JB Journals.ASM.org

COMMENTARY

Regulation by the Modulation of Gene Expression Variability

David Dubnau

Public Health Research Institute, New Jersey Medical School, Rutgers University, Newark, New Jersey, USA

Classically, transcription is regulated so that the average expression per cell changes, often with a distribution that extends across the population. Roggiani and Goulian (M. Roggiani and M. Goulian, J. Bacteriol. 197:1976–1987, 2015, doi:http://dx.doi.org/10.1128/JB.00074-15) have shown that this is what happens when the *torCAD* operon of *Escherichia coli* is induced anaerobically by the addition of trimethylamine-*N*-oxide (TMAO). However, when the same inducer is added to aerobically growing cells, only a subset of the cells respond, although the mean expression per cell is similar to that obtained anaerobically. Thus, in the presence of oxygen, the variance but not the expression mean is altered. The regulation of gene expression variance appears to be due to noise in the phosphorelay that governs *torCAD* transcription.

Bacteria respond to environmental signals by a variety of mechanisms, most commonly adjusting the transcriptional output of appropriate genes in appropriate ways. The classical *Escherichia coli lacZ* paradigm made satisfying sense; in response to the presence of a delicious sugar, the bacteria (all of them in the population), transcribe the genes that make the utensils needed for dinner. The prescient study by Novick and Weiner (1) alerted us to interpret ensemble measurements carefully because they can hide dramatic heterogeneity on the single-cell level, even in the "simple" *lacZ* system. It is now widely appreciated that the randomness of chemical interactions can be harnessed to produce important cell-to-cell phenotypic divergence, and so, populations of bacteria, like people, live in an Orwellian world in which not all cells are equal. Phenotypic heterogeneity in clonal populations of bacteria, when coupled with the appropriate circuitry (notably, positive feedback) can cause these populations to bifurcate into subpopulations with dramatically different gene expression profiles. The various developmental states of the Gram-positive model organism *Bacillus subtilis* provide several well-studied examples of this during the development of biofilms, spores, genetic competence, and the reversible transitions between motile and chained sessile cells (2, 3). As noted above, the stochastic nature of chemical interactions, e.g., the encounters of promoters with cognate transcription factors, lies at the root of these divergences in gene expression, and it is intuitively obvious that the importance of this "noise" is inversely related to the abundance of the interactants. In other words, when a transcription factor is present in very few copies per cell, the phenotypic consequences of cell-to-cell variation in its productive binding to a promoter may become more important than it would if the factor were abundant.

The divergence of populations into subtypes is likely to be adaptive. A good example is the occurrence of antibiotic-tolerant subpopulations, so-called persister cells, which are widely considered to represent a bet-hedging strategy that prepares clonal populations for encounters with toxic chemicals and possibly other insults. And so, it must be that the mechanisms that produce these divergent subtypes are subject to selection, leading to the conclusion that the magnitude of gene expression noise must itself evolve. Such considerations have led to important theoretical investigations of the selective pressures that adjust the proportions of, for example, persister cells (for example, see reference 4).

In this issue, Roggiani and Goulian (5) report an unanticipated wrinkle in all of this. They have discovered that, in response to oxygen, *E. coli* adjusts not the average transcription level per cell of an operon that encodes trimethylamine-*N*-oxide (TMAO) reductase but rather the variance of expression. In other words, cell-to-cell variation is regulated in response to oxygen availability. TMAO reductase is encoded by the *torCAD* operon, which is switched on by the phosphorylated form of TorR, a response regulator protein. TorR-P is produced by a phosphorelay involving TorS, a hybrid transmembrane sensor kinase with three phosphorylation sites and the periplasmic protein TorT, which binds to and responds to the presence of TMAO (6, 7). As a result, the system is off when TMAO is absent and on when it is present, in which case this molecule can serve as an alternative to oxygen for respiration. There are other systems in which alternative electron acceptors are induced when conditions are appropriate, and these conditions usually involve the absence of a preferred acceptor, usually oxygen. But not this system, because *torCAD* is induced by TMAO even under oxic conditions and to about the same extent as during anoxia when the population average is measured with a *lacZ* reporter (8). However, Roggiani and Goulian have now found that, on the single-cell level, the heterogeneity of *torCAD* transcription was high in the presence of oxygen, while the population distribution of gene expression was relatively narrow in its absence. (The authors measured the standard deviation divided by the mean of the distributions, a widely used metric of heterogeneity.)

When two copies of the *torCAD* promoter were fused to two fluorescent protein variants in the same cells, a strong correlation of their expression levels was observed, indicating that the noise-generating apparatus was operating in *trans*. When TorT and TorS expression levels were increased artificially or when a *torR* mutant that did not require phosphorylation was introduced, cell-to-cell

Accepted manuscript posted online 30 March 2015

Citation Dubnau D. 2015. Regulation by the modulation of gene expression variability. J Bacteriol 197:1974–1975. doi:10.1128/JB.00235-15.

Editor: R. L. Gourse

Address correspondence to dubnauda@njms.rutgers.edu.

doi:10.1128/JB.00235-15

variation was similar to the level observed in the absence of oxygen. These clever experiments strongly suggest that the source of variation resides in the phosphorelay. This is reminiscent of the *B. subtilis* Spo0A-P phosphorelay, which has been described as a noise generator (9).

Many questions remain, as usual. How does cell-to-cell variation in *torCAD* transcription affect protein levels? How do the cells sense oxygen, and how does the regulation of heterogeneity work? Is it via control of TorS and TorT levels? And then the ultimate question that motivates us as biologists is: what is it for? Why has the system evolved in this way? What is the advantage of increasing heterogeneity when oxygen is around? Interestingly, the total cost of expressing the *tor* operon is about the same in the presence or absence of oxygen. But when oxygen is present, only some cells carry the burden. Is this a bet-hedging strategy, as is often suggested in such cases, or is something else going on here? It makes sense that under conditions of anoxia and in the presence of TMAO, all of the cells express *torCAD* because only in this way can each cell enjoy the derived benefit. But perhaps under oxic conditions, the reduction of TMAO serves a public function. As Roggiano and Goulian point out, it has been suggested (8) that trimethylamine may serve to reduce environmental acidification. The cost of this mutually enjoyed benefit might be borne by a few altruistic cells.

ACKNOWLEDGMENT

Work on the regulation of gene expression in my laboratory is supported by GM057220.

REFERENCES

1. **Novick A, Weiner M.** 1957. Enzyme induction as an all-or-none phenomenon. Proc Natl Acad Sci U S A **43:**553–566. http://dx.doi.org/10.1073/pnas.43.7.553.
2. **Lopez D, Vlamakis H, Kolter R.** 2009. Generation of multiple cell types in Bacillus subtilis. FEMS Microbiol Rev **33:**152–163. http://dx.doi.org/10.1111/j.1574-6976.2008.00148.x.
3. **Mirouze N, Dubnau D.** 2013. Chance and necessity in *Bacillus subtilis* development. Microbiol Spectr **1:**TBS-0004-2012. http://dx.doi.org/10.1128/microbiolspectrum.TBS-0004-2012.
4. **Kussell E, Kishony R, Balaban NQ, Leibler S.** 2005. Bacterial persistence: a model of survival in changing environments. Genetics **169:**1807–1814. http://dx.doi.org/10.1534/genetics.104.035352.
5. **Roggiani M, Goulian M.** 2015. Oxygen-dependent cell-to-cell variability in the output of the *Escherichia coli* Tor phosphorelay. J Bacteriol **197:**1976–1987. http://dx.doi.org/10.1128/JB.00074-15.
6. **Baraquet C, Theraulaz L, Guiral M, Lafitte D, Mejean V, Jourlin-Castelli C.** 2006. TorT, a member of a new periplasmic binding protein family, triggers induction of the Tor respiratory system upon trimethylamine *N*-oxide electron-acceptor binding in Escherichia coli. J Biol Chem **281:**38189–38199. http://dx.doi.org/10.1074/jbc.M604321200.
7. **Jourlin C, Ansaldi M, Mejean V.** 1997. Transphosphorylation of the TorR response regulator requires the three phosphorylation sites of the TorS unorthodox sensor in Escherichia coli. J Mol Biol **267:**770–777. http://dx.doi.org/10.1006/jmbi.1997.0919.
8. **Ansaldi M, Theraulaz L, Baraquet C, Panis G, Mejean V.** 2007. Aerobic TMAO respiration in Escherichia coli. Mol Microbiol **66:**484–494. http://dx.doi.org/10.1111/j.1365-2958.2007.05936.x.
9. **Chastanet A, Vitkup D, Yuan GC, Norman TM, Liu JS, Losick RM.** 2010. Broadly heterogeneous activation of the master regulator for sporulation in Bacillus subtilis. Proc Natl Acad Sci U S A **107:**8486–8491. http://dx.doi.org/10.1073/pnas.1002499107.