The stability of cell populations with a burdensome gene circuit depends on a fitness-entropy compensation mechanism

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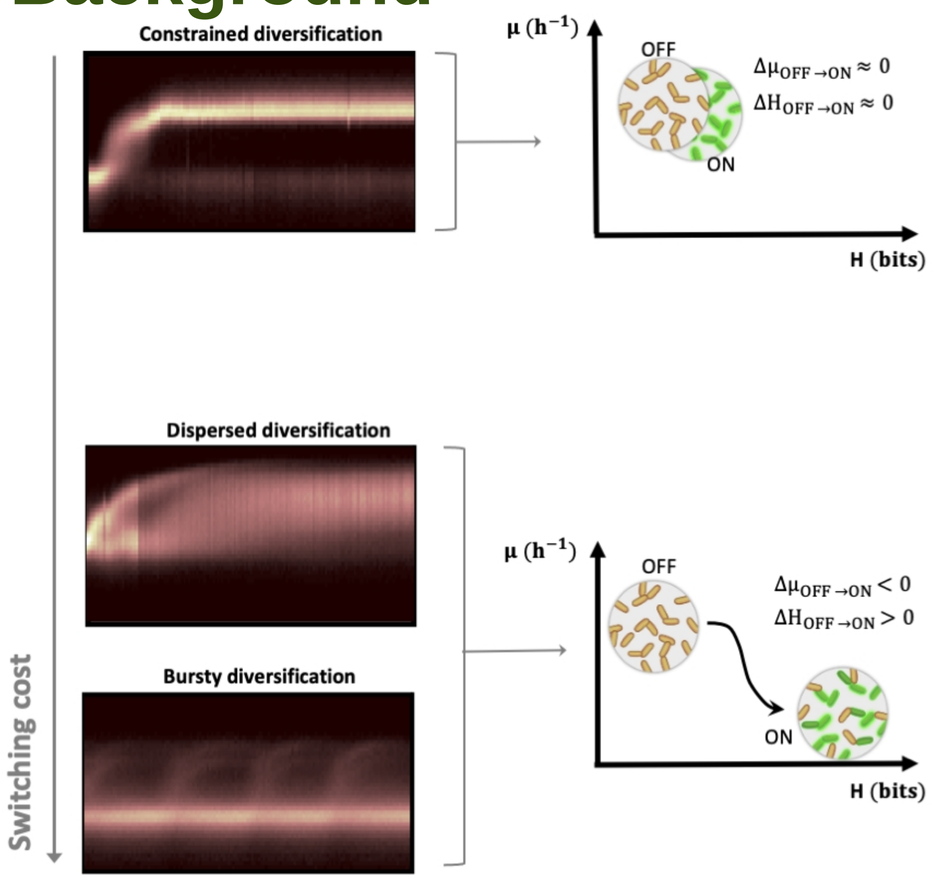
Introduction

Understanding gene expression dynamics at a single cell resolution is crucial for the effective control of a population phenotype. Our previous work revealed a connection between gene expression heterogeneity and fitness cost; as the cost associated with gene activation increases, so does population heterogeneity [1]. We observed that the activation of burdensome gene circuits is accompanied by an increase of phenotypic heterogeneity, measured here as the information entropy.

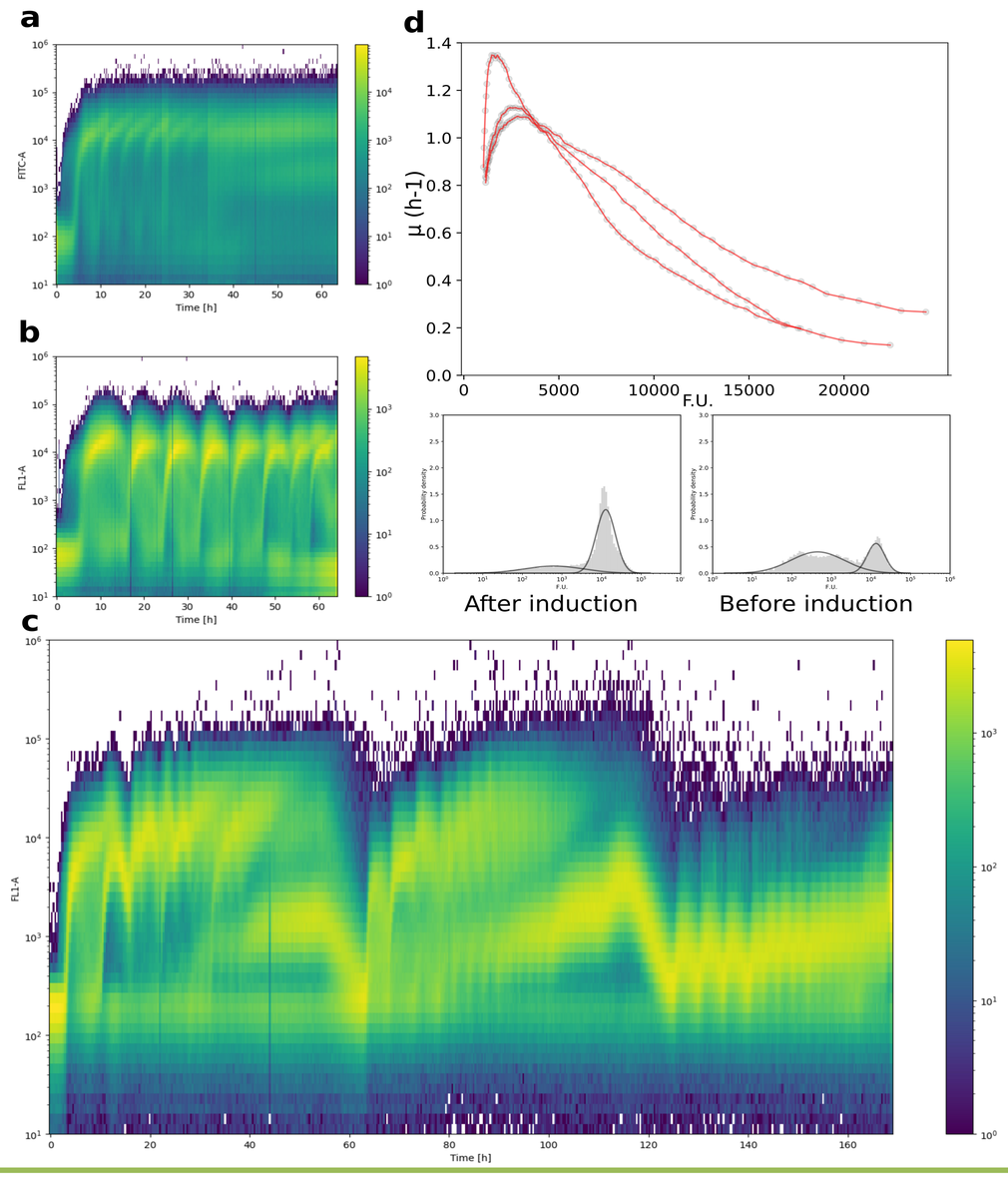
[1] Henrion, L., Martinez, J.A., Vandenbroucke, V. et al. Fitness cost associated with cell phenotypic switching drives population diversification dynamics and controllability. Nat Commun 14, 6128 (2023). https://doi.org/10.1038/s41467-023-41917-z

Background



We have previously observed that as the fitness cost associated with the activation of a gene circuit increases, the population becomes more and more heterogeneous. Based on this, we identified three diversification regimes: 1) constrained for low switching cost; 2) dispersed for intermediate cost; 3) bursty, where the population synchronizes itself, for high fitness cost, such as sporulation in *Bacillus subtilis*. Interestingly, applying a periodic induction profile instead of continuous one has been shown to homogeneize the induction of systems associated with a high fitness cost (similar to a high production load).

Results



a) Chemostat cultivations of *E. coli* BL21 are characterized by a significant degree of heterogeneity in induction (up to 10,000x) of the T7 expression system.

b) When applying a pulse-based strategy, the population is globally more homogeneous, and diversification occurs during the de-induction phase.

d) Analysis of the growth rate per fluorescence level reveals a "fitness entropy compensation" mechanism where cells that were less induced overgrow the others, thus amplifying population heterogeneity.

c) Trying to circumvent this phenomenon by pulsing faster and faster leads to the occurrence of a sub-population composed of a mutant of BL21 with a weakened lac promoter.

Outlook

In a continuous cultivation set-up, an expression system cannot be both strong (fitness cost) and homogeneously express across the population. Heterogeneity arises simply because less induced cells grow faster than fully induced ones. In a way, phenotypic heterogeneity safeguards the population and we have shown that trying to eliminate it leads to genotypic escape. Maybe we should stop trying to eliminate it and instead accept that a pocket of heterogeneity is beneficial. Another approach could consist into exploiting it to produce genetically distinct sub-populations with a differentiation system such as Bxb1. Such method would make population induction tunable by ajusting the proportion of “producers” and more genetically stable by maintaining “growers”.

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