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SOME PROBLEMS OF MOLECULAR BIOLOGY AND PHYSICS

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THIS article will touch upon some aspects of molecular biology involving the processes of synthesis or autoreproduction of the two most important polymers of living nature, the proteins and the nucleic acids. In living nature, the proteins play the major role. On the one hand, the proteins are the catalysts of the material-exchange reactions (enzymes), and on the other hand, they are the mechanochemical machines that perform the transformation of chemical energy into mechanical work.

As is widely known now, the proteins are copolymers of the twenty amino acids. They consist of polypeptide chains composed, in a common degree of complexity, of several hundred residues. The smallest protein, insulin, consists of only 51 residues. The sequence of the twenty amino acids along the protein chain is defined with mathematical exactitude, and even the replacement of a single residue out of the several hundred arises in nature from an inheritable mutation. Such a genetic injury, as it is commonly called, often affects its function, e.g., its ability to act as an enzyme, i.e., a specific catalyst accelerating one particular reaction in the material exchange of the cell.

At present, about ten proteins have been completely investigated analytically, i.e., their structural formulas have been determined. One protein, insulin, has been synthesized by classical chemical methods, and it showed the same properties and could perform the same biological function as natural insulin. Thus the problems of protein structure are in principle solved, and contain no major secrets. It is now a question of working out the details.

Among the fundamental questions now facing molecular biology, we must assign first place to the biosynthesis of proteins.

How is the strictly determined chain of a protein produced? How do false residues appear in it?

As we know, the principal role in the biosynthesis of proteins is played by the nucleic acids, which contain information on the structures of all the proteins of the cell, i.e., on all the details of the sequence of the various residues along all the protein chains of the cell. The number of the latter is of the order of a thousand. The nucleus of the cell contains DNA, which contains all the necessary information on the structure of all the proteins, but does not in itself participate in the synthesis of proteins. Matrix RNA (mRNA) is copied from it (the DNA), and is transferred from the nucleus to the cytoplasm of the cell. It becomes attached to special round bodies, the ribosomes, of which we shall speak later, and there the formation of new proteins takes place. Hence, the starting point of the synthesis of protein is the synthesis of mRNA from DNA; thus the information is transferred from its storehouse in the nucleus to the workshop, i.e., the ribosomes. The DNA of the nucleus is the memory element of the machine, or as it were, the magnetic tape, since the memory is recorded one-dimensionally, just as on a magnetic tape. The mRNA is the working copy, that goes directly into the job. If we can compare the DNA with the tracing cloth that is kept in the design office, the mRNA is a blueprint taken from it, going directly into the shop, and like any such working object, it doesn't last long, but soon wears out and gets out of order.

In order to follow the entire chain of events, we shall first take up the synthesis of the nucleic acids, DNA copied from DNA, mRNA from DNA, etc. This process is much simpler in principle than the synthesis of protein from mRNA, since both polymers, the one being copied and the one being synthesized, are homologous. However, even here we encounter a physical principle thus far unknown in synthetic chemistry. The point is that an absolutely ordered copolymer is synthesized with a mathematically determined sequence of the four types of nucleotides along the chain. Chemistry knows how to construct copolymers, but they are always statistical structures. Even if there is any definite regularity in the sequence of the units, e.g., unit A prefers to have unit B as its neighbor, then such simple rules can be formulated only statistically. The mean fluctuation in the sequence of A and B units can be of the order of 10^{-2}. In a nucleic acid, the position of each unit among a thousand is determined with colossal exactitude. Copying takes place with complete identification. This does not mean that errors never occur. Errors, i.e., a false insertion of a nucleotide unit, actually occur with a low probability of the order of 10^{-13}—10^{-15}. However, this is now a catastrophe, or mutation. Such a polymer continues to be copied with the error. It brings about various modifications of one of the proteins of the cell, most often harmful, but rarely useful.

How does the exact literal copying of the DNA chain take place? It is a synthesis on a linear matrix, or form imposing its structure, i.e., its definite sequence of units, on the second chain being synthesized.

Matrix synthesis is a new concept without precedent in classical chemistry. In the synthesis of the nucleic acids, whether DNA from DNA, or RNA from DNA, or

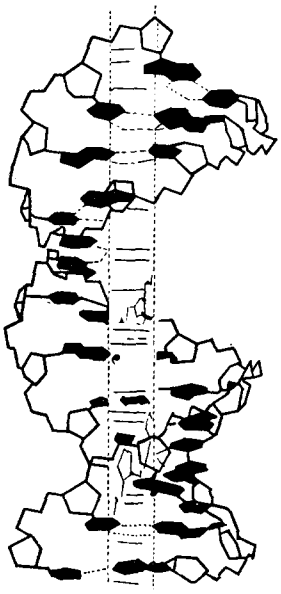

FIG. 1. A model of the Watson-Crick double helix. The purine and pyrimidine base side-groups are in black. The five-membered deoxyribose rings are in outline. The central rod is imaginary.

RNA from RNA, the principles of the matrix synthesis are determined by the Watson-Crick model of the spatial structure (Fig. 1). The principles of the spatial structure of the nucleic acids are well known, and have been illustrated in detail in the pages of this journal.[1]

Pair complementarity in itself determines the action of each individual chain as a matrix. This involves the complementarity of nucleotides necessary for formation of hydrogen bonds between the bases, i.e., the complementarity of thymine or uracil to adenine (A–T or A–U pairing) and of cytosine to guanine (G–C pairing). If a given base must always be paired with a strictly complementary one, A always with T, and G always with C, then evidently the sequence of residues in one chain exactly determines the sequence in the second complementary chain that forms a Crick-Watson helix with the first chain. Thus we can easily envision how the synthesis of nucleic acids takes place on a matrix.

The double helix separates, not as a whole, but by formation of loops. The exposed single chains adsorb monomers from the surrounding medium, and the synthesis of the two complementary chains takes place (Fig. 2). As a result, four chains are formed from the two. What has taken place is commonly designated by the term "semiconservative reduplication."

This is the general principle of the duplication of DNA, as well as of the copying of mRNA from DNA. In the copying of mRNA from DNA, the monomers are ribonucleotides, i.e., the second chain differs chemically from the first, but this does not alter the principle of action of the matrix. Here we must emphasize the difference in action of a catalyst and a matrix. All of the reactions under discussion are catalytic, i.e., enzymatic. The catalyst determines which chemical bonds are broken and which are formed anew in each

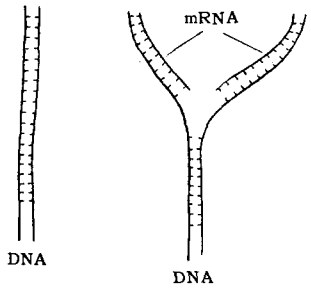

FIG. 2. Diagram of the reduplication of DNA or the synthesis of mRNA from DNA.

elementary event. However, the sequence of the residues in the polymer chain is not at all determined by the catalyst. Any one of the four possible residues is equally suitable to the catalyst. Only the presence of the matrix imposes a strictly determined sequence on the polymer being synthesized.

Let us take up some curious details of this phenomenon. A very interesting problem not yet fully solved is that of the unwinding of the DNA double helix during the synthesis of the two complementary satellite strands. The double helix must separate in the synthesis of the new chains, so that hydrogen bonds can be formed with the monomers. In unwinding thus, it must rotate with high velocity. According to the calculations of Delbruck based on the known rate of synthesis of DNA, it rotates with a velocity of 200—300 revolutions per second in bacteria and viruses (Fig. 3).[2] The fact has seemed especially strange that, as shown by experiment, the DNA of bacteria, viruses, and phages proves to form a ring. That is, it is closed on itself (Fig. 4).[3] Here, unfolding seems to be totally impossible topologically. The problem of experimentally studying the process of rotation of DNA during its duplication has not yet been solved, although it is being discussed very vigorously. In our laboratory, we have tried to understand what the situation is regarding the closure of the chromosomes, which would seem hopelessly to prevent reduplication. The following experiment was set up.[4] Radioactive bacteriophage T_2 was grown with very "hot" DNA (it was tagged with P^{32} with a specific activity of 100 millicuries/mg). This virus was used to infect bacterial cells, introducing into each of them one molecule of DNA having a characteristic molecular weight (130

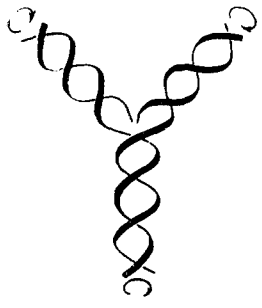

FIG. 3. Diagram of the unwinding of the DNA double helix.

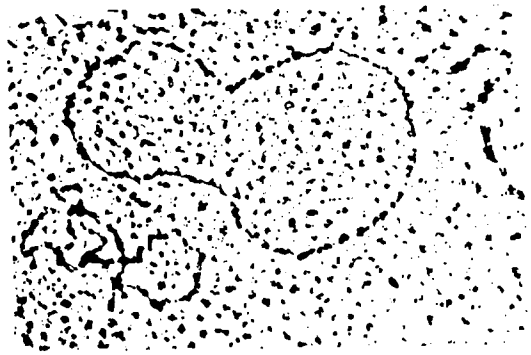

FIG. 4. Electron micrograph of a DNA chain from polyoma virus.

million). The infection of the bacteria consists in the penetration of the DNA molecules of the virus into the cells. Thereupon, synthesis begins within the cell, first of a series of proteins, the information for which is contained in the virus DNA, and then the DNA itself is multiplied. Finally, beginning in 12—15 minutes, complete phages are assembled within the cell. In about 20—25 minutes, the envelope of the cell breaks and the entire "crop" of phages emerges.

We have opened the cells and measured the molecular weights of the radioactive DNA alone, i.e., the maternal DNA, with the ultracentrifuge at various stages of the development of the new generation of phages at definite intervals of time after infection. None of the newly synthesized DNA was visible, since it contained phosphorus as P^{31}, whereas we were tracing the sedimentation of radioactive polymer. The following result was obtained. At the first instant after infection, the molecular weight of the maternal DNA had the original value, 130×10^6. However, after a short time, it began to decompose into definite fragments of weight about 35×10^6 (Fig. 5). One might ask whether the synthesis of the new DNA makes use of the 35×10^6 fragments, or only of the original large molecules. To answer this, the inverse experiment was set up. The infection was performed with non-radioactive phage, while the bacteria were grown on a medium containing P^{32}. The experiment showed that the newly-synthesized phage DNA has the molecular weight of the fragments, i.e., 35×10^6; only at the moment of final assembly of the ripe phage particles does DNA of high molecular weight appear in the cell, assembled on the average from four of the units that serve as matrices for synthesis and reproduction (Fig. 6). Thus, the replication of the DNA is preceded by fragmentation, and the difficulty involving the closure of the phage DNA vanishes. However, the problem of what makes the double chain unwind by rapid rotation remains unsolved.

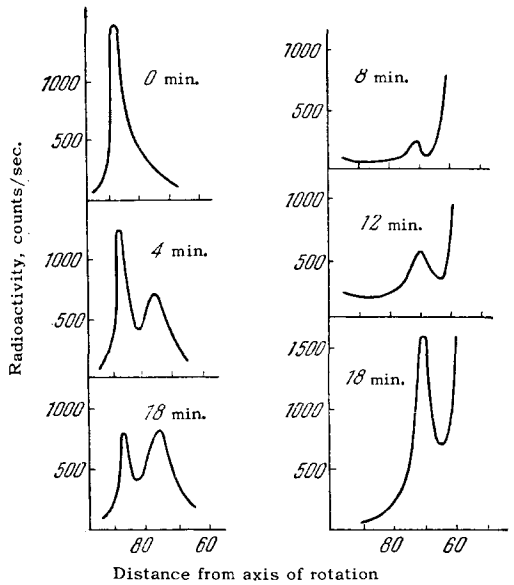

FIG. 5. Study of the DNA of T_2 bacteriophage during the process of intracellular phage growth. The experiment is performed by opening the infected cells at definite periods (0, 4, and 18 min after infection), and ultracentrifuging the phage DNA. Left: the direct experiment, in which the maternal-phage DNA was labeled with P^{32}. Right: the inverse experiment, in which the newly synthesized daughter-phage DNA was labeled.

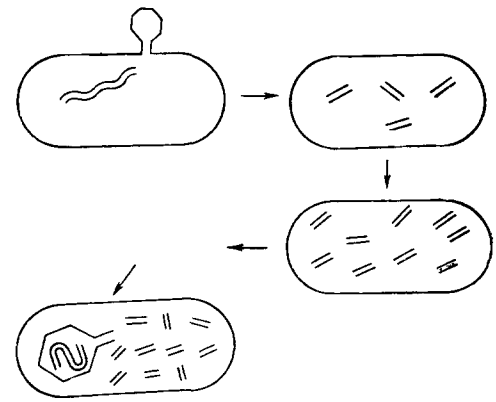

FIG. 6. Diagram of the intracellular growth of phage.

Another curious problem involving DNA is that of the appearance of mutational injuries. Using the phenomenon of transformation, one can study chemical mutagenesis as an ordinary reaction, avoiding all complications.

What does the transformation of bacteria consist in (Fig. 7)? Let us assume that we have a strain of bacteria (e.g., the hay bacillus = Bacillus subtilis) having a hereditary injury in its DNA, i.e., in a chromosome.

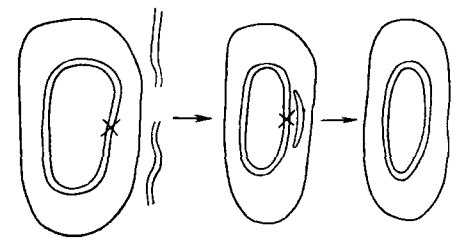

FIG. 7. Diagram of the phenomenon of transformation of bacteria.

For example, let the cell be incapable in itself of synthesizing the amino-acid tryptophan. This means that it does not have complete information on the structure of some one of its enzyme proteins necessary for the synthesis of tryptophan. Such a strain will grow in a medium having an obligatory supply of tryptophan. We can isolate the DNA from complete cells capable of independent production of tryptophan. We can dissolve this purified DNA and add it to a suspension of our defective cells. Then, experiment shows that it penetrates the cell envelope, enters the chromosome, and confers on the cells the ability to make tryptophan. That is, it converts them into complete cells. This phenomenon is the simplest and most elementary example of transfer of inheritable traits by means of molecules of a pure substance, polymeric DNA.

Now we shall show how we can use transformation to study mutations. Let us isolate the DNA from any strain of bacteria, purify it as much as possible, and then treat it with some mutagenic agent. Actually, in the laboratory we have treated the DNA alternatively with nitrous acid, hydroxylamine, or ultraviolet light.[5] Then the injured DNA molecules were introduced into cells, and we examined whether the chemical processes gave rise to new properties in the cells. Thus it turns out, i.e., by treating the isolated DNA rather than the cells, and then demonstrating new characteristics, that we can actually obtain mutants.

Let us examine the kinetics of the reaction of DNA with some mutagenic factor. We shall show that this is a first-order reaction, i.e., that the mutation arises from a single event of successful interaction of the DNA with HNO_2 or NH_2OH or a light quantum. This would be simple if a second, much more probable process of inactivation of the DNA by the action of the same factors were not superimposed on the mutagenesis reaction. We must not forget that there is a tremendous variety of possibilities in the reactions of the nucleic acids, and most of the processes will simply put the DNA molecule out of action. Let us assume that the DNA molecule has undergone z chemical injuries distributed at random along its chain. Obviously, its incorporation into a chromosome can give a positive effect only if a fragment is incorporated that contains an active region (e.g., bears information on the enzyme synthesizing tryptophan) but contains none of the z chemical injuries that the particle has undergone. We can state that the number of cells transformed by DNA that has undergone z "hits" will be

$$n = Af(z), \tag{1}$$

where A is a coefficient containing the probability that the DNA molecule will enter the cell, interact with the chromosome, etc. The quantity f(z) is a function of the number z of injuries, depending on the length of the region of the molecule remaining uninjured. The number of inactivating hits z will increase in proportion to the time: $z = k_1 t$. We shall assume that one effective event of reaction within the given region of the DNA is necessary for mutation. Then the number y of mutational "injuries" will increase linearly with the time: $y = k_2 t$. In order that the mutational "injury" should be manifested upon transformation in the form of a new organism, or mutated cell, the molecule must enter the cell and the appropriate region of the DNA must be incorporated into the chromosome. Here we must take into account the fact that, besides the mutational "injuries," various inactivating "injuries" of the DNA molecule occur much more often. Evidently, we can derive the following formula for the number of mutants:

$$n_{mut} = Af(z)k_2 t, \tag{2}$$

where f(z) and A are the same quantities as in Eq. (1).

Dividing n_{mut} by n, we have

$$\frac{n_{mut}}{n} = k_2 t = \frac{k_2}{k_1} z.$$

Hence, the ratio of the number of mutants manifested by the transformation to the number of transformants formed by the action of the DNA that have been inactivated under the same experimental conditions will increase linearly with the time only if mutagenesis is a one-hit process. Figure 8 shows that the results of the measurements fully confirm this hypothesis. In addition, we see here that the effectiveness of the mutagenic factor, as defined by k_2/k_1, i.e., the ratio of the mutagenesis constant to the inactivation constant, is greatest of all for hydroxylamine, 2.5 times smaller for nitrous acid, and 15 times smaller for the action of ultraviolet radiation.

Independently of us, Gierer has shown that mutagenesis is a first-order reaction also when HNO_2 acts on the RNA of tobacco mosaic virus. This means that a chemical change in one residue of the polynucleotide chain is the basis of chemical mutagenesis. This result agrees with what we know about the structure of mutated proteins. In them, it has been found by analysis that always only one residue in the polypeptide chain is injured. That is, one normal residue has been replaced by another one not characteristic of the given protein.

Now let us proceed from discussion of DNA to the second stage, the formation of mRNA from DNA, i.e., the transformation of potential into actual information. We can formulate the basic question here as follows. Is the information for protein synthesis taken from both DNA chains, or only from one? In other words, is mRNA synthesized from both complementary DNA matrices? This problem is very important. The point is that the two complementary DNA chains are not identical. On the other hand, they define each other. That is, they contain the same information, but the structure of the proteins is coded differently in them. Hence it seemed at first that only one of the chains bears genetic information, i.e., is actually copied in forming mRNA. A rather simple experiment was set up in our

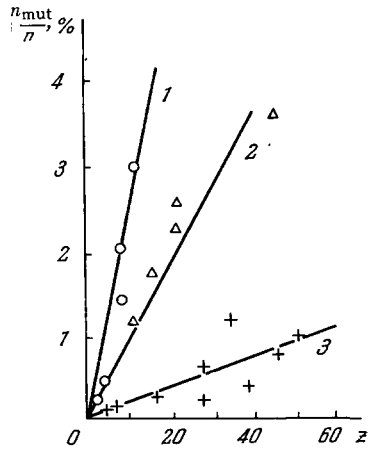

FIG. 8. Relative yield of the number of mutants as a function of the number of inactivating injuries in the DNA molecule. Line 1 – action of hydroxylamine; 2 – action of nitrous acid; 3 – action of ultraviolet light.

laboratory to solve this problem.[6] Again we used the phenomenon of transformation of bacteria. Let us assume that we have a strain of bacteria (hay bacillus) having two hereditary injuries, e.g., it is incapable of synthesizing two amino-acids, tryptophan and histidine. Such a strain will grow only in a medium containing the free amino-acids tryptophan and histidine. We can take the DNA from an unimpaired strain of bacteria, and isolate and purify this DNA, and transform cells with both traits at once. It was shown that both genetic markers, tryptophan and histidine, i.e., both regions in the DNA bearing information on the corresponding enzymes, occur in series within one DNA macromolecule. Hence, the transformation with both traits takes place simultaneously. This is very important, since it permits us to perform the following experiment. Let us take DNA from two "halfway" strains. One of them can synthesize tryptophan but not histidine, and the other can synthesize histidine but not tryptophan (Fig. 9). Let us isolate the DNA from each of them, mix them in solution, and heat until the double helices melt (90°C) and then separate into individual strands; then let us slowly cool the mixture as though annealing it. Then we will obtain the re-formation of the DNA double helices,[7] and half of the re-formed helices will be hybrids that we have made artificially, and which bear one active marker on each chain. Special experiments using an isotopic marker (N^{15}) on half of the DNA before melting and annealing showed that such a "hybridization of molecules" actually takes place and is fully effective.[8] We might ask

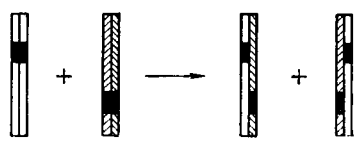

FIG. 9. Diagram of the formation of "hybrid" DNA double chains.

whether we can transform doubly defective cells using such molecular hybrids. That is, will the cells that have obtained a DNA molecule consisting of two non-identical strands live and grow? If this is so, then mRNA must be copied from both DNA strands, rather than from one. The experiment gave this answer unequivocally. And since no two codes for reading the two complementary chains have been found, this means that an additional complementary copying must occur in the cell, this time of RNA from RNA. Of the two primary mRNA strands, one is the positive, as it were, and the other is the negative. However, a second process must occur in the cytoplasm of the cell, the formation of a positive from the negative, i.e., another complementary copying of RNA from RNA. Interestingly, in the last year biochemists have discovered such enzymes in various cells of plants, animals, and bacteria. Our experiments have specified a useful job for these enzymes to do.

I shall spend some time now on the details of the process of synthesis of the protein chain. Here mRNA is used as the matrix determining the sequence of the amino-acids in the protein chain. As we know, there is a special code relating the sequence of nucleotides in the nucleic acid with that of the amino-acids in the protein. I shall not take up here the problem of studying the code, since a special article has already been devoted to this problem in this journal.[1] I shall only mention that a triplet of nucleotides in the mRNA chain codes one residue in the protein. Thus the most economical code is realized in nature, since the nucleic acid chain consists of four types of monomers, while the protein chain consists of twenty types (one can make up $4^3 = 64$ triplets out of four letters, i.e., quite a sufficient number to represent twenty amino acids; the number of possible doublets would be only $4^2 = 16$, i.e., would not satisfy the requirement).

However, the physical accomplishment of protein synthesis introduces not cybernetic, but purely structural complications. It would seem physically unthinkable to apply structural requirements on the dimensions of the protein chain with a three-symbol code. One amino-acid residue occupies 3.6 Å, while three nucleotide residues occupy at least 21 Å. Besides, it is incomprehensible how one could picture the molecular forces regulating this selective machine. How can an amino-acid residue be determined by three nucleotide residues so unlike it? To solve this puzzle, Crick advanced a hypothesis consisting in the idea that there must also exist special small molecules, or adapters, that transfer the amino-acids and attach them to the matrix, which is the mRNA. It has now been determined that the amino-acids are first attached to a special small transfer-RNA chain consisting of only 70–80 nucleotide residues. There are just as many transfer RNA's as there are amino-acids. It is precisely with the aid of this transfer RNA that the selection of the amino-acids on the mRNA chain occurs.

Each triplet of nucleotides on the mRNA codes a particular amino-acid, or is a codon. The codon joins by hydrogen bonds, using the principle of complementarity, to a triplet of residues of the transfer RNA, or the so-called anti-codon. Thus each amino-acid finds its place on the matrix. At each given instant of time, the already-synthesized polypeptide chain must be attached to the matrix, i.e., to the mRNA, through the terminal transfer-RNA molecule, together with another amino-acid through its own transfer RNA. This is the instant of formation of the next peptide bond, or the formation of the next link of the chain. Figure 10 depicts the gist of our knowledge about this process plus a certain amount of imagination, since the exact position of the anti-codons in the structure of the transfer RNA is not yet clear. It has been shown recently that each ribosome contains a single point at which the polypeptide chain is joined on through the transfer RNA. Consequently, the ribosome is an apparatus in which a single protein chain is synthesized at a time.

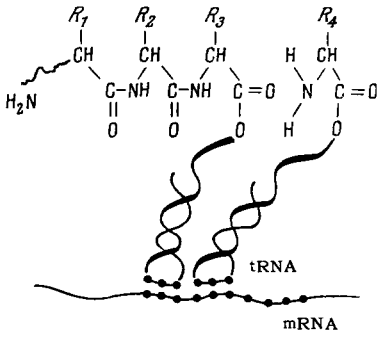

FIG. 10. Diagram of the linkage of the growing polypeptide chain to the matrix.

What is the internal structure of the ribosomes, and how is it related to their functions? The ribosomes are the universal workshops for protein synthesis. Precisely what protein is being synthesized in them is determined by what mRNA is attached to them. It has been shown by a special experiment that the ribosomes show no specialization. They synthesize at each particular moment the type of protein that the cell requires. And if the cell is sick, if it is being parasitized by a virus, then the ribosomes of the cell are busy synthesizing the proteins of the virus (e.g., bacteriophage) instead of the characteristic proteins of the cell. This is the information, or if one prefers, misinformation that the ribosomes receive in the form of the corresponding mRNA chains. In themselves, the ribosomes consist of two unequal parts, a larger one with a sedimentation constant of 50 Svedberg units and a smaller one with a sedimentation constant of 30 units. Both subunits are combined into one unit, but can be separated by making the concentration of magnesium ions in the surrounding medium 100 times smaller than in the cell (i.e., 10^{-4} M instead of 10^{-2} M). The dissociation of the ribosomes into two subunits is a reversible

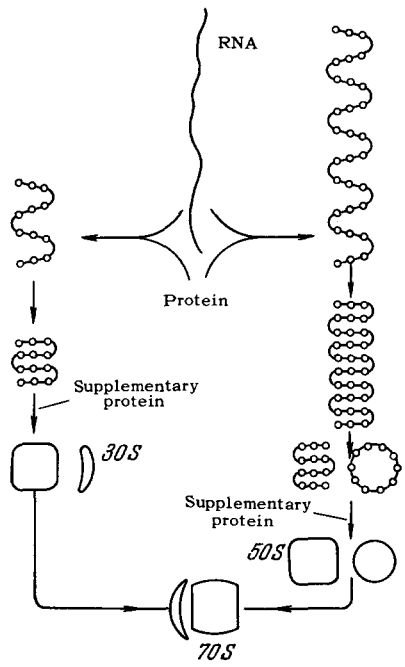

FIG. 11. Diagram of the construction of the ribosome from RNA and protein. The diagram shows the configuration of both subunits and the topology of packing of the RNA chain. The numbers 30S, 50S, and 70S are sedimentation constants.

process. If the magnesium concentration is raised, they unite again. The ribosomes consist of one-half protein and one-half RNA of special types. One of the subunits contains one chain of ribosomal RNA having a molecular weight of 1,200,000, and the smaller subunit contains an RNA chain having a molecular weight of about 600,000. Both types of ribosomal RNA are synthesized, like all other types of RNA, by complementary copying of special regions of the DNA in the chromosome. What is the function of the ribosomal RNA? This is one of the puzzles not yet solved. In the ribosome, the RNA and protein, as is generally said, are integrated into a three-dimensional structure. The most detailed information on the internal structure of the ribosomes has been obtained by A. S. Spirin and N. A. Kiselev by electron microscopy of ribosomes and their parts.[9] On the basis of their experiments, we can reconstruct the ribosomes with a certain degree of likelihood (Fig. 11). Here we see that the two subunits are formed like a vessel with a cover or envelope which can open as the concentration of magnesium in the medium is lowered. The walls of the ribosome are a mosaic of packed zigzag RNA chains and globular protein molecules. We can draw an analogy between the structure of the ribosomes and of certain viruses.

There is another curious detail in the process of protein synthesis. The mRNA chain has a high molecular weight (up to 10—15 million), as is quite natural, since it is a copy of a DNA molecule. Its length can be as great as one micron, while the diameter of a ribo-

some is 170 Å. A single mRNA molecule contains information on a series of protein chains, and here we see that several (up to 8—10) ribosomes attach themselves to an mRNA chain at equal distances, and from all appearances, move along it, adding on at one end and sliding to the other. This is how the so-called polysomes arise (Fig. 12).[10] By working with isotopically-labeled ribosomes, this picture can be confirmed experimentally. All of this looks as if the ribosomes move along the mRNA strand, read the information stored in it, and transform it into the material polypeptide chains of a protein. Polysomes are now being isolated in many laboratories by means of ultracentrifuges and are being observed in the electron microscope (Fig. 13).[11] It is not clear how the movement of the ribosomes along the mRNA chain takes place. In addition, the details of the process of linkage of amino-acids into a single chain, i.e., the details of the final process of synthesis of the peptide bonds, are still unclear.

FIG. 12. Diagram of the formation of polysomes.

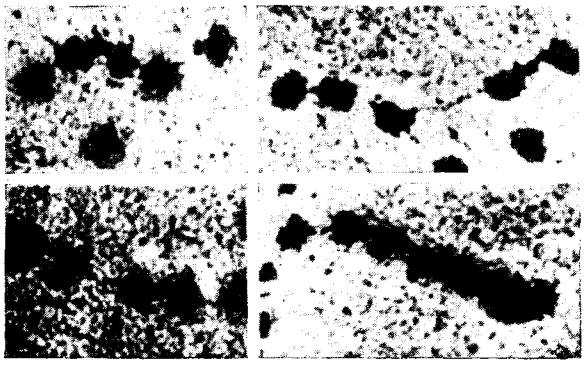

FIG. 13. Polysomes in the electron microscope.

In order to study this stage, it is important to be able to isolate the intermediate product of the synthesis, i.e., the protein chain while it is not yet complete, and is attached by a chemical bond to RNA. Various laboratories in the world have searched during recent years for this intermediate compound. In our laboratory[12] we have learned how to obtain it and identify it with the aid of a method of zone electrophoresis in a heavy-water gradient that we have developed. Imagine a column filled with a mixture of light and heavy water, having pure D_2O at the bottom, and a linearly declining concentration of D_2O above that up to the meniscus. Such a column has a linearly declining density gradient, which greatly stabilizes the liquid against any convection currents. At a certain point in the column (Fig. 14), one introduces a thin zone of the product containing the intermediate compound of protein biosynthesis. Here the synthesized polypeptide is labeled with highly radioactive amino-acids (C^{14} of high specific

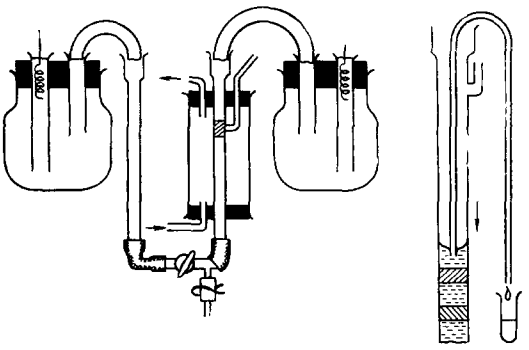

FIG. 14. Apparatus for zone electrophoresis in a density gradient. It is shown at the right how samples are removed from the electrophoresis vessel.

activity). Let us choose the electrophoresis conditions such that (pH = 4.5) the proteins are practically uncharged, while the nucleic acids bear one electronic charge per residue of the chain. Under these conditions, the proteins do not move in the electric field, and their zone is stationary, while the nucleic acids migrate very quickly to the anode. How will the intermediate product of synthesis behave? It migrates to the anode, only somewhat more slowly than the RNA, but in addition it is labeled with radioactive amino-acids. Thus we measure it as polypeptide (Fig. 15).

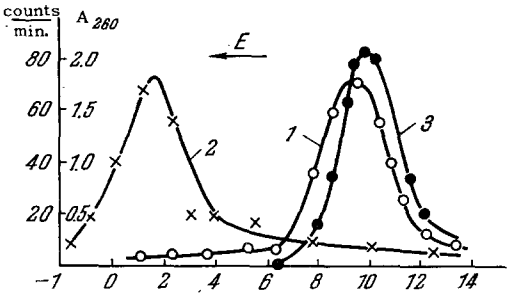

FIG. 15. Isolation of polynucleotide-peptide labeled with C^{14} and amino-acids by zone electrophoresis. 1 – Polynucleotide-peptide zone; 2 – the result of a control experiment in which the bond between the polypeptide and polynucleotide chains has been broken (enzymatically); 3 – zone of transfer RNA added as a control. In the latter, the concentration was measured from the absorbance at 260 mμ, rather than from the radioactivity. The zero point on the abscissa corresponds to the point of introduction of the sample into the electrophoresis vessel.

We call this substance polynucleotide-peptide. If we break the bond between the two polymers, for which purpose one generally takes a rather alkaline medium or a special enzyme, the radioactive protein no longer migrates. Hence the zone-electrophoresis method discriminates very well between protein, nucleic acid, and polynucleotide-peptide. In order to study the composition of the intermediate product, a double label was introduced. The polypeptide was labeled as before with C^{14}, and the polynucleotide with P^{32}. It is not hard to count them separately, using the difference in energy of the electrons from C^{14} and P^{32}. What could be determined concerning the structure of the intermediate

complex? Apparently, in addition to transfer RNA, it also contains ribosomal RNA, i.e., the substance comprising 50% of the mass of the ribosome. Up to now it has not been known what useful function this polymer serves in nature. If it is contained in the intermediate product of protein synthesis, this means that the ribosomal RNA starts or initiates the process of synthesis of the protein chain. Another fact is in accord with the existence of two types of RNA bound to the nascent protein chain. There are two types of bonds between RNA and the protein, a more labile one destroyed by 10^{-3} M alkali, and another more stable one resisting this concentration of alkali for tens of hours. At the present moment, intensive work is proceeding in order to confirm finally by the most direct means that the intermediate compound has such a structure. Thus it will be possible to understand how the entire process of protein polymerization takes place, since an understanding of the initiation of this process may prove to be the key to knowing the entire mechanism of the final reaction in protein synthesis, i.e., the linkage of the amino-acids with one another by peptide bonds.

On the other hand, we know from the experiment of producing ordinary polymers that the initiation reaction involves the possibility of expediently regulating the rate of synthesis of the polymer chains. Hence it is not ruled out that in the case of protein synthesis as well, a regulation of the amount of synthesized protein is possible at the level of the ribosomes.

The problem of automatic regulation of the synthesis of enzymes is another fundamental problem of molecular biology deserving especial examination.

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