OTS: 60-11,848

JPRS: 2958

30 June 1960

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NEW YORK 17, N. Y.

JPRS: 2958
CSO: 4107-N

ON THE ROLE OF RIBONUCLEIC ACIDS IN PROTEIN SYNTHESIS

[Following is the translation of an article by F. B. Straub of the Institute of Medicinal Chemistry, Budapest University, in Voprosy Meditsinskoy Khimii (Problems in Medicinal Chemistry), Vol. VI, No. 2, March-April 1960, pages 115-120.]

Less than 20 years ago Casperson[1] and Brachet[2] were the first to note a relationship between the amounts of ribonucleic acid (RNA) in the cell and the rate of protein formation. An elaborate quantitative study by Davidson[3] clearly demonstrated this parallelism within various tissues and organisms.

Today we have a much greater knowledge on the role of RNA in the biosynthesis of proteins. Upon the formation of a new idea certain difficulties have to be overcome before one can give his studies a perspective direction. It was true also in this case. As usually, one of the difficulties was the lack of a reliable method for a quantitative determination of RNA and its separation from desoxyribonucleic acid (DNA). But there was also another more serious difficulty, namely, a deeply implanted idea that by synthesizing such a specific substance as a protein, the genes, i. e. the nucleic matter, otherwise spoken of as DNA, could be directly controlled. However, by 1950 the work mentioned above had convinced biochemists that the biosynthesis of cytoplasm proteins

is primarily associated with the ribonucleic acids and is only indirectly controlled by the nucleus and DNA.

Jeener and Jeener[4] showed by very convincing experiments that in the presence of thermobacteria the synthesis of RNA and DNA can be selectively suppressed. They were successful in showing that the synthesis of RNA was associated with an increase in the mass of cytoplasm, whereas the synthesis of DNA is associated only with cellular division. At the same time Price[5] for the first time pointed out the increase in RNA formation in presence of an enzyme synthesis induced by microorganisms.

One of the clearest arguments against the participation of DNA and in favor of the participation of RNA in protein synthesis is presented by Cohen and Barner[6]. They used a mutant of E. coli which grows only in the presence of thymine; without thymine the growth of the culture is inhibited; however, its capability of formation of the induced enzyme under induction conditions is well preserved. Under these conditions the synthesis of DNA is prevented, whereas the regeneration of RNA is continued. Approximately at this time Pardee[7] conducted experiments with a mutant lacking in uracil. In the presence of this organism and in absence of uracil, i. e. under such conditions that the organism is unable to synthesize RNA, the inductive synthesis of enzymes is prevented.

Kramer and Straub[8] came to the conclusion that after the induction of the B. Cereus cell with penicillin and up to the time of the appearance of the induced enzyme (penicillinase) a formation of a new specific form of RNA must take place. Analogous results were presented also by Hunter and Butler[9] and Spiegelman[10] and coworkers.

A different kind of experimental evidence on the role of RNA in protein synthesis and its independence from DNA can be found in the laboratory work of Brashe[11]. A monocellular alga Acetabularia mediterranea can be successfully divided into nucleicmatter-containing and

non-containing parts. The part containing no nucleic matter is able to live for several months, during which time it can bring about an increase in the mass of cytoplasm. A study on the rate of protein synthesis using labeled aminoacids did not show any substantial difference between the intact cells and the cells devoid of nucleus.

Another experimental approach to this problem is based on the work of Hultin[12], Borsook[13] and Allfrey and coworkers[14]. In their work the problem of the intracellular localization of the biosynthesis of the proteins was investigated. Labeled amino acids were injected intraperitoneally into animals, at various time intervals they were killed and then fractions of nucleus, mitochondria, microsome and cellular fluid were isolated from their liver tissue. It was found that within a few minutes after introduction of the labeled amino acid the concentration of the "label" in the protein of the microsome fraction was higher than in all other fractions; the nuclear matter was strongly lagging behind other remaining fractions in inclusion of the "label". As it is known, the microsome fraction contains the greatest quantity of RNA; more than 50 percent of all RNA of the cell is concentrated in the microsomes. More recent investigations by Palade[15] showed that the microsome fraction contains cytoplasmic network and a multitude of small compact corpuscles, called the microsome granules (or ribosomes). Since the cytoplasmic network is basically built up of lipoproteins the ribosomes contain RNA in the form of the ribonucleoprotein. The fraction of microsome obtained by centrifugation of the homogenate for one hour at 105,000 g. (after preliminary separation of the fraction which precipitates at 10,000 g.) can be further fractionated using detergents, e.g. desoxycholate. Furthermore, the granules of the microsome can be separated from the cytoplasmic network; centrifuging at 100,000 g. the micro-

some granules precipitate, but the substance of the cytoplasmic network remains in solution. The enclosed "label" appears mainly in the granules of the protein portion of the ribonucleicprotein. The granules contain RNA and protein approximately in the ratio 1:1. Experiments of this kind were carried out mainly with the liver cells, and, although in other kinds of cells the ratio is somewhat different, the high rate of inclusion of the "label" into the ribonucleicprotein of the microsome was found everywhere. From this a conclusion was made that the proteins of the cytoplasm are sythesized in the microsome granules and are subsequently carried to the other parts of the cell.

We shall not discuss here if the conclusion in this form is correct. However, we must note that up to now the labeled protein of the microsome granules had not been identified with any of the known proteins. On the other side, Bates and Simpson[16] observed the synthesis of the cytochrome C in mitochondria of the cardiac muscle to proceed, as far as they could see, without the participation of the microsome. Nevertheless, the fact remains that the microsomes are very active in the inclusion of labeled amino acids into their ribonucleicproteins.

Furthermore, the inclusion of labeled amino acids into isolated fractions of the cell <u>in vitro</u> was investigated. This method meets some objections, since it is possible that the inclusion of labeled amino acids <u>in vitro</u> may not be identified with the synthesis of proteins. Investigation according to this method originated mainly in Zamechnik's laboratory; this work may appear to be the first clue in solving the puzzle of the mechanism of the biosynthesis of protein. First of all it was established that the fraction obtained from liver cell homogenate or from cells of ascitic tumors after removal of the nucleus and mitochondria was capable to include labeled amino acids into the protein[17].

As the energy source necessary for the synthesis is considered ATP and the system which regenerates the latter. Under these conditions the fraction containing the microsomes and the cellular fluid includes the "label" into the protein part of the ribonucleic protein of the microsome granule.

This is a very successful system since by using it it was found to be possible to separate the components participating in the complex process. Hoagland[18] showed the presence of a number of enzymes in the cellular fluid which activate discrete amino acids. Activation takes place by the way of a reaction proposed by Lipman[19]. ATP reacts with the amino acid cleaving off pyrophosphate and forming a mixed anhydride of the amino acid and adenylic acid. This activation of amino acids at the carboxyl group is now accepted as the first of the basic steps in the mechanism of the biosynthesis of protein.

Enzymes activating the acyl group can be precipitated from the cellular fluid at pH 5.2. The precipitate contains a great part of the acyl-activating enzyme and also a quantity of RNA. This fraction is called the pH 5-enzyme. RNA in this fraction represents practically all the RNA remaining in solution after the precipitation of the microsome. It is called S-RNA (soluble RNA). It was found that it has a low molecular weight - its molecule contains only 30 - 40 mononucleotide groups. Hoagland and coworkers discovered the important role of this S-RNA in the inclusion process. Amino acids with an activated carboxyl group or amino acyladenylates react with S-RNA, whereupon the amino acid radical attaches to the RNA. Such RNA - amino acid complexes have been isolated. If such a complex adds to the microsomes then the labeled amino acid residue must be transported from the S-RNA into the microsome protein. For such a transport the presence of guanosine polyphosphate is required. There is still much to be done in the

clarification of details for this course of the reaction, however, its main steps are corroborated by work in many other laboratories. The process can be expressed in the form of the following scheme:[22]

amino acid $\xrightarrow{\text{pH 5-enzyme}}$ aminoacyl - AMP $\xrightarrow{E_2}$ aminoacyl - RNA

$\xrightarrow{E_3}$ microsome RNA - protein.

In this scheme E_2 and E_3 are specific enzymes differing from the pH 5-enzyme.

The discovery of the RNA - amino acid complex as being the intermediate product in the protein synthesis revived interest in RNA chemistry. A few years ago Dounce[23], Keil[24], and also Koningsberger[25] found in commercial preparates of RNA a stably bound component giving a color reaction with ninhydrin and yielding a series of amino acids after hydrolysis. However, not all amino acids were detected. Whether or not the presence of the amino acids stably bound with the RNA in such preparates indicates addition as the result of an activation reaction of the amino acids remains unclear.

The soluble RNA makes up only a part of all the RNA of the cell. However, this fraction is by no means homogeneous. Hoagland[20] and Schweet[26] showed that the inclusion of the various amino acids into the S-RNA proceeds additively, in other words one or another amino acid is detained by the different molecules of the RNA. Holley [27]recently reported that by the counercurrent-distribution method the S-RNA can be fractionated so that the alanine and tyrosine enclosing types of the molecules are separated.

The nature of the bond between the amino acid and the RNA is not yet clear. Lipmann and coworkers[28] isolated from the hydrolysate of the labeled amino acid - S-RNA complex an ether of the amino acid and the adenylic

acid in which the amino acid residue is linked with the hydroxyl group at the C - 2 or C - 3 atom of the ribose residue of the adenylic acid occupying the end position in the polynucleotide chain of the S-RNA. The properties of the aminoacyladenylates constitute difficulties in this scheme of study: anhydrides of a similar kind are very reactive molecules; they, as was observed by Berg[29], hydrolyse in solution at pH 6 in the course of a few minutes; on the other hand, they can react with any compounds that have an amino group, forming acylated derivatives. According to Fruton[30] the amino acids of the amino acid adenylates when added to the homogenate are inclosed into the proteins at the N - end of the peptide chain. Moldave pointed out that the adenylates of the amino acids can spontaneously regroup themselves into complex ethers of adenylic acid and amino acid similar to the ones found by Lipmann. This difficulty was soon realized and resolved by assuming that the amyloacyladenylates do not exist in a free form in solution, but only in a combined form. Putting aside the question about the nature of the bond between the amino acid and the RNA, we note that there is no discrepancy in the conclusion that the amino acid attaches to the terminal nucleotide of the soluble RNA and that this terminal nucleotide always seems to be the adenylic acid. In a similar complex the bond of the aminoacyl residue is such that it can form the peptide bond with a decrease in the free energy of the system.

I do not want to go into details of the speculative assumptions that arise on the basis of these discoveries. Crick[32], author of many clever hypotheses, proposed that the soluble RNA plays a role of an adapter, i. e. that it transports the amino acid residue to a predestined location in the matrix. Assumed that this takes place in the last step of the scheme cited by me above, evidently, the RNA - amino acid complex reacts (with the participation

of guancsine triphosphate) with the microsome RNA, which,as is assumed, plays the role of matrix. Besides the fact that the RNA labeled with the C^{14}-amino acid transports this "label" to the protein part of the microsome protein we do not know anything else about this process.

Up to now we were not concerned whether or not the mechanism of the biosynthesis of protein is connected with the RNA metabolism. Not too much data were obtained from the results of measuring the rates of RNA restoration, if we disregard the facts that the RNA is somehow restored and that various RNA fractions have various circulation rates. But this is not surprising if we take into account that we always have the substance as a complex mixture of the various RNA molecules. Consequently, the question of whether or not the RNA metabolism is connected with the synthesis of pretein can be approached only by adapting a simplified system. This system is represented by the supernatant fraction of the sucrose homogenate in which only the soluble RNA can be found. In Zamechnik's laboratory it was found(Hecht and coworkers[33]) the C^{14}-adenylic acid from ATP is actively included into the soluble RNA of the supernatant fraction. The inclusion of adenine triphosphate is speeded up in the presence of cytidine triphosphate, the addition of uridine triphosphate, however, is not detectable. It was found that this inclusion does not indicate the synthesis of new molecules of the RNA de novo, only an addition of a few nucleotides to the already existing polynucleotide chain of a nucleic acid molecule. From similar experiments carried out by Harbers and Heidelberger[34] follows that there are two different forms of the S-RNA molecule having the following nucleotide segments at the end:

...C ----- C ----- A and ... U ----- U -----G

These terminal groups of the RNA molecule, evidently are subjected to a rapid interchange in this soluble system. The first of them ending in ...C ----- C ----- A probably participates in a reaction with the activated amino acids. The role of the molecule of the other type has not yet been explained.

Another line of investigation also leads to the conclusion that the RNA metabolism is dependent on the presence of amino acids. Gros[35] and others noted that in E. coli cells an accumulation of RNA takes place if chloromycetin is present and the medium contains amino acids; no RNA is accumulated if amino acids are absent. A similar phenomenon was observed by Munro[36] who found that the change in the RNA content of the liver was dependent on the amino acid content in the diet. These phenomena, evidently, are not connected with the processes of synthesis of purine and pyrimidine bases.

There are several explanations of data obtained by Gros. Some assume that the RNA forming in the presence of chloromycetin is a waste product of metabolism and not a natural or a physiological RNA. We do not agree with such an opinion. I propose that this phenomenon is connected with the process of protein synthesis; in my opinion the accumulation of the intermediate product is dependent on the suppression of the protein synthesis. This opinion is based on our own investigations on amylase synthesis in cell-less extracts. Chloromycetin suppresses the formation of amylase from the protein-predecessor. In this case we discovered the accumulation of a [illegible] specific form of RNA (in the system under investigation no increase in the total quantity of the RNA takes place, it rather decreases [illegible] due to the presence of RNA-ase). If the RNA accumulated in the presence of chloromycetin is extracted and purified and then added to a cell-less extract of the pancreas it brings about the formation of amylase from the [illegible] protein

predecessor. We succeeded in showing that such a specific RNA can be prepared by incubating pancreatic RNA with the pH 5-enzyme of the liver, ATP, arginine, and threonine. We propose (while this has not yet been proven) that the specific RNA, which brings about the formation of amylase from the protein-predecessor, represents by itself an RNA - amino acid complex accumulating only in the presence of chloromycetin.

A few years ago, based mainly on the work by Spiegelman[37] and Pardee[38], a hypothesis was formed according to which on every molecule of the protein to be synthesized initially synthesizes a new RNA molecule which disintegrates after having fulfilled its role in the protein synthesis. This hypothesis of the "compatible synthesis" died a natural death; when it was investigated it could neither prove nor disprove the restoration of all cellular RNA. However, the results obtained with the soluble RNA, on whose chain terminus several nucleotides undergo a rapid exchange indicates the possibility that the RNA participates in the protein synthesis not as a passive templet, but as a labile organic material whose metabolism is closely connected with its functions.

Five years ago we could only say that RNA is more directly connected with the protein synthesis than is DNA. Reports of the last five years, as we have shown in several examples, represent a more detailed information on the mechanism of RNA participation in this process. The basic attention was paid to the role of the soluble RNA in the first stage of the protein synthesis. However, we were inclined to assume that also other types of RNA have some part in this process and that distinct types of RNA have distinct roles. Since we have a basis to think that the synthesis of a specific form of protein occurs in several successive stages it is reasonable to assume that in the most of these stages the decisive role may be played by specific RN acids.

According to a widely spread opinion the high specificity of proteins is determined by the characteristic ribonucleicacid specificity which serves the matrix in the synthesis of the protein. If the synthesis occurs in several steps then it is more likely that information about the structure can not be obtained in one step and from a single ribonucleicacid. We have several proofs that it really is so. Such an idea is in agreement with the contemporary views in genetics. We now know that the view assuming the existence of a discrete gene for each enzyme is not acceptable; the formation of an enzyme is dependent on several genetic loci which do not depend on each other. This is equivalent to the assumption that distinct RNacids active in different stages of the protein synthesis must be participating in the formation of one form of protein. This does not mean that for the available number of cellular proteins the cells must contain a still greater number of differing RNA molecules, because one and the same RNA molecule can participate in the formation of many differing proteins.

Looking forward we see before us two problems which, evidently, will be resolved within the near future. The first problem, a methodical one, consists of the separation of the RNA mixture into components of an identifiable molecular type. The second one consists of the determination of the mode in which RNA fulfills its function. It is possible that here we will discover something similar to the action of the enzymes. Apparently, we find ourselves at the source of knowledge about the biological role of the nucleic acids.

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