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Sheldon Nicol
Illinois Wesleyan University

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by

Sheldon Nicol

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The unprecedented growth of interest in protein synthesis among biochemists is both a result of, and has contributed to, our rapidly advancing knowledge of the chemistry and metabolism of the nucleic acids. Over the past two decades, evidence has accumulated which points to an intimate association between cellular nucleic acid and protein synthetic activity. A large number of experiments in bacterial systems have shown that ribonuclease disrupts the cell's protein synthetic machinery and that ribonucleic acid (RNA) can frequently restore it. Studies on bacterial transformation (Hotchkiss, 1957) and the discovery of the autonomous infectivity of tobacco mosaic virus RNA (Gierer and Schramm, 1956) unequivocally establish that nucleic acids alone contain the necessary information in their structure to direct the synthesis of new and genetically significant proteins. A large body of information on the fate of C^{14}-amino acids in whole animals demonstrates conclusively that the initial and major site of incorporation of amino acids into protein are the cellular ribonucleoprotein particles (ribosomes). Thus it was clear, before cell-free systems has received much scrutiny, that nucleic acids had some intimate directive role in converting amino acids to protein and that the study of protein synthesis was inseparable from a study of the nucleic acids.

The history of nucleic acid study began in the 1860's, when Mendel set forth evidence, from his observations of inheritance in peas, that genetic information is carried in discrete units from one generation to another, and when Miescher isolated a substance from the nuclei of cells

that he called nuclein and that is today known as desoxyribonucleic acid (DNA). He knew that he had discovered a novel substance containing nitrogen and phosphorus, and he was well aware of its location in the nucleus. But he had no idea of its function, because the role of the nucleus in heredity was unknown at the time, even to Mendel. It was well over half a century later, long after the death of both men, before their work could be fused. The fusion required much more nucleic acid and protein chemistry than existed in Miescher's time and a vast amount of new biology, including the later unearthing of Mendel's work.

During the last thirty years of the nineteenth century, work in biology, led by Weismann, suggested the continuity of the germ plasm and implied that the nucleus of the cell plays a central role in heredity. Attention soon focused on the chromosomes. Since sperm and egg nuclei provide equal complements of autosomal chromosomes and since there is precisely equal cleavage and chromosome distribution in cell division, it seemed clear that chromosomes are concerned with heredity. With the rediscovery of Mendel's work and the growth of genetics, biologists talked less about the germ plasm and more about the genes as distinct units of germinal material. It became increasingly clear that each gene is derived from a pre-existing gene. The genes were located on chromosomes, which now, far more conclusively than before, were shown to contain the materials determining heredity. Meanwhile the chemistry of nucleic acids was making progress. But there was little contact between the two movements. The now standard color test for DNA was first demonstrated in 1914 by Feulgen, but was not used until ten years later to stain cells and show that the chromosomes are loci of DNA concentration in the nucleus.

The evidence that genetic information is carried by DNA came in the

late 1940's from a number of sources. The DNA content of nuclei in germ cells and various somatic cells was measured and found, in a given organism, to be constant per set of chromosomes. This constancy pointed to DNA as the essential material of the genes. Numerous experiments with pneumococci showed that hereditary traits could be transmitted from one strain of bacteria to another by transferring, to cells of the latter, DNA extracted from the former. These experiments proved conclusively that DNA is the carrier of genetic information.

These developments made a great impression on geneticists, for they went a long way toward answering questions concerning the nature of the gene. The impact on biochemists was far greater; biochemists could now study the molecular basis of gene action, tracing the effect of DNA in discrete observable events in the synthesis of molecules composing the cell. In the last twenty years, it has become possible to answer, at the molecular level, some of the fundamental questions of genetics and cell biology. The most significant advance came in 1953, when two English scientists, J. D. Watson and F. H. C. Crick, after work with x-ray crystallography, devised a new molecular structure for the DNA molecule, based on a double helix model (Watson and Crick, 1953).

For many years, the hypothesis of the tetranucleotide sturcture, which proposed by Levine, was first proposed by Levine, was accepted. According to this hypothesis, nucleic acid results from polymerization of tetranucleotides. These polymers are aggregates of four nucleotides bound together by phosphate-ester linkages, each of which results from the combination of phosphoric acid with a carbohydrate residue and a different nitrogenous base. Tetranucleotide structure was based upon the following facts: (1) hydrolysis of nucleic acids seemed to always liberate equimolecular amounts of the

four constituent bases, and always in the ratio of two purines to two pyrimidines, (2) early determinations of the molecular weight of the acid gave figures which seemed to indicate a molecule of about tetranucleotide size, and (3) titration of the acid indicated four or five free acid hydrogens in the phosphate radicals. This was best explained by a structure in which one mononucleotide is linked to another through a phosphate ester linkage from the phosphate of one nucleotide to a hydroxyl group of the sugar of another. It had even seemed possible on the basis of their relative rates of hydrolysis to decide which nucleotides were the outer ones and which were the central ones. In accordance with the available evidence, various structures were proposed for the tetranucleotide, including open chain compounds and giant rings.

The Watson-Crick model for the molecular structure of DNA is based on the construction of molecular models arranged to conform to the physical and chemical characteristics of the DNA molecule. In the proposed structure, two molecular strands are coiled around a common axis to form a fairly rigid helix. Each chain is a long polynucleotide, resulting from the linkage of many individual nucleotide units. The nucleotides are formed by the combination of molecules of desoxyribose sugar with phosphoric acid molecules and nitrogenous bases.

The nitrogenous base is of one of two types: purine or pyrimidine. Purine bases are double-ring compounds with two nitrogen atoms in each ring. There are two purine bases in DNA: adenine and guanine. Guanine contains a hydroxyl group on the six-membered ring and can thus be distinguished from adenine. Pyrimidine bases are single-ring compounds containing two nitrogen atoms. Two pyrimidine bases are found in DNA: thymine and cytosine. Cytosine, because it possesses an amine group,

can be distinguished from thymine. In the T2, T4, and T6 bacteriophages of Escherichia coli, 5-hydroxy-methyl-cytosine takes the place of cytosine and in wheat germ about one-fourth of the cytosine is replaced by methyl-cytosine. Glucose has been reported as a constituent of the DNA of certain bacteriophages (Sinsheimer, 1954). The purine or pyrimidine base is attached to the sugar at the one-carbon and the phosphate to the three-carbon. The nucleotides are linked together by phosphate ester groups in such a way that the three-carbon of one sugar is linked to the five-carbon of the next.

The unique feature of the Watson-Crick structure is the arrangement of the bases. The two chains are joined together by hydrogen bonds which join the opposing nucleotide bases. The phosphates and sugar groups are on the outside of the helix; the bases are on the inside and directed perpendicularly to the fiber axis. Bonding occurs only between adenine and thymine and between cytosine (or a substituted cytosine) and guanine. Adenine-thymine bonds contain two hydrogen bonds, while cytosine-guanine bonds contain three hydrogen bonds. Hydrolysis of DNA preparations yields the desoxynucleotides of adenine, guanine, cytosine, and thymine. There are differences in the base ratios of DNA preparations from different species; that is, the molar amounts of adenine, guanine, cytosine, and thymine are not 1:1:1:1 as required by the old tetranucleotide hypothesis of DNA structure, but there are definite analytical regularities. The ratio of adenine to thymine and guanine to cytosine seems always to equal one. The proportions and sequence of nucleotide components, however, vary and are characteristic for each species. This is evident from the so-called dissymetry ratio, which is the ratio of adenine and thymine to

guanine and cytosine. The ratio changes from 1.85 in the sea urchin Paracentrotus lividus to 0.42 in the avian tubercle bacillus and 1.53 in man (Chargaff, 1957).

There is no known restriction on the sequence of bases in one chain but it is obvious that the chains are complementary; that is, once the sequence of bases in one chain is known, the necessity for specific pairing determines the sequence of bases in the second chain. Each nucleotide pair is rotated 36 degrees with respect to its neighbor and successive base pairs are 3.3 Angstroms apart (DeRobertis, 1958). The DNA molecule is very long (about 30,000 nucleotides), quite narrow (about 20 Angstroms in diameter), and has a molecular weight of several million (Strauss, 1960).

The experimental evidence, other than x-ray data, which supports the double helical model, falls into two categories: the chemical evidence and the physico-chemical evidence. The chemical evidence shows that the molar ratio of purine and pyrimidine bases is very close to unity for all sources of DNA. There is now much physical evidence to support a two-chain structure. Titration curves suggest that the bases form hydrogen bonds and that these are bonds within the structure. Light scattering, viscosity, and sedimentation measurements show that DNA in solution is highly extended, but not completely straight, and that its diameter is compatible with the double helix model. Studies of the rates at which the structure is broken down by gamma rays (Cox et al., 1955), acid (Thomas and Doty, 1956), and enzymatic attack (Thomas, 1956), are consistent with there being two strands in the DNA, so that the molecule does not come apart until there are breaks in both backbones almost opposite one another.

DNA structure combines symmetry and pseudo-symmetry, repitition and non-repitition. The phosphate-sugar backbone repeats regularly, both chemically and structurally. This repitition necessarity implies that the phosphate-sugar groups are related by symmetry, in this case by a screw axis, and it is this which makes the backbone a simple helix. Again, the two separate phosphate-sugar backbones are related to each other by symmetry, in this case by two-fold rotation axes perpendicular to the fiber axis. The arrangement of the bases, however, does not repeat, and only shows pseudosymmetry; that is, the region occupied by a pair of bases is fixed, and successive regions are related to each other by symmetry, but there is no restriction on which pair of bases occurs at any point, as long as one of the allowed pairs is used. It remains to be seen whether there are structural reasons for the particular base pairings.

It should be noted that while x-ray diffraction shows that a substantial portion of the DNA must be in the double helix form it is an extremely poor method for deciding how much of the DNA is in this configuration. The titration curves and the analytical data suggest that the great majority of bases are paired. However, it seems certain that the molecule is folded in its biological condition, and that there may be occassional regions where the configuration is somewhat modified. Degradation of DNA with the crystalline enzyme desoxyribonuclease results in a large proportion of dialyzable fragments, but a non-dialyzable fraction remains, whose composition is significantly different from the original DNA and the dialyzate. This indicates a complex and asymmetrical organization of the DNA molecule.

Dried sodium salts of DNA form fibers with intense negative birefringence and structural viscosity. Electron microscopy shows that DNA

precipitates contain long branching and anastomosing fibrous processes, further indicating the considerable molecular asymmetry. This molecular asymmetry and the tendency of DNA particles to become oriented under stretching is also revealed by the dichroism shown in ultra-violet light.

Nucleoproteins result from the combination of nucleic acids and proteins and, in certain cells, constitute the major part of the solid material. Nucleic acids combine with the proteins by ionic bonds which are dissociated with relative ease. It is suspected that a basic protein lies in the narrow groove that spirals around the DNA molecule and that the component basic amino acids of the protein neutralize the phosphoric acid residues of the DNA. Two main types of basic proteins have been found associated with chromosomes: one of low molecular weight (protamine) and one of high molecular weight (histone). In addition, there occurs an acidic protein of high molecular weight.

Evidence suggests that the protamine chain is wound helically around the DNA structure in the smaller of the two grooves between the backbones. Models show that an extended polyarginine chain can be fitted in this groove without difficulty, with the positively charged basic groups of the side-chains going alternately up and down to the negatively charged phosphate groups of the DNA backbones. In nucleoprotamine there appears to be one arginine for every phosphate, yet only two-thirds of the protamine side-chains are basic. This suggests that the polypeptide chain is folded whenever the non-polar amino acids occur. Model building shows that it is difficult to construct a fold with one non-polar residue, but relatively easy with two in succession. Data on the amino acid sequence shows that the non-polar residues do occur in pairs. It is not yet clear whether the interaction between the non-polar residues and the bases of

the DNA structure is specific or non-specific, nor whether the non-polar folds go inwards or outwards. The main features of the preliminary x-rays are that (1) the DNA - or at least part of it - maintains its characteristic structure; and that (2) some larger repeating structure is also present. These results are obtained with nuclei, swollen in water and drawn into fibers, and also with artificial combinations of DNA with lysine-rich histone. One equatorial spacing (of about 60 Angstroms) changes little on drying; another, around 40 Angstroms, alters with humidity (Crick, 1957). These results show that nucleohistone has a structure of some sort.

In the chromosome, each of the ultimate fibrils consists of two complementary DNA molecules intertwined about the basic protein. These fibrils are 3-4 mu in diameter and several microns long. Bundles of these then form the microscopically visible chromonema of 0.5-1 micron in diameter. Whether or not a single DNA molecule extends the entire length of the chromosome is a matter of debate. The chromonema undergoes a characteristic coiling cycle which may be the result of cyclical changes in the chemical links between DNA and its basic protein partner. The coiling behavior of chromosomes may be experimentally modified by treatment with metabolic poisons like potassium cyanide. When at a relatively extended state, the chromonema has a series of microscopically visible beadlike swellings along its length called chromomeres. Chromomeres may represent regions where the elementary chromosome fibrils form compact gyres instead of running along in an easy spiral path roughly parallel to the axis of the chromonema.

The fractionation of DNA by various means has yielded fractions of different composition indicating that chemical heterogeneity does occur.

Physical heterogeneity has been observed in observation with ultracentrifuging at very low concentrations. A range of sedimentation constants, often extending form S=10 to 40 or more, is observed in a single specimen (Shooter and Butler, 1955). Therefore, there must be considerable variation of particle configuration of DNA specimens. It has not yet been possible to prepare DNA specimens by fractional centrifugation in sufficient quantity to determine their composition. It appears that DNA does not exist as fairly straight rods of constant diameter and different length, but rather that some kind of aggregation occurs.

RNA is, in general, structurally similar to DNA. It is a long polynucleotide but is generally single-stranded. In its backbone it contains the sugar ribose instead of desoxyribose. The bases linked to the ribose units can be any of four different types, all similar to those found in DNA except uracil, which is found instead of DNA's thymine. RNA is abundant in the cytoplasm, particularly in the ribosomes.

DNA can be topographically located within the cell by means of the Feulgen nucleal reaction. This method consists of treating the tissue with Schiff's reagent (basic fuchsin bleached with sulfurous anhydride, leucobase) after acid hydrolysis. The parts of the cell containing DNA give a positive reaction (they combine with and recolor the basic leucofuchsin). This reaction is entirely negative with RNA. The mechanism of Feulgen's reaction is not well understood, but it is considered to occur in two different stages. In the first one, purine bases are split through acid hydrolysis, setting free aldehyde groups in the DNA molecule and producing a compound sometimes called thymic acid. During the second stage, the colored compound is synthesized by means of a chemical reaction between the aldehyde groups and the decolored fuchsin. When

used with the cell, the Feulgen reaction is positive in the nucleus and negative in the cytoplasm. In the nucleus, the nucleolus is negative and chromonemata (especially the chromocenters) are intensely positive.

In ultra-violet light, nucleic acids show a characteristic absorption peak at 2600 Angstroms due to the presence of purine and pyrimidine bases. Ultra-violet microspectrophotometry permits the localization of the two types of nucleic acid without distinguishing between them, while the nucleal reaction of Feulgen shows DNA presence.

Some time ago it was thought that the double-stranded DNA structure occurred in all organisms. Recently, however, there have been reports of a virus which has a single-stranded DNA structure, judged from its susceptibility to inactivation by phosphorus-32 decay (an efficiency of one inactivation per disintegration which is about ten times greater than the efficiency of inactivation of the double-stranded phage DNA) and by the unusual physical properties of the nucleic acid (Sinsheimer, 1959). The finding of a virus with a single-stranded DNA implies that the genetic information can be obtained in a single strand.

DNA is located exclusively in cell nuclei. The amount of DNA per cell nucleus is relatively constant. Although the content of RNA and of protein changes drastically with changes in the physiological condition of the cell, the DNA content remains constant as would be expected if DNA content reflects the gene number. It is evident that DNA is inert and does not participate in cell metabolism.

Analyses performed in a variety of animals shows that among mammals, in spite of large differences in size of the different species, the variations in DNA content are small, while in different species of birds

and fishes the content definitely varies. Among invertebrates, the lowest DNA values are found in the most primitive animals such as sponges and coelenterates. The values of DNA in lung fishes, amphibians, reptiles, and birds suggest that during evolution there has been a decline in DNA content (Mirsky and Ris, 1951).

The real experimental evidence that forces exclusive consideration of DNA as the genetic material comes from studies with microorganisms, particularly from studies on the transforming principles of bacteria. Pneumococci ordinarily possess a smooth polysaccharide coat. Occassionally after culture in vitro, cells are obtained which yield colonies in which the polysaccharide is not produced and which have a rough appearance. This transformation is irreversible; rough cells never spontaneously develop the ability The substance responsible for transformation has been shown to be DNA. Transformation is not restricted to the capsular polysaccharides; transformations of drug resistance, biochemical characteristics, etc. have been achieved.

The evidence is quite good that the transforming principle is really DNA uncontaminated by protein (Zamenhof, 1957). It is therefore possible to eliminate the possibility that protein plays a specific role in the transformation process. Although many characteristics may be transformed, in general they are transformed only one at a time. Only a portion of the total potential material contained in the donor DNA is actually incorporated into the host at one time. Transformation therefore does not involve a complete substitution of one set of genes for another. Transformation represents an unusual but definite method of gene transfer. The DNA of the donor, when introduced into the recipient cell, becomes a part of the recipient genetic material.

Although the exact mechanism of DNA synthesis is still subject to speculation, some basic facts have been demonstrated. Most interpretations are influenced by the Watson-Crick model, which gives use to the theory of replication by a template (model) process by which each strand acts as a mold for a newly synthesized one. This mechanism can be compared with photographic reproduction, in which a negative is made first and from this a positive print. Since one of the polynucleotide chains determines the synthesis of the other during interphase, all that is needed to set the template mechanism into action is the separation of each strand. This hypothesis presupposes that the simple nucleotides fall into phase and that the parent helix of DNA unwinds while the daughter helices are formed and unwound. Calculations indicate that energy requirements for such an uncoiling are not too great. Nonetheless, a detailed model for the duplication of the coiled DNA as it occurs in vivo is difficult to make even though there have been several attempts.

In nuclei of living, proliferating cells, a Feulgen-negative, ultraviolet absorbing component has been found which is absent in non-dividing cells. It is postulated that this component comprises relatively unpolymerized DNA precursors that are lost by fixation and hydrolysis. This component exists in amounts approximately equal to the DNA content; it also doubles during interphase.

Certain agents interfere with the process of chromosome replication by changing the metabolism of DNA and protein. Among these are the competitors in the metabolism of folic acid, which is a precursor of coenzymes acting in the synthesis of purines. Other competitors act on the purine mechanism directly. Some agents may produce a chromosome rupture like that caused by ionizing radiation. A deviation from normal synthesis

may originate from an interference with the spiralization cycle.

One of the surprising developments of the past few years has been the description of an enzyme system that synthesizes DNA (Kornberg, 1957). The synthesis is detected by the incorporation of labelled desoxyribonucleotides into DNA using a polymerase enzyme preparation obtained from Escherichia coli cells. A net synthesis of 50% or better has been reported. The enzyme system has the following characteristics: all four desoxyribonucleotide triphosphates are required for appreciable synthesis (the diphosphates will not substitute), magnesium ion is required, the system is essentially irreversible (as would be required to explain the metabolic stability of DNA), and about 1% of the maximum reaction rate is obtained when a single nucleotide is eliminated from the reaction mixture.

Polymerized DNA is required to serve as a primer for the reaction, apparently to serve as the template upon which complementary chains are constructed. Overall proportions of the bases in the product DNA parallel the base composition of the primer and the sequence of the nucleotides in the enzymatically synthesized DNA is the same as in the primer DNA. Incorporation ratios of the bases are not changed by variations of their concentrations in the reaction mixture, as is the case for the polynucleotide phosphorylase which synthesizes an RNA (Ochoa and Heppel, 1957). The activity of the primer is increased by heating, indicating that it is a single strand of DNA which serves as a model.

In cells of higher organisms, DNA synthesis is restricted to the interphase period in which the chromosomes are still in their extended form and are not easily visible. Studies using the autoradiograph method indicate that DNA synthesis occurs during the middle of the interphase

period (Taylor, 1957). There must be some sort of signal to set DNA synthesis going since this synthesis is not a continuous activity of the cell in higher organisms. Presumably the synthesis of DNA requires the activation of the Kornberg enzyme but there is no knowledge of what sort of signal is required.

DNA itself cannot be the template material for protein synthesis. The major site of protein synthesis in most organisms is the small particle fraction of the cell, a region devoid of DNA. It is possible to dissociate protein synthesis from DNA synthesis; that is, inhibition of the synthesis of DNA by ultra-violet light, x-ray irradiation, mitomycin, or thymine starvation does not inhibit protein synthesis (Spiegelman, 1957). The high concentration of RNA in the cytoplasm suggests that this substance has a major role in protein synthesis. RNA concentration is always high in rapidly growing cells and in glands in which enzymes are being produced. Cells involved in activities that do not include protein synthesis contain very small quantities of RNA. Ribonuclease, an enzyme that inactivates RNA, inhibits protein synthesis.

The relationship between the genes, RNA, and protein synthesis is now apparent. The DNA in the genes controls the production of a specific template or messenger RNA which is sent out to the cytoplasm (to the ribosomes) to direct specific protein synthesis. The template RNA molecules are produced from DNA molecules by specific base pairings just as in DNA synthesis. The amino acids are carried to the high molecular weight ribosomal template RNA molecules by low molecular weight transfer RNA molecules.

Protein synthesis appears to occur mostly in the small particle (ribosome) fraction of the cytoplasm. There have been reports of protein

synthesis by nuclei (Allfrey, Mirsky, and Osawa, 1957) and in Azobacter a protein synthesis reaction seems to occur in a system other than the ribosomes (Beljanski and Ochoa, 1958) but in higher organisms the synthesis of protein seems to take place mainly in the ribosomes. Ribosomes appear as uniform, round, electron-dense particles having an average diameter of 100 to 200 Angstroms. The ribosomes contain 80 to 90% of the total cellular RNA (Hoagland, 1955). They exist apparently free in the cytoplasm in some tissues (notably bacteria and rapidly growing mammalian cells), perhaps sometimes attached to the limiting membrane of the cell (bacteria), but usually are found in association with lipoprotein-rich membranous material of the cytoplasm. The ribosomes, in natural association with this membrane-like material comprise what has been variously named the cytoplasmic ground substance, ergastroplasm, or endoplasmic reticulum by electron microscopists. The individual molecules of template RNA on the ribosome may be bound to each other and to the particle protein by magnesium ions and base interaction (Hoagland, 1955).

The best demonstration that RNA is the template molecule for protein synthesis is the ability of tobacco mosaic virus RNA to direct the formation of a specific protein and of the RNA from certain animal viruses to infect and lead to the production of complete virus-containing protein (Sprunt et al., 1959). There are many other experiments which show that RNA formation must accompany protein formation. For example, although thymine-requiring bacteria can still form induced enzymes in the absence of growth factor (Cohen and Barner, 1955), adenine- or uracil-requiring bacteria cannot. Inhibition of bacteria with the RNA analogue 5-hydroxyuridine inhibits formation of beta- galactosidase (Spiegelman, et al., 1959).

1955). Although protein formation depends on RNA synthesis, the synthesis on RNA can occur in the absence of protein synthesis.

The direct relationship between DNA and RNA has been demonstrated by experiments with cell-free systems. The enzyme RNA polymerase, when placed in a cell-free medium containing the four nucleotide units of RNA, fails to induce RNA synthesis even when RNA is added as a primer. Addition of a small quantity of DNA, however, results in rapid synthesis of RNA. Thus it is actually DNA that stimulates RNA synthesis. It is apparent that the four nitrogenous bases appear in the synthetic RNA in the same proportions as they occur in the DNA molecule used as a primer, with uracil substituted for thymine. Further evidence of the specificity of the reaction is obtained by using as primers two synthetic DNA polymers, one containing only thymine (poly-T) and one containing alternate units of adenine and thymine (AT copolymer). With poly-T as the DNA primer, the resulting RNA contains only thymine's regular partner, adenine. With AT copolymer as the primer, the RNA consists of a copolymer of uracil and adenine in perfectly alternating sequence. This specific incorporation takes place even though all four bases are present during the reaction (Hurwitz and Furth, 1962). The control over RNA synthesis by DNA is also seen in living cells. When bacterial viruses infect bacteria, an RNA is formed that resembles the virus DNA and not that of the host in its base composition (Volkin, Astrachan, and Countryman, 1958).

One can conjecture that the double helix of the DNA molecule partially unwinds in the course of the reaction so that ribonucleotides (in triphosphate form and with the aid of the enzyme RNA polymerase) can pair off against the nucleotides in one strand of the DNA. After the comple-

mentary pairing has taken place, two of the phosphate groups depart, allowing the ribonucleotides to link up into an RNA polymer. The RNA then pulls away from the DNA matrix, and the DNA reverts to its previous double-stranded form.

The idea of a messenger RNA sent out from the genes to the ribosomes has been confirmed by experiments with T4 virus. Colon bacilli were grown on substances containing heavy isotopes of nitrogen and carbon. The cells were then infected with T4 virus and simultaneously transferred to a medium containing ordinary nitrogen and carbon. Immediately after infection the cells were exposed briefly to radioactive phosphorus to label the messenger RNA produced by the virus DNA. After centrifugation, the two ribosome fractions were examined for radioactive messenger RNA. It was found that only the heavy ribosomes (those present before infection) were radioactive (Brenner, Jacob, and Meselson, 1961). Therefore, no new ribosomes were synthesized after infection. Ribosomes, then, are only machines responsible for synthesizing protein. Not until the machine has been supplied with the proper instructions, supplied by messenger RNA, can a given protein be synthesized.

Developments in understanding the cell-free system illuminate another, and more direct, participation of RNA in protein synthesis. It is known that the soluble, nonparticulate cell fraction (pH 5 fraction), found necessary for the overall incorporation of amino acids into ribosome protein, contains enzymes that catalyze the carboxyl activation of amino acids by formation of enzyme-bound amino acyl adenylate compounds from ATP (adenosine triphosphate) and amino acids. It is found that this same species of RNA is uniquely capable of reaction with amino acids in a highly specific manner. The amino acid activating enzymes not only

catalyze the activation of amino acids, but also attach the amino acid thus activated to the terminal nucleotide of specific, soluble, low molecular weight RNA molecules. These molecules, once charged with amino acid, are able to transfer the amino acid to the protein of the ribonucleoprotein particles. Ultracentrifugal and electrophoretic analyses of transfer RNA (sRNA) preparations indicated that they are heterogeneous. It is apparent that sRNA must consist of a number of distinct species since amino acids are attached to sRNA additively and terminally.

A characteristic of transfer RNA's is their content of a relatively high proportion of unusual bases, particulary pseudo-uridine, of which one or two molecules per chain are present (Dunn, Smith, and Spahr, 1960). The amino acid-carrying terminal is uniformly cytidilic, cytidilic, adenylic acid in all 20 amino acid-specific sRNA's, while the phosphate terminal of the chain appears to be uniformly guanylic acid. The molecular weight of transfer RNA is, with little variation, 20,000-30,000, corresponding to 65-85 nucleotides. In comparison, the high molecular weight template RNA molecules have a molecular weight of 0.5-1 x 10^6 (Lipman, 1960).

Protein synthesis is often measured by the incorporation of radioactive amino acids into the ribosome fraction. The complete system contains a preparation of ribosomes, two nucleotides (ATP and guanosine di- or tri-phosphate), pH 5 enzyme, salts, and medium of the proper concentration. Although many other systems have been used, this system seems to be giving the best results.

There is evidence that the incorporation of amino acids into the ribosomes represents true protein synthesis even though no net increase in the protein is observed. The incorporated amino acids are linked

by peptide bonds. Schweet et al. (1958) have studied the incorporation of labelled amino acids into the ribosomes of reticulocytes. The amino acids are incorporated into protein in a ratio characteristic of the composition of hemoglobin, the protein produced by these cells in vivo, and are not incorporated in the ratio of the total amino acids in the ribosome protein. This indicates that a specific protein is being formed by the system. It is important to show that amino acid incorporation really represents protein synthesis since, in some systems, it was thought that the process of amino acid incorporation could be dissociated from true protein synthesis although the processes were related.

The first process in protein synthesis consists of the activation of amino acids. The process of peptide formation from free amino acids is not spontaneous and the amino acids must therefore be converted to compounds with a higher chemical potential. The lengthening of peptide chains itself does not necessarily require large amounts of energy and can occur by transpeptidation. This process depends on a supply of peptides and can be catalyzed by the proteolytic enzymes. It is not known whether it has any physiological function. Since the amino acids contain both amino and carboxyl groups and since both types of groups can be activated, it was not obvious how the activation would take place.

The evidence now is that activation occurs by reaction of the carboxyl groups of the amino acids. This was suspected because of the behavior of systems that form simple peptides and could therefore be considered as models for protein synthesis. Such systems are the synthesis of pantoic acid, the acetylation of sulfonilamide, and the synthesis of the tri-peptide glutathione. In all these syntheses it is the carboxyl group which is activated, either by combination with coenzyme A or by

the formation of an adenylic acid derivative. At present it is believed that the initial reaction of protein synthesis is the combination of the constituent amino acids with ATP to form an adenylic acid derivative of the amino acids in which the carboxyl group of the amino acid is combined with the adenyl portion of the ATP.

In the presence of enzyme and amino acid, phosphorus-32 labelled ATP will exchange with non-labelled pyrophosphate. One evidence that this reaction is involved in protein synthesis is the demonstration that all 20 of the common amino acids can be activated (Lipman, 1958); another is the behavior of certain analogues of trypophan. Certain analogues of the amino acids are actually incorporated into protein. Sharon and Lipman (1957) demonstrated that those tryptophan analogues which were incorporated into protein were activated by the tryptophan-activating enzyme. Those analogues which were not incorporated were not activated but instead inhibited enzyme action. This correlation indicates that the activation reaction is a part of the protein synthesis mechanism. Activation occurs in the soluble or supernatant fraction of the cell.

Activating enzymes are specific; that is, each amino acid is activated by its own single specific enzyme. Several relatively pure activating enzymes have now been isolated and the work continues. The fact that amino acids do not compete for enzyme sites indicates that in all likelihood the remaining enzymes will be located. There is general agreement that activation is entirely independent of RNA. This statement is based on the fact that ribonuclease does not affect the activation of amino acids by crude or purified activating enzyme preparations; and that the more highly purified activating enzymes do not contain measurable RNA and are fully active in the absence of RNA. The activated

amino acyl adenylate intermediate is very firmly bound to its specific enzyme and dissociates to only a very small extent in the absence of an acceptor for the amino acid. Thus the activating enzyme and its bound amino acyl adenylate can be thought of as acting effectively as a single unit. The transfer of amino acid to sRNA is mediated directly by the activating enzyme without the involvement of other enzymes.

In the second phase of protein synthesis, the amino acid becomes attached in an energy-rich ester bond to the terminal adenosine of the corresponding sRNA (Leahy, Glassman, and Schweet, 1960). This RNA must have built into its nucleotide sequence a specificity for the particular amino acid to which it becomes attached on the particular activating enzyme. It is important that the reactivity of an sRNA with its corresponding amino acid varies with enzymes from different species. The specificity of transfer RNA for a particular amino acid, expressed by its affinity to the activating enzyme, is therefore not homogeneous for all living organisms. It is now well known that the amino acid is joined in ester linkage on the sRNA to one of the adjacent free hydroxyl groups of ribose of a terminal adenosine of sRNA.

The third step in the synthesis of proteins is the transfer of the amino acid from the sRNA to the template RNA of the ribosome. It has been demonstrated with isolated RNA-amino acid complexes that guanosine triphosphate is essential for this transfer reaction. The transfer RNA molecule combines with its complementary sequence of nucleotides in the template RNA molecule. The fact that sRNA appears to remain intact and reacts in a cyclic, coenzymatic fashion, deserves mention. A very broad compatibility is seen for incorporation into protein on the ribosome in contrast to the species-limited sRNA specificity for activation enzymes.

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A species-independence in this last phase indicates a universal amino acid code throughout the organismic world. The amino acid-specific RNA, therefore, seems to carry two apparently separate specificities: (1) for the activating enzyme, and (2) for position on the ribosome. Work with the electron microscope has provided a direct and remarkable view of ribosomes in the act of synthesizing the protein hemoglobin. It appears that several ribosome particles are linked together at the time of synthesis, probably by messenger RNA.(Warner, Knopf, and Rich, 1963). It has been proposed that the ribosome attaches to the messenger RNA at one end and travels across to the other end. In the process, the ribosome synthesizes hemoglobin according to the code it reads off the RNA molecule.

The coding problem can be explicitly stated as the problem of how the sequence of the four bases in RNA determines the sequence of the 20 amino acids in the protein. The problem has two aspects, one general and one specific. Specifically one would like to know just what sequence of bases codes each amino acid. The more general aspect of the coding problem has to do with the length of the genetic coding units, the way they are arranged in the DNA molecule, and the way in which the message is readout.

The simplest sort of code would be one in which a small group of bases stands for one particular acid. This group can scarcely be a pair, since this would yield only 16 possibilities, and at least 20 are needed. More likely the shortest code group is a triplet, which would provide 64 possibilities. A group of bases that codes one amino acid is called a codon. Early coding schemes suggested that adjacent codons overlap. One consequence of such a code is that only certain amino acids can

follow others. Another consequence is that a change in a single base leads to a change in three adjacent amino acids. Recent evidence makes an overlapping code seem unlikely. In the first place, there seems to be no restriction of amino acid sequence in any of the proteins so far examined. It has also been shown that typical mutations change only a single amino acid in the polypeptide chain of a protein. Although it is theoretically possible that the genetic code may be partly overlapping, it is more likely that adjacent codons do not overlap at all.

Since the backbone of the DNA molecule is completely regular, there is nothing to mark off the code into groups. To solve this difficulty, various ingenious solutions have been proposed. It was thought, for example, that the code might be designed in such a way that if the wrong set of triplets were chosen, the message would always be complete nonsense and no protein would be produced. But it now looks as though the message begins at a fixed starting point, probably one end of the gene, and is simply read three bases at a time. If the reading started at the wrong point, the message would fall into wrong sets of three and would then be hopelessly incorrect.

If this idea were correct, it would immediately explain why the addition or deletion of a base in most parts of the gene would make the gene completely non-functional, since the reading of the genetic message from that point onward would be totally incorrect. In experiments with T4 bacteriophage, single mutations were always without function although, if certain pairs of them were put together, the gene would work. If a mutation causing deletion of a base and one causing addition of a base occur close to one another on the DNA molecule, the gene will still be functional. If the addition and deletion are too far apart, the combin-

ation will not work.

To understand this, the code must again be referred to. There are 64 possible triplets but only 20 amino acids to be coded. Conceivably two or more triplets may stand for each amino acid. On the other hand, it is reasonable to expect that at least one or two triplets may represent some other meaning, such as a signal to start or to stop protein formation. Although such hypothetical triplets may have a meaning of some sort, they have been named nonsense triplets. It is possible that the misreading produced in a region lying between an addition and a deletion might give rise to a nonsense triplet, in which case the gene might not work. Investigations with a number of addition-with-deletion combinations in which the intervening distance was relatively short have shown that these combinations were indeed inactive when they might be expected to function. Presumably an intervening nonsense triplet is to blame. In confirmation of this hypothesis, it has been possible to modify such nonsense triplets by mutagens that turn one base into another, thereby restoring the gene's activity. At the same time it has been possible to locate the position of nonsense triplet. It is probable that the message is read from a fixed point, most likely one end of the gene.

So far, all the evidence has fitted very well into the general idea that the message is read off in groups of three, starting at one end. The same results should have been obtained, however, if the message had been read off in groups of four, or indeed in groups of any size. To test this, three addition mutations were put fairly close together in one gene. Taken either singly or in pairs, these mutations will destroy the function of the gene. But when all three are placed in the same gene, the function reappears. The same results have been obtained by using three base dele-

tions (Crick, 1962). The explanation is obvious. One addition will put the reading out of phase. A second addition will give the other wrong reading. But if the code is a triplet code, a third addition will bring the message back in phase, and from then on to the end it will be read correctly. Although the most likely explanation is that the message is read three bases at a time, this is not completely certain. The reading could be in multiples of three.

The field of cellular protein synthesis and the inseparable field of nucleic acid chemistry will continue to be widely studied and new information will certainly be discovered at an increasingly rapid pace. At the present time, the coding problem is probably drawing the most interest. Discovery of the codes for specific amino acids will allow experimentation with alternation, by chemical means, of genetically inherited characteristics. A problem in mapping amino acid codes is the difficulty in nucleic acid degradation. This difficulty could possibly be alleviated by separation of different pure DNAs. Chromatographic methods occupy a rather unique position among recent developments in the field of separation methods. The term chromatography now covers several highly efficient laboratory techniques: adsorption, partition, ion exchange, molecular sieve, gas, and electrochromatography.

For my experiments with DNA, I used ion exchange columns. The principle of ion exchange chromatography is based on the common phenomenon of a solid material exchanging ions with a solution. To be useful as an ion exchanger, a material must be ionic in nature and at the same time highly permeable. Synthetic ion exchangers are, therefore, cross-linked organic and inorganic polyelectrolytes. Ion exchange resins are elastic gels that absorb

water and other polar solvents and in doing so swell considerably. The interior of a resin bead in some ways resembles a drop of concentrated electrolyte solution. The properties of the polymer network, including its low dielectric constant, affect the ion exchange behavior, and, in addition, the polymer acts to a certain extent as a solvent or adsorbent for certain compounds.

Ion exchange columns are usually operated by elution development. In elution chromatography, the substance to be separated is first sorbed on a narrow band of resin at the top of the column. Ions of this substance are then eluted, or carried down the column, by a second species of ions which competes for bonds on the resin. Separation is accomplished by varying the concentration of the competing ions. Fractions are collected at the bottom of the column.

The application of ion exchange elution chromatography to analytical and preparative problems in the nucleic acid field directly followed the invention of the technique and its application to inorganic cations (Tompkins, Khym, and Cohn, 1947) and was actually the way in which true elution chromatography by exchange was introduced into biochemistry. The complexes between the polyvalent inorganic cations and the polybasic organic acids that performed so spectacularly on the first test of ion exchange chromatography may be considered as amphoteric, inasmuch as the degree and kind of charge may be varied continuously over a wide range by pH adjustment. Since the nucleic acids are also amphoteric, it was apparent that the principle of charge control by pH control could be directly applied to them without the necessity of adding complexing ions. It was also apparent that separations by

either anion or cation exchange were feasible. The first application of ion exchange chromatography to the nucleic acid field (Cohn, 1949) touched off a series of discoveries of new nucleotides, both those derived from nucleic acid structure, as well as those that exist free in nature. It also made available a chemical technique of broad usefulness in a wide variety of analytical and preparation problems.

In my experiments, I sought to effect a separation of DNA, by ammonium hydroxide elution, on an ion exchange column. The column was prepared by pouring a slurry of Dowex-50, a commercial cation-type exchange resin, into a buret plugged at the bottom by a wad of glass wool. The column was washed with distilled water to assure a neutral pH. A small quantity of DNA, in solution, was administered to the top of the column. To accomplish elution, increasing concentrations of ammonium hydroxide were added to the top of the column. Fractions were collected at the bottom of the column (elution rate = 2 ml./min.) in weighed sample bottles. After the contained solution was evaporated in an oven, each sample bottle was weighed and the mass of the DNA was computed. After each sample was eluted, the column was regenerated by passing through dilute hydrochloric acid and then rinsing with distilled water until a neutral pH was obtained.

For the first column, approximately 0.3 g. of commercially-prepared DNA was dissolved by heating in a 0.05 M. solution of NH_4OH and sorbed on the column (25 mm.). The following schedule of elution concentrations was used: fractions 1-4, 0.05 M. NH_4OH; fraction 5, 0.10 M.; fraction 6, 0.20 M.; fractions 7-10, 0.50 M.; fractions 11-13, 1.00 M.; fractions 14-15, 2.00 M.; fraction 16,

14.80 M. Seventeen ml. fractions were taken and the following residues were obtained:

fraction 1 - 0.1238 g.
2 - 0.0601
3 - 0.0038
4 - 0.0037
5 - 0.0032
6 - 0.0027
7 - 0.0037
8 - 0.0063
9 - 0.0039
10 - 0.0017
11 - 0.0031
12 - 0.0245
13 - 0.0047
14 - 0.0037
15 - 0.0012
16 - 0.0023

For the next column, 0.1 g. of DNA was dissolved by heating in 0.02 M. NH_4OH and sorbed on the column (35 mm.). In an attempt to resolve the large initial DNA residue (peak) obtained in the first column into possible component peaks, the following elution schedule was used: fractions 1-3, 0.02 M. NH_4OH; fractions 4-6, 0.05 M.; fraction 7, 0.10 M.; fraction 8, 0.20 M.; fractions 9-11, 0.50 M.; fractions 12-14, 1.00 M.; fractions 15-16, 2.00 M.; fractions 17-18, 14.80 M. Seventeen ml. fractions were collected and the following residues were obtained:

fraction 1 - 0.0641 g.
2 - 0.0035
3 - 0.0015
4 - 0.0009
5 - 0.0010
6 - 0.0010
7 - 0.0006
8 - 0.0006
9 - 0.0013
10 - 0.0015
11 - 0.0018
12 - 0.0019
13 - 0.0025
14 - 0.0025
15 - 0.0085

fraction 16 - 0.0033 g.
17 - 0.0013
18 - 0.0014

For the third column, 0.1 g. of DNA was dissolved in hot water and sorbed on the column (40 mm.). In still another attempt to resolve the initial peak into possible component peaks, the following elution schedule was used: fractions 1-6, 0.02 M. NH_4OH; fractions 7-9, 0.05 M.; fractions 10-12, 0.50 M.; fractions 13-16, 1.00 M.; fractions 17-18, 2.00 M.; fractions 19-22, 7.40 M. Fractions of 6 ml. were collected and the following residues were obtained:

fraction 1 - 0.0019 g.
2 - 0.0028
3 - 0.0424
4 - 0.0022
5 - 0.0009
6 - 0.0013
7 - 0.0008
8 - 0.0006
9 - 0.0010
10 - 0.0007
11 - 0.0005
12 - 0.0009
13 - 0.0010
14 - 0.0016
15 - 0.0015
16 - 0.0016
17 - 0.0023
18 - 0.0019
19 - 0.0016
20 - 0.0012
21 - 0.0002
22 - 0.0000

In all three columns, two apparent peaks are noted. A large peak is present at very low NH_4OH concentrations and a smaller peak is present at 1.00 M. NH_4OH elution. There are no other noticeable or consistent peaks present. The small fluctuations present in the residues from the third column are probably due to the small fractions. The two peaks represent DNAs of different chemical properties

and, with different elution schedules, could possibly be broken down into smaller peaks.

Separation of DNAs by chromatography has been previously accomplished by various means but, because buffer solutions are usually used, the DNA fractions are mixed with salt residues which make base sequence determination be degradation difficult. DNA separation by ammonium hydroxide elution has definite applications because, after evaporation, collected fractions contain only DNA, on which degradation experiments can be performed.

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