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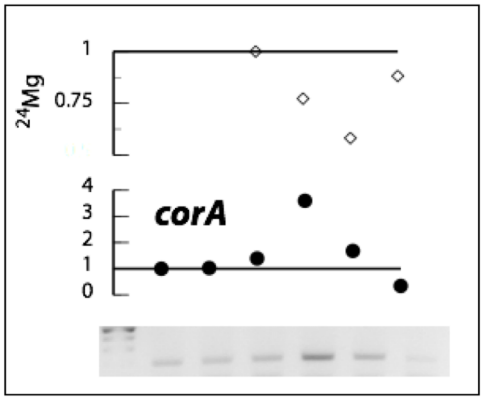
Metal demands during protein overexpression in bacteria

"For Biocatalysts Ltd, this study highlighted the importance of metal supplementation in commercial fermentation processes to maximise enzyme activity and yield."

Peter Chivers, Durham University; Mark Blight, Biocatalysts Ltd



Cellular Mg content is inversely correlated with changes in transcript levels of the *corA* gene, which is associated with Mg uptake.

OUTCOMES: Transcript levels and metal content of *E. coli* cells were measured at different time points post-induction during a representative fermenter run. Increased transcript levels of genes important for Mg, Fe, Mn, and Ni acquisition were observed in the latter half of the protein overexpression time course (≥ 9 h post-induction). These increases correlated with decreases in total cellular metal content for each metal, consistent with metal deficiency sensed by metal-responsive transcriptional regulators. These deficiencies have potential effects on translational efficiency (Mg), synthesis of non-natural amino acids that affect the fidelity of tRNA charging (Ni), and posttranslational processing of newly synthesized polypeptides (Fe and Mn). No evidence for Zn-deficiency or Cu-stress was detected based on transcript levels and metal content. These results suggest straightforward strategies — namely metal supplementation — to ensure metal supply is maintained during the protein overexpression time course.

INITIAL AIMS: Protein overexpression is a major facet of industrial biotechnology, yet the capacity of host organisms to overexpress proteins is not naturally optimized. Transition metals are key components of the cellular protein synthesis machinery. This project will explore the changes in metal demands brought about by protein overexpression in *Escherichia coli*, a widely used platform for biologics production. Metal allocation and use will be determined by transcript analysis to monitor changes in the expression of metal-regulated genes and measurement of cellular metal content to establish links between changes in gene expression and metal supply.

- Supplementing growth media with metals could improve the quality and/or quantity of protein synthesis