# Perspectives

## Anecdotal, Historical and Critical Commentaries on Genetics

*Edited by James F. Crow and William F. Dove*

## The Genome of Bacteriophage T4: An Archeological Dig

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> The major task for the elderly is to create meaning out of what they have done with their lives. Take what they tell you with a grain of salt.
>
> Anonymous Old Sage

I was asked to share my reflections about an article that appeared over 40 years ago in the 1963 *Cold Spring Harbor Symposium for Quantitative Biology*. The article (Epstein *et al.* 1963) described the phenotypes of 130 conditional-lethal mutants of T4, 69 amber mutants, and 61 temperature-sensitive (ts) lethal mutants and presented a genetic map of these mutations. The mutations were located in 47 different genes, 35 of which appeared to be involved in morphogenesis of the phage particles. When these morphogenesis mutants were grown under restrictive conditions, most of the infective processes appeared to continue normally; there was nuclear breakdown followed by rapid DNA synthesis and cell lysis, but no viable phage were released. Only phage components were produced: heads, or tails, or heads and tails, or particles lacking tail fibers. The locations of these genes are not random on the linkage map. Genes with similar phenotypes are clustered. Mutants defective in each of the other 12 genes showed aberrations in DNA synthesis. They also showed gross defects in cell lysis and phage component production as well. These genes also showed clustering in the genome. We argued that a significant fraction of the essential genes of T4 had been identified.

When I received the invitation to write this *Perspectives* article, I had almost nothing at hand except my memories. I could not find any of my reprints, and my science files had long since disappeared. I retired in 1990, and for the last 14 years I have engaged almost entirely in nonscientific matters. I recall with some embarrassment that 20 or so years ago the Genetics Society of America asked that I give my papers to the University Library for safekeeping. I then looked at my old notebooks from the 1950s and 1960s—loose-leaf binders with the pages falling out, many undated, just lists of numbers. They were total gibberish to me. I hid them away then and they eventually disappeared. To write this retrospective, I contacted Bill Dove, one of the *Perspectives* editors, and he sent me a copy of the Cold Spring Harbor article. I was relieved to find that I could still understand the material. My friend and colleague for 50 years, Frank Stahl, also came to my aid and sent me copies of my grant reports for 1961–1963. He also sent me our correspondence from the 1950s and 1960s.

It has been claimed that one of the tasks for the elderly is to create meaning out of what they have done with their lives. I can certainly see that urge now in me, and so I have approached the preparation of this article with considerable interest. I have sifted through the few documents made available to me, and I have dug into my memories and the memories of those who collaborated with me in this work.

### THE CONTEXT

In 1960, when Dick Epstein and I started to work with conditional-lethal mutants, our knowledge of the genome of T4 was quite sketchy. Until 1960 we had been living with the view that T4 had three linkage groups, perhaps three or more DNA molecules, which duplicated, recombined, and somehow assorted themselves properly into phage heads at maturation. Figure 1 is the "historical" T4 linkage map I drew for our grant report in 1961. It consisted basically of the three linkage groups presented by Doermann and Hill (1953), and most of the mutations were plaque morphology mutations whose phenotypic basis was not known. The lone

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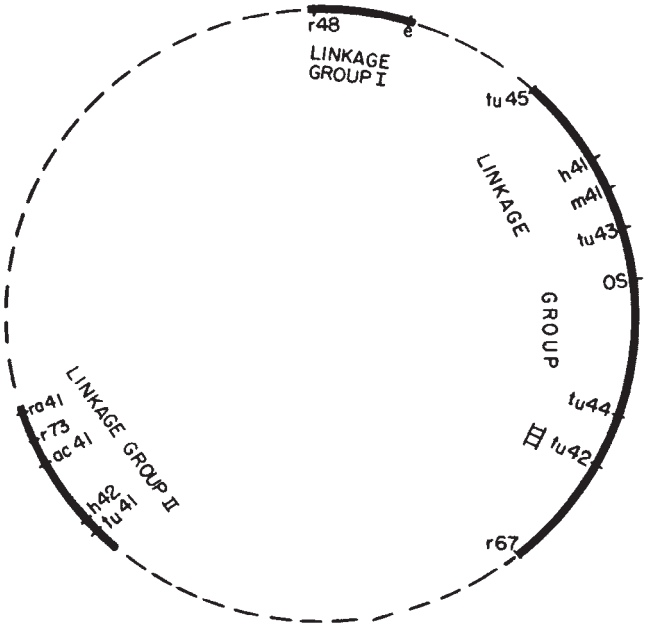


Figure 1.—The historical map of T4. The linkage map, composed of three presumably independent linkage groups, shows the disposition of available genetic markers as of 1959. The map has been extracted from a grant report for 1961 submitted by Max Delbrück and Bob Edgar to the National Foundation for Infantile Paralysis.

exception on the map was the gene coding for the phage endolysin, gene e, discovered by George Streisinger in 1959. In 1960, Streisinger and Bruce demonstrated that all the genes of T4 formed one linkage group by establishing the linkage II-I-III. In 1961 I collaborated with George (Streisinger *et al.* 1964) to demonstrate that there was linkage between linkage groups II and III, establishing that the T4 genetic map was circular. At about the same time we learned that the DNA of T4 was just one single molecule and not many small ones.

## THE HISTORY

**1960:** When I left the University of California—Santa Cruz in 1990 and cleared out my office, I had saved a few scientific books. One was *Phage and the Origins of Molecular Biology*, a collection of articles honoring Max Delbrück's 60th birthday in 1966. I read again my article in the book (Edgar 1966) about the discovery of conditional lethals. It described how Dick Epstein had discovered the amber mutants in early 1960 or late 1959 and how, during the summer of 1960 at Cold Spring Harbor, Alan Campbell and I had come up with the strategy of looking for conditional lethals. It also describes how I had started to look for temperature-sensitive lethal mutations in the fall of 1960. Both Alan Campbell (1993) and Millard Susman (1995) have written *Perspectives* articles for Genetics that mention that encounter. Frank Stahl's 1995 *Perspectives* article for Genetics about the ambers is detailed and excellent. Curiously, my article for the Delbrück Festschrift, probably written in 1965, made no mention of the 1963 Cold Spring Harbor article that was the first publication about conditional lethals in T4 and their usefulness for the study of morphogenesis.

Millard Susman and Charley Steinberg were Max Delbrück's last phage graduate students, and both were authors on the Cold Spring Harbor article. They helped Dick and me with the conditional-lethal work in many ways. Charley died in 1999, and a *Perspectives* article about him appeared in Genetics (Wu and Lindahl 2001). Recently Millard wrote to me about his experiences working with me and Dick and Charley:

> One evening, Dick, Charley, and I did 100 crosses to map a bunch of amber mutants. Dick prepared everything. He made all the mixtures of parental phages, labeled all the plates (I think), and measured the broth in all the dilution tubes. Charley and I were just extra hands. It was exciting. Dick started a new cross every 30 sec, and we did the standard assays of unadsorbed phage, IC's, and such. At the end, we had huge piles of plates, all of which Dick counted. At the end, we all believed that we had completed the experiment without any mistakes. That experiment, done in the quiet of the evening hours at Caltech, remains in my memory as one of the glorious events in my life as a biologist.
>
> When I wrote a 'Perspectives' article on the Cold Spring Harbor Phage Course, I wrote that I remembered that day when you told me, on the beach at the end of Bungtown Road, at Cold Spring Harbor, that you were planning a new initiative when we returned to Caltech. You were going to try to isolate temperature-sensitive mutations, and you were doing it because the Horowitz and Leupold experiment (Horowitz and Leupold 1951) suggested that such mutations could be found in many genes that encoded essential enzymes. You asked me not to tell Charley what you were planning, because he would think of a million reasons not to search for such mutations. I have no doubt that my memory is clear on all of that. Your logic was unassailable, and I admired your plan to look for ts mutants. And I understood why you might be fearful of Charley's skepticism, because, as much as I loved Charley, I was permanently humbled by him.

As best I can recall, when I returned to Caltech in the fall of 1960, Dick Epstein was already in the process of moving to the University of California—Los Angeles, where he continued his work of isolating, mapping, and characterizing amber mutants. While there, he collaborated with others at the Massachusetts Institute of Technology on the first paper on the ambers, indicating that one of the amber mutants (gene 42 am N122) was defective for production of the phage-specific deoxycytidylate hydroxymethylase (Wiberg *et al.* 1962). Meanwhile, I started the search for temperature-sensitive mutants. My first search was, as I remember, successful, and only then did I tell Charley. His response was, yes, he had thought of looking for them but doubted very much that any would be stable enough to work with. Like Millard, I, too, was a bit intimidated by Charley!

I spent the autumn of 1960 happily isolating and mapping ts mutants. During the winter of 1960–1961,

Eduard Kellenberger, a leading electron microscopist from Geneva, came to Caltech for a time and helped us get our gene physiology studies started. Eduard had been a student of Jean Weigle's while Jean was still a physics professor in Geneva. Jean had retired and moved to Pasadena, California, and was working on phage in Max's lab when I first met him in the late 1950s. It was during Eduard's visit that we did most of the physiological experiments on the mutants. For those cells that went through the infective process normally except for failing to release live phage, we used electron microscopy to look in cell lysates for the presence of phage particles. I remember the thrill of seeing on the microscope screen for the first time, not phage particles, but component parts only. In some mutant lysates, we saw only heads, in others only tails, and in still others, only heads and tails but unassembled.

Eduard has recently written me:

> I was not familiarized with your beautiful system, before I arrived in Caltech. I participated a little bit on the early screens of mutants: I introduced the optical microscopy of nuclear breakdown (with the method of equalizing refractive indexes) and the chemistry of DNA synthesis. The method of studying *in situ* lysates by electron microscopy and of agar-filtration I had developed earlier; I think that at Caltech we used mainly the second method for studying lysates as a whole.
>
> I had been proposed as a candidate for a position as Electron Microscopist at Caltech but I declined. I guess that Max Delbrück and Jean Weigle considered your work so important that they decided to invite me for a visit of a few months to help you in electron microscopy. I also taught Alex Lielausis in the methods of E.M. (so that your studies could continue after I left).

In retrospect I do believe that the whole story would have turned out quite differently had Eduard not visited Caltech when he did. He not only set us up to do the physiology experiments on the ts mutants and to continue them after he left, but also invited Dick to join him in Geneva where the work on the amber mutants continued with vigor.

I believe I was the only phage geneticist working at the time to have been trained as a biologist rather than as a physicist or chemist. In fact, when I was an undergraduate, there was no such field as biology. There was botany and there was zoology. Both of these disciplines consisted almost exclusively of taxonomy and morphology. I took genetics as a junior and loved it. I got my first college A in genetics while flunking organic chemistry. I did take and pass medical school biochemistry while in graduate school, but just the lectures, not the lab. I remember that there was only one lecture on nucleic acids and that was entirely on gout! The year was 1953, and the Watson-Crick paper (Watson and Crick 1953) was just hitting the newsstands. As a consequence of my background, I maintain that I was very comfortable doing what geneticists do, namely, crosses, but very uncomfortable doing what chemists do, namely, muck with chemicals and extracts. In the summer of 1953 or 1954, I remember very clearly being cornered by Jim Watson at a party on the porch of Blackford Hall at Cold Spring Harbor. Jim tried to persuade me that the future of genetics was with chemistry and that I should learn some. Well, he was right, of course. The glory days of "pure genetics," exemplified by studies like those of Benzer (1955) and of Crick *et al.* (1961) with the rII system, were just about over. If Eduard had not visited Caltech, I believe I would have kept my lab focused solely on genetics. We would have isolated and mapped thousands of ts mutants, and others would have explored the phenotypes. Eventually, someone would have written a review article much like the Cold Spring Harbor article.

After reading these comments, Frank Stahl wrote,

> Your belief about your colleagues (at least one of them) is wrong. My AB was in Biology, and it was of the old-fashioned kind. I studied only enough Math, Chemistry and Physics to meet the very minimal Harvard requirements for a Biology degree. (However, my degree—in 1951—was in Biology, since Harvard did not grant separate Botany and Zoology degrees. So, I guess, my degree was not of the MOST old-fashioned kind.)

**1961:** By the fall of 1961, Dick had moved to Geneva, and the work on the ambers was again at full throttle. At the end of 1961, I drew the map of T4 shown in Figure 2 for our grant report. Dick Epstein had by then mapped 26 amber mutants, and I had mapped 28 ts mutations. Thirteen of the identified genes contained both amber and ts mutations. We had also shown the relation between these conditional lethals and the plaque morphology mutations upon which the genetic map had previously been built. Thanks to Eduard, all the ts mutant phenotypes had been determined.

After a time, the more mutants we isolated, the fewer new genes we discovered. We tried various strategies to trick shy genes into showing themselves. None of these strategies were very successful. By the time we ran out of steam, we had isolated ~400 ts mutants and had identified 37 genes (Edgar and Lielausis 1964). We put extensive effort into creating an integrated map of the amber and ts mutations (Edgar *et al.* 1964). Frank Stahl developed a mapping function to correct for negative interference, and we applied this to our mapping data to create a genetic map that we hoped was congruent with the underlying physical structure (Stahl *et al.* 1964). We also had intragenic recombination data for 20 of the genes (Bernstein *et al.* 1964). These data gave us some idea about the sizes of the genes.

**1962:** I think it was in the fall of 1962 that I traveled to Geneva to write the Cold Spring Harbor article with Dick and Eduard. We managed to pool our data and write the article. As I recall, I spent about a month in Geneva. I do clearly remember that as my departing plane climbed out of the clouds, I could see the sun shining on snowy Mont Blanc and realized that in my

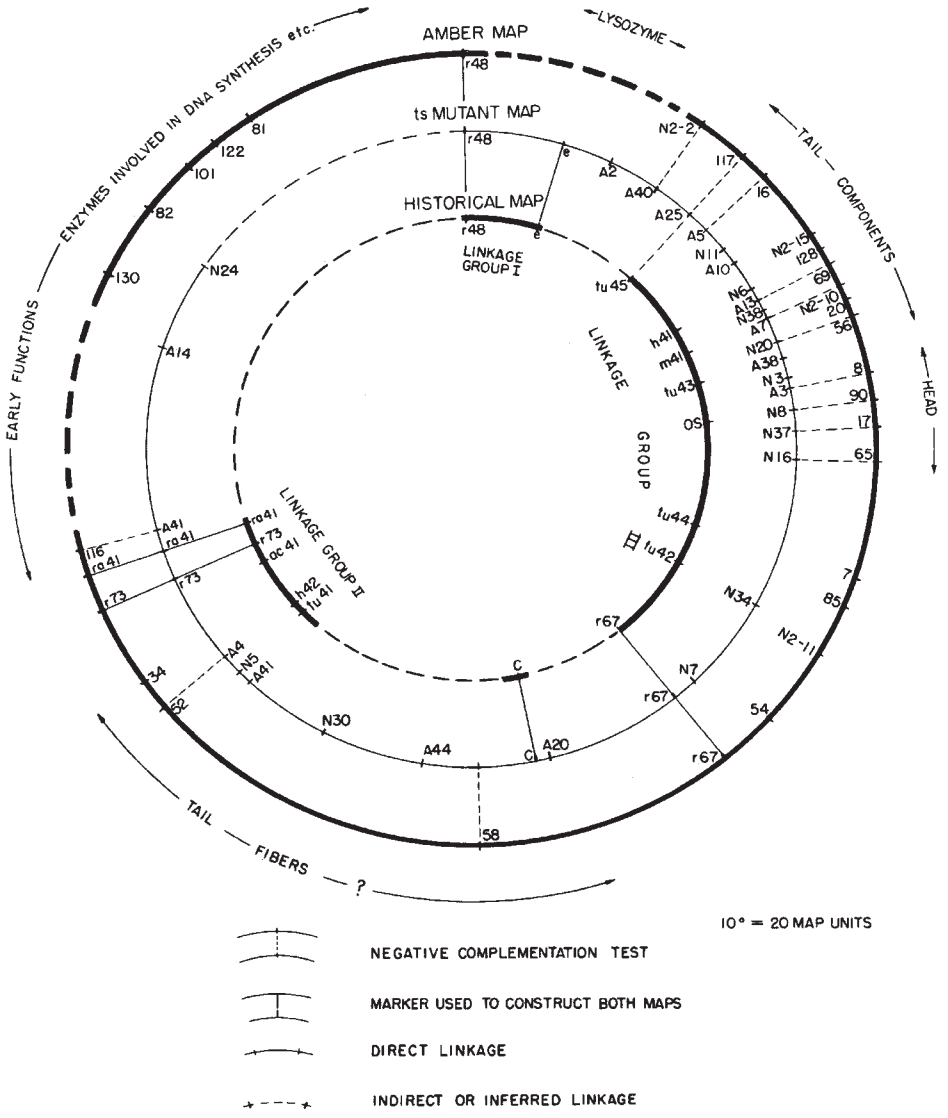


FIGURE 2.—A map of T4D comparing the linkages of historical markers with amber and temperature-sensitive mutants. This map was drawn for a grant report for 1961 submitted by Max Delbrück and Bob Edgar to the National Foundation for Infantile Paralysis.

month in Geneva I had never seen the sun. Somehow, the joy and warmth of the time with these colleagues and our work together had made me forget my sunny California home. The summer of 1963 finally arrived, and it was time to present this paper on our research. In those days, giving an invited paper at the Cold Spring Harbor Symposium was like being on the Ed Sullivan TV show. It was prime time. Dick gave the presentation.

The biggest surprise for me about the work was the large number of genes that we found that were apparently involved in the formation of the phage particles. At the time it was widely believed that the phage perhaps contained only three to four proteins. Yet we had found 35 genes apparently needed just for the assembly of the particle. This result pleased me immensely. It demonstrated the power of genetics to analyze cellular processes. Biochemical methods alone were inadequate. This had already been shown in the work of BEADLE and TATUM (1941) and others in the genetic dissection of metabolic pathways in Neurospora. Genetic analysis revealed enzymatic steps undetected by biochemical methods alone. Now we could use genetic dissection to analyze the formation of macromolecular structures, such as phage, using methods similar to those of the biochemical geneticists. Shortly after this work was completed, Bill Wood and I used these methods to work out some of the phage assembly steps *in vitro* (EDGAR and WOOD 1966). With Bill's advice and encouragement, I was finally mucking about with chemicals and extracts!

**1965:** Two years after our article was published, I was offered the National Academy of Sciences Award in Molecular Biology. The award stated: "For his development and application of the method of 'conditional lethal mutants' for the analysis of the genetic control of morphogenesis at the molecular level."

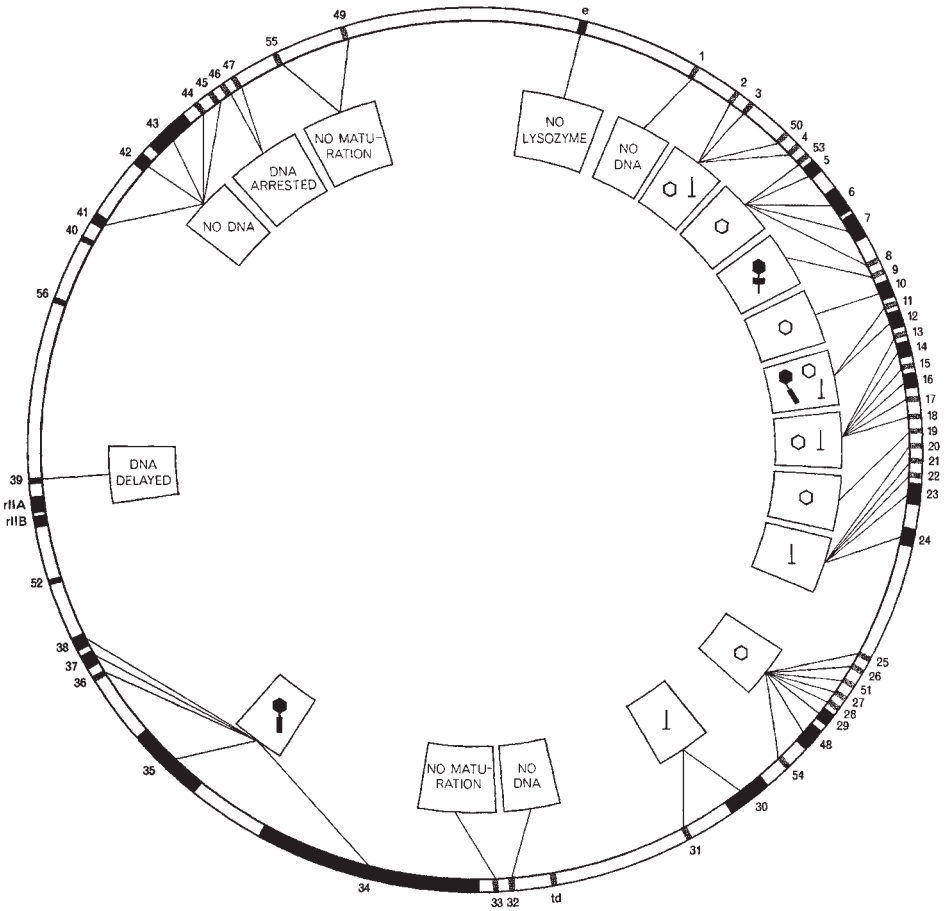


Figure 3.—The map of the genes of T4D identified by conditional-lethal mutations. This map was drawn in 1965 for a Scientific American article (Edgar and Epstein 1965). The map was drawn by using the mapping function developed by Stahl *et al.* (1964). Those genes whose extent was determined by intragenic mapping are indicated by solid bars. The locations of genes for which no intragenic data were available are indicated by shaded bars. The defective phenotypes are indicated in boxes.

When I was told of the award, my first reaction was dismay. I felt it should have been given to Dick Epstein and me jointly and probably Eduard as well. After all, it was for our work presented at the Cold Spring Harbor Symposium in 1963. I told Ray Owen, our department chairman, that I could not accept the award unless it was offered to both Dick Epstein and me. Shortly thereafter I was called into the office of Harold Brown, the president of Cal Tech. He sought to persuade me to accept the award; even though I was in awe of him at the time, I remained committed to my decision that we receive the award jointly or I would not accept it. S. E. Luria also called and urged me to accept the award. A few days later Max Delbrück told me he had received a call from the president of the National Academy of Sciences. The president said that, because of my recalcitrance, they had decided not to give an award that year. It turned out no such call was made. Max loved practical jokes, at least when he made them.

Eventually I relented and accepted the award. With my prize money, I decided to get something I would never otherwise buy. I settled on a harpsichord. I could barely play it, but I loved to tune it, a marvelously meditative exercise. Frank Stahl claims that I gave half the money to Dick, but I do not remember if I did.

In a recent e-mail Dick states,

> I never knew that you were in such an agonizing situation in regards to the award and am very touched by your thinking of refusing it. Frank is right about your sharing the prize with me. I was never very clear (until now) from your correspondence exactly what the award (US Steel award?) was all about, but I was delighted to get the money.

The last map we published was in a 1965 Scientific American article (Edgar and Epstein 1965). By that time we had analyzed >1000 amber and ts mutants and identified 56 genes. The map is shown in Figure 3. For some genes many mutations were found, and so the extent of the gene could be determined from intragenic mapping. These genes are represented by solid bars in Figure 3. From this we surmised that the genes we had identified encompass about half of the genetic map.

**2004:** While writing this article, I became curious to learn about the present state of knowledge of the T4 genome. I found the map of T4 in Figure 4 posted on the T-even website. It is from Kutter *et al.* (1994). A review of the T4 genome was published in 2003 by Miller *et al.* The DNA sequence was completed only recently owing to the difficulty of working with its odd chemical structure. It is estimated that ~300 genes are packed into

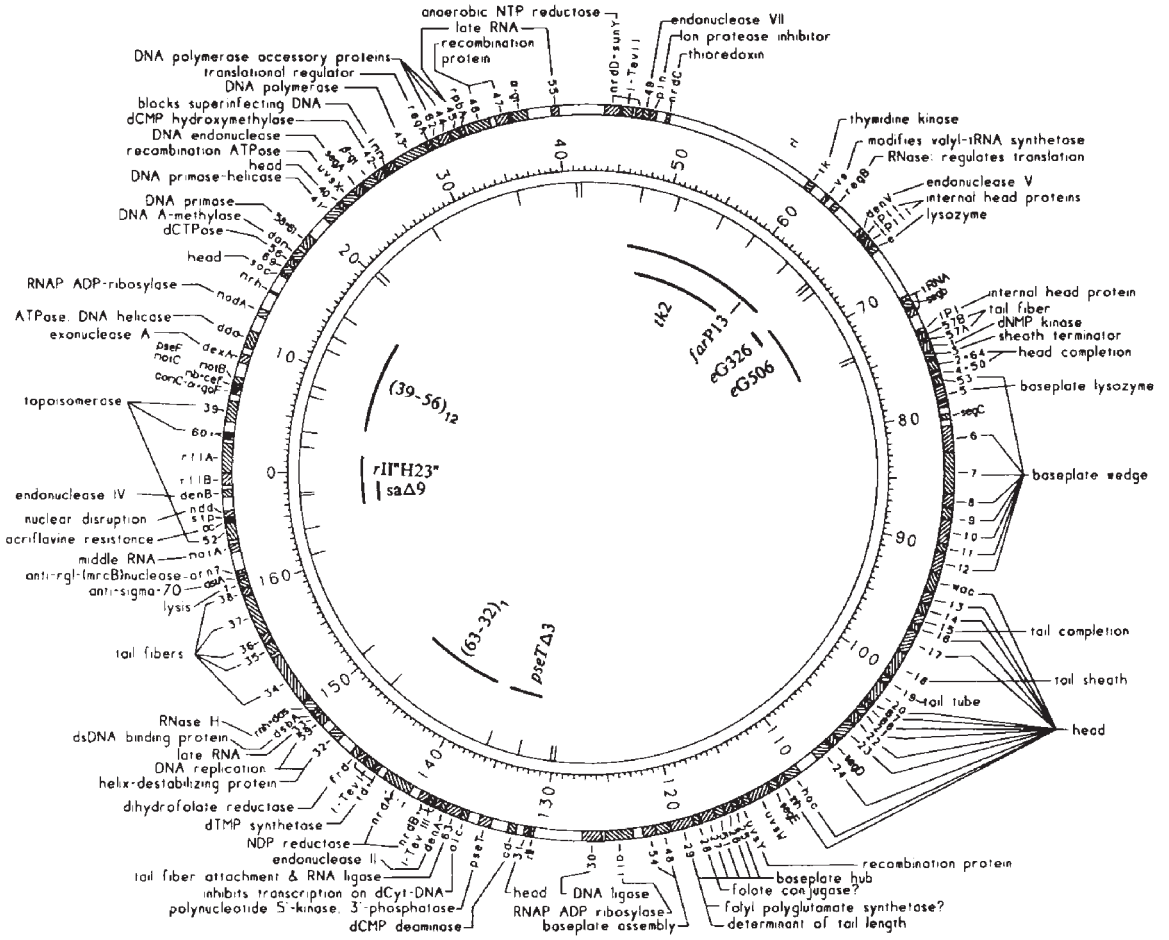


FIGURE 4.—The map of T4 as of 1994. This figure was taken from KUTTER *et al.* (1994) and is shown at http://phage.bioc.tulane.edu/html/t4gmap.html.

the 168,903-bp genome. The functions and properties of 156 genes have been characterized by mutation and/or by the properties of cloned gene products.

The authors of the review article (MILLER *et al.* 2003, pp. 104–106) state,

> Only 62 of the T4 genes are 'essential' under standard laboratory conditions (rich medium, aeration, 30 to 37°); mutants altered in a few other genes produce very small plaques under standard conditions. Many of these key genes are much larger than the average T4 gene; together, they occupy almost half of the genome. They include genes that encode proteins of the replisome and of the nucleotide-precursor complex, several transcriptional regulatory factors, and most of the structural and assembly proteins of the phage particle. Most of these genes were first identified by the isolation of amber or temperature-sensitive conditional-lethal mutations and were assigned numbers before their functions were determined.

I take great satisfaction in learning that we had accomplished what we had hoped. We had identified most of the essential genes of the phage, and these genes covered about half the genome. Frank's mapping function worked well.

## AFTERTHOUGHTS

In the article I wrote for the Delbrück Festschrift in 1965, I claimed that amber mutants were named to honor Harris Bernstein's mother, yet in 1995 Frank claimed that they were named for Harris himself. Last week I found Harris by searching the Internet and made contact with him after 40 years. [The week before, I had discovered that he was, in fact, a coauthor with me (BERNSTEIN *et al.* 1964).] Regarding the naming of the ambers, this is what Harris wrote me:

> In 1960 when I was a biology graduate student at Cal Tech, I was friends with Charley Steinberg (also a grad student) and Dick Epstein (a post-doc). I was doing my thesis on Neurospora, and Charley and Dick were working on phage T4 with Bob Edgar, who was then an Assistant Professor. At the time Charley, Dick and myself were all single, and were in the habit of working late into the night, but occasionally taking time off to see a movie. One evening, I was bored with my own work, and wandered down to the basement of Church Laboratory where the phage lab was located, with the hope of convincing my friends to go to a local movie. However, they were well into a phage mutant hunt, and suggested that, rather than hanging around kibitzing, I help them out. My help

> consisted of flaming a wire loop to sterilize it, then using it to repeatedly pick phage plaques from among hundreds on one petri dish and then inoculating two other petri dishes which had been seeded with host bacteria. After some time I asked them to explain what they were doing. As I remember it, I thought their idea to look for certain conditional lethal mutants sounded good and expressed enthusiasm (although I no longer remember what their original purpose was, and probably didn't fully understand it at the time). In any case, they were pessimistic about the outcome, and we agreed as sort of a joke that if the experiment did work out, they would name mutants after my mother. In later years Charley remembered it somewhat differently. A popular racy novel in the fifties was called "Forever Amber," and sometimes they referred to me in a kidding way as "immer wieder Bernstein," the German translation of the book title. Charley's memory was that they promised to name any mutants they found after me as an inducement to help them pick plaques. The next day it was clear that they had found the new mutant class they had decided to name amber mutants. Since I pretty consistently had heated my wire loop excessively, I had fried most of phage plaques I had picked, and so I had actually contributed nothing to the experiment. The significance of the amber mutants as nonsense mutants was not realized until several months later, but they, along with the temperature sensitive mutants of Bob Edgar, allowed phage workers to identify virtually all the essential phage genes, whereas previous work was pretty much limited to the rII genes and a few other genes.

After sharing Harris's reflection with Millard Susman, he responded,

> I'm glad to see that there is still controversy over the naming of the "amber" mutations. At the age of 69, I am not prepared to tell you that I am absolutely confident that my memory of events occurring 40 years ago is accurate, but I believe I was present when Harris walked into the lab to invite Dick and Charley to go to a movie with him. At least that is what I have been telling students in my classes for the past 35 years or so. According to my version of the story [in agreement with Harris], the promise made to Harris was that the mutants would be named for him, not for his mother. I remember that I was surprised when I later heard Dick tell his version of the story.

However, in a recent e-mail Dick has written in response,

> According to me, Harris is right about the naming of the ambers. When Frank was writing his article, Charley and I presented different versions of the incident. Charley and I never discussed the differences, but I was happy that Harris (who should know) confirms my version. I suspect that Charley was concerned that "naming the mutants after his mother" was indecorous.

And so, appropriately, Dick has the last word, probably.

Another comment Millard Susman made in his correspondence with me was the following:

> There was always a moral dimension to my teaching, and I liked to point out that you and Dick were generous with your mutants, sharing them freely with anyone who wanted to do research on the genetic control of phage development. That seems to me a model of appropriate scientific practice. Thanks to your generosity, the field progressed with breathtaking speed. Now, of course, the legal offices at research institutions limit the distribution of such materials, and researchers are acutely aware of the commercial potential of their research. Too bad.

Yes indeed, too bad. I guess these days mutants are patented. I do not really know, but back in "the good old days" we (at least Dick and I and our friends) gave mutants to anyone who asked for them. There was "a gentleman's agreement" to respect each other's territory. I expected a request for a mutant to include a description of what use was going to be made of it. If that did not impinge upon my immediate plans, off it went. We, as geneticists, knew that the growth of the field was dependent on collaboration. Although competition and secrecy were common practices in other fields, they were not countenanced among geneticists. As Frank has pointed out to me, "This cooperative attitude was thought by some to be one of Max's great contributions to phage research."

I have thought a lot about the issue of the relationship between Dick and me. Were we collaborating or competing? He had found the amber mutants. I was motivated to look for the temperature-sensitive mutants in part because of envy. But the ambers were his to exploit and develop as he saw fit. We talked freely about our ideas and our plans and were already old and close friends. The end result was a collaborative study that worked beautifully even though we were, for most of the time, thousands of miles apart.

In the 1970s I switched to "the worm," *Caenorhabditis elegans*. I chose the worm because Sydney Brenner's vision for the worm was the same as mine had been for T4. In fact, he told me that he had spent a year unsuccessfully working toward that goal with T4 just a few years before our work with conditional lethals. He had hoped that small plaque mutants (minutes) would turn out to be "leaky" lethal mutations that he could use much as we had used conditional lethals. He then turned to eukaryotes and chose the worm because of its excellent genetics and small number of cells (Brenner 1974). The genome of the worm is a much larger genome than that of T4, but still small for a eukaryote. Thus, the worm genetics project had to become a cottage industry, each lab working on a part of the worm. Many American postdoctoral fellows cycled through Brenner's lab and came home to set up worm labs. Our collective goal was to understand the whole worm. To be successful, we were dependent on labs sharing their findings with other labs. Fortunately, many of us had already collaborated, knew the value of collaboration, and thus passed on this way of working in the labs under our supervisions. We even sometimes called the worm a "eukaryotic T4" because so many of the early worm workers had started with T4, *e.g.*, Sidney Brenner, myself, Bill Wood, Bob Horvitz, Sam Ward, and Dick Russell.

Yesterday, I awoke from my regular afternoon nap

(ah, the joys of retirement!) with a vivid memory buried for decades. In the 1960s, every fall the Biology Division at Caltech held a tennis tournament. In 1969, to my great surprise Bill Wood asked me if I would be his doubles partner. I rarely tried to play tennis, while Bill was the best. He had been on the Harvard varsity tennis team and was then the Pasadena, California, champion. I was touched. We had been close partners in the lab working on phage assembly *in vitro*, so why not be partners on the tennis court? He told me we would do fine if I just served as carefully as I could and then stayed out of his way. We made it all the way to the finals. Our opponents were to be Max Delbrück and Sam Ward. Max was quite good as a tennis player and Sam was better, almost as good as Bill. He was also Bill's graduate student and working on T4 assembly. Bill is of modest height, Sam is ~6′ 4″ and very competitive, but try as he might he never had beaten Bill.

The day of the finals arrived, and Max got a phone call at 4 AM from Sweden. He had won the Nobel Prize. Later, when reporters asked him about his reaction to winning the prize, he replied that mainly he was trying to keep his composure because he had a very important tennis match coming up later in the day. Bill and I arrived at the courts with our bottles of Gatorade to find the place so jammed with observers and reporters we had a hard time getting in. That day Max and Sam could do no wrong. And we tried, we really tried. I can still see Sam looming over the net and Max in the backcourt with a smile of satisfaction on his face. Bill and I were trounced.

The father of T4 had his day on court.

I am very grateful to Frank Stahl for providing me with documents, sound advice, and useful recollections. I especially wish to thank Dick Epstein, Eduard Kellenberger, and Millard Susman, fellow coauthors of the original article, for reading various drafts of this article and for unearthing documents, facts, and memories that they shared with me. I also thank them and Harris Bernstein for permission to freely quote from their e-mail correspondences.

## LITERATURE CITED

Beadle, G. W., and E. L. Tatum, 1941 Genetic control of biochemical reactions in Neurospora. Proc. Natl. Acad. Sci. USA **27:** 499–506.

Benzer, S., 1955 Fine structure of a genetic region in bacteriophage. Proc. Natl. Acad. Sci. USA **41:** 344–354.

Bernstein, H., G. H. Denhardt and R. S. Edgar, 1964 Intragenic complementation among temperature sensitive mutants of bacteriophage T4D. Genetics **51:** 987–1002.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

Campbell, A., 1993 Thirty years ago in Genetics: prophage insertion into bacterial chromosomes. Genetics **133:** 433–438.

Crick, F. H. C., L. Barnett, S. Brenner and R. J. Watts-Tobin, 1961 The general nature of the genetic code. Nature **192:** 1227.

Doermann, A. H., and M. B. Hill, 1953 Genetic structure of bacteriophage T4 as described by recombination of factors influencing plaque morphology. Genetics **38:** 79–90.

Edgar, R. S., 1966 Conditional lethals, pp. 166–170 in *Phage and the Origin of Molecular Biology*, edited by J. Cairns, G. S. Stent and J. B. Watson. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Edgar, R. S., and R. H. Epstein, 1965 The genetics of a bacterial virus. Sci. Am. **212:** 70–78.

Edgar, R. S., and I. Lielausis, 1964 Temperature-sensitive mutants of bacteriophage T4D: their isolation and genetic characterization. Genetics **49:** 649–662.

Edgar, R. S., and W. B. Wood, 1966 Morphogenesis of bacteriophage T4 in extracts of mutant-infected cells. Proc. Natl. Acad. Sci. USA **55:** 498–505.

Edgar, R. S., G. H. Denhardt and R. H. Epstein, 1964 A comparative genetic study of conditional lethal mutations of bacteriophage T4D. Genetics **49:** 635–648.

Epstein, R. H., A. Bolle, C. M. Steinberg, E. Kellenberger, E. Boy De La Tour *et al.*, 1963 Physiological studies of conditional lethal mutants of bacteriophage T4D. Cold Spring Harbor Symp. Quant. Biol. **28:** 375–394.

Horowitz, N. H., and O. Leupold, 1951 Some recent studies bearing on the one gene-one enzyme hypothesis. Cold Spring Harbor Symp. Quant. Biol. **33:** 677–687.

Kutter, E., B. Guttman and K. Carlson, 1994 The transition from host to phage metabolism after T4 infection, pp. 343–346 in *Molecular Biology of Bacteriophage T4*, edited by J. D. Karam, J. W. Drake and K. N. Kreuzer. ASM Press, Washington, DC.

Miller, E. S., E. Kutter, G. Mosig, F. Arisaka, T. Kunisawa *et al.*, 2003 Bacteriophage T4 genome. Microbiol. Mol. Biol. Rev. **67:** 86–156.

Stahl, F. W., 1995 The amber mutants of phage T4. Genetics **141:** 439–442.

Stahl, F. W., R. S. Edgar and J. Steinberg, 1964 The linkage map of bacteriophage T4. Genetics **50:** 539–552.

Streisinger, G., and V. Bruce, 1960 Linkage of genetic markers in phages T2 and T4. Genetics **45:** 1291–1296.

Streisinger, G., R. S. Edgar and G. H. Denhardt, 1964 Chromosome structure in phage T4. I. Circularity of the linkage map. Proc. Natl. Acad. Sci. USA **51:** 775–779.

Susman, M., 1995 The Cold Spring Harbor Phage Course (1945–1970): a 50th anniversary remembrance. Genetics **139:** 1101–1106.

Watson, J. D., and F. H. C. Crick, 1953 Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid. Nature **171:** 737–738.

Wiberg, J. F., M. L. Dirksen, R. H. Epstein, S. E. Luria and J. M. Buchanan, 1962 Early enzyme synthesis and its control in *E. coli* infected with some amber mutants of bacteriophage T4. Proc. Natl. Acad. Sci. USA **48:** 293.

Wu, G. E., and K. F. Lindahl, 2001 Memories of a mentor: Charley Steinberg. Genetics **157:** 927–932.