INVESTIGATING CELLULAR RESOURCE ALLOCATION IN MAMMALIAN CELL LINES

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Introduction

Because of the limited availability of resources within living cells, these need to be efficiently distributed. Whenever an exogenous gene construct is introduced, it sequesters transcriptional and translational machinery that would otherwise be destined to the production of host genes[1]. As a result, an increase in the construct's expression is linked to a reduction in the production of host proteins (gene coupling)[2], and changes in growth rate[3]. While this process has been widely described in bacteria, **further research needs to be done in mammalian cells.**

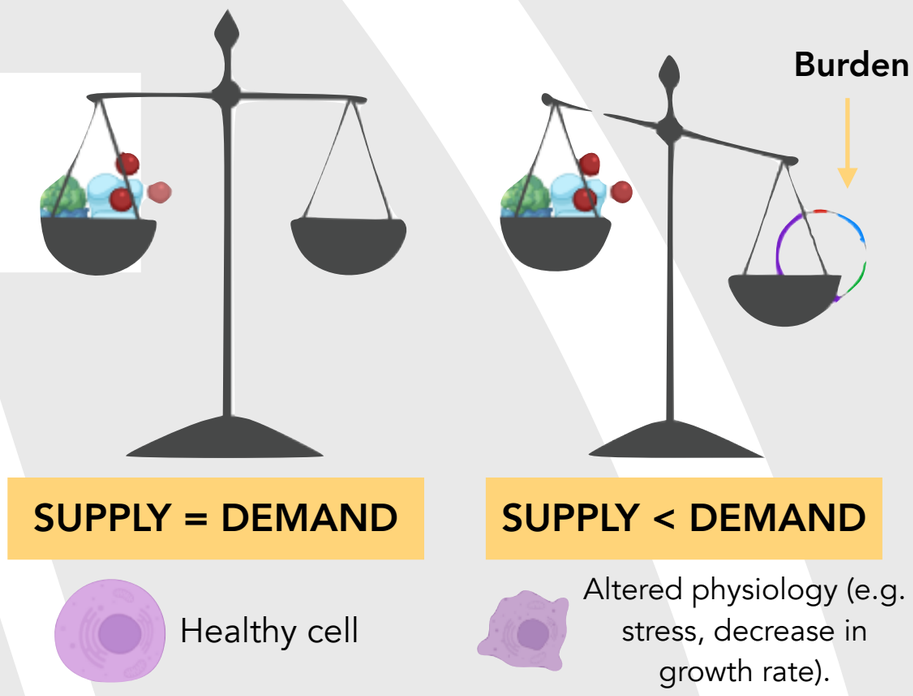

Figure 1. *Genetic circuits are burdens for the host cell.* The introduction of an exogenous gene construct generates an imbalance between the supply and demand of resources, which could negatively affect the host cell's physiology.

AIMS

- Describe the impact that exogenous gene constructs have on mammalian cell's growth rate.
- Identify the major constraints in cellular resource allocation: are RNA polymerases more restricting than ribosomes or *vice versa*?

Materials and Methods

Model organism: Chinese Hamster Ovary (CHO) cell line

- Construction of a vector expressing two fluorescent reporter proteins, one constitutive (GFP) and one inducible (RFP), through Golden Gate Assembly techniques. Expression levels of GFP will be measured as different doses of RFP inducer are administered.
- Analysis of intracellular components (RNA and proteins) and growth rate when translation or transcription is inhibited. Changes in growth will be measured through a colorimetric assay, correlating the acidification of the medium with the Population Growth Factor.
- The measurements will be repeated at least three times and verified by statistical analysis.

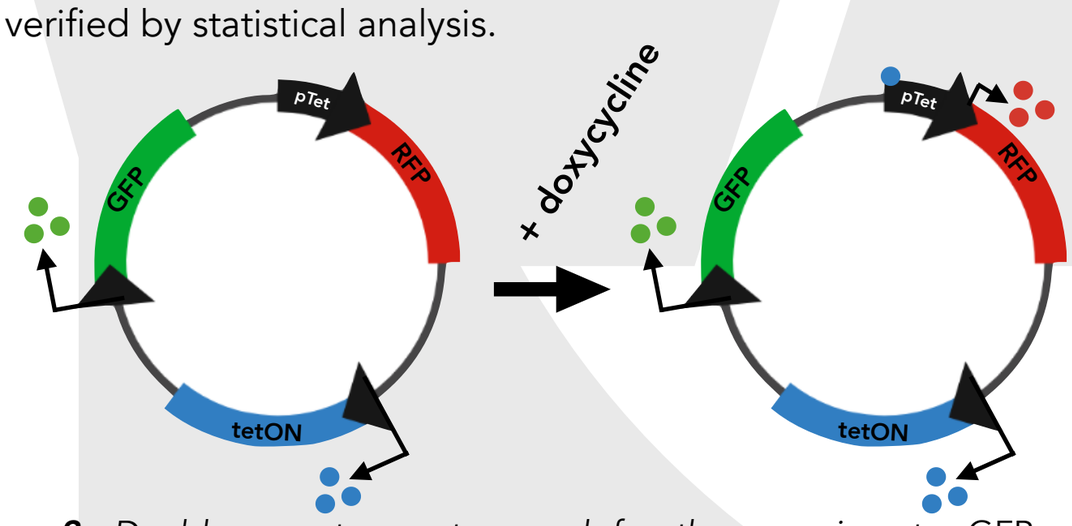

Figure 2. *Double reporter vector used for the experiments.* GFP will be constitutively expressed, while RFP will be only synthesised when doxycycline is administered.

Risks and mitigation

- If the plasmid is not correctly assembled, a simpler system could be used, subtracting the constitutive gene. In this case, the burden of the construct will only be measured in terms of growth rate.
- To prevent plasmid loss due to evolutionary pressure, viral origins of replication could be used to increase the copy number.

Expected results

Similarly to that observed in bacteria, GFP expression levels (symbolising host genes) are expected to decrease as RFP (symbolising genetic circuits) is induced, together with the growth rate.

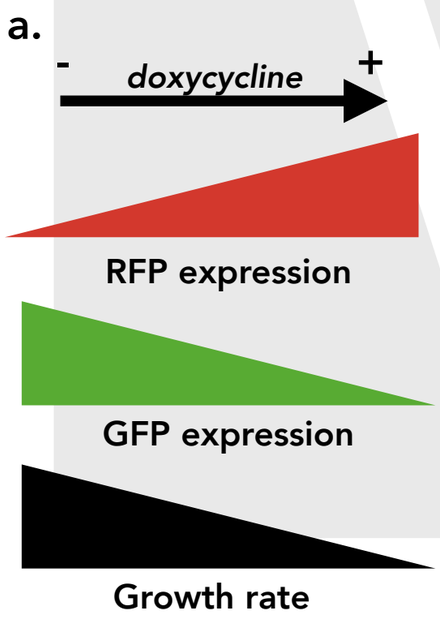

Figure 3. *Expected effect of inducible RFP on constitutive GFP and growth rate, as a result of gene coupling (a). When the burden of the genetic construct is reduced, no effect on GFP expression or growth rate should be observed (b).*

Future perspectives

Once the effect of gene coupling in mammalian cells has been experimentally determined, the goal is to produce constructs that generate a lower (ideally none) burden on the cell. These could be achieved by altering regulating sequences (e.g. Ribosome Binding Sites), knocking out non-essential genes or introducing enzymes that would only transcribe the synthetic construct.

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