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Dynamic pathway regulation: recent advances and methods of construction

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Microbial cell factories are a renewable source for the production of biofuels and valuable chemicals. Dynamic pathway regulation has proved successful in improving production of molecules by balancing flux between growth of cells and production of metabolites. Systems for autonomous induction of pathway regulation are increasingly being developed, which include metabolite responsive promoters, biosensors, and quorum sensing systems. Since engineering such systems are dependent on the available methods for controlling protein abundance in the desired host, we review recent tools used for gene repression at the transcriptional, post-transcriptional and post-translational levels in *Escherichia coli* and *Saccharomyces cerevisiae*. These approaches may facilitate pathway engineering for biofuel and biochemical production.

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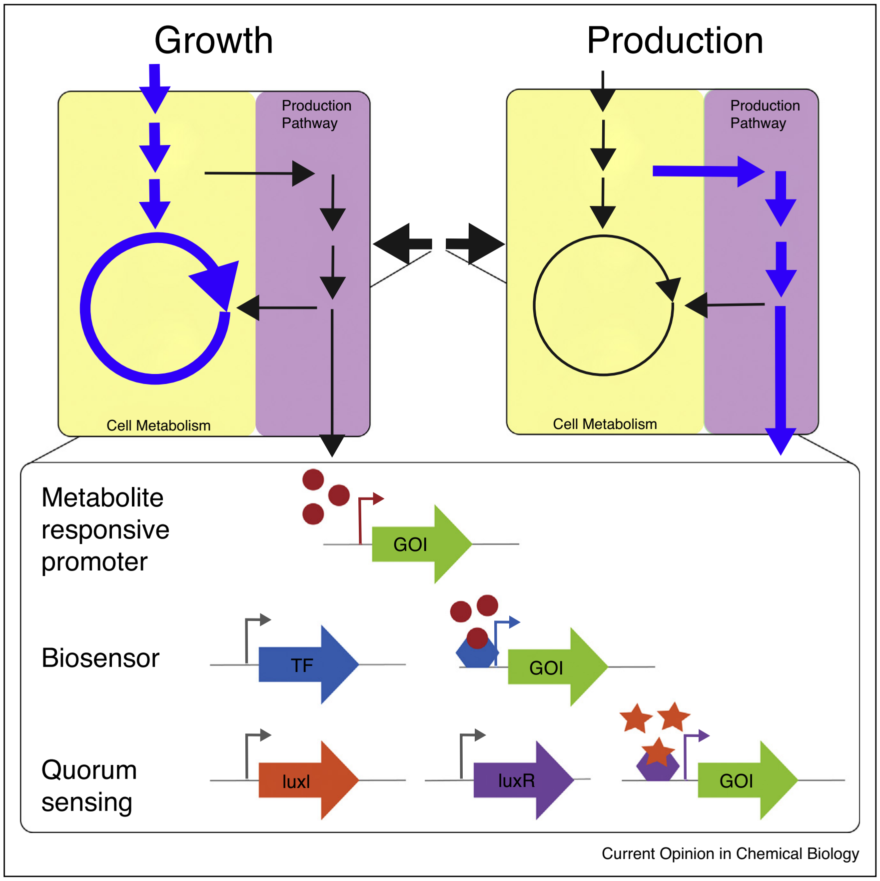
Introduction

Microorganisms are promising hosts for the production of valuable chemicals, such as fuel alternatives [1,2], flavors [3], and polymer [4] and pharmaceutical [5] precursors. The immense diversity in the physiology of different microbes offers tremendous potential in using microbial cell factories given a desired product, starting material or growth condition. For example, the yeast *Saccharomyces cerevisiae*'s native ability to produce ethanol efficiently from glucose has rendered it the ideal host for bioethanol production [6]. *Pseudomonas putida* that naturally degrades aromatic acids is a suitable host for using lignocellulosic biomass as a starting material [7], while methanotroph bacteria provides a biological means to the upgrading of methane [8], which is the least expensive source of carbon. For growth at a desired condition, environmental strains can also be isolated and further engineered, such as a *Bacillus megaterium* strain capable of growing at sterile supercritical CO_2 conditions [9]. With the abundance of microorganisms to choose from, the limitation then lies in the availability of genomic sequence-to-function information for the microorganism and molecular biology methods for host DNA manipulation. Once the desired host has been chosen, metabolic engineering of the microorganism for production of molecules then typically involves pathway engineering and host engineering. Pathway engineering includes expressing the required enzymes, whether native or heterologous, for the conversion of substrate to product. Host engineering involves manipulation of the rest of the cell, such as removing competing or regulatory pathways to increase flux to the desired product. Often times, these methods consist of static changes, such as constitutive overexpression of pathway enzymes and deletion of competing enzymes, both of which are also generally optimized separately. Although these conventional strategies have proven to be successful in improving titers and yields, these methods are constrained by the interconnectivity of pathways in cells and a finite amount of cellular resources. To that end, dynamic regulation of pathways has emerged as a preferred method to better balance flux between growth of the cell and production of the desired molecule (Figure 1). Here, we report recent developments in applying dynamic pathway regulation to improve production of molecules in bacteria and yeast. Then, we review current methods used to engineer pathway regulation and address their limitations. These methods show promise for improving the productivity of microbes for synthesis of biofuels and bioproducts.

Dynamic pathway regulation by exogenous inducers

Regulation of pathways can be broadly divided into two categories: upregulating pathway enzymes to direct flux into the production pathway and downregulating competing enzymes that draw flux into native cell metabolism. Upregulation of pathway enzymes is well established and typically achieved by using inducible promoter systems. In downregulating competing enzymes, an effective flux control point is first identified. This control point is typically an endogenous enzyme that catalyzes an irreversible reaction in cell metabolism that is also located where the production pathway intersects with cell metabolism. Placing this enzyme under dynamic control then

Figure 1



Dynamic pathway regulation balances flux between growth of cells and production of desired metabolites. Once sufficient biomass and cellular resources have accumulated from an initial growth phase, substrate flux can then be diverted into the production pathway. An effective flux control point at the intersection of cell metabolism and production pathway is identified and the expression of this enzyme is controlled in a dynamic manner. Autonomous induction of pathway regulation has recently been developed using metabolite responsive promoters, biosensors and quorum sensing systems. Metabolite responsive promoters are often derived from native promoters that can be implemented simply by promoter replacement of the gene of interest (GOI) without additional expression of transcription factors. Biosensors are engineered from transcription factors that bind to their associated promoters upon interaction with the metabolite of interest. Placing the GOI under this promoter then enables metabolite dependent gene expression. Quorum sensing systems, such as the LuxR system, consist of a synthase that produces acyl-homoserine lactone (AHL) that serves as a proxy for cell mass. Controlling the expression of the GOI using a repressor that is responsive to AHL then enables cell density dependent expression of the GOI.

enables flux redirection from cell metabolism into the production pathway at intermediate times in the fermentation by addition of an exogenous inducer or carbon source. Recent applications of dynamic regulation include the production of isopropanol in *E. coli*, where a toggle switch was designed to simultaneously increase flux through the isopropanol pathway and decrease flux into the TCA cycle, leading to a 4-fold improvement in isopropanol titers upon induction with IPTG [10]. In the yeast *S. cerevisiae*, native carbon source responsive promoters are typically used for inducible expression or repression of genes. For example, HXT and GAL promoters were used to control expression of pathway genes to increase flux of farnesyl pyrophosphate (FPP) for carotenoid production [11]. The same approach could provide yield improvements for isoprenoid-derived biofuels. The HXT1 promoter that is induced at high glucose concentrations and repressed at low glucose concentrations was also used to improve fatty alcohol production by regulating free fatty acid and acyl-CoA pools [12]. The SUC2 sucrose responsive promoter was used to induce RNA interference system for gene repression [13]. Since these promoters are responsive to glucose, the application of these methods is restricted by carbon source. To circumvent this limitation, synthetic promoter systems such as the doxycycline responsive tetracycline transactivator protein (tTA) has been used to control glucose flux into glycolysis, leading to improved yields of gluconic acid and isobutanol from glucose by 50-fold and 3-fold [14].

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