Supporting Information

Sánchez-Romero and Casadesús 10.1073/pnas.1316084111

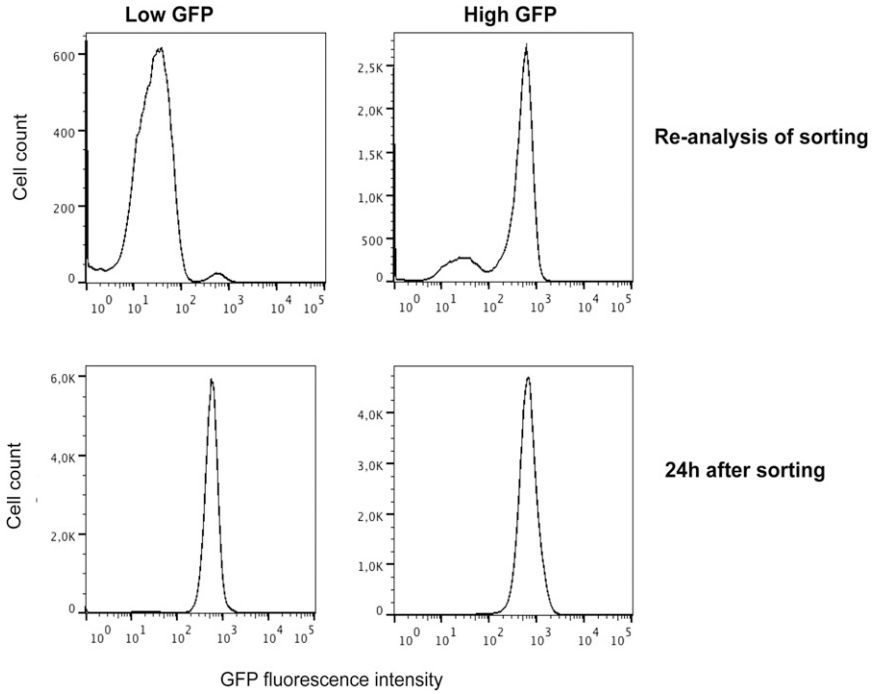

Fig. S1. Reversibility of *ompC* down-regulation, monitored by flow cytometry analysis. Aliquots from exponential *Salmonella enterica* cultures were washed and resuspended in PBS to a final concentration of 5×10^6 cells/mL Cells were sorted using a MoFloTM XDP cytometer (Beckman Coulter). Immediately before sorting, 5×10^6 cells were analyzed for GFP expression. Based on this analysis, a gate was drawn for each population to include the 10% of cells expressing GFP at low level, and a gate that covered the 10% of cells expressing GFP at high level. From each gate, 1×10^6 cells were collected into a sterile Eppendorf. After sorting, cells were spun at 3,400 × *g* for 10 min and traces of FACS buffer were removed. An aliquot of sorted cells was run again through the cytometer to test the purity of the preparation. Sorted cells were resuspended in 1 mL LB broth and grown overnight. The following day aliquots from two subpopulations of sorted cells were taken, washed, and resuspended in PBS to analyze GFP expression. A representative experiment is shown. Down-regulation of *ompC* expression was found to be reversible, thus confirming its nongenetic origin.

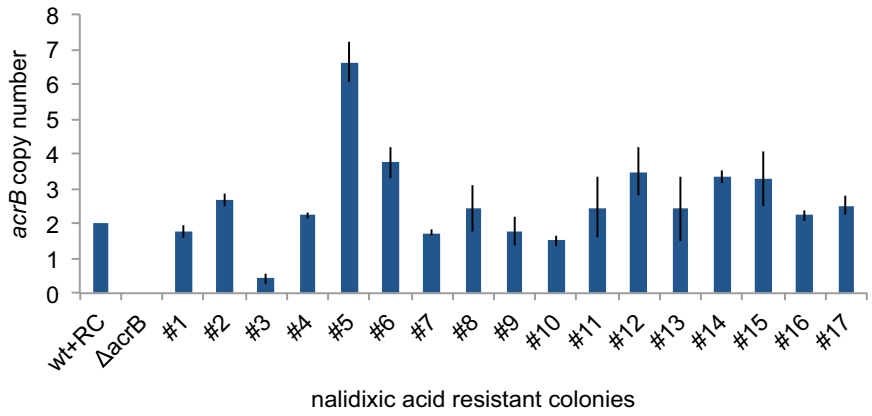

Fig. S2. Analysis of *acrAB* gene dosage in nalidixic-acid resistant derivatives of *S. enterica* SL1344. Colonies grown in LB agar plus nalidixic acid were used in *acrB* copy number assays. The copy number of the *acrB* gene was determined using the method of Lee et al. (1). Cells were suspended in sterile water and boiled for 5 min. This solution was used as template for real-time PCR. All real-time PCR runs were performed in triplicate using a LightCycler 480 II (Roche). Reaction mixtures were prepared using the SYBR Green Master Mix (Takara) in a total volume of 10 μL on a 480-well optical reaction plate (Roche) containing 5 μL SYBR, 0.5 DYE II (Takara), 4 μL DNA, and two gene-specific primers at a final concentration of 0.2 mM each. Real-time cycling conditions were as follows: (*i*) 95 °C for 10 min and (*ii*) 40 cycles at 95 °C for 15 s, 60 °C for 1 min. After the amplification, a melting peak analysis with a temperature gradient of 0.1 °C/s^{-1} from 60 to 95 °C was performed to confirm that only specific products were amplified. Gene-specific primers were designed with PRIMER3 software (http://primer3.sourceforge.net): RT-acrB-rev: 5′CGC GAT CGA TAA AGT CGT TT 3′ and RT-acrB-for: 5′CGC TGG GCG TAT CTA TTA GC 3′. The primer set specific for the *pipA* gene of *Salmonella* (pipA-F: 5′ATT CCC GAA CAT GCA CCA A 3′ and pipA-R: 5′ GTT TAT GGC AAG GCT GTC ATG A 3′) was used for normalization, and as an internal control. ΔCt values were calculated by substracting the *pipA* gene Ct from from the *acrB* gene Ct for each replicate. The average ΔCt from the 3 replicates was then calculated. *S. enterica* cultures growing with rifampicin and cephalexin for 3 h (wt + RC) was used as the calibrator for the *acrB* gene dosage assay, and the ΔCt's from all of the remaining DNA samples were normalized to the wt + RC Ct. A DNA sample showing increased *acrB* gene dosage (#5) was detected.

1. Lee C, Lee S, Shin SG, Hwang S (2008) Real-time PCR determination of RNA gene copy number: Absolute and relative quantification assays with *Escherichia coli*. *Appl Microbiol Biotechnol* 78(2):371–376.

Table S1. Fluctuation in the frequencies of rifampicin-resistant isolates obtained upon plating approximately 10^8 colony-forming-units of *S. enterica* SL1344 on LB + rifampicin (100 μg/mL)

Number of colonies	Individual cultures	Single culture
	80	26
	65	48
	13	38
	17	17
	8	44
	125	30
	40	42
	212	32
	32	40
	0	42
	3	30
	19	38
	74	48
	0	24
	129	18
	141	34
	43	50
	89	26
	2	27
	4	16
Average	54.8	33.5
SD	59.1	10.5
Coefficient of variation	1.07	0.31

Table S2. Fluctuation in the frequencies of kanamycin-resistant isolates obtained upon plating approximately 10^8 colony-forming-units of *S. enterica* SL1344 on LB + kanamycin (25 μg/mL)

Number of colonies	Individual cultures	Single culture
	1	21
	8	29
	8	38
	18	17
	59	24
	25	19
	110	21
	24	22
	0	31
	40	22
	1	40
	33	15
	43	28
	15	24
	0	27
	2	30
	32	18
	40	24
	1	20
	1	23
Average	23.05	26.45
SD	27.14	6.53
Coefficient of variation	1.17	0.24

Table S3. Fluctuation in the frequencies of nalidixic acid-resistant isolates obtained upon plating 10^8 colony-forming-units of *S. enterica* SL1344 on LB + nalidixic acid (10 μg/mL)

Number of colonies	Individual cultures	Single culture
	3	9
	50	10
	8	4
	18	7
	9	8
	25	9
	0	7
	14	8
	0	11
	64	12
	31	10
	23	15
	3	8
	15	8
	18	10
	2	13
	0	8
	28	6
	3	10
	19	18
Average	15.65	9.55
SD	17.29	3.17
Coefficient of variation	1.10	0.33

Table S4. Frequencies of stable and unstable kanamycin-resistant isolates obtained upon plating approx. 10^8 colony-forming-units of *S. enterica* SL1344 on LB + kanamycin (25 μg/mL)

Number of colonies	Total number	Stable isolates	Unstable isolates
	34	24	10
	9	3	6
	13	6	7
	29	20	9
	88	NT	NT
	5	0	5
	16	6	10
	4	0	4
	20	17	3
	38	29	8
	92	NT	NT
	6	1	5
	7	1	6
	79	71	8
	16	6	10
	189	NT	NT
	224	NT	NT
	98	NT	NT
	100	NT	NT
	23	19	4
Average	54.50	14.60	7.13
SD	62.32	18.29	2.69
Coefficient of variation	1.14	1.24	0.37

NT, not tested.