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Poly(ADP-Ribosyl)ation of Transcription Factor Yin Yang 1 under Conditions of DNA Damage

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Under conditions of severe DNA damage the nuclear enzyme poly(ADP-ribose) polymerase 1 (PARP-1) is activated, catalyzing the modification of proteins by forming and attaching to them poly(ADP-ribose) chains. A specific physical interaction between PARP-1 and transcription factor Yin Yang 1 (YY1) *in vitro* was shown previously, which had important consequences for the activities of both proteins. It is demonstrated here that YY1 and PARP-1 form complexes *in vivo*. YY1 was transiently poly(ADP-ribosyl)ated immediately after genotoxic treatment of HeLa cells. The narrow time frame of the modification coincides with that known for the activation of PARP-1 under these conditions. This immediate modification correlated with a decreased affinity of YY1 to its cognate DNA binding sites.

Key Words: **PARP; YY1; poly(ADP-ribosyl)ation; transcriptional silencing.**

Transcription factor Yin Yang 1 (YY1) is a multifunctional zinc finger protein with uncommon properties (1–3). This 44 kDa zinc finger-containing protein can act as a transcriptional repressor, activator, or an initiator element binding protein that directs and initiates transcription *in vitro* (reviewed in (4–6)). The transcriptional activity of YY1 can be regulated by viral oncoproteins such as adenovirus E1A, via the E1A associated protein p300 (7), suggesting a possible role for YY1 in cell proliferation. Recently, YY1 has been shown to be essential for mouse early embryonic development (8). In *Drosophila*, a putative YY1 homolog has been identified and shown to be a member of the Polycomb Group protein family (9). However, little is known about the mechanisms that underlie the crucial role of YY1 in regulating important biological functions.

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Recently, YY1 has been shown to physically interact with the poly(ADP-ribose) polymerase (PARP-1) (10–12). PARP-1 is a key enzyme that is involved in regulating several nuclear processes, such as DNA repair, replication, and recombination, as well as transcription and apoptosis (reviewed in (13–15)). In response to the appearance of DNA lesions, generated either directly by genotoxic agents or indirectly following enzymatic incision of a DNA-base lesion the enzymatic activity of PARP-1 is induced. PARP-1 catalyzes the transfer of ADP-ribose moieties to protein acceptors and synthesis of poly(ADP-ribose) using NAD^+ as a substrate, with the main reaction being automodification of PARP-1 (16). This nuclear post-translational modification has been considered to function in cellular surveillance of genotoxic stress. *In vivo*, PARP-1 is associated with components of the base excision repair (BER) pathway (17, 18) and plays an important role in regulating BER (19). PARP-1 function was also implicated in the regulation of RNA polymerase II-dependent transcription (20–22). Catalytically active PARP-1 may cause silencing of transcription, involving modifications of transcription factors by poly(ADP-ribosyl)ation which interferes with their DNA-binding activity (11). An example is the regulation of YY1 by PARP-1. PARP-1 induces poly(ADP-ribosyl)ation of YY1 which hinders the ability of YY1 to bind its recognition sequences (11). However, the effect of this physical interaction on PARP-1 was less clear.

In this report, we demonstrate a physical interaction between both proteins *in vivo*, specifically under conditions of DNA damage. Following a treatment of HeLa cells with methyl-*N'*-nitro-*N'*-nitrosoguanidine (MNNG) YY1 was transiently poly(ADP-ribosyl)ated concomitantly and binding of YY1 to its recognition sequences was affected.

MATERIALS AND METHODS

Cell culture and treatment. HeLa cells were maintained at 37°C in a humidified atmosphere containing 5% Dulbecco's minimal Eagle medium (Sigma) supplemented with 10% fetal bovine serum, 20 mM

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L-glutamine, and 500 μg/ml Streptavidin/Penicillin. Treatment of nonconfluent growing cells was performed. Stock solution of MNNG was dissolved in dimethyl sulfoxide and serially diluted with phosphate-buffered saline (PBS) immediately before use. 500 μM MNNG was added to cultured cells at a time period as indicated. After treatment, cells were washed twice with PBS, and the cells were scraped of the plates in PBS. From harvested cells nuclear extracts were prepared (23).

Immunoprecipitation/Western blot. Nuclear HeLa cell lysates were immunoprecipitated with protein A Sepharose and antibodies (anti-PARP-1 rabbit antibodies or as control anti-c-Jun rabbit antibodies (Santa Cruz, CA)) or anti-YY1 agarose-coupled rabbit antibodies (C-20 AC, Santa Cruz, CA) for 1 h at 4°C in a buffer containing 10 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20. After five washes, precipitates were run on an 8% SDS–polyacrylamide gel and transferred to nitrocellulose. Western blot analyses was performed with anti-PARP-1 goat antibodies or anti-YY1 monoclonal antibodies (H-10, Santa Cruz, CA) and with secondary antibodies coupled to alkaline phosphatase (Santa Cruz, CA).

Isolation of poly(ADP-ribosyl)ated proteins. Modified proteins were purified by the method of Adamietz and Hilz (24) with some modifications. Nuclear extracts prepared (23) from typically three 100-mm Falcon tissue culture dishes were immediately precipitated with 20% trichloroacetic acid (TCA). All following procedures were carried out at 4°C. The precipitate was collected by centrifugation, washed twice with 5% TCA and 70% ethanol and then resuspended in 300 μl 6 M guanidine hydrochloride, 100 mM 3-(*N*-Morpholino)propanesulfonic acid (Mops) (pH 7.0). The pH was adjusted to pH 9.0 by addition of NaOH and the sample was immediately loaded onto a 200 μl boronyl column (Pierce) equilibrated with 6 M guanidine hydrochloride, 100 mM Mops (pH 8.9). Incubation at a pH $>$ 8 was kept to a minimum (less than 30 min) to avoid hydrolysis of the alkaline-sensitive protein modification. The column was washed with 600 μl 6 M guanidine hydrochloride, 100 mM Mops (pH 8.9), 1 ml 6 M guanidine hydrochloride, 100 mM Mops (pH 8.0), and 200 μl 6 M guanidine hydrochloride, 100 mM Mops (pH 4.5). Modified proteins were subsequently eluted with 400 μl 3 M ammonium acetate (pH 5.0). After precipitation by the addition of 1 ml ethanol modified proteins were collected by centrifugation, washed twice with 70% ethanol and redissolved in 1 M hydroxylamine (pH 7.0) and incubated for 1 h at 37°C. Then proteins were subjected to SDS–PAGE, followed by transfer to nitrocellulose (Schleicher & Schuell). Detection of YY1 was performed by western analyses with affinity purified rabbit antibodies directed against YY1, kindly provided as a gift by Dr. B. Lüscher, Hannover.

Electrophoretic mobility shift assay. ^{32}P labeling of the YY1 oligonucleotide (25) GGCTCCGCGGCCATCTTGGCGGCT and AP-1 oligonucleotide CGCTTGATGAGTCAGCCGGAA (26) and EMSAs with HeLa nuclear extracts prepared (23) were performed as described previously (10). In brief, in a 10 μl binding reaction nuclear HeLa extracts (8 μg of protein) were incubated with 5 ng of ^{32}P-labeled duplex YY1 oligonucleotide for 10 min at room temperature in 12 mM Hepes, pH 7.9, 1 mM EDTA, 5 mM $MgCl_2$, 0.2 mM dithiothreitol, 10% ficoll, 100 μg/ml bovine serum albumin, 500 ng poly(dI-dC), and 400 ng of an unrelated oligonucleotide to inhibit unspecific DNA-binding activities. Protein-DNA complexes were then separated in a 4% polyacrylamide gel. After electrophoresis the gels were autoradiographed. All data presented are representative of at least three independent sets of experiments.

RESULTS

Coimmunoprecipitation experiments were performed to examine a physical association of YY1 and PARP-1 in the living cell (Fig. 1). Nuclear extracts were prepared (23) from nonconfluent HeLa-cells and protein complexes were precipitated with polyclonal antibodies directed against the two proteins. Apparently the PARP-1 antibodies exhibited some unspecific reactivity to higher molecular weight proteins (see the smear of higher molecular weight above the position of PARP-1 in Fig. 1, lane 2). However, a highly specific agarose-coupled YY1 antibody was used in the coimmunoprecipitation experiments (Fig. 1, lanes 3 and 6). Western blot analyses showed that agarose-coupled YY1 antibodies were able to coimmunoprecipitate PARP-1 from HeLa nuclear extracts (Fig. 1, lane 3). Consistent with this result, PARP-1 antibodies, but not c-Jun antibodies used as control, were able to coimmunoprecipitate YY1 from HeLa nuclear extracts (Fig. 1, lanes 5–7). Thus, endogeneous complexes of YY1 and PARP-1 are present in nuclear extracts of HeLa cells.

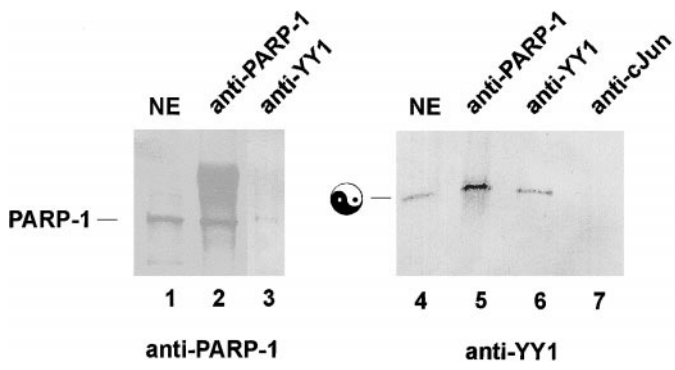

FIG. 1. Existence of a YY1-PARP-1 complex in HeLa cells. Immunoprecipitation experiments were performed as described under Materials and Methods. HeLa nuclear lysates were immunoprecipitated with an antibody directed against the protein indicated above the lanes and subjected to Western blot analyses with anti- PARP-1 (lanes 1–3) or anti- YY1 (lanes 4–7) antibodies. In lanes 1 and 4 pure nuclear HeLa extracts (NE, 1 μg protein) were applied to the gel. The positions of YY1 (☯) and PARP-1 are indicated.

YY1 has been shown previously to be poly(ADP-ribosyl)ated by PARP-1 *in vitro* (11, 12). Using an affinity chromatography approach (24) the modification of YY1 in HeLa cells was examined. As described under Materials and Methods, HeLa nuclear extracts were prepared and subjected to chromatography using boronic acid as an affinity resin which retains proteins modified with poly(ADP-ribose). After elution of modified proteins from the affinity column, ADP-ribose polymers were detached by treatment with neutral hydroxylamine. Proteins were then separated by SDS–PAGE, transferred to nitrocellulose, and subjected to western analyses with specific antibodies directed against YY1. As shown in Fig. 2, poly(ADP-ribosyl)-ation of YY1 was undetectable in growing, untreated HeLa cells (Fig. 2, lane 1). It is well established that genotoxic stress situations such as treatment with alkylating agents result in the induction of the catalytic activity of PARP-1 (27, reviewed in 13–15). Therefore, HeLa-cells were treated with MNNG and the status of

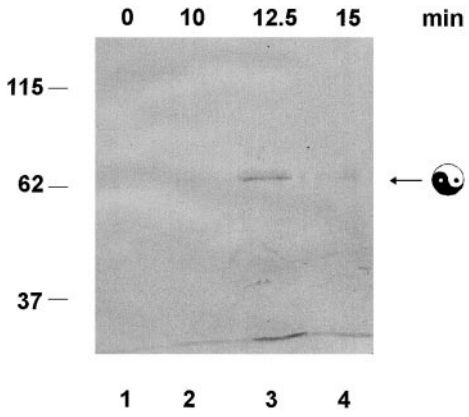

FIG. 2. YY1 is poly(ADP-ribosyl)ated after MNNG treatment. HeLa cells were treated with 500 μM MNNG for 0, 10, 12.5, or 15 min as indicated. Nuclear extracts were immediately prepared and proteins modified with poly(ADP-ribose) were isolated using an affinity support (see Materials and Methods). After detachment of poly(ADP-ribose), isolated proteins were separated by SDS–PAGE and transferred to nitrocellulose. Immunostaining was performed with anti-YY1 antibodies. Relative molecular weights of marker proteins (numbers on the left) and the position of YY1 (☯) are indicated.

YY1 poly(ADP-ribosyl)ation was examined during a time course of 15 min posttreatment. Surprisingly, significant amounts of modified YY1 isolated from HeLa cells were detected only 12.5 min after the treatment with MNNG (Fig. 2, lane 3). This modification was transient, because a significantly reduced level of poly(ADP-ribosyl)ated YY1 was detected 15 min after MNNG-treatment (Fig. 2, lane 4). Proteolytic cleavage and cell-death occur only after a long-term exposure (longer than 15 min) to MNNG (28) and the PARP-1 levels were unchanged during the time course examined. Thus, the reduced level of modified YY1 15 min after MNNG-treatment (Fig. 2, lane 4) is consistent with previous observations that the half-life of poly(ADP-ribose) synthesized following genotoxic treatment is rather short (29, 30). No modification of YY1 was detected in MNNG-treated cells, cultured in the presence of 3-aminobenzamide, an inhibitor of poly(ADP-ribosyl)ation (data not shown).

It was demonstrated previously that specific DNA binding of some transcription factors including YY1 could be prevented by poly(ADP-ribosyl)ation (11). Therefore, nuclear extracts were prepared from untreated or MNNG-treated HeLa cells, and specific YY1-DNA binding activity was analyzed in EMSAs (Fig. 3). Nuclear proteins were subjected to EMSAs using a YY1 (Fig. 3, lanes 1 and 2) or an AP-1 (Fig. 3, lanes 3 and 4) -specific oligonucleotide probe as outlined under Materials and Methods. The signals of specific YY1-DNA complexes obtained with extracts of untreated cells are significantly more intense as compared to MNNG-treated cells (compare in Fig. 3, lanes 1 and 2). Previously was shown that c-Jun was not affected by poly(ADP-ribosyl)ation in their specific DNA binding ability (11). Consistent with those findings, in EMSA using the AP-1 oligonucleotide specific for c-Jun binding, the formation of AP-1 complexes was insensitive to a treatment with MNNG (Fig. 3, lanes 3 and 4).

DISCUSSION

Covalent modifications of DNA binding proteins are thought to be involved in the regulation of processes such as gene transcription and DNA repair. In the present study the poly(ADP-ribosyl)ation of the transcription factor YY1 under conditions of DNA damage caused by alkylating agents is observed. YY1 was poly(ADP-ribosyl)ated in a strict and narrow timeframe after the introduction of DNA lesions, coinciding with the activation of PARP-1. In addition, specific DNA binding of YY1 is repressed after induction of PARP-1 activity.

Previous studies described a function of PARP-1 in the regulation of transcription (reviewed in 14). PARP-1 plays dual roles in transcription, depending on the presence of DNA strand breaks. Catalytically inactive PARP-1 promotes activator-dependent transcription by interacting with RNA pol II-associated factors (21, 31). A number of reports revealed that several transcription factors, among them Oct-1 (32) and B-Myb (33) may form stable complexes with PARP-1. Moreover, PARP-1 deficiency correlated with an al-

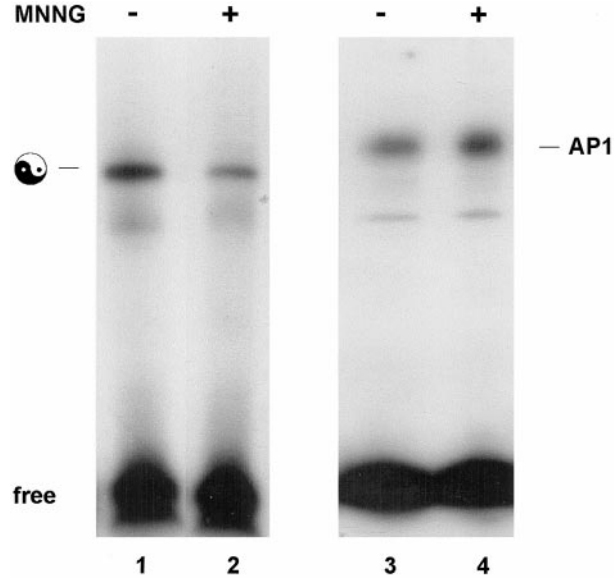

FIG. 3. Specific DNA binding ability of YY1 is reduced after MNNG treatment. Nuclear extracts were prepared from untreated (−) or MNNG-treated (+) HeLa cells. Identical amounts of nuclear extracts were incubated with ^{32}P-labeled YY1 oligonucleotide (lanes 1 and 2) or ^{32}P-labeled AP-1 oligonucleotide (lanes 3 and 4) and analyzed in EMSA as described under Materials and Methods. The positions of specific DNA-YY1 (☯), DNA-AP-1 complexes, and unretarded oligonucleotides (free) are indicated.

tered expression of some genes that may contribute to genomic instability, cancer, and aging (34). Enzymatic modifications of transcription factors are one of several molecular mechanisms for transcriptional silencing. Poly(ADP-ribosyl)ation of some transcription factors, among them P53 (35) and NF-κB (36), affects their specific DNA binding ability. In this study the specific poly(ADP-ribosyl)ation of a transcription factor following DNA damage is demonstrated *in vivo.* Poly(ADP-ribosyl)ation of YY1 was only detectable within a narrow time-window (Fig. 2). This is presumably due to the fact that the half-life of synthesized poly(ADP-ribose) is rather short (30), and that about 30 minutes after the introduction of DNA lesions PARP-1 activity is down-regulated (19). This transient nature of YY1 modification coincides with the time-dependent occurrence of poly(ADP-ribose) in cells in response to alkylating treatment, as for example reported by Malanga and Althaus (37), and also with the conditions required for transcriptional silencing (11, 22). The observed effects seem to be specific, since other transcription factors such as c-Jun are not affected by poly(ADP-ribosyl)ation (11).

In conclusion, in this study a new role of PARP-1 in regulating trancription in situations of DNA damage has been uncovered. Covalent modification of transcription factors with poly(ADP-ribose) could be an efficient mechanism to prevent the expression of damaged genes.

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