Direct observation of single stationary-phase bacteria reveals a surprisingly long period of constant protein production activity

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Exponentially growing bacteria are rarely found in the wild, as microorganisms tend to spend most of their lifetime at stationary phase. Despite this general prevalence of stationary-phase bacteria, they are as yet poorly characterized. Our goal was to quantitatively study this phase by direct observation of single bacteria as they enter into stationary phase and by monitoring their activity over several days during growth arrest. For this purpose, we devised an experimental procedure for starving single *Escherichia coli* bacteria in microfluidic devices and measured their activity by monitoring the production rate of fluorescent proteins. When amino acids were the sole carbon source, the production rate decreased by an order of magnitude upon entry into stationary phase. We found that, even while growth-arrested, bacteria continued to produce proteins at a surprisingly constant rate over several days. Our identification of this newly observed period of constant activity in nongrowing cells, designated as constant activity stationary phase, makes possible the conduction of assays that require constant protein expression over time, and are therefore difficult to perform under exponential growth conditions. Moreover, we show that exogenous protein expression bears no fitness cost on the regrowth of the population when starvation ends. Further characterization of constant activity stationary phase—a phase where nongrowing bacteria can be quantitatively studied over several days in a reproducible manner—should contribute to a better understanding of this ubiquitous but overlooked physiological state of bacteria in nature.

bacterial metabolism | constant activity stationary phase | CASP

In his thesis, Jacques Monod (1) pioneered the precise and quantitative analysis of bacterial cultures. He focused on mathematically defining and measuring the growth rate of exponentially growing bacterial populations. In parallel to Leo Szilard and coworker (2), he developed the chemostat (3), which allows the maintenance of constant growth conditions in bacterial cultures over time. These conceptual and experimental tools laid the foundation for quantitative studies of growing bacteria, which could be compared and reproduced. In contrast to these advances in the characterization of growing bacteria, the stationary phase, which is the prevalent state of bacteria in nature, is far from being characterized in a similarly satisfactory manner (4). From an ecological point of view, the quantitative study of nongrowing bacteria is highly relevant: ecosystems such as soil, sea water, and biofilms are dominated by nongrowing bacteria. Nongrowing bacteria have been recently linked to persistent infections, which represent a growing health issue (5–7), therefore an understanding of the physiology of these bacteria, both in vitro and in vivo (8) should have important clinical implications.

Typically, stationary phase is attained when a culture exhausts nutrients and its OD remains constant. Starved microorganisms need to respond to a wide range of stresses (4), such as nutrient limitation, high concentrations of organic acids, osmotic and oxidative stress, and pH changes, depending on the specific conditions that have led to stationary phase. The constant OD measured at stationary phase may result from lack of growth or, alternatively, the result of a dynamic balance between growth and death. The term "stationary phase" is thus used to designate very different conditions that may be only remotely connected (9, 10). Nevertheless, several studies have addressed the question as to whether bacteria do in fact produce proteins during stationary phase, as reviewed in (11). Upon entry into stationary phase, the decrease in growth rate is accompanied by an 80% reduction of protein synthesis, compared with cells in exponential growth (12). Shaikh et al. (13) showed that *Escherichia coli* cells are indeed able to produce new proteins during stationary phase on minimal medium with glucose by reusing amino acids derived mostly from degraded proteins synthesized during the exponential growth phase. Microarrays analysis revealed that *E. coli* can maintain some activity even after 20 h of starvation in sea water (14). Studies of longer term survival at stationary phase in rich media focused on genetic changes acquired by a subpopulation of surviving cells that confer a growth advantage at stationary phase, named GASP (15). Most studies focused on changes in gene expression, metabolism, and morphology that occur at the onset of stationary phase, but relatively few follow the dynamics of cultures over days of growth arrest (16). Furthermore, most studies follow cultures at only a few points over time (13, 17–19). Finally, the question of whether the activity measured at the population level reflects the behavior of the entire bacterial population, or is rather the result of nontrivial activity of subpopulations (20), could not be determined in the absence of direct monitoring of single cells under starvation.

Our goal, therefore, was to make possible the quantitative and reproducible characterization of the potential for transcription and translation activity of bacteria over many hours of starvation, down to the single cell level. For this purpose we developed methodology suitably designed for the continuous monitoring of

Significance

Although most laboratory work is performed on dividing bacteria, in the wild bacteria spend most of their lifetime in the stationary phase, scarcely dividing. To study the stationary phase, we developed a method of tracking nondividing bacteria at the single cell level and characterizing their response to external stimulation. While following bacteria under growth-arrest conditions, we found that they maintain surprisingly constant activity for several days after entering the stationary phase. These bacteria were able to produce proteins without significant cost to their viability or regrowth dynamics. Moreover, this constant-activity stationary phase enables the measurement of different bacterial characteristics, which are difficult to perform under exponential growth conditions.

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single planktonic bacteria as they enter stationary phase, and for following their activity over several days of starvation, under the microscope and under controlled starvation conditions.

Results

Starvation of Single Cells in Microfluidic Devices. We addressed this challenge by designing and fabricating a microfluidic setup, based on chambers previously described (ref. 21 and *Materials and Methods*). In brief, the bacteria to be observed are trapped in a patterned polydimethylsiloxane (PDMS) layer (typically, in lines the width of a bacterium); medium flows in microfluidic channels (flow channels), but is separated from the trapped bacteria layer by a transparent membrane, such that nutrients, inducers, etc., can diffuse through the membrane to the trapped bacteria without disturbing their position. We reproduced, under the microscope, the starvation dynamics of batch cultures. This was done by using the flow channels to flow a parallel batch of bacterial culture (flow bacteria) grown from exponential to stationary phase. The bacteria trapped in the patterned layer could thus be tracked individually while being subjected to the same conditions as those of a batch culture grown to stationary phase (see illustration in Fig.1*A*, comparison with batch culture growth in *Supporting Information*). We used M9 medium supplemented solely with amino acids, which resulted in a sharp entry into stationary phase and minimal loss of viability (Fig. 1*K* and *Supporting Information*). After an initial phase of exponential growth, we were able to directly observe the trapped bacteria as they were starved and to determine whether the typical constant OD observed at stationary phase is caused by entry into dormancy or by equal single cell probabilities to die or grow. As seen in Fig. 1 *G–J*, most of the bacteria arrested their growth upon entry into stationary phase. Apart from a minority of lysing cells (the number of lysing cells varied between experiments, but always less than 7%), the bacteria were shown to be alive (*Supporting Information*). Extremely slow growth (>20 h per division) was observed in only a small percentage (less than 5%) of bacteria.

Observation of Constant Promoter Activity in Nongrowing Single Cells. To quantitatively measure the activity of these nongrowing bacteria, similarly to the approach adopted in refs. 16 and 21), we examined the dynamics of the promoter activity (PA) of engineered promoters. In general, the PA can be written as the product of two terms: $PA(t) = p1(t) \cdot p2(t)$ (22). Here, $p1(t)$ is a factor common to many promoters and depends on the transcription and translation capabilities of the cell, for example on the total number of available polymerases, the number of available ribosomes, etc., whereas $p2(t)$ represents the specific regulation of the promoter. Our goal was to characterize $p1(t)$ at stationary phase down to the single cell level. For this purpose, we used strong engineered promoters with simple regulation for which PA is proportional to $p1(t)$ only (16). Note that similar results were obtained with two different inducible systems, which were chosen, after induction, to reflect the behavior of unregulated promoters. We measured the fluorescence of single *E. coli* bacteria bearing plasmids with fluorescent reporters under two different inducible promoters, the synthetic *Ptac* promoter, a functional hybrid derived from the *trp* and *lac* promoters, induced by Isopropyl β-D-1-thiogalactopyranoside (IPTG) (23), and *PLtet* promoter, induced by anhydrous tetracycline (aTc) (24), both nonmetabolized inducers (*Materials and Methods* and Fig. 2 *B–C*). Our aim was to evaluate whether the nongrowing bacteria—that is, the majority of the cells

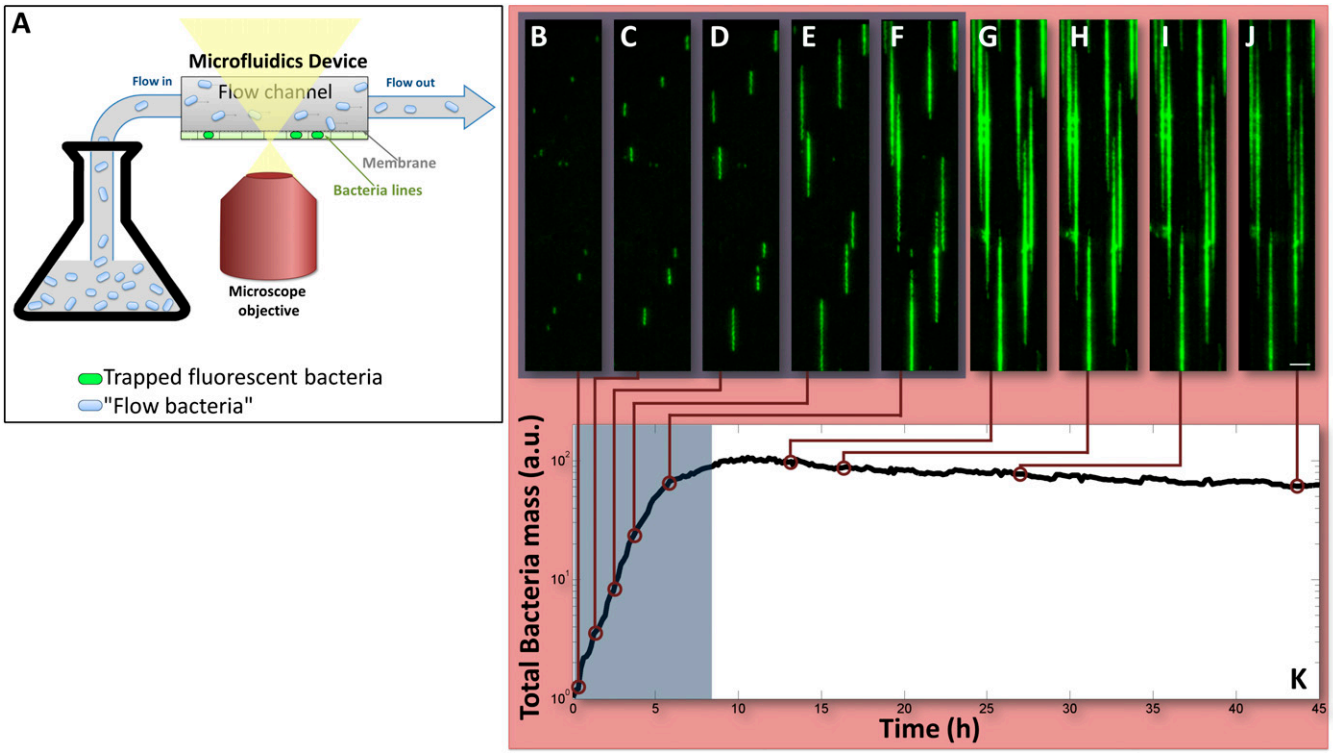

Fig. 1. Direct observation of growth arrest in single cells. (*A*) Schematic view of the microfluidic device and the experimental setup for growth arrest. The device consists of several layers: a thin patterned PDMS layer with microscopic lines for trapping bacteria; a cellulose membrane; a thicker PDMS layer with microfluidic flow channels. A batch culture is pumped into the flow channels to create stationary phase conditions for the trapped bacteria under observation. (*B–J*) Fluorescence imaging of the growth arrest of single cells in the microfluidics device. Constitutively GFP expressing bacteria trapped in the microfluidics device grow along the bacteria lines (*B–F*), and then enter stationary phase, where growth arrest can be directly visualized (*G–J*). Bar = 10 μm. (*K*) Growth curve in the microfluidic device. The total area of the bacteria in the microfluidic device is shown, as extracted from the total area of the fluorescence signal. The apparent decrease during more than 35 h of stationary phase is due to photo-bleaching. The gray shaded area marks the exponential phase of growth.

being observed—respond to an external induction signal and start protein production or whether only the minority of very slowly growing cells is responsible for the protein production at stationary phase.

Typical induction dynamics in the microfluidic device under stationary phase conditions are shown in Fig. 2 *D–J* (time-lapse) and Fig. 2*K*. The bacteria constitutively expressed mCherry, whereas the GFP fluorescence was induced after about 15 h of starvation. A large majority (>90%) of the starved bacteria responded to the induction signal, ruling out the hypothesis that metabolic activity during stationary phase is restricted to a small population of growing cells. We noticed, however, that the induction dynamics at the single cell level was linear for many hours, despite the fact the single bacteria did not grow. This observation suggested that nongrowing starved cells are able to produce proteins at a constant rate for many hours.

Measurements of Constant Activity over Several Days in Batch Cultures. To verify that the promoter activity remains indeed constant over many hours, and to avoid the limitations imposed by the degradation of fluorophore and/or bleaching, we set up an additional experiment, this time in batch cultures. Bacteria were grown to stationary phase in identical parallel cultures in 96-well plates with their fluorescence level being continuously monitored. To accurately measure the slope of the fluorescence induction, inducers were added by automatic injection into different wells at different time points that spanned the whole growth curve—namely, from the exponential phase until deep into stationary phase (illustration of the experimental design is shown in Fig. 3*A*). Each well was subjected to the inducer at a different time, *t*. Note that in contrast to the single-cell experiments in which the induction signal was applied only once, *PA(t)* in the batch culture was measured as the initial slope of the fluorescence increase following each induction. The initial slope is a direct measurement of the *PA* and is independent of assumptions on the dynamics of fluorophore degradation at the stationary phase. Representative results of such experiments are shown in Fig. 3 *B–D*. Using this setup, we were able to monitor the initial response of the bacterial culture upon induction over several days.

As seen in Fig. 3*C*, the induction curves abruptly change upon entry into stationary phase (25), with a decrease of the PA by one order of magnitude (Fig. 3*D*). In accordance to the single-cell observations during stationary phase, the induction curves stay parallel despite the absence of growth, namely the bacteria maintain a constant *PA* for tens of hours of starvation.

Similar results were obtained with two different inducers controlling the expression of different fluorophores by different promoters, showing that the surprisingly constant rate of protein production is not restricted to one particular type of inducible system. We also verified that the results do not depend on whether the inducible system is on a plasmid or inserted into the chromosome (*Supporting Information*, Fig. S2). The cultures maintained a constant ability to respond to external signals, to activate transcription and to produce proteins over several days of starvation, without significant decrease in viability (Fig. S3).

Taken together, the single-cell induction curves in microfluidic devices and the batch culture measurements show that nongrowing stationary phase bacteria can maintain a constant activity level over more than 60 h of starvation. We coined the expression constant activity stationary phase (CASP) to designate this physiologically distinct period in bacterial dynamics that differs from other starvation conditions in which activity and viability may decrease by orders of magnitude (15).

Effect of Protein Production at CASP. Protein expression levels depend on the product of two terms: consumable resources (amino acids, nucleotides, etc.) and machinery (ribosomes, RNA polymerases, etc.) (26). The constant activity at CASP suggests

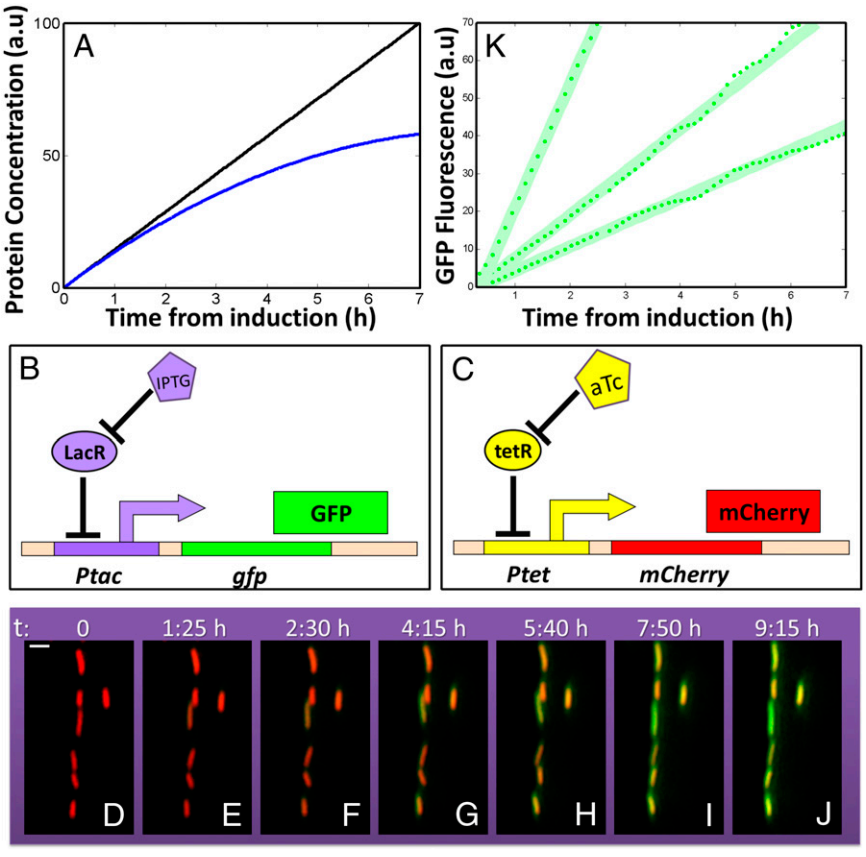

Fig. 2. Protein induction dynamics in single cells. (*A*) Illustration of possible induction dynamics in nongrowing cells. Blue line: promoter activity decays with time during stationary phase. Black line: constant promoter activity. (*B*) *Ptac* inducible construct used to measure the protein induction dynamics. The *gfp* under the *tac* promoter is induced by IPTG. The construct was used on a medium copy plasmid (33) (*C*) *Ptet* inducible construct used to measure the protein induction dynamics. mCherry fluorescence protein under the *tet* promoter is induced by aTc. The construct was used on a medium copy plasmid (24) or integrated into the chromosome. (*D–J*) Induction of single cells in the microfluidic device. Bacteria were induced to express mCherry before stationary phase and kept at stationary phase under the microscope. GFP was induced during stationary phase. Bar = 2 μm. (*K*) Induction dynamics of three representative single bacteria in the microfluidic device. Each line represents the induction of a different bacterium at CASP.

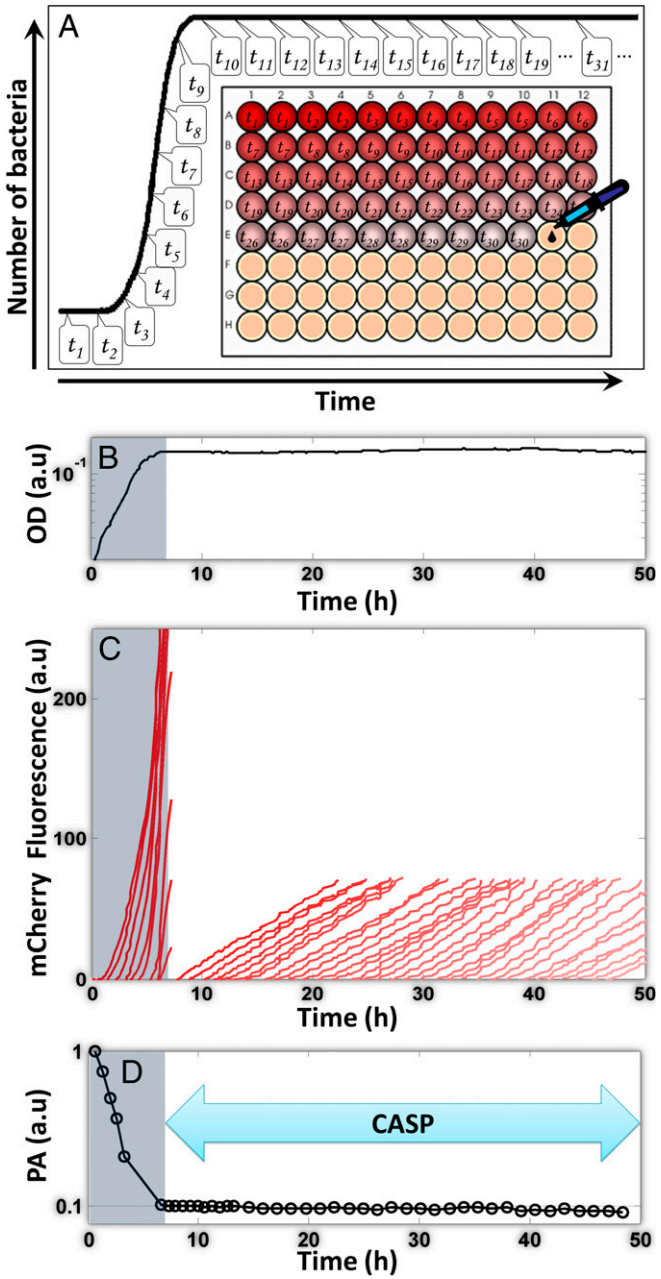

Fig. 3. Protein induction dynamics in batch culture. (*A*) Illustration of the experimental design. Bacteria are grown in 96-well plate to stationary phase. Inducers were added by an automatic injector to different wells at different time points during growth and stationary phase, while monitoring red and green fluorescence. (*B*) A representative growth curve (OD) of the bacteria in one well. The gray shaded area marks the exponential phase of growth. (*C*) The mCherry fluorescence signal for different wells. Each line represents the total fluorescence signal in one well. (*D*) Promoter activity. The promoter activity along the growth curve was calculated from the fluorescence signal. Constant promoter activity at CASP can be observed for tens of hours.

that both terms remain constant. To determine whether limited consumable resources are depleted during CASP or remain constant, we compared the production rates of two cultures (Fig. 4*A*): the first culture (blue) was initially induced with IPTG to continuously produce GFP early during CASP (at t_1) and thereafter, at $t_2 = t_1 + 30$ h, with aTc to start producing mCherry proteins. The second culture (gray) was subjected to the two inducers simultaneously at t_2 only. The production rate of mCherry for both cultures was found to be similar, irrespective of whether the culture had been producing GFP for 30 h (Fig. 4*B*, blue curve, PA = 2.37 ± 0.04) or not (Fig. 4*B*, gray curve, PA = 2.4 ± 0.05), indicating that depletion of resources is not a limiting factor and the amount of resources can be considered an infinite reservoir for protein production during tens of hours of CASP.

Next, we addressed the question of whether the two induction processes may compete for the expression machinery, namely whether the expression of one fluorophore decreases the concurrent expression of the second (26). We compared two parallel cultures at stationary phase (Fig. 4*C*): the first culture (red line), was induced with only one inducer to produce mCherry, while the second culture (gray line) was induced at the same time to produce both mCherry and GFP. We found that the production rate of the singly induced cultures was 10% higher compared with the doubly induced cultures (Fig. 4*D*). Assuming a fixed total machinery concentration, we could calculate (*Supporting Information*) that the gratuitous protein production competes for about 20% of the total production machinery (Fig. 4*E*).

We asked whether this significant reallocation of the protein production machinery may affect the fitness of the bacteria at CASP. Typically, the measure of fitness is the growth rate in exponentially growing cells and fitness cost is defined as a decrease in growth rate. To evaluate the fitness cost of gratuitous protein production at CASP, we first needed to define a fitness parameter in nongrowing cells. We chose to define the fitness of CASP bacteria by evaluating their ability to regrow upon dilution in fresh medium and defined fitness as $T_{regrowth}$, the typical time for the culture to grow by a factor of 20, once the culture is diluted into fresh medium. In fact, $T_{regrowth}$ regroups several factors that may be affected by protein expression at stationary phase before dilution: the viability of the culture, the lag time following exposure to fresh medium, and the initial growth rate upon dilution (*Supporting Information* and Fig. S4). Note that the shorter the $T_{regrowth}$, the higher the fitness of the population: if two populations with identical exponential growth rates but different $T_{regrowth}$ are mixed, the one with the shorter $T_{regrowth}$ would prevail. We evaluated the cost of protein production at CASP by comparing the $T_{regrowth}$ of two cultures: a culture subjected to an inducer at CASP showing a constant rate of fluorescence increase for 50 h (Fig. 4*F*, *Inset*); and another culture kept under the same conditions but without inducer. The $T_{regrowth}$ of both cultures was the same (Fig. 4*F*) suggesting that the constant gratuitous protein production during CASP has no measurable effect on the fitness of the population, despite the burden of 20% on the protein production machinery. Similar results were obtained when fitness was assayed under harsher conditions (*Supporting Information*, Figs. S5 and S6).

Precise Measurements at CASP. In contrast to experiments conducted under exponential growth conditions—for which special devices such as chemostats are required for maintaining constant conditions over days—experiments at CASP can be easily conducted in standard 96-well formats. A more thorough analysis will be required of other parameters at CASP to evaluate their constancy. Nevertheless, for experiments in which the crucial parameter to be maintained constant is the *PA*, measurements at CASP are clearly advantageous, as demonstrated by the following example.

We measured at CASP the Hill coefficient of the promoters studied above. Typical induction curves at different inducer concentrations are shown in Fig. 5 *A* and *C* (for P_{tac} and P_{tet}, respectively). The linearity of the induction curves made possible a straightforward and precise measurement of the promoter activity. We measured a Hill coefficient of 1.6 ± 0.2 for the P_{tac} promoter and of 3.1 ± 0.4 for the P_{tet} promoter (Fig. 5 *B* and *D*). Whereas the P_{tac} promoter behaved as expected in accordance with (27), the P_{tet} promoter curve was asymmetric, departing from the expected curve at low concentrations of aTc (Fig. 5 *B* and *D*). This discrepancy probably reflects the titration of the aTc inducer

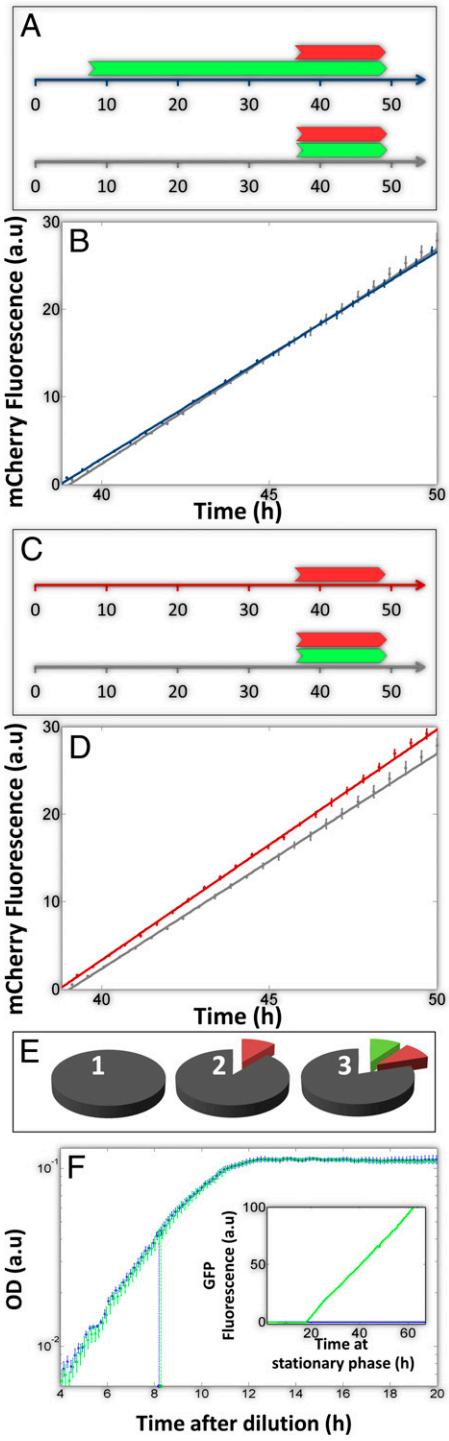

Fig. 4. Evaluating the cost of protein production at CASP. (*A* and *B*) Consumable resources does not deplete during CASP. (*A*) Two parallel cultures were induced to produce GFP and mCherry at different time points. (*B*) The *mCherry* induction was identical despite the fact the one culture (blue line) produced GFP 30 h before the *mCherry* induction, and the other (gray line) received the two inducers together. (*C–E*) Measurement of the difference in the machinery occupancy during CASP. (*C*) Two parallel cultures were induced to produce mCherry or GFP and mCherry at the same time point. (*D*) The production rate of the singly induced cultures was higher by 10% compared with the doubly induced cultures. (*E*) Machinery occupancy during CASP: in 1 no external induction was made, 100% of the machinery is directed to cell metabolism. In 2, *mCherry* is induced, 89% of the total machinery of the cell remains for metabolism. In 3 the production of mCherry and GFP together, occupies about 20% of the total machinery

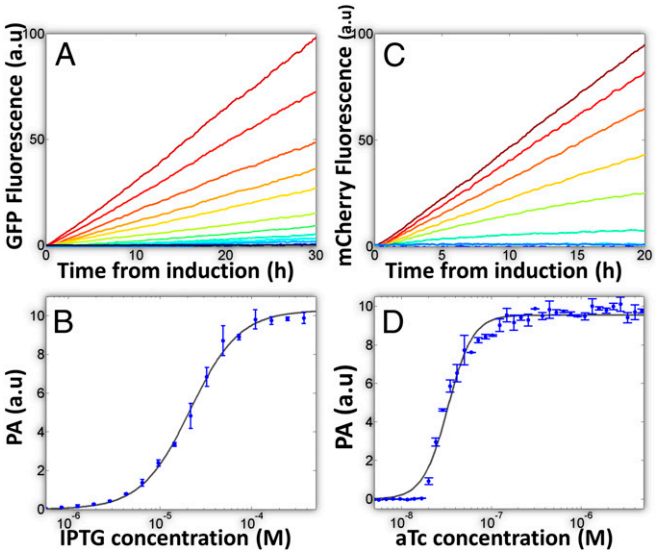

Fig. 5. Quantitative measurements at CASP. (*A*) GFP induction at CASP by different levels of IPTG. Each color describes an IPTG concentration (same range as in *B*), where dark blue designate 0 M, and dark red 10^{-3} M. (*B*) Binding curve for IPTG. PA extracted from *A*. The gray line is the Hill curve fitted with Hill coefficient of 1.62. Error bars: std from triplicates. (*C*) mCherry induction at CASP by different levels of aTc . Each color describes an aTc concentration (same range as in *D*) where dark blue designate 0 M, and dark red $5{\cdot}10^{-5}$ M. (*D*) Binding curve for aTc. PA extracted from *C*. The gray line is the Hill curve fitted with Hill coefficient of 3.13. Error bars: std from duplicates. Note that the curve does not fit the low concentration data.

molecules by the Tet repressors (28) and was made apparent by our precise PA measurements at CASP.

Discussion

Using a starvation protocol in microfluidics devices, we were able to observe the entry of single bacteria into stationary phase and monitor, thereafter, their behavior over many hours of starvation. Whereas the commonly held view is that at stationary-phase bacteria fade away toward inevitable demise, our results have shown that nongrowing bacteria can maintain a constant protein-expression level over several days. The sharp decrease in bacterial activity at the onset of stationary phase, probably by the activation of the stringent response (29, 30), enables the maintenance of resources essential for extended bacterial survival under starvation conditions, and leads to the absence of fitness cost even after many hours of gratuitous protein production. The question of how the bacterial capability of protein expression is maintained constant at CASP is yet to be clarified, but it suggests that nutrients are not the limiting factor that can account for the absence of growth. Further work will also be required to determine whether CASP is under tight regulation, or is the byproduct of one major limiting step in the metabolism. Further studies may reveal pathways specifically evolved to be active during deep stationary-phase conditions. As stationary phase is the common state of bacteria in the wild, specific genes may prove to be essential for maintaining CASP over several days. Energy conservation

(*Supporting Information*). (*F*) Cost effect. Experimental measurements of the regrowth of CASP cultures upon dilution into fresh medium. Cultures maintained at CASP, with (green) and without (blue) GFP production were diluted into fresh medium without inducer and their regrowth was compared. No measurable difference in the $T_{regrowth}$ was observed, indicating that gratuitous protein production during CASP exerts no measurable fitness cost. Error bars: SE of six repeats. *Inset*: GFP fluorescence levels of the two cultures during CASP before dilution into fresh medium.

requires the eventual end of CASP, the timescale for which remains yet to be determined.

In general, the ability to reproducibly and quantitatively measure bacterial activity at CASP opens up the door to further quantitative characterizations of bacteria in the nongrowing state. The difficulty with many strategies for the treatment of infection lies in that bacteria in the wild are often growth-arrested, and most assays in this field are performed on growing cells. For example, antibiotic efficacy is typically tested on exponentially growing cells, and many antibiotics fail in killing nongrowing persister cells (31). Understanding the special physiology required for CASP maintenance in cells at growth arrest may pave new ways of targeting nongrowing persister bacteria, believed to be a major source of failure in the treatment of various infections.

Materials and Methods

Bacterial strains and plasmids are listed in Table S1. All experiments were started from identical frozen aliquots that were washed twice (2,500 × *g*, 10 min) and diluted 1:2 ·10^2–10^4 into fresh medium.

Media and Drugs. M9 0.1% casamino acids- M9 (Difco M9 Minimal Salts, 5x), supplemented with antibiotics for plasmids maintenance (ampicillin: 100 μg/mL, kanamycin: 30 μg/mL).

Inducers. Anhydrous tetracycline (AcrosOrganics) was added to a final concentration of 0.2 μg/mL. Isopropyl β-D-1-thiogalactopyranoside (Ornat) was added to a final concentration of 10 μg/mL.

Microscopy. Microscopy was performed using a Leica DMIRE2 inverted microscope system with automated stage (Ludl) and shutters (Uniblitz). The microscope was placed in a large incubator box at 32 °C (Life Imaging Systems) that controls the temperature to an accuracy of 0.1 °C. All microfluidic supply lines and vessels were kept inside the incubator during the experiments. Autofocus and image acquisition were done using custom macros in Scope-Pro (Media Cybernetics) to control the microscope, stage, shutters, and camera. Several different locations were monitored in parallel on the same device. Images were acquired using a 63× long-range air objective, and a cooled CCD camera (−75 °C) (Orca II, Back-illuminated, Hamamatsu) and processed with ImageJ and Matlab.

Microfluidic Devices. The microfluidic devices were fabricated as previously described (21, 32). In brief, the microfluidic devices consist of several layers clamped together: a thin patterned PDMS layer (Sylgard 184, Dow Corning) with microscopic grooves or rectangular boxes made by soft lithography techniques using a mold of AZ4110 (Clariant); a cellulose membrane [Spectra/Por 4 dialysis membrane, molecular weight cut off (MWCO): 12–14,000; extensively washed in double distilled water (DDW)] a thicker PDMS layer with flow channels patterned using a mold of SU-8 2100 (MicroChem).

Protocol for starving bacteria in the microfluidic device. A batch culture of nonfluorescent bacteria (MG5) was grown under continuous stirring. This culture was continuously flow in the microfluidic channels (flow bacteria), separated from the trapped bacteria by a membrane. Thus, the trapped bacteria under observation were exposed to the same medium as the flow bacteria. To reach stationary phase under the microscope, the batch culture started as an exponential culture and, after a few hours of growth, reached stationary phase.

The flow rate was set to have the microfluidic flow channels washed once per second. Application and removal of inducers occurred within a minute. It should be noted that the parallel flow of bacteria at stationary phase in the microfluidic channels above the membrane may present several drawbacks that have to be addressed: to avoid high fluorescence background, the flow bacteria did not contain the fluorescent reporters; also, we found out that the oxygen content was a crucial parameter in keeping constant stationary phase conditions, so that the flow bacteria had to come from a continuously shaken culture; and, finally, in the induction of *tet* promoter with aTc, we used bacteria bearing the tetracycline efflux pump to avoid accumulation and titration of the aTc inducer molecules in the bacteria that changes the effective concentration of aTc in the medium.

Batch culture experiments. Batch cultures were grown in 96-well plates in a plate reader (PerkinElmer, Victor3) at 32 °C with shaking. With OD and fluorescence being measured continuously every 5 min (filter wavelengths; GFP: 480 nm/535 nm mCherry: 535 nm/625 nm). The reader is equipped with two injectors, which were controlled by our custom software to inject different wells at defined time points.

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