

Dynamic control of the *rpoS* regulon in *E. coli* populations

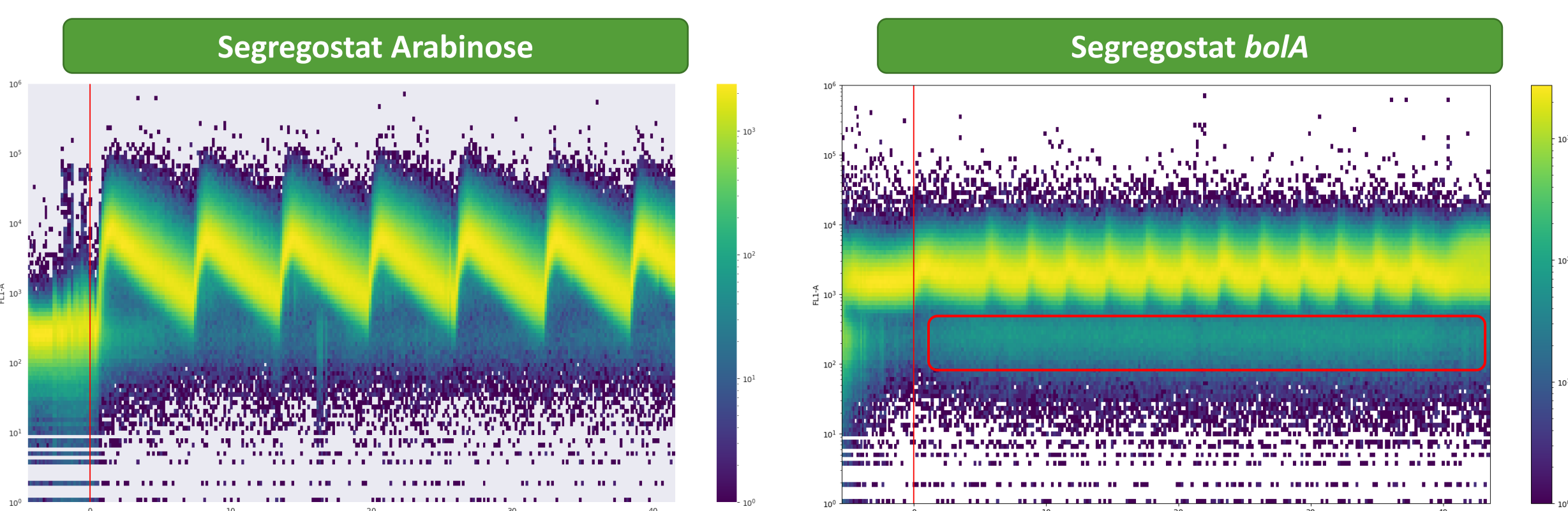
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Introduction

Bacteria undergo alternating periods of rapid growth and nutrient deprivation during which additional stresses may occur. To face those environmental challenges, they rely on effective sensing and responding strategies leading to physiological adaptations. They also count on cell-to-cell heterogeneity resulting in sub-populations of cells differently prepared for future stress, with thus more or less chance to survive. Many stress-related genes have nontrivial expression dynamics. In addition to being upregulated during entry into stationary phase, they are among the noisier genes. They are thus involved in both survival strategies. That has an impact on the population survival in nature but can have implications for bioprocesses as well. In this work, we will study the general stress response in *Escherichia coli*, focusing on the *rpoS* regulon and the control of related genes expression. Through the Segregostat the population can be controlled to oscillate between two phenotypes, active growth, and stress resistance. This feature will be exploited in two applications: the control of persister cells upon antibiotic treatment and microbial population robustness enhancement for a given bioprocess.

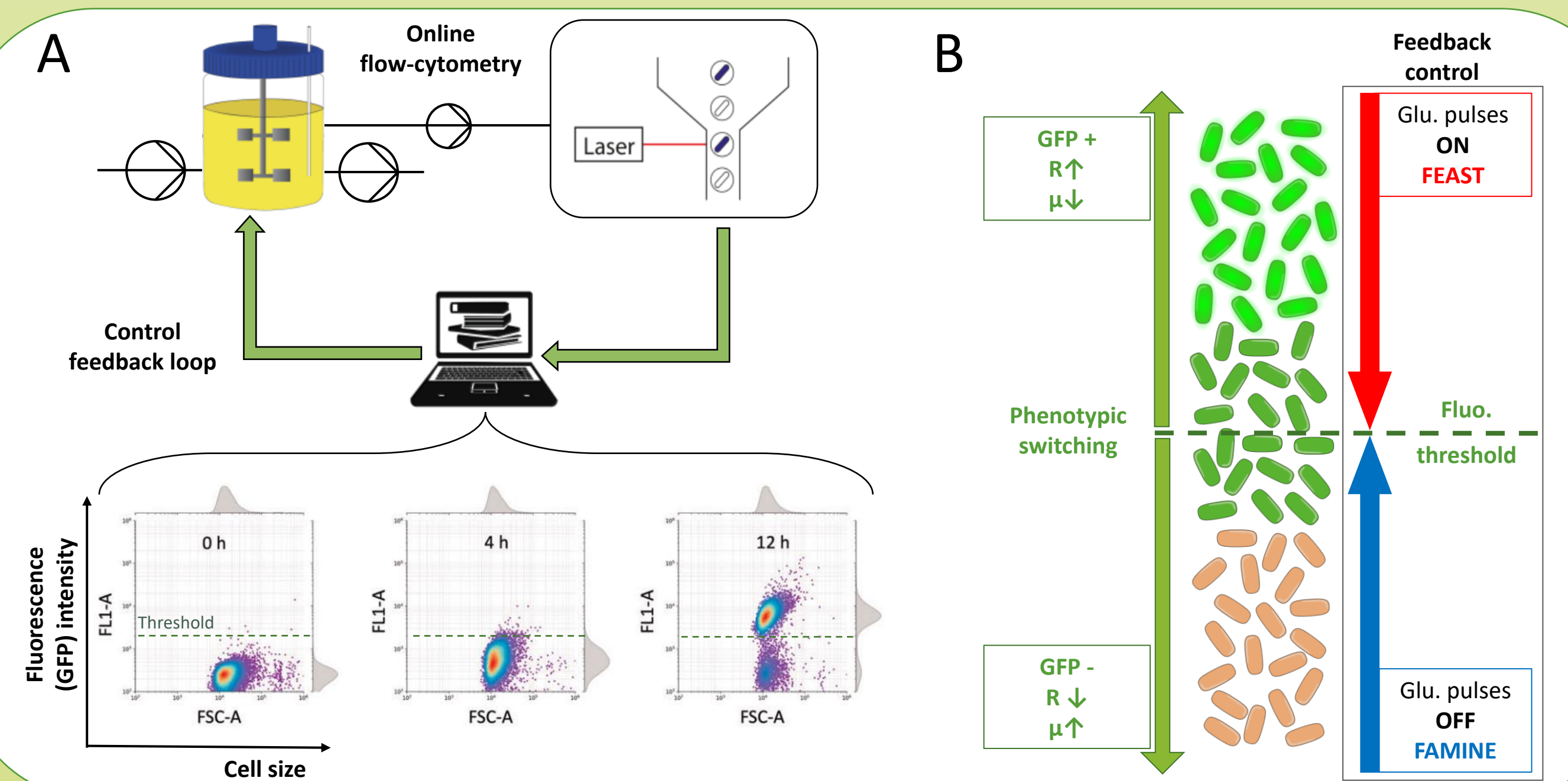
Preliminary results



It is possible to synchronize the expression of *bolA* in continuous culture by pulsing glucose at a specific frequency (determined automatically by the Segregostat). But this gene keeps a stochastic component in comparison to other controlled genes. [1]

- Do different stress response related genes keep a stochastic component when dynamically controlled?
- Is it due to specific regulatory components?

The Segregostat



A The Segregostat is a cell-machine interface allowing the control of the cell population through adjustment of environmental conditions based on the phenotypic profile of the population. [1]
B *E. coli* stress response to nutrient availability (based on a stress transcriptional reporter, e.g., *P_{bolA}:gfpmut2*) was controlled through glucose pulses.

Control of stress-related genes

A. Reporters screening

Screening of ±30 representative transcriptional reporters

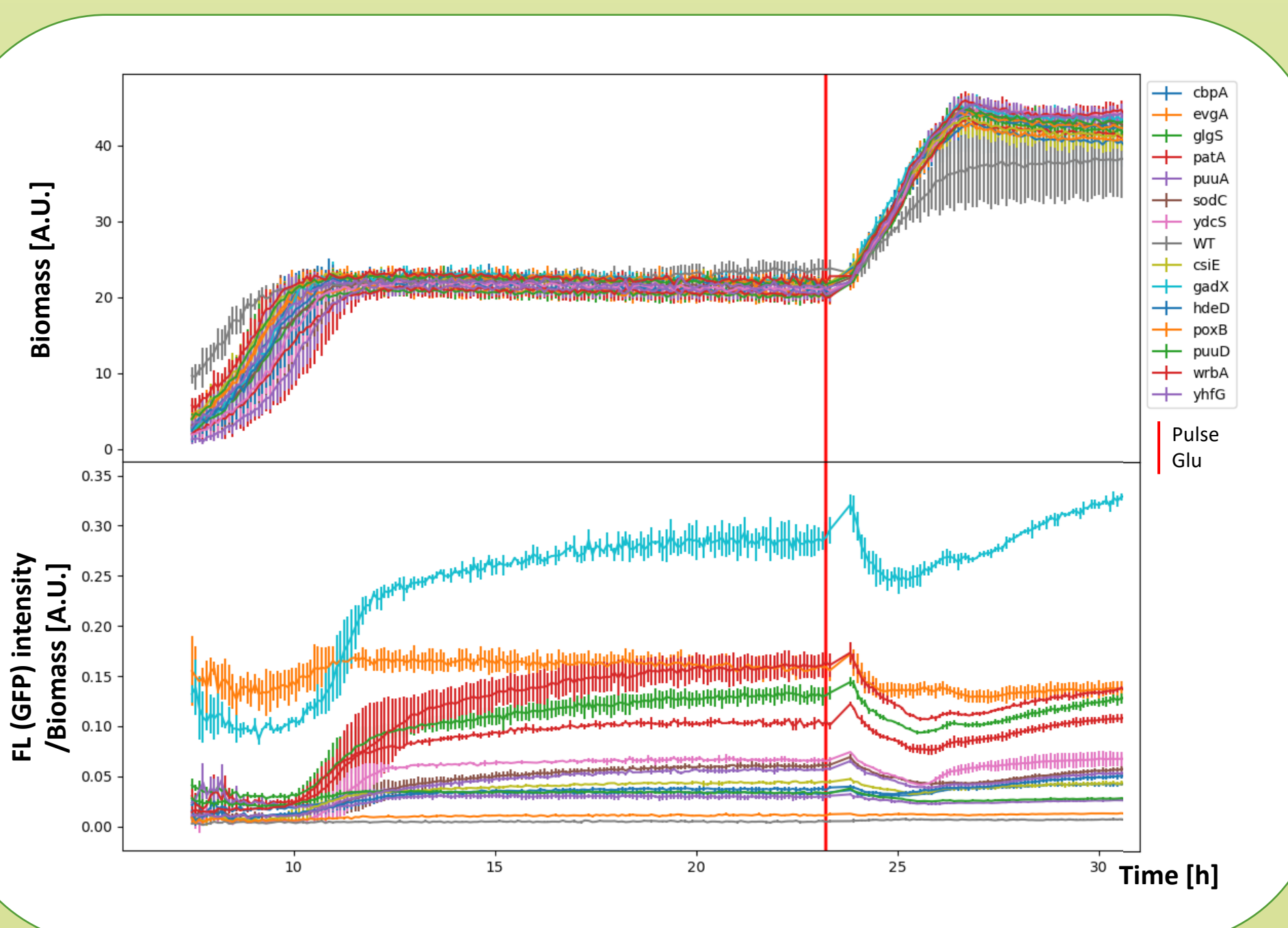
Control frequency : $f(\text{dynamic range of reporters})$ [1]

◆ Those reporters have most probably different control frequencies. True?

Not a unique control frequency for one gene, but several [1]

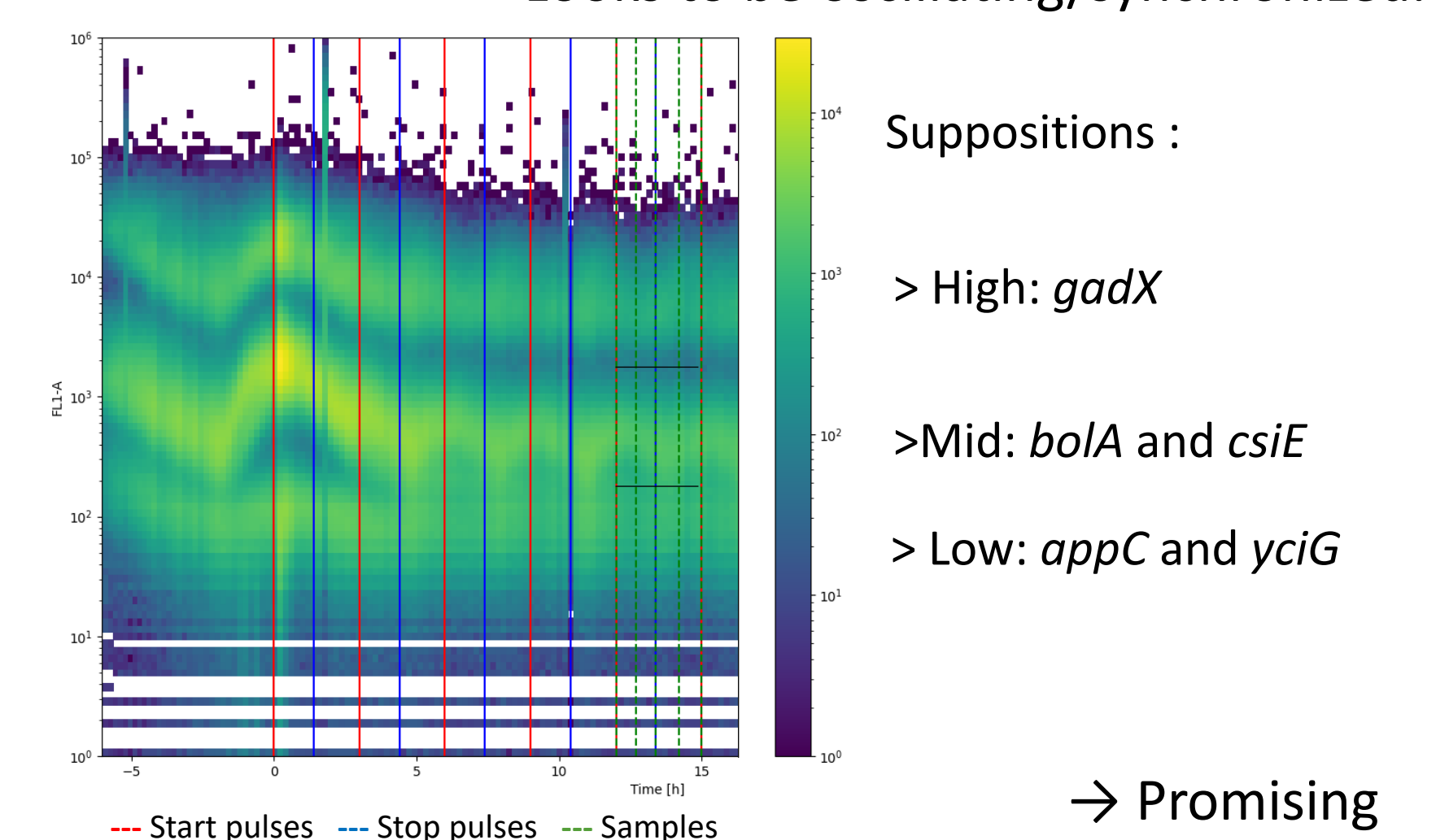
- ◆ Is there control frequencies common to several genes?
- ◆ Is there a control frequency that can optimize the synchronization of a maximum of genes of the same regulon?

► Useful for B. and C.



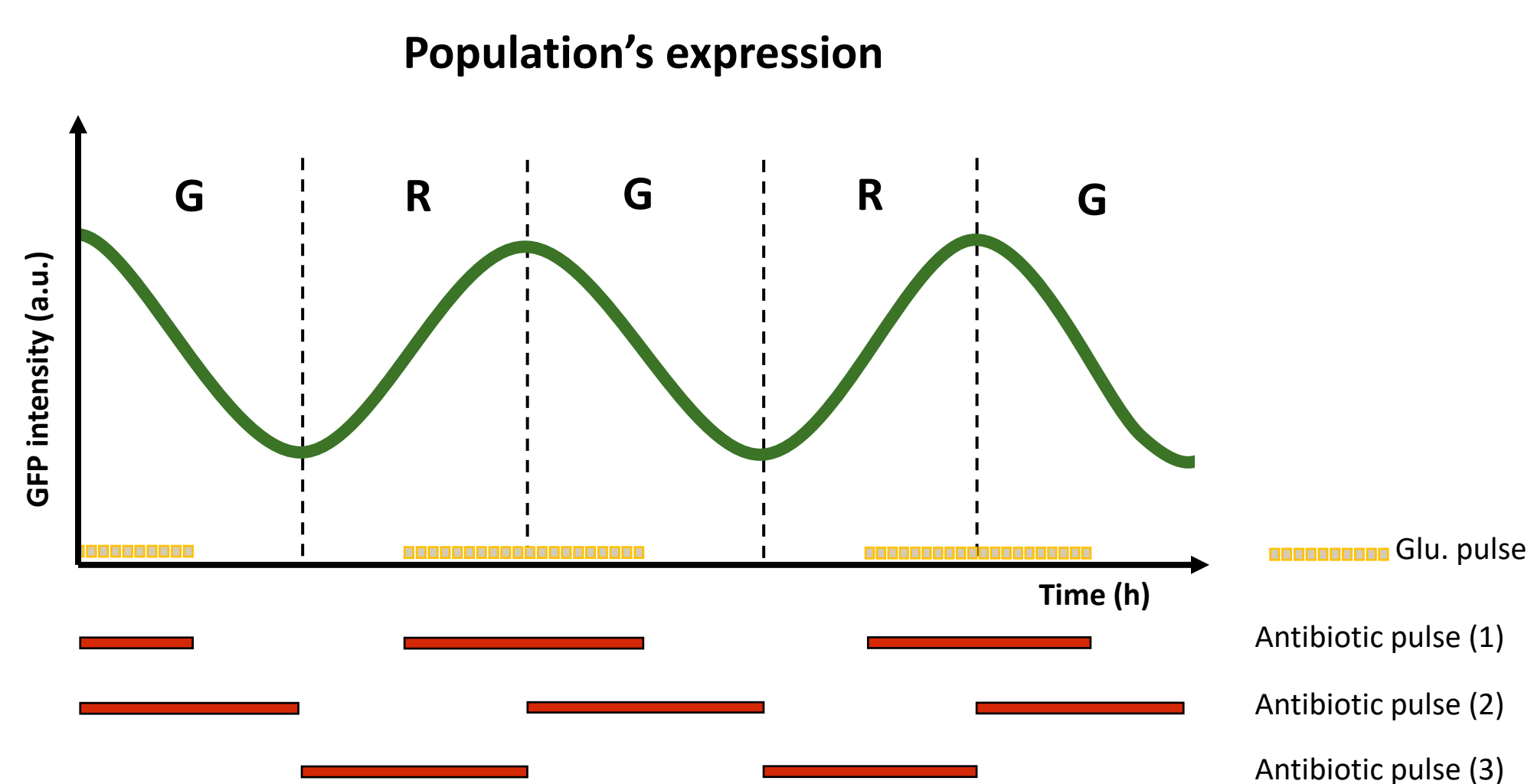
E. coli PARTY

5 reporters, 3 subpopulations
Looks to be oscillating/synchronized.



→ Promising

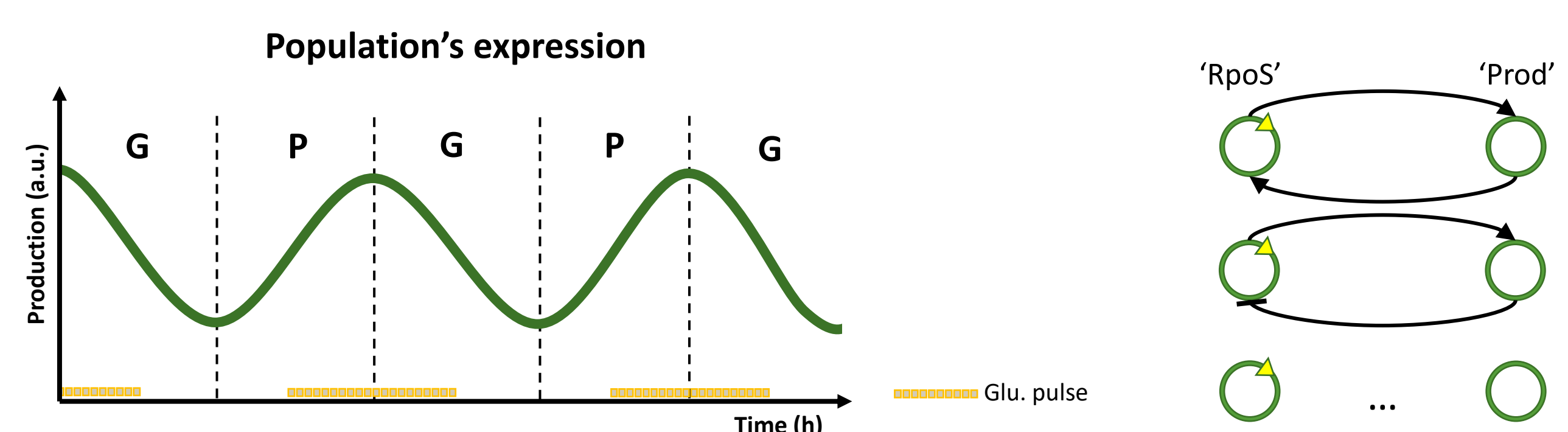
B. Study antibiotic persistence



- Segregostat to study antibiotic persistence
- Antibiotics pulses // control of Growth(G)/Resistance(R)
- Control through antibiotic pulsing - reverse ecology?

Antibio. could activate SR. Need to characterize:
(i) the antibio. persist. ((antibio.)/viability) at different level of stress,
(ii) how [antibio.] induces stress response.

C. Exploit stress to produce



Decoupling G & R//production(P)

"2-phase" + "dynamic control" in batch continuous culture

Different possibilities:

plasmid couples to link stress and prod

Link with inducer system (e.g., intermediary compound as inducer)

...

- ◆ If production under stress, would the resources be sufficient to produce efficiently?