Effects of Divalent Metal Cations on Composition and Neoplasia-specific Antigenicity of Chromatins

Sherry L. F. Dupere,2 Steve Holland,3 Stephen Gawne,3 Kevin E. Cancelliere,3 B. Ann Sedita,3 Phylis J. Dale,3 Ellen D. Jarrell,3 and Timothy E. O’Connor2,4


ABSTRACT

The antigenicity and composition of chromatins differ markedly in chromatin preparations obtained by different procedures. Rat Novikoff hepatoma chromatin (NC) obtained by the “salt precipitation” and the micrococcal nuclease digestion procedures using significant levels of EDTA and NaCl each shows a common complement fixation (CF) capacity, exceeding chromatin preparations obtained from normal rat liver when tested with rabbit antiserum raised to dehistonized NC. In contrast, “structured” NC preparations, which have been postulated to retain a native physical conformation, show minimal CF capacity when tested with the same antiserum but show high CF following elution of histones. While further progressive elution of non-histone proteins (NHPs) did not alter the CF capacity per unit DNA, the completely separated DNA and NHP fractions each showed minimal CF. The data suggest that the antigens detected in the CF assay predominantly represent an artifactual but specific complex of DNA and NHP arising from a denaturation of the native chromatin following elution of metal ions or histones. A qualitatively similar profile of NHPs in salt-precipitated NCs shows a range of total protein/DNA ratios, suggesting that the NHPs found in chromatin preparations may not be intrinsic to the native chromatin structure.

INTRODUCTION

Chromatin preparations have usually been found to be composed of DNA, histones, a large number of NHPs, and minor amounts of RNA. While many aspects of chromatin structure have been clarified, a number of questions await elucidation. One unresolved issue is the molecular nature of the tissue-specific and neoplasia-specific chromatin antigens which have been detected by a number of immunological procedures. A second important question is the extent to which NHPs, which have been implicated in chromatin function through a demonstrable role in genome regulation, are also involved as essential components in the native chromatin structure.

Early studies reported the presence of tissue-specific (7) and neoplasia-specific (33) CF antigens of chromatins (39) and dehistonized chromatins (33). These antigens are thought to be composed of nucleoprotein complexes of DNA with residual fractions of NHPs which resist elution with urea/salt mixtures (4). It should be noted that the levels of urea/salt used would elute the HMG proteins (15) which have been shown to be associated with DNAses I sensitivity and related activation of chromatins for transcription (36). Subsequently, Yeoman et al. (38) described an immunoprecipitable antigenic glycopeptide in NC which was not detectable in normal rat liver chromatin. Recently, the application of the sensitive immunoprecipitation of mixed proteins to blots of electrophoretically separated NHPs of NC and normal rat liver chromatins indicated an association of specific antigenicity with a few NHPs which differed between the 2 chromat in preparations (14). While noting this, we seemed improbable to us that the observed consistent antigenic differences between chromatins of neoplastic and normal tissues were due solely to a small subset of antigenic NHPs. The xenogeneic host could be expected to mount a predominant but variable response to antigens of the many NHPs shared by both normal and neoplastic tissues rather than invariably respond in CF reactions to antigens corresponding to the few differing NHPs in these preparations. In evaluating these considerations, we particularly noted the earlier observations of only minor differences in NHP composition of chromatins of various normal tissues (10) and of mouse Friend cells before and after induction of erythroid differentiation with dimethylsulfate (25).

The idea that NHPs contribute to the function of chromatins is based on observations of the tissue-specific transcriptional properties of reconstituted chromatins (1, 6); on minor, but presumably significant, variations in composition of the large number of NHPs observed in various tissues and tumors (10, 25); on the tissue-specific (7) and neoplasia-specific antigenicities of chromatins (39) and dehistonized chromatins (33); on the binding of HMG proteins associated with transcriptional regulation (36); and on changes in NHPs involved in chromosome decondensation (21).

The corollary concept that NHPs are components which are intrinsic in the higher order structure of native chromatins appears at variance with both biological and physicochemical experimental evidence. Unlike the stable histones synthesized in the S-phase of the cell cycle (16), the NHPs are a heterogeneous group with diverse half-lives and synthesis times (2). Thus, the expected stability and coupling of synthesis to the DNA and histone components, which are essential to the chromatin structure, are lacking. It is of interest that SV40, which contains histone-like proteins, is devoid of NHPs with the possible exception of a structural antigenic protein (20). Previous studies on the composition of chromatins prepared from rat liver with minor varia-

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5 The abbreviations used are: NHP, non-histone protein; CF, complement fixation; HMG, high-mobility group; NC, Novikoff rat hepatoma chromatin; PAP, peroxidase antiperoxidase; HC, chromatin fraction containing DNA and subset of NHPs; PMSF, phenylmethylsulfonyl fluoride; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; RS, 10 mM NaCl, 1.5 mM MgCl2, and 10 mM Tris-HCl, pH 7.6; SDS, sodium dodecyl sulfate; P/DNA, total protein/DNA ratio.
6 Received June 28, 1982; accepted July 13, 1983.
tions in the salt precipitation isolation procedure showed large variations in total protein/DNA ratios (8). Recent evidence suggests that chromatin, as isolated by micrococcal enzyme digestion of intact nuclei, are composed of polynucleosomal (11) or polychromatosomal (30, 32) 10-nm fibers of histone/DNA complexes which can undergo conversion in the presence of Mg²⁺ to rigid 30-nm solenoidal structures (23). No evidence is available which documents NHPS in these chromatin structures.

The profound differences observed in the physical properties of polychromatosomes in the presence and absence of divalent metal cations (23) suggested that the rate of removal of divalent cations by chelating agents during chromatin extraction could profoundly influence the composition and properties of the chromatin preparation. These speculations led to a reexamination of the antigenic and structural properties of chromatin prepared by: (a) a salt precipitation procedure (22); (b) a micrococcal nuclease digestion procedure (31); and (c) a minimal denaturation procedure reported to provide a structured chromatin which, on the basis of digestion with micrococcal nuclease and melting point, approximates native chromatin (37).

MATERIALS AND METHODS

Animals. Sprague-Dawley rats (male, over 3 months old) were obtained from Charles River Breeders, Wilmington, Mass.

Experime ntal Tumors and Tissues. Novikoff rat hepatoma (a gift from Dr. Sidney Weinhouse, Fels Research Institute, Philadelphia, Pa.) was passaged weekly as an ascites tumor in Sprague-Dawley rats. The cells were washed extensively at 0–4° in TNKM buffer, pH 7.4 (38), and frozen at −20° until used. Normal rat livers were washed extensively in Tris/0.9% NaCl solution, pH 7.6, buffer and frozen at −20°.

Preparation of Chromatins. Conventional salt-precipitated chromatins were prepared by the procedure of Marushige and Bonner (22) from Novikoff and normal rat liver nuclei isolated by the procedure of Yeoman et al. (38). Dehistonized chromatins were prepared by treating chromatins with urea/salt/phosphate buffer as described by Chytil and Spelsberg (7). The HC fraction of chromatins was obtained using the method described by Chiu et al. (4). Micrococcal nuclease chromatin (nucleosomol oligomer) was prepared by the method of Todd and Garrard (31). Preparation of structured chromatins involved a modification of the earlier procedures of Hancock (17) and Yaneva and Dessev (37); Novikoff hepatoma ascites cells were washed once in 5 volumes of ice-cold Solution A [0.25 M sucrose, 5 mM EDTA, and 1 mM PMSF (Sigma Chemical Co., St. Louis, Mo.), and 4 mM HEPES, pH 8.0] followed by 3 washes in 25 volumes of Solution B [0.25 M sucrose, 0.1 mM EDTA, 1 mM PMSF, and 4 mM HEPES, pH 8.0] and then in 3 washes of ice-cold Solution B. After centrifugation at 2000 × g, the pellets were suspended in 2 volumes of Solution C (2% Nonidet P-40 detergent, 0.2 mM EDTA, 1 mM PMSF, and 4 mM HEPES, pH 8.0) and stirred for 15 min on ice at each step. After centrifugation at 2000 × g, the pellets were suspended in 2 volumes of Solution D (0.25 M sucrose, 0.1 mM EDTA, 0.1 mM PMSF, and 4 mM HEPES, pH 8.5) and centrifuged at 1000 × g for 30 min. The supernatant fluid was carefully aspirated, and the walls of each tube were wiped with tissue to avoid contamination of the pellet with residual detergent. The clear, gelatinous pellets were suspended in a small amount of Solution E (5 μM EDTA, 1% mercaptoethanol, and 4 mM HEPES, pH 7.8), with gentle agitation to prevent shearing, and dialyzed against this same buffer for 4 hr at 4°.

Preparation of chromatins was carried out by the paraequilibrium salt-precipitation method. After being washed in isotonic TNKM medium, the cells were replaced hourly in media containing 25, 50, 75, and 100%, respectively, of RSB buffer. The resulting highly swollen cells were then lysed by the addition of 0.5% Nonidet P-40 and moderate stirring. The nuclei were separated by centrifugation, washed in 75 mM NaCl/25 mM EDTA, pH 8.0, and digested overnight in the NaCl/EDTA medium. Further steps of preparation and purification of the chromatin conformed to the procedure of Marushige and Bonner (22) with the modification that 1 mM PMSF and 1% mercaptoethanol were incorporated into all media.

Aliquots from all chromatin preparations were extensively dialyzed against 10 mM Tris-HCl, pH 8.0, buffer to remove PMSF and mercaptoethanol for biochemical analyses. DNA content was determined by the diphenylamine procedure described by Shattkin (29), and protein content was determined by the procedure of Bradford (3).

Immunological Studies. Antiserum to chromatin preparations were obtained from young adult male rabbits immunized with NC, dehistonized NC, and HC chromatins fractions. The immunization schedule involved 4 inoculations at 2-week intervals of 200 μg DNA content of antigen into the left foot pad, right foot pad, and twice into the thigh muscle. The foot pad inoculations incorporated complete Freund’s adjuvant, while the 2 subsequent inoculae into the thigh muscle used incomplete Freund’s adjuvant. Serum was first harvested one week after subsequent times booster shots with the antigen. Sera were frozen until used. They were then thawed, heat-inactivated at 58–60° for 30 min, and stored at 50% glycerol at 4°. The complement fixation reaction of sera with the various antigens was conducted according to the procedure of Wasserman and Levine (34). For this assay, all chromatins samples (antigens) were dialyzed extensively against 10 mM Tris-HCl, pH 8.0, 1 mM PMSF, and 1% mercaptoethanol prior to CF testing. A significant loss of antigenicity was noted when antigens were dialyzed in the absence of PMSF.

DNase I Digestion of Chromatins. Chromatin, at a DNA concentration of 1000 μg/ml in RSB buffer, was treated with DNase I (5 μg/ml at 37°). Acid-soluble DNA content was determined by the procedure described by Garel et al. (12). Assays in the absence of added Mg²⁺ were performed in RSB minus MgCl₂. Structured chromatin undergoes slow solubilization by endogenous DNase (19) over a period of days; therefore, it is necessary to perform all measurements on freshly prepared chromatins.

SDS-Polyacrylamide Gel Electrophoresis of Proteins from Chromatin Fractions. Residual proteins were removed from all chromatins and chromatins fractions for SDS-polyacrylamide gel electrophoresis using SDS according to the procedure of Elgin and Bonner (10), concentrated by lyophilization, and reconstituted with the sample buffer prior to electrophoresis. slab gel SDS-polyacrylamide gel electrophoresis was carried out according to the procedure of Neville and Glossman (24) using a 3% acrylamide stacking gel and a 10% acrylamide separating gel. Aliquots of 25 μg total protein were applied to each track. Molecular weight standards included carbonic anhydrase (M, 28,000), creatine kinase (M, 42,000), and human serum albumin (M, 68,000).

RESULTS

An examination of the CF activity of salt-precipitated NC, dehistonized NC, the HC fraction of NC, and the corresponding fractions of normal rat liver chromatins on treatment with rabbit antiserum against the HC fraction of NC is presented in Chart 1. The xenogeneic antiserum to the HC chromatin fraction (Chart 1a) clearly discriminate between the Novikoff and normal rat liver chromatin fractions but show only low and nonspecific reaction with either separated DNA or NHPS of the NC. Thus, the preponderant neoplasia-specific CF activity requires the presence of both DNA and the NHPS in a nucleoprotein complex. Although differences in the protein composition may be present, the contribution of protein antigens per se to the observed differences appear to be quantitatively minor. In accordance with the method of Chiu et al. (4, 18), our data indicate only minor differences in the CF capacity of the NC, dehistonized NC, and the HC Novikoff fractions (containing 100, 30, and 15% of the total NC protein,
Metal Cations in Chromatin Antigenicity and Composition

Table 1

<table>
<thead>
<tr>
<th>Compositions of chromatins and chromatin fractions</th>
<th>P/DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt-precipitated NC preparations</td>
<td>1.5-2.0</td>
</tr>
<tr>
<td>Chytil-Spelsberg dehistonization of NC preparations</td>
<td>0.2-0.5</td>
</tr>
<tr>
<td>&quot;HC&quot; fraction of NC preparations</td>
<td>0.2-0.3</td>
</tr>
<tr>
<td>Salt-precipitated NC &quot;paraequilibrium&quot; preparations</td>
<td>0.9-1.2</td>
</tr>
<tr>
<td>Salt-precipitated normal rat liver chromatins preparations</td>
<td>1.6-2.2</td>
</tr>
<tr>
<td>Chytil-Spelsberg dehistonization of normal rat liver chromatins preparations</td>
<td>0.2-0.5</td>
</tr>
<tr>
<td>&quot;HC&quot; fraction of normal rat liver salt-precipitated chromatin preparations</td>
<td>0.2-0.25</td>
</tr>
<tr>
<td>&quot;Structured&quot; NC preparations</td>
<td>2.0-3.0</td>
</tr>
<tr>
<td>Chytil-Spelsberg dehistonization of structured NC preparations</td>
<td>0.25-0.5</td>
</tr>
</tbody>
</table>

respectively, as shown in Table 1) when they are compared per unit content of DNA (Chart 1b). Similar results were obtained (data not shown) when antisera raised to dehistonized NC were used.

Therefore, the present and earlier CF observations (4, 6, 33) cannot be used to predict the presence of neoplasia-specific antigen in NC in vivo. Judged by a wide melting point range and the production of a heterogeneous mixture of polynucleotide fragments on digestion with micrococcal nuclease (37), salt-precipitated chromatins are denatured. Accordingly, we compared the immunochemical properties of salt-precipitated NC (22), NC chromatins prepared by micrococcal nuclease digestion of Novikoff hepatoma nuclei and subsequent extraction with hypotonic EDTA (31), and structured chromatins made by extraction of Novikoff hepatoma or normal rat liver cells with nonionic detergent in the presence of hypotonic media in a modification of the procedures of Hancock (17) and Yaneva and Dessev (37).

It has been suggested that structured chromatins represent a minimal deviation from native chromatins in view of their sharp melting point and homogeneous micrococcal nuclease digestion pattern (37). As shown in Table 2, the structured NC and normal rat liver chromatin each demonstrated low CF activity when tested with antisera to either salt-precipitated NC or derived dehistonized NC. However, the salt-precipitated NC and NC obtained by micrococcal nuclease digestion of Novikoff nuclei each showed high CF activity. Novikoff hepatoma nuclei on lysis in hypotonic EDTA also showed high CF activity. When structured NC and normal rat liver chromatin were dehistonized by the procedure of Chytil and Spelsberg (7) or by extraction with 0.4 M H2SO4 (data not shown), the residues in each case showed enhanced antigenicity. The neoplasia specificity of the dehistonized NC was evident, since the increase in antigenicity shown by the dehistonized NC was much greater than that shown by the dehistonized normal liver chromatin. Rabbits immunized with structured NC yielded sera which showed only borderline reaction with the structured NC but did specifically react with salt-precipitated NC at a medium titer (50% CF at 1/400 dilution; data not shown). Electron microscopic observations by T. Seed, Argonne National Laboratory, of dehydrated preparations of structured NC (data not shown) revealed highly porous nuclear-type bodies with fibrillar inner structures as described previously by Hancock (17). These observations suggest that the low antigenicity of structured chromatin does not arise from inaccessibility to either antibody or complement, although the possibility that the observed porous structures represent electron microscopic preparation artifacts should be considered.

Since the preparation of structured chromatin involved the use of HEPES buffer, the possible role of this reagent in masking antigenic sites was examined. A salt-precipitated NC preparation was dialyzed for 48 hr against several changes of Solution E containing HEPES buffer. After dilution in 0.9% NaCl solution, as specified in the Wasserman-Levine CF assay (34), the untreated and HEPES-treated NC samples showed no significant differences when tested with 1/3200 dilution of antisera to dehistonized NC (56% CF with treated versus 45% CF with untreated). Maturation of avian reticulocytes to erythrocytes has been shown to be associated with chromatin condensation and...
a loss in reticulocyte-specific chromatin CF activity, but the specific CF activity can be restored in the erythrocyte chromatin by sonication (26, 27). Sonication of a structured NC preparation (1 min with Bronson Instrument Sonifier at 2.5 amp) resulted in a marginal increase in CF activity (sonicated sample, 27% CF activity; untreated sample, 11% CF with 1/1600 dilution of antiserum to dehistonized NC).

Since the isolation of structured chromatin differs from the other procedures in the use of very low levels of chelating agents, the role played by possible elution of metal ions in generating antigenic sites was considered. Flame atomic absorption spectrophotometry revealed the presence of Ca²⁺ and Mg²⁺ ions in structured NC at levels of 2000 and 600 ng/mg DNA, respectively, whereas salt-precipitated NC contained approximately 30 ng/mg of each cation. To further test for the presence of intrinsic divalent cation, the structured NC preparation was subjected to digestion with DNAse I, which is sensitive to the presence of Mg²⁺. Structured NC yielded 3% soluble DNA fragments on equilibrium exposure to DNAse I in the absence of added divalent cation to serve as cofactor. Salt-precipitated NC showed no detectable solubilization under these conditions. In contrast, structured NC showed 27% and salt-precipitated NC showed 49% equilibrium enzymatic solubilization of DNA in the presence of added 3 mM MgCl₂.

The preparation of the antigenic salt-precipitated chromatins involved the rapid lysis of cells in hypotonic media containing the detergent Nonidet P-40 and the use of a Tekmar Tissuemizer (28), followed by extraction of chromatin from the nuclei with NaCl/EDTA and subsequent purification in sucrose media (22). All of these operations were conducted as rapidly as possible, and 1.0 mM PMSF was incorporated into all media to minimize action of nuclease (13). As shown in Table 1, the total P/DNA ratios of these chromatins ranged from 1.5 to 2.0. The effect of added Mg²⁺ on the viscosity of structured NC suggested that rapid chromatin extraction procedures might not allow sufficient time for complete elution of divalent cations from regions of high viscosity within the nucleus, resulting in a temporary gradient of divalent cation concentration. We speculated that the Mg²⁺ gradient could result in the transformation of a native linear polychromatosome structure to a tangled 3-dimensional network which could entrap nucleosol proteins. The entangled chromatin network would persist after complete elution of divalent cations from regions of high viscosity within the nuclei, resulting in a temporary gradient of divalent cation concentration. In an attempt to provide a greater opportunity for divalent cation migration, we explored a paraequilibrium method which involved a slower elution of metal ions during hypotonic swelling of the cells and a subsequent overnight incubation of the nuclei with EDTA. Chromatins prepared by this procedure showed lower P/DNA ratios (range, 0.9 to 1.2) which approximated the P/DNA value expected for a polychromatosome structure devoid of NHPs and having 160 nucleotide base pairs of DNA associated with each chromatosome (30). Nevertheless, on reaction with antisera to dehistonized chromatins, these chromatins showed CF activities per unit of DNA comparable to those shown by salt-precipitated chromatins having higher P/DNA values (data not shown).

The NHP content of chromatins and of chromatin fractions was examined after removal of DNA by the simultaneous electrophoresis of equal amounts of the residual protein mixtures in different tracks in slab gels (Fig. 1) or in separate column gels (data not shown). In spite of loading of each track with an equal quantity of protein, the stained gels revealed that equal quantities of the protein mixtures did not enter the gel tracks during

Table 2

<table>
<thead>
<tr>
<th>Serum designation</th>
<th>Test antigens</th>
<th>CF activity (%) at following serum dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/50</td>
</tr>
<tr>
<td>Raised to salt-precipitated NCs</td>
<td>XI-16A</td>
<td>81</td>
</tr>
<tr>
<td>XIV-40-10-S3</td>
<td>XI-17B</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>XI-23B</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>XI-24C</td>
<td>69</td>
</tr>
<tr>
<td>Raised to dehistonized salt-precipitated Novikoff chromatin</td>
<td>XI-16A</td>
<td>84</td>
</tr>
<tr>
<td>XIV-54-4-S3</td>
<td>XI-17B</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>XI-23B</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>XI-24C</td>
<td>88</td>
</tr>
<tr>
<td>Raised to dehistonized salt-precipitated Novikoff chromatin</td>
<td>XI-16A</td>
<td>67</td>
</tr>
<tr>
<td>XIV-54-7-S3</td>
<td>XI-17B</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>XI-23B</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>XI-24C</td>
<td>63</td>
</tr>
<tr>
<td>IV-12-4-S3</td>
<td>Salt-precipitated NC XIII-131B</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>Structured NC XVII-24C</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>Dehistonized structured NC XVII-26B</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Micrococcal nuclease NC XVII-33B</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Novikoff hepatoma nuclei lysed in hypotonic EDTA medium</td>
<td>85</td>
</tr>
</tbody>
</table>

* Each antigen contains 12.5 μg DNA/ml.
* Prepared from the NC preparation XI-16A by the Chytil-Spelsberg procedure (7).
* Prepared by application of the Chytil-Spelsberg procedure to the normal rat liver chromatin XI-23B.
* Prepared from structured NC XVII-24C.
the subset of NHPs required for this activity. Our electrophoretic observations on the association of the HMG proteins with DNAse I-specific digestion (36), which has been linked to activation of the chromatin for tissue-specific transcription (35). Additionally, the tissue-specific (7) and neoplasia-specific CF activities of chromatins (39) and dehistonized chromatins (33) can be interpreted as supporting the concept. Particularly pertinent is the recent observation on the immunospecificity of separated NHPs as tested by the sensitive PAP procedure (14).

The present report addresses questions on the nature of the molecular species associated with the immunospecific CF antigens of NC and the role of NHPs as intrinsic components in native NC structure. Our repetition of earlier experiments by Wakabayashi and Hnilica (33) and Chiu et al. (4–6) on various NC fractions and our new observations on CF activity of micrococal nuclease digestion NC preparations are in full accord with the earlier findings, demonstrating the presence in NC of immunospecific CF antigens when compared to corresponding chromatin fractions of normal rat liver. Several features of our data merit comment. Our extensive quantitative CF assays, performed simultaneously with the same serum on different Novikoff hepatoma and normal rat liver chromatin fractions, indicate that the various NC fractions of markedly different protein/DNA ratios show identical specific CF capacities per unit DNA, while the separated DNA and NHPs show negligible CF capacities. This suggests that the CF antigens of NC are composed of an NHP-DNA complex. Thus, our data are in accord with the earlier observation of abolition of reticulocyte-specific CF activity on digestion with either Pronase or DNAse I (18).

In view of the low antigenicity of native DNA, the CF activity in such complexes might readily be assigned to specific conformations of the NHPs, shifting the focus of attention delineating the subset of NHPs required for this activity. Our electrophoretic studies suggest that NC chromatin fractions having a range of protein/DNA ratios and common CF antigenicity per unit DNA contain different amounts of a common profile of NHPs. Therefore, our findings do not document an antigenically active fraction of NHPs involved in CF.

Clearly, these findings on CF should not be extrapolated to an interpretation of specific nuclear antigens associated with neo-
plasia or tissue differentiation as detected by the quite different methodologies of immunoprecipitation (38), the PAP procedure on electrophoretically separated NHPs (14), or application of the PAP procedure to fixed tissues (18).

Structured NC chromatin, which approximate native chromatin in melting behavior and micrococcal nuclease digestion pattern (37), do not show significant CF activity but do yield preparations with high CF activity following dehistonization. Our observations could be interpreted on the basis of either antigen masking or true absence of CF antigen in the structured chromatin preparation. Antigen masking is possible in light of recent observations on the complete restoration of reticulocyte-specific chromatin CF antigens upon shearing of the condensed and transcriptionally inactive chromatin of mature erythrocytes derived from the transcriptionally active reticulocytes (26, 27). In contrast, sonication of structured NC, as derived from the transcriptionally active Novikoff hepatoma nucleus, gave only a minor increase in CF antigenicity. Exposure to HEPES buffer was not found to mask CF antigens of salt-precipitated NC preparations. Electron microscopic examination suggested that structured NC has an open structure that should be accessible to both antibody and complement component.

In considering the nature of the CF chromatin antigens, we note differences in (a) the level of EDTA as used in the preparation of structured NC and salt-precipitated NC; (b) the Mg2+ content of these chromatin preparations as measured by flame photometry and indirectly by effects of DNase I digestion; (c) the finding that salt-precipitated chromatin are denatured as judged by melting point and micrococcal nuclease digestion patterns (37); and (d) recent studies on the effects of Mg2+ on the physical structure of polychromatosomes (23). While our data do not exclude masking of antigens in structured chromatin, these considerations and the absence of significant CF activity in the isolated NHPs lead us to raise the possibility that CF activity is generated by a conformational change in the chromatin associated with denaturation and involves removal of Mg2+ or dehistonization. It should be noted that detection of CF antigens in this and previous studies (4–7, 33, 39) used antisera raised to immunogens derived from salt-precipitated chromatin, which is demonstrably denatured (37).

Chromatin preparations are generally effected as rapidly as possible in order to obviate the effects of intrinsic protease digestion (13). Rapid chromatin extraction procedures may not provide sufficient time for complete elution of divalent cations from regions of high viscosity within the nucleus. Therefore, we visualize the temporary establishment of a Mg2+ gradient and the development of both flexible fiber and solenoidal forms of polychromatosomes (23, 30, 32). Earlier electron microscopic observations of coaxially wound solenoids (9) with diameters in excess of 30 nm, and of structures that we now interpret as the transcriptionally active Novikoff hepatoma nucleus, gave only a minor increase in CF antigenicity. Exposure to HEPES buffer was not found to mask CF antigens of salt-precipitated NC preparations. Electron microscopic examination suggested that structured NC has an open structure that should be accessible to both antibody and complement component.

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