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Anti-Pdc1p Nanobody as a Genetically Encoded Inhibitor of Ethanol Production Enables Dual Transcriptional and Post-translational Controls of Yeast Fermentations

Background/Objective

By tuning gene expression to divide microbial fermentation systems into two stages, growth and production, dynamic control improves titers during the chemical production stage. However, most dynamically-controlled systems rely on transcriptional controls of metabolic enzymes, leaving existing intracellular enzymes unregulated and so are limited in their ability to switch off metabolic pathways. Nanobodies, which are antibody fragments derived from camelids, can inactivate existing intracellular enzymes, enabling post-translational control of cell growth. Here, we developed a novel two-layer transcriptional/post-translational control system using optogenetics and a nanobody for the dynamic control of yeast fermentations.

Approach

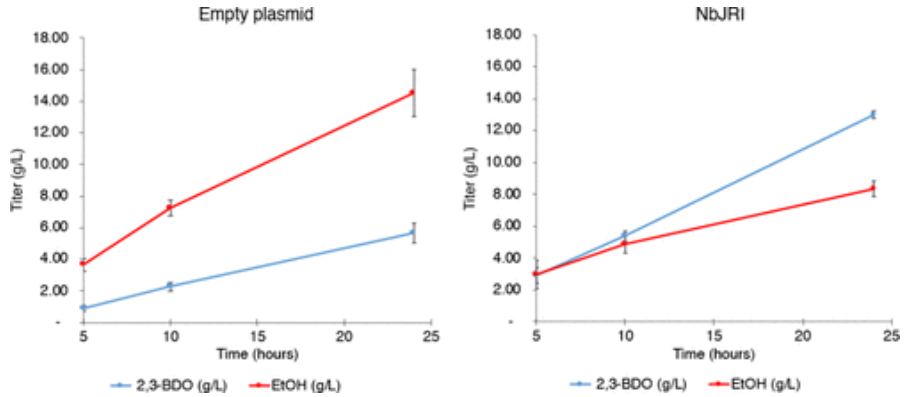
The optogenetic system uses blue light to transcriptionally activate the major pyruvate decarboxylase, *PDC1,* required for cell growth and concomitant ethanol production. Switching to darkness transcriptionally inactivates PDC1 and activates the anti-Pdc1p nanobody, NbJRI, to inhibit Pdc1p accumulated during the growth phase. *Saccharomyces cerevisiae* was engineered with this dynamically-controlled system on a plasmid to produce chemicals 2,3-butanediol (2,3-BDO) and citramalate.

Results

This system improved the production of 2,3-BDO and citramalate by up to 100 and 92% compared to using transcriptional controls alone in dynamic two-phase fermentations. This study also established the NbJRI nanobody as an effective genetically encoded inhibitor of Pdc1p that can enhance the production of pyruvate-derived chemicals, and more generally, regulate ethanol production, one of the most active metabolic pathways known.

Significance/Impacts

This study suggests that nanobodies in general could be used to post-translationally control just about any metabolic pathway in nature to develop dynamic controls of microbial fermentations.



Kinetic characterization of nanobody-induced metabolic rewiring.

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