Short Communication

Mysterious Role of H3K56ac in Embryonic Stem Cells

(H3K56ac / p300/CBP / pluripotency / chromatin)

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Abstract. Posttranslational modifications of histones belong to epigenetic mechanisms that regulate gene expression by chromatin structure changes. Generally, histone acetylation reduces its positive charge and consequently weakens the stability of the nucleosome. Acetylation of lysine 56 on histone H3 is implicated in the processes associated with loosened chromatin structure. H3K56ac is a mark for histones with high nucleosome turnover in the nuclear processes such as gene transcription, DNA replication and reparation in yeasts. During evolution, the main H3K56ac regulatory pathway was lost and the level of H3K56ac remained very low in mammalian cells. Moreover, the function of this modification still remains unclear. In this minireview, we summarize the recent knowledge of the ambiguous role of H3K56ac in mammalian embryonic stem cells.

Introduction

Unlike N-end lysines, lysine 56 is positioned at the amino-terminal α-N helix in the globular, central part of histone H3. This area is in contact with a place where DNA enters and exits the nucleosome (Hyland et al., 2005; Ozdemir et al., 2005). Acetylation of histones in the lateral surface of nucleosomes can directly influence histone-DNA interactions and weaken the nucleosome stability even in the absence of potential binding proteins (Fenley et al., 2010; Tropberger and Schneider, 2013). The lysine residue stabilizes the helical structure of α-N helix by its positive charge. After histone acetylation, DNA is more accessible for specific proteins as a consequence of increased conformation entropy in α-N helix (Xu et al., 2005). The nucleosome remodelling activity is first modestly in hands (SWI/SNF or RSC) and then even dramatically enhanced (INO80) after recruitment of chromatin remodellers on the chromatin composed of H3K56ac nucleosomes (Neumann et al., 2009; Watanabe et al., 2013). Artificially prepared chromatin containing H3K56ac histones showed no evidence of affecting the nucleosome stability. On the contrary, H3K56ac noticeably increased nucleosome breathing, the state of nucleosome with partially unwrapping DNA (Neumann et al., 2009). H3K56ac incorporated into nucleosomes facilitates partial DNA unwrapping from the histone octamer and uncovers DNA regulatory binding sites for other proteins (Neumann et al., 2009). In conclusion, H3K56ac relaxes chromatin fibres not by affecting the nucleosome stability, as is the case of acetylation on N-tail lysines, but mostly by changing the chromatin dynamics.

H3K56ac regulation in mammalian cells

H3K56ac is a common and relatively well-described histone modification in yeasts. Histones with such a modification are mainly a mark of newly synthesized nucleosomes (Hyland et al., 2005; Masumoto et al., 2005; Han et al., 2007a; Li et al., 2008). H3K56ac is involved in nuclear processes that need to rebuild impaired nucleosomes during DNA replication and reparation, or replenish histones behind processing RNA polymerases (Recht et al., 2006; Han et al., 2007b; Tsubota et al., 2007; Wurtele et al., 2012). H3K56ac also serves as a marker, favouring sister chromatid recombination before other DNA reparation mechanisms when enabling recognition of newly synthetized chromatid by DNA repairing complexes (Muñoz-Galván et al., 2013). Moreover, the modification is also important for protecting DNA against small insertions/deletions or hyperamplification of ribosomal RNA (Ide et al., 2013; Kadyrova et al., 2013). Participation in many different nuclear processes points to the importance of H3K56ac in the yeasts.

In *Saccharomyces cerevisiae*, the transfer of the acetyl group to H3K56 is catalysed by HAT Rtt109 in complex with the H3-H4 dimer (Fillingham et al., 2008; Kolonko et al., 2010). Because of the lack of Rtt109 ho-

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Abbreviations: ESCs – embryonic stem cells, H3K56ac – histone H3 lysine 56 acetylation, HATs – histone acetyltransferases, HDACs – histone deacetyltransferases, TSS – transcription start site.

mology protein and the modification rarity, first researchers had not predicted the presence of H3K56ac in mammalian cells (Xu et al., 2005; Bazan, 2008; Drogaris et al., 2012).

In the mammalian system (Fig.1A), three HATs were identified as enzymes responsible for transfer of the acetyl group to H3K56ac: p300, CBP and Gcn5. HATs from the p300-CBP co-activator family contain motifs that are structurally close to Rtt109 (Das et al., 2009). Both p300 and CBP can transfer the acetyl group to lysine residues on H3 and H4, even on H3K56 (Das et al., 2009; Vempati et al., 2010; Henry et al., 2013; Li et al., 2014). Through acetylation, p300/CBP orchestrates tissue- or signal-dependent gene expression and consequently regulates different cellular processes such as proliferation, differentiation, pluripotency or apoptosis (Das et al., 2009; Zhong and Jin, 2009; Goodman and Smolik, 2000; He et al., 2011; Xu et al., 2011). p300/CBP also promotes acetylation of K56 on free histones. Similarly to p300, the CBP bromodomain interacts with H3 and catalyses the acetyl group transfer to K56 only if the CBP protein is auto-acetylated (Filippakopoulos et al., 2012; Das et al., 2014). Gcn5 is a member of several co-activator complexes implicated in transcription regulation and acetylation of H3K56 (Brownell et al., 1996; Kuo et al., 1996; Kong et al., 2011). As a part of SAGA and ATAC complexes, Gcn5 is involved in H3K56 acetylation in mammalian cells (Fillingham et al., 2008; Tjeertes et al., 2009; Jin et al., 2011).

Two mammalian sequence homologous proteins, ASF1A and ASF1B, are responsible for incorporation of H3K56ac into the nucleosome (Yuan et al., 2009). ASF1 is a modulator of K56 acetylation specificity in both mammalian and yeasts cells. ASF1 is necessary for H3K56ac interaction with p300/CBP. ASF1 probably directs H3 histone into the HAT domain of the enzyme (Das et al., 2014). ASF1A enhances the level of H3K56ac in a dose-dependent manner not only for free histones, but also for chromatin-bound histones (Groth et al., 2007). Another chaperone, CAF1, is responsible for correct incorporation of H3K56ac histones into the newly folded nucleosome (Das et al., 2009). CAF-1 also communicates with the replication complex through the p150 subdomain and PCNA protein (Shibahara and Stillman, 1999). However, there is no clear evidence that CAF-1 is involved in the incorporation of H3K56ac into chromatin during S phase like in the yeasts.

K56 deacetylation in mammalian cells is catalysed by many different HDACs: HDAC1, HDAC2, and members of the sirtuin family: SIRT1, SIRT2, SIRT3, and SIRT6 (Das et al., 2009; Michishita et al., 2009; Yang et al., 2009; Miller et al., 2010; Vempati et al., 2010).

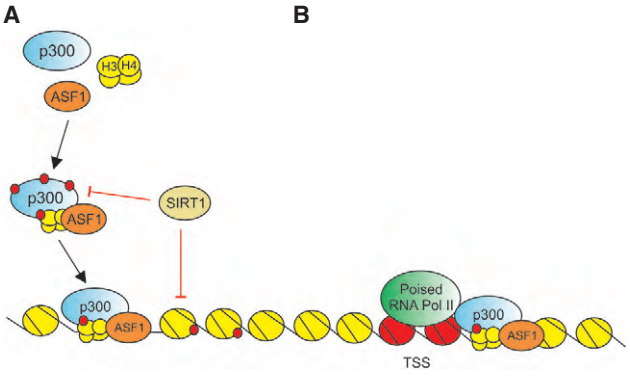

Fig. 1. Possible p300-dependent regulation of H3K56ac in mammalian cells

A) The complex composed of acetylated p300, ASF1 and histone octamer catalyses the transfer of an acetyl group (red dot) to H3K56. Together with another chaperone and CAF1, the H3K56ac is incorporated into a new nucleosome. The p300 activity, as well as that of H3K56ac, is regulated by HDACs (for example SIRT1). **B**) H3K56ac in the regulation of poised RNA polymerase II. The first nucleosome downstream to TSS is targeted by p300. H3 is then acetylated on K56 and the modification promotes interaction with chromatin remodelling complexes followed by nucleosome decomposition.

H3K56ac and pluripotency

The chromatin of pluripotent embryonic stem cells (ESCs) has an *in vivo* potential to activate the development of all cell lineages. In contrast to differentiated cells, the ESC chromatin is homogenous, highly dynamic and structurally distinct (Meshorer and Misteli, 2006; Efroni et al., 2008). Nucleosomes contain a larger amount of acetylated H3 and interact loosely with heterochromatic binding proteins, such as HP1 or histone H1 (Meshorer et al., 2006; Bártová et al., 2008; Krejcí et al., 2009; Bhattacharya et al., 2009). Generally, ESCs contain the largest proportion of transcriptionally active DNA with little restriction for chromatin integration proteins (Meshorer and Misteli, 2006; Gaspar-Maia et al., 2011).

Even in ESCs, the level of H3K56ac remains very low. What is the role of K56ac during the cell pluripotency regulation? Studies of human ESCs confirm an important role for H3K56ac in transcription induction of the most active genes regulated by SOX2, OCT4 and less by NANOG (Xie et al., 2009; Tan et al., 2013). Almost 79 % of highly active genes regulated by at least one of SOX2, NANOG or OCT4 contain nucleosomes with K56ac at promoter sites. Surprisingly, modifications were also created in co-localization with inactive gene promoters, but mostly on promoters with bound poised RNA polymerase II. Poised RNA polymerase II is poised on transcription start sites (TSS) of the genes that are important for the fast entry to differentiation. During differentiation, H3K56ac marks relocate from pluripotency-related gene promoters to promoters of tissue-specific developmental genes (Xie et al., 2009). Moreover, H3K56ac together with H3K18ac and H4K20me1 overlap with replication origins in ESCs, but not in differentiated cells (Li et al., 2014). K56ac-modified H3 histones also probably promote interaction of Oct4 with destabilized nucleosomes. Oct4 strongly interacts with H3K56ac in comparison with other ace-

tylation sites or unmodified H3. Loss of Asf1 in ESCs results in a decreasing level of K56ac, down-regulation of pluripotent markers and induction of differentiation (Tan et al., 2013).

One of the sources of pluripotent stem cells, known as induced pluripotent stem cells (iPS), originates from somatic cells by activation of dedifferentiation processes. H3K56ac involvement in the mechanisms stabilizing the pluripotent state raises the question whether we can use the induction of H3K56ac for enhancing cell dedifferentiation. Modulation of SIRT1 activity showed the opposite effect. H3K56ac deacetylation activity induction by resveratrol increases efficiency of iPS formation, and vice versa, the opposite effect was observed during SIRT inhibition by NADH (Lee et al., 2012).

H3K56ac is important for the maintenance of a pluripotent state. Co-occupancy with highly transcribed genes and Oct4 indicates the role of H3K56ac in increasing RNA Pol II turnover in promoter areas. Moreover, the different composition of the replication origin in ESCs and differentiated cells shows a possible link to the existence of a novel chromatin re-modelling complex regulating the start of DNA replication on H3K56ac destabilized chromatin.

Discussion

In the last five years, research has answered a number of key questions about the presence of H3K56ac in mammalian cells. We already know the H3K56ac's major regulatory pathway, its chromatin binding sites and how K56 affects the nucleosome structure. However, we do not know yet what is the role of H3K56ac during DNA replication and transcription and why the level of H3K56ac is low in mammalian cells. Other questions are raised about the role of H3K56ac in the cell development. Why is this mark present in high levels in pluripotent cells in comparison to differentiated cells? Is Oct4 stabilized on chromatin by interaction with H3K56ac? Is this mark associated with the poised RNA polymerase II on promoters or with the release of the enzyme from the promoter site? Nucleosomes tend to settle into chromatin in a sequence-dependent manner. In mammalian cells, the translational position of histone is managed by approximately 10-bp periodicity of purine (RR) with anti-phased pyrimidine (YY) nucleotides (Kato et al., 2003; Segal et al., 2006; Albert et al., 2007; Chung and Vingron, 2009). Strong periodicity was observed in promoters of tissue-specific genes associated with high CpG islands' presence. The first nucleosome downstream to TSS is targeted by p300. Consequently, the catalysed H3K56ac on the nucleosome induces changes in the rotational setting of the first nucleosome. The rotational change position of the histone octamer towards DNA induces nucleosome disassembly and promotes the elongation phase of transcription (Fig. 1B). This model can explain the rapid activation mechanisms of paused RNA polymerase II (Williams et al., 2008; Hebert and Roest Crollius, 2010).

Research of core histone modifications is relatively new, with promising results in stem cell biology. Even at low levels, H3K56ac shows a specific function in the transcription regulation during pluripotency. However, we do not know yet the role of H3K56ac. Use of novel laboratory methods enabling *in vivo* synthesis of modified proteins could provide the answer to our question.

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