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Visualizing translational errors – one cell at a time

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Abstract

Physiological heterogeneity among single cells with identical genetic information has been observed in a large number of bacterial phenotypes, including growth, stress responses, cell size, and antibiotic tolerance. Despite the widespread observation of this phenomenon in bacterial populations, not much is known about the molecular mechanisms behind phenotypic heterogeneity. Currently, our understanding is primarily limited to transcriptional profile of single cells using fluorescence reporters. Although the development of these tools has been extremely informative, it cannot fully explain the heterogeneity seen in populations. In a recent publication, Fan et al. have developed a dual-fluorescent reporter system that is capable of quantitatively measuring translational fidelity in single cells. It is shown that translational fidelity is heterogeneous and affects the growth characteristics of single cells. The development of tools for analysis of molecular heterogeneity downstream of transcription may play an important role in advancing our understanding of the physiology of bacterial populations.

Keywords

Protein Synthesis; Genetic Code; Single Cell

Gene expression heterogeneity within bacterial populations is best understood at the transcriptional level. Gene expression was first shown to be stochastic by Elowitz et al. (Elowitz, et al. 2002), where their work shows that two mostly identical copies of reporter genes are expressed at different levels in the same cell, especially when the transcriptional level becomes low. Later work demonstrates that transcription of individual genes is bursty with distinct on and off time periods, resulting in heterogeneous mRNA levels among single cells in a population (Golding, et al. 2005, Muramoto, et al. 2012, Raj, et al. 2006, Taniguchi, et al. 2010). Such work has led to a hypothesis that gene expression heterogeneity is a ‘bet-hedging’ mechanism that allows bacterial populations to survive a wide range of environments (Ackermann 2015). In *Escherichia coli*, the transcriptional noise of each promoter has been characterized (Silander, et al. 2012). It is shown that different promoters exhibit various levels of heterogeneity, and interestingly, stress response promoters appear to be the most heterogeneous. This provides further evidence that heterogeneity of gene expression is important for bacterial adaptation to various stress conditions.

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Transcription is the first step in gene expression, but is not fully responsible for the ultimate levels of proteins in the cell. As such, heterogeneity of transcription may not be fully representative of the phenotypic heterogeneity observed in bacterial populations. Changes in mRNA stability, translation, and protein stability within individual cells are also expected to play important roles in defining gene expression noise and consequently the phenotypic heterogeneity. However, it has been challenging to experimentally measure these sources of gene expression noise due to underlying fluctuations in transcription. Additionally, a variety of more-subtle mechanisms can affect transcript levels, including sub-cellular localization of mRNA and ribonucleases (Redder 2016), further complicate investigations into the heterogeneity of transcription.

As the last step in gene expression, changes in protein synthesis can have a large impact on cell physiology. For instance in *Salmonella*, decrease in protein synthesis via treatment with ribosomal inhibitors results in drastic changes in gene expression and bacterial behavior (Brunelle, et al. 2014). At the single cell level, altering protein synthesis rates has been shown to generate physiologically distinct subpopulations, e.g., bacterial persisters due to increased HipA toxin activity that decreases translation (Germain, et al. 2015). Changes in ribosomal stalling and rescue affect both translation and mRNA stability (Hayes and Sauer 2003, Jin, et al. 2016), however whether this affects single cell heterogeneity in a population is currently unknown.

In addition to changes in protein synthesis rate, changes in translational fidelity also affect cell behavior. Incorporation of noncognate amino acids into proteins leads to alteration of protein activity or activation of stress responses, which can positively or negatively impact cell physiology (Fan, et al. 2017b, Pan 2013, Pouplana, et al. 2014, Reynolds, et al. 2010). In the past decade, there has been increasing evidence that translational fidelity is regulated by environmental stresses, such as nutrient starvation (Ballesteros, et al. 2001, Zaborske, et al. 2014), oxidative stress (Bullwinkle, et al. 2014, Ling and Söll 2010, Netzer, et al. 2009), and temperature shift (Meyerovich, et al. 2010, Schwartz and Pan 2016), and that increased translational errors facilitate bacterial survival under severe oxidative and antibiotic stresses (Fan, et al. 2015, Javid, et al. 2014, Su, et al. 2016). To understand the heterogeneity of translational fidelity and its role in environmental adaptation, sensitive reporters to quantitate translational errors in individual cells are needed, which have been recently developed by several groups for stop codon readthrough (Fan, et al. 2017a), frameshifting (Fan, et al. 2017a, Rakauskaite, et al. 2011), and missense errors (Gomes, et al. 2016, Su, et al. 2016).

To separate transcriptional noise from translational noise, we have developed a dual-fluorescent reporter fusion system to measure levels of stop codon readthrough and frameshifting errors in single bacterial cells (Fan, et al. 2017a). Like transcription rates, ribosomal accuracy is also shown to be heterogeneous in bacterial populations. Interestingly, this reporter system has also revealed that increased errors during protein synthesis may positively affect a subpopulation of cells, as cells with higher UGA stop codon readthrough recover from stationary phase faster than cells with low read-through.

The molecular mechanisms that affect the heterogeneity of stop codon readthrough are still not fully understood. When a ribosome encounters a stop codon in *E. coli*, the codon is

recognized by release factor 1 (RF1) or 2 (RF2), then release factor 3 catalyzes the release of the ribosome and RF1 or RF2 complex from the mRNA (Youngman, et al. 2008). Meanwhile, charged tRNAs can compete with the release factors and allow further elongation along the mRNA. Population-based experiments suggest that increased levels of tRNA enhance stop codon readthrough (Fan, et al. 2017a). It is reasonable to hypothesize that variation in the levels of tRNAs and release factors among single cells accounts for the heterogeneity of stop codon readthrough in a population, which needs to be directly tested in future studies.

The current reporter systems for translational fidelity contain several technical limitations. For example, translational errors that have very low rates, such as most missense errors and readthrough of more stringent stop codons like UAA, cannot be quantified in individual cells at present. Also, quantitation of multiple types of errors within a single cell remains challenging, due to the limited number of orthogonal fluorescence reporters currently available. The development of more sensitive and versatile fluorescence reporters is therefore warranted.

Increasing evidence suggests that phenotypic heterogeneity within communities is critical for bacterial adaption to environmental changes. The recently developed tools to quantitate translational errors in single cells can be applied to address many key questions in future studies. For instance, what causes noisy translational errors in single cells? How do environmental conditions affect the level and heterogeneity of translational errors? What is the role of translational fidelity during bacteria-host interaction? We are therefore entering an exciting new era of understanding the physiological impact of translational fidelity.

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Figure 1. Noise in gene expression impacts phenotypic heterogeneity

Fluctuation of the protein levels among individual cells of the genetically-identical population is a cumulative result from gene expression noise at various steps, including transcription by the RNA polymerase (RNAP), mRNA degradation, translation, and protein degradation. Gene expression noise contributes to the phenotypic heterogeneity that diversifies individual cells and provides a bet-hedging mechanism for the population to quickly adapt to changing environments.