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Stochasticity in cellular metabolism and growth: Approaches and consequences

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Abstract

Advances in our ability to zoom in on single cells have revealed striking heterogeneity within isogenic populations. Attention has so far focussed predominantly on underlying stochastic variability in regulatory pathways and downstream differentiation events. In contrast, the role of stochasticity in metabolic processes and networks has long remained unaddressed. Here we review recent studies that have begun to overcome key technical challenges in addressing this issue. First findings have already demonstrated that metabolic networks are stochastic in nature, and highlight the plethora of cellular processes that are critically affected by it.

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Stochasticity and metabolism

Elucidating the role of molecular stochasticity in metabolic processes is a central issue in cellular physiology. It is key to understanding cellular homeostasis, and could help explaining heterogeneous phenotypes ubiquitously observed across all domains of life, ranging from persistence to cancer [1,2]. Stochasticity in metabolism could underlie bet-hedging strategies, in which distinct sub-populations anticipate future environmental change [3,4]. On the other hand, metabolic stochasticity could limit optimal growth and require regulatory mechanisms to ensure homeostasis [5]. More generally, as metabolism ultimately drives all cellular processes, fluctuations and instability could impact a myriad of phenomena ranging from the cell cycle to differentiation events. So far however, stochastic variability is commonly considered to have negligible effects in metabolic networks, as reflected by current theoretical models [6]. Indeed, metabolic fluctuations may be insignificant because of averaging over the many reaction events underlying metabolism in cells, chemical equilibration, metabolite secretion, or a lack of limiting steps within metabolic pathways [6–13].

At the practical level, quantifying any type of metabolic fluctuations comes with its own specific challenges. In contrast to regulatory proteins within signaling networks, which can be tagged fluorescently, metabolites are difficult to visualize at the single-cell level. Metabolites can be quantified by single-cell mass spectrometry [14], but so far not dynamically in time. Spectroscopic methods can follow metabolite abundance in time, but only for specific highly abundant molecules such as lipids [15]. FRET and fluorescent sensors hold a lot of promise, but remain limited to some metabolites and cannot yet quantify stochastic fluctuations [16–20].

Recently, important progress has been made in developing novel approaches that circumvent these limitations. In this review, we will examine these new efforts, their first findings, as well as related theoretical modeling. We will also cover recent work that is addressing the impact metabolic variability has on other cellular phenomena.

Enzyme expression generates metabolic noise

Early single-cell experiments showed how the expression of transcription factors fluctuate and propagate to downstream genes [21–23]. Similarly, such expression noise in key metabolic enzymes could generate variations in the flux of the reaction they catalyze, even if reaction-event noise averages out [24]. Moreover, if these flux variations propagate down-stream along the pathway, they could produce variations in the rate of cellular growth. A recent study by Kiviet et al. [25] was based on this premise. While such an approach presents the challenge of quantifying enzyme expression and cellular growth with high accuracy, it avoids the need to measure fluctuations in metabolite concentrations.

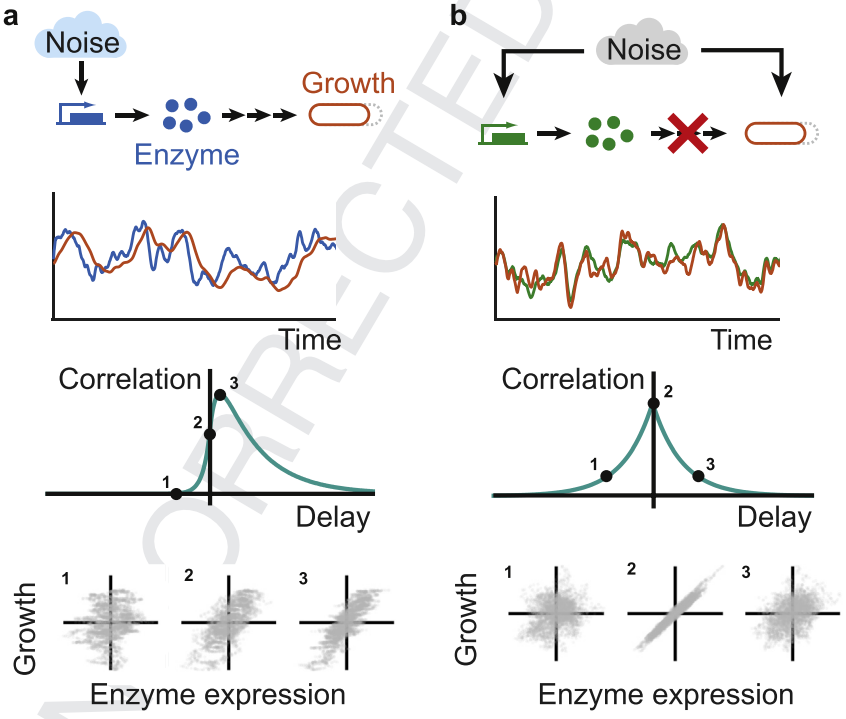
Growth was quantified by following the size of individual cells by time-lapse microscopy. Specifically, using the known overall shape of *Escherichia coli* – a rod capped with half-domes – its length could be determined to

below the diffraction limit, which may be compared to how fluorophores are positioned in super-resolution microscopy [25]. Currently, a range of different single-cell image analysis approaches are available [26–33], including ones utilizing machine learning [34–36]. Cellular growth has also been quantified by measuring cellular dry mass [37], and by using AFM-like cantilevers [38], as will be discussed more exhaustively below.

The data on the instantaneous cellular growth rate appeared correlated with the expression of metabolic enzymes [25]. However, such correlations could signal that growth fluctuations perturb expression, rather than the other way around. Time dependent correlation analysis can be used to address this issue [21,22] (Figure 1). This approach showed that the correlations were on average stronger after a certain delay, consistent with enzyme production fluctuations happening first, and growth fluctuations happening some time later (Figure 1a). In line with the idea that enzyme (expression) fluctuations affect the flux of the reaction they catalyze, this delay was observed only for genes that were considered limiting, such as *gltA* and *icd* in acetate media, and *pfkA* and *icd* in lactose media.

Interestingly, even when considering non-limiting genes, the expression rate was still strongly correlated with growth – however the correlations were now instantaneous and did not show a delay (Figure 1b). It suggested that more generally, proteins are expressed significantly faster in cells that transiently grow faster, which is actually not unreasonable given that some cells grow twice as fast others for almost a full generation, and expression needs diverse metabolites. Put differently, fluctuations in growth-controlling factors, which may be anything from ribosomes to ATP, are also a source of gene expression noise [39]. In turn, metabolic fluctuations may thus affect processes that are controlled by gene expression, such as differentiation events [40,41]. Metabolic noise can be compared to other noise sources

Figure 1



Fluctuations from enzyme expression to metabolism, and from metabolism to enzyme expression. Expression measurements of a single metabolic enzyme and growth rates in individual cells can be used to reveal metabolic stochasticity. Two key modes of noise transmission have been observed, which can act both individually and jointly, and may interact. (a) Noise in the expression of a single enzyme (blue trace), result in fluctuations in metabolic flux that are transmitted through the metabolic network and affect growth with some time delay (orange trace). The delay can be quantified by cross-correlation analysis. The cross-correlation curve illustrates that on average, current enzyme expression correlates better with growth some time later, as illustrated by the expression-growth scatter plots. Note that the sources of expression noise here are not only intrinsic, or caused by molecular processes specific to one gene. They also include extrinsic or transmitted noise from other processes, such as transcription factor, polymerase, or metabolic factors such as amino acid abundance, which may affect expression but not growth. Noise sources that affect both expression and growth are discussed in panel b. (b) Noise sources within the metabolic network that perturb both expression (green trace) and growth (orange trace). Fluctuations in components that affect both expression and growth, such as ATP and other central metabolites, could define such sources of noise. In contrast to panel a, the cross-correlation here is symmetric because expression and growth respond approximately equally fast to the fluctuations. Note that the resulting expression noise may affect growth (panel a), or may not (this panel) – for instance because the expressed enzyme is not metabolically active or because it is abundant and hence does not limit growth.