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Intranuclear Localization of Histone Acetylation in *Physarum polycephalum* and the Structure of Functionally Active Chromatin

JAAP H. WATERBORG* AND HARRY R. MATTHEWS

Cell and Molecular Biology Laboratory, University of Sussex, Brighton BN1 9QG, UK and Department of Biological Chemistry, Medical School, University of California at Davis, Davis, CA 95616 USA

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Abstract

Based on studies of histone acetylation in vivo in *Physarum polycephalum,* we present the following hypotheses: (1) Transcription-specific histone acetylation on histones H3 and H4 is a localized process at the nuclear matrix; (2) Histone acetylation in the S phase, which is specific for newly synthesized histones, occurs in an intranuclear nonlocalized process.

These hypotheses can explain: (1) the histone specificity of histone acetylation that is dependent on the functional state of the chromatin; (2) the apparent absence of turnover of histone acetylation in the bulk of the chromatin despite a definite low level of steady-state acetylation of all four core histones in bulk chromatin; (3) the pattern of butyrate-induced hyperacetylation observed for active and inactive chromatin.

Index Entries: Intranuclear localization, of histone acetylation; localization; intranuclear, of histone acetylation; histone acetylation, intranuclear localization of; acetylation, intranuclear localization of histone; *Physarum polycephalum,* histone acetylation and chromatin structure in; chromatin structure, in *Physarum polycephalum*.

*Author to whom all correspondence and reprint requests should be addressed.

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Introduction

Recent experiments on histone acetylation in vivo using the acellular slime mold *Physarum polycephalum* have yielded results that have important implications for any model that attempts to describe in detail the structure of chromatin within the eukaryotic nucleus. The results obtained have a bearing on the structure of active and inactive chromatin and on the interaction between active chromatin and the nuclear matrix.

Histone acetylation was studied in the naturally synchronous macroplasmodia of *Physarum* by very short in vivo pulses with radioactive acetate. High resolution acid–urea–Triton gel electrophoresis separates the acetylated histone species, which are then detected by staining and fluorography. Distinctly different patterns of histone acetylation were observed in the G2 phase of the cell cycle from those seen in S phase (*1*). Further analysis showed that two types of histone acetylation could be distinguished, one specific for transcriptionally active chromatin and one specific for chromosome replication [submitted paper (*2*)]. The differences between the two patterns, in combination with other published and unpublished observations, suggest that histone acetylation specific for transcriptionally active chromatin occurs in association with the nuclear matrix.

Materials and Methods

Labeling of Physarum

Physarum polycephalum, strain M3C, was cultured as microplasmodia and macroplasmodia as described before (*1, 3*). Macroplasmodia were radioactively labeled on 0.5 mL pulse medium with 2.1 mCi [^{3}H]-Na acetate (1.6–4 Ci/mmol) for 5 min (*1*) or with 0.5 mCi [^{3}H]-lysine (68.4 Ci/mmol) for 20 min. Pulse labeling in S phase started at 20 min after the second metaphase following fusion of microplasmodia. Pulse labeling in the G2 phase was done approximately 5 h after the second mitosis. Cell cycle times ranged from 8 to 10.5 h.

Inhibitors

Cycloheximide, 100-fold concentrated stock solution in water, was used at 10 μg/mL both in the pulse medium and during a 15 min pre-incubation (*4, 5*). Fluorodeoxyuridine was used at 5 μg/mL, with a 30 min pre-incubation period, in the presence of uridine (100 μg/mL) and folic acid (40 μ*M*) to prevent inhibition of RNA synthesis (*6, 7*). A 50-fold concentrated stock solution was made in 0.1*M* $NaHCO_3$. Cordycepin was added from a 25-fold concentrated stock solution in 25% aqueous ethanol and was used at 200 μg/mL (*8*) during the 60 min pre-incubation and during the labeling. All stock solutions were sterilized by filtration.

Isolation of Nuclei and Histones

Nuclei were isolated and histones prepared as described by Mende et al. (*3*). The histones were separated on 15% acid–urea–Trinton X-100 gels (15 × 30 cm, 0.5 mm thickness) according to Bonner et al. (*3, 9*). Histones prepared from one-quarter of a macroplasmodium (approximately 25 million nuclei) were loaded in each gel lane. Gels were stained with Coomassie Blue, destained, and photographed prior to impregnation with PPO (2,5-diphenyloxazole) for fluorography at −70°C with preflashed Kodak XAR-5 film, as described previously (*1*).

Fluorographs and Polaroid negatives of stained gels were scanned with a Cary 210 spectrophotometer equipped with a gel scanner and a digital interface port. The data were collected directly into a Hewlett-Packard 9845S computer through a 16-bit parallel interface. Scans of stain and label were aligned using interactive computer graphics and the alignment was confirmed visually by superimposing the fluorograph and the dried gel. Gaussian distributions were fitted to some of the scans using an interactive program developed for this purpose. The programs for scanning, aligning scans, and Gaussian fitting, in BASIC, are available on request from Dr. H. R. Matthews.

Isolated nuclei from macroplasmodia labeled in G2 phase with [^{3}H]-Na acetate in the presence of cycloheximide were digested with micrococcal nuclease and the digested chromatin was fractionated on sucrose gradients, as described by Johnson et al. (*10*). Fractions containing lexosomes (these are labile nucleosomes derived from transcriptionally active chromatin by limited digestion with micrococcal nuclease previously called "peak A particles"), mononucleosomes, oligonucleosomes (n = 2–5) were pooled separately, dialyzed and lyophilized, and then electrophoresed on 17.5% SDS gels as described previously (*3*). Histones were also prepared from the nuclei after removal of the digested chromatin and electrophoresed in parallel. The gels were stained with Coomassie Blue, fluorographed, scanned, and the specific radioactivities of histones H3 and H4 were determined.

Results and Discussion

G2 Phase Histone Acetylation.

During the G2 phase of the cell cycle, a very simple pattern of acetate turnover on histones is seen: only histone H4 and H3 are acetylated (*1*). A large number of observations correlate this acetylation with transcriptional activity of the chromatin.

1. The level of modification of the histones is high for both these histones, predominantly di-, tri-, and tetra-acetylated (Fig. 1) (*1, 2, 11, 12*). Multi-acetylation of histones, in particular of H4, correlates very well with transcription, whereas histone acetylation in general does not correlate well (*13, 14*).

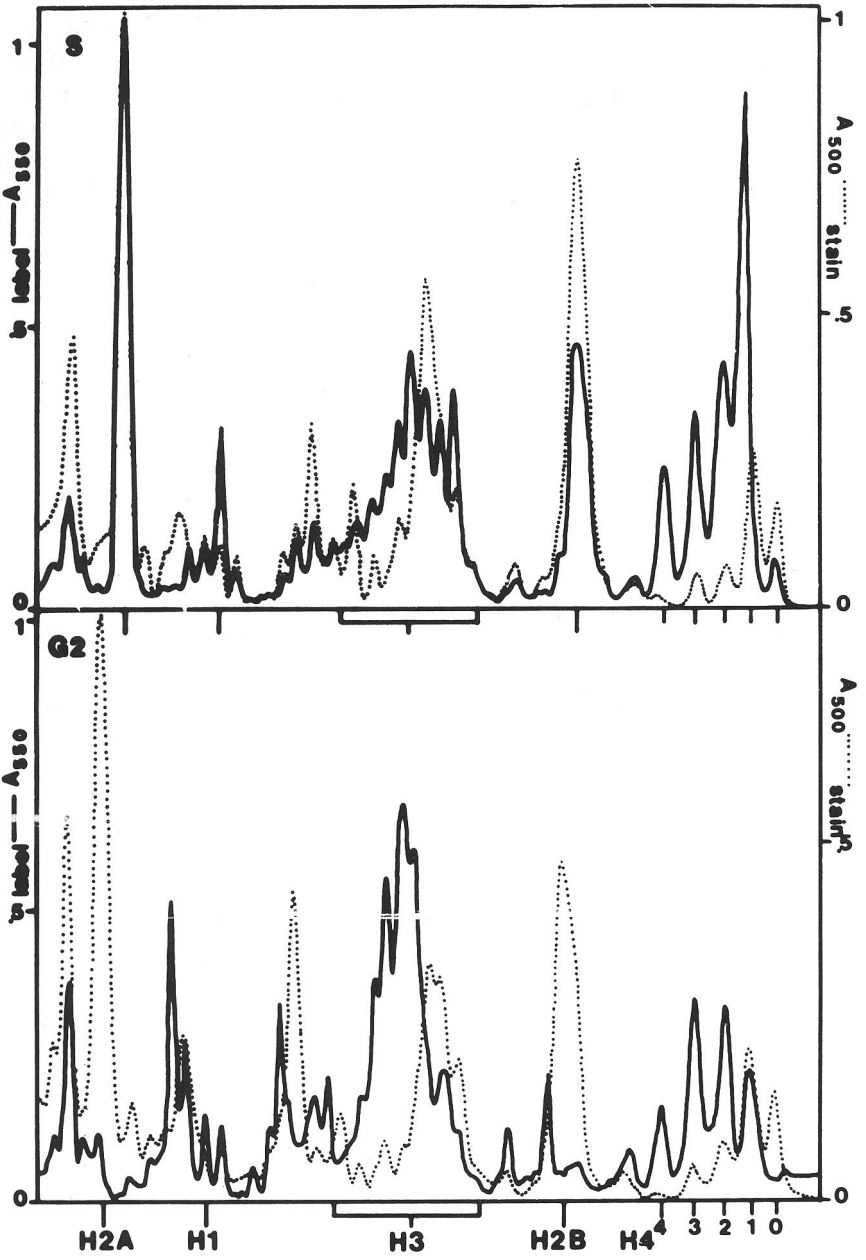

Fig. 1. Histone acetylation in S phase and G2 phase in *Physarum*. Macroplasmodia were radioactively labeled in S phase (top panel) and G2 phase (bottom panel) with [^{3}H]-NaAc. Acid–urea–Triton gel electrophoresis was from left to right. The Coomassie Blue-stained gel patterns (dotted lines) and fluorography patterns (continuous line) were aligned by computer (see Materials and Methods). The position of H2A, H1, H3, H2B, and H4, non- through tetra-acetylated, is indicated.

2. Comparison of the stain and the radioactive patterns for H3 and H4 (Fig. 1) shows that only a fraction of these histones is involved in this modification reaction (*2*).

3. The rate of acetate turnover on H4 and H3 is extremely rapid, nearing equilibrium by 10 min (*1*). Apparently, histones H2A and H2B do not exchange acetate to any detectable extent within 10 min (*2*).

4. The acetate labeling of H4 and H3 is severely inhibited when RNA synthesis is inhibited to a major extent by the transcription inhibitor cordycepin (Fig. 2) (*2*).

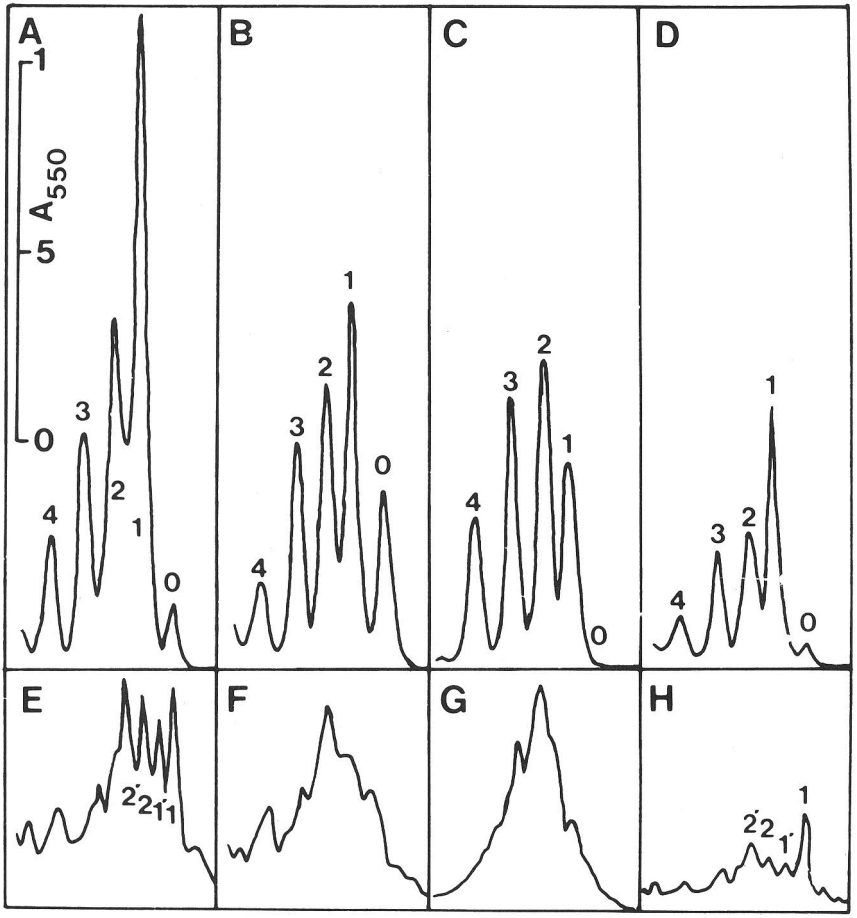

Fig. 2. Acetate incorporation in histones H4 and H3 in the presence of inhibitors. Plasmodia were grown for a short time in the presence of a specific inhibitor and then pulse-labeled for 5 min with [^{3}H]-NaAc in S phase. The histones were analyzed by acid–urea–Triton gel electrophoresis. The fluorography pattern of H4 (A–D) and of H3 (E-H) after electrophoresis from left to right are given after labeling in the absence of any inhibitor (A, E), labeling in the presence of fluorodeoxyuridine (B, F), of cycloheximide (C, G), or of cordycepin (D, H). The position of non- through tetra-acetylated H4 (0–4) and of mono- and di-acetylated H3 are indicated as 1 and 2 for the higher mobility form of H3 and 1′ and 2′ for the lower mobility form.

5. In lexosomes, labile nucleosomes derived from transcriptionally active chromatin by limited digestion with micrococcal nuclease, previously called peak A particles (*15*), the specific radioactivity of acetate-labeled H4 and H3 is greater than that in mononucleosomes. This parallels the enrichment of lexosomes by transcribed sequences, such as ribosomal DNA, compared to mononucleosomes in general (Table 1) (*2*).

Observations 1 through 5 support the idea that the rapid turnover of acetate on H4 and H3 is specific for transcriptionally active chromatin. No acetate turnover occurs on H2A or H2B. The data also show the absence of acetate turnover on the nucleosomal core histones in the bulk of the chromatin.

S Phase Histone Acetylation

During the S phase of the cell cycle, when gene transcription occurs in a process coupled to the progress of DNA replication (*16*), a G2-like pattern of acetylation of H4 and H3, predominantly at the higher levels of modification, seems to be superimposed on a pattern specific for S phase (*2*). This S phase-specific acetylation pattern shows acetylation of histones H2A and H2B (Fig. 1), of mono- and di-acetylated H4 (Fig. 3) and of mono-and di-acetylated H3 (Fig. 4). H3 in *Physarum* exists in two forms that differ slightly in their mobility on acid–urea–Triton gels (*2*) and thus it shows four S phase-specific modified forms (Fig. 4). In addition, irreversible acetate labeling is seen at the amino terminal residue of newly synthesized H1 and H4 (*1, 3, 11*). A number of observations, including radioactive labeling of the newly synthesized histones with tritiated lysine, indicate that all the S phase-specific acetylation occurs exclusively on newly synthesized histones.

6. The rate of accumulation of acetate label in the S phase-specific forms of the histones seems to be constant for labeling periods of up to 30 min (*1*) (Waterborg

Table 1
Specific Radioactivity of G2 Phase Acetate-Labeled Histones H3 and H4[a]

	Specific activity of H3 + H4 arbitrary units	Area fluorography peak/area Coomassie peak
Time of digestion, min	2	16
Enzyme concentration, U/mL	50	70
Lexosomes	3.23	1.41
Mononucleosomes	1.14	1.36
Oligonucleosomes	0.93	1.05
Residual chromatin (matrix)	1.98	2.09

[a]Macroplasmodia were radioactively labeled for 10 min in G2 phase with [^{3}H]-NaAc in the presence of cycloheximide (10 μg/mL). *Physarum* nuclei were digested with micrococcal nuclease at 37°C to a limited extent (2 min at 50 U/mL) and more extensively (16 min at 70 U/mL). The digests were fractionated by sucrose gradient centrifugation (*10*). Histones were prepared from the gradient fractions containing lexosomes, mononucleosomes, and oligonucleosomes, and from the nuclei from which the digested chromatin had been removed. The histones were separated on SDS polyacrylamide gels. The specific radioactivity of the histones was estimated from the Coomassie Blue staining and the fluorography of the gels.

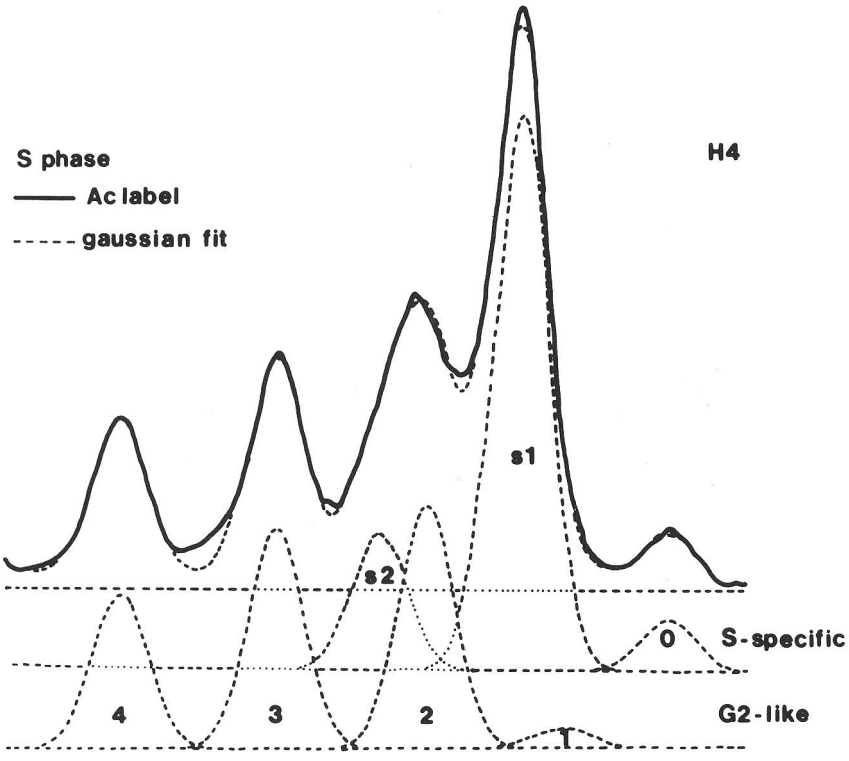

Fig. 3. Acetylation pattern of *Physarum* histone H4 in S phase. The pattern of acetylation of H4 in a S phase fluorogram (Fig. 1) was analyzed by fitting Gaussian distributions to the scan data. The G2 phase-like acetyalted forms (*1–4*) and the non-acetylated form (0) co-electrophorese with the bulk forms of H4. The S phase-specific mono- and di-acetylated forms (s1, s2) display a reduced mobility. The solid line is the scan of the fluorograph. The broken line superimposed on the solid line is the sum of the three S phrase-specific Gaussian peaks illustrated below and the four G2-like Gaussian peaks shown at the bottom.

and Matthews, unpublished). This suggests that the reversible acetylation of the histones in the S phase-specific patterns actually shows net incorporation of acetate and that it is not purely a rapid turnover of acetate as seen for the G2 phase pattern.

7. The S phase-specific pattern of histone acetylation in the absence of a G2-like pattern was observed after S phase labeling with radioactive lysine in soluble histones (*2*). The modification by acetylation of these newly synthesized histones occurred presumably on the new histones prior to chromatin formation.

8. Inhibition of histone synthesis by cycloheximide completely abolished the S phase pattern of acetylation (Fig. 2). The G2-like pattern is unaffected (*2*).

9. The S phase pattern of H4 is exclusive to newly synthesized H4 that displays a reduced mobility in the acid–urea–Triton polyacrylamide gel system (*2, 11*). No S phase-specific acetylation is seen on pre-existing H4 (Fig. 3).

10. The incorporation of acetate in the S phase-specific pattern of acetylation for all the histones displayed a short lage phase identical to that seen for the irre-

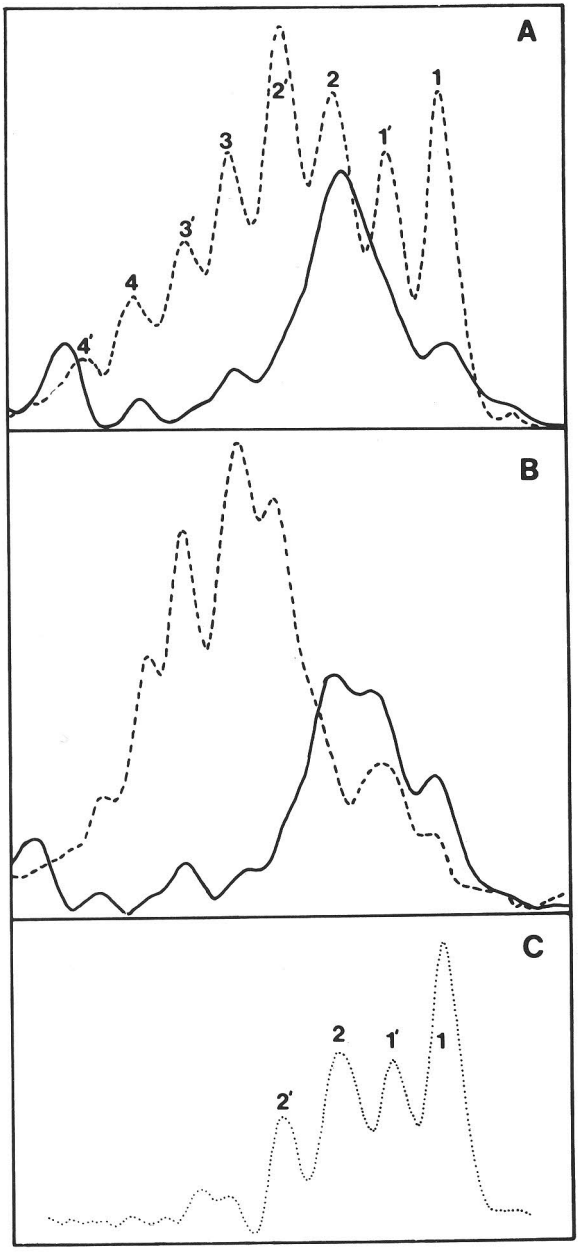

Fig. 4. Acetylation of H3 in S phase and G2 phase. Detail of the H3 region of Fig. 1. The solid line is a scan of the stained gel. The broken line is a scan of the fluorograph. The top panel (A) gives the S phase and the middle panel (B) the G2 phase pattern. The H3 bands are numbered according to the number of acetyl-lysines per molecule (1 through 4 represent one subfraction of H3, 1′ through 4′ represent the other subfraction of H3). The scans of the S phase and G2 phase fluorographs were aligned using H2A and H2B bands as markers and scaled so that the H2A and H2B bands overlapped exactly. The G2 phase scan was then subtracted, point by point, from the S phase scan and the resulting difference scan is shown in C (dotted line).

versible amino terminal acetylation of newly synthesized H1 and H4. The G2 phase acetylation did not display any lag in label incorporation (*1*) (Waterborg and Matthews, unpublished).

Observations 6 through 10 indicate that the S phase-specific acetylation of the core histones is specific for newly synthesized histones. This has been most directly proven for H4. The complete disappearance of the S phase-specific acetylation pattern for all the core histones by cycloheximide suggests that it is true for all core histones (*2*). Thus the core histones synthesized in S phase as unmodified polypeptides acquire some level of acetylation, possibly already resembling the steady-state level of acetylation seen in the chromatin, prior to their deposition on the DNA in newly assembled nucleosomes.

Inhibition of DNA synthesis by fluorodeoxyuridine (FUdR) or hydroxyurea (HU) slightly affected the pattern of acetylation on new H4 and H3. The pattern of modification of both histones shifted towards a lower level, both qualitatively and quantitatively (Fig. 2). The S phase acetylation of H2A and H2B and the G2 phase pattern of all histones remained unaffected (*2*). This indicates that the complete acetylation of newly synthesized H4 and H3 depends on continuation of DNA synthesis. However, this does not necessarily mean that this acetylation occurs on all the new nucleosomes at the replication fork. H4 and H3 in nucleosomes assembled on transcriptionally active genes may immediately start to participate in the G2-like turnover of acetate. It could be this acetylation that is affected by the inhibition of DNA synthesis because inhibition of DNA synthesis will also directly inhibit the tightly coupled process of transcription of newly synthesized chromatin (*16*).

No acetate turnover is apparent on pre-existing nucleosomes when they participate in replicating chromatin structures. However, it is possible that pre-existing nucleosomes on active genes may turn over acetate groups on H4 and H3 while they pass through the replicating process.

What do these observations imply for the structure of chromatin?

How must we envision the acetate turnover in transcriptionally active chromatin by histone acetyltransferases and deacetylases?

Chromatin Structure

Some relevant aspects of the chromatin structure, discussed in detail elsewhere (*13*) include:

1. Functionally inactive chromatin is observed in most studies as a chromatin fiber of approximately 30 nm diameter in which the nucleosomes are arranged in a solenoid or superbead conformation, depending on the method of preparation (*17, 18*); however, see re00 *19*.

2. Chromatin in a potential or actual state of transcription has a conformation in which individual nucleosomes as "beads-on-a-string" are not in contact with each other, but are separated by linker DNA (*20*).

3. The conversion from 30 nm fiber to "beads-on-a-string" is thought to be caused by an increase in the steady-state level of acetylation in the amino terminal random coil regions of the core histones, to a multi-acetylated level. This abolishes the positive charge of these regions of the histones so that their binding to

DNA, presumably the DNA of neighboring nucleosomes in the 30 nm fiber, is lost and the nucleosomes lose internucleosomal contact (*13, 14, 21*).

Previously it was thought that all four core histones participate in this process in the same way. However, the acetylation data obtained in the G2 phase of the *Physarum* cell cycle suggest that only H4 and H3 play a major role (*1*). Histones H4 and H3 differ from H2A and H2B in several ways. Generally the level of acetylation seen on H4 and H3 is significantly higher (*13*). Multi-acetylation in H4 and H3 occurs on all amino terminal lysines (*11, 12*), but in H2A and H2B on only a limited number of them (*13*). Thus the positive charge of the amino termini of these histones is never completely negated as it is for H4 and H3.

4. In chromatin in general, no differences are apparent between the amino terminal regions of the core histones. They all seem to be mobile, as seen by NMR in nucleosomes, or at least are located in such a way on the surface of chromatin, inactive or active, that they are readily digestible by proteolytic enzymes (*13, 22–28*).

5. The increased sensitivity of transcribing and replicating chromatin to digestion by nucleases implies that these states of chromatin are in an open, extended conformation (*13, 29*). Polymerase and ligase enzymes can work within this chromatin and so, supposedly, can the enzymes involved in the turnover of acetate on the histones.

How then can we explain why the nuclear histone acetyltransferase and deacetylase enzymes do not give any measurable turnover of acetate on the histones in inactive chromatin?

How can the extremely rapid turnover in active chromatin be restricted to H4 and H3?

Specificity of Histone Acetylation for Transcription

The specificity for H4 and H3 may reside within the acetyltransferase enzyme (histone acetyltransferase A/DB) in the way it interacts with nucleosomes. On histones free in solution, the enzyme will acetylate all potential sites in the amino terminal regions of all four core histones. However, it acetylates preferentially H4 and H3 when nucleosomes are given as substrate, especially under conditions that may mimic a nucleosome destabilized in a way that may exist in active chromatin, e.g., by polyamines (*30–33*). Histone deacetylase enzymes so far have failed to show any preference for specific histones.

The absence of acetylation or deacetylation of histones in inactive chromatin in the presence of apparently readily accessible substrate sites can only be explained if these enzymes are not freely present in the nucleoplasm. They must be sequestered or localized in such a way that they are only in contact with specific subsets of chromatin.

It has been known since the start of the study of histone acetyltransferase that only extractions of nuclei by high ionic strength will solubilize this enzyme, acetyltransferase A/DB (*13*). It binds tightly to naked DNA during chromatography over DNA-cellulose columns (*34, 35*). Isolated nucleosomes seem to contain the enzyme bound to or within their structure (*32, 36*).

Quantitative measurements of histone deacetylase have proven to be very difficult. Solubilization of the enzyme, e.g., by high salt extraction and sonication of nuclei of *Physarum,* has always led to a rapid decline in the total activity observed (Waterborg and Matthews, unpublished). Only the recent introduction of a peptide substrate for this enzyme (*37*) makes it possible to measure the total amount of nuclear histone deacetylase activity (*38*). Our observations suggested that the enzyme only remained stable if retained within the larger sedimentable structure of the nucleus. High salt extractions and sonication always solubilized the DNA much more readily than the deacetylase activity (Waterborg and Matthews, unpublished). The nuclear deacetylase in calf thymus has also been observed in heterogeneous complexes with molecular weights as high as 600,000 D (*39*). Recently, Hay and Candido reported that the histone deacetylase activity in HeLa cell nuclei was localized within the salt-stable nuclear matrix structure (*40*).

DNA replication in eukaryotic cells is localized on the nuclear matrix (*41–45*). Very recently, several studies have suggested that the same may be true for transcription (*46–50*). The ovalbumin and conalbumin genes in chicken are not localized near the nuclear matrix when in brain, a tissue that does not express these genes. However, in chicken oviduct where they are expressed, they are found attached to the nuclear matrix at multiple points. These two genes selectively and reversibly associate with the matrix upon estrogen stimulation and withdrawal (*50*).

These observations lead us to propose that the specificity of histone acetylation does not reside in the chromatin structure itself, but in its intranuclear localization at or near the nuclear matrix. Thus, the turnover of acetate on histones H4 and H3 is a localized process defined by the presentation of a gene to be activated (chosen and transported by a yet unknown mechanism) to the nuclear matrix. There the localized enzymes increase the steady-state level of acetylation of H4 and H3. This then converts the structure into a more open and readily transcribable one of nucleosomal "beads-on-a-string." Subsequently, this matrix-associated chromatin displays the rapid turnover of acetate that is correlated with transcriptionally active chromatin. The rate of this turnover may actually be correlated with the movement of RNA polymerases. When transcription in *Physarum* is stopped by cordycepin, a significant drop in the incorporation of acetate during a short pulse was noted (Fig. 2). However, the inhibition of acetate turnover was only partial and did not change with increasing dose (50–200 μg/mL) (*2*). This may indicate that part of the acetate turnover is dependent on actual transcription.

The notion that transcriptionally active chromatin with rapid acetate turnover is localized at or very near the nuclear matrix is supported by the observation in *Physarum* that the specific activity of G2 phase acetate-labeled H4 and H3 in the chromatin remaining at the nuclear matrix was increased after micrococcal nuclease digestion. This mimics the behavior of the lexosomes (Table 1).

The assumption that it is at the matrix that the steady-state level of histone acetylation in chromatin may increase to a multi-acetylated state under a constant turnover of acetate implies that the balance between acetylation and deacetylation results in net acetate incorporation. This may be based on the relative amounts of the two enzymes. However, external factors may be involved. It has been found

that High Mobility Group proteins 14 and 17 (HMG 14 and 17) may inhibit histone deacetylase activity (*51*). These proteins have been preferentially located within active chromatin (*13, 51*) and they have recently been reported to be preferentially bound to the nuclear matrix (*52*).

Specificity of Histone Acetylation in S Phase

The localized process of chromatin acetylation, specific for H4 and H3, must clearly be different from that by which the newly synthesized histones, all four core histones, acquire their S phase-specific acetylation prior to histone deposition (*2*). Cytoplasmic histone acetyltransferase B will acetylate newly synthesized H4. However, it is inactive on the other core histones (*32, 53–55*) and no other cytoplasmic or free nuclear acetyltransferases are known (*13*). We suggest that the new histones enter the nucleoplasm in an unacetylated state, with the probable exception of H4. In the nucleoplasm, the new histones, free in solution, interact *at random* with the nuclear acetyltransferase A/DB and nuclear deacetylase at their fixed locations. In this way they acquire a low but significant level of acetate modification. The specificity of acetyltransferase A/DB for H4 and H3, as seen with nucleosomes, is absent on these free histones as it is in the in vitro assays for acetyltransferase activity with soluble histone substrates. From the moment that the new histones interact with each other at the replication fork to form new nucleosomes, the level of acetylation is fixed because the new chromatin in general, like the bulk of the pre-existing chromatin, will not be involved in acetate turnover.

The observation that newly synthesized histone H3 has the same level of acetylation as pre-existing H3 (*55–56*) supports this interpretation. In Physarum the level of S phase-specific acetylation of H4 and H3 is also similar to that observed for the steady-state level of acetylation of these histones (Figs. 1, 3, and 4) (*2*). The same is true for the level of histone acetylation at the replication fork of SV40 and HeLa cell chromatin, which is as low as that of the bulk of the chromatin (*57, 58*).

It was found, as noted above, that pre-existing nucleosomes passing through the replication fork, although structurally open and localized at or near the matrix, do not show turnover of acetate to any measurable extent. It has also been noted that maturation of newly formed chromatin does not depend on histone deacetylation (*59*). However, new H4 and H3 showed a limited turnover of acetate dependent on continuation of DNA (or RNA) synthesis, as indicated by the fluorodeoxyuridine and hydroxyurea inhibition data obtained for *Physarum* (Fig. 2). One should also expect that new H4 and H3 that participates in chromatin on active genes will show as rapid a turnover of acetate as the pre-existing nucleosomes on such genes.

The Slow Turnover of Acetate in Chromatin

The methodology used in the study of histone acetylation in *Physarum* cannot detect the metabolism of acetate that occurs in slow processes (*1*). The observed localizations of nuclear acetyltransferase and deacetylase enzymes does not pre-

clude that they may exist to a minor extent free in solution within nuclei. This could give rise to a slow and general turnover of acetate groups on all nuclear chromatin. Such turnover would not be detected in *Physarum*. Chalkley and coworkers have reported that in HTC cells in addition to a very rapid process of deacetylation (half-life, 3 min), deacetylation also occurs at a much slower pace (half-life, 30 min) (*60, 61*). Addition of butyrate, a potent inhibitor for histone deacetylase (*62*), will cause a virtually instantaneous rise in acetylation to a hypermodified state for transcriptionally active chromatin. The bulk of the chromatin increases its level of acetylation (half-life, 3–5 h) only very slowly, and some parts of the inactive chromatin do not increase at all (*63, 64*). Thus, if a slow turnover of acetate exists, the use of butyrate indicates that its rate of turnover is much lower than the process occurring at the matrix on transcriptionally active chromatin.

The only chromatin that also acquires a hyperacetylated state rapidly in the presence of butyrate, is newly replicated chromatin (*65*). As reasoned above, this is to be expected since the newly synthesized histones acquire prior to deposition a much higher level of acetylation through random interaction with acetyltransferases because the deacetylation reaction is blocked. Thus the newly formed chromatin is already hyperacetylated upon formation and may, like bulk chromatin, increase further.

The slow turnover of acetate on chromatin not localized at the matrix could be functionally important. It may be the mechanism through which a switched-off gene that is released from the matrix in a highly acetylated state may slowly return to a much lower level of modification that is determined by the random interaction with acetyltransferase and deacetylase enzymes. Thus it could return from the potentially active conformation of "beads-on-a-string" to an inactive 30-nm chromatin fiber.

Acknowledgments

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