Biochemical and Biophysical Research Communications 397 (2010) 112–116

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Histone acetylation facilitates association of nucleosomes with SET domain of ALL-1 methyltransferase in vitro

Wladyslaw A. Krajewski *, Oleg L. Vassiliev [1]

Institute of Developmental Biology of Russian Academy of Sciences, Moscow 119334, Russia

ARTICLE INFO

Article history:
Received 20 April 2010
Available online 20 May 2010

Keywords:
Nucleosomes
Histone acetylation
SET domain
Transcription
Active chromatin

ABSTRACT

The inheritable methylation pattern of gene activity, created upon cell differentiation, is further maintained by the "SET" (methyltransferase)-domain proteins. However, it is still not clear how SET-proteins can decide on the required gene activity state and the way their chromatin association is maintained. Here we have found that high levels of histone acetylation – the hallmarks of active chromosome regions in vivo – can increase the affinity of reconstituted nucleosomes to the SET domain of ALL-1 histone methyltransferase in a defined system in vitro.

1. Introduction

DNA in nucleus is wrapped around a protein-based structure termed chromatin. The basic unit of chromatin is the nucleosome, formed by a tetramer of histone proteins H3–H4, flanked on either side with a histone H2A–H2B dimer [1]. The flexible N-terminal tails of histones are subjected to various modifications, including methylation, acetylation, ubiquitination, etc. [2]. This inheritable "histone code" [2,3], a major component of which is histone methylation, define whether a chromatin region will be recognized as active or repressed by the transcription machinery. For example, methylation of lysine 4 of histone H3 is associated with gene activation, while methylation of H3 lysines 9 or 27 is associated with silencing.

The stable pattern of chromatin methylation is maintained by the evolutionarily conserved Polycomb (PcG) and trithorax (trxG) group proteins [4]. In general, PcG proteins control repression of genes in cells where these genes must remain inactive during development, while trxG proteins maintain active gene states. The PcG and trxG proteins are present in all cells, but they selectively repress or activate each homeotic gene only in cells descended from cells in which primary transcription machinery had initiated such repression or activation in the early embryo; when the primary regulation system decay, the PcG and trxG proteins assume further control of gene activity. The PcG and trxG proteins typically share a common 130-amino-acid histone methyltransferase domain "SET", through which they methylate chromatin for transcription and silence [3]. However, it remains unclear how SET-proteins can decide on the required gene activity state and the way they target their chromosomal loci.

The SET domains are usually located at the amino- or carboxy-terminus of a protein (except the Ash1, in which SET domain is located in the middle of the protein); apart with highly-conserved SET domain, a less conserved 50–80-amino-acid pre- and post-SET regions may be recognized, respectively, at the amino- and carboxy boundaries of SET – these regions are often included in the definition of "SET domain" (Fig. 1A). It has been shown that SET domains can efficiently bind N-terminal tails of histones H3 and this histone-binding is critically important for the functioning of SET-proteins [5]. We found, however, that in "classical" nucleosome the histone–SET interactions are restricted, implying that nucleosome must be structurally altered for the association with SET domain [6].

From literature, active chromatin regions possess increased overall sensitivity toward DNase I and exhibit more diffused MNase digestion pattern, that are considered to be intrinsic features of the active chromatin structure and are attributed to the differential packaging of DNA into nucleosomes [7,8]. It is notable that various types of histone methylation are hallmarks of not only gene-regulatory and transcription initiation regions but also of the entire transcribed genes [9]. It can be presumed that active chromatin form more "open" configuration in which histone termini

Abbreviations: BSA, bovine serum albumin; DNase I, deoxyribonuclease I; MNase, micrococcal nuclease; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecylsulphate; aa, amino-acids; bp, base pairs.

* Corresponding author. Address: Institute of Developmental Biology, ul. Vavilova 26, Moscow 119334, Russia. Fax: +7 499 135 80 12.
E-mail address: wkrajewski@hotmail.com (W.A. Krajewski).
[1] Permanent address: Shemyakin Institute of Bioorganic Chemistry, ul. Miklukho-Maklaya 16/10, Russian Academy of Sciences, Moscow V-437, Russia.

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.05.080

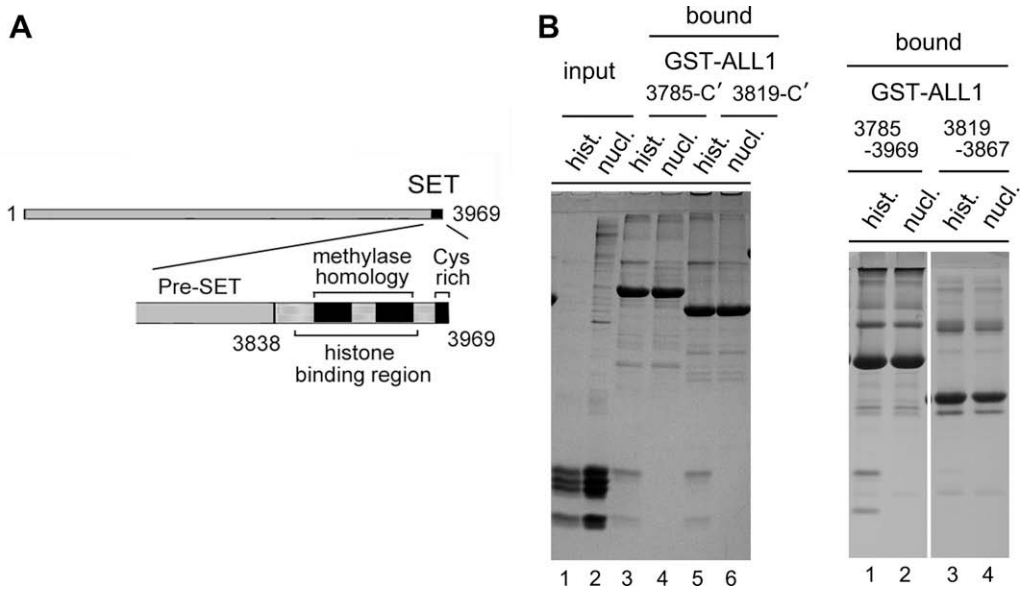

Fig. 1. SET domain of ALL-1 binds histones, but not nucleosomes. (A) Domain structure of ALL-1 C-termini. (B) Immobilized GST–SET polypeptides, spanning intact (lanes 3–6 at left and 1 and 2 at right) or truncated (at right, 3 and 4) histone-binding region, were incubated with isolated HeLa histones or nucleosomes; bound proteins were resolved by 15% SDS–PAGE and stained with Coomassie. Lanes 1 and 2 show protein content of input histones and nucleosomes, respectively.

are more accessible to SET domains. This, in principle, may underlie the mechanisms of SET domain discrimination of active and repressed chromatin.

It had been shown that acetylation of the N-terminal lysines of histones, the other major type of chromatin modifications, is essential for the establishment of a transcriptionally-competent state of chromatin, histone acetylation is also associated with overly more accessible chromatin structure [10,11]. It is conceivable, that the N-acetylation, that quenches positive charges in the histone termini with consequent weakening of electrostatic interaction of histone tails with DNA [12–15], could therefore facilitate opening up of the chromatin and promote targeting of SET-proteins. In this respect it is notable, that chromatin regions containing highly acetylated isoforms of histones H3 and H4 are the preferential targets of histone methyltransferases in vivo (review in [16]). Histone acetyltransferases were identified as components of purified complexes of Drosophila trithorax (dCBP) [17] and its human homolog ALL-1 (TAFII250) [18] and were shown to co-localize with many Trithorax and ALL-1 binding sites in vivo (ibid), suggesting that histone methyltransferases and acetyltransferases act synergistically in the epigenetic maintenance of gene expression states by mechanisms that remain unclear.

Here, using a defined system, we have shown that high levels of histone acetylation can increase affinity of reconstituted nucleosomes to the SET domain of ALL-1 histone methyltransferase.

2. Materials and methods

2.1. Nucleic acid and protein procedures

PCR fragments, encoding the desirable polypeptides, were cloned in-frame into the NdeI–EcoRI sites of the pGEX-2TX-derived plasmid pGEX-2TKN [5]; the encoded residues are indicated on the figures. DNA template for in vitro assembly of nucleosome was 292 bp fragment of the 601 positioning sequence [19], retrieved by BamH1 digestion from plasmid pGEM3Z601R [20]. Recombinant GST-fusion proteins were expressed in *Escherichia coli* Origami B strain (Novagen) and purified through immobilization on glutathion-Sepharose as described in [5]. Purified HeLa histones and nucleosomes were prepared as described in [21]. Hyperacetylated histones were purified from CV1 cells grown in the presence of histone deacetylase inhibitor Trichostatin A as described in [22] and assayed on Triton–Acetic Acid–Urea gels (ibid); normal CV1 histones were purified from TSA-untreated CV1 cells by the same procedure.

2.2. Nucleosome reconstitution and GST–SET pull-down assays

Nucleosomes were reconstituted by stepwise dilution of 2 M NaCl mixture of purified histones and DNA (at starting concentrations of 0.5 μg/μl each) as described in [21], except the dilution buffer contained 50 μg/μl BSA and glycerol was omitted from the final dilution buffer. To verify reconstitutions, nucleosome aliquots (typically 5–7 μl) were mixed with 1/5th volume of 60% sucrose/0.01% xylene cyanole in 1× TE (pH 7.6) and resolved on 5.5% native PAGE (acrylamide to bisacrylamide = 29:1) in 0.5 TBE buffer and stained with ethidium bromide. The GST pull-down assays were performed as described in [5], except the binding reaction was performed for 1–1.5 h at RT and NaCl concentrations in washing buffers were 0.65 and 0.5 M for histone- and nucleosome-binding assays, respectively. On Fig. 3B (right) nucleosomes samples contained purified Isw2 at 1:30 M ratio (control lanes from nucleosome remodeling/GST–SET binding assay). In histone-binding assays the washed GST–SET beads were mixed with 1 vol 2× SDS sample buffer, resolved on 13% SDS–PAGE and stained with Coomassie. In nucleosome-binding assays the washed GST–SET beads were extracted with phenol–chloroform, DNA was ethanol-precipitated, resolved on 5.5% native PAGE and stained with ethidium bromide.

3. Results and discussion

The human ALL-1 protein is a Trithorax class histone H3 lysine 4 methyltransferase [23]; ALL-1 positively regulates homeobox and other genes, which are regulators of development in multicellular organisms. Katsani et al. [5] had shown, that SET domain of Trithorax can tightly bind to the N-termini of histones H3, and that a point mutation in the SET domain that changes glycine 3601 to serine and results in arrested homeotic development and lethality [24], also selectively interferes with histone-binding, suggesting

that histones are a critical target during developmental gene regulation by Trithorax. We found [6], however, that SET domains of ALL-1 and Trithorax can bind only purified histone H3–H4 tetramers but not histones in nucleosomes (Fig. 1B), that is likely due to a limited accessibility of H3 termini in "canonical" nucleosomes [25] and implies that nucleosome structure must be altered for association with SET [6]. Here we attempted to approach whether histone acetyl-lysine modification that weakens histone–DNA contacts, can facilitate nucleosome–SET interaction in a defined system of pure components.

3.1. Hyperacetylated core histone can assemble positioned nucleosomes in vitro

Nucleosomes were assembled using purified histones from CV1 cells and DNA of the full-size (292 bp) "601" high-affinity nucleosome positioning sequence [19]. The full-size 601 DNA consists of a "minimal" 147 bp nucleosome positioning sequence flanked by 65 and 73 bp DNA spacers, which further enhance the ability of minimal 601 DNA to position a histone octamer.

Nucleosomes with elevated levels of histone acetylation were reconstituted using core histones extracted from CV1 (green monkey kidney) cells grown in the presence of histone deacetylase inhibitor Trichostatin A [26], which induces accumulation of highly acetylated histone species. The CV1 cells exhibit unusually high TSA-induced acetylation for both histone H2A/H2B dimers and H3/H4 tetramers. The Fig. 2A shows histone acetylation isoforms resolved on Triton–Acid–Urea (TAU) gel [27]. The well-resolved isoforms of H4 and H2B may be used as a convenient indicator for the degree of acetylation. While "normal" H4 and H2B are predominantly unmodified, the bulk of these histones from TSA-treated cells are tetraacetylated. Prominent differences in isoform composition can also be seen for histones H3 and H2A, although isoform separation there is not as good. It is notable, that in Drosophila embryo extract the hyperacetylated CV1 histones can assemble RNA pol II transcriptionally-competent chromatin, possessing high conformational flexibility of DNA and increased accessibility for transcription factors [28,29].

Nucleosomes were reconstituted by stepwise dilution of 2 M NaCl mixture of histones and DNA to a final concentration of 100 mM NaCl. The hyperacetylated nucleosomes, similar to normal, formed a defined band of 601-positioned nucleosome on native gel (Fig. 2B). However, acetylated nucleosomes migrated slower and formed fuzzier band than normal nucleosomes. It was shown that nucleosome electrophoretic mobility is greater when a histone octamer is positioned close to the end, than to the center of a DNA fragment [30] due to the kink that DNA forms at the entry-exit point of the nucleosome (Fig. 2B, lanes M1 and M2). The slower migration of hyperacetylated nucleosomes is likely a result of altered paths of the DNA external to the 146-bp core DNA due to the partial release of the DNA coils constrained at its entry and exit regions of the nucleosome and/or increase in the effective radius of a nucleosome (see discussion in [13]) that are caused by loosened contacts between acetylated histone termini and DNA [10,12,15,25]. More diffused band of hyperacetylated nucleosome may be explained by higher flexibility of extranucleosomal DNA (ibid) as well as by the heterogeneity in the acetylation levels of histones used to assemble nucleosomes (see Fig. 2A).

Hyperacetylated nucleosomes, comparably to normal ones, also contain a higher proportion of histone octamers assembled off-center of the minimal 601 sequence (Fig. 2B). This is consistent with the observation that acetylation of histone lysines reduces

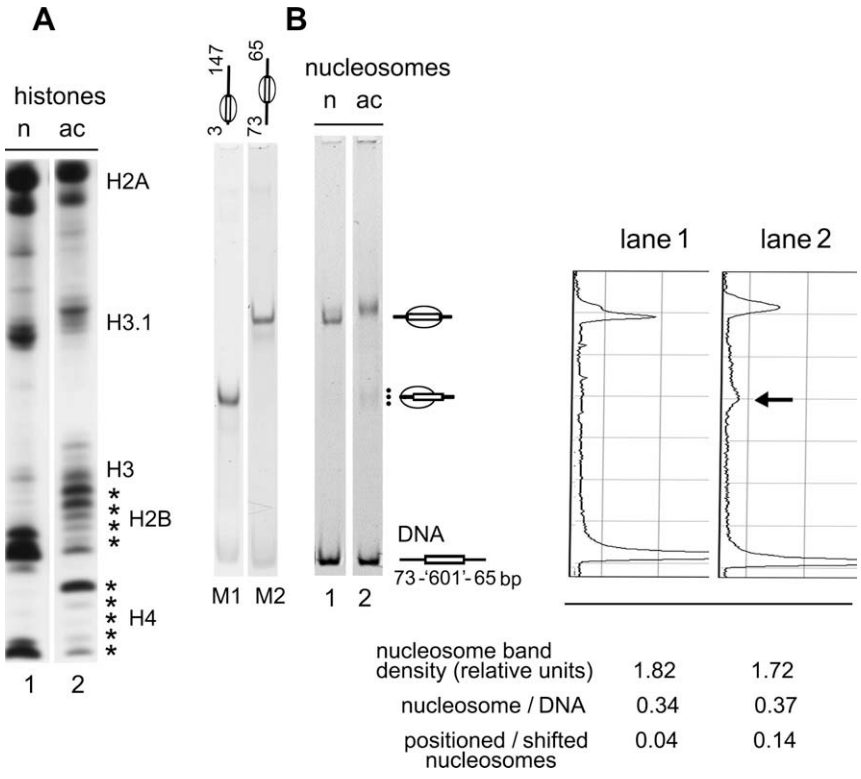

Fig. 2. Hyperacetylated histones from Trichostatin A-treated CV1 cells can assemble positioned nucleosomes on the "601" DNA template in vitro. (A) Normal (n) and (ac) acetylated histones, resolved by 12% Triton/Acetic acid/Urea PAGE. Asterisks indicate acetylated isoforms of histones H4 and H2B. (B) reconstituted nucleosomes resolved by 5% native PAGE. The "–O–" and "O—" indicate histone octamers located, respectively, in the center or at the edge of DNA template, the latter nucleosome population is indicated by an arrow on the gel scan profile at right. Markers of nucleosome mobility M1 and M2 show nucleosomes assembled, respectively, in the center or on the end of the DNA fragment. At bottom – estimates of the nucleosome band gray values, the ratio of nucleosomes to unassembled DNA, and the share of "601-positioned" nucleosomes to nucleosomes shifted towards the side of DNA template.

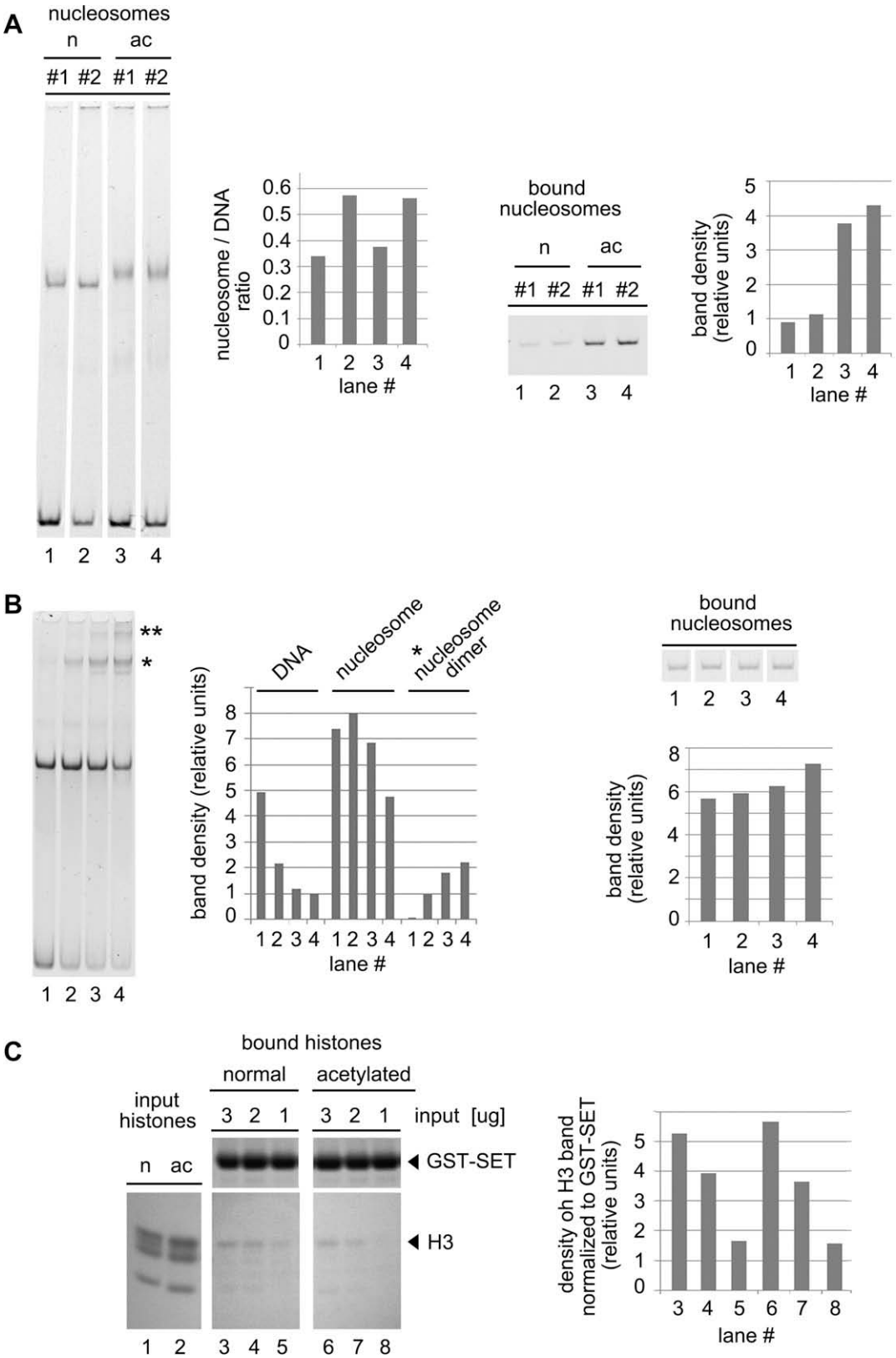

Fig. 3. Hyperacetylated nucleosomes possess an increased affinity to ALL-1 SET domain. (A) Normal (n) and acetylated (ac) nucleosomes samples (#1 and #2) with different degree of assembly (native PAGE is shown at left), at equalized nucleosome concentration were mixed for 1 h with immobilized GST–SET (aa: 3819–3969). Amount of nucleosomes in the reaction was about 6-fold more than loaded on the gel at left. Bound material was deproteinized, resolved on 5% PAGE and stained with ethidium (right panel). (B) Nucleosomes assembled from normal HeLa histones at different histones–DNA ratios (shown at left; the graph depicts amounts of assembly products), were assayed for binding to immobilized GST–SET as above (right panel). (C) Equal amounts of normal and acetylated CV1 histones were incubated with immobilized GST–SET (aa: 3785–3969), bound proteins were resolved by 15% SDS–PAGE and stained with Coomassie.

the energy of octamer binding to 601 sequence [15] as well as some earlier studies showing that increased acetylation of nucleosomes is often correlated with loss of positioning (reviewed in [11]).

3.2. SET domain of ALL-1 methyltransferase possesses increased affinity to hyperacetylated nucleosomes

Interactions of nucleosomes with ALL-1 SET domain were examined in a pull-down assay using immobilized GST-tagged 151 amino-acids portion of ALL-1 C-termini (Fig. 3A). To evaluate the effects from variations in nucleosome reconstitution, we compared binding of GST–SET to nucleosomes with different degrees of assembly (samples #1 and #2). Nucleosomes samples resolved on native gel are shown on the left panel of Fig. 3A, the diagram shows the ratios of assembled nucleosomes to unassembled DNA. Normal and hyperacetylated nucleosomes were incubated with GST–SET beads, the beads were then extensively washed in a buffer containing 0.5 M NaCl/0.2% NP-40. Bound nucleosomes were extracted with phenol–chloroform, deproteinized DNA was resolved on 5% polyacrylamide gel and stained with ethidium bromide. The amount of bound nucleosomes was about four times greater in case of hyperacetylated nucleosomes, than in case of normal nucleosomes (Fig. 3A, at right).

For an additional test for the effects of nucleosome assembly conditions on the nucleosomes pull-down by GST–SET, we reconstituted a series of nucleosomal samples using increasing amounts of normal HeLa histones (Fig. 3B, left panel) that results in formation of visible amounts of nucleosome dimers/aggregates (indicated by asterisk(s); the diagram shows the relative compositions of assembly products). However, these variations in nucleosome assembly did not significantly affect the efficiency of nucleosome–SET binding (Fig. 3B, right panel).

The unmodified and acetylated histones H3 bind to GST–SET with similar efficiency (Fig. 3C), therefore, the elevated affinity of hyperacetylated nucleosomes to the ALL-1 SET domain is likely a result of more exposed histone H3 termini in extensively acetylated nucleosomes [14,31].

Therefore, although the mechanisms of the molecular discrimination and epigenetic inheritance of gene activity states by the trxG and PcG proteins are not fully understood, the accumulated data suggest that histone recognition constitutes an essential step during the in vivo control of gene expression by the SET-containing proteins. The in vivo binding of SET domain proteins to their chromosomal sites must involve the targeting of SET-proteins to nucleosomes through accessible histone tails. Acetylation of chromatin may promote the accessibilization of histone N-termini, and in this way contribute to the SET domain recognition of specific configuration of active chromatin. Although the increase in affinity of acetylated nucleosome to SET domain, observed in vitro, was only incremental, these differences may be essential for the specific targeting of SET domains to acetylated chromosome regions in vivo.

Acknowledgments

The studies were supported by the grants from the Russian Foundation for Basic Research (08-04-01010), the Leukemia Research Foundation (Evanston, IL) and the Association for International Cancer Research (06-466).

References

[1] K. Luger, A.W. Mader, R.K. Richmond, D.F. Sargent, T.J. Richmond, Crystal structure of the nucleosome core particle at 2.8 Å resolution, Nature 389 (1997) 251–260.
[2] T. Kouzarides, Chromatin modifications and their function, Cell 128 (2007) 693–705.
[3] T. Jenuwein, The epigenetic magic of histone lysine methylation, FEBS J. 273 (2006) 3121–3135.
[4] A. Breiling, L. Sessa, V. Orlando, Biology of polycomb and trithorax group proteins, Int. Rev. Cytol. 258 (2007) 83–136.
[5] K.R. Katsani, J.J. Arredondo, A.J. Kal, C.P. Verrijzer, A homeotic mutation in the trithorax SET domain impedes histone binding, Genes Dev. 15 (2001) 2197–2202.
[6] W.A. Krajewski, J.C. Reese, SET domains of histone methyltransferases recognize ISWI-remodeled nucleosomal species, Mol. Cell. Biol. 30 (2010) 552–564.
[7] F. Esposito, R.R. Sinden, DNA supercoiling and eukaryotic gene expression, Oxf. Surv. Eukaryot. Genes 5 (1988) 1–50.
[8] N. Gilbert, B. Ramsahoye, The relationship between chromatin structure and transcriptional activity in mammalian genomes, Brief. Funct. Genomic. Proteomic. 4 (2005) 129–142.
[9] B. Li, M. Carey, J.L. Workman, The role of chromatin during transcription, Cell 128 (2007) 707–719.
[10] J. Ausio, Histone variants – the structure behind the function, Brief. Funct. Genomic. Proteomic. 5 (2006) 228–243.
[11] S.Y. Roth, J.M. Denu, C.D. Allis, Histone acetyltransferases, Annu. Rev. Biochem. 70 (2001) 81–120.
[12] J. Ausio, K.E. Van Holde, Histone hyperacetylation: its effects on nucleosome conformation and stability, Biochemistry 25 (1986) 1421–1428.
[13] V.G. Norton, K.W. Marvin, P. Yau, E.M. Bradbury, Nucleosome linking number change controlled by acetylation of histones H3 and H4, J. Biol. Chem. 265 (1990) 19848–19852.
[14] W.R. Bauer, J.J. Hayes, J.H. White, A.P. Wolffe, Nucleosome structural changes due to acetylation, J. Mol. Biol. 236 (1994) 685–690.
[15] M. Manohar, A.M. Mooney, J.A. North, R.J. Nakkula, J.W. Picking, A. Edon, R. Fishel, M.G. Poirier, J.J. Ottesen, Acetylation of histone H3 at the nucleosome dyad alters DNA–histone binding, J. Biol. Chem. 284 (2009) 23312–23321.
[16] J.C. Rice, C.D. Allis, Histone methylation versus histone acetylation: new insights into epigenetic regulation, Curr. Opin. Cell Biol. 13 (2001) 263–273.
[17] S. Petruk, Y. Sedkov, S. Smith, S. Tillib, V. Kraevski, T. Nakamura, E. Canaani, C.M. Croce, A. Mazo, Trithorax and dCBP acting in a complex to maintain expression of a homeotic gene, Science 294 (2001) 1331–1334.
[18] T. Nakamura, T. Mori, S. Tada, W. Krajewski, T. Rozovskaia, R. Wassell, G. Dubois, A. Mazo, C.M. Croce, E. Canaani, ALL-1 is a histone methyltransferase that assembles a supercomplex of proteins involved in transcriptional regulation, Mol. Cell 10 (2002) 1119–1128.
[19] A. Thastrom, L.M. Bingham, J. Widom, Nucleosomal locations of dominant DNA sequence motifs for histone–DNA interactions and nucleosome positioning, J. Mol. Biol. 338 (2004) 695–709.
[20] M. Carey, B. Li, J.L. Workman, RSC exploits histone acetylation to abrogate the nucleosomal block to RNA polymerase II elongation, Mol. Cell 24 (2006) 481–487.
[21] T. Owen-Hughes, R.T. Utley, D.J. Steger, J.M. West, S. John, J. Cote, K.M. Havas, J.L. Workman, Analysis of nucleosome disruption by ATP-driven chromatin remodeling complexes, Methods Mol. Biol. 119 (1999) 319–331.
[22] W.A. Krajewski, P.B. Becker, Reconstitution and analysis of hyperacetylated chromatin, in: P.B. Becker (Ed.), Chromatin, Humana Press, Totowa, New Jersey, 1999, pp. 195–206.
[23] E. Canaani, T. Nakamura, T. Rozovskaia, S.T. Smith, T. Mori, C.M. Croce, A. Mazo, ALL-1/MLL1, a homologue of Drosophila TRITHORAX, modifies chromatin and is directly involved in infant acute leukaemia, Br. J. Cancer 90 (2004) 756–760.
[24] T.R. Breen, Mutant alleles of the Drosophila trithorax gene produce common and unusual homeotic and other developmental phenotypes, Genetics 152 (1999) 319–344.
[25] K. Luger, T.J. Richmond, The histone tails of the nucleosome, Curr. Opin. Genet. Dev. 8 (1998) 140–146.
[26] M. Yoshida, S. Horinouchi, T. Beppu, Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function, Bioessays 17 (1995) 423–430.
[27] C.R. Alfageme, A. Zweidler, A. Mahowald, L.H. Cohen, Histones of Drosophila embryos. Electrophoretic isolation and structural studies, J. Biol. Chem. 249 (1974) 3729–3736.
[28] W.A. Krajewski, P.B. Becker, Reconstitution of hyperacetylated, DNase I-sensitive chromatin characterised by high conformational flexibility of nucleosomal DNA, Proc. Natl. Acad. Sci. USA 95 (1998) 1540–1545.
[29] K.P. Nightingale, R.E. Wellinger, J.M. Sogo, P.B. Becker, Histone acetylation facilitates RNA polymerase II transcription of the Drosophila hsp26 gene in chromatin, EMBO J. 17 (1998) 2865–2876.
[30] G. Meersseman, S. Pennings, E.M. Bradbury, Mobile nucleosomes – a general behavior, EMBO J. 11 (1992) 2951–2959.
[31] J. Ausio, Histone variants – the structure behind the function, Brief. Funct. Genomic. Proteomic. 5 (2006) 228–243.