Preliminary Notes

On the mechanism of incorporation of adenylic acid from adenosine triphosphate into ribonucleic acid by soluble mammalian enzyme systems

In a previous publication[1] it was shown that a dialyzed extract of an acetone powder prepared from the soluble cytoplasmic fraction of rat liver is capable of incorporating uridine-5'-monophosphate in internucleotide linkage into a material possessing the properties of ribonucleic acid (RNA). Similar experiments performed with 8-^{14}C-adenosine-5'-triphosphate and ^{32}P-adenosine-5'-triphosphate (Adenine-ribose-^{32}P-P-P) have shown that (1) adenylic acid (AMP) is incorporated into RNA or into a RNA-like material, (2) an internucleotide linkage is formed, (3) AMP is attached to a terminal monoesterified cytidylic acid of RNA, (4) the enzyme systems involved are specific for this reaction and will not attach AMP to other nucleotides in RNA.

These conclusions are based on the following experimental results. Incubation of 8-^{14}C-adenosine-diphosphate alone with the enzyme system does not result in any incorporation; however, incorporation occurs in the presence of creatine phosphate and creatine kinase. Under such conditions, the incorporated radioactivity is associated with the acid-insoluble fraction. The incorporated radioactivity can be extracted from the acid-insoluble fraction with 10% NaCl. It can be precipitated with 3 volumes of ethyl alcohol from the 10% NaCl solution and is non-dialyzable. Upon alkaline hydrolysis of the salt-extracted RNA, 90–95% of the recovered radioactivity is associated with 8-^{14}C-adenosine (Table I). When ^{32}P-adenosine-triphosphate, specifically labeled with ^{32}P in the first phosphate adjacent to the ribose, is incubated with the soluble mammalian enzyme system, 90–95% of the ^{32}P recovered after alkaline hydrolysis is found associated wth 2'- and 3'-cytidylic acid.

TABLE I

RADIOACTIVITY IN ADENOSINE AND IN THE NUCLEOTIDES ISOLATED AFTER ALKALINE HYDROLYSIS OF RNA*

The incubation mixture contained: 0.5 ml enzyme[1], 0.08 ml ATP, 0.05 ml 0.20 M creatine phosphate, 0.05 ml creatine kinase (2 mg/ml), 0.15 ml 0.25 M tris(hydroxymethyl)aminomethane buffer, pH = 7.4, 0.1 ml 0.33 M $MgCl_2$, 0.1 ml 0.20 M phosphate buffer, pH = 7.4. Incubation time, 30 min.

Substrate	*Results expressed as % of the total radioactivity recovered after column chromatography*[4,5]				
	Adenosine	*Cytidylic acid*	*Adenylic acid*	*Uridylic acid*	*Guanylic acid*
8-^{14}C-ATP	90–95	0	< 1	0	0
^{32}P-ATP	0	90–95	< 1	< 1	< 1

* Materials. 8-^{14}C-ATP (Schwartz laboratories), 100,000 c.p.m./0.08 ml, specific activity, 0.8 μc/mg, final concentration, $2.2 \cdot 10^{-3}$ M. ^{32}P-ATP, 400,000 c.p.m./0.08 ml, specific activity, $18 \cdot 10^6$ c.p.m./mg, final concentration, $2.5 \cdot 10^{-4}$ M. Creatine kinase, prepared by the method of KUBY, NODA AND LARDY[6] up to the point prior to crystallization.

Since the ^{32}P is associated with cytidylic acid after alkaline hydrolysis, it may be inferred that the ^{32}P-AMP is attached to cytidylic acid in internucleotide linkage. Since the radioactivity due to ^{14}C-AMP is recovered only as 8-^{14}C-adenosine after alkaline hydrolysis, it is suggested that the ^{14}C-AMP is linked to a monoesterified terminal cytidylic acid and that ^{14}C-AMP has now become a terminal monoesterified nucleotide. Implicit in this latter finding is the conclusion that the AMP-portion of adenosine triphosphate will not attach to any significant extent to a terminal monoesterified adenylic acid, since in the latter circumstance some of the 8-^{14}C-AMP should be recovered as a 2'- and 3'-phosphate after alkaline hydrolysis. Fig. 1 explains this formulation. In this figure the vertical lines indicate the positions of sodium hydroxide cleavage.

The present results may offer an explanation for the results obtained by HEIDELBERGER *et al.*[2]. These investigators have shown that when AM^{32}P is incubated in the presence of a rat liver homogenate, the AM^{32}P is incorporated into RNA and that after alkaline hydrolysis the ^{32}P is found attached to cytidylic acid 2'- and 3'-phosphate. It would be interesting to know whether in those experiments also the AM^{32}P had attached to a terminal monoesterified cytidylic acid.

The requirement in this reaction for ATP rather than ADP need not *necessarily* be interpreted to mean that the triphosphates are the precursors for this incorporation, since phosphatases are

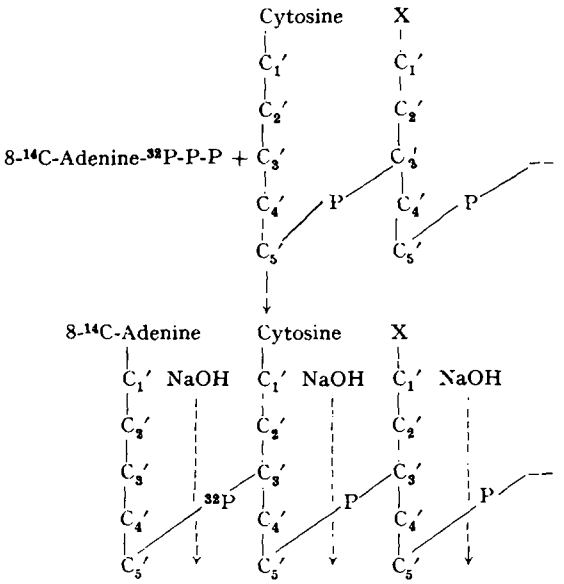

Fig. 1

present in this preparation. A point of difference between the mammalian system under investigation and the bacterial system developed by GRUNBERG-MANAGO AND OCHOA[3] appears to be a specificity apparent in the mammalian system and the lack of specificity encountered in the bacterial system. Also, in the mammalian system evidence is provided only for the incorporation of nucleotides into "RNA-like material", rather than net synthesis as evidenced by the bacterial system.

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