Cross-regulation between proteome reallocation and metabolic flux redistribution governs bacterial growth transition kinetics

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Abstract:

Bacteria need to adjust their metabolism and protein synthesis simultaneously to adapt to changing nutrient conditions. It's still a grand challenge to predict how cells coordinate such adaptation due to the cross-regulation between the metabolic fluxes and the protein synthesis. Here we developed a dynamic Constrained Allocation Flux Balance Analysis method, which integrates flux-controlled proteome allocation and protein limited flux balance analysis. This framework can predict the redistribution dynamics of metabolic fluxes without requiring detailed enzyme parameters. We reveal that during nutrient up-shifts, the calculated metabolic fluxes change in agreement with experimental measurements of enzyme protein dynamics. During nutrient downshifts, we uncover a switch of metabolic bottleneck from carbon uptake proteins to metabolic enzymes, which disrupts the coordination between metabolic flux and their enzyme abundance. Our method provides a quantitative framework to investigate cellular metabolism under varying environments and reveals insights into bacterial adaptation strategies.

Introduction

Microbes are always exposed to changing conditions in their natural habitats. To respond to fluctuating environments such as nutrient shifts, microbes redistribute their metabolic flux to synthesize amino acids and other internal metabolites, which are supplied as the precursors for the biosynthesis of protein. The variation in metabolic processes would further lead to the rebalance in allocation of protein synthesis among the catabolic proteins, ribosomal proteins, metabolic enzymes and others [1]. On the other hand, the changes of protein allocation inversely alter the amino acids synthesis through the metabolic network [2]. Therefore, the cross-regulation between the cellular metabolic fluxes and proteome allocation under the changing environments provide a highly dynamical, intricate, and complex response.

Genome-scale metabolic network models (GEMs) are powerful tools for simulating cellular metabolism under different conditions. One of the most widely used methods for GEMs simulation is flux balance analysis (FBA), which optimizes a predefined objective function (such as biomass or metabolite production) subject to mass balance and capacity constraints [3]. FBA assumes that the fluxes including nutrient uptake are at steady state, which limits its applications especially when the extracellular environment changes over time. Dynamic FBA (dFBA) extends FBA by incorporating the temporal variations of extracellular nutrients and metabolites, and simulate the metabolic adaptation of cells in response to these changes [4]. However, dFBA still neglects the effects of enzymatic constraints, which may be significant for energy metabolism[5, 6], metal utilization [7] and biomass productions [8].

Enzymatic constraints are essential for realistic simulation of metabolic models, but they depend on many parameters that vary over time and are hard to measure. Recent advances in proteomics data availability have enabled the integration of enzyme concentrations into (GEMs), but other parameters, such as enzyme turnover rates or binding affinities, are still uncertain or unknown [8]. Moreover, these parameters are not constant, but change dynamically in response to environmental fluctuations. Therefore, the accuracy and applicability of such enzyme-constrained GEMs are limited by the availability and validity of these parameters. One way to overcome this challenge is to infer the dynamics of enzyme parameters from molecular interactions and use them to predict the metabolic fluxes under changing conditions. However, this approach is computationally expensive and usually requires detailed knowledge of the gene regulatory network that controls enzyme expression.

Recent studies of quantitative bacterial physiology have revealed that bacterial gene expression is coordinated by functional sectors that respond to environmental changes [1, 9, 10]. The overall proteome can be coarse-grained into four major sectors based on their functions: carbon uptake (C-sector, ϕ_C), metabolism (E-sector, ϕ_E), translation (R-sector, ϕ_R), and housekeeping (Q-sector, ϕ_E) [11]. These sectors have different protein fractions that are regulated by the translational activity of the ribosome (σ), which in turn depends on the protein synthesis flux (v_R) and the ribosome fraction. The global regulation of proteome resource allocation in steady-state growth follows the 'growth law' [6, 11, 12], while the flux-controlled

regulation (FCR) can predict the kinetics of the protein reallocation without detailed metabolic fluxes under various carbon shift conditions [1].

Here, we developed a novel computational framework that integrates coarse-grained model of flux-controlled proteome allocation and genome-scale metabolic network model of flux balanced analysis to predict the metabolic flux redistribution during nutrient shifts. We applied our framework to analyze the temporal changes of individual metabolic fluxes of *Escherichia coli* during growth shifts between co-utilized carbon substrates. By comparing our predicted kinetics of metabolic fluxes with omics data of enzyme kinetics, we identified the bottleneck reactions among the metabolic network that limit the adaptation dynamics to nutrient changes. From a coarse-grained view, we found that the limiting factor for metabolic fluxes switched from carbon uptake proteins ϕ_C to metabolic enzymes ϕ_E during carbon downshift. This switch resulted in a transient increase in the growth rate that was previously overlooked by experiments. Our results reveal the cross-regulation between proteome reallocation and metabolic flux redistribution plays an important role in the bacterial adaptation to environmental changes. Our framework provides a quantitative tool to investigate cellular metabolism under varying environments and reveals insights into bacterial adaptation strategies.

Materials and methods

Construction of the dCAFBA model

In this study, we performed all the model simulations in a proteome allocation model introduced by Mori et al. [13], which is constructed based on the *E.coli i*JR904 genome-scale metabolic model [14], including 761 metabolites and 1075 reactions. Following the recent studies on bacterial proteome [6, 11, 12], the entire cellular proteome was divided into four coarse-grained functional sectors in the proteome , which are carbon-scavenging proteins (C-sector) that includes proteins devoted to carbon import and transport from extracellular, metabolic enzymes (E-sector) that consists of biosynthetic enzymes, translational proteins (R-sector) that comprises ribosomal and its affiliated proteins, and house-keeping proteins (Q-sector) whose express keeps to be constant at various growth conditions. In the resulting proteome allocation model, there is a single reaction (carbon transport reaction) in C-sector. In total, 615 reactions belong to E-sector. 458 reactions were assigned to Q-sector. The fractions of the four proteome sectors should sum up to be 1, i.e. $\emptyset_C + \emptyset_E + \emptyset_R + \emptyset_Q = 1$.

The study of Erickson et al. [1] has shown that the protein synthesis flux of C sector and R sector are regulated by the translational activity $\sigma(t)$ with regulation functions of $\chi_C(\sigma)$ and $\chi_R(\sigma)$ in the form of $\frac{d\phi_C}{dt} = \chi_C(\sigma) \cdot \sigma(t)$ and $\frac{d\phi_R}{dt} = \chi_R(\sigma) \cdot \sigma(t)$, where the regulation functions of protein allocations $\chi_C(\sigma)$ and $\chi_R(\sigma)$ are: $\chi_C(\sigma) = (1 - \sigma\chi_R/\lambda_C)\emptyset_{C,max}$, $\chi_R(\sigma) = \emptyset_{R,0}/(1 - \sigma/\gamma)$. $\lambda_C, \gamma, \phi_{C,max}, \phi_{R,0}$ are constants measured by the steady state protein allocations

under different growth mediums [1] (see methods parameter table). The dynamics of the metabolic enzymes $\phi_E(t)$ can be written as the rest of the protein partition: $\phi_E(t) = 1 - \phi_C(t) - \phi_R(t) - \phi_Q$. With this knowledge, we can get the protein dynamics as long as the translational activity $\sigma(t)$ is deduced.

Since the translational activity $\sigma(t) \equiv v_R(t)/\phi_R(t)$ is defined by the protein synthesis rate ($v_R(t)$) per ribosome ($\phi_R(t)$). We then assume all the synthesized amino acids from the metabolic network are assembled into protein synthesis, so that the protein synthesis rate is proportional to the amino acid synthesis rate $v_R(t) = \alpha v_{aa}(t)$ with a constant α to represent the synthesis flux of amino acids. As a result, the dynamics of $\sigma(t)$ can be expressed as:

$$\frac{d\sigma(t)}{dt} = \alpha \frac{1}{\phi_R(t)} (\frac{dv_{aa}}{dt} - v_{aa}(t)\sigma(t)(\chi_C - \chi_R) \quad (1)$$

This equation describes how the amino acid synthesis flux $v_{aa}(t)$ regulates the dynamics of protein allocation $\phi_C(t), \phi_R(t), \phi_E(t)$ via translational activity $\sigma(t)$. It is also important to formulize how protein allocation constrains the metabolic flux distribution through the metabolic network. To do so, we simply set the upper boundaries on the carbon uptake flux v_C and the sum of all the absolute metabolic fluxes $\sum_i |v_i|$ by:

$$v_{C,j}(t) \leq \emptyset_{C,j}(t)/w_{C,j} \quad (2)$$

$$\sum_i |v_i(t)| \leq \emptyset_E(t)/w_E$$

where i, j are the index of metabolic reactions in E-sector and index of carbon exchange reactions, respectively. $w_{C,j}$ and w_E characterizes the carbon uptake protein and metabolic enzymes needed to carry one unit flux, which are determined by fitting the growth rates at steady state and protein partitions at corresponding growth medium (see methods and parameter table). With protein allocation constrains, we can calculate the flux distribution $v_i(t)$ by optimizing the biomass synthesis flux λ.

Based on experimental findings [12], $\emptyset_C$ was assumed to depend linearly on the carbon intake flux v_C, i.e. $\emptyset_C = \emptyset_{C,0} + w_C v_C$, where $\emptyset_{C,0}$ represents a growth rate-independent offset and is assumed to be 0 in this work, unless otherwise stated elsewhere. w_C characterizes the efficiency of the carbon catabolic protein for carbon intake. v_c is the carbon influx that carbon transport reaction carries.

Similarly, the proteome fraction of the E-sector is written as $\emptyset_E = \emptyset_{E,0} + w_E v_i$, where $\emptyset_{C,0}$ is the growth rate-independent offset and is also assumed to be 0 in this work. The weight w_E stands for the efficiency of a metabolic enzyme for carrying one unit of metabolic flux v_i. In this study, $\emptyset_Q$ of 0.45 reported in a previous work [11] has been exploited. Other parameters for simulating the cell growth during the shift are listed in Table S2.

Model simulations

All simulation was performed using FBA as implemented in the COBRA toolbox executable in MATLAB. The GLPK Optimizer solver in combination with the

toolbox was used to solve the linear programming problems. In this study, the biomass equation was used to obtain the optimal solution of the metabolic model as described elsewhere [3]. Mathematically, the dCAFBA can be represented as follows:

$$\begin{aligned} &\text{Maximize: } \lambda \\ \text{Subject to } &\sum_i S_{\mu i} v_i = 0 \quad \forall \mu \\ &l_i \ll v_i \ll \mu_i \quad \forall i \\ &l \ll v_{C,j} \ll \frac{\emptyset_{C,j}}{w_{C,j}} \quad \forall j \\ &\sum_i w_E |v_i| \leq 1 - \emptyset_C - \emptyset_R - \emptyset_Q \end{aligned}$$

where λ is the flux that the biomass reaction carries, representing the growth rate, S_{ui} is the stoichiometric matrix, is a vector of all metabolites (u) and reactions (i), l_i and u_i represent lower and upper bounds for the flux of each reaction v_i, respectively. v_c is the carbon influx that carbon transport reaction carries, $\emptyset_C$ represents proteins associated with carbon intake and transport. The constants of the substrates used in this work are given in Table S3.

Efficiency of enzyme utilization

The relative efficiency $\epsilon_i(t)$ of the enzyme utilization was defined by the ratio between fluxes $v_i(t)$ and the enzyme partitions $\phi_i(t)$ of each reaction i normalized by the pre-shift enzyme efficiency $\epsilon_i(t) = \frac{v_i(t)}{\phi_i(t)} / \frac{v_i^{pre}(t)}{\phi_i^{pre}(t)}$ where v_i^{pre} and ϕ_I^{pre} denotes the fluxes and enzyme partitions in the pre-shift steady stat. In our calculation, the fluxes of a given reaction was predicted by the dCAFBA model while the protein partition of the enzymes are measured by Erickson *et al.*.

Construction of the simplified model

The modified FCR model is built on the published FCR model [1] by introducing the dynamics of E protein: $\phi_E(t) = 1 - \phi_R(t) - \phi_C(t) - \phi_Q$, shown in Figure 4b. We assume the protein synthesis flux v_R to be the amino acid synthesis flux v_{aa}, the translational activity σ in the context can be described as $\sigma(t) = \frac{v_R}{\phi_R} = \frac{v_{aa}(t)}{\phi_R(t)}$. According to the results of our dCAFBA model, we set constraints on the amino acids synthesis flux v_{aa} either by the amount of carbon uptake proteins ϕ_C or the amount of metabolic proteins ϕ_E with a simple piece-wise function shown in equation (3).
When $\mu_f \phi_{C2}(t) \leq \beta \phi_E(t)$, the differential equation for σ can be expressed as $\frac{d\sigma}{dt} = \sigma\left(\mu_f \chi_C - \sigma \chi_R\right)$. While, if $\mu_f \phi_{C2}(t) > \beta \phi_E(t)$, and considering that $\sigma \phi_R = \beta \phi_E = \beta(1 - \phi_R - \phi_C - \phi_Q)$, the differential equation for σ is written as $\frac{d\sigma}{dt} = \sigma\left(\beta\left(1 - \phi_Q\right) - (\beta \chi_C + \beta \chi_R + \sigma \chi_R)\right)$.

The constants appearing in the modified coarse-grained model β, h are determined by fitting to the growth rate at steady state. Moreover, the modified coarse-grained model requires growth-shift-specific parameters: the initial growth rate λ_i and the final growth rate λ_f. The initial growth rate λ_i and the final growth rate λ_f determine the parameters μ_i and μ_f appearing in the differential equation of σ.

The initial condition of the translational activity $\sigma(0)$ is determined by the type of shifts. For up-shift between co-utilized carbon sources, the initial condition $\sigma(0)$ equals the initial σ of the pre-shift, which is determined by the initial growth rate λ_i. For downshift between co-utilized carbon sources, the initial condition $\sigma(0)$ is determined by the carbon influx of the remaining substrate, which is calculated from the fraction of catabolic protein ϕ_C of the remaining substrate, the fraction of ribosomal protein ϕ_R and μ_f.

Results

Construction of the cross-regulated dynamical model

We developed a proteome-constrained metabolic model based on FCR and FBA to simulate the metabolic flux kinetics of *Escherichia coli* during carbon substrate shifts. Specifically, we used the *E. coli i*JR904 genome-scale metabolic model [14] as the basis for metabolic network, and introduced two coarse-grained constraints into the fluxes depending on their types of reactions (e.g. $v_C \leq \phi_C / w_C$ for the carbon uptake flux (v_C), and $\sum_i |v_i| \equiv v_E \leq \phi_E / w_E$ for the sum of all absolute metabolic fluxes (v_i), where w_C and w_E were the protein efficiencies of the carbon uptake and metabolic proteins) (Fig. 1a). Therefore, we can obtain the flux distribution v_i using the FBA by optimizing the biomass synthesis flux λ. We then calculated the total amino acid synthesis flux (v_{aa}) and considered it as the total protein synthesis flux (v_R) under flux balance assumption. The total protein synthesis flux was further partitioned into the synthesis fluxes for the carbon uptake and translation according to FCR [1] (See Methods).

Under steady-state growth condition, the partition of protein synthesis fluxes equals to the protein fractions of each sector. During the nutrient shift, we adopted a quasi-equilibrium assumption that the metabolic fluxes reached a balanced state at each time step, as they adapt much faster than protein synthesis and growth mediated dilution. We then obtained the dynamics of flux distribution by growth-rate optimization and extracted the instantaneous amino acid synthesis flux $v_{aa}(t)$ to further determine the protein reallocation kinetics (see Method). Therefore, this iterative framework enabled us to calculate the flux redistribution processes without the requirement of detailed knowledge about protein synthesis cost.

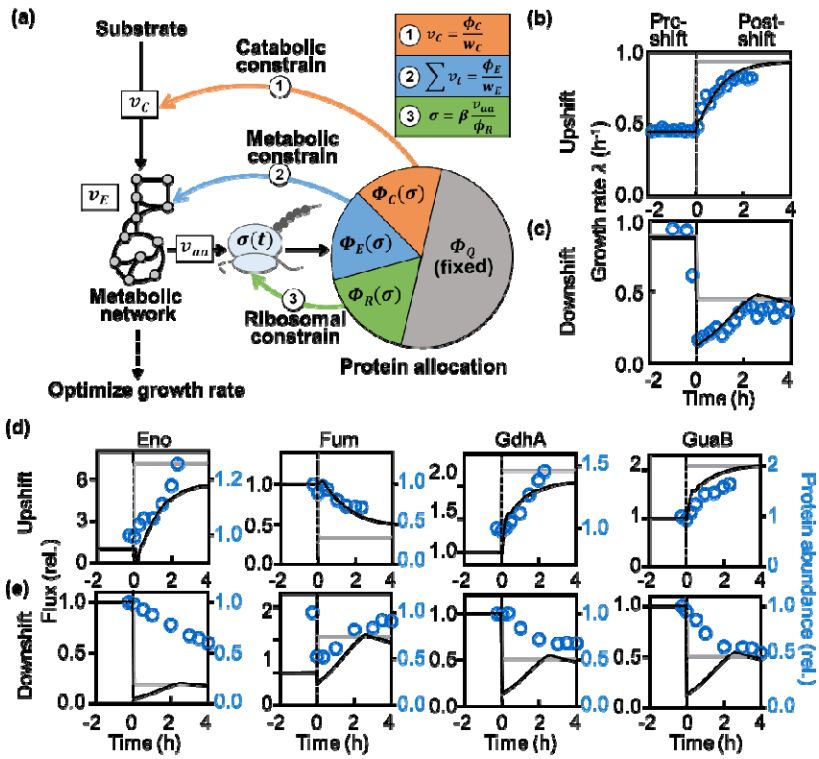

Figure 1. The dCAFBA model and its prediction during nutrient shift. (a) Schematic view of the model where the metabolic network (left) and the protein allocation are cross regulated. The carbon influx , constrained by protein partition of carbon uptake proteins , was converted to the synthesis flux of amino acids through the metabolic network which is constrain by the partition of enzyme proteins . It then determines the translational activity of ribosome which then regulates the protein synthesis flux that are allocated to coarse-grained protein sectors such as carbon intake protein (C), metabolic protein (E), ribosomal protein (R) proteins and houses-keeping proteins (Q, fixed) via the regulatory function and (see method). The dynamics of this model is assumed to be determined through while the fluxes in the metabolic network are in quassi-steady stat. The growth rate predicted by this model during upshift of gluconate added in medium of succinate (b black line) and during downshift of glucose depletion in medium of succinate and glucose (c black line) are compared to experimental data (blue circles, adopted from Erickson *et al* [1]) and the dFBA model (gray lines). The metabolic fluxes of typical reactions predicted by the dCAFBA model (black lines) during upshift (d) and downshift (e) are compared to the dynamics of related enzyme abundance (Eno for enolase; Fum, for fumarase; GdhA for glutamate dehydrogenase; GuaB for Inosine-5'-monophosphate dehydrogenase) obtained from Erickson *et al* [1]. (blue circles) and the prediction of the dFBA model (gray lines).

Kinetics of metabolic fluxes during nutrient shifts

The dCAFBA model can first recapture the transition kinetics of the growth rate

and protein allocations during nutrient shifts. The growth rate is defined as the flux of biomass synthesis, which depends on the availability and utilization of nutrients. When bacterial population experience a growth up-shift (e.g., gluconate added in medium of succinate in Fig. 1b d & Fig. S1a, and another example in Fig. S2), they gradually increase their growth rate to a new steady state in a few hours (about 2-3 generations). In contrast, when they experience a downshift (e.g., glucose depleted from medium of glucose and succinate in Fig. 1c e & Fig. S1b, and another example in Fig. S3), they quickly drop their growth rate and gradually recover to a lower post-shift state. During the transitions, cells change their gene expression patterns in response to changes in their growth conditions. Our dCAFBA model can also recapture the relaxation kinetics of protein allocations, specifically in carbon uptake (ϕ_C), translational (ϕ_R) and metabolic (ϕ_E) proteins (Fig. S1-S3). These transition kinetics are not easily captured by the classic dFBA model. dFBA model considers the nutrient availability as the limiting factor that governs the growth transition kinetics, thereby it only predicts immediately jumps of growth rate after nutrient shift (the grey lines in Fig. 1bc).

As an important improvement over the FCR model, our dCAFBA model can predict the transition kinetics of individual metabolic fluxes. Unlike the dFBA model, which suggests abrupt changes in the metabolic fluxes (the grey lines in Fig. 2a, b), our model revealed that the fluxes in the metabolic network would undergo a relaxation transition accompanied by the kinetics of enzymes during the carbon shifts. These detailed predictions of the transition kinetics of individual metabolic fluxes allows for closer examination of the transition kinetics of fluxes in the metabolic network, which further suggested that the transitions kinetics were not synchronized over the entire metabolic network.

To illustrate, we examined the reactions of the key pathways in the metabolic network, such as such as the reactions catalyzed by enolase (Eno) of the glycolysis pathway, fumarase (Fum) of the tricarboxylic acid cycle, glutamate dehydrogenase (GdhA) of the amino acid synthesis pathway, and insine-5'-monophosphate dehydrogenase (GuaB) of the nucleotide synthesis pathway. In both the upshift and downshift cases, the predicted transition time for these four reactions were consistent with the experimentally measured enzyme dynamics. In the upshift case (Fig. 2a), the predicted fluxes of Eno, GdhA and GuaB increased gradually after a minor perturbation induced by the carbon shifts, while the flux of Fum decreases gradually. The metabolic fluxes of these reactions changed synchronously with the experimentally measured enzyme abundance related to these reactions [1]. In the downshift case (Fig. 2b), the fluxes of all these reactions dropped abruptly after the carbon shift, and then increased smoothly until 2.4 hours and then decreased again to a steady value. The overall changes (from 0 to 4 hours) of the fluxes of the four reactions qualitatively coordinated with the changes of their related enzymes, where Eno, GdhA and GuaB decreased and Fum increased. However, the abrupt drop was not observed in the measured protein abundances, and the magnitude of the drop varied from one reaction to another. These complex behaviors highlight the need for a comprehensive understanding of the relationship between the dynamics of reaction

flux and enzyme expression levels.

Correlations between fluxes and enzymes

Based on the our model predictions, we further systematically examined the dynamic relationship between metabolic fluxes and their corresponding enzymes by calculating the temporal Pearson correlation coefficient (PCC) between each metabolic flux and its experimentally measured enzyme abundance after nutrient shift (Fig. S4). A positive PCC value indicates that the metabolic flux changes in the same direction as its reaction enzyme, while a negative PCC value indicates the opposite. Under different steady-state growth conditions, the majority of fluxes are positively correlated to the related enzyme abundance [15]. However, this relation is not always maintained especially during growth transition.

The temporal flux-enzyme correlation coefficients over the entire metabolic network revealed distinct patterns during the carbon up-shift (Fig. 2a) and downshift (Fig. 2b). In the up-shift case, most reactions (Fig. 2c) exhibited positive flux-enzyme correlations, except for a few reactions related to the TCA cycle and ATP production pathways. The well-coordinated dynamics between metabolic fluxes and their enzyme abundance indicates that during carbon upshift the carbon influx is surplus while enzymatic machinery limits the biomass synthesis fluxes, which requires both enzyme abundance and metabolic fluxes to gradually increase to adapt to their new steady state. In contrast, when cells underwent carbon downshift, most reaction fluxes became negatively correlated with the changes of enzyme abundance (Fig. 1d). The negative correlation was a result of a gradual increase of fluxes after an abrupt drop at nutrient shift, while the related enzyme concentrations gradually decrease to the post-shift lower concentration state (Fig. 1e). This indicates that after downshift the cells experienced a shortage of carbon influx which immediately results a drop in metabolic fluxes thereby the enzymes are surplus. These difference between up-and-down shifts further suggests that the efficiency of enzyme utilization would vary during the growth transitions.

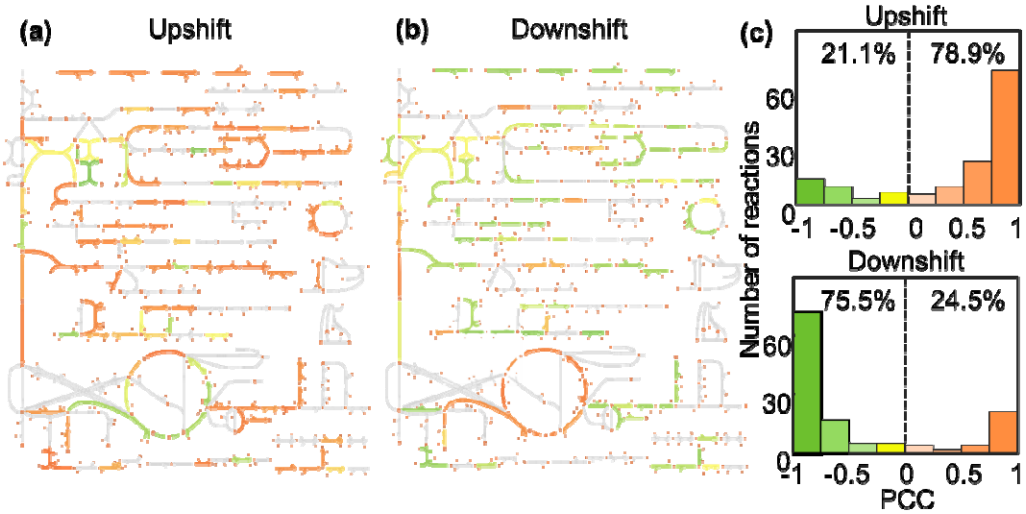

Figure 2. Illustration of the Pearson correlation coefficient (PCC) between dynamics

of the metabolic flux predicted by dCAFBA model and enzyme abundance during the up-shift case of gluconate addition in medium of succinate(a) and the downshift cases of glucose depletion from medium of glucose and succinate (b). The calculation of PCC was presented in method. Colors from Green to red represent the PCC value from -1 to 1. Gray color represents no data (nd). The hist gram of the PCC values showed that 78.9% of the reactions have positive correlated dynamics between fluxes and enzyme abundance in the upshift case while only 24.5% of them are positively correlated in the downshift cases (c).

Bottleneck reactions during nutrient shifts

With the detailed prediction of the flux kinetics from the dCAFBA model, we are able to quantify the relative efficiency of enzyme utilization during the growth shifts, to determine the bottleneck reactions during nutrient shifts. The relative efficiency of enzyme utilization is defined as the ratio of the actual flux to the maximum possible flux given the enzyme abundance (See Methods). During carbon up-shift, the reactions within glycolysis pathways exhibited a high level of efficiency in enzyme utilization, indicating that these enzymes play a limiting role in the kinetics of metabolic fluxes (Fig. S5). However, in the downshift case, most of them showed low efficiency during the first 2-3 generation-time. The relative efficiency of enzyme utilization increased over time, as the fluxes of reactions adjusted to the post-shift steady state after the initial drops while the enzyme abundance decreased gradually (Fig. 3a). These results suggested the metabolic machinery is not always optimal during nutrient downshift, due to the temporal constraints in nutrient influx.

We further examined the temporal dynamics of the key catalytic reactions and their corresponding enzymes during the carbon downshift of glucose depletion in medium of glucose and succinate. After glucose was rapidly depleted, the carbon influx switched to succinate uptake. The post-shift dynamics of succinate influx showed two phases (Fig. 3b): it first increased rapidly as the gene expression of succinate uptake proteins increased, and then decreased gradually to the new steady-state. The downstream reactions, such as succinate catalysis and malate synthesis (SUCC, SUCD and FUM), followed the same flux dynamic pattern. However, the fluxes of other reactions in the TCA cycle and biomass synthesis showed a different temporal pattern. They first dropped due to the lack of substrate supply, and then increased as the supply from upstream reactions gradually recovered. After 2-3 generation-time, those fluxes reached a peak and gradually adapted to the new steady state, as the limit factor shifted from succinate uptake to enzyme abundance in each reaction. This reduction in downstream fluxes fed back to upstream reactions, causing the decrease of succinate influx despite the continuous accumulation of its uptake proteins (Fig. S1). In summary, the flux pattern of the whole metabolic network during the downshift can be divided into several sectors (e.g. carbon intake and biosynthesis) depending on the limits of the reactions over time.

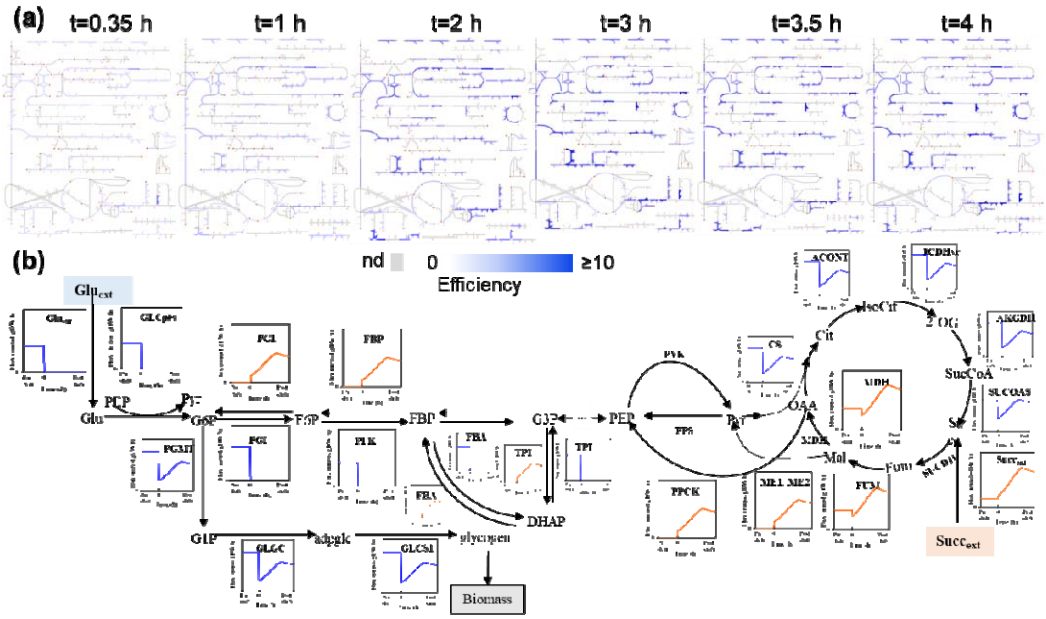

Figure 3. (a) Dynamics of the protein efficiencies during carbon downshift. The deepness of the blue color represents the efficiency of enzyme utilization in the central metabolic network (see methods for detailed calculation). (b) The dynamics of the selected reaction fluxes in the glycolysis, gluconeogenesis and TCA pathways were plot. The abbreviations for metabolites are as follows: Gluext, extracellular glucose; Glu, glucose; G1P, glucose-1-phosphate; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; FBP, fructose-1,6-biphosphate; G3P, glyceraldehyde-3-Phosphate; DHAP, dihydroxyacetone phosphate; PEP, phosphoenol pyruvate; Pyr, pyruvate; OAA, oxalacetic acid; Cit, citrate; IsoCit, threo-isocitrate; Succ, succinate; Succext, extracellular succinate; SucCoA, succinyl-CoA; Fum, fumarate; Mal, malate; 2-OG, 2-oxoglutarate. The abbreviations for are as follows: GLCpts, glucose transporter; PGI, glucose-6-phosphate isomerase; PFK, phosphofructokinase; FBA, fructose bisphosphate aldolase; TPI, triose phosphate isomerase; PPS, phosphoenolpyruvate synthase; PYK, pyruvate kinase; CS, citrate synthase; ACONT, aconitase; ICDHyr, isocitrate dehydrogenase; AKGDH, 2-Oxogluterate dehydrogenase; SUCOAS, succinyl CoA synthetase; SUCD1i, succinate dehydrogenase; FUM, fumarase; MDH, malate dehydrogenase; PPCK, phosphoenolpyruvate carboxykinase; ME1/ME2, PGMT, Phosphoglucomutase; GLGC, glucose-1-phosphate adenylyltransferase; GLCS1, glycogen synthase.

Limitation switch during carbon downshift

To address if the limitation switch depends on the specific reactions, we further proposed a coarse-grained picture of the metabolic network during the nutrient-shift (See Methods). The changes of fluxes in the metabolic network can be categorized into two sectors, one that followed the dynamics of carbon intake flux , which was limited by the dynamics of the proteome allocation to the carbon uptake proteins , and another that followed the overall metabolic flux defined by the sum of all the metabolic fluxes and was limited by the dynamics of the proteome

allocation to the metabolic enzymes . Under the flux balance assumption, the optimal flux distribution was constrained by the maximal fluxes of the two sectors that they could carry ( ).

In the upshift case, the metabolic flux was limited by the maximal enzymatic flux , as the influx of high-quality carbon source increased the supply of substrates for central metabolism, while the downstream metabolic machinery was insufficient. Therefore, the cell increased the expression of metabolic enzymes , while the carbon uptake sector decreases. This resulted the decrease of and the increase of , until a new balanced state was reached that allowed an optimal growth.

During carbon downshift, the cell experienced a bottleneck switch in the metabolic flux distribution (Fig. 4a & Fig. S7). At first, the glucose depletion caused an abrupt drop in both the fluxes and the maximal carbon influx, due to the limited pre-expressed uptake proteins for the new carbon source. Then, the carbon uptake protein sector gradually recovered, along with the increase of flux . After 2-3 generation time, the carbon influx was no longer the limiting factor, while the enzyme flux reached its maximum flux as the metabolic sector decreased due to the growth-mediated dilution. As a result, the maximum growth rate occurred at the time of limitation switch from carbon-uptake limitation to enzymatic limitation.

Further analysis suggested that the time of limitation switch was determined by the protein efficiencies ( ). Lower uptake protein efficiency required less protein to carry sufficient uptake flux to supply the metabolic system, leading to an earlier limitation switch. In contrast, higher efficiency of metabolic enzymes required more enzymes to carry the same metabolic fluxes in the pre-shift condition, resulting in a longer time for to decrease to match the recovery of carbon influx (Fig. S8).

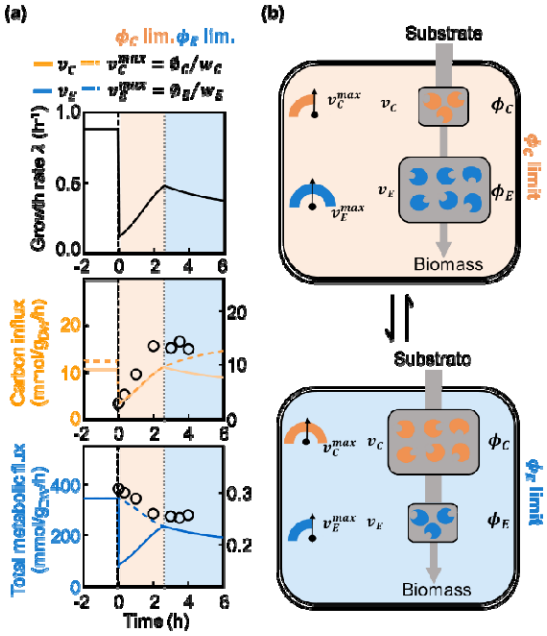

Figure 4. limitation switch during carbon downshift upon depletion of glucose at t=0

in medium of glucose and succinate. (a) The post-shift dynamics of the growth rate λ predicted by the dCAFBA model (upper panel) is divided into the carbon uptake protein limited region (ϕ_C limit, colored in orange) and the enzyme limited region (ϕ_E limit, colored in blue) at the peak of growth rate. These two regions have distinct dynamics relation between the actual carbon influx (solid orange line, v_C) v.s. the maximal carbon influx (dashed orange line, v_C^{max}) (middle panel), and the dynamic relation between the actual total metabolic flux (solid blue line, v_E) and the maximum of metabolic fluxes (dashed blue line, v_E^{max}) (lower panel). In this prediction, the following parameters were used: w_{c1}= 1.96 x 10^{-2} gh/mmol and w_E= 8.3 x 10^{-4} gh/mmol. (b) The $\emptyset_C$ limit and $\emptyset_E$ limit represent weather sequenced fluxes of the coarse-grained reactions v_C and v_E is limited by the maximal flux v_C^{max} or v_E^{max} that can be carried by $\emptyset_C$ and $\emptyset_E$.

Discussion

Bacterial cells respond to environmental perturbations by globally regulating their growth rate, gene expression and metabolism. Recent quantitative studies of bacterial physiology have provided a coarse-grained picture that recapture the kinetics of proteome reallocation during nutrient shifts, yet it is still challenging to investigate the dynamics of metabolic network. Here, we combined flux balance analysis with the global regulation of proteome allocation to quantitatively predict flux redistribution processes. This method allowed comparison between fluxes and enzyme abundance of each reaction to determine the bottleneck of metabolic network during nutrient shift. We further adopted a coarse-grained approach to describe the proteome-remodeling kinetics and optimize the growth-rate to determine the flux distribution, which does not require the detailed parameters of enzymes [16, 17]. This enabled us to predict the redistribution kinetics of metabolic fluxes by only two free parameters about the efficiency of uptake proteins and metabolic enzymes (w_C and w_E).

We identified the bottleneck of the system by comparing the dynamics of predicted flux redistribution and measured proteomic relocation during nutrient shifts. In the up-shift cases, we showed that the accumulation of enzyme expression limited the dynamics of the flux redistributions, while the limiting factor switched from carbon uptake proteins to enzyme proteins during downshift. A further simplified model by coarse graining the total of metabolic fluxes to a single flux of v_E (see Method) could also recapture the limitation switch as well as the overshoot of growth rate that are widely observed (Fig. S9) in the downshift condition (Fig. S10), suggesting the switch of metabolic bottleneck is a consequence of the conservative global regulatory control.

Quantitative understanding of the cross-regulatory kinetics between proteome reallocation and flux redistribution is a prerequisite for predicting cellular dynamics, such as the protein expression dynamics that are relevant to stress response [18] and infectious diseases [19]. Our study also provided a framework, which could be helpful to design genetic circuits that dynamically control the key enzyme expression to optimize the production of metabolites [20-24].

Acknowledgement

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Author contributions

X.F. & Y.B. conceived the project. H.Y. performed all the numerical simulations All the authors analyzed the results and wrote and revised the paper.

Data availability

Data supporting the findings of this study are available within the main text and Supplementary Information. The Custom-made simulation code is available at github: https://github.com/BaiYangBqdq/dCAFBA

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Supplemental tables

Table S1: Description of variables

symbol	description
σ	translational activity
χ_{Rb}	regulation function of ribosomal protein allocation
χ_C	regulation function of catabolic protein allocation
ϕ_{Rb}	fraction of ribosomal protein over total protein
ϕ_C	fraction of catabolic protein over total protein
ν_{aa}	protein synthesis flux
ν_C	carbon uptake rate
λ	cell growth rate

Table S2: Description of parameters. Default values were used unless otherwise stated.

symbol	description	value	source
γ	Inverse slope of growth laws	13.52 h^{-1}	[1]
λ_C	Strain specific constant of growth laws	1.17 h^{-1}	[1]
$\phi_{Rb,0}$	A growth rate-independent offset of R sector	0.049	[1]
ϕ_Q	Fraction of house-keeping protein	0.45	[11]
$\phi_{C,max}$	Maximum of proteome attainable to C, E and R sectors	0.44	[12]
β	conversion factor between moles and gram	0.1968 g/mmol	
$h_{1,2}$	carbon-specific allocation		
ω_E	enzyme cost per unit metabolic influx	8.3×10^{-4} gh/mmol	[13]
ω_C	enzyme cost per unit carbon influx	See Table S3	

Table S3: The constants of the substrates used in the simulation of dCAFBA.

Substrate1	Substrate2	λ_1 (h^{-1})	λ_2 (h^{-1})	λ_{12} (h^{-1})	ω_{C1}	ω_{C2}	h_1	h_2
succinate	glucose	0.45	0.91	0.92	1.96×10^{-2}	4.3×10^{-3}	0.62	1
	gluconate	0.45	0.91	0.94	1.96×10^{-2}	2.29×10^{-3}	0.63	1
pyruvate	gluconate	0.60	0.91	0.97	5.17×10^{-3}	2.39×10^{-3}	0.77	1

Supplemental figures

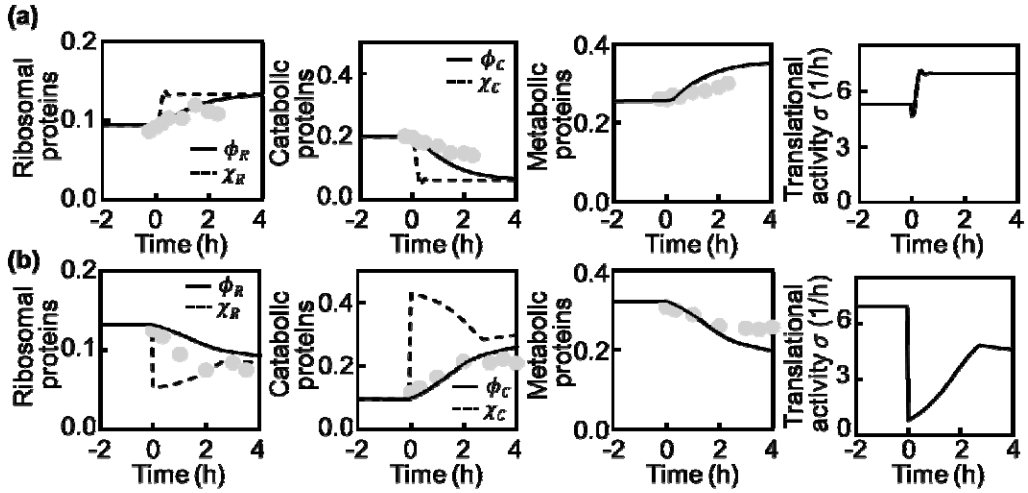

Figure S1. Regulatory functions and protein fractions of catabolic proteins, ribosomes, metabolic proteins and translational activity during the upshift (a) and downshift (b) of Figure 1. Dots, experimental data obtained from Erickson et al., 2017. Black lines, simulations of dCAFBA.

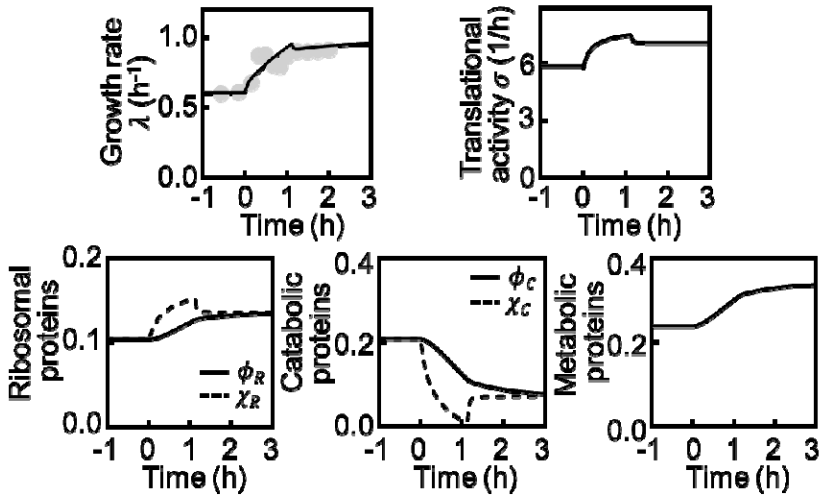

Figure S2. Growth rate , translational activity ,and regulatory functions and protein fractions of catabolic proteins, ribosomes as well as metabolic proteins during the upshift of gluconate addition in medium of pyruvate. Dots, experimental data obtained from Erickson et al., 2017, if available. Black lines, simulations of dCAFBA.

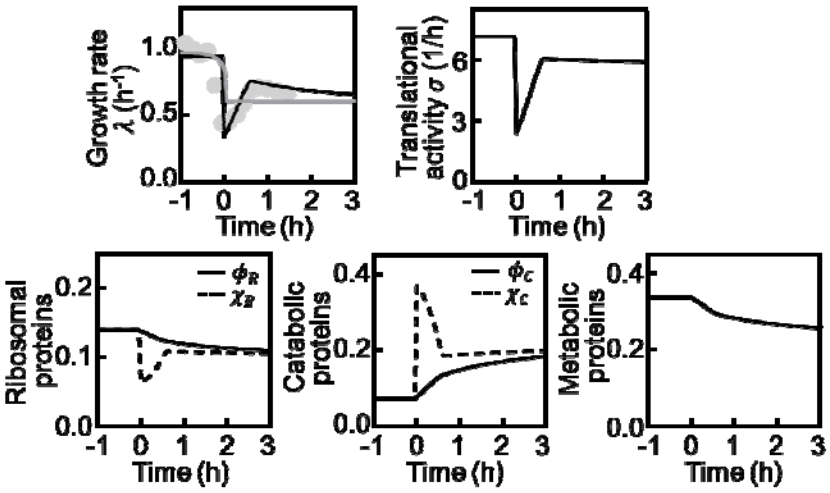

Figure S3. Growth rate , translational activity ,and regulatory functions and protein fractions of catabolic proteins, ribosomes as well as metabolic proteins during the downshift of gluconate depletion in medium of gluconate and pyruvate. Dots, experimental data obtained from Erickson et al., 2017, if available. Black lines, simulations of dCAFBA.

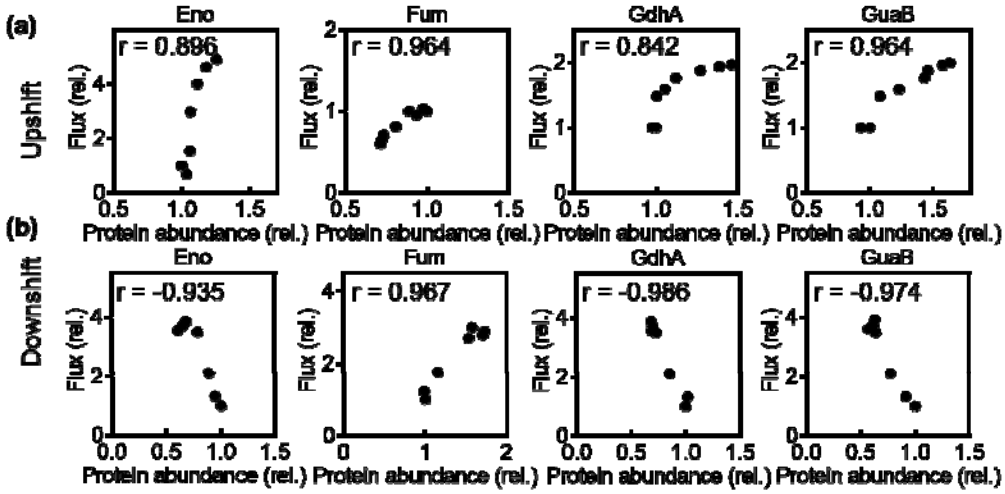

Figure S4. Correlations between predicted metabolic fluxes and experimentally measured enzyme abundances of selected reactions during up-shift (a) and downshift (b). Each dot represents the flux and enzyme abundance at a time point. (Eno for enolase; Fum, for fumarase; GdhA for glutamate dehydrogenase; GuaB for Inosine-5'-monophosphate dehydrogenase)

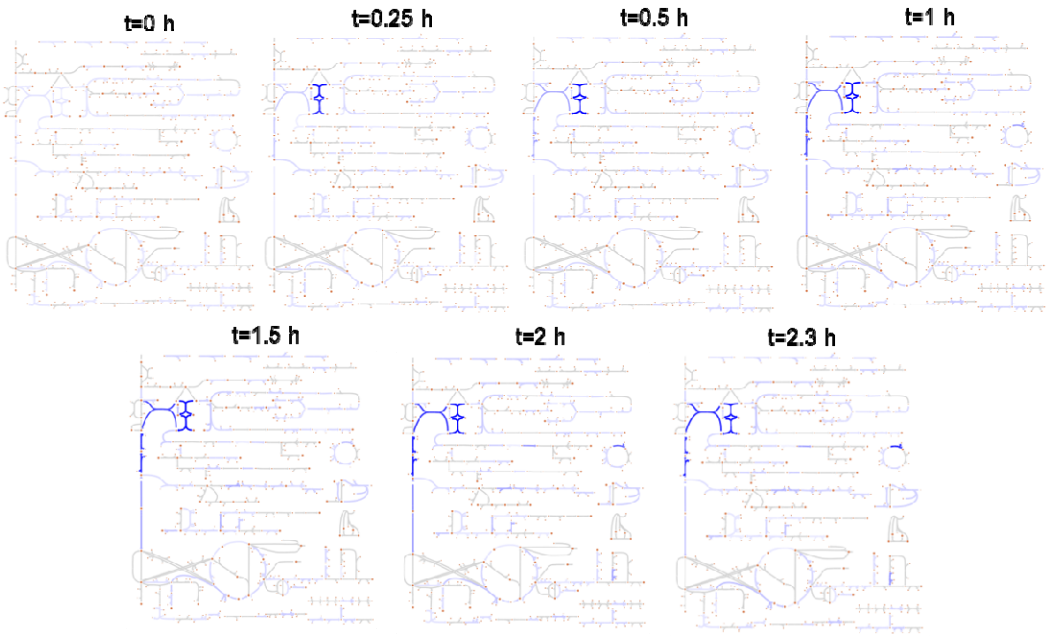

Figure S5. Dynamics of the protein efficiencies during carbon upshift. The deepness of the blue color represents the value of protein efficiency calculated by where is the predicted flux of a given reaction in the metabolic network and is the protein partition of the enzyme of the related flux measured by Erickson *et al.*.

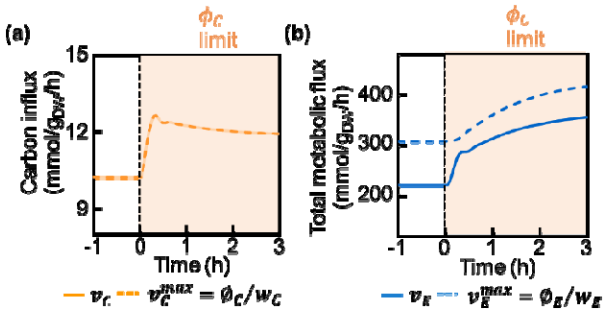

Figure S6. In the upshift case, comparisons between carbon influx (solid orange line,) v.s. the maximal flux the carbon uptake protein can carry (dashed orange line,) and total metabolic flux (solid blue line,) v.s. the maximal metabolic flux the enzyme proteins can carry (dashed blue line,) showed that the post shift period dynamics was limited by carbon intake proteins.

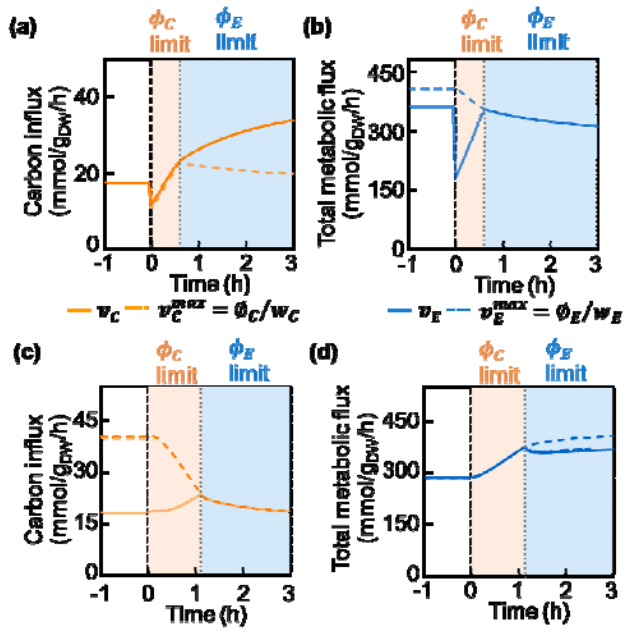

Figure S7. Comparisons between the actual flux (solid lines) and the maximal flux determined by enzyme abundance (dashed lines), for carbon intake flux (orange lines) and the total metabolic flux (blue lines), in the case of downshift upon depletion of gluconate in medium of gluconate and pyruvate (a, b) and upshift upon addition of gluconate in medium of pyruvate (c, d). Orange and blue region represent the period was limited by the carbon intake proteins (limit) or by the enzyme proteins (limit).

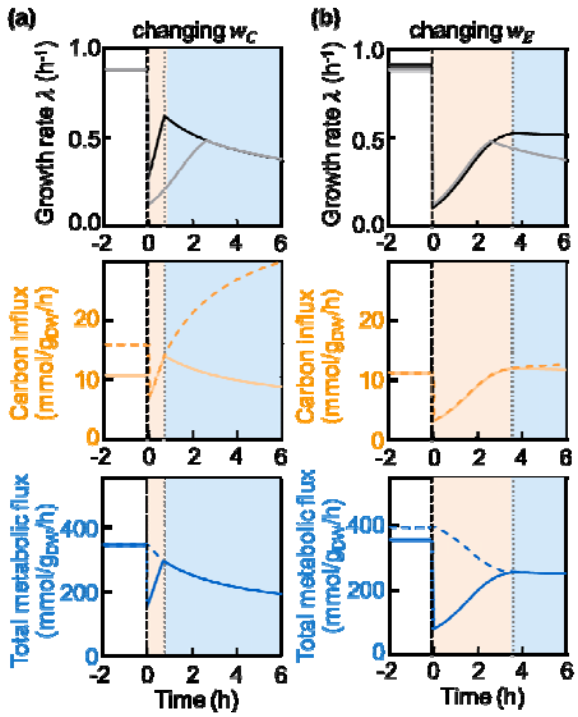

Figure S8. Effect of key parameters (a) and (b) in the downshift case upon depletion of glucose in medium of glucose and succinate glucose. The gray lines used the value of and as in figure 4. (a) Decreasing , , decreased the period of limit. While decreasing , , increased the period of limit.

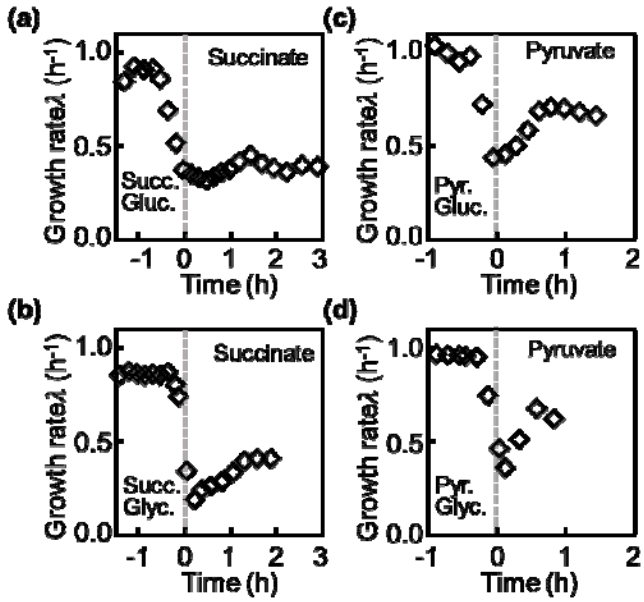

Figure S9. Overshoot of growth rate reported in other downshift co-utilized carbon substrates. E.coli grown on succinate (succ.) (a, b) or pyruvate (pyr.) (c, d). Combined with either gluconate (gluc.) (a, c) or glycerol (glyc.) (b, d) . At around t=0, gluconate or glycerol were depleted. Data was summarized from Erickson *et al.* [1].

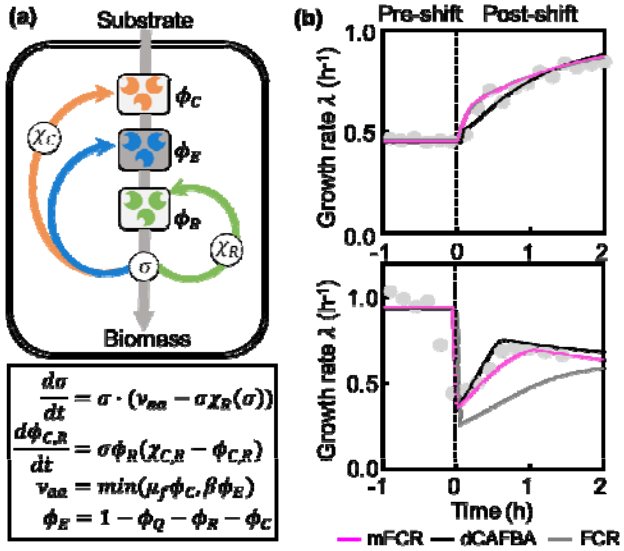

Figure S10. Model of modified FCR. (a) Schematic view of the simplified model integrating ϕ_E which is a modification of the FCR model. The Carbon substrates are imported by carbon uptake proteins ($\emptyset_C$), which are then converted into central precursors by metabolic enzymes ($\emptyset_E$). These produced precursors are consumed by the ribosome ($\emptyset_R$) for protein synthesis which determines the translational activity σ. Finally, the protein synthesis flux is allocated to coarse-grained sectors of catabolic (C), ribosomal (R) proteins via the regulatory functions χ_C and χ_R, respectively. (b) Comparisons of predicted growth rate $\lambda(t)$ by modified FCR, dCAFBA and FCR models in up-(left) and downshift (right). The magenta, black, grey curves are predictions of the mFCR, FCR and dCAFBA models, respectively. Grey dots indicate experimental data obtained from Erickson et al., 2017. The upshift case is gluconate addition in medium of succinate and the downshift case is gluconate depletion in medium of pyruvate and gluconate.