Magnesium controls carbon flux to biomass or fermentation products.

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Project Goals: The goal of this project is to determine how protein acetylation affects metabolism in engineered microorganisms. Lysine acetylation is a common post-translational modification that eukaryotes, archaea, and bacteria employ to regulate protein activity. Multiple studies have recently shown that lysine acetylation predominantly targets metabolic enzymes – in fact, most metabolic enzymes are subject to lysine acetylation. We hypothesize that bacteria employ lysine acetylation as a global mechanism to regulate metabolism in response to their energy and redox status. Our previous work suggests that lysine acetylation may be an attractive and innovative target for metabolic engineering. We are investigating how lysine acetylation affects fuel production in engineered microorganisms. The significance of this work is that it will address a fundamental gap in our understanding of bacterial metabolism and identify new approaches for overcoming the problems associated with the production of advanced biofuels.

N^{ε}-lysine acetylation is a posttranslational modification that occurs within all three domains of life. The acetylation reaction occurs through the donation of an acetyl group from a donor molecule onto a susceptible lysine of a protein or peptide. This modification neutralizes the positive charge of the lysine side chain and increases its size. Acetylation of residues required for catalytic function can render an enzyme inactive. Additionally, neutralization of the positive charge can disrupt salt bridges necessary for protein-protein interactions. In *E. coli*, acetylation is known to be catalyzed by two mechanisms. One, the canonical enzymatic mechanism, utilizes the only known lysine acetyltransferase, YfiQ, to catalyze the donation of the acetyl group of an acCoA molecule onto a lysine. The other and more predominant mechanism employs acetyl phosphate (acP), the intermediate of the acetate fermentation (AckA-Pta) pathway, to donate its acetyl group onto proteins non-enzymatically. Therefore, conditions that promote acetate fermentation invariably lead to protein acetylation.

Previously, our lab and others have found that *E. coli* grown in carbon excess leads to high acetylation levels due to the production of acetate[1,2]. In this work, we report that carbon is directed into biomass rather than acetate when magnesium, the limiting nutrient in our experiments, is in excess. We found that *E. coli* grown in tryptone broth buffered to pH7 (TB7) supplemented with 0.4% glucose grew to an OD_{600} value of ~3. When we further supplemented the growth medium with 1 mM magnesium sulfate ($MgSO_4$), *E. coli* had an extended exponential phase and reached an OD_{600} that was 2-3 times greater. Six additional carbon sources showed the same magnesium-induced biomass increase. We also found that cells grown in media where

tryptone was replaced with either casamino acids or peptone exhibited the same effect. Even the common laboratory medium LB was found to benefit from magnesium supplementation when provided with excess carbon. More significantly, magnesium-induced biomass increase was accompanied by a significant reduction in acetylation as measured by Western blot analysis.

These results demonstrate that magnesium indirectly affects protein acetylation by determining whether carbon flux is diverted to biomass or acetate. The mechanism is likely related to ribosome abundance, because magnesium is known to increase the stability of the ribosomes[3]. We are currently testing this hypothesis. Collectively, these results provide a deeper understanding of how different media formulations influence bacterial metabolism and physiology, and demonstrate how *E. coli* regulates its metabolism accordingly.

References

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