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# Segregation of rapidly acetylated histones into a chromatin fraction released from intact nuclei by the action of micrococcal nuclease

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ABSTRACT

It has been previously shown that micrococcal nuclease digestion and subsequent fractionation of hen oviduct nuclei generates fractions enriched (first supernatant fraction - 1SF) and depleted (second supernatant fraction - 2SF) in ovalbumin genes, while a third fraction, the pellet fraction, contains about the same level of this gene as whole chromatin (Bloom and Anderson (1978) Cell 15, 141-150). We have utilized this fractionation method in an attempt to assess the extent and kinetics of histone acetylation associated with chromatin from the 1SF, 2SF, and pellet fraction.

Hepatoma Tissue Culture (HTC) cells were labelled for 30 minutes in vivo with $^{3}$H-acetate, nuclei isolated and the chromatin fractionated. The specific activity of the histones in the 1SF was slightly greater than that of the 2SF (1.2 to 1.6 fold difference) independent of the length of nuclease digestion. If the labelling period is followed by short (10 to 60 minute) treatment of the cells with sodium butyrate, the more rapidly as well as more extensively acetylated histones are also preferentially found in the 1SF. This is in part the result of segregation of chromatin particles into the 1SF as the histones associated with these particles become hyperacetylated. That is, the extent of histone acetylation regulates the distribution of chromatin in the 1SF, 2SF and pellet fraction.

## INTRODUCTION

Acetylation of histones occurs at more than one rate in eukaryotic cells (1), as does the subsequent deacetylation of these proteins (2,3). Although the function of histone acetylation is unknown, it has been postulated that this modification might be necessary for proper deposition of newly synthesized histone on the DNA (4,5) and for expression of genetic information (6,7). In the latter case, it has been argued that acetylation of histones might weaken histone-DNA contacts within the nucleosome or affect interactions between nucleosomes thereby allowing access to, and transcription of, appropriate regions of chromatin. As a result of these arguments, many of the recent studies on histone acetylation have been to ascertain the relationship between this modification and gene expression.

Three nucleases, DNase I, micrococcal nuclease and DNase II appear to

show specificity for active genes and for chromatin containing more highly acetylated histones. Gottesfeld et al. (8) developed a method of fractionating chromatin into active and inactive regions after DNase II digestion, and histone H4 appears to be hyperacetylated in the active fraction (9). DNase I more readily attacks the DNA in active regions of the genome as compared to the bulk of the chromatin (10-14) rendering the DNA acid soluble, and it was subsequently shown that this enzyme also readily attacks chromatin containing hyperacetylated histones (15-18). In these experiments, histone deacetylation was blocked in vivo (19-22) to increase the degree of histone acetylation (23,24). The regions most readily attacked by DNase I in cells not treated with sodium butyrate also appear to contain more highly acetylated histones as compared to the bulk of the chromatin histones (25,26), as well as being enriched for transcriptionally active DNA (27).

Micrococcal nuclease releases nucleosome size transcriptionally active DNA slightly faster than nucleosome size whole DNA (28-31). Conversely, it has been reported that this enzyme generates nucleosomes containing highly repetitive and satellite DNA more slowly than those containing bulk DNA (32). Monosomes obtained from sodium butyrate treated cells contain more highly acetylated histones compared to larger oligomers, when these structural units are isolated from very short micrococcal nuclease digests (15). More extensive digestion of sodium butyrate treated cells (15), or untreated cells (26,33), does not reveal any striking release of monosomes containing more highly acetylated histones. Therefore the use of micrococcal nuclease appears to depend on the extent of digestion or as with DNase II, may depend on the ability to fractionate chromatin into active and inactive components after digestion.

Fractionation methods have been particularly useful after digestion with micrococcal nuclease. For example, fractionation of chromatin after digestion can be effected by using the presumed differential solubility of nucleohistone particles at different salt concentrations (34), releasing into the supernatant an active chromatin fraction (35-37) containing more highly acetylated H4 (38). Bloom and Anderson (29) have utilized this approach, fractionating hen oviduct nuclei to obtain a monosome fraction (1SF) enriched for the transcriptionally active ovalbumin gene and a second fraction (2SF), consisting predominantly of nucleosome oligomers, depleted in this gene. The fractionation method appears to be related to genetic activity since the 1SF from hen liver is not enriched for the ovalbumin

gene, a gene not expressed in this tissue. Similarly the 1SF from hen oviduct is not enriched in globin genes.

Although many recent reports have investigated the relationship between chromatin digestion kinetics, chromatin fractionation and the extent of histone acetylation, there have been no reports suggesting that chromatin may be fractionated into classes which contain histones varying in their rates of acetylation. Recently, Cousens et al. (1) reported a convenient method for assessing rates of histone acetylation, and we have therefore determined whether chromatin particles containing histones with particular rates of acetylation are preferentially segregated into any of the fractions obtained by the method of Bloom and Anderson (29). As a result of short treatment of the cells with sodium butyrate, we find that the 1SF contains the majority of the histone class with a more rapid rate of acetylation. We also find that the fractionation itself depends, at least in part, on the extent of histone acetylation.

## MATERIALS AND METHODS

### Treatment of Cells

Hepatoma Tissue Culture (HTC) cells were grown in suspension culture in Swim's S-77 medium supplemented with 5% calf and 5% fetal calf serum as described by Oliver et al. (39). Cycloheximide treatment (10 mg/l, Sigma), $^{3}H$-acetate labelling and sodium butyrate treatment were carried out *in vivo* at cell densities of 4-6 x $10^5$ cells/ml as described in the figure legends. Ice was added directly to suspended cells to terminate incubations before nuclear isolations. The cells were then pelleted at 3000 x g for 3 minutes and resuspended in ice cold nuclear isolation buffer (0.25 M sucrose, 60 mM KCL, 15 mM NaCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 6 mM Na-butyrate, 10 mM MES (2(N-morpholino)ethane sulfonic acid), pH 6.5, 0.1 mM PMSF (phenylmethylsulfonylfluoride), 0.5% Triton X-100. Sodium butyrate (6 mM) is included throughout the nuclear isolation and digestion to prevent histone deacetylation (40).

### Isolation, Digestion and Fractionation of Nuclei

Nuclei were isolated by two washes in nuclear isolation buffer (vortexing and centrifuging at 3000 x g), and digested at 37° in the same buffer without Triton X-100. Micrococcal nuclease (Worthington) was used at a concentration of 0.5 units enzyme/50 µg DNA and the reaction quenched with 1/10 volume of 20 mM EGTA, pH 6.5. The resultant digested material was fractionated according to Bloom and Anderson (29) and the fractions

designated 1SF, 2SF and pellet according to their nomenclature. The time course of the digestion was monitored by release of chromatin particles into the 1SF and by the release of acid soluble oligonucleotides (11). Nuclei remain intact until ruptured by the addition of 5 mM EDTA, pH 7 to obtain the 2SF (29).

Isolation and Electrophoresis of Histone, DNA and Nucleohistone

Histones: Histones for Triton acid-urea slab gels were isolated by the method of Panyim and Chalkley (41). The acid-urea slab gels contained 8 M urea and 0.37% Triton X-100 as described by Alfageme et al. (42). Gels were stained in methanol/acetic acid/water (4/1/5) containing 0.25% coomassie blue for 6 hr and destained overnight in the above solution without coomassie blue. The method of Bonner and Laskey (43) and Laskey and Mills (44) was used to treat gels for fluorography of the $^{3}H$ labelled histones. Hypersensitized Kodak X-Omat R film was exposed to the vacuum-heat dried gels in x-ray film holders at -70°. Microdensitometer scans of the fluorograms and measurement of the area under the curve for a given histone band were obtained using a Model 2955 scanning densitometer (Transidyne, Ann Arbor, Michigan).

Histones for SDS gels generally were obtained by precipitation of the samples, in digestion buffer, with two volumes of ethanol, followed by centrifugation and washing and drying the pellet. In Figure 2, however, histone in the 1SF, 2SF and pellet was obtained by acid extraction (41). Pellets were resuspended in SDS sample buffer and electrophoresed directly on 18% polyacrylamide gels containing SDS (45,46). If necessary, histone specific activities (cpm $^{3}H$/relative amount of protein) were determined as described previously (26).

DNA: To obtain DNA from the 1SF, 2SF and pellet fraction, samples were digested with micrococcal nuclease without PMSF present and fractionated into 1SF, 2SF and pellet. The pH was readjusted to 8.5 with 1 M Tris, SDS added to 1%, Proteinase K (E. Merck, Darmstadt, Germany) added to 50 µg/ml and the samples incubated for 3 hr at 37°. DNA was extracted 2X with chloroform/isoamyl alcohol (24/1), precipitated with two volumes of ethanol and resuspended in 10 mM Tris, 2 mM EDTA, pH 7.5. RNA was removed by digestion with RNase A (Worthington, 50 µg/ml) for 30 minutes at 37°. DNA was extracted 1X with chloroform/isoamyl alcohol, precipitated with two volumes of ethanol after the addition of NaCl to 0.15 M, and the precipitate washed with ethanol and dried. The double stranded DNA was electrophoresed on 8% polyacrylamide slab gels according to the method of

Loening (47). The sample buffer was simply the tray buffer containing 10% glycerol and 0.005% bromphenol blue as tracking dye. Gels were stained with ethidium bromide (1 mg/liter) and photographed under ultraviolet illumination.

Nucleohistone: Nucleohistone was electrophoresed as described previously (26).

## RESULTS

### Micrococcal Nuclease Digestion Kinetics

Digestion kinetics of HTC nuclei similar to those described by Bloom and Anderson (29) for hen oviduct nuclei were established using micrococcal nuclease at a concentration of 0.5 units per 50 µg of DNA at 37° (Figure 1). About 1% of the DNA is rendered acid soluble every six minutes of digestion even after long digestion times (50 minutes). Within three to five minutes of incubation with the enzyme, the maximum amount of material in the 2SF is obtained. This amount remains relatively constant with continued digestion. The quantity of DNA in the 1SF, in contrast to the 2SF, did not plateau, but continued to increase during the incubation. Although nucleohistone is apparently released into the 1SF after one minute, core size DNA did not appear in this fraction until about three minutes after the addition of enzyme (data not shown). Except for experiments conducted during the time course of digestion, incubations of five to ten minute duration were used for the work to be described. This represents a digestion in which about 1% of the DNA is rendered acid soluble, 7-11% of the DNA is found in the 1SF and a maximal amount (30-40%) of the DNA is in the 2SF. Short digestion times are convenient but do not appear to be critical for the fractionation. The division of chromatin into differentially soluble components may be independent of the extent of digestion (34) and subsequent experiments appear to substantiate this result (see Figure 3). As determined by light microscopy, nuclei remain intact during the digestion and subsequent centrifugation to obtain the 1SF.

### Composition of Chromatin Fractions

The 1SF, 2SF and pellet fraction DNA sizes obtained after digestion and fractionation of HTC cell nuclei are analogous to those found by Bloom and Anderson (29) using hen oviduct nuclei. The DNA, nucleohistone and histone compositions of the fractions are shown in Figure 2. The 1SF consists almost entirely of core particles (Figure 2B - slot 1). Some ethidium bromide staining material remains at the top of the nucleohistone gel,

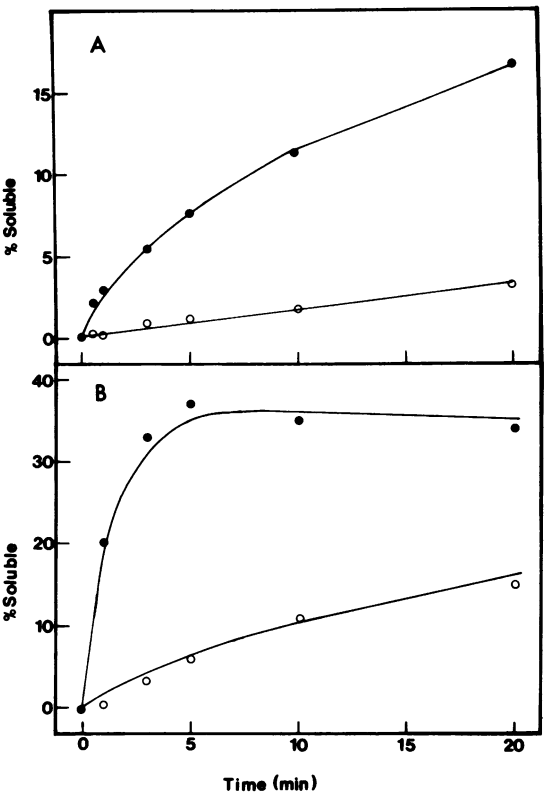



Figure 1. Micrococcal Nuclease Digestion Kinetics. Hepatoma Tissue Cultue (HTC) cell nuclei at a concentration of 6 Abs.(260 nm) units per ml were digested with 0.5 units of micrococcal nuclease per 50 µg DNA. Digestions were monitored by (A) the percent of total absorbance at 260 nm released into the supernate (1SF(●)) and by the percent of the total DNA rendered acid soluble (o), and by (B) the percent of total absorbance at 260 nm in the 2SF(●) and 1SF(o).

but second dimension SDS histone gel electrophoresis revealed no histone and a considerable amount of nonhistone protein at the gel top (data not shown). This material may be ribonucleoprotein associated with the HTC nucleus. Very little H1 histone was present in the 1SF (Figure 2C -slot 1) and the DNA size was primarily 140-145 base pairs although traces of dimer and trimer size DNA were present (Figure 2A - slot 2). Marker DNA (Figure 2A -slot 1) was prepared by micrococcal nuclease digestion of chromatin stripped of H1 and non-histone proteins (48) and sizing the resulting main

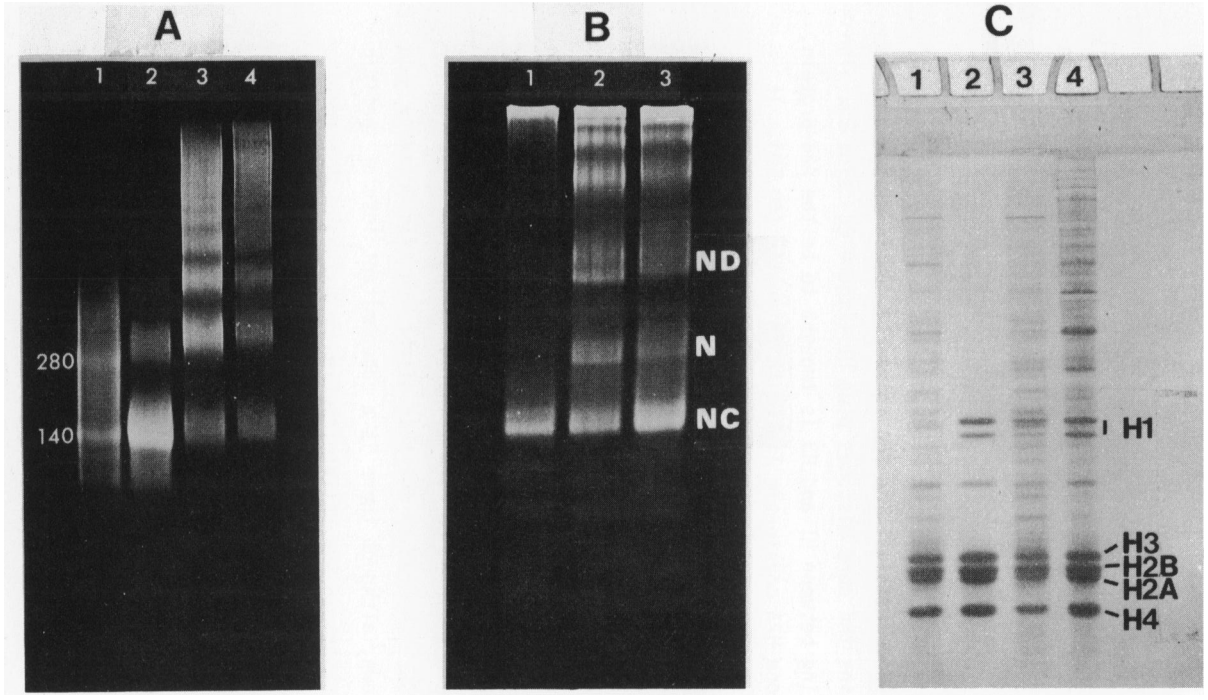



Figure 2. Composition of Chromatin Fractions. HTC nuclei were digested for 5 minutes with micrococcal nuclease (1.1% of the DNA rendered acid soluble) and fractionated according to the method of Bloom and Anderson (29). The composition of the fractions was assayed by electrophoresis of the (A) DNA, (B) nucleohistone and (C) proteins. (A) DNA sizes: slot 1- DNA standard containing 140 and 280 base pairs of DNA, slot 2- 1SF, slot 3- 2SF and slot 4- pellet DNA. (B) Nucleohistone particles: slot 1- 1SF, slot 2- 2SF and slot 3- pellet nucleohistone particles (NC- nucleosome core, N- nucleosome, ND- nucleosome dimer). (C) Histones: slot 1- 1SF, slot 2- 2SF, slot 3- pellet fraction histones and slot 4- proteins from whole nuclei.

bands (140 and 280 base pairs) with known DNA sizes obtained from the Eco RII restriction nuclease digestion products of SV40 DNA. The small amount of dimer DNA in the 1SF sized somewhat larger than the 280 base pair marker DNA (presumably 'spacerless dimer' from the stripped chromatin) yet somewhat smaller than dimer DNA from the 2SF and pellet fraction. The non-histone proteins found in the 1SF (Figure 2C- slot 1) are not necessarily associated with the 1SF DNA since the predominant protein-DNA complex in the 1SF is the nucleosome core particle. The 2SF and pellet fraction appear to be similar in overall composition except for the non-histone proteins found in the pellet fraction (Figure 2C- slot 3). Again many of these proteins may not be associated with DNA, but may simply be part of the nuclear membrane or matrix. The 2SF is nearly devoid of non-histone proteins and contains the core histones plus H1 (Figure 2C- slot 2). The band electrophoresing between H1 and H3 is thought to be the A24 protein, consisting of histone H2A covalently linked to ubiquitin (49,50). It appears to be equally distributed among the fractions.

Histone Acetylation in the Chromatin Fractions

Histones were labelled for 30 minutes with $^{3}$H-acetate *in vivo*, the chromatin fractionated after micrococcal nuclease digestion, and the specific activities of the histones determined as in Figure 3. A slight but reproducible difference between the specific activity of the histones in the 1SF and 2SF is found. As shown in the figure, the specific activity difference does not appear to be a function of the length of digestion though the specific activities do rise slightly during the digestion for both the 1SF and 2SF. The reason for this slight specific activity increase is not known, although there is a concommitant loss of specific activity of the histones in the pellet fraction. Obviously this result indicates that histones found in the 1SF are either more extensively or more rapidly acetylated (or both) then those in the 2SF, and it would appear that any nuclease digestion time could be used to investigate these differences.

That histones can be acetylated at different rates has been recently reported by Cousens et al. (1). We have utilized their method to assay the various fractions for both extent of acetylation and for the presence of histones modified at different rates. HTC cells were labelled with $^{3}$H-acetate for 30 minutes, the radiolabel removed, and the cells chased in the presence of 50 mM sodium butyrate to block histone deacetylation. One can then study the rate at which histones are modified by following

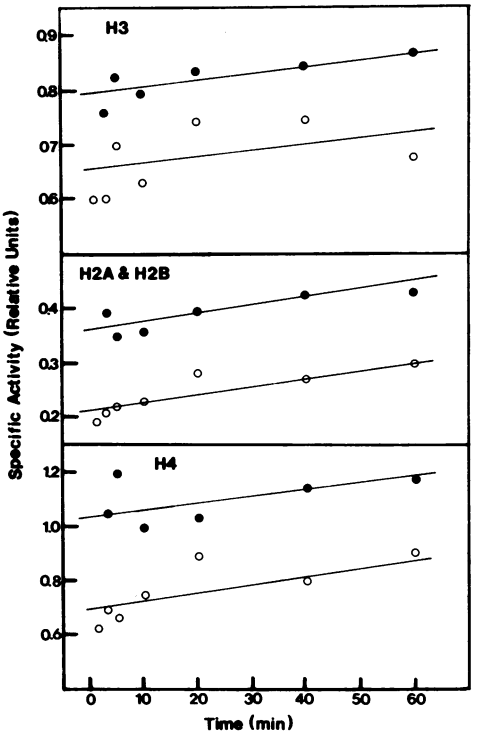
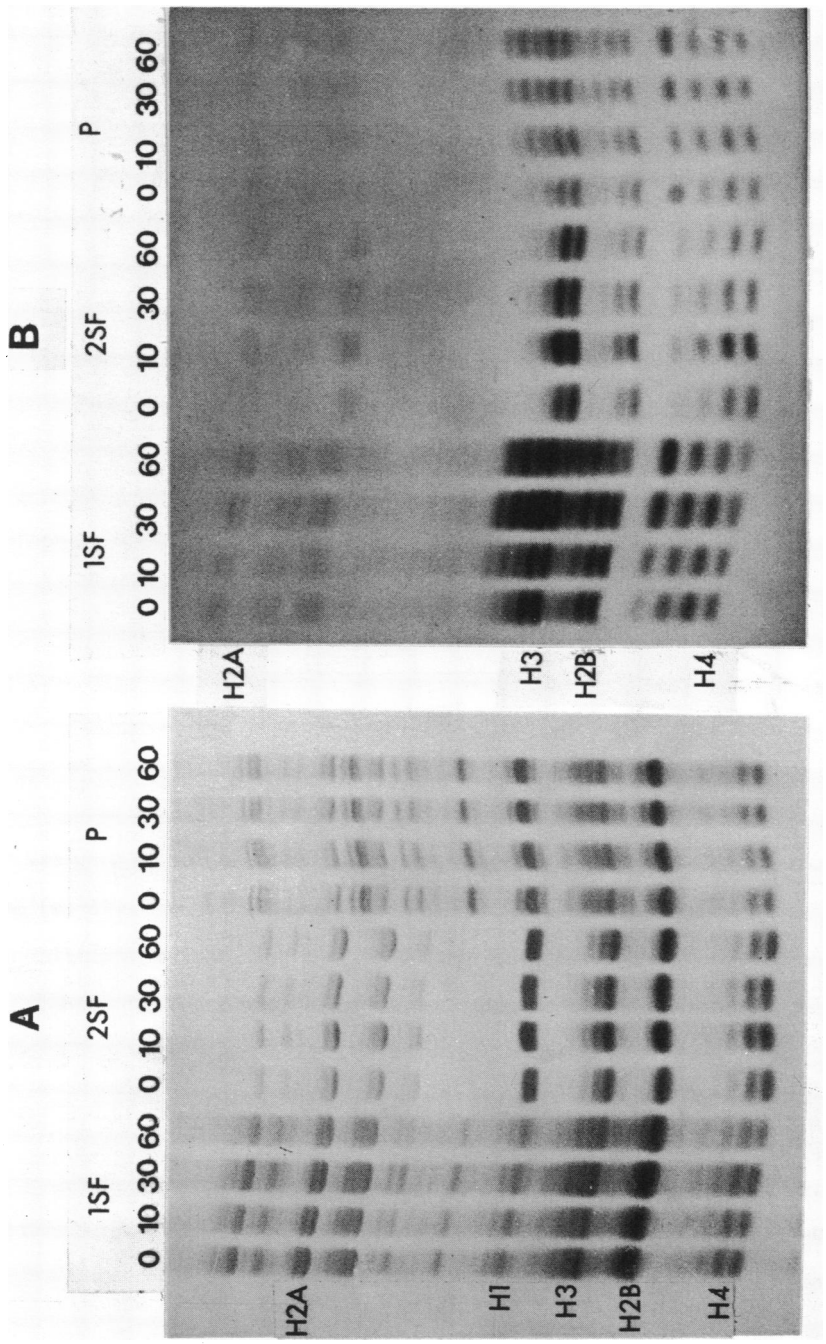



Figure 3. Comparison of the Specific Activities (cpm $^{3}$H-acetate/amount of protein) of the Histones in the 1SF and 2SF During the Time Course of Micrococcal Nuclease Digestion. HTC cells (1 liter) were resuspended in 50 ml of medium containing 5 mCi of $^{3}$H-acetate and labelled for 30 minutes. Isolated nuclei were digested with micrococcal nuclease (0.5 units/50 µg DNA) for the times indicated, and the histones obtained from the 1SF(●) and 2SF(o) as described in the Materials and Methods. Samples were electrophoresed on 18% polyacrylamide SDS histone gels and the specific activity (relative units) of H3, H2 (H2A+H2B) and H4 determined as in Nelson et al. (26). Electrophoresis was in triplicate to obtain mean values for each specific activity.

radiolabel moving to higher levels of histone acetylation. If the period of sodium butyrate treatment is sufficiently short, only those histones which are rapidly acetylated will be elevated to the highest levels of histone acetylation. In a separate report (Covault and Chalkley, manuscript in preparation), all rapid histone modification is complete within 1 hour after exposure to 50 mM sodium butyrate. Therefore to detect which chromatin fractions contain the most rapidly acetylated histones, incubation


A
1SF
2SF
P
0 10 30 60 0 10 30 60 0 10 30 60
H2A
H1
H3
H2B
H4
B
1SF
2SF
P
0 10 30 60 0 10 30 60 0 10 30 60
H2A
H3
H2B
H4

periods in 50 mM sodium butyrate should not exceed 60 minutes. Incubation times (in 50 mM sodium butyrate) were chosen to be 0, 10, 30 and 60 minutes.

The results of such an experiment are shown in Figure 4. The left hand panel (protein stained gel) shows the levels of histone modification in the various fractions as a function of the length of the chase in sodium butyrate. As the treatment in sodium butyrate proceeds and hyperacetylation develops, a small increased degree of acetylation shows up in histones in the 1SF (and pellet fraction) whereas histones in the 2SF seem unaffected. This result is illustrated more dramatically in the fluorogram of the same gel shown in the right hand panel of Figure 4. Histones which have been rapidly hyperacetylated are enriched in the 1SF and are nearly absent from the 2SF. This is confirmed graphically in Figure 5A where the percentage of radiolabel in tetra-acetylated H4 (H4Ac4) for each fraction is plotted as a function of the length of sodium butyrate treatment. As shown in the graph, the pellet fraction consists of a mixture of rapidly and slowly acetylated histones as is the case with whole chromatin (data not shown). It is also evident from Figure 5 that prior to sodium butyrate treatment, histones in the 1SF are slightly more highly acetylated than those in the 2SF, as evidenced by 1) an increased ratio of monoacetylated to parental histone in the protein stained gel and 2) the greater radioactivity in the higher levels of acetylation (in the fluorogram).

Figure 4. Histone Acetylation in the 1SF, 2SF and Pellet Fraction as Assayed by Sodium Butyrate Treatment of Cells after Labelling for 30 Minutes with $^{3}$H-acetate. HTC cells ($4 \times 10^{5}$ cells/ml) were treated for 30 minutes with 10 mg/ml cycloheximide (to terminate protein synthesis (Balhorn et al.(53)) and remove the possibility of incorporating $^{3}$H-acetate into newly synthesized histone either as a permanant N-terminal modification or on residues perhaps necessary for histone deposition) and resuspended in 80 ml of the same medium containing 20 mCi of $^{3}$H-acetate for 30 minutes. Cells were washed 2X at 4° with the above medium (without radiolabel) containing 50 mM sodium butyrate(1) and one-fourth of the cells pelleted and resuspended in nuclear isolation buffer to represent 0 minutes of sodium butyrate treatment. The remainder of the cells were resuspended in the original culture medium (without radiolabel) containing 50 mM sodium butyrate and incubated at 37° for 10, 30 and 60 minutes. Nuclei were isolated, digested for 10 minutes with micrococcal nuclease, fractionated, and the histones isolated from the 1SF, 2SF and pellet fraction for each time of sodium butyrate treatment. Histones were electrophoresed on a Triton acid-urea slab gel and the bands visualized by Coomassie Blue staining. Tritium in each band was visualized by fluorography. (A) Photograph of gel, (B) fluorograph. Numbers indicate minutes of sodium butyrate treatment.

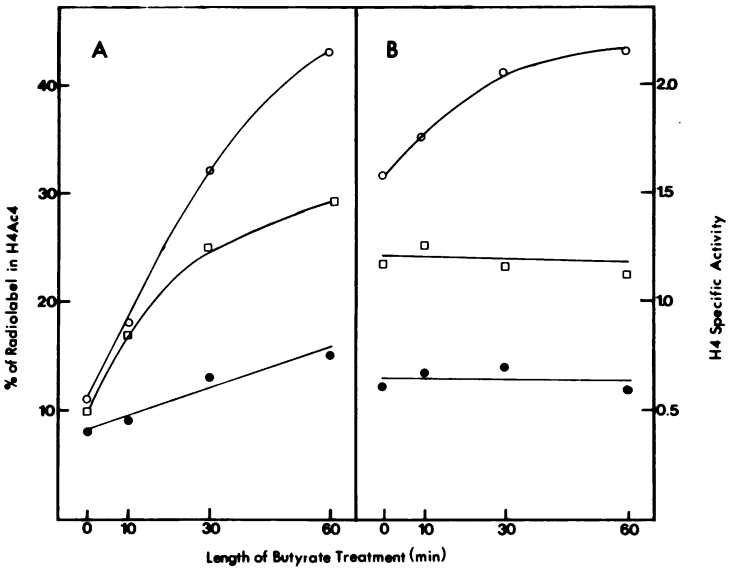



Figure 5. Percent of Radiolabel in Tetra-acetylated H4 and Specific Activity of H4 as a Function of the Length of Sodium Butyrate Treatment. (A) The percent of radiolabel in tetra-acetylated H4 was determined by scanning the fluorogram in Figure 4 in the H4 region for the 1SF(o), 2SF(•) and pellet fraction(□) after 0, 10, 30 and 60 minutes of treatment with 50 mM sodium butyrate. (B) The specific activity of H4 in each fraction after sodium butyrate treatment was determined as in Figure 3. Symbols represent the same fractions as in part (A).

## Both Kinetic Forms of Histone Acetate are Found in the 1SF

The evidence presented above indicates that after relatively short exposure of cells to sodium butyrate, fractionation of chromatin leads to enrichment in the 1SF of rapidly acetylated forms of histone and that the slowly acetylated forms are sequestered into the 2SF. We wanted to determine whether the selective solubility of chromosomal material into the 1SF is a reflection of properties possessed specifically by that fraction of chromatin containing rapidly acetylated histones, or if it is simply a consequence of more extensive histone acetylation, irrespective of rate. In the latter case, both rapidly and slowly hypermodified forms of histone will be found in the 1SF, though obviously after short exposure to sodium butyrate, the higher levels of acetylated histone will necessarily be enriched in the rapid form of the modification.

In order to ask if the slow form of the modification is also present

in the 1SF we have devised a protocol to remove a substantial part of the label contributed by the faster component. HTC cells were labelled with $^{3}$H-acetate for 30 minutes, the radiolabel removed, but prior to the 50 mM sodium butyrate treatment a chase of 10 minutes in the absence of sodium butyrate was interposed. The level of radioactivity associated with histones immediately after the intervening chase is shown in Figure 6B and may be compared with the control (Figure 6A) in which no intervening chase was present. As is evident in Figure 6B, those histones which were more rapidly acetylated during the sodium butyrate treatment are the same histones which are most rapidly deacetylated (and lose radiolabel) during the intervening chase. As a result of the exposure to sodium butyrate after the intervening 10 minute chase, radioactivity is again shifted to more highly modified forms in the 1SF (Figure 6B), but at a rate more characteristic of the slower forms of modification. In contrast, the radioactivity distribution in the 2SF seems to be independent of the intervening chase period. Thus we conclude that the 1SF contains both kinetic forms of histone acetate whereas the 2SF seems to contain primarily the slow turnover form. The main conclusion from these experiments is that the 1SF is enriched in those histones with a greater degree of modification.

Increased Yield of 1SF from Chromatin Containing Hyperacetylated Histones

An unexpected result of these experiments was found by examining Figure 4. Even though the amount of histone loaded on each gel slot for the 1SF (Figure 4A) was relatively constant, the amount of radiolabelled histone on the gel increases as a result of the sodium butyrate treatment (Figure 4B). This suggests that the specific activity of the histones in the 1SF increases during the one hour time course of sodium butyrate treatment. This observation was verified by electrophoresis of the samples shown in Figure 4 on SDS histone gels. The specific activity of the histones was determined as a function of the length of sodium butyrate treatment. As shown in Figure 5B, the specific activity of H4 in the 1SF increased by about 40% during the exposure to sodium butyrate. A reasonable explanation of this result is that chromatin particles containing rapidly acetylated (and therefore more highly labelled) histones are recruited into the 1SF (from the 2SF and pellet fraction) as they become highly acetylated following sodium butyrate treatment. This is in agreement with the result that more extensively modified histones are preferentially found in the 1SF. In other words, the release of chromosomal particles into the 1SF may be in part a function of the extent of histone

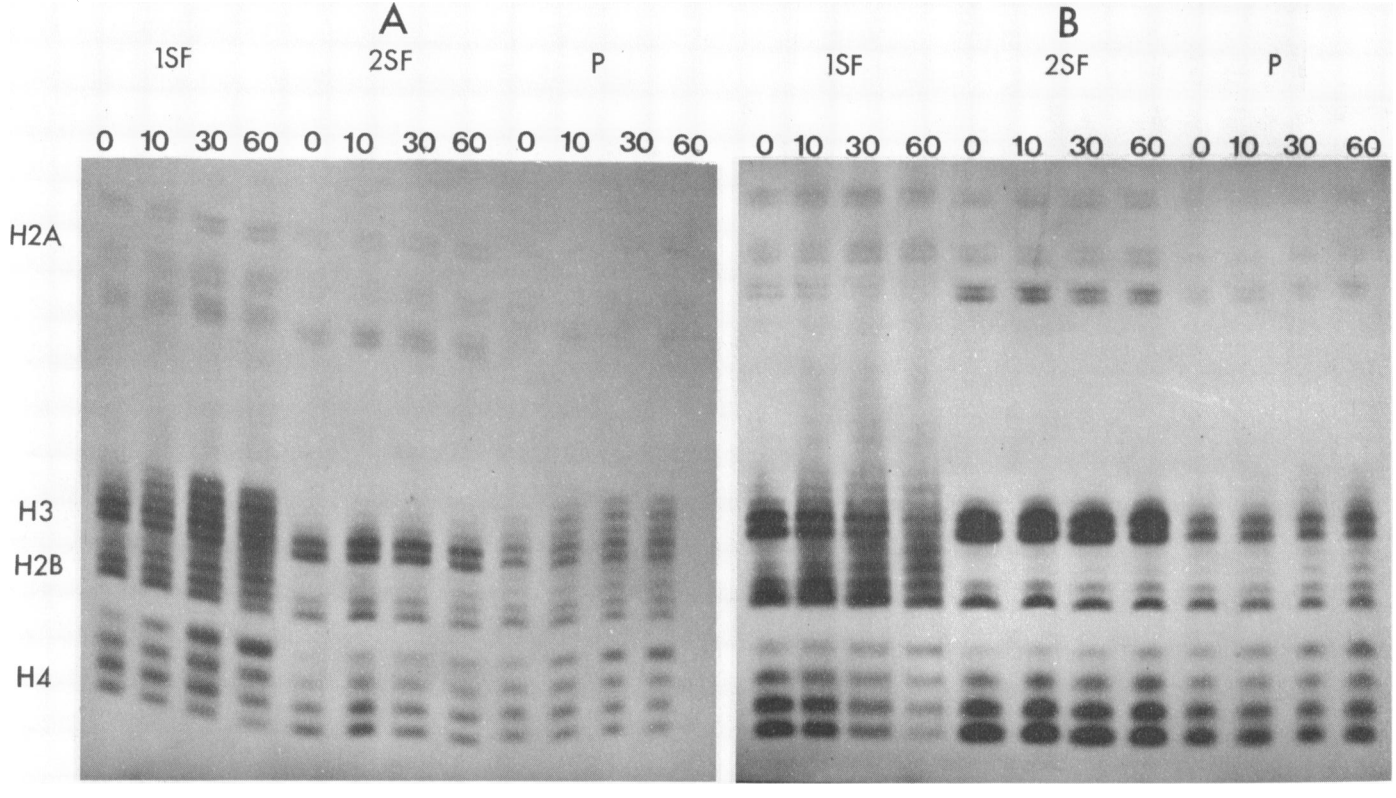



Figure 6. Histone Acetylation in the 1SF, 2SF and Pellet Fraction as Assayed by Sodium Butyrate Treatment of the Cells, Including an Intervening Chase in Fresh Medium. Cells and samples were treated as in Figure 4. HTC cells were labelled for (A) 30 minutes with $^{3}$H-acetate before sodium butyrate treatment and for (B) 30 minutes with $^{3}$H-acetate followed by a 10 minute chase in fresh medium (containing cycloheximide) before sodium butyrate treatment. Only the fluorograms are shown. Numbers indicate minutes of sodium butyrate treatment.

acetylation. This result implies that during an extended exposure to sodium butyrate, as a significant portion of histone becomes hyperacetylated, the amount of material segregated into the 1SF should increase.

To test this idea, the effect of histone hyperacetylation on the amount of material fractionated into the 1SF, 2SF and pellet fraction was examined. Two liters of HTC cells were divided in half and one group kept as control while the other was treated with 6 mM sodium butyrate for 14 hours at 37° to effect extensive hyperacetylation (23,24). Nuclei were isolated and digested as in Figure 1. As reported previously (15,17), the rate of release of acid soluble oligonucleotides was the same from control and sodium butyrate treated cells during the digestion with micrococcal nuclease (Figure 7). On the other hand, the amount of nucleosomal material which was soluble in the digestion buffer and was therefore released into the 1SF was

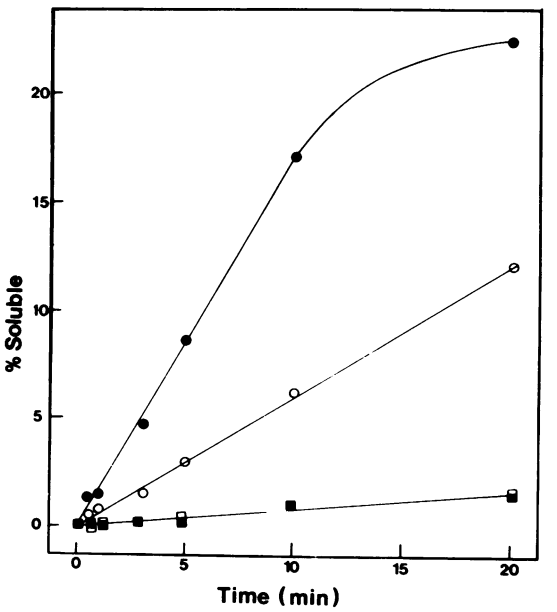



Figure 7. Percent of the DNA Rendered Acid Soluble and Percent Released into the 1SF from Control and Sodium Butyrate Treated Cells. One liter of HTC cells (4 x $10^5$ cells/ml) was treated for 14 hours with 6 mM sodium butyrate to enhance the levels of histone acetylation. Another liter of cells was incubated without treatment as control. Nuclei were isolated and digested as in Figure 1. Squares- Percent acid soluble of control (□) and sodium butyrate treated (■) cells. Circles- Percent of total absorbance at 260 nm in the 1SF from control(o) and sodium butyrate treated(●) cells.

substantially increased when cells were treated with 6 mM sodium butyrate. A two to three fold increase of material absorbing at 260 nm was noted in the 1SF from sodium butyrate treated cells (Figure 7). Measurements of the percent of absorbance at 260 nm in the 2SF and the pellet fraction indicate that some DNA is removed from both fractions to account for the increase in material in the 1SF. These results were substantiated further by demonstrating that the amount of chromosomal material in the 1SF could be directly related to the length of prior exposure to sodium butyrate. For example, following a 10 minute micrococcal nuclease digestion, the amounts of nucleosomal material soluble after 0, 4 and 14 hours of sodium butyrate treatment were respectively; 6, 12 and 17%.

## DISCUSSION

A chromatin fractionation method described by Bloom and Anderson (29) yields a subset of nucleosomes soluble in the nuclease digestion buffer (the 1SF), the majority of which have been trimmed to core size by the action of micrococcal nuclease. Conversely, the 2SF consists predominantly of nucleosome oligomers containing all five histones. The extent of histone acetylation plays an important role in determining the amount of nucleohistone in the 1SF. Increased histone acetylation as a result of blocking deacetylation *in vivo* promotes the release of nucleosomal material into the 1SF even though the extent of digestion of nuclei containing hyperacetylated histones, as measured by the percentage of DNA rendered acid soluble, is the same as that found for control cell nuclei. The fractionation, based to a considerable degree on the extent of histone acetylation, can then be utilized to partially separate chromatin particles containing rapidly acetylated and deacetylated histones from the more slowly turning over forms. Nucleosomes enriched in rapidly acetylated histones are found in the 1SF after short (30 to 60 minute) exposures to sodium butyrate (see Figure 4). This is because they contain the most extensively modified histones after these times of treatment with sodium butyrate. On the other hand, the 2SF contains primarily slowly modified 'hypoacetylated' histone. Since the 2SF consists chiefly of nucleosome oligomers, we conclude that nucleosomes with the slow kinetic form of histone acetate must exist contiguously *in vivo*.

It is apparent from Figure 4 that not all the histone in the 1SF is extensively modified, and it is therefore necessary to account for the presence of some unmodified parental histone in the 1SF. At this time a

satisfactory explanation is not available, but this data may suggest that the extent of histone acetylation is not the only variable involved in the chromatin fractionation. Certainly the extent of digestion regulates the amount of nucleosomal material in the 1SF. Particle composition (ie, $\pm$ H1) or digestion conditions may also determine solubility properties. Nevertheless hyperacetylation does alter the distribution of chromatin among the 1SF, 2SF and pellet fraction allowing up to three fold more nucleosomal material to be sequestered into the 1SF as a result of extended sodium butyrate treatment. If indeed histone acetylation per se can alter the solubility properties of chromatin, then this implies that histone acetylation may alter the ability of nucleosomal particles to interact with one another. This leads to, but of course does not prove the notion that more soluble chromatin is less compacted and more accessible to nucleases. It should be pointed out that a change in nucleosome compaction to allow transcription does not presume dramatic differences in chromatin morphology (such as hetero- versus euchromatin). Chromatin structural alterations need occur only very locally within the genome, suggesting rapid histone acetylation and deacetylation as a candidate for such a local event.

Although the nature of the DNA (ie, transcriptionally active or inactive) in the chromatin fractions from Hepatoma Tissue Culture cells has not been investigated in this paper, the results suggest a novel approach to test the possible relationship between gene expression and rapid histone acetate turnover. In the absence of sodium butyrate, both the 1SF and 2SF are likely to contain histones of the rapid kinetic variety either in the highly modified (fractionating into the 1SF) or less modified form (in which case they will probably fractionate into the 2SF). The effect of short exposure to sodium butyrate is to shift all the rapid kinetic forms of histone acetate to hypermodified species, allowing them to segregate into the 1SF. Clearly then if one wanted to compare sequences of DNA in association with relatively pure rapid kinetic form histones, with sequences of DNA in association with slow forms, one should compare DNA in the 1SF before sodium butyrate treatment with DNA in the 2SF after short (30 to 60 minute) exposure to sodium butyrate.

Inherent in the above discussion is the notion that histone acetylation may be playing some role in eukaryotic transcriptional processes. It seems certain that the total degree of acetylation (40-50%) is too great to account for an immediate and intimate involvement in RNA synthesis. However, the amount of histone involved in rapid acetylation may be closer

to the amount of the genome generally considered to be active at any given instant (51,52). The correlation between rapidly acetylated histone and active ovalbumin genes is also intriguing. Both fractionate into the 1SF. Thus we currently subscribe to the possibility that the rapid form of histone acetylation might be involved directly in transcription (perhaps in a permissive role). The slower form of the modification may be no more than a low level recognition of the sites recognized more rapidly during transcription.

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