Analysis of stochasticity of gene expression in single cells of *Mycoplasma gallisepticum*

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Abstract. Genetically identical organisms under the same conditions usually have differences in the level of expression of the same genes. This phenomenon is called genetic noise, and it is characteristic of all organisms. In this paper, we studied the stochasticity of gene expression in *Mycoplasma gallisepticum* at the level of single cells using fluorescence microscopy. The results show that *M. gallisepticum* demonstrates high level of variations in gene expression: even in case of strong promoters and ribosome-binding sites, some cells demonstrate negligible expression level. Such high stochasticity in gene expression may have significant impact on the life of this reduced bacterium.

1. Introduction

Genetically equal organisms under the same conditions always demonstrate differences in the expression level of the same genes. This phenomenon is called gene expression noise [1]. In varying degrees, it is characteristic of all organisms. Gene expression noise causes non-genetic differences in gene expression in identical individuals, which leads to phenotypic differences in cell culture. It may lead to harmless properties of some individuals, like color, body size and the shape of fingerprints. But sometimes gene expression noise affects the fate of the cell. Bacteria demonstrate non-genetic resistance to antibiotics, and one of origins of this phenomenon is dormancy which is partially based on noisy expression of some genes, for instance, in toxin-antitoxin systems [2, 3]. In this work, we studied stochasticity in the level of gene expression in single cells of *Mycoplasma gallisepticum*, which belongs to the *Mollicutes*. *Mollicutes* evolved from gram-positive bacteria; they miss the cell wall. Their genome is substantially reduced and consists of 0.58-2.20 Mbp. Mycoplasmas are among the most simply organized bacteria, they are close to the concept of "minimal" cell [4, 5]. Thus, their physiology is significantly simplified in comparison to most other bacterial cells, which makes *Mollicutes* an interesting model for research. In addition, mycoplasmas are the causative agents of numerous diseases. They are able for a long time to be in a state imperceptible to the immune system.

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of the host. In particular, *M. gallisepticum* causes respiratory infections in poultry and causes serious economic damage to poultry farms around the world [6].

In this paper, we were able to directly measure the number of fluorescent protein in single cells of *M. gallisepticum*. Results of this work show that *M. gallisepticum* demonstrates high level of gene expression noise for all combinations of promoters and ribosome-binding sites used in this study.

2. Materials and methods

*M. gallisepticum* S6 cells (with appropriate genetic construction with mMaple2 fluorescent protein gene) were added at a ratio of 1:10 to a custom-made fresh medium consisting of tryptose (20 g / l), NaCl (5 g / l), Tris (3 g / l), KCl 1.3 (g / l), yeast dialysate (5 % v/v), horse serum (10% v/v), glucose (1% w/v) at pH 7.4 and were grown at 37 °C. To visualize the differences in the expression level, we used a photo-convertible fluorescent protein mMaple2. The study was carried out on a Nikon Ti-E microscope, which was equipped with sCMOS Andor Zyla 4.2 camera.

To assess the influence of different promoters and ribosome binding sites (RBSs) on expression of mMaple2, several strains with promoters and RBSs of different strengths were previously constructed. The obtained values of fluorescence intensity were compared with the concentration of mMaple2 protein using a calibration procedure. It was obtained from an estimated number of cells in sample and an average concentration of protein in them using Western blotting.

Data with a quantitative assessment of the rate of accumulation of fluorescent protein were obtained when taking cells on a microscope for 6 h with a period of 30 min. Previously accumulated fluorescent protein was photobleached. To assess the expression level of the mMaple2 protein, which is under the control of an inducible promoter activated under oxidative stress, *M. gallisepticum* was cultivated in a medium with the addition of oxidized glutathione. After that, long-term observation under a microscope was carried out for 12 h with a period of 30 min without photobleaching.

To obtain the values of fluorescence intensity in cells, images obtained from the microscope were processed in the ImageJ program. The background fluorescence was subtracted from the values. With the help of preliminary calibration, the intensity was transferred to the quantitative assessment of protein number in the cell.

3. Results and discussion

To evaluate the level of expression, in current study several combinations of promoters and ribosome binding sites of different strengths were studied. Using the calibration, we were able to convert the fluorescence intensity into the number of the fluorescent protein mMaple2 in each *M. gallisepticum* cell (see Figure 1). The results indicate that there are significant variations in the expression level of the mMaple2 fluorescent protein, which is under the control of various combinations of promoters and ribosome binding sites (RBSs): some cells show the expression level indistinguishable from zero, while other cells show a significant expression level (see Figure 2). Also we followed the expression dynamics of the mMaple2 protein gene under the control of several combinations of promoters and ribosome binding sites of different strengths (see Figure 3).

In addition, we followed the expression dynamics of the mMaple2 protein, whose gene is under the control of an inducible promoter activated by oxidative stress. For this purpose, long-term observation of *M. gallisepticum* cells cultured in medium with the addition of oxidized glutathione was carried out under a microscope. It has been established that, under these conditions, a substantial accumulation of the mMaple2 protein occurs in the cells, with significant differences in the level of expression.

The data obtained show that *M. gallisepticum* cells demonstrate high level of stochasticity in gene expression. This phenomenon can be of great importance for the physiology of this organism, including the cause of non-genetic resistance to antibiotics. It is well-known that gene expression noise may affect cell fate, for instance, in *Bacillus subtilis* it triggers the cell between competent and non-competent states [7]. In a competent state cell becomes capable of taking up foreign DNA and thereby may have opportunity to survive in adverse conditions. Moreover, gene expression noise is considered to be one of important factors which cause resistance to antibiotics [8]. Stochasticity in
gene expression may help cell to passively resist drugs by altering bacterial growth rate (for instance, dormant state is characterized by decreased growth rate as well as decreased susceptibility to antibiotics) as well as to actively resist antibiotics using elevated levels of efflux pumps or enzymes. In addition, stochasticity in the expression of vital genes can contribute to the function of the most important molecular mechanisms. For example, the rate of cell division of mycoplasmas and, accordingly, the process of their reproduction can theoretically depend on the stochastic expression of the \textit{ftsZ} gene.

\textbf{Figure 1.} The number of mMapple2 fluorescent protein molecules in single \textit{M. gallisepticum} cells (xN clone). Count corresponds to the number of cells with such a molecules number.
Figure 2. Stochasticity of mMaple2 expression at the single-cell level. Top – images in transmitted light, demonstrating cell morphology; bottom – images in mMaple2 fluorescence channel. Left – cells of xN clone with high expression level of mMaple2; right – cells of 1-1 clone with medium expression level of mMaple2.
Figure 3. Dynamics of mMaple2 expression at the single-cell level. a, b – single cells of xN clone (strong constitutive promoter combined with strong RBS); c – A1 clone (less strong constitutive promoter combined with strong RBS); d – YebC clone (oxidative stress promoter which was induced by oxidized glutathione).

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