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Proteins with sulfhydryl groups in nuclei and nuclear matrix of normal and tumour cells

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The results of many investigations revealed essential role of sulfhydryl groups (SH) for functions of cell nuclei [1–5], especially for organization of their skeletal structures called nuclear matrix [6–8].

The nuclear matrix is operationally defined as the residual structure after treatment of nuclei with DNase I followed by buffered salts (NaCl, $NH_4(SO_4)_2$, $MgCl_2$) or detergent (lithium 3,5-diiodosalicylate) to remove chromatin and loosely bound proteins [7–11]. Nuclear matrix presents mainly proteinaceous structure consisting of residual components of nuclear envelope (lamina and nuclear pore complex), remnants of nucleoli, and an internal fibrogranular network [12–14]. In contrast to the lamina, the structural basic of residual nucleoli and internal network is far from clear [13, 15, 16]. The internal fibrogranular network is especially labile [8, 11, 15].

For this reason a stabilization step is included in recent nuclear matrix isolation procedures [8, 17]. For stabilization of nuclear structure different approaches have been applied, i.e. oxidation with sodium tetrathionate [6, 8], fixation with acrolein [18], treatment with Cu^{2+} [19] or incubation at 37°C or 43°C [8, 10, 15]. Molecular mechanisms responsible for stabilization are poorely understood. However, its omission results in dissociation of the internal fibrogranular network during nuclear matrix isolation [17]. It has been suggested that stabilization by incubation at 37°C acts through oxidation of SH groups to disulfides [20].

In identification of free SH groups in proteins or those engaged in S-S bridges (after their reduction) histochemical, electrophoretic or cytofluorimetric determinations of protein-bound thiols are usually performed [19–23].

The aim of the present studies was to compare electrophoretic behaviour (one- and two-dimensional gel electrophoresis) of nuclear and nuclear matrix proteins originating from hamster Kirkman-Robbins hepatoma and liver for identification of the components containing SH groups.

Nuclei were prepared from hepatoma and livers by the sucrose method [24]. Nuclear matrix from the examined cells were isolated by the recently described technique developed in Berezney's laboratory [8]. It involves stabilization of nuclei at 37°C for 60 min followed by treatment with DNase I (50 mM Tris/HCl, pH 7.4, 1 mM PMSF, 5 mM $MgCl_2$, 4°C, 30 min) and extraction with buffered 0.25 M ammonium sulfate.

Proteins of nuclei and nuclear matrix from normal and tumour cells were separated by SDS-polyacrylamide gel electrophoresis (8%) one- and two-dimensional technique [25, 26] followed by staining with Coomassie Brilliant Blue R-250 [27] and silver nitrate [28] (Fig. 1a, b

[1]Abbreviations: IFPA, isoelectric focusing in polyacrylamide gel; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

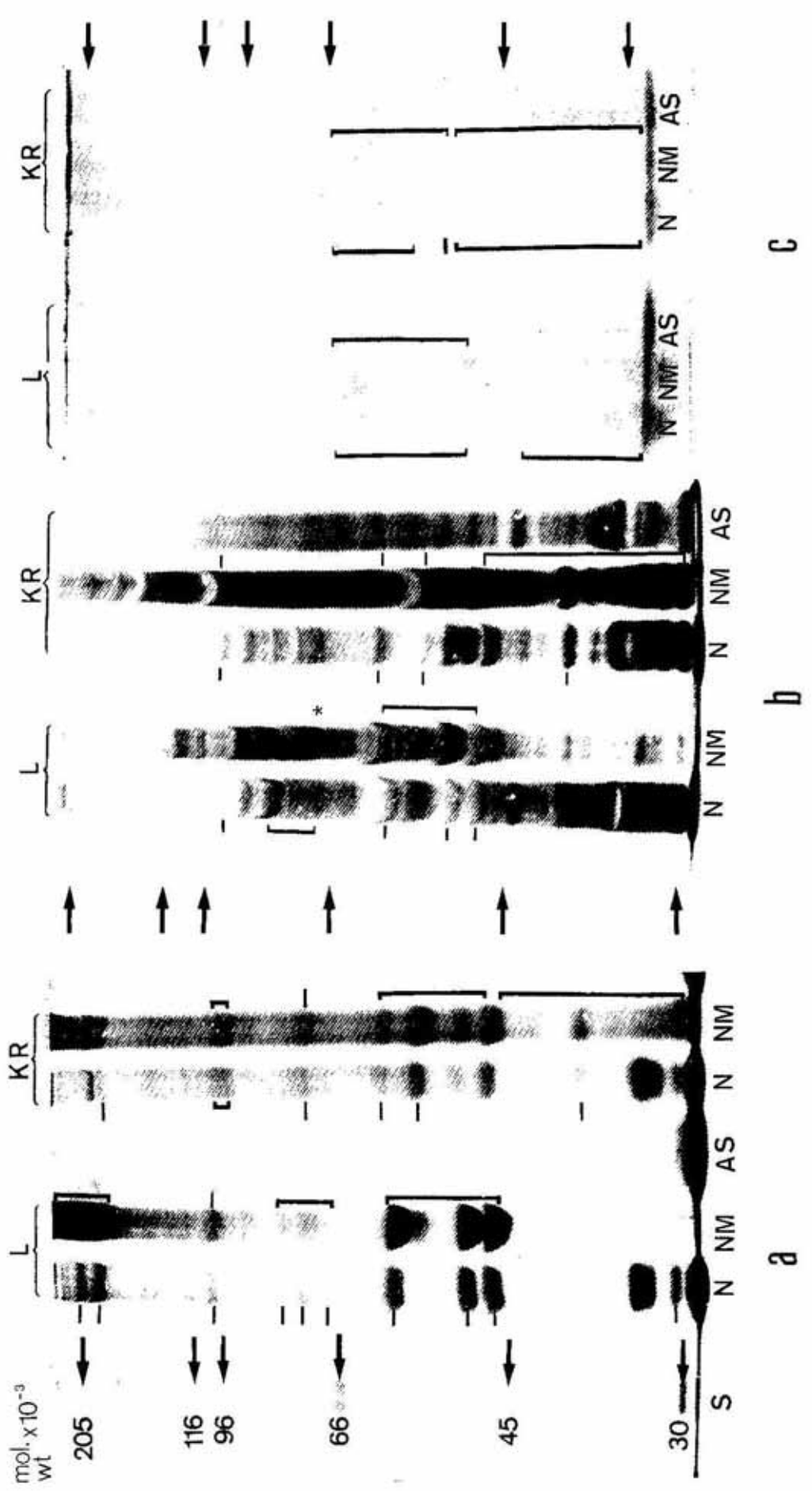

Fig. 1. One-dimensional SDS-PAGE of nuclear (N) and nuclear matrix (NM) proteins from hamster liver (L) and Kirkman-Robbins hepatoma (KR) stained with Coomassie Brilliant Blue R-250 (a), silver nitrate (b) or by the DDD-Fast Black K technique (c).
In some cases ammonium sulfate extracted proteins (AS) were stained; s — standard proteins; * — BSA (bovine serum albumin).

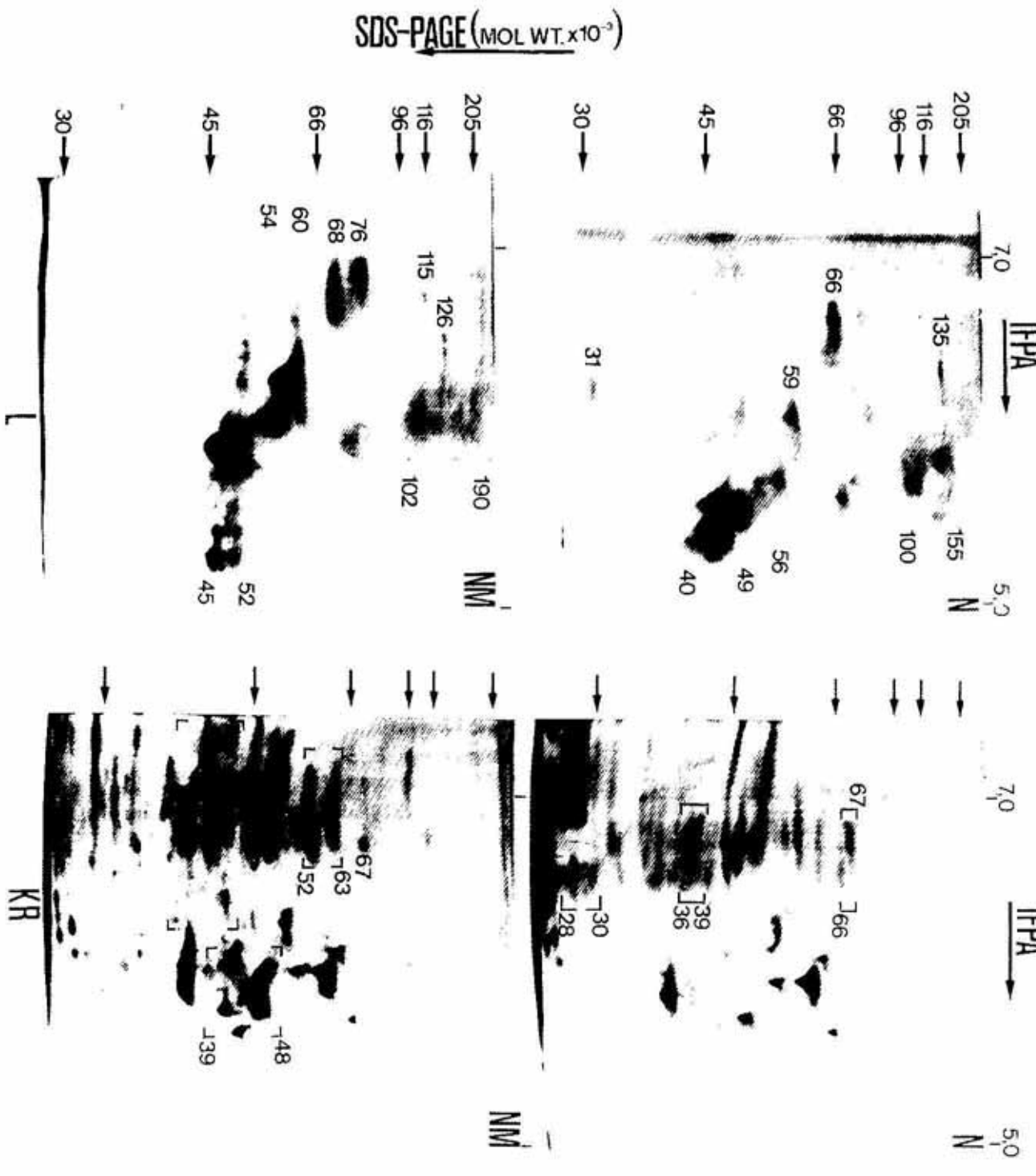

Fig. 2. Two-dimensional electrophoresis (IFPA in first, and SDS-PAGE in second dimension) of nuclear (N) and nuclear matrix proteins (NM) from hamster liver (L) and Kirkman-Robbins hepatoma (KR) stained with silver nitrate.

and Fig. 2). Using the electrophoretic approach followed by the two step reaction with a high specific reagent 2,2'-dihydroxy-6,6'-dinaphthyl disulfide (DDD) and the dye Fast Black K [21] we were able to determine distribution of SH groups in the investigated proteins of normal liver and hepatoma (Fig. 1c).

Comparison of electrophoretic patterns of nuclear and nuclear matrix proteins from hamster liver and hepatoma cells reveals many common components, among which enzymes, regulatory and structural proteins can be expected [7, 12]. However, as it can be seen in Fig. 1a, b and Fig. 2 there are some qualitative and quantitative differences in the profile of nuclear and nuclear matrix proteins between normal and neoplastic cells, especially in the regions of gels corresponding to the following molecular weight: 36000–39000; 41000–47000; 50000–64000; 66000–78000; 94000–106000; 140000–175000 and 180000–210000. In electrophoretic profiles of nuclear matrix proteins of both types of cells, the components of molecular weight of 35000–45000 and 60000–80000 are enriched (especially well visible in electrophoretic patterns of proteins stained with silver nitrate).

According to the present knowledge in this molecular weight ranges hnRNP core proteins (hnRNPs) and the lamins could be expected [6, 8, 13, 29]. Recently some attempts have been done in the search of specific proteins in nuclear skeleton to find potential neoplastic markers [30–32].

Most of the nuclear and nuclear matrix proteins contain sulfhydryl groups. By specific staining of SH groups in the proteins separated by SDS-PAGE some differences in their distribution were observed in liver and hepatoma nuclear and skeletal structures (Fig. 1c). The main differences were seen among nuclear polypeptides of molecular weight of 31000–39000 and 46000–61000. Elevation of some components with sulfhydryl groups in nuclear matrix of both cell types, especially in the areas of gels with molecular weight 46000–61000 and 30000–45000; 46000–65000 in normal and tumour cells, respectively, also was noticed. The two-dimensional electrophoretical analysis of nuclear and nuclear matrix proteins containing sulfhydryl groups from examined cells is in progress.

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