Asymmetric Disposal of Individual Protein Aggregates in *Escherichia coli*, One Aggregate at a Time

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Escherichia coli cells employ an asymmetric strategy at division, segregating unwanted substances to older poles, which has been associated with aging in these organisms. The kinetics of this process is still poorly understood. Using the MS2 coat protein fused to green fluorescent protein (GFP) and a reporter construct with multiple MS2 binding sites, we tracked individual RNA-MS2-GFP complexes in *E. coli* cells from the time when they were produced. Analyses of the kinetics and brightness of the spots showed that these spots appear in the midcell region, are composed of a single RNA-MS2-GFP complex, and reach a pole before another target RNA is formed, typically remaining there thereafter. The choice of pole is probabilistic and heavily biased toward one pole, similar to what was observed by previous studies regarding protein aggregates. Additionally, this mechanism was found to act independently on each disposed molecule. Finally, while the RNA-MS2-GFP complexes were disposed of, the MS2-GFP tagging molecules alone were not. We conclude that this asymmetric mechanism to segregate damage at the expense of aging individuals acts probabilistically on individual molecules and is capable of the accurate classification of molecules for disposal.

Escherichia coli is a well-established and simple model in aging studies. Stewart and colleagues demonstrated that two apparently identical sister cells resulting from cell division are functionally asymmetric (14). This asymmetry is representative of the aging of the cells, since unwanted protein aggregates tend to concentrate at the older pole of the mother cell. This can be observed as an accumulation of cell constituents with limited diffusion and a long half-life at the old pole of the mother cell, resulting in larger old poles and, consequently, a cumulatively slower growth of the daughter cells receiving these substances. Additional evidence of this phenomenon of asymmetric segregation of protein aggregates at older poles and its association with cellular aging in *E. coli* was presented previously (9), where it was shown that this pattern of segregation of unwanted substances appears to occur by a common mechanism irrespective of the unwanted substance segregated. The kinetics of this process is yet poorly understood.

To better understand the mechanism of the preferential accumulation of unwanted protein aggregates at older poles in *E. coli*, it is essential to detect and track these aggregates individually. Although the detection of individual molecules in bacteria has been elusive and difficult, a recent technique has been developed that, provided the proper automated image segmentation algorithms, allows the tracking of single RNA molecules in *E. coli* by tagging them with multiple fluorescent proteins. This technique may be used for the purpose of tracking unwanted aggregates in *E. coli*. The technique uses the RNA bacteriophage MS2 coat protein fused to green fluorescent protein (GFP) and a reporter construct with multiple MS2 binding sites (5). The RNA-MS2-GFP complexes can be tracked for several hours in live cells due to the substantially extended lifetime of the aggregate (5), allowing observations of how these aggregates are partitioned in cell division (11).

Through the controlled coexpression of the MS2-GFP constructs, defined focal points of RNA-MS2-GFP complexes can be observed by fluorescence microscopy. The expression of the RNA

target is controlled by the synthetic promoter $P_{lac/ara-1}$, which contains a fusion of the operator *lacO1* with *araI1* and *araI2*, which can be bound by the repressor LacI and the activator 1- β -D-arabinofuranosyl cytosine (araC), respectively. The addition of isopropyl- β -D-thiogalactopyranoside (IPTG) and arabinose induces the transcription of the RNA target. The binding of the tagging molecules to the target RNA is usually fast, making them visible during transcription elongation (5). By both *in vitro* and *in vivo* characterizations of the RNA-protein complex, it was shown previously that for weak transcriptional activity, each fluorescent RNA-MS2-GFP spot is generally comprised of one RNA molecule and approximately 70 molecules of MS2-GFP (6).

We have studied the spatial kinetics of RNA-MS2-GFP complexes and their partitioning in cell division with a previously proposed method to detect RNA molecules (5). We first determined where novel RNA-MS2-GFP complexes first appear in individual cells, and we then tracked their positions in those cells over time. Each complex tends to travel to a pole and remain there. Evidence is also provided to show that the individual tagging molecules of MS2-GFP do not tend to aggregate or accumulate at the poles. In conclusion, we discuss how the RNA-MS2-GFP complexes are disposed of and how the disposal mechanism is capable of accurate classification for disposal.

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MATERIALS AND METHODS

Measurements of RNA-MS2-GFP complexes in *E. coli*. The method for RNA detection and quantification was first proposed Fusco et al. (4) and was characterized for *E. coli* by Golding and Cox (5). This method exploits the ability of the bacteriophage MS2 coat protein to tightly bind specific RNA sequences. Golding and Cox demonstrated the high-resolution detection of single RNA transcripts with 96 tandem repeats of the MS2 binding sites in *E. coli* by using dimeric MS2 fused to GFP (MS2-GFP fusion protein) as a detection tag. Their method uses the controlled expression of two genetic constructs. The first construct is a medium-copy vector that expresses the fused MS2-GFP protein, whose promoter (PtetO) is regulated by a tetracycline repressor. The second construct is a single-copy F-based vector, with a $P_{lac/ara-1}$ promoter (12) controlling the production of the transcript target, which codes for red fluorescent protein 1, followed by an array of 96 MS2 binding sites. Both constructs were generously provided by I. Golding (University of Illinois).

Cells with both MS2-GFP and transcript target plasmids were grown overnight at 37°C in LB supplemented with the appropriate antibiotics. On the following day, cells were diluted in fresh medium plus antibiotics. To induce the production of MS2-GFP, 100 ng/ml anhydrotetracycline (IBA GmbH, Göttingen, Germany) was added to the diluted bacterial culture. The expression of the target RNA was induced by the addition of IPTG (0.25 to 2 mM; Fermentas, Finland) and L-arabinose (0.335 to 13.4 mM; Sigma-Aldrich, Schnelldorf, Germany) to the cultures. Cells were subsequently incubated with these inducers at 37°C for 1 h with shaking to a final optical density (600 nm) of ~0.4.

Following induction, 8 μ l of culture was placed onto a microscopic slide between a coverslip and a 0.8% LB-agarose gel pad set. Cells were visualized by microscopy using a Nikon Eclipse (TE-2000-U; Nikon, Japan) inverted confocal laser scanning microscope equipped with a 100 $\times$ (1.49-numerical-aperture [NA]) objective. GFP fluorescence was measured by using a 488-nm laser (Radius 405 laser; Coherent, Inc., Santa Clara, CA) and a 500- to 530-nm detection filter. Images of cells were taken with a Nikon Digital Sight camera (Nikon) and acquired with Nikon EZ-C1 FreeViewer software, version 3.30 (Nikon Corp.). For each concentration of inducers, images were acquired from two independent experiments to test for consistency.

Images of cell populations were taken approximately 1 h after induction. For the time series recordings of individual cells, images were taken approximately 7 min after induction, one every minute, over a period of approximately 2 h. We observed that on the slide, the cell division time was approximately 90 min, likely due to the imaging.

Detection of cells, MS2-GFP-RNA spots in cells, and RNA molecules from spots. We detected cells from raw images according to a method described previously (15). This method divides a grayscale image into three classes: background, cell border, and cell region. This method then exploits an iterative cell segmentation process that identifies and segments clumped cells based on size and edge information. We found that the cell detection performance degraded in regions where several cells were clumped together. This can be avoided by applying a threshold based on cell size and discarding the cells whose sizes go beyond the threshold.

After the detection of the cells, we detected the RNA-MS2-GFP complexes in each cell. We segmented the RNA-MS2-GFP spots with a kernel density estimation method for spot detection proposed previously (1). The kernel density estimation, also known as the Parzen window, estimates the probability density function over the image from local information. The method processes an image, f , by filtering it with a desired kernel, as follows:

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

where h is the smoothing parameter or bandwidth, (k, l) represents the pixel location inside the kernel, card is the cardinality of the set, and $K(u)$ is the kernel. We used a Gaussian kernel according to an implementation described previously (3) and then applied a thresholding method de-

scribed previously by Otsu (13) to segment RNA-MS2-GFP spots from the kernel density estimated image, highlighting the spots. Finally, the number of RNA molecules in each spot was quantified by adopting a spot intensity distribution slicing approach proposed previously (6).

The quantification of individual RNA molecules is not trivial, since RNA-MS2-GFP complexes can be colocalized (6). The number of GFP molecules attached to an RNA molecule at any given moment can vary from 40 to 100 (70 on average) (5). However, in general, the first peak of the distribution of intensities of many spots from cells on the same slide corresponds to individual RNA-MS2-GFP complexes (6). From that, the intensity of a single RNA-MS2-GFP complex can be obtained. Subsequent peaks in the distribution of intensities correspond to spots consisting of multiple RNA-MS2-GFP complexes. The number of RNA-MS2-GFP complexes in a spot can then be estimated by normalizing the intensity of the spot to the intensity of a single RNA-MS2-GFP complex (6).

Analytical estimation of the expected difference in the number of RNA molecules between the two poles, $\langle |\Delta N| \rangle$, assuming a biased binomial partitioning. We used $|\Delta N|$, the absolute difference between the number of RNA-MS2-GFP complexes in each pole of the cell, as a measure of the degree of bias of the partitioning distribution. Assuming that the partitioning of the RNA-MS2-GFP complexes between the two daughter cells follows a biased binomial distribution with a given p , the expected $|\Delta N|$ for a given total number (N) of RNA-MS2-GFP complexes in a cell can be calculated as follows:

$$E_{|\Delta N|}(N, p) = \sum_{k=0}^N \left| 2k - N \right| \binom{N}{k} p^k (1-p)^{N-k} \quad (2)$$

RESULTS

Detection of RNA-MS2-GFP complexes in *E. coli*. To analyze the dynamics of the production and movement of RNA-MS2-GFP complexes in the cells, we measured their numbers and their locations over time in individual *E. coli* cells. Cells were imaged every minute over a period of approximately 2 h following the induction of the target RNA and of the MS2-GFP tagging proteins. In Fig. 1, we show an example of an image where several *E. coli* cells each express one or more target RNAs, along with the same image following the segmentation process. In Fig. 2, we show a sequence of three images taken with the same measurement to show how the RNA-MS2-GFP complexes move along the major axis of the cell.

We first studied the location where RNA-MS2-GFP complexes first appeared in the cell. The RNA-MS2-GFP complexes are visible shortly after the target RNA is transcribed (5), and thus, the location where they are first seen should be very near the F plasmid. In *E. coli*, F plasmids preferentially reside near the center of the cell, migrating to the first-quarter and third-quarter positions along the cell's major axis following its duplication (7, 8). Consequently, the RNA molecules should be produced and first appear near the cell center or at the first-quarter or third-quarter position. We recorded the position along the major axis where each RNA-MS2-GFP complex was first detected. The results shown in Fig. 3 demonstrate that spots were first observed at these positions, in agreement with the expected location of the F plasmid. Namely, most spots appeared close to the center, and a few appeared close to the first-quarter and third-quarter positions.

We determined how many RNA-MS2-GFP complexes were present in each spot from the intensity of the fluorescence when they first appeared (see Materials and Methods). All spots that appeared during the time series measurements clearly contained a single target RNA. A previous assessment (5) also reported that RNA-MS2-GFP complexes at the midcell generally contain only a single target RNA.

FIG 1 RNA-MS2-GFP complexes in *E. coli* cells. Shown are an unprocessed image of RNA-MS2-GFP complexes in *E. coli* cells, with an arrow pointing to an individual spot (left), and the corresponding segmented image showing the detected cells (gray) and the spots (white) within (right). The expression of the target RNA was induced with 1 mM IPTG and 6.7 mM arabinose.

Migration of newly produced RNA-MS2-GFP complexes within single cells. We tracked the movement of newly produced RNA-MS2-GFP complexes. All but 5% traveled independently to one of the poles of the cell during the observation period, in agreement with previously reported observations (5). After an RNA-MS2-GFP complex reached a pole, while brief incursions to the midcell regions were occasionally observed, it tended to remain there, in agreement with previously reported observations (5). Notice that even when only one tagged RNA was present in the cell, it traveled to and remained at a pole most of the time, implying that this dynamic is not due to interactions between RNA-MS2-GFP complexes.

To further verify that RNA-MS2-GFP complexes preferentially locate at the poles, we imaged 762 cells 1 h after the induction of the target gene by IPTG and arabinose. We tested different concentrations of inducers to examine whether the spatial distribution would differ in shape with the mean expression level. No differences were found, and we thus merged the results of these tests. Figure 4 shows the distribution of the distances of all detected individual RNA-MS2-GFP complexes to the center of the cells, normalized by half the cell length from this set of measurements. The distribution is bimodal, with a strong peak centered at 0.8, showing that most spots were located at the poles, and a small “peak” at the midcell region, likely due to the contribution of RNA-MS2-GFP complexes visible while the target RNA was being transcribed and anchored to the plasmid (5). We note a striking similarity between this distribution and the one reported previ-

ously by Lindner et al. (9) of the spatial distribution of a fluorescently tagged chaperone (IbpA) involved in aggregate processing.

Next, from time series measurements, we measured the time between the productions of individual RNA-MS2-GFP complexes and recorded the pole to which each complex traveled. Examples of these measurements are shown in Table 1. Table 1 shows the moment when a spot was first observed, how many new RNA-MS2-GFP complexes appeared in each cell, to which pole each RNA-MS2-GFP complex traveled, and how long it took them to travel there. The mean time between the productions of consecutive RNA-MS2-GFP complexes was approximately 2,000 s, as these cells were subjected to only half the maximum induction (see Materials and Methods). The time to reach a pole was found to be much lower than the mean interval between production events, indicating that newly created RNA-MS2-GFP complexes reached a pole prior to the transcription of the next target RNA (described in more detail below). Finally, it is apparent that while the choice of pole to which the RNA-MS2-GFP complexes travel in each cell is probabilistic, it is strongly biased toward one of the poles of the cell.

The fact that the time interval between transcription events is much larger than the time each RNA-MS2-GFP complex takes to, independently, reach a pole indicates that the accumulation of the RNA-MS2-GFP complexes at the cell poles is not the result of aggregation with other complexes. This is further supported by the fact that even when only one target RNA molecule exists in the

FIG 2 Spatial kinetics of RNA-MS2-GFP complexes in *E. coli* cells. Sequences of images of a cell with RNA-MS2-GFP complexes are shown. The arrows point to an RNA spot as it is formed in the midcell region and moves from there to a pole. In this case, the RNA-MS2-GFP complex took approximately 1,000 s to reach the pole since it was created. The expression of the target RNA was induced with 1 mM IPTG and 6.7 mM arabinose.

FIG 3 Spatial distribution of RNA-MS2-GFP complexes in cells when first observed. Shown are the numbers of newly created RNA-MS2-GFP complexes and the distance between where they were first observed and the midcell point along the major axis of the cell. The distance is normalized by half the cell length. These data are an example of results from observations of 12 cells.

cell, the RNA-MS2-GFP complex still travels to and then remains at one of the poles most of the time.

Next, we investigated whether there is a bias in the choice of pole, as the results in Table 1 suggest. In Fig. 5, we show the progression in time of the mean absolute difference between the numbers of RNA-MS2-GFP complexes in each pole of each cell (denoted by $\langle |\Delta N| \rangle$), taken over all cells for each time point (50 cells were observed, each of which produced at least one RNA-MS2-GFP complex during the observation time). For comparison, we show how this quantity would vary over time, assuming an unbiased binomial partitioning of RNA molecules by the two poles ($p = 0.5$), a totally biased partitioning ($p = 1.0$), and, finally, a partitioning that follows a binomial distribution with a bias of a p value of 0.85 toward one of the poles, which was found to fit the measurements well. In all analytical estimations, we assumed the same total number of RNA-MS2-GFP complexes as those detected in the measurements at each moment in time. We therefore conclude that RNA-MS2-GFP complexes are partitioned in a biased fashion, with a bias close to 0.85, which appears to be consistent over time.

FIG 4 Spatial distribution of RNA-MS2-GFP complexes in a cell population. Shown is the distribution of the distances of RNA-MS2-GFP complexes to the center of the cells, normalized by half the cell length (data from 218 cells not subject to inducers, 198 cells subjected to 0.05 mM IPTG and 0.335 mM arabinose, and 346 cells subjected to 0.25 mM IPTG and 1.675 mM arabinose).

TABLE 1 Examples of the spatial kinetics of individual RNA-MS2-GFP complexes and choice of pole^a

Cell	Time(s) when each new spot appeared after induction (s)	No. of spots that went to pole 1	No. of spots that went to pole 2	Time(s) to reach pole (s)
1	558, 1,578, 6,444	3	0	1,083, 182, 0
2	NA	0	0	NA
3	NA	0	0	NA
4	918, 2,480, 3,918, 5,479	3	1	0, 60, 0, 121
5	619, 2,960, 3,258	3	0	0, 58, 0
6	798, 1,220	2	0	0, 180
7	918, 2,718	2	0	1,742, 181
8	NA	0	0	NA
9	1,518, 5,178, 7,279	2	1	0, 184, 59
10	2,718	1	0	0
11	678, 2,239, 2,899, 7,760	3	0	60, 0, 239
12	1,760, 4,100	1	0	178

^a The times when RNA-MS2-GFP complexes first appeared, to which poles they traveled (the numbering of the poles is arbitrary), and the times to reach the pole are shown. One RNA-MS2-GFP complex in cell 11 and another in cell 12 were not observed to reach a pole, as they were created at a late stage in the measurement. Poles are numbered by convention, with pole “1” being the one to which most of the RNA traveled.

In Fig. 6, we show the mean difference in RNA-MS2-GFP complexes between poles in a population of 367 cells, 60 min after induction, as a function of the number of RNA-MS2-GFP complexes in each cell (N). For comparison, we show the expected value of $|\Delta N|$ as a function of N , assuming a binomial partitioning of RNA-MS2-GFP complexes, with biases of 0.5, 0.85, and 1.0 (see Materials and Methods). The results are in clear agreement with the temporal measurements, since, again, a good fit was obtained with highly biased binomial pole-to-pole partitioning ($p = 0.85$). The results also show that the bias is independent of N , the number of RNA-MS2-GFP complexes in a cell, as would be expected if the bias results from an asymmetric segregation of aggregates.

FIG 5 Temporal evolution of the pole-to-pole bias in the number of RNA-MS2-GFP complexes. Shown are the mean differences over time between numbers of RNA-MS2-GFP complexes in each pole ($\langle |\Delta N| \rangle$) averaged over 50 cells, each of which produced more than 1 spot during the observation time.

FIG 6 Cellular pole-to-pole bias in numbers of RNA-MS2-GFP complexes versus the total number of RNA-MS2-GFP complexes. Shown are the mean differences between the numbers of RNA-MS2-GFP complexes in each pole of individual cells ($\langle \Delta N \rangle$) (solid black line) as a function of the total number of RNA-MS2-GFP complexes in the cell (N). Errors bars show the standard deviations. The expected $\langle \Delta N \rangle$ values, assuming an unbiased binomial partitioning ($p = 0.5$) (dashed line), a biased binomial partitioning ($p = 0.85$) (solid gray line), and a totally biased partitioning ($p = 1.0$) (dotted line), are shown for comparison. Data are from 80 cells treated with 0.5 mM IPTG and 3.35 mM arabinose, 101 cells treated with 1.0 mM IPTG and 6.7 mM arabinose, and 186 cells treated with 2.0 mM IPTG and 13.4 mM arabinose.

Note that the observations are from three measurements, in which the cells were subjected to different induction levels, and no significant difference was found in the mean biases observed for the three sets of cells. Due to this, we again merged the results from the three measurements. In conclusion, we find that RNA-MS2-GFP complexes were partitioned in a biased fashion, with a bias that is consistent both over time and with respect to the number of previously segregated complexes.

It is possible, given the long division time of the cells, that the segregation of the F plasmids to the first and third quarters of the cell following its duplication could affect the results. Namely, it is conceivable that an RNA-MS2-GFP complex produced from an F plasmid at the quarter region would preferentially migrate to the nearest pole. If only one of the F plasmids expresses, this could be responsible for some bias in the RNA-MS2-GFP complex partitioning between the poles. We therefore tested for a correlation between the side of the cell where an RNA-MS2-GFP complex first appears (when it appears at a quarter region) and the pole to which it travels.

From two independent time series, we tracked 17 such RNA-MS2-GFP complexes that first appeared at the first- or at the third-quarter position. We then registered to which pole they traveled. The Pearson correlation coefficient between the initial and the final normalized locations along the cell major axis was -0.1 , indicating that there is no strong correlation between where RNA-MS2-GFP complexes appeared and the pole to which they traveled. Interestingly, these results also show that if an F plasmid was off-center prior to duplication and segregation, it would still not be a source of the observed bias in pole choice by the RNA-MS2-GFP complexes.

We noticed that the cells' division time on the slide is longer (approximately 90 min) than the division time in liquid culture (see Materials and Methods). We verified whether the stress to which the cells are subjected, which affects the division time, would also affect the partitioning of the RNA-MS2-GFP com-

plexes by the two poles of the cells. For this, for the same induction strength, we imaged several cell populations at different moments after being placed under the microscope, since prior to this, they had a normal division time of approximately 35 min (when in liquid culture) and were thus not under stress. We found no difference in the value of the bias of the partitioning by the poles, and therefore, we can conclude that this phenomenon is not significantly affected by the stress to which the cells are subjected.

It is of importance to determine the kinetics of the MS2-GFP tagging molecules alone. It was shown previously that MS2-GFP distributes itself homogeneously in the cell cytoplasm (5). We verified this result in detail both in many cells of several populations and over time (results not shown). Namely, we analyzed the fluorescence intensity distribution in multiple cells in which the expression of the target RNA was not induced, to determine the *in vivo* spatial distribution of MS2-GFP reporter proteins alone. From image analyses of images taken of many such cells over time, we found that the spatial distribution of MS2-GFP remained uniform over time. From this, it is possible to conclude that only RNA-MS2-GFP complexes are sent to and remain at the poles, with the choice of pole being biased. The same behavior was not observed for the MS2-GFP molecules alone.

Finally, we note that analyses of the spatial distribution of several RNA molecules using other tagging methods that do not rely on MS2-GFP (such as fluorescence *in situ* hybridization [FISH]) have provided evidence that RNA molecules alone are not generally located at the cell poles (10). From this, we conclude that only the RNA-MS2-GFP complexes are disposed of by the cells, while the tagging molecules alone or the RNA alone is not. This suggests that this disposal mechanism is capable of accurately distinguishing protein aggregates from functional proteins.

DISCUSSION

The appearance and inheritance of spontaneous protein aggregates within lineages of *E. coli* cells grown under nonstressed conditions were monitored previously by using time-lapse microscopy and a fluorescently tagged chaperone (IbpA) involved in aggregate processing (9). These measurements revealed that these aggregates accumulated upon cell division in cells with older poles. The authors of that study suggested the existence of an asymmetric strategy whereby dividing cells segregate damage at the expense of aging individuals. Several examples exist of such mechanisms in organisms other than *E. coli*.

Since RNA-MS2-GFP complexes are not native to *E. coli*, it may be that the cell recognizes them as an undesirable substance. Due to this, here we used the MS2-GFP-tagging method to observe the kinetics of this disposal mechanism at the single-event level, as it allows the observation of individual tagged RNA molecules.

We observed that the disposal mechanism acts on individual aggregates and that the choice of pole is probabilistic and biased. Since the cells do not accumulate MS2-GFP molecules at the poles, even though these protein complexes are also not indigenous to the cells, it is possible that this mechanism is capable of targeting very specific protein complexes.

Several questions remain unanswered. For example, the choice of pole was found to be well fit by a binomial distribution, with a bias of 0.85. It would be of great interest to determine whether this bias is similar in all cases, e.g., for all protein aggregates, or if it varies depending on the aggregate that is being segregated. Other

partitioning schemes may also exist (e.g., remaining at the cell center, near the point of division, rather than moving to the poles). Also, is the bias observed a demonstration of some inefficiency of this mechanism, a “safety precaution” against the absolute segregation of substances wrongly identified as being harmful, or a compromise between energy costs and the removal of harmful substances? Finally, what are the mechanisms responsible for targeting specific protein complexes and for segregating them? Future studies are needed to address these questions and may be of relevance for an understanding of the adaptability of these organisms and how they cope with aging.

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REFERENCES

1. Chen TB, Lu HH, Lee YS, Lan H-J. 2008. Segmentation of cDNA microarray images by kernel density estimation. *J. Biomed. Inform.* 41: 1021–1027.
2. Reference deleted.
3. Devroye L, Györfi L. 1996. A probabilistic theory of pattern recognition, 1st ed. Springer-Verlag, New York, NY.
4. Fusco D, et al. 2003. Single mRNA molecules demonstrate probabilistic movement in living mammalian cells. *Curr. Biol.* 13:161–167.
5. Golding I, Cox EC. 2004. RNA dynamics in live *Escherichia coli* cells. *Proc. Natl. Acad. Sci. U. S. A.* 101:11310–11315.
6. Golding I, Paulsson J, Zawilski SM, Cox EC. 2005. Real-time kinetics of gene activity in individual bacteria. *Cell* 123:1025–1036.
7. Gordon GS, et al. 1997. Chromosome and low copy plasmid segregation in *E. coli*: visual evidence for distinct mechanisms. *Cell* 90:1113–1121.
8. Gordon S, Rech J, Lane D, Wright A. 2004. Kinetics of plasmid segregation in *Escherichia coli*. *Mol. Microbiol.* 51:461–469.
9. Lindner AB, Madden R, Demarez A, Stewart EJ, Taddei F. 2008. Asymmetric segregation of protein aggregates is associated with cellular aging and rejuvenation. *Proc. Natl. Acad. Sci. U. S. A.* 105:3076–3081.
10. Llopis PM, et al. 2010. Spatial organization of the flow of genetic information in bacteria. *Nature* 466:77–81.
11. Lloyd-Price J, et al. 2012. Probabilistic RNA partitioning generates transient increases in the normalized variance of RNA numbers in synchronized populations of *Escherichia coli*. *Mol. Biosyst.* 8:565–571.
12. Lutz R, Bujard H. 1997. Independent and tight regulation of transcriptional units in *Escherichia coli* via the LacR/O, the TetR/O and AraC/I1-I2 regulatory elements. *Nucleic Acids Res.* 25:1203–1210.
13. Otsu N. 1979. A threshold selection method from gray-level histograms. *IEEE Trans. Syst. Man Cybern.* 9:62–66.
14. Stewart EJ, Madden R, Paul G, Taddei F. 2005. Aging and death in an organism that reproduces by morphologically symmetric division. *PLoS Biol.* 3:295–300.
15. Wang Q, Niemi J, Tan C-M, You L, West M. 2010. Image segmentation and dynamic lineage analysis in single-cell fluorescence microscopy. *Cytometry A* 77:101–110.