CHAPTER 4

DNA Damage Signaling through Poly(ADP-Ribose)

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Abstract

Several lines of evidence reveal that poly(ADP-ribose)polymerase-1 (PARP-1) operates in a DNA damage signaling network. Poly(ADP-ribose) metabolism induced by DNA damage participates in DNA repair and contributes to downstream mechanisms leading to cell cycle arrest, cell survival, cell death, or cell transformation. An important element of these multiple actions is the recruitment of DNA damage checkpoint proteins coordinating DNA repair with downstream events. The focus of this overview is the mechanism by which poly(ADP-ribose)—attached to the automodified PARP-1—interacts with DNA damage checkpoint proteins and how it may reprogram the functions of specific protein domains. Several proteins of the genome surveillance system, e.g., p53, p21, DNA-PK, NF-κB, XRCC1, and XPA are targets of such regulation. In all cases studied, a specific 'polymer-binding' sequence motif of 20 to 26 amino acids is targeted by poly(ADP-ribose) and this motif overlaps with important functional domains responsible for protein-protein or protein-DNA interactions, nuclear import or export, enzymatic catalysis, or protein degradation.

Introduction

In eukaryotic cells, DNA damage may induce a several thousand fold stimulation of poly(ADP-ribose) metabolism. A few restrictions and rules apply: yeast does not express such a response, and in all other eukaryotes tested so far, the most effective types of DNA damages are those that are substrates for the DNA base excision repair pathway. By far the largest amount of ADP-ribose is processed through the catalytic domains of two nuclear enzymes: poly(ADP-ribose)polymerase-1, (PARP-1), and its catabolic counterpart, poly(ADP-ribose)glycohydrolase (PARG). Other members of the growing PARP family may contribute to this metabolism, albeit to a much lesser extent and with mechanisms that await further elucidation (for reviews see refs. 1,2).

The poly(ADP-ribose) metabolism arising from the cooperation of PARP-1 and PARG is involved in DNA base excision repair and in DNA damage signaling to cell survival/cell death pathways.[1,2] The present review focuses on a particular aspect of poly(ADP-ribose) signaling: when PARP-1 is activated, it catalyzes poly(ADP-ribose) synthesis on itself ('automodification'). The polymers on PARP-1 can then recruit other proteins into multiprotein complexes and reprogram their domain functions.

The Different Steps of Signaling

A reasonable understanding on how poly(ADP-ribose) may exert its signal functions has been achieved. A growing number of DNA damage checkpoint proteins containing a poly(ADP-ribose)-binding sequence motif of 20 to 26 amino acids have been identified.[3,4]

Poly(ADP-Ribosyl)ation, edited by Alexander Bürkle.

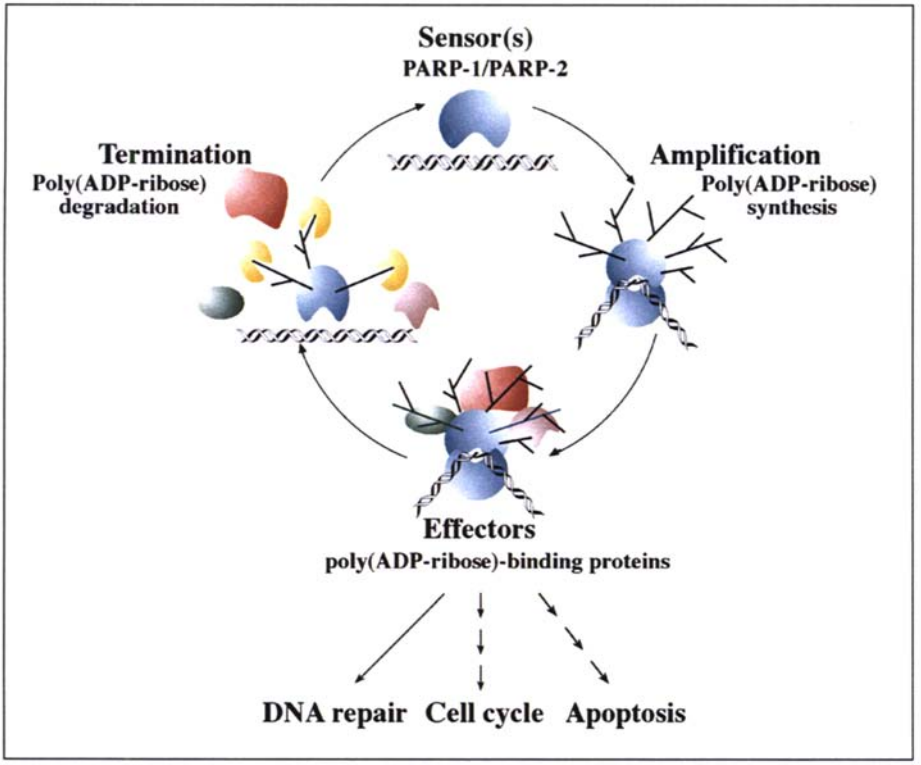

Figure 1. The major steps of the DNA strand break signaling model. See text for details.

PARP-bound polymers bind strongly, but noncovalently to these sequences and reprogram the resident domain functions. How do the polymers get in touch with these proteins? The PARP-1/PARG system has all the hallmarks of a DNA strand break based signal transduction mechanism, with poly(ADP-ribose) playing the effector role.[4] The model features the following steps (Fig. 1): PARP-1 has a high binding affinity for DNA ends and acts as a DNA damage sensor.[5] Binding leads to a more than 500-fold activation of PARP-1's catalytic activity[6] and the original signal (i.e., the lesion on DNA) is transduced into protein-bound ADP-ribose polymers of various size (ranging from few to over 200 units) and structural complexity (linear or branched), clustered at the site of damage. PARP-1 acts as a homodimer[7,8] and in DNA damaged cells it serves as a major acceptor of poly(ADP-ribose), i.e., it catalyzes its automodification at multiple sites simultaneously,[9] thus leading to signal amplification. The automodified PARP-1 stays in the vicinity of the DNA strand break.[10,11] Another nuclear member of the PARP family, PARP-2, is also able to catalyze DNA damage-dependent automodification[12] and can act as a catalytic homodimer, or together with PARP-1 as a heterodimer,[13] in the response to genotoxic stress. The effector step of signaling consists in the selective recruitment of poly(ADP-ribose)-binding proteins to the vicinity of DNA breaks.[3,4,10,11] These proteins may either directly participate in DNA base excision repair or coordinate repair with chromatin unfolding, cell cycle progression, and cell survival or cell death pathways. The relative affinity and local availability of poly(ADP-ribose) binding partners, associated with the extent of DNA damage, may determine the type of response and the signaling outcome. For instance, PARP-1 automodification may allow the rapid recruitment of the BER complex as a primary response to the DNA strand breaks,[10,11] and then recruit p53 and modulate its multiple signal functions leading to cell cycle arrest or cell death.[14]

The amount of XRCC1, the scaffold protein on which the BER complex is assembled[15] and also a poly(ADP-ribose) binder,[3] might determine the number of repair foci and the threshold above which survival/death programs are activated.

Finally, signal termination is achieved after poly(ADP-ribose) degradation by PARG. This enzyme disengages poly(ADP-ribose)-bound proteins and reverses the automodification status of PARP-1/PARP-2, which are now ready for a new round of DNA strand break binding. DNA damage induced polymers are degraded several hundred fold faster than constitutive polymers of undamaged cells.[16] By virtue of dynamic and reversible automodifications, PARP-1 and PARP-2 can rapidly change the spectrum of partner proteins for recruitment into multiprotein complexes. PARPs may acquire increased binding affinity and/or new partner specificity upon automodification (vide infra).

The following sections summarize some of the evidence leading to this model. A focal question was: which proteins become targets of poly(ADP-ribose)-binding and what are the consequences of polymer-binding on specific domain functions of these proteins?

Protein Targeting by Poly (ADP-Ribose)

How can poly(ADP-ribose) target proteins in a chromatin environment? (ADPribose) polymers are variously sized acidic molecules, some of them containing branches.[17] The ribose-phosphate-phosphate-ribose backbone of poly(ADP-ribose) has a higher negative charge density than DNA and therefore may attract basic proteins from DNA.[18,19] The helical conformation[20] and the branched structure of long polymers, might also be involved in conferring some binding specificity.[21] The first evidence that poly(ADP-ribose) might play a role beyond that of a posttranslational protein modification was presented by Ohashi et al reporting that the activity of DNA ligase in reconstituted chromatin could be stimulated by either polymer addition or by PARP-1-bound polymers, synthesized in the course of the incubation in vitro.[22] It turned out that histones bind directly to poly(ADP-ribose) or PARP-1-bound polymers and this can cause the release of DNA from nucleosomal core particles.[19,21,23-25] The observation that poly(ADP-ribose) degradation by PARG restores the nucleohistone structure led to the mechanistic model of a histone shuttle.[24] Thus, the PARP-1/PARG system has the capacity to target histones for reversible dissociation from DNA. The potential biological relevance of this phenomenon lies in the fact that poly(ADP-ribose), by virtue of its affinity for histones, might act in vivo as a catalyst of nucleosomal unfolding, by transiently displacing histones from DNA and hence facilitating DNA access to repair proteins in localized areas of the chromatin.[24] Indeed, histones exhibit a high preference for poly(ADP-ribose)-binding in the presence of DNA; a polymer of 40 ADP-ribose residues is sufficient to dissociate the entire histone complement of a chromatosome. Histones display different affinity for poly(ADP-ribose), the hierarchy of binding being H1>H2A>H2B=H3>H4. For all of them, however, interaction is far stronger and more specific than would be expected on the basis of electrostatic interactions. For instance, poly(ADP-ribose)-bound histones resist phenol partitioning, strong acids, detergents, and high salt concentrations.[21] An additional element of specificity is represented by the fact that protein basicity and/or DNA binding ability are not sufficient to confer affinity for poly(ADP-ribose). On the other hand, size and branching of (ADP-ribose) polymers are important determinants of binding, as branched polymers are a highly preferred target, followed by long linear molecules.[21] These classes of polymers are also synthesized in vivo and their levels increase in response to DNA damage.[26-29] The amazingly high specificity of histone-poly(ADP-ribose) interactions could be explained by the discovery that binding only occurs at specific histone domains (C-terminus of histone H1 and N-terminal tails of core histones)[30] and within such domains, only at distinct sequences that define, over a stretch of 20-26 amino acids, a highly homologous binding motif.[3] Amino acid conservation within these sequences entails the physicochemical properties of specific residues rather than their identity; the binding motif comprises at its C-terminal part a block of regularly spaced hydrophobic and basic residues, that by mutational analysis have been found to be critical for binding;[3] the interaction with poly(ADP-ribose) is

further strengthened by, but not absolutely dependent on, flanking arginines or lysines and a cluster of basic amino acids at the N-terminus. These rules initially defined in the authors' laboratory,[3,4] have recently been confirmed by Poirier's group.[31] Screening of protein sequence databases with the polymer-binding consensus motif has led to the identification of other potential poly(ADP-ribose) interaction partners (Table 1), many of which are directly involved in the cellular response to DNA damage, at the level of damage recognition and processing (i.e., XPA, XRCC-1, MSH6, DNA ligase III, DNA polymerase ε) and/or in later events responsible for cell cycle regulation/apoptosis (i.e., p53, p21, NF-kB, iNOS, DNA-PK, caspase activated DNase). In view of the widespread occurrence of PARPs in different cellular compartments,[1,2] it is noteworthy that MARCKS (Myristoylated-Alanine-Rich-C-kinase-Substrate) and MRP (MARCKS-related protein), proteins regulating rearrangements of the actin cytoskeleton, also carry a poly(ADP-ribose) binding motif in their effector domain.[33]

For most of the proteins listed in (Table 1), actual poly(ADP-ribose) binding has been biochemically confirmed by photoaffinity labeling and/or by a polymer blot binding assay using synthetic peptides, covering the putative interaction sequences, and/or on full length proteins, immobilized on nitrocellulose. Recently, a combination of liquid-phase isoelectric focusing for protein extract fractionation, poly(ADP-ribose) binding testing by the blot assay, and MALDI-TOF mass spectrometry for protein identification, has allowed large scale screening and characterization of various heterogeneous nuclear ribonucleoproteins (hnRNPs) as poly(ADP-ribose) interaction partners.[31] These hnRNPs share a highly homologous poly(ADP-ribose) binding motif in the RNA recognition domain.

In addition to the proteins listed in (Table 1), for which the binding sites have been elucidated, the following polymer-binding proteins have been identified: caspase 7,[34] the 20S proteasome,[35] the telomere binding protein TRF-2,[36] lamins[31] and several other nuclear and nuclear matrix proteins, whose identity has yet to be established.[29,37] It is predictable that the in silico approach combined with biochemical testings,[3,4] and protein fractionation techniques in conjunction with mass spectrometry[31] will reveal a much larger family of polymer-binding proteins. The biological relevance of poly(ADP-ribose) interaction with the identified protein targets should, however, be tested with appropriate functional assays.

Polymer-Binding 'Reprograms' Domain Functions of Proteins

How does polymer-binding affect domain functions in proteins? - Firstly, the binding of poly(ADP-ribose) to the polymer-binding consensus motif is very strong.[3,21] Secondly, in almost all cases studied so far, the polymer-binding sequence overlaps with strategic functional domains in the target protein.[3,4,14,31,33,36,38] Thirdly, polymer binding may enhance and inhibit separate domain functions in the same protein.[38] The tumor suppressor protein p53 is a particularly well studied example: the polymer-binding sites colocalize with the sequence specific DNA binding domain (residues 153-181 and 231-256) and with the C-terminal domain (amino acids 326-351), containing nuclear localization signal, nuclear export signal and tetramerization funtion.[14] p53 plays a key role in transduction pathways induced by several types of cellular stress, by regulating the expression of gene products that can either lead to cell cycle arrest in G1, thereby preventing the replication of DNA before the damage is repaired, or cause cell death by apoptosis.[39] In vitro studies have demonstrated that poly(ADP-ribose) binding at the target sites is able to block (or reverse) p53 association both with ssDNA and, at higher concentrations, with a ds-oligonucleotide containing a p53 consensus sequence.[14] Thus, p53 may differentially respond to DNA damage-induced poly(ADP-ribose): at low levels of DNA damage, a few polymers clustered on PARP-1/PARP-2 could block the ssDNA binding function and favour the transcriptional activity of p53. Conversely, high amounts of poly(ADP-ribose), associated with excessive DNA damage and massive NAD^+ consumption, could inhibit p53 activities completely, and thus contribute to directing cells towards caspase-independent programmed cell death[40,41] or necrosis.[42] Hence, poly(ADP-ribose) metabolism might operate as a dual mechanism that activates p53-dependent and p53-independent

Table 1. Poly(ADP-ribose) binding proteins

Target Proteins	SwissProt Accession N°	Binding Sequences	Structural Features/Function of Target Sites
		h x bx hh bb hh b	
H351-72	P16106	I R R Y Q K S T E L L I R K L P F Q R L V R	
H416-40	P02304	K R H R K V L R D N I Q G I T K P A I R R L A R R	In all core histones the binding sites overlap
H2A11-36	P02261	R A K A K T R S S R A G L Q F P V G R V H R L L R K	the N-terminus of the histone fold
H2B23-47	P02278	K K D G K K R K R S R K E S Y S I - Y V Y K V L K Q	
p53156-181	P04637	R V R A M A I Y K Q S Q H M T E - V V R R C P H H E R	Sequence-specific
p53234-256		Y N Y M C N S S C M G G M N R R P I L T I I T	DNA binding domain
p53329-351		T L Q I R G R E R F E M F R E L N E A L E L K	oligomerization domain
p21140-163	P38936	R K R R Q T S M T D F Y H S K R R L I F S K R K	PCNA binding domain/potential NLS
NF-kB179-199	Q00653	K E L K K V M D L S I V R L R F S A F L R	Rel homology domain
iNOS505-525	P35228	K R R P K R R E I P L K V L V K A V L F A	calmodulin binding domain
CAD148-169	O76075	R F Q S K S G Y L R Y S C E S R I R S Y L R	
XP-A215-237	P23025	K Q K K F D K K V K E L R R A V R S S V W K R	TFIIH interaction
XRCC-1379-400	P18887	R I V R K E W V L D C H R - M R R R L P S R R	BRCT domain
DNA lig III12-34	P49916	K R G T A G C K K C K E K I V K - G V C R I G K	Zinc finger
DNA pol ε691-709	Q07864	K R R L A D Y C R K A Y K K I H I T K	
DNA-PKcs 2728-2752	P78527	K V A R K R K R M V T G N G S L K R K S S R K	KIP binding domain
MSH6295-317	P52701	R R R F M R D Q E K L S L M Y A R K G V A E Q K R	
Ku70243-263	P12956	R K V R A K E T R K R A L S R - L K L K L N K	DNA binding domain
MARCKS151-175	P29966	K K K K R F S F K K S F K L S G F S F K K N K K	Effector domain
TERT962-983	O14746	R G F K A G R N M R R K L F G V L R L K C H	
hnRNP A299-120	P22626	R E E S G K P G A H V T V K K L F V G G I K	RNA binding domain
topo I261-280	P11387	A K M L D H E Y T T K E I F R K N F F K	"cap" region
topo I532-551		K D S I R Y Y N K V P V E K R V F K N L	catalytic site
topo I669-688		K A D A K V M K D A K T K K V V E S K K	linker domain
***topo II** α971-990	P11388	K M T E E K L A E A E R V G L H K V F K	catalytic domain
****CENPA**57-74	O35216	R K K P F S M V V R E I C E K F S R	
****CENPB**583-597	P27790	R K N H A R Q A G V R G L G H	

See text for references. *Malanga and Althaus, unpublished. **See reference 32.

pathways to ensure either repair or disposal of cells with compromised genome functions. To add to this complexity, PARP-1 may also catalyze the covalent modification of p53, albeit with poor efficiency. The precise location of this heteromodification is not known, however, it blocks sequence-specific binding leading to transcriptional inactivation of p53.[43,44]

Upregulation of p53 has been demonstrated even in the absence of DNA damage.[45] Microinjection of cells with an antibody targeting the C-terminal part of the protein suffices to activate p53-dependent gene expression.[45] Thus, PARP-1-bound polymers extending from DNA breakage sites may directly activate p53 by neutralizing the inhibitory influence of the C-terminal domain on p53 transcriptional activity. In agreement with this scenario, poly(ADP-ribose) synthesis participates in p53 induction following γ-irradiation and in the expression of p53-responsive genes (i.e., mdm2, p21) as well as in p53-dependent cell cycle arrest in the G1 phase.[46-48]

Yet another pathway has been put forward with regards to p53 stabilization. Regulation of p53 protein occurs mainly at the posttranslational level. A functional link between PARP-1 and p53 is suggested by the fact that both in vitro and in vivo, the two proteins form complexes and this depends on p53 phosphorylation and involves the N-terminal and central domains of PARP-1 and the central and C-terminal domains of p53.[49,50] Furthermore, basal p53 levels are significantly reduced in chinese hamster cell lines with defective poly(ADP-ribosyl)ation and in embryonic fibroblasts from PARP-1 knockout mice.[49-51] In the latter case, p53 depletion could be counteracted by treatment with leptomycin B, an inhibitor of nuclear export. This points to an impairment of p53 basal stability, rather than reduced p53 gene expression, in the absence of PARP-1. Thus, in DNA damaged cells, automodified PARP-1 may regulate the nucleocytoplasmic shuttling of p53, such that larger amounts of the protein accumulate in the nucleus.

Poly(ADP-ribose) could also affect cellular responses to DNA damage in another way. The poly(ADP-ribose) binding site of the cell cycle regulator p21 lies within a highly conserved region responsible for PCNA binding. Mutations in this site abolish the interaction with PCNA and expose p21 to proteasome-dependent degradation.[52] Lack of binding to PCNA of mutated p21 has also been found to cause cell cycle arrest.[53] Thus one can speculate on a dual effect of poly(ADP-ribose) binding to p21: it might protect p21 from proteolytic degradation while at the same time distract it from interacting with PCNA; PCNA may then become available for DNA repair synthesis in the PCNA-dependent long-patch branch of the BER pathway. In living cells, this BER subpathway is in fact greatly impaired in the absence of catalytically active PARP-1.[54,55] It should be noted that another player of long patch BER, DNA polymerase ε, is also a poly(ADP-ribose) binding partner in vitro, suggesting that the polymer may help recruit this protein to sites of initiated DNA repair. Moreover, both p21 and PCNA have been shown to form dynamic complexes with native PARP-1 in vitro and in vivo;[56] p21 sequestration by PARP-bound poly(ADP-ribose) in DNA damaged cells might mimic the effect of mutated p21 and lead to cell cycle arrest.

Thus, by targeting key regulators of survival/death pathways, poly(ADP-ribose) may alert the cell to the status of genotoxic stress and elicit a response that is commensurate with the extent of damage. It is relevant in this regard that, besides p53 and p21, other components of stress signaling systems (NF-κB, iNOS, DNA-PK) as well as effectors of the apoptotic response (caspase 7, caspase-activated-DNase) are poly(ADP-ribose) binding partners. Poly(ADP-ribosyl)ated PARP-1 has been identified as the preferred substrate for caspase 7 rather than the native enzyme.[34] On the other hand, automodified PARP-1 can also favour the elimination of oxidatively damaged proteins by direct interaction with and activation of the nuclear 20S proteasome.[35] In K562 human leukemic cells, the degradation of oxidized histones is an early response to DNA damage (starting within 5 minutes after induction of oxidative stress) and is stimulated by poly(ADP-ribose).[37]

PARP-mediated nuclear proteasome activation might operate in parallel with removal of DNA lesions by the BER pathway to ensure fast and efficient restoration of the native chromatin

structure. The central role of poly(ADP-ribose) in the recruitment/assembly of the BER multiprotein complex, initially only hypothesized on the basis of the high affinity of key components of the BER machinery for poly(ADP-ribosyl)ated PARP-1/PARP-2 (i.e., XRCC1,[3,13,58] DNA ligase III,[3,59] DNA polymerase ε[3]) has recently been shown in vivo.[10,11] Okano et al[10] and El-Khamisy et al[11] could elegantly demonstrate the rapid recruitment of XRCC1 to sites of poly(ADP-ribose) formation. Polymer synthesis and recruitment were detectable within less than 2 minutes and were abrogated by chemical or genetic inactivation of PARP-1. Moreover, in these studies, XRCC1 recruitment to repair foci was absent following mutations in the poly(ADP-ribose)-binding site. This site lies within a BRCT domain, which has been found in a large number of proteins involved in DNA repair and cell cycle control, and is situated between the binding sites of DNA polymerase β and DNA ligase III. Thus, poly(ADP-ribose)-bound XRCC1 is still able to interact with its protein partners to form a functional repair complex.

Recently, an additional mechanism whereby PARP-1/PARP-2 may participate in the maintenance of genomic stability has been suggested by the observation that automodified PARPs are potent modulators of DNA topoisomerase I activity.[38] Topoisomerase I is involved in the control of DNA supercoiling, allowing topological changes that are necessary for DNA transactions.[60] In the course of the reaction, eukaryotic topoisomerase I cleaves one strand of the DNA substrate and remains covalently bound to it through a 3'-phospho-tyrosine linkage. After DNA rotation around the intact strand, the DNA backbone integrity is restored, and the enzyme is released, in a reverse transesterification reaction, with the 5'-hydroxyl end of the cleaved strand acting as a nucleophile. Under normal conditions, DNA religation is fast; however, when acting on damaged DNA, topoisomerase I may get trapped in the complex with nicked DNA (stalled topoisomerase I).[61] Stalled topoisomerase I is a threat to genomic stability as it can be converted into DNA strand breaks and irreversible enzyme-DNA crosslinks upon collision with replication forks or elongating RNA polymerases (topoisomerase I-induced DNA damage).[61] In vitro, both the forward and reverse transesterification reactions can be modulated by poly(ADP-ribose), through physical interactions with specific domains of topoisomerase I. DNA cleavage is in fact inhibited, while the religation activity of the enzyme blocked in a ternary complex with nicked DNA and the anticancer drug camptothecin, is resumed in the presence of poly(ADP-ribose).[38] Thus, in living cells, PARP-bound poly(ADP-ribose) might have a protective effect against secondary topoisomerase I-induced DNA damage, by preventing the enzyme from starting its catalytic cycle on damaged DNA, and also reactivating stalled topoisomerase I and inducing rapid resealing of the cleaved DNA strand.

Concluding Remarks

In conclusion, the available data support a model in which poly(ADP-ribose) synthesis on PARP-1/PARP-2 following genotoxic insult by chemicals (alkylating or oxidizing agents) or γ-radiation, serves as a DNA strand break signal transduction mechanism. The variety of poly(ADP-ribose) interaction partners, their local availability and relative affinities for the polymer may confer a wide range of signaling options in different cell types and under different stress conditions. The primary effects of the PARP-automodification reaction may be local, involving changes in chromatin architecture (by poly ADP-ribose targeting of histones), recruitment of the BER machinery (by specific binding of XRCC1 and/or other DNA repair proteins), or removal of damaged nuclear proteins (by direct activation of the nuclear 20S proteasome). Inhibition of topoisomerase I activity on damaged DNA and reactivation of the stalled enzyme in the vicinity of DNA lesions may be part of this early attempt to restore genomic integrity. Secondarily, if the extent of damage exceeds the capacity of the repair systems, the status of genomic stress can be signaled to downstream effectors of the DNA damage response and initiate survival/death pathways. In this scenario, the size and complexity of poly(ADP-ribose) might also play a role in determining the type of response; for instance, interaction with the proteasome has been reported to be restricted to long poly(ADP-ribose) molecules,[35] while XRCC-1 has been shown to interact preferentially with oligo(ADP-ribosyl)ated PARP-1.[58]

Finally, the recruitment mechanism relying on ADP-ribose polymers as the 'fishing rods' may have a more general application in cellular signaling. All members of the PARP family studied so far, can modify themselves and thus recruit other proteins analogous to the example shown for XRCC1. Moreover, poly(ADP-ribose) becomes attached to other proteins in the course of the heteromodification reaction, catalyzed by several members of the PARP family. The best studied example is PARP-1, which can heteromodify several nuclear proteins, primarily histones, but also enzymes (DNA polymerases, RNA polymerases, topoisomerases, nucleases) and transcription factors (p53, Fos, TFIIF, YY-1) (ref. 1,62).

Hetero(ADP-ribosyl)ation, however, entails a limited fraction of the cellular poly(ADP-ribosyl)ation capacity and may play a major role in cellular processes other than DNA repair. Covalent histone modification, which involves essentially short ADP-ribose oligomers,[63-65] may destabilize inter- and intra-nucleosomal DNA-protein interactions as it has been shown for acetylation, phosphorylation, ubiquitination.[66] In fact, ADP-ribosylation of histone H1 and core histones has been demonstrated to cause relaxation of polynucleosomal preparations and partial unfolding of mononucleosomes respectively (for review see ref 67). Oligo(ADP-ribosyl)ation of histones and other proteins might also work together with other posttranslational modifications[63,68] (and with PARP-automodification) to establish altered chromatin structures and/or to modulate protein-protein interactions in multiprotein complexes, allowing fine-tuning of regulated gene expression. The close correlation between poly(ADP-ribosyl)ation, chromatin structure and programmed gene expression has found recent corroboration in the work of Tulin and Spradling on Drosophila polytene chromosomes.[18] In such processes poly(ADP-ribose) is likely to play a role both as covalent modifier and noncovalent interaction partner for chromatin proteins.

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