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Cell Biology

Nuclear matrix proteins reflect cell type of origin in cultured human cells

(nonhistone nuclear proteins/cell-type-specific proteins)

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ABSTRACT **The low abundance proteins of the nuclear matrix (NM) were separated from the intermediate filament (IF) proteins and analyzed by two-dimensional gel electrophoresis. Three human breast carcinoma lines had virtually identical patterns of 37 NM proteins. In contrast, cell lines derived from diverse tissues had qualitatively different NM protein patterns. Together, the five cell types examined here had a total of 205 distinguishable NM proteins with 125 of these proteins unique to a single cell type. The remaining NM proteins were shared among cell types to different degrees. Polyclonal antisera, obtained by immunization with total NM proteins as antigens, preferentially stained the nuclear interior and not the exterior IF. These observations suggest that the NM proteins, localized to the interior of the nucleus, vary in a cell-type-specific manner.**

The existence of a nuclear matrix (NM) with a discrete protein composition has been demonstrated in several studies (1–14). Berezney and Coffey (15) showed that the majority of proteins of the NM (80%) behaved as acidic nonhistone proteins in classical nuclear protein fractionation schemes (16). The matrix proteins include the relatively well characterized lamins and ribonucleoprotein (RNP) complex proteins together with a poorly characterized group of low abundance proteins (1–3, 8, 11–14, 17–19). The detection of the latter was somewhat dependent on the conditions of preparation as summarized by Berezney (20).

The nuclear lamins, described by Gerace, Blobel, and others (21–25), are major components of the NM as prepared by different methods from a variety of cell types (10, 12–14, 17–19). There are nuclear components associated with but probably distinct from the NM. These include heterogeneous nuclear RNA (hnRNA) and the proteins of the RNP complex (26, 27), whose extensive association with the NM (4, 14, 28–36) may reflect the proposed role of the NM in RNA splicing (37). Also, the intermediate filaments (IF) are tightly associated with the NM. Together, the matrix and IF constitute, in *in situ* preparations, a structural complex, which has been designated the NM–IF scaffold (11, 14, 19). In contrast to structures associated with the matrix, there is another class of fibers in the matrix interior composed of a discrete set of poorly characterized nonhistone nuclear proteins, which appear to be constituents of the matrix itself (3, 4, 8, 10–12, 14, 17, 38–40). These interior proteins have been separated and characterized in this report.

Kaufmann and Shaper (12) have shown that the complex pattern of nonlamin NM proteins corresponds to the proteins in filament structures of the interior NM that are less stable than the lamina–pore complex. Similarly, monoclonal antibodies to individual NM proteins that stain with both punctate and filamentous patterns in interior and peripheral regions of the nucleus indicate the existence of a distinct set of interior NM proteins (41–46).

We have previously described a relatively gentle procedure for the isolation of the NM–IF (11, 14). In this report, we further separated the NM proteins from the much larger mass of IF proteins. Once separated in this manner, the NM was seen to include many proteins specific to the cell type. These cell-type-specific proteins are largely masked in conventional nuclear preparations by the much more abundant nonhistone chromatin proteins that are highly homologous in most cell types.

MATERIALS AND METHODS

Cell Culture and Labeling. The human cell lines used in this study were breast carcinoma cell lines MCF7 (HTB 22), BT-20 (HTB19), T-47D (HTB 133), diploid fibroblasts, glioblastoma line A-172 (CRL 1620), adrenal cortex adenocarcinoma SW-13 (CCL 105), duodenal adenocarcinoma HuT 80 (HTB 40). These cells were grown to a density of $\approx 4 \times 10^6$ cells per 100-diameter plastic tissue culture plate. Cell monolayers were grown at 37°C in Dulbecco's medium supplemented with 10% fetal bovine serum (GIBCO) in a humidified atmosphere of 5% CO_2/95% air. Protein was labeled for 1 hr in 25 μCi of L-[^{35}S]methionine per ml (8.3 mCi/ml; 1064 Ci/mmol; 1 Ci = 37 GBq, New England Nuclear) at 37°C in Dulbecco's medium and 10% fetal bovine serum that lacked methionine.

Purification of NM Proteins. Cells were extracted in a series of buffers containing detergents and nucleases to produce the NM–IF scaffold as has been described (11, 14). The NM–IF scaffolds were solubilized in a disassembly buffer (8 M urea/20 mM Mes, pH 6.6/1 mM EGTA/1 mM phenylmethylsulfonyl fluoride/0.1 mM $MgCl_2$/1% 2-mercaptoethanol) according to the method of Zackroff *et al.* (47). The samples were dialyzed for 12 hr at 20°C against 1000 vol of assembly buffer (0.15 M KCl/25 mM imidazole hydrochloride, pH 7.1/5 mM $MgCl_2$/2 mM dithiothreitol/0.125 mM EGTA/0.2 mM phenylmethylsulfonyl fluoride). The reassembled filaments were harvested by centrifugation (150,000 × *g*, 90 min, 20°C). The supernatants containing the NM proteins were precipitated in 4 vol of acetone, and the dried pellets were resuspended in O'Farrell sample buffer (48).

Electrophoresis. One-dimensional polyacrylamide gels and immunoblots were prepared as described (14). Two-dimensional gel electrophoresis was performed essentially by the method of O'Farrell (48) with equilibrium isoelectric focusing gels containing 2% of a 4:1 mixture of pH 3.5–10 and pH 5–7 Ampholines (LKB, Bromma, Sweden). Isoelectric points were determined using a calibration standard (pI range, 4.7–10.6) obtained from BDH. NM proteins (200,000 cpm) were separated and the dried gels were exposed for 24, 48, and 96 hr. The developed x-ray films were photographed and

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Abbreviations: NM, nuclear matrix; IF, intermediate filament(s); RNP, ribonucleoprotein.

enlarged. The positions of proteins from three exposures of the same gel were transferred to acetate transparency sheets. Comparisons of individual cell types were done using the results of at least three separate experiments. Monoclonal anti-cytokeratin (clone K8.13), specific for cytokeratins 8 and 18, was obtained from ICN Immunobiologicals (Lisle, IL).

Immunofluorescence. Antiserum to total NM proteins from MCF7 breast carcinoma cells was obtained after successive injections of 400 μg of purified NM protein in RIBI adjuvant (RIBI Immunochem, Hamilton, MT) into BALB/c mice. Monoclonal antiserum to cytokeratins 8 and 18 is described above. Immunofluorescence microscopy of purified NM–IF scaffold preparations was performed as described (11).

RESULTS

The purified NM–IF scaffold (11, 14) was isolated and the more abundant IF proteins were selectively removed. To effect this separation, the NM–IF fraction was first solubilized in a disassembly buffer containing 8 M urea and the urea was then gradually removed by dialysis. The IF proteins repolymerized (47) and were separated from the soluble NM proteins almost quantitatively by ultracentrifugation (Fig. 1). When the NM–IF proteins from MCF7 breast carcinoma cells were separated, >95% of the cytokeratins 8, 18, and 19 (49, 50) and actin pelleted with the IF fraction (Fig. 1). The supernatant NM proteins were composed of the nuclear lamins and a complex population of low abundance proteins that were revealed when the more abundant cytokeratins of the NM–IF fraction were removed.

Immunofluorescence showed that the proteins of the NM fraction were largely localized to the nuclear interior and were therefore relatively free of proteins derived from the IF. Antiserum was obtained by injecting mice with purified NM proteins as described. The fluorescence pattern was localized predominantly in the interior nuclear region of purified NM–IF scaffold structures from MCF7 cells (Fig. 2*a*). For comparison, staining with an antibody to the cytokeratin proteins labels IF in the cytoplasmic space in the same preparation (Fig. 2*b*).

A small amount of the protein found in the NM fraction apparently originates in other nuclear fractions. We have designated only those proteins detected exclusively in the NM fraction as belonging to the NM. The two-dimensional gel electropherogram of NM proteins from MCF7 breast carcinoma cells consists of 56 protein spots, of which 37 are found only in the NM fraction (Fig. 3*b*). Proteins that are not unique to the matrix can be seen in the basic region of the gel (pI, 7.0–8.3), indicated by brackets, which contains the RNP complex proteins (14, 51). While most of the RNP complex proteins are removed from the NM–IF scaffold by the digestion with RNase A, a small amount often remains as part of the NM fraction. All the protein spots designated as RNP components in Figs. 3 and 4 have been identified in the RNase A-released fraction of nuclear proteins (14). The core hnRNP proteins (27) are extremely basic (pI, >8.4) and do not appear in the gel system used here. For the purposes of this study, the RNP complex proteins are not considered as NM proteins.

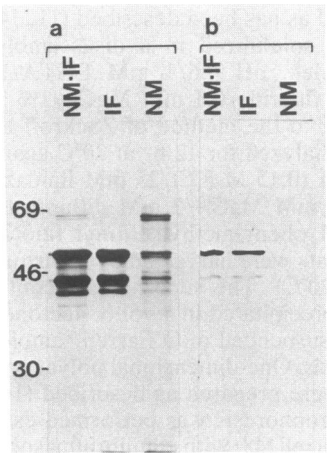

FIG. 1. $NaDodSO_4$/PAGE and immunoblot analysis of proteins obtained after separation of NM and IF proteins. (*a*) Electropherogram showing the separation of the NM–IF proteins into distinct IF and NM fractions. The NM proteins are a minor subset of the NM–IF proteins. (*b*) Immunoblot analysis of an identical gel using a monoclonal antibody to cytokeratins 8 and 18 shows that virtually all the cytokeratins present in the NM–IF are removed from the NM after dialysis and centrifugation of the repolymerized filaments. Numbers on left represent kDa.

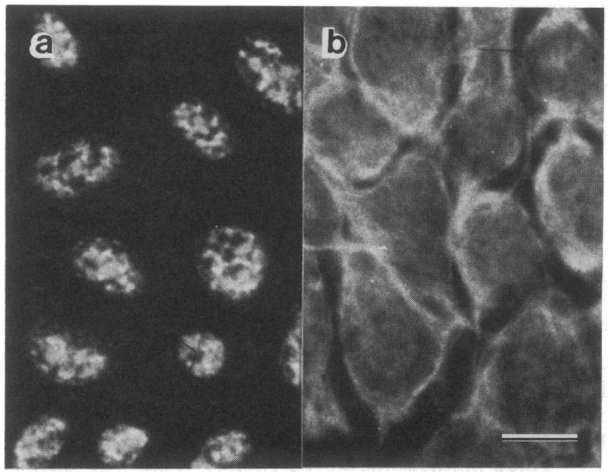

FIG. 2. Immunofluorescence micrographs showing the localization of NM antigens (*a*) compared with the localization of cytokeratins 8 and 18 (*b*) in MCF7 breast carcinoma cells. The fluorescent pattern indicates that antisera to the NM proteins (*a*) preferentially stain the interior nuclear matrices of MCF7 cells (11, 41, 42, 45). The cytokeratin immunofluorescence (*b*) indicates the location of IF in the cytoplasmic space. (Bar = 20 μm.)

The nuclear lamins described by Gerace and others (21–25) are major components of NM–IF scaffold (14). These proteins, identified by electrophoretic mobility and immunoreactivity on immunoblots (not shown), partition entirely with the NM fraction of every human cell type examined to date. The lamins A (70 kDa; pI, 7.63), B (67 kDa; pI, 5.94), and C (60 kDa; pI, 7.63) are represented by shaded spots and are indicated in all gels (Figs. 3 and 4).

The IF proteins and actin, although largely removed by the repolymerization and centrifugation steps, appear as minor components of the NM fraction. These proteins have been identified on the basis of published electrophoretic mobilities and by immunoblot analysis by using the appropriate antisera (data not shown). For the breast cell NM proteins shown in Fig. 3, cytokeratins 8 (52 kDa; pI, 6.06–5.95), 18 (45 kDa; pI, 5.95), and 19 (41 kDa; pI, 5.67) (50) and actin (43 kDa; pI, 5.93) are identified in each gel electropherogram and composite drawing. Actin was identified by its unique molecular mass and isoelectric point. There are also a few NM proteins that are detectable in the chromatin fraction. These proteins are indicated by shaded circles in the schematic diagram (Fig. 3*d*).

The RNP complex proteins, actin, vimentin, vimentin-related proteins (52, 53), the cytokeratins, as well as the lamins and those proteins detected in other cellular fractions, are all represented by shaded spots in Figs. 3 and 4. Those proteins represented by open and solid circles are tentatively identified as true NM proteins. The open circles identify NM

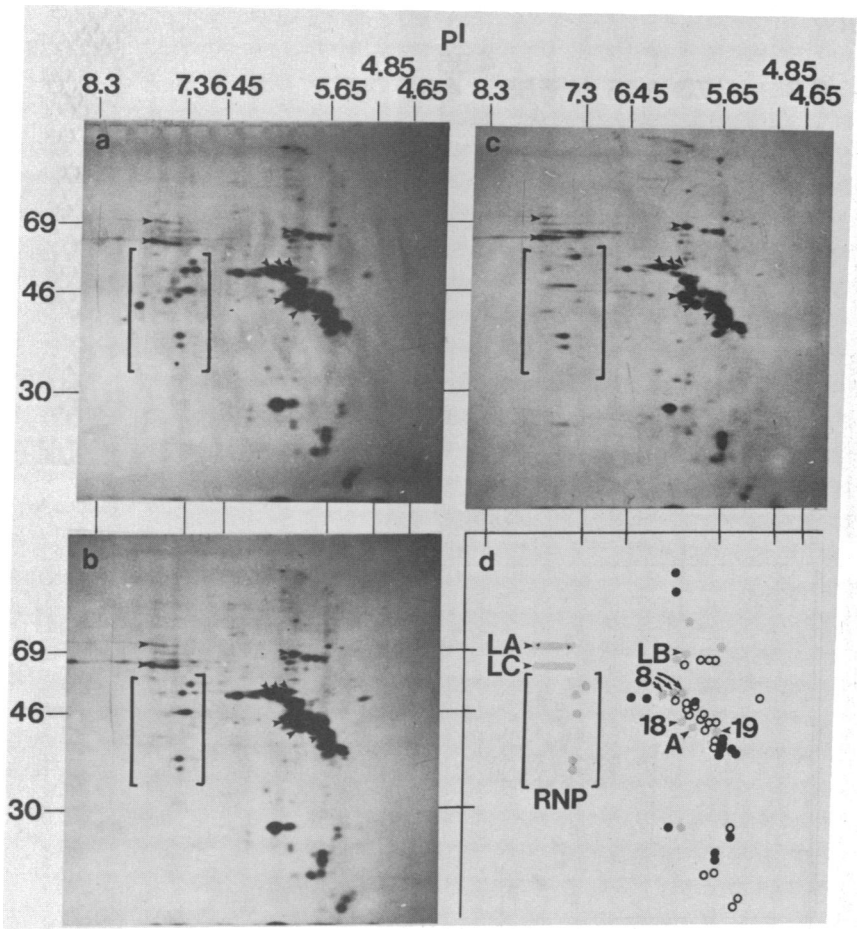

FIG. 3. Comparison of the protein composition of the NM fractions of three breast carcinoma cell lines. The NM proteins from three breast carcinoma cell lines, T-47D (*a*), MCF7 (*b*), and BT-20 (*c*), were examined by equilibrium two-dimensional gel electrophoresis. The positions of lamins A, B, and C (LA, LB, and LC); cytokeratins 8, 18, and 19 (nos. 8, 18, and 19); and actin (A) were determined by two-dimensional immunoblots with the appropriate antibodies (not shown). The proteins of the NM fraction also observed in the RNP fraction (14) focus in the basic region of the gel and are indicated by brackets (RNP). All proteins indicated by shaded circles in the diagram (*d*) have been identified above or are observed in the chromatid fraction and are not considered as NM proteins in this study. The proteins represented by open circles are NM proteins that have been observed in at least one of the other four cell types examined in this study (Fig. 4). Proteins represented by solid circles are those observed only in cells derived from breast tissue. The patterns of NM proteins from all three breast carcinoma lines are essentially identical. Numbers on left represent kDa.

proteins that are common to two or more of the five cell types examined here. The solid circles indicate those NM proteins that appeared in only one of the five cell types. The true NM proteins of three breast carcinoma cell lines, presumably of similar epithelial origin, exhibit a superimposable pattern of 37 NM proteins (Fig. 3). Of these 37 proteins, 16 (solid circles, Fig. 3) are observed only in the breast lines and not in any of the other four cell lines. This result suggests that cells of the same tissue of origin have a similar unique NM protein composition.

In contrast to the constancy in a single cell type, the NM proteins from four additional cell types show a strong cell-type dependence (Fig. 4). There are 47 NM proteins in the human diploid fibroblast line. Of these, only five NM proteins are specific to the fibroblast NM (Fig. 4*e*). A greater diversity of NM protein composition is observed in the other cell lines examined (Fig. 4). The glioblastoma, duodenal adenocarcinoma, and adrenal cortex adenocarcinoma lines have 79, 84, and 78 total NM proteins, respectively. Of these NM proteins, 30, 42, and 32, respectively, are present only in one of the five cell types examined.

DISCUSSION

While several reports identify individual NM proteins using monoclonal antibodies (41–46), the minor proteins of the NM have not been characterized as a group. In this report, we have described a procedure for isolating the low abundance NM proteins. Analysis of these proteins by two-dimensional gel electrophoresis has provided several unanticipated findings. The NM proteins from cell lines derived from the same cell type display almost identical two-dimensional electrophoretic patterns (Fig. 3) and appear to be invariant within a cell type. The NM proteins from diverse cell types consist of two populations: those that are expressed in several cell lines and those that are expressed in a cell-type-specific manner. In the five different cell types examined, the total number of NM proteins identified ranged from 37 to 84 per cell line excluding the cytokeratins, vimentin, lamins, and RNP complex proteins. Of the 204 proteins identified as NM proteins among five different cell types, 125 (61%) of these proteins were observed in only one cell type.

Because these proteins are present in low abundance, it is possible that they are present in other cellular fractions but are obscured by the more abundant proteins. Monoclonal antibodies specific for individual NM proteins can be used to study the distribution of these proteins in all cellular fractions and to determine the nature of the association of these proteins with the NM.

The NM proteins within an individual cell type range in size from 20 to 200 kDa. The fractions were prepared with protease inhibitor present and afforded completely reproducible patterns. This observation argues against proteolysis contributing to the diversity of NM proteins in different cell types. Some of the alterations in protein pattern might be due to posttranslational modifications of the NM proteins. Analysis of phosphoproteins and glycoproteins in the NM fractions of several cell lines (data not shown) suggests that these modifications are not responsible for the majority of the protein differences observed in NM fractions. The most likely explanations of the cell-type-specific expression of NM proteins are the expression of many different genes for NM proteins or the alternative splicing of transcripts from a more limited number of genes for NM proteins.

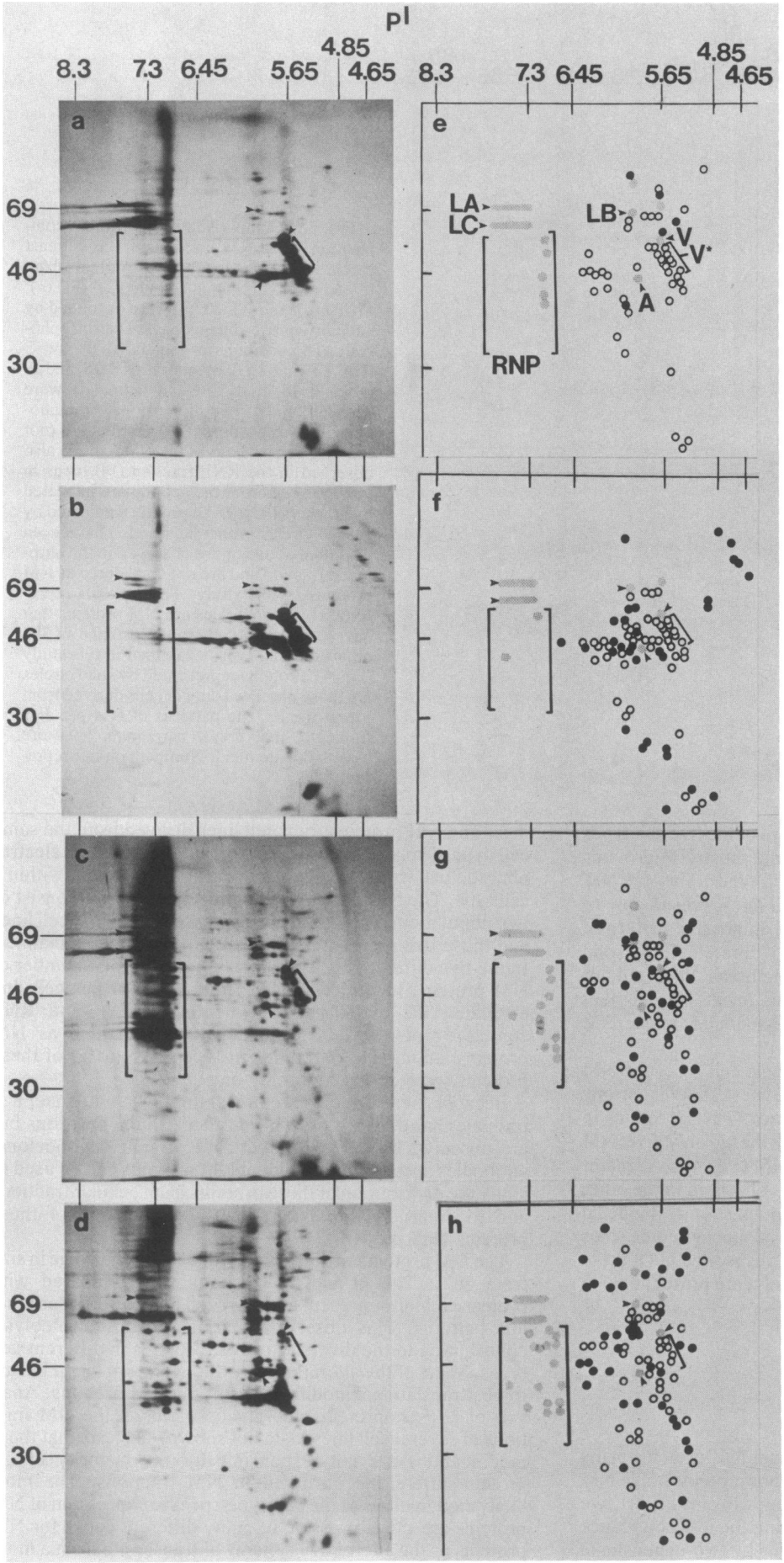

FIG. 4. Comparison of the protein composition of the NM fractions of cell lines derived from four tissue types. The NM proteins are shown as electropherograms of two-dimensional gels (*a–d*) and schematic diagrams (*e–h*). The cell lines examined were human diploid fibroblasts (*a* and *e*), glioblastoma line A-172 (*b* and *f*), duodenal adenocarcinoma line HuT 80 (*c* and *g*), and adrenal cortex adenocarcinoma SW-13 (*d* and *h*). The lamins (LA, LB, and LC), actin (A), and the RNP complex proteins (RNP, brackets) were identified as described above. Vimentin (V) and a cluster of vimentin-related proteins (V*, bracket) were identified by using a two-dimensional immunoblot (not shown). All proteins indicated by shaded circles in the diagrams (*e–h*) have been identified above or are observed in the chromatin fraction and are not considered as NM proteins in this study. The proteins represented by open circles are NM proteins that have been observed in at least one of the other four cell types examined in this study (Fig. 3). Proteins represented by solid circles are those observed only in one cell type. Numbers on left represent kDa.

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