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DEGREE OF CONSERVATION OF HELA INTERPHASE NONHISTONE ANTIGENS IN METAPHASE AND WITH CHROMATIN FROM NON-HUMAN CELLS

Kenneth W. Adolph

Department of Biochemistry
University of Minnesota Medical School
Minneapolis, Minnesota 55455

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SUMMARY: Immunological procedures were applied to determine the degree of conservation of the nonhistone proteins of HeLa interphase chromatin. Polyacrylamide gels were overlaid with antiserum to HeLa interphase chromatin, and ^{125}I-Protein A was used to detect bound antibodies. With two-dimensional gels, more than 80 interphase species were found as components of metaphase chromosomes. The degree of conservation of HeLa nonhistone antigens with other chromatin sources was calculated through densitometry of autoradiograms and stained gels. Chromatin was obtained from chicken erythrocytes, Novikoff rat hepatoma cells, and mouse L cells, and the presence of one-quarter to one-third of the nonhistone antigens of HeLa chromatin was demonstrated.

The organization of chromatin in the interphase nucleus and the structure of metaphase chromosomes result from the interaction of three classes of macromolecules: DNA, histones, and nonhistone proteins. Attempts to discover the structural roles of nonhistones during the cell cycle are hindered by the variety of species that are present (1). This investigation therefore combined the use of antibodies to nonhistones with techniques of polyacrylamide gel electrophoresis to detect the conservation of species. Two fundamental questions were posed. To what extent are nonhistones of HeLa interphase chromatin found as components of metaphase chromosomes? Are nonhistones of HeLa chromatin preserved among the nonhistones of chromatin from different animals cells?

The immunological procedures began with the separation of nonhistone samples on both one-dimensional and two-dimensional SDS-polyacrylamide gels. The gels were then directly overlaid with antiserum, or the purified IgG fraction, to HeLa interphase chromatin. ^{125}I-labeled Staphylococcus aureus Protein A was used to locate by autoradiography antibodies bound to proteins in

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the gels. Direct overlay of gels has proven to be a sensitive and reliable method to detect antigens and specific binding proteins (2-4). It was therefore employed rather than procedures in which proteins are transferred to an immobilizing support such as nitrocellulose (5-7). In addition to HeLa cells, chromatin was obtained from chicken erythrocytes, Novikoff rat hepatoma cells, and mouse L cells.

Applying these procedures demonstrated that nonhistone antigens of HeLa interphase chromatin are not only present, but are almost completely conserved among the nonhistones of metaphase chromosomes. HeLa nonhistone antigens are preserved, to a lesser extent, as components of chromatin from non-human cells.

MATERIALS AND METHODS

Preparation of Chromatin, Metaphase Chromosomes, and Nuclear Scaffolds

HeLa S-3 cells were maintained in suspension culture in minimum essential medium containing 5% fetal bovine serum.

Nuclei from HeLa, Novikoff rat hepatoma N1S1-67, and mouse L929 cells were obtained as previously described (8). To prepare chromatin, nuclei were incubated with 10 units/ml micrococcal nuclease for 5 min at 37°C, after which chromatin fragments were released by resuspending the nuclei in 5 mM EDTA, 0.1% NP40. HeLa nuclear scaffolds were obtained by treating nuclei with DNAase I (100 μg/ml) for 60 min on ice, before adding an equal volume of 4 M NaCl, 20 mM Tris-HCl pH 7.4, 20 mM EDTA, 0.2% Ammonyx LO (8).

HeLa metaphase chromosomes were isolated by arresting cells in mitosis with colchicine, and then disrupting the mitotic cells in a buffer of 50 mM NaCl, 5 mM Hepes pH 7.4, 5 mM $MgCl_2$, 0.5 mM $CaCl_2$, 0.1 mM PMSF after adding 0.5% NP40 and 0.1% sodium deoxycholate (9).

Antiserum Preparation

For chromatin, New Zealand White rabbits (male, 4-6 months) were initially injected with chromatin (4mg/ml total chromatin protein) emulsified with complete Freund's adjuvant. A similar amount of protein in incomplete Freund's adjuvant was used for subsequent injections. The IgG fraction of antiserum to HeLa interphase chromatin was purified by ammonium sulfate precipitation and DEAE-cellulose chromatography (10).

Detection of Conserved Proteins

SDS-polyacrylamide gel electrophoresis followed the conditions given by Laemmli (11). For two-dimensional separation, non-equilibrium pH gradient electrophoresis (12) was used in the first dimension and 8% SDS-polyacrylamide slab gels were used in the second. The procedure of Merril *et al.* (13) was employed to silver stain gels.

One-dimensional and two-dimensional polyacrylamide gels were directly overlaid with antiserum and with ^{125}I-labeled Protein A following electrophoresis of the various samples. The gels were first fixed in 50% methanol, 10% acetic acid (4 h). They were equilibrated in a buffer of 150 mM NaCl, 50 mM Tris-HCl pH 7.4 for 16 h, and then covered with antiserum (1:10 diluted) for 24 h. ^{125}I-Protein A (1 μCi/ml) in 2% bovine albumin, 150 mM NaCl, 50 mM Tris-HCl pH 7.4, 0.1% NaN_3 was then spread over the gels. After 24 h, they were extensively washed in buffer.

RESULTS

Immunological Detection of the Conservation of Nonhistone Antigens Using One-Dimensional and Two-Dimensional Gels

Fig. 1A contains a 12.5% SDS-polyacrylamide gel stained with Coomassie brilliant blue, and Fig. 1B contains an 8% stained gel. A large number of nonhistone species are found with each sample, but the distributions of species do not have a close resemblance, even though some coincidences in the positions of bands may be seen.

Fig. 1C includes an autoradiogram of a similar 8% gel overlaid with antiserum to HeLa interphase chromatin and with ^{125}I-Protein A. Lane 1 of the autoradiogram indicates that antibodies were raised to many nonhistones of chromatin. As lane 2 demonstrates, not only are numerous molecular weight species of

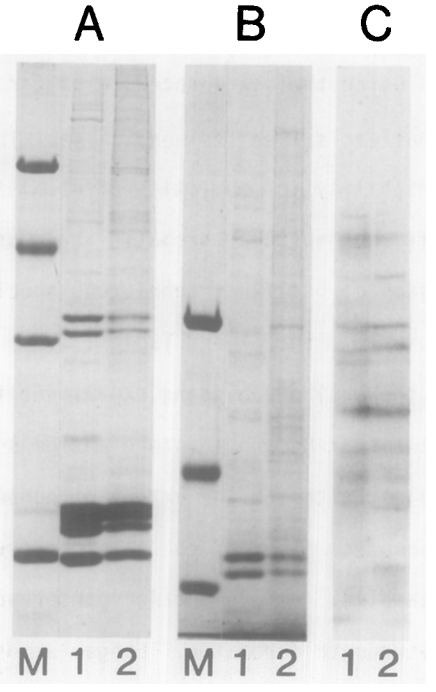


Figure 1. Detection of conservation of HeLa nonhistone antigens by direct overlay of polyacrylamide gels with antiserum and ^{125}I-Protein A. (A) Nonhistones of interphase chromatin (lane 1) and metaphase chromosomes (lane 2) separated in a 12.5% SDS-polyacrylamide gel. The gel was stained with Coomassie brilliant blue. Lane M includes molecular weight markers of bovine albumin (66,000), ovalbumin (45,000), carbonic anhydrase (30,000) and lysozyme (14,000). (B) A stained 8% gel with the same samples. (C) Autoradiogram of a similar 8% gel treated with antiserum to HeLa interphase chromatin and then with ^{125}I-Protein A.

interphase chromatin nonhistones conserved in metaphase, but the relative intensities of the bands are similar. Thus, both the fraction and amounts of species which are conserved from interphase to metaphase are similar.

Experiments which substituted pre-immune serum for immune serum demonstrated that the binding was specific.

In Fig. 2, the proteins of isolated HeLa metaphase chromosomes were separated on two-dimensional gels. The protein staining pattern of metaphase chromosome nonhistones is in Fig. 2A, and an autoradiogram of the antiserum overlay experiment is in Fig. 2B. Over eighty distinct spots can be counted on the autoradiogram. Two-dimensional gels confirm the results with one-dimensional gels that interphase chromatin nonhistones are substantially conserved in metaphase.

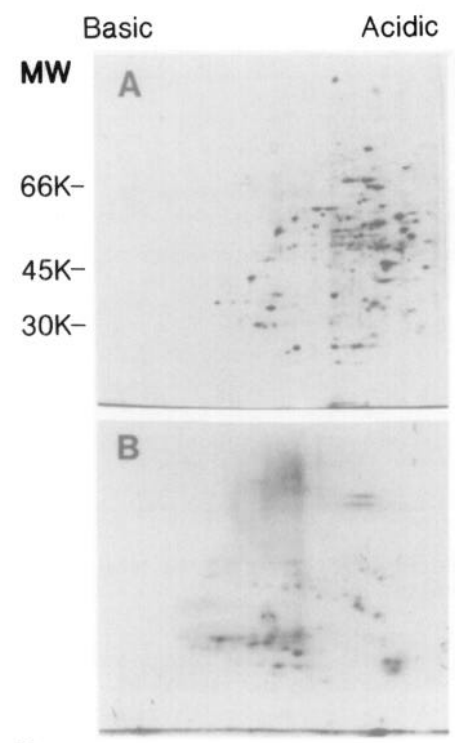
Presence of HeLa Chromatin Nonhistones with Chromatin from Other Animal Cells

Chromatin was prepared from chicken erythrocytes, Novikoff rat hepatoma cells, and mouse L cells using the same procedure as for HeLa chromatin. Fig. 3A includes an 8% gel that was silver stained. A striking feature of the chromatin samples is the variability in mobilities of H1 histones. The amounts of nonhistones with Novikoff rat hepatoma chromatin and mouse L cell chromatin are comparable to the HeLa sample, but fewer nonhistone species are found with chicken erythrocyte chromatin (Fig. 3A and Table I).

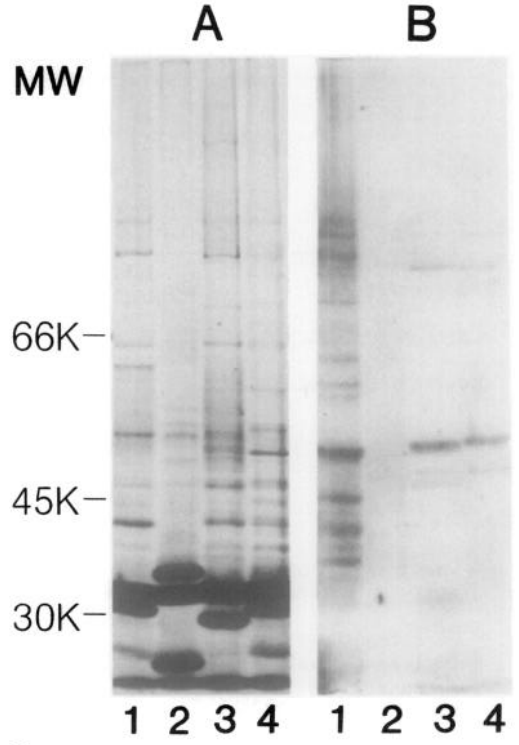
An autoradiogram of the antibody binding experiment is in Fig. 3B. The autoradiogram demonstrates that HeLa nonhistone antigens are present with the non-human chromatin samples, although the extent of conservation is less than for the HeLa metaphase samples. Many of the same conserved species are found with the rat and mouse samples. However, major conserved species are not observed for chicken erythrocyte chromatin. Longer exposures than in Fig. 3B show numerous minor species.

Calculation of Fraction of Conserved Nonhistones

Densitometry of autoradiograms and stained gels was used to determine the extent of conservation of HeLa nonhistones. For each sample the fraction of the total nonhistone density on autoradiograms was calculated with respect to the



(2)



(3)

Figure 2. Immunological procedure with two-dimensional gels of HeLa metaphase chromosome nonhistones. The proteins of isolated metaphase chromosomes were separated by non-equilibrium pH gradient electrophoresis and in 8% SDS-polyacrylamide slab gels. (A) Silver stained pattern of the metaphase nonhistones. (B) Autoradiogram showing the metaphase species that are conserved from interphase chromatin.

Figure 3. Detection of antigenic determinants of HeLa chromatin nonhistones among the nonhistones of chromatin from other species. Chromatin was prepared from chicken erythrocytes, Novikoff rat hepatoma cells, and mouse L cells and the proteins were electrophoresed in SDS-polyacrylamide gels. (A) An 8% gel, stained with silver, of the proteins of (1) HeLa interphase chromatin, (2) chicken erythrocyte chromatin, (3) Novikoff rat hepatoma chromatin, and (4) mouse L cell chromatin. (B) Autoradiogram of a similar 8% gel that was subjected to the immunological procedure. In this experiment, a purified IgG preparation was used instead of whole antiserum.

total density for HeLa chromatin. Densitometry of stained gels was used to normalize the values. Both the total amount of core histones and the total amount of nonhistones were measured as the basis of the normalization. The first column of Table I includes values normalized with respect to the amount of core histones. These values demonstrate that the overall degrees of conservation for Novikoff rat hepatoma cells (36%) and mouse L cells (33%) are similar, while the degree of conservation for chicken erythrocyte chromatin is lower (25%).

TABLE I

Conservation of HeLa Chromatin Nonhistones with Chromatin from Other Species

Source of chromatin sample	Fraction of Conserved Nonhistones[a] Normalized to[b]		Ratio of nonhistones to histones plus nonhistones[c]
	core histones	total nonhistones	
HeLa cells	1.00	1.00	.10
Chicken erythrocytes	.25	.52	.06
Novikoff rat hepatoma cells	.36	.43	.10
Mouse L cells	.33	.36	.11

[a] The fraction of conserved nonhistones was calculated with respect to the total density on autoradiograms for the HeLa chromatin control.

[b] Densitometry of gels stained with Coomassie brilliant blue was used to normalize the values. Normalization was based on the total amount of core histones and also on the total amount of nonhistones. The estimated uncertainty of these values is ±7%.

[c] Stained gels were also used to estimate the ratio of histones to histones plus nonhistones.

The values for the fraction of conserved nonhistones were also normalized with respect to the total amount of nonhistones. The data in the second column of Table I reveals that the low degree of conservation for chicken erythrocyte chromatin given in column 1 (25%) is partly due to the low nonhistone content of this chromatin. Normalizing to total nonhistone content raises this value to 52%. The values for the rat and mouse samples are increased only moderately by this procedure.

DISCUSSION

Why is the conservation of nonhistone proteins significant? The reason is that nonhistones have structural roles in organizing the eukaryotic genome through the cell cycle. This is in addition to the functional roles of nonhistones as, for example, regulators of gene expression. The conservation of nonhistones becomes important in understanding which structural roles at both the short-range and higher-order levels of organization are also found with metaphase chromosomes.

Using one-dimensional gels, the conservation of nonhistone antigenic determinants of HeLa interphase chromatin among the nonhistones of isolated metaphase chromosomes was quantitated from densitometer scans of autoradiograms and stained gels (14). 92% conservation was found for chromatin prepared from isolated metaphase chromosomes. Two-dimensional gels (Figure 2) showed at least 80 conserved proteins. This substantial extent of conservation suggests that many basic features of the levels of structure influenced by nonhistone interactions are the same during metaphase.

Autoradiograms also showed that HeLa nonhistone antigens are found with chromatin from other sources (Figure 3), although the degree of conservation is less than with HeLa chromatin. Calculations from densitometer scans demonstrated that one-quarter (chicken erythrocytes) to one-third (Novikoff rat hepatoma cells and mouse L cells) of nonhistone antigens are conserved. This result suggests that the conserved proteins have similar structural and functional roles with chromatin from the different species.

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