32. Miyoshi, K., Okada, T.N., Siomi, H. & Siomi, M.C. *RNA* **15**, 1282–1291 (2009).
33. Rüdel, S. *et al. Nucleic Acids Res.* **39**, 2330–2343 (2011).
34. Eulalio, A., Huntzinger, E. & Izaurralde, E. *Nat. Struct. Mol. Biol.* **15**, 346–353 (2008).
35. Till, S. *et al. Nat. Struct. Mol. Biol.* **14**, 897–903 (2007).
36. Djuranovic, S. *et al. Nat. Struct. Mol. Biol.* **17**, 144–150 (2010).
37. Behm-Ansmant, I. *et al. Genes Dev.* **20**, 1885–1898 (2006).
38. Iwasaki, S. *et al. Mol. Cell* **39**, 292–299 (2010).
39. Iki, T. *et al. Mol. Cell* **39**, 282–291 (2010).
40. Kwak, P.B. & Tomari, Y. *Nat. Struct. Mol. Biol.* **19**, 145–151 (2012).
41. Liu, Q. *et al. Science* **301**, 1921–1925 (2003).

PARP pairs up to PARsylate

Nicolas Coquelle & J N Mark Glover

New structural analyses suggest two different models for poly(ADP-ribose) polymerase 1 (PARP1) activation by single- and double-strand DNA breaks, providing evidence for PARP1 activation in *cis* and in *trans*.

All living organisms maintain elaborate systems to recognize and repair damage to their genetic material. In multicellular organisms, defects in these systems can lead to the accumulation of DNA mutations and, ultimately, cancer. Although substantial insight has been gained into the signaling mechanisms triggered by different DNA lesions, surprisingly little is known about how some of the most crucial forms of DNA damage are initially recognized. These forms include breaks in the DNA backbone, such as single-stranded breaks, which are thought to arise with great frequency, and the more rare, but potentially more deleterious, double-stranded breaks[1]. PARP1 is a major sensor of DNA strand breaks, and binding to these lesions activates the enzyme to catalyze the formation of poly(ADP-ribose) chains from NAD^+ (ref. 2). Two new studies[3,4] look at the core DNA-binding domain of human PARP1 but propose differing models for how binding to DNA strand breaks leads to activation of PARP1 catalytic activity.

PARP1 is a complex, multidomain enzyme (**Fig. 1**) consisting of three zinc finger modules (ZnF1–ZnF3), a BRCT (BRCA1 C-terminal) domain, a domain rich in tryptophan, glycine and arginine residues (the WGR domain) and a C-terminal catalytic domain composed of a helical subdomain and a core catalytic subdomain that carries out the polymerization of ADP-ribose chains (the ART, or ADP-ribose transferase domain). The multidomain nature of PARP1 protein complicates its structural analysis, but major insights have come from the study of the individual domains. Recent structures of isolated ZnF1 and ZnF2 showed that each of these domains is capable of binding double-stranded DNA ends in similar ways using a conserved α2-α3 loop to pack against the exposed terminal base pair[5]. That work left open the issue of why PARP1 contains two apparently redundant DNA-binding zinc fingers.

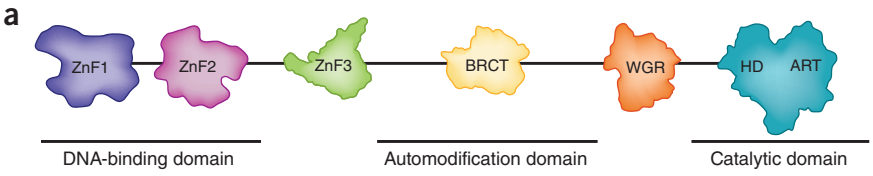

Figure 1 The molecular architecture of human PARP1. The DNA-binding domain comprises both ZnF1 and ZnF2, the automodification domain encompasses the BRCT domain and the surrounding linker regions, and the catalytic domain (CAT) is composed of a helical subdomain (HD) and the ART subdomain.

In this issue, Ali *et al.*[3] now answer this question through their determination of the crystal structure of the intact ZnF1-ZnF2 DNA-binding domain of PARP1 bound to a DNA end with a single-nucleotide 5′ overhang (**Fig. 2a**). This structure shows that ZnF1 and ZnF2 cooperate to recognize the DNA end, with ZnF2 binding DNA in a way similar to that observed in the structure of the isolated ZnF2 domain. In contrast, ZnF1 binds to the opposite side of the DNA such that its α2-α3 loop does not directly contact the DNA but instead lies over the top of the analogous loop of ZnF2 and interacts with this loop through a small hydrophobic interaction surface. The authors developed an assay to detect PARP1 binding to UV-laser–induced DNA damage foci in human cells and show that mutations that affect the DNA binding of either ZnF1 or ZnF2 abrogate the accumulation and retention of PARP1 at these sites. Notably, mutations of residues that mediate interactions between the two zinc fingers also block PARP1 binding to nuclear foci, showing that ZnF1 and ZnF2 cooperate to bind damaged DNA in cells.

Interestingly, the arrangement of the two zinc fingers on the DNA end is such that it is highly improbable that the two domains come from the same polypeptide, and Ali *et al.*[3] propose that PARP1 heterodimerizes on the damaged DNA, with ZnF1 contributed from one protomer and ZnF2 contributed from the other. Consistent with this model, quantitative gel mobility shift assays showed that two molecules of PARP1 are needed to fully bind a single DNA end. Furthermore, the authors carried out a series of affinity pulldown experiments to show that the PARP1 DNA-binding domain can interact with its dimerization partner on a DNA end in a way that involves specific in-*trans* interactions between ZnF1 and ZnF2. In addition, they also show that deletion of either ZnF1 or ZnF2 from full-length PARP1 impairs DNA-dependent catalytic activity and that this activity can be rescued by the addition of the missing zinc finger in *trans*. These data strongly suggest not only that dimerization is needed for the recognition of damaged DNA but also that this mode of DNA recognition is required to drive catalysis by PARP1.

In another report, Langelier *et al.*[4] propose a quite different model for PARP1 activation that involves recognition of the DNA end by a single PARP1 protomer. In that model, the DNA end is recognized directly by ZnF1, which catalyzes the collapse of the otherwise extended PARP1 structure around the DNA end, leading to conformational changes that ultimately enhance the flexibility and activity of the ART domain (**Fig. 2b**). The model is derived largely from a crystal structure of the separate ZnF1, ZnF3 and WGR–helical subdomain–ART domains bound to a blunt-ended DNA double-strand break. In that structure, ZnF1 binds the DNA end in a manner similar

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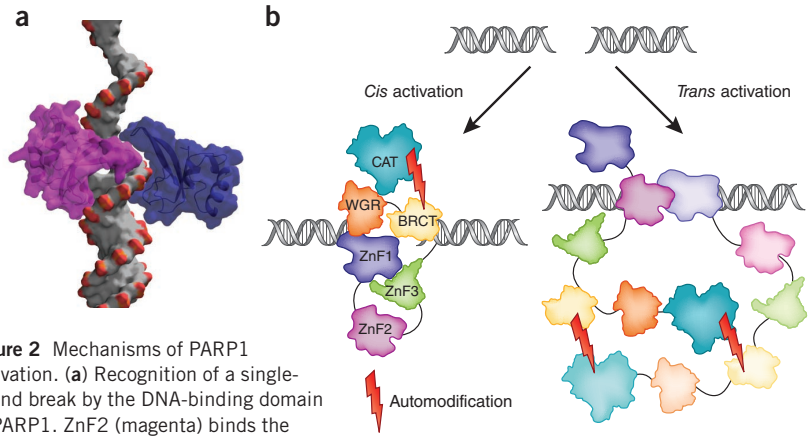

Figure 2 Mechanisms of PARP1 activation. (**a**) Recognition of a single-strand break by the DNA-binding domain of PARP1. ZnF2 (magenta) binds the minor groove of the DNA and caps the broken strand, whereas ZnF1 (blue) binds the major groove of the DNA. The tips of these zinc fingers also interact. The DNA single-strand break shown here was modeled on the DNA used in the crystal structure described by Ali *et al.*[3]. (**b**) *Cis* compared to *trans* automodification models for human PARP1. In the *cis* activation model proposed by Langelier *et al.*[4], a single PARP1 protomer collapses on a DNA end, triggering intramolecular interactions and conformational changes that increase the flexibility of the catalytic domain to induce automodification. In the *trans* activation model proposed by Ali *et al.*[3], two copies of PARP1 heterodimerize at the site of damage, enabling the modification of one protomer by the catalytic domain of its dimer partner.

to the way in which ZnF2 recognizes DNA in the structure described by Ali *et al.*[3]; ZnF3 and the WGR domain also make limited contacts with the DNA backbone, as well as with ZnF1. Langelier *et al.*[4] argue that these interactions drive a conformational change in the catalytic domain that leads to an enhancement of the flexibility and catalytic activity of this domain. Although the PARP1 automodification domain is not present in the structure described by Langelier *et al.*[4], the relative arrangement of the ZnF3, WGR and catalytic domains suggest that a flexible automodification domain might be able to access the catalytic active site in *cis* without the need to invoke a catalytic dimer.

Ali *et al.*[3] clearly validate the key role of ZnF2 in DNA recognition and show that dimerization of the DNA-binding domain facilitates the recognition of strand discontinuities in DNA. The implication is that dimer formation could activate in-*trans* automodification, wherein the catalytic domain of one protomer acts on the automodification domain of the other protomer (**Fig. 2b**). Although this is an attractive idea, further work will be required to determine the relative contributions of *cis* compared to *trans* models of automodification.

The discovery that the chemical inhibition of PARP1 can lead to dramatic synthetic lethality in cells deficient in the human breast cancer tumor suppressors, BRCA1 and BRCA2, has led to the idea that these inhibitors could provide powerful new therapies for cancer patients with mutations in either BRCA1 or BRCA2 (refs. 6,7). Indeed, the results of phase 2 clinical trials have generally provided support for the clinical efficacy of PARP1 inhibitors, even in patients with cancers that have become resistant to other therapies[8]. Understanding the structural mechanisms of PARP1 activation will be key to guide the development of new inhibitors for this crucial DNA-damage response enzyme.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Ciccia, A. & Elledge, S.J. *Mol. Cell* **40**, 179–204 (2010).
2. Luo, X. & Kraus, W.L. *Genes Dev.* **26**, 417–432 (2012).
3. Ali, A.A. *et al. Nat. Struct. Mol. Biol.* **19**, 685–692 (2012).
4. Langelier, M.F., Planck, J.L., Roy, S. & Pascal, J.M. *Science* **336**, 728–732 (2012).
5. Langelier, M.F., Planck, J.L., Roy, S. & Pascal, J.M. *J. Biol. Chem.* **286**, 10690–10701 (2011).
6. Farmer, H. *et al. Nature* **434**, 917–921 (2005).
7. Bryant, H.E. *et al. Nature* **434**, 913–917 (2005).
8. Lord, C.J. & Ashworth, A. *Nature* **481**, 287–294 (2012).

Nef-arious goings-on at the Golgi

James H Hurley & Juan S Bonifacino

HIV-1 avoids the immune detection of infected cells by preventing class I molecules of the major histocompatibility complex (MHC-I) bound to viral peptides from reaching the cell surface. A new structure shows how Nef turns MHC-I from a noncargo into a cargo for the clathrin adaptor AP-1, thus directing MHC-I to the lysosome instead of the plasma membrane.

In the late 1980s, HIV-1 infection was effectively a death sentence. Since then, the three enzymes encoded by the HIV-1 genome, protease, reverse transcriptase and integrase, have become some of the most intensively scrutinized proteins ever studied because they were the clear first lines of attack against the virus. A structural analysis of these enzymes accelerated the discovery of the inhibitors that make up the highly effective antiviral cocktail treatment currently in use[1]. Now that HIV-1 can be treated effectively, at least in the developed world, attention has turned to the issue of resistance to the currently used antiviral drugs. Ultimately, it is hoped that HIV-1 infection can be eradicated from patients. The challenges of combating drug resistance and eradicating infection will require new lines of attack.

With a genome coding for just 15 proteins, HIV-1 makes for itself a virtue of necessity by adeptly turning myriad host cell processes to its own ends. The host cell machinery is involved in many steps of the viral life cycle, including cellular entry, genome integration, transcription, RNA export, translation and the assembly and release of viral particles. All of these viral processes are accomplished while dodging the restriction factors of the host cell, as well as evading detection and destruction by the immune system. The dependence of HIV-1 on so many host factors[2] is a vulnerability for the virus given that the host proteins are not subject to the same selective pressures that yield drug-resistant variants of viral proteins. Thus, in recent years, the field of HIV-related structural biology has shifted much of its focus to understanding

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