Supporting Information

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SI Materials and Methods

The plasmid pBAD18-*lacZ*481 (1) was used to create *toxT* 5′ UTR translational fusions. The *toxT* 5′ UTR region from +1 to +71 with respect to the transcription start site (2) was PCR amplified from *Vibrio cholerae* genomic DNA using primers NheI-*toxT* and *toxT*-EcoRI (Table S1), digested with NheI and EcoRI and ligated into pBAD18-*lacZ*481 digested similarly. This created plasmid pBO1296, which transcribes *lacZ* from the pBAD promoter that is translated from the native *toxT* 5′ UTR. *toxT* 5′ UTR mutations U5C and/or U7C were introduced by site-directed mutagenesis (QuikChange, Stratagene; Table S1 provides a complete list of primers) into pBO1296, resulting in pBO1826 (*toxT* 5′ UTR U5C), pBO1829 (*toxT* 5′ UTR U7C), and pKEK1807 (*toxT* 5′ UTR U5C, U7C).

toxT translated from its native 5′ UTR was PCR amplified with oligos WT 5′ UTR Fw NheI and ToxT Rev SphI, then digested with NheI and SphI, and finally ligated into pBAD18-lacZ481 that had been digested similarly to create plasmid pKEK1682, which transcribes *toxT* with its native UTR from the pBAD promoter. *toxT* 5′ UTR mutations U5C and/or U7C were introduced into pKEK1682 by site-directed mutagenesis.

pKEK87 contains *toxT* transcribed from the pBAD promoter and translated from the *araBAD* 5′ UTR (3). *toxT-FLAG* translated from the *toxT* 5′ UTR was created by PCR amplification of pKEK1682, pKEK1683, pKEK1684, and pKEK1685 with oligos ToxT-FLAG Fw and ToxT-FLAG Rev, followed by digestion with DpnI and transformation into DH5α to create pKEK1733 (native 5′ UTR-*toxT*-FLAG), pKEK1734 (U5C UTR *toxT*-FLAG), pKEK1735 (U7C UTR *toxT*-FLAG), and pKEK1736 (U5C, U7C UTR *toxT*-FLAG). The U5C and/or U7C mutations were introduced by site-directed mutagenesis (Stratagene QuikChange) into plasmid pKEK1445 [*toxT* with 1-kb flanking regions in pKAS46 (4)] to create plasmids pKEK1468, pKEK1469, and pKEK1808.

The plasmids used in RNA structure probing experiments were created by PCR amplification of the *toxT* 5′ UTR with primers T7-*toxT* and *toxT*-runoff-SP, and ligation into pUC18 to create plasmid pBO1299. This introduced a T7 promoter sequence upstream and an MlsI site downstream of the *toxT* 5′ UTR. The U5C, U7C mutations were introduced into pBO1299 by site-directed mutagenesis resulting in plasmid pBO1845.

1. Waldminghaus T, Fippinger A, Alfsmann J, Narberhaus F (2005) RNA thermometers are common in alpha- and gamma-proteobacteria. *Biol Chem* 386(12):1279–1286.
2. Yu RR, DiRita VJ (1999) Analysis of an autoregulatory loop controlling ToxT, cholera toxin, and toxin-coregulated pilus production in *Vibrio cholerae*. *J Bacteriol* 181(8): 2584–2592.
3. Klose KE, Mekalanos JJ (1998) Distinct roles of an alternative sigma factor during both free-swimming and colonizing phases of the *Vibrio cholerae* pathogenic cycle. *Mol Microbiol* 28(3):501–520.
4. Skorupski K, Taylor RK (1996) Positive selection vectors for allelic exchange. *Gene* 169(1): 47–52.

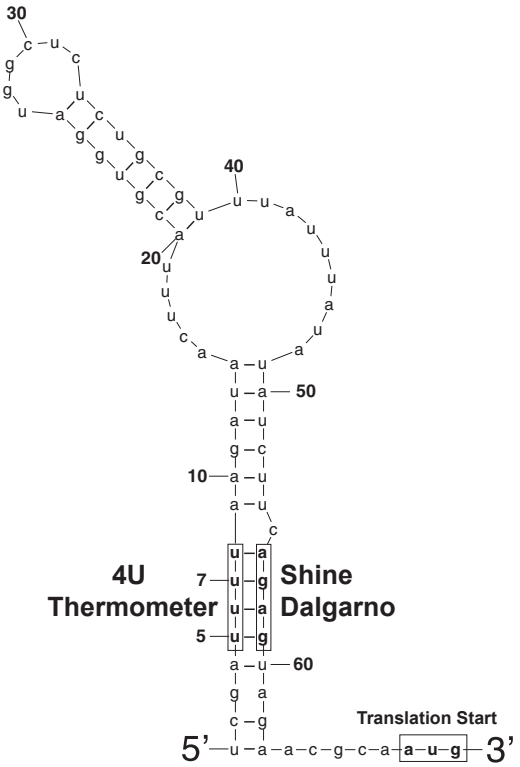

Fig. S1. The predicted structure of the *toxT* 5′ UTR. Secondary structure derived from Mfold (1), putative fourU thermometer, Shine-Dalgarno (SD) sequence, start codon, and the U5 and U7 residues are indicated.

1. Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res* 31(13):3406–3415.

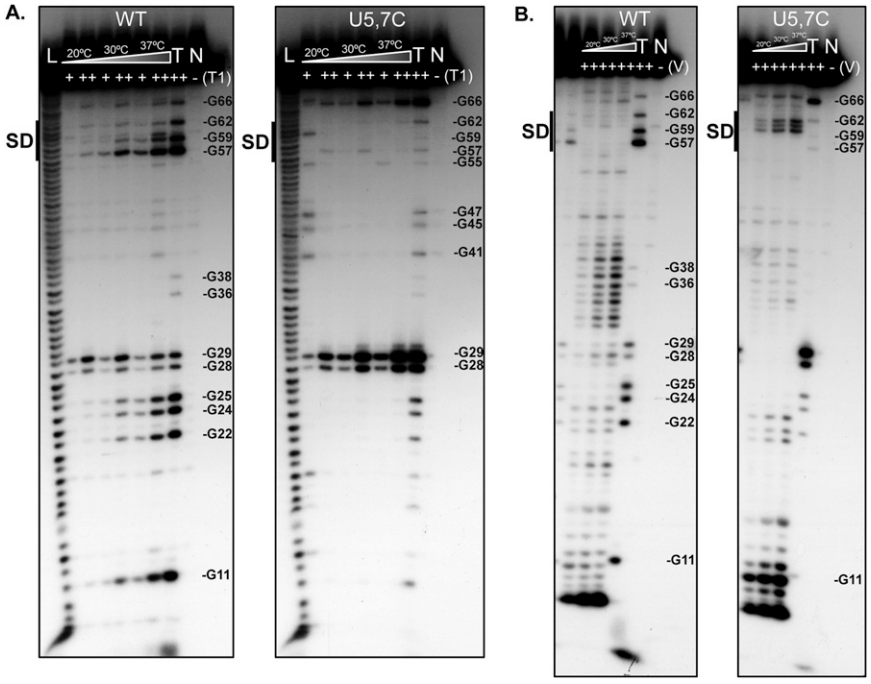

Fig. S2. The *toxT* 5′ UTR fourU RNA thermometer facilitates temperature-dependent access to the SD sequence. (*A*) Enzymatic cleavage of 5′ ^{32}P-end-labeled *toxT* RNA was carried out at 20 °C, 30 °C, and 37 °C. RNA fragments were separated on 15% polyacrylamide. (*A*) RNase T1 (cuts 3′ of single-stranded guanines) was used at the indicated concentrations [0.01 U (+) and 0.1 U (++)]. (*B*) RNase V1 (cuts double-stranded, stacked regions) was used at 0.1 U. Lane L is the alkaline ladder, lane N contains no RNase, and RNA was cleaved with 0.1 U T1 at 42 °C in lane T. WT indicates the native *toxT* 5′ UTR, and U5, 7C indicates the U5C U7C *toxT* 5′ UTR. The SD sequence is labeled, and select nucleotides are noted. These are the full gel images that correspond to Fig. 2.

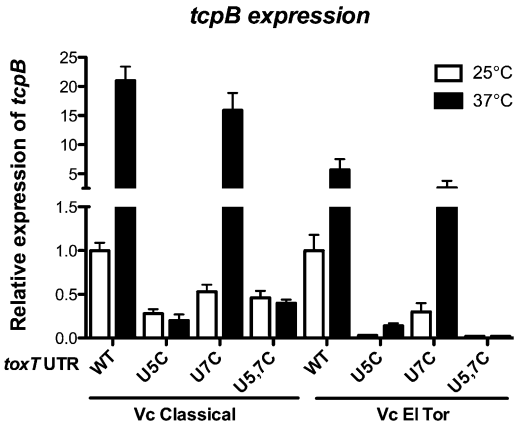

Fig. S3. The *toxT* 5′ UTR fourU RNA thermometer facilitates temperature-dependent expression of *tcpB*. *V. cholerae* O1 Δ*toxT* strains KKV2288 (Classical) and KKV2426 (El Tor) carrying plasmids pKEK1682 (5′-*toxT* UTR-*toxT*), pKEK1683 (5′-U5C *toxT* UTR-*toxT*), pKEK1685 (5′-U7C *toxT* UTR- *toxT*), or pKEK1686 (5′-U5C U7C *toxT* UTR- *toxT*) were grown at 25 °C and 37 °C in the presence of 0.1% arabinose and harvested at identical cell densities (OD_{600} = 0.6). mRNA abundance for *tcpB* was determined by quantitative RT-PCR (*Materials and Methods*). These results are from the same strains shown in Fig. 4. *tcpB* transcription was higher at 37° than at 25° in strains expressing the native UTR-*toxT*, ($P < 0.001$ classical and $P < 0.01$ El Tor). The U5C and U5C U7C UTRs caused significant decreases in *ctx* transcription ($P < 0.001$ at 37 °C for both classical and El Tor). The U7C UTR had little effect on *ctx* transcription (no significant difference for classical or El Tor).

Table S1. Oligonucleotides used in this study

Name	Sequence
U5C pKAS Fw	GTTATGTAGTTTTATATTAACTTCGACTTTAAGATAACTTTACGTGGATGGCT
U5C pKAS Rev	AGCCATCCACGTAAAGTTATCTTAAAGTCGAAGTTAATATAAAACTACATAAC
U7C pKAS Fw	GTTATGTAGTTTTATATTAACTTCGATTCTAAGATAACTTTACGTGGATGGCTCTCT
U7C pKAS Rev	AGAGAGCCATCCACGTAAAGTTATCTTAGAATCGAAGTTAATATAAAACTACATAAC
U5,7C pKAS Fw	GTAGTTTTATATTAACTTCGACTCTAAGATAACTTTACGTGGATG
U5,7C pKAS Rev	CATCCACGTAAAGTTATCTTAGAGTCGAAGTTAATATAAAACTAC
WT5′UTRFw NheI	GCGCGCTAGCTCGATTTTAAGATAACTTTACGTGGATGGC
U5C Fw NheI	GCGCGCTAGCTCGACTTTAAGATAACTTTACGTGGATGGC
U7C Fw NheI	GCGCGCTAGCTCGATTCTAAGATAACTTTACGTGGATGGC
U5,7C Fw NheI	GCGCGCTAGCTCGACTCTAAGATAACTTTACGTGGATGGC
ToxT Rev SphI	CGCGGCATGCTTATTTTTCTGCAACTCCTGTCAAC
pBAD U5,7C Fw	CCGTTTTTTTGGGCTAGCTCGACTCTAAGATAACTTTACGTGGATGGC
pBAD U5,7C Rev	GCCATCCACGTAAAGTTATCTTAGAGTCGAGCTAGCCCAAAAAAACGG
ToxT-FLAG Fw	GATTACAAGGATGACGATGACAAGTAAAAGCTTGGCTGTTTTGGCGGATGAGAGAAGATT
ToxT-FLAG Rev	TTACTTGTCATCGTCATCCTTGTAATCTTTTTCTGCAACTCCTGTCAACATAAATAAATA
ToxT-FLAG KEK87 Fw	TTTATGTTGACAGGAGTTGCAGAAAAAGATTACAAGGATGACCATGACAAGTAATCTAGAGTCGACCTGCAGGCATGCAAG
ToxT-FLAG KEK87 Rev	CTTGCATGCCTGCAGGTCGACTCTAGATTACTTGTCATCGTCATCCTTGTAATCTTTTTCTGCAACTCCTGTCAACATAAA
ToxT Fw RT	GGCGTTGGGCAGATATTTGT
ToxT Rev RT	TCCTCGAGACTCTAGTTCTTTTTTCA
NheI-*toxT*	TTTGCTAGCTCGATTTTAAGATAAC
toxT-EcoRI	TTTGAATTCAGACATAATGTTAACTCC
toxT-U5C-fw	TCGACTTTAAGATAACTTTACGTGG
toxT-U5C-rv	CCACGTAAAGTTATCTTAAAGTCGA
toxT-U7C-fw	TCGATTCTAAGATAACTTTACGTGG
toxT-U7C-rv	CCACGTAAAGTTATCTTAGAATCGA
toxT-U5,7C-fw	TCGACTCTAAGATAACTTTACGTGG
toxT-U5,7C-rv	CCACGTAAAGTTATCTTAGAGTCGA
T7-*toxT*	GAAATTAATACGACTCACTATAGGGTCGATTTTAAGATAACTTTACG
toxT-runoff-SP	TGGCCATTGCGTTCTACTCTGAAG

Table S2. Strains and plasmids used

Strain/plasmid	Description	Reference
Strain		
Top10	*E. coli* strain	Invitrogen
DH5α	*E. coli* strain	(1)
SM10 λpir	*E. coli* mating strain	(2)
O395	*V. cholerae* O1 classical biotype	(3)
C6706	*V. cholerae* O1 El Tor biotype	(4)
KKV598	O395 Δ*lacZ*	(5)
KKV1678	C6706 Δ*lacZ*	This study
KKV2288	KKV598 Δ*toxT*::Cm	This study
KKV2333	O395 *toxT* 5′ UTR U5C	This study
KKV2368	O395 *toxT* 5′ UTR U7C	This study
KKV2372	KKV2426 with restored wildtype *toxT*	This study
KKV2289	KKV2288 with restored wildtype *toxT*	This study
KKV2382	C6706 *toxT* 5′ UTR U5C, U7C	This study
KKV2425	O395 *toxT* 5′ UTR U5C, U7C	This study
KKV2426	C6706 Δ*toxT*::Cm	This study
Plasmid		
pBAD18	Arabinose-inducible transcriptional fusion vector	(6)
pBAD24	Arabinose-inducible translational fusion vector plasmid	(6)
pBAD18-*lacZ* 481	LacZ translational fusion vector	(7)
pBO1296	pBAD18-*lacZ* 481 with native *toxT* 5′UTR	This study
pBO1299	pUC18 with native *toxT* 5′ UTR for structure probing	This study
pBO1826	pBAD18-*lacZ* 481 with *toxT* 5′ UTR U5C	This study
pBO1829	pBAD18-*lacZ* 481 with *toxT* 5′ UTR U7C	This study
pBO1845	pUC18 with *toxT* 5′ UTR U5C, U7C for structure probing	This study
pKAS46	conjugative suicide plasmid	(8)
pKEK87	pBAD24 with *toxT*	(9)
pKEK1445	pKAS46 with *toxT* + 1kb flanking regions	(10)
pKEK1468	pKEK1445 with *toxT* 5′ UTR U7C	This study
pKEK1469	pKEK1445 with *toxT* 5′ UTR U5C	This study
pKEK1808	pKEK1445 with *toxT* 5′ UTR U5C, U7C	This study
pKEK1682	pBAD18 with native *toxT* 5′ UTR-*toxT*	This study
pKEK1683	pBAD18 with *toxT* 5′ UTR U5C-*toxT*	This study
pKEK1684	pBAD18 with *toxT* 5′ UTR U7C-*toxT*	This study
pKEK1685	pBAD18 with *toxT* 5′ UTR U5C, U7C-*toxT*	This study
pKEK1733	pBAD18 with native *toxT* 5′ UTR-*toxT* -FLAG	This study
pKEK1734	pBAD18 with *toxT* 5′ UTR U5C-*toxT*-FLAG	This study
pKEK1735	pBAD18 with *toxT* 5′ UTR U7C-*toxT*-FLAG	This study
pKEK1736	pBAD18 with *toxT* 5′ UTR U5C, U7C-*toxT*-FLAG	This study
pKEK1789	pKEK87 with *toxT*-FLAG	This study
pKEK1807	pBAD18-*lacZ* 481 with *toxT* 5′ UTR U5C, U7C	This study
pKEK1808	pKEK1445 with *toxT* 5′ UTR U5,7C	This study

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3. Mekalanos JJ, Collier RJ, Romig WR (1979) Enzymic activity of cholera toxin. II. Relationships to proteolytic processing, disulfide bond reduction, and subunit composition. *J Biol Chem* 254(13):5855–5861.
4. Thelin KH, Taylor RK (1996) Toxin-coregulated pilus, but not mannose-sensitive hemagglutinin, is required for colonization by *Vibrio cholerae* O1 El Tor biotype and O139 strains. *Infect Immun* 64(7):2853–2856.
5. Correa NE, Lauriano CM, McGee R, Klose KE (2000) Phosphorylation of the flagellar regulatory protein FlrC is necessary for *Vibrio cholerae* motility and enhanced colonization. *Mol Microbiol* 35(4):743–755.
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7. Waldminghaus T, Fippinger A, Alfsmann J, Narberhaus F (2005) RNA thermometers are common in alpha- and gamma-proteobacteria. *Biol Chem* 386(12):1279–1286.
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9. Klose KE, Mekalanos JJ (1998) Distinct roles of an alternative sigma factor during both free-swimming and colonizing phases of the *Vibrio cholerae* pathogenic cycle. *Mol Microbiol* 28(3):501–520.
10. Childers BM, et al. (2011) N-terminal residues of the *Vibrio cholerae* virulence regulatory protein ToxT involved in dimerization and modulation by fatty acids. *J Biol Chem* 286(32): 28644–28655.