Direct association of LIS1, the lissencephaly gene product, with a mammalian homologue of a fungal nuclear distribution protein, rNUDE[1]

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Abstract LIS1 is a product of the causative gene for type I lissencephaly characterized by a smooth brain surface due to a defect in neuronal migration during brain development and a regulatory subunit of platelet-activating factor acetylhydrolase (PAF-AH). It is also a mammalian homologue of the fungal nuclear distribution (*nud*) gene, *nudF*, which controls the migration of fungal nuclei. Using the two-hybrid system, we identified a novel LIS1-interacting protein, rat NUDE (rNUDE), and found that it is a mammalian homologue of another fungal *nud* gene product, NUDE, and *Xenopus* mitotic phosphoprotein 43 which is phosphorylated in a cell cycle-dependent manner. rNUDE and the catalytic subunits of PAF-AH interact with the N- and C-termini of LIS1, respectively. However, these proteins, instead of simultaneously binding to LIS1, appeared to bind to LIS1 in a competitive manner. These results suggest that LIS1 functions in nuclear migration by interacting with multiple intracellular proteins in mammals. **© 2000 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

Key words: LIS1; Platelet-activating factor; Platelet-activating factor acetylhydrolase; NUDF; NUDE

1. Introduction

LIS1 is a causative gene for lissencephaly type I [1,2], a brain malformation characterized by widespread agyria of the brain. This disorder is thought to be due to a defect in the neuronal migration process during brain development [3]. Mice homozygous for a null *Lis1* mutation die early in embryogenesis soon after implantation [4]. Heterozygous and compound heterozygous mice have dosage-dependent defects in neuronal migration and neurogenesis [4]. LIS1 was also identified biochemically from bovine brain extracts as the regulatory subunit β of platelet-activating factor acetylhydrolase (PAF-AH) [5,6]. PAF-AH contains two additional catalytic subunits, α1 and α2, which form a heterodimer and homodimers depending on the tissue and developmental stages [5–8]. Involvement of the catalytic subunits of PAF-AH in neuronal migration is also suggested by the observations that LIS1 and the two catalytic subunits are highly expressed in actively migrating neurons [9], and switching of the dimer from α1/α2 to α2/α2 occurs during brain development [8].

Genetic analysis of nuclear migration in fungi has led to the identification of genes required for nuclear migration and positioning [10,11]. These include genes encoding α-tubulin (a component of microtubules) [12], cytoplasmic dynein heavy and light chains (components of a microtubule-associated mechanical motor) [13,14], ARP1 (a component of the dynactin complex, which functions as a dynein activator) [11], and some other proteins of unknown function [15,16]. Among them, interestingly, *Aspergillus nidulans* NUDF is a fungal homologue of LIS1 [16]. This finding provided a clue to understanding the function of LIS1, namely, it may also be involved in nuclear movement during neuronal migration [17]. However, it is unknown how LIS1 affects nuclear movement during neuronal migration.

To gain more insight into the cellular function of LIS1, we screened molecules that interact with LIS1, other than those previously reported, by a yeast two-hybrid system. Here we identify a protein that interacts directly with LIS1 and found that it is a mammalian homologue of both NUDE, a product of another fungal nuclear movement gene, and a *Xenopus* mitotic phosphoprotein 43, which is phosphorylated in a cell cycle-dependent manner.

2. Materials and methods

2.1. Strains and media

Yeast strains (HF7C; *MATa ura3-52 his3-200 lys2-801 ade2-101 trp1-901 leu2-3, 112 gal4-542 gal80-538 LYS2::GAL1-HIS3 URA3::(GAL4 17mers)$_3$-CYC1-lacZ*) were grown at 30°C in rich medium (1% yeast extract, 2% Bacto-peptone, 2% glucose) or in synthetic minimal medium with the appropriate supplements.

2.2. Two-hybrid screen

The pGBT9-LIS1 plasmid was constructed by inserting the *Eco*RI/*Sal*I fragment of the pFASTBac1-LIS1 plasmid [18] into the pGBT9 plasmid (Clontech, Palo Alto, CA, USA). The resulting plasmid expresses LIS1 as a fusion protein with the DNA binding domain of Gal4. The yeast reporter strain HF7C, which contains the reporter genes LacZ and HIS3 downstream of the binding sequences for Gal4, was sequentially transformed with the pGBT9-LIS1 plasmid and with a rat liver cDNA library in pACT2 vector (Clontech) using the lithium acetate method. Double transformants were plated on synthetic medium lacking histidine, leucine and tryptophan. The plates were incubated at 30°C for 3 days. His$^+$ colonies were patched on selective plates and assayed for β-galactosidase activity by a filter assay (reference). Plasmid DNA was prepared from colonies displaying a His/LacZ phenotype and used to transform *Escherichia coli* competent cells (HB101, Takara, Kyoto, Japan). The cDNA inserts from specific clones were sequenced with the Sanger dideoxy-termination method

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[1] The nucleotide sequence data reported in this paper have been submitted to the GenBank data base under the accession number AF240463 for rNUDE.

Abbreviations: PAF, platelet-activating factor; PAF-AH, platelet-activating factor acetylhydrolase; *nud*, nuclear distribution gene; rNUDE, rat NUDE

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using the ABI377 system (Perkin-Elmer, Branchburg, NJ, USA). The sequence of one clone (clone 17) contained the full-length cDNA encoding rat NUDE (rNUDE). Protein sequence comparisons were carried out with CLUSTALW software.

2.3. Plasmid construction and DNA transfection

To prepare cDNAs encoding rNUDE with Myc-tag at the N-terminus, we first introduced oligonucleotides into mammalian expression vector pcDNA3 to generate the Myc-tag (pcDNA3-N-Myc). cDNAs encoding rNUDE with *Hin*dIII and *Not*I sites at the 5′- and 3′-ends, respectively, were generated by the polymerase chain reaction (PCR). The PCR product generated was inserted into the *Hin*dIII and *Not*I sites of pcDNA3-N-Myc. The resulting plasmid was designated pcDNA3-Myc-rNUDE.

To prepare cDNAs encoding LIS1 and the mutant LIS1, which lack the N-terminal 65 amino acids, with T7-tag at the N-terminal, we first introduced oligonucleotides to generate the T7-tag into the *Kpn*I/*Eco*RI sites of mammalian expression vector pcDNA3 (pcDNA3-N-T7) as described above. cDNA fragments encoding the entire or mutated (amino acids 66–410) bovine LIS1 with *Eco*RI and *Sal*I sites [18] were inserted into *Eco*RI and *Xho*I sites of pcDNA3-N-T7. The resulting plasmids were designated pcDNA3-T7-LIS1 and pcDNA3-T7-LIS1Δ65, respectively.

Plasmid DNAs were purified on a Qiagen Matrix Column (Qiagen, Hilden, Germany). For transfection, CHO-K1 cells were grown to 80% confluence in 100 mm dishes. cDNA (6 μg) and LipofectAMINE (40 μl) (Life Technologies, Inc.) were separately diluted in OPTI-MEM (Life Technologies, Inc.) to 600 μl volumes, mixed, and incubated at room temperature for 30 min. Cells were washed once with OPTI-MEM and were added with the DNA–lipid complexes. After 4 h incubation, the medium of the cells was replaced with Ham's-F12 supplemented with 20% fetal calf serum (FCS). The cells were further incubated for 20 h at 37°C, suspended in fresh medium (Ham's-F12 supplemented with 10% FCS), and harvested after 48 h.

2.4. Immunoprecipitations

The transfected CHO-K1 cells (10^7 cells) were mixed with 1 ml of buffer A (50 mM Tris, 5 mM EDTA, 150 mM sodium chloride, 10 mM sodium fluoride, 1 mM pervanadate, 1% Triton X-100, pH = 7.4) containing 1 mM phenylmethanesulfonyl fluoride and 5 μg/ml aprotinin, and were incubated on ice for 1 h with occasional vortexing. Cell lysates were prepared by centrifuging the cells at $8000 \times g$ at 4°C for 20 min. 1 ml of the cell lysate was precleared with 100 μl of a 50% suspension of protein G-Sepharose beads (Amersham-Pharmacia) for 1 h at 4°C under continuous rotation. Beads were eliminated by centrifugation, and the supernatants were incubated with 20 μg of the

A

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rat NUDE            1 MEDSGKT-FGSEEEETNYWRD-LAMTYKQRAENTQEELREFQEGSREYEAELETQLQQAETRNRDLLSENNRLRMELESVKEKFEMQHSEGYRQISALEDDLAHTKAIKDQLQKYIRELE  118
Xenopus MPP43       1 MDDLENNIFNSVEEEILYWKS-VAMKYKQCSEEAQQELQEFQEASREYEAELEAQLLQTEGRNRDLFSENNRLRMELDAIKEKYEEQHSENYVQISTLEGDLSQTKAVRDQLQKYIRELE  119
A.nidulans NUDE    1 MPSADEP-SSTRTNGTSSRSDQLA-YYKKQYEQLESELADFQASSRELEAELEKEIEASEKRERQLKEKVDNLRYEVEEWKSKYKQSKSEASTAQNALQKEITSLRDANLTLQLKLRDTE  118
N.crassa RO-11      1 MAADV-P-GSPLAKNATTEEA-LA-WYKSQYEELEQELKEFQQSSKELEAELEKDLDAADKRERALQQKAEGLSYEVEEWKRKYKESKSEANAAQSALEKEITALRETNRTLQLKLRDIE  116
                      * .     ...  ... ..   .*  **.  *    ** .**. *.*.***** .. ... *.*.*   . .*..*..  *.*.. . ** . . . ..*. ..  ...   .**   *..*

rat NUDE          119 QANDDLERAKRATIMSLEDFEQRLNQAIERNAFLESELD--E--KENLLESVQRLKDEARDLRQELAVQQKQDKPRTPMPSSGETKRTDMAVQATGSAPSTPITH----QGSSSGLNTPE  230
Xenopus MPP43     120 QANDDLERAKRATIMSLEDFEQRLNQAIERNAFLENELD--E--KENLLESVQRLKDEARDLRQELAVQQKQEKPKSNMVSPETERM-DTSVQASIAIPSAPLTP-LSQRGCASTLTSPL  233
A.nidulans NUDE 119 VANDDYERQARHTTSSLEDMESKYNQALEREVLLDMEYKQGEQERESLRIENQRLRDELNDLKIETEIVQERLRNNNRRRRPAPLGRSPSTPHT-PEIFDRS-------PGEST-VSSP-  228
N.crassa RO-11   117 VANDDFERQARNTSSSLEDLESKYNVAIERAVMMEEEIKIGEQERERLRVEAQRLREELSDLKIEAEILQSKLRKHQARGHLTQITTTIAPAPASP-LSTASS-PLVSTPPDTKSLSTID  234
                      .****.**..*.*. **** *  .*.*.**..... *..  *  .* *. ..***..*..**..*... * .                .    . . ..    . . . . .

rat NUDE          231 TFRCGLGSPSSGTPLTPAARISALNIVGDLLRKVGALESKLASCKNFMYDQSPSRKSGPALGRGTKNRD-GIDRRPGST---AVGDKGSGKRLEFAK---PSSQLSSPALPSTQGVVKLL  343
Xenopus MPP43     234 SFRTSLDDGYSGTPLTPCARISALNIVGDLLRKVGALESKLASCRNFVHEQSPNRPLTSVSARMKKTRE-GIENRLSIASGSSVE-KGLIKRLEFGSF--PS-NTPMQGMHSPQGVVKMI  348
A.nidulans NUDE 229 LFSTP-------PT---KLSLT-LASATATPPSPPMSETSSSMRKSLTAASGFPLQKASASE------SFGTRSLYGNRPQRFQAHSRATSYAFSNGRSTPSATTT-RPSLPKANNTTAN  330
N.crassa RO-11   235 TLSEVQDP-PSPPMSDASLGKG-LRASRSTPVKQTASRPGGC-RTPKTSISKSAAAKSSAQATHKANQSFSENNITPK-PKPLSSSTSSQSNRPSNGRFNTNSYPMVRTPSSRPSGARAA  350
                                          *

rat NUDE          344 L                                                                                                                    344
Xenopus MPP43     349 I                                                                                                                    349
A.nidulans NUDE 331 -RPSG----IPKSGSLHQIRGLIGKMQKLEERVQSAKSKLPPPSETASRASSRAG-SMLDASPGAATIAMRRDTRKRLSGSSFSS-SVRDGDGAPSYVTSSR--PSYGTRTQGDSRPSSR  441
N.crassa RO-11   351 ERPRAPVHRIPPSNSLTHIRTLTAQMQKLEARVHSARSKLPATAQTPPKQSPRFSGG-IGGLAAT--VAMRGKKQRGVGAST-SSLNLLDDDNASDISGT-HSNPDFRSSTLNLSKHIPR  465

A.nidulans NUDE 442 TSFSSSLSHSTHPSVTPSNRPESRQSRTKT-PLGHYSTNPTTESRRPRSSLSNPA-GQSTPINGMTYIDEDEDLAEQFNMRATISSTRPTRLPSFSNPAFSTPTGLKKRSTSGMSGIPAP  554
N.crassa RO-11   466 LSTSG-VSRIAFGPLPIRH-PASAAASTTSTSTATA-TNVDSEVSRPSSSRASSSGYGR--PISRADSHSTAASGY-MPSSSS--RPISRTSLPGHGTRTPVGSWPRSSGNLSAYGHGPAH  574

A.nidulans NUDE 555 RTLRRGNTMGPPKTKPKPVAGDLGETF                                                                                              586
N.crassa RO-11   575 SQASISYSTAEEDELTDDGQRELRSKTTPARRRTGTLSARDAPDHHHTGIPIPGSGGNRRQSGSSSASRSSVTGSLSLRRQSNAAALGHGHGHNAGYGHGHGTVPGGTTVRKVVDLGETY  694
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B

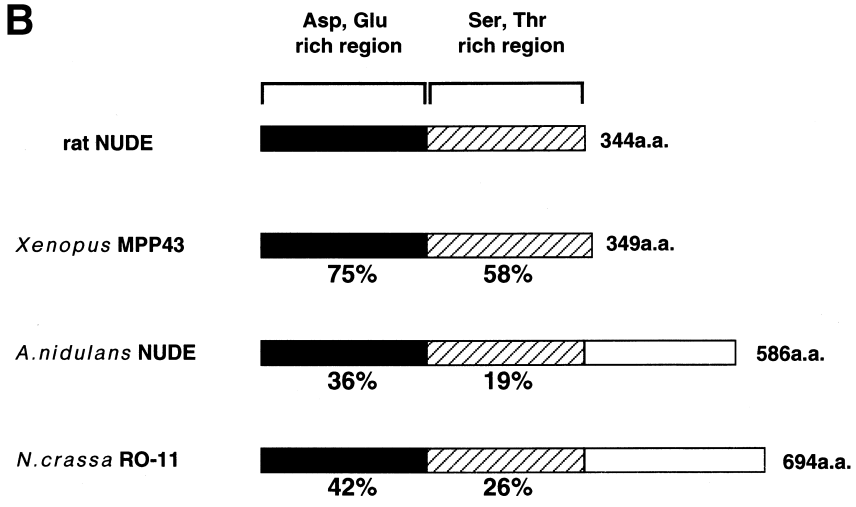

Fig. 1. Amino acid comparison of rNUDE and related proteins. A: Comparison of amino acid sequences of rNUDE with *X. laevis* mitotic phosphoprotein 43, *A. nidulans* NUDE and RO-11. The multiple sequence alignment was performed using the CLUSTALW program (EMBL). Residues conserved among all four proteins are labeled with an asterisk. B: Schematic representation of rNUDE and related proteins. The filled and hatched bars indicate Asp/Glu- and Ser/Thr-enriched regions, respectively. Numbers under the bars indicate the percent identities of corresponding regions compared with rNUDE. The numbers of amino acids of each protein are indicated at the right.

anti-Myc monoclonal antibody 9E10 (Sigma) and 40 μl of a 50% suspension of protein G-Sepharose beads for 1 h under similar conditions. Immune complexes bound to protein G were sedimented by rapid centrifugation, and beads were washed three times with 1 ml of buffer A. The pellets were resuspended in 60 μl of sample loading buffer containing 0.1 M β-mercaptoethanol and boiled for 5 min. Proteins were separated by sodium dodecyl sulfate (SDS)–PAGE on 10% gels and subjected to Western blotting using anti-LIS1 or anti-T7-tag monoclonal antibodies as described previously [8].

2.5. Pull-down assay using GST-α1 and α2

For preparation of GST-tagged α1 and α2, cDNAs encoding rat α1 or α2 were inserted into the *Eco*RI/*Sal*I sites of pGEX4T-1 vector (Amersham-Pharmacia Biotech). Recombinant proteins were expressed and purified according to the manufacturer's protocol. 10 μg of purified GST-tagged α1 or α2 proteins was coupled to 25 μl of GSH-Sepharose 4B (Amersham-Pharmacia Biotech). The cell lysates of CHO-K1 cells transiently transfected with cDNA for T7-LIS1 or T7-LIS1Δ65 (600 μg each) were mixed with the GSH-Sepharose beads. After washing the beads with buffer A, binding of T7-tagged proteins to the beads was detected by Western blot analysis using anti-T7 monoclonal antibody.

2.6. Northern blot analysis

Rat Multiple Tissue Northern blots were purchased from Clontech (Palo Alto, CA, USA), DNA probes (coding region) were labeled by random priming with [α-^{32}P]dCTP and hybridization was carried out at 65°C for 4 h in a Rapid-hybridization buffer (Amersham-Pharmacia Biotech, Uppsala, Sweden). Each blot was rinsed with 2×SSC at room temperature for 5 min, washed twice with 0.5×SSC containing 0.1% SDS at 65°C for 30 min and then autoradiographed using Kodak X-Omat AR film at −80°C with an intensifying screen for 24 h. Finally, each blot was re-hybridized with a β-actin cDNA probe (Clontech, Palo Alto, CA, USA) as an internal standard.

3. Results

3.1. Screening of a rat liver cDNA library with LIS1

In this study, we attempted to isolate proteins other than the catalytic subunits of PAF-AH that interact with LIS1. We chose the liver cDNA library since the liver expresses very low levels of the catalytic subunits but relatively high levels of LIS1 expression [8,19]. Plasmid pGBT9, which contains the entire region of LIS1 fused to the Gal4 DNA binding domain, was used as bait to screen a rat liver cDNA library using the yeast two-hybrid system. Analysis of a total of 1.7×10^6 clones resulted in the identification of approximately 400 candidates as determined by the His$^+$ screen. However, most of these were eliminated upon testing for their ability to activate the β-galactosidase gene. Among the 20 positive clones, two groups with overlapping sequences derived from the same genes were found to interact strongly with the LIS1 as determined by the intensity of the blue color indicator (data not shown). One group (two clones) encodes the α2 subunits of PAF-AH. In the other group (four clones), the longest clone, cl.17 containing the full-length cDNA, was composed of a total of 2153 nucleotides and encoded 344 amino acid residues (Fig. 1A). The sequence of the clone around the ATG matches the Kozak consensus initiation sequence, and stop codons are present in this reading frame within the upstream 5′-non-coding region (data not shown). The open reading frame predicted a 344 amino acid polypeptide with a calculated mass of 38.2 kDa (Fig. 1A). The N-terminal half of the protein is enriched with Glu and Asp, which account for nearly 25% of the amino acids of this region, while the C-terminal half is enriched with Ser and Thr, which account for about 20% of the amino acids.

Data base searches using the BLAST algorithm revealed that the sequence showed a significant homology to a fungal nuclear migration protein, *A. nidulans* NUDE (GenBank accession number AF085679), *Neurospora crassa* RO-11 (GenBank accession number AF015560) [20] and *Xenopus* mitotic phosphoprotein 43 (MPP43) (GenBank accession number U95097) [21] (Fig. 1B). All three proteins also possess Glu/Asp-rich and Ser/Thr-rich domains, and fungal NUDE and RO-11 contain extra sequences in their C-terminal regions, which exhibit no homology with any mammalian proteins (Fig. 1B). Although the properties of the *nudE* gene have not been characterized yet, the base sequence was described in the GenBank data base as a novel gene involved in the distribution of nuclei in fungi (nuclear distribution gene; *nud* gene) that was isolated as a multicopy suppressor of the *nudF* mutation in *A. nidulans*. The *ro-11* gene, isolated from *N. crassa*, was also identified as a novel gene required for nuclear migration [20]. These data strongly suggest that the isolated protein that interacts with LIS1 by the yeast two-hybrid system is also involved in nuclear movement in mammalian cells. Therefore, we designated it rNUDE. MPP43 was identified by screening for phosphorylated proteins during mitosis by electrophoretic mobility shifts and was suggested to be phosphorylated by the cyclin-dependent kinase cdc2 during mitosis (M phase) [21]. rNUDE also possesses eight potential phosphorylation sites (SP or TP) that can be phosphorylated by a cyclin-dependent kinase, cdc2, in the second half of the Ser/Thr-rich region.

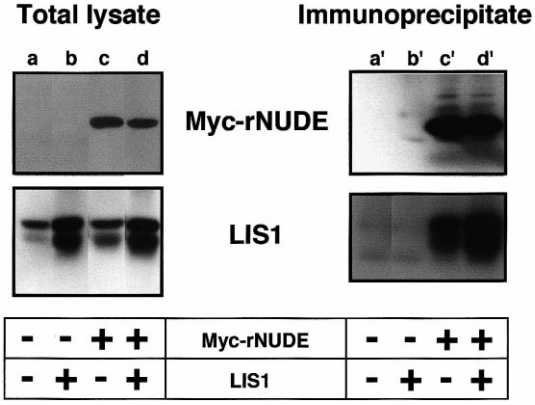

Fig. 2. Interaction of rNUDE with LIS1 in mammalian cells. CHO-K1 cells were transiently transfected with the indicated cDNAs. Left panels: cell lysates (a–d) were subjected to Western blot analysis using anti-Myc and anti-LIS1 monoclonal antibodies. Right panels: cell lysates were immunoprecipitated and the immunoprecipitates (a′–d′) were subjected to the same Western blot analysis.

3.2. Interaction of rNUDE with LIS1 in mammalian cells

The interaction of rNUDE with LIS1 was examined in CHO-K1 cells transfected with Myc-rNUDE cDNA. As shown in Fig. 2, an antibody against Myc co-precipitated endogenous LIS1 from the supernatant of CHO-K1 cells transfected only with rNUDE cDNA (lane c′). When the Myc-rNUDE cDNA was co-transfected with LIS1 cDNA, more LIS1 protein was recovered from the immunoprecipitate (lane d′). These results indicate that rNUDE interacts with LIS1 in the cells.

Next, the domain in LIS1 that is responsible for interaction with rNUDE was examined through deletion mutants of LIS1. LIS1 is composed of 410 amino acid with a coiled-

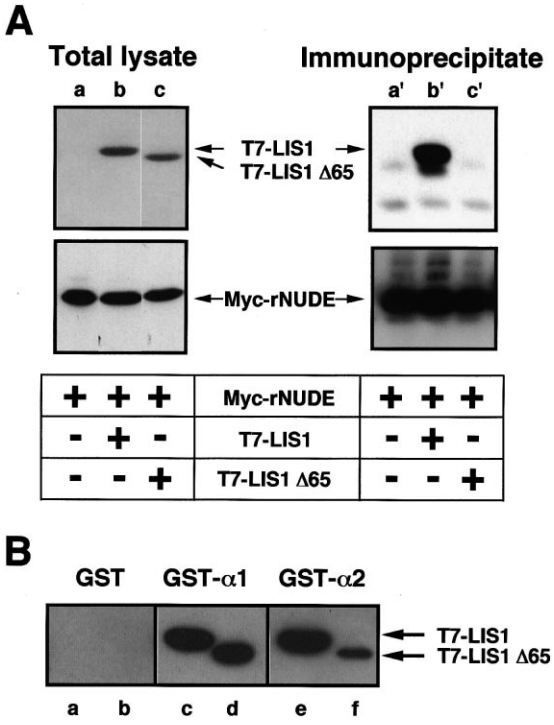

Fig. 3. Domains of LIS1 required for binding to rNUDE or to the α1 and α2 catalytic subunits of PAF-AH. A: The N-terminal 65 amino acid residues of LIS1 are required for binding of LIS1 to rNUDE. Lysates of CHO-K1 cells that had been transiently transfected with the indicated cDNAs were immunoprecipitated with anti-Myc monoclonal antibody. Expressions of T7-LIS1 and Myc-rNUDE in cell lysates (a–c) and immunoprecipitates (a′–c′) were detected by Western blot analysis using anti-T7 and anti-Myc antibodies, respectively. B: The N-terminal 65 amino acid residues of LIS1 are not required for binding of LIS1 to α1 or α2. Lysates of CHO-K1 cells that had been transiently transfected with cDNA for T7-LIS1 (a, c and e) or T7-LIS1Δ65 (b, d and f) were subjected to GSH-Sepharose coupled with GST (a and b), GST-α1 (c and d) or GST-α2 (e and f). Binding of T7-tagged proteins to the beads was detected by Western blot analysis using anti-T7 monoclonal antibody.

coil basic motif at the N-terminal domain and with seven-WD repeats in its C-terminal domain. We made various N-terminal and C-terminal deletion constructs of LIS1. Among the deletion mutants, the construct lacking the N-terminal 65 amino acid (ΔN65LIS1) was found to be stably expressed in CHO cells, but this mutation abolished binding to rNUDE (Fig. 2A, lane c′), and the expression level was slightly lower than that of native LIS1 (Fig. 3A, lane c). On the other hand, ΔN65LIS1 still possessed an ability to bind the catalytic subunits of PAF-AH. Native LIS1 and ΔN65LIS1 were first expressed in CHO cells, and the cell lysate was applied to a GST-α1 (α2) fusion protein column. As shown in Fig. 3B, native LIS1 and ΔN65LIS1 bound to the GST-α1 affinity column to a similar degree (lanes c and d), whereas ΔN65LIS1 bound to the GST-α2 column but to a significantly lesser extent than the native protein (lanes e and f). This suggests that the sites in the LIS1 that bind to these two catalytic subunits do not completely overlap. In any case, the data shown in Fig. 3 suggest that the binding sites in LIS1 for rNUDE and the α subunits are not identical and that the N-terminal region of LIS1 is essential for rNUDE binding but not for binding the catalytic subunits of PAF-AH.

3.3. Competitive binding of the PAF-AH catalytic subunits and rNUDE to LIS1

Next we examined whether the LIS1–α subunit–rNUDE ternary complex can be formed in vitro and in vivo. When Myc-rNUDE cDNA was transfected in CHO cells, the anti-Myc antibody precipitated endogenous LIS1 but not the endogenous α subunits of PAF-AH, even though CHO cells express significant levels of the catalytic subunits (data not shown). The same result was obtained using CHO cells transfected with the α2 cDNA and Myc-rNUDE cDNA. T7-tagged α2 and Myc-rNUDE cDNAs were co-transfected into CHO cells and the resulting cell lysates were immunoprecipitated with the anti-Myc antibody. The anti-Myc antibody precipitated endogenous LIS1 together with Myc-rNUDE, but not T7-α2 (Fig. 4A, lane b′), even though T7-tagged α2 has the capacity to bind to LIS1 (data not shown). The same

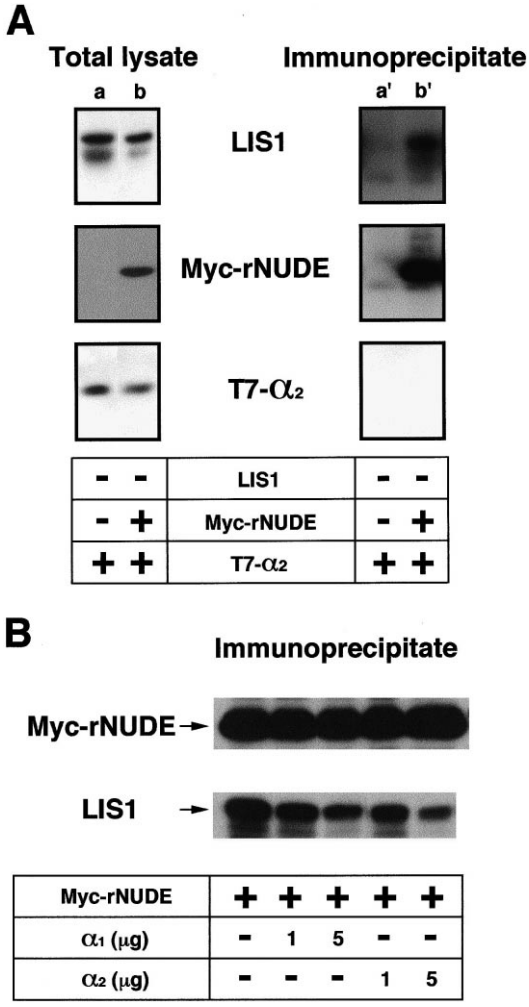

Fig. 4. Competitive binding of the PAF-AH catalytic subunits and rNUDE to LIS1. A: A rNUDE–LIS1–α2 complex is not detected in cells transfected with rNUDE and T7-α2 cDNAs. Lysates of CHO-K1 cells that had been transiently transfected with the indicated cDNAs were immunoprecipitated with anti-Myc monoclonal antibody. Expression of each protein in the cell lysates and immunoprecipitates was detected by Western blot analysis using anti-LIS1, anti-T7 and anti-Myc monoclonal antibodies. B: α1 and α2 subunits of PAF-AH compete with rNUDE in LIS1 binding. α1 or α2 recombinant proteins were added to a cell lysate of CHO-K1 cells that had been transfected with rNUDE cDNA. The lysate was then immunoprecipitated as in A.

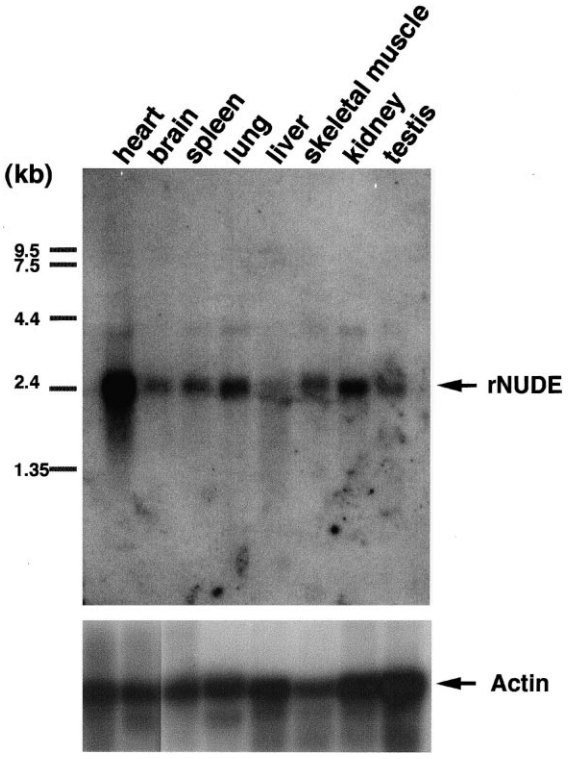

Fig. 5. Northern blot analysis of rNUDE mRNAs in various adult rat tissues. 2 μg of poly(A)$^+$ RNA transferred to nylon membrane (Rat Multiple Tissue Northern, Clontech) was hybridized to DNA probes, specific for rNUDE and β-actin. Lane 1, heart; 2, brain; 3, spleen; 4, lung; 5, liver; 6, skeletal muscle; 7, kidney; 8, testis.

data were obtained using T7-α1 (data not shown). Next, we prepared cell lysates from CHO cells transfected with Myc-rNUDE cDNA, added varying amounts of the recombinant α1 or α2 to the lysate, and then performed immunoprecipitation using anti-Myc antibody. As shown in Fig. 4B, addition of the recombinant α1 or α2 reduced the amounts of LIS1 in the anti-Myc immunoprecipitates (Fig. 4B). In this experiment, the α1 and α2 recombinant proteins were again not detected in the immunoprecipitates (data not shown). Together, these data indicate that rNUDE and the catalytic subunits of PAF-AH cannot associate with LIS1 simultaneously, but rather they compete for the binding sites on LIS1.

3.4. Tissue and cellular distribution of rNUDE

Expression of rNUDE mRNA in various rat tissues was examined by Northern blot analysis employing cDNA corresponding to the coding region of each subunit as a probe. Transcripts encoding rNUDE consisting of messages of 2.4 kb were seen in all tissues, with highest in testis and heart, respectively (Fig. 5). We also tested various human cell lines for NUDE mRNA expression and found that the message was expressed in all human cell lines tested including HL-60, HeLa S3, K-562, MOLT-4, Raji, SW480, A549 and G361 (data not shown). These data suggest that as in the case of LIS1, NUDE is expressed ubiquitously in most of mammalian tissues and cells.

4. Discussion

In this study, using the yeast two-hybrid system, we have isolated a cDNA encoding a novel 42 kDa protein from rat liver that directly interacts with LIS1. We named it rNUDE, since this protein has significant amino acid sequence homology with the product of the *A. nidulans nudE* gene. As mentioned above, *nudE* gene was isolated as a multicopy suppressor of the *nudF* mutation in *A. nidulans*, and only the base sequence was described in the GenBank data base as a novel gene involved in the distribution of nuclei in fungi. The present data demonstrated for the first time that NUDE interacts directly with LIS1 in mammals and that NUDE and the catalytic α subunits of PAF-AH bind to LIS1 in a competitive manner.

The N-terminal half of rNUDE is rich in Asp and Glu residues and may form an α-helical coiled-coil structure, since the corresponding region of the RO-11, *Neurospora* homologue of NUDE was predicted to form such a structure [20]. The N-terminal side of LIS1 is also predicted to form a coiled-coil structure and is abundant in Lys and Arg residues, suggesting that the N-terminal basic domain of LIS1 electrostatically interacts with the N-terminal acidic domain of rNUDE. Consistent with this idea, deletion of the N-terminal 65 amino acids of LIS1, which removes about half of the N-terminal coiled-coil domain, completely abolished the binding to rNUDE. In contrast to the binding of rNUDE to LIS1, the binding of the catalytic subunits of PAF-AH to LIS1 is relatively complicated. Deletion of 65 N-terminal amino acids of LIS1 did not affect the binding of α1 to LIS1, but significantly reduced the binding of α2, suggesting that a part of the N-terminal domain of LIS1 participates in the binding of α2 but not α1. Moreover, as shown in Fig. 4B, the amount of LIS1 bound to Myc-rNUDE in the presence of α2 is lower than that in the presence of α1, suggesting that α2 displaces rNUDE from LIS1 more efficiently than α1. We have previously demonstrated that LIS1 accelerates the PAF-AH activity of the α2/α2 homodimer, but slightly suppressed the activity of the α1/α1 homodimer [18]. A small but detectable difference in the binding site of LIS1 between the α1 and α2 subunits may explain, at least in part, the differential regulation of the catalytic dimers by LIS1.

Patients with Miller–Dieker lissencephaly are heterozygous for the LIS1 mutation [2], suggesting that the failure of neuronal migration is caused by haploinsufficiency of LIS1. Using a gene-targeting system, Hirotsune et al. also showed that the neuronal-specific role for LIS1 in neural cell migration is dosage-sensitive [4]. Thus, the intracellular level of LIS1 is critical for proper functioning of this protein in the cells. In this context, it is interesting to note that the catalytic α subunits of PAF-AH compete with rNUDE for the binding to LIS1 (Fig. 4). In other words, the level of the PAF-AH catalytic subunits or the affinity of these subunits for LIS1 may determine the level of LIS1 available for the rNUDE binding.

Genetic analysis of nuclear movement in fungi has shown that the motor for nuclear migration in *A. nidulans* appears to be cytoplasmic dynein, and that microtubules and the dynactin complex, which is required for cytoplasmic dynein to move intracellular vesicles along microtubules, are also involved [12–14,26]. It has been suggested that fungal NUDF influences the dynein–dynactin system [14], but there is no clear indication of how NUDF affects the dynein motor. The *A. nidulans nudE* gene was characterized as a multicopy suppressor of the *nudF* mutation. In addition, mutations in the heavy chain of cytoplasmic dynein (*nudA*) suppress the *nudF* muta-

tion [14]. These results together with our present study strongly indicate that *nudE* is located genetically between *nudF* and *nudA*. If this system can be applied to higher organisms, LIS1 may regulate dynein motor through rNUDE during nuclear migration. It can be speculated that during cell migration, dynein motor complex may directly drive the nucleus like other intracellular vesicles along the microtubules, and that LIS1 together with rNUDE may regulate the process.

rNUDE (Fig. 5) as well as LIS1 [19,23] are distributed ubiquitously in most tissues and cells, suggesting that these proteins have other non-specialized functions in addition to neuronal migration. In fact, very recently, Liu et al. reported direct evidence for a role of LIS1 in cell division in *Drosophila* [27]. Interestingly, rNUDE also showed significant sequence homology with mitotic phosphoprotein 43 from *Xenopus laevis* [21]. The *Xenopus* protein was identified in a screen for phosphorylated proteins during mitosis and was suggested to be phosphorylated by cyclin-dependent kinase cdc2 during mitosis (M phase) [21]. rNUDE contains eight cdc2-dependent phosphorylation sites. LIS1 is also phosphorylated, and interestingly the phosphorylated LIS1 is mainly found in the microtubule-associated protein fraction [28]. Thus it is interesting to assume that the binding of rNUDE, PAF-AH catalytic subunit and microtubules to LIS1 is differentially regulated by phosphorylation, and this experiment is now in progress in our laboratory.

5. Uncited references

[22,24,25]

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