XX Congreso Nacional de Biotecnología y Bioingeniería

11-15 de septiembre del 2023. Ixtapa Zihuatanejo, Guerrero

ENGINEERING PROTEOMIC RESOURCE ALLOCATION FOR SYNTHETIC BIOLOGY: NOVEL APPROACHES FOR E. COLI AS AN IMPROVED HOST

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Palabras clave:Synthetic biology, cell engineering, proteome allocation

Introduction. The efficient allocation of cellular resources is a fundamental challenge in synthetic biology. Wild-type organisms allocate abundant cellular resources for survival in changing environments, which reduces the productivity of engineered functions. We compared bacterial complexity reduction approaches using E. coli as a model organism and showed that resource reallocation efforts can be optimized if they are focused on a few genes producing highly expressed dispensable proteins. We propose that resource allocation reduction strategies should be focused on the proteome rather than the genome[1]. We formalized the calculations of the saved resources of eliminating genes when those genes are transcribed and translated, and calculated the theoretical liberated resources in terms of energy and proteome liberation for a defined growth environment. Our analysis showed that the amount of saved resources is much larger for translation than for transcription or DNA replication. Using a genome-scale model of metabolism and expression (ME-model)[2], we were able to account for "distant" processes, such as the need for macromolecular machinery (such as ribosomes) that carry out each process, thus incurring a cost. We found that even a 1% reduction in unused proteome can increase the heterologous proteome fraction by 10%. Our goal is to determine and implement the best strategy for improving resource allocation in minimized cells.

Methods. To optimize resource allocation, we developed a new top-down cell engineering strategy for E. coli using the transcriptional regulatory network (TRN) as a control layer for proteome allocation. The design algorithm is based on modifying the transcriptional regulatory network. Our method, ReProMin[3], identifies the minimal set of genetic interventions that maximizes savings in cell resources that are typically used to express non-essential genes.

Results. To this end, we categorize transcription factors (TFs) according to the essentiality of the genes they regulate, and we use available proteomic data to rank them based on their proteomic balance, defined as the net proteomic mass they release. Using a combinatorial approach, we design the removal of TFs that maximizes the release of the proteomic mass and validate our model predictions experimentally. This approach involves identifying the minimal set of genetic interventions that maximize the savings in cell resources. We designed the combinatorial removal of transcription factors that maximize the release of resources, resulting in a strain containing only three mutations, theoretically releasing 0.5% of its proteome. The resulting strain had a higher proteome budget, increased production of an engineered metabolic pathway, and showed that the regulatory interventions are highly specific.

Conclusions. This work demonstrates that by reducing the expression of dispensable proteins, it is possible to increase the availability of resources for the expression of desired functions, opening new possibilities for the engineering of cellular metabolism. Our approach shows that combining proteomic and regulatory data is an effective way of optimizing strains using conventional molecular methods. The resulting strains exhibited an increased availability of cellular resources to express engineered synthetic biology functions.

Acknowledgements. CONACyT Paradigmas y Controversias de la Ciencia 2022, proyecto: 319352.

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