Loss of phenotypic inheritance associated with ydcl mutation leads to increased frequency of small, slow persisters in Escherichia coli

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Antibiotic tolerance or persistence was first described by Hobby et al in 1942 (1). Hobby observed that when a genetically homogenous Streptococcus culture was exposed to the bactericidal action of penicillin, a small number of cells [subsequently termed “persisters” by Bigger in 1944 (2)] survived the treatment. The phenomenon is an example of phenotypic variation as persisters are genetically identical to nonpersister cells. Persistence has been described in nearly all known microbes and bacteria survive sterilization, prolongs antibiotic treatment and contributes to the development of genetic antimicrobial drug resistance (AMR). In this study we performed single-cell tracking of wild-type and high-persister mutant strains of Escherichia coli to identify factors that correlate with persistence. We found, as expected, persistence correlated with slow growth, but also with small birth size. We investigated intergenerational (mother–daughter) and intragenerational (sister–sister) phenotypic inheritance of growth parameters and discovered the mutant phenotype was associated with lower levels of phenotypic inheritance and identified the gene responsible, the transcription factor ydcl. Targeting pathways involved in persistence could reveal approaches to impeding persistence and the development of AMR.

Significance

Persistence, the phenomenon whereby a small subpopulation of bacterial cells survive sterilization, prolongs antibiotic treatment and contributes to the development of genetic antimicrobial drug resistance (AMR). In this study we performed single-cell tracking of wild-type and high-persister mutant strains of Escherichia coli to identify factors that correlate with persistence. We found, as expected, persistence correlated with slow growth, but also with small birth size. We investigated intergenerational (mother–daughter) and intragenerational (sister–sister) phenotypic inheritance of growth parameters and discovered the mutant phenotype was associated with lower levels of phenotypic inheritance and identified the gene responsible, the transcription factor ydcl. Targeting pathways involved in persistence could reveal approaches to impeding persistence and the development of AMR.

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The authors declare no competing interest.

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Data deposition: Individual sequencing read data for the E. coli HipQ mutant and parental strain are publicly available at National Center for Biotechnology Information (NCBI) BioSample, https://www.ncbi.nlm.nih.gov/biosample (accession nos. SAMN13648605 [E.coli HipQ mutant strain] and SAMN13648604 [E. coli parental strain]).

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the recently characterized LysR family transcriptional regulator ydcI (14).

Several models of growth and division of cells have been proposed recently (15–18). These include models where cells measure a fixed increase in length between division (i.e., the adder model) (15), sense absolute time [i.e., the timer model (16)], or sense absolute size [i.e., the sizer model (16–18)]. To examine how persistence fits into these paradigms we performed single-cell studies using a microfluidics platform (Fig. 1 and SI Appendix, Fig. S1A) to measure steady state growth and division of individual cells of both the wild-type (WT) and the high-persister HipQ mutant (Fig. 1 A–C and SI Appendix, Fig. S1B).

Growth characteristics (division time $T$, elongation rate $g$, size at birth $L_0$, size at division $L_f$, and size extension $\Delta$) of both WT and HipQ mutant cells were recorded before antibiotic exposure (SI Appendix, Table S1). The values of both $\mu$ (Fig. 1B) and $g$ (Fig. 1C) varied widely between generations but, at steady state, appeared constrained in a bounded range of values. We found that cells add an approximately constant length prior to division and hence were behaving consistently with the adder model only (Fig. 1D, equation in Fig. 1E, and SI Appendix, Fig. S1 C and D). The average value or distribution of measured parameters did not differ significantly between WT and HipQ mutant strains (SI Appendix, Table S1 and Fig. S2 A–F).

We next examined inheritance of phenotypic variation, measuring sister–sister (S–S) correlations within a generation and mother–daughter (M–D) correlations (SI Appendix, Tables S2 and S3, respectively) between generations. As found previously (19, 20), M–D correlations of $T$ or $\Delta$ were close to zero, indicating that there was virtually no memory of these parameters between mother and daughter cells, but moderate levels of M–D correlations were observed in WT cells on $L_f$, $L_j$, and $g$ (SI Appendix, Table S3), demonstrating that limited phenotypic information is passed between generations. Despite low M–D correlations on $T$ or $\Delta$, high correlations were found for both these parameters between sisters (SI Appendix, Table S2) in WT. Surprisingly, the HipQ mutant strain exhibited statistically significant loss of M–D correlations on elongation rate, $g$, which were less than half the value for the WT (Fig. 2 C, D, and F); also S–S correlations on division time $T$ (Fig. 2 A, B, and E) and (although nonsignificant) elongation rate $g$ were reduced (SI Appendix, Table S2). The HipQ mutant strain thereby exhibits the phenotype of reduced phenotypic inheritance (RPI) with statistical significance of 0.014 and 0.03 for $T$ and $g$, respectively, for the HipQ mutant versus WT.

To investigate if and how RPI affects persister formation we characterized persister cells (identified as surviving initial antibiotic exposure but being killed by antibiotic after regrowth; Fig. L4 and Movie S1) and their relatives. Persisters tended to be

![Fig. 1](https://example.com/fig1.png)

**Fig. 1.** Single-cell analyses of *E. coli* persisters using microfluidics and image tracking. (A) Single-cell data showing persister (yellow arrow). (B) WT growth rate (2,172 cells) with black bars indicating steady state and red line indicating mean. (C) Elongation rate trajectories. Blue circles indicate divisions, with cell numbers. (D) Growth characteristics of cells for the adder model (WT are shown as solid and HipQ mutant are shown as clear circles, with persisters’ mothers shown as solid triangles). Data represent three experiments (WT = 1,710, mutant = 1,638 cells) with error bars representing $n$ = 3. Solid linear fitting line is for WT ($\Delta = (0.0563 \pm 0.0731)L_0 + (2.4646 \pm 0.2730)$), and dashed line is for mutant ($\Delta = (0.0042 \pm 0.0641)L_0 + (2.1654 \pm 0.2355)$). (E) Adder model equation.
born with small $L_0$ (Fig. 3A) and grew at slower elongation rates $g$ (Fig. 3B), a difference that was statistically significant when compared to the rest of the population and with persister's mothers (Fig. 3A and B). Since growth of persisters includes a period of anomalous elongation during exposure to antibiotic it was not possible to measure $L_f$, $T$, or $\Delta$; however, the average age

Fig. 2. S–S and M–D growth parameters at the population level. S–S T in (A) WT and (B) HipQ mutant. $g$ between mother and daughter for (C) WT and (D) mutant. Correlation coefficients (CC) with SDs for HipQ mutant for (E) S–S $T$ and (F) M–D $g$. Pearson's CC following slow stratification of $T$ and showing $L_0$ and $g$ between (G) S–S and (H) M–D with WT in black and mutant in gray. Data represent three independent experiments (WT = 1,710 and mutant = 1,638 cells).
Fig. 3. Persisters are small and slow, with the HipQ mutant gene identified as ydcI. (A) Size at birth ($L_0$) and (B) elongation rate ($g$) measurements for normal (N, $n = 1,644$), persister’s mother (PM, $n = 14$), persister (P, $n = 17$), and persister’s sister (PS, $n = 14$) and (C) size at division ($L_f$) and (D) $\Delta$ for N and PM, from at least 3 individual experiments for N and for over 14 for P, PS, and PM. Statistics were calculated using Student’s t test with Welch’s correction (absolute effect size of Cohen’s standardized mean difference was over 0.9 for all statistically significant comparisons). Percentage survivors at 24 h over time 0 for ampicillin (100 $\mu$g/mL) for (E) WT, $\Delta$ydcI, and $\Delta$ybal and (F) HipA7 mutant and WT and HipQ mutant and WT (representative of three experiments). Ampicillin (100 $\mu$g/mL) kill curve for (G) parental Keio strain, ydcI, and yabI and (H) hipB whole-gene knockout mutants ($n = 3$ with SE bars). Correlation coefficients (CC) for ydcl mutant ($n = 525$ individual cells) and WT ($n = 624$ cells) for (I) S–S T and (J) M–D g.
of the persister population, prior to antibiotic addition, was about twice that of the nonpersister population, indicating that their division time is lengthened to compensate for their slower elongation rate, as predicted by the adder model (Fig. 1E and SI Appendix, Table S4).

The character of persistence thereby appears to be solely due to the persister cell’s acquisition of extreme values of $L_0$ and $g$. The reduced cell length at birth appears to be phenotypically inherited from the persister cell’s mother that tended to be also small at birth and grew at slower rates to achieve a smaller cell size at division (Fig. 3 C and D) to thereby generate smaller daughters (Fig. 3A). We hypothesized that the character of persistence would correlate across sisters; however, surprisingly, given the high degree of correlation for all measured parameters between sisters, the sister of a persister was generally not a persister. However, when cells were stratified according to division time into fast-, medium-, and slow-replicating cells, significantly lower M-D (Fig. 2G) and S-S (Fig. 2H) correlations on $L_0$ and $g$ were found for slow-growing HipQ mutant cells compared to WT (SI Appendix, Fig. S1 E-H), indicating that their RPI is enhanced in the slow-growing cells that are the progenitors of persisters, leading to lower sister correlations.

We next hypothesized that reduced M-D phenotypic inheritance of $g$ found in the HipQ mutant is responsible for generating its high levels of persisters. To test this hypothesis, we made the assumption that fluctuations in values of $g$ averaged over the cell cycle can be described phenomenologically as a Brownian motion with a friction term mimicking the resistance of $g$ again undergoing large jumps. This corresponds to the Ornstein-Uhlenbeck (OU) process (21), which is the simplest process allowing explicit control of the noise intensity $D$ on $g$ and the correlation time $\tau$ of the fluctuations in $g$. Our assumption was that the HipQ mutant is characterized by a smaller correlation time $\tau$ than the WT. This model minimally extends equation 1 in Fig. 1 to include dynamics across generations (as it stands, equation 1 in Fig. 1 only relates cell features within the same generation). The capability of this model to give rise to the biphasic behavior of the corresponding killing curves will depend on mechanisms governing the behavior across generations of all other quantities present in equation 1 in Fig. 1, requiring a population model that currently does not exist. We exemplify the qualitative behavior of the OU process for distinct values of the correlation time $\tau$ in SI Appendix, Fig. S1K. The RPI phenotype of the HipQ mutant has thus been explained by a slightly longer tail in the distribution of elongation rate $g$ (SI Appendix, Fig. S1K; although not visible in our population data, most likely hidden in the noise) to thereby generate more slow-growing progenitors of persisters. To identify the mutation (22) responsible for persistence in the HipQ mutant, we whole-genome sequenced the WT and HipQ mutant and identified two single-nucleotide polymorphisms (SNPs) with nonsynonymous mutations in genes of unknown function, ydcI and ybal (23, 24). The SNP in ydcI results in a change from nonpolar (alanine) to a charged amino acid (glutamic acid), so is likely to have phenotypic consequences, whereas the ybal mutation is a more conservative valine to glutamic acid change. To identify which mutation was responsible for the persister phenotype, we obtained WT and gene deletion strains of each gene from a mutant library (25) and found that the $\Delta$ydcI mutant, but not the $\Delta$ybal mutant, exhibited high levels of persisters similarly to HipQ (Fig. 3 E–H), implicating this gene as the cause of the persister phenotype. Single-cell studies carried out on the $\Delta$ydcI mutant determined that the mutant strain also exhibited reduced S-S correlations for $T$ similar to the HipQ mutant (Figs. 3I and 2E). Reduced M-D correlations for $g$ were not observed for the $\Delta$ydcI mutant; however, the WT (which is a different strain from the HipQ WT) had a much lower M-D correlation of $g$ than observed with the HipQ WT (Figs. 3J and 2F). E. coli ydcI is a recently characterized LysR family transcriptional regulator with a predicted role in pH homeostasis (14). The trigger for ydcI is unknown but may be linked to stress, e.g., pH, and was also previously identified as differentially expressed in a DosP (phosphodiesterase) mutant with reduced tryptophanase activity that was associated with increased levels of persistence (26) and in Salmonella enterica is required for resistance to acid stress (27).

Discussion

In this study, we have demonstrated that in E. coli the HipQ high-persistence phenotype is caused by a mutation in the gene ydcI, which is associated with the phenotype of RPI. This gene affects inheritance of phenotypic variation in bacteria, and this link with RPI remains to be determined in other bacterial species, such as in Mycobacterium tuberculosis, where the importance of persisters is undisputed. Persistence in tuberculosis has long been observed in humans and in the mouse model of infection (28–30). Vilchez and colleagues have established both an in vitro model of tuberculosis persistence (31) and dual reporter mycobacteriophages to observe mycobacterial persister cells (32). Interestingly, enhancing respiration in M. tuberculosis via the addition of N-acetylcysteine or vitamin C prevented the formation of persisters (31). Other studies have also linked changes in metabolic status to persistence, growth rate, cell size, and asymmetric growth in mycobacteria (26, 33, 34), as in E. coli (35, 36). A recent report also links genome replication with cell division in an updated adder model in E. coli (37), a subject which is linked to cell cycle control and the inherent asymmetry in highly heterogeneous bacteria (34). The prospective link with RPI remains to be elucidated in tuberculosis or other bacterial infections or when using different antibiotics. In addition, phenotypic variation and its inheritance are of fundamental importance in many other biological phenomena from development, to cancer, epigenetics, and evolution (38, 39). Characterizing the mechanistic link between the ydcI gene and RPI may shed light on the underlying mechanisms accounting for phenotypic variation of characters, such as persistence, and may provide ways to target persister cells.

Materials and Methods

Bacterial Strains and Culturing. The E. coli HipQ parental WT (MG1655) and HipQ mutant strains were obtained from Balaban et al. (8), and the E. coli $\Delta$ydcI and $\Delta$ybal strains plus parental (BW25113) were obtained from the Keio collection, https://csgc.biology.yale.edu/KeioList.php (22), with the pyb5b4 vector used for complementation. LB Lennox (Sigma-Aldrich) was used for all liquid growth media, and cultures were maintained at 37 °C plus shaking. Technical agar no. 3 (Sigma-Aldrich) was added to LB for solid media. Ampicillin (Sigma-Aldrich) was used at a final concentration of 100 $\mu$g/mL. For batch culture kill curves, aliquots were taken from a culture at optical density (OD) 1.2 to 1.4 and incubated with ampicillin for up to 48 h, at room temperature, with colony-forming units measured at time 0 and then at named intervals.

Sequencing. Whole-genome sequencing was carried out by Edinburgh Genomics. Briefly, the sequencing libraries were prepared using the Nextera XT kit (Illumina) and sequenced on a HiSeq 2500 using the sequencing by synthesis v4 chemistry. The resulting sequences were aligned to the E. coli K12 reference genome (U00009.2) using Burrows Wheeler alignment to generate the binary alignment map files. Whole genome sequences are available as BioSample accession nos. SAMN13648604 and SAMN13648605 (https://www.ncbi.nlm.nih.gov/biosample).

Microfluidics Platform. Bacterial cells in log phase (OD of 1.2) were grown as above and diluted 1:5 and filtered five times using a 24 gauge needle before loading into the CellASIC ONIX Microfluidic Platform with the Bacterial Plate (with pressurized height of 0.7 μm) and at a flow rate of ~10 μL/hr as described in ref. 40. Imaging was performed under a Nikon A1M, Eclipse Ti-E confocal microscope with an environmental chamber, motorized stage, and perfect focus system (PFS). Automated multiarea imaging with a 40× air objective lens Nikon (Nikon Apo λ), a numerical aperture of 0.95 (resolution of ~0.32 μm with PFS (resolution of ~0.25 μm). LB only was used for growth experiments (8 to 10 generation) and for persister cell discovery the stages were as follows: grow, LB for 2 h; kill, LB plus ampicillin (100 μg/mL) for 6 h; regrow, LB for 6 h; and rekill, LB plus ampicillin (100 μg/mL) for 6 h. For the growth period, images were taken every ~6 s and every 10 mins for the remaining stages.
Modeling. We assume that fluctuations of the cell cycle averaged $g$ are described by the OU process:

$$
\frac{dg}{dt} = -\frac{1}{\tau} g + D\frac{1}{\tau^2} \xi(t),
$$

where $g$ is the average, $\xi(t)$ is Gaussian white noise, $D$ is noise intensity, and $\tau$ is the correlation time. The $g$ correlation function results in

$$
\langle g(t)g(0) \rangle = D \exp \left( -\frac{t}{\tau} \right),
$$

and the stationary probability distribution is

$$
\rho(g) = \frac{1}{\sqrt{2\pi D/\tau}} \exp \left( -\frac{g^2}{2D/\tau} \right).
$$

with $\tau^2 = D/\pi$. Reduced M-D correlations in HipQ mutant imply a reduced correlation time $\tau$ and as a consequence a broader Gaussian distribution. The increased population in the tails of this distribution corresponds to an increased number of persisters.

Data Availability Statement. Keio strains are publicly available at https://cgsc.biology.yale.edu/KeioList.php (25). Single-cell movies are available in Movie S1. Individual sequencing read data for the E. coli HipQ mutant and parental strain are publicly available at NCBI BioSample (https://www.ncbi.nlm.nih.gov/biosample) as SAMN13648604 and SAMN13648605, respectively.

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