CHROMATIN

# A ubiquitin crowbar opens chromatin

Monoubiquitylation of histone H2B is found to disrupt condensation of chemically defined chromatin fibers. A novel fluorescence-based assay is used in concert with analytical ultracentrifugation to uncover the synergistic roles of histone acetylation and ubiquitylation on chromatin dynamics.

Craig L Peterson



Eukaryotic genomes must be condensed into compact chromatin structures to fit within the limited confines of the nucleus. Consequently, these condensed structures limit access to the underlying DNA and are inherently repressive to essential nuclear functions, such as transcription, DNA repair and replication. Given that these processes function efficiently within a chromatin environment, it should come as no surprise that mechanisms exist within cells that ensure that condensed chromatin structures have dynamic properties. In this issue, a study by the Muir laboratory uses a chemical biology approach to demonstrate that the post-translational modification of a chromatin component, histone H2B, with the ~8.5-kDa polypeptide ubiquitin, is sufficient to interfere with the formation of condensed chromatin fibers[1].

At a basic level, chromatin consists of linear arrays of nucleosomes. Each nucleosome building block contains ~147 base pairs of DNA wrapped nearly twice around an octamer of core histones (H2A, H2B, H3, H4). The histones contain 15–38-residue N-terminal and/or C-terminal 'tail' domains that protrude from the nucleosomal surface and play key roles in nucleosome-nucleosome interactions that drive intramolecular condensation and cross-fiber interactions. The histone tails also contain sites for a plethora of post-translational modifications that control the structure and biological function of chromatin fibers. One prevalent mark is the monoubiquitylation of a lysine residue within the C-terminal tail of H2B (uH2B). H2B ubiquitylation is associated with transcriptionally active chromatin[1,2], and recent work indicates that uH2B facilitates DNA repair as well[3]. How uH2B contributes to formation of permissive chromatin environments has not been clear, but work presented in the recent study by the Muir group appears to have provided a straightforward answer[1].

One technical challenge that has limited studies on histone ubiquitylation has been the lack of methodologies for generating large quantities of recombinant, site-specifically ubiquitylated protein. Previous analyses of simple histone marks, such as histone acetylation, used expressed protein ligation (EPL) to generate semi-synthetic histones[4]. Ubiquitylation is a more difficult modification, however, as it involves formation of a branched polypeptide (**Fig. 1a**). Previously, Muir and colleagues devised sophisticated EPL techniques for generating uH2B, but the yields were low and the method unlikely to be recapitulated in groups with less chemical biology expertise[5]. More recently, they developed a simplified method for large-scale ubiquitylation of H2B, using a site-specific disulfide linkage[6] ($uH2B_{SS}$; **Fig. 1**). With this method, it is then a rather simple procedure to reconstitute recombinant, homogeneous nucleosomal arrays in which each nucleosome contains two copies of uH2B.

Fierz *et al.*[1] have now applied this approach to determine the effect of H2B ubiquitylation on the folding dynamics of nucleosomal arrays. Biophysical analyses

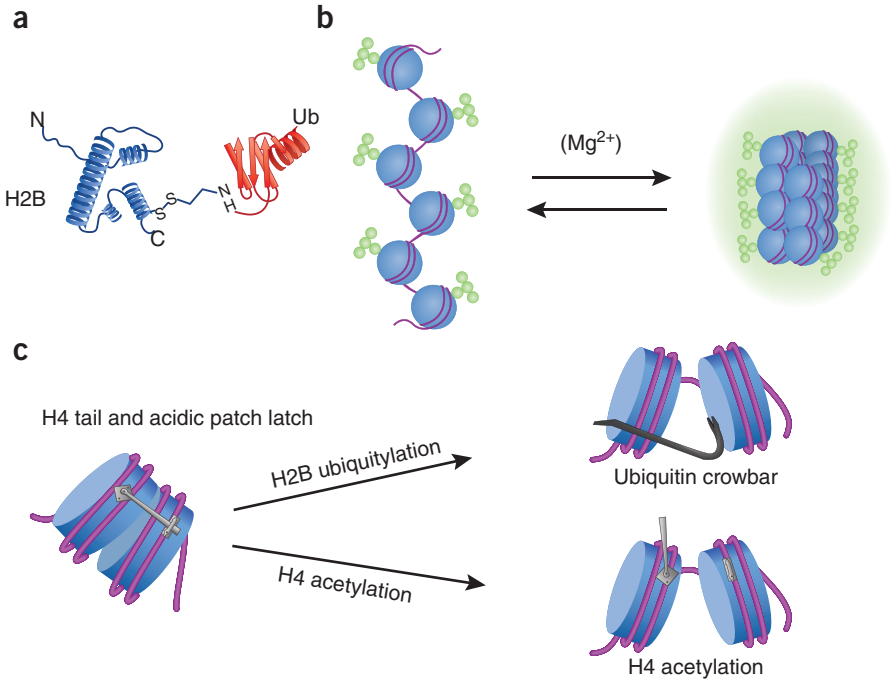


**Figure 1 |** H2B ubiquitylation disrupts nucleosomal array condensation. (**a**) $uH2B_{SS}$ is a branched chain polypeptide. H2B sequences are shown in blue; ubiquitin sequences are shown in red, attached to H2B residue 120 via a disulfide linkage. Note that the C terminus of H2B is at residue 125. (**b**) Homo-FRET analysis of chromatin folding. Green dots denote fluorescein moieties attached to a C-terminal cysteine on the H2A tail. In the context of an extended filament, little homo-FRET occurs. As fibers fold in $MgCl_2$, internucleosomal distances are much shorter, leading to energy transfer between fluorescein groups. (**c**) Disruption of chromatin folding by H4 acetylation or H2B ubiquitylation. The H4 N-terminal tail interacts with an acidic patch on the H2A/H2B dimer surface to form a nucleosome-nucleosome contact that is essential for array condensation. This chromatin 'latch' is disrupted by acetylation of H4 Lys16. The C-terminal tail of H2B lies at the interface of each pair of nucleosome disks within a folded fiber. uH2B may disrupt fiber folding by disrupting nucleosome-nucleosome stacking interactions.

of model nucleosomal arrays commonly employ sedimentation velocity analyses in a modern analytical ultracentrifuge. In such studies, arrays of 11–12 nucleosomes adopt an extended "beads-on-a-string" structure in low-salt buffers and form more compact fibers in the presence of ~1 mM $MgCl_2$. At higher concentrations of $MgCl_2$, arrays participate in cross-fiber interactions that form very large structures. Remarkably, incorporation of uH2B into nucleosomal arrays prevented formation of the condensed structures and inhibited array oligomerization[1]. Indeed, the uH2B-mediated defects in fiber folding are reminiscent of those caused by histone H4 acetylation (H4ac)[7], another mark of 'open' chromatin (**Fig. 1**).

To gain a higher-resolution view of chromatin fiber dynamics, Muir and colleagues adapted a fluorescence homo-resonance energy transfer method (homo-FRET) that monitors internucleosomal distances within arrays[1]. In this method, a fluorescein group is coupled to an engineered cysteine residue within the C-terminal tail of histone H2A. When assembled into a nucleosomal array, the fluorescein chromophores on different nucleosomes undergo homo-FRET as arrays compact in the presence of $MgCl_2$ (**Fig. 1**). When used in conjunction with computational modeling, the homo-FRET data support a model in which fibers condense through heterogeneous intermediates. The homo-FRET method also confirms that uH2B disrupts fiber folding. Analysis of chromatin that contains both uH2B and H4ac indicates that these two marks function through distinct mechanisms, with H4ac having a dominant role. Interestingly, analysis of cross-fiber interactions demonstrated a synergistic and dramatic disruption when nucleosomes contained both uH2B and H4ac.

Of the >50 known histone modifications, only four histone 'marks' have been shown to have an inherent ability to impact the condensation of nucleosomal arrays[1,7–9]. Consequently, their presences at chromosomal loci provide strong predictors of chromatin structure. Previous studies of uH2B led to the prevailing view that it functions by directing the subsequent methylation of histone H3 by Set1 and Dot1 methyltransferases[2,10]. The results from the Muir group require a reassessment of that view. In particular, roles for uH2B in driving chromatin unfolding at target loci must be analyzed in the absence of redundant H4 acetylation. Given that modification of H2B with the ubiquitin-like protein, Hub1, does not yield an equivalent disruption of chromatin condensation[1], future studies are likely to focus on identifying domains and surfaces of ubiquitin that mediate this novel function.

*Craig L. Peterson is in the Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, Massachusetts, USA.*
*e-mail: craig.peterson@umassmed.edu*


## References

1. Fierz, B. *et al. Nat. Chem. Biol.* **7**, 113–119 (2011).
2. Sun, Z.W. & Allis, C.D. *Nature* **418**, 104–108 (2002).
3. Chernikova, S.B., Dorth, J.A., Razorenova, O.V., Game, J.C. & Brown, J.M. *Radiat. Res.* **174**, 558–565 (2010).
4. Chatterjee, C. & Muir, T.W. *J. Biol. Chem.* **285**, 11045–11050 (2010).
5. McGinty, R.K., Kim, J., Chatterjee, C., Roeder, R.G. & Muir, T.W. *Nature* **453**, 812–816 (2008).
6. Chatterjee, C., McGinty, R.K., Fierz, B. & Muir, T.W. *Nat. Chem. Biol.* **6**, 267–269 (2010).
7. Shogren-Knaak, M. *et al. Science* **311**, 844–847 (2006).
8. Lu, X. *et al. Nat. Struct. Mol. Biol.* **15**, 1122–1124 (2008).
9. Watanabe, S. *et al. Biochim. Biophys. Acta* **1799**, 480–486 (2010).
10. Kim, J. *et al. Cell* **137**, 459–471 (2009).



## Competing financial interests

The author declares no competing financial interests.




POST-TRANSLATIONAL MODIFICATIONS

# S-linked sugars lost and found

The vast majority of core structures of protein and peptide glycosylation motifs belong to either O-linked or N-linked glycans. A recent publication describes the structure and biosynthesis of an unusual S-linked glycan linkage in the antibacterial glycopeptide sublancin.

Róbert Šardzík, Peter Both & Sabine L Flitsch

Protein glycosylation motifs are highly conserved in all higher organisms, with the majority belonging to N-glycans (**1**), O-glycans (**2**) or GPI anchors. Though these modifications are well known, the existence of thioglycosides (S-glycans) in natural proteins and peptides has been controversial. The most prominent report of an S-glycan, from Lote and Weiss[1] 40 years ago, described S-glycopeptide structures isolated from human urine and blood. Since then, however, no further natural S-glycopeptides have been found. A BLAST search (http://blast.ncbi.nlm.nih.gov/) of the reported S-glycopeptide sequence CEHSHDGA reveals that the sequence does not appear to be part of the human proteome.

In the meantime, S-glycosides have become popular as hydrolytically stable biosimilars to their equivalent O-glycosides, and there is a large body of literature on their chemical synthesis[2]. As a result, S-glycosides have been tested against many glycosidases and are generally found to be stable analogs with applications in X-ray crystallography and potentially for carbohydrate-based therapeutics and vaccines. Interestingly, however, the stability toward glycosidases is not universal; for example, Macauley and colleagues[3] have found an *O*-GlcNAcase that can cleave both S- and O-glycosides at similar rates. The practical importance of S-glycosides has also prompted studies aimed at their enzymatic synthesis using glycosyltransferases, which have been successful, albeit at much lower catalytic efficiencies[4,5]. Thus, the biosynthetic machinery for the synthesis and degradation of S-glycosides appears to be available, but evidence of such linkages in natural proteins has been elusive.

This gap has now been filled by a paper published in this issue[6], which describes a novel structure for the antimicrobial glycopeptide sublancin containing a glucosyl cysteine linkage (**Fig. 1**) and also identifies the glycosyltransferase responsible for the formation of the S-glycan linkage. Sublancin was isolated over ten years ago from *Bacillus subtilis*[7] and belongs to a structurally diverse class of post-translationally modified peptide natural products that are produced by microorganisms and are a rich source for antimicrobial agents. Sublancin was originally thought to belong to the lantibiotics, a known class of peptides that contain a characteristic thioether cross-linking motif. However, a number of discrepancies made scientists go back and re-examine the structure. The starting point was a search of the *B. subtilis* genome, which