Molecular Biology Through Discovery
Jones OW & Nirenberg MW (1962). *Qualitative Survey of RNA Codewords.*
Proc Natl Acad Sci USA 48:2115-2123

I. Introduction

In October 1957, the world was astounded when the Soviet Union launched into orbit Sputnik, the world's first artificial satellite. The space race that followed culminated in 1969 with the event that permanently reversed the meaning of the ancient phrase, "You have as much chance of doing that as going to the moon!" Four years after Sputnik, another race began, this one aimed at ending the status of the gene as an unknowable abstraction that connects heredity to cellular function. Again, the race began in the Soviet Union.

The fifth International Congress of Biochemistry was held in Moscow, August 1961. It was a huge meeting – many thousands of attendees – and the first major scientific meeting hosted in the Soviet Union. It had a second significance. Biology in the Soviet Union was still in the grip of Lysenkoism, which rejected any dissent from the official position of environmentally acquired inheritance, particularly Mendelian genetics, Morgan's notion of the gene, and molecular genetics in general. This Congress gave Soviet molecular biologists ammunition to exposing Lysenkoism by showcasing the advances in molecular biology in the past 10 years.

But what has been remembered from the Moscow Congress was a talk attended by almost no one.[1] One person who ***was*** there, Matt Meselson, was astonished by what he heard. The speaker was Marshall Nirenberg, a little known researcher at the National Institutes of Health (not in those days the major hub of research as it is now). After the talk, Meselson prevailed upon Francis Crick, who was the chair of the final session of the meeting to give Nirenberg a wider audience. Nirenberg repeated his presentation at that session, this time with hundreds listening.

What Nirenberg said was that he and Heinrich Matthaei (**Fig. 1**) had succeeded in translating an artificial RNA, poly-U, in a cell free system and had determined that the product was poly-phenylalanine. That, coupled with the consensus belief that the code was triplet,[2] meant that the first codon had been discovered: UUU, encoding phenylalanine.

Figure 1: Heinrich Matthaei (left) and Marshall Nirenberg (right), 1961. (Courtesy of N. MacIvar, National Institutes of Health)

This announcement gave those in attendance (in Meselson's words) "*…the immediate itch to get out of Moscow, to get back to the lab.*"[1] In the next six years, there was an explosion of research activity, and by 1967, two years before the landing on the moon, the genetic code that governs the translation of DNA of all life on earth had been fully elucidated.

We'll now examine one of many articles from that period as an example of the types of experiments that led to the breaking of the code.

II. Breaking of the code: An examination of Jones et al (1962)

This would be a good time to get the full text of Jones & Nirenberg (1962).

II.A. Introduction

As you skim the article (always a good initial tactic) you'll see that it has four tables that look very similar to each other, at least in format, plus two summary tables. That's it. The work is based on a single sort of experiment, repeated four times. All we need to do is to understand that one experiment.

That experiment, and all others during the first heady years, made use of an enzyme, polynucleotide phosphorylase (PNP), discovered a few years earlier by Severo Ochoa and Marianne Grunberg-Manago. This enzyme is able to synthesize RNA given free ribonucleotides (we now know that it functions within the cell not in RNA synthesis but in RNA degradation). PNP plus the nucleotide triphosphate UTP yielded poly-U, and this was the source of the RNA that produced the results Nirenberg reported in Moscow.[3] You might think it an obvious strategy to use PNP to synthesize poly-A, poly-C, and poly-G, throw them into the in vitro protein synthesis system and deduce three more codons,… but this strategy worked poorly, if at all.

SQ1. From your current knowledge of the genetic code, what proteins would you expect from translation of the four possible homopolymers, poly-A, poly-C, poly-G, and poly-U?

It turned out that poly-G interacts with itself and could not be translated.[4] The system translated poly-A to poly-lysine (unbeknownst to the many who tried), but the product escaped detection, because poly-lysine is so basic that it did not precipitate under the standard conditions.[5] Poly-C did lead to a minor enrichment in proline incorporation, but the effect was slight, for unknown reasons. As a result, the initial experiments focused on poly-U and other RNAs in which U predominated.

We know now that the problems were all of merely a technical nature, but at that time the authors were concerned that maybe they were encountering a rule of Nature: perhaps all codons contain U.

With that in mind, take a look at the Introduction to the article.

SQ2. What was the question addressed by this article?

II.B. The experiment

From Tables 1 through 4, you can gather that the experiments measured amino acid incorporation (into protein) in the presence of some polynucleotide (RNA). We'd like to know how was protein synthesis accomplished and what kind of RNA was being used. Unfortunately, the article does not speak directly to these questions. Instead, the Materials and Methods section, first paragraph, points us to other articles. Let me save you a trip this time (and see Ref 6 for an illustration of the procedure).

The experiment relies on adding artificial RNA, so that means that all reactions must take place in a test tube, not in a cell. How do you get protein synthesis to work in a test tube? At this time, protein synthesis was pretty much a mystery. Therefore, the best they could do was to break open cells and try to keep the cell extract sufficiently happy to continue synthesizing proteins, as it did when the cell was alive. Protein synthesis was therefore a black box. But this black box was not good enough. The cell extract comes with its own RNA, which would mask the effect of the added artificial RNA. The solution of Nirenberg and others was to destroy the ***DNA*** in the extract (by the enzyme deoxyribonuclease) and wait. After thirty minutes, the endogenous RNA had disappeared, since mRNA is short-lived, and with no DNA to transcribe more RNA, the cell extract was largely mRNA-free.

SQ3. Why not add an enzyme (ribonuclease) to destroy the RNA directly?

After the waiting period, artificial RNA was added, along with all nineteen amino acids and one radioactively labeled amino acid. The experiment was repeated 20 times, with a different labeled amino acid each time. After a suitable period to allow protein synthesis to take place, strong acid was added to precipitate protein, leaving unincorporated amino acids in solution. The amount of radioactivity in the precipitate was counted and indicated the amount of the specific amino acid that had been incorporated into protein.

The artificial RNA was made by PNP, provided with nucleotides in the desired proportions. PNP dutifully synthesized RNA according to these proportions, incorporating the nucleotides at random. For example, if PNP were given ATP and CTP in the ratio of 1:5, then something like the following was produced:

CCCCCCACCCCCCACCCCAAAACCCCCCCCCCCCACCCCCACCCCACCCC

SQ4. Is this sequence consistent with ATP and CTP in the ratio of 1:5?

II.B. A result

Consider Table 3, the experiment designated "J108" (sixth column).

SQ5. Bearing in mind how the artificial RNA was made, how do you interpret "A 80" "C 20"?

SQ6. List the triplet codons that are composed solely of A's and C's?

SQ7. Given an RNA that's composed solely of A's and C's, in the ratio of 80:20, list the probability of encountering each of the possible triplet codons.

SQ8. Suppose that there are 707 units of codons in an artificial RNA composed solely of A's and C's in the ratio of 80:20. Calculate how many units you would expect of each of the possible triplet codons.

SQ9. Using your current knowledge of the genetic code, assign amino acids to each of the codons you listed in SQ7 and, using your answer to SQ8, determine the number of units of each amino acid you'd expect upon translation of the artificial RNA.

SQ10. Take a look at the results of experiment J108. What do the numbers signify? Compare them to your predictions from SQ9.

SQ11. What amino acids in experiment J108 have non-zero values that you predict should not have them? Can you think of any reason why they do?

It's time now to take a look at the last column, entitled "Minus polynucleotide control". These values were obtained by running the same protein synthesis system but this time with no added RNA.

SQ12. How do you account for these numbers? Why is there any incorporation of amino acid at all?

SQ13. Reinterpret the numbers for experiment J108, in light of the minus polynucleotide control and the footnote at the bottom of the table. What do the numbers mean? What makes them suspicious?

SQ14. Draw what conclusions you can regarding associations between specific codons and specific amino acids.

This strategy was used in a large number of experiments, but you can see that it can take you only so far. The next technical advance came from Gobind Khorana (**Fig. 2**), who pioneered the synthesis of short oligonucleotides and used them to synthesize artificial RNA's composed of repeating units, e.g. poly(AC) = "ACACACACACACAC…". These were very useful in obtaining further codon assignments.[7,8]

SQ15. How could such artifical RNA's go beyond what was possible with the tools of Jones & Nirenberg (1962)?

SQ16. Try it yourself! Use the tools of Nirenberg and Khorana to deduce the genetic code of an alien organism. See *Alien Genetic Code*.[9]

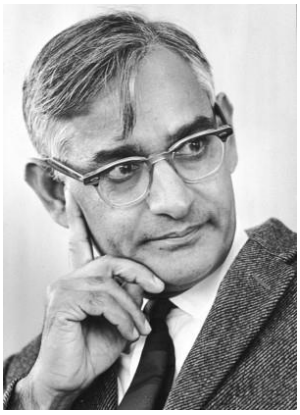

Figure 2. H. Gobind Khorana.
(courtesy of Wisconsin State Journal)

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