Regulation of two highly similar genes, omcB and omcC, in a 10 kb chromosomal duplication in Geobacter sulfurreducens

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The Fe(III)-reducing micro-organism Geobacter sulfurreducens requires an outer-membrane c-type cytochrome, OmcB, for Fe(III) reduction, but a related cytochrome, OmcC, which is 73% identical to OmcB, is not required. The omcB and omcC genes are part of a tandem chromosomal duplication consisting of two repeated clusters of four genes. The 2.7 kb sequences preceding omcB and omcC are identical with the exception of a single base pair change. Studies that combined genetic, Northern blotting and primer extension analyses demonstrated that both omcB and omcC are transcribed as monocistronic and polycistronic (orf1-orf2-omcB/omcC) transcripts. All of the promoters for the various transcripts were found to be located within the 2.7 kb identical region upstream of omcB and omcC. The sequences of the promoter regions for the two monocistronic transcripts are identical and equidistant from the omcB or omcC start codons. The promoters for the two polycistronic transcripts, in contrast, are distinct. One is specific for transcription of orf1-orf2-omcB and the other is associated with transcription of orf1-orf2-omcC. Studies with an RpoS-deficient mutant suggested that transcription from all four promoters is RpoS dependent under one or more growth conditions. Deletion of orfR, a gene immediately upstream of orf1-orf2-omcB that encodes a putative transcriptional regulator, significantly lowered the omcB transcription when Fe(III) was the electron acceptor and partially inhibited Fe(III) reduction. In contrast, levels of omcC transcripts were unaffected in the orfR mutant. These results indicate that omcB and omcC operons represent a rare instance in which duplicated operons, located in tandem on the chromosome, have different transcriptional regulation.

INTRODUCTION

Micro-organisms in the family Geobacteraceae are the predominant organisms in a wide variety of environments in which dissimilatory metal reduction plays an important role in the degradation of organic matter or the bioremediation of organic or metal contaminants (Lovley, 1991, 2000). Understanding the mechanism of Fe(III) reduction and the regulation of this process in Geobacteraceae is necessary in order to model these important environmental processes (Lovley, 2002, 2003). Microbial reducible Fe(III) is primarily present in the form of insoluble Fe(III) oxides in most soils and sediments (Lovley, 1991; Lovley et al., 2004). The mechanisms for electron transfer to Fe(III) appear to be significantly different in phylogenetically distinct Fe(III) reducers (Childers et al., 2002; Nevin & Lovley, 2000, 2002a, b). Present evidence suggests that Geobacter species must directly contact Fe(III) oxides in order to reduce them, and that electron transfer to Fe(III) oxides takes place at or near the outer membrane (Lovley et al., 2004). This is consistent with the fact that Geobacteraceae have the ability to transfer electrons onto electrodes, which also represent an insoluble, extracellular electron acceptor (Bond et al., 2002; Bond & Lovley, 2003).

Most studies investigating the mechanism of Fe(III) reduction in Geobacteraceae have been conducted with Geobacter sulfurreducens, because it is closely related to the Geobacteraceae that predominate in many subsurface environments and can readily be cultured in the laboratory. Furthermore, the complete genome sequence and a genetic system are available (Coppi et al., 2001; Methe et al., 2003) for this organism. OmcB is an outer-membrane c-type cytochrome that is required for Fe(III) reduction in G. sulfurreducens (Leang et al., 2003). Deleting omcB greatly diminished Fe(III) reduction, and expressing omcB in trans restored Fe(III) reduction in proportion to the amount of OmcB produced. In contrast, deleting the gene for OmcC,
another outer-membrane c-type cytochrome with 73% amino acid identity to Omcb, had no effect on Fe(III) reduction (Leang et al., 2003). This difference in the function of these very similar c-type cytochromes is even more surprising when it is considered that omcB and omcC are contained within a 10 kb chromosomal duplication. The 2.7 kb sequences upstream of omcB and omcC are identical with the exception of a single base-pair change, and code for three consecutive open reading frames (orfR/S, orf1 and orf2), a putative transcriptional regulator (OrfR/S), a protein of unknown function (Orf1) and a putative c-type periplasmic cytochrome (Orf2) (Leang et al., 2003). In order to gain additional insight into the divergent functions of omcB and omcC, the transcriptional regulation of these genes was investigated. The promoter/operator regions of omcB and omcC were determined. In addition, the effects of two transcriptional regulators, RpoS and OrfR, on omcB and omcC expression were evaluated. The results of this study demonstrate that the transcriptional regulation of omcB and omcC differs substantially, further suggesting that the products of these closely related genes have different functions.

**METHODS**

**Bacterial strains and culture conditions.** Escherichia coli strain JM109 [endA1 recA1 gyrA96 thi hsdR17(rK, mK) relA supE44 Δ(lac–proAB) (F’ traD36 proAB lacZΔM15)] (Yanisch-Perron et al., 1985) was cultured in LB medium at 37°C with shaking. Targeted gene disruption experiments were performed on G. sulfurreducens strain DL1 (Caccavo et al., 1994) to produce strains DL10 (orfR::gm) and DL11 (orf1::orf2-omicc::kan). RpoS-deficient mutant DLCN16 (rpoS::kan) was obtained from our laboratory culture collection (Nunez et al., 2004). G. sulfurreducens strains were cultured anaerobically at 30°C in either acetate/fumarate (NBAF) or Fe(III) citrate (FWAFC) medium as previously described (Coppi et al., 2001).

DNA and RNA manipulations. Genomic DNA was extracted with the MasterPure Complete DNA & RNA Purification kit (Epicentre Technologies). Plasmid DNA and PCR products were purified with the Qiagen mini plasmid purification kits and Qiagen PCR purification kits, respectively. DNA cloning and other manipulations were carried out according to the methods outlined by Sambrook et al. (1989). Restriction enzymes and other DNA-modifying enzymes were purchased from New England Biolabs. Probes for Southern or Northern blot analysis were labelled with [α-32P]dCTP with the NEBlot kit (New England BioLabs). [α-32P]dCTP was purchased from PerkinElmer Life and Analytical Sciences. Qiagen Taq DNA polymerase was used for all PCR amplifications.

Total RNA was isolated from mid-exponential-phase cultures with RNasey Midi kits (Qiagen) followed by treatment with RNase-free DNase (Ambion). Northern blot analyses were performed with the Northern Max-Gly system (Ambion) using dsDNA probes according to the manufacturer’s instructions. The omcB-specific probe (435 bp) was amplified with primers 8916 (5'-GGACTCGGGACCATCAAGG-3') and 8908-2 (Leang et al., 2003). An omcC-specific probe (553 bp) was amplified with primers 8914 (5'-GCCAGAGTGAGGCCAGCA-3') and 8909 (+285 to +302 of the omcC gene, and 8914 (Chin et al., 2004).

**Single-step gene replacement (Fig. 1).** Genes were deleted with single-step gene replacement as previously described (Lloyd et al., 2003). To disrupt the orf1::orf2-omicc cluster, a 2.28 kb linear DNA fragment was generated by recombinant PCR (Lloyd et al., 2003; Murphy et al., 2000) from three primary PCR products: (1) the sequence upstream of orf1 of the omcC cluster [560 bp, −1138 to −578, amplified with primers Triple1 (5'-GAAAGCATCATGCTTCC-3') and Triple2 (5'-GTATGCTTCTTCGAGAAG-3')]; (2) the 3' end of omcC [606 bp, +1471 to +2077, amplified with primers 8901 and 8902 (Leang et al., 2003)]; and (3) a kanamycin resistance (kanR) cassette [1114 bp, amplified with primers Triplekan (5'-CTTCGGAAGAAAGCGATCATCCTGGAGTAGTGCAGC-3') and 8905 (Leang et al., 2003), using plasmid pBBR1MCS-2 (Kovach et al., 1995) as template]. Recombinant PCR was carried out with these three PCR products as templates with distal primers Triple1 and 8901. PCR conditions were as previously described (Lloyd et al., 2003), except that the annealing temperature was 58°C.

![Fig. 1](https://www.microbiologyresearch.org/) Organization of the omcB and omcC clusters, mutation schemes, and predicted promoters for transcription of omcB and omcC. The omcB and omcC clusters are indicated by boxes with diagonal lines. Identical 2.7 kb sequences upstream of both omcB and omcC genes are represented by a thick black bar. Gene replacement is indicated by a horizontal bar. The transcriptional orientation of the gmR or kanR cassettes is indicated by a bold arrow. The 5' of orf1 was deleted and replaced with a gmR cassette, resulting in strain DL10 (orfR::gm), and the orf1-orf2-omicc cluster was replaced with a kanR cassette, resulting in strain DL11 (orf1-orf2-omicc::kan). The predicted promoters for the omcB (P2 and P3) and omcC (P1 and P3) clusters are indicated with arrows followed by dotted wavy lines representing the omcB or omcC transcripts.
To disrupt the orfR gene, a similar strategy was used. Three primary PCR reactions were performed to amplify a 1-8 kb liner DNA fragment: (1) the upstream sequence of the orfR gene [507 bp, −522 to −16], amplified with primers OP1-for (5′-GGGAGTACAACCTCTCG-3′) and OrfR-a (5′-GCTGATCTATGAACGGCAGCTG-3′); (2) the 3′ end of the orfR gene [517 bp, +284 of the orfR gene to +30 of the orf1 gene, amplified with primers OrfR-b (5′-CCGCTATCCAGCTGAAAGC-3′) and Orf1-C (5′-GGAGCGCTCGATGAAAGC-3′); and (3) the gentamicin resistance (gmR) cassette

AAGGAC-3′

orf1

inserting a 1 kb PCR fragment consisting of 800 bp of orf1 gene, amplified with primers Orf1-rev (5′-CGAACTTACCTCTGTCAGCTG-3′) and OrfR-for (5′-CGAAGCAGCTGATGGTCTTG-3′) and Gmrev (5′-CGCAAGCAGCTGAAAGGCCGCTG-3′), using plasmid pBSL141 (Alexeyev et al., 1995) as template. Recombinant PCR was performed as described above by combining all the three primary products with distal primers OP1-for and Orf1-C. PCR conditions were identical, except that the annealing temperature was 55 °C.

Electroporation, mutant isolation and genotype confirmation were performed as previously described (Coppi et al., 2001; Lloyd et al., 2003). One of each of the mutants, designated DL10 (orf-r::gm) and DL11 (orf-l2-omcr::kan), was chosen as the representative strain (Fig. 1).

**Expression of orfR in trans.** The complete orfR coding sequence was amplified with primers OrfRepSphI-F (5′-GGGAGCAGCTCAAGCAGCTGATGGTCTTG-3′, Sph site italicized) and OrfRepSphII-R (5′-GGGAGCAGCTCAAGCAGCTGATGGTCTTG-3′, SphII site underlined) under the following conditions: 96 °C, 40 s followed by 25 cycles of 96 °C, 40 s; 58 °C, 1 min; 72 °C, 1 min; and a final extension at 72 °C for 10 min. The PCR product of the orfR coding sequence was digested with SphI and BglII and inserted into the SphI and BglII sites of the expression vector pCM66 (Marx & Lidstrom, 2001) via ligation; the resulting plasmid was designated pCM-orfR. The orfR gene was then sequenced to screen for PCR artifacts.

Following electroporation of strain DL10 with pCM-orfR, a kanamycin-resistant transformant was isolated and designated DL10/pCM-orfR. The simultaneous presence of both the plasmid pCM-orfR and the orfR::gm mutation in this strain were confirmed by PCR.

**Primer extension analysis.** Total RNA was isolated from _G. sulfur-reducens_ as described above. For identification of the P1 and P2 promoters, primer extension experiments were performed at 55 °C using ThermoScript reverse transcriptase (Invitrogen) with primer Orf1-C (complementary to +13 to +31 of orf1 in both the _omcrC_ and _omcrB_ clusters; sequence described above). To define the P3 promoters, primer extension experiments were carried out at 42 °C using RAV2 reverse transcriptase (Amersham Biosciences) with primer OP2b-rev (5′-CGAAGCTGACTTCTTGACC-3′, complementary to the 5′ end of orf1) and short (primer OP2b-rev, 5′-CGAAGCTGACTTCTTGACC-3′) transcripts of the _omcrB_ and _omcrC_ clusters. Two primer extension products were

epifluorescence microscopy (Lovley & Phillips, 1988). Protein concentration was determined by the bicinchoninic acid method with BSA as a standard (Smith et al., 1985).

**RESULTS**

**Transcriptional organization and initiation sites of the _omcrB_ and _omcrC_ clusters**

Northern blot analyses with _omcrB_- or _omcrC_-specific probes revealed two transcripts of about 5 and 2.5 kb for both the _omcrB_ and _omcrC_ genes (Fig. 2a, b; lanes 1 and 2). In accordance with previously reported protein data (Leang et al., 2003), both genes were expressed when either fumarate or Fe(III) was the terminal electron acceptor. The _omcrB_ transcripts were about six to eight times higher during growth with Fe(III) than they were with fumarate (Fig. 2a, lanes 1 and 2). In contrast, the levels of _omcrC_ transcripts were nearly equivalent during growth on either electron acceptor (Fig. 2b, lanes 1 and 2).

Primer extension studies were performed to define the transcriptional initiation sites of the _omcrB_ and _omcrC_ clusters. The primers used to synthesize cDNA for these transcripts were complementary to the 2.7 kb identical sequence found in both the _omcrB_ and _omcrC_ clusters, and were designed to amplify both the long (primer Orf1-C, 5′-GGGAGCAGCTGAGCTGACC-3′, complementary to the 5′ end of orf1) and short (primer OP2b-rev, 5′-CGAAGCTGACTTCTTGACC-3′) transcripts of the _omcrB_ and _omcrC_ clusters. Two primer extension products were
positions of the identical sequences of the \textit{omcB} and \textit{omcC} clusters, the start sites for P1 were located 540 bp downstream of the beginning of the identical sequences, 590 bp downstream of the OrfR start codon and 444 bp downstream of the OrfS start codon (Fig. 3c). The mRNA start site for P3 was within the \textit{orf2} coding regions, 307 nt upstream of the \textit{omcB} and \textit{omcC} start codons. These results, combined with results based on Northern blot analyses, indicated that the long transcript (5 kb) resulted from the co-transcription of the \textit{orf1}, \textit{orf2} and \textit{omcB}/\textit{omcC} genes, but not the \textit{orfR} or \textit{orfS} genes. The short transcripts, in contrast, were monocistronic (2-5 kb), consisting solely of \textit{omcB} or \textit{omcC}.

The finding that promoters P1 and P2 were located in the duplication region of the \textit{orf1-orf2-omcB} and \textit{orf1-orf2-omcC} clusters led to the question of whether there is a difference in transcriptional start sites for the 5 kb transcripts for the two clusters. In order to evaluate this, an approximately 5 kb chromosomal DNA fragment containing the \textit{orf1-orf2-omcC} cluster was deleted and replaced with a kanamycin-resistance cassette (Fig. 1). The \textit{omcC} cluster-deficient mutant was designated DL11 (\textit{orf1-orf2-omcC::kan}). As expected, the deletion of the \textit{orf1-orf2-omcC} cluster did not affect the abilities of cells to grow in medium containing acetate as the electron donor and either fumarate or Fe(III) citrate as the electron acceptor (data not shown). This result is not surprising given the presence of a second copy of \textit{orf1} and \textit{orf2} upstream of \textit{omcB} and the fact that \textit{OmC} is not essential for fumarate or Fe(III) reduction (Leang et al., 2003).

Primer extension analysis indicated that the remaining \textit{orf1-orf2-omcB} cluster in strain DL11 (\textit{orf1-orf2-omcC::kan}) was preferentially transcribed from P2 (Fig. 4a, lanes 3 and 4). Transcriptional initiation from P1 was greatly decreased in strain DL11 with respect to wild-type. These results suggest that expression of the \textit{orf1-orf2-omcB} cluster is initiated from promoter P2, whereas P1 is mainly responsible for expression of the \textit{orf1-orf2-omcC} cluster (summarized in Fig. 1).

The P1, P2 and P3 regulatory regions are RpoS dependent

The -35 sequences of P1, P2 and P3 (Fig. 3) are GC rich, similar to the promoters which are recognized by RNA polymerase containing RpoS in \textit{E. coli} (Wagner, 2000). In order to determine whether transcription from P1, P2 and P3 was RpoS-dependent, Northern blot analyses were carried out on the wild-type strain and an RpoS-deficient mutant (DLCN16) (Nunez et al., 2004). The \textit{omcC} transcripts were hardly detectable (0 to about 7 % of wild-type intensity) in the RpoS-deficient mutant supplied with either fumarate or Fe(III) as the electron acceptor (Fig. 2b, lanes 3 and 4). Expression of \textit{omcB} in the RpoS-deficient mutant was also very low (3 % of wild-type level) during growth on Fe(III) as the electron acceptor (Fig. 2a, lane 4). However, during growth on fumarate (Fig. 2a, lane 3), the levels of the
omcB transcripts were much higher (about 500%) in the RpoS-deficient mutant than the wild-type strain.

Similar results were also obtained from primer extension analyses. No products corresponding to P1, P2 or P3 were detectable when total RNA was isolated from the Fe(III)-grown, RpoS-deficient mutant (Fig. 4a, b, lane 6). Primer extension products corresponding to P2 and P3 were detected with the fumarate-grown RpoS-deficient mutant, while P1 was hardly detectable (Fig. 4a, b, lane 5). This result further supports the hypothesis that P1 is the primary promoter for the omcC cluster, whereas P2 serves as the promoter for the omcB cluster.

Identification and characterization of a potential transcriptional regulator of the omcB and omcC clusters

The ORF (647 bp) upstream of the orf1-orf2-omcC::kan, DL11 (orf1-orf2-omcC::kan), DLCN16 (RpoS-deficient mutant) (Nunez et al., 2004) and wild-type. Primer extension products were derived from total RNA (40 μg) isolated from cells grown in medium with either fumarate (odd lanes) or Fe(III) citrate (even lanes) as sole electron acceptor. Lanes 1 and 2, wild-type (DL1); lanes 3 and 4, DL11 (orf1-orf2-omcC::kan); lanes 5 and 6, RpoS-deficient mutant. (a) Promoters P1 and P2 from long transcripts; (b) Promoter P3 from short transcripts.

In order to determine whether OrfR functions as a transcriptional regulator of omcB and/or omcC expression, an OrfR knockout mutant was constructed (DL10, orfR::gm). The OrfR-deficient mutant (DL10) grew as well as the wild-type when fumarate was supplied as the electron acceptor (data not shown). The Fe(III) reduction rate of the OrfR-deficient mutant (DL10) was approximately 55% of that of the wild-type when cells were grown in medium containing Fe(III) as the electron acceptor (Fig. 5). When orfR was reintroduced into the OrfR-deficient mutant in trans, growth of the complemented strain (DL10/pCM-orfR) on Fe(III) was similar to that of the wild-type (Fig. 5).

Associated with the inhibition of Fe(III) reduction in the OrfR-deficient mutant was a decrease in levels of both polycistronic and monocistronic omcB mRNA (Fig. 2a, lane 6) to only 42% of wild-type. In contrast, the mutant had levels of omcC mRNA that were similar to wild-type during growth on Fe(III) (Fig. 2b, lane 6). During growth on fumarate, the levels of omcC transcripts in the mutant were slightly higher than in wild-type (Fig. 2b, lane 5), whereas the levels of omcB transcripts in the mutant were similar to wild-type (Fig. 2a, lane 5).

DISCUSSION

Not only are the functions of OmcB and OmcC different (Leang et al., 2003), but also, as shown in this study, the regulation of the omcB and omcC genes is different. As discussed in detail below, the expression of omcB and omcC

![Fig. 4. Primer extension analyses comparing DL11 (orf1-orf2-omcC::kan), DLCN16 (RpoS-deficient mutant) (Nunez et al., 2004) and wild-type. Primer extension products were derived from total RNA (40 μg) isolated from cells grown in medium with either fumarate (odd lanes) or Fe(III) citrate (even lanes) as sole electron acceptor. Lanes 1 and 2, wild-type (DL1); lanes 3 and 4, DL11 (orf1-orf2-omcC::kan); lanes 5 and 6, RpoS-deficient mutant. (a) Promoters P1 and P2 from long transcripts; (b) Promoter P3 from short transcripts.](http://mic.sgmjournals.org)

![Fig. 5. Fe(III) reduction of wild-type (■), DL10 (orfR::gm) (▲) and the complemented strain DL10/pCM-orfR () in medium containing acetate as the electron donor and Fe(III) citrate as the electron acceptor. Data are means ± SD of triplicate values.](http://mic.sgmjournals.org)
is under the control of RpoS and possibly a putative transcriptional regulator, OrfR. The results suggest that expression of omcB is correlated to Fe(III) respiration whereas the expression of omcC is not. In addition, this study aids the further understanding of the controls of dissimilatory metal reduction, as this is believed to be the first report on the regulation of genes whose products are involved in Fe(III) reduction in G. sulfurreducens.

**Transcription of omcB and omcC**

The results suggest that, under the conditions evaluated, both omcB and omcC are expressed and are transcribed in a similar manner, as both a monocistronic (omcB/C) and a polycistronic (orf1-orf2-omcB/C) transcript. The combination of Northern blot and primer extension analyses identified the 2.5 kb transcripts of the omcB and omcC genes. The results suggest that the transcription initiation site for both of the two monocistronic transcripts is P3, which is found 307 bp upstream of both omcB and omcC and is located within a 2.7 kb duplication region that is virtually identical in both the omcB and omcC clusters. The mRNA start sites for the long transcripts, P1 and P2, are also located within the 2.7 kb identical region. The finding that the primer extension product P1 was missing when the orf1-orf2-omcC cluster was deleted from the genome suggests that the polycistronic orf1-orf2-omc transcript is initiated from P1, whereas orf1-orf2-omcB is initiated from P2. The reasons for this are as yet unknown, but it is possible that there are transcriptional regulators which recognize divergent sequences upstream of the omcB or omcC cluster but not the other.

Other results also suggest that expression of the omcB and omcC genes is regulated differently. Levels of both omcB transcripts are higher when Fe(III) serves as the electron acceptor than during growth on fumarate, whereas those of omcC are similar. In addition, the absence of two transcriptional regulators, RpoS and OrfR, had different effects on omcB and omcC expression under specific conditions. In the presence of fumarate as an electron acceptor, elimination of rpoS led to an increase in omcB expression but a dramatic decrease in omcC expression. During growth on fumarate, deletion of orfR increased the expression of the long omcC transcript but did not affect omcB expression. Likewise, during growth on Fe(III), omcB expression decreased whereas omcC expression was relatively unaffected.

During growth on Fe(III), omcB and omcC transcripts were hardly detectable in the RpoS-deficient mutant, suggesting that RpoS regulates transcription of all four promoters, P1, P2, P3, and P4. Regulation of omcB and omcC expression by RpoS is consistent with the fact that the sigma factor RpoS of E. coli and other Gram-negative bacteria (Nunez et al., 2004), and that the -35 regions of P1, P2, P3, and P4 are GC rich (4/6, 6/6 and 4/6 respectively), similar to those recognized by RNA polymerase containing RpoS in E. coli. However, a potential alternative explanation for the observed results is that other regulator(s), which might be RpoS dependent, control the transcription initiation of omcB and/or omcC operons. For example, during growth on fumarate, omcC transcript levels in the RpoS mutant were higher than in the wild-type, whereas transcription of omcC still appeared to be inhibited. This suggests that at least one other regulator, which may be RpoS dependent, functioned as a repressor of omcB transcription in the absence of Fe(III) and further emphasizes that the primary function of OmcB is probably electron transfer to Fe(III).

Other potential global regulators appear to be involved in regulation of the omcB and/or omcC operons. Whole-genome microarray analyses have revealed that a mutant of G. sulfurreducens in which fur (ferrous uptake regulator) was deleted had higher levels of omcB transcripts, but not omcC transcripts, during growth on fumarate with limited iron (R. O’Neil, unpublished results). A potential fur box was found 80 bp upstream of the P1 transcriptional start site (J. Krushkal, personal communication). Furthermore, omcB/omcC expression may also be related to the level of ppGpp in the cells (L. DiDonato, unpublished results). In the absence of RelA (ppGpp synthetase I) during growth on fumarate, the expression of omcB was up-regulated whereas that of omcC was not.

Another factor potentially controlling transcription of omcB, and hence Fe(III) reduction, appears to be the product of orfR. The notable effect of deleting orfR was a partial inhibition of Fe(III) reduction associated with lower levels of omcB transcripts. Deleting orfR did not have a significant effect on levels of omcC transcripts during growth on Fe(III). This suggests that the product of orfR may be an activator for omcB transcription, but only during growth on Fe(III), and further emphasizes that the function of OmcB is related to Fe(III) reduction. The combination of results suggests that OrfR may function as a modulator to fine tune the expression of the omcB/C operon under different growth conditions. Clearly, the mechanisms by which OrfR functions in regulating the expression of these genes warrant further study.

The genome of G. sulfurreducens contains genes for over 100 c-type cytochromes (Methe et al., 2003) and many of these appear to have arisen as the result of gene duplications. The high degree of identity between all of the components of the omcB and omcC operons (79–100%) suggests that this duplication occurred on a relatively recent evolutionary time scale. However, the regulation of omcB and omcC is markedly different. The results presented here, as well as functional analysis of other c-type cytochrome genes in G. sulfurreducens (L. DiDonato, unpublished results), suggest that many of the cytochromes with similar gene sequences have different functions and/or are differentially regulated. Investigation into the function and regulation of these duplicated genes will aid further understanding of the physiology of G. sulfurreducens.
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