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REVIEW

Poly(ADP-ribosyl)ation in regulation of chromatin structure and the DNA damage response

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Abstract Poly(ADP-ribose) (PAR) is a post-translational modification of proteins and is synthesised by PAR polymerases (PARPs), which have long been associated with the coordination of the cellular response to DNA damage, amongst other processes. Binding of some PARPs such as PARP1 to broken DNA induces a substantial wave of PARylation, which results in significant re-structuring of the chromatin microenvironment through modification of chromatin-associated proteins and recruitment of chromatin-modifying proteins. Similarly, other DNA damage response proteins are recruited to the damaged sites via PAR-specific binding modules, and in this way, PAR mediates not only local chromatin architecture but also DNA repair. Here, we discuss the expanding role of PAR in the DNA damage response, with particular focus on chromatin regulation.

Introduction

Poly(ADP-ribose) (PAR) is a dynamic and abundant post-translational modification, with a wide array of nuclear and cytoplasmic functions in fundamental biological processes such as transcription, replication, cell cycle progression and division, ageing, intracellular transport and apoptosis/necrosis (Abd Elmageed et al. 2012; Ame et al. 2004; D'Amours et al. 1999; Gibson and Kraus 2012; Kraus 2008; Schreiber et al. 2006; Virag et al. 2013). However PAR has been most extensively studied as a mediator of the DNA damage response, where it is an essential regulator of chromatin architecture, DNA repair and transcription.

The founding member of the PAR polymerase or PARP (ARTD) family, PARP1, was discovered five decades ago, and the family now comprises 17 members in humans as determined from homology to PARP1 (Ame et al. 2004; Chambon et al. 1963; Otto et al. 2005). Whilst some PARPs share PARylating activity with PARP1 (such as PARP2 and tankyrases), many PARPs are mono(ADP-ribose) transferases or are capable only of making very short ADP-ribose oligomers, and for some (e.g. PARPs 9 and 13), ADP-ribosylation activity has not yet been reported (Gibson and Kraus 2012; Otto et al. 2005). PARPs transfer the first ADP-ribose group from the universal co-enzyme NAD+ onto a protein acceptor, primarily on glutamate and aspartate residues (Chapman et al. 2013; Matic et al. 2012; Riquelme et al. 1979; Sharifi et al. 2013; Tao et al. 2009). Following the establishment of the initial ester bond, some PARPs (such as PARP1/2 and tankyrases) repeatedly transfer additional ADP-ribose units via a unique 2′,1″-O-glycosydic ribose–ribose bond, eventually producing long chains of PAR (Alvarez-Gonzalez and Jacobson 1987; Gibson and Kraus 2012; Ruf et al. 1998). PAR chains were reported to reach up to 200 ADP-ribose units in length and were suggested to occasionally contain branching (Juarez-Salinas et al. 1982; Miwa et al. 1979).

Like other post-translational modifications, PAR cellular levels are dynamic and tightly controlled, with the half-life ranging from 1 to 6 min (Alvarez-Gonzalez and Althaus 1989). Reversal of signalling and recycling of modified proteins are important requirements for any post-translational modification, and persistence of PAR signalling after the appropriate cellular response has been achieved would be particularly detrimental to the cell, since depletion of cellular NAD+ leads to necrosis (Ha and Snyder 1999) and excessive protein-free PAR can trigger apoptosis in a process called parthanatos (Wang et al. 2011; Barkauskaite et al. 2013). The major enzyme for the removal

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of cytoplasmic and nuclear PAR is PAR glycohydrolase (PARG) (Dunstan et al. 2012; Miwa and Sugimura 1971; Kim et al. 2012; Slade et al. 2011; Ueda et al. 1972). PARG efficiently reverses PARylation by cleaving the unique O-glycosidic ribose–ribose bonds within the PAR chains, releasing free ADP-ribose (Slade et al. 2011; Ueda et al. 1972). Recent reports also suggest that a second hydrolase, ARH3, exhibits the analogous activity (Mueller-Dieckmann et al. 2006; Niere et al. 2012). However, both PARG and ARH3 cannot hydrolyse the proximal ADP-ribose unit from a PAR chain that is directly linked to the modified proteins since the biochemistry of the connection to acidic amino acid residues is distinct from the ribose–ribose bonds within the chain (Mueller-Dieckmann et al. 2006; Slade et al. 2011). Most recently, three enzymes—terminal ADP-ribose protein glycohydrolase (TARG1/C6orf130) (Sharifi et al. 2013), MacroD1 and MacroD2 (Jankevicius et al. 2013; Rosenthal et al. 2013; Barkauskaite et al. 2013;)—were all shown to possess this long-sought enzymatic activity to cleave mono(ADP-ribosyl)ated protein substrates, participating in what is postulated to be the rate-limiting step in PAR hydrolysis (Wielckens et al. 1982).

Cellular deficiency in PARPs, PARG and more recently TARG1 has been shown to cause substantial DNA repair defects and, in the case of PARG and TARG1, neurodegeneration (Hanai et al. 2004; Sharifi et al. 2013). Inhibition of PARP1 with PARP inhibitors (which have received significant attention for their potential applications in cancer therapy (Bryant et al. 2005; Farmer et al. 2005; Rouleau et al. 2010)) or its depletion with siRNA leads to single-strand (SSBR) and double-strand break repair (DSBR) defects (Boulton et al. 1999; Dantzer et al. 2000; Ding et al. 1992; Fisher et al. 2007). Similar effects are observed upon depletion of the PAR-hydrolysing enzymes (Ame et al. 2009; Koh et al. 2004; Sharifi et al. 2013), and concurrent depletion of PARP1 and PARG does not produce a cumulative effect (Feng and Koh 2013). Moreover, PARP1−/− and PARP2 −/− mice exhibit significant genomic instability (Schreiber et al. 2002; Trucco et al. 1998) (and in the absence of p53, an increase in spontaneous tumour development (Nicolas et al. 2010)), whilst loss of both PARP1 and PARP2 or PARG in vertebrates causes embryonic lethality (Koh et al. 2004; Menissier de Murcia et al. 2003). These phenotypes support a fundamental and vital role for PARylation in the maintenance of genome stability.

The highly abundant chromatin-associated PARP1 is responsible for production of about 90 % of cellular PAR (Ludwig et al. 1988; Yamanaka et al. 1988). Upon binding to DNA breaks, PARP1 is dramatically activated, leading to extensive PARylation of chromatin-associated proteins such as histones and especially PARP1 itself (Ogata et al. 1981), which regulates many downstream events during the DNA damage response process (Fig. 1). The emergent PAR scaffold also promotes recruitment (and in some cases, modification) of an array of DNA repair factors and chromatin-modifying proteins, usually via one of four distinctly evolved PAR-binding domains (Žaja et al. 2013) (see later). Chromatin-modifying proteins such as ALC1, APLF and CHD4 generally improve chromatin accessibility and in some cases down-regulate transcription, whilst other proteins such as XRCC1 and p53 have roles in the repair of the DNA breaks and checkpoint signalling (Ahel et al. 2009; Malanga and Althaus 2005; Pleschke et al. 2000). Usually, the net result of PARylation at DNA damage sites is therefore the efficient repair of the damaged DNA and cell survival, although in cases where DNA damage is excessive, PARylation can induce apoptosis.

PARP1 is activated by binding to damaged DNA

PARP1 binds both to nucleosomes and to various types of damaged DNA. In its inactive form, the majority of PARP1 is associated with nucleosomes, producing compact, transcriptionally inactive chromatin (Ji and Tulin 2010) (Fig. 2). This is supported by in vitro experiments in which addition of recombinant PARP1 to purified chromatin promotes a more condensed conformation (Wacker et al. 2007). Similarly, at heterochromatic regions such as telomeres and centromeres, inactive PARP1 helps to maintain a repressive DNA state (Gomez et al. 2006; Kanai et al. 2003).

However, PARP1 and PARP2 (Ame et al. 1999) are activated several hundred-fold by binding to broken DNA ends. The recent solution of PARP1-DNA structures has provided a more detailed understanding of the interaction of PARP1 with DNA (Ali et al. 2012; Eustermann et al. 2011; Langelier et al. 2011, 2012). Langelier et al. show that interaction of PARP1 with a DNA double-strand break causes conformational changes which result in re-organization of the DNA-binding zinc finger domains 1 and 3, and WGR domains. Subsequent destabilization of the catalytic PARP domain causes increased active site flexibility and is suggested to be responsible for the dramatic increase in activity of PARP following binding to DNA breaks (Langelier et al. 2012). Given the significance of PARylation in the response to different types of DNA damage, it will be important to qualify further the interactions of PARP1 with other types of damaged DNA. In this vein, Clark et al. investigated the binding of PARP1 to different DNA substrates in vitro and revealed the necessity for linker DNA and at least one free DNA end for activation of PARP1 by nucleosome binding (Clark et al. 2012).

However, additional mechanisms of PARP activation other than binding to DNA damage exist. For example, interactions with other proteins such as histones (Pinnola et al. 2007), binding to non-B DNA structures (Lonskaya et al. 2005) and post-translational modifications of PARPs (such as phosphorylation (Kauppinen et al. 2006) and mono(ADP-ribosyl)ation (Mao et al. 2011)) have been suggested to induce PARylation (Schreiber et al. 2006; Szabo et al. 2006) (Fig. 1).

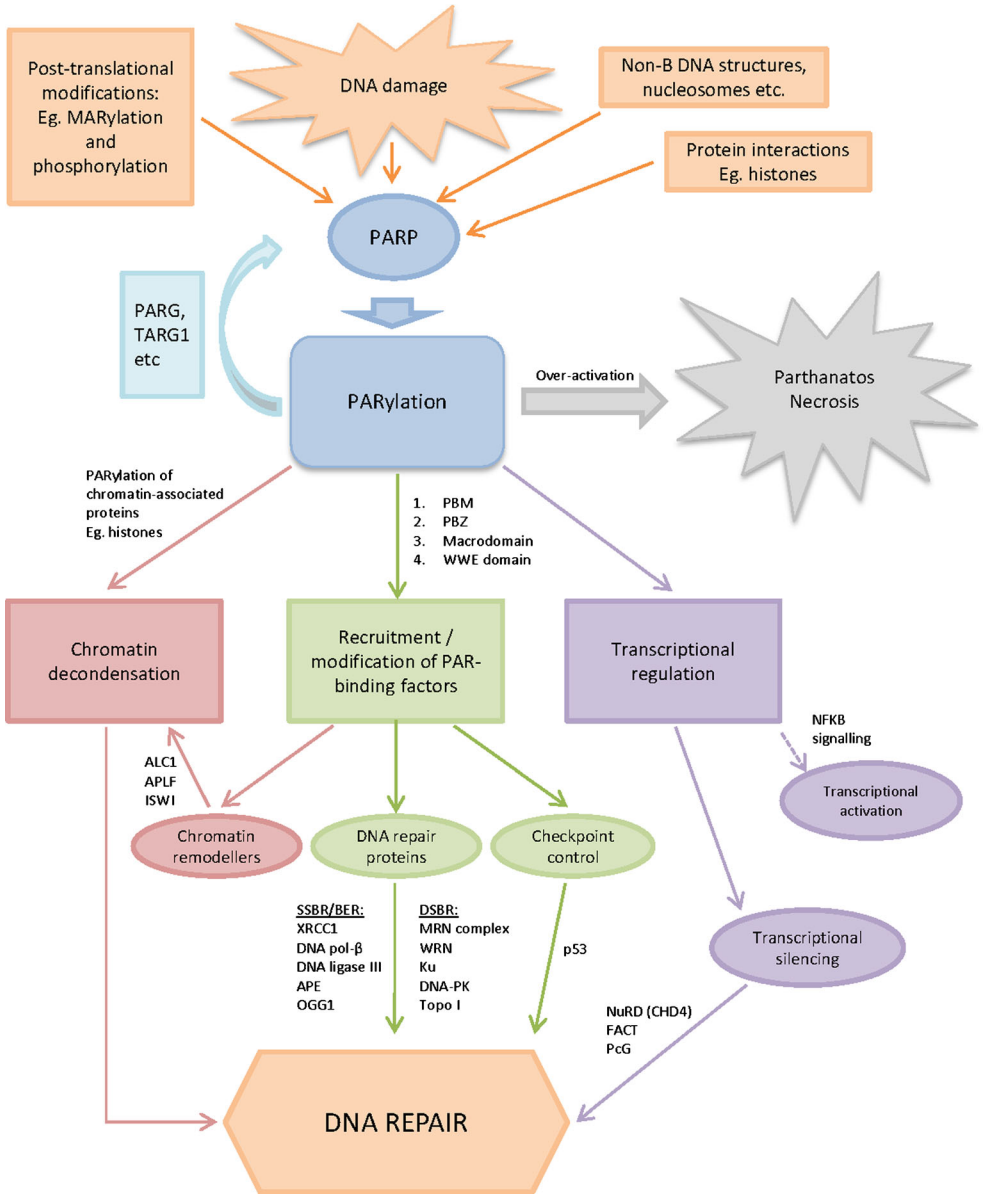

Fig. 1 Overview of PARylation in the DNA damage response. PARPs are activated by a variety of stimuli, the most significant of which is binding to damaged DNA (although protein interactions, post-translational modifications and binding to other DNA structures have also been implicated in their activation). Activation of PARP leads to a massive increase in cellular PAR, which is quickly catabolised by PARG although complete reversal requires TARG or MacroD1/2 activity. The roles of PARylation in the DNA damage response can be grouped into three overlapping classes: chromatin de-condensation, recruitment and/or modification of PAR-binding factors and transcriptional regulation. Chromatin de-condensation is facilitated by PARylation of chromatin-associated proteins including histones and PARP1 itself, causing their eviction from DNA. PAR-binding factors are recruited mostly via one of the four classes of PAR-binding domain: the PAR-binding motif (PBM), PAR-binding zinc finger (PBZ), macrodomain and WWE domain. Some (e.g. ALC1, APLF, ISWI) are involved in remodelling of chromatin. Others are involved in DNA repair (e.g. XRCC1, DNA pol-β, the MRN complex, Topo I) or generalised DDR signalling (e.g. p53). Finally, transcriptional regulation mostly involves the recruitment or modification of proteins involved in transcriptional silencing (e.g. CHD4, PcG proteins and the FACT complex), although PAR-mediated activation of transcription also occurs—for example, in NFκB-mediated inflammation signalling. Notably, excessive PARylation leads to cell death by parthanatos or necrosis pathways, helping to maintain the genomic integrity of an organism by the sacrifice of heavily damaged cells

Recruitment/modification of DNA damage response proteins

Extensive PARylation by PARP1 produces a significant local scaffold for the recruitment of a vast array of DNA repair factors, chromatin remodellers and other proteins involved in the maintenance of genome integrity. The significance of PAR for the rapid recruitment of proteins is evidenced by the evolution of at least four PAR-binding modules (reviewed in (Žaja et al. 2013)), although the presence of other domains,

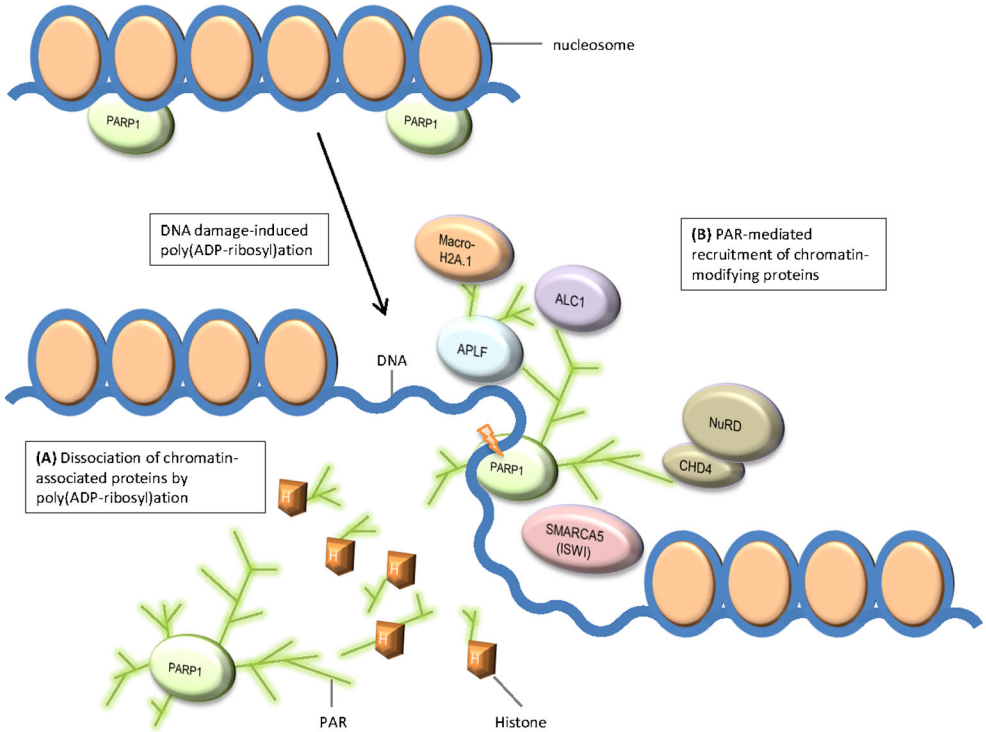

Fig. 2 Chromatin re-organization by DNA damage-activated PARP1. Inactive PARP1 associates with chromatin, producing a repressive and compact chromatin structure. Upon sensing DNA damage, PARP-mediated PARylation promotes relaxation of chromatin by: (*A*) direct PARylation of chromatin-associated proteins such as histones and PARP1 itself, causing their eviction from DNA and (*B*) facilitating recruitment of chromatin-modifying proteins. Proteins recruited to the local PAR scaffold include chromatin remodellers such as ALC1 and SMARCA5/SNF2H, histone chaperones and histone variants such as APLF/MacroH2A and proteins that help to maintain a transcriptionally repressive environment in the open chromatin (for example, CHD4/NuRD complex). The net result is dynamic changes in chromatin architecture surrounding the damaged DNA, which aids its efficient repair

such as a BRCT domain, in PARP1 does enable interactions with the unmodified protein.

To date, there are four known classes of PAR-binding modules: PAR-binding linear motifs (PBMs), macrodomains, PAR-binding zinc fingers (PBZs) and the WWE domains (Žaja et al. 2013). PBMs were first observed as specific non-covalent association sites between PAR and p53 and were later defined to contain a consensus sequence of basic and hydrophobic residues (Althaus et al. 1999; Malanga et al. 1998; Pleschke et al. 2000). Macrodomains are found throughout all kingdoms of life (Pehrson and Fried 1992). Notably, this module is present in the chromatin-modifying proteins ALC1 (Ahel et al. 2009; Gottschalk et al. 2009) and macroH2A (Timinszky et al. 2009), as well as the main ADP-ribose hydrolysing enzymes—PARG (Slade et al. 2011), TARG1 (Peterson et al. 2011; Sharifi et al. 2013) and MacroD1/2 (Chen et al. 2011; Jankevicius et al. 2013). On the other hand, PBZs are found in only three human proteins: histone chaperone APLF, CHFR and SNM1A, although they are more common in some other eukaryotes (Ahel et al. 2008; Eustermann et al. 2010; Mehrotra et al. 2011; Oberoi et al. 2010; Rulten et al. 2008). The most recently identified PAR-binding module, the WWE domain, links PARylation and ubiquitylation pathways and was identified in a PAR-directed E3 ligase, Iduna (RNF146), which targets PARylated proteins for proteasomal degradation (Aravind 2001; He et al. 2012; Kang et al. 2011; Wang et al. 2012). Collectively, PAR-binding modules enable proteins to be recruited to sites of PAR synthesis, such as DNA lesions, in a highly regulated manner.

Recruitment of DNA repair machinery

The first direct evidence that PAR provides a scaffold for the recruitment of DNA repair complexes was provided in 1998, when an interaction between SSBR protein XRCC1 and PARP1 (and later PARP2) was identified (Masson et al. 1998; Schreiber et al. 2002). Later, it was shown that XRCC1 accumulated at sites of PAR synthesis, suggesting PAR-mediated recruitment of XRCC1 to DNA damage sites with the aid of the conserved PAR-binding motif described earlier (El-Khamisy et al. 2003; Okano et al. 2003; Pleschke et al. 2000). Despite lacking any known catalytic activity, XRCC1 is a linchpin of the SSBR pathway (Caldecott 2003), mediating recruitment and assembly of the SSBR machinery including DNA polymerase-β, OGG1, AP endonuclease and DNA ligase

III—some of which also interact directly with PAR (Dantzer et al. 2000; Leppard et al. 2003; Noren Hooten et al. 2011; Simsek et al. 2011). Notably, in many cases, PARP1 not only interacts with but also modifies DNA repair proteins, although in most cases, the significance of this is unclear (Ahel et al. 2008; Masson et al. 1998).

As we have already seen, PARP1 is activated by binding not only to DNA SSBs but also to DSBs, and as such, it is implicated in the repair of DSBs by classical/alternative non-homologous end-joining (c/aNHEJ) and homologous recombination (HR) pathways (Pears et al. 2012). PARP1 is involved in the early recruitment of the MRN complex to DSBs (Haince et al. 2008) and interacts with cNHEJ components such as WRN (Adelfalk et al. 2003), Ku (Li et al. 2004) and DNA-PK (Spagnolo et al. 2012). However, cNHEJ is normal in PARP1-deficient cells (Yang et al. 2004), leading to postulation that other PARPs may also be responsible for ADP-ribosylation in cNHEJ (see below). Instead, PARP1 is suggested to facilitate aNHEJ by promoting synapsis of DNA ends and recruiting a novel complex of XRCC1, DNA ligase III and polynucleotide kinase (Audebert et al. 2004, 2006). PARylation by PARP1 appears to have little direct effect on HR since homology-directed repair is normal in PARP-depleted cells (Schultz et al. 2003), although deletion of PARP1 in chicken DT40 cells (which lack functional PARP2) did result in reduced HR (Hochegger et al. 2006). However, PARP1 has been strongly implicated in HR-mediated repair and reactivation of stalled replication forks, thus facilitating faithful DNA replication (Berti et al. 2013; Sugimura et al. 2008; Yang et al. 2004). Indeed, PARP1 promotes recruitment of MRE11 and RAD51 specifically in response to severe stalled replication forks (Bryant et al. 2009; Haince et al. 2008). Furthermore, it was shown that ablation of PARP1 causes sensitivity to Topo I inhibitors and, later, that PARP1/PAR have roles in the release of Topo I from stalled forks (Ray Chaudhuri et al. 2012).

PAR also interacts with proteins from different signalling networks involved in the DDR. For example, the fundamental DDR transcription factor and checkpoint control protein, p53, has been known for some time to interact with PAR (Malanga et al. 1998) and to be a PARylation target of PARP1 (Mendoza-Alvarez and Alvarez-Gonzalez 2001; Valenzuela et al. 2002; Wesierska-Gadek et al. 1996), supporting a physical and functional interaction with PAR or PARylated PARP1. The physiological role of this interaction has remained controversial, although in 2007, Kanai et al. showed that DNA damage-induced PARylation of p53 by PARP1 leads to its accumulation in the nucleus by blocking its interaction with the nuclear export factor Crm1 (Kanai et al. 2007).

Although PARP1 has received the most attention in terms of DNA damage, the roles of other PARPs in the DNA damage response have also been uncovered. PARP2, for example, shares many of the functions of PARP1—most notably in its engagements with the same SSBR machinery (Yelamos et al. 2008). Furthermore, some authors suggest that PARP2 is specialised for later steps of the repair pathway and perhaps in the repair of gaps and flap structures rather than conventional SSBs (Kutuzov et al. 2013; Mortusewicz et al. 2007). On the other hand, PARP3 is activated by binding to DSBs in vitro and is now emerging as an important component of the NHEJ repair pathway (Boehler et al. 2011; Rulten et al. 2011). It has been shown that PARP3 promotes recruitment of APLF to sites of DSBs, a function analogously performed by PARP1 in SSBR (see below) (Fenton et al. 2013; Rulten et al. 2011). Finally, Tankyrase 1 has also been implicated in DNA repair in a function independent of telomere length maintenance. Although very poorly understood, it appears to have a role in stabilization of DNA-PKcs in NHEJ (Dregalla et al. 2010). For a more thorough review of PARPs/PAR and DNA repair, see De Vos et al. (2012).

PARylation of chromatin-associated proteins promotes chromatin re-organization

A fundamental requirement of an efficient DNA damage response is the re-organization of chromatin, such that the extensive DNA repair machinery can access the damaged site. Chromatin plasticity is regulated by a variety of factors including post-translational modifications (such as PARylation, acetylation, phosphorylation, SUMOylation and ubiquitination), ATP-dependent chromatin remodellers and histones/histone variants.

Modification of chromatin-associated proteins with PAR (Fig. 2 (A)) has long been suggested to promote a relaxed chromatin conformation. As far back as 1982, it was shown in vitro that PAR and PARylated histones facilitate nucleosome disassembly (Aubin et al. 1982; Poirier et al. 1982). Soon after, ADP-ribosylation of histones was described in vivo (Krupitza and Cerutti 1989), and since then, this modification has been shown to mainly occur on arginine and glutamate residues (Ogata et al. 1980; Ushiroyama et al. 1985)—although more recently, lysine residues have also been suggested as ADP-ribosylation sites (Messner et al. 2010). Yet PARP1 itself is the most significant acceptor of PAR and is also released from DNA following the accumulation of negatively charged PAR polymers. Moreover, it was shown that PARP1 can directly regulate chromatin structure without modification of core histones (Kim et al. 2004), and so the physiological significance of such trans-modifications in the regulation of chromatin structure is open to debate.

A useful model organism for studying PAR-mediated regulation of chromatin structure has been *Drosophila melanogaster*, where the ability of PARP1 to relax chromatin structure by ADP-ribosylation has been demonstrated in native chromatin at puff loci (Tulin and Spradling 2003). Puff loci are local loosenings of the polytene chromatin structure associated with transcription (for example, the

Hsp70 chaperone), which usually appear under stress conditions such as heat shock treatment. Tulin et al. showed that puffs acquire elevated levels of ADP-ribose polymers and that PARP is required for induction of hsp70 expression after heat exposure (Tulin and Spradling 2003). Some years later, PARylation of histones was suggested to account for the rapid loss of nucleosomes at hsp70 gene after heat shock (Petesch and Lis 2008). Recently, the recruitment and activation of PARP1 at stress-induced chromatin loci have also been associated with its co-localization with histone variant H2Av (the homolog of human H2Az/H2Ax) (Kotova et al. 2011). In H2Av *Drosophila* mutants, PARylation is severely reduced, indicating that H2Av is required to regulate the enzymatic activity of PARP1 in vivo.

Recruitment of chromatin-modifying proteins

In addition to direct modifications of histones, PARP1/PAR also contribute to chromatin re-organization by facilitating recruitment of chromatin-modifying proteins such as chromatin remodellers and histone chaperones (Fig. 2 (B)). One of the best understood chromatin remodellers which requires PAR for its function and efficient recruitment to DNA damage sites is ALC1. ALC1 (also known as CHD1L) is a macrodomain-containing SNF2-like ATPase and is recognized as a possible oncogene since it is found in excess in hepatocellular carcinoma cells and because overexpression of ALC1 in mice induces spontaneous tumours (Chen et al. 2009; Ma et al. 2008). ALC1 is rapidly recruited to DNA breaks in a PAR-dependent manner (Ahel et al. 2009; Gottschalk et al. 2009), and ALC1-depleted cells show sensitivity to DNA-damaging agents (Ahel et al. 2009). Furthermore, the ATP-dependent nucleosome remodelling activity of ALC1 is stimulated by PARP1 and NAD+ (Ahel et al. 2009; Gottschalk et al. 2009). More recently, it was shown that ALC1 activation depends on the formation of a stable, PAR-mediated ALC1–PARP1–nucleosome intermediate (Gottschalk et al. 2012) and that ALC1 stably binds PARylated PARP1 via its macrodomain region (Ahel et al. 2009; Gottschalk et al. 2012). Altogether, these results support the hypothesis that PARylated PARP1 plays a unique role as an allosteric effector of ALC1 chromatin remodelling to promote PAR-dependent chromatin relaxation at DNA damage sites.

SMARCA5/SNF2H, the catalytic subunit of ISWI chromatin remodelling complexes, is also recruited to DSBs in a PARP1-dependent manner. SMARCA5 accumulation at DNA damage sites is significantly reduced, but not abolished, in PARP1-depleted/inhibited cells, whereas depletion of PARP2 does not have any notable effect (Smeenk et al. 2013). Remarkably, not only the recruitment and distribution of SMARCA5 along damaged chromatin loci but also its interaction with RNF168 (an E3 ubiquitin ligase involved in DSB repair (Doil et al. 2009)) depends on PARP1 activity. Indeed, in vivo studies show that SMARCA5 recognizes and binds to PAR polymers on RNF168 (Smeenk et al. 2013). These data suggest a physical and functional association between DNA damage-induced PARylation, chromatin remodelling and the signalling cascade that is initiated by ubiquitin ligases. Interestingly, another E3 ligase, CHFR, contains a PBZ domain for interaction with PAR (Ahel et al. 2008) and was recently shown to modulate the early stages of the DNA damage response by ubiquitination (and subsequent targeting for proteasomal degradation) of chromatin-associated PARP1 and, possibly, PARylated histones, thereby promoting chromatin relaxation (Kashima et al. 2012; Liu et al. 2013).

In addition to chromatin remodelling proteins, the histone chaperone APLF is also recruited to DNA damage sites via an interaction with PAR that is mediated by its PBZ domains (Ahel et al. 2008; Iles et al. 2007; Mehrotra et al. 2011). Abolishment of PAR synthesis delays and sustains APLF recruitment (Bekker-Jensen et al. 2007), and down-regulation of APLF leads to cellular sensitivity to DNA damaging agents (Iles et al. 2007). APLF was shown to bind strongly to histones particularly in the response to DNA damage, where it might promote histone removal and recruitment of histone variants such as MacroH2A1 (Mehrotra et al. 2011). PAR-mediated recruitment of the macrodomain-containing protein MacroH2A1.1 to laser-induced DNA damage sites had previously been demonstrated, and cells expressing this immobile, chromatin-associated histone variant were shown re-organize chromatin structure in response to PARylation (Timinszky et al. 2009; Xu et al. 2012).

In addition to promoting chromatin relaxation, chromatin-modifying proteins are also implicated in the establishment of a transcriptionally repressed state. CHD4, a member of the SNF2/RAD54 helicase family, is also recruited to DNA damage sites in a PAR-dependent manner (Polo et al. 2010). CHD4 represents the ATPase catalytic subunit of the NuRD chromatin remodelling complex that plays an important role in epigenetic transcriptional repression. Recent data have implicated CHD4 particularly in the response to DNA damage, since it accumulates at laser-induced DNA damage sites and its depletion results in delayed repair of DSBs (Larsen et al. 2010; Pan et al. 2012; Polo et al. 2010). Its localization to DNA damage sites is reliant upon PAR, since PARP1/2 ablation completely abrogates CHD4 accumulation at laser-microirradiated sites. Furthermore, the *Drosophila* CHD4 orthologue, dMi-2, was recently shown to possess PAR-binding activity and to rely on that activity for its rapid localization to heat shock loci (Murawska et al. 2011).

Another protein complex involved in chromatin remodelling and transcriptional repression at DNA damage sites is the Polycomb group (PcG) proteins, protein complex 1 (PRC1) and 2 (PRC2). These complexes were shown to be

recruited to DNA damage sites in a PAR-dependent manner (Chou et al. 2010). Together with the NuRD complex, they are responsible for producing a transcriptionally repressive chromatin structure, thereby facilitating DNA repair.

PAR and transcriptional regulation

In the response to DNA damage, generalised shutdown of transcription is an important mechanism to prevent further damage caused by collision of transcription/repair complexes or unwinding of damaged DNA (Beneke 2012). We have already discussed how various PAR-dependent chromatin remodelling complexes, such as the CHD4/NuRD and PcG complexes, establish a chromatin microenvironment that is transcriptionally repressive. In addition, PARylation of Spt16, a component of the pro-transcriptional FACT complex, causes its dissociation from DNA, further inhibiting transcription at DNA damage sites (Heo et al. 2008; Huang et al. 2006). Furthermore, interaction of PAR with transcription factors such as p53 can affect their function and in this way might affect transcription on a more global scale following DNA damage.

In addition to controlling the establishment and maintenance of a transcriptionally repressive state, PAR also has activating roles in transcription in the response to DNA damage. For example, PARP1 functions as a trans-activator of transcription with NFκB, inducing the expression of pro-inflammatory genes particularly after acetylation by p300 and CREB-binding protein (Chang and Alvarez-Gonzalez 2001; Hassa and Hottiger 1999; Hassa et al. 2001, 2005). Whilst the direct trans-activator function of PARP1 was shown not to require PAR, a second mechanism of NFκB activation, in which DNA damage-activated and auto-modified PARP1 facilitates translocation of NFκB from the cytoplasm to the nucleus, was PAR-dependent (Stilmann et al. 2009).

It should be noted that PARP1 and PAR are also heavily implicated in general transcriptional regulation in the absence of DNA damage. For example, as far back as 1983, PARP1 was isolated as TFIIC, a transcription factor shown to inhibit random transcription at nicked DNA by RNA polymerase II (Slattery et al. 1983), and PARylation of TATA-binding protein and TFIIF causes their eviction from transcription complexes (Oei et al. 1998; Rawling and Alvarez-Gonzalez 1997). A thorough evaluation of the roles of PARP1/PAR in transcriptional regulation is beyond the scope of this review.

PAR and apoptosis

PAR not only facilitates repair of DNA lesions, but also acts as a DNA damage sensor and mediator of the life-or-death decision of the cell (Luo and Kraus 2012). At low DNA damage loads, PARylation by PARP1 facilitates DNA repair via the processes discussed in this review. However, following excessive DNA damage, cells undergo cell death pathways rather than attempting repair. Perhaps most significantly, PAR contributes to parthanatos (or PAR-mediated cell death) via an apoptosis-inducing factor (AIF)-mediated mechanism (Barkauskaite et al. 2013; Cregan et al. 2004; David et al. 2009; Wang et al. 2009, 2011). PARP1 activation by excessive DNA damage leads to the PAR-mediated release of AIF from the mitochondria, from where it translocates to the nucleus and induces cell death by DNA fragmentation (Wang et al. 2009). This release is triggered by the physical interaction of PAR with the PBM recently identified in AIF (Wang et al. 2011).

PARP1 appears to de dispensable for caspase-mediated apoptosis, despite its cleavage into 89-kDa and 24-kDa fragments by activated caspases being a hallmark of this process (Lazebnik et al. 1994; Wang et al. 1997). However, PARP1 over-activation contributes significantly to necrosis by depletion of the cellular NAD+ pool and subsequent energy crisis-induced cell death (Ha and Snyder 1999). As such, PARylation is at the forefront of both DNA repair and cell death pathways, contributing extensively to the organism's genomic integrity.

Concluding remarks

PAR and the proteins involved in its metabolism are now well established as central mediators of the DNA damage response. Within seconds of DNA damage induction, the chromatin microenvironment becomes a hive of biochemical signals, of which PAR is arguably one of the most significant. PARP1/PAR have diverse and extensive roles in mediating chromatin re-organization, recruiting various DNA damage response factors, regulating transcription and, in some cases, inducing apoptosis. One of the pressing questions in the field is how the large-scale recruitment of such a vast array of protein factors, each with diverse and important roles, to highly localised DNA lesions is coordinated such that steric hindrance is avoided. Accurate metabolism and catabolism of the transient PAR signal, spatio-temporal specificity of different PAR-binding domains and the multiplicity of PARPs could provide answers that help to develop our understanding of the field. Moreover, a better understanding of PAR biology in the DNA damage response might lead to improved efficacy of PARP inhibitors in cancer therapy.

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