Explorations in the land of DNA and beyond

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Like many of my colleagues, I have liked science for as long as I can remember. Though not college educated, my parents were always encouraging, as were two uncles who knew a little chemistry. When I was still in grammar school, the basement of our house in Los Angeles became a sizeable laboratory where I built radios, spectroscopes and other instruments, purified radium and rare-earth elements from carnotite and monazite ores by fractional crystallization and ion-exchange chromatography, and synthesized for neighbors the recently discovered insecticide DDT.

The war, though far away, was nevertheless the focus of attention then, and it seemed wrong to spend high school vacations solely in recreational activities. So I took a job washing glassware at a vitamin factory and attended summer school classes to shorten the time required to earn a diploma and begin college. To my surprise, however, academic credits were not enough: California law required 3 full years of high school physical education. Then I learned of a college that took students without requiring the last 2 years of high school—the University of Chicago.

Upon entering the College of the University of Chicago in 1946, expecting to study chemistry and physics, I found that undergraduate programs in specialized subjects had been abolished in favor of a mandatory curriculum based on classical writings in the humanities and the social and natural sciences. I count this elementary education in what is uninformatively called the 'liberal arts' a piece of good fortune, even though it got me only a Ph.B. (bachelor of philosophy), no good at all for admission to a graduate school in science. After 3 years, I left Chicago and spent the next 6 months traveling around Europe, reading and discussing with friends what might lie ahead for America and the world. It was 1949—the devastation of the war was still evident and the Cold War was starting up.

The following year, with almost no undergraduate science credits, I entered the California Institute of Technology, a freshman all over again. I took Linus Pauling's general chemistry course and did a research project for him to determine the accessibility of the heme group of the hemoglobin of the marine worm *Urechis* to a series of increasingly large alkyl isocyanides that I synthesized. When at the end of the year I showed Pauling my only partly complete results, expecting a stern response, he beamed his broad smile and said that the important thing for a student to learn from such work was that it can take much longer to complete than expected.

I was dissatisfied with undergraduate life at Cal Tech. Except for general chemistry, the courses I took seemed based too much on memory and, after Chicago and half a year in Europe, the other students (all males and 4–5 years younger) seemed uninterested in the world outside. Eventually, after a year back at Chicago taking chemistry and math courses and a year at Berkeley as a physics graduate student, through great good luck I became Linus Pauling's last graduate student at Cal Tech. Initially, he suggested that for my dissertation research I should determine the structures of some tellurium minerals by means of X-ray diffraction. He may have been only half-serious, because he went on to caution me that some chemists working with tellurium compounds had acquired a horrible halitosis called 'tellurium breath,' isolating them from society and driving some of them to suicide. By then wondering how ordinary atoms could be put together to make self-replicating structures, I asked to be assigned instead a molecule composed of biologically more important atoms. So Pauling suggested another project that he must have had up his sleeve all along: the X-ray diffraction determination of the structure of *N*,*N*′-dimethylmalonamide. The idea was to test the prediction from his resonance theory that its two amide groups are planar, as of course they turned out to be.

I remember being disappointed at the time by Erwin Schrödinger's little 1945 book, *What Is Life?*[1]. A founder of quantum mechanics, Schrödinger had left his native Austria soon after the German occupation in 1938 and had become a professor at Trinity College, Dublin. After pointing out that heat motion would make assemblages of independent molecules too unstable to account for the stability of genes, he concluded that genes, or maybe even entire chromosomes, are huge molecules, the stability of which derives from their covalent bonds, and that their variety corresponds to their various stable isomeric states. Though couched in quantum-mechanical terms, the concept boiled down to what for chemists is a truism—that only covalent bonds can account for the stability of the hereditary substance. Yet coming from Schrödinger and written so gracefully, with a moving epilog on determinism and free will, it influenced some of the pioneers of molecular biology to enter the field.

Schrödinger addressed the question of the chemical stability of the genetic material but said nothing about how it might replicate. It was Pauling, in 1948, who grasped the answer, published at the University of Nottingham as the 21st Sir Jesse Boot Foundation Lecture entitled "Molecular Architecture and the Processes of Life":

> *In general, the use of a gene or virus as a template would lead to the formation of a molecule not with identical structure but with complementary structure. It might happen, of course, that a molecule could be at the same time identical with and complementary*

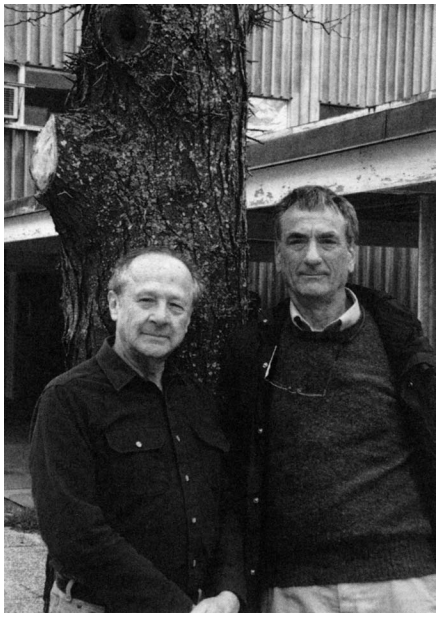

Figure 1 Matthew Meselson and Frank Stahl in 1996, standing at the place they met in 1954 at Woods Hole. From ref 2.

to the template on which it is moulded. However, this case seems to me to be too unlikely to be valid in general, except in the following way. If the structure that serves as a template (the gene or virus molecule) consists of, say, two parts, which are themselves complementary in structure, then each of these parts can serve as the mould for the production of a replica of the other part, and the complex of two complementary parts thus can serve as the mould for the production of duplicates of itself.

Pauling attributed the templating specificity involved in gene duplication, as well as in certain other manifestations of biological specificity, to detailed structural complementarity and the cooperative action of individually rather weak interactions, such as van der Waals attraction and hydrogen bonding. Later, it was learned that the great fidelity of gene duplication is achieved by the repeated application of such interactions in certain error-correction mechanisms.

Despite Pauling's insight requiring the gene to be made of two complementary parts, he proposed a three-strand structure for DNA (and RNA). From an X-ray diffraction measurement of the spacing between DNA molecules in a DNA fiber (assuming a hexagonal lattice), the fiber's density, and the nucleotide molecular weight (but making no allowance for the contribution of water), he calculated the distance per residue along the molecule to be almost exactly one-third of the 3.4-Å repeat seen in the diffraction pattern. He interpreted the result to mean that the molecule consisted of three chains coiled about one another. The calculation appears on a page dated 26 November 1952 in one of his research notebooks, followed by the notation, "Perhaps we have a triple-chain structure!" Of course, the three-chain structure he and Corey published three months later was wrong. Soon after receiving from James Watson and Francis Crick a prepublication copy of their April 1953 letter to *Nature* describing their double-helical model of DNA, Pauling wrote to his son Peter, "The structure seems to me to be a very interesting one and I have no strong argument against it. I do not think their arguments against our structure are strong ones, either." He went on to write that if the specimens from which his data came contained about 30% water, the DNA molecules in them would have only two chains.

My first knowledge of the great discovery of Watson and Crick came through an act of violence. Sometime in 1953, after the publication of the double helix, I went for the first time to talk with Max Delbrück. Immediately he asked what I thought of the papers Jim and Francis had published in *Nature* in April and May. When I replied that I had not heard of them, Max hurled a stack of reprints at me and shouted, "The most important development in biology in a decade and you don't know about it? Read these and don't come back until you have!" I took this as an invitation to come back. When I did, Max spoke of his difficulty in imagining that the two polynucleotide chains wound around each other could come apart during replication without breaking—an understandable dilemma, because DNA topoisomerases were then unknown. How this question led to the invention of equilibrium density-gradient centrifugation that formed the second half of my doctoral dissertation, and to the experiment that Frank Stahl and I then did using ^{15}N as a density label, demonstrating the semiconservative replication of DNA, is told in detail in a book by Frederick Lawrence Holmes (**Fig. 1**)[2–4].

Density-gradient centrifugation proved useful for attacking other fundamental problems posed by the double-helical structure of DNA. Jean Weigle and I used it in 1960 to show that genetically recombinant chromosomes of phage lambda contain segments of parental DNA consistent with a break-and-join model of recombination rather than with the template-switching model called copy choice[5]. Earlier that year, Cedric Davern and I showed by a density-transfer experi-

Figure 2 François Jacob, Max Delbrück, Matthew Meselson, Ronald Rolfe (partly hidden from view), Gunther Stent and Sydney Brenner in Pasadena, June 1960. From ref. 16.

ment that ribosomal RNA molecules of *Escherichia coli* are stable for many generations[6]. Achieving the necessary resolution for both of these experiments required greater density labeling than could be achieved with ^{15}N alone and became possible only with the availability of isotopically pure ^{13}C. This was made by isotope diffusion in Moscow specifically for me at the request of Pauling. It was essential also for the experiment conceived by Sydney Brenner, who came to Cal Tech with François Jacob to join with me for a few weeks in 1960 to test the messenger hypothesis of Jacob and Monod (**Fig. 2**).

It was known that T-even phage infection of *E. coli* brings about the synthesis of a short-lived RNA with base composition like that of the phage, shuts down most other RNA synthesis and diverts amino acid incorporation to the massive synthesis of phage proteins. The messenger hypothesis predicted that the short-lived phagelike RNA and the newly synthesized phage proteins would be found on ribosomes that were already present before phage infection. The protocol was to infect bacteria grown in ^{13}C ^{15}N heavy-isotope medium with phage T4, immediately transfer the culture to light-isotope medium containing ^{32}P or ^{35}S to label RNA or protein, and then determine the distribution of the radioisotopes after CsCl density-gradient centrifugation. The position of ribosomes was determined by their UV adsorption. François, suffering from World War II shrapnel wounds in his legs, kept overall watch on the proceedings. I mainly ran the centrifuge, and Sydney worked furiously not only on the messenger experiment but also on helping me late at night to score phage plaques from my lambda recombination experiments. The chief obstacle we had to overcome was the instability of ribosomes in the CsCl density gradients. Only in the last few days of their planned stay, after I had to leave on a trip east, did Sydney find that greatly increasing the magnesium ion concentration would stabilize the particles and make the experiment work. The newly made RNA (and protein, as later found by Sydney in Cambridge) were found to be associated with the heavy ribosomes, just as predicted[7]. The experiment I had done with Davern had left the possibility that ribosomal RNA could be informational, even though there was evidence that at least some informational RNA, particularly that for β-galactosidase, was short-lived. Finding phage RNA and phage protein on ribosomes that had been made before there was any phage information in the cell effectively ruled this out and confirmed the messenger hypothesis.

Upon moving to Harvard in 1961, I continued to study mechanisms of genetic recombination[8] and later worked on host-controlled restriction and modification of DNA and on DNA mismatch repair, leading to the prediction and then the demonstration, with Miro Radman, of methyl-directed DNA mismatch repair[9,10]. In attempting to isolate an activity responsible for DNA restriction in *E. coli* K12, I discovered an ATP requirement for degradation of unmodified DNA in extracts of restricting cells. Attempts at purification led to loss of activity, however. At that point, Bob Yuan came to our lab and joined me in attempting purification of the enzyme. From unpublished observations of Bill Wood on the effect of methionine deprivation on restriction, we got the idea that methionine might also be required. By adding both ATP and methionine we could continue the purification, but the activity soon decreased again. At that point, Bob realized that *S*-adenosylmethyltransferase in the partially purified preparation might be making *S*-adenylmethionine (SAM) from methionine and ATP. At first, we thought that only SAM would be needed, but we soon found that SAM and ATP are both needed, allowing us to purify the endonuclease to homogeneity[11,12]. We discovered to our surprise that, even in the limit digest, not all lambda molecules are broken at the same sites. The argument followed from the sedimentation distribution of the digested lambda pieces. For example, although the size of some pieces was in the range of 40% of a lambda chromosome, they accounted for less than 40 mass % of the digest. This lack of site specificity, alas, made *Eco*K and the other type I endonucleases we studied useless for genetic engineering. We also found that the enzyme nicks before it makes a double-strand break and that hybrid DNA made by annealing modified and unmodified strands is neither nicked nor cleaved, meaning that the enzyme looks at both chains before deciding what to do, providing a basis for protecting a cell from its own restriction system. Werner Arber's Nobel account of his outstanding work on restriction and modification acknowledges our work on *Eco*K as having set the basis for his *in vitro* studies of these processes.

Sometime around 1970 I read an article that asked not how genetic recombination works but why it exists. There are many hypotheses but no general agreement about what makes sexual reproduction so nearly universal in animals and plants, and what causes the relatively early extinction of nearly all lineages that abandon it—a fundamental problem in biology. Although the genetic recombination that accompanies sexual reproduction can generate variants of increased fitness, it also can tear apart well-adapted gene combinations, and it imposes various other costs, including those of producing and maintaining males. A possible experimental approach to the problem is the study of rotifers of the class Bdelloidea, a highly unusual group of diminutive freshwater invertebrates of worldwide distribution comprising some 370 described species in which, despite much study, males, hermaphrodites and meiosis are unknown, and which has nevertheless persisted and successfully evolved for tens of millions of years. Bdelloid eggs are produced from oocytes by two mitotic divisions, without chromosome pairing and without reduction in chromosome number—each oocyte yielding one egg and two polar bodies. Since I first learned of these creatures in 1989, we have conducted a series of experiments to provide molecular genetic tests of their ancient asexuality and to explore what may have allowed them to dispense with sex without going extinct[13–15]. One of the several unusual characteristics we have discovered is their apparent lack of retrotransposons, mobile genetic elements that are found in nearly all of the numerous and diverse eukaryotes in which they have been sought. Such elements are generally deleterious, and one class of them, the non-LTR (non–long terminal repeat) retrotransposons, seems to be as ancient as meiosis itself. Transposable elements are capable of autonomous increase within the genomes they inhabit, but their number can be kept in check by mechanisms whose effectiveness increases disproportionately as their copy number increases. In sexual species, this can occur by purifying selection through ectopic crossing-over, leading to inviable aneuploidies, and by homologous recombination if the deleterious effects of insertions interact synergistically. Because asexual organisms lack both of these mechanisms and would also lack any other control mechanisms that depend on meiosis, we have wondered whether an important cause of the early extinction of asexual organisms and, correspondingly, of the relative advantage of sexual reproduction is the ability of sex to combat the unchecked increase of deleterious retrotransposons. If so, much evolutionary theory would need to be re-examined, providing new insights into many issues, among them the causes of extinction of asexual lineages and, perhaps, even that of some species that reproduce sexually.

1. Schrödinger, E. *What Is Life?* (Macmillan, New York, 1945).
2. Holmes, L.F. *Meselson, Stahl, and the Replication of DNA* (Yale University Press, New Haven, 2001).
3. Meselson, M., Stahl, F. & Vinograd, J. Equilibrium sedimentation of macromolecules in density gradients. *Proc. Natl. Acad. Sci. USA* **43**, 581–588 (1957).
4. Meselson, M. & Stahl, F. The replication of DNA in *E. coli*. *Proc. Natl. Acad. Sci. USA* **44**, 671–682 (1958).
5. Meselson, M. & Weigle, J. Chromosome breakage accompanying genetic recombination in bacteriophage. *Proc. Natl. Acad. Sci. USA* **47**, 857–868 (1961).
6. Davern, C.I. & Meselson, M. The molecular conservation of RNA during bacterial growth. *J. Mol. Biol.* **2**, 153–160 (1960).
7. Brenner, S., Jacob, F. & Meselson, M. An unstable intermediate carrying information from genes to ribosomes for protein synthesis. *Nature* **190**, 576–581 (1961).
8. Meselson, M. The molecular basis of genetic recombination in *Heritage from Mendel* (ed. Brink, R.A.) 81–104 (University of Wisconsin Press, Madison, 1967).
9. Wagner, R. & Meselson, M. Repair tracts in mismatch DNA heteroduplexes. *Proc. Natl. Acad. Sci. USA* **73**, 4135–4139 (1976).
10. Meselson, M. Methyl-directed repair of DNA mismatches in *The Recombination of Genetic Material* (ed. Low, K.B.) 91–113 (Academic Press, San Diego, 1988).
11. Meselson, M. & Yuan, R. DNA restriction enzyme from *E. coli*. *Nature*, **217**, 1110–1114 (1968).
12. Meselson, M., Yuan, R. & Heywood, J. Restriction and modification of DNA. *Annu. Rev. Biochem.* **41**, 447–466 (1972).
13. Mark Welch, D.B. & Meselson, M. Evidence for the evolution of Bdelloid rotifers without sexual reproduction or genetic exchange. *Science* **288**, 1211–1215 (2000).
14. Mark Welch, J.L., Mark Welch, D.B. & Meselson, M. Cytogenetic evidence for asexual evolution of Bdelloid rotifers. *Proc. Natl. Acad. Sci. USA* **101**, 1618–1621 (2004).
15. Arkhipova, I. & Meselson, M. Transposable elements in sexual and ancient asexual taxa. *Proc. Natl. Acad. Sci. USA* **97**, 14473–14477 (2000).
16. Judson, H.F. The Eighth Day of Creation (Cold Spring Harbor Laboratory, New York, 1996).

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