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Abstract

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DIFFERENCE IN THE HOMOLOGY OF TWO NUCLEAR NONHISTONE PROTEIN FRACTIONS AS COMPARED BY POLYACRYLAMIDE GEL ELECTROPHORESIS

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Abstract. The extent of homology between two protein fractions was compared by simple electrophoretic analysis. Nuclear proteins of several rodent cells of different origins were fractionated into acid-soluble and acid-insoluble fractions. The two protein fractions were subjected to polyacrylamide gel electrophoresis in separate gel systems, and protein bands with identical mobilities were sought either in all possible combinational pairs of cell types or in all cell types. The paired and overall homology indices calculated from these data and chi-square testing of the results indicated that acid-soluble nuclear nonhistone proteins are more homologous than acid-insoluble nuclear proteins. Several factors which might have affected the results were discussed.

Key words : nuclear proteins, protein homology, polyacrylamide gel electrophoresis.

The heterogeneity of nuclear nonhistone proteins has been analyzed by polyacrylamide gel electrophoresis (1-5). Some of the proteins were tissue or species specific although most of the major components were common (3, 4). The conclusions were based on the assumption that proteins having the same mobility in a given gel system are homologous. The results may differ, however, if the conditions for electrophoretic separation are changed. A relatively low degree of protein homology was observed between different tissues when the separation was based on the combination of two independent physical parameters, e.g., isoelectric point and molecular weight (5). Thus, it may not be reasonable to discuss the homology of a particular protein pair by comparing their mobilities in a single one-dimensional gel system. Sequential determination of individual proteins would be required to demonstrate homology in the evolutional context.

The extent of overall homology of two protein groups can be reasonably estimated by comparing the electrophoretic patterns of protein groups from different sources. For this purpose, conventional one-dimensional polyacrylamide gel systems with high resolution are adequate. In this study, nuclear proteins of several cell types were fractionated into acid-soluble and acid-insoluble groups and differences in the homology of the two groups were examined by polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

Nuclei were prepared from mouse ascites sarcoma cells (SR-C3H/He and Ehrlich) and cultured cells (murine leukemia and XC). Washed cells were treated with a hypotonic solution and disrupted in 2.2 M sucrose solution containing 5 mM $MgCl_2$ and 10 mM Tris-HCl (pH 7.8) using a Waring type blender. The homogenate was centrifuged at 40,000 × g for 60 min and the pelleted nuclei were washed once with an isotonic medium. Liver cell nuclei were prepared by the same procedure except that the hypotonic treatment was omitted. The purified nuclei (essentially free from cytoplasmic contamination), which contained 500 μg of DNA were extracted on ice with 0.25 ml of 0.2 M H_2SO_4 for 3.5 h with occasional vortexing. After centrifugation at 2,200 × g for 10 min, the supernatant (0.1 ml) containing acid-soluble nuclear proteins was dialyzed against 0.9 M acetic acid. The residual material (nuclei) was reextracted with 2 ml of 0.2 M H_2SO_4, followed by washing with 2 ml of 10 mM Tris-HCl (pH 7.8), and then lyzed with 0.2 ml of 2 % sodium dodecylsulfate (SDS). The solubilized protein was dialyzed against 2 % SDS for 7 h at room temperature.

For analysis of the acid-soluble proteins, electrophoresis was carried out on 15 % polyacrylamide slab gels (15 × 15 × 0.1 cm) containing 2 M urea and 0.9 M acetic acid (acid-urea gel, Ref. 6). The dialyzed samples (15 μl) were mixed with 5 μl of 10 M urea and applied to each slot of the gel which had been prerun for 11 h at 15 mA. Electrophoresis was performed at 4 ℃ for 7.5 h (15 mA per gel). The acid-insoluble nuclear proteins (35 μl of the dialyzed lysate) were separated in 10 % polyacrylamide slab gels containing 0.1 % SDS by the method of Laemmli (7). Marker proteins were run simultaneously for molecular weight estimation. After the electrophoresis, gels were stained with 0.1 % Coomassie blue in 25 % trichloroacetic acid, destained with 8 % acetic acid, and dried between cellophane sheets. All discernible protein bands except those of histones which are readily identifiable by their large quantity were marked with a marker pen on dried gels. In each lane of both gels (SDS- and acid-urea gels), the total number of marked bands was counted.

RESULTS

Nuclear nonhistone proteins were prepared from six different rodent cells including cells transformed by retroviruses: SR-C3H/He cells (8), mouse leukemia cells (9), and XC cells. Nuclear proteins were fractionated into acid-soluble and acid-insoluble proteins by extracting the nuclei directly with 0.2 M H_2SO_4. Acid-soluble nuclear proteins from various cells were subjected to electrophoresis in separate tracks on an acid-urea polyacrylamide slab gel. About 30-40 nonhistone protein bands were reproducibly separated, and their mobilities were easily compared between tracks (Fig. 1, left panel). For all combinational pairs of the cell types, the number of bands with equal mobilities (common bands) was counted (Table 1). If the same number of bands were observed in all cell types, the degree of polypeptide homology between cell types i and j would be simply expressed by the number of bands common to i and j (Nij). Since the total number of bands in different cell types generally differs, Nij has to be normalized according to the mean of the total band numbers to obtain a more appropriate index for the combinational homology. Thus, we used a homology index defined as $Nij/(Ni \times Nj)^{1/2}$, where Ni and Nj stand for the total number of bands in cell types i and

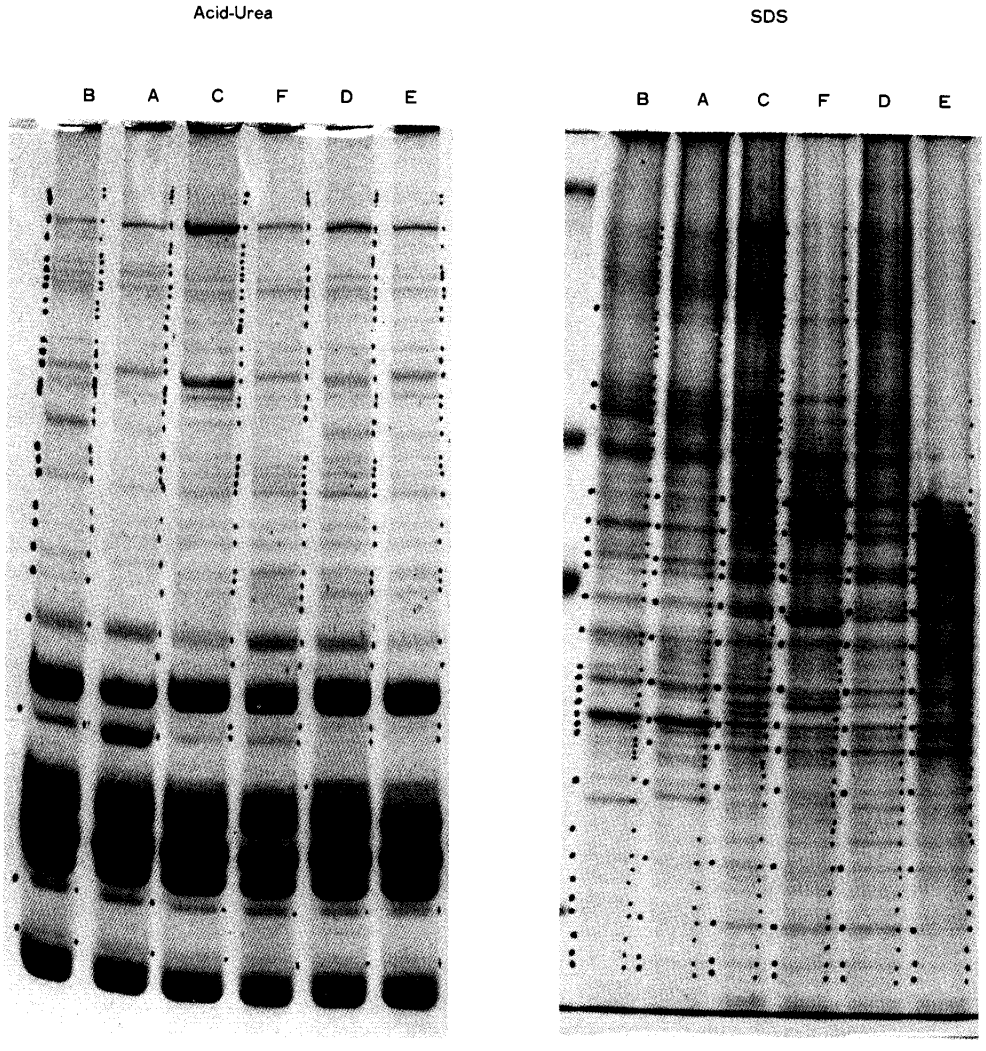

Fig. 1 Electrophoretic separation of acid-soluble and residual nuclear proteins in acid-urea and SDS-polyacrylamide gels. Cell types from which these protein fractions were prepared are given in the footnote for Table 1. Stained bands that are discernible by the naked eye are marked on the right side of each track. On the leftmost side are marked protein bands common to all cell types. Note that histones (the five heavy bands seen in the acid-urea gel) are not incorporated into the homology calculation.

j, respectively. Although the geometrical mean is used here, the conclusions were not affected by adopting the arithmetical mean. The homology indices of acid-soluble proteins separated by acid-urea gel were calculated using the above formula for all possible combinations of the cell types (Table 1). Electrophoresis of residual nuclear proteins in 10 % SDS-polyacrylamide gel gave clear resolution of 50-60

TABLE 1. PAIRED COMPARISON OF THE HOMOLOGY OF ACID-SOLUBLE AND RESIDUAL NUCLEAR NONHISTONE PROTEINS

	A	B	C	D	E	F
A	55 \ 33	48 (0.88)	39 (0.72)	46 (0.80)	34 (0.71)	37 (0.71)
B	29 (0.89)	54 \ 32	44 (0.81)	40 (0.70)	43 (0.90)	36 (0.69)
C	29 (0.83)	27 (0.78)	54 \ 37	46 (0.81)	38 (0.80)	40 (0.77)
D	30 (0.90)	29 (0.88)	32 (0.90)	60 \ 34	34 (0.68)	44 (0.80)
E	29 (0.88)	26 (0.80)	31 (0.89)	32 (0.96)	42 \ 33	34 (0.74)
F	29 (0.87)	27 (0.82)	31 (0.87)	31 (0.91)	31 (0.93)	50 \ 34

The electrophoretic patterns of nuclear proteins from A, mouse liver; B, rat liver; C, SR-C3H/He cells; D, murine leukemia cells; E, Ehrlich ascites cells and F, XC cells were compared. The number of common bands between all possible combinational pairs are shown. Data for the acid-soluble proteins are in the lower left and those for the residual proteins are in the upper right part of the table. The figures along the diagonal line represent the total number of bands. The homology indices were calculated as in the text and are shown in parentheses. Values for corresponding combinations appear in symmetrical positions on either side of the diagonal.

TABLE 2. OVERALL COMPARISON OF THE HOMOLOGY OF ACID-SOLUBLE AND RESIDUAL NUCLEAR NONHISTONE PROTEINS.

Polypeptide band	Nuclear protein fraction	
	Acid-soluble	Residual
Common[a]	156	156
Total[b]	203	315

a Total number of protein bands common to all 6 cell types ($26 \times 6 = 156$ for both fractions).
b Total number of protein bands in the gel.

bands (Fig. 1, right panel). The polypeptide homology indices of the residual proteins are also shown in Table 1. In both protein groups, more than 70% of the bands were common polypeptides for each pair. This result is consistent with the limited heterogeneity of nonhistone proteins reported previously (3, 4). The homology indices of the acid-soluble nuclear nonhistone proteins were greater than those of the residual proteins in 13 out of 15 corresponding combinations ($x^2 = 6.7$, $p < 0.01$).

Bands common to all cell types were also sought in each group. In both protein groups 26 bands were common to all cell types (Table 2). The degree

of overall homology defined here as the total number of common bands divided by the total number of bands in all cell types was 0.77 for acid-soluble, and 0.50 for residual nonhistone protein groups. Chi-square testing of the results (Table 2) showed that the deviation was statistically significant ($x^2 = 38.5$, $p < 0.001$), clearly demonstrating the higher homology of the acid-soluble nuclear nonhistone proteins.

DISCUSSION

In this study, different gel systems were used for acid-soluble and residual nuclear proteins. In the acid-urea gel used to analyze the acid-soluble proteins, mobility of proteins depends on two factors: positive charge density (10) and molecular weight of proteins. The latter factor is based on the sieving effect of the polyacrylamide gel matrix albeit there is no quantitative correlation between mobility and molecular weight such as is seen in the SDS-polyacrylamide gel system. Since two independent physical properties are involved in the separation mechanism of the acid-urea gel, the present conclusion that the acid soluble nuclear proteins are more homologous than the residual proteins should be more reliable than in the case where only SDS-polyacrylamide gel electrophoresis is used for both protein groups.

Although being homologous, some proteins varied greatly in amount among different cells. Proteins of low abundance may not be detectable in all cell types even if they were completely conserved. The degree of overall homology would be underestimated in such a case. On the other hand, the homology would be overestimated when protein bands are so closely located in the gel that those with similar mobilities are misidentified as homologous. There is little possibility of this in the present experiment, however, because of the relatively low average band density(2.2 and 3.7 bands per cm for the acid-urea gel and the SDS-gel, respectively). It should be pointed out that the resolution power of the acid-urea gel used in this study is comparable to that of the SDS-gel as indicated by the similar band density, which is reciprocally related to the average band distance, and the similar average band width in both gel systems (Fig. 1).

It was noted that apparent homology of the residual nuclear proteins with molecular weights higher than 10^5 was relatively low compared to smaller proteins. Only 3 common polypeptides were found in this molecular weight region. A similar observation has been made by Elgin and Bonner (3). Because of the possible occurrence of aggregated polypeptide bands in the upper part of the gel, this may be only an apparent effect. The observed difference in homology of the two protein groups was significant even when the calculation was made after omission of the high molecular weight bands in the residual proteins ($x^2 = 4.92$, $p < 0.05$).

The nuclear acid extract contains many nonhistone protein species including nucleoplasmic and nucleolar acidic proteins as well as chromosomal nonhistone

proteins, some of which are now well characterized. The highly conserved nature of the acid soluble nuclear proteins including histones suggests that these proteins are involved in "house keeping" metabolism or the general structure of nuclei.

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