

# Nucleosomal histones of transcriptionally active/competent chromatin preferentially exchange with newly synthesized histones in quiescent chicken erythrocytes

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The incorporation of newly synthesized histones among various chromatin fractions isolated from non-replicating cell-cycle-phase- $G_0$  chicken immature erythrocytes was investigated. We find that newly synthesized erythroid-specific histone H1 variant, H5, is incorporated randomly into chromatin. In contrast, newly synthesized nucleosomal histones H2A, H2A.Z, H2B, H3.3, and H4 are preferentially found in a fraction that is highly enriched in active/competent gene chromatin fragments and depleted in repressed gene chromatin. Moreover, ubiquitinated species of histones H2A and H2B and hyperacetylated species of H4 and H2B, which are complexed to active DNA, are labelled. These observations provide evidence that newly synthesized histones preferentially exchange with the nucleosomal histones of transcriptionally active/competent chromatin domains. The results of this study suggest that nucleosomes of active chromatin may be inherently less stable than bulk nucleosomes *in vivo* and have implications for chromatin remodelling.

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## INTRODUCTION

The nucleosome, which is the basic repeating structural unit of eucaryotic chromatin, is composed of 146 bp of DNA wrapped around a histone octamer consisting of two molecules each of the histones H2A, H2B, H3, and H4. Histone H1, which is located at the position where the DNA enters and exits the nucleosome, has a key role in the higher-order compaction of the chromatin fibre. Transcriptionally active DNA is also associated with nucleosomal histones, but the structure of transcriptionally active/competent chromatin differs from that of bulk chromatin (e.g., active/competent gene chromatin is sensitive to DNAase I).

Biochemical analysis of transcriptionally active/competent gene-enriched chromatin fractions has demonstrated that the compositional features of active/competent gene chromatin differ from those of bulk (Gross & Garrard, 1987). The active/competent gene-enriched, low-salt-soluble polynucleosome fraction of chicken immature erythrocytes has been most informative about the biochemical composition of active/competent chromatin. This chromatin fraction contains only 2% of the total nuclear DNA, but approx. 15% of the total active sequences and approx. 40% of the total competent sequences. In contrast, only 0.3% of the total repressed gene sequences are present in this fraction (Delcuve & Davie, 1989). The active/competent gene-enriched polynucleosomes are enriched in highly acetylated species of histones H2B and H4 (Ridsdale & Davie, 1987; Delcuve & Davie, 1989), ubiquitinated (u) and polyubiquitinated species of H2A and more strikingly uH2B (Nickel *et al.*, 1989; Delcuve & Davie, 1989), and the histones H3 and H4 actively undergoing methylation (Hendzel & Davie, 1989). The analysis of active/competent gene-enriched chromatin fractions isolated from a variety of sources has provided evidence that active/competent DNA is complexed with highly acetylated histones and ubiquitinated histone H2B (Ip *et al.*, 1988; Lin *et al.*, 1989; Nickel *et al.*, 1989). Notable among these studies is the direct

demonstration that highly acetylated histones are associated with active, but not repressed, DNA of chicken erythroid cells (Hebbes *et al.*, 1988).

The majority of histone synthesis and incorporation into chromatin is tightly coupled to DNA synthesis during the S-phase of the cell cycle (Wu *et al.*, 1986). However, several investigators have demonstrated that a basal amount of histone synthesis occurs in both  $G_1$  and  $G_0$  phases of the cell cycle (Waithe *et al.*, 1983; Wu *et al.*, 1983a). Non-S-phase histone synthesis differs qualitatively from S-phase synthesis. The proportion of the histone H2A variants H2A.X and H2A.Z ( $G_1$  only) and the histone H3 variant H3.3 ( $G_0$  and  $G_1$ ) synthesized is increased relative to the S-phase synthesis pattern (Sariban *et al.*, 1985). Although all four core histones are synthesized to a similar extent in the absence of DNA replication, newly synthesized histones H2A and H2B are preferentially incorporated into chromatin by exchanging with nucleosomal histones. The newly synthesized histones H3 and H4 partition to a greater extent with a soluble histone pool that is not tightly associated with chromatin (Wu *et al.*, 1983b; Louters & Chalkley, 1985; Bonner *et al.*, 1988; Jackson, 1990). Once incorporated into chromatin, the newly synthesized histones are stable for at least several days in  $G_0$ -phase cells (Wu *et al.*, 1982).

Louters & Chalkley (1985) postulated that newly synthesized histones were preferentially exchanging with transcriptionally active chromatin regions. To test this hypothesis, we determined the distribution of newly synthesized histones among chromatin fractions isolated from immature chicken erythrocytes. This cell population consists almost entirely of non-replicating, terminally differentiated erythrocytes arrested in  $G_0$  phase of the cell cycle (Williams, 1972). We demonstrate that newly synthesized histone H5, a histone H1 variant, is deposited randomly on to chromatin. In contrast, newly synthesized histones H2A, H2A.Z, H2B, H3.3, and H4 co-fractionate with transcriptionally active and competent DNA. Moreover, newly synthesized histones H2B and H4 are hyperacetylated and newly synthesized histones H2A and

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Abbreviations used: H1 (etc.), histone H1 (etc.); u, ubiquitinated; AUT, acetic acid/6.7 M-urea/0.375% Triton X-100; PMSF, phenylmethanesulphonyl fluoride; HMG, high-mobility group.

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H2B are ubiquitinated, consistent with these histones being localized in transcriptionally active/competent chromatin domains. These observations provide support for the hypothesis that newly synthesized histones preferentially exchange with nucleosomal histones of transcriptionally active/competent chromatin. The results suggest that active/competent regions of chromatin may be remodelled by exchanging pre-existing nucleosomal histones with a newly synthesized histone pool whose composition varies from the S-phase pattern.

## MATERIALS AND METHODS

### Isolation and incubation of immature chicken erythrocytes

Immature chicken erythrocytes were isolated as described by Hendzel & Davie (1989). Cells were resuspended to  $2.5 \times 10^8$  cells/ml in Dulbecco's modified Earle's medium, pH 7.5, deficient in lysine and supplemented with 10 mM-sodium butyrate. Cells were preincubated for 30 min with  $5 \times 10^{-6}$  M-aphidicolin to inhibit DNA synthesis (Affolter *et al.*, 1987). Aphidicolin and sodium butyrate were found not to influence labelling of total acid-soluble nuclear proteins. Aphidicolin was included, however, to prevent labelling of the few replicating cells (e.g. erythroblasts) present in the peripheral erythrocytes of anaemic birds (Williams, 1972). Sodium butyrate, an inhibitor of histone deacetylase, was included to maintain both the high level of acetylated histones that are complexed to active/competent DNA and the solubility of active/competent gene polynucleosomes in 0.15 M-NaCl (Ridsdale *et al.*, 1990). For some incubations, actinomycin D (15  $\mu$ g/ml) was added during the preincubation period to inhibit transcription. L-[4,5- $^3$ H]lysine monohydrochloride (85 Ci/mmol; Amersham International) was added to the cell suspension to a final concentration of 100  $\mu$ Ci/ml and cells were further incubated for 60 or 90 min at 37 °C. Incubations of 90 min were typically used because the greater amount of labelled histones incorporated into chromatin after this labelling period facilitated their detection among the chromatin fractions. The duration of labelling did not affect either the spectrum of labelled histones incorporated into chromatin or the distribution of the labelled histones amongst the various chromatin fractions. Cells were collected by centrifugation and stored at -70 °C.

### Nuclei isolation and digestion, and chromatin fractionation

Nuclei were isolated and digested as described previously (Delcuve & Davie, 1989) except that the micrococcal-nuclease digestion time was reduced to 20 min. A portion of the digested nuclei was saved (T). Digested nuclei were collected by centrifugation and resuspended in 10 mM-EDTA (pH 7.5)/1 mM-phenylmethanesulphonyl fluoride (PMSF) and left on ice for at least 30 min to release chromatin fragments into solution. The soluble ( $S_E$ ) and insoluble ( $P_E$ ) fractions were separated by centrifugation at 12000 g for 10 min. NaCl was added to the  $S_E$  fraction to a final concentration of 150 mM, and after centrifugation at 12000 g for 10 min, an insoluble ( $P_{150}$ ) and a soluble fraction ( $S_{150}$ ) were obtained. The salt-soluble chromatin fragments of fraction  $S_{150}$  were separated by gel-exclusion chromatography on a Bio-Gel A-5m column, yielding fractions  $F_I$ ,  $F_{II}$ ,  $F_{III}$ , and  $F_{IV}$  (Delcuve & Davie, 1989). Fractions  $F_I$  and  $F_{II}$  contain the salt-soluble polynucleosomes, whereas fraction  $F_{IV}$  has the mononucleosomes. The percentage of DNA in each fraction was determined by diphenylamine assay (Giles & Myers, 1965).

### Preparation and analysis of protein samples

Nucleosomal histones were isolated by the following procedures. Chromatin fractions were made 0.2 M with respect to  $H_2SO_4$ , and the acid-soluble proteins were isolated (Nickel *et al.*,

1987). Alternatively, chromatin fractions  $S_E$ ,  $P_{150}$ ,  $S_{150}$ , and pooled Bio-Gel A-5m column fractions were prepared by elution from a Bio-Gel HTP hydroxyapatite column as described by Hendzel & Davie (1989) with the following modifications. Fraction  $P_{150}$  was resuspended in water and loaded directly on to the column. Fractions  $S_E$ ,  $S_{150}$  and pooled Bio-Gel A-5m fractions were loaded directly on to the column. All fractions were washed with 0.45 M-NaCl/0.1 M-potassium phosphate, pH 6.7, before elution of the histones with 2.0 M-NaCl/0.1 M-potassium phosphate, pH 6.7. Treatment of chromatin with 0.45 M-NaCl before eluting the histones removes any non-nucleosomal histones that may be bound to the surface of chromatin (Seale, 1981; Jackson, 1990). Additionally, this procedure removes the majority of non-histone proteins as well as the H1 histones and some of the histone H5. The removal of these proteins facilitates the analysis of the nucleosomal histones resolved on AUT (acetic acid/6.7 M-urea/0.375% Triton X-100) gels. SDS/PAGE and AUT/PAGE was performed as described by Nickel *et al.* (1987). Fluorography was performed as described by Hendzel & Davie (1989). Enrichments of H2A were determined by comparing peak heights of densitometer readings of the fluorograms for each fraction on equivalently loaded AUT polyacrylamide gels. Hydroxyapatite-prepared histones were used for determination of labelled H2A enrichments in the various chromatin fractions. H2A was chosen because it is well resolved from other labelled species. The enrichment of newly synthesized H2A in the 0.15 M-NaCl-soluble polynucleosomes represents a mean of enrichments of fractions  $F_I$  and  $F_{II}$ .

## RESULTS

### Distribution of newly synthesized nucleosomal histones in chicken erythrocyte chromatin

Immature chicken erythrocytes were incubated with L-[4,5- $^3$ H]lysine for 60 min to label the newly synthesized histones. The labelled chromatin was fractionated by a low-ionic-strength procedure. Four fractions were obtained:  $P_E$  (EDTA-insoluble chromatin),  $S_E$  (EDTA-soluble chromatin),  $S_{150}$  (EDTA-soluble, 0.15 M-NaCl-soluble chromatin), and  $P_{150}$  (EDTA-soluble, 0.15 M-

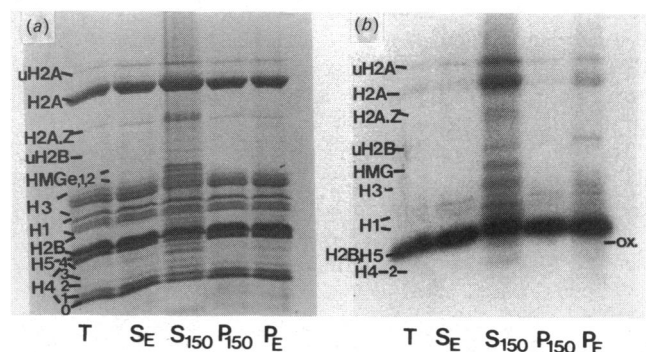
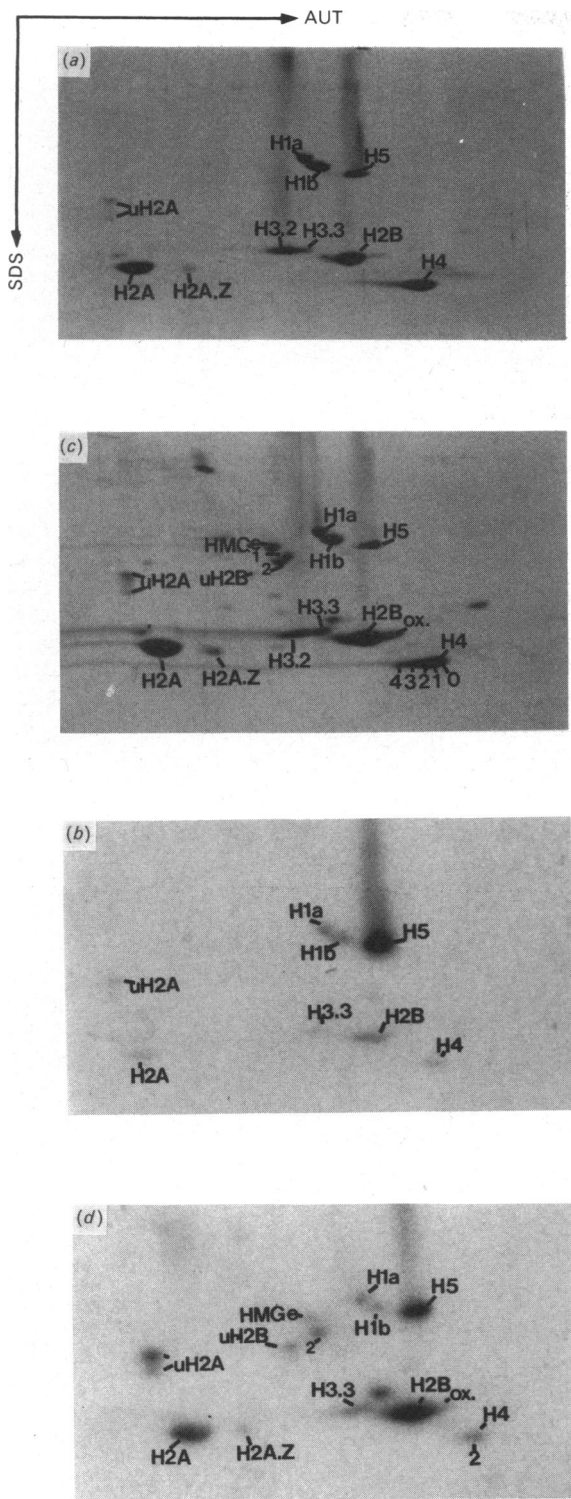


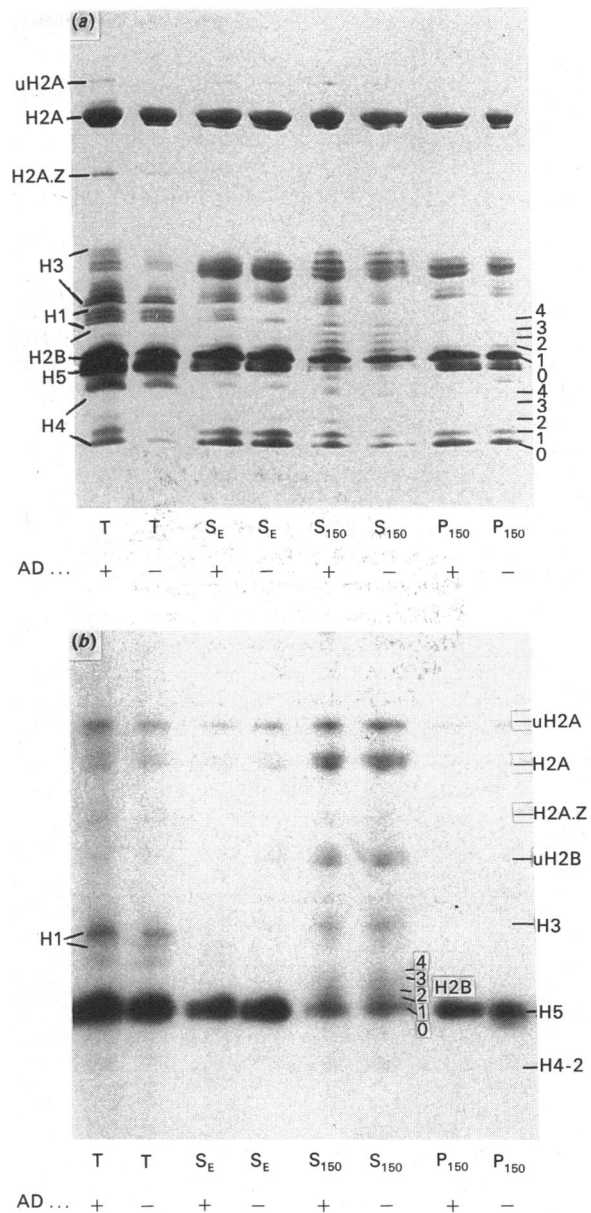
Fig. 1. Distribution of newly synthesized histones among the chromatin fractions

Acid-soluble proteins of immature chicken erythrocytes incubated with [ $^3$ H]lysine (60 min) were isolated from the chromatin fractions. The proteins (10  $\mu$ g) were electrophoretically resolved on 15% polyacrylamide/AUT gels. (a) Shows the Coomassie Blue-stained gel pattern and (b) shows the accompanying fluorogram. The ubiquitin adducts of histones H2A and H2B are denoted as uH2A and uH2B respectively. The acetylated species of histone H4 are denoted as 0, 1, 2, 3, and 4, representing the un-, mono-, di-, tri-, and tetra-acetylated species respectively. Oxidized histone H2B is indicated as 'ox.'



**Fig. 2. Active/competent gene-enriched chromatin fraction  $S_{150}$  is enriched in newly synthesized nucleosomal histones**

Acid-soluble proteins of unfraktionated chromatin (T) and the salt-soluble, active/competent gene-enriched chromatin fraction  $S_{150}$  were electrophoretically resolved by two-dimensional gel electrophoresis (AUT into SDS). (a) and (c) are the Coomassie Blue-stained gel patterns containing proteins of T and  $S_{150}$  respectively. (b) is the accompanying fluorogram of (a), and (d) is the fluorogram for (c). The ubiquitin adducts of histones H2A and H2B are denoted as uH2A and uH2B respectively. The acetylated species of histone are denoted as 0, 1, 2, 3, and 4, representing the un-, mono-, di-, tri-, and tetra-acetylated species respectively. 'ox.' indicates oxidation of histone H2B. Note that on the first-dimension AUT gel, oxidized H2B co-migrates with tetra-acetylated H4.



**Fig. 3. Inhibition of transcription does not affect nucleosomal histone exchange**

Immature erythrocytes were incubated for 90 min with [ $^3$ H]lysine in the presence (15  $\mu$ g/ml; +) or absence (-) of actinomycin D (AD). Acid-soluble proteins of unfraktionated chromatin (T) and hydroxyapatite-prepared histones of the chromatin fractions were electrophoretically resolved on 15%-polyacrylamide AUT gels. (a) Shows the Coomassie Blue-stained gel pattern and (b) shows the accompanying fluorogram. The ubiquitin adducts of histones H2A and H2B are denoted as uH2A and uH2B respectively. The acetylated species of histones H2B and H4 are denoted as 0, 1, 2, 3, and 4, representing the un-, mono-, di-, tri- and tetra-acetylated species respectively.

NaCl-insoluble chromatin); they contained approx. 10, 90, 12 and 78% respectively of the total nuclear DNA. Transcriptionally active gene sequences (e.g. histone H5 and  $\beta$ -globin) partition with chromatin fragments that are soluble in 0.15 M-NaCl (fraction  $S_{150}$ ) and with chromatin fragments associated with the EDTA-insoluble residual nuclear material (fraction  $P_E$ ). Competent genes (e.g.  $\epsilon$ -globin and vimentin), which are sensitive to DNAase I but transcriptionally inactive, are highly enriched in fraction  $S_{150}$ , but not fraction  $P_E$ . Repressed genes (e.g.

vitellogenin), which are not enriched in fraction  $S_{150}$  or  $P_E$ , are located primarily in fraction  $P_{150}$  (Delcuve & Davie, 1989).

Fig. 1 (lane T) shows that newly synthesized histone H5 is the principal histone synthesized and associated with chicken immature erythrocyte chromatin, in agreement with the results of Affolter *et al.* (1987) (see also Figs. 2a and 2b). Labeled histone H5 is located in all of the chromatin fractions. In contrast with histone H5, the content of labeled nucleosomal histones (e.g. H2A) in the various chromatin fractions is not equivalent, with the nucleosomal histones of chromatin fractions  $S_{150}$  and  $P_E$  being labeled to the greatest extent.

Fig. 2 shows the labeled proteins of fractions  $S_{150}$  and T resolved by two-dimensional gel electrophoresis. In fraction  $S_{150}$ , histones H1, H5, H2A.Z, H3, H2B, and H4 and ubiquitinated species of histones H2A and H2B (denoted uH2A and uH2B respectively) are labeled, with histones H2A and H2B being labeled to a greater extent than H3 and H4. The species of histone H3 that incorporated label was identified as H3.3 and acetylated species thereof. The identification of the labeled histone H3 variant as H3.3 was determined by electrophoretically resolving the histones on long AUT/polyacrylamide gels, which separate the acetylated species of H3.3 from the un-, mono- and di-acetylated forms of H3.2 (results not shown).

It has been demonstrated that newly synthesized histones can bind to the surface of chromatin without being incorporated into nucleosomes (Seale, 1981; Jackson, 1990). Thus the acid-soluble labeled proteins present in the chromatin fractions shown in Fig. 1 may be a combination of nucleosomal and surface-bound histones. The surface-bound histones can be removed from chromatin with 0.45 M-NaCl (Seale, 1981). In order to analyse only those newly synthesized histones that have exchanged with nucleosomal histones, hydroxyapatite-bound chromatin was washed with 0.45 M-NaCl and the histone octamer was recovered in 2 M-NaCl. It should be noted that 0.45 M-NaCl removes both H1 histones (excluding H5) and high-mobility-group (HMG) proteins from chromatin.

Immature chicken erythrocytes were labeled for 90 min in the presence or absence of actinomycin D, and the chromatin was

fractionated. Except for fraction T (acid-extracted nuclear histones), histones were isolated from hydroxyapatite-bound chromatin. In agreement with the results of Louters & Chalkley (1985), Fig. 3 shows that incubation of cells with actinomycin D did not affect the distribution of labeled histones among the various chromatin fractions. Fig. 3 also shows that, similarly to the results shown in Fig. 2, fraction  $S_{150}$  has the greatest level of labeled nucleosomal histones. This is a representative result of nine experiments. Thus the observation that fraction  $S_{150}$  has the greatest amount of labeled nucleosomal histones appears to be independent of the time that cells were incubated with label and the method of histone isolation.

Fig. 4 shows densitometric scans of fluorograms (continuous lines) and Coomassie Blue-stained gel patterns (broken lines) containing histones of chromatin fractions  $S_E$ ,  $S_{150}$  and  $P_{150}$ . Fraction  $P_{150}$  has the lowest level of labeled nucleosomal histones, with histone H2A of fraction  $P_{150}$  being labeled approx. 0.4-fold that of H2A in fraction  $S_E$ . (Because fraction T was not isolated by hydroxyapatite chromatography,  $S_E$  was used as a reference for determining enrichments of labeled histones in the chromatin fractions.) Fraction  $S_{150}$ , which is enriched approx. 6-fold in active and 4.3-fold in competent genes (Delcuve & Davie, 1989), is enriched approx. 8.3-fold compared with fraction  $S_E$  in labeled H2A. In fraction  $S_{150}$ , histones H2A, H2B and their ubiquitinated forms are the major labeled histones. Note that the acetylated forms of H2B are labeled. Label is also incorporated into H2A.Z, H3 and the diacetylated species of H4. This is a representative result of eight experiments.

#### Newly synthesized nucleosomal histones are highly enriched in 0.15 M-NaCl-soluble polynucleosomes

Chromatin fraction  $S_{150}$  contains both transcriptionally active/competent and repressed DNA. Active/competent DNA is found in the salt-soluble polynucleosomes, whereas the repressed DNA is localized in the mononucleosomes (Delcuve & Davie, 1989). Size fractionation of the salt-soluble chromatin fragments of fraction  $S_{150}$  by gel-exclusion chromatography on a Bio-Gel A-5m column allows the isolation of the highly

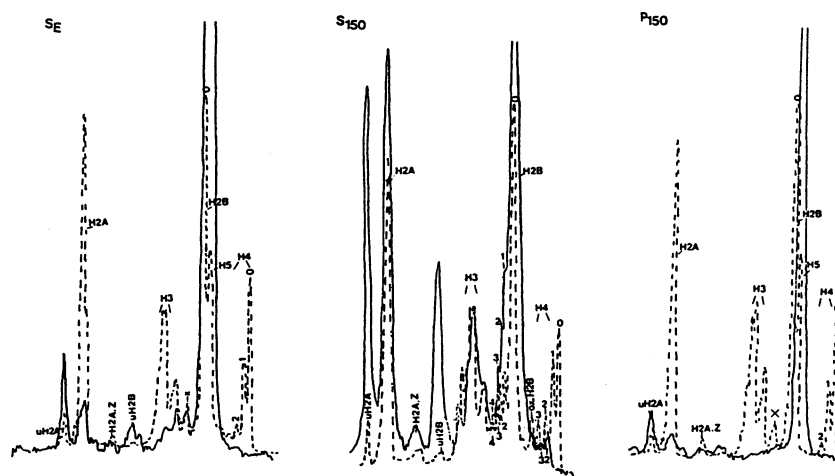
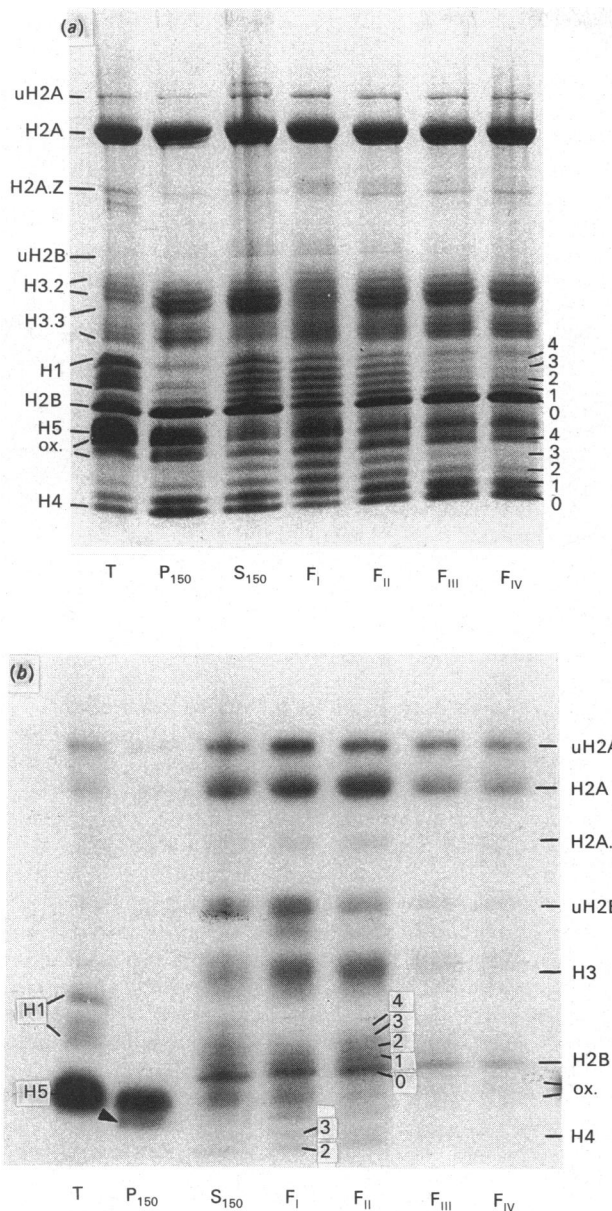


Fig. 4. Fraction  $S_{150}$  is highly enriched in newly synthesized labeled nucleosomal histones

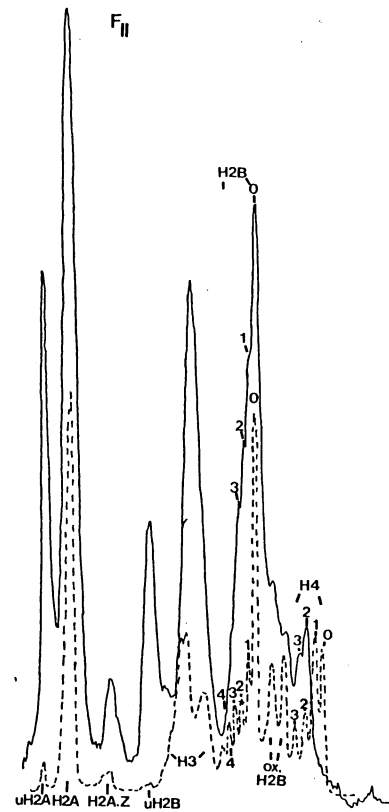
Hydroxyapatite-prepared histones of chromatin fractions  $S_E$ ,  $S_{150}$ , and  $P_{150}$  were electrophoretically resolved on a 15%-polyacrylamide/AUT gel. Densitometric scans of fluorograms (continuous lines) and Coomassie Blue-stained gel patterns (broken lines) containing these histones are shown. The densitometer was adjusted to maximum sensitivity for scanning the fluorograms in order to produce adequate signals from fraction  $P_{150}$ . x indicates the migration of an unknown labeled protein. The ubiquitin adducts of histones H2A and H2B are denoted as uH2A and uH2B respectively. The acetylated species of histones H2B and H4 are denoted as 0, 1, 2, 3, and 4, representing the un-, mono-, di-, tri-, and tetra-acetylated species respectively.



**Fig. 5. Nucleosomal histones of highly active/competent gene-enriched salt-soluble polynucleosomes are highly enriched in newly synthesized histones**

Acid-soluble proteins of unfractionated chromatin (T) and hydroxyapatite-isolated histones of the chromatin fractions and size-fractionated salt-soluble chromatin fragments of fraction  $S_{150}$  (fractions  $F_I$ ,  $F_{II}$ ,  $F_{III}$  and  $F_{IV}$ ) were electrophoretically resolved on 15%-polyacrylamide/AUT gels. (a) Shows the Coomassie Blue-stained gel pattern and (b) shows the accompanying fluorogram. The ubiquitin adducts of histones H2A and H2B are denoted as uH2A and uH2B respectively. The acetylated species of histones H2B and H4 are denoted as 0, 1, 2, 3, and 4, representing the un-, mono-, di-, tri- and tetra-acetylated species respectively. 'ox.' indicates oxidized H2B. The arrow below H5 in (b), lane  $P_{150}$ , shows the position of modified species of H5 which co-migrates with H5 on SDS/polyacrylamide gels.

active/competent gene-enriched polynucleosomes. These polynucleosomes, which are present in column fractions  $F_I$  and  $F_{II}$ , contain approx. 2% of the total nuclear DNA and, relative to total (or to  $S_E$ ) DNA, these fractions are enriched 7.6-fold (15.1-fold) and 26-fold (25.9-fold) in active and competent DNA respectively (Delcuve & Davie, 1989).



**Fig. 6. Newly synthesized histones of fraction  $F_{II}$  are acetylated and ubiquitinated**

Hydroxyapatite-prepared histones of chromatin fraction  $F_{II}$  were electrophoretically resolved on a 15% polyacrylamide/AUT gel. Densitometric scans of a fluorogram (continuous lines) and a Coomassie Blue-stained gel pattern (broken lines) are shown. The ubiquitin adducts of histones H2A and H2B are denoted as uH2A and uH2B respectively. The acetylated species of histones H2B and H4 are denoted as 0, 1, 2, 3, and 4, representing the un-, mono-, di-, tri- and tetra-acetylated species respectively. 'ox. H2B' is oxidized H2B.

To determine whether the labelled nucleosomal histones co-fractionated with the active/competent gene-enriched polynucleosomes, chromatin fragments of fraction  $S_{150}$  were size-resolved; four fractions,  $F_I$ ,  $F_{II}$ ,  $F_{III}$  and  $F_{IV}$ , of decreasing chromatin fibre lengths, were obtained. Fig. 5 shows that the labelled nucleosomal histones are enriched in the salt-soluble polynucleosomes (fractions  $F_I$  and  $F_{II}$ ). Fraction  $P_{150}$ , which contains 78% of the total nuclear DNA, incorporates the least label. The salt-soluble polynucleosomes ( $F_I$  and  $F_{II}$ ) are enriched 17.0-fold in labelled H2A compared with  $S_E$ . Of the salt-soluble chromatin fractions, the histones of fractions  $F_{IV}$ , which consists primarily of mononucleosome-size particles, are the least labelled. This is a representative result of six experiments.

Fig. 6 shows a densitometric scan of a fluorogram (continuous lines) and Coomassie Blue-stained gel pattern (broken lines) containing histones of chromatin fraction  $F_{II}$ . The following histones are labelled: uH2A, H2A, H2A.Z, uH2B, H3, H2B and its acetylated species (mono-, di-, tri- and tetra-acetylated forms), and di- and tri-acetylated H4. Approx. 66% of the labelled H2B and 51% of total H2B of fraction  $F_{II}$  are acetylated. This is a representative result of six experiments. Peptide mapping of uH2A demonstrated that both H2A and ubiquitin are labelled, with approx. 62% of the label being incorporated into H2A (results not shown). After correcting for the contribution of



labelled ubiquitin to the total labelling of uH2A, we calculate that approx. 32% of the labelled H2A is ubiquitinated. In contrast, the scan of the Coomassie Blue-stained gel pattern shown in Fig. 6 shows that approx. 8% of the total H2A associated with these chromatin fragment is ubiquitinated.

## DISCUSSION

Our results show that newly synthesized histone H5 is incorporated into all regions of the chromatin of chicken erythrocytes which are arrested in  $G_0$  phase of the cell cycle. The incorporation of newly synthesized histone H5 into chromatin may occur by exchange with pre-existing H5 (Louters & Chalkley, 1985) or represent new deposition of this histone on to the chromatin fibre.

The present study provides evidence that newly synthesized histones H2A, H2A.Z, H2B, H3.3, and H4 exchange preferentially with the nucleosomal histones of transcriptionally active/competent chromatin domains. The following observations support this conclusion. First, enrichment of labelled nucleosomal histones paralleled the enrichment of active DNA in chromatin fractions  $S_{150}$  (salt-soluble chromatin fragments) and  $F_I/F_{II}$  (salt-soluble polynucleosomes). The enrichment of labelled histone H2A in fractions  $S_{150}$  and  $F_I/F_{II}$  of 8.3-fold and 17.0-fold respectively is similar to the 6.0-fold and 15.1-fold enrichment of active DNA sequences in these fractions. Secondly, hyperacetylated species of histones H2B and H4 are labelled. Zhang & Nelson (1986, 1988) demonstrated that, in immature chicken erythrocytes, 3.7% of the modifiable histone lysine sites are undergoing acetylation and deacetylation. Thus approx. 1–2% of the genome is composed of dynamically acetylated and deacetylated histones, and these histones are complexed with active, but not repressed, DNA (Hebbes *et al.*, 1988; Ridsdale *et al.*, 1990). In the presence of sodium butyrate, which was used in our cell incubations, the dynamically acetylated histones become hyperacetylated (tri- and tetra-acetylated forms). The observation that the hyperacetylated species of H2B and H4 of the active/competent gene-enriched polynucleosomes (fractions  $F_I$  and  $F_{II}$ ) are labelled suggests that newly synthesized histones have exchanged with nucleosomal histones complexed to active/competent DNA. Furthermore, the observation that ubiquitinated histone H2B, which is preferentially located in transcriptionally active chromatin (Nickel *et al.*, 1989), is labelled provides evidence that newly synthesized histones are localized in transcriptionally active/competent chromatin regions.

The amount of labelled uH2A and uH2B in the histones of salt-soluble polynucleosomes is quite striking, with approx. 32% of the labelled H2A being ubiquitinated. In contrast, 8% of the total H2A of the salt-soluble polynucleosomes is ubiquitinated. Other investigators have observed that the ratio of uH2A/H2A in  $G_1$ -phase cells or in cells where DNA replication has been inhibited increases 4–6-fold compared with the S-phase cells (Jackson & Chalkley, 1985; Jackson, 1990). Jackson (1990) has demonstrated that, in the absence of replication, the pool of excess histones is susceptible to extensive modification by ubiquitin, resulting in an increase in the amount of the newly synthesized uH2A and uH2B incorporated into chromatin. These results suggest that newly synthesized H2A and H2B are ubiquitinated before exchange with the nucleosomal histones in the  $G_0$ -phase chicken erythrocytes.

Consistent with the results of others (Louters & Chalkley, 1985; Bonner *et al.*, 1988), we find that more newly synthesized histones H2A and H2B are incorporated into chromatin than newly synthesized histones H3 and H4. A model has been proposed in which the passage of the RNA polymerase displaces one H2A/H2B dimer (Baer & Rhodes, 1983; Loidl, 1988).

Although our results do not exclude this possibility, we demonstrate that the incorporation of newly synthesized H2A/H2B and other nucleosomal histones into chromatin is not dependent upon on-going transcription. Exchange of newly synthesized histones with nucleosomal histones of transcriptionally active/competent chromatin may reflect instability in the active/competent nucleosomes. Ridsdale *et al.* (1988) demonstrated that the nucleosomes of highly active/competent gene-enriched chromatin fragments (fractions  $F_I$  and  $F_{II}$ ) were more readily dissociated than bulk nucleosomes by the intercalating agent ethidium bromide. Nacheva *et al.* (1989) reported differences in histone binding to DNA with the hsp70 gene upon induction of transcription. Using a low-salt fractionation procedure that enriches for active genes, these investigators demonstrated that cross-linking efficiencies of histones to DNA were reduced in transcriptionally active gene-enriched chromatin relative to bulk. Interestingly, they observed that histones H2A and H2B had a greater reduction in cross-linking efficiency to DNA than histones H3 and H4. This may result in histone-DNA complexes of transcriptionally active chromatin existing in a more dynamic state than bulk stable nucleosomes. The instability of the nucleosome within transcriptionally active domains may be necessary to facilitate efficient transcription.

Our results have implications for mechanisms of chromatin remodelling. If nucleosomal histone exchange occurs in the  $G_1$  phase preferentially with active/competent chromatin regions, as we have demonstrated for  $G_0$ -phase erythrocytes, then this event could account for the enrichment of the histone variants H2A.Z and H3.3 (which are preferentially synthesized in  $G_1$ ) in transcriptionally active/competent chromatin as reported by Ridsdale & Davie (1987). Additionally, early S-phase replication of active chromatin regions has been demonstrated (Hatton *et al.*, 1988). This may result in the acquisition of histones from a pool enriched in the histone variants preferentially synthesized in the  $G_1$  phase (H2A.X, H2A.Z, and H3.3). Therefore this difference may also contribute to the remodelling of active/competent chromatin.

This work was supported by a grant from the Medical Research Council of Canada (MA-9186) and a Medical Research Council of Canada Scholarship (to J.R.D.).

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Received 25 January 1990/19 April 1990; accepted 2 May 1990