# II.K.7 Genetics and Molecular Biology of Hydrogen Metabolism in Sulfate-reducing Bacteria

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## Objectives

The energy transduction systems of the anaerobic sulfate-reducing bacteria of the genus *Desulfovibrio* are to be elucidated. The target strain is *D. alaskensis* G20 because of its robust fermentative growth on pyruvate that should allow mutant analysis of sulfate respiration. Mutations of various transmembrane complexes are to be analyzed to determine their role in the energy budget of the cell. The development of genetic transfer systems for manipulation of the G20 strain is necessary for this analysis.

## Technical Barriers

The assumption that pyruvate fermentation generates ATP only through substrate-level phosphorylation may be inaccurate. Thus learning the pathway(s) of reoxidation of the reduced ferredoxin produced in pyruvate oxidation is likely to reveal electron transfer complexes pumping protons that contribute to a proton gradient used for ATP synthesis. The gene transfer capacity of the G20 strain through transformation has proven recalcitrant. Gene transfer by conjugation is more time consuming but it allows the generation of mutants to proceed.

## Abstract

Energy generation by the strictly anaerobic sulfate-reducing bacteria has been a controversial topic because of the unique feature that sulfate is the only inorganic electron acceptor that requires an energy-consuming activation before reduction. During the initial stages of growth of batch cultures that are respiring sulfate with organic acids as electron donors, hydrogen is both produced and then consumed. This observation led to the interesting hydrogen-cycling hypothesis (Odom and Peck, 1981) as a mechanism for contributing to the energy budget of the cells. We seek to elucidate the role of hydrogen in the energy budget of these bacteria through genetic approaches. Exploration of electron flow in the *cycA* mutant of the G20 strain, I2, that lacks the type 1 tetraheme cytochrome $c_3$ (Tp1- $c_3$) has revealed an apparent minor role for fumarate as a terminal electron acceptor even during sulfate respiration. Further growth results with this mutant have shown that high pyruvate concentrations transiently inhibit sulfate respiration. In studies to determine the enzymes and compartments for hydrogen production during the hydrogen transient observed, it has been observed that putative cytoplasmic hydrogenases are not conserved among species of *Desulfovibrio*. Examination of our library of nearly 7000 random transposon mutants of the *D. vulgaris* Hildenborough strain constructed in cells grown on lactate/sulfate medium revealed that the cytoplasmic CO-associated hydrogenase complex was not interrupted by transposons. This result was interpreted to mean that this enzyme complex plays an important role in the metabolism of cells grown on these substrates. Interestingly this hydrogenase has been shown to be among a family of transmembrane hydrogenases that oxidize reduced ferredoxin generating hydrogen and pumping a proton. We will continue to generate mutations in genes encoding transmembrane complexes to elucidate their role(s) in energy generation in the sulfate-reducing bacteria.

## Progress Report

1. Studies with the *Desulfovibrio* G20 tetraheme cytochrome $c_3$ (Tp1- $c_3$) mutant, CycA-, showed that sulfate respiration was transiently inhibited with electrons from pyruvate (Giles et al. under revision).
2. The alternative respiration of fumarate by G20 is robust (Fig. 1A) and analyses of excreted metabolites showed that some electrons are diverted to produce succinate even during sulfate reduction or pyruvate fermentation by the wild type G20 strain. CycA- mutant and a fumarate reductase transposon mutant are unable to respire fumarate (Fig. 1A).
3. Microarray and proteomics (Fig. 2) analysis of G20 and I2 revealed the surprising result that genes for fumarate respiration and, therefore, the enzymes were significantly decreased in the I2 mutant regardless of the growth mode. This suggests a redox control on transcription of these genes affected by the absence of the periplasmic cytochrome.
4. A putative two component regulatory operon was found upstream of the fumarate reductase operon (Fig. 3). A transposon mutation in the histidine kinase gene in that operon was tested for growth on fumarate and found to be unable to disproportionate fumarate

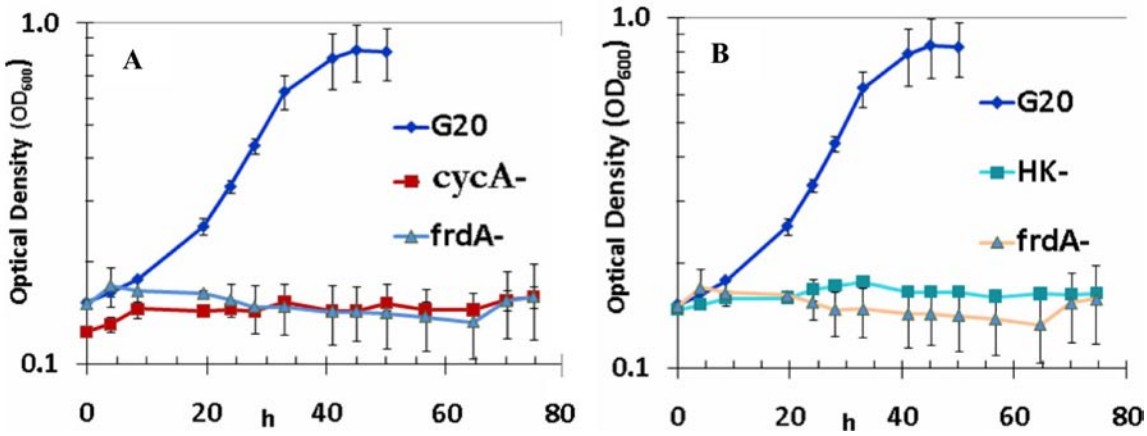


**FIGURE 1.** Growth by disproportionation of 60 mM Fumarate by *D. alaskensis* G20, a fumarate reductase (*frdA*) transposon mutant, the I2 mutant (*cycA*), and a transposon mutation in the histidine kinase (HK) upstream of the fumarate reductase operon.

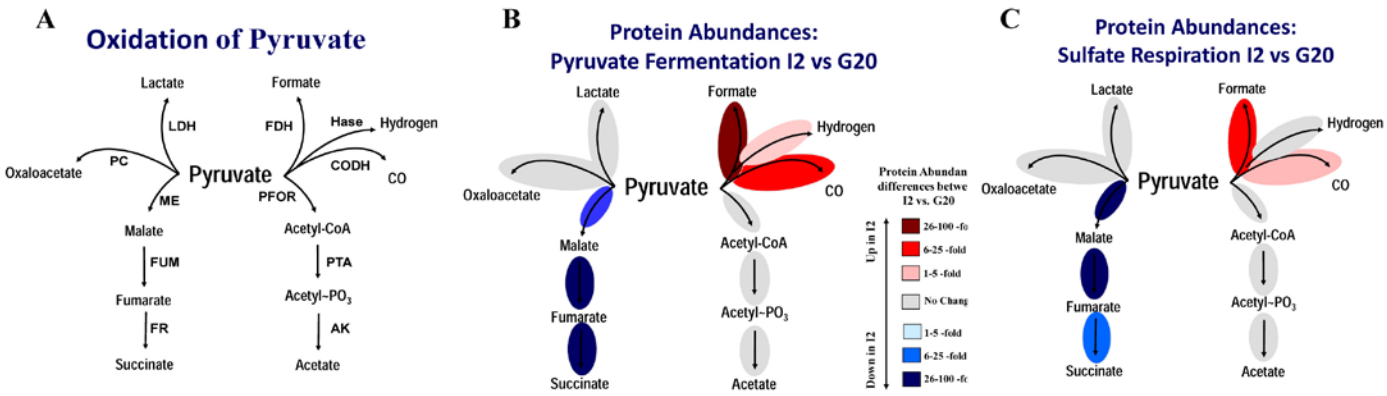


**FIGURE 2.** A) Metabolic fates of pyruvate in *Desulfovibrio.* LDH, lactate dehydrogenase; PC, pyruvate carboxylase; ME, malic enzyme; FUM, fumarate hydratase; FR, fumarate reductase; FDH, formate dehydrogenase; Hase, hydrogenases; CODH, CO dehydrogenase; PFOR, pyruvate formate oxidoreductase; PTA, phosphotransacetylase; AK, acetate kinase. B) Oval color represents the magnitude and direction of difference between protein abundances in I2 versus G20 for pyruvate fermentative cells. Grey is no difference. C) Colors as in B) for I2 versus G20 for lactate/sulfate grown cells.

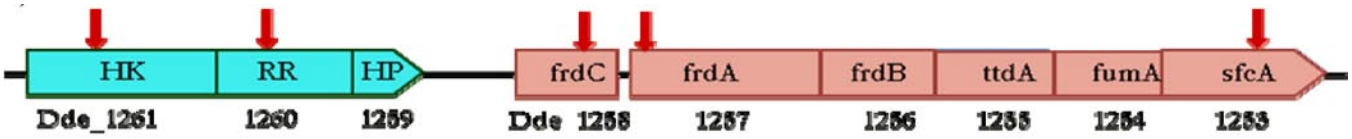


**FIGURE 3.** The fumarate reductase operon arrangement in *D. alaskensis* G20 in red and the upstream regulatory region (aqua). Red arrows are the relative positions of transposon insertion sites. HK, histidine kinase; RR, response regulator; HP, hypothetical protein. *frdCAB* encode a putative fumarate reductase, *ttdAfumA* encode the two subunits of a putative fumarase, and *sfcA*, a malic enzyme.

(Fig 1B). We will test to determine whether this is a redox sensing regulator affected by the redox changes occurring when the periplasmic tetraheme cytochrome is missing.

5. Over 7000 transposon mutations of *D. vulgaris* Hildenborough have been located through sequencing in collaboration with Adam Deutschbauer at LBNL. All selections have been performed with respiration of sulfur oxides and lactate as carbon source and electron donor. If randomly positioned, we would expect nearly two insertions per kb. When the insertion positions were examined, it was surprising that the genes encoding the CO-associated hydrogenase, covering about 7.5 kb, (but not the CO dehydrogenase enzyme) lacked transposons

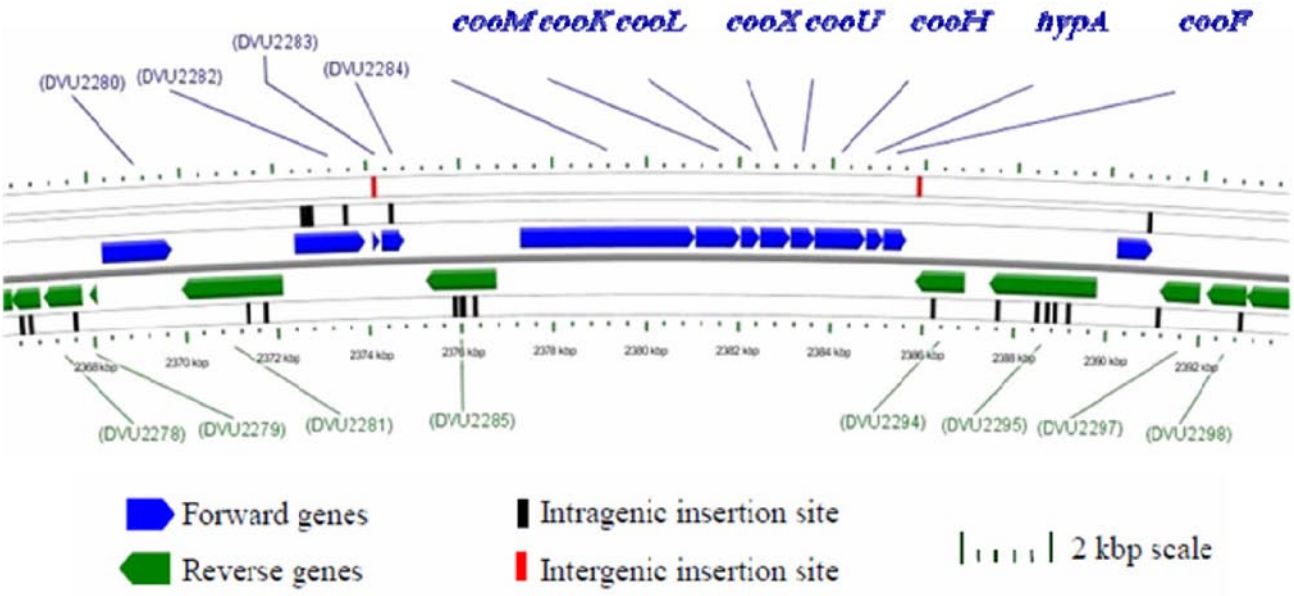


**FIGURE 4.** *D. vulgaris* chromosomal map in the region of the CO-associated cytoplasmic hydrogenase. Generated with CGView. CooMKLXUHhypAcooF had no transposon mutations.

(Fig. 4). We interpret this result as suggesting that strains with interruptions in these genes are not readily recovered in our procedures or that this enzyme complex is needed for growth under the conditions of selection. This hydrogenase has been predicted to be involved in energy conservation, acting as a primary proton pump in a ferredoxin-dependent electron transport system (Welte et al., 2010).

## Future Directions

1. In *D. vulgaris*, we can construct multiple mutations in a single strain and propose to delete both the CO-associated as well as the Ech hydrogenase because these are the two candidates for cytoplasmic hydrogenases. The results of this experiment may allow us to resolve the debate about the hydrogen cycling contribution to the energy budget.
2. Candidates for energy conserving, ferredoxin-dependent complexes in G20 are not as obvious. There are now over 12,000 transposon mutants and no predicted cytoplasmic hydrogenase that lacks mutations. We will explore the heterodisulfide reductase complex mutants for growth rate comparisons with wild type cells to determine whether the mutants appear to be impaired because of an energy defect.

## References

**1.** Odom, J.M., and H.D. Peck, Jr. 1984. Hydrogenase, electron-transfer proteins, and energy coupling in the sulfate-reducing bacteria *Desulfovibrio*. Annu. Rev. Microbiol. 38:551-592.

**2.** Welte, C., C. Krätzer, and U. Deppenmeier. 2010. Involvement of Ech hydrogenase in energy conservation of *Methanosarcina mazei*. FEBS J. 277:3396-3403.

## Publication list (including patents) acknowledging the DOE grant or contract

**1.** Wall, J.D., A.P. Arkin, N.C. Balci, and B.J. Rapp-Giles, Genetics and genomics of sulfate respiration in *Desulfovibrio*. Springer-Verlag, Berlin, Heidelberg, Microbial Sulfur Metabolism (**1**):1-12 (2009)

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