Probabilistic RNA partitioning generates transient increases in the normalized variance of RNA numbers in synchronized populations of Escherichia coli†

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We explore the effects of probabilistic RNA partitioning during cell division on the normalized variance of RNA numbers across generations of bacterial populations. We first characterize these effects in model cell populations, where gene expression is modeled as a delayed stochastic process, as a function of the synchrony in cell division, the rate of division, and the RNA degradation rate. We further explore the additional variance that arises if the partitioning is biased. Next, in Escherichia coli cells expressing RNA tagged with MS2d–GFP, we measured the normalized variance of RNA numbers across several generations, with cell divisions synchronized by heat shock. We show that synchronized cell populations exhibit transient increases in normalized variance following cell divisions, as predicted by the model, which are not observed in unsynchronized populations. We conclude that errors in partitioning of RNA molecules generate diversity between the offspring of individual bacteria and thus constitute a form of reproductive bet-hedging.

Introduction

Phenotypic diversity aids bacterial populations in coping with environmental fluctuations.1 Evidence suggests that the diversity of a monoclonal cell population can change under different environmental conditions.2,3 Noise in gene expression is a major source of this diversity since it generates cell to cell variability in RNA and protein numbers.4 To some extent, this noise is sequence dependent, as it varies from gene to gene.5

There are other sources of cell to cell diversity in RNA and protein numbers. A recent work6 mathematically demonstrated that stochastic partitioning of RNA and proteins in cell division contributes to the variance of these molecules in a population. However, it is still uncertain if the partitioning of these molecules is subject to any means of internal control. Experimental verification of their results6 may shed light on this question, but is not yet available.

The extent to which deviations from a perfectly equal partitioning of RNA and protein molecules can affect the diversity of their numbers in a cell population depends on several parameters including, but not limited to, the degradation rate of the RNA, the generation time of the cells, and the level of synchronization of the cell cycles. Some of these parameters may be sequence dependent, causing the effects to differ between RNAs.

Here, we study the cell to cell diversity in RNA numbers in populations of dividing Escherichia coli cells, as a function of the variables listed above. An additional factor may be relevant in generating diversity during cell division. Internally, E. coli cells are not spatially homogeneous. For example, different plasmids preferentially localize in different regions of the cell,7 and RNA molecules do not diffuse considerably from their point of transcription, leading to the spatial organization of translation and RNA decay within the cell.8 Additional factors may contribute to asymmetry in divisions.9 We thus also consider the possibility that the RNA partitioning, while probabilistic, may be biased.

We address the following question: to what extent does the probabilistic nature of the RNA partitioning affect the variance of RNA numbers of populations of dividing cells?

Using a delayed stochastic model of the expression dynamics of the P_{lac/ara} promoter, we first characterize the normalized variance in RNA numbers that can realistically arise from stochastic partitioning during cell division as a function of the RNA degradation rate, the mean division time, the level of synchrony, and the strength of the bias in partitioning.
Next, we measure absolute RNA quantities in vivo in DH5α-PRO E. coli cells that have been synchronized by heat shock. We compare with measurements from a cell population not subject to heat shock and with the predictions from the model.

Methods

Cells, plasmids and chemicals

The method of RNA detection and quantification was proposed by Fusco et al. and characterized in E. coli by Golding and Cox. The E. coli strain DH5α-PRO (identical to DH5α-Z1) contains two constructs. The first is the bacterial expression vector PROTET-K133 carrying a single chain MS2 dimer (MS2d) fused with green fluorescent protein (MS2d–GFP). This vector has a promoter, P_{lacO1}, inducible by anhydrotetracycline (aTc; IBA GmbH, Göttingen, Germany). The second construct is a pIG–BAC (P_{lacO1}–mRFP1–MS2-96bs) vector, a bacterial artificial chromosome based on F factor replication with an array of 96 MS2d binding sites under the control of the P_{lacO1} promoter. The constructs were generously provided by Dr Ido Golding, University of Illinois, USA. The mRNA target is inducible by isopropyl-β-D-1-thiogalactopyranoside (IPTG) (Fermentas, Finland) and/or l-arabinose (Sigma-Aldrich, Schnelldorf, Germany).

In vivo measurements of tagged RNA molecules in E. coli

Cells were grown overnight at 37 °C in LB supplemented by the appropriate antibiotics. The next day, cells were diluted in fresh medium plus antibiotics. To induce production of MS2d–GFP, 100 ng mL⁻¹ aTc and 0.1% l-arabinose were added to the diluted bacterial culture. Cells were then incubated with these inducers at 37 °C with shaking for 45 min to a final optical density (OD-600 nm) of ~0.4. Afterwards, expression of the target RNA was induced by 1 mM IPTG. For imaging, 8 μL of culture were placed on a microscopic slide between a cover slip and 0.8% LB-agarose gel pad set at specific points in time after induction by IPTG. Epifluorescence microscopy was used to minimize the risk of not detecting spots. Measurements were done with a B-2A filter (EX 450-490, DM 505, BA 520), Nikon DS-Fil camera and NIS-Elements F software (version 2.20, Nikon Corp).

Cells were imaged 30, 45, 60, 75, 90, 105 and 120 minutes after induction. Cells were taken from the liquid culture at these moments and immediately placed under the microscope and imaged over a period of ~10 minutes. In this way, we aimed to measure the RNA numbers and spatial distributions in individual cells as if they were in liquid culture.

Synchronizing cell divisions by heat shock

In one experiment, cell division times were synchronized by heat shock as described by Lomnitzer and Ron. The cells were grown overnight as described above, and then subjected to 45 °C for 15 min prior to induction by IPTG.

Division times of heat shocked synchronous cells were determined by the OD of the liquid culture. The OD was measured from at least two samples every ten minutes from 30 to 120 minutes after addition of the inducers. The OD was then averaged over the samples. Dilutions were used so that the OD remained smaller than 0.4. From OD measurements it is possible to estimate the mean division time. 13

Detecting cells and quantifying tagged RNA molecules from the images

We detected cells from raw images using the method proposed by Wang et al. This method divides a grayscale image into three classes: background, cell border and cell region. It then exploits an iterative cell segmentation process that identifies and segments clumped cells based on the size and edge information (Fig. 1). Cell detection performance degrades if several cells are clumped together. This can be avoided by a threshold based on the cell size and discarding the “cells” whose size is beyond the threshold.

After detecting the cells, we detect RNA molecules tagged with MS2d–GFP. We segment the RNA spots with the kernel density estimation method for spot detection. This method estimates the probability density function over the image from local information. The process translates an image f by filtering it with a desired kernel as follows:

\[
\hat{f}(i,j) = \frac{1}{\text{card}(C(i,j))h} \sum_{(k,l) \in C(i,j)} K\left(\frac{f(i,j) - f(k,l)}{h}\right)
\]

where h is the smoothing parameter or bandwidth, (k, l) represents pixel location inside the kernel, card is the cardinality of the set, and K(α) is the kernel. We used a Gaussian kernel and applied Otsu’s thresholding method to segment RNA spots from the kernel density estimated image, highlighting the spots.

Finally, the number of RNA molecules in each spot is quantified by assuming that the first peak in the distribution of intensities of many RNA spots from cells on the same slide corresponds to individual RNA molecules. Subsequent peaks in the distribution of intensities correspond to spots of multiple RNA molecules. A sample intensity distribution is shown in Fig. S5 (ESI†).

The MS2 binding sites of each RNA molecule may not be saturated at all times. This is likely one of the sources of variance of each peak (along with the movements of the tagged RNA molecules along the z-axis, for example). Nevertheless, these sources of noise are not strong enough to prevent a clear distinction between individual peaks (see Fig. S5, ESI†), and thus it does not significantly affect the accuracy of the quantification of the number of mRNAs in each spot.

Another possible source of error in the RNA quantification method would be the occurrence of recombination events that would lower the number of MS2 binding site repeats.

![Fig. 1 Unprocessed image of tagged RNA molecules in E. coli cells from fluorescence microscopy (left) and the corresponding segmented image with the detected cells (grey) and RNA spots (white) (right).](image-url)
The variability in the number of binding sites in different cells used for the measurements was tested by amplifying the target gene from several colonies after many divisions, and determining its length by electrophoresis. For this, we designed primers to amplify the region containing MS2 binding sites (ESI†). The results (Fig. S9, ESI†) show that there is no significant variation between samples and within samples, as the bands are all in the same region and the width of each band is equal to the width of the bands of the ladder. Given the length of each MS2 binding site (~45 nucleotides long, see supplementary material from ref. 11), a significant diversity in the number of binding sites ought to be detectable. This allows us to conclude that errors in quantifying RNA numbers due to variability in light intensity arising from errors in recombination are negligible in our measurements.

The copy number and partitioning of F-plasmids are stringently controlled by internal cellular mechanisms. However, a fraction of inaccurate F-plasmid distributions have been reported. For example, in the case of the tet-O-array derivative (pDAG480) during growth at 20 °C in LB medium, 5.7 ± 1.0% per generation (standard error of three measurements) are usually lost. In the case of ΔσpC mini-F (pDAG115), this percentage is 4.8 ± 0.6%. In both cases, ~5% of the cells in the next generation did not contain the plasmid. Following these measurements, we considered 5% of all cells as outliers in each experiment, since this quantity is in good agreement with the observed outliers in the number of RNAs per cell.

It is noted that the MS2d-GFP tagging proteins are expressed from a strong promoter (P_{tetO-1}) on a high-copy number plasmid (PROTET-K133). Within the duration of our measurements we observed that there was always enough MS2d-GFP in the cells to properly detect all target RNA molecules. This was assessed by measuring the background fluorescence over time (Section S5, ESI†). This also shows that if partitioning errors in the MS2d-GFP exist, they are negligible for our measurements. Our assessment is in agreement with previous reports.11

Modelling populations of cells with stochastic gene expression and probabilistic RNA partitioning during cell division

The delayed stochastic modelling strategy of gene expression 19 accounts for the stochasticity of the chemical interactions and the duration of complex steps in gene expression. It was shown to match gene expression dynamics at the single molecule level.20 We use it to model the expression of the target RNA.

In E. coli cells DH5α-PRO, transcription is regulated by a repressor (LacI) and inducers (IPTG and arabinose).21 We designed a delayed stochastic model19 of the gene expressing the target RNA (see ESI†). The model explicitly represents the promoter and the binding/unbinding of the activators and repressors that modify the probability that an RNAP will bind to the promoter and initiate transcription. The model includes the effects of the promoter open complex formation, and the time required for the polymerase to produce the final RNA. The simulation of multiple cells, subject to divisions, is implemented in CellLine.23

In perfectly synchronized cell populations we imposed that all cells divided simultaneously, while in asynchronous populations the time until the first division was scaled by a uniform random number in the range [0,1) (half-open interval including 0, but excluding 1). Subsequent divisions occur at regular intervals, as determined by the division time. When a division occurs, all RNA molecules in the mother cell are partitioned between the daughter cells by generating a random number N_t following the binomial distribution B(N,p), where N is the number of RNA molecules in the mother cell and p is the partitioning bias. One daughter cell inherits N_t RNA molecules, while the other inherits N − N_t.

Results

Behaviour of the model

The effect of probabilistic RNA partitioning on the cell-to-cell diversity in RNA numbers in a population depends on several factors. It depends on the rate by which RNA molecules degrade, which “dissipates” the effects of errors in partitioning between daughter cells. It depends on the mean lifetime of the cells since each division can contribute to the diversity in RNA numbers of the population.6 The degree of synchrony of cell divisions affects the evolution of the diversity in RNA numbers in the population. Finally, we consider the effects of having a bias in the probabilistic partitioning towards one of the daughter cells.

The simulations allow us to explore what diversity in RNA numbers is achievable by varying these four parameters in realistic ranges (present methods to detect individual RNA molecules in vivo do not allow some of these parameters to be varied, such as the degradation rate of the target RNA). To quantify cell to cell diversity in RNA numbers, we use the normalized variance (CV², variance over the mean squared) of the RNA numbers in each cell.6

The model of transcription was tuned so that the mean number of target RNAs at 60 min matched the measured RNA production. From measurements of 1000 cells, 1 hour after full induction, we observed that each cell contained, on average, 3.36 tagged RNA molecules (data not shown).

Interestingly, we also observed that tagged RNA molecules tended to be located in the first or third quarter of the cell (Fig. 1; Fig. S6, ESI†). They were also distributed asymmetrically along the major axes of the cells. The absolute difference between the numbers of RNA molecules in each side was consistent with a binomial distribution with p equal to 0.85. Finally, in E. coli, mean division time and mean RNA lifetimes vary, respectively, from 20 to 60 min and from 3 to 20 min.24

Given the above, we model populations of dividing cells and measure the CV² of RNA numbers 60 min after induction, as we vary each of the aforementioned parameters within realistic intervals. Unless stated otherwise, we set the RNA degradation rate to 0.1 min⁻¹, the division time to 30 min, the bias in RNA partitioning to 0.85, and asynchronous divisions (Table 1, case 1).

In each row of Table 1, besides the parameter values, there is the mean number of RNA molecules per cell and the CV² of RNA numbers at 60 min. The results of each row are from model populations with 10 000 cells per generation, simulated for 60 min. From Table 1, the CV² of RNA numbers 60 min
after induction of the model cell populations varies widely as the parameters are varied. In some cases this change does not imply significant changes in the mean RNA level.

Case 2 (synchronous divisions) of the table has a notably higher CV\(^2\) than case 1 (asynchronous divisions). This is because the measurement of CV\(^2\) occurs immediately after a synchronized division in case 2. A more detailed temporal analysis shows this.

In Fig. 2, we plot the CV\(^2\) of RNA numbers over time for the cells in cases 1 and 2 (until the second synchronized division of cells of case 2). For comparison, we also plot the CV\(^2\) of RNA numbers over time for cells with synchronous divisions but unbiased RNA partitioning. The results show that synchronized divisions generate transient increases in CV\(^2\) of RNA numbers that can be strongly enhanced if there is a biased partitioning of RNA molecules at cell division. Asynchronous divisions cause the CV\(^2\) to not fluctuate with time and to be slightly higher than the CV\(^2\) of synchronous populations prior to a division. Comparing cases 3 and 4 (Table 1), we observe that, in the absence of synchronization in divisions, the stronger the bias in RNA partitioning when cells divide, the higher is the CV\(^2\).

The rate of RNA degradation has an interesting effect. Decreasing it leads to an increase in mean RNA levels, which decreases CV\(^2\). However, if the RNA lifetime is of the same order of magnitude as that of the cell lifetime, CV\(^2\) can “accumulate” across generations. To show this, in Fig. 3, we plot the value of CV\(^2\) over time, for synchronous divisions, of three cell populations differing in RNA degradation rates.

Two of the rates are within realistic intervals, while one rate (900 min\(^{-1}\)) is set abnormally weak so as to ease the visualization of the effect (note the inversions between the CV\(^2\) of the three populations following the divisions). Such an abnormally weak rate of degradation could be accomplished artificially by, e.g., coating the RNA with viral coat proteins.

From Fig. 3 it is obvious that decreasing cell division time has two effects. First, there are more frequent partitions of RNA molecules, thus contributing more frequently to CV\(^2\). When the mean division time is small enough to be of the same order of magnitude as the mean RNA lifetime, then cumulative effects in CV\(^2\) appear (increasing CV\(^2\) from one generation to the next). This is shown in Fig. 3, for the two populations with slower RNA degradation rates.

Finally, we investigated the range of the effects of probabilistic RNA partitioning on the CV\(^2\) of RNA numbers of a population. Within realistic intervals, case 9 has all parameters set so as to minimize CV\(^2\), while in case 10, all parameters are set to maximize the CV\(^2\). These cases show that a dynamic range of ~16 fold is achievable by varying these parameters within realistic intervals.

**Table 1** Effects of RNA probabilistic partitioning in division, for different mRNA degradation rates, division times, degrees of synchrony in division, and partitioning biases, on the mean and CV\(^2\) of RNA numbers of model cell populations. Each case represents a different set of model parameters. Parameters that are blank in the table were set to their value in case 1

<table>
<thead>
<tr>
<th>Case</th>
<th>RNA degradation rate/min(^{-1})</th>
<th>Division time/min</th>
<th>Synchrony</th>
<th>Partitioning bias</th>
<th>Mean no. RNAs at 60 min</th>
<th>CV(^2) at 60 min</th>
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<tr>
<td>1</td>
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<tr>
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</tr>
<tr>
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<td></td>
<td></td>
<td></td>
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<td>3.36</td>
<td>0.33</td>
</tr>
<tr>
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<tr>
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<td>0.77</td>
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<tr>
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<td>0.33</td>
</tr>
<tr>
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<td></td>
<td>60</td>
<td>Async</td>
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<td>3.70</td>
<td>0.25</td>
</tr>
<tr>
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<td>0.85</td>
<td>6.53</td>
<td>0.15</td>
</tr>
<tr>
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<td>1/3</td>
<td>20</td>
<td>Sync</td>
<td>1</td>
<td>0.59</td>
<td>2.42</td>
</tr>
</tbody>
</table>

**Fig. 2** CV\(^2\) of RNA numbers over time of three cell populations (cases 1 and 2 in Table 1) and a population similar to case 2 but with unbiased partitioning (not in Table 1). Population 1 has synchronous divisions and biased partitioning, population 2 has asynchronous divisions and biased partitioning, and population 3 has synchronous divisions and unbiased partitioning. In all cases the mean division time is 30 min. Data are from 10000 model cells per generation of each population.

**Fig. 3** CV\(^2\) of RNA numbers over time of three cell populations that differ in RNA degradation rates (in min\(^{-1}\)).
causes an observable effect on real cell populations, we imaged *E. coli* cells from a population whose cell divisions were synchronized by heat shock (see Methods). These cells express RNA target for MS2d–GFP, allowing the detection of individual RNA molecules. The CV^2^ of RNA numbers was measured every 15 minutes from multiple cells. Results are shown in Fig. 5 (circles). For comparison, we repeated the experiment without the heat shock (crosses in Fig. 5). Each data point was obtained by observing on average 140 cells.

From OD measurements (data not shown) we calculated the mean division time to be approximately 51.5 minutes in the first two divisions in the synchronous population. This long division time is likely due to the high metabolic costs of the processes induced by aTc, IPTG, and arabinose. We verified the mean division time and the degree of synchrony by recording division times of cells under the microscope for two hours following the heat shock (data in Fig. S7, ESI†). Asynchronous cells were found to have a similar mean division time.

To verify that the synchronization by heat shock does not introduce abnormal RNA distributions, we measured the RNA positions along the major axes of the cells. The distributions for the synchronous and asynchronous cases are shown in Fig. 4. The distributions are identical, indicating that the heat shock does not significantly affect the RNA localization in the cells. Also, we verified that there are no significant changes in the RNA spatial distribution over time (data not shown).

Finally, we also verified that the absolute difference between the numbers of RNA molecules on each side of the cells is similar in the synchronous and asynchronous cases. We found them to be consistent with binomial distributions with *p* = 0.92 and 0.87 for the synchronous and asynchronous cases, respectively.

The normalized variance in RNA numbers of the synchronized cell population varies over time (Fig. 5), strongly increasing at specific time points, in particular between 45 and 60 min, and between 90 and 105 min. These increases occur where they would be expected if there are synchronized divisions roughly every 50 min. These transient increases would not be possible if cell divisions were not synchronized or if the equal partitioning of RNA molecules was perfect.

To verify that this trend in CV^2^ is enhanced by the biased RNA partitioning, we compared the distributions of RNA numbers in the cell population at 45 min and at 60 min after a heat shock (Fig. 6). Between these two time points, an event (synchronized divisions) occurred that reshaped the distribution, from unimodal to bimodal. The cells were partitioned into two subpopulations: one with low numbers of RNA molecules, the other with high numbers.

To determine the degree of agreement between model and measurements, we modelled two cell populations under the same conditions as the two measurements. Divisions are set to occur at the same rate as in the measurements (every 51.5 min). RNA molecules are not subject to degradation since, in the experimental setting, RNAs are virtually immortalized by the tagging molecules. We simulated two populations of 10,000 model cells for 3 generations. The CV^2^ of RNA numbers of the two model cell populations are shown in Fig. 5. The model cell populations differ in that in one case the divisions are synchronous (solid line), while in the other they are fully asynchronous (dashed line). As in the measurements, the variation of CV^2^ over time differs significantly. Namely, in the synchronous cell populations, the CV^2^ of RNA numbers...
varies over time, sharply increasing after each division event. This is then followed by a decrease until the next synchronized division event occurs. These model cells behave identically to those whose dynamics is shown in Fig. 2, except that in the former, the RNA does not degrade.

Comparing the two measurements with their respective models, we find that the normalized variance in RNA numbers in the model cells accurately follows the trend of the measurements in both cases. Models and measurements only differ in that the CV^2 is slightly lower in the model cells, both in the synchronous and the asynchronous cases. This is expected given that the measurements have additional sources of diversity such as cell death, errors in counting of RNA molecules, and variability in the amount of inducers absorbed by each cell.

Conclusions and discussion

We studied the normalized variance of RNA numbers in model cell populations of *E. coli* across generations. In addition to the inherent stochasticity in RNA transcription and degradation, the diversity is found to depend on the probabilistic nature of RNA partitioning. The contribution from this source was found to depend on the mean cell division time, the degree of synchrony in cell division, and it can be further enhanced if there is bias in the partitioning. RNA degradation limits the extent to which the probabilistic partitioning affects the overall diversity in RNA numbers, as it tends to reduce the effects of errors in partitioning.

These results confirm a previous study on the relevance of the probabilistic nature of RNA partitioning at cell division on the overall diversity of RNA numbers of a bacterial population. We add to this study in that the numerical simulations of the realistic models of gene expression and dynamics of cell divisions allowed us to quantify the dynamic range (~16 fold) of normalized variance that is realistically obtainable by populations of *E. coli* cells. By inducing synchrony alone, we found from the simulations that the variation should be on the order of 3-fold, which is expected to be detectable in measurements of cell-to-cell diversity in RNA numbers, e.g., using the MS2d–GFP system for tagging RNA molecules.

We further extend the results of Huh and Paulsson by showing with the simulations that synchronous divisions allow transient increases in normalized variance, which can be enhanced by any bias in the partitioning. The amplitude of these transient increases is also tunable in a wide range, with the parameters described above within realistic ranges.

We provide experimental verification of this result. Our measurements show that the degree of synchrony in cell division is non-negligible and causes transient increases in the normalized variance of RNA numbers, irrespective of the existence of a bias in partitioning, which can enhance the amplitude of the transient increase. Comparing the distributions in RNA numbers of the synchronous cell population, before and after the division, allows the contribution of the partitioning events to the variance in RNA numbers to be estimated.

Cell synchrony can be induced by many types of stress such as starvation, and can be stably maintained through several generations. Interessingly, cell-to-cell variability is likely to be most advantageous under stress conditions, and it was under these conditions where we observed the strong transient increases in the normalized variance of RNA numbers. In our case, the amplitude of these transient increases was enhanced by the observed bias in RNA partitioning.

We note that the comparison between the distributions of RNA numbers before and after divisions in the synchronous cell population shows that the bias in partitioning observed in our measurements is a phenomenon that occurred in most cells, rather than a rare event, as it caused the population to split into two subpopulations, distinct in the number of RNA molecules in each cell. This suggests that we are observing a phenomenon of biased partitioning towards one of the daughter cells, or a phenomenon of unbiased partitioning with high variance (e.g. if the RNA molecules clumped together and the clumps were partitioned binomially).

Either case could be explained by the mechanism described by Lindner and colleagues of an asymmetric strategy, whereby dividing cells segregate damage at the expense of aging individuals. Since the tagged RNA complexes are not naturally occurring in *E. coli*, it may be that the cell recognizes these complexes as an undesirable substance. If so, it is possible that we are observing, for the first time at the single molecule level, a mechanism by which dividing cells accumulate unwanted substances at the older poles.

Our results cannot be used to show that RNA is partitioned in a biased fashion in *E. coli*, and it was not our intention to do so since the spatial kinetics of the target RNA in our measurements are likely altered by the tagging. Our goal here was to quantitatively investigate the effects of probabilistic partitioning, whether biased or not, on cell-to-cell diversity in RNA numbers in a cell population over time. The experimental results confirmed the model’s predictions of the effects of synchrony and bias in partitioning, which is independent of whether the bias is, in this case, natural or artificial.

The ability of *E. coli* to spatially organize RNA molecules was recently demonstrated by Llopis and colleagues. This organization may cause some RNAs to be partitioned in a biased fashion. Our study provides the means to predict the consequences of such partitioning mechanisms in cell to cell diversity in RNA numbers.

In any case, our measurements are an example of the ability of bacteria to spatially organize macromolecules (RNA tagged with MS2d–GFP). Further, we show that this process is discriminative, since MS2d–GFP is homogeneously distributed in the absence of the target RNA. From an evolutionary point of view, what is relevant is that errors and biases in partitioning of RNA molecules generate diversity between the offspring of individual bacteria. It thus constitutes a form of reproductive bet-hedging.

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Notes and references