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Here I describe early research on RNA structure and the discovery of the DNA-RNA hybrid helix, a key component of information transfer. More than 50 years ago it was realized that the interaction between DNA and RNA was at the core of molecular biology. The problem was chemical in nature: could two different types of molecules interact and serve in the transmission of biological information?

In the 1950s it was widely assumed that “DNA makes RNA, RNA makes protein.” This was not based on experimental evidence that DNA and RNA could combine but was more in the nature of an intuitive belief. However, by early 1960 I was finally able to carry out a direct experiment, the first DNA-RNA hybridization. In 1960, messenger RNA was still 1 year in the future, and there was not a great deal of understanding of the major components of information transfer. The DNA double helix proposed by Watson and Crick in 1953 (1) clearly suggested that information was contained in the order of nucleotides, but during the 1950s our understanding of RNA was fragmentary.

The origins of the 1960 experiment go back to the mid-1950s. In 1954, while at Caltech, Jim Watson and I had been trying to find out if RNA by itself could form a double helix, but the fiber diffraction studies of RNA gave inconclusive results (2, 3). The fuzzy diffraction patterns all looked alike, but unlike DNA, the RNA base ratios were all different in the samples we examined. It was clear that RNA was more complex than DNA, and its structure was unknown. In their 1953 paper Watson and Crick (1) pointed out that it was probably impossible to form their double helix with ribose due to van der Waals interference of the 2’-OH with the structure. Thus, it was likely to be different.

A New Type of Polymer Chemistry

The research changed dramatically at the National Institutes of Health (NIH) in 1956 when David Davies and I began working with synthetic polyribonucleotides made using the polynucleotide phosphorylase enzyme discovered by Grunberg-Manago and Ochoa (4). When we mixed together polyriboadenylic acid (poly(rA)) and polyribouridylic acid (poly(rU)) and pulled fibers, they yielded a clear diffraction pattern of a double helix (5). The two molecules had combined to make an RNA double helix! This was a new type of chemical reaction, one that involved thousands of units binding specifically to each other in an extended array. Furthermore, no polymer reaction had ever been seen in which the monomers from two polymers bound together with great spec-
Two weeks after sending off the 1956 *Journal of the American Chemical Society* note, I wrote a letter to my postdoctoral mentor, Linus Pauling, describing these results. The letter reveals a sense of incredulity on my part that this reaction could happen and that it was “completely reproducible.” The experimental demonstration that these molecules could form a double helix seems obvious today. However, it was a considerable surprise at that time and was often greeted with skepticism. Most biochemists felt that a double helix could only be made by an enzyme, such as the one discovered by Arthur Kornberg and associates, which appeared to replicate the DNA double helix (6). Polymer chemists felt that very long molecules involving thousands of nucleotides would probably become entangled and could not sort themselves out to form a regular double helix. Still other researchers, on theoretical grounds, felt that two highly negatively charged polymers were unlikely to combine to make a single structure.

However many scientists were receptive. In the early fall of 1956, a McCollum Pratt meeting was organized at Johns Hopkins University in Baltimore around the subject of the “Chemical Basis of Heredity.” It was an excellent meeting with all of the major research workers in the field. In my talk (7) I included a discussion of the specificity of the interaction and the fact that these long molecules seek each other out in solution and adopt this elongated helical form. Julian Huxley, a prominent scientist and writer, came up to me after my talk and warmly congratulated me for having discovered “molecular sex.”

Gradually, the idea became fixed in the thinking of biologists and biochemists that these long nucleic acid molecules had considerable flexibility and could be made to form helical structures in solution. Although no one used the phrase at the time, this was the first hybridization reaction, and it represented a paradigm shift in the way chemists and biochemists thought about macromolecular nucleic acids.

**Two Different Structures**

Watson and Crick (1) pointed out that RNA would not form the DNA structure. This was confirmed as the RNA x-ray diffraction pattern revealed significant differences from the DNA double helix pattern. The diameter of the RNA double helix was 6 Å larger than the DNA double helix (7, 8). Analysis of the diffraction pattern revealed that the first layer line of the RNA-RNA duplex was stronger than the second—a reversal from that seen in the diffraction pattern of the DNA-DNA duplex (7, 8). Another significant difference was that alterations in relative humidity changed the DNA double helix, producing both the A and B forms, whereas the RNA double helix seemed invariant to changes in relative humidity.

The reaction between poly(rA) and poly(rU) was associated with a drop in optical density in the ultraviolet (9), a property that could be used to identify the 1:1 stoichiometry and analyze the reaction quantitatively (10). Only much later was it realized that the RNA duplex structure was close to the dehydrated A conformation of DNA duplex. However, by 1960, it was clear that DNA and RNA duplexes were significantly different.

In 1957, together with Felsenfeld, we discovered that the poly(rA)-poly(rU) duplex could take on a third strand of poly(rU) to form a triple helix (11). We pointed out that this could be associated with forming two hydrogen bonds between the incoming uracil O4 and N3 and adenine N6 and N7, an interaction of bases that was confirmed 2 years later by Hoogsteen in a single crystal x-ray analysis (12). Between 1956 and 1960 a number of experiments were carried out with polynucleotides of different composition (13–15), and their interactions could generally be explained in terms of the ability of the bases to form at least two hydrogen bonds in the center of the molecule.

Could DNA and RNA interact? Polymers of ribonucleotides were available because of the discovery of polynucleotide phosphorylase (4). This made it possible to produce polynucleotides with a variety of compositions, but there was no analogous method of producing DNA polymers. The question “How does DNA make RNA?” remained an open issue. Several biochemists were trying to isolate the enzyme known as RNA polymerase which is dependent upon a DNA template. Experiments by Stevens, Hurwitz, and Weiss developed preparations that had some activity, but they were not purified enough to show what was actually happening. In a reflective article published in 1959 called “An Analysis of the Relation between DNA and RNA,” I surveyed the various possibilities for DNA-RNA interactions (16). In particular, I asked whether RNA synthesis could be based on a double-stranded primer or a single-stranded primer of DNA. The discovery of several triple helical complexes of RNA molecules made it reasonable to consider a model in which RNA nucleotides were assembled by binding in a sequence-specific manner to double-stranded DNA. The analysis showed that such a model was unlikely because there were not enough stereospecific interactions to specify an RNA molecule. I concluded that it was likely to be based on forming an RNA strand on a single-stranded primer (16). These conclusions were fortified by the recent discoveries that denatured DNA could provide a primer for Kornberg’s DNA polymerase enzyme (17), and fur-
thermore, Sinsheimer had discovered that the virus 8X174 had a single-stranded genome (18) and it was a primer for the DNA polymerase. This reinforced the idea that single strands were adequate templates. In the same article (16), I speculated that RNA was likely to be the first polynucleotide molecule in the origin and early evolution of life and also pointed out the possibility of an enzyme that used a single-stranded RNA primer to make a DNA strand; it was called reverse transcriptase when it was discovered 10 years later.

**Formation of a Hybrid Helix**

Could a DNA-RNA hybrid helix form and have the stability needed so that it could be used for information transfer in view of the different physical properties and geometries of the RNA and the DNA duplex? I was finally able to address this problem with the chemical synthesis of oligodeoxythymidylic acid by Khorana and colleagues (19). Khorana kindly gave me a reaction mixture, which I fractionated and took the longer molecules to see if they would react with polyriboadenylic acid. The experiment worked (20)! The discovery in 1960 that these could form a double helix represented the first experimental demonstration that a hybrid helix could be a method for the transfer of information from DNA to RNA. The evidence was based on measurements of the hypochromism, which occurs when helical molecules are formed, and the changes in sedimentation rate associated with complex formation. These results indicated that DNA could make RNA by using a single-stranded template to make a complementary RNA strand, and it was a model for RNA polymerase activity. However, the discovery of messenger RNA was still 1 year in the future.

It is interesting that 1 year later in 1961, with a more highly purified preparation of RNA polymerase and using as a template the same oligodeoxythymidylicate synthesized by Khorana, Hurwitz was able to synthesize polyriboadenylic acid using his enzyme preparation (21). This proved that a single-stranded template was adequate for RNA polymerase.

The publication in the summer of 1960 (20) was the first demonstration of DNA-RNA hybridization although that term had not yet been invented. That particular hybridization is still widely used today in that immobilized oligo(dT) molecules are used to isolate eukaryotic messenger RNA through their poly(rA) tails. At the same time in 1960, Marmur, Doty, and colleagues found they could take denatured DNA molecules and hold them for a prolonged period at an intermediate temperature, called an annealing temperature, thereby allowing the DNA molecules to find each other and re-form a double helix (22, 23). After presenting my work on DNA-RNA interactions at the Gordon Conference in 1960, Sol Spiegelman and Ben Hall came up to me and said they were inspired to try that reaction with a viral system. One year later they combined my work with the annealing work of Marmur and Doty and found that a newly synthesized RNA strand from T2 virus infection could be similarly annealed with DNA from the virus to make a hybrid helix (24). Thus, by 1961 both DNA-DNA and RNA-DNA hybridizations were available for a variety of biological studies.

The significance of the discovery of the DNA-RNA hybrid helix is relevant not only to understanding the activity of RNA polymerase, but it also explains how reverse transcriptase works, as well as telomerase, retrotransposons, and a variety of other interactions in which DNA and RNA strands combine. Today, fluorescence in situ hybridization is used in which DNA-RNA hybrids are formed to identify specific areas of the genome. Similarly, microarray gene expression profiling studies use DNA-RNA hybrid formation and are dependent on this 1960 discovery.

The full structural analysis of how these RNA and DNA chains accommodated each other was not revealed until much later. In 1982 the first DNA-RNA single-crystal hybrid molecule was solved in my laboratory (25). It showed that a DNA decamer containing four ribonucleotides formed a hybrid segment that stabilized the entire molecule in the A conformation. In 1992, we carried out a single-crystal x-ray analysis of an Okazaki fragment in which the nucleating ribonucleotides that initiate DNA synthesis in DNA replication were crystallized on DNA (26). Again, the presence of a few ribonucleotides was sufficient to convert the entire fragment into the A conformation.

The central role of the ribose ring puckers that differ in DNA and RNA is widely understood today. The van der Waals crowding of the ribose C2’ oxygen determines the RNA pucker in the RNA double helical A conformation. This interaction provides a sufficient energy barrier to prevent a change in pucker in double helical RNA, in contrast to the facile manner with which the deoxyribose in DNA changes its ring pucker on lowering the water content to form A-DNA or to accommodate the presence of RNA in a hybrid double helix.

The hybrid DNA-RNA helix remains the bedrock of information transfer in biological systems. Indeed, the existence of a hybrid helix seems so obvious today that young research workers simply take it for granted. There is little realization of the extent to which, almost a half-century ago, scientists wrestled with problems of understand-
ing how different polymers can react together to make a stable structure. The roots of our understanding of hybrid helix formation go back to 1960 and even further to the mid-1950s.

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Discovery of the Hybrid Helix and the First DNA-RNA Hybridization
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doi: 10.1074/JBC.X600003200

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