Poly(ADP-ribose) Binds to Specific Domains in DNA Damage Checkpoint Proteins*

Poly(ADP-ribose) is formed in possibly all multicellular organisms by a family of poly(ADP-ribose) polymerases (PARPs). PARP-1, the best understood and until recently the only known member of this family, is a DNA damage signal protein catalyzing its automodification with multiple, variably sized ADP-ribose polymers that may contain up to 200 residues and several branching points. Through these polymers, PARP-1 can interact noncovalently with other proteins and alter their functions. Here we report the discovery of a poly(ADP-ribose)-binding sequence motif in several important DNA damage checkpoint proteins. The 20-amino acid motif contains two conserved regions: (i) a cluster rich in basic amino acids and (ii) a pattern of hydrophobic amino acids interspersed with basic residues. Using a combination of alanine scanning, polymer blot analysis, and photoaffinity labeling, we have identified poly(ADP-ribose)-binding sites in the following proteins: p53, p21CIP1/WAF1, xeroderma pigmentosum group A complementing protein, MSH6, DNA ligase III, XRCC1, DNA polymerase ε, DNA-PKcs, Ku70, NF-κB, inducible nitric-oxide synthase, caspase-activated DNase, and telomerase. The poly(ADP-ribose)-binding motif was found to overlap with five important functional domains responsible for (i) protein-protein interactions, (ii) DNA binding, (iii) nuclear localization, (iv) nuclear export, and (v) protein degradation. Thus, PARPs may target specific signal network proteins via poly(ADP-ribose) and regulate their domain functions.

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Four different poly(ADP-ribose) polymerases (PARPs)1 have been identified in multicellular organisms to catalyze poly(ADP-ribose) [PAR] synthesis from NAD+ (1). The best understood member of the PARP family is PARP-1. As a highly conserved, multimodular 113-kDa protein, PARP-1 shares many hallmarks with proteins of the cellular DNA damage signal network (2). Like ATM, ATR, DNA-PK, and p53 (3), PARP-1 binds to and is activated by DNA strand breaks and interacts with several other DNA damage checkpoint proteins. In addition, PARP-1 can regulate its protein and DNA interactions by catalyzing its automodification with multiple PAR molecules. An alternative product of the PARP-1 gene is sPARP-1, a 55.3-kDa protein with nuclear localization and sequence identity to the catalytic domain of PARP-1 (4). It is also activated by DNA damaging agents but apparently does not require DNA strand breaks for activation. Tankyrase, another member of the PARP family, is a multimodular 142-kDa protein with a catalytic domain homologous to PARP-1 (5). In vitro, tankyrase catalyzes its automodification as well as the modification of the telomere-specific protein TRF1 in a DNA-independent manner. The third member of the PARP family, PARP-2, is a 62-kDa protein (6, 7). It is activated by DNA strand breaks, but its function is unknown. Finally, vault PARP, a 193-kDa protein, has recently been identified as a protein component of vaults (8), large ribonucleoprotein complexes of unknown function (9). Vault PARP catalyzes the poly(ADP-ribose)ylation of a major vault protein.

The presence of a larger family of PARPs in multicellular organisms illustrates the potential importance of PAR in regulating cellular functions. We have previously shown that the noncovalent binding of PAR to p53 (10) or members of the MARCKS protein family (11) may drastically alter several domain-specific functions of these proteins. Apart from its specificity, PAR binding to proteins is exceptionally strong. For example, we have previously found that PAR-histone complexes resist phenol partitioning, strong acids, chaotropes, detergents, and high salt concentrations (12). These observations led us to postulate the involvement of a specific sequence motif in PAR binding to proteins. The results of the present study confirm the presence of a PAR-binding consensus sequence in a family of important DNA damage checkpoint proteins.

EXPERIMENTAL PROCEDURES

Peptides and Proteins—The following peptides derived from human proteins were used (Chiron Mimotopes, Clayton, Victoria, Australia): MARCS effector peptides, alanine substituted as described in Fig. 1B; CDN1 (9–32), RNPGCSSKACRRLFPGVDSQUEL; CDN1 (140–163), BKREQRTSMDDPFIHISRKRLLFSKRK; XPA (25–46), RASIERRQRPMMLRLQARLAAR; XPA (215–237), KQKKFDKVKELRRAVRSSVRK; H2A (227–249), RKRKFQDADYQLLRL; KU70 (295–317), KVARKRKMRVNGSLRK; DN3 (12–34), KRRGATCCKCERKVKVRGC; DN3 (779–800), SRKAPPSFSSTKAEGLSNS; DPOB (26–48), KNVQAHIYNATRKAASVIAKY; DPOB (39–61), RKAAAVMPLHPIKSGAAEKK; Ku70 (243–264), BKVR4AKTRKR4SLRLKLKLN; Ku86 (124–145), KKFRRHIEIFTDLSRSFKSQ; DNA-PKcs (3728–2752), RRRFMQRQIEKLSMYARKVGEKQFK; HB2 (179–199), KELKVMDSLVRFFSAP; NOS2 (505–525), KRRKRRREILVKLVLFLA; caspase-activated DNase (CAD) (148–169), RFQSKSG.
the PAR-binding MARCKS effector peptide. A, polymer blot analysis of alanine substituted peptides under high stringency (1 M NaCl) conditions. The sequence of the MARCKS effector peptide M with three clusters of basic amino acids (clusters 1–3) is indicated. The basic amino acids of one (M1–M3) or two clusters (M4–M6) were substituted by alanine and the hydrophobic positions were subjected to polymer blot analysis (left panels). The PAR binding of the effector peptide with hydrophobic residues or serines changed to alanine was tested as indicated in the right panels. The autoradiograms of the polymer blot assays of the different peptides (0.5 μg each) are shown. B, the sequences of all synthetic peptides are listed, and the residues that were changed to alanine are underlined. C, alignment of the PAR-binding core histone sequences with the MARCKS effector peptide. The PAR-binding sequences are given with the N-terminal residue numbers. The hydrophobic positions are shown in bold against a dark gray background, and the basic residues against a light gray background. The putative PAR-binding consensus motif is indicated (a, hydrophobic aa; b, basic aa).

Polymers analyses—Polymers analysis was performed as described (16). Pure peptides or proteins were dot-blotted directly onto nitrocellulose membrane. The membrane was rinsed with three changes of TBST (10 mM Tris, pH 7.4, 0.15 M NaCl, 0.05% Tween 20). The incubation mixtures (15 μl) were prepared as described (13), vascularized with a N-terminal His tag (14), and the hMutS heterodimer, hMSH2, and hMSH6 were purified from a baculovirus system as described (15).

Polymer blot analysis—Polymer blot analysis was performed as described (16). Pure peptides or proteins were dot-blotted directly onto nitrocellulose membrane. The membrane was rinsed with three changes of TBST (10 mM Tris, pH 7.4, 0.15 M NaCl, 0.05% Tween 20). (32P)PAR (0.5 μCi/nmol ADP-ribose; 0.5–1.0 nmol of total ADP-ribose; mean PAR size, 20 residues) were diluted to 10 ml with TBST and added to the nitrocellulose. For incubation for 1 h at room temperature with gentle agitation, the membrane was washed with TBST containing 1 M NaCl to eliminate unspecific binding. The results of polymer blot analysis were only considered positive if PAR binding resisted the high stringency (polymer blot analysis). The autoradiograms of the polymer blot assays of the different peptides (0.5 μg each) are shown. B, the sequences of all synthetic peptides are listed, and the residues that were changed to alanine are underlined. C, alignment of the PAR-binding core histone sequences with the MARCKS effector peptide. The PAR-binding sequences are given with the N-terminal residue numbers. The hydrophobic positions are shown in bold against a dark gray background, and the basic residues against a light gray background. The putative PAR-binding consensus motif is indicated (a, hydrophobic aa; b, basic aa).

Characterization of a PAR-binding Peptide by Alanine Scan—The starting point for our studies was a 25-amino acid region encompassing the MARCKS/MARCKS-Related Protein effector domain. Using site-directed mutagenesis, we have previously demonstrated that this domain is both necessary and sufficient for noncovalent PAR binding to MARCKS proteins (11). A series of mutated effector peptides was generated, dot-blotted onto nitrocellulose membranes, and examined for PAR binding (polymer blot analysis) under high stringency (1 M NaCl) conditions (Fig. 1). As expected, PAR bound strongly to the native effector domain (M; Fig. 1A), and a 7-fold excess of competing poly(A) (11) or a 10-fold excess of sonicated calf thymus DNA (17) did not reduce PAR binding (cf. “Experimental Procedures”). When clusters of basic amino acids were substituted by alanines individually (M1–M3) or in pairs (M4–M6), PAR binding was not abolished (Fig. 1A). Notably, alanine substitution of two clusters with up to 10 basic residues re-
FIG. 2. PAR binding to p21. A, polymer blot analysis of p21 and two p21 peptides. The autoradiogram reveals an interaction of p21 (0.5 μg) with PARP-1-bound [32P]PAR (left panel; lysozyme, negative control). The right panel shows polymer blot analysis of the indicated p21 peptides (0.5 μg) with free [32P]PAR. B, saturation of photoincorporation of photoactive polymers into p21 protein. 1.2 pmol of [32P]PAR containing azido groups was photolysed after addition of increasing amounts of p21 (0.1–1.1 μg). The photolabelled protein was separated from unbound probe by SDS-PAGE. The autoradiogram is shown, and the incorporation of bound probe by SDS-PAGE. The autoradiogram was shown by scanning densitometry of the autoradiographic bands. C, protection against photolabeling of p21 by photoactive PAR in the presence of an increasing amount of PAR as competitor. The amount of 2-azido-[32P]PAR13/14-mer was 1.1 pmol, and the molar ratio of unlabeled to photoactive probe was increased up to 40. Incorporation of photoactive probe into p21 protein (22 pmol) was followed by autoradiography and quantified by scanning densitometry. The position of the unbound photoactive PAR is indicated.

duced the binding only slightly (Fig. 1A, M4–M6), suggesting that positively charged amino acids do not significantly contribute to PAR binding. Changing all hydrophobic amino acids of the effector peptide to alanine virtually abolished PAR binding (Fig. 1A, M13), and became undetectable when an additional six N-terminal lysines were substituted by alanines (M12). Alanine substitution of just three (M14) or four phenylalanines (M16) at a time drastically reduced PAR binding and became undetectable by polymer blot analysis when the N-terminal lysine cluster was replaced as well (M15). An altered version of the peptide, in which the serine residues in addition to cluster 1 of basic amino acids was replaced by alanines, was less informative (Fig. 1A, M11). Taken together, these results demonstrate the importance of selected hydrophobic amino acids for PAR binding.

Strategy for the Identification of PAR-binding Proteins—With the sequence motif of the MARCKS effector peptide at hand, we searched for the occurrence of similar motifs in other proteins. Fig. 1C shows a motif alignment with sequences identified in the core histones H2A, H2B, H3, and H4 (16). A common feature of the sequences is the presence of a pattern of hydrophobic amino acids interspersed with basic residues, and a cluster of basic amino acids at the N-terminal side of the peptides. All these peptides tested positive in the polymer blot assay (data not shown). We used the C-terminal part to construct amino acid profiles with various combinations and expansions of the hydrophobic amino acid spectrum of the histones. These profiles were used to search the SWISSPROT data bank with the generalized sequence profile method (19). From the matches, the proteins that were likely to interact with PAR judging from their physiological function and subcellular localization were investigated for the additional presence of a cluster of basic residues at the N-terminal side. Another strategy was to search for the PAR-binding motif in proteins that were known or suspected to interact with PAR-1. Thus, the PAR-binding motif in each protein was typically detected by sequence analysis and biochemical testing.

PAR Binds to the Cyclin-dependent Kinase Inhibitor p21—The cyclin-dependent kinase (Cdk) inhibitor p21 directly mediates growth arrest by inhibiting the kinase activity of a wide range of cyclin-Cdk complexes (20, 21). Since PARP-1 has been implicated as a DNA damage checkpoint protein affecting cell cycle activity (22, 23), we tested p21 as a PAR-binding protein. As shown in Fig. 2A, PARP-1-bound PAR interacted noncovalently with human p21 in the polymer blot assay. Examination of the p21 primary structure revealed a stretch of amino acids similar to the putative PAR-binding motif. A synthetic peptide corresponding to this C-terminal region (aa 140–163) was found to bind PAR in the polymer blot assay, whereas a peptide from the N-terminal part of p21 (aa 9–32), which did not show the putative PAR-binding motif, was not able to interact with PAR polymers (Fig. 2A).

In a parallel approach, photoaffinity labeling was used to confirm the results obtained with blot assays. PAR containing a photoactive azido group were prepared (18) and radiolabeled with 32P. p21 protein was incubated with [32P]2-azido-PAR of two size classes (13/14-mer) and analyzed by SDS-PAGE after UV irradiation. The autoradiogram in Fig. 2B shows that p21 was labeled in a UV-dependent manner. The C-terminal p21 peptide (aa 140–163) could also be photolabeled, whereas the N-terminal peptide (aa 9–32) showed no photoinsertion (data not shown). To establish that the observed photolabeling of p21 was specific, increasing amounts of p21 protein were photolabeled with a constant amount of photoactive probe. The photoincorporation was dose-dependent as shown in the autoradiogram of Fig. 2B. A quantitative analysis of the autoradiogram by scanning densitometry showed that saturation of binding was reached at a molar ratio of p21 to photoactive PAR13/14-mer of approximately 30 (Fig. 2B, bottom graph). Finally, the specificity of photolabeling was examined in the presence of unlabeled competitor PAR polymers without azido groups (Fig. 2C). Photoincorporation of azido-PAR was inhibited in a dose-dependent manner, when increasing amounts of unlabeled PAR of the same size range were added (Fig. 2C, autoradiogram). Quantification by scanning densitometry revealed an almost complete inhibition at a competitor PAR/photactive PAR-ratio of 10 (Fig. 2C, bottom graph). Taken together, these results indicate that the noncovalent interaction of PAR with p21...
PAR binding to XPA. A. Polymer blot analysis of XPA and XPA peptides. 2.5 µg of XPA and 2.5 µg of the indicated XPA peptides were dot-blotted onto nitrocellulose membrane and analyzed by polymer blot assay under high stringency (1 M NaCl) conditions. The autoradiogram is shown. B. Photoaffinity labeling of XPA and XPA peptides. Whole XPA protein (22 pmol, left panels) or XPA peptides (2 µg, right panels) were photolabeled with 1.1 pmol of photoactive [32P]PAR and analyzed by SDS-PAGE. The Coomassie-stained gels were subjected to autoradiography. C. Saturation of [32P]2-azido-PAR photoincorporation into XPA. Increasing amounts of XPA (0–1.7 µg) were photolyzed after addition of 1.1 pmol of photoactive [32P]PAR15/16-mer. The photolabeled protein was separated from unbound probe by SDS-PAGE. Incorporation of [32P]PAR was quantified by scanning densitometry of the autoradiographic bands. D. Protection against photolabeling of XPA (22 pmol) by photoactive PAR in the presence of an increasing amount of unlabeled PAR. The amount of photoactive [32P]PAR11/12-mer was 1.5 pmol, and the molar ratio of unlabeled to photoactive probe is indicated. Incorporation of [32P]PAR into XPA was quantified by scanning densitometry of the autoradiographic bands obtained after SDS-PAGE and autoradiography.

DNA Damage Recognition Factors—The nucleotide excision repair protein XPA recognizes a wide variety of DNA lesions and recruits other proteins to repair damaged DNA (14, 24, 25). The presence of a PAR-binding sequence motif at the C terminus made XPA a candidate for polymer interaction. XPA protein and two selected XPA peptides were subjected to polymer blot analysis. Fig. 3A shows that PAR bound to human XPA and a C-terminal synthetic peptide covering residues 215–237, whereas a N-terminal peptide was negative (aa 25–46). These results could be confirmed using photoaffinity labeling of XPA protein and XPA peptides with photoactive [32P]PAR (Fig. 3B). The specificity of PAR binding to XPA was demonstrated by two experiments. First, with increasing amounts of XPA, the photoincorporation increased and reached saturation at a molar ratio of XPA to photactive PAR15/16-mer of about 40 (Fig. 3C). Second, increasing amounts of unlabeled PAR without azido groups as competitor decreased the extent of incorporation of a constant amount of photoactive [32P]PAR11/12-mer into the XPA protein (Fig. 3D). Protection of photoincorporation was almost complete at a ratio of competitor PAR to photactive PAR polymers of about 10, reflecting the specificity of the noncovalent interaction.

During mismatch repair, the heterodimer hMutSα is involved in the recognition of G/T mismatches and 1-nucleotide insertion-deletion mismatches (26). By polymer blot assay, we observed a noncovalent interaction between PAR and the hMutSα heterodimer, which was due to PAR binding to MSH6 (Fig. 4A, left panel). The primary sequences of both proteins were examined for the presence of the PAR-binding motif. A PAR-interaction for a MSH6 peptide (aa 295–317) could be confirmed experimentally, but not for a MSH2 peptide (aa 227–249; Fig. 4A, right panel).

Base Excision Repair Proteins—PARP-1 is activated after the incision step of the base excision repair (BER) pathway, which results in formation of a DNA single-strand break. Therefore, we searched for putative PAR-binding domains in proteins known to act in the BER process. The PAR binding of synthetic peptides derived from different BER proteins was examined by polymer blot analysis (Fig. 4B). An XRCC1 peptide (aa 379–400) could interact noncovalently with PAR as predicted. Likewise, PAR binding was predicted and confirmed for aa positions 12–34 in DNA ligase III (Fig. 4B). A peptide derived from DNA polymerase β, the enzyme most likely responsible for long-patch repair synthesis in BER (27), bound to PAR, whereas two overlapping peptides from the N terminus of DNA polymerase β, the enzyme catalyzing short patch repair synthesis, were negative in the polymer blot assay (Fig. 4B). In this way, functional consensus PAR-binding motifs were identified in XRCC1, DNA ligase III, and DNA polymerase β.

DNA-dependent Protein Kinase—Both DNA-PK and PARP-1 are activated by DNA strand interruptions and probably participate in DNA repair. DNA-PK is an abundant nuclear serine/threonine protein kinase consisting of three subunits. The catalytic subunit DNA-PKcs of 470 kDa shares a domain of homology with members of the phosphatidylinositol 3-kinase family, and the heterodimer Ku70/Ku86 is important for DNA binding (28–30). We searched the primary structure of these proteins for the presence of PAR-binding sequences and tested them by polymer blot analysis and photoaffinity labeling. Positive results were obtained for two peptides derived from DNA-PKcs and Ku70 (Fig. 4C), whereas the Ku86-derived peptide was negative by blot analysis. In agreement with this, no
Photolabeling was detected with the Ku86 peptide, whereas the peptides from Ku70 and DNA-PKcs were positive in the photolabeling assay (data not shown).

**Cell Death and Replication Life-span Regulators—PARP-1** has been implicated in necrotic cell death (31, 32) as well as in death by apoptosis (33, 34). Therefore, several cell death regulating proteins were examined for the presence of PAR-binding sequences. The NF-xB/Rel family of transcription factors interacts with PARP-1 protein (35, 36). A PAR-binding site could be confirmed experimentally by polymer blot analysis for the p52 subunit of NF-xB homo- and heterodimers (KBF2, aa 179–199; Fig. 4D). The inducible NO synthase (iNOS), which is transcriptionally regulated by NF-xB, contains a PAR-binding sequence motif (NOS2, aa 505–525) in the calmodulin-binding domain. This was confirmed using the polymer blot assay (Fig. 4D). CAD is responsible for apoptotic DNA degradation (37). Sequence analysis and biochemical testing confirmed the presence of a functional PAR-binding motif (aa 148–169; Fig. 4D). Likewise, telomerase, a reverse transcriptase regulating telomere length and replication life-span of cells (38), was found to contain a PAR-binding sequence motif in the catalytic subunit (TERT, aa 962–983; Fig. 4D).

**Refinement of the PAR-binding Motif**—From the alignment of the PAR-binding sequences, it is apparent that the PAR-binding domains do not contain a single invariant amino acid, but there is a clear consensus pattern of residues with conserved properties in some positions (Fig. 5). The typical PAR-binding motif comprises approximately 20 amino acids and contains two conserved regions: (i) a cluster rich in positive residues and (ii) the consensus pattern -hxxhxxxxh-, where h indicates residues with hydrophobic side chains, b stands for a preference for basic amino acids, and x is any amino acid. The number of basic residues in the consensus pattern varies between 2 and 4. The consensus sequence presented in Fig. 5 may be a good guide for identification and future classification of PAR-binding proteins.

**Discussion**

Our study demonstrates the presence of a PAR-binding site in several proteins of the DNA damage signal network. A common function of these proteins is that they all contribute to the maintenance of genomic stability in cells. The PAR-binding sequences were found to overlap with five functionally important domains, responsible for (i) protein-protein interactions, (ii) DNA binding, (iii) nuclear localization signaling, (iv) nuclear export signaling, and (v) protein degradation. In p53 protein, three important functional domains are targets for noncovalent PAR binding: the sequence-specific DNA binding domain, the nuclear export signal and the oligomerization domain (Fig. 5; Ref. 10). The PAR-binding site of p21 lies within a highly conserved region responsible for PCNA binding (39–41). Mutations in this site (M147A, D149A, F150A) abolish PCNA binding and expose p21 to proteasome-dependent degradation (42). Moreover, p21 effects cell cycle arrest following various types of DNA damage by forming quaternary complexes with PCNA, Cdk, and cyclins (21, 43). The participation of PARP-1 activity in G1 and G2 cell cycle checkpoints has been demonstrated (22, 44).

**Fig. 4. Identification of polymer-binding proteins and peptides under high stringency (1 M NaCl) conditions.** A, MSH2, MSH6, and hMutSα proteins. Peptides (0.5 m lysozyme: negative control). B, PAR-binding peptides from BER proteins. Peptides (0.5 m each) were derived from MSH2 and MSH6 (2.5 and 0.5 m, respectively) were also subjected to polymer blot analysis (right panel; lysozyme: negative control). B, PAR-binding peptides from BER proteins. Peptides (0.5 m each) were derived from XRCC1, DNA ligase III (DNL3), DNA polymerase β (DPOB), and ε (DPOE) were tested for PAR binding. C, peptides from Ku70, Ku86, and DNA-PKcs were examined by polymer blot analysis and the autoradiograms are shown. D, PAR-binding sites in NF-xB p52 (KBF2), iNOS (NOS2), CAD, and TERT were identified by polymer blot assay of the indicated peptides (0.5 m). The N- and C-terminal residue numbers of the peptides are given in A–D.

**Fig. 5. Alignment of PAR-binding sequences with the consensus PAR-binding motif.** A common feature of the peptide sequences is the presence of hydrophobic amino acids (b; ACCVILM-FYW) spaced by basic amino acids (K/RH) and additionally an accumulation of basic residues at the N-terminal side of the motif (KR). Conserved hydrophobic residues are indicated in bold against a dark gray background, and neighboring basic amino acids as well as the residues corresponding to the basic block at the N-terminal part are shown against a light gray background. The positions that are similar in at least 50% of the sequences are indicated (bold asterisk, hydrophobic aa; lightface asterisk, basic aa). The near-terminal acidic amino acid at the beginning of the sequence (from the SWISSPROT or TrEMBL data base) are listed: MACS, MARCKS protein; CDN1, p21; DNL3, DNA ligase III; DPOE, DNA polymerase ε; NOS2, iNOS; KBF2, NF-xB p52; TERT.

**Consensus**

[... K/R ...] h.b.h.b.h.b.h.b.h.b. ...
In the DNA-PK complex, PAR-binding sequences were identified in DNA-PKcs (aa 2728–2752) as well as in the Ku70 subunit (aa 243–264). The PAR-binding site of Ku70 covers one of the nine conserved regions (region V, aa 243–261) and is in close proximity to a leucine zipper-like region (aa 214–242). The core region of Ku70 (253–430) is involved in DNA end binding and heterodimerization (45). Covalent modification of DNA-PKcs by PARP-1 has been shown recently to stimulate DNA-PK activity (46). The covalent modification of DNA-PKcs by PAR could play a role in enhancing the interaction between DNA-PKcs and the Ku70/Ku86 heterodimer via the noncovalent PAR-binding site found in the Ku70 protein. Moreover, the formation of PARP-Ku70/Ku86 complexes has been demonstrated by co-immunoprecipitation from nuclear extracts and these complexes specifically bind to matrix attachment regions flanking the immunoglobulin μ heavy chain enhancer (47). Although the mechanistic complexities of these interactions remain to be elucidated, molecular genetic evidence suggests an important role of PARP-DNA-PK-interactions in maintaining genomic integrity (48).

A conserved PAR-binding sequence is also present in several DNA repair proteins. In the damage recognition protein XPA, it is located in the C-terminal portion interacting with TFIIF during nucleotide excision repair (49). In the DNA mismatch recognizing protein hMutSα, a heterodimeric protein consisting of MSH2 and MSH6 (26), a PAR-binding domain was discovered in MSH6 but not in MSH2. Thus, PAR binding could interfere with the DNA damage recognition step of nucleotide excision and mismatch repair.

Several proteins of the BER pathway were also found to contain a PAR-binding sequence. In XRCC1, it maps to a BRCT domain, which is present in a large number of DNA repair and cell cycle checkpoint proteins (50, 51). The BRCT domain of XRCC1 (aa 301–402) has recently been demonstrated to bind preferentially to oligo-ADP-ribosylated PARP-1 protein (52). It is very likely that this involves the PAR-binding site of XRCC1 (aa 379–400). Thus, the DNA damage stimulated automodification of PARP-1 may prompt the formation of a PARP-1–XRCC1 complex, whereby XRCC1 is recruited into the complex by PAR polymers. The PAR-binding site of XRCC1 is situated between the binding sites for DNA polymerase β and DNA ligase III (53, 54), leaving these domains available for recruitment of these other binding partners. Moreover, DNA ligase III contains a PAR-binding site of its own; it maps to the N-terminal domain and could modulate interactions with XRCC1 at the DNA strand break. The binding site for XRCC1 lies within the C terminus of DNA ligase III, which also contains a BRCT domain (50, 54). Additionally, a PAR-binding site was identified in DNA polymerase ε (aa 691–709). An attractive model can be proposed in which the PAR-binding motifs contained within XRCC1, DNA ligase III, and DNA polymerase ε are involved in recruiting the proteins to interact at DNA strand breaks. The stimulation of BER synthesis in vitro in the presence of NAD+ supports this idea (55, 56).

The PAR-binding site in the p52 subunit of NF-κB falls within the Rel homology domain responsible for sequence-specific DNA binding, dimerization, nuclear localization, and protein interactions (57). The site (aa 179–199) is at the transition between insert region (aa 141–187) and the N-terminal core domain (aa 38–140 and 188–220) within the Rel homology domain. This region presents a potential interaction surface to other proteins (58). The conserved hydrophobic motif can be found also in p50, suggesting that this NF-κB subunit may also have PAR-binding potential. Co-immunoprecipitation of PARP-1/NF-κB complexes has been reported (35), and PARP-1-deficient cells are defective in NF-κB-dependent transcriptional activation and show a down-regulation of iNOS after genotoxic stress (35, 36). Induction of iNOS expression is abolished by inhibitors of PARP-1 (59). A PAR-binding site is also present in iNOS (aa 505–525), which overlaps with the calmodulin-binding site (aa 509–529). A putative calmodulin-binding domain can be found in all members of the NOS family. By analogy to the MARCKS protein family, calmodulin-iNOS interactions could be regulated by PAR polymers (11).

The PAR-binding site in telomerase localizes to the TERT catalytic subunit at position aa 962–983. This site could be targeted by tankyrase, a recent addition to the PARP family (5). Tankyrase catalyzes its automodification in a DNA-independent manner, and these PAR polymers could directly interact with telomerase and regulate its activity. This could play a role during the inappropriate expression of telomerase during carcinogenesis, allowing cells to overcome the limited replication life-span and progress toward malignancy (for review, see Ref. 60). No obvious functional consequence can be derived for the PAR-binding site in CAD, a caspase-activated DNA-catalyzing DNA degradation during apoptotic cell death. The site is located at position aa 148–169, between a regulatory domain (aa 1–83) and the catalytic domain (aa 290–345; Ref. 61).

In conclusion, the identification of specific PAR-binding sites in several proteins of the cellular signal network suggests that these proteins may be interaction partners of the PARP protein family. Many of these proteins are like PARP-1 members of the DNA damage signal network. By targeting specific domains in these proteins, PAR could regulate protein-protein or protein-DNA interactions, protein localization, or protein degradation. PAR could also play a chaperone function in the DNA damage signal network by facilitating the temporary formation of multiprotein complexes. It remains to be seen whether such complexes are formed in vivo. Preliminary results of co-immunoprecipitation experiments are compatible with this possibility. For example, PARP-MSH6 complexes can be immunoprecipitated with PAR (or MSH6 or PARP) antibodies from TK6 human cell lines.2

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