Survival of the quickest – Identification of time-optimal regulatory strategies of metabolism in *Escherichia coli*

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1 Introduction

While the ability of microorganisms to adapt to specific environmental conditions has been studied with much detail in the past, specific mechanisms to cope with changing environments only recently have received increasing attention (Buescher et al., 2012; Schuetz et al., 2012; Wessely et al., 2011). In two recent studies, work from my group has focused on the identification of specific regulatory strategies that allow microorganisms to reduce their response time when facing a change in nutritional conditions (Wessely et al., 2011; Bartl et al., 2013). Being able to quickly adjust fluxes through metabolic pathways is of central importance in order to reduce lag times in case of deprivation of an essential nutritional constituent or during major growth-transitions such as the exit from stationary phase (Geisel et al., 2011; Geisel, 2011; Schuetz et al., 2012).

2 Results

A minimal regulatory strategy

In the first study (Wessely et al., 2011), we investigated the coexpression of enzymes belonging to the same pathway on the level of the entire metabolism in *Escherichia coli*. While we found a large number of subsystems of metabolism in which most pathways showed a strong coexpression we also identified several subsystems in which pathways appeared not to be co-regulated. In order to understand how metabolic pathways could be controlled without a consistent regulation across all enzymes, we used dynamic optimization to identify a regulatory strategy that allows to precisely control the flux through a metabolic pathway with a minimal amount of transcriptional regulatory interactions. To this end, we studied a prototypical example pathway comprising five reactions governed by irreversible Michaelis-Menten-Kinetics that convert a buffered substrate into a product that is drained with varying dilution rates in the course of the simulation. Using dynamic optimization we searched for a time-course of the enzymes that maintains the concentration of the product in a narrow predefined range while minimizing a weighted sum of initial enzyme concentrations and the amount of regulation. The amount of regulation was measured as the deviation of enzyme concentrations from their initial value.

The results of the optimization showed that, in case of a low weight of initial enzyme concentrations, it is optimal to transcriptionally control a metabolic pathway only in the initial and terminal step of the pathway. We call this program of regulation "sparse transcriptional regulation". With an increasing weight of initial enzyme concentrations, we observed a shift from sparse transcriptional regulation to the regulation of all enzymes within the pathway, which we called

"pervasive transcriptional regulation". To test the predictions of the optimization approach, we analyzed the pathway-position dependent occurrence of transcriptional regulatory interactions. We could confirm that within the subsystems of metabolism in which we didn't find a co-expression of all enzymes within a pathway, there was a significant increase in the frequency of transcriptional regulatory interactions at the beginning and end of pathways. Moreover, we observed a sparse transcriptional regulation in particular for pathways consisting of lowly abundant enzymes.

We explained the optimality of these different programs of pathway activation by a trade-off between response time and protein cost. A sparse transcriptional regulation of a pathway allows the organism to quickly adjust the flux through a pathway since only the concentration of key enzymes needs to be adjusted. However, it entails a high protein cost since enzymes at intermediate positions within these pathways are expressed constitutively. In contrast, a pervasive transcriptional regulation entails a slow response time since the concentrations of all enzymes need to be adjusted but proteins are only produced if they are needed. Thus, depending on the requirement for a rapid response or a minimization of protein cost, either a sparse or pervasive transcriptional control is optimal. In consequence, it is optimal to sparsely regulate metabolic pathways with a low protein cost (e.g. in co-factor synthesis) while it is optimal to pervasively control metabolic pathways with a high protein cost (e.g. in amino acid biosynthesis). A notable exception is the pentose phosphate pathway that has a high protein cost but shows a pattern of sparse transcriptional regulation. This pathway produces reduction equivalents in the form of NADPH that are required by a large number of other pathways. In consequence, being able to quickly adjust the flux through the pentose phosphate pathway appears to outweigh the high protein cost.

Optimal programs of pathway activation

In a second study, we analyzed how a pathway is optimally activated in the light of limitations of the cellular protein synthesis capacity (Bartl et al., 2013). To this end, we studied a simple metabolic pathway consisting of four enzymatic steps that convert a buffered substrate into a product that is limiting for growth. Starting from an initially inactive pathway, we investigated how the individual enzymes are optimally activated given two constraints on enzyme synthesis to minimize the time until a resumption of growth. These two constraints are a maximal synthesis rate of the individual enzymes and the free protein synthesis capacity that puts an upper limit on the total amount of enzymes that can be synthesized at the same time. While the former constraint is strongly influenced by the amount of protein that is required, the free protein synthesis capacity is influenced by the number of free ribosomes. With an increasing enzyme synthesis rate relative to free protein synthesis capacity, we found a shift from the optimality of a simultaneous activation of all enzymes over a sequential activation of groups of enzymes to a sequential activation of individual enzymes within the pathway. Thus, we found, in contrast to previous works, that a sequential activation of enzymes is only optimal in the case of high protein costs. Moreover, we found that large differences in the abundance of proteins within a pathway lead to the optimality of a accelerated activation of highly abundant enzymes while the induction of lowly abundant enzymes are delayed.

In order to validate the predictions by the optimization approach, we studied the operonic structure of a large number of metabolic pathways across 550 prokaryotes from the MicroCyc collection of metabolic pathways (Vallenet et al., 2013). The operonic organization of the genes of a metabolic pathway allowed us to deduce the particular regulatory program that is used for its control since enzymes within an operon are activated almost concomitantly. Thus, in accordance with our predictions we expected operon sizes to decrease with increasing protein abundance and to increase with increasing protein synthesis capacity. Moreover, we expected highly abundant enzymes within a pathway to be more often coexpressed with earlier enzymes of the same path-

way while we expect lowly abundant enzymes to be more often coexpressed with later steps of a metabolic pathway. Of the 99 pathways with sufficient data of all organisms across the MicroCyc collection, we could confirm for 21 that the dependence between protein abundance and protein synthesis capacity followed our predictions. Notably we found only two cases in which we found significant correlations opposite to our predictions. Moreover, we could confirm that highly abundant proteins are more often coexpressed with earlier enzymes of a pathway while lowly abundant enzymes tend to be coexpressed with later steps.

3 Discussion

These two studies show that with an increasing abundance of proteins within a pathway, the complexity of the transcriptional regulatory programs used for its control drastically increases. For pathways with lowly abundant proteins a focused regulation of key steps is optimal, while a high protein cost entails the optimality of distinct activation times of individual enzymes. Apart from the identification of optimal programs of pathway control, results from these studies are also of importance to identify pathways that are differentially expressed between conditions and for our understanding of the evolution of operons. Relating to the identification of differentially expressed genes, our results imply that, depending on the transcriptional regulatory program used to control a metabolic pathway, a differential activity of a metabolic pathway might only be obvious from changes in the first and/or terminal step. Concerning the evolution of operons, the abundance of enzymes within a pathway as well as the the capacity of the protein synthetic machinery can change in the evolutionary history of an organism. Thus, also the optimal operonic organization of the enzymes of a pathway changes which could partially explain the high evolutionary plasticity of operons even between closely related species (Price et al., 2006).

References

M. Bartl, M. Kötzing, S. Schuster, P. Li, and C. Kaleta. Dynamic optimization identifies optimal programs for pathway regulation in prokaryotes. Nat Comm, 2013. revised version submitted.

J. M. Buescher, W. Liebermeister, M. Jules, M. Uhr, J. Muntel, E. Botella, B. Hessling, R. J. Kleijn, L. L. Chat, F. Lecointe, U. Mäder, P. Nicolas, S. Piersma, F. Rügheimer, D. Becher, P. Bessieres, E. Bidnenko, E. L. Denham, E. Dervyn, K. M. Devine, G. Doherty, S. Drulhe, L. Felicori, M. J. Fogg, A. Goelzer, A. Hansen, C. R. Harwood, M. Hecker, S. Hubner, C. Hultschig, H. Jarmer, E. Klipp, A. Leduc, P. Lewis, F. Molina, P. Noirot, S. Peres, N. Pigeonneau, S. Pohl, S. Rasmussen, B. Rinn, M. Schaffer, J. Schnidder, B. Schwikowski, J. M. V. Dijl, P. Veiga, S. Walsh, A. J. Wilkinson, J. Stelling, S. Aymerich, and U. Sauer. Global network reorganization during dynamic adaptations of *Bacillus subtilis* metabolism. Science, 335(6072):1099–1103, Mar 2012.

N. Geisel. Constitutive versus responsive gene expression strategies for growth in changing environments. PLoS One, 6(11):e27033, 2011.

N. Geisel, J. M. G. Vilar, and J. M. Rubi. Optimal resting-growth strategies of microbial populations in fluctuating environments. PLoS One, 6(4):e18622, 2011.

M. N. Price, A. P. Arkin, and E. J. Alm. The life-cycle of operons. PLoS Genet, 2(6):e96, Jun 2006.

R. Schuetz, N. Zamboni, M. Zampieri, M. Heinemann, and U. Sauer. Multidimensional optimality of microbial metabolism. Science, 336(6081):601–604, May 2012.

D. Vallenet, E. Belda, A. Calteau, S. Cruveiller, S. Engelen, A. Lajus, F. L. Fèvre, C. Longin, D. Mornico, D. Roche, Z. Rouy, G. Salvignol, C. Scarpelli, A. A. T. Smith, M. Weiman, and C. Médigue. Microscope–an integrated microbial resource for the curation and comparative analysis of genomic and metabolic data. Nucleic Acids Res, 41(Database issue):D636–D647, Jan 2013.

F. Wessely, M. Bartl, R. Guthke, P. Li, S. Schuster, and C. Kaleta. Optimal regulatory strategies for metabolic pathways in *Escherichia coli* depending on protein costs. Mol Syst Biol, 7:515, 2011.