Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Controlling and exploiting cell-to-cell variation in metabolic engineering

Tiebin Wang[1,3] and Mary J Dunlop[2,3]

Individual cells within a population can display diverse phenotypes due to differences in their local environment, genetic variation, and stochastic expression of genes. Understanding this cell-to-cell variation is important for metabolic engineering applications because variability can impact production. For instance, recent studies have shown that production can be highly heterogeneous among engineered cells, and strategies that manage this diversity improve yields of biosynthetic products. These results suggest the potential of controlling variation as a novel approach towards improving performance of engineered cells. In this review, we focus on identifying the origins of cell-to-cell variation in metabolic engineering applications and discuss recent developments on strategies that can be employed to diminish, accept, or even exploit cell-to-cell variation.

Addresses
[1] Molecular Biology, Cell Biology & Biochemistry Program, Boston University, Boston, MA, USA
[2] Department of Biomedical Engineering, Boston University, Boston, MA, USA
[3] Biological Design Center, Boston University, Boston, MA, USA

Corresponding author: Dunlop, Mary J (mjdunlop@bu.edu)

Current Opinion in Biotechnology 2018, **57**:10–16

This review comes from a themed issue on **Energy biotechnology**

Edited by **Cynthia Collins** and **Danielle Tullman-Ercek**

https://doi.org/10.1016/j.copbio.2018.08.013

Introduction

As microscopy, flow cytometry, and other single-cell measurement technologies advance, researchers have begun to compare measurements of individual cells to bulk population averages. These studies have revealed that variability between cells can be significant, suggesting that population-level averages may obscure underlying heterogeneity [1–4]. Cell-to-cell variation can be caused by many factors including genetic differences, phenotypic heterogeneity, and differences in the local microenvironment. In natural contexts, this type of variability can play an important functional role, such as reducing burden of costly protein expression or increasing survival in changing environments [5–7]. In this review, we discuss the origins of variation relevant to metabolic engineering and highlight recent examples of strategies to control diversity that hold promise for improving production yields.

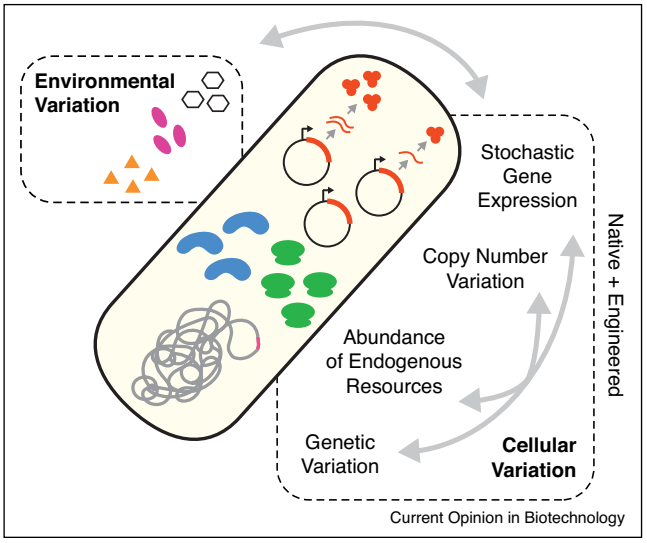
Despite the prevalence of cell-to-cell variation in nature, it is not traditionally studied in the context of metabolic engineering applications. The reasons behind this are practical, as it can be technically challenging to measure single-cell level effects, and reporters and methods for quantifying metabolically-relevant states often do not exist. However, the importance of understanding cell-to-cell differences is highlighted by recent metabolic engineering studies that have shown that cell-to-cell variation can impose a significant impact on production. For example, Xiao *et al.* demonstrated that 15% of cells in an isogenic *Escherichia coli* population of free fatty acid producers were responsible for over half of the total product [8••]. In another study, approximately a third of cells in cultures of the production host *Bacillus megaterium* were shown to persist in a low production state, regardless of culturing conditions [9]. These studies suggest that managing cell-to-cell-variation may offer a potential approach for further optimization of production pathways, which can be used in concert with traditional metabolic engineering strategies.

In this review, we discuss the origins of cell-to-cell variation in metabolic engineering and strategies to control variability. We divide the origins of variation into environmental and cellular categories, the latter of which includes variation due to native and engineered components, and the interplay between them. We then discuss strategies for controlling and exploiting variation in metabolic engineering contexts. These range from diminishing, to accepting, to actively creating variation within populations of cells. Finally, we describe technological advances that would help to facilitate quantification and the engineering of control strategies.

Origins of cell-to-cell variation in metabolic engineering

Cell-to-cell variation in metabolic engineering applications can be divided into two categories. First, environmental variation, which is due to the impact of gradients in local conditions, such as nutrient availability or extracellular product levels. Second, cellular variation, which is due to properties internal to the cell, such as

Figure 1



Origins of cell-to-cell variation in metabolic engineering. Sources of variation can be divided into environmental and cellular variation. Environmental variation originates from heterogeneity in the local environment, such as due to poor-mixing in a large-scale bioreactor. Cellular variation can result from both native and engineered pathways due to genetic diversity or phenotypic heterogeneity. Significant interplay exists between environmental and cellular variation, and between native and engineered pathways.

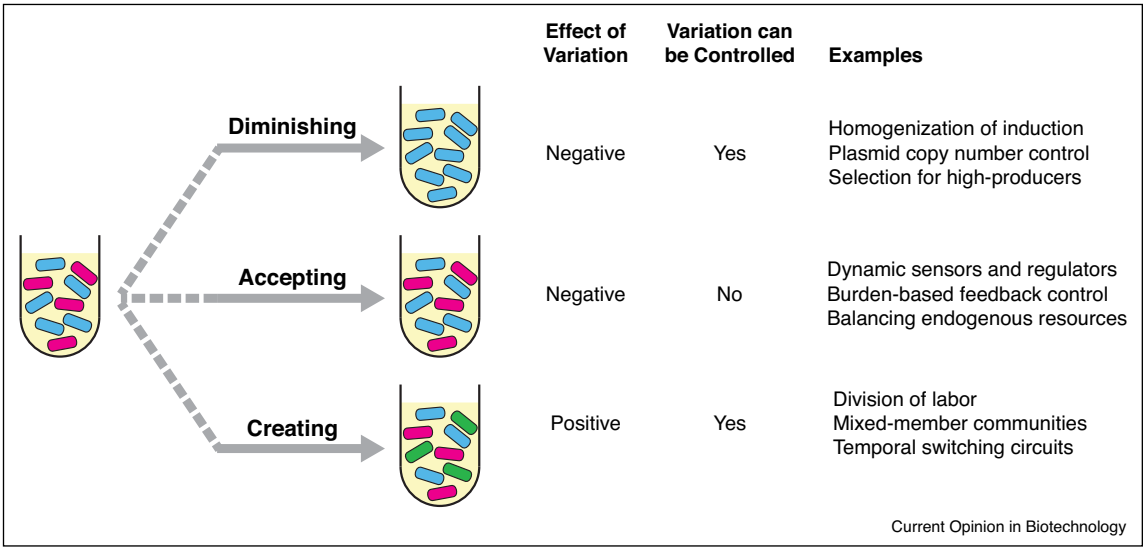
heterogeneity in cellular resources or intracellular product levels (Figure 1).

Although we generally assume that production environments are homogeneous, several studies have demonstrated that this assumption is not entirely valid as volume scales are increased. Within industrial scale bioreactors, mixing becomes challenging due to high viscosities and large volumes [10]. Consequently, production variation can occur even within isogenic cell populations due to the fact that cells are exposed to different local conditions within the same bioreactor. Most approaches for increasing homogeneity within scaled-up bioreactors are mechanical and aim to ensure even mixing [11–13], however it is also possible to design genetic circuits that work to mitigate the effects of this nonuniformity.

Cellular variability is impacted by both native properties, such as ribosome and ATP levels, and heterologous factors, such as expression of a burdensome, non-native enzyme. In addition, diversity can arise due to the interplay between these native and engineered components. For instance, some cells may have a higher capacity for expression of a synthetic circuit than others due to single-cell level differences in transcription or translation machinery.

Examples of endogenous sources of cell-to-cell variation are genetic diversity and phenotypic heterogeneity in expression of native pathways. Genetic diversity can arise from mutations accumulated during the production process, which can lead to production differences between cells [14••]. Alternatively, genetic differences may be specifically engineered, such as in applications that employ different stains or species in co-cultures for biosynthesis [15••]. In contrast to variation due to genetic changes, phenotypic heterogeneity, which is commonly

Figure 2



Strategies for coping with cell-to-cell variation in metabolic engineering. Depending on the circumstances, the optimal engineering strategy may be to diminish variation, accept variation but mitigate its negative impact, or create and exploit cell-to-cell variation.