Effect of Acetylation of Histone H4 on Communication in Chromatin

E. V. Nizovtseva, N. S. Gerasimova, and V. M. Studitsky

Cancer Epigenetics Team, Fox Chase Cancer Center, Philadelphia, PA 19111 United States

Bioengineering Department, Biological Faculty, Moscow State University, Moscow 119234 Russia

*e-mail: vasily.studitsky@fccc.edu

Received May 22, 2019; revised August 3, 2019; accepted September 2, 2019

Abstract—Long-distance interaction plays an important role in the regulation of eukaryotic genes. Chromatin structure is involved in the process, but the role of histone modifications has not been studied. In the present work, the role of acetylation H4K16 (H4K16-Ac) to enhancer-promoter communication (EPC) was analyzed. This modification is associated with euchromatin and is involved in the decompaction of chromatin fibers. We have shown that the effect of H4K16-Ac on EPC in vitro depends on the level of chromatin assembly. EPC in chromatin, which lacks nucleosomes at random positions on DNA, is inhibited in the presence of H4K16-Ac. At the same time, EPC in chromatin, in which nucleosomes occupy all available positions on DNA, is somewhat stimulated in the presence of H4K16-Ac.

Keywords: chromatin, transcription, regulation, enhancers, histones, modifications, acetylation.

DOI: 10.3103/S0096392519040114

Transcription of the majority of eukaryotic genes is regulated by enhancers, which are the DNA sequences that bind the transcription factors and upregulate transcription from remote promoters [1, 2]. To be activated, genes should be located within the region of a so-called “open chromatin” or euchromatin [3], which is the DNA-histone complex characterized by relaxed structure and certain histone modifications (for example, H4K16-Ac and H3K27-Ac) [4]. It is also known that most enhancers provide in cis activation of their target promoters, and the DNA fragment dividing them forms a loop [4]. These and other studies showed the importance of chromatin structure to support interactions between the enhancer and promoter [2, 5]. Moreover, it was shown that DNA condensation during the chromatin modeling and, thus, the decrease in the average distance between the enhancer and chromatin are not the only reasons why chromatin stimulates interactions between the genomic regions [6].

To understand the exact mechanisms of the influence of individual chromatin components on its plasticity and dynamics, as well as the possibility to sustain effective enhancer-promoter interaction (EPI), we have developed an in vitro transcription system that allowed quantitative analysis of the EPI rate. We obtained a template that contained a promoter and a remote enhancer. A long fragment of DNA between them included 13 “601” synthetic sequences going one after another, characterized by high affinity to histones and providing accurate assembly of nucleosomes and formation of the chromatin fiber [7, 8]. This experimental system allowed us to demonstrate the important role of the terminal regions (“tails”) of the core histones and free DNA regions [9] in sustaining of effective EPI [10].

The present study was aimed to determine the effect of covalent modification of one of the histones (H4K16-Ac) on the EPI in chromatin. It was previously shown that this modification is associated with euchromatin [11] and affects its structure via decondensation of the chromatin fiber [12].

MATERIALS AND METHODS

Proteins. All proteins of the transcription complex were purified as described in [13].

Chromatin template assembly. Unmodified histone octamers, as well as the octamers with histone H4 acetylated by Lys-16, were obtained as described in [14]. Chromatin with histone terminal domains removed was obtained by treatment of chromatin isolated from chicken erythrocytes with trypsin [15].

Nucleosomes were assembled on the linearized pYP05 plasmid at different octamer and template ratios (0.9 : 1 and 1 : 1) using the gradient dialysis against the buffer that contained from 2 M to 10 mM NaCl [16].

Assessment of the quality of chromatin template assembly. Verification of the expected positions of
nucleosomes on the used templates was performed by
the analysis of the enzyme cleavage sensitivity. The
assembled chromatin was incubated with the AluI and
Scal restriction endonucleases (New England Biolabs, United States). DNA was purified and used as
the template for polymerase chain reaction (PCR)
with a single radioactively labeled primer (method of
primer elongation) and DNA Taq-polymerase (New
England Biolabs, United States). The primer was
annealed right prior the promoter [7].

Transcription. Conditions for the in vitro transcription
were optimized to provide maximal use of the
chromatin template. Transcription was performed as
described in [13].

A closed initiation complex (RPC) was assembled
on the template. One transcription round was carried
out in 50 μL reaction mixture, which contained Tris-
acetate buffer (50 mM, pH 8.0), 100 mM potassium
acetate, 8 mM magnesium acetate, 27 mM ammoni-
um acetate, 0.7% PEG-8000, 0.2 mM dithiothreitol,
1 mM chromatin or DNA, 10 nM RNA polymerase,
300 nM sigma-factor 54 and the NrcC and NtrB pro-
tein regulators of nitrogen metabolism in concentra-
tions of 120 and 400 nM, respectively. All components
were first mixed together in the transcription buffer
(total volume 40 μL) and incubated at 37°C for 15 min
in order to form the closed complex. Next, 5 μL of
40 mM ATP dissolved in the transcription buffer were
added to the reaction mixture to a final concentration
of 4 mM. The reaction was carried out for 2 min at
37°C in order to form the open initiation complex (RPO).
Then, a mixture of four ribonucleosidetriphosphates
(NTP) (4 mM each) in the transcription buffer supple-
mented with 2.5 μCi [α-32P]-GTP (3000 Ci/mM) and
2 mg/mL heparin was added to the reaction in order to
initiate elongation and limit the number of transcrip-
tion cycles to one round. The reaction was carried out
at 37°C for 15 min and was stopped by the phenol :
chloroform mixture (1 : 1). Radioactively labeled
RNA was purified and analyzed in denaturating poly-
acrylamide gel. The gel was dried and transferred onto
the radiosensitive screen, which was then scanned.
The data obtained were analyzed with the OptiQuant
(PerkinElmer, USA) program. All experiments were
repeated thrice. The mean value of the transcription
efficiency and the standard deviation were calculated.

RESULTS AND DISCUSSION

In the present study, we used the experimental in vitro system, which allowed us to perform quantitative
analysis of the EPI efficiency on the polynucleosome
templates with precisely positioned nucleosomes,
which spontaneously formed chromatin fibers under
physiological conditions [7, 8]. To perform the experi-
ments, we used either the templates totally covered
with nucleosomes (Figs. 1a, 1e, template 13) or the
templates that lacked one of the nucleosomes at a ran-
don position (Fig. 1e, template 13-1). These tem-
plates contained either intact histones or histone H4
acetylated at Lys-16. The level of nucleosome assem-
by was assessed by the analysis of sensitivity of free
DNA fragments and fragments bound to proteins to
the AluI restriction endonuclease (Figs. 1b, 1c). The
presence and location of DNA breaks were estimated
by the method of primer elongation.

Studies carried out with the previously developed
in vitro transcription system, which allowed us to
assess quantitatively the EPI rate on the chromatin
template (Fig. 1d), showed that incomplete saturation
of DNA with nucleosomes (Fig. 1e, template 13—
one of thirteen histone octamers is absent) leads to an
inhibitory effect of H4K16-Ac on EPI (Figs. 1e, 1f,
template 13-1). This results in the distortion of the
structure of chromatin fiber, because the H4K16-Ac
distorts the contacts between the positively charged N-
terminal domain of the H4 and negatively charged
H2A/H2B part of the neighbor nucleosome. The
activation is less expressed on the templates with a
higher level of nucleosome assembly (Figs. 1e, 1f,
template 13). The data obtained are consistent with
the data on the role of histone terminal domains in
maintaining EPI [10].

The nucleosome free regions of DNA work as
“joints” and enable the compact chromatin fibril to
make a turn or loop in order to maintain the effective
EPI [9]. Therefore, this structure is characterized by
optimal dynamics and remains compact. Acetylation
of the H4K16 leads to chromatin decondensation and
decreases the rate of EPI (Fig. 1f, template 13-1).

A higher level of chromatin assembly (Fig. 1, tem-
plate 13) results in the formation of more rigid chro-
matin fiber, which is characterized by a nonoptimal
dynamics and a higher level of compaction. Acetyla-
tion of the H4K16 distorts the level compaction but,
most probably, increases flexibility of the fibril
because of opening of free DNA regions. All these
events lead to a small upregulation effect of the
H4K16-Ac on the EPI at the saturated level of chro-
matin assembly (Figs. 1e, 1f, template 13). It is note-
worthy that, previously, a decrease in the EPI effi-
ciency was observed if the N-terminal domains of his-
tones were removed on template 13 (Figs. 1a, 1e) [2].
This resulted in loss of not only compactness but also
several electrostatic interactions inside the chromatin
fiber, which, apparently, affect the sustainment of
effective EPI. Hence, the H4K16-Ac on template 13
induces an effect opposite to that observed after the
removal of the N-terminal domains of histones.

The data obtained support the hypothesis that the
H4K16-Ac provides controversial effects on the EPI
in chromatin depending on the level of its assembly.
This is consistent with the data obtained in vivo [17],
which showed that the H4K16-Ac is located inside rel-
atively small regions of active chromatin and, appar-
ently, opens access to the transcription factor binding
sites and provides the chromatin fiber with flexibility.
**Fig. 1.** Role of the H4 histone acetylation at Lys-16 (H4K16-Ac) on the enhancer-promoter interaction (EPI) in chromatin.

(a) Polynucleosome transcription template scheme containing enhancer and promoter. (b) The experimental scheme for assessment of the chromatin template assembly. The polynucleosome template, which contained 13 repeated motives of 177 b.p., which included the 601 (601(177)×13) sequence highly affine to histones, was assembled on the linearized plasmid and incubated with the *Alu*I restriction endonuclease. Purified DNA was used as the template in order to perform elongation of the radioactively labeled primer. (c) The characteristics of the nucleosome saturated templates (13) and the templates that lacked one of the nucleosomes at a random position (13-1) obtained by the method of protection against endonuclease cleavage with *Alu*I. The analysis of primer elongation products was performed by electrophoresis in denaturing polyacrylamide gel. The radioactive signal was detected by the radiosensitive scree and fluorescence reading system. (d) The experimental scheme for the EPI rate measurement in chromatin in vitro. RP3 and RP0-closed and open complexes, respectively. (e) The effect of the H4K16-Ac on the EPI in chromatin depends on saturation of DNA with nucleosomes. (f) Quantitative analysis of the transcripts shown on the panel (e). Statistical analysis of the data of three independent experiments; standard deviation is shown.
similar to that of a “joint” in order to provide effective EPI. Therefore, the rate of EPI in chromatin may increase in two ways: removal of individual nucleosomes [9] or via the acetylation at the H4K16-Ac as shown in the present study.

FUNDING

This work was supported by the Russian Scientific Foundation, project no. 19-74-30003.

COMPLIANCE WITH ETHICAL STANDARDS

This study used neither animals nor people as subjects.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Wasylyk, B., Wasylyk, C., and Chambon, P., Short and long range activation by the SV40 enhancer. Nucleic Acid Res., 1984, vol. 12, no. 14, pp. 5589–5608.
2. Kulaeva, O.I., Nizovtseva, E.V., Polikanov, Y.S., Ulianov, S.V., and Studitsky, V.M., Distant activation of transcription: Mechanisms of enhancer action, Mol. Cell. Biol., 2012, vol. 32, no. 24, pp. 4892–4897.
3. Gilbert, N., Boyle, S., Fiegler, H., Woodfine, K., Carter, N.P., and Bickmore, W.A., Chromatin architecture of the human genome: Gene-rich domains are enriched in open chromatin fibers, Cell, 2004, vol. 118, no. 5, pp. 555–566.
4. Spitz, F., Gene regulation at a distance: From remote enhancers to 3D regulatory ensembles, Semin. Cell Dev. Biol., 2016, vol. 57, pp. 57–67.
5. Nizovtseva, E.V., Todolli, S., Olson, W.K., and Studitsky, V.M., Towards quantitative analysis of gene regulation by enhancers, Epigenomics, 2017, vol. 9, no. 9, pp. 1219–1231.
6. Rubtsov, M.A., Polikanov, Y.S., Bondarenko, V.A., Wang, Y.H., and Studitsky, V.M., Chromatin structure can strongly facilitate enhancer action over a distance, Proc. Natl. Acad. Sci. U.S.A., 2006, vol. 103, no. 47, pp. 17690–17695.
7. Polikanov, Y.S. and Studitsky, V.M., Analysis of distant communication on defined chromatin templates in vitro, Methods Mol. Biol., 2009, vol. 543, pp. 563–576.
8. Maeshima, K., Rogge, R., Tamura, S., Joti, Y., Hikima, T., Szerlong, H., Krause, C., Herman, J., Seidel, E., DeLuca, J., Ishikawa, T., and Hansen, J.C., Nucleosomal arrays self-assemble into supramolecular globular structures lacking 30-nm fibers, EMBO J., 2016, vol. 35, no. 10, pp. 1115–1132.
9. Nizovtseva, E.V., Clauvelin, N., Todolli, S., Polikanov, Y.S., Kulaeva, O.I., Wengryn, S., Olson, W.K., and Studitsky, V.M., Nucleosome-free DNA regions differentially affect distant communication in chromatin, Nucleic Acid Res., 2017, vol. 45, no. 6, pp. 3059–3067.
10. Kulaeva, O.I., Zheng, G., Polikanov, Y.S., Colasanti, A.V., Clauvelin, N., Mukhpadhyay, S., Sen Gupta, A.M., Studitsky, V.M., and Olson, W.K., Inter-nucleosomal interactions mediated by histone tails allow distant communication in chromatin, J. Biol. Chem., 2012, vol. 287, no. 24, pp. 20248–20257.
11. Bone, J.R., Lavender, J., Richman, R., Palmer, M.J., Turner, B.M., and Kuroda, M.I., Acetylated histone H4 on the male X chromosome is associated with dosage compensation in Drosophila, Genes Dev., 1994, vol. 8, no. 1, pp. 96–104.
12. Shogren-Knaak, M., Ishii, H., Sun, J.M., Pazin, M.J., Davie, J.R., and Peterson, C.L., Histone H4-K16 acetylation controls chromatin structure and protein interactions, Science, 2006, vol. 311, no. 5762, pp. 844–847.
13. Polikanov, Y.S., Rubtsov, M.A., and Studitsky, V.M., Biochemical analysis of enhancer-promoter communication in chromatin, Methods, 2007, vol. 41, no. 3, pp. 250–258.
14. Dann, G.P., Liszczak, G.P., Bagert, J.D., Muller, M.M., Nguyen, U.T.T., Wojcik, F., Brown, Z.Z., Bos, J., Panchenko, T., Pollock, S.B., Diehl, K.L., Al- lis, C.D., and Muir, T.W., ISWI chromatin remodelers sense nucleosome modifications to determine substrate preference, Nature, 2017, vol. 548, no. 7669, pp. 607–611.
15. Polach, K.J., Lowary, P.T., and Widom, J., Effects of core histone tail domains on the equilibrium constants for dynamic DNA site accessibility in nucleosomes, J. Mol. Biol., 2000, vol. 298, no. 2, pp. 211–223.
16. Walter, W. and Studitsky, V.M., Construction, analysis, and transcription of model nucleosomal templates, Methods, 2004, vol. 33, no. 1, pp. 18–24.
17. Taylor, G.C., Eskeland, R., Hekimoglu-Balkan, B., Pradeepa, M.M., and Bickmore, W.A., H4K16 acetylation marks active genes and enhancers of embryonic stem cells, but does not alter chromatin compaction, Genome Res., 2013, vol. 23, no. 12, pp. 2053–2065.

Translated by Mikhail Bibov