

Mason Talk - 1/15

We have obtained a stable, cell-free E. coli system which incorporates C¹⁴ ~~valine~~ ^{amino acid} 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;

that is, a requirement for ~~all~~ ^{messenger or template} RNA, needed even in the presence

~~of excess soluble RNA and ribosomes.~~ ^{such as E. coli messenger RNA and tRNA, rRNA, and such} Naturally occurring RNA₃ ~~and~~

~~with~~ 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 constituents 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.

addition of microgram quantities ^{Poly U} to reaction 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've tested every C^{14} amino acid ^{individually} and poly U stimulates the incorporation only of phenylalanine.

Randomly mixed polymers of adenylic & uridylic acids were completely ^{inactivated} in stimulated incorporation. ~~When not mixed~~
~~Poly U & poly A, doubly & triply stranded helices are~~
~~formed. A solution of poly U & Poly A, which does~~
~~pair to form double & triple stranded helices, had~~
~~no activity whatsoever, suggesting that single-~~
~~strandedness is a necessary requisite for activity.~~

The phenylalanine incorporation ~~required~~ ^{required} ~~both~~
~~ribosomes & 100,000 xg supernatant solution~~ ^{are}
~~required for incorporation.~~ ^{The} ~~phenylalanine~~
~~incorporation~~ ^{is} ~~stimulated~~ ^{inhibited} ~~by~~ ^{promycin & 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

B. th. ribosomes + 100,000 xg supernatant solution were required for incorporation
E. coli in which incorporation of amino acids into protein was

dependent upon the addition of template RNA. ~~Amino acid~~ incorporation *iso*

required ATP and an ATP-generating system, and was inhibited by

The product of the reaction was partially characterized;
puromycin, chloramphenicol and RNAase. ~~Addition of poly-U resulted~~

and it appears to be polyphenylalanine.

~~in the incorporation of phenylalanine alone into a protein resembling~~

These results are in press & will be published shortly in the Proc. of the Nat. Acad. Sci.

~~polyphenylalanine.~~ Poly-U appears to function as a synthetic template,

or messenger RNA, in this system.

One or more nucleic acid residues, then, appear to be the code for phenylalanine