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Patterns of histone acetylation in *Physarum polycephalum*

H2A and H2B acetylation is functionally distinct from H3 and H4 acetylation

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Histone acetylation has previously been correlated with both chromosome replication and transcription. We present evidence that (a) confirms both correlations in the true slime mold, *Physarum polycephalum* and (b) shows that quite a different pattern of acetate turnover is associated with replication compared with transcription. The pattern asssociated with replication involves turnover of acetate on all four core histones on species containing one or two acetates per molecule. This pattern was resolved from the transcription-associated pattern by three different procedures: (a) detailed analysis of gels of histones pulse-labelled with acetate; (b) the pattern of acetylation of histones pulse-labelled with [^{3}H]lysine; and (c) the pattern of acetylation of soluble histones. The pattern associated with transcription is restricted to histones H3 and H4 and occurs mostly on highly acetylated species. This pattern was resolved by (a) analysis of gels of histones pulse-labelled with acetate; (b) the pattern of histone acetylation in G2 phase of the cell cycle; and (c) the pattern of histone acetylation in the presence of cycloheximide.

The nucleosome is recognized as the basic structural unit of chromatin. The electron microscope appearance and biochemical properties of nucleosomes vary according to the current function of the DNA in the nucleosome but the structural features of these variations and the signals that control them are still under investigation (for a review see Igo-Kemenes et al. [1]). Some years ago it was proposed that histone acetylation was involved in transcription (e.g. [2,3]) or replication (e.g. [4,5]) and a number of attempts has been made to reconstitute nucleosomes with acetylated histones with a view to determining the effect of acetylation on nucleosome structure. Most of the observed effects have been minor [6–13]. This may be due to inadequate reconstitution procedures, insensitive tests, use of the wrong pattern of acetylation or absence of continuous turnover of acetate. In order to focus the reconstitution studies, it is important to determine the actual patterns of acetylation associated with chromosome replication and transcription *in vivo*. Previous work has generally not studied the differences between replication-associated and transcription-associated acetylation in the same system and various patterns have been reported. In the present study we have identified and described two distinct patterns of acetylation *in vivo* in the same system, thus eliminating possible confusion due to cell-specific variations. We have collected evidence that one pattern is associated with newly synthesized histones while the other is associated with a sub-set of pre-existing histones and correlates with transcription.

MATERIALS AND METHODS

Labeling of Physarum

Physarum polycephalum, strain M3C, was cultured and nuclei and histones were isolated as described [14–19]. Care was taken during histone isolation to maintain the ratio of 1 ml Bio-Rex 70 resin per extract from 10^9 nuclei. To increase the column volume for elution of histones from the resin from 0.02–0.10 ml to approximately 1 ml, an appropriate amount of swollen Sephadex G-25 fine was added to the Bio-Rex 70 resin before equilibration with the histones.

Histone isolation from cytoplasm followed the same protocol except for the following changes. The first post-nuclear supernatant was respun for 15 min at 7000 × *g* at 4 °C to remove all nuclei and nuclear fragments. The supernatant was made 40% (w/v) in guanidine hydrochloride and 0.1 M potassium phosphate pH 6.8 by adding dry salt and concentrated KOH solution. It was spun for 10 min at 30000 × *g*, acidified to 0.25 M HCl and respun for 30 min at 400000 × *g* [16]. The supernatant was diluted to 5% guanidine hydrochloride with 0.1 M potassium phosphate buffer pH 6.8 and equilibrated with 1.25 ml fresh Bio-Rex 70 resin per macroplasmodium. Histones were eluted from the resin with 40% guanidine hydrochloride in phosphate buffer.

Macroplasmodia were labeled on 0.5 ml pulse medium with 2.1 mCi sodium [^{3}H]acetate (NEN 1.6–3.0 Ci/mmol or ICN 4 Ci/mmol) for 5 min, unless stated otherwise [19]. For [^{3}H]lysine-labeling experiments macroplasmodia were grown in a low-lysine medium containing Bacto-soytone (Difco) as suggested by Turnock et al. [20]. Macroplasmodia grown in this same medium were labeled with 0.5 mCi [^{3}H]lysine (NEN 68.4 Ci/mmol) for 20 min, essentially following the protocol for acetate labeling. Pulse labeling in S phase started at 20 min after the second or third metaphase after fusion of microplasmodia. Pulse labeling in G2 phase was done approximately 5 h after the second mitosis. Cell cycle times ranged from 8 h to 10.5 h. For chase experiments after lysine pulse-labeling, the macroplasmodia were transferred to fresh soytone growth medium to which 0.15 mg/ml lysine had been added [21].

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 [22,23]. Puromycin was used

in the same way [24]. The DNA synthesis inhibitor 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 μM) to prevent inhibition of RNA synthesis [25,26]. A 50-fold concentrated stock solution was made in 0.1 M $NaHCO_3$. Hydroxyurea was used at 50 mM [27] or at 1 mM [28], as a 50-fold concentrated stock solution in water, filter-sterilized, with a 30-min pre-incubation. Cordycepin (3′-deoxyadenosine) was added from a 25-fold concentrated stock solution in 25% ethanol and was used at 200 μg/ml (except when noted) [29] during the 60-min pre-incubation and during the labeling. All stock solutions were sterilized by filtration.

Separation of histones

Physarum histones, isolated from macroplasmodial nuclei, were separated on 15% acid/urea/Triton X-100 gels (15 × 30 cm, 0.5 mm thickness) according to Bonner et al. [30,16]. On analytical gels up to 20 samples of histones from approximately 25×10^6 nuclei (1/4 macroplasmodium) were run while on a preparative scale histones from 5×10^8 nuclei were separated on one gel. Gels were Coomassie-stained, destained and photographed prior to impregnation with PPO (2,5-diphenyloxazole) for fluorography at −70 °C [31,32] with preflashed Kodak XAR-5 film, as described [19].

Fluorographs and Polaroid negatives of stained gels were scanned with a Cary 210 spectrophotometer equipped with a gel scanner and a digital interface port. 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 confirmed visually by superimposing fluorograph and dried gel. Gaussian curves were fitted to some of the scans using an interactive program developed for this purpose. The programs for scanning, filtering data, aligning scans, integration of peak areas and Gaussian fitting, in BASIC, are available on request [33].

RESULTS

Two patterns of acetylation

Histones were isolated from *Physarum* plasmodia pulse-labeled with [^{3}H]acetate for 5 min in either S phase or G2 phase of the naturally synchronous mitotic cycle. The histones were analyzed by gel electrophoresis; Fig. 1 shows both the pattern of Coomassie blue stain and the pattern of radioactivity obtained. The pattern of stained bands is similar to that previously reported [15–18] for histones from *Physarum* microplasmodia. A number of non-histone bands is visible. These bands may represent other chromosomal proteins, for example high-mobility group (HMG) proteins, H1° or ubiquitin-histone conjugates as discussed in [16] or they may be other nuclear proteins involved, for example, in the nuclear matrix. None of these proteins appears to undergo rapid acetylation. We observed only minor quantitative differences in the patterns of stain produced by histones from S or G2 phase; for example, the pattern of histone H1 is different. This difference reflects a change in the phosphorylation of H1, which changes through the cell cycle as previously described [58,59]. Notice that H1 is only labelled with acetate in S phase. This small amount of label presumably represents labelling of *N*-acetylserine in H1 and is analogous to the labelling of *N*-acetylserine in H4 which gives rise to a small amount of label in ac_0H4. In the case of H1, only a single band is labelled, implying that only one electrophoretic species of H1 was being synthesized at that time and suggesting that the other species of H1 seen in the stain pattern are due to post-synthetic modification. The patterns of radioactivity are very different between S and G2 phases, particularly in the absence of labeling of H2A and H2B in G2 phase and in the degree of labeling of monoacetylated histone H4 and mono- and di-acetylated histone H3. These results confirm the labelling results previously reported [19] and extend them by providing higher resolution and showing both stain and label patterns from the same gel lanes.

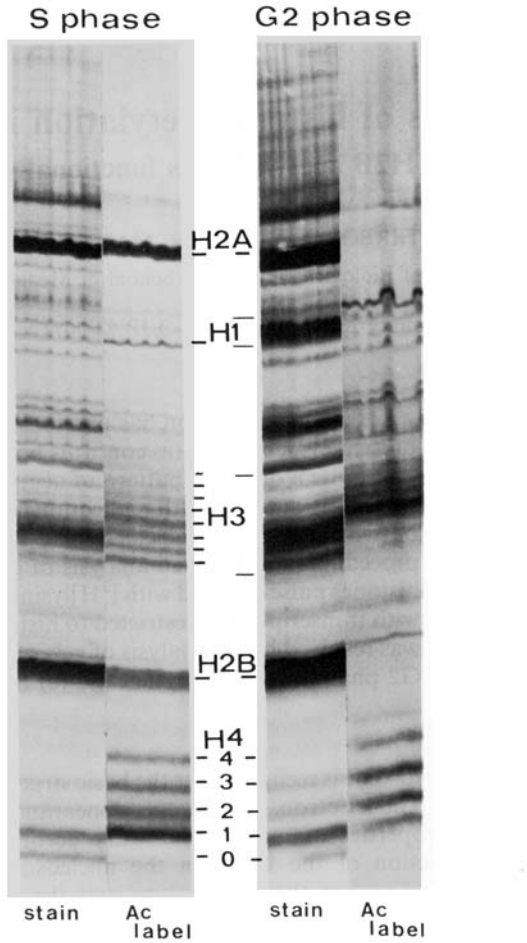

Fig. 1. *Acetylation of* Physarum *histones in S and G2 phase.* Macroplasmodia were labeled with [^{3}H]acetate for 5 min in either S phase or G2 phase. Histones were isolated and analyzed by acid/urea/Triton gel electrophoresis. The gel was stained and photographed and then subjected to fluorography. The tracks shown are, from left to right, S phase histone stain pattern and fluorograph, then G2 phase histone stain pattern and fluorograph. The histone bands H1–H4 are identified and the different acetylated forms of H4 are numbered 0 to 4 according to the number of acetylated lysines per molecule. Label in H1 and in ac_0H4 in S phase is due to labeling of *N*-acetylserine

The higher resolution led to the observation that labeled ac_1H4 in S phase did not exactly co-migrate with its stained equivalent. The difference is small and not easy to see in comparing prints as in Fig. 1 but it was clearly seen when the fluorograph was placed over the stained, dried, gel and was found reproducibly. It can also be clearly seen by comparing a

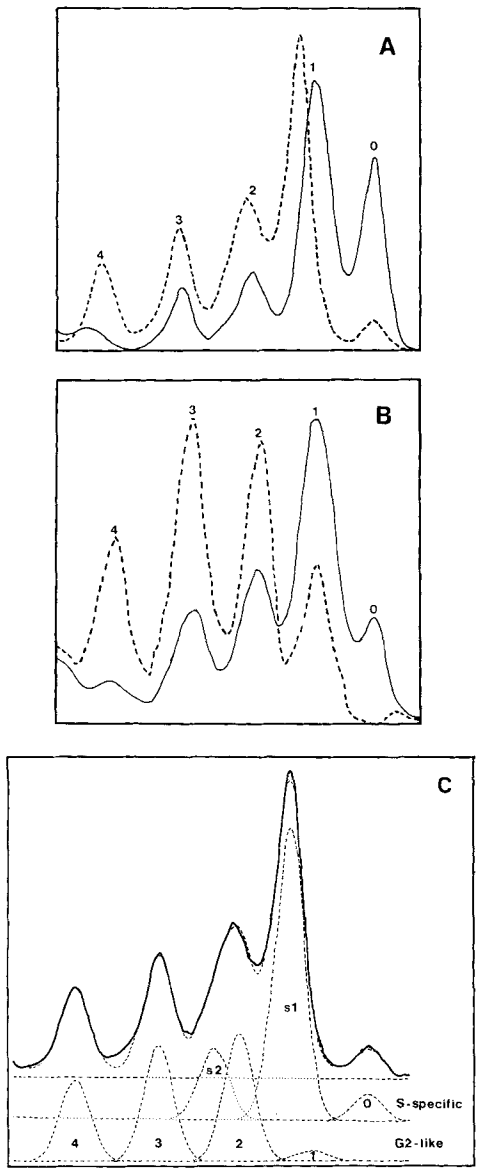

Fig. 2. *Detail from the H4 region of gels similar to those of Fig. 1.* (A) S phase. (B) G2 phase. (——) Scan of the stained gel; (– – –) scan of the fluorograph. Only the H4 region is shown; the H4 bands are numbered according to the number of acetyllysines per molecule, electrophoresis was from left to right. (C) (——) Scan of the S phase fluorograph (same as the broken line in A); the broken line superimposed on the solid line is the sum of the three new histone Gaussian peaks illustrated below and the four G2-like Gaussian peaks shown at the bottom

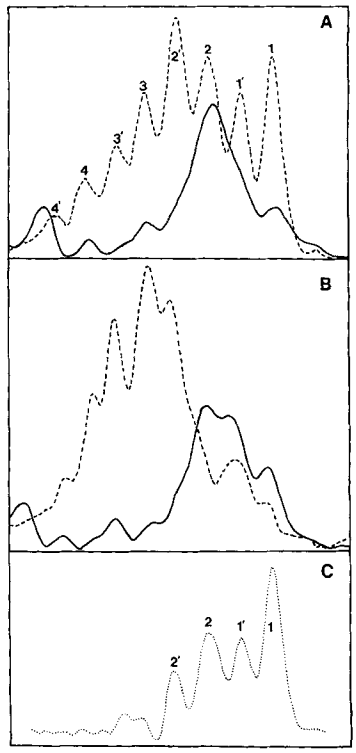

Fig. 3. *Detail from the H3 region of gels similar to those of Fig. 1.* (A) S phase. (B) G2 phase. (——) Scan of the stained gel; (– – –) scan of the fluorograph. Only the H3 region is shown; the H3 bands are numbered according to the number of acetyllysines per molecule (1 –4 represent one sub-fraction of H3, 1′ –4′ represent the other sub-fraction of H3); electrophoresis was from left to right. (C) The scans of the S phase and G2 phase fluorographs were aligned using the 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. The numbers refer to ac_1H3 and ac_2H3 as in A

scan of the fluorograph with a scan of a negative of a photograph of the stain patterns. Examples of such scans are shown in Fig. 2. These scans were obtained by linking a gel scanner to a computer [33]. The scans were then aligned using interactive computer graphics and linear transformations so that the peaks corresponding to histone H2A and H2B were accurately superimposed. When this was done, the results shown in Fig. 2 were obtained. Notice that in G2 phase (Fig. 2B) the three major peaks derived from labeled H4 (1 –3 acetates per molecule) are exactly superimposed on the corresponding three peaks of stained H4 while in S phase the ac_1H4 peak of label is clearly displaced from the corresponding peak of stained H4 and the ac_2H4 peaks are slightly displaced. These results confirm those obtained by laying the fluorograph over the stained gel. (In both sets of scans we have ignored the stain in the region of ac_4H4 since this region is very easily affected by a minor band that runs close to the position of ac_4H4 as previously noted by Chahal et al. [34]. The label in ac_0H4 in S phase is due to labeling of *N*-acetylserine during histone synthesis.)

The scans of the fluorographs shown were analyzed in terms of overlapping Gaussian peaks. The G2-phase scans were always described accurately by the sum of four Gaussian peaks but the S phase pattern of radioactivity was composed of two groups of underlying peaks. The first group, shown as Gaussians 1 –4 in Fig. 2C, is qualitatively similar to the complete pattern seen in G2 phase (Fig. 2B) and will be termed the G2-like component of the S phase pattern; the second group, shown as Gaussians 0, S1 and S2 in Fig. 3, comprises bands seen only in S phase and experiments described below show this is due to acetylation of recently synthesized histone. This group of bands will be termed new histone acetylation. The sum of the G2-like component and the new histone component accounts quantitatively for the observed S phase pattern of radioactivity as shown by superimposing the sum and the actual scan, as in the upper part of Fig. 2C. The root-mean-square difference between the sum of Gaussians and the actual scan was an absorbance of approximately ± 0.02 which

is comparable with the estimated experimental errors in fluorography and scanning in each of the three experiments analysed in this way.

In the case of H3, the stain and radioactivity patterns co-migrated in both S and G2 phases (Fig. 3A, B). H3 occurs as two sub-fractions in *Physarum* which may be due to sequence differences or to another, unknown, post-synthetic modification. Label was not observed in non-acetylated H3 since it does not have an *N*-acetyl terminus [16, 35]. In Fig. 3A the numbers 1–4 and 1′–4′ indicate the number of acetate groups per molecule on each of the two sub-fractions of H3. Fig. 3C shows the result of subtracting the scan of the G2 phase fluorograph from the scan of the S phase fluorograph, point by point. This subtracted scan represents a predicted new histone pattern for H3. It is not expected to be quantitatively accurate since the G2-like component of the S phase pattern of acetylation may not be identical to the G2 phase pattern of acetylation particularly because the rate of transcription and number of sequences being transcribed vary between S and G2 phases. However, the result does predict that the H3 molecules with low acetate content are characteristic of new histone acetylation and that H3 moelcules with high acetate content are characteristic of G2-like acetylation, similar to the patterns for H4.

The S and G2 patterns were observed previously but the present analysis suggests that the underlying patterns are the new histone pattern and the G2-like pattern, with the observed S phase pattern being the sum of the new histone pattern and the G2-like pattern. The remainder of this study is concerned with experiments to test this conclusion. Firstly, the new histone pattern was separated from the G2-like pattern experimentally by (a) looking at soluble histones and (b) looking at histones labelled with a short pulse of [^{3}H]lysine. Secondly, the G2-like pattern was separated from the new histone pattern, in S phase, by inhibiting protein synthesis.

The new histone pattern

A very small proportion of the S phase acetate-labeled histone is recovered in the wash fractions consisting of cytoplasm and any components removed from nuclei by the homogenizing medium. The amounts were too small to be seen except with acetate labeling. In S phase, this soluble fraction shows a pattern of acetate label similar to the new histone pattern and the G2-like components are missing. Fig. 4 shows how each of the core histones is labeled but highly acetylated forms of H3 and H4 are absent. In addition, the position of ac_1H4 corresponds to the slow or new histone position. We interpret the soluble fraction of histones as being newly synthesized histones in the process of being deposited on chromatin, although we were unable to achieve sufficient specific activity in the lysine labeling to confirm this. Fig. 4 provides confirmation that the new histone pattern of acetylation exists separately from the G2-like pattern. No soluble histones could be detected in G2 phase.

The S phase pattern of acetylation was probed further by pulse-labeling with [^{3}H]lysine to label newly synthesized histones. The labeled histones were isolated and analyzed by gel electrophoresis and fluorography. Fig. 5A (upper scan) shows a scan of the H4 region of the fluorograph. [^{3}H]Lysine is found in the bands containing 0, 1 or 2 acetates per molecule only and the position of the ac_1H4 band corresponds to its position in the new histone pattern of acetate label (Fig. 2C) and does not correspond to the position of stained ac_1H4 (not shown). Thus, the pattern of H4 pulse-labeled with [^{3}H]lysine

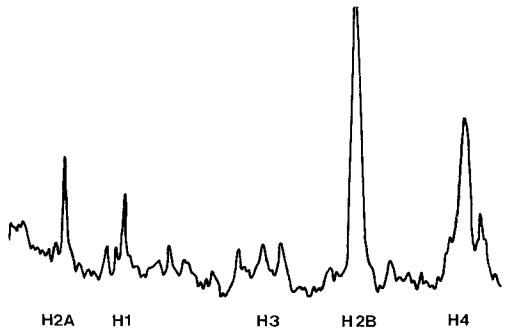

Fig. 4. *Soluble histones.* Histones were isolated from the nuclear isolation medium and analyzed by gel electrophoresis. The [^{3}H]acetate radioactivity was detected by fluorography and the fluorograph was scanned. Radioactive labeling was as in Fig. 1

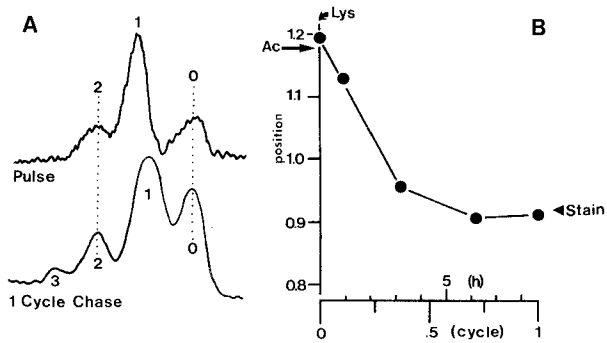

Fig. 5. *Lysine pulse-chase experiment (H4). Physarum* macroplasmodia were pulse-labeled with [^{3}H]lysine for 20 min in S phase and then transferred to medium containing excess non-radioactive lysine for various periods of time. Histones were isolated and subjected to Triton/acid/urea gel electrophoresis and fluorography. The patterns of stain were similar to those shown in Fig. 1. (A) Scans of two fluorographs: the upper scan shows lysine incorporation into histone H4 during the pulse, with no chase; the lower scan shows incorporation after the same pulse but followed by a chase of one cell cycle. The one-cycle chase pattern is very similar to the stain pattern (cf. Fig. 2A solid line). The ac_1H4 band changed position with respect to the adjacent ac_0H4 and ac_2H4 bands during the chase. (B) The position of the ac_1H4 band as a function of chase period time (0–9 h) or as a fraction of the cell cycle (0–1 cycle). On the abscissa, zero would represent the position of ac_0H4, 2.0 would represent the position of ac_2H4 and 1.0 is the position halfway between ac_0H4 and ac_2H4. The arrows labeled Ac, Lys and stain show the position of ac_1H4 when observed as acetate pulse-labeled material (5-min pulse), [^{3}H]lysine pulse-labeled material or as stained material, respectively

corresponds qualitatively with the new histone components of [^{3}H]acetate-labeled H4. We interpret these results to mean that the complete S phase pattern of acetylation is composed of two functional components. The first component, G2-like, is also present in G2 phase and probably represents acetylation of histone H4 in chromatin that is being transcribed or is open for transcription. The second component, new histone, is acetylation of newly synthesized histone H4.

The time course of conversion of newly synthesized ac_1H4 from the new histone electrophoretic mobility to the mobility of the bulk of the ac_1H4 was determined by a pulse-chase experiment. Fig. 5A (lower scan) shows that after a one-cycle chase [^{3}H]lysine is found in a pattern qualitatively similar to that of stained H4. Fig. 5B shows that the electrophoretic mobility of pulse-labeled ac_1H4 increased steadily for approxi-

mately 4 h after the time of the pulse until it reached the mobility of stained ac_1H4 and then remained constant. The molecular basis of this mobility change has not been determined at this time but *Physarum* H4 is post-transcriptionally modified by methylation of Lys-20 and Lys-79 [36] and the time course is consistent with methylation [37].

Fig. 5A (lower scan) shows [^{3}H]lysine in 'old' histone. This includes both active and inactive chromatin. Comparison of this figure with Fig. 2C shows an excess of ac_1H4 in the 'old' histone compared with the G2-like pattern, even after accounting for the number of acetates per molecule. This implies that inactive chromatin largely contains H4 in the ac_1H4 form that is not turning over acetate.

Table 1. *Specific activity of histone H4 with different levels of acetate content (G2 phase)*
Plasmodia were pulse-labelled with [^{3}H]acetate as described in Methods

Acetate content	Protein (area of stain peak)	Radioactivity (area of fluorograph peak)	Relative specific activity
mol/mol H4	%		
0	29.1	0.0	0
1	44.7	16.7	0.37
2	13.2	29.6	1.12
3	9.2	33.7	1.22
4	3.8	20.0	1.31
Total	100	100	1.00

The G2-like pattern

We showed above that the G2 pattern of acetylation persisted through S phase. Since this pattern occurs alone quite naturally in G2 phase when chromosome replication is complete we were able to analyze the pattern in a little more detail. In particular, Table 1 shows the specific activity of each species of H4 in G2 phase. As expected, ac_0H4 is not labelled. The most interesting result is that the highly acetylated species, ac_2H4, ac_3H4 and ac_4H4 show a fairly uniform level of acetylation while ac_1H4 is being acetylated at about a third the rate, on average. This is the same as the result previously observed using [^{3}H]lysine labelling (Fig. 5, above) and supports the conclusion that inactive chromatin largely contains H4 in the ac_1H4 form that is not turning over acetate.

Specific inhibitors of replication, transcription and translation were used to test the proposed functional correlation of G2-like and new histone acetylation with transcription and replication respectively. *Physarum* plasmodia were labeled with [^{3}H]acetate for 5 min in either S phase or G2 phase in the presence of various inhibitors (experimental details are provided in Methods) and the histones were isolated and analyzed by gel electrophoresis. Fig. 6 shows examples of the gel patterns obtained. The lanes shown are taken from a single gel that included a complete set of patterns for each of the inhibitors used in both S and G2 phases. The gel was stained and fluorographed. None of the inhibitors significantly affected the overall histone composition as revealed by the patterns of stain. Inhibition of DNA replication was achieved with either hydroxyurea [27] or fluorodeoxyuridine [25,26]. Very low levels of hydroxyurea (1 mM) which cause inhibition of S phase histone synthesis in CHO (Chinese hamster ovary) cells without affecting basal histone synthesis [28] had no

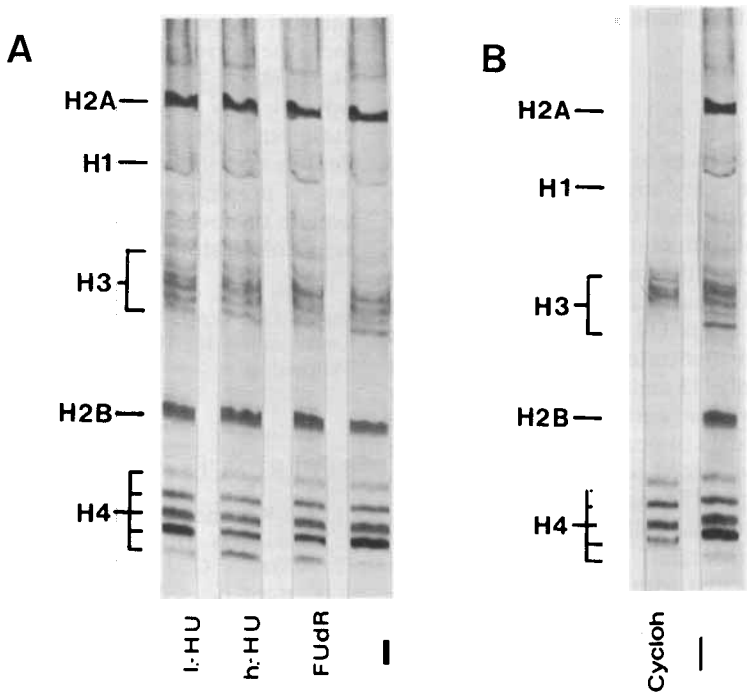

Fig. 6. *Acetate incorporation in the presence of inhibitors. Physarum* plasmodia were grown for a short time in the presence of a specific inhibitor and then pulse-labeled with [^{3}H]acetate. Histones were isolated and analyzed by acid/urea/Triton gel electrophoresis. The figure shows key lanes of the fluorograph of the gel. All the lanes shown are of histones labeled in S phase. (A) The effect of inhibitors of DNA synthesis. The lanes contained, from left to right, histones from plasmodia grown in the presence of hydroxyurea (1 mM), hydroxyurea (50 mM), fluorodeoxyuridine or no inhibitor, respectively. (B) The effect of inhibition of protein synthesis. The left lane contained histones from plasmodia grown in the presence of cycloheximide; the right lane is a control, without inhibitor

significant effect on the pattern of acetylation in S or G2 phase. Fluorodeoxyuridine (5 μg/ml) or higher levels of hydroxyurea (50 mM) also did not affect the pattern of acetylation in G2 phase. In S phase, acetylation of H2A and H2B was not affected and there were only minor changes in H3. H4 acetylation showed a quantitative change in the region dominated by the new histone component of the H4 acetylated pattern, with less label in ac_1H4 and much more label in ac_0H4. Both replication inhibitors produced the same effects (Fig. 6A).

The effects of cycloheximide (10 μg/ml) were dramatic, in S phase. Firstly, the 'background' of lightly labeled proteins disappeared; secondly, the labeling of H1, H2A and H2B stopped; and thirdly, the labeling of H3 and H4 closely resembled their G2 phase patterns of acetylation (Fig. 6B). Under the conditions used, cycloheximide inhibits DNA synthesis as well as protein synthesis [22,23], but the relatively mild effects of inhibiting DNA synthesis alone (as above) suggest that most or all, the effects of cycloheximide are due to inhibition of protein synthesis. This suggests that acetylation of newly synthesized histones accounts for all the acetylation of H2A and H2B, for the new histone component of H4 and for a component of H3 which is analogous to the new histone component of H4. Puromycin does not affect acetylation patterns, consistent with its inability to inhibit protein synthesis in *Physarum*.

Cordycepin (200 μg/ml) [29] is an inhibitor of RNA synthesis and it is the only inhibitor that affected the G2 phase pattern of acetylation, which was reduced in intensity. The extent of the reduction was independent of cordycepin concentration in the range 50 – 200 μg/ml and of the additional presence of cycloheximide at 10 μg/ml. In S phase, cordycepin at the same concentrations produced a similar overall reduction in acetylation. This may reflect an indirect effect of cordycepin on translation since [^{3}H]lysine incorporation into histones was reduced by a similar amount.

DISCUSSION

The main point of this paper is to show that the two proposed functions for histone acetylation may occur simultaneously in the same cell type and that the patterns of acetylation associated with each function are different. The pattern associated with newly synthesized histones involves acetate turnover on mono-and di-acetylated forms of each of the four core histones. In the case of H4 this confirms earlier studies [4,38,39]. The pattern associated with transcribing chromatin is quite different. Only H3 and H4 turnover acetate rapidly and the turnover occurs mostly in the highly acetylated forms. Turnover on ac_1H4 appears to occur in the bulk of the chromatin. Other reports have noted a difference between acetylation of H3 and H4 and H2A and H2B [40 – 46,8]. Notably, Vidali et al. [40] found that calf thymus nuclei only acetylated H3 and H4 and Marzluff and McCarty [46] identified H3 and H4 as associated with transcription.

The resolution and description of these two patterns is of specific importance for reconstitution experiments but some other conclusions are noteworthy. It has been suggested that acetylation acts by reducing the interaction of the N-terminal regions of core histones with DNA, thus relaxing the higher order packing of nucleosomes and allowing transcription or replication to occur [3,7,8,13,19,34,47]. The present results severely restrict this hypothesis. The data imply that no acetylation of pre-existing histones is needed to 'open out' the chromatin structure for replication. This is in accord with the reconstitution results of McGhee et al. [13] who showed that chromatin with hyperacetylated histones formed a 30-nm fibre structure in almost the same fashion as control chromatin. (Hyperacetylation was produced by butyrate treatment which probably acts initially at the replication fork since it prevents de-acetylation of all four core histones (new histone pattern) and since the DNA sequences associated with hyperacetylation are scattered at random throughout the genome [48]). The present data support a role for histone acetylation in nucleosome assembly and maturation extending the original hypothesis [4] to all four core histones.

In the case of the G2 pattern, we recently showed that the acetylated histones were preferentially released by micrococcal nuclease just as active ribosomal genes are released and that peak A particles or lexosomes [49 – 52] were highly enriched in acetylated histones [53]. The G2 pattern may represent the pattern of acetylation in transcriptionally active chromatin. This possibility raises some interesting questions [54]. For example, do H3 and H4 stabilize the inactive nucleosome while H2A and H2B stabilize the active nucleosome?

This paper has been concerned with acetate turnover. We attempted to study the acetate content of the histones by analysis of the patterns of stain. In the case of H4, the resolution of acetylated species was fully adequate but we were prevented from making a satisfactory analysis by a minor band overlapping the ac_4H4 band. This band was previously reported by Chahal et al. [34] but Loidl et al. [55] recently claimed to be able to analyze such gels. The reason for the discrepancy is not clear but it is possible that Loidl et al. [55] failed to resolve the minor band from ac_4H4 and thus they failed to observe the very low levels of ac_4H4 found by Chahal et al. [34] in mid-G2 phase and mitosis [34]. The low value in mitosis was previously observed also in mammalian cells by Gurley et al. [56]. Loidl et al. [55] also mistakenly attributed the correlation of ac_2H4 with replication to Chahal et al. In fact, the correlation is based on a number of other studies [4,38,39,57]. The data from the present study (not shown) do not show any major changes in ac_2H4 content through the cell cycle but the results are not accurate enough to confirm or deny a correlation of ac_2H4 content with replication. The results presented do, however, clearly define the patterns of acetate turnover associated with new histone and with a subset of old histone.

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