Using a Synthetic Probe to Study the Robustness of the Segregation Process of Protein Aggregates in *Escherichia coli*

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Abstract—**Even though the processes of protein production and folding are not immune to errors, *Escherichia coli* lineages are capable to maintain a stable cell lineage, provided viable environmental conditions. One of the internal processes that makes this possible consists of segregating unwanted protein aggregates to the cell poles by nucleoid exclusion, which, combined with cell divisions, generates asymmetries in the aging process of the population, with some individuals aging faster while others exhibit rejuvenation. A recent study showed that this process is not immune to sub-optimal temperature conditions due to increased cytoplasm viscosity, which weakens the anisotropy in aggregate displacements at the nucleoid borders. This was made possible by the usage of a synthetic fluorescent probe, consisting of a RNA sequence with multiple binding sites for the MS2-GFP synthetic protein, which can be tracked in time-lapse microscopy images. Here we provide a description of the findings from these measurements and investigate with an *In Silico* model the consequences in the context of cell lineages.**

Keywords-segregation; polar retention; protein aggregates; cold temperature conditions; synthetic probes; cell lineages.

I. INTRODUCTION

Escherichia coli are able to segregate unwanted protein aggregates to the cell poles by nucleoid exclusion. This process is essential for cell lineages to generate cells that are free from aggregates. Such 'rejuvenated' cells have been shown to exhibit faster division time than 'older' cells, where aggregates accumulate at, and are thus essential for the maintenance of vitality of the lineages [1].

The exclusion of aggregates from midcell is made possible by the presence of the nucleoid at midcell, which causes anisotropy in the dynamics of the aggregates that generates the preference for polar localization [2].

Recent studies, making use of a synthetic fluorescent probe that allows observing the processes of segregation and retention with single aggregate sensitivity, showed that at lower temperatures, the degree of viscosity of the cytoplasm increases, which hampers the anisotropy [3]. These synthetic probes are ideal in that they behave similarly to natural aggregates, have long life-times with highly stable fluorescence levels, and are robust to photobleaching [2]. In addition, and contrary to natural aggregates, the synthetic aggregates have all the same fluorescence level and do not interact with one another or with other cellular components, facilitating their counting from the images.

Here, based on the empirical data that was obtained by observing cells containing these probes and placed in environments at different temperatures while under microscope observation, we investigate the long-term consequences to future cell generations of the temperature-dependence of the aggregate segregation and subsequent polar retention processes, following the occurrence of sub-optimal conditions.

II. PREVIOUS FINDINGS

In our previous study [3], we compared the efficiency with which aggregates are segregated to and retained at the cell poles by the nucleoids in optimal and in sub-optimal temperature conditions.

Observing cells with one nucleoid, and by probing the positioning of both nucleoids and aggregates, we found that at lower temperatures the aggregates are not preferentially located at the poles. Results are shown in Table I.

From the table, note how the relative concentration of aggregates at the poles is close to 1 (corresponding to uniform distribution along the major cell axis) for low temperatures. Meanwhile, at the higher temperatures, it is much larger than 1. Note also how, according to the Kolmogorov-Smirnov test, the behavioral change is statistically significant between 24 and 37 degrees.

TABLE I.   AGGREGATES AT THE POLES

T (°C)	**Concentration of Aggregates at the poles**		
	Relative nucleoid length	*Relative concentration of Aggregates at poles*	*P value of KS test*
10	0.63	1.32	
24	0.56	1.09	0.11
37	0.53	1.86	< 0.01
43	0.47	1.79	0.05

Next, in cells with two nucleoids, the concentration of aggregates in between the nucleoids was measured. From Table II, the relative concentration of aggregates in between nucleoids in cells close to division decreases significantly as the temperature increases [3]. Thus, one can conclude that the relative concentration of the aggregates at the poles is increasing with increasing temperature.

TABLE II.   AGGREGATES IN BETWEEN NUCLEOIDS

T (°C)	Concentration of Aggregates in between nucleoids in cells close to dividing		
	Relative nucleoids length	*Relative concentration of Aggregates in between nucleoids*	*P value of the permutation test*
10	0.75	0.85	
24	0.68	0.78	< 0.01
37	0.72	0.69	< 0.01
43	0.70	0.68	< 0.01

III.   RESULTS AND DISCUSSION

Note that, in division, while the aggregates at the poles will remain at the old pole of the cells of the new generation, those at midcell will be at the new poles. As such, changes in the fractions at midcell prior to division should affect the distributions of aggregates in individual cells of future generations. In particular, we hypothesized that at lower temperatures, as the ability of cells to exclude the aggregates to the poles is significantly reduced, future cell generations will have more homogenous distributions of unwanted protein aggregates, which is expected to hamper the rejuvenation process of the lineage. To validate our hypothesis, we developed a simple stochastic model.

In this model, we start with a cell near division (generation 0), with 200 aggregates whose location (in between nucleoids or at the poles) is defined by the empirical values in Table II. Then, the cell divides and the aggregates are placed in the 'old' and 'new' pole of the two daughter cells, in accordance with their location in the mother cell prior division (i.e., in the pole or in between nucleoids, respectively). In this regard, the aggregates that were at midcell were placed randomly in either daughter cell. Note that, at this stage (i.e., generation 1) all aggregates are at the poles in all cells. Finally, these daughter cells also divide, producing four cells (generation 2). Two of these cells will inherit the original poles of the mother cell, while the remaining ones will inherit only poles generated during the two division processes. Meanwhile the partitioning processes of the aggregates follow the same rules as before.

Using this model, we compared the outcomes at different temperatures, by setting different concentrations of aggregates in between nucleoids of the original mother cell in accordance with the empirical values in Table II. Namely, for each condition, we obtained the mean and standard deviation of the numbers of aggregates in individual cells in the last generation from 10.000 independent simulations. Results are shown in Table III.

TABLE III.   AGGREGATES IN THE LAST GENERATION

T (°C)	Distributions of aggregates in cells of the last generation	
	Mean number of Aggregates per cell	*Standard deviation of the number of aggregates per cell*
10	50	10.5
24	50	12.2
37	50	16.7
43	50	17.4

From Table III, first, as expected, temperature does not affect the mean number of aggregates in each cell (50 as we started with 200 and 2 rounds of division took place). Also, we find that as temperature increases (and thus, the relative concentration of aggregates at the poles decreases), as expected, the variability in the aggregates numbers in cells of future generations increases. The decrease at lower temperatures, most likely, will result in the hampering of the rejuvenation process of the lineage in these conditions.

We conclude that the effects of lower temperatures at the single cell level have long term consequences in the functioning of cell lineages aging and rejuvenation processes.

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