



Research Article

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My Discovery: Matthaei and Nirenberg's Poly-U has No Triplet Nature

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Abstract

This article reports my findings as follows:

a) Till now, there is no report indicating that phenylalanine has a chemical property of the “3 of what”. This indicates that the triplet nature of poly-U is unnecessary for phenylalanine incorporation into protein;

b) Obviously, the insertion or deletion of one or two uracil molecule from poly-U will not cause Matthaei and Nirenberg's experimental result to change to non-phenylalanine amino acids, which strictly rejects the Crick-Brenner “insertion or deletion” genetic experiments;

c) Clearly, the triplet nature of poly-U is ultimately expressed by the anti-codon region of phe-tRNA, not by the internal chemical structure of poly-U itself;

d) Using simple mathematics, both UUUU.....UUUUUU and phe-phe-phe.....phe-phe are mathematical lines made up of identical points, there is no math theorem to prove that UUUU.....UUUUUU is made up of many UUUs. These findings indicate that Matthaei and Nirenberg did not decipher the first genetic code UUU in 1961.

Keywords: Matthaei and Nirenberg, Poly-U, Triplet nature

Significance Statement

In 1962, Dr. Crick stated in his Nobel Lecture: “The breakthrough in the coding problem has come from the discovery, made by Nirenberg and Matthaei” [1,2]. Today, poly-U's triplet nature has become the most fundamental theory in molecular biology textbooks. However, chemists and biologists can easily judge the differences between UUUU.....UUUUUU and UUU-UUU-U.....UUU-UUU-UUU. This article might launch a large campaign of revising Watson's series textbooks of <Molecular Biology of the Gene>.

On the Triplet Nature of Poly-U

In the research article <General Nature of the Genetic Code for Proteins> published on Nature in Dec 30, 1961, Crick and Brenner were the first to claim the discovery of the triplet nature of poly-U, writing that “at the recent Biochemical Congress at Moscow, the audience of Symposium I was startled by the announcement of Nirenberg that he and Matthaei had produced *polyphenylalanine* (*this is, a polypeptide all the residues of which are phenylalanine*)

by adding polyuridylic acid (that is, an RNA the bases of which are all uracil) to a cell-free system, which can synthesize protein. This implies that a sequence of uracils codes for phenylalanine, and our work suggests that it is probably a triplet of uracils" [3].

One year after the discovery of triplet nature of poly-U and the discovery of first genetic code UUU, the double helical model of DNA obtained the Nobel Prize for medicine or biophysics in 1962. On the Nobel Prize rewarding ceremony, Crick delivered a speech <On the Genetic Code> which precisely described the "UUU" and predicted the future $4 \times 4 \times 4 = 64$ triplets. For the relationship between the triplet nature and Crick-Brenner's experiment, in March 1963, Crick stated "the main evidence for a wholly triplet code is from

the genetic work on the rII gene of phage T₄, and this can only be accepted with reservations" [4]. H. G. Wittman from the Max-Planck Institute for Biology also stated in 1963 that "the investigation of Crick et al. with chemically induced mutants of bacteriophage T₄ indicate that an amino acid is determined by a group of three adjacent nucleotides, i.e., a triplet" [5].

About UUU and "UUU-UUU-UUU-UUU-UUU"

Looking back the history, the original and theoretical idea of the triplet nature of nucleic acids was initiated by G. Gamow, et al. [6] shown in (Figure 1). George Gamow, "Possible relation between deoxyribonucleic acid and protein structures," Nature [6]. © 1954 Macmillan Magazines Ltd. Reprinted by permission.

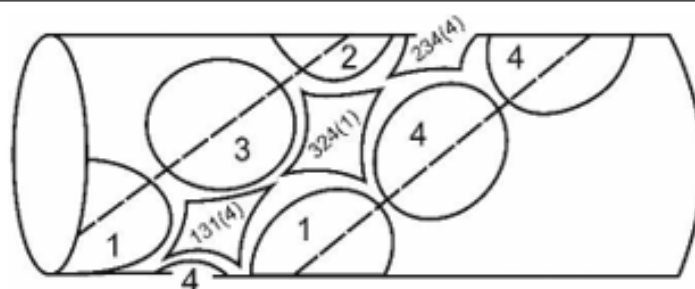


Figure 1: "Shows schematically the structure of the deoxyribonucleic acid molecule as derived by Watson and Crick" [6].

Notes:

- Even today, if the Figure captions by Gamow are not followed, I think it would be impossible for any chemist to recognize this diagram as the Watson-Crick DNA model. Clearly, the chemical properties of adenine ($C_5H_5N_5$), guanine ($C_5H_5N_5O$), cytosine ($C_4H_5N_3O$), and thymine ($C_5H_6N_2O_2$) are more complicated than the numbers 1, 2, 3 and 4.
- Crucially, no chemists or biologists agree that the number combinations •••••131(4) •324(1) •234(4) ••••• in this diagram express the chemical and biological properties of amino acids. However, connecting Matthaei and Nirenberg's "if poly-U↑, then poly-phe↑" statement with Gamow's diamond code diagram leads to the following result: if there is "•••••UUU(U) • UUU(U) • UUU(U) •••••", then there comes "•••••phe • phe • phe •••••". This makes Gamow's 131(4) •324(1) •234(4) sequence accurate for "UUU(U) • UUU(U) • UUU(U)" as well as for "phe • phe • phe."

According to the numerous textbooks, Gamow's deduction

of triplet nature has gradually formed into a piece of common knowledge as the description here: "since there are only four nucleotides, a code of single nucleotides would only represent four amino acids, such that A, C, G and U could be translated to encode amino acids. A doublet code could code for 16 amino acids (4×4). A triplet code could make a genetic code for 64 different combinations ($4 \times 4 \times 4$) genetic code and provide plenty of information in the DNA molecule to specify the placement of all 20 amino acids". Obviously, Gamow's calculation about the triplet nature was based on the digitalized double helical model of DNA. Crick and Brenner's experiments of triplet nature was not based on exact DNA model. In March 1963, by using Crick and Brenner's experimental conclusion, Nirenberg in his article <The Genetic Code: II> [7] separated UUUUUUUUUUUUU into "UUU-UUU-UUU-UUU-UUU" [7] (Figure 2) to explain the chemical triplet structure of poly-U, see (Figure 2). poly-U was written in the form of "UUU-UUU-UUU-UUU-UUU" [7] by Nirenberg in March 1963. Marshall W. Nirenberg, <The Genetic Code: II>, Scientific American, 1963 (March), 208(3): p86. Reprinted by permission. © 1963 SCIENTIFIC AMERICAN, INC.

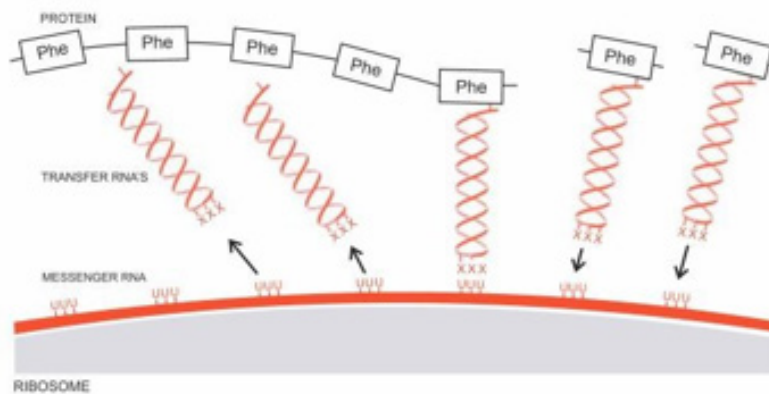


Figure 2: Poly-U was written in the form of “UUU-UUU-UUU-UUU-UUU” [7] by Nirenberg in March 1963. Marshall W. Nirenberg, <The Genetic Code: II>, Scientific American, 1963 (March), 208 [3]. Reprinted by permission. © 1963 SCIENTIFIC AMERICAN, INC.

Notes:

- a) In this figure, Nirenberg drew a big gap between every two triplets of UUU on UUUUUUUUUUUUUU chain. Poly-U then changes to a three-gap-three-gap-three pattern. The presence of gaps indicates that every UUU on the poly-U is separated from other UUU's and clearly visible (not invisible). However, "gaps" demand the chemical structure of poly-U to be evenly separated by every three digits to ensure the triplet nature of poly-U is obtained when testing the chemical structure (rather than Crick-Brenner's triplet nature genetic experiments).
- b) Notably, if poly-U can be visualized with numerous gaps, then poly-C, poly-A, poly-G, poly-T, and all other nucleotide chains must also be visualized similarly, signifying that the single-stranded nucleotide sequences must be chemically separated by every three digits. This implies that Nirenberg began to play a big-brother role in the history of molecular biology.

The first time the statement “a codon for phenylalanine is composed of a group of three uridylic acid residues (UUU)” [8] appeared in university textbooks was in the first edition of J.D. Watson’s *<Molecular Biology of the Gene>* published on July 21, 1965, which he used to teach the undergraduate course at Harvard University. Notably, in Watson’s books, “UUU” sometimes indicates “poly-U”, alternatively, “poly-U” sometimes indicates “UUU”.

Triplet Nature of Poly-U Cannot be Established in Chemistry

Here, I list three different linear expansion forms of poly-U molecules:

- a) Case I: poly-U having no triplet nature (assuming to ignore Crick's comma or comma-less theory) U U U U U U U U U U U U
U U U U U U U U U U U U U U U U U U U U U U U U U U U U U U
U U U U U U U U U U U U U U U U U U U U U U U U U U U U U U
U U U U U U U U U U U U U U U U U U U U U U U U U U U U

UUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUU
UUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUU

- b) Case II: poly-U having full triplet nature (assuming to agree with Nirenberg's chemical diagram in 1963)
- U U U -U U U- U
U U -U U U- U U U -U U U -U U U- U U U- U U U- U U U- U
U U - U U U- -U U U- U U U -U U U -U U U- U U U- U
U U-..... U U U -U U U- U U U U- U U U- U U U- U U U -U U
U -U U U- U U U -U U U- -U U U- U U U -U U U -U U U
- c) Case III: poly-U having occasional triplet nature (assuming to agree that only triplet "UUU" carries confidential information), i.e.: only triplet UUU carries the genetic information, or only triplet UUU is the unit of information.
- U U U U U U- U U- U U U U- U U U U
U U- U U- U- U U U(informational region)- U U U U U U U- U U- U U
U U- U U U U U U- U U- U U U(informational region) - U U U U U U
U-..... U U- U U U U- U U U U U U- U U- U U U U- U U U U U U- U U- U
U U U- U U U U U U- U U- U U U U- U

The Full Triplet Nature of Poly-U (Case I) Cannot be Established in Chemical Science

Similar to the fact that in the field of chemistry, $\text{CH}_3\text{-CH}_2\text{-CH}_3$ (propane) has three carbon atoms and $\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_3$ (butane) has four carbon atoms, “pU-pU-pU” [9] (triplet) represents three uracil residues while “pU-pU-pU-pU” (quadruplet) represents four uracil residues. No qualified chemists would intentionally use an overlapping reading method to read $\text{CH}_3\text{-CH}_2\text{-CH}_2\text{-CH}_3$ (butane) as two “ $\text{CH}_3\text{-CH}_2\text{-CH}_3$ ” (propane) or use a nonoverlapping reading method to read $\text{CH}_3\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_3$ (hexane) as two “ $\text{CH}_3\text{-CH}_2\text{-CH}_3$ ”(propane); thus, no qualified biochemists will consider an overlapping reading method as a biological property of UUUU (so as to read UUUU as two rough triplets of UUU), neither consider a nonoverlapping reading method as a biological property of UUUUUU(so as to read UUUUUU as two accurate triplets of UUU)! Thus, Matthaei and Nirenberg cannot discover the triplet nature residing in “U U U U U U U U U” form of poly-U! As shown

(Figure 3) in, the chemical highlights of Matthaei and Nirenberg's poly-U experiment merely reflected a linear correlation [10,11] between poly-U and poly-Phe ("if Poly U↑, then poly Phen↑" reaction), no chemists would see the triplet nature of poly-U in such a chemical reaction figure (Figure 3). Nirenberg, M.W. and Matthaei,

J.H. The dependence of cell-free protein synthesis in *E. coli* upon naturally occurring or synthetic polyribonucleotides. Proc Nat Acad Sci U.S (1961). [11] Reprinted with permission from Proc Nat Acad Sci U.S Vol. 47. © 1961 by PNAS.

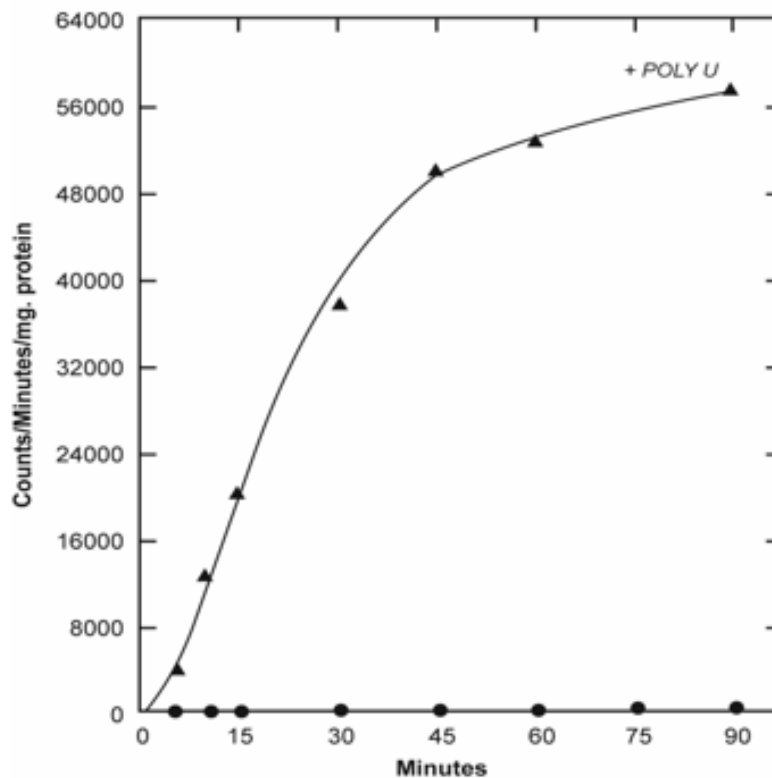


Figure 3: Stimulation of U-C14-L-phenylalanine incorporation by polyuridylic acid.

● Without polyuridylic acid; ▲ with addition of 10 µg polyuridylic acid.

On the other hand, in inorganic chemistry, there truly exist some "dots" in the chemical formulas to denote the "loose chemical bonds", such as the copper sulfate penta-hydrate: $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. If using "chemical dot" to denote the "triplet nature" of nucleotide chain "UUUU UUUU U UUUUUUUUU", we get the below chemical writing form:

"•UUU•UUU•UUU•UUU•UUU•UUU•UUU•UUU•UUU•
UUU•UUU•UUU•UUU•UUU•UUU•UUU•UUU•UUU•UUU•
UUU• UUU•UUU•UUU•UUU••UUU•UUU• UU •UUU•
UUU•UUU•UUU•UUU• UUU•UUU•UUU•UUU• UUU•UUU• UUU•
UUU•UUU• UUU•UUU• UUU• UUU•UUU•UUU•UUU•"

If referencing the function of "chemical dots" in the copper sulfate penta-hydrate: $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, I find the difference between "having triplet nature" and "not having triplet nature" is like

i) nonoverlapping or overlapping reading. i.e: both overlapping reading $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and non-overlapping reading $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ are wrong chemical hypotheses, because the chemical

structure of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ is a question of stereo configuration, but not a linear order;

- ii) comma or comma-less, i.e: both " $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ has comma" and " $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ has not comma" are wrong hypotheses, because "the chemical dot" here is not a comma in mankind's written language, it is just a symbol of chemical structure;
- iii) start codon or stop codon, i.e.: both " $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ has a starting point Cu" and " $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ has a stopping point H_2O " are wrong chemical hypotheses.

In addition, in molecular biology, the phrase UUU has an anti-codon GAA, soon we find: iv) the chemical concept of the "anti-molecule of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ " in " $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ " cannot be established in chemistry, because " $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ " is not a linear sequence; and v) the chemical concept of the "degeneracy of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ " in " $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ " cannot be established in chemistry, because there are no "residues" to replace "the third position O_4 " and to make " $\text{CuS} (?) \cdot 5\text{H}_2\text{O}$ " 100%

similar as " $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ". --Please note: the degeneracy of UUU to UUC exactly means that "third position U in Crick reading chemistry is 100% as same as the third position C in Crick reading chemistry".

Crick and Brenner's Experimental Conclusion "Matthaei-Nirenberg's Poly-U has A Triplet Nature" Cannot be Established in Chemical Science.

The deletion or insertion of one or two uridylic acids in the Crick-Brenner phage T_4 experiments would not lead to the synthesis of an amino acid other than phenylalanine. In fact, in two classic research articles titled <General Nature of the Genetic Code for Proteins> and <The Dependence of Cell-Free Protein Synthesis in *E. coli* upon Naturally Occurring or Synthetic Polyribonucleotides>, it was revealed that neither the Matthaei-Nirenberg nor the Crick-Brenner experiments had ever located a UUU site on their genetic maps. On one hand, poly-U cannot substitute the rII region of phage T_4 because the genetic nature of the rII region of cistron B within phage T_4 is unrelated to Matthaei-Nirenberg's synthetic poly-U. On the other hand, poly-phe of the Matthaei-Nirenberg experiment is completely different from Crick-Brenner's r-plaque on *E. coli* B that is recognized (attacked) by phage T_4 .

On the side of poly-Phenylalanine, the different molecular formations of Matthaei-Nirenberg's poly-phe do not conform to Crick-Brenner's "wild-type r-plaque," "pseudo-type r-plaque," or "true wild-type r-plaque" [3]. The genetic classification of Crick-Brenner's r-plaque is incapable of describing the chemical conformation of Matthaei-Nirenberg's polyphenylalanine. (Note: this signifies that the success of the "if poly-U $\uparrow$, then poly-phe $\uparrow$ " reaction was not due to the triplet nature of poly-U). Thus, even if the genetic relationships between the types of r-plaques on *E. coli* K and B strains are sufficient to predict whether the rII region of cistron B within phage T_4 exhibits a triplet property, Matthaei and Nirenberg should have still used the chemical conformation of poly-phe to determine whether their synthetic poly-U exhibits a triplet property (a three-digit reading frame). For a better trustworthy methodology of science, Matthaei and Nirenberg should have researched for the three-digit reading frame (triplet UUU) of poly-U independently. Using the triplet property of the rII region of cistron B (even if it truly exists in the processes of phage- attacking bacteria) to represent the triplet property of synthetic poly-U does not follow the appropriate scientific methodology. --- To this aspect, we say it is a joke when Crick and Brenner used the imagined chemical features of the rII region of cistron B of the phage T_4 to deduce the chemical structure of Matthaei and Nirenberg's poly-U!

The Lack of the "3 What" Of Phenylalanine in Chemistry Implies that the "Triplet Nature of Poly-U" has No Connection with Phenylalanine Incorporating into Protein

According to today's textbook knowledge, the final usage of UUUUU.....UUUUU's triplet nature is to explain the protein synthesis of phenylalanine incorporating into protein, which asserts that in chemistry, the incorporation of phenylalanine (a

single molecule $\text{C}_9\text{H}_{11}\text{NO}_2$) into poly-phe (amino acid polymer type of protein) requires only a triplet UUU (and not doublet UU or quadruplet UUUU). As a chemistry-based inference methodology, if the incorporation of a single phenylalanine $\text{C}_9\text{H}_{11}\text{NO}_2$ into poly-phe truly needs a triplet UUU, there must be chemical properties regarding "3 what of phenylalanine $\text{C}_9\text{H}_{11}\text{NO}_2$ " residing on the phenylalanine molecule $\text{C}_9\text{H}_{11}\text{NO}_2$. Thus, before using UUU to design a poly-phe protein synthesis route, chemists and biologists should first answer what is the "3 what of phenylalanine $\text{C}_9\text{H}_{11}\text{NO}_2$ " in pure chemistry. If there is no chemical concept of the "3 what of phenylalanine" (or the "3 what" of alanine/ the "3 what" of glycine, etc.), then all the triplet nature of UUUUU.....UUUUU aiming at phenylalanine can be considered a joke in phenylalanine chemistry, i.e., the three-digit reading on UUUUU.....UUUUU does not reflect whatever chemical nature of phenylalanine if there are no chemical discoveries about the three-digit reading property on phenylalanine molecule $\text{C}_9\text{H}_{11}\text{NO}_2$.

Here I draw a simple diagram below:

-U-U-U-U-U-U-U-U-U-U-U-U-U-U-U-U-U-U-

a) : Can anybody see the triplet nature of phenylalanine in this sequence of U?

-Phe-Phe-Phe-Phe-Phe-Phe-Phe-Phe-Phe-Phe-Phe-Phe-Phe-Phe-Phe-Phe-Phe-

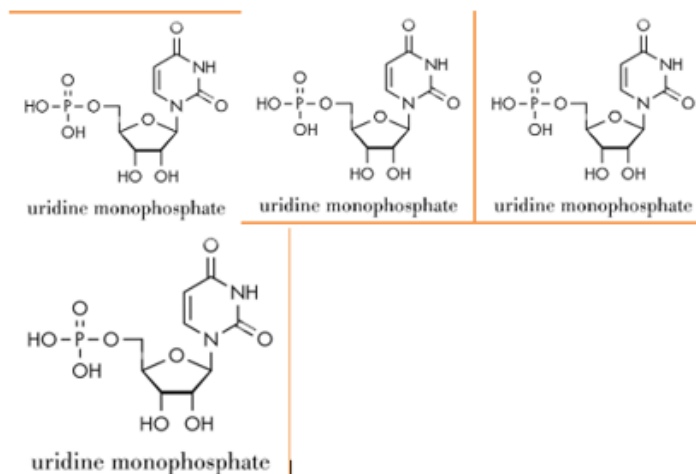
b) : Can anybody find the triplet nature of $\text{C}_9\text{H}_{11}\text{NO}_2$ in this Phe sequence? Why does the sequence of Phe not have a triplet nature?

-Phe-Val-Ala-Gln-Phe-Val-Ala-Gln-Phe-Val-Ala-Gln-Phe-Val-Ala-Gln-Phe-Val-Ala-Gln-

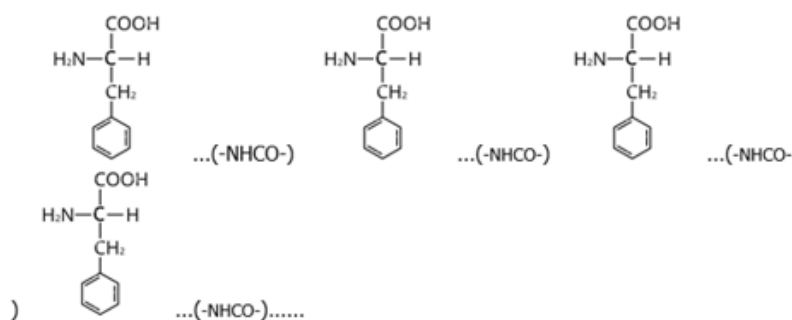
c) : Assuming $\text{C}_9\text{H}_{11}\text{NO}_2$ (phenylalanine) has a chemical nature of the "3 what," why do $\text{C}_5\text{H}_{11}\text{NO}_2$ (valine) and $\text{C}_2\text{H}_5\text{NO}_2$ (glycine), which are quite different from $\text{C}_9\text{H}_{11}\text{NO}_2$, also have a chemical nature of the "3 what"? Why do other amino acids not exhibit a chemical nature of the "4 what" or the "5 what"?

(Figure 4) Skeptical questions regarding the triplet nature experiments. This diagram is drawn by author YANG Xing Yang.

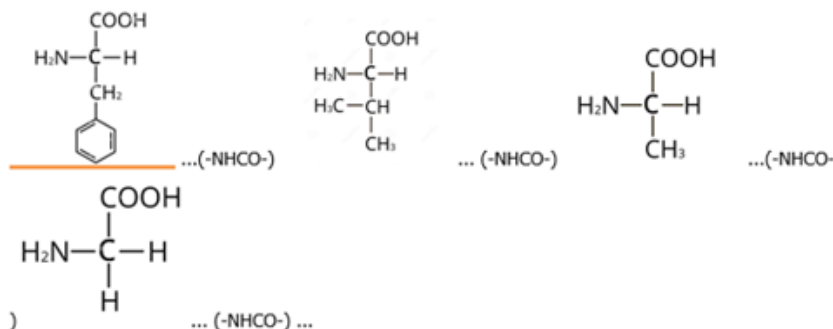
Caution: any person's experiments to test the triplet nature of poly-U will pave a way to test the numerous gaps among several two-neighbor triplets. If there is no evidence regarding regular gaps, it is impossible for natural triplets to be present in poly-U. This is similar the story of emperor (poly-U)'s new clothes (triplet nature). The weavers claimed that they knew how to weave clothes with fabrics of the most beautiful colors; however, they announced that this wonderful cloth would be invisible to everyone who was unfit for the job the emperor promised. Therefore, when coming across triplet nature experiments, it is better not to watch their experimental procedures or listen to their beautiful wordings, you will only need to survey their experimental conclusions and judge whether they are treating "non-three nucleotides" (such as non-three uracil) as "three nucleotides" (such as three uracils).



(a): Can anybody see the triplet nature of phenylalanine in this sequence of Us?



(b): Can anybody find the triplet nature of $C_9H_{11}NO_2$ in this Phe sequence? Why does the sequence of Phe not have a triplet nature?



(c): Assuming $C_9H_{11}NO_2$ (phenylalanine) has a chemical nature of the "3 what," why do $C_5H_{11}NO_2$ (valine) and $C_2H_5NO_2$ (glycine), which are quite different from $C_9H_{11}NO_2$, also have a chemical nature of the "3 what"? Why do other amino acids not exhibit a chemical nature of the "4 what" or the "5 what"?

Figure 4: Skeptical questions regarding the triplet nature experiments. This diagram is drawn by author YANG Xing Yang.

In mathematics, "triplet nature" signifies the "3 what" (i.e: 3 of what). As shown in Figure 4(a), if a triplet nature truly exists in -U-U-U-U-.....U-U-U-U-U-, it must also exist in -Phe-Phe-Phe-Phe-Phe-Phe-Phe-Phe-.....-Phe-Phe-Phe-Phe-Phe-Phe-Phe-

Phe-Phe-, as shown in Figure 4(b), because both sequences are composed of the same mathematical points. Worst of all, if the chemical nature of phenylalanine is "3 what", then that of glycine cannot be "3 what" because the residue of glycine (+H) comprises a

single hydrogen atom. Therefore, it is unreasonable to hypothesize that the “3 what” of glycine is derived from that of phenylalanine according to the triplet structure of phe’s code UUU. (Notably, no genetic code chemists have conducted experiments to use the “3 what” of phenylalanine to infer the “3 what” of glycine. From my perspective, this is unfair to protein chemical science). The “3 what” can never be universal in amino acid chemistry! (Notably, in the Crick $4 \times 4 \times 4$ <the Genetic Code> table, not only does the genetic code of phenylalanine require a triplet nature but also so does the genetic code of all the amino acids). Regarding nucleotide chains, the triplet feature is not a necessary chemical rule to bridge Matthaei–Nirenberg’s poly-U with their poly-phe because of the following reasons: (i) *the triplet feature of UUU differs from the three-gap-three feature of poly-U’s “•UUU•UUU•..... UUU•UUU•UUU.”* The obvious difference between the two can be observed from the exponential function mentioned below: $y = (U + C + A + G)^3$, $y = (U^3 + C + A + G)^3$, and $y = (poly-U + C + A + G)^3$; and (ii) a phenylalanine molecule ($C_9H_{11}NO_2$) cannot generate the triplet structure of $C_4H_4N_2O_2$ – $C_4H_4N_2O_2$ – $C_4H_4N_2O_2$ (UUU) and an uracil molecule ($C_4H_4N_2O_2$) cannot generate the triplet structure of $C_9H_{11}NO_2$ – $C_9H_{11}NO_2$ – $C_9H_{11}NO_2$ (phe-phe-phe).

Moreover, in the field of chemistry, because poly-U is devoid of Gamow’s and Crick’s specific calculation premise, neither of their $4 \times 4 \times 4 = 64$ calculation for the sum of the genetic code nor Crick–Brenner’s phage T4 studies regarding the triplet nature are capable of directly separating Matthaei and Nirenberg’s poly-U as “•UUU•UUU•..... UUU•UUU•UUU•UUU•.” Thus, without considering •UUU•UUU•..... UUU•UUU•UUU’s triplet segment UUU, the understanding of UUUUUUUU’s triplet nature is a play on words that challenges the intellectual limits of human scientists, chemists, geneticists, biologists, and mathematicians.

UUUUU.....UUUUU’s Triplet Nature Seriously Violates “Gamow’s Triplet Calculation Formula $4 \times 4 \times 4 = 64$ ” and “Crick’s Concept of Genetic Information”

“One out of four bases specifies one amino acid” violates Gamow’s triplet calculation formula $4 \times 4 \times 4 = 64$ that “three out of four bases specifies one amino acid”: About the triplet nature relating to Gamow’s diamond code On one hand, Gamow’s calculation is about “four different bases” and not about “one bases repeatedly use three times”, here, “triplet nature of UUUUU..... UUUUU” is about “one base”; On the other hand, Gamow’s calculation is about the genetic functions of Watson-Crick double helical DNA model and not about the genetic functions of a single nucleotide chain like UUUUU.....UUUUU. The deduction of the “triplet nature of UUUUU.....UUUUU” must be different from “the triplet nature of Watson-Crick double helical DNA model”. - - This is the key point in my article.

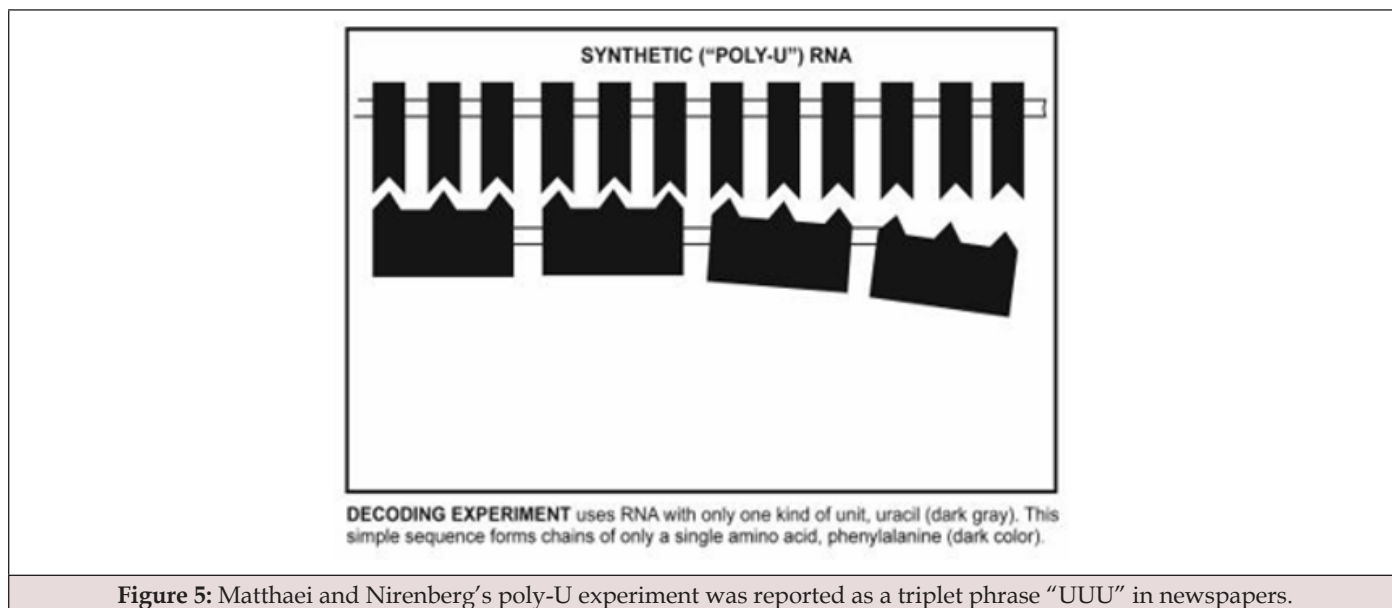
“The Accumulation of One Base Repetition Carries the Genetic Message” Violates Crick’s Information Theory that

“The Order of Four Different Bases” Carries the Genetic Information: Crick’s concept of “the genetic information” in “central dogma” and “sequence hypothesis” means “the order of four bases ---T,C,A,G”, and does not mean “the triplet nature of nucleic acids”. If there are T,C,A,G four different bases, there must be many different orders among each other, these orders are the “messages” clearly, Crick’s “information flow” means “order flow or chemical structure flow”, it is not science. However, in the Crick-Brenner triplet nature experiment, “3” and “not 3” became the judge of “having information” and “not having information”. In this situation, the order of four different bases is no longer the genetic message anymore. If having a triplet nature, then UUUUU.....UUUUU is an “informational molecule”; If not, not the “informational molecule”. The academic significance of UUUUU.....UUUUU’s “triplet nature” is used for fixing the concept of “genetic information”, and furtherly to fix the concept of “natural information matter” clearly, “the number of residues” in chemical science is never the criteria to distinguish a “informational molecule” and “non-informational molecule”.

“How A Sequence of Four Things (Nucleotides) can Determine A Sequence of Twenty Things (Amino Acids)” [12] Is Called The “Coding Problem” In Molecular Biology: To cite Matthaei and Nirenberg’s experiments to establish the “4-letter language of nucleic acids” [13], to show that “the four-letter language embodied in molecules of nucleic acid controls the 20-letter language of the proteins” [14], and to illustrate the chemical process of “translation from the four-unit language of the polynucleotides to the twenty-unit parlance of the proteins” [15], I advocate that the chemical structure of mRNA molecules must include adenine ($C_5H_5N_5$), guanine ($C_5H_5N_5O$), cytosine ($C_4H_5N_3O$), and uracil ($C_4H_4N_2O_2$), therefore, poly-U could not be mRNA. Not being mRNA here means not being the representative of double helical DNA.

The “Triplet Nature” of UUUUU.....UUUUU In Protein Synthesis is Exactly the Technical Application of “The 3 Bases Segment of the Anti-Codon Region on Trna Molecule”

See (Figure 5), the triplet nature of UUUUU.....UUUUU was illustrated as external non-UUU triplet block [16] which is like a three-digit calibration of the Vernier caliper used to measure the number of digits on UUUUU.....UUUUU and then the three-digit calibration of the Vernier caliper was considered the triplet nature of UUUUU.....UUUUU U, this triplet imagination was published in public media as early as January 5, 1962 (Figure 5): <NIH Researchers Crack the Genetic Code>. [16]. Reprinted by permission. © 1962 Medical World News. Thus, it is obviously not that the triplet nature of UUUUU.....UUUUU comes from UUUUU.....UUUUU itself! This finding further supports Crick et al.’s research outcome in 1957 that the sequences UUU (in the form of other triplets), CCC, AAA, and GGG “must be nonsense” [12].



The “Triplet Nature” of UUUUU.....UUUUU is Not Capable of Deducing the Number System “3, 4, 20, 64, 4 $\rightleftharpoons$ 20, 4 $\rightleftharpoons$ 64, 3 $\rightleftharpoons$ 20, 3 $\rightleftharpoons$ 64, 20 $\rightleftharpoons$ 64”

“The Genetic Code” is a systematic concept in the history of molecular biology. There is a transferring mechanism about the number system “3, 4, 20, 64, 4 $\rightleftharpoons$ 20, 4 $\rightleftharpoons$ 64, 3 $\rightleftharpoons$ 20, 3 $\rightleftharpoons$ 64, 20 $\rightleftharpoons$ 64” from Matthaei - Nirenberg chemical reaction “if Poly U $\uparrow$, then poly Phen $\uparrow$ ” to Crick table’s “UUU coding for Phenylalanine”. “Triplet nature” superficially changes UUUUU.....UUUUU to UUU and roughly provides the “evidence of 3”. However, we find there are still many questions left as below:

- a) Problem 1, the “triplet nature” of UUUUU.....UUUUU does not meet the needs of “the degeneracy nature of UUU” , i.e.: from UUU to UUC or from “1/64” as UUU to “2/64” as UUC; For example, Crick and Brenner announced that “multiple sets of three letters stood for a particular amino acid” [12], however, Crick and Brenner’s experiment did not conclude the “degeneracy” of UUUU UU.....UUUU as below:

UUC-UUC-UUC--UUC-UUC-UUC-UUC-UUC-UUC-UUC-UUC-UUC-UUC-

..... UUC-UUC-UUC-UUC-UUC-UUC-UUC-UUC-UUC
-UUC-UUC-U

U C- (coding for phenylalanine)

- b) Problem 2, the “triplet nature” of UUUUU.....UUUUU is never possible to deduce “20 different types of ties between amino acids and nucleic acids”. In Gamow’s Nature article “Possible relation between deoxyribonucleic acid and protein structures”, see (Figure 6), “choosing 3” is the minimum guarantee to build 1-on-1 numerical relations between “20 diamond holes observed by Gamow” and “20 amino acids

chosen by Gamow". If discarding Gamow's 20 diamond holes and turning to believe Crick's "triplet nature" of UUUUU.....UUUUU, nobody knows how can UUUUU.....UUUUU precisely transfer to "20 diamond holes".

- c) (Figure 6). The 20 different types of diamond holes in the Watson-Crick DNA model [6]. George Gamow, <Possible relation between deoxyribonucleic acid and protein structures>, [6] © 1954 Macmillan Magazines Ltd. Reprinted by permission. Note: These 20 diamonds observed by Gamow, which contains a numerical conversion system (4-nucleotide converse to 4-letter language, 20-amino acid converse to 20-letter language, 64-trinucleotide chemical science converse to 64-codon genetic science) and a biological translation system (4-nucleotide chemistry translates into 20-amino acids chemistry by 64-types of “trinucleotide translates to aa”), was the theoretical origin of the Matthaei-Nirenberg poly-U experiment.
- d) Problem 3, the “triplet nature” of UUUUU.....UUUUU has no functions to predict and confirm the nucleotide orders of the phe-tRNA molecule. In the theory of protein synthesis, the final explanation of poly- U’s “triplet nature” is to schedule the natural protein synthesis depending upon the participation of phe-tRNA molecule, in which there is a special zone called the anticodon region in the RNA chain of the phe-tRNA molecule to pass the “genetic message” on phenylalanine. When considering the biological function of tRNA for protein synthesis, scientists take the different orders of the tRNA nucleotide chain as the first concern, it comes to such a consensus: the different orders of the tRNA nucleotide chains govern the “specific matches between the tRNA’s anticodon region and its amino acid”. Therefore, it is clear, if Crick-Brenner’s experiments truly aim at helping Matthaei and

Nirenberg to establish “UUU codes for Phenylalanine”, their experimental conclusion “genetic information is triplet bases” should use to determine “UUU’s anticodon GAA and the exact sequence order of the phe-tRNA’s nucleotide chain”. However,

by checking Crick-Brenner’s “triplet nature” experimental process, we find Crick and Brenner have never attempted to fix the order of the soluble RNA (the original version of tRNA in Matthaei and Nirenberg’s experiment).

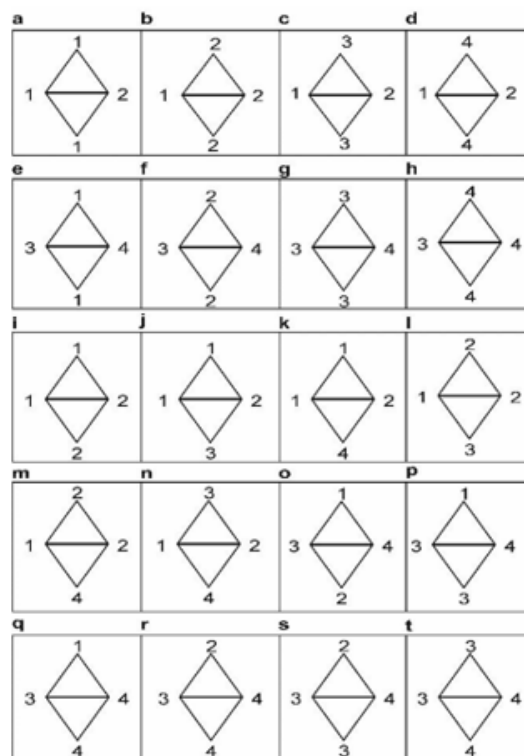


Figure 6: The 20 different types of diamond holes in the Watson–Crick DNA model [6]. George Gamow, <Possible relation between deoxyribonucleic acid and protein structures>, Nature 173 (1954): 318. © 1954 Macmillan Magazines Ltd. Reprinted by permission.

Nobody can see definite “first U, second U, and third U” on Matthaei -Nirenberg’s UUUUU.....UUUUU

Looking into Crick’s $4 \times 4 \times 4$ <the Genetic Code> Table, see (Table 1): Table 1: Crick’s $4 \times 4 \times 4$ <the Genetic Code> (Table 1) [17,18,19] CSHL Quant Biol. 31(1966): p1. Reprinted with permission from CSHL Quant. Biol., Vol. 31. © 1966 by CSHL Quant. Biol., www.cshl.edu. Notes: a) “The arrangement of this table, whose significance for biology has been compared to that of the Periodic Table of Elements for Chemistry, was suggested by Crick” [18]; b) the byline “F. H. C. Crick” under the table’s caption in the screenshot was originally from the document CSHL Quant. Biol. 31 (1966) itself. C) This table was originally termed as <the Genetic Code> in the articles and lectures by Crick; hereafter, it has been referred

to as the “Crick’s $4 \times 4 \times 4$ <the Genetic Code> Table” to address both Crick’s historical contribution to <the Genetic Code> and its mathematical structure $4 \times 4 \times 4$, although most current textbooks and papers refer to this table as “the Standard Genetic Code (SGC)” compared with the derived versions of the genetic code table. We find the “triplet nature” of poly-U seems to support Crick’s $4 \times 4 \times 4$ <the Genetic Code> Table by writing UUUU.....UUUU to - U U U -U U U-..... U U U -U U U-, however, the introduction of “triplet nature” brings many academic troubles to Matthaei-Nirenberg chemical reaction “if Poly U $\uparrow$, then poly Phen $\uparrow$ ”. For example, in Crick’s $4 \times 4 \times 4$ <the Genetic Code> table, each UUU has been divided into “U(first letter) U(second letter) U (third letter)”. Yet, checking Matthaei-Nirenberg experiment, it is never possible to check or see the order difference as “position 1, position 2, position 3” as below:

THE GENETIC CODE					
1st↓2nd→	U	C	A	G	↓3rd
U	PHE	SER	TYR	CYS	U
	PHE	SER	TYR	CYS	C
	LEU	SER	Ochre	?	A
	LEU	SER	Amber	TRP	G
C	LEU	PRO	HIS	ARG	U
	LEU	PRO	HIS	ARG	C
	LEU	PRO	GLUN	ARG	A
	LEU	PRO	GLUN	ARG	G
A	ILEU	THR	ASPN	SER	U
	ILEU	THR	ASPN	SER	C
	ILEU	THR	LYS	ARG	A
	MET	THR	LYS	ARG	G
G	VAL	ALA	ASP	GLY	U
	VAL	ALA	ASP	GLY	C
	VAL	ALA	GLU	GLY	A
	VAL	ALA	GLU	GLY	G

This table shows the “best allocations” of the 64 codons at the time of the Symposium. Some of these allocations are less certain than others. The two codons marked ochre and amber are believed to signal the termination of the polypeptide chain. The codons suspected of being concerned with chain initiation are not indicated here. The evidence used to produce this table comes mainly from *E. coli*. It is likely that the genetic code in other organisms is either very similar or identical to that shown here. (FHC Crick)

In addition, neither the Crick team nor the Nirenberg team had ever assumed that the “triplet nature” of UUUUU.....UUUUU was the sufficient chemical premise for the occurrence of “if Poly U ↑, then poly Phen ↑” chemical reaction. More ironic, once the occurrence of “if Poly U ↑, then poly Phen ↑” chemical reaction does need a “chemical triplet separation (triplet nature)” in chemistry, the “triplet nature” of UUUUU.....UUUUU should have been presented before the Matthaei-Nirenberg experiment, instead of after the Matthaei-Nirenberg experiment. If considering it with communication techniques, the introduction of “triplet nature” to UUUU.....UUUUU was exactly a sudden change of UUUU.....

In chemistry, whether the nucleotide sequence UUUU.....UUUU UUU has a triplet nature or doublet nature, the question about “triplet nature” is of chemical structure. If it has a triplet nature, the chemical structure of poly-U is as “UUU-UUU-UUU--UUU-UUU”; If it has not, UUUU.....UUUU UUU is still UUUU.....UUUU UUU. “Triplet nature” or “not triplet nature” has no

connection with “information”.

In contrast, “codons are the units of information in mRNA” [20] is a concept of communication activity. It involves two set of “molecular communication”, one is the communication between RNA and DNA, and the other is between RNA and protein. According to Crick’s “central dogma”, mRNA’s genetic information comes from double helical DNA and UUUU.....UUUU UUU’s genetic information comes from TTTTTTTTTT.....TTTTTTTTT, therefore we find: if Crick-Brenner team and Matthaei-Nirenberg team truly want to infer the “genetic information of UUUU.....UUUU UUU”, their attention must focus on many judges like: judging “whether TTTTTTTTTT.....TTTTTTTTT has genetic information”, judging “whether TTTTTTTTTT.....TTTTTTTTT passes its message to UUUU.....UUUU UUU”, and judging “whether TTTTTTTTTT.....TTTTTTTTT has a triplet nature”, and not judging “whether UUUU.....UUUU UUU sequence has a chemical structure of UUU-UUU-UUU--UUU-UUU”.

“Discovering the Triplet Nature of Poly-U” Might be A Hyped Science During the Cold War Period

Clearly, in Matthaei-Nirenberg experimental systems, Matthaei and Nirenberg never acknowledged the triplet nature of poly-U nor implied that poly-U might exhibit a triplet nature (i.e., a three-digit reading frame). Following the Moscow International Congress and after the publication of their PNAS article, Matthaei and Nirenberg still wrote phe’s genetic code as “UUU•••” [21] and not as the triplet letter “UUU.” Following the Crick–Brenner triplet nature experiment,

Nirenberg in March 1963 noted “in 1954, the theoretical physicist George Gamow, now at the University of Colorado, pointed out that the code words in such a dictionary would have to contain at least three bases” [7], which indicates that Nirenberg’s understanding about “triplet nature of poly-U” came from Gamow’s diamond code scheme, instead of Crick-Brenner’s experimental result. It is quite clear that Crick and Brenner’s triplet nature experiment was not the best way to judge if UUU.....UUUU nucleotide chain has a triplet nature. If one wants to distinguish chemical UUU.....UUUU from chemical UUU-UUU-UUU--UUU-UUU, they can just check the chemical structure of UUU.....UUUU directly, no one agrees that triplet nature of poly-U has a connection with phage T₄.

Just two weeks after Matthaei and Nirenberg published their PNAS paper, Crick wrote Nirenberg, encouraging him to “show that the coding ratio is 3” (say, triplet nature, see (Figure 7)); On January 4, 1962, see (Figure 8a,8b), in another letter to Nirenberg, Crick admitted that “the English papers have made rather a fuss about our Nature paper, which was published on Saturday, but as far as I have been able I have stressed that it is your discovery which was the real break-through”. The most striking event, the news article regarding “-UUU—UUU—UUU-” was published on December 24, 1961, see (Figure 9a,9b,9c) [22], which was before Nirenberg formulated the phrase UUU in 1962 and before the publication of the experimental results of “3 Uracil” by Crick and Brenner on December 30, 1961.

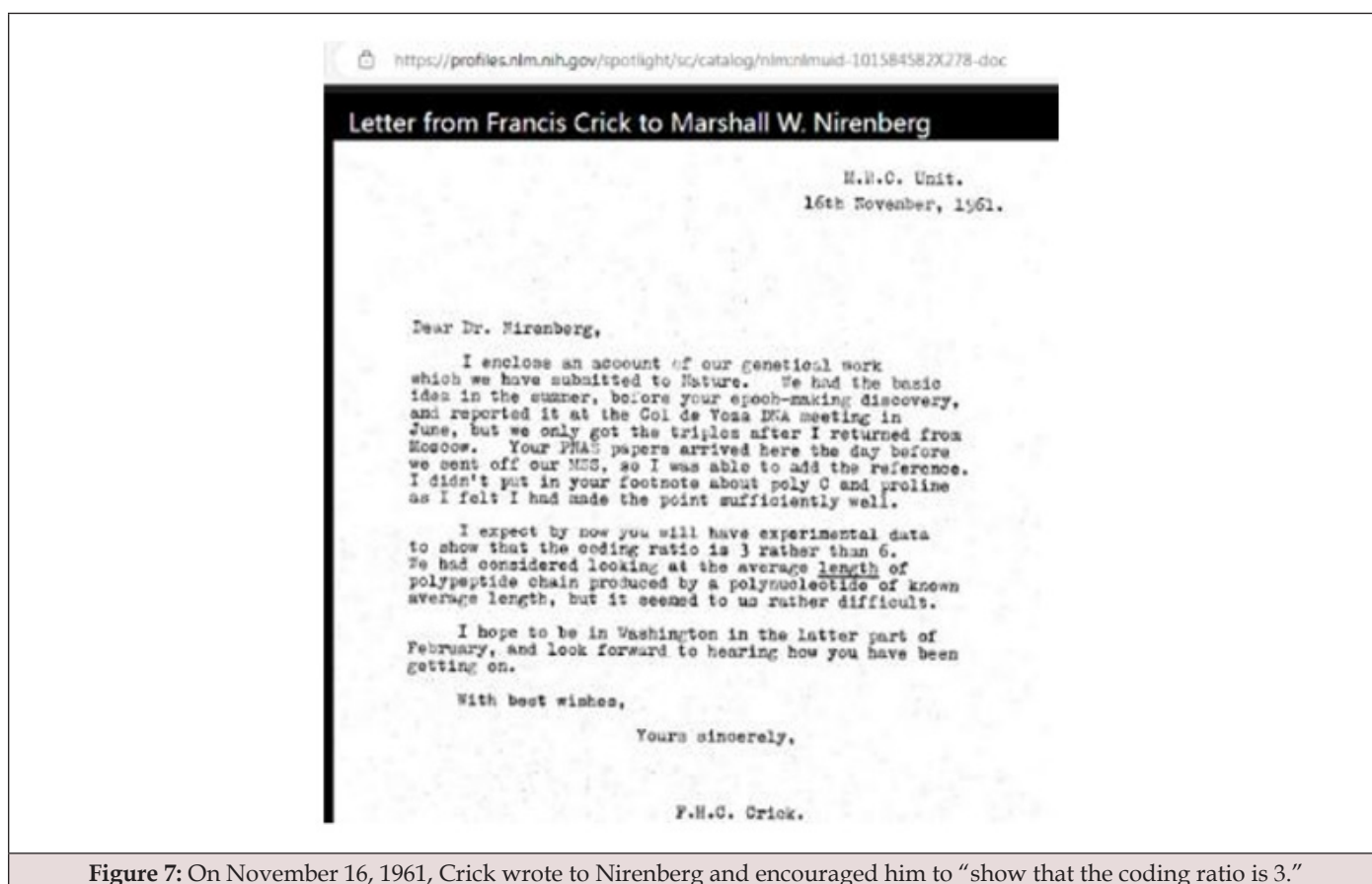


Figure 7: On November 16, 1961, Crick wrote to Nirenberg and encouraged him to “show that the coding ratio is 3.”

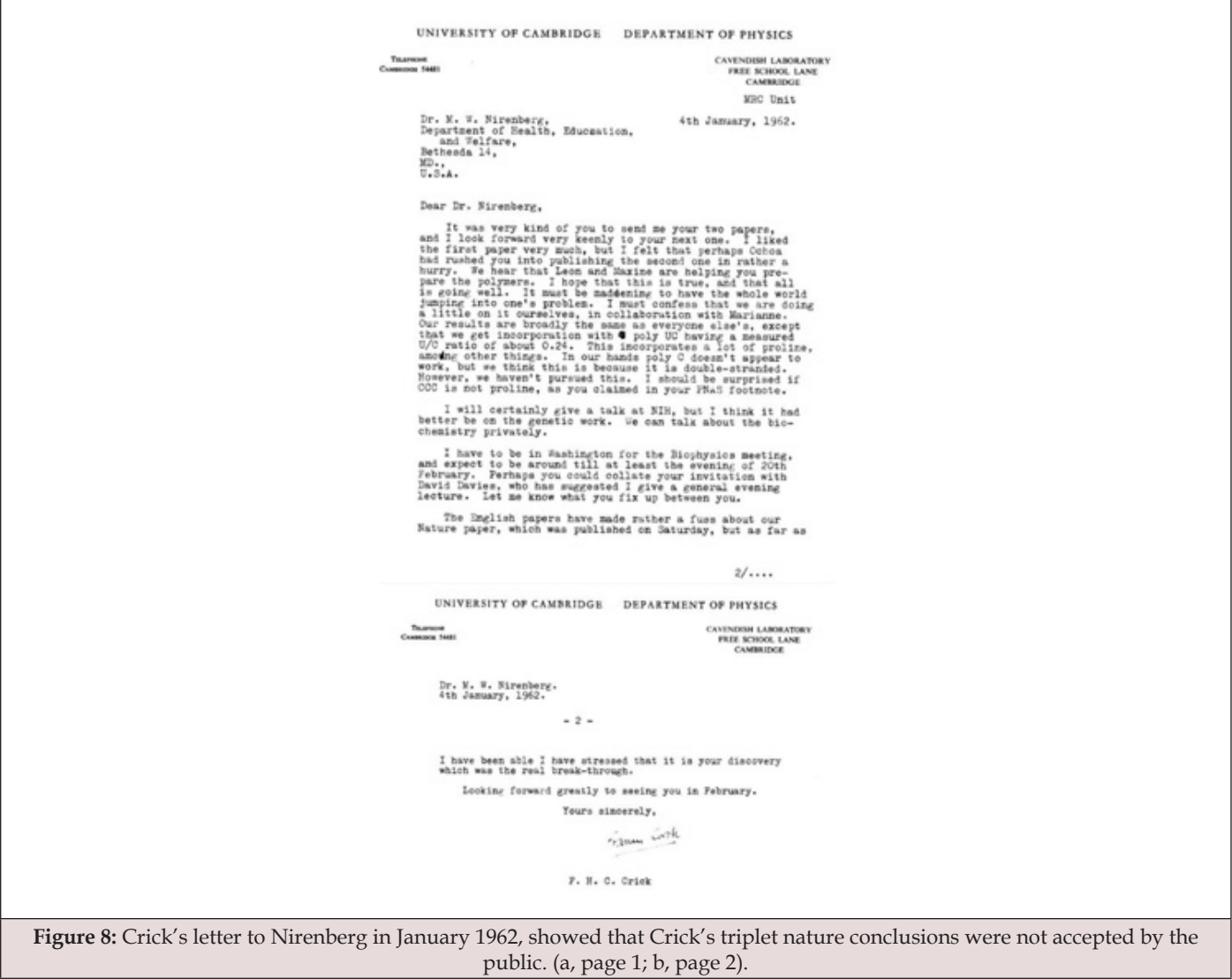


Figure 8: Crick’s letter to Nirenberg in January 1962, showed that Crick’s triplet nature conclusions were not accepted by the public. (a, page 1; b, page 2).

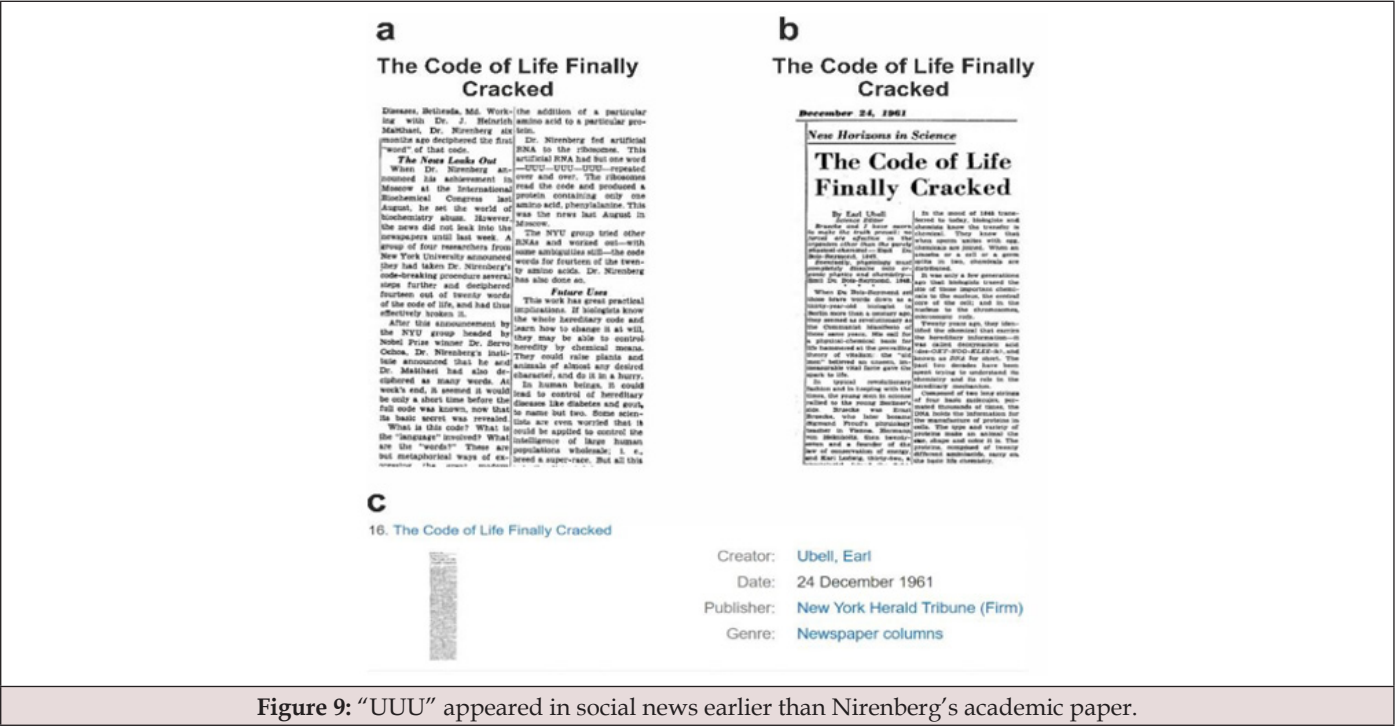


Figure 9: “UUU” appeared in social news earlier than Nirenberg’s academic paper.

- a) The phrase “UUU” published in a newspaper in 1961 [22];
- b) News report about Matthaei–Nirenberg’s “triplet UUU” published on December 24, 1961 [22].
- c) The date of the news was “December 24, 1961” which was earlier than the official publication of Crick–Brenner’s research article on “triplet nature”.

Conclusion

In Watson’s <Molecular Biology of the Gene> (first edition, as an example), to relate the triplet UUU with the Matthaei–Nirenberg poly-U experiment, a whole chapter is dedicated to layout space (Chapter 9, p255–296) to set the stage for introducing the Crick–Brenner’s phage T₄ experimental conclusion “the group number 3 comes from the genetic experiments described in Chapter 9” [8] and “THE GENETIC CODE IS READ IN GROUPS OF THREE” [8]. That is unfair for both double helix DNA and poly-U. The popularized textbook knowledge that “Nirenberg and Matthaei therefore concluded that the triplet codon UUU encodes phenylalanine” [23] or “Nirenberg and Matthaei had thus shown that the codon UUU specifies phenylalanine” [24] is certainly a misleading statement! The textbook writers roughly follow Watson’s 1965 textbook, misleading students into believing that the Matthaei and Nirenberg experiment deduced the triplet codon UUU, this constitutes the key problem of present-day university education regarding the first genetic code UUU!

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Conflict of Interest

None.

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