IN MEMORIAM

Recollections of Arthur Kornberg (1918–2007) and the beginning of the Stanford Biochemistry Department

Arthur Kornberg died on October 26, 2007. He was one of the most remarkable scientists of our time. His discovery of DNA polymerase I (Bessman et al. 1958; Lehman et al. 1958a) and his demonstration that it faithfully copies the base sequence of a template DNA strand (Lehman et al. 1958b) led to his being awarded the Nobel Prize immediately in 1959. Earlier, the prevailing view was that enzymes function in directing a cell's metabolism and in producing the energy a cell needs, while DNA synthesis is part of the mystery of life. In a Cold Spring Harbor Symposium paper on the structure of DNA (Watson and Crick 1953), the authors stated, "It is not obvious to us whether a special enzyme would be required to carry out the polymerization or whether the existing helical single chain could effectively act as an enzyme." Arthur's work with DNA polymerase I changed all this, placing self-replication of genes on an equal biochemical footing with energy metabolism and putting an end to vitalism. His discovery launched a new gold rush, pointing the way as scientists raced to find enzymes like RNA polymerase, which copies the sequence of a DNA strand into RNA, and restriction enzymes, which turned out to be specific DNA endonucleases.

Arthur was born on March 3, 1918. He came from a Jewish immigrant working-class family. His father had no formal education but could speak at least six languages. Arthur was precocious and graduated from the Abraham Lincoln High School in New York at age 15. When he

graduated from City College of New York at age 19, he received the best science degree in his class. In college, he worked evenings, weekends, and holidays selling men's clothing, and he saved enough to pay for the first two years of medical school at the University of Rochester.

Arthur has described his story and his accidental entry into research work in an *Annual Reviews* chapter (Kornberg 1989a). As a medical student, he was intrigued to find symptoms of a mild jaundice in himself, which proved to be Gilberts syndrome, a difficulty in metabolizing bilirubin. His paper describing a survey of people with this slight metabolic disorder was read by Rolla Dyer, the Director of the National Institutes of Health (NIH), and this led to his being recalled to the NIH from sea duty in the Public Health Service in 1942. Arthur's first research involved searching for missing nutritional factors in a synthetic diet fed to rats. He was converted to searching instead for enzymes while doing postdoctoral work in 1946 with Severo Ochoa at New York University. He became a passionate advocate of using enzymes to deconstruct how cells work. His creed was simple and positive: "If a cell can do it, then a biochemist can do it and I can do it." His book about his scientific career was named "For the Love of Enzymes" (Kornberg 1989b), and he wrote guidelines for beginners on the "Ten Commandments" of using enzymes to dissect biological processes (Kornberg 2000).

Arthur had a commanding presence; when he said something, people listened. I remember the story of Arthur speaking at a congressional hearing. After the hearing, one committee member was surprised to learn that another member had changed his vote and asked him why he did this. "I don't want to be called a fool by Arthur Kornberg" was the reply. Dan Koshland, a longtime friend of Arthur's, was speaking at an event Arthur had organized. He began by saying "I never say 'no' to Arthur Kornberg." On the other hand, Arthur would put you immediately at ease in a personal conversation. A friend remarked, "He made you feel his whole attention was focused on you."

One of Arthur's many remarkable abilities was department building. He left NIH in 1953 to become Chair of a new Department of Microbiology at Washington University, St. Louis, where he was once briefly a postdoctoral fellow with Carl and Gerty Cori. Arthur greatly admired them (Kornberg 2005), and the prospect of renewing contact was a strong attraction. One of his first major appointments was Melvin Cohn, who had spent several years at the Institut Pasteur, Paris, working with Jacques Monod on untangling the story of induced enzyme synthesis in *Escherichia coli*. Mel had a strong background in immunology from his early work with A.M. Pappenheimer Jr., and in St. Louis he began a study of antibody synthesis in single cells. The other members of the department in St. Louis who would later move to Stanford with Arthur were Paul Berg and Bob Lehman, who had been postdoctoral fellows with Arthur, and Dave Hogness and Dale Kaiser, who both came from the Institut Pasteur.

When Arthur was offered the Chair of Biochemistry at Stanford in 1958, he did not say yes but he did not say no. Instead, he replied, "I must return to St. Louis to consult my colleagues." The occasion for forming a new Biochemistry Department was the move of the Stanford Medical School from San Francisco to the main Stanford campus near Palo Alto. Stanford also appointed Joshua Lederberg as Chair of a new Genetics Department; his office at Stanford would be close to Arthur's. When the department at St. Louis moved to Stanford in June, 1959, I joined it as a physical biochemist, coming from the University of Wisconsin.

The new science of molecular biology was just coming into existence in 1959, and the level of excitement in our department was intense. The central dogma, DNA makes RNA makes proteins, was widely accepted, but many of the biochemical steps were mysteries. In St. Louis, Paul Berg had begun a study of the enzyme-aminoacyl adenylates that are intermediates in the synthesis of aminoacyl tRNAs (Berg 1961), and Paul continued to analyze steps in protein synthesis. Arthur's work on the enzymatic synthesis of DNA had shown the necessity of characterizing the nucleases of *E. coli*, the enzymes that degrade

DNA, and Bob Lehman had begun this work in St. Louis. Dave Hogness and Dale Kaiser had undertaken a study of bacteriophage lambda as a model for how a small genome goes through its life cycle. They were developing methods for making this study at the DNA and mRNA levels. Two postdoctoral fellows from Australia, Ross Inman and Gerry Wake, joined me in an effort to test whether the newly synthesized DNA made by Pol I remains base-paired with its template strand, by using DNA melting curves to distinguish double helices that contain 5-bromouracil in one strand.

Arthur thought carefully about details of organizing the department as well as settling it into the life of the medical school and the university. His plan was to create the ideal workplace. The research groups would cooperate with each other and nothing would interfere with research, although the department took pride in the teaching of its popular course in general biochemistry. Arthur sometimes discussed with Wallace Sterling, the President of Stanford, his ideas about how medical science should develop at Stanford. He twice invited President Sterling to an informal lunch with the Biochemistry faculty so that we would know him, too.

Here are some of the innovations Arthur made when the department started in 1959. Students and postdoctoral fellows were mixed together in common laboratories so that the different research groups would be familiar with each other's research work. Rare enzymes were shared, and major instruments were made available to everyone. Research grants were shared. Each faculty member was expected to bring in the amount of money spent by his group, but strict accounting was not required and there were no financial deadlines. The entire department attended Tuesday/Thursday noon seminars. At first, only faculty members and visiting scientists gave talks, but later postdoctoral fellows and senior students were added. The department admitted only four new students each year for a faculty of seven, and prospective students were screened rigorously. Many of the early students became well-known scientists. Group sizes were kept small. Faculty members worked in the laboratory themselves and taught students by the apprentice method. Faculty meetings were held only when there was something important to decide; Arthur made lesser decisions himself. Issues were decided at faculty meetings by consensus; once an issue had been discussed, there was no need to vote. How did all this work in practice? Fabulously, according to postdoctoral fellows who, when they started new jobs and left, wistfully recalled their days at Stanford.

Of the original seven faculty members, only one ever left. In 1959, I could sense tension between Arthur and Mel Cohn over how to tackle biological problems. Mel spoke enthusiastically about Gestalt biology, the view that one cannot understand a biological problem by

dissecting it into parts. Arthur advocated using chemistry, specifically enzymes, as the basic tool for solving biological problems. In 1962, Mel Cohn left Stanford for the newly formed Salk Institute, and in 1963–64, Lubert Stryer and George Stark joined our department. They soon were doing innovative experiments, especially developing new methods that attracted wide attention. Lubert's experiments on fluorescence energy transfer (Stryer and Haugland 1967) led to the development of FRET (fluorescence resonance energy transfer) as a major biophysical tool. George developed widely used methods for analyzing transcription and translation experiments on paper: the Northern method (Alwine et al. 1977) for mRNAs and the Western method (Renart et al. 1979) for proteins. Although both Lubert and George eventually left our department, they retained close ties. In 1971, Ron Davis joined the department and, like the original six faculty, never left Stanford. Ron brought with him electron microscopy of DNA, a powerful tool that soon was used widely in the department.

The early Stanford Biochemistry Department was a community where everyone shared and rejoiced in the discoveries made by others. In 1959, the whole department would gather at Arthur's home one evening a month to listen to the latest findings of one research group. Within a few years, we could not all fit into Arthur's living room and we tried meeting at a room on the Stanford campus, but the atmosphere was not the same. Then, in 1972, we began twice-a-year meetings at Asilomar, lasting a few days, at which all research groups reported. In those days, there was always at least one surprising discovery at every meeting.

By 1970, solutions to the major problems of how DNA makes RNA makes proteins were clear at least in outline, and our faculty members were moving on to new problems. Arthur had found that DNA replication is a multi-enzyme problem even in simple viral systems, and he began to work out the detailed enzymatic mechanisms of DNA replication, first of phages M13 and ϕ x174, then of *E. coli* itself. Paul Berg undertook the study of animal viruses and soon was involved with problems of recombinant DNA. Bob Lehman began to study genetic recombination at the DNA level, using both enzymatic and genetic tools. Dave Hogness undertook the analysis of development in *Drosophila* at the DNA and mRNA levels by studying developmental mutants that make monsters with extra legs or wings. Dale Kaiser chose a bacterial system, *Myxococcus*, to work out a genetic analysis of development in a prokaryotic system. Even Arthur tested the waters of investigating development at the enzymatic level by using bacterial spores as a possible model system. However, his DNA replication work pushed spores aside. I began a search for structural intermediates in protein folding and the mechanism of folding.

A dramatic moment came in 1969 when John Cairns announced that he and his assistant had found a viable mutant of *E. coli* that lacks detectable activity of the enzyme DNA polymerase I (De Lucia and Cairns 1969). A search for other DNA polymerases soon followed. Amazingly, it was Arthur's son, Tom, working with Malcolm Gefter at Columbia, who found first DNA polymerase II and then DNA polymerase III, the enzyme that replicates the *E. coli* chromosome. The story is summarized by Arthur in his scientific memoir (Kornberg 1989a). Tom was a music student at Juilliard with a promising career as a cellist ahead of him. At the time when Cairns's mutant became public knowledge, Tom had suffered an injury to his hand that interfered with his cello playing. Without formal training in biochemistry, Tom joined Gefter's laboratory and succeeded where others were having little luck. Another dramatic moment came in 2006 when Arthur's oldest son, Roger, received the Nobel Prize in Chemistry for his work on the molecular basis of eukaryotic transcription (Kornberg 2007). Arthur's third son, Ken, is an architect known for his designs of scientific laboratories.

Here are two examples of research that took off because of Arthur's policy of mixing research groups in common laboratories. In 1968, Immo Scheffler, a student in my group who was analyzing DNA helix-coil transitions in hairpin helices, shared a lab with Toto Olivera, a postdoctoral fellow in Bob Lehman's group who was examining the enzymatic properties of *E. coli* DNA ligase. Toto and Immo found that ligase will close $d(TA)_n$ oligonucleotides into single-strand circular molecules (Olivera et al. 1968) if they are large enough ($n = 16$ or greater). Then, Immo and Elliot Elson, who was working with Immo on the analysis of the hairpin melting curves, found they could use the circular oligonucleotides, which make double hairpin helices with a four-base loop at each end, to obtain detailed information about the role of small loops in DNA melting (Scheffler et al. 1970). A second example is provided by Doug Vollrath, a student in Ron Davis's group who in 1986 shared a laboratory with Gil Chu, a postdoctoral fellow in Paul Berg's group. Gil has a Ph.D. in physics from the Massachusetts Institute of Technology. Doug was trying to obtain better-resolved agarose gel patterns for giant DNA molecules by using the new technique of applying an alternating electric field, which takes advantage of the strong dependence of the DNA reorientation time on molecular weight. Gil realized he could address the problem of getting straight, regular DNA bands that are well resolved by solving equations from basic electrical theory. His solution requires multiple electrodes whose voltages can be individually controlled. The result was beautifully resolved DNA band patterns (Chu et al. 1986).

As an example of how the policy of sharing enzymes worked, Dale Kaiser recalls Arthur's gift in 1965 of two

purified enzymes that made possible the work from Dale's laboratory on the cohesive ends of phage lambda DNA (Strack and Kaiser 1965). The two enzymes were *E. coli* exo III, which degrades DNA strands from the 3' end, and *E. coli* DNA Pol I, which copies the base sequence of a template strand by synthesizing DNA at the 3' end of a primer strand. The high specificities of the two enzymes are important in interpreting the results. Strack and Kaiser had available an infectivity assay that measures the ability of purified lambda DNA to produce phage in *E. coli* when inactive helper phage is also added. It was known that purified lambda DNA has cohesive sites (Hershey et al. 1963) that function in forming DNA dimers and trimers. Strack and Kaiser found that both exo III and Pol I inactivate lambda DNA, as tested by the infectivity assay, and inner genetic markers are lost after enzyme treatment at the same rate as outer markers, indicating that each of the two enzymes inactivates lambda DNA in an all-or-none process. Their results fit a model in which dangling single-strand ends protrude at each end of double-stranded lambda DNA and provide the cohesive sites found by Hershey and coworkers (1963). Exo III degrades progressively from the 3' end while pol I uses the dangling 5' end as a template to extend the 3' end until only double-stranded DNA remains, which prevents the lambda DNA from circularizing.

In 1990, at age 72, Arthur started a new research field, the enzymatic synthesis and degradation of polyphosphate, which involved investigating the biological functions of polyphosphate. Arthur and his late wife, Sylvie, had discovered polyphosphate synthesis in *E. coli* back in 1956 (Kornberg et al. 1956). Polyphosphate contains high-energy phosphate bonds and can be used to make ATP from AMP. Thus, polyphosphate is a storage form of reserve energy for bacterial cells and can be expected to play a vital role in the life cycle of bacteria. Arthur found numerous cases in which this is true (Kornberg 1999). Notably, in pathogenic bacteria polyphosphate metabolism is commonly required for virulence.

Arthur was proud of the scientific achievements of all the research groups in our department, and he took a personal interest in everyone who passed through. Many of the graduates are keenly aware of Arthur's role in making the department a great place to do research. For my part, I know deep down it was the Stanford environment that attracted first-rate people to my group, and it was Arthur who was chiefly responsible.

When Arthur died of respiratory failure in October, he was 89 years old, and he had been ill for about one week. Earlier, he was actively directing research on polyphosphate. The Stanford Biochemistry Department remembered Arthur with a 4-hour "teach-in." Students and

faculty selected favorite papers from Arthur's list of 463 publications and gave short 5- to 10-minute summaries. Arthur would have liked that.

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