., Tolic, N., Anderson, G.A., and Bruce, J.E. (2009) Identification of protein-protein interactions and topologies in living cells with chemical cross-linking and mass spectrometry. *Mol Cell Proteomics* **8:** 409–420.