Histone hyperacetylation can induce unfolding of the nucleosome core particle

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ABSTRACT

A direct correlation exists between the level of histone H4 hyperacetylation induced by sodium butyrate and the extent to which nucleosomes lose their compact shape and become elongated (62.0% of the particles have a length/width ratio over 1.6; overall mean in the length/width ratio = 1.83 ± 0.48) when bound to electron microscope specimen grids at low ionic strength (1mM EDTA, 10mM Tris, pH 8.0). A marked proportion of elongated core particles is also observed in the naturally occurring hyperacetylated chicken testis chromatin undergoing spermatogenesis when analyzed at low ionic strength (36.8% of the particles have a length/width ratio over 1.6). Core particles of elongated shape (length/width ratio over 1.6) generated under low ionic strength conditions are absent in the hypoacetylated chicken erythrocyte chromatin and represent only 2.3% of the untreated HeLa S3 cell core particles containing a low proportion of hyperacetylated histones. The marked differences between control and hyperacetylated core particles are absent if the particles are bound to the carbon support film in the presence of 0.2 M NaCl, 6mM MgCl₂ and 10mM Tris pH 8.0, conditions known to stabilize nucleosomes. A survey of the published work on histone hyperacetylation together with the present results indicate that histone hyperacetylation does not produce any marked disruption of the core particle 'per se', but that it decreases intranucleosomal stabilizing forces as judged by the lowered stability of the hyperacetylated core particle under conditions of shearing stress such as cationic competition by the carbon support film of the EM grid for DNA binding.

INTRODUCTION

ϵ -amino acetylation is a reversible, post-synthetic enzymatic modification of the core histones which occurs at specific lysine residues of the N-terminal tails of the histones H4, H3, H2A and H2B (1–3). The result is a neutralization of the positive charge of the affected lysine residues and therefore a reduction of the net positive charge of the N-terminal tails of the eight core histones in the nucleosome. It has long been speculated that this

modification might represent a mechanism of changing inter- or intra-nucleosomal interactions and in turn of altering or modulating chromatin structure (1–10). The acetylation of histones 'in vivo' correlates with several types of transitions in chromatin structure such as those accompanying transcription (3, 4, 6, 11–18), histone deposition during DNA replication (6, 19–25), the displacement of histones from spermatin nuclei during spermatogenesis (26–33) or during sporulation in *Physarum* (34–36). However, experiments 'in vitro' with acetylated chromatin have been difficult to interpret so far and a role for histone acetylation in regulating chromatin structure remains controversial (reviewed in 10). Using the high-resolution analytical electron microscopy technique, electron spectroscopic imaging (ESI) (37, 38), we have previously reported that treatment of HeLa S3 cells with sodium butyrate caused the core particles isolated from their chromatin to adopt a slightly elongated shape (length/width ratio of 1.52 ± 0.19) (10). In order to gain further insight into this phenomenon we have performed a systematic study of core particles with different levels of histone hyperacetylation. In addition we have compared the effects that a low ionic strength (1 mM EDTA, 10 mM Tris, pH 8.0) versus a higher one (0.2 M NaCl, 6mM MgCl₂, 10mM Tris, pH 8.0, has, at the time of binding of the core particles to the carbon support film, on the morphological changes detected by ESI. We show that a correlation exists between the level of histone hyperacetylation induced by sodium butyrate and the length/width ratio of the core particles when they are bound to the specimen film at low ionic strength but not at 0.2 M NaCl, 6mM MgCl₂, 10mM Tris, pH 8.0. In addition, we show that core particles of elongated shape generated at low ionic strength but not at 0.2 M NaCl, 6mM MgCl₂, 10 mM Tris, pH 8.0, are also present in naturally occurring hyperacetylated chicken testis chromatin and are absent in hypoacetylated chicken erythrocyte chromatin. A current survey of the physical properties that are altered 'in vitro' following histone acetylation is presented (table I) and a model to help unify the apparently controversial set of results related to histone hyperacetylation is proposed.

MATERIALS AND METHODS

Nuclei from HeLa cells and chicken erythrocytes were prepared as described (10). Nuclei from rooster testes were prepared by

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Fig. 1: Preparation and characterization of the core particles. A scheme is presented indicating the basic steps of the preparation, control and use of the core particles (see methods section for details). Left: DNA length control in hyperacetylated (H) or control (C) HeLa S3 core particles. 'M' indicated marker. Right: Protein controls. Polyacrylamide-SDS (PA-SDS), polyacrylamide-triton-urea (PA-triton-urea) or two dimensional electrophoresis (PA-triton-urea/PA-SDS) of the proteins from hyperacetylated (H) or control (C) HeLa S3 core particles.

homogenizing fresh, chilled, scissor-minced mature testes in 5 vol. of ice cold 3mM $MgCl_2$, 1mM $CaCl_2$, 25 mM KCl, 0.12 mM spermine, 1mM PMSF, 10 mM sodium butyrate, 0.05% Nonidet NP-40, 0.25M sucrose, filtered through 3 layers of cheesecloth and centrifuged at 4000 rpm (JS7.5 Beckman rotor) for 15 min at 4°C. The pellet was resuspended and washed in the above medium except that the detergent was omitted. Finally the nuclear pellet was resuspended in the above medium containing 2M sucrose, layered on the top of a 2.2 M sucrose cushion (the remainder of the components were identical) and centrifuged at 20,000 rpm in a Beckman SW-27 rotor at 4°C for 60 min. The nuclear pellet was resuspended in micrococcal nuclease digestion media (10) and supplemented with the following protease inhibitors: 0.1 mM PMSF, Leupeptin (5 μ g/ml), pepstatin A (5 μ g/ml) and aprotinin (100U/ml). The micrococcal nuclease digestion, core particle isolation and electrophoretic analysis of DNA and protein were performed as described (10).

Preparation of specimen grids and image analysis was performed as described (10). The carbon support films were prepared by electron beam bombardment, collected on freshly-cleaved mica and picked up on the surface of distilled water with 1000-mesh copper electron microscope grids. The particles were bound to the grids either in the presence of low ionic strength

buffer, 1mM EDTA, 10 mM Tris pH8, or medium ionic strength buffer 0.2 M NaCl, 6mM $MgCl_2$, 10mM Tris pH8. Each of the experiments was performed three separate times (starting from three independent batches of cells) and the core particles were processed and analyzed in a double blind manner in which the observations of the length/width ratio of the particles were made and recorded without identification of the samples and then later correlated with the acetylation levels (determined by two-dimensional electrophoresis: PA-triton-urea/PA-SDS) and the ionic strength conditions.

Phosphorous imaging

Net phosphorous distributions within particles were obtained as described previously (38). Reference images were recorded at 120eV energy loss and phosphorous enhanced images were recorded at 160eV, with an energy window of 15eV. After normalization over regions of the background and alignment of the digitized regions of the micrograph pairs to within a pixel by the computer, a net phosphorous map is generated by subtraction. To relate the DNA distribution to the total particle, the net phosphorous distribution is represented in levels of red from black to bright red and is superimposed on the reference image, in which greater mass is represented by darker gray levels.

Mass and Length to Width Measurements

To give confidence that particles of equal molecular weight were being compared, mass histograms of the nucleosome from each of the experimental and control preparations were obtained. Nucleosome core particles were spread onto thin carbon support films of approximately 2.5 nm thickness. Images were recorded using low energy loss electrons, corresponding to 120eV. The condenser was adjusted to give uniform illumination over the field and micrographs were recorded to give an optical density over the background of 0.4. Because an entire micrograph could not be digitized with sufficient resolution, regions of the micrographs were digitized in a raster fashion. Each particle in each region corresponding to approximately 1500×1500 nm was selected by the computer. Occasionally, large clumps of particles were rejected from the analysis, an operation that occurred with equal frequency with all the specimens examined. Particles were delineated by choosing boundaries corresponding to 10% above the optical density of the surrounding support film using software provided on the IBAS (Kontron) computer. Histograms of the experimental and control fractions were normalized with respect to the mass of the carbon support films since these samples were spread on grids from one carbon film. This provided an arbitrary, but relative, mass unit. The nucleosome particles prepared from chicken erythrocyte nuclei were spread onto a second preparation of carbon. Normalization of this histogram to the control histogram was accomplished using short strands of naked DNA co-spread with the nucleosomes on each grid. Length to width measurements were obtained using Kontron software also available on the IBAS computer.

RESULTS

Preparation of the Nucleosomal Core Particles

Treatment of HeLa cells with sodium butyrate leads to the accumulation of hyperacetylated species of histone H4 (Fig. 1) (39, 40), the effect being due to inhibition of the chromatin-bound histone deacetylase. The extent of acetylation depends on the nature of the cell line (41), the butyrate concentration used, the length of the treatment and the phase at which the cells are treated with butyrate (eg. early-mid, mid or mid-late logarithmic growth). To avoid confusion between the hyperacetylated histone species and other proteins migrating at the same position (e.g. histone variants), we have routinely determined the hyperacetylation level of histone H4 using two-dimensional electrophoresis (1st dimension: PA-triton-urea, 2nd dimension: PA-SDS). At the core particle level, the only differences found between butyrate-treated and control samples were those attributable to histone acetylation (Fig. 1). No differences were observed either in the sucrose gradient profiles or in the DNA lengths of the different type of core particles (Fig. 1). Untreated HeLa S3 core particles had 1.2 acetyl residues/histone H4, and other batches of butyrate-treated HeLa S3 core particles were prepared containing either 2.2 or 3.0 acetyl residues/histone H4.

Length/width ratio of the different preparations of core particles

When bound to carbon support films on electron microscope specimen grids at low ionic strength (1mM EDTA, 10mM Tris pH8), chicken erythrocyte nucleosomes with 0.6 acetyl residues/histone H4 had a length/width ratio of 1.30 ± 0.11 and untreated HeLa S3 cells with 1.2 acetyl residues/histone H4 had a length/width ratio of 1.23 ± 0.10 (fig. 2, first two columns).

Although the average values for the untreated HeLa S3 cells are slightly lower than for chicken erythrocyte, the differences are not significant since they lie within the resolution limit (10% measurement error or 0.1 units in the length/width ratio). A more informative way to search for differences in the two populations is to consider only the particles having markedly elongated shapes (length to width ratio over 1.6): No such images are present in the hypoacetylated chicken erythrocyte core particles, and only 2.3% of such elongated shapes were present in control HeLa S3 core particles containing a very low proportion of hyperacetylated histones. In contrast with this minimal structural variety found in the core particles with low acetylation levels (Between 0.6 and 1.2 acetyl residues/histone H4), a significant increase in the length/width ratio is observed when the acetylation level is raised from 1.2 to 2.2 acetyl residues per histone H4 by means of *in vitro* treatment of the cells with sodium butyrate (Fig. 2, third column). The length to width ratio increases even further when the acetyl residues/histone H4 to increase to 3.0 (Fig.2, fourth column). It is important to note that in this case the large standard deviation in the length/width ratio of the two hyperacetylated populations does not reflect a distribution spread from error measurement but a real distribution in the length to width ratio in the specimens (error due to measurement is only 0.1 units in the length/width ratio). Such a wide distribution in the length to width ratio almost certainly has its origin in the distribution in the number of acetyl residues per core particle. Such a range in the acetylation level in histone H4 is obvious from examination of the hyperacetylated histone sample (H) (see Fig 1). All these results are summarized in Fig.2, bottom panel. Some representative samples from these specimens shown in Fig.2 were selected for further analysis.

Fig.3 illustrates the protein density, phosphorous densities and the combined phosphorous plus protein density of hyperacetylated HeLa S3 core particles (panel A of Fig.3) and hypoacetylated chicken erythrocyte core particles (panel B of Fig. 3). The number of front views in the hyperacetylated sample (panel A, Fig. 3) is very low (14%, 11/80), the majority of these particles having an elongated shape composed of 3–4 domains. In panel B (Fig. 3) the first image shown is a front view of the chicken erythrocyte core particle and the second and third images correspond to side views. The number of front views greatly exceeds the number of side views (75 to 4 or 95%).

The marked differences in the length to width ratio between the hyperacetylated and control core particles are absent when the various particles are bound to the carbon support film at higher ionic strength in the presence of Mg^{2+} ions (0.2 M NaCl, 6mM $MgCl_2$, 10mM Tris, pH 8.0) (Fig.4). The overall average in the length to width ratio in the hyperacetylated population (3.0 acetyl/histone H4) at low ionic strength (1.83 ± 0.48) is reduced to 1.27 ± 0.18 when bound under the latter conditions. Also the number of elongated particles over 1.6 (length/width ratio) present in the hyperacetylated population at low ionic strength (78.0%) decreases to only 4.6% at 0.2 M NaCl, 6mM $MgCl_2$, 10 mM Tris, pH 8.0. (Fig 4). Particles of markedly elongated shape (length to width ratio over 1.6) generated at low ionic strength but not at 0.2 M NaCl, 6 mM $MgCl_2$, 10 mM Tris, pH 8.0 are also present in the naturally occurring hyperacetylated chromatin from chicken testes undergoing spermatogenesis (Fig. 4B). At low ionic strength the overall average in the length to width ratio of these particles is 1.66 ± 0.63 (50.1% of the particles have a length to width ratio over 1.6) and at medium ionic strength the overall mean in length to width ratio comes down

Fig. 2: Direct analysis by electron spectroscopic imaging (ESI) of different pools of core particles containing increasing levels of histone acetylation. **A.** Histograms of the distribution in the length to width ratio in different preparations of core particles whose source indicated at the top; Control: HeLa S3 core particles containing 1.2 acetyl residues/histone H4. Hyperacetylated 1: Butyrate-treated HeLa S3 core particles containing 2.2 acetyl residues/histone H4. Hyperacetylated 2: Butyrate-treated HeLa S3 core particles containing 3.0 acetyl residues/histone H4. **B.** Histogram of the mass distributions in the different preparations of core particles indicated at the top of the figure. **C.** General views of the different particles preparations indicated at the top. Scale bar is 120 nm. **D.** Correlation between the extent of histone H4 acetylation in the different types of cores and the length/width ratio adopted by the specimens when bound to the carbon support film on electron microscope grids at low ionic strength.

to 1.24 (none of the particles has a length to width ratio over 1.6) (Fig. 4B).

No significant differences in the length/width ratio between low salt and medium salt are observed in the hyperacetylated

chicken erythrocyte chromatin (medium salt length/width ratio = 1.34 ± 0.18 ; low salt length/width ratio = 1.30 ± 0.11). Unexpectedly, the control HeLa S3 core particles showed a slight overall increase in the length to width ratio when the ionic strength

Fig.3: Electron spectroscopic imaging of core particles bound to the carbon support film at low ionic strength (10 mM Tris pH8, 1 mM EDTA). Scale bar is 100Å. A. Hyperacetylated (3.0 acetyl residues/histone H4) particles prepared from HeLa cells. The first column (column 1)(the one containing the scale bars) illustrates the protein density, the second column the phosphorous density and the third column the combined phosphorous plus protein density. In column number 4 a possible path for the DNA is suggested (other interpretations are possible). B. Chicken erythrocyte core particles (0.6 acetyl residues/histone H4). Column 1: protein-density. Column 2: phosphorous density. Column 3: phosphorous plus protein density. Column 4: possible path for the DNA. The first row shows a frontal view of the core particle. This donut-shaped image represents 87% of the particles. The second and third rows show side views of the core particle. Those images are rare, present only in 13% of the particles. C. Cartoon illustrating possible theoretical transitions of a canonical core particle which would respect the integrity of the domains created by the H3-H4 dimers or tetramer and the H2A H2B dimers.

was raised (length to width ratio in low salt = 1.23 ± 0.10 ; length/width in medium salt = 1.31 ± 0.23). The differences are small and lie within the standard deviation, though they are present even when only the particles with a marked elongated shape are considered (length/width over 1.6); 2.3% of elongated shapes at low salt increases to 11.9% at medium salt.

Mass distribution in the different types of core particles

The relative masses corresponding to the different samples are essentially indistinguishable (Fig. 2). This indicates that only images corresponding to the density expected for a single core particle were used to obtain the length/width ratio measurements.

Fig.4: Electron spectroscopic imaging of hyperacetylated core particles bound to the carbon support film at low ionic strength (1mM EDTA, 10mM Tris pH8) versus 0.2 M NaCl, 6mM MgCl₂, 10 mM Tris pH8.0. A. Photomicrographs: Shape of the hyperacetylated core particles (Acetyl residues per histone H4 = 3.0) bound to the E.M. grids at 0.2M NaCl, 6mM MgCl₂, 10mM Tris pH8.0 (left) or low ionic strength (right). The particles have been selected to represent the same statistical composition as that found when analyzing a population of > 200 particles. The histograms corresponding to the length/width ratios and to the mass distributions are shown next to the corresponding photomicrographs. B. Photomicrographs: Rooster testis core particles bound the E.M. grids in the presence of 6 mM MgCl₂, 0.2 M NaCl, 10 mM Tris pH8.0 (top panel) and in the presence of 1 mM EDTA, 10 mM Tris, pH8.0 (bottom panel). The top panel shows the typical morphology of the core particle found when analyzing a population of > 200 particles. In the bottom panel, four elongated core particles are shown at the right (in the heavy lined box). This type of elongated particle (length to width ratio = 2.84 ± 0.42) represents 14.3% of the preparation of rooster testes core particles. For the remaining 85.7% of the particles, six examples shown in the left side of the bottom panel. The histograms corresponding to the length/width ratios and to the mass distributions are shown next to the corresponding photomicrographs.

DISCUSSION

Correlation between the degree of histone acetylation and structure of the core particle determined by electron spectroscopic imaging

We have shown that the level of histone H4 hyperacetylation induced by 'in vivo' treatment of HeLa cells in culture with butyrate correlates with the adoption of an elongated shape by the core particle when bound to the carbon film on electron microscope grids at low ionic strength (Figs. 2–4) for example,

62.0% of the particles have a length/width ratio over 1.6; overall mean = 1.83 ± 0.48 when the acetylation level reached 3.0 acetyl residues/histone H4.

Six of the elongated images were selected for further analysis (Fig 3A) and subjected to measurements of protein density (first column of Fig 3A) and Phosphorous density (second column of Fig 3A). In the third column of Fig 3A the combined phosphorous plus protein densities are shown, and in the fourth column of Fig 3A one of the possible interpretations of the path of the DNA is shown. In an attempt to find out whether specific structural

MEASUREMENT OR TECHNIQUE		DIFFERENCE BETWEEN CONTROL AND HYPERACETYLATED AND/OR INTERPRETATION OF THE RESULTS	REF.
Neutron scattering	CP	No detectable unfolding of the hyperacetylated particles free in the solution. No evidence for unwinding of the DNA ends	81
Hydroxylapatite dissociation chromatography	CH	Hyperacetylated histone H4 is eluted at lower NaCl than non-acetylated or hypoacetylated H4, suggesting that hyperacetylated H4 has a decreased stability in nucleosomes	79
Non denaturing electrophoresis	CP	No differences	79
	CP	Hyperacetylated particles migrate slower	81
In Situ flow cytometry	NU	Lowering of the heat stability of the nucleosomes by about 5° C	82
Protamine-mediated histone displacement	NU	Hyperacetylated H4 is displaced preferentially over hypo or non-acetylated H4 as analyzed by two dimensional gel electrophoresis. Suggests either increased accessibility of those regions of the nuclei containing hyperacetylated histones or a decreased individual stability of hyperacetylated histone H4 or hyperacetylated core particles	10
	CH	Hyperacetylated H4 is displaced preferentially over hypo or non-acetylated H4 in S-phase Physarum chromatin as analyzed by PA-triton-urea electrophoresis	35
	CH	2mM sodium butyrate added during 1h. to plasmodia results in blockage of the subsequent protamine-mediated histone displacement	35
	CP	Core particle pools containing hyperacetylated histones are disassembled with preference over hypoacetylated or non-acetylated core particles	10
Electron microscopy	NU	Treatment with sodium butyrate inhibits the condensation of interphase chromatin	57
Electron spectroscopic imaging	CP	Butyrate induced histone hyperacetylation to a level of 2.2 acetyl residues/histone H4 correlates with the adoption of a length/width ratio of 1.52±0.19 in the core particle	10 (Fig. 2)
	CP	The adoption of elongated shapes in the core particles depends on the degree of histone hyperacetylation reached. Hyperacetylation of 3.0 acetyl residues/histone H4 correlates with a length/width ratio of 2.06±0.50 (values ranging between 1.25 to 3.46)	(Fig. 2-4)
	CP	14.3% of the testis core particle preparation display a length/width ratio of 2.84±0.42. This shape does not appear if the particles are bound to the grids at 0.2 M NaCl, 6 mM MgCl ₂ , 10 mM Tris pH 8.0	(Fig. 4)
"in vitro" nucleosome core particle reconstitution	CH	Histone acetylation reduces nucleosome core particle linking number change	56
DNaseI	NU	Increase sensitivity	83
	NU	Increase sensitivity	84
Fluorescence anisotropy	CP	Small change in tyrosine fluorescence anisotropy which might indicate, at most, a slightly looser structure in the hyperacetylated cores over the pH range 5.5-8.5	85
CD	CP	Small change in CD near 283nm which might indicate a slightly looser structure of the hyperacetylated cores	85

Table I: Update of the previous summary (10) of the *in vitro* physical properties of the nucleosome core particle related to histone hyperacetylation. 'CP' indicates that the measurement has been performed using isolated core particles, 'CH' indicates chromatin and 'NU' indicates nuclei.

patterns are present in these images we have included, in Fig 3C, a cartoon that illustrates possible theoretical transitions of a canonical core particle preserving at the same time the integrity of the H3-H4 dimer or tetramers and of the H2A-H2B dimers. At the present we still cannot define a model of the structure of a typical hyperacetylated-elongated core particle, however, clear cut conclusions that can be drawn from these images (Fig 3A) is that they are elongated and show a domain structure within their overall shapes. However, the elongated structures depicted by the images presented in this paper do not necessarily occur in solution in the absence of other destabilizing factors. The findings are significant because they show real differences in the stability of hyper- versus hypo-acetylated core particles, i.e. hypoacetylated particles are not elongated under the conditions that reveal elongated structures with the hyperacetylated samples. In addition, electron spectroscopic imaging provides a new approach to study the structure of the nucleosome: further analysis of the different structures induced by acetylation should make it possible to obtain a picture of the domains of the nucleosome and the transitions which can be induced by different agents controlling chromatin structure. For comparison, Fig 3B shows the structure of a chicken erythrocyte, both in front view (first row) and side view (second and third rows). We have observed that the proportion of front views (95%) greatly exceeds the number of side views (5%) in the chicken erythrocyte core particles. Thus, the chicken erythrocyte core particle binds with

strong preference by the side that yields front views and which yields the most stable interaction with the carbon support film. This is best explained if the nucleosome is a flat disk so that our results support the flat disk model (48) and argue against an alternative model (49). Further, the measurement of the dimensions of the front and side views in the chicken erythrocyte core particles indicate that they are 11.0 nm in diameter and 6.5 nm in thickness, measurements which strongly indicate that, at least when bound to the carbon support film and analyzed by this direct visualization technique (without staining or shadowing), the nucleosome is a flat disk.

A significant proportion of elongated particles is also observed in fields of nucleosomes from naturally occurring hyperacetylated chicken testis chromatin undergoing spermatogenesis (28,29) under low ionic strength conditions (36.8% of the particles have a length/width ratio over 1.6). Core particles of such a markedly elongated shape generated under low ionic strength conditions are absent in hypoacetylated chicken erythrocyte chromatin and represent only 2.3% of control Hela S3 core particles (from cells untreated with butyrate) and containing a low proportion of acetylated histones (Fig. 2).

The overall correlation between the acetylation level of the different pools examined and the length to width ratio adopted by the core particles at low ionic strength is shown at the bottom of Fig. 2. The shape of the curve suggests that a minimum threshold level of histone hyperacetylation, between 1.2 and 2.2 acetyl residues per histone H4 is needed to destabilize the nucleosome under this low ionic strength condition. A threshold level has also been suggested to account for differences in electrophoretic migration of hyperacetylated core particles (8,88,89). A critical finding is that the adoption of this elongated shape by the hyperacetylated core particles is prevented if the binding of the particles to the electron microscope grids is performed at medium ionic strength, in the presence of Mg²⁺ (0.2M NaCl, 6mM MgCl₂, 10mM Tris pH8.0)(Fig. 4). We think that this latter observation is very important to help unify the apparent variety of results related to the effect of histone hyperacetylation upon chromatin or nucleosome structure (10) (Table I). Most of the 'in vitro' experiments have been performed with core particles in solution using a wide variety of conditions such as presence or absence of divalent cations, EDTA, different ionic strengths, pH, acetylation levels, temperature and core particle concentration. As derived from Figs. 2-4 and by other observations (42-47, 86, 87), the structure, compactness or stability of the nucleosome is sensitive to all these variables. Keeping this in mind, the results obtained (Fig. 2-4) (Table I) and ref. (10) indicate that histone acetylation, by itself, does not disrupt the nucleosome structure or change its morphology to a markedly detectable level in solution under conditions of ionic strength reasonably close to physiological. The question is why do we then detect these marked morphological differences between hyperacetylated and control core particles at low ionic strength? Our interpretation is that we have introduced destabilizing factors to the nucleosome structure, namely, by binding the core particles to a positively charged specimen support film. At low ionic strength, this binding is likely to result in a competition between the positively charged support film and the histones for DNA binding, which in turn would lead to a destabilization of the hyperacetylated core particles as compared to control core particles. This underlying lowered stability of the hyperacetylated core particles manifests itself by the adoption of the elongated shapes we observe (Fig. 2).

A key question is whether the marked differences in shape detected *in vitro* at low but not at more physiological ionic strengths between hypo- and hyperacetylated core particles reflect similar changes in the stability of nucleosomes *in vivo*. Nucleosomal core particles *in vivo* are not isolated as they are in the *in vitro* experiments performed in solution, but are constrained by a variety of potential destabilizing factors including superhelical stress forces on the DNA (56, 68–71) and the competing action of DNA binding proteins such as regulatory DNA binding proteins (trans-acting factors) (72–75), the positively charged protamines or transition proteins (in mammals) associated with nucleosome disassembly during spermatogenesis (26–29, 76–78, 92–95), or those involved in the replicational or transcriptional machinery (68–69). Such *in vivo* destabilizing factors could further decrease the stability of nucleosomes in certain regions of chromatin and, therefore, induce, when histone hyperacetylation also occurs, structural transitions similar to those presented in this paper (Figs. 2–4).

A similar partially reversible destabilization of core particles at low ionic strength has also been observed when the N-terminal tails of the histones are removed (43), or in the Mg^{++} soluble fraction (transcriptionally active chromatin) from *Physarum polycephalum* (50). Also nucleosomes with altered structural properties have been reported to be present during transcription (51–55). Recently it has been reported that histone acetylation reduces the linking number change when acetylated nucleosomes are bound to topologically constrained DNA (56). This indicates that the DNA is less constrained within the nucleosome beaded structure when the histones are hyperacetylated and this can be interpreted either by an intranucleosomal effect of histone hyperacetylation or by an internucleosomal effect. The limited number of changes observed in the physical properties of the core particle in solution in the absence of factors destabilizing the nucleosome structure are interpreted by Norton et al (56) in support of an internucleosome effect. The results of our protamine competition experiments on intact nuclei (10) are compatible with both hypotheses, but the data obtained with core particles (lacking a superstructure) (Figs. 2–4 and 10) indicate that histone acetylation also has a significant effect in decreasing intranucleosomal stabilizing forces. A possible explanation for the changes induced by histone acetylation would be that nucleosome stabilizing interactions provided by the histone N-terminal tails interacting with the DNA (5), would be decreased as a result of the neutralization of the positive charge of the lysine residues by acetylation. The immediate consequence would be that these tails would 'lift' partially from the core. This is supported by studies with isolated histone tails which show that their weak binding to DNA is abolished upon acetylation (5).

Histone acetylation could also be regarded as a signal for a subsequent structural transition. This signal could change intranucleosomal interactions (see above), and thus provide or remove sites for the binding of other proteins such as histone H1 (57). Such sites could also be recognized by proteases (58) or other enzymes able to modulate the chromatin structure of the specific domains modified by acetylation. 'Signals' provided by acetylation are likely to be highly specific as suggested by the non-random utilization of the acetylation sites (33, 59–62, 90) and the specific pattern and levels of acetylation correlated with different cellular processes. For example, histone H4 di-acetylation is involved in histone deposition in replicating chromatin (6, 19–25, 63), whereas histone H4 hyperacetylation

is involved in transcription (3, 4, 11–18, 63) and nucleosome disassembly during spermatogenesis (10, 26–33, 64).

Finally, we must also consider the high turnover of acetyl residues concomitant with histone hyperacetylation during transcription (65, 16) or spermatogenesis (28), which suggests that acetylation might not be merely a 'static' phenomenon, but may cause the production of 'waves' of changing chromatin structure which in turn could rapidly and reversibly expose sites in chromatin domains to DNA binding proteins (28,65).

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