THE UPTAKE OF RIBONUCLEOTIDE 5'-TRIPHOSPHATES BY NUCLEAR RIBOSOMES

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Ribosomes extracted from isolated nuclei of calf thymus cells (Wang, 1961; Pogo, et al., 1962; Frenster, et al., 1961) are capable of incorporating labelled amino acids into protein constituents in the presence of nuclear pH 5 enzyme, ATP, an ATP generating system and GTP. With the aid of a procedure similar to those employed with calf thymus, ribosomes with similar characteristics can be extracted from nuclei of Landschutz ascites tumour cells. Besides being able to incorporate amino acids into protein constituents these ribosomes can catalyse the incorporation of labelled nucleotide units from ribonucleoside 5'-triphosphate such as UTP and ATP, into their RNA component.

EXPERIMENTAL

Nuclei previously isolated from Landschutz ascites tumour cells in 0.25M sucrose-3.3mM $MgCl_2$ were extracted at 0° with 10mM Tris-HCl buffer pH 7.6 - 0.5mM $MgCl_2$ in an M.S.E. "Nelco" blender at full speed for 1 min. and the suspensi centrifuged at 15,000xg. for 20 min. to yield an opalescent extract. A crude ribonucleoprotein fraction was collected by centrifuging this extract at 105,000xg for 2 hrs. at 0°. This fraction was then suspended in ice-cold 10mM Tris-HCl buffer pH 7.6 - 0.5mM $MgCl_2$ and sodium deoxycholate was added to a final concentration of 1%. After 20 min., the suspension was clarified by centrifugation at 15,000xg for 10 min. and the supernatant fluid was centrifuged for a further 2 hr. at 105,000xg. The clear pellets obtained were pooled, suspended in 0.1mM $MgCl_2$ and dialysed for 3 hr. against the same medium. The solution was then clarified at 15,000xg for 20 min. and the clear supernatant fluid containing the nuclear ribosomes was used immediately.

These particles contained equal amounts by weight of RNA and protein and a trace amount of DNA (<1%) which was difficult to remove. They exhibited sedimentation properties which were sensitive to the magnesium ion concentration of the medium. For example at pH 7.6, in 1mM $MgCl_2$ the main species showed sedimentation constants of 55 S and 69S, whereas in 0.1mM $MgCl_2$ the main componen was 30 S. Higher concentrations of magnesium ions caused irreversible precipitation of the ribosomes.

UTP labelled with ^{32}P in the α-phosphate group were prepared as described by Burdon and Smellie (1961). ATP-8-^{14}C was purchased from Schwarz laboratories Inc. (4.13 μc/mg.).

RESULTS AND DISCUSSION

Figure 1 shows the time course of the incorporation of ATP and UTP into the RNA component of Landschutz ascites tumour cells. Optimal nucleotide incorporation was achieved only when the ratio of magnesium ions was to ribonucle 5'-triphosphate was 2, although an excess of magnesium ions was less inhibitory than a deficiency.

If the incorporation of nucleotide units from ribonucleoside 5'-triphosphate involved the liberation of pyrophosphate, it would be reasonable to expect that the addition of pyrophosphate might inhibit the reaction. The uptake of both ATP and UTP was strongly inhibited by the addition of pyrophosphate in experiment designed to eliminate secondary effects on the magnesium ion concentration of the medium. Under the same conditions addition of comparable amounts of orthophosphate had a much smaller effect. Labelled ribonucleoside 5'-diphosphat could not replace the corresponding 5'-triphosphates as substrates for these reactions.

The nucleotide incorporation showed no requirement for added primer, either RNA or DNA, andprior treatment of the nuclear ribosomes with RNase (60 μg/mg at pH 7.6 for 1 hr. at 37°) had no effect, although prior treatment with trypsin (120 μg/mg at pH 7.6 for 1 hr. at 37°) or with heat (100° for 3 min. at pH 7.6) or with EDTA (2.8mM) was inhibitory.

Sonic disruption of the nuclear ribosomes (carried out at 0° in 0.1mM $MgCl_2$ using a Mullard ultrasonic drill for 2 min. at 50W; 20kc) resulted in a diminished ability to incorporate ribonucleotides (Fig. 1) which could be further reduced by RNase treatment. Normal levels of ribonucleotide incorporation could only be restored by the addition of certain specific polyribonucleotides to the reaction medium.

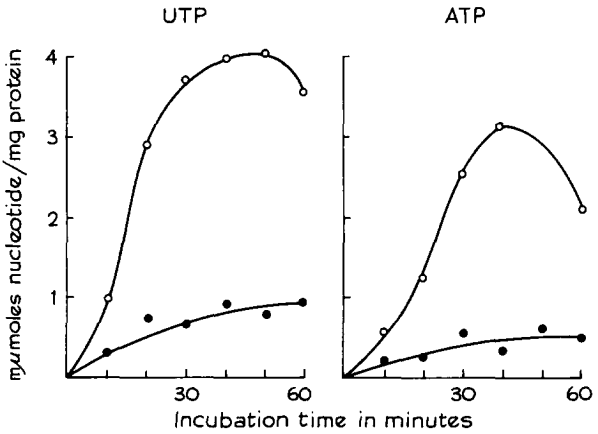

Figure 1. The time course of ribonucleotide 5'-triphosphate incorporation into RNA by normal and disrupted nuclear ribosomes. Each reaction mixture (0.25 ml.) was 0.1M with respect to Tris-HCl buffer (pH 7.6) and contained 75 mμmoles labelled ribonucleotide 5'-triphosphate, 150 mμmoles $MgCl_2$, 90 μg normal (o) or sonically disrupted (•) nuclear ribosomes. Incubation at 37° and analytical procedure as described by Burdon and Smellie (1961).

Table I shows that polyadenylic acid (poly A) restored the level of ATP uptake while polyuridylic acid (poly U) is required for optimal UTP incorporation. The sonic disruption process also released potent RNA degrading enzymes (cf. Elson 1958) and phosphatases capable of cleaving ribonucleoside 5'-triphosphates to the corresponding 5'-di- and 5'-monophosphates.

The uptake of labelled ATP by unsonicated nuclear ribosomes was depressed in the presence of other non-labelled ribonucleoside 5'-triphosphates, such as GTP, CTP or UTP. Using 0.1 μmole GTP, CTP or UTP per 0.075 μmole ATP-8-^{14}C the observed percentage inhibitions were as follows: with GTP, 40; with CTP, 46; with UTP, 71. Similarly, the incorporation of labelled UTP was diminished in the presence of non-labelled ATP (by 82%), CTP (by 59%) or GTP (by 45%).

TABLE I. The effect of synthetic polyribonucleotides on the uptake of labelled ribonucleoside 5'-triphosphates into RNA by disrupted nuclear ribosomes.

Added polyribonucleotide	Ribonucleotide incorporated (mμmoles/mg protein/10 min.)	
	^{14}C-ATP	^{32}P-UTP
none (control)	0.38	0.84
poly A	0.87	0.45
poly U	0.31	1.21
poly AUGC (1:1:1:1)	0.00	0.30

Reaction mixtures (0.25 ml.) contained 0.5 μmoles $MgCl_2$, 75 mμmoles labelled nucleotide, 114 μg disrupted nuclear ribosomes and were 0.1M with respect to Tris-HCl buffer (pH 7.6). 50 μg of synthetic polymers were added where indicated Incubation at 37° and analytical procedure as described by Burdon and Smellie (19

However, in similar experiments using ribonucleoside 5'-di- and 5'-monophosphates in place of the corresponding 5'-triphosphates no such depressions were observed. Although the nature of this effect is unknown, indirect action owing to an effect on magnesium ion concentration can be ruled out.

The possibility of competition at the active site of a ribosomal enzyme without subsequent incorporation was investigated. Nuclear ribosomes were allowed to incorporate UTP in the presence and absence of other non-radioactive ribonucleoside 5'-triphosphates. The ribosomal RNA so labelled was subjected to alkaline hydrolysis and the products examined for radioactivity. Since the ^{32}P incorporated with a uridylate unit is released upon alkaline hydrolysis with the adjacent nucleotide unit in the polyribonucleotide chain (see Burdon and Smellie, 1961), the results shown in Table II indicate that uridylate units derived from UTP were primarily incorporated adjacent to one another to form stretches of polyuridylic acid, despite the presence of non-labelled ATP, GTP and CTP.

Such an observation together with the fact that sonically disrupted nuclear ribosomes require preformed sequences of uridylate units for the optimal utilisation of UTP (Table I) suggest that the newly incorporated uridylate units are attached to the ends of existing ribosomal polyribonucleotide chains terminating in polyuridylate sequences. Similarly it seems possible that

TABLE II. The distribution of ^{32}P amongst the ribonucleoside 3'(or 2')-monophosphates formed on the alkaline hydrolysis of ribosomal RNA after the incorporation of ^{32}P-UTP.

Additions	Per cent total RNA radioactivity			
	AMP	CMP	GMP	UMP
None (control)	6	5	9	80
ATP	12	4	8	76
ATP + GTP + CTP	5	0	8	87

Each reaction mixture (5 ml.) contained 1.5 μmoles ^{32}P-UTP, 12 μmoles $MgCl_2$, 3 mg nuclear ribosomes and was 0.1M with respect to Tris-HCl buffer (pH 7.6). Where indicated 2 μmole portions of non-radioactive ribonucleotides were added. Incubation was for 30 min. at 37° and the analytical procedure was as described by Burdon and Smellie (1961).

adenylate units are attached to existing ribosomal polyribonucleotides ending in polyadenylate sequences. The insensitivity of these reactions to the action of RNase could be ascribed to the location of these sequences at specialised sites on the ribosome inaccessible to RNase. However, further study is required to determine the precise role that endogenous ribosomal RNA might play in the ribonucleotide incorporation reactions. A system with certain similarities has been described in calf thymus nuclei by Edmonds and Abrams (1960). With ATP as substrate, the system, which may be particle bound, catalyses the formation of polyadenylate sequences in the absence of primer RNA.

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