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Poly(ADPribosyl)ation System in Transcriptionally Active Rat Testis Chromatin Fractions

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Abstract The rat testis chromatin fractions (soluble, S, and insoluble, P) were prepared by mild digestion of nuclei with DNAase I. They appeared to be different in specific biochemical features such as their transcriptional competence and protein patterns, the latter indicating, according to results previously obtained, that the testis-specific H1t is preferentially associated to the soluble fraction, whereas the other H1 variants are localized in the pellet. S and P chromatins also differed in the distribution of the poly(ADP-ribosyl)ating system, (poly(ADP-ribose)polymerase, reaction product and acceptor proteins), detected by incubating nuclei with ^{32}P-NAD. The ^{32}P-modified H1s and core histones of both fractions, known as specific ADPribose target proteins, were separated by high performance liquid chromatography and it was demonstrated that the H1 variants from S and P are differently ADPribosylated, being H1t always the best acceptor, and that most of the ADPribosylated variants were solubilized after DNase I treatment. The further digestion of P chromatin with the nuclease produced a fraction (pP) devoid of most DNA, but particularly enriched in transcriptionally competent tracts. The low DNA content of pP chromatin, which reflects the typical feature of a nuclear matrix, corresponded to a relevant poly(ADPribosyl)ation, the highest as compared to S and P fractions. Moreover, long and branched chains of poly(ADP-ribose) were found associated to pP sample which resemble the products determined in the soluble chromatin.

Key words: testis, transcription, poly(ADPribose), poly(ADPR)polymerase, DNA repair

The DNA coiling into eukaryotic chromatin requires several organizational levels to achieve the highest degree of condensation found in transcriptionally inactive regions [van Holde, 1988]. A main question is how the complex architecture of chromatin can be modulated to establish and maintain particular states of gene activity. A number of biochemical features have been proposed as characterizing active chromatin, as has the presence of sites hypersensitive to DNAase I [Wu, 1980]. Noncanonical nucleosomes [Ausio, 1992; Weintraub and Groudine, 1976] and selective reduction in the levels of linker histones [Liao and Cole, 1981; Huang and Cole, 1984; Cohen and Shefferey, 1985] also accompany gene derepression. A nonuniform distribution of somatic and tissue-specific H1 variants along hetero- and euchromatin is among the features modulating the differential activation of gene expression [Yu and Bender, 1995; Zlatanova and van Holde, 1992]. The small structural differences between the subtypes of the linker histone can justify their different abilities to interact with DNA [Thomas and Khabaza, 1980; De Lucia et al., 1994].

Most active gene–enriched chromatin fractions contain increased levels of specific variant histone species [Gross and Garrard, 1988], which, in addition, undergo post-translational modification(s) [Boulikas, 1990].

The modification of the linker H1 by the polyanion poly(ADP-ribose) alters drastically chromatin conformation [Boulikas, 1990, 1992]. Poly(ADP-ribose) can be of various complexity (linear, made of short or long polymers, branched), probably as a consequence of a different involvement of the synthesizing enzyme, the poly(ADPR)polymerase (PARP), in various events of DNA metabolism, especially in DNA repair [Lautier et al., 1993; Mathis and Althaus, 1990; Satoh and Lindahl, 1992]. The chromatin

Abbreviations used: ADPR, ADPribose; HMG, high-mobility group proteins; PARP, poly(ADPR)polymerase; PMSF, phenylmethylsulfonylfluoride; RP, reverse phase; TBS, Tris-buffered saline.

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structure and the nuclear enzyme activities are regulated also by the high rates of turnover of the poly(ADPR)polymer [Boulikas, 1992].

Poly(ADPR)polymerase activity has been found preferentially associated to transcriptionally active chromatin domains, and the best acceptors of its product (H1, core histones, HMGs, PARP itself) are involved in transcriptional and post-transcriptional processes [Hough and Smulson, 1984; Levy-Wilson, 1981].

This paper presents evidence that both the levels of poly(ADPR)polymerase and the patterns and distribution of ADPR polymers differ in rat testis chromatin fractions, which can be distinguished by different contents of transcriptionally active tracts.

MATERIALS AND METHODS

Materials

U^{14}C-NAD$^+$, nicotinamide (U^{14}C)adenine dinucleotide ammonium salt, 248 mCi/mmol, ^{32}P-NAD$^+$, nicotinamide adenine dinucleotide di(triethylammonium)salt adenylate-^{32}P (1,000 Ci/mmol), and [α-^{32}P]CTP (3,000 Ci/mmol) were supplied by Amersham International plc; DNAase I (EC 3.1.21.1), phenylmethyl sulfonyl fluoride (PMSF), leupeptin, chimostatin, antipain, pepstatin, spermine, and spermidine were obtained from Sigma Chemical Company. Electrophoretic molecular weight markers were purchased from Pharmacia; X-Omat RP films were from Kodak; and nitrocellulose filters (type BA85) were from Schleicher and Schuell. Filters purchased from Millipore (0.45 μm pore size, type HA) were used for enzymatic assay.

Preparation of Chromatin Fractions

Nuclei from 60-day-old rat testes were isolated as described by Faraone-Mennella et al. [1993] in the presence of 0.15 mM spermine, 0.75 mM spermidine, and 1 mM EDTA/EGTA. This treatment preserves the integrity of chromatin structure [Liao and Cole, 1981]. Isolated nuclei were resuspended in 15 mM Tris-HCl, pH 7.5, and 0.25 M sucrose, and digested with DNAase I (41 U/mg DNA) in the presence of 0.66 mM $MnCl_2$ for 2 min at 30°C. Nuclease activity was stopped by addition of 7.5 mM Na-EDTA. Digested nuclei were suspended in 10 mM Tris–HCl buffer, pH 7.5, 1 mM Na-EDTA, and 1 mM phenylmethylsulfonil fluoride (lysis buffer), and lysed by incubation for 1 hr at 0°C with intermittent gentle agitation by passage through a Pasteur pipette [Huang and Cole, 1984]. Lysed nuclei were centrifuged for 20 min at 12,000*g* in a JA-21 rotor (Beckman). The supernatant was collected and the pellet re-extracted twice as above in lysis buffer. The pooled supernatants are referred to as "soluble chromatin" (fraction S) and the material remaining after extraction is indicated as fraction P or "insoluble fraction." Fraction pP was obtained by digesting twice P chromatin with DNAase I (800–1,000 U/mg DNA) in 60 mM Tris–HCl buffer, pH 7.5, containing 60 mM NaCl and 20 mM $MgCl_2$ at 37°C for 1 hr.

All procedures were performed at 4°C unless noted. Proteinases were irreversibly inhibited by the presence of 1 mM PMSF in all steps except for nuclei digestion/lysis and the pP fraction preparation, in which leupeptin 10 μg/ml, chimostatin 10 μg/ml, antipain 5 μg/ml, and pepstatin 5 μg/ml were added.

Nuclear Protein Extraction and Reverse-Phase HPLC

Acid-soluble proteins from nuclei, fraction S, and fraction P were extracted twice in 0.2 M H_2SO_4 [Nicholas and Goodwin, 1982] with 1 hr stirring at 4°C, and centrifuged at 9,250*g* in a JA-21 rotor (Beckman) for 15 min. The extracts were pooled and proteins precipitated with 20% trichloroacetic acid. Precipitates were collected by centrifugation at 16,500*g* for 20 min, and washed with acetone.

HPLC was performed on a C_4 column (Vydac ODS, 0.5 μ particles, 0.5 × 25 cm) according to De Lucia et al. [1994].

Gel Electrophoresis, Autoradiography, and Densitometric Analysis

Acid-soluble proteins of chromatin fractions S and P were analyzed using slab gels on 12% polyacrylamide in the presence of 0.1% SDS as described by Nicholas and Goodwin [1982]. Densitometry was performed by a Cellomatic densitometer (model CGA 2). For scanning, the gel was cut above the H1 region and just below core histones. The ^{32}P-labeled electrophoresed samples were exposed to X-Omat (Kodak) films for a time ranging between 12 and 72 hr.

Poly(ADPribose)polymerase Assay

Poly(ADPribose)polymerase (PARP; E.C. 2.4.2.30) activity was assayed according to Faraone-Mennella et al. [1993) *in 80 mM Tris–HCl,*

pH 8.0, containing 0.64 mM ^{14}C-NAD (10,000 cpm/nmol), 14 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 4 mM NaF, 40 U DNAase I (Sigma), 10 μg calf thymus DNA, 10 μg rat testis histone H1, and, as enzyme source, an amount of nuclei or chromatin fraction corresponding to 1 μg of DNA (final volume 0.25 ml). Incubation was performed for 10 min at 25°C and the reaction was stopped with ice-cold trichloroacetic acid (25% final concentration). The radioactivity present in the acid-insoluble material was collected on a HAWP (0.45 μm) filter and determined in a Beckman LS 1701 liquid scintillation spectrometer. One enzymatic unit was defined as the enzyme activity catalyzing the incorporation per minute at 20°C of 1 μmol of ADP-ribose into acid-insoluble material.

Blotting Experiments

Activity- and immunoblot were performed according to Simonin et al. [1991]. Aliquots of nuclei and of fractions S and P were suspended in 25 mM Tris–HCl, pH 8.0, containing 50 mM glucose, 10 mM EDTA, and 1 mM PMSF, and sonicated 60 sec pulse at 180 V. The crude extract, after incubation at 65°C for 15 min in 16 mM Tris–HCl, pH 6.8, 2 M urea, 2% 2-mercaptoethanol, and 1% SDS, was separated on 10% polyacrylamide slab gel in 0.1% SDS and electrotransferred onto nitrocellulose filters.

For activity-blot experiments, proteins transferred onto nitrocellulose filters were incubated for 1 hr at room temperature in renaturation buffer [50 mM Tris–HCl, pH 8.0, 100 mM NaCl, 1 mM DTT, 0.3% (v/v) Tween 20, 20 μM Zn(II)acetate, 2 mM $MgCl_2$] containing DNAase I–activated calf thymus DNA (2 μg/ml), and for 1 hr in the same buffer containing ^{32}P-NAD (1 μCi/ml; specific activity 1,000 Ci/mmol). The blots were then washed extensively with the renaturation buffer, dried, and analyzed by autoradiography.

For immunoblot experiments, nitrocellulose sheets were treated for 3 hr with the blocking solution [50 mM Tris–HCl buffer (pH 8.0), 150 mM NaCl, 0.5% (v/v) Tween 20, and 3% (w/v) gelatin]. Incubation with rabbit anti-human PARP polyclonal antibodies (50 μg/ml) was performed for 15 hr at room temperature in the same solution supplemented with 0.3% gelatin. The blots were then washed several times with TBS-Tween, and antibody binding was detected using phosphatase-conjugated goat anti-rabbit IgG second antibody [Harlow and Lane, 1988].

Poly(ADP-ribosyl)ation Reaction

Intact nuclei were resuspended (1/1; w/v) in 0.25 M sucrose containing 10 mM Tris–HCl buffer (pH 8.0), 5 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 50 mM NaCl, 5 mM NaF, 1 mM PMSF, and 10 μg/ml leupeptin, and incubated with 0.2 mM ^{32}P-NAD (22,000 cpm/nmol) for 20 min at 20°C. The reaction was terminated by diluting with the same buffer and chilling on ice. The nuclei, collected by centrifugation at 15,000*g* in a JA-21 rotor (Beckman) for 15 min at 4°C, were washed with incubation buffer to remove unbound radioactivity. The different chromatin fractions were then prepared from the incubated nuclei by the previously described procedure.

Analysis of Reaction Products

Intact ^{32}P-poly(ADPribose) moieties incorporated into the proteins of fractions S, P, and pP were detached by incubation of modified proteins at 60°C for 3 hr with 10 mM Tris, 1 mM EDTA, and 8 mM NaOH, pH 11. Samples were extracted with phenol/$CHCl_3$/isoamyl alcohol (49:49:2, v/v/v), dried in Speed-vac, dissolved in 50% urea, 25 mM NaCl, and 4 mM EDTA, pH 7.5, and then analyzed on 20% polyacrylamide slab gel (20 × 25 × 0.1 cm) as described by Panzeter and Althaus [1990].

Other Methods

Protein concentration was determined by the commercial method of Pierce using bovine serum albumin as standard. DNA content was determined on the basis of the absorbance at 260 nm (1.0 A_{260} = 50 μg/ml DNA) or by the diphenylamine method [Burton, 1968]. RNA synthesis was followed by incubation of nuclei in the presence of ^{32}P-CTP according to Greenberg and Ziff [1984] and the ^{32}P-RNA was measured as 25% trichloroacetic acid-insoluble radioactivity.

RESULTS

Characterization of Chromatin Fractions

In both soluble (S) and insoluble (P) chromatin fractions from rat testis DNAase–digested nuclei, differing for DNA contents (Table I), the analysis of H_2SO_4-soluble proteins showed qualitative and quantitative differences. In fact, the electrophoretic patterns of S and P acid-soluble proteins revealed the presence of considerable amounts of nonhistone proteins in the soluble fraction, which, in contrast, was quite devoid of H1 histone (Fig. 1A). A quantitative comparison

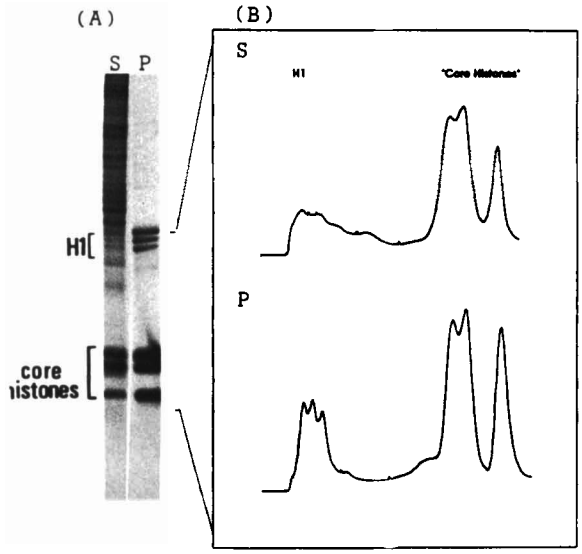

Fig. 1. SDS-PAGE of acid-soluble proteins associated with S and P chromatin. A: Electrophoretic pattern of proteins (80 μg) in the H_2SO_4 extracts. The gel was stained in 0.05% Coomassie. B: Densitometric scanning of the gel corresponding to the tract between H1 and core histones.

TABLE I. Distribution of DNA, Proteins, and Newly Synthesized ^{32}P-RNA in Rat Testis Chromatin Fractions

	Total DNA[a] (%)	Total proteins (%)	^{32}P-RNA[c] (%)
Nuclei	100	100	100
Fraction S	27	72	55
Fraction P	73	28	45
Fraction sP[b]	66	12	16
Fraction pP[b]	7	16	29

[a]The DNA concentration in nuclei was assumed as 100%. Measured by A_{260} (1.0 A_{260} = 50 μg DNA/ml) the value was 1.0 ± 0.2 mg DNA/gm tissue (mean of seven independent experiments); by Burton procedure it was 0.83 ± 0.18 mg DNA/gm tissue (five different experiments).
[b]sP (soluble fraction) and pP (insoluble fraction) were obtained after further digestion of P chromatin with DNAase.
[c]Measured as acid-insoluble radioactivity. Aliquots of each sample were precipitated by 25% trichloroacetic acid (final concentration) in the presence of 5 mM Na pyrophosphate, filtered through Millipore filters (0.45 μ), washed with 10% trichloroacetic acid, and counted for radioactivity.

of S and P in regard to H1 content was obtained by densitometric scanning of the tracts of the gels corresponding to the migration regions of H1 and core histones (Fig. 1B). The H1/core histones ratio was calculated to be 0.27 for S and 0.46 for P, clearly indicating a lower H1 content in the soluble fraction. Previous analyses had also shown a different distribution of H1 variants between S and P fractions. The tissue-specific H1t was more abundant in S than in P,

TABLE II. Distribution of PARP Activity and ^{32}P-ADPR in Rat Testis Chromatin Fractions*

	PARP activity		^{32}P-ADPR	
Chromatin	(%)	mU/mg$_{DNA}$	(%)	(cpm/mg protein)
Nuclei	100	3.5	100	55,280
Fraction S	57	7.4	13	46,116
Fraction P	43	2.1	87	58,000
Fraction sP	ND	—	29	11,000
Fraction pP	ND	—	58	190,400

*The data are the mean of the values from two different preparations. Fractions sP and pP were obtained by two sequential and extensive digestions of fraction P with DNAse I.

whereas the somatic H1a was mainly associated with the insoluble chromatin [De Lucia et al., 1994]. From RP-HPLC data, the H1a/H1t ratio was calculated to be 1.6 in S and 2.8 in P.

The further characterization of chromatin fractions was achieved by measuring the difference in transcriptional activity/competence of S and P preparations (Table I). By incubating rat testis nuclei with ^{32}P-CTP the synthesis of new RNA was followed. The corresponding S and P fractions, obtained as described above, showed that the S fraction, containing about 30% DNA (Table I), was enriched in ^{32}P-RNA. The results regarding sP and pP fractions are discussed below.

The further digestion of P precipitate with DNAse I solubilized, in the sP supernatant, most DNA (66%), but not the ^{32}P-RNA, which remained associated to pP by 29%.

Poly(ADPRibosyl)ation System in S and P Chromatin Fractions

The results obtained from the analysis of the poly(ADPR)polymerase system (i.e., enzyme levels, acceptor proteins, and the kind of products) were different for rat testis S and P chromatin fractions.

About 57% of poly(ADPR)polymerase activity was associated with the soluble chromatin fraction (Table II), containing almost 30% total DNA (Table I). The specific activity of the S fraction was the highest when referred to DNA. The activity-blot analysis (Fig. 2A) further confirmed that the enzyme was preferentially associated with the soluble fraction. The incubation of the same filter in the presence of polyclonal anti-PARP antibodies (Fig. 2B) gave evidence that the high levels of PARP activity associated with the S fraction corresponded to a larger content of the enzyme as compared to the P

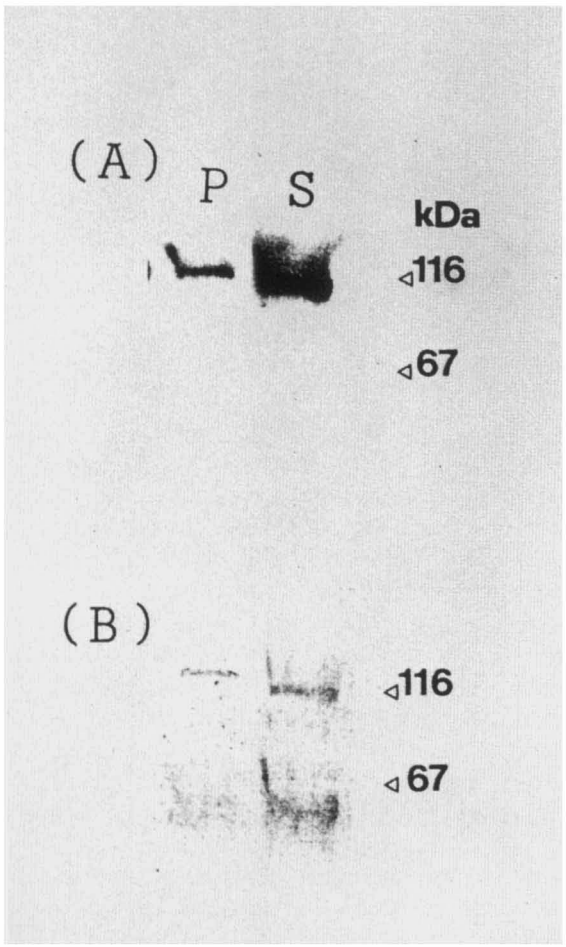

Fig. 2. Activity- (A) and immunoblot (B) of rat testis poly(ADPR)polymerase. Soluble (S) and insoluble (P) fractions were analyzed. Seventy microgram proteins were loaded on the gel.

fraction. The 60 kDa band is a proteolytic fragment of PARP previously described [Lamarre et al., 1988].

The analysis of radioactivity distribution in different chromatin fractions, isolated from rat testis nuclei incubated with ^{32}P-NAD followed by DNAase I digestion, indicated a preferential association of radioactivity with the P (87%) as compared to the S fraction (Table II).

The further extensive digestion of fraction P with DNAase I produced a chromatin fraction (pP) characterized by a highly nuclease-resistant DNA. The very low amount of residual DNA corresponded to 5–10% of total DNA (Table I). However, the removal of most DNA did not cause solubilization of radioactivity, which was mainly found in the pP pellet (58%); the recovery was from 50% to 74% for different preparations (Table II). Thus, the high specific radioactivity of the P fraction is only apparent, since the removal of bulk chromatin (sP) gave rise to the pP pellet with the highest levels of labeling.

Therefore, a valid comparison of chromatin fractions with regard to distribution of ^{32}P-poly-ADPribose must take into account that most radioactivity is associated with S and pP, the two fractions able to synthesize RNA to a larger extent (Table I). The last one, once we removed all nuclease-soluble DNA, was still characterized by high levels of transcriptional activity/competence (Table I).

Highly heterogeneous polymers of ADPribose were found in S and P fractions without significant differences between the two chromatins (Fig. 3, lanes 1, 2, 4).

However, after further treatment of P with nuclease, the resulting soluble (sP) and insoluble (pP) fractions revealed highly different patterns of polymers (Fig. 3, lanes 3, 5). While short oligomers were associated with bulk chromatin, removed by DNAase I digestion, the pP fraction was particularly enriched in long polymeric chains (above 20 residues) and branched products, the latter evidenced by the strong labeling on the top of the gel. Thus, the longest polymers present in the P fraction were strictly associated with pP.

Analysis of Acid-Soluble ADPR-Acceptor Proteins From Different Chromatin Fractions

When both chromatin fractions were isolated from nuclei previously incubated with ^{32}P-NAD, the acid-soluble proteins in the S fraction were labeled at a higher specific activity than those in the P fraction. Electrophoretic and autoradiographic analyses revealed that histone H1 and core histones were the main ADPR protein acceptors in both chromatin fractions (Fig. 4). In fraction S, the band above molecular weight 94,000 is likely the automodified PARP.

Analysis by RP-HPLC of the acid-soluble proteins of S and P chromatins clearly evidenced that H1 variants, particularly H1t, are the best targets of poly(ADPR), with a specific radioactivity higher than for core histones (Table III). Moreover, in S chromatin, the ADPribosylation levels of H1 variants were two or three times higher than in P, clearly indicating that the modified proteins are mostly soluble.

DISCUSSION

The presented results indicate a preferential association of poly(ADPR)polymerase to rat tes-

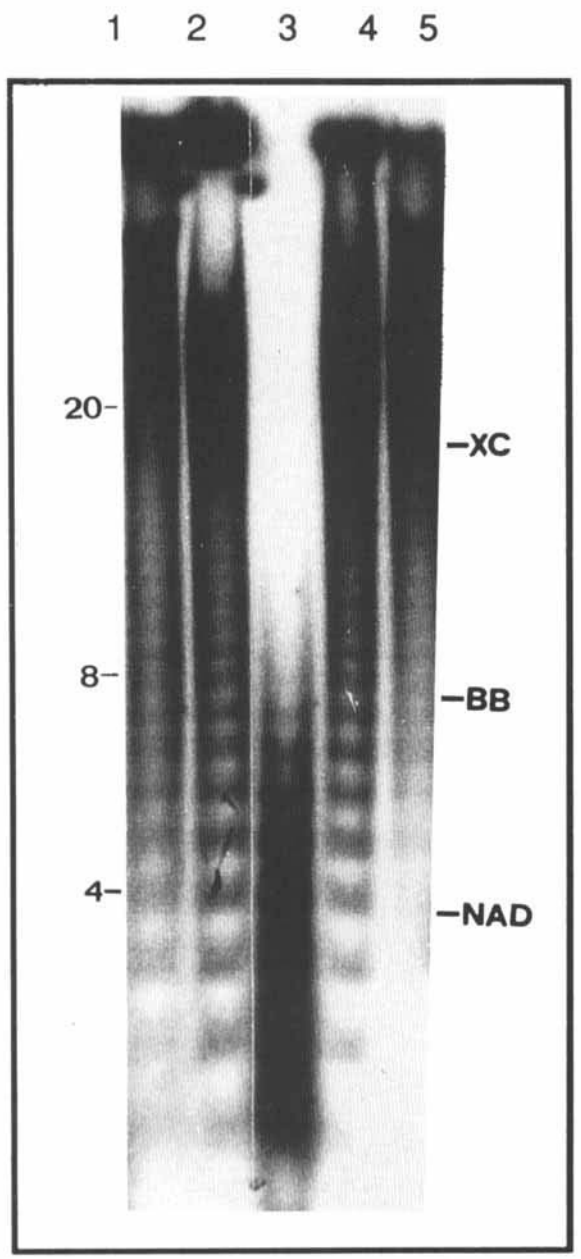

Fig. 3. Autoradiography of poly(ADP-ribose) associated with different chromatin fractions. **Lane 1**, fraction S; **lanes 2,4**, fraction P; **lane 3**, fraction sP; **lane 5**, fraction pP. About 2,000 cpm were loaded for each sample. XC, xylene cyanol; B, bromophenol blue. The lengths of polymers in terms of ADPribose residues are indicated to the left.

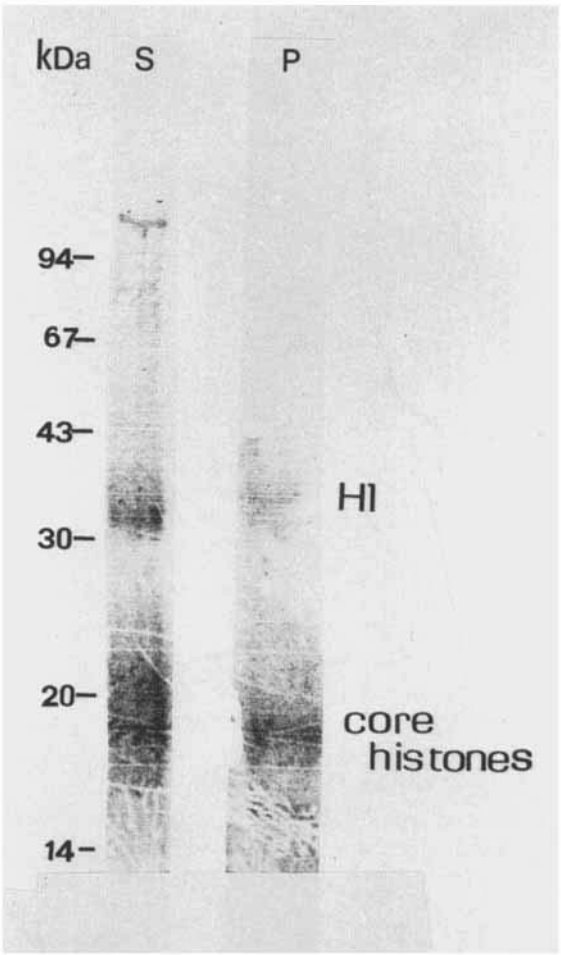

Fig. 4. Autoradiography of acid-soluble proteins from rat testis S and P fractions. One hundred and eighty microgram proteins for each sample were run on SDS-PAGE under the conditions of Figure 1.

tis S chromatin. This fraction exhibits a protein pattern (presence of nonhistones, partial H1 depletion, variable content of H1 subtypes) already described for transcriptionally active/competent chromatin domains [Cohen and Shefferey, 1985; Ausio, 1992]. The transcriptional competence of S chromatin was also confirmed by in vitro transcription experiments, following the distribution of newly synthesized ^{32}P-RNA among chromatin fractions.

The enrichment of PARP in the S fraction and the evidence of both a nonuniform distribution of H1 variants and their different degree of ADPribosylation in the S and P fractions suggest that this modification plays a specific role in various functional features of chromatin, possibly involved in processes taking place in particular chromatin domains [Hough and Smulson, 1984; Levy-Wilson, 1981]. Poly-ADPribosylation has been related to several nuclear functions, which, as it is known, are accompanied by local changes in chromatin organization. The highly ADPribosylated proteins in the S fraction might reflect a more decondensed structure of this chromatin. Poly-ADPribosylation can be considered, together with the poor presence of H1 in S, among the putative factors which might contribute to the functionality of active chroma-

TABLE III. ADPribosylated Histones From S and P Chromatin Fractions*

Histone	Specific Radioactivity	
	Fraction S	Fraction P
H1a	0.92	0.38
H1d-e	0.78	0.25
H1c	0.74	0.25
H1t	1.62	0.75
H2B	0.20	0.10
H2A + H4	ND[a]	0.05
H3	0.18	0.04

*Acid-soluble extracts from both S and P fractions were analyzed by RP-HPLC, and radioactivity associated to the peaks corresponding to histones was measured. The values in the table were calculated as ratio between each measured radioactivity and the peak area recorded by the integrator.
[a]ND = not detectable.

tin. The finding that rat testis H1 variants undergo ADPribosylation to a different extent and the testis-specific H1t is both preferentially modified and enriched in the S fraction might have a particular functional meaning. During spermatogenesis it appears in pachytenic spermatocytes, where tissue-specific genes are highly expressed. However, the specific involvement of H1 variants in transcriptional processes needs to be further investigated.

The modification reaction might be involved not directly in transcription, but in associated events such as DNA repair [Althaus and Richter, 1987; Selby and Sancar, 1993]. It was also reported that human hereditary disorders such as Cockayne's syndrome and Xeroderma Pigmentosum are related to deficiency of a strand-specific repair mechanism [Hanawalt, 1994]. An altered poly(ADPribose) system is also detectable in these patients [Althaus and Richter, 1987; Selby and Sancar, 1993].

Particularly interesting are the results regarding fraction pP. Although this chromatin fraction was not prepared by high salt extraction, it can be functionally compared to nuclear matrix, as it contains a DNA highly resistant to nuclease digestion, which represents 10% or less of total DNA. As it is known, the matrix-associated DNA anchors the chromatin domains to this nuclear fraction, which is considered an important control center of nuclear processes, including transcription. In fact, many active genes, particularly those tissue specific, are localized in the nuclear matrix.

The possibility that the pP fraction represents a subnuclear fraction functionally comparable to the nuclear matrix finds further support in the results reported by Shefferey and Cohen [1985] and Stratling et al. [1986]. These authors prepared, by extensive DNAase I digestion, an analogous nuclear fraction, which was characterized as functionally active. Following their procedure, we were able to obtain, starting from rat testis nuclei incubated with ^{32}P-CTP, pP fraction, which, together with the soluble chromatin S, was particularly enriched in newly synthesized ^{32}P-RNA . These results, besides confirming the S fraction as active/competent chromatin, reveal that the pP fraction represents an actual site of RNA synthesis.

The finding that ^{32}P-ADPR remains associated with the pP fraction after removal of bulk chromatin by extensive nuclease digestion confirms the results already described for rat testis nuclear matrix preparations [Quesada et al., 1994]. High levels of poly(ADPR) were observed in nuclear matrix to variable extent depending on the cell cycle stages [Cardenas-Corona et al., 1987]; the authors suggested that long polymers of ADPribose might mediate transient interactions of proteins with the nuclear matrix.

Whether the large amount of radioactivity found in the pP fraction corresponds to polymers noncovalently bound to nuclear matrix components, or to polymers covalently attached to tightly bound or matrix-intrinsic proteins, as we previously proposed [Quesada et al., 1994], has to be further investigated.

However, the presence of long and branched polymers in the functionally active S and pP fractions as compared to the short oligomers of bulk chromatin might have a precise functional meaning. The length of ADPribose polymers might represent an important task in regulating, through poly-ADPribosylation, the functionality of these chromatin fractions. This nonrandom quantitative and qualitative distribution of products might depend on both localization of PARP in chromatin and turnover rate of poly-ADPribose. We have previously demonstrated a preferential association of PARP with the S fraction, and that a tightly bound form of this enzyme is present in the nuclear matrix [Quesada et al., 1994].

In light of these results, we suggest a preferential association of the ADPribosylating system with the chromatin fractions (S and pP) which are potentially involved in the transcriptional process. As stated before, it must be considered

a possible, more specific involvement of ADPribosylation in additional events flanking the transcriptional process, such as the recently reported DNA repair mechanism associated with DNA chains under transcription [Hanawalt, 1994].

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