Vol. 185, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1992 Pages 414-419

ACETYLATION AND METHYLATION OF HISTONES H3 AND H4 IN CHICKEN IMMATURE ERYTHROCYTES ARE NOT DIRECTLY COUPLED

Michael J. Hendzel and James R. Davie*

Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada, R3E 0W3

Received April 13, 1992

SUMMARY: The relationship between histone methylation and dynamic histone acetylation was investigated. Previously, we demonstrated in chicken erythrocytes that dynamically acetylated histones H3 and H4 of transcriptionally active gene chromatin were selectively methylated. However, methylation of these histones was not dependent upon their acetylated states. Here, we tested the hypothesis that methylation tags these histones for participation in dynamic acetylation. Using an inhibitor of protein methylation, adenosine dialdehyde, we show that the processes of histone methylation and dynamic acetylation are not directly coupled. Our results suggest that the selective methylation of dynamically acetylated chromatin reflects features of the organization of transcriptionally active gene chromatin.

In chicken immature erythrocytes, approximately 4% of the modifiable histone lysine sites participate in active acetylation and deacetylation (1). We have shown that the dynamically acetylated histones H3 and H4 of transcriptionally active erythroid chromatin were selectively methylated (2-5). Consistent with our results, it has been demonstrated that acetylated histones of K562 erythroleukemia cells were preferentially methylated (6). These results indicate that there exists a coupling between histone acetylation and methylation. Changing the level of hyperacetylated histones affected the solubility of the active gene chromatin in 0.15 M NaCl, with induction of hyperacetylation increasing the solubility of active gene chromatin (4). However, increasing or decreasing the levels of the acetylated histone forms did not affect ongoing histone methylation or alter the selective methylation of histones with the potential to become hyperacetylated (3). These observations indicated that acetylated histones were not better predisposed to becoming methylated than

*To whom correspondence should be addressed.

0006-291X/92 $4.00

unacetylated histones. However, the results suggested that methylation tags the histones that are to participate in dynamic acetylation. This hypothesis was tested by incubating chicken immature erythrocytes in the presence of adenosine dialdehyde, an inhibitor of protein methylation (7).

MATERIALS AND METHODS

Isolation and incubation of chicken erythrocytes. Immature chicken erythrocytes were isolated from phenylhydrazine-induced anaemic Adult White Leghorn chickens as described (2). Cells were washed once with an isotonic buffer (130 mM NaCl, 5.2 mM KCl, 7.5 mM $MgCl_2$, 10 mM Hepes, pH 7.2) and once in Swim's S-77 medium pH 7.2 (acetate labelling) or Dulbecco's modified Earle's medium pH 7.5 (methionine labelling). Cells were then resuspended at one-third volume packed erythrocytes and two-thirds volume media and pre-incubated for 30 min in the presence or absence 10 μM adenosine dialdehyde (Sigma) and 20 μM cycloheximide. For methylation studies, 10 mM sodium butyrate was added to the labelling medium. Sodium [^{3}H] acetate (1.9 Ci/mmol; New England Nuclear) or L-methyl [^{3}H] methionine (85 Ci/mmol, New England Nuclear) was then added to a final concentration of 0.1 mCi/ml, and the cells were incubated for 15 min (acetate labelling) or 1 h (methionine labelling). For acetylation studies, cells were collected by centrifugation and washed once in Swim's S-77 media containing 0.1 mM sodium acetate and 10 mM sodium butyrate. Subsequently, cells were resuspended in the same media with sodium acetate and sodium butyrate and incubated a further 60 min in the presence or absence of adenosine dialdehyde. Cells were then collected by centrifugation and stored at -70°C until use.

Nuclei isolation, digestion, and chromatin fractionation. Nuclei were isolated and digested, and chromatin was fractionated as described previously (2) except that digestions with micrococcal nuclease were typically shortened to 20 min duration.

Preparation and analysis of protein samples. Histones were isolated from the various chromatin fractions by extraction with 0.4 N H_2SO_4 as described (8). Polyacrylamide gel electrophoresis was performed as described (8). Fluorography was performed as described (2).

RESULTS

To study the effects of adenosine dialdehyde on histone methylation, chicken immature erythrocytes that were pre-incubated with cycloheximide were incubated for 60 min with L-methyl [^{3}H] methionine and 10 mM sodium butyrate in the presence or absence of adenosine dialdehyde. Fig. 1 shows that adenosine dialdehyde effectively inhibited the incorporation of methyl label into histones H3 and H4.

To study the effect of adenosine dialdehyde on dynamic histone acetylation, chicken immature erythrocytes were pulse-labelled with [^{3}H] acetate for 15 min followed by a 60 min chase in Swim's medium containing sodium butyrate with or without adenosine dialdehyde. Fig. 2B, lane T, shows that during the chase period in the presence or absence of adenosine dialdehyde a portion of the metabolically

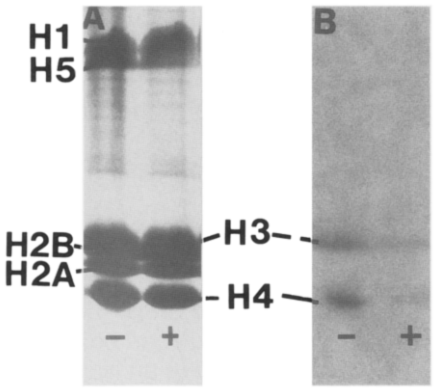

Fig. 1. Adenosine dialdehyde is a potent inhibitor of histone methylation. Acid soluble proteins (10 μg) of nuclei isolated from chicken immature erythrocytes that were labelled with L-[^{3}H]-methionine following preincubation in the presence or absence of 10 μM adenosine dialdehyde were electrophoretically resolved on a SDS/15% polyacrylamide gel. **(A)** and **(B)** show the Coomassie Blue-stained pattern and the accompanying fluorogram, respectively.

active histone H4 population became hyperacetylated, with approximately 31.1% and 29.2% of the labelled histone H4 of adenosine dialdehyde-treated and untreated cells, respectively, being tetraacetylated. We have shown previously that the histone H3 variants H3.2 and H3.3 were actively acetylated and methylated, with histone H3.3 being acetylated and methylated to a greater level than histone H3.2, the major histone H3 variant in chicken erythrocytes (2,5). Fig. 2B, lane T, shows that adenosine dialdehyde treatment did not affect the preferential acetate labelling of histone H3.3.

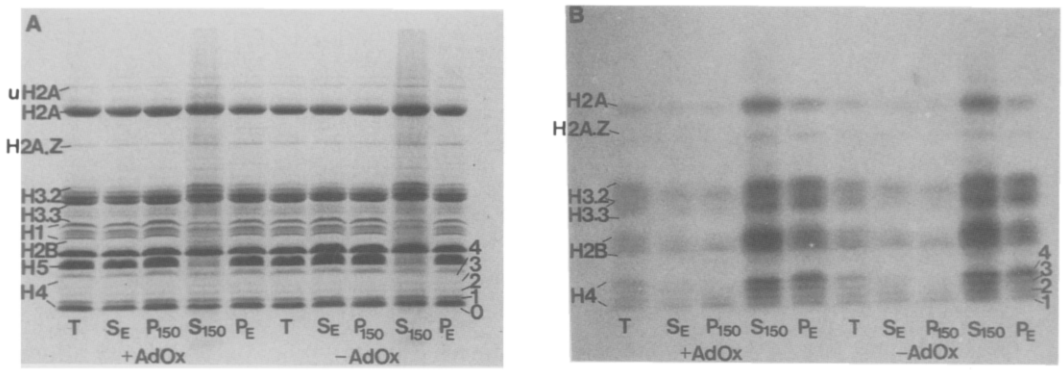

Fig. 2. Adenosine dialdehyde does not affect histone acetylation. Acid soluble proteins (10 μg) of chromatin fractions isolated from chicken immature erythrocytes that were labelled with [^{3}H]-acetic acid following preincubation in the presence or absence of 10 μM adenosine dialdehyde were electrophoretically resolved on an acetic acid-urea-Triton X-100/15% polyacrylamide gel. **(A)** and **(B)** show the Coomassie Blue-stained pattern and the accompanying fluorogram, respectively.

Chicken immature erythrocyte chromatin was fractionated by a procedure that separates chromatin fragments differing in their content of transcriptionally active and repressed DNA sequences. Transcriptionally active DNA sequences were enriched in the 0.15 M NaCl-soluble chromatin fragments (fraction S_{150}) and chromatin fragments associated with the residual nuclear material (fraction P_E) (9). Fig. 2B shows that the extent of the labelling of the histones was the highest for the histones of chromatin fractions S_{150} and P_E. Treating the erythrocytes with adenosine dialdehyde before and during the pulse labelling and chase did not affect the partitioning of the labelled histones among the various chromatin fractions (Fig. 2B). Furthermore, the monoacetylated histone H4 species of fraction P_{150} and the tetraacetylated H4 species of chromatin fractions S_{150} and P_E were the major labelled histone H4 forms of adenosine dialdehyde-treated and untreated cells. For treated and untreated cells, approximately 34.7 and 34.1%, respectively, of the labelled H4 of fraction S_{150} was tetraacetylated, while approximately 39.4 and 41.6%, respectively, of the labelled H4 of fraction P_E was tetraacetylated.

DISCUSSION

Our previous studies demonstrated that dynamically acetylated histones that were bound to transcriptionally active DNA were preferentially methylated (2,3). Possible explanations for this observation include (a) histone acetylation acts as a signal for the histone methyltransferase, (b) histone methylation triggers histone acetylation, (c) histone acetyltransferases, deacetylases and methyltransferases are co-localized in specific chromatin regions, and (d) selective methylation of dynamically acetylated histones reflects the nuclear organization of transcriptionally active chromatin. We have shown that unacetylated histones H3 and H4, which did not participate in dynamic acetylation and were associated with transcriptionally active/competent chromatin, were methylated (3). This observation shows that acetylation of a histone is not a necessary tag for the histone to be methylated. Furthermore, changing the steady state acetylation level of the dynamically acetylated histones affected the 0.15 M NaCl solubility of transcriptionally active chromatin and, presumably, its compaction (4). However, the selective methylation of the dynamically acetylated histones was not altered, suggesting that altering the level of compaction of transcriptionally active gene chromatin did not change the accessibility of these histones to the histone methyltransferase. The results presented here show that inhibition of methylation with adenosine dialdehyde does not affect dynamic acetylation of histones H3.2, H3.3 and H4 in chicken immature erythrocytes,

demonstrating that histone methylation is not an essential tag for acetylation. Thus, our observations present evidence that acetylation does not favorably predispose histones H3 and H4 for methylation, and vice versa histone methylation does not prime these histones for acetylation. In chromatin fractionation experiments, we found that histone acetyltransferase and deacetylase activities did not partition identically among the chromatin fractions. Histone methyltransferase, but not deacetylase, activity was enriched in the 0.15 M NaCl-soluble, transcriptionally active gene-enriched chromatin, while histone deacetylase and, to a much lesser degree, methyltransferase activities were enriched in the low salt insoluble, transcriptionally active gene-enriched chromatin fraction (fraction P_E) which also contained the residual nuclear material (2,5). It should be noted that greater than 75% of the total histone deacetylase activity was located in this fraction. We conclude that the two processes, ongoing histone methylation and dynamic histone acetylation, are coupled due to the organization of transcriptionally active gene chromatin within the chicken immature erythroid nucleus. Recently, we reported that histone acetyltransferase and deacetylase were components of the internal nuclear matrix (5,11, Hendzel and Davie, unpublished results). We postulate that histone acetyltransferase and deacetylase are involved in the localization of transcriptionally active gene chromatin to specific nuclear compartments by transiently interacting with histones that are complexed with transcriptionally active DNA. The association of histone methyltransferase with transcriptionally active gene nucleosomes (2) would result in the observed linkage between dynamic acetylation and ongoing methylation.

ACKNOWLEDGMENTS

This project was supported by a grant from the Medical Research Council of Canada (MA-9186) and by the awards of a Medical Research Council Studentship to M.J. Hendzel and a Medical Research Council Scientist to J.R. Davie.

REFERENCES

1. Zhang, D.-E. and Nelson, D.A. (1986) Biochem. J. 240, 857-862.
2. Hendzel, M.J. and Davie, J.R. (1989) J. Biol. Chem. 264, 19208-19214.
3. Hendzel, M.J. and Davie, J.R. (1991) Biochem. J. 273, 753-758.
4. Ridsdale, J.A., Hendzel, M.J., Delcuve, G.P. and Davie, J.R. (1990) J. Biol. Chem. 265, 5150-5156.
5. Hendzel, M.J., Delcuve, G.P. and Davie, J.R. (1991) J. Biol. Chem. 266, 21936-21942.

6. Reneker, J. and Brotherton, T.W. (1991) Biochemistry 30, 8402-8407.

7. Najbauer, J. and Aswad, D.W. (1990) J. Biol. Chem. 265, 12717-12721.

8. Nickel, B.E., Roth, S.Y., Cook, R.G., Allis, C.D. and Davie, J.R. (1987) Biochemistry 26, 4417-4421.

9. Delcuve, G.P. and Davie, J.R. (1989) Biochem. J. 263, 179-186.

10. Chan, S., Attisano, L. and Lewis, P.N. (1988) J. Biol. Chem. 263, 15463-15465.

11. Hendzel, M.J. and Davie, J.R. (1992) Biochem. Biophys. Acta in press.