

Conservation of interphase chromatin nonhistone antigens as components of metaphase chromosomes

Kenneth W. Adolph

Department of Biochemistry, University of Minnesota Medical School, Minneapolis, MN, USA

Received 24 August 1983; revised version received 29 September 1983

The degree of conservation of HeLa interphase chromatin nonhistone antigens among the nonhistones of isolated metaphase chromosomes was determined with immunological procedures. Proteins were separated on SDS-polyacrylamide gels and electrophoretically transferred to diazophenylthioether (DPT)-paper, which was then overlaid with antiserum to chromatin from interphase nuclei. The bound antibodies were detected with ^{125}I -labeled protein A. Alternatively, polyacrylamide gels were directly overlaid with antiserum and with ^{125}I -protein A. Densitometry of autoradiograms and stained gels revealed the degree of conservation of nonhistone antigenic determinants from interphase to metaphase to be over 90% for chromatin.

Nonhistone Chromatin Metaphase chromosome HeLa cell Antiserum Electrophoresis

1. INTRODUCTION

The degree of conservation of interphase non-histone proteins on metaphase chromosomes is difficult to estimate by direct comparison of polyacrylamide gel patterns [1]. Coincidences in the positions of species may be noted, but can only suggest that the species may represent identical polypeptide chains. Immunological procedures have therefore been applied. The procedures use the high resolution of SDS-polyacrylamide gel electrophoresis to separate the nonhistones, and use the specificity and convenience of antibodies to detect the conserved species.

Several techniques have been reported for the transfer of proteins from SDS-polyacrylamide gels to immobilizing supports [2-9]. *Staphylococcus aureus* protein A, labeled with ^{125}I , is a powerful reagent for locating specific antibodies bound to the immobilized proteins. But the detection of antigens does not require that proteins be trans-

ferred from polyacrylamide gels. Direct overlay of gels with antiserum and ^{125}I -protein A is a simple and sensitive method for the identification of antigens [10,11].

These methods were applied to determine the conservation of HeLa interphase chromatin non-histone antigens. As would be expected, the nonhistones were found to vary in their antigenicity, and so the distinction must be made between the conservation of nonhistone antigens and the conservation of nonhistone polypeptides. Nevertheless, the results provide convincing evidence that interphase nonhistone antigens are substantially conserved as components of metaphase chromosomes.

2. MATERIALS AND METHODS

2.1. Preparation of chromatin and metaphase chromosomes

HeLa S-3 cells were culture in suspension at 37°C at a concentration of $2-5 \times 10^5$ cells/ml by daily dilution in minimum essential medium (Joklik modified) containing 5% fetal bovine serum (Flow). Nuclei from HeLa cells were obtained as in [12]. HeLa metaphase chromosomes were isolated from metaphase-arrested cells using a

Abbreviations: SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride; NP40, Nonidet P40; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BSA, bovine serum albumin

buffer containing divalent cations [13]. Chromatin was prepared by treatment of nuclei and metaphase chromosomes with 10 units/ml micrococcal nuclease for 5 min at 37°C. After the treatment, chromatin was obtained by resuspending nuclei in 5 mM EDTA, 0.1% NP40.

2.2. Preparation of antiserum

Antiserum was obtained from New Zealand White rabbits (male, 4–6 months) by intramuscular injection. The initial injections consisted of 0.8 ml chromatin (4 mg/ml total chromatin protein) which was emulsified with an equal volume of complete Freund's adjuvant. Subsequent injections at 2-week intervals contained a similar amount of protein but incomplete Freund's adjuvant. A positive antiserum response was detected by Ouchterlony analysis after 7 weeks.

2.3. Immunological detection of proteins

Separation of proteins by SDS-polyacrylamide gel electrophoresis was performed as in [14].

Proteins of the various samples were electrophoretically transferred from polyacrylamide gels to diazophenylthioether (DPT)-paper [3]. Electrophoretic transfer was performed in 25 mM sodium phosphate (pH 6.5), 4°C, for 3 h at 5 V/cm. The paper was covered with rabbit antiserum [diluted 1:10 in 150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 0.1% NaN₃] for 16–24 h at 25°C. After washing with 150 mM NaCl, 50 mM Tris (pH 7.4), the paper was reacted for 16–24 h with 1.0 μ Ci/ml of ¹²⁵I-labeled protein A (Amersham) in 2% bovine albumin (Armour, immunoglobulin-free), 150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 0.1% NaN₃. Non-specific background binding was reduced by washing the paper with a solution containing 1 M NaCl [2].

Polyacrylamide gels were also directly overlaid with antiserum and ¹²⁵I-labeled protein A [10]. Following electrophoresis, SDS-polyacrylamide gels were fixed for 4 h in 50% methanol, 10% acetic acid, and equilibrated for 16 h in buffer [150 mM NaCl, 50 mM Tris-HCl (pH 7.4)]. The gels were then covered for 24 h at 25°C with rabbit antiserum diluted 1:10 in this buffer containing 0.1% NaN₃. Non-specific binding to the gels were reduced by washing with the 150 mM NaCl, 50 mM Tris-HCl (pH 7.4) buffer. The gels were then overlaid for 24 h with 1.0 mCi/ml of ¹²⁵I-protein

A in 2% bovine albumin, 150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 0.1% NaN₃. They were then extensively washed in buffer.

3. RESULTS

3.1. Conservation of HeLa chromatin nonhistone antigens from interphase to metaphase

Fig. 1 shows the results with 12.5% polyacrylamide gels. This percentage of acrylamide resolves proteins ranging in M_r from 10 000 to over 200 000, and, in particular, the core histones are resolved. Fig. 1A includes a stained gel. Although coincidences in the M_r values of certain bands can be observed between samples, the complexity of the protein compositions prevents any definite conclusions regarding the conserva-

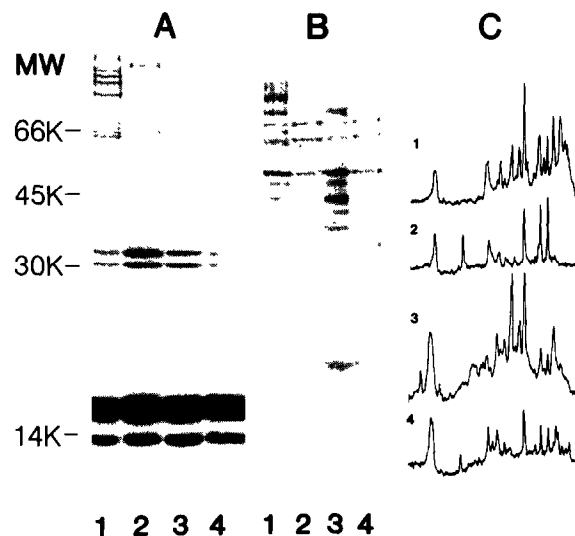


Fig. 1. Conservation of HeLa nonhistones detected by electrophoretic transfer of proteins from polyacrylamide gels to DPT-paper. (A) Coomassie blue-stained, 12.5% SDS-polyacrylamide gel containing samples of (1) nuclei, (2) isolated metaphase chromosomes, (3) interphase chromatin, and (4) chromatin from metaphase chromosomes. The M_r markers indicate the positions of bovine serum albumin (66 000), ovalbumin (45 000), carbonic anhydrase (30 000), and lysozyme (14 000). (B) Autoradiogram of a transfer to DPT-paper treated with antiserum and ¹²⁵I-protein A. The proteins were electrophoresed in a similar 12.5% gel before transfer. (C) Densitometer scans of the 4 lanes of the autoradiogram in (B).

tion of nonhistones.

Immunological procedures were therefore employed, and the results are presented in fig. 1B. In this experiment, the proteins were electrophoretically transferred to DPT-paper and the paper was treated with whole antiserum against interphase chromatin and with ^{125}I -labeled protein A. The presence of bands in all 4 columns of this autoradiogram demonstrates that interphase chromatin nonhistones are conserved in metaphase. Lane 3, containing interphase chromatin, is the control and shows the large number of distinct bands that are detected by this method. Many of these bands are also present with metaphase chromosomes and metaphase chromatin. Fig. 1C contains densitometer scans of the 4 lanes of the autoradiogram in fig. 1B.

The number of bands which is observed is the result of various factors, including the antigenicity of the chromatin proteins, the extent of recovery of antigenic determinants of the chromatin proteins, the extent of recovery of antigenic determinants after SDS denaturation, and the efficiency of binding of immunoglobulins and ^{125}I -protein A to proteins on DPT-paper.

A direct immunological procedure is to overlay polyacrylamide gels, after removing SDS, with antiserum and ^{125}I -protein A. The method should produce a more reliable estimate of the degree of nonhistone conservation, since non-quantitative transfer and incomplete binding to DPT-paper are avoided. The autoradiogram of an 8% gel is shown in fig. 2B. (The stained pattern of a similar 8% gel is in fig. 2A.) A large number of bands are seen along lane 3, the control lane in which interphase chromatin antiserum is reacted against interphase chromatin proteins. As was found with the DPT-paper experiments, many interphase nonhistones are conserved as metaphase species. The specificity of binding is demonstrated in fig. 2C. The experiment was performed with pre-immune serum and no binding of ^{125}I -protein A was detected.

Two-dimensional polyacrylamide gels provide a more accurate measure of the number of proteins detected by these procedures. Although the autoradiograms in fig. 1, 2 seem to show 1 or 2 dozen bands, autoradiograms of two-dimensional gels reveal at least 80 species with the control of HeLa chromatin and a comparable number with metaphase chromosomes (unpublished).

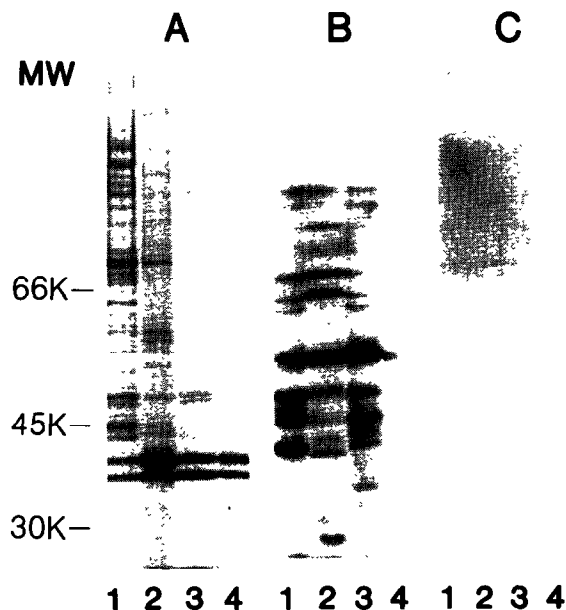


Fig. 2. Direct overlay of polyacrylamide gels with chromatin-specific antiserum and ^{125}I -labeled protein A. Samples of (1) nuclei, (2) isolated metaphase chromosomes, (3) interphase chromatin, and (4) chromatin from metaphase chromosomes were separated in 8% SDS-polyacrylamide gels, and the gels were covered with antiserum and then with ^{125}I -protein A. (A) Coomassie blue-stained, 8% gel of the 4 HeLa samples. (B) Autoradiogram of a polyacrylamide gel overlaid with immune serum to interphase chromatin. (C) Autoradiogram of a gel identical to the one in (B) except that pre-immune serum was used.

3.2. Quantitation of degree of conservation

The degree of conservation was quantitated from densitometer scans of autoradiograms and stained gels. The results of these calculations are given in table 1. In these calculations, the total nonhistone area of the densitometer scans was measured, and expressed as a fraction of the value for HeLa chromatin. To compare meaningfully different samples, two methods were used to normalize the measurements. Normalization was either with respect to the total amount of core histones, determined from densitometer scans of gels stained with Coomassie brilliant blue, or with respect to the total amount of nonhistones, also measured from scans of stained gels. The data in the first column of table 1, normalized with respect to core histones, demonstrate that over 90% of

Table 1

Degree of conservation of HeLa interphase chromatin nonhistone antigens

Sample	Degree of conservation ^a		Non-histones/histones plus non-histones
	Normalized to core histones	Normalized to total non-histones	
HeLa chromatin	1.00	1.00	0.10
HeLa metaphase chromosomes	0.88	0.30	0.23
HeLa chromatin from metaphase chromosomes	0.92	0.85	0.13

The conservation of nonhistone antigenic determinants of HeLa chromatin was quantitated from densitometer scans of autoradiograms of 8% polyacrylamide gels overlaid with antiserum to interphase chromatin and with ¹²⁵I-protein A. The total density under the nonhistones was measured for each sample and expressed as a fraction of the total density for the control of HeLa interphase chromatin. To compare accurately different samples, the value was normalized either with respect to the amount of core histones in each sample or with respect to the total nonhistone content. These amounts were determined from densitometer scans of 8% and 12.5% polyacrylamide gels stained with Coomassie brilliant blue. The ratio of nonhistones/histones plus nonhistones was also calculated from densitometer scans of gels stained with Coomassie blue

^aThe uncertainty of these values is estimated to be $\pm 7\%$

nonhistone antigenic determinants of interphase chromatin are found with metaphase chromatin.

The third column of table 1 shows that the ratio of nonhistones/histones plus nonhistones differs considerably for the various samples. In particular, metaphase chromosomes are enriched in nonhistone proteins. To take account of these differences, the calculations were also normalized with respect to the total amount of nonhistones, and these values are included in table 1. The value for metaphase chromosomes is significantly lower (30% conservation), which indicates that HeLa metaphase chromosomes have abundant proteins unique to the structure of metaphase chromosomes.

4. DISCUSSION

Antiserum to HeLa interphase chromatin was prepared in rabbits and reacted in overlay experiments with the nonhistones of HeLa interphase and metaphase samples. The electrophoretic transfer procedure was more sensitive for high-percentage (12.5%) acrylamide gels, presumably because the small pore size of the polyacrylamide matrix of high-percentage gels prevented efficient binding of antibody molecules.

To calculate accurately the degree of conservation of nonhistones (table 1), autoradiograms from direct gel overlay experiments (with 8% gels) were used. This was because quantitative electrophoretic transfer to DPT-paper or nitrocellulose is difficult to achieve, especially for high-*M_r* proteins. Several transfer buffers were tried in addition to 25 mM sodium phosphate (pH 6.5), but did not improve the extent of transfer. Furthermore, DPT-paper and nitrocellulose have limited binding capacities. This was seen, by staining gels following transfer, in the accumulation of stained protein at the interface between gel and paper.

The results of the experiments with transfer to DPT-paper (fig.1) and direct antiserum overlay (fig.2) demonstrate that HeLa interphase chromatin nonhistones are largely conserved in metaphase. For metaphase chromatin, the degree of conservation was calculated to be over 90%. Thus, although chromatin is considerably more condensed during mitosis, many of the fundamental structural roles of nonhistone proteins are likely to be preserved.

ACKNOWLEDGEMENTS

This research was supported by a grant from the National Institutes of Health and by funds from the Biomedical Research Support Grant of the University of Minnesota Medical School.

REFERENCES

- [1] Adolph, K.W. and Phelps, J.P. (1982) *J. Biol. Chem.* 257, 9086-9092.
- [2] Renart, J., Reiser, J. and Stark, G.R. (1979) *Proc. Natl. Acad. Sci. USA* 76, 3116-3120.

- [3] Alwine, J.C., Kemp, D.J., Parker, P.A., Reiser, J., Renart, J., Stark, G.R. and Wahl, G.W. (1979) *Methods Enzymol.* 68, 220-242.
- [4] Bittner, M., Kupferer, P. and Morris, C.F. (1980) *Anal. Biochem.* 102, 459-471.
- [5] Stellwag, E.J. and Dahlberg, A.E. (1980) *Nucleic Acids Res.* 8, 299-317.
- [6] Symington, J., Green, M. and Brackmann, K. (1981) *Proc. Natl. Acad. Sci. USA* 78, 177-181.
- [7] Towbin, H., Staehelin, T. and Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* 76, 4350-4354.
- [8] Burnette, W.N. (1981) *Anal. Biochem.* 112, 195-203.
- [9] Bowen, B., Steinberg, J., Laemmli, U.K. and Weintraub, H. (1980) *Nucleic Acids Res.* 8, 1-20.
- [10] Burridge, K. (1978) *Methods Enzymol.* 50, 54-64.
- [11] Adair, W.S., Jurivich, D. and Goodenough, U.W. (1978) *J. Cell Biol.* 79, 281-285.
- [12] Adolph, K.W. (1980) *J. Cell Sci.* 42, 291-304.
- [13] Adolph, K.W. (1980) *Chromosoma* 76, 23-33.
- [14] Laemmli, U.K. (1970) *Nature* 227, 680-685.