Perspectives

Anectdotal, Historical and Critical Commentaries on Genetics

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Hershey

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ALFRED Day Hershey died at his home, in the Village of Laurel Hollow, New York, on May 22, 1997 at the age of 88. Seven weeks later, a number of Al's friends met at Cold Spring Harbor to commemorate his life. The tributes paid on that occasion have informed this *Perspectives*, and some of them are cited in what follows. Full copies are available from the Cold Spring Harbor Laboratory.

Most students of biology know of Hershey—his best known experiment is described in texts of both biology and genetics. This work (Hershey and Chase 1952a) provided cogent support for the hypothesis that DNA is the conveyor of genetic information.

The subject of the "Hershey-Chase experiment" was the bacteriophage T2, composed half of protein and half of DNA, a combination compatible with any of the three competing views of the chemical basis of heredity. T2, like many other phages, is a tadpole-shaped virus that initiates an attack on a bacterium by sticking to it with the tip of its tail. The Hershey-Chase experiment used DNA-specific and protein-specific radioactive labels to show that the DNA of the virus then entered the bacterium while most of the protein could be stripped from the surface of the cell by agitation in a Waring Blender. Such abused cells produced a normal crop of new phage particles. Previous evidence implicating DNA in heredity had shown that a property of the surface coat of the pneumococcus bacterium could be passed from one strain to another via chemically isolated DNA. The observation by Hershey and Chase justified the view that the entire set of hereditary information of a creature was so encoded. This work counted heavily in making Hershey a shareholder, with Max Delbrück (1906–1981) and Salvadore E. Luria (1912–1991), of the 1969 Nobel prize for Physiology or Medicine.

In 1934, Al earned his Ph.D. in the Departments of Chemistry and Bacteriology at Michigan State College with a thesis that described separations of bacterial constituents, identified by the quaint definitions of the times. Except for its evident care and industry, the work was unremarkable, merely part of an ongoing study ". . . to arrive ultimately at some correlation between the chemical constitution of [Brucella species], and the various phenomena of specificity by them" (Hershey 1934).

Al then assumed an instructorship in Bacteriology and Immunology at Washington University in St. Louis, where he collaborated with Professor J. Bronfenbrenner. From 1936 to 1939, their papers reported studies on the growth of bacterial cultures. Al certainly had the background for this work; his thesis research had involved not only the preparation of liver infusion growth medium (from scratch, as was routine in those days) but also the testing of 600 better defined media, none of which supported growth of Brucella. From 1940 to 1944, his experiments dealt with the phage-antiphage immunologic reaction and with other factors that influenced phage infectivity. During both those periods, about half of the 28 papers bearing Hershey's name were sole-authored. (It was apparently here that Al learned how to handle phage. It may also have been here that Al acquired the idea that authorship belongs to those who do the experiments and should not reflect patronization, rank, title, or even redaction of the manuscript.) Some of these papers may have been important contributions to the understanding of antigen-antibody reactions. To this reviewer they appear original, thoughtful, and quantitative, especially those on the use of phage inactivation to permit the study of the antigen-antibody reaction at "infinite" dilution of antigen (*e.g.*, Hershey 1941). But, of course, they interested an audience that did not include many geneticists or others interested in biological replication (except, perhaps, for Linus Pauling). It took Max Delbrück to move Al in that direction.

As recounted by Judson (1996), Delbrück was attracted by Al's papers. Perhaps he liked their mathematical, nonbiochemical nature. He must have liked their originality, logical precision, and economy of presentation. Max invited Al to Nashville in 1943 and recorded the following impression: "Drinks whiskey but not tea. Simple and to the point . . . Likes independence." Al's first "interesting" phage papers appeared soon thereafter (Hershey 1946, 1947).

A *sine qua non* for genetical investigation is the availability of mutants. The ease with which large numbers of phage particles can be handled facilitated the discovery and characterization of mutants that were easily scored. Al recognized that the high infectivity of phage and the proportionality of plaque count to volume of suspension assayed allowed for quantification of mutation far exceeding that possible in most other viral systems. Al measured mutation rates, both forward and back, and demonstrated the mutational independence of *r* (rapid lysis) and *h* (host range). He succeeded also in showing (in parallel with Delbrück) that these mutationally independent factors could recombine when two genotypes were grown together in the same host cells (Hershey 1946, 1947). Thus, "Phage Genetics" was born as a field of study, and it became conceivable not only that the basic question of biological replication could be addressed with phage but so could phenomena embraced by the term "Morgan-Mendelism."

Al continued the formal genetic analyses of T2 with investigations of linkage. Hershey and Rotman (1948) demonstrated that linkage analysis would have to take into account the production of recombinant particles containing markers from three different infecting phage genotypes. The same authors (1949) used "mixed indicators" to enumerate all four genotypes from two-factor crosses involving *h* and *r* mutants. That trick made it feasible to analyze fully the yields from individual mixedly infected bacterial cells. The signal finding was that all four genotypes of phage could be produced by an individual cell but that the numbers of complementary recombinants, which were equal on the average, showed little correlation from cell to cell. This demonstration of apparent nonreciprocality in the exchange process leading to recombination raised the specter that "crossing over" in phages would prove to be fundamentally different from that occurring in meiosis. The desire to unify this and other apparently disparate properties of phage and eukaryotic recombination into a single theoretical framework motivated subsequent studies of recombination by other investigators.

Delbrück tried to make such a unification by algebraic legerdemain and Papal Bull (see below). He formalized phage recombination as a succession of meiosis-like, pairwise exchanges between linear linkage structures (Visconti and Delbrück 1953). The resulting algebra embraced some of the major ways in which phage linkage data differed from meiotic data. In particular, it rationalized the "negative interference" between crossovers and the appearance of progeny particles that had inherited markers from three different infecting phage types. Visconti and Delbrück assumed that the exchange process involved physical breakage of chromosomes (DNA duplexes) and reciprocal reunion of the resulting fragments. They blamed the failure to see correlated numbers of complementary types in "single bursts" on vagaries of replication and packaging (into proteinaceous "heads") of the complementary recombinant chromosome types subsequent to their formation. Delbrück stopped thinking about phage genetics after Charley Steinberg and I (1958) showed him that his final expressions were independent of both of his two assumptions, reciprocal and break-join. A fully satisfactory conclusion to these issues for T2 came only with Mosig's and Albert's elucidation of the nonreciprocal, replicative mechanism by which recombinants are formed in T-even phages (for review, see Mosig 1994). More recent advances have established that the various recombinational mechanisms employed by bacteria and their viruses and by eukaryotes are, in fact, pleasingly similar, depending on homologous strategies and enzymes.

By most criteria, individual T2 particles are "haploid"—they contain but one set of genetic material. However, "heterozygous" particles, which contain two different alleles at a single locus, were described by Hershey and Chase (1952b) at the 1951 Cold Spring Harbor Symposium. After the elucidation of DNA as a duplex molecule (Watson and Crick 1953), it was possible to propose heteroduplex models for those "heterozygotes." Such models played a central role in all subsequent thinking about recombination, especially that involving relationships between meiotic crossing over and gene conversion.

In 1958, Hershey, like Levinthal (1954) before him, expanded on the Visconti-Delbrück analysis in an effort to connect observations on heterozygotes, which had molecular implications, with formal concepts proposed to deal with the populational aspects of phage crosses. The effort provided few, if any, answers, but clarified the ambient questions, at least for Al.

In 1950, Al left St. Louis to join the staff of the Carnegie Institution of Washington at Cold Spring Harbor. That move put him at the geographical center of the embryonic field of microbial/molecular genetics, and he soon became the intellectual center of its phage branch. At this time, the fruits of a collaboration conducted at St. Louis were published (Hershey *et al.* 1951). This work showed that phage particles were "killed" by the decay of the unstable isotope ^{32}P incorporated within their DNA. After the central importance of DNA to the phage life cycle (and to genetics) had been demonstrated, this "suicide" technique was exploited in other labs in efforts to analyze the phage genetic structure and its mode of replication. Like most early experiments in "radiobiology," these analyses were fun but not much more. From this time on, Al's studies became more down-to-earth (and successful) as he turned from mathematically based genetic analyses to serious studies of phage structure and the biochemistry of phage development. There is no doubt, however, that these studies were informed by Al's acute awareness of the genetical and radiobiological facts that had to be explained. These new studies were jump-started by the "blender" experiment, described above.

Several subsequent papers refined the conclusions of the blender experiment by showing, for instance, that *some* protein is injected along with the phage DNA (Hershey

1955). With Watson-Crickery well established by this time, these studies were interesting but not threatening to the view that the genetic substance was DNA. During this period, Al's lab published works that described DNA and protein production, and relations between them, in infected cells. They provided the biochemical counterpart of the genetically defined notion of a pool of noninfective, "vegetative" phage (Visconti and Delbrück 1953; Doermann 1953). This change of emphasis allowed Hershey (1956) to write:

> I have proposed the ideas that the nucleic acid of T2 is its hereditary substance and that all its nucleic acid is genetically potent. The evidence supporting these ideas is straightforward but inconclusive. Their principal value is pragmatic. They have given rise to the unprecedented circumstance that chemical hypotheses and the results of chemical experiments are dictating the conditions of genetic experiments. This development I regard as more important than the bare facts I have presented, which may yet prove to be of little or no genetic interest.

Biochemical studies on phage development were clouded by the lack of understanding of phage genome structure. It was not even clear how many "chromosomes" (DNA molecules) a phage particle contained. Furthermore, although Watson and Crick had specified what any short stretch of DNA should look like (plectonemically coiled complementary polynucleotide chains), they had been understandably proud of the fact that their model was structurally coherent in the absence of any specification of longitudinal differentiation. For them, it was enough to say that therein lay genetic specificity. For Al, that was not enough, and his lab pursued studies dedicated to the physical description of phage DNA. The results of these studies were succinctly reviewed by Hershey (1970a) in his Nobel Lecture. I'll briefly summarize my view of them, dividing the studies by phage type.

Al developed and applied chromatographic and centrifugal methods to the analysis of T2 chromosome structure (*e.g.*, Mandell and Hershey 1960; Hershey and Burgi 1960). This work systematized our understanding of the breakage of DNA during laboratory manipulation and had its denouement in the demonstration that a T2 particle contains just one piece of DNA (Rubenstein *et al.* 1961) with the length expected of a linear double helix (Cairns 1962). That conclusion was in apparent contradiction to genetic demonstrations that T4 chromosomes contained more or less randomly located physical discontinuities (Doermann and Boehner 1963). A major insight into the structure of T-even phage chromosomes resulted from attempts to reconcile the apparently contradictory physical and genetic descriptions of T-even chromosomes. The basic idea, elaborated and confirmed in a series of papers orchestrated by George Streisinger, was that the nucleotide sequences in any clone of phage particles were circularly permuted and that the sequence at one end of a given chromosome was duplicated at the other end (the chromosomes were "terminally redundant"). The predicted circular linkage map provided an elegant frame for displaying the functional organization of the T4 chromosome, as revealed by the pioneering studies of Epstein *et al.* (1963).

The terminal redundancies of the T-even phage chromosomes provided an additional physical basis for Al's heterozygotes. (See Streisinger 1966 for references and a more detailed recounting.) These insights were exploited and elaborated upon by Gisela Mosig, who spent the years 1962–65 in Al's lab. There she combined her genetic savvy of T4 with studies on the structure of the truncated, circularly permuted DNA molecules that she discovered in certain defective T4 particles. Those studies formed the basis for an elegant demonstration of the quantitative relations between the linkage map of T4 (as constructed from recombination frequencies) and the underlying chromosome (Mosig 1966). Fred Frankel (1963) and Rudy Werner (1968), in Hershey's lab, examined the intracellular state of T-even phage DNA. Their discovery that it was a network undermined analyses of phage recombination as a series of tidy, pairwise, meiosis-like "matings," and well-aimed triparental crosses by Jan Drake (1967) killed the pairwise mating idea once and for all.

Meselson and Weigle (1961) demonstrated that phage λ DNA, like that of *Escherichia coli* (Meselson and Stahl 1958), is replicated semiconservatively, in agreement with Watson and Crick's proposal that the replication of DNA involves separation of the two complementary strands. However, uncertainties about the structure of the semiconserved entities identified by Meselson prevented those experiments from being taken as proof of the Watson-Crick scheme. Careful measurements of the molecular weight of λ DNA (Hershey *et al.* 1961) demonstrated that there was just one molecule per particle. That conclusion, combined with Cairns' autoradiographic measurement of the length of λ DNA (1961), established that λ's semiconservatively replicating structure is, indeed, a DNA duplex, putting the issue to rest.

The chromosome of λ also provided a surprise (Hershey *et al.* 1963; Hershey and Burgi 1965). Though the chromosomes in a λ clone are all identical (*i.e.*, not permuted), each chromosome carries a terminal 12-nucleotide-long segment that is single-stranded and is complementary to a segment of the same length carried on the other end. The complementary nature of the segments gives λ "sticky ends." These ends anneal at the time of infection, circularizing the chromosome, which can then replicate in both theta and sigma modes. The demonstration of a route by which the λ chromosome can circularize provided physical substance to Campbell's (1962) proposal that the attachment of λ prophage to the host chromosome involves crossing over between the host chromosome and a (hypothesized) circular form of λ. And, of course, the understanding of λ's sticky ends, whose annealing creates *cos*, is exploited by today's gene cloners every time they work with a cosmid.

The nonpermuted character of λ's chromosome made it susceptible to analyses prohibited in T-even phage. For instance, Skalka *et al.* (1968) demonstrated the mosaic nature of the chromosome; major segments differed conspicuously from each other in their nucleotide composition. (That conclusion foreshadowed our current understanding of the role of "horizontal transmission" in prokaryotic evolution.) Hershey's lab demonstrated that these differing segments had distinguishable annealing ("hybridization") behavior. They exploited those differences to identify the approximate location of the origin of replication (Makover 1968) and to identify regions of the chromosome that were transcribed when λ was in the prophage state (Bear and Skalka 1969).

Al appreciated that progress in science depends on the development of new methods. Among those to which Al's lab made important contributions were fixed-angle Cs gradients, methylated albumin columns for fractionating DNA, methods of handling DNA that avoid breakage and denaturation, as well as methods that would break phage chromosomes into halves and quarters, and the calibration of methods for measuring molecular weights of DNA. Al confessed that the development of a method was painful; his view of heaven was a "place" where a new method, finally mastered, could be applied over and over. Bill Dove quoted Al as saying, "There is nothing more satisfying to me than developing a method. Ideas come and go, but a method lasts."

Al occasionally blessed us with his thoughts about the deeper significance of things. His papers "Bacteriophage T2: parasite or organelle" (1957), "Idiosyncrasies of DNA structure" (1970a), and "Genes and hereditary characteristics" (1970b) delighted his contemporaries and can still be read with pleasure and profit.

But how many people really knew Al? From his works, we can say he was interested in this or that, but such a contention might leave the impression that we have adequately summarized his interests. That is hardly likely. Each of Al's contributions was truly original—he never copied even himself! Consequently, each paper was a surprise to us. We can surmise, therefore, that his published works do not begin to saturate the library of ideas available to him. His papers must be but a small sampling of his scientific thoughts.

And the rest of his mind? Who knows? Al exemplified reticence. His economy of speech was greater even than his economy of writing. If we asked him a question in a social gathering, we could usually get an answer such as "yes" or "no." However, at a scientific meeting one might get no answer at all, which was probably Al's way of saying, in the fewest possible words, that he had no thoughts on that subject.

Encounters with Al were rare, considering that he worked at Cold Spring Harbor, which hosted hundreds of visitors every summer. That's because Al spent his summers sailing in Michigan, and, except at occasional Symposia or the annual Phage Meetings, which came early and late in the season, he was not to be seen.

Thus, most of us who valued Al as a colleague and acquaintance didn't really know him. I am one of those, and I suppose that status qualifies me for this assignment; the Al about whom I write is the same Al that most other people did not really know, either.

The Phage Church, as we were sometimes called, was led by the Trinity of Delbrück, Luria, and Hershey. Delbrück's status as Founder and his *ex cathedra* manner made him the Pope, of course, and Luria was the hard working, socially sensitive priest/confessor. And Al was the saint. Why? How could we canonize Al when we hardly knew him?

Maybe some of the following considerations apply: The logic of Al's analyses was impeccable. He was original, but the relevance of his work to the interests of the rest of us was always apparent; he contributed to and borrowed from the communal storehouse of understanding, casual about labeling his own contributions but scrupulous about attributing the ones he borrowed. He was industrious (compulsively so—each day he worked two shifts). He was a superb editor (*e.g.*, Hershey 1971) and critic, devastatingly accurate but never too harsh; he deplored that gratuitous proliferation of words which both reflects and contributes to sloppiness of thought. And his suggestions were always helpful.

Does that qualify him for sainthood? It would do if he was in all other respects perfect. And he may have been. Who could tell? Who among us knew this quiet man well enough to know if there was a dark side? Perhaps canonization was a mark of our deep respect for this quintessential scientist. Maybe, by canonizing Al we could accept the relative insignificance of our own contributions. Maybe we were just having fun.

But, in his papers, Saint Al was *there.* He talked to the reader, explaining things as he saw them, but never letting us forget that he was transmitting provisional understanding. We got no free rides, no revealed truths, no invitation to surrender our own judgment. And we could never skim because *every* word was important. I think this style reflected his verbal reticence, which, in turn, mirrored his modesty. Examples: "Some clarification, at least in the mind of the author, of the concepts 'reversible' and 'irreversible' has been achieved" (Hershey 1943). "On this question we have had more opportunity in this paper to discover than to attack difficulties" (Hershey 1944). Al's modesty was dramatically documented by Jim Ebert, who recalled that Al, whose research support was guaranteed by the Carnegie Institution, argued with Carnegie directors for the right to apply for National Institutes of Health support so that he might benefit from the critiques of his peers.

In science, Al appeared to be fearless. Fearlessness and modesty might seem an unlikely combination. Not so. Modesty is kin to a lack of pretense. In the absence of pretense, there is nothing to fear.

Tastes of the many flavors of Hershey's mind and the accomplishments of his laboratory can be best gained from the Annual Reports of the Director of the Genetics Research Unit, Carnegie Institution of Washington Yearbook. The principal investigators of this unit were Hershey and Barbara McClintock. In 1963 Al wrote, "Our justification for existence as a Unit, however, resides in the value of our research. We like to think that much of that value is as unstatable and as durable as other human produce that cannot be sold. Some can be put on paper, however. That we offer with the usual human mixture of pride and diffidence." Those who worked with Hershey at Cold Spring Harbor included Phyllis Bear, Elizabeth Burgi, John Cairns, Connie Chadwick, Martha Chase, Carlo Cocito, Rick Davern, Gus Doermann, Ruth Ehring, Stanley Forman, Fred Frankel, Dorothy Fraser, Alan Garen, Eddie Goldberg, June Dixon Hudis, Laura Ingraham, Nada Ledinko, Cy Levinthal, Shraga Makover, Joe Mandell, Norman Melechen, Teiichi Minagawa, Gisela Mosig, David Parma, Catherine Roesel, Irwin Rubenstein, Ann Skalka, Mervyn Smith, George Streisinger, Neville Symonds, Jun-ichi Tomizawa, Nick Visconti, Bob Weisberg, Rudy Werner, Frances Womack, and Hideo Yamagishi.

In his retirement, Al cultivated an interest in computers, and he renewed his youthful interest in music. He is survived by his wife, Harriet Davidson Hershey, and by his son, Peter.

LITERATURE CITED

Bear, P. D., and A. Skalka, 1969 The molecular origin of lambda prophage mRNA. Proc. Natl. Acad. Sci. USA **62:** 385–388.

Cairns, J., 1961 An estimate of the length of the DNA molecule of T2 bacteriophage by autoradiography. J. Mol. Biol. **3:** 756–761.

Cairns, J., 1962 Proof that the replication of DNA involves separation of the strands. Nature **194:** 1274.

Campbell, A., 1962 Episomes. Adv. Genet. **11:** 101–145.

Doermann, A. H., 1953 The vegetative state in the life cycle of bacteriophage: evidence for its occurrence and its genetic characterization. Cold Spring Harbor Symp. Quant. Biol. **18:** 3–11.

Doermann, A. H., and L. Boehner, 1963 An experimental analysis of bacteriophage T4 heterozygotes. I. Mottled plaques from crosses involving six *r*II loci. Virology **21:** 551–567.

Drake, J. W., 1967 The length of the homologous pairing region for genetic recombination in bacteriophage T4. Proc. Natl. Acad. Sci. USA **58:** 962–966.

Epstein, R. H., A. Bolle, C. M. Steinberg, E. Kellenberger, R. S. Edgar *et al.*, 1963 Physiological studies of conditional lethal mutants of bacteriophage T4D. Cold Spring Harbor Symp. Quant. Biol. **28:** 375–392.

Frankel, F., 1963 An unusual DNA extracted from bacteria infected with phage T2. Proc. Natl. Acad. Sci. USA **49:** 366–372.

Hershey, A. D., 1934 The chemical separation of some cellular constituents of the Brucella group of micro-organisms. Ph.D. thesis, Michigan State College. Published in co-authorship with R. C. Huston and I. F. Huddleson as Technical Bulletin No. 137 of the Michigan Agricultural Experiment Station.

Hershey, A. D., 1941 The absolute rate of the phage-antiphage reaction. J. Immunol. **41:** 299–319.

Hershey, A. D., 1943 Specific precipitation. V. Irreversible systems. J. Immunol. **46:** 249–261.

Hershey, A. D., 1944 Specific precipitation. VI. The restricted system bivalent antigen, bivalent antibody, as an example of reversible bifunctional polymerization. J. Immunol. **48:** 381–401.

Hershey, A. D., 1946 Mutation of bacteriophage with respect to type of plaque. Genetics **31:** 620–640.

Hershey, A. D., 1947 Spontaneous mutations in bacterial viruses. Cold Spring Harbor Symp. Quant. Biol. **11:** 67–77.

Hershey, A. D., 1955 An upper limit to the protein content of the germinal substance of bacteriophage T2. Virology **1:** 108–127.

Hershey, A. D., 1956 The organization of genetic material in bacteriophage T2. Brookhaven Symp. Biol. **8:** 6–14.

Hershey, A. D., 1957 Bacteriophage T2: parasite or organelle, pp. 229–239 in *The Harvey Lectures, Series LI.* Academic Press, New York.

Hershey, A. D., 1958 The production of recombinants in phage crosses. Cold Spring Harbor Symp. Quant. Biol. **23:** 19–46.

Hershey, A. D., 1963 Annual Report of the Director of the Genetics Research Unit, Carnegie Institution of Washington Yearbook **62:** 461–500.

Hershey, A. D., 1970a Idiosyncrasies of DNA structure (Nobel Lecture). Science **168:** 1425–1427.

Hershey, A. D., 1970b Genes and hereditary characteristics. Nature **226:** 697–700.

Hershey, A. D. (Editor), 1971 *The Bacteriophage Lambda*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Hershey, A. D., and E. Burgi, 1960 Molecular homogeneity of the deoxyribonucleic acid of phage T2. J. Mol. Biol. **2:** 143–152.

Hershey, A. D., and E. Burgi, 1965 Complementary structure of interacting sites at the ends of lambda DNA molecules. Proc. Natl. Acad. Sci. USA **53:** 325–328.

Hershey, A. D., and M. Chase, 1952a Independent functions of viral protein and nucleic acid in growth of bacteriophage. J. Gen. Physiol. **36:** 39–56.

Hershey, A. D., and M. Chase, 1952b Genetic recombination and heterozygosis in bacteriophage. Cold Spring Harbor Symp. Quant. Biol. **16:** 471–479.

Hershey, A. D., and R. Rotman, 1948 Linkage among genes controlling inhibition of lysis in a bacterial virus. Proc. Natl. Acad. Sci. USA **34:** 89–96.

Hershey, A. D., and R. Rotman, 1949 Genetic recombination between host-range and plaque-type mutants of bacteriophage in single bacterial cells. Genetics **34:** 44–71.

Hershey, A. D., M. D. Kamen, J. W. Kennedy and H. Gest, 1951 The mortality of bacteriophage containing assimilated radioactive phosphorus. J. Gen. Physiol. **34:** 305–319.

Hershey, A. D., E. Burgi, H. J. Cairns, F. Frankel and L. Ingraham, 1961 Growth and inheritance in bacteriophage. Carnegie Institution of Washington Year Book **60:** 455–461.

Hershey, A. D., E. Burgi and L. Ingraham, 1963 Cohesion of DNA molecules isolated from phage lambda. Proc. Natl. Acad. Sci. USA **49:** 748–755.

Judson, H. F., 1996 *The Eighth Day of Creation* (Expanded Edition). Cold Spring Harbor Laboratory Press, New York, p. 35.

Levinthal, C., 1954 Recombination in phage T2: its relationship to heterozygosis and growth. Genetics **39:** 169–184.

Makover, S., 1968 A preferred origin for the replication of lambda DNA. Proc. Natl. Acad. Sci. USA **59:** 1345–1348.

Mandell, J. D., and A. D. Hershey, 1960 A fractionating column for analysis of nucleic acids. Anal. Biochem. **1:** 66–77.

Meselson, M., and F. W. Stahl, 1958 The replication of DNA in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **44:** 671–682.

Meselson, M., and J. J. Weigle, 1961 Chromosome breakage accompanying genetic recombination in bacteriophage. Proc. Natl. Acad. Sci. USA **47:** 857–868.

Mosig, G., 1966 Distances separating genetic markers in T4 DNA. Proc. Natl. Acad. Sci. USA **56:** 1177–1183.

Mosig, G., 1994 Homologous recombination, pp. 54–82 in *Bacteriophage T4*, edited by J. D. Karam *et al.* ASM Press, Washington, DC.

Rubenstein, I., C. A. Thomas, Jr., and A. D. Hershey, 1961 The molecular weights of T2 bacteriophage DNA and its first and second breakage products. Proc. Natl. Acad. Sci. USA **47:** 1113–1122.

Skalka, A., E. Burgi and A. D. Hershey, 1968 Segmental distribution of nucleotides in the DNA of bacteriophage lambda. J. Mol. Biol. **34:** 1–16.

Steinberg, C., and F. Stahl, 1958 The theory of formal phage genetics. Appendix II in Hershey (1958).

Streisinger, G., 1966 Terminal redundancy, or all's well that ends well, pp. 335–340 in *Phage and the Origins of Molecular Biology*, edited by J. Cairns, J., G. S. Stent and J. D. Watson. Cold Spring Harbor Laboratory, New York.

Visconti, N., and M. Delbrück, 1953 The mechanism of genetic recombination in phage. Genetics **38:** 5–33.

Watson, J. D., and F. H. C. Crick, 1953 A structure for deoxyribonucleic acid. Nature **171:** 737–738.

Werner, R., 1968 Initiation and propagation of growing points in the DNA of phage T4. Cold Spring Harbor Symp. Quant. Biol. **33:** 501–507.