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Organelle evolution, fragmented rRNAs, and Carl

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I am honored to have been asked to contribute to this memorial issue, although I cannot claim to have known Carl Woese well. Carl's insights and the discoveries that his research group made over the years certainly stimulated my own research program, and at several points early on, interactions with him were pivotal in my career. Here I comment on these personal dealings with Carl and emphasize his influence in two areas of long-standing interest in my lab: organelle evolution and rRNA evolution.

Organelle Evolution

I first heard of Carl Woese when Linda Bonen, a recent member of Carl's group, moved to Halifax in the early 1970s to join the lab of Ford Doolittle (like me, a newly minted faculty member in the Department of Biochemistry at Dalhousie University), bringing with her the then cutting-edge technique of T1 oligonucleotide fingerprinting.[1] As applied in the Woese lab to bacterial small subunit (SSU; 16S) rRNAs,[2] this technique generated catalogs of RNase T1 oligonucleotides that could be compared, allowing evolutionary relationships to be deduced from molecular sequence data. Ford and Linda (currently Professor in the Department of Biology at the University Ottawa) decided to use the T1 cataloging approach to test the endosymbiont hypothesis of organelle origins, recently resurrected by Lynn Margulis[3] but quite contentious.[4] They chose to analyze the chloroplast and cytoplasmic SSU rRNAs (16S and 18S, respectively) of a marine red alga, *Porphyridium* sp., observing extensive similarity between the T1 oligonucleotide catalogs of *Porphyridium* chloroplast and several bacterial 16S rRNAs, but little similarity between the 16S catalogs (both chloroplast and bacterial) and that of *Porphyridium* 18S rRNA: results strongly supportive of an endosymbiotic origin of the red algal plastid 16S rRNA gene from a bacterial progenitor.[5] In the same issue of the *Proceedings of the National Academy of Sciences USA* (PNAS), the Woese lab reported a fingerprint analysis of the chloroplast 16S rRNA of another alga, *Euglena gracilis*, drawing similar conclusions.[6] These two papers arguably constituted the earliest and most compelling molecular evidence supporting a bacterial origin of the chloroplast and its genome.

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At the time, my lab had begun a project to isolate and characterize the mitochondrial nucleic acids of land plants, little studied up to that point. Our initial results with the plant mitochondrial system were similar to those reported by Leaver and Harmey,[7] but quite different from what had been seen in other mitochondrial systems (notably animal and fungal) up to that point. Wheat mitochondrial SSU and large subunit (LSU) rRNAs were essentially the same size as their cytoplasmic counterparts (18S and 26S, respectively), and a mitochondrial 5S rRNA (not found in animals or fungi) was present. Only the absence of a mitochondrial 5.8S rRNA convinced us that we were not dealing with contaminating cytoplasmic rRNAs. Following the success of the fingerprint approach with chloroplast 16S rRNAs, we initiated a collaboration with Ford and Linda (spearheaded by my first graduate student, Scott Cunningham) to carry out T1 oligonucleotide fingerprinting of wheat mitochondrial and cytoplasmic rRNAs. This study demonstrated that wheat mitochondrial 26S, 18S, and 5S rRNAs were indeed distinct in sequence from their cytoplasmic counterparts.[8] Subsequent in-depth cataloging of wheat mitochondrial SSU rRNA clearly demonstrated the bacterial character of this RNA species, the results arguing in favor of an endosymbiotic origin of mitochondria.[9] Later work on the post-transcriptional modification pattern of wheat mitochondrial SSU rRNA reinforced this conclusion.[10]

After gene cloning arrived on the scene, David Spencer, a research assistant in my lab, determined the complete sequence of the wheat mitochondrial SSU rRNA gene and another research assistant, Murray Schnare, and I modeled its secondary structure. The resemblance to *E. coli* 16S rRNA, both in primary sequence and secondary structure, was striking, Our choice of the wheat mitochondrial system for these studies turned out to be fortuitous, in that the mitochondrial genome of land plants evolves far more slowly in sequence than mtDNA in other eukaryotic groups; accordingly, the mtDNA-encoded rRNAs of plants more convincingly retain vestiges of their evolutionary ancestry than do their orthologs from other eukaryotes such as animals and fungi.

By this time I had a passing acquaintance with Carl and thought he might be able to help us get a paper reporting these results into PNAS. When I phoned and asked for Carl, the voice at the other end replied gruffly, "He's not here." I politely asked that a message be relayed to Carl that Mike Gray from Dalhousie had called and would like to speak with him, at which point "the voice" replied, "Oh, hi Mike." We then carried on a normal conversation as if nothing unusual had just occurred.

In 1984, sponsorship by a member of the National Academy of Sciences (NAS) was a prerequisite for submission to PNAS.

Although Carl was not at that time an NAS member, he prevailed on Ralph Wolfe (who was) to act as sponsor. We sent off a draft of our manuscript, and in due course I received a somewhat disapproving phone call from Carl, who thought that our choice of title and the overall thrust of the discussion hadn't captured the evolutionary significance of the work as well as it could have done. Happily, after a re-write following Carl's suggestions, the paper passed muster with him and with reviewers, and was published.[11]

Later that year, while I was on sabbatical leave at Stanford University, Carl phoned to say that he'd been comparing the T1 oligonucleotide catalog of the wheat mitochondrial SSU rRNA with the 16S rRNA catalogs of various bacteria. He'd concluded that *Agrobacterium tumefaciens* (a member of the α subdivision of purple bacteria,[12] now designated "α-Proteobacteria") had the most similar catalog, and he urged that we sequence the *A. tumefaciens* 16S rRNA gene. However, if we didn't wish to do so, his group would go ahead. As Carl's lab was in a better position to do the sequencing, I suggested he proceed. The resulting paper, based on phylogenetic reconstructions using the wheat mitochondrial SSU rRNA sequence in combination with the *A. tumefaciens* and other bacterial 16S rRNA sequences, demonstrated a specific affiliation between the mitochondrial and α-proteobacterial sequences: the first solid molecular sequence data supporting an origin of mitochondria from within α-Proteobacteria,[13] an affiliation that had been suggested previously on biochemical grounds.[14] Since 16S rRNA sequences had pointed to a specific phylogenetic relationship of the chloroplast with Cyanobacteria, an evolutionary connection between mitochondria and a different bacterial group (α-Proteobacteria) clearly argued for separate endosymbiotic events in the evolutionary origin of these two key eukaryotic organelles.[15]

By 1989, Carl was an NAS member in his own right, and he agreed to sponsor another of our papers on mitochondrial evolution. Although the high degree of sequence divergence of non-plant mitochondrial rRNA sequences make their overall alignment with orthologous rRNAs problematic, a solution was provided by the recognition that SSU and LSU rRNAs contain a highly conserved "universal core" of primary sequence and secondary structure.[16,17] Basing sequence alignments and phylogenetic reconstructions on these core elements allows the inclusion of even very divergent mitochondrial sequences. A collaboration during the 1980s with David Sankoff and the late Bob Cedergren at the University of Montreal used this approach to explore organismal relationships, with particular emphasis on organelles.[18,19] A consistent finding in these analyses was an anomalous branching position of plant vs. non-plant (particularly green algal) mitochondrial sequences, leading us to suggest[20] that "the rRNA genes in plant mitochondria may be of more recent evolutionary origin than the rRNA genes in other mitochondria": a suggestion that had obvious implications for monophyletic vs. polyphyletic origins of mitochondria. Although this "secondary acquisition" proposal[20] was consistent with available data, and we had taken pains to try to show that the anomalous branching position of plant mitochondria was not a methodological artifact, we were not entirely comfortable with the suggestion, pointing out that additional comparative data were of key importance in the ultimate validation or rejection of this hypothesis. In time, additional non-plant mitochondrial rRNA and genome sequences (including more conservative green algal ones) failed to validate the secondary acquisition hypothesis, instead strongly supporting the now-current view that all mitochondrial genomes and their encoded rRNAs trace their origin to a single endosymbiotic event.[21] Nevertheless, we were grateful at the time that Carl felt that the idea and the data we presented in support of it were of sufficient interest and merit that he was prepared to sponsor our paper for PNAS.

As far as I'm aware, Carl published only one other paper dealing explicitly with the origin of organelles.[22] In it, he posited that "the mitochondrion did not originate from an endosymbiosis, 1–2 billion years ago, involving an aerobic bacterium. Rather, it arose by endosymbiosis in a much early [sic], anaerobic period, and was initially a photosynthetic organelle, analogous to the modern chloroplast." Certainly, the idea that the mitochondrion originated very early in the eukaryotic lineage, perhaps even coincidentally with the origin of the eukaryotic cell itself, has gained support in recent years,[21] and the fact that the α-proteobacterial lineage contains photosynthetic members[12] leaves open the possibility that the mitochondrial endosymbiont was indeed photosynthetic.

Fragmented rRNAs and rRNA Evolution

Although Carl is arguably best known for the discovery of archaebacteria,[23] and for the progenote[24,25] and three-domains-of-life[26,27] concepts, he was also deeply interested throughout his career in the origin and evolution of the translation system per se. His RNA-centric view of the primordial translation system[28-30] paralleled the advent of the "RNA world" hypothesis[31-34] and the ultimate realization that the ribosome is fundamentally an RNA machine.[35] Carl's views were particularly influential in attempts by my group to make sense of several unusual (indeed bizarre) ribosomal systems in which the functional SSU and/or LSU rRNAs are not single, covalently continuous molecules but comprise a number of separate, interacting pieces. In certain of these cases, the fragmented rRNAs result from the excision of transcribed spacer sequences from an initially high molecular weight pre-rRNA (as occurs, e.g., in the generation of 5.8S rRNA in the LSU of eukaryotic cytosolic ribosomes).[36-38] However, in other examples, coding modules corresponding to the rRNA fragments are separated from one another, scrambled in the genome containing them, and interspersed with tRNA and protein-coding genes.[39-42] Nevertheless, in all of these cases of fragmented rRNAs, the mature small rRNA fragments have the potential to interact through complementary base-pairing to form a three-dimensional complex that reconstitutes the essential universal core of a conventional ribosome: akin to putting together the pieces of a jigsaw puzzle. Accordingly, we were led to suggest that the primordial ribosome might have consisted of a complex of this sort, with individual small RNAs contributing specific functional elements.[40]

So, how could such an arrangement of small, non-covalently associated rRNA pieces have evolved into the large, covalently continuous rRNA species that are characteristic of most contemporary ribosome systems? Again, work from Carl's lab[43] and our own collaboration with Robin Gutell on the modeling of rRNA secondary structure[44] provided insight. In 16S–18S and 23S–28S rRNAs, the highly conserved sequence elements that comprise the universal core are dispersed throughout the primary sequence and separated from one another by variable regions, so-called because they differ extensively in length, base composition, and potential secondary structure. The fact that excised spacer sequences in fragmented rRNA systems coincide with variable regions in conventional SSU and LSU rRNAs suggested to us that failure to excise spacers would effectively convert them to variable regions, providing a possible mechanism by which adjacent rRNA modules might have been stitched together at the genome level in the course of rRNA evolution. These ideas prompted us to propose a model of rRNA gene evolution in which various fragmented rRNA systems serve as modern recapitulations of different postulated stages of rRNA evolution.[45,46]

Envoi

My face-to-face meetings with Carl were largely limited to various meetings and conferences, although I did have the opportunity of paying a visit to his lab in 1990 as a member of the Advisory Board for the rRNA Database Project (RDP),[47] which Carl was overseeing at the time. I recall both Carl's enthusiasm for the project and the courtesy and hospitality he extended to us. Carl Woese was unquestionably a giant in the field of Microbiology. The depth and breadth of his legacy will continue to challenge and refine our ideas about organismal origins and phylogenetic relationships, as well as the origin and evolution of the genetic information transfer system that underpins these relationships.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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