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INCREASE IN INITIATION SITES FOR CHROMATIN DIRECTED RNA SYNTHESIS BY ACETYLATION OF CHROMOSOMAL PROTEINS

H.Oberhauser, A.Csordas, B.Puschendorf and H.Grunicke

Institut für Medizinische und Klinische Chemie,
University of Innsbruck, Innsbruck, Austria

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SUMMARY

Treatment of rat liver chromatin with o.7 mM acetic anhydride(1) leads to an approximately twofold increase in initiation sites for DNA-dependent RNA polymerase from E. coli. With reconstituted chromatin, in which only the histone moiety was acetylated, again a twofold increase in initiation sites could be observed, compared to control chromatin which had undergone the dissociation and reassociation procedure, but which had not been exposed to acetic anhydride.

INTRODUCTION

Histone acetylation has been correlated to regulation of gene expression in eukaryotic cells (reviewed (2)). However, the biological relevance of this reaction remained obscure. Numerous observations suggest that histone acetylation may be a prerequisite for the derepression of genes (3,4). If this is the case, one may expect histone acetylation to increase the number of initiation sites for RNA synthesis. The studies presented here have been performed in order to verify this hypothesis.

MATERIALS AND METHODS

Chromatin preparation:

Female Sprague-Dawley rats, 2oo - 3oo g body weight were stunned and killed by cervical dislocation.

All steps were carried out between o - 4°C. Nuclei were prepared according to Blobel & Potter(5), washed 3 times in 8o volumes of o.15 M NaCl - o.o1 M Tris pH8.o and centrifuged each time at 1ooo g for 1o minutes.

The nuclei were then suspended in cold bidistilled water and homogenized in a loose fitting Teflon homogenizer (1ooo rpm, 1o strokes). After 2o minutes incubation on ice, chromatin was sedimented for 15 minutes at 12ooo g and again taken up in cold bidistilled water with a Teflon homogenizer (5oo rpm, 5 strokes, loose fitting pistill).
Chromatin was kept unfrozen on ice. The DNA content of the chromatins used was o.2 - o.6 mg DNA/ml. E. coli RNA poly-

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merase holoenzyme was prepared according to Burgess(6). A frozen cell mass of E. coliB harvested in midlog phase was obtained from E.Merck, Darmstadt, Germany.

RNA synthesis without reinitiation(7):
Chromatin (containing 2 µg of DNA) was preincubated with varying amounts of RNA polymerase from E. coli (or approx. 1o µg of enzyme were preincubated with varying amounts of chromatin) at 37°C for 15 minutes in 2oo µl of preincubation buffer containing 12.5 µM of Tris-HCl pH7.9, o.25 µM of $MnCl_2$, 12.5 µM of $(NH_4)_2SO_4$ pH7.9 and o.5o µM of 2-mercaptoethanol. At the end of the preincubation period, RNA synthesis was started with the addition of 37.5 nM each of ATP, GTP, CTP and (^{3}H)UTP (115 dpm/pmol) and 1o µg of rifampicin (Boehringer Mannheim GmbH, Germany) in 5o µl and incubated for an additional 15 minutes. RNA synthesis was stopped by the addition of 1oo µl of o.5% bovine serum albumin and 1o ml cold 5% trichloroacetic acid containing o.o1 M sodium pyrophosphate. After 15 minutes the precipitate was sedimented by centrifugation, washed twice with 1o ml of cold 5% trichloroacetic acid, dissolved in o.5 ml of o.2 N NaOH and counted in toluene - based scintillation fluid (2 vol. toluene - scintillation fluid + 1 vol. Triton X-1oo) in a Beckman liquid scintillation counter.

Chemical acetylation of chromosomal proteins:
This was carried out according to the method of Wong and Marushige(8) with o.7 mM acetic anhydride (purchased from E.Merck, Darmstadt, Germany). The acetylation of the histone fraction was performed with o.7 mM acetic anhydride at a protein concentration of o.6 mg/ml. For lower protein concentrations, the acetic anhydride concentration was appropriately reduced.

Chromatin dissociation and reconstitution:
Chromatin was dissociated according to Tsai et al.(9). Urea was "ultra pure" from Schwartz/Mann Corporation (USA). Buffers containing urea were used within 24 hours. Chromatin was reconstituted according to the O'Malley procedure(9) by first gradually removing NaCl and then urea.

Five ml volume of chromatin were taken for dissociation and the reconstituted complexes were taken up in 5 ml bidistilled water with a Teflon homogenizer (5oo rpm, 1o strokes).

The DNA content of chromatin was determined by the Keck procedure(1o).

Protein concentration was determined according to Lowry(11) with bovine serum albumin (Sigma) as a standard.

RESULTS

Fig.1 demonstrates chromatin directed rifampicin-resistant RNA synthesis as a function of the amount of RNA polymerase. In these experiments chromatin was preincubated with RNA polymerase for 15 minutes at 37°C. RNA synthesis was then initiated by the addition of nucleosidetriphosphates and rifampicin. Rifampicin was used to prevent reinitiation. All curves exhibit a transition point at which high affinity

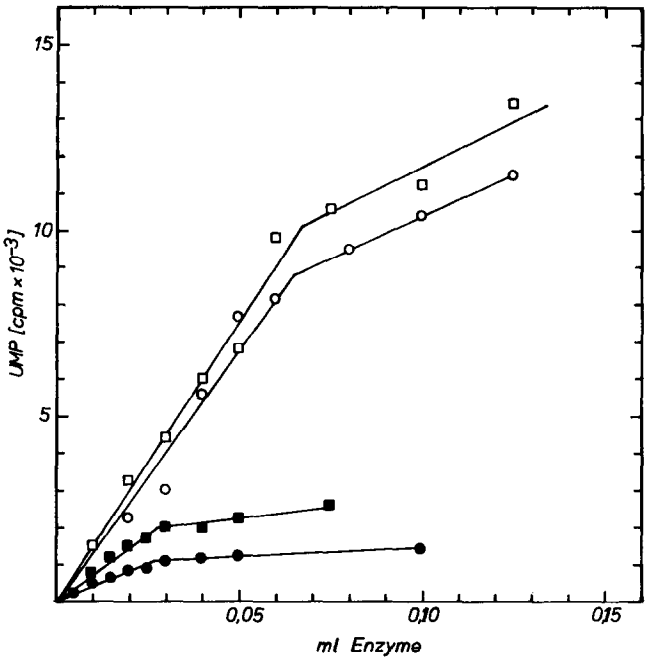

Fig.1
Effect of acetylation on rifampicin-resistant RNA synthesis on native and reconstituted chromatin.

Increasing amounts of E. coli RNA polymerase were preincubated for 15 minutes at 37°C with rat liver chromatin (o.oo2 mg DNA/ml). After preincubation RNA synthesis was initiated by the addition of nucleosidetriphosphates and rifampicin. RNA synthesis was measured as described under "Materials and Methods".

—●— : native chromatin; —■— : reconstituted control chromatin; —○— : chromatin treated with o.7 mM acetic anhydride; —□— : reconstituted chromatin with chemically acetylated histone fraction.

binding sites are saturated. With acetylated chromatin, higher amounts of enzyme are needed to reach a transition point - thus, indicating that the number of initiation sites had been increased.

The same results are obtained if a fixed amount of RNA polymerase is titrated against increasing amounts of chromatin (Fig.2). Under these conditions, an increase in initiation sites on chromatin results in a shift of the transition point to the left - i.e. to lower amounts of template.

In order to evaluate the contribution of histone to the observed increase in binding sites, chromatin was dissociated

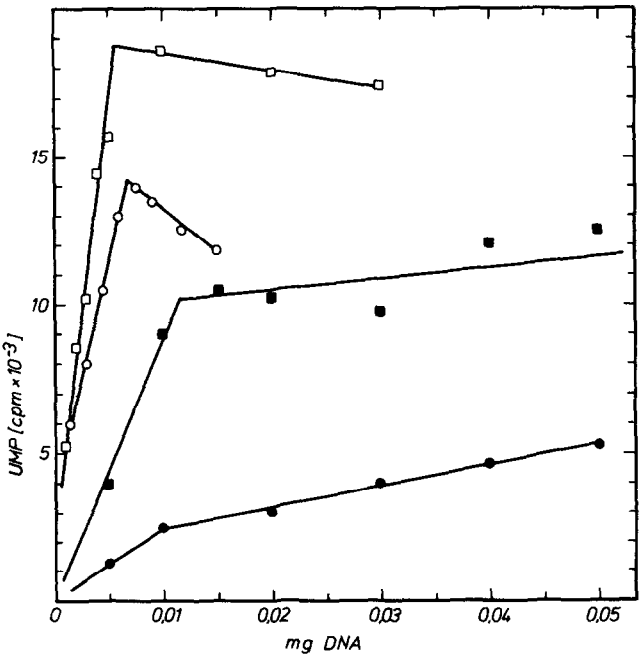

Fig.2
Effect of acetylation on rifampicin-resistant RNA synthesis on native and reconstituted chromatin.

Increasing amounts of chromatin were preincubated with E. coli RNA polymerase. After preincubation, RNA synthesis was initiated by the addition of nucleosidetriphosphates and rifampicin. RNA synthesis was measured as described under "Materials and Methods".

—●— : native chromatin; —■— : reconstituted control chromatin; —○— : chromatin treated with o.7 mM acetic anhydride; —□— : reconstituted chromatin with chemically acetylated histone fraction.

into nonhistone, histone and DNA fractions. The isolated histone fraction was then acetylated and reconstituted with DNA and nonhistone proteins to yield a reconstituted chromatin in which only the histone fraction had been acetylated. It should be noted at this point, that the histone fraction obtained by the procedure used in these experiments is contaminated with some nonhistone proteins (approx.5% of nonhistone protein). This point will be considered in the discussion. Fig.1 demonstrates that with nonacetylated reconstituted chromatin a transition point is reached at the same enzyme concentration which is required to obtain a transition point

Table 1

Effect of histone acetylation on the protein content of reconstituted chromatin.

Chromatin was dissociated into "Nonhistone proteins", "Histones" and "DNA" as described under "Materials and Methods". The isolated histone fraction was acetylated with o.7 mM acetic anhydride and the chromatin reconstituted as described under "Materials and Methods". The reconstituted complexes were sedimented and the supernatants measured at O.D. 26o and O.D. 28o nm for nonreassociated materials.

Chromatin	Protein/to DNA (mg/mg)	Supernatant O.D.26o nm	O.D.28o nm
reconstituted control	1.41	o.143	o.143
reconstituted control	1.46	o.18o	o.127
reconstituted acetylated	1.57	o.176	o.127
reconstituted acetylated	1.37	o.167	o.127

with native chromatin. On the other hand, reconstituted chromatin containing acetylated histones required twice as much enzyme for saturation. The extent of this increase in initiation sites is very close to the increase which is observed when total native chromatin is acetylated. These results are confirmed by the experiment included in fig.2. In this experiment a fixed amount of enzyme is titrated against increasing amounts of chromatin. There is an increase in the number of initiation sites which leads to a shift in the transition point to the left.

It seemed possible, that acetylated histones do not bind to DNA as easily as unmodified histones and that this may cause a lower histone content in the reconstituted chromatin containing the acetylated histones. In order to check this possibility, the protein content of the reconstituted chromatin was measured. As shown in Table 1 the gross chemical composition of the chromatin reconstituted in the presence of acetylated histones is the same as the composition of chromatin reconstituted with unmodified histones. Thus, there is no indication for a loss of a major amount of histone protein in reconstituted chromatin containing acetylated histones.

DISCUSSION

The studies presented here demonstrate that chemical acetylation of chromatin leads to an increase in initiation sites for RNA

synthesis. Recently Marushige reported that acetylation of histone side chains stimulates the rate of chain elongation during transcription of chromatin without increasing the number of available sites for RNA chain initiation(12).

We can confirm the stimulation of elongation rate by chemical acetylation but our experiments also show a dramatic increase in available initiation sites for RNA synthesis after acetylation of histones. It should be emphasized, however, that Marushige used chromatin from calf thymus, whereas our experiments were performed with rat liver chromatin. Perhaps this explains the apparent inconsistencies.
By using chromatin dissociation and reconstitution techniques it could furthermore be demonstrated that acetylation of the histone fraction alone has roughly the same effect as acetylation of total chromatin. The histone preparation in these studies is contaminated with some nonhistone proteins (approx. 5% of total nonhistone protein). Thus, it cannot be excluded that the acetylation of these nonhistone proteins is responsible for the observed effects. This possibility, however, seems unlikely, as recent data from our laboratory demonstrate that acetylation of the other nonhistone protein fractions has no effect on chromatin directed RNA synthesis (manuscript in preparation).

Enzymatic acetylation of histones is known to be highly specific with regard to the number and location of internal lysyl residues (13-19). This site specificity is not obtained by chemical acetylation and it may therefore be questioned whether studies with this technique bear any relevance to in vivo processes. It should be emphasized, however, that in our experiments the extent of acetylation is comparable to the content achieved in vivo(12). The results presented here demonstrate that a change in the number of acetyl groups within the biologically occurring range is capable of opening new initiation sites for *E. coli* RNA polymerase.

REFERENCES

1. Simpson, R.E. (1971) Biochemistry 10, 4466-4470.
2. Allfrey, V.G. (1971) in: "Histones and Nucleohistones" (D.M.P. Phillips, Ed.), Plenum Press, London, New York, pp. 241.
3. Sarkander, H.I., Fleischer-Lambropoulos, H. and Brade, W.P. (1975) FEBS-Lett. 52, 40-43.

4. Sarkander, H.I. and Knoll.Köhler, E. (1978) FEBS-Lett. 85, 3o1-3o4.
5. Blobel, G. and Potter, V. (1966) Science 154, 1662.
6. Burgess, R.R. (1969) J.Biol.Chem. 244, 616o-6167.
7. Tsai, M.J., Twole, H.C., Harris, S.E. and Bert W. O'Malley (1976) J.Biol.Chem. 251, 4713-4721.
8. Wong, T.K. and Marushige, K. (1976) Biochemistry 15, 2o41.
9. Tsai, S.Y., Harris, S.E., Tsai, M.J. and Bert O'Malley (1976) J.Biol.Chem. 251, 4713-4721.
1o. Keck, K. (1956) B.B.A. 63, 446-451.
11. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and.Randall, R.J. (1951) J.Biol.Chem. 193, 265-275.
12. Marushige, K. (1976) Proc.Natl.Acad.Sci. 73, 3937.
13. Gershey, E.L., Vidali, G. and Allfrey, V.G. (1968) J.Biol. Chem. 243, 5o18.
14. Vidali, G., Gershey, E.L. and Allfrey, V.G. (1968) J.Biol. Chem. 243, 6361.
15. De Lange, R.J., Frambrough, D.M., Smith, E.L. and Bonner, J. (1969) J.Biol.Chem. 244, 319.
16. Candido, E.P.M. and Dixon, G.H. (1971) J.Biol.Chem. 246, 3182.
17. Candido, E.P.M. and Dixon, G.H. (1972) J.Biol.Chem. 247, 3868.
18. Candido, E.P.M. and Dixon, G.H. (1972) Proc.Natl.Acad.Sci. 69, 2o15.
19. De Lange, R.J., Hooper, J.A. and Smith, E.L. (1972) Proc. Natl.Acad.Sci. 69, 882.