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GUEST COMMENTARY

Lysogeny at Mid-Twentieth Century: P1, P2, and Other Experimental Systems

Giuseppe Bertani*

Biology Division, California Institute of Technology, Pasadena, California 91125

Most of us doing research have a preferred material, a set of well-tried techniques, a standing list of unsolved problems, ways of looking at or of doing things, which we share to a large extent with colleagues in the same laboratory and others in the same area of specialization, be they friends, former associates, or competitors. All this and more is encompassed by the concept of "experimental system" as introduced and used by Hans-Jörg Rheinberger (76, 77), a historian of science. His concept, rather flexible and rich in metaphors, may be easily adapted to the present narrative, which proceeds from a personal view rather than from a critical historical examination. A careful restriction of material, techniques, and nomenclature allows more constructive interactions between different laboratories and different generations of scientists in the same field. Of course, carried out to excess, this process will stifle developments in new areas and defer some discoveries. Max Delbrück, who in the early forties had forcefully advocated the study of bacteriophage as the royal road to the secrets of replication and recombination, was quite outspoken about the necessity for workers on that road to use a common material (the T phages in *Escherichia coli* B) and precisely standardized techniques (1). Of course, the T phages are generally virulent, take-no-prisoner parasites of bacteria and thus could not instruct us about the more shadowy interactions between "weaker" bacteriophages and their bacterial hosts: about lysogeny, where infection meets heredity.

The term lysogenic—generating lysis—was applied very early after the discovery of bacteriophages and was used at first in broadly descriptive, uncritical ways. On the other hand, bacterial cultures that spontaneously (i.e., in the absence of obvious infection from the outside) produced bacteriophage, yet grew well, without gross evidence of lysis, were isolated as early as 1922. Varied interpretations were debated back and forth for several years, quite fiercely so in the francophone medical research community. While the development of the concept has been well summarized (68), it deserves a serious look from the point of view of the history and philosophy of science. The flavor of the debates is conveyed by one of the participants, Paul Flu (42; see also reference 84). Eugène Wollman (90, 91) of the Institut Pasteur was one of the few who saw at the time the genetic implications of concepts of lysogeny. Genetics was then rather underdeveloped in the Latin countries of Europe, immunology being the star of the day in medical quarters. Also, the debate only rarely focused on one type of experiment with well-defined and generally available material. In the early thirties a definition of lysogeny was reached (26) that is still valid, obviously without modern molecular implications. Anlage, a German word used in embryology, was applied by Burnet and McKie (27) to what we now call prophage. The concept of Anlage or prophage was based on the fact that no one had succeeded, using a variety of methods, in demonstrating the presence of infectious phage particles inside a lysogenic bacterium. World War II interrupted much of this work. The Wollmans died, and Flu barely survived, in Nazi concentration camps. Burnet took up more medically oriented work.

I encountered lysogeny in early 1949 while at Cold Spring Harbor as a research fellow in Milislav Demerec's laboratory. I was studying spontaneous and induced mutation in a streptomycin-dependent *E. coli* B strain. I had never worked with bacteria before. Puzzled once by a colony that looked sectorially nibbled, I showed it to Evelyn Witkin and to the late Gus Doermann, who, fortunately for me, had their laboratories just next to my room. They suggested the obvious explanation, phage contamination, but one of them added "There is also something called lysogeny. . ." (Actually, at that time the English word in use was lysogenesis or lysogenicity.) I had never heard of lysogeny before (although I had been exposed to Paul Buchner's [25] works on endosymbioses) and started digging for more information in the library.

At about the same time, unknown to me, two important events had happened. In Madison, Esther and Joshua Lederberg, in the course of their work on the K-12 strain of *E. coli*, then the only bacteria—apart from *Pneumococcus*—known to exchange genetic material, isolated a mutant that unexpectedly lysed when in contact with the parent strain. They came to the conclusion that the original K-12 strain was lysogenic for a previously unsuspected phage, which they named lambda, thinking it might be something like Tracy Sonneborn's kappa factor in *Paramecium*. This was briefly communicated in the first issue (January 1950) of Witkin's informal Microbial Genetics Bulletin; actual publication came much later (58). In Paris, André Lwoff, a well known protozoologist and bacterial physiologist, took up the question of how phage particles are produced by lysogenic bacteria. The problem was bravely attacked by direct micromanipulation of individual, or small numbers of lysogenic bacteria, the oversized *Bacillus megaterium*, cultured in a droplet of broth under the microscope. At

* Mailing address: 156-29 Biology Division, California Institute of Technology, Pasadena, CA 91125. Phone: (626) 395-8851. E-mail: gbertani@earthlink.net.

intervals, the liquid surrounding the bacteria was removed, replaced with fresh broth, and then tested for presence of bacteriophage. At the same time, the number of bacteria in the droplet was recorded, and the droplet was examined for bacteria that might be lysing. A strong result was that when the droplet contained phages, these were present in large numbers, as one might expect from the sudden lysis of one or more bacteria. Conversely, it was shown that the bacteria could grow and divide several times without producing any phage. However, the correlation of phage production with cell lysis could not be immediately demonstrated. This was later clarified by the finding that *B. megaterium* cells had the property of lysing spontaneously and nonproductively, leaving a visible cell ghost, while lysis linked to phage production was extremely rapid and left no microscopically visible traces of the bacterial bodies (69, 70).

In the fall of 1949 I joined Salvador Luria at Indiana University in Bloomington. As we were making work plans, I proposed that I study lysogeny. Luria was not too happy about it, as he had in mind other problems relating to the development of the virulent phage T2 (on which however I did work for several months). Perhaps—I am guessing—he had not mentioned lysogeny in his research grant proposal. . . . He was nevertheless very cooperative and promised to try to obtain some strains that I could use to study lysogeny. In fact, in January 1950, we received from the Lederbergs indicator strains that could be used with K-12 and also the classical Lisbonne strain, a lysogenic *E. coli* strain isolated in the early twenties by M. Lisbonne and L. Carrère, together with its *Shigella* indicator. I immediately started working on both sets of strains, looking at their cultural properties and trying to optimize growth and plaque formation. The obvious advantage with K-12 and lambda was that one could combine the study of phage with that of bacterial recombination, as the Lederbergs in fact had also begun to do. The alternative required the use of *Shigella*, officially a pathogen, which would call for more stringent lab safety precautions than the use of *E. coli*. Unfortunately, a few weeks later, Luria met Joshua Lederberg at some meeting and understood that he and Esther would have much preferred if I did not work on lambda. I was rather displeased at the time, although I recognized that the Lederbergs' request was within their rights, as their discovery of lambda had not yet been published in the open literature. Luria, Jim Watson (then in his last year of graduate school at Bloomington), and I were sharing a smallish laboratory, from which a corner had been cut out for Luria's desk. One late afternoon we had a serious discussion on how to proceed in view of the Lederbergs' request. I remember Jim declaring at the top of his voice that he would not want to be in a lab where one used routinely the presumably pathogenic *Shigella*. Nevertheless, Luria convinced me to leave lambda alone, at least for a time, and I accepted the challenge of using some extra care in handling *Shigella*. Looking back, however, it seems that this minor episode never let me develop the proper "feeling for the organism" (43) with respect to lambda.

Using the Lisbonne strain (symbolized for brevity as Li) and *Shigella*, I set out to investigate essentially the same problem as Lwoff. Having suffered the fatigues of micromanipulation in the course of my thesis work (4), the idea of using his approach never crossed my mind. Besides, phage production in lysogenic *B. megaterium* is unusually high (e.g., one free phage particle for every two bacteria) as compared to other lysogenic strains (a few percent free phage), so that the direct microscopic approach would hardly be successful with most strains. I first isolated from *Shigella* a streptomycin-resistant mutant (later known as *Sh/s* or Sh-16) and showed that the phage produced by the Li lysogen was unaffected by streptomycin. Next I set up what I called a "modified single burst" experiment, in which exponentially growing lysogenic bacteria (washed to eliminate any free phage) were distributed among a set of tubes, and after further incubation the whole content of each tube was plated with the streptomycin-resistant indicator and a drop of streptomycin. This technique scores only the free phage present at the time of plating and not phage that the lysogenic bacteria would produce, in the absence of streptomycin, on the plates. If the phage were produced continuously during the lysogen growth, the plates would have randomly distributed plaques. If lysogenic cultures would produce phage in "bursts," as when a phage-sensitive cell is infected by a virulent phage, most plates would have no plaques and a few would have a large number of plaques, that is, presumably, all the phage progeny of one bacterium. Once the parameters were adjusted, the experiment worked beautifully (5). It confirmed that phage production by a lysogen was discontinuous, involving rare, large bursts of phage. A day's work thus allowed one to measure the frequency of spontaneous phage production, even down to very low levels (one burst per 45,000 cell generations for strain Li), and the average burst size: more than one could obtain in months of micromanipulation. But there was a surprise in store: while the phage recovered from cultures of strain Li was known to be rather heterogeneous as to plaque size, in my experiment the plaques looked different in different bursts. Further investigation showed that strain Li did indeed produce three immunologically distinct types of phages, which I named P1, P2, and P3, all three able independently to establish lysogeny in the *Shigella* strain and that as a rule they were produced in homogeneous bursts, independently of each other (5). I continued working with the phages from strain Li, giving particular attention to P2, in which it was relatively easy to recognize various plaque-type mutants.

Meanwhile, at the Pasteur, Lwoff and several collaborators had been looking for conditions that might affect the "decision" of a lysogenic cell to shift from normal growth to the suicidal production of a burst of phage. After the failure of various chemical treatments, they obtained a dramatic success: exposure to a very small dose of UV light caused nearly all the bacteria in their lysogenic *B. megaterium* cultures to lyse and produce a burst of phage (71). The discovery of UV induction, as the new phenomenon was called, attracted much attention to lysogeny. Even Delbrück, who only a few years earlier had expressed doubts about it (39), embraced lysogeny, mostly, one should add, through the enthusiasm of his colleague, Jean Weigle, professor of physics at the University of Geneva, who had just then taken early retirement and committed himself to research on phage (83). It was soon found that also a phage in *Pseudomonas* (51) and lambda in *E. coli* K-12 (89) could be induced by UV light. On the other hand, my attempts to induce strains Li or *Sh(P2)* (i.e., *Shigella* isolates made lysogenic for P2) were totally negative. This was disappointing but was also

the first indication that there might be basic biological differences between lysogenic systems.

Several interesting questions about lysogeny were open. In an established lysogenic bacterium would there be one prophage copy or many copies? If just one, how would it segregate regularly at cell division? And what was the modus operandi of immunity to superinfection? Was immunity due to a diffusible, prophage-specific product ("immunity substance", "repressor"), which would block the development of a superinfecting phage, or was it the result of some necessary interaction with a special bacterial site? Preliminary bacterial crosses by Esther Lederberg, mentioned in her 1950 report, had indicated some kind of linkage, albeit complex, between lambda lysogeny and certain genetic markers in K-12. The possibility of chromosomal control of a population of cytoplasmic elements (as in the case of kappa in *Paramecium*) was not excluded. I used P2 plaque-type mutations as markers in superinfection of *Sh(P2)* lysogenic cells, trying to follow the fate of the superinfecting phage. The latter, it turned out, was not degraded or rejected by the immunity system of the lysogen; it was only blocked in its replication (as "superinfection preprophage") and distributed randomly to the daughter cells as the lysogen continued to grow and divide. When a cell that carried it happened to lyse, the superinfecting phage would participate in the burst essentially on equal footing with the prophage. On rare occasions, the resident prophage (or some of its genes) would be replaced by the superinfecting type. Still more rarely a stable doubly lysogenic strain would be established. A P2 mutant (the clear plaque type "weak virulent") that had lost the ability to lysogenize could be carried in the blocked state for several cell generations and, rarely, even behave like a second prophage, i.e., be inherited by all progeny cells, as long as the original prophage (turbid plaque type) was present; it behaved as expected of a mutant with a recessive mutation affecting immunity. These results (6, 7, 8, 9, 10), while open to more complex interpretations, supported well both the idea of one prophage, or exceptionally two, per bacterial cell (more precisely, per nucleoid) and the concept of a specific prophage product interacting with superinfecting phage. Meanwhile, a new strain of *E. coli*, later called *C* (16), appeared on the scene, indirectly the result of a finding by Cavalli and Heslot (35). Strain C was sensitive to lambda and to all phages from strain Li, gave decent plaques with P2, and furthermore (35) would cross with K-12 strains. Gradually, I shifted from *Shigella* to strain C for most of the work with P2, and then bacterial crosses to establish the chromosomal location of prophage could be performed (10, 14). While I here emphasize work with P2, most readers will know that during that time (1951 to 1957) rapid progress was made in the understanding of the genetics of K-12 and lambda, as then reviewed (53) by two of its main contributors. It became quite clear that the lambda prophage itself was actually anchored at a specific site, thought at first to be the only one possible, on the bacterial chromosome. A detailed linkage analysis (28) and the then new notions of chromosome circularity led to Allan Campbell's proposal of his well-known integration model.

Outside of lysogeny, P1 and P2 also contributed more generally to the early progress in bacterial genetics. (Little was ever done with P3.) An example is the discovery of "host-controlled variation", now more commonly called "restriction and modification." I noticed it in P2 (using strain B as the restricting host, *Shigella* being the standard host) and did not know what to make of it. Jean Weigle, with whom I often corresponded, noticed it in lambda (using strain C as the permissive host, K-12 being the standard host). Being aware of my results, he immediately recognized the similarities of the two findings. Shortly before that, a minor laboratory accident, as told by Luria (66), had led to the discovery of another, albeit more complex case of host-controlled variation (67). Although no satisfactory mechanistic explanation was in sight at the time, Jean and I were encouraged by the parallelism between our two, totally independent "systems" and decided to publish our findings together (16). It rarely happens that a new phenomenon, observed in two different systems, in different laboratories, is described in the same paper, in a comparative manner. This strengthened the evidence and hinted at the generality of the phenomenon, while scoring a point for cooperation versus competition in science and human affairs. (A similar case, several years later, was that of a paper by René Thomas and Elizabeth Bertani [87], which reported parallel experiments with lambda and with P2 to more precisely define the mode of action of the immunity repressor.) Growing P2 in *E. coli* B led to another unexpected finding: the presence of a defective prophage (related to P2 but with a different immunity specificity) in this most traditional phage host strain (37). Today defective prophages are an almost daily finding in genomic analyses of bacteria.

P1 also gave some surprises. Its establishment of lysogeny in *Shigella* was almost absolutely controlled by temperature (17): very high frequencies of lysogenization and no immediate phage production when the infected bacteria were kept at 20°C after infection, 100% lysis with phage production at 40°C. This property was apparently lost as P1 was "adapted," through at least two mutational steps, *k* and *c* (60, 88), to grow more efficiently on K-12, and no one has returned to the study of the original wild type in *Shigella*. The reason for this neglect of course is the fact that P1 was found capable of transduction and began to be used almost exclusively in K-12 derivatives. Transduction, discovered in 1951 by Zinder and Lederberg in *Salmonella* (94), and correlated to phage P22, allowed genetic analysis of closely linked mutations but no gross mapping. On the other hand, fine-structure mapping was still impracticable with *E. coli* K-12. The credit for discovering this capacity of P1 goes to Ed Lennox, a physicist then in the process of converting to biology, who one morning in early 1954 entered our laboratory proclaiming: "Joe, let's try and see if your phages can transduce!" I had by then some auxotrophic mutants of strain C that could be used to test the idea. We did the experiment the following day, and it turned out that indeed P1 (but not P2) was a very efficient transducer. I still have copy of a letter to Jean Weigle, dated 16 April, where I overenthusiastically wrote: "We spent a week of great excitement (i.e., Ed Lennox and I). My phage P1 can transduce. P1 grown on Shiga can transduce the Arginine character in *coli* C, a Galactose marker also in *coli* C, and a Streptomycin marker in *coli* B. Enough?. . .It will be possible to study transduction and genetic recombination in the same organism; compare the temperature effect on lysogenization and perhaps on transduction; perhaps succeed in transducing prophage P2 through phage P1; et cetera et cetera." The finding was reported at the Oak Ridge

meeting on genetic recombination that same spring (59) and was followed by the very comprehensive paper of Lennox (60). Both Lennox (60) and Jacob (52) demonstrated cotransducibility of lambda prophage with bacterial markers. Later, P2 prophages at three different sites were cotransduced and oriented by means of P1 (31).

While older geneticists were infecting and crossing, the methods of what is now molecular biology came on the scene, on the heels of electron microscopy and ultracentrifugation. Over a few decades the enthusiasm for lambda as an experimental system made lambda the paradigm for lysogeny (47, 48, 75, 82). It is now so well known down to the molecular level that it is usefully modeled in silico (2). P1 also attracted numerous followers, at first as a tool in transduction and later in its own right (93), because of its unusual chromosomal behavior (50). A few, including myself, continued working on P2, although at times with a feeling of isolation and the worry that perhaps its differences from lambda were not sufficiently important to justify the effort. This, I believe, turned out not to be the case (12).

Particles of P2 and lambda differ structurally. Unlike lambda, P2 DNA replication follows a typical rolling-circle model throughout its reproductive cycle (54, 64, 73, 79). This had been suggested by two striking findings, the strict requirements of P2 DNA replication for the host cell Rep function (32) and for a *cis*-acting phage protein (62), as is the case for the very different, virulent phage, ϕX174. Encapsidation occurs directly from monomeric circles (74). Several P2-specific attachment sites, different from that of lambda, exist on the bacterial chromosome (3, 14, 33, 55). Genetic recombination in P2 mixed infections occurs with extremely low frequency (11, 15): the P2 genetic map (13, 61) had to be obtained with the help of UV irradiation to stimulate recombination. The P2 regulatory circuit, lysis versus lysogeny, is simpler (78) than that of lambda. The P2 prophage integration mechanism, while a typical site-specific recombination, has special features (20, 30, 46), in part responsible for blocking detachment of the prophage on derepression. No case of specialized transduction is known in P2; on the other hand, its complementary event—eduction of a bacterial marker—has been described (56). A great boost for P2 studies was the discovery in 1966 by Erich Six (81) of the remarkable satellite phage P4 and its complex interactions ("transactivation") with P2 and P2-like phages (22, 29, 36, 65). A number of very intriguing observations, peculiar to P2, remain incompletely understood and deserve further study: striking metabolic effects on the frequency of lysogenization (18, 19); cell sensitization to a small molecular product (21); the complex interactions between P2 nonessential gene *old*, the host cell, and a coinfecting phage lambda (24, 44, 63, 72, 80); and others. Based on the results of screens of coliforms in Paris and Los Angeles (23, 53) and the Dhillons' extensive work in Hong Kong (40), it became clear that—to the extent that modern notions of segmental evolution (57) allow it— P2 and lambda are representative of two main groups of temperate phages for such bacteria. Within the P2-like group, phage 186 was studied in great genetic and molecular detail by Barry Egan and his students (41, 92); phage 299 was studied to a lesser extent by E. I. Golub (45). More recently, other phages obviously related to P2 have been encountered in several other species besides *E. coli* and as defective prophages in DNA sequencing studies (34).

The above recollections seem to indicate that at about the middle of the last century, starting before the formulation of the DNA structure and independently of the introduction and diffusion of "molecular" methods of analysis, there was a confluence of the rigorous approach of phage work with more traditional bacteriological perspectives, energized by the new remarkable findings about gene transfer. Studies of lysogeny were rather central in this process of broadening interest with respect to problems and materials. As a result, several new "experimental systems" à la Rheinberger were developed in the fifties and sixties, lambda being the most successful. One is tempted to generalize these observations and suggest that it is the way of scientific progress to alternate between periods of broad and somewhat haphazard exploration and periods of highly focused in-depth analysis of particular problems or materials. On the one hand, as Francis Crick once wrote (38), "Few molecular biologists would care to be caught studying the colour of butterflies' wings. . . ." The tendency in molecular biology (as it was, mutatis mutandis, in classical genetics) is for one to analyze the experimental material to the lowest possible structural level and thus invest heavily in one's system. On the other hand, the naturalist looks open mindedly for what may happen to be there and how it might be related to what has already been seen: in a way, he scouts for new experimental systems.

A comment may be made concerning induction: not lambda's, that of philosophers. It is hard to see how our intelligent species would have ever evolved without trusting induction, yet there are everyday examples of the risks of excessive reliance upon inductive predictions. After the *B. megaterium* phage, a phage in *Pseudomonas*, and lambda in K-12 were found to be inducible by UV light, it was hard to believe that P2 was not. How do you prove a negative? Similarly, after seeing that both lambda and P2 prophages were present in single copies, physically integrated in the bacterial chromosome at specific sites, it was very surprising when P1 was found to be present as one unattached copy and yet be regularly transmitted to the bacterial progeny without losses (50) or Mu to be capable of inserting itself anywhere in the chromosome (85, 86). Perhaps most surprising, after the early efforts had convinced everyone that lysogens produce phage through the lysis of individual cells, was the discovery that filamentous phages are indeed continuously secreted by growing cells, without lysis (49).

Postscript. My first paper on lysogeny (5), describing the modified single-burst experiment and the isolation of P1, P2, and P3, also contained the formula of the LB medium which I had concocted in order to optimize *Shigella* growth and plaque formation. Its use has since become very popular. The acronym has been variously interpreted, perhaps flatteringly, but incorrectly, as Luria broth, Lennox broth, or Luria-Bertani medium. For the historical record, the abbreviation LB was intended to stand for "lysogeny broth."

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