JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
pubs.acs.org/JACS
Communication

Synthesis of ADP-Ribosylated Histones Reveals Site-Specific Impacts on Chromatin Structure and Function

Nir Hananya, Sara K. Daley, John D. Bagert, and Tom W. Muir*

Cite This: J. Am. Chem. Soc. 2021, 143, 10847−10852
Read Online

ABSTRACT: ADP-ribosylation of nuclear proteins is a critical feature of various DNA damage repair pathways. Histones, particularly H3 and H2B, are major targets of ADP-ribosylation and are primarily modified on serine with a single ADP-ribose unit following DNA damage. While the overall impact of PARP1-dependent poly-ADP-ribosylation is heavily investigated, very little is known about the specific roles of histone ADP-ribosylation. Here, we report the development of an efficient and modular semisynthetic route to full-length ADP-ribosylated histones H3 and H2B, chemically installed at specific serine residues. The modified histones were used to generate various chemically defined ADP-ribosylated chromatin substrates, which were employed in biophysical assays. These studies revealed that ADP-ribosylation of serine-6 of histone H2B (H2BS6ADPr) inhibits chromatin folding and higher-order organization; notably, this effect was enhanced by ADP-ribosylation of H3S10. In addition, ADP-ribosylated nucleosomes were utilized in biochemical experiments employing a panel of lysine methyltransferase enzymes, revealing a context-dependent inhibition of histone H3K9 methylation. The availability of designer ADP-ribosylated chromatin described here is expected to facilitate further biochemical and structural studies regarding the roles of histone ADP-ribosylation in the DNA damage response.

Cells are constantly exposed to exogenous and endogenous genotoxic agents which induce various DNA lesions, including DNA breaks.[1] Since single- or double-strand DNA breaks pose a threat to genome integrity and cell viability, cells have evolved various repair pathways to correct such damage.[2,3] One of the first responders to broken DNA is poly(ADP-ribose)polymerase 1 (PARP1)—this enzyme is activated upon binding to DNA breaks and, subsequently, catalyzes the mono- and poly-ADP-ribosylation of a multitude of nuclear proteins.[4,5] The physiological roles of ADP-ribosylation include modulation of chromatin structure and recruitment of proteins that participate in DNA repair.[6,7] The importance of protein ADP-ribosylation in various DNA repair pathways makes PARP1 an important target for cancer therapy.[8] Because of the biological and clinical significance of ADP-ribosylation, it is essential to understand the cellular pathways involved in its regulation and delineate its function within the DNA repair process.

Histone proteins are major targets of ADP-ribosylation, and several serine residues, H2BS6, H3S10, and to a lesser extent H3S28, are the most abundant ADP-ribosylation sites within core histones.[4,9,10] While for many years it has been assumed that the major final output of PARP1 signaling is poly-ADP-ribosylation, recent studies show that for nuclear proteins in general, and histone proteins in particular, a dedicated glycohydrolase rapidly trims this initial polymeric modification back to the mono-ADP-ribosyl unit and that this is thought to be the predominant species upon DNA damage.[11,12] Importantly, while the overall impact of PARP1 activation upon DNA damage is the subject of intensive investigation, very little is known about the specific roles of histone ADP-ribosylation, in large part due to the lack of molecular tools to study biochemical effects (Figure 1). In contrast to most other known histone post-translational modifications, currently there exists no in vitro strategy for the preparation of chemically defined chromatin containing a native ADP-ribose (ADPr) modification. This is particularly striking considering the enormous value of such "designer" chromatin substrates for studying attendant biochemistry.[13−15] This problem motivated us to develop a method for generating chemically defined ADP-ribosylated histones, which were further used to assemble designer ADP-ribosylated chromatin substrates. These reagents were employed in various biophysical and biochemical assays to explore the effects of histone ADP-ribosylation.

Inspired by recent progress on the generation of O-linked ADP-ribosylated peptides using synthetic[16−19] or enzymatic[11] means, we imagined that native full-length ADP-ribosylated histones could be accessible through a semisynthetic approach,[20] that is, by traceless native chemical ligation of an ADP-ribosylated peptide α-thioester with a recombinant fragment containing an N-terminal cysteine. H2B contains an alanine at position 17, suggesting that native H2BS6ADPr could be generated by ligation of an $H2B_{(1-16)}$S6ADPr synthetic peptide α-thioester with a recombinant $H2B_{(17-125)}$ containing an A17C mutation, followed by desulfurization to convert the cysteine ligation "scar" to a native alanine residue.

Received: May 26, 2021
Published: July 15, 2021

https://doi.org/10.1021/jacs.1c05429
J. Am. Chem. Soc. 2021, 143, 10847−10852

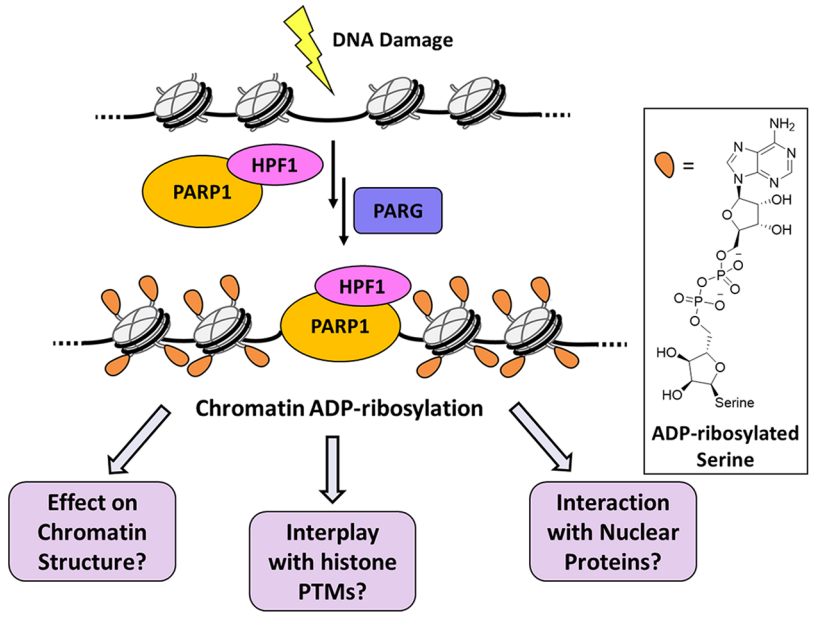

Figure 1. Histones become mono-ADP-ribosylated in response to DNA damage. Binding of histone PARylation factor 1 (HPF1) to PARP1 is required for ADP-ribosylation of serine residues on histones H3 and H2B. The action of poly(ADP-ribose) glycohydrolase (PARG) rapidly trims initial poly-ADP-ribosylated products back to mono-ADP-ribosylated histones (inset). The impact of this modification on chromatin structure and function is poorly understood.

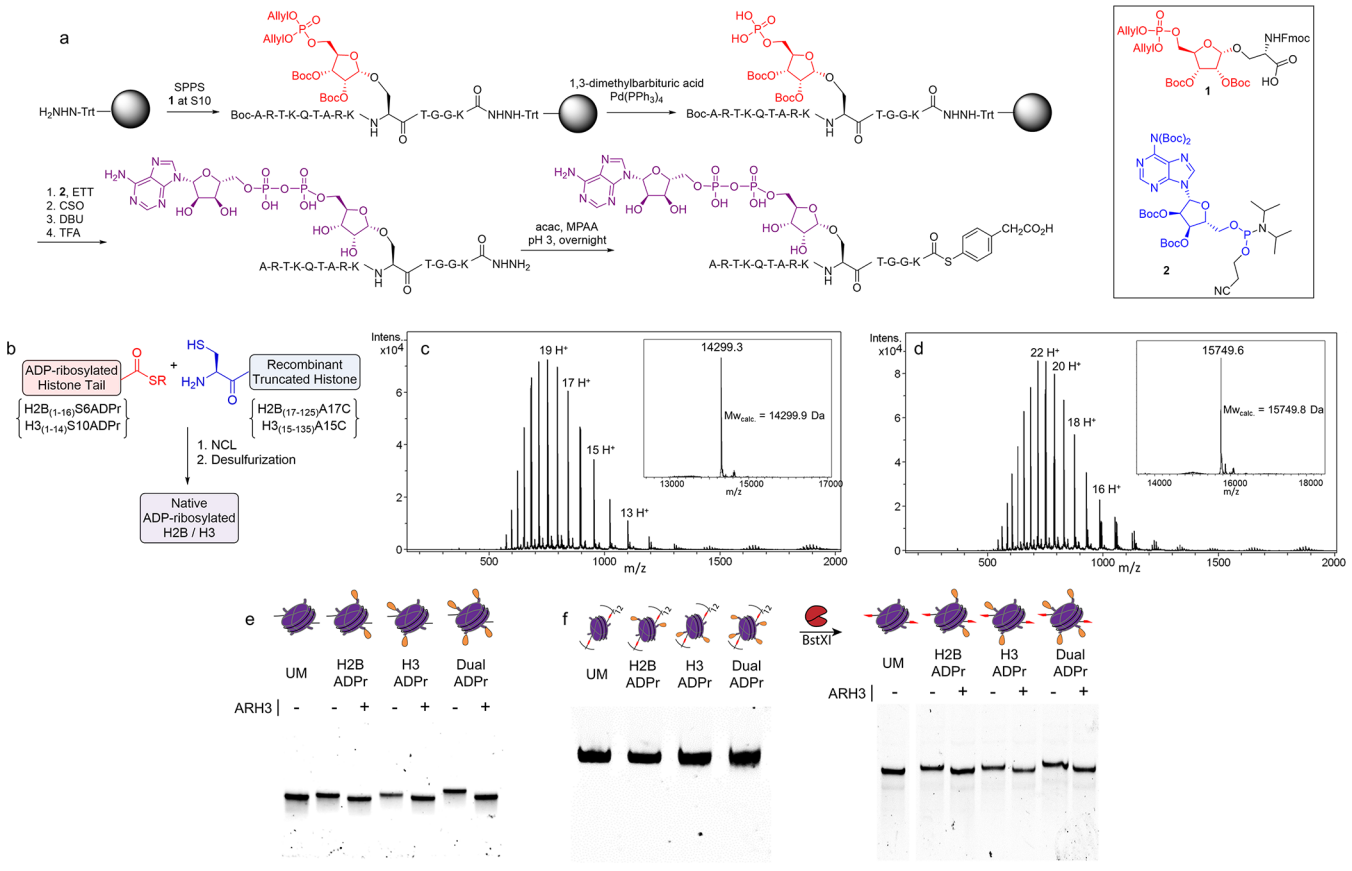

Figure 2. Synthesis of ADP-ribosylated "designer" chromatin. (a) Scheme showing the chemical synthesis of ADP-ribosylated synthetic peptide α-thioesters ($H3_{1-14}$ sequence is depicted). Key building blocks **1** and **2** are shown in the inset. (b) Generation of ADP-ribosylated histones by traceless native chemical ligation. (c, d) ESI-MS characterization of H2BS6ADPr (c) and H3S10ADPr (d). Inset: Deconvoluted MS. (e) Native PAGE of indicated reconstituted MNs. Treatment with the ADP-ribosylhydrolase, ARH3, efficiently removes the modification as indicated by a change in migration on the gel. (f) Left: Analysis of the ADP-ribosylated 12-mer arrays by native gel electrophoresis on an agarose-acrylamide composite gel. Right: Native PAGE of the same arrays following restriction enzyme digestion to MNs and treatment with ARH3. UM: Unmodified.

https://doi.org/10.1021/jacs.1c05429
J. Am. Chem. Soc. 2021, 143, 10847−10852

Similarly, native H3S10ADPr could be generated by ligation of an $H3_{(1-14)}$S10ADPr synthetic peptide α-thioester with a recombinant $H3_{(15-135)}$ containing an A15C mutation, followed by desulfurization.

To prepare the requisite ADP-ribosylated peptide α-thioesters, we designed a modified 'on-resin pyrophosphate formation' strategy,[16,17] as depicted in Figure 2a. A hydrazine linker, a thioester surrogate, was loaded on a trityl resin.[21] Standard Fmoc-based SPPS was used to synthesize the peptide sequence, in which the phosphoribosylated serine derivative **1** (see Supporting Information for synthetic details) was incorporated at the appropriate position (S6 for H2B, S10 for H3). The phosphate group in compound **1** is protected with allyl moieties, which enabled Pd-catalyzed selective deprotection of the phosphate following peptide chain synthesis. Subsequently, the pyrophosphate bond was formed on-resin through a reaction between the free phosphate and the Boc-protected adenosine-phosphoramidite **2** (see Supporting Information for synthetic details), followed by phosphite oxidation. The 2-cyanoethyl group was removed by DBU, and subsequent cleavage and side-chain deprotection with TFA yielded the ADP-ribosylated peptide hydrazide. Hydrazides are often converted to thioesters via an acyl azide intermediate through treatment with nitrous acid;[21] however, the adenine moiety contains an aromatic amine and is not compatible with such oxidizing conditions. Therefore, we employed a recent, milder method in which the peptide hydrazide is reacted with acetylacetone (acac) to provide an acyl pyrazole intermediate.[22] This active species further reacts with 4-mercaptophenylacetic acid (MPAA), generating the corresponding thioester. The overall synthetic workflow provided highly pure ADP-ribosylated peptide α-thioesters: $H2B_{(1-16)}$S6ADPr and $H3_{(1-14)}$S10ADPr (Figure S1), in 26% and 12% yield, respectively.

Following successful synthesis of the ADP-ribosylated peptide α-thioesters, we turned to the preparation of full-length ADP-ribosylated histones. The N-terminal cysteine-containing fragments $H2B_{(17-125)}$A17C and $H3_{(15-135)}$A15C were expressed in *Escherichia coli* as reported previously.[23,24] Native chemical ligation of the peptide thioesters with the corresponding recombinant fragments, followed by free-radical-mediated desulfurization of the cysteine ligation "scar" proceeded smoothly, affording the native full-length ADP-ribosylated proteins—H2BS6ADPr and H3S10ADPr (Figures 2b−-d and S2).

Next, we utilized the modified histone proteins for the reconstitution of ADP-ribosylated chromatin substrates, starting with mononucleosomes (MNs). To this end, we refolded three different ADP-ribosylated histone octamer complexes, which contained either ADP-ribosylated H2B, ADP-ribosylated H3, or both, in addition to a control octamer complex in which all histones are unmodified. The various octamers were mixed with the "Widom 601" nucleosome-positioning DNA sequence[25] and salt gradient dialysis was employed to reconstitute MNs. Analysis by native polyacrylamide gel electrophoresis (PAGE) indicated that the bulky, anionic ADP-ribose moiety did not impede efficient MN formation, although, as expected, the modified MNs migrate slightly slower compared to the unmodified MN (Figures 2e and S3). To evaluate the authenticity of the ADP-ribosylated MNs, we treated them with the ADP-ribosylhydrolase, ARH3, which removes the ADP-ribose mark from serine residues.[26] Treatment with ARH3 converted all ADP-ribosylated MNs to a faster-migrating species that corresponds to the unmodified MN (Figure 2e), which indicates that the ADP-ribosylated MNs contain the native α-anomeric serine-ADP-ribose linkage.[17] In addition, all MNs were tested in a fluorescence-based thermal stability assay (Figure S4).[27] The ADP-ribosylated MNs exhibited a melting temperature similar to that of the unmodified MN, further indicating that the presence of the ADP-ribose moiety does not affect proper nucleosome assembly or thermal stability.

In addition to MNs, the modified histones were utilized to prepare ADP-ribosylated 12-mer nucleosome arrays; such reagents are useful for gathering information about the regulation of chromatin folding and compaction.[28,29] To this end, the various histone octamer complexes were mixed with a DNA template that harbors 12 copies of the "Widom 601" sequence, separated by a 35-bp linker DNA. Salt gradient dialysis and subsequent Mg^{2+}-induced precipitation provided pure 12-mer arrays (Figure 2f). Digestion of the arrays to MNs with BstXI restriction enzyme yielded the expected ADP-ribosylated MNs, as indicated by native PAGE (Figure 2f).

With the designer chromatin substrates in hand, we set out to explore the biophysical and biochemical effects of histone ADP-ribosylation. The first question we aimed to address is whether the modification alters chromatin higher-order structure. It is well established that PARP1-mediated poly-ADP-ribosylation induces chromatin relaxation,[30] thereby facilitating the accessibility of DNA repair factors to the damage site.[31] However, it is unknown whether chromatin relaxation requires long poly(ADP-ribose) chains or whether histone mono-ADP-ribosylation is sufficient to impede chromatin compaction. This question is of particular importance since mono-ADP-ribosylation is the primary form of histone ADP-ribosylation in PARP1-dependent DNA damage signaling.[11] Furthermore, it is not clear whether the site-specificity of histone ADP-ribosylation is important in determining its potential impact on chromatin decompaction. To address these questions, we performed sedimentation velocity analysis of the various 12-mer nucleosome arrays in the absence or presence of 1 mM Mg^{2+} ions. Without Mg^{2+} supplementation, the various nucleosome arrays adopt an extended structure and sediment similarly at around 32 S (Figure 3a). As expected,[32] the addition of 1 mM Mg^{2+} to the unmodified array induced the formation of a more compact structure that sediments at 45 S (Figure 3a). Remarkably, whereas the H3ADPr array behaves similarly to the unmodified control, the H2BADPr array sediments significantly slower (41 S) upon Mg^{2+} addition (Figure 3a). The dual-modified ADPr array is even less compacted and sediments at 36 S, a surprising result given that the H3ADPr modification on its own has no obvious effect. Notably, pretreatment of the same batch of modified arrays with ARH3 resulted in sedimentation behavior similar to the unmodified control (Figure S5).

To further explore the impact of ADP ribosylation on chromatin structure, we measured the effect of the modification on Mg^{2+}-mediated array self-association, as indicated by the decrease in the material that remained in the supernatant upon centrifugation.[33] As before, we observed differential behavior depending on which histone the ADP-ribose mark was located; H3ADPr arrays again behaved similarly to the unmodified control, whereas attachment of the modification to H2B inhibited array self-association as reflected by the higher Mg^{2+} concentration needed to precipitate the sample (Figure 3b). Also consistent with the

https://doi.org/10.1021/jacs.1c05429
J. Am. Chem. Soc. 2021, 143, 10847−10852

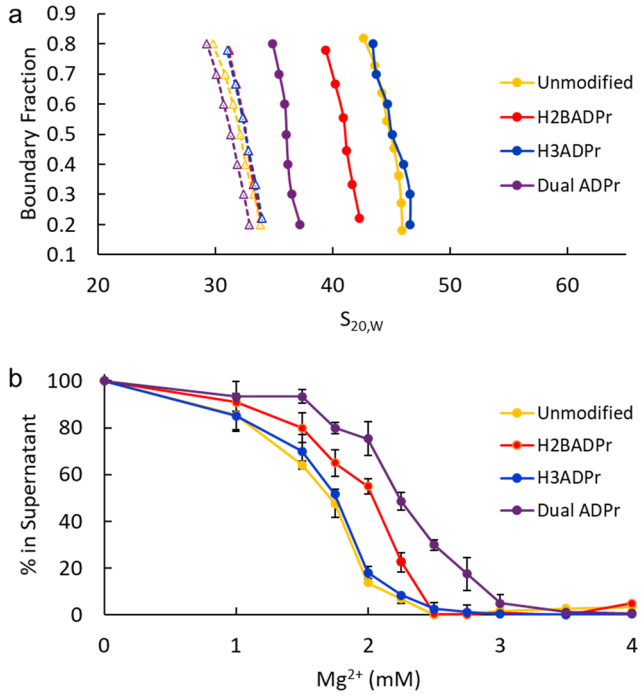

Figure 3. Effect of ADP ribosylation on higher-order chromatin structure. (a) Integrated sedimentation coefficient distributions of indicated 12-mer nucleosome arrays in the presence (solid lines) or absence (dashed lines) of 1 mM Mg^{2+}, as determined by sedimentation velocity experiments and van Holde-Weischet analysis. (b) Mg^{2+}-mediated self-association of the various arrays, determined by the percentage of material remaining in solution following centrifugation. Error = S.D., n = 3.

trend observed in sedimentation velocity analysis, an even bigger inhibitory effect was observed for arrays containing the modification on both H2B and H3. Thus, using two quite different measures of chromatin compaction, we observe that the inhibitory effect of ADP ribosylation is enhanced when it is present on the two histone tails, even though as a single modification an effect is only observed when attached to H2B. The physical basis of this apparent nonadditive behavior warrants further study, including whether the presence of the H3ADPr modification somehow enhances the existing inhibitory effect of H2BADPr or conversely whether the latter somehow turns on an otherwise latent property of the former. Regardless, our data clearly suggest that the mono-ADP-ribosylation of histones is sufficient to inhibit chromatin fiber compaction and interarray interactions. These findings imply a role of histone mono-ADP-ribosylation in chromatin relaxation in the context of DNA repair.

Our next goal was to explore the interplay between histone ADP-ribosylation and other histone post-translational modifications. Specifically, we asked how the ADP-ribosylation of H3S10 affects lysine methylation by different lysine methyltransferases (KMTs) that modify the H3 tail. We, therefore, employed the ADP-ribosylated MNs in histone methyltransferase experiments with the following KMTs: (i) MLL1 (H3K4 KMT), (ii) G9a (H3K9 KMT), (iii) PRC2 (H3K27 KMT), and (iv) NSD2 (H3K36 KMT). Methylation of H3K4, H3K27, and H3K36 was detected by Western blot, as the antibodies against these methylation sites are insensitive to H3S10 ADP-ribosylation (Figure S6). H3K9 methylation was detected by autoradiography since the ADPr moiety on H3S10 is expected to interfere with antigen recognition by the H3K9me antibody. ADP-ribosylation of H3S10 inhibits G9a-mediated H3K9 methylation; a decrease of 85% in the autoradiography signal is observed with H3ADPr- and

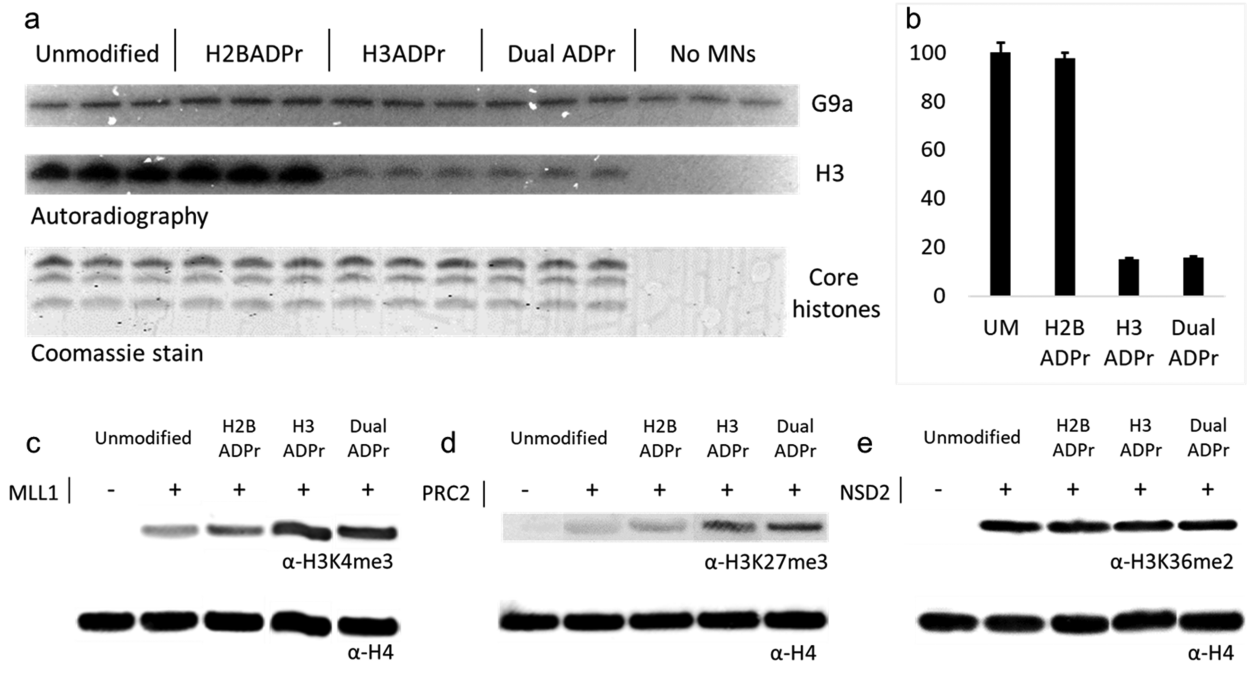

Figure 4. Effect of ADP ribosylation on histone methyl transferase activity (a) Autoradiogram showing G9a-mediated H3K9 methylation of the various MNs. Coomassie stain of core histones was used as a loading control for the MNs. (b) Quantification of the autoradiogram in panel a: error = S.D., n = 3. (c–e) Representative Western blots showing the impact of ADP ribosylation on the methyltransferase activity of MLL1 (c), PRC2 (d), and NSD2 (e). In each case, H4 was used as a loading control.

https://doi.org/10.1021/jacs.1c05429
J. Am. Chem. Soc. 2021, 143, 10847–10852

H3ADPr/H2BADPr-MNs (Figure 4a and b). By contrast, H2BS6 ADP-ribosylation on its own does not affect G9a activity. This suggests that the inhibitory effect of H3S10 ADP-ribosylation on G9a activity occurs in cis and is presumably due to an inability of G9a to engage its target lysine (which is the preceding amino acid in the substrate sequence). Previous work employing an ADP-ribosylated peptide, showed that H3S10 ADP-ribosylation interferes with H3K9 methylation carried out by the fungal KMT, Dim5.[34] Our data extends this to the human H3K9 KMT, G9a, using a physiologically relevant substrate, an ADP-ribosylated MN. Notably, we and others have previously shown that modifications on H3K9 inhibit H3S10 ADP-ribosylation by the PARP1/HPF1 complex.[24,34] Thus, there exists a reciprocal negative crosstalk between modifications on these adjacent amino acids. In contrast to G9a-mediated H3K9 methylation, the methylation of H3K4 and H3K27 is moderately increased by H3S10 ADP-ribosylation (Figures 4c,d and S7). This effect may be related to modulation of the H3-tail dynamics by ADP-ribosylation, as H3-tail modifications that weaken its binding to nucleosomal DNA increase accessibility to tail-interacting proteins.[35] Consistent with this idea, we did not observe a significant effect of histone ADP-ribosylation on the methylation of H3K36 (Figures 4e and S7), which we note resides at the base of the H3 N-terminal tail and hence might be expected to be less affected by changes in dynamics. Further research is required to explore the possible effect of ADP-ribosylation on histone tail dynamics and how this might modulate the interaction of the tail with chromatin modifiers including those relevant to DNA repair pathways.

In conclusion, we developed an efficient synthesis of ADP-ribosylated peptide α-thioesters, which enabled production of native full-length ADP-ribosylated histones through protein semisynthesis. ADP-ribosylated H2B and H3 were utilized to generate chemically defined ADP-ribosylated mononucleosomes and nucleosome arrays, which were employed in various biophysical and biochemical experiments. These investigations revealed that (i) histone mono-ADP-ribosylation is sufficient to impede chromatin compaction and histone PTM deposition and (ii) the specific site of ADP-ribosylation is critical in determining these effects. This work sheds light on a role of histone mono-ADP-ribosylation in DNA repair and also highlights the value of designer ADP-ribosylated chromatin substrates in exploring the effects of this unique modification. More generally, we imagine that by extending the scope of protein semisynthesis to ADP-ribosylated targets, the procedures described herein will help illuminate the roles of this fascinating and poorly understood post-translational modification.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.1c05429.

Experimental details, supplementary figures, synthetic protocols, and NMR spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Tom W. Muir − *Department of Chemistry, Princeton University, Princeton, New Jersey 08544, United States;* orcid.org/0000-0001-9635-0344; Email: muir@princeton.edu

Authors

Nir Hananya − *Department of Chemistry, Princeton University, Princeton, New Jersey 08544, United States*

Sara K. Daley − *Department of Chemistry, Princeton University, Princeton, New Jersey 08544, United States*

John D. Bagert − *Department of Chemistry, Princeton University, Princeton, New Jersey 08544, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.1c05429

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Merck Research Laboratories for financial support (LKR 179713). N.H. is a Robert Black Fellow of the Damon Runyon Cancer Research Foundation, DRG-2425-21. We thank E. Ge for providing PRC2, and other members of the Muir lab for helpful discussions.

■ REFERENCES

(1) Jackson, S. P.; Bartek, J. The DNA-Damage Response in Human Biology and Disease. *Nature* **2009**, *461* (7267), 1071−1078.

(2) Caldecott, K. W. Protein ADP-Ribosylation and the Cellular Response to DNA Strand Breaks. *DNA Repair* **2014**, *19*, 108−113.

(3) Palazzo, L.; Mikolčević, P.; Mikoč, A.; Ahel, I. ADP-Ribosylation Signalling and Human Disease. *Open Biol.* **2019**, *9* (4), 190041.

(4) Hendriks, I. A.; Larsen, S. C.; Nielsen, M. L. An Advanced Strategy for Comprehensive Profiling of ADP-Ribosylation Sites Using Mass Spectrometry-Based Proteomics. *Mol. Cell. Proteomics* **2019**, *18* (5), 1010−1026.

(5) Buch-Larsen, S. C.; Hendriks, I. A.; Lodge, J. M.; Rykær, M.; Furtwängler, B.; Shishkova, E.; Westphall, M. S.; Coon, J. J.; Nielsen, M. L. Mapping Physiological ADP-Ribosylation Using Activated Ion Electron Transfer Dissociation. *Cell Rep.* **2020**, *32* (12), 108176.

(6) Liu, C.; Vyas, A.; Kassab, M. A.; Singh, A. K.; Yu, X. The Role of Poly ADP-Ribosylation in the First Wave of DNA Damage Response. *Nucleic Acids Res.* **2017**, *45* (14), 8129−8141.

(7) Ray Chaudhuri, A.; Nussenzweig, A. The Multifaceted Roles of PARP1 in DNA Repair and Chromatin Remodelling. *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 610.

(8) Lord, C. J.; Ashworth, A. PARP Inhibitors: Synthetic Lethality in the Clinic. *Science* **2017**, *355* (6330), 1152−1158.

(9) Leidecker, O.; Bonfiglio, J. J.; Colby, T.; Zhang, Q.; Atanassov, I.; Zaja, R.; Palazzo, L.; Stockum, A.; Ahel, I.; Matic, I. Serine Is a New Target Residue for Endogenous ADP-Ribosylation on Histones. *Nat. Chem. Biol.* **2016**, *12*, 998.

(10) Palazzo, L.; Leidecker, O.; Prokhorova, E.; Dauben, H.; Matic, I.; Ahel, I. Serine Is the Major Residue for ADP-Ribosylation upon DNA Damage. *eLife* **2018**, *7*, e34334.

(11) Bonfiglio, J. J.; Leidecker, O.; Dauben, H.; Longarini, E. J.; Colby, T.; San Segundo-Acosta, P.; Perez, K. A.; Matic, I. An HPF1/PARP1-Based Chemical Biology Strategy for Exploring ADP-Ribosylation. *Cell* **2020**, *183* (4), 1086−1102. e23.

(12) Hendriks, I. A.; Buch-Larsen, S. C.; Prokhorova, E.; Rebak, A. K. L. F. S.; Ahel, I.; Nielsen, M. L. The Regulatory Landscape of the Human HPF1- and ARH3-Dependent ADP-Ribosylome. *bioRxiv*, 2021.01.26.428255, January 27, 2021. DOI: 10.1101/2021.01.26.428255 (accessed 2021-05-26).

(13) Müller, M. M.; Muir, T. W. Histones: At the Crossroads of Peptide and Protein Chemistry. *Chem. Rev.* **2015**, *115* (6), 2296−2349.

(14) Pick, H.; Kilic, S.; Fierz, B. Engineering Chromatin States: Chemical and Synthetic Biology Approaches to Investigate Histone Modification Function. *Biochim. Biophys. Acta, Gene Regul. Mech.* **2014**, *1839* (8), 644−656.
(15) Lobbia, V. R.; Trueba Sanchez, M. C.; van Ingen, H. Beyond the Nucleosome: Nucleosome-Protein Interactions and Higher Order Chromatin Structure. *J. Mol. Biol.* **2021**, *433* (6), 166827.
(16) Kistemaker, H. A. V; Nardozza, A. P.; Overkleeft, H. S.; van der Marel, G. A.; Ladurner, A. G.; Filippov, D. V. Synthesis and Macrodomain Binding of Mono-ADP-Ribosylated Peptides. *Angew. Chem., Int. Ed.* **2016**, *55* (36), 10634−10638.
(17) Voorneveld, J.; Rack, J. G. M.; Ahel, I.; Overkleeft, H. S.; van der Marel, G. A.; Filippov, D. V. Synthetic α- and β-Ser-ADP-Ribosylated Peptides Reveal α-Ser-ADPr as the Native Epimer. *Org. Lett.* **2018**, *20* (13), 4140−4143.
(18) Liu, Q.; van der Marel, G. A.; Filippov, D. V. Chemical ADP-Ribosylation: Mono-ADPr-Peptides and Oligo-ADP-Ribose. *Org. Biomol. Chem.* **2019**, *17* (22), 5460−5474.
(19) Voorneveld, J.; Rack, J.; van Gijlswijk, L.; Meeuwenoord, N.; Liu, Q.; Overkleeft, H.; van der Marel, G.; Ahel, I.; Filippov, D. Molecular Tools for the Study of ADP-Ribosylation: A Unified and Versatile Method to Synthesise Native Mono-ADP-Ribosylated Peptides. *Chem. - Eur. J.* **2021**, DOI: 10.1002/chem.202100337.
(20) Thompson, R. E.; Muir, T. W. Chemoenzymatic Semisynthesis of Proteins. *Chem. Rev.* **2020**, *120* (6), 3051−3126.
(21) Fang, G.-M.; Li, Y.-M.; Shen, F.; Huang, Y.-C.; Li, J.-B.; Lin, Y.; Cui, H.-K.; Liu, L. Protein Chemical Synthesis by Ligation of Peptide Hydrazides. *Angew. Chem., Int. Ed.* **2011**, *50* (33), 7645−7649.
(22) Flood, D. T.; Hintzen, J. C. J.; Bird, M. J.; Cistrone, P. A.; Chen, J. S.; Dawson, P. E. Leveraging the Knorr Pyrazole Synthesis for the Facile Generation of Thioester Surrogates for Use in Native Chemical Ligation. *Angew. Chem., Int. Ed.* **2018**, *57* (36), 11634−11639.
(23) Chiang, K. P.; Jensen, M. S.; McGinty, R. K.; Muir, T. W. A Semisynthetic Strategy to Generate Phosphorylated and Acetylated Histone H2B. *ChemBioChem* **2009**, *10* (13), 2182−2187.
(24) Liszczak, G.; Diehl, K. L.; Dann, G. P.; Muir, T. W. Acetylation Blocks DNA Damage−Induced Chromatin ADP-Ribosylation. *Nat. Chem. Biol.* **2018**, *14* (9), 837−840.
(25) Lowary, P. T.; Widom, J. New DNA Sequence Rules for High Affinity Binding to Histone Octamer and Sequence-Directed Nucleosome Positioning. *J. Mol. Biol.* **1998**, *276* (1), 19−42.
(26) Fontana, P.; Bonfiglio, J. J.; Palazzo, L.; Bartlett, E.; Matic, I.; Ahel, I. Serine ADP-Ribosylation Reversal by the Hydrolase ARH3. *eLife* **2017**, *6*, e28533.
(27) Taguchi, H.; Horikoshi, N.; Arimura, Y.; Kurumizaka, H. A Method for Evaluating Nucleosome Stability with a Protein-Binding Fluorescent Dye. *Methods* **2014**, *70* (2), 119−126.
(28) Shogren-Knaak, M.; Ishii, H.; Sun, J.-M.; Pazin, M. J.; Davie, J. R.; Peterson, C. L. Histone H4-K16 Acetylation Controls Chromatin Structure and Protein Interactions. *Science* **2006**, *311* (5762), 844−847.
(29) Gibson, B. A.; Doolittle, L. K.; Schneider, M. W. G.; Jensen, L. E.; Gamarra, N.; Henry, L.; Gerlich, D. W.; Redding, S.; Rosen, M. K. Organization of Chromatin by Intrinsic and Regulated Phase Separation. *Cell* **2019**, *179* (2), 470−484.
(30) Poirier, G. G.; de Murcia, G.; Jongstra-Bilen, J.; Niedergang, C.; Mandel, P. Poly(ADP-Ribosyl)Ation of Polynucleosomes Causes Relaxation of Chromatin Structure. *Proc. Natl. Acad. Sci. U. S. A.* **1982**, *79* (11), 3423−3427.
(31) Smith, R.; Lebeaupin, T.; Juhász, S.; Chapuis, C.; D'Augustin, O.; Dutertre, S.; Burkovics, P.; Biertümpfel, C.; Timinszky, G.; Huet, S. Poly(ADP-Ribose)-Dependent Chromatin Unfolding Facilitates the Association of DNA-Binding Proteins with DNA at Sites of Damage. *Nucleic Acids Res.* **2019**, *47* (21), 11250−11267.
(32) Ausió, J. Analytical Ultracentrifugation and the Characterization of Chromatin Structure. *Biophys. Chem.* **2000**, *86* (2), 141−153.
(33) Schwarz, P. M.; Felthauser, A.; Fletcher, T. M.; Hansen, J. C. Reversible Oligonucleosome Self-Association: Dependence on Divalent Cations and Core Histone Tail Domains. *Biochemistry* **1996**, *35* (13), 4009−4015.
(34) Bartlett, E.; Bonfiglio, J. J.; Prokhorova, E.; Colby, T.; Zobel, F.; Ahel, I.; Matic, I. Interplay of Histone Marks with Serine ADP-Ribosylation. *Cell Rep.* **2018**, *24* (13), 3488−3502.
(35) Morrison, E. A.; Bowerman, S.; Sylvers, K. L.; Wereszczynski, J.; Musselman, C. A. The Conformation of the Histone H3 Tail Inhibits Association of the BPTF PHD Finger with the Nucleosome. *eLife* **2018**, *7*, e31481.

https://doi.org/10.1021/jacs.1c05429
J. Am. Chem. Soc. 2021, 143, 10847−10852