

## Discovering new ADP-ribose polymer cycles: protecting the genome and more

Multicellular organisms possess multiple mechanisms to maintain genomic integrity. When these mechanisms fail, the results can be catastrophic, ranging from unlimited cell proliferation in the case of metastatic cancer to the massive cell death that occurs as a result of reperfusion injury following cardiac or cerebral ischemia. Central to the mechanisms that maintain genomic integrity is the modification of nuclear proteins by ADP-ribose (ADPR) polymers<sup>1</sup>. ADPR polymers have a wide range of sizes but can be more than 100 residues and contain multiple points of branching<sup>2</sup> (Fig. 1). These structural features and the resulting high density of negative charge make them well suited for modulating the properties of DNA-binding proteins, by competing with DNA for interaction with DNA-binding proteins. Individual polymers have a very transient existence due to the closely coordinated polymer synthesis from nicotinamide adenine dinucleotide (NAD) by poly(ADP-ribose) polymerases (PARPs) and hydrolysis to free ADPR by poly(ADP-ribose) glycohydrolase (PARG)<sup>1</sup> (Fig. 1).

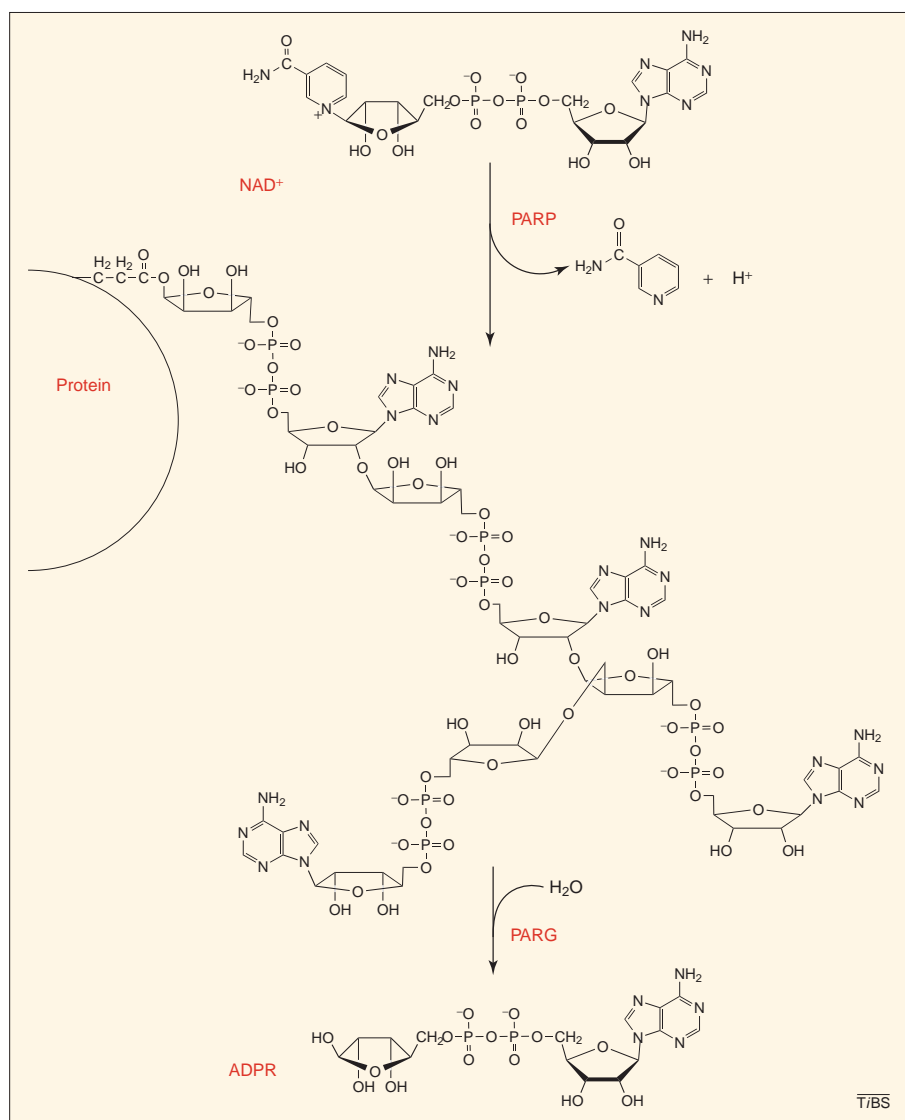
PARPs belong to a larger family of cell-signaling enzymes that catalyze the transfer of ADPR from NAD to acceptors. Other members of this group are protein-mono-ADP-ribosyltransferases<sup>3</sup> and cyclic ADP-ribose synthases<sup>4</sup>. Although it has been realized for some time that ADPR polymer cycles are involved in the maintenance of genomic integrity, an expanded involvement of this protein modification in cellular functions is suggested by the recent discoveries of multiple members of the PARP family.

### PARP-1

The best understood and, until recently, the only known member of the PARP family is now referred to as PARP-1. PARP-1 is a highly conserved 113-kDa multidomain protein containing a DNA-binding domain with two zinc finger motifs, a nuclear location signal (NLS), a combined BRCA1 C terminus (BRCT)/polymer automodification domain and a catalytic domain<sup>5</sup>. The pres-

ence of DNA strand breaks strongly activates PARP-1, and many studies (reviewed in Ref. 1) have provided evidence that, in concert with other DNA break sensors such as p53 and DNA protein kinase, PARP-1 participates in modulating DNA base excision repair, apoptosis and necrosis. Studies of mouse strains with a disrupted PARP-1 gene<sup>6-8</sup> have generated much useful information concerning PARP-1 functions. Under conditions

of low or moderate DNA damage, PARP-1 functions as a survival factor in concert with many other DNA damage response checkpoint genes. Consequently, PARP-1 knockout (KO) animals exhibit genomic instability in response to alkylating agents and  $\gamma$ -irradiation. On the other hand, when excessive DNA damage occurs, such as in the case of ischemia reperfusion injury, PARP-1 functions to promote cell death probably because its activity is not limited by caspase cleavage. Thus, PARP-1 KO animals are highly resistant to the tissue-damaging effects of oxidative stress<sup>1</sup>. The recent observation that cells derived from PARP-1 KO animals still generate ADPR polymers<sup>9</sup> suggested the presence of multiple PARP activities in mammalian cells. In the last few months, tankyrase, PARP-2, V-PARP and a putative PARP-3 have been discovered.



**Figure 1**

The synthesis of ADP-ribose (ADPR) polymers from oxidized nicotinamide adenine dinucleotide (NAD<sup>+</sup>) catalyzed by poly(ADP-ribose) polymerase (PARP) and hydrolysis of polymers to ADPR catalyzed by poly(ADP-ribose) glycohydrolase (PARG).

### Tankyrase

The discovery of a component of chromosome termini (telomeres) containing PARP activity<sup>10</sup> provides a new paradigm for the involvement of ADPR polymer cycles in the maintenance of genomic integrity. Tankyrase, a 142-kDa protein, has a catalytic domain homologous to PARP-1 but, otherwise, has a very different domain structure. Most notably, tankyrase contains 24 tandem repeats of the ankyrin motif, a 33-amino-acid-sequence motif that often links membrane proteins to the cell cytoskeleton. In contrast to PARP-1, tankyrase does not appear to require DNA for activity and it interacts with and catalyzes polymer modification of the telomere-specific protein TRF1 *in vitro*. Telomeres are the terminal regions of chromosomes that contain unique repetitive DNA sequences and G-rich, single-stranded overhangs that are stabilized by the telomere-specific proteins TRF1 and TRF2 (Ref. 11). Recently, a model for a telomere loop structure has been proposed<sup>11</sup>, in which TRF1 binds to and aligns double-stranded telomere DNA into a structure (t-loop) that allows the G-rich overhangs to invade and form a displacement loop

(D-loop) with double-stranded telomere DNA. D-loop formation is promoted or stabilized by TRF2, or both. An attractive feature of the loop model is that it provides an explanation for how telomeres escape detection by DNA-damage checkpoint proteins but it seems logical that this structure must be disassembled and reassembled to allow telomere replication and, when telomerase is present, telomere length stabilization. The telomere loop model suggests a role for tankyrase and PARG in disassembly and reassembly of the loop as suggested in Fig. 2. Tankyrase-catalyzed addition of negatively charged polymers to TRF1 results in disassociation of TRF1 from telomere DNA and disassembly of the loop. In turn, PARG-catalyzed degradation of TRF1-associated polymers allows reassembly of the loop.

### PARP-2

Three recent publications have reported the isolation of partial<sup>12,13</sup> and full-length<sup>14</sup> cDNAs from mouse and human encoding a protein with considerable homology to the catalytic domain of PARP-1. This protein, termed PARP-2, is a 62-kDa protein that contains an NLS and is activated by DNA breaks, although

its DNA-binding domain is very different from that of PARP-1 (Ref. 14). Function(s) for PARP-2 are not yet known but its nuclear location and activation by DNA breaks<sup>14</sup> suggest that it is probably responsible for the polymer synthesis observed in cells derived from PARP-1 KO mice<sup>9</sup>. The activation of PARP-2 by DNA damage might allow it to compensate partially for the absence of PARP-1 in PARP-1 KO animals. An intriguing possibility is that PARP-2 is involved in the repair of DNA damage in chromatin (e.g. telomeres), where access to DNA-damage checkpoint proteins (e.g. p53, PARP-1) must be avoided<sup>11</sup>.

### V-PARP

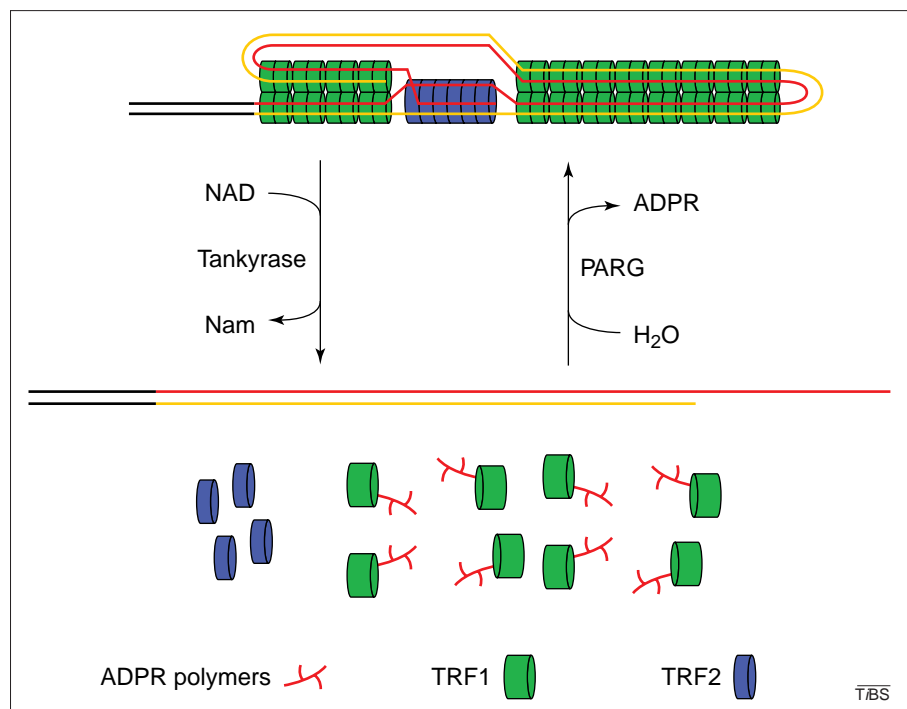
Another protein that contains a domain homologous to the catalytic domain of PARP-1 (Ref. 15) has been identified recently as one of three proteins present in vaults<sup>16</sup> (i.e. large ribonucleo-protein complexes of unknown function located primarily in the cytoplasmic compartment<sup>17</sup>). V-PARP is a 193-kDa protein containing BRCT and inter-alpha trypsin inhibitor domains and several putative NLS sequences. It also contains a domain that interacts with the major protein component of vaults (MVP). MVP is a substrate for V-PARP-catalyzed modification by ADPR polymers *in vitro*. It is interesting that the third protein component of vaults is a protein (TEP-1) that is also a component of the telomerase complex. Not all V-PARP is associated with vaults, and it is intriguing that V-PARP, but not the other components of vaults, localizes to the mitotic spindle. Similar to tankyrase, V-PARP does not appear to require DNA for activity. While the localization of V-PARP in mitotic spindles suggests a role in maintaining genomic integrity, the primarily cytoplasmic location of vaults<sup>17</sup> indicates that ADPR polymer cycles might not be restricted to the cell nucleus.

### Still more PARPs?

The presence of an additional human cDNA sequence with high sequence homology to PARP-2 has been reported<sup>13</sup>. If this protein (PARP-3?) is shown to have PARP catalytic activity, it will represent the fifth known member of the PARP family in mammalian cells.

### PARG

The isolation and characterization of cDNAs encoding PARG has provided the first detailed information concerning the enzymatic activity that catalyzes the hydrolysis of ADPR polymer



**Figure 2**

A speculative scheme for the involvement of tankyrase and poly(ADP-ribose) glycohydrolase (PARG) in the disassembly and reassembly of a telomere loop structure. The black lines represent non-telomere DNA and the yellow and red lines represent C-rich and G-rich strands of telomere DNA, respectively. The G-rich overhang is stabilized by the telomere-specific proteins TRF1 and TRF2 to form a loop structure. Tankyrase-catalyzed addition of negatively charged ADP-ribose (ADPR) polymers to TRF1 results in dissociation of TRF1 from telomere DNA and disassembly of the loop. In turn, PARG-catalyzed degradation of TRF1-associated polymers allows reassembly of the loop. Nam refers to nicotinamide.

residues<sup>18,19</sup>. PARG from mammals is a highly conserved protein of 111 kDa containing an N-terminal putative regulatory domain, a centrally located putative NLS and a C-terminal catalytic domain<sup>19</sup>. PARG is encoded by a single-copy gene and its amino acid sequence shows little or no homology with other known proteins<sup>18</sup>. This raises the possibility that a single gene product catalyzes the hydrolysis arm of polymer cycles initiated by several different PARPs. A definitive answer to the question of whether multiple PARG gene products are present or whether other activities can replace PARG in polymer degradation awaits characterization of cells and, if viable, animals in which the known PARG gene is inactivated. The presence of multiple forms of PARG (Ref. 20) is consistent with the possibility that different PARG isoforms serve as partners to different PARPs.

#### Implications for health and disease

Early studies (reviewed in Ref. 1) revealed that PARP inhibitors enhance the cytotoxic effects of DNA damage repaired by the DNA base excision repair pathway and several different PARP inhibitors are under evaluation as chemosensitizing or radiosensitizing agents in cancer chemotherapy. More recent studies (also reviewed in Ref. 1) demonstrating the remarkable resistance of PARP-1 KO animals to myocardial infarction, stroke, shock, diabetes and neurodegeneration have identified PARP-1 as an attractive target for the development of new approaches to treat these clinical conditions. The unlimited proliferation potential of cancer cells is closely linked with their ability to maintain stable telomere structures. Thus, the disruption of telomere stability by targeting tankyrase suggests a new approach for cancer treatment. The upregulation of vaults in multidrug-resistant cancers<sup>21</sup> might also make V-PARP a target for cancer therapeutics. Most inhibitors developed to inhibit PARP-1 act at or near the nicotinamide region of the NAD-binding site of the enzyme, which is likely to have a similar structure in all PARPs. This is supported by the observations that 3-aminobenzamide inhibits the activity of each of the four known PARPs<sup>5,10,14,16</sup>. The discovery of multiple PARPs now dictates the design of inhibitors specific for different PARPs for both elucidation of function and therapeutic targeting of ADPR polymer cycles. Finally, PARG may be an important therapeutic target. The

dynamic nature of polymer cycles indicates that PARPs and PARG function coordinately, thus inhibition (or activation) of PARG is expected to affect functions modulated by polymer cycles. The low homology of PARG to other known proteins and its structurally unique substrate suggest that highly selective PARG inhibitors could be developed. Furthermore, since each of the known PARPs undergo automodification, modulation of PARG might be an effective way to modulate these enzymes.

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## Covers

In the September 1999 issue, Birkbeck College Crystallography Dept should also have been thanked for providing materials for the cover.

The cover of the October 1999 issue was inspired by a design supplied by A. F. Oberhauser. We apologize for these omissions.

