Optimality and robustness in quorum sensing (QS)-mediated regulation of a costly public good enzyme

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Bacteria secrete a variety of public good exoproducts into their environment. These exoproducts are typically produced under the control of quorum sensing (QS), a signaling mechanism by which bacteria sense and respond to changes in their density. QS seems to provide an advantageous strategy to regulate these costly but beneficial exoproducts: it delays production until sufficiently high cell density, when the overall benefit of exoproducts outweighs cost of their production. This notion raises several fundamental questions about QS as a general control strategy adopted by bacteria. How much delay is advantageous? Under what conditions does QS-mediated regulation become advantageous? How does this advantage depend on the kinetic properties of QS? How robust is a given QS system to the stochastic events that occur over bacterial lifecycles? To quantitatively address these questions, we engineered a gene circuit in *Escherichia coli* to control the synthesis and secretion of a costly but beneficial exoenzyme. We show that exoenzyme production is overall advantageous only if initiated at a sufficiently high density. This property sets the potential advantage for QS-mediated regulation when the initial density is low and the growth cycle is sufficiently long compared with the exoenzyme response time. This advantage of QS-mediated regulation is robust to varying initial cell densities and growth durations, and it is particularly striking when bacteria face uncertainty, such as from stochastic dispersal during their lifecycle. We show, however, that, for QS to be optimal, its kinetic properties must be appropriately tuned; this property has implications for antibacterial treatments that target QS.

bacterial communication | social evolution | synthetic biology

Bacteria produce and secrete a wide variety of exoproducts into their environment (1–4). For the producing bacterial population, these exoproducts are costly to produce, but after release, they benefit all cells in the local environment and hence, are referred to as public goods (2, 5, 6). As an example, consider an exoproduct that provides benefit by degrading a stress-causing agent in the environment. For a fixed per-cell production rate of the exoproduct, the cost per cell is fixed. However, the benefit of the exoproduct, reflected in any reduction of the stress-causing agent's concentration, should increase with exoproduct concentration, which in turn, increases with the cell density. At a high cell density, the secreted exoproduct would be at a sufficiently high concentration to remove the stress within a short time. In contrast, with a low-density population, the concentration of the exoproduct in the environment is low, which will prolong the stress removal period. Intuitively, undertaking the cost of production under stress would only be advantageous if the cell density is above a sufficiently high critical density.

This notion captures the potential advantage of sensing cell density: to delay production of costly exoproducts until an appropriately high density is reached, where the benefit of exoproducts can outweigh cost of their production (Fig. 1*A*). Consistent with this finding, many bacterial exoproducts (1–3, 5, 7, 8) are regulated by quorum sensing (QS), a cell–cell signaling mechanism that enables bacteria to respond to changes in their density by producing and sensing small molecules (9). Of particular relevance in this regard is the recent work by Darch et al. (10) based on the model QS bacterium *Pseudomonas aeruginosa*. Using a QS mutant, where density-dependent activation was abolished, the study showed that the benefit of producing an exoproduct (one that is typically produced under QS-mediated control) increased in a density-dependent manner, whereas the benefit of a private intracellular good did not (10).

Knowing that exoproduct benefit is density-dependent (10, 11) and that QS provides a density-sensing mechanism (9) raises several fundamental questions on QS as a general control strategy to regulate production of exoproducts in bacteria. How much delay is advantageous, and what are the QS characteristics to achieve this delay? What happens when production is activated earlier or later? When critical bacterial lifecycle events, such as the advent of stress and population dispersal (transition from high to low density), occur stochastically, is QS still beneficial and if so, to what extent? In a particular environment, how do signaling parameters of the QS machinery affect the overall benefit?

These questions can be answered using a biological system, where the result of different activation strategies (differing in the timing of activation) can be consistently compared based on the specific impact of exoenzyme production. Here, we take a synthetic biology approach (12–14), where a synthetic circuit provides a well-defined system to study its corresponding natural counterpart with a focus on the key fundamental parameters. Over the years, several studies have taken advantage of this approach to address a range of biological questions, such as the role of autoregulation (15), the propagation of noise in gene networks (16), and the more recent demonstration of Simpson's paradox in cooperative populations (13, 17).

In this study, we considered three control strategies (Fig. 1*B*) that span a wide range of control in regulating a costly but beneficial exoenzyme in engineered *Escherichia coli* (Fig. 1*C*). The first strategy is not to produce the exoenzyme (OFF), where cells avoid the cost of production but grow slower under stress. The second strategy is to produce the exoenzyme always at a high rate (ON). With this strategy, cells incur high production cost, but the secreted exoproduct can provide benefit by actively relieving the stress. The third strategy is QS-mediated control, where cells only produce the exoenzyme at a sufficiently high density.

Results and Discussion

Synthetic System for Exoenzyme Production. We implemented the three control strategies in a common synthetic gene circuit (Fig. 1*C*) using the well-characterized LuxR/LuxI QS system of *Vibrio fischeri*. Circuit-carrying cells, when induced, can produce and secrete BlaMs (Fig. S1), a modified β-lactamase exoenzyme that degrades a β-lactam antibiotic in the culture 6-aminopenicillanic

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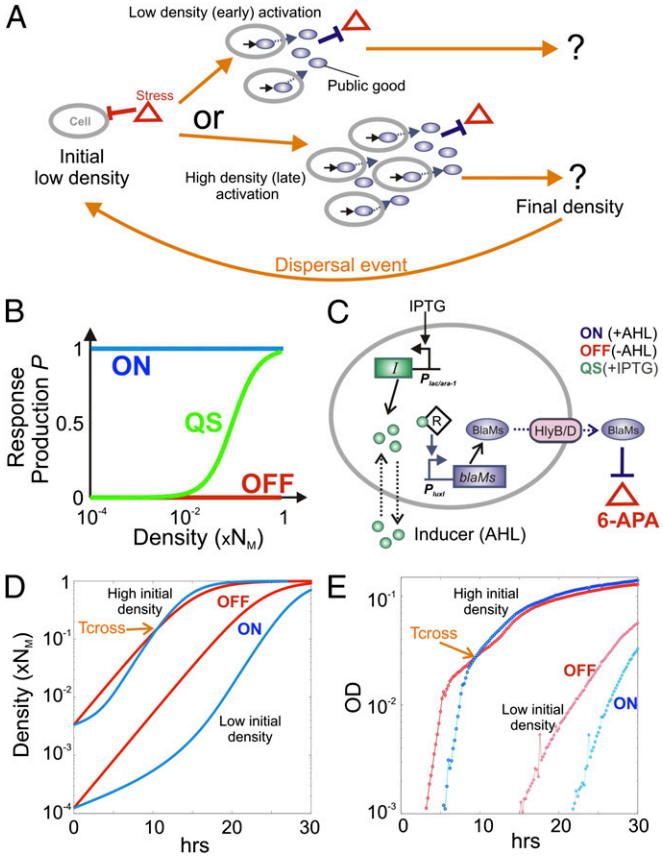

Fig. 1. Strategies for production of exoproducts. (*A*) Lifecycle of a bacterial species that can produce and secrete exoproducts. The schematic depicts distinct strategy choices to activate exoproduct synthesis during population growth: early activation at low cell density or late activation at high cell density. Exoproduct production comes at a cost, but it can benefit by degrading stress-causing agents in the environment. Once secreted, exoproduct concentration in the environment increases with cell density. Thus, benefit per cost for an individual cell increases with cell density. A dispersal event marks the transition from the final high density to low initial density. (*B*) Under QS control, production is activated in a density-dependent manner: OFF at low densities and activated to ON at sufficiently high densities. Cell density is shown scaled to the carrying capacity N_M of the bacterial environment. (*C*) A synthetic gene circuit that implements three strategies to control exoenzyme production. LuxR (R) and hemolysin transporters HlyB and HlyD are constitutively produced in the circuit. AHL can bind to cytoplasmic LuxR and activate BlaMs production. BlaMs is cytoplasmic until secreted into the environment by the HlyB-HlyD transport machinery, where it degrades 6-APA. For the OFF case, no AHL is added. For the ON case, AHL is added exogenously. For the QS case, IPTG at appropriate concentration is added. IPTG drives expression of LuxI (I) that catalyzes AHL synthesis. At a low cell density, AHL concentration is low both inside and outside the cells. A sufficiently high cell density leads to sufficient accumulation of intracellular AHL, leading to exoenzyme production. Plasmids pExoI and pRTrans (Table S1) carry the entire circuitry (Fig. S1 shows BlaMs construction and function). (*D*) Simulated growth of ON and OFF populations starting from either high or low initial cell density in the presence of antibiotic. (*E*) Measured growth dynamics starting at high and low initial density in the presence of 25 μg/mL 6-APA. Absorbance (OD) readings below 0.001 are not reliably detectable. ON and OFF cultures were split from the same dilution and hence, had the same initial density.

acid (6-APA) (18), which mimics stress. Exogenously added acyl-homoserine lactone (AHL) can bind to and activate LuxR, which in turn, activates BlaMs production from the P_{LuxI} promoter. Expression of transporter genes *hlyB* and *hlyD* enables extracellular secretion of BlaMs (18) (Fig. S1). Here, the AHL-induced circuit realizes the ON strategy; the uninduced circuit realizes the OFF strategy. For QS-mediated control, AHL can be autonomously produced by the cells through LuxI, the expression of which is controlled by isopropyl β-D-thiogalactoside (IPTG) (Fig. S2). Under QS, AHL concentration increases with cell density and will activate expression of BlaMs only at a sufficiently high density. Expression of LuxR and the Hly transporter proteins is regulated in the same way for all three strategies.

The following characteristics of our system are critical to address the questions on QS control. (*i*) The specific circuit configuration and the ability to chemically induce the desired control strategy (ON, OFF, or QS in Fig. 1 *B* and *C*) allow multiple strategies to be compared based on the specific impact of exoenzyme production. (*ii*) The exoenzyme BlaMs acts as an inducible public good exoproduct. When secreted into the environment, it degrades β-lactams, such as 6-APA, but provides no protection when within cells (Fig. S1). (*iii*) The cell density at which exoproduct synthesis is initiated by QS can be modulated, allowing comparison across a spectrum of strategies, all within the same system (Fig. S2). (*iv*) By adjusting the 6-APA concentration, the relative ratio of cost to benefit can be changed, and the role and importance of appropriate activation timing can be observed. (*v*) High temporal resolution measurement of bacterial growth provides an insight into the reaction and transport dynamics of exoproduct and its impact over the bacterial lifecycle.

We developed a mathematical model for this system that, at its core, accounts for the combined effect of the cost and benefit of exoenzyme production; exoenzyme cost is incorporated through a reduction in growth rate proportional to the extent of maximal activation, whereas its benefit is indirectly accounted for by the increase in growth rate when secreted exoenzyme degrades antibiotic (*SI Text* and Table S2). We have neglected the cost of QS per se, because this cost is much less than the cost caused by production of exoproducts (5, 19). This assumption is also consistent with measurements with our synthetic system (*SI Text*).

Exoenzyme Production Is Overall Beneficial only at Sufficiently High Density. We simulated growth of ON and OFF populations starting from the same initial density in the presence of stress. We first compared their growth when their initial density was high (Fig. 1*D*). Initially, the ON population grows slower than the OFF population. Because of the cost of producing the exoenzyme and the inherent delay between exoenzyme production and antibiotic degradation, the collective benefit does not instantly exceed the cost of production. As the exoenzyme degrades the antibiotic, the growth rate of the ON population gradually increases and eventually exceeds that of the OFF population. After sufficient time (*Tcross*), the ON population reaches and outgrows the OFF population, making ON an advantageous strategy. Experimentally, we monitored the growth of ON and OFF populations when a sublethal concentration of 6-APA was applied at a high initial density (300-fold dilution of an overnight culture to $\sim 3 \times 10^5$ cells/mL). Consistent with simulation, the ON population eventually recovered from an initial delay to exceed the OFF population (Fig. 1*E* and Fig. S3, *Tcross* ~ 10 h).

If the initial density is sufficiently low, however, simulation shows that the ON population will never win during the growth period, despite its ability to actively degrade the antibiotic (Fig. 1*D*). Higher dilution of the secreted exoenzyme results in little benefit for the ON cells, but the exoenzyme synthesis incurs the same cost as seen earlier. As such, eventual benefit from exoenzyme production cannot make up its initial growth disadvantage compared with the OFF population, making OFF the advantageous strategy. Again, consistent with simulations, when 6-APA at the same concentration as before was applied at a low initial density (24,300-fold dilution of an overnight culture to $\sim 4 \times 10^3$ cells/mL), the ON population was unable to outgrow the OFF population during the growth period (Fig. 1*E*).

The dependence of accrued benefit per cost invested on cell density results in *Tcross* increasing rapidly as the initial density is decreased and approaching the entire growth period observed during which the ON population cannot outgrow the OFF population (Fig. S3*B*). Thus, whether the production of a costly exoenzyme can eventually benefit the population depends on both the initial cell density and duration of growth: both must be sufficiently large for production to become beneficial at the population level. This finding suggests that a critical intermediate density (N_{crit}) exists as a transition point at which production becomes advantageous in the bacterial lifecycle (Fig. S3*B*). For a population starting growth at a low density (below the transition point), a control mechanism that only activates production (OFF to ON) after this critical density would be advantageous. QS is an implementation of such a control strategy (*SI Text*).

QS Provides an Appropriate Density-Dependent Control Strategy. To examine the effectiveness of QS as an optimal control strategy, we simulated growth of three populations starting from a low initial density in the presence of the stress (Fig. 2*A*). We chose a QS system that activates production at a sufficiently high density (reaching half-maximal activation at $10^{-1} \times N_M$, where N_M is the carrying capacity) (Fig. 1*B*). For the same low initial density as before, the ON population cannot outgrow the OFF population. The QS population initially grows similarly to the OFF population, because neither incurs significant cost of exoenzyme production. At a sufficiently high density, however, the QS population can trigger expression of the exoenzyme. At this high density, the net benefit of the exoenzyme will exceed its production cost (with a brief time delay, during which the QS population was losing slightly to the OFF population). The overall benefit of the exoenzyme enables the QS population to outgrow the OFF population.

To test this prediction experimentally, we compared growth of three bacterial populations starting at the same low initial density (~4×10^3 cells/mL) in the presence of 25 μg/mL 6-APA, with BlaMs synthesis OFF, always ON, or controlled by QS. For the ON population, the concentration of the exogenously added AHL was comparable with the AHL concentration measured in the QS culture at a high density (~9 nM) (Fig. S2*C*). Again, because of the low initial starting density, the OFF population always outgrew the ON population. As predicted by modeling, the QS population initially grew similar to the OFF population but outgrew the latter at high density (Fig. 2*C*).

In general, stress can occur at variable densities and the subsequent growth duration can also vary. Given such variability, is QS still advantageous? To address this question, we simulated QS, ON, and OFF populations starting at varying initial densities and compared them all throughout their growth period (Fig. 2*B*). When starting at low initial densities (below $10^{-4} \times N_M$), growth of the QS population largely follows the growth of the OFF population until a sufficiently high density, where QS-mediated activation allows the QS population to overtake OFF. At these low initial densities, ON is always the losing strategy, never being able to overtake OFF or QS during the growth period. Starting from high initial densities (above $10^{-3} \times N_M$), exoenzyme synthesis is beneficial, and the ON population grows slower than OFF (Fig. 2*B*, below the orange curve) for a short period before overtaking it (Fig. 2*B*, above the orange curve). Here, QS activates exoenzyme synthesis within a short period of initiating growth and overtakes both ON and OFF populations. Thus, OFF is the losing strategy for a high initial density (Fig. 2*B*, the region above the orange curve). Regardless of the initial density, however, QS only loses to OFF for a brief period after activation (Fig. 2*B*, pink region). This region is an inherent feature reflecting the dynamics of the enzymatic process: although cost is incurred immediately during production, the beneficial reduction in antibiotic requires the dynamical processes of transport and enzymatic reaction (*SI Text* and Fig. S4 have more detailed analysis on where QS would not be the best strategy).

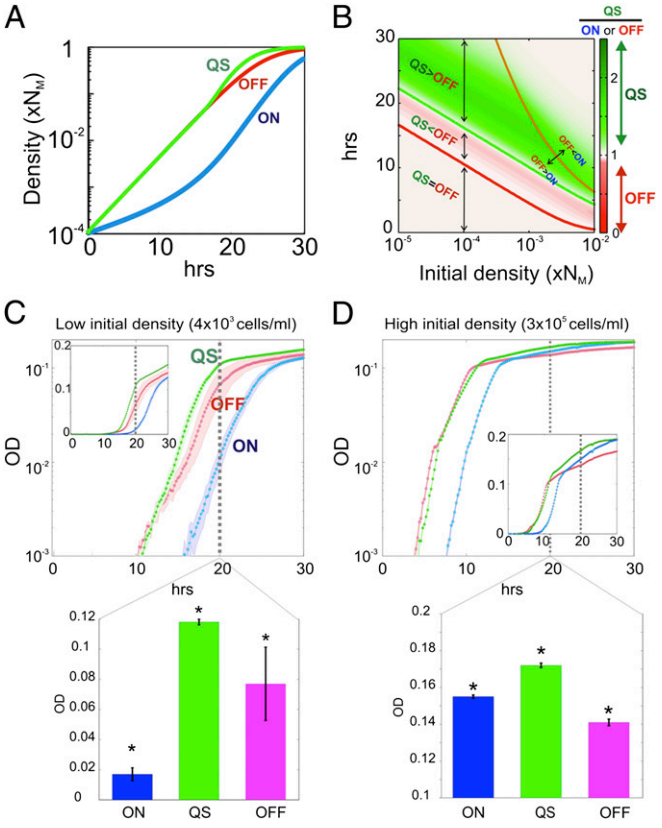

Fig. 2. Density-dependent activation through QS, (*A*) Simulated growth of QS, ON, and OFF populations starting from a low initial density in the presence of stress. (*B*) Simulations of QS, ON, and OFF populations were carried out over a range of initial densities (onset of stress), and the densities of all three were compared at each time point. The color intensity at each dot in the grid ($10^3 \times 10^3$ data points) represents the ratio of cell density of QS against the greater of ON or OFF at the corresponding time. Colored lines are shown to help demarcate the regions where a particular strategy is best. To demarcate the regions, a difference of 1% in cell density at any point was considered significant. Moving vertically from any initial density, the red line indicates transition from QS = OFF (before QS activation) to OFF > QS (pink region; immediately after QS activation). The next transition occurs at the green line, where QS > OFF after the benefit of exoproduct action at high density. The orange line marks *Tcross*, where ON outgrows OFF (Fig. S3*B*). Fig. S4 shows extended simulations examining the effect of stress level and other QS systems that activate at higher or lower densities. (*C* and *D*) Growth of QS, ON, and OFF populations starting from low (*C*) and high (*D*) initial densities in the presence of 25 μg/mL 6-APA. *Insets* show the corresponding results in linear scale to highlight the cross-over of ON and OFF. (*Upper*) Each data point is the average of four replicates, and shaded area spans the SD. Initial densities (cells per milliliter) were estimated from the dilution level and density of the overnight culture. Vertical stippled line marks the 20-h point where the three are compared below. (*Lower*) Bar graphs compare growth of QS, ON, and OFF at the 20-h point. *Significant difference from each of the others (one-way ANOVA and Tukey–Kramer method multiple comparison with $\alpha = 0.01$). Fig. S5 shows observations at other initial densities and corresponding results in the absence of 6-APA.

Overall, it emerges that QS is advantageous or at least, nonlosing for all combinations of initial densities and growth periods (Fig. S4 has QS comparison across varying stress levels and activation densities). This predicted property was confirmed experimentally (Fig. 2 *C* and *D* and Fig. S5) by comparing the three populations over different initial densities during the growth period. As expected, in the absence of the 6-APA, OFF was always advantageous, regardless of initial density (Fig. S5). In the

presence of 6-APA, QS emerged as the winning strategy over the majority of the observed growth period, regardless of initial density, and was never the losing strategy. At low initial densities (below ~10^4 cells/mL), as before, ON was the losing strategy, whereas QS was the best strategy (Fig. 2*C*). At high initial densities (above ~10^5 cells/mL), exoenzyme production by both QS and ON is beneficial, with OFF as the losing strategy (Fig. 2*D*).

QS-Mediated Regulation Is Robust to Stochastic Dispersal Events. We have shown that QS is an advantageous control strategy when considering a single bacterial population starting growth from a defined initial density. However, an event, such as dispersal, that restarts the growth cycle (Figs. 1A and 3A) from low cell numbers after reaching a high cell density (biofilm formation, fruiting body development, or sporulation) can result in variable initial densities (20). Consequently, the postdispersal global population will consist of subpopulations with a large spread in the distribution of their initial densities (Fig. 3*B*). Aside from being implicated in the dispersal event (21, 22), QS is suggested to be critical during initiation of growth from low numbers after dispersal (20). To examine whether and to what extent QS remains advantageous in such an event, we mimicked dispersal through extreme dilution (13). High dilution (5×10^7-fold) of an overnight culture into a 96-well plate resulted in high stochastic variability in the seeding density of individual wells with about 2–3 cells/well (estimated from the number of empty wells) (13) (*SI Text*). Here, each well acted as a subpopulation with a variable initial density. We exploited this variability to examine whether QS, which is driven by the kinetics of signal production, diffusion, and sensing and hence, could be sensitive to environmental fluctuations, would remain effective as a controller. Consistent with simulations (Fig. 3*C*), we found that, despite the stochasticity in the initial density, the overall QS population outgrew the overall OFF population (Fig. 3*D*), akin to the result seen earlier in the absence of variability in seeding.

We note, however, that the stochasticity that we generate is not a universal number, and biologically, its magnitude may vary from the magnitude generated experimentally (Fig. 3*B*). In lieu of this finding, we investigated how the extent of variability in initial density affects QS. Using simulations, we compared the growth of two QS populations starting at the same mean initial density but with different degrees of variability in the densities of their subpopulations (Fig. 3*E*). Experimentally, we similarly compared two QS populations by using the dilution level to manipulate the degree of variability in subpopulation densities (Fig. 3*B*, *SI Text*, and Fig. S6).

Surprisingly, both simulations (Fig. 3*E*) and experiments (Fig. 3*F*) showed that the high-spread population significantly outgrew the low-spread population for a large period. The reason behind this counterintuitive result is in the particular way in which QS operates: starting from a sufficiently low initial density, target gene activation is solely dependent on a critical cell density. During growth under stress, a high-spread population will have more subpopulations at higher and lower density than the low-spread population (Fig. 3*B*). As such, after a sufficiently long growth period, the higher-density subpopulations will be the first to reach a density at which exoenzyme synthesis would be beneficial, and hence, they will be the first to exploit the benefit of exoenzyme secretion. Thus, beyond showing that QS control is advantageous and robust over the bacterial lifecycle, our results show that the benefit of QS control is only enhanced by the variability in initial density generated by stochasticity in dispersal.

QS Kinetics Need to Be Appropriately Tuned for Optimal Regulation. Lastly, we note that QS systems in nature are tremendously diverse in terms of both their molecular implementations and their activation properties. For instance, the density at which QS triggers its target gene can span four orders of magnitude (23). This

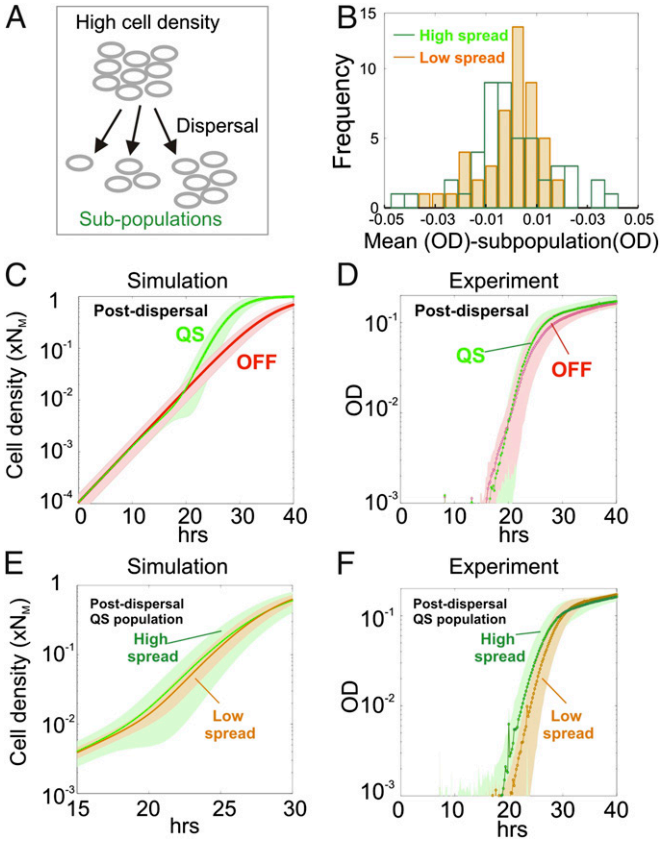

Fig. 3. QS-based control is robust to stochasticity caused by dispersal. (*A*) Schematic showing stochastic dispersal of a high-density population generating subpopulations with a wide distribution of initial seeding densities. (*B*) Histogram showing the typical distributions generated experimentally within the wells of 96-well plates after extreme dilution of an overnight culture. Note that higher dilution (green; 5×10^7-fold of overnight culture) results in higher spread than the lower dilution (orange; 5×10^6-fold of overnight culture). (*C*) Simulation results showing growth QS and OFF populations after dispersal event. Initial densities for 200 subpopulations were chosen from a Poisson distribution (a Poisson parameter $\lambda = 2$ was used with population mean shifted to 10^{-4}). (*D*) Combined growth dynamics of QS and OFF populations after high dilution in the presence of 25 μg/mL 6-APA show that QS remains advantageous. Each dot is OD averaged over 48 wells (subpopulations), and the shaded area spans the SD. (*E*) Simulation results showing growth of QS populations with high and low spread after dispersal event. Simulations were performed as described in *C*. To compare the difference between populations with high (green) and low (orange) initial spreads, λ values of 2 and 20 were chosen to capture the 10-fold difference in experimental dilution. A shorter time span (15–30 h) is used, so that the differences in growth are more visible. (*F*) Combined QS population growth in the presence of 25 μg/mL 6-APA comparing the cases of high or low spread in the initial density across subpopulations. Each dot is OD averaged over 48 wells (subpopulations), and the shaded area spans the SD. Additional results from replicate experiments are shown in Fig. S6.

diversity raises another fundamental, unaddressed question with regard to the advantage of QS control: how do two distinct QS systems compare when one activates production at a lower density than the other? We previously developed a metric sensing potential (v) that uniquely captures the dominant activation properties of QS (*Materials and Methods*) (23). The larger the value of v, the lower the density at which a QS system activates (Fig. 4*A*). At the extreme, an infinitely large v represents a system that is always activated (ON), and $v = 0$ represents one that is always inactive (OFF). Thus, using v as a metric allows us to examine a continuous range of QS systems.

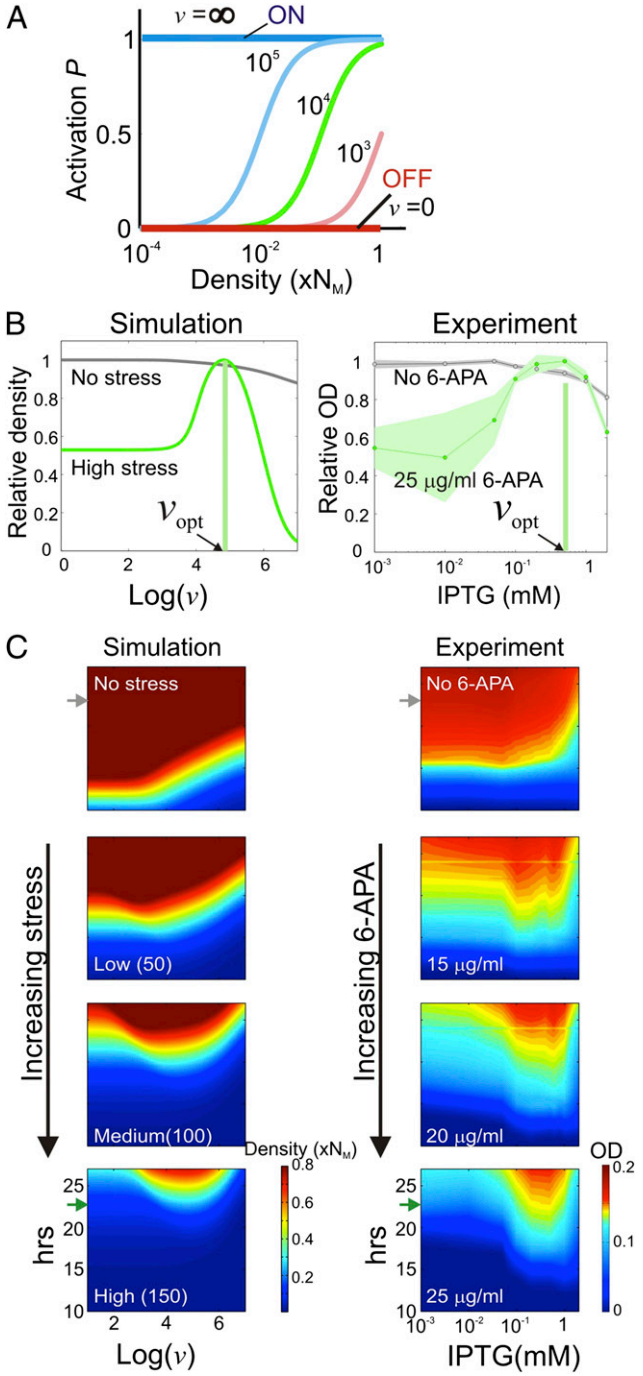

Fig. 4. Optimal tuning of QS to maximize bacterial growth, (*A*) Density-dependent activation of production governed by the potential *v* of a QS system. QS systems that activate early (high *v*), at an intermediate density, and late (low *v*) during growth are shown. (*B*) Optimal QS potential as predicted by simulation (*Left*) and measured experimentally (*Right*). Cell densities (normalized to the highest value) at 22.5 h were used to compare the different potentials in the presence (green) and absence (gray) of stress. (*C*) Simulated (*Left*) and experimentally observed (*Right*) bacterial growth starting from a low initial density over a range of *v* and increasing stress (from top to bottom). Both simulations and experiments show biphasic dependence of growth on *v* in the presence of stress. Values in parentheses indicate the level of stress (for simulation) and corresponding concentration of 6-APA used in the experiment. The horizontal arrow at 22.5 h indicates the point in time at which growth was compared in *B*. Increasing 6-APA increases the benefit per cost of exoenzyme production (Fig. S2*D*). Fig. S7 compares the cost of exoenzyme production with QS-based signaling in our system.

In the absence of stress, simulations predict growth to be a decreasing function of *v* (Fig. 4*B*, *Left*, gray). This decrease reflects the corresponding increase in cost accrued with early activation during growth in the absence of any benefit. In contrast, in the presence of stress, simulations predict growth to be a biphasic function of *v* (Fig. 4*B*, *Left*, green) with a finite potential (v_{opt}) at which growth is maximal. This biphasic function results from the opposing roles of cost, which increases with early activation, and benefit, which increases with late activation. v_{opt} represents the QS-mediated activation path (Fig. 4*A*) during the lifecycle that is closest to the theoretical optimal path (*SI Text* discusses the derivation of optimal control strategy).

To examine this finding experimentally, we modulated the strength of QS using IPTG. Increasing IPTG leads to faster AHL production and shifts QS activation to lower density, which corresponds to a higher sensing potential (Fig. S2). We then compared growth of QS populations under different levels of IPTG in the presence and absence of antibiotic. Consistent with simulation results, biphasic growth dependence was seen only in the presence of 6-APA, and an intermediate level of QS strength (0.5 mM IPTG) proved optimal. In the absence of 6-APA, growth decreased monotonically with increasing IPTG, reflecting the accrued cost without benefits (Fig. 4*B*, *Right*).

Noting that the optimal sensing potential (v_{opt}) represents a tradeoff between the costliness of early activation and the loss of obtainable benefit in late activation, we examined the interplay between the level of stress and QS characteristics. In simulations (Fig. 4*C*, *Left*) and experiments (Fig. 4*C*, *Right*), we used cell densities to define the growth landscape. In the absence of stress, growth initially does not change significantly with increasing IPTG (Fig. 4*C*, *Right*, row 1), which is reflected by the relatively flat growth landscape (until 12 h or OD < 0.05). This result indicates that the cost of LuxI production through IPTG is small compared with the cost of exoenzyme synthesis and secretion as assumed in our model (Fig. 4*C*, *Left*, row 1). After this period, growth starts to decrease with IPTG, and the steepness of the decline increases with time. This finding reflects the cost of exoenzyme synthesis and secretion after the onset of QS-controlled activation in the absence of any benefit—increasing IPTG leads to earlier activation and hence, higher accrued cost with time. In the presence of stress, however, the growth landscape becomes biphasic, and its shape changes predictably with the level of stress (Fig. 4*C*, rows with antibiotics). With increasing stress, the biphasic peak of the landscape (which determines the optimal QS characteristics) becomes increasingly sharp and shifts to higher *v*; earlier activation is favored with higher stress. These landscape changes show that the optimal QS characteristics, as determined by the overall benefit of late vs. early activation, depend on the specific parameters of the stress–response scenario.

When stress onset is stochastic in timing and magnitude, *v* for the species may not be exactly at the optimal level for the specific case, and its position on the landscape would determine the overall advantage of QS. This scenario is relevant where QS inhibition is being examined as an alternative intervention strategy against QS bacterial pathogens (24–26). Given the biphasic relationship between growth and *v*, inhibition of signaling, which reduces *v* (Fig. S2), may counterintuitively increase overall growth (Fig. 4*B*) (for example, from 2 to 0.25 mM IPTG in the presence of 6-APA). Depending on factors such as stress level and extent of signal disruption, this result could similarly occur when an inhibitor that targets signal production is used as an antibacterial treatment, and it may lead to misinterpretation of the effects of the intervention strategy. With stress that is constant in magnitude and regular over bacterial lifecycles, QS characteristics would be directed to v_{opt} (Fig. 4 *B* and *C*), with which overall growth during the lifecycle is maximal (*SI Text* has additional notes on evolution of QS characteristics). This notion has been speculated

based on the observation that natural QS systems seem to be tuned for their specialized niches (27, 28). Overall, our study shows that, when density critically determines whether production of a costly exoproduct is overall beneficial, QS provides a robust and effective density-dependent control mechanism.

Materials and Methods

Growth Conditions. Cells were grown in TBK media [10 g tryptone, 7 g KCl, buffered with 100 mM 3-(N-morpholino) propanesulfonic acid, MOPS] at 30°C with pH balanced to 7.0 using KOH. Cells transformed with circuit-carrying plasmids (pExoI and pRTrans) were grown from single clones overnight for 14 h. Plasmids were maintained with 100 μg/mL chloramphenicol and 50 μg/mL kanamycin. 1 mL overnight culture was spun down, washed, and suspended in 1 mL distilled water. Washed cells were diluted ranging from 300- (lowest dilution and highest initial density) to 24,300-fold (threefold dilution series and five levels) into prepared media with 25 ng/mL anhydrotetracycline (aTc) to induce production of LuxR and transporter. To induce LuxI for QS, 2 mM IPTG (in water) or appropriate dilution was used. An equivalent amount of water was added as control in the absence of IPTG. TBK media supplemented with 0.1% Arabinose and 25 ng/mL aTc were used for all comparisons of QS, ON, and OFF populations. $3OC_6$AHL (K3007; Sigma) was prepared as a stock solution of 20 mM by adding 4.5 mg to 1 mL ethyl acetate. This solution was subsequently diluted by adding 5 μL stock solution to 995 μL media, giving 100 μM final concentration. Lower concentrations were prepared using serial dilutions in media. 6-APA solution was freshly prepared for each experiment by dissolving 50 mg 6-APA (A70909; Sigma) powder in 1 M HCl. Subsequent dilutions were made in water such that the effective HCl solution concentration was always 0.1 M. As a control, 0.1 M HCl was added to wells where no 6-APA was added, and 200 μL prepared cultures were laid out in triplicates in 96-well microplates (Corning); 50 μL mineral oil were added to prevent evaporation, and the plate was incubated in the plate reader (Victor3; Perkin-Elmer) at 30 °C. Readings of absorbance at 600 nm (OD) and GFP fluorescence (when applicable) were taken every 10 min with periodic shaking (5-s orbital shaking followed by 5-s pause).

Plasmids and Strains. A two-plasmid system was used throughout this study. pExoI (p15A, Kan^R) is responsible for IPTG-inducible production of LuxI and BlaMs (under P_{LuxI}) inducible by AHL-bound LuxR. pRTrans (ColE1, Cm^R) expresses LuxR and the transporter genes under an aTc-inducible promoter. It also constitutively expresses the *tetR* repressor for low, tightly controlled expression. The transporter gene cassette was derived from plasmid pVDL9.3, and 25 ng/mL aTc were added to the media for expression of LuxR and the transporter. Table S1 has a list of strains and plasmids used in our study. Strains used were JM109 [*endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB*$^+$Δ*(lac-proAB)* e14$^-$ [F′ *traD36 proAB*$^+$ *lacIq lacZΔM15*] *hsdR17*($r_K^-m_K^+$)], MG1655 (F$^-$ λ$^-$ *ilvG*$^-$ *rfb-50 rph-1*), and MC4100Z1, which is MC4100 [*araD139* (*argF-lac)205 flb-5301 pstF25 rpsL150 deoC1 relA1*] with a Z1 cassette for lacIq, tetR, and spect(R).

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