In the early part of the 20th Century, the nature of nucleic acid and what its role was within the cell were a bit of a mystery. DNA itself was first isolated as far back as 1869 by the Swiss chemist Johann Friedrich Miescher. He separated nuclei from the cytoplasm of cells and then isolated an acidic substance from these nuclei that he called nuclein. Chemical tests by Miescher showed that nuclein contained large amounts of phosphorus and no sulphur, characteristics that differentiated it from proteins. The first step in determining the structure of nucleic acid (either DNA or RNA) would be to identify its precise composition. RNA was considered a more approachable target for composition analysis because the simple treatment of RNA with hydroxide rapidly and completely hydrolysates the molecule to its individual component nucleotides. DNA, on the other hand, is resistant to such treatment.

In 1909, Phoebus Levene observed that yeast ribonucleic acid was composed of approximately equal amounts of the two purines (adenine and guanine) and the two pyrimidines (cytosine and uracil). Based on this, and by determining the type of linkages that joined the nucleotides together, Levene and Henry Simms proposed a tetranucleotide structure to explain the chemical arrangement of nucleotides within nucleic acids. In this theory, they proposed a very simple four-nucleotide unit that was repeated many times to form long nucleic acid molecules. The simplistic nature of the tetranucleotide structure led to the widespread acceptance that nucleic acids would be incapable of providing the chemical variation expected of the genetic material. We would have to wait until the seminal experiments of Oswald Avery, Colin MacLeod and Maclyn McCarty were published in 1944 to discover otherwise.

In the late 1940s and early 1950s, Roy Markham and John D. Smith, working at the Agricultural Research Council’s Virus Research Unit at the Molteno Institute, in Cambridge, published a raft of papers — predominately in the Biochemical Journal — in which they described techniques for the analysis of nucleotides formed by the hydrolysis of RNA. In these articles, they described how to separate, by paper electrophoresis, single nucleotides and small oligonucleotides obtained from the hydrolysis of RNA from the genomes of plant and animal viruses. They showed that under the influence of a high voltage, the ribonucleotides and oligoribonucleotides migrate across the moist paper with mobilities that decrease with increasing size. The methods they described represented the first analysis of individual components of nucleic acid. These papers not only paved the way for the more precise analysis of RNA and DNA molecules, but also provide intriguing insights into the structure of RNA.

Figure 1. The transphosphorylation (top) and hydrolysis (bottom) reactions catalysed by ribonucleases
and Lord Alexander Todd, was correct. The hydrolysis reaction proceeds in two steps: transphosphorylation of RNA to form a 2′,3′-cyclic phosphodiester (N_p) intermediate and hydrolysis of this cyclic intermediate to form a 3′-phosphomonoester (N_p) (Figure 1). The second of these papers investigated the smaller products of ribonuclease digestion. Several dinucleotides were identified and it was discovered that the cyclic forms of the dinucleotides were formed first and that the subsequent hydrolysis was slow.

Subsequent work from Markham's laboratory showed that the natural configuration of the purine nucleotides in RNA was 3′–5′ rather than the alternative suggestion of 2′–5′. Further evidence for this type of link was obtained from subsequent studies of the action of nucleases on mononucleotide esters carried out by others.

Reading through the Markham and Smith papers is an absolute joy. I came away with an overwhelming feeling of pioneering work taking place. The deductions of the linkages that must occur between nucleotides within the RNA chain based on their breakdown products was certainly an important advance in understanding a long, repetitive molecule. Others who subsequently solved the three-dimensional structure of nucleic acids may have attracted bigger accolades, but, without the pioneering work to determine the precise chemical linkages, those structures may not have been calculated until much later.

The first of this pair of papers that appeared in the Biochemical Journal (‘The structure of ribonucleic acid. I. Cyclic nucleotides produced by ribonuclease and by alkaline hydrolysis’) describes the methods employed for the analysis of the products of the digestion of RNA both with ribonuclease and through hydrolysis with alkali. The cyclic nucleotides (2′,3′-monohydrogen phosphate esters of nucleosides) produced by this treatment and analysed by ‘paper strip electrophoresis’ proved that the previously published chemical scheme for the alkali hydrolysis of RNA, by Daniel Brown and Lord Alexander Todd, was correct. The hydrolysis reaction proceeds in two steps: transphosphorylation of RNA to form a 2′,3′-cyclic phosphodiester (N_p) intermediate and hydrolysis of this cyclic intermediate to form a 3′-phosphomonoester (N_p) (Figure 1). The second of these papers investigated the smaller products of ribonuclease digestion. Several dinucleotides were identified and it was discovered that the cyclic forms of the dinucleotides were formed first and that the subsequent hydrolysis was slow.

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References

1. Miescher, F. (1871) Über die chemische Zusammensetzung der Eiterzellen. Hoppe-Seyler's medicinisch-chemische Untersuchungen 4, 441–460
On a personal note, reading through these classic papers makes me realize just how lucky we are to be able to practice science today. The description of a how to build a 1000 V power supply for the electrophoresis experiments seems alien to those of us who have gone through our entire scientific careers by being able to dip into catalogues to buy the ‘standard’ molecular biology equipment off-the-shelf. In addition, the electrophoresis apparatus itself — being constructed of three museum jars with paper wicks sticking out of them — has a distinctly Heath Robinson feel to it and the chromatograms produced have a distinctly smeary nature to them. However, as with many pioneering experiments, the interpretation of what may not necessarily be the clearest data can lead to important breakthroughs.

The methods initiated by Markham and Smith were widely and rapidly disseminated. Most famously, perhaps, the paper chromatography method was employed by Erwin Chargaff to perform experiments to address the chemical composition of DNA at Columbia University, New York. He hydrolysed DNA into its constituent nucleotides by treatment with strong acid, and then separated the nucleotides using similar methods to those described by Markham and Smith. As we all know, Chargaff’s experiments showed that the relative ratios of the four bases in DNA were not equal, but were also not random. The number of adenine (A) residues in all DNA samples was equal to the number of thymine (T) residues, while the number of guanine (G) residues equalled the number of cytosine (C) residues. This finding, combined with the X-ray diffraction data from Maurice Wilkins and Rosalind Franklin, formed the basis of Watson and Crick’s 1953 iconic double-helical DNA structure model. Much later, the procedures developed within the pages of Markham and Smith’s papers were modified into the separation of nucleic acid fragments by gel electrophoresis for the analysis and sequencing of both RNA and DNA.