Supplementary Materials for Hwang et al. (2014)

Supplementary text

Thiamine pyrophosphate biosynthesis homologs of Hfx. volcanii identified by comparison to bacterial and yeast pathways. Reconstruction of thiamine biosynthesis in halophilic archaea has been previously reported [1, 2]. Here we update the annotation of the complete genome sequence of Hfx. volcanii DS2 [3] with respect to homologs related to bacterial and eukaryotic enzymes of de novo and salvage pathways used for thiamine pyrophosphate (TPP) biosynthesis. For details and references to support biological function of these enzymes see Fig. S1 and Table S1-2. Conserved homologs were identified based on clustering to proteins classified with thiamine biosynthetic pathways in InterPro [4] and NCBI Conserved Domain Databases (CDD) [5] as well as 3D structural homology modeling using Phyre2 [6].

De novo biosynthesis of thiamine. De novo biosynthesis of thiamine (Fig. S1A-B) involves separate synthesis of two intermediate molecules: 4-amino-hydroxymethyl-2-methylpyrimidine pyrophosphate (HMP-PP) and 4-methyl-5-(β-hydroxyethyl)thiazole phosphate (HET-P or THZ-P). Once formed, HMP-PP and HET-P are condensed to generate thiamine monophosphate (TMP). TMP is then phosphorylated to TPP.

(a) HMP-PP synthesis. In bacteria, HMP-PP synthesis is described as starting from 5-amino-1-(5-phospho-D-ribosyl)imidazole (AIR), an intermediate of purine biosynthesis that is generated by PurM. AIR is converted to 4-amino-hydroxymethyl-2-methylpyrimidine phosphate (HMP-P) in a complex rearrangement by the radical SAM enzyme ThiC, the key enzyme for the bacterial pathway. HMP-P is phosphorylated by ThiD leading to HMP-PP, which is one substrate of the condensation reaction. In yeast, the key enzyme THI5p mediates the synthesis of HMP-P from histidine and pyridoxal-phosphate (PLP). HMP-P is subsequently phosphorylated to HMP-PP by THI21 and THI20 paralogues, which have N-terminal domains related to bacterial ThiD that are important for function. As can be seen by the provided ORF codes, Hfx. volcanii encodes a homolog to each of these enzymes with exception of the key enzyme THI5. THI5 pyrimidine synthase family (IPR027939) members are found in some archaea and bacteria but not Hfx. volcanii.

(b) HET-P synthesis. HET-P is generated in bacteria from iminoacetate (derived from glycine or tyrosine), 1-deoxy-D-xylulose 5-phosphate (DXP), and a sulfur atom in activated form. These intermediates are condensed to a tautomer of hydroxyethylthiazole phosphate (cTHZ*P) by ThiG and then tautomerized by Tenl to HET-P, the other substrate of the condensation reaction. Generation of iminoacetate from glycine requires ThiO and from tyrosine requires ThiH (not shown). As can be seen by the absence of ORF codes, there are no homologs to any of these enzymes (ThiG, ThiO, Tenl, or ThiH) in Hfx. volcanii. Note that the description of sulfur activation and eukaryotic synthesis of HET-P is described in later sections.

(c) Condensation of HMP-PP and HET-P. Two alternate enzymes (ThiE and ThiN) catalyze the condensation of HMP-PP and HET-P to thiamine monophosphate (TMP) in bacteria. Yeast encode a bifunctional THI6p that has a ThiE-type TMP synthase fused to a C-terminal ThiM domain, which salvages HET-P by phosphorylation of 4-methyl-5-(β-hydroxyethyl)thiazole (THZ). Hfx. volcanii genome codes for both ThiE- and ThiN-type TMP synthases with the ThiN homolog (HVO_0662) fused to an N-terminal helix-turn-helix (HTH) DNA binding domain (Fig. S2) suggesting it may function in transcriptional regulation of the pathway. After condensation, TMP is phosphorylated by Thil (bacteria) or hydrolyzed and then pyrophosphorylated by THI80p (yeast) to the final product thiamine pyrophosphate (TPP).

Similarly to other archaea, Hfx. volcanii codes for a Thil (not a THI80p) homolog.
**Thiamine salvage.** Microbes have evolved transporters and kinases to uptake and salvage thiamine derivatives present in the environment (Fig. 1C). In bacteria, an ABC-type transporter (ThiBPQ) is used for the uptake of thiamine and TPP and appears conserved in *Hfx. volcanii*. The putative transmembrane protein HVO_0023 of the UPF0118 superfamily may associate with this ABC-type thiamine transporter based on genome neighborhood linkage. *Hfx. volcanii* is also predicted to uptake thiamine precursors by a symport mechanism based on coding sequence overlap of HVO_B0379 (PtuP2, a Na+/solute symporter homolog) with HVO_B0380 (TenA2, a homolog of bacterial TenA and yeast THI20 C-terminal domain thiaminase II enzymes). Thiaminase II cleaves thiamine related compounds including those generated by YlmB-mediated deformylation to generate hydroxymethylpyrimidine (HMP). HMP is successively phosphorylated through a series of ThiD mediated kinase reactions to synthesize HMP-PP. *Hfx. volcanii* has homologs to all of these enzymes (TenA, YlmB and ThiD) suggesting it can synthesize HMP-PP by a salvage pathway. *Hfx. volcanii* also appears to salvage HET-P through phosphorylation of THZ based on identification of the ThiM homolog HVO_2667. Thiamine pyrophosphokinase (TPK) enzymes of the IPR006282 family that convert thiamine to TPP were restricted to bacteria and eukaryotes with no homologs identified in *Hfx. volcanii* or other archaea.

**Sulfur activation for the thiazole ring.** In the bacterial pathway, sulfur is provided for ThiG in an activated form, as thiocarboxylate on the C-terminal glycine of the carrier protein ThiS. Generation of this thiocarboxylate starts with activation of ThiS by adenylation, which is catalyzed by ThiF. The adenylate is then exchanged against a sulfur atom provided by Thil. Homologs for all of these proteins are identified in *Hfx. volcanii*. ThiS has a ubiquitin-fold, and its *Hfx. volcanii* structural homologs (SAMP1, HVO_2619; SAMP2, HVO_0202; SAMP3, HVO_2177 with a corrected start codon to result in a 92 aa protein) were shown to be covalently attached to target proteins in a process called sampaylation [7, 8]. SAMP1 and SAMP2 were also shown to be involved in sulfur chemistry, SAMP1 participating in biosynthesis of molybdopterin while SAMP2 participates in thiolation of tRNA [9]. *Hfx. volcanii* has only a single E1-type enzyme (UbaA, HVO_0558) which belongs to the ThiF/MoeB/HesA family and adenylates all three SAMPs based on its requirement for SAMP function [8, 9]. Thus, we have the rare opportunity to determine if any of the SAMPs are involved in sulfur chemistry of thiamine biosynthesis by analyzing a Δ*ubaA* strain (this study).

An eventual involvement of the This-ThiF homologs, SAMP(1-3)-UbaA, would require sulfur transfer from Thil (HVO_1651). However, *Salmonella enterica* Thil provides sulfur for thiamine biosynthesis via its rhodanese domain, a domain also occurring in the *E. coli* ortholog [10]. This rhodanese domain is found in a minority of the Thil homologs and is missing from HVO_1651, making involvement on HVO_1651 in thiamine biosynthesis rather unlikely. In addition to thiamine biosynthesis, *S. enterica* Thil is also involved in thiolation of tRNA, a function which requires only the two N-terminal domains [10]. Thus, nearly all of the proteins named “thiamine biosynthesis protein Thil” in the databases are concluded to be completely unrelated to thiamine biosynthesis but instead are involved in generation of the modified tRNA base 4-thiouridine [11]. Similarly to the methanogen homolog MMP1354, this is also the likely function of HVO_1651 as we find it is not required for growth of *Hfx. volcanii* in the absence of thiamine (data not shown).
### Supplementary Tables

**Suppl. Table S1. Haloferax volcanii DS2 gene homologs of thiamine (vitamin B1) metabolism and transport.**

| Gene  | Hfx. volcanii ORF locus tag, aa | Hfx. volcanii ORF Gi, UniProtKB | Thiamine biosynthesis function | Highly conserved domain(s), E value | Hfx. volcanii ORF aCOG / COG | Evidence (organism, ref.) |
|-------|---------------------------------|---------------------------------|---------------------------------|-----------------------------------|-------------------------------|--------------------------|
| thiQ  | HVO_0020, 361 aa                | Gi:292654197, UniProtKB:D4GYL5 | ABC thiamine transporter ATPase | COG3842: PotA, ABC-type spermidine/putrescine transport systems, ATPase components, 1.03e-123 | arCOG00177, COG3842         | B, *S. typhimurium* ABC transporter (ThiBPQ) required for transport of thiamine and TPP [12] |
| thiP  | HVO_0021, 573 aa                | Gi:292654198, UniProtKB:D4GYL6 | ABC thiamine transporter permease | COG1178: ThiP, ABC-type Fe3+ transport system, permease component, 2.55e-65 | arCOG00163, COG1178         |                                                      |
| thiB  | HVO_0022, 378 aa                | Gi:292654199, UniProtKB:D4GYL7 | ABC thiamine transporter substrate-binding protein | COG4143: ABC-type thiamine transport system, periplasmic component, 1.80e-85 | arCOG00226, COG4143         |                                                      |
| thiF  | HVO_0558 (UbaA), 270 aa         | Gi:292654724, UniProtKB:D4GSF3 | Thiazole biosynthesis adenyllytransferase | cd00757: ThiF/MoeB/HesA family, 3.70e-102 | arCOG01676, COG0476         | B, *E. coli* ThiF [13] |
| thiN  | HVO_0662, 299 aa                | Gi:292654826, UniProtKB:D4GSS2 | TMP synthase (ThiN) with N-terminal helix-turn-helix (HTH) domain | pfam10120: Putative aldolase; Members of this family of archaeal and bacterial proteins are likely to be aldolases, 1.12e-47 | arCOG00021, COG1992         | B, *T. maritima* ThiN domain [14] A, *P. caldifontis* ThiN domain [15] |
| THI4  | HVO_0665, 307 aa                | Gi:292654829, UniProtKB:D4GSSS5 | Suicide thiamine thiazole synthase | PRK04176: ribulose-1,5-biphosphate synthetase, provisional, 1.22e-124 | arCOG00574, COG1635         | E, *S. cerevisiae* THI4p [16] |
| thiL  | HVO_1651, 391 aa                | Gi:292655800, UniProtKB:D4GZL6 | Thiamine/ thioruridine biosynthesis | COG0301: ThiL, thiamine biosynthesis ATP pyrophosphatase, 6.12e-112 | arCOG0038, COG0301         | B, *S. enterica* Th rhodanese-like (RHD) domain [10, 17] |
| thiC  | HVO_1861, 297 aa                | Gi:292655996, UniProtKB:D4GSW4 | TMP kinase | cd02194: ThiL (TMP kinase) plays a dual role in de novo biosynthesis and in salvage of exogenous thiamine, 2.60e-66 | arCOG00638, COG0611         | B, *S. typhimurium* ThiL [19] |
| purM  | HVO_1557, 324 aa                | Gi:292655707, UniProtKB:D4GYY6 | AIR synthase | IPR004733, phosphorosbyformylglycinamidine cyclophosphylase family (HVO_1557 is only *Hfx. volcanii* member) | arCOG00536, COG0309         | B, *E. coli* PurM [23, 24] |
| thiS  | HVO_2619 (SAMP1), 47aa          | Gi:292656738, UniProtKB:D4GUF6 | Thiamine biosynthesis sulfur carrier protein | Ubiquitin-fold superfamily, small ubiquitin-fold archael | arCOG00536, COG1977 arCOG00535, | B, *B. subtilis* and *E. coli* ThiS [25, 26] |

**Notes:**
- “...” indicates that the gene is not present in the listed organism.
- “...” indicates that the gene is present in the listed organism but not fully characterized.
- “...” indicates that the gene is present in the listed organism and fully characterized.

**References:**
- [10, 17]...
| **thiD** | HVO_2666, 279 aa | GI:292656785, UniProtKB:D4GV38 | HMP and HMP-P kinase (ThiD) (or synonym pyridoxine, pyridoxal, and pyridoxamine kinase, PdxK) | cd01169: HMP and HMP-P kinase, 4.66e-81 | arCOG00020, COG0351 | B, B. subtilis ThiD (PdxK) [31] E. S. cerevisiae THI21p and THI20p (ThiD domains) [32, 33] |
| **thiM** | HVO_2667, 298 aa | GI:292656786, UniProtKB:D4GV40 | THZ kinase | cd01170: THZ kinase, catalyzes the phosphorylation of the hydroxyl group of THZ, 1.02e-47 | arCOG00019, COG2145 | B, E. coli ThiM [34] E. A. thaliana THIMp (potential origins from mitochondria/chloroplast) [35] |
| **thiE** | HVO_2668, 214 aa | GI:292656787, UniProtKB:D4GV42 | TMP synthase | cd00564: TMP synthase, Ten, 6.21e-47 | arCOG01089, COG352 | B, B. subtilis ThiE [36, 37] |
| **tenA** | HVO_B0381, 221 aa HVO_B0380, 261 aa | GI:292494313, UniProtKB:D4GQ27 GI:292494312, UniProtKB:D4GQ26 | Thiaminase II | Thiaminase II (IPR027574); COG0819: TenA, THI-4, PQQ family, 1.34e-73 and 1.37e-36 | arCOG01128, COG0819 | B, B. subtilis TenA [38, 39] E. S. cerevisiae THI20p [40] |
| **ylmB** | HVO_B0002, 385 aa | GI:292493940, UniProtKB:D4GP03 | AMPF deformylase? | Acetylornithine deacetylase/succinyl-diaminopimelate desuccinylase family (IPR010182) | arCOG01107, COG0624 | B, B. subtilis BsYlmB [39]; Predicted AMPF deformylase based only on co-clustering with BsYlmB to IPR010182 and genomic linkage with HVO_B0381 (tenA1) and HVO_B0380 (tenA2) |
| **SSSF protein** | HVO_B0379, 509 aa | GI:292494311, UniProtKB:D4GQ25 | Sodium:solute symporter family (SSSF) (IPR001734) protein | cl00456: SLC5-6-like_sbd Superfamily, 3.70e-87 | arCOG01319, COG0591 | Predicted uptake of thiamine-related compounds based on clustering to SSSF and coding sequence overlap with HVO_B0380 (tenA2) |
| **THI5** | -- | -- | -- | -- | -- | E. S. cerevisiae THI5p [41] |
| **thiG** | -- | -- | thiazole synthase | IPR008867: thiazole biosynthesis family | -- | B, B. subtilis ThiG [26] |
| **tenI** | -- | -- | thiazole tautomerase | cd00564: TenI TMP synthase | -- | B, B. subtilis TenI [42] |
| **thiO** | -- | -- | glycine oxidase | TIGR02352: thiamin_ThiO glycine | -- | B, B. subtilis ThiO [43, 44] |
|   |   |   |                                                                 |                                                                 |   |
|---|---|---|----------------------------------------------------------------|----------------------------------------------------------------|---|
|   | thiH | -- | tyrosine lyase                                                  | cd01335: Radical_SAM superfamily; PRK09240: thiamine biosynthesis protein ThiH | B, E. coli ThiH [45, 46] |
|   | THI6 | -- | bifunctional TMP diphosphorylase / hydroxyethyl-thiazole kinase | N-terminal domain COG0352: ThiE, TMP synthase; C-terminal domain COG2145: ThiM, hydroxyethylthiazole kinase, sugar kinase family | E, S. cerevisiae THI6p [47, 48] |
|   | THI80 | -- | thiamine pyrophosphokinase                                      | COG1564: THI80 Thiamine pyrophosphokinase; cd07995: Thiamine pyrophosphokinase (TPK, EC:2.7.6.2) | E, S. cerevisiae THI80 [49] |
|   | tpk  | -- | thiamine pyrophosphokinase                                      | (IPR006282) Thiamine pyrophosphokinase (EC:2.7.6.2); TPK catalytic domain (IPR007371) | E, S. cerevisiae TPK [50]; B, S. aureus TPK [51] |

\(^a\) ThiH gene homologs not predicted or not applicable. COG and aCOG classification according to Wolf et al. [52].

\(^b\) Abbreviations: A, Archaea; B, Bacteria; E, Eukarya; ADP-thiazole (ADT); deoxy-D-xylulose 5-phosphate (DXP); 5-aminomimidazole ribotide or 5-aminol-(5-phospho-D-ribosyl)imidazole (AIR); S-adenosyl-methionine (SAM); thiazole tautomer (R,Z)-2-(2-carboxy-4-methylthiazol-5(2H)-ylidene)ethyl phosphate (cTHZ*-P); pyridoxal 5'-phosphate (PLP), nicotinamide adenine dinucleotide (NAD); 4-methyl-5-(β-hydroxyethyl)thiazole (THZ); 4-methyl-5-(β-hydroxyethyl)thiazole phosphate (THZ-P; synonym 4-methyl-5-(β-hydroxyethyl)thiazole phosphate, HET-P); thiamine monophosphosphate (TMP); thiamine pyrophosphate (TPP); hydroxymethylpyrimidine (HMP); 4-amino-hydroxymethyl-2-methylpyrimidine pyrophosphate (HMP-PP); 4-amino-hydroxymethyl-2-methylpyrimidine phosphate (HMP-P); N-[4-amino-2-methylpyrimidin-5-yl]methyl]formamide (AMPF); 4-amino-5-aminomethyl-2-methylpyrimidine (AAMP); Salmonella typhimurium (S. typhimurium); Escherichia coli (E. coli); Thermotoga maritima (T. maritima); Pyrobaculum calidifontis (P. calidifontis); Saccharomyces cerevisiae (S. cerevisiae); Salmonella enterica (S. enterica); Bacillus subtilis (B. subtilis); Haloferax volcanii (Hfx. volcanii); Staphylococcus aureus (S. aureus); Arabidopsis thaliana (A. thaliana).

\(^c\) While UbaA shares 39% amino acid identity (over a query coverage 90%) with E. coli ThiF, UbaA is not required for thiamine biosynthesis (this study) and instead functions with the ubiquitin-fold SAMPs in the formation of ubiquitin-like isopeptide bonds, the thiolation of tRNA, and the biosynthesis of molybdopterin (MPT) [8, 9].

\(^d\) HVO_1651 is related to Thil but devoid of the rhodanes domain (RHD). HVO_1651 is not required for thiamine biosynthesis (this study) and likely functions in tRNA modification based on analogy to methanogens [18].

\(^e\) Hfx. volcanii SAMPs are Ub-fold proteins structurally related to ThiS [28-30] and function with UbaA in sulfur transfer and protein modification [8, 9, 27]. However, SAMPs do not appear to be linked with thiamine metabolism based on analysis of UbaA (this study).
**Suppl. Table S2.** Yeast THI4p active site cysteine (Cys205) is conserved among select archaeal Th4 homologs.

| Eukaryotes |  |  |
|---|---|---|
| F32318 | Saccharomyces cerevisiae ScTHI4p | VTAANGTCQMDPNVIELAG |
| Q38814 | Arabidopsis thaliana AtTHI4p | VAQNHHTQCMDPNVMEAKI |

| Euryarchaeota – Haloarchaea |  |  |
|---|---|---|
| D4GSS5 | Haloferax volcanii (HVO_0665) | VHALPREITVDPIAVESDL |
| B0R844 | Halobacterium salinarum R1 | VHALPREITVDPIALEADV |
| B9LMD6 | Halorubrum lacusprofundi | VHALPREITVDPIAVESEL |
| C7NOF3 | Halorhodobacter utahensis | VHALPREITVDPIAVESKL |
| C7NVN8 | Halomicrobium mukohatae | VHALPREITVDPIAVEADL |
| E2RLQ7 | Haloterrigena rakuyahensis | VHALPREITVDPIAVESDL |
| E2QUG8 | Halorhodobacter tiamatea | VHALPREITVDPIAVESDL |
| F7PLY7 | Halorhabdus tepidus | VHALPREITVDPIAVESDL |
| F8D479 | Halopiger xanaduensis | VHALPREITVDPIAVESDL |
| G0LHM9 | Haloquadratum walsbyi DSM16854 | VHALPREITVDPIAVESDL |
| G2M276 | Haloquadratum walsbyi DSM16790 | VHALPREITVDPIAVESDL |
| G4G9D1 | Natronobacterium gregoryi | VHALPREITVDPIAVESDL |
| G4GE57 | Natrinema pellirubrum | VHALPREITVDPIAVESDL |
| Q18KP1 | Haloquadratum walsbyi DSM16790 | VHALPREITVDPIAVESDL |
| Q3IMI0 | Natronomonas pharaonis | VHALPREITVDPIAVESDL |
| Q3V7Z9 | Haloarcula marismortui | VHALPREITVDPIAVESDL |

| Euryarchaeota – select species of methanogens and pyrococci |  |  |
|---|---|---|
| F4HJT0 | Pyrococcus sp. (strain NA2) | VSALPRQITVDPIAVESKI |
| F8AJA7 | Pyrococcus yayanosii (strain CH1) | VSALPRQITVDPIAVESKI |
| F6D358 | Methanobacterium sp. strain SW | VSALPRAITVDPIAVESKI |
| F1O6Y7 | Methanobacterium sp. (strain AL-21) | VSALPRAITVDPIAVESKI |
| H8I7V6 | Methanocella conradii | VSALPRAITVDPIAVESKI |
| K2QAU0 | Methanobacterium formicicum | VSALPRAITVDPIAVESKI |

| Thaumarchaeota |  |  |
|---|---|---|
| A0RUI0 | Cenarchaeum symbiosum strain A | VSALPRAITVDPIAVESKI |
| A9A48S | Nitrosoarchaeum limnia | VSALPRAITVDPIAVESKI |
| B3T7X2 | uncultured marine crenarchaeote | VSALPRAITVDPIAVESKI |
| B6M1J9 | Methanosarcina acetivorans (MA_2851) | VSALPRAITVDPIAVESKI |
| A0B980 | Methanosaeta thermophila | VSALPRAITVDPIAVESKI |

| Crenarchaeota - Aeropyrum |  |  |
|---|---|---|
| Q9Y9Z0 | Aeropyrum pernix | VQGLPRAITVDPVGRLAEY |

| Archaea - with ‘histidine-containing’ TH4 homologs |  |  |
|---|---|---|
| Q58018 | Methanoalkalococcus jannaschii(MJ0601) | IERAK---HIDPLTIRSKV |
| Q5CD25 | Thermococcus kodakaraensis (TK0434) | VMTGG---HDVDPLVEAKF |
| Q8TM19 | Methanosarcina acetivorans (MA_2851) | VTTQR---HDVDPLMRKIN |
| A0B080 | Methanoseta thermophila | ADMA---HDVDPLAIRAV |
| A1W13 | Pyrobasilicatium | IQMS---HDVDPLTAQKA |
| A2BJG4 | Hyperthermus butylicus | VEAG---HDVDPIYIEAR |
| A2SQ47 | Methanococcus maripaludis | VVREG---HDVDPLSFRKI |
| A3CS64 | Methanothrix marina | VDMAG---HDVDPLTMABC |
| A3DK5S | Methanopyrus kandleri | IFEAG---HDVDPLTFVEAKF |
| A3NP61 | Pyrobaculum calidifontis | IQMS---HDVDPLQZQA |
| A4FVG7 | Methanococcus maripaludis | IEKAG---HDVDPLTISAKY |
| A4WLY7 | Pyrobasilicatium | IQMS---HDVDPLTMKA |
| A4Y7V7 | Metallosphaeroides sedula | TMAV---HDVDPLTSAKA |
| A6U67 | Methanococcus vannielii | IEKAG---HDVDPLTISAKY |
| A6UV59 | Methanococcus aeolicus | IKNAG---HDVDPLTISAKY |
| A6VGT9 | Methanococcus maripaludis | IEKAG---HDVDPLTISAKY |
| A8A290 | Tignicoccus hospitalis | IEJAG---HDVDPIFFKE |
| A9M926 | Caldivirga maquilingens | IQMAG---HDVDPLTSKESN |
| A9A9W1 | Methanococcus maripaludis | IEKAG---HDVDPLTISAKY |
| B1L513 | Korarchaeum cryptofilum | VLLAG---HDVDPLTISAKY |
| B1YDXX | Thermoproteus neutrophilus | IQMS---HDVDPLQZQA |
| B1D4X8 | Desulfurococcus kamchatkensis | VQLS---HDVDPLTSKESN |
| Protein ID | Organism                       | Sequence Alignment |
|------------|--------------------------------|--------------------|
| C3MQY1     | Sulfolobus islandicus          | TQMAS---HVDPIFISAKA|
| C3MWW5     | Sulfolobus islandicus          | TQMAS---HVDPIFISAKA|
| C3N6N6     | Sulfolobus islandicus          | TQMAS---HVDPIFISAKA|
| C3N745     | Sulfolobus islandicus          | TQMAS---HVDPIFISAKA|
| C3NG16     | Sulfolobus islandicus          | TQMAS---HVDPIFISAKA|
| C4K1A7     | Sulfolobus islandicus          | TQMAS---HVDPIFISAKA|
| CS6A00     | Thermococcus gammatolerans     | VMRTG---HVDPLTVEARF|
| C79PGQ     | Methanocaldococcus fervidus    | IEKAG---HIDPLTSKSI |
| C9RDQ9     | Methanocaldococcus vulcani     | IERAG---HIDPLTRSKV |
| D0K5C6     | Sulfolobus solfataricus        | TQMAS---HVDPIFISAKA|
| D2P013     | Sulfolobus islandicus          | TQMAS---HVDPIFISAKA|
| D2REC7     | Archaeoglobus profundus       | TFMAG---HVDPLVLRSKV|
| D3S3G6     | Ferrolobus pacidus             | VQ1AG---HVDPLMIESKA|
| D3SSY8     | Methanocaldococcus sp.         | IERAG---HIDPLTRSKV |
| D3EH9H     | Methanohalophilus mahli        | VE1GR---HVDPLTRSRL |
| D5V99M     | Methanocaldococcus infermus    | IERAG---HVDPLAESKV |
| D7DYA4     | Staphylythermus helenicus      | IYEAG---HVDPPYEIEANA|
| D7D7D7     | Methanococcus voltae           | IKAAG---HVDPHYYIEANA|
| D7E696     | Methanolobum evestigatum       | VSIAN---HVDPLTRAKV |
| D9PUB7     | Methanothermobacter marburgensis| VEMAG---HVDPLTVRAAGA|
| EOSQ9J     | Ignisphaera aggregans          | VVMSG---HVDPLFITSRA|
| E1QR50     | Vulcanisaeta distributa       | VMAG---HVDPPFIEAKA|
| E1RE05     | Methanoplanus petrolearius     | VEMAG---HVDPLMTKTKV|
| E3GXE6     | Methanothermus fervidus        | AEMAK---HVDPLVFISAKA|
| F0NKN1     | Sulfolobus islandicus          | TQMAS---HVDPIFISAKA|
| F0QW10     | Vulcanisaeta moutnovski        | IQAAN---HVDPPFIEAKA|
| F0T955     | Methanobacterium sp. strain AL-21| VMSSG---HVDPLTRSKA|
| F1KN2T     | Archaeoglobus veneficus        | VEMAG---HVDPLTRSRK |
| F2L34J     | Thermoproteus uzoniensis       | IQMAG---HVDPLVZTAKA|
| F4B7H4     | Acidianus hospitalis           | TCMAG---HVDPLFITSRA|
| F4BUD4     | Methanosaeta concilii          | AEMAG---HVDPPCIRARY|
| F4FZ8X     | Metallosphaera cuprina         | TMAG---HVDPLFITSRA |
| F4HLX9     | Pyrococcus sp. strain NA2      | VLMTG---HVDPLTRVEAKY|
| F6BCS4     | Methanoterris veneficus        | IEGAG---HIDPITIYAK |
| F6BQUU     | Methanoterris igneus           | IEKAG---HIDPITIYAK |
| F6D5C3     | Methanobacterium sp. strain SW | VEMAG---HVDPLTRSRK |
| F7XMG6     | Methanosalms zilliae           | VGIKG---HVDPLTRSKV |
| F8A1S2     | Pyrococcus yamanoi            | VRMAG---HVDPLTVREAKF|
| F8AL44     | Methanothermococcus okinawensis| IDKAG---HVDPLTINAKY|
| G0EF7R     | Pyrolobus fumarii IA           | VGIAN---HVDPLMEFKA |
| G0HLR3     | Thermococcus sp. strain        | VMAG---HVDPLTVREAKF|
| G27657     | Methanothermabacter thermotrophicus| VEMAG---HVDPLTRARAA|
| G29556     | Archaeoglobus fulgidus         | VEGAG---HVDPLFITSRA|
| G50982     | Pyrococcus horikoshii         | VLMAG---HVDPLTIEAKY|
| G12O93     | Methanococcoideae burtonii     | VE1GK---HVDPLAIRSK |
| G2PM60     | Methanospirillum hungatei      | VEATG---HVDPLITGCM |
| G4NE31     | Methanascarcina barkeri       | VTVQR---HVDPLMRTKLN|
| G4JAF8     | Sulfolobus acidocaldarium      | TQMA---HVDPLFITSKA |
| G6LQK8     | Methanococcus maripaludis      | IEKAG---HVDPLFITSKA|
| Q80QBS     | Methanosarcina mazei           | VTTQR---HVDPLMRTKLN|
| Q8T67S     | Methanopyrus kandleri          | VKAAN---HVDPLALEAYE |
| Q8U0Q5     | Pyrococcus furiosus            | VKMG---HVDPLTVREAKY|
| Q8Z2MS     | Pyrobaculum aerophilum         | IQMG---HVDPLVYQAKA|
| Q975R0     | Sulfolobus tokodai            | TCMAG---HVDPLFITSKA|
| Q97YS9     | Sulfolobus solfataricus        | TQMAS---HVDPIFISAKA|
| Q9V0OJ     | Pyrococcus abyssi              | VLMVG---HVDPLTVREAKY|

Archaea - with 'proline-containing' THI4p homologs

| Protein ID | Organism                       | Sequence Alignment |
|------------|--------------------------------|--------------------|
| B5ID81     | Aciduliprofundus boonei        | -VIGE---HIDPLSITYAKY|
| B5IDDO     | Aciduliprofundus boonei        | -VIGE---HIDPLSITYKY |

Select bacteria

| Protein ID | Organism                       | Sequence Alignment |
|------------|--------------------------------|--------------------|
| Q9MWZP4    | Thermotoga maritima            | VMRTG---HVDPLTVREAKF|

4Residues in analogous position to conserved active site cysteine (Cys205) of ScTHI4p are highlighted (i.e., cysteine residues in red, histidine residues in black, and proline residues in blue). Hfx. volcanii HVO_0665 is the THI4p homolog of this study. MJ0601 and MA_2851 are described as a D-ribose-1,5-bisphosphate isomerases [53]. TK0434 is annotated as a putative ribose 1,5-bisphosphate isomerase but was demonstrated to lack this activity in vitro [54]. Note that select species of methanogens and pyrococci have two THI4 homologs including one with a conserved active site cysteine and another with a histidine in this position. UniProtKB/Swiss-Prot numbers are listed for each protein sequence. Gaps introduced to optimize multiple amino acid sequence alignment are indicated by -. 

7
Figure S1 [cont].
Figure S1. De novo biosynthesis (A, B) and salvage (C) pathways of thiamine metabolism. Thiamine biosynthetic enzymes are summarized in Table S1 with reference to function, gene locus tag and UnitProtKB accession numbers. Enzymes of bacteria and yeast are indicated in blue and purple, respectively. Hfx. volcanii ORF code homologs associated with thiamine metabolism based on homology are indicated in green (where X indicates no homolog detected and ? is used when the enzyme is yet unassigned), while those based on gene synteny and limited homology are indicated in orange. THI4-SH indicates the catalytic cysteine side chain. THI4-C=CH indicates the dehydroalanine form of the enzyme after sulfur transfer. The sulfur atom associated with formation of the thiazole ring is highlighted in red. Abbreviations: 4-amino-5-aminomethyl-2-methylpyrimidine (AAMP); ADP-thiazole (ADT); 5-amino-1-(5-phospho-D-riboyl)imidazole (AIR, synonym 5-aminoimidazole ribotide); N-[4-amino-2-methylpyrimidin-5-yl]methylformamide (AMPF); deoxy-D-xylulose 5-phosphate (DXP); 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP); 4-amino-5-hydroxymethyl-2-methylpyrimidine phosphate (HMP-P); 4-amino-5-hydroxyethyl-2-methylpyrimidine phosphate pyrophosphate (HMP-PP); nicotinamide adenine dinucleotide (NAD); pyridoxal 5'-phosphate (PLP); S-adenosyl-methionine (SAM); 4-methyl-5-β-hydroxyethylthiazole (THZ); 4-methyl-5-β-hydroxyethylthiazole phosphate (THZ-P or HET-P); thiazole tautomer (RZ)-2-(2-carboxy-4-methylthiazol-5(2H)-ylidene)ethyl phosphate (cTHZ*-P); thiamine monophosphate (TMP); thiamine pyrophosphate (TPP). Hfx. volcanii ORFs not listed in Table S1 include: HVO_0109 [D4GQ28, SufS-type cysteine desulfurase][55]; HVO_0001 [D4GQ22, Orc1-type DNA replication protein]; HVO_B0382 (D4GQ23, TATA-box-binding protein 3 or Tbp3). Hfx. volcanii homologs of bacterial glycine oxidase (ThiO), thiazole synthase (ThiG), thiamine pyrophosphokinase (TPK) and thiazole tautomerase (TenI) in addition to yeast HMP-P synthase (THI5) and thiamine pyrophosphokinase (THI80) were not detected. Conversion of ADT to THZ-P is predicted to be catalyzed by a NUDIX hydrolase domain enzyme that has yet to be identified [56]. Members of the RHD (IPR001763) and NUDIX hydrolase domain (IPR000086) are common in Hfx. volcanii. HVO_1651 is related to bacterial Thi but devoid of the rhodanese-like domain (RHD), which alone mediates the catalytic function of Thi in thiamine biosynthesis [10, 11].
Suppl. Figure S2. Domain organization and 3D-structural modeling suggest *Hfx. volcanii* HVO_0662 functions as transcriptional regulator of thiamine metabolism. (A) HVO_0662 is organized as fusion of an N-terminal helix-turn-helix (HTH) DNA binding domain and C-terminal ThiN domain based on InterProScan domain recognition [4]. Protein homologs with this HTH-ThiN domain configuration are widespread in halophilic archaea and identified in species of Crenarchaeota (*Pyrobaculum*, *Thermofilum*, *Sulfolobus*, *Metallosphaera*, *Caldivirga*, *Hyperthermus*, *Vulcanisaeta* and *Acidianus*) and Euryarchaeota (*Thermococcus* and *Pyrococcus*). Other archaea and bacteria are predicted to encode ThiD-ThiN protein fusions. Overview (B) and selected close-up views (C) of the 3D structural models of HVO_0662 HTH (left) and ThiN (right) domains generated at 97.7% and >99.9% confidence, respectively, by Phyre2 based fold-recognition and model building [6]. Models were overlaid onto x-ray crystal structures with PDB, UniProtKB reference and amino acid residue numbering as indicated. The ThiN domain of the ThiDN fusion protein, TM0790, from the bacterium *Thermotoga maritima* catalyzes thiamine phosphate synthase (TPS) activity in vitro and complements an *E. coli* ΔthiE strain for thiamine auxotrophy (although additional factors in *E. coli* cell lysate as well as the N-terminal ThiD domain were important for full activity) [14]. The archaeal ThiDN (of *Pyrobaculum caldifontis*) is multifunctional in formation of TMP from HMP and THZ-P in the presence of Mg-ATP [15], and its ThiN domain is a functional analog of the bacterial ThiE, catalyzing formation of TMP with release of PPI from HMP-PP and THZ-P (also known as HET-P or 4-methyl-5-(β-hydroxyethyl)thiazole phosphate) [15]. X-ray crystal structure (PDB 2P89) guided site-directed mutagenesis [15] suggest the *Pyrococcus* ThiN R320 and H341 are involved in the catalytic reaction (residues of structural analogy are predicted for HVO_0662; R183 and H204, respectively). Whether HTH-ThiN fusion proteins such as HVO_0662 synthesize TMP and/or bind intermediates/products of thiamine biosynthesis to modulate transcription remains to be determined.
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