A Normally Masked Nuclear Matrix Antigen That Appears at Mitosis on Cytoskeleton Filaments Adjoining Chromosomes, Centrioles, and Midbodies

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Abstract. mAbs were generated against HeLa nuclear matrix proteins and one, HIB2, which selectively stained mitotic cells, was selected for further study. Western blot analysis showed HIB2 antibody detected a protein of 240 kD in the nuclear matrix fractions. The HIB2 antigen was completely masked in immunofluorescently stained interphase cells. However, removing chromatin with DNase I digestion and 0.25 M ammonium sulfate extraction exposed the protein epitope. The resulting fluorescence pattern was bright, highly punctate, and entirely nuclear. Further extraction of the nuclear matrix with 2 M NaCl uncovers an underlying, anastomosing network of 9-13 nm core filaments. Most of the H1B2 antigen was retained in the fibrogranular masses enmeshed in the core filament network and not in the filaments themselves.

The HIB2 antigen showed remarkable behavior at mitosis. As cells approached prophase the antigen became unmasked to immunofluorescent staining without the removal of chromatin. First appearing as a bright spot, the antibody staining spread through the nucleus finally concentrating in the region around the condensed chromosomes. The antibody also brightly stained the spindle poles and, more weakly, in a punctate pattern in the cytoskeleton around the spindle. As the chromosomes separated at anaphase, HIB2 remained with the separating daughter sets of chromosomes. The HIB2 antigen returned to the reforming nucleus at telophase, but left a bright staining region in the midbody. Immunoelectron microscopy of resinless sections showed that, in the mitotic cell, the HIB2 antibody did not stain chromosomes and centrioles themselves, but decorated a fibrogranular network surrounding and connected to the chromosomes and a fibrogranular structure surrounding the centriole.
Materials and Methods

Cell Culture

HeLa cells were grown either in suspension culture (CCL 2.2) in Joklik-modified minimal essential medium containing 7% (vol/vol) horse serum or on monolayers (CCL 2) in DME containing 10% (vol/vol) FBS. MCF-7 breast adenocarcinoma cells (HTB 22) and CaSkii cervical carcinoma cells (CRL 1550) were cultured in Dulbecco's medium containing 10% (vol/vol) Horse Serum. The W12 cervical dysplasia cell line (Stanley, 1989) was a gift of Dr. Charles P. Stanley. Mouse myeloma hybridoma cells were grown in RPMI 1640 medium containing 10% (vol/vol) fetal bovine serum (FBS). Mouse myeloma cells and hybridoma cells were cultured in RPMI 1640 medium containing 10% (vol/vol) FBS. HeLa cells were maintained in RPMI 1640 medium containing 10% (vol/vol) FBS. All media were supplemented with 2 mM L-glutamine and 100 U/ml penicillin/streptomycin.

Cell Extraction

The in situ sequential extraction of cultured cells to reveal the nuclear matrix has been described in previous publications (Capco et al., 1982; Fey et al., 1986). After a wash in PBS, cells were extracted in cytoskeleton buffer: 10 mM Pipes, pH 6.8, 100 mM NaCl, 300 mM sucrose, 3 mM MgCl2, 1 mM EGTA, 4 mM vanadyl riboside complex, 1.2 mM phenylmethylsulfonyl fluoride, and 0.25% (vol/vol) Triton X-100. For the production of ascites fluid, Balb/C mice were injected at 2-wk intervals with 100-400 µg of HeLa nuclear matrix proteins in the Ribi Adjuvant System (Ribi Immunocor Biociences, Hamilton, MT), which is a mixture of monophosphoryl lipid A and trehalosedimycolate. Mouse serum obtained from immunized animals was used as a positive control. The cells from the nonsecreting mouse myeloma line SP2/0-Ag14 were cultured in RPMI 1640 medium containing 10% (vol/vol) FBS and 2 mM L-glutamine.

mAb Generation

Mouse myeloma cells and hybridoma cells were cultured in RPMI 1640 containing 10% (vol/vol) FBS. Babl/C mice were injected at 2-wk intervals with 100-400 µg of HeLa nuclear matrix proteins in the Ribi Adjuvant System (Ribi Immunocor Biociences, Hamilton, MT), which is a mixture of monophosphoryl lipid A and trehalosedimycolate. Mouse serum obtained from immunized animals was used as a positive control. The cells from the nonsecreting mouse myeloma line SP2/0 Ag14 were cultured in RPMI 1640 medium containing 10% (vol/vol) FBS and 2 mM L-glutamine.

Western Blot Analysis of the H1B2 Antigen

HeLa cells were fractionated by the sequential procedure described above. Fractions with either 0.25 M ammonium sulfate or 2 M NaCl were dialyzed against PBS and nuclear fractions were treated with Benzonase (E. Merck) to remove interfering nucleic acids. Proteins were separated by SDS-PAGE (Laemmli, 1970) before semi-dry transfer to HyBond ECL nitrocellulose (Amersham Corp., Arlington Heights, IL) for 1.5 h at 20 V in 48 mM Tris, 39 mM glycine, 0.13 M sodium deoxycholate, and 20% methanol.
Nickerson et al. Relocalization of a Nuclear Matrix Protein at Mitosis

Cells were grown on glass slides. They were extracted as described above before fixation with either 3.7% formaldehyde or 3.7% para-formaldehyde in PBS for 30 min at room temperature. These extracted and fixed cells were washed with PBS and blocked with 10% (vol/vol) goat serum, 10% (vol/vol) FBS, or 2% (wt/vol) BSA in saline for 30 min at room temperature. Staining with monoclonal first antibodies and rhodamine-conjugated goat anti-mouse IgG plus IgM plus IgA (Cappel-Organon Teknika Corp., West Chester, PA) was done at room temperature in saline containing 1% normal goat serum, except when the mAb was present in culture supernatants. After staining, coverslips were mounted with Aquamount mounting medium (Lerner Laboratories, Pittsburgh, PA) before viewing with both epifluorescence and phase contrast optics.

Results

The HIB2 mAb was prepared using mice immunized with purified nuclear matrix proteins from HeLa (CCL2.2) cells. Hybridomas were screened first by ELISA using isolated HeLa nuclear matrix proteins, and then by immunofluorescent staining of HeLa (CCL2) cells grown on microscope slides. Hybridoma clones producing ELISA-positive antibodies with interesting fluorescence patterns were selected for further cloning and study.

HIB2, an IgM, was chosen for further study because it reacted strongly in ELISA assays and, under appropriate conditions, selectively stained the nuclear matrix of HeLa cells. The antigen, present in many different human epithelial cell lines, showed a striking redistribution during mitosis (to be discussed below). While many nuclear matrix proteins are specific to cell type and stage of differentiation, others are invariant from cell to cell (Fey and Penman, 1988; Stuurman et al., 1989; Stuurman et al., 1990; Dworetzky et al., 1990). The HIB2 antigen has been found in HeLa, MCF-7, CaSki, SiHa, and W12 cells and may be a universal component of the basic nuclear matrix.

Identification of the HIB2 Antigen

Western blots of HeLa cell fractions showed that the HIB2 antigen was a nuclear matrix protein of ~240 kD (Fig. 1). A small fraction of this protein was removed with the chromatin by DNase I digestion and 0.25 M ammonium sulfate extraction (Fig. 1, lane 3). More was removed by 2 M NaCl extraction of the complete nuclear matrix (Fig. 1, lane 4), but most of the high molecular weight HIB2 antigen was retained with the HeLa core filament network (Fig. 1, lane 5). The location of the HIB2 antigen was entirely nuclear as shown by immunofluorescent light microscopy and immunogold EM (Figs. 2-4). Staining cells at different stages of our sequential fractionation confirmed the results of Western blots. The antigen was primarily in the nuclear matrix fraction and not in the chromatin. Furthermore, the antigen appeared largely associated with the core filament subfrac-
Figure 2. Immunofluorescence localization of HIB2 in CaSki cell interphase nuclei. All the immunofluorescence photomicrographs were taken and printed at the same exposure to make quantitative comparison possible. (a) When cells were extracted with 0.5% Triton X-100 in cytoskeletal buffer the nuclei were only slightly stained. (b) To uncover the nuclear matrix, chromatin was removed from 0.5% Triton X-100 extracted cells by digestion with DNase I and extraction with 0.25 M ammonium sulfate. After this treatment, HIB2 stained the nucleus with a punctate pattern. (c) Cells extracted to reveal the complete nuclear matrix as in part b were further extracted with 2 M NaCl to uncover the network of core filaments (He et al., 1990). The pattern and intensity of HIB2 immunofluorescence was unchanged in most cells. A few cells had less intense, finer, and more dispersed spots of fluorescence.

Immunofluorescence Localization of the HIB2 Antigen in Interphase Nuclei

The HIB2 antibody did not stain the nuclei of unextracted cells since the antigen is masked during interphase. Extraction of cells with 0.5% Triton X-100 removed soluble proteins but did not unmask the nuclear antigen (Fig. 2 a). Only a low intensity, uniform nuclear fluorescence was detected. Occasional nuclei had a more intense fluorescence in a single spot or a small region of the nucleus. As shown below these are probably cells nearing mitosis.

The HIB2 epitope was revealed by the removal of chromatin. The cells were digested with DNase I and extracted with 0.25 M ammonium sulfate. This procedure removed essentially all of the chromatin leaving only the nuclear matrix in the nuclear interior (Fey et al., 1986; He et al., 1990). Fig. 2 b shows the punctate staining pattern of the HIB2 antigen in the nuclear matrix prepared in this way.

This complete nuclear matrix can be separated into two distinct parts. The thick nuclear matrix fibers are comprised of a complex set of proteins, some of which are cell type specific. These are assembled around a highly branched network of 9- and 13-nm core filaments which may serve as cores in the thick filaments. In a previous report (He et al., 1990), we showed that extracting the complete nuclear matrix with 2 M NaCl removes most of the outer proteins of the interior nuclear matrix, revealing the inner network of core filaments. This filament fraction, with a relatively simple protein composition, contains nearly all of the nuclear RNA. There are at least two components, the filament network itself and dense masses of fibrogranular material enmeshed in the filaments. These dense masses contain the hnRNP proteins and may be the location of hnRNA.

Much of the HIB2 antigen was associated with the nuclear matrix core filament network. The percentage remaining after 2 M NaCl extraction to uncover the core filaments varied with cell type. In MCF-7 cells about half of the total fluorescence remained in the core filaments while in CaSki cells the core filaments retained nearly all the fluorescence (Fig. 2c). In HeLa cells, as shown by Western blotting (Fig. 1), most of the antigen is retained with the core filaments. Immunoelectron microscopy (Fig. 4) of CaSki cells extracted to reveal the nuclear matrix core filaments showed that the HIB2 antigen was associated principally with the granular material enmeshed in the core filament network and not with the core filaments themselves.

Immunoelectron Microscopy HIB2 Antigen at Interphase

The conventional technique for high resolution localization of structural proteins by EM is to stain epon or Lowicryl embedded thin sections with antibodies. However, while traditional embedded-section EM is an adequate technique for viewing organelles and membrane systems which are best seen in cross-section, it is not suitable for studying the cytoskeleton or nuclear matrix. In embedded sections only the stained surface of the section is seen; the filaments of the structural networks appear only in cross-section unless they
are both close to and parallel to the surface of the section, something which occurs rarely.

Sample preparation techniques developed in this laboratory are better suited for electron microscopy of the filamen-

tous skeletons of detergent extracted cells (Capco et al., 1982). In this procedure, adapted from that of Wolosewick (1978), extracted cells are embedded in the removable resin diethylene glycol distearate, sectioned and the embedding

Figure 3. Stereo electron micrograph of HIB2 immunolocalization in the nuclear matrix of W12 cervical dysplasia cells. W12 cells were extracted with 0.5% Triton X-100 in cytoskeletal buffer and their chromatin was removed by DNase I digestion and 0.25 M ammonium sulfate extraction. After fixation, the matrix preparation was treated with HIB2 antibody and then with a colloidal gold-coupled second antibody. This resinless section shows part of the nucleus bounded by the nuclear lamina (L). The thick fibers of the nuclear matrix were strongly decorated with 5-nm gold beads showing the location of the HIB2 antigen. The cytoskeleton, consisting principally of intermediate filaments (IFs), was not stained. Bar, 0.2 μm.

Figure 4. HIB2 distribution in the network of nuclear matrix core filaments as seen in immunostained resinless sections. The low magnification electron micrograph (a) of a CaSki cell nuclear matrix made by the DNase I-0.25 M ammonium sulfate method which was further extracted with 2 M NaCl to reveal the network of 10 nm core filaments. A higher magnification view of the same cell is shown in b. The double arrowheads point to residual masses that were retained with the network and stained heavily with HIB2 antibody and second antibody conjugated 5-nm gold beads. Single arrowheads show the 10-nm core filaments which were, with few exceptions, devoid of stain. The core filament network was connected to the nuclear lamina (L) which also anchored the intermediate filaments of the cytoskeleton (IF). The marked rectangle in panel a shows the position of the higher magnification view shown in b. Bars: (a) 0.2 μm; (b) 0.1 μm.
Figure 5. The dynamic redistributions of the HIB2 antigen through mitosis in CaSki cells. CaSki cells were synchronized by single thymidine block and were extracted with 0.5% Triton X-100 in cytoskeletal buffer before fixation and immunofluorescent staining. The pairs of micrographs illustrate different mitotic stages seen in immunofluorescence and in phase contrast. Cells in interphase which were extracted in this way had very weak nuclear staining (Fig. 2 a). Only removal of chromatin by the DNase I-0.25 ammonium sulfate procedure uncovered the antigen (Fig. 2 b). As seen in this figure, the HIB2 antigen was uncovered in mitotic cells without this extraction. (a) Cells approaching prophase had only a single small area of the nucleus stained. (b) At a more advanced premiotic stage cells had more nuclear staining. There was a range of different staining patterns from cells showing only a single nuclear spot to cells with staining of larger nuclear areas to cells with staining over the whole nucleus. (c) In prophase, as chromosomes began to condense, the labeling was very intense throughout the whole nucleus. Note the strong staining of the aster to the left of the nucleus. (d) At prometaphase there was intense HIB2 staining in the area around the condensed chromosomes. The staining was not exactly coincident with the chromosomes. While the chromosomes appeared to form a halo, there was HIB2 staining in the center where chromosomes were absent. (e) In metaphase, as the sets of chromosomes lined up at the metaphase plate, intense HIB2 staining could be seen around the chromosomes. The area around the centriole at the spindle poles was stained and there was a punctate staining in the cytoskeletal region. (f) In anaphase cells the perichromosomal region retained a high degree of stain. The punctate cytoskeletal staining remained. (g) In cells at cytokinesis there was HIB2 staining in the reforming daughter nuclei. The punctate cytoskeletal staining was stronger than at earlier mitotic stages; there were fewer but larger and more intensely stained spots. The midbody between the daughter cells stained intensely.
Figure 6. High resolution localization of HIB2 antigen in the perichromosomal region of an anaphase cell. CaSki cells were extracted with 0.5% Triton X-100 in cytoskeletal buffer before fixation, immunogold staining with HIB2 and a 5-nm gold bead–conjugated second antibody, and resinless section EM. The chromosomes (Chr) were enmeshed in a fibrogranular network (double arrowheads) which was intensely stained with the HIB2 antibody. Spindle microtubules, connected to the chromosomes and marked by arrows, were unstained except where they connected to the fibrogranular network. Note the fine detail of the microtubule–chromosome connections at the kinetochore afforded by the resinless section technique. The insert shows the same cell at lower magnification with a rectangle marking the location of the higher magnification view. Bar, 0.2 μm.

Unmasking of the HIB2 Antigen as Cells Approach Prophase

A previous study (Chaly et al., 1984) demonstrated that nuclear matrix proteins redistributed in a variety of patterns during mitosis. Some were associated with the chromosomes at mitosis, including one on the periphery of the chromosomes, while others dispersed throughout the cytoplasm.

The behavior of HIB2 antigen, studied in synchronized CaSki cells, was unlike that of any previously described nuclear matrix protein. First, the normally masked antigen became increasingly accessible as the cell approached mitosis. During interphase, the HIB2 antigen could be stained only after DNase I digestion and 0.25 M ammonium sulfate extraction had unmasked the antigenic site. As cells approached prophase, the antigen became increasingly exposed and could be immunostained without salt extraction (Fig. 5). The antigen was first seen in one small region of the nucleus (Fig. 5a). The position of the HIB2 staining did not correspond to the nucleoli, as seen in the corresponding phase-contrast image, and may represent a previously uncharacterized premitotic nuclear structure.

In a few cells a second HIB2 spot appeared (Fig. 5b) and in some cells the fluorescence had spread to larger regions of the nucleus. Finally, regions of HIB2 immunofluorescence filled the whole nucleus. Later in mitosis, when the chromosomes condensed in prophase, as seen by phase-contrast mi-
croscopy, the HIB2 staining surrounded but was not limited to the chromosomes (Fig. 5 c). This was more clearly seen in the prometaphase CaSki cell of Fig. 5 d which has a ring of condensed chromosomes and an empty central region. The HIB2 immunofluorescence was present in the central region as well as in the doughnut of surrounding chromosomes, so even at this low resolution the antigen surrounded but was not coincident with the chromosomes. The asters, precursors of the centrioles, were stained with HIB2. This is clearly seen in the prophase cell of Fig. 5 c. Pericentriolar staining persisted throughout the rest of mitosis.

Redistribution of the HIB2 Antigen during Mitosis: Identification of Perichromosomal Filaments

At metaphase, as the chromosomes assembled at the metaphase plate, the HIB2 immunofluorescence appeared to surround the chromosomes (Fig. 5 e). Some antigen was also present in many small, bright specks throughout the cell. This punctate material must be bound to the cytoskeleton since the extraction with 0.5 % Triton X-100 had not released it.

As the chromosomes separated in anaphase, HIB2 fluorescence separated into two distinct perichromosomal regions with an unstained space between the separating daughter chromosomes (Fig. 5 f). The images suggest that the antigen containing material moved with the two daughter sets of chromosomes. At cytokinesis (Fig. 5 g), as the two daughter nuclei were reforming, HIB2 was distributed throughout the new nuclei, but small patches of fluorescence remained associated with the cytoskeleton. These may have derived from the more numerous and less intensely staining cytoplasmic spots of the metaphase cell (Fig. 5 e). The smaller spots of the metaphase cell may have coalesced into the larger, more intensely stained spots of the anaphase cell. A striking example of the relocation of HIB2 at mitosis was the bright fluorescence that remained in the midbody, the last connection between the daughter cells.

Immunofluorescence light microscopy showed HIB2 staining around, but not exactly coincident with, the chromosomes (Fig. 5). When seen at the higher resolution made possible by EM (Fig. 6), the localization of HIB2 in the perichromosomal region of mitotic cells was both on and around the chromosomes. The chromosomes were surrounded by a fibrogranular network containing the HIB2 antigen. The filaments of the fibrogranular material were ~9–14 nm in diameter and were clearly distinct from the microtubules of the spindle apparatus. These CaSki cells were treated with 5 μg/ml taxol before and during extraction to preserve the spindle microtubules. The HIB2 antigen was not associated with those microtubules but was, instead, located in the fibrogranular network surrounding the chromosomes and occasionally at the surface of the chromosomes. The absence of the HIB2 antigen in the microtubule spindle was verified by immunostaining HeLa cells with rabbit anti- tubulin and monoclonal mouse HIB2 antibodies. The tubulin and HIB2 staining patterns were not coincident.

The fibrogranular network containing the HIB2 antigen (Fig. 6) consisted, in part, of 9–14 nm filaments which interconnected adjacent chromosomes and were occasionally decorated with the HIB2 antibody. A similar network of filaments and associated granular material surrounded the centrioles (Fig. 7). This network was also stained by the HIB2 antibody. This fibrogranular material may comprise the pericentriolar "cloud" seen indistinctly in the conventional epon-embedded section.
Immunofluorescence light microscopy showed HIB2 staining in the midbody between daughter cells at cytokinesis. The midbody, connecting cells, contains remnants of the pole to pole spindle fibers and, as shown by the stereoscopic micrograph of Fig. 8, also contained thinner filaments of ~12-17 nm containing Hlb2. The stereoscopic view shows clearly that these filaments are distinct from the microtubule bundles passing through the midbody and represent a previously undetected mitotic structure.

**Altered Lability of HIB2 at Mitosis**

The appearance of HIB2 immunofluorescence in unextracted nuclei was an unambiguous sign of approach to mitosis. This emergence of the HIB2 epitope appeared to reflect a found reorganization of cell architecture and not simply a conformational change of HIB2 itself. Another observation supports this view; much of the HIB2 antigen became extractable with 0.25 M ammonium sulfate, even without a DNase I digestion, as cells approached mitosis. Later in mitosis, extraction of CaSki cells with 0.25 M ammonium sulfate removed the fluorescence from the chromosome region, even without a prior DNase I digestion. Not all of HIB2 was removed by this salt treatment; some remained specifically at the midbody and in the pericentriolar filaments near the spindle poles.

**Discussion**

Nuclear matrix architecture is complex; its characterization
will require the coordinated use of microscopic, biochemical, and immunological techniques to reveal the organization of the cell nucleus. These same techniques, applied to mitotic cells, may detect previously unseen structural networks that facilitate the disassembly and reassembly of non-chromatin nuclear structures at mitosis.

Our approach to nuclear matrix characterization is to generate mAbs against nuclear matrix proteins and to use those antibodies to characterize the antigen. The antibodies also serve to localize the antigen in the cell by immunofluorescence microscopy and, at much higher resolution, by immunogold EM of resinless sections. Conventional EM can show little of the nuclear interior and there were previously no completely satisfactory methods for fractionating the nucleus. The more recently developed techniques of embedment-free EM and nuclear fractionation (Capco et al., 1982, 1984; Fey et al., 1986, He et al., 1990) have given us a clearer understanding of nuclear matrix structure and composition. More recently we have used antibodies and colloidal gold-conjugated second antibodies to label nuclear proteins in resinless sections of extracted cells (Nickerson et al., 1990). This report describes our first application of this combination of techniques for the study of nuclear architecture.

The 240-kD HIB2 protein, normally masked in the detergent-extracted interphase nucleus, was uncovered and made accessible to the antibody either by digestion with DNase I and extraction with 0.25 M ammonium sulfate or by entrance into mitosis. Once the nuclear matrix had been exposed by DNase I digestion and 0.25 M ammonium sulfate extraction, it stained with the HIB2 antibody in a speckled pattern. The mechanism by which the DNase I- 0.25 M ammonium sulfate treatment unmasked HIB2 is unknown. It seems likely that the extraction removed structural components that normally cover the HIB2 antigen.

The rearrangements of the HIB2 antigen with the cell cycle may show the redistribution of the nuclear matrix during mitosis. The HIB2 antigen gradually became unmasked and could be stained without the removal of chromatin as the cell approached prophase. The structural rearrangements preparing the cell for mitosis were also uncovering the antigen. Fluorescence appeared first in a small, single region of the nucleus and later spread throughout the entire nucleus. In some cells a second spot was seen, but whether this is a normal or requisite stage is not known.

When the nuclear envelope disassembled into vesicles and the nuclear matrix rearranged at prometaphase, the HIB2 protein was found in a fibrogranular network surrounding the chromosomes and in a pericentriolar structure surrounding the mitotic spindle pole. This latter fibrogranular structure can be seen in conventional embedded sections only as a pericentriolar "cloud". Previous reports from this laboratory (Capco and Penman, 1983; Wagner et al., 1986) have described nonmicrotubular filaments associated with the spindle. The HIB2 antigen was associated with extensive perichromosomal and pericentriolar networks containing thin filaments and granules, providing the first clue as to their composition.

As the cell began cytokinesis HIB2 fluorescence was, as expected, seen in the reforming daughter cell nuclei. More surprising was the HIB2 staining of the midbody. The midbody consists of at least 35 proteins (Mullins and McIntosh, 1982). Some midbody proteins are associated with other mitotic structures before telophase. A mAb raised against a mitotic scaffold fraction (Cooke et al., 1987) stained peptides of 135 and 155 kD which relocated from the chromosome scaffold to the midbody during telophase. Two proteins of CHO cells, with molecular masses of 95 and 105 kD, are located throughout the spindle, although at higher concentrations in equatorial regions, and these become midbody proteins at telophase (Sellito and Kuriya, 1988).

The protein detected by the HIB2 mAb is a component of the nuclear matrix. The presence of the HIB2 antigen in the matrix was shown by Western blots (Fig. 1), immunofluorescence light microscopy (Fig. 2), and immunogold EM (Fig. 3). Furthermore, the HIB2 antigen was retained with the network of 9 and 13 nm core filaments that underlies the complete nuclear matrix (He et al., 1990). Treatment of the complete nuclear matrix with 2 M NaCl leaves most of the HIB2 protein with the core filaments in CaSki and HeLa cells. Western blotting of HeLa fractions with the HIB2 mAb reproducibly detected a band at 240 kD (Fig. 1). This high molecular protein is present in all nuclear fractions, but principally in the core filament network (Fig. 1, lane 5).

The characterization and localization of structural nuclear proteins will provide important information about nuclear matrix architecture and function. These studies will also yield useful insights into the ultrastructural dynamics of the cell in mitosis.

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