Archives of Biochemistry and Biophysics 591 (2016) 1–6

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

# Mobilization of hyperacetylated mononucleosomes by purified yeast ISW2 in vitro

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ARTICLE INFO

*Article history:*
Received 25 August 2015
Received in revised form
2 December 2015
Accepted 10 December 2015
Available online 12 December 2015

*Keywords:*
Nucleosomes
Histone acetylation
ISWI remodeling
Transcription
Active chromatin

ABSTRACT

Catalytic activity of ISWI chromatin remodelers, which regulate nucleosome positioning on the DNA, depends on interactions of the putative acidic patch in ISWI helicase domain with the N-termini of nucleosomal H4 – such, that removal of H4 termini abolishes ISWI remodeling. Acetylation of H4 termini is also known to disrupt H4 interactions with acidic protein surfaces, and thus, histone acetylation could potentially impede ISWI functions. Since active chromatin in vivo is hyperacetylated, it is important to clarify if ISWI activities can function on the in vivo hyperacetylated nucleosomes. We evaluated if purified yeast ISW2 can act on mononucleosomes in which all four core histones are highly acetylated. Mononucleosomes were assembled using purified histones from mammalian CV1 cells grown in the presence of deacetylase inhibitor Trichostatin A (TSA). The CV1 cell line is characterized by fast kinetic of accumulation of highly acetylated histone isoforms in response to TSA treatment. However, such 'native' histone hyperacetylation had no apparent effects on the nucleosome remodeling propensities, suggesting that histone hyperacetylation does not necessarily block ISWI functions and that ISWI enzymes can function on active chromatin as well.



## 1. Introduction

The basic unit of chromatin, the nucleosome – an octamer of histones H2A, H2B, H3 and H4 wrapped in ~1.7 coils of 147 bp DNA segment [1] – restricts accessibility and dynamics of underlying DNA [2,3]. Disposition of nucleosomes on the DNA can be regulated by the ATP-utilizing chromatin remodeling complexes, divided into ISWI, SWI/SNF, CHD, INO80 and ATRX subfamilies based on the similarity of their ATPase subunits [4,5].

The ISWI (Imitation Switch) enzymes share a conserved catalytic domain of ATP-dependent DNA helicases [6] and generally relocate nucleosome along DNA [7]. The helicase ATPase activity critically depends on the interactions of its conserved acidic amino acids cluster ('acidic patch') with the basic K16-R19 'patch' in H4 termini [4,5,8–10] emerging the nucleosome core at ~20 bp away from the nucleosome dyad axis [11,12]. Removal of H4 N-termini severely inhibits nucleosome mobilization by CHRAC, ACF, dISWI, NURF, NoRC, SNF2H, and ISW2 [9,10,13–16], and, reciprocally, the ATPase activity of Drosophila ISWI could be stimulated by a mixture of DNA and H4 tail polypeptides [9,17].

The H4 basic patch K16–K20 also promotes polynucleosome compaction by interacting with the acidic patch in the H2A/H2B dimer of adjoining nucleosome (reviewed in [18]). Deletion of H4 basic region severely impacts folding and self-association of nucleosome arrays [19]. Acetylation of H4 termini also interferes with polynucleosome folding/self-association by disturbing interactions between H4 basic patch and H2A/H2B acidic patch [18] – such as, acetylation of 30% H4 K16 lysines reduces polynucleosome compaction greater than removal of H4 termini [20]. Thus, by analogy, H4 acetylation could potentially affect ISWI binding and catalytic activity by impeding interactions of H4 basic patch with the acidic patch in ISWI helicase. Indeed, SNF2H binds non-acetylated, but not tetraacetylated H4 tail polypeptides [16]. Acetylation of H4K12, K16 or K5 compromises the ability of synthetic H4 tail polypeptides to stimulate dISWI ATPase on the DNA [9] or to compete for dISWI ATPase activity on nucleosomes [21]. ACF only poorly mobilized nucleosomes containing semi-synthetic H4 with uniformly acetylated H4K16 [22]. On the other hand, in Drosophila

*Abbreviations:* bp, base pairs; BSA, bovine serum albumin; MW, molecular weight; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonyl fluoride; RE, restriction endonuclease; SDS, sodium dodecyl sulfate; TSA, Trichostatin A.

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http://dx.doi.org/10.1016/j.abb.2015.12.002
0003-9861/

embryo cell-free system (- in which chromatin assembly is largely governed by ISWI activities, such as ACF, CHRAC, etc) the in vivo hyperacetylated and unmodified histones (purified from TSA-treated and untreated CV1 cells, respectively) equally efficiently formed nucleosome arrays possessing identical nucleosome spacing and density [23,24]. Of note, removal of histone tails by trypsin severely impacted the regularity of nucleosome arrays assembled in this system [25]. In addition, hyperacetylated nucleosomes – either purified from butyrate-treated HeLa cells or reconstituted with histones from butyrated Drosophila SL2 cells – stimulated ATPase activity of NURF similarly as unmodified nucleosomes [26], whereas trypsin cleavage of histone termini abolished NURF activity (ibid). Thus, it could be supposed that depending on the context histone acetylation could have different effects on ISWI remodeling. In contrast to histone tail deletion, the in vivo acetylation does not necessarily abolish ISWI functions. In general, inhibitory effects of acetylation were observed with synthetic acetylated H4 tail polypeptides to simulate acetylated nucleosomes, or using nucleosomes with semi-synthetic H4 acetylated at K16. However, no noticeable effects of acetylation were observed with natively hyperacetylated histones/nucleosomes, although these experiments utilized poorly fractionated cell-free system or were evaluating only the ISWI ATPase activity (but not the nucleosome repositioning as such).

The in vivo functionally-active chromatin is characterized by multiple histone acetylation marks on all four core histones, but, eventually, it seems to be also associated with ISWI activities. This raises a question of whether 'native' histone acetylation could abolish nucleosome mobilization. We evaluated cumulative effects of high acetylation levels of all four core histones on the nucleosome mobilization by purified yeast ISW2 using defined in vitro system with nucleosomes assembled from natively hyperacetylated histones. For comparison, in the Supplementary Information section we have shown remodeling of hyperacetylated nucleosomes with purified yeast SWI/SNF - another yeast chromatin remodeler, which activity does not depend on histone termini.

## 2. Materials and methods

### 2.1. DNA templates and histones for nucleosome assembly, nucleosome reconstitution

DNA template for nucleosome assembly – the 285 bp DNA fragment containing 'full-size' 601 sequence [27] – is BamH1-BamH1 fragment of the plasmid pGEM3Z601R [28]. Hyperacetylated and unmodified histones were purified from Trichostatin A-treated and untreated CV1 cells, respectively, and assayed on Triton- Acetic Acid -Urea gels as described in [23,24]. HeLa cell histones and nucleosomes were purified as described in [29]. Nucleosomes were reconstituted by stepwise dilution of 2M NaCl mixture of purified histones and DNA and analyzed on native PAGE as described before [29–31].

### 2.2. Purification of ISW2, nucleosome remodeling and nuclease assays

Yeast ISW2 was purified from *Saccharomyces cerevisiae* extracts through 3xFLAG tag as described in [31]. ATPase activity was assayed as described in [31,32]: 5 μL reactions contained 50 ng or 100 ng of each substrate (HeLa core histones or nucleosomes, linear PGEMEX1 DNA or HeLa nucleosomal DNA or buffer only), 1-6 ng ISW2, 0.25–0.35 μCi of [$\gamma$-$P^{32}$] ATP and 100 μM of ATP. Nucleosome remodeling reactions (10 μL) were performed in buffer A (10 mM Tris–HCl (pH 7.4), 1 mM EDTA, 100 mM NaCl, 4 mM $MgCl_2$, 2 mM DTT, 0.025% NP-40, 50 μg/mL BSA, and 0.5 mM PMSF and 1 mM ATP) and contained 100 ng of assembled DNA and about 5 ng of ISW2. After incubations at 25 °C for 1 h, reactions were resolved on native PAGE and stained with ethidium bromide. Restriction endonuclease (RE) assays were performed as described before [31,33]: remodeling reactions (20 μL) were terminated with 10 milliunits of apyrase for 15 min at 25 °C, then samples were digested with 10U of RE for 30 min at 25 °C. DNA was purified, resolved in 7% PAGE and stained with ethidium bromide.

## 3. Results and discussion

To evaluate whether high levels of 'native' histone hyperacetylation can abolish nucleosome mobilization, we used mononucleosomes reconstituted from in vivo hyperacetylated histones and a full-size 285 bp '601' DNA [30], which contains a core 147 bp nucleosome positioning sequence flanked by 65 and 73 bp DNA spacers [27]. The extended extranucleosomal spacers promote nucleosome repositioning rather than other nucleosome alterations. Hyperacetylated histones were purified from CV1 cells grown in the presence of deacetylase inhibitor Trichostatin A (TSA) [34]. Green monkey kidney CV1 cell line exhibit extremely fast accumulation of highly-acetylated isoforms of core histone [23,24,30]. Histone acetylation was analyzed in Triton-Acid-Urea gels, which resolves histones according to their net charge [35]. The bulk of histones H4 from TSA-treated cells was tetraacetylated, whereas most of H4 from untreated cells was unmodified (Fig. 1A). It is of note, that in Drosophila embryo extract the hyperacetylated CV1 histones assembled RNA Pol II transcriptionally-competent chromatin with increased nuclease sensitivity and high conformational flexibility of nucleosomal DNA, which are characteristics of active chromatin in vivo [23,24].

Nucleosomes were reconstituted by dilution of histones-DNA mixture from 2M NaCl [29,30]. Assembly products were analyzed in native PAGE (Fig. 1B). Hyperacetylated nucleosomes formed a defined band (indicative of positioned nucleosomes), which was slightly more diffused and slower-migrating than unmodified nucleosomes. These not very apparent differences could reflect

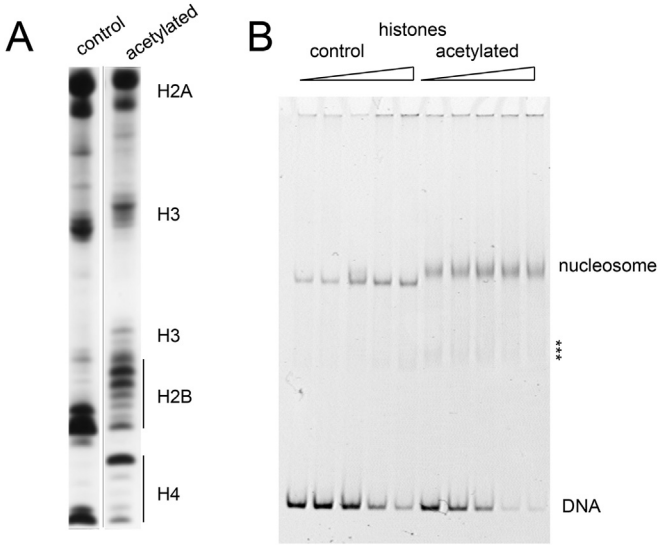


**Fig. 1.** (A) Hyperacetylated and unmodified ('control') histones purified from TSA-treated and untreated CV1 cells, respectively, resolved in 12% Triton/Acetic acid/Urea PAGE. (B) Nucleosomes assembled from unmodified and acetylated CV1, resolved by 5% native PAGE and stained with ethidium bromide. The asterisks indicate positions of the minor subpopulation of nucleosomes assemble out of center of the minimal 601 DNA sequence.