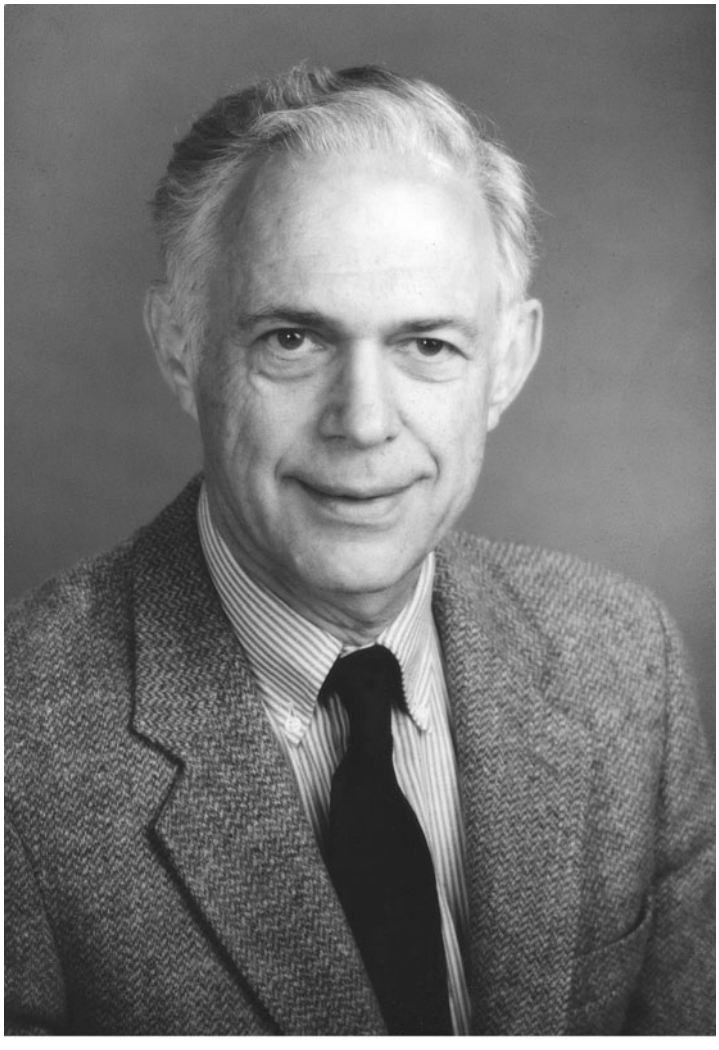

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Phage Integration and Chromosome Structure. A Personal History

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Annu. Rev. Genet. 2007. 41:1–11

First published online as a Review in Advance on April 27, 2007

The *Annual Review of Genetics* is online at http://genet.annualreviews.org

This article's doi: 10.1146/annurev.genet.41.110306.130240

0066-4197/07/1201-0001$20.00

Key Words

lysogeny, transduction, conditional lethals, deletion mapping

Abstract

In 1962, I proposed a model for integration of λ prophage into the bacterial chromosome. The model postulated two steps (*i*) circularization of the linear DNA molecule that had been injected into the cell from the phage particle; (*ii*) reciprocal recombination between phage and bacterial DNA at specific sites on both partners. This resulted in a cyclic permutation of gene order going from phage to prophage. This contrasted with integration models current at the time, which postulated that the prophage was not inserted into the continuity of the chromosome but rather laterally attached or synapsed with it. This chapter summarizes some of the steps leading up to the model including especially the genetic characterization of specialized transducing phages (λ*gal*) by recombinational rescue of conditionally lethal mutations.

The serendipitous discovery of the conditional lethals is also described.

PRELUDE

gal: a cluster of genes whose products are needed to metabolize the sugar galactose

This chapter covers part of my career around 1960 and the questions that occupied my attention at that time. A brief summary of my previous life indicates where I was coming from. At the end, I'll say something about the subsequent 40 years.

In high school I was inclined toward creative writing, but I thought a career in science should provide more secure employment. It was a fortunate decision, because I realized in retrospect that I have virtually no talent for effective original writing. My best subject in school was mathematics. As a potential scientist, I suffered from an inability to do much of anything right in the laboratory. But I also felt that experimental science kept me in touch with reality, whereas mathematics invited me to dwell within a dream world of abstractions.

So in 1946 I enrolled as a chemistry major at the University of California. My basic attitudes toward scientific thinking were strongly shaped by the Berkeley chemists. Probably my favorite professor was William Giauque, who won a Nobel Prize for low temperature chemistry during my senior year. I had never encountered anyone so completely immersed in science. He seemed a worthy role model (and not because of his Nobel). Outside of the Chemistry Department, my imagination was stimulated by the biochemically oriented microbiologists—especially Mike Doudoroff and Roger Stanier. Inspired by them, I decided in my senior year to apply to graduate school in microbiology. Roger recommended several places, and also directed me toward C.B. van Niel's course at the Hopkins Marine Station that summer (1950). van Niel became a second role model. I then became a graduate student of Sol Spiegelman's at the University of Illinois. Before I arrived, I.C. Gunsalus and Salva Luria had been recruited to Illinois, which then arguably had the best bacteriology department in the world (at least for basic, nonmedical bacteriology.) Graduate school application was much less organized than now, and I had little idea of what I was getting into. I had no special inclination for research. I liked learning and enjoyed being a student and taking classes. I've read many accounts from scientists (including some of my friends) who were motivated by ambition for research success from an early age. Others of us just stumbled into research careers.

My favorite professors at Berkeley had all been research-oriented, but that was not what had drawn me to them. I cared very little about how much they had accomplished. What I liked was that they seemed to have thought deeply about the basic issues in their fields. It took me much longer to appreciate fully the linkage between depth of understanding and hands-on involvement in research.

In Urbana the Spiegelman lab was next to the Luria lab. Salva's interests were turning toward temperate phages, and Joe Bertani, then a research associate in Salva's lab, was performing many of the pioneering experiments on lysogeny. I finished my thesis with Sol in 1953, then started my first academic appointment as Instructor in Bacteriology at the University of Michigan Medical School (an appointment interrupted almost immediately by two years of military service). At Michigan, I started out working on yeast (a continuation of my doctoral research), but spent the summer of 1956 with Joe Bertani in Pasadena (where he had moved to the Cal Tech phage group). I arrived with a plan for some experiments on restriction/modification (which produced some very preliminary results), but then became engrossed with specialized transduction of the *gal* operon by phage λ.

SPECIALIZED TRANSDUCTION

At that time, Jean Weigle had joined the Cal Tech phage group, but spent his summers in Geneva (where he held a professorship in physics). When I arrived in Pasadena for the summer, he had left behind some preliminary notes that Joe thought I might follow up on. Specialized transduction had been

discovered by Larry Morse (16), but Jean wanted to look into it further (19). I was unaware at the time that he had also encouraged Werner Arber in Geneva to investigate the same system. Eventually, Werner and I came, quite independently, to very similar conclusions. Two such parallel studies are about the right number to provide corroboration but avoid needless duplication.

On returning to Michigan, I postponed further work on yeast (for 50 years now) and dedicated myself full time to the study of specialized transduction. I had a small NIH grant to support the yeast work, so I informed NIH in my annual renewal that I was now working mainly on phage. I am eternally grateful to have started my career at a time when such flexibility was routine.

My initial work, paralleled by that of Arber (3), examined the effect of multiplicity of infection on the frequency and nature of the transductants (5)—something that neither Morse nor Weigle had done. The results indicated that transduction was carried out by chimeric phages that had lost some of their own genes while acquiring the nearby *gal* genes. The phage DNA that was missing included the *h* gene, which was in the middle of the phage genetic map. The finding heightened my consciousness that most previous students of lysogeny had treated the prophage as an indivisible unit (analogous to the concept of the gene that prevailed in the first half of the twentieth century), whereas it might sometimes be more relevant to try tracing the origins of the individual genes of the prophage.

Two years in the Michigan Medical School was enough for me, and I resigned the following year, never again to serve on a medical school faculty. Although many medical schools have become more egalitarian than they were in 1957, the basic system is hierarchical. Historically, medical schools grew up around hospitals, which needed a chain of command from chief surgeon through scrub nurses. Department chairmen frequently served for life, ruling their own little fiefdoms. The tradition of arts colleges comes from clerical institutions, which of course had their own discipline but adapted more readily to a democratic structure.

I was not offered any position in arts colleges at that time, but Miloslav Demerec, Director of the Carnegie Institution Department of Genetics at Cold Spring Harbor, wanted a one-year appointment to replace George Streisinger, who was spending the year abroad. Cold Spring Harbor was a marvelous place to do research, free from other obligations, and the senior people—Demerec, Barbara McClintock and Al Hershey—provided inspiration and moral support.

The initial task I set myself that year was to find out more precisely which segment of the λ genome was missing from λ*gal*. I knew that the *h* gene was gone and that some other genes were present. Dale Kaiser had made a good map of λ, but the number of available genetic markers was very limited. So I decided to make more.

Jean Weigle had discovered that, if both λ and *Escherichia coli* are exposed separately to UV light and then the λ is plated on the irradiated host, a substantial fraction of the observed plaques are mutant. The mechanism was unknown. One favored hypothesis at the time was recombinational rescue from a postulated prophage homolog in the host, but it is now understood as inducible SOS mutagenesis arising during radiochemical repair (9). My intention was to collect small plaque mutants, map them by phage crosses, then see whether their wild-type alleles could be rescued from λ*gal*.

SOS mutagenesis: induction of mutations by action of the products of some of the bacterial genes inducible by DNA damage

CONDITIONAL LETHALS

I did not get that far, because of a chance discovery. I was routinely plating phage on strain C600, where λ made nice large plaques. However, one day I ran out of C600 plating bacteria, so I replated my latest mutants on W3350, the *gal* strain used as recipient in transduction. Both C600 and W3350

are *E. coli* K-12 derivatives, bearing various laboratory-induced mutations.

To my dismay, there were no plaques on the plates the next morning. But checking things out showed that the result was real: Some of my small plaque mutants really did not plate on W3350. Primed by Benzer's rII work, I realized that these "host-dependent" mutants might be very useful in determining the gene content of λ*gal*. I'll finish up with what became of mutants, then come back to their use with λ*gal*.

The mutations were widely distributed across the λ map and seemed to belong to many "cistrons," in Benzer's lexicon. Benzer's work was invaluable to me because it provided a simple operational protocol for classifying my mutants by recombination and complementation. In 1959, after a year at Cold Spring Harbor and a subsequent year in Paris, I stopped by Cold Spring Harbor on the way to my new academic post in Rochester. I encountered Bob Edgar, who updated me on some Cal Tech T4 results of Dick Epstein's. Epstein had isolated T4 mutants, which he called *amber*, that plated on a K-12 strain but not on *E. coli* B. Like my λ mutants, they were widely distributed around the map and defined many complementation groups. Furthermore, Epstein's mutants plated on certain K-12 derivatives such as C600 and not on others, such as W3350. They plated only on those strains where my mutants (which I had sent to Jean Weigle at Cal Tech) also plated. Bob was enthusiastic about spending the rest of his life identifying all the essential functions of T4.

It didn't work out quite that way. In a few years, the Cal Tech group had classified a number of the functions (10) and many other labs were getting into the act. No technical breakthrough belongs to its discoverers for very long. Following my conversation with Bob, I isolated many more amber mutants, and also some thermosensitive mutants, verified that the amber mutants were responding to a suppressor mutation in permissive strains like C600, and wrote up the results (6). When I sent the manuscript to Bob, he wrote back something like "Great minds move in the same tracks." He too had been isolating thermosensitive T4 mutants to complement the amber work (8).

Determination of phage gene functions was not my primary goal. Luckily, many other workers became interested in λ, and the genetic hierarchy of λ development was elucidated. To younger investigators, I recommend sharing stocks freely. Not only is science advanced that way, but the donor of stocks can keep abreast of what's going on in the field.

I also recommend free sharing of ideas, as with Bob Edgar and me in 1959. Our 1959 conversation was very fruitful both for us and for other workers in the field. Before appearing to seek the last word on that exchange, I realize in retrospect that neither of us was paying full attention to what the other said. For example, I clearly remember mentioning the work of Horowitz & Leupold (13) on thermosensitive mutants, whereas Bob seems later to have rediscovered the concept without that prior knowledge. On the other hand, I can't really remember what Bob thought at the time about the mechanism of amber suppression, which later proved to be through compensating errors between the cell's RNA dictionary and nonsense mutations in phage genes (18). Bob was probably thinking along those lines, but I tended to block out hypotheses that seemed unnecessary for reaching the goals under discussion. I could imagine a number of mechanisms for genetic suppression that would affect some, but not all, alleles of many genes; and discovering which one was responsible in the case at hand did not seem like a high priority.

Amber mutants have of course become one of the workhorses of molecular biology. I've since read numerous second-hand accounts about their discovery and use. Some of these read as though someone had started with a knowledge of missense suppression, then used it to design a mutant screen. In fact, the whole program could go on before the mechanism was thoroughly understood. Certainly neither Epstein nor I started out to design

such a mutant screen. As indicated earlier, I just happened upon the λ mutants; whereas the first T4 ambers came from an attempt to verify an erroneous hypothesis derived from radiobiology.

MAPPING OF λ*gals*

After that long digression, I return to my own use of the mutants from 1958 onward. In his deletion mapping of T2rII, Seymour Benzer had defind the methodology. No tedious conventional crosses to see what part of the λ genome was present in λ*gal*; simply by spotting a mutant lysate and a lysogen for the defective λ*gal* on a lawn of the nonpermissive host W3350, irradiating lightly to lift prophage immunity and stimulate recombination, I could test whether the wild-type allele of the mutant was present in λ*gal*.

The results were clearcut. In my first λ*gal*, some genes were present and others absent. This was true of every λ*gal*. But each λ*gal* had a unique gene content. All λ*gal* were missing a "core region" including the *h* locus (and the sites within the corresponding J gene defined by conditional lethals) and some adjacent loci. Thus each λ*gal* had lost a connected segment of the λ chromosome. So λ*gal*s could be used to construct a deletion map of λ, similar to Benzer's deletion map of rII. The uniqueness of each λ*gal* was corroborated by physical studies (20) showing that each λ*gal* had a different buoyant density, which implied a different DNA content.

I'll return to the origin of λ*gal* shortly, I was unable to square the results with the models that were then most popular, but I didn't have a better one in mind.

THE INTEGRATION MODEL

The "better model" resulted from a different activity. Around 1960, Ernst Caspari (then the chair of my Department) invited me to write a review for "Advances in Genetics." I chose to write on "Episomes" (a term that had recently been proposed by Jacob and Wollman for elements like λ and F that can be either autononous or chromosome associated). This gave me a reason to spend more time thinking about the literature in the field and whether I really understood it.

Review writing is sometimes undervalued by the research community. Deans and promotion committees, for example, may view it as a means to expand one's bibliography without accomplishing anything. And indeed some reviews fit that description. But it can also provide a time for fresh creative thinking if one tries to frame a satisfactory scheme to explain the available facts in an area wider than one's own research. So I approached the topic with an open mind, hoping I would learn something. The most important ideas in the review developed as I was writing.

One question that I re-examined was the attachment of episomes to chromosomes. The model then favored, proposed by Jacob and Wollman, was that prophages (although they could be mapped among chromosomal markers) were not inserted into the chromosome but were instead synapsed with them because of substantial homology. On this idea, λ*gal* should arise by a double crossover that utilizes λ homology on both sides of *gal*.

One paper that had appeared shortly before my review challenged this concept. Calef & Licciardello (4) crossed lysogenic strains genetically marked in both prophage and chromosome. Their results fit the hypothesis that λ prophage was inserted into the chromosome, between *gal* and *trp*, but required the gratuitous assumption that the gene order in the prophage had changed from the phage order (m5 co mi) to co mi m5. Playing with the possibilities, I noted that, if the phage genome were to circularize and then to insert by reciprocal crossing over between m5 and co, their result would be obtained. At about the same time, Frank Stahl mailed me a manuscript called "Some Circular Thoughts about Chromosomes," which postulated, for rather arcane reasons, that all chromosomes in bacteria, including phage chromosomes, should be circular.

F: a conjugative plasmid (one whose bearer can act as genetic donor in bacterial conjugation)

bio: a cluster of genes whose products are needed for biosynthesis of the vitamin biotin

PI: a bacteriophage able to package bacterial DNA and deliver it to other bacteria

Reassessing, I finally concluded that Jacob and Wollman had arrived at their picture of lysogeny by opposing a model (insertion) that made very specific predictions against a loosely defined model of lateral attachment or synapsis with many more degrees of freedom. Then, any result that contradicted the simplest version of the insertion model was taken as evidence for lateral attachment. To my mind, insertion (even with the gratuitious assumption of circularization) was still simpler than lateral attachment. Insertion would then take place by a crossover between two circles that had some homology (very little, as it later turned out) at the crossover site. I also postulated that Jacob and Wollman's other prototypic episome—the fertility plasmid F—was circular when autonomous and integrated into the chromosome by crossing over.

IMMEDIATE AFTERMATH

After the review appeared, it received a mixed reaction. The most sympathetic came from those like Luria, who regarded the model as beautiful but wrong (because of the Jacob/Wollman evidence.) François Jacob read the manuscript before publication, and provided detailed and encouraging comments. On the other hand, I kept hearing reports that Eli Wollman had a bacterial gene (I presume it was *bio*) that he'd mapped between *gal* and λ prophage but was not included in λ*gal* (although he could get λ*bio* phages that do not include *gal*)—a strong argument against the model, at least as it applied to λ*gal*.

New supporting evidence came from Wu & Kaiser (21), who showed that λ DNA actually could circularize as postulated, and from Luria's and Yanofsky's labs. June Rothman (then a student with Luria) did PI transductions with marked λ prophages that supported the gene order I had proposed and also placed the λ prophage between *gal* and *bio* (17). And Franklin et al., using a λ that had been placed in an abnormal location near *trp*, performed a deletion mapping of the prophage together with nearby bacterial markers (11). Later on, Sankar Adhya, in my lab, selected chlorate-resistant mutants of a normal λ lysogen and mapped deletions penetrating the prophage (2). The chlorate resistance stems from deletion of genes whose products catalyze steps in molybdopterin synthesis, two clusters of which, then called *chl*D and *chl*A, bracket λ prophage.

All these mapping experiments were facilitated by the availability of conditionally lethal mutants, which I had furnished to Rothman and Franklin. Where the Calef/Licciardello experiments used three phage genetic markers and looked at various recombinant prophages by examining plaque morphology, with the conditional lethals the genotype at each prophage locus could be assessed by a simple spot test.

Some years later, Enrico Calef called my attention to a cross between lysogenic strains published by Jacob and Wollman several years before Calef/Licciardello, which could be read as supporting the Calef/Licciardello map; i.e., if you looked at the most common genetic classes and assumed they were single crossovers, the Calef/Licciardello map fell out. **Table 1** shows the data. The critical classes are those showing recombination within the prophage, where the singles (S2 and S3) outnumber the triples. In the same experiment, Jacob and Wollman also scored some recombinants that were donor type for both flanking markers (*gal* and 21) and found that 127/135 of these were donor type for the prophage marker (noncrossover class), whereas even the pure recipient type prophage (double crossover) was very rare 1/122).

So why didn't Jacob and Wollman interpret their result that way? Their genetic acumen is unquestionable. Or why didn't any of the rest of us who read their paper (except for Calef) do so? At the time, one thing that was known for sure about the λ genome was the order of genes, as determined from phage crosses. The map was linear, *m5* was at one end, *co* was in the middle, and *mi* was at the right. This was a rock on which to

Table 1 Recombinational map from a cross between lysogenic strains of *E. coli* [a]

Recombinant genotype[b]					Crossover class[c]	No. recombinants
Gal (1)	Co (2)	mi (3)	m5 (4)	21		
(D)	D	D	D	(R)	S4	276
(D)	R	R	R	(R)	S1	57
(D)	D	D	R	(R)	S3	12
(D)	R	R	D	(R)	T134	1
(D)	D	R	D	(R)	T234	3
(D)	R	D	R	(R)	T123	1
(D)	R	D	D	(R)	T124	2
(D)	D	R	R	(R)	S2	6

[a]Hfr donor was *gal*$^+$ (λm5co$^+$ mi$^+$) 21$^-$Sm^s; F$^-$ recipient was *gal*$^-$(λm$_5$$^+$ co mi) 21$^+$ Sm^R (Sm is unlinked to the region shown). Selection was for Gal + SmR. Only *gal*$^+$ 21$^+$ recombinants are shown. Data from (14).

[b]Genes are shown in the order they occur on the lysogenic chromosome. D: donor type; R: recipient type.

[c]S: single; T: triple; regions 1,2,3,4 shown in column 1.

build. A single result putting the markers of the prophage in a different order seemed inadequate to show that the prophage was actually rearranged. So instead the assumption was that the gene order was the same in the prophage as in the phage, but that recombination frequencies were distorted by interactions with the chromosome.

One problem was that, in both the Calef/Licciardello and the Jacob/Wollman crosses, there were only three genetic markers on the prophage. Since there was no expected orientation, the result boiled down to the fact that, of the three markers used, the wrong one was in the middle. How much more obvious might the cyclic permutation have been had the prophage been marked at five or six sites instead of only three! This was one dividend from the use of conditional lethals. Thus, Rothman had prophages marked at 4 or 5 sites. To do the equivalent with conventional markers required distinguishing 16 or 32 phenotypic classes—difficult when the phenotypes are limited to plaque morphology.

As to Wollman's mapping of *bio* between *gal* and λ prophage, I never saw published data. There were notes in the *Comptes Rendus* referring to future notes that would document that conclusion. I presume that it was based on 2 factor crosses, and that the *gal-bio* distance came from crosses between nonlysogens. I agree very much with Wollman's logic. The expectation that λ*gal* should include all genes between *gal* and λ prophage was critical; a single exception could have killed the model. I wrote Wollman about some later results, such as Rothman's, but I never received a reply.

ROOTS AND RAMIFICATIONS

So that's the story of one segment of my career. Some additional comments seem in order, referring to issues already mentioned.

Relation to Chromosome Structure

From the outset, many geneticists (including myself) found insertion more appealing than synapsis or sticking. But the more appealing an idea is, the more wary one should be of data that seem to support it. It helps to understand why the idea was so attractive.

The roots of my own preference for insertion go to some fundamental concepts of chromosome structure and how they relate to lysogeny. All my conditioning, mostly from Luria, Bertani, and Lwoff, strongly emphasized that prophages behaved in most respects like parts of the bacterial chromosome.

Hfr: a bacterial strain where the F plasmid has inserted into the chromosome, creating a strain that gives a *H*igh *f*requency of *r*ecombinants in bacterial crosses

At least at a gross level, λ prophage could be mapped; and an observer unaware that the phage genome had entered the cell from outside would see the prophage as just another chromosomal segment. So I rejected the notion that prophages were attached to chromosomes in a different manner than one chromosomal gene is connected to another; if prophage could add by synapsis, some of the genes already classifed as "chromosomal" must have added that way in the past. In principle, I was open to the possibility that chromosomes were more complex than it seemed. After all, most chromosomal mapping had been performed in an era where genes could be treated as beads on a chromosomal string. Maps were said to be linear, but the evidence spoke only to the unidimensionality of the string. The internal structure of the beads began to be accessible in the 1950s, culminating in Benzer's work on T4rII. Benzer's results strongly indicated the linearity of short segments of the genome, going down to intragenic segments, but stopped short of integrating these short segments into the continuity of the chromosome.

In 1960, DNA chemistry was less advanced than it is today. Serious evidence-based proposals were being floated that some cellular DNA was four-stranded rather than two-stranded (7) and that chromosomes were constructed from short DNA segments joined by protein linkers (12). Much of that ambiguity has passed with technical improvements and with sequencing projects that assemble DNA segments into complete molecules. Thus we can now assert with strong confidence that the genes of the *E. coli* chromosome lie on one continuous circular molecule and that each of the four *Drosophila* chromosomes contains a single linear DNA molecule. These facts change our perspective but don't completely resolve all the older questions.

Insertion of λ prophage is an established fact. The molecular proof started with the sequencing of junction fragments by Landy & Ross (15), who initially got the fragments from λ*gal* and λ*bio* rather than from lysogenic chromosomes. I doubt that many genomicists of the younger generation will have the patience to go through the whole tortuous path of genetic logic that I have presented here, when the same conclusions come out of simple sequencing. But we may still ask at this point: Suppose the synapsis model is right—not for λ, but for some other proviruses. After all, diversity is the general rule in living systems; usually every imaginable possibility eventually shows up somewhere. The question for the current molecular geneticist is then, if there are proviruses attached by synapsis, would they be identified by current methods?

Such a provirus, being unconnected to the chromosome by covalent bonds, would probably be classified as a plasmid. There are of course prophages such as P1 that are perpetuated as autonomous plasmids, unlinked to any chromosomal genes. But a synapsed prophage would show linkage to its chromosomal homolog. Genomics has effectively redefined "chromosome." Classically, chromosomes are visible structures containing RNA and protein as well as DNA. Most of that DNA comprises a continuous double helix. When DNA is isolated in preparation for sequencing, any noncovalent bonds are ruptured so that only the covalent connections are recorded. But is there evidence at any level for DNA segments (not necessarily prophages) that are associated with a specific part of the chromosome by noncovalent bonds? If they existed, would they have been found?

I think they would have been found because genetics would have put them on the chromosome and genomics would have excluded them from it. That assumes that the elements in question are recognizable by some phenotypic effects.

Some eukaryotic viruses are chromosome-associated. For example, the DNAs of Epstein Barr Virus and Bovine Papillomavirus are attached to chromatin by specific viral proteins; when the gene encoding the protein is deleted, viral genomes are no longer segregated regularly at mitosis (1). However, the only known

persistent eukaryotic viruses that map to a specific chromosomal location are those, like retroviruses, that are inserted.

The location of F′ elements in a cell that contains a complete chromosome is also relevant. The F′ can certainly recombine with its chromosomal homolog, as evidenced by chromosome mobilization; and a strain where the F′ has actually inserted seems a reasonable intermediate in mobilization. However, I don't know of strains where the integrating crossover has been shown to take place at a specific site within the homologous region. Both in insertion and excision, there must be a stage of synapsis preceding recombination, and if that stage is long, the F′ could spend most of its time neither in the chromosome nor fully autonomous.

Experimental Basis of the Model

For a few years after it was proposed, my model was sometimes described as "purely theoretical," as opposed, I guess, to other models that are more directly data-based. This did not bother me at the time. I myself regarded all models as tentative and subject to revision. But in retrospect, what was special about my model seems primarily a matter of presentation. If one compares my results on λ*gal* with, say, Benzer's "deletion mapping" of *r*II mutants, both data sets can be used in equivalent arguments; each λ*gal*, like each deletion, representing a connected segment of the lysogenic chromosome. It didn't occur to me to make that argument at the time partly because the same review where I proposed insertion was also the first place I had suggested how λ*gal* might originate. Both ideas were new to me and I still felt uncertain about them.

λ*gal*s result from heterologous breaking and joining of prophage and bacterial DNA, creating one "novel joint" characteristic of each λ*gal.* I still remember a conversation with Max Delbruck on the subject. At first, he seemed very interested in the insertion model, but then at one point he asked about the genesis of λ*gal*s. I said I did not really understand it, and that I could only postulate some kind of looping out followed by breakage and joining of heterologous DNA. (I suspect that my actual words were much less coherent than what I've just written.) Max then proclaimed that the idea was "very ugly" and that neither it nor the model should be discussed further. But of course if you look at Benzer's deletion mapping (which Max seemed to accept), the molecular mechanisms that generate most deletions are equally obscure ("ugly?"). Benzer did not make the tactical mistake of trying to explain how deletions arise. Both deletions and λ*gal*s are produced by events that are much rarer than homologous recombination, so that crosses with conventional markers can delineate the endpoints.

F′: a derivative of F that has incorporated a segment of bacterial DNA

Tandem Duplications as a Source of Instability

Another reaction came from Bill Hayes. Bill wrote a highly (and deservedly) regarded book, *The Genetics of Bacteria and Their Viruses*, whose definitive nature has not since been equaled. He covered the insertion model in the 1964 version of the first edition. (His viewpoint was modified substantially by the second (1968) edition.) I felt honored to be included. Despite some obvious skepticism about whether λ and F are really inserted, he kept returning to the model to account for some of the facts, especially about F′s. At least the model had explanatory value. But one of his assumptions surprised me; if he doubted whether λ was really inserted, he had no doubts about λ*gal*. He thought it was not inserted, for reasons too obvious to require discussion.

As far as I can tell, Hayes' view of λ*gal* (which I also encountered in other bacterial geneticists at the time) stemmed from the fact that λ*gal* could rather easily be lost from a cell that carries it. Plasmids could be lost; integrated elements should be stable. My own thinking was that, since a strain with an integrated λ*gal* has two copies of the *gal* operon

in close proximity and in the same orientation, recombination between the two can loop out the λ*gal*, leaving one copy of *gal* behind.

Such thinking was not common among bacterial geneticists at the time. As a graduate student at Illinois, I was fortunate to have taken a cytogenetics course from Marcus Rhoades, where we worked out many of the consequences of homologous recombination between and within chromosomes. I mistakenly assumed a readership to whom this was, likewise, classroom stuff. I've sometimes thought that my main contribution to prokaryotic genetics could be characterized simply as applying the principles of eukaryotic cytogenetics to bacteria.

AFTERWORD

After 1965, λ insertion attracted the interest of a host of talented investigators who probed the genetics and biochemistry in depth. These included Bob Weisberg, Howard Nash, Kiyoshi Mizuuchi, and Art Landy, to mention just a few. I have continued to work in the area, looking especially at regulation. I've also dedicated some time to biotin biosynthesis, evolution of λ related phages, and genomics. In most of these areas, students and collaborators contributed heavily to my lab's output. I won't try to list them, because they're not part of the story I set out to tell here. At the moment, my long-time collaborator Alice del Campillo Campbell and I are studying the evolution of the phage-coded enzymes needed for insertion and excision, and their regulation.

DEDICATION

This chapter is dedicated to the memory of Esther M. Lederberg (1922–2006) whose early work laid some of the ground for the author's research described herein.

SUMMARY POINTS

1. Bacteriophage λ is perpetuated in lysogenic cells as a prophage inserted into the chromosome.
2. The gene order of the inserted prophage is a cyclic permutation of that in the free phage particle.
3. Specialized tranducing phages, such as λ*gal* played a critical role in demonstrating insertion before direct sequencing of bacterial genomes became possible.

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. Abroi A, Ilves I, Kivi S, Ustav M. 2004. Analysis of chromatin attachment and partitioning functions of bovine papillomavirus type I E-2 protein. *J. Virol.* 78:2100–13
2. Adhya S, Cleary P, Campbell A. 1968. A deletion analysis of prophage lambda and adjacent genetic regions. *Proc. Natl. Acad. Sci. USA* 61:956–62
3. Arber W. 1958. Transduction des caractères *gal* par le bactériophage lambda. *Arch. Sci. (Geneva)* 11:259–358

4. Calef E, Licciardello G. 1960. Recombination experiments on prophage host relationships. *Virology* 12:81–103
5. Campbell A. 1957. Transduction and segregation in *Escherichia coli* K12. *Virology* 4:366–84
6. Campbell A. 1961. Sensitive mutants of bacteriophage l. *Virology* 14:22–32
7. Cavalieri LF, Funston R, Rosenberg BH. 1961. Multi-stranded deoxyribonucleic acid as determined by x-irradiation. *Nature* 189:833–34
8. Edgar RS, Liclausis I. 1964. Temperature sensitive mutants of bacteriophage T4D: their isolation and genetic characterization. *Genetics* 49:649–62
9. Ennis DG, Fisher B, Demiston S, Mount DW. 1985. Dual role for *Escherichia coli* Rec A protein in SOS mutagenesis. *Proc. Natl. Acad. Sci. USA* 82:3325–29
10. Epstein RH, Bolle A, Steinberg CM, Kellenberger E, Boy dela Tour E, et al. 1963. Physiological studies of conditional lethal mutants of bacteriophage T4D. *Cold Spring Harbor Symp. Quant. Biol.* 18:375–92
11. Franklin NC, Dove WF, Yanofsky C. 1965. The linear insertion of a prophage into the chromosome of *E. coli* shown by deletion mapping. *Biochem. Biophys. Res. Commun.* 18:910–23
12. Freese E. 1958. The arrangement of DNA in the chromosome. *Cold Spring Harbor Symp. Quant. Biol.* 23:13–18
13. Horowitz NH, Leupold U. 1951. Some recent studies bearing on the one gene-one enzyme hypothesis. *Cold Spring Harbor Symp. Quant. Biol.* 16:65–74
14. Jacob F, Wollman EL. 1957. Genetic aspects of lysogeny. In *The Chemical Basis of Heredity*, ed. WD McElroy, B Glass, pp. 468–98. Baltimore: Johns Hopkins Press
15. Landy A, Ross W. 1977. Viral integration and excision: structure of the lambda att sites. *Science* 197:1147–60
16. Morse M, Lederberg E, Lederberg J. 1956. Transduction in *Escherichia coli* K-12. *Genetics* 41:121–56
17. Rothman JL. 1965. Transduction studies on the relation between prophage and host chromosome. *J. Mol. Biol.* 12:892–912
18. Stretton AO, Brenner S. 1965. Molecular consequences of the amber mutation and its suppression. *J. Mol. Biol.* 12:456–65
19. Weigle JJ. 1957. Transduction by coliphage l of the galactose marker. *Virology* 4:14–25
20. Weigle J, Meselson M, Paigen K. 1959. Density alterations associated with transducing ability in the bacteriophage lambda. *J. Mol. Biol* 1:379–86
21. Wu R, Kaiser AD. 1968. Structure and base sequence of the cohesive ends of bacteriophage lambda DNA. *J. Mol. Biol.* 35:523–37