Moscow Talk - Aug 15

We have obtained a stable, cell-free E. coli system which incorporates C^{14} amino acids into protein at a rapid rate, and which has many characteristics of protein synthesis. Conditions have been found which demonstrate a novel characteristic of this system; messenger in template. This work was published by Nirenberg, Matthaei, Jones, Rottman, and Weaver in the Proc. Nat. Acad. Sci. that is, a requirement for RNA, needed even in the presence of excess soluble RNA and ribosomes. Naturally occurring RNA; such as E. coli messenger RNA and TMV RNA, and a synthetic polynucleotide, were active in this system. The synthetic polynucleotide appears to contain the code for the synthesis of a protein containing only one amino acid.

First slide, please. Each reaction mixture contains these constitutents in micromoles per milliliter. The enzyme extract consisted of E. coli ribosomes and 100,000 x g supernatant solution. Complete details are presented in Biochemical and Biophysical Research Communications 4 (1961).

In slide No. 2, counts per minute per milligram protein is plotted against time and minutes. In the absence of added DNAase, valine was rapidly incorporated into protein. At the end of 90 minutes

~~We have been~~

The synthetic polynucleotide is polyuridylic acid. Poly U

Addition of microgram quantities of Poly U to reticulocyte mixtures results in a 500 to a 1000-fold stimulation in incorporation of C^{14}-phenylalanine into a protein which strongly resembles polyphenylalanine. We've tested many other synthetic polynucleotides. Only poly U stimulates phenylalanine incorporation.

The incorporation is also extremely specific. We have individually tested every C^{14}-amino acid and poly U stimulates the incorporation only of phenylalanine.

~~Randomly linked polymers of adenylic & uridylic acids were completely inactive.~~ When ~~Poly U & poly A doubly & triply stranded helices are formed~~. A solution of poly U & Poly A, which loses ~~gains~~ to form doubly & triply stranded helices, had no activity whatever, suggesting that single-strandedness is a necessary requisite for activity.

~~The Phenylalanine incorporating system required Ribosomes + 100,000 Kg supernatant solution are required for incorporation. The phenylalanine incorporation is inhibited by puromycin & chloramphenicol.~~

The mechanism of the incorporation process is under current investigation. Attempts are also being made to determine other letters of the code.

In summary, a stable, cell-free system has been obtained from *E. coli* in which incorporation of amino acids into protein was dependent upon the addition of template RNA. Amino acid incorporation required ATP and an ATP-generating system, and was inhibited by puromycin, chloramphenicol and RNAase. Addition of poly-U resulted in the incorporation of phenylalanine alone into a protein resembling polyphenylalanine. Poly-U appears to function as a synthetic template, or messenger RNA, in this system.