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Global profiling of histone modifications in the polyomavirus BK virion minichromosome

Chiung-Yao Fang [a], Cheng-Huang Shen [b], Meilin Wang [c], Pei-Lain Chen [d], Michael W.Y. Chan [e], Pang-Hung Hsu [f,*], Deching Chang [e,**]

[a] Department of Medical Research, Chia-Yi Christian Hospital, Chia-Yi, Taiwan
[b] Department of Urology, Chia-Yi Christian Hospital, Chia-Yi, Taiwan
[c] Department of Microbiology and Immunology, Chung Shan Medical University, Taichung, Taiwan
[d] Department of Medical Laboratory Science and Biotechnology, Central Taiwan University of Science and Technology, Taichung, Taiwan
[e] Institute of Molecular Biology, National Chung Cheng University, 168 University Rd., Min-Hsiung, Chia-Yi 621, Taiwan
[f] Department of Bioscience and Biotechnology, National Taiwan Ocean University, 2 Pei-Ning Rd., Keelung 202, Taiwan

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ABSTRACT

During polyomavirus infection, the viral DNA adopts histones from host cells and forms minichromosomes as an important part of the viral life cycle. However, the detailed mechanisms of this histone incorporation remain unclear. Here, we profiled the histone posttranslational modifications (PTMs) in BKPyV minichromosomes and in the chromatin of BKPyV host cells. Through Triton-acetic acid-urea (TAU)-PAGE separation followed by nanoflow liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) analysis, we identified different kinds of PTMs on histones from BKPyV minichromosomes and from host cells. We observed not only the common PTMs on histones such as acetylation, methylation, phosphorylation, ubiquitination, and formylation but also several novel PTM sites. Our results also confirmed that the BKPyV minichromosome is hyperacetylated. Our detailed histone PTM profiles for the BKPyV minichromosome provide insights for future exploration of the underlying mechanisms and biological relevance of these histone PTMs.

Introduction

The basic structure of chromatin is the nucleosome, assembled from 146 bp of DNA and an octamer complex of core histone proteins containing two copies of H2A, H2B, H3, and H4 (Kornberg, 1974; Kornberg and Lorch, 1999; Luger et al., 1997). The N-terminus of the histones extends outside the histone octamer and is the major region where posttranslational modifications (PTMs) take place (Cheung et al., 2000a). To date, more than eight types of histone PTMs have been reported, including acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP-ribosylation, deimination, and proline isomerization (Kouzarides, 2007). Histone PTMs at certain loci affect the higher-order structures of chromatin and serve as signals for protein recognition. In addition, histone PTMs play major roles in several important cellular processes, such as recruitment of effector proteins to DNA regions, chromatin compaction, and regulation of gene transcription, as well as regulation of the cell cycle and cell growth, differentiation, and apoptosis (Jenuwein and Allis, 2001; Strahl and Allis, 2000; Wang and Patel, 2011). Therefore, the identification of PTMs on histones is a pivotal aspect in the study of gene regulation.

During infection by viruses, the host cells' machinery is utilized for viral propagation and completion of the viral life cycle. Besides depending on functions that are controlled by the host chromatin, viruses have also developed unique mechanisms to regulate chromatin structure and function as a way to control their life cycles. Previous studies have reported the assembly of viral nucleosomes in several different viruses, such as polyomaviruses (Prieto-Soto et al., 1983), simian virus 40 (SV40) (Griffith, 1975), human and bovine papillomaviruses (HPV and BPV) (Favre et al., 1977; Rosl et al., 1986), adenovirus (Tate and Philipson, 1979), latent Epstein-Barr virus (EBV) (Shaw et al., 1979), herpes simplex virus (HSV)-1 (Deshmane and Fraser, 1989), and hepatitis B virus (HBV) (Bock et al., 1994). Importantly, the histone PTMs on viral nucleosomes are highly correlated with the stage of the viral life cycle. For example, the genome of HSV-1 forms an ordered nucleosome structure when the virus infects a sensory neuron and exists in a latent state (Deshmane and Fraser, 1989) but not when it enters the lytic stage (Leinbach and Summers, 1980; Muggeridge and Fraser, 1986), and the HSV-1 genome has been

* Corresponding author. Tel.: +886 2 2462 2192x5567; fax: +886 2 2462 2320.
** Corresponding author. Tel.: +886 5 2720411x66500; fax: +886 5 2722871.
E-mail addresses: phsu@ntou.edu.tw (P.-H. Hsu), biodcc@ccu.edu.tw (D. Chang).

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shown to exhibit differential levels of the histone activation marks, histone H3 Lys9 and Lys14 acetylations, during the latent and lytic stages of infection (Kent et al., 2004). Similarly, highly acetylated histones H3 and H4 in the minichromosome has been found to represent the active transcription or replication state of HBV (Belloni et al., 2009; Pollicino et al., 2006), and the viral life cycle of HPV has also been correlated with dynamic changes in histone PTMs (Wooldridge and Laimins, 2008). The polyomaviral minichromosome is assembled when host histones are encapsulated into the virion and are wrapped up by the viral genome (Tan, 1977; Waga and Stillman, 1998). Polyomaviruses regulate host cell chromatin and alter viral minichromosome status in the course of completing their life cycle, suggesting similar mechanisms of chromatin regulation for both the viral minichromosomes and their host cells. Although some histone PTMs of polyomavirus have been investigated (Balakrishnan et al., 2010; Balakrishnan and Milavetz, 2005; Milavetz, 2004; Milavetz et al., 2012), the global profiling of histone modifications in polyomavirus remains absent.

The human polyomavirus BK (BKPyV) is found in the kidney and causes polyomavirus-associated nephropathy in renal transplantation patients (Ramos et al., 2002; Ramos and Hirsch, 2006). The presence of virally encoded miRNA in the latent stage of BKPyV infection indicates that the BKPyV life cycle is regulated at the epigenetic level (Broekema and Imperiale, 2013). Mass spectrometry, a detection method of high accuracy and sensitivity, has been intensively used in recent years in the identification of protein PTMs (Britton et al., 2011; Garcia et al., 2006; Gilmore and Washburn, 2007). Our recent evidence that the BKPyV genomic DNA is not being methylated during infection (Chang et al., 2011) raises the possibility that histone PTMs may play pivotal roles in the BKPyV life cycle. In the current study, we profiled the histone PTMs on BKPyV minichromosomes by performing Triton-acetic acid-urea (TAU)-PAGE separation followed by mass spectrometry. The results we obtained can serve as the foundation for future mechanistic studies.

Results

TAU-PAGE analysis of histones in BKPyV virions and host cells

The purification profile of BKPyV virions is shown in Supplementary Fig. 1, including the $OD_{260/280}$ ratio, particle density and hemagglutination assay (HA) (Supplementary Fig. 1A). The purity of BKPyV virions is demonstrated by SDS-PAGE and Coomassie blue staining (Supplementary Fig. 1B). According to our previous results of Western blot (Fang et al., 2010), the additional bands are fragments of capsid proteins. In order to profile the histone PTMs present in BKPyV minichromosomes and in BKPyV's host cells, we employed TAU-PAGE to separate the modified histone species, followed by mass spectrometry analysis to identify the sites and types of the PTMs. Acid-extracted histone samples from BKPyV virions and uninfected host Vero cells were subjected to TAU-PAGE separation. As visualized by Coomassie blue staining, the overall pattern of histone bands was different between BKPyV minichromosomes and host chromatin (Fig. 1A). The banding profiles of individual histone subunits were identified by western blotting with specific antibodies against histones H2A (Fig. 1B), H2B (C), H3 (D), and H4 (E). We found that histone subunits extracted from BKPyV virions showed higher band positions on TAU gels than those extracted from host cells. We also found that H3 and H4 in BKPyV were higher amount than in Vero. To examine the differences in histone patterns between BKPyV virions and host cells, the major bands observed in TAU-PAGE were subjected to in-gel digestion and LC–MS/MS analysis to identify the PTMs they contained.

Identification of PTMs on histone H2A

Histone H2A, a subunit of the core histone octamer, consists of a main globular domain with extended tails on both N- and C-termini. Both histone tails can extend outside of the nucleosomal space and help maintain nucleosome structure (Luger et al., 1997). In this study, one additional band for histone H2A in BKPyV virion extract was identified as compared with Vero cell extract (Fig. 1B). N-terminal acetylation of histone H2A was further demonstrated only in BKPyV virions (Table 1). Most of the PTMs identified on histone H2A were located in the N- and C-terminal tails (Fig. 2). Acetylation marks were identified on three H2A lysine residues, Lys5, Lys9, and Lys36. We further determined that these three acetylations occurred sequentially: Lys36 was the first to be acetylated, followed by Lys5 and Lys9. The acetylation for N-terminal and Lys5 in histone H2A was demonstrated in Supplementary Fig. 2. The double acetylation of Lys5 and Lys9 was found in the hyperacetylated form of H2A in both Vero and BKPyV virions (Supplementary Fig. 3). In addition, we identified one formylation mark on Lys36, but found no correlation between acetylation and formylation at Lys36. Several PTMs were identified on Lys118 of H2A, including mono-methylation, di-methylation, and ubiquitination. A previous study has correlated H2A ubiquitination at Lys119 with gene inactivation (Zhou et al., 2008). The ubiquitination of Lys118 was found in both host and BKPyV H2A (Supplementary Fig. 4); however, the ubiquitination on Lys119 was only found in host H2A but not on BKPyV H2A (Supplementary Fig. 5).

Identification of PTMs on histone H2B

The histone H2B bands observed in TAU-PAGE (labeled 0 through 7 in Fig. 1C) were trypsin-digested and analyzed by LC–MS/MS. We identified the following PTMs on the histone H2B of BKPyV virions (with those PTMs not found on host cell H2B indicated in italics): acetylations at the *N-terminus* and on Lys5, Lys11, Lys12, Lys15, Lys16, Lys20, Lys23, *Lys34*, *Lys46*, *Lys116*, and Lys120; methylations on Lys5 and Arg72; a di-methylation on Lys108; a phosphorylation on *Ser6*; a formylation on *Lys34*; and ubiquitinations on *Lys108* and Lys120 (Table 2). Several PTMs on H2B of BKPyV virions were characterized, such as acetylations at the N-terminus (Supplementary Fig. 6), acetylation on Lys34 (Supplementary Fig. 7), Lys46 (Supplementary Fig. 8), Lys116 (Supplementary Fig. 9). A novel serine phosphorylation site, Ser6, was identified on BKPyV H2B (Supplementary Fig. 10).

Hyperacetylated H2B was previously found in SV40 and has been suggested to help maintain the SV40 minichromosome in an active state (Chestier and Yaniv, 1979). Our current mass spectrometry analysis identified more than 9 acetylation marks on lysine residues in BKPyV H2B. These acetylation marks were mainly located in the N-terminal tail and the core domain of H2B (Table 2 and Fig. 3). After examining the results for the different H2B subspecies, we concluded that the acetylations on H2B took place sequentially. Lys20 was the first to be acetylated, followed by the N-terminus and Lys16. The next acetylations occurred at Lys5, Lys34, and Lys120. In the final stage, Lys12, Lys23, Lys46, and Lys116 were acetylated (Fig. 3, bands H2B-0 through H2B-3). In addition, H2B extracted from BKPyV virions had a higher level of acetylation than that extracted from host cells. For example, 9 lysine residues were found to be acetylated in BKPyV H2B band 3, but only 6 acetylated lysines were characterized in host H2B band 4. The biological significance of H2B hyperacetylation in BKPyV needs to be further studied.

Other PTMs were also detected on histone H2B besides acetylation. One of them was a new serine phosphorylation site, Ser6, on BKPyV H2B (band 3 seen in TAU-PAGE). Although the biological function of this serine phosphorylation is still unclear, our data showed that it may be correlated with Arg72 methylation on