RESULTS
Results

In the present study the nuclear proteins were fractionated from whole brain (B), neuronal (N) and glial (G) nuclei using the procedure of Thompson (1973). The nuclei were subjected to nuclease digestion (DNase 1 and RNase A) for brief periods of time as indicated in the methods and were resolved on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels (Fig. 1). The pattern in general showed specific association of proteins with neuronal and glial fractions. In the glial fraction a protein with a molecular weight approximately 50 kDa (possibly GFAP) and a 32 kDa protein were found enriched, while another protein with 198 kDa was enriched in the neuronal fraction which could possibly be one of the members of neurofilamental triplet proteins (NFTs) basing on its molecular weight. The proteins enriched in neuronal and glial fractions could be observed together in whole brain fraction, which constitutes the nuclear protein fraction from the whole brain. The sodium dodecyl sulfate polyacrylamide gel electrophoretic pattern in overall suggests that, the total nuclear protein from whole brain fraction can be very clearly fractionated into neuron and glial fractions. The protein pattern in all these fractions matches with the pattern shown by Thompson (1973). Further, it indicates the presence of a heterogeneous type distribution of nuclear proteins in different cell types of brain.

The nuclear matrices were prepared from neuronal and glial nuclei by two different ways as described in the methods. Following the steps involved in the protocol the nuclear matrices were prepared using high salt method (HSM) and low salt method (LSM) as suggested and the matrices were subjected to the analysis by scanning electron microscopy (SEM) and biochemical characterization. The data from the scanning electron...
microscopic studies (Fig. 2) suggest that nuclear matrix preparation whether by high salt method or low salt method do not show any morphological variation even though studies by others (Verheijen et al. 1988) using transmission electron microscopy (TEM) have shown wide variety of changes in the constitution of nuclear matrix preparation by high salt method and low salt method. The scanning electron microscopic pictures of nuclear matrix isolated by high salt method or low salt method showed that the shape of the nucleus was not altered even though most of its constituents were extracted while preparing the matrices, which agrees with earlier observations.

Fig. 3 shows the protein composition of nuclear matrix in neuronal and glial nuclei. The sodium dodecyl sulfate polyacrylamide gel electrophoresis pattern showed slight variation which can mainly be attributed to the method of nuclear matrix preparation. In general, low salt method preparation yielded a matrix having relatively less protein composition than by high salt method. However, lamins were retained irrespective of high salt method or low salt method preparation of nuclear matrix. Since, the neuronal and glial fractions and the matrix protein composition in high salt and low salt methods suggested wide variations, a comparison of age dependent variations in nuclear proteins was done by isolating the nuclei from the brains of rats aged 10, 45 and 180 days. Further, the differences were not really obvious when analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Therefore, the proteins were resolved on two dimensional electrophoresis (2DE) analysis, which involves separation of proteins by isoelectricfocussing (IEF) as well as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).
Earlier studies by others also showed that the high salt and low salt extracted matrices contain 6.5 and 1.9% of protein respectively of the corresponding nuclear proteins suggesting that the nuclear matrices prepared by low salt method are relatively depleted structures compared to high salt extracted matrices (Smith et al. 1987). This is because of the methodological variations such as the presence of lithium 3, 5-diiodosalicylate, a chaotropic agent which allows stabilization of nuclear matrices. The data suggests that nuclear matrix preparation by low salt method (LSM) involving lithium diiodosalicylate (LIS) extraction efficiently depletes the non-histone chromosomal proteins (NHCPs) from the nucleus.

Neuronal and glial non-histone chromosomal proteins were analyzed from the brains of 10, 45 and 180 days aged rats. In Fig. 4. the top panels A, B and C show the pattern of non-histone chromosomal proteins from the neuronal cells. In over all the data suggest, that by the time the rat ages (development reaches) 45 days there are many proteins which seem to have been translocated into the nuclei. The 45 day samples accrue many number of peptides below the molecular weight range of 36 kDa. By the time the rat is 180 days old; even though the low molecular weight proteins are retained, it is observed in general that 180 day neuronal nuclei contain relatively less number of proteins than 45 days sample. The panels D, E and F show the pattern of non-histone chromosomal proteins of glial nuclei. The non-histone chromosomal protein patterns of 10 and 45 days samples of glial nuclei do not show much variation, excepting for a slight quantitative increase in very few proteins. The number of non-histone chromosomal proteins between 10 and 45 days old samples remain almost unchanged. On the controversy, the 180 day old samples show up with many new proteins, which appear to be translocated into the glial nuclei of 180 days rat.
The nuclear matrices prepared from 10 and 45 day old neurons by low salt method and high salt method are shown in Fig. 5. At the age of 180 days most of the nuclear matrix proteins contained lamins A, B and C. More or less similar features are seen in case of 10 day old samples also. But the lamins were very faintly expressed in the nuclear matrix prepared by low salt method of 10 day old neurons. The non-lamin proteins associated with nuclear matrix were in general more at the age of 180 days, particularly in the nuclear matrix prepared by low salt method. It is rather intriguing to explain more number of nuclear proteins associated with matrix as the lithium diiodosalicylate extraction is known to deplete most of the proteins.

Fig. 6. represents nuclear matrix proteins from the glial cells of 45 and 10 day old rat. Similarly, the 180 day old rat neuronal and glial nuclear and nuclear matrix proteins pattern were shown in Fig. 7. In the nuclear matrix prepared by high salt method and low salt method the lamins A, B and C could be identified in 45 days as well as 10 day samples. However the lamin association with the nuclear matrix in glial cells appears to be rather selective. In the high salt method preparation of 180 day old glia, lamin B and C alone are seen but not A, while in low salt method sample appears to contain only lamin B. On the other hand the high salt method samples of 10 day glia contain all the lamins but the low salt method samples contain lamins A and C but not B. Apart from the selective association of lamins to the nuclear matrix the low salt method and high salt method preparations contain several other matrix associated proteins as can be noticed from the figure. In general the lamins were more associated with high salt extracted matrix when compared to the low salt extracted matrix of glia. Though in 10 day old preparation of high salt method lamin A, B and C could be noticed, in 45 days old sample only B and C could be identified. In low salt method preparations lamin A or B alone could be identified; suggesting that low
salt method preparation might possibly deplete selective lamins. In addition to the alterations in the lamin composition, the non-lamin proteins associated with matrix appear to be differing in high salt method and low salt method, which is an interesting aspect in the present study. Since the matrix composition was shown to vary by the age, the method (high salt and low salt) and in different cell types (neurons and glia), only 45 days aged rats were chosen for further study. Since most of the proteins were in acidic range, the diethylaminoethyl (DEAE) cellulose column chromatography was employed to separate the matrix proteins.

In an attempt to fractionate nuclear matrix proteins, the nuclear matrix proteins were solubilized in 5 M urea and subjected to DEAE cellulose chromatography. The proteins were eluted with increasing concentrations of NaCl in a step gradient of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 M. The proteins in the eluates were precipitated with acetone after extensive dialysis. The protein fractions resolved from high salt matrix preparation of neurons after DEAE are shown in Fig. 8, while low salt matrix preparation of neurons subjected to DEAE chromatography under similar condition are shown in Fig. 9. The chromatograms at optical density at 280 nm peaks during step wise gradient elution shown in panel A. The peak fractions, when analyzed in sodium dodecyl sulfate polyacrylamide gel electrophoresis showed 2 to 3 bands; but showed several bands on two dimensional electrophoretic analysis. The protein patterns from different fractions of high salt method showed mostly lamins, while the low salt method preparation yielded a better resolved protein separation in the sense that it had more of non-lamin proteins separated particularly in 0.4 M and 0.6 M elutes. Since most of these proteins were present in small amounts, the fractions were pooled and analyzed by two dimensional electrophoresis followed by silver staining. Though the nuclear matrix proteins particularly in low salt method preparation could be separated through DEAE
column, the step wise gradient elution failed to yield a fraction which is entirely homogeneous and made of a single protein. Consequently, most of the proteins were resolved in groups and could be identified in two dimensional gel electrophoretic gels in Fig. 8.

The high and low salt matrix proteins from glial cells were subjected to DEAE chromatography under similar conditions as described for neuronal matrix preparation. The DEAE chromatograms for glial high salt method (Fig. 10) and glial low salt method (Fig. 11) indicate that the 0.6 M fraction eluated from glial high salt method preparation contain lamins and very low molecular weight proteins which could possibly be some small ribonuclear proteins (RNPs) basing on their positions in a two dimensional electrophoresis (2DE). Though the small ribonuclear proteins were separated as early as 0.2 M, their separation was complete only in 0.6 M NaCl buffer. The 1.0 M eluate still showed up few lamins.

The low salt method preparation of glia during DEAE cellulose chromatography yielded fractions having a heterogeneous group of proteins which look like high mobility group (HMG) type, most of them were in high molecular weight range. The results from the DEAE cellulose chromatography of glial high salt and low salt matrix preparations indicate that like in the case of neuronal matrix preparation glial high salt method preparation yielded a protein separation, which is better resolved in low molecular weight range. Conversely, the glial low salt method preparation yielded chromatograms having high mobility group type of proteins associated with the nuclear matrix. These results indicate that the separation of nuclear matrix proteins by DEAE column chromatography differs depending on high salt and low salt preparation of matrices and the composition of proteins separated by DEAE chromatography differs quantitatively as well as qualitatively.
Since the DEAE chromatography was rather unsuccessful in terms of separating nuclear matrix proteins, the preparation was further subjected to high performance liquid chromatography (HPLC) on a Shimpak PA-DEAE column of Shimadzu SCL-6AV using binary linear gradient elution at a pH gradient of 2.6-7.0 with the buffer system citric acid and sodium phosphate. The peak fractions were collected and the protein was precipitated with acetone and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. Patterns of high performance liquid chromatography fractions for high and low salt preparation have been shown in Fig. 12 (HPLC profiles of neuronal nuclear matrix proteins prepared by high and low salt method). The high performance liquid chromatography even though has yielded separation into peaks during binary linear gradient elution the sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of these fractions very clearly indicated that each of these peaks contain groups of proteins, most of them concentrated at high molecular weight range. An exactly similar kind of patterns were obtain from the high and low salt nuclear matrix preparation from glial cells as shown in Fig. 13. The high performance liquid chromatographic separation pattern also supports the results obtained in DEAE chromatography that the matrix proteins can not be resolved into individual protein by DEAE chromatography and therefore a combination of approaches have to be formulated in order to separate the matrix proteins.

Since most of the nuclear matrix proteins are implicated to be associated with replication forks during DNA replication, an attempt was made to identify single strand deoxyribonucleic acid (ssDNA) binding proteins on single strand DNA cellulose column. The single strand DNA binding of neuronal nuclear matrix proteins extracted by low salt method (Fig. 14) were eluted with increasing concentration of NaCl in a step
gradient of 0.1, 0.2, 0.4, 0.6 and 1.0 M and the proteins in the eluates were precipitated with acetone and solubilized in O'Farrel buffer and subjected to two dimensional electrophoresis. The 0.1 and 0.2 M fractions had groups of proteins while 0.4 M fraction did not show up any proteins. On the other hand 0.6 and 1.0 M fraction had several groups of proteins. Usually in a DNA cellulose chromatography, proteins eluted between 0.2 to 0.8 M are suspected to involve in regulatory function, while proteins eluted above 1.0 M might involve in structural organization of chromatin. In the present study presence of several groups of proteins in 0.6 and 1.0 M fractions of neuronal nuclear matrix proteins extracted by low salt method suggest that most of the nuclear matrix proteins might have a regulatory role.

Differing with this pattern, the DNA binding nuclear matrix proteins of glial cells showed (Fig. 15) almost no protein in fractions of above 0.4 M NaCl. The lamins were eluted at 0.2 M ionic strength itself and further elution yielded not many proteins unlike in the case of neuronal nuclear matrix prepared by low salt method with respect to single strand DNA binding protein. Though a clear function can not be attributed it can be speculated that neurons being non-replicating cells, most of the single strand DNA binding proteins of neuronal nuclear matrix prepared by low salt method observed at 0.6 and 1.0 M elutes might possibly involve in DNA repair or recombination and transcription. Under the condition of single strand DNA cellulose chromatography the results very clearly show that the neuronal nuclear matrix proteins prepared by low salt method were found to bound to single strand DNA, significantly better than the glial nuclear matrix proteins prepared by low salt method.

The immuno cross reactivity of different antibodies was checked by Ouchterlony (Fig. 16) and further by western analysis. The nuclear and matrix proteins have been separated on sodium dodecyl sulfate poly-
acrylamide gel electrophoresis and transferred on to nitrocellulose paper, which was subjected to western blotting analysis using different polyclonal antibodies as probes.

Anti serum to total nuclear matrix proteins of NHSM (neuronal nuclear matrix proteins extracted by high salt method), NLSM (neuronal nuclear matrix proteins extracted by low salt method), GHSM (glial nuclear matrix proteins extracted by high salt method) and GLSM (glial nuclear matrix proteins extracted by low salt method) was produced in rabbits by injecting total nuclear matrix protein. The cross reactivity of all the four antibodies (NHSM-antibody, NLSM-antibody, GHSM-antibody and GLSM-antibody) is shown in Fig. 17. The panel-A shows the immune reactivity of NHSM-antibody with all the nuclear and nuclear matrix preparations. In general, the antibodies raised for low salt extracted nuclear matrix preparations of neuronal and glia showed relatively intense immune reactivity. On the other hand, the high salt extracted nuclear matrix antibodies showed reactivity with polypeptides having a molecular weight range from 25 to 120 kDa. Further, an interesting feature was observed by the low salt matrix antibodies of neuronal as well as glia (Panel-B and D in lanes 4 and 5). Low salt matrix antibodies react with a polypeptide around 70 kDa, which could not be identified in the immunoblots treated with high salt matrix antibodies of neuronal and glia. The immunoblots further suggest the existence of a common group of nuclear matrix proteins irrespective of the cell type and method of matrix preparation. However, the identification of specific polypeptide reactivity with low salt matrix antibodies indicates a possibility for the presence of either cell or / and matrix method dependent enrichment of specific matrix proteins in the nuclei. This data agrees with the earlier reports in the literature (Stuurman et al., 1990 ; Dworetzky et al., 1990).
The tissue specific and brain region specific immune reactivity of the four antibodies is shown in Fig. 18. Considering the regional heterogeneity of the brain, the different regions of the brain (cerebellum, brain stem, cerebral cortex, hippocampus and mid brain), the brain stem showed very high reactivity, whereas mid brain showed least reactivity. In all the five different regions of the brain, three major NHSM reactive polypeptides with a molecular weight 105, 38 and 20 kDa were identified. Cerebral cortex, brain stem and hippocampus possessed another protein of 50 kDa. The NHSM antibodies were least reactive with non-neuronal tissues examined in the present study (such as liver, heart, kidney, muscle and lung). Kidney showed very faint immune reactivity particularly with a polypeptide around 80 kDa, while heart also showed a very minor protein around 70 kDa.

The NLSM antibody was reactive with a polypeptide around 38-40 kDa from all the regions of the brain. This polypeptide further showed intense reactivity with brain stem and hippocampus rather than other regions of the brain. However the NLSM antibodies did not react with any other polypeptides either from the brain or from other tissues examined.

The GHSM antibodies showed an immune reactive pattern which is almost similar to that of NHSM antibody with minor variations. The variations include the 20 kDa polypeptide seen in NHSM was not reactive with GHSM antibody. The 80 kDa polypeptide band was more intense in GHSM (in contrast to NHSM). The tissue pattern showed the presence of 90 kDa protein in heart more prominently than in NHSM. More than that appearance of 40 kDa protein in heart also observed in GHSM. Three different proteins (90, 80, 75 kDa) in muscle and 90 kDa protein in lung were noticed.
The GLSM antibodies showed an immune reactive pattern which is close to the pattern of NLSM antibodies. The 90 kDa protein band was reactive in brain stem, hippocampus and very faintly in cerebral cortex. There was no immune reactivity with the other tissue proteins.

The immunological data using NHSM and GHSM antibodies show that there is a regional heterogeneity of nuclear matrix protein distribution with in the brain mostly with two different categories of matrix proteins such as a large fraction of common nuclear matrix proteins and a small fraction of very specific nuclear matrix proteins. This diversity also is extended to non-neuronal tissues as very few polypeptides reactive to NHSM and GHSM antibodies are identified from other tissues such as heart, kidney and muscle. The NLSM and GLSM antibodies were more specific and limited in their distribution. They were reactive and present in almost all regions of brain but specific only to brain and these are either not reactive or atleast reactive with non-neural tissues, suggesting that the NLSM and GLSM antibodies are almost specific polyclonal antibodies to brain.

Using these polyclonal antibodies, the cross reactivity was checked with different cancer cell lines of neuronal as well as non-neuronal origin. The sodium dodecyl sulfate polyacrylamide gel electrophoretic pattern of neuronal nuclear, C6, SK-N-SH, U-373MG, IMR-32 and Neuro-2A is shown in Fig. 19. The C6 and U-373MG are of glioblastoma in origin while SK-N-SH and Neuro-2A are Neuroblastoma, IMR-32 is a neuroblastoma mixed with fibroblast. The sodium dodecyl sulfate polyacrylamide gel electrophoretic analysis showed a different pattern in IMR-32 compared to other cell lines. The nuclear proteins from all these cell lines were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoretic analysis and transferred on to an immunoblot in quadruplicates. These blots were probed with all the four different antibodies
(NHSM-Ab, NLSM-Ab, GHSM-Ab and GLSM-Ab) (Fig. 20). The NHSM and NLSM antibodies showed no reactive species of polypeptides in all the tumor cell lines. However, GHSM and GLSM antibodies showed a immune reactive specific pattern with different tumor cell lines. The GHSM antibodies showed reactive polypeptides of 66, 45 and 36 kDa proteins in C6, SK-N-SH and U-373MG tumor cells. Of the three proteins the 36 kDa peptide showed an intense staining. The tumor cell lines IMR-32, Neuro-2A showed no cross reactivity with these antibodies. Interestingly the GLSM antibodies showed immune reactivity with same tumor cell types but it was with a 29 kDa protein but not with 66, 45 and 36 kDa reactive species. The GHSM antibody recognized a 45-50 kDa protein in Neuro-2A tumor cell line, which can be recognized by GLSM anti-bodies. On the whole the data suggests two polypeptides 36 and 29 kDa are very prominently recognized by the GHSM and GLSM antibodies, in neuroblastoma and astrocytomas, suggesting differential expression of nuclear matrix proteins in different tumor cell line which could be of basic importance in identification and classification of tumors.
Fig. 1: Protein patterns of the nuclear fractions. Nuclear fractions were electro-phoresed on SDS-PAGE gels as described in materials & methods and stained with silver nitrate.

Mr = Molecular weight markers (bovine albumin-66000 ; egg albumin-45000 ; glyceraldehyde-3-phosphate dehydrogenase-36000 ; carbonic anhydrase-29000 ; trypsinogen-24000 ; soyabean trypsin inhibitor-20100 ; α-lactalbumin-14200).

B = Whole brain nuclear proteins

N = Neuronal nuclear proteins

G = Glial nuclear proteins.

a = 50 kDa (GFAP)

b = 190 kDa (NFP)
Fig. 2: Scanning electron micrographs of nuclei and nuclear matrices. Nuclear matrices were isolated and prepared as described in experimental procedures.

Panel A = Nuclei from neurons

Panel B = Nuclear matrix from neurons prepared using high salt extraction method

Panel C = Nuclear matrix from neurons prepared using low salt extraction method

Panel D = Nuclei from glial cells

Panel E = Nuclear matrix from glia prepared by high salt extraction method

Panel F = Nuclear matrix from glia prepared using low salt extraction method.

Magnification on SEM 6000 X. (Note that the nuclear shape is retained despite high salt and low salt extraction methods to isolate nuclear matrices)
Fig. 3: SDS-PAGE analysis of the nuclei and nuclear matrix associated proteins in neurons and glia

NN = Neuronal nuclear proteins

NISM = Neuronal nuclear matrix prepared by high salt extraction method
NLSM = Neuronal nuclear matrix prepared using low salt extraction method

GN = Glial nuclear proteins

CHSM = Glial nuclear matrix prepared by high salt extraction method
GLSM = Glial nuclear matrix prepared using low salt extraction method.

Mr = Molecular weight markers (X 10^{-3} daltons)

Panel A = in 18 % gel
Panel B = 12.5 % gel
Fig. 4: Developmental expression of neuronal and glial nuclear proteins. Silver stained two dimensional polyacrylamide gel electrophoretic patterns of proteins from

A = Neuronal nuclei of rat brain (10 days old)
B = Neuronal nuclei of rat brain (45 days old)
C = Neuronal nuclei of rat brain (180 days old)
D = Glial nuclei of rat brain (10 days)
E = Glial nuclei of rat brain (45 days old)
F = Glial nuclei of rat brain (180 days old)

Molecular weight (MW X $10^{-3}$ daltons) shown on the right side of the figure while IEF pattern runs acidic to basic from left to right on the abcissa of each panel.
Fig. 5: Developmental expression of neuronal nuclear matrix proteins. Silver stained two-dimensional polyacrylamide gel electrophoretic patterns of proteins from:

A = Neuronal nuclei of rat brain (45 days)

B = Neuronal nuclear matrix isolated by HSM (45 days old)

C = Neuronal nuclear matrix isolated by LSM (45 days old)

D = Neuronal nuclei of rat brain (10 days)

E = Neuronal nuclear matrix isolated by HSM (10 days old)

F = Neuronal nuclear matrix isolated by LSM (10 days old)

a = Lamin A

b = Lamin B

c = Lamin C

Molecular weight (MW X 10^{-3} daltons) shown on the right side of the figure while IEF pattern runs acidic to basic from left to right on the abscissa of each panel.
Fig. 6: Developmental expression of glial nuclear matrix proteins: silver stained two dimensional polyacrylamide gel electrophoretic patterns of proteins from

A = Glial nuclei of rat brain (45 days)
B = Glial nuclear matrix isolated by HSM (45 days old)
C = Glial nuclear matrix isolated by LSM (45 days old)
D = Glial nuclei of rat brain (10 days)
E = Glial nuclear matrix isolated by HSM (10 days old)
F = Glial nuclear matrix isolated by LSM (10 days old)

\begin{itemize}
  \item a = Lamin A
  \item b = Lamin B
  \item c = Lamin C
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Molecular weight (MW $\times 10^{-3}$ daltons) is shown on the right side of the figure while IEF pattern runs acidic to basic from left to right on the abcissa of each panel.
Fig. 7: Developmental expression of neuronal and glial nuclear and nuclear matrix proteins: silver stained two dimensional polyacrylamide gel electrophoretic patterns of proteins from

A = Neuronal nuclei of rat brain (180 days old)
B = Neuronal nuclear matrix isolated by HSM (180 days old)
C = Neuronal nuclear matrix isolated by LSM (180 days old)
D = Glial nuclei of rat brain (180 days)
E = Glial nuclear matrix isolated by HSM (180 days old)
F = Glial nuclear matrix isolated by LSM (180 days old)

Molecular weight (MW $\times 10^{-3}$ daltons) is shown on the right side of the figure while IEF pattern runs acidic to basic from left to right on the abcissa of each panel.
Fig. 8: Isolation and fractionation of proteins from neuronal nuclear matrix extracted by high salt method from 45 days old rat brain. The nuclear matrix protein sample was applied on to a pre-equilibrated DEAE column with equilibration buffer and bound proteins were eluted in a non-linear gradient fractionation with increasing ionic strength buffers (0.1 to 1.0 M). Elutes were collected in 1 ml fractions and the absorbance profile at A 280 nm is shown in panel A. Other panels show two dimensional gel analysis of 0.2, 0.4, 0.6, 0.8 and 1.0 M peak fractions as denoted in the panels.
Fig. 9: Isolation and fractionation of proteins from neuronal nuclear matrix extracted by low salt method from 45 days old rat brain. The nuclear matrix protein sample was applied on to a **pre-equilibrated** DEAE column with equilibration buffer and bound proteins were eluted in a non-linear gradient fractionation with increasing ionic strength buffers (0.1 to 1.0 M). Elutes were collected in 1 ml fractions and the absorbance profile at A 280 nm is shown in panel A.

Other Panels show two dimensional gel analysis of 0.1, 0.2, 0.4 and 0.6 M peak fractions as denoted in the panels.
Fig. 10: Isolation and fractionation of proteins from glial nuclear matrix extracted by high salt method from 45 days old rat brain. The nuclear matrix protein sample was applied on to a pre-equilibrated DEAE column with equilibration buffer and bound proteins were eluted in a non-linear gradient fractionation with increasing ionic strength buffers (0.1 to 1.0 M). Elutes were collected in 1 ml fractions and the absorbance profile at A 280 nm is shown in panel A.

Other panels show two dimensional gel analysis of 0.1, 0.2, 0.4, 0.6 and 1.0 M peak fractions as denoted in the panels.
Fig. 11: Isolation and fractionation of proteins from glial nuclear matrix extracted by low salt method from 45 days old Rat brain. The nuclear matrix protein sample was applied on to a pre-equilibrated DEAE column with equilibration buffer and bound proteins were eluted in a non-linear gradient fractionation with increasing ionic strength buffers (0.1 to 1.0 M). Elutes were collected in 1 ml fractions and the absorbance profile at A 280 nm is shown in panel A.

Panel B = shows SDS-PAGE analysis of the elutes in different fractions.

BLC = Before loading on to the column

A = After washing with equilibration buffer

5-85 = correspond to fraction numbers

M.wt = Molecular weight markers (bovine albumin-66000; egg albumin-45000; glyceraldehyde-3-phosphate dehydrogenase-36000; trypsinogen-24000).

Other Panels show two dimensional gel analysis of 0.1, 0.2, 0.4 and 0.6 M peak fractions as denoted in the panels.
Fig. 12: Nuclear matrix proteins were prepared and analysed by high performance liquid chromatography employing Shimpack PA-DEAE column. The proteins were eluted in binary linear gradient mode with citric acid-phosphate buffer system.

Panel A = Chromatogram of proteins associated with neuronal nuclear matrix prepared by high salt method.

Panel B = SDS-PAGE analysis of peak fractions. The number corresponds to the peak fraction in chromatogram of Panel A.

Panel C = Chromatogram of proteins associated with neuronal nuclear matrix prepared by low salt method.

Panel D = SDS-PAGE analysis of peak fractions. The number corresponds to the peak fraction in chromatogram of panel C.
**CALCULATION REPORT**

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Fig. 13: Nuclear matrix proteins were prepared and analysed by high performance liquid chromatography employing Shimpack PA-DEAE column. The proteins were eluted in binary linear gradient mode with citric acid-phosphate buffer system.

Panel A = Chromatogram of proteins associated with glial nuclear matrix prepared by high salt method.

Panel B = SDS-PAGE analysis of peak fractions. The number corresponds to the peak fraction in chromatogram of panel A.

Panel C = Chromatogram of proteins associated with glial nuclear matrix prepared by low salt method.

Panel D = SDS-PAGE analysis of peak fractions. The number corresponds to the peak fraction in chromatogram of panel C.
** CALCULATION REPORT **

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6  26.683  18604  65   5  19.4565
7  30.674  25119  372  35  35.9153

TOTAL: 69941  961 100

** CALCULATION REPORT **

** TIME **  ** AREA **  ** HEIGHT **  ** M **  ** CONC **
1  0.235  460  104  0.0474
2  15.86  31192  575  45.9408
3  17.573  111116  379  19.4312
4  18.744  23147  476  34.0076

TOTAL: 7846  1534  100

** COLUMN **:
- SHIMPACK PA-200 (P-805A)
- Solvent: 0.1 M citric acid and 0.2 M NaNO₃, pH gradient from 2.6 to 7.0
- ELUENT: 280 nm
- SAMPLE: GLIA NUCLEAR MATRIX BY LOW-SALT METHOD

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- SHIMPACK PA-200 (P-805A)
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- ELUENT: 280 nm
- SAMPLE: GLIA NUCLEAR MATRIX BY LOW-SALT METHOD
Fig. 14: single strand DNA–cellulose chromatography of proteins from neuronal nuclear matrix extracted by low salt method from 45 days old rat brain. The nuclear matrix protein sample was applied on to a pre-equilibrated ssDNA cellulose column with equilibration buffer and bound proteins were eluted in a non-linear gradient fractionation with increasing ionic strength buffers (0.1 to 1.0 M). Elutes were collected in 1 ml fractions and the absorbance profile at A 280 nm is shown in Panel A.

Other Panels represent two dimensional gel analysis of the 0.1, 0.2, 0.6 and 1.0 M peak fractions.

Mr represents molecular weight markers
Fig. 15: single strand DNA–cellulose chromatography of proteins from *glial* nuclear matrix extracted by low salt method from 45 days old rat brain. The nuclear matrix protein sample was applied on to a **pre**-equilibrated ssDNA cellulose column with equilibration buffer and bound proteins were eluted in a non-linear gradient fractionation with increasing ionic strength buffers (0.1 to 1.0 M). Elutes were collected in 1 ml fractions and the absorbance profile A 280 nm is shown in Panel A.

Panel B shows the SDS-PAGE analysis of the elutes in different fractions. Numbers 4-65 correspond to the respective fractions.

Other Panels represent two dimensional gel analysis of the 0.1, 0.2, 0.4 and 0.6 M peak fractions.
Fig. 16: **Immunodiffusion**

Panel A = NHSM antibody

Panel B = NLSM antibody

Panel C = GHSM antibody

Panel D = GLSM antibody

1 = Neuronal nuclear proteins

2 = Neuronal nuclear matrix proteins extracted by high salt method

3 = Neuronal nuclear matrix proteins extracted by low salt method

4 = Glial nuclear proteins

5 = Glial nuclear matrix proteins extracted by high salt method

6 = Glial nuclear matrix proteins extracted by low salt method
Fig. 17: Western blot analysis of Nuclear and Nuclear matrix associated proteins with the following antibodies.

Panel A = Samples treated with NHSM antibody

Panel B = Samples treated with NLSM antibody

Panel C = Samples treated with GHSM antibody

Panel D = Samples treated with GLSM antibody

In each Panel the numbers pertain to:

1 = Neuronal nuclear proteins (NN)

2 = Neuronal nuclear matrix prepared by High salt extraction method (NHSM)

3 = Neuronal nuclear matrix prepared using Low salt extraction method (NLSM)

4 = Glial nuclear proteins (GN)

5 = Glial nuclear matrix prepared by High salt extraction method (GHSM)

6 = Glial nuclear matrix prepared using Low salt extraction method (GLSM).

MW = Molecular weight markers (bovine albumin–66000; egg albumin–45000; chymotrypsinogen–25000; soyabean trypsin inhibitor–20100; cytochrome–C–12300).

a = 70 kDa protein
Fig. 18: Tissue specificity of Nuclear matrix proteins. Western blot analysis.
Nuclear proteins were isolated from the tissues:

1 = Cerebellum
2 = Brain stem
3 = Cerebral cortex
4 = Hippocampus
5 = Mid brain
6 = Liver
7 = Heart
8 = Kidney
9 = Muscle
10 = Lung

and were separated on SDS-PAGE gels, electroblotted and immunostained with antibodies in:

Panel A = NHSM antibody treated blot
Panel B = NLSM antibody treated blot
Panel C = GHSM antibody treated blot
Panel D = GLSM antibody treated blot

MW = Molecular weight markers
a = 105 kDa protein
b = 38 kDa protein
c = 20 kDa protein
d = 90 kDa protein
e = 80 kDa protein
f = 75 kDa protein
g = 68 kDa protein
h = 50 kDa protein
i = 40 kDa protein
Fig. 19: Nuclear protein patterns from normal cells and tumor cell lines. Nuclear proteins were electrophoresed on SDS-PAGE gels as described in materials & methods and stained with silver nitrate.

NN = Rat Neuronal nuclei
GN = Rat Glial nuclei
C6 = Rat C6 glioma cell line

SK-N-SH = Epithelial like human neuroblastoma (neuroblastoma / metastasis to bpne)
U-373MG = Epithelial like human glioblastoma (glioblastoma / astrocytoma)
IMR-32 = Human Neuroblastoma (Fibroblast mixture cell type)
Mr = Molecular weight markers (MW)
Fig. 20: Immuno reactivity of the four antibodies with tumor cell line

Panel A = Treated with NHSM antibody
Panel B = Treated with NLSM antibody
Panel C = Treated with GHSM antibody
Panel D = Treated with GLSM antibody

GN = Glial nuclear proteins

C6 = Rat C6 glioma nuclear proteins

SK-N-SH = Epithelial like human neuroblastoma nuclear proteins

U-373MG = Epithelial like human glioblastoma nuclear proteins

IMR-32 = Neuroblastoma and Fibroblast mixture cell type nuclear proteins

Neuro-2A = Neuroblastoma nuclear proteins

Mr = Molecular weight markers (bovine albumin-66000; egg albumin-45000; glyceraldehyde-3-phosphate dehydrogenase-36000; carbonic anhydrase-29000; trypsinogen-24000; soyabean trypsin inhibitor-20100; \( \beta \)-lactalbumin-14200).