Differential expression of two \( bc_1 \) complexes in the strict acidophilic chemolithoautotrophic bacterium \textit{Acidithiobacillus ferrooxidans} suggests a model for their respective roles in iron or sulfur oxidation

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Three strains of the strict acidophilic chemolithoautotrophic \textit{Acidithiobacillus ferrooxidans}, including the type strain ATCC 23270, contain a petIIABC gene cluster that encodes the three proteins, cytochrome \( c_1 \), cytochrome \( b \) and a Rieske protein, that constitute a \( bc_1 \) electron-transfer complex. RT-PCR and Northern blotting show that the petIIABC cluster is co-transcribed with cycA, encoding a cytochrome \( c \) belonging to the \( c_4 \) family, sdrA, encoding a putative short-chain dehydrogenase, and hip, encoding a high potential iron–sulfur protein, suggesting that the six genes constitute an operon, termed the petII operon. Previous results indicated that \textit{A. ferrooxidans} contains a second pet operon, termed the petI operon, which contains a gene cluster that is similarly organized except that it lacks hip. Real-time PCR and Northern blot experiments demonstrate that petI is transcribed mainly in cells grown in medium containing iron, whereas petII is transcribed in cells grown in media containing sulfur or iron. Primer extension experiments revealed possible transcription initiation sites for the petI and petII operons. A model is presented in which petI is proposed to encode the \( bc_1 \) complex, functioning in the uphill flow of electrons from iron to NAD(P), whereas petII is suggested to be involved in electron transfer from sulfur (or formate) to oxygen (or ferric iron). \textit{A. ferrooxidans} is the only organism, to date, to exhibit two functional \( bc_1 \) complexes.

INTRODUCTION

The cytochrome \( bc_1 \) complex is a central component of the energy transduction of the biosphere and is involved in almost all respiratory (aerobic as well as anaerobic) and photosynthetic electron-transfer chains (Hunte \textit{et al.}, 2003 and references therein). In prokaryotes, the \( bc_1 \) complex, while common, is not universal. The complex is composed of three subunits, a cytochrome \( c_1 \), a cytochrome \( b \) and a Rieske iron–sulfur protein, that transfer electrons from a membrane-localized quinone to a small soluble redox protein such as cytochrome \( c \), plastocyanin, or a high-potential iron–sulfur protein (HiPIP) (Menin \textit{et al.}, 1998; Hunte \textit{et al.}, 2003 and references therein).

Reverse electron flow from cytochrome \( c \) through the cytochrome \( bc_1 \) complex to quinone has also been reported in a variety of chemolithoautotrophic organisms. During autotrophic growth of these bacteria, the energetic substrate has to provide electrons for reduction of NAD(P) to NAD(P)H, which is required for CO\( _2 \) fixation and other anabolic processes. When the midpoint potential of the electron donor is more positive than that of the NAD(P)/NAD(P)H couple, the reduction of NAD(P) requires energy. Depending on the level at which the electrons enter the respiratory chain, they have to be transported ‘uphill’ to NAD(P) by reverse electron transport through the cytochrome \( bc_1 \) complex, the quinone pool and the NAD(P)H dehydrogenase (Griesbeck \textit{et al.}, 2000). This uphill (reverse) electron transport uses the proton-motive force.
force generated by hydrolysis of ATP derived from electron donor oxidation.

In the strictly acidophilic chemolithoautotrophic Gram-negative bacterium *Acidithiobacillus ferrooxidans*, a cytochrome bc₁ complex was shown by spectroscopic techniques to function in reverse in ferrous-iron-grown cells, even in the presence of thiosulfate, while it functioned in the normal (downhill) direction in sulfur-grown cells (Brasseur et al., 2002, 2004). This raised questions regarding the mechanism regulating the flow of electrons either uphill or downhill in the same complex. A candidate operon, termed petI, was identified in *A. ferrooxidans* ATCC 33020 and ATCC 19859 (Levicán et al., 2002) that could potentially encode a bc₁ complex, and a second distinct operon, termed petII, was experimentally validated in *A. ferrooxidans* ATCC 33020 (Bruscella et al., 2005) and bioinformatically detected in the type strain *A. ferrooxidans* ATCC 23270 (Brasseur et al., 2002), raising the possibility that one of the two distinct bc₁ complexes might be involved in the uphill flow of electrons and the other in the downhill flow (Brasseur et al., 2004). The aim of the research described in this paper was to deepen our understanding of the petII operon and to study the expression of both the petI and petII operons in response to ferrous iron and sulfur in order to gain further insight into the respiratory chains in which these two cytochrome bc₁ complexes are involved and how their synthesis might be regulated.

**METHODS**

**Bacterial strains, plasmids and growth conditions.** *Acidi-thiobacillus ferrooxidans* ATCC 33020, ATCC 19859 and ATCC 23270 were obtained from the American Type Culture Collection. *A. ferrooxidans* was grown at 30 °C under oxic conditions in ferrous iron or sulfur medium as described previously (Yarzábal et al., 2003).

Escherichia coli strain TG1 (supE hsdS thi Δlac-proAB) F− traD36 proAB lacIΔlacZΔM15) was used for phagemid propagation and was grown on LB medium (Ausubel et al., 1992). The phagemid SK+ Bluescript was purchased from Stratagene.

**Analytical methods.** The ferrous iron concentrations were determined by the o-phenanthroline method (Muir & Anderson, 1977).

**DNA manipulations.** General DNA manipulations were performed according to Ausubel et al. (1992). Before manipulations, *A. ferrooxidans* cells were washed several times in basal salt solution corresponding to the medium in which they were grown, in order to remove ferrous iron precipitates or sulfur aggregates. Genomic DNA from *A. ferrooxidans* was prepared using the Nucleospin Tissue kit (Macherey-Nagel), according to the manufacturer’s instructions for bacterial DNA extraction. Taq polymerase purchased from Eppendorf was used for PCR. The oligonucleotides were obtained from Sigma-Genosys Corporation. The nucleotide sequences of the cloned fragments were determined from both strands by GENOME Express.

**Plasmid construction.** In order to synthesize the *hip* and *cycA1* RNA probes used for the Northern blot experiments, an internal fragment of each gene was amplified by PCR (Table 1) and cloned into the EcoRV restriction site of the SK+ Bluescript vector, between the T7 and T3 promoters.

**RNA manipulations.** Total RNA was extracted from 500 ml ferrous iron- or sulfur-cultures at different stages of growth (early exponential, mid exponential, late exponential or stationary phase) with the High Pure RNA isolation kit (Roche) as described previously (Guiliani et al., 1997). For RT-PCR and real-time PCR, total RNA was treated twice with DNase I (Roche) and DNA contamination was checked by PCR.

**Northern blotting.** Formaldehyde gels were used for Northern blotting, as described by Ausubel et al. (1992). RNA was transferred by capillary action to positively charged nylon membranes purchased from Roche. RNA was UV cross-linked to the membrane with the Stratalinker from Stratagene. DIG-labelled *hip* and *cycA1* RNA probes were obtained by *in vitro* transcription performed on SK-*hip* or SK-*cycA1* plasmids, linearized with EcoRI or HindIII restriction enzymes, using T7 or T3 RNA polymerase and DIG-UTP from the Strip-EZ kit (Ambion). Prehybridization and hybridization steps were performed under high-stringency conditions with the DIG-labelled *hip* and *cycA1* RNA probes. Detection was performed by chemiluminescence with CSPD (Roche).

**Reverse transcriptase-PCR (RT-PCR).** Coupled RT-PCR experiments were performed with the Promega Access RT-PCR system. RT-PCR was carried out in two steps: (i) the reverse transcription was done on approximately 1 μg total RNA (DNA free) extracted from ferrous-iron-grown cells of *A. ferrooxidans*, with the Omniscript RT kit purchased from Qiagen; (ii) routine PCR amplification, with the oligonucleotides of interest (Table 1), was done using the cDNA obtained as matrix, as described above. For each RT-PCR experiment, three controls were used: one without template to detect potential contamination, one with genomic DNA as a positive control for PCR amplification and one with RNA not treated with reverse transcriptase to check for DNA contamination during RNA preparation.

**Real-time PCR.** The *rrs* gene encoding the 16S rRNA has been shown to be expressed at the same (constitutive) level under both conditions of growth examined (on ferrous iron and sulfur media) (Yarzábal et al., 2004) and was used as a reference standard. Equal amounts of total RNA, extracted from ferrous iron- and sulfur-grown cells at different stages of growth, were retrotranscribed with the Superscript II reverse transcriptase (Invitro Life Technologies) at 42 °C for 50 min, followed by 15 min at 70 °C to inactivate the enzyme. Real-time PCR quantification was performed on the total cDNA obtained, using the LightCycler instrument and the LightCycler Fast Start DNA master (plus) SYBR Green I kit, with external standards, as described in Roche Molecular Biochemicals technical note no. LC 11/2000 and Yarzábal et al. (2004). Real-time PCR experiments were performed several times, using RNA samples from at least two independent cultures. The sequences of the oligonucleotide primers are given in Table 1.

**Primer extension.** Primer extension was performed with Superscript II reverse transcriptase (Invitro Life Technologies) as follows: extension at 42 °C or 50 °C for 50 min, followed by heating at 70 °C for 15 min to inactivate the enzyme. The oligonucleotides used (Table 1) were [32P]ATP-labelled with T4 polynucleotide kinase from Biolabs. The experiments were done in duplicate, using RNA samples from independent cultures.

AJ413192 (petC1 internal region), AJ413171 (intergenic region petC1–resB), AJ413193 (resB internal region) and AJ413194 (intergenic region resB–resC).


**Bioinformatic techniques.** Potential sigma-70-like promoters were detected using an HMM model trained on A. ferrooxidans sigma-70-like promoters (M. Santa Ana, J. Valdes, M. Chacon, T.

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**Table 1. Oligonucleotides used in this study**

<table>
<thead>
<tr>
<th>Primer (names used in Figs 1 and 2)</th>
<th>Sequence</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip9</td>
<td>AgTgAgAAACTgAaAAgAAAC</td>
<td>hip</td>
</tr>
<tr>
<td>Hip10</td>
<td>gCgggCATCggCgTAAAC</td>
<td>hip</td>
</tr>
<tr>
<td>cycA1-1</td>
<td>gCTCCCTATCTGGTCAAAC</td>
<td>cycA1</td>
</tr>
<tr>
<td>cycA1-2</td>
<td>CTgCTgTGACCTgCCAAG</td>
<td>cycA1</td>
</tr>
<tr>
<td>HipC4-1</td>
<td>AgAgAATAgTgggATAACgg</td>
<td>cycA2</td>
</tr>
<tr>
<td>Ext HC4-1</td>
<td>CgCCTTggTATAgTTgTggg</td>
<td>petII 5’ untranslated region</td>
</tr>
<tr>
<td>ResC4-1</td>
<td>gCATgCACCCATACC</td>
<td>cycA1</td>
</tr>
<tr>
<td>II Ext RC4-1</td>
<td>ACCATCTTACCCACC</td>
<td>petII 5’ untranslated region</td>
</tr>
</tbody>
</table>

**Expression plasmid construction**

(for in vitro transcription for RNA probes)

**Primer extension**

| HipC4-1                             | AgAgAATAgTgggATAACgg | cycA2 |
| Ext HC4-1                           | CgCCTTggTATAgTTgTggg | petII 5’ untranslated region |
| ResC4-1                             | gCATgCACCCATACC | cycA1 |
| II Ext RC4-1                        | ACCATCTTACCCACC | petII 5’ untranslated region |

**PCR, RT-PCR and real-time PCR**

*The superscript letters indicate the use(s) of the primer: a, PCR; b, RT-PCR; c, real-time PCR.

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AJ413192 (petC1 internal region), AJ413171 (intergenic region petC1–resB), AJ413193 (resB internal region) and AJ413194 (intergenic region resB–resC).


**Bioinformatic techniques.** Potential sigma-70-like promoters were detected using an HMM model trained on A. ferrooxidans sigma-70-like promoters (M. Santa Ana, J. Valdes, M. Chacon, T.
L’Heureaux, E. Jedlicki, & D. S. Holmes, unpublished results). Potential transcription factor binding sites were searched for using MAT inspector (Cartharius et al., 2005) and information theory (Schneider, 1999). Potential rho-independent translational stop sites were detected according to de Hoon et al. (2005).

RESULTS

Genetic organization and conservation of the petII operon in multiple strains of A. ferrooxidans

Using information derived from the fully sequenced genome of the type strain A. ferrooxidans ATCC 23270 (http://www.tigr.org/), DNA primers were designed for each gene and intergenic region of the petII operon. The sizes of the resulting amplified fragments suggest that the organization of the petII operon previously described in the type strain of A. ferrooxidans ATCC 23270 (Brasseur et al., 2002) is conserved in A. ferrooxidans ATCC 33020 (Fig. 1a, b) and A. ferrooxidans ATCC 19859 (data not shown). The sequences of the PCR fragments obtained from the ATCC 33020 strain display higher identity to the petII than to the petI locus from ATCC 23270 (data not shown). Taken together, these results indicate that, as in ATCC 23270, there are two pet loci in ATCC 33020 and ATCC 19859.

The petII gene cluster constitutes an operon

RT-PCR experiments were performed with convergent oligonucleotide primers designed from two adjacent genes of the ATCC 23270 petII locus (see Table 1 and Fig. 1) on total RNA extracted from ATCC 33020 grown with ferrous iron (Fig. 1c). RT-PCR products of the expected size were generated between each gene pair of the petII cluster, suggesting that it is an operon.

A candidate transcriptional start site of the petII operon was detected by reverse-transcriptase-mediated primer extension experiments (Fig. 2a). This was compared to the predicted start site of the petI operon using similar techniques (Fig. 2b and data not shown). In both cases putative sigma-70-like promoter sequences were detected by bioinformatic techniques upstream of the respective transcriptional start sites (Fig. 2c). Transcription from the petI promoter was detected only in iron-grown cells and transcription from the petII promoter in iron- and in sulfur-grown cells (Fig. 2a, b), which was in agreement with
Northern blot hybridization and real-time PCR experiments described below.

**Regulation of the petI and petII operons**

Northern blotting and real-time PCR experiments were carried out to determine the relative levels of expression of the petI and petII operons in cells grown in either iron or sulfur medium.

Antisense RNA probes complementary to the cycA1 and hip genes were hybridized to total RNA extracted from *A. ferrooxidans* ATCC 33020 grown in iron or sulfur medium. The largest transcript detected with the cycA1 probe was 4000 nt long (Fig. 3a), which suggested that the petI operon includes transcripts that correspond to the full length operon, confirming results obtained from RT-PCR experiments (Levican *et al.*, 2002). Smaller transcripts were also observed. Because the cycA1 probe corresponds to the first gene of the petI operon, these data suggest that the largest transcript is processed. With the hip probe, the largest transcript detected was 4600 nt long (Fig. 3b), again potentially corresponding to a full-length transcript of the operon. Smaller transcripts were also observed, perhaps resulting from RNA processing or transcription from internal promoters. However, no internal promoters were detected upstream of hip by reverse transcriptase-mediated primer extension experiments (data not shown), reducing the likelihood of this explanation.

The results of Northern blotting experiments indicated that the petI and petII operons are differentially expressed. Using the same amount of total RNA, the hybridization signal is clearly more intense for cycA1 when cells are grown in iron versus sulfur medium (Fig. 3a). However, hip appears to be transcribed under both conditions, with slightly more transcripts in sulfur medium (Fig. 3b). This suggests that the petI and petII operons are regulated in response to the energetic substrate.

To corroborate the Northern blot results, the amount of transcripts corresponding to petI and petII was quantified at all stages of the cell growth by real-time PCR. The results are shown in Table 2. The petI and petII pattern of expression in response to the energetic substrate was similar to that detected by Northern blotting, with much higher expression of petI in iron- than in sulfur-grown cells and expression of petII in sulfur- and in iron-grown cells. In addition, several interesting points were noticed: (i) as shown for the rus operon (Yarzabal *et al.*, 2004), the amounts of all the petI transcripts decreased significantly after 3 days of growth in ferrous-iron-grown cells when complete oxidation of
Two bc₁ operons in Acidithiobacillus ferrooxidans

ferrous iron to ferric iron had occurred; (ii) the petII operon was expressed in ferrous iron-grown cells, mainly in the early exponential phase; (iii) hip transcripts were clearly more abundant in sulfur- than in iron-grown cells. hip may have an additional regulatory mechanism, which we propose is at a post-transcriptional level because no internal promoter could be detected upstream of hip by primer extension (data not shown).

**Table 2.** Quantification of the petI and petII transcripts by real-time PCR in ferrous iron (F)- and sulfur (S)-grown cells at different stages of growth

Total RNA was extracted from ferrous-iron-grown cells at 17 h (early-exponential phase), 1 day (mid-exponential phase), 2 days (late-exponential phase) and 3 days (stationary phase). Total RNA was extracted from sulfur-grown cells at 1 day (early-exponential phase), 2, 3 and 4 days (mid-exponential phase), and 5 days (late-exponential phase). All values (except for rrs, encoding the 16S rRNA) are expressed as n-fold relative to 16S rRNA (×10⁶). The data are representative of real-time PCR experiments performed on at least two independent cultures. The experiment shown used single cultures and the values are the mean of duplicate assays that almost always varied by less than 25%.

<table>
<thead>
<tr>
<th>Gene</th>
<th>F</th>
<th>Growth conditions:</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17 h</td>
<td>1 d</td>
<td>2 d</td>
</tr>
<tr>
<td>rrs</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>sdrA1</td>
<td>600.9</td>
<td>761.2</td>
<td>302.1</td>
</tr>
<tr>
<td>petA1</td>
<td>609.9</td>
<td>627</td>
<td>149.7</td>
</tr>
<tr>
<td>petB1</td>
<td>1119</td>
<td>1383.1</td>
<td>360.2</td>
</tr>
<tr>
<td>petC1</td>
<td>39.8</td>
<td>348.6</td>
<td>23.9</td>
</tr>
<tr>
<td>cycA2</td>
<td>87</td>
<td>553.9</td>
<td>14.3</td>
</tr>
<tr>
<td>sdrA2</td>
<td>154.7</td>
<td>573.1</td>
<td>16.6</td>
</tr>
<tr>
<td>petA2</td>
<td>103.9</td>
<td>704.3</td>
<td>21.8</td>
</tr>
<tr>
<td>petB2</td>
<td>143.5</td>
<td>639.2</td>
<td>33.8</td>
</tr>
<tr>
<td>petC2</td>
<td>84.5</td>
<td>331.3</td>
<td>11.4</td>
</tr>
<tr>
<td>hip</td>
<td>158.4</td>
<td>959.3</td>
<td>134.3</td>
</tr>
</tbody>
</table>

**Fig. 3.** Northern blot hybridization analysis. Total ATCC 33020 A. ferrooxidans RNA from early-exponential iron-grown cells or mid-exponential sulfur-grown cells probed with DIG-labelled cycA1 (a) or hip (b) RNA. Arrows show the largest transcripts. The positions and sizes of the RNA ladder (Invitrogen) are indicated on the left of each blot.
To explore possible mechanisms involved in the differential expression of the petI and petII operons depending on energy source, the 5’ untranslated regions of both operons were compared using MAT inspector. No obvious conserved regulatory motifs were detected.

**DISCUSSION**

**Two differentially expressed operons encoding bc1 complexes in A. ferrooxidans**

Bioinformatic analysis, PCR experiments and DNA sequence analysis indicate that the genomes of three strains of *A. ferrooxidans*, ATCC 19859, ATCC 33030 and the type strain ATCC 23270, contain two operons, petIABC and petIIABC, encoding two complete cytochrome bc1 complexes. Both are cotranscribed with cycA and sdrA, which encode a c4-type cytochrome and a short-chain dehydrogenase respectively.

Data from primer extension, Northern blotting and real-time PCR experiments (Figs 2 and 3, Table 2) and preliminary transcriptome analysis (Quatrini et al., 2006) demonstrate that petI and petII are differentially transcribed depending on the growth conditions. The petI operon is principally transcribed only when ferrous iron is provided in the growth medium as an energy and electron source. In contrast, the petII operon is transcribed in sulfur- or iron-grown cells. Transient transcription is observed from the petII operon when cells are first placed in ferrous iron medium but at later stages of cell growth significantly less expression is detectable (Table 2), a situation that is reminiscent of the transient expression of the rus operon (Yarzábal et al., 2003, 2004). Although no explanation for this low amount of transient expression has been experimentally validated, it has been speculated that, in the case of the rus operon, it could be a response to an increase in particular nutrients to allow quick adaptation to the environment, perhaps mediated by a Fis-like protein (Yarzábal et al., 2004). However, no obvious Fis-binding DNA motif, which is particularly degenerate (Hengen et al., 1997), was detected in the proposed regulatory region of petII.

Given that petI is induced in ferrous iron medium, a computational search was carried out for known iron-regulated transcription factor binding sites in the region upstream of cycA1 where a sigma-70-like promoter is predicted (Fig. 2c). No obvious Fur-binding site (Lavrarr & McIntosh, 2003) could be detected, although the *A. ferrooxidans* genome is known to encode Fur and has been shown experimentally to have Fur-binding sites upstream of known iron-regulated genes (Quatrini et al., 2005). Computational analysis also failed to detect significant similarity in the *A. ferrooxidans* genome with the two-component system PmrA–PmrB (Wosten et al., 2000), and no cis-acting iron-responsive operators (IROs) of the RirA regulatory system (Todd et al., 2005) could be detected upstream of either the petI or the petII operons, leaving open, for the moment, the question of how these operons are differentially regulated.

**Proposed role for the redox proteins encoded by the petI and petII operons**

*A. ferrooxidans* is the first organism described so far to have two complete and functional bc1 complexes, raising the...
question of why this is necessary. Genetic evidence has been presented for the existence of a cytochrome-containing complex functioning exclusively during iron oxidation (Cabrejos et al., 1999; Levican et al., 2002) and it has been reported that a bc1 complex functions only in reverse in iron-grown cells (Elbehti et al., 2000), even in the presence of an appropriate substrate (Brasseur et al., 2002). On the other hand the existence of a bc1 complex has been proposed to be involved in the aerobic and anaerobic oxidation of sulfur and formate processes (Corbett & Inglewed, 1987; Pronk et al., 1991) and a bc1 complex has been shown recently to function in direct mode in sulfur-grown cells (Brasseur et al., 2004). This raises the possibility that two operationally independent bc1 complexes are needed to cope with iron and sulfur oxidation, respectively: one for uphill flow during iron oxidation and the other for downhill flow during sulfur oxidation. A corollary of this is that neither of the two complexes can switch the direction of electron flow. Whether this imposition comes from intrinsic mechanistic differences in the sequence and structure of the two bc1 complexes that specify unidirectional flow of electrons or from the action of additional, as yet unknown, structural or regulatory components that could channel electrons to the correct bc1 complex, remains to be determined.

Our hypothesis is that the bc1 complex encoded by petI is the one functioning in reverse and transfers the electrons from ferrous iron to NAD(P), while the bc1 complex encoded by petII is the one functioning directly, transferring electrons from sulfur to oxygen and possibly involved in the aerobic and anaerobic oxidation of sulfur and formate described by Pronk et al. (1991).

Together with the three subunits of the bc1 complex, the petI and petII operons encode other redox proteins. Because these genes are in the same transcriptional unit, these proteins are probably involved in the same electron-transfer chain. The sdrA1 gene has been predicted to encode a short chain dehydrogenase (Levican et al., 2002) but its function remains unknown. The cytochrome c4 encoded by the cycA1 gene has been proposed to belong to the electron-transfer chain between ferrous iron and oxygen, and more precisely to receive the electrons directly from ferrous iron and to transfer them to rusticyanin (Giudici-Orticoni et al., 2000). However, according to the data presented here, this cytochrome c4 is more likely to be involved in the reverse electron pathway between ferrous iron and NAD(P) because cycA1 belongs to the petI operon, and because the rus operon contains another cytochrome c4 encoding gene (cyc1) that has been suggested (Appia-Ayme et al., 1999) to assume the role postulated by Giudici-Orticoni et al. (2000). In the reverse electron pathway the bc1 complex receives the electrons from a cytochrome c and transfers them to the quinol pool. We suggest that this cytochrome c is the cytochrome c4 encoded by the cycA1 gene (Fig. 4). Oxygen and NAD(P) reduction have been proposed to be coupled to balance the reducing equivalent from ferrous iron between the two pathways: the exergonic one, through the aa3-type oxidase towards oxygen, and the endergonic one, through a bc1 complex toward NAD(P). As previously reported (Brasseur et al., 2004), we propose that the branching point is at the level of rusticyanin, which can give electrons to two different cytochromes c2: CycA1 encoded by the petI operon or Cyc1 encoded by the rus operon. In the former case, electrons are transferred to NAD(P), while in the latter case, they are transferred to oxygen (Fig. 4).

When functioning in direct mode, the bc1 complex receives electrons from the quinol pool and transfers them either to a membrane-bound cytochrome c and/or to a soluble redox protein such as rusticyanin, or a high potential iron–sulfur protein (HiPIP) that subsequently passes the electrons to the terminal oxidase where oxygen reduction takes place (Hunte et al., 2003 and references therein; Bonora et al., 1999; Pereira et al., 1999). Since a membrane-bound cytochrome c (CycA2) and a soluble HiPIP are encoded by the petII operon, we suggest that these two redox proteins transfer electrons between integral membrane complexes: bc1 and a terminal reductase, in particular a quinol oxidase (Brasseur et al., 2004). An interesting possibility is that the electrons are transferred either to the cytochrome CycA2 or to the HiPIP Hip depending on the environmental conditions (Fig. 4). Since hip is transcribed more in sulfur- than in ferrous iron-growth conditions it is proposed that Hip is involved in the electron transfer from sulfur.

ACKNOWLEDGEMENTS

We are grateful to Y. Denis (IBSM, Transcriptome Unit, Marseille, France) for much helpful advice regarding real-time PCR. Preliminary sequence data were obtained from The Institute for Genomic Research website at http://www.tigr.org. Sequencing of A. ferrooxidans was accomplished with support from the US Department of Energy. C. A.-A. acknowledges the support of a post-doctoral fellowship from the EEC. This study was partly supported by ‘BIOMINE’ European project (sixth PCRD no. NM2.ct.2005.500329). Additional funding was received from Fondecyt 1056003 and a Microsoft sponsored Research Award.

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reverse but not in the forward direction. Is there a second bc complex? Biochim Biophys Acta 1555, 37–43.


Edited by: J. Green