

Accepted Manuscript



Adaptive response of yeast cells to triggered toxicity of phosphoribulokinase

Catherine Rouzeau, Adilya Dagkesamanskaya, Krzysztof Langer, Jérôme Bibette, Jean Baudry, Denis Pompon, Véronique Anton-Leberre

PII: S0923-2508(18)30099-8

DOI: [10.1016/j.resmic.2018.06.002](https://doi.org/10.1016/j.resmic.2018.06.002)

Reference: RESMIC 3676

To appear in: *Research in Microbiology*

Received Date: 5 March 2018

Revised Date: 15 June 2018

Accepted Date: 20 June 2018

Please cite this article as: C. Rouzeau, A. Dagkesamanskaya, K. Langer, J. Bibette, J. Baudry, D. Pompon, V. Anton-Leberre, Adaptive response of yeast cells to triggered toxicity of phosphoribulokinase, *Research in Microbiology* (2018), doi: 10.1016/j.resmic.2018.06.002.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Adaptive response of yeast cells to triggered toxicity of phosphoribulokinase

ACCEPTED MANUSCRIPT

Catherine Rouzeau^a, Adilya Dagkesamanskaya^a, Krzysztof Langer^b, Jérôme Bibette^b, Jean Baudry^b, Denis Pompon^a and Véronique Anton-Leberre^{a*}

^aLISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France

^bLaboratoire Colloïdes et Matériaux Divisés, from the Institute of Chemistry, Biology and Innovation (CBI) - ESPCI ParisTech, CNRS – UMR 8231, PSL* Research University, 10 rue Vauquelin, 75005 Paris, France

Correspondance to: V. Anton-Leberre, LISBP - INSA Toulouse - 135 avenue de Rangueil - 31077 Toulouse CEDEX 04, France - Email: veronique.leberre@insa-toulouse.fr.

Abstract

Adjustment of plasmid copy number resulting from the balance between positive and negative impacts of borne synthetic genes, plays a critical role in the global efficiency of multistep metabolic engineering. Differential expression of co-expressed engineered genes is frequently observed depending on growth phases, metabolic status and triggered adjustments of plasmid copy numbers, constituting a dynamic process contributing to minimize global engineering burden. A yeast model involving plasmid based expression of phosphoribulokinase (PRKp), a key enzyme for the reconstruction of synthetic Calvin cycle, was designed to gain further insights into such a mechanism. A conditional PRK expression cassette was cloned either onto a low (ARS-CEN based) or a high (2-micron origin based) copy number plasmid using complementation of a *trp1* genomic mutation as constant positive selection. Evolution of plasmid copy numbers, PRKp expressions, and cell growth rates were dynamically monitored following gene de-repression through external doxycycline concentration shifts. In the absence of RubisCO encoding gene permitting metabolic recycling, PRKp expression that led to depletion of ribulose phosphate, a critical metabolite for aromatic amino-acids biosynthesis, and accumulation of the dead-end diphosphate product contribute to toxicity. Triggered copy number adjustment was found to be a dynamic process depending both on plasmid types and levels of PRK induction. With the ARS-CEN plasmid, cell growth was abruptly affected only when level PRKp expression exceeded a threshold value. In contrast, a proportional relationship was observed with the 2-micron plasmid consistent with large copy number adjustments. Micro-compartment partitioning of bulk cultures by embedding individual cells into inverse culture medium/oil droplets, revealed the presence of slow and fast growing subpopulations that differ in relative proportions for low and high copy number plasmids.

Keywords: plasmid burden, cell toxicity response, population heterogeneity, metabolic engineering

Download English Version:

<https://daneshyari.com/en/article/8842849>

Download Persian Version:

<https://daneshyari.com/article/8842849>

[Daneshyari.com](https://daneshyari.com)