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Erythroid-specific gene chromatin has an altered association with linker histones

J.A.Ridsdale, J.B.Rattner[1] and J.R.Davie

Department of Biochemistry, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, R3E OW3 and [1]Department of Anatomy and Cell Biology, University of Calgary, Calgary, Alberta T2N 4N1, Canada

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ABSTRACT

The chromatin of several genes was assayed for sensitivity to DNAase I and for solubility as polynucleosomes in 0.15 M NaCl. The degree of solubility of chromatin fragments as polynucleosomes in 0.15 M NaCl correlates well with the sensitivity to DNAase I for several genes. Chromatin of repressed, housekeeping and erythroid-specific genes can be distinguished as distinct groups by the degree to which they display these properties. NaCl precipitation of chromatin fragments stripped and then reconstituted with varying quantities of H1 and H5 (linker) histones indicate that the polynucleosomes of erythroid-specific genes have altered interaction with these histones. Linker histones interacted with bulk chromatin and in the chromatin of the repressed ovalbumin and vitellogenin genes to form salt precipitable structures. Chromatin of erythroid-specific genes (histone H5 and β-globin) as well as that of the histone H2A.F gene was resistant to linker histone induced precipitation.

INTRODUCTION

Gene expression in eucaryotes is thought to be regulated by, at least, the interaction of trans-acting factors with cis-acting DNA sequences, and the availability of the template to the transcription machinery, i.e., the gene is localized in a transcriptionally competent chromatin domain. The specific interaction of DNA binding proteins with cis-acting DNA sequences is probably a key step towards establishing the transcriptionally competent chromatin domain. It is clear from experiments with transgenic mice that cis-acting DNA sequences are sufficient for the tissue-specific expression of some genes (1-3). Present evidence indicates that the interaction of trans-acting factors with cis-acting DNA sequences has a key role in establishing but not maintaining the transcriptionally competent chromatin structure (4,5).

Transcriptionally competent gene chromatin is in a state which is sensitive to attack by DNAase I relative to the bulk of chromatin. The region of DNAase I sensitivity extends well beyond the coding region of the gene (6). Sensitivity to DNAase I suggests an open or exposed chromatin structure which would be accessible to the transcription machinery. The repressed class of chromatin structures may be effectively invisible to the elements involved in transcriptional regulation.

The factors involved in maintaining the transcriptionally competent chromatin structure are poorly understood. Factors thought to be involved include post-synthetic histone modifications (e.g. histone acetylation and ubiquitination; 4,7-10) and the loss of histone H1. Since these processes are reversible, the maintenance of the transcriptionally competent chromatin structure should be considered as a dynamic process (9,10). One of the key steps in this process is thought to be the disruption of histone H1-H1 contacts

essential for the cooperative binding of the H1 histone to chromatin (11). Several lines of evidence suggest a role for H1 histones in gene repression (12,13). Chromosomal components, which interfere with the binding of H1 histones to the nucleosome, will prevent the chromatin fiber from forming higher order, repressed chromatin structures.

Recently, we described the isolation and characterization of a rare class of chicken erythrocyte chromatin which is highly enriched in transcriptionally competent β-globin gene sequences and soluble at physiological ionic strength (10). This atypical chromatin contained significantly elevated levels of acetylated histones H4, H2A.Z and H2B, and ubiquitinated histone species of H2B and H2A, and was associated with a lower level of H1 histones (H1 and H5) than that found in unfractionated chromatin. In this current work we present evidence that the chromatin of repressed genes, erythroid-specific genes and housekeeping genes can be distinguished in terms of the degree of polynucleosome solubility which correlated to the extent of DNAase I sensitivity. We also present evidence that the differences in polynucleosome solubility is due to an altered association of the atypical chromatin fibril with H1 and H5 histones. Furthermore, we present evidence of structural alterations of the core particle of the competent enriched chromatin.

MATERIALS AND METHODS

Isolation and digestion of nuclei with micrococcal nuclease

Red blood cells from adult white Leghorn chickens were collected in 75 mM NaCl, 25 mM EDTA, washed of the buffy coat and stored at -70°C. Nuclei were isolated and digested with micrococcal nuclease as previously described (10).

Digested nuclei were resuspended into an equal volume of 10 mM EDTA, pH 7.2, 1 mM PMSF and left on ice for 2 hours to release chromatin fragments into solution. Insoluble nuclear debris was removed by centrifugation (12,000 g x 20 min). 80.3 ± 3.8% (n=4) of the total nuclear 260 nm absorbing material was recovered in the supernatant.

Fractionation of chromatin fragments

For NaCl precipitation the EDTA supernatant (SEDTA) was diluted to a 260 nm absorbance of 30 units/ml by the addition of 10 mM EDTA, and NaCl was added with vigorous stirring to 0.15 M from a 4 M stock. The resulting precipitate was collected immediately by centrifugation (12,000 g x 20 min).

To obtain a highly competent-gene enriched fraction the supernatant from the NaCl precipitation (10.8 ± 0.4% of SEDTA, n=4) was concentrated against polyethylene glycol and applied to a 2.5 x 110 cm Bio-Gel A-5m column in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.15 M NaCl and the polynucleosome fraction (of approximately octamer size and larger) collected.

Digestion of nuclei with DNAase I

Nuclei were digested with DNAase I as described by Villeponteau et al. (14). Nuclei were suspended in RSB (10 mM NaCl, 10 mM Tris-HCl, pH 7.5 and 3 mM $MgCl_2$) to a concentration of 13 A_{260} units/ml. DNAase I (Sigma) was added to 1μg/ml and the nuclei were digested for 10 min at 37°C. Digestion was terminated by the addition of EDTA to 10 mM and placing the digest on ice. DNA was prepared for slot blot analysis as described (10).

Preparation and reconstitution of stripped chromatin fragments and linker histones

Dry CM Sephadex (30 mg/ml) was added to SEDTA and then NaCl to 0.35 M from a 4 M stock (with stirring) and the suspension was stirred at 4°C for 1 hour. The chromatin fragments stripped of the linker histones were collected by filtration. The CM Sephadex was first washed with 0.6 M NaCl and then 1.0 M NaCl. The second wash contained the H1 and H5 histones, in proportions equivalent to that found in nuclei, which were essentially free from other proteins. The linker histone preparation was dialyzed against water, lyophilized and stored as a concentrated solution at -20°C. The stripped chromatin was dialyzed against 1 mM EDTA, 1 mM PMSF stored at 4°C and used within a few days.

Stripped chromatin was reconstituted with varying quantities of the mixed H1 and H5 preparation essentially according to the method of Nelson et al. (15). Both chromatin and histones were dialyzed against 10 mM Tris-HCl, pH 8.0 and 0.5 M NaCl. Varying quantities of the histones were added to a constant amount of chromatin and dialysis was continued for 2 hours, then for one hour against 0.25 M NaCl, 1 mM EDTA and then against 1 mM EDTA overnight, both buffered with 10 mM Tris-HCl, pH 8.0.

Reconstituted material was fractionated by the addition of NaCl to 0.15 M as described above.

Electrophoresis and blotting

For Southern blotting equal quantities of chromatin samples were dialyzed against water and lyophilized. The lypophylized powder was redissolved in DNA sample buffer containing 2% SDS. Electrophoresis was on 1% agarose gels in standard electrophoresis buffer containing 0.1% SDS. After electrophoresis gels were stained for about 45 min in 1 μg/ml ethidium bromide for photography. DNA denaturation and blotting was carried out by standard procedures (10).

For slot blotting the DNA samples were purified as described (10). The DNA was then made to 0.3 M NaOH and incubated at 37°C for 30 minutes and neutralized by the addition of an equal volume of 2 M ammonium acetate, pH 7.0 and applied to nitrocellulose using a Schleicher and Schuell slot blotting manifold.

Hybridization with nick translated probes was carried out as described by Thomas (16). The cloned DNA probes used were: pCBG 14.4 an unique intronic sequence of the adult β-globin gene (17) which was acquired from H. Martinson. pVTG 412, obtained from H. Weintraub, recognizes the 5' region of the chicken vitellogenin gene (18). pH2A.F, received from J.R.E. Wells, is a cDNA clone of the gene of the histone H2A variant H2A.Z (19). pchV2.5B/H from A. Ruiz-Carrillo is a 2.5 kbp fragment of the histone H5 gene (20). pOV12 is the ovalbumin gene received from M.-J. Tsai (21). pCkTk and pMyc3.2SST, genomic clones for the thymidine kinase and c-myc genes respectively, were acquired from M. Groudine. pVIT, a genomic clone of the vimentin gene (22), was a gift from B.M. Paterson.

Quantitation of Data

Slot blots were loaded with varied quantities of purified DNA and hybridized. The autoradiograms were scanned with a densitometer and the areas under the curves integrated to quantitate the extent of hybridization. The relation between signal and the amount loaded was essentially linear. The DNAase I sensitivity of the various gene chromatins were determined as described by Villeponteau et al. (14). All hybridization data for DNAase I treated nuclei were first normalized to the hybridization of total genomic DNA (from micrococcal nuclease treated nuclei) and then calculated as a percentage of ovalbumin hybridization to identical slots.

Protein content was measured by densitometric scanning of Coomassie blue stained polyacrylamide gels.

Electron Microscopy

Samples of salt soluble polynucleosomes and total chromatin were prepared for electron microscopy by two methods. In the first method the sample was applied directly to a 200 mesh copper electron microscopy grid with a carbon film. The sample was allowed to adhere to the grid for one minute and then air dried and processed as described below. In the second method the sample was layered over a 0.1 M sucrose cushion made up in the same salt concentration as the sample (salt soluble polynucleosomes; 0.15 M NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA: total chromatin; 10 mM EDTA, pH 7.2) and deposited onto a carbon coated electron grid in a tabletop centrifuge operated at 3000 g for 2 minutes. The grids were washed in a wetting agent, 0.5% Photoflow (Kodak) to remove the sucrose. This protocol has been used routinely in the study of higher order chromatin structure (23). Samples were stained in 2% ethanolic uranyl acetate, rotary shadowed with platinum:palladium 80:20 and examined in a Hitachi H 500 electron microscope operated at 50 Kv. It was found that both procedures produced comparable results.

Treatment of core particles with ethidium bromide

Core particles from EDTA released chromatin (SEDTA) and from salt soluble polynucleosomes were prepared by a method similar to that of Libertini and Small (24). H1 and H5 histones were removed with CM Sephadex as described above and the stripped chromatin was dialyzed against 10 mM Tris-HCl, pH 8.0 and 1 mM $CaCl_2$. The chromatin was made to 10 A_{260} units/ml and digested with 10 units/ml of micrococcal nuclease for approximately 10 min (digestion times were optimised for each preparation). In some cases the core particles were further purified by gel filtration on Bio-Gel A-5m in 10 mM Tris-HCl, pH 8.0 and 1 mM EDTA. Treatment of the core particles with ethidium bromide and analysis of particle integrity by electrophoresis was carried out as described by McMurray and van Holde (25).

RESULTS

The level of polynucleosome solubility defines distinct classes of developmentally regulated genes

Mature chicken erythrocyte chromatin fragments were separated into two fractions based on their solubility properties in 0.15 M NaCl. The salt soluble chromatin fraction consists primarily of mononucleosomes while the majority of the polynucleosomes are salt insoluble. We have demonstrated that the small percentage of polynucleosomes, which are salt soluble, are highly enriched in β-globin gene sequences (10). Figure 1 shows the hybridization of a variety of additional cloned probes to fractions obtained by chromatin precipitation with 0.15 M NaCl. Examination of the extent of hybridization in the oligonucleosome region of the salt soluble chromatin lanes reveals three distinct classes: (1) class 1 gene chromatin, which includes H5 and H2A.F gene sequences, is enriched several fold over total in the salt soluble oligonucleosomes, (2) class 2 gene chromatin, which includes c-myc, vimentin and thymidine kinase, is not enriched, but shows a limited extent of hybridization to salt soluble oligonucleosomes, and (3) class 3 gene chromatin, which includes the repressed ovalbumin gene sequences, is virtually absent in the salt soluble oligonucleosomes.

Nuclei were digested with DNAase I as described by Villeponteau et al. (14). The digested DNA was placed onto nitrocellulose and hybridized to the indicated gene probe. The intensities of hybridization were

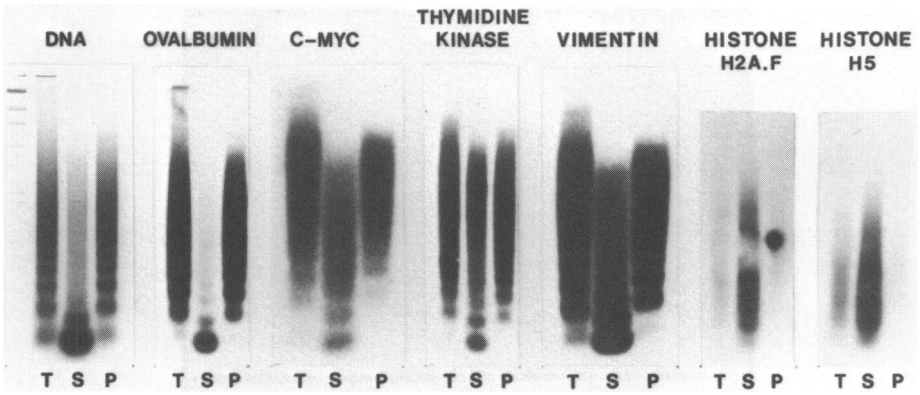

Figure 1. Polynucleosome solubility of chromatin of different genes. Purified DNA from total EDTA released chromatin fragments (T), and those soluble (S) or precipitable (P) in 0.15 M NaCl were electrophoretically resolved on a 1% agarose gel containing 1 μg/ml ethidium bromide, transfered to nitrocellulose and hybridized to various cloned DNA probes. Each lane contained 5 μg of DNA.

qantified by densitometer scanning and expressed as a percentage of ovalbumin probe hybridization (14). The correlation between DNAase I sensitivity and salt solubility properties of the chromatin of the various genes is shown in Figure 2. Class 1 gene chromatin (e.g., H5, β-globin and H2A.F gene chromatin), which is potentially transcriptionally active (19,26), has the greatest sensitivity to DNAase I as well as greatest salt

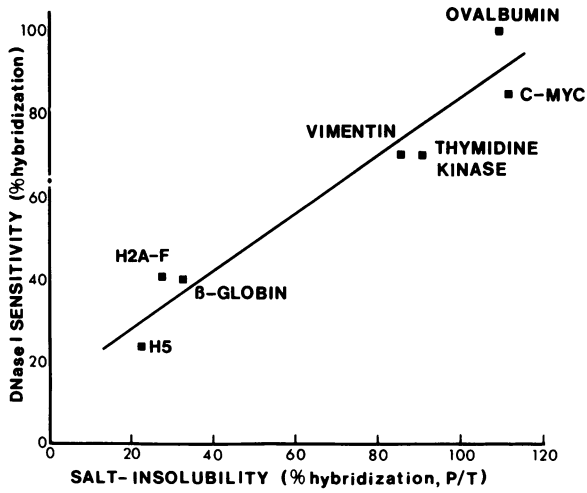

Figure 2. The relationship between DNAase I sensitivity and polynucleosome insolubility. The DNAase I sensitivity of various nuclear genes relative to the sensitivity of ovalbumin (100%) was determined as described in the Materials and Methods section. Salt insolubility is the ratio of the hybridization of DNA isolated from the 0.15 M NaCl insoluble chromatin (P) to that isolated from total EDTA released chromatin (T).

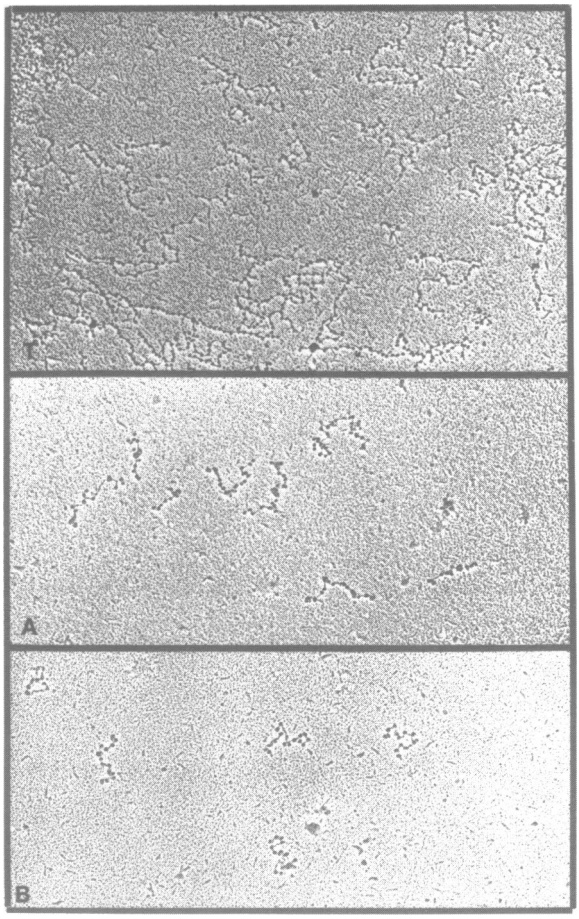

Figure 3. Electron micrographs of chromatin fragments. EDTA released chromatin fragments (T) and salt soluble polynucleosomes isolated by gel exclusion chromatography on Bio-Gel A-5m (A and B) were examined by electron microscopy. A and B are approximately equivalent to column fractions I and II (see reference 10).

solubility suggesting a relationship between these two properties. Class 2 gene chromatin (e.g., that of the thymidine kinase and c-myc genes, housekeeping genes which are not expressed in these cells (27)) has a low to moderate sensitivity to DNAase I and is only partially soluble in 150 mM NaCl. Class 3 gene chromatin, which contains repressed gene sequences like those of ovalbumin, is salt insoluble and relatively insensitive to DNAase I digestion.

The nucleosome density of the salt soluble polynucleosomes is unaltered

Total chicken erythroid chromatin fragments and salt soluble polynucleosomes obtained by gel exclusion chromatography of salt soluble chromatin, were examined by electron microscopy (Figure 3). The salt soluble

polynucleosomes have the same apparent nucleosome density as that of total chromatin fragments. Nucleosome free stretches of DNA were not observed in the salt soluble polynucleosomes. The occasional irregularities in the linker length can be accounted for by stretching during specimen preparation. At this level of resolution the structure of the competent gene enriched chromatin did not differ from that of total chromatin.

Erythroid-specific gene polynucleosomes are resistant to exogenously added linker histone induced precipitation

Analysis of the protein content of the salt soluble polynucleosomes demonstrated that these chromatin fragments are depleted approximately 30% in the linker histones, H1 and H5, relative to levels found in bulk chromatin (10). In order to test whether the chromatin of erythroid-specific genes had an altered binding of linker histones, H1/H5 stripped total chromatin was reconstituted with varying quantities of purified linker histones (see Materials and Methods section) and then separated into 0.15 M NaCl soluble and insoluble fractions. Figure 4, DNA panel, shows the size distribution of the DNA fragments which remain salt soluble. Without the addition of linker histones, all of the chromatin fragments remain soluble. The amount of polynucleosomes present in the salt soluble fraction declined with increasing linker histone added until the mononucleosome sized fragment predominated. The blot hybridized with the ovalbumin probe shows a decline in the extent of hybridization with increasing amount of linker histone added. The size distribution of the soluble ovalbumin chromatin fragments parallels that of total chromatin. In marked contrast the degree of hybridization to the histone H5 gene probe shows an increasing enrichment in the soluble chromatin as the amount of linker histones is added until an apparent maximum is reached, and then begins to decline slightly. The size distribution of the chromatin fragments that remain soluble is quite different than that of the bulk with H5 gene sequences being localized in oligonucleosomes. β-globin and histone H2A.F gene probes yielded the same results as the histone H5 probe with the salt soluble oligonucleosomes being enriched in these sequences (not shown). These observations indicate that class 1 gene chromatin, but not class 3 gene chromatin, has the inherent property to resist linker histone induced precipitation.

We have shown previously that the largest chromatin fragments, which are soluble in 0.15 M NaCl, are highly enriched in β-globin gene sequences (10). These salt soluble polynucleosomes are characterized by having a large number of modified and variant core histone species. The polynucleosome fraction from the reconstituted material, which is salt soluble, contains linker histones and has core histone modifications and variants which are similar in type and extent to those found in native chromatin. Most noticeable amongst these are significant increases in the levels of acetylation, ubiquitinated H2B and the variants H3.3 and H2A.Z (not shown).

Core particles of salt soluble polynucleosomes are sensitive to ethidium bromide induced dissociation

McMurray and van Holde (25) reported that binding of ethidium bromide to chicken erythrocyte core particles results in a dissociation of the structure which is reversible, time dependent and independent of DNA concentration. Total dissociation was complete within 25 h and required 2 moles of ethidium bromide per mole of base pair (25). We studied whether the nucleosome core particles of salt soluble polynucleosomes responded to the ethidium bromide induced dissociation in the same manner as that of core particles of total chromatin. Nucleosome core particles from total and salt soluble polynucleosomes were prepared by removing the linker histones with CM-Sephadex and digesting the stripped fragments with micrococcal nuclease. The DNA fragment size of the core particles thus prepared is shown in Figure 5A. The extent of dissociation of

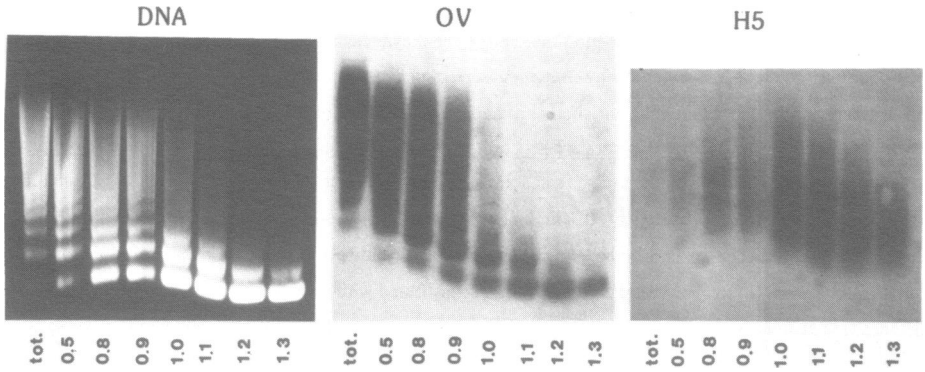

Figure 4. Hybridization of DNA fragments isolated from salt soluble reconstituted chromatin. Chromatin, stripped of and reconstituted with H1 and H5 histones, was precipitated with 0.15 M NaCl. The total reconstituted material (tot.) and the soluble fraction from reconstitutions carried out with varying quantities of H1 and H5 (given as the fractions of H1 and H5 levels in EDTA soluble chromatin) were hybridized with DNA probes for histone H5 (H5) and ovalbumin (OV) genes.

nucleosome core particles of total (T) or salt soluble polynucleosomes (I) at various concentrations of ethidium bromide was determined by electrophoretic analysis (Figure 5B). In the absence of ethidium bromide both types of core particle migrated as a single band, with the nucleosome core particles of salt soluble

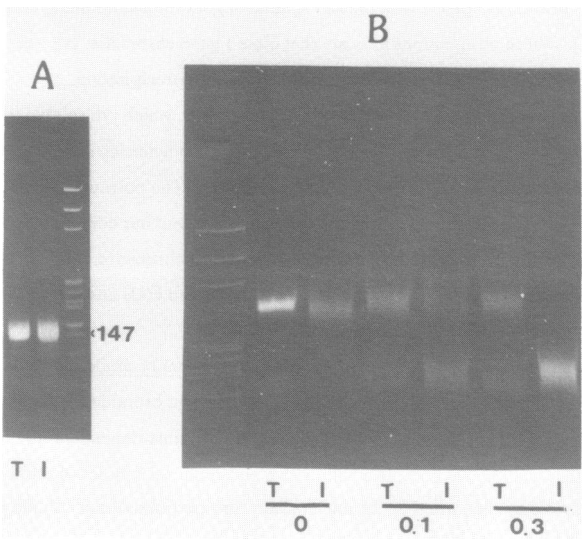

Figure 5. Core particle dissociation by ethidium bromide. A: 4.5% polyacrylamide gel electrophoresis of DNA from the core particle preparation of total (T) chromatin and salt soluble polynucleosomes (I). B: Electrophoretic analysis of core particles preincubated with ethidium bromide (described in reference 25). The number beneath each pair of lanes is the molar ratio of ethidium bromide:base pair DNA.

polynucleosomes (I) showing a greater dispersity than those from total chromatin. This dispersity could be a consequence of the high level of acetylated histone species present in the core particles of salt soluble polynucleosomes (10,28,29). At an ethidium bromide:core particle base pair molar ratio of 0.10 a dissociation of the T core particles occurs to a small extent as seen by the appearance of free DNA. McMurray and van Holde (25) calculated a 6% level of dissociation under these conditions. In contrast, at least 50% of the I core particles have dissociated. Increasing the ratio of ethidium bromide:base pair DNA to 0.3 resulted in the complete dissociation of the I particles and approximately 10% of the T particles.

Initially the core particle-ethidium bromide complexes were incubated for approximately 15 h before analysis. Reduction in the incubation time did not alter the extent of dissociation of the I particles. Moreover, at ratios of 0.3 complete dissociation was observed when electrophoretic analysis was carried out immediately after mixing, indicating that ethidium bromide induced dissociation of I core particles is essentially immediate.

DISCUSSION

DNAase I sensitive genes are preferentially localized in salt soluble chromatin

The results presented here indicate that the chromatin fragments that are soluble as polynucleosomes in 0.15 M NaCl are from the same genomic chromatin domains which are sensitive to DNAase I. Moreover, these chromatin fragments exclude the cooperative interaction amongst H1 histones which lead to the formation of higher order structure.

Analysis of the DNAase I sensitivity and salt solubility properties of various gene chromatins indicated the differential chromatin organization of erythroid-specific, housekeeping and repressed genes. Erythroid-specific gene sequences (H5 and β-globin) and histone H2A.F gene sequences are sensitive to DNAase I and also found enriched in chromatin which is salt soluble as polynucleosomes. The relationship between DNAase I sensitivity and salt solubility indicates that the biochemical features of these gene chromatins may determine both properties. Our results suggest that the salt solubility properties of the chromatin of erythroid-specific genes are a consequence of an altered association with linker histones (H1/H5). This altered association, which may give rise to a reduction in the steady state levels of these proteins complexed to the transcriptionally competent chromatin regions, would probably result in the destabilization of linker histone dependent higher order structure. Also, the salt soluble polynucleosomes are enriched in acetylated and ubiquitinated histones (7-10). Since the N-terminal tails of the histones are thought to be involved in the formation of higher order structures, the acetylation of the lysine residues within this region of the molecule may lead to the weakening of these interactions (30). The housekeeping genes (c-myc, thymidine kinase) also have, to some extent, an atypical chromatin structure. It is interesting to note that the chromatin structures of housekeeping and erythroid-specific genes can also be distinguished by in situ nuclear nick-translation. The labeled chromatin regions are localized at the borders of condensed chromatin masses along interchromatin channels communicating with the nuclear periphery (31). Since the labeling of chromatin by this technique requires access by both DNAase I and E. coli DNA polymerase I, our results predict that only regions with the atypical structure will be available to both enzymes. Indeed, erythroid-specific gene

chromatin is preferentially labeled by this technique (31). We expect that histone H2A.F gene chromatin would also be selectively labeled. Housekeeping genes, such as thymidine kinase, have only a very limited solubility as polynucleosomes and are labeled to a considerably lower extent than erythroid-specific chromatin regions.

The atypical, open chromatin structure might be expected to facilitate the transcription process. It should be noted that although the mature erythrocyte has very low levels of mRNA, at least some of the erythroid-specific genes are in a transcriptionally ready state (26).

Erythroid-specific gene chromatin is resistant to H1/H5 induced salt precipitation

Reconstitution of stripped chromatin with varying amounts of linker histone demonstrated that an inherent property of the erythroid-specific and histone H2A.F gene chromatins alters H1/H5 induced precipitability in 0.15 M NaCl. A simple explanation based on our results is that the chromatin of erythroid-specific (and H2A.F) genes has a reduced affinity or altered mode of binding to the linker histones, as opposed to the ovalbumin gene chromatin which avidly associates with H1 and H5. This hypothesis is in agreement with the observation by Weintraub (12) that ovalbumin gene chromatin exchanged exogenously added histones H1 and H5 while β-globin gene chromatin does not.

Caplan et al. (32) observed that the sedimentation behaviour of EcoRI produced globin gene fragments was consistent with an altered association with linker histone. These authors argue that the sedimentation behaviour of this gene chromatin is due to the presence of the DNAase I hypersensitive regions which are localized at the 3' and 5' ends of the gene. Such nuclease hypersensitive sites are probably not responsible for the solubility of the competent gene enriched polynucleosomes for several reasons : 1) these sites are also hypersensitive to micrococcal nuclease (33,34) and would likely be cleaved in our preparation, 2) assuming such sites are not preferentially cleaved, then it would follow that thymidine kinase gene chromatin, which has a 5' hypersensitive site (35), would be preferentially salt soluble, but it is not, and 3) examination of the highly competent gene enriched salt soluble polynucleosome fraction by electron microscopy indicated a typical "beads-on-string" appearance, nucleosome density, and no indication of nucleosome free regions. This chromatin fraction maintained an extended appearance even in 0.15 M NaCl while total solubilized chromatin is highly aggregated under these conditions (not shown). It is likely that the observed extended configuration in NaCl of this chromatin fraction could account for any observed alterations in the hydrodynamic properties of β-globin chromatin. It is unlikely, however, that nucleosome free regions, in bulk, can account for the altered solubility of this chromatin fraction.

Both the stripped chromatin and linker histone preparation used for reconstitution were free of detectable nonhistone proteins. Therefore, the observed differences of the ovalbumin and H5 gene chromatins are most likely the result of differences in nucleosome core structure.

Structural alterations of nucleosome core particles in salt soluble polynucleosomes

Nucleosome structure of the salt soluble polynucleosomes was analyzed by ethidium bromide induced dissociation. The nucleosome core particles derived from the salt soluble polynucleosmes were sensitive to ethidium bromide induced dissociation. Complete dissociation of these core particles occurs at a much lower stoichiometric ratios of ethidium bromide:base pair of DNA compared to those required for bulk core particles

(0.3 vs 2.0 (25)). It is possible that the increased sensitivity to ethidium bromide induced dissociation may be due, in part, to increased levels of acetylation. Recently Oliva et al. (36) have demonstrated that histone hyperacetylation is associated with an elongated shape of the nucleosome. Also, several studies indicate that hyperacetylation loosens the histone-DNA contacts which may facilitate the ethidium bromide induced dissociation process (see various references listed in reference 36). This alteration in nucleosome shape may influence the interaction of histone H1 with the nucleosome.

In summary, our results suggest that the competent chromatin state is due to an altered association of chromatin domains with linker histones and that this altered association itself results from differences in the structure of the nucleosome core.

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