The *trpE* gene negatively regulates differentiation of heterocysts at the level of induction in *Anabaena* sp. strain PCC 7120

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Supporting Information

Table S1. Oligonucleotide primers used in this study.

Oligonucleotide[a]	Sequence
233-F3-EcoRI	TATAGAATTCCTTATCAACCTATTTAGTGG
233-R1-BamHI	TATAGGATCCTTTGAGCATTTTCTACTAGG
alr3233-up-F	GGATCCGAGTGTTCAGTATTAAATTGTTGAATTAC
alr3233-up-R	CTAAAGTTTGCCCGGGCACCTCGGTTGTGCTACGAGATACGCG
alr3233-dn-F	AACCGAGGTGCCCGGGCAAACTTTAGAAACATCAATTTACTC
alr3233-dn-R	GAGCTCACGGTTGGCGCAGCAGTTAATTGGCGTTGTAAAG
araC-dBam-OEX-F	AGATTAGCAGATCCTACCTGACGC
araC-dBam-OEX-R	GTAGGATCTGCTAATCTTATGGATA
araC-XhoI-F	ATATACTCGAGTTATGACAACTTGACGGCTACATCATTCAC
K12-PlacZ-F	TATCTCACTCGCAATCAAATTCAGCC
K12-PlacZ-R	AATCCTGTTTGATGGTGGTTAACGGC
PmrBAD-BaS-SacI-R	TATATGAGCTCATCCCGGGATGGATCCACTCCATCCAAAAAAACGG GTATGGAGAAACAG
PpetE-EcoRI-SmaI-BamHI-R	TATATGGATCCATCCCGGGATGAATTCTCCTAACCTGTAGTTTTATTT TTCTTATTTC
PpetE-SacI-F	ATATAGAGCTCGCTGAGGTACTGA
PpetE-XhoI-F	TATATCTCGAGGCTGAGGTACTGAGTACACAGC
PpetE-YFP-OEX-F	GAGAACGCCATGAGCAGCGGCGCCCTGCTG
PpetE-YFP-OEX-R	CTGCTCATGGCGTTCTCCTAACCTGTAGTT
PtrpE-BamHI-F	ATATAGGATCCTTCAGTTACCAATAGAAACTATCAGCCATATAG
PtrpE-OEX-R	CCTTCTTAAATCTAGAGTTACCAAGGGTTGTGTAAGCAAGTGTATAG

PtrpE-Rmut-OEX-F	TTCGTTTGGCACAAAAGAAATCAGCGATCGCCAAAAGTAGGGC
PtrpE-Rmut-OEX-R	TTCTTTTGTGCCAAACGAATTCAACAATCAAACCAATTTCACTATCT CCAAAAGAAG
PtrpE-XhoI-F	ATATACTCGAGTTCAGTTACCAATAGAAACTATCAGCCATATAG
R-bind 29mer top	GTAGGCGAGGGGTCTAACCCCTCATTACC
R-bind 29mer btm	GGTAATGAGGGGTTAGACCCCTCGCCTAC
R-bsmut 29mer top	GTAGGCGATTTGTCTAACAAATCATTACC
R-bsmut 29mer btm	GGTAATGATTTGTTAGACAAATCGCCTAC
trpE-110	TTGAATGATGGGTTACACCCCTCAATCAG
trpE-110-C	CTGATTGAGGGGTGTAACCCATCATTCAA
trpE-BamHI-F	ATATAGGATCCATGAATTACTATACACTTGCTTACACAACC
trpE-155-F	CTGTAGGAAATGTTTTGTGCTG
trpE-155-R	TGGAGGGAGTATAGCGCGAACGG
trpE-BglII-R	AGATCTAGAGTAAATTGATGTTTCTAAAGTTTGGG
trpE-down-out	GGTAAGGATTTTGCTGCAAAGCTGTCAAG
trpE-Rmutfull	TTGAATTCGTTTGGCACAAAAGAAATCAG
trpE-Rmutfull-C	CTGATTTCTTTTGTGCCAAACGAATTCAA
trpE-int-R	CCTGTGATGTAGTCATGATTGAGGCTG
trpE-NdeI-F	CATATGAATTACTATACACTTGCTTACACAACC
trpE-out-F	GCTATCATCTTCAATAACCAGGATTG
trpE-SacI-R	ATATAGAGCTCCTAAGAGTAAATTGATGTTTCTAAAGTTTGGG
trpE-up-out	GGGGATAAAACATCGTACAATTCCTC
YFP-SacI-R	ATATAGAGCTCTCAGCTGGTGTCTCCGGAAC

[a]Oligonucleotides are shown in the 5’ to 3’ direction.

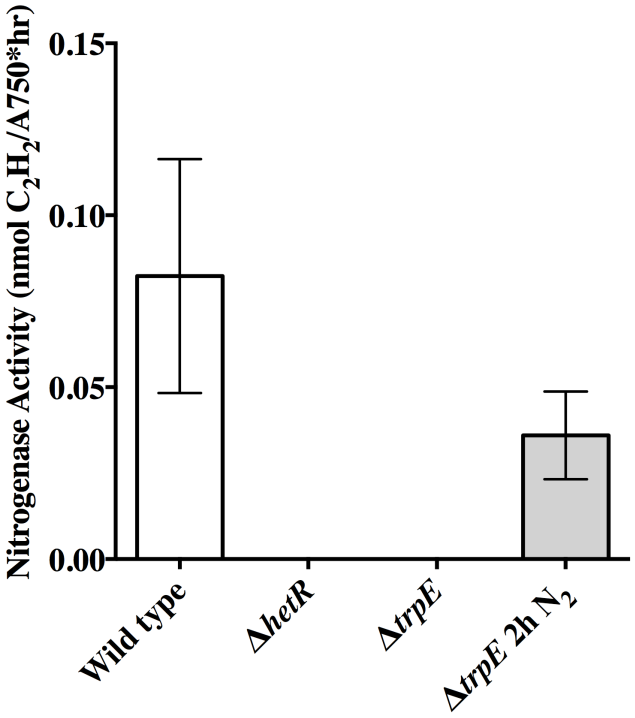

Figure S1. Heterocysts produced by the Δ*trpE* mutant only fix in the absence of combined nitrogen. Nitrogenase activity was assessed by acetylene reduction assay from cultures of the wild type and the Δ*hetR* mutant (UHM103) maintained in the absence of combined nitrogen and the Δ*trpE* mutant (UHM335) grown continuously on nitrate or 2 h after the removal of nitrate (2h N_2). Assays were run in triplicate. Error bars represent the standard deviation.

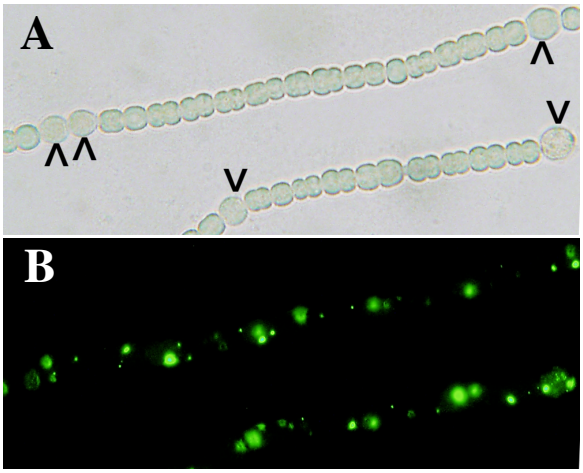

Figure S2. Subcellular localization of TrpE. Bright-field (A) and fluorescence (B) micrographs of the Δ*trpE* mutant containing a plasmid bearing a C-terminal translational fusion of TrpE to YFP that was expressed from the *petE* promoter, induced with 2 μM copper. Fluorescence was false-colored green. Images were taken 24 hours after the removal of combined nitrogen following growth on nitrate. Carets indicate heterocysts.

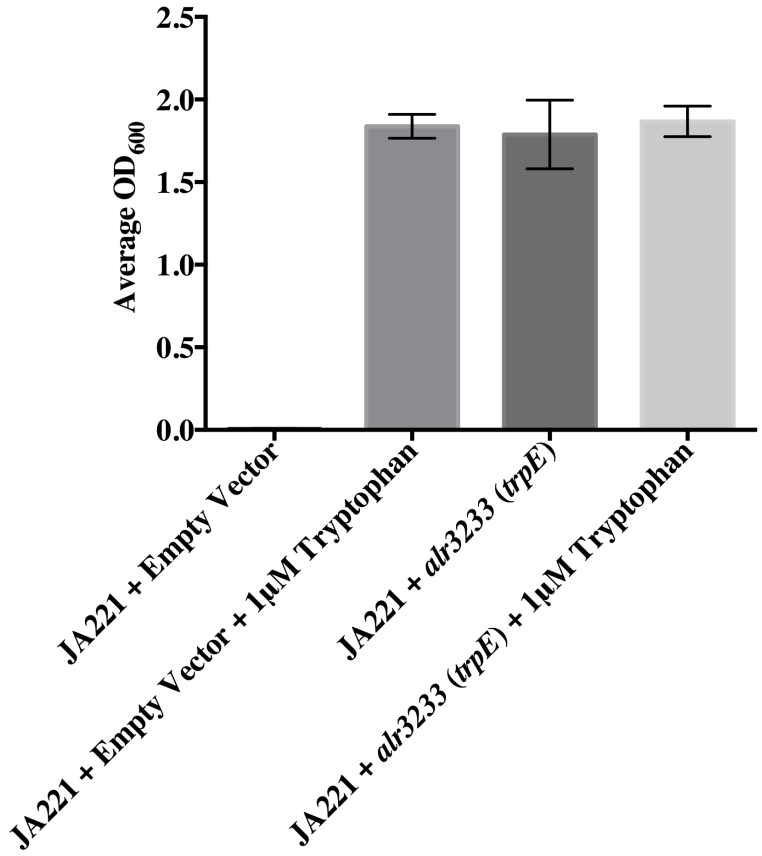

Figure S3. The *alr3233* gene can complement an *E. coli* strain JA221 (a Δ*trpE* mutant). JA221 harboring a plasmid with *alr3233* (*trpE*) controlled by the arabinose-inducible *araBAD* promoter or an empty vector was grown in M9 minimal medium either with or without 1 μM L-tryptophan. Cultures were inoculated at an optical density at 600 nm (OD_{600}) of 0.05, expression was induced with the addition of a final concentration of 0.2% L-arabinose, and the final OD_{600} was recorded after overnight growth. All treatments were conducted in triplicate. Error bars represent the standard deviation.

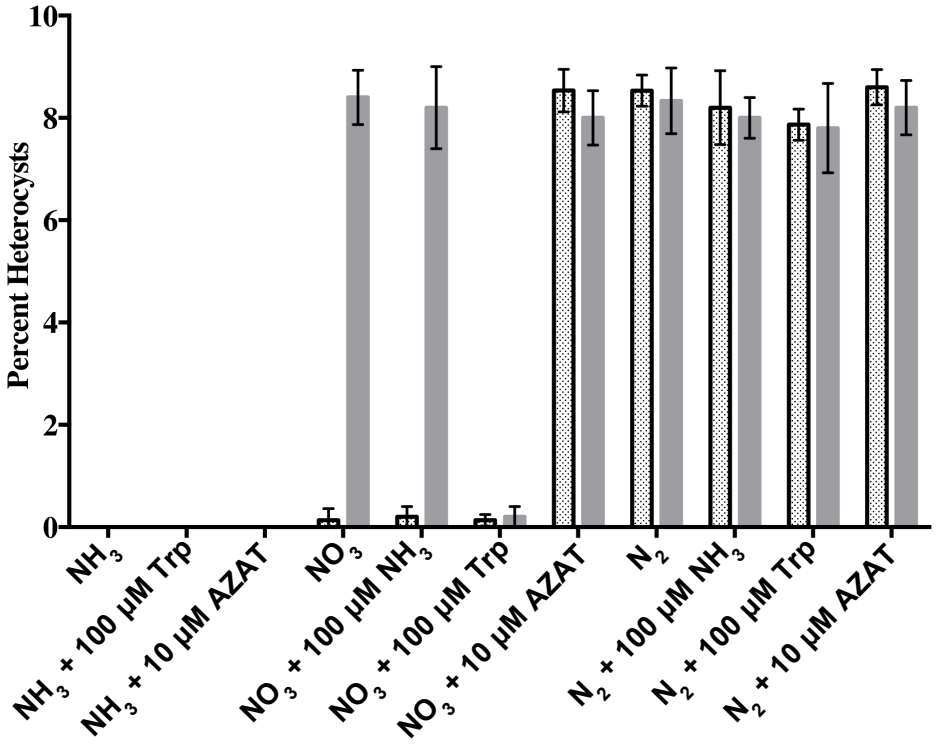

Figure S4. Tryptophan complements the Δ*trpE* mutant (UHM335), and DL-7-azatryptotphan (AZAT) promotes differentiation. The wild type (dotted bars) and UHM335 (grey bars) were incubated with either 10 µM AZAT, 100 µM DL-tryptophan (Trp), or 100 µM ammonia (NH_3) during growth on medium containing 6mM NH_3, 17.6 mM nitrate (NO_3), or lacking a source of combined nitrogen (N_2). Average heterocyst percentages were determined from counts of 500 cells in triplicate. Error bars represent the standard deviation.