J Mol Evol (2016) 83:169–175
DOI 10.1007/s00239-016-9767-3

ORIGINAL ARTICLE

The RNA World at Thirty: A Look Back with its Author

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Received: 15 September 2016 / Accepted: 15 November 2016 / Published online: 19 November 2016

Abstract Thirty years ago, molecular biologist Walter Gilbert published his RNA world hypothesis, which posited that early in evolution living systems were composed entirely of RNA. Proposed in the immediate wake of the discovery that certain RNA molecules were capable of catalyzing biological reactions, the hypothesis ascribed both of life's essential functions, namely carrying information and catalysis—respectively, performed by DNA and proteins in most modern life systems—to RNA, which were labeled as ribozymes. In the years since its inception, the RNA world has been greeted with equal parts enthusiasm and opposition from the origins of life research community, of which Gilbert neither was, nor really became, a part. For this special historical issue of the *Journal of Molecular Evolution*, Gilbert agreed to revisit his hypothesis and share his memories about the theory's origins and his insights into its fate in the years since he first published his idea.

Keywords RNA world hypothesis · Walter Gilbert · Origins of life · Ribozymes · History of science

Introduction: Gilbert's One Short Argument

Charles Darwin's *Origin of the Species*, his "one long argument" for evolution by natural selection, has been long and rightly touted as a masterpiece of persuasion through logical progression. If you are on board with him at the outset—and really, how could you not be as he reeled you in with the easily identifiable descriptions of domestic breeding practices also known as artificial selection?—there is really no help for it, you will be so until the end, when he provided his entirely heterodox conclusion for the times: that man, monkeys, or even mangoes for that matter, all of the extant life forms on earth were descended from a common ancestor. For at no point in his argument did he make a leap that can elicit the "But wait, that doesn't make sense" reaction; his progression from step to step is that logically airtight.

Unlike Darwin's treatise, Walter Gilbert's 1986 paper describing a different level of origins and evolution—those of life and the "RNA world," respectively—is a very short argument: just a single page long, it probably has fewer words than the *Origin of Species* has pages! But it shares one of the most signature features of the older treatise, in that despite its undeniable drawbacks, it "appears to be an outright logical inevitability," to borrow the characterization of modern day computational biologist, Eugene Koonin (Bernhardt 2012, p. 8). Beginning as Darwin did, with a set of life's conditions that are easily recognized or grasped, Gilbert's argument leads the reader, step by logical step, through a thought experiment to a startling, rather unbelievable endpoint. Like Lucy—or perhaps, more appropriately given the context, LUCA[1]—the "girl with kaleidoscope eyes" of the Beatles' song; one is suddenly thrust willy nilly into the sky with diamonds!

The familiar starting point of Gilbert's argument is the division of life's labors in modern living systems on earth,

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[1] LUCA is the acronym for the Last Universal Common Ancestor, a term first coined in 1996 by a group of origins of life researchers at a meeting, "The Last Common Ancestor, and beyond" at Treilles Foundation in France (see EMorel 2013).

whereby nucleic acids carry the information and proteins supply the enzymatic or catalytic activities required for metabolism and replication (Eigen 1971; Eigen et al. 1981). Based on this state of affairs, Gilbert explained, when one thought about the origin of life scenarios in terms of molecules performing these functions, “One imagined that the first self replicating systems consisted of both RNA and protein.” Apart from a minor quibble on the specifics—why, for example, we might ask, does he favor RNA over DNA when the latter is clearly what is doing the work now?—one cannot really poke holes in the grander logic of his scenario for life, namely that be it protein or nucleic acid, one without the other is incomplete.

So far so good. The scenario spelt out by Gilbert in his first two sentences is a perfectly logical one and was, in fact, a pithy statement of what most of the research community interested in questions of the nature and origins of life more or less took for granted until a few years prior to the publication of his paper. Of course, buried in this brief description is the history of a long impasse that this view of life had created for those interested in doing research on the origins of life, namely the disagreement over what appeared first in earth’s earliest life forms. This impasse—aptly labeled as a chicken versus egg conundrum (Eigen 1971)—had taken different forms since the early twentieth century, articulated variously in cellular: cytoplasmic versus nucleocentric; disciplinary: biochemical versus genetic; functional: metabolic or catalytic versus replication or information; and macromolecular: protein versus nucleic acid, terms on the question of which had come first (see Kamminga 1980, 1988; Podolsky 1996).

Matters changed for the origins of life research community during the 1980s when two groups of scientists working on different problems in different places, stumbled upon the fact that there existed certain RNA molecules that carried the properties of catalysis (Kruger et al. 1982; Guerrier-Takada et al. 1983; Sankaran 2012). Dubbed as “ribozymes,” for their *ribo*nucleic acid composition and en*zyme* functions, the existence these molecules had immediate implications for origins of life theorizing because they offered a way around the dichotomy, as Gilbert was quick to realize. Barely 3 years after the first ribozyme discoveries then, he articulated a scenario for the origins of life that did away with the chicken and egg problem that had been confounding the origins of life research community for so long. If, as Gilbert proposed, there were two known enzymic activities associated with RNA, then surely there could be more? Sure, you (the reader) think, that’s certainly plausible, I’m with him… and so you read on. “And,” he adds, “if there are activities among these RNA enzymes, or ribozymes, that can catalyze the synthesis of a new RNA molecule from precursors and an RNA template…” Why not? You interrupt your reading mid-sentence to ask yourself. After all, enzymes are know to catalyze a whole slew of reactions; his examples are no more incredible than those already discovered. So you continue to read to see what he supplies for the “then” end of this if–then scenario, which logically enough contends that “there is no need for protein enzymes at the beginning of evolution.” From there the conclusion that “One can contemplate an RNA world, containing only RNA molecules that serve to catalyze the synthesis of themselves,” does not require a major leap of faith. Thus, just like that, you stepped from a path entirely logical into a realm quite fantastical, that of the “RNA world,” a world that you would have likely dismissed out of hand had it been presented without the context Gilbert provided.

It would be nice if one could spin out the analogies between the *Origin of Species* and the RNA world into a full-fledged story in honor of the 30th anniversary of the latter, but to do so would be forced and artificial. Save for the logical progression of their arguments, both of which concerned the topic of life’s evolution, neither the ideas and nor their authors have much in common. Darwin, as is well known, had spent 20 years developing his argument for natural selection and would remain engaged with it for the remainder of his career, revising newer editions of the *Origin* and publishing others books on the topic as well. Gilbert, in contrast, was not an obvious candidate for inventing the RNA world. A theoretical physicist who had made the shift to molecular biology in the early 1960s, he was, and perhaps still is, best known for developing a rapid technique for sequencing DNA (Maxam and Gilbert 1977, 1980), for which he shared 1980 Nobel Prize in Chemistry with biochemists Fred Sanger and Paul Berg. He is also the person who coined the terms “exons” and “introns” for the different portions of a contiguous sequence of DNA which are, respectively, retained and removed during the process of DNA transcription (Gilbert 1978). At the time he published the RNA world paper, his principal interests were in understanding the structure and evolution of genes and genomes (see, for example, Gilbert 1978, 1980; Lomedico et al. 1979; Perler et al. 1980; Church and Gilbert 1984; Lonberg and Gilbert 1985), and problems about the origins of life and chemical evolution do not seem to be among the problems that loomed large in his research landscape. Even after publishing the RNA world paper and witnessing the immense attention it received, Gilbert never made it or the origins of life his primary research focus, with the exception of some work on modeling the formation of primordial cells (Jay and Gilbert 1987) and on relating gene structure to the RNA world (see Gilbert and de Souza 1999). But the impact of his hypothesis has been so profound and far-reaching, that it was inevitable that he would be called upon to comment on the RNA world as it turns 30. Earlier this year, for instance, he was a participant in a panel discussion on its origins organized by Nathaniel Comfort, Astrobiology Chair at the Library of Congress's John W. Kluge Center in Washington, DC (See webcast on The Origins of the RNA World 2016). For this special historical issue of the *Journal of Molecular Evolution*, Gilbert agreed to a conversation with a historian to share some of his insights on how the RNA world came to be and where it is today.

The Idea and its Antecedents

If Gilbert was an unexpected inventor of the RNA world, the "News and Views" section of *Nature*, where his paper was published, is perhaps an even less obvious venue for the publication of such a startling new scientific hypothesis. As indicated in *Nature*'s website, this section of the journal is not a place for publishing original scientific research (see http://www.nature.com/authors/author_resources/article_types.html). Rather it is a section that contains short commentaries which consider the implications of recent scientific findings usually reported in the sections of the journal containing peer-reviewed research papers. Geared for wider audiences, these commentaries are normally—but not always—commissioned by the editors of the journal, typically to authors who are not directly involved in the basic research on which they are commenting but are recognized as broad experts in the field. As a freer forum that allows for opinion and conjecture, this section is not subject to peer review.

Gilbert's paper is something of departure from the "News and Views" norm because although he fits the author profile as a non-participant in the actual research, the article "was not an invited piece," but one that he wrote of his own accord. As to what prompted him to write it, he is very clear.

> The thing that triggered it was Cech's article on self-splicing RNA [Zaug and Cech, 1986] and therefore realization that RNA could be an enzyme.

These enzymes, the ribozymes themselves, had been "completely unexpected" finds by two groups working on unrelated problems, and the close proximity in the timing of the two discoveries had also been simply a matter of coincidence (Pace and Marsh 1985). Neither group was engaged in origins of life research at the time, but the implications of their findings for the field were so evident that both discussed the issue in their papers. Cech's group in Colorado, who had discovered the ribosomal RNA capable of excising pieces of itself, had concluded their report with the prediction that "The finding of self-splicing RNA adds a new dimension to discussions about possible roles for RNA early in evolution" (Kruger et al. 1982, p. 155). Sidney Altman and his group at Yale, who had discovered that the catalytic portion of the ribonuclease P enzyme was in fact an RNA and not a protein subunit, were even more explicit in their statement and, in fact, came close to making the very same prediction as Gilbert regarding the possibility of an RNA world:

> If proteins were relative latecomers in the evolution of macromolecules, then primeval manipulations of nucleic acids may have been carried out *entirely or predominantly* by catalytic nucleic acids themselves (Guerrier-Takada et al. 1983, p. 855, emphasis added).

But neither group elaborated further on the issue, and thus, the task of giving the RNA world its full form, complete with its name, was left to Gilbert.

While Gilbert's position an outsider might explain why he was able to take a broader, more comprehensive view of the field and synthesize the work of the insiders into formulating a new hypothesis about life's origins, it nevertheless raises the question as to why the papers on RNA catalysis caught his eye to begin with. Undoubtedly his interest in the structural organization of genes would have played a role in attracting his attention:

> Pieces about that thought that RNA could be an enzyme pursued an interest of mine, the possibility that very earliest organisms used an intron exon structure to put together information in useful forms. So, if what Cech had found was really a self-splicing intron, one could think about using such self-splicing RNAs as carriers to splice exons out of one RNA molecule and splice them into another.

Gilbert's account may also help explain why in retrospect, he tends to identify the discoveries of Cech's group over those of Altman's Lab, as the spring-board for his thinking. Although he cited the work of both groups fairly even-handedly in his original paper, it is clear that the self-splicing RNA exons discovered by the Cech group had more immediate implications for his own interests, due to which they have lingered longer in his memory. In addition, the discovery of the capacity of the intron to catalyze the polymerization of RNA strands (Zaug and Cech 1986) had a more direct bearing on the RNA world itself. As he explains:

> I got excited about the idea of RNA enzymes because I realized that if RNA was copying itself, it could have been both the first *genetic material and enzymatic material* (emphasis added).

Gilbert's description takes one back nearly 100 years, when another Harvard scientist, physicist, and psychologist Leonard Troland, speculating on the origins of life, had posited the following:

> Let us suppose that there suddenly appears at some point in the ocean body one molecule of a catalytic substance [that has a] specific catalytic effect upon the reaction which was responsible for its *own* initial production. In other words, it is not only catalytic, but also *autocatalytic*. Such an agent, doubly specific, may be called a *genetic enzyme* (Troland 1916, p. 380, emphases in original).

Despite the fact that his description echoes that of Troland so closely, Gilbert did not cite this earlier work and, in fact, does not seem to have known about Troland's role in origins of life research. Such a gap, he says, is not unusual, for with few exceptions, "Scientists are not reading history. [We] stop reading text books relatively early and the papers go back only a few years." The bibliography of his paper certainly upholds his claims about scientists concerns with immediacy: eight of the nine works cited were five or fewer years old at the time, and each was included because it provided support for the different steps in his carefully constructed argument for the RNA world. With specific regard to Troland, Gilbert's view about scientists and history certainly rings true, for with the exception of Herman Muller, a key figure in debates on the origins of life, there is little reference to his work in the scientific literature (Muller 1922, 1947). Furthermore, as historian Iris Fry has remarked, Troland had provided an "abstract model" (Fry 2006, p. 27). Therefore, it is not surprising that he was not on the radar of a bench scientist such as Gilbert, whose interests in origins of life were peripheral to his main research in any case.

Gilbert's comment about history would also help explain his omission of a quartet of works on early life written in the 1960s (Rich 1962; Woese 1967; Crick 1968; Orgel 1968). Written by eminent molecular biologists who were also active in origins of life research, these papers hold an important place in most histories of origins of life theorizing:

> As a group [they] form an intellectually coherent set of hypotheses that signal the scientific shift that was leading to the hegemony of nucleic acid studies over biochemical approaches and that has ever since shaped mainstream origin-of-life research (Lazcano 2012, p. 413).

Despite being personally acquainted with Crick, Orgel, and Rich by then, Gilbert acknowledges that "I didn't know of Francis's paper for some reason. [Although] I was doing molecular biology at the time—in 1968 I was working on DNA replication in Paris—I probably didn't read Alex's, Leslie's or Woese's papers either."

The earliest of these articles—a 1962 essay on the subjects of evolution and biological information transfer by the American biophysicist Alexander Rich—is in fact, regarded by many as the first conceptualization of the RNA world (Lazcano 2012; Neveu et al. 2013; Lehman 2015). In contrast to the ribozyme discovery papers of Cech and Altman, where the topic of the origins of life were raised only in the final sections, Rich, best known for his contributions to nucleic acid chemistry and structure, had constructed a systematic argument for an RNA-first scenario in the origins of life in his article.

> [A] reasonable description of a primitive "living" system might be one in which there is an autocatalytic replication of an information-containing polymer which utilizes monomeric components from the environment and incorporates them into replicas of itself. This system, once started, would then be subject to all the modifying influences of the environment and we could call this the beginning of life (Rich 1962, p. 112).

With this basic structure in place, he went on to suggest that because,

> The sequence of amino acids in proteins is, in a sense, a derivative of the sequence information encoded in the order of nucleotides in some part of the nucleic acids, […] it may be more reasonable to consider a theory of the origin of life in which the nucleic acids were developed as the primary agents (p. 113).

Eventually, Rich speculated, there would evolve a "primitive, self-replicating nucleic acid system […] in which a modification of the replicating ability of the nucleic acids is associated with the polymerization of a polypeptide chain" (p. 118). Citing experimental evidence for the chemical polymerization in vitro of ribonucleosides—i.e., monomers or subunits that make up RNA—in preference over deoxyribonucleosides (Schramm et al. 1962 as cited by Rich 1962, p. 114), together with the existence of RNA viruses as evidence for the ability of RNA to carry genetic information, he suggested that

> [I]t may be reasonable to speculate that the hypothetical stem or parent polynucleotide molecule was initially an RNA-like polymer which was able to convey genetic information as well as organize the amino acids into a specific sequence to make proteins (pp. 123–24).

Reading over Rich's essay, Gilbert feels that although it might be cited in support of an "RNA first" scenario in the evolution of early life, it did not presage the RNA world hypothesis. The paper talks about "RNA as primary to DNA, of systems where RNA came first—before DNA—but not about systems with *only* RNA."

The idea of an RNA world is similarly missing from the three 1968 papers as well, says Gilbert, pointing out furthermore that their focus was on the origins of the genetic code rather than of life itself. While they all discussed the place of nucleic acids in the process, only "Crick came closer to saying something about RNA," he concedes. "tRNA looks like Nature's attempt to make RNA do the job of a protein" Crick had observed previously (1966, p. 7), and in his 1968 paper had speculated that:

> If indeed rRNA and tRNA were essential parts of the primitive machinery, one naturally asks how much protein, if any, was then needed. It is tempting to wonder if the primitive ribosome could have been made entirely of RNA (Crick 1968, p. 371).

But Gilbert stresses that Crick and the others were "speaking of efforts to get the code in place before you have the enzymology. Whereas in my paper I wrote that the enzymes—the fancy enzymology—was already there."

In a retrospective on origins of life research 25 years after they had published their paper, Crick and Orgel "confessed" that they had missed the mark somewhat in anticipating the RNA world:

> We discussed a number of hypothetical schemes for the origins of our genetic system and touched on each of the major features of the RNA world hypothesis. However, we […] took it for granted that RNA-based catalysis was necessarily less efficient than protein-based catalysis [which] led us to underestimate the potential complexity of an RNA world (Orgel and Crick 1993, p. 238).

Consequently, Gilbert can justifiably boast to the claim that his "*Nature* paper was first clear statement that if I had an RNA enzyme that is copying itself, then I have the genetic material [as well as enzymology] and the enzyme is mutating to better behavior."

Still a Happening World? The Status Quo and Future Prospects

Today, 30 years after it was first proposed, the RNA world lives on in the origins of life research community, albeit in a hotly debated, highly contentious atmosphere. Some have loved the idea, others have strongly objected to it, and some, even seem to do both: consider for instance, New Zealand biochemist Harold Bernhardt's charge that the RNA world hypothesis is the "worst theory of the early evolution of life, except for all the others." According to him, the hypothesis, "Although far from perfect or complete, is the best we currently have to help understand the backstory to contemporary biology" (Bernhardt 2012, p. 1).

Anthony Poole, another origin of life researcher from New Zealand, suggests a possible reason for the strong division of opinion on the viability of the RNA world hypothesis:

> I think there has historically been two literatures, with limited overlap, since many chemists have been heavily focused on using in vitro selection [to help] define the parameters for the RNA world, while biologists have looked at whether reconstruction of RNA relics reveals aa historical trace, and how the transitions from RNA to proteins and DNA would have occurred (A.M. Poole, Personal communication via e-mail, 20 July 2016).

Gilbert clearly identifies with the biological side of this divide. He readily accepts that "I am not really a chemist. I call myself a molecular biologist. The main question [for us] is, how do chemical molecules make life happen." His hypothesis, he explains, is

> An origin of life theme based more on an evolutionary attitude than a chemical attitude. The critical thing is that the catalytic system could evolve, not the nature of the underlying catalysis.

It is certainly true that many of the opponents of the RNA world hypothesis have objected to the idea on the basis of chemistry. According to the Swedish microbial geneticist Charles Kurland, "RNA world is an expression of the infatuation of molecular biologists with base pairing in nucleic acids played out in a one-dimensional space with no reference to time or energy." But he adds,

> This is not chemistry. This is genetics. And, when true believers apply their genetic dogma to studies of chemical mechanism, the result is […] a five order of magnitude kinetic discrepancy described as a 'fundamental' similarity.

Consequently, Kurland concluded,

> Regardless of its spontaneous appeal, I suggest that RNA world should now take its place on the shelf of 'nice ideas' along with Aristotle's identifications of whales as fish and the worker bee as a male (Kurland 2010, p. 870).

Meanwhile, proponents of the RNA world, such as Gilbert's colleague Jack Szostak, cite features of modern living systems as evidence for their support of the hypothesis. For example, "When modern cells make proteins, they first copy genes from DNA into RNA and then use the RNA as a blueprint to make proteins. This last stage could have existed independently at first." Even more compelling is the fact that ribozymes "serve a pivotal role in modern cells."

> The structures that translate RNA into proteins are hybrid RNA-protein machines, and it is the RNA in them that does the catalytic work. Thus, each of our cells appears to carry in its ribosomes "fossil" evidence of a primordial RNA world (Ricardo, and Szostak 2009, p. 56).

The idea that living cells carry relics or "molecular fossils" (Jeffares et al. 1998) of the RNA world had, in fact, already been described in detail by Gilbert in his original paper. In a nutshell, he proposed that:

> [T]he RNA world is one of replicating molecules that reassort exons by transposable elements created by introns. […] The relic of this process is the intron/exon structure of genes, left imprinted on DNA from the RNA molecules that earlier encoded proteins, a residue of the basic mechanism of RNA recombination (Gilbert 1986).

But relics, however compelling their evidence, do not recreate the past; they only give hints as to what might have been. Thus far, no one has seen an actual living system—natural or synthetic—that is completely based in RNA. "There is no such thing in reality. No one has yet shown a complete in vitro system for fully RNA-based replication, which I had conjectured in my paper," says Gilbert, who also confesses that he is "disappointed that a self-replicating RNA has not yet been synthesized/discovered in the laboratory" in the years since he predicted his hypothesis. But given the explosion of virtually every field of biology that he has observed since his entry into the discipline, he is optimistic.

"I expect it to emerge eventually," he says, affirming too, that "The RNA World is still very exciting."

Acknowledgements This list of people to thank for bringing this paper to fruition is long, but must begin with Prof. Wally Gilbert who very generously shared a Sunday talking with me about the RNA World and sharing his art as well. The second person is Dr. Antonio Lazcano who was kind enough to forward the call for papers for this special historical issue based on a few email exchanges due to which he learned of my interest in the history of the RNA World. Profuse thanks also to my international network of friends with interests in this area, many of whom I have only met virtually: notably Anthony Poole, Scott Podolsky, and Susie Fisher for their input and insights on various drafts of this paper, and Dhananjay Bambah-Mukku for keeping me in touch with the academic world.

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