COINCIDENCE OF SUBNUCLEAR DISTRIBUTION OF POLY(ADP-RIBOSE) SYNTHETASE AND DNA POLYMERASE β IN NUCLEI OF NORMAL AND REGENERATING LIVER

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1. Introduction

An increase of hepatic poly(ADP)-ribosylation of predominantly non-histone chromatin proteins occurs at an early pre-cancerous state following treatment with dimethyl nitrosamine [1]. This increase in poly(ADP)-ribosylation is specific for the pre-cancerous state and an opposite effect is induced by growth hormone [2]. In search of the mechanism of the dimethyl nitrosamine-induced increase in poly(ADP)-ribosylation it was apparent that no measurable DNA fragmentation was detectable in vivo [1] therefore, the putative stimulatory effect of this process on poly-(ADP-ribose) synthetase activity [3] seemed unlikely. Two distinct types of mechanisms are recognized that are germane to the carcinogenicity of dimethyl nitrosamine:

(1) Covalent modification of DNA and of other macromolecules [4–7]. A subsequent excision repair of modified DNA is also generally known but the cellular physiology of this process is poorly understood.

(2) Cancer promotion, which is the second broadly defined stage of the 2-step processes of carcinogenesis [8]. Partial hepatectomy or cell death-induced by CCl_4 and subsequent regeneration [9,10] are powerful promotors and can initiate carcinogenesis following an otherwise ineffective dose of carcinogen. Dimethyl nitrosamine alone at a certain dose produces cell death and induces regeneration, thus requires no promotor [11] like partial hepatectomy. The dose of dimethyl nitrosamine used in [1] is therefore likely to have produced both stages of carcinogenesis. To determine whether or not the promotor process was responsible for the increase in protein poly(ADP)-ribosylation [1] we determined the effect of surgically-induced liver regeneration [12] on both poly(ADP-ribose) synthetase activity and on DNA synthesis. Instead of assaying whole nuclei, we have chosen to determine the membrane association of these 2 systems in normal and regenerating liver, because in both prokaryotes [13–15] and eukaryotes [16,17], 'M-band'-associated DNA and RNA synthetase activities are directly relevant to cell division. In mitochondria, the protein ADP-ribosylating system is significantly associated with the mitochondrial 'M-band' fraction and ADP-ribosylation in mitochondria inhibits DNA-polymerase γ [18,19]. The subnuclear association of the poly(ADP)-ribosylating system with the 'M-band' fraction had not been studied.

2. Experimental

Male Sprague-Dawley rats (150 g body wt) were used throughout and they were deprived of food 16 h prior to the experiment. Liver regeneration was induced surgically as in [12] and livers from 6–8 rats were pooled in each experimental group. At 22 h after the removal of 75% of liver tissue, the liver mass was completely regenerated, therefore this time was chosen for nuclear assays. At this time, the ratio of liver wt/100 g body wt was 1.4 in controls (sham operated) and 1.8 in experimental animals, and nuclear DNA/protein ratio was 0.3 in controls and 0.4 in the experimental group. The NAD^+ content of normal and regenerating livers at 22 h was identical (588 nmol/g liver in controls and 577 nmol/g in regenerating liver) as determined fluorometrically [20]. Liver nuclei were isolated and assayed for DNA, protein, and poly(ADP-ribose) synthetase as in [1] and the incor-

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poration of [^{3}H]thymidine phosphate into DNA was determined as in [21]. The nuclear 'M-band' was isolated as the Mg^{2+}–lauroylsarcosinate complex by a discontinuous sucrose gradient centrifugation method [22], following the incubation of whole nuclei with either [^{14}C]adenine-labelled NAD^+ or methyl [^{3}H]-thymidine triphosphate under appropriate conditions [1,21]. In all assays parallel agreed within 5%.

3. Results and discussion

As shown in table 1, [^{3}H]thymidine phosphate incorporation in the 'M-band' fraction, isolated from nuclei of regenerating liver was increased by 49% compared to controls. The thymidine phosphate incorporation related enzymatic activity was therefore membrane-associated to ~50% during regeneration. The following arguments support the view that the increased rate of thymidine phosphate incorporation into 'M-band' associated DNA is most probably an expression of a process that is equivalent to DNA repair, rather than replication. Thymidine phosphate incorporation into DNA in this system was insensitive to *N*-ethylmaleimide (table 1 legend) therefore the enzyme assayed under these conditions was most likely DNA polymerase β that is known to catalyze DNA repair synthesis [23]. DNA polymerase α, the enzyme primarily involved in cellular replication related DNA synthesis, is localized in eukaryotes in the perinuclear region [24]. Thus, liver nuclei isolated from sheared homogenates are likely to be deficient in DNA-polymerase α. Since active cellular proliferation leading to complete liver regeneration takes place in our model, it is obvious that DNA polymerase α must be participatory. However, its intracellular topography that is distinct from polymerase β [24] predicts the possibility of differential control of the two DNA polymerases. It is apparent from table 2 that the subnuclear localization of poly(ADP-ribose) synthetase is nearly identical with that of DNA polymerase β, and that poly(ADP-ribose) synthetase is also augmented in the 'M-band' fraction of regenerating liver. The increase in poly(ADP-ribose) synthetase in nuclei of pre-cancerous livers [1] was 37%, the same as the observed augmentation of poly(ADP)-ribosylation in the 'M-band' of nuclei isolated from regenerating livers. Because the steady state [NAD^+] was unchanged during liver regeneration under our conditions, it is predictable that rates of in vivo poly(ADP)-ribosylation of intranuclear systems, that are structurally closely associated with the poly(ADP-ribose) synthesizing system, were unimpaired as far as substrate requirement for poly(ADP)-ribosylation was concerned, and therefore a modification of DNA polymerase was probable also in vivo. As illustrated in table 3 incubation of liver

Table 1
[^{3}H]Thymidine phosphate incorporation into DNA of whole liver nuclei and nuclear fraction [22]

No.	Conditions	Total nuclei	Soluble fraction	'M-band'
1	Control (pooled from 6 rats)	45.3 X 10^3 dpm 18.9 pmol	26.6 X 10^3 dpm 11.1 pmol	18.6 X 10^3 dpm 7.8 pmol
2	Regenerated liver (pooled from 8 rats)	55.2 X 10^3 dpm 23 pmol	27.3 X 10^3 dpm 11.4 pmol	27.8 X 10^3 dpm 11.6 pmol
	% Change in 2 cf. 1	+21%	+3%	+49%

Liver nuclei, equivalent to 500 μg protein (150 μg DNA)/test were incubated with 0.027 mM [^{3}H]thymidine triphosphate (spec. act. 790 dpm/pmol) and 0.1 mM desoxynucleotide triphosphates each (dGTP, dCTP, dATP) in the presence of 1 mM DTT, 5 mM ATP, 6 mM $MgCl_2$ in 300 μl final vol. made up to 0.1 M Tris–HCl pH 7.5 at 30°C for 10 min with agitation. Incorporation into DNA was determined as in [21]. The distribution of labelled DNA was determined in each nuclear fraction [22]. Identical results were obtained when 0.1 mM *N*-ethyl maleimide was present during the reaction

Table 2
Protein-bound ^{14}C-labelled poly(ADP-ribose) formation in whole liver nuclei and nuclear fractions [22]

No.	Conditions	Total nuclei	Soluble fraction	'M-band'
1	Control (pooled from 6 rats)	83.0 X 10^3 dpm 6.9 nmol	58.7 X 10^3 dpm 4.9 nmol	24.3 X 10^3 dpm 2.0 nmol
2	Regenerating liver (pooled from 9 rats)	97.5 X 10^3 dpm 8.1 nmol	64.2 X 10^3 dpm 5.4 nmol	33.3 X 10^3 dpm 2.8 nmol
	% Change in 2 cf. 1	+17%	+9%	+37%

Poly(ADP)-ribosylation of nuclear proteins was assayed [1] by incubation of nuclei (500 μg protein) at 30°C for 10 min with 0.5 mM N[^{14}C]AD$^+$ (9600 dpm/nmol) in the presence of 10 mM $MgCl_2$, 0.1 mM Tris–HCl (pH 7.8), 2.5 mM DTT, 5 mM EDTA, 1 mM phenyl-methane sulfonyl fluoride in 250 μl final vol. Determination of poly(ADP)-ribosylation products in subnuclear fractions [22] was done as in [1]

nuclei with NAD$^+$ inhibited both 'M-band' associated and free forms of DNA polymerase β. The inhibition by NAD$^+$ was prevented by inhibitors of poly(ADP)-ribosylation [19]; therefore, the inhibitory component of the system was poly(ADP-ribose). Our results explain the apparent inhibition of DNA synthesis (assayed as thymidine uptake) in liver nuclei by poly-(ADP)-ribosylation [25] and conversely inhibitors of poly(ADP)-ribosylation to enhance (de-inhibit) sister chromatide exchange in human lymphoblastoid cells [26,27] or to accelerate unscheduled DNA-synthesis that was induced by *N*-methyl-*N*^{1}-nitro *N*-nitrosoguanidine [28]. These seemingly diverse reactions are likely to involve the catalytic activity of DNA polymerase β which by virtue of its selective localization [24] is sensitive to control by poly(ADP)-ribosylation, as shown here. Our results also show that the promotor process of regeneration can increase poly(ADP)-ribosylation and thus impair DNA repair by DNA-polymerase.

Table 3
The effect of poly(ADP)-ribosylation of liver nuclei on [^{3}H]thymidine phosphate incorporation into DNA

No.	Conditions	Total nuclei	Soluble fraction	'M-band'
1	Control (preincubation without NAD$^+$)	16.7 X 10^3 dpm 7.0 pmol	12.8 X 10^3 dpm 5.4 pmol	3.9 X 10^3 dpm 1.6 pmol
2	After poly(ADP)-ribosylation	10.1 X 10^3 dpm 4.2 pmol	7.5 X 10^3 dpm 3.1 pmol	2.6 X 10^3 dpm 1.1 pmol
	% Change in 2 cf. 1	−40%	−42%	−34%

The effect of poly(ADP)-ribosylation was determined by preincubation of nuclei (500 μg protein/test) with and without 1 mM unlabelled NAD$^+$ for 5 min at 30°C followed by the assay of thymidine phosphate incorporation into DNA as in table 1 legend except for 5 min, i.e., table 2 contains 5 min rates. Preincubation of nuclei (±NAD$^+$) without ATP and substrates results in some loss of DNA polymerase β activity and dissociation from the membrane-bound to soluble form (c.f. table 1)

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