

Supplementary Materials: The *Escherichia coli* COG1738 Member YhhQ Is Involved in 7-Cyanodeazaguanine (preQ₀) Transport

Rémi Zallot, Yifeng Yuan and Valérie de Crécy-Lagard

Z mobilis	1	--MEMMTAVKGRNVVEATAQETDRPRFSEI	A FREGKRTGTATMKGKVLTTPAMPVGTATVKALEK
E coli	1	-----M A F E I	DITDGRAFRGTFEDRCVWETPFCMPVGTATVKGM
H sapiens	1	-----MA G -AATQASLESAPRIMSLVA	ECSRARA RARACNLPHCTVATVMPVGTATMKGT
C elegans	1	-----M R Y D V	L ERCAFARRNLSLPHSIVETPVEMPVGTATMKGV
C diphtheriae	1	-----M D T D P E I R T E L D	D P G H E G R T C V W T P H C D I N T P A P F T P V A T Z A T V K E T
A coleocanis	1	-----M G -VTDNLDPERIDAQRCPE TARVDEVPVIETPYDKPLERTCGLTTAHCQIETPAPF PVGTAAVKGVL	
B breve	1	MTSDVLNHFIC-AQACKP-GDRSAFIFETITRLPSAANGLGRGARYERTGTTAHTPHGCIATTPAPF PVGTAAVKGVL	
consensus	1	g kfei	a kaRtG 1 phg i TPafmPVgt atvKgl
Z mobilis	67	DEVRATCILLLNTVHLJARPCBERHAKLGGHLHSVYCAADPPLLTDSGGCVQMSLSSH	
E coli	43	PEEVATGAQIILANTVHLJLRFGEIENKLIGLHLFEMWKPLLTDSGGCVQFSLGDI	
H sapiens	59	TEQDLGCRICLQLNTVHLJLRFGEIENKLIGLHLFEMWKPLLTDSGGCVQFSLGDI	
C elegans	43	PEQWLSMDCRILQLNTVHLJLRFGEIENKLIGLHLFEMWKPLLTDSGGCVQFSLGDI	
C diphtheriae	51	PQCDRTGQAILENNTVHLJLRFGEIENKLIGLHLFEMWKPLLTDSGGCVQFSLGDI	--DIR
A coleocanis	70	PEMDIGQACIILNNTVHLJLRFGEIENKLIGLHLFEMWKPLLTDSGGCVQFSLGDI	AEQEK
B breve	77	PEQKELGAQOCLLNAHHLJLRFGEIENKLIGLHLFEMWKPLLTDSGGCVQFSLGDI	
consensus	81	peqv lgaqiiLgNtyHlwlrPG eii agglhkfmnW rpiltTDSGGCVQfsLg 1	
Z mobilis	126	TKQSEEVGTVFKSHIDCSRHMLSPERSBIOHLLGSDIVMAFDCTPTYPATPSRAASSMERSMRWAIRGRDRED	
E coli	102	--RKREBQGVHFRNPINCDPFLDPEKSMQIQLGSDIVMFEDCTPTYPADWDIAKRMBSLIRWAIRESRERFD	
H sapiens	118	-SEVIEEGVFRSEYDGNETLSPKSVIQNALGSDIVMOIDDWSVSTVTCPRVEEAMRSIRWLIRCLIAAHQ	
C elegans	102	--MTVDENGVNEPESHTEEMMAPEKSMQIQLGSDIVMOIDDVHIVII TCDIVK3AHRSIRWLIRCKVAHT	
C diphtheriae	128	APKDRYAAVDEBGVDPFSTIDGSNHRSPDEPVSKCIOIBPLGADINFADEDTLILVUTROYQESSTERHWRARCILHD	
A coleocanis	150	EAVKRSRKSPDEBGVVRSPHHDCDTHRFNPPIISKIQHBLGADINFADEDTLILVUTROYQESSTERHWRARCILHD	
B breve	154	AKGKERMMAWDEDGVTPFSTIDCAHRSPEBISNGCIOIBPLGADINFADEDTLILVUTROYQESSTERHWRARCILHD	
consensus	161	vdBeGV Frspldg h lspErSi Ioh 1gaDivmafDevtsvl t y esmersmRWakRcidan	
Z mobilis	199	SAK---QAEAAALFGCQGSWEENRQOSRDNAAE-----CFDGVVCCIAAGCQDEBMRPVIDBSVPMLEDE	
E coli	175	SLG----NKAFLGIIQGSVIEDLRDISVKEIWDI-----CFDGVAVVGLAVGSEPKADMRIIHEHCPQIAD	
H sapiens	191	----RPDKONLFAIIQGSVADLRATCLEEMTK-----DVGCFALCGESKSCOFMRVALTSRILKED	
C elegans	175	----RD-DOAEMPLIQQGNIILSRKECAKEAK-----PKVGHACGCCGSEKOHFWVVAACOAALPH	
C diphtheriae	208	RLFTREREQKPLQBLIGCVUQCAQEDLRLQAVRGLLIDDEKEADNDGRGPFCGEIIGCA--IEKENLCTIIVGNCDEBILQH	
A coleocanis	230	KLTAEQPERPYDQCLGVLQCAOBELRERTRIIRMEFE-----GCFDGYGLGGA--IEKENLCTIIVSNLITSELBS	
B breve	234	RLFATRIGKPLQALYGVVQGAN-EDLRLRRAASOASL-----DFDGVIIGCA--IEKKRILGDTCAWICDAMES	
consensus	241	I qalfgiiQGgvfedLrk ak 1 ei gfdGyaiggl geekd m rvvafvc 1P d	
Z mobilis	266	KPRYLMGVCPDDLVGVERGCLDMFDCVLPTRSCRNQAFATNDGP-INIRNARFSEDLKMPIDSECHCIVQKMSRAYHH	
E coli	239	KPRYLMGVCPDDLVGVERGCLDMFDCVMPTRNARNGHLEVTDGV-VIIRNAKYSLDTPDPLDPCDCTCRNMSRAYHH	
H sapiens	254	KPRYLMGVCPDDLVGVALGGDMDFDCVFPTRIAAREGSALVPTGN-LOLEKKVFERDGPIDECTCTCQKRSRAELHA	
C elegans	237	IPRYLMGVCPDDLVICSFGLDMFDCVCPTRIAAREGSALVPTGN-TAMVRGGLMOLNOKRMKDEDPIDKKCENCTKNNTRAYHS	
C diphtheriae	285	KPRYLMGVCPDDLVITENGCADDFDCVCPTRIQRGCVVTIDGR-VNLIAAREKEDBQVDEBFGC-PIAEYSRAYHH	
A coleocanis	302	KPRYLMGVCPDDFFRAIEGATDFDCVCPTRIQRGCVVTIDGR-VNLIAAREKEDBQVDEBFGC-PIAEYSRAYHH	
B breve	301	IPRYLMGVIASDDFAQEVNGGDFDCVCPTRIQRGCVVTIDGR-YNIRRAHRSDFCPLAACCDCTCQHYSRAYDH	
consensus	321	kPrylmGv pdDiv ave GaDmFDCV PtR aRng lft dG inirnarfk df pld ec c tc kysrAyih	
Z mobilis	345	LIFACBILGAMWPHHNIABYCQLIOKERDSITSECRPSOFAODERARVEPRNS-----	
E coli	318	LD-CANLILGARLNATHNHRYQYRLAGRRAIPEEGLKLSFVTDFTYORQGREVPFLNVD-----	
H sapiens	333	LLSDNTAAHLHLITVHNIAQOCLMSA/RTSIVEKRBEDFVRFYCAYEDP-----	TLCPTWATDALASVG
C elegans	317	IIVGKE-TIVGCHLVSVHNHKQHLLLRD/RCALQSNSVSBGFLKPFYDLYPPIOSENPSKQDSEKMRREVPOQWRDAVIDHMG	
C diphtheriae	363	LFKAAPBPLGGCQCLGMWVIAWMQCLIDNRSAINNQDPEAIRDPEFLGCVYI-SKCGPAAGRSGGMYIGV-----	
A coleocanis	381	LFATKQILISLIAHTHNEHSTWLLDTIREBAKNREVLACKEEVLOREYSEKKC-----	
B breve	380	LLAARAFGCFMLIATHHNEHSTWLLDTIREBAKNREVLACKEEVLOREYSEKKC-----	
consensus	401	Iir e lg I tiHHn f l Lm ir al g fe f dflaryya	
Z mobilis	-----		
E coli	-----		
H sapiens	400 ITLG-		
C elegans	396 YKLDF		
C diphtheriae	-----		
A coleocanis	-----		
B breve	-----		
consensus	481		

Figure S1. Sequence alignment of tRNA guanine⁽³⁴⁾ transglycosylase (TGT) proteins. Bacteria that do not have queuosine biosynthesis capability, but have TGT, and not QueA (tRNA preQ₁₍₃₄₎ S-adenosylmethionine ribosyltransferase-isomerase (EC 2.4.99.17)), QueG or QueH (tRNA epoxyqueuosine⁽³⁴⁾ reductase (EC 1.17.99.6)) (with species names in red), have non-canonical substrate binding residues in their TGT sequence. For reference, the substrate binding pockets of the different TGT families that have been well characterized are presented in red boxes [32]. Alignment realized with Clustal Omega [50]. Abbreviations: Z mobilis: *Zymomonas mobilis*; E coli: *Escherichia coli*; H sapiens: *Homo sapiens*; C elegans: *Caenorhabditis elegans*; C diphtheriae: *Corynebacterium diphtheriae*; A coleocanis: *Actinomyces coleocanis*; B breve: *Bifidobacterium breve*.

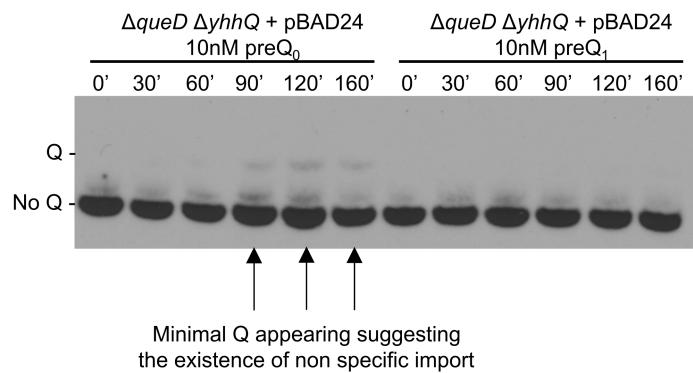


Figure 3. Non-specific transport of 7-cyano-7-deazaguanine (preQ₀). Detection of Queuosine in tRNA^{AspGUC} as a representative of the salvage of the Q precursors preQ₀ and 7-aminomethyl-7-deazaguanine (preQ₁). *E. coli* bulk tRNA was separated on an 8 M urea, 8% polyacrylamide gel containing 0.5% 3-(acrylamido)phenylboronic acid and transferred to a nylon membrane. The transferred tRNA was probed with a biotinylated primer, and detected by Northern blot. At long incubation times, Q is detected in tRNAs extracted from the $\Delta queD \Delta yhhQ$ strain (carrying pBAD24), when fed with 10 nM preQ₀, suggesting the presence of low-affinity transporters.