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SOME PROBLEMS OF THE INTERCONNECTION OF THE BIOSYNTHESIS OF PROTEIN AND NUCLEIC ACIDS

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Until recently problems of protein and nucleic acid synthesis, although extensively discussed in the literature, have been discussed, so to speak, unilaterally from the point of view of the influence of nucleic acids on protein synthesis. The extensive review of the literature in Brachet's (1957) book, which was published in 1957, and Loftfield's (1958) review have been written specifically in this way.

During the past two or three years, however, a number of works has appeared which show that the problem is not so simple as it appeared, and the relationships between nucleic acid and protein in which the protein was regarded, speaking mathematically, as a function, while the nucleic acid was regarded as the argument apparently does not correspond altogethe

to reality.

These problems, as far as we know, have not been discussed extensively in the literature, unless we consider the summaries of Chantrenne (1958a) in which he literally devoted several lines to them.

We are not proposing to summarize and generalize all the material on this problem. It is still contradictory in large part, and apparently the time for such a summary has not come as yet. Our purpose is to show new facts which make it possible to gain a different viewpoint of certain aspects of the interconnection of the protein and nucleic acid synthesis.

Mention should be made of the fact that in the subsequent presentation we shall utilize many works in which the investigations were carried out by means of tagged atoms. Surely, the uptake of the label does not, by far, always mean a synthesis de novo. However, at the present time, in the majority of cases we are still unable to differentiate the renewal of a molecule from its synthesis. Therefore, for convenience and simplicity of presentation we shall speak of synthesis, meaning both processes mentioned by this term.

The Interrelationship Between the Biosynthesis of DNA and Protein

The evaluation of what the nature of the influence of DNA on protein synthesis is may be expressed on the basis of an analysis principally of the following four groups of works: 1) Investigation of the Influence of Phage DNA on the Reproduction of Phage; 2) Study of the Transforming

Effect of DNA; 3) Works on the Study of Protein Synthesis in Microorganisms; 4) Investigations on Protein Synthesis in the Cell.

1) As is well known from a large number of works, phage DNA, being incorporated into the microbial body, produces a large number of phages in it. Recently, it has been shown very clearly that under ordinary conditions protein synthesis in the cell infected by phage proceeds continuously. Directly after the infection RNA is formed; however, the synthesis of some kind of proteins precedes it. This RNA, apparently, also exerts a further influence on the change in protein synthesis. As has been found by immunological methods, the protein is first synthesized, which is different from the cell protein and from the phage protein. The latent period necessar for the synthesis of new DNA following infection is apparently utilized so that the incorporated phage DNA change its metabolism in such a way that it directs it chiefly toward the reproduction of itself (Hershey, Melechen, 1957 Astrachan, Volkin, 1957, 1959; Volkin, Astrachan 1957; Cohen 1957, 1958; Watanabe, 1957, Astrachan, 1958).

Jeener (1957), on the basis of his works, draws the conclusion that the mechanisms of synthesis of the phage protein and the phage DNA are independent. True, Stent (1958a) presented Brown's report in which Brown observed the in vitro formation of small, yet noticeable quantities of phage antigen after the addition of purified phage DNA to preparations of destroyed bacterial cells, but the details of this work are not as yet known, and

therefore, it would be premature to draw any kind of conclusions at this point. At the same time, it remains unclear how pure the phage DNA incorporated in the microbe is. Ain all probability, it contains the so-called "internal" protein, which contains basic amino acids (Spuzizen, 1957; Levine, Barlow Van-Vunkac, 1958).

As is seen from the material presented, there is still no basis for speaking of the direct influence of DNA macromolecules or of the processes of its synthesis on protein synthesis in the phenomenon of bacteriophagia.

2) Numerous works on the transforming effect of DNA on microbes are generally known. They might be able to give some information on the influence of DNA on protein synthesis. However, first of all, it is far fr being clear how pure the DNA is which is used for the transformation: according to recent data, it still contains some protein (Zamenhof, 1957), true enough, very small quantity (0.02 percent); secondly, data in the literature attest to the fact only that under the influence of DNA (in cases of transformation) a change occurs in metabolism, apprently including also a change in protein synthesis, but the biochemical routes of these changes are not as yet known.

It is very possible that RNA plays a part in the change in direction of protein synthesis. In any case, as far as we know, there is still no direct proof of the direct influence of foreign DNA on protein synthesis in the transformation phenomenon (Timakov and Skavronskaya, 1958; Khesin, 1958; Ephrussi-Taylor, 1957; Hotchkiss, 1957; Goodal, Harrison, 1957; Hotchkiss, 1957).

3) Among the works on the study of the mechanism of protein synthesis in microorganisms we should dwell on the extensive investigations of Gale (1957)and Folkes (1953a, 1955a, b, 1958a, b) which were performed on killed staphylococci. According to their data, after the removal of the nucleic acids the uptake of tagged amino acidis stimulated to varying degrees by the addition of both DNA and RNA of the same origin. However, this uptake is stimulated also by products of enzymic decompositi of nucleic acids. Here, among the products of enzymic hydrolysis of RNA a factor of unknown origin is contained which stimulates the uptake of a number of amino acids. However, it does not replace the nucleic acids in increasing catalase activity or the mixture of purines and pyrimidines needed for the formation of galactosidase. Therefore, the material of these research workers do not give us the basis for drawing any conclusion concerning the direct specific effect of DNA on protein synthesis.

Quite recently, a number of other works appeared which showed that DNA synthesis has is not connected directly with protein synthesis. For example, it was shown that the induced synthesis of enzymes proceeds in the absence of DNA (Landman, Spiegelman, 1955). According to Chantrenne and Devreux (1958) low concentrations of 8-azoguanine had no influence on DNA synthesis in B. cereus and suppressed protein synthesis. On the other hand, the suppression of DNA synthesis does not interfere with the

protein synthesis (Barner, Cohen, 1958). Okazaki and Okazaki (1958), on the basis of a study of DNA, RNA and protein synthesis in Lactobacillus acidophilus, conclude that protein synthesis does not depend on DNA formation.

Pardee and Prestidge (1958) believe that DNA does not determine the rate of synthetic processes in the cell at all. A. Spiegelman (1957) points out that the thymidine-requiring mutant of E. coli synthesizes various enzymes in the absence of DNA synthesis. This speaks for the fact that proteins and DNA synthesis may be disassociated.

According to information presented by the same author, 99 percent of the DNA can be removed from protoplasm without any loss of its capacity of forming enzymes, which would speak for the absence of any influence of the DNA molecule on protein synthesis.

Data on the influence of DNAase on the uptake of tagged amino acids in the microorganisms are entirely unclear. Thus, in one case, through the example of fragments of E. coli, it was shown that the addition of DNAase inhibits the uptake (Nisman, Hirsch, Marmur, Causin, 1955) of amino acids into proteins; ~~under the influence of DNAase~~ in others, on the other hand, it was found that this uptake was stimulated by the effect of DNAase (Jaster, 1953; Beljanski, 1954).

Thus, we see that ~~&~~ even in this case the data presented do not give us any direct proof of the direct effect of DNA on protein

synthesis.

4) Usually, reference is made to the experiments of Allfrey, Mirsky and Osawa for proving the participation of DNA in protein synthesis. here, protein synthesis in cell nuclei, which had been inhibited by DNAase, was restored by a fresh portion of DNA. However, these experiments have been submitted to another treatment. The fact has been established by these same authors that the removal of DNA from the nucleus stops ATP synthesis. The addition of DNA, which restores protein synthesis, also restores ATP synthesis. Therefore, the inhibition and recovery of protein synthesis after the removal and addition of DNA may be explained by the fact that it influences the energy provision for the synthesis and not the protein synthesis directly. Chantrenne (1958), for example, adheres to this viewpoint.

In addition, these experiments strangely speak about the absence of a specific influence of DNA on synthesis. Thus, protein synthesis may be restored by the addition of a fresh portion of DNA of any origin, by RNA, by a dialyzed mixture of products of enzymic decomposition of DNA, and by dialyzed RNA with a low degree of polymerism (Allfrey, Mirsky, Osawa, 1955; Mirsky, Osawa, Allfrey, 1957a; Allfrey, Mirsky, 1956; Allfrey, Mirsky, Osawa, 1957).

In addition, Allfrey and Mirsky (1958a, b) in their latest works showed that the uptake of tagged amino acids can be restored to normal by

the addition of polyadenine or by polyanionites such as heparin, polyethylenesulfate, chondroitin sulfate, and does not require the addition of DNA. This is a very important fact, on which we shall dwell in detail somewhat later.

According to the data of other authors, RNA of the nucleolus rather than the DNA of the nucleus is responsible for the uptake of tagged amino acids in the nuclear proteins, apparently (Mazia, Prescott, 1955).

It should also be mentioned that extensive experimental works performed on cells from which the nuclei had been removed and summarized by Brachet in his monograph, show in all evidence that protein synthesis may proceed entirely successfully in the absence of DNA.

We should like to mention, finally, numerous works carried out on various biological objects which show that after ultra-violet or X-irradiati DNA synthesis is inhibited without the inhibition of protein synthesis or the synthesis of RNA (Brachet, 1957).

Thus, if we sum up all of the material presented, it may be said th we still do not have at our disposal any strict proof of the fact that DNA synthesis is directly related to protein synthesis. On the other hand, all the existing data rather speak for the fact that protein synthesis and DNA synthesis are not connected or are not very well connected with each o If the DNA macromolecule controls protein synthesis, this control is, to a considerable degree, indirect. It is curious to note that in those

cases where the influence of the DNA molecule on protein synthesis might have been specific (bacteriophagia, transformation) it has not been shown. In those cases where this influence is supposed to have been shown it is not specific, and the DNA molecule may be replaced by DNA of other origin, or by RNA, or simply by another polymer.

We should now like to try to analyze the problem of whether protein influence DNA synthesis. Until recently there was almost complete agreement with regard to this matter: it was accepted that protein has no relationship to DNA synthesis. Is this really the case?

Recently, a number of works has appeared which were carried out on bacteria infected by phage or treated with mustard gas or irradiated with ultra-violet rays; in these works it was shown that protein synthesis plays a direct part in DNA synthesis.

Cohnen in 1948 found that for the DNA synthesis in a cell infected by phage a preliminary protein synthesis is necessary. These data were confirmed by Burton in 1955, by Tomizawa and Sunakawa (1956), by Crawford in 1957, by Harold and Ziporin in 1958 (1958a, b) and Drakulic and Errer in 1959.

In almost all of these works the experiments were performed the same way. In one way or another the protein synthesis was inhibited in the microbial cell, and as a result of this no DNA synthesis was observed. However, all that was necessary to do for the synthesis of a new phage

DNA to occur was to add, for example, chloramphenicol, and this phenomenon occurred five minutes after the phage was incorporated into the cell. Tomizawa and Sunakawa (1956) conclude that protein synthesis is necessary for the beginning of DNA synthesis but it is not required for its continuation.

Harold and Ziporin (1958a), on the basis of similar experiments on E. coli treated with mustard gas or irradiated with ultra-violet rays, believe that the quantity of protein synthesized before the addition of the inhibitor determines the rate of DNA synthesis. The protein synthesis, they assert, is an integral part of DNA reduplication.

Drakulic and Errera (1959), who showed directly that DNA is synthesized in the presence of previously synthesized protein, possibly histone, came to the same conclusions. Doudney (1959) also finds that the synthesis of protein and RNA is required for the biosynthesis of DNA. Okazaki and Okazaki (Okazaki E., Okazaki R., 1959) conclude that protein synthesis is necessary for DNA synthesis; an amino acid insufficiency inhibits DNA synthesis. The synthesis of nucleic acids proceeds only in the presence of all the essential amino acids (Gale, Folkes, 1958a). Authors working on entirely different biological objects -- tissue cells (Harris, 1959) -- come to approximately the same conclusions.

Such references may be multiplied; we can refer, for example, to works in which the nature of proteins affecting DNA synthesis is

elucidated; they, as might have been expected, are enzymes. Kornberg and co-authors (Kornberg, Zimmerman, Koruber, Josse, 1959) found that after infection by T-2 phage E. coli forms three new enzymes, which can be detected even four minutes after the infection.

The nature of the bases taken up into the phage DNA during its synthesis, is regulated by a specific kinase system (Samerville, Greenberg 1959); here, each of the four nucleotides has its own kinase which regulate the rate of production of the triphosphates for the polymerization system (Keir, Smellie, 1959). If too much triphosphate has been formed, special enzymes exist which transform it into monophosphates and, in this way, excrete them from the synthesizing polymer system, as has been shown throug the example of desoxycytidin triphosphate (Koerner, Smith, Buchmann, 1959). In this way, the enzyme systems participate in the regulation of the specif icity of DNA synthesis.

Finally, recent works by Kornberg, Lehman, Bessman, Simms and other (Kornberg, Lehman, Bessman, Simms, 1956; Kornberg, Lehman, Simms, 1956; Schachman, Lehman, Bessman, Adler, Simms, Kornberg, 1958; Adler, Bessman, Lehman, Schachman, Simms, Kornberg, 1958; Bessman, Lehman, Adler, Zimmerman Simms, Kornberg, 1958a; Lehman, Bessman, Simms, Kornberg, 1958; Bessman, Lehman, Simms, Kornberg, 1958) showed directly, on the one hand, that the enzyme-polymerases that they isolated participate in DNA synthesis; on the other hand, however, it was shown that for the occurrence of

DNA synthesis by means of this enzyme, in any case in vitro, the presence of a "primer" is necessary in the form of polymeric nucleic acid, whereby the "priming" material can be obtained from tissues of the highest animals and can contribute to the DNA synthesis of a bacterial enzyme system. The polymeric and architectonic nature of the primer and its tertiary structure play an essential part in the process of synthesis. Thus, in the case of depolymerization of the "primed" DNA it cannot carry out its part, and synthesis stops. Insignificant influences on the DNA, let us say a small quantity of DNAase, leading to a change (apparently a compression) of its tertiary structure, lead to the stimulation of synthesi Here, as has been shown, the newly synthesized DNA is, judging by the relat ship of the nitrogen bases, identical with the primed DNA, no matter where it was taken from, that is, apparently the effect of the enzyme is not specific.

Thus, we see that for DNA synthesis a matrix is necessary in the form of polymeric priming DNA; however, at the same time, the possibility cannot be excluded, in any case in vivo, that enzyme systems have an influence in determining the specificity of the DNA synthesized.

The Interrelationship Between RNA and Protein Synthesis

Investigations on the influence of RNA on protein synthesis have been carried out very extensively in all the recent years, and tremendou literature exists on this subject which requires a special detailed

analysis. In this section, we are attempting chiefly to sum up the existing data. For the purpose of facilitation of the presentation we, as in the preceding section, shall analyze, perhaps somewhat arbitrarily, the works dealing with this subject in the following groups and shall analyze each of them separately: 1) investigations on the infectivity of virus RNA; 2) study of the significance of RNA and its synthesis in systems which synthesize proteins; 3) works on the mechanism of influence of RNA on protein synthesis.

1) As has been established in the works of Schramm, Fraenkel-Konrat through the example of TMV /tobacco mosaic virus/, the RNA of this virus is infective and produces a multiplication of the TMV in the tobacco leaves after their infection; hence, also the synthesis of specific virus protein. However, in order that we may speak of the influence of RNA on the protein synthesis in this case the question should be elucidated as to how pure the RNA preparations are with respect to protein (since there is no DNA contained in the TMV at all). In the literature this has been subjected to a lively discussion repeatedly in recent time. However, in a discussion at the Fourth International Congress of Biochemists (Tovarnitskiy, Tikhonenko, 1959) data were presented by Fraenkel-Konrat which showed that in RNA preparations with which he worked there are no determinable quantities of protein; only short-chain peptides, consisting of several amino acids, exist in them.

Therefore, the existing experimental material makes it possible to speak of the direct influence of the TMV RNA on protein synthesis

2) For the purpose of elucidating the influence of RNA on protein synthesis numerous research workers are extensively utilizing ribonucleas If, parallel with this, the participation of DNA in protein synthesis is excluded by one means or another, such experiments well illustrate the direct participation of RNA in protein synthesis: for example, the experiments of Straub and Ulmann (1957) on protein synthesis using a desiccated acetone extract from the pigeon pancreas. The works of Kramer and Straub (1956) on the synthesis of penicillinase by microbes, the works of Groth (1956), eener (1955), Nisman, Hirsch, Marmur (1955) on microbes, of Beljanski (1954) of Fraser and Mahler (1957), of Landman and Spiegelman (1955) on protoplasts, of Brachet (1957) Ameba, etc. in which protein synthesis was stopped under the influence of ribonuclease, show the direct participation of polymeric RNA in protein synthesis. It may also be mentioned that in cell granules under the influence of RNAase the uptake of tagged amino acids is stopped (Daly, Allfrey, Mirsky, 1955; Zamecnik, Kellek, 1954; Webster, Johnson, 1955; Zubovskaya and Tongur, 1959) or protein synthesis stops, which is also evidence on behalf of the conclusion drawn. In many of the works mentioned the protein synthesis began again after the addition of polymeric RNA.

In certain cases the stimulating effect of

of products obtained from the enzymic decomposition of RNA was shown on protein synthesis or on the uptake of tagged amino acids int proteins. However, even here, apparently, in many cases polymeric RNA formed from the added fragments has an influence on protein synthesis.

Engler and Schramm (1959) mention that before the onset of synthesis of TMV protein a certain quantity of virus RNA must be formed first.

In addition, there is an abundance of indirect data in the literature obtained by means of RNAase and showing the participation of the polymeric RNA molecule in protein synthesis. These are works on the inhibiti of growth and multiplication of cells by means of RNAase; they are of spec interest, but the analysis of them, transcends the limits of this article.

Therefore, as follows from the material presented, it has been shown by a number of experiments on the most diverse biological systems that the polymeric RNA molecule is necessary for protein biosynthesis. Bhargawa, Simkin and Work (1958), for example, even conclude that protein synthesis is unrelat to RNA synthesis.

The experiments of Webster (1956) may be mentioned, however, in which a mixture of four nucleotides increased the uptake of tagged glutamic acid into proteins of cytoplasmic particles and RNA synthesis. In the experiments of Jeener (1958), blockage of RNA synthesis also blocked the synthesis of protein in lysogenic bacteria infected with phage.

Numerous experiments of other authors have shown that after the blockage of RNA synthesis by analogues of the nitrogen bases the synthesis of adaptive enzymes is also blocked (Pardee, 1955; Spiegelman, Holvorson, Ben-Ishai, 1955; Spiegelman, 1957). All this proves that RNA synthesis is necessary for protein synthesis.

Loftfield (1958) believes that 8-azoguanine blocks the synthesis of the adaptive enzymes (β-galactosidase), but the formation of the enzymes of which it is constituted (glucozymase) is not inhibited by it. He believes that stable RNA, which is present in the cell, is not sensitive to azoguanine and is responsible for the synthesis of the constitutive enzymes. The synthesis of functional, not stable, RNA, is necessary for the synthesis of adaptive enzymes, is stopped by azoguanine However, quite recently Dutton and co-authors (Dutton, Dutton, George, 1958) after the suppression of RNA synthesis by azaguanine, observed a cessation of synthesis of the constitutive proteins also. In connection with what has been stated a number of authors go even further and believe that the pre-existing RNA does not participate at all in protein synthesis (Ogata, Shimiza, Togashi, 1958).

As we see, a contradiction has been created in the experimental dat Chantrenne (1958) attempts to resolve this contradiction in the belief that the metabolism of RNA precursors (in which molecules are formed which are smaller than RNA) participates in the formation of proteins along with

the polymeric RNA.

Loftfield (1958) notes another possibility: he believes that there is no conclusive proof that protein synthesis is accompanied by the simultaneous synthesis of the RNA molecule -- simply, the short life of the RNA molecule in a number of cases makes a resynthesis of it necessary for the continuation of production of certain proteins.

We shall return to this matter somewhat later.

3) Solid data on behalf of the participation of RNA in protein synthesis have been presented in the works of Hoagland (1958) and related investigations. These works not only speak of the direct part of RNA in protein synthesis but also show the routes of participation of the nucleic acid in this process.

The system which takes up the amino acids consists of the following components: 1) amino acids tagged for carbon; 2) ATP; 3) enzymes soluble at a pH of 5: enzymes are included among them which activate the amino acids and other soluble enzymes necessary for the process; 4) soluble RNA included in the pH-5-enzyme; 5) microsomal ribonucleoprotein particles; 6) guanosine triphosphate.

The tagged amino acids are activated by the pH-5 enzyme, combining with ATP; through this process a pyrophosphate is produced. The activated amino acids are accepted by the soluble RNA which exist in the form of free polynucleotides and are transferred directly to the ribonucleoprotein

particles. They are the site of formation of a peptide linkage, whereby the RNA particles bind the soluble RNA with the amino acids accepted on it only in the presence of guanosine triphosphate, the role of which is not clear. A similar mechanism of synthesis apparently exists not only in cells of mammals but also in plants, bacteria, yeasts, etc., Hoagland, 1958; Offengand, Bergman, Berg, 1958; Commoner, Tung-yoe Wang, Sherer, 1959; Bosch, Bloemendal, Slugser (1959). Here, note should be made of the following essential details (Hoagland, 1958).

Every amino acid has its own activating enzyme and its own RNA, with which it is bound (Davis, Novelli, 1956; Schweet, Glassman, Allen, 1958; Smith, Cordes, Schweet, 1959; Holley, Merill, 1959). The soluble RNA is not specific, that is, it possesses the same properties regardless of the object from which it was isolated. This circumstance has given us the basis for supposing the existence of a universal RNA entity which is included in different viruses and cytoplasmic nucleoproteins of plants and animals (Ping-Joo Chang, 1957).

After the separation of RNA and the pH-5-enzyme protein its activity is restored if the soluble RNA of a dog's or rat's liver is combined with the pH-5-enzyme protein isolated from a guinea pig, but the RNA of viruses, yeasts, and microbes do not recover the lost activity (Schweet, Glassman, Allen, 1958). The soluble RNA is unique in its capacity of binding activated amino acids and cannot be replaced by

any other polymeric RNA.

The amino acids are bound to the RNA additively, by a covalent linkage (Gutfreund, Traser, Shimizu, 1956). The reaction of binding the amino acids is reversible. The soluble RNA is an obligatory component of systems which take up amino acids into their proteins (Nohava, Ogata, 1959). The bound amino acids are localized on the second or third ribose hydroxyl group. The RNAase inhibits the activation of the amino acids (Ogata, Nohava, 1957; Ogata, Nohava, Marita, 1957). The degree of uptake is determined by factors which are different from those determining the rate of activation (Gutfreund, 1958). The microsomal fraction which takes up the amino acids can be converted into a lyophilized powder with maintena of its activity (Sachs, 1957).

The composition of the terminal groups of the soluble RNA is the factor participating in the binding of the amino acids (Hoagland, 1958). In the soluble RNA two cytosin nucleotides follow the terminal adenine nucleotide. Specificity of their configuration is essential for the combination of amino acids with the RNA. The amino acids combine with the 2' or 3' hydroxyl groups of the terminal adenine nucleotide (Hecht, Stephenson, Zamecnik, 1958, 1959; Preiss, Berg, Ofengand and others, 1959)

These facts are very important; they will be discussed somewhat later.

Therefore, the direct and immediate participation of both the

polymeric RNA molecule and, apparently, of its synthetic processes in the synthesis of protein can be considered a solidly established fact.

Now, let us try to analyze whether and in what way protein and the processes of its synthesis influence RNA synthesis. Unfortunately, the direct works investigating this problem are very few; however, the influence of protein on RNA synthesis has nevertheless been dealt with in a number of investigations, and at the present time, we already have at our disposal material which permits us to draw some conclusions.

As has been found in cells infected with phage, protein synthesis precedes RNA synthesis characteristic of the infected cells (Astrachan, Volkin, 1959).

In works of recent years it has been shown that the uptake of uracil and adenine in RNA is stimulated by a mixture of amino acids, and it is inhibited by the analogues as well as by chloramphenicol, a nucleus which specifically stops protein synthesis (Webster, 1957c). As Clark and others have pointed out (Clark, Naismith, Munro, 1957), the quantity of RNA in the rat liver is determined by its supply of amino acids essential for protein synthesis.

It was shown later that RNA synthesis inhibited by chlormycetin can be restored to normal by the addition of amino acids (Gros and Gros, 1958). A deficiency of tryptophane in the mixture of amino acids in the nutrition of rats inhibited the uptake of glycine and orotic acid in

the liver RNA. The stability of the RNA, as was shown, depends on a complete set of amino acids to be utilized for protein synthesis (Munro, Clark, 1959). The data presented might be multiplied, but there is no need for this; they all indicate that the presence of amino acids is essential for RNA synthesis, that is, apparently, they are obligatory components of RNA synthesis.

On the other hand, it is well known that chloramphenicol added in small concentrations blocks protein synthesis without stopping RNA synthesis, that is, it is possible to separate both syntheses in this way (Icas, Braverman, 1957). Apparently, the chloramphenicol reacts with the RNA, interfering with its influence on protein synthesis (Ramsey, 1958) However, even after the addition of this toxin, the amino acids nevertheless influence the synthesis of nucleic acid. In case of phage infection, which induces the synthesis of new RNA, the addition of chloramphenicol prior to the infection blocks protein and DNA synthesis without touching RNA synthesis. After the addition of chloramphenicol and following the infection even a stimulation in RNA synthesis is observed (Watanabe, Kiho, Miura, 1958). However, observations on isolated thymus nuclei showed that chloramphenicol blocks both the uptake of tagged amino acids and proteins and of orthophosphate into the RNA and DNA (Breitman, Webster 1958). In the process of inhibiting protein synthesis, azoguanine in Bacillus cereus (Chantrenne and Devreux, 1958) does not affect RNA

synthesis or the synthesis of DNA or hexosamine; higher concentrations of this compound partially inhibit the synthesis of DNA also.

Apparently, the synthesis of protein and RNA synthesis proceed at different rates. In E. coli mutants the ratio between the quantity of protein formed and RNA is 3-4:1, and the blockage of RNA synthesis produces also a blockage in protein synthesis, but the inhibition of protein synthesis is not reflected in RNA synthesis (Ben-Ishai and Volcean, 1956). It has been shown in the microsomal particles that the uptake in RNA occurs more slowly than in protein (Balis, Somarth, Peterman, Hamilton, 1958; Bhargawa, Simkin, and Work, 1958). Reid and Stevens,(1958) also point out that in the microsomes the ratesof renewal of RNA and protein are not correlated with each other. If this is actually so, the supposition remains,despite the fact that both syntheses have common precursors in the form of amino acids, that they diverge from each other at an early stage of synthesis; in this way stoppage of the process of protein synthesis has no influence on RNA synthesis.

Recently, however, it has been shown that apparently the blockage of protein synthesis still, in some way or other, influences either the quality of the RNA or the general properties of the system being studied. It has been shown that RNA synthesized in cases of blockage of protein synthesis by chloramphenicol is different from the usual RNA in its biological properties: as has been mentioned in the literature, it is

not stable, and even its physico-chemical properties, such as molecular weight and electrophoretic mobility, are not similar to the analogous properties of ordinary RNA, although, apparently the nucleotide composition of both RNA's are the same (Lombard, Chargaff, 1957; Horowitz, Lombard, Chargaff, 1958; Gale, Folkes, 1958a, 1958b). Attempts have been made to explain the instability of this RNA by the fact that it is unable to enter complexes, for example, with proteins, and therefore it is more accessible to enzymic degradation (Horowitz, Lombard, Chargaff).

It was later found that after the removal of chloramphenicol, which had been added for the purpose of blocking protein synthesis, a reduction should occur in the quantity of nucleic acid accumulated to a normal level, and only then do the growth and multiplication of bacterial cells begin; possibly this arises from the fact that the normal quantitative interrelationships between protein and nucleic acid are disturbed, as is supposed by the authors of these experiments (Hahn, Chechten, Celdowski, Hopps, Ciak, 1957), but it is also possible that in this case the RNA synthesized is not entirely the usual.

It has been shown, finally, that the RNA which accumulates under conditions of methionine starvation synthesizes proteins poorly (Borek, Rockenbach, Ryan, 1956).

Recently, new data have been reported concerning the nature

of the influence of chloramphenicol on processes of protein and RNA synthesis (Aronson, Spiegelman, 1958). It has been shown that protein synthesized in the presence of small doses of chloramphenicol is different from ordinary protein in certain properties, which speaks, it would appear, for the direct influence of this toxin on protein synthesis. Large doses of chloramphenicol completely suppress protein synthesis, but in this case the amino acids no longer influence the RNA synthesis.

RNA synthesized in the presence of chloramphenicol represents one of the stages in the formation of ribonucleoprotein and is not some unusual kind of RNA. The synthesis of this RNA occurs also in the absence of chloramphenicol; the RNA included in the ribonucleoprotein particles and the RNA not included in them can be separated by sedimentation. After the removal of the toxin the unstable RNA rapidly changes into the ordinary stable form. The effect of chloramphenicol consists simply in the blockage of the transition of the unstable form of RNA into the stable form. If this is actually so, then, apparently, only stable ribonucleoprotein particles are associated with protein synthesis. The unstable RNA which accumulates after the effect of chloramphenicol does not participate in protein synthesis.

In summing up, it may be said that the material presented is quite contradictory and at the present time permits us to speak, but only with a certain reserve, of the influence of protein synthesis

on RNA synthesis.

At the same time, the data of Ochoa, Grunberg-Manago, Beers concerning the synthesis of compounds which are similar to RNA in many properties by means of an enzyme which they isolated -- polynucleotide phosphorylase -- are well known (Grunberg-Manago, Ochoa, 1955; Grunberg-Manago, Ortiz, Ochoa, 1955; Beers, 1956; Grunberg-Manago, 1958).

True, even purified preparations of polynucleotide phosphorylase contain about three percent RNA; however, there is no doubt of the fact that synthetic processes proceed under the influence of this enzyme. This indicates that, probably, the polymeric protein molecule participates directly in ribonucleic acid synthesis as an enzyme.

In the dv development of these works Herbert (1958) showed that the enzyme system of a soluble fraction of a homogenate of rat liver is responsible for the uptake of C^{14} adenine nucleotide into the monoesterified end groups of the RNA molecule. The enzyme system of the nuclear fraction of the homogenate stimulates the uptake of the nucleotide into the inner portion of the molecule. Apparently, in addition to polynucleotide phosphorylase, at least one other enzyme system exists which accounts for the build-up of the ends of the RNA molecule, utilizing nucleoside triphosphates for this purpose. This reaction is different from the synthesis of polyribonucleotides as represented by Grunberg-Manago, Ochoa (Hecht, Zamecnik, Stephenson, Scott, 1958).

The terminal portions of the RNA molecule probably are, in general, more labile than its"core", and it must be supposed that the uptake of precursors at the ends of RNA does not reflect the dynamic state of the entire molecule and possibly does not mean a pure synthesis of it, but at the same time this uptake must somehow change the information contained in the RNA molecule (Harber, Heidelberger, 1959).

Discussion

Thus, we see that the syntheses of DNA, RNA and protein are interconnected, and probably the relationship of RNA and protein synthesis is a closer one than that of DNA and protein.

Apparently, the influence of DNA on protein synthesis is very indirect and occurs through metabolism, which is indicated by a number of works previously quoted. Okazaki, F. and Okazaki, R., (1958) even believed that in certain cases DNA synthesis proceeds completely independently of RNA and protein synthesis.

The relationship of protein and RNA synthesis has been studied to a much greater extent. It should be supposed that both syntheses have common precursors. Recently, Holvorson (1958) gave the following approximate schema for this relationship:

	Nucleic acid	Protein
	↓ ↑	↑ ↓
	Purines	Amino Acids
	↑ ↓	↑ ↓
Xanthine, hypoxanthine + NH_3 +		α-Ketoacids

Webster (1957c) believes that this relationship is effected by means of a combination of activated amino acids with nucleoside diphosphates and later, depending on the method of decomposition of this compound, decomposition products proceed to the synthesis either of protein or of nucleic acid, for example: if a rupture of the pyrophosphate linkage occurs, synthesis of nucleic acid occurs, etc.

According to the data of Hoagland (1958), this relationship can be effected through the pH-5 enzyme, which activates not only the amino acids but also the rapid uptake of nucleoside monophosphates into the RNA of the pH-5 enzyme. Hecht, Stephenson, Zamecnik,(1958) showed that this uptake of end nucleotides correlates with the uptake of amino acids. Von der decken, Hultin (1958) found that the soluble fraction of a rat liver homogenate can not only bring amino acids to the nucleoprotein of the microsomes but also nucleoside triphosphates, and here the amino acids activate this process somewhat.

Webster has shown that nucleoproteins from destroyed microsomes of the pea sprout can catalyze the following: the uptake of amino acids into proteins, the uptake of nucleoside of the 5' phosphates into their RNA, the activation of 12-amino acids, and, depending on the amino acids, the exchange of AMP with ATP, which indicates the close connection of these processes.

Other authors believe that the synthesis of protein and RNA are concurrent processes and that the existing nucleotide-amino-acid complexes can be synthesized immediately into nucleoproteins (Mandel, Weill, Ledig, Busch, 1959).

Therefore, an interrelationship not only between the biosynthesis of RNA and protein has been established but the specific mechanisms of this relationship have been outlined.

At the same time, it has been noted in a number of works, as we have shown in literature already quoted, that such a relationship is not always a direct one (Barner, Cohen, 1958; Ben-Ishai, Volcean, 1956; Balis, Samarth, Petermaren, Hamilton, 1958; Breitman, 1958; Webster, 1959; Watanabe, Kiho, Miura, 1958).

We believe that at the present time it would be somewhat premature to discuss these problems. Here, it is important to note the following fact, which is of great significance, we believe. An interrelationship exists between the biosynthesis of protein and nucleic acids, and this association is carried out at a low molecular-weight level, through metabolism, as well as at the level of the matrix-macromolecule.

Certainly, the reservation should be made that the systems of RNA-protein and DNA-protein can not apparently be regarded as functioning entirely independently of each other; it must be supposed that they are connected through the RNA-DNA system.

Those investigators who believed that RNA is synthesized on the matrix of DNA, which transmits its code to it, and then that the protein synthesis is carried out on the RNA which has been coded in this way are hardly right, however. In any case, we now have data indicating that in a number of cases

RNA is the precursor of DNA and that the synthesis of RNA is accomplished before the synthesis of DNA (Harris, 1959; Sisken, 1959), and here in a number of cases the relationship of these syntheses occurs through the low polymerized precursor products, which goes both into RNA synthesis and into the synthesis of DNA, where it is quite difficult to speak of the transmission of information (Astrachan, 1958; Astrachan, Volkin, 1957, 1959; Fraser, Mahler, 1957). At the same time, it has been found that syntheses of RNA and DNA compete with one another at a low molecular-weight level (Okazaki, F. Okazaki, H., 1958). The capacity of RNA of anuclear cells of taking up phosphorus is evidence that synthesis of RNA is accomplished in the absence of DNA. Finally, the statement of Tamm and Osterhout (1959) to the effect that RNA of the host cell plays a determinative part in the multiplication of certain viruses containing DNA is very important. We may refer also to the data of Danielly, Lorch, Ord and Wilson, obtained in amebae containing nuclei transplanted from another species, constituting evidence to the effect that the influence of cytoplasm is predominant in the determination of physiological and morphological indices of these amebae.

Zubay (1958), on the basis of a detailed analysis of data in the literature, comes to the conclusion that the sequence of nucleotides in the RNA molecule can not be entirely determined

by the DNA matrix, but rather requires the specific participation of enzymes for RNA synthesis.

Therefore, the relationship may hold not only in the direction from DNA to RNA but also, on the other hand, from RNA to DNA, whereby this relationship may be carried out at a low molecular-weight level with the participation of enzymes.

Thus, we conclude that the interrelationship of the biosyntheses of nucleic acids and protein proceeds along two channels or, in other words, is accomplished by two methods: at a low molecular-weight level by means of the utilization of common precursors for synthesis, which is very convenient since both syntheses have a common metabolic reserve; and at the macromolecular level by means of the direct influence of a polymeric molecule on synthesis. Along this line, we believe, it is necessary to analyze the certain contradiction in the data with respect to the influence of RNA on protein synthesis presented in the previous chapter.

Such a functioning of synthetic systems with a "double drive" makes them more autonomous and resistant to the influence of the environment. As a matter of fact, the common metabolic reserve is, on the one hand, a kind of buffer between the synthetic processes and the environment; in other words, the synthetic processes mediate its influence

through the metabolic cauldron. Here, as recent data have shown (Cowie, McClure, 1959), apparently two functionally different amino-acid reserves exist -- the concentration and the conversion reserves. The first is formed of exogeneous amino acids and at a definite concentration of them can produce material for the conversion reserve. Here, a preparation of amino acids occurs for protein synthesis. However, the conversion reserve can be formed also in the absence of a concentration reserve; it is capable of synthesizing amino acids for itself.

On the other hand, the common metabolic reserve makes it possible for the organism to "maneuver", or, in other words, makes possible the synthesis of the polymer specifically (RNA or DNA proteins) which it needs particularly at the time. If under the influence of some kind of artificial conditions one kind of synthesis begins to predominate markedly over the other and the regulation of synthetic processes is disturbed, the polymer synthesized in excessive quantities degenerates because of its instability, giving decomposition products to the general metabolic cauldron for a new synthesis. The conception of the dynamic state of synthetic processes in which the rates of degradation and synthesis correspond to each other which was developed by Holvorson (1958) seems to us most fitting from this point of view.

The transmission of information, it seems to us, proceeds along several channels: on the one hand, it is assured by the polymeric nature of the molecule; the information is reproduced by a new synthesis according to a pattern obtained from precursors existing in the metabolic cauldron; on the other hand, according to Hoagland's schema (1958), protein synthesis is accomplished in two stages, so to speak, and apparently two forms of soluble RNA exist for the transfer of each amino acid (Goldthwait, 1958).

It has been shown recently that some amino acids influence the activation of other amino acids (Nisman, 1959) that is, the combination of them with metabolic RNA; in addition, such a combination depends not only on the interrelationship between the amino acids existing in the substrate but also on their absolute concentration (Fraser, Shimizu, Gutfreund, 1959); therefore, depending on the presence of various metabolic RNA's in the substrate, the concentration and interrelationship of the amino acids, different quantities of amino acids will be transferred to the polymeric RNA, and a definite amino-acid composition of the protein will be assured, that is, at this stage of synthesis the transmission of information is brought about by the metabolic cauldron, which provides for the presence of the necessary kinds of RNA and amino acids, and at the same time it is embedded into

the structure of the metabolic RNA, since, probably, specifically this structure produces the specificity of the relationship with a definite amino acid. Here, we again see the principle of the double linkage.

The second stage of synthesis occurs on a polymeric patterned RNA, to which the metabolic RNA brings the amino acids. The patterned RNA provides for the sequence of arrangement of the amino acids, communicating the rest of the information necessary for the inclusion of it into the protein structure.

This system of two-stage synthesis has been observed not only in cells of highly organized animals but also in plants and microorganisms. The justifiability of this system has been confirmed by works in which peptides with activated carboxyl groups were found in microsomes and microbes (Koningsberger, Van der Grinten, Overbeek, 1957; Van der Griuten, Schnurs, Koningsberger, 1958; Gilbert, Jemm, 1958; Bernlohr, Webster, 1959; Anderson, Albright, 1958). These works would seem to indicate that the transfer of activated amino acids can be accomplished by oligo- and polynucleotides.

Apparently, the Hoagland system is not universal. The formation of the peptide linkage may be observed directly in phosphorylated yeast RNA after the addition of 21 [?] amino-acids to it, which would speak against the two-stage

synthesis system (Dounce, Hawtrey, Gutsche, Richards, 1958).

In addition, the activation of amino acids is not always completed by their uptake into proteins (Heller, Szafranski, Subkowski, 1959) and, on the other hand, protein synthesis may proceed without activation of amino acids in the absence of pH-5 enzyme, as has been shown on mitochondria (Greengard, 1959, Greengard, Campbell, 1959; Reis, Coote, Work, 1959), on rat liver microsomes (Cohn, 1959; Zalta, Khouvine, 1959) on membranes of Alcaligenes faecalis (Beljanski, Ochoa, 1958), and on ribonucleoprotein pea particles (Webster, 1959).

Apparently, it may be supposed that several routes exist for protein synthesis. Specifically, the uptake of amino acids into the microsomes without the participation of soluble RNA of the pH-5 enzyme is possible; this latter is replaced by the cell sap which does not contain RNA but probably possesses the necessary set of enzymes and cofactors specific for the uptake of various amino acids; the same thing may be observed also in the synthesis of protein by nuclei and mitochondria (Rendi, 1959; Rendi, Campbell, 1959).

The enzymic synthesis of peptides from amino acids which we recently observed (Connel, Watson, 1958) confirms the multiplicity of the routes of proteinbiosynthesis. In any case, with any mechanism of protein synthesis, in order to change its nature and the information determined by it either

the structure of the polymer and, by the same token, the information transmitted in it should be changed or the interrelationship and activity of substances in the metabolic reserve needs to be changed radically without interferring with synthetic processes. It is very difficult to do either one. This explains the conservatism of synthetic processes.

Finally, there is still a great deal that remains unclear: how, for example, does the reorganization of proteins included directly into a tumor occur (Sinclair, Leslie, 1959) ?

In conclusion, we should like to discuss certain problems with respect to the specificity of synthesis, directing attention to new facts and without giving ourselves the problem of clarifying the entire specificity problem.

Protein synthesis proceeds in two stages or in one: in either case the specificity of the assembly of amino acids is apparently determined to a considerable degree by the matrix on which this assembly is accomplished. The matter of specificity of these matrices or surfaces is not altogether clear. Based on general physicochemical concepts it may be supposed that any surface possessing suitable properties (charge, etc) may carry out of the function collecting various substances from the environment. The role of the matrix in the synthesis of lignin-like compounds may be carried out, for example,

by cellulose or chitin (Siegel, 1957). However, such an assembly is not always specific. Specifically from this point of view, it seems to us, the experiments of Allfrey and Mirsky (1958) quoted at the beginning of the article should be considered; in these, the uptake of tagged amino acids into proteins was restored to normal after the replacement of DNA which had been removed by heparin, polyethylene sulfate, or chondroitin sulfate. Incidently, is not this an explanation for the anticarcinogenic effect of heparin, which, by becoming a distinct analogue of DNA, distorts and thereby nterrupts the metabolism?

Certainly, such a nonspecific assemblage, even from purely kinetic considerations, should be carried out much more easily than a directed specific synthesis. In summarizing the data concerning the influence of DNA on protein synthesis we noted that where this influence should be specific it was not noted, and where it was noted it was not specific, that is, in other words, it seems to us that in the latter case specificall a nonspecific assemblage of amino acids is observed by a polymer as a surface. Surely, such a collection should occur relatively easily, and it is easier to observe it.

Incidently, not so long ago, a number of works appeared in which it was noted that apparently no absolute specificity of protein synthesis exists, and it is possible to observe the

uptake of amino-acid analogues into the protein molecule, which does not interferewith the completion of synthesis (Vaughan, Steinberg, 1958). In the amylase of B. subtilis the methionine in it was replaced by ethionine to the extent of more than one third during the synthesis of the amylase; however, its electrophoretic properties and activity were preserved (Joshida, 1958; Joshida, Jamasaki, 1959); this replacement had no influence on the growth of the culture or the formation of amylase.

Analogues of amino acids do not affect the formation of bacterial flagellae, which are indistinguishable morphological serologically and functionally from the controls (Kerridge, 1959).

After uptake into E. coli proteins norleucine and parafluorophenylalanine did not essentially affect their molecular specificity or physicochemical properties. The synthesis of protein, despite the presence of analogues, continued, but the enzymic power of the synthesized proteins was reduced (Cowie, 1959).

Certainly, the uptake of amino-acid analogues into the protein molecule should not always occur without a trace, so to speak. Naturally, after being taken up, in a number of cases they alter, as might be expected, the properties and structure of the proteins (Munier, Cohen, 1959).

The data presented show that the matrix mechanism may "err", confirming an absence of any principle of absolute specificity. Approaches to the explanation of the degree of error through the analysis of processes of biosynthesis from the kinetic point of view are given by Pasynskiy (1960). Incidently, is not the absence of absolute specificity in synthesis one of the sources of variation? In considering the synthesis of nucleic acids, we note this phenomenon. The DNA which contains 5-bromuracil preserves its transforming activity (true, it is quantitatively altered) (Ephrati-Elizur, Zamenhof, 1959).

These data as well as considerations previously expressed make us conclude that it is impossible to regard the matrices of macromolecules outside of their relationship to processes occurring around them. Pasynskiy (1960), who showed the need for a kinetic approach to biosynthetic processes, is absolutely right. Incidently, his ₤ viewpoint has been solidly confirmed in the work of Rendi and Campbell, (1959) who found that the degree of uptake of amino acids into proteins is determined by the rate of enzymic reactions rather than by the number of points of combination of the amino acid with the RNA of the pH-5 enzyme. Therefore, we have gone from the principle of mechanical autoreproduction of molecules of nucleic acids, which was quite widespread previously, to a

more complex but also to a truer dynamic process of synthesis of them, in which, along with the matrix, the enzymic reactions and the precursor compounds, which also participate in determining the specificity of synthesis and hence, in the transmission of information, play a part.

The problems of specificity of synthesis of nucleic acids have been studied by Sekigucki and Sibatani,(1958a, b, 1959). These authors showed that apparently specificity is not so essential for the synthesis of DNA and RNA. In any case, the uptake of tagged phosphorus into nucleic acids inhibited by the partial removal of the latter was renewed after the addition of nucleic acids of another origin and even of chondroitin sulfate, which indicates, as has been correctly written, a physicochemical rather than a biological role (certainly in this case) on the part of the substances added. It is interesting to note that after the removal of more than 80 percent of the DNA of the nucleus by means of DNAase it is impossible to restore the uptake into DNA, in contrast to RNA, and here the major part of the phosphorus taken up into the RNA is not preexistent in the DNA.

Approximately the same results were obtained by Allfrey and Mirsky,(1958): the uptake of C^{14} adenosine and C^{14} orotic acid into RNA is reduced after treatment of the nuclei

with DNAase, but it may be restored to normal after the addition of DNA of another origin or of polyethylene sulfonate. It is interesting to note that in these cases the addition of RNA and DNA of another origin, as well as of polyethylene sulfonate, restores the aerobic phosphorylation which had been inhibited by DNAase. Do not these facts indicate that RNA synthesis is autonomous to a considerable degree and independent of DNA and that the information obtained by RNA during synthesis is not transmitted from the DNA or, in any case not always from the DNA as we mentioned above? Surely, it would be very important to elucidate the nature of the nucleic acids synthesized in these cases.

It was not without reason that Stent (1958b) under pressure of the facts, advanced a new hypothesis of DNA reduplication, according to which the ribonucleoprotein may serve as a matrix for DNA synthesis and, by this means, transmit genetic information. Such a possibility has been confirmed in recent experimental works (Doudney, Haas, 1959).

Therefore, we see that problems of transmission of information, and of specificity of collection are not so clear as they seem to be recently. New data which have been accumulated cause us to make a more cautious approach to these problems and outline possible routes for a certain revision of the ideas which have been built up.

Certainly, we in the present article have not touched on all the problems concerning the phenomena under analysis. We have only attempted to point to certain new facts and to the trend of evolution of our views on the biosynthetic processes of protein and nucleic acids which they are roducing.

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