Supporting information for Mo and Burkholder (2010)

Tables

Figures

References

Table S1. Plasmids used in this study.

pCRII-TOPO	Vector for TOPO cloning; Kan^R, Amp^R (Invitrogen).
pCR2.1-TOPO	Vector for TOPO cloning; Kan^R, Amp^R (Invitrogen).
pDG1662	*amyE*:: (*cat*); Amp^R, Spc^R, Cm^R (2) *amyE* integration plasmid.
pDR111	*amyE*:: (*spc* $P_{spank(hy)}$, *lacI*); Amp^R, Spc^R Kindly provided by David Rudner (unpublished) – pDR111 is a derivative of the $P_{spac\text{-}hy}$ plasmid pJQ43 (3) and contains a second *lacO* operator site, bound by Lac repressor, to achieve better repression in the absence of the inducer IPTG.
pGEM-CAT	Plasmid containing *cat* cassette; Cm^R, Amp^R (4)
pRDC19	*thrC*:: (*erm xylR* P_{xyl}); Amp^R, Spc^R, Erm^R (1)
pSB243	pBluescript II SK- derived plasmid containing *erm* cassette; Erm^R, Amp^R (S.J. Biller, unpublished).
pAM45	pDR111-derived plasmid expressing *yneA* from the $P_{spank(hy)}$ promoter. The *yneA* ribosome binding site and open reading frame were amplified by PCR from JH642 genomic DNA using oligonucleotides OAM63 and OAM64 and inserted into pDR111 after digestion of both plasmid and insert with HindIII and SphI.
pAM67	pDG1662-derived plasmid expressing *yneA* from the $P_{spank(hy)}$ promoter. A ($P_{spank(hy)}$-*yneA lacI*)-containing DNA fragment was obtained by digesting pAM45 with EcoRI and BamHI and ligated into pDG1662, digested with the same enzymes.
pAM72	*thrC*:: (*erm* $P_{spank(hy)}$ *lacI*); Amp^R, Spc^R, Erm^R – Derivative of pRDC19 carrying the $P_{spank(hy)}$ promoter instead of P_{xyl}. A ($P_{spank(hy)}$- *lacI*)-containing DNA fragment was obtained by digesting pDR111 with EcoRI and BamHI and ligated to the pRDC19 backbone, digested with the same enzymes to drop out (*xylR* P_{xyl}).

pAM73	pRDC19-derived plasmid expressing *yneA-phoA* from the $P_{spank(hy)}$ promoter. Leaderless *'phoA* (without signal peptide) was amplified by PCR from *E. coli* N99 genomic DNA using oligonucleotides OAM76 and OAM77 and cloned into pCRII-TOPO using the TOPO-TA kit (Invitrogen). *'phoA* was then digested out of pCRII-TOPO with HindIII and BamHI and ligated into pRDC19, digested with the same enzymes, resulting in pAM68 (cloning intermediate not listed in this table). The *yneA* ribosome binding site and open reading frame was amplified by PCR from JH642 genomic DNA using oligonucleotides OAM63 and OAM78 and cloned into pCRII-TOPO using the TOPO-TA kit (Invitrogen). *yneA* was then digested out of pCRII-TOPO with HindIII and NotI and cloned into pAM68, digested with the same enzymes, resulting in pAM70 (cloning intermediate not listed in this table). *yneA-'phoA*-containing DNA fragment was obtained by digesting pAM70 using HindIII and SpeI and ligated into pAM72 digested with HindIII and NheI.
pAM148	pRDC19-derived plasmid expressing *yneA-S-flag-His10* from the $P_{spank(hy)}$ promoter. A mulit-cloning site following by the coding sequence for the FLAG epitope tag (MCS-*flag*) was generated by annealing oligonucleotides OAM123 and OAM124 and ligating into pAM73 digested with NotI and SphI, dropping out *'phoA*, resulting in pAM99 (cloning intermediate not listed in this table). The coding sequence for a his_{10} epitope tag was introduced by annealing oligonucleotides OAM156 and OAM157 and ligating into pAM99 digested with SpeI and SphI, resulting in pAM118 (cloning intermediate not listed in this table). The coding sequence for the S epitope tag was introduced by annealing oligonucleotides OAM225 and OAM226 and ligating into pAM118 digested with AsiSI and NheI.
pAM157	Vector for integrating *yneA-S-flag-His10* by single crossover at the *yneA* locus. *'yneA* (starting at bp 31)-*S-flag-His10* amplified by PCR from pAM148 using oligonucleotides OAM232 and OAM233 and ligated into pSB243 and digestion of both plasmid and insert with HindIII and XhoI.
pAM160	Vector for integrating *lytE-'phoA* by single crossover at the lytE locus. Leaderless *'phoA* (without signal peptide) was amplified by PCR

from *E. coli* N99 genomic DNA using oligonucleotides OAM73 and OAM74 and cloned into pCR2.1-TOPO using the TOPO-TA kit (Invitrogen). *'phoA* was then digested out of pCR2.1-TOPO with SalI and NheI and ligated into pGEM-CAT, digested with SalI and XbaI, resulting in pAM158 (cloning intermediate not listed in this table). *'lytE* (starting from bp 519) was amplified by PCR from JH642 genomic DNA using oligonucleotides OAM231 and OAM222 and inserted into pAM158 after digestion of both plasmid and insert with HindIII and SalI.

pAM45- , pAM73-, and pAM148-derived plasmids expressing mutant alleles of *yneA*, *yneA-phoA*, or *yneA-S-flag-His10* from the $P_{spank(hy)}$ promoter, respectively, were made using a Quikchange site-directed mutagenesis kit (Stratagene) and the oligonucleotides indicated.

Plasmid			Mutation	Oligonucleotides
yneA	*yneA-phoA*	*yneA-S-flag-His10*		
pAM89		pAM150	SSGQ to ASAA	OAM113 and OAM114
pAM115		pAM166	D97A	OAM150 and OAM151
pAM129	pAM133		E6A	OAM166 and OAM167
pAM162	pAM184		L25A	OAM204 and OAM205
pAM163			S26A	OAM206 and OAM207
pAM169	pAM185		S7A	OAM172 and OAM173
pAM170	pAM186		I8A	OAM174 and OAM175
pAM171			I9A	OAM176 and OAM177
pAM172	pAM188		F10A	OAM178 and OAM179
pAM173			V11A	OAM180 and OAM181
pAM174	pAM190		L13A	OAM182 and OAM183
pAM175	pAM191		F14A	OAM184 and OAM185
pAM176	pAM192	pAM199	T15A	OAM186 and OAM187
pAM177			V16A	OAM188 and OAM189
pAM178	pAM193		I17A	OAM190 and OAM191
pAM180	pAM194		S19A	OAM194 and OAM195
pAM181	pAM195	pAM200	V21A	OAM196 and OAM197
pAM182	pAM196		I22A	OAM198 and OAM199
pAM183	pAM197		L23A	OAM200 and OAM201

Table S2. Oligonucleotides used in this study.

Primer	Sequence
OAM63	5’-GCGAAGCTTATGCGAACAAACATTCCTGTTG-3’
OAM64	5’-GCGGCATGCCTATCTTACAGTTGCTAATTC-3’
OAM73	5’-GCGGTCGACCCTGTTCTGGAAAACCGG-3’
OAM74	5’-GCGGCTAGCTTATTTCAGCCCCAGAGCGGCTTTC-3’
OAM76	5’-GCCATCAAGCTTGCGGCCGCTCCTGTTCTGGAAAACCGGG-3’
OAM77	5’-CGCATTGGTCGACTTATTTCAGCCCCAGAGCGG-3’
OAM78	5’-CGCATGTGCGGCCGCTCTTACAGTTGCTAATTC-3’
OAM113	5’-GTCATATACAGCCAGCGCCGCAGAGCTTAATC-3’
OAM114	5’-GATTAAGCTCTGCGGCGCTGGCTGTATATGAC-3’
OAM123	5’-GGCCGCAGGCGATCGCGCTAGCGATTATAAAGATGACGATGACAAAACTAGTGCATG-3’
OAM124	5’-CACTAGTTTTGTCATCGTCATCTTTATAATCGCTAGCGCGATCGCCTGC-3’
OAM150	5’-GAAAAAGAAGCATCAGGCTGCATATGAATTAG-3’
OAM151	5’-CTAATTCATATGCAGCCTGATGCTTCTTTTTC-3’
OAM156	5’-CTAGTCATCACCATCACCATCACCATCACCATCACTAGGCATG-3’
OAM157	5’-CCTAGTGATGGTGATGGTGATGGTGATGGTGATGA-3’
OAM166	5’-CATGAGTAAAGCATCTATTATTTTTGTC-3’
OAM167	5’-GACAAAAATAATAGATGCTTTACTCATG-3’
OAM172	5’-CATGAGTAAAGAAGCTATTATTTTTGTCG-3’
OAM173	5’-CGACAAAAATAATAGCTTCTTTACTCATG-3’
OAM174	5’-GAGTAAAGAATCTGCTATTTTTGTCGG-3’
OAM175	5’-CCGACAAAAATAGCAGATTCTTTACTC-3’
OAM176	5’-GTAAAGAATCTATTGCTTTTGTCGGTCTG-3’
OAM177	5’-CAGACCGACAAAAGCAATAGATTCTTTAC-3’
OAM178	5’-GAATCTATTATTGCTGTCGGTCTGTTTAC-3’
OAM179	5’-GTAAACAGACCGACAGCAATAATAGATTC-3’
OAM180	5’-CTATTATTTTTGCCGGTCTGTTTAC-3’
OAM181	5’-GTAAACAGACCGGCAAAAATAATAG-3’
OAM182	5’-CTATTATTTTTGTCGGTGCGTTTACAGTG-3’
OAM183	5’-CACTGTAAACGCACCGACAAAAATAATAG-3’
OAM184	5’-GTCGGTCTGGCTACAGTGATTTTG-3’
OAM185	5’-CAAAATCACTGTAGCCAGACCGAC-3’
OAM186	5’-CGGTCTGTTTGCAGTGATTTTGAGC-3’
OAM187	5’-GCTCAAAATCACTGCAAACAGACCG-3’
OAM188	5’-GGTCTGTTTACAGCGATTTTGAGCGCG-3’
OAM189	5’-CGCGCTCAAAATCGCTGTAAACAGACC-3’
OAM190	5’-CTGTTTACAGTGGCTTTGAGCGCGG-3’
OAM191	5’-CCGCGCTCAAAGCCACTGTAAACAG-3’
OAM194	5’-CAGTGATTTTGGCCGCGGTTATCCTTATG-3’
OAM195	5’-CATAAGGATAACCGCGGCCAAAATCACTG-3’

OAM196	5’-GATTTTGAGCGCGGCTATCCTTATGCTG-3’
OAM197	5’-CAGCATAAGGATAGCCGCGCTCAAAATC-3’
OAM198	5’-GAGCGCGGTTGCCCTTATGCTGTC-3’
OAM199	5’-GACAGCATAAGGGCAACCGCGCTC-3’
OAM200	5’-GCGCGGTTATCGCTATGCTGTCATATAC-3’
OAM201	5’-GTATATGACAGCATAGCGATAACCGCGC-3’
OAM204	5’-GTTATCCTTATGGCGTCATATACAAGC-3’
OAM205	5’-GCTTGTATATGACGCCATAAGGATAAC-3’
OAM206	5’-CCTTATGCTGGCATATACAAGCAGC-3’
OAM207	5’-GCTGCTTGTATATGCCAGCATAAGG-3’
OAM222	5’-GCGGCTAGCGTCGACGAATCTTTTCGCACCGAGGTAAC-3’
OAM225	5’-CGCAAGGAGACTGCTGCAGCAAAATTCGAGAGACAACATA TGGACTCTG-3’
OAM226	5’-CTAGAGAGTCCATATGTTGTCTCTCGAATTTTGCTGCAGCA GTCTCCTGCGAT-3’
OAM231	5’-GCGAAGCTTAAACGGCTTAAAATCAG-3’
OAM232	5’-GCGAAGCTTGTCGGTCTGTTTACAGTGATTTTG-3’
OAM233	5’-CGCCTCGAGCTAGTGATGGTGATGGTGATG-3’

Restriction sites used for cloning are underlined.

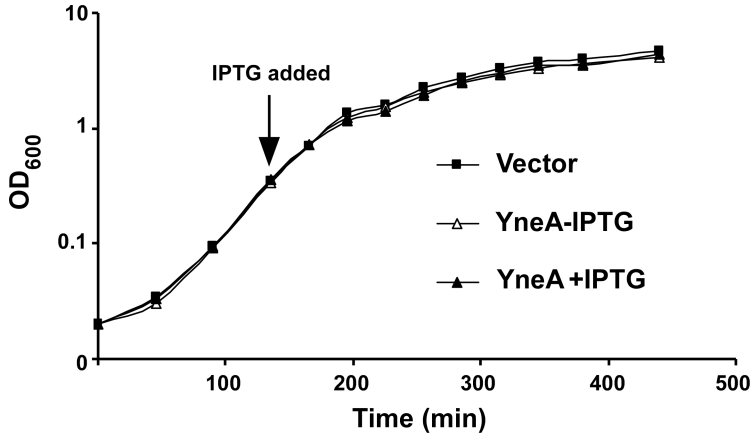

Fig. S1. Cells overexpressing *yneA* cease exponential growth at the same time as uninduced and vector control cells.
Overnight cultures of AM62 (*amyE*::*cat*) and AM93 (*amyE*::P$_{spank(hy)}$-*yneA*) were diluted back to OD_{600} ~ 0.02, grown at 37 °C in LB to exponential phase (OD_{600} = 0.3-0.5), and induced with 1 mM IPTG (time of addition indicated by arrow on graph). Cell density was measured at the time points indicated.

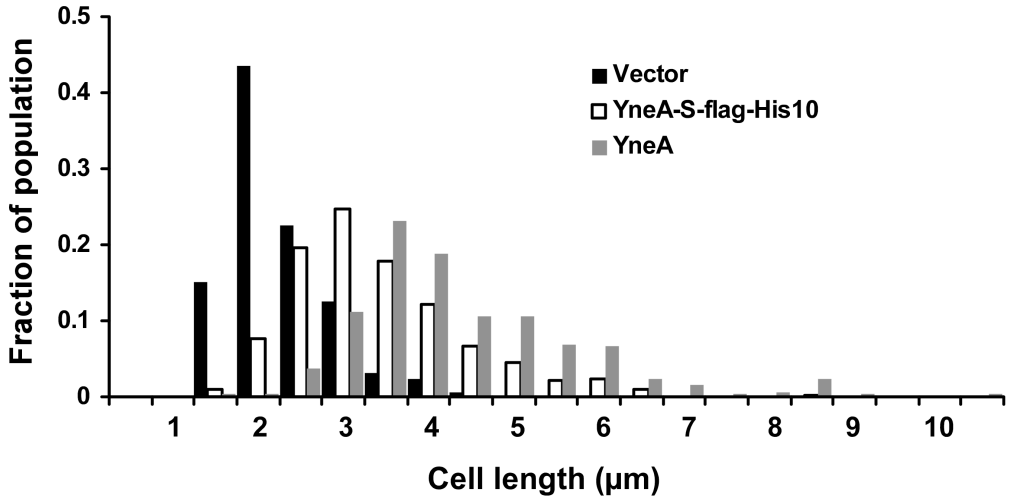

Fig. S2. YneA-S-flag-His10 is partially functional.
Overnight cultures of AM93 (*amyE*::P$_{spank(hy)}$-*yneA*), AM199 (*thrC*::P$_{spank(hy)}$-*yneA-S-flag-His10*), and AM206 (*thrC*::*erm*) were diluted back to OD_{600} ~ 0.02, grown at 37 ^{o}C in LB to exponential phase (OD_{600} = 0.3-0.5), and induced with 1 mM IPTG. 2 hours after induction of *yneA-S-flag-His10*, cells were stained with FM4-64 and DAPI and visualized. Cells lengths were quantified for at least 300 cells for each strain.

References.

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