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Research paper

Ribosome profiling reveals an adaptation strategy of reduced bacterium to acute stress

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ABSTRACT

Bacteria of class Mollicutes (mycoplasmas) feature significant genome reduction which makes them good model organisms for systems biology studies. Previously we demonstrated, that drastic transcriptional response of mycoplasmas to stress results in a very limited response on the level of protein. In this study we used heat stress model of *M. gallisepticum* and ribosome profiling to elucidate the process of genetic information transfer under stress. We found that under heat stress ribosomes demonstrate selectivity towards mRNA binding. We identified that heat stress response may be divided into two groups on the basis of absolute transcript abundance and fold-change in the translatome. One represents a noise-like response and another is likely an adaptive one. The latter include ClpB chaperone, cell division cluster, homologs of immunoblocking proteins and short ORFs with unknown function. We found that previously identified read-through of terminators contributes to the upregulation of transcripts in the translatome as well. In addition we identified that ribosomes of *M. gallisepticum* undergo reorganization under the heat stress. The most notable event is decrease of the amount of associated HU protein. In conclusion, only changes of few adaptive transcripts significantly impact translatome, while widespread noise-like transcription plays insignificant role in translation during stress.

1. Introduction

Recent studies have demonstrated that bacteria induce a complex response to a particular stress [1] [2] [3], [4]. This response involves numerous genes not obviously related to the particular stress. This phenomenon may result from either imperfect transcriptional control or a complex adaptive response. Bacterial transcription seems to produce a significant amount of noise [5] [6], likely due to imperfect transcriptional control. At the same time, the adaptive value of numerous changes occurring upon conditional shift can be negligible or even negative, as measured for *Shewanella oneidensis* [3].

These observations may lead to opposite conclusions. One can speculate that the substantial amount of response at the gene expression level represents noise and that only a few changes are adaptive. The noise imposes burden on cell metabolism and may even impede an adaptive response. However, the evolutionary development of perfect control is probably not worth the effort dedicated to achieving this goal [3]. Another concept supposes that cells in their real environment are subjected to complex challenges and evolved to simultaneously respond to numerous factors. Therefore, the response that is barely adaptive in a tube may be robust in a real niche of a given bacterium and may not be fully understood under artificial conditions. At the same time, several environmental cues can be interpreted in the context of the complex response and can lead to pre-adaptation to the condition that is not physically linked with the particular condition, but rather is correlated with it. For example, heat stress may be interpreted by a pathogenic bacterium as a signal of upcoming inflammation.

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The degenerative evolution of class Mollicutes bacteria, particularly mycoplasmas, resulted in the loss of most of the conserved transcriptional control systems. Transcriptomic studies of mycoplasmas revealed several mechanisms of indirect transcriptional regulation, including conditional terminators and spurious promoters [1] [2], [7]. However, the observed response demonstrated a noise-like pattern rather than an adaptive pattern. Heat stress in *Mycoplasma gallisepticum* results in the activation of numerous low abundance transcripts, including antisense mRNAs and the coding transcripts for small ORFs. Small ORFs have been shown to have an important function in mycoplasmas [8]. The general upregulation of sub-optimal promoters and the widespread deregulation of transcription terminators, which allow transcription to continue further downstream of CDSs, support an idea of noise-like response. Despite the numerous transcriptional changes, the proteome was shown to be mostly unaffected [2], [9]. However, the quantification of low fold changes and changes of low-abundance proteins in complex samples, such as a whole cellular proteome, is difficult [10]. Therefore, changes important from the adaptive point of view may have been undetected. The most striking feature of these findings is that cells ultimately survive the stress [11]. This survival, in the presence of minor proteomic changes, may be explained by the remodeling of protein complexes. Extensive studies of higher organisms have revealed that the variable subunits of the defined protein complexes may significantly contribute to the cell phenotype without changing the primary players [12]. The dynamic effects may contribute to the regulation without employing the activity of the regulators [13]. General cell simplification likely increases the role of regulatory mechanisms other than the direct regulation of transcription. One can speculate that the deregulation at the level of transcription is countered at the level of translation. The majority of transcriptional changes are discarded, whereas few key modulators are differentially translated, making the ribosome the primary regulator of cell homeostasis.

High-throughput technologies have revealed an increasing number of cases of low correlation between mRNA and protein levels in bacteria such as *E. coli* [14], *Desulfovibrio vulgaris* [15] and *Lactococcus lactis* [16]. The regulation of the translation of single genes or operons is well documented. However, discordance between transcription and translation on a genome-scale level must depend on a global regulation. Translational regulation may be implemented via antisense RNAs, secondary structures within mRNAs [17] [18], and regulatory proteins associated with ribosomes. For example, a subpopulation of *E. coli* ribosomes lacking S1 protein preferably translate leaderless mRNAs [19]. The stoichiometry of the ribosome components may vary in different subpopulations of the ribosomes within a cell and lead to the functional difference in higher organisms [20]. Translational regulation may involve the moonlighting activity of proteins whose known functions are not related to translation. For example, the glycogen-synthase of *Saccharomyces cerevisiae* may regulate the translation of several mRNAs [21].

A noise-like stress response coupled with stress survival in genome-reduced organisms is a phenomenon that has to be understood. Therefore, we analyzed the translatome (the pool of ribosome-bound mRNA) and the ribosomal proteome of *M. gallisepticum*. Using previous data collected for the *M. gallisepticum* transcriptome, we studied genome-scale regulation at the translational level. We used absolute copy number values, fold change mRNA data and ribosome-bound mRNA and proteomics data to distinguish between noise-like and adaptive responses.

2. Materials and methods

2.1. Cell culture

Mycoplasma gallisepticum S6 was cultured in liquid medium containing tryptose (20 g/L), Tris (3 g/L), NaCl (5 g/L), KCl (5 g/L), yeast dialysate (5%), horse serum (10%) and glucose (1%) at pH = 7.4 and 37 °C in aerobic conditions and were exposed to stress conditions as previously described [11]. Briefly cells were exposed to sublethal heat stress at 46 °C for 30 min. The dose of stress and absence of massive cell death was previously determined by CFU test.

2.2. Isolation of ribosomes and ribosome-bound RNA

Isolation of ribosomes was performed as previously described [22]. Chloramphenicol was added to a final concentration of 100 μg/ml to 12 ml of a mid-exponential culture of *M. gallisepticum* to quench translation. The cells were incubated for 5 min on ice and harvested by centrifugation at 4500 g for 20 min at 4 °C. Cells from a total culture volume of 50 ml were resuspended in 500 μl of buffer containing 20 mM HEPES, 100 mM NaCl, 6 mM $MgCl_2$, 2 mM spermidine, 100 μg/ml chloramphenicol, 5 μl protease inhibitor (GE Healthcare), and 200 μm RNAse inhibitor (Thermo Scientific), pH 7.5. The cells were lysed by adding 15 μl of 10% NP-40 solution. Lysates were frozen at −75 °C for at least 1 h and thawed. Lysates were centrifuged at 20,000 g for 20 min at 4 °C to remove debris. Then, the lysates were fractionated by centrifugation in a step sucrose gradient of 10–50% with a step of 10%. The gradient was formed using a pipet in a 5 ml polycarbonate tube. The sucrose gradient was created in the same buffer as was used for cell lysis but without NP-40 or protease or RNAse inhibitors. Fractionation was performed using an Optima (Beckman Coulter) centrifuge with MLS 50 bucket rotor (Beckman Coulter) at 50,000 rpm (average relative centrifugal force was 200,620 g) for 1 h at 4 °C. Finally, 200 μl fractions were collected by pipet.

To isolate RNA, 400 μl of TRIzol LS reagent was added to 200 μl of each given fraction. The mixture was extracted with 200 μl of chloroform. The aqueous phase was purified from tRNA using a PureLink RNA Mini Kit (Ambion). Three biological replicates of control and heat stress conditions were obtained.

2.3. Preparation of the RNA-Seq libraries

Preparation of the libraries was performed as previously described [2]. Ribosome-bound RNA was fragmented into 200 b.p. by chemical fragmentation with $ZnSO_4$ buffer and was subsequently end-repaired using T4 polynucleotide kinase (Thermo Scientific). cDNA preparation was performed according to the standard protocol for SOLiD library preparation. Amplified ds-cDNA was subjected to normalization twice with DSN (double-strand specific nuclease, Evrogen). Samples were prepared in three biological replicates, with one technical replicate per one biological replicate.

2.4. Sequencing and data analysis

Sequencing was performed with a SOLiD 5500 (Life Technologies) platform using the SOLiD EZ Bead E80 System and SOLiD ToP Sequencing Kit, MM50 (Applied Biosystems). Read mapping was performed using Bowtie software. Quantitative analysis was performed using R packages as previously described [2].