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Transcriptional Regulation of the Phosphotransacetylase-Encoding and Acetate Kinase-Encoding Genes (*pta* and *ack*) from *Methanosarcina thermophila*

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Phosphotransacetylase and acetate kinase catalyze the activation of acetate to acetyl coenzyme A in the first step of methanogenesis from acetate in *Methanosarcina thermophila*. The genes encoding these enzymes (*pta* and *ack*) have been cloned and sequenced. They are arranged on the chromosome with *pta* upstream of *ack* (M. T. Latimer, and J. G. Ferry, J. Bacteriol. 175:6822–6829, 1993). The activities of phosphotransacetylase and acetate kinase are at least 8- to 11-fold higher in acetate-grown cells than in cells grown on methanol, monomethylamine, dimethylamine, or trimethylamine. Northern blot (RNA) analyses demonstrated that *pta* and *ack* are transcribed as an approximately 2.4-kb polycistronic message and that the regulation of enzyme synthesis occurs at the mRNA level. Primer extension analyses revealed a transcriptional start site located 27 bp upstream from the translational start of the *pta* gene and 24 bp downstream from a consensus archaeal boxA promoter sequence. S1 nuclease protection assays detected transcripts with four different 3′ ends, each of which mapped to the beginning of four consecutive direct repeats. Northern blot analysis using an *ack*-specific probe detected both the 2.4-kb polycistronic transcript and a smaller 1.4-kb transcript which is the estimated size of monocistronic *ack* mRNA. A primer extension product was detected with an *ack*-specific primer; the 5′ end of the product was in the intergenic region between the *pta* and *ack* genes but did not follow a consensus archaeal boxA sequence. This result, as well as detection of an additional 1.4-kb mRNA species, suggests processing of the polycistronic 2.4-kb transcript.

Methanogenic microbes represent the largest and most diverse group in the domain *Archaea*. Most methane producers utilize only one energy-yielding pathway, the reduction of CO_2 to methane; however, *Methanosarcina* species can also obtain energy for growth by reducing the methyl groups of acetate, methanol, or methylamines to methane. Methanol provides more energy for growth than acetate; thus, the methanosarcina primarily utilize methanol before switching to acetate when grown in the presence of both substrates (14, 16). Two-dimensional polyacrylamide gel electrophoresis reveals that nearly 100 polypeptides are synthesized in acetate-grown *Methanosarcina thermophila* that are not present in methanol-grown cells (5). The extent of this regulation is unprecedented among methanogenic microbes and presents an opportunity to investigate regulation of gene expression in the *Archaea*.

The pathway for acetate conversion to methane is well characterized in *M. thermophila* (4). Sequential reactions catalyzed by phosphotransacetylase and acetate kinase activate acetate to acetyl coenzyme A which initiates the pathway (Fig. 1). The synthesis of both enzymes is elevated when acetate, rather than methanol, is used as the growth substrate (1, 9); however, the mechanism of this growth substrate regulation has not been investigated. The genes (*pta* and *ack*) encoding both these enzymes have been cloned and sequenced (Fig. 1) (8). Only one copy of each gene is present per chromosome as determined by Southern blot analysis (8). Here we show that *pta* and *ack* constitute a single transcriptional unit that is regulated at the mRNA level by the growth substrate. We also report transcriptional mapping of the 5′ and 3′ ends of mRNA, identification of promoter sequences, and potential transcription termination sites.

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MATERIALS AND METHODS

Materials. DNA restriction endonucleases and the 0.24- to 9.5-kb RNA molecular weight markers were obtained from GIBCO/BRL. The Sequenase version 2.0 sequencing kit was purchased from United States Biochemicals. The Klenow fragment of *Escherichia coli* DNA polymerase I, S1 nuclease, and the random priming kit were obtained from Boehringer Mannheim. The avian myeloblastosis virus reverse transcriptase enzyme was purchased from Promega Corp. Radiolabeled nucleotides were supplied by DuPont-New England Nuclear Corp. Kodak imaging film X-AR was used for autoradiography. The QIAGEN Plasmid Midi Kit was obtained from QIAGEN Inc. Bovine serum albumin and yeast tRNA were supplied by Sigma Chemical Co. The Bradford protein dye reagent was obtained from BioRad. All other materials were of reagent grade and are commercially available.

Plasmids and routine molecular biology methods. Plasmid DNA was purified by using a QIAGEN Plasmid Midi Kit. Routine molecular biology methods were performed as described by Sambrook et al. (12). Plasmid pUC19/ack has been described previously (8) and contains a PCR-derived *M. thermophila ack* sequence subcloned into the *Sma*I site of pUC19. Plasmid pML150 contains the *M. thermophila pta* and *ack* genes subcloned into the *Eco*RI site of pUC19. Plasmid pML150 was used as the template for double-stranded sequencing by the dideoxynucleotide termination method (13) using Sequenase version 2.0. Oligonucleotides ack1, ack2, pta1, and pta2 served as sequencing primers (see below).

Enzyme assays. *M. thermophila* cells were harvested, and the cell pellet was resuspended in 100 mM Tris (pH 7.5) buffer containing 50 mM KCl and 2 mM dithiothreitol. Chloroform was added to make the cells permeable, the cells were vortexed, and assays were performed in triplicate. Acetate kinase activity was determined by the hydroxamate assay which detected the formation of acetylphosphate from acetate and ATP (1). Phosphotransacetylase activity was assayed by detecting acetyl coenzyme A formation from acetylphosphate and HS-coenzyme A (9). Protein concentrations were determined by the Bradford method (3), using protein dye reagent (BioRad) and bovine serum albumin as the standard. Activities are expressed as micromoles per minute per milligram and represent an average of at least three independent experiments.

RNA isolation. *M. thermophila* cultures were harvested, and the RNA was isolated by the procedure described by Sowers et al. (15) with the following modification. After the RNA was initially isolated, it was resuspended in 4 M

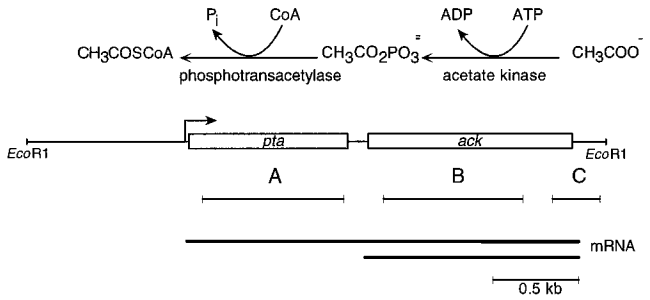

FIG. 1. Organization of the *M. thermophila pta-ack* region. The first two reactions in the pathway of acetate conversion to methane, catalyzed by acetate kinase and phosphotransacetylase, are shown from right to left. The bars labeled A and B represent *Eco*RI-*Pst*I DNA fragments used as probes in Northern blot hybridizations (Fig. 2). The bar labeled C refers to the *Cla*I-*Hin*cII DNA fragment used as a probe in S1 nuclease mapping (Fig. 5). Transcripts detected by Northern blot analyses are represented by the thick lines. The direction of transcription (┌) is indicated. CoA, coenzyme A.

LiCl to remove any remaining cell debris. The RNA was pelleted and stored in isopropanol at −20°C.

Northern blot (RNA) analyses. Northern blot analyses were performed as described by Sambrook et al. (12). RNA fractionation was performed on 1.3% (wt/vol) agarose gels containing 6% (vol/vol) formaldehyde. The fragments used for Northern blot hybridization consisted of probe A (an 896-bp *Eco*RI-*Pst*I fragment that spanned codons 16 to 310 of *pta*) (Fig. 1) and probe B (a 792-bp *Eco*RI-*Pst*I fragment that spanned codons 75 to 331 of *ack*) (Fig. 1). Fragments were labeled with [α-^{32}P]dATP by the random-priming methods as described by the supplier. Hybridization reactions were performed at 42°C in a solution containing 5× Denhardt's reagent (12), 6× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA [pH 7.7]) (12), 0.5% (wt/vol) sodium dodecyl sulfate (SDS), 50% formamide, and 50 ng of the radiolabeled probe. The final wash of the filters was at 65°C in 0.2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.1% SDS (wt/vol).

Primer extensions. The 5′ ends of the *pta* and *ack* mRNAs were mapped with the following oligonucleotides: pta1 (5′-GTTAAGTTTCTTTGCTCTTTCACT-3′, nucleotides 52 to 75 relative to the identified transcriptional start site of *pta*), pta2 (5′-CAAGGATCTTGGCAGCTGCCTG-3′, nucleotides 118 to 139), ack1 (5′-CCTATCCTCTCGCAAAGACCTAC-3′, nucleotides 1215 to 1237), and ack2 (5′-CCAGTACTTTCATGTGTAAACC-3′, nucleotides 1125 to 1146). Primer extension analysis was based on a procedure described by Alam et al. (2). Briefly, RNA (10 to 20 μg) isolated from acetate-grown *M. thermophila* cells was pelleted and the pellet was resuspended in a solution consisting of 2 μmol of primer and 7.5 μM (each) dGTP, dCTP, and dTTP. The RNA was denatured by boiling, 5× avian myeloblastosis virus reverse transcriptase annealing buffer was added, and the mixture was incubated for 10 min at 42°C. [α-^{35}S]dATP (1 μl of 1,000 to 1,500 Ci/mmol) was added along with 12 U of avian myeloblastosis virus reverse transcriptase. The reaction was incubated at 42°C for 1 h and electrophoresed on an 8% (wt/vol) polyacrylamide sequencing gel.

S1 nuclease protection experiments. The procedure used in S1 nuclease protection experiments was a modification of a protocol described by Sambrook et al. (12). The 379-bp *Cla*I-*Hin*cII fragment (Fig. 1) used to protect the RNA was from the plasmid pUC19/ack (see above) and contained nucleotides 2231 to 2573 relative to the transcriptional start site of the *pta* gene. The coding strand was labeled on the 3′ end by filling in the 5′ overhand with [α-^{32}P]dCTP and deoxynucleotides with the Klenow fragment of *E. coli* DNA polymerase I. The probe was denatured in 0.1 volume of 2 N NaOH–2 mM Na_2EDTA for 5 min at 25°C, extracted with phenol-chloroform-isoamyl alcohol (25:24:1), and precipitated. The probe (50 ng) was then added to *M. thermophila* RNA (10 to 20 μg) isolated from acetate-grown cells, to *Saccharomyces cerevisiae* tRNA (22 μg), or to buffer alone. The mixture was precipitated, the pellets were resuspended in 40 μl of formamide and 10 μl of 5× hybridization salts (0.2 M piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) [PIPES] [pH 6.4], 5 mM Na_2EDTA [pH 8], and 2 M NaCl), incubated at 4°C for 3 h to allow easier resuspension of the pellets, vortexed, denatured at 65°C for 10 min, and then allowed to hybridize at 42°C overnight. Approximately 2 U of S1 nuclease was used to digest the nucleic acids for 30 min at 33°C. The reactions were run on an 8% polyacrylamide sequencing gel next to a DNA ladder obtained from sequencing reactions using pML150 as a template and oligonucleotide Ack1 as a primer. The sizes of the protected fragments were estimated by comparison to the unrelated DNA sequencing ladder.

TABLE 1. Specific activities of phosphotransacetylase and acetate kinase in cell extracts from *M. thermophila* grown on different substrates

Growth substrate	Sp act (U/mg)[a]	
	Pta	Ack
Acetate	59 ± 10	11 ± 3
Methanol	7.0 ± 2	<1[b]
Trimethylamine	6.5 ± 1	<1
Dimethylamine	5.6 ± 1	<1
Monomethylamine	5.7 ± 1	<1

[a] One unit equals 1 μmol of acetylphosphate produced per min at 37°C. Values are the means ± standard deviations of three determinations.
[b] Below the limit of detection (1 U/mg).

RESULTS

Regulation of enzyme synthesis. The specific activities of phosphotransacetylase and acetate kinase in extracts of acetate-grown cells (Table 1) were approximately 8- and 11-fold higher than for cells grown with methanol, in agreement with previous reports (1, 9). Additionally, the specific activities of both enzymes in extracts of acetate-grown cells were at least ninefold higher than for cells grown with trimethylamine, dimethylamine, or monomethylamine as the energy source. These results suggest that the levels of phosphotransacetylase and acetate kinase are greater in acetate-grown cells than in cells grown on all other substrates.

Northern blot analyses (Fig. 2) indicated that both the *pta*- and *ack*-specific probes (Fig. 1) hybridized to an approximately 2.4-kb transcript which is of sufficient size to include *pta* and *ack*, suggesting that the genes are cotranscribed. These results also demonstrated that the steady-state levels of transcript were severalfold greater in acetate-grown cells than in cells grown on all other substrates, suggesting that regulation of phosphotransacetylase and acetate kinase synthesis occurs, at least in part, at the mRNA level. The *ack*-specific probes also revealed accumulation of an mRNA species of approximately 1.4 kb, the appropriate size for a monocistronic *ack* transcript. It is unlikely that this mRNA was a result of transcription from another copy of the *ack* gene, because Southern blot analysis shows only one copy of the *ack* and *pta* genes present per chromosome (8). Smaller *ack*-specific species were also observed; however, they were variable in size and amount, depending on the RNA sample and growth substrate, which sug-

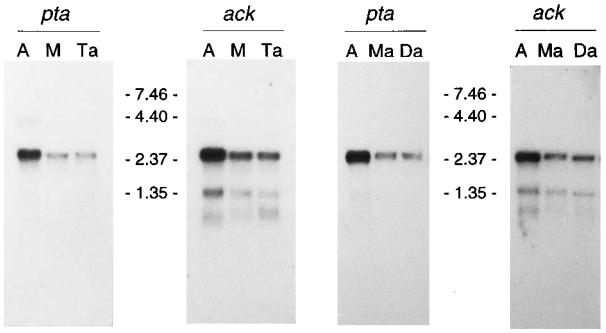

FIG. 2. Northern blot analyses of the *pta-ack* region of *M. thermophila*. Total RNA (20 μg) prepared from *M. thermophila* was hybridized with either a *pta*-specific probe (probe A in Fig. 1) or an *ack*-specific probe (probe B in Fig. 1). RNA from cells grown on acetate (lanes A), methanol (lanes M), trimethylamine (lanes Ta), dimethylamine (lanes Da), or monomethylamine (lanes Ma). A 0.24- to 9.5-kb RNA ladder was used for molecular size determination.

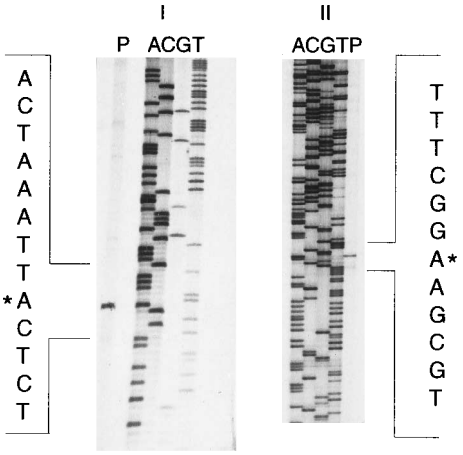

FIG. 3. Mapping of the 5′ ends of the transcripts. Lane P, primer extension products obtained with RNA isolated from acetate-grown *M. thermophila* and oligonucleotide primers Pta1 (gel I) and Ack1 (gel II). Lanes A, C, G, and T, products of DNA sequencing reactions using pML150 as a template and the same oligonucleotides used for primer extension. The asterisk indicates the 5′ nucleotide of the primer extension product.

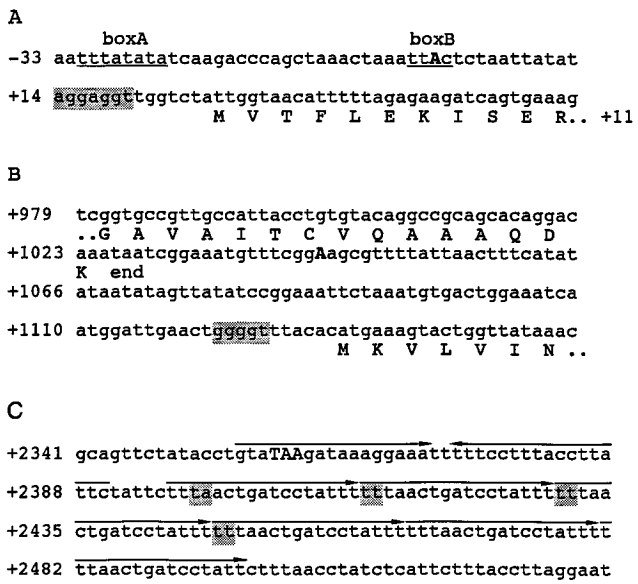

FIG. 4. Partial sequence of the region containing the *pta* and *ack* genes showing sequences relevant to transcription and translation. The entire sequence of this region has been published elsewhere (9). The nucleotide sequence is numbered with respect to the transcriptional start site of *pta*. The amino acid sequence (single-letter symbols) is shown directly below the first base of each codon. (A) Upstream sequence and part of the coding region of the *pta* gene. The start site of transcription is shown with an uppercase boldface A nucleotide. The boxA and boxB sequences are underlined, and the potential ribosome binding site is shaded. (B) Intergenic region between *pta* and *ack*. The potential endonuclease processing site determined by primer extension analysis is shown with an uppercase boldface A nucleotide. A potential ribosome binding site is shaded. The stop codon of phosphotransacetylase is represented by the word "end." (C) Sequence at the 3′ end of the *ack* gene. The stop codon of *ack* is shown in uppercase letters. The inverted and tandem repeats are indicated with arrows. The 3′ ends of the transcript, as determined by S1 nuclease protection analyses, are shaded.

gests they may have resulted from degradation of the 1.4-kb, *ack*-specific, mRNA species. No transcripts smaller than 2.4 kb were detected with the *pta*-specific probe.

5′ and 3′ end mapping of the mRNA. Primer extension analyses were performed to determine the 5′ ends of the mRNA. One major transcriptional initiation site was identified by using a primer specific for *pta* (Fig. 3). The 5′ end mapped to the A nucleotide located 27 bp upstream of the *pta* translational start site (Fig. 4A). This result was independently confirmed by using another primer (Materials and Methods). The results indicate that transcription initiation occurs 24 bp downstream from the end of an 8-bp sequence (Fig. 4A) with 100% identity to the consensus archaeal boxA promoter sequence [5′-TTTA(T/A)ATA-3′] (11). Typically, transcription of genes from the methanogenic *Archaea* initiates 22 to 27 bp downstream of boxA at a purine-pyrimidine dinucleotide, ideally in a boxB sequence (consensus 5′-ATGC-3′) (11). The transcriptional start site for the 2.4-kb mRNA occurs at a purine-pyrimidine dinucleotide in a region with 50% identity to the consensus boxB sequence (Fig. 4A).

Primer extension analyses with an *ack*-specific primer produced one major product (Fig. 3). The 5′ end mapped to the A nucleotide located 90 bp upstream of the *ack* translational start site and 16 bp downstream of the *pta* stop codon (Fig. 4B). This result was confirmed by using a second primer (Materials and Methods). The results further support that the 1.4-kb *ack* mRNA species detected by Northern blot analysis is monocistronic. The 5′ end of the primer extension product was not found within a purine-pyrimidine dinucleotide, and no consensus boxA sequence was located at the expected distance (22 to 27 bp) upstream of it. Although transcription initiation at this site cannot be ruled out, the results are more consistent with endonucleolytic cleavage of the primary 2.4-kb transcript.

Both thymine-rich sequences and stem-loops are postulated to function as transcription termination sites in the *Archaea* (11). Sequences located immediately downstream of the *ack* stop codon contain a 32-bp inverted repeat, with the potential to form a stem-loop structure (free energy of formation = −9.3 kJ/mol, as predicted by the Genetics Computer Group FOLD program) (17), followed by six thymine-rich tandem repeats (5′-TTTAACTGATCCTATTT-3′) (Fig. 4C). The 3′ ends of the transcripts were determined by S1 nuclease protection assays (Fig. 5). Four 3′ ends were localized to within 2 bp which mapped to the beginning of the four consecutive direct repeats immediately following the inverted repeat (Fig. 4C and 5). The majority of the mRNA population had a 3′ end within the repeated sequence closest to the stop codon of the *ack* gene. No protected fragments were detected in this region when yeast tRNA was substituted for *M. thermophila* RNA or when no RNA was included.

DISCUSSION

The results presented here indicate that *pta* and *ack* are cotranscribed and that growth substrate regulation of phosphotransacetylase and acetate kinase occurs at the mRNA level. The *cdhA* gene, encoding the 89-kDa subunit of the carbon monoxide dehydrogenase complex, is also regulated by acetate at the mRNA level (15). This complex is the central enzyme in the pathway of methanogenesis from acetate (4). Besides the consensus archaeal promoter sites, no conserved sequences were found when comparing the upstream regions of these three genes. The transcriptional mapping of the *pta* and *ack* genes provides a basis for future in vitro transcription studies to determine *cis*-acting elements and *trans*-acting factors required for regulation.

The 2.4- and 1.4-kb mRNAs have multiple 3′ ends which occur at approximately the same site within four thymine-rich tandemly repeated sequences (5′-TTTAACTGATCCTATTT-

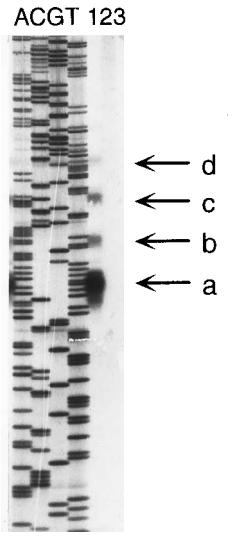

FIG. 5. Mapping of the 3′ ends of the transcripts. A *Cla*I-*Hin*cII DNA fragment was used in S1 nuclease protection experiments (Fig. 1) with RNA isolated from acetate-grown *M. thermophila* (lane 1), yeast tRNA (lane 2), or no RNA (lane 3). The sizes of the protected fragments were determined by comparison with an unrelated DNA ladder generated by sequencing reactions shown in lanes A, C, G, and T. The 3′ ends of the protected fragments indicated by letters correspond to the nucleotides shown in Fig. 4C as follows: a, +2398 and +2399; b, +2413 and +2414; c, +2430 and +2431; d, +2447 and 2448.

3′) (Fig. 4C and 5). These sequences are perfectly conserved, suggesting a necessary function. Transcription termination in the *Archaea* most often occurs in thymine-rich stretches (11), which suggests that the tandemly repeated sequences are transcriptional termination signals. Multiple transcription termination sites within tandemly repeated sequences have not been reported in the *Archaea*. Six tandem repeats of a 14-bp sequence and three tandem repeats of a 29-bp sequence occurs between the *carB* and *argG* genes of *Methanosarcina barkeri*; however, no function has been proposed for this region (11). An inverted repeat occurs 6 bp upstream from the first tandemly repeated sequence at the 3′ end of the *ack* gene of *M. thermophila*; however, it is not known if this putative stem-loop structure functions as a rho-independent terminator, as proposed for the few examples in the *Archaea* in which an inverted repeat is located in the 3′ end of a transcript (11).

Although *pta* and *ack* are cotranscribed, one or more additional mechanisms may be necessary to adjust the relative amounts of the gene products to meet physiological requirements. Considering that phosphotransacetylase is a monomer and acetate kinase is a homodimer and that the turnover number for phosphotransacetylase (9) is greater than that of acetate kinase (1), it may be most efficient to the cell if the *ack* gene product were synthesized at a level that was greater than the *pta* gene product. One way to coordinate the amounts of the two enzymes is at the mRNA level. The detection of a 1.4-kb *ack*-specific mRNA species in addition to the 2.4-kb transcript suggests increased expression of *ack* relative to that of *pta*. The absence of a consensus archaeal promoter upstream of the 5′ end of the *ack*-specific mRNA suggests that initiation of a 1.4-kb transcript is unlikely; thus, an endonucleolytic cleavage of the 2.4-kb primary transcript, as well as greater stability of the 1.4-kb *ack*-specific species over the *pta*-specific species, is a consideration. Although the putative stem-loop structure at the 3′ end of *ack* could potentially function as a transcription termination signal, it may also help to stabilize the 1.4-kb *ack*-specific species by acting as a barrier to 3′ exonucleases. Other factors could contribute to differential regulation of enzyme levels including translational regulation and enzyme stability. For example, differential regulation of synthesis could occur by use of the less common UUG codon encoding the N-terminal methionine for phosphotransacetylase instead of the more common AUG codon used to encode the N-terminal methionine of acetate kinase (Fig. 5). Translational initiation at non-AUG codons has been shown to be less efficient than at AUG codons in both the *Eucarya* and *Bacteria* domains (6, 7, 10); however, it is not known if this is also a mechanism of regulation in the *Archaea*.

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REFERENCES

1. **Aceti, D. J., and J. G. Ferry.** 1988. Purification and characterization of acetate kinase from acetate-grown *Methanosarcina thermophila*. J. Biol. Chem. **263:**15444–15448.
2. **Alam, J., R. A. Whitaker, D. W. Krogmann, and S. E. Curtis.** 1986. Isolation and sequence of the gene for ferredoxin I from the cyanobacterium *Anabena* sp. strain PCC 7120. J. Bacteriol. **168:**1265–1271.
3. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
4. **Ferry, J. G.** 1993. Fermentation of acetate, p. 304–334. *In* J. G. Ferry (ed.), Methanogenesis. Ecology, physiology, biochemistry & genetics. Chapman and Hall, New York.
5. **Jablonski, P. E., A. A. DiMarco, T. A. Bobik, M. C. Cabell, and J. G. Ferry.** 1990. Protein content and enzyme activities in methanol- and acetate-grown *Methanosarcina thermophila*. J. Bacteriol. **172:**1271–1275.
6. **Klionsky, D. J., D. G. Skalnik, and R. D. Simoni.** 1986. Differential translation of the genes encoding the proton-translocating ATPase of *Escherichia coli*. J. Biol. Chem. **261:**8096–8099.
7. **Köpke, A. K. E., and P. A. Leggatt.** 1991. Initiation of translation at an AUA codon for an archaebacterial protein gene expressed in *E. coli*. Nucleic Acids Res. **19:**5169–5172.
8. **Latimer, M. T., and J. G. Ferry.** 1993. Cloning, sequence analysis, and hyperexpression of the genes encoding phosphotransacetylase and acetate kinase from *Methanosarcina thermophila*. J. Bacteriol. **175:**6822–6829.
9. **Lundie, L. L., and J. G. Ferry.** 1989. Activation of acetate by *Methanosarcina thermophila*. J. Biol. Chem. **264:**18392–18396.
10. **Peabody, D. S.** 1989. Translation initiation at non-AUG triplets in mammalian cells. J. Biol. Chem. **264:**5031–5035.
11. **Reeve, J. N.** 1993. Structure and organization of genes, p. 493–527. *In* J. G. Ferry (ed.), Methanogenesis. Ecology, physiology, biochemistry & genetics. Chapman and Hall, New York.
12. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
14. **Smith, M. R., and R. A. Mah.** 1978. Growth and methanogenesis by *Methanosarcina* strain 227 on acetate and methanol. Appl. Environ. Microbiol. **36:**870–879.
15. **Sowers, K. R., T. T. Thai, and R. P. Gunsalus.** 1993. Transcriptional regulation of the carbon monoxide dehydrogenase gene (*cdhA*) in *Methanosarcina thermophila*. J. Biol. Chem. **268:**23172–23178.
16. **Zinder, S. H., and A. F. Elias.** 1985. Growth substrate effects on acetate and methanol catabolism in *Methanosarcina* sp. strain TM-1. J. Bacteriol. **163:** 317–323.
17. **Zucker, M., and P. Stiegler.** 1981. Optional computer folding of large RNA sequences using thermodynamics and auxiliary information. Nucleic Acids Res. **9:**133–148.