COMMENTARY

Stress management strategies in single bacterial cells

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Microbial genomes are highly adaptable to changing environmental conditions (1). Mechanisms such as duplications, deletions, and horizontal gene transfer events generate diversity in bacterial gene repertoires. In effect, most free-living bacterial populations contain myriads of individual cells that differ in gene content. Some bacterial species contain open pangenomes in which each strain contains a unique subset of genes, whereas others have closed pangenomes in which the total number of genes in the population is finite (2). Thus, each bacterial population contains hundreds or thousands of genes in low abundance, any of which can rapidly spread in the population to match a new environmental challenge, given appropriate selective constraints. On top of this variability in gene content, positive or diversifying selection on, for example, genes for cell surface proteins that are targets for bacteriophages can generate dramatic gene sequence diversity among individual cells in the population despite otherwise nearly identical genomes. However, none of these responses depends on a learning process in which an individual cell temporarily changes its behavior without genetic modification to ensure a higher chance to survive when faced with a similar challenge in the near future. In PNAS, Mathias and Ackerman (3) explore the question of memory on a single-cell level.

Short-Term Memory in Bacterial Populations

Whether bacterial cells can "memorize" past events, and whether they can forget, and if so, how quickly, are controversial. Part of the problem is that it is not entirely clear how to define a bacterial cell "memory." Although it has been shown that bacterial behavior can be modified to reflect preceding events, the time elapsed between the events is normally very short, counting in seconds or minutes, and, as such, may not be classified as a "true memory." Fluctuations in nutrient supply or other nonlethal stress events have been shown to trigger short-term history-dependent changes in cellular functions under laboratory settings (4, 5). Mechanistically, the responses operate through the transmission of cytoplasmic proteins with lifetimes longer than the generation of a bacterial cell. For example, the stability of proteins encoded by the lactose operon reduced the lag phase during growth in media in which the concentrations of glucose and lactose fluctuated (4). In principle, such effects are to be expected for any transport protein, regulatory system, or enzyme with a stability that exceeds the lifetime of the cell. These short-term changes in cell functionality have been referred to as a "passive, physiological, or phenotypic memory."

However, the short-term memory effects should perhaps better be viewed as stress management strategies to cope with rapidly changing growth conditions. In contrast, constitutive expression of the induced proteins would provide a long-term true memory, but at a metabolic cost that would give the constitutive cells a fitness disadvantage under conditions in which the expressed molecules would not be needed (4). By transiently expressing these molecules, a short-term fitness advantage can be achieved, whereas the costs are kept at a minimum. How the cell balances prolonged protein expression against the metabolic costs is likely to depend on how frequently the stimuli appear over a given time scale in the natural habitat of the bacterial population. One hypothesis is that protein expression levels and lifetimes are evolutionarily tuned not only to the immediate growth conditions but also to the physiological memory of past events (4).

Memory-Like Response Regulation in Natural Ecosystems

The observations made from laboratory experiments raise questions about the extent to which bacterial memories operate in the natural environment. In the natural ecosystem, bacterial responses to stimuli and stresses are much more complicated than in well-defined laboratory experiments (as discussed in 5). In the simplest case scenario, the bacterial cell responds directly to an altered condition, for example, by up-regulating the lactose operon when the nutritional supply of lactose increases. If the up-regulated proteins are more stable than the life span of the cell, they may persist and facilitate a rapid response if the stimuli reappear shortly

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Author contributions: S.G.E.A. wrote the paper.
The author declares no conflict of interest.

See companion article on page 4224.

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thereafter, as observed in the experimental test systems (4, 5). However, the environmental fluctuations between two different states may be erratic, and thereby difficult to predict. In such cases, stochastic switching between alternative states may be the best strategy to meet the challenges.

A predictable but more complicated scenario is that a preceding signal provides indications of another, subsequent environmental change. One such example is provided by the entrance of *Escherichia coli* into the digestive tract, in which an initial rise in temperature is followed by a reduced concentration of oxygen. Interestingly, when tested on *E. coli* in the laboratory, either of these changes induced the expression of genes needed to cope with both a rise in temperature as well as a drop in oxygen levels (5). However, the responses were decoupled when the signals were presented out of phase, suggesting that the bacterium senses and responds to the frequency at which the environmental challenge is presented.

Another predictable pattern is asymmetrical response regulation in which one stimulus activates two responses but another stimulus activates only one of the responses (5). For example, lactose can induce both the lactose and the maltose operons in *E. coli*, whereas maltose only induces the maltose operon. This example is also of direct relevance for *E. coli* in its ecological context because the exposure to lactose precedes the exposure to maltose in the digestive tract. As expected, the WT *E. coli* strain displayed a fitness advantage if growth on maltose was preceded by growth on lactose (5). However, after 500 generations of growth on lactose without exposure to maltose, the maltose operon was no longer activated by lactose. Thus, the asymmetrical cross-talk was lost under prolonged growth in environments in which no such cross-talk was favorable. This loss of a linked response regulatory system suggests that the ability to predict frequently encountered changes in the growth environment and adjust the metabolism accordingly is a costly system to maintain, and thereby easily lost.

The examples given above demonstrate that memory-like responses to fluctuating growth conditions are operating on bacterial populations to adjust cellular activities appropriately. In the short term, history-dependent behavior at the single-cell level results from the persistence of proteins over one or more bacterial generations, which enables repeated responses to the same stressful event. In the long term, gene expression levels and gene regulatory circuits may be modified by genetic changes to reflect frequently encountered environmental fluctuations. However, no experiments have yet provided evidence for a single-cell memory not associated with genetic modifications that act in the long term.

Caulobacter crescentus as a Model for Studies of Memory-Like Processes

In PNAS, Mathis and Ackerman (3) used *Caulobacter crescentus* as the model organism to study the responses of single cells to repeated exposure to sodium chloride using microfluidic devices. *C. crescentus* is adapted to aquatic environments and evolutionarily related to free-living Alphaproteobacteria that are abundant in marine, freshwater, and soil ecosystems. *C. crescentus* has a unique lifestyle and is frequently used as a model organism for studies of the cell cycle (6). It divides in an asymmetrical manner, in which a surface-attached stalked cell generates a stalked daughter cell that remains attached to the surface and a motile daughter cell that disperses into the water. The motile cell differentiates subsequently into a stalked cell that attaches to the surface and initiates replication and cell division. Once attached to the surface, the stalked cells are unable to escape from stressful changes in the environment. Because of these unique lifestyle characteristics, it was argued that *C. crescentus* should be a useful model bacterium for studies of long-term memory effects in single cells.

It has been shown previously that the exposure of *C. crescentus* to osmotic stress increases the expression of an alternative sigma factor, σ^T, which is involved in the regulation of the general stress response (7). This response is short-lived, however, and persists only for as long as the increased expression level of the alternative sigma factors persists. To test for such short-lived effects, *C. crescentus* was exposed to 80 mM sodium chloride during "a warning event," and immediately thereafter to "a stressful event" with an even higher concentration of 100 mM. As expected, the prior exposure to salt enabled a higher proportion of cells to survive the second stress event, most likely due to a temporarily altered expression level of key proteins in stress response regulation. These results are fully consistent with previous studies in *C. crescentus* and other bacterial species.

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In PNAS, Mathis and Ackermann (3) extended the experiments to test also for long-lived memory effects. In these experiments, the time between the warning and the stressful event ranged from 45 to 160 min, which is far too long for changes in protein expression levels to remain. Surprisingly, the results showed that a prior warning effect influenced the response to the subsequent stressful event. However, the learning process did not simply improve the fraction of surviving cells after the stress event as might be anticipated. Rather, the survival rate fluctuated with peaks every 80 min. Mathis and Ackermann (3) hypothesize that the cell cycle became synchronized during the warning event, as a result of which all cells in the population were at the same stage of cell division when subsequently exposed to the stressful event. If bacterial cells in different stages of the life cycle were sensitive to the high salt concentration to different extents, periodic fluctuations in survival rates might be anticipated. Indeed, an analysis of the cell division activity confirmed that cells that had just divided or were about to divide had a higher survival probability than those cells that were in the middle of cell division. Mechanistically, it was hypothesized that the observed synchronization upon exposure to an increased salt concentration was due to an induced degradation of the *dnaA* gene with the aid of the Lon protein (8), which halted the initiation of DNA replication.

Thus, the mechanisms involved in the learning processes from a warning event are strikingly different if the time elapsed between the warning and the stressful event is short vs. long. If the waiting time is long, the warning event did not simply train the population to cope better with the second stressful event. Therefore, it should not be considered a cellular memory. Rather, the effects observed seem to reflect a more general stress management strategy, in which cell division was blocked in each individual cell upon the first encounter of the stressful event and not resumed until the stressor was removed again. Thus, whether temporary, long-term memory effects operate on single bacterial

cells remains an open question. Irrespectively, it is important to learn more about how individual bacterial cells respond to repeated exposure to the same stressor. Such knowledge may assist in the development of efficient antibiotic treatment schemes, and is thus potentially of great clinical relevance.

Acknowledgments

The author's research is supported by the Swedish Research Council (Grants 349-2007-8732 and 621-2014-4460) and the Knut and Alice Wallenberg Foundation (Grants 2011.0148 and 2012.0075).

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