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Interaction of the Transcription Factor YY1 with Human Poly(ADP-Ribosyl) Transferase

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Poly(ADP-ribosyl) transferase (ADPRT) is a nuclear enzyme that catalyzes the synthesis of ADP-ribose polymers from NAD^+ as well as the transfer of these polymers onto acceptor proteins. The function of ADPRT is thought to be related to a number of nuclear processes including DNA repair and transcription. The transcription factor Yin Yang 1 (YY1) is a potent regulator of RNA polymerase II (Pol II)-dependent transcription. In this study Alu-retroposon-associated binding sites for YY1 located in the distal region of the promoter of the human ADPRT gene have been identified suggesting a possible involvement of this protein in the regulation of ADPRT-gene expression. In the presence of the recombinant automodification domain of the ADPRT the formation of specific YY1 complexes, detected in gel-shift experiments, was strongly inhibited, indicating that this domain of the enzyme may interact directly with YY1. In accordance with this result YY1 was specifically precipitated from nuclear extracts by ADPRT immobilized on sepharose. These results suggest a direct ADPRT–YY1 interaction which may be of impotrance in the regulation of Pol II-dependent transcription. They also indicate that in some human promoters this regulation may be mediated by retroposons of the Alu family.

Nuclear NAD^+-dependent poly (ADP-ribosyl) transferase (ADPRT; EC 2.4.2.30) is a highly abundant protein which modifies proteins by forming poly(ADP-ribose) chains. The posttranslational transfer of ADP-ribose moieties from NAD^+ to proteins is thought to be involved in the regulation of processes such as cell proliferation, differentiation, neoplastic transformation and DNA repair (reviewed in 1). The human ADPRT gene was localized to chromosome 1(q41-q42) (2) and its genomic structure was established (3). Changes in the level of the ADPRT mRNA have been observed during lymphocyte activation (4) and liver regeneration (5) and it was suggested that expression of ADPRT is mainly regulated at the level of transcription (6). Although several putative binding sites for transcription factor SP1 are present within the first GC-rich 600 bp sequence upstream of the RNA polymerase II (Pol II) transcription start site of the human ADPRT promoter (Genbank Acc. M60436), there exist no TATA and CCAAT boxes (6–9). The distal part of the ADPRT promoter includes Alu-sequences which were implicated in the regulation of the promoter (8, 9). The presence of Pol II transcription-modulating elements was originally detected in a green monkey Alu repeat (10) and then demonstrated in human Alu consensus sequences (11). The number of examples demonstrating Alu-associated Pol II regulatory elements in human promoters is growing (12).

The nuclear protein Yin Yang 1 (YY1; other names such as δ, NF-E1, UCRBP and CF1 have also been used in the literature) has been described to be a regulator of Pol II-dependent transcription which may act as a repressor as well as an activator (13, 14). These opposite effects of YY1 on transcription may be mediated by specific protein-protein interactions, for example, by interaction of YY1 with the mammalian homolog of the yeast global transcriptional regulator RPD3 (15).

In the present study Alu-retroposon-associated binding sites for the transcription factor YY1 were identified in the distal region of the human ADPRT promoter. It is also shown that the binding of YY1 to its cognate binding sequence may be repressed by the ADPRT automodification domain (amino acids 337-573) via direct interaction of YY1 and ADPRT. This is consistent with the view that ADPRT may be a secondary factor in regulation of Pol II transcription (16, 17).

MATERIALS AND METHODS

Reagents were purchased from Sigma unless otherwise noted. HeLa nuclear extracts were obtained from Promega. [γ-^{32}P]ATP was

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supplied by NEN, DuPont. Oligonucleotides were synthesized by Bio TeZ, Berlin-Buch GmbH. Polyclonal antibodies developed in rabbit and raised against human YY1 were provided by Santa Cruz Biotech. Co.. Recombinant His-tagged ADPRT-automodification domain (amino acids 337-573) was overexpressed in *E. coli* and purified as described previously (18).

Electrophoretic mobility shift assay. The upper strand 5′ to 3′ sequences of the oligonucleotides used in this study are given in Fig. 1B. ^{32}P-Labeling of the oligonucleotides and band-shift assays with HeLa nuclear extracts were performed as described previously (19, 20). In brief, 5 μg of nuclear HeLa extracts were incubated with 5 ng of ^{32}P-labeled duplex YY-oligonucleotide for 30 min at room temperature in 12 mM HEPES buffer, pH 7.9, 1 mM EDTA, 5 mM $MgCl_2$, 0.2 mM dithithreitol, and 10% Ficoll containing 1 μg of the double-stranded poly (dI-dC), and 100 μg/ml bovine serum albumin. In some experiments further additions were made as indicated in the legends to the Figures were present. After incubation compexes were separated in a 4% PAG, containing 23 mM Tris-HCl pH 8.2, 23 mM boric acid, and 0.5 mM EDTA.

Gel electrophoresis and electrophoretic transfer. Proteins were separated on 6 × 10 cm SDS-PAGE minigels according to Laemmli (21), except that the samples were not boiled. Electrotransfer of proteins onto nitrocellulose sheets and immunostaining with antibodies raised against YY1 were performed as described by Towbin et al. (22).

Affinity precipitation of HeLa nuclear proteins with ADPRT-sepharose. Purified His-tagged pADPRT was immobilized on CL-4B sepharose (Pharmacia Biotech) using the cyanogen bromide method, and employed for affinity precipitation with HeLa nuclear extracts as described previously (18).

Affinity purification of antibodies against ADPRT. Polyclonal antibodies developed in rabbit and raised against the automodification domain of human ADPRT (amino acids 337-573) were affinity-purified according to a method of Wofsy and Burr (23) using human full-length ADPRT immobilized on CL-4B sepharose (18).

RESULTS

A schematic representation of the human ADPRT promoter is depicted in Fig. 1A. Within the 5′ flanking region of the human ADPRT gene four Alu repeats could be identified (Alu 1-4 in Fig. 1A). All of these elements are about 300 basepairs long and share about 70% homology with other human Alu repeats.

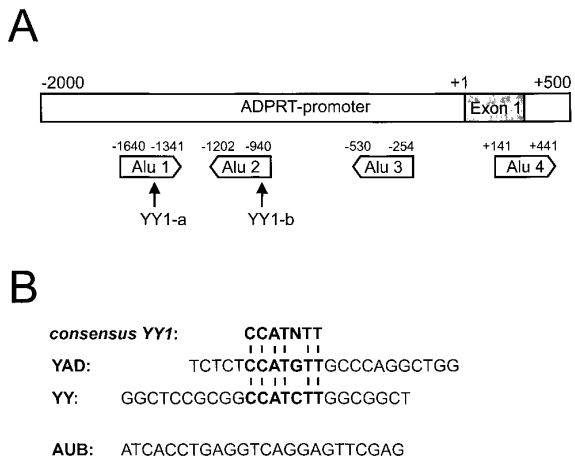

FIG. 1. (A) Schematic representation of the human ADPRT-promotor. The positions of Alu repeats and Alu-associated potential binding sites for transcription factor YY1 are shown. (B) 5′ to 3′ sequences of oligonucleotides used in this study. The YY1 consensus sequence in the YY oligonucleotide and the putative YY1 binding site in the YAD oligonucleotide are outlined in bold.

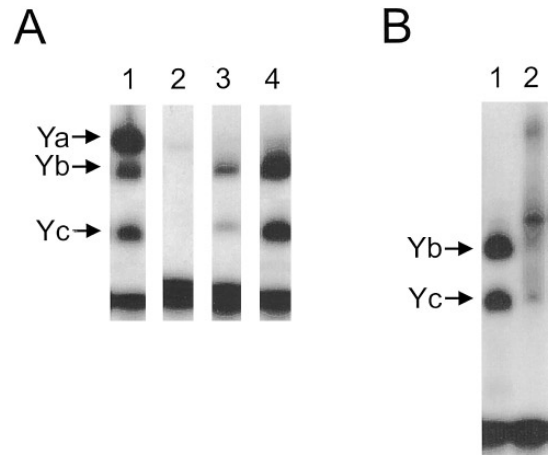

FIG. 2. Electrophoretic mobility shift assay of complexes formed by transcription factor YY1. The autoradiograms of complexes of ^{32}P-labeled duplex YY oligonucleotide (Fig. 1B) with HeLa nuclear proteins after separation in a 4% PAG are shown. The positions of three different complexes Ya, Yb, and Yc are indicated on the left. (A) Before electrophoresis the binding reaction was carried out as described under Materials and Methods in the absence (lane 1) or the presence of 2 μg of each unlabeled duplex YY oligonucleotide (lane 2), unlabeled duplex YAD oligonucleotide (lane 3), and unlabeled duplex AUB oligonucleotide. (B) Before electrophoresis the binding reaction was carried out as described under Materials and Methods with 2 μg of unlabeled AUB oligonucleotide in the absence (lane 1) or the presence of 2 μg of antibodies raised against YY1 (lane 2).

Analysis of the ADPRT promoter region revealed two putative YY1-recognition sites (Fig. 1A, YY1-a and YY1-b), which differ only slightly from the YY1-consensus sequence CCATNTT, described earlier (24). To investigate a possible involvement of YY1 in the regulation of ADPRT expression gel-shift experiments were performed, using a ^{32}P-labeled YY1 consensus oligonucleotide as a probe (YY in Fig. 1B). When incubated with HeLa nuclear extracts, the YY-oligonucleotide was present within three different complexes, termed Ya, Yb, and Yc (Fig. 2A, lane 1). Three different oligonucleotides (Fig. 1B) were tested in their ability to compete with the formation of the three complexes. As expected, in a gel-shift experiment in the presence of an excess of unlabeled YY oligonucleotide none of the three complexes could be observed (Fig. 2A, lane 2). Another oligonucleotide (YAD in Fig. 1B), corresponding to the sequence-952 to -974 upstream of the initiation site of the ADPRT-gene and containing the putative YY1 binding site YY-b (Fig. 1A) was also tested. It was able to supress the formation of the complexes, although, especially with regard to Yb, to a smaller extent (Fig. 2A, lane 3). In a gel-shift experiment carried out in the presence of an excess of a control oligo-

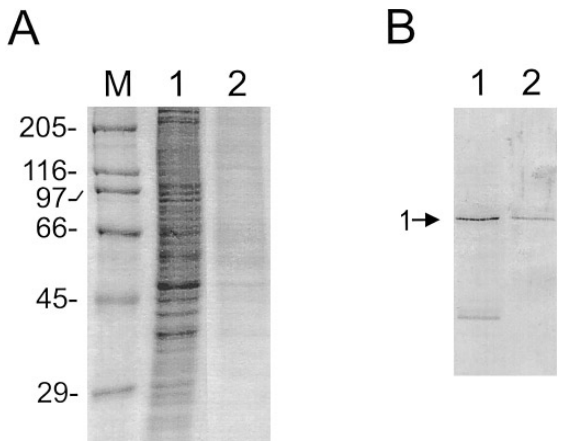

FIG. 3. YY1 interacts directly with full length ADPRT immobilized on sepharose. HeLa nuclear extracts (1 mg) in 500 μl TBST buffer were incubated with 25 μl of ADPRT–sepharose for 1 h at 4°C. After 5 washings proteins bound to the ADPRT–sepharose were extracted with SDS–PAGE sample buffer for 10 min at 25°C and separated by electrophoresis. **A,** 10% SDS–PAGE of untreated HeLa nuclear extract containing 100 μg of protein (lane 1), and the proteins precipitated with ADPRT–sepharose (lane 2) stained with Coomassie Blue. Relative molecular sizes of marker proteins (M) are indicated ($M_r \times 10^{-3}$). **B,** an identical gel was transblotted onto nitrocellulose and immunostained with antibodies raised against YY1. The position of YY1 is indicated.

nucleotide without any YY1-sites (AUB, Fig. 1A), the complexes Yb and Yc remained unaffected (Fig. 2A, lane 4). Under these conditions the formation of Ya was completely inhibited (Fig. 2A, lane 4), indicating that Ya is a sequence-unspecific complex. Thus, all subsequent gel-shift experiments were carried out with an excess of AUB-oligonucleotide to prevent the formation of the unspecific complex Ya. The presence of YY1 itself within the complexes Yb and Yc was demonstrated, using polyclonal antibodies raised against a C-terminal peptide of YY1. As shown in Fig. 2B, both complexes were supershifted with these antibodies.

In independent experiments YY1 was found to exhibit direct protein-protein interactions with the ADPRT enzyme. Purified recombinant His-tagged ADPRT was immobilized on sepharose, as described previously (18). In affinity precipitation experiments ADPRT-sepharose was incubated with nuclear HeLa extracts. After several washings, proteins bound to the ADPRT-sepharose were extracted with SDS-PAGE sample buffer and separated by electrophoresis (Fig. 3). After Western blotting and immunostaining YY1 was found to precipitate with ADPRT-sepharose (Fig. 3B, lane 2). Similar results were obtained using the recombinant His-tagged ADPRT-automodification domain immobilized on sepharose in affinity chromatography with nuclear extracts from human placenta (data not shown). After preincubation of this ADPRT-domain with HeLa nuclear extracts, in gel-shift experiments. The formation of Yb and Yc was completely inhibited (Fig. 4, lane 2). Under these conditions the formation of the unspecific complex Ya appeared to be stimulated, although an excess of AUB-competitor oligonucleotide was present (Fig. 4, lane 2). The significant influence of the ADPRT-automodification domain on YY1-complex formation was substantially reversed in the presence of polyclonal antibodies raised against this domain (Fig. 4, lane 3). These results indicate, that the automodification domain of ADPRT interferes with binding of YY1 to its DNA recognition site.

DISCUSSION

The present study demonstrates, that the transcription factor YY1, known as a Pol II-dependent transcriptional regulator (13–15), interacts directly with the ADPRT enzyme as well as with specific DNA-elements within the ADPRT-promoter region. The presence of the recombinant ADPRT-automodification domain inhibits binding of YY1 to its DNA-recognition site in gel-shift experiments (Fig. 4), suggesting a concerted action of the ADPRT enzyme and YY1 in Pol II-dependent transcription. In earlier studies the distal region of the ADPRT promoter containing negative transcriptional elements was proposed to be involved in the autoregulation of ADPRT expression (8, 9). Here, it is shown that this region contains two YY1-binding sites located within two Alu-associated elements as it has

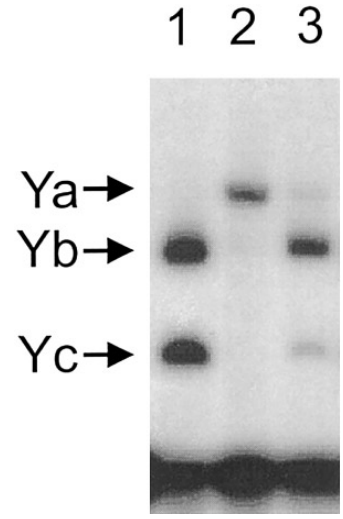

FIG. 4. Interaction of the transcription factor YY1 with the automodification domain of ADPRT (AMD). The autoradiogram of complexes of ^{32}P-labeled YY oligonucleotide (Fig. 1B) with HeLa nuclear proteins after separation in a 4% PAG is shown. The positions of three different complexes Ya, Yb, and Yc are indicated on the left. Before electrophoresis the binding reaction was carried out as described under Materials and Methods with 2 μg of unlabeled duplex AUB oligonucleotide (lane 1). HeLa nuclear extracts were preincubated for 15 min at room temperature with 3 μg of the purified recombinant AMD in the absence (lane 2) or the presence of antibodies raised against the AMD (lane 3).

been demonstrated by Humphrey et al. for other human Alu-retroposons (25). Therefore, the proposed autoregulation may possibly be attributed to a direct interaction between ADPRT and YY1. The ability of YY1 to induce DNA bending may regulate transcription by changing the spatial relationships between transcription activators and components of the basal transcription apparatus (26). In line with this suggestion, a similar mechanism including DNA-bending by the ADPRT enzyme was proposed in a model for the autoregulation of ADPRT-expression (8, 9).

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