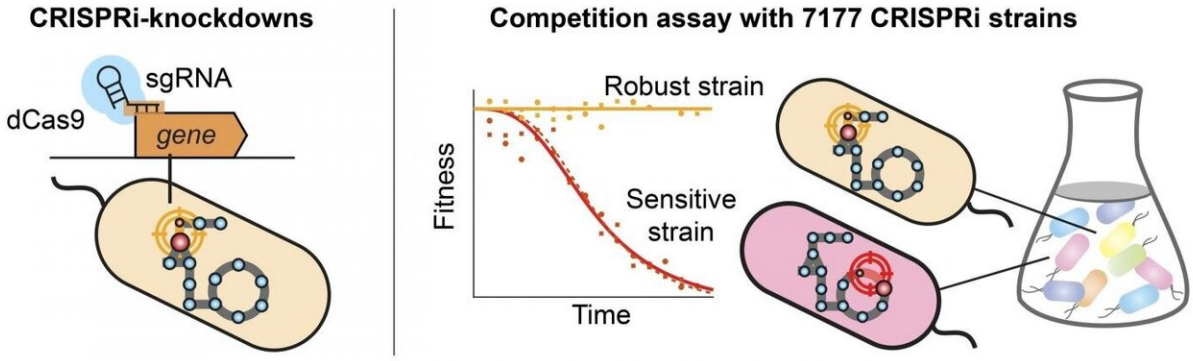
CRISPRi screens reveal sources of metabolic robustness in E.coli

November 24 2020



CRISPR interference uses a deactivated Cas9 protein (dCas9) and a single guide RNA (sgRNA) and enables specific knockdowns of enzymes in the metabolic network of bacteria. Deep sequencing can track growth of 7177 CRISPRi in parallel and informs about robustness of each CRISPRi strain. Credit: Max Planck Institute for terrestrial Microbiology/Link

Metabolic robustness, the ability of a metabolic system to buffer changes in its environment, is not always a welcome feature for microbiologists: it interferes with metabolic engineering or prevents that antibiotics kill bacteria. Therefore it is important to understand the mechanisms that enable metabolic robustness. A massively parallel CRISPRi screen

demonstrated that *E. coli* metabolism is very robust against knockdowns of enzymes, and multi-omics data revealed the mechanisms behind it. In the future, the researchers want to apply this knowledge to build better models of metabolism, which enable rational-design of industrial microbes.

In their natural habitat, bacteria like *E. coli* are confronted with constant changes in the composition of nutrients. But under laboratory conditions, they can also be real specialists, and grow for instance on a single carbon source like glucose. To do so, their metabolic network must synthesize all cellular building blocks from scratch. This task requires that hundreds of enzyme-catalyzed reaction in the metabolic network work at the right pace, and that no reaction accidently falls below a critical threshold. Otherwise, a single bottleneck in the network may have wide-spread consequences and eventually stop cellular growth.

To understand how *E. coli* accomplishes this task, researchers led by Dr. Hannes from the Max Planck Institute for Terrestrial Microbiology applied the CRISPR interference (CRISPRi) technology. By inducing knockdowns of each protein in the metabolic network of *E. coli*, they created a CRISPRi library with 7177 strains. Deep sequencing of the library during a pooled competition assay allowed the researchers to track fitness of each CRISPRi strain for 14 hours. The results of this massively parallel CRISPR screen were somewhat surprising. While knockdowns of only seven genes—keypoints in the metabolic network, like biosynthesis of deoxynucleotides for DNA synthesis—caused immediate and strong fitness defects, hundreds of other knockdowns had little effects.

As Dr. Hannes Link explains: "Our results demonstrated that *E. coli* cells accomplish a very high metabolic robustness. In general, robustness enables living organisms to survive despite external and internal disturbances, and there are different mechanisms that mediate it, such as

feedback mechanisms or redundancy. In this context, organisms are always in a trade-off situation: either they express high enzyme concentrations, which is costly; or the express low enzyme concentrations which can limit the metabolic capacity. For us researchers, robustness is not always a welcome feature in bacteria, for example in the course of biotechnological applications, if we want to engineer metabolism to overproduce chemicals with bacteria. Therefore it is important to understand how *E. coli* accomplishes this task."

To answer this question, the team measured the proteome and metabolome of 30 CRISPRi strains. In some strains the proteome responses revealed mechanisms that actively buffered the CRISPRi knockdowns. For example, knockdown of homocysteine transmethylase (MetE) in the methionine pathway caused a compensatory upregulation of all other enzymes in the methionine pathway. In other words, *E. coli* cells sensed that the knockdown caused a bottleneck in methionine biosynthesis and then mounted a very precise and local response around the methionine pathway. The other 30 CRISPRi strains revealed similar buffering mechanisms that were surprisingly specific, but whether all metabolic pathways are equipped with such precise and localized buffering mechanisms remains open. Therefore, the Link Lab is currently innovating new mass spectrometry methods to probe the complete metabolism of the complete CRISPRi library.

This comprehensive approach creates new possibilities for the development of industrially useful microbes, as Dr. Hannes Link points out: "In the future, we want to use these data to construct metabolic models that are dynamic and predictive. We used a very small dynamic model in the current study, but building larger models remains one of the big challenges. Such models would allow us to engineer *E. coli* cells that stop growing upon a certain signal and then concentrate all metabolic resources on the synthesis of a desired chemical. This controlled decoupling of growth from overproduction would break new ground in

metabolic engineering and opens new applications in industrial biotechnology."

More information: Stefano Donati et al, Multi-omics Analysis of CRISPRi-Knockdowns Identifies Mechanisms that Buffer Decreases of Enzymes in E. coli Metabolism, *Cell Systems* (2020). DOI: 10.1016/j.cels.2020.10.011

Provided by Max Planck Society