Structure
Previews

2005), indicating that binding of E6 to PDZ domains might be implicated in the development of cervical cancer. Remarkably, E6 was found to bind to PTPN3, a very close paralog of PTPN4, via the PDZ domain of PTPN3 (Jing et al., 2007). Indeed, the last five residues at the C terminus of HPV18 E6 read "RETQV," which is a very close sequence to the last five residues of the optimized PTPN4-binding cell killing peptide of Babault et al. (2011) (RETEV). It will be very interesting to investigate whether the cell-penetrating PTPN4-binding peptides developed by Babault et al. interfere with the interaction of HPV E6 with PTPN3 and/or

promote the induction of apoptosis of HPV-positive cancer cell lines, such as HeLa, SiHa, or CaSki.

REFERENCES

Babault, N., Cordier, F., Lafage, M., Cockburn, J., Haouz, A., Prehaud, C., Rey, F.A., Delepierre, M., Buc, H., Lafon, M., et al. (2011). Structure 19, this issue, 1518–1524.

Doyle, D.A., Lee, A., Lewis, J., Kim, E., Sheng, M., and MacKinnon, R. (1996). Cell 85, 1067–1076.

Javier, R.T. (2008). Oncogene 27, 7031–7046.

Jing, M., Bohl, J., Brimer, N., Kinter, M., and Vande Pol, S.B.J. (2007). J. Virol. 81, 2231–2239.

Liu, H., Golebiewski, L., Dow, E.C., Krug, R.M., Javier, R.T., and Rice, A.P. (2010). J. Virol. 84, 11164–11174.

Nourry, C., Grant, S.G., and Borg, J.P. (2003). Sci. STKE 179, RE7.

Préhaud, C., Wolff, N., Terrien, E., Lafage, M., Mégret, F., Babault, N., Cordier, F., Tan, G.S., Maitrepierre, E., Ménager, P., et al. (2010). Sci. Signal. 3, ra5.

Simonson, S.J.S., Difilippantonio, M.J., and Lambert, P.F. (2005). Cancer Res. 65, 8266–8273.

Songyang, Z., Fanning, A.S., Fu, C., Xu, J., Marfatia, S.M., Chishti, A.H., Crompton, A., Chan, A.C., Anderson, J.M., and Cantley, L.C. (1997). Science 275, 73–77.

te Velthuis, A.J., Sakalis, P.A., Fowler, D.A., and Bagowski, C.P. (2011). PLoS ONE 6, e16047.

PARG: A Macrodomain in Disguise

Markus Hassler,[1,2] Gytis Jankevicius,[1,2] and Andreas G. Ladurner[1,2,*]
[1]Genome Biology Unit and Structural and Computational Biology Unit, European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany
[2]Department of Physiological Chemistry, Ludwig Maximilian University, Adolf-Butenandt-Strasse 5, 81377 Munich, Germany
*Correspondence: andreas.ladurner@med.lmu.de

Our understanding of poly-ADP-ribosylation as a posttranslational modification was limited by the lack of structural information on poly-ADP-ribose (PAR) hydrolysing enzymes. A recent study in *Nature* (Slade et al., 2011) reports the structure of PAR glycohydrolase (PARG), revealing unexpected similarity to the ubiquitous ADP-ribose-binding macrodomains.

Poly-ADP-ribosylation is a reversible posttranslational modification (PTM) that occurs mostly in response to cellular stress (e.g., DNA strand breaks) and is catalyzed by members of the PAR polymerase (PARP) family. Utilizing NAD^+ as a substrate, PARP enzymes form complex branched PAR polymers covalently attached to target proteins, such as nuclear histones. The best-characterized member of the PARP family is PARP-1, which catalyzes more than 90% of the PAR synthesis that occurs rapidly in response to different types DNA damage. Although still lacking a complete understanding, the mechanistic and functional aspects of PAR synthesis have been extensively characterized, and structural information for all domains of PARP-1 is available (Langelier et al., 2011). Much progress is also being made with regard to how PAR functions in

cells and how this PTM is recognized by various cellular machineries.

Although PARP-1 and PARylation have been known for over forty years, it is the discoveries in recent years of macrodomains and PAR-binding zinc fingers (PBZ) as readout modules for ADP-ribose (Karras et al., 2005; Ahel et al., 2008) that has ignited interest in the field and given us badly needed new tools for this elusive PTM. Crystal structures of several macrodomain proteins bound to ADP-ribose provide atomic insight into how these globular protein modules appear designed to recognize ADP-ribose moieties deep within their extended nucleotide binding pocket. Further, NMR structures of PBZ domains have been highlighted an alternative mechanism of PAR recognition (Karras et al., 2005; Timinszky et al., 2009; Eustermann et al., 2010).

In sharp contrast, we had no structural insight into the mechanism of how PAR chains can be rapidly removed from the modified proteins. While it was known that PARG enzymes catalyze the breakdown of PAR into individual ADP-ribose moieties and that the PARG null mutation causes lethality in mouse embryos (Koh et al., 2004), underscoring the physiological importance of PAR catabolism, no structures on PARG existed. There was also uncertainty in the community as to whether PARG may be able to cleave off terminal ADP-ribose moieties (exo-), "internal" *O*-glycosidic bonds (endo-activity), and/or also remove the ADP-ribose moiety from mono-ADP-ribosylated side chains (Koch-Nolte et al., 2009).

In a recent *Nature* report, Slade et al. (2011) were able to gain high-resolution

structural information on the enzymes that catalyze PAR breakdown reactions by determining the crystal structure and the mechanism of catalysis of a bacterial enzyme with PARG activity. Similar to its anabolic counterpart PARP-1, PARG orthologs have been described for almost all eukaryotic organisms. Certain filamentous fungi and bacteria, however, lack obvious PARG orthologs. Now, Slade et al. (2011) reveal the existence of a domain termed DUF2263 that is a distant relative of the eukaryotic PARG fold. While the structure and enzymatic analysis leaves open a few questions, the report by Slade et al. resolves issues about PARG specificity and reveals a striking structural similarity to the conserved and widespread family of ADP-ribose-binding macrodomain modules (Karras et al., 2005; Kustatscher et al., 2005), as was initially predicted for human PARG using sensitive fold recognition algorithms (unpublished research by Fernando Bazan cited in Koch-Nolte et al., 2009). Slade et al. (2011) use biochemical assays to identify PAR glycohydrolase activity of these "PARG-like" domains from a number of organisms. Consistently, overexpression experiments with human PARP-1 and the bacterial PARG enzymes in yeast confirm that the newly identified PARG-like enzymes suppress PAR formation.

The presence of proteins with PARG activity in bacteria is puzzling. Clearly, the new data reveal that enzymes capable of degrading PAR appeared earlier than previously thought, raising questions on the evolutionary origin(s) of PARylation pathways. The function of PAR metabolism in bacteria might be related to DNA damage responses, as illustrated by the upregulation of the PARG homolog in the radiation resistant bacteria *Deinococcus radiodurans* (Liu et al., 2003). Further work is needed to shed light on the nature of PAR and/or ADP-ribose-related metabolism in bacteria.
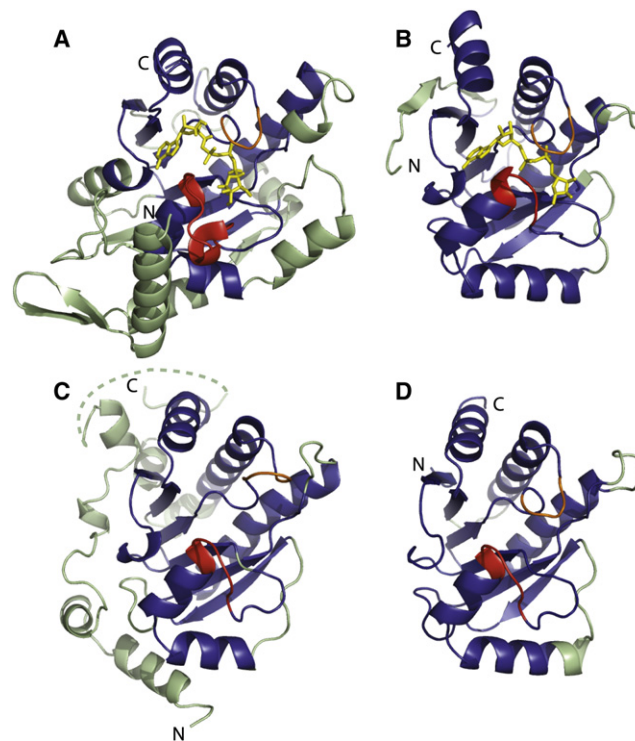

Figure 1. Comparison of *T. curvata* PARG in Complex with ADP-Ribose with Three Representative Macrodomain Structures
Cartoon view of (A) PARG with ADP-ribose (PDB ID 3SIG), (B) macroH2A1.1 with ADP-ribose (PDB ID 3IID), (C) MacroD1 (PDB ID 2X47), and (D) YmdB (PDB ID 1SPV) showing the core α-β-α sandwich fold typical of macrodomains in blue. Extended noncanonical structural features are shown in pale green. ADP-ribose is shown in yellow. The signature GGG-X$_{6-8}$-QEE loop in PARG and the Gly-rich loops typical in macrodomains are shown in red. The conserved GVFG stretches are shown in orange.

The structure of PARG extends the list of the known functions of macrodomains with those of a glycohydrolase and is proof of the versatility of this simple fold, which typically consists of a six-strand β sheet sandwiched by five characteristic α helices (Figure 1). *E. coli* YmdB and human MacroH2A1.1, for example, represent such canonical macrodomains. Human MacroD1 has a 55 amino acid α-helical extension at the N terminus and now *Thermomonospora curvata* PARG shows yet another type of N-terminal helical extension. Several macrodomain proteins not only efficiently, rapidly, and transiently recognize PARylated proteins, mono-ADP-ribose, phospho-ADP-ribose, and the Sir2/sirtuin NAD metabolite *O*-acetyl-ADP-ribose, but they have also been reported to possess catalytic activity (Timinszky et al., 2009; Karras et al., 2005; Kustatscher et al., 2005; Chen et al., 2011). This raises a question if the known crystal structures can provide insight into the functional versatility of macrodomains.

The catalytic activity of PARG strongly depends on the two Glu residues in the GGG-X$_{6-8}$-QEE motif (E114 and E115 in *T. curvata* PARG) (Figure 1A, red loop). Although both Glu residues are essential for activity, only E115 is proposed, based on structural evidence, to be directly involved in the catalysis. E115 and an ordered water molecule are in an ideal position to cleave an ADP-ribose moiety via the formation of an oxocarbenium intermediate, providing a plausible mechanism for the hydrolytic mechanism of PAR breakdown.

Comparing PARG to MacroD1, one of the enzymes recently shown to efficiently deacetylate *O*-acetyl-ADP-ribose, we find the close structural and evolutionary relationship between macro and PARG domains. For example, the residues in MacroD1 that are proposed to catalyze the acetyl hydrolysis (N174, D184) are located in the vicinity of a Gly-rich loop, which in macrodomains forms the rim of the ADP-ribose cavity analogous to the signature GGG-X$_{6-8}$-QEE loop in PARG (Figures 1A and 1C, red). Furthermore, the diphosphate-binding loop, which flanks the other side of the ADP-ribose-binding cavity, is highly conserved between PARG and macrodomains (GVFG motif, orange loops in Figure 1). In both PARG-ADP-ribose and macrodomain-ADP-ribose structures, ADP-ribose is recognized in an almost identical manner. The presence of a 2′-OH-linked ADP-ribose group on ribose would be prohibited without major structural rearrangements, providing us with evidence that PARG is an exo- rather than an endoglycohydrolase (Figures 1A and 1B). Slade et al. (2011) also show that the enzyme is not able to efficiently remove the last ADP-ribose moiety. For DUF2263, this answered a few long awaited questions, but how similar are *T. curvata* and metazoan PARG likely to be? It is expected

that human PARG domains will closely resemble and therefore also have a fold based on the macrodomain. The key sequence motifs mentioned above are highly conserved and a homology search for human PARG (http://www.sbg.bio.ic.ac.uk/~phyre/) indeed confirms the close relationship to *T. curvata* PARG.

Important questions remain open. Only a subset of the macrodomains are catalytically active and some are not even capable of binding ADP-ribose or related nucleotide ligands (Kustatscher et al., 2005). For example, the ligands for the histone variants macroH2A1.2 and macroH2A2 remain completely unknown, despite the high conversation of these histones across vertebrate evolution. On the issue of PAR degradation as a regulatory posttranslational modification, the question of which enzyme(s) may specifically remove the "final" ADP-ribose moiety from posttranslationally modified proteins remains open. The hunt for such enzymes and for physiological PARP-family targets is made more complex by the fact that there is evidence to support both glutamate and lysine residues as

key ADP-ribose acceptors. There can be much confidence, however, that such open questions will soon be addressed.

In conclusion, after more than forty years of research into ADP-ribosylation signaling, the paper by Slade et al. (2011) has provided us with detailed structural insight into PARG enzymes, a plausible PAR degradation mechanism, and revealed a surprising relation to the macrodomain module. As the ADP-ribosylation field shifts into a higher gear with this and other recent progress, the stage looks set for further surprises. Other macrodomains in disguise may abound, promising to reveal new molecular and physiological roles for this nucleic acid with signaling functions.

REFERENCES

Ahel, I., Ahel, D., Matsusaka, T., Clark, A.J., Pines, J., Boulton, S.J., and West, S.C. (2008). Nature *451*, 81–85.

Chen, D., Vollmar, M., Rossi, M.N., Phillips, C., Kraehenbuehl, R., Slade, D., Mehrotra, P.V., von Delft, F., Crosthwaite, S.K., Gileadi, O., et al. (2011). J. Biol. Chem. *286*, 13261–13271.

Eustermann, S., Brockmann, C., Mehrotra, P.V., Yang, J.-C., Loakes, D., West, S.C., Ahel, I., and Neuhaus, D. (2010). Nat. Struct. Mol. Biol. *17*, 241–243.

Karras, G.I., Kustatscher, G., Buhecha, H.R., Allen, M.D., Pugieux, C., Sait, F., Bycroft, M., and Ladurner, A.G. (2005). EMBO J. *24*, 1911–1920.

Koch-Nolte, F., Haag, F., Guse, A.H., Lund, F., and Ziegler, M. (2009). Sci. Signal. *2*, mr1.

Koh, D.W., Lawler, A.M., Poitras, M.F., Sasaki, M., Wattler, S., Nehls, M.C., Stöger, T., Poirier, G.G., Dawson, V.L., and Dawson, T.M. (2004). Proc. Natl. Acad. Sci. USA *101*, 17699–17704.

Kustatscher, G., Hothorn, M., Pugieux, C., Scheffzek, K., and Ladurner, A.G. (2005). Nat. Struct. Mol. Biol. *12*, 624–625.

Langelier, M.F., Planck, J.L., Servent, K.M., and Pascal, J.M. (2011). Methods Mol. Biol. *780*, 209–226.

Liu, Y., Zhou, J., and Omelchenko, M. (2003). Proc. Natl. Acad. Sci. USA *100*, 4191–4196.

Slade, D., Dunstan, M.S., Barkauskaite, E., Weston, R., Lafite, P., Dixon, N., Ahel, M., Leys, D., and Ahel, I. (2011). Nature, in press. Published online September 4, 2011. 10.1038/nature10404.

Timinszky, G., Till, S., Hassa, P.O., Hothorn, M., Kustatscher, G., Nijmeijer, B., Colombelli, J., Altmeyer, M., Stelzer, E.H.K., Scheffzek, K., et al. (2009). Nat. Struct. Mol. Biol. *16*, 923–929.

It Takes Two to Get3

Irmgard Sinning,[1,*] Gert Bange,[1] and Klemens Wild[1]
[1]Heidelberg University Biochemistry Center (BZH), INF 328, 69120 Heidelberg, Germany
*Correspondence: irmi.sinning@bzh.uni-heidelberg
DOI 10.1016/j.str.2011.10.001

Tail-anchored (TA) membrane proteins perform essential cellular functions. They are posttranslationally inserted into the endoplasmic reticulum (ER) membrane by interaction of the Get3 chaperone with the Get1/2 receptor. Two independent structural and functional analyses of the Get3/receptor complex by Stefer et al. and Mariappan et al. now provide insights into TA protein insertion.

In the textbooks, insertion of membrane proteins into the ER is mediated by the universally conserved signal-recognition particle (SRP), which relies on the presence of an N-terminal signal sequence (Grudnik et al., 2009). In eukaryotes, however, about 5% of all membrane proteins, including the SNARE or Bcl-2 family proteins, carry their targeting signal within a single transmembrane domain present at their C terminus and are therefore termed

tail-anchored (TA) proteins. They are subject to the recently identified GET (guided entry of TA proteins) pathway (reviewed in Simpson et al., 2010). The GET machinery comprises at least five components (Get1–5) that mediate the three main steps of TA protein insertion: Get4/5 assisted loading of the Get3 ATPase with a TA protein, docking of the Get3/TA protein complex to the Get1/2 receptor at the ER, and subsequent insertion. The Get3 ATPase

forms the core of the GET machinery, and a series of Get3 crystal structures suggests that the Get3 dimer oscillates between an "open" and a "closed" state by a nucleotide-dependent rotation of the two subunits (Simpson et al., 2010). While the dimer is clamped together at the bottom by a zinc ion, the TA protein is expected to bind to a hydrophobic pocket on top of the ATPase domain in the TA protein binding domain (TABD),