I’ll Have the Chopped Liver Please, or How I Learned To Love the Clone

A recollection of some of the events surrounding one of the pivotal experiments that opened the era of DNA cloning

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In February 1973, Herb Boyer from the University of California, San Francisco, called me to say the initial “cloning” experiments had worked. Indeed, the simple plasmid splicing experiment between pSC101 and RSF-1010 was barely noticed in light of their current massive success in cloning eukaryotic DNA sequences. In any event, we agreed that Maggie So from my lab would visit both Boyer’s lab and Stanley Cohen’s laboratory at Stanford University, Stanford, Calif., the following spring to try our hand at identifying the bacterial genes involved in enterotoxin production in enterotoxin (ENT) plasmids. Subsequently, S., with help from Boyer and Mary Betlach, did clone the first virulence determinant of a bacterium, the heat-stable enterotoxin of *Escherichia coli*. This was the final push I needed to begin to abandon 15 years of work on plasmid biology and to return to my first love—bacterial pathogenicity. We subsequently cloned a number of virulence determinants from a variety of pathogenic bacteria.

What was the genesis of this scientific achievement? In November 1972 I went to Honolulu for the first time. Two years earlier I visited Seattle and took a position at the University of Washington, but before that I had never been west of the Mississippi. When I arrived in Honolulu in the early afternoon, I ran into Boyer. We had first met when he was a graduate student with Ellis Englesberg at the University of Pittsburgh, Pa. Boyer was hard at work trying to decipher the biochemical basis of restriction modification. He became interested in this subject because Englesberg’s beloved ara locus was linked to the restriction modification locus in *E. coli* K-12 and *E. coli* B. Our friendship had been cemented as much from a common scientific interest, as well as from a common love of cats. Boyer and his wife Gracie owned two seal point Siamese, cats named Watson and Crick (guess which one had the crooked tail).

Boyer and I spent several hours on the beach drinking Blue Hawaiians and talking about the focus of the meeting, R-factors (actually, the new term was R-plasmids). After dinner, we sat in the lobby again contemplating yet another Blue Hawaiian when Cohen appeared. I had known Cohen since 1966 when we met at the first international meeting on R-factors held at Georgetown University, Washington, D.C., under the auspices of the U.S. Food and Drug Administration. Within minutes, Charlie Brinton and his wife Ginger also joined our threesome. Brinton was the “father” of bacterial pili; he was a professor of biophysics at the University of Pittsburgh, and, of course, he knew Boyer. I knew Brinton through my mentor, Lou Baron, at Walter Reed. Brinton, Baron, and I had published several papers together mapping the chromosomal location of the pil locus, next to the ara region. Brinton was a big man with a prodigious appetite. He had determined that the dining room was closed and invited us to join him in search of a snack.

The R-plasmid field was just becoming a major scientific arena and had caught the attention of the medical field, as well as the biomedical field of research. Plasmids were a hot topic in 1972. Plasmids! The name had been coined by Joshua Lederberg to
describe the ephemeral F-factor of *E. coli* K-12. R-factors, discovered in Japan in 1958, were transmissible extrachromosomal elements but had never been observed to integrate into the host chromosome like F-factor. The existence of R-plasmids, as they eventually became called, was introduced to the Western world by Tsutomu Watanabe in a review article published in 1961 in *Bacteriological Reviews*. The presence of R-plasmids in the Western world was discovered shortly thereafter by Naomi Datta in England, Gerhard Lebek in Germany, and by David H. Smith, Ed Hook, and Fred Gill in the United States. The F factor was and is a marvel to the world of basic science. Transmissible sex!

R-plasmids had caused a revolution in medicine. Transmissible antibiotic resistance was one of the first concrete examples where the burgeoning fields of molecular biology and microbial genetics could actually contribute to understanding the treatment of infectious diseases in humans and how bacteria become resistant to therapy. At one level, we understood that R-plasmids are composed of DNA, are relatively small in relationship to the bacterial chromosome, and encode multiple antibiotic resistance. However, in another context we knew relatively little about the biology of plasmids; what we knew was descriptive. We lacked the biochemical knowledge to understand how these extrachromosomal elements are transferred from cell to cell, how they replicate in a wide variety of bacterial species, and how they mediate antibiotic resistance. At the experimental level, there was a good deal of frustration. The resolving power of bacterial genetics and what passed for molecular biology at the time did not provide enough information, nor did there appear to be a major breakthrough in the offing.

With all of this to talk about, Boyer, Cohen, the Brintons, and I set out to find something to eat on a Sunday night in Waikiki. We walked down a dark, quiet road and found a commercial area where, on a corner, a bright sign an-
Further Thoughts

Boyer's and my laboratory enjoyed several years of fruitful collaboration. Perhaps the most significant work we did together was the construction of the pBR series of plasmids as cloning vectors that was spearheaded by Francisco Bolivar in Boyer's lab and Jorge Croza from my lab. During the course of our construction of the pBR plasmids, a serendipitous observation led to the development of the agarose gel electrophoresis method for the characterization of plasmid DNA and, subsequently, to the use of this method for molecular epidemiology.

I suppose it was equally serendipitous that the plasmid nomenclature committee established during the Hawaiian meeting went on to become the plasmid working group that reported to the historic Asilomar meeting on the potential biohazards of recombinant DNA cloning. Because of our role on this committee, Roy Curtiss, Don Helinski, and I asked to be part of the first NIH recombinant DNA committee. The deliberations of the plasmid working group subcommittee and its recommendations became the foundation for the first version of NIH recombinant DNA guidelines. But that is also another story, and one recently reported by Don Fredrickson, former director of NIH.

There is no doubt a practical method for cloning DNA would have been forthcoming whether or not there had been a plasmid meeting in Hawaii. Yet, a continuing theme in science is that it takes a combination of observation and good luck to bring the right concurrence of ideas and people together. I have felt over the years that this occurred on a Sunday evening in Honolulu. Perhaps I put more emphasis on it because I was a participant (at least in a limited way) in this event. However, I think individuals almost 30 years later who may occasionally wonder how it happened can profit from the lesson of this story of scientific interchange and collaboration, and historians might like the recollections of an eyewitness to scientific history.

pable of autonomous replication and carry antibiotic resistance genes. Yet, we had never successfully been able to transfer the smaller molecular species as an autonomous element from Proteus. Thus, our observation that R-plasmids might be cotransferred was all theoretical and based on tracings of pictures taken by a Model E ultracentrifuge of DNA at equilibrium in a CsCl density gradient. However, within the past year there was considerable excitement generated by the description of naturally occurring, small self-replicating R-plasmids in Salmonella by E. S. Anderson and by Roy Clayes, who was scheduled to present at the meeting the next day. Cohen and Chang's transformation experiment to isolate pSC101 surely came as a surprise, but their results provided direct evidence that R-plasmids are indeed composed of cotransferred replicons that are capable of being present as composites in a large transmissible element or dissociating as a much smaller nontransmissible element and a separate conjugative element.

When my turn came to speak, I described how Pat Guerry and I had worked with Bob Hedges and Datta on the DNA homology among plasmids of different incompatibility groups. We were also beginning to focus on the transmissible enterotoxin plasmids described by H. Williams Smith that our plasmid hybridization studies showed were "F-like" sex factors. In parallel with Jan Van Embden, we also had come upon small, nontransmissible R-plasmids. In particular, we had worked with one encoding sulfonamide-streptomycin resistance. This plasmid was known as Sex (for extrachromosomal streptomycin resistance) but eventually became known as RSF1010.

Boyer's laboratory recently had discovered a restriction enzyme, EcoRI. Previously, he had spent a good deal of effort on a restriction enzyme EcoRII, which appeared to give numerous cuts, seemingly at random, in DNA. EcoRII had been discovered in 1971 in Boyer's laboratory by Bob Yakamori as an R-plasmid-encoded restriction enzyme, but unlike EcoRI, showed only a limited pattern of cleavage in nucleic acid from a number of diverse sources. Boyer had solicited plasmid DNA from pSC101 from Cohen and RSF1010 from my laboratory as one of
the means of investigating the specificity of the enzymatic cleavage properties of the EcoRI enzyme.

In the deli Boyer described to all a finding that he had revealed to me privately that afternoon: EcoRI cleaved pSC101 and RSF1010 only once along the entire chromosome.

Our discussions revolved around whether an enzyme like EcoRI could be responsible for recombining plasmids of different incompatibility groups with one another or was responsible somehow for the distribution of antibiotic-resistance markers among plasmids. Understand that at this time we were unaware of genetic transposition of antibiotic resistance genes. This was not discovered until several years later by Datta, Alan Jacobs, and Bob Hedges.

However, as Boyer continued to sing the praises of EcoRI, there came a point in the conversation when he reiterated that pSC101 could be cut only once by the enzyme and that this also was true of RSF1010. In what seemed an unconnected thought, Boyer then repeated Janet Merz’s observation about “sticky” DNA ends and some of the work that Peter Lobund was doing with P22 phage in Dale Kaiser’s lab to form enzymatic joining of DNA molecules. The conversation to that point had been animated and often consisted of four people talking simultaneously. However, the juxtaposition of the facts about EcoRI, its behavior on small plasmids, that common “sticky ends” were formed by EcoRI cleavage, and that plasmid DNA could now be easily genetically transformed caused a silence to fall over the table. It was much too obvious to slip by unnoticed.

Cohen said in a slow, clear voice, “That means...” Boyer didn’t let him finish, “That’s right, it should be possible.” Sometimes in science, as in the rest of life, it is not necessary to finish a sentence or thought. The experiment was straightforward enough. Mix EcoRI-cut pSC101 and RSF1010, heat and anneal the two, and there should be a proportion of recombiant plasmid molecules formed that could be isolated by Cohen and Chang’s transformation method. However, the larger implications of the work were not lost on us that evening. The excitement was palpable, and the idea of isolating DNA fragments randomly cleaved with EcoRI was quickly obvious. The idea of joining distinct DNA species had been at the cutting edge of molecular biology and was, in fact, the focus of Berg’s group, as well as those of Kaiser and Lobund, but here was a direct way to do the experiment.

On the way back to the hotel that night we discussed the experiment and its ramifications should it succeed. I think it fair to say that we all felt there was a high likelihood this simple idea was going to work.

In retrospect, it is extraordinary that we didn’t discuss this set of experiments again for the next two days. The meeting began the next morning, and our time was taken fully with the realities of the moment, rather than with experiments of the future. On the last day of the meeting, a Wednesday morning, there was a long discussion about what to do about plasmid taxonomy. Someone asked, “Just how many plasmids with the name R1 are we willing to tolerate in the literature? How should plasmids be named?” Richard Novick and Roy Curtiss, in particular, took a lead in these discussions. There was near-unanimous consent (I actually dissented) that a committee should be formed to consider a uniform nomenclature for plasmids and episomes. Novick was appointed the chair, together with Cohen, Curtiss, Clowes, Datta, Donald Helsinki, and me, as punishment for my dissent. The meeting adjourned on that note.

Boyer and I saw each other that evening. He had met briefly with Cohen and they had discussed the logistics of the experiments they planned to perform together upon their return to the Bay Area. Boyer and I agreed to meet the next morning and travel to the airport together. We then spent the next hour or so sitting in a quiet spot discussing the splicing experiment and its implications. He asked what role I wanted to play in the planned work to splice together pSC101 and RSF1010. It seemed to me that all I had to offer was some purified plasmid DNA from RSF1010 and this was not a major contribution that required coauthorship. However, by now I anticipated that by cleaving larger
plasmid DNA with EcoRI, we might be able to focus on individual fragments that contained genes of interest.

At that moment, I was not thinking about cleaving large bacterial chromosomes of interest or even considering eukaryotic DNA but rather about analyzing plasmid DNA. I was most interested in the plasmids encoding enterotoxin described by H. Williams Smith in pigs and calves, and we had just showed that plasmids isolated from E. coli from cases of human traveler’s diarrhea were very similar. Thus, as Boyer and I left for the Honolulu airport, we agreed he would call me if the gene splicing experiments worked, and we would try to extend the work to understand the nature of the enterotoxin genes carried by the Ent plasmids.

The papers by Cohen and Boyer in the following year (1973) are widely viewed as among the most important scientific contributions of the 20th century. They are seminal papers in the discovery of gene cloning. The parallel work of Paul Berg, Dale Kaiser, Janet Mertz, Peter Lobbund, Ron Davis, and many others outside the UCSF-Stanford conglomerate has been widely recognized. Prizes have been awarded, although Cohen and Boyer did not receive the Nobel Prize for their contribution. However, in my view, Cohen and Boyer performed the most clear-cut gene splicing experiments and the most convincing. They reduced it to practice, and it is essentially the cloning method we use today in the laboratory.

On the way home from Hawaii, I mulled over what the consequences would be if the experiments that Cohen and Boyer planned to do were successful. I knew that if these experiments worked, the science I practiced would never be the same. I had a similar realization when I first viewed the DNA sequencing facilities at The Institute for Genomic Research, but that is another story.

SUGGESTED READING