Supplementary Information

References

1. Overgaard, M., Borch, J., and Gerdes, K. (2009) RelB and RelE of Escherichia coli Form a Tight Complex That Represses Transcription via the Ribbon–Helix–Helix Motif in RelB. *J Mol Biol,* **394**(2), 183–196.

2. Maisonneuve, E., Castro-Camargo, M., and Gerdes, K. (2013) (p) ppGpp controls bacterial persistence by stochastic induction of toxin-antitoxin activity. *Cell,* **154**(5), 1140–1150.

3. Li, G.-W., Burkhardt, D., Gross, C., and Weissman, J. S. (2014) Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources. *Cell,* **157**(3), 624–635.

4. Cataudella, I., Sneppen, K., Gerdes, K., and Mitarai, N. (2013) Conditional cooperativity of toxin-antitoxin regulation can mediate bistability between growth and dormancy. *PLoS Comput Biol,* **9**(8), e1003174.

1 Supplementary Tables

Table S1. Parameter values in the model

Parameter	Meaning	Value	Remark
K_o	Dissociation constant between A_2T and promoter	3 μm^{-3}	Assumed. This parameter is insensitive to persister properties (Fig. S7).
β_T	Maximal reduction of toxin production by free toxins	20	Assumed. This parameter is insensitive to persister properties (Fig. S7).
β_A	Maximal reduction of antitoxin production by free toxins	10	Experimental measurements gave $\beta_A/\beta_T \approx 0.5$ (Table I).
β_0	Maximal growth reduction by free toxins	200	Assumed. This parameter is insensitive to persister properties (Fig. S7).
K_t	Association constant between A_2 and T, and between A_2T and T	3 μm^3	Ref. [1] measured that the dissociation constant is $0.33nM$. Here we use that $1nM$ approximately corresponds to 1 molecule per μm^3. This parameter is insensitive to persister properties (Fig. S7).
$d_{A,low}$	Degradation rate of antitoxins with low (p)ppGpp level	$2.9 * 10^{-4} s^{-1}$	The half life of antitoxins in low (p)ppGpp level is approximately 40 min.
$d_{A,high}$	Degradation rate of antitoxins with high (p)ppGpp level	$5.8 * 10^{-4} s^{-1}$	The half life of antitoxins in high (p)ppGpp level is approximately 20 min (fitted to the RelB measurement in Ref. [2]).
d_T	Degradation rate of toxins	$3.9 * 10^{-5} s^{-1}$	Assume that the half life of toxins is 300 min.
D	Michaelis-Menten constant for toxins' activity	200 μm^{-3}	Assumed. This parameter is insensitive to persister properties (Fig. S7).
Γ_0	Dilution rate of cells	$7.8 * 10^{-4} s^{-1}$	The doubling time of cells with low (p)ppGpp level is assumed to be 40 min. So we have $\Gamma_0/(1 + \beta_0 \sum_i [T_f^{(i)}]/(\sum_i [T_f^{(i)}] + D)) = \ln(2)/2400 s^{-1}$. Under high (p)ppGpp level, we assume that cells do not grow, corresponding to $\Gamma_0 = 0$.
σ_A	Maximal production rate of antitoxin dimers	$12 \mu m^{-3} s^{-1}$	Equation for antitoxins is in the steady states with low (p)ppGpp level.
σ_T	Maximal production rate of toxins	$2.1 \mu m^{-3} s^{-1}$	Equation for toxins is in the steady states with low(p)ppGpp levels.
$[A]_0$	Steady state level of antitoxins with low (p)ppGpp level	200 μm^{-3}	Assumed.
$[T]_0$	Steady state level of toxins with low (p)ppGpp level	59 μm^{-3}	We choose the ratio of production rates to be $A : T = 6 : 1$ based on ribosome profiling data [3]. The degradation rate of antitoxin is $1/(40min) + d_A$ and the rate of toxin is $1/(40min) + d_T$. So the ratio of degradation rates is $A : T = (1/(40min) + d_A)/(1/(40min) + d_T) \approx 1.76$. Therefore, the steady state level satisfies that $[A]_0 : [T]_0 \approx 3.4 : 1$.
r_+	Transition rate of (p)ppGpp from low level to high level	$2.9 * 10^{-8} s^{-1}$	Fitted
r_-	Transition rate of (p)ppGpp from high level to low level	$2.9 * 10^{-5} s^{-1}$	Fitted

2 Supplementary Figures

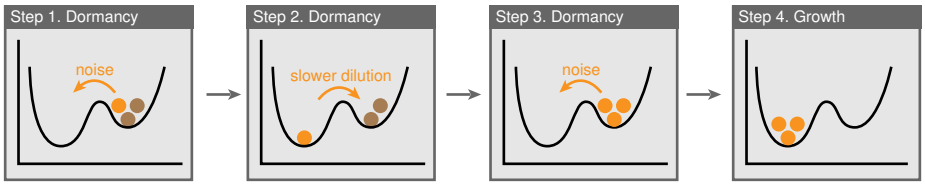

Figure S1. Schematic description of stochastic transition from dormancy to growth following bistability-based mechanism. If molecular noises induce one or few TA systems to a temporarily high-antitoxin-low-toxin level (step 1), cellular growth remains repressed as the other TA systems keep producing free toxin proteins. This slow growth rate may drive the switched TA systems back to dormancy state since toxins are long-lived and are sensitive to cell dilution while antitoxins are not [4] (step 2). Therefore, a successful transition requires a simultaneous switching of all TA systems to the growth state (step 3-4).

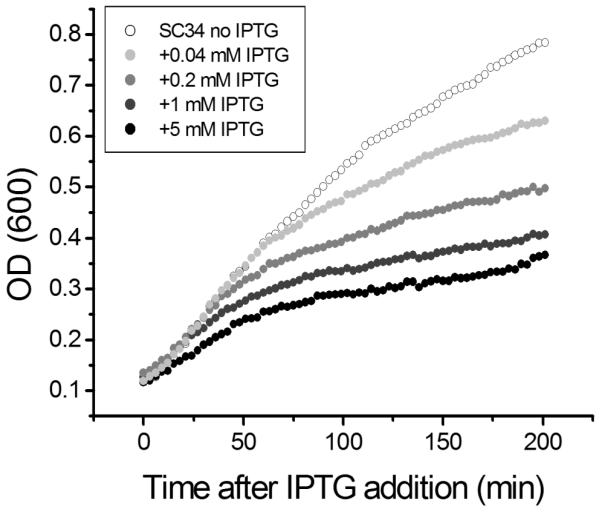

Figure S2. The effect of RelE expression on the growth of SC34 cells. SC34 cells containing the pSEM3187 plasmid were grown at 37°C in LB zeocin (70 μg/ml) medium to $OD_{600} \sim 0.1$ in a FLUOstar Omega Microplate Reader (BMG Labtech) before IPTG was added to the cultures (0 time) at different concentrations.

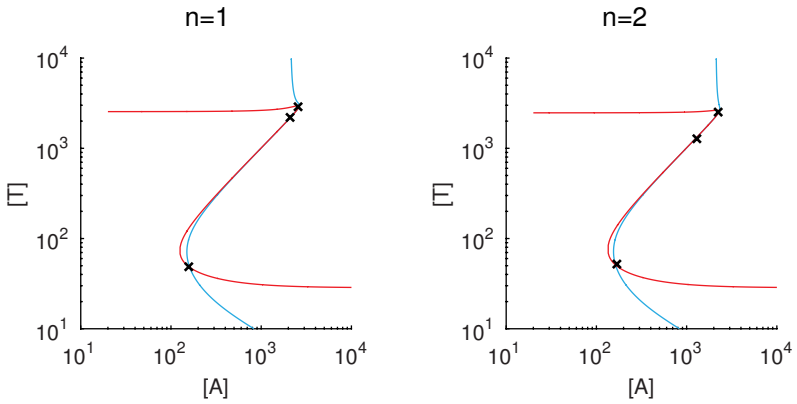

Figure S3. Steady states and nullclines of the model for coupled TA systems without (p)ppGpp fluctuation (related to Fig. 3) The steady states (black cross) and nullclines (blue for $\mathrm{d}[A]/\mathrm{d}t = 0$ and red for $\mathrm{d}[T]/\mathrm{d}t = 0$) are computed by assuming that all TA systems share the same concentrations. n represents the number of TA systems.

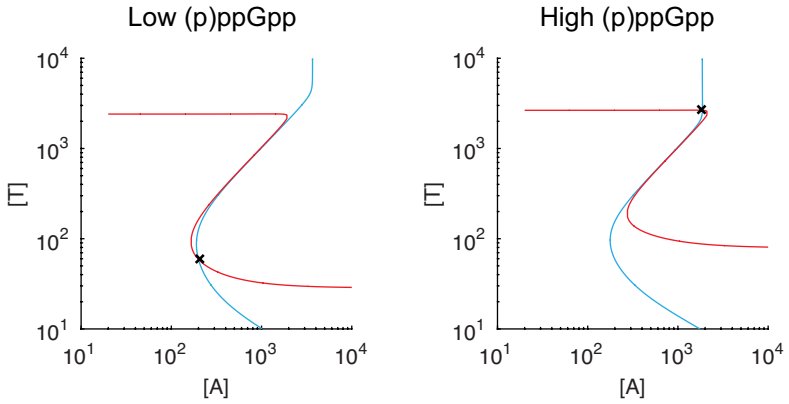

Figure S4. Steady states and nullclines of the model for 10 coupled TA systems with (p)ppGpp fluctuation (related to Table S1). The figures are produced as described in Fig. S3.

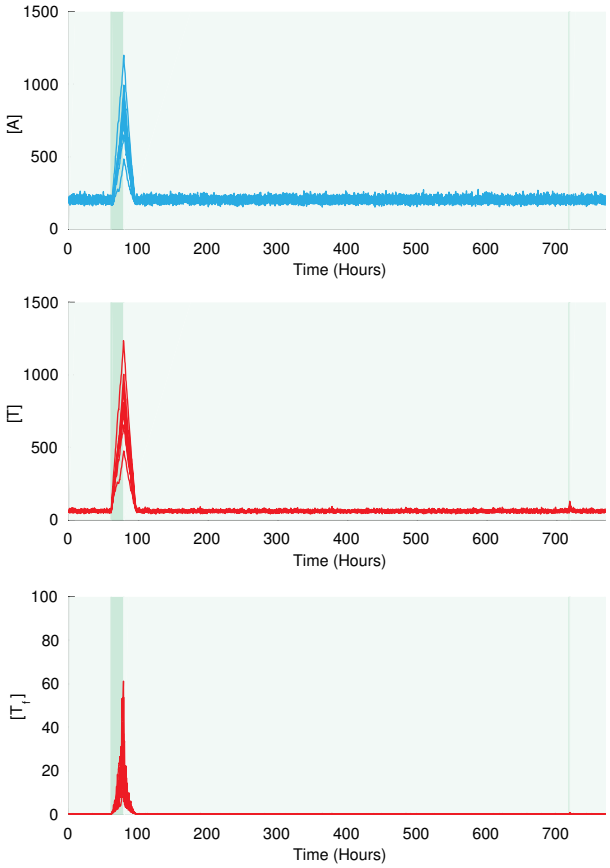

Figure S5. Sample simulation trajectory of the model with 10 coupled TA systems (related to Fig. 4a)

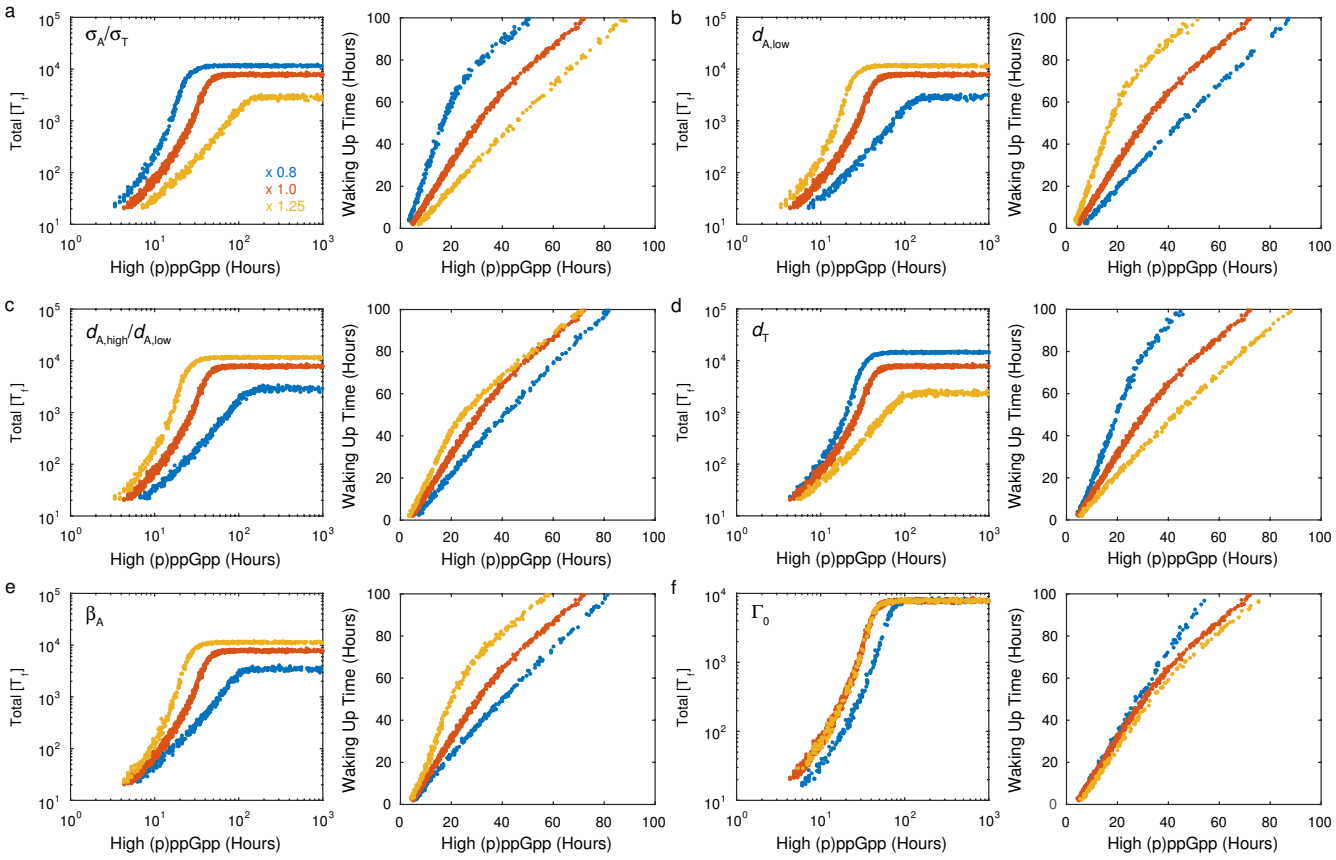

Figure S6. Sensitive parameters of the model for coupled TA systems with (p)ppGpp fluctuation (related to Table S1) We implement the model with parameter values listed in Table S1 and modulate the value of the parameter indicated in the figure. We simulate the model and produce the dependency of the amount of accumulated free toxins (left) and waking up times (right) on the duration of (p)ppGpp fluctuation following the same procedure for Fig. 5. Blue dots: parameter value reduces by 1.25 folds; red dots: parameter value is not changed; yellow dots: parameter value increases by 1.25 folds. (a) Change in the ratio of production rates (σ_A/σ_T). We keep the value of σ_T and change the value of σ_A accordingly. (b) Change in the degradation rates of antitoxins under low (p)ppGpp levels ($d_{A,low}$). We keep the value of $d_{A,high}/d_{A,low}$ unchanged. (c) Change in the effect of (p)ppGpp on antitoxin degradation rates ($d_{A,high}/d_{A,low}$). We keep the value of $d_{A,low}$ and change $d_{A,high}$ accordingly. (d) Change in the degradation rate of toxins (d_T). (e) Change in the translation inhibition on antitoxins (β_A). (f) Change in the value of dilution rate (Γ_0).

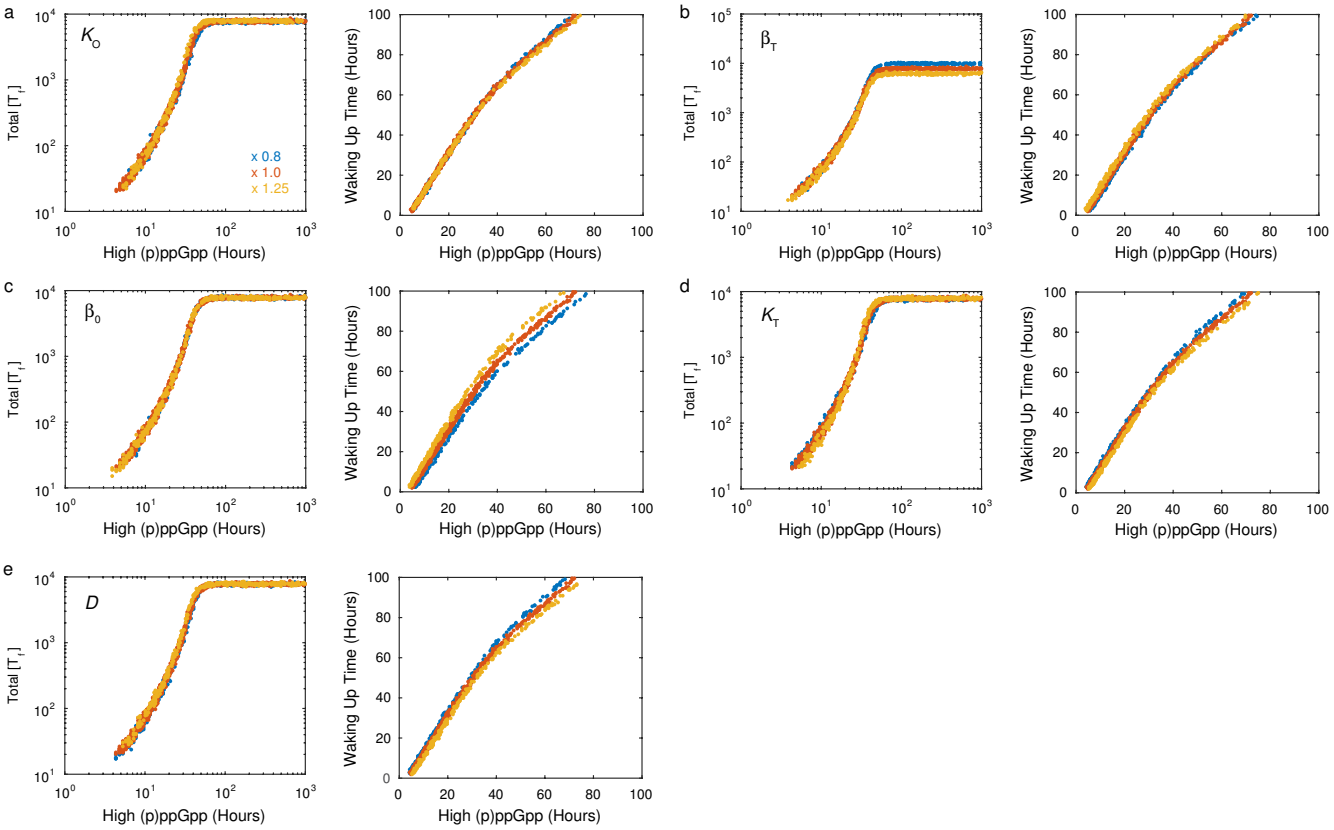

Figure S7. Insensitive parameters of the model for coupled TA systems with (p)ppGpp fluctuation (related to Table S1). We follow the same procedure as described in Fig. S6 caption. (a) Change in the dissociation constant between DNA and trimers A_2T (K_o). (b) Change in the translation inhibition on toxins (β_T). We keep the values of the ratios β_A/β_T and β_0/β_T and change the value of β_T accordingly. (c) Change in the translation inhibition on cellular growth (β_0). (d) Change in the association constant between antitoxins and toxins (K_T). (e) Change in the Michaelis-Menten constant for toxins' activity (D).

3. Sequences of the plasmid constructs

pSEM3187

pMB1 origin
rop
lacI
zeocin^{R}
rrnBT$_1$*T*$_2$
synthetic promoter with a lac operator
Ribosome binding site and relE ORF
Stop codon
Start codon

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Sequence of the mCherry-YFP fragment in pSEM4063

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start codon
stop codon

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Sequence of the mCherry-YFP fragment in pSEM4049

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start codon
stop codon

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     GGTGACAACG TTAGGTTATG GGTTGATGTG CTTTGCTAGA TACCCAGATC ACATGAAAAG     1020
     GCATGACTTC TTTAAATCTG CAATGCCAGA AGGTTACGTC CAAGAACGTA CTATTTTCTT     1080
     TAAAGATGAC GGTAATTATA AAACTAGGGC TGAAGTTAAA TTCGAAGGTG ACACACTTGT     1140
     AAATCGAATA GAGTTAAAGG GGATTGATTT CAAAGAGGAT GGTAATATTC TAGGCCATAA     1200
     ACTTGAATAT AACTATAATT CACACAACGT TTACATTACC GCCGACAAGC AGAAGAATGG     1260
     AATCAAAGCC AATTTTAAGA TTAGACACAA TATTGAGGAT GGTGGAGTAC AGCTTGCTGA     1320
     TCATTACCAA CAAAATACCC CGATCGGTGA TGGACCAGTT TTGCTACCCG ATAACCATTA     1380
     TCTGTCCTAT CAAAGCAAAT TGTCAAAAGA TCCTAACGAA AAAAGAGACC ACATGGTACT     1440
     CTTGGAATTT GTAACAGCTG CTGGGATTAC ACATGGCATG GATGAACTAT ACAAAGGTTC     1500
     TGGAACCGCA TAATAAGTCG AC                                              1522
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