Analysis of Nucleotide Sequence-Dependent DNA Binding of Poly(ADP-ribose) Polymerase in a Purified System†

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ABSTRACT: The enzymatic transfer of ADP-ribose from NAD to histone H_1 [defined as trans(oligo-ADP-ribosylation)] or to PARP-1 [defined as auto(poly-ADP-ribosylation)] requires binding of coenzymic DNA. The preceding paper [Kun, E., et al. (2004) *Biochemistry 43*, 210−216] shows that oligonucleotides of dsDNA can serve as coenzymic DNA for PARP-1 trans- or auto-modification activity. Results of DNA−protein binding (EMSA) experiments reported here demonstrate that short DNA oligonucleotides containing the 5′-TGTTG-3′ nucleotide sequence motif preferentially bind to cloned PARP-1 *in vitro*. The same nucleotide sequence motif is responsible for striated myocyte-selective transcription of a contractile protein gene encoding cardiac troponin T (cTnT). Results of experiments reported here demonstrate that mutation of this motif also abolishes the differentiation-dependent activation of the transfected cTnT promoter in myoblasts cultured *in vitro*, indicating that nucleotide sequence-dependent binding of PARP-1 to promoter DNA of the cTnT gene is also necessary for differentiation-dependent activation. Thus, PARP-1 has two types of dsDNA binding activity: (1) nucleotide sequence-dependent binding, analyzed here with EMSA experiments, and (2) coenzymic binding, measured catalytically, which does not depend on the nucleotide sequence of the dsDNA. We hypothesize that the well-known association of PARP-1 with chromatin can be attributed to its stable binding to chromosomal dsDNA, some portion of which is likely to be nucleotide sequence-dependent binding. According to this hypothesis, the distribution of this protein-modifying enzyme in chromatin may be targeted to specific genomic loci and vary according to cell type and developmental stage.

Poly(ADP-ribose) polymerase (PARP-1,[1] EC 2.4.2.30) is a nuclear enzyme thought to be present in all eukaryotic cell types above *Saccharomyces*. At approximately 0.5×10^6 molecules per nucleus, PARP-1 is the second most abundant non-histone nuclear protein (*1*). PARP-1 uses NAD as a substrate to catalyze the addition of poly(ADP-ribose) [$(ADPR)_n$] to acceptor proteins. "Auto-modification" refers to addition of $(ADPR)_n$ to one PARP-1 molecule within a homodimer. "Hetero-" or "trans-modification" occurs when PARP-1 forms a complex with a second acceptor protein such as histone H_1, the most frequent acceptor, with octameric histones (histones within nucleosomes, but not free histones), or with a variety of other non-histone proteins, including a growing list of transcription factors. The secondary structure of $(ADPR)_n$ chains predicts that proteins so modified will be altered in their binding to other proteins or DNA (*2*, *3*). PARP-1 catalytic activity absolutely requires DNA as a coenzyme.

A special significance of this system is that the ADPR donor is NAD, a nucleotide intimately connected to cellular redox systems, which delicately regulate NAD/NADH ratios. Since *only* NAD is the PARP-1 substrate, the redox state of the cell defines, in part, the active versus inactive state of the PARP-1, thereby linking this powerful epigenetic switch to cellular metabolism. Under some circumstances, PARP-1 can become "superactivated", a condition that can lead to diminution of the level of NAD to levels incompatible with cell survival. In cells with extensive DNA breakage, because of ionizing radiation or alkylating drugs, the abnormal binding of PARP-1 to the broken ends of "damaged" DNA leads to an increase in the amount of auto$(ADPR)_n$, ultimately depleting cellular NAD and becoming a component of the cell death pathway (*4*). The role of PARP-1 in these aspects of DNA damage and apoptosis has been extensively studied and its pathophysiologic importance recognized.

The pathophysiologic interpretation fails to predict any physiologic role of PARP-1. DNA breaks are rapidly repaired in normal cellular DNA, so under normal circumstances, this highly abundant chromatin constituent would be inactive if it depended exclusively upon such breaks for its functionality. Nonetheless, $(ADPR)_n$ is known to exist in normal cells (*3*),

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[1] Abbreviations: cTnT, cardiac troponin T; dsDNA, double-stranded DNA; EMSA, electrophoretic mobility shift assay; MCAT core motif, 5′-CATTCCT-3′ nucleotide sequence, a TEF-1 binding site; MCAT-1 element, 23-nucleotide segment of DNA containing a PARP-1 binding site immediately adjacent to a TEF-1 binding site; PARP-1, poly(ADP-ribose) polymerase 1; TEF-1, transcription enhancer factor 1; ADPR, ADP-ribose subunit; $(ADPR)_n$, poly(ADP-ribose) polymer containing n subunits ($n > 3$).

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and many physiological roles have been proposed for PARP-1 in normal cells (see ref *52*). There is substantial evidence indicating association of PARP-1 with a number of transcriptional factors, exerting both positive and negative regulation (*5*). PARP-1 has also been implicated as playing a role in DNA replication, a function supported by its colocalization with DNA polymerase (*6*) and localization at DNA replication sites (*7*). The conceptual difficulty in this field has been the persistent reference to "broken DNA" as the exclusive coenzyme of PARP-1 and a "DNA strand break" as the sole site in DNA to which a PARP-1 molecule binds.

The hypothesis that broken DNA is the exclusive coenzyme for PARP-1 enzymatic activity was recently disproven using oligomeric double-strand DNAs (dsDNA) that lacked either single-strand breaks or 5′- and 3′-termini. The results showed that intact dsDNAs serve as efficient coenzymes of poly(ADP-ribose) polymerase-catalyzed adenosine diphosphoribose transfer from NAD^+ to histone H_1 (trans-modification) or to PARP-1 (auto-modification) (*8*). More importantly, from a perspective of DNA binding, the K_a for binding of PARP-1 to dsDNA was found to be at least 100 times lower than that for broken DNA in the trans-modification of histone H_1. These results demonstrate that physiologically occurring dsDNAs are more efficient PARP-1 coenzymes than DNA containing single-strand breaks and, therefore, provide a basis for understanding the physiological role of PARP activity in normal, intact cells.

Recent reports suggest that PARP-1 binds to dsDNA in a nucleotide sequence-dependent fashion. Bacterially expressed human PARP-1, and PARP-1 in nuclear extracts from chick embryos, bind to a restricted region of the MCAT-1 element of the transcriptional promoter of the cTnT gene (*9*). PARP-1 and PARP-1−VP 16 fusion proteins bind to the HTLV-I Tax responsive element (*10*). PARP-1 in chromatin extracts associates with DNA fragments from the PARP-1 gene transcriptional promoter (*11*). The relationship between coenzymic dsDNA binding to PARP-1, measured by enzymatic activity, and nucleotide sequence-dependent binding of dsDNA is unknown.

The preceding paper (*52*) demonstrates that DNA of any nucleotide sequence, including AT homopolymers, serves efficiently as coenzymes for both trans- and auto(poly-ADP-ribosylation). Here we report experiments in which the nucleotide sequence-dependent binding of purified human PARP-1 to dsDNA *in vitro* is analyzed directly using electrophoretic mobility shift assays (EMSAs). The PARP-1 dsDNA binding preference is also analyzed indirectly, through analysis of PARP-1-dependent transcriptional promoter activity in transfection experiments. The results indicate that the presence of PARP-1 in chromatin can be attributed to its direct binding to chromosomal DNA and that a portion of that binding occurs at preferred sites such as that identified here. Nucleotide sequence-dependent binding of PARP-1 to chromosomal DNA may be involved in the regulation of other genes, and the results presented here provide a basis for testing PARP-1's role in chromatin structure and function.

MATERIALS AND METHODS

Synthetic DNA Probe for EMSA Analysis of PARP-1−DNA Binding. The synthetic, 42-nucleotide dsDNA polynucleotides (Qiagen) used as a probe in these studies had an upper strand nucleotide sequence of 5′-GAGTGTTG**CATTCCT**CTCTGGGCGCCGGG*CAggtaCCT*GCTG-3′. This nucleotide sequence matches that of the chicken cTnT gene promoter region (nucleotides −102 to −59, relative to the transcription initiation site; see refs *12*−*14*). It contains an intact core TEF-1 binding motif (bold type) within the MCAT-1 element (underlined). The MCAT-2 motif nucleotide sequence (italicized) has been altered (lowercase) in a manner that disrupts its core TEF-1 recognition motif. Complementary strands of the probe were annealed and radiolabeled at their 3′-termini with [α-^{32}P]dCTP with Klenow fragment.

Table 1: Nucleotide Sequences of Oligonucleotides Used as Coenzymes and EMSA Competitors[a]

A. competitors with naturally-occurring MCAT-flanking regions.			
1.	MCAT 1	5'	TGCAAGTGTTG**CATTCCT**CTCTG 3'
2.	MCAT 2	5'	TGCGCCGGGCA**CATTCCT**GCTGC 3'
3.	SV40-CAT	5'	TGCCTGACACA**CATTCCT**CAGCT 3'
B. mutant oligonucleotide competitors			
1.	k1	5'	TGCAAGCGTTG**CATTCCT**CTCTG 3'
2.	k2	5'	TGCAAGTATTG**CATTCCT**CTCTG 3'
3.	k3	5'	TGCAAGTGCTG**CATTCCT**CTCTG 3'
4.	k4	5'	TGCAAGTGTCG**CATTCCT**CTCTG 3'
5.	k5	5'	TGCAAGTGTTA**CATTCCT**CTCTG 3'
6.	k6	5'	TGCAAGCGTTA**CATTCCT**CTCTG 3'
7.	k7	5'	TGCAAGTATCG**CATTCCT**CTCTG 3'
8.	k8	5'	TGCAAGTGCTGA**ATTCCT**CTCTG 3'
9.	k9	5'	TGCAAGATAGC**CATTCCT**CTCTG 3'
10.	k10	5'	GATCACTGTTGACCTCGACACAT 3'
11.	k13	5'	TGCAATATAGC**CATTCCT**CTCTG 3'
12.	k14	5'	TGCAGTATAGC**CATTCCT**CTCTG 3'
C. non-specific control oligonucleotide			
	NSC	5'	TGGTCGTATCTTCACCGTATCTG 3'
D. promoter mutations			
1.	m3	5'	TGCAAGGTTTG**CATTCCT**CTCTG 3'
2.	m4	5'	TGCAAGTGGGG**CATTCCT**CTCTG 3'

[a] Complementary oligonucleotides were annealed prior to use in catalytic or EMSA competition experiments, but only the upper strand nucleotide sequence is shown here. The nucleotides of the core motif for TEF-1 binding are shown in bold type. Nucleotides that vary with respect to the MCAT-1 oligonucleotide sequence are underlined. NSC represents the nonspecific control oligonucleotide.

EMSA and EMSA Competition Experiments. EMSA and EMSA competition experiments were carried out as described previously (*9*, *15*) using the 42 bp probe described above. For competition EMSA experiments, double-stranded DNA oligonucleotides (23 bp, Operon or Invitrogen) were synthesized with the nucleotide sequence compositions listed in Table 1. Each binding and binding competition assay mixture (70 μL) in 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 10 mM $MgCl_2$, 10 mM DTT, and 10% (v/v) glycerol contained 35 fmol of the ^{32}P-labeled probe (final concentration of 100 nM) and unlabeled competitor DNA at molar ratios with the probe indicated in the figures. To initiate the binding or binding competition reactions, 0.5 μg of PARP-1

(Trevigen) was added to the mixture (final PARP-1 concentration of ~60 nM), and after incubation for 20 min at room temperature, the PARP-1/DNA mixtures were loaded onto a 6 or 8% native polyacrylamide gel (60/1 acrylamide/bisacrylamide ratio), electrophoresed in 0.5× TBE buffer for approximately 2 h, dried under vacuum, and then autoradiographed with an intensifying screen at −80 °C overnight. The relative band intensity for competition titration experiments was measured using NIH Image and scanned autoradiographs.

Promoter−Reporter Plasmid Constructs. The promoter−reporter constructs used in this study contain 129 nucleotides of the chicken troponin T gene promoter that contains the probe sequence outlined above. The selective expression of this promoter construct was initially established using promoter−reporter constructs in Bluscript K/S+ with this promoter linked to the chloramphenicol acetyltransferase reporter gene (pBS.CAT) containing 38 nucleotides of exon 1 (*12*, *14*). Luciferase reporter constructs were generated by digestion of the above promoter inserts with *Xho*I, located in the polylinker, and *Nae*I, which cuts at nucleotide 15 of exon 1, and subcloned into the PGL2 plasmid (Promega, Madison, WI). The same pattern of regulation of this promoter was recently demonstrated *in vivo* using transgenic zebrafish (*16*).

Cell Culture and Transfection. The QM-7 myogenic cell line was cultured under growth and differentiation conditions (*17*) and transfected with promoter−reporter plasmids as described previously (*9*, *12*, *15*, *16*, *18*) except for the use of Effectene transfection reagent (Qiagen). Cell lysis and luciferase assays were conducted using reagents according to the manufacturer's instructions (Promega).

RESULTS

EMSA Analysis of Nucleotide Sequence-Dependent Binding of PARP-1 to dsDNA. The binding of purified PARP-1 to dsDNA oliognucleotides was analyzed in an EMSA system which allows bound and unbound DNA to be electrophoretically separated (Figure 1). After nondenaturing acrylamide gel electrophoresis, labeled probe DNA migration is shifted, indicating the presence of a stable PARP-1−dsDNA complex (Figure 1, lane 1). When a 100- or 200-fold excess of unlabeled probe DNA is added prior to electrophoresis, the labeled PARP-1−dsDNA complex is almost completely displaced (Figure 1, lanes 10 and 11). Similarly, addition of the same molar ratio of a shorter (23 bp) oligonucleotide containing the MCAT-1 element nucleotide sequence (Table 1A, line 1) also effectively competes for PARP-1 binding (Figure 1, lanes 8 and 9). By contrast, the control 23 bp oligonucleotide containing a nucleotide sequence unrelated to that present in the MCAT-1 element (Table 1C) is unable to compete for PARP-1 binding (Figure 1, lanes 6 and 7).

The displacement of labeled MCAT-1 dsDNA−PARP-1 complexes by an increase in the molar ratio of the unlabeled 23 bp MCAT-1 competitor oligomer is quantitatively indistinguishable from that obtained with self-competition using the full 42 bp probe (in Figure 2A, compare empty squares and circles). Maximal displacement of labeled probe is observed when unlabeled competitor dsDNA is present between a 100- and 200-fold excess in both cases. At these

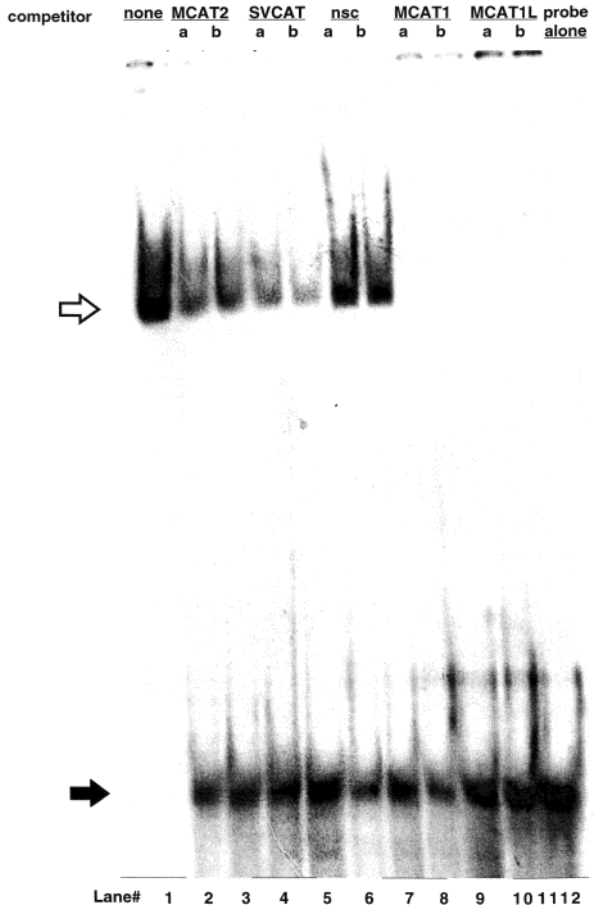

FIGURE 1: EMSA competition by naturally occurring nucleotide sequences. Competitor DNAs: lane 1, none; lanes 2 and 3, MCAT-2; lanes 4 and 5, SV 40-CAT; lanes 6 and 7, NSC; lanes 8 and 9, MCAT-1; lanes 10 and 11, MCAT-1L (L designates the 42 bp probe); and lane 12, probe alone. The ratio of competitor to probe DNA was 100 in lanes 2, 4, 6, 8, and 10 and 200 in odd-numbered lanes. All competitors were 23mer dsDNAs except in lanes 10 and 11, in which the 42mer MCAT-1 dsDNA was used. The probe was radiolabeled MCAT-1 42mer. Competitor DNA sequences are given in Table 1.

same molar ratios, the nonspecific competitor displaces only approximately one-third of the labeled MCAT-1 dsDNA−PARP-1 complex [Figure 2A (●)]. When represented on double-reciprocal plots, displacement of the probe indicates a K_a of approximately 100 nM, similar to the K_m obtained for coenzymic binding of dsDNA during PARP-1 catalysis (see ref *52*). These results demonstrate that PARP-1 stably binds to dsDNA and preferentially binds to a nucleotide sequence common to both the 23 and 42 bp oligonucleotides, specifically those contained within the MCAT-1 element.

The 23 bp MCAT-1 element is part of the transcriptional control region of a contractile protein gene. It contains the optimal binding site (5′-CATTCCT-3′, MCAT motif) for transcriptional enhancer factor 1 (TEF-1) family of DNA-binding proteins (*15*, *19*−*22*). Previous experiments had suggested that PARP-1 and TEF-1 bind to the MCAT-1 element in a cooperative fashion (*9*). To determine if the TEF-1 binding site plays a primary role in the binding of purified PARP-1, competition experiments were performed with oligonucleotides containing optimal TEF-1 binding sites but with different flanking regions found in other naturally occurring TEF-1 binding elements (in Table 1A, compare rows 2 and 3 with row 1). Each of these competitors was

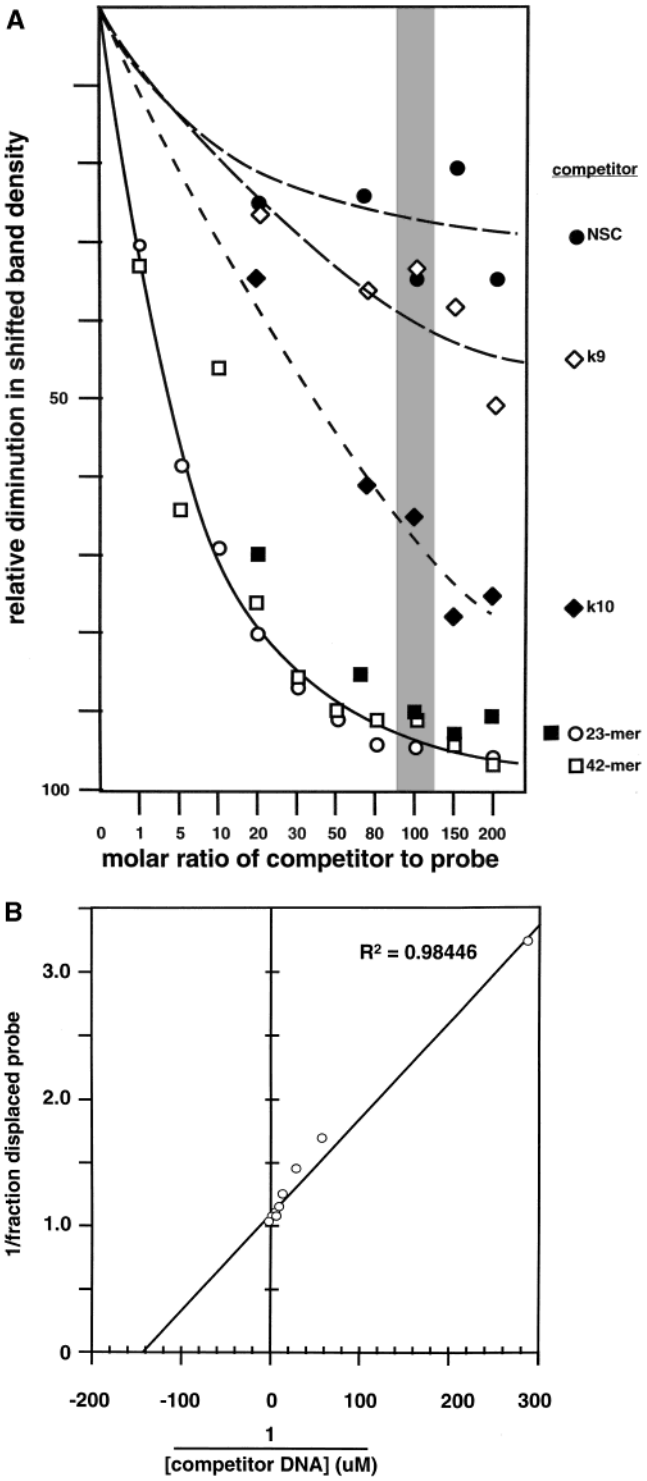

FIGURE 2: Comparison of competitive displacement of PARP-1−MCAT-1 binding complexes by oligonucleotides with varying nucleotide sequences. (A) Competition by oligonucleotides as indicated at the right. The nucleotide sequence of each competitor oligonucleotide is given in Table 1. (B) Double-reciprocal plot of competition by 23mer MCAT-1 oligonucleotide (empty circles from panel A) as computed by KaleidaGraph. The nonreciprocal value for the *x* intercept is 69 nM, and the R^2 value for this solution is 0.98.

found to compete less effectively for PARP-1 binding in EMSA analysis than either the 42 bp probe or the 23 bp dsDNA oligonucleotide MCAT-1 element competitor (in Figure 1, compare lanes 2−5 with lanes 8−11). This shows that specific binding of PARP-1 to MCAT-1 dsDNA depends

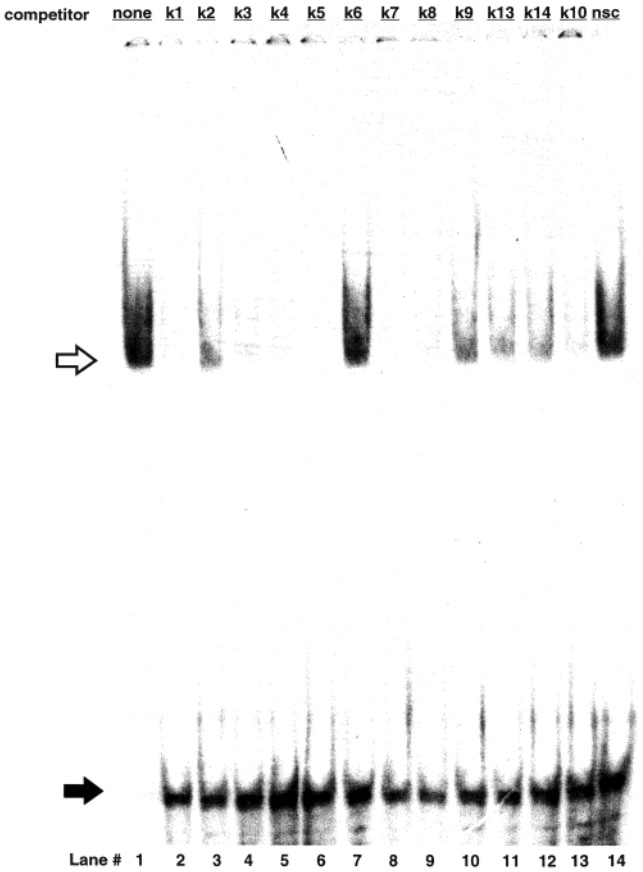

FIGURE 3: EMSA competition by synthetic oligonucleotide sequences. Competitor DNAs: lane 1, no competitor; lanes 2−13, mutant oligonucleotides (Table 1B); and lane 14, nonspecific control oligonucleotide (Table 1C). All competitor dsDNAs were 23mer oligonucleotides at a molar ratio of oligonucleotide to probe of 100. Other details as in the legend of Figure 1.

primarily upon nucleotides lying outside the TEF-1 binding site.

We focused on the 5′-flanking region of the MCAT-1 element because previous work indicated that alteration of these nucleotides inhibited low-mobility complex formation (attributable, in part, to PARP-1 binding) in EMSA competition experiments using nuclear extracts (*9*, *15*). A series of 23 bp oligonucleotide dsDNAs with variations in the nucleotide sequence of the 5′-flanker (Table 1B) were used for EMSA competition experiments (Figure 3). A 23 bp oligonucleotide probe containing a complete alteration of the TEF-1 binding site and a single nucleotide immediately upstream of the 5′-TGTTG-3′ flanking sequence (k10, Table 1B) competed for PARP-1 binding almost as effectively as the intact MCAT-1 element competitor [Figure 3 (lane 13) and Figure 2 (◆)]. On the other hand, an oligonucleotide competitor in which all five nucleotides immediately flanking the 5′-end of the TEF-1 binding site were changed to an unrelated sequence arrangement (k9, Table 1B) competed poorly for PARP-1−MCAT-1 probe binding [Figure 3 (lane 10) and Figure 2 (◇)]. Oligonucleotide competitors that included the k9 changes and with additional changes upstream (k13 and k14, Table 1B) competed no less efficiently than k9 itself (in Figure 3, compare lanes 11 and 12 to lane 10). These results support the conclusion that it is the 5′-TGTTG-3′ pentanucleotide sequence that is primarly

responsible for nucleotide sequence-dependent binding of purified human PARP-1 to the MCAT-1 element.

Competition experiments employing oligonucleotides in which individual nucleotides were changed within the 5′-TGTTG-3′ motif either singly or in pairs (Figure 3, lanes 2−9) indicate that some positions and/or residues may be more important than others for PARP-1 binding. A G → A replacement at position 2 (Table 1B, k2, row 2) reduced EMSA competition to an extent almost comparable to that with modification of the entire 5′-TGTTG-3′ sequence (in Figure 3, compare lane 3 to lane 10). Interestingly, methylation of the G residue at position 2 also blocks formation of a low-mobility complex with nuclear extracts that is dependent upon PARP-1 binding (*9*, *15*), indicating the importance of this particular residue. Single-nucleotide substitutions at other positions had a much weaker effect on EMSA competition (Figure 3, lanes 2 and lanes 4−6). Additional experiments (not shown), in which each nucleotide position in the $T_1G_2T_3T_4G_5$ motif (where the position is indicated with a subscript) was replaced with all three alternative nucleotides and the effect on PARP-1−MCAT-1 binding analyzed by EMSA competition experiments as described above, indicated that the relative importance of each position for PARP-1 binding is as follows: $G_2 > T_3 > G_5 > T_1 > T_4$.

Changing two nucleotides at once within the 5′-TGTTG-3′ motif indicates that this motif may not be unique for PARP-1 binding. Substitutions at positions 1 and 5 (Table 1B, rows 1 and 5) have little effect upon EMSA competition when present singly but in combination (Table 1B, row 6) profoundly reduce the level of competition (in Figure 3, compare lanes 2 and 6 with lane 7). Conversely, the substantial reduction in the level of competition resulting from G → A substitution at position 2 (Table 1B, row 2) can be almost completely compensated by a simultaneous substitution at position 4 (Table 1B, row 7; in Figure 3, compare lane 3 with lane 8). These results support the conclusion that PARP-1 may preferentially bind to more than one nucleotide sequence motif (see Discussion) and/or that PARP-1 binding to dsDNA may be more sensitive to DNA structure than to the identity of individual nucleotides *per se*.

Functional Analysis of Nucleotide Sequence-Dependent Binding of PARP-1 during Muscle Differentiation in Vitro. The same pentanucleotide identified above has been shown to control the striated myocyte-selective transcription of the chicken cTnT gene, both *in vitro* (*15*) and in an *in vivo* transgenic zebrafish model (*16*). A muscle cell culture model was used here to determine if this pentanucleotide sequence is also involved in the developmental activation of an MCAT-1-dependent promoter during the early steps of muscle differentiation. During the *in vitro* differentiation of skeletal muscle, mononucleated myoblasts proliferate and then fuse to form multinucleated myotubes, which then become contractile. Myoblasts do not express contractile protein genes, but after fusion, the myotube nuclei activate a cohort of contractile protein genes (*23*, *24*), including the cTnT gene. The cTnT gene is expressed transiently during early stages of skeletal muscle differentiation (*14*, *25*) and then transcriptionally repressed in midfetal development (*26*, *27*).

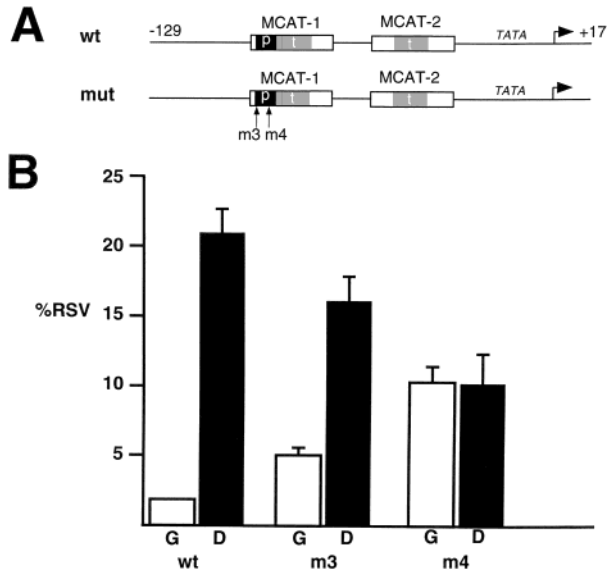

FIGURE 4: Effect of PARP-1 binding and activity on transcription in differentiating muscle. (A) Structure of wild-type and mutant promoters. The top model is the wild-type promoter fragment of the chicken cTnT gene containing 129 nucleotides upstream of the transcription initiation site (right-angle arrow). The positions of the MCAT-1 and MCAT-2 elements (for the nucleotide sequences, see Table 1A) are indicated as the TATA box. The bottom model shows the positions of mutations m3 and m4, indicated by vertical arrows. The nucleotide sequence of each mutation is given in Table 1C. Activity of wt and mutant promoters after transfection into QM7 muscle cultures under growth (G, white bars) or differentiation (D, black bars) conditions. Promoter activity was determined by a luciferase assay and indexed relative to that of RSV-driven luciferase. Error bars show the standard error of the mean.

In cell culture, the switch between myoblast mitotic expansion (growth) and the onset of myoblast fusion (differentiation) can be controlled by adjusting the medium serum content and myoblast cell density. Thus, expression of a transfected promoter−reporter gene construct can be assayed both before and after differentiation to determine its pattern of differentiation-dependent regulation. A luciferase reporter construct under the transcriptional control of a promoter fragment of the cTnT gene (Figure 4A) was transfected into cultures of the QM7 myoblast cell line under growth or differentiation conditions. Luciferase activity directed by the wild-type promoter fragment is low in myoblasts under growth conditions but increases dramatically after these myoblasts are induced to differentiate (Figure 4B, wt G and D, respectively). An identical promoter construct, but with alteration of two nucleotides in the center of the five-nucleotide PARP-1 binding site (m4, Table 1D), is equally active in myoblasts and myotubes. Therefore, substantial alteration of the pentanucleotide sequence of the PARP-1 binding site converts this developmentally regulated promoter into one that is developmentally unregulated.

An intermediate level of regulation, slightly elevated in myoblasts and slightly repressed in myotubes, is obtained after an inversion the first nucleotide of the PARP-1 binding site with its upstream nearest neighbor (m3, G and D, respectively, in Figure 4B; Table 1D). Alteration of the first nucleotide of the PARP-1 binding site slightly diminishes the affinity of PARP-1−dsDNA binding (Figure 2, k10). This is consistent with the notion that regulatory strength may be

related to the affinity with which PARP-1 binds to dsDNA with a varying nucleotide sequence composition.

DISCUSSION

The experiments reported here show that purified PARP-1 binds to dsDNA *in vitro* in a stable and nucleotide sequence-dependent fashion. The five-nucleotide site of nucleotide sequence-dependent binding of purified PARP-1 observed here has a 5′-TGTTG-3′ sequence and is identical to the site necessary for striated myocyte-selective transcription controlled by the cTnT gene promoter. A two-nucleotide point mutation within the same pentanucleotide site abolishes cell-selective transcription in embryo cell culture (*9*, *15*) and in transgenic zebrafish embryos (*16*). Artificial promoter constructs, consisting of five MCAT-1 repeats located upstream of a heterologous proximal promoter region, show the same strict cell selectivity but have promoter strength in muscle cells increased to a level comparable to that of viral promoters (*15*). Similar artificial promoters with mutation of the PARP-1 pentanucleotide binding site are cell non-specific, comparable to TEF-1-dependent promoters lacking the PARP-1 pentanucleotide flanking sequences (*15*, *16*). The binding interactions between purified PARP-1 and the 5′-TGTTG-3′ dsDNA pentanucleotide sequence in dsDNA oligos observed here *in vitro* are sufficient to fulfill the regulatory roles attributed to this site *in vivo*.

A binding site for members of the TEF-1 family of transcriptional regulatory proteins lies immediately downstream of the PARP-1 site. Apparent cooperative binding occurs when both PARP-1 and TEF-1 are mixed with MCAT-1 dsDNA (*9*), which is reminiscent of the cooperative binding of histones and PARP-1 to DNA (*28*). Such cooperative binding between proteins with different nucleotide sequence preferences suggests that such cobinding *in vivo* not only can provide additional stability and regulatory complexity but also adds a second combinatorial component that could affect PARP-1 locus-selective binding in chromatin.

A Broad Role for PARP-1 in Gene Regulation. Other recently published reports also implicate PARP-1 in gene regulation by way of nucleotide sequence-dependent binding to DNA (*10*, *11*, *29*) and with binding site sequence preferences that differ in varying degree from those of the 5′-TGTTG-3′ motif identifed here. PARP-1 has been shown to bind in a nucleotide sequence-dependent fashion to the Tax response element (TRE) which contains the 5′-GTTG-3′ nucleotide sequence in the critical binding region, matching four of five nucleotides identified here as the PARP-1 binding site in the MCAT-1 element (*30*). PARP-1 binds to a promoter region of the Reg gene (which promotes pancreatic β cell replication) that is not at all or only slightly homologous with the pentanucleotide PARP-1 dsDNA binding site reported here (*29*). PARP-1 binds to negatively supercoiled matrix attachment sequences (*31*) which also are not at all or only slightly homologous with this pentanucleotide. Competition EMSA experiments presented here, using oligos with single- or double-nucleotide substitutions, also support the conclusion that more than one sequence motif may be preferentially recognized by PARP-1. PARP-1 has been shown to avidly bind structurally complex DNAs (*32*), and it is possible, therefore, that it recognizes a structural feature common to dsDNAs with differing nucleotide sequences. More detailed experiments will be necessary to determine the full range of DNA binding properties and preferences of PARP-1.

A growing body of evidence further indicates that PARP-1 binds directly, and in the absence of DNA, to cellular transcription factors such as NF-kappa B (*33*−*36*), AP-2 (*37*), B-MYB (*38*, *39*), basic helix−loop−helix protein E47 (*40*), YY-1 (*41*, *42*), MAX (*43*), and viral activators (*44*, *45*). In fly pupae, PARP-1 has been implicated in transcriptional derepression by modulating the chromatin conformation in polytene chromosomes, and the extent of chromosome "puffing" at specific loci is reduced in PARP-1 knockout flies (*46*, *47*). Murine PARP knockout models also show defects in transcriptional regulation (*48*−*50*). Genetic knockout of PARP-1 in flies has no effect on larval development but is required for pupal development (*51*).

While these numerous reports support the notion that PARP-1 is involved in the regulation of gene expression, the mechanistic basis for such regulation has been unclear partly because of the perceived need for broken or damaged DNA for activation of PARP-1 enzymatic activity, and the implicit assumption that PARP-1's association with native ("undamaged") chromatin occurs via protein−protein interactions alone. The results of this study overcome these difficulties by showing that PARP-1 binds directly to dsDNA and in a manner that shows a preference for a particular nucleotide sequence motif. Previous results, and the preceding paper (*52*), show that dsDNA, regardless of its nucleotide sequence composition, is far more efficient as a coenzyme for PARP-1 enzymatic activity than broken or damaged DNA (*8*). Taken together, these findings show that PARP-1 preferentially interacts with dsDNA for both its coenzymic and nucleotide sequence-dependent DNA binding activities.

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