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Identification of lysines 36 and 37 of PARP-2 as targets for acetylation and auto-ADP-ribosylation

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ABSTRACT

Poly-ADP-ribose polymerase-2 (PARP-2) was described to regulate cellular functions comprising DNA surveillance, inflammation and cell differentiation by co-regulating different transcription factors. Using an *in vitro* and *in vivo* approach, we identified PARP-2 as a new substrate for the histone acetyltransferases PCAF and GCN5L. Site directed mutagenesis indicated that lysines 36 and 37, located in the nuclear localization signal of PARP-2, are the main targets for PCAF and GCN5L activity *in vitro*. Interestingly, acetylation of the same two PARP-2 residues reduces the DNA binding and enzymatic activity of PARP-2. Finally, PARP-2 with mutated lysines 36 and 37 showed reduced auto-mono-ADP-ribosylation when compared to wild type PARP-2. Together, our results provide evidence that acetylation of PARP-2 is a key post-translational modification that may regulate DNA binding and consequently also the enzymatic activity of PARP-2.

1. Introduction

Poly-ADP-ribosylation was postulated to function either as reversible covalent post-translational modification of proteins or as non-covalent attachment of free poly-ADP-ribose polymers to proteins (reviewed in Hassa et al., 2006; Hassa and Hottiger, 2008). The enzyme responsible for the synthesis of poly-ADP-ribose was termed poly-ADP-ribose synthetase (PARS) or poly-ADP-ribose polymerase (PARP). PARP-1 has been initially thought to be the only existing enzyme with poly-ADP-ribosylation activity in mammalian cells. However, five additional *parp*-like genes encoding *"bona fide"* PARP enzymes have been identified in recent years (reviewed in Hassa and Hottiger, 2008). These poly-ADP-ribose polymerases (PARP-1 to PARP-6) comprise an ancient family of enzymes, which share a highly conserved catalytic domain (reviewed in Hassa et al., 2006; Hassa and Hottiger, 2008). The active site, also commonly accepted as the 'PARP signature', is formed by an evolutionarily well-conserved sequence of approximately 50 amino acids (residues 859–908 of hPARP-1). The 'PARP signature' contains the NAD acceptor sites and critical residues involved in the initiation, elongation and branching of poly-ADP-ribose polymers (Marsischky et al., 1995; Rolli et al., 1997; Ruf et al., 1998 and reviewed in Hassa et al., 2006; Hassa and Hottiger, 2008). Among the six PARP family members, PARP-2 bears the strongest resemblance of PARP-1 (60% identity in the catalytic domain) (Ame et al., 1999). PARP-2 and PARP-1 can homo- and heterodimerize and display partially redundant functions as indicated by the embryonic lethality of the *parp1-parp2*-double gene disruption in mice (Menissier de Murcia et al., 2003 and reviewed in Schreiber et al., 2006).

Mouse PARP-2 was described as a 66 kDa nuclear chromatin and nuclear matrix associated protein (Ame et al., 1999 and reviewed in Hassa et al., 2006; Hassa and Hottiger, 2008). The amino-terminal part of PARP-

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2 has no significant homology with PARP-1 or with any other PARP family member. These structural differences between PARP-1 and PARP-2 could indicate different substrate specificities of the two proteins (Ame et al., 1999; Johansson, 1999). The amino-terminal region of human and mouse PARP-2 shows high sequence variability and is rich in basic amino acids (27% Lys or Arg). PARP-2 contains an amino-terminal SAP/SAF motif/module (named after scaffold-associated protein/scaffold-associated factor SAF-A/B, Acinus and PIAS, Aravind and Koonin, 2000), a previously undetected eukaryotic module proposed to be involved in sequence- or structure-specific DNA and RNA binding and often associated with different domains involved in the assembly of pre-mRNA processing complexes (Aravind and Koonin, 2000). Similar to PARP-1, PARP-2 was suggested to synthesize poly-ADP-ribose polymers in a DNA-dependent manner (Ame et al., 1999). However, the exact co-enzyme(s) for PARP-2 remains to be elucidated. PARP-2 also displays auto-modification properties similar to PARP-1 and may account for the residual poly-ADP-ribose synthesis observed in *parp-1* knockout cells. PARP-2 has been described in different functions, which are mainly regulated by protein-protein interactions, and it interacts with other proteins mainly through its amino-terminal domain (aa 1–208) (reviewed in Hassa et al., 2006; Hassa and Hottiger, 2008). Similar to PARP-1, PARP-2 is mostly expressed in actively dividing tissues during mouse development, however to a much lower extent (reviewed in Hassa et al., 2006; Hassa and Hottiger, 2008). Unlike PARP-1, the physiological functions of PARP-2 are not yet understood (reviewed in Hassa et al., 2006; Hassa and Hottiger, 2008).

Recently, protein acetylation, mediated by histone acetyltransferases (HATs), such as p300/CBP (CREB-binding protein) and PCAF (p300/CBP-associated factor), has been proposed as a new mechanism for modulating the enzymatic activities of various enzymes, including acetyltransferases, DNA polymerases, DNA nucleases and kinases (Hasan et al., 2002, 2001; Mittal et al., 2006; Sun et al., 2007; Sun et al., 2005). HATs transfer the acetyl group from Acetyl coenzyme A to the epsilon-amino group of a lysine residue on proteins (Yang, 2004b). The steady-state acetylation level of proteins is accomplished by the action of histone acetyltransferases (HATs) and histone deacetylases (HDACs) (Narlikar et al., 2002; Cheung et al., 2000).

PCAF was originally identified as a CBP/p300-binding protein (Yang et al., 1996). PCAF and GCN5L show sequence conservation in the regions responsible for the HAT activity (Candau et al., 1997; Wang et al., 1998). The nuclear histone acetyltransferases PCAF and GCN5L have been shown to acetylate nucleosomal histones *in vitro* and *in vivo*. PCAF as well as GCN5L exist as components of large multisubunit HAT/chromatin remodeling complexes (Ogryzko et al., 1996; Forsberg et al., 1997). These complexes possess global histone acetylation activity and locus-specific co-activator functions together with FAT activity on non-histone substrates. There are large numbers of known substrates for PCAF and GCN5L including many non-histone proteins, however, histones are considered to be their major targets (for a review see Yang, 2004a). Thus, the biological functions of PCAF and GCN5L cover a wide range of tasks and render them indispensable for the normal function of cells. In vertebrates, it became clear that the two proteins are not expressed in every cell type and even if they are coexpressed in the same cell, their relative expression level can vary substantially.

We have recently reported that PARP-1 can be specifically acetylated by p300/CBP. Here, we provide evidence that PARP-2 is acetylated in mammalian cells and *in vitro* and that the two lysine residues K36 and K37 are the major direct target sites for PCAF and GCN5L-mediated acetylation. Interestingly, acetylation of PARP-2 strongly reduces the DNA binding and enzymatic activity of PARP-2. Finally, PARP-2 with mutated lysines 36 and 37 showed reduced auto-mono-ADP-ribosylation when compared to wild type PARP-2, indicating that lysines 36 and 37 may also serve as target sites for auto-ADP-ribosylation of PARP-2.

2. Materials and methods

2.1. Plasmids

Mammalian expression vectors for wild type mPARP-2 and different mPARP-2 mutants (K19/20R, K36/37R, K19/20/36/37R, K36R and K37R) were obtained by cloning the corresponding PCR products into pphCMV-HA. Baculovirus expression vectors for His-tagged mPCAF and hGCN5L were obtained by cloning the corresponding PCR products into pBACPAK8 (Clontech Laboratories). Baculovirus expression vectors for His-tagged wild type PARP-2 and the different PARP-2 mutants were obtained by cloning the corresponding PCR products into pphBACPAK9-MC-PrSc-HA-HIS or pphBACPAK9-MC-PrSc-HIS. The corresponding baculoviruses were generated using the BACPAK6-based bacmid system from Clontech Laboratories. All PARP-2 mutants were generated by a site directed mutagenesis procedure and confirmed by sequencing. Mammalian expression vectors for mPCAF and hGCN5L were obtained by cloning the corresponding PCR products into pphCMVkm (Hassa et al., 2005). The bacterial expression vectors for GST-HAT full-length fusion proteins (mPCAF and hGCN5L) were obtained by cloning the corresponding PCR products into pGex6P1.

2.2. Expression and purification of recombinant proteins

Recombinant hp300, mCBP, mPCAF and hGCN5L, as well as mPARP-2 full-length, fragments and mutant proteins were expressed as carboxy-terminal His-tagged proteins in *Sf21* insect cells as described previously (Hassa et al., 2003, 2005). Recombinant proteins were purified by standard nickel-NTA affinity chromatography according to the manufacturer's protocol in the presence of 1 M NaCl/0.5% NP-40. GST-tagged proteins were expressed in *E. coli* strain BL21-DE3-Gold as described previously (Hassa et al., 2003, 2005). Recombinant histones H2A/H2B (refolded) were a kind gift from Tobias Stuwe (European Molecular Biology Laboratory (EMBL), Gene Expression Unit, 69117 Heidelberg, Germany). All purified proteins were analyzed by Coomassie staining and confirmed by Western blot analysis using the corresponding antibodies.