Retraction

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Retraction for "Bacterial persistence by RNA endonucleases," by Etienne Maisonneuve, Lana J. Shakespeare, Mikkel Girke Jørgensen, and Kenn Gerdes, which was first published July 25, 2011; 10.1073/pnas.1100186108 (*Proc Natl Acad Sci USA* 108: 13206–13211).

The authors wish to note the following: "In this article, we reported that successive deletion of 10 toxin-antitoxin (TA) modules of *Escherichia coli* K-12 progressively reduced the level of spontaneously formed antibiotic-tolerant persisters. After the publication of the article, we became aware that crucial strains including some of the TA module deletion series were infected with bacteriophage phi80, a notorious laboratory contaminant. We thus reconstructed and analyzed all strains in a phi80-free background to examine the effect of lysogenization on spontaneous persister formation. We found that major parts of our original data (Figs. 2, 3, and 4, and Figs. S6 and S7) were affected by phi80 carriage and our new data (1) no longer support that successive deletion of TA loci progressively reduces persistence. The conclusion that ectopic overproduction of individual TA-encoded mRNase toxins dramatically increases antibiotic tolerance is still valid (Fig. 1, Figs. S3 and S4). We believe that the most appropriate course of action is to retract the paper. We offer our sincerest apologies to the scientific community for these inadvertent errors and for any inconvenience they may have caused."

1. Harms A, Fino C, Sørensen MA, Semsey S, Gerdes K (2017) Prophages and growth dynamics confound experimental results with antibiotic-tolerant persister cells. *MBio* 8: e01964-17.

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RETRACTION

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Bacterial persistence by RNA endonucleases

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Bacteria form persisters, individual cells that are highly tolerant to different types of antibiotics. Persister cells are genetically identical to nontolerant kin but have entered a dormant state in which they are recalcitrant to the killing activity of the antibiotics. The molecular mechanisms underlying bacterial persistence are unknown. Here, we show that the ubiquitous Lon (Long Form Filament) protease and mRNA endonucleases (mRNases) encoded by toxin-antitoxin (TA) loci are required for persistence in *Escherichia coli*. Successive deletion of the 10 mRNase-encoding TA loci of *E. coli* progressively reduced the level of persisters, showing that persistence is a phenotype common to TA loci. In all cases tested, the antitoxins, which control the activities of the mRNases, are Lon substrates. Consistently, cells lacking *lon* generated a highly reduced level of persisters. Moreover, Lon overproduction dramatically increased the levels of persisters in wild-type cells but not in cells lacking the 10 mRNases. These results support a simple model according to which mRNases encoded by TA loci are activated in a small fraction of growing cells by Lon-mediated degradation of the antitoxins. Activation of the mRNases, in turn, inhibits global cellular translation, and thereby induces dormancy and persistence. Many pathogenic bacteria known to enter dormant states have a plethora of TA genes. Therefore, in the future, the discoveries described here may lead to a mechanistic understanding of the persistence phenomenon in pathogenic bacteria.

RelE | RelB | MqsR | MazF | drug tolerance

It has been known for many years that bacteria generate persisters tolerant to antibiotics (1, 2). Persisters are cells that have entered a nongrowing dormant state that protects them from the lethal action of many antibiotics and other harmful environmental insults. The drug tolerance of persisters and their common presence may contribute to the intractability of chronic and recurrent infections (3). It has been proposed that bacterial persistence depends on epigenetic processes that reflect fortuitous deterioration toward cell death. Another view conceives that bacterial persistence is a programmed phenomenon with a genetic basis that has evolved to allow the organisms to survive sudden environmental insults. Consistent with this view, persister cells are generated stochastically at a constant low frequency from exponentially growing populations of *Escherichia coli* cells (4).

A genetic basis for bacterial persistence was initially proposed by the observation that certain mutations in the *hipA* gene of *E. coli* induced a high level of persisters (5). Later analyses showed that High persistence protein A (HipA) is a "toxin" encoded by the type II *hipAB* toxin-antitoxin (TA) locus. HipB is the corresponding antitoxin that combines with and neutralizes HipA (6, 7). HipA is thought to inhibit translation by phosphorylation of the essential translation factor Elongation Factor-Tu (7). Generally, prokaryotic TA loci code for two components: a toxin that inhibits cell growth and an antitoxin that counteracts the toxin. In type I TA loci, the antitoxins are small antisense RNAs that repress translation of the toxin genes (8, 9), whereas in type II loci, the antitoxins are proteins that combine with and neutralize the toxins (10). Type III TA loci encode small RNA antitoxins that combine with and neutralize protein toxins (11).

Based on toxin sequence similarities, type II loci have been divided into gene families (10, 12). RelE of *E. coli* belongs to a well-described toxin superfamily with many homologs in both bacteria and archaea (13). RelE is a riboendonuclease that cleaves mRNA positioned at the ribosomal A site, between the second and third bases of the A-site codon (14, 15). Therefore, ectopic production of RelE rapidly shuts down translation and halts cell growth (16). Interestingly, RelE from archaea cleaves mRNA at the ribosome in *E. coli*, whereas RelE from *E. coli* cleaves mRNA positioned at mammalian and mitochondrial ribosomes (17, 18).

In addition to *hipAB, E. coli* K-12 has 10 type II TA loci, all of which encode mRNA endonucleases (mRNases) (Fig. S1). In all cases, ectopic production of the mRNases leads to a rapid degradation of mRNA and shut-down of translation (14, 19–21). Six of the mRNases (RelE, YoeB, HigB, YhaV, YafO, and YafQ) cleave mRNA positioned at the ribosomal A site (14, 15, 21–24), whereas the other 4 (MazF, ChpB, MqsR, and HicA) cleave RNA site-specifically, independent of the ribosomes (19, 20, 25). The 10 mRNases and their cognate antitoxins are encoded by bicistronic operons that are autoregulated by the antitoxins, which bind to operator sequences in the TA promoter regions (10). The TA complexes bind stronger and cooperatively to the operators; thus, the mRNases themselves function as corepressors of transcription (26). Interestingly, if an mRNase is in excess of its cognate antitoxin, the mRNase destabilizes the promoter–operator complex, and thereby induces TA operon transcription (26). The TA operons of *E. coli* that encode mRNases are induced by amino acid starvation via a Lon (Long Form Filament)-dependent mechanism (21, 27, 28). Direct evidence of Lon degradation of antitoxins has been obtained for RelB, MqsA, and YefM (27, 29) (Fig. S2), whereas indirect evidence has been obtained in the cases of MazE, MazE-2 (ChpBI), HicB, YafN, and YgjM (HigA) (19, 28). Hence, Lon controls the activities of the mRNases by controlling the levels of the antitoxins.

The first indication that the mRNases might be involved in persistence came from the observation that their ectopic overproduction not only very efficiently inhibited translation (consistent with mRNA cleavage) but induced dormancy from which the cells could be rapidly resuscitated by the induction of cognate antitoxin genes (21, 30, 31). Cells overproducing TA-encoded mRNases were, similar to persister cells, tolerant to antibiotics (32–35). Moreover, cell populations enriched for persisters had increased levels of TA mRNAs (33, 34), and deletion of *yafQ* (encoding a RelE homolog) resulted in reduced persistence of cells in a biofilm but not in planktonic cells (32). Interestingly, the type I TA locus *tisAB/istR* (36) was required for persister generation after induction of the SOS response (37, 38). We show here that type II TA loci encoding mRNases are responsible for persister cell formation during exponential growth and during the stationary phase. The requirement for Lon protease in persister generation supports a simple model in which

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The authors declare no conflict of interest.

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stochastic activation of the mRNases in a small fraction of the cells induces dormancy and, hence, persistence.

Results

Ectopic Production of mRNases Induces Persistence. Persisters are typically measured as the fraction of cells in a culture surviving prolonged antibiotic treatment. Treatment of an exponentially growing culture of wild-type (*wt*) *E. coli* K-12 cells with either the fluoroquinolone ciprofloxacin or the β-lactam ampicillin produced typical biphasic survival kinetics with an initial rapid killing of the bulk of the cells and a persister subpopulation reaching around 10^3–10^4 cells/mL (Fig. 1 *B* and *C*). Using this approach, we measured the levels of persisters after overproduction of five different mRNases (RelE, YafO, MqsR, HigB, and MazF). Indeed, in all

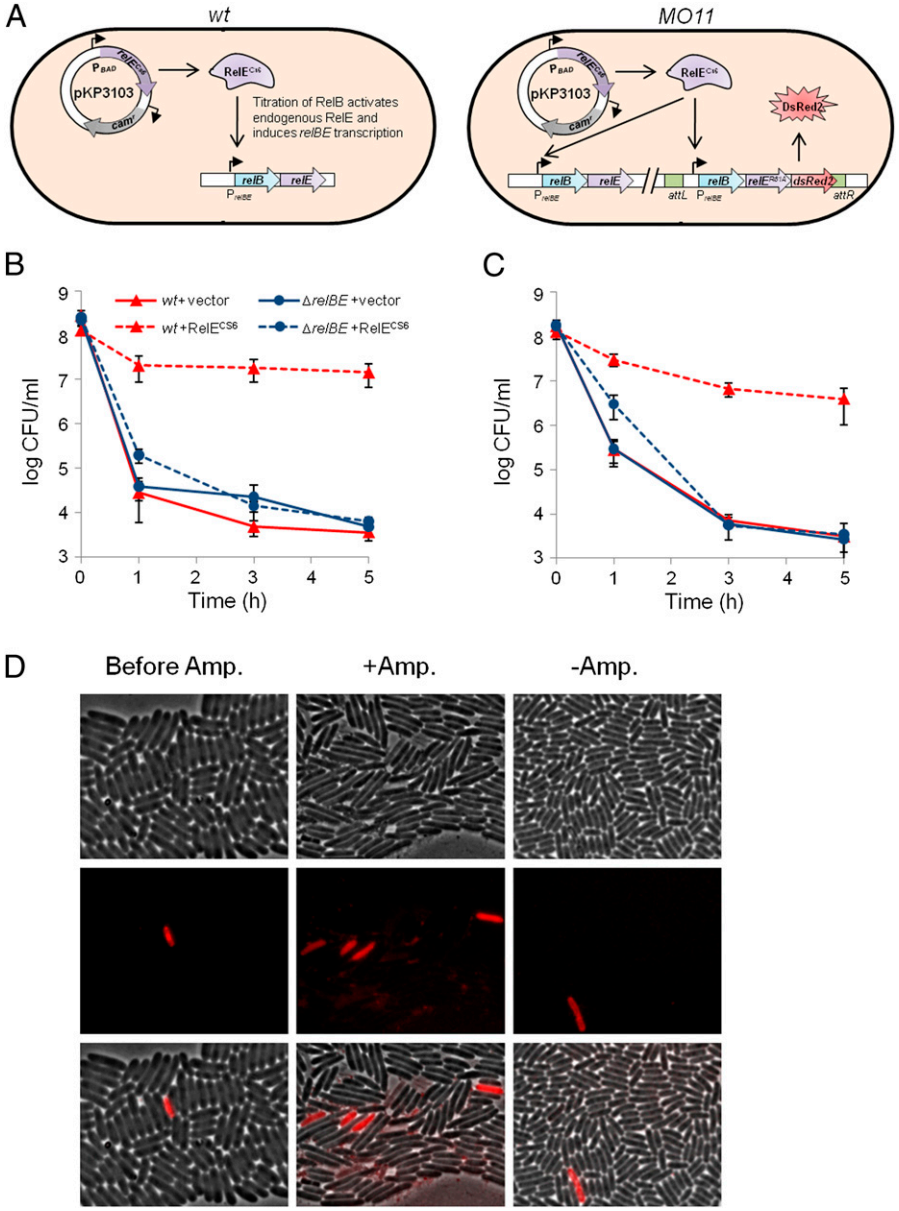

Fig. 1. Activation of endogenous RelE induces persister cell formation. (*A*) Genetic setup of the strains used to induce endogenous RelE encoded by the *relBE* operon in MG1655 (*wt*, *relBE*$^+$) and MO11 (*relBE*$^+$*relBE*^{R81A}*::dsRed2*) strains. (*Left*) Ectopic expression of the nontoxic variant RelE^{cs6} (from pKP3103) in *wt* cells induces RelE activity and transcription of the endogenous *relBE* operon by titration of RelB. (*Right*) Ectopic expression of RelE^{cs6} induces RelE activity and production of DsRed2 because MO11 contains a *relBE*^{R81A}*::dsRed2* operon fusion inserted at *attB*. Mutant *relE*^{R81A} encodes a nontoxic RelE variant, such that the two strains (*wt* and MO11) express comparable amounts of active RelE in the induction experiment. Exponentially growing cultures of MG1655 (*wt*) and its Δ*relBE* derivative overexpressing RelE^{cs6} from plasmid pKP3103 (pBAD33*::his6::relE*^{cs6}) were exposed to 1 μg/mL ciprofloxacin (*B*) or 100 μg/mL ampicillin (*C*) after 45 min of induction by arabinose (0.2%), followed by quenching of induction by glucose (0.2%) (Details are provided in *SI Materials and Methods*). The two strains harboring the empty vector plasmid (pBAD33) were included for comparison. Cells were grown in LB containing chloramphenicol (50 μg/mL) at 37 °C. The numbers of surviving cells were determined by plating on solid medium. The graphs show averages of five independent experiments; error bars indicate the SDs. (*D*) Activation of transcription of endogenous *relBE* at the single-cell level by induction of *relE*^{cs6} detected by dsRed2 fluorescence. Exponentially growing cultures of MO11 strain (Fig. 1*A*) overexpressing RelE^{cs6} from plasmid pKP3103 before (*Left*) and after 5 h of ampicillin treatment (100 μg/mL; *Center*) are shown. (*Right*) Cells are shown after 5 h without ampicillin as a control. Induction of *relE*^{cs6} was as described in *B* and *C*. Images represent phase contrast (*Top*), fluorescence (*Middle*), and merged (*Bottom*) images, respectively. Amp., ampicillin.

cases, toxin overproduction strongly increased the persister fraction with the two different antibiotics, thus supporting that the initial observations (32–35) are generally valid (Fig. S3).

Endogenously Encoded RelE Can also Induce Persistence. Although overproduction of mRNases appeared to induce a high level of persisters, certain unrelated proteins that become toxic when produced from plasmids have similar effects (35). Using *relBE* as a model system, we asked if an mRNase encoded by a TA locus could be activated to generate persisters. In exponentially growing cells, RelB is in excess of RelE, and RelE is thus kept inactive (26). We used a nontoxic variant of RelE (called RelE^{cs6}) that lacks mRNA cleavage activity but forms a stable complex with RelB to titrate endogenous RelB, and thereby activate endogenous RelE (26). As shown schematically in Fig. 1*A* (*Left*), ectopic production of RelE^{cs6} in MG1655 (*wt*) also induces transcription of the *relBE* operon as the result of titration of RelB (26). Consistent with titration of RelB, overproduction of RelE^{cs6} in the *wt* strain dramatically increased the persister fraction for both antibiotics tested (up to 4,000-fold) (Fig. 1 *B* and *C*). In contrast, no increase in persisters was observed when RelE^{cs6} was overproduced in an isogenic strain lacking the *relBE* locus 3 and 5 h after onset of antibiotic treatment. We note that when RelE^{cs6} was overexpressed in the Δ*relBE* strain, the initial slope was slightly reduced. This could be attributable to a slightly reduced growth rate of the cells because of overexpression of RelE^{cs6} which, in turn, might have influenced the antibiotic sensitivity of the bulk of the cells. Nevertheless, this minor increase disappeared at later time points, and the effect of overproducing RelE^{cs6} on persistence was fully convincing.

To obtain information on transcription of the endogenously encoded *relBE* locus in single cells, we inserted into the chromosome a *relBE^{R81A}::dsRed2* fusion into *wt* strains, resulting in strain MO11. A schematic drawing of the relevant genes of MO11 is shown in Fig. 1A (*Right*). The change of the conserved arginine at +81 to alanine renders RelE almost totally inactive (30), and we thereby secured that only the native *relBE* locus was functional.

Exponentially growing cells of MO11 (*relBE^{+}relBE^{R81A}::dsRed2*) did not produce fluorescent cells at a detectable level (<0.001%, n > 100,000 cells). We then titrated RelB by overexpressing RelE^{cs6} for 45 min as described above. We now observed a low number of fluorescent cells (0.083%, n > 2,000), consistent with titration of RelB and induction of *relBE* transcription (Fig. 1*D*, *Left*). As seen, *relBE* transcription was highly heterogeneous. After 5 h of ampicillin treatment, we observed an ~20-fold enrichment in the fraction of fluorescent cells (1.8%, n > 1,000), showing that cells in which *relBE* transcription had been induced to a high and detectable level were more tolerant to the antibiotic. This increase in the frequency of fluorescent cells after ampicillin treatment is visualized in Fig. 1*D* (*Middle*). No such increase was seen without ampicillin (Fig. 1*D*, *Right*). Thus, activation of RelE and persister cell formation are correlated.

To substantiate further that the high level of persistence seen with MO11 (*relBE^{+}relBE^{R81A}::dsRed2*) after induction of *relE^{cs6}* was attributable to activation of endogenous RelE, we performed a similar experiment with the isogenic Δ*relBE* derivative of MO11. As seen from Fig. S4, a much larger proportion of the cells of strain MO12 (MO11Δ*relBE*) became fluorescent after induction of *relE^{cs6}* (52.2%, n > 2,000; compared with 0.083% with MO11).

TA Loci Encoding mRNases Are Required for Persistence. A difficulty in investigating the role of mRNases encoded by TA loci is the multiplicity of the genes. For instance, deletion of single genes encoding mRNases (*relE*, *mqsR*, *yoeB*, *yafQ*, or *mazF*) had no effect on persister formation (33, 34). We extended this analysis by showing that deletion of any single TA locus of *E. coli* K-12 encoding an mRNase did not significantly reduce the level of

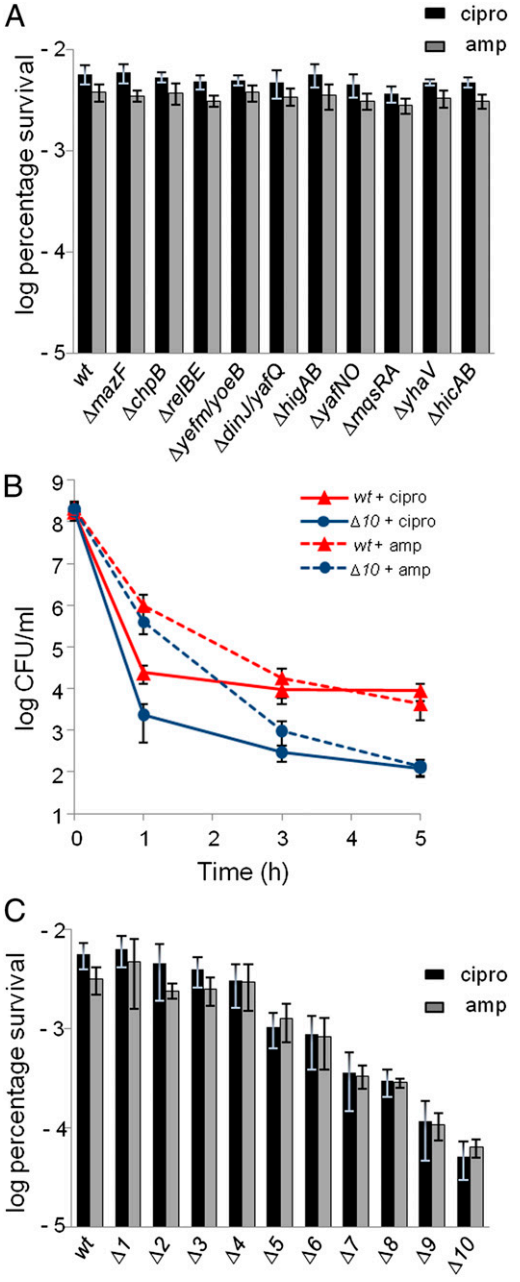

Fig. 2. TA-encoded mRNases are required for persister cell formation. (*A*) Cells of MG1655 (*wt*) and isogenic deletion strains (*mazF*, *chpB*, *relBE*, *yefM yoeB*, *higBA*, *dinJ yafQ*, *yafNO*, *mqsRA*, *yhaV*, and *hicAB*) were exposed to 1 μg/mL ciprofloxacin (black bars) or 100 μg/mL ampicillin (gray bars) in exponential growth phase. The percentage of survival after 5 h of antibiotic treatment was compared with that of the *wt* strain (log scale). amp, ampicillin; cipro, ciprofloxacin. (*B*) Exponentially growing cultures of MG1655 (*wt*) and MG1655 Δ*10TA* strains were exposed to 1 μg/mL ciprofloxacin or 100 μg/mL ampicillin. Surviving cells were determined as in Fig. 1. (*C*) Exponentially growing cells of MG1655 carrying increasing numbers of TA locus deletions were exposed to 1 μg/mL ciprofloxacin (black bars) or 100 μg/mL ampicillin (gray bars). Percentage of survival after 5 h of antibiotic treatment was compared with the *wt* strain (log scale). The bars show averages of at least three independent experiments; error bars indicate the SD. Genotypes: Δ*1TA* = MG1655ΔchpB; Δ*2TA* = Δ*1TA* Δ*mazF*; Δ*3TA* = Δ*2TA* Δ*relBE*; Δ*4TA* = Δ*3TA* Δ(*yefM yoeB*); Δ*5TA* = Δ*4TA* Δ(*dinJ yafQ*); Δ*6TA* = Δ*5TA* Δ*higBA*; Δ*7TA* = Δ*6TA* Δ(*prlF yhaV*); Δ*8TA* = Δ*7TA* Δ*yafNO*; Δ*9TA* = Δ*8TA* Δ*mqsRA*; Δ*10TA* = Δ*9TA* Δ*hicAB*. The entire genotypes of the strains are listed in Table S2.

persisters (Fig. 2*A*). In a second approach, we constructed a strain devoid of all 10 TA loci encoding mRNases (*SI Materials and Methods*). Exponentially growing cells of the Δ*10TA* strain generated a dramatic 100- to 200-fold reduction of persisters with both antibiotics (Fig. 2*B*). Deletion of the 10 TA loci did not affect the growth-rate of the strain (Fig. S5). A clear reduction of the number of persisters was also seen with stationary cell cultures treated with ciprofloxacin (Fig. S6). Importantly, the minimal inhibitory concentrations (MICs) of ciprofloxacin and ampicillin for the Δ*10TA* strain were similar to those of the *wt* strain (Table S1).

Next, we measured the persister levels generated by strains carrying deletions of 1 to 10 TA loci (Fig. 2*C*). Although the combined deletion of the first 4 TA loci (*mazEF*, *chpB*, *relBE*, and *yefM yoeB*) did not significantly reduce persistence, additional deletions were accompanied by a progressive reduction of persisters. To investigate if persister formation depended on particular TA loci, we constructed a deletion series using the reverse order of deletion. As shown in Fig. S7, a highly similar and progressive reduction of persistence was observed. These results show that TA loci encoding mRNases contribute cumulatively to the formation of persisters.

Lon Protease Is Required for Persistence. The fact that Lon degrades the antitoxins predicted that cells lacking Lon should produce a reduced level of persisters because such cells cannot activate the mRNases at a normal rate. Consistently, cells carrying a *lon* deletion showed a massive decrease in the persister fraction after ciprofloxacin treatment (250-fold) and also a significant decrease after ampicillin treatment (10-fold) (Fig. 3). This decrease was entirely specific to Lon, because none of the other five protease-deficient mutant strains that we tested exhibited reduced levels of persisters (Fig. 3). We also combined the Δ*lon* allele with the Δ*10TA*. As seen in Fig. 3, the *lon* deletion significantly reduced the level of persisters generated by the Δ*10TA* strain. To ensure that these effects were not indirect, we measured the MICs of the strains. The MICs of both antibiotics of the Δ*lon* and Δ*10TA* Δ*lon* strains were similar to those of the *wt* strain (Table S1).

Persister Cell Formation Depends on Lon-Mediated Degradation of the Antitoxins. To investigate further the involvement of Lon in persister cell formation, we used controlled overexpression of Lon from a plasmid (*SI Materials and Methods*). Previous studies showed that overproduction of Lon severely inhibited cell growth (39) and that deletion of five TA loci reduced Lon toxicity (40). To minimize artifacts attributable to Lon toxicity, we used a low level of inducer that did not change the cell growth rate. Lon expressed from the plasmid was functional because its presence complemented the mucoidy and the length phenotype of cells carrying a *lon* deletion. The modest overproduction of Lon in the *wt* strain resulted in a 70-fold increase in the level of persisters (Fig. 4). Overproduction of Lon in the Δ*lon* strain produced a similar level of persisters. However, neither the overproduction of a Lon mutated in the ATPase domain (Lon^{K362Q}) (41) nor the overproduction of a proteolytically defective Lon (Lon^{S679A}) (41) increased the level of persisters significantly (Fig. 4). These results show that it is Lon activity, per se, that induces the high level of persistence.

Finally, we considered the obvious possibility that persister cell formation in the Lon overproduction experiments depended on Lon-mediated degradation of the antitoxins that counteract the activities of the mRNases. To test this, Lon was overproduced in the Δ*10TA* strain. In this case, the level of persisters increased fourfold only (Fig. 4). This increase should be compared with the 70-fold increase seen when Lon was overexpressed in the *wt* strain (Fig. 4). This result strongly suggests that persister cell formation depends on Lon-mediated degradation of the TA-encoded antitoxins.

Discussion

The findings presented here yield unique mechanistic insights into the phenomenon of persister cell formation in *E. coli*. Simultaneously, our observations allow us to propose a long sought after common function to TA loci. The combined observations that overproduction of mRNases induced a persister-like state (32, 35) and that mRNase-induced stasis was reversible (30) prompted us to investigate the suggested connection between persistence and TA loci. We found strong evidence that TA loci encoding mRNases and the persistence phenomenon are intimately connected: Activation of endogenously encoded RelE and ectopic production of five mRNases induced antibiotic tolerance (Fig. 1

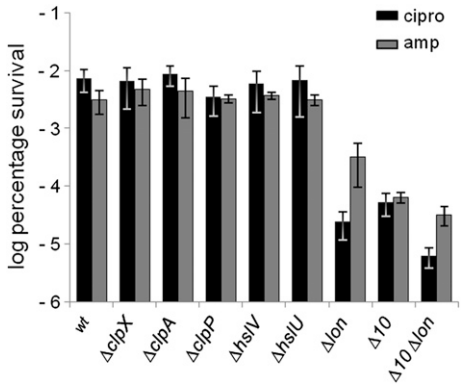

Fig. 3. Lon protease is required for persister formation in *E. coli*. MG1655 (*wt*) and isogenic deletion strains (Δ*lon*, Δ*clpP*, Δ*clpA*, Δ*clpX*, Δ*hslV*, Δ*hslU*, Δ*10TA*, and Δ*10TA*Δ*lon*) were exposed to 1 μg/mL ciprofloxacin (black bars) or 100 μg/mL ampicillin (gray bars) in exponential growth phase. The percentage of survival after 5 h was compared with the *wt* strain (log scale). The graphs show averages of at least three independent experiments; error bars indicate the SD. amp, ampicillin; cipro, ciprofloxacin.

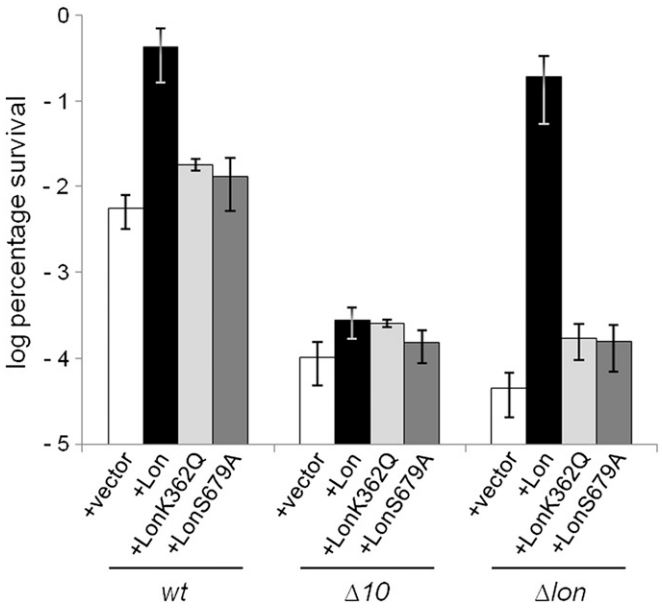

Fig. 4. Lon activates TA-encoded mRNases to generate persisters. Exponentially growing cells of MG1655 (*wt*), Δ*10TA*, and Δ*lon* overexpressing Lon protease or its mutant forms Lon^{K362Q} and Lon^{S679A} from plasmid pCA24N were exposed to 1 μg/mL ciprofloxacin (details are provided in *SI Materials and Methods*). The percentage of survival after 5 h was compared with that of a control strain carrying the plasmid vector (pCA24N) (log scale). The graphs show averages of four independent experiments; error bars indicate the SD.

GENETICS

and Fig. S3). Most importantly, however, deletion of all 10 TA loci encoding mRNases resulted in a dramatic 100- to 200-fold reduction of persister cell formation (Fig. 2*B*). This reduction was not attributable to deletion of a single TA locus. Rather, deletion of more and more TA loci gradually reduced the persister level (Fig. 2*C*). This result showed that the TA loci of *E. coli* had a cummulative effect on the level of persisters. In our initial deletion series, deletion of the first 4 TA loci did not significantly reduce the persister level (Fig. 2*C*, *Δ1TA* to *Δ4TA*). One reason for this could be that, by coincidence, none of the first 4 TA loci deleted (*mazF*, *chpB*, *relBE*, and *yefM yoeB*) were involved in persister cell formation. However, we find it more likely that the high number of TA loci yields such a high redundancy that the effect of the initial deletions was too insignificant to be detected in our assay. This view was supported by our second deletion series, which was generated by deleting the TA loci in the reverse order compared with our initial series (Fig. S7). In this case, inactivation of 2 TA loci (by deleting *hicAB* and *mqsR*) from the *wt* strain had no effect, whereas additional deletions again produced a gradual reduction of persisters, very similar to that of the initial deletion series. Inactivation of these 2 TA loci had a gross effect in the initial series (*Δ9* and *Δ10* in Fig. 2*C*). Thus, in the persistence phenotype, TA loci are clearly redundant, because several loci could be deleted without any measurable effect in our persistence assay, irrespective of the order of deletion (Fig. 2*C* and Fig. S7). At present, we do not understand the mechanistic basis for this redundancy. Independent stochastic induction of each TA locus present in a cell should predictably yield a straightforward additive contribution by each locus irrespective of the total number of TA loci present. Therefore, the result raises the possibility of the presence of an unknown activating signal that is transmitted to (or sensed by) each TA locus at a certain probability. The more TA loci present, the less the chance will there be that the signal escapes being transmitted to at least 1 TA locus. Thus, if a cell is equipped with TA loci, such that the activating signal leads to persistence with a probability of 95%, adding 1 more TA locus to that cell will only result in a slightly elevated level of persistence (e.g., 95–97%), and therefore not be detected in our persistence assay. We are now trying to determine if such a signal exists.

The antitoxins that regulate the activities of the mRNases are Lon substrates (21, 27, 28). Consequently, Lon is required for activation of the mRNases. Our results therefore predicted that cells lacking Lon should exhibit a reduced level of persisters. Indeed, this was the case (Fig. 3). A direct connection between Lon and TA loci in persister cell formation was supported by the observation that Lon overproduction induced very high levels of persisters in *wt* cells but not in cells lacking the 10 mRNases (Fig. 4). The simultaneous requirement for Lon and TA loci in persister formation led us to propose a simple model in which Lon activates the mRNases in a small fraction of cells by degradation of the antitoxins. Lon protease is a heat shock protein and is responsible for the degradation of ~50% of defective proteins in *E. coli* (42, 43). Because environmental factors affect Lon activity, it is possible that the levels of persisters can be modulated by the environment via Lon.

The biological function(s) of type II TA loci have been intensely debated, but it has not been possible to assign a single common function to them (10, 44–46). However, the observations described here, particularly the coherent patterns seen in Fig. 2*C* and Fig. S7, suggest a common function of TA loci in persistence. On the other hand, these observations, of course, do not exclude that particular TA loci have been recruited to perform other functions, as recently proposed for the *mqsRA* locus of *E. coli* (47). The entirely different tertiary folds of the translational inhibitors encoded by type II TA loci, such as RelE, MazF, and HicA, indicate that they have independent evolutionary origins (7, 15, 48–51). Therefore, the common genetic organization, transcriptional regulation, and cellular targets (i.e., translation) of type II TA loci suggest that these gene families emerged by convergent evolution. Because TA loci encoding mRNases and other types of inhibitors of translation are highly abundant in bacteria and archaea, our observations raise the possibility that the mechanism of persister generation revealed here is more general. *Mycobacterium tuberculosis*, well known for its long-term persistence in the human body (52–54), has at least 88 TA loci, the majority of which encode inhibitors of translation (55, 56). It is now important to learn if TA loci are also central to the persistence of pathogenic bacteria.

Materials and Methods

Persistence Assay Persistence was measured by determining the number of cfu/mL after exposure to 1 μg/mL ciprofloxacin or 100 μg/mL ampicillin (Sigma). Overnight cultures were diluted 100-fold in 10 mL of fresh medium and incubated for 2.5 h at 37 °C with shaking (typically reaching ~2×10^8 cfu/mL). Aliquots of 5 mL were then transferred into 28 × 114-mm Sarstedt tubes, and antibiotics were added. Tubes were placed with shaking at 37 °C for 5 h. For determination of cfu, 1-mL aliquots were removed at the indicated time and cells were spun, resuspended in fresh medium, serially diluted, and plated on solid medium. Persisters were calculated by dividing the number of cfu/mL in the culture after 5 h of incubation with the antibiotic by the number of cfu/mL in the culture before adding the antibiotic. A comprehensive description of materials and methods used is provided in *SI Materials and Methods*. Table S2 lists strains and plasmids used, while Table S3 lists the DNA oligonucleotides that we used.

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