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Design and application of genetically-encoded malonyl-CoA biosensors for metabolic engineering of microbial cell factories

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ABSTRACT

Malonyl-CoA is the basic building block for synthesizing a range of important compounds including fatty acids, phenylpropanoids, flavonoids and non-ribosomal polyketides. Centering around malonyl-CoA, we summarized here the various metabolic engineering strategies employed recently to regulate and control malonyl-CoA metabolism and improve cellular productivity. Effective metabolic engineering of microorganisms requires the introduction of heterologous pathways and dynamically rerouting metabolic flux towards products of interest. Transcriptional factor-based biosensors translate an internal cellular signal to a transcriptional output and drive the expression of the designed genetic/biomolecular circuits to compensate the activity loss of the engineered biosystem. Recent development of genetically-encoded malonyl-CoA sensor has stood out as a classical example to dynamically reprogram cell metabolism for various biotechnological applications. Here, we reviewed the design principles of constructing a transcriptional factor-based malonyl-CoA sensor with superior detection limit, high sensitivity and broad dynamic range. We discussed various synthetic biology strategies to remove pathway bottleneck and how genetically-encoded metabolite sensor could be deployed to improve pathway efficiency. Particularly, we emphasized that integration of malonyl-CoA sensing capability with biocatalytic function would be critical to engineer efficient microbial cell factory. Biosensors have also advanced beyond its classical function of a sensor actuator for *in situ* monitoring of intracellular metabolite concentration. Applications of malonyl-CoA biosensors as a sensor-invertor for negative feedback regulation of metabolic flux, a metabolic switch for oscillatory balancing of malonyl-CoA sink pathway and source pathway and a screening tool for engineering more efficient biocatalyst are also presented in this review. We envision the genetically-encoded malonyl-CoA sensor will be an indispensable tool to optimize cell metabolism and cost-competitively manufacture malonyl-CoA-derived compounds.

1. Introduction

The field of metabolic engineering has witnessed rapid advancements, further consolidating it as an enabling technology for engineering biological cell factories for producing value-added chemicals and bio-products. The three pillars of metabolic pathway engineering are to achieve high titers, yield and productivity of desired product without detrimental effects on cell growth. Hence, it is critical to develop metabolite-based biosensors to execute feedback control and decouple metabolite production from cell growth. A recent crucial contribution by synthetic biology to engineer more efficient microbial cell factory is the development of transcriptional factor (TF)-based biosensors. Whether coupled with a readable output to enable high-throughput screening or integrated into a genetic circuit to dynamically regulate key metabolic pathways, TF-based biosensors are an indispensable tool for redistributing carbon flux and adapt cell metabolism to the changing environment (Liao and Oh, 1999; Mainguet and Liao, 2010; van der Meer and Belkin, 2010). Such synthetic biosensors are derived from naturally evolved transcriptional factors that propagate changing environmental signals or cellular status into a transcriptional

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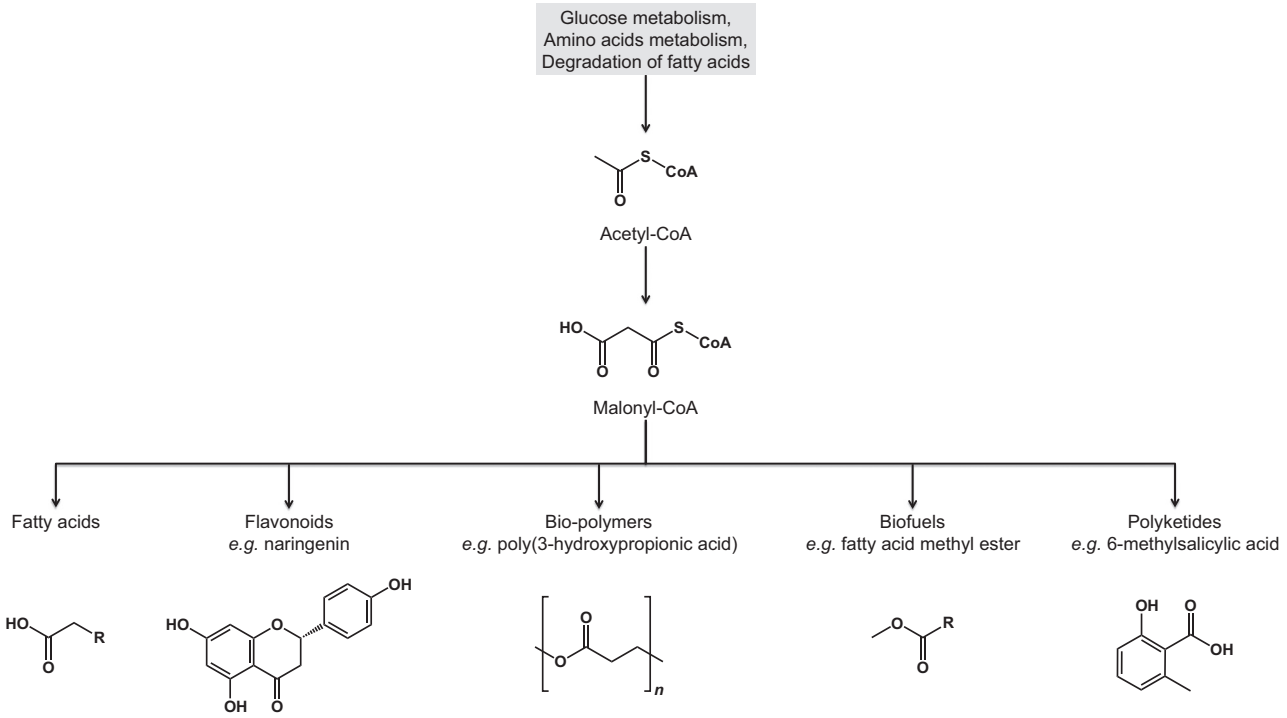

Fig. 1. Compounds derived from malonyl-CoA. Malonyl-CoA, a direct product of acetyl-CoA, can be used as a precursor for the synthesis of fatty acids, flavonoids, bio-polymers, biofuels, and polyketides.

output or cellular phenotype that promotes either cell viability, survival or metabolic economics (Harrison and Dunlop, 2012; Liu et al., 2015). Structural feature of this biosensor is generally divided into two parts: a metabolite-responsive transcriptional regulator and a fluorescence-coupled or fitness-related read-out module (Harrison and Dunlop, 2012; Rogers et al., 2015). This enables metabolic engineers to efficiently quantify varying concentration of cellular metabolites in contrast to the laborious, time-consuming and low throughput analytical methods such as HPLC and LC-MS (Dietrich et al., 2013; Liu et al., 2015). Metabolite-sensing genetic circuits have been reported for sensing various metabolites including macrolides (Mohrle et al., 2007), acetyl phosphate (Farmer and Liao, 2000), farnesyl pyrophosphate (Dahl et al., 2013), 3-hydroxypropanoic acid (Rogers et al., 2015; Rogers and Church, 2016), 1-butanol (Dietrich et al., 2013), and more recently, malonyl-CoA (Ellis and Wolfgang, 2012).

The use of biosensors for *in vivo* detection and/or quantification of metabolites essentially creates an input-output communication platform within biological cells. This platform has predominantly been exploited to monitor metabolite levels in real-time, especially for the detection of accumulated intermediates or metabolites present in relatively low abundance. Access to such crucial information allows metabolic engineers to gain a deep understanding of kinetics and regulatory mechanisms underlying the engineered metabolic network. In turn, this enables the design of more robust and effective metabolic intervention strategies to maximize production titers of metabolites of interest. That said, biosensors have also found front-end applications as dynamic metabolic pathway regulators and back-end applications as screening devices. When applied to dynamic metabolic pathway regulation, biosensors allow the cell to probe the exact metabolic state and actuate pathway expression that compensates for the metabolic activity of the engineered pathway, and improves the overall productivity and fitness of the engineered cell factory (Xu et al., 2014b). Biosensors can also be developed into a high-throughput screening platform by coupling with a readable output such as fluorescence. This approach is often used to select for high-producing genetic variants or to identify process conditions leading to high product titers (Dietrich et al., 2010, 2013; Williams et al., 2016). These applications potentially help to accelerate design-build-test cycles in the engineering of metabolic pathways by facilitating genotype manipulation-phenotype evaluation processes (Rogers et al., 2015; Rogers and Church, 2016).

Over all, many of these applications require that such sensors are orthogonal (without sensor cross-talk) and tunable, allowing for customized biosensor output relative to expected concentrations of metabolites for a range of physiological conditions. This constraint could be decomposed into three pillars: specificity, sensitivity and dynamic range, which are the primary design considerations for their proper function inside the cell. In this review, we explore the fundamental design principles and strategies in modifying and tuning the metabolite-responsive transcription factor (MRTF), using malonyl-CoA biosensor as a prototype. We also explore how these genetically encoded circuits have been successfully applied as a tool in metabolic engineering to dynamically regulate intracellular malonyl-CoA metabolite pools and improve production of malonyl-CoA-derived chemicals in notable host microorganisms.

2. Malonyl-CoA – a vital metabolite

In practically every living system, a portion of the acetyl-CoA flux from the central metabolic pathway is diverted to malonyl-CoA for fatty acid and lipid membrane synthesis, with the aid of acetyl-CoA carboxylase (ACC). This suggests the vital roles that malonyl-CoA plays in cell metabolism and structure. Specifically, malonyl-CoA is a rate limiting substrate for fatty acid synthesis, which in turn, is pivotal for maintaining cell membrane integrity and energy conservation (Schujman et al., 2003, 2006, 2008). In mammals, malonyl-CoA has been identified as a crucial fatty acid oxidation regulator which inhibits the mitochondrial carnitine palmitoyltransferase (CPT) − an enzyme involved in fatty acid uptake in the heart and skeletal muscle (Folmes and Lopaschuk, 2007; Foster, 2012). This makes it an effective therapeutic target molecule for treating diseases caused by poor or excessive fatty acid uptake. This has also attracted medical interest in drug development to control malonyl-CoA metabolism at the enzymatic