



Perspective

The early history of the genetics of photosynthetic bacteria: a personal account

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Abstract

The development of genetics as a tool for the study of photosynthesis is recounted, beginning in the period when no genetic exchange mechanism was known for any photosynthetic microorganism, and ending with the sequencing of the key genes for photosynthesis.

The challenge

I first heard about photosynthetic bacteria from Sam Kaplan. I was nearing the end of my thesis research at Western Reserve University with Harriet Ephrussi-Taylor and looking for a postdoctoral position. Sam had just joined the Western Reserve faculty, fresh from his own postdoctoral with Sydney Brenner. While in England, Sam had been convinced by June Lascelles to set up his own lab to study the regulation of membrane formation in *Rhodospseudomonas sphaeroides*, later renamed *Rhodobacter sphaeroides*. Sam quickly moved on from Western Reserve to the University of Illinois, and I joined him as a postdoc in 1967. We were both trained in biochemical genetics, and we had lots of ideas about how to begin using those tools to pick apart the regulatory story of the membrane-associated photosynthetic apparatus of *R. sphaeroides*. Unfortunately, there was no known way of moving genes from one cell to another for any photosynthetic bacterium, so genetics was not possible. We conferred with William (Bill) Sistrom, who had been working on the problem for several years, and he seemed to have tried most of the obvious ways of developing genetics in *R. sphaeroides*. Convinced that this ground was fairly well plowed, Sam and I went on to look at some of the developmental phenomena that could be studied without genetics. Sam was very interested in

global regulatory strategies, and we had a look at the translational apparatus to see if the numerous changes that occurred during a switch from aerobic to phototrophic growth were controlled by underlying changes in tRNA, ribosomes, or the like. We showed that the ribosomes of *R. sphaeroides* were very different from those of *E. coli* (Marrs and Kaplan 1970), but our work did not find anything that seemed to explain the large change in gene expression that accompanied the induction of photosynthetic membrane synthesis.

I went from the University of Illinois at Urbana to Stanford University to do a postdoc with Charlie Yanofsky, and I set aside the photosynthetic bacterial system to look at the regulation of mRNA synthesis and degradation in the *trp* operon in *E. coli*. As my time with Charlie drew to a close, I was having no great success in finding a faculty position. I wanted to start my own research into the regulation of gene expression in photosynthetic bacteria, and I cold-called Howard Gest at Indiana University, since he was a major figure in the field. Howard quickly suggested that I come to his lab and start developing biochemical genetic studies there until I could find a permanent home of my own. It is typical of Howard that he would reach out to help someone, and this was important mentoring for me. Even when my household possessions arrived in Bloomington ahead of me, and Howard was unexpectedly pressed into advancing the money for my

move without really knowing where I was, his support never faltered. When I finally appeared in Howard's lab, I expected to be asked to work on the biochemical physiology of photosynthetic microbes. Instead, Howard guided me to use my know-how of microbial genetics on problems related to respiratory and photosynthetic electron transport. *Rhodobacter capsulatus* was the organism of choice in Howard's lab, and it was very similar to *R. sphaeroides* with which I had worked under Kaplan's tutelage. Howard's guidance opened up a fruitful field at the interface between microbial genetics and electron transport physiology and biochemistry (Marrs et al. 1972; Marrs and Gest 1973a, b).

The breakthrough

Although much can be learned from the study of mutants without the tools of genetic exchange, when I set up my own laboratory at Saint Louis University School of Medicine in 1972, high on my list of priorities was the development of true genetic tools for any nonsulfur purple photosynthetic bacterial (NSPPB) species. I was a new faculty member without grant support, so I was free to try any approach, as long as it did not cost much. I decided that it was needlessly limiting to try and guess how photosynthetic bacteria exchanged genes in nature. What we should do is set up a screen so that we could tell when genes were being exchanged, and then figure out the mechanism later. Sandy Bilyeu, Nien-Tai Hu, and I went to Forest Park in Saint Louis and collected soil and pond samples for the isolation of new strains of NSPPB. We isolated one NSPPB from each enrichment. We did not bother to determine which species we had isolated, because we could work with whichever one showed genetic exchange. From each isolate we selected one rifampicin- and one streptomycin-resistant mutant. We then mixed the antibiotic-resistant derivatives pair wise, one strep-resistant strain A with one rif-resistant strain B and so forth, and allowed them to grow together into stationary phase. These mixed cultures were plated on nutrient agar containing the two antibiotics simultaneously. If any pair of strains could exchange genes, we would find more double-resistant mutants than the spontaneous mutation controls, which had been grown without mates. Once this design was set up, it was Sandy's responsibility to plow through the many pair-wise combinations – many weeks of repetitive work. After about a month of negative results, I was ready to move on to some-

thing else. I feared that my new technician would tire of doing the same thing over and over. But Sandy said she enjoyed the predictability of these well-defined experiments, and she wanted to stick it out until we had done all the combinations. Within the week we found our first hit, the first genetic exchange system to be described for any NSPPB (Marrs 1974).

What ensued was one of those all-too-brief periods of ecstatic research, where almost every day brought new revelations. Did the cells need to be in contact for gene exchange to occur? No. Did DNase block the transfer? No. Did the genetic material get shed into the medium by the bacteria? Yes. Did it behave like discrete particles? Yes, it sedimented in a tight band at 70S in sucrose density gradients. 70S? That is too small for known transducing phages. Yes, 70S, the same size as a ribosome. OK, so it was small, but let's see the plaques. No plaques. Try again, there must be plaques. No plaques, no plaques, no plaques. And no lysis upon production of the particles, and all genes seemed to move at the same frequency (Marc Solioz did most of this work). This was truly a strange, new genetic exchange mechanism. And by the way, the species that was doing this was my old friend, *R. capsulatus* (Solioz et al. 1975).

R. capsulatus über alles

This was totally a roll of the dice, but scientists seem to love conspiracy theories as much as the next person. There was some rivalry between those working with *R. sphaeroides* and those who used *R. capsulatus*, and some insisted that I had developed the system in *R. capsulatus* because I wanted to give an advantage to those workers over the other camp. It would have been nice to be able to have that much power over nature, but that was not the case. Then there was the confusion around strains of *R. capsulatus*. Years earlier, Howard Gest had worked at Washington University in Saint Louis, and isolated the 'St. Louis' strain of *R. capsulatus*, with which he continued to work. Then I came along, out of Howard's lab, and started working at Saint Louis University with new isolates of *R. capsulatus*, also isolated there, but not related to the 'St. Louis' strain.

A new sort of sex

Fellow microbial geneticists were of two minds about our discovery. Some were intrigued by the novelty of

this strange genetic exchange system. Charlie Steinberg, a senior editor for the prestigious journal *Genetics*, noted that Saint Louis University was a Jesuit institution and offered immediate publication, if I would call the new process trans-substantiation (offer declined). We decided to call the process capsduction, in honor of the species in which it was discovered, and we called the particles that carried the genes gene transfer agents (GTAs), since that was all we knew about them. This is where I ran afoul of a second school of microbial geneticists, who were mostly virologists. To their way of thinking, this was clearly phage-mediated transduction (nothing new), and the fact that I could not find any associated virus did not bother them one whit. It just proved that I was not much of a virologist.

What further annoyed my virologist colleagues was that I had the temerity to propose that far from being by-products of some hidden phage, GTAs were the evolutionary ancestors of phage. Here we had an urphage, a prophage. The GTA particulate gene shipping system clearly was of use to *R. capsulatus*: it gave them sex. One can imagine the evolution of something as useful as sex. And once Nature had designed a system of little genetic lifeboats, one can also imagine them evolving into something self-serving and malignant – voilà – phage. Needless to say, this added insult to injury among my virologist friends, who somehow figured that, since they discovered phages first, phages must have evolved first. In fact, it is most difficult to resolve this issue experimentally, and it remains unresolved till today (Lang and Beatty 2001).

So now there was a genetic exchange system for *R. capsulatus*, and we could begin analyzing how the genes for photosynthesis were arranged on the chromosome, as a first step toward understanding the regulation of gene expression. One of the beauties of working with the NSPPBs was that one could isolate mutants in pigment synthesis, recognized by their colors, and these mutants could be easily propagated by aerobic growth, even if the mutation blocked photosynthesis. We quickly established that there was genetic linkage between the genes for bacteriochlorophyll synthesis and those for carotenoid synthesis (Yen and Marrs 1976). In fact, we were able to argue that these genes were located very close together on the chromosome, because we had laboriously determined the amount of DNA carried by the GTA particles to be about 5000 base pairs. If two genetic markers were separated by more than the amount of DNA carried by a single GTA particle, no genetic linkage would be

seen, and the average gene is about 1000 base pairs long (Solioz and Marrs 1977).

We were better able to determine the DNA capacity of GTA particles through the clever work and persistence of Bill Yen, a postdoc who had done his PhD work with Howard Gest. When Bill started in the lab, we only had bioassays as a tool for characterizing GTA. We could not see it in the electron microscope or directly visualize its protein or nucleic acid. This is really a tribute to the sensitivity of genetics, where one detects single molecules, but it was quite a hurdle for understanding the nature of the GTA mechanism. Bill decided that we needed more GTA to resolve these questions, and he set out to discover ‘over-producer’ mutants of *R. capsulatus* that would make more GTA. This involved developing an intricate and precise plating system in which we could visualize by bioassay the productivity of each clone of mutagenized *R. capsulatus* cells. Bill continued to optimize the assay and screen with it, until one day he placed in front of me, without a word, a plate containing about a thousand tiny colonies of normal type, and one colony that was clearly pumping out hundreds of times more GTA than the rest. With the over-producer in hand, we quickly cranked out the complete characterization of the GTA particle (Yen et al. 1979).

Another sort of sex

The genetic mapping of the genes for photosynthesis proceeded apace, but it was hampered by the short pieces of DNA transferred by the GTA. I decided to see if we could interest *R. capsulatus* in some alternative sexual modes. A family of promiscuous plasmids or R-factors had been discovered in *Pseudomonas aeruginosa* that occasionally mobilized chromosomal genes. We were able to mate one of these antibiotic-resistance-carrying plasmids into *R. capsulatus*, and we discovered that it did indeed cause chromosomal genes to move between *R. capsulatus* cells in a kind of mating activity requiring cell-to-cell contact. To our delight, we discovered that the promiscuous plasmid moved the chromosomal genes of its host by integrating a portion of the host chromosome into itself, forming a new larger plasmid or R-prime-factor. By setting up the right genetic crosses, we could create R-primes that carried big blocks of DNA with the genes for photosynthesis included. These R-primes were very unstable when propagated in *R. capsulatus*, and until we learned the trick of moving these R-prime-factors in *Escherichia coli*, we did not learn much



Figure 1. The author (Barry MARRS), 2001.

from them. But once we moved the R-primes into this microbial genetics workhorse, brave new worlds opened up to us. We had just cloned the genes for photosynthesis (MARRS 1981)!

The sequence of things

Teaming up with Dean Taylor in Stan Cohen's lab, we were able to do restriction mapping of the photosynthetic gene cluster, and we could assign genetic markers to different restriction fragments by complementation studies. We could align the genetic and physical maps of the region (Taylor et al. 1983). After I described this work at a seminar at the University of California at Berkeley, Doug Youvan and his mentor John Hearst proposed that we team up to study and sequence the genes for photosynthesis (Youvan and MARRS 1984). I was not much interested in sequencing, so I readily agreed to supply the clones for them to sequence, including some clones that might carry reaction center protein genes. It turned out that Hartmut Michael, at the Max Planck Institute in Martinsreid, Germany, was working on crystallizing the photosynthetic reaction centers from a related phototroph, and sequence information was needed to complete the work. Hearst's group, which I was supporting, got into a kind of race with another group, Mel Simon and JoAnn Williams working at Cal Tech, who were

going after the sequence of *R. sphaeroides* reaction centers, using a very different approach to cloning the genes. I believe both sequences were completed within a few months of each other, and this information enabled Michael to solve the crystal structure for which he, along with J. Deisenhofer and R. Huber, won the Nobel Prize in Chemistry in 1988. This was a turning point for work with the genetics of NSPPB, launching it from a little-known backwater of microbiology into the spotlight, and bringing to a close, to my mind, the 'early days' in this field.

At the suggestion of the editors, I have included a recent photograph of myself (Figure 1).

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