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HipA-mediated antibiotic persistence via phosphorylation of the glutamyl-tRNA-synthetase

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Bacterial persistence has been shown to be an underlying factor in the failure of antibiotic treatments. Although many pathways, among them the stringent response and toxin–antitoxin modules, have been linked to antibiotic persistence, a clear molecular mechanism for the growth arrest that characterizes persistent bacteria remained elusive. Here, we screened an expression library for putative targets of HipA, the first toxin linked to persistence, and a serine/threonine kinase. We found that the expression of GltX, the *glutamyl-tRNA-synthetase*, reverses the toxicity of HipA and prevents persister formation. We show that upon HipA expression, GltX undergoes phosphorylation at Ser239, its ATP-binding site. This phosphorylation leads to accumulation of uncharged $tRNA^{Glu}$ in the cell, which results in the activation of the stringent response. Our findings demonstrate a mechanism for persister formation by the *hipBA* toxin–antitoxin module and provide an explanation for the long-observed connection between persistence and the stringent response.

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The phenomenon of bacterial persistence to antibiotics has been extensively studied in the past decade[1,2]. Persistence is observed when a fraction of a clonal bacterial population survives extensive antibiotic treatments that have efficiently killed off the majority of the population[3]. In contrast to resistance, persistence is a transient phenotype and not linked to genetic alterations[4]. Persistence can be viewed as a powerful survival strategy of bacteria within a genetically uniform population to cope with unpredictable stress[1,2] through the stochastic formation of a small subpopulation of transiently dormant bacteria refractive to many antibiotics that target actively growing cells, as well as to phages[5] and temperature[6]. Although other phenotypes have been linked to persistence, dormancy is the most commonly reported survival factor[1]. Several toxin–antitoxin (TA) modules have been linked to the persistent phenotype[7,8], with *hipAB* being the first one directly correlated to this phenotype[9]. Although understanding of the environmental conditions needed to trigger persistence through TA modules is still lacking, the *hipBA* module and its high-persistence mutant, *hipA7*, constitute a 'model system' for studying persistence that appears upon starvation, namely Type I persistence. HipA is a toxin and a serine/threonine kinase that is capable of auto-phosphorylation[10] and the phosphorylation of EF-Tu[11], causing growth arrest and persister formation when the HipA level is higher than HipB. Ectopic expression of HipA arrests cell growth and mediates the formation of persister cells[12]. Using this system, we have previously shown that the key feature of persistence, namely the coexistence of dormant and actively growing cells, is due to a threshold mechanism resulting from the titration of HipA by HipB[13]. This threshold is a general feature of TA modules. However, the molecular mechanism through which HipA arrests growth and controls the duration of the growth arrest remains unclear. The observation that mutants in the stringent response pathway create less persisters than the wild-type *Escherichia coli* cells[14,15] suggests that the stringent response plays an important role in persister formation[16,17]. In agreement with this understanding, activation of the stringent response by HipA has recently been reported[18]. The absence of induction of the stringent response upon inhibition of EF-Tu[19] suggests that the phosphorylation of EF-Tu may be only part of the explanation for the growth arrest observed upon *hipA* expression.

Our goal was to further investigate the connection between HipA, growth arrest and the stringent response. We found that HipA activates the stringent response by phosphorylation of GltX, which is an aminoacyl $tRNA^{Glu}$ synthetase, at its ATP-binding site. This phosphorylation inactivates GltX, leads to increased levels of uncharged $tRNA^{Glu}$, thus activating RelA, and increases (p)ppGpp levels.

Results

HipA7 cold sensitivity phenotype is suppressed by GltX. In order to search for the mediating mechanism between HipA expression and (p)ppGpp induction, we took advantage of the *MG1655A7* (Supplementary Table S1) mutant[9]. Two point mutations were found in *hipA7* (ref. 14) that trigger high persistence and cold sensitivity[6,9]. Using the cold-sensitive phenotype for selection, we transformed *MG1655A7* cells with a genomic *E. coli* library[20]. The purified plasmids, which were collected from the colonies that overcame the cold-sensitive phenotype, were found to be in two groups: plasmids containing the *hipAB* operon together with part of the *yneO* gene, which is a pseudogene (10 clones) and plasmids containing the *gltX* gene (9 clones), a glutamyl-tRNA-synthetase, together with YfeD, which is predicted to be an antitoxin from an uncharacterized TA system (Supplementary Fig. S1). We cloned the *gltX* gene separately and found that *MG1655A7* bacteria overexpressing GltX were the only one able to grow at 20 °C.

GltX prevents ppGpp synthesis upon HipA overexpression. In order to test whether the growth arrest caused by HipA is directly related to the activation of the stringent response, we transformed $hipB^+$ cells (MGNR— Supplementary Table S1) with a plasmid

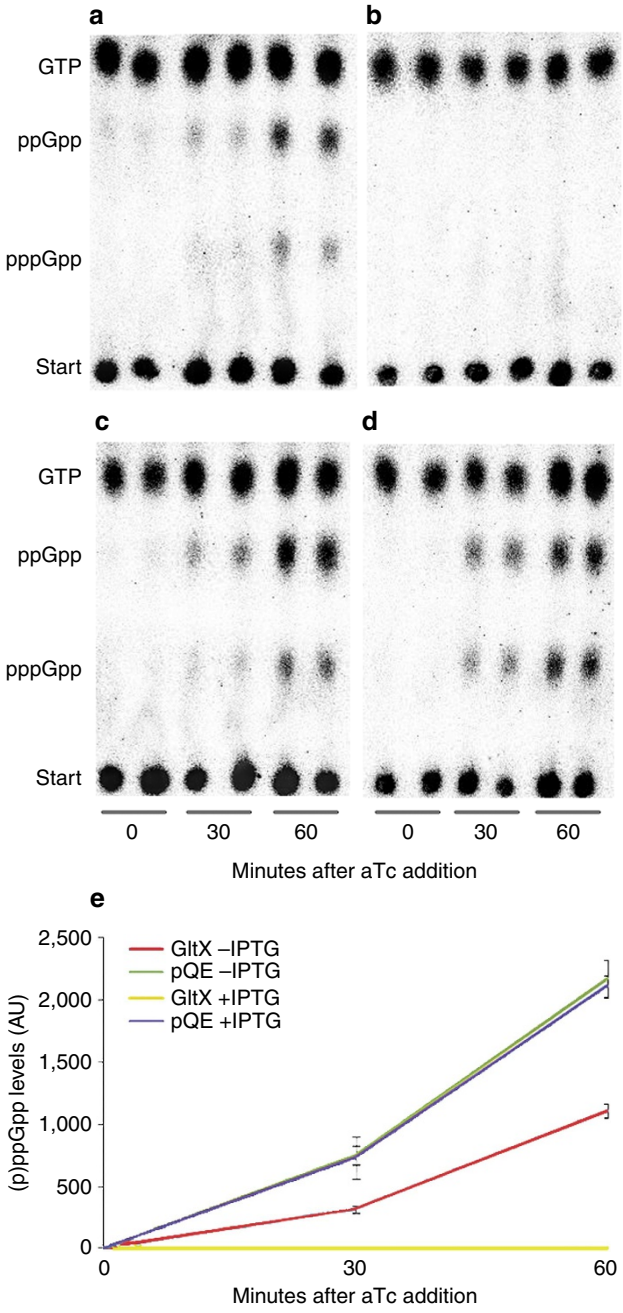

Figure 1 | Formation of (p)ppGpp following overexpression of HipA. MGNR cells overexpressing HipA were grown in 100 µl medium (as described in the Methods section). Samples of 10 µl were separated by thin-layer chromatography. Representative autoradiogram of PEI thin-layer chromatography of the accumulation of (p)ppGpp in MGNR cells overexpressing HipA with different levels of GltX: (**a**) $GltX^+$ cells. (**b**) $GltX^{++}$ cells. (**c**) $GltX^{wt}$ cells carrying pQE_{30} empty vector (**d**) $GltX^{wt}$ cells carrying pQE_{30} empty vector with the addition of IPTG. (**e**) Quantification of **a**–**d**. **a**- Red; **b**- Yellow; **c**- Green; **d**- Purple. Error bars represent s.d. of duplicates from two different experiments.

carrying pTet-*hipA-mcherry*[13], induced the overexpression of HipA and monitored the levels of ppGpp and pppGpp (collectively named (p)ppGpp) which are the hallmarks of stringent response activation[21]. Clear (p)ppGpp synthesis was observed 30 min after the induction of HipA in agreement with a recent report[18] (Fig. 1a), indicating that HipA expression activates the stringent response.

In order to determine whether GltX prevents the activation of the stringent response that is induced by HipA, we added another plasmid controlling the level of *gltX* (pTac-*gltX*) (Supplementary Table S2). The Tac promoter is known to be leaky, resulting in three levels of GltX expression: (a) 'GltX^{wt}': expression from the native chromosomal gene; (b) 'GltX^{+}':GltX^{wt} together with leaky expression from pTac-*gltX*; and (c) 'GltX^{++}':GltX^{wt} together with maximal induction from pTac-*gltX*. We monitored (p)ppGpp synthesis after the induction of HipA in the presence (Fig. 1b) and absence (Fig. 1a,c,d) of overexpressed GltX. We found that (p)ppGpp synthesis was completely abolished in GltX^{++} cells despite the overexpression of HipA. The inhibition of (p)ppGpp synthesis upon HipA induction by GltX is dependent on the levels of HipA. In the present experiment, the levels of HipA were maximal (see Materials and Methods), and thus, the leakiness from pGltX (GltX^{+}) is not sufficient to completely reduce (p)ppGpp synthesis (Fig. 1a,e). Maximal levels of GltX abolished the effect of HipA on (p)ppGpp synthesis (Fig. 1b,e).

GltX prevents growth arrest mediated by HipA. We hypothesized that the prevention of (p)ppGpp synthesis by GltX

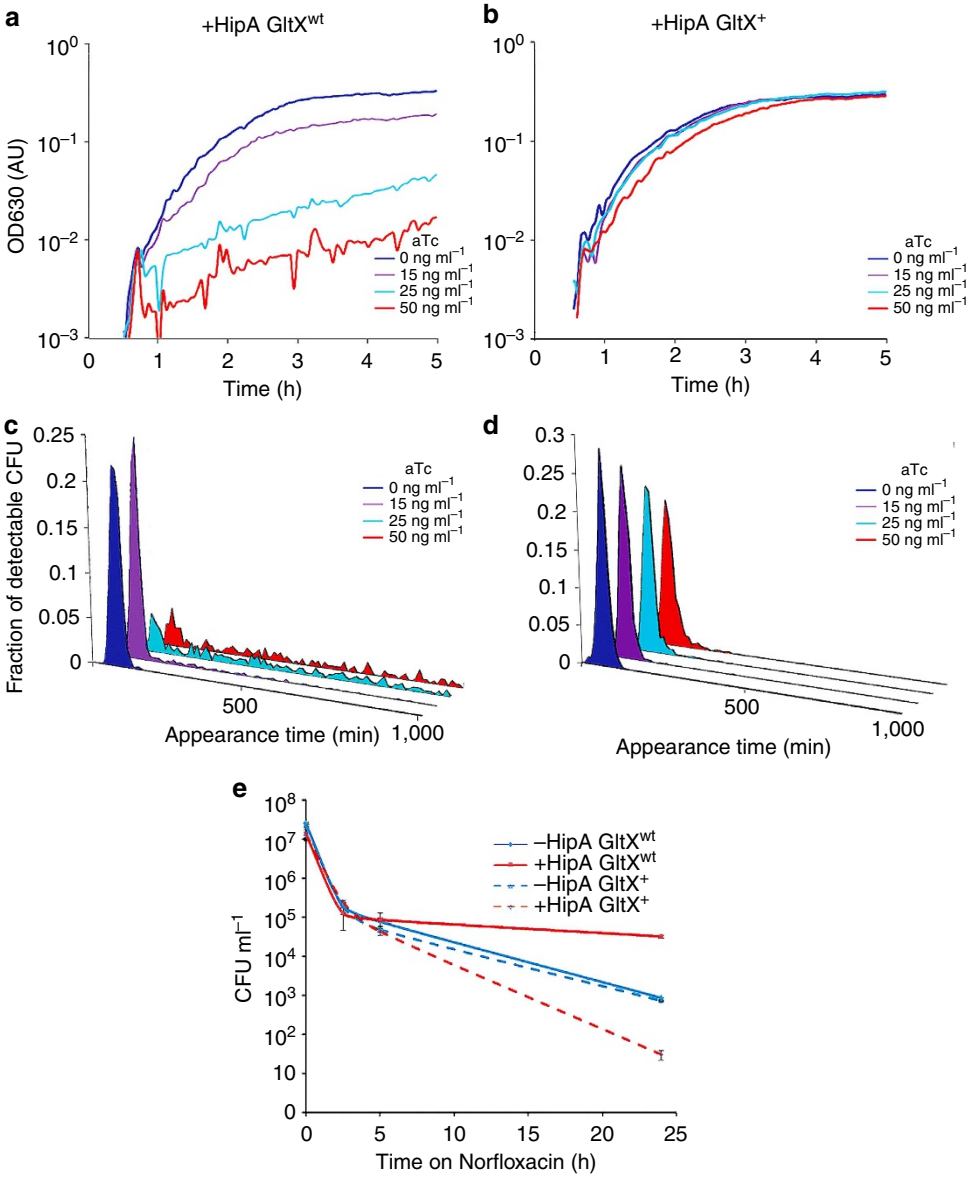

Figure 2 | The effect of GltX on growth arrest and persistence. (**a**) Growth curve of *hipB*$^{+}$ cells carrying the HipA plasmid only. (**b**) Growth curve of *hipB*$^{+}$ cells carrying HipA plasmid together with pGltX. The optical density was measured in a multiplate reader (Victor2, Perkin-Elmer) every 2.5 min. Statistical information is shown in Supplementary Fig. S5. (**c**,**d**) Cells from **a** or **b**, respectively, were plated on LB 3 h after aTc addition. The appearance time of colonies was continuously monitored by the ScanLag system. The histograms show the fraction of CFUs detected at each time point. (**e**) Survival curve of cells under Norfloxacin treatment. Solid lines—cells carrying pHipA and pQE$_{30}$ (GltX^{wt} cells) without IPTG addition; Dashed lines—cells carrying pHipA and pGltX (GltX^{+} cells). Blue—without the addition of ATC; Red—with 4 ng ml^{-1} aTc. Four independent assays show significant lower persistence in GltX^{+} conditions, with a *P*-value of 0.017, using the *t*-test. Error bars represent s.d. of duplicates from three different experiments.

overexpression would also abolish the growth arrest caused by HipA, as well as persister formation. We first measured the effect of co-expressing GltX on growth upon HipA overexpression with anhydrotetracycline (aTc). GltX^{+} cells grew normally despite the overexpression of HipA, whereas GltX^{wt} cells were growth arrested (Fig. 2a,b). We previously showed that the growth arrest inflicted by HipA is transient and results in an extended lag time. The expression level of HipA above a threshold determines the duration of the lag time and thus the level of persistence[13]. We measured the effect of GltX on the persistence induced by HipA by monitoring the lag time distribution by the ScanLag method[22], with and without overexpression of GltX. As previously reported, cells expressing HipA above the threshold exhibited an extended lag with a broad distribution of colony appearance—a 'tail' which represents the persisters subpopulation. In contrast, GltX^{+} cells expressing low and medium levels of HipA did not exhibit the extended growth arrest. Only at very high levels of HipA expression the lag time was again extended despite the presence of extra GltX (Fig. 2c,d). Thus, GltX prevents the formation of growth-arrested cells caused by the accumulation of HipA.

GltX reduces antibiotic persister levels. The above described phenotype implies that GltX should also reduce persistence to antibiotic treatments. To test this prediction, we monitored the survival under Norfloxacin treatment of cultures overexpressing HipA, with and without the overexpression of GltX (Fig. 2e). We found that the high-survival level acquired by cultures over-expressing HipA disappeared in GltX^{+} cells. These results show

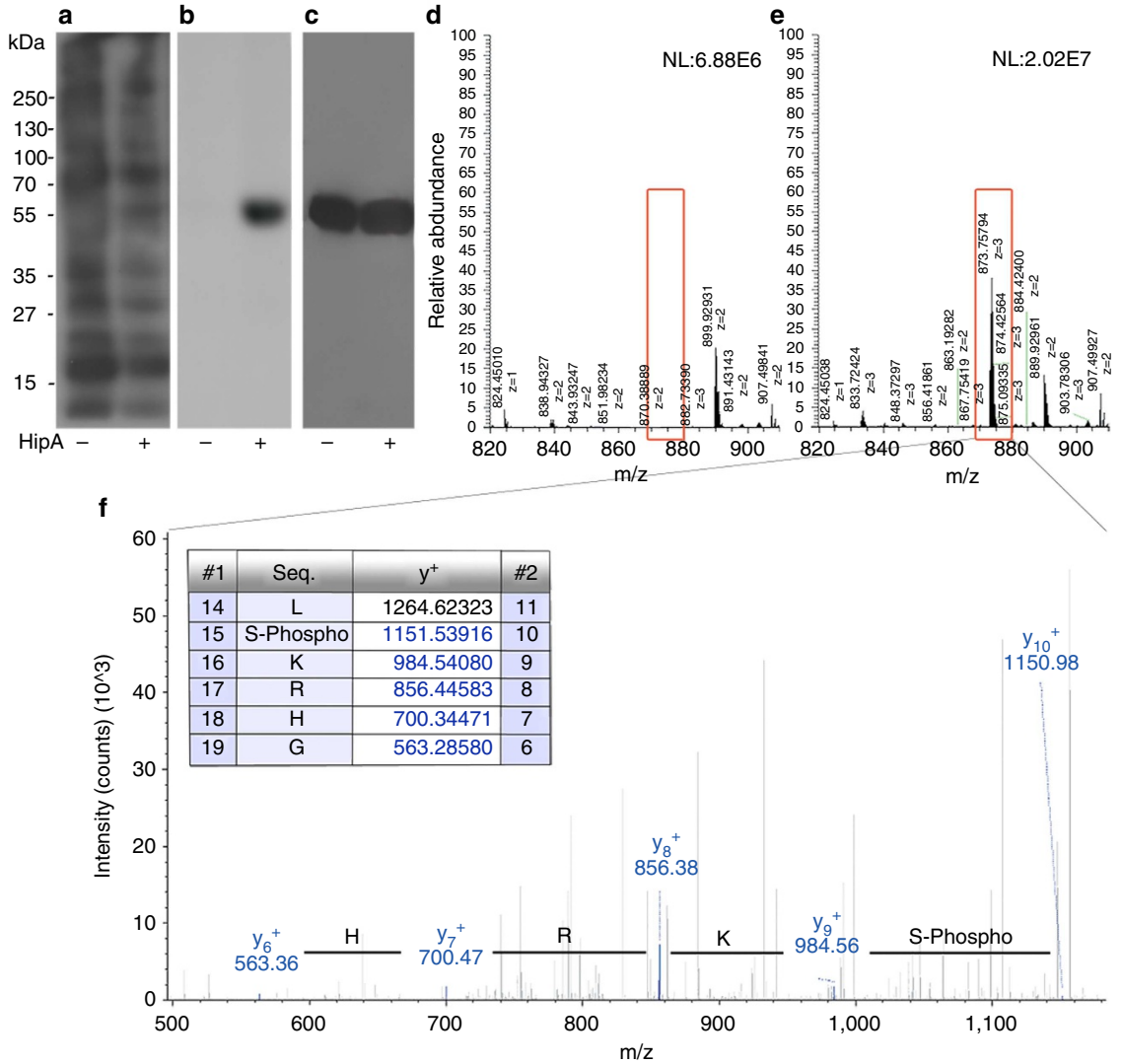

Figure 3 | Phosphorylation of GltX in the presence of HipA. (**a**–**c**) *hipB*$^{+}$ cells carrying two plasmids expressing HipA and his-GltX grown in the presence of ^{32}P. All cells were GltX^{+} $^{+}$. (−) refers to cells that do not express HipA; (+) refers to cells overexpressing HipA by the addition of aTc. (**a**) Whole-cell extract was separated by electrophoresis, transferred to a PVDF membrane and exposed to a hyper sensitive film. (**b**) His-GltX was separated from the whole-cell extract by Ni-bids, further treated as in (**a**). (**c**) Western blot analysis of the membrane in **b** using an anti-his antibody. (**d**,**e**) MS analysis MS of purified GltX as described in **b**. (**d**) MS analysis of GltX without the induction of HipA. (**e**) MS analysis of GltX with the induction of HipA. Red box marks the location of the peptide containing the phosphorylated serine. (**f**) MS-MS analysis of the phosphorylated peptide of GltX, by the Protein Discoverer 1.3 software, using a cutoff of 0.8 Da. The table and the spectrum show the 'y' singly charged ions of the phosphorylated peptide. In the spectrum the distance between the peaks represent the (m/z) of each amino acid. The spectra of the whole phosphorylated and non-phosphorylated peptides are shown in Supplementary Fig. S2.

that increased levels of GltX preclude the onset of the stringent response activated by HipA, preventing growth arrest and persister formation.

GltX is phosphorylated upon induction of HipA. A common method to induce the stringent response is by inhibiting tRNA-synthetases[23], suggesting that HipA may inhibit GltX. HipA was shown to be a serine/threonine kinase capable of auto-phosphorylation[10] and the phosphorylation of other targets in the cell[11]. In order to determine whether GltX is phosphorylated as a result of HipA overexpression, cell cultures containing plasmids overexpressing both HipA and GltX were grown in the presence of ^{32}P and the labelled protein profile analyzed by gel electrophoresis. A band the size of GltX (56 kDa) can be seen in the whole-cell extract only upon HipA overexpression (Fig. 3a). Stronger support for the phosphorylation of GltX in the presence of high HipA levels is found in purified his-GltX. A clear radioactive band was seen in the purified fraction at the size of 56 kDa, again only in cells that overexpressed HipA (Fig. 3b). We verified that his-GltX was equally expressed in both cultures, namely in cells that did and did not express HipA, by western blot analysis to the same membrane as in Fig. 5b (Fig. 3c). Using Mass Spectrometry (MS) analysis, we confirmed that GltX is phosphorylated upon HipA overexpression (Fig. 3d,e). Interestingly, we found by MS-MS that the phosphorylation site of GltX is a serine (Ser239) (Fig. 3f and Supplementary Fig. S2). This residue is part of the KKLSKR motif in the ATP-binding site of GltX[24], suggesting that HipA may inactivate GltX by phosphorylation and is in agreement with predictions by Sekine *et al.*[24], that a mutation at this site would change the conformation of the ATP-binding site thus leading to GltX inactivation. As further corroboration with this prediction, we show that the induction of HipA leads to the accumulation of uncharged $tRNA^{Glu}$, whereas overexpression of GltX ($GltX^{++}$) prevents this accumulation (Supplementary Fig. S3).

Discussion

Our findings as well as similar results obtained while this work was under revision[25] can serve to fill in a missing link in the cycle describing the formation of persisters by the *hipBA* TA module (Fig. 4). Overexpression of HipA inactivates GltX by phosphorylating its ATP-binding site, leaving GltX unable to covalently link the glutamate amino acids to their cognate $tRNA^{Glu}$ (Supplementary Fig. S3). Increased levels of uncharged $tRNA^{Glu}$ in the cells trigger RelA to synthesize (p)ppGpp, activating the stringent response[26] and thus leading to dormancy and persistence. This pathway now closes the loop of persister formation when combined with the results of an earlier model[27], suggesting that (p)ppGpp is the regulator of persistence by activating TA loci[28] via PolyP and Lon[29,30], which has been shown to degrade HipB[31]. We had shown that a triggering mechanism leading to an imbalance of activation of HipA toxicity is needed to bring the bacterial population closer to the threshold and generate Type I persisters. Typically, Type I persistence is triggered by starvation conditions. When *MG1655A7* cells are maintained at strictly exponential growth, persistence vanishes[10,32]. We propose that an initial activation of the stringent response by starvation increases the level of (p)ppGpp, and activates the persistence cycle in the cells in which free HipA is near the threshold (Fig. 4). Free HipA causes the phosphorylation of GltX, further triggering the stringent response due to the presence of uncharged tRNAs as well as increasing cellular (p)ppGpp levels[26]. The interplay between the stringent response and the action of the HipA toxin suggests new ways to target persister cells by interfering directly with the stringent response. Indeed, the use of novel analogues of ppGpp designed to inhibit RelA enzymes that interfere with long-term bacterial survival strategies[33] may thus present a promising candidate for new specific anti-persister therapies[34].

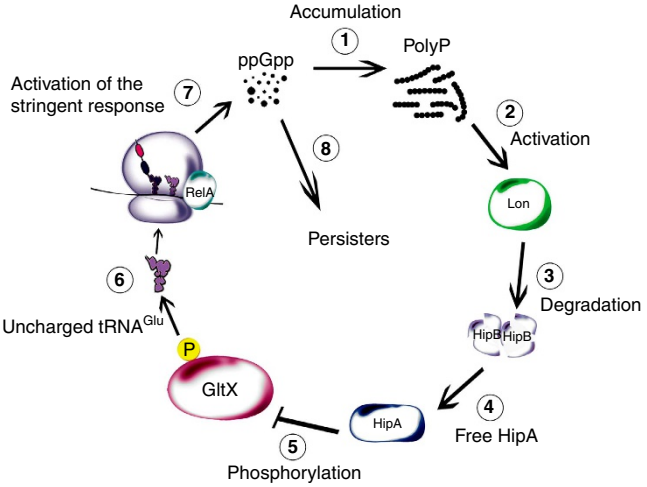

Figure 4 | The persistence cycle through the activation of the stringent response. (**1**) Increased levels of ppGpp at the entrance to stationary phase lead to accumulation of inorganic polyphosphate (polyP)[29]. (**2**) PolyP promotes Lon degradation activity[27,30]; (**3**) HipB, is degraded by Lon[31] leading to (**4**) free active HipA. (**5**) Free HipA inactivates GltX by phosphorylation of its ATP-binding site (ser239). (**6**) Phosphorylated GltX is unable to charge $tRNA^{Glu}$ with glutamate, leading to increased levels of uncharged $tRNA^{Glu}$. (**7**) RelA is activated by uncharged $tRNA^{Glu}$ to synthesize more (p)ppGpp, closing the cycle and (**8**) leading to growth arrest and persister formation. Note that HipA action on GltX is probably only one of the pathways closing the cycle.

The persistence cycle activates the stringent response, together with growth arrest, in a runaway process that further sharpens the threshold separating persisters from normal cells. We have added the positive feedback of the free HipA level on the degradation rate of HipB, via the inactivation of GltX, to our previous model of the *hipBA* TA module describing the persistence level as a function of the total toxin level in cells, A_T (ref. 13) (Supplementary Fig. S4). The analytic solution of the model (Supplementary Table S3) now predicts the level of uncharged $tRNA^{Glu}$, a proxy for the persistence level, as a function of the levels of HipA and GltX and reproduces the reduction in persistence that we observed upon GltX overexpression (Supplementary Fig. S4). Note that while the HipA–GltX interaction represents the first closure of the persistence cycle, it is likely, in light of the identical persistence level of *hipA* deletion mutant[35,36], that several other parallel ways may close the cycle in a similar way.

The model proposed in Fig. 4 describes the induction of Type I persistence by any step that may start the persistence cycle, in particular starvation and activation of the stringent response. Type I persistence triggered by starvation is the most common form of persistence[1]. For example, it was shown recently in *Pseudomonas aeruginosa* that the response to starvation actively mediates antibiotic tolerance and biofilm formation[15]. Our results show that the mutual activation of the stringent response and the *hipBA* TA locus can provide a mechanism for Type I persistence, by inactivation of aminocyl-tRNA synthetases, a mechanism which may be used by other TA modules in parallel persister activation cascades[37] that converge on the activation of the stringent response. This is consistent with the observation that Type I persistence decreases in cells impaired for the stringent response

both in planktonic cells[14,38,39] and in biofilms[15]. The novel HipA target revealed in our study therefore serves to uncover another piece in the puzzle of HipA-mediated persistence.

Methods

Media and reagents. Bacterial strains used in this study are described in Supplementary Table S1. Plasmid construction is described in Supplementary Table S2. All *E. coli* strains were grown at 37 °C in LB medium unless indicated differently. The antibiotics used for selections were used at the following concentrations: ampicillin 100 μg ml^{-1}, kanamycin 50 μg ml^{-1}, tetracycline 20 μg ml^{-1}, and chloramphenicol 34 μg ml^{-1}.

***In vivo* (p)ppGpp accumulation assay.** Δ*hipAhipB*$^{+}$ (MGNR) cells carrying pZA21HipA-mcherry plasmid[13] and pGltX plasmid, as indicated in the text, were grown in MOPS glucose minimal medium[40] supplemented with all amino acids. At OD_{600} 0.1, cells were labelled with $H_3^{32}PO_4$, and 1.2 μg ml^{-1} of isopropylthiogalactoside (IPTG) was added when necessary for the induction of GltX and incubated for 1 h, after which HipA was induced by the addition of 50 ng ml^{-1} of aTc. Samples were withdrawn 30 and 60 min after addition of aTc. Reactions were stopped by the addition of 25 μl of formic acid per 100 μl reaction volume. Aliquots (10 μl) of each reaction were loaded and separated on Cellulose PEI TLC plates (Merck) using 1.5 M KH_2PO_4 as mobile phase. Plates were autoradiographed using the Fijix Bas100 Phosphor Imager (Japan). (p)ppGpp signal was measured using TINA 2.0 software (Raytest, Strauben-Hardt).

Library screen. MG1655A7 cells were transformed with a genomic *E. coli* library mix[20]. Cells were plated on ampicillin plates and incubated at 20 °C for 48 h. As controls, we used the MGH strain that did not contain any plasmid, and the MGH strain containing an ample pBR vector. Plasmids were purified from the colonies that succeeded to grow at 20 °C. MGH cells were transformed again, this time with each of the purified plasmids and incubated at 20 °C for 48 h to verify that the phenotype is restored. Plasmids capable of restoring the cold sensitivity phenotype were sequenced.

Cloning of genes from the library screen. The *GltX* and *YfeD* genes were cloned separately into the pQE_{30} vector under a his-tag with the primers:
FW-5′-ACGAGAGCTCATGAAAATCAAAACTCGCTT-3′,
REV-5′-ATATGTCGACTTACTGCTGATTTTCGCGTT-3′,
FW-5′-ATACGAGCTCATGAAAAGATTACGCAATAA-3′ and
REV-5′-TTAAGTCGACTTATGCTGATTCGTCAATAT-3′, respectively. As templates, we used the plasmids purified from the library screen.

Protein preparation and analysis. MGNR cells carrying relevant plasmids were grown in MOPS glucose minimal medium[40] supplemented with all amino acids. At OD_{600} 0.1, cells were labelled with $H_3^{32}PO_4$ and 1.2 μg ml^{-1} of IPTG was added for the induction of GltX when necessary. After 1 h of incubation, HipA expression was induced by the addition of 50 ng ml^{-1} of aTc. After another 45 min, cells were harvested by centrifugation and lysed by resuspension in a BugBuster solution (Novagen). His-tagged GltX was purified from the lysate, as described before[33]. For analysis of the protein samples from whole cells, lysate and purified GltX were separated by 12% SDS-polyacrylamide gel electrophoresis and transferred to PVDF membrane (Millipore Bedford). The membrane was autoradiographed by using Hyper Sensitive films (GE Health Care) for 4 h. The same membrane was processed for immunoreaction using mouse anti-His antibody (GE Health Care) diluted 1:10,000. Immunoreactive proteins were detected using a chemiluminescence kit (Biological Industries) according to the manufacturer's protocol.

Growth curves. *hipB*$^{+}$ cells (MGNR) carrying pZA21HipA plasmid and either an empty pQE_{30} vector or a pGltX plasmid were grown in LB medium at 37 °C with shaking. The optical density was measured in a multiplate reader (Victor2, Perkin-Elmer) every 2.5 min. At OD_{630} of 0.01, aTc was added at indicated concentrations to induce the expression of HipA. After 3 h, cells were diluted and plated on LB Petri dishes containing Kanamycin and Ampicillin and colony formation was monitored at 32 °C using the ScanLag setup[22].

Death curves. *hipB*$^{+}$ cells (MGNR) carrying pZA21HipA plasmid and either an empty pQE_{30} vector or a pGltX plasmid were grown in LB medium at 3 °C with shaking. At OD_{600} of 0.2, aTc (0 ng ml^{-1} or 4 ng ml^{-1}) was added to induce the expression of HipA. After 3 h, cells were diluted into fresh medium containing Norfloxacin (8 μg ml^{-1}). Cells were diluted on LB plates (without Norfloxacin) 0, 2.5, 5 and 24 h after the dilution into Norfloxacin. Colony forming units were counted after 12, 26 and 56 h of incubation at 37 °C. Until the addition of Norfloxacin, all strains, with and without the addition of aTc, showed equal growth curves, unless indicated otherwise. Statistical analysis was performed by the calculation of the percent of the survival cells after 24 h on Norfloxacin from four different death curves.

MS measurements and protein identification. His-GltX was purified as described in 'Protein labelling, purification and analysis', and separated by 12% SDS-polyacrylamide gel electrophoresis. We have purified GltX from cells over-expressing HipA and from cells that did not express HipA at all. The MS/MS study was done on these two protein samples. The proteins were reduced (3 mM dithiothreitol), modified with 12 mM iodoacetamide and digested in-gel with trypsin and chymotrypsin (Promega) separately at a 1:50 enzyme-to-substrate ratio in 10 mm ammonium bicarbonate and 10% acetonitrile.

The resulting peptides were resolved by reverse-phase chromatography on 0.075 × 200-mm fused silica capillaries (J&W) packed with Reprosil reversed-phase material (Dr Maisch GmbH, Germany). The peptides were eluted with 65 min linear gradients of 5–45% and 15 min at 95% acetonitrile with 0.1% formic acid in water at flow rates of 0.25 μl min^{-1}. Mass spectrometry was performed by an ion-trap mass spectrometer (Orbitrap XL, Thermo) in a positive mode using repetitively full MS scan followed by collision-induced dissociation of the seven most dominant ions selected from the first MS scan. In order to analyze phosphopeptides, multistage activation was employed.

The mass spectrometry data were analyzed using the Proteome discoverer 1.3 (ThermoFisher Inc.) using both Sequest and Mascot search engines, searching against the *E. coli* section of the NCBI_NR database and versus the specific sequence of the protein. Identifications were filtered according to mass accuracy and 1% false discovery rate.

Northern blot analysis. Δ*hipA hipB*$^{+}$ cells (MGNR) cells carrying pZA21HipA-mcherry plasmid[13] and pGltX plasmid, as indicated in the text, were grown in LB medium with the appropriate antibiotics. At OD_{600} 0.3, 1.2 μg ml^{-1} of IPTG was added when necessary for the induction of GltX and incubated for 30 min, after which HipA was induced by the addition of 20 ng ml^{-1} of aTc for 40 min. RNA was purified using the RNeasy kit (Qiagen) at pH 5.5. The samples were fractioned on a 18% polyacrylamide gel (19:1 acrylamide:bisacrylamide) containing 8 M urea. The samples were then transferred onto a Hybond + membrane (GE Healthcare, Germany). $tRNA^{Glu}$ probe % 5′-(GCGGTGTCCTGGGCCTCT)-3′ was labeled and detected using ECL DIRECT Nucleic Labeling and Detection System (GE Healthcare, Germany).

Cloning GltX into pSA11. The *GltX* gene was cloned into EcoRI/XbaI site of pSA11 vector. GltX was amplified by GltX-FW: 5′-TCTCTCTCGAATTCAG-GAGGAGCTCATCATGAAAATCAAAACTCGCTTCG-3′ GltX-Rev: 5′-TCTCTCTCTCTAGACCGTCTCGATATTGACGAATCA-3′.

As template, we used the plasmids purified from the library screen.

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Author contributions

N.Q.B and G.G. designed the project. I.K. performed most of the experimental work. E.R. performed the survival and persisters assays. N.W. derived the model and equations. I.R. was responsible for the cloning of the different genes. I.K., N.Q.B and G.G. wrote the manuscript.

Additional information

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