DOE F 4300.3
(09-93)
(All other editions are obsolete)

U.S. Department of Energy

OMB Control No.
1910-0500
OMB Burden Disclosure
Statement on Page 4

Semi-Annual Summary Report of DOE-Owned Plant and Capital Equipment (P&CE)

Contractor Name Univ of Maryland Biotechnology Institute Contract No. DE-FG02-93ER20106

Address 701 E. Pratt St., Baltimore, MD 21202

Location of Property (City, State) _____

Contracting Office _____

1	2	3	4	5	6	7	8	9
Asset Type Code	Beginning Balance As of _____		Acquisitions		Dispositions		Ending Balance As of _____	
	No. of Items	$	No. of Items	$	No. of Items	$	No. of Items	$
No capital equipment was purchased under the above-referenced award.								
Total Plant and Capital Equipment								

Prepared By Mildred Homa Manager, Contracts/Grants 410-234-8805 Mildred Homa
name (printed), title, telephone number, signature

Date of Last Physical Inventory of Capital Equipment _____

Contracting Officer Representative Signature _____

(Attach results of latest Physical Inventory if conducted since last reporting period)

Summary of Acquisitions & Dispositions by Type of Transactions

Acquisitions from Column (5) above

1. Purchases:
   a. P&CE Budgeted Items ..... $ _____
   b. Operating Expense Budgeted Items ..... $ _____
2. Fabrications
   a. Beginning Balance ..... $ _____
   b. Additions - P&CE Budgeted ..... $ _____
   c. Additions - Operating Expense Budgeted ..... $ _____
   d. Completions ..... ($ _____) $ _____
   e. Ending Balance ..... $ _____
3. No-Charge-Transfers from Other DOE Offices or Contractors ..... $ _____
4. No-Charge-Transfers from Other Federal Agencies ..... $ _____
5. Other (Explain) ..... $ _____
6. Total Acquisitions ..... $ _____

Dispositions from Column (7) Above

7. Sales (Salvage Credit of $) ..... $ _____
8. Trade-ins (Salvage Credit of $) ..... _____
9. No-Charge-Transfers to Other DOE Offices or Contractors ..... $ _____
10. No-Charge-Transfers to Other Federal Agencies ..... $ _____
11. Other (Explain) ..... $ _____
12. Total Dispositions ..... $ _____

Note: Detail lines 1 through 12 above in accordance with the following columnar headings. Attach extra sheets if necessary.

Printed with soy ink on recycled paper

DOE F 2050.11
(2-87)
(Previous GC-792)

OMB Control No.
1910-0800

PATENT CERTIFICATION

Kevin Sowers, Ph.D. - University of Maryland
Contractor Biotechnology Institute

☐ Interim Certification
☒ Final Certification

DE-FG02-93ER20106
DOE Prime and/or Subcontract Nos.

Contractor hereby certifies that:

1. All procedures for identifying and disclosing subject inventions as required by the patent clause of the contract have been followed throughout the reporting period.

2. There were no subcontracts or purchase orders involving research, development, and demonstration except as follows: [State none when applicable.]
None.

3. No inventions or discoveries were made or conceived in the course of or under this contract other than the following *(Certification includes* ☐*, does not include* ☐ *all subordinates):*

[State none when applicable.] None.

TITLE	INVENTOR	DATE REPORTED	DOE "S" NO.*

4. The completion date of this contract is as follows: 08/31/2008

5. The following period is covered by this certification:

May 20 1993 to August 31, 2008
Month Day Year Month Day Year

Kevin Sowers, Ph.D.-University of Maryland
Contractor Biotechnology Institute

701 E. Pratt St.

Baltimore, MD 21202
Address

Signature

04/03/2013
Date of Certification

* Also include Subcontract No. If available

FINANCIAL STATUS REPORT

(Short Form)

(Follow instructions on the back)

1. Federal Agency and Organizational Element to Which Report is Submitted	2. Federal Grant or Other Identifying Number Assigned By Federal Agency	OMB Approval No.	Page of
Dept. Of Energy	DE-FG02-93ER20106	0348-0038	pages

3. Recipient Organization (Name and complete address, including ZIP code)
University of Maryland Biotechnology Institute 701 E. Pratt St., Baltimore, MD 21202

4. Employer Identification Number	5. Recipient Account Number or Identifying Number	6. Final Report	7. Basis
526002033	5-27203	☒ Yes ☐ No	☐ Cash ☒ Accrual

8. Funding/Grant Period *(See instructions)* From: (Month, Day, Year)	To: (Month, Day, Year)	9. Period Covered by this Report From: (Month, Day, Year)	To: (Month, Day, Year)
05/20/93	08/31/08	05/20/93	08/31/08

10. Transactions:	I Previously Reported	II This Period	III Cumulative
a. Total outlays		410,807	410,807
b. Recipient share of outlays		0	0
c. Federal share of outlays		410,807	410,807
d. Total unliquidated obligations		0	0
e. Recipient share of unliquidated obligations		0	0
f. Federal share of unliquidated obligations		0	0
g. Total Federal share *(Sum of lines c and f)*		410,807	410,807
h. Total Federal funds authorized for this funding period		410,807	410,807
i. Unobligated balance of Federal funds *(Line h minus line g)*		0	0

11. Indirect Expense	a. Type of Rate *(Place "X" in appropriate box)*			
	☐ Provisional	☒ Predetermined	☐ Final	☐ Fixed
	b. Rate	c. Base	d. Total Amount	e. Federal Share
	48.5%	275,628	133,680	133,680

12. *Remarks: Attach any explanations deemed necessary or information required by Federal sponsoring agency in compliance with governing legislation.*

13. Certification: **I certify to the best of my knowledge and belief that this report is correct and complete and that all outlays and unliquidated obligations are for the purposes set forth in the award documents.**

Typed or Printed Name and Title	Telephone (Area code, number and extension)
Mildred L. Homa, Manager, Contracts and Grants	410-234-8805
Signature of Authorized Certifying Official	**Date Report Submitted**
Mildred Homa	4/3/2013

U.S. DEPARTMENT OF ENERGY JUNE 2005

FINANCIAL ASSISTANCE PROPERTY CLOSEOUT CERTIFICATION

Award Number	Recipient *(Name and address)*
DE-FG0293ER2016	University of Maryland Biotechnology Institute, 701 E. Pratt St., Ste 200, Baltimore, MD 21202

The purpose of this report is to facilitate the closeout of the Award. Based on the records maintained by the Recipient in accordance with the Property Management standards set forth in the Award, the following data reflects the Recipient's closeout inventory of real and personal property that was provided by the Department of Energy (DOE) or partially or wholly acquired with project funds.

I. EQUIPMENT

A. **Federally-Owned:** *(Government Furnished Equipment): (10 CFR 600.133(a), 600.232, 600.322, or Federal Demonstration Partnership (FDP) General Terms and Conditions No. 33, as applicable):* XNo ☐ Yes

*(If **yes,** attach property inventory list that includes item description, manufacturer, model, serial number, original acquisition date, original acquisition cost and disposal condition code per the Federal Management Regulation 102-36.240)*

B. **Equipment Acquired with Award Funds where Title Vests in the Recipient with further obligations to DOE:**
(10 CFR 600.133, 600.134, 600.232, or 600.321, as applicable)

XNo ☐ Yes

If **yes,** does the equipment have a per unit fair market value of $5,000 or more? ☐ No ☐ Yes

*(If **yes**, attach a property inventory list that includes item description, manufacturer, model, serial number, original acquisition date, original acquisition cost, disposal condition code per the Federal Management Regulation 102-36-240 and one of the disposition codes listed below)*

(1) The property will continue to be used for the purposes authorized in the Award.

(2) The property is no longer needed for the purposes of the Award, and will be used on another Federally sponsored activity *(List Activity and Federal Agency):*

(3) The Recipient wishes to retain the property and compensate DOE for its share of the current per unit fair market value.
(Identify the fair market value on the attached property inventory list and describe how the value was determined).

(4) The property is no longer needed for the purposes of the Award or other Federally sponsored activities and the Recipient requests DOE disposition instructions.

II. SUPPLIES *(10 CFR 600.135, 600.233, 600.324, or FDP General Terms and Conditions No. 35, as applicable)*

Does the residual inventory of unused supplies exceed $5,000 in total aggregate value? X No ☐ Yes *(if **yes**, check block below)*

☐ The supplies will be used on another Federally sponsored activity *(List Activity and Federal Agency).*

☐ The supplies will be sold or retained for use on non-Federally sponsored activities and the Recipient will compensate DOE for its share of the sales proceeds (or estimate of current fair market value). Attach a list of the supplies and complete the following Worksheet:

Sale proceeds or estimate of current fair market value	$ 0
Percentage of Federal participation	%
Federal share	$ 0
Selling and handling allowance	$ 0
Amount to be remitted to DOE	$ 0

U.S. DEPARTMENT OF ENERGY

FINANCIAL ASSISTANCE
PROPERTY CLOSEOUT CERTIFICATION

III. REAL PROPERTY: *(Real Estate - 10 CFR 600.132, /600.231, 600.321, or FDP General Terms and Conditions No. 32, as applicable)* X No ☐Yes *(If **yes**, complete A –C)*

A. Description of Real Property:

Not applicable.

B. Complete Address of Real Property:

Not applicable

C. Period of Federal Interest in the Property: From _____ **To** _____ (Unless the award specifies otherwise, the Federal Interest in the property ends when the award project period ends.)

D. Disposition Preference Request. If the period of Federal Interest in the property exceeds the project period, check one of the following blocks to indicate your disposition preference:

☐ Transfer property to another Federal award.

☐ Sell and compensate DOE.

☐ Return to DOE.

☐ Retain title and compensate DOE for its share of the current fair market value of the property.

Certification: I certify to the best of my knowledge and belief that all information presented in this report is true, correct and complete, and constitutes a material representation of fact upon which the Federal government may rely.

Name	Signature	Title	Date
Mildred Homa	Mildred Homa	Manager, Contracts and Grants	4/3/13

U.S. DEPARTMENT OF ENERGY

FINANCIAL ASSISTANCE PROPERTY CLOSEOUT CERTIFICATION

To be completed by the Department of Energy:

DOE PROPERTY DISPOSITION

☐ **Negative Report**

☐ **Real Property:**

☐ **Equipment:**

☐ **Supplies:**

Property Management Official Name	Signature	Date

FINAL REPORT

Grant: DE-FG02-93ER20106
Title: Physiology and Genetics of Biogenic Methane-Production from Acetate
P.I.: Kevin R. Sowers
Review Period: May 1, 2004 to April 31, 2008 (includes 1 yr NCE)

PUBLICATIONS AND PRESENTATIONS

This progress report describes our research accomplishments from May 1, 2004 to April 31, 2008, encompassing the 3 years of DOE funding and a one year no-cost extension. **This research project is acknowledge in the following peer-reviewed journals:**

1. **Apolinario-Smith, E., K.M. Jackson, and K.R. Sowers.** 2005. Development of a plasmid-mediated reporter system for in vivo monitoring of gene expression in the archaeon *Methanosarcina acetivorans*. Appl. Environ. Microbiol. 71: 4914-4918.

2. **Maeder, D.L., I. Anderson, T. Brettin, D. Bruce, P. Gilna, C. S. Han, A. Lapidus, W.W. Metcalf, E. Saunders, R. Tapia, and K.R. Sowers.** 2006. The *Methanosarcina barkeri* genome: comparative analysis with *Methanosarcina acetivorans* and *Methanosarcina mazei* reveals extensive rearrangement within methanosarcinal genomes. J. Bacteriol. 188: 7922-7931.

3. **MacAuley, S.R., S.A. Zimmerman, E.E. Apolinario, C. Evilia, Y.-M. Hou, J.G. Ferry, K.R. Sowers.** 2009. The Archetype γ-Class Carbonic Anhydrase (Cam) Contains Iron when Synthesized *in vivo* in *Methanosarcina acetivorans*. Biochem. 48(5): 817-819.

4. **Anderson, K.L., E.E. Apolinario, S.R. MacAuley, and K.R. Sowers**. 2009. Localization of a regulatory region within the 5' untranslated leader region of an archaeal CO dehydrogenase/acetyl-coenzyme synthase. J. Bacteriol. 191(22): 7123-7128.

5. **Menezes, S., K. Gaston, K. Krivos, E.E. Apolinario, N.O. Reich, K.R. Sowers, P.A. Limbach, J.J. Perona.** 2011. Formation of m^2G6 in *Methanocaldococcus jannaschii* tRNA catalyzed by the novel methyltransferase Trm14. Nucl. Acids Res. 39(17): 7641-7655

Three book chapters have been written that include data generated by this project:

1. **Sowers, K.R. and K. Anderson.** 2007. Molecular Genetics of Archaea. In: R. Cavicchioli (ed.), Archaea: Molecular Cell Biology. American Society for Microbiology, Washington, D.C. , pp. 463-477. ISBN: 978-1-55581-391-8.

2. **Sowers, K.R., S. DasSarma and P. Blum.** 2007. Gene transfer in Archaea. In: C. A. Reddy, T. J. Beveridge, J. A. Breznak, G. A. Marzluf, and T. M. Schmidt (ed.), Methods for General and Molecular Microbiology. American Society for Microbiology, Washington, D. C. ISBN: 978-1-55581-223-2.

3. **Sowers, K.R. and J.E.M. Watts.** 2006. Methods for the study of strictly anaerobic microorganisms. *In:* F. A. Rainey and A. Oren (ed.), *Methods in Microbiology – Extremophiles,* v. 35. Elsevier/Academic Press, Oxford, pp. 739-764. ISBN: 0-12-521537-1.

Findings from this research have been presented wholly or in part at the following professional meetings and invited presentations:

1. Biotechnological Advances in the Study of the Methanogenic Archaea. Chinese National Academy of Sciences, Beijing, China. November 5, 2008 (invited).
2. Applications of Marine Microbes for Bioremediation and Bioenergy. 2008 Joint EC-US/CIESM Workshop on Marine Genomics. Fontvielle, Monaco. October 12-14, 2008 (invited).
3. Regulatory elements in the UTR of methanosarcinal carbon monoxide dehydrogenase (cdh). K.L. Anderson*, E.A. Apolinario-Smith, and K.R. Sowers. Gordon Research Conference on the Molecular Basis of Microbial One-Carbon Metabolism. July 20-25, 2008. Lewiston, ME.
4. How methanogens get turned on: a model for the study of archaeal gene transcription. K.R. Sowers. Center for Advanced Research in Biotechnology, Shady Grove, MD. Oct. 15, 2007 (invited).
5. A third alternative for protein expression: the methanogenic Archaea. K.R. Sowers. Annual Meeting of the Society for Industrial Microbiology, Denver, CO. July 29-Aug 2, 2007 (invited).
6. Regulation of Biogenic Methane Production from Acetate by Marine Methanosarcina Species. K.L. Anderson*, E.A. Apolinario-Smith, and K.R. Sowers. Gordon Research Conference on Archaea: Ecology, Metabolism and Molecular Biology. August 19-24, 2007, Andover, NH.
7. The Na^+/H^+ Mrp antiporter in Methanosarcina acetivorans. R. Jasso-Chavez*, E.A. Apolinario-Smith, K.R. Sowers and J.G. Ferry. Gordon Research Conference on Archaea: Ecology, Metabolism and Molecular Biology. August 19-24, 2007, Andover, NH.
8. Regulation of biogenic methane production from acetate by marine Methanosarcina species. K.L. Anderson*, E.E. Apolinario, S.R. MacAuley, and K.R. Sowers. 8th International Marine Biotechnology Conference, Eilat, Israel. March 11-16, 2007.
9. Regulation of Gene Expression for Carbon monoxide Dehydrogenase (cdhA) from Methanosarcina species. Kimberly L. Anderson*, Ethel E. Apolinario-Smith, Sheridan R. MacAuley, and Kevin R. Sowers. 106th Ann. Mtg. Amer. Soc. Microbiol. May 21-25, 2006. Orlando, Fl.
10. *Methanosarcina barkeri* Genome: Comparative Analysis with *M. acetivorans* and *M. mazei* reveals extensive rearrangement within methanosarcinal genomes. Dennis L. Maeder, Alla Lapidus, Elizabeth Saunders†, Cliff S. Han, Roxanne Tapia, Thomas S. Brettin, David C. Bruce, Paul Gilna, Iain Anderson, William W. Metcalf and Kevin R. Sowers*. 1st Annual Joint Genome Institute User Meeting March 29-April 1, 2006. Walnut Creek, CA.
11. Terrestrial analogues for Martian life: Novel Survival Strategies Among Psychrotolerant Archaea. I. N. Reid*, J.A. Muller, A. Colman, K.R.Sowers, W.B. Sparks and Shiladitya DasSarma. Astrobiology Science Conference. March 26-30, 2006. Washington, DC.
12. Marine Bioremediation: Dechlorination, Methanogenesis and Mars. COMB Retreat, Edgewater, Maryland. October 14, 2005.
13. Mechanisms of Gene Expression for Carbon Monoxide Dehydrogenase (cdhA) from *Methanosarcina thermophila*. K.L. Anderson*, E.A. Apolinario-Smith, and K.R. Sowers.

Marine Estuarine Environmental Sciences Annual Colloquium. October 7-9, 2005. Baltimore, MD.

14. Mechanisms of Gene Expression for Carbon Monoxide Dehydrogenase (*cdh*A) from *Methanosarcina thermophila*. K.L. Anderson*, E.A. Apolinario-Smith, and K.R. Sowers. Gordon Research Conference on Archaea: Ecology, Metabolism and Molecular Biology. August 14-18, 2005, Oxford, England.
15. Terrestrial analogues for extraterrestrial life. N. Reid*, K.R. Sowers, H.D. Shukla, S. MacAuley, T. Miller, R. Suvanasuthi, R. Belas, A. Colman, F.T. Robb, P. DasSarma, J.A. Müller, F. Chen, R. Cavicchioli, S. DasSarma, W.B. Sparks, S. Lubow, M. McGrath, M. Livio, J. Valenti. NASA Astrobiology Institute Biennial Meeting, Boulder, CO. Apr 10-14, 2005.
16. Antarctic Extremophiles and Extraterrestrial Life. 2005. Astrobiology Afternoon, Space Telescope Science Institute, Baltimore, MD. Jan 20, 2005. (Invited)
17. Marine Microbial Processes from Earth to Mars. K.R. Sowers. American Type Culture Collection, George Mason University, Manassas, VA. Dec 9, 2004. (Invited)
18. Marine Bioremediation: Dechlorination, Methanogenesis and Mars. K.R. Sowers. Michmoret. Conference on Marine Biotechnology, Michmoret, Israel. Nov 22-23, 2004. (Invited)
19. A system for inducible overexpression of recombinant protein in *Methanosarcina acetivorans.* S.R. MacAuley*, M. Harmon, Y.-M. Hou and K.R. Sowers. 5th International Conference on Extremophiles. Cambridge, MD. September 19 – 23, 2004.
20. A system for inducible overexpression of recombinant protein in *Methanosarcina acetivorans.* S.R. MacAuley*, M. Harmon, Y.-M. Hou and K.R. Sowers. Marine Estuarine Environmental Sciences Colloquium. Cambridge, MD. September 17 – 18, 2004

RESEARCH ACCOMPLISHMENTS

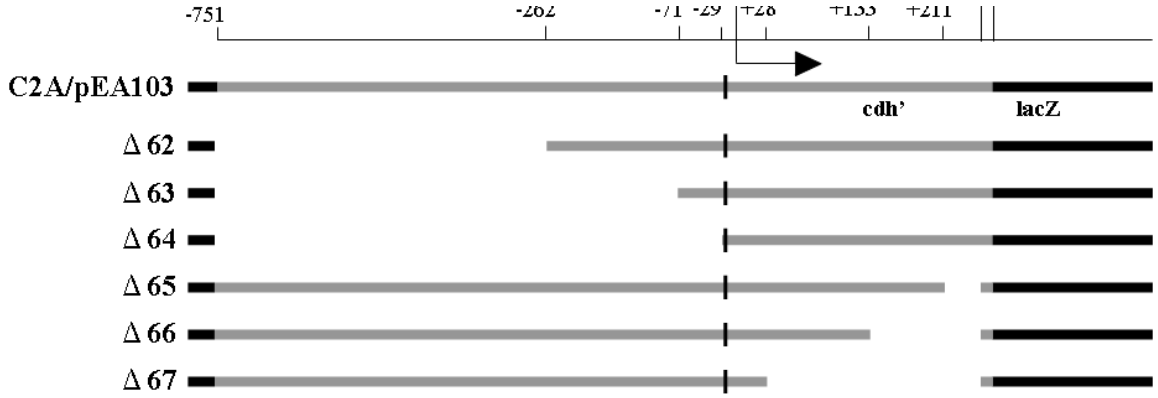
Specific research goals. The specific goal of this project is to identify the mechanisms that regulate the aceticlastic pathway in *Methanosarcina* using the highly regulated CO dehydrogenase catabolic system as a model system. We proposed to to identify DNA and protein components required for the regulation of aceticlastic catabolism. In addition to identifying components required for the regulation of transcription initiation, we proposed to determine whether expression of *cdh* is mediated by coordinate interaction of other processes such as transcription elongation rate and mRNA stability. The overall goal was to identify an archaeal paradigm for catabolic acetate metabolism. Highlights of the study are described below.

Objective 1. Determine the role of trans-acting elements in regulation of cdh transcription initiation.

We constructed and tested lacZ reporter fusions for the three TATA binding proteins using a reporter plasmid constructed for the project (see manuscript) and found that TBP1 is regulated in response to growth substrate. We also conducted deletions in conserved consensus regions identified by aligning (carbon monoxide dehydrogenase (*cdh*) genes from three methanosarcinal species and identified regions both upstream and downstream of the promoter that affected gene regulation. We also showed by Surface Plasmon Resonance analysis that there is differential binding of proteins to the carbon monoxide promoter region in response to

growth on different substrates. Gel-binding assays that indicated differential protein binding in a region upstream of the promoter. To identify sequences involved in CODH/ACS regulation a series of deletions were generated in the CODH/ACS *lacZ* reporter construct upstream of the transcriptional start site and downstream in the 371 bp 5' leader region between the transcription start site and the translation start site of the CODH/ACS operon. Progressive deletions were made from -751 to within 262 and 71 bases upstream of the transcription start site to detect both distant and near putative *cis*-regulatory elements (Figure 1). To detect putative *cis*-regulatory elements in the 5'-UTR progressive downstream deletions were made immediately upstream of the predicted ribosomal binding site of the *cdh*ABCDE operon to within 211, 133 and 14 bases of the transcription start site. Deletion of DNA sequences upstream of the promoter (Δ62 or Δ63) did not appear to have an effect on expression of β-galactosidase compared with wild type

Figure 1. Map of deletions upstream and downstream of the *Methanosarcina thermophila* TM1 *cdhA* transcriptional start site Locations of the deletions are shown in relation to the transcriptional start site of *cdhA* indicated by arrow. The vertical line in each deletion construct indicates the position of the promoter sequence



sequence in cells grown with all substrates, indicating that upstream *cis* elements were not involved in regulation of transcription initiation in response to substrate. Deletion of (Δ64) resulted in extremely low β-galactosidase levels, which is expected since the deleted sequence included the CODH/ACS promoter. Expression with deletion 65, which had 124 bases deleted near the 3' end of the leader sequence, was not significantly different from wild type when cells were grown on TMA. There was a difference, however, when cells were grown on methanol and on acetate. Some deletions downstream of the transcription start site within the 5' leader sequence did have an effect on expression during methylotrophic growth. Table 1 shows that Δ66 and Δ67 had only a 4- and 13-fold difference in expression, respectively, between acetate and methanol grown cells compared to the 61-fold difference observed in wild type constructs. The same was true for cells grown on TMA, where Δ66 and Δ67 were 7- and 5-fold higher, respectively, compared to a 20-fold difference for the wild type construct. However, the relative expression between acetate and each of the two methylotrophic substrates was different between deletions 66 and 67, which suggests a difference in regulation by these substrates. Regardless of the differential deletion effects observed between methylotrophic substrates, *these data show unequivocally that the 5' leader region is involved in regulation of expression based on*

substrate, and that the sequence within +28 to +211 base pairs of the 5' leader sequence has a critical role in the regulation of CODH/ACS expression.

Table 1. β-galactosidase levels and fold differences for deletion constructs on different substrates.

	Substrate			Fold Difference	
Construct	Acetate	Methanol	TMA	Acetate vs Methanol	Acetate vs TMA
WT/pEA103	23.5±1.9	0.4±0.03	1.2±0.4	61.7±6.1	20.5±4.8
Δ62	18.3±2.1*	0.3±0.05*	1.2±0.05	67.2±11.4	16.0±1.7
Δ63	23.1±2.41	0.3±0.02	1.2±0.06	70.0±7.4	18.6±1.9
Δ64	ND	ND	ND	2.5±0.6	1.1±0.2
Δ65	14.1±0.3*	0.2±0.02*	1.1±0.2	68.5±4.7	13.4±1.7
Δ66	18.6±2.8	4.5±0.1*	2.9±0.5*	4.1±0.6	6.6±1.3
Δ67	16.5±0.5*	1.2±0.03*	3.7±0.5*	13.3±0.4	4.5±0.6

*Indicates values that are significantly different ($p<0.05$) from the wild type values
ND indicates values that were below the limits of detection

Objective 2. Characterize the role of transcription elongation and mRNA turnover in cdh regulation.

As described above there were three possible scenarios that our research could reveal depending on whether *cdh* is regulated at the levels of pre-initiation complex formation, transcription initiation/elongation or mRNA processing. As proposed we determined whether regulation occur by differential control of transcription initiation or elongation. We conducted RT-PCR experiments to detect nascent message, which would indicate early termination of transcription processivity, but the results were inconclusive. We then conducted *in vitro* termination experiments in collaboration with Tom Santangelo and John Reeve to find evidence of differential transcription termination. The experiments indicated that processivity of RNAP was affected by the 371 bp leader sequence between the start of transcription and the structural gene. Deletions in the leader sequence were observed to relaxed early termination, but we still observed multiple early termination events in the region. As both these approaches were inconclusive, we investigated the effects of deletions in the leader sequence on expression of CO dehydrogenase/acetyl-coenzyme A synthase using qRT-PCR. A 10- to 15-fold difference in transcript level was observed within 358 bases of the 5' end in cells grown on acetate and methanol. However, transcript levels 405 bases downstream of the 5' end showed a significantly greater difference, increasing to 68-fold difference between acetate and methanol-grown cells. These results suggest that termination of elongation occurred between 358 and 405 bases downstream of the 5' end of the *cdh* transcript during methylotrophic growth. Another possible cause for the change in transcript level could be differential processing of mRNA. While experiments with actinomycin D ruled out differential mRNA decay, we could not rule out the possibility of differential processing of mRNA, as the addition of actinomycin D could have disrupted a processing event. The mRNA might be cleaved between 358 and 405 bp during methylotrophic growth, causing the difference in transcript levels between acetate and methylotrophic growth. In addition to the difference noted between 358 and 405 bp, a ten-fold difference in transcript levels between cells grown on acetate and methanol was observed from +70 to +358 bases downstream of the transcription start site. This observation indicates that differential transcript initiation also affects CODH/ACS expression. *Termination of*

transcriptional elongation in sequences distal to the regulatory CODH/ACS 5' leader sequence is consistent with regulatory mechanisms involving changes in secondary structure, such as attenuation or riboswitches. Another possible mechanism for termination of transcription would involve factor-dependent termination, such as seen in bacterial systems involving the termination factor rho. Under methylotrophic substrates, this termination factor might bind to the RNA, translocating along the RNA until it encounters the transcription complex and causes transcription to terminate. Under aceticlastic conditions, a conformational change in the RNA would prevent termination by the terminating protein. This model is also consistent with the results of this study.

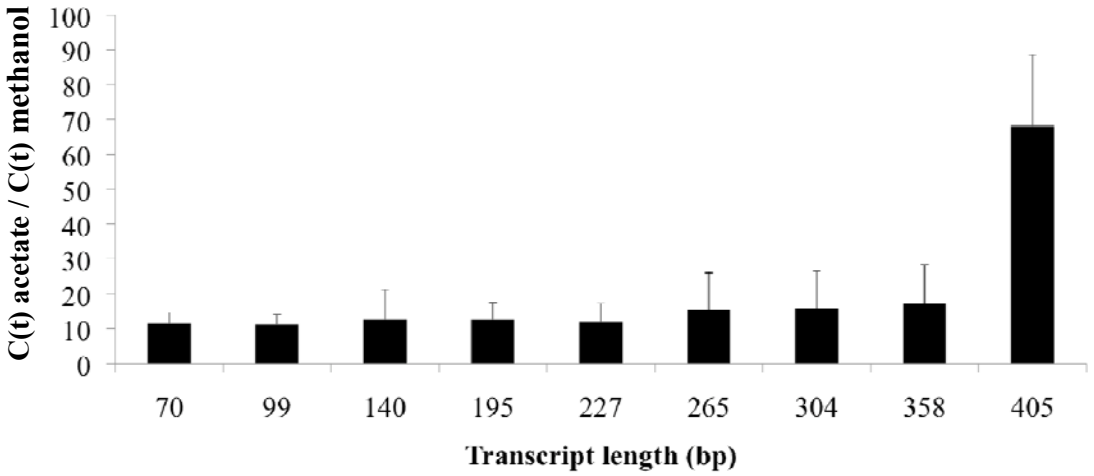


Figure 2. Differences in levels of transcripts of varying lengths. Fold differences were calculated from the C(t) values for RNA from acetate- versus methanol-grown cells. Positions of forward and reverse primers are indicated at top of figure

Summary.

5' leader regions identified within the Archaea include the 113 bp leader region identified upstream of a DEAD-box RNA helicase in the Antarctic methanogen *Methanococcoides burtonii* and the methyltransferase genes in *Methanosarcina* spp. In both of these examples, the UTR was implicated in regulation, although the role of the leader region in regulation was not confirmed. The system studies in this project appears to involve sequences downstream of the start of transcription, well into the 5' leader sequence. Our results also indicate that the 5' leader region has a role in regulation of CODH/ACS post initiation via termination of elongation during methylotrophic growth. This conclusion is supported by several observations. First, post-transcriptional regulation by differential translation was ruled out, as the fold difference of the protein levels and the transcript levels were not significantly different. Second, *cdh* transcript stability was similar in cells grown aceticlastically and methylotrophically, ruling out differential mRNA degradation as a possible mechanism. Finally, a significant difference in transcript levels was observed 405 bases downstream of the 5' end of the transcript. The results indicate that methanosarcinal CODH/ACS expression is controlled by at least one mechanism at the level of transcription elongation, as part of the regulatory strategy employed by these methanogenic Archaea to efficiently direct carbon and electron flow in anaerobic consortia during fermentative processes. A proposed model supported by the results of this project is shown in Figure 3. *To the best of our knowledge this is the first evidence of regulation at the level of transcriptional elongation by a 5' leader region as a mechanism for gene regulation in the Archaea.* This

project set the stage for future studies that will focus on identification of *trans*-acting elements and putative secondary structures to characterize the paradigm for catabolic CODH/ACS regulation in these Archaea.

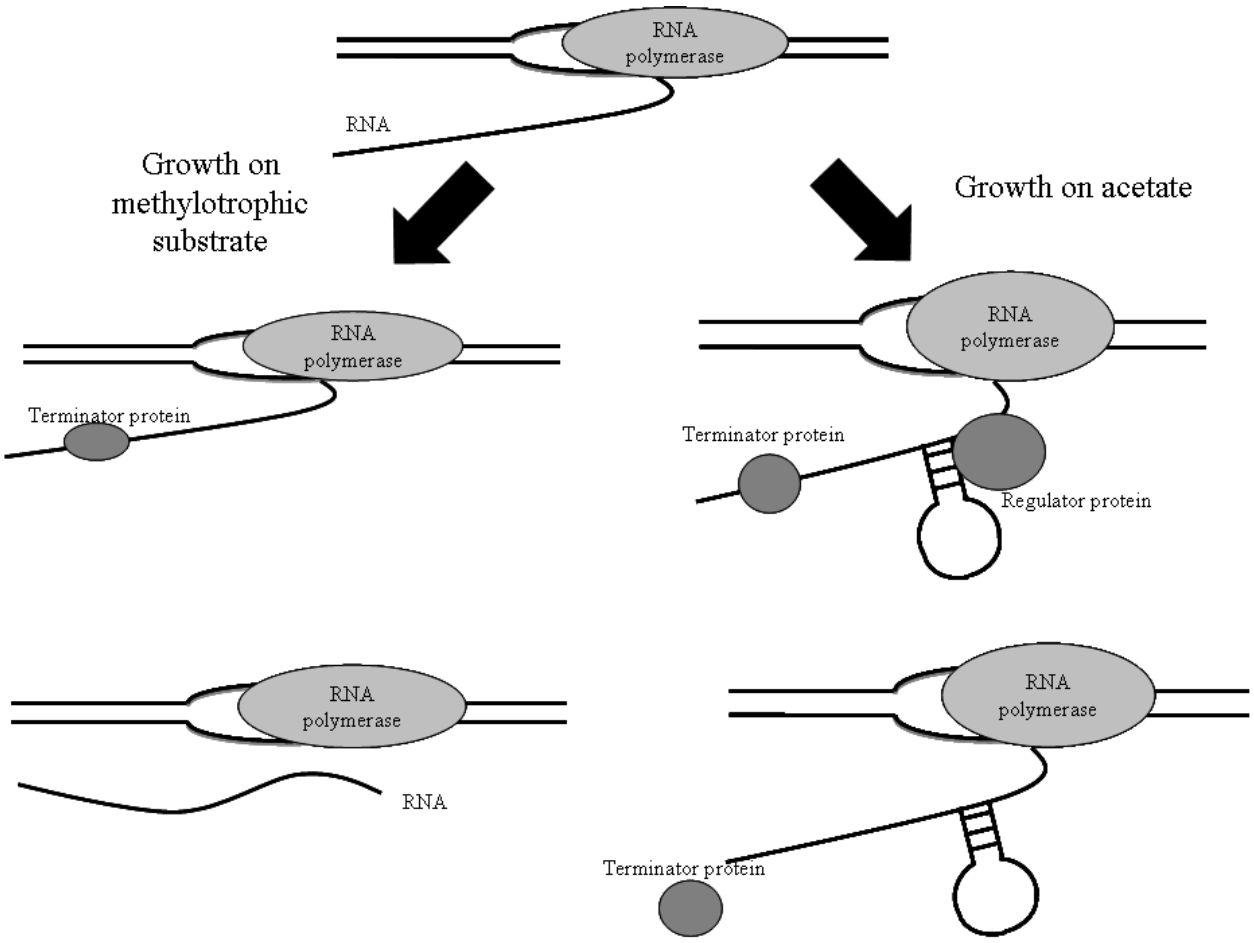


Figure 2. Proposed model for the regulation of *cdhABCDE*. The steps on the left illustrate what occurs during methylotrophic growth. Under these conditions, a terminator protein binds to the newly synthesized RNA, interfering with the RNA polymerase and terminating transcription. The steps on the right illustrate what occurs during aceticlastic growth. Under this condition, the terminator protein still bind, but secondary structures which form within the RNA, due to a regulator protein, prevent termination of transcription.