Paper 1

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-018-07702-z OPEN

Escherichia coli can survive stress by noisy growth modulation

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Gene expression can be noisy, as can the growth of single cells. Such cell-to-cell variation has been implicated in survival strategies for bacterial populations. However, it remains unclear how single cells couple gene expression with growth to implement these strategies. Here, we show how noisy expression of a key stress-response regulator, RpoS, allows *E. coli* to modulate its growth dynamics to survive future adverse environments. We reveal a dynamic positive feedback loop between RpoS and growth rate that produces multi-generation RpoS pulses. We do so experimentally using single-cell, time-lapse microscopy and microfluidics and theoretically with a stochastic model. Next, we demonstrate that *E. coli* prepares for sudden stress by entering prolonged periods of slow growth mediated by RpoS. This dynamic phenotype is captured by the RpoS-growth feedback model. Our synthesis of noisy gene expression, growth, and survival paves the way for further exploration of functional phenotypic variability.

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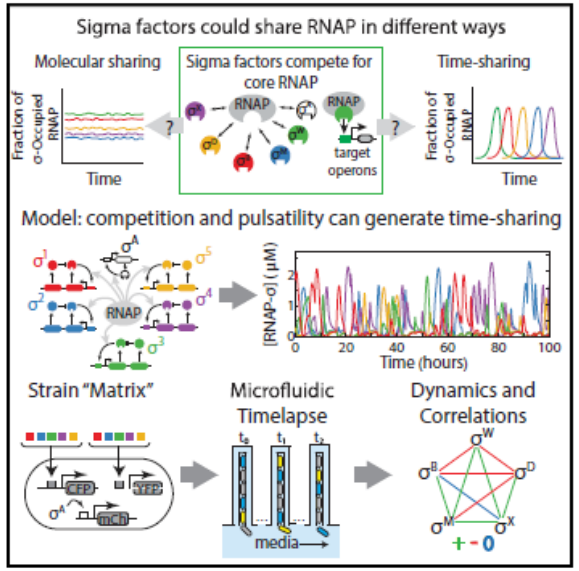
Paper 2

Cell Systems

Molecular Time Sharing through Dynamic Pulsing in Single Cells

Graphical Abstract



Highlights

- Alternative sigma factors activate in repetitive pulses under constant conditions
- Time-lapse movies reveal positive and negative dynamic correlations
- Sigma factors appear to compete with different strengths for core RNAP
- Modeling shows competing pulsatile sigma factors can dynamically "time share" RNAP

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In Brief

Cellular regulatory factors often compete for limited amounts of core enzymes. Sharing is typically assumed to involve statically partitioning core enzyme molecules. In contrast, using time-lapse movies, we find that *Bacillus subtilis* alternative sigma factors, which compete for core RNA polymerase, activate dynamically in stochastic, repetitive, hour-long pulses. Using mathematical modeling, we show how such pulsatile competitive circuits can effectively time share, or take turns using, core polymerase under similar conditions. Time-sharing represents an alternative mode of resource sharing in cells.

Park et al., 2018, Cell Systems 6, 216–229
February 28, 2018
https://doi.org/10.1016/j.cels.2018.01.011

CellPress

Paper 3

Probing Gene Expression in Live Cells, One Protein Molecule at a Time

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We directly observed real-time production of single protein molecules in individual *Escherichia coli* cells. A fusion protein of a fast-maturing yellow fluorescent protein (YFP) and a membrane-targeting peptide was expressed under a repressed condition. The membrane-localized YFP can be detected with single-molecule sensitivity. We found that the protein molecules are produced in bursts, with each burst originating from a stochastically transcribed single messenger RNA molecule, and that protein copy numbers in the bursts follow a geometric distribution. The quantitative study of low-level gene expression demonstrates the potential of single-molecule experiments in elucidating the workings of fundamental biological processes in living cells.

The central dogma of molecular biology states that DNA is transcribed into mRNA, which is then translated into protein. Ever since the pioneering work on the *lac* operon (*1*), our knowledge of gene expression has come primarily from genetic and biochemical studies (*2*–*4*) conducted with large populations of cells and molecules. Recently, many in vitro single-molecule experiments have probed real-time dynamics and yielded valuable mechanistic insights into macromolecules (*5*–*8*), including transcriptional (*9*) and translational (*10*) machineries. In order to understand the workings of these machineries in their physiological contexts, we set out to probe gene expression at the single-molecule level by real-time monitoring of protein production in live cells.

Gene expression is often stochastic (*11*–*14*), because most genes exist at single or low copy numbers in a cell. Some genes are expressed at high levels and others at low levels. The mRNA expression can now be tracked in a single cell with single-molecule sensitivity (*15*, *16*). The protein expression has been traditionally characterized by averages of cell populations, in which stochasticity is masked. More information is available from both the distribution of expression levels among a cell population (*17*–*19*) and the temporal evolution of a single cell by using fluorescent reporters (*20*). However, these studies have been restricted to high expression levels because of the low sensitivity for protein detection, yet many important proteins are produced at small copy numbers (*21*, *22*). Here, we demonstrate probing protein expression in individual *Escherichia coli* cells under the control of a repressed *lac* promotor, one molecule at a time (*23*).

The most popular reporters for monitoring gene expression in live cells are green fluorescent protein (GFP) and its derivatives, such as yellow fluorescent protein (YFP) (*24*–*26*). We use a YFP variant, Venus, as the reporter because of its short maturation time (*27*). However, it is difficult to image a single GFP or YFP molecule in cytoplasm, because its fluorescence signal spreads to the entire cytoplasm by fast diffusion during the image acquisition time and is overwhelmed by cellular autofluorescence. On the other hand, single YFP fusion protein molecules on cell membranes can be detected (*28*, *29*) because their diffusion is slowed. Therefore, we designed a fusion protein consisting of Venus and a membrane protein, Tsr, as the reporter for monitoring *lac* promoter activity. A well-studied methylation-dependent chemotaxis receptor protein (MCP) (*30*), Tsr contains two transmembrane domains and is fused to the N terminus of Venus.

We constructed an *E. coli* strain SX4 in which a single copy of the chimeric gene *tsr-venus* was incorporated into the *E. coli* chromosome, replacing the native *lacZ* gene. The endogenous *tsr* gene of *E. coli* was left intact. Because the *tsr* gene is highly expressed (*30*), a small amount of exogenous Tsr-Venus poses minimal perturbation to cells' normal functions. Western assay of induced SX4 cells showed the presence of Venus only in the membrane fraction and not in the cytoplasmic fraction, suggesting efficient membrane localization of Tsr-Venus. We also compared the levels of induced expression of Tsr-Venus and Venus in two strains, both under the control of the *lac* promoter [Supporting Online Material (SOM) Text and fig. S1]. No notable difference was observed, indicating that the introduction of the *tsr* sequence does not change the yield of Venus production, which is not the case for many other membrane-targeting sequences that we tested.

We first show the ability to detect single Tsr-Venus fluorescent protein molecules expressed in SX4 cells (Fig. 1). Figure 1A shows two diffraction-limited fluorescent spots [full width at half maximum (FWHM) ~ 300 nm] in the left cell. A line cross section of the fluorescence image along the cells' long axes shows the signal distinctly above the cells' autofluorescence background (Fig. 1C). We attribute each fluorescent peak to an individual Tsr-Venus molecule on the basis of abrupt disappearance of the signal upon photobleaching, which is characteristic of single molecules. Figure 1D shows such a photobleaching time trace. Had the signal arisen from multiple molecules, its disappearance would be in multiple steps. In addition, the fluorescence intensity of each peak is consistent with in vitro measurements of purified single Venus molecules (fig. S2).

A sketch of our live-cell experiment is shown in Fig. 2. Upon an infrequent and spontaneous

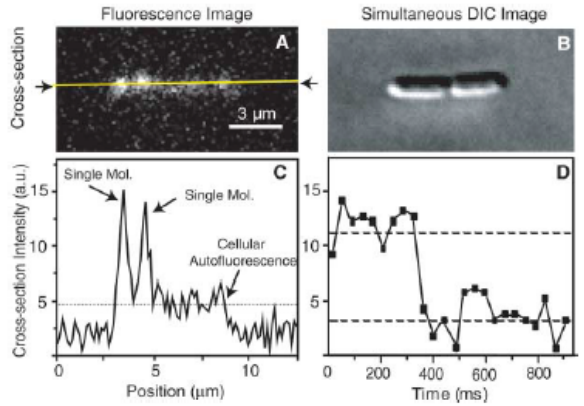

Fig. 1. Single-molecule detection of a fluorescent fusion protein, Tsr-Venus, in live *E. coli* cells. **(A)** Fluorescence and **(B)** DIC images of two *E. coli* cells (strain SX4) expressing Tsr-Venus. Two single fusion protein molecules were detected as diffraction-limited fluorescent spots (FWHM at ~300 nm) in the left cell. The fluorescence image is taken with 514-nm laser excitation and a 100-ms exposure time at 0.3 kW/cm². **(C)** Line cross section of the fluorescence signal along long axes of the two *E. coli* cells. a.u., arbitrary units. **(D)** Fluorescence time trace of a single Tsr-Venus molecule in an *E. coli* cell, showing abrupt photobleaching (40-ms exposure at 0.5 kW/cm²).

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Paper 4

Article

TRANSPARENT PROCESS OPEN ACCESS

molecular systems biology

Growth-dependent heterogeneity in the DNA damage response in *Escherichia coli*

Sebastián Jaramillo-Riveri[1,†], James Broughton[1,†], Alexander McVey[1], Teuta Pilizota[1], Matthew Scott[2] & Meriem El Karoui[1,*]

Abstract

In natural environments, bacteria are frequently exposed to sub-lethal levels of DNA damage, which leads to the induction of a stress response (the SOS response in *Escherichia coli*). Natural environments also vary in nutrient availability, resulting in distinct physiological changes in bacteria, which may have direct implications on their capacity to repair their chromosomes. Here, we evaluated the impact of varying the nutrient availability on the expression of the SOS response induced by chronic sub-lethal DNA damage in *E. coli*. We found heterogeneous expression of the SOS regulon at the single-cell level in all growth conditions. Surprisingly, we observed a larger fraction of high SOS-induced cells in slow growth as compared with fast growth, despite a higher rate of SOS induction in fast growth. The result can be explained by the dynamic balance between the rate of SOS induction and the division rates of cells exposed to DNA damage. Taken together, our data illustrate how cell division and physiology come together to produce growth-dependent heterogeneity in the DNA damage response.

Keywords bacterial physiology; DNA repair; single-cell
Subject Categories DNA Replication, Recombination & Repair; Microbiology, Virology & Host Pathogen Interaction
DOI 10.15252/msb.202110441 | Received 10 May 2021 | Revised 13 April 2022 | Accepted 27 April 2022
Mol Syst Biol. (2022) 18: e10441

Introduction

Bacteria are remarkable in their capacity to respond favourably to different environmental conditions, including variations in nutrient availability and perturbations from many different stresses such as oxidative damage or temperature changes. Natural environments vary in their levels of nutrients, affecting the growth of micro-organisms. For example, *Escherichia coli* has been estimated to divide every 3 h inside the intestine, whereas estimates for division time in the urine (bladder) are about 20–30 min (Myhrvold *et al*, 2015; Forsyth *et al*, 2018). These variations in growth rate can have important consequences for bacterial stress response because they impose constraints on the capacity of bacteria to modify their proteomes (Hui *et al*, 2015). This is particularly true for stresses induced by exposure to antibiotics, as the targets of most antibiotics are growth-related processes (Lewis, 2013) and variations in growth rate correlate with molecular and physiological changes in bacteria (Bremer & Dennis, 2008). For example, the analysis of the interplay between growth-related changes and the response to antibiotics has been useful in gaining a quantitative understanding of how bacteria respond to ribosome-targeting antibiotics (Greulich *et al*, 2015; Pinheiro *et al*, 2021). Yet, the connection between growth-related changes and the response to other stresses, such as DNA damage, has not been explored.

DNA damage is one of the most ubiquitous types of stress encountered by bacteria. It can arise from external sources such as exposure to UV light or to DNA damaging agents, for example, quinolone antibiotics (Gutierrez *et al*, 2018). Impaired DNA replication leads to the accumulation of DNA Double Strand Breaks (DSBs) at inactivated replication forks, providing a direct link between the cell cycle and DNA damage. Spontaneous DSBs have been linked to stalling of the replisome by obstacles, and/or a replication fork encountering DNA nicks and gaps (Kuzminov, 2001; Michel *et al*, 2004, 2018). DNA replication is also involved in the formation of DSBs after exposure to quinolones (Pohlhaus & Kreuzer, 2005; Drlica *et al*, 2008). DSBs are the most deleterious type of DNA damage as they lead to loss of genetic information. They are repaired by homologous recombination where the broken chromosome is repaired using an intact homologous copy as a template. Homology search is catalysed by RecA which forms a nucleoprotein filament on single-strand DNA and promotes strand invasion after a homologous copy has been found (Del Val *et al*, 2019). This also leads to the induction of the SOS response (see below).

Changes in growth rates have important consequences on DNA replication in bacteria. In *E. coli*, in rich nutrient conditions, replication of the chromosome is estimated to take about 40 min, and segregation/septation to take another 20 min, for a cell cycle time of approximately 60 min (Bremer & Dennis, 2008). When cells divide faster than 60 min, they initiate several overlapping rounds of DNA replication (a process referred to as "multifork

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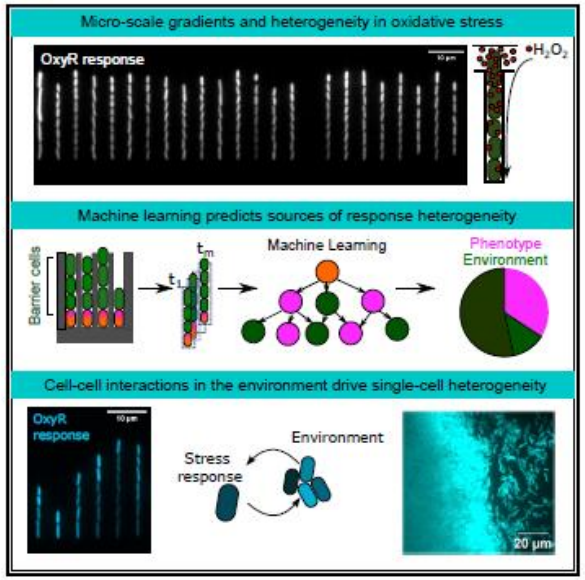
Paper 5

Cell Reports

Phenotypic heterogeneity in the bacterial oxidative stress response is driven by cell-cell interactions

Graphical abstract



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In brief

Clonal populations of bacteria often display heterogeneous phenotypes under stress. Here, using single-cell imaging and machine learning, Choudhary et al. show that heterogeneity in the response of *E. coli* cells to oxidative stress arises from short-range cell-cell interactions. This leads to a collective protection of the population.

Highlights

- Machine-learning model predicts single-cell heterogeneity
- Oxidative stress response heterogeneity is driven by cell-cell interactions
- Population protection increased by gradual adaptation of neighboring "barrier" cells
- Spatial H_2O_2 gradients give rise to cellular heterogeneity in mutagenesis

Choudhary et al., 2023, Cell Reports 42, 112168
March 28, 2023
https://doi.org/10.1016/j.celrep.2023.112168

CellPress

Paper 6

Article

TRANSPARENT PROCESS OPEN ACCESS

molecular systems biology

Tunable phenotypic variability through an autoregulatory alternative sigma factor circuit

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Abstract

Genetically identical individuals in bacterial populations can display significant phenotypic variability. This variability can be functional, for example by allowing a fraction of stress prepared cells to survive an otherwise lethal stress. The optimal fraction of stress prepared cells depends on environmental conditions. However, how bacterial populations modulate their level of phenotypic variability remains unclear. Here we show that the alternative sigma factor σ^V circuit in *Bacillus subtilis* generates functional phenotypic variability that can be tuned by stress level, environmental history and genetic perturbations. Using single-cell time-lapse microscopy and microfluidics, we find the fraction of cells that immediately activate σ^V under lysozyme stress depends on stress level and on a transcriptional memory of previous stress. Iteration between model and experiment reveals that this tunability can be explained by the autoregulatory feedback structure of the *sigV* operon. As predicted by the model, genetic perturbations to the operon also modulate the response variability. The conserved sigma-anti-sigma autoregulation motif is thus a simple mechanism for bacterial populations to modulate their heterogeneity based on their environment.

Keywords *Bacillus subtilis*; microbial systems biology; single-cell time-lapse microscopy; stochastic gene expression; stress priming

Subject Category Microbiology, Virology & Host Pathogen Interaction

DOI 10.15252/msb.20209832 | Received 3 July 2020 | Revised 28 May 2021 | Accepted 1 June 2021

Mol Syst Biol. (2021) 17: e9832

Introduction

Cells live in a changeable environment and experience a wide range of environmental stresses. Bacterial populations have evolved strategies to survive these stresses. One strategy is for all cells to immediately respond to stress with the activation of the relevant stress response pathway (Hilker *et al*, 2016). Alternatively, a bacterial population can generate a broad range of cellular states, which allows it to hedge its bets against the changeable environment (Veening *et al*, 2008b). Noise in gene expression has been proposed as a mechanism for generating phenotypic variability in genetically identical cells (Raj & van Oudenaarden, 2008; Martins & Locke, 2015). This phenotypic variability has also been shown to be affected by changes in the cellular environment, such as a shift in stress level or growth conditions (Megerle *et al*, 2008; de Jong *et al*, 2012; Mitosch *et al*, 2019), as well as by previous 'priming' stresses (Mitosch *et al*, 2017). However, how the bacterial population regulates individual cell decisions to modulate the fraction of cells that enter an alternative transcriptional state remains unclear (Fig 1A).

The σ^V mediated lysozyme stress response pathway in *Bacillus subtilis* is an ideal model system to examine how bacterial populations can tune their phenotypic variability. σ^V is an extracytoplasmic function (ECF) alternative sigma factor. Alternative sigma factors replace the 'housekeeping' sigma factor, σ^A, in the RNA polymerase holoenzyme and redirect it to regulons that control distinct regulatory programmes. They have already been shown to display a high level of gene expression variability in *B. subtilis* (Locke *et al*, 2011; Young *et al*, 2013; Cabeen *et al*, 2017; Park *et al*, 2018), and the σ^V activation pathway is both well characterized and specific to one stress condition, which greatly simplifies analysis of its activation.

σ^V is the only pathway activated in response to C-type lysozyme (Guariglia-Oropeza & Helmann, 2011; Ho *et al*, 2011; Ho & Ellermeier, 2012). Lysozyme is produced by animals as part of their innate immune system and kills bacteria by cleaving the peptidoglycan of the cell wall between the *N*-acetylmuramic acid residue and the β-(1,4)-linked *N*-acetylglucosamine (Lal & Caplan, 2011). In its inactive form, σ^V is bound to its anti-sigma factor RsiV, a transmembrane protein (Fig 1B). If lysozyme is present, RsiV binds to lysozyme (Hastie *et al*, 2014; Hastie *et al*, 2016) and activates a signal transduction cascade to release σ^V. First RsiV undergoes a conformational change that allows signalling peptidases to cleave RsiV at site-1 (Hastie *et al*, 2014; Castro *et al*, 2018; Lewerke *et al*, 2018) (Fig 1B). *Bacillus subtilis* has five type 1 signal peptidases, of which the two major peptidases are SipS and SipT (Tjalsma *et al*, 1998). Either SipS or SipT is sufficient for site-1 cleavage (Castro *et al*, 2018; Ho & Ellermeier, 2019). The truncated RsiV can then be cleaved by RasP (a site-2 protease), which results in the release of σ^V (Hastie *et al*, 2013; Hastie *et al*, 2014) (Fig 1B).

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