INCORPORATION OF THE ADENINE RIBONUCLEOTIDE INTO RNA BY CELL FRACTIONS FROM E. COLI B

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Reactions involving the incorporation of ribonucleotides into RNA with nucleoside triphosphates serving as precursors have been described with enzyme fractions from different sources. The reactions have been found to involve principally the incorporation of nucleotides into terminal positions of RNA. Many reports have concerned the incorporation of CMP and AMP into terminal positions of the RNA of the 105,000 x g supernatant fraction, S-RNA, to form S-RNA-CMP-CMP-AMP (Canellakis, 1957; Edmonds and Abrams, 1957; Herbert, 1958; Hecht, Zamecnik, Stephenson, and Scott, 1958; Goldthwait, 1959; Harbers and Heidelberger, 1959; Canellakis and Herbert, 1960; Preiss and Berg, 1960). A separation of enzymes from calf thymus nuclei and E. coli (Hurwitz, Bresler, and Kaye, 1959; Alexander, Bresler, Furth, and Hurwitz, 1960), which catalyze the incorporation of individual ribonucleotide residues from nucleoside triphosphates into principally terminal positions of an RNA fraction which is added to the incubation mixtures, has been described. Incorporation of nucleotide precursors into internucleotide linkage has, however, been reported for certain animal systems (Edmonds and Abrams, 1957; Chung and Mahler, 1959; Chung, Mahler, and Enrione, 1960; Edmonds and Abrams, 1960). The incorporation of a ribonucleotide residue from a nucleoside triphosphate precursor in these systems shows no requirement for all of the other nucleoside triphosphates. An incorporation of the CMP residue from CTP

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into RNA of intact nuclei has been described briefly (Weiss and Gladstone, 1959) and has been found to be stimulated by the presence of all four ribonucleoside triphosphates.

The purpose of this report is to describe an enzyme system in cell fractions of E. coli B extracts which catalyzes the incorporation of the adenine ribonucleotide with ATP serving as precursor into an RNA fraction

Table I

Incorporation of Isotope from ATP-α-P^{32} and ADP-α-P^{32} into RNA

	Additions	cpm Incorporated
	ATP	790
	ATP, phosphocreatine, creatine kinase	880
	ATP, UTP, CTP, GTP	3920
	ATP, phosphocreatine, creatine kinase, UTP, CTP, GTP	6850
*	ATP, phosphocreatine, creatine kinase, UTP, CTP, GTP	1590
	ADP	690
	ADP, UDP, CDP, GDP	1800
	ADP, UTP, CTP, GTP	3120
	ADP, phosphocreatine, creatine kinase, UDP, CDP, GDP	6700
	ADP, phosphocreatine, creatine kinase, UTP, CTP, GTP	6940
*	ADP, UDP, CDP, GDP	680

The incubation mixtures contained in a total volume of two ml: Magnesium acetate, 0.01 M; Tris buffer, pH 7.4, 0.011 M; potassium phosphate buffer, pH 7.0, 0.0017 M; and 1 ml of a 30,000 x g supernatant fraction, containing 6.4 mg of protein per ml. Additions were: ATP-α-P^{32}, 0.0005 M, 1 x 10^6 cpm; ADP-α-P^{32}, 0.0005 M, 1 x 10^6 cpm; UTP, CTP, GTP, each 2.5 x 10^{-4} M; UDP, CDP, GDP, each 2.5 x 10^{-4} M; phosphocreatine, 0.005 M; creatine kinase, 200 μg. The mixtures were incubated for 10 minutes at 37°, diluted to 6.5 ml with 0.01 M magnesium acetate, 0.001 M Tris buffer, pH 7.4, and centrifuged for two hours at 40,000 rpm in the 40.3 rotor of the Spinco preparative ultracentrifuge. The pellets were suspended in buffer, washed six times with cold 0.2 N $HClO_4$, dissolved in 0.5 N KOH, and incubated for 18 hours at 37°. The alkaline hydrolysates were acidified with $HClO_4$ and centrifuged, and the supernatant solution was neutralized with KOH. An aliquot of the supernatant solution was plated and counted.

Minus incubation for 10 minutes at 37°.

which shows sedimentation properties similar to that of the small ribonucleoprotein particles. For the incorporation of the adenine nucleotide, CMP, UMP, and GMP precursors are each required, and the incorporation of AMP is more than 90% into internucleotide linkage in the RNA.

In Table I are presented results of a study of the incorporation of ATP-α-P^{32} and ADP-α-P^{32} into RNA by a 30,000 x g supernatant fraction of alumina-ground E. coli cells. The material analyzed was sedimented from the reaction mixtures by centrifugation at 115,000 x g for two hours. Isotope from ATP is incorporated only in the presence of the other nucleoside triphosphates. The incorporation is stimulated by the presence of the triphosphate-generating system, phosphocreatine and creatine kinase. ADP-α-P^{32} is poorly incorporated into this fraction under the same conditions, even in the presence of the other nucleoside diphosphates, unless the ATP-generating system is used.

Analysis of a labelled sedimentable product formed with the 30,000 x g supernatant fraction has shown: It is RNase labile; isotope from ATP-C^{14} is recovered after alkaline hydrolysis in 3'(2')-AMP and adenosine in the ratio of 25:1; isotope from ATP-α-P^{32} is recovered after alkaline hydrolysis in 3'(2')-AMP, 3'(2')-UMP, 3'(2')-CMP, and 3'(2')-GMP in the ratio of 1:.45:.55:.60.

Incorporation of isotope from ATP into acid-insoluble, alkali-sensitive material can also be demonstrated with the supernatant fraction obtained by centrifuging the 30,000 x g supernatant fraction for two hours at 105,000 x g. With the 105,000 x g supernatant fraction, omission of UTP, CTP, or GTP from the incubation mixtures inhibits the rate of incorporation of isotope by 90%. UDP, CDP, and GDP replace the nucleoside triphosphates in stimulating the ATP incorporation. The triphosphate-generating system still stimulates the reaction two-fold. The incorporation is not inhibited by 0.01 M inorganic phosphate. It is inhibited 100% by both RNase and DNase.

Since the product formed with the 30,000 x g supernatant fraction was completely sedimented by centrifugation at 115,000 x g for two hours, it

seemed likely that it was not identical with the major share of the RNA of the 105,000 x g supernatant fraction. Centrifugation of a labelled 105,000 x g supernatant fraction through a sucrose density gradient gave the results shown in Table II. The isotope is distributed in the region of small ribonucleoprotein particles (Roberts, Britten, and Bolton, 1958). This distribution may indicate incorporation of isotope into small particles in the 105,000 x g supernatant fraction, formation or extension of long RNA chains, or binding of a free RNA to small particles. The product is, however, differentiated from the large share of RNA of the 105,000 x g supernatant fraction. It is of interest to point out that Roberts, Britten, and Bolton (1958) have found that in growing cultures of E. coli B, inorganic P^{32} is most rapidly incorporated into small ribonucleoprotein particles.

Table II

Density Gradient Centrifugation of a Labelled 105,000 x g Supernatant Fraction

ml from bottom of tube	μmoles of RNA	cpm incorporated
.33	< .004	63
.66	"	72
1.00	"	99
1.33	"	149
1.66	"	141
2.00	"	146
2.33	"	131
2.66	"	119
3.00	"	80
3.33	"	39
3.66	.011	15
4.00	.036	20
4.33	.012	10

ATP-α-P^{32}, 0.0005 M, 2 x 10^5 cpm, was incubated with 0.1 ml of a 105,000 x g supernatant fraction, .45 mg of protein, with phosphocreatine, creatine kinase, nucleoside triphosphates, Mg++, and buffer as shown in Table I, in a total volume of 0.2 ml for 10 minutes at 37°. The reaction mixture was layered on a 4 ml of a 5% to 20% sucrose gradient solution and centrifuged at 37,000 rpm in the swinging bucket rotor of the Spinco preparative ultracentrifuge for 2 hours. Aliquots were collected from a pinhole in the bottom of the centrifuge tube, and assayed for acid-insoluble radioactivity and for RNA (orcinol determination). Reovery of RNA was 75%.

By centrifuging the 105,000 x g fraction at 115,000 x g for five hours, a pellet is obtained which incorporates labelled ATP at the same rate as the 105,000 x g supernatant fraction. The pellet contains 20-25% of the protein, 100% of the DNA, and 40% of the RNA of the 105,000 x g supernatant fraction. A pH 5.0 precipitate of the 115,000 x g sedimentable fraction has 75% of the ATP-incorporating activity. Less than 10% of the polynucleotide phosphorylase activity of the 105,000 x g supernatant fraction is found in this pH 5.0 precipitate.

Further study of the components of the incorporation system is in progress.

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