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Bet-hedging: Bacterial ribosome dynamics during growth transitions

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It is known that bacteria reduce their ribosome numbers during nutrient starvation. New research shows that this regulation leads to the formation of two subpopulations with distinct ribosomal RNA levels. The distinct levels affect the growth recovery when nutrients become available, suggesting a possible bet-hedging strategy.

Bacteria respond dynamically to their fluctuating environments as they strive to maximize their fitness. When nutrients are plentiful, bacteria grow exponentially; in contrast, nutrient scarcity and other adverse conditions force them to cease growth and enter a non-dividing state until nutrients become available. Of course, a strain of bacteria is rarely alone, and often competes for nutrients with other species. Quickly utilizing nutrients to initiate growth is an essential trait for survival in fluctuating environments. Ribosome abundance and activity have been found to be correlated with the growth rate[1,2]. Thus, adjusting ribosome abundance and activity is crucial for adaptation to a new environment.

The ribosome is the cell's major workhorse for protein synthesis. This, in turn, affects the cell's biomass and population growth, as it enables the synthesis of enzymes crucial for generating cellular components. Due to its importance for active growth, the ribosome is the most abundant macromolecular machine in the cell during exponential growth[3], whereas its abundance decreases drastically during nutrient starvation[4].

Bacterial ribosomes consist of 55 ribosomal proteins and 3 ribosomal RNAs (rRNAs)[5], making up approximately 37% of the cell's dry mass during the exponential growth phase[3]. As such, the ribosome is the major consumer for not only materials but also energy; protein synthesis accounts for approximately 50% of the energy consumption during exponential growth[6]. Therefore, cells mitigate this burden by controlling the synthesis of new ribosomes and even degrade the large fraction of non-active ribosomes during the transition from a fast-growing state to dormancy[7,8]. Since the synthesis of ribosomal proteins would be extremely costly during nutrient depletion, cells must be careful when degrading ribosomes. But what would be the optimal strategy? In this issue of *Current Biology*, Ciolli Mattioli *et al.* reveal how the coordination between ribosome degradation and downregulation of rRNA synthesis generates heterogeneity in rRNA levels in *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) during nutrient depletion[9] (Figure 1).

Traditionally, experiments were carried out in bulk cultures, which can only allow measurement of population-averaged responses. However, in reality, bacterial populations exhibit remarkable heterogeneity, particularly during growth transitions[10]. This study shows that ribosomes are no exception. Ciolli Mattioli *et al.* carried out fluorescence *in situ* hybridization targeting rRNAs (rRNA-FISH) to measure the rRNA abundance in individual cells. As a population entered stationary phase, rRNA abundance decreased, as expected. However, whereas rRNA abundance decreased uniformly in *Escherichia coli* and *Staphylococcus aureus*, *S.* Typhimurium exhibited a heterogeneous decrease, giving rise to two distinct subpopulations characterized by different rRNA levels: $16S^{high}$ and $16S^{low}$, corresponding to cells with high and low 16S rRNA levels, respectively.

S. Typhimurium causes a typhoid-like infection in mice and is commonly used as a model organism for studying typhoid fever. Notably, this bacterium infects macrophages, eventually spreading throughout the body and causing systemic infections[11]. Nutrients available inside the macrophages are limited[12,13], and a majority of cells exhibits growth arrest[14]. Not surprisingly, the fraction of $16S^{high}$ cells decreased during infection, suggesting a link between ribosome abundance and the bacterium's response to the intracellular environment.

What could be the implications for this heterogeneity? Given that rRNA levels correspond to the cell's capacity for protein synthesis, the authors hypothesized that such heterogeneity would affect the ability to resuscitate from dormancy. To test this hypothesis, the authors used carbon- and phosphate-limited conditions instead of using complex rich media. During phosphate limitation, the majority of cells reduced rRNA levels ($16S^{low}$), whereas carbon limitation yielded a higher fraction of $16S^{high}$ cells. As expected, cultures with a higher fraction of $16S^{high}$ cells (carbon limited) showed increased protein synthesis. Given the important role of ribosomes in cell growth, cells with low levels of rRNAs are likely to exhibit a longer lag time or even be unable to recover from dormancy when nutrients become available[4,15]. Consistent with this prediction, a culture grown in a condition that yielded a lower fraction of $16S^{high}$ cells (phosphate limited) resulted in a longer lag time. Although FISH was critical

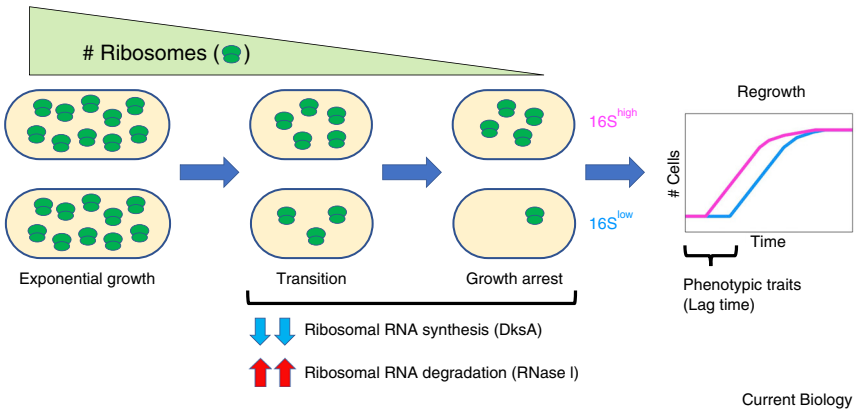

Figure 1. Bimodal distribution of ribosomes within a population of *Salmonella* Typhimurium in adverse environments.
During nutrient starvation or infection of host cells, two distinct subpopulations with different ribosomal RNA (rRNA) levels emerge in an *S.* Typhimurium population. This bimodal distribution is controlled by the synthesis and degradation of rRNAs. The resulting heterogeneity in rRNA levels ($16S^{high}$ and $16S^{low}$) leads to variations in phenotypic traits such as protein synthesis and the recovery time from dormancy when the environment becomes favorable for growth.

to the observation of this heterogeneity, its procedure naturally involves chemical fixing of cells. Therefore, although heterogeneity was confirmed by assaying individual cells, it was not possible to directly measure the effects of rRNA levels on the lag time at single-cell resolution.

How do bacteria generate the bimodality in ribosome abundance? During starvation, bacteria reduce ribosome abundance through a combination of mechanisms, including downregulation of ribosome biosynthesis, active degradation of ribosomes[7,8], and likely reductive cell division as well[10]. To assess the impact of active ribosome degradation on the bimodal ribosome distribution, the authors developed theoretical models of ribosome reduction during the transition. These models included a dilution model in which ribosome reduction occurs through the shutdown of rRNA synthesis and partitioning of the ribosome pool by cell division, as well as a dilution model that included active degradation. The models suggest that the reduction in ribosome abundance observed during the transition cannot be solely explained by the dilution alone, suggesting that active degradation is a key factor in this process.

To identify the genetic factors responsible for ribosome bimodality, the authors conducted a genome-wide screen using Tn-seq in combination with sorting of $16S^{high}$ cells. Among the candidate genes identified, the authors confirmed the role of DksA (a stringent response factor known to influence rRNA synthesis) and RNase I (RNA degradation) on ribosome bimodality. The absence of $16S^{low}$ cells in these mutant strains was correlated with the uniform short lag time during nutritional upshift, indicating the direct link between DksA, RNase I, and ribosome bimodality.

Hibernation factors such as RMF and HPF have been reported to be essential for preservation of ribosomes during prolonged starvation in a wide range of bacterial species[16]. Interestingly, hibernation factors did not influence the ribosome bimodality in *S.* Typhimurium in this paper. One possible reason for this discrepancy is that the measurements were made during transition, whereas previous studies of hibernation factors were conducted in deep stationary phase.

Previous studies have shown how the lag time affects population survival under various environments. Non-growing cells in the lag phase can tolerate antibiotic treatment (persisters)[17,18]. In contrast, cells with shorter lag time can quickly occupy the environment when nutrients become available. Indeed, both dormancy and active division have crucial roles in pathogenesis of *S.* Typhimurium[19,20]. It is plausible that *S.* Typhimurium strategically employs the bimodal ribosome distribution as a bet-hedging, risk mitigation strategy, enhancing overall adaptability and resilience of a population in fluctuating environments. This suggests that factors influencing ribosome bimodality could serve as potential drug targets to reduce heterogeneity, making cells more sensitive to ribosome-targeting drugs or eliminating the cell's ability to resuscitate from dormancy by further reducing ribosome abundance.

This study generates many other interesting questions. How do cells control the degradation of ribosomes? Clearly, complete degradation of ribosomes would be highly unfavorable. When do they decide to cease the degradation? Is there a way to predict which cells would have high levels of ribosomes prior to the transition? Exploring the real-time kinetics of ribosome degradation at single-cell resolution would be an interesting avenue of research.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Insect neurobiology: What to do with conflicting evidence?

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Sensory systems gather information from the environment so the nervous system can formulate appropriate responses. But what happens when sensory information is inconsistent? A new study demonstrates how flies respond to incompatible visual evidence of their own motion.

In poor weather, when clouds and fog obscure the ground, amateur pilots sometimes believe their plane is level when it is actually banking and losing altitude. Pulling the yoke back to regain elevation instead leans further into the turn, reducing lift and hastening the drop. These 'graveyard spirals' are just as dangerous as they sound[1], so when visibility is low experienced pilots disregard their own senses and rely solely on their instruments. When your visual and vestibular systems disagree with your attitude indicator, knowing what to believe can determine whether your landing is soft and safe, or abrupt and unpleasant[2]. The stakes are just as high for animals in the wild, which routinely contend with incomplete or seemingly contradictory sensory information. In this issue of *Current Biology*, Tanaka *et al.*[3] report that fruit flies presented with simultaneous, conflicting visual cues about their own turning seem to weigh both supporting and opposing evidence, much like trained pilots, when deciding how to stabilize.

For collecting evidence about the environment, your visual system provides a wealth of information[4]. By responding to the incoming direction of light, vision reveals external objects, both near and inconceivably distant, along with their structure and motion. Vision is a highly informative sense[5]: as long as there is light and a clear line of sight, you get fast and precise updates about your external world[6]. Beautiful, but the external world can be a confusing mess. To navigate through an airport, for example, you must analyze quickly changing scenes of corridors, turns, crowds, officials, lights, and signs, and then integrate them into behaviors you hope will lead to your gate. What demands attention? What do you