Short Communication

Poly(ADP-ribose) polymerase-1 silences retroviruses independently of viral DNA integration or heterochromatin formation

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PARP-1 silences retrotransposons in *Drosophila*, through heterochromatin maintenance, and integrated retroviruses in chicken. Here, we determined the role of viral DNA integration and cellular heterochromatin in PARP-1-mediated retroviral silencing using HIV-1-derived lentiviral vectors and Rous-associated virus type 1 (RAV-1) as models. Analysis of the infection of PARP-1 knockout and control cells with HIV-1 harbouring WT integrase, in the presence or absence of an integrase inhibitor, or catalytic-dead mutant integrase indicated that silencing does not require viral DNA integration. The mechanism involves the catalytic activity of histone deacetylases but not that of PARP-1. In contrast to *Drosophila*, lack of PARP-1 in avian cells did not affect chromatin compaction globally or at the RAV-1 provirus, or the cellular levels of histone H3 N-terminal acetylated or Lys27 trimethylated, as indicated by micrococcal nuclease accessibility and immunoblot assays. Therefore, PARP-1 represses retroviruses prior to viral DNA integration by mechanisms involving histone deacetylases but not heterochromatin formation.

The cellular response to invading genomes is evolutionarily conserved, suggesting an early origin of these mechanisms (Ali *et al.*, 2013; Gasiunas *et al.*, 2014; Pinsker *et al.*, 2001; Weitzman *et al.*, 2010). A variety of DNA and RNA viruses activate DNA repair pathways in the absence of host DNA damage (Hoelzer *et al.*, 2008; Lilley *et al.*, 2005; 2011; Ohsaki *et al.*, 2004; Orzalli *et al.*, 2013; Pinsker *et al.*, 2001; Ross *et al.*, 2009; Schreiner *et al.*, 2013; Weitzman *et al.*, 2010), recruiting DNA repair proteins to their genomes (Lilley *et al.*, 2005; 2011; Ohsaki *et al.*, 2004; Orzalli *et al.*, 2013; Ross *et al.*, 2009; Weitzman *et al.*, 2010), and evolving dependency of this cellular response for efficient viral replication (Cooper *et al.*, 2013; Koyama *et al.*, 2013; Lilley *et al.*, 2005; 2011; Ohsaki *et al.*, 2004; Orzalli *et al.*, 2013; Ross *et al.*, 2009; Sakurai *et al.*, 2009; Schreiner *et al.*, 2013; Weitzman *et al.*, 2010). For example, the DNA damage sensor poly(ADP-ribose) polymerase-1 (PARP-1) (Ji & Tulin, 2010; Krishnakumar & Kraus, 2010) is recruited to the Kaposi's sarcoma-associated herpesvirus and Epstein–Barr virus genomes and impairs viral replication by modifying viral proteins involved in genome replication and partitioning (Ohsaki *et al.*, 2004), whereas hepatitis B virus depends on PARP-1 for efficient transcription (Ko *et al.*, 2013). In addition, PARP-1 represses the expression of retrotransposons in *Drosophila* (Kotova *et al.*, 2010; 2011; Tulin *et al.*, 2002) and retroviruses in avian cells (Bueno *et al.*, 2013). Conflicting data regarding a role for PARP-1 in HIV DNA integration exist (Ariumi *et al.*, 2005; Baekelandt *et al.*, 2000; Gaken *et al.*, 1996; Ha *et al.*, 2001; Kameoka *et al.*, 2004; 2005; Siva & Bushman, 2002), which is not surprising considering that, in mammals, this enzyme is the founding member of a family of 18 proteins (Ame *et al.*, 2004) with significant functional redundancy (Ame *et al.*, 2004; Boehler *et al.*, 2012; Krishnakumar & Kraus, 2010; Ménissier de Murcia *et al.*, 2003; Wang *et al.*, 1995). Therefore, simpler organisms lacking this redundancy are advantageous to the study of PARP-1 (Bueno *et al.*, 2013; Kotova *et al.*, 2010; Pinnola *et al.*, 2007; Tulin *et al.*, 2002). One of these cellular models is the chicken B lymphoblastoid cell line DT40 that, within the PARP family, only expresses PARP-1 (Hochegger *et al.*, 2006). These cells are a reliable model to study the role of PARP-1 in retroviruses, since it supports the early phase of the life cycle of gamma retrovirus- and lentivirus-derived viral vectors (Barr *et al.*, 2005; Beitzel & Bushman, 2003; Bueno *et al.*, 2013; Mitta *et al.*, 2004, 2005; Randow & Sale, 2006).

We took advantage of the DT40 cellular model to understand better the mechanism of action of PARP-1 in retroviral silencing (Bueno *et al.*, 2013), by determining whether PARP-1-mediated silencing requires viral DNA integration. Since PARP-1 represses integrated retro-elements, it is possible that integration-induced DNA damage recruits PARP-

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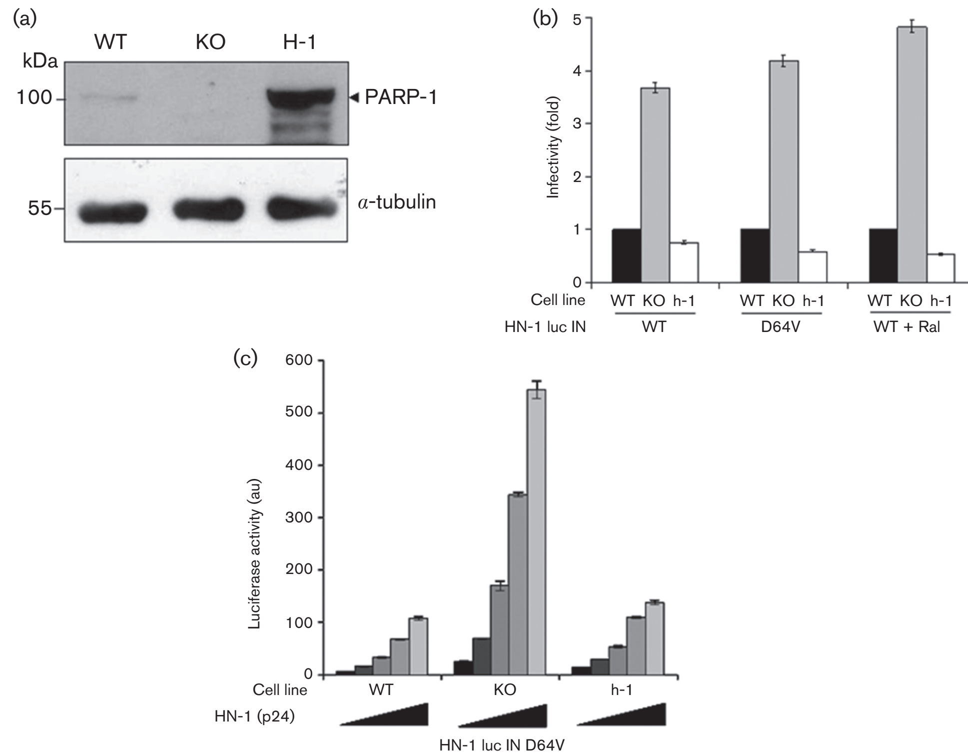

Fig. 1. Effect of PARP-1 on HIV-1 infection. (a) Immunoblot analysis of PARP-1 expression in DT40-derived cells detected with an anti-human PARP-1 monoclonal antibody (C2-10) that modestly cross-reacts with avian PARP-1. (b and c) DT40-derived cells were infected with VSV-G-pseudotyped HIV-1-derived viral vectors expressing luciferase from an internal CMV promoter. ATP-normalized luciferase was determined in cells infected with integrating (HIV luc IN WT) or non-integrating (HIV luc IN D64V or HIV luc IN WT in the presence of Raltegravir) 4 and 2 days post-infection, respectively. ATP-normalized luciferase was expressed relative to DT40 WT levels (b) or as arbitrary units (AU) (c). SD values indicate the variability of one experiment; data are representative of three (b) or one (c) independent experiments.

1 to the invading genome causing transcriptional repression, as described for other types of DNA damage (Chou *et al.*, 2010). Using procedures previously described (Bueno *et al.*, 2013), DT40 cells WT, PARP-1 knockout (KO) or KO cells engineered to express human PARP-1 (h-1) (Hochegger *et al.*, 2006) (Fig. 1a) were infected with p24-normalized amounts of vesicular stomatitis virus glycoprotein G (VSV-G)-pseudotyped, single-round infection HIV-1-derived viral vectors harbouring integrase WT (HIV luc IN WT) or a catalytic-dead mutant (HIV luc IN D64V) that expresses luciferase from an internal human cytomegalovirus immediate early gene (CMV) promoter. Cells were also infected with HIV luc IN WT in the presence of the integrase inhibitor Raltegravir (60 nM). Due to the transient nature of the expression of unintegrated retroviral genomes (Butler *et al.*, 2001; Kilzer *et al.*, 2003; Schneider *et al.*, 2012; Yu *et al.*, 2008), luciferase was measured in cells infected with integrated retroviruses (HIV luc IN WT) 4 days post-infection, to allow elimination of the transgene expression from unintegrated viral genomes, and in cells infected with unintegrated retroviruses (HIV luc IN D64V or WT plus Raltegravir) 2 days post-infection, to prevent loss of the unintegrated viral genomes. In support of this rationale, luciferase expression in HIV luc IN D64V-infected cells decreased by more than 90 % by day 4, being undetectable by day 6 post-infection; this rate of decay was similar in cells expressing or not PARP-1 (data not shown). Luciferase values were normalized to ATP levels to standardize for cell viability and number, and the normalized values were expressed relative to those found in DT40 WT cells to calculate fold infectivity. As expected (Bueno *et al.*, 2013), PARP-1 KO cells infected with HIV luc IN WT expressed 3–4-fold more luciferase than infected cells expressing chicken (WT) or human (h-1) PARP-1 (Fig. 1b). Similar results were obtained with HIV luc IN D64V (Fig. 1b, c) and with HIV luc IN WT in Raltegravir-treated cells (Fig. 1b). Analysis of three independent experiments using a two-tailed Student's t-test for independent samples (hereafter referred as statistical analysis) showed significant differences between the infectivity of DT40 WT and KO cells ($p<0.01$) and KO and h-1 cells ($p<0.001$) independently of the integration capacity of the HIV-1 analysed, indicating that viral DNA integration is not a prerequisite for PARP-1-mediated retroviral silencing.

PARP-1 has been reported to silence integrated retroviruses and retrotransposons through epigenetic mechanisms implicated in the formation and maintenance of the host heterochromatin (Bueno *et al.*, 2013; Kotova *et al.*, 2010, 2011, 2011; Tulin *et al.*, 2002). Unintegrated and integrated retrovirus genomes are associated with chromatin (Kantor *et al.*, 2009; Schneider *et al.*, 2012; Sloan & Wainberg, 2011); therefore, PARP-1 could silence these through similar mechanisms. However, potential variations in the chromatin organized at these two forms of viral genome could determine differences in the mechanisms implicated. To evaluate these alternatives, we determined the role of histone deacetylation and DNA methylation in PARP-1-mediated silencing of unintegrated HIV-1.

DT40 WT, KO and h-1 cells were infected with HIV-1 p24-normalized amounts of HIV luc IN D64V or IN WT viruses, and 2 or 4 days later, respectively, the cells were treated or not with the histone deacetylase inhibitor sodium butyrate (SB, 5 mM) for 24 h and then luciferase and ATP levels were measured. Statistical analysis of three independent experiments demonstrated significant differences in the infectivity of untreated DT40 WT and KO cells ($p<0.005$) and KO and h-1 cells ($p<0.001$) with either HIV luc IN D64V or WT virus. However, SB treatment increased luciferase activity at a higher magnitude in cells expressing PARP-1 infected with either integrating or non-integrating viruses, rendering these differences statistically nonsignificant ($p<0.005$) (Fig. 2a), and indicating that histone deacetylases mediate PARP-1 silencing of integrated and unintegrated HIV-1-derived vectors.

To evaluate the role of DNA CpG methylation in PARP-1-induced retroviral silencing, DT40-derived cells were infected with HIV luc IN D64V in the presence or not of the DNA methylation inhibitor 5-azacytidine (30 μM), and ATP and luciferase were measured 36 h later. 5-Azacytidine augmented retroviral luciferase expression, as expected (Schneider *et al.*, 2012), but in a similar magnitude in cells expressing or not PARP-1 (Fig. 2c). Consequently, statistical analysis of two independent experiments showed significant differences between the infectivity of 5-azacytidine-treated or 5-azacytidine-untreated DT40 WT and KO ($p<0.005$), and KO and h-1 cells ($p<0.001$). These results indicated that, in contrast to integrated retroviruses (Bueno *et al.*, 2013), the effect of DNA CpG methylation on the expression of unintegrated retroviruses is PARP-1 independent, suggesting important differences in the PARP-1-silencing mechanism of integrated and unintegrated retroviruses.

Furthermore, we determined the role of PARP-1 enzymatic activity in retroviral silencing. Non-infected DT40 cells were treated with DMSO or the nicotinamide-mimetic PARP inhibitor 1,5-isoquinolinediol (200 μM, inhibitor IV), and poly(ADP-ribose) (PAR) levels were measured 24 h later by ELISA (Trevigen). DMSO-treated WT and h-1 cells expressed 53.71 and 32.87 ng ml^{-1} of PAR, respectively, but this was undetectable in KO cells (<10 pg ml^{-1}, ELISA's detection limit), and inhibitor IV reduced PAR levels by 17- and 22-fold in DT40 WT and h-1 cells, respectively (Fig. 2d).

DT40 WT, KO and h-1 cells were treated with DMSO or inhibitor IV at the time of infection with HIV luc IN WT or D64V, and the input virus and drugs were removed 24 h later. Two (HIV luc IN D64V) or four (HIV luc IN WT) days after infection, luciferase and ATP were determined. Results (Fig. 2e) indicated that PARP-1 inhibition did not increase luciferase expression in infected DT40 WT or h-1 cells. Statistical analysis of two independent experiments revealed significant differences between DT40 WT and KO ($p<0.005$) or KO and h-1 ($p<0.005$) infected with HIV luc IN WT or D64V in the presence or not of inhibitor IV, suggesting that PARP-1 silences both integrating and non-integrating retroviruses in a catalytic-independent manner. Considering the essential role of the enzymatic activity of PARP-1 in DNA repair (Bürkle & Virág, 2013; Rass *et al.*, 2012), these data also suggest that PARP-1 silences retroviruses independently of DNA damage which is supported by the fact that PAR levels were 1.1-fold higher in non-infected than in HIV luc IN WT-infected h-1 cells, 24 h post-infection.

Similar to the mechanism implicated in PARP-1-mediated silencing of retrotransposons in *Drosophila*, PARP-1 silences retroviruses in avian cells in a catalytic-independent manner potentially through epigenetic modifications [Fig. 2e and (Bueno *et al.*, 2013)]. In *Drosophila,* lack of PARP-1 globally decreases heterochromatin levels, as evaluated by micrococcal nuclease (MNase) digestion, causing transcriptional de-repression of retro-elements (Kotova *et al.*, 2010, 2011; Tulin *et al.*, 2002). MNase cleaves the genomic DNA in the internucleosomal space, and its accessibility to this target is largely influenced by the degree of compaction of the chromatin (Chung *et al.*, 2010). Therefore, we used this method to determine whether the role of PARP-1 in heterochromatin maintenance is conserved through evolution from insect to avian and humans.

Chromatin from DT40-derived cells was digested as described previously (Pinnola *et al.*, 2007) using several MNase amounts (12.5, 25 and 50 U), incubation times (5, 10 and 15 min), and temperatures (19 °C and 37 °C), and then the digested DNA was purified and analysed by agarose electrophoresis. Results using these conditions were similar to those obtained with 50 U of MNase for 15 min at 37 °C (Fig. 3a). and indicated no differences in the susceptibility of DT40 chromatin to MNase digestion as a function of PARP-1 levels. These findings indicated that, in contrast to *Drosophila* (Kotova *et al.*, 2010, 2011; Tulin *et al.*, 2002), the avian or human PARP-1 proteins do not affect the global abundance of heterochromatin in avian cells.

To further define the potential role of PARP-1 in regulating global levels of heterochromatin in avian cells, we determined the abundance of histone H3 post-translational modifications implicated in transcriptional regulation. Modification of histone H3 activity through position-specific methylation or

acetylation plays a fundamental role in the regulation of gene expression (Jenuwein & Allis, 2001; Kimura, 2013). In addition, the histone deacetylase inhibitor SB that reverts PARP-1-mediated retroviral silencing (Fig. 2a, b) is known to increase the cellular levels of N-terminal acetylated histones H3 and H4 (Drogaris *et al.*, 2012). Therefore, histone H3 N-terminal acetylated (H3Ac), a marker of actively transcribed chromatin (Morales & Richard-Foy, 2000), and Lys27 trimethylated (H3K27me3) that is enriched in heterochromatin regions (Kim & Kim, 2012) were measured by immunoblot analysis with specific rabbit polyclonal antibodies (06-599 and 07-449, respectively, Millipore). Total histone H3 was also detected (clone A3S. 05-928, Millipore) as a loading control. Results (Fig. 3b) showed similar levels of H3Ac and H3K27me3 in the cells analysed, indicating that human or avian PARP-1 did not affect global levels of epigenetic marks implicated in transcriptional regulation in chicken cells. The lack of evolutionary conservation of the role of PARP-1 in regulating cellular levels of heterochromatin is expected considering the low conservation of chicken and *Drosophila* PARP-1 proteins (43 % identity and 60 % similarity) [ClustalW2 (Larkin *et al.*, 2007) and BLASTP 2.2.19$^+$ (Altschul *et al.*, 1997)].

To define whether PARP-1 affects chromatin organization in proviruses, we explored the effect of MNase digestion on the integrity of the LTRs of the Rous-associated virus type 1 (RAV-1) provirus. This virus was used to generate DT40 cells (Baba *et al.*, 1985), and PARP-1 seems to repress its expression since KO cells produce higher levels of RAV-1 than WT or h-1 cells (Bueno *et al.*, 2013). Genomic DNA was isolated from DT40-derived cells and subjected or not to MNase digestion using suboptimal (6.25 U for 15 min at 29 °C) or optimal (50 U for 30 min at 37 °C) conditions (Fig. 3c), and then used for real-time PCR analysis (Garcia-Rivera *et al.*, 2010) with two sets of primers, DG24/DG25 or DG30/DG31. Primers DG24 (5′ TAGTCTTATGCAATACTCTAATGC 3′) and DG25 (5′ GGAAGGCAACAGACGGGTCTAA 3′) bind to nucleotides 5–28 and 179–200, respectively, in the U3 region, and primers DG30 (5′ TTATTAGGAAGGCAACAGACG 3′) and DG31 (5′ GCACATGCATGAAGCAGAAGGCTT 3′) bind to nucleotides 149–169 and 324–347 in the U3 and U5 sequences,

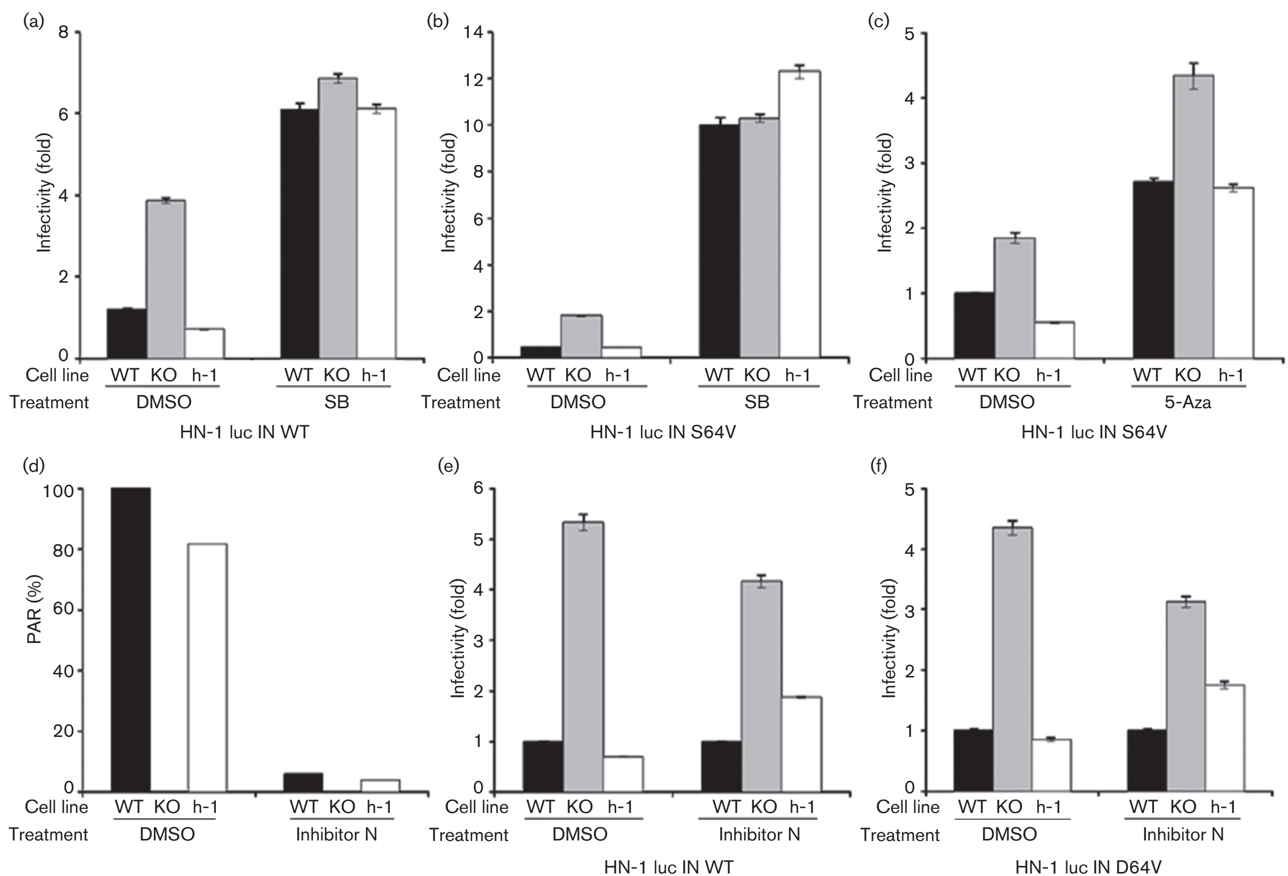

Fig. 2. Effect of sodium butyrate (SB) (a and b), 5-azacytidine (5-Aza) (c) and PARP-1 inhibition (e and f) on HIV-1 infection. Analysis of infection was carried out at 5 (a), 3 (b and c), 4 (e) or 2 (f) days post-infection. Fold infectivity in panels a–c and e and f was calculated by expressing ATP-normalized luciferase levels of each cell line relative to those found in untreated or DMSO-treated DT40 WT cells. (d) Effect of PARP-1 inhibition on cellular PAR levels. Values are expressed as percentage of the PAR levels detected in untreated DT40 WT cells. SD indicates the variability corresponding to one experiment; data are representative of three (a and b) or two (c–f) independent experiments.

respectively, amplifying an overlapping region that spans 96 % of both RAV-1 LTRs. Data (Fig. 3d) indicate the relative fold change for each primer set, experimental conditions, and cell lines calculated by using the ΔC_T method, and normalized for the fold change found in the respective cell line's undigested DNAs. Suboptimal conditions of MNase treatment increased detection of the LTRs as compared to non-digested samples, possibly due to a noted increase in the solubility of the template DNA. In contrast, at optimal conditions, LTR levels decreased for all the cell lines evaluated, being more marked in DT40 WT cells. However, levels of LTR in MNase-digested DNA did not correlate with the cellular levels of PARP-1 or RAV-1, indicating that the accessibility of the proviral LTRs to MNase is not influenced by PARP-1 levels. These data suggest that PARP-1 does not repress RAV-1 expression by organizing compacted chromatin at the provirus.

In summary, our results further support the importance of DNA repair proteins in the cellular response to invading genomes (Bueno *et al.*, 2013; Cooper *et al.*, 2013; Lilley *et al.*, 2005, 2011; Ohsaki *et al.*, 2004; Orzalli *et al.*, 2013; Pinsker *et al.*, 2001; Ross *et al.*, 2009; Sakurai *et al.*, 2009; Tulin *et al.*, 2002; Weitzman *et al.*, 2010), and demonstrate that PARP-1 restricts in a catalytic-independent manner the expression of retroviruses before viral DNA integration occurs. The enzymatic activity of histone deacetylases, but not of DNA methylases, mediates PARP-1 silencing of non-integrated retroviruses, indicating that PARP-1 silences retroviruses before and after integration by different mechanisms (Bueno *et al.*, 2013).

Our data also indicate a major distinction between the mechanism implicated in PARP-1-mediated silencing of retrotransposons in *Drosophila* (Kotova *et al.*, 2010, 2011; Tulin *et al.*, 2002) and retroviruses in avian cells (Bueno *et al.*, 2013). Although, in both cases, PARP-1-mediated silencing is independent of its catalytic activity, it involves the organization of heterochromatin at the retro-element genome in *Drosophila* but not in avian cells.

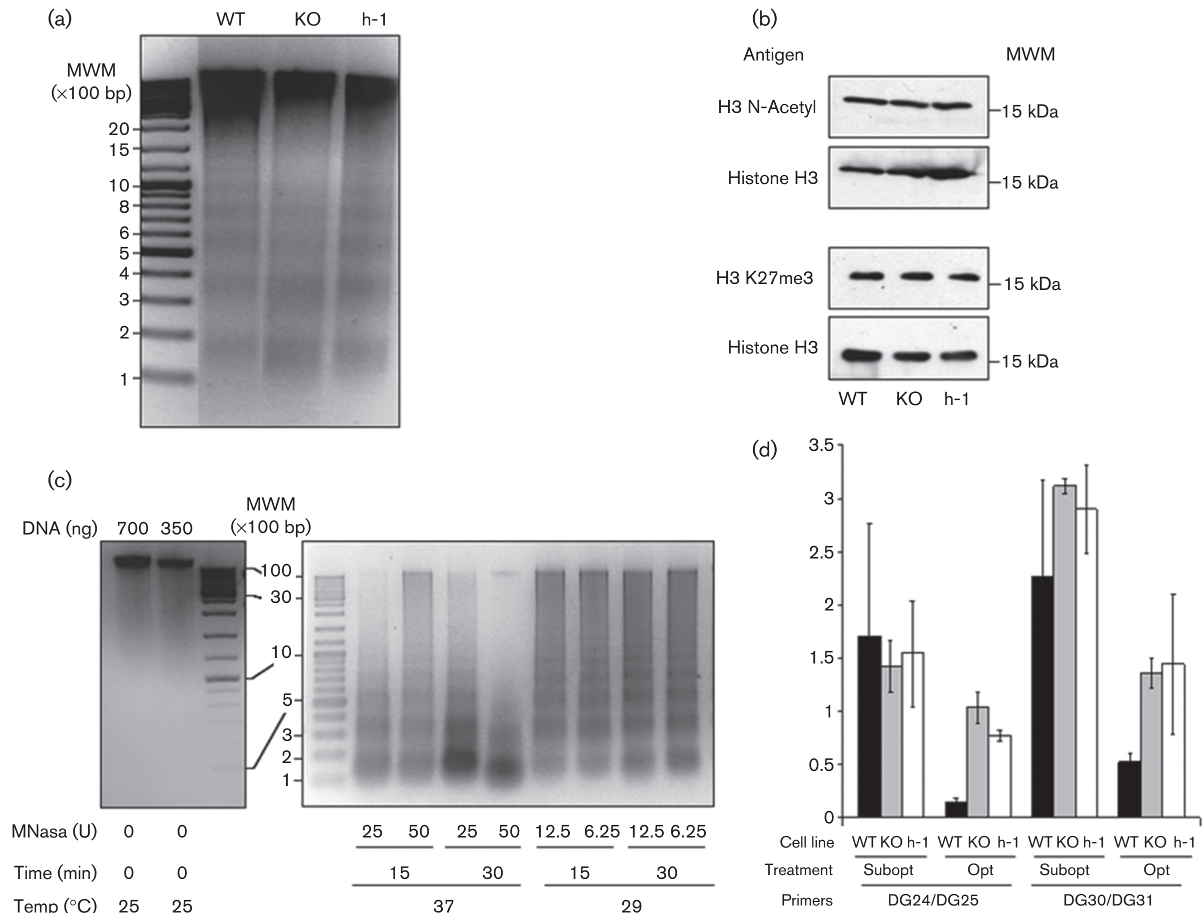

Fig. 3. Effect of PARP-1 on heterochromatin maintenance in avian cells. (a) MNase chromatin accessibility assay. Agarose gel electrophoresis analysis of DNA obtained after MNase digestion of chromatin isolated from DT40-derived cells. (b) Immunoblot analysis of histone H3 total and post-translational modifications in DT40-derived cells. (c) MNase undigested (left panel) or digested (right panel) DNA isolated from DT40 WT cells. (d) Real-time PCR quantification of Rous-associated virus type 1 LTRs. Data are representative of six (a), two (b) or one (c) independent experiments. Means and SDs in panel d were calculated from two MNase digestion and real-time PCR independent experiments.

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REFERENCES

Ali, S. S., Whitney, J. C., Stevenson, J., Robinson, H., Howell, P. L. & Navarre, W. W. (2013). Structural insights into the regulation of foreign genes in *Salmonella* by the Hha/H-NS complex. *J Biol Chem* **288**, 13356–13369.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., Lipman, D. J. & Schaffer, A. A. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.

Amé, J. C., Spenlehauer, C. & de Murcia, G. (2004). The PARP superfamily. *Bioessays* **26**, 882–893.

Ariumi, Y., Turelli, P., Masutani, M. & Trono, D. (2005). DNA damage sensors ATM, ATR, DNA-PKcs, and PARP-1 are dispensable for human immunodeficiency virus type 1 integration. *J Virol* **79**, 2973–2978.

Baba, T. W., Giroir, B. P. & Humphries, E. H. (1985). Cell lines derived from avian lymphomas exhibit two distinct phenotypes. *Virology (Auckl)* **144**, 139–151.

Baekelandt, V., Claeys, A., Cherepanov, P., De Clercq, E., De Strooper, B., Nuttin, B. & Debyser, Z. (2000). DNA-dependent protein kinase is not required for efficient lentivirus integration. *J Virol* **74**, 11278–11285.

Barr, S. D., Leipzig, J., Shinn, P., Ecker, J. R. & Bushman, F. D. (2005). Integration targeting by avian sarcoma-leukosis virus and human immunodeficiency virus in the chicken genome. *J Virol* **79**, 12035–12044.

Beitzel, B. & Bushman, F. (2003). Construction and analysis of cells lacking the HMGA gene family. *Nucleic Acids Res* **31**, 5025–5032.

Boehler, C., Gauthier, L. R., Mortusewicz, O., Biard, D. S., Saliou, J. M., Bresson, A., Sanglier-Cianferani, S., Smith, S., Schreiber, V., Boussin, F. & Dantzer, F. (2012). Poly(ADP-ribose) polymerase 3 (PARP3), a newcomer in cellular response to DNA damage and mitotic progression. *Proc Natl Acad Sci U S A* **108**, 2783–2788.

Bueno, M. T., Reyes, D., Valdes, L., Saheba, A., Urias, E., Mendoza, C., Fregoso, O. I. & Llano, M. (2013). Poly(ADP-ribose) polymerase 1 promotes transcriptional repression of integrated retroviruses. *J Virol* **87**, 2496–2507.

Bürkle, A. & Virág, L. (2013). Poly(ADP-ribose): PARadigms and PARadoxes. *Mol Aspects Med* **34**, 1046–1065.

Butler, S. L., Hansen, M. S. & Bushman, F. D. (2001). A quantitative assay for HIV DNA integration *in vivo*. *Nat Med* **7**, 631–634.

Chou, D. M., Adamson, B., Dephoure, N. E., Tan, X., Nottke, A. C., Hurov, K. E., Gygi, S. P., Colaiacovo, M. P. & Elledge, S. J. (2010). A chromatin localization screen reveals poly (ADP ribose)-regulated recruitment of the repressive polycomb and NuRD complexes to sites of DNA damage. *Proc Natl Acad Sci U S A* **107**, 18475–18480.

Chung, H. R., Dunkel, I., Heise, F., Linke, C., Krobitsch, S., Ehrenhofer-Murray, A. E., Sperling, S. R. & Vingron, M. (2010). The effect of micrococcal nuclease digestion on nucleosome positioning data. *PLoS One* **5**, e15754.

Cooper, A., García, M., Petrovas, C., Yamamoto, T., Koup, R. A. & Nabel, G. J. (2013). HIV-1 causes CD4 cell death through DNA-dependent protein kinase during viral integration. *Nat New Biol* **498**, 376–379.

Drogaris, P., Villeneuve, V., Pomiès, C., Lee, E. H., Bourdeau, V., Bonneil, E., Ferbeyre, G., Verreault, A. & Thibault, P. (2012). Histone deacetylase inhibitors globally enhance h3/h4 tail acetylation without affecting h3 lysine 56 acetylation. *Sci Rep* **2**, 220.

Gaken, J. A., Tavassoli, M., Gan, S. U., Vallian, S., Giddings, I., Darling, D. C., Galea-Lauri, J., Thomas, M. G., Abedi, H. & other authors (1996). Efficient retroviral infection of mammalian cells is blocked by inhibition of poly(ADP-ribose) polymerase activity. *J Virol* **70**, 3992–4000.

Garcia-Rivera, J. A., Bueno, M. T., Morales, E., Kugelman, J. R., Rodriguez, D. F. & Llano, M. (2010). Implication of serine residues 271, 273, and 275 in the human immunodeficiency virus type 1 cofactor activity of lens epithelium-derived growth factor/p75. *J Virol* **84**, 740–752.

Gasiunas, G., Sinkunas, T. & Siksnys, V. (2014). Molecular mechanisms of CRISPR-mediated microbial immunity. *Cell Mol Life Sci* **71**, 449–465.

Ha, H. C., Juluri, K., Zhou, Y., Leung, S., Hermankova, M. & Snyder, S. H. (2001). Poly(ADP-ribose) polymerase-1 is required for efficient HIV-1 integration. *Proc Natl Acad Sci U S A* **98**, 3364–3368.

Hochegger, H., Dejsuphong, D., Fukushima, T., Morrison, C., Sonoda, E., Schreiber, V., Zhao, G. Y., Saberi, A., Masutani, M. & other authors (2006). Parp-1 protects homologous recombination from interference by Ku and ligase IV in vertebrate cells. *EMBO J* **25**, 1305–1314.

Hoelzer, K., Shackelton, L. A. & Parrish, C. R. (2008). Presence and role of cytosine methylation in DNA viruses of animals. *Nucleic Acids Res* **36**, 2825–2837.

Jenuwein, T. & Allis, C. D. (2001). Translating the histone code. *Science* **293**, 1074–1080.

Ji, Y. & Tulin, A. V. (2010). The roles of PARP1 in gene control and cell differentiation. *Curr Opin Genetics Dev* **20**, 512–518.

Kameoka, M., Nukuzuma, S., Itaya, A., Tanaka, Y., Ota, K., Ikuta, K. & Yoshihara, K. (2004). RNA interference directed against poly(ADP-ribose) polymerase 1 efficiently suppresses human immunodeficiency virus type 1 replication in human cells. *J Virol* **78**, 8931–8934.

Kameoka, M., Nukuzuma, S., Itaya, A., Tanaka, Y., Ota, K., Inada, Y., Ikuta, K. & Yoshihara, K. (2005). Poly(ADP-ribose)polymerase-1 is required for integration of the human immunodeficiency virus type 1 genome near centromeric alphoid DNA in human and murine cells. *Biochem Biophys Res Commun* **334**, 412–417.

Kantor, B., Ma, H., Webster-Cyriaque, J., Monahan, P. E. & Kafri, T. (2009). Epigenetic activation of unintegrated HIV-1 genomes by gut-associated short chain fatty acids and its implications for HIV infection. *Proc Natl Acad Sci U S A* **106**, 18786–18791.

Kilzer, J. M., Stracker, T., Beitzel, B., Meek, K., Weitzman, M. & Bushman, F. D. (2003). Roles of host cell factors in circularization of retroviral DNA. *Virology (Auckl)* **314**, 460–467.

Kim, J. & Kim, H. (2012). Recruitment and biological consequences of histone modification of H3K27me3 and H3K9me3. *ILAR J* **53**, 232–239.

Kimura, H. (2013). Histone modifications for human epigenome analysis. *J Hum Genet* **58**, 439–445.

Ko, H. L., Ng, H. J., Goh, E. H. & Ren, E. C. (2013). Reduced ADP-ribosylation by PARP1 natural polymorphism V762A and by PARP1 inhibitors enhance hepatitis B virus replication. *J Viral Hepat* **20**, 658–665.

Kotova, E., Jarnik, M. & Tulin, A. V. (2010). Uncoupling of the transactivation and transrepression functions of PARP1 protein. *Proc Natl Acad Sci U S A* **107**, 6406–6411.

Kotova, E., Lodhi, N., Jarnik, M., Pinnola, A. D., Ji, Y. & Tulin, A. V. (2011). Drosophila histone H2A variant (H2Av) controls poly(ADP-ribose) polymerase 1 (PARP1) activation in chromatin. *Proc Natl Acad Sci U S A* **108**, 6205–6210.

Koyama, T., Sun, B., Tokunaga, K., Tatsumi, M. & Ishizaka, Y. (2013). DNA damage enhances integration of HIV-1 into macrophages by overcoming integrase inhibition. *Retrovirology* **10**, 21.

Krishnakumar, R. & Kraus, W. L. (2010). The PARP side of the nucleus: Molecular actions, physiological outcomes, and clinical targets. *Mol Cell* **39**, 8–24.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. & Higgins, D. G. (2007). Clustal W and clustal X version 2.0. *Bioinformatics* **23**, 2947–2948.

Lilley, C. E., Carson, C. T., Muotri, A. R., Gage, F. H. & Weitzman, M. D. (2005). DNA repair proteins affect the lifecycle of herpes simplex virus 1. *Proc Natl Acad Sci U S A* **102**, 5844–5849.

Lilley, C. E., Chaurushiya, M. S., Boutell, C., Everett, R. D. & Weitzman, M. D. (2011). The intrinsic antiviral defense to incoming HSV-1 genomes includes specific DNA repair proteins and is counteracted by the viral protein ICP0. *PLoS Pathog* **7**, e1002084.

Ménissier de Murcia, J., Ricoul, M., Tartier, L., Niedergang, C., Huber, A., Dantzer, F., Schreiber, V., Amé, J. C., Dierich, A. & other authors (2003). Functional interaction between PARP-1 and PARP-2 in chromosome stability and embryonic development in mouse. *EMBO J* **22**, 2255–2263.

Mitta, B., Weber, C. C., Rimann, M. & Fussenegger, M. (2004). Design and *in vivo* characterization of self-inactivating human and non-human lentiviral expression vectors engineered for streptogramin-adjustable transgene expression. *Nucleic Acids Res* **32**, e106.

Mitta, B., Weber, C. C. & Fussenegger, M. (2005). *In vivo* transduction of HIV-1-derived lentiviral particles engineered for macrolide-adjustable transgene expression. *J Gene Med* **7**, 1400–1408.

Morales, V. & Richard-Foy, H. (2000). Role of histone N-terminal tails and their acetylation in nucleosome dynamics. *Mol Cell Biol* **20**, 7230–7237.

Ohsaki, E., Ueda, K., Sakakibara, S., Do, E., Yada, K. & Yamanishi, K. (2004). Poly(ADP-ribose) polymerase 1 binds to Kaposi's sarcoma-associated herpesvirus (KSHV) terminal repeat sequence and modulates KSHV replication in latency. *J Virol* **78**, 9936–9946.

Orzalli, M. H., Conwell, S. E., Berrios, C., DeCaprio, J. A. & Knipe, D. M. (2013). Nuclear interferon-inducible protein 16 promotes silencing of herpesviral and transfected DNA. *Proc Natl Acad Sci U S A* **110**, E4492–E4501.

Pinnola, A., Naumova, N., Shah, M. & Tulin, A. V. (2007). Nucleosomal core histones mediate dynamic regulation of poly(ADP-ribose) polymerase 1 protein binding to chromatin and induction of its enzymatic activity. *J Biol Chem* **282**, 32511–32519.

Pinsker, W., Haring, E., Hagemann, S. & Miller, W. J. (2001). The evolutionary life history of P transposons: from horizontal invaders to domesticated neogenes. *Chromosoma* **110**, 148–158.

Randow, F. & Sale, J. E. (2006). Retroviral transduction of DT40. *Subcell Biochem* **40**, 383–386.

Rass, E., Grabarz, A., Bertrand, P. & Lopez, B. S. (2012). [Double strand break repair, one mechanism can hide another: alternative non-homologous end joining]. *Cancer radiothérapie: journal de la Société française de radiothérapie oncologique*. **16**, 1–10.

Ross, P. J., Kennedy, M. A. & Parks, R. J. (2009). Host cell detection of noncoding stuffer DNA contained in helper-dependent adenovirus vectors leads to epigenetic repression of transgene expression. *J Virol* **83**, 8409–8417.

Sakurai, Y., Komatsu, K., Agematsu, K. & Matsuoka, M. (2009). DNA double strand break repair enzymes function at multiple steps in retroviral infection. *Retrovirology* **6**, 114.

Schneider, W. M., Wu, D. T., Amin, V., Aiyer, S. & Roth, M. J. (2012). Mulv IN mutants responsive to HDAC inhibitors enhance transcription from unintegrated retroviral DNA. *Virology (Auckl)* **426**, 188–196.

Schreiner, S., Kinkley, S., Bürck, C., Mund, A., Wimmer, P., Schubert, T., Groitl, P., Will, H. & Dobner, T. (2013). SPOC1-mediated antiviral host cell response is antagonized early in human adenovirus type 5 infection. *PLoS Pathog* **9**, e1003775.

Siva, A. C. & Bushman, F. (2002). Poly(ADP-ribose) polymerase 1 is not strictly required for infection of murine cells by retroviruses. *J Virol* **76**, 11904–11910.

Sloan, R. D. & Wainberg, M. A. (2011). The role of unintegrated DNA in HIV infection. *Retrovirology* **8**, 52.

Tulin, A., Stewart, D. & Spradling, A. C. (2002). The Drosophila heterochromatic gene encoding poly(ADP-ribose) polymerase (PARP) is required to modulate chromatin structure during development. *Genes & Development* **16**, 2108–2119.

Wang, Z. Q., Auer, B., Stingl, L., Berghammer, H., Haidacher, D., Schweiger, M. & Wagner, E. F. (1995). Mice lacking ADPRT and poly (ADP-ribosyl)ation develop normally but are susceptible to skin disease. *Genes & Development* **9**, 509–520.

Weitzman, M. D., Lilley, C. E. & Chaurushiya, M. S. (2010). Genomes in conflict: maintaining genome integrity during virus infection. *Annu Rev Microbiol* **64**, 61–81.

Yu, S. S., Dan, K., Chono, H., Chatani, E., Mineno, J. & Kato, I. (2008). Transient gene expression mediated by integrase-defective retroviral vectors. *Biochem Biophys Res Commun* **368**, 942–947.