DNA-DEPENDENT SYNTHESIS OF POLYURIDYLIC ACID

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A procedure, similar to that employed by Sibatani, et al. (1962) with isolated calf thymus nuclei, has been used to isolate various fractions from Landschutz ascites tumour cell nuclei. The nuclei (previously isolated by density centrifugation in 0.25M sucrose - 3.3mM $MgCl_2$ after osmotic disruption of the intact cells) were extracted with 0.01M tris-HCl buffer pH 7.6 - 0.5mM $MgCl_2$ to yield nuclear sap and nuclear ribosomes (Burdon, 1963; Burdon, Wykes and Wilkie, 1963). These ribosomes are capable of attaching uridylate units derived from UTP to the ends of existing ribosomal polyribonucleotides terminating in polyuridylate sequences and similarly adenylate units derived from ATP to ribosomal polyribonucleotides ending in polyadenylate sequences (Burdon, 1963).

After the removal of the nuclear ribosomes and nuclear sap, the nuclear residue was homogenised in 20 vol. 1M NaCl and the resulting suspension stirred for 1 hour. The viscous solution was then centrifuged at 40,000 x g for 1 hour. The supernatant fluid, a source of DNA-dependent RNA polymerase (Burdon, Wykes and Wilkie, 1963), was removed and the sediment stirred for 1 hour with 20 vol. 1M NaCl. The insoluble material was collected once more by centrifugation, suspended in 0.01M tris-HCl buffer pH 7.6 (to a protein concentration of 6-8 mg./ml.) and dialysed against the same buffer to remove salt. Sibatani, et al. (1962) refer to such a 1M NaCl insoluble fraction as the "nucleolar fraction". For some studies an extract of this fraction was made by submitting it to sonic vibration (20 kc:50W) for 2 min. at 0°, using

a Mullard ultrasonic drill, centrifuging the resulting suspension at 105,000 x g for 1 hour and collecting the supernatant fluid (1-2 mg. protein/ml.).

RESULTS

Table I shows that both the 1M NaCl insoluble fraction, and the extract prepared from it, catalyse the incorporation uridylate units from UTP^{32} into polyribonucleotide material in a reaction which shows no requirement for the other three ribonucleoside 5'-triphosphates. Preincubation of the 1M NaCl insoluble fraction with DNase decreases its ability to incorporate UTP, but this can be restored by the addition of DNA from Landschutz ascites cells. The extract made from the 1M NaCl insoluble fraction allowed this requirement for DNA to be studied in more detail. The addition of heat denatured DNA is required for optimal activity of the system which is unaffected by Actinomycin D. However pretreatment of the heat denatured DNA with nitrous acid decreased its stimulatory effect.

To examine the nature of the product of this reaction, both the 1M NaCl insoluble fraction and the extract prepared from it were allowed to incorporate radioactively labelled UTP (in the case of the extract, heat denatured ascites cell DNA was added to the reaction mixture). The polyribonucleotide material so labelled was then isolated and subjected to alkaline hydrolysis and the products examined for radioactivity. With UTP^{32} as substrate, it was evident from the percentage of ^{32}P recovered in UMP-3'(or 2') as compared with the other ribonucleoside 3' (or 2')-monophosphates, that 95-97% of the newly formed phosphodiester linkages are between UMP residues in polyuridylic acid chains. After the incorporation of ^{14}C-UMP residues into polyribonucleotide material by the 1M NaCl insoluble fraction, 1% of the radioactivity was recovered in uridine. This indicates a chain length of polyuridylic acid of approximately 100 nucleotides, although with the extract as source of enzyme, the chain length, calculated on the same basis, was only in the region of 20 nucleotides in the presence of added heat denatured Landschutz ascites cell DNA.

TABLE I

Expt. No.	Source of enzyme	Conditions	μμmoles UMP^{32} residues incorporated into polyribonucleotide/mg. protein
1	1M NaCl insol. fraction	Normal (control)	95
		+ ATP, GTP & CTP	15
		pretreated with DNase	40
		DNase pretreatment + native DNA	100
		pretreated with RNase	97
		RNase pretreatment + yeast RNA	94
		+ Actinomycin D (20 μg)	97
2	Extract of 1M NaCl insol. fraction	Normal (control)	158
		+ yeast RNA	148
		+ polyadenylic acid	53
		+ polyuridylic acid	71
		+ polycytidylic acid	123
		+ native DNA	106
		+ heated (heat denatured) DNA	312
		+ native DNA + ATP, GTP & CTP	88
		+ heated DNA + ATP, GTP & CTP	96
		+ native DNA + Actinomycin D (20 μg)	106
		+ heated DNA + Actinomycin D (20 μg)	306
3	Extract of 1M NaCl insol. fraction	Normal (control)	80
		+ heated DNA	409
		+ nitrous acid treated DNA (5 min.)	206
		+ nitrous acid treated DNA (15 min.)	110
4	Extract of 1M NaCl insol. fraction	+ heated DNA	300
		+ heated DNA + EDTA (12.5mM)	194
		+ heated DNA + $(NH_4)_2SO_4$ (10% satd.)	282
		+ heated DNA + UTP	10
		+ heated DNA + UDP	200
		+ heated DNA + pyrophosphate (1.5mM)	5
		+ heated DNA + orthophosphate (1.5mM)	150

Each incubation mixture (final volume 0.4 ml.) contained 50 μmoles tris-HCl buffer pH 7.6, 0.1 μmole UTP-α-^{32}P, 2 μmoles $MgCl_2$, 1 μmole $MnCl_2$, 1 μmole 2'-mercaptoethanol, 50 μg bentonite (prepared by the method of Fraenkel-Conrat, Singer and Tsugita, 1961) and 1.25 mg. protein (1M NaCl insol. fraction) or 0.1-0.3 mg. protein (extract of 1M NaCl insol. fraction). Where indicated the following were also added: 60 μg portions of nucleic acid preparations and 0.1 μmole portions of ATP, GTP, CTP, UTP and UDP. DNase pretreatment of the 1M NaCl insol. fraction was carried out as follows: 30 μg for 1 hour at 37° at pH 7.6 in 2.17mM $MgCl_2$. RNase pretreatment was carried out as follows: 30 μg for 1 hour at 37° at pH 7.6. Native and heat denatured (heated) Landschutz ascites tumour cell DNA was prepared by the method of Keir, Binnie and Smellie (1962). Nitrous acid treatment of the heated DNA was carried out at 37° as described by Litman (1961). Incubation was at 37° for 15 min. after which 60 μmoles sodium pyrophosphate were added to reduce non-specific binding of UTP-α-^{32}P to protein and/or nucleic acid components (Kammen, Klemperer and Canellakis, 1961) and then the analytical procedures of Burdon and Smellie (1961) carried out.

The ability of various nucleic acid preparations to support polyuridylic acid synthesis catalysed by the extract of the 1M NaCl insoluble fraction is shown in Table I. Neither RNA nor polyuridylic acid itself can replace the DNA requirement. To determine whether the heat denatured ascites cell DNA was required only to initiate the polyuridylic acid synthesis, an attempt was made to destroy the priming DNA after some polyuridylic acid formation had already occurred. Table II shows that treatment of the DNA in the reaction mixture with DNase depressed further synthesis of polyuridylic acid. Thus it appears that the heat denatured ascites cell DNA is required not only for the initiation of the polyuridylic acid synthesis but also for the continued synthesis of the polynucleotide.

TABLE II

The Effect of DNase addition during polyuridylic acid synthesis

Tube		μμmoles UMP^{32} residues incorporated/mg. protein
1	Incubated for 5 min. at 37°	101
2	Incubated for 15 min. at 37°	501
3	Incubated for 5 min. at 37° then heated to 100° for 2 min. 30 μg DNase were then added and the mixture incubated for 4 min. at 37°. After this 0.25 mg. of fresh enzyme preparation was added and the mixture incubated for a further 10 min. at 37°.	210
4	Incubated for 5 min. at 37° then heated to 100° for 2 min. 0.25 mg. of fresh enzyme was then added and the mixture incubated at 37° for a further 10 min.	455

Each incubation mixture contained 50 μmoles tris-HCl buffer pH 7.6, 0.1 μmole UTP-α-^{32}P, 2 μmoles $MgCl_2$, 1 μmole $MnCl_2$, 1 μmole 2'-mercaptoethanol, 50 μg bentonite, 0.25 mg. enzyme protein (extract of 1M NaCl insol. fraction) and 60 μg heat denatured Landschutz ascites tumour cell DNA. Subsequent treatment was as indicated after which 60 μmoles sodium pyrophosphate were added (see Table I) and the analytical methods of Burdon and Smellie (1961) used.

DISCUSSION

It appears therefore that the 1M NaCl insoluble fraction from the nuclei of Landschutz ascites cells contains an enzyme system catalysing the DNA-

dependent synthesis of polyuridylic acid. It is unlikely that this polyuridylic acid is a special feature of the DNA-dependent RNA polymerase itself, since the addition of the other three ribonucleoside 5'-triphosphates is inhibitory, as is ammonium sulphate addition which stimulates DNA-dependent RNA polymerase (Goldberg, 1961).

Systems, with certain similarities, catalysing the DNA-dependent formation of polyadenylic acid, have already been isolated from hen liver cell nuclei (Chambon, Weill and Mandel, 1963) and from E. coli (Chamberlin and Berg, 1962; Goldberg, et al., 1962; Hurwitz, et al., 1962).

Although the mechanism for the DNA-dependent polyuridylic acid formation is not yet understood, the observations that Actinomycin D has no effect on the reaction, whereas nitrous acid treatment of the heat denatured DNA depresses its priming ability, suggest that sequences of deoxyadenylate residues in the DNA might prime the formation of corresponding sequences of uridylate residues.

The possible "nucleolar" origin of this polyuridylic acid synthesising system (Sibatani, et al., 1962) is of some interest, since the studies of Perry (1962) on fibroblasts and of Birnsteil and Chipchase (1963) on pea nuclei point to the nucleolus as a possible site of ribosomal RNA synthesis, and nuclear ribosomes can incorporate uridylate units as polyuridylate extensions of existing ribosomal polyribonucleotides terminating in polyuridylate sequences (Burdon, 1963).

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