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Production of chemicals using dynamic control of metabolic fluxes

Peng Xu

Engineered microbial cell factories are constantly experiencing metabolic imbalance due to nutrients depletion, metabolites buildup, evolutionary pressure or genetic instability. It is important to equip the engineered cell factory with sensor-regulator system to enable cell adjust metabolism and respond to the changing environment. Dynamically allocating cellular resources and optimally controlling pathway expression have proved as promising strategies to manage the tradeoff between cell growth and product formation as well as improve the cost-competitiveness of industrial fermentation. With metabolite-responsive transcriptional factors as basic tools, metabolic engineers are well positioned to engineer robust cell factories that achieve self-adaptation or autonomous control for both biotechnological and biomedical applications. In this review, we present promising dynamic control strategies that have been successfully applied to pathway optimization and chemical manufacturing.

Address
Department of Chemical, Biochemical and Environmental Engineering, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250, USA

Corresponding author: Xu, Peng (pengxu@umbc.edu)

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Introduction

Metabolic engineering is an enabling technology to construct efficient microbial cell factories and a major driver for next-generation bio-economy [1]. The three pillars of metabolic engineering are the titer, yield and rate (TYR), which have become the benchmarks to evaluate the cost-competitiveness of engineered cell factory. By engineering heterologous pathways or optimizing endogenous metabolism, metabolic engineers have now been able to manufacture a large portfolio of commodity chemicals [2,3], novel materials [4], sustainable fuels [5,6••] and pharmaceuticals [7,8] from renewable feedstocks.

To construct efficient microbial cell factory with improved TYR metrics, most of metabolic engineering efforts has been focused on selecting efficient biocatalysts and examining precursor/cofactor requirements. Reverse metabolic engineering by investigating substrate–product stoichiometric relationship is perhaps the most effective strategy to infer genetic engineering targets and improve pathway efficiency. To overcome kinetic or thermodynamic barriers, a number of strategies have been adopted by metabolic engineering community, including overexpression of rate-limiting steps [9], deletion of competing pathways [10•], managing ATP level [11,12], recycling NADPH [13,14] and precursor metabolites [12], and so on. Despite most of these strategies are useful, engineering microbial overproduction phenotypes remains a daunting task, as engineered cell factory constantly experiences environmental perturbations and eventually loses cellular fitness and production phenotype. For example, precursor flux improvement by overexpression of heterologous pathways may not be accommodated by downstream pathways [15]; intermediate accumulation or depletion may compromise cell viability and pathway productivity [16]; and overexpressed heterologous pathway may penalize the cell with additional energy cost and elicit cellular stress response [17].

Under growth conditions (bioreactor or flasks), engineered strains with highly intervened regulatory and metabolic networks are incapable of adapting themselves to the changing environment and maintaining metabolic homeostasis. The source of metabolic heterogeneity could arise from a multitude of factors, including substrate inhibition, the buildup of toxic intermediary metabolites, product toxicity, nutrient depletion, genetic instability, evolution pressure or other stress factors [18–20]. To enable proper response and combat metabolic heterogeneity, it is essential to rewire transcriptional regulatory networks and have the cell autonomously adjust pathway expression and adapt the metabolic activity to the changing environment [21]. Past few years' achievements have witnessed the application of dynamic control theory to maximize pathway efficiency. There have been a few excellent reviews that focused on dynamic pathway regulation in past series [22,23]. In this review, we intend to provide an updated summary of the recent achievements that apply dynamic control theory to optimize cell metabolism.

Filling the gap between naturally existing regulatory network and synthetic gene network

Microbes have evolved exquisite regulatory control mechanisms to precisely sense the surrounding environment and adjust cell metabolism to survive and flourish. In terms of anabolic metabolism (i.e. tryptophan biosynthetic pathway) in *E. coli*, biosynthetic gene clusters are typically organized in a repressible operon form, where the end-product functions as a co-repressor to activate the repressor protein and shut down the biosynthetic gene cluster when there is sufficient amount of the end-product. In terms of catabolic metabolism (i.e. lactose degradation pathway), carbohydrate degradation gene clusters are typically organized in an inducible operon form, where the substrate functions as an inducer to deactivate the repressor protein and turn on the catabolic gene cluster. Apart from transcriptional regulation, allosteric feedback inhibition/activation, gene attenuation and post-translational modification also provide alternative strategies for microbes to prioritize cellular activity and dynamically allocate cellular resources [24].

The existence of various naturally-occurring regulatory mechanisms is perhaps best manifested with the tryptophan operon. Tryptophan (Trp) is one of the 20 amino acids that play important roles in cellular function and protein synthesis. Trp biosynthesis is regulated by a complex feedback control system that comprises enzyme feedback inhibition, transcriptional repression, and ribosome-mediated gene attenuation (Figure 1a). Transcriptional repression provides the forefront ON/OFF control to adjust the gene expression of the Trp biosynthetic gene cluster (*TrpEDCBA*, Figure 1a). The presence of tryptophan activates the Trp repressor protein (encoded by *TrpR*) to shut down the transcription of *TrpEDCBA*. Gene attenuation provides a second layer fine-tuning of the expression of *TrpEDCBA*. High level of tryptophan facilitates the premature termination of transcription and tunes down gene expression. Feedback inhibition provides an instantaneous control that is used to adjust enzyme activity in response to the fluctuating metabolites pool size. These three-layer of control ensures a fast and robust response of Trp operon gene expression when environmental perturbation arises.

In terms of engineering genetic regulation, one has to consider the time-responsiveness, design space and specificity (orthogonality) of the various genetic control elements. Feedback inhibition relies on allosteric regulation with time scale from milliseconds to a few seconds, it is very useful to introduce transient oscillatory pattern of metabolites pool change [25]. Translational regulation could happen in a few minutes [26], which is much faster than transcriptional regulation (Figure 1b). Design space is primarily related with how many possible variations that one ligand can interact with the genetic control elements. Due to the fact that only four base pairs (ATGC) in DNA versus twenty amino acids in protein, transcriptional regulation should have a design space smaller than enzyme allosteric regulation. Considering the flexibility of RNA folding, the design space of transcriptional regulation should also be smaller than translational regulation (riboswitch or riboregulatory). Given the fact that there are numerous reports about enzyme promiscuity and the presence of antimetabolite or antagonist, transcriptional factors in general are more specific to a small metabolite than enzymes or riboswitches does. It is not surprising that most of the genetic switch is developed on the basis of transcriptional regulation, including the well-known genetic toggle switch [27], repressilator [28] and metabolator [29].

Dynamic control to coordinate metabolic flux

Nature utilizes a number of frequently occurring regulatory motifs to regulate gene expression [30]. Negative autoregulation speeds up the response time of transcription networks (Figure 2a) and reduces the cell–cell variation of protein level [31]. On the contrary, positive regulation slows the response time of gene expression and tends to increase cell–cell variability [32]. Positive feedback has been the source for complex gene expression dynamics including bistability and hysteresis in synthetic gene networks [33–37]. Other network motifs (Figure 2b) include the incoherent feedforward loop which generates pulse-like signal. This incoherent feedforward loop has been used to engineer biofuel tolerance phenotype ensuring just-in-time gene expression of the biofuel exporter protein [38]. The single-input module (SIM) network motif allows for sequential activation of gene expression and dynamic allocation of cellular resources for arginine biosynthesis [39].

Mimicking the natural gene regulatory network, metabolic engineers and synthetic biologists have been able to exploit the metabolites-responsive transcriptional factors (MRTFs) as basic control elements to reprogram cellular activity. This is commonly achieved by integrating sensor-actuator systems with various cell chassis to dynamically control the expression of biocatalytic enzymes and drive carbon flux toward the target pathway. These biosensors translate a metabolite concentration signal to a transcriptional output and drive the expression of the designed genetic/biomolecular circuits to compensate the activity loss of the engineered biosystem [6••,40•]. A few of the sensor design principles, including how to tweak the specificity, orthogonality and dynamic output range, have been formulated recently [41–46]. These endeavors greatly expanded our capability in engineering artificial TFs as novel sensors to control and optimize cell metabolism.

The primary MRTFs are transcriptional repressor or activators. Repressor or activator protein typically transduces a C-terminal ligand-binding activity to the N-terminal DNA-binding activity [47]. Upon interaction with a small molecule or environmental stress signal, TFs will undergo