SUPPLEMENTAL INFORMATION

Structure and Mutational Analysis of the Archaeal GTP:AdoCbi-P Guanylyltransferase (CobY) from *Methanocaldococcus jannaschii*: Insights into GTP Binding and Dimerization.

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Running title: Structure and mutational analysis of an archaeal GTP:AdoCbi-P guanylyltransferase

Table S1. List of strains and plasmids used in these studies.

Strain or plasmid	Genotype	Reference or source[2]
***Salmonella enterica*[1]**		
TR6583; formerly SA2929	*metE205 ara-9*	K. Sanderson *via* J. Roth
Derivatives of TR6583		
JE8268	Δ*cob1315* Δ*ycfN112*[3]	(*1*)
Derivatives of JE8268		
JE8269	/ pT7-7	(2)
JE8335	/ pCOBY14	(*2*, *3*)
JE9293	/ pCOBY42	
JE9294	/ pCOBY43	
JE9299	/ pCOBY44	
JE9305	/ pCOBY45	
JE9306	/ pCOBY39	
JE10882	/ pCOBY48	
JE10883	/ pCOBY49	
JE10884	/ pCOBY50	
JE10885	/ pCOBY51	
JE10886	/ pCOBY52	
JE10887	/ pCOBY53	
JE12119	/ pCOBY57	
JE12120	/ pCOBY58	
JE12121	/ pCOBY59	
JE12503	/ pCOBY56	
JE12504	/ pCOBY60	
Escherichia coli		
BL21-CodonPlus® (DE3)-RIL	B F$^-$ *ompT hsdS* (r_B^-, m_B^-) *dcm*$^+$ *tet gal* λ(DE3) *endA* Hte (*argU ileY leuW cat*)	Stratagene
Plasmids		
pT7-7	*bla*$^+$; cloning vector	Derivative of pT7-1 (*4*)
Derivatives of pT7-7		
pCOBY14	*M. jannaschii cobY*$^+$ in pT7-7 *bla*$^+$	(*5*)
pCOBY39	*M. jannaschii cobY1*, encodes CobY^{G153D}	

Derivatives of pCOBY14		
pCOBY42	*M. jannaschii cobY2,* encodes CobY^{G8D}	
pCOBY43	*M. jannaschii cobY3*, encodes CobY^{P20A}	
pCOBY44	*M. jannaschii cobY4*, encodes CobY^{N177R}	
pCOBY45	*M. jannaschii cobY5*, encodes CobY^{P94A}	
pCOBY48	*M. jannaschii cobY6*, encodes CobY^{P54A}	
pCOBY49	*M. jannaschii cobY7*, encodes CobY^{D101G}	
pCOBY50	*M. jannaschii cobY8*, encodes CobY^{E18D}	
pCOBY51	*M. jannaschii cobY9*, encodes CobY^{T53S}	
pCOBY52	*M. jannaschii cobY10*, encodes CobY^{Y80F}	
pCOBY53	*M. jannaschii cobY11*, encodes CobY^{D83G}	
pCOBY56	*M. jannaschii cobY12*, encodes CobY^{S50A}	
pCOBY57	*M. jannaschii cobY13*, encodes CobY^{T56A}	
pCOBY58	*M. jannaschii cobY14*, encodes CobY^{S100A}	
pCOBY59	*M. jannaschii cobY1395,* encodes CobY^{T180A}	
pCOBY60	*M. jannaschii cobY15*, encodes CobY^{N179D}	
pCOBY61	*M. jannaschii cobY16,* encodes CobY$^{L38K\ K41D}$	
pCOBY62	*M. jannaschii cobY17,* encodes CobY$^{L38K\ L41D}$	
pCOBY63	*M. jannaschii cobY18,* encodes CobY$^{I144D\ F146D}$	

[1] All strains are derivatives of *S. enterica* serovar Typhimurium strain LT2 and carry a null allele of the *metE* gene, which encodes the Cbl-independent methionine synthase (MetE) enzyme. In the absence of Cbl, *metE* mutants are methionine auxotrophs. When Cbl is available, *metE* mutants use the Cbl-dependent methionine synthase (MetH) enzyme to methylate homocysteine to methionine (*6*).

[2] Strains were constructed for this study unless otherwise indicated.

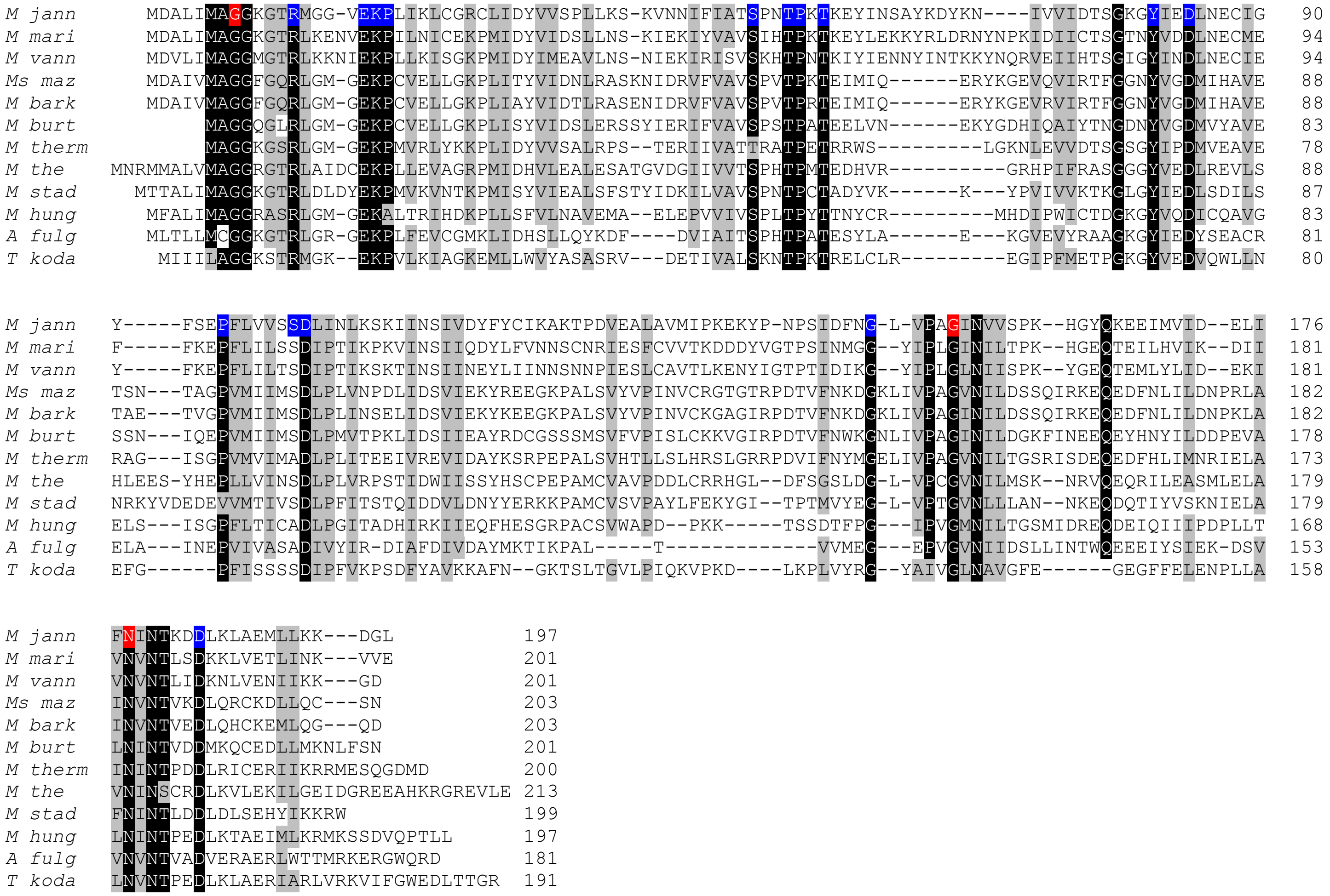
[3] In the text, this strain is referred to as Δ*cobU* Δ*ycfN*.

Table S2. List of primers used for site-directed mutagenesis.

Plasmid	Amino Acid Change	Forward Primer Sequence	Reverse Primer Sequence
pCOBY42	G8D	5’- GGA TGC TCT AAT TAT GGC T**GA** **T**GG TAA AGG AAC AAG AAT GGG TGG AG -3’	5’- CTC CAC CCA TTC TTG TTC CTT TAC C**AT** **C**AG CCA TAA TTA GAG CAT CC -3’
pCOBY50	E18D	5’- CAA GAA TGG GTG GAG TT**G** **AT**A AGC CGT TGA TTA AGC -3’	5’- GCT TAA TCA ACG GCT T**AT** **C**AA CTC CAC CCA TTC TTG -3’
pCOBY43	P20A	5’- GGG TGG AGT TGA AAA G**GC** **G**TT GAT TAA GCT TTG TGG -3’	5’- CCA CAA AGC TTA ATC AA**C** **GC**C TTT TCA ACT CCA CCC -3’
pCOBY56	S50A	5’- GTA AAT AAT ATA TTT ATC GCT ACC **GCT** CCA AAT ACA CCA AAG -3'	5’- CTT TGG TGT ATT TGG **AGC** GGT AGC GAT AAA TAT ATT ATT TAC -3'
pCOBY51	T53S	5’- CGC TAC CTC TCC AAA T**TC** **A**CC AAA GAC AAA GG -3’	5’- CCT TTG TCT TTG G**TG** **A**AT TTG GAG AGG TAG CG -3’
pCOBY48	P54A	5’- CGC TAC CTC TCC AAA TAC A**GC** **G**AA GAC AAA GGA ATA TAT AAA TTC AGC -3’	5’- GCT GAA TTT ATA TAT TCC TTT GTC TT**C** **GC**T GTA TTT GGA GAG GTA GCG -3’
pCOBY57	T56A	5’- CCA AAT ACA CCA AAG **GCA** AAG GAA TAT ATA AAT TCA GC -3'	5’- GCT GAA TTT ATA TAT TCC TT**T** **GC**C TTT GGT GTA TTT GG -3'
pCOBY52	Y80F	5’- GTT ATA GAC ACA TCT GGT AAA GGT **TTT** ATA GAA GAT TTA AAT GAA TGT ATT GG -3’	5’- CCA ATA CAT TCA TTT AAA TCT TCT AT**A** **AA**A CCT TTA CCA GAT GTG TCT ATA AC -3’
pCOBY53	D83G	5’- CTG GTA AAG GTT ATA TAG AA**G** **GC**T TAA ATG AAT GTA TTG G -3’	5’- CCA ATA CAT TCA TTT AA**G** **CC**T TCT ATA TAA CCT TTA CCA G -3’

pCOBY45	P94A	5'- GTA TTG GAT ATT TCT CAG AAG CGT TCT TAG TTG TTA GCT CTG AC -3'	5'- GTC AGA GCT AAC AAC TAA GAA CGC TTC TGA GAA ATA TCC AAT AC -3'
pCOBY58	S100A	5'- CCA TTC TTA GTT GTT AGC **GCT** GAC TTA ATT AAC TTA AAA TCG -3'	5'- CGA TTT TAA GTT AAT TAA GTC **AGC** GCT AAC AAC TAA GAA TGG -3'
pCOBY49	D101G	5'- CCA TTC TTA GTT GTT AGC TCT **GGC** TTA ATT AAC TTA AAA TCG -3'	5'- CGA TTT TAA GTT AAT TAA **GCC** AGA GCT AAC AAC TAA GAA TGG -3'
pCOBY39	G153D	5'- CGG CTT AGT TCC TGC A**GA C**AT AAA TGT TGT ATC CCC -3'	5'- GGG GAT ACA ACA TTT AT**G TC**T GCA GGA ACT AAG CCG -3'
pCOBY44	N177R	5'- GGT TAT TGA TGA GCT GAT ATT C**CG C**AT AAA TAC TAA AGA TG -3'	5'- CAT CTT TAG TAT TTA T**GC G**GA ATA TCA GCT CAT CAA TAA CC -3'
pCOBY60	N179D	5'- GAG CTG ATA TTC AAT ATA **GAT** ACT AAA GAT GAT TTA AAA CTT GC -3'	5'- GCA AGT TTT AAA TCA TCT TTA GT**A** **TC**T ATA TTG AAT ATC AGC TC -3'
pCOBY59	T180A	5'- GAT GAG CTG ATA TTC AAT ATA AAT **GCT** AAA GAT GAT TTA AAA CTT GCT G -3'	5'- CAG CAA GTT TTA AAT CAT CTT T**AG** **C**AT TTA TAT TGA ATA TCA GCT CAT C- 3'
pCOBY61	L38K K41D	5'- CTT ATA GAT TAT GTT GTC TCT CCC TTA **AAG** AAA TCA **GAT** GTA AAT AAT ATA TTT ATC GCT ACC TCT CC -3'	None
pCOBY62	L38D L41D	5'- CTT ATA GAT TAT GTT GTC TCT CCC TTA **GAT** AAA TCA **GAT** GTA AAT AAT ATA TTT ATC GCT ACC TCT CC -3'	None
pCOBY63	I144D F146D Y163R	5'- CA AAA GAA AAA TAT CCA AAC CCC TCA **GAC** GAT **GAC** AAC GGC TTA GTT CCT GCA GGG -3' 5'- GTT GTA TCC CCA AAG CAT GGA **CGT** CAA AAA GAA GAA ATT ATG GTT ATT GAT G -3'	None

Bold type indicates the codon changed. Plasmids were constructed using method A, with the exception of plasmids pCOBY61-63, which were constructed using method B; both methods are described under Experimental Procedures.



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M jann           MDALIMAGGKGTRMGG-VEKPLIKLCGRCLIDYVVSPLLKS-KVNNIFIATSPNTPKTKEYINSAYKDYKN----IVVIDTSGKGYIEDLNECIG   90
M mari           MDALIMAGGKGTRLKENVEKPILNICEKPMIDYVIDSLLNS-KIEKIYVAVSIHTPKTKEYLEKKYRLDRNYNPKIDIICTSGTNYVDDLNECME   94
M vann           MDVLIMAGGMGTRLKKNIEKPLLKISGKPMIDYIMEAVLNS-NIEKIRISVSKHTPNTKIYIENNYINTKKYNQRVEIIHTSGIGYINDLNECIE   94
Ms maz           MDAIVMAGGFGQRLGM-GEKPCVELLGKPLITYVIDNLRASKNIDRVFVAVSPVTPKTEIMIQ------ERYKGEVQVIRTFGGNYVGDMIHAVE   88
M bark           MDAIVMAGGFGQRLGM-GEKPCVELLGKPLIAYVIDTLRASENIDRVFVAVSPVTPRTEIMIQ------ERYKGEVRVIRTFGGNYVGDMIHAVE   88
M burt                MAGGQGLRLGM-GEKPCVELLGKPLISYVIDSLERSSYIERIFVAVSPSTPATEELVN------EKYGDHIQAIYTNGDNYVGDMVYAVE   83
M therm               MAGGKGSRLGM-GEKPMVRLYKKPLIDYVVSALRPS--TERIIVATTRATPETRRWS---------LGKNLEVVDTSGSGYIPDMVEAVE   78
M the         MNRMMALVMAGGRGTRLAIDCEKPLLEVAGRPMIDHVLEALESATGVDGIIVVTSPHTPMTEDHVR----------GRHPIFRASGGGYVEDLREVLS   88
M stad          MTTALIMAGGKGTRLDLDYEKPMVKVNTKPMISYVIEALSFSTYIDKILVAVSPNTPCTADYVK------K---YPVIVVKTKGLGYIEDLSDILS   87
M hung           MFALIMAGGRASRLGM-GEKALTRIHDKPLLSFVLNAVEMA--ELEPVVIVSPLTPYTTNYCR---------MHDIPWICTDGKGYVQDICQAVG   83
A fulg           MLTLLMCGGKGTRLGR-GEKPLFEVCGMKLIDHSLLQYKDF----DVIAITSPHTPATESYLA------E---KGVEVYRAAGKGYIEDYSEACR   81
T koda            MIIILAGGKSTRMGK--EKPVLKIAGKEMLLWVYASASRV---DETIVALSKNTPKTRELCLR---------EGIPFMETPGKGYVEDVQWLLN   80

M jann        Y-----FSEPFLVVSSDLINLKSKIINSIVDYFYCIKAKTPDVEALAVMIPKEKYP-NPSIDFNG-L-VPAGINVVSPK--HGYQKEEIMVID--ELI  176
M mari        F-----FKEPFLILSSDIPTIKPKVINSIIQDYLFVNNSCNRIESFCVVTKDDDYVGTPSINMGG--YIPLGINILTPK--HGEQTEILHVIK--DII  181
M vann        Y-----FKEPFLILTSDIPTIKSKTINSIINEYLIINNSNNPIESLCAVTLKENYIGTPTIDIKG--YIPLGLNIISPK--YGEQTEMLYLID--EKI  181
Ms maz        TSN---TAGPVMIIMSDLPLVNPDLIDSVIEKYREEGKPALSVYVPINVCRGTGTRPDTVFNKDGKLIVPAGVNILDSSQIRKEQEDFNLILDNPRLA  182
M bark        TAE---TVGPVMIIMSDLPLINSELIDSVIEKYKEEGKPALSVYVPINVCKGAGIRPDTVFNKDGKLIVPAGINILDSSQIRKEQEDFNLILDNPKLA  182
M burt        SSN---IQEPVMIIMSDLPMVTPKLIDSIIEAYRDCGSSSMSVFVPISLCKKVGIRPDTVFNWKGNLIVPAGINILDGKFINEEQEYHNYILDDPEVA  178
M therm       RAG---ISGPVMVIMADLPLITEEIVREVIDAYKSRPEPALSVHTLLSLHRSLGRRPDVIFNYMGELIVPAGVNILTGSRISDEQEDFHLIMNRIELA  173
M the         HLEES-YHEPLLVINSDLPLVRPSTIDWIISSYHSCPEPAMCVAVPDDLCRRHGL--DFSGSLDG-L-VPCGVNILMSK--NRVQEQRILEASMLELA  179
M stad        NRKYVDEDEVVMTIVSDLPFITSTQIDDVLDNYYERKKPAMCVSVPAYLFEKYGI--TPTMVYEG-L-VPTGVNLLLAN--NKEQDQTIYVSKNIELA  179
M hung        ELS---ISGPFLTICADLPGITADHIRKIIEQFHESGRPACSVWAPD--PKK-----TSSDTFPG---IPVGMNILTGSMIDREQDEIQIIIPDPLLT  168
A fulg        ELA---INEPVIVASADIVYIR-DIAFDIVDAYMKTIKPAL-----T-------------VVMEG---EPVGVNIIDSLLINTWQEEEIYSIEK-DSV  153
T koda        EFG------PFISSSSDIPFVKPSDFYAVKKAFN--GKTSLTGVLPIQKVPKD----LKPLVYRG--YAIVGLNAVGFE------GEGFFELENPLLA  158

M jann        FNINTKDDLKLAEMLLKK---DGL            197
M mari        VNVNTLSDKKLVETLINK---VVE            201
M vann        VNVNTLIDKNLVENIIKK---GD             201
Ms maz        INVNTVKDLQRCKDLLQC---SN             203
M bark        INVNTVEDLQHCKEMLQG---QD             203
M burt        LNINTVDDMKQCEDLLMKNLFSN             201
M therm       ININTPDDLRICERIIKRRMESQGDMD         200
M the         VNINSCRDLKVLEKILGEIDGREEAHKRGREVLE  213
M stad        FNINTLDDLDLSEHYIKKRW                199
M hung        LNINTPEDLKTAEIMLKRMKSSDVQPTLL       197
A fulg        VNVNTVADVERAERLWTTMRKERGWQRD        181
T koda        LNVNTPEDLKLAERIARLVRKVIFGWEDLTTGR   191
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Figure S1. Alignment of putative CobY proteins. Conserved residues are outlined in black; conservative changes are shown in gray. *M jann*, *Methanocaldococcus jannaschii* DSM 2661; *M mari*, *Methanococcus maripaludis* S2; *M vann*, *Methanococcus vannielii* SB; *Ms maz*, *Methanosarcina mazei* Gö1; *M bark*, *Methanosarcina barkeri* str. *fusaro*; *M burt*, *Methanococcoides burtonii* DSM 6242; *M therm*, *Methanothermobacter thermautotrophicus* str. ΔH; *M the*, *Methanosaeta thermophila* PT; *M stad, Methanosphaera stadtmanae* DSM 3091; *M hung, Methanospirillum hungatei* JF-1; *A fulg*, *Archaeoglobus fulgidus* DSM 4304; *T koda*, *Thermococcus kodakarensis* KOD1. Site-directed substitutions were made at the conserved residues highlighted in blue; conserved residues highlighted in red inactivated the enzyme.

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