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Synthetic Posttranslational Modifications: Chemical Catalyst-Driven Regioselective Histone Acylation of Native Chromatin

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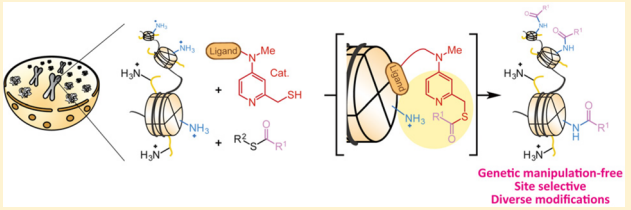
Supporting Information

ABSTRACT: Posttranslational modifications (PTMs) of histones play an important role in the complex regulatory mechanisms governing gene transcription, and their dysregulation can cause diseases such as cancer. The lack of methods for site-selectively modifying native chromatin, however, limits our understanding of the functional roles of a specific histone PTM, not as a single mark, but in the intertwined PTM network. Here, we report a synthetic catalyst DMAP-SH (DSH), which activates chemically stable thioesters (including acetyl-CoA) under physiological conditions and transfers various acyl groups to the proximate amino groups. Our data suggest that DSH, conjugated with a nucleosome ligand, such as pyrrole-imidazole-polyamide and LANA (latency-associated nuclear antigen)-peptide, promotes both natural (including acetylation, butyrylation, malonylation, and ubiquitination) and non-natural (azido- and phosphoryl labeling) PTMs on histones in recombinant nucleosomes and/or in native chromatin, at lysine residues close to the DSH moiety. To investigate the validity of our method, we used LANA-DSH to promote histone H2B lysine-120 (K120) acylation, the function of which is largely unknown. H2BK120 acetylation and malonylation modulated higher-order chromatin structures by reducing internucleosomal interactions, and this modulation was further enhanced by histone tail acetylation. This approach, therefore, may have versatile applications for dissecting the regulatory mechanisms underlying chromatin function.



INTRODUCTION

Posttranslational modifications (PTMs) of histones play an important role in the complex regulatory mechanisms governing gene transcription. Dysregulation of histone PTMs can result in the development of diseases such as cancer.[1] Lysine acylation on histones, which is a representative PTM in chromatin, affects gene transcription and chromatin structure. For example, acetylation, butyrylation, and crotonylation on histone tails have been shown to directly stimulate transcription.[2−4] Ubiquitination of H2A and H2B influences gene transcription and chromatin structure.[5−7] Although other types of lysine acylation, such as malonylation,[8] have been recently identified at various sites on histones,[9] their functions are largely unknown.

Several powerful techniques that allow site-selective incorporation of noncanonical amino acids in recombinant proteins have furthered our understanding of the function of histone PTMs. These include protein semisyntheses using native chemical ligation,[10−14] ribosome-mediated protein syntheses using expanded genetic code,[15−17] and recently reported chemistry-coupled posttranslational mutagenesis.[18,19] Installation of a PTM in recombinant nucleosomes using these techniques enabled us to explore how the PTM modulates the biochemical function of the nucleosomes.[20] In addition, because a specific histone PTM often functions in a dynamic and cooperative network with other PTMs, examining a PTM in native chromatin that contains diverse histone PTMs helps better understand how the biological properties of native chromatin are regulated by the PTM network.[21] For such a purpose, protein semisynthesis has been extended to histone ubiquitination within native chromatin.[22] However, this technique is restricted to histone tail regions and requires high-level expression of histone mutant proteins, which would potentially disturb the network of histone PTMs in chromatin. Protein synthesis by genetic code expansion has allowed site-specific incorporation of histone acetylation in human cells.[23] However, an amber codon suppression strategy of this technique provokes genome-wide alteration of translation termination sites, preventing simple understanding of the

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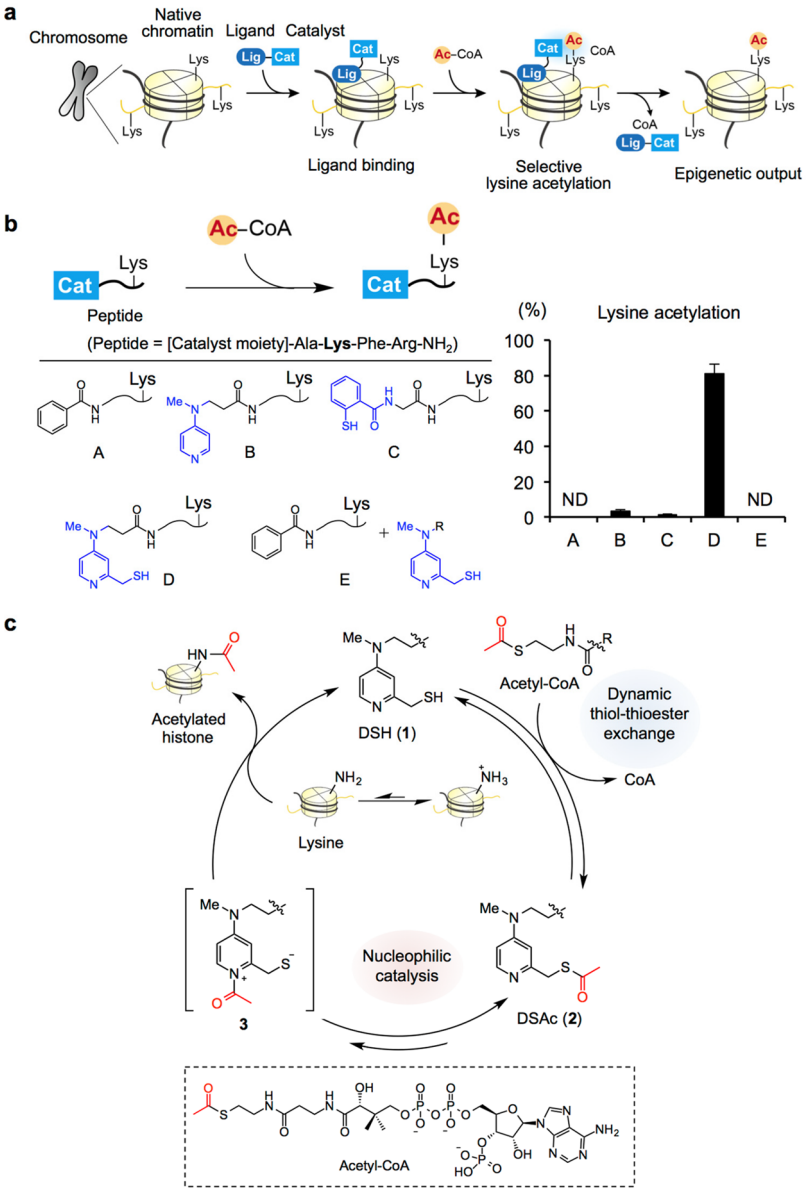

Figure 1. Development of a DMAP-SH (DSH) catalyst for regioselective acetylation. (a) Ligand-directed catalysis to achieve lysine residue-selectivity in native chromatin. A catalyst motif conjugated with a ligand that binds to a specific region in a nucleosome enables selective acetylation of a proximal lysine residue using an acetyl donor, acetyl-CoA, leading to an epigenetic output. (b) Peptide model studies for identifying an efficient lysine acetylation catalyst moiety. Peptides (0.1 mM) containing a catalyst moiety and a substrate lysine were mixed with acetyl-CoA (1 mM) under aqueous buffered conditions (pH 7.5) at room temperature (rt) for 8 h, and acetylation yields were determined by HPLC measurements. (A) No catalyst, (B) DMAP, (C) thiosalicylamide, (D) DSH, and (E) DSH and lysine in a different peptide. Detailed structures of the peptides are shown in Figure S1. ND denotes not detected. Error bars represent standard deviations of three independent experiments. (c) A plausible mechanism for lysine acetylation by DSH (**1**). Ac, acetyl.

effects of the incorporated histone PTMs. This technique also depends on the availability of an orthogonal tRNA synthetase−tRNA pair, which limits the scope of PTMs. Therefore, a versatile method that allows site-selective introduction of a specific histone PTM, such as lysine acylation, in recombinant nucleosomes as well as in native chromatin without genetic manipulations is required for a better understanding of how the PTM functions in the intertwined PTM network in chromatin.

Herein, we report a synthetic catalyst DMAP-SH (DSH), which activates chemically stable thioesters including acetyl-CoA under physiological conditions. Our data suggest that DSH promotes a variety of regioselective histone acylations both in recombinant nucleosomes and in native chromatin, when conjugated with a proper ligand. To investigate the validity of our method, we used DSH to promote histone H2B lysine-120 (K120) acylation, the function of which is largely unknown, and revealed that H2BK120 acetylation and malonylation modulate higher-order chromatin structures by reducing internucleosomal interactions. Thus, this approach may have versatile applications for dissecting regulatory mechanisms underlying chromatin functions.

RESULTS

Development of a Catalyst Moiety Activating Acetyl-CoA. In living cells, histone acetyltransferases (HATs) catalyze acetyl transfer reactions from acetyl-CoA to specific lysine

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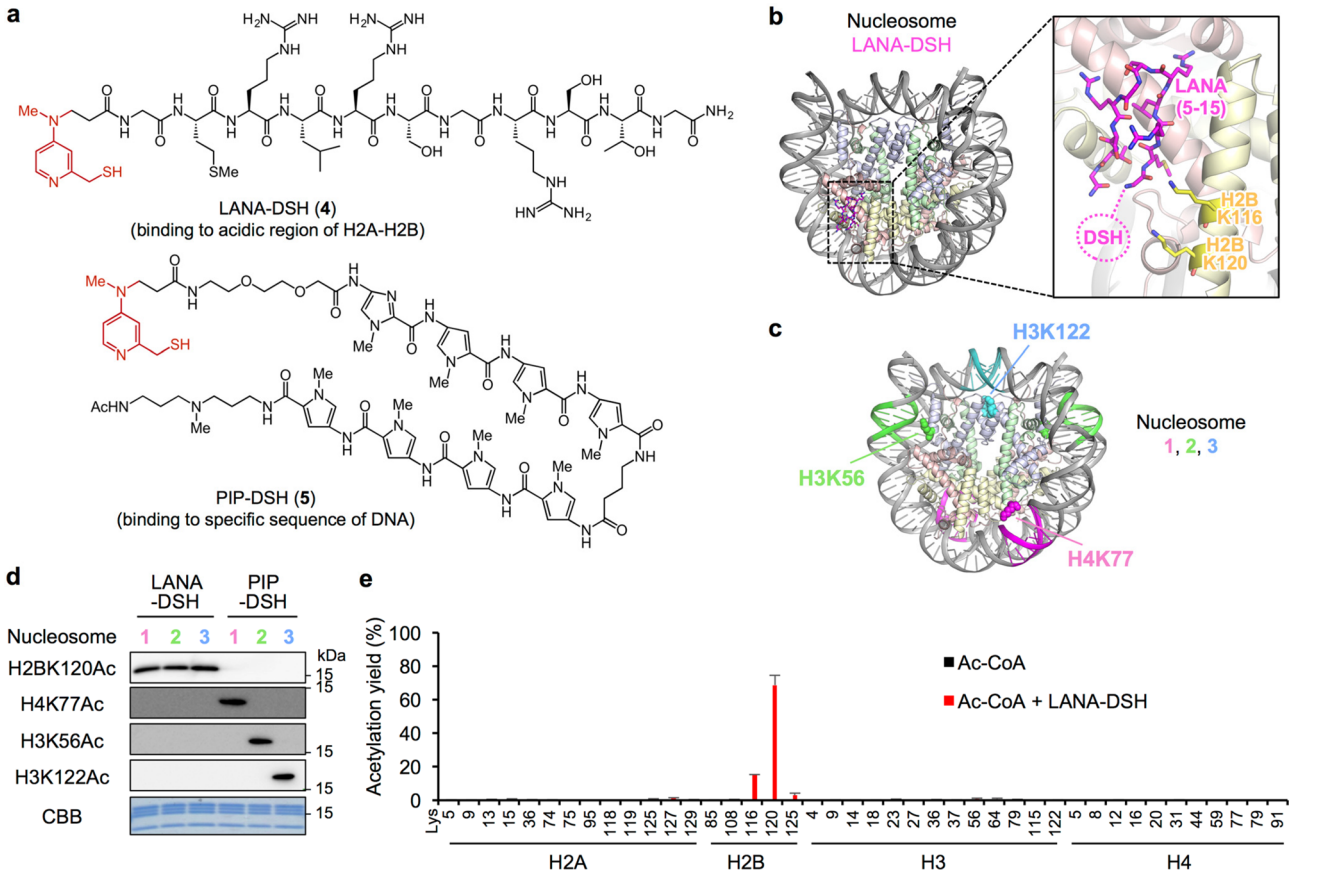

Figure 2. Regioselective histone acetylation in recombinant nucleosomes by ligand-conjugated DSH. (a) Chemical structures of LANA-DSH (**4**) and PIP-DSH (**5**). (b) An X-ray crystal structure of the LANA-DSH−nucleosome complex. Because the electron density of the DSH moiety was not observed, its estimated position is depicted with a dashed circle (PDB: 5GTC). (c) Positional relationships between PIP-DSH-binding DNA sequences and proximal lysine residues in nucleosomes 1, 2, and 3, which are highlighted in the same color, and mapped in a single nucleosome structure (PDB: 3LZ0; magenta, nucleosome 1; green, nucleosome 2; cyan, nucleosome 3). (d) DNA sequence-dependency of histone acetylation by LANA-DSH and PIP-DSH using the nucleosomes 1, 2, and 3. Each nucleosome (33 ng/μL DNA, 0.37 μM) was reacted with LANA-DSH (2 μM) or PIP-DSH (5 μM) in the presence of acetyl-CoA (1 mM) and Tris(2-carboxyethyl)phosphine (TCEP, 0.1 mM) at 37 °C for 8 h, and acetylated lysines were detected by immunoblotting using residue-specific antiacetyl lysine antibodies. Protein amount is indicated by Coomassie Brilliant Blue (CBB) staining. (e) LC−MS/MS analysis of acetylation yield of each lysine residue on a nucleosome (33 ng/μL DNA, 0.37 μM) treated with acetyl-CoA (1 mM) and TCEP (0.1 mM) with and without LANA-DSH (2 μM) at 37 °C for 2 h. Each number on the *x*-axis indicates the position of a lysine residue on histones. The list of chemical yields is shown in Table S2. A representative LC−MS/MS data set is shown in Table S4. Error bars represent standard deviations of three independent experiments. Ac, acetyl.

residues in target proteins.[24] HATs accelerate lysine acetylation by enhancing the reactivity of the amino groups of lysine residues.[24] We conceived that activation of the acetyl donor side, acetyl-CoA, would be more feasible for an artificial small-molecule catalyst. We reasoned that regioselectivity of lysine acetylation could be achieved by employing a ligand-directed approach,[25,26] which allows delivery of a catalyst to a target site (Figure 1a). To develop a catalyst that can activate acetyl-CoA and exclusively acetylate a lysine residue proximal to the catalyst, we undertook a quantitative investigation using a peptide containing both a catalyst moiety and a lysine residue as a model of ligand-directed acetylation of a protein (Figure 1b). Lysine acetylation of peptides lacking any catalyst moiety or containing a 4-(dimethylamino)pyridine (DMAP) moiety, which is a well-known nucleophilic acyl transfer catalyst,[27] barely proceeded (Figure 1b, conditions A and B, and Figure S1a,b).

Therefore, to more efficiently activate acetyl-CoA, we conceived the use of a thiol-thioester exchange[28,29] to receive the acetyl group from acetyl-CoA into the catalyst moiety. A thiosalicylamide moiety, which is reported to transfer an acetyl group from cellular metabolites, presumably acetyl-CoA, to a target protein,[30,31] was tried, but the lysine acetylation did not proceed efficiently (Figure 1b, condition C, and Figure S1c). This would be due to high acidity of the thiol moiety in the thiosalicylamide, which shifts an equilibrium of the thiol-thioester exchange to the side of acetyl-CoA. We, therefore, designed DMAP-SH (DSH, **1**), in which a mercaptomethyl group (less acidic than a thiophenol) was conjugated to the 2-position of DMAP (Figure 1b,c). We postulated facile thiol−thioester exchange between the aliphatic thiol moiety and acetyl-CoA, and subsequent intramolecular acetylpyridinium formation through nucleophilic catalysis. HPLC measurements supported the notion that the thiol−thioester exchange between DSH and acetyl-CoA proceeded and generated DMAP-SAc (DSAc, **2**, Figure 1c) under neutral conditions (Figure S2). Remarkably, acetylation of the lysine residue on the model peptide with DSH as a catalyst moiety proceeded efficiently under physiological conditions (Figure 1b, condition D, and Figure S1d). Importantly, intermolecular acetyl transfer

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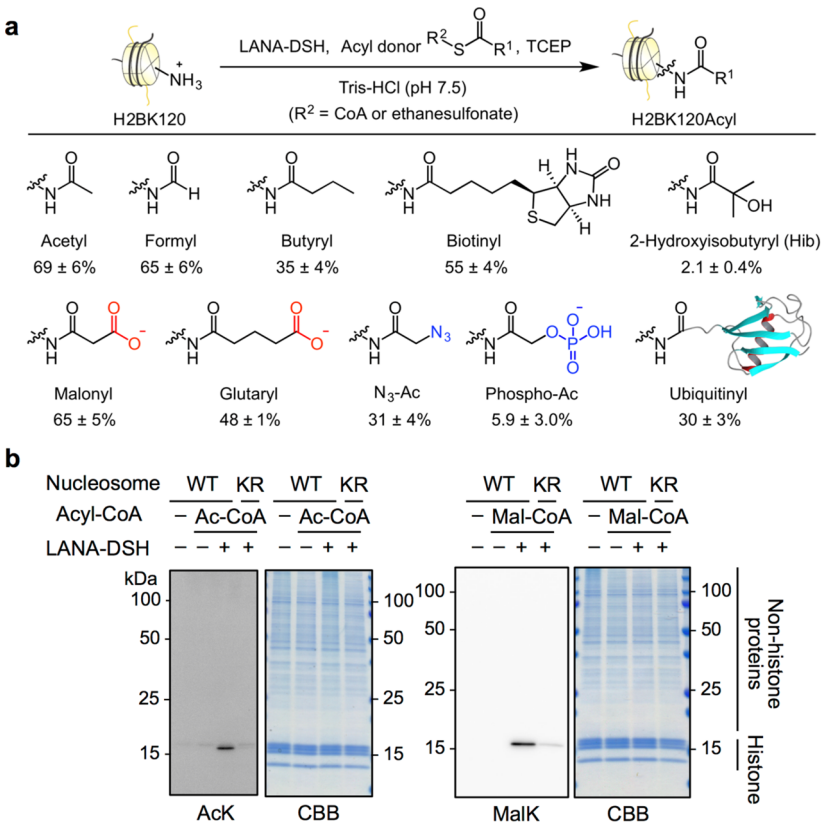

Figure 3. Regioselective introduction of various histone acylations in recombinant nucleosomes by LANA-DSH. (a) Scope and chemical yields of H2BK120 acylations. A recombinant nucleosome (33 ng/μL DNA, 0.37 μM) was incubated with LANA-DSH and an acyl donor in the presence of TCEP (0.1 mM), and the acylation yield at H2BK120 was determined by LC−MS/MS analysis. Detailed reaction conditions are summarized in the Supporting Information. The mean chemical yields are shown with standard deviations of three independent experiments. The acetylation yield shown is the same as that reported in Figure 2e. The structure of ubiquitin is obtained from PDB (1UBQ). (b) Histone- and H2BK120-selective acetylation and malonylation of recombinant nucleosomes by LANA-DSH. A mixture of wild-type (WT) or H2BK120R (KR) mutant nucleosomes (33 ng/μL DNA, 0.37 μM) and HeLa cell extract (to evaluate protein selectivity) was incubated with LANA-DSH and acyl-CoAs in the presence of TCEP (0.1 mM), and acylated lysines were detected by immunoblotting using the indicated antibodies. Protein amount is indicated by CBB staining. Other reaction conditions are the same as in (a). For histone-selectivity in other acylations promoted by LANA-DSH, see Figure S7. N_3-Ac, azidoacetyl; Phospho-Ac, phosphoryloxyacetyl; Ac, acetyl; Mal, malonyl.

from DSAc to a lysine residue in a different peptide did not proceed, suggesting that proximity effects dominated the reactions (Figure 1b, condition E, and Figure S1e). This feature is advantageous for avoiding undesired acetylation of off-target lysines. The DMAP moiety is important for efficient acetyl transfer, as was evident from the results that lysine acetylation of a peptide with a simple cysteine residue or a simple benzyl thiol as a catalyst moiety proceeded less efficiently (Figure S1f−h).

We examined the reaction profiles of lysine acetylation promoted by DSH. The reaction rates increased with temperature, but there was no obvious peptide concentration dependency (Figure S3a,b). One DSH moiety promoted more than two lysine acetylations, indicating that DSH undergoes catalytic turnover (Figure S3c). Sufficient lysine selectivity was observed, while cysteine was acetylated without DSH via direct thiol−thioester exchange from acetyl-CoA (Figure S4). The cysteine acetylation would not be a critical side reaction, however, because most cysteine residues are buried inside proteins by the formation of disulfide bonds, and also the acetylation is reversible as long as there are no proximal nucleophiles. A hypothetical reaction mechanism (Figure 1c) of DSH-mediated lysine acetylation is (1) dynamic thiol−thioester exchange between acetyl-CoA and the mercaptomethyl group, generating DSAc (**2**); (2) enhanced acetylation activity by the generation of acetylpyridinium intermediate **3** through acetyl group migration; and (3) transfer of the activated acetyl group to the amino group of a lysine, producing *N*-acetyl lysine. Density functional theory (DFT) calculations suggested that conjugation of the mercaptomethyl group to DMAP markedly decreases the activation energy to form acetylpyridinium ion from methyl thioacetate (the highest activation energies of 33.5 kcal/mol vs 19.0 kcal/mol for direct and stepwise activation by DMAP and DSH, respectively; Figure S5), supporting the postulated reaction mechanism in Figure 1c.

Regioselective Histone Acetylation in Recombinant Nucleosomes by DSH. We then synthesized DSH derivatives containing ligands that bind to specific nucleosome regions. Because DSH selectively acetylates proximal lysine residues (Figure 1b), we expected that a limited number of lysine residues located near the ligand-binding site would be selectively acetylated when DSH is conjugated with ligands. Kaposi's sarcoma-associated herpesvirus LANA (latency-associated nuclear antigen) is an 1162-amino acid viral protein interacting with an acidic patch of H2A-H2B via its *N*-terminus.[32] Thus, we synthesized LANA-DSH (**4**, Figure 2a) by conjugating DSH with the *N*-terminus of LANA (residues 5−

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15), the minimum chromosome-binding region of LANA.[32] X-ray crystallography analysis confirmed that LANA-DSH binds to the acidic patch (Figure 2b and Table S1).[32] Although the electron density of the DSH moiety was not determined, the structural information suggests that the DSH moiety of LANA-DSH is proximally located to H2BK120 on the disk surface of the nucleosome (Figure 2b). We used pyrrole-imidazole polyamide (PIP)-DSH (**5**) as an alternative catalyst containing a PIP ligand moiety that binds to DNA with a 5′-WGWWWW-3′ (where W is A or T) sequence[33] (Figure 2a). To evaluate the DNA sequence-dependency of lysine residue selectivity, we prepared three kinds of recombinant nucleosomes in which the recognition DNA sequence by PIP-DSH was proximal to three different lysine residues on the lateral surface of the nucleosome (H4K77, H3K56, and H3K122 in nucleosomes 1, 2, and 3, respectively, Figure 2c; for DNA sequences, see the Supporting Information).[34]

Western blot analysis using residue-specific antiacetyl lysine antibodies indicated that acetyl-CoA with LANA-DSH (2 μM) acetylated H2BK120 regardless of the DNA sequence in the nucleosomes (0.37 μM, Figure 2d). On the other hand, acetyl-CoA with PIP-DSH (5 μM) acetylated lysine residues proximal to its recognition DNA sequence; H4K77, H3K56, and H3K122 of nucleosomes 1, 2, and 3 (0.37 μM) were selectively acetylated, respectively (Figure 2d). Liquid chromatography tandem mass spectroscopy (LC−MS/MS) analysis revealed that acetyl-CoA with LANA-DSH acetylated H2BK120 in high yield and selectivity (69 ± 6%: Figure 2e, red bars), while no significant acetylation of any lysine residue proceeded in the absence of LANA-DSH (Figure 2e, black bars; see Table S2 for each value). Acetylation at H2BK116 was detected to a minor extent (15.1 ± 0.3% yield) under acetyl-CoA + LANA-DSH conditions due to its proximity to H2BK120 (Figure 2b). LC−MS/MS analyses of the nucleosomes treated with acetyl-CoA + PIP-DSH indicated that PIP-DSH promoted the target acetylation with a moderate regioselectivity (Figure S6, Table S3). This moderate regioselectivity could be due to the nonoptimized PIP-sequence and linker length. These findings indicate that DSH promotes acetylation of lysine residues at predictable sites in nucleosomes by conjugating with suitable ligands.

Expansion of Histone Acylation Types. Other acylation types, such as malonylation, are observed in cellular chromatins.[9] DSH activated acyl-CoAs containing a variety of acyl groups and promoted the corresponding acylations in a peptide-model substrate (Figure S3d). Moreover, treatment of a recombinant nucleosome with LANA-DSH and acyl-CoAs led to acylations (e.g., formylation, butyrylation, biotinylation, 2-hydroxyisobutyrylation, malonylation, and glutarylation) at H2BK120 (Figure 3a). Those acylations were significantly suppressed in the H2BK120R mutant nucleosome, in which H2BK120 was replaced with arginine (R), suggesting that the acylations were H2BK120-selective (Figure 3b and Figure S7a). In addition, acylations of nonhistone proteins hardly proceeded when using a mixture of a recombinant nucleosome and HeLa cell extract as substrates, indicating high histone-selectivity (Figure 3b and Figure S7a). LANA-DSH can also transfer non-natural acyl groups (azidoacetyl and phosphoryloxyacetyl) from the corresponding acyl-CoAs to the H2BK120 residue (Figure 3a). Further, using ubiquitin-MES (mercaptoethanesulfonate) thioester as an acyl donor, LANA-DSH can efficiently promote monoubiquitination at H2BK120 in a nucleosome, which is genuinely the same structure as that observed in cells (Figure 3a and Figure S7c,d). Our results indicate that ligand-conjugated DSH can introduce various types of biologically relevant acylations at predictable lysine residues, illustrating its utility for regioselective acylations of histones.

Biochemical Properties of H2BK120-Acylated Nucleosomes. H2BK120 acetylation and other acylations, such as ubiquitination and malonylation, are observed in higher eukaryotes.[9] H2BK120 ubiquitination is known to promote gene transcription through structural changes of chromatin[5,35] and/or downstream histone lysine methylations.[7,13] While acetylation of H2BK120 is enriched in core promoter regions of actively transcribed genes,[36,37] it remains to be elucidated how this acetylation modulates the properties of chromatin. The functional roles of H2BK120 malonylation[38] are not known. As LANA binds not only to recombinant nucleosomes but also endogenous chromatin[32] containing polynucleosomes with various native PTMs, we envisioned that acylation by LANA-DSH could be used as a versatile approach to study the function of H2BK120 acylations.

We first produced H2BK120-acetylated (69 ± 6%) and -malonylated (65 ± 5%) nucleosomes by treating recombinant nucleosomes with LANA-DSH and the corresponding acyl-CoAs. A nucleosome thermal stability assay[39] indicated that neither acetylation nor malonylation of H2BK120 affected nucleosome stability (Figure 4a). On the other hand, Mg^{2+}-promoted nucleosome self-association[40] was significantly inhibited by LANA-DSH-mediated acetylation or malonylation of H2BK120 (Figure 4b). The effect of malonylation was greater than that of acetylation (Figure 4b). The inhibitory effects on nucleosome self-association were significantly suppressed by H2BK120R mutation (Figure S8), indicating that H2BK120 acylation was responsible for these effects. These results suggest that the H2BK120 acylation attenuates internucleosomal interactions without affecting the stability of individual nucleosomes.

Regioselective Histone Acylation in Native Chromatin. Next, to evaluate the effects of H2BK120 acetylation and malonylation on native chromatin, we treated purified nuclei with LANA-DSH and the corresponding acyl-CoAs (Figure 5a). We initially found that addition of only acetyl-CoA promoted histone tail acetylation (Figure 5b and Figure S9a), probably mediated by endogenous HATs in the purified nuclei. Several types of HAT recognize acetyl-CoA through the CoA moiety,[24] and therefore we hypothesized that endogenous HAT-mediated acetylation could be suppressed when thioesters lacking the CoA moiety were used as an acetyl donor. As expected, p300 HAT hardly promoted histone acetylation of recombinant nucleosomes using TEG-Ac (**6**, Figure 5c and Figure S9b). In the purified nuclei, the increase of histone tail acetylation by TEG-Ac was also barely detectable (Figure 5b and Figure S9a). LANA-DSH, however, activated TEG-Ac, promoting selective acetylation of H2BK120 in the presence of other nucleoplasmic proteins (Figure 5b, acetic anhydride was used as a nonselective acetylation control).

We then treated the nuclei with micrococcal nuclease (MNase) to isolate nucleosomal arrays, the average of whose length was around 3-kb (Figure 5d,e). LC−MS/MS analysis indicated that treatment with both LANA-DSH and TEG-Ac significantly increased H2BK120 acetylation, but did not significantly increase histone tail acetylation (Figure 5f, see Figure S9c for other residues). Acetylation by LANA-DSH reduced Mg^{2+}-dependent sedimentation of the nucleosomal arrays (Figure 5g). Taken together with the results using

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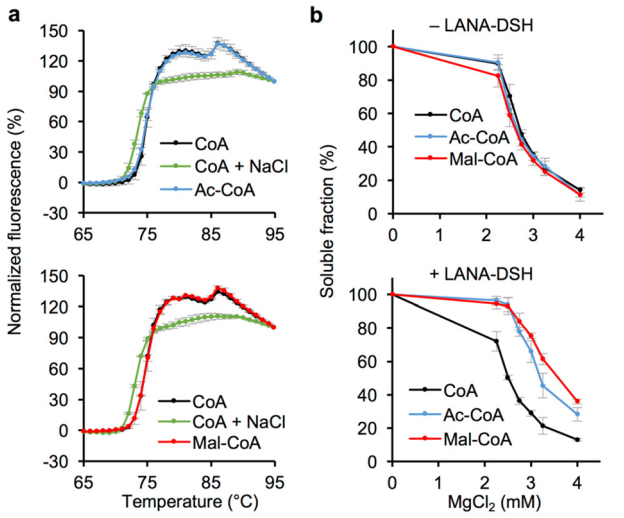

Figure 4. Biochemical properties of H2BK120-acylated nucleosomes. (a) Thermal stability assay of H2BK120-acylated nucleosomes. Relative fluorescence intensity was plotted for every 0.53 °C (from 65 to 95 °C). The concentration of nucleosomes in the assay is 300 ng/μL DNA (3.4 μM). The result using acetylated nucleosome (H2BK120Ac: 69 ± 6%) is shown in the upper panel, and that using malonylated (H2BK120Mal: 65 ± 5%) is shown in the lower panel. NaCl (100 mM) was used as a positive control, and products of reaction using CoA, instead of acyl-CoAs, were used as a negative control. Error bars represent the range of two independent experiments. (b) Mg^{2+}-promoted sedimentation assay of H2BK120-acylated nucleosomes. The concentration of nucleosomes in the assay is 120 ng/μL DNA (1.3 μM). The amount of nucleosomes in the supernatant after incubation with $MgCl_2$ was analyzed. The results using nucleosomes incubated without LANA-DSH (H2BK120 acylations were not detected) are shown in the upper panel, and those using nucleosomes acylated by LANA-DSH (H2BK120Ac, 69 ± 6%; H2BK120Mal, 65 ± 5%) are shown in the lower panel. For control experiments using H2BK120R mutant nucleosomes, see Figure S8. Error bars represent standard deviations of three independent experiments. Ac, acetyl; Mal, malonyl.

recombinant nucleosomes (Figure 4), it suggests that H2BK120 acetylation can attenuate self-association of the nucleosomal arrays.

Acetylation of H4K16 has been reported to modulate higher-order chromatin structures, probably by disrupting specific binding of the H4 tail to the pocket within the H2A–H2B acidic patch.[41,42] Consistent with this, treatment of HeLa cells with sodium butyrate (a histone deacetylase inhibitor) promoted H4K16 acetylation and reduced Mg^{2+}-dependent sedimentation of the nucleosomal arrays (Figure 5h–j, see CoA + LANA-DSH condition) as observed previously.[43] Intriguingly, we found that acetylation by TEG-Ac and LANA-DSH further reduced the sedimentation of the nucleosomal arrays isolated from sodium butyrate-treated cells (Figure 5h–j and Figure S9d), suggesting that the effect of H2BK120- and histone tail-acetylation on the self-association of the nucleosomal arrays could be cooperative.

When the purified nuclei were treated with malonyl-CoA and LANA-DSH, a protein corresponding to histone H2B was efficiently and selectively malonylated, while treatment with only malonyl-CoA exhibited no detectable malonylation (Figure S10a,b; compound 7 was used as a nonselective malonylation control). LC–MS/MS analysis suggested that malonylation by LANA-DSH was H2BK120-selective (Figure S10c). Similarly to acetylation, malonylation by LANA-DSH also reduced the sedimentation of the nucleosomal arrays isolated from sodium butyrate-untreated and -treated cells (Figure S10c–e), suggesting that H2BK120 malonylation can also attenuate self-association of the nucleosomal array. Taken together, these results suggest that H2BK120 acylations modulate higher-order structures of native chromatin, and that the effect is likely cooperative with those of histone tail acetylation.

DISCUSSION

In this study, we developed a novel synthetic acylation system involving a DSH catalyst and an acyl-donor, as a useful method to evaluate functions of divergent lysine acylations. This is the first synthetic system to promote regioselective histone acylations of native chromatin without genetic manipulations. While both HATs and DSH use an acyl-CoA as an acyl source, the mechanism of acyl-transfer and the types of acylations that can be introduced differ. Mechanistic studies of some HATs indicated that ammonium groups of lysine residues are activated via deprotonation with a glutamate residue in the reaction cavity of HATs, which triggers nucleophilic attack of the lysine amino group to acyl-CoA.[24] In contrast, DSH activates the acyl-CoA side through a dynamic thiol–thioester exchange, followed by presumable nucleophilic catalysis (Figure 1c). It is noteworthy that HATs catalyzing anionic acylations have not yet been reported, suggesting that each HAT promotes a narrow scope of acylations. In contrast, DSH is versatile, and can promote a wider range of acylations, including anionic acylations and even non-natural acylations. Importantly, direct and regioselective introduction of the anionic acylations on lysines in proteins, such as histones, has not yet been reported.[44] The modularity of our catalyst is advantageous. The lysine residue-selectivity as well as protein selectivity of our catalysts can be easily modulated in a predictable manner simply by changing the ligand conjugated to the DSH moiety (Figure 2 and Figure S11). Therefore, synthetic acylation approaches using DSH catalysts targeting nucleosomes through regions other than the acidic patch or DNA, such as histone tails,[45] or nonhistone proteins would also be versatile tools for elucidating the roles of biologically relevant acylations of histone as well as nonhistone proteins, when such a ligand is available.

We demonstrated, for the first time, the effects of histone lysine malonylation at a specific site in chromatin structures, and provided a functional comparison between acetylation and malonylation. Our data suggest that acetylation of H2BK120, in concert with histone tail acetylation, attenuates the self-association of nucleosomal arrays, consistent with the fact that H2BK120 acetylation coexists with a series of histone tail acetylations at active gene promoter regions.[37] Interestingly, malonylation of H2BK120 induced slightly stronger suppression of internucleosomal interactions than acetylation, suggesting that H2BK120 malonylation might be also associated with active gene transcription. The distribution of H2BK120 malonylation in chromatin needs to be determined for further characterization. Because H2BK120 is located near the acidic patch, changing the size of the lysine residue and/or its positive charge to neutral or negative may inhibit interactions between a neighboring H4 tail and the acidic patch, which is important for internucleosomal interactions.[46] Although LANA-DSH promoted acetylation of H2BK116 to a slight extent and the biochemical characterization is based on a mixture of

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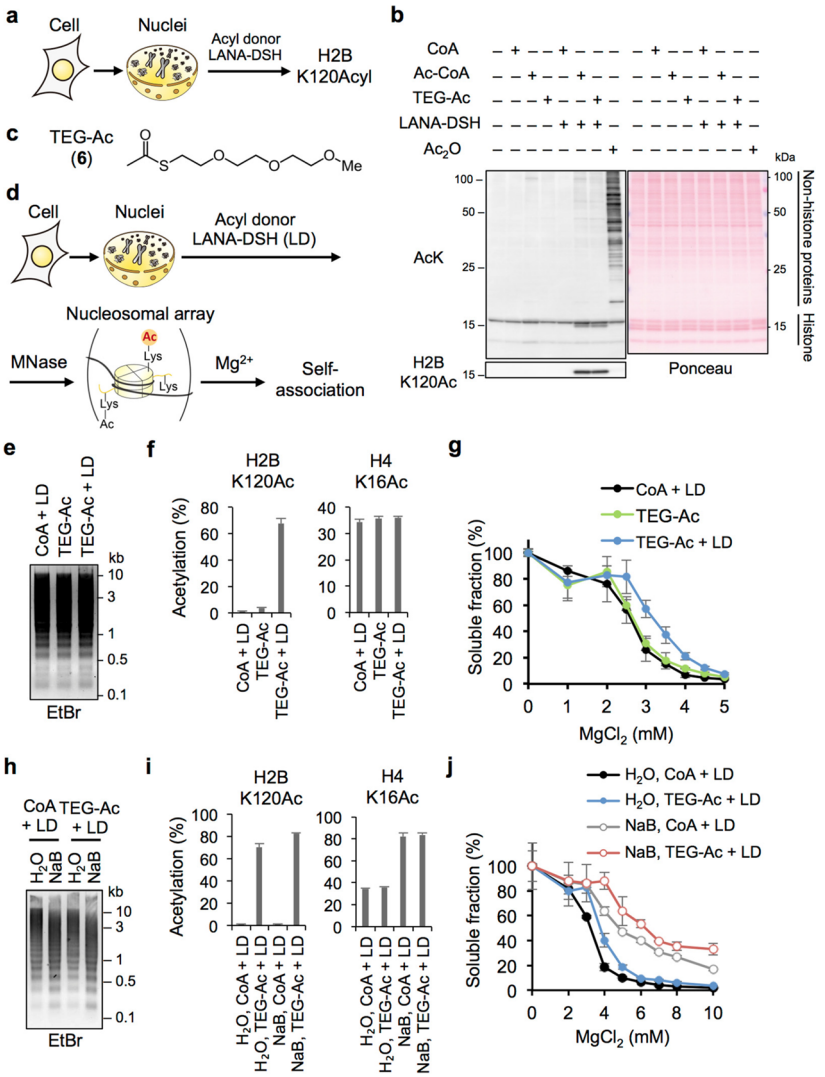

Figure 5. Regioselective histone acetylation in intact nuclei by LANA-DSH and biochemical properties of the acetylated native chromatin. (a) Schematic for histone acylation in intact nuclei by LANA-DSH. (b) Histone-selective acetylation of intact nuclei by LANA-DSH and acetyl donors. Purified nuclei were incubated with the indicated acetyl donor (2 mM) with and without LANA-DSH (0.1 mM) in the presence of TCEP (0.7 mM) at 37 °C for 4 h, and acetylated lysines were detected by immunoblotting using indicated antibodies. Protein amount is indicated by Ponceau staining. Acetic anhydride (0.5 mM) was used as a nonselective acetylation control. (c) The chemical structure of TEG-Ac (**6**). (d) Schematic for Mg^{2+}-promoted sedimentation assay of native chromatin acetylated by LANA-DSH. (e–j) Mg^{2+}-promoted sedimentation assay of native chromatin acetylated by LANA-DSH with (h–j) or without (e–g) pretreatment with sodium butyrate (5 mM) for 24 h before preparation of the nuclei. Purified nuclei were acetylated as in (b). Agarose gel electrophoresis image of DNA purified from the nucleosomal arrays (e,h) and percentage of the acetylated lysine in the sum of the acetylated and unmodified lysines at selected residues of the nucleosomal arrays determined by LC–MS/MS analysis (error bars represent standard deviations of three independent experiments.) (f) and (i) are shown. DNA was stained with ethidium bromide (EtBr). The amount of chromatin in the supernatant after incubation with $MgCl_2$ was plotted as a function of the concentration of $MgCl_2$ (error bars represent standard deviations of three A_{260} measurements.) (g,j) Ac, acetyl; LD, LANA-DSH; NaB, sodium butyrate.

heterogeneously modified nucleosomes, proper control experiments using easily accessible H2BK120R mutant nucleosomes enabled the functional studies of the target H2BK120 acylations.

In principle, our system for regioselective histone acylation can be expanded to living cells or even organisms. We have already confirmed that lysine acetylation of recombinant nucleosomes by LANA-DSH proceeds even in the presence of the physiological concentration of glutathione, which is a cellular thiol nucleophile and can interfere with the thiol–thioester exchange between DSH and the acetyl donor, albeit with a decreased efficiency (Figure S12). Although acetyl-CoA is not membrane-permeable, *N*,*S*-diacetylcysteamine, which is a permeable,[47] truncated acetyl-CoA surrogate and is activated by DSH (Figure S4), could be used as an exogenous acetyl donor. Because of the high affinity of LANA-peptide to the acidic patch, LANA-DSH may remain bound to the acidic patch after promoting the acyl transfer reaction and affect downstream biological outputs. Future investigations will be directed at improving the properties of the synthetic catalysts, such as cell permeability, intracellular stability, and reversible histone binding, toward developing a new method to dissect epigenetic mechanisms in living systems. This direction of the research is now undergoing in our laboratory. Additionally, such a method may open new therapeutic strategies in which synthetic acylation would be used to restore the correct network of PTM, leading to cures for diseases caused by the dysregulation of histone PTM.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.7b02138.

Synthetic protocols, spectroscopic characterizations, experimental details, Figures S1−12, and Tables S1−S3 (PDF)

Representative LC−MS/MS data (PDF)

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Notes

The authors declare no competing financial interest.

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