ENZYMATIC SYNTHESIS OF RNA[1]

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This report concerns the synthesis of ribonucleic acid, RNA, by an enzyme system from pea embryos. The enzyme, as prepared, requires as substrate the four riboside triphosphates, ATP, CTP, GTP and UTP, and contains not only protein but a substantial fraction of the cellular DNA.

Preparation of enzyme. Embryonic axes were obtained by removal of the cotyledons from 40 hour germinating pea seedlings (var. Alaska). The tissue (ca. 200 gm) previously sterilized with chlorox was ground by hand at 4°C with 1.5 volumes of homogenizing medium (0.25 M sucrose, 0.001 M $MgCl_2$ and 0.05 M Tris buffer, pH 8.0) in a prechilled mortar. The homogenate was filtered through miracloth in a basket centrifuge to remove debris and the resulting filtrate centrifuged at 4,000 x g for 30 minutes. The pellet from this centrifugation consists of a layer of starch covered by a brownish layer. The latter, which contains the enzymatic activity, was separated by scraping and washed twice by centrifugation (10,000 x g, 10 minutes) in the grinding medium. This was followed by two similar washings in 0.25 M sucrose to remove excess $MgCl_2$. The final pellet was suspended in 0.05 M Tris (pH 8.0) and dialysed for 30 minutes against 500 ml of Tris buffer, 0.05 M, pH 8.0.

The enzyme thus prepared yields, from 200 gm of fresh tissue, approximately 80 mg of protein (determined by biuret), 8 mg of DNA (deter-

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mined by diphenylamine, Burton, 1956) and 1.6 mg of RNA (determined by orcinol, Dische and Schwarz, 1937). It has been shown by Ts'o and Sato (1959) that by the above procedure over 90% of the DNA of the seedling tissue is recovered in the present fraction.

Assay for incorporation of ATP ($8\text{-}C^{14}$) into RNA. The reaction was stopped after incubation by the addition of 5 ml cold 5% TCA. The acid-insoluble precipitate was washed twice with ethanol ether (3:1) and then extracted 2x with 5 ml portions of 10% NaCl, pH 8.0, at 100°C and for 30 minutes. At each extraction, 4 mg of carrier RNA was added. The combined NaCl extracts were then equilibrated with 2 mg of unlabeled ATP for 30 minutes at room temp., the RNA precipitated by addition of absolute alcohol (final conc. 70%) and the precipitate washed twice with cold 5% TCA. The precipitate was then redissolved in 1 ml of water with the aid of a trace of ammonia. Radioactivity of the RNA was then determined in a gas flow counter on 0.2 ml aliquots.

Assay for PP^{32} - nucleotide exchange reaction. After incubation the reaction was stopped by the addition of 0.5 ml of cold 14% TCA. The acid-soluble supernatant was adjusted to pH 4.0 by the addition of 0.1 M sodium acetate. Nucleotides were absorbed on 160 mg of charcoal. The charcoal was further washed 2x with 3×10^{-2} M $Na_4P_2O_7$, 2x with water and once with 95% ethyl alcohol. The washed charcoal was suspended in 1 ml of 95% ethyl alcohol and its radioactivity assayed in a gas flow counter.

Results. The data of table 1 show that the enzyme preparation catalyses the incorporation of the label of $8\text{-}C^{14}$ ATP into material insoluble in cold TCA and referred to as RNA. This incorporation is rapid, particularly during the first 2 minutes (at 37°C) although it continues slowly for longer periods.

Incorporation of the label of ATP into cold TCA insoluble material is dependent in this system on the simultaneous presence of the three riboside triphosphates, CTP, GTP and UTP. Omission of any one or all of

Table 1

Incorporation of ATP ($8\text{-}C^{14}$) into RNA by pea embryo enzyme as a function of time[1]

Time of incubation: min	0	1	2	4	10	30
Incorporation of ATP into RNA: μμ moles	16	57	145	153	162	225

Incubation at 37°C

[1]System consists (in μmoles/ml) of: Tris buffer pH 8.0, 100; $MgCl_2$, 5; cysteine, 10; KCl, 60; NaF, 20; ATP ($8\text{-}C^{14}$), 1.5 (2 μcuries); GTP, 1; CTP, 1; UTP, 1; and 0.5 ml enzyme in total volume of 1 ml. 0.5 ml enzyme contained 3.5 mg protein, 0.4 mg DNA and 0.1 mg RNA.

these three nucleotides reduces the incorporation of the label of ATP into RNA by a factor of four or more as is shown in the data of table 2.

Table 2

Incorporation of ATP ($8\text{-}C^{14}$) into RNA by pea embryo enzyme as a function of the presence of the other riboside triphosphates

System[1]	Incorporation of ATP into RNA μμ moles
Complete	201
Complete, minus CTP	55
Complete, minus GTP	34
Complete, minus UTP	47
Complete, minus CTP, GTP, UTP	51
Complete, 0°C	20

[1]Complete reaction mixture as in table 1. 0.5 ml enzyme contains 5 mg protein, 0.65 mg DNA, 0.17 mg RNA. Incubation for 4 minutes at 37°C.

That the labeled product formed possesses the properties of RNA is indicated by the fact that it is non-dialysable but is converted into dialysable material by the action of crystalline RNAase as is shown by the

experiment of table 3.

Table 3

Behavior of the enzymatically synthesized incorporation product of ATP ($8\text{-}C^{14}$) toward dialysis and RNAase treatment

Treatment of product[1]	Radioactivity in product; cpm	
	Before dialysis	After dialysis
None	820	690
RNAase[2]	800	54

[1] Product used consisted of purified RNA from enzymatic reaction mixture.

[2] RNAase treatment consisted of 0.4 mg enzyme incubated with carrier containing (20 mg) labeled RNA for 24 hours at 25°C.

The labeled product together with the carrier RNA, isolated from the enzymatic mixture, was exhaustively dialysed for 12 hours against 0.1 M NaCl and 0.5 M Tris buffer pH 7.5 and then further dialysed for 12 hours against distilled water to free it of salt. The data of table 3 show that while the labeled product is initially non-dialysable, it is almost wholly converted to dialysable material by the reaction of RNAase.

The present preparation catalyses a further reaction, namely the exchange of P^{32}-labeled pyrophosphate into the ribonucleoside triphosphates, as is shown in table 4. This typical experiment was done as follows: Enzyme was incubated with P^{32}-labeled pyrophosphate and in the presence either of single or of all four of the riboside triphosphates. After an incubation time of 30 minutes, the reaction was stopped with cold TCA and the ribonucleoside triphosphates isolated and counted. The data of table 4 show that exchange of pyrophosphate into the ribonucleoside triphosphates takes place to a small extent in the presence of a single or of three triphosphates but is increased by over 10 fold in the presence of all four, differing in this respect from the riboside triphosphate-pyrophosphate exchange reaction reported by Chung et al. (1960). It would appear that the

Table 4

Exchange of P^{32}-labeled pyrophosphate into nucleotide triphosphates by RNA synthesizing enzyme preparation of pea embryos

System[1]	Total radioactivity in isolated riboside triphosphates cpm/1 ml reaction mixture
Complete	9,770
Complete, minus 4 nucleotides[2]	570
Complete, minus GTP, CTP, UTP	960
Complete, minus ATP, UTP, GTP	620
Complete, minus GTP, CTP, UTP; 4x ATP	920
Complete, zero time	100

[1] Complete system consisted (in μmoles/ml) of: Tris buffer pH 8.0, 100; $MgCl_2$, 5; cysteine, 10; KCl, 60; NaF, 20; ATP, 3; GTP, 3; CTP, 3; UTP, 3; P^{32} pyrophosphate, 1.5 (6×10^5 cpm) and 0.5 ml enzyme in a total volume of 1 ml. 0.5 ml enzyme contained 3.5 mg protein, 0.28 mg DNA and 0.08 mg RNA. Incubation for 30 minutes at 37°C.

[2] The four nucleotides were added after the reaction had been stopped with TCA.

present reaction is dependent upon the presence of the four triphosphates.

Discussion. The enzyme system prepared as described above contains the bulk of the DNA of the tissue as well as protein and a small amount of RNA. It appears that although the nuclei of the cells of the tissue are ruptured in the grinding process, the enzyme preparation is nonetheless enriched in certain of the nuclear constituents as represented by the DNA and impoverished in certain of the (principally) non-nuclear constituents as represented by the RNA. The characteristics of the synthesis of RNA by the present system are similar in respect to dependence on the four riboside triphosphates, to those found by Weiss (1960) for RNA synthesis by an enzyme obtained from isolated nuclei of liver.

Although the enzyme, as prepared, does contain endogenous RNA, we have no evidence as to whether or not this material is essential to the RNA synthesizing capacity of the system. That the DNA of the preparation is of

importance to the activity of the system, as it is in the case of the E. coli system of Hurwitz et al. (1960), is indicated by the fact that the partial removal of the DNA by DNAase has resulted in partial inactivation of its RNA synthesizing capacity.

The present enzyme system not only requires the presence of the four riboside triphosphates as the condition of RNA synthesis but in addition possesses the ability to equilibrate pyrophosphate into the riboside triphosphates. These facts suggest that RNA synthesis may proceed in the present instance from the riboside triphosphates and with the elimination of pyrophosphate.

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