Activity and Transcriptional Regulation of Bacterial Protein-Like Glycerol-3-Phosphate Dehydrogenase of the Haloarchaea in *Halofax volcanii*^†^

Katherine S. Rawls, Jonathan H. Martin, and Julie A. Maupin-Furlow*

**University of Florida, Department of Microbiology and Cell Science, Gainesville, Florida 32611-0700**

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Glycerol is a primary energy source for heterotrophic haloarchaea and a major component of “salty” biodiesel waste. Glycerol is catabolized solely by glycerol kinase (encoded by *glpK*) to glycerol-3-phosphate (G3P) in *Halofax volcanii*. Here we characterized the next critical step of this metabolic pathway: the conversion of G3P to dihydroxyacetone phosphate by G3P dehydrogenase (G3PDH). *H. volcanii* harbors two putative G3PDH operons: (i) *glpA1B1C1*, located on the chromosome within the neighborhood of *glpK*, and (ii) *glpA2B2C2*, on megaplasmid pHV4. Analysis of knockout strains revealed that *glpA1* (and not *glpA2*) is required for growth on glycerol. However, both *glpA1* and *glpA2* could complement a *glpA1* knockout strain (when expressed from a strong promoter in trans) and were required for the total G3PDH activity of cell lysates. The *glpA1B1C1, glpK, glpF* (encoding a putative glycerol facilitator), and *ptsH2* (encoding a homolog of the bacterial phosphotransferase system protein Hpr) genes were transcriptionally linked and appeared to be under the control of a strong, G3P-inducible promoter upstream of *glpA1*. Overall, this study provides fundamental insights into glycerol metabolism in *H. volcanii* and enhances our understanding of central metabolic pathways of haloarchaea.

Glycerol is a highly abundant energy source in hypersaline environments as a result of leakage from and lysis of *Dunaliella* cells, which are known to accumulate glycerol in molar quantities as an organic, osmotic solute (3, 5, 7, 32). Thus, glycerol is a primary energy source for heterotrophic members of this community.

In biological systems, glycerol is metabolized to dihydroxyacetone phosphate (DHAP) by one of two routes: (i) phosphorylation by glycerol kinase and subsequent conversion of sn-glycerol-3-phosphate (G3P) into DHAP through G3P dehydrogenase (G3PDH) or (ii) oxidation by glycerol dehydrogenase to form dihydroxyacetone (DHA), which is subsequently phosphorylated by an ATP-dependent or phosphoenolpyruvate:phosphotransferase system (PEP:PTS)-dependent DHA kinase to form DHAP. Once generated from glycerol, DHAP can be channeled into metabolic intermediates, including pyruvate, G3P, and/or sn-glycerol-1-phosphate (G1P).

Recently, we demonstrated through *Halofax volcanii* that haloarchaea require glycerol kinase (encoded by *glpK*) for the catabolism of glycerol (27). These results suggest that (i) G3PDH is needed for glycerol metabolism and (ii) the homologs of bacterial PEP:PTS-dependent DHA kinase are not needed for glycerol catabolism in *H. volcanii* but may serve in the metabolism of DHA overflow products generated by other members of the hypersaline community, such as *Dunaliella salina* (4).

In this study, we investigated the oxidation of G3P to DHAP by G3PDH, a metabolic step likely to be central to glycerol catabolism and subsequent to the phosphorylation of glycerol by glycerol kinase in haloarchaea. Since archaea use G1PDH (encoded by *egsA*) to convert DHAP to G1P for the biosynthesis of phospholipids, G3PDH homologs are not common in this domain (19, 23). In contrast, bacteria and eukaryotes use G3PDH to synthesize G3P for the backbones of their membrane lipids. Although previous work has demonstrated an archaeal G3PDH, this enzyme (GspA) has an unusual preference for NADP" (26). Furthermore, GspA is not a bacterial protein-like G3PDH; instead, it is a close homolog of the products of open reading frames (ORFs) from only a few archaea (*Archaeoglobus fulgidus, Methanothermobacter thermautotrophicus, Aeropyrum pernix*).

Here we provide evidence that bacterial protein-like G3PDH homologs are common among haloarchaea and are required for the catabolism of glycerol in *H. volcanii*. We also demonstrate that the central G3PDH activity of *H. volcanii* is encoded by a glycerol metabolic operon on the main chromosome that includes not only *glpA1B1C1* (encoding G3PDH complex I) but also the downstream *glpK* (encoding glycerol kinase), *glpF* (encoding a putative glycerol facilitator), and *ptsH2* (encoding a bacterial protein-like PTS Hpr homolog) genes. This glycerol metabolic operon is under transcriptional control from a strong G3P-inducible promoter (*P*1*glyA1*) upstream of *glpA1* and possibly from downstream promoters (*P*2*glyF*). A large transcript (spanning the entire operon) that may undergo nucleolytic cleavage into shorter transcripts of differential stability was detected. Our findings not only provide the first molecular characterization of a bacterial protein-like G3PDH complex in archaea but also shed light on the central pathway of glycerol metabolism in haloarchaea.
MATERIALS AND METHODS

Materials. Biochemicals used for analysis of G3PDH activity were purchased from Sigma-Aldrich (St. Louis, MO). Other organic and inorganic molecular grade chemicals were from Fisher Scientific (Pittsburgh, GA) and Bio-Rad (Hercules, CA). Desalted oligonucleotides were from Integrated DNA Technologies (Coraville, IA). DNA polymerases and modifying enzymes were from New England Biolabs (Ipswich, MA).

Strains, media, and plasmids. Strains, oligonucleotide primers, and plasmids are summarized in Tables S1 and S2 in the supplemental material. Escherichia coli TOP10 was used for routine recombinant DNA experiments. H. volcanii strains were transformed (18) with plasmid DNA isolated from E. coli GM2163 by use of the QIAprep spin miniprep kit (Qiagen, Valencia, CA). E. coli strains were grown at 37°C (200 rpm) in Luria-Bertani medium supplemented with 100 mg per liter ampicillin as needed. H. volcanii strains were grown at 42°C (200 rpm) in Casamino Acids (CA) and minimal medium (MM) with formulae according to The Halohandbook (12) except that MM was supplemented with glycerol (Gly MM), glucose (Glu MM), and/or succinate (Suc MM) at 20 mM each. Cultures were harvested at an OD of 0.5 or when the OD increased by 0.1 to 0.2 (5-FOA) (50 μg/ml) for growth in the presence of 5-FOA, respectively. An overnight culture was inoculated into a final volume of 5-FOA (50 μg/ml) and uracil (10 and 50 μg/ml) for growth in the presence and absence of 5-FOA, respectively) were included as needed, and tryptophan (Tryp) (80 μg/ml) was added where indicated. Uracil and 5-FOA were solubilized in 100% (vol/vol) dimethyl sulfoxide (DMSO) at 50 μg/ml to 100 μg/ml prior to addition to the growth medium. For anaerobic growth on glycerol, H. volcanii strains were grown twice aerobically on YPC medium (12) to log phase (12 h) in 10-ml screw-cap tubes (200 rpm) and were inoculated at 1% (vol/vol) for anaerobic growth in 10-ml screw-cap tubes on YPC medium supplemented with 100 mM DMSO and 20 mM Gly MM. Strains were grown in 13- by 100-mm tubes (200 rpm) and were inoculated at 1% (vol/vol) for growth with 50 μg/ml 5-FOA and 100 μg/ml uracil (5-FOA) and uracil (50 μg/ml). For Northern blot analysis, RNA was transferred to a BrightStar-Plus nylon membrane (Ambion, Austin, TX) by upward capillary action overnight using 20× SSC, cross-linked using a UV Stratalinker 2400 (Stratagene), and hybridized overnight at 50°C with DIG-labeled double-stranded DNA (dsDNA) probes specific for glpA2 and glpA1. PCRs for the generation of the probes were performed with the primers listed in Table S2 in the supplemental material and Taq DNA polymerase according to the supplier’s recommendations with the following modifications: 3% (vol/vol) DMSO was included, and the 1× DIG deoxyribonucleotidetriphosphate mixture (catalog no. 1277085, Roche) was supplemented with a solution of mixed deoxyribonucleotides (New England Biolabs) to 0.1 mM. For hybridization, membranes with the cross-linked RNA samples were equilibrated in high-sodium dodecyl sulfate (SDS) buffer (5× SSC, 2% [wt/vol] blocking reagent, 0.1% [wt/vol] Nonidet P-40, 0.2% [wt/vol] SDS, 50% [vol/vol] formamide) (2 h, 50°C), followed by incubation with 100 ng labeled probe in 1 ml of high-SSD buffer (16 h, 20°C). Membranes were washed with 2× SSC supplemented with 0.1% (wt/vol) SDS (twice, for 30 min each time) and with 0.1× SSC supplemented with 0.1% (wt/vol) SDS (twice, for 15 min each time) at 50°C. Hybridization membranes were detected by a chemiluminescent (CSPD) digoxigenin immunosassay (Roche).

Transcriptional reporter construction and assay. A plasmid-based reporter system was used to analyze transcription (10) from promoter regions upstream of glpA1, glpK, and inAA that were fused to the Halofex alcaligenes-derived bgaH gene encoding β-galactosidase (for details, see Tables S1 and S2 in the supplemental material). The promoter activity of each construct was determined by an assay of the β-galactosidase activity of log-phase cells as described previously (17). One unit of β-galactosidase activity is defined as the amount of enzyme catalyzing the hydrolysis of 1 μmol o-nitrophenyl-β-D-galactopyranoside (ONPG) min−1 with a molar extinction coefficient for o-nitrophenol of 3,300 M−1 cm−1.

HPLC analysis of glycerol and glucose. At various time points, culture broths (1 ml) of both the parent (H26) and glpA1 mutant (KS11) strains growing on 5 mM Gly Glu MM (MM supplemented with glycerol and glucose at 5 mM each) were withdrawn and centrifuged (10 min at 10,000 × g and 4°C). Supernatant fractions were filtered and analyzed by high-performance liquid chromatography (HPLC) using a Bio-Rad HPX-87H column with a 4 mM H2SO4 eluent.

DNA sequencing. Sanger automated DNA sequencing was performed using an Applied Biosystems model 3130 genetic analyzer (ICCRB Genomics Division, University of Florida).

RESULTS AND DISCUSSION

Bacterial G3PDH homologs in halaarchaeal. Glycerol metabolism in H. volcanii requires the phosphorylation of glycerol to G3P by a bacterial protein-like glycerol kinase (encoded by glpK) (27). To analyze the subsequent step, the oxidation of G3P to DHAP, homologs of bacterial G3PDH enzymes known to catalyze this reaction were identified in H. volcanii and other halaarchaeal genomes. This included the identification of halaarchaeal homologs of all three subunits of the anaerobic G3PDH complex (GlpA, GlpB, and GlpC) of bacteria (see Fig. S1 in the supplemental material). Homologs of the G3PDH catalytic subunit A, GlpA, were also identified in archaeal of
the classes Thermoplasmata and Thermoprotei (see Fig. S1 in the supplemental material). In contrast to the halophilicarchae, the latter archaea do not encode homologs of the bacterial GlpB or GlpC proteins, which (in addition to GlpA) are essential for the anaerobic growth of E. coli on G3P (31). Thus, among the archaea, only the halophilicarchae appear to encode bacterial protein-like G3PDH complexes in addition to homologs of bacterial glycerol kinase and PTS components, including a putative PEP:PTS-dependent DHA kinase (27).

Most halophilicarchae encode two GlpA-related proteins (GlpA1 and GlpA2) and single GlpB and GlpC homologs. Typically, the halophilicarchae genes (gpaA1 and gpaA2) encoding the GlpA homologs are located on the main chromosome, with gpaA1 organized in an apparent gpaA1 gpaB1C1 operon and gpaA2 in a separate chromosomal region (see Fig. S1 in the supplemental material). H. volcanii is exceptional in that it harbors two apparent gpaABC operons, with (i) gpaA1 located on the main chromosome upstream of gpaB1C1-glpK-glpF-ptsH2 and (ii) gpaA2 located on megaplasmid pHV4 upstream of gpaB2C2 (Fig. 1). While most of the halophilicarchae GlpA homologs cluster phylogenetically into distinct GlpA1 and GlpA2 lineages, the H. volcanii GlpA homologs cluster together in the halophilicarchae GlpA1 lineage (see Fig. S1). Consistent with this relationship, H. volcanii GlpA1 and GlpA2 are similar in amino acid length and harbor a C-terminal bacterioferritin-associated ferredoxin (BFD)-like [2Fe-2S] domain common to bacterial GlpA proteins (see Fig. S2 in the supplemental material). The GlpA2 homologs of other halophilicarchae are missing the BFD-like domain. Although the physiological role of BFD domains remains unclear, this protein may serve as a general redox enzyme and/or a regulatory component of iron storage and mobilization (15). Thus, based on its genome sequence, H. volcanii harbors two separate gpaABC operons that may encode functional G3PDH complexes.

**Knockout of gpaA1 and gpaA2 from the H. volcanii genome.** To investigate the function of the halophilicarchae G3PDH homologs, gpaA1 and gpaA2 were targeted for markerless deletion from the genome of H. volcanii H26 (designated the wild-type strain throughout this study). Genes encoding GlpA homologs were selected for knockout, since subunit A is required for the catalytic activity of G3PDH in E. coli (9). Markerless deletion was confirmed by PCR, Southern blotting, and DNA sequence analysis (see Fig. S3 in the supplemental material).

**Requirement of gpaA1 for glycerol metabolism.** The gpaA1 (KS11) and gpaA2 (KS10) mutant strains were compared to the wild type with respect to growth on glycerol and glucose (Gly MM and Glu MM, respectively). Since G3PDH gene expression is often regulated by environmental conditions (14), strains were examined for growth on glycerol by using oxygen as well as dimethyl sulfoxide as a terminal electron acceptor. While all strains grew similarly to the wild type on glucose, the gpaA2 mutant grew similarly to the wild type on glycerol, the gpaA1 mutant was unable to grow on glycerol (Fig. 2A). The gpaA1 mutant was complemented by expressing gpaA1 or gpaA2 in trans from a strong Halobacterium salinarum tRNA P2 promoter with the pHV2-based plasmid pJAM2696 or pJAM2711, respectively (Fig. 2A; see also Table S1 in the supplemental material). HPLC analysis of cell culture broth revealed that the gpaA1 mutant could not utilize glycerol and consumed only glucose during growth in a medium with glycerol and glucose (Fig. 2B). This contrasted with the behavior of wild-type cells, which utilized both glycerol and glucose, with an apparent preference for glycerol, as observed previously (27). Together, these results reveal that gpaA1 (like glpK-encoded glycerol kinase [27]) is needed for growth on glycerol and for glycerol metabolism. The ability of gpaA2 to trans-complement the gpaA1 mutant suggests that GlpA2 is a functional homolog of GlpA1. Since the genomic copy of gpaA2 is not required for growth on glycerol and does not compensate for the loss of gpaA1 when controlled by the gpa2 native promoter, it is likely that the genomic copy of gpaA2 is not transcribed under these conditions (even in the absence of gpaA1). Thus, gpaA2 transcript levels were examined by RT-PCR for wild-type and mutant strains, including KS10 (ΔgpaA2) and KS11 (ΔgpaA1), with and without trans-complementation by gpaA2 (pJAM2711). Cells were grown on medium with glycerol and glucose; the latter carbon source was included to allow for the growth of KS11. With this approach, gpaA2 transcripts were readily detected in the gpaA1 mutant trans-complemented with gpaA2 (KS11/pJAM2711) but were not detected when gpaA2 was present only in a genomic copy or was deleted (H26, KS10, and KS11) (see Fig. S4 in the supplemental material).
findings explain why glpA1 (and not glpA2) is required for standard growth on glycerol and why glpA2 complements the glpA1 knockout when expressed in trans from a strong promoter. Whether glpA2 is induced by other environmental conditions is unclear.

G3PDH activity levels are altered by the carbon source. To further investigate G3PDH, its specific activity was determined in lysates of H. volcanii cells (H26, the wild-type strain) grown to log phase on a medium containing glycerol, glucose, or glycerol plus glucose. G3PDH activity was 2.7-fold higher in cells grown on glycerol than in cells grown on glucose alone (Table 1). However, as with the H. volcanii glycerol kinase (27), the levels of G3PDH activity were not significantly reduced in cells grown with both glucose and glycerol. This contrasts with the findings for many bacteria and yeast that glycerol catabolism (including the levels of glycerol kinase and G3PDH activities) is substantially reduced by the addition of glucose to the growth medium (22, 28).

GlpA1 and GlpA2 are required for full G3PDH activity. We next examined whether glpA1 and/or glpA2 was responsible for the G3PDH activity observed. Cell lysates were prepared from glpA1 and glpA2 mutants (grown on glycerol and/or glucose) and were assayed for G3PDH activity compared to that for the wild type. Neither single knockout of glpA1 nor single knockout of glpA2 had a notable impact on the low levels of G3PDH activity detected in glucose-grown cells (Table 1). However, when cells were grown in the presence of glycerol, the G3PDH activity of the glpA1 mutant remained low, at levels 3.7-fold lower than those for the wild-type strain under these growth conditions (Table 1). In contrast, knockout of glpA2 had only a slight impact (a 1.4-fold reduction from the wild-type level) on the high-levels of G3PDH activity observed in cells grown on glycerol (with or without glucose) (Table 1). Both the glpA1 and glpA2 mutant strains were complemented by providing a copy of the respective gene in trans, confirming that the reductions in G3PDH activity observed on glycerol were not due to polar effects of the mutations (Table 1). The G3PDH activity of the glpA1 mutant was also restored to wild-type levels by providing glpA2 in trans (Table 1), again suggesting that glpA2 is a functional analog of glpA1. To further investigate the roles of glpA1 and glpA2, a double knockout of both glpA1 and glpA2

![FIG. 2. Catabolism of glycerol requires the G3PDH homolog GlpA1 in H. volcanii.](http://jb.asm.org/)

**TABLE 1.** G3PDH-specific activity of the parent strain and mutant strains deficient in the synthesis of glycerol kinase or G3PDH subunit A homologs

| Strain | Gly MM | Gly MM | Gly Glu MM |
|--------|--------|--------|------------|
| H26 (parent) | 76 ± 10 | 28 ± 4 | 67 ± 10 |
| ΔglpA1 mutant | No growth | 19 ± 1 | 18 ± 1 |
| ΔglpA2 mutant | 55 ± 6 | 24 ± 2 | 47 ± 6 |
| ΔglpA1 ΔglpA2 mutant | No growth | UD | UD |
| ΔglpK mutant | No growth | 19 ± 4 | 21 ± 1 |
| ΔglpA1/glpA2 strain | 70 ± 9 | 26 ± 6 | 68 ± 9 |
| ΔglpA1/glpA2 strain | 67 ± 3 | 28 ± 5 | 67 ± 4 |
| ΔglpA2/glpA2 strain | 73 ± 9 | 28 ± 5 | 72 ± 7 |
| ΔglpK/glpK strain | 72 ± 8 | 27 ± 3 | 67 ± 5 |

*a* The ΔglpK strain is deficient in the synthesis of glycerol kinase; the ΔglpA1 and ΔglpA2 strains are deficient in the synthesis of G3PDH subunit A homologs. slashes are used to indicate strains with plasmids expressing glpK, glpA1, or glpA2 in trans.

*b* G3PDH activity was determined for cell lysates as described in Materials and Methods. Cells were grown to log phase in minimal medium (MM) with Gly, Glu, or both as indicated. No growth, mutant strains that did not grow on Gly MM, UD, undetectable levels of activity. Experiments were performed in biological triplicate, and the means ± standard deviations were calculated. No activity was detected for controls with no substrate or with boiled cell lysates.
was constructed (KS12) and was found to be devoid of any detectable G3PDH activity, even when cells were grown in the presence of glycerol (Gly Glu MM) (Table 1). Thus, GlpA1 and GlpA2 are required for the full G3PDH activity of *H. volcanii*, and the levels of the GlpA1-dependent G3PDH activity are substantially increased by the addition of glycerol to the growth medium.

**G3P is needed for enhanced levels of GlpA1-dependent G3PDH activity.** Glycerol catabolism is often intricately coordinated by a number of mechanisms in prokaryotic and eukaryotic cells. In *E. coli*, the glp regulon (mediating glycerol and G3P catabolism) is controlled at the transcriptional level by anaerobic conditions, catabolite repression, and the inducer G3P, which binds the DeoR-type regulator GlpR and alleviates transcriptional repression of the *glp* regulon (14, 33). To improve our understanding of the inducers of glycerol catabolism in *H. volcanii* that may be responsible for the enhanced levels of GlpA1-dependent G3PDH activity observed during growth on glycerol (with or without glucose) compared to growth on glucose alone, the G3PDH activity of a *glpK* (glycerol kinase) mutant was determined. Consistent with the possibility that G3P regulates the levels of G3PDH produced in the cell, the *glpK* mutant (unable to synthesize G3P) displayed a lower level of G3PDH activity than the wild-type strain during growth in the presence of glycerol (Table 1). G3PDH activity was restored to wild-type levels in the *glpK* mutant by trans-complementation with *glpK* (Table 1). Thus, we speculate that G3P, generated by GlpK during the presence of glycerol, alleviates the transcriptional repression of *glpA1* and enhances the levels of G3PDH in *H. volcanii*.

**Intergenic regions upstream of *glpA1* and *glpK* can drive transcription.** To further examine the regulation of glycerol catabolism in *H. volcanii*, genomic regions immediately upstream of the translational start codons of *glpA1* (*P* _glpA1_) and *glpK* (*P* _glpK_) (310 and 354 bp, respectively) were fused to the _H. alicantei_ _bgaH_ gene, encoding β-galactosidase. Transcription, driven by promoter elements within these regions, was monitored by an assay of β-galactosidase activity in lysates prepared from cells carrying these transcription fusions. With this approach, transcription from _P_ _glpA1_ and _P_ _glpK_ was found to be higher than that from the vector control, which retained a Shine-Dalgarno sequence upstream of _bgaH_, served as a negative control.

### Table 2. Transcription from the *glpA1*, *glpK*, and *tnaA* promoter regions based on a β-galactosidase reporter gene

| Medium and strain | Sp act of β-galactosidase (mU · mg⁻¹) with the following promoter^a^: | Vector (none) |
|-------------------|---------------------------------------------------------------------|--------------|
|                   | P1 _glpA1_ (310 bp) | P2 _glpA1_ (354 bp) | P1 _tnaA_ (321 bp) | P2 _tnaA_ (551 bp) |
| Gly MM H26 (parent) | 310 ± 5 | 16 ± 2 | ND | 260 ± 10 | 8.1 ± 0.1 |
| ∆glpK mutant | No growth | No growth | No growth | No growth | No growth |
| ∆glpA1 mutant | 300 ± 7 | 18 ± 2 | ND | 260 ± 3 | 8.7 ± 0.9 |
| ∆glpR mutant | No growth | No growth | No growth | No growth | No growth |
| Glu MM H26 (parent) | 38 ± 0.1 | 22 ± 0.7 | ND | 250 ± 7 | 7.3 ± 0.9 |
| ∆glpK mutant | 30 ± 1 | 25 ± 2 | ND | 250 ± 8 | 6.5 ± 0.7 |
| ∆glpA1 mutant | 35 ± 3 | 21 ± 1 | ND | 260 ± 3 | 9.2 ± 1 |
| ∆glpR mutant | 36 ± 0.5 | 23 ± 0.6 | ND | 250 ± 4 | 8.3 ± 0.9 |
| Gly Glu MM H26 (parent) | 280 ± 8 | 14 ± 2 | ND | 260 ± 2 | 8.1 ± 0.05 |
| ∆glpK mutant | 28 ± 2 | 22 ± 3 | ND | 250 ± 5 | 8.3 ± 0.6 |
| ∆glpA1 mutant | 270 ± 9 | 18 ± 0.5 | ND | 240 ± 6 | 7.3 ± 0.5 |
| ∆glpR mutant | 270 ± 8 | 19 ± 1 | ND | 250 ± 7 | 8.0 ± 0.5 |
| Succ MM (H26 [parent]) | ND | ND | 35 ± 6 | 260 ± 20 | 8.0 ± 0.08 |
| Succ MM (H26 [parent]) | ND | ND | 1,700 ± 50 | 230 ± 30 | 7.4 ± 0.07 |

^a Determined from the lysates of cells grown to log phase in minimal medium (MM) as indicated. No growth, mutant strains that did not grow on Gly MM; ND, not determined. Experiments were performed in biological triplicate, and the means ± standard deviations were calculated.

^b The parental strain H26 and the ∆glpK, ∆glpA1, and ∆glpR mutant strains were transformed with a plasmid carrying the promoter region of *glpA1* (*P* _glpA1_) or *glpK* (*P* _glpK_) transcriptionally fused to the β-galactosidase *bgaH* reporter gene. The tryptophan-inducible promoter _P_ _tnaA_ and the strong promoter _P_ _tnaA_ were included for comparison. Promoter fusions included the start codon and genomic region immediately upstream of each target gene. The length of the promoter fusion is given in parentheses after the promoter designation. Plasmid vector pJAM2715, containing only a Shine-Dalgarno sequence upstream of _bgaH_, served as a negative control.
G3P is an inducer of transcription from a promoter upstream of *glpA1*, our reporter assays revealed that transcription from *P1*/*glpA1* in a *glpK* knockout strain was constitutive and was 10-fold lower than that of the wild type (and the *glpA1* mutant) when cells were grown in the presence of glycerol (Table 2). Expression from *P2*/*glpK* and the *P2*/*ptsH2* control was also constitutive but was not altered by any of the genomic mutations or growth conditions examined (Table 2). Thus, in the absence of G3P (in the *glpK*-deficient strain), transcription from *P1*/*glpA1* is reduced to constitutive and basal levels. These results are consistent with the *E. coli* model, in which G3P serves as the inducer for the glycerol metabolic operon (9). In *E. coli*, G3P relieves the transcriptional repression mediated by GlpR (21), thus allowing expression of the *glp* regulon, including the *glpD*, *glpTQ*, and *glpKF* operons, when cells are grown in the absence of glucose and the presence of glycerol.

**GlpR is not required for G3P induction of *glpA1* transcription.** To directly examine whether *H. volcanii* GlpR modulates transcription from either *P1*/*glpA1* or *P2*/*glpK*, the transcriptional reporter constructs of these promoter regions were analyzed in a *glpR* knockout strain (KS8) (Table 2). In contrast to the *E. coli* model, we do not predict repression of the *H. volcanii* glycerol metabolic operon by a DeoR/GlpR-type regulator. This hypothesis is based on our previous data (27), which demonstrate that during growth on glycerol, the single DeoR/GlpR-type regulator of *H. volcanii* controls both fructose and glucose metabolic enzymes through transcriptional repression of *phkB* (encoding phosphorfructokinase) and *kdglK* (encoding 2-keto-3-deoxy-6-gluconate kinase) (25). In agreement with our prediction that *H. volcanii* GlpR is not required for the regulation of G3PDH gene expression, transcription from both *P1*/*glpA1* and *P2*/*glpK* was independent of the *glpR* mutation (Table 2). This apparent difference in the regulation of the glycerol metabolic operons between *H. volcanii* and *E. coli* is consistent with the distant phylogenetic relationship between these two microbes and their disparate habitats. Glycerol is a major source of carbon in many of the hypersaline environments where *H. volcanii* thrives, whereas it plays a more limited role in the ecosystems of *E. coli*. Furthermore, *H. volcanii* has an apparent preference for glycerol over glucose, while *E. coli* does not.

**Transcriptional organization of the *glp* operon.** Due to the close proximity of *glpA*/*B*/*C1*, *glpK*, *glpF*, and *ptsH2* on the chromosomes of *H. volcanii* (Fig. 1) and other halophagids, we investigated whether these genes formed an operon(s). Initial analysis was performed by RT-PCR using total RNA extracted from wild-type cells grown on glycerol (Gly MM). Primers were designed to anneal within the coding regions of neighboring genes, including *glpA1*, *glpB1* (13-bp overlap in the coding sequence), *glpB1* and *glpC1* (3-bp overlap in the coding sequence), *glpK* and *glpF* (4-bp intergenic region), *glpF* and *ptsH2* (2-bp overlap in the coding sequence), and *glpC1* and *glpK* (363-bp intergenic region) (Fig. 1); the latter primer pair is from our previous work (27). In each case, a single RT-PCR product with a molecular size and DNA sequence consistent with cotranscription of these neighboring genes was detected (Fig. 3A).

To further examine the transcripts generated from the glycerol metabolic operon, total RNA (extracted from wild-type cells grown on glycerol with or without glycerol) was analyzed by Northern blotting using probes specific for *glpA1* and *glpK*. With this approach, *glpA1*-specific transcripts of ∼4.3 kb, a size consistent with the *glpA1B1C1* coding region, were readily detected in cells grown on glycerol plus glucose (Fig. 3B). Less-prominent *glpA1*-specific transcripts of ∼7.7 kb were also observed in these cells, suggesting that the *glpA1B1C1* operon is cotranscribed with its downstream gene neighbors (Fig. 3B). In addition to these *glpA1*-specific transcripts, *glpK*-specific transcripts of multiple lengths were identified in cells grown on glycerol and glucose, including highly abundant transcripts of ∼2.5 kb as well as less abundant transcripts of ∼4.3, 6.3, and 7.7 kb (Fig. 3B). Although most of the transcripts that hybridized to the *glpA1*- and *glpK*-specific probes were not observed...
in cells grown on glucose alone, the glpK-specific transcripts of ∼2.5 kb were detected at low levels in glucose-grown cells. Based on its size, the latter transcript (present in cells grown in the presence or absence of glycerol) may be the cotranscript of glpK and glpF detected by RT-PCR (Fig. 3A), since these genes span 2.6 kb of the genome. Production of glpKF transcripts in the absence of glycerol may be physiologically advantageous, since both gene products (a glycerol kinase and a putative glycerol facilitator) are predicted to be required early in glycerol catabolism and produce the internal G3P needed to induce the system. The molecular mechanism(s) responsible for generating the glpAI- and glpK-specific transcripts of various lengths remains unclear. However, our results suggest that transcription of the glycerol metabolic operon of H. volcanii is driven by more than one promoter element (the G3P-inducible promoter P1_{glpAI} and the constitutive promoter P2_{glpK}) and that the primary transcript(s) from this operon may be cleaved into shorter transcripts of differential stability. While mechanisms of RNA degradation in archaea have only recently been studied (13), examples of RNase-mediated endonucleolytic cleavage of primary transcripts into shorter transcripts with different half-lives are well characterized in bacteria (24).

Conclusions. Here we demonstrate that H. volcanii requires G3PDH encoded by an operon on the main chromosome for the catabolism of glycerol. Although two genomic regions (glpA1B1C1 on the main chromosome and glpA2B2C2 on megaplasmid pHV4) are predicted to encode homologs of all three subunits of the anaerobic G3PDH of bacteria, we demonstrate that G3P is dissimilated primarily through the GlpA1-containing G3PDH. GlpA2, though a functional complement to GlpA1 and required for the basal levels of G3PDH activity observed in a glpAI knockout strain, is not required for growth on glycerol. Interestingly, GlpA1 is needed for the enhanced levels of G3PDH activity observed when cells are grown on glycerol (with or without glucose) compared to growth on glucose alone. Promoter fusions to bgaH (Table 2) reveal that the genomic region upstream of glpA1 (P1_{glpA1}) harbors a strong promoter element that is tightly controlled and is induced by growth in the presence of glycerol. This increase in P1_{glpA1}-mediated transcription is likely responsible for the differences observed between G3PDH activity during growth on glycerol and that in its absence. G3P appears to serve as the inducer of P1_{glpA1}-mediated transcription, based on the requirement of glycerol kinase (and not glpA1-encoded G3PDH) for this induction during growth on glycerol. In contrast to the Escherichia coli model, this induction is not subject to regulation by a DeoR/GlpR-type repressor; instead, it is mediated by an uncharacterized protein in H. volcanii. Interestingly, transcription from the intergenic region between bgaC1 and glpK (P2_{glpK}) occurs only at basal levels and is not responsive to growth on glycerol. Further examination of the glpA1B1C1, glpK, glpF, and ptsH2 genes by RT-PCR analysis and Northern blotting revealed that all six genes are cotranscribed with their neighboring genes. Distinct transcripts of various lengths are generated from this operon, not all of which are predicted based on transcription from P1_{glpA1} and P2_{glpK}. Interestingly, we observed previously by RT-quantitative PCR (qPCR) that glpA1- and glpK-specific transcripts are at differential levels (78- and 9-fold, respectively) in glycerol-grown versus glucose-grown cells (27). The regulatory process(es) responsible for controlling the levels of these transcripts has yet to be fully elucidated. However, our current results reveal multiple mechanisms that may be utilized by the cell to control transcription of this glycerol metabolic operon, including (i) G3P-inducible P1_{glpA1}-driven and constitutive P2_{glpK}-driven transcription and (ii) posttranscriptional processing of a primary transcript (spanning the entire 7.7-kb operon) into smaller transcripts that may be of differential stability.

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ERRATUM

Activity and Transcriptional Regulation of Bacterial Protein-Like Glycerol-3-Phosphate Dehydrogenase of the Haloarchaea in Haloferax volcanii

Katherine S. Rawls, Jonathan H. Martin, and Julie A. Maupin-Furlow

University of Florida, Department of Microbiology and Cell Science, Gainesville, Florida 32611-0700

Volume 193, no. 17, p. 4469–4476, 2011. Page 4469, column 2, lines 10 and 11: “GspA” should read “GpsA.”
Page 4471, column 1, line 14: “glpBIC2” should read “glpBIC1.”