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INTERACTIONS OF ACETYLATED HISTONES WITH DNA AS REVEALED BY UV LASER INDUCED HISTONE-DNA CROSSLINKING

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The interaction of acetylated histones with DNA in chromatin has been studied by UV laser-induced crosslinking histones to DNA. After irradiation of the nuclei, the covalently linked protein-DNA complexes were isolated and the presence of histones in them demonstrated immunochemically. When chromatin from irradiated nuclei was treated with clostripain, which selectively cleaved the N-terminal tails of core histones, no one of them was found covalently linked to DNA, thus showing that crosslinking proceeded solely via the N-terminal regions. However, the crosslinking ability of the laser was preserved both upon physiological acetylation of histones, known to be restricted to the N-terminal tails, and with chemically acetylated chromatin. This finding is direct evidence that the postsynthetic histone acetylation does not release the N-terminal tails from interaction with DNA.

Twenty five years since Allfrey et al.(1) first correlated the postsynthetic histone acetylation with one of the properties, acquired by chromatin upon activation, the precise role of this modification is still unknown. The current concept for changes in chromatin structure as a result of weaken histone-DNA interactions has not gained experimental support both at the level of the nucleosome and the higher order chromatin structure (2-8). The only paper suggesting an opening of nucleosome core particles upon acetylation is that of Bode et al (9), showing that core particles with at least 10 acetyl groups migrated slower in non-denaturing gel than their less acetylated or unmodified counterparts. The increased frictional ratio of the hyperacetylated core particles they attributed to an opening of their structure. Reduced electrophoretic mobility of highly acetylated core particles was observed also by Imai et al (8), but their neutron scatter studies failed to reveal a detectable unfolding of these particles free in solution. According to these authors, the increased frictional resistence of the hyperace-

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tylated core particles might be due to release of the N-terminal domain of the core histones. Such a possibility was suggested also by Ausio and van Holde to account for the small effect of acetylation on some hydrodynamic and optical properties of nucleosomes(7).

One approach to study the interactions of hyperacetylated N-terminal tails with DNA is offered by the method of UV laser-induced crosslinking histones to DNA we recently developed (10). In this paper, using clostripain as an agent for selective cleavege of N-terminal tails of core histones, we show that this is the region of the molecule, which is involved in the crosslinking. We took advantage of this finding to study the efficiency of crosslinking histones to DNA as a function of histone acetylation.

MATERIALS AND METHODS

Isolation of nuclei and chromatin. Calf thymus nuclei were prepared essentially by the method of Blobel and Potter (11) Chromatin was isolated from the nuclear pellet after mild treatment with micrococcal nuclease (0.5 units/A_{260}, 3 min, at 37°C) and lysis in 3mM NaCl, 0.25mM EDTA. Histone H1-depleted chromatin was obtained using the ion-exchange resin Dowex AG 500 Wx2 as described in (12). Nuclei from Friend erythroleukemia cells were isolated according to (13). To preserve the acetylated state of histones, all solutions contained 5mM sodium butyrate.

Treatment of chromatin with clostripain. Proteolysis of H1-depleted chromatin by clostripain was carried out following the protocol in (14).

Elecrophoretic analysis. Native and proteolysed histones were analysed by electrophoresis in 18% polyacrylamide gel, containing SDS (15).Acetylated histones were electrophoresed in 15% polyacrylamide gel, containing 0.9N acetic acid, 8M urea, 0.37% Triton X100 (16).

Histone acetylation in chromatin. In vivo acetylated chromatin was isolated from Friend erythroleukemia cells, grown for 24 h in the presence of 20mM sodium n-butyrate. The viability of the cells was determined by the trypan-blue exclusion test. Chemically acetylated chromatin was prepared by treatment with acetic anhydride as described in (17).

UV laser crosslinking procedure. The source of UV radiation was a home made passively mode-locked picosecond Nd:YAG laser. Irradiation of the samples at 266nm was carried out in rectangular fused silica cuvettes at pulse duration 30 ps, pulse energy 4mJ, diameter of the beam 0.5 cm, repetition rate 0.5 Hz. The optical density of the solutions was kept in the range $1<A_{260}<5$. During irradiation the sample was continuously stirred. The intensity of irradiation was controlled by focussing and defocussing fused silica lenses. The energy of the radiation was measured with pyroelectric detectors calibrated with a Model Rj 7200 energy meter (Laser Precision Corp.) and the electric signal transmitted to an Apple II microcomputer for further processing and handling.

Separation of the crosslinked histone-DNA complexes. To isolate the crosslinked material, the irradiated nuclei were made 1% in Sarkosyl, sonicated for 90 sec and centrifuged through a preformed CsCl gradient (10).

Antibodies and Immunodot analysis. Antibodies to H2A, H2B and H4 were raised as described in (10) and immunospecifically purified

from IgG by affinity chromatography (18). Monoclonal antibody to H3 was the gift of Dr E.Jockers-Wretou (Institut Pasteur, Athens, Greece). The specificity of each antibody preparation was tested by enzyme linked immunosorbent assay (ELISA). The reaction of the antibodies with the crosslinked protein-DNA complexes was analysed by dot immunoassay as described in (10).

RESULTS

Crosslinking of histones to DNA in clostripain-digested chromatin

Treatment of chromatin with clostripain resulted in progressive proteolysis of core histones, leading to a limit digestion, characterized by two bands on SDS-polyacrylamide gel electrophoresis (Fig.1). The result is in complete agreement with the data of Dumius-Kervabon (14), according to which the slow migrating band represent the resistant fragments of H2A, H2B and H3, and the fast one correspond to H4.

Chromatin was irradiated with the UV laser and then treated with clostripain at the conditions of limit digestion. The covalently crosslinked protein-DNA complexes were isolated and the presence of each core histone analysed by dot immunoassay, using specific antibodies to each individual histone. As Fig. 1 shows, no one of the core histones was identified in the dotted material, i.e. crosslinking proceeded exclusively via the N-terminal tails.

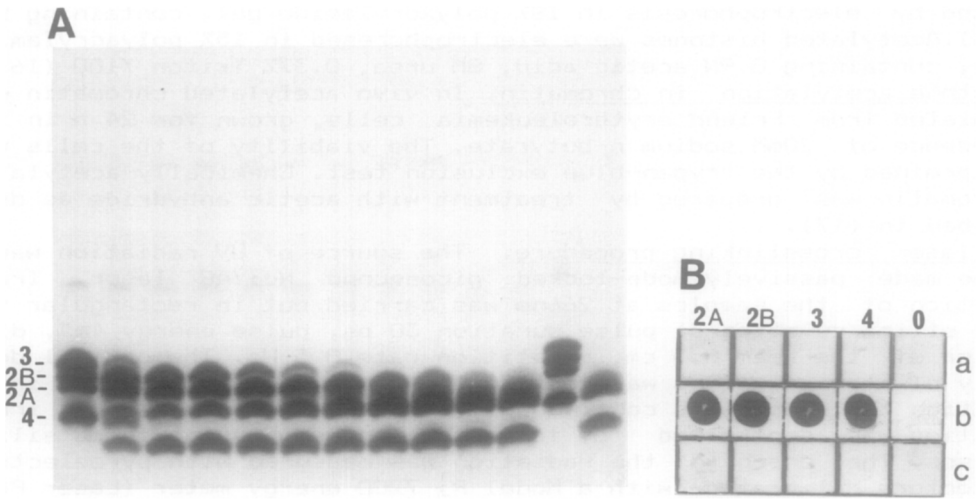

Fig.1. (A). SDS-18% polyacrylamide gel electrophoresis of histones, isolated from clostripain-digested H1-depleted chromatin from calf thymus as a function of time (1,3,5,10,20,30,45,60,90,120,210 min, slots from left to right,respectively); slots 1 and 12: native histones. (B). Immunodot assay of the reaction of the antibodies to H2A, H2B, H3 and H4, and non-immune IgG(O) with (a) control, non-irradiated calf thymus nuclei, (b) irradiated nuclei, and (c) irradiated and clostripain-digested chromatin.

Crosslinking of histones to DNA in chromatin, containing in vivo acetylated histones

Friend erythroleukemia cells were grown in the presence of sodium n-butyrate to obtain physiologically acetylated histones. The pattern of acetylation is shown in Fig.2. Nuclei from these cells were irradiated with the laser and the isolated crosslinked material was analysed for the presence of histones. All core histones were found covalently linked to DNA (Fig.2), the efficiency of crosslinking being similar to that of the control, non acetylated chromatin.

Crosslinking of core histones to DNA in chemically acetylated chromatin

Histone H1-depleted chromatin from calf thymus was treated with acetic anhydride to chemically acetylate the histones. The condi-

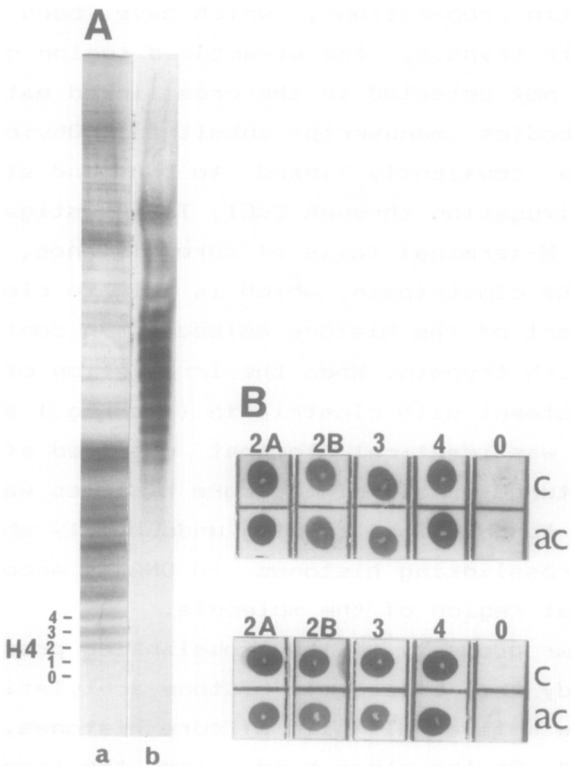

Fig.2.(A) 15% polyacrylamide-acetic acid-urea-Triton X-100 gel electrophoresis of chromatin from Friend erythroleukemia cells, grown in the presence of butyrate (a) and histones isolated from chemically acetylated calf thymus H1-depleted chromatin (b). Acetylated forms of H4 in (a) are shown by numbers, indicating the number of acetyl groups. (B). Immunodot assay of the reaction of the antibodies to H2A, H2B, H3 and H4, and non-immune IgG (0) with control, irradiated nuclei (c); irradiated nuclei, isolated from butyrate-treated Friend cells (ac, upper pannel); irradiated, chemically acetylated H1-depleted chromatin from calf thymus (ac, lower pannel).

tions of acetylation we used (1.8mM acetic anhydride, pH 8.5,30 min at 4°C) were reported to modify about 50% of the lysine residues with no evidence for dissociation of histones (21).The extent of acetylation is well demonstrated with histone H4 (Fig.2): the zone of migration of this histone is free of proteins; the first bands of acetylated histone molecules appeared behind this zone. When such a chromatin was irradiated with the UV laser, all core histones were identified in the isolated protein-DNA complexes.

DISCUSSION

In a previous paper we showed that UV laser irradiation of isolated nuclei and whole cells crosslinked covalently histones to DNA (10). Meanwhile we were able to demonstrate that the crosslinking proceeded solely via the non-structured tails of the histone molecule: in chromatin preparations, which have been irradiated and then treated with trypsin, the structured region of all core histone species was not detected in the crosslinked material as probed by specific antibodies (manuscript submitted). Obviously, this domain has not been covalently linked to DNA and stripped from it during the centrifugation through CsCl. To investigate the involvement of C- and N-terminal tails of core histones, we used another endopeptidase, the clostripain, which is able to cleave selectively the N-terminal part of the histone molecule, in contrast to the effect observed with trypsin. When the irradiation of the nuclei was followed by treatment with clostripain (see Fig.1 and ref.14), the result obtained was identical to that observed after trypsinization: the structured domain of the core histones was not found covalently linked to DNA. This finding undoubtedly shows that the UV laser induced crosslinking histones to DNA is accomplished solely via the N-terminal region of the molecule.

This peculiar property of the crosslinking procedure offers an approach to study the effect of histone acetylation, known to be restricted to the N-terminal tails of core histones, on their interaction with DNA. On the other hand, since the laser-induced covalent crosslinking of a particular protein to DNA requires this protein to be bound to DNA, the efficiency of crosslinking histones to DNA addresses directly the question of the interaction of acetylated histones with DNA, respectively the release of N-terminal tails, supposed by some authors (7,8) to account for the increased frictional ratio of hyperacetylated core particles. To this end we studied the efficiency of crosslinking histones to DNA in chromatin

from Friend erythroleukemia cells, grown in the presence of n-butyrate. It has been shown that such a treatment enhanced the level of histone acetylation (19) through the inhibition of histone deacetylase (5,20), so that histones are acetylated in their original in vivo sites. When the acetylated chromatin was crosslinked by UV laser irradiation, all core histones were identified in the covalent protein-DNA complexes with no evidence for quantitative differences in the efficiencies of crosslinking of acetylated and non acetylated chromatin (Fig.2B). The extent of acetylation, estimated by scanning the pattern of H4 was 2.3-2.7 acetyl groups per molecule. Such a value is close to that obtained by Imai et al (8) and higher than the critical level of 10 acetyl groups per core particle, reported by Bode et al (9) to affect its electrophoretic behaviour. Nevertheless, the core histones have been crosslinked to DNA, i.e. their N-terminal tails have not been released from interaction with DNA.

The efficiency of crosslinking was studied also with chemically acetylated chromatin. To avoid dissocoation of histones as a result of the treatment with acetic anhydride (21,22), acetylation was performed under mild conditions, reported to modify about 50% of lysine residues in histones (20). Having in mind the number of lysines in the N-terminal tail of the molecule (4,6,5,5 for H2A,H2B,H3 and H4, respectively; see ref.14) such an acetylation is at least as high as that we obtained in vivo,except that H2A will be modified too. Again, acetylation did not prevent core histones from crosslinking to DNA.

Histone acetylation,therefore, does not release the N-terminal tails from intercations with DNA. Very recently,based on the melting properties of core particles reconstituted from histones with excised N-terminal tails, Ausio et al. claim that hyperacetylation should be expected to weaken but not to abolish tail-DNA interactions (23). Our direct experimental data support such an assumption and do not substantiate the above mentioned suggestions, that the increased frictional ratio of hyperacetylated core particles should be attributed to released N-terminal tails.

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