A Post-translational Metabolic Switch Enables Complete Decoupling of Bacterial Growth from Biopolymer Production in Engineered Escherichia coli

Most of the current methods for controlling the formation rate of a key protein or enzyme in cell factories rely on the manipulation of target genes within the pathway. In this article, we present a novel synthetic system for post-translational regulation of protein levels, FENIX, which provides both independent control of the steady-state protein level and inducible accumulation of target proteins. The FENIX device is based on the constitutive, proteasome-dependent degradation of the target polypeptide by tagging with a short synthetic, hybrid Nla/SsrA amino acid sequence in the C-terminal domain. Protein production is triggered via addition of an orthogonal inducer (i.e., 3-methylbenzoate) to the culture medium. The system was benchmarked in Escherichia coli by tagging two fluorescent proteins (GFP and mCherry), and further exploited to completely uncouple poly(3-hydroxybutyrate) (PHB) accumulation from bacterial growth. By tagging PhaA (3-ketoacyl-CoA thiolase, first step of the route), a dynamic metabolic switch at the acetyl-coenzyme A node was established in such a way that this metabolic precursor could be effectively redirected into PHB formation upon activation of the system. The engineered E. coli strain reached a very high specific rate of PHB accumulation (0.4 h\(^{-1}\)) with a polymer content of ca. 72% (w/w) in glucose cultures in a growth-independent mode. Thus, FENIX enables dynamic control of metabolic fluxes in bacterial cell factories by establishing post-translational synthetic switches in the pathway of interest.