Thiamine pyrophosphate (TPP) is an essential cofactor for all forms of life. In Salmonella enterica, the thiH gene product is required for the synthesis of the 4-methyl-5-β-hydroxyethyl-thiazole monophosphate moiety of TPP. ThiH is a member of the radical S-adenosylmethionine (AdoMet) superfamily of proteins that is characterized by the presence of oxygen labile [Fe-S] clusters. Lack of an in vitro activity assay for ThiH has hampered the analysis of this interesting enzyme. We circumvented this problem by using an in vivo activity assay for ThiH. Random and directed mutagenesis of the thiH gene was performed. Analysis of auxotrophic thiH mutants defined two classes, those that required thiazole to make TPP (null mutants) and those with thiamine auxotrophy that was corrected by either L-tyrosine or thiazole (ThiH* mutants). Increased levels of AdoMet also corrected the thiamine requirement of members of the latter class. Residues required for in vivo function were identified and are discussed in the context of structures available for AdoMet enzymes.

Thiamine pyrophosphate is an essential cofactor that stabilizes acyl carbanions generated by several enzymes in carbohydrate metabolism such as transketolase, α-ketoacid decarboxylase, α-ketoacid dehydrogenase, and acetolactate synthase. The biosynthesis of thiamine pyrophosphate involves the separate formation of the 4-amino-5-hydroxymethyl-2-methylpyrimidine pyrophosphate (HMP-PP)1 and thiazole monophosphate (THZ-P) moieties (Fig. 1). These moieties are then coupled and phosphorylated, forming thiamine pyrophosphate (1). In Escherichia coli and Salmonella at least seven genes, thiFSGH, thiL, iscS, and ddx, are required to convert 1-deoxy-D-xylulose phosphate, L-cysteine and L-tyrosine to THZ-P (2–4). Many of the mechanistic aspects of this conversion have been worked out in vitro (5–8). Our understanding of thiazole biosynthesis has increased dramatically with the reconstitution of the pathway in cell-free extracts by two groups (9, 10). Although these groups used proteins from Bacillus subtilis and E. coli, the proposed mechanisms are similar. The primary difference between these pathways is the generation of the glycine imine intermediate from which the C-2 and N-3 of the thiazole product are derived. In B. subtilis, ThiO catalyzes the oxidation of glycine to form the imine (11), whereas in E. coli, the imine intermediate is proposed to be the product of ThiH (10). In the case of ThiH, it is suggested that an interaction between S-adenosylmethionine (AdoMet), and the [Fe-S] cluster allows the formation of the 5-deoxyadenosyl radical, generating a tryosyl radical that results in the glycine imine via a series of steps (10).

Genetic studies identified a connection between thiamine biosynthesis and [Fe-S] cluster metabolism in Salmonella enterica and suggested that ThiH was the weak link in thiazole synthesis (12). ThiH is a 45-kDa protein that was identified as a member of the radical AdoMet superfamily of proteins (13). Proteins in this superfamily are characterized by a conserved cysteine motif (CXXCXXX) that provides three cysteine ligands for a labile [Fe-S] cluster. The characterized proteins of this family generate radical species by reductive cleavage of AdoMet at the [Fe-S] cluster, and structural and biochemical data have shown that the AdoMet molecule binds either the iron that is not liganded by the protein or one of the μ3-bridging sulfides of the cluster (14–17). Evidence for the presence of an [Fe-S] cluster in ThiH has been provided by genetic (18, 19) and biochemical (20) studies. However, data directly tying the [Fe-S] cluster to enzymatic activity have not been reported.

ThiH homologs are not prevalent in genomes of microorganisms that synthesize thiamine. Organisms that are able to synthesize thiamine yet lack thiH (e.g. B. subtilis and Rizobium etli) have thiO, which encodes a glycine oxidase (11, 21). The remaining genes involved in thiamine synthesis are more widely conserved (22, 23). The difference(s) between the chemistries and metabolic requirements of the ThiO and ThiH pathways in vivo are not clear.

Our interest in thiazole biosynthesis focuses on understanding the integration of this pathway with the metabolic network of the cell. The work herein was initiated to better understand both the direct and indirect requirements for ThiH activity in vivo. A series of thiH mutations were generated, and the mutant phenotypes were characterized. Data obtained support a role for an [Fe-S] cluster and AdoMet in the function of ThiH and define other critical residues that suggest structural features of the protein.

EXPERIMENTAL PROCEDURES

Strains, Media, and Chemicals

The strains used in this study are derived from S. enterica LT2 and are listed with their respective genotypes in Table I. Minimal media was NCE (24) supplemented with MgSO4 (1 mM) and glucose (11 mM). The final concentrations of L-tyrosine, and thiamine were 0.1 mM and 400 mM, respectively. Rich media was Difco nutrient broth (8 g/liter) with NaCl (5 g/liter). Solid media contained 1.5% agar. The final concentrations of antibiotics were: chloramphenicol, 20 μg/ml; ampicillin, 40 μg/ml; kanamycin, 50 μg/ml. All chemicals were purchased from...
Sigma Chemical Co. 4-Amino-5-hydroxymethyl-2-methylpyrimidine was purified from spent medium as described previously (25) and was provided where indicated.

**Mutagenesis and Molecular Biology Techniques**

**Plasmids**—Plasmid DNA was obtained using Promega Miniprep kits (Madison, WI). The appropriate strains were transformed with relevant plasmid DNA by electroporation using an *E. coli* Pulser (Bio-Rad Instruments) for 3 s at 2 kV. After an hour of incubation, the mixture was plated on nutrient agar plates with the relevant antibiotic.

**PCR Mutagenesis**—For PCR mutagenesis plasmid pThiH1 (wild type thiH in pSU19 (26)) was used as a template. Gene specific primers (Integrated DNA Technologies, Coralville, IA) with the following sequence were used in amplification reactions with Vent (exo-) polymerase (New England Biolabs, Beverly, MA): NdeF3P, 5'-GAAGGAAGATATACATATGAAAACCTTCACCGAC-3' and BamR2P, 5'-AGGATATCCATACCGCTTGCAGAAG-3'. The mutagenized population of thiH fragments was gel purified and ligated into pSU19 that had been digested with SmaI and treated with calf intestinal phosphatase (Promega, Madison, WI). Ligation mixtures were transformed into strain DM2275 (thiH) and plated on nutrient broth-chloramphenicol. The chloramphenicol resistant transformants were scored for their nutritional requirement by replica printing to minimal media, minimal media with L-tyrosine, and minimal media with thiamine. All plasmid-containing strains were reconstructed, and the phenotypes were verified.

**Site-directed Mutagenesis**—Two different methods for site-directed mutagenesis were used. One method involved the generation of two PCR products using either Nde3P or BamR2P primers with a primer with the desired mutation. Both fragments were gel purified and used together in a PCR reaction with the external Nde3P and BamR2P primers, generating a full-length thiH that was then ligated into pSU19. A second method was performed as described in the QuikChange XL site-directed mutagenesis kit (Stratagene, La Jolla, CA). Mutant plasmids were confirmed by sequence analysis.

**DNA Sequencing**—DNA sequencing was performed at the University of Wisconsin-Madison Biotechnology Center-Nucleic Acid Facility. DNA sequence analysis program BLAST was used to compare the sequences to wild type thiH sequence from the database (27).

**Phenotypic Analysis**

**Liquid Growth**—Nutritional requirements were determined by replica printing on solid media and liquid growth analysis. For the latter, strains were grown overnight in nutrient broth (plus chloramphenicol

---

**Fig. 1. Biosynthesis of thiamine pyrophosphate in *Salmonella enterica*.** Gene products involved in each reaction are indicated next to the relevant arrows. The specific reaction catalyzed by ThiG and ThiH in *Salmonella* have not been defined. A mechanism to generate THZ-P has been proposed (10) as shown: the deoxyadenosyl radical (DOA) generates a tyrosyl radical that with conformational changes prior to cleavage generates the corresponding quinone methide 3 and a glycinyl radical 4. It is not well understood which electron acceptor will allow this radical to lose an electron, but an iminium ion will be formed after this reaction 5. The condensation between 1-deoxy-x-xylulose phosphate, the thiocarboxylate, and the iminium ion will occur leading to the formation of an intermediate that is believed to be the substrate of ThiG 6. Condensation will be achieved, and THZ-P would be generated. The formation of HMP is shown as well; the reaction involves a complex rearrangement of 5-aminoimidazole ribotide to generate 7. ThiC is the only required gene product that has been identified to catalyze this reaction. ThiD catalyzes the phosphorylation of 7, generating HMP-PP. Condensation between THZ-P and HMP-PP occurs with the activity of ThiE prior to phosphorylation by ThiL generating thiamine pyrophosphate.
ing motif found in S-adenosylmethionine methyltransferases (13). In this motif, residues Gly-130 and Glu-131 are conserved among the 20 ThiH homologs considered in this analysis. Alanine substitutions at G130A or E131A inactivated ThiH. In addition, an E131K substitution mutation was isolated in a screen for randomly generated for thiH null alleles.

**Critical Residues Are Dispersed Throughout ThiH**—Random mutagenesis identified amino acid substitutions that eliminated *in vivo* function of ThiH. Only alleles that resulted in stable proteins (as determined by immunoblot analysis) were considered. Residues critical for function were distributed throughout the protein (Fig. 2A). As expected, substitutions that eliminated *in vivo* function of the enzyme primarily changed conserved or invariant residues; a notable exception was the E187D substitution. At this position, a glutamic acid residue is found in only 3 of 20 ThiH homologs, and yet in *S. enterica* ThiH reduction of the side chain of this residue by one methylene group generated a null phenotype. The null phenotype of this conservative substitution was confirmed by creating the same allele by site-directed mutagenesis.

**Multiple Residue Changes That Generate a ThiH* Phenotype**—We identified previously a unique class of thiH alleles that encode proteins (referred to as ThiH*) that are functional in *vivo* when tyrosine is present (12). The mutations of this class described previously generated mutant proteins ThiH124Y or ThiH*P347S (12). Additional plasmids encoding thiH* alleles of this class were generated in this work (Fig. 2).

**Substitution of Residues Result in a Null or ThiH* Phenotypes**—Two residues, Cys-285 and Ala-61 (Fig. 2), were of interest because substitutions at these residues were found in proteins that caused null or ThiH* phenotypes. In the case of residue Cys-285, a change to histidine eliminated function, whereas a change to an alanine resulted in the ThiH* phenotype. Strikingly, only 11 of 20 homologs have a cysteinyl residue at this position, and the remaining 9 have an alanine.

An alanine to threonine change at position 61 resulted in a null phenotype, whereas the ThiH* phenotype was observed with a change to serine and only when another mutation was present, P334Q. thiH alleles with either one of these single substitutions encoded functional proteins.

**AdoMet Synthase in Multicopy Restores Thiamine Synthesis to thiH Mutants**—We noted that the H124Y mutation was juxtaposed to the AdoMet binding motif (Fig. 2A), raising the possibility that a ThiH* phenotype reflected weak AdoMet binding. To pursue this hypothesis, the wild type allele of the gene encoding S-adenosylmethionine synthase (*mshA*) was introduced into two mutant strains, DM4104 and DM4106, that have a ThiH* phenotype and contain ThiH124Y and ThiH*P347S mutant proteins, respectively. The resulting strains were monitored for thiamine-independent growth. As shown in Fig. 3, increased levels of MetK restored thiamine synthesis in these two strains. The induction of *mshA* in plasmid pMETK2 increases AdoMet synthase activity ~5-fold.²

**ThiH* Phenotype Caused by Other Metabolic Functions Is Distinct from That of thiH Alleles**—Mutations in loci involved in Fe-S cluster metabolism (*gshA, apbC, apbE, isca*) result in ThiH* phenotype (12, 18, 19). The induction of *mshA* in *gshA, apbC*, or *apbE* mutant strain backgrounds failed to allow significant growth in the absence of tyrosine or THZ (data not shown). This result suggested that the ThiH* phenotype could be generated by at least two mechanisms. This interpretation was consistent with the observation that suppressors of the *gshA, apbC*, or *apbE* mutant strains (anaerobic growth, overproduction of YggX) were unable to restore thiamine synthesis

² P. Frey, personal communication.

---

**Table I**

| Strains and plasmids | | |
|----------------------|------------------|------------------|
| Strain | Genotype | |
| DM1 | Wild type | |
| DM460 | thiH910::MudJ | |
| DM1774 | apbC55::Tn10D(zxx-8077):Tn10D(Cm) | |
| DM2275 | thiH942::Tn10D(Tc) | |
| DM4104 | zii-8093::Tn10D thiH1105 (encodes ThiH*H1124V) | |
| DM4106 | zii-8093::Tn10D thiH1106 (encodes ThiH*P347S) | |
| DM5784 | apbE42::Tn10D(Tc) zii-8077::Tn10D(Cm) | |
| DM6668 | gshA101::Tn10D(Tc) zii-8077::Tn10D(Cm) | |
| DM7407 | zii-8077::Tn10D(Cm) yggX* zii-8093::Tn10D(Tc) thiH1105 | |
| DM7408 | gshA101::Tn10D(Tc) thiH910::MudJ | |
| DM4710 | thiH910::MudJ | |
| DM4711 | thiH910::MudJ | |
| DM7421 | gshA101::Tn10D(Tc) zii-8077::Tn10D(Cm)pPL1 | |
| DM4722 | gshA101::Tn10D(Tc) zii-8077::Tn10D(Cm)p META2 | |
| DM4723 | apbC55::Tn10D(Tc) zii-8077::Tn10D(Cm)pPL1 | |
| DM4724 | apbC55::Tn10D(Tc) zii-8077::Tn10D(Cm)p META2 | |
| DM4727 | zii-8093::Tn10D thiH1105pMETA2 | |
| DM4728 | zii-8093::Tn10D thiH1105pMETA2 | |
| DM4729 | zii-8093::Tn10D thiH1106pPL1 | |
| DM4730 | zii-8093::Tn10D thiH1106pMETA2 | |
| DM4731 | thiH942::Tn10D(Tc)pPL1 | |
| DM4732 | thiH942::Tn10D(Tc)p META2 | |

**Plasmid Insert Parent vector Antibiotic resistance**

- PMETK2 MetK pPL1 Kanamycin
- pThiH*I ThiH pSU19 Chloramphenicol
in strains with a thiH* allele (data not shown).

Plasmids containing each of nine alleles of thiH generating a ThiH* phenotype were transformed into gshA, apbE, or apbC mutant backgrounds. Growth of the resulting strains in medium supplemented with thiamine or L-tyrosine was assessed. The resulting data showed that each of the 27 resulting strains had a null mutant phenotype (i.e., requirement for THZ or thiamine). In medium with or without tyrosine, the cell density (A$_{650}$) of cultures of 26 of these strains was between 0.02 and 0.13 after 18 h of incubation with no further increase observed after 24 h of incubation. A culture of the remaining strain reached an A$_{650}$ of 0.3. In the presence of thiamine, all cultures reached high cell densities (A$_{650}$ > 0.7) after 18 h of incubation. Control experiments confirmed that a thiH null mutant (DM460) carrying each of these plasmids individually displayed a ThiH* phenotype, as reflected by a final A$_{650}$ ranging from 0.04 to 0.15 and 0.61 to 1.3 in minimal medium or minimal medium supplemented with tyrosine or thiamine, respectively. Finally, when the only source of ThiH was encoded by a plasmid carrying the wild type allele of thiH, strains with lesions in the gshA, apbE, and apbC genes retained a ThiH* phenotype (data not shown).

**Oligomers Formed by Different ThiH* Mutant Proteins Restore Function**—Four plasmids carrying thiH alleles encoding ThiH* proteins were transformed into a strain carrying a chromosomal thiH allele that encodes a ThiH* protein (DM4104). Growth of the resulting strains was assessed, and the results are summarized in Table II. Some combinations of the chromosomal and plasmid-encoded ThiH* proteins allowed thiamine-independent growth. Of the four alleles tested, three allowed...
ThiH in Salmonella

![Graph](http://www.jbc.org/)

**Figure 3.** Increased AdoMet synthase activity restores thiamine synthesis to thiH<sup>+</sup> mutants. Strains were grown at 37°C in minimal glucose medium as described under “Experimental Procedures,” and growth was monitored over time as A<sub>650</sub>. Strains shown are: A, DM4104 (ThiH<sup>H124Y</sup>); B, DM406 (ThiH<sup>P347T</sup>); and C, DM2275 (thiH:<sup>Tn10</sup>). Each panel shows data from the relevant strain with plasmid pMETK2 (triangles) and with an empty vector (squares) in minimal medium (open symbols). Filled symbols represent growth in the presence of 100 nM thiamine.

**Table II**

Function is restored by combinations of thiH alleles

| Recipient strain | Plasmid-encoded protein | Minimal | Tyrosine | Thiamine |
|------------------|-------------------------|---------|----------|----------|
| DM4104 None      | 0.11 ± 0.01             | 0.71 ± 0.1 | 0.80 ± 0.1 |
| DM4104 ThiH<sup>306K</sup> | 0.30 ± 0.02             | 0.67 ± 0.1 | 0.88 ± 0.1 |
| DM4104 ThiH<sup>E55G</sup> | 0.75 ± 0.1              | 0.71 ± 0.2 | 0.89 ± 0.1 |
| DM4104 ThiH<sup>312V,A373P</sup> | 0.59 ± 0.1             | 0.62 ± 0.1 | 0.79 ± 0.1 |
| DM4104 ThiH<sup>H124Y</sup> | 0.23 ± 0.04             | 0.70 ± 0.1 | 0.88 ± 0.1 |
| DM4104 ThiH<sup>V350I</sup> | 0.71 ± 0.02             | 0.71 ± 0.1 | 0.85 ± 0.1 |
| DM4104 ThiH<sup>C285A</sup> | 0.20 ± 0.05             | 0.68 ± 0.1 | 0.90 ± 0.1 |
| DM4104 ThiH<sup>A50T</sup> | 0.22 ± 0.04             | 0.70 ± 0.1 | 0.84 ± 0.1 |

growth on minimal medium when present in a strain containing the ThiH<sup>H124Y</sup> mutant protein. A similar pattern of growth was seen with the same plasmids present in a strain containing the ThiH<sup>P347T</sup> mutant protein (data not shown). As expected, strains carrying the same allele on the chromosome and plasmid maintained a requirement for thiazole or tyrosine. This result is consistent with (but not conclusive of) the functional form of ThiH being a multimer and does not exclude its interaction with other proteins as suggested (20).

**DISCUSSION**

Although the *E. coli* ThiH has been purified (20) and thiazole synthesis has been reconstituted in two systems (9, 10), no activity assay for purified ThiH has yet been described. To circumvent the lack of an *in vitro* ThiH activity assay we took an *in vivo* approach to gain insights into the mechanism of function of this protein in *S. enterica*. The *in vivo* characterization of mutant ThiH proteins described here provides the basis for future work aimed at integrating the results of *in vivo* and *in vitro* studies of ThiH function.

**Structural Features Critical for ThiH Function**—Sequence analysis of ThiH identifies a CXXCXXXC<sup>292</sup> motif and residues of a weakly defined AdoMet binding motif (LLVTGE-HQAKV<sup>136</sup>) that together result in the assignment of ThiH to the AdoMet radical superfamily (13). The identification of substitutions at these residues that eliminate ThiH function support a role for AdoMet and the [Fe-S] cluster in enzyme mechanism. Among radical AdoMet proteins there is a near consensus requirement for an aromatic residue in the position adjacent to the third cysteinyl residue of this motif (e.g. X<sup>290</sup>). In *S. enterica* ThiH this residue is a tyrosine, and a substitution by alanine or phenylalanine inactivates the protein. In the published structures of biontin synthase (BioB) (15), coproporphyrinogen III oxidase (HemN) (16), and pyruvate-formate-lyase activate this residue (Tyr, Phe, and Tyr respectively) interacts with the adenine moiety of AdoMet. In the case of BioB this interaction could be explained by a hydrogen bond between the hydroxyl group of the tyrosyl residue and the 3'-hydroxyl group of the adenine moiety of AdoMet. The fact that phenylalanine cannot substitute for tyrosine at position 91 in the *S. enterica* ThiH suggests a critical role for the hydroxyl group of the tyrosine. In HemN and pyruvate-formate-lyase activates the aromatic interaction between the purine moiety of AdoMet and the respective phenylalanine residue is responsible for stabilization. Two residues within the putative AdoMet binding motif, namely Gly-130 and Glu-131, were found to be critical for function. Structural data available for HemN suggests that these residues interact with the adenine moiety of AdoMet.

Residue Glu-187 is critical for ThiH function in *S. enterica*. This may be a specific feature of a few ThiH homologs because a glutamyl residue is found at this position in only 3 of the 20 homologs analyzed. The structural role of residue Glu-187 is so critical that shortening the side chain of this residue by a single carbon eliminates enzyme activity. We predict that the Glu-187 residue is also critical for the function of the other two ThiH homologs in which it is present.

Comparison of the primary sequence of ThiH with those of radical AdoMet proteins, HemN and BioB, for which crystal structures are available identified a number of residues that are conserved in the active site. Residues Cys-89 and Tyr-91 are conserved in all radical AdoMet proteins (13). Our mutational analysis supports the important roles of these residues in enzyme function.

**The ThiH<sup>+</sup> Phenotype Provides Insight on ThiH Structure and Function in Vivo—Mutants that require tyrosine, THZ, or thiamine are said to have a ThiH<sup>+</sup> phenotype. Our working model suggests that a ThiH<sup>+</sup> phenotype may result from disrupting one of two structural features of the ThiH protein, either AdoMet binding or [Fe-S] cluster integrity. We suggest that correction of the ThiH<sup>+</sup> phenotype by increased levels of AdoMet supports the idea of AdoMet stabilizing the former class of mutant proteins. The lesions in the two mutants that were suppressed by increased AdoMet levels are consistent with this hypothesis, in particular the H124Y substitution, which is directly adjacent to the AdoMet binding motif.

Mutants defective in *apbC, apbE*, or *gshA* functions are compromised in the assembly and/or repair of Fe-S clusters (12, 18), and thus we have proposed that the ThiH<sup>+</sup> phenotype in strains lacking these functions reflects a compromised Fe-S cluster (12). We propose that some thiH lesions that generate

---

3 Available at www.proteinexplorer.org.
the ThiH* phenotype do so by disrupting the structure of the Fe-S cluster. The effect of such mutations would not be corrected by increased levels of AdoMet as is the case for mutations affecting AdoMet binding.

Significantly, tyrosine restores ThiH function in both classes of proteins suggesting that the binding of tyrosine may provide stability lost by reduced binding of AdoMet or a destabilized Fe-S cluster. There is precedent for a stabilizing role of substrate in radical AdoMet enzymes. In the case of BioB there is >20-fold enhancement of AdoMet binding when the substrate (dethiobiotin) is bound (15). Noteworthy was the finding that tyrosine did not restore function to a ThiH protein that was simultaneously compromised in both AdoMet and Fe-S cluster binding, perhaps indicating the severity of the structural defects in this situation.

Analysis of the BioB and HemN structures show that the C-terminal portion of the protein is partially folded back with residues of the N-terminal in close proximity. We envision an active site of ThiH that is defined by residues involved in the binding of the Fe-S cluster and AdoMet and suggest that tyrosine has contacts near both the C and N termini indicating a folded structure similar to that of BioB (15). This conformation is consistent with the mutant analysis of thiH, particularly the finding that two substitutions (A61S/P334Q and A51V/A373P) are found in some strains with a ThiH* phenotype. In the case of ThiHA61S/P334Q neither substitution alone disrupted function of proteins suggesting that the binding of tyrosine may provide stability lost by reduced binding of AdoMet or a destabilized Fe-S cluster. There is precedent for a stabilizing role of substrate in radical AdoMet enzymes. In the case of BioB there is >20-fold enhancement of AdoMet binding when the substrate (dethiobiotin) is bound (15). Noteworthy was the finding that tyrosine did not restore function to a ThiH protein that was simultaneously compromised in both AdoMet and Fe-S cluster binding, perhaps indicating the severity of the structural defects in this situation.

Acknowledgments—We thank Perry Frey for valuable discussion and biochemical suggestions. We acknowledge Nicole Baun and Jorge Escalante-Semerena for providing pMETK2 prior to publication.

REFERENCES

1. Begley, T. P., Downs, D. M., Ealick, S. E., McLafferty, F. W., Van Loon, A. P., Taylor, S., Campobasso, N., Chiu, H. J., Kinsland, C., Reddick, J. J., and Xi, J. (1999) Arch. Microbiol. 171, 293–300

2. Vander Horn, P. B., Backstrom, A. D., Stewart, V., and Begley, T. P. (1993) J. Bacteriol. 175, 982–992

3. Lauhon, C. T., and Kambampati, R. (2000) J. Biol. Chem. 275, 20996–21013

4. Palenchar, P. M., Buck, C. J., Cheng, H., Larson, T. J., and Mueller, E. G. (2000) J. Biol. Chem. 275, 8283–8286

5. Begley, T. P., Xi, J., Kinsland, C., Taylor, S., and McLafferty, F. (1999) Curr. Opin. Chem. Biol. 3, 623–629

6. Taylor, S. V., Kelleher, N. L., Kinsland, C., Chiu, H. J., Costello, C. A., Backstrom, A. D., McLafferty, F. W., and Begley, T. P. (1998) J. Biol. Chem. 273, 16555–16560

7. Kambampati, R., and Lauhon, C. T. (2000) J. Biol. Chem. 275, 10727–10730

8. Sprenger, G. A., Schorken, U., Wiepert, T., Grolle, S., de Graaf, A. A., Taylor, S. V., Begley, T. P., Bringer-Meyer, S., and Sahm, H. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12857–12862

9. Park, J. H., Dorrestein, P. C., Zhai, H., Kinsland, C., McLafferty, F. W., and Begley, T. P. (2003) J. Bacteriol. 185, 11789–11817

10. Leonardi, R., and Roach, P. L. (2004) J. Biol. Chem. 279, 17054–17062

11. Settembre, E. C., Dorrestein, P. C., Park, J. H., Augustine, A. M., Begley, T. P., and Ealick, S. E. (2003) J. Bacteriol. 185, 2971–2981

12. Grahn, J., Webb, E., Beck, B., and Downs, D. (2000) J. Bacteriol. 182, 5180–5187

13. Sofia, H. J., Chen, G., Hetzler, B. G., Reyes-Spindola, J. F., and Miller, N. E. (2001) Nucleic Acids Res. 29, 1097–1106

14. Chen, D., Walsey, C., Hoffman, B. M., and Frey, P. A. (2003) J. Am. Chem. Soc. 125, 11788–11797

15. Berkovich, F., Nicolet, Y., Wan, J. T., Jarrett, J. T., and Brennan, C. L. (2004) Science 303, 76–79

16. Logan, D. T., Anderson, J., Sjoberg, B. M., and Nordlund, P. (1999) Science 283, 1499–1504

17. Cooper, M. M., Cooper, N. J., Hong, W., Shokes, J. E., Broderick, W. E., Broderick, J. B., Johnson, M. K., and Scott, R. A. (2003) Protein Sci. 12, 1573–1577

18. Skovran, E., and Downs, D. M. (2003) J. Bacteriol. 185, 98–106

19. Skovran, E., and Downs, D. M. (2000) J. Bacteriol. 182, 3986–3993

20. Leonard, R., Fairhurst, S. A., Kriek, M., Lowe, D. F., and Roach, P. L. (2003) FEBS Lett. 539, 95–99

21. Miranda-Rios, J., Morera, C., Taboada, H., Davalos, A., Encarnacion, S., Mora, J., and Soberon, M. (1997) J. Bacteriol. 179, 6887–6893

22. Morett, E., Korbel, J. O., Rajan, E., Saah-Bincon, G., Olvera, L., Olivera, M., Schmidt, S., Snel, B., and Bork, P. (2003) Nat. Biotechnol. 21, 790–795

23. Rodionov, D. A., Vitreschak, A. G., Mironov, A. A., and Gelfand, M. S. (2002) J. Bacteriol. 184, 4984–4995

24. Vogel, H. J., and Bonner, D. M. (1956) J. Biol. Chem. 218, 97–106

25. Petersen, L. A., and Downs, D. M. (1997) J. Bacteriol. 179, 4894–4900

26. Bartholome, B., Jube, J., Martinez, E., and de la Cruz, F. (1991) FEBS Lett. 279, 149–153

27. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410

28. Lutz, B., and Bujard, H. (1997) Nucleic Acids Res. 25, 1203–1210

29. Cooper, N. J., Booker, S. J., Ruzicka, F., Frey, P. A., and Scott, R. A. (2000) Biochemistry 39, 15668–15673

30. Way, J. C., Davis, M. A., Morisato, D., Roberts, D. E., and Kleckner, N. (1984) Gene 289, 369–379

31. Castilho, B. A., Olsson, P., and Casadaban, M. J. (1984) J. Bacteriol. 158, 488–495
Mutational Analysis of ThiH, a Member of the Radical S-Adenosylmethionine (AdoMet) Protein Superfamily
Norma C. Martinez-Gomez, Matt Robers and Diana M. Downs

J. Biol. Chem. 2004, 279:40505-40510. doi: 10.1074/jbc.M403985200 originally published online July 22, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M403985200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 31 references, 17 of which can be accessed free at http://www.jbc.org/content/279/39/40505.full.html#ref-list-1