Selective Solubilization of β-Globin Oligonucleosomes at Low Ionic Strength†

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ABSTRACT: We [Rocha, E., Davie, J. R., van Holde, K. E., & Weintraub, H. (1984) *J. Biol. Chem. 259*, 8558-8563] have previously reported that the transcriptionally competent β-globin gene domain is selectively enriched in chromatin fractions eluted with solutions of approximately physiological ionic strength from micrococcal nuclease digested mature chicken erythrocyte nuclei. In this report, we demonstrate that β-globin chromatin is eluted as oligonucleosomes while vitellogenin, a transcriptionally inactive gene, is eluted as mononucleosomes as is the bulk of sequences found in this fraction. Following removal of the salt, the eluted chromatin was made 100 mM KCl and separated into aggregation-prone and aggregation-resistant fractions. Globin sequences were present in both fractions and had the greatest enrichment in the aggregation-prone fraction which contained H1 and H5, H1 being more abundant. A procedure is presented in which H1 is selectively removed from the erythrocyte nuclei. Following the selective removal of H1 and subsequent fractionation, globin but not vitellogenin oligonucleosomes were present in the aggregation-resistant chromatin fraction. The results indicate the β-globin domain is a mosaic of aggregation-resistant and aggregation-prone regions with the latter being associated with H1 and H5. Vitellogenin sequences were associated principally with aggregation-prone regions complexed with H5.

The H1 (or linker) histones seem to have a key role in the maintenance of the transcriptionally repressed state by stabilizing a highly condensed chromatin folding (Weintraub, 1985). In the chicken erythrocyte, the preponderance of the linker histones is H5 which appears to give a high degree of stability to higher order structures and may give rise to the transcriptional inactivity of these cells (Thomas & Rees, 1983; Thomas et al., 1985; Bates et al., 1981; Mazen et al., 1982). Histone H5 is accumulated up to a H5/H1 ratio of slightly greater than 2 (0.9 mol of H5/nucleosome and 0.4 mol of H1/nucleosome; Bates & Thomas, 1981) during the terminal stages of erythrocyte differentiation. One of the intriguing questions yet to be answered is whether histone H5 replaces histone H1 in specific locations along the chromatin fiber. Mazen et al. (1982) addressed this question by studying the distribution of histone H5 along polynucleosome chains of chicken erythrocyte chromatin with anti-H5 antibodies and by immunoelectron microscopy. These investigators demonstrated that there existed histone H5- and H1-enriched domains. However, this study did not determine whether the histone H5 molecules were preferentially localized with specific DNA sequences.

Cole and co-workers have demonstrated that the distribution of H1 is nonuniform in chromatin (Huang & Cole, 1984; Jin & Cole, 1986). Huang and Cole (1984) separated two regions of chromatin by their solubility properties at physiological ionic strength: an aggregation-resistant region (chromatin soluble at physiological ionic strength) depleted in H1 and an aggregation-prone region (chromatin insoluble at physiological ionic strength) containing more H1 than the former region. In a recent study, Jin and Cole (1986) reported that H1 exchange was limited to their respective regions: H1 exchange would not occur between aggregation-resistant and aggregation-prone regions.

Several workers have shown that a chromatin fraction apparently less compact than the bulk is enriched in globin sequences (Weintraub, 1984; Kimura et al., 1983). Weintraub (1984) electrophoretically resolved two populations of supranucleosome particles, one of which contained globin genes and another which contained vitellogenin sequences. The integrity of vitellogenin supranucleosomes was not dependent on the continuity of the DNA strand. Apparently, this structure was held together by the interactions of contiguous H1 histones. The globin gene containing supranucleosomes which contained H1 and H5 differed from the vitellogenin supranucleosomes in that it did not have the DNA size independent stability nor did it take up exogenously added histone H1 as did the vitellogenin supranucleosome species. Kimura et al. (1983) used a different approach to study the distribution of the H1 histones in chicken erythrocyte chromatin by determining the sedimentation rates of various gene chromatin fragments in sucrose gradients. They found the erythrocyte-specific retardation of β-globin gene chromatin to be consistent with a partial depletion of H1 histones. Our previous studies (Rocha et al., 1984) demonstrated that the globin gene domain was selectively enriched in the chromatin fractions eluted with low salt (50-200 mM NaCl) from micrococcal nuclease treated nuclei while transcriptionally inactive genes, vitellogenin and ovalbumin, were concentrated in the less salt soluble fractions. The results of these experiments are in accord with those of Kimura et al. (1983), who suggested the chromatin of the β-globin may be depleted in the H1 histones.

In this report, we have further investigated the distribution and type of linker histone complexed with various gene chromatins (β-globin gene domain and vitellogenin gene). To this end, we redesigned the chromatin fractionation procedure and studied the extraction/solubility properties of the various gene chromatins isolated from nuclei that had histone H1 selectively removed.

Materials and Methods

Isolation and Digestion of Nuclei. Red blood cells from adult white Leghorn chickens were collected in 75 mM

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NaCl/25 mM (ethylenedinitrilo)tetraacetic acid (EDTA),[1] washed of the buffy coat, and stored at −70 °C. Nuclei were isolated by washing 3 times in RSB (10 mM NaCl, 10 mM Tris-HCl, pH 7.5, 3 mM $MgCl_2$, and 1 mM PMSF) including 0.25% NP-40 in the first two washes. Nuclei were resuspended in the digestion buffer (1 M hexylene glycol, 10 mM PIPES, pH 7.0, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 1% thiodiglycol, and 1 mM PMSF) to a DNA concentration of 2.5 mg/mL and incubated with micrococcal nuclease at 37 °C. Digestion was stopped by the addition of EGTA to 10 mM, and the nuclei were collected by centrifugation. The digestion conditions were such that the leakage of chromatin fragments into the supernatant did not occur (Rocha et al., 1984). All buffers contained 30 mM sodium butyrate. The extent of micrococcal nuclease digestion was as described in figure legends.

Citric Acid Buffer Treatment for Removal of H1. Isolated nuclei were washed twice in 0.25 M sucrose, 25 mM KCl, 1 mM $CaCl_2$, and 1 mM $MgCl_2$ in citric acid/phosphate buffers, adjusted to varying pH, at 4 °C for 30 min (Lawson & Cole, 1979). These nuclei were equilibrated in digestion buffer for 1 h before digestion with micrococcal nuclease.

Salt Fractionation of Digested Nuclei. Salt fractionation was carried out as described (Rocha et al., 1984). Micrococcal nuclease digested nuclei were collected by centrifugation, resuspended in 50 mM Tris-HCl, pH 7.0, 2 mM $MgCl_2$, 75 mM KCl, 10 mM EGTA, 1% thioglycol, 30 mM sodium butyrate, 1 mM PMSF, and 200 mM NaCl, and left on ice for 30 min. When collected by centrifugation, the pellet is fraction P0.2. The supernatant (S0.2) was dialyzed against 1 mM EDTA, pH 7.0, and then made to 100 mM KCl to precipitate chromatin containing H1 and H5. The precipitated chromatin (S0.2 ppt.) was collected by centrifugation (12000*g* for 20 min) and resuspended in 1 mM EDTA. The supernatant (S0.2 sup.) was dialyzed against 1 mM EDTA.

Soluble chromatin was also prepared in a low ionic strength buffer by resuspending digested nuclei in 10 mM EDTA/1 mM PMSF and left on ice for 2 h. The nuclear debris was removed by centrifugation (12000*g* for 20 min). The resulting supernatant contained 63% of the total nuclear 260-nm-absorbing material. This solubilized chromatin (SEDTA) was treated with KCl as above to obtain soluble and insoluble fractions.

DNA Preparation, Blotting, and Hybridization. DNA was prepared from each fraction as described previously (Davie & Saunders, 1981) and was used for dot blot and Southern blot analysis. DNA was prepared for dot blot analysis as in Maniatis et al. (1982) and applied to nitrocellulose (Schleicher & Schuell, BA-83) using the Bio-Rad Bio-dot apparatus. 20 × SSC (1 × SSC: 150 mM NaCl and 15 mM sodium citrate) was used for rinsing the wells before and after DNA application. DNA (10 μg/lane) was electrophoresed on 1% agarose minigels. Southern transfer was carried out as in Maniatis et al. (1982). Hybridization of the filters was performed as described by Thomas (1979). The cloned DNA probes used were pCBG 14.4 (a unique adult β-globin sequence) and pCBG 18.7 (an embryonic ε-globin sequence) (Villeponteau et al., 1982). Both clones were acquired from H. Martinson. pVTG 421, obtained from H. Weintraub, recognizes the 5′ region of the chicken vitellogenin gene (Burch & Weintraub, 1983). The distribution and amount of a specific probe sequence in the chromatin fractions were determined by integrating the densitometric scans of each dot as described by Ferenz and Nelson (1985).

[1] Abbreviations: EDTA, (ethylenedinitrilo)tetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate; NP-40, Nonidet P-40; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

Table I: Distribution of Chromatin in Different Fractions

fraction	distribution of 260-nm-absorbing material (% of total)	
	+H1	−H1[a]
pellet P0.2	80.6 ± 0.5	76.2 ± 2.6
supernatant S0.2	9.3 ± 0.9	12.0 ± 2.7
KCl soluble S0.2 sup.	8.1 ± 0.1	10.2 ± 0.2
KCl insoluble S0.2 ppt.	1.2 ± 0.1	1.8 ± 0.2

[a] Nuclei treated with citric acid/phosphate buffer, pH 2.4.

Gel Electrophoresis. The proteins in each fraction were isolated by acid extraction (0.4 N H_2SO_4) and centrifuged, and the supernatant was dialyzed sequentially against 0.1 N acetic acid and H_2O. The proteins were electrophoretically resolved on a 15% polyacrylamide–SDS gel (Davie & Saunders, 1981) and stained with Coomassie blue.

Results

β-Globin Oligonucleosomes Are Enriched in Low-Salt-Eluted Chromatin Fractions. Our previous results (Rocha et al., 1984) demonstrated that the β-globin domain was enriched in the low-salt-eluted chromatin fractions (50, 100, and 200 mM NaCl eluted fractions). The fractionation procedure has been modified such that the micrococcal nuclease digested nuclei are extracted directly with a 200 mM NaCl containing buffer. This treatment liberated about 10% of the 260-nm-absorbing material (Table I) which consisted predominantly of mononucleosomes (Figure 1, DNA, lane c). This chromatin fraction (S0.2) was enriched in β-globin sequences (approximately 4–5-fold over total) containing about 45% of the total globin sequences. The vitellogenin sequences were present at approximately the same level as the bulk of DNA in this fraction S0.2.

The hybridization results suggest the monomers were extracted with a low degree of sequence discrimination as they contained both globin and vitellogenin sequences (Figure 1). The most striking observation was that, although the amount of dinucleosomes and longer chromatin fragments in the salt extract was minor, they hybridized strongly to the globin probe and minimally to vitellogenin. An embryonic globin gene (ε-globin) gave the same hybridization pattern as β-globin (Figure 1). This observation suggested that the β-globin domain in mature chicken erythrocyte nuclei was selectively eluted as oligomer-sized chromatin fragments.

β-Globin Domain Is Associated with Aggregation-Resistant and Aggregation-Prone Regions. The 200 mM NaCl extracted fraction (S0.2) contained the four core histones (H2A, H2B, H3, and H4), an enrichment of the HMG proteins (1, 2, E, 14, and 17), and a reduced amount of the H1 histones (H1 and H5) with an increased relative content of H1 to H5 (Figure 1, protein). It should be noted that we have previously reported that HMG proteins 1 and 2 and to some extent 14 and 17 are extracted from undigested nuclei at this ionic strength (Rocha et al., 1984). Since the major nucleosome species in this fraction was core particle sized (146 base pairs), this would explain the depletion of the H1 histones in this fraction since this nucleosome species is prone to losing its H1 histones (Jin & Cole, 1986). To ascertain the extent to which the H1 histones were associated with extracted globin oligomers, this chromatin fraction was separated into an aggregation-resistant fraction (depleted in the H1 histones) and

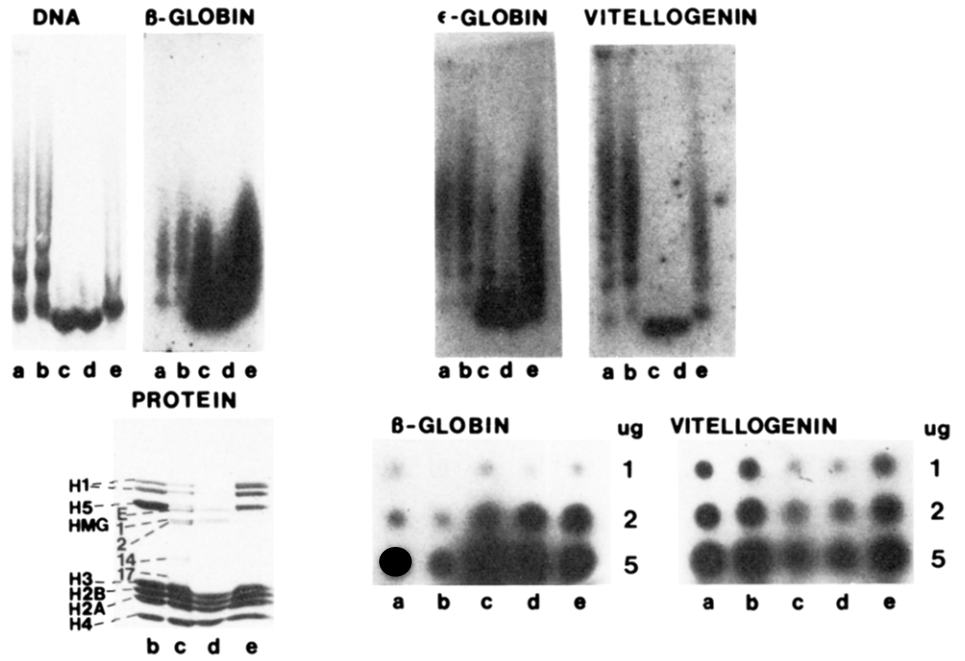

FIGURE 1: β-Globin domain chromatin is preferentially extracted from micrococcal nuclease digested nuclei as oligonucleosomes. Mature chicken erythrocyte nuclei were resuspended in digestion buffer to a DNA concentration of 2.5 mg/mL and incubated with micrococcal nuclease (50 A_{260} units/mL) at 37 °C for 25 min. DNA prepared from each fraction was used for Southern blot and dot blot analysis as described under Materials and Methods. The DNA (10 μg/lane) was electrophoresed in 1% agarose minigels and stained with ethidium bromide. The cloned DNA probes used were as follows: β-globin, pCBG 14.4; ϵ-globin, pCBG 18.7 (this plasmid contains sequences which hybridize to repetitive elements (Villeponteau et al., 1982); vitellogenin, pVTG 421. These panels show the autoradiograms of the blots after hybridization with the radiolabeled DNA probe. The acid-extracted proteins were electrophoretically resolved on a 15% polyacrylamide–SDS minislab gel and stained with Coomassie blue. The lanes correspond to the following: a, total; b, fraction P0.2; c, fraction S0.2; d, fraction S0.2 sup.; e, fraction S0.2 ppt.

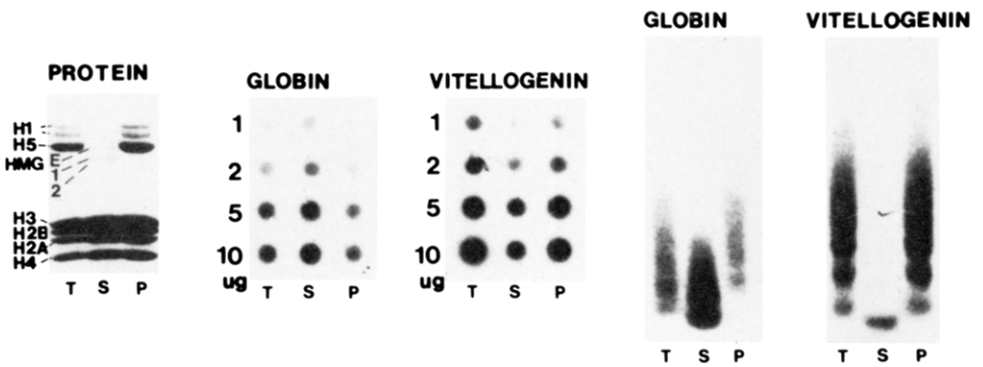

FIGURE 2: β-Globin chromatin fragments are enriched in an aggregation-resistant, KCl-soluble fraction. Nuclei were digested as described in Figure 1 and resuspended in 10 mM EDTA, pH 7.0. The solubilized chromatin (T) was made 100 mM in KCl and centrifuged. The KCl-soluble, aggregation-resistant fraction (S) contained 30% of the 260-nm-absorbing material in fraction T while the KCl-insoluble, aggregation-prone chromatin (P) contained 70% of the 260-nm-absorbing material. The globin probe was pCBG 14.4, and vitellogenin was pVTG 421. The acid-extracted proteins were electrophoretically resolved on a 15% polyacrylamide–SDS minislab gel.

an aggregation-prone fraction (Huang & Cole, 1984; Jin & Cole, 1986). This was achieved by making the dialyzed salt eluted fraction to 100 mM KCl (Simpson, 1978). The aggregation-prone, precipitated fraction (S0.2 ppt.) contained both H1 and H5 with the relative content of H1 being greater than in total chromatin, and the aggregation-resistant, soluble fraction (S0.2 sup.) was depleted in the H1 histones and enriched in the HMG proteins (Figure 1). The β-globin oligonucleosomes were greatly enriched (about 8–9-fold) in the precipitate. The ϵ-globin oligomers distributed in the same manner as the β-globin fragments (Figure 1).

To determine whether the 200 mM NaCl extracting buffer was causing a redistribution of the H1 histones contributing to the generation of the KCl-soluble globin chromatin, the chromatin fragments were released from micrococcal nuclease digested nuclei into a low ionic strength buffer. The chromatin fragments were split into an aggregation-resistant, KCl-soluble fraction and the aggregation-prone, KCl-insoluble fraction. As expected, the H1 histones were depleted in the aggregation-resistant fraction (Figure 2). Again, aggregation-resistant oligomers were enriched in globin sequences and depleted in vitellogenin (Figure 2). These results indicated that the extraction of the nuclei with the 200 mM NaCl solution was not responsible for the presence of the globin oligonucleosomes in the aggregation-resistant chromatin fraction.

Histone H1 Can Be Selectively Extracted from Chicken Erythrocyte Nuclei. In order to ascertain the role of H1 in the fractionation of the globin and vitellogenin sequences, we wanted a procedure which would selectively remove H1 from the chicken erythrocyte nuclei. Lawson and Cole (1979) developed a procedure which selectively released histone H1 from HeLa nuclei without disruption of the sequential arrangement of nucleosomes on DNA. We determined that for chicken erythrocyte nuclei resuspended in a citric acid/sodium

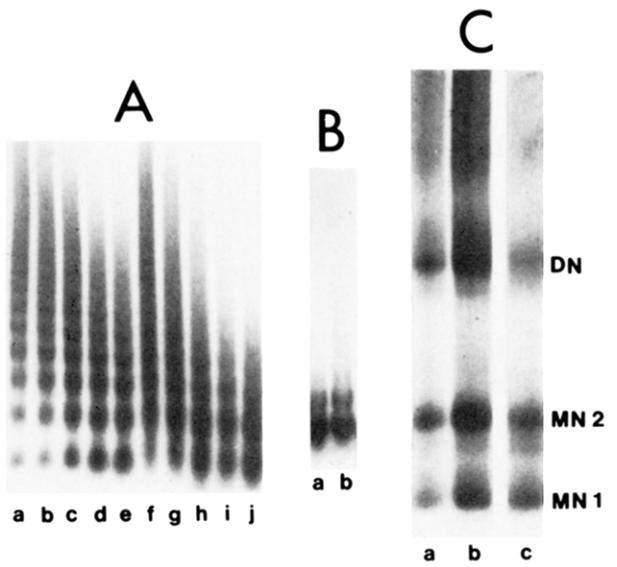

FIGURE 3: Structure of the nucleosomes is not disrupted by treatment of nuclei with citric acid/phosphate, pH 2.4, buffer. (A) Untreated nuclei (lanes a–e) or nuclei treated with citrate acid/phosphate buffer, pH 2.4 (lanes f–j), were digested for various times with micrococcal nuclease (50 A_{260} units/mL) at 37 °C. DNA was isolated and electrophoretically resolved on a 1% agarose gel which contained ethidium bromide. The times of digestion were the following: a, 10 min; b, 15 min; c, 20 min; d, 30 min; e, 40 min; f, 5 min; g, 10 min; h, 15 min; i, 20 min; j, 30 min. (B) Untreated nuclei (lane b) or nuclei treated with acid phosphate buffer (lane a) were digested with micrococcal nuclease (100 A_{260} units/mL) at 37 °C for 25 or 20 min, respectively. The DNA fragments were electrophoretically resolved on a 1% agarose gel. (C) Chromatin fragments from treated (lanes a and b) or untreated nuclei (lane c) were electrophoretically resolved on a 4% polyacrylamide/0.5% agarose composite gel. The gel was stained with Coomassie blue.

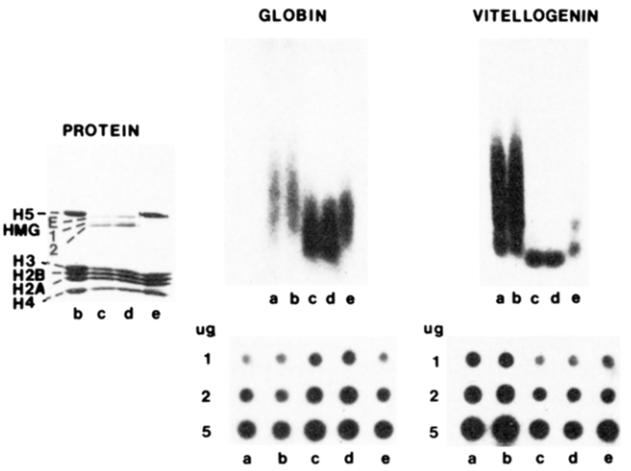

FIGURE 4: Fractionation of globin and vitellogenin sequences after removal of H1 from chicken erythrocyte nuclei. Acid phosphate (pH 2.4) treated nuclei were digested with micrococcal nuclease (50 A_{260} units/mL) at 37 °C for 20 min. Chromatin fractionation and DNA isolation were performed as described under Materials and Methods. The DNA was analyzed by Southern blot and dot blot hybridization analysis for the presence of β-globin sequences (pCBG 14.4) and vitellogenin sequences (pVTG 421). Acid-extracted proteins were electrophorically resolved on a 15% polyacrylamide–SDS minislab gel. The lanes correspond to the following: a, total; b, fraction P0.2; c, fraction S0.2; d, fraction S0.2 sup.; e, fraction S0.2 ppt.

phosphate buffer, pH 2.4, the selective removal of H1 appeared to be optimal and no detectable H5 was extracted from the nuclei. In addition to H1, HMG 14 and 17 and some high molecular weight proteins were extracted (not shown).

The integrity of the chromatin of the acid-treated nuclei was checked by electrophoretic analysis of the DNA fragment pattern after micrococcal nuclease digestion (Figure 3). Although the digested citrate/phosphate-treated nuclei yielded an electrophoretic banding pattern that was somewhat less distinct than that of control nuclei, it still clearly gave the characteristic nucleosomal DNA repeat. The initial rate of nuclease digestion of the acid-treated nuclei was greater than that of the untreated nuclei by about 2.3-fold. To further test for nucleosome integrity after acid treatment, the treated or untreated nuclei were extensively digested with micrococcal nuclease. When the size distribution of DNA fragments was examined on agarose gels (Figure 3B) or 4% polyacrylamide-nondenaturing gels (not shown), the monomeric-size DNA fragment predominated in both cases. Fragments migrating more rapidly than the monomeric fragment were found in the same abundances for both samples. The chromatin fragments from both samples were inspected by electrophoresis on agarose/polyacrylamide gels which resolve the nucleoprotein complexes (Figure 3C). The H5–nucleosomes and nucleosomes without linker histones isolated from acid-treated nuclei migrated as discrete particles similar to their counterparts from untreated nuclei. Thus, by these different criteria, irreversible disruption of the nucleosome by this acid treatment was not occurring.

Removal of H1 Alters the Distribution of the Globin Sequences. Following treatment with the acid buffer, the nuclei were digested with micrococcal nuclease and subsequently extracted with 200 mM NaCl. The removal of H1 did not markedly affect the amount of material that could be extracted from the nuclei (Table I) or the distribution of the β-globin and vitellogenin sequences among the various sized chromatin fragments in the supernatant S0.2 fraction (comparing Figure 1 to Figure 4, lanes a–c). Note that globin but not vitellogenin sequences were located in the extracted oligonucleosomes. The relative nuclease sensitivities of the globin and vitellogenin chromatin did not markedly alter after H1 was removed (Figure 4, compare lanes a). As described above, the 200 mM NaCl extract was separated into two fractions, and the distribution of the globin and vitellogenin sequences was determined. When H1 was removed, the enrichment of the globin sequences in the aggregation-prone chromatin fraction was less pronounced compared to what was observed in the previous experiments (compare Figure 1 to Figure 4 β-globin, lane e). This was also observed for the vitellogenin sequences in the aggregation-prone fraction although the reduction of hybridization to the various sized DNA fragments was less acute than that observed for globin. It should be noted that the distribution of the 260-nm-absorbing material in the aggregation-prone (S0.2 ppt.) and aggregation-resistant (S0.2 sup.) fractions did not change significantly (Table I).

Discussion

Rocha et al. (1984) previously demonstrated that the β-globin domain chromatin was preferentially extracted by low ionic strength buffers (buffers containing 50 and 100 mM NaCl). The principal nucleosome species in these fractions was the mononucleosome, and we (Rocha et al., 1984) assumed that the globin enrichment in the low-salt fractions was due to selective extraction of the globin mononucleosomes. Our present analysis demonstrates that although the salt-extracted nucleosomes are primarily of monomer size, the globin nucleosomes are present as oligomers. Globin oligomers can also be extracted by 100 mM NaCl buffers (B. E. Nickel and J. R. Davie, unpublished experiments). Moreover, both β- and ε-globin oliogmers are selectively extracted by the low-salt buffers. Therefore, the results of these and previous (Rocha

et al., 1984) experiments suggest the β-globin domain is preferentially extracted from micrococcal nuclease digested nuclei as oligomers.

Our results demonstrate that β-globin chromatin [a transcriptionally competent, DNase I sensitive gene (Rocha et al., 1984; Bloom & Anderson, 1979)] is present in both aggregation-resistant and aggregation-prone fractions. The aggregation-prone chromatin fraction (S0.2 ppt.) was greatly enriched in globin sequences and in H1. After removal of histone H1 from the nuclei prior to fractionation, the extent of globin enrichment in this fraction diminished. Although the content of the vitellogenin sequences in the S0.2 ppt. fraction from the citrate phosphate treated nuclei was less than that for untreated nuclei, the decline in vitellogenin sequences in this fraction was not as acute as that observed for globin sequences. This observation suggests that a portion of the β-globin domain is present in H1-enriched chromatin regions. Our analysis indicates that the β-globin domain in mature chicken erythrocyte is a mosaic of aggregation-resistant and aggregation-prone regions. The aggregation-resistant chromatin may be either depleted in the H1 histones and/or contain a full complement of H1 histones which are bound differently to a chromatin which resists aggregation (Jin & Cole, 1986; Weintraub, 1984). The aggregation-prone regions are associated with both H1 and H5. The pattern of distribution of the vitellogenin sequences, a transcriptionally inactive, DNase I insensitive gene, is not markedly affected by the removal of H1. For example, the removal of H1 did not result in the solubilization of vitellogenin oligonucleosomes in the supernatant S0.2. Moreover, when EDTA-released chromatin from H1-depleted nuclei was separated into aggregation-resistant and aggregation-prone fractions as described in Figure 2, globin but not vitellogenin oligonucleosomes were found in the aggregation-resistant chromatin fraction (not shown). These observations suggest the vitellogenin gene is principally associated with aggregation-prone chromatin regions which are predominantly H5 rich.

It has been suggested that the β-globin genes are selectively soluble at approximately physiological ionic strength as an indirect result of their greater nuclease sensitivty and smaller average size: the smaller fragments may lose the histones H1 and H5 to the larger chromatin fragments (Komaiko & Felsenfeld, 1985). To test this hypothesis, chicken erythrocyte chromatin was released from micrococcal nuclease digested nuclei into low ionic strength buffers and separated into aggregation-resistant and aggregation-prone regions (100 mM KCl soluble and insoluble, respectively) which is a similar procedure used by Jin and Cole (1986). It is important to note that the vitellogenin and globin chromatin are digested at similar rates by micrococcal nuclease (Bloom & Anderson, 1979). If the small fragments lost their H1 histones to the longer fragments, these smaller fragments would become aggregation resistant, and one should observe both small globin and vitellogenin fragments in similar concentrations in the KCl-soluble fraction. Clearly, this did not occur (Figure 2). It is conceivable that globin chromatin due to its composition is more prone to losing its H1 histones, or a portion of the globin chromatin is not associated with these histones. Since we isolated salt-extracted globin oligonucleosomes containing H1 histones (S0.2 KCl ppt., Figure 1), this result argues that the globin oligonucleosomes are capable of retaining their H1 histones and that there may indeed be a portion of the globin chromatin which is depleted in the H1 histones.

Ferenz and Nelson (1985) and Nelson et al. (1986) demonstrated that globin oligonucleosomes were selectively eluted from micrococcal nuclease digested immature chicken erythrocyte nuclei into solutions which were of approximately physiological ionic strength. Thus, our results are in accord with theirs. It should be noted that the β-globin chromatin in the immature erythrocyte is actively transcribed and in a micrococcal nuclease sensitive state (Bloom & Anderson, 1979). For the cells used in our study, the globin chromatin is not transcribed and has lost its sensitivity to micrococcal nuclease. (The globin chromatin is still in a DNase I sensitive state which is identified as "competent".) Therefore, although the active and competent β-globin chromatins differ in micrococcal nuclease sensitivities, both chromatins must share similar compositional features which allow them to be extracted as oligomers at physiological ionic strength. Ferenz and Nelson (1985) and Nelson et al. (1986) have presented strong evidence to suggest the enhanced solubility of the β-globin oligomers is due to selective histone hyperacetylation of this gene chromatin. We (Rocha et al., 1984) have previously reported that the histones in the low-salt-eluted fractions containing the globin oligomers were enriched in the hyperacetylated histone H4 species. An enrichment of the hyperacetylated H4 species was also observed in the aggregation-resistant, KCl-soluble (S0.2 sol.) fraction (not shown). However, other studies with late-stage trout testis chromatin suggest that histone hyperacetylation is not sufficient to promote increased solubility (see Discussion; Rocha et al., 1984). Our analyses indicate that increased solubility at physiological ionic strength of the globin oligonucleosomes is dependent not only on histone hyperacetylation but also on the depletion of the H1 histones as well.

In summary, we find that the β-globin chromatin domain is a mosaic of aggregation-resistant and aggregation-prone regions. Such a chromatin composition may reflect a destabilized higher order structure which could readily explain the preferential solubility of globin oligonucleosomes at physiological ionic strength, the altered sedimentation rates of erythrocyte β-globin chromatin (Kimura et al., 1983), and the destabilized supranucleosome structure of β-globin chromatin (Weintraub, 1984).

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REFERENCES

Bates, D. L., & Thomas, J. O. (1981) *Nucleic Acids Res. 9*, 5883–5894.

Bates, D. L., Butler, P. J. G., Pearson, E. C., & Thomas, J. O. (1981) *Eur. J. Biochem. 119*, 469–476.

Bloom, K. S., & Anderson, J. N. (1979) *J. Biol. Chem. 254*, 10532–10539.

Burch, J. B. E., & Weintraub, H. (1983) *Cell (Cambridge, Mass.) 33*, 65–76.

Davie, J. R., & Saunders, C. A. (1981) *J. Biol. Chem. 256*, 12574–12580.

Ferenz, C. R., & Nelson, D. A. (1985) *Nucleic Acids Res. 13*, 1977–1995.

Huang, H., & Cole, R. D. (1984) *J. Biol. Chem. 259*, 14237–14242.

Jin, Y., & Cole, R. D. (1986) *J. Biol. Chem. 264*, 3420–3427.

Kamaiko, W., & Felsenfeld, G. (1985) *Biochemistry 24*, 1186–1193.

Kimura, T., Mills, F. C., Allan, J., & Gould, H. (1983) *Nature (London) 306*, 709–712.

Lawson, G. M., & Cole, R. D. (1979) *Biochemistry 18*, 2160–2166.
Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Mazen, A., DeMurcia, G., Bernard, S., Pouyet, J., & Champagne, M. (1982) *Eur. J. Biochem. 127*, 169–176.
Nelson, D. A., Ferris, R. C., Zhang, D., & Ferenz, C. P. (1986) *Nucleic Acids Res. 14*, 1667–1682.
Rocha, E., Davie, J. R., van Holde, K. E., & Weintraub, H. (1984) *J. Biol. Chem. 259*, 8558–8563.
Simpson, R. T. (1978) *Biochemistry 17*, 5524–5531.
Thomas, J. O., & Rees, C. (1983) *Eur. J. Biochem. 134*, 109–115.
Thomas, J. O., Rees, C., & Pearson, E. C. (1985) *Eur. J. Biochem. 147*, 143–151.
Thomas, P. S. (1979) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5201–5215.
Villeponteau, B., Landes, G. M., Pankratz, M. J., & Martinson, H. G. (1982) *J. Biol. Chem. 257*, 11015–11023.
Weintraub, H. (1984) *Cell (Cambridge, Mass.) 38*, 17–27.
Weintraub, H. (1985) *Cell (Cambridge, Mass.) 42*, 705–711.

Competition for Formation of Nucleosomes on Fragmented SV40 DNA: A Hyperstable Nucleosome Forms on the Termination Region†

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ABSTRACT: We have studied the relative abilities of different simian virus 40 (SV40) DNA segments to reconstitute into nucleosomes in vitro. The SV40 genome was separated into 15 discrete fragments by restriction endonuclease digestion and reconstituted with calf thymus core histones under conditions of varying histone-to-DNA ratios. Three fragments show very different abilities to form nucleosomes when low histone-to-DNA ratios require all fragments to compete for available histones. Two of these fragments, both from within protein-coding regions, are significantly underreconstituted. The third fragment, covering the SV40 termination region, competes much more effectively for histones than the other 14 fragments. The fragment containing the SV40 origin region formed nucleosomes with about average probability. Overall, the SV40 fragments differed by approximately an order of magnitude in their abilities to support nucleosome formation in vitro. The stability of the nucleosomes was measured by challenge with high concentrations of the destabilizing reagent heparin. The fragment that reconstituted most effectively also formed nucleosomes that were unusually stable to heparin challenge. These observations are intriguing since this fragment contains the sequences where replication of SV40 DNA commonly terminates and where early messenger RNA synthesis may terminate as well. The existence of unique hyperstable nucleosomes in this region suggests the interesting possibility that such nucleosomes may assist in termination events by assisting in the pausing of replication or transcription complexes.

It is generally accepted that chromatin structure may modulate processes that are basic to gene expression, such as transcription and replication. However, there is still controversy over the question of how chromatin structure is controlled or maintained. A list of parameters that may affect the structure of chromatin would include DNA sequence, chemical modifications of DNA and histones, the presence of nonhistone proteins and histone variants, and the state of torsional stress of the DNA. An understanding of the relative importance of these parameters to the maintenance of a specific chromatin configuration in vivo would enhance our understanding of how gene expression is regulated.

The chromatin structure of the simian papovavirus virus, SV40,[1] has been studied extensively. Late after infection, replicated SV40 DNA associates with host cellular histones to form a repeating structure of nucleosomes. This viral nucleoprotein complex is called the minichromosome and has served as a convenient model for the chromatin of higher eukaryotes. Several studies have shown that in a subpopulation of SV40 minichromosomes nucleosome placement is nonrandom with respect to DNA sequence (Persico-DiLauro et al., 1977; Nedospasov & Georgiev, 1980; Cereghini et al., 1982). In these complexes, a nucleosome-free region of approximately 300–400 base pairs exists at map positions 0.66–0.75 (Varshavsky et al., 1978, 1979; Scott & Wigmore, 1978; Waldeck et al., 1978; Saragosti et al., 1980; Jacobovits et al., 1980; Beard et al., 1981; Robinson & Hallick, 1982). Since this gap is positioned over the origin of the replication, the binding sites for the viral regulatory protein, T antigen, and the promoters for early and late SV40 mRNAs, it is presumed that this structure is relevant to the processes of DNA replication and transcription.

The parameters responsible for establishing the nucleosome-free gap in SV40 chromatin are not fully understood.

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[1] Abbreviations: bp, base pair(s); EDTA, (ethylenedinitrilo)tetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; SV40, simian virus 40; Tris, tris(hydroxymethyl)aminomethane.

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