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Dissecting specific and global transcriptional regulation of bacterial gene expression

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Gene expression is regulated by specific transcriptional circuits but also by the global expression machinery as a function of growth. Simultaneous specific and global regulation thus constitutes an additional—but often neglected—layer of complexity in gene expression. Here, we develop an experimental-computational approach to dissect specific and global regulation in the bacterium *Escherichia coli*. By using fluorescent promoter reporters, we show that global regulation is growth rate dependent not only during steady state but also during dynamic changes in growth rate and can be quantified through two promoter-specific parameters. By applying our approach to arginine biosynthesis, we obtain a quantitative understanding of both specific and global regulation that allows accurate prediction of the temporal response to simultaneous perturbations in arginine availability and growth rate. We thereby uncover two principles of joint regulation: (i) specific regulation by repression dominates the transcriptional response during metabolic steady states, largely repressing the biosynthesis genes even when biosynthesis is required and (ii) global regulation sets the maximum promoter activity that is exploited during the transition between steady states.
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Introduction

Specific transcription factors (TFs) regulate fundamental biological functions including metabolism, development and differentiation (Kalir *et al*, 2001; Zaslaver *et al*, 2004; Egli *et al*, 2008; Segal *et al*, 2008). Departing from discovery and intuitive reasoning, the current focus is on understanding the quantitative and dynamic responses of transcriptional circuits to perturbations, for example to uncover DNA repair dynamics (Ronen *et al*, 2002) or to design *de novo* synthetic circuits (Cox *et al*, 2007). A hallmark of such studies is the use of mathematical models to place experimental measurements on firm theoretical footing (Kim *et al*, 2009). Typically, the dialogue between theory and experiment considers only interactions strictly within the transcriptional circuit, such as gene–TF or ligand-TF interactions. Transcriptional circuits are thus implicitly considered to function independently of their host organism status, an assumption that is both simplistic and incomplete. In fact, early (Bremer and Dennis, 1996; Schaechter *et al*, 1958; Maaløe, 1979) and recent works (Scott *et al*, 2010; Scott and Hwa, 2011) demonstrated that the overall process of gene expression in bacteria is tightly coupled to the physiological growth status of the cell. Specifically, growth-dependent parameters related to cellular physiology and the global expression machinery, such as transcription rate and gene copy number increase due to multiple replication forks, strongly link gene expression to the growth rate (Klumpp *et al*, 2009). Because environmental perturbations typically trigger both a change in growth rate and a specific transcriptional response, simultaneous regulation of gene expression by transcriptional circuits and the global expression machinery constitutes an unavoidable—but often neglected—layer of complexity in gene expression. To fully understand the principles by which bacterial gene expression is a function of the specific ('by the transcriptional circuit') and the global ('by the expression machinery') regulation, the contribution of expression machinery must therefore be quantified and included into analysis of gene expression regulation.

The expression machinery is a complex molecular network that performs the necessary steps of gene expression from transcription to translation, a process that involves hundreds of components and interactions (Thiele *et al*, 2009; Lerman *et al*, 2012). As a consequence, quantification of expression machinery activity cannot be obtained by simply measuring component abundances, such as RNA polymerases (RNAP) or

ribosomes, but must be achieved by model-based interpretation of expression data. The natural starting point to understand expression machinery regulation is studying constitutive ('not specifically regulated') gene expression. Foundational results from *Escherichia coli* and *Salmonella* demonstrated expression of constitutive genes to be a function of the specific growth rate as the only parameter of gene expression, established at the level of transcription that increases with growth rate (Schaechter *et al*, 1958; Maaløe, 1979; Neidhardt *et al*, 1990; Scott and Hwa, 2011). Consequently, proposed models of constitutive gene expression focus at the promoter level and postulate mechanisms of growth rate-dependent increase in transcription, for example, by an increase in the pool of RNAPσ^{70} holoenzyme that is available to initiate transcription (Liang *et al*, 1999; Klumpp and Hwa, 2008). So far, two major limitations hampered the inclusion of global regulation into the analysis of transcriptional circuits. First, growth rate dependency of gene expression has so far only been shown for steady-state growth, hence it remains unclear whether this relationship is applicable to the general case of dynamic changes in growth rate. Second, determining the impact of expression machinery regulation on individual genes requires promoter-specific parameters that are difficult to estimate. Extending our quantitative understanding of global expression machinery regulation to dynamic changes in growth rate and obtaining the relevant governing parameters are necessary to unravel the interplay between transcriptional circuits and the global expression machinery in regulating dynamic cellular processes.

Here, we develop an approach to quantify global expression machinery regulation in the bacterium *E. coli*. We employ fluorescent reporters to quantify promoter activity (Zaslaver *et al*, 2006) as a measure of gene expression for a set of constitutive and specific regulated promoters. Using a Michaelis–Menten type rate law to capture the promoter-specific growth rate dependency, we can quantitatively describe and predict constitutive promoter activity not only during steady state but also during dynamic changes in growth rate. We then unravel the joint regulation by a transcriptional circuit and the global expression machinery for the specific case of arginine biosynthesis (Lim *et al*, 1987; Charlier *et al*, 1992). Accurate model-based predictions of the complex temporal responses to simultaneous perturbations in arginine availability and growth rate indicate that we achieved a quantitative understanding of both specific and global transcriptional regulation processes. We conclude that our approach allows including the unavoidable and ubiquitous global regulation in the analysis and simulation of bacterial gene expression.

Results

A model for specific and global regulation of bacterial gene expression

Here, we present a model of bacterial gene expression to interpret promoter activity data obtained from plasmid-borne fluorescence promoter reporters in the bacterium *E. coli* (Zaslaver *et al*, 2006). Promoter activity (pa) is routinely determined as the production rate of a stable green fluorescent protein (GFP) expressed as a promoter fusion from a reporter plasmid, normalized by the optical density of the cell population (OD) (Zaslaver *et al*, 2006), and thus constitutes the aggregated output of all steps in the gene expression cascade. To relate this phenomenological measurement to specific and global regulatory mechanisms in the gene expression cascade, we used a standard model of bacterial gene expression (Klumpp *et al*, 2009) in which GFP concentration is determined by transcription rate (α_m), translation rate (α_p), gene copy number (g), cell volume (v), dilution by growth rate (μ), mRNA (β_m) and protein (β_p) degradation.

All above parameters could influence the measured promoter activity and thereby, in principle, must be accounted for in the mechanistic interpretation of experimental results. During exponential growth, however, mRNA degradation β_m (Klumpp *et al*, 2009) and translation rate α_p (Liang *et al*, 1998, 2000; Klumpp *et al*, 2009) were previously shown to be constant and GFP degradation β_p to be negligible compared with GFP dilution due to cell growth ($\beta_p \ll \mu$) (Zaslaver *et al*, 2006). Although gene copy number (g) and cell volume (v) are functions of the growth rate (Klumpp *et al*, 2009; Bremer and Dennis, 1996), analysis of their reported dependency on the growth rate, complemented with plasmid copy numbers measured by us and others (Bollenbach *et al*, 2009), revealed a constant plasmid concentration for the plasmids used in this study (see Supplementary information Text 2; Supplementary Figure S6). Thus, the only growth-dependent parameter of the expression machinery that affects promoter activity is the transcription rate α_m.

Superimposed to global transcriptional regulation that effects essentially all genes is specific regulation of genes through TFs that can regulate the recruitment of RNAP (Bintu *et al*, 2005) as well as other steps in transcription (Garcia *et al*, 2012). Promoter activity under joint global and specific transcriptional regulation can thus be expressed as function of the growth rate (μ) and of the activity of the specific TFs (TFA), with a proportionality term to transcription rate that is given by the constant plasmid concentration (g/v), translation rate (α_p), and mRNA degradation (β_m):

$$\mathrm{pa} = \frac{d\mathrm{GFP}}{dt \times \mathrm{OD}} = \alpha_m(\mu, \mathrm{TFA}) \times \frac{g \times \alpha_p}{v \times \beta_m}$$

For practical purposes, the above relationship implies that to dissect specific and global regulation an explicit formulation of the transcription rate α_m is necessary. As a first step toward dissecting the two regulatory sources, we develop a transcription rate function α_m for promoters that are solely under global regulation.

In exponential growth, global regulation has been shown to increase transcription rate as a function of the growth rate by a trend well represented by a Michaelis–Menten rate law (Liang *et al*, 1999; Klumpp and Hwa, 2008). Mechanistic interpretations of this observed relationship suggested a growth rate-dependent increase in the availability of the RNAPσ^{70} holoenzyme that is free to initiate transcription (Liang *et al*, 1999; Klumpp and Hwa, 2008), from now on referred to as free RNAP. For a constitutive promoter, we thus expect promoter activity to be described by a Michaelis–Menten type rate law as a function of the growth rate (μ) and two promoter-specific

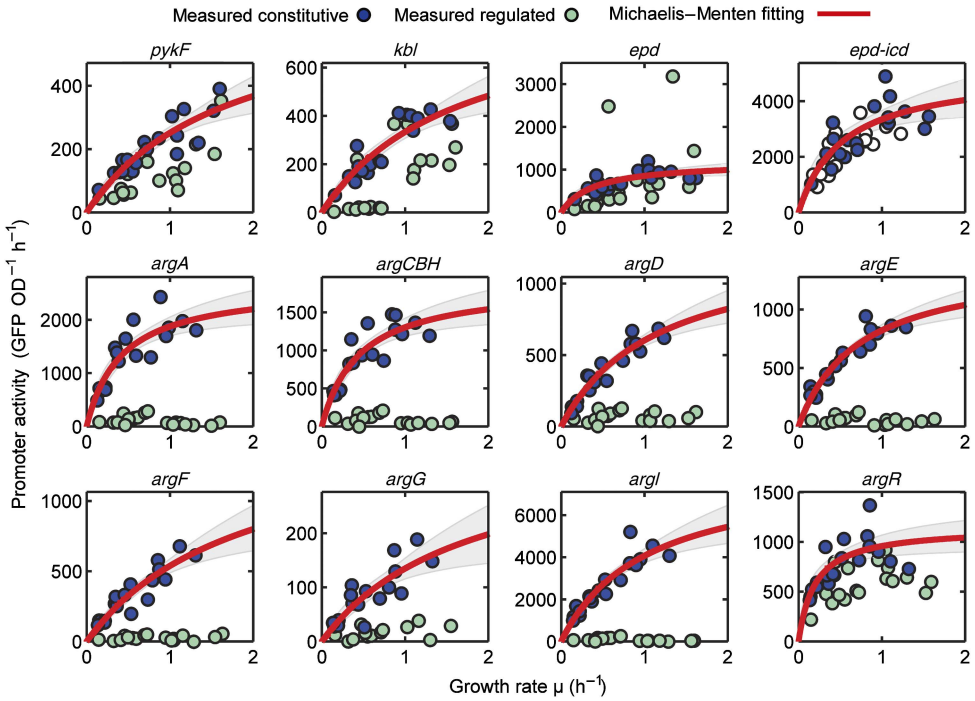

Figure 1 Global expression machinery regulation of promoter activity during exponential growth. Promoter activity of constitutive (blue dots) and native, specific regulated (green dots) promoters as a function of the steady-state growth rate under 18 nutritional conditions. Red lines show optimal least-square fitting of a Michaelis–Menten rate law. Grey shaded areas illustrate the margins of fits within 20% of the optimal sum squared error. Constitutive promoter activity was measured in wild type, except for promoters in the arginine pathway which were measured in the ΔArgR background strain. In the case of the *epd-icd* promoter, promoter activity was also measured in a ΔArgR strain (white dots) to evaluate ΔArgR knockout effects on promoter activity and growth rate.

parameters V_{max} and K_m:

$$pa = V_{max} \times \frac{\mu/K_m}{1+\mu/K_m} \propto \alpha_m(\mu, -) \qquad (2)$$

In the above relationship, V_{max} quantifies the maximal promoter activity sustained by the promoter and K_m the growth rate at which promoter activity is half-maximal. Similar to standard representations of specific transcriptional regulation (Liao *et al*, 2003; Nachman *et al*, 2004), the global regulation is therefore described by the set of promoter-specific parameters V_{max} and K_m, as well as by a variable signal, the expression machinery activity, captured here in the form of growth rate (μ). To dissect the contribution of global regulation to promoter activity, Equation (2) should be validated against systematic experimental data and the corresponding V_{max} and K_m parameters should be quantified. In the next section, we test Equation (2) and estimate V_{max} and K_m parameters from quantitative promoter activity and growth rate data for 12 novel constitutive promoters.

Quantification of global expression machinery regulation

To quantify the global expression machinery regulation using the developed model of constitutive promoter activity in Equation (2), we constructed 12 constitutive promoter-GFP reporter strains that are not regulated by specific TFs anymore. Specifically, we choose three promoters in central carbon metabolism (*pykF*, *kbl* and *epd*) and eight promoters in arginine biosynthesis for which well-characterized specific TFs and RNAP binding sites were known (Rex *et al*, 1991; Ramseier *et al*, 1995; Bledig *et al*, 1996; Charpentier *et al*, 1998). Three constitutive promoter reporters were constructed for the *pykF*, *kbl* and *epd* promoters by replacing their specific TF binding sites that did not overlap with the RNAP binding site with a non-functional sequence. A fourth constitutive promoter was obtained by replacing the RNAP binding site of the *epd* promoter with the RNAP binding site of the *icd* promoter to generate the constitutive hybrid *epd-icd* promoter. Additionally, we made all eight promoters of the arginine biosynthesis pathway constitutive by transforming their promoter reporter plasmids (Zaslaver *et al*, 2006) into a strain with a deletion of their only regulator, the repressor ArgR (termed as ΔArgR) (Baba *et al*, 2006). Expression from these 12 constitutive promoter-GFP constructs is thus exclusively subject to global regulation (Supplementary Table S1). We determined constitutive promoter activity and growth rate in the 12 strains during exponential growth under 18 nutritional conditions with growth rates between 0.2 and 1.5 h^{-1}; that is, minimal medium with acetate, pyruvate, galactose, fructose, glucose, gluconate, glycerol, mannose or succinate, either with or without supplemented amino acids (Supplementary Table S2).

The activity of these 12 constitutive promoters varied up to four-fold across conditions and showed a positive correlation with the growth rate, a trend that was well recapitulated by the least square fit to Equation (2) with a mean percentile error of 16% (Figure 1; Supplementary Figure S7). The estimated promoter-specific constitutive parameters varied between 373

and 8217 GFP OD^{-1} h^{-1} for the V_{max} values and between 0.20 and 1.90 h^{-1} for the K_m values (see Table I). The two parameters V_{max} and K_m were independent of each other (Pearson correlation −0.2, *P*-value 0.45), and their estimation was robust to point elimination (up to 3 points removed, Supplementary Figure S8) and measurement noise (up to 20% additional noise, Supplementary Figure S9).

We evaluated the parameter space of V_{max} and K_m pairs that fit data within 5, 10 and 20% of the optimal sum square error and found that promoters have distinct parameter spaces and thus are arguably promoter specific even under parameter estimation uncertainty (Supplementary Figure S10). The different K_m parameters suggest a promoter-specific component (Liang *et al*, 1999), rather than solely an unspecific one (Klumpp and Hwa, 2008), in the non-linear relationship between promoter activity and growth rate. Following previously proposed mechanistic interpretations, this finding suggests a saturation trend of promoters by the free RNAP concentration at higher growth rates (Liang *et al*, 1999). To confirm that this Michaelis–Menten type relationship is specific to constitutive promoters, we constructed or retrieved (Zaslaver *et al*, 2006) the existing transcriptionally regulated GFP reporters of the constitutive promoters (see Supplementary Table S1), and measured their activity during steady-state exponential growth under the 18 previously employed conditions (Figure 1). Visual inspection and Pearson correlation on the linearized Lineweaver-Burk double reciprocal revealed that the Michaelis–Menten type relationship between growth rate and promoter activity was statistically significant (*P*-value < 0.01) for all constitutive and only for two, presumably weakly or growth-dependent specifically regulated promoters (Supplementary Figure S11).

Table I Promoter-specific constitutive parameters and ArgR repressor dissociation constants

Promoter	V_{max} (GFP OD^{-1} h^{-1})	K_m (h^{-1})	K_r (−)
argA	2660	0.42	1
argCBH	1886	0.45	1.52
argD	1296	1.15	5.25
argE	1414	0.72	1.75
argF	1558	1.90	1.43
argG	373	1.77	2.85
argI	8217	1.02	0.60
argR	1146	0.20	25.70
pykF	683	1.72	—
kbl	877	1.63	—
epd	1185	0.39	—
epd-icd	5104	0.53	—

Consistent with existing data (Liang *et al*, 1999; Klumpp *et al*, 2009; Scott and Hwa, 2011), the growth rate-dependent expression machinery regulation recapitulates the activity of constitutive promoters in steady state. This relationship, however, has not yet been tested during dynamic changes in growth rate, where an array of regulatory mechanisms, such as the alarmone ppGpp or alternative sigma factors, could possibly affect the activity of the expression machinery (Chang *et al*, 2002; Traxler *et al*, 2006). According to our model of global regulation, activation of alternative regulatory mechanisms would be observable as a disruption of the growth rate dependency of constitutive promoter activity. To identify the possible occurrence of such regulatory mechanisms, we monitored promoter activity and growth rate of the 12 constitutive promoters throughout the entire batch growth cycle under the 18 previously described conditions, from inoculation to stationary phase. In addition, we imposed a shift in growth rate by a diauxic nutritional downshift from glucose to succinate. We simulated constitutive promoter activities using time-course measured growth rates and the earlier determined promoter-specific V_{max} and K_m parameters within Equation (2); (Figure 2; Supplementary Figure S12). Throughout the whole time course of the investigated conditions, measured and simulated promoter activities exhibited a

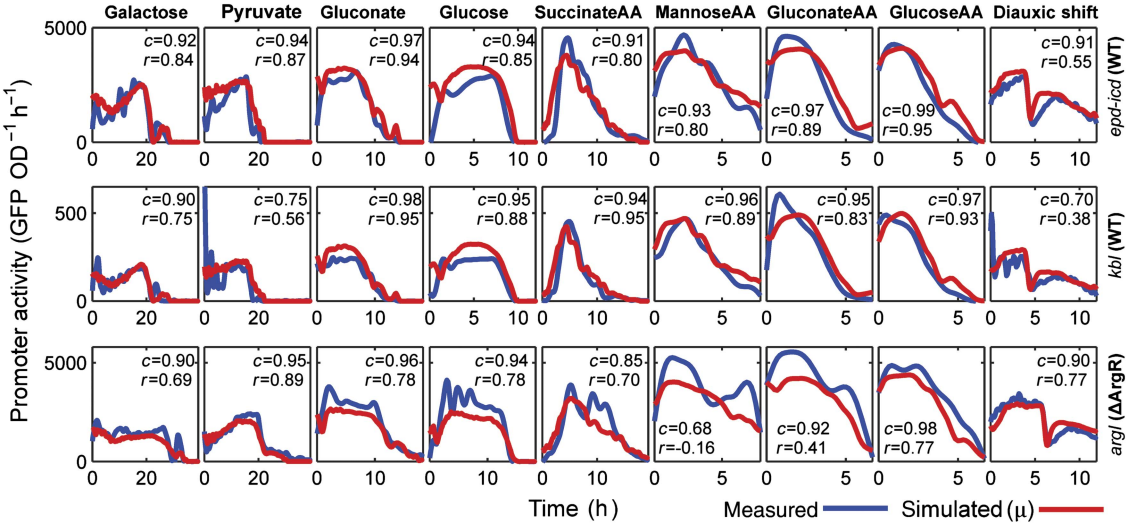

Figure 2 Global expression machinery regulation of promoter activity during dynamic changes in growth rate. Measured (blue) and simulated (red) constitutive promoter activities for three representative promoters and nine growth conditions in rows and columns, respectively. Simulations are based on time-course measured growth rate and the constitutive, promoter-specific V_{max} and K_m parameters inferred in steady state. The Pearson correlation (*c*) and the coefficient of determination (*r*) between measured and simulated promoter activities are given in each box. The *epd-icd* and *kbl* promoters were measured in the wild-type background and the *argI* promoter was measured in the ArgR knockout background. Except for the diauxic shift, growth conditions are ordered by increasing maximum growth rate.

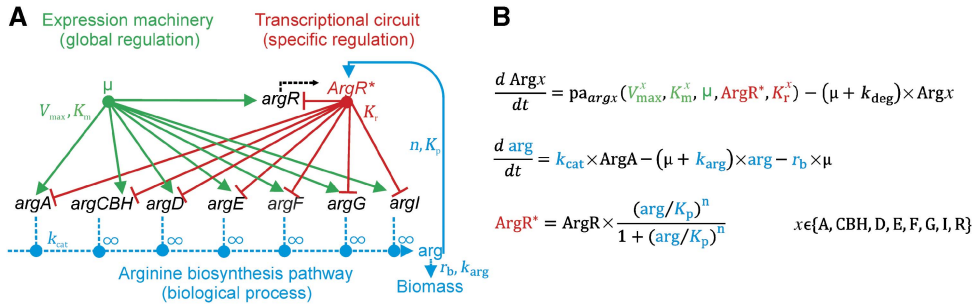

Figure 3 Model of specific and global regulation of the arginine biosynthesis pathway in *E. coli*. (**A**) Schematic representation of specific transcription (red lines) and global expression machinery (green lines) regulation of gene expression in the arginine biosynthesis pathway (blue lines). The pathway synthesizes arginine through linear reactions catalyzed by enzymes transcribed from seven σ^{70} promoters. The transcriptional circuit is coupled to arginine metabolism through activation of the ArgR repressor by arginine (blue line). Global expression machinery regulation regulates constitutive promoter activity as a function of growth rate. Parameters that govern the interaction strength between molecular components as defined in our model are shown. (**B**) Ordinary differential equation model for the arginine biosynthesis pathway including metabolic biosynthesis and feedback, transcriptional regulation and expression machinery regulation.

surprising agreement not only in their trend of higher activity at higher growth rate (Pearson correlation = 0.87 and P-value $< 10^{-9}$, see Supplementary Table S4), but also in their quantitative levels ($R^2 = 0.75$, see Supplementary Table S5). This consistency indicates that our constitutive promoters are predominantly regulated by the global expression machinery in a strictly growth rate-dependent relationship.

Thus, we confirmed and extended previous results based on fewer promoters and conditions that global regulation of promoter activity in steady state is relevant and can be quantitatively described by a Michaelis–Menten type rate law as a function of growth rate and two promoter-specific parameters V_{max} and K_m (Liang *et al*, 1999). Our observation of different K_m values is consistent with the notion that the increase in promoter activity is the result of a promoter-specific response to growth rate increases (Liang *et al*, 1999). Further, we demonstrated that this quantitative relationship holds more generally also during dynamic changes in growth rate. For practical purposes, our findings indicate that growth rate, an easily accessible experimental parameter, can be employed as an approximation to quantitatively predict the global regulation of constitutive promoters given the parameters V_{max} and K_m.

Quantifying specific transcriptional regulation of the arginine repressor circuit

Equipped with our approach to quantify global regulation, we next aimed at understanding the interplay of specific and global transcriptional regulation in controlling biological function. As a test case, we focused on the single input repressor circuit that controls arginine biosynthesis through a feedback loop from the pathway product to the repressor ArgR in *E. coli* (Figure 3A; Lim *et al*, 1987; Charlier *et al*, 1992). We developed a promoter activity equation using the thermodynamic framework (Buchler *et al*, 2003; Bintu *et al*, 2005) that includes regulation by the ArgR repressor along with the constitutive characterization by V_{max} and K_m parameters (see Table I). To link the global regulation with the repression mechanisms known to operate on the arginine promoters (Charlier *et al*, 1992), we interpreted growth rate as a proxy to the free RNAP concentration (free RNAP $\propto \mu$), (Klumpp *et al*, 2009; Liang *et al*, 1999). Under this assumption and given that ArgR and RNAP binding is mutually exclusive due to steric hindrance by overlapping ArgR and RNAP binding sites (Charlier *et al*, 1992), transcriptional regulation by *arg* promoters can be written as a function of the condition- and time-dependent arginine repressor activity ArgR* and its promoter-specific binding dissociation constant, K_r:

$$\mathrm{pa}_{\mathrm{arg}x} = V^x_{\max} \times \frac{\mu/K^x_m}{1 + \mu/K^x_m + \mathrm{ArgR}^*/K^x_r} \propto \alpha_m(\mu, \mathrm{ArgR}^*) \qquad (3)$$

where V^x_{max} and K^x_m are the constitutive parameters and K^x_r the repressor binding affinity for the arginine promoter arg$x$ with $x \in \{A, CBH, D, E, F, G, I, R\}$. Under the above mechanistic interpretation, the parameter K_m becomes a measure of the affinity between free RNAP and the promoter. As becomes apparent from Equation (3), the expression machinery activity given by the growth rate sets the maximum promoter activity that is achievable at a given point, in the following referred to as promoter capacity. The repressor activity ArgR* modulates promoter activity between promoter capacity and full repression.

We inferred the promoter-specific repressor affinities K_r and the condition-dependent repressor activity ArgR* from activities of the 8 regulated promoters under the 18 steady-state growth conditions by least square fitting minimizing the overall percentage error (see Supplementary information, Text 5). Specifically, we used the previously estimated V_{max} and K_m parameters (see Table I) and the measured growth rate in Equation (3). Since ArgR* activity and the K_r parameters can be scaled, we set the repressor affinity for the *argA* promoter to unity. As expected, substantially lower ArgR* activity was found in conditions with biosynthetic production of arginine compared to those with externally supplemented arginine (Supplementary Figure S13c). Repressor affinity varied at most three-fold among the seven enzymatic promoters, but was ten-fold weaker for its own *argR* promoter (see Table I; Supplementary Figure S13a). Consistent with an optimally efficient response to arginine depletion (Zaslaver *et al*, 2004), such lower affinity for the *argR* promoter ensures basal repressor expression even under full repression. A striking quantitative aspect was much lower promoter activity than promoter capacity (Figure 1; Supplementary Figure S14), showing that these promoters are strongly repressed, even

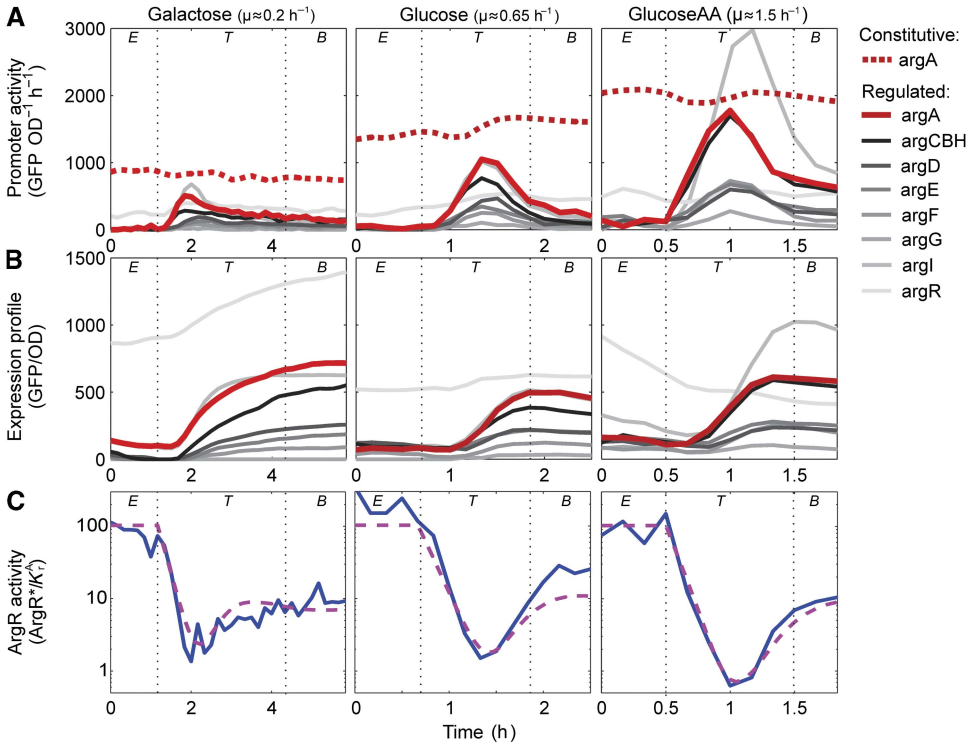

Figure 4 Specific and global regulation of the arginine pathway upon dynamic arginine depletion during exponential growth. The three growth phases during the switch from external uptake to biosynthesis of arginine are separated by vertical dotted lines: growth on externally supplemented (E), transition from uptake to biosynthesis (T) and steady biosynthesis (B) of arginine. (**A**) Activities of the eight arginine promoters in response to depletion of externally supplemented arginine during exponential batch growth. The red continuous line represents the measured regulated promoter activity and the red dotted line represents the simulated growth rate-dependent promoter capacity for *argA*. (**B**) The fluorescence expression profile, a proxy to enzyme concentration, of the eight arginine biosynthesis enzymes as measured during the shifts. (**C**) Activity of the arginine repressor ArgR (blue continuous line) as inferred from measured promoter activity of the eight arginine promoters and as fit by the ODE model (purple dashed line).

when cells actually need to synthetize arginine. Calculations assuming mutual exclusion of ArgR and RNAP, as described above, showed the ArgR repressor to be bound to enzymatic promoters >80% of the time during arginine biosynthesis (Supplementary Figure S13b).

Why have the arginine promoters evolved promoter capacities that are much larger than the promoter activity required in either the repressed or the unrepressed steady-state regime? To test whether this apparent excess of promoter capacity is exploited during dynamic metabolic adaptations, we determined activities of the eight regulated arginine promoters upon arginine depletion during exponential growth of *E. coli* batch cultures growing at $0.2\,h^{-1}$ (galactose), $0.65\,h^{-1}$ (glucose) and $1.5\,h^{-1}$ (glucose supplemented with amino acids) (Figure 4A; Supplementary Table S6). We thereby triggered a dynamic response of pathway-specific transcriptional regulation by adding a small amount of arginine that runs out during growth, while maintaining the expression machinery regulation at three different activity levels. During the transition from external supplementation to intracellular biosynthesis of arginine, the activity of the arginine promoters transiently peaks far above the steady-state levels (Figure 4A; Supplementary Figures S15 and S16). The only exception was the *argR* promoter with a higher baseline level and a lower peak, presumably due to its lower repressor affinity. Calculating promoter capacity of the eight arginine promoters from growth rate and the constitutive parameters V_{max} and K_m using Equation (2) revealed that the regulated promoter activity approaches the promoter capacity during the transient adaptation (Figure 4A; Supplementary Figures S15 and S16). Notably, the promoter activity bursts at fast growth could not be achieved by the promoter capacity set by the expression machinery regulation at slow growth. Thus, the promoter capacity is only transiently exploited during adaptation and the growth rate-dependent expression machinery regulation ensures increasing promoter capacity for rapid enzyme synthesis at higher growth rates.

These dynamic patterns of promoter activity bursts achieve a smooth increase and constant steady-state concentration of the biosynthesis enzymes at all growth rates (Figure 4B), indicating that increasing promoter capacity counter balances the faster dilution of enyzmes in more rapidly dividing cells. Consistent with the postulated just-in-time regulatory program (Zaslaver *et al*, 2004), we observed an ordering of enzyme concentration that follows the pathway order with *argA* > *argCBH* > *argD* > *argE* > *argF* > *argG* and the isoenzyme *argI* as the only exception (Figure 4B). However, we did not find evidence for the proposed ordering of response times (Zaslaver *et al*, 2004); that is, all enzymes reached half-maximum concentration roughly at the same time for a given growth rate (Figure 4B). Overall, our results highlight two distinct principles of arginine pathway regulation. First, the

specific repressor circuit determines the fraction of promoter capacity that is exploited. Full derepression is confined to short transient phases whereas in steady state the biosynthesis promoters are largely repressed, even when required. Second, the global regulation by the expression machinery sets a growth-dependent promoter capacity that allows higher bursting of promoter activity during fast growth, to rapidly produce enzymes and counter balance the increased growth dilution.

Predicting the response to simultaneous dynamic-specific and global perturbations

With a quantification of the specific and global regulation interplay at the promoter level in hand, we finally attempted to obtain a comprehensive, pathway-level understanding of regulation in the arginine pathway (Figure 3A) by investigating the response to simultaneous and orthogonal dynamic perturbations in the specific and global regulation. For this purpose, we developed an ordinary differential equation (ODE) model that includes arginine production, consumption and feedback to the repressor ArgR (Figure 3B). Since we focus on gene expression regulation rather than detailed pathway biochemistry, the ArgA-catalyzed first of the linear reactions converting glutamate to arginine was modelled as rate-limiting, assuming the other reactions to operate instantaneously. The regulatory and metabolic pathway structure was converted into a set of ODEs that describe concentrations of enzymes and repressor as dictated by their promoter activity, arginine concentration and repressor activity. The model has 30 parameters, 24 of which were previously inferred as the 8 promoter-specific V_{max} and K_m parameters and ArgR dissociation constants K_r (see Table I, Supplementary Table S7). To infer the six missing parameters, we fitted the ODE model to reproduce the ArgR activity underlying the three arginine shifts by least-square criteria (Figure 4C). ArgR activity was obtained from the measured promoter activity using Equation (3) and, as expected, showed an initially high activity and a transient minimum that increases to reach a second slightly lower steady-state level. As the combined output of arginine production, consumption and repressor activation, ArgR activity represents an ideal readout to infer the relevant metabolic-feedback parameters in the model. We fitted the ODE model to the dynamic ArgR activity from the onset of promoter upregulation, thus discarding the initial fully repressed state that is not informative, and identified a parameter set that optimally reproduces the dynamic ArgR activity (parameter values $k_{cat} = 0.32$, $k_{deg} = 0.64$, $k_{arg} = 1.3$, $r_b = 68$, $n = 2.6$ and $K_p = 148$, initial conditions ArgR = 800, ArgA = 0, arg = 71).

To challenge our parameterized ODE model, we investigated the pathway response to a novel scenario on which the model has not been trained: simultaneous and orthogonal dynamic perturbations in both specific and global regulation. In a set of experiments, *E. coli* cultures experienced arginine depletion at different phases of growth during a diauxic shift from the preferred carbon source glucose to succinate, whose consumption is repressed in the presence of glucose. The obtained promoter activities of arginine promoters for the entire 7 h of growth are consistent with the above principles (Figure 5A); that is, a transient burst in arginine promoter activity that increases with the growth rate, and a growth rate-dependent promoter capacity set by the expression machinery that is essential for reaching the observed burst levels. To test whether the quantitative knowledge acquired by our approach can capture such a complex transcriptional response, we predicted *in silico* the regulatory response of arginine promoters to both simultaneous perturbations using the ODE model without any refitting of model parameters. The inputs given were the measured growth rate during diauxic growth and the 12 onset times at which arginine runs out, as identified from the experimental data (see Supplementary information, Text 7 for simulation details). The simulated promoter activities accurately reproduced the measured data (Figure 5A and B; Supplementary Figures S17–S19). Since our mathematical model of arginine pathway regulation is precise in recapitulating the dynamic responses, we conclude that our approach is able to achieve a satisfactory quantitative understanding of joint specific and global regulation.

Discussion

Cellular functions arise from the coordinated interplay between molecular networks. While reconstruction of topologies is thriving, the challenge shifts to identify the principles of network coordination (Gerosa and Sauer, 2011; Kotte *et al*, 2010). Here, we unravelled two principles of coordination between a repressor circuit with a single input and the global expression machinery for the conjoint control of arginine biosynthesis. First, substantially reduced ArgR repressor activity occurs only transiently during a switch from external uptake to arginine biosynthesis. Together with a strong affinity of the repressor for enzymatic promoters, this leads to strongly repressed arginine promoters, even when cells need to produce arginine, intervened by only short bursts of near maximum expression at the onset of the transition. Second, the global expression machinery sets a growth rate-dependent maximum expression level that we interpreted as promoter capacity, such that transient promoter activity can peak higher when derepressed at fast growth. Thus, specific regulation by repression is dominant during metabolic steady states, and global regulation becomes relevant during transitions between metabolic steady states. Together, these two coordination principles between the metabolic-repressor circuit and the global expression machinery enable rapid pathway induction at any growth regime. Strong deficiencies in the growth rate of the ArgR knockout strain during biosynthetic conditions (Supplementary Figure S1) might thus be explained by an abnormal upregulation of enzyme levels, since derepressed biosynthesis genes are quantitatively tuned to foster enzyme bursts and not to maintain steady-state levels.

The uncovered coordination principles are likely to be general for the large class of biosynthetic pathways that are controlled by single input repressor circuits, because metabolic pathways are unavoidably challenged with the growth dilution of enzymes during transient upregulation (Chubukov *et al*, 2012). Quantitative functioning of the abundant single input repressor circuit motif (Alon, 2007) was previously assessed through parameterizing interactions (Ronen *et al*, 2002;

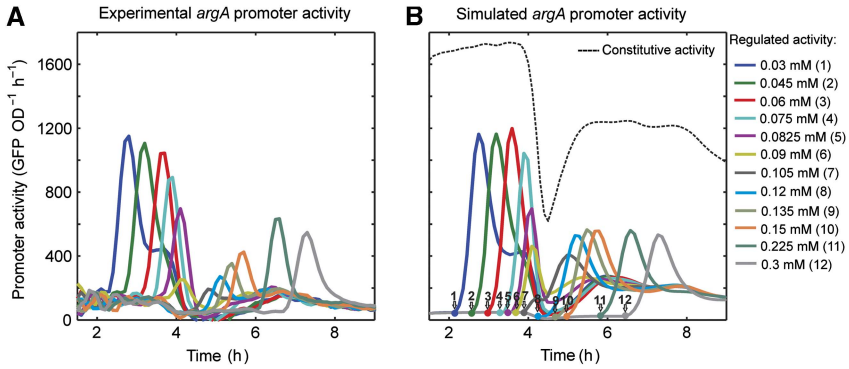

Figure 5 Measured and simulated argA promoter activity under simultaneous dynamic perturbations in specific and global regulation. In 12 independent experiments, a different arginine concentration (see legend) was supplemented and cells depleted it at different time points during the course of growth in a diauxic shifts from glucose to succinate. (**A**) Promoter activity of the argA promoter as measured in the 12 experiments. (**B**) Promoter activity of the argA promoter as simulated by the ODE model starting simulation at each of 12 different upregulation onset times (see legend). The simulated constitutive promoter activity for argA promoter is shown (dotted black line).

Chubukov *et al*, 2012) or quantifying regulator abundances (Garcia and Phillips, 2011). Here, we added a layer of complexity that was previously neglected by explicitly including the global expression machinery regulation. Our results demonstrate that single input repressor circuit performance is not simply dictated by its own input–output relation (Garcia and Phillips, 2011), but also by the activity of the global expression machinery that sets the promoter capacity. Thus, understanding the quantitative role of network motifs in gene regulation (Alon, 2007) might require to characterize their relationship with global regulation by the expression machinery. As an example for the much larger class of biosynthetic genes, we found specific regulation to dominate arginine biosynthesis through a single-input repressor. By focusing on one enzyme-encoding and two TF genes, a recent paper postulates predominance of global regulation in central metabolism with only a minor, fine-tuning contribution from the pleiotropic TFs Crp and Fis (Berthoumieux *et al*, 2013). The here proposed approach can now be used to evaluate how common such specific and global transcriptional paradigms of regulation are across cellular functions and transcriptional circuit configurations.

Akin to activity inference of metabolic (Sauer, 2006) and transcription (Liao *et al*, 2003; Buescher *et al*, 2012) networks, the activity of the global expression machinery was accessible only by model-based interpretation of a measurable proxy, in our case through GFP-promoter fusions of synthetic constitutive promoters (Zaslaver *et al*, 2006). For data interpretation, we used a model based on foundational work showing (i) that steady-state expression of constitutive genes is strictly growth rate dependent (Schaechter *et al*, 1958; Maaløe, 1979; Neidhardt *et al*, 1990; Scott and Hwa, 2011) and (ii) that a Michaelis–Menten type rate law describes steady-state constitutive promoter activity dependency on growth rate (Liang *et al*, 1999; Klumpp and Hwa, 2008). We extended this work by demonstrating that the governing V_{max} and K_m parameters are promoter specific and that the phenomenological growth rate dependency largely applies also to dynamic changes in growth. Specifically, we found that V_{max} and K_m estimates obtained from steady-state data can be used to predict constitutive promoter activity from experimentally accessible growth rates. Akin to enzymes, promoter regions could thus be routinely characterized by their V_{max} and K_m values and made available in databases of standardized biological parts (Canton *et al*, 2008). Such promoter parameters could also potentially be determined *in vitro* (Maslak and Martin, 1994) or inferred computationally from *in vivo* expression and sequence information (Nachman *et al*, 2004; Pan *et al*, 2007; Hardiman *et al*, 2010; Brewster *et al*, 2012).

To include global regulation within transcriptional circuits, we leveraged a previously proposed mechanistic interpretation in terms of free RNAP availability and promoter saturation (Liang *et al*, 1999; Klumpp and Hwa, 2008) which is consistent with the physiological and gene expression parameters of *E. coli* reported in the literature (Klumpp *et al*, 2009). This view is also consistent with the reported growth dependency of parameters in the gene expression cascade (Klumpp *et al*, 2009), which highlights transcription rate as the main driver of growth dependency in gene expression. An implication is that the translation rate is growth independent, even though the ribosome content is known to increase with faster growth (Bremer and Dennis, 1996). Several authors proposed a constant-free ribosome concentration as a likely explanation (Liang *et al*, 1998; Liang *et al*, 2000; Klumpp *et al*, 2009). However, it can be argued that the translation rate has not been characterized with the systematic rigor of the other parameters (Liang *et al*, 1998, 2000) and a growth rate-dependent translation rate might eventually be included within formulations of constitutive gene expression. Our approach and the relative conclusions on the mainly repressed biosynthesis promoters with only transient expression bursts are independent of this putative influence of the translation rate, as is the phenomenological shown predictive power for constitutive and regulated promoter activity. Solely the mechanistic interpretation of increased constitutive gene expression in terms of increased promoter capacity might have to be amended by a growth-dependent translation rate. This amendment would require to additionally dissect the individual contributions of the possibly tightly and complexly coupled processes of transcription and translation in global regulation (Proshkin *et al*, 2010).

Overall, we developed an experimentally validated approach to include global expression machinery regulation in the analysis and simulation of bacterial gene expression.

This is particularly relevant for promoter activity data that is increasingly employed to quantify transcriptional regulation (Kalir *et al*, 2001; Setty *et al*, 2003; Bollenbach *et al*, 2009; Zaslaver *et al*, 2009), but less so for the growth-independent mRNA abundance (Klumpp *et al*, 2009) as a measure of gene expression. Unless global regulation is properly considered, inferring specific transcriptional regulation from promoter activities obtained at different growth rates will be misleading. While some authors have used constitutive reporters to normalize promoter activity data (Davidson *et al*, 2010; Sasson *et al*, 2012), we developed a superior approach based on first principles to explicitly include the promoter-specific growth dependency into models, under the constraint that parameters in the gene expression system can be expressed as a function of the growth rate. Our approach not only allows to pin-point the regulatory role of global expression machinery instead of concealing it under a heuristic normalization, but additionally allows precise simulation of a pathway under joint specific and global regulation. We envisage that the here described principles will help including the ubiquitous and unavoidable role of global regulation in studies aiming for holistic understanding of interacting cellular networks (Buescher *et al*, 2012; Karr *et al*, 2012; Lerman *et al*, 2012).

Materials and methods

Strains, plasmids and media

The *E. coli* K-12 strain BW25113 was used throughout. The *argR* deletion mutant was obtained from a knockout library (Baba *et al*, 2006) and deprived of its kanamycin resistance as previously described (Datsenko and Wanner, 2000). GFP-based promoter reporter plasmids were obtained from a library (Zaslaver *et al*, 2006) or constructed by PCR following the procedures of the original study (Zaslaver *et al*, 2006) (see Supplementary Table S1). Growth experiments were performed in M9 minimal medium to which carbon sources were added from sterilized stock solutions (adjusted to pH 7) to a final concentration $5\,g\,l^{-1}$ (see Supplementary information, Text 1). For amino-acid addition, a stock solution containing all 20 amino acids (adjusted to pH 7) was added to yield final concentrations as described elsewhere (Zaslaver *et al*, 2004), unless stated otherwise (see Supplementary Table S2). All chemicals were purchased from Sigma-Aldrich unless stated otherwise.

Cultivation

M9 medium batch cultures in 96 deep-well format plates (Kuehner AG, Birsfeld, Switzerland) were inoculated 1:50 from LB precultures and incubated overnight at 37°C under shaking. Subsequently, 96-well flat transparent plates (Nunc, Roskilde, Denmark) containing M9 medium (fill volume 200 μl) were inoculated 1:200 with overnight cultures and sealed with parafilm to reduce evaporation. On-line measurements of optical density at 600 nm (OD_{600}) and fluorescence (excitation wavelength: 500 nm, emission wavelength: 530 nm) were performed at 37°C with shaking using a plate reader (TECAN infinite M200, Tecan Group Ltd, Männedorf, Switzerland) at 10 min intervals.

Data processing and modelling

Fluorescence (GFP) and OD_{600} measurements were analyzed using custom MATLAB software. Both signals were processed in a well-specific manner to obtain promoter activity ($d\mathrm{GPF}/(dt \cdot \mathrm{OD})$), growth rate ($d\ln(\mathrm{OD})/dt$) and expression profile (GFP/OD) time-course data. We note that GFP values represent raw reading values from the measuring device and are thus in arbitrary units, not in protein counts. OD and GFP signals were normalized to blanks by subtracting values before inoculation of cells and smoothed using a moving average window with size 3. GFP and ln(OD) derivatives were obtained by two-point finite difference numerical approximation. Promoter activity and expression profile signals were corrected for fluorescence background by subtracting the corresponding signal of the promoter-less plasmid reporter strain p139 (Zaslaver *et al*, 2006). Promoter activity and growth rate values for steady-state growth were calculated as the average value in the time range visually identified as exponential phase. In arginine depletion experiments, signals from different promoters were aligned by the growth rate to correct for differences in inoculation OD. Estimation of parameters was performed using the *fmincon* MATLAB function.

Data availability and supplementary information

Experimental measurement data sets, the ODE model (in MATLAB script format) and Supplementary information are available at the Molecular Systems Biology website (www.nature.com/msb). Supplementary data set 1 contains steady-state measurements for constitutive and regulated promoters (Figure 1; Supplementary Figures S1 and S14; Supplementary Table S4; Supplementary Table S5), Supplementary data set 2 contains time-course measurements for constitutive promoters (Figure 2; Supplementary Figures S2, S3 and S12), Supplementary data set 3 contains measurements of arginine shifts during exponential growth for the arginine promoters (Figure 4; Supplementary Figures S15 and S16) and Supplementary data set 4 contains measurements of the double perturbation experiments for the arginine promoters (Figure 5; Supplementary Figure S17).

Supplementary information

Supplementary information is available at the *Molecular Systems Biology* website (www.nature.com/msb).

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Author contributions: LG conceived and designed the study, performed the computational analysis, and wrote the manuscript. KK performed the experimental work, contributed to designing the study and writing the manuscript. MH supervised the study. US supervised the study and wrote the manuscript. All authors read and approved the final paper.

Conflict of interest

The authors declare that they have no conflict of interest.

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