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TRANSCRIPTIONAL PROPERTIES OF OLIGONUCLEOSOMAL TEMPLATES CONTAINING ACETYLATED $(H3 \cdot H4)_2$ TETRAMERS

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SUMMARY Direct chemical acetylation of an oligonucleosomal template for bacteriophage T7 RNA polymerase is accompanied by a substantial increase in its capability to support RNA synthesis. The template was assembled from a plasmid, containing a promoter and a terminator for T7 RNA polymerase, plus one $(H3 \cdot H4)_2$ tetramer and two H2A·H2B dimers for each 200 base pairs of DNA. Under the employed conditions, acetylation modifies in a preferential way the lysine residues located in the amino-terminal domains of core histones. When the template is assembled with acetylated tetramers and untreated dimers, its efficiency in promoting RNA synthesis is also largely increased. Since a previous work reported transcriptional stimulation upon acetylation of H2A·H2B dimers [Puerta et al. (1995) Biochem. Biophys. Res. Commun. 210, 409], the trancriptional repression brought about by core histone octamers seems to require that the amino-terminal domains of both $(H3 \cdot H4)_2$ tetramers and H2A·H2B dimers are not acetylated.

Eukaryotic DNA binds core histone octamers to produce the structural units of chromatin, the nucleosomal cores (1). Histone $(H3 \cdot H4)_2$ tetramers play a dominant role in inducing nucleosomal DNA structure, while the two H2A·H2B dimers complete the nucleosomal core by binding at opposite sites of the previous $(H3 \cdot H4)_2$-DNA structure (2-4). The H2A·H2B dimers appear to be present in the nucleosomal particle in a more dynamic equilibrium than the $(H3 \cdot H4)_2$ tetramers, being more easily released and interchanged (5,6). Each of the four core histones comprises a globular and an amino-terminal domain . The globular domains are mainly responsible for the structural organization of the nucleosomal core (4,7-9), while the amino terminal tails, which contain a high proportion of basic amino acid residues, are flexible and

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highly mobile, and bind to negatively-charged DNA increasing the thermal stability of the particle (8,10).

The amino-terminal domains of core histones are physiologically acetylated, a modification that by affecting their interaction with DNA can potentially modulate nucleosomal structure and its capability to bind other molecules (11,12). Although efforts to find a direct relationship between physiological acetylation and transcription have been unsuccessful (13-17), chemical acetylation of the amino-terminal tails of core histones has been correlated with a significant increase in the efficiency of nucleosomal cores as "in vitro" transcription templates (18). Because of the different structural properties of $(H3{\cdot}H4)_2$ tetramers and H2A·H2B dimers, modification of their corresponding amino-terminal domains might affect the structural and functional properties of the nucleosomal unit in different ways. Previous work from our laboratory shows that acetylation of histone H2A·H2B dimers facilitates transcription by T7 RNA polymerase of an oligonucleosomal template (19). Using the same "in vitro" transcription system, our new results indicate that acetylation of the $(H3{\cdot}H4)_2$ is also accompanied by a substantial transcriptional stimulation.

MATERIALS AND METHODS

Transcription Templates. Transcription templates were assembled on plasmid pGEMEX-1 (Promega Biotec), which contains 4.0 kilobases and includes one promoter and one terminator region for bacteriphage T7 RNA polymerase, the terminator being separated from the initiation site by a coding region of aproximately 1.1 kilobases. Core histone octamers were prepared from chicken erythrocyte nuclei by extraction with 4 M NaCl after removal of histones H1 and H5 with 0.7 M Na Cl (20). Dimers H2A·H2B and tetramers (H3·H4)2 were isolated from core histone octamers by chromatography on Sephadex G-100 (20). The acetylated $(H3{\cdot}H4)_2$ tetramers were obtained from nucleosomal cores treated with acetic anhydride. Nucleosomal cores were obtained from chicken erythrocytes as previously described (21). Acetylation of nucleosomal cores (0.1-0.2 mg of DNA/ml) took place at room temperature, in 0.2 M N-[tris(hydroxymethyl)methyl]glycine (Tricine) (pH 8.2), by treatment with 5 μmol of acetic anhydride/ml (18). The modified nucleosomal cores were dissociated into $(H3{\cdot}H4)_2$-DNA particles and H2A·H2B dimers in the presence of 0.34 M NaCl and 4 M urea (22), and the $(H3{\cdot}H4)_2$-DNA particles were separated from the H2A·H2B dimers by centrifugation at 20°C in a linear 5-20% sucrose gradient containing the dissociating solution. The $(H3{\cdot}H4)_2$-DNA particles were dialyzed against 0.1 M potassium phosphate (pH 6.8) and 2 M NaCl, and the acetylated histone tetramers were separated from DNA by chromatography on a hydroxylapatite column (23). The isolated $(H3{\cdot}H4)_2$ tetramers were concentrated by ultrafiltration and dialyzed at 4°C for 12-16 h against 10 mM Tris-HCl (pH 8.2), 1 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride.

Histone-DNA complexes were assembled from the chosen plasmid and histone components by dialysis against decreasing salt concentrations (24). pGEMEX-1 (60 μg of DNA/ml) was mixed at 4°C with the required amount of $(H3.H4)_2$ tetramers (intact or acetylated) and the complementary amount of H2A.H2B dimers (intact or acetylated), in 10 mM Tris-HCl (pH 7.8), 2 M NaCl, and 0.25 mM EDTA. Two H2A.H2B dimers were added for each $(H3.H4)_2$ tetramer. To prepare directly acetylated oligonucleosomal templates, the histone-DNA complexes assembled with intact $(H3{\cdot}H4)_2$ tetramers and H2A·H2B dimers were treated with acetic anhydride as indicated for nucleosomal cores.

The nucleo-histone complexes were digested with micrococcal nuclease (Boehringer Mannheim) at 20°C. The complexes (35 μg of DNA/ml) were digested, for different times, with 0.4 units of nuclease/μg of DNA. The reaction was ended by addition of EDTA to a final concentration of 5 mM. The DNA was isolated and subjected to electrophoresis in a 8% polyacrylamide gel containing TBE buffer solution (25).

Transcription Assays. Transcription by bacteriophage T7 RNA polymerase (Boehringer Mannheim)(6.7 units/μl) was estimated by measuring the radioactivity from [^{3}H]UTP incorporated into acid-insoluble RNA (24). The transcription templates (7.5 ng of DNA/μl) were preincubated at 37°C for 5 min with 0.4 mM each of ATP, CTP, GTP, and UTP (containing 33 nCi of [^{3}H]UTP/μl) in 40 mM Tris-HCl (pH 8.0), 6 mM $MgCl_2$, 2 mM spermidine, 10 mM dithiothreitol, and human placental ribonuclease inhibitor (1 unit/μl). Transcription was initiated by addition of RNA polymerase, incubation taking place at 37°C.

To determine the size of the transcription products, the reaction took place as described above, except that 10 μCi of [α-^{32}P]CTP were included in each assay mixture instead of [^{3}H]UTP. The final concentration of UTP was 0.4 mM, and that of [α-^{32}P]CTP was 0.1 mM. After incubation for 10 min, the reaction was ended by addition of 10 mM EDTA (pH 7.4), 100 mM NaCl, and 14 μg of salmon DNA/ml. The reaction products were separated from unreacted [α-^{32}P]CTP by centrifugation of the assay mixture through Sephadex G-50 columns equilibrated with 10 mM Tris-HCl (pH 8.0), 0.1 M Na Cl, and 1 mM EDTA. RNA was purified by phenol/chloroform extraction and precipitation with ethanol and subjected to electrophoresis in gels containing 4% polyacrylamide and 8 M urea (25).

RESULTS

To investigate the transcriptional effects caused by acetylation of the lysine residues located in the histone octamer, the oligonucleosomal template assembled from pGEMEX-1 plus histone $(H3{\cdot}H4)_2$ tetramers and H2A·H2B dimers (one tetramer and two dimers per 200 base pairs) was directly acetylated with acetic anhydride. Treatment took place under conditions producing extensive acetylation of the histone amino-terminal tails but small modification of the corresponding globular domains (18). Figure 1 shows the level of RNA synthesized by T7 RNA polymerase using the described acetylated template as well as the corresponding untreated control and naked plasmid. Acetylation is accompanied by a substantial increase in the capability of the oligonucleosomal template to support RNA synthesis, in agreement with results previously obtained with nucleosomal cores (18). Since transcription of pGEMEX-1 by T7 RNA polymerase is inhibited by histone octamers predominantly at the level of initiation (24), acetylation of histone octamers in the oligonucleosomal templateseems to allow efficient transcriptional initiation. Previous results, using the same "in vitro" transcription system, show that chemical acetylation of the H2A·H2B dimers employed to assemble the oligonucleosomal template facilitates its transcription (19).

To find out whether acetylation of the $(H3{\cdot}H4)_2$ tetramers has also an effect on transcription, histone-DNA complexes containing acetylated $(H3{\cdot}H4)_2$ tetramers and untreated H2A.H2B dimers were assembled. The acetylated tetramers were obtained from chemically

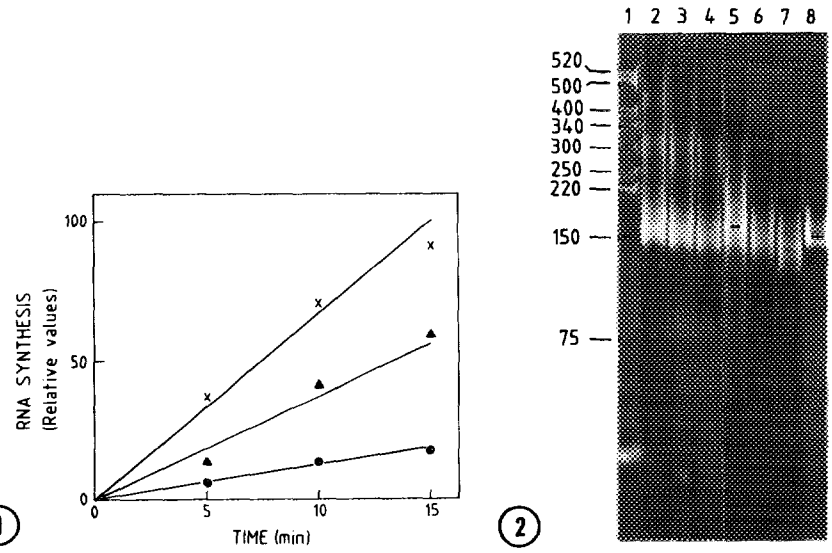

Fig. 1. **RNA synthesis obtained with oligonucleosomal templates treated with acetic anhydride.** The incorporation of [^{3}H]UTP into RNA was determined after the indicated incubation times. The transcription templates were naked DNA (×) and DNA associated to histone octamers untreated (●) or directly acetylated by treatment with acetic anhydride (▲).

Fig. 2. **Electrophoresis of DNA from histone-DNA complexes digested with micrococcal nuclease.** DNA obtained after digestion of DNA associated to core histone octamers containing untreated (lanes 2-4) or acetylated (lanes 5-7) $(H3{\cdot}H4)_2$ tetramers. Samples were digested for 10 min (lanes 2,5), 20 min (lanes 3,6), or 40 min (lanes 4,7). DNA from nucleosomal cores (lane 8) and DNA fragments obtained by digestion of pBR 322 with PstI and HinfI were also included (lane 1). Numbers to the left indicate DNA size in base pairs.

acetylated nucleosomal cores in which 85% of the lysine residues present in the histone amino-terminal tails were acetylated but only 30% of those located in the globular domains (18). Micrococcal nuclease digestion of the oligonucleosomal templates containing the acetylated $(H3{\cdot}H4)_2$ tetramers gives protected DNA fragments similar in size to those obtained from the corresponding untreated complexes (Fig. 2). The DNA in the acetylated preparation appears more susceptible to digestion than that in the untreated one. A moderate relaxation of the nucleosomal unit was also observed in the acetylated nucleosomal cores from which the employed acetylated $(H3{\cdot}H4)_2$ tetramers were obtained. These acetylated nucleosomal cores show lower thermal stability than the corresponding untreated particles (18).

To evaluate the transcriptional properties of the oligonucleosomal templates containing acetylated $(H3{\cdot}H4)_2$, the level of RNA synthesis was determined after different incubation times and the transcription products were analyzed by electrophoresis on polyacrylamide gels. Figure 3 shows that the oligonucleosomal templates containing acetylated $(H3{\cdot}H4)_2$ tetramers support a high level of RNA synthesis as compared with the corresponding nonacetylated control. The main transcription product obtained with the acetylated template has a size of approximately

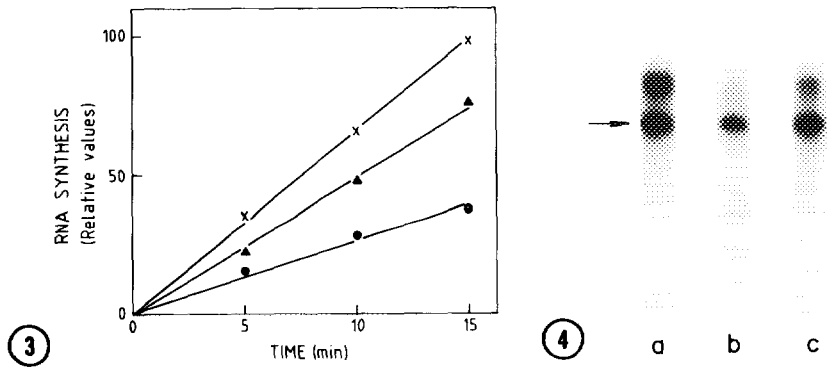

Fig. 3. **RNA synthesis obtained with oligonucleosomal templates containing acetylated $(H3\cdot H4)_2$ tetramers.** The incorporation of [^{3}H]UTP into RNA was determined after the indicated incubation times. The transcription templates were naked DNA (×) and DNA associated to histone octamers containing untreated (●) or acetylated (▲) $(H3\cdot H4)_2$ tetramers.

Fig. 4. **Electrophoresis of the transcription products obtained with different templates.** The transcription templates were naked DNA (a) and DNA associated to histone octamers containing untreated (b) or acetylated (c) (H3·H4)2 tetramers. The arrow indicates the position corresponding to RNA chains of 1,100 nucleotides, which is the size of the RNA molecules initiated at the promoter and terminated at the terminator region.

1,100 nucleotides (Fig. 4), which corresponds to transcription of the coding region of pGEMEX-1, from the promoter to the terminator. Only small amounts of larger RNA chains are observed, in contrast with the transcription products found with the naked plasmid. Apparently, the presence of histone octamers increases the efficiency of the termination signal, in agreement with previous results (19,24). No RNA chains smaller than 1,100 were detected with any of the employed templates, indicating that inhibition is not caused by premature chain termination.

DISCUSSION

The physiological acetylation of cores histones has been associated with transcriptional active chromatin (11,12). Early results supported this view by showing that chemical acetylation of chromatin is accompanied by a large increase in RNA synthesis (26,27). With chemically acetylated nucleosomal cores as transcription templates, transcriptional stimulation is correlated to acetylation of the histone amino-terminal domains (18). The failure to observe any stimulatory effect of "in vivo" acetylation on "in vitro" RNA synthesis (13-17) was attributed to the low level of acetylation in the employed "hyperacetylated" templates relative to the maximal physiological acetylation possible (17). The particular lysine residues which must be acetylated to promote transcription might not be modified in the "hyperacetylated" templates.

With the oligonucleosomal templates used in the present work, direct acetylation of the histone-DNA complex is accompanied by substantial stimulation in RNA synthesis. Acetylation

was conducted under conditions producing preferential modification of the amino-terminal tails but small acetylation of the globular domains (18). When the oligonucleosomal templates are assembled with either acetylated H2A·H2B dimers plus untreated $(H3·H4)_2$ tetramers (19) or untreated dimers plus acetylated tetramers (present results), significant increases in the level of RNA synthesis are observed. Apparently, the amino-terminal tails of core histones play an inhibitory role in transcription, presumably by their electrostatic interaction with DNA. This transcriptional repression seems to require that the amino-terminal domains of both $(H3·H4)_2$ tetramers and H2A·H2B dimers are not extensively acetylated. Acetylation of the amino-terminal domains would prevent inhibition by weakening their binding to DNA. The acetylation state of the core histone octamer might control trancriptional activity "in vivo" by affecting the interaction of DNA and the amino-terminal tails of core histones with different structures, including the transcriptional machinery. Although our results do not show significant transcriptional differences between templates with either acetylated $(H3·H4)_2$ tetramers or acetylated H2A·H2B dimers, "in vivo" acetylation of tetramers might play a role different from that corresponding to the acetylation of dimers, thus increasing the possibilities of transcriptional control.

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