Programmable control of acetate metabolism in *Escherichia coli*

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Abstract

We are creating a genetic circuit that detects and responds to acetate production, a common problem in industrial fermentations of E. coli

This circuit integrates three metabolite signals, computes the cause of acetate production, and produces outputs that counteract these conditions

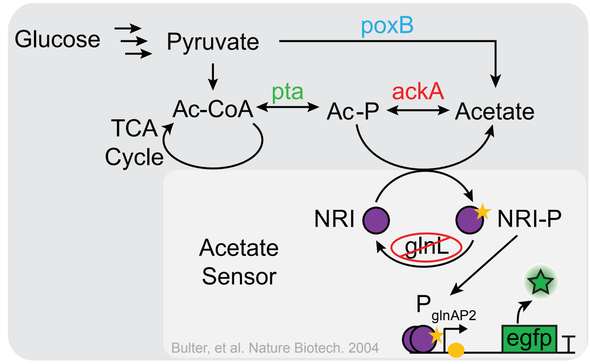
Genetic control enables individual cells to respond to the stresses they experience during industrial fermentation, which can improve growth and product yields

Sensing and reducing native acetate production

Δpta ΔpoxB eliminates acetate production, but slows growth rate

We will try to use feedback to regulate *pta* and *poxB* to eliminate acetate production w/o slowing growth

NRI acts as a strong acetate sensor in Δ*glnL* strains



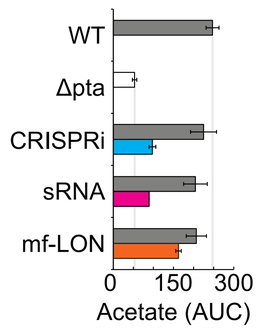
Controlling transcription, translation, and degradation of *pta* reduces acetate production

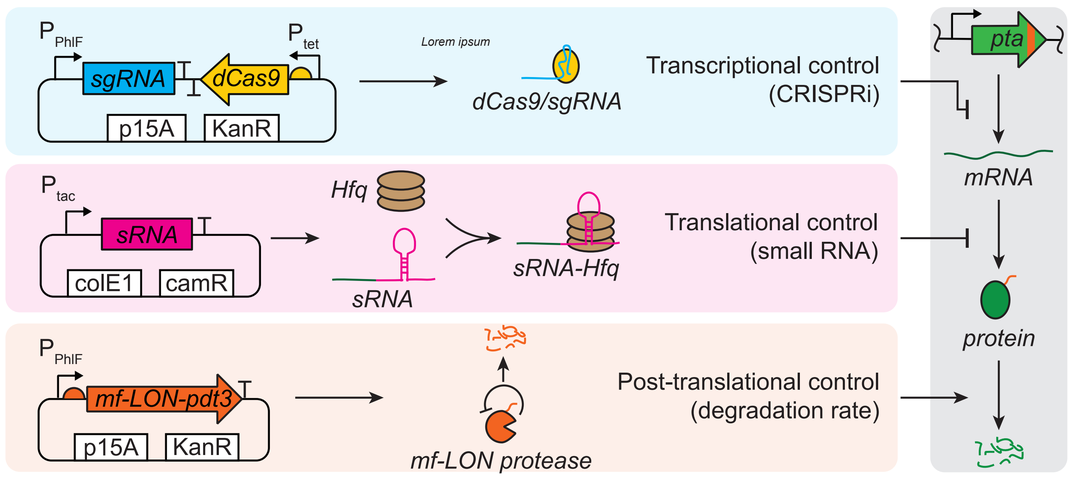
Developed inducible repression of acetate production genes

- CRISPRi[3], small RNA's, and *Mesoplasma florum* LON protease[4] act against native *pta* gene expression

Each approach downregulated target genes, but displayed different dynamics

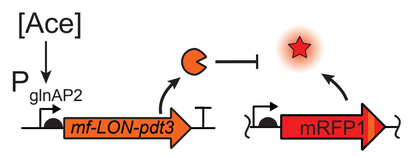
We chose to use the mf-LON output due to its rapid degradation of the target gene.



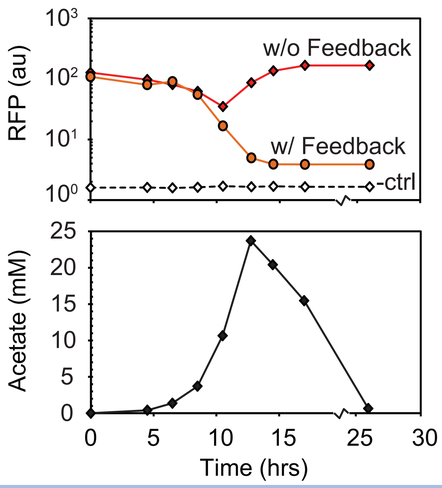


Direct feedback reduces acetate production

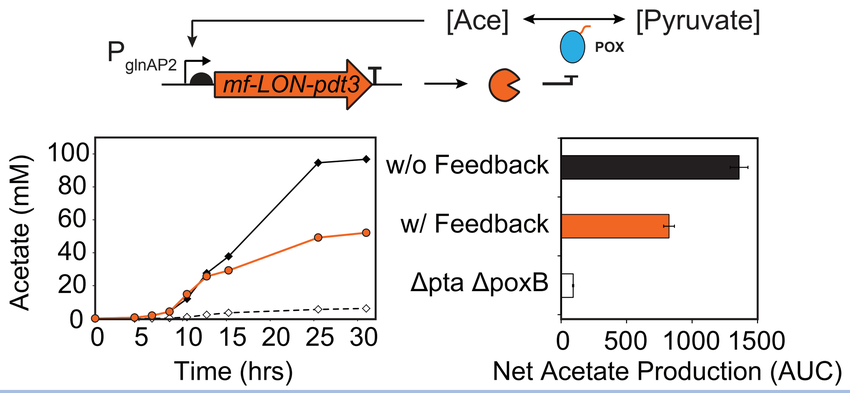
We used the acetate sensor (P_{glnAP2}) to drive expression of the mf-LON protease.



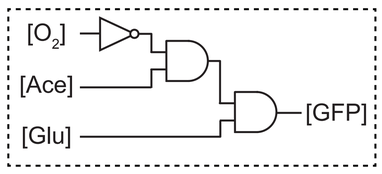
We observed strong, real-time control of RFP abundance in response to an endogenous increase in acetate production.



When mf-LON was targeted at the poxB enzyme (activated late in growth), acetate accumulation was reduced by 40%.

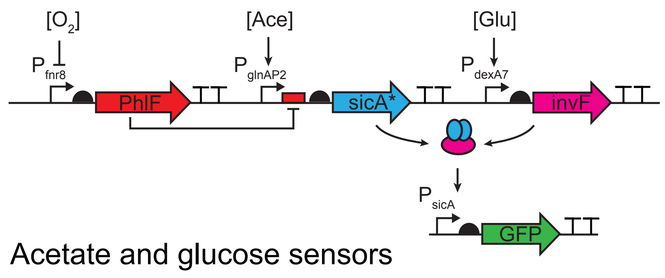


A digital circuit responds to metabolic cell state

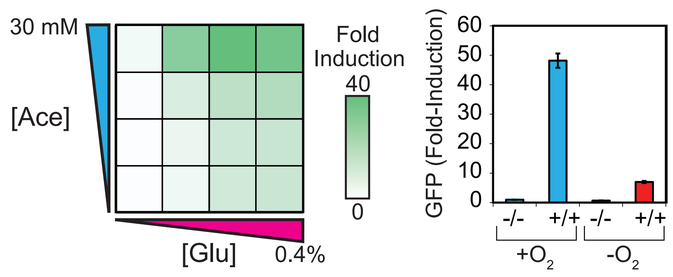


We built a genetic program that integrates signals for acetate, glucose, and oxygen which

- Activates GFP production only when acetate is made in response to glucose
- Deactivates GFP production in microaerobic conditions



Acetate and glucose sensors drive an activator/chaperone system (invF/sicA*), forming an AND gate. A native oxygen sensor drives production of a PhlF repressor that downregulates the acetate-sensitive promoter.



The system is sensitive to low glucose levels and shows up to 20-fold activation 7.5 mM acetate.