JOURNAL OF BACTERIOLOGY, Feb. 1999, p. 697–699 Vol. 181, No. 3
0021-9193/99/$04.00+0

GUEST COMMENTARY

Growth in Studying the Cessation of Growth

ROBERTO KOLTER*

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

Having been asked to write a guest commentary for the *Journal of Bacteriology* as part of the year-long commemoration of the American Society for Microbiology (ASM) centennial, how does one proceed? Having been asked to write about the change in mind-set that led molecular microbiologists to begin exploring the molecular mechanisms at play in the stationary phase of the bacterial life cycle, what can one say? I accepted the challenge of writing something different for the journal, something beyond the traditional research papers and minireviews. After all, the journal for which I have served as editor for the last 5 years is changing in two important ways in celebration of the centennial of the ASM. First, it is dressing up with a color photo on the cover. The journal will now be stunning on the outside as well as the inside. Second, the opening pages of each issue will now include, for this year at least, occasional guest commentaries infusing the journal with a little history, some personal perspective, and perhaps even some lightness.

So, what really did happen in the minds of molecular microbiologists during the last 15 years that led to an opening of perceptions, to a switch in view, to a realization that stationary phase, whatever that meant, was something worth investigating, worth pursuing? What happened? Did anything really happen? Was there really a change in perception? I do remember that a literature search using the key term "stationary phase" in 1983 yielded very few articles, most of them related to matrix selection in liquid chromatography. A similar search today yields thousands of references, the vast majority focused on some aspect of bacterial physiology. When the latest issue of the *Journal of Bacteriology* landed on my desk and I perused its abstracts, it was not too surprising that close to a dozen articles made mention of the stationary phase. This attention to postexponential physiology seems nothing other than natural today, yet less than 15 years ago rather few molecular geneticists paid much attention to this aspect of the bacterial life cycle. How was it that the molecular biology of stationary phase grew over the last two decades? An accurate recounting of the events is certainly much more than can be done within the context of this essay. I am thus left with the (admittedly much easier and personally more enjoyable) task of relating how, as one of the individuals involved in the work, my own ideas about stationary phase changed during that time and how interactions with others helped bring those changes about.

What was the purpose of our work on stationary-phase physiology? One argument which I tried to make was that in the natural setting bacteria seldom encounter such plentiful supplies of nutrients and such benign environments as a culture of *Escherichia coli* encounters in the laboratory while growing exponentially at 37°C. This idea was based on no more evidence than the fact, learned in high school microbiology, that if *E. coli* cells could grow unrestricted in exponential phase they would equal the mass of the earth in less than 2 days. Thus, the naive hypothesis was that stationary phase in the laboratory, rather than exponential phase, more closely resembled what bacteria experienced in their natural environments. This argument was perhaps poorly developed and, in addition, it would not have seemed the least bit unexpected or earth-shattering to microbial ecologists and environmental microbiologists. But most molecular microbiologists studying *E. coli* in the early 1980s had a different mind-set, one that has indeed changed dramatically in the last 15 years. Marty Dworkin recently related to me what might best encapsulate the change in mentality. Since the mid 1950s bacterial physiologists and geneticists had been greatly influenced by the seminal work of Jacques Monod. When Monod said that the purpose of an *E. coli* cell was to make another *E. coli* cell, people listened. The physiology of the growing cell ruled the hearts and minds of those studying *E. coli*. But, Marty Dworkin remarked, a change has occurred and a new phrase can now be heard, "the purpose of an *E. coli* cell is also to survive in the absence of growth."

My graduate training on plasmid molecular biology placed me rather far from the field of stationary-phase physiology. But in 1980, soon after finishing my thesis, a series of events planted the seeds in my mind that would, once I had started my own laboratory, generate the initial impetus behind our studies on stationary phase. Reading the plasmid literature I came across a paper describing microcin-plasmids (12). Further investigation of the microcin literature brought me to encoding the initial report on microcins, a new family of low-molecular-weight antibiotic compounds from enterobacteria (1). Having worked with plasmids, it would have been impossible not to be acquainted with the colicins. But the microcins seemed different, much more like the conventional antibiotics produced by diverse species of *Streptomyces* and *Bacillus*. The thought that *E. coli* produced conventional antibiotics left me wondering, and this wondering led to a conversation with John Ingraham, whom I had had the fortune of meeting while he was on sabbatical at the University of California, San Diego. The discussion somehow turned to microcins, their similarities to antibiotics, and to the question of whether they might also be produced predominantly during stationary phase, such as is the case for most antibiotics. At that time John made, almost in passing, a statement that stuck with me for several years. The transition from exponential growth into stationary phase must be accompanied by major changes in the cell, for he recalled how, 20 years before, he had observed the complete conversion of the unsaturated membrane fatty acids to their cyclopropyl derivatives as cultures entered stationary phase. And yet no

* Mailing address: Department of Microbiology and Molecular Genetics, Harvard Medical School, 200 Longwood Ave., Boston, MA 02115. Phone: (617) 432-1776. Fax: (617) 738-7664. E-mail: kolter@mbcrr.harvard.edu.

one seemed to be studying the molecular biology of stationary phase. The seeds were planted; it would be several years before they sprouted.

One of the first things I did upon arriving at Harvard Medical School in 1983 was to contact someone working with microcins. As I discussed this possible topic with Jon Beckwith, he suggested I contact Felipe Moreno, who coincidentally had just finished a short visit in Jon's laboratory. Thus started what would prove to be one of the most fruitful and enjoyable collaborations I have had to date. Felipe welcomed me as a collaborator to explore the regulation of the synthesis of microcin B17. It soon became evident that microcin B17 activity in culture supernatants increased by several orders of magnitude as cells entered stationary phase. Transcriptional fusions to the microcin B17 production genes also showed dramatic increases upon the cessation of growth (6). We thus had the tools necessary for the study of the regulation of stationary phase-inducible gene expression. Almost at the same time, Matin and coworkers reported the isolation of carbon starvation-inducible fusions as part of a study analyzing the global changes in patterns of proteins made as a consequence of starvation (5). Less than a year later, Nancy Connell from my laboratory, in collaboration with Zhiyi Han and Felipe Moreno, published the sequence of the first stationary phase-inducible promoter, the microcin B17 promoter (3). It was as if stationary-phase physiology had just obtained a learner's permit to conduct experiments in the field of *E. coli* molecular biology.

The identification and characterization of stationary phase-inducible promoters was just the first step in the process of changing of mentalities among bacterial geneticists. The complete switch would come about only with the identification of *rpoS*, the gene encoding an alternative sigma factor that controls the expression of many genes induced at the onset of stationary phase. This process took over 10 years to reach maturity and occurred along two seemingly unrelated paths that eventually converged. One path focused on the mechanisms that protect the cell against the damaging effects of hydrogen peroxide and near-UV light. The second path came from studies that were initially centered around the synthesis of the periplasmic acid phosphatase of *E. coli*.

Working on the biochemistry and genetics of catalases from *E. coli*, Loewen and Triggs in 1984 described a new locus, *katF*, which, along with *katE*, controlled the synthesis of the hydroperoxidase HPII (9). At the time of that publication, however, it was not possible to ascertain whether *katE* or *katF* encoded HPII. In 1981, Tuveson had already described a gene, *nur*, which controlled near-UV sensitivity (23). Given that near-UV generates intracellular hydrogen peroxide, it was not too surprising when Sammartano et al. reported that *nur* and *katF* were two alleles of the same gene (16). The simplest interpretation at that time was that the gene encoded HPII. It was not until 1988, when Mulvey et al. cloned both *katE* and *katF*, that it became apparent that *katE* encoded HPII, and therefore by inference, it was likely that *katF* encoded a positively acting regulatory factor required for HPII activity (11).

The second path that would lead to the eventual identification of *rpoS* resulted from Paul Boquet's investigation of acid phosphatase expression. Boquet and coworkers had noticed that expression of this phosphatase varied greatly depending on the strain background. Using this strain variability as a source of alleles, they identified a regulatory locus, *appR*, that controlled acid phosphatase activity. The initial characterization of *appR* mutants, published in 1986, showed them to have physiological pleiotropy (21). The *appR* mutants were able to reverse the inability to grow on succinate conferred by *cya* or *crp* mutations. This phenotype led them to hypothesize that the mutant *appR* product(s) functioned as weak substitutes for a functional cyclic AMP (cAMP)-cAMP receptor protein complex, suggesting that AppR had a global regulatory role. While formally not quite correct, this hypothesis was the first formulation that this locus encoded a global regulator.

While the two paths (*appR* and *katF*) remained disconnected, two papers, coincidentally appearing in May of 1989, revealed that the products of these genes were *trans*-acting positive regulatory factors affecting the expression of multiple genes. In one paper Sak et al. reported that *katF* mutations affected the expression of exonuclease III, the product of the *xthA* gene, in addition to their effect on the expression of *katE* (15). However, given that both *katE* and *xthA* were known to be involved in cellular recovery from oxidative damage, the inference was that *katF* was responding specifically to this type of damage. The second paper perhaps pointed more clearly to the global regulatory role of the *appR* gene product. It is also a finding with its roots close to my own laboratory. Paul Boquet had visited Jon Beckwith's laboratory, next door to mine, and had related to me both the identification of *appR* and the fact that the acid phosphatase activity that *appR* controlled was maximal during stationary phase. Soon thereafter, Lola Díaz-Guerra spent a few months in my laboratory studying the expression of microcin C7 (her husband, José Luis San Millán, was a postdoc next door with Jon Beckwith). Lola and Jose Luis told me they had noticed that microcin C7 production was highly variable, depending on strain background, and like microcin B17 production, was maximal during stationary phase. The similarities between the expression of microcin C7 and acid phosphatase were too great to miss, and I suggested that they test the effects of *appR* alleles on microcin C7 gene expression. At the very same time that *katF* control of *katE* and *xthA* was reported, Díaz-Guerra et al. reported that *appR* controlled the transcription of several genes involved in the synthesis of microcin C7, for the first time linking this regulator with stationary phase-inducible transcription (4).

The connection between *katF* and *appR*, however, was not recognized in publications for some time yet. Before that, however, came a key paper further describing *katF*. In December of 1989 Mulvey and Loewen published the sequence of *katF* (10). The conclusion was incontrovertible; the similarity between the predicted *katF* gene product and sigma-70 and sigma-32 strongly suggested that *katF* encoded an alternative sigma factor. Caution had to be exercised, and everyone concerned with this gene continued to refer to it as encoding a putative sigma factor. However, everyone involved operated under the assumption that another alternative sigma factor had been identified. But was it involved solely in the response to oxidative damage, or was it a central regulator of stationary-phase gene expression? The answer to that came when *appR* and *katF* were recognized as one and the same. Again coincidentally, two papers linking the two genes appeared in different journals in January 1991. Touati et al. had compared the phenotypes of *appR* and *katF* mutants and found them to be identical (22). Working independently, Lange and Hengge-Aronis had identified a carbon starvation-inducible fusion, *csi2::lacZ* (8). Analysis of the pleiotropic properties of the mutant as well as genetic mapping led these investigators to propose that *appR*, *katF*, and *csi2* were allelic. In addition, two-dimensional gels and analysis of glycogen production revealed a much greater pleiotropy caused by mutations in this locus. Feeling that a critical mass of evidence was now in hand, they baptized the gene *rpoS* and its product as sigma S. While it would be 2 1/2 years before we could remove the "putative" from "putative sigma factor" (when the biochemical demonstration that the *rpoS* gene product functioned as a sigma

factor in vitro was published [19]), the facts were clear—stationary phase had its very own sigma factor. In the eyes of molecular biologists and biochemists alike, stationary phase as a field had earned its driver's license, we were now able to conduct experiments with their approval.

Within months literally dozens of laboratories jumped into the studies of sigma S-dependent transcription. A literature search today reveals nearly 250 *rpoS*-related papers published between 1991 and 1998. As a result, we now have a wealth of knowledge regarding the *rpoS* regulon, the mechanism of action of sigma S, and the mechanism by which sigma S activity is regulated. The key remaining challenge is defining the precise signaling pathways leading from nutrient depletion to sigma S activation. Ironically, Lange and Hengge-Aronis and our group showed that the microcin B17 promoter, the first starvation-inducible promoter described, was independent of *rpoS* (2, 7). Having attention so heavily focused on studies of *rpoS*-dependent genes has somewhat obscured the fact that the transcription of a large number of genes is induced at the onset of stationary phase, independent of *rpoS*.

The excitement surrounding the unfolding of the *rpoS* story was quite apparent at the time. Nonetheless, I have felt for a long time that there is much more to stationary phase than the analysis of transcription regulation. Nothing convinced me more of that than the discovery of population takeovers occurring during stationary phase. These unexpected findings came from control experiments performed while we were searching for mutations that affected stationary-phase survival (20). In mid-1989, Antonio Tormo began a series of mixing experiments with "young" and "aged" cultures and was surprised to observe that the surviving cells from aged cultures could grow and take over young cultures. Quite fittingly, I presented these preliminary observations at a meeting honoring John Ingraham on the occasion of his retirement in September 1989. Buoyed by John's and Fred Neidhardt's enthusiastic reception of the results I encouraged a new graduate student in the laboratory, Mechas Zambrano, to pursue these observations. Working closely with Debby Siegele, Mechas figured out the mechanism of the takeovers: the aged cultures contained mutants that could grow in stationary phase as the parental cells lost viability. When we finally published the results of the characterization of these so-called GASP mutants we certainly looked at stationary-phase cultures in a very different light; far from being stationary, these cultures were remarkably dynamic (24). The process of getting to that new mind-set had involved confronting many unexpected results. But it had also involved taking time to go back and read the old literature. Interest in the survival of bacteria during stationary phase was new to us molecular geneticists. But by exploring the literature we discovered, much to our delight, that bacterial physiologists had been fascinated by this topic since the beginning of the century. In September 1990 my entire laboratory went off to Maine and retreated at seaside for the first of what would become the annual "Kolter Lab Maine Event." During that time, besides discussing the results at hand, we also read and discussed key papers on starvation survival dating back more than half a century. Thus, we became acquainted with wonderful papers exploring the life and death of bacteria in stationary phase by Shearer, Steinhaus, Ryan, Postgate, and others (13, 14, 17, 18). In an era of on-line searches, it is easy for almost anyone to miss being exposed to the excellent science that was done prior to 1966. Of all the events that most influenced my view of stationary phase, I would place the discussions that took place that week in Maine at the top of the list. It was a wonderful lesson that taught me that, in order to be able to look freshly at the challenges of the day, it is always an excellent idea to stop and see what others saw long before one opened one's eyes.

REFERENCES

1. **Asensio, C. J., C. Perez-Diaz, M. C. Martinez, and F. Baquero.** 1976. A new family of low molecular weight antibiotics from enterobacteria. Biochem. Biophys. Res. Commun. **69:**7–14.
2. **Bohannon, D. E., N. Connell, J. Keener, A. Tormo, M. Espinosa-Urgel, M. M. Zambrano, and R. Kolter.** 1991. Stationary-phase-inducible "gearbox" promoters: differential effects of *katF* mutations and the role of σ^{70}. J. Bacteriol. **173:**4482–4492.
3. **Connell, N., Z. Han, F. Moreno, and R. Kolter.** 1987. An *E. coli* promoter induced by the cessation of growth. Mol. Microbiol. **1:**195–201.
4. **Díaz-Guerra, L., F. Moreno, and J. L. S. Millán.** 1989. *appR* gene product activates transcription of microcin C7 plasmid genes. J. Bacteriol. **171:**2906–2908.
5. **Groat, R. G., J. E. Schultz, E. Zychlinsky, A. Bockman, and A. Matin.** 1986. Starvation proteins in *Escherichia coli*: kinetics of synthesis and role in starvation survival. J. Bacteriol. **168:**486–493.
6. **Hernandez-Chico, C., J. L. San Millan, R. Kolter, and F. Moreno.** 1986. Growth phase and OmpR regulation of transcription of the microcin B17 genes. J. Bacteriol. **167:**1058–1065.
7. **Lange, R., and R. Hengge-Aronis.** 1991. Growth phase-regulated expression of *bolA* and morphology of stationary-phase *Escherichia coli* cells is controlled by the novel sigma factor σ^S (*rpoS*). J. Bacteriol. **173:**4474–4481.
8. **Lange, R., and R. Hengge-Aronis.** 1991. Identification of a central regulator of stationary phase gene expression in *E. coli*. Mol. Microbiol. **5:**49–59.
9. **Loewen, P. C., and B. L. Triggs.** 1984. Genetic mapping of *katF*, a locus that with *katE* affects the synthesis of a second catalase species in *Escherichia coli*. J. Bacteriol. **160:**668–675.
10. **Mulvey, M. R., and P. C. Loewen.** 1989. Nucleotide sequence of *katF* of *Escherichia coli* suggests KatF protein is a novel σ transcription factor. Nucleic Acids Res. **17:**9979–9991.
11. **Mulvey, M. R., P. A. Sorby, B. L. Triggs-Raine, and P. C. Loewen.** 1988. Cloning and physical characterization of *katE* and *katF* required for catalase HPII expression in *Escherichia coli*. Gene **73:**337–345.
12. **Pérez-Díaz, J. C., and R. R. Clowes.** 1980. Physical characterization of plasmids determining synthesis of a microcin which inhibits methionine synthesis in *Escherichia coli*. J. Bacteriol. **141:**1015–1023.
13. **Postgate, J. R., and J. R. Hunter.** 1962. The survival of starved bacteria. J. Gen. Microbiol. **29:**233–263.
14. **Ryan, F. J.** 1959. Bacterial mutation in stationary phase and the question of cell turnover. J. Gen. Microbiol. **21:**503–539.
15. **Sak, B. D., A. Eisenstark, and D. Touati.** 1989. Exonuclease III and the hydroperoxidase II in *Escherichia coli* are both regulated by the *katF* product. Proc. Natl. Acad. Sci. USA **86:**3271–3275.
16. **Sammartano, L. J., R. W. Tuveson, and R. Davenport.** 1986. Control of sensitivity to inactivation by H_2O_2 and broad-spectrum near-UV radiation by the *Escherichia coli katF* locus. J. Bacteriol. **168:**13–21.
17. **Shearer, C.** 1917. On the toxic action of dilute pure sodium chloride solutions on the meningococcus. Proc. R. Soc. **89:**440.
18. **Steinhaus, E. A., and J. M. Birkeland.** 1939. Studies on the life and death of bacteria. I. The senescent phase in aging cultures and the probable mechanisms involved. J. Bacteriol. **38:**249–261.
19. **Tanaka, K., Y. Takayanagi, N. Fujita, A. Ishihama, and H. Takahashi.** 1993. Heterogeneity of the principal sigma factor in *Escherichia coli*: the *rpoS* gene product, σ^{38}, is a principal sigma factor of RNA polymerase in stationary phase *Escherichia coli*. Proc. Natl. Acad. Sci. USA **90:**3511–3515.
20. **Tormo, A., M. Almirón, and R. Kolter.** 1990. *surA*, an *Escherichia coli* gene essential for survival in stationary phase. J. Bacteriol. **172:**4339–4347.
21. **Touati, E., E. Dassa, and P. L. Boquet.** 1986. Pleiotropic mutations in *appR* reduce pH 2.5 acid phosphatase expression and restore succinate utilization in CRP-deficient strains of *Escherichia coli*. Mol. Gen. Genet. **202:**257–264.
22. **Touati, E., E. Dassa, J. Dassa, P. L. Boquet, and D. Touati.** 1991. Are *appR* and *katF* the same *Escherichia coli* gene encoding a new sigma transcription factor? Res. Microbiol. **142:**29–36.
23. **Tuveson, R. W.** 1981. The interaction of a gene (*nur*) controlling near-UV sensitivity and the *polA1* gene in strains of *E. coli* K-12. Photochem. Photobiol. **33:**919–923.
24. **Zambrano, M. M., D. A. Siegele, M. Almirón, A. Tormo, and R. Kolter.** 1993. Microbial competition: *Escherichia coli* mutants that take over stationary phase cultures. Science **259:**1757–1760.

The views expressed in this Commentary do not necessarily reflect the views of the journal or of ASM.