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The linkage between nutrient supply, intracellular enzyme abundances and bacterial growth: New evidences from the central carbon metabolism of *Corynebacterium glutamicum*

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ABSTRACT

Corynebacterium glutamicum serves as important production host for small molecular compounds that are derived from precursor molecules of the central carbon metabolism. It is therefore a well-studied model organism of industrial biotechnology. However, a deeper understanding of the regulatory principles underlying the synthesis of central metabolic enzymes under different environmental conditions as well as its impact on cell growth is still missing. We studied enzyme abundances in *C. glutamicum* in response to growth on: (i) one limiting carbon source by sampling chemostat and fed-batch cultivations and (ii) changing carbon sources provided in excess by sampling batch cultivations. The targeted quantification of 20 central metabolic enzymes by isotope dilution mass spectrometry revealed that cells maintain stable enzyme concentrations when grown on D-glucose as single carbon and energy source and, most importantly, independent of its availability. By contrast, switching from D-glucose to D-fructose, D-mannose, D-arabitol, acetate, L-lactate or L-glutamate results in highly specific enzyme regulation patterns that can partly be explained by the activity of known transcriptional regulators. Based on these experimental results we propose a simple framework for modeling cell population growth as a nested function of nutrient supply and intracellular enzyme abundances. In summary, our study extends the basis for the formulation of predictive mechanistic models of bacterial growth, applicable in industrial bioprocess development.

1. Introduction

A number of studies have been conducted to elucidate the interconnections between nutrient supply, enzyme synthesis, metabolic fluxes and growth of a bacterial cell population (Fig. 1). Based on extensive, quantitative omics datasets from several model organisms, mainly two different working hypotheses on the underlying regulatory mechanism have been formulated.

For example, it was shown that *Escherichia coli* actively regulates enzyme levels to maintain a stable metabolic state in response to changes in growth rate under defined D-glucose conditions (Ishii et al., 2007). Moreover, intracellular levels of metabolites did not change significantly with growth rate. Recently, the proteomic responses of *E. coli* to different growth pertubations were studied by modulating carbon and nitrogen availability as well as translational activity (Hui et al., 2015). The analysis of the proteomic data revealed six groups of enzymes with distinct modes of gene expression in response to the applied limitations. Again, the concentrations of these so called 'coarse-grained enzymes' correlated linearly with the growth rate. Noteworthy, the authors quantified native proteins by applying isotope dilution mass spectrometry coupled to high performance liquid chromatography (IDMS-LC–MS/MS). No differentiation between the total amount of an enzyme and its active fraction was made. Thus, post-translational enzyme modifications (PTMs such as phosphorylation or acetylation) were neglected. Proteome reallocation in *E. coli* as a function of growth rate was justified by a potential minimization of protein synthesis costs among different protein groups (Peebo et al., 2015).

From a stringent mechanistic viewpoint this would mean that the adaptation of the cells metabolic fluxes in response to nutrient availability (resulting in a specific growth rate) is solely driven by the distinct fine-tuning of (active) enzyme concentrations, while corresponding metabolite pools are not affected. In other words, flux regulation at the metabolite level, which is based on local substrate concentrations, metabolite–protein interactions (including gene

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Nomenclature

$\dot{V}_{\{in,out\}}$	volume flow in and out of the bioreactor ($l_{reactor}\ h^{-1}$)	D	dilution rate (h^{-1})
$\hat{V}_{cell}$	specific cell volume ($l_{cell}\ g_{biomass}^{-1}$) or ($l_{cell}\ l_{biovolume}^{-1}$)	h_{reg}	regulation function (–)
μ	specific growth rate (h^{-1})	m_S	amount of substrate ($mmol_{substrate}$)
c_E	concentration of enzyme ($mmol_{enzyme}\ l_{cell}^{-1}$)	m_X	biomass ($g_{biomass}$) or biovolume ($l_{biovolume}$)
c_R	concentration of regulator ($mmol_{regulator}\ l_{cell}^{-1}$)	V_R	volume of bioreactor ($l_{reactor}$)
c_S	concentration of substrate ($mmol_{substrate}\ l_{reactor}^{-1}$)	v_{deg}	rate of enzyme degradation ($mmol_{enzyme}\ l_{cell}^{-1}\ h^{-1}$)
c_X	concentration of biomass ($g_{biomass}\ l_{reactor}^{-1}$) or biovolume ($l_{biovolume}\ l_{reactor}^{-1}$)	v_{syn}	rate of enzyme synthesis ($mmol_{enzyme}\ l_{cell}^{-1}\ h^{-1}$)
		$v_{S,upt}$	specific substrate uptake rate ($mmol_{substrate}\ l_{cell}^{-1}\ h^{-1}$)

expression regulation, PTMs and allosteric regulation of enzymatic activity), can be neglected. Motivated by this viewpoint (denoted as working hypothesis H_0 in the following), an extension of the classical flux balance analysis approach termed 'Constrained Allocation Flux Balance Analysis' was proposed and simulation results show remarkable consistence with experimental data (Mori et al., 2016).

However, detailed studies on the role of transcription in regulating metabolic fluxes came to somehow contrary results across different microbes (Kochanowski et al., 2013). Significant mismatches between the changes in enzyme concentrations (inferred from transcript measurements) and metabolic fluxes were reported for the reactions of central carbon metabolism in *Bacillus subtilis* (Chubukov et al., 2013). This nonlinear behavior was explained by the fact that most central metabolic enzymes are available in excess and changes in corresponding fluxes are not primarily realized through transcriptional regulation, i.e. by modification of enzyme concentrations. In addition, local substrate availability was also insufficient to explain the observed changes in metabolic fluxes across conditions. Hence, the authors argued that both PTMs and allosteric regulation of enzyme activity are most likely the predominant control mechanisms of metabolic fluxes in bacteria.

Although substantial progress has been made in unraveling PTMs in bacteria (Dworkin, 2015; Carabetta and Cristea, 2017), it is still unclear to which extent such modifications affect metabolic fluxes and, moreover, cell growth. Interestingly, a recent large-scale analysis of PTMs in *E. coli* under D-glucose limiting conditions showed that phosphorylation and acetylation are relatively scarce. Even on very abundant proteins (including some central metabolic enzymes) these modifications tend to occur at low frequencies (Brown and Eikmanns, 2017). For the eukaryotic model organism *Saccharomyces cerevisiae*, which is known to be strongly regulated by a diverse set of PTMs, Hacket and coworkers could recently show that the concentrations of local substrates and allosteric metabolite effectors have a much higher impact on metabolic fluxes than the concentrations of the respective enzyme catalyst's (Hackett et al., 2016). This finding (denoted as working hypothesis H_1 in the following) was explained by the reverse argument from above, i.e. for the cell it can be 'cheaper' to make excess enzyme than building a complex machinery for a fine-tuned control of individual enzyme concentrations.

Following quantitative omics studies we also observed vast overexpression of specific central metabolic genes in *Corynebacterium glutamicum*. For example, the expression of the citrate synthase gene *gltA* had to be reduced by more than 90% (following promoter engineering) to cause a significant change in metabolic flux over the TCA-cycle and, thus, a measurable decrease in the specific growth rate (van Ooyen et al., 2012). Moreover, we found much higher concentrations for the enzymes pyruvate kinase and phosphoenolpyruvate carboxylase than would be required to meet stable metabolic flux demands on defined D-glucose media (Voges et al., 2015).

Within this study, we conducted a series of quantitative proteomic experiments with wild-type *C. glutamicum* to learn more about the linkage between nutrient supply, intracellular enzyme abundances and bacterial growth in this model host. In particular, we wanted to see whether our data can support one of the two diverging working hypotheses introduced above.

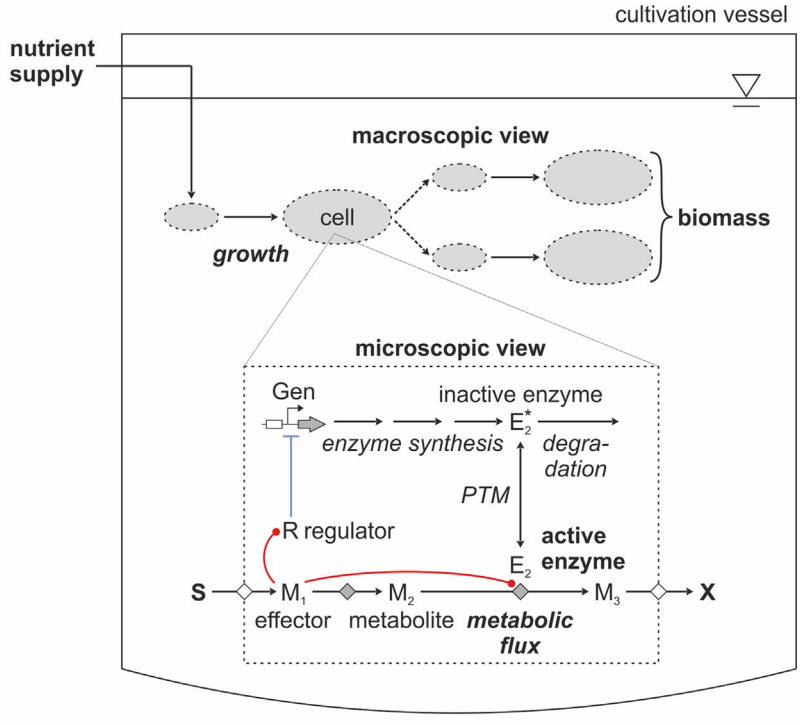

Fig. 1. Macroscopic and microscopic view of cell population growth in a defined environment such as a cultivation vessel. Cells respond to changes in extracellular nutrient supply via different regulatory mechanism exerted at the level of enzyme synthesis and activity. Many of these mechanisms are based on metabolite–protein interactions, however, the knowledge on which effectors and regulatory proteins/enzymes interact with each other is still very limited. Finally, metabolic fluxes are the ultimate manifestation of all regulatory events controlling cellular metabolism and, hence, largely determine the specific growth rate of a cell population.