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ON ULTRAVIOLET LIGHT AND THE ORIGIN OF RIBOSOMES

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Letter to the Editor

ON ULTRAVIOLET LIGHT AND THE ORIGIN OF RIBOSOMES

While there are many steps which remain to be elucidated, laboratory studies of the last decade and a half have made it plausible that amino acids, nucleoside phosphates and their respective polymers were present in some fair abundance in the eobiochemical soup, the aqueous medium in which life arose (Horowitz, Drake, Miller, Orgel, and Sagan, 1970; Calvin, 1969; Kenyon and Steinman, 1969). The question of the possible primitive interaction of these two categories of molecules in the absence of the contemporary genetic transcription apparatus has been raised in the context of studies on the origin of genetic code (Woese, 1965; Woese, 1967; Sagan, 1965). Despite the possibility that the contemporary code may be an evolutionary accident frozen into its present form because any significant deviations from it are deleterious, the direct steric interaction of amino acids with nucleic acids is nevertheless considered to be the best experimental approach to the problem of the origin of the code and has been incisively discussed in the literature (Crick, 1968).

The underlying assumption in such an approach is that the interactions between amino acids and nucleic acids leading to protein synthesis were not covalent but were, rather, "weak," involving van der Waals forces and hydrogen bonding, and were readily broken after peptide synthesis had occurred.

The importance of nucleoproteins in the earliest epochs of chemical evolution was raised many years ago (Sagan, 1957). The nucleopeptides which gave rise to ribosomes would have been those which catalyzed protein synthesis most effectively. The development of ribosomes from such nucleoproteins must have occurred via several stages of increasing complexity. The many functions (Nomura, et. al., 1969) of ribosomal component factors and enzymes currently known to be involved in protein synthesis must have been accrued gradually as complexity increased. The earliest adapter molecules would be expected to have arisen during the development of the ribosome and to have evolved their ability to assist the specificity and efficiency of the amino acid-codon interaction during protein synthesis at that time. Once components such as the adapter had arisen, further evolution of the ribosome towards optimum utilization of the entire protein synthesis apparatus can be expected. Thus, the earliest catalytically active nucleo-peptides must have undergone many changes before ribosomes appeared.

In the absence of ozone and molecular oxygen in the primitive reducing atmosphere, the upper surface of the Eobiochemical Soup was probably bathed in ultraviolet light in the 2400 to 2900 Å wavelength interval (Sagan, 1971); and since this is the wavelength region where purines and pyrimidines, as well as aromatic side groups of amino acids, strongly absorb, it has been suggested (Sagan, 1957) that ultraviolet light was instrumental in the synthesis of nucleic acids, polypeptides

and their interaction products in primitive times. A related suggestion (Sagan, 1957) that ultraviolet-induced excited states of purines and pyrimidines were instrumental in the origin of nucleoside phosphates has received some experimental confirmation (Sagan, 1965; Ponnamperuma, Mariner, and Sagan, 1963; Ponnamperuma, Sagan, and Mariner 1963). [Excited states of nucleotides and singlet energy transfer in polynucleotides have been studied (Guéron, Eisinger, and Shulman, 1967; Eisinger and Shulman, 1968)]. Once formed the high energy phosphates might have been general energy donors for polynucleotide (Ponnamperuma, Mariner, Sagan, 1963; Ponnamperuma, Sagan, Mariner, 1963) and polypeptide (Paecht-Horowitz, Berger, and Katchalsky, 1970; Rabinowitz, Flores, Krebsbach, and Rogers, 1969) synthesis, e.g., by amino acid activation.

Recently, an article appeared "On the Origin of the Genetic Code: Photochemical Interaction between Amino Acids and Nucleic Acids not Requiring Adapters" (Lesk, 1970). This article independently takes up the possible importance of U.V. light in the interaction of amino acids and polynucleotides. U.V. light, it is suggested, produced a photochemically specific combination of amino acids and nucleic acids prior to protein synthesis. However, the experiments referred to by Lesk (1970) indicate that the interaction between amino acids and nucleic acids caused by U.V. light is not weak or ionic (i.e., reversible), as would be required for a functioning primordial code, but that, instead, the interaction is covalent. The photochemically induced interactions of nucleic acids with amino acids

cited by Lesk do not in fact result in a reversible nucleic acid - amino acid interaction but rather in an irreversible link (Smith, 1968; Smith and Muen, 1968). The dihydrouracil - amino acid products isolated are bonded via the amino group side chain (Smith, 1969) or amino group at the α-position. Thus, at early times, amongst a plethora of polymeric compounds, there should have been a significant abundance of nucleoproteins. Such covalent bond formation between amino acids and nucleic acids is not likely to be of direct importance in understanding the origin of the genetic code. However, covalent bond formation of this sort does fulfill requirements for some of the early steps leading to the origin of ribosomes, by the following arguments.

Both nucleic acids and proteins are present in ribosomes. Transfer-RNA predominantly interacts with the larger subunit of the ribosome and the messenger-RNA interacts with both subunits probably at the interface of the two subunits. Stretches of nucleic acids in internal regions of the ribosome could loosely interact with transfer-RNA or messenger-RNA [triple stranded RNA complexes and "Hoogsteen" pairing are known (Davies, 1967, Hoogsteen, 1963; Blake, Massovlié, and Fresco, 1967)]. The 5S ribonucleic acids in several bacteria and humans have been sequenced and their secondary structure thought possibly to resemble the clover-leaf model for the structure of transfer-RNA's (Dubay and Weissman, 1971). Since this RNA is isolated from ribosomes the function of ribosomal RNA may be more than the simply structural role that is usually postulated. On the other hand the possibility

that ribosomal RNA is largely a molecular relic from eobiochemical times has also been raised (Sagan, 1971).

Why are proteins necessary? Ribosomal proteins may influence recognition efficiency (Davies, Gilbert, and Gorini, 1964) and may play several additional roles in ribosomal function. During the synthesis of a peptide bond, two or three GTP molecules are cleaved to GDP (Lengyel, 1969). There are indications that energy from these high energy phosphate bonds is utilized in binding the peptidyl-transfer-RNA to the ribosome; in transfer of the peptide from the peptidyl-transfer-RNA to the amino acid on the amino acyl-transfer-RNA, to form the new peptide bond; in movement of the enlarged peptidyl-transfer-RNA at the binding site to the empty peptide site after release of the now free transfer-RNA; in movement of the ribosome along the messenger-RNA codon by codon; and in facilitating the arrival of a new amino acyl-transfer-RNA (Lengyel, 1969). Since these energy transfers and motions in space, are all specific, it is most reasonable that ribosomal proteins acting in an allosteric capacity (Davies, Gilbert and Gorini, 1964; Shuman and Simpson, 1969; Hill, 1969) are responsible for these functions. Some experimental support for their specificity exists (Lengyel, 1969). For example, omission of one of the twenty proteins in reconstituted 30S ribosomal subunits leads to the loss of ribosomal function (Kurland, 1970; Nomura, et. al., 1969) in a reconstituted protein-synthesizing system.

The differences in sedimentation properties and RNA content of the ribosomes of chloroplasts, mitochondria, and prokaryotes

compared with eukaryotes [or even of sea urchin and mammalian ribosomes (Lehninger, 1970)] as well as the electrophoretic properties of the proteins (Lehninger, 1970) are suggestive of ribosomal evolution -- despite the fact that very little evolution in overall ribosomal architecture is apparent (Pinder, Gould, and Smith, 1969).

We propose that in eobiochemical times nucleoproteins were able to enhance the rate of protein synthesis, and that the bonding between the protein and nucleic acid components involved covalent, as well as the ionic and weak interactions mentioned above. Long timescales for reactions may have compensated for low efficiencies (Black, 1970) as has been discussed for enzymes.

The change from covalently bonded nucleoproteins in the earliest ribosomes to the contemporary ionic and weak interactions may lie in the evolutionary advantage of a relatively simple and general apparatus for protein synthesis. It would be more costly for cells to maintain a separate apparatus for the synthesis of all the other enzymes and proteins, none of which are linked to nucleic acids, than to maintain one general mechanism.*

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*There are contemporary cases of proteins to which low molecular weight molecules are covalently attached. But most prosthetic groups are not covalently bound to the polypeptide chain (Fruton and Simmonds, 1960). The majority of coenzymes

do not bind covalently, including nicotinamide adenine dinucleotide, flavin mononucleotide, flavin adenine dinucleotide, thiamin pyrophosphate (Shifrin and Kaplan, 1960) and pteridine derivative cofactors (Kaufman, 1967). Pyridoxal phosphate is reversibly bound to enzymes via an imine (Schiff base) link (Fischer, Kent, Snyder, and Krebs, 1958; Ivanov and Karpeisky, 1969). Biotin (Hofmann, 1940) and lipoic acid (Daigo and Reed, 1962; Schmidt, Graffen, Atland, and Werner Goedde, 1969) are covalently attached to enzymes. [However, one case has recently been found where biotin participates as a coenzyme in the free form (Stoll, Ryder, Edwards, and Lane, 1968)]. In addition to the biotin and lipoic acid enzymes the following three enzymes have been shown to contain covalently bound molecules: (active) phosphorylase a contains four inorganic phosphates per enzyme tetramer (Cori, 1945; Fischer and Krebs, 1955), (inactive) glucagon synthetase D contains inorganic phosphate molecules (Friedman and Larner, 1963), (inactive) glutamine synthetase b contains twelve adenosine monophosphate residues per enzyme molecule (Mecke, Wulff, Liess, and Holzer, 1966; Holzer, 1969). As noted in the relevant references, specific enzymes have been shown to attach the cofactors to the polypeptide chains. The biotin and lipoic acid may be remnants of the change-over mentioned above, since they participate in the enzymatic activity. However, the covalently attached molecules in phosphorylase, glucagon synthetase, and glutamine synthetase are only involved in the regulation of their enzymatic activities and may have been accrued since eobiochemical times.

Recently, evidence has been obtained that two of the thirty proteins of the 50S component of bacterial ribosomes copurify with RNA (Nomura and Erdman, 1970) even though there is no RNA associated with the other purified proteins. However, these are apparently not due to covalent links (Nomura, 1971).

In the primitive environment, the evolutionary and energetic cost of maintaining both covalent and weak and ionic bonding between macromolecules in primitive ribosomes would have been lower, because U.V. light was a means for the synthesis of covalent linkages between nucleic acids and amino acids or proteins. When U.V. radiation was no longer available, due to the transition from a reducing to an oxidizing atmosphere, it would have been much more costly to maintain a dual protein synthetic apparatus. At this time a single apparatus was selected to synthesize the enzymes, as well as ribosomes, in which the proteins and nucleic acids were associated by ionic and weak interactions. In this manner we anticipate contemporary ribosomes to have arisen.

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