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Bacterial sugar utilization gives rise to distinct single-cell behaviors

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Abstract

Inducible utilization pathways reflect widespread microbial strategies to uptake and consume sugars from the environment. Despite their broad importance and extensive characterization, little is known how these pathways naturally respond to their inducing sugar in individual cells. Here, we performed single-cell analyses to probe the behavior of representative pathways in the model bacterium *Escherichia coli*. We observed diverse single-cell behaviors, including uniform responses (D-lactose, D-galactose, N-acetylglucosamine, N-acetylneuraminic acid), “all-or-none” responses (D-xylose, L-rhamnose), and complex combinations thereof (L-arabinose, D-gluconate). Mathematical modeling and probing of genetically modified pathways revealed that the simple framework underlying these pathways—inducible transport and inducible catabolism—could give rise to most of these behaviors. Sugar catabolism was also an important feature, as disruption of catabolism eliminated tunable induction as well as enhanced memory of previous conditions. For instance, disruption of catabolism in pathways that respond to endogenously synthesized sugars led to full pathway induction even in the absence of exogenous sugar. Our findings demonstrate the remarkable flexibility of this simple biological framework, with direct implications for environmental adaptation and the engineering of synthetic utilization pathways as titratable expression systems and for metabolic engineering.

INTRODUCTION

All forms of life must obtain sources of carbon and energy from the environment in order to survive and replicate. Within microorganisms, a prevalent strategy is the use of inducible utilization pathways that uptake and consume exogenous sugars. These pathways encode transporters and enzymes that are expressed only in the presence of the sugar (Figure 1A). This framework can be viewed as a cost-saving measure to conserve resources when the sugar is absent (Dekel and Alon, 2005). However, this framework also imparts positive and

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negative feedback (Figure 1B) (Krishna *et al.*, 2007). Positive feedback emerges from the sugar inducing the expression of transporters, which in turn import additional sugar molecules into the cell. Similarly, negative feedback emerges from the sugar inducing the expression of catabolic enzymes, which in turn shunt the intracellular sugar into metabolism.

Coupling positive and negative feedback has been shown to impart unique properties not ascribed to either mode of feedback alone (Thomas, 1978; Tian *et al.*, 2009; Shah and Sarkar, 2011; Afroz and Beisel, 2013). For instance, natural and synthetic gene regulatory networks that feature positive and negative feedback have been reported to exhibit ranging behaviors (Brandman and Meyer, 2008), such as stable oscillations within synthetic transcriptional networks (Atkinson *et al.*, 2003; Stricker *et al.*, 2008; Tigges *et al.*, 2009; Prindle *et al.*, 2012; Biliouris *et al.*, 2012), excitability within the uptake of foreign DNA (Süel *et al.*, 2006), and bistability with limited memory within the transcriptional response to D-galactose in yeast (Acar *et al.*, 2005; Avendaño *et al.*, 2013).

Although inducible utilization pathways inherently couple positive and negative feedback, these pathways have been assumed to only exhibit uniform behaviors (Ninfa and Mayo, 2004; Smits *et al.*, 2006; Krishna *et al.*, 2007). This assertion can be traced to (i) the predominant use of bulk characterization techniques (Kuhlman *et al.*, 2007; Kaplan *et al.*, 2008), (ii) single-cell analyses of the D-lactose and L-arabinose utilization pathways deprived of sugar catabolism (Novick and Weiner, 1957; Cohn and Horibata, 1959; Siegele and Hu, 1997; Khlebnikov *et al.*, 2001; Morgan-Kiss *et al.*, 2002; Ozbudak *et al.*, 2004; Megerle *et al.*, 2008), and (iii) limited mathematical modeling without any ensuing experimental validation (Krishna *et al.*, 2007). The prevailing notion is that, in the absence of catabolism, these pathways exhibit “all-or-none” responses in which pathways are maximally induced (“all”) or negligibly induced (“none”) in single cells; in the presence of catabolism, the “all-or-none” response is lost, yielding a uniform response among all cells in the population. However, the only single-cell analyses of a native utilization pathway (L-arabinose) revealed a bimodal response even in the presence of sugar catabolism (Khlebnikov *et al.*, 2001; Morgan-Kiss *et al.*, 2002). Therefore, what remains unclear is how different utilization pathways respond to their inducing sugar in individual cells and the role of sugar catabolism in the response.

Here, we investigated the single-cell behaviors of representative inducible utilization pathways within the model bacterium *E. coli*. Surprisingly, the assessed pathways exhibited varying single-cell behaviors, including uniform responses, “all-or-none” responses, and varying combinations thereof. Within the diversity of responses, we found that the simple coupling of inducible transport and inducible catabolism could give rise to most of these behaviors. Furthermore, sugar catabolism was an important contributor to each behavior, where loss of catabolism eliminated tunable induction and enhanced memory of previous conditions. These findings demonstrate the remarkable flexibility of this simple biological framework and suggest that individual pathway behaviors can be tailored—either through evolution or through engineering—to sugars present in the environment.

RESULTS

Varying single-cell behaviors in sugar utilization in *E. coli*

We focused on inducible utilization pathways in *E. coli* K-12 substrain MG1655 because this bacterium possesses at least 16 pathways containing both inducible transport and inducible catabolism and virtually all of the pathway components have been identified and well characterized (Table S1). In particular, we concentrated on eight pathways (lactose, D-galactose, N-acetylglucosamine, N-acetylneuraminic acid, D-xylose, L-rhamnose, L-arabinose, and D-gluconate) that have undergone extensive characterization. To monitor the single-cell response in each pathway, we placed the green fluorescent protein gene (*gfp*) under the control of a pathway promoter on a low-copy plasmid (Zaslaver *et al.*, 2006). The use of this plasmid parallels many other studies of gene regulation in single cells (Khlebnikov *et al.*, 2001; Morgan-Kiss *et al.*, 2002; Ozbudak *et al.*, 2004) and reflects a trade-off between a strong fluorescence signal and titration of the pathway components (Del Vecchio *et al.*, 2008). MG1655 cells transformed with each plasmid were grown in M9 minimal medium with 0.4% glycerol (M9 glycerol) with varying concentrations of the sugar before being subjected to flow cytometry analysis (Figure S1A). The cells were grown in exponential phase for 20 hours to a low final density ($ABS_{600} \sim 0.002$) to help ensure that the fluorescence values approached a stable distribution (Figure S1B) and to minimize depletion of the sugar in the medium (Ozbudak *et al.*, 2004; Avendaño *et al.*, 2013). Dot plots of the resulting distributions were generated from the fluorescence histograms similar to previous work (Pelet *et al.*, 2011). The dot plots communicate unimodal or bimodal distributions as well as the fluorescence and relative size of each sub-population (Figure S1A).

Remarkably, the assessed utilization pathways exhibited ranging responses (Figure 2). The D-lactose, D-galactose, N-acetylglucosamine, and N-acetylneuraminic acid utilization pathways exhibited graded responses wherein all cells exhibited uniform fluorescence levels at all sugar concentrations. The D-xylose and L-rhamnose utilization pathways exhibited “all-or-none” responses wherein the sugar concentration only affected the relative fraction of cells in the fully induced or uninduced states (Ozbudak *et al.*, 2004). The L-arabinose utilization pathway exhibited a bimodal response at low sugar concentrations but then a graded response at higher sugar concentrations once the entire population was induced. Finally, the D-gluconate utilization pathway exhibited a graded response at lower sugar concentrations only to bifurcate into induced and uninduced sub-populations at higher sugar concentrations. The responses appeared to be independent of the specific promoter selected, as another promoter for D-xylose (P_{araF}) and three other pathway promoters for L-arabinose (P_{araE} , P_{araF} , P_{araJ}) exhibited the same qualitative response (compare Figure 2 and Figure S2A). The responses also did not correlate with the doubling time of each strain in the presence or absence of sugar (Table S2), downplaying the potential contribution of growth rate. Finally, the responses did not appear to be influenced by the copy number of the reporter plasmid based on single integrants of the P_{araB} reporter, although lower fluorescence levels complicated interpretation of the response (Figure S2B).

A simple model predicts varying behaviors from the underlying framework

The observed behaviors may be due to attributes unique to each pathway, such as feedback through central metabolism (e.g. carbon catabolite repression), the number of transport systems and enzymatic routes, or the presence of additional regulatory mechanisms. However, another possibility is that the shared framework of inducible transport and inducible catabolism underlies many of these behaviors. To explore the potential contribution of this framework, we developed a simple deterministic model of a generalized utilization pathway (see Supplementary Information, Table S3). This pathway consisted of one transporter and one enzyme whose expression is induced by a sensory activator in the presence of intracellular sugar (Figure 3A). To simplify the analysis, the model (i) assumed similar dilution rates for the transporter, enzyme, and sugar through cell division and (ii) neglected sugar-dependent differences in growth rate. Within the model, the strength of positive and negative feedback were captured by the maximal activities of the fully expressed transporter (α) and enzyme (γ), respectively. Unlike previous models of sugar utilization (Carrier and Keasling, 1999; Ozbudak *et al.*, 2004; Krishna *et al.*, 2007; Santillán and Mackey, 2008; Stamatakis and Mantzaris, 2009; Savageau, 2011; Yildirim, 2012), this model is not focused on any single pathway, accounts for inducible sugar catabolism, and does not overly simplify the mechanisms of sugar import and catabolism.

The model predicted graded as well as bistable responses that principally depended on the strengths of inducible transport (positive feedback) and inducible catabolism (negative feedback). As shown in the phase diagram in Figure 3B, graded responses were predicted for weak transport activity (low α) as well as for strong catabolic activity (high γ), whereas bistable responses were predicted for strong transport activity and weak catabolic activity (high α and low γ). These behaviors follow from positive feedback driving bistability and negative feedback countering bistability as predicted for transcriptional networks (Tian *et al.*, 2009). Excitability or oscillations were also predicted for unequal dilution rates of the transporter, enzyme, and intracellular sugar (Figure S3). Similar behaviors were also observed for a sensory repressor (Figure S4, Supplementary Information) or for two transporters (Afroz *et al.*, 2014). These predictions suggest that the combined influence of inducible import and catabolism of an exogenous sugar can give rise to different emergent behaviors.

The model also predicted unique features of the response curves—the relationships between extracellular sugar and enzyme levels—within the phase diagram. First, minimal transport activity was necessary to achieve a reasonable dynamic range, defined here as the ratio of enzyme levels with saturating or no extracellular sugar (Figure 3B, dashed blue line). This minimal value always fell within the bistable region for lower catabolic activities ($\gamma < 0.048$), suggesting that sugar catabolism principally determines whether an inducible pathway will exhibit graded or bistable behavior. Second, we observed that modulating the catabolic activity had other effects on the response (Figure 3C). In particular, increasing the catabolic activity within the bistable regime (i) expanded the tunability of the induced state and (ii) reduced the width of the bifurcation region, representing the range of extracellular sugar concentrations in which two stable enzymes levels were possible. In contrast, altering transport activity had a minor impact on the tunability of the induced state as well as the

width of the bifurcation region (Figure 3D). These predictions mirror most of the observed behaviors (Figure 2), supporting the contribution of positive and negative feedback.

Sugar catabolism allows tunable induction

To probe model predictions, we experimentally investigated how sugar catabolism contributes to the observed response. We focused on all but the D-lactose and D-gluconate utilization pathways (Figure 4): the D-lactose pathway was previously interrogated in the absence of catabolism using the non-hydrolyzable lactose analog TMG (Novick and Weiner, 1957; Cohn and Horibata, 1959; Ozbudak *et al.*, 2004), whereas the D-gluconate pathway contains redundant catabolic steps through the two D-gluconate kinases GntK and IdnK (Istúriz *et al.*, 1986; Vivas *et al.*, 1994). For each evaluated pathway, we disrupted sugar catabolism by deleting the catabolic genes, which eliminated the ability of *E. coli* to grow on each sugar (data not shown). This disruption generally reduced growth in the presence of the sugar (Table S2), as expected when inducing pathway expression without any fitness benefit. We then generated response curves for the resulting catabolism-deficient (*E*) strains under the same growth conditions as that of the wild type (WT) strains (Figure 2). Note that the induction time of 20 hours was sufficient to achieve a stable distribution as determined for L-arabinose (Figure S1B).

Flow cytometry analysis of the catabolism-deficient strains revealed two general responses lacking tunable induction (Figure 4): (i) an “all-or-none” response in which cells were either negligibly or fully induced (N-acetylneuraminic acid, D-xylose, L-rhamnose, L-arabinose), and (ii) full induction independent of the sugar concentration (D-galactose, N-acetylglucosamine). The “all-or-none” response paralleled the simulated response curves in the absence of sugar catabolism ($\gamma = 0$) and previous single-cell studies of pathways lacking catabolism (Novick and Weiner, 1957; Siegle and Hu, 1997; Ozbudak *et al.*, 2004). This response also lacked tunability of the induced state, in line with setting $\gamma = 0$ at different points within the predicted phase diagram (Figure 3). Note that the loss of tunability for L-arabinose cannot be attributed to self-catabolite repression (Katz and Englesberg, 1971), as the presence of 2 mM cyclic AMP in the medium did not alter the qualitative response to L-arabinose (Figure S2C). Furthermore, excision of the resistance cassette used to delete the catabolic operons had no impact on the response (Figure S5). We thus attribute these behaviors to inducible transport driving bistability based on the loss of catabolism.

The D-galactose and N-acetylglucosamine utilization pathways lacking catabolism exhibited full induction even in the absence of exogenous sugar (Figure 4). A distinguishing feature of both pathways is that *E. coli* can synthesize each inducing sugar (Wu and Kalckar, 1966; Wu and Wu, 1971; Vogler *et al.*, 1989). In particular, N-acetylglucosamine can be synthesized through the sequential activities of L-glutamine:D-fructose-6-phosphate aminotransferase (GlmS) and N-acetylglucosamine deacetylase (NagA), whereas D-galactose is an inadvertent bi-product of the Leloir pathway. To explore the impact of endogenous biosynthesis, we modified our mathematical model to allow for constitutive production of intracellular sugar (Figure 5A, Supplementary Information). The model predicted that sugar biosynthesis elevated expression levels in the absence of exogenous sugar and converted a bistable response into a graded response (Figure 5B). Both predictions emerge from the

synthesized sugar inducing transporter expression even in the absence of exogenous sugar, thereby breaking positive feedback. The model also predicted that disrupting sugar catabolism elevated expression levels in the absence of exogenous sugar, which was magnified with increasing catabolic activity (Figure 5C). In this case, catabolism restrains build-up of the endogenous sugar, which would otherwise drive full induction of the pathway. These predictions closely match the experimental observations for D-galactose and N-acetylglucosamine (Figure 4), although leaky promoter activity may also contribute to the basal expression. Overall, catabolism appears to be a critical component of utilization pathways that respond to sugars synthesized by the cell.

Sugar catabolism reduces the extent of hysteresis

Another intriguing model prediction was that sugar catabolism reduces the extent of hysteresis—or memory of previous conditions—by counteracting bistability. In the case of inducible utilization pathways, hysteresis would reflect initially induced cells being more sensitive to the sugar than initially uninduced cells (Novick and Weiner, 1957). To experimentally test this prediction, we focused on the D-xylose and L-arabinose utilization pathways because they exhibited a clear bimodal response in the absence of sugar catabolism and were fully induced at high sugar concentrations. For each pathway, we included either no sugar or sufficient sugar to induce the entire cellular population in the overnight cultures of WT and *Δ* cells. The cells were then washed to remove residual sugar, back diluted into varying sugar concentrations, and grown for 20 hours prior to flow cytometry analysis (Figure 6). To quantify the extent of hysteresis, we calculated the ratio of concentrations to achieve ~50% induced cells with or without pre-incubation of the sugar.

Paralleling model predictions, we found that deleting the catabolic genes substantially increased the extent of hysteresis for both pathways (Figure 6). In particular, deleting the catabolic genes increased the extent of hysteresis by a factor of over 100 for D-xylose (concentration ratios of 9.9 ± 3.0 and $1,770 \pm 570$ for the WT and *Δ**xyxAB* strains, respectively; average $\pm$ S.E.M., $n = 3$) and by a factor of over 4 for L-arabinose (concentration ratios of 1.0 ± 0.1 and 4.1 ± 0.6 for the WT and *Δ**araBAD* strains, respectively, average $\pm$ S.E.M., $n = 3$). The extent of hysteresis between these pathways was quite distinct, particularly in the absence of sugar catabolism (concentration ratios of 1,770 for D-xylose and 4.1 for L-arabinose). Interestingly, this difference cannot be readily attributed to the strength of positive feedback (α), which was predicted to have little effect on the width of the bifurcation region (Figure 3D). Instead, sensitivity analysis identified the Hill coefficient for transporter expression (n_1) as the largest contributor. This would suggest that apparent cooperativity in the expression of pathway transporters, such as through DNA looping (Kuhlman *et al.*, 2007), accounts for the extent of hysteresis between pathways (Figure S6).

As an alternative means to evaluate hysteresis, we sorted bimodal populations of WT and *Δ**araBAD* cells into induced and uninduced sub-populations and monitored each sub-population in the same medium over time monitored by flow cytometry analysis (Figure S7). The WT sub-populations converged on similar fluorescence distributions, while the *Δ**araBAD* sub-populations maintained fixed fluorescence distributions despite active growth

of the cultures (Figure S7). We thus conclude that a distinct contribution of sugar catabolism is reducing memory of previous conditions.

DISCUSSION

We found that inducible utilization pathways exhibit diverse single-cell responses. This finding directly challenges the repeated assertion that all natural utilization pathways exhibit uniform behaviors (Ninfa and Mayo, 2004; Smits *et al.*, 2006; Krishna *et al.*, 2007) and underscores the need for single-cell analyses when studying inducible sugar utilization in the microbial world (Lubelska *et al.*, 2006; Johnsen *et al.*, 2009; Horák, 2013). Our findings also highlight the limitations of drawing conclusions about natural pathways from studies that neglect sugar catabolism. For instance, we found that the lactose utilization pathway naturally exhibits a graded response, yet this pathway is widely associated with an “all-or-none” response that emerges only in the absence of catabolism (Novick and Weiner, 1957; Ozbudak *et al.*, 2004).

Our model predictions suggest that the combination of inducible transport and inducible catabolism in itself can give rise to diverse emergent behaviors. One ramification is that pathways may be tailored to conditions in which the inducing sugar is naturally found. For instance, some pathways may exhibit bimodal responses to sugars whose concentrations fluctuate erratically (Acar *et al.*, 2008; Bennett *et al.*, 2008), whereas other pathways may exhibit graded responses to sugars that are present more consistently. Alternatively, the bimodal responses may allow coordinated sugar utilization while conserving resources (Kovárová-Kovar and Egli, 1998) or may promote heterogeneity as part of sugar-dependent processes such as virulence (Muñoz-Elías and McKinney, 2006; Rojo, 2010), biofilm formation (Sadykov *et al.*, 2011; Chai *et al.*, 2012), or the uptake of foreign DNA (Lo Scrudato and Blokesch, 2012). The complexity of the responses may be even further enhanced in the presence of multiple sugars because of carbon catabolite repression (Kaplan *et al.*, 2008; Görke and Stülke, 2008), cross-regulation between pathways (Desai and Rao, 2010), or promiscuity of some transport systems (Zhu and Lin, 1986; Kornberg and Lourenco, 2006). As one example, such responses would impact commensal *E. coli* as it consumes N-acetylneuraminic acid, N-acetylglucosamine, D-gluconate, and other sugars during gut colonization (Chang *et al.*, 2004).

Our deterministic modeling considered a simplified pathway of one transporter and one enzyme to draw general insights into inducible sugar utilization. However, each natural pathway is likely influenced by additional regulatory factors, such as auto-repression by the regulator (Hahn and Schleif, 1983; Weickert and Adhya, 1993; Egan and Schleif, 1993), multiple transport systems (Yildirim, 2012), signaling through downstream metabolic products (Katz and Englesberg, 1971), or gene repression by regulatory RNAs (Beisel and Storz, 2011; Papenfort *et al.*, 2013). Furthermore, differential growth on the sugar (Table S2) can lead to faster or slower dilution of the cell components (Tan *et al.*, 2009; Klumpp *et al.*, 2009). Many of these factors would impart positive or negative feedback that may influence the single-cell behaviors. For instance, we speculate that the bimodal and non-monotonic response to D-gluconate (Figure 2) could emerge in part from (i) D-gluconate lowering cAMP levels (Epstein *et al.*, 1975), which would downregulate pathway

expression, and (ii) the overlapping metabolic routes involved in D-gluconate catabolism (Wu and Wu, 1971; Vogler *et al.*, 1989). Beyond the framework considered in this work, other known frameworks of inducible utilization pathways exist, such as pathways induced by metabolic intermediates (e.g. maltose, L-fucose; see Table S1). These alternative frameworks may result in disparate behaviors that warrant further investigation and may expand the diversity of single-cell responses associated with inducible sugar utilization.

Our insights are expected to inform the engineering of utilization pathways. Sugar utilization represents the first step in most endeavors within metabolic engineering (Alper and Stephanopoulos, 2009) and environmental bioremediation (Ramos *et al.*, 2011). Depending on the abundance and identity of the sugar, only a fraction of the population may be actively importing and consuming the sugar. Any existing or imported utilization pathway would require interrogation at the single-cell level and potential manipulation to ensure a uniform response—for instance, by strengthening negative feedback or by disrupting positive feedback (Khlebnikov *et al.*, 2001; Morgan-Kiss *et al.*, 2002; Afroz *et al.*, 2014). Similar issues may be encountered when co-opting natural utilization pathways as titratable expression systems (Guzman *et al.*, 1995; Cardona *et al.*, 2006). Finally, the combination of inducible transport/synthesis and export/degradation offers a simple framework for the construction of synthetic gene networks. The existing toolkit in synthetic biology is principally composed of gene regulatory devices such as transcription factors or regulatory RNAs. Therefore, the development of synthetic inducible pathways would greatly expand this toolkit and offers means to generate tunable or memory-based devices.

EXPERIMENTAL PROCEDURES

Bacterial strains and plasmids

All of the strains were derivatives of *E. coli* K-12 substrain MG1655 (obtained from S. Gottesman, National Cancer Institute). All strains, plasmids, and oligonucleotides used in this work are reported in Tables S4, S5, and S6, respectively. To generate the deletion strains, the chloramphenicol resistance cassette encoding the *cat* gene was amplified by PCR from pKD3 using primers with gene-specific homology regions at the 5' ends (Datsenko and Wanner, 2000). The resulting linear PCR products were recombined into NM500 harboring the mini- λ cassette (Court *et al.*, 2003) in order to generate NM500 *araBAD::cat*, NM500 *xylAB::cat*, NM500 *rhaBADM::cat*, NM500 *galKM::cat*, NM500 *nagB::cat*, and NM500 *nanA::cat*. Each resistance cassette was then transferred to MG1655 by P1 transduction. The resistance cassette in MG1655 *nagB::cat*, MG1655 *nanA::cat*, MG1655 *araBAD::cat*, and MG1655 *xylAB::cat* was excised using pCP20 (Cherepanov and Wackernagel, 1995), thereby generating MG1655 *nagB*, MG1655 *nanA*, MG1655 *araBAD - cat*, and MG1655 *xylAB -cat* respectively. Successful recombinants and transductants were verified by PCR and by sequencing.

All of the reporter plasmids were based on the pUA66 plasmid encoding the *gfpmut2* variant of the green fluorescent protein gene downstream of a multi-cloning site (Zaslaver *et al.*, 2006). The plasmid also encodes a kanamycin resistance cassette and the low-copy sc101 origin-of-replication. Promoter regions from the *E. coli* genome are inserted into the multicloning site, resulting in a fluorescent readout of transcriptional activity. Most of the

plasmids were purchased through OpenBiosystems from the *E. coli* promoter collection originally developed by the Alon laboratory (Zaslaver *et al.*, 2006). The rest of the plasmids (pUA66ParaE, pUA66ParaF, pUA66PxylA, pUA66PxylF) were unavailable in the collection. To generate pUA66PxylF, the promoter region was amplified from the MG1655 chromosome and cloned into the BamHI/XhoI restriction sites in pUA66. To generate pUA66ParaE, pUA66ParaF and pUA66xylA, the promoter regions were also amplified from the MG1655 chromosome by PCR. For these promoters, the primers were designed with 5' ends that overlapped with the pUA66 plasmid. The backbone of pUA66 was amplified by PCR in two fragments (primers pUA66.fwd1/pUA66.rev1 and pUA66.fwd2/pUA66.rev2) from pUA66 DNA linearized with XhoI. The amplified PCR products were assembled using the Gibson method (Gibson, 2011). All cloned plasmids were verified by colony PCR and by sequencing.

To integrate the *P_{araB}-gfp* construct into the *E. coli* MG1655 genome, we amplified the reporter construct from pUA66ParaB by PCR and cloned the construct into pCAH63 (Haldimann and Wanner, 2001) in place of *uidAf* by the Gibson method. The resulting pCAH63-ParaB plasmid was then integrated into the *E. coli* genome as described previously with slight modifications (Haldimann and Wanner, 2001). Briefly, the plasmid was transformed at 30°C into MG1655 cells carrying the helper plasmid pINT-ts. The transformed cells were recovered at 37°C for one hour and then at 42°C for 30 minutes before plating on LB agar containing chloramphenicol to select for integrants at 37°C. Colonies were then restreaked on LB agar at 42°C to clear the helper plasmid. Individual colonies were then replicate plated on LB agar containing chloramphenicol or ampicillin. Colonies that were resistant to chloramphenicol and sensitive to ampicillin were screened by PCR to identify single integrants (Haldimann and Wanner, 2001).

Growth conditions and media

All strains were streaked from freezer stocks onto LB plates and single colonies were inoculated into 2 ml of LB media (10 g/L tryptone, 5 g/L yeast extract, 10 g/L sodium chloride). Each reporter plasmid was transformed into the indicated strain by electroporation followed by preparation of freezer stocks. For the flow cytometry experiments, freezer stocks of cells with the reporter plasmid were streaked to isolation on LB agar (LB media with 1.5% agar) supplemented with 50 µg/ml of kanamycin. Individual colonies were then inoculated into M9 minimal medium (1X M9 salts (Difco), 2 mM magnesium sulfate, 100 µM calcium chloride, 0.001% thiamine hydrochloride) with 0.4% glycerol and 50 µg/ml of kanamycin (M9 glycerol) and shaken at 250 RPM and 37°C. The cultures were grown to a final turbidity of $ABS_{600} \sim 1.0$ as measured on a Nanodrop 2000c spectrophotometer (Thermo Scientific). These cultures were back diluted into 2 ml of M9 glycerol with varying concentrations of the indicated sugar and grown for 20 hours at 250 RPM and 37°C prior to flow cytometry analysis. Back dilutions ($\sim 1:10^6$ for 20-hour induction experiments) were made such that the cell densities prior to flow cytometry analysis were $ABS_{600} \sim 0.002$. Based on the measured doubling times (Table S2) and back dilutions for each strain, the cultures appeared to be in exponential phase growth for the vast majority of the incubation time. For the time-course experiments in Figure S1A, overnight cultures were back diluted to achieve a final turbidity of $ABS_{600} \sim 0.002$ for each indicated incubation time. To measure

hysteresis, the indicated concentration of sugar was included in the overnight cultures. The overnight cultures then were pelleted by centrifugation and resuspended in M9 glycerol two times prior to back dilution for the flow cytometry experiments.

Flow cytometry and generation of dot plots

Fluorescence measurements were performed on a BD Accuri C6 flow cytometer equipped with a CFlow plate sampler, a 488 nm laser, and a 530 ± 15 nm bandpass filter. Cultures were directly run on the cytometer without any dilutions or media changes. Events reflecting cells were gated based on forward scatter (FSC-H) and side scatter (SSC-H) using lower cutoffs of 11,500 A.U. and 500 A.U., respectively. The specific gate was set using DRAQ5 red dye to ensure that only cells and not debris appeared within the gate. At least 2,000 gated events were collected for each measurement based on the low cell density and the location of the gate. The green fluorescence of gated events was measured using the FL1 channel and recorded as FL1-H. The fluorescence distributions were fit with unimodal or bimodal normal distributions implementing the MATLAB function *fit*. The mean and area of each distribution were converted into dots. The mean determined the vertical location of the dot, whereas the area determined the size of the dot. The diameter of each dot directly scaled with the area under each normal distribution. Figure S1A illustrates the process of generating dot plots from the events recorded on the flow cytometer.

Cell sorting

Cells were grown for 20 hours in M9 minimal medium with 0.4% glycerol containing the indicated concentration of L-arabinose to give roughly 50% induced cells and $ABS_{600} \sim 0.002$. Cells were then sorted into uninduced and induced populations on a MoFlo cell sorter (Beckman Coulter) with 1X PBS as the sheath fluid. Importantly, FACSFlow, the standard sheath fluid for Beckman Coulter, was not used because it arrested growth of *E. coli* unless extensively diluted. At least 1,000,000 cells in each population were collected. Sorted cells were back diluted different amounts into medium with the designated concentration of L-arabinose. Each back-diluted culture was grown until reaching an ABS_{600} of ~ 0.002 and then subjected to flow cytometry analysis.

Mathematical modeling

Details on the deterministic model can be found in Supplemental Information, including its derivation and parameter values. Briefly, the model comprises three differential equations that capture sugar import and catabolism and the inducible expression of the transporter and the enzyme. All simulations were conducted in Matlab using the Euler method and the arc-length continuation method.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Inducible sugar utilization. (A) Inducible utilization pathways are commonly composed of a set of transporters and catabolic enzymes whose expression is induced in the presence of the recognized sugar. (B) The pathways harbor a combination of positive (green plus) and negative (red dash) feedback. Positive feedback emerges from the sugar inducing the expression of the transporters, leading to additional import of the sugar. Negative feedback emerges from the sugar inducing the expression of the enzymes, leading to breakdown of the sugar.

Figure 2.

Varying single-cell responses to different sugars in *E. coli*. MG1655 cells harboring a transcriptional reporter plasmid were grown in M9 glycerol with varying concentrations of the indicated sugar for 20 hours in exponential phase to a low cell density and then subjected to flow cytometry analysis. The promoter used to drive GFP expression is specified. Each dot represents the mean fluorescence and the relative number of cells in the induced (black) and uninduced (white) subpopulations (see Figure S1A). Only one dot is shown for unimodal distributions. The diameter of each dot scales with the fraction of cells in that population. Each dot plot is representative of at least three biological replicates conducted on separate days.

Figure 3.

A simple model predicts varying responses from coupling inducible transport and inducible catabolism. (A) Major reactions considered by the deterministic model. Extracellular sugar (S_0) is imported into the cell by the transporter (T). The intracellular sugar (S) can reversibly bind to the sensory activator or can undergo irreversible degradation by the enzyme (E). The activator bound to the sugar (R) upregulates the expression of the transporter and the enzyme. See Supplementary Information for more details on the model. (B) Phase diagram based on the maximal activities of the fully expressed transporter (α) and enzyme (γ). See Table S3 for values of fixed parameters (with $\nu = 0$). Monostable regions represent parameter values in which the model predicts one stable enzyme level for any extracellular sugar concentration at steady-state. Bistable regions represent parameter values in which the model predicts two stable and one unstable enzyme levels for at least one extracellular sugar concentration at steady-state. The dashed blue line represents parameters that achieve 95% of the maximal dynamic range, where the dynamic range is defined as the ratio of enzyme levels for saturating extracellular sugar and no sugar. Proceeding to the right of this line further increases the dynamic range. (C) Bifurcation curves representing the steady-state relationship between enzyme levels and extracellular sugar concentration for a given set of parameter values. Different curves are shown whereby the only altered parameter value is the maximal enzyme activity (γ). Dashed red lines indicate steady-state enzyme levels that are unstable. (D) Bifurcation curves whereby the only altered parameter value is the maximal transporter activity (α). See Supplementary Information for more details about the model.

Figure 4.

Single-cell responses in the absence of sugar catabolism. The specific genes deleted from each pathway and the promoter driving GFP expression are indicated. Note that the lactose utilization pathway was not evaluated because its behavior was previously probed in the absence of sugar catabolism through the use of the non-hydrolyzable inducer TMG (Ozbudak *et al.*, 2004). The D-gluconate utilization pathway was also not evaluated because of redundancy in D-gluconate catabolism. See Figure 2 for the experimental growth conditions and an explanation of the dot plots. All dot plots are representative of at least three biological replicates conducted on separate days.

Figure 5.

Impact of endogenous biosynthesis of the sugar on the predicted response. (A) The model was modified to allow for constitutive biosynthesis of the intracellular sugar (v , yellow arrow). (B) Bifurcation curves for the predicted enzyme levels (E) in the presence of sugar catabolism ($\gamma = 0.1$) for different rates of endogenous sugar production. See Figure 2 for more information about the bifurcation curves. (C) Predicted ratio of enzyme levels without ($\gamma = 0$) or with ($\gamma > 0$) sugar catabolism in the absence of exogenous sugar ($S_0 = 0$). The ratio peaks at intermediate rates of endogenous sugar biosynthesis and then begins to decline as enzyme levels approach full induction ($E = 1$) even in the presence of sugar catabolism. All plots represent $\alpha = 0.1$ and all other parameter values are reported in Table S3. See Supplementary Information for more details about the model.

Figure 6.

Extent of hysteresis for the D-xylose and L-arabinose utilization pathways with intact or disabled sugar catabolism. Overnight cultures of wild type cells (WT) or cells lacking the catabolic genes (*xylAB* or *araBAD*) were incubated with or without sufficient sugar to induce the entire population (1 mM of D-xylose or 100 μM of L-arabinose for WT, 0.1 mM of D-xylose for *xylAB*, 10 μM of L-arabinose for *araBAD*). The cultures were then washed and grown in M9 glycerol with the indicated concentration of sugar for 20 hours. See Figure 2 for an explanation of the dot plots. The gray regions represent the extent of hysteresis based on the extrapolated sugar concentration required to induce ~50% of the population. All dot plots are representative of at least three biological replicates conducted on separate days.