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In Vitro Exchange of Nucleosomal Histones H2a and H2b†

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ABSTRACT: We have asked whether exogenous, radiolabeled histones can exchange with nucleosomal histones in an in vitro system. Using two different electrophoretic techniques, we were able to separate the histones contained in nucleosomes from those histones which were simply bound to the surface of the chromatin. Fluorography was used to determine which of the exogenous histones exchange with the nucleosomal histones. We observed substantial exchange of histones H1, H2a, and H2b when the chromatin and exogenous histones were incubated under approximately physiological conditions. We have also observed a small amount of exchange of H2a and H2b, as well as a substantial exchange of H1, from one chromatin fragment to another. Other conditions affecting the exchange of histones H2a and H2b are also reported.

The nucleosome is a fundamental unit of chromatin structure. Initially, the nucleosome was viewed as a largely static structure with histone octamers remaining intact and associated with the same stretch of DNA throughout the lifetime of the cell. However, the need for a measure of plasticity in this structure is likely in view of the nuclear metabolic events which require DNA as a template. One measure of the dynamic nature of the nucleosome structure would be the ability of histones to exchange in and out of the nucleosome. We have investigated this possibility directly.

Three lines of evidence led us to expect that exogenous histones might exchange into nucleosomes. First, it is now clear that there is a measure of continued histone synthesis throughout the cell cycle (Groppi & Coffino, 1980; Shenin & Lewis, 1980; Tarnowka et al., 1978; Waithe et al., 1983; Wu & Bonner, 1981). These histones synthesized in the absence of DNA replication have been shown to be associated with chromatin, which may indicate that they have exchanged with nucleosomal histones (Nadeau et al., 1978; Russev et al., 1980; Russev & Hancock, 1981). Second, newly synthesized histones H1, H2a, and H2b are not deposited specifically on new DNA but are somewhat randomized, leading to the suggestion that these histones can exchange with a histone pool, possibly nucleosomal in origin (Jackson & Chalkley, 1981a,b). Third, it has been clearly demonstrated that histone H1 can exchange in vitro at relatively low ionic strength (Caron & Thomas, 1981).

In this report, we have investigated the possibility of in vitro exchange between exogenous core histones and nucleosomal histones. By two separate methods, one based on isolation of the internal octamer and the other on isolation of nucleosomes, we conclude that core histones H2a and H2b, as well as histone H1, can undergo substantial exchange. In addition to observing the exchange of exogenous histones with nucleosomal histones, we have also observed a very small amount of exchange of histones H2a and H2b, as well as an extensive exchange of H1, from chromatin to chromatin.

Materials and Methods

Generation and Isolation of [^{3}H]Lysine-Labeled Histones. HTC cells were grown for 2–3 generations in Swims S-77 with serum plus 2–3 mCi of [^{3}H]lysine per L. Cells were collected by centrifugation at 400*g* for 5 min and frozen. The cells were washed 4 times in washing buffer [10 mM tris(hydroxymethyl)aminomethane (Tris),[1] pH 7.5, 10 mM $MgCl_2$, 0.25

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M sucrose, and 0.5% Triton], the nuclei were resuspended in 50 mM Tris, pH 7.4, and 1 mM PMSF, and the histones were extracted by adjusting the nuclei suspension to 2.0 M NaCl. To isolate the [^{3}H]lysine-labeled histones, the extract was layered on 20% sucrose, 2.0 M NaCl, 20 mM Tris, pH 7.4, and 1 mM PMSF for centrifugation at 150000*g* and 4 °C for 20 h. The upper portion containing the extracted protein was dialyzed against 10 mM TEA, pH 7.4, and 0.1 mM PMSF and frozen for future use. The concentration of histones was estimated by the absorbance at 230 nm. Core histones were obtained for some experiments by first washing the nuclei with 0.6 M NaCl to remove H1. The chromatin was then pelleted and resuspended in 50 mM Tris, pH 7.4, 1 mM PMSF, and 2.0 M NaCl to extract the core histones. These core histones were isolated by centrifugation as previously outlined.

For some experiments, histones were extracted by acid rather than salt. In this procedure, the nuclei were adjusted to 0.4 N H_2SO_4 and sonicated, and the precipitated DNA was pelleted by centrifugation at 20000*g* at 4 °C for 30 min. The soluble histones were decanted and dialyzed against 10 mM TEA, pH 7.4, and 0.1 mM PMSF. These samples were also frozen for later use.

Isolation of Chromatin. Nuclei were isolated from frozen HTC cells by washing 4 times with washing buffer and 1 time with digestion buffer (10 mM $MgCl_2$, 10 mM Tris, pH 7.4, and 1 mM $CaCl_2$). The nuclei were resuspended in digestion buffer at about 40–60 A_{260} units·(OD-mL)$^{-1}$ (DNA concentration = 2–3 mg/mL). The nuclei were digested with micrococcal nuclease at 0.005–0.01 unit·(OD-mL)$^{-1}$ for 30 min at 37 °C to solubilize the chromatin (exchange also occurred with undigested chromatin, but the yield of octamer was much reduced). The digestion was stopped by adjusting the EDTA concentration to 20 mM. The nuclei were pelleted, resuspended in 0.2 mM EDTA, pH 8.0, and dialyzed overnight against 0.2 mM EDTA in order to rupture the nuclei. To generate a greater quantity of soluble chromatin for either nucleoprotein gel analysis or exchange of histones from chromatin to chromatin, we increased the micrococcal nuclease concentration to 0.25–0.5 unit·(OD-mL)$^{-1}$.

Exchange of Exogenous Histone with Nucleosomal Histone. To allow exchange, radiolabeled histones were mixed with chromatin at various ionic conditions. The usual mixing procedure was to add the salt as a 10× stock (1.0 M NaCl and 0.8 M NaH_2PO_4, pH 7.4) and histones (usually 0.1 or 0.2 times the amount of nucleosomal histone) at the same time. The addition was done slowly with constant vortexing. Other methods of mixing, such as adding the salt and histones separately (in either order) or mixing the chromatin and histones and then dialyzing to the final salt concentration, did not alter the results. The mixture of salt, histones, and chromatin was then maintained at either 4, 25, or 37 °C for the stated time. Following this incubation, two assays for exchange were performed, one based on the isolation of the formaldehyde-cross-linked protein octamer and the other based on isolation of nucleosomes.

(*a*) *Isolation of Cross-Linked Octamer.* Following the exchange incubation, the pH was adjusted to 9.0 by the addition of an equal volume of 200 mM sodium borate, and formaldehyde was then added (final concentration 1%). After 15–18 h (at 4 °C), the cross-linked octamer was extracted by addition of H_2SO_4 (final concentration 0.4 N). The precipitated DNA was removed by centrifugation (20000*g* for 10 min). The supernatant containing the octamer was dialyzed against 4 mN H_2SO_4 to remove excess formaldehyde, and the octamer was then precipitated by dialysis against ethanol. The octamer was dissolved in sample buffer [20 mM Tris, pH 7.4, 1% SDS, 2.5% glycerol (v/v), and 0.005% bromophenol blue] and electrophoresed on an SDS–acrylamide gel [modified from Laemmli (1970)]. The electrophoresis buffer was 0.1% SDS, 0.20 M glycine, and 0.25 M Tris, pH 8.3, and the separating gel was 18% acrylamide, 0.09% methylenebis(acrylamide), 0.1% SDS, and 0.75 M Tris, pH 8.8. The stacking gel was 2.5% acrylamide, 0.125% methylenebis(acrylamide), 0.1% SDS, and 0.125 M Tris, pH 6.8. Electrophoresis was at 180 V for 18 h at 4 °C. Following electrophoresis, the slab gels were stained in 0.1% Coomassie brilliant blue R, 40% methanol, and 10% acetic acid for 2–3 h and destained with 40% methanol and 10% acetic acid. The gels were impregnated with PPO as outlined by Laskey & Mills (1975), and the gel was dried and fluorographed as previously outlined (Jackson et al., 1981). The destained gels and fluorograms were scanned in a RFT-II scanning densitometer for calculations of specific activities.

To reverse the cross-links and isolate the core histones, the octamer region was cut out and soaked in 2.5 M Tris, pH 7.4, and 1% SDS at 25 °C for 60 min. The mixture was adjusted to 0.5 M BME and heated to 95 °C for 30 min. The acrylamide strip was then polymerized directly into a second SDS–acrylamide gel for separation of individual histones.

The cross-links of the acid-insoluble chromatin were reversed by resuspending the pellet from the octamer extraction in 4.0 M guanidine hydrochloride, 50 mM Tris, pH 8.0, and 0.5 M BME. The suspension was neutralized with additional Tris buffer and heated to 95 °C for 30 min. The reversed products were prepared for electrophoresis by dialysis against 20 mM BME and addition of 10× SDS gel sample buffer.

(*b*) *Isolation of Nucleosomes.* Following the incubation, the exchange mixture was dialyzed against 10 mM MES, pH 6.5, and 2 mM EDTA and adjusted to 5% glycerol and 0.005% bromophenol blue. The micrococcal nuclease generated chromatin fragments were electrophoretically separated on a 4.5% acrylamide, 0.1% methylenebis(acrylamide), 10 mM MES, pH 6.5, and 2 mM EDTA gel (electrophoresis at 120 V and 4 °C for 12 h). This gel was stained with ethidium bromide and either directly prepared for fluorography or electrophoresed in the second dimension on an SDS gel prior to preparation for fluorography.

Exchange of Histones from Chromatin to Chromatin. HTC cells grown for 2–3 generations in 2.0 mCi of [^{3}H]-lysine/L were harvested. Undigested radiolabeled chromatin was isolated from these cells by washing 4 times with washing buffer and 1 time with 10 mM Tris, pH 8.0, and 10 mM EDTA and finally rupturing the nuclei by resuspension in water. This large chromatin was mixed with an equal quantity of micrococcal nuclease digested chromatin. The ionic condition was adjusted to 180 mM (100 mM NaCl and 80 mM NaH_2PO_4, pH 7.4) by adding an appropriate amount of a 10× concentrate slowly with constant vortexing. Following the incubation, the labeled undigested chromatin was pelleted by centrifugation at 20000*g* for 10 min at 4 °C. The soluble chromatin was manipulated to isolate either the nucleosomes or the cross-linked octamer by the methods outlined above.

Results

Assay for the Exchange of Core Histones. In order to study the possible exchange of exogenous and nucleosomal histones,

[1] Abbreviations: PMSF, phenylmethanesulfonyl fluoride; TEA, triethanolamine; MES, 2-(*N*-morpholino)ethanesulfonic acid; BME, β-mercaptoethanol; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; PPO, 2,5-diphenyloxazole.

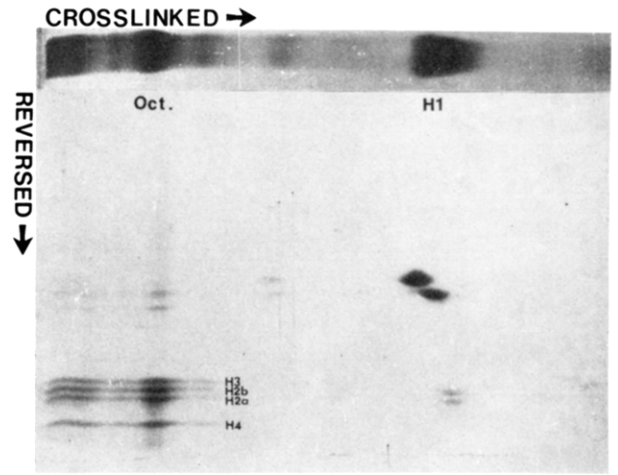

FIGURE 1: Cross-linked proteins formed when chromatin is reacted with formaldehyde at pH 9.0. Chromatin, which had been solubilized by limited micrococcal nuclease digestion, was adjusted to 100 mM sodium borate, pH 9.0, and treated with 1% formaldehyde. The cross-linked protein products released upon extraction in 0.4 N H_2SO_4 of this fixed chromatin were separated on an SDS–acrylamide gel and are shown horizontally. The cross-links were reversed and the individual histones separated on a second SDS–acrylamide gel (vertical separation).

it is essential to have some means of distinguishing between those histones which come from the nucleosomal core and those which are merely associated with the surface of the chromatin. It has been previously shown that when chromatin is fixed with formaldehyde at pH 7.4, all the core histones become cross-linked to DNA. However, when the fixation is performed at pH 9.0, the core histones are cross-linked internally to give octamers, but the octamers do not become cross-linked to DNA (Jackson, 1978). Subsequently, a simple acid extraction leads to the isolation of cross-linked core octamers. This is shown in Figure 1 in which the products of a fixation at pH 9.0 are analyzed in a gel, indicating that a good yield of the core octamer is obtained (horizontal separation at the top of Figure 1). The components of the octamer can be examined after reversing the cross-links by heating, and these are shown in the second dimension of Figure 1.

This approach provides us with a means of identifying core histones within the nucleosomal structure. However, when histones are added exogenously, there is evidence that they bind to the surface of the chromatin in the form of histone octamers (Eisenberg & Felsenfeld, 1981; Stein, 1979; Voordouw & Eisenberg, 1978). Therefore, we first asked whether such external octamers might also be released from chromatin after formaldehyde fixation at high pH, in which case it would be impossible to distinguish between exchange and simple association to the surface of the chromatin. Chromatin was first fixed with formaldehyde at pH 7.4 so that internal octamers could not be extracted away from the DNA. After removal of surplus formaldehyde by dialysis against diethylamine-containing buffer, ^{3}H-labeled core histones were added and incubated at the conditions used to allow exchange (see below). Following incubation, all of the added histones remained extractable with acid. Subsequently, this mixture was treated with formaldehyde at pH 9.0. The cross-linked material was then extracted by the standard protocol and assayed by SDS electrophoresis. As shown in Figure 2A, both the coomassie-stained gel and the fluorogram revealed that no detectable yield of extractable octamer is found under these conditions. We then took the acid-insoluble, cross-linked products and reversed the cross-links. These products were separated by electrophoresis and analyzed by Coomassie

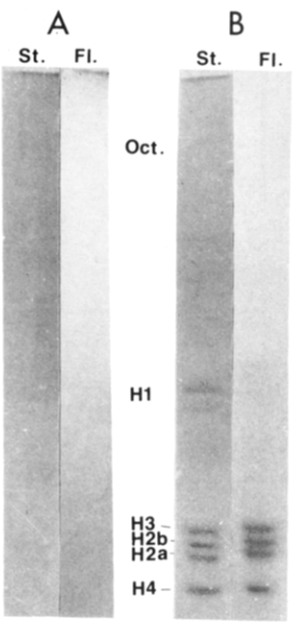

FIGURE 2: Nonextractability of surface-bound histones. Chromatin was cross-linked with formaldehyde at pH 7.4 and the excess formaldehyde removed. This cross-linked chromatin was then incubated with [^{3}H]lysine-labeled core histones at 25 °C and 180 mM salt (100 mM NaCl and 80 mM NaH_2PO_4, pH 7.4) for 2.0 h. The incubation was adjusted to pH 9.0 with sodium borate and reacted with 1% formaldehyde. The mixture was extracted with acid and the soluble protein electrophoresed on an SDS–acrylamide gel (stained and fluorographed gels are shown in panel A). The acid-insoluble chromatin was heated to reverse the cross-links, and the histones were separated on an SDS–acrylamide gel (shown in panel B).

staining and fluorography (Figure 2B). These results indicate that while the exogenous histones did not form isolatable octamers, they did bind to chromatin as expected. Therefore, if exogenous histones are bound to the surface of chromatin as octamers, they, unlike true nucleosomal octamers, become fixed to the chromatin during reaction with formaldehyde at pH 9.0.

This then provides us with an approach to analyze for exchange from the ^{3}H-labeled external histone to the internal octamer. Fixation at pH 9.0 is followed by acid extraction and an assay of the amount and nature of radiolabeled histone isolated with the internal octamer.

Conditions Favoring Exchange of Core Histones. We were now in a position to investigate more closely the conditions required for the exchange of core histones with external histones. Chromatin, initially in 10 mM TEA, pH 7.4, was adjusted to the desired ionic strength and temperature for the required times. Exchange was terminated by dilution with cold water, and the pH was then rapidly adjusted to 9.0 and formaldehyde added. The results of such a series of exchange reactions are summarized in Figure 3. Panel A shows the time course of the exchange obtained when the incubations are held at 25 °C and 180 mM salt concentration. The results indicate that exchange is rapid with maximum exchange occurring within 2 h ($\simeq$35%). The dependence of exchange on temperature is shown in panel B. In this experiment, the incubation time and salt concentration were held constant (25 min and 180 mM). An increase in the temperature from 4 to 37 °C caused a 45% increase in exchange (from 14.5% to 21%). Panel C shows the effect on exchange of increasing salt concentration when incubations are at 25 °C for 25 min. Increasing the salt concentration from 10 to 360 mM increased exchange from 1.5% to 30%, indicating that core histone exchange is very sensitive to salt concentration.

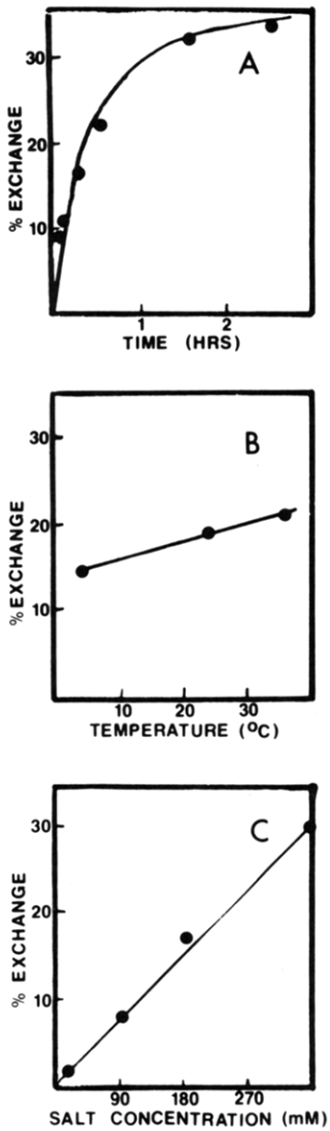

FIGURE 3: Conditions affecting the degree of histone exchange. Soluble chromatin was incubated with [^{3}H]lysine-labeled histones (0.1× the amount of nucleosomal histone) under the conditions specified. Following the incubation, the octamers were isolated, their specific activity was determined, and the percent exchange was calculated [100% exchange is the calculated specific activity if the exogenous histones were to completely randomize with nucleosomal histones ($^1/_{11}$ × the specific activity of the added histones)]. Panel A shows the results obtained when the incubation is at 25 °C and 180 mM salt, pH 7.4, for various lengths of time. Panel B shows the results of incubations at 180 mM salt, pH 7.4, for 25 min and at either 4, 25, or 37 °C. Panel C shows the results obtained from incubations at 25 °C for 25 min and at varied salt concentrations (adjusted by the addition of appropriate volumes of stock buffer; 1.0 M NaCl–0.8 M NaH_2PO_4, pH 7.4).

Exchange of Histones H2a and H2b. In addition to understanding the conditions favoring core histone exchange, we were also interested to see which histones were exchanging. Core octamers were isolated after exchange with ^{3}H-histones, the cross-links were reversed, and the histones which had composed the internal octamer were separated on a second SDS gel. The results are shown in Figure 4, in which we see that exchange has been limited to histones H2a and H2b. In all the incubation conditions we tested in Figure 3, we have been able to demonstrate exchange only of histones H2a and H2b.

Several other parameters have been varied, and since the presentation of the results is the same as in Figures 1–3, we do not present data. We have used both acid- and salt-extracted histones in these exchange experiments. Both types of histones show exchange; however, salt-extracted histones appear to exchange to a somewhat greater degree. Experiments adding ATP, DNA, or urea to the exchange reaction indicate that these factors do not improve the degree of exchange. Chromatin of larger size (~9–10 nucleosomes) shows an approximately 3-fold greater ability to exchange H2a and H2b than mononucleosomes. If an analysis for exchange is conducted in the presence of radiolabeled histones H2a and H2b without any added histones H3 and H4, very little radioactivity is found in the octamer region, indicating that all four core histones must be added together in order to produce significant exchange.

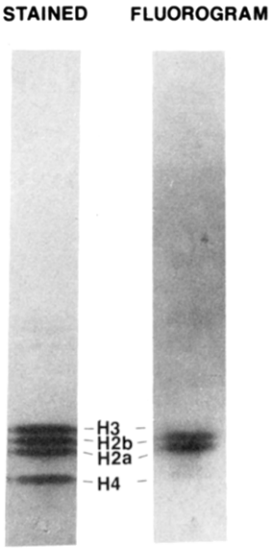

FIGURE 4: Identification of exchanged core histones. Soluble chromatin and [^{3}H]lysine-labeled histones were incubated in 180 mM salt, pH 7.4, at 25 °C for 2.0 h and then cross-linked with 1% formaldehyde at pH 9.0. The cross-linked octamer was separated (see Figure 1) and cut out of the first SDS gel; the cross-links were reversed and the core histones separated on a second SDS–acrylamide gel. This stained gel and its fluorogram are shown.

Analysis of Exchange by Nucleoprotein Gels. We have established the strong possibility of exchange of exogenous histones into the nucleosomal cores of chromatin. When chromatin is digested by micrococcal nuclease, a series of oligomer nucleoproteins is produced which can be resolved electrophoretically. Therefore, if exchange has occurred, the distribution of added radiolabeled histone should parallel that of the nucleoprotein particle upon such electrophoretic analysis. Chromatin which had been digested with micrococcal nuclease was incubated with exogenous [^{3}H]lysine-labeled histones as previously described. The chromatin nucleosomal fragments were then separated on a nucleoprotein gel and the histones associated with particular particles analyzed in a second-dimension SDS gel. The results of such an analysis are shown in Figure 5. The distribution of the histones assayed by the Coomassie stain accurately reflects the positions of the various nucleoprotein species as previously described by ourselves and others (Jackson et al., 1981; Rill & Nelson, 1977). Fluorography of this gel corroborates the earlier results that histones H2a and H2b are primarily involved in core histone exchange. At this time, it is not clear whether the small amount of labeled H4 that appears diffusely in the higher oligomer region of the gel represents a small measure of exchange or is a reflection of smaller oligomers which have reduced mobility due to the charge-neutralizing effect of surface-bound H4.

In this experiment, radiolabeled H1 was present in the added

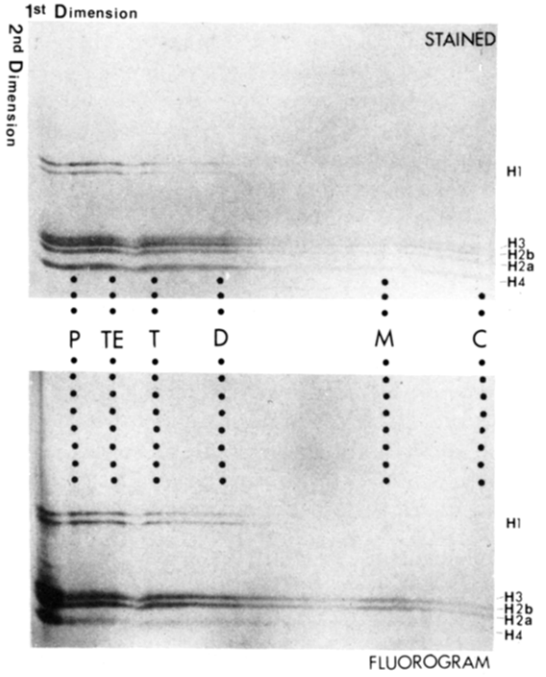

FIGURE 5: Two-dimensional gel electrophoresis of nucleosomes. Chromatin, which had been digested with micrococcal nuclease, was incubated with [^{3}H]lysine-labeled histones in 180 mM salt, pH 7.4, at 25 °C for 2.0 h. The nucleosomal fragments were then separated on a 4.5% acrylamide gel and the histones separated in the second dimension on an SDS–acrylamide gel. The stained second-dimension SDS gel and its fluorogram are shown. The various nucleosomal fragments are indicated as follows: C, core; M, monomer; D, dimer; T, trimer; TE, tetramer; P, pentamer. Their locations were determined from the more visible bands of the first-dimension gel.

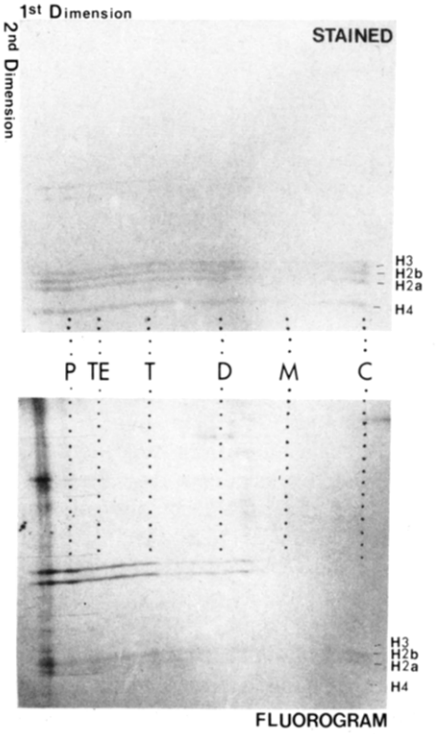

FIGURE 6: Exchange of histones from chromatin to chromatin. Large, undigested [^{3}H]lysine-labeled chromatin was incubated with small, soluble chromatin in 180 mM salt, pH 7.4, at 25 °C for 75 min. The large chromatin was pelleted by centrifugation, and the soluble chromatin fragments were separated in the first dimension on a 4.5% acrylamide gel. The proteins were separated in the second dimension on an SDS–acrylamide gel. The stained SDS gel and its fluorogram are shown. The various nucleosomal fragments are indicated as follows: C, core; M, monomer; D, dimer; T, trimer; TE, tetramer; P, pentamer. Their locations were determined from the more visible bands of the first-dimension gel.

histone, and it is apparent from these results that histone H1 exchanges efficiently under these conditions (180 mM buffer, 25 °C, 2 h) (Figure 5). We have also observed a rapid and complete exchange of H1 in conditions of much lower ionic strength (50 mM buffer, 5 min, 4 °C) (data not shown). This was expected in view of results obtained earlier by Caron & Thomas (1981).

Exchange of Core Histones from Chromatin to Chromatin. The exchange reactions reported above were done by adding extra histone. We were interested in determining if exchange could occur without the driving force of additional histone. In order to test this, we needed to mix two different chromatin fractions and to show that subsequently they could be efficiently separated. We mixed [^{3}H]thymidine-labeled large, undigested chromatin (obtained by disrupting nuclei with EDTA) with unlabeled oligonucleosomes obtained by a limited micrococcal nuclease digestion. The mixture was adjusted to the exchange conditions of 100 mM NaCl–80 mM NaH_2PO_4, pH 7.4, and maintained at 25 °C for 75 min. The material was then centrifuged, and the amount of [^{3}H]thymidine remaining in the supernatant was determined. We observed that 1.7% of the radiolabel remained in the supernatant. The experiment was then repeated by using [^{3}H]lysine-labeled histone in the large chromatin. After the exchange period, the supernatant (small) chromatin was separated and analyzed on nucleoprotein gels as shown in Figure 6. The fluorogram reveals a substantial shift of radiolabeled H1 from the large to the small chromatin. We estimate that approximately 50% of the H1 was exchanged during the 45-min incubation. The data also indicate that H2a and H2b have exchanged, though to a much lesser degree, such that 3–5% of the H2a and H2b in the large chromatin has been reassociated with the smaller material. However, if the larger chromatin is prewashed with 180 mM salt before exchange, then the amount of H2a and H2b migrating is reduced to background level (data not shown.)

We have also looked for histone exchange in the reciprocal experiment where the small chromatin contains the labeled histones. Under these conditions, we can demonstrate H1 migration but cannot clearly demonstrate movement of core histones.

Discussion

The data presented in this paper demonstrate that exogenously added histones H1, H2a, and H2b exchange in vitro with nucleosomal histones. The exchange of these histones occurs under ionic conditions similar to physiological values and well below those required to dissociate histones from DNA. The exchange of H1 was not surprising in view of earlier work (Caron & Thomas, 1981; Cremisi & Yaniv, 1980). However, the in vitro exchange of core histones is of special interest since it has not previously been reported.

The conclusion that exogenous histones H2a and H2b will exchange with nucleosomal histones is based on two methods of analysis. In the first method, we isolated the cross-linked core octamer, reversed the cross-links, and separated the histones by electrophoresis. From the fluorogram of this gel, we concluded that histones H2a and H2b, but not histones H3 and H4, had exchanged (Figure 4). In the second method, we separated the nucleosomal particles on a nucleoprotein gel and separated the histones in the second dimension on an SDS gel. From the fluorogram of this gel, we also concluded that histones H2a and H2b had exchanged (Figure 5). Even

though exogenous histones are known to form octamers bound to the surface of nucleosomes (Eisenberg & Felsenfeld, 1981; Stein, 1979; Voordouw & Eisenberg, 1978), we are confident that the isolated radioactive H2a and H2b are not from these surface octamers. First, the isolated octamers contained radioactive H2a and H2b and not H3 and H4 whereas we would expect surface-bound octamers to contain radioactivity in all four core histones. Second, control experiments using chromatin from which the histones could not exchange indicated that all surface-bound octamers become cross-linked to chromatin after fixation at pH 9.0 and therefore are not isolated by a subsequent acid extraction (see Figure 2).

The amount of histone involved in exchange increases with increasing time, temperature, and ionic strength. Calculations indicate that H2a and H2b exchange to the same extent (see Figure 4). Although under these conditions H2a/H2b and H3/H4 exist in solution as dimers and tetramers, respectively, there is strong evidence that they bind together as an octamer on the surface of nucleoproteins (Stein, 1979). Thus, the observation that the exchange of H2a and H2b requires the presence of H3 and H4 may reflect an exchange of H2a and H2b from an external octamer to an internal octamer. The result that oligonucleosomes show exchange about 3-fold more efficiently than mononucleosomes may thus simply reflect differences in binding external octamer, though this has not been proven in this work.

We do not know whether in vitro exchange reflects in vivo exchange mechanisms (as may occur during histone deposition). However, there is some evidence that exchange does occur in vivo. First, deposition of some of the newly synthesized histones H2a and H2b, though not newly synthesized H3 and H4, appears to involve exchange (Jackson & Chalkley, 1981a,b). Second, there is a measure of continued histone synthesis throughout the cell cycle (Groppi & Coffino, 1980; Wu & Bonner, 1981). And third, histones synthesized in the absence of DNA replication are also deposited into chromatin and nucleosomal structures (Nadeau et al., 1978; Russev & Hancock, 1981; Gurley & Hardin, 1969). We have observed that histones H2a and H2b synthesized in either G_1 or in the presence of hydroxyurea are incorporated into nucleosomal octamers (L. Louters, and R. Chalkley, unpublished results). This evidence indicates that cells possess some mechanism by which core histones exchange, but whether this involves mechanisms similar to those for S-phase histone deposition is unresolved.

We also provide evidence that histones H1, H2a, and H2b exchange from one chromatin fragment to another. While the extensive exchange of H1 corroborates earlier work (Caron & Thomas, 1981), a very limited exchange of H2a and H2b does seem to occur at higher ionic conditions (180 mM). The exchange (2–3%) seems to involve a "pool" of H2a and H2b that can be easily removed by washing with 180 mM buffer, pH 7.4. The fact that radiolabel is found in H2a and H2b and not H3 and H4 does, however, provide support for the idea that real exchange, albeit to a very small extent, seems to be occurring under these conditions.

One point of great interest is whether exogenous histones exchange equally well with all regions of chromatin or if the exchange process is selective. For example, the amount of exogenous histone added in these exchange reactions usually varied from about 0.1 to 0.2 times the amount of histone present in the chromatin. Therefore, a 35% exchange of the exogenous histone could indicate one of two extreme possibilities (or a continuum between). Either the total genomic chromatin exchanges to an extent of 3.5–7% [35%x (0.1 or 0.2)] or a specific 3.5–7% region of the chromatin selectively exchanges. If the latter possibility were true, one ought to be able to saturate the exchange process. We have tested this idea by increasing the amount of exogenous histones added to the exchange incubations (range from 0.05 to 0.4×). The results show similar degrees of exchange (~35%) which indicates that if there is a select portion of chromatin where histone exchange occurs, it is greater than 7%. There are some technical problems with these experiments, however. As one increases the amount of exogenous histone greater than 0.4×, the chromatin precipitates. This complicates both exchange and isolation of the cross-linked octamer and nucleosomes. Therefore, while these experiments suggest that greater than 7% of the chromatin is involved in exchange, they do not eliminate the possibility that the exchange of histones H2a and H2b could be selective for certain chromatin regions.

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