A Novel Discovery: Poly-U in 1961 Matthaei-Nirenberg Experiment Cannot Act as mRNA

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***Abstract*—This study reports novel discoveries as (i) poly-U described by Matthaei and Nirenberg in 1961 could never satisfy the versatility requirements of mRNA, (ii) the 5' to 3' reading direction of poly-U does not comply to mRNA's unidirectional reading direction of 5' to 3', (iii) Matthaei and Nirenberg did not and could not experimentally distinguish "messenger poly-U" from "non-messenger poly-U", (iv) Matthaei-Nirenberg's poly-U was not a discovery of natural nucleic acid molecule, (v) poly-U is a "one-letter language" instead of "four-letter language" stated in Crick's "coding problem", i.e., each triplet segment in poly-U has a math model of "1 × 1 × 1 = 1", yet that of mRNA is "4 × 4 ×4 = 64".**

***Keywords*—Matthaei and Nirenberg, poly-U, messenger RNA**

I. Introduction

In 1961, Matthaei and Nirenberg conducted a cell-free protein synthesis experiment and announced that "Polyuridylic acid appears to function as a synthetic template or messenger RNA" [1] (p. 189), [2] (p. 1601). Due to this, and the news story of Matthaei and Nirenberg deciphering the first genetic code UUU, one year later, Watson-Crick model of DNA double helix obtained the Nobel Prize in 1962. Therefore, Crick admitted in his Nobel Lecture that: "The breakthrough in the coding problem has come from the discovery, made by Nirenberg and Matthaei" [3].

I double-checked Matthaei and Nirenberg's Moscow Congress presentation and their PNAS paper and found that the key experimental evidence for "poly-U functioning as a mRNA" was stated in the figures and tables below. Fig. 1 reflected a linear correlation between reactant poly-U and product poly-Phe, i.e., "if poly-U↑, then poly-phe↑" reaction; Table I concluded that "poly-U only stimulated poly-Phe incorporating into protein and did not stimulate other amino acids", and Table II concluded that "poly-Phe only accepted the stimulation of poly-U to participate in synthesizing proteins, and other polynucleotides did not stimulate the incorporation of phenylalanine". For the data listed in Table III, it had shown that "poly-U stimulated a small amount of many other amino acids incorporating into protein, such as leucine, isoleucine, threonine, methionine, arginine, histidine, lysine, valine, tryptophan, and proline", however, we do not know why Matthaei and Nirenberg did not change the chemical reaction mixtures to increase the incorporation amounts of non-phenylalanine amino acids stimulated by poly-U, and do not know why Matthaei and Nirenberg roughly made a conclusion as "the specificity of phenylalanine incorporation in cell-free protein synthesis system, merely depends on the stimulation of poly-U" (Please note: "The specificity of one amino acid stimulated by poly-U" implies that the specificity of total twenty amino acids require the stimulation of exact 20 polynucleotides, and "the specificity of poly-U stimulation corresponding to phenylalanine" implies that "the specificity of total 4 bases" only corresponds to "total 4 species of amino acids", instead of "total 20 species of amino acids". The notion of "specificity" is a theoretical mistake).

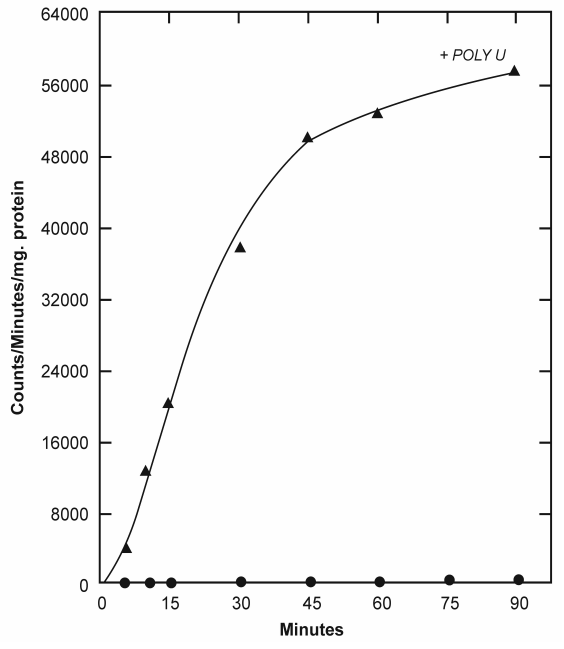

Fig. 1. Stimulation of U-C14-L-phenylalanine incorporation by polyuridylic acid. ● without polyuridylic acid; ▲ 10 μg polyuridylic acid added. Source: Nirenberg and Matthaei [2] (p. 1598).

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In addition, Ochoa, the Nobel Laureate of 1959, promptly agreed in 1961 that the Matthaei-Nirenberg experiment demonstrated "poly U acts as messenger RNA in this system" [4] (p. 1938). Watson, the first author to include Matthaei-Nirenberg experiment in a college textbook, stated in 1965, "Poly U was the first synthetic polynucleotide discovered to have mRNA activity" [5] (p. 466), [6] (p. 369). Crick, the initiator of "Crick's 4 × 4 × 4 <the Genetic Code> Table", again highly confirmed in 1966 Cold Spring Harbor Symposia on Quantitative Biology entitled The Genetic Code that "the breakthrough came, as we all know, by the discovery by Nirenberg and Matthaei (1961) that poly U could act as a messenger" [7] (p. 6).

TABLE I. POLYNUCLEOTIDE SPECIFICITY FOR PHENYLALANINE INCORPORATION

Experiment No.	Additions	Counts/min/mg protein
1	None	44
	+10 μg polyuridylic acid	39,800
	+10 μg polyuridylic acid	50
	+10 μg polyuridylic acid	38
	+10 μg polyuridylic acid	57
	+10 μg polyadenylic -uridylic acid (2/1 ratio)	53
	+10 μg polyuridylic acid +20 μg polyadenylic acid	60
	Deproteinized at zero time	17
2	None	75
	+10 μg UMP	81
	+10 μg UDP	77
	+10 μg UTP	72
	Deproteinized at zero time	6

Source: Nirenberg and Matthaei [2] (p. 1595).

TABLE II. CHARACTERISTICS OF POLYURIDYLIC ACID-DEPENDENT PHENYLALANINE INCORPORATION

Additions	Counts/min/mg protein
Minus polyuridylic acid	70
None	29,500
Minus 100,000 × g supernatant solution	106
Minus ribosomes	52
Minus ATP, PEP, and PEP kinase	83
0.02μ moles puromycin	7,100
0.31μg moles chloramphenicol	12,550
6 μg RNAase	120
6μg DNAase	27,600
Minus amino acid mixture	31,700
Deproteinized at zero time	30

Source: Nirenberg and Matthaei [2] (p. 1599).

TABLE III. SPECIFICITY OF AMINO ACID INCORPORATION STIMULATED BY POLYURIDYLIC ACID

Experiment No.	C14-amino acids present	Additions	Counts/min/mg protein
1	Phenylalanine	Deproteinized at zero time	25
		None	68
		+10 μg polyuridylic acid	38,300
2	Glycine, alanine, serine	Deproteinized at zero time	17
	aspartic acid	None	20
	glutamic acid	+10 μg polyuridylic acid	33
3	Leucine, isoleucine, threonine	Deproteinized at zero time	73
	methionine, arginine, histidine	None	276
	lysine, tyrosine, tryptophan	+10 μg polyuridylic acid	899
4	proline, valine		
	S35-cysteine	Deproteinized at zero time	6
		None	95
		+10 μg polyuridylic acid	113

Source: Nirenberg and Matthaei [2] (p. 1599).

II. MY DISCOVERY: THE CHEMICAL BASES OF POLY-U ARE SERIOUSLY AGAINST THAT OF RNA

See below Table IV, the documents in 1959 showed that Ochoa classified poly-U and RNA as two different polyribonucleotides. RNA in Ochoa's research paper is completely different from poly-A, poly-U, poly-C, and poly-I.

TABLE IV. SPECIFICITY OF PRIMING BY POLYNUCLEOTIDES

Polymer synthesized	Effect of					
	Poly A	Poly U	Poly C	Poly I	Poly AU	RNA (natural or synthetic)
Poly A	−	−	−	0	+	+
Poly U	−	−	+	0	+	+
Poly C	−	−	+			−
Poly G	0	0	+			
Poly I	−	0	+	+		
Poly AU					+	
Poly AGUC	0	0	+			+

+ Denotes priming, − denotes inhibition, 0 denotes no effect. Blank spaces, no information.

Note: In Ochoa's Nobel Speech of 1959, Poly U was different from neither Poly AGUC nor RNA (natural or synthetic).

Also, see the below Table V, the structural requirement of Crick's 4 × 4 × 4 <the Genetic Code> Table is the obvious mathematical calculation formula "4 × 4 × 4 = 64" [8] (pp. 416–417), [9] (p. 1231), [10] (p. 68), [11] (p. 82), [12] (p. 169), [13] (p. 136), [14] (p. 529), [15] (p. 46) in accordance with every "triplet segment X (4 different letters) Y (4 different letters) Z (4 different letters)" on RNA. However, clearly,

every triplet segment on poly-U, or "UUUUUU……UUUUUU" sequence, is merely the case of "1 × 1 × 1 = 1", and not "4 × 4 × 4 = 64 different mathematical cases" on RNA.

TABLE V. THE GENETIC CODE [16] (P. 1), [17] (P. 368), [18] (P. 548), [19] (P. 1124)

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

Source: CSHL [16] (p. 1); Crick [17] (p. 368); Reprinted with permission from CSHL [16]. © 1966 by CSHL: 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] (pp. 547–548; b) the byline "F. H. C. Crick" under the table's caption in the screenshot was originally from the documents [16] itself. c) this table was originally named as <the Genetic Code> in Crick's articles and lectures, hereafter I refer to it as "Crick's 4 × 4 × 4 <the Genetic Code> Table" to address both Crick's historical contribution to it and the table's mathematical structure 4 × 4 × 4. d) most of today's books and papers refer to this table as "the Standard Genetic Code (SGC)" in comparison with derived versions of this table.

Strikingly, the basic chemical concept of nucleic acids in Crick's "coding problem" is also named the "4-letter language of nucleic acids" [20] (p. 35), [21] (p. 55) or as "four-unit language of the polynucleotides" [22] (p. 1328), obviously, "1-letter language" or "UUUU UUUU……UUUUUU" is quite different from "4-letter language" or "f (U, C, A, G) = RNA". In the meanwhile, it is true that there is a special case called that "three consecutive uracil UUU" on "UU UU UUUU……UUUUUU" happens to be the same as "three consecutive uracil UUU" on "f (U, C, A, G) = RNA", however, the methods to look for "the natural connections between a triplet UUU and a singlet phe" is to check how and why an individual "phenylalanine" must be equal (located the same sites of a sequence) to individual "UUU", and not to "pile" or "aggregate" individual phenylalanine into "polyphenylalanine" that Matthaei-Nirenberg experiments had done in 1961.

Finally, for establishing the idea of "code" or "not-code", molecular biologists always say that "the sequence of four bases 'codes' the sequence of the twenty amino acids" [23] (p. 158), instead of saying that "the aggregation of one base (million times of repetition of one nucleoside base)codes the aggregation of one amino acids (million times of repetition of one amino acids)" in accordance with millions of chemical reaction events like Matthaei-Nirenberg's experimental type of "if reactant A↑, then product B↑" (see Fig. 1).

III. MY DISCOVERY: POLY-U HAS NO CAPABILITY TO BEAR SO MANY THEORETICAL CONCEPTS THAT "MRNA" MUST BEAR

In today's worldwide college textbooks like <Biochemistry> <Molecular Biology> <Organic Chemistry> <Genetics> etc., most fundamental theories and hypotheses of genetics and biochemical sciences are based on the concept of mRNA molecules. These include the (a) "central dogma" [proposed before the poly-U experiment), (b) "sequence hypothesis" [proposed before the poly-U experiment), (c) concepts of start and stop codons and classification of RNA coding and noncoding regions [proposed after the poly-U experiment), (d) frame shift theory (which was proposed after the poly-U experiment) and its basic concept, i.e., the reading frame on mRNA, (e) nonoverlapping (which "implies that there must be some way of determining which triplets in a sequence are coding triplets and which are not" [24] (p. 689)) and overlapping reading discussions, (f) "Wobble Pairing Hypothesis" and Wobble interaction with tRNA molecules, (g) concept of the "collinearity" of nucleic acids with polypeptide chains, (h) precise enzymatic sites of ribosomes for protein synthesis, (i) taxonomy of organisms such as prokaryotic and eukaryotic cells, and (j) "RNA world hypothesis" [25] (p. 3458) (which asserts that "RNA is an extremely complex molecule" [26] (p. 2)). Thus, based on chemical theories and chemical reaction studies, the synthetic poly-U described in Matthaei-Nirenberg experiments could never satisfy the aforementioned versatility requirements of mRNA.

For example, assuming one biologist has already planted poly-U molecule into the unknown "RNA world" of the living organisms, no biochemists of today would believe that both Matthaei and Nirenberg could have experimentally succeeded in ignoring "millions of non-coding RNA" and in turn in confirming "poly-U is the best coding RNA".

IV. MY DISCOVERY: POLY-U'S CHEMICAL FUNCTIONS ARE NEVER EQUAL TO ONE STRAND OF THE WATSON-CRICK MODEL OF DNA

In the history of "mRNA hypothesis", Crick's "central dogma" assumption was the "theoretical host" of "mRNA hypothesis" and Jacob's "experimental conclusion about mRNA" was considered as the "evidence for existing mRNA molecule".

A. Poly-U Is not Possible to Replace "The Step of RNA" in Crick's "Central Dogma" Triangle

In Crick's "central dogma" triangle [27] (p. 562) (see Fig. 2(a, b)), mRNA is depicted as an intermediate molecule between a double helix and protein. "Whether poly-U has carried the confidential genetic message" was exactly decided by "poly-T", which is said to represent one single strand of Watson-Crick model of DNA, and not decided by "the aggregation reaction of phenylalanine in Matthaei-Nirenberg chemical reaction system". Therefore, we say, if Matthaei and Nirenberg experiments were eventually designed to deduce that "uracil was considered to be informatically analogous to thymine in DNA" [27] (p. 561), their experimental process should be focused on "how poly-T produces poly-U in a chemical reaction", and on "how one single strand of Watson-Crick model of DNA could be sufficiently equal to poly-T". This means that "even if poly-U does not cause the occurrence of a chemical reaction producing poly-Phe", as long as Matthaei and Nirenberg experimentally proved that "poly-U has successfully received the message from poly-T", then, "poly-U is a messenger RNA" can be established.

Clearly, Matthaei and Nirenberg did not demonstrate that the sequential information of poly-U originated from poly-T (or other source molecules) nor did they show that the sequential information of the letter C (or A and G) on mRNA originated from the sequential information of the letter C (or A and G) on DNA.

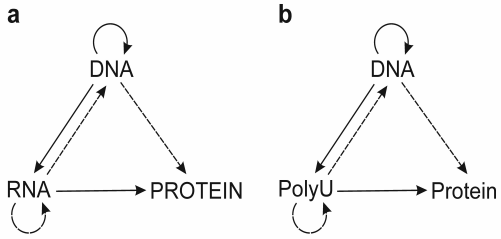

Fig. 2. Poly-U cannot replace the intermediate molecule (RNA or mRNA) in the "Central Dogma" diagram.

Crick Francis. <The Central Dogma of Molecular Biology>. Nature, 227: p. 562 (Aug. 8, 1970). Reprinted with permission from Nature, vol. 227. © 1970.

1. The 1970 version of the "central dogma". [27] (p. 562);

2. The poly-U version of the "central dogma" made by author Xingyang Yang.

Note: i) Matthaei and Nirenberg did not provide any evidence to prove that the message of poly-U came from poly-T, i.e., there was no "if poly-T↑, then poly-U↑" chemical reaction experiment, neither did they prove that poly-T was some sort of DNA in 1961, i.e., there was no "if DNA↑, then poly-U↑" chemical reaction experiment. ii) "RNA" in 1970 Crick's "central dogma" was not named "mRNA".

B. Poly-U Does not Conform with the Discovery of mRNA in Jacob's Experiments

In April 1961, Jacob-Monod experiments defined "mRNA" [28] (pp. 349–350) as "Messenger RNA should have a base composition reflecting base composition of DNA", however, uracil of poly-U is none of four bases on DNA; Jacob-Monod experiments also defined "mRNA" [28] (pp. 349–350) as "Messenger RNA should be found associated with ribosomes", however, Matthaei-Nirenberg's poly-U was clearly an artificial polymer, and was not extracted from the natural ribosomes.

Historically, it was after the publication of Matthaei–Nirenberg PNAS paper that Nirenberg planned to learn more regarding mRNA from Jacob's lab [29] letter; this indicates that in 1961, Nirenberg himself was unsure regarding whether poly-U was an mRNA.

V. My Discovery: The Poly-U's 5' to 3' Reading Is Totally Different from mRNA's 5' to 3' Reading

The very classical textbooks teach us "the direction of mRNA reading is 5' to 3'" [6] (p. 332), through which the "different orders of 64 genetic codons" in Crick's 4×4×4 <the Genetic Code> Table can be assured. For example, assuming there is an mRNA as the below:

5' U C A G C C C A G U U U C A G A G C U C U C U C A A A A G A C G G A U G 3'

When reading it from 5' to 3', it has triplet codes as U C A, G C C, C A G, U U U, C A G, A G C, U C U, C U C, A A A, A G A, C G G, A U G;

When reading it from 3' to 5', we get the different triplets as GUA, GGC, AGA, AAA, CUC, UCU, CGA, GAC, UUU, GAC, CCG, ACU.

However, for the nucleotide sequence "UUUUUU……UUUUUU", it has no differences between reading from 5' to 3' and reading from 3' to 5', as below:

Reading from 5' to 3', it is as:

U U U U U U……U U U U U U

Reading from 3' to 5', it is also:

U U U U U U……U U U U U U

More important, it is clear that the exampled mRNA sequence "U C A G C C C A G U U U C A G A G C U C U C U C A A A A G A C G G A U G" has a dramatic feature of sequential order, yet "UUUUUU……UUUUUU" has no the sequential order.

VI. My Discovery: Matthaei-Nirenberg Experiments Could not Distinguish "Messenger Poly-U" from "Non-messenger Poly-U"

It is a disastrous experiment for Matthaei and Nirenberg to distinguish "messenger poly-U" from "non-messenger poly-U".

The concept of theoretical mRNA, which includes "informational RNA" [22] (p. 1329), intelligence RNA, spy RNA, coding RNA, template RNA [1] (p. 184), [2] (p. 1601), [6] (p. 326) (or format RNA), intermediate RNA, puppet RNA, and mRNA tape, involves a message or messenger (intelligence or spy, information or information provider, code word or code word designer, and empty template or empty template creator); if Matthaei and Nirenberg truly wanted to establish poly-U as an mRNA (or messenger poly-U), then their experimental direction based on routine scientific methodologies should have focused on distinguishing

"messenger poly-U" (similar to cipher words written on a sheet of paper) from "non-messenger poly-U" (similar to an empty sheet of paper). In other words, Matthaei and Nirenberg should have experimentally established the concepts of "non-messenger RNA" [30] (p. 273), [31] (p. 7536) or "non-coding RNA" [32] (p. 3), [33] (p. 1) in advance and then clarified that poly-U (or some regions of poly-U) cannot exist as a non-messenger, noncoding, or noninformational molecule to push forward the relevant question about what is "mRNA defined poly-U" (i.e., what is an mRNA poly-U? what is mRNA type informational poly-U?) or about what is "poly-U defined mRNA" (i.e., what is poly-U mRNA? What is poly-U type informational mRNA?). Assuming that Matthaei and Nirenberg have known that poly-U carries certain information or message (say, mRNA poly-U or poly-U mRNA), it would signify that poly-U was not a noncoding or non-message molecule. Thus, the following paradox surfaces: how can a chemist determine whether poly-U's message originates externally and why does poly-U not generate information or genetic message by itself? If poly-U generates a message naturally (say, self-message), it will be an intelligence provider (say, a message source or a "self-message molecule") but not an intermediary molecule that is similar to mRNAs (say, "messenger molecule" or "postman molecule"). If poly-U does not generate a message naturally and truly carries an additive message coming from the external molecules (say, the "additive message" from outside the poly-U), Matthaei and Nirenberg would have had to face several difficult experiments before being able to claim that poly-U is similar to an mRNA-like molecule, akin to a molecule between a double helix and protein: (1) Matthaei and Nirenberg should have conducted experiments to demonstrate that the messages of poly-U must be originated exclusively from poly-T (say, a "natural informational molecule"); (2) they should have experimentally demonstrated that "the natural message" of poly-T was generated by poly-T only (confirming that poly-T itself is a natural informational molecule but not an intermediate informational molecule); and (3) they should have experimentally provided an unidirectional route that allowed poly-T (confirmed that it is a nonintermediate informational molecule) to pass its natural message on to poly-U (assuming it is not a natural informational molecule) but not to pass the natural message on to any other molecules in the living cells; (4) very importantly, they should have experimentally ensured that the message carried by poly-U (assuming poly-U has already obtained the message exclusively from poly-T) can never pass back to poly-T (confirmed that poly-T can only send message out and cannot receive the message from outside). However, Matthaei and Nirenberg would never have been able to conduct so many unknown experiments to distinguish between informational poly-U (including self-informational molecule poly-U and intermediate informational molecule poly-U) and the noninformational poly-U.

From the perspective of interpersonal communication, no matter Matthaei and Nirenberg concluded poly-U was a natural self-informational molecule or poly-T was a natural self-informational molecule, they all mean to conclude "the existence of a natural self-informational molecule", which means to decipher "the language communication between one stone and another stone".

VII. My Discovery: Matthaei and Nirenberg Had not Collected the Genetic Data to Allocate Poly-U (but not Triplet UUU) in Any Wild Organisms or Natural Organelles

As we all know, Matthaei-Nirenberg's poly-U was a synthetic molecule but not a discovery of the natural nucleic acid molecule.

On one hand, looking back the history, within the short duration between August 10, 1961 (5th Moscow conference) and October 15, 1961 (official publication of Matthaei and Nirenberg's PNAS article), Matthaei and Nirenberg would have had no time to collect genetic data to evince the hypothesis that the biological message (genetic information) of their synthetic poly-U molecule came from the chromosome or DNA of any living cell. And, they had no scientific evidence to prove that living organelles harbored such synthetic poly-U molecules. According to the science history, until February 1963 (2 years after the Matthaei-Nirenberg poly-U experiment), "no naturally occurring RNA has been observed with such a large fraction of U" [34] (p. 774). Thus, it is highly likely that Matthaei-Nirenberg's poly-U was not derived from living organisms! It was only an artificial chemical.

On the other hand, multiple biofunctions of mRNA molecules must be realized by "codon-anticodon" pairings between mRNA and tRNA molecules. With no presence of phe-tRNA, it is not possible for "mRNA poly-U" to flow its message to phenylalanine successfully. However, I have checked Matthaei and Nirenberg's papers of 1961 many times, I have not seen any clues in their experimental process to hint at the necessity of "creating UUU's anti-codon". This implies that the synthesis of "poly-Phe" in Matthaei-Nirenberg experimental system is possibly not chemically constituted by one-after-one phenylalanine molecule "to feed the phenylalanine chain" growing on ribosomes, as what is being taught in the current biological textbooks according to Crick's "sequence hypothesis".

VIII. My Discovery: Matthaei and Nirenberg Did not Provide Evidence to Prove that "Poly-phe Has Received Whatever Message from Poly-u" Except for "if Poly-U↑, then Poly-phe↑" Reaction

There is quite a strange academic phenomenon. In both Matthaei and Nirenberg's Moscow Congress report and PNAS paper, I do not see their attention in analyzing the chemical reaction mechanisms within "if poly-U↑, then poly-phe↑" reaction, furtherly, I do not see their discussions relating to the diseases "Phenylketonuria [pKU)" [35] (pp. 44–54) caused by "the gathering of phenylalanine in human body". On the contrary, the two researchers from National Institute of Health announced "The artificial molecule – poly-U" was a "mRNA", which hints that "poly-U is a chemical information

transmitter" and "poly-phe is a chemical signal receiver"! Since it has been a "Rosetta Stone" event in the history of science, let's take a look and check whether Matthaei and Nirenberg had ever designed "the route of the information flow from poly-U to poly-Phe" in their experimental process.

We suppose there happened truly a "communication process" between poly-U and poly-Phe in the Matthaei-Nirenberg experiments, as the communication chart shown in Fig. 3, poly-U can be postulated as the signal transmitter at the input end to provide "information or message", poly-Phe then works as the signal receiver at the output end behind the "processing box". Immediately, we find that "Matthaei's and Nirenberg's success in polyphenylalanine synthesis" only reflects the occurrence of chemical reaction from reactant polyuridylic acid to product polyphenylalanine, but not reflecting that (1) poly-U has ever transmitted signals to whatever chemical matters; (2) processing box has ever transferred the "natural message of poly-U" from "analog signal" into "digital signal"; (3) poly-Phe has ever received whatever signals sent out by the processing box.

Fig. 3. The simplest communication process assumed between poly-U and poly-Phe.

The worst of all, according to the academic research "UUU sending a message to phenylalanine" explained by Crick's 4 × 4 × 4 <the Genetic Code> Table, the correct solution for judging "whether or not poly-U sends a message to poly-Phe" is to check whether or not the output end, the "terminal apparatus" poly-Phe, has successfully received a message, but not to check whether or not poly-U sends a message. However, the biological history told us that Crick and his team focused on "confirming the information flow of Matthaei and Nirenberg's poly-U" in such a wrong way: (1) not checking if the poly-phe in Matthaei and Nirenberg experiments has ever received whatever message during the whole process, but deducing whether poly-U carries a message; (2) not checking if "ribosomal RNA, ATP, ATP-generating system, the mixture of amino acids" presented in Matthaei and Nirenberg's experiment process has ever "hear" (or "seen") the message sent out by poly-U molecule, but by checking whether the cistron A and cistron B on the rII region of phage T4 that was outside of Matthaei and Nirenberg's chemical reaction mixtures to conclude that "Matthaei and Nirenberg's poly-U has a triplet message". ---This is quite a ridiculous method! This is never an effective way to judge "whether Matthaei and Nirenberg's poly-U carries a natural message"! Even if poly-U carries a "UUU message", the determination that poly-U carries a "UUU message" is still invalid for proving "poly-Phe has received a message from poly-U". In general, if poly-Phe does not carry a message, we do not say "poly-Phe has received a message from external chemical matter".

In chemical science, the mechanism of linear synthesis of "phe-phe-phe……-phe-phe" from countless single molecules of "phenylalanine" is the process of "losing a residue of −H or --OH" from different single phenylalanine. If one must relate "the chemical reasons why poly-Phe has been produced in the 1961 Matthaei-Nirenberg experiment" with the term "message in the concept of mRNA", we say, "losing a residue of −H or OH" might be those sorts of message. However, how can a chemical matter concept of UUU make one phenylalanine lose a residue of "−H" and make the next one lose a residue of "--OH" under the same chemical reaction conditions? Therefore, "UUU sending a message to phenylalanine" cannot be established in chemical science.

IX. My Discovery: Highly Repettive Uracil of Poly-U Violates the Genetic Law "Highly Repettive DNA Does Not Code for Protein"

"Highly repetitive DNA does not code for protein" is common genetic knowledge, and it has been proved by many scientists. This implies that "theoretical mRNA" cannot be highly repetitive "nucleotide bases", and cannot be "fully repetitive U" or "fully repetitive C or A or G". Now we see, poly-U is not only a highly repetitive sequence, but also the fully repetitive sequences. To this aspect, we say "poly- U cannot act as mRNA".

X. Discussion

In April 1961, Jacob experimentally concluded that "messenger RNA should be found associated with ribosomes"; In 1970, Crick did not use "messenger RNA" in his "Central Dogma" triangle; In 2001, some experts announced that "it should be clarified that nowadays DNA and RNA are considered as synonymous" [36] (p. 215). In contrast, the synthetic poly-U in Matthaei-Nirenberg experiments was not associated with ribosomes, does not match "Central Dogma", and is not considered as DNA synonymous, therefore, I advocate that Matthaei and Nirenberg's synthetic poly-U in 1961 was not sort of messenger RNA.

The final academic purpose that Matthaei and Nirenberg announced "poly-U functions as messenger RNA" in 1961 was to help Dr. Crick and Dr. Watson to establish a "4-letter nucleic acids control 20-letter protein" scheme before Watson-Crick Model of double helix obtained the Nobel Prize in 1962, I advocate that the chemical structure of "mRNA" molecules must include total four bases of adenine (C5H5N5), guanine (C5H5N5O), cytosine (C4H5N3O), and uracil (C4H4N2O2). This way, for poly-U or "UU UU UUUU……UUUUUU", as one-letter languages, I suggest kicking them out of the concept of "mRNA".

XI. Unanswered Questions

Question one: What is the "natural message" if Matthaei-Nirenberg's experiment had proved that "the message of poly-U" came from "poly-T"? Assuming there exist "non-order natural message" in oligopolymers like poly-U, poly-C, poly-A, poly-G, poly-I, and poly-T

in the living organisms, are there any types of "noise message" in poly-U, poly-C, poly-A, poly-G, poly-I, and poly-T? Why all the "natural messages" are effective messages? And, why do other organic chemicals like alkane or alkene not have a "natural message"?

Question two: As we all know, "mRNA" was a key "theoretical molecule" assumed by Crick in the 1950s to explain the genetic functions of the Watson-Crick model of DNA, easily it comes out a simple question: If Crick did not initiate the concept of "theoretical molecule mRNA" to separate nucleic acids and proteins in the cell circumstances, would other biochemists be possible to design the chemical reactions that the " A-T pairing and C-G pairing nucleic acids" directly stimulate the amino acids incorporation into protein? (we assume that the "cell-free protein synthesis experiments in the 1960s" could not conform to the natural law of bio-protein synthesis). In biochemical mechanisms, except for the Watson-Crick model of DNA molecules, can we find any other type of chemical reactant that carries the "natural information"? and, it has a "natural postman" who delivers such "natural information" to its specific consumers (information consumers)?

Question three: Following Matthaei-Nirenberg's announcement "poly-U acting as a messenger RNA", after the 1961 Moscow 5TH International Congress of Biochemistry, the Crick team particularly conducted a famous genetic experiment [9] (p. 1232) to "prove" that the "genetic message" of UUUUU…UUUUU is of "3 bases". Based on Crick's "DISCOVERY that genetic message is of 3 bases", the story of Matthaei and Nirenberg discovering "poly-U acting as messenger RNA" was immediately changed to the story of "Matthaei and Nirenberg discovering the first genetic code UUU". In nowadays college textbooks, the sentence "Matthaei and Nirenberg discovering the first genetic code UUU" has been sufficiently widespread in all kinds of bio-textbooks, therefore, my question is: Why doesn't the poly-U carry a genetic message if it is written in the form of UUUUUUUUUUUU even if it acts as a messenger RNA? Why DOES the poly-U carry the genetic message (and it is the secondhand message) only when it is written in the form of UUU-UUU-UUU-UUU-U? (Welcome the influential chemists to follow up with my next paper "What is the triplet nature of Matthaei and Nirenberg's poly-U?")

Question four: According to the official website of the American Chemical Society, and the official site of the NIH, the Matthaei and Nirenberg chemical reaction "if poly-U↑, then poly-phe↑" was completed in May 1961, however, many "additive concepts" to Matthaei and Nirenberg's poly-U, such as "degeneracy", "collinearity", "universality", "specificity", "evolution", "anticodon", "frozen accidents", were gradually added to "messenger" poly-U after May 1961, why was that? Was it because of the "August 1961's Moscow 5th International Congress of Biochemistry?" Therefore, it comes out another question "Did the Matthaei and Nirenberg experiment crack the first code UUU before the Moscow congress?" or after the Moscow congress? Or on the Moscow congress venue?

XII. Conclusion

For Matthaei and Nirenberg's occurrence of "if poly-U↑, then poly-phe↑" reaction, poly-U truly works as a chemical reactant in comparison with "poly-phe is a chemical product" announced by Matthaei and Nirenberg; For Crick's theory of Central Dogma, poly-U was the worst evidence to support the "information flow from natural nucleic acid molecules to natural proteins" because poly-U was lack of the orders of adenine, guanine, cytosine, and uracil. I suggest biochemists select other biochemicals to promote Crick's "Central Dogma" assumption.

Conflict of Interest

The author declares no conflict of interest.

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