POLY(ADP-RIBOSE) METABOLIZING ENZYMES IN NUCLEI AND NUCLEOLI OF *TETRAHYMENA PYRIFORMIS*

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1. Introduction

Poly(ADP-ribose) is formed in the nuclei of eucaryotic cells from NAD^+ by the enzyme poly(ADP-ribose)polymerase and is degraded at the glycosidic bonds by poly(ADP-ribose)glycohydrolase. The physiological function of poly(ADP-ribose) in intact cells is unknown. Possible functions include a role in cell proliferation, differentiation, DNA replication, repair or transcription. The subject has been reviewed [1,2]. We have chosen a specific defined gene system to study the physiology of poly(ADP-ribose). The system chosen is the ribosomal genes of exponentially growing *Tetrahymena*. It is a well defined, specific gene system in which both replication and transcription may be studied. Indeed the average transcriptional template activity of *Tetrahymena* nucleoli is at least 25-fold greater than that of whole nuclei [3]. We have asked whether poly(ADP-ribose) is made in isolated nucleoli. We show that not only is it synthesized in association with those ribosomal genes, but that the specific activity of both the synthetic and elongating enzymes in nucleoli is about the same as for the bulk of the chromosomal DNA. Thus, the very high level of ribosomal transcriptional activity is not associated with a correspondingly high activity of poly(ADP-ribose) enzymes, although these enzymes occur in purified nucleoli.

Abbreviations: ADPR, ADP-ribose; PR-AMP, phosphoribosyl-AMP; Ado, adenosine

2. Materials and methods

Cultures of *Tetrahymena pyriformis* GL were grown and nuclei prepared as in [4]. The nucleoli were extracted and purified by the sucrose cushion technique in [4] except that in the cushion 1 mM $MgCl_2$ replaced EDTA. The nucleoli were diluted with 3 vol. 'cushion buffer' (10 mM Tris-buffer, pH 8.0, 140 mM NaCl, 10 mM $MgCl_2$, 1 mM 2-mercaptoethanol) and collected by centrifugation at 10 000 × *g* for 30 min. The nucleoli were resuspended in 'cushion buffer' to give a 10–15-times concentrated suspension. At least 90% DNA in the nucleolar preparation was rDNA as checked by agarose gel electrophoresis [4].

2.1. *Enzyme assays*

Poly(ADP-ribose)polymerase was assayed as in [5], but at 18°C. Poly(ADP-ribose)glycohydrolase was assayed as in [6] using poly(ADP-ribose) synthesized from isolated nuclei and purified as in [7]. RNA polymerase was assayed as in [4]. The poly(ADP-ribose)polymerase products were analyzed as in [8] and the glycohydrolase products by the method in [6] except that cellulose polythyleneimine plates were used for the chromatograms. DNA was estimated by the Burton method [9] and protein by the Lowry method [10].

2.2. *Metrizamide gradients*

Nucleolar material, purified as above, was placed at the bottom of a 15–45% (w/v) linear metrizamide gradient in 'cushion buffer'. Centrifugation at

10 000 × *g* for 2 h in a Beckman SW-27 rotor banded nucleoli at their equilibrium position. Fractions (3 ml) were collected and diluted with 3 vol. 'cushion buffer' and centrifuged for 30 min at 10 000 × *g*. Each pellet was resuspended in 200 μl 'cushion buffer'; 150 μl were used to assay for poly(ADP-ribose)polymerase and 35 μl for RNA polymerase.

3. Results and discussion

The possible involvement of poly(ADP-ribose) metabolism in the control of rRNA synthesis in *Tetrahymena* was investigated by measuring the activity of poly(ADP-ribose)polymerase and glycohydrolase in both purified nuclei and nucleoli from exponentially growing cells. There is no significant difference in the specific activity of either of the two enzymes in nuclei compared to nucleoli (table 1). Thus, very active rRNA synthesis is not paralleled by an increased poly(ADP-ribose) metabolism. On the other hand, the results do not exclude a difference in the actual level of poly(ADP-ribosylation) of chromosomal proteins in intact cells. The products of the two enzymes from both sources were analyzed by thin-layer chromatography as described in section

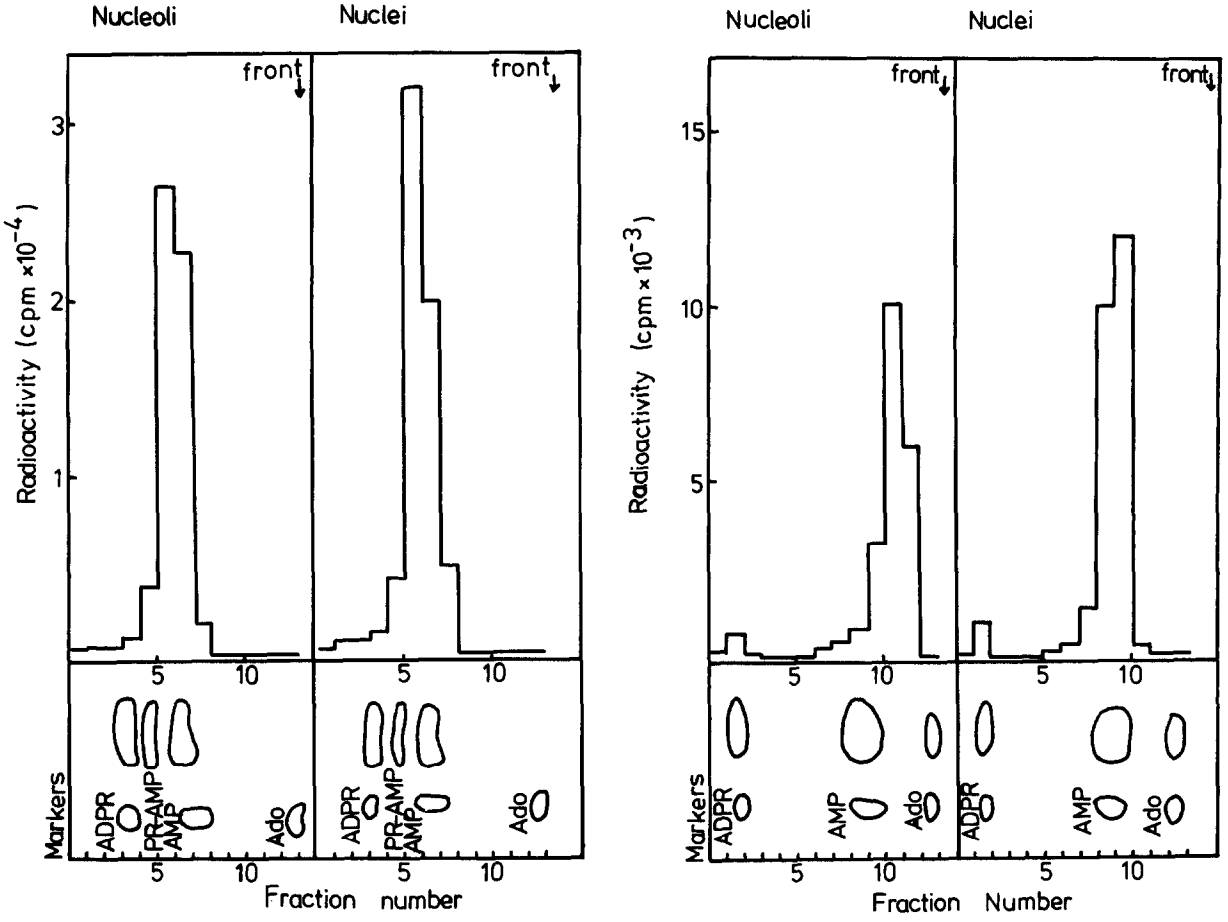

Fig.1. Analysis of the products of the two enzymes on PEI cellulose thin-layer chromatography. In fig.1a the products of the polymerase activity were analyzed after snake venom phosphodiesterase digestion. Non-radioactive markers of ADPR, AMP and Ado were used. Solvent LiCl. In fig.1b the products of the glycohydrolase activity were analyzed. The markers were ADPR, AMP and Ado. The same solvent was used as above.

Table 1
Specific activity of enzymes of poly(ADP-ribose) metabolism in nuclear and nucleolar fractions of *Tetrahymena*

	Nuclei	Nucleoli
Volume (ml)	13	1.2
Protein (mg)	84	0.23
DNA (mg)	18.7	0.23
Poly(ADP-ribose) polymerase		
Total enzyme activity mUnits	26.8	0.27
Specific activity mUnits/mg protein	0.326	0.20
mUnits/mg DNA	1.42	1.2
Poly(ADP-ribose) glycohydrolase		
Total enzyme activity mUnits	38.80	0.72
Specific activity mUnits/mg protein	0.46	0.44
mUnits/mg DNA	2.06	3.20

One unit of poly(ADP-ribose)polymerase activity is defined as 1 μmol NAD^+ incorporated into acid-insoluble material in 1 min at 18°C. One unit of poly(ADP-ribose)glycohydrolase activity is defined as 1 nmol poly(ADP-ribose) (20 ADP-ribose units chain length) degraded in 5 min at 37°C

2. These analyses confirm the identification of the two enzymes, because the polymerase generates a product from which PR-AMP is obtained after digestion with snake venom phosphodiesterase (fig.1a). The polymer chain length as estimated according to [11], was 1.1 units for both fractions. The glycohydrolase generates ADP-ribose (fig.1b); these products were treated with alkaline phosphatase without any change in their chromatographic properties, thus confirming their identification. As is seen from the amount of AMP produced after glycohydrolase hydrolysis, there were also other hydrolases present in both our nuclear and nucleolar preparations and in particular a phosphodiesterase or a nucleotide pyrophosphatase. There is, however, clearly some ADP-ribose detectable.

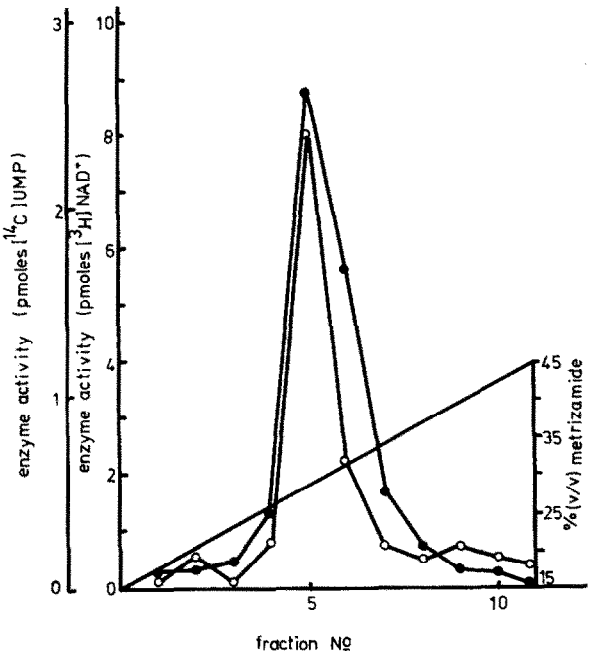

Fig.2. Association of poly(ADP-ribose)polymerase activity with purified nucleoli (r-chromatin). Purified nucleoli were centrifuged to equilibrium in metrizamide gradients. RNA polymerase (•—•) and poly(ADP-ribose)polymerase (○—○) activities were assayed in each fraction.

In order to rule out the possibility that the poly(ADP-ribose)polymerase and glycohydrolase had been lost from the nucleoli during the purification steps, nuclei were taken through the same purification procedure. No loss of either enzyme activity was observed; the specific activity of both enzymes remained the same after the extra purification steps.

Table 2
Inhibition of poly(ADP-ribose)polymerase activity by nicotinamide and thymidine

	% Inhibition of enzyme activity			
	Nicotinamide		Thymidine	
	53 μm	132 μm	53 μm	132 μm
Nuclei	48.5	95	51	96
Nucleoli	60	94	65	94

The association of the poly(ADP-ribose)polymerase activity with the nucleoli was further confirmed by centrifuging the nucleoli to equilibrium in metrizamide gradients. Both the poly(ADP-ribose)polymerase and RNA polymerase activities coincide with the DNA peak (fig.2) which was located by trichloroacetic acid precipitation. The poly(ADP-ribose)polymerase activities of nuclei and nucleoli were further characterized by inhibition studies. As seen from table 2 both nicotinamide and thymidine inhibit the nuclear and nucleolar activities to about the same extent, indicating that the same enzyme is present in the two locations.

Nuclei were extracted with 500 mM NaCl [5] in 50 mM Tris/Cl, pH 8.0, containing 3 mM $MgCl_2$. The nuclear salt extract needed additional DNA and histones for poly(ADP-ribose)polymerase activity; the enzyme extract was stable for 5 days (100%, activity at 4°C), and 18°C was the optimum temperature. At 25°C the enzyme activity was 10–15% lower. Poly(ADP-ribose)glycohydrolase activity was also present in salt extracts. The enzyme in nuclei and nucleoli hydrolyzed about 35% original [^{3}H]poly-(ADP-ribose) radioactivity in 60 min.

Poly(ADP-ribose)polymerase activity in the presence and in the absence of active RNA synthesis was investigated [4]. Both nuclei and nucleoli were incubated with the components required for active RNA synthesis as shown [4]. In the presence of active RNA synthesis the poly(ADP-ribose)polymerase activity in both nuclei and nucleoli was the same as in the absence of RNA synthesis.

We conclude from these studies that in *Tetrahymena* the poly(ADP-ribose) metabolizing enzymes are evenly distributed between whole nuclei and the transcriptionally very active nucleoli. There is therefore no evidence for a direct involvement of these enzyme activities in transcription.

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