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THE CLINICAL CENTER
DIVISION OF RESEARCH GRANTS

December 13, 1955

Mrs. Maxine F. Singer
Department of Biochemistry
Yale University
333 Cedar Street
New Haven, Connecticut

Dear Mrs. Singer:

Here are the "Project Suggestions" that I promised you:

Project: Net synthesis of polynucleotide material has been demonstrated in several bacterial systems, with nucleoside 5'-diphosphates as precursor compounds (1). No comparable success has been achieved with soluble extracts of animal tissues although the results of isotope incorporation experiments are encouraging. Potter ?

I propose to study nucleic acid synthesis in animal tissues by searching for a soluble system catalyzing the phosphorolysis or pyrophosphorolysis of polynucleotides. A number of possible substrates include virus, yeast or animal ribonucleic acid, the various polymers described by Ochoa and Manago (1) or the small polynucleotides terminated with a 5'-phosphomonoester group which have recently been obtained by Heppel, Ortiz and Ochoa (2). There is reason to believe that polynucleotides terminated with a 5'-phosphomonoester end group are more apt to be involved in nucleic acid biosynthesis than the "3'-ended" polynucleotides which result from the action of pancreatic ribonuclease on RNA.

The problem outlined above is difficult because the nucleic acid synthesis system is probably weak compared with the activity of interfering nucleases, which act on polynucleotides, and the many enzymes which would remove nucleoside 5' diphosphates. However, studying the reaction in the direction of polynucleotide phosphorolysis should offer a good chance of success. With purification the various interfering activities should be removed and the reaction mechanism can then be examined in detail.

(1) M. Grunberg-Manago and S. Ochoa. J. Am. Chem. Soc. 77, 3165 (1955).

(2) L. Heppel, P. J. Ortiz, and S. Ochoa, Science, In Press.

An Alternative Suggestion: It has been suggested that ribonucleic acid synthesis may take place in the nucleus and that the newly synthesized polymer is then transferred to the cytoplasm. Accordingly it becomes of interest to study the enzymes of nuclei preparations which act on ribonucleic acid and ribopolynucleotides.

The most active diesterase activity of nuclei is the hydrolysis of cyclic mononucleotides and cyclic polynucleotides to give the non-cyclic derivatives (1). This activity is far greater than the rate of hydrolysis of various internucleotidic phosphodiester linkages. The hydrolysis is very curious in that it yields exclusively nucleoside-2' phosphates, which were once considered artefacts of alkaline hydrolysis. It would be of considerable interest to purify this activity and to study it in detail.

Another strange property of relatively crude liver nuclei preparations is to hydrolyze yeast RNA to give exclusively nucleoside-3' phosphates, whereas the "5'-ended" polymers of Ochoa are split to give exclusively nucleoside-5' phosphates and small polynucleotides terminated with a phosphomonoester group at C-5'. Study of this phenomenon should be very profitable indeed.

(1) L. Heppel and H. Kaplan. Unpublished.

Suggestion (1) could be expanded. Point out that enzymatic and other studies of large polymers are complicated by the uncertain nature of the material isolated. With small, chemically defined polynucleotides of different types (i.e., 5'-ended and 3'-ended) one might have a better chance of working out the reaction mechanism for polynucleotide synthesis and degradation.

I hope these rather tentative suggestions are useful. I sent on the sponsor's form to Dr. Scantlebury.

We all enjoyed your visit very much and hope that your fellowship applications will be favorably acted upon. Please let me know how everything goes.

Sincerely yours,

Leon A. Heppel

Leon A. Heppel

LAH:lh