Toxin/Antitoxin Systems Induce Persistence and Work in Concert with Restriction/Modification Systems to Inhibit Phage

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SUMMARY

Myriad bacterial anti-phage systems have been described and often the mechanism of programmed cell death is invoked for phage inhibition. However, there is little evidence of "suicide" under physiological conditions for these systems. Instead of death to stop phage propagation, we show here that persister cells, i.e., transiently-tolerant, dormant, antibiotic-insensitive cells, are formed and survive using the *Escherichia coli* C496_10 tripartite toxin/antitoxin system MqsR/MqsA/MqsC that degrades mRNA. Specifically, MqsR/MqsA/MqsC inhibited T2 phage by one million-fold and reduced T2 titers by 500-fold. Further evidence of the formation of persister cells during T2 phage attack in the presence of MqsR/MqsA/MqsC include the single-cell physiological change of reduced metabolism (via flow cytometry) and heterogeneous resuscitation. Critically, we found restriction-modification systems (primarily EcoK McrBC) work in concert with the toxin/antitoxin system to inactivate phage, likely while the cells are in the persister state. Hence, phage attack invokes a stress response similar to antibiotics, starvation, and oxidation, which leads to persistence, and this dormant state likely allows restriction/modification systems to clear phage DNA.

INTRODUCTION

15 Phage attack, resulting in up to 40% cell death[13], is the universal constraint on bacterial immortality; hence, bacteria have developed numerous phage-inhibition systems. For phage inhibition, there is little evidence that these systems cause cell death; instead, it is much more likely these systems invoke dormancy[31, 44]. Bacterial cell dormancy has several potential benefits: (i) allowing time for other host-encoded, phage-inhibition systems to function, (ii) slowing viral replication, and (iii) accommodating for
20 spacer accumulation for CRISPR-Cas[44]. In fact, the very existence of spacers in CRISPR-Cas type III and VI systems; i.e., those shown to induce dormancy by targeting host RNA, demonstrate cells must survive phage attack to obtain spacers. Also, growth-inhibited cells have more spacer acquisition[9]. In contrast, programmed cell death during phage attack is paradoxical from the perspective of individual cells and has yet to be proven beneficial for kin.

25 Given that cells enter a state of dormancy known as persistence to survive numerous stresses (e.g., antibiotics[15], starvation[17], oxidation[16]) through guanosine pentaphosphate and tetraphosphate (p)ppGpp signaling[19], which leads to dimerization of their ribosomes to cease translation[21, 41, 50], we reasoned that phage attack is another form of extreme stress, so it should lead to persistence. Also, phage-inhibition systems include toxin proteins of toxin/antitoxin systems, which are known to induce persistence by up to
30 14,000-fold[7, 16, 46]. For example, after transcription shutoff by T4 phage activates the Hok/Sok toxin/antitoxin system, Hok damages the cell membrane to cease cell metabolism to halt phage propagation, in the first link of a toxin/antitoxin system to phage inhibition (1996)[32]. Similarly, phage attack activates various toxins to reduce cell metabolism including those of the CBASS (phospholipases, nucleases, and pore-forming proteins[22]), RADAR (RdrB converts ATP to ITP[11]), and Thoeris (ThsA degrades NAD^{+}[23])
35 phage inhibition systems.

Therefore, based on these two insights (extreme stress produces persisters and phage-inhibition systems utilize toxins to reduce metabolism), we investigated whether persister cells are formed as a result of inducing the *bona fide Escherichia* spp. phage inhibition system, the tripartite toxin/antitoxin/chaperone system *E. coli* C496_10 MqsR/MqsA/MqsC[4, 45], which utilizes RNase MqsR[5, 35] upon phage attack. We

40 confirmed that the MqsR/MqsA/MqsC, produced from its natural promoter via the same system used to find it dramatically inhibits T2 phage[45], and discovered this system converts *E. coli* into the persister state to inhibit phage. In addition, we discovered that restriction/modification systems assist the MqsR/MqsA/MqsC toxin/antitoxin system to inhibit T2 phage. To our knowledge, this is the is the first connection of phage inhibition with persistence, and the first non-CRISPR-Cas system to be linked to
45 restriction/modification systems.

RESULTS

Given ours and others' extensive experience with *E. coli* persister cells[16, 20, 38-41, 49] and given our discovery of the MqsR/MqsA toxin/antitoxin system[5, 35], we chose a phage exclusion system of *Escherichia* spp. based on MqsR/MqsA to investigate the hypothesis that persister cells are formed upon phage attack.
50 We also used the same expression system that was used to demonstrate phage inhibition and used T2 phage since it was inhibited best by the MqsR/MqsA/MqsC system[10, 45], and produced MqsR/MqsA/MqsC in a background that lacks this system (K-12).

MqsR/MqsA/MqsC inhibits T2 phage. As published previously[45], T2 phage was efficiently inhibited by the MqsR/MqsA/MqsC TA system in our hands; i.e., MqsR/MqsA/MqsC increased cell viability by 10^6-
55 fold compared to an empty plasmid control (**Table S1**). Corroborating the phage inhibition, the number of T2 phage decreased 500-fold in the presence of MqsR/MqsA/MqsC: $6 \pm 2 \times 10^5$ pfu/mL were obtained for MqsR/MqsA/MqsC whereas $2.6 \pm 0.5 \times 10^8$ pfu/mL were obtained for the empty plasmid when T2 was added initially at 7.14×10^6 pfu (MOI of 0.1). Therefore, MqsR/MqsA/MqsC actively inhibits T2 phage under these conditions.

60 **Cells surviving T2 phage attack with MqsR/MqsA/MqsC are persisters.** Critically, treatment with T2 phage induced persistence in 8% of the cells producing MqsR/MqsA/MqsC (**Table S1**); i.e., these cells survived treatment of ampicillin at 10X the minimum inhibitory concentration (MIC). In contrast, cells bearing the empty plasmid were completely eradicated by ampicillin after exposure to T2 phage indicating no cells entered the persister state without MqsR/MqsA/MqsC. Normally, persister cells are formed at less

65 than 1% of the population[27, 28], so the number of persisters created by MqsR/MqsA/MqsC during T2 phage attack is striking (~10%). Similar results were seen with a different class of antibiotic, ciprofloxacin (10X MIC), in that 0.28% cells with the MqsR/MqsA/MqsC phage inhibition system were persisters after T2 attack (**Table S1**), whereas no cells survived T2 and ciprofloxacin in the absence of MqsR/MqsA/MqsC (**Table S1**). Moreover, the increase in persistence by MqsR/MqsA/MqsC was not due to a difference in
70 growth since *mqsRAC*$^{+}$ had virtually no impact on the specific growth rate in the absence of phage (**Fig. S1**, μ = 1.7 ± 0.1/h, μ = 1.62 ± 0.06/h, and μ = 1.74 ± 0.09/h for MG1655, MG1655/pCV1, and MG1655/pCV1-mqsRAC, respectively).

Corroborating that MqsR/MqsA/MqsC converts *E. coli* into persister cells, we assayed single cell resuscitation on LB gel pads after 1 h of T2 attack (0.1 MOI) and found significant heterogeneity in waking
75 with four phenotypes including 36 ± 13% immediate waking, 28 ± 5% lags in waking, 18 ± 7% elongation, 6% waking then death) whereas 97 ± 3% of the sample of exponentially-growing cells wake uniformly without elongation, lags, or death (**Fig. 1**, **Table S2**). Heterogeneity in resuscitation of single-cell persisters was discovered by us[18] and confirmed by other laboratories[12, 34, 37, 48]; hence, after T2 attack, cells become persistent based on their antibiotic tolerance to two different classes of antibiotics and based on their
80 heterogeneity in resuscitation. Further evidence of persistence after T2 phage attack includes metabolic staining with flow cytometry revealed that after 1 h of T2 attack, *E. coli* cells had significantly reduced metabolism compared to exponentially-growing cells (**Fig. S2**).

Persistence cell formation is rapid upon phage attack. To investigate how rapidly persister cells form upon phage attack, we investigated induction of the three loci required to inactivate ribosomes: *raiA, rmf,*
85 and *hpf*[41]. Using qRT-PCR, we found no induction of these loci for the cells producing the MqsR/MqsA/MqsC phage inhibition systems, which indicates the cell uses existing proteins to become dormant rapidly (i.e., in msec), rather than relying on expression of ribosome inactivation genes once phage attacks. However, for cells that lack this phage inhibition system, *raiA* was induced 4-fold at 15 min after phage attack, which indicates these cells are stressed as they unsuccessfully try to thwart T2 phage (**Table**
90 **S3**). Bacteriophage T2 takes 60 minutes for its burst size, with a latent period of around 20 min and a rise

period of 10 min[26].

Restriction/modification assists MqsR/MqsA/MqsC during persistence. Since cells with MqsR/MqsA/MqsC weather T2 attack by rapidly becoming persistent and resuscitate, which indicates inactivation of phage, we hypothesized that another system works while the cells are dormant to clear T2 phage. Therefore, we investigated whether cells that lack the restriction systems/DNA processing enzymes encoded by *mrr, mcrB,* and *recD* are susceptible to T2 attack and found that inactivation of the McrBC system was most important for defending against T2 since its inactivation reduces cell viability by 100,000-fold (**Table S4A**). Critically, we found that the MqsR/MqsA/MqsC works with the McrBC restriction/modification system to defend against T2 phage since cells deficient in McrBC had 10-fold less survival in the presence of MqsR/MqsA/MqsC (**Table S4B**) as well as a 4-fold increase in PFU (**Table S4C**). Therefore, MqsR/MqsA/MqsC works in concert with restriction/modification systems to inhibit T2 phage.

DISCUSSION

Our results demonstrate phage inhibition systems form persister cells. Moreover, this persistence allows at least some of the cells to survive phage attack by providing time for phage DNA degradation by restriction/modification systems. Hence, our results suggest toxin/antitoxin systems act first to reduce metabolism, followed by restriction/modification systems, to undermine phage attack. Similarly, CRISPR-Cas has been shown to act first, followed by restriction/modification systems in *Listeria* spp[47]. Our results showing synergy between toxin/antitoxin and restriction/modification systems are significant since both of these phage defense systems are found in nearly all bacteria (unlike many other phage inhibition systems such as CRISPR-Cas, CBASS and retrons that are found in 39%, 13% and 11% of procaryote genomes, respectively[43]), which suggests this interaction is widespread and may be dominant for phage inhibition. Our results also provide the first reproducible link between toxin/antitoxin systems and persister cells under physiological conditions (rather than through overproduced toxins and discredited deletion strains[42]).

These results also have implications for other phage inhibition systems in that, rather than causing cell

death as frequently claimed with little evidence[1, 24, 25, 29] (as also claimed for the MqsR/MqsA/MqsC system studied here without evidence[45]) or claimed after non-physiological overexpression[10], these anti-phage systems probably induce dormancy; i.e., persistence, which allows the cell to thwart the phage in the same manner as we studied here. Along with the formation of persister cells, some cells indubitably succumb to
120 phage attack; however, our results indicate the primary purpose of the anti-phage systems is not what has been termed 'abortive infection' in which the phage inhibition system kills the cells[29, 45], but instead, these systems protect the cells and allow them to survive phage attack. Similar results showing dormancy have been seen with type VI CRISPR-Cas and restriction/modification systems[47].

Corroborating our findings, many phage-inhibition systems reduce energy metabolites like ATP
125 (RADAR[11] and Detocs[36]) and NAD^+ (Thoeris[23]), and the reduction of energy metabolites like ATP consistently induces persistence[8, 21]. Furthermore, reduction of ATP by toxin/antitoxin systems leads to persistence[6].

Central to persister cell formation is accumulation of (p)ppGpp[21, 41, 50]; hence, it is expected that if cells prevent phage propagation by becoming persisters, they will do so via an increase in (p)ppGpp. Therefore,
130 to counter this bacterial defense, if our hypothesis/data are correct in this report, phages should try to reduce (p)ppGpp levels. Critically, this has been recently shown with *Pectobacterium* spp. phage PcCB7V, which encodes the nucleotide pyrophosphatase MazG that is enriched against TIR-STING anti-phage systems and reduces (p)ppGpp[14]. In addition, these authors demonstrated that (p)ppGpp is increased during phage attack[14].

135 Our model (**Fig. 2**) is that by invoking persistence (i.e., dormancy) through phage attack, back-up phage inhibition systems like restriction modification systems can degrade the phage genome since restriction enzymes do not require any resources from the cell for activity; i.e., Gibbs free energy is less than zero for phage DNA degradation due to the increase in entropy, so degradation of the phage genome can occur while the cells sleep. This general concept of allowing back-up DNA-targeting enzymes to function while the cell
140 is dormant has been proposed for the type III/VI CRISPR-Cas systems[30, 44] and demonstrated while this work was being prepared for type VI CRISPR-Cas in *Listeria* spp.[47]; however, the benefit of dormancy has

not been suggested for the plethora of recently-discovered, non-CRSPR-Cas phage inhibition systems. Here we demonstrate that (i) dormancy in the form of persistence is a key aspect of phage inhibition of non-CRISPR-Cas systems and that (ii) restriction/modification is utilized during the dormant state.

Since phages rarely eradicate populations, a logical extension of our results is that for Bacteria and Archaea, phage attack should lead to a small population of surviving cells in the persister state. Hence, for medical applications, a combination treatment will likely be required that recognizes that persister cells will develop during phage therapy. Therefore, compounds such as mitomycin C, which is capable of killing a wide range of cells in the persister state[20], will likely be required.

Since the cells are becoming dormant (i.e., persisters) rather than dying, our results also suggest that after phage attack, the cells which survive phage, resuscitate upon detecting nutrients, as we have shown using single cell microscopy for persister cells[40, 41, 50], by using chemotaxis proteins and sugar transport membrane proteins, by reducing cAMP and ppGpp internal signals, and by increasing HflX activity to convert dimerized ribosomes (100S ribosomes) into active 70S ribosomes.

Finally, our results show that, like myriad other stresses such as starvation[17], oxidation[16], and antibiotic challenge[21], bacteria form persister cells to survive the extreme stress of phage attack. Hence, the formation of persister cells is a consistent and conserved means to weather stress, which we suggest as our **Conserved Bacterial Stress Survival** (**CBaSS**) theory.

MATERIALS AND METHODS

Bacteria and growth conditions. Bacteria and plasmids are shown in **Table S5**, and cells were cultured at 37°C in LB supplemented with 30 µg/ml of chloramphenicol (to retain plasmids). The complete MqsR/MqsA/MqsC plasmid-based system (pCV1-*mqsRAC* and pCV1 empty plasmid control) was verified via Plasmidsaurus sequencing.

Persister formation assay and phage titers. Single colonies were cultured overnight in LB-chloramphenicol, then diluted 100X and grown to a turbidity of 0.5 at 600 nm. T2 phage was then added (multiplicity of infection ~ 0.1) for one hour, then the cells were washed twice with phosphate buffered

saline (PBS, 8 g of NaCl, 0.2 g of KCl, 1.15 g of Na_2HPO_4, and 0.2 g of KH_2PO_4 in 1000 mL of dH_2O) and cultured for three hours with LB-ampicillin (100 µg/mL, 10 MIC) or LB-ciprofloxacin (5 µg/mL, 10 MIC) to kill non-persister cells[21]. Cells were washed twice with PBS and enumerated on LB-Cm plates to measure
170 persistence. To determine phage titers, after 30 minutes of phage contact, supernatants were diluted with phage buffer (0.1 M NaCl, 10 mM $MgSO_4$ and 20 mM Tris-HCl pH 7.5), and plaques were enumerated on top agar, TA, (1% tryptone, 0.5% NaCl, 1.5% agar lower layer and 0.4% agar top layer) double layer plates.

Persister resuscitation assay. Single-cell microscopy of resuscitating persister cells was performed as described previously[18] using LB agarose gel pads that were observed up to 3 h via a light microscope (Zeiss
175 Axioscope.A1, bl_ph channel at 1000 ms exposure). The persister cells were generated by the addition of T2 phage at 0.1 MOI for 1 h. The microscope was maintained at 37ºC by placing it in a vinyl glove box (Coy Labs), which was warmed by an anaerobic chamber heater (Coy Labs, 8535-025).

Metabolic activity via flow cytometry. To ascertain metabolic activity, *E. coli* MG1655/pCV1-*mqsRAC* was grown to a turbidity of 0.5 at 600 nm and T2 phage was added (MOI 0.1) for 1 h. Cells were washed,
180 resuspended in PBS, stained for metabolic activity (BacLight™ RedoxSensor™ Green Vitality Kit, Thermo Fisher Scientific Inc., Waltham, MA, USA), which measures the cellular redox state. The fluorescence signal of 100,000 cells was analyzed by flow cytometry (Beckman Coulter LSRFortessa) using the FL1 (530 ± 30 nm).

qRT-PCR. To quantify transcription after T2 phage attack (15 min at MOI 0.1) in the presence of
185 MqsR/MqsA/MqsC, RNA was isolated by cooling rapidly using ethanol/dry ice in the presence of RNA Later (Sigma) using the High Pure RNA Isolation Kit (Roche). The following qRT-PCR thermocycling protocol was used with the iTaq™ universal SYBR® Green One-Step kit (Bio-Rad): 95 ºC for 5 min; 40 cycles of 95 ºC for 15 s, 60ºC for 1 min for two replicate reactions for each sample/primer pair. The annealing temperature was 60ºC for all primers. Primers are shown in **Table S6**.

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Figure Legends

Fig. 1. Heterogenous single-cell resuscitation after phage attack. Representative images (from seven independent cultures) of resuscitation of cells producing MqsR/MqsA/MqsC (**A**) and exponential cells (**B**) after 1 h as determined with light microscopy (Zeiss Axio Scope.A1) using LB agarose gel pads. The persister cells were generated by the addition of T2 phage at 0.1 MOI for 1 hour. Cells with the empty plasmid are not shown due to the cellular debris that stems from complete eradication by T2 phage. Black arrows indicate cells with immediate waking, yellow arrows indicate cells with delayed waking, red arrows indicate cells that wake then die, and blue arrows indicate cells that elongate. Black arrows were not added to exponential cultures pictures because all of them divide. Data for percentages are shown in **Table S5**.

Fig. 2. Schematic illustrating phage inhibition systems invoke dormancy. Our results indicate that after phage attack, bacteria become persister cells (i.e., dormant), through RNase decay via toxin MqsR of the MqsR/MqsA/MqsC tripartite toxin/antitoxin system. During this period of inactivity, restriction/modification systems, which do not require cell resources, degrade phage DNA. Upon favorable conditions (nutrients provided), the persister cells resuscitate, having survived the phage attack.

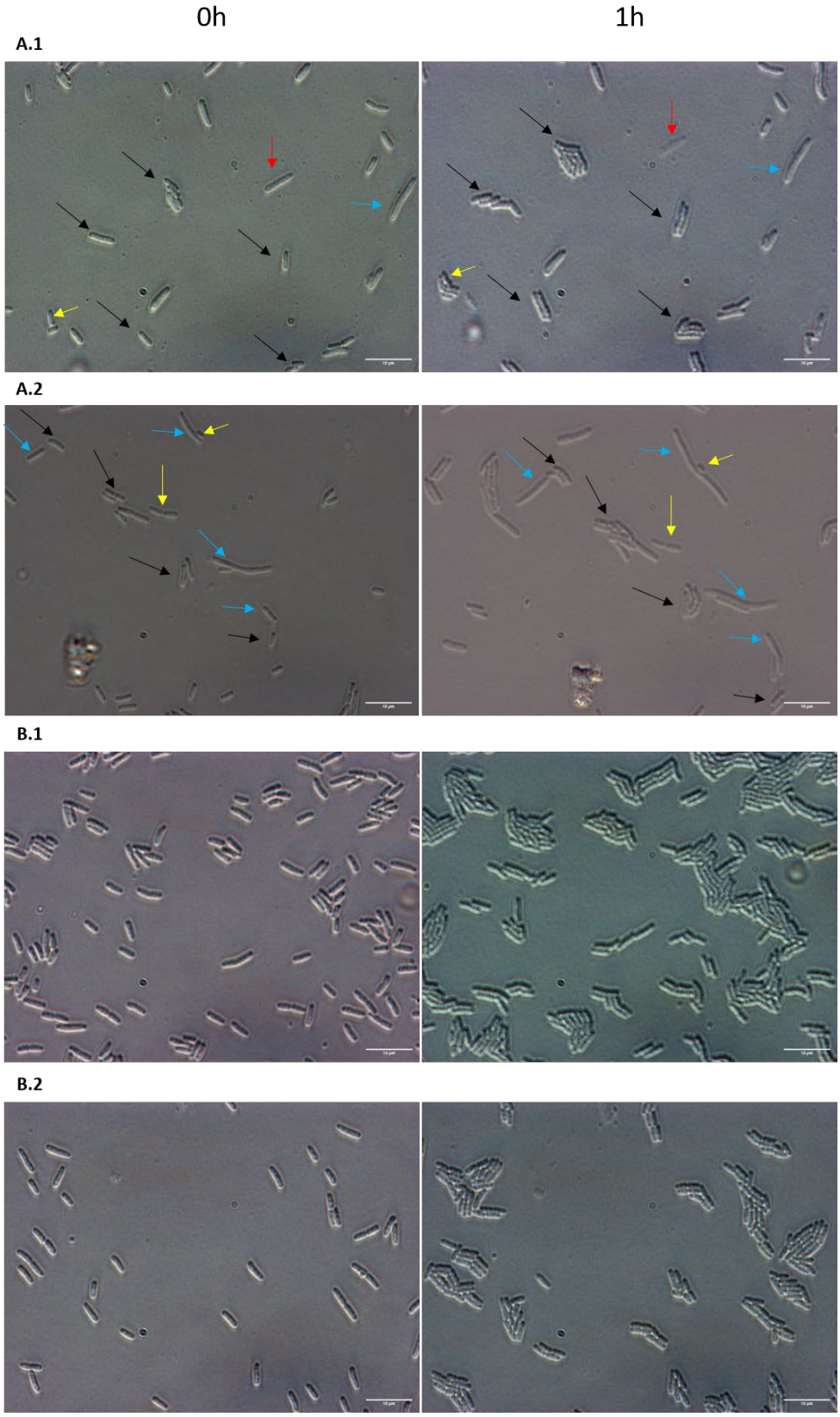

Figure 1

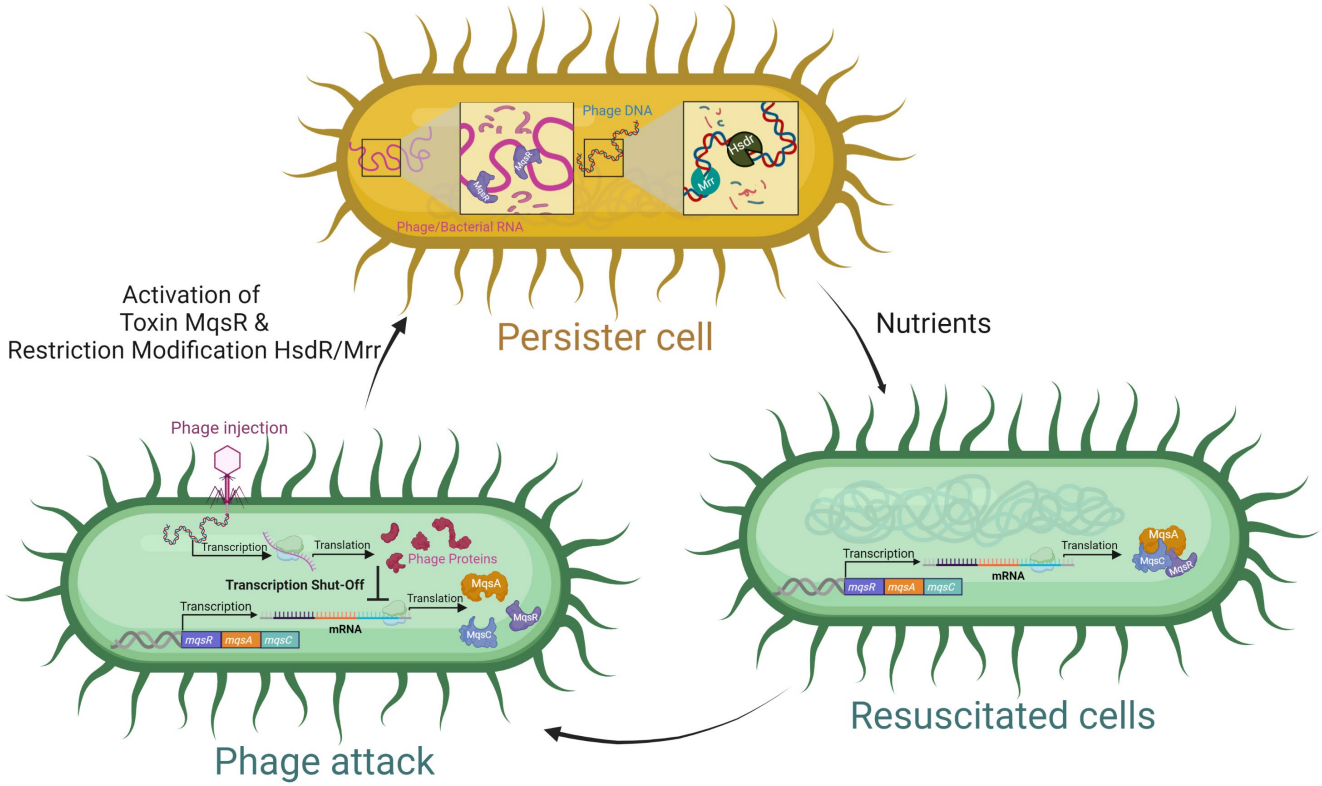
Phage DNA
Phage/Bacterial RNA
Persister cell
Activation of
Toxin MqsR &
Restriction Modification HsdR/Mrr
Nutrients
Phage injection
Transcription
Translation
Phage Proteins
Transcription Shut-Off
MqsA
MqsR
MqsC
mRNA
mqsR
mqsA
mqsC
Phage attack
Resuscitated cells

Figure 2

Supporting Information

Toxin/Antitoxin Systems Induce Persistence and Work in Concert with Restriction/Modification Systems to Inhibit Phage

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Table S1. MqsR/MqsA/MqsC inhibits T2 phage. Cells were contacted with T2 phage at 0.1 MOI for 1 h and with antibiotics (10 MIC) for 3 h. Bold results indicate million-fold difference for inhibiting T2 phage. 'Std Dev' indicates standard deviation.

Strain	Condition	CFU/ml average	Std Dev	Survival %
MG1655/pCV1	No phage	2×10^9	5×10^8	100%
	Phage T2	**3.4×10^2**	8×10^1	100%
	No phage + 100 µg/ml ampicillin	1×10^8	1×10^8	7%
	No phage + 5 µg/ml ciprofloxacin	2.4×10^6	1×10^6	0.2%
	Phage T2 + 100 µg/ml ampicillin	0.00	0.00	0%
	Phage T2 + 5 µg/ml ciprofloxacin	0.00	0.00	0%
MG1655/pCV1-*mqsRAC*	No phage	1.5×10^9	2×10^8	100%
	Phage T2	**4×10^8**	1×10^8	100%
	No phage + 100 µg/ml ampicillin	2.5×10^8	0.7×10^8	16%
	No phage + 5 µg/ml ciprofloxacin	3×10^6	1×10^6	0.2%
	Phage T2 + 100 µg/ml ampicillin	4×10^7	1×10^7	8%
	Phage T2 + 5 µg/ml ciprofloxacin	1×10^6	4×10^5	0.3%

Table S2. Percentage of persister cell waking after treatment with T2 phage (0.1 MOI) for 1 h.

Sample	No of cells	% of waking in 0.5 h (No)	% of waking in 1 h (No)	% of lagged waking (No)	% Elongated cells (No)	% of dead (No)	Unmodified cells (No)
1	33	-	88 % (29/33)	9 % (3/33)	9% (3/33)	1 % (1/33)	
2	25	20 % (5/25)	24 % (6/25)	-	16% (4/25)	12 % (3/25)	40 % (10/25)
3	27	41 % (11/27)	37 % (10/27)	-	11% (3/27)	7 % (2/27)	7 % (2/27)
4	65	58 % (38/65)	28 % (18/65)	-	20% (13/65)	1 % (1/65)	8 % (5/65)
5	23	26 % (6/23)	22 % (5/23)	30 % (7/23)	35% (8/23)	4 % (1/23)	17 % (4/23)

Table S3. Phage attack does not induce ribosome inactivation proteins. Fold changes for the wild-type producing MqsR/MqsA/MqsC after 15 min of T2 attack are relative to the wild-type strain with the empty plasmid with 15 min of T2 attack as determined by qRT-PCR. Cycle numbers (C_t) are indicated for each sample including that for the target genes as well as that of the house-keeping gene, *rrsG,* which was used to normalize the data. Fold were calculated as described earlier[33]:

$$2^{-(C_{t\ target_cas2+} - C_{t\ purM_cas2+})} / 2^{-(C_{t\ target_cas2-} - C_{t\ purM_cas2-})}$$

Gene	***rrsG***		***rmf***		***hpf***		***raiA***	
Strain	MG1655 /pCV1	*MG1655 /pCV1-mqsRAC*	MG1655 /pCV1	*MG1655 /pCV1-mqsRAC*	MG1655 /pCV1	*MG1655 /pCV1-mqsRAC*	MG1655 /pCV1	*MG1655 /pCV1-mqsRAC*
C_T	4.6 ± 0.5	4.48 ± 0.05	16.9 ± 0.6	17.01 ± 0.2	15.3 ± 0.6	15.2 ± 0.1	12.8 ± 0.2	14.7 ± 0.1
ΔC_T			12.3 ± 0.1	12.6 ± 0.2	10.7 ± 0.2	10.7 ± 0.1	8.2 ± 0.3	10.2 ± 0.2
$\Delta\Delta C_T$			0.3 ± 0.1		0.02 ± 0.02		2.0 ± 0.1	
fold			1.2		1.05		4.0	

Table S4. MqsR/MqsA/MqsC inhibits T2 phage through cooperation with restriction/modification systems. (**A**) Survival after 30 min of T2 phage infection at 0.1 MOI. (**B**) BW25113 and BW25113 *mcrB* cells producing MqsR/MqsA/MqsC from its native promoter were contacted with T2 phage at 0.1 MOI for 1 h and with ampicillin (10 MIC) for 3 h. Bold indicates CFU comparison of persister cells. (**C**) Phage titers after 30 min of infection at 0.1 MOI**.** Std Dev' indicates standard deviation. Part **A** includes results from two independent cultures whereas parts **B** and **C** include results from four independent cultures each.

A

Strain	CFU/ml average	Std Dev
BW25113	1.4×10^6	3×10^5
BW25113 Δ*mrr*	7.8×10^4	2×10^4
BW25113 Δ*recD*	4.0×10^6	4×10^5
BW25113 Δ*mcrB*	1.7×10^2	1×10^2

B

Strain	Condition	CFU/ml average	Std Dev
wild-type/pCV1-*mqsRAC*	No phage	1.7×10^9	3×10^8
	Phage T2	3.8×10^8	3×10^7
	No phage + 100 µg/ml Ampicillin	2×10^8	3×10^7
	Phage T2 + 100 µg/ml Ampicillin	**1.7×10^6**	5×10^5
ΔmcrB/pCV1-*mqsRAC*	No phage	7.6×10^8	2×10^8
	Phage T2	1.3×10^7	6×10^6
	No phage + 100 µg/ml Ampicillin	1.9×10^7	5×10^6
	Phage T2 + 100 µg/ml Ampicillin	**2×10^5**	2×10^5

C

Strain	PFU/ml average	Std Dev	CFU/ml average	Std Dev
BW25113/pCV1-*mqsRAC*	7.4×10^5	1.6×10^5	1.8×10^8	7×10^7
BW25113 *ΔmcrB*/pCV1-*mqsRAC*	2.9×10^6	9.4×10^5	3.2×10^7	1×10^7

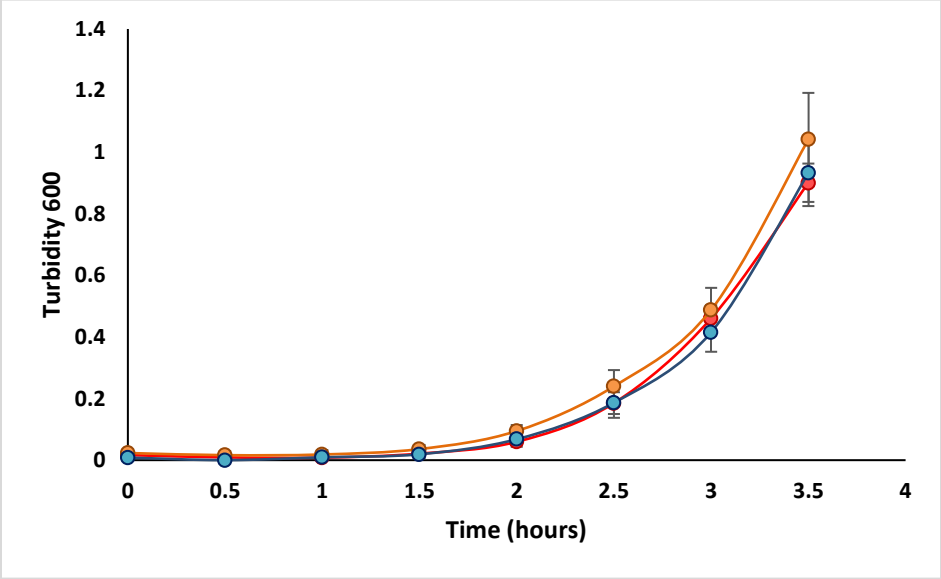
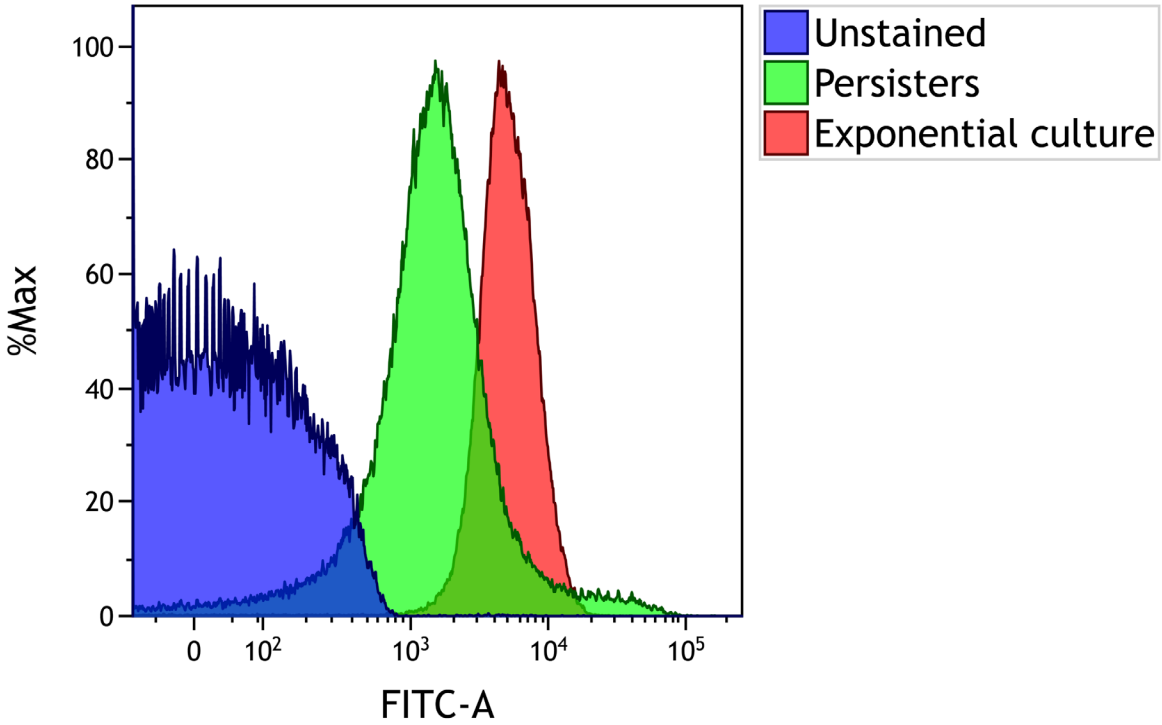
Table S5. ***E. coli*** **bacterial strains and plasmids utilized.** Cm^R is chloramphenicol resistance, and Kan^R is kanamycin resistance.

Strains and Plasmids	Features	Source
Strains		
MG1655	F^- lambda- *ilvG- rfb*-50 *rph*-1	[3]
BW25113	*rrnB3 ΔlacZ4787 hsdR514 Δ(araBAD)567 Δ(rhaBAD)568 rph-1*	[2]
BW25113 *mcrB*	BW25113 *mcrB* Ω Kan^R	[2]
BW25113 *recD*	BW25113 *recD* Ω Kan^R	[2]
BW25113 *mrr*	BW25113 *mrr* Ω Kan^R	[2]
Plasmids		
pCV1	Cm^R	[45]
pCV1-*mqsRAC*	P_{mqsRAC}::*mqsRAC;* Cm^R	[45]

Table S6. qRT-PCR primers

Gene	Primer pair	Length	Sequence (5'→3')	T_m (ºC)	Product size
rrsG ***(housekeeping)***	*rrsG* Fow	20 bp	TATTGCACAATGGGCGCAAG	56.9	234 bp
	rrsG Rev	20 bp	ACTTAACAAACCGCCTGCGT	50.0	
raiA	*raiA* Fow	25 bp	CCAGCAAACAAATGGAAATTACTCC	61.3	122 bp
	raiA Rev	22 bp	TTGTGGCTCTTTGGACAGAATG	60.8	
rmf	*rmf* Fow	24 bp	AACGGGCACATCAACGTGGTTATC	64.6	100 bp
	rmf Rev	20 bp	CCTCCCAGCCATTGTGACCT	64.5	
hpf	*hpf* Fow	20 bp	TCGAGATCACCGAGGCACTG	64.5	119 bp
	gpf Rev	24 bp	GGTGTGGGTGACTTTCTCCACTTT	64.6	
hflX	*hflX* Fow	22 bp	TTCCGCCGGTGTCGAAGCATTG	66.4	124 bp
	hflX Rev	20 bp	CGAAGCACCCGTCGCTTTGA	64.5	

Supplemental Figure S1. Growth of four independent colonies of each strain in LB at 37ºC. 'MG1655' is the wild-type host (red), 'MG1655/pCV1' is the host with the empty plasmid (negative control) (orange) and 'MG1655/pCV1-mqsRAC' is the host with the MqsR/MqsA/MqsC tripartite toxin/antitoxin system (blue).



Supplemental Figure S2. Metabolic activity via flow cytometry. MG1655/pCV1-mqsRAC was grown to a turbidity of 0.5 at 600 nm and T2 phage was added (MOI 0.1) for 1 h, then stained for metabolic activity using the BacLight™ RedoxSensor™ Green Vitality Kit ('persister' cells) and compared to exponentially-growing cells stained with the same kit ('Exponential culture'). RedoxSensor Green measures the cellular redox state. 'Unstained' indicates results with an overnight, unstained sample. '%Max' indicates the relative number of cells (100,000 cells total), and 'FITC-A' is indicative of the intensity of the RedoxSensor Green fluorescence. One representative image of two independent cultures shown.