deviation from ideality became obvious at a virus concentration in the cell of about 0.3 percent and higher. Although the pattern of apparent deviation from ideality was similar in tilted and untilted cells, the fringe number at the equilibrium speed yielded a lower molecular weight with the untilted cell in the comparison runs E and F.

The molecular weights obtained in runs A and B (tilted cells) are probably quite close to the true particle weight of these rod samples. The modified equation, however, includes an approximation, due to the tilting which has, as an upper limit, an error of $\pm$ 1 percent. The uncertainty in the determination of c_0 is of this same order, while that from the determination of $\bar{v}$ is not yet well defined. The precision of the data from the magnetic ultracentrifuge for identical runs on small molecules is $\pm$ 0.5 percent. Assuming that the s_{20} value for these rods at 0.1 percent concentration is accurate, introduction of the mean molecular weight from experiments A through D (41.6×10^6) into the Svedberg equation yields a diffusion coefficient at this concentration of 0.403×10^{-7} cm^2/sec. This value is in good agreement with that determined on these same rods by free diffusion experiments (*10*).

From these preliminary studies it appears possible to define in a thermodynamically satisfactory manner the activity of large rods and to adduce shape information from a few experiments. For this purpose a more precise definition of components as well as other refinements may be desirable (*11*).

F. N. WEBER, JR., R. M. ELTON
H. G. KIM, R. D. ROSE
Department of Physics, University of Virginia, Charlottesville
R. L. STEERE
Plant Virology Laboratory, U.S. Department of Agriculture, Beltsville, Maryland
D. W. KUPKE
Department of Biochemistry, School of Medicine, University of Virginia

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13 May 1963

Molecular Model for Protein Synthesis

Abstract. *Molecular models constructed for RNA, adapter RNA, and the ribosome are consistent with available physical-chemical data. These components can be assembled to produce a stereochemically sound model for the template mechanism in protein synthesis.*

The arrangement of amino acids in proteins is one of nature's greatest complexities (*1*). Yet man has, within the short span of a decade, almost completely solved its mystery. The theory of how amino acids are ordered was proposed by Crick (*2*), and is shown in Fig. 1. Amino acids are linked to specific adapter-RNA molecules; these become transferred to the ribosome, where the adapter RNA's are joined by hydrogen bonds to complementary sites on an RNA template—called messenger RNA—and the amino acids are placed in the proper juxtaposition for peptide synthesis. The latter is a sequential process, proceeding from the N-terminal to the C-terminal amino acid (*3*).

A great deal is known about the molecular structures of adapter RNA, messenger RNA, and the ribosome. As a result it has been possible to consider more constructively the exact nature of the template mechanism for protein synthesis (*4*). First let us consider the molecular structure of adapter RNA.

The x-ray diffraction pattern of total adapter RNA bears a strong resemblance to the diffraction pattern given by DNA (*5*). The sharpness of the main equatorial reflection indicates a regularity in packing between molecules paralleled only by the best samples of DNA which have been studied. The orientation observed for the equatorial 23 Å and meridional 3.3 Å spacing strongly confirm this double-helical nature. It has been argued by Doty *et al.* (*6*) that hyperchromasy may be taken as evidence for the existence of a helical secondary structure for polynucleotides in solution. Such studies on adapter RNA suggest that it maintains its double-helical configuration in solution since on heating from room temperature in physiological saline, the optical density of adapter RNA rises gradually until there has been an increase of about 30 percent. A regular double helix of high molecular weight, like DNA, does not increase in optical density until a temperature of about 80°C is reached, at which point it increases abruptly by about 40 percent. The smaller and more gradual increase in hyperchromasy shown by adapter RNA might be caused by irregularities in the base pairing. Alternatively, it might be the result of its short double helix, which, even if perfectly regular, would include only about 30 base pairs.

Recently, Spencer and his co-workers (*7*) succeeded in crystallizing a soluble RNA fraction from yeast, which they believe to be the adapter RNA. The regularity of the x-ray diffraction patterns of such material has led them to propose that adapter RNA from yeast has completely regular Watson-Crick type base pairing. But small irregularities in base pairing would be very difficult to detect from such preliminary observations, and we believe that the exact nature of adapter RNA base pairing is still uncertain.

In solution, the molecular weight of adapter RNA determined by ultracen-

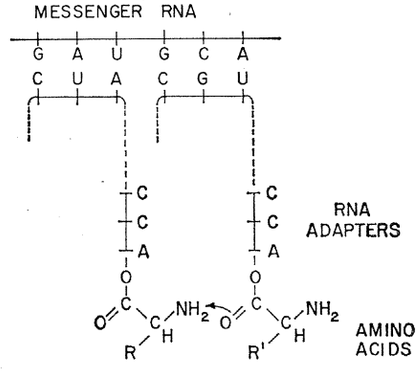

Fig. 1. The function of adapter RNA in protein synthesis. Amino acids are linked to specific adapter RNA's. These become transferred to the ribosome, where the adapter RNA's form hydrogen bonds at complementary sites on the template, placing the amino acids in the proper juxtaposition for peptide synthesis.

trifugation techniques is about 24,000, indicating a chain length of about 67 nucleotides. There is correspondingly one nucleosidic terminal group for each 24,000 molecular-weight units. This led us to suggest (*5*), about 3 years ago, that the structure consists of a single polynucleotide chain with a bend approximately in the middle; the two halves of the chain are wound around each other in a helical manner. In this model for adapter RNA, the two halves of the chain are situated so that hydrogen bonding between 6-keto and 6-amino bases could take place, as in DNA. The two halves are each only about 30 nucleotides long—about three turns of a double helix.

These and other known properties of adapter RNA have been incorporated into the model illustrated in Fig. 2 (left). All adapter RNA's have the terminal sequence: amino acid-adenylic acid-cytidylic acid-cytidylic acid on one end, and guanine on the other (*8*). Enzymatic degradation experiments indicate that the A-C (adenine-cytosine) terminal grouping is more susceptible to attack than the rest of the molecule (*9*), suggesting that this terminal grouping may be exposed, as it would be if it were unpaired and not hydrogen bonded.

With two free nucleotides on the amino acid end, the third nucleotide, which is a C, could make a base pair of the Watson-Crick type with the G (guanine) from the other end of the adapter RNA. In making the proposed bend in the middle of the adapter molecule, a minimum of three nucleotide residues must be placed in the unpaired state, as first pointed out by Fresco, Alberts, and Doty (*10*). These structural features can be correlated with the known function of adapter RNA in protein synthesis. The three unpaired nucleotides in the bend could conveniently serve as the coding trinucleotide sequence which is presumed to react by specific base pairing with the messenger RNA template. The unpaired A-C grouping on the end, carrying the amino acid, may give amino acids on adjacently adsorbed adapter RNA's sufficient freedom of movement to make contact.

Figure 2*b* is a space-filling model of Fig. 2*a*. Only one of the three turns of double helix is shown. The model was constructed with Courtaulds atomic models which have nonbonding van der Waals radii accurate to about ± 0.1 Å. A plastic collar is supplied with these

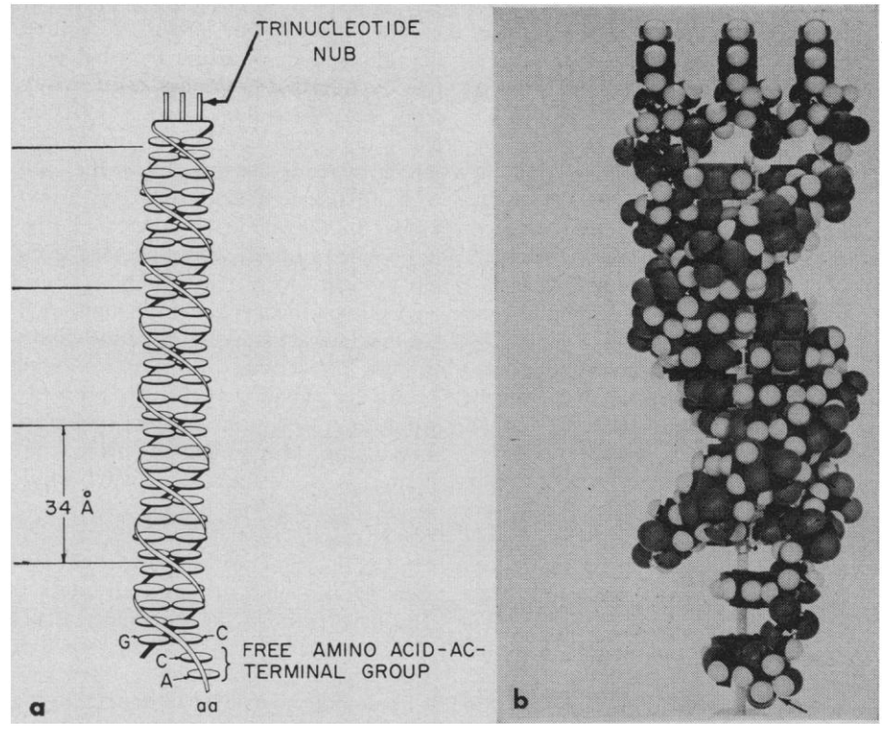

Fig. 2. (*a*) Proposed structure for adapter RNA. A single polynucleotide chain interacts with itself by forming a bend near the middle. Hydrogen bonding takes place between the nucleotides from the two halves of the chain and results in the formation of a double-helix structure. In the bend, three bases are of necessity unpaired. The guanine from one end of the chain forms a base pair of the Watson-Crick type with the cytosine third from the other end. This leaves two nucleotides on the end bearing the amino acid in the flexible state. The molecule is about 100 Å long and 20 Å wide. (*b*) A space-filling molecular model. Only one of the three turns of double helix are shown. Position for attachment of amino acid is indicated by arrow.

models which restricts the degree of bond-angle distortion between covalently linked atoms to 6 degrees from normal. These were used in construction, but they were removed before photography for purely asthetic reasons. For convenience, and from ignorance, all of the bases in the double-helix portion of the structure are represented by base pairs of the Watson-Crick type. The fact that this structure can be built from accurate atomic models, with bond-angle restrictions, is strong evidence that such a structure is possible.

The template for protein synthesis, called messenger RNA, is separable from the ribosome (*11, 12*). We shall assume that messenger RNA, when active, exists in the form of a single polynucleotide chain with no intra- or intermolecular hydrogen bonding—except, of course, that made with adapter RNA's.

With these structures of adapter RNA and messenger RNA in mind, we can now consider a detailed stereochemical model for their interaction during protein synthesis. There is experimental evidence that in such interaction Watson-Crick base pairing occurs. Thus, hypoxanthine can form the same pair of hydrogen bonds with cytosine as does guanine, and it can replace guanine in synthetic messenger RNA. Uracil can be partially replaced by 5-fluorouracil, but not at all by *N*-methyl uracil (*13*). Only the latter compound has one of its hydrogen bonding sites blocked. Ordinarily, Watson-Crick base pairing results in double-helix formation between the interacting polynucleotides, but we fail to see how such a configuration could be a useful intermediate in protein synthesis. An extended search with atomic models led us to propose that base pairing takes place in the manner indicated in Figs. 3*a* and 3*b* (*4*). A template structure similar to this has been independently proposed by Zamecnik (*14*). In this model the bases of the two molecules interact with the polynucleotide chain in the completely extended configuration producing a hydrogen-bonded step-ladder-like structure analogous to the extended beta configuration in proteins.

This is accomplished by orienting the polynucleotide chain of the messenger RNA in such a way that all the bases are situated on one side, and the phosphate groups on the other, with a residue-repeating distance of 6.8 Å along the chain. The three alleged coding nucleotides in the bend of the adapter RNA quite naturally adopt a similar extended configuration. The translational distance per three nucleotides for extended messenger RNA would be 20.4 Å, which is very nearly the distance of close approach of adjacent adapter RNA double helices. Amino acids on adjacent adapter molecules could not interact unless provided with a flexible extension. The postulated A-C terminal grouping of the RNA provides the minimum number of unpaired nucleotides to give the necessary reach.

The sequence of nucleotides in a synthetic messenger RNA has been related to the sequence of amino acids in the product polypeptide chain. Coupling this information with the fact that peptide synthesis proceeds sequentially from the N-terminal portion (*3*) has led to the conclusion that the messenger RNA is read, starting from the end with an unesterified 3′-hydroxyl group (*15*). In the model illustrated in Fig. 3, peptide synthesis would proceed from left to right.

Although the stepladder configuration for two interacting polynucleotide chains implies no serious distortion of bond angles or bond lengths, it probably would not form in aqueous solution, where the normal double-helix configuration is possible. The greater stability of the latter with the same hydrogen bonds results from the fact that adjacent base pairs are close-packed and their hydrophobic surfaces can interact. The base pairs in the stepladder structure are separated by molecular cavities 3.4 Å wide, which could be filled with a monomolecular layer of water. In the case of the template we are considering, double-helical interaction would not be possible because the coding nucleotides on the adapter are rigidly held in the extended chain configuration. Presumably the interacting portion of the messenger RNA molecule is also, in some way, held in this state. The above stepladder structure suggests, at least in part, the role of the ribosome in protein synthesis.

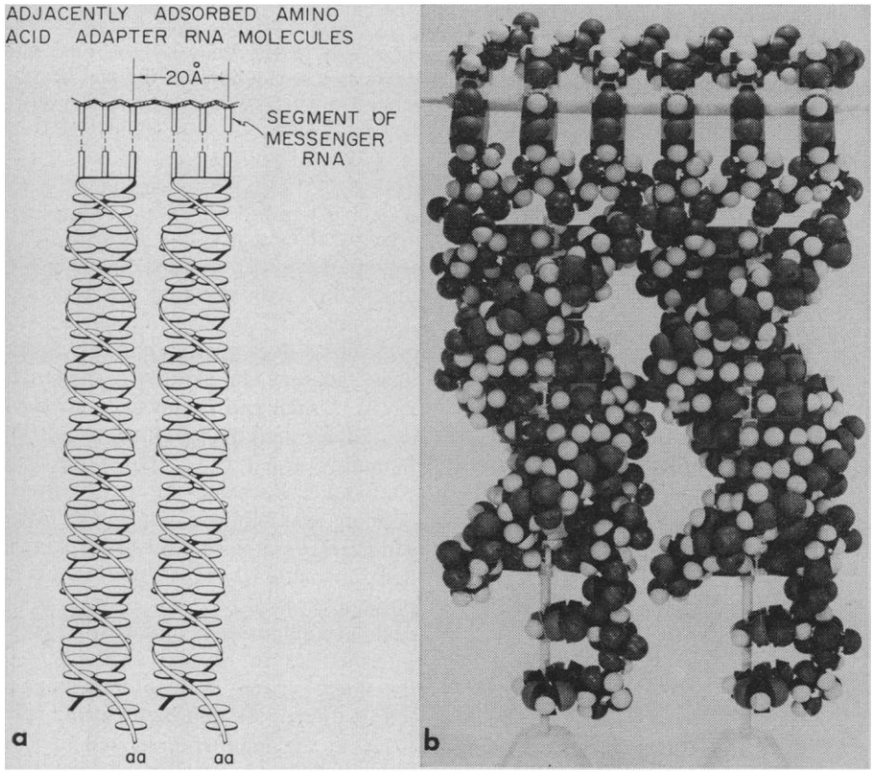

Fig. 3. (*a*) Proposed structure for adapter-RNA–messenger-RNA complex. The polynucleotide chain of the messenger RNA is in the completely extended configuration with the bases situated on one side of the chain and the phosphate groups on the other. The residue-repeating distance is 6.8 Å, making the coding triplet-repeating distance 20.4 Å. The three alleged coding nucleotides in the adapter RNA adopt a similar extended configuration. The bases from the two polynucleotides interact, producing a hydrogen bonded stepladder-like structure. Adjacently adsorbed adapter RNA's are nearly close-packed. The necessary "reach" between amino acids is provided by the flexible A-C terminal group. (*b*) A space-filling molecular model. The adapters have been foreshortened as in Fig. 2 (right). Amino acids are not shown.

The specific interaction between adapter RNA and messenger RNA, leading to peptide synthesis, only takes place when this system is in contact with the ribosome (*12*). Present indications are that ribosomes from a common source are alike, and that they must therefore also serve some nonspecific function common to the synthesis of all proteins. Ribosomes from most sources have, under some conditions, a single predominant species sedimenting in the range 70*S* to 85*S*. The 70*S* ribosomes from *Escherichia coli* are composed of two ribonucleoprotein subunits, a 30*S* and a 50*S* subunit, with molecular weights of 0.9×10^6 and 1.5×10^6 respectively (*16*). Each of the subunits contains by weight about two-thirds RNA and one-third protein. The 70*S* particle can dimerize to form a 100*S* particle in which the two 30*S* subunits are joined. Electronmicroscopy studies (*17*) indicate that the 100*S* ribosome has the approximate contour of a prolate ellipsoid with major and minor axes of 190 Å and 75 Å, respectively. In the region between the 50*S* and 30*S* subunits there is a cleft about 25 Å across. The interconnected 30*S* subunits have a wedge-shaped cavity on one side.

Both the 50*S* and the 30*S* particles are required in protein-simulated polypeptide synthesis in vitro (*4, 18, 19*). Which of the larger particles are active in protein synthesis is not yet decided (*19, 20*), but at present it seems likely that the 70*S* ribosome is the smallest potentially effective unit. This does not mean that the 100*S* particle could not synthesize protein, or that clusters of several 70*S* particles held together by a single polynucleotide chain of messenger RNA could not be equally functional (*21*).

Several other properties of the ribosome are of direct concern to our model. (i) Ribosomes in the process of protein synthesis do not dissociate as readily into their respective 50*S* and 30*S* subunits as do those ribosomes which are inactive (*18, 19*). Evidently

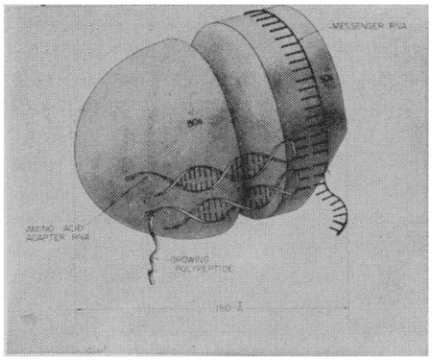

Fig. 4. Model of a 70*S* ribosome to demonstrate the role of the ribosome in protein synthesis. The ribosome provides a stabilizing surface for maintaining the messenger RNA in a configuration that would be thermodynamically unstable in solution and for maintaining the reacting adapter molecules in parallel register. Only part of the messenger is bound at one time. The dimensions of the ribosome were determined from electron microscopy.

there is something additional holding them together. (ii) Messenger RNA is dissociable from the ribosome, and ribosomes may associate with more than one messenger RNA with varying molecular weight (*11, 12*). (iii) The lifetime of the ribosome in bacteria is much greater than that of the messenger RNA (*22*). (iv) The 30*S* subunit and the 70*S* particle have been shown (*23*) to form a complex with synthetic messenger RNA under conditions where the 50*S* subunit does not. (v) The 30*S* subunit in *E. coli* contains a latent ribonuclease (*24*). (vi) In reticulocytes where the messenger RNA does not turn over, there is no evidence for a latent structural RNAse on the ribosome (*4*). (vii) Observations iii to vi support the idea that messenger RNA is bound to the 30*S* subunit.

The site of binding to the ribosome must allow the messenger RNA's of various size to be accommodated. In most cases, messenger RNA molecules would be too large for the entire molecule to be in direct contact with the ribosome simultaneously. Probably only part of the messenger is bound at one time, as is suggested in Fig. 4. Adjacently adsorbed adapters might, for greater stabilization, be adsorbed to the outer surface of the 50*S* particle, and the polypeptide synthesis would thus be more closely associated with the 50*S* ribosome. The function of the ribosome in this model is to provide a "stabilizing surface" for maintaining the messenger RNA in a configuration that would be thermodynamically unstable in solution and for maintaining the reacting adapter molecules in parallel register. The 50*S* and 30*S* subunits in the active particle would be more firmly held together than in the inactive 70*S* ribosome by virtue of the linked-adapter RNA which is connected to both the growing end of the polypeptide chain and the messenger RNA.

I have attempted to link structural and biochemical information into one coherent pattern, and to utilize the resulting knowledge in the production of a stereochemically sound model for the template mechanism in protein synthesis (*25*).

GEOFFREY ZUBAY
Biology Department, Brookhaven National Laboratory, Upton, New York

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Photosynthetic Mutants Separate Electron Paramagnetic Resonance Signals of Scenedesmus

Abstract. *Current schemes for the mechanism of the photosynthesis reaction imply a two-part cycle; one part produces a strong photoreductant, the other functions to release molecular oxygen. Wild-type* Scenedesmus *exhibits the two light-induced electron-paramagnetic resonances typical of green algae and chloroplasts of higher plants. These resonances indicate the presence of unpaired electrons in at least two sites. By means of mutants which are blocked in one or the other part of the dual cycle, the narrow, rapidly decaying signal can be correlated with the photoreductant part, and the broad, slowly decaying signal can be correlated with the part releasing molecular oxygen.*

Most current schemes for the photosynthesis reaction imply the cooperation of two more or less separate photochemical reactions. One of these is believed to produce a strong photoreductant while the other is functional in the release of molecular oxygen (*1*). Since in such systems there is necessarily the transfer of electrons, the participation of free radicals in the overall process is to be expected. The occurrence of photoinduced unpaired electrons has been amply demonstrated (*2–4*) by means of electron paramagnetic resonance (EPR) spectroscopy. At least two separate light-induced signals may be observed in algal species and chloroplasts; these signals may be differentiated on the basis of their kinetics, form, and position in the electromagnetic spectrum. There has been, however, no rigorous demonstration of a relationship between these free radicals and photosynthesis as it occurs in natural systems. We now present evidence that the narrow, rapidly decaying (*R*) signal, with a *g*-value (*5*) of 2.0025, is correlated with the CO_2-fixing portion of the photosynthetic cycle, and that the broad, structured, slowly decaying (*S*) signal is visible evidence of the events leading to O_2 production.

An almost complete separation of the two signals, heretofore observed only in combination in intact systems, has been achieved by use of two classes of photosynthetic mutants of the green alga *Scenedesmus obliquus*. The photochemical characteristics of one of these