Growth rate controls the sensitivity of gene regulatory circuits

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Abstract Unicellular organisms adapt to their changing environments by gene regulatory switches that sense chemical cues and induce specific target genes when the inducing signal is over a critical threshold. Using mathematical modeling we here show that, because growth rate sets the dilution rate of intracellular molecules, the sensitivity of gene regulatory switches generally decreases with growth rate, independent of their precise architecture. We confirm the modeling predictions by experimentally demonstrating that the concentration of inducer required for activating the *lac* operon in *E. coli* decreases quadratically with growth rate at the population level, and that growth-arrested cells become hyper-sensitive to inducer at the single-cell level. Moreover, we establish that this growth-coupled sensitivity allows bacteria to implement concentration-dependent sugar preferences, in which a new carbon source is used only if its concentration is high enough to improve upon the current growth rate of the cells. Using microfluidics in combination with time-lapse microscopy, we validate experimentally that this strategy governs how mixtures of glucose and lactose are used in *E. coli* and that the central regulator CRP plays a key role in implementing this strategy. Overall growth-coupled sensitivity provides a general mechanism through which cells can 'mute' external signals in beneficial conditions when growth is fast, and become highly sensitive to alternative nutrients or stresses when growth is slow or arrested.

Introduction

From bacteria to humans, most biological organisms sense signals in their environment and employ systems for processing this information to adapt their behavior. One challenge for such information processing systems is that optimal responses to environmental signals are often highly context-dependent. For example, whether it is worthwhile to pursue a particular nutrient when detecting it in the environment will crucially depend on whether other, better nutrients are also available. While the central nervous systems of multi-cellular eukaryotes obviously enable complex context-dependent responses, it is currently unclear to what extent bacteria are also capable of context-dependent responses to environmental stimuli, or how their relatively simple regulatory circuitry would implement such context-dependence.

A number of studies over the last decade has shown that bacteria obey several so called "growth laws" which determine how major aspects of cell physiology and proteome allocation vary with the growth rate during exponential growth [1–4]. Although the global effects of these growth laws on gene expression are broadly understood [5], to what extent growth rate affects the functioning and information processing of the regulatory circuitry of cells has so far not been explored. Here we use a combination of theoretical modeling and single-cell experiments with *E. coli* to show that growth rate is a key contextual parameter

for the response of regulatory circuits and controls their sensitivity to external signals. In particular, while fast growing cells are relatively insensitive, slow growing or growth arrested cells become hyper-sensitive to signals in their environment.

The origin of this *growth-coupled sensitivity* (GCS) lies in the effects of dilution by growth. For any intracellular molecule that is stable relative to the doubling time of the cell, the rate at which its concentration decays is dominated by dilution, and thus equal to the growth rate. Consequently, the intracellular steady-state concentration of molecules that are being produced at a fixed rate will be inversely proportional to growth rate. This not only applies to constitutively expressed genes, as has been confirmed experimentally [2,5], but also to, for example, molecules that are imported into the cell by membrane-bound transporters. Consequently, the behavior of gene regulatory circuits is expected to be intrinsically coupled to changes in growth rate, since the concentrations of their protein players (e.g. transcription factors) as well as of signalling molecules either activating or repressing them are affected by dilution due to growth.

To explore such growth-coupled effects on regulatory circuitry, we focus on regulatory switches in which a positive feedback loop is coupled to a signal. These regulatory switches typically switch from an uninduced ('off') to an induced ('on') state when the intracellular concentration of the activating signal surpasses a critical concentration. Such regulatory switches are involved in many biological functions including the lysis-lysogeny switches employed by phages [6], the regulatory circuitry involved in carbon source utilization [7–9], competence [10], sporulation [10], and virulence [11]. In addition, a large fraction of the two-component signaling systems used by prokaryotes involve positive feedback loops and behave as regulatory switches.

Results

We first investigated how growth rate might affect the functioning of regulatory switches using simple mathematical models. For a minimal bistable system consisting of an operon containing a transcription factor (TF) that activates its own expression, the parameter regime for which the system exhibits bistability has been shown to depend on growth rate [5]. We here show that this simple positive feedback loop behaves as a regulatory switch as a function of growth rate, i.e. even without any coupling to an external signal (Fig. 1A-C; SI 1.1). As growth rate is decreased, the operon goes from being stably switched off at high growth rates, to bistable at moderately slow growth, to being stably switched on at very slow growth. Moreover, the growth rates at which these transitions occur can be tuned by the basal expression level of the promoter (Fig. 1B-C). Thus, the natural coupling to growth rate due to dilution turns a simple positive feedback loop into a regulatory switch that senses the cell's growth rate. Although we are not aware of examples of regulatory switches that are solely controlled by growth rate, it is conceivable that phages could use such systems to switch on their lytic cycle as the growth rate of their host bacteria drops below a critical value.

In many known examples of regulatory switches, an operon activating its own expression is coupled to an external signal. For example, the regulatory circuits

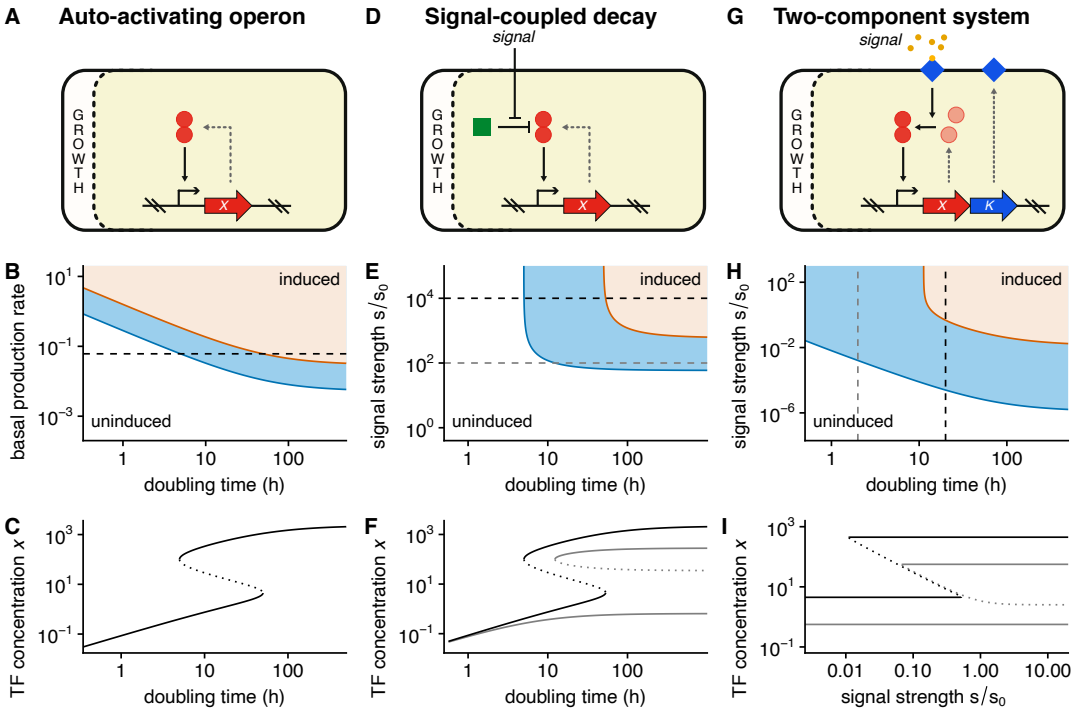

Figure 1. Theoretical analysis of gene regulatory switches exhibiting growth-coupled sensitivity due to growth rate dependent dilution. (A) A minimal regulatory switch circuit consisting of a TF (red circles) that positively regulates its own expression, coupled to growth rate through dilution of the TF. (B) Phase diagram of the auto-activating TF as a function of the doubling time and basal expression rate of the operon. Cells are uninduced in the white area of the plot, stably induced in the red area, and bistable in the blue area. (C) Induction curves showing steady-state TF concentration X as a function of doubling time for a basal expression corresponding to the dotted line in panel B. The solid lines show the stable steady-states and the dotted line the unstable steady-state. Note that the system is bistable for doubling times between 5 and 50 hours. (D) A regulatory switch in which an auto-activating TF (red circles) is coupled to an external signal through active degradation by a protease (green square) whose activity is in turn repressed by the external signal. (E) Phase diagram for the regulatory switch with signal-coupled decay as a function of the doubling time and signal strength s relative to the signal strength s_0 at which protease activity is at half-maximum. (F) Induction curves showing TF concentration as a function of doubling time at high signal strength (black curves and black dotted line in panel E) and intermediate signal strength (gray curves and gray dotted line in panel E). Note that at the intermediate signal strength, the system remains bistable for arbitrarily large doubling times. (G) Two-component system regulatory switch. Here the auto-activating operon contains a TF gene X (red circles) and a gene for a membrane-bound kinase K (blue diamonds). Kinase activity depends on an external signal and phosphorylation of the TF leads to its activation through dimerization. (H) Phase diagram of the two-component system as a function of doubling time and signal strength. Note that the minimal signal strength at which bistability sets in decreases approximately quadratically with doubling time. (I) Induction curves showing TF expression as a function of signal strength either at relatively fast growth (gray curves and grey dotted line in panel H) or at slow growth (black curves and black dotted line in panel H). The solid lines show stable steady states and the dotted lines unstable steady states. Note that at the faster growth rate the system remains bistable for arbitrarily high signal strengths. See SI for detailed parameter settings of each circuit.

that implement competence and sporulation in *B. subtilis* have at their core an auto-activating TF that is coupled to an external signal through repression of a protease that determines the TF's decay rate (Fig. 1D) [10]. The phase diagram of this type of circuit shows that it causes cells to only commit to sporulation/competence when both the external signal and the doubling time are over a critical value (Fig. 1E-F; SI 1.2). Thus, even though this regulatory circuit only interacts with a single signal, the coupling to growth rate makes the response of this system context-dependent, effectively integrating two signals. Switching can either occur by varying the signal strength at a given growth rate, or by varying the growth rate at a given signal strength. Moreover, the switching behavior as a function of one of these variables depends on the value of the other variable. For example, while at high signal strengths the system eventually stably switches on at sufficiently slow growth, at lower signal strength the system remains bistable even at growth arrest (Fig. 1F), illustrating how such circuits can be selected to function in one regime or the other. More generally, by tuning the parameters of the system, cells can implement stochastic responses in which only a subset of the population commits at intermediate growth rates or signal strengths. Indeed, it is well-established that competence is only induced in a subset of cells but not in the whole population while sporulation occurs in the majority of cells [12].

The most common form of regulatory switches in bacteria involve two-component systems in which a TF positively regulates its own expression and is coupled to an external signal through phospho-relay by a membrane-bound kinase (Fig. 1G). In this simple example we assume the TF dimerizes upon being phosphorylated by the membrane-bound kinase, which in turn is activated by an external signal. In the parameter regime chosen for this example, the system switches from off to bistable, and then to stably on as a function of signal strength at low growth rates (Fig. 1H-I, black curves), but remains bistable for arbitrarily high signal strengths at high growth rate (Fig. 1H-I, gray curves). In addition, the threshold level of the signal at which the system becomes bistable increases roughly quadratically with growth rate (Fig. 1H; SI 1.3). That is, as doubling time increases by ten-fold, the threshold level of the signal decreases by hundred-fold, showing that the sensitivity of the regulatory circuit strongly decreases with growth rate.

While these three simple examples only scratch the surface of the possible ways in which growth rate can affect the functioning of regulatory circuits, they illustrate that theoretical modeling predicts that the natural coupling to growth rate through dilution can profoundly affect the sensitivity of gene regulatory switches.

To investigate experimentally whether regulatory switches in bacteria indeed exhibit growth rate dependent sensitivity, we focused on the *lac* operon of *E. coli*, which is arguably the archetypical example of a regulatory switch. The *lac* operon consists of three genes which are involved in the metabolism of galactosides such as lactose. Its expression is repressed by the TF LacI and can be induced by lactose or artificial galactosides such as TMG (which are not metabolized): when TMG is present, it inhibits DNA binding of the repressor LacI, leading to increased expression of the operon, including the transporter LacY, which increases transport of TMG into the cell, creating a positive feedback loop (Fig. 2A). Consequently, the *lac* operon can switch

from an uninduced to an induced state when the TMG concentration exceeds a threshold value. We adapted a simple mathematical model of the *lac* operon [7] to model the effects of dilution (see SI 2) and found that it predicts that the critical level of TMG decreases quadratically with the doubling times of the cells (Fig. 2B).

Interestingly, this prediction reconciles observations in the literature that may have appeared contradictory. In particular, whereas Choi et al. [13] found critical levels of *lac* expression of several hundred molecules for cells growing in M9 media with glycerol and amino acids, our previous single-cell experiments showed that for growth arrested cells any nonzero *lac* expression is sufficient to induce the system when lactose is present [9]. Indeed, at parameter settings that match observations on the *lac* operon (see SI), the *lac* expression at the unstable steady-state that separates the induced and uninduced states in the bistable region is predicted to be $100 - 200$ molecules when cells are doubling every $1 - 2$ hours, but at the same inducer level cells are guaranteed to induce at low growth rates or growth arrest (Fig. 2C).

One prediction of our theory is that, due to GCS, inducer concentrations exist that are too low to cause induction in growing cells, but that should cause induction when cells are growth arrested, even transiently. To test this prediction, we compared the responses of the *lac* operon to switches to a given TMG concentration (20 µM TMG) in 2 different media. Using microfluidics in combination with time-lapse microscopy [9,14], we monitored growth and *lac* operon expression in single cells carrying a LacZ-GFP fusion at the native locus. Cells were initially grown on glycerol minimal media and were then switched either to the same media supplemented with 20 µM TMG, or to lactulose minimal media supplemented with the same concentration of TMG (Fig. 2D). Notably, since lactulose requires LacZ to be metabolized, cells can only grow on lactulose if the *lac* operon is induced, but lactulose does not itself induce the *lac* operon [15]. Under the first switch, cells continue to grow at the same rate and we observe that 20 µM of TMG is not sufficient to cause induction of the *lac* operon in any of the cells during the entire experiment (Fig. 2D). A very different behavior is observed for the second switch. First, since the *lac* operon is repressed under growth on glycerol, the switch to lactulose causes almost all cells to immediately go into growth arrest (Fig. 2D, Fig. S4C). However, several hours later we observe that the large majority of cells have induced their *lac* operon and have recommenced growth. Thus, the transient growth arrest led to the induction of the *lac* operon in the majority of cells, demonstrating experimentally that cells become more sensitive to inducers of the *lac* operon when they are growth-arrested.

We next set out to quantify how the critical concentration of the inducer depends on growth rate: we grew batch cultures in media with different carbon sources to modulate growth rate and, in each of the growth media, measured *lac* expression as a function of TMG concentration using Miller assay (Fig. 2E). Since we wanted to test how the sensitivity to the TMG inducer depends on growth rate due to dilution and since the *lac* operon is known to also be indirectly regulated by growth rate through CRP activity, we used a $\Delta cyaA$ $\Delta cpdE$ mutant strain in which CRP activity is kept constantly high by knocking out the synthesis and degradation of cAMP and by supplementing 1mM of extracellular cAMP [16,17]. Fitting Hill functions to the observed induction

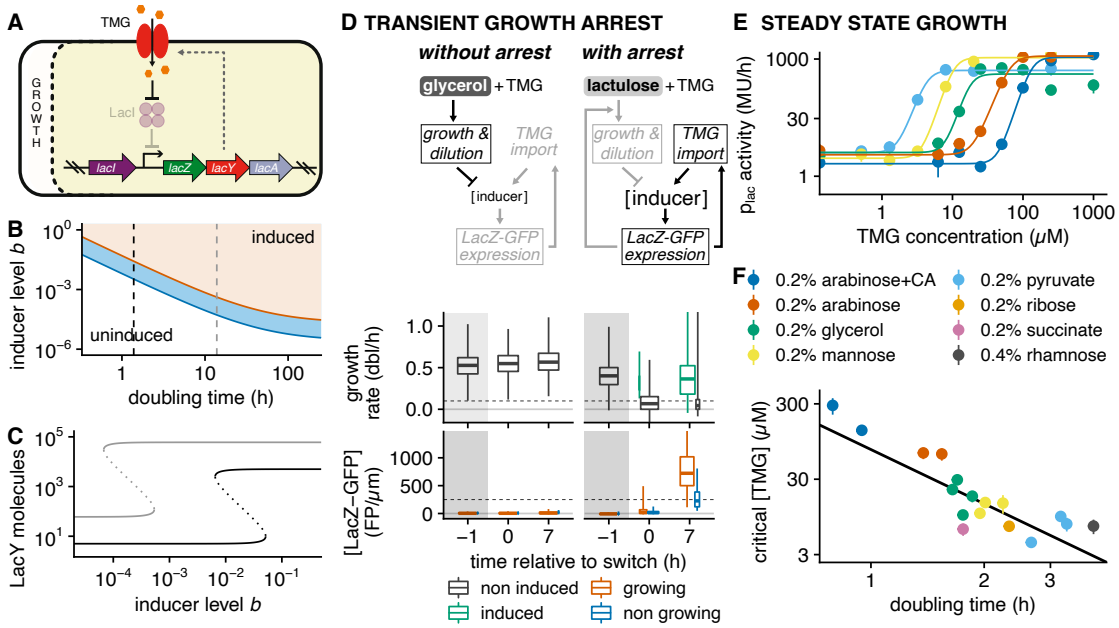

Figure 2. Growth-coupled sensitivity of the *lac* operon. **A**. Schematic of the *lac* operon's positive feedback circuit coupled to the artificial inducer TMG. Note that the intracellular concentration of TMG is set by the balance between import by LacY and dilution due to growth, which leads to GCS. **B**. Theoretical phase diagram of *lac* operon expression as a function of doubling time and external inducer level (white: repressed, blue: bistable, orange: induced). The critical inducer level decreases quadratically with the doubling time over a wide range of growth rates. **C**. Theoretically predicted induction curves of LacY expression as a function of inducer level at a $1-2$ hour doubling time typical for growth in minimal media with lactose (black) and at a ten-fold higher doubling time (gray). See SI for detailed parameter values in B and C. **D**. Experimental validation that transient growth arrest increases the *lac* circuit's sensitivity to TMG. Cells are initially grown in the DIMM microfluidic device without TMG and then switched to media that contain 20 µM of TMG and nutrients that either do not require *lac* operon expression (glycerol; left half), or nutrients that require *lac* operon expression but do not themselves induce *lac* operon expression (lactulose; right half). Note that in the latter cases cells enter growth arrest upon the switch because the *lac* operon is initially repressed. The box-whisker plots show distributions of instantaneous growth rates (upper) and LacZ-GFP concentration (lower) immediately before, immediately after, and 7 h after the switch (≥ 300 cells per time point). Note that cells were stratified by induction (upper) or growth rate (lower); see also Fig. S5. **E**. Induction curves of the *lac* operon as a function of TMG concentration in cells growing exponentially in M9 minimal media supplemented with different carbon sources, and where CRP activity is locked on (using a $\Delta cyaA$ $\Delta cpdA$ mutant supplemented with 1mM cAMP). Only one representative biological replicate is shown for each condition (all replicates are shown in Fig S8); points and errors bars show the mean and standard error of three technical replicates; solid lines show fits to Hill functions; MU: Miller Units. **F**. The critical concentration of TMG (estimated from fits as in E) as a function of doubling time shows an approximately quadratic decrease (exponent -2.4 ± 0.4) as predicted by our model (B).

curves (Fig. 2E), we estimated the critical TMG concentration in each media. Remarkably, we found that the critical concentration at which the *lac* operon induces indeed decreases approximately quadratically with doubling time as predicted by the model (Fig. 2F), with an almost 100-fold change in critical concentration between the fastest and slowest growth conditions. This confirms that the sensitivity of the *lac* operon to its inducers indeed depends strongly on the growth rate of the cells.

While the foregoing experiments establish that regulatory switches such as the *lac* operon exhibit GCS, it is unclear to what extent evolution has exploited this GCS to tune regulatory responses in an adaptive manner. For example, the current predominant view is that *E. coli* has a fixed hierarchy of preferences for carbon sources, so that when a mixture of such carbon sources is available, cells first exhaust the preferred carbon source before consuming the less preferred one [8,18]. Indeed, when grown in batch on a mixture of glucose and lactose, *E. coli* cells first consume glucose before starting to consume lactose. However, such a fixed hierarchy cannot always be optimal. For example, since it is well known that the growth rate that can be achieved on a given carbon source is a hyperbolic 'Monod' function of its concentration [19], the growth rates that can be attained on different carbon sources crucially depend on their concentrations, so that it cannot be optimal to always prefer a given carbon source over another independent of concentration. Instead, an optimal choice of carbon source should be concentration-dependent and, ideally, cells should opt for whatever carbon source maximizes growth rate.

For example, if cells are growing on carbon source A at rate λ_A, and an alternative carbon source B appears at concentration c_B, then cells should ideally only switch to consuming B if the growth rate λ_B that they can attain on B is larger than the current growth rate λ_A. Since the growth rate λ_B increases with concentration c_B, the minimal concentration $c_B(\lambda_A)$ that is required to ensure $\lambda_B \geq \lambda_A$ is an increasing function of the current growth rate λ_A, and given by the inverse of the hyperbolic Monod function. As we have seen above, GCS does naturally cause the critical concentration for inducing the regulatory switch to increase with growth rate, and this raises the question whether GCS might be tuned so as to achieve such optimal carbon source switching. As detailed in section 3 of the SI, this is indeed possible and is realized when the expression $y_h(\lambda)$ of the *lac* operon at full induction decreases linearly with growth rate λ as follows

$$y_h(\lambda) = y_0 \left(1 - \frac{\lambda}{\lambda_*}\right), \tag{1}$$

where the expression at growth arrest y_0, and the growth rate λ_* at which *lac* expression vanishes depend on the parameters of the *lac* system (see SI section 3). Remarkably, recent work in the context of general bacterial growth laws has shown that cAMP and CRP regulate the *lac* operon in precisely this manner, i.e. the expression at full induction decreases linearly with growth rate, reaching zero at a maximal growth rate λ_* [3]. When regulated in this manner, the theory predicts that the center of the bistable regime of the regulatory switch for lactose perfectly tracks the Monod function, i.e. the minimal concentration needed to achieve a given growth rate λ on lactose (Fig. 3A).

These theoretical results suggest that *E. coli* may be exploiting GCS to switch between carbon sources in a concentration-dependent manner such that

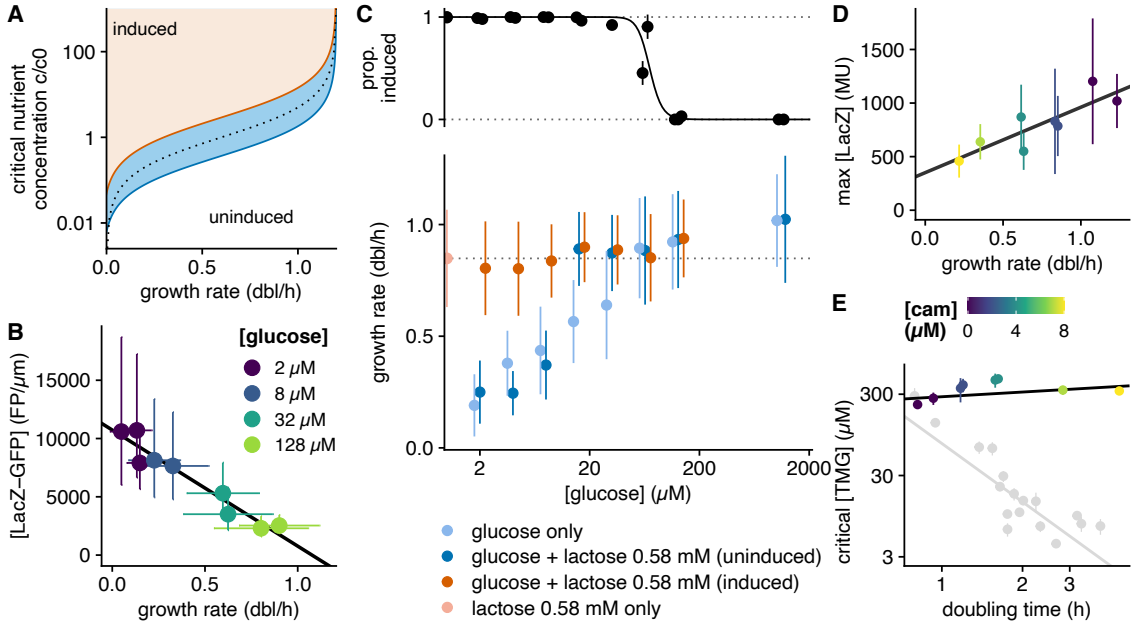

Figure 3. Growth-coupled sensitivity implements concentration-dependent sugar preferences. **A**. Phase diagram of the *lac* operon regulatory switch when *lac* expression at full induction is regulated by CRP so as to decrease linearly with growth rate. The center of the bistable region (blue) perfectly tracks the inverted Monod function (dotted line). **B**. Mean and standard-deviations of the distributions of fully-induced *lac* expression and growth rate in single cells grown in media with different concentrations of glucose (in colors) and supplemented with 200 µM IPTG. The linear fit shows that these single-cell results confirm previous observations that CRP regulation causes fully-induced *lac* expression to decrease linearly with growth rate. **C**. Fraction of single cells with induced *lac* operon when growing on mixtures of saturating lactose (0.58 mM) and different glucose concentrations (2 µM to 1.11 mM) (upper panel), and distributions of growth rates (mean ± s.d.) of the corresponding induced and uninduced subpopulations (lower panel, orange and dark blue; note that even when the fraction of induced cells is almost 1, enough uninduced cells remain to measure the distribution of growth rates). For comparison, the distributions of single-cell growth rates on media with only glucose at different concentrations (light blue) and on media with only lactose (light orange) are also shown. Bacteria were grown in a modified version of the DIMM microfluidic device where media are flown through the growth channel during the whole experiment (1 to 3 independent replicates with more than 70 cells per condition; median ≈ 600 cells); no growth is observed in absence of carbon source (Fig. S11). **D**. Expression of the fully-induced *lac* operon as a function of growth rate when cells are exposed to subinhibitory levels of chloramphenicol at different concentrations (in colors). **E**. The critical level of TMG inducer is largely independent of growth rate when growth rate is modulated by chloramphenicol (colored points and black line for the linear fit). For comparison, critical TMG levels when growth rate is modulated by changing nutrients (same data as in Fig. 2F) is shown in light grey. In D and E, 2 to 8 µM of chloramphenicol were added to M9 + 0.2 % arabinose + 0.1 % casaminoacids; points and errors bars show the mean and standard error of three technical replicates; MU: Miller Units.

the resulting growth rate is maximized. For example, in contrast to the current view that *E. coli* always prefers to consume glucose when grown on a mixture of glucose and lactose, the theory predicts that whenever the glucose concentration is sufficiently low, i.e. when the growth rate that can be attained consuming glucose is lower than the growth rate that can be attained consuming lactose, the *lac* operon will be induced. However, testing such predictions is challenging because substantial reductions in growth rate generally only occur when glucose concentration becomes so low that cell density in batch cultures is too small to quantify using standard techniques. To overcome this limitation we modified our DIMM microfluidic device such that growth media continuously flow through the channels in which the bacteria grow, thereby ensuring an homogeneous growth environment and avoiding nutrient gradients that would otherwise arise within dead-end channels [20].

Using this device, we could grow bacteria at different glucose concentrations ranging from the usual 0.2 % (11.1 mM) down to 5500 times less, at a mere 2 µM. We observed a large range of growth rates (from 1.06 dbl/h down to 0.19 dbl/h) and provided, to our knowledge, the first experimental confirmation that growth rate increases as Monod curve with glucose concentration at the single-cell level (Fig. 3C, light blue symbols, and Fig. S12). Second, we measured fully-induced *lac* operon expression at each glucose concentration by adding 200 µM ITPG and confirmed that average LacZ-GFP decreases linearly with growth rate (Fig. 3B), similar to what has been reported based on bulk measurements where the availability of glycolytic sugars was modulated by titrating their importers [3].

We then monitored growth and *lac* operon expression on mixtures of lactose and glucose, with glucose varying over the same range of concentrations and lactose at saturating concentration (0.58 mM i.e. 0.2 %) (Fig. 3C). When glucose concentrations are very low, almost all cells induce their *lac* operon and grow at rates similar to growth on media containing only lactose. However, small fractions of cells with uninduced *lac* operon remain and we observed that their growth rates match the growth rates observed when growing on the same concentration of glucose only. As the concentration of glucose increases, the growth rates of the uninduced cells increases and, around a glucose concentration of 50 µM, the distributions of growth rates of the induced cells and uninduced cells become virtually identical. Remarkably, it is exactly at this critical concentration that we also see the fraction of induced cells drop sharply, and at higher glucose concentrations virtually all cells are uninduced (Fig. 3C). That is, these single cell experiments confirm our theory that, in contrast to the classical diauxie picture, there is no fixed sugar hierarchy but *E. coli* induces its *lac* operon in a concentration-dependent manner so as to always grow on the carbon source that maximizes growth rate. These results suggest that, more generally, *E. coli* exploits GCS to implement a strategy by which induction of operons for alternative carbon sources is both growth rate and concentration dependent, and tuned so that only the catabolic operon of the carbon source that is able to support the highest growth rate is induced.

Finally, although above we have considered changes in growth rate due to changes in nutrient quality, growth rate can of course also be modulated by other environmental factors, e.g. by stresses that inhibit replication or translation. While, as discussed above, the critical inducer concentrations of optimally tuned regulatory switches for catabolic operons should depend on

growth rate when growth rate is modulated by nutrient quality, the critical concentration should ideally remain unchanged when growth rate is modulated by other environmental factors. This suggests a challenge for our GCS theory because changes in growth rate will affect dilution rate independent of what environmental factors cause these changes in growth rate.

One way to overcome this challenge is by modifying the way CRP regulates the expression $y_h(\lambda)$ of the fully-induced *lac* operon. As shown in section 4 of the SI, in order for the critical inducer concentration to remain unchanged when growth rate is modulated by environmental factors other than nutrient quality, the expression $y_h(\lambda)$ should *increase* linearly with growth rate, rather than decreasing linearly as occurs when growth rate is modulated by nutrient quality. Remarkably, studies on resources allocation in the context of bacterial growth laws have established that CRP regulation exhibits exactly this behavior. In particular, when growth rate is decreased by translation inhibition using sub-lethal doses of chloramphenicol, CRP activity *decreases* and, consequently, the expression of CRP targets increases linearly with growth rate under translation inhibition [3].

We confirmed that, when grown on different subinhibitory concentrations of chloramphenicol, the expression $y_h(\lambda)$ of the fully-induced *lac* operon indeed increases roughly linear with growth rate (Fig. 3D). Moreover, using the experimental approach described above to measure *lac* promoter activity during balanced exponential growth, we confirmed experimentally that the critical concentration of TMG is indeed independent of the growth rate in this case (Fig. 3E). This confirms that CRP regulation not only optimally tunes the growth rate dependence of critical inducer concentration under changes in nutrient quality, but also ensures that the critical concentration remains unchanged when growth rate is modulated by translation inhibition.

Discussion

We here studied the effects of growth rate through dilution on the functioning of gene regulatory circuitry, focusing on the behavior of gene regulatory switches. Although the effects of growth rate on gene regulatory switches can be complex and dependent on the precise circuitry and its parameters, the default behavior is that gene regulatory switches decrease their sensitivity to their inducers with growth rate. While the impact of growth rate that had already been reported for synthetic gene circuits [21,22], we here show that all native regulatory circuits are subject to GCS. This GCS allows cells to respond to external signals in a context-dependent manner, e.g. to only respond to a particular signal when growth rate is below a critical value, or to scale the critical level of an external signal with growth rate. Indeed, for the *lac* operon in *E. coli* we confirmed experimentally that GCS causes critical inducer levels to increase approximately quadratically with growth rate.

More strikingly, in contrast to the currently predominant view that there is a fixed hierarchy of sugar preferences, our analysis suggests that *E. coli* exploits GCS in combination with regulation by CRP to implement optimal concentration-dependent sugar preferences. In this strategy, the regulatory circuitry ensures that when different sugars are present at different concentrations, only the catabolic genes for the sugar that maximizes growth rate at

these concentrations are switched on. We confirmed this experimentally using single-cell experiments with mixtures of glucose and lactose, showing that the critical concentration for *lac* operon induction is exactly where the single-cell distributions of growth rates on glucose and lactose match. It will be interesting to explore to what extent this regulatory strategy also applies to other sugar mixtures. It is of course well-known that certain sugar mixtures are not used sequentially but in parallel [18], and we foresee that such situations may simply correspond to bistable regimes of the regulatory switches.

In addition, we found that CRP regulation also ensures that when growth rate is modulated by factors other than nutrient quality, i.e. translation inhibition, the effect of GCS is buffered and the critical inducer concentration remains independent of growth rate. Although it is tempting to speculate that evolution may have tuned CRP regulation to specifically implement the optimal concentration-dependent sugar preferences that we observed here, it should be noted that the theoretical models that have been proposed to explain the so-called bacterial growth laws argue that the growth rate dependence of CRP regulation and even the Monod equation itself follow from the necessity to balance catabolic and anabolic intracellular fluxes [2,3]. It is thus conceivable that the optimal concentration-dependent regulation of carbon source preference reported here is in fact an emergent property of regulatory switches operating within the context of these growth laws.

Apart from the specific regulation of carbon source preferences that we investigated here, the fact that GCS causes regulatory circuits to become less sensitive with increasing growth rate is likely adaptive in general for bacteria. That is, GCS causes fast growing cells to stabilize their current state by effectively muting their response to fluctuations in external signals and causes slowly growing cells to become highly sensitive to external signals. This view is consistent with recent work from our lab that shows that gene expression noise in *E. coli* results to a large extent from the propagation of noise through the gene regulatory network, and that noise levels systematically decrease with growth rate [23]. This suggests a general strategy in which GCS causes slowly growing cells to more actively explore alternative gene expression states and we show elsewhere that such behavior is highly adaptive for bet-hedging strategies [24].

While we have here focused on the behavior of gene regulatory switches in bacteria, the coupling of gene regulatory circuits to growth rate through dilution is so general that it likely affects the operation of gene regulatory circuits across organisms and GCS might also play a role in development and cell differentiation in multi-cellular eukaryotes. For example, since even a transient decrease in growth rate can cause regulatory switches to induce, it is conceivable that modulation of growth rate could be used in development to induce particular cell fate commitments, and behavior suggestive of this mechanism has been observed for the commitment to neurogenesis of neural progenitors [25,26]. Exploring how GCS may have been exploited in the regulatory circuitry implementing the development and cell differentiation in multi-cellular eukaryotes is a fascinating area for future study.

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