

# Carl Woese

## A structural biologist's perspective

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Not long after Carl Woese died, I received a message from Robin Gutell asking if I would contribute an article to this issue of *RNA Biology*. While my admiration for Carl's contributions to biology knows no bounds, I did not know him well personally. For that reason I advised Robin to strike my name off the list of contributors and replace it with that of someone who is better qualified than I am, but he persisted, and here we are. I guess Robin thought it would be useful to hear from one of those who admired Carl from afar.

The naïve outsider might find it surprising that a structural biologist like me, who worries about the minutia of the three-dimensional structures of biological macromolecules, would ever have had anything in common with a big-picture, evolutionary biologist like Carl, but I did. What we shared was an interest in the structures of RNAs, especially rRNAs.

### Meeting Carl

None who knew Carl will be surprised to learn that scientific meetings contributed next to nothing to our scientific interactions. The meeting of the ribosome fraternity that was held in Madison, WI, in 1979 is the only one I can think of that we both attended, and there Carl gave a talk in which he vehemently criticized the members of that community, to which I belong, for its collective failure to develop a theory about ribosomes and the mechanism of protein synthesis robust enough to guide the research we were doing.<sup>1</sup> It baffled me. How do you come up with a theory about something you hardly understand, and what good would it do if you did? The theory that was guiding my work at the time, if you can call it that, was that it would be a Good Thing to know more about the three-dimensional structure of the ribosome. The Madison meeting may have been the only ribosome meeting Carl ever attended; no doubt he found us dull company, but it was not because of any lack of interest on our part in what Carl had to say.

Mike Yarus and I were co-chairs of the 1994 Nucleic Acids Gordon Conference. Sometime in 1993, we had a telephone

conversation in which Mike suggested that we ask Carl to give a talk, and said that if I concurred, he would extend an invitation. I did concur, but I remember Mike saying that if Carl actually came to the meeting it would be like seeing Bigfoot. Sure enough, Carl did not show up, and we did not see Bigfoot.

Bob Zimmermann and I tried again in 2007. This time, the event being organized was a ribosome meeting, and the venue was West Falmouth, MA. We knew that Carl would be on Martha's Vineyard at the time of the meeting, only a few miles away, and we told Carl he could talk in whatever session he wanted, that we would arrange to have him picked up in Woods Hole at the ferry dock, and would deliver him back to the ferry afterwards at the time of his choosing. Meals and drinks would be free, and there would be no registration fee. Short of hiring him a helicopter, I don't know what else we could have done to sweeten the offer. Once again: no dice.

The only one-on-one conversation I ever had with Carl occurred during a seminar visit I made to Urbana-Champaign in the early 2000s. We talked about all kinds of things: science, politics, the politics of science, and much else. It was wonderful. I would have happily spent the rest of the day with Carl, but after the allotted 45 min had passed, I was hustled off to my next appointments, about which I now remember *absolutely* nothing.

### RNA Structure

From my narrow perspective, Carl's most important contribution to science was the method he developed for deducing RNA secondary structures from sequences. This problem was first addressed in the paper published by Robert Holley and his colleagues in the spring of 1965 that reported the sequence they had just obtained for yeast alanine tRNA.<sup>2</sup> Guided by the knowledge that RNAs form stem-loop structures, and the notion that more base pairs are better than fewer base pairs, Holley's group advanced three proposals for the secondary structure of their tRNA, one of which was the now iconic cloverleaf. By the following June, there were several more tRNA sequences available, all of which were compatible with the cloverleaf. Furthermore, in every case, as far as numbers of base pairs were concerned, the cloverleaf was the best possible secondary structure, or so close to it as made no obvious difference (see ref. 3). Since it was already clear that, to first order, the ribosome treats all tRNAs alike, they had to have similar three-dimensional structures, and hence, the cloverleaf model had to be right.

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It was not until the sequence of the 5S rRNA from *E. coli* was published a few years later<sup>4</sup> that the shortcomings of the maximum base-pairing concept became clear. That sequence is compatible with many different stem loop secondary structures, none of which is anywhere near as extensively base paired as the tRNA cloverleaf, and it was far from obvious which one was best. As additional 5S sequences became available, and partial nuclease digestion experiments were done to probe their structures, three-stem models for the secondary structure of 5S rRNA began appearing in the literature that we now know are fundamentally correct (see ref. 5). About the same time, a less extensively base-paired model of the three-stem type was advanced by Carl and George Fox.<sup>6</sup> What distinguished their analysis of the 5S problem from everything that had gone before was their total abandonment of the maximum base pairing principle. They argued that homologous RNAs must have similar structures because they perform the same function. It follows that the correct secondary structure for all of the members of some family, which is to say the secondary structure that will be biologically relevant, must be compatible with the sequences of all of them, no matter what.

In my estimation, the secondary structure models proposed for the two large rRNAs around 1980 were/are the most important products of Fox-Woese reasoning. Sequences, of course, are the sine qua non for this kind of science, and in the early 1970s, there were no complete sequences available for the large rRNAs. Nevertheless, by the time the Fox-Woese model for 5S rRNA appeared, an effort had been under way for several years to sequence 16S rRNA directly, and one lived in hope. However, in the end, the first complete rRNA sequences were obtained by Harry Noller and his colleagues using a different approach. They sequenced the DNA of rRNA genes, rather than the RNAs themselves (see ref. 7). (NB: It may be instructive to point out that the only DNA sequencing devices available in 1980 were graduate students, and postdocs.) Using Harry's complete sequences for the 16S rRNA from two different bacterial species, the large collection of 16S oligonucleotide sequences Carl and his colleagues had accumulated (see below), and Fox-Woese logic, Harry and Carl produced a model for the secondary structure of 16S rRNA.<sup>7</sup> Shortly thereafter, a similar model was obtained for 23S rRNA.<sup>8</sup>

It is impossible to overstate the impact these secondary structure models had. They explained a lot of what was already known about the ribosome and rRNAs, and once they appeared, the days of thinking about the large rRNAs as shapeless, formless strings of nucleotides were over forever. This development, coupled with the discovery of ribozymes a year or two later,<sup>9,10</sup> gave new life to an idea that had been around in the field for years, namely that rRNAs are the functional heart of the ribosome.

The larger the number sequences for a family of homologous RNAs there are to analyze, the more accurate the secondary structure model that can be obtained for them. Thus, as sequences accumulated, the models Harry and Carl had first proposed matured. (This enterprise has consumed the energies of Robin Gutell for most of his career.) The definitive test of their accuracies came in the summer of 2000 when the first atomic resolution crystal structures were published for the two ribosomal

subunits.<sup>11,12</sup> Both models passed with flying colors. While it is true that the crystal structures revealed the existence of small numbers of base pairs that were not then included in the models, every secondary structure element the models called for exists in the ribosome. What more could anyone have asked?

## Ribosomes and Evolution

All of the above notwithstanding, when histories of 20th century biology are written, it is unlikely that they will say much about Carl's contributions in the area of RNA folding. What they will surely emphasize instead is the revolutionary impact his work had on our understanding of evolution. It is hard to grasp the magnitude of his achievement in this arena if you do not know what people of my generation (and his) were taught about evolution. In those days, half a century ago, biology was divided into two parts: plants (botany) and animals (zoology). Introductory zoology invariably included an account of the evolution of multicellular animals, which was not too different from what students are taught today. Introductory botany included something similar for higher plants. And then there were the unicellular organisms. Some of them seemed to be plants, but there were many others that even a naïve student like me could see instructors would rather not have had to talk about. Prominent among these problematic organisms were the bacteria, which were then commonly classified as fungi. (I suspect that order name *Actinomyces*, which is applied to filamentous eubacterial species like those belonging to the genus *Streptomyces* is a relic of that misunderstanding. The suffix - *myces* comes from the Greek word for fungus.) As for the evolutionary relationships that might exist between higher plants, higher animals, and anything else, who knew? Thankfully, this cramped, fragmented view of the biological universe has been swept away, and Carl did a lot of the sweeping.

It is obvious from the published record that it was Carl's interest in evolution that motivated his concern with RNA sequences, not his interest in RNA secondary structure as such. Carl was not the first to realize that inferences about evolutionary relationships can be drawn from sequence comparisons, but as far as I know, he was the first to recognize that comparisons of rRNA sequences were likely to be uniquely powerful in this regard. His argument was that the first organisms to appear that had any kind of genetic systems at all, must have contained the enzymes that are the progenitors of the ribosomes we know today, which may well have been RNA molecules. Thus, comparisons of rRNA sequences were likely to reveal important information about the earliest and most profound branchings in the tree of life. This reasoning led Carl and his collaborators to begin sequencing the large oligonucleotides found in enzymatic digests of the 16S and 18S rRNAs prepared from many prokaryotic species. If I had been asked at the time, which I might have been, but happily for the progress of science I was not, I would have dismissed this undertaking as beyond hopeless. Complete sequences are one thing, but how are you going to learn anything from sequencing fragments that together constitute only a few percent of the molecules from which they derive? Nevertheless, in a few years, statistical analysis of

sequences of just this sort led Carl to the conclusion that there are two kinds of prokaryotes.<sup>13</sup> We refer to them today as the *Archaea* and the *Bacteria*, and when it comes to their rRNA sequences, they are as different from each other as they both are from the *Eucarya*.<sup>14</sup> The complete sequences of rRNA we have today, of there are now thousands, as well as an abundance of data of other sorts, support this conclusion.

Not only did this work revolutionize our understanding of prokaryotic biology, it demonstrated that comparisons of rRNA sequences could shed light on the evolutionary relationships that exist within both prokaryotic groups, which was a problem that had previously been thought insoluble.<sup>15</sup> Not only that, rRNA sequence comparisons could yield plausible evolutionary schemes that encompassed all the organisms now found on this planet: plants, animals, bacteria, the lot. Nothing has been the same since.

It is highly unlikely that the last word has been written about the evolution of life on Earth, and most particularly about the events that occurred in its earliest stages, which were the ones that appear to have interested Carl the most. Also, it would be a mistake to think that Carl's views about evolution were warmly received in all quarters when they were first published, or even that they are universally accepted today. For example, 15 y ago, I read an article by Ernst Mayr that questioned Carl's conclusion that the *Archaea* should be considered a group coequal with the *Bacteria* and the *Eucarya*.<sup>16</sup> This was no small matter. Mayr was arguable the most distinguished systematic biologist of his time, and among other things, he accused Carl of being an amateur. (One is reminded of Chargaff's famous comment that, "Molecular biology is essentially the practice of biochemistry without a license."<sup>17</sup>) It was indeed true that Carl had not

been trained as a systematic biologist, but in my estimation, his rebuttal of Mayr's article was devastating nonetheless.<sup>18</sup>

Less than a decade later, Mayr gave a lecture on evolution at Yale that was intended for a general audience, to which I went to out of curiosity. I came away with the impression that Mayr was still unhappy about the directions sequence comparisons were taking his discipline. There were times during the hour that I thought I could hear distant echoes of disputes about the future of biology that had occurred in faculty meetings of the Biology Department at Harvard decades earlier. I thought that Mayr and his disciples should have been applauding Carl and his molecular biological allies for giving them the most powerful tool they will ever have for solving the problems they care about, but apparently, emotionally at least, this was still not possible for Mayr. The plain fact is that the methods Carl helped develop gave new life to a part of biology that, at the time Carl entered it, most of us thought a moribund backwater, plagued by pedantic disputes that would never be resolved.

Taxonomy, or systematic biology, if you prefer, is the most ancient part of the science of biology. It is what Linnaeus did. Done properly, as Darwin realized, the classification/naming schemes we devise for organisms should reflect their evolutionary relationships. They should thus encapsulate the history of life, the elucidation of which remains one the central objectives of biology. We are fortunate that in our lifetimes we got to witness what can happen when an intellect as powerful and as unique as Carl's was applied to a scientific problem that grand.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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