A Pathway and Genetic Factors Contributing to Elevated Gene Expression Noise in Stationary Phase

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ABSTRACT Previous studies have identified factors associated with transcription and translation efficiency, such as promoter strength and mRNA sequences, that can affect stochasticity in gene expression. Here we present evidence for a pathway and associated genetic factors (namely, the ribosome modulation factor RMF and ppGpp) in *Escherichia coli* that contribute to heightened levels of gene expression noise during stationary phase. Endogenous cellular mechanisms that globally affect gene expression noise, such as those identified in this study, could provide phenotypic diversity under adverse conditions such as stationary phase.

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In a previous study, we predicted and measured the level of gene expression noise in a synthetic gene circuit under different experimental conditions (1). We also carried out stochastic simulations with a molecular kinetic model designed to represent the behavior of our synthetic gene network. Interestingly the stochastic model predicted that when cell division is stopped, fluctuations in protein levels expressed from high copy number plasmids increases. This increase in gene expression noise occurs because the random partitioning of plasmids between daughter cells during cell division tends to reduce plasmid copy number variability within the cell population. This prediction was validated by comparing noise in gene expression from cells undergoing exponential growth to that of cells grown in minimal media or cells in stationary phase. Surprisingly, the gene expression noise measured from cells in stationary phase was even higher than that predicted by our stochastic model. We established that stationary phase gene expression is noisier than exponential phase gene expression, and found that gene expression from our network was even noisier in stationary phase than was predicted by the model. Specifically, there was an increase of 0.1 in the coefficient of variation (CV, standard deviation divided by the mean) in our experimental measurements in stationary phase, which we could not account for with our model. We thus sought to address the source of this noise by identifying genetic factors that contribute to this variation.

In narrowing the field of potential genetic factors that may alter gene expression noise, we considered that the transition to stationary phase is governed by activation of several global regulators that cause cell-wide changes in gene expression, in particular, the ribosome modulation factor RMF and guanosine 3′,5′-bis(diphosphate) (ppGpp). RMF accumulation causes sequestration and inactivation of ribosomal subunits, decreasing the translational capacity of the cell (2,3), while ppGpp accumulation causes downregulation of transcription, translation, and DNA synthesis (Fig. 1 *a*).

We hypothesized that RMF activity, during stationary phase, increases gene expression noise by decreasing the level of translationally active ribosomes. Deleting *rmf* should thus lead to decreased gene expression noise.

Using the same gene circuit as in our previous study (Supplementary Material Fig. S1), we measured gene expression noise from the green fluorescent protein (GFP), indirectly induced with arabinose. We found that deleting the *rmf* gene led to decreased variation in *gfp* expression (an ~0.05 decrease in CV) during stationary phase, consistent with the above hypothesis (Fig. 1 *b*). We suspect that a higher level of translational efficiency in the *rmf* deletion mutant is responsible for the decreased variation. In cells without RMF, more ribosomes are active during stationary phase which likely increases the translation of protein. When protein translation is increased and the level of transcription remains steady, the protein production CV will be reduced (4,5). We used mathematical modeling to provide further support for this relationship between translational efficiency and gene expression noise (Box 1). In addition, we observed a higher mean GFP fluorescence in the *rmf* deletion mutant (Supplementary Material Fig. S2), substantiating the notion that there is increased translational efficiency resulting from the *rmf* deletion.

Given our finding that RMF only contributes a 0.05 increase in CV, we suspected that there might be other factors in addition to RMF, such as ppGpp, that contribute to gene expression noise during stationary phase. ppGpp-mediated downregulation of transcription, in addition to translation, could lead to increased gene expression noise. *Escherichia coli* has two ppGpp synthetases, encoded by *relA* and *spoT*. The *spoT* gene product also has ppGpp degradase activity (6). Deleting

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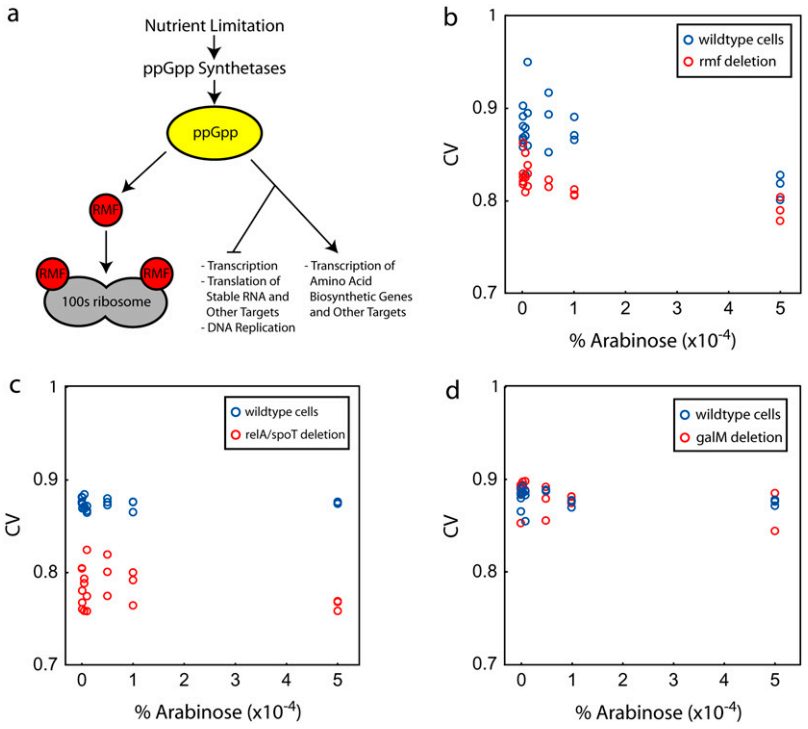

FIGURE 1 Graphical representation of a stationary phase-related gene cascade and coefficient of variation (CV) results for deletion mutants and wild-type cells. (*a*) A simplified model of a stationary phase-related gene cascade. Proteins produced by the *relA* and *spoT* genes mediate the production of ppGpp, which in turn activates the *rmf* gene. RMF protein causes ribosomes to become inactivated. (*b*) CV versus arabinose levels for our reporter system with (*blue*) and without (*red*) the *rmf* gene. (*c*) CV versus arabinose levels for our reporter system with (*blue*) and without (*red*) the *relA* and *spoT* genes. (*d*) CV versus arabinose levels for our reporter system with (*blue*) and without (*red*) the *galM* gene.

both *relA* and *spoT* renders cells unable to make ppGpp, which should lead to decreased gene expression noise.

As shown in Fig. 1 *c*, deleting *relA* and *spoT* decreased gene expression noise substantially. The double mutant has a CV that is 0.1 lower than that of cells with *relA* and *spoT*. Part of this effect is likely due to RMF, which is stimulated by ppGpp accumulation; other ppGpp-stimulated factors likely also contribute. It is important to note that the increase in stationary phase noise in both the RMF and ppGpp deletion strains is beyond that which can be accounted for by the measured decrease in the mean expression level, indicating nontrivial contributions from stationary phase-associated factors that are in addition to those causing downregulation of transcription and translation.

We also created and tested a deletion strain of the *galM* gene as a control to show that the above findings are not the result of nonspecific artifacts of the gene deletions. The expression of *galM* is not dependent on growth phase, and it does not exert transcriptional or translational control on the genes involved in our reporter system. We measured GFP expression from the *galM* deletion and wild-type strains in stationary phase and found that there was no change in gene expression noise due to the *galM* deletion (Fig. 1 *d*). In addition, to demonstrate that the *rmf* and *relA*/*spoT* deletions are altering gene expression noise only in stationary phase, we measured the CV of GFP production in both deletion strains and the wild-type strain during exponential phase, and found little difference between them (Supplementary Material Fig. S3).

Previous work has shown, by directly manipulating mRNA sequences and DNA sequences regulating specific genes under study, that the biochemical processes of transcription and translation affect gene expression noise (4,7–12). Our present work provides evidence for a cellular pathway and associated genetic factors (Fig. 1 *a*) that globally affect gene expression noise in a growth-phase specific manner, through their influence on transcription and translation. Phenotypic diversity arising from such noise effects (13), could confer a survival advantage under extreme conditions, such as stationary phase (14–17).

BOX 1

In the *rmf* deletion mutant, there are more active ribosomes to carry out translation compared to wild-type cells; this increases the translation rate while transcription remains steady. We hypothesized that increased translational efficiency in protein production can decrease GFP expression noise in our system. To test this hypothesis, we used a model including mRNA transcription, translation, and degradation, similar to previous work (18):

$$\phi \underset{\mu}{\overset{\lambda}{\leftrightarrow}} mRNA \tag{1}$$

$$\phi \overset{\lambda_2 \mathrm{mRNA}}{\underset{\mu_2}{\leftrightarrow}} protein \tag{2}$$

$$CV^2 = \frac{\mu\mu_2}{\lambda(\mu+\mu_2)} + \frac{\mu\mu_2}{\lambda\lambda_2}. \tag{3}$$

The terms λ and λ_2 in the above equations represent transcription and translation of GFP, respectively, while μ and μ_2 represent the degradation of GFP mRNA and GFP protein, respectively. The exact analytical form of the squared CV of GFP production (Eq. 3) can be derived based on the model represented by Eqs. 1 and 2. From the derivation (Eq. 3), we can see that when translation (λ_2) increases and transcription (λ) is held steady, the CV of the protein production decreases. This analysis supports our hypothesis that increasing translational activity (e.g., by deleting RMF and increasing the number of active ribosomes in stationary phase) can lead to reduced gene expression noise.

SUPPLEMENTARY MATERIAL

To view all of the supplemental files associated with this article, visit www.biophysj.org.

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