PROPOSED TRAINING

(a) Characteristics of RNA coding units

A cell-free E. coli amino acid incorporating system dependent upon the addition of messenger or template RNA has been obtained (Nirenberg and Matthaei, Proc. Nat. Acad. Sci., 47, 1588 (1960)). Both naturally occurring and synthetic polyribonucleotides have been found to direct protein synthesis in this system. The purpose of this investigation will be to compare the characteristics of naturally occurring and synthetic polyribonucleotide coding units. These characteristics will include (a) the coding ratio, i.e., the number of nucleotides comprising one coding unit (b) specificity of coding units, i.e., whether two or more coding units direct the incorporation of one amino acid into protein, and (d) the universality of coding units, i.e., whether a coding unit corresponding to an amino acid is the same in different species.

Polyuridylic acid serves as template RNA by directing the cell-free synthesis of polyphenylalanine (Nirenberg and Matthaei, Proc. Nat. Acad. Sci., 47, 1588 (1960)). Also, randomly mixed polynucleotide direct the incorporation of other amino acids into protein. Thus, the nucleotide composition of RNA coding units corresponding to 15 amino acids has been determined (Martin, Matthaei, Jones and Nirenberg, Biochem. Biophys. Res. Comm. (in press)).

The addition of tobacco mosaic virus RNA to the cell-free E. coli system greatly stimulated incorporation of amino acids into protein. The soluble C^{14}-protein formed during the course of the reaction has many properties characteristic of tobacco mosaic virus protein. For example, it is antigenic and is precipitated by tobacco mosaic virus antibody; it may be purified by DEAE column chromatography in the same manner as tobacco mosaic virus protein; a peptide can be isolated from the middle of the molecule after digestion with trypsin which contains C^{14}-amino acid in the proper place (Tsugita, Frankel-Conrat and Nirenberg, unpublished results). However, the product of the reaction did not combine with tobacco mosaic virus RNA to form infective virus. The product appears to be similar, but not identical to tobacco mosaic virus protein. Attempts will be made to compare the finger-prints of the product of the reaction and authentic tobacco mosaic virus protein and thus determine the precise differences between them. These studies should show whether coding units contained in tobacco mosaic virus RNA can direct the synthesis of similar proteins in both tobacco plants and E. coli. In other words, a direct comparison may be obtained between the function of RNA coding units in two different species.

Soluble RNA has been shown to be an intermediate in polyphenylalanine synthesis (Nirenberg, Matthaei and Jonas, Proc. Nat. Acad. Sci. (in press)). Since soluble RNA appears to be a cofactor which functions as an "adapter" carrying an amino acid to its proper place on tempate RNA, a variant soluble RNA base-pairing with a different code letter of template RNA would substitute one amino acid for another during protein synthesis. It is possible that in species other than E. coli, polyuridylic acid may be either meaningless or may serve as a template for a different amino acid. Soluble RNA will be purified from different species and will be charged enzymatically with different C^{14}-amino acids. The C^{14}-amino acyl soluble RNAs will be used in a purified E. coli amino acid incorporating system and synthetic polyribonucleotides will be added to direct amino acid incorporation. It is hoped that data obtained from these experiments will show whether soluble RNA from different species can recognize the same RNA coding unit. These data, as well as the experiments mentioned previously, may indicate whether RNA coding units in different species are the same.

Randomly mixed polyurydilic-guanylic acid directs the incorporation of a number of amino acids including leucine and valine. The amounts of leucine and valine incorporated into protein were equal and were proportional to the guanylic acid content of each polymer. Thus, the number of coding units prevailing in leucine per polymer were the same. However, omission of C^{12}-valine did not affect the incorporation of C^{14}-leucine, which demonstrated that the coding units for valine and leucine are different. Degeneracy in this system was demonstrated by the finding that both polyuridylic-guanylic acid and polyuridylic-cytidylic acid stimulated the incorporation of leucine into protein.

The specificity and degeneracy of RNA coding units in tobacco mosaic virus RNA will be investigated in a similar manner. Thus, the information concerning the code which has been obtained using synthetic polynucleotides may be directly compared with the coding characteristics of a viral RNA.

(b and c) This type of training in enzymology and biochemical genetics will aid Dr. Byrnes in his future research. Upon completion of his fellowship, Dr. Byrnes should be exceptionally well qualified to do independent research and to teach.

Facilities Available

The Laboratory of Molecular Biology in the Institute of Arthritis and Metabolic Diseases, National Institutes of Health, will contain all of the equipment necessary for this type of work. This laboratory is staffed by people trained in different disciplines,

such as crystallography, physical chemistry, biophysics, organic chemistry, biochemistry and genetics. Interaction and exchange of information between such people should be valuable and Dr. Byrnes should obtain training of unusually broad nature. Frequent interdisciplinary seminars help to maintain a stimulating intellectual and research environment.

FUNDS REQUESTED

An annual stipend for Dr. Byrnes of $6,500. It will not be necessary to receive the institutional allowance in partial payment of costs incurred by the National Institutes of Health in pursuance of Dr. Byrnes' research and training. Part of these funds, however, may be an aid to Dr. Byrnes in defraying tuition expenses of elective courses given at the National Institutes of Health if Dr. Byrnes wishes to participate in them.