Reflections
A PAPER IN A SERIES COMMISSIONED TO CELEBRATE THE CENTENARY OF THE JBC IN 2005

JBC Centennial
1905–2005
100 Years of Biochemistry and Molecular Biology

Early Steps on the DNA Ladder—A Recollection

Robert Sinsheimer
From the Department of Molecular, Cell, and Developmental Biology, University of California, Santa Barbara, California 93106-9610

I have participated in and observed more than 60 years of the “golden age” of biology. What has most impressed me is the cumulative nature of this enterprise: discovery producing discovery producing new techniques producing further discovery, in ever cumulative progression. Our prizes and awards recognize landmarks in this process but most significant is the ever continuing and broadening stream of advances.

Pre-war Years: 1937–1942

In my undergraduate years at MIT, immediately prior to World War II, I enrolled in the newly reformulated program in quantitative biology. Two concepts seized my imagination: viruses and genes. Viruses seemed to occupy some nebulous realm between living and non-living. Genes seemed to be the essential key to biology, much as the atom was to chemistry and the atomic nucleus was to the physics of the day.

To an MIT student, steeped in physics, chemistry, and mathematics, the then intangible nature of the gene seemed a tantalizing problem. By default, genes were hypothesized to be proteins because no other substances seemed to afford the requisite complexity and variety. However, as yet no one knew the complete composition of any one protein, much less its three-dimensional structure.

On the other hand, one of the few clues to the physical nature of the gene (the ultraviolet action spectrum for the induction of mutations) paralleled the absorption spectrum of the nucleic acids (1, 2).

Graduate School Years: 1946–1949

When I returned to biology at the end of the War, two papers from the wartime years stood out for me. The demonstration by Avery, McLeod, and McCarty (3) that the transforming principle is DNA was dramatic, and the brilliant experiments of Beadle and Tatum (4) demonstrated that genes could specify the structure of enzymes (known to be proteins), thereby linking genes directly to the metabolism of the cell.

My graduate mentor was John Loofbourow (5), a physicist with a background in spectroscopy, who had turned his attention to biophysical problems. I learned much from him, including a tradition of venturesome self-reliance.

Iowa State Years: 1949–1957

In my first faculty post at Iowa State, I set out to learn as much as I could about DNA. At Iowa State as a biophysicist in a physics department I was quite isolated, but indeed almost all of the few DNA researchers of the time were isolated. (Isolation was not all bad; it provided time to develop one’s own ideas and to work at a steady pace.) In 1950, the first Gordon Conference on Proteins and Nucleic Acids drew only some 78 participants from the United States and abroad.
The summer Gordon Conferences (held in New Hampshire) and the annual FASEB meetings provided the few opportunities to counter the isolation of Iowa. At the summer conferences I met and conferred with, among others, Paul Doty, Rollin Hotchkiss, John Edsall, Erwin Chargaff, and Maurice Wilkins and, later, James Watson and Francis Crick and (as proteins were then included) Fred Sanger, John Kendrew, Max Perutz, and Aaron Klug (Fig. 1, a and b). The FASEB meetings provided contact with the biochemists including Arthur Kornberg, Paul Berg, Stanford Moore, William Stein, and Paul Zamecnik.

The extant techniques of DNA research were primitive and no ancillary industry existed, as today, to provide components and instruments.

Having very little funds, I took advantage of my location as a biophysicist in the physics department to make use of its excellent machine shop and its sophisticated research equipment. We designed and built a time-controlled fraction collector for column fractionations and a versatile light-scattering instrument for determination of molecular weight and size.

To study the basic components of DNA (the deoxyribonucleotides) I needed to prepare DNA and to devise means to digest it quantitatively. We prepared calf thymus DNA (6) from thymus glands obtained directly from the slaughterhouse in Des Moines. To digest it completely (which had never been done) we used pancreatic deoxyribonuclease (commercially available) and venom phosphodiesterase (for which we devised a method of purification from snake venom (7)). The deoxyribonucleotides were then fractionated quantitatively on an ion exchange column (8) as was pioneered by Waldo Cohn (9). This result in turn permitted us to establish the first spectrophotometric standards for the deoxyribonucleotides.

We obtained infrared spectra of the nucleotides in H₂O and D₂O to determine that they were in the amino and keto forms in solution (10). Seeking to attack the mutation question, we irradiated the deoxyribonucleotides with ultraviolet light and followed the changes in UV absorption and column behavior (11, 12).

We also observed that the deoxyribonuclease digestion alone cleaved just 25% of the phosphodiester bonds. Column fractionation revealed that about 1% of the product was
mononucleotides and about 16% was mixed dinucleotides (13, 14). We were able to column fractionate these into the various possible combinations. We noted that methylcytosine occurred in dinucleotides only in combination with guanine.

 Chargaff's data (15) on the molar equalities of A and T and of G and C (plus mC) in DNA were becoming available. Our dinucleotides proved that these equalities were not the result of any simple sequential pairing of A and T or G and C.

To proceed further in nucleic acid research I needed to be able to study function in a biological system. For this I turned to my other long standing interest, viruses. For an RNA virus I used tobacco mosaic virus, which I could grow in the University's greenhouses (to the dismay of the botanists). We grew tobacco mosaic virus in tobacco and purified it, and from this we prepared its RNA. By light scattering we demonstrated that the RNA of the virus comprised one molecule of molecular weight of about 2 million (16, 17). Subsequently we demonstrated that the infectivity of the RNA was associated exclusively with the 2 million molecular weight molecule (18, 19).

For a DNA virus, bacteriophage was the obvious choice. Thanks to Max Delbruck, I was able to spend 6 months at Caltech in the first half of 1953. This was of course the time period of the breakthrough in DNA structure analysis with, first, Pauling's failed proposal of a three-stranded helix (20) and then the Watson-Crick double helix (21). The latter was first known from a letter to Delbruck that spring and then at the Cold Spring Harbor Symposium in June.

At Caltech one met legends like George Beadle and Henry Sturtevant and Linus Pauling and legends to be like Ed Lewis and visitors such as Francois Jacob and Andre Lwoff.

Back at Iowa State, I broadened my DNA studies to include (for comparison) DNA from wheat germ and the phage DNAs. We discovered the glucosylation of cytosine (22) in the DNA of the T-even phages (variant in different strains (23)). However, I focused most of my attention on a small bacteriophage discovered earlier in Paris, φX174 (24). I reasoned that a
small phage would have less nucleic acid and thus be more amenable to analysis without fragmentation by the techniques then available and as well would have fewer genes, thereby easing the dissection of its functions.

As with any new virus we had to work out the conditions for growing it and storing it and purifying the particles. Fortunately we had by then acquired an ultracentrifuge (courtesy of the National Science Foundation).

Using the electron microscope in the department, I obtained pictures of the purified virus showing it to be a particle ~25 nm in diameter. Light scattering provided a particle weight of about 6.2 million for the virus and confirmed the particle diameter. This particle weight together with the phosphorous content suggested a weight of 1.7 million for the DNA, but the sedimentation constant of the DNA (23.8 S) was anomalous for that molecular weight.

Caltech Years: 1957–1977

At this time (1957) I moved to a faculty position at Caltech. Once re-established there, I quickly confirmed (by light scattering) the molecular weight of the DNA to be 1.7 million (about 5400 nucleotides). Then, using the techniques we had earlier developed, we established that the nucleotide composition did not fit the Chargaff ratios. The DNA was single-stranded and dX was the first of a new class of viruses (25, 26).

We went on to demonstrate that the DNA was infective (27) and that it was circular (28, 29, 30), to learn how it was replicated (31), and to ascertain its genetic map (32). In collaboration with Arthur Kornberg we demonstrated that his purified DNA polymerase could successfully replicate the DNA and produce synthetic infective molecules (33). Subsequently, Fred Sanger used dX DNA to produce the first complete genome sequence (34).

In 1959 the Journal of Molecular Biology began publication, and in 1960, I chaired the Gordon Conference on Proteins and Nucleic Acids. It was the last year the two fields would meet together.

With the double helix and DNA polymerase (35), with Sanger’s sequence of insulin (36), with Kendrew’s X-ray diffraction-derived structure of myoglobin (37), and Perutz’s structure for hemoglobin (38) molecular biology had arrived. The unending wave of progress had been launched. How far we have come!

Address correspondence to: sinsheim@lifesci.ucsb.edu.

REFERENCES

1. Stadler, L. J., and Uber, F. M. (1942) Genetic effects of ultraviolet radiation in maize. IV Comparison of monochromatic radiations. Genetics 27, 84–118
2. Hollaender, A., and Emmons, C. W. (1941) Wavelength dependence of mutation production in ultraviolet with special emphasis on fungi. Cold Spring Harbor Symp. Quant. Biol. 9, 179–186
3. Avery, O. T., McLeod, C. M., and McCarty, M. (1944) Studies on the chemical nature of the substance inducing transformation of pneumococcal types. J. Exp. Med. 79, 137–158
4. Beadle, G. W., and Tatum, E. L. (1941) Genetic control of biochemical reactions in Neurospora. Proc. Natl. Acad. Sci. U. S. A. 27, 499–506
5. Loofbourow, J. R. (1940) Borderland problems in biology and physics. Rev. Mod. Phys. 12, 267–358
6. Mirsky, A. E., and Pollister, A. W. (1946) Chromosin, a desoxyribose nucleoprotein complex of the cell nucleus. J. Gen. Physiol. 30, 117–148
7. Sinsheimer, R. L., and Koerner, J. F. (1952) A purification of venom phosphodiesterase. J. Biol. Chem. 198, 293–296
8. Sinsheimer, R. L., and Koerner, J. F. (1951) Ion exchange separation of deoxyribonucleotides. Science 114, 42–43
9. Cohn, W. E. (1950) The anion-exchange separation of ribonucleotides. J. Am. Chem. Soc. 72, 1471–1478
10. Sinsheimer, R. L., Nutter, R. L., and Hopkins, G. R. (1955) Infrared absorption spectra of pyrimidine nucleotides used to replicate the DNA and produce synthetic infective molecules (33). Subsequently, Fred Sanger used dX DNA to produce the first complete genome sequence (34).

In 1959 the Journal of Molecular Biology began publication, and in 1960, I chaired the Gordon Conference on Proteins and Nucleic Acids. It was the last year the two fields would meet together.

With the double helix and DNA polymerase (35), with Sanger’s sequence of insulin (36), with Kendrew’s X-ray diffraction-derived structure of myoglobin (37), and Perutz’s structure for hemoglobin (38) molecular biology had arrived. The unending wave of progress had been launched. How far we have come!

Address correspondence to: sinsheim@lifesci.ucsb.edu.

REFERENCES

1. Stadler, L. J., and Uber, F. M. (1942) Genetic effects of ultraviolet radiation in maize. IV Comparison of monochromatic radiations. Genetics 27, 84–118
2. Hollaender, A., and Emmons, C. W. (1941) Wavelength dependence of mutation production in ultraviolet with special emphasis on fungi. Cold Spring Harbor Symp. Quant. Biol. 9, 179–186
3. Avery, O. T., McLeod, C. M., and McCarty, M. (1944) Studies on the chemical nature of the substance inducing transformation of pneumococcal types. J. Exp. Med. 79, 137–158
4. Beadle, G. W., and Tatum, E. L. (1941) Genetic control of biochemical reactions in Neurospora. Proc. Natl. Acad. Sci. U. S. A. 27, 499–506
5. Loofbourow, J. R. (1940) Borderland problems in biology and physics. Rev. Mod. Phys. 12, 267–358
6. Mirsky, A. E., and Pollister, A. W. (1946) Chromosin, a desoxyribose nucleoprotein complex of the cell nucleus. J. Gen. Physiol. 30, 117–148
7. Sinsheimer, R. L., and Koerner, J. F. (1952) A purification of venom phosphodiesterase. J. Biol. Chem. 198, 293–296
8. Sinsheimer, R. L., and Koerner, J. F. (1951) Ion exchange separation of deoxyribonucleotides. Science 114, 42–43
9. Cohn, W. E. (1950) The anion-exchange separation of ribonucleotides. J. Am. Chem. Soc. 72, 1471–1478
10. Sinsheimer, R. L., Nutter, R. L., and Hopkins, G. R. (1955) Infrared absorption spectra of pyrimidine nucleotides in H₂O and D₂O solution. Biochim. Biophys. Acta 18, 13–27
11. Sinsheimer, R. L. (1954) The photochemistry of uridylic acid. Radiat. Res. 1, 505–513
12. Sinsheimer, R. L. (1955) The photochemistry of cytidylic acid. Radiat. Res. 6, 121–125
13. Sinsheimer, R. L., and Koerner, J. F. (1952) Di-desoxyribonucleotides. J. Am. Chem. Soc. 74, 283
14. Sinsheimer, R. L. (1954) The action of pancreatic desoxyribonuclease I. Isolation of mono- and dinucleotides. J. Biol. Chem. 206, 445–459
15. Chargaff, E. (1955) Isolation and composition of the deoxypentose nucleic acids and of the corresponding nucleoproteins. In The Nucleic Acids (Chargaff, E., and Davidson, J. N., eds) Vol. I, pp. 307–371, Academic Press, New York
16. Northrop, T. G., and Sinsheimer, R. L. (1954) Light scattering by tobacco mosaic virus nucleic acid. J. Chem. Phys. 22, 705–707
17. Hopkins, G. R., and Sinsheimer, R. L. (1955) Visible and ultraviolet light scattering by tobacco mosaic virus nucleic acid. Biochim. Biophys. Acta 17, 470–484
18. Cheo, P. C., Friesen, B. S., and Sinsheimer, R. L. (1959) Biophysical studies of infectious ribonucleic acid from tobacco mosaic virus. Proc. Natl. Acad. Sci. U. S. A. 45, 305–313
19. Friesen, B. S., and Sinsheimer, R. L. (1959) Partition cell analysis of infective tobacco mosaic virus nucleic acid.
References: Early Steps on DNA Ladder

20. Pauling, L., and Corey, R. B. (1953) Proposed structure for the nucleic acids. Proc. Natl. Acad. Sci. U. S. A. 39, 84–97
21. Watson, J. D., and Crick, F. H. C. (1953) Molecular structure of nucleic acids. Nature 171, 737–738
22. Sinsheimer, R. L. (1954) Nucleotides from T2r bacteriophage. Science 120, 551–553
23. Sinsheimer, R. L. (1956) The glucose content of the deoxyribonucleic acids of certain bacteriophages. Proc. Natl. Acad. Sci. U. S. A. 42, 502–504
24. Sertic, V., and Bulgakov, N. (1935) Classification et identification des typhi-phages. Comptes Rendus de la Soc. de Biol. 119, 1270–1272
25. Sinsheimer, R. L. (1959) Sedimentation and properties of bacteriophage φX174. J. Mol. Biol. 1, 37–42
26. Sinsheimer, R. L. (1959) A single-stranded deoxyribonucleic acid from bacteriophage φX174. J. Mol. Biol. 1, 43–53
27. Guthrie, G. D., and Sinsheimer, R. L. (1960) Infection of protoplasts of Escherichia coli by subviral particles of bacteriophage φX174. J. Mol. Biol. 2, 297–305
28. Fiers, W., and Sinsheimer, R. L. (1962) The structure of the DNA of bacteriophage φX174 III. Ultracentrifugal evidence for a ring structure. J. Mol. Biol. 5, 424–434
29. Kleinschmidt, A. K., Burton, A., and Sinsheimer, R. L. (1963) Electron microscopy of the replicative form of the DNA of bacteriophage φX174. Science 142, 961
30. Freifelder, D., Kleinschmidt, A. K., and Sinsheimer, R. L. (1964) Electron microscopy of single-stranded DNA: circularity of DNA of bacteriophage φX174. Science 146, 254–255
31. Sinsheimer, R. L., Starman, B., Nagler, C., and Guthrie, S. (1962) The process of infection with bacteriophage φX174. I. Evidence for a "replicative form." J. Mol. Biol. 4, 142–160
32. Benbow, R. M., Hutchison, C. A., III, Fabricant, J. D., and Sinsheimer, R. L. (1971) The genetic map of bacteriophage φX174. J. Virol. 7, 549–558
33. Goulian, M., Kornberg, A., and Sinsheimer, R. L. (1967) Enzymatic synthesis of DNA XXIV. Synthesis of infectious phage φX174 DNA. Proc. Natl. Acad. Sci. U. S. A. 58, 2221–2228
34. Sanger, F., Air, G. M., Barrell, B. G., Brown, R. I., Coulson, A. R., Fiddes, J. C., Hutchison, C. A., III, Slocombe, P. M., and Smith, M. (1977) Nucleotide sequence of bacteriophage φX174 DNA. Nature 265, 687–695
35. Lehman, I. R., Zimmerman, S. B., Adler, J., Bessman, M. J., Simms, E. S., and Kornberg, A. (1958) Enzymatic synthesis of deoxyribonucleic acid. 5. Chemical composition of enzymatically synthesized deoxyribonucleic acid. Proc. Natl. Acad. Sci. U. S. A. 44, 1191–1196
36. Sanger, F. (1952) The arrangement of amino acids in proteins. Adv. Protein Chem. 7, 1–28
37. Kendrew, J. C., Dickerson, R. E., Strandberg, B. E., Hart, R. G., Davies, D. R., Phillips, D. C., and Shore, V. C. (1960) Structure of myoglobin: a three-dimensional Fourier synthesis at 2-Å resolution. Nature 185, 422–427
38. Cullis, A. F., Muirhead, H., Perutz, M. F., Rossman, M. G., and North, A. C. T. (1962) The structure of haemoglobin. IX. A three-dimensional Fourier synthesis at 5.5-Å resolution: description of the structure. Proc. R. Soc. Lond. A 265, 161–187
Early Steps on the DNA Ladder—A Recollection
Robert Sinsheimer

J. Biol. Chem. 2004, 279:40247-40251.
doi: 10.1074/jbc.X400004200 originally published online May 28, 2004

Access the most updated version of this article at doi: 10.1074/jbc.X400004200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 38 references, 18 of which can be accessed free at http://www.jbc.org/content/279/39/40247.full.html#ref-list-1