

Cracking the Genetic Code

By the early 1960s molecular biologists had adopted the so-called “central dogma,” which states that DNA directs synthesis of RNA (transcription), which then directs assembly of proteins (translation). However, researchers still did not completely understand how the “code” embodied in DNA and subsequently in RNA directs protein synthesis. To elucidate this process, Marshall Nirenberg embarked upon a series of studies that would lead to solution of the genetic code.

Background

Proteins are made from combinations of 20 different amino acids. The genes that encode proteins—that is, specify the type and linear order of their component amino acids—are located in DNA, a polymer made up of only four different nucleotides. The DNA code is transcribed into RNA, which is also composed of four nucleotides. Nirenberg’s studies were premised on the hypothesis that the nucleotides in RNA form “codewords,” each of which corresponds to one of the amino acids found in protein. During protein synthesis, these codewords are translated into a functional protein. Thus, to understand how DNA directs protein synthesis, Nirenberg set out to understand the relationship between RNA codewords and protein synthesis.

At the outset of his studies, much was already known about the process of protein synthesis, which occurs on ribosomes. These large ribonucleoprotein complexes can bind two different types of RNA: messenger RNA (mRNA), which carries the exact protein-specifying code from DNA to ribosomes, and smaller RNA molecules now known as transfer RNA (tRNA), which deliver amino acids to ribosomes. tRNAs exist in two forms: those that are covalently attached to a single amino acid, known as amino-acylated or “charged” tRNAs, and those that have no amino acid attached called “uncharged” tRNAs. After binding of the mRNA and the amino-acylated tRNA to the

ribosome, a peptide bond forms between the amino acids, beginning protein synthesis. The nascent protein chain is elongated by the subsequent binding of additional tRNAs and formation of a peptide bond between the incoming amino acid and the end of the growing chain. Although this general process was understood, the question remained: How does the mRNA direct protein synthesis?

When attempting to address complex processes such as protein synthesis, scientists divide large questions into a series of smaller, more easily addressed questions. Prior to Nirenberg’s study, it had been shown that when phenylalanine-charged tRNA was incubated with ribosomes and polyuridylic acid (polyU), peptides consisting of only phenylalanine were produced. This finding suggested that the mRNA codeword, or codon, for phenylalanine is made up of the nucleotides containing the base uracil. Similar studies with polycytidylic acid (polyC) and polyadenylic acid (polyA) showed these nucleotides containing the bases cytosine and adenine made up the codons for proline and lysine, respectively. With this knowledge in hand, Nirenberg asked the question: What is the minimum chain length required for tRNA binding to ribosomes? The system he developed to answer this question would give him the means to determine which amino-acylated tRNA would bind which mRNA codon, effectively cracking the genetic code.

The Experiment

The first step in determining the minimum length of mRNA required for tRNA recognition was to develop an assay that would detect this interaction. Since previous studies had shown that ribosomes bind mRNA and tRNA simultaneously, Nirenberg reasoned that ribosomes could be used as a bridge between a known mRNA codon and a known tRNA. When the three components of protein synthesis are incubated together in vitro, they should form a complex. After devising a method to detect this complex, Nirenberg could then alter the size of the mRNA to determine the minimum chain length required for tRNA recognition.

Before he could begin his experiment, Nirenberg needed both a means to separate the complex from unbound components and a method to detect tRNA binding to the ribosome. To isolate the complex he exploited the ability of nylon filters to bind large RNA molecules, such as ribosomes, but not the smaller tRNA molecules. He used a nylon filter to separate ribosomes (and anything bound to the ribosomes) from unbound tRNA. To detect the tRNA bound to the ribosomes, Nirenberg used tRNA charged with amino acids that contained a radioactive label, ^{14}C . All other components of the reaction were not radioactive. Since only ribosome-bound tRNA is retained by the nylon

membrane, all radioactivity found on the nylon membrane corresponds to tRNA bound to ribosomes. Now, a system was in place to detect the recognition between an mRNA molecule and the proper amino-acylated tRNA.

To test his system, Nirenberg used polyU as the mRNA, and ^{14}C -phenylalanine-charged tRNA, which binds to ribosomes in the presence of polyU. Ribosomes were incubated with both polyU and ^{14}C -phenylalanine tRNA for sufficient time to allow both molecules to bind to the ribosomes; the reaction mixtures were then passed through a nylon membrane. When the membranes were analyzed using a scintillation counter, they contained radioactivity, demonstrating that in this system polyU could recognize phenylalanine-charged tRNA. But was this recognition specific for the proper amino-acylated tRNA? As a control, ^{14}C -lysine and ^{14}C -proline-charged tRNAs also were incubated with polyU and ribosomes. After the reaction mixtures were passed through a nylon filter, no radioactivity was detected on the filter. Therefore, the assay measured only specific binding between an mRNA and its corresponding amino-acylated tRNA.

Now, the minimum chain length of RNA necessary for proper amino-acylated tRNA recognition could be determined. Short oligonucleotides were tested for their ability to bind ribosomes and recognize the appropriate tRNA.

When UUU, a trinucleotide, was used, tRNA binding to ribosomes could be detected. However, when the UU dinucleotides was used, no binding was detected. This result suggested that the codon required for proper recognition of tRNA is a trinucleotide. Nirenberg repeated this experiment on two other homogeneous trinucleotides, CCC and AAA. When these trinucleotides were independently bound to ribosomes, CCC specifically recognized proline-charged tRNA and AAA recognized lysine-charged tRNAs. Since none of the three homogeneous trinucleotides recognized other charged tRNAs, Nirenberg concluded that trinucleotides could effectively direct the proper recognition of amino-acylated tRNAs.

This study accomplished much more than determining the length of the codon required for proper tRNA recognition. Nirenberg realized that his assay could be used to test all 64 possible combinations of trinucleotides (see Figure 4.1). A method for cracking the code was available!

Discussion

Combined with the technology to generate trinucleotides of known sequence, Nirenberg's assay provided a way to assign each specific amino acid to one or more specific trinucleotides. Within a few years, the genetic code was cracked, all 20 amino acids were assigned at least one trinucleotide, and 61 of the 64 trinucleotides were found to correspond to an amino acid. The final three trinucleotides, now known as "stop" codons, signal termination of protein synthesis.

With the genetic code cracked, biologists could read the gene in the same manner that the cell did. Simply by knowing the DNA sequence of a gene, scientists can now predict the amino acid sequence of the protein it encodes. For his innovative work, Nirenberg was awarded the Nobel Prize for Physiology and Medicine in 1968.

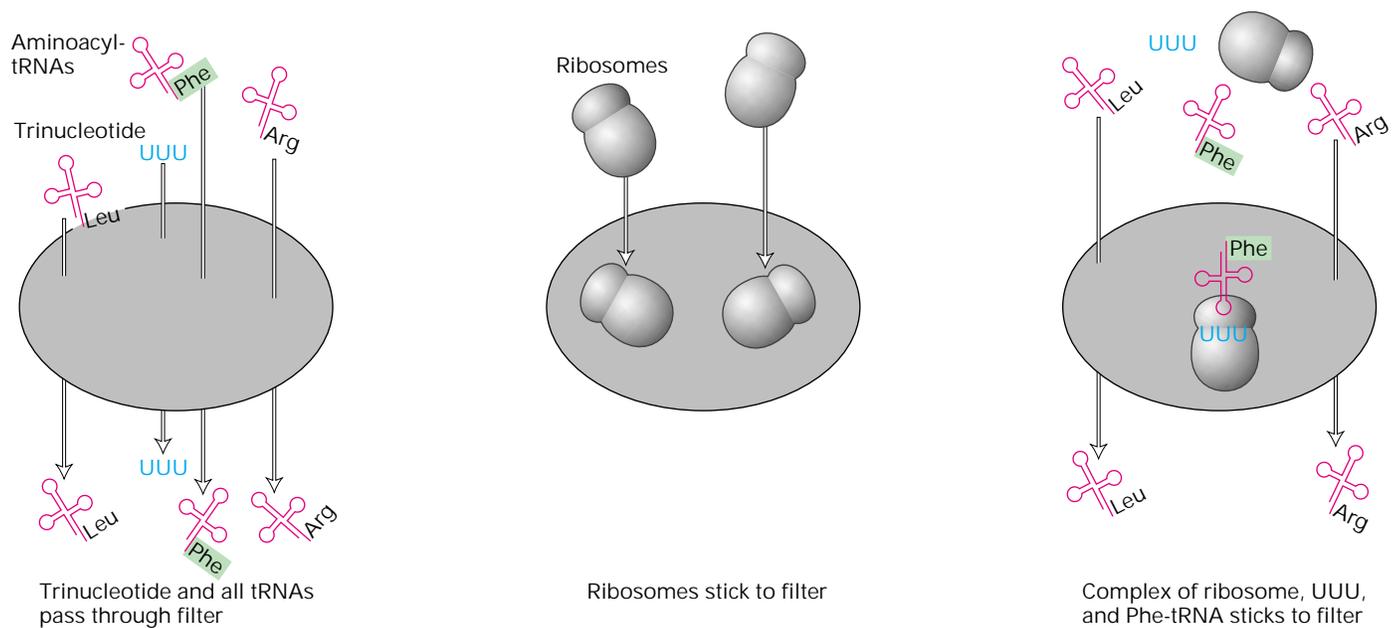


Figure 4.1

Assay developed by Marshall Nirenberg and his collaborators for deciphering the genetic code. They prepared 20 *E. coli* extracts containing all the aminoacyl-tRNAs (tRNAs with amino acid attached). In each extract sample, a different amino acid was radioactively labeled (green); the other 19 amino acids were present on tRNAs but remained unlabeled. Aminoacyl-tRNAs and trinucleotides passed through a nylon filter without binding (*left panel*); ribosomes, however, bind to the filter (*center panel*). Each of the 64 possible trinucleotides was tested separately for its ability to attract a specific tRNA by adding it with ribosomes to different extract samples. Each sample was then filtered. If the added trinucleotide causes the radiolabeled aminoacyl-tRNA to bind to the ribosome, then radioactivity is detected on the filter; otherwise, the label passes through the filter (*right panel*). By synthesizing and testing all possible trinucleotides, the researchers were able to match all 20 amino acids with one or more codons (e.g., phenylalanine with UUU as shown here). [From H. Lodish et al., 1995, *Molecular Cell Biology*, 3rd ed. W. H. Freeman and Company. See M. W. Nirenberg and P. Leder, 1964, *Science* 145:1399].