SYNTHETIC ACTIVITY OF POLYRIBONUCLEOTIDE-NUCLEOTIDYL TRANSFERASE ON THE PREIMPLANTATION STAGES OF DEVELOPMENT OF RAT EMBRYOS

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The homogenate obtained from 5-day rat embryos (blastocyst) was found to catalyze the incorporation of ADP-C^{14} into acid-insoluble material. Incorporation of ATP-C^{14} was much less than that of ADP-C^{14} under the same conditions. The presence of polyribonucleotide-nucleotidyl transferase in rat embryos is postulated in the preimplantation stages of development.

Synthetic activity of polyribonucleotide-nucleotidyl transferase (polyribonucleotide: orthophosphate-nucleotidyl transferase, 2.7.7.8; PNPase) has not hitherto been identified in animal tissues [4]. Only three not very convincing reports on the presence of such PNPase activity in animal tissues can be found in the literature [2, 3, 6]. The absence of synthetic PNPase activity in animal tissues can be attributed to the following reasons: 1) loss of this enzyme activity in the course of its isolation and purification; 2) the presence of an inhibitor of this enzyme activity in animal tissues; 3) disappearance of the synthetic activity of the enzyme during evolution.

To examine the third possibility the investigation described below was carried out in order to study the synthetic activity of PNPase in rat embryos in the preimplantation stage of development.

EXPERIMENTAL METHOD

Female rats were autopsied on the 5th day of pregnancy. The cornua of the isolated uterus were washed out with medium No. 199 to obtain the embryos. By the 5th day of pregnancy (examination under the MBS-1 stereoscopic microscope) the embryos consisted of a blastocyst formed from 26-60 blastomeres. The total number of embryos was counted. The zona pellucida was destroyed with pronase. The embryos were then rinsed with medium No. 199 to remove the pronase and collected with a microsyringe. The embryos were disintegrated by freezing and thawing three times in the microsyringe. The resulting homogenate was transferred to tubes containing 50 μg deoxyribonuclease dissolved in distilled water.

TABLE 1. Incorporation of ADP-C^{14} and ATP-C^{14} into Acid-Insoluble Material and Phosphorolysis of Poly-A Catalyzed by Enzymes of Homogenate of 5-Day Rat Embryos

Reaction	Substrate	Number of embryos	Sample radioactiv. aft. deduct. of cntrl. (pulses/min per sample)	Enzyme activity (in nmoles/h per sample)
Polymerization	ADP-C^{14}	45	188	0,765
		95	341	1,483
	ATP-C^{14}	97	110	0,48
Phosphorolysis	Poly- A	40	600	13,2

PNPase activity in the homogenate of the rat blastocysts was determined by the ADP-C^{14} polymerization reaction of phosphorolysis of polyadenylic acid (poly-A) in the presence of orthophosphate-P^{32}, as described previously [1]. Unlike in the previous experiments the incubation mixture for the polymerization reaction additionally contained 100 μg poly-A and 100 μg bovine albumin. Besides the

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usual components [1], the incubation mixture for the phosphorolysis reaction contained 200 μg bovine albumin.

EXPERIMENTAL RESULTS AND DISCUSSION

As shown in Table 1 the enzymes of the rat blastocyst homogenate catalyzed the incorporation of ADT-C^{14} into acid-insoluble material. With an increase in the number of blastocysts from which the homogenate was obtained from 45 to 95 the incorporation of ADP-C^{14} was almost doubled.

Only one enzyme catalyzing the polymerization of nucleoside-diphosphates and, in particular, of ADP is at present known, namely PNPase.

The incorporation of ADP-C^{14} into TCA-insoluble material in these experiments suggests that an enzyme analogous to PNPase exists in homogenized rat blastocysts.

Another possibility could be that the homogenized rat blastocysts contain enzymes phosphorylating ADP to ATP, which is then incorporated into acid-insoluble material through the operation of enzyme systems other than PNPase. One such system could be DNA-dependent RNA-polymerase and poly-A-polymerase. This is all the more likely because poly-A-polymerase, catalyzing the synthesis of poly-A from ATP, has now been found in animal tissues [5].

Attempts were therefore made to detect the synthetic activity of the blastocyst homogenate using ATP-C^{14} as substrate. These experiments showed (Table 1) that ATP-C^{14} was incorporated under the experimental conditions used into acid-insoluble material to a much lesser degree than ADP-C^{14}. These results confirm the hypothesis that PNPase is present in blastocysts.

Since PNPase catalyzes not only the polymerization of nucleoside diphosphates but also the phosphorolysis of polyribonucleotides, tests were carried out to detect any possible phosphorolytic activity of the blastocyst homogenate. These experiments showed that on incubation of poly-A and orthophosphate-P^{32} with the blastocyst homogenate the label was incorporated into material adsorbed on Norite (Table 1). The results of the experiments to study incorporation of orthophosphate-P^{32} into material adsorbed on Norite do not themselves prove, of course, that this incorporation is due to the action of PNPase present in the blastocysts. However, comparison of these results with those of experiments on the incorporation of ADP-C^{14} and ATP-C^{14} suggests that PNPase is present in the preimplantation stages of embryogenesis in rats.

For the final verification of this hypothesis it is necessary next to isolate and identify the polyribonucleotide synthesized under the experimental conditions used. The final elucidation of the reasons why synthetic activity of PNPase is absent in adult animals and present in the early stages of embryogenesis could shed some light on the differentiation of tissues and the role of PNPase in this process.

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