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The rise and fall of poly(ADP-ribose): An enzymatic perspective

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ABSTRACT

Human cells respond to DNA damage with an acute and transient burst in production of poly(ADP-ribose), a posttranslational modification that expedites damage repair and plays a pivotal role in cell fate decisions. Poly(ADP-ribose) polymerases (PARPs) and glycohydrolase (PARG) are the key set of enzymes that orchestrate the rise and fall in cellular levels of poly(ADP-ribose). In this perspective, we focus on recent structural and mechanistic insights into the enzymes involved in poly(ADP-ribose) production and turnover, and we highlight important questions that remain to be answered.

1. Introduction

Cells respond instantaneously to DNA damage with post translational modifications of proteins that repair DNA damage, alter gene expression, or control passage through the cell cycle. The covalent modification of these proteins induce a dynamic network of protein–protein interactions and regulates enzymatic activities, broadly changing cellular physiology and serving to integrate myriad responses to DNA damage that dictate outcomes for DNA repair, cell survival, and responses to chemotherapy. One of the most prodigious posttranslational modifications caused by DNA damage is the poly-(ADP-ribosylation) of proteins, catalyzed by members of the poly-(ADP-ribose) polymerase (PARP) superfamily of NAD^+ dependent ADP-ribosyltransferases [1]. Poly-(ADP-ribose) (PAR) is a large, negatively-charged and branched polymer that can exceed the mass of the unmodified protein. PARylation creates binding sites for PAR-specific binding proteins [2,3] and changes the electrostatic properties of the modified protein, with the notable capacity to change DNA binding properties of enzymes, histones, and structural proteins [4]. PARP-1 itself is the target of most of the poly-(ADP-ribosylation) (PARylation) occurring in response to DNA damage. Automodification of PARP-1 increases its association with a variety of repair and signaling proteins that are recruited to sites of DNA damage by PARP-1 activity [3,5]. In turn, some of these proteins are PARylated by PARP-1.

PARP enzymes responding to damage can consume substantial amounts of cellular NAD^+ within minutes, changing a cell's metabolic status while modifying vast numbers of proteins, many of which have been recently identified by proteomic surveys [6,7]. For most of these proteins, the effects of PARylation remain to be functionally characterized. These studies are complicated by the fact that PAR modifications turn over rapidly due to the activity of poly-(ADP-ribose) glycohydrolase (PARG) and mono-(ADP-ribose) glycohydrolases (MARGs) [8,9]. Both the synthesis and turnover of poly-(ADP-ribose) appear to be important for normal responses to DNA damage. In this short perspective, we will review the recent literature on the structures and functions of DNA damage-dependent PARPs and PARG, and then speculate about how these activities may be tied mechanistically to various disease processes and the resulting opportunities for therapeutic intervention.

2. Structure and mechanism of DNA damage-dependent PARPs

Three members of the PARP superfamily are catalytically activated through interaction with DNA damage: PARP-1, PARP-2, and PARP-3. PARP involvement in the cellular response to DNA damage has long been appreciated and continues to actively develop [10,11]. A general model that has collectively emerged indicates that the DNA-damage dependent PARPs act early in the process of damage detection, which promptly results in PARP catalytic activation and a burst of PAR production. PARP presence and activity at the damage site then can contribute to the efficiency of the

Abbreviations: PAR, poly(ADP-ribose); PARP, PAR polymerase; PARG, PAR glycohydrolase; MARG, mono-(ADP-ribose) glycohydrolase; PARylation, poly(ADP-ribosylation).

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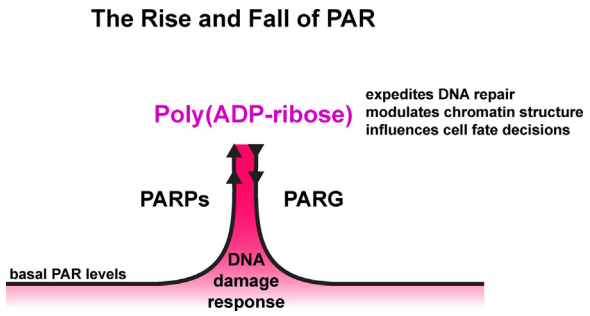

Fig. 1. The rise and fall of poly(ADP-ribose). The ADP-ribose posttranslational modification regulates many fundamental aspects of human biology. During the DNA damage response, there is an acute and transient burst of poly(ADP-ribose) production and turnover that facilitates repair and contributes to important cell fate signaling events.

repair process and the repair pathway choice. A key role of the DNA-damage dependent PARPs and the PAR modification they produce is to recruit repair factors to the site of damage. Several motifs and domains have been identified in repair proteins that mediate the interaction with PAR and the recruitment to sites of PAR synthesis [12,13]. In addition to PAR serving as a recruiting platform, PAR modification of repair and chromatin-associated factors in the vicinity of a damage site is expected to change the catalytic properties of targeted proteins, and the local structure of chromatin [10]. However, detailed insights into PAR-mediated regulation of protein function are lacking in general. And although a general model for PARP contribution to the DNA damage response has formed, the molecular details of PARP involvement are not clearly established, which has limited our understanding of PARP's contribution to specific steps of repair, and the contribution of different PARPs to repair pathway choice. Over recent years, structural and biochemical studies have provided key insights into the early stages of PARP-1 involvement in DNA repair: The detection of DNA damage, and the allosteric coupling of damage detection to acute levels of PAR production. Here we will provide an overview of these important insights into PARP-1 mechanism, and we will indicate some of the key questions that remain to be answered (Fig. 1).

The DNA-damage dependent PARPs have similar catalytic domain structures, but they differ somewhat in the domains that contact DNA damage (Fig. 2) [13]. In the catalytic domain, they share a conserved structural feature known as the helical domain (HD) [14] (also referred to as the PARP regulatory domain- PRD). The HD is only found in the DNA-damage dependent PARPs, and it plays an important role in regulating PARP catalytic activity, as described later. The HD is adjacent to the ADP-ribosyl transferase (ART) fold, which is common to all PARP family members. The ART contains the binding site for NAD^+, which donates ADP-ribose, and a second binding site for an ADP-ribose unit, which accepts the next ADP-ribose during the PAR extension reaction that can result in both linear and branched polymers [15,16] (Fig. 2). Detailed structural views of PAR biosynthesis (NAD^+ binding, initiation on target protein, polymer extension) have not been obtained, thus our complete understanding of PAR synthesis is limited. The NAD^+ binding sites for the DNA-damage dependent PARPs are similar and have the conserved His-Tyr-Glu (HYE) amino acids that define catalytically active PARP members capable of forming PAR (as opposed to mono-ADP-ribose) [17,18]. The acceptor binding sites vary between PARP-1, PARP-2, and PARP-3 and this is likely to influence the type of polymer formed (e.g., polymer length, number of branch points). For example, PARP-3 has an Arg residue in the acceptor site where PARP-1 and PARP-2 have a Met residue, which is expected to contribute to the binding pocket for the adenosine base of an acceptor ADP-ribose modification [16]. Presumably this

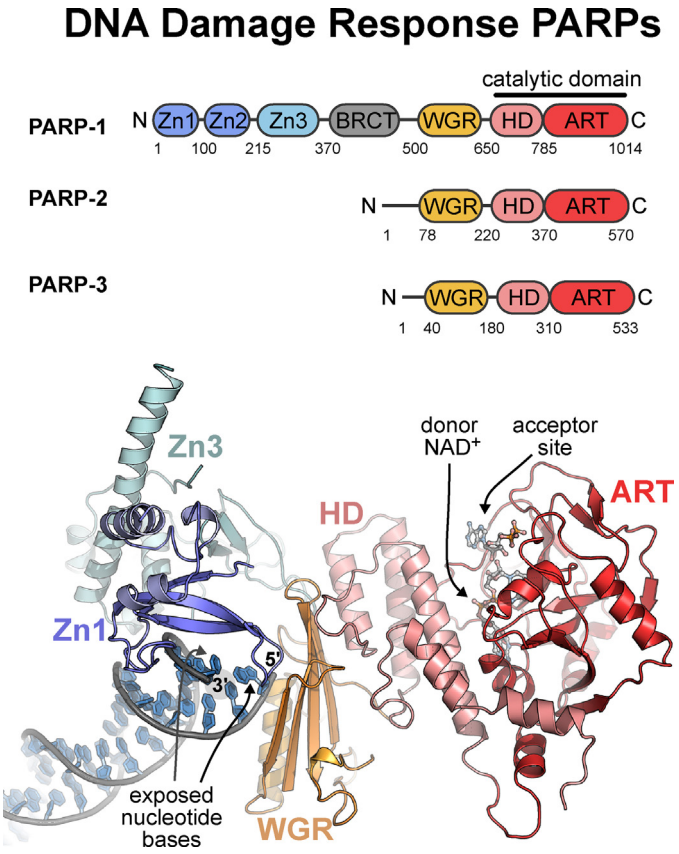

Fig. 2. DNA damage response PARPs. Three human PARP enzymes are catalytically activated through binding to DNA damage: PARP-1, PARP-2, and PARP-3. The WGR domain and the HD region of the catalytic domain are defining and unique features of the DNA damage-dependent PARPs. PARP-1 consists of multiple domains that assume an active conformation upon binding to DNA damage. Zinc finger domains 1 and 3 (Zn1 and Zn3) interact with a DNA break and pack against the WGR domain, which serves as an intermediary between the C-terminal catalytic and N-terminal DNA binding domains, and allosterically couples damage detection to catalytic activation.

change in sequence perturbs the binding site and contributes to the smaller size of polymer produced by PARP-3 [18]. It is not understood how the differences in the structure of PAR produced might differentially influence downstream signaling to repair pathways, and it will be important to resolve this issue.

3. Mechanism of PARP-1 activation

Outside of the catalytic domain, the DNA-damage dependent PARPs also have in common a Trp-Gly-Arg (WGR) domain that is essential to damage-dependent activation, and is the most defining feature of the DNA-damage dependent PARPs. A crystal structure that contained the essential domains of PARP-1 in complex with DNA damage provided the first views of the WGR domain contacts with DNA (Fig. 2). The structure indicated that conserved regions of the WGR make sequence-independent contacts with the DNA backbone near the 5′ terminus [19]. The importance of these contact residues to catalytic activation was confirmed through mutagenesis. Although their are no structures for PARP-2 and PARP-3 in complex with DNA damage, it is interesting to note that their activation levels are sensitive to modifications to the 5′ terminus of the DNA, such as phosphorylation [20], suggesting that their WGR domains have specialized interactions with the 5′ terminus. PARP-1 in contrast is relatively insensitive to the detailed composition of the break site, consistent with the PARP-1 complex structure in which the 5′ terminus is not directly contacted [19]. The bio-