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Rapamycin-binding FKBP25 associates with diverse proteins that form large intracellular entities

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ABSTRACT

In this paper, we show some evidence that a member of the FK506-binding proteins, FKBP25 is associated to diverse components that are part of several different intracellular large-molecular mass entities. The FKBP25 is a high-affinity rapamycin-binding immunophilin, which has nuclear translocation signals present in its PPIase domain but it was detected both in the cytoplasm compartment and in the nuclear proteome. Analyses of antiFKBP25-immunoprecipitated proteins have revealed that the endogenous FKBP25 is associated to the core histones of the nucleosome, and with several proteins forming spliceosomal complexes and ribosomal subunits. Using polyclonal antiFKBP25 we have detected FKBP25 associated with polyribosomes. Added RNAs or 0.5 M NaCl release FKBP25 that was associated with the polyribosomes indicating that the immunophilin has an intrinsic capacity to form complexes with polyribonucleotides via its charged surface patches. Rapamycin or FK506 treatments of the polyribosomes isolated from porcine brain, HeLa and K568 cells caused a residual release of the endogenous FKBP25, which suggests that the immunophilin also binds to some proteins via its PPIase cavity. Our proteomics study indicates that the nuclear pool of the FKBP25 targets various nuclear proteins that are crucial for packaging of DNA, chromatin remodeling and pre-mRNA splicing whereas the cytosolic pool of this immunophilin is bound to some components of the ribosome.

1. Introduction

FKBP25 is a 25-kDa rapamycin-binding immunophilin that has been purified from bovine brain [1]. It is an abundantly expressed intracellular protein that has two distinct domains, namely an N-terminal positively charged RNA/DNA binding domain [2] and a C-terminal PPIase domain (FKBD) [1,3], with a unique charged loop that may bind to diverse negatively charged biopolymers [4]. Mammalian cells express at least 15 isoforms of the FKBPs, some of which are strong binders of rapamycin, namely FKBP12A, FKBP12B, FKBP13 and FKBP25 [5].

The immunosuppressive macrolide rapamycin (sirolimus) was first described for its potential to stop proliferation of certain cancerous cells [6], although some cancers are refractory to the drug [7]. Rapamycin itself can bind to prokaryotic ribosomes [8], whereas in eukaryotes it has an influence on autophagy [9], stimulation of ribosome biogenesis [10], regulation of cell size [11], and quiescence of some organisms [12]. Rapamycin affects diverse cellular activities including the signaling cascade downstream from IL-2 receptor in T-cells, which is believed to be a crucial step causing immunosuppression in humans [13]. In *Saccharomyces cerevisiae*, the ScFKBP12/rapamycin complex inhibits the activity of one of the two isoform of the large multi-domain protein kinase called target of rapamycin (TOR) [14]. Although, the TOR kinase and its allosteric inhibitor, the FKBP12A/rapamycin complex, have functional inputs in cells starting from the unicellular *S. cerevisiae* to the mammalian cells, substrates of this kinase and its modulating co-factors have only been partially established [14–16]. As to whether diverse molecular events induced by rapamycin and diverse rapalogs in the cell [5–16], and its influence on several systemic events such as anticancer action [16–18] or slowing senescence of organisms [12] are all under the control of TOR kinase, or whether some of those rapamycin-induced events are dependent on the other FKBP/rapamycin complexes [5] require further study.

In this communication we show that the endogenous FKBP25 is associated to some components that belong to three different supra-macromolecular entities, namely polyribosomes, nucleosomes and spliceosomal complexes. Our findings are discussed in

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terms of putative functions of the endogenous FKBP25 that may control fine spatial features via cis/trans switch of X-Pro epitopes in diverse intracellular targets.

2. Methods

2.1. Materials

Acrylamide, *N*,*N*′-methylenebisacrylamide, ammonium persulfate, *N*,*N*,*N*′,*N*′-tetramethylenediamine (TEMED), reduced glutathione, chymotrypsin, $(dT)_{12}$-affinity gel, cyclosporine A (CsA), rapamycin, ascomycin, FK506, Triton X100, Tween-20, *N*-octyl glucoside (NOG), deoxycholic acid disodium salt (DOC), and Succ-ALPF-pnA were purchased from Sigma. Molecular mass markers were purchased from BioRad. RNAse inhibitor RNAsin was bought from Promega. 3,3′-Diaminobenzidine tetrahydrochloride (DABT, Fluka) was used for staining immunoblots treated with anti-rabbit IgG peroxidase conjugate (Sigma). HeLa, K568 and U937 (culture of macrophages containing 5×10^9 cells) were purchased from CilBiotech SA (Belgium). Nitrocellulose (Westran S) and PVDF membranes were purchased from Whatman and Millipore, respectively. All other analytical grade chemicals were purchased from Sigma. Rabbit polyclonal antiFKBP25 was prepared from natural FKBP25 isolated from bovine brain [1].

2.2. Fractionation of proteins from HeLa, K568 and U937 cells

Initial steps of fractionation of cells were made according to the method of Chen and colleagues [19]. Briefly, either HeLa or K568 cells (5×10^9) were washed in PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4). PBS and all the other buffers were prepared in sterilized water treated with 0.1% of DEPC. Cells were dispersed in a hypotonic solution (Hs1 buffer: 30 mM Tris–HCl pH 7.5, 10 mM KCl, 5 mM $MgCl_2$, 10 mM DTT) at 4 °C and passed three times via a needle 25G in the presence of the 'Complete protease' inhibitor cocktail tablet (Roche). Nuclei were spun down in JA20 rotor at 1000×*g* at 4 °C during 10 min through the hypotonic buffer containing 24% sucrose. Cytosolic fraction was spun down at 14,000×*g* in JA20 rotor (Beckman) during 10 min (post-mitochondrial supernatant). The supernatant was layered on a discontinuous sucrose gradient (5 ml of 24%, 5 ml of 36% and 5 ml of 48% sucrose in Hs1 buffer). The tubes were spun down during 120 min at 50,000×*g* in a SW28 rotor (Beckman L70 centrifuge). The resulting pellet containing polyribosomes was frozen at −20 °C. Separately, the nuclear and cytosolic extracts were spun down at 14,000×*g* and loaded onto $(dT)_{12}$-cellulose beads (Sigma). The cellulose beads were washed with Hs2 buffer and the bound proteins were eluted with high-salt buffer Hs3 (10 mM Tris–HCl pH 7.5, 1 mM EDTA, 0.5 M NaCl). Before being loaded onto the immobiline gels, the samples were concentrated and desalted in an Amicon cell using a 3 kDa cut-off membrane.

2.3. Preparation of nuclear extracts at two different conditions

U937 or K567 cells (5×10^9) were lyzed as described above. Nuclei were washed two times in the hypotonic buffer containing 0.5% Triton X100 and once in detergent-free buffer and divided into two roughly equal parts. One part was re-suspended in a low-salt aqueous solution (Hs2 buffer: 20 mM Tris–HCl pH 7.5, 100 mM KCl, 2 mM $MgCl_2$, 2 mM EDTA, 0.5 mM EGTA, 10 mM DTT, plus protease inhibitors). The second part was dispersed in buffer Ns1 (20 mM Tris–HCl pH 7.5, 250 mM KCl, 250 mM NaCl, 2 mM $MgCl_2$, 0.2 mM EDTA, 25% glycerol). The suspension was sonicated twice during 30 s at maximum tip setting and the nuclear debris was spun down at 24,000×*g* in 50.2 TI rotor during 1 h. In each case, the pellet was gently shaken during 2 h at 7 °C. The slurry was dialyzed in Ns2 buffer (15 mM Hepes pH 7.5, 60 mM KCl, 15 mM NaCl, 0.5 mM sucrose) to which was added 2 mM $CaCl_2$ and treated with 50 units of Micrococcal nuclease (Thermo Scientific) at 36 °C during 5 min. Digestion was stopped with 10 mM EDTA and each preparation was spun down at 45,000×*g* during 2 h. The supernatant was cleared through 0.22 μM filter and treated with polyclonal antiFKBP25. The mixture was gently shaken at 7 °C during 6 h and Protein-A Sepharose beads (Sigma) were added. After additional 2 h of equilibration, the mixture was spun down at 3500×*g*, and the pellet was washed 3 times with the dialysis buffer +0.05% Tween-20. The beads were treated with 8 M urea and loaded onto either 1D 12% SDS/PAGE or onto 17 cm immobiline 6–11 strips. Proteins at first IEF dimension were focalized during 55,000 Vh and resolved on 20 × 20 cm 15% SDS/PAGE gels.

2.4. Preparation of ribosomal subunits from HeLa and K568 cells

The resulting pellet from sucrose gradient containing polyribosomes was dispersed in Hs4 buffer and the mixture was gently shaken during 20 min at 4 °C. The suspension was centrifuged during 10 min at 70,000×*g* in 50.2 TI rotor (Beckman) and the resulting pellet was again re-suspended in the above buffer and gently shaken during 20 min at 4 °C. The suspension of polyribosomes was again centrifuged at 70,000×*g* in the same rotor during 10 min. The combined supernatant was adjusted to 200 mM KCl (Hs5 buffer) and loaded on $(dT)_{12}$ affinity gel. The gel was gently shaken during 90 min at 4 °C. The affinity gel was washed twice with Hs5 buffer and the proteins were eluted with 10 mM Tris–HCl, pH 7.5 kept at 65 °C. The fractions obtained were resolved on SDS–PAGE and transferred onto PVDF membrane that was kept with anti-FKBP25 at room temperature during 6 h and developed with DAB.

2.5. Acid-induced dissociation of polyribosomes

Soluble fraction of ribosomal proteins was prepared according to the published procedure [20]. The fraction containing the polyribosome particles from gradient ultracentrifugation was dispersed in Hs6 buffer (12.5 mM Tris, pH 7.8, and 50 mM magnesium acetate). Two volumes of glacial acetic acid were added and the mixture was gently shaken during 2 h. The precipitated material containing RNA and residual proteins was removed by centrifugation at 20,000×*g* during 20 min. The RNA pellet was washed twice with Hs5 buffer. The combined mixture of proteins was dialyzed twice against 2 liters of buffer (Hs7 buffer: 25 mM Tris, pH 7.8, 5 mM $MgCl_2$ and 1 mM DTT), concentrated and resolved on 2D gels.

2.6. Treatment of polyribosomes with diverse immunosuppressive drugs

The 80S fraction of polyribosomes were dispersed in two different buffers, namely Hs4 buffer (30 mM EDTA, 0.5% Triton X-100, 25 mM Tris–HCl pH 7.5) and binding buffer Bs1 (100 mM NaCl, 50 mM Tris–HCl pH 7.5, 2 mM $CaCl_2$ and 2 mM $MgCl_2$). Polyribosomal slurry was gently shaken during 10 min at 4 °C. The polyribosomes were centrifuged at 15,000×*g* and dispersed again in Hs4 buffer to which were added the following components: (a) rapamycin at 5.5 μM; (b) FK506 at 5.5 μM; (c) 0.5 M NaCl; (d) an excess of polyRNAs from calf thymus. The samples were gently shaken during 3 h at 4 °C followed by centrifugation at 15,000×*g* in JA20 rotor (Beckman). As control, the same quantity of polyribosomes was shaken in Hs4 and Bs1 in the presence of 0.5% Triton X100. The soluble aliquots and the solid particles were dissolved in the same volume of Laemmli buffer, resolved on 12% SDS–PAGE, blotted onto nitrocellulose and treated with antiFKBP25.

2.7. Gel electrophoresis, blotting and sequencing of proteins

Immobiline gels (General Electric) 3–10L and 6–11L (18 cm) were used. Protein samples were dissolved in the lyse buffer containing 8 M urea, 2% (v/v) Triton X100, 2% carrier ampholytes 7–9 with 100 mM DTT for normal 2D gels and without DTT for samples obtained from immunoprecipitation experiments. IEF/SDS–PAGE gels (12%, size 20 × 20 cm) were carried as described [21]. For Western blotting the gels were transferred onto nitrocellulose membranes and treated with antiFKBP25 in 1% (v/w) solution of casein in 1X PBS. Protocols for immunoprecipitation and in-gel trypsin digestion, protein identification by MALDI-TOF PMF and MALDI-TOF/TOF peptide sequencing are described in Supplementary data

3. Results

3.1. Association of the endogenous FKBP25 with polyribosomes

Polyribosomes were isolated as shown at the right panel of Fig. 1 and their components were resolved on 2D gels (Fig. 2A and B). Proteomic approach combining MS, and MS/MS of randomly chosen spots of proteins revealed several sequences belonging to the ribosomal proteins, namely 60S acidic ribosomal protein P2 (gi:4506671), 40S ribosomal protein S12 (gi:36146), 60S acidic ribosomal protein P0-like (gi:4506667) (Fig. 2A and B and Figs. Fs1A, Fs1B and Fs1C with Table Ts1 in Supplementary material).

Immunoblotting with antiFKBP25 has revealed that the endogenous FKBP25 was detected in the pellet containing polyribosomes (line 1 in Fig. 3 – part A). The endogenous FKBP25 remained associated with ribosomal subunits after the EDTA-induced dissociation of the polyribosomes (line 2 in Fig. 3 – part A). In another experiment, the polyribosomes were dissociated into multiple components upon their treatment with 67% acetic acid. The dissociated ribosomal components were dialyzed against Hs7 buffer and fractionated on 2D gel (Fig. Fs2A, Supplementary material). Immunoblotting with antiFKBP25 showed that the endogenous hFKBP25 was present among the soluble proteins (line 1 in Fig. 3 – part B) but some quantity of it was associated with the remaining mRNA pellet (line 2 in Fig. 3 – part B). To better characterize potential targets of the endogenous FKBP25, we made the following three steps. Firstly, we made immunoprecipitation of proteins present in the fraction containing soluble proteins from the acetic acid-induced dissociation of polyribosomes. In order to increase the chance for co-immunoprecipitation of other proteins with the endogenous hFKBP25, we added RNA- and DNA-free recombinant mFKBP25 (10 μM). Immuno-precipitated proteins were resolved on 2D gels (Fig. Fs3B, Supplementary material) and identified with MS. Besides the mFKBP25 and hFKBP25, several ribosomal proteins were identified in the immunoprecipitated fraction of proteins, namely ribosomal protein P2 (gi:4506671) and 40S ribosomal (scar) protein S4 (gi:337930). Secondly, freshly prepared polyribosomes (line 1, Fig. 3 –part C) were dissociated in 30 mM EDTA into ribosomal subunits and proteins loosely bound were washed off with 200 mM KCl. The ribosomal pellet was treated with 5.5 μM rapamycin. Immunoblotting with antiFKBP25 showed that a small fraction of the endogenous hFKBP25 was released (line 2, Fig. 3 – part C) but a substantial amount of it was still bound to the dissociated ribosomal subunits (line 3, Fig. 3 – part C). Thirdly, polyribosomes were dispersed in Bs1 buffer and treated with 0.5 M NaCl or an excess of RNAs. The immunoblotting with antiFKBP25 revealed that both NaCl and RNAs caused a full release of the immunophilin (Fig. 3 – part D).

Similar set of experiments was made on the polyribosomes isolated from porcine brain. Immunoblotting with antiFKBP25 revealed that the endogenous porcine FKBP25 (pFKBP25) was associated with the fraction containing polyribosomes. In this tissue, the pellet with polyribosomes contained more diversified set of proteins than in the case of the polyribosomes isolated from HeLa or K568 cells, probably due to a greater complexity of the brain tissue that is composed of diverse cells and their connective networks. Analyses of fractions obtained from a size exclusion column loaded with the isolated polyribosomes revealed that the endogenous pFKBP25 was localized in flow-through fraction containing the heavy particles (Fig. Fs3). Although, Triton X100 treatment did not displace the endogenous pFKBP25 from the brain's

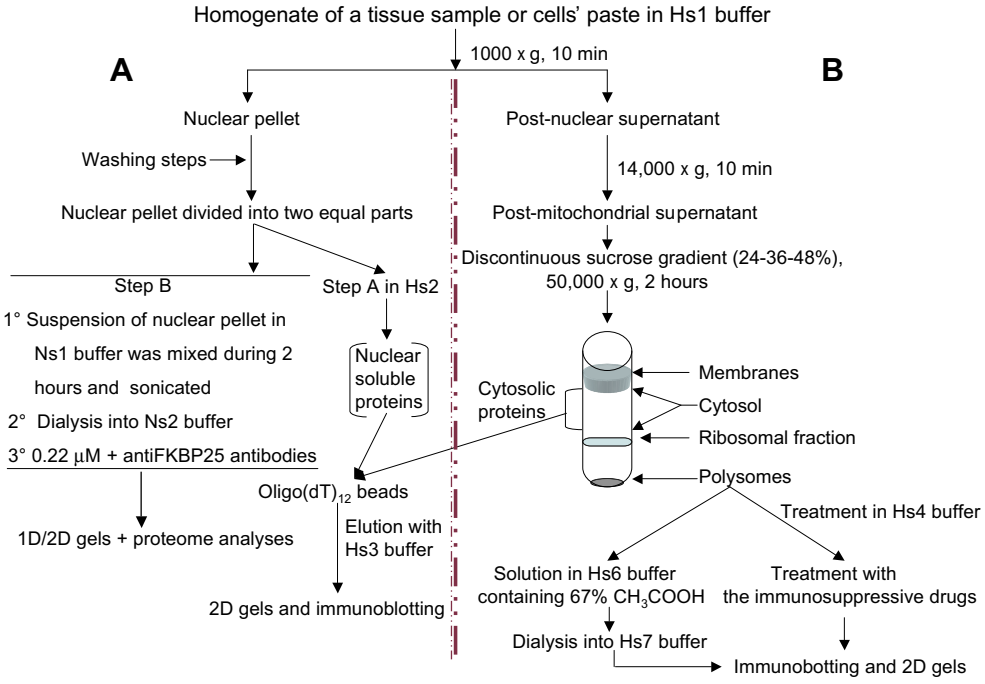

Fig. 1. Diagram of the steps used for isolation and enrichment of various organelles and fractions from tissues and cells.

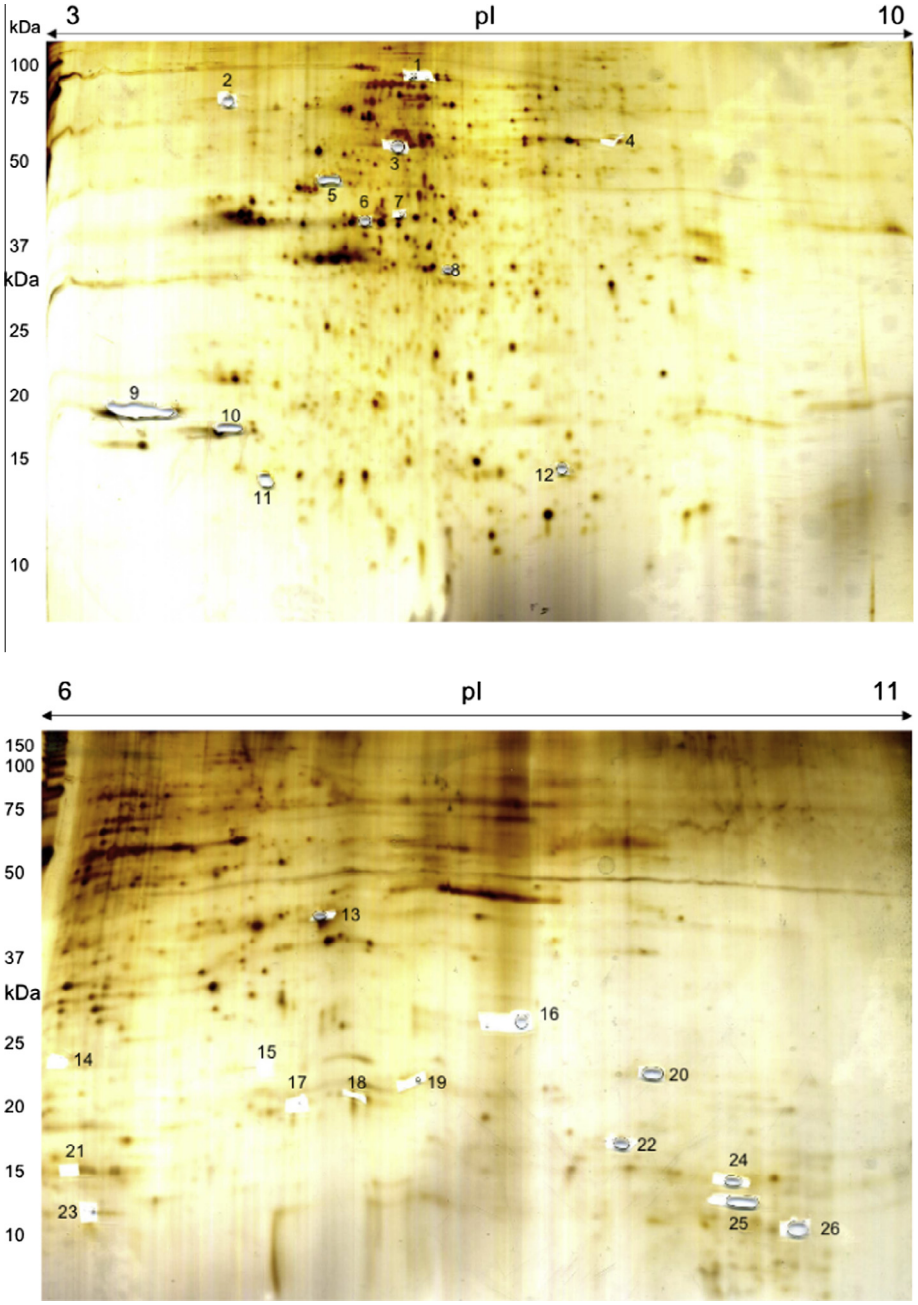

Fig. 2. 2D gels of proteins co-migrating with polyribosomes; (A) pI 3–10; (B) pI 6–11 (see also Figs. Fs1A and Fs1B, and Table Ts1, Supplementary data).

polyribosomes but deoxycholic sodium salt (DOC) treatment released the protein that was found at the top of the sucrose gradient (data not shown). Treatment of the polyribosomes with 5.5 μM of rapamycin or 5.5 μM of FK506 showed that both drugs were capable to release a residual amount of the endogenous pFKBP25 (Fig. Fs4).

3.2. FKBP25 binds to some proteins that form nucleosomes and spliceosome

Nuclear extracts of proteins isolated from U937 (1×10^9 cells) were treated with polyclonal antiFKBP25 and the immunoprecipitated proteins were bound to (Protein A)-Sepharose affinity beads. The pellet was solubilized in 8 M urea and loaded onto SDS–PAGE (12%) in non-reducing Laemmli buffer and onto 6–11 pI (18 cm) immobiline strips. The large 150 kDa band containing the polyclonal antibodies trapped on the affinity beads do not overlap with the low molecular mass bands of low molecular mass proteins. Using several different 2D gels (3–10 and 6–11) we were able to characterize the endogenous FKBP25 with several other proteins. At the lower part of the gel, namely from about 30 to 7 kDa were found several bands whose sequencing by MS/MS is shown in Table 1. All the components of the core nucleosome (H2A, H2B, H3 and H4) were detected. Also, linker histone H1, ribosomal proteins (L13a; NP_036555.1), spliceosome-associated serine/arginine-rich splicing factor 3 (NP_003008.1), and (L7; NP_000962.2) were co-immunoprecipitated with the endogenous hFKBP25.

As some spliceosomal proteins are bound to poly(A)$^+$ mRNAs, we incubated nuclear extracts from HeLa and K568 cells with $(dT)_{12}$-affinity beads. The affinity-entrapped proteins (see the left side of Fig. 1) were fractionated on 2D gels (Fig. 4A). Proteomic analyses (MS-PMF and MS/MS of several tryptic fragments)

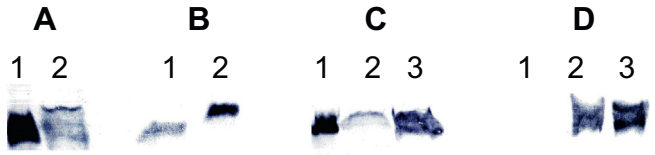

Fig. 3. Detection of the endogenous hFKBP25 in polyribosomes isolated from HeLa cells on Western blots using antiFKBP25 antibody using: (A) 1, polyribosomal pellet; 2, ribosomal pellet after polyribosomes were treated with 0.2 mM KCl and 30 mM EDTA; (B) Polyribosomes dissolved in acetic acid: 1, hFKBP25 in soluble fraction; 2, hFKBP25 remaining in the insoluble pellet. (C) Rapamycin treatment of ribosomes: 1, hFKBP25 remaining in ribosomal pellet after polyribosomes were treated with 0.2 mM KCl and 30 mM EDTA; 2, hFKBP25 released from ribosomal pellet treated with 5.5 μM rapamycin; 3, hFKBP25 remaining in ribosomal pellet after treatment with rapamycin. (D) 1, ribosomal pellet after treatment with polyRNA; 2, hFKBP25 released from ribosomal pellet with an excess of polyRNA; 3 ribosomal pellet after treatment with 0.5 mM NaCl; 4, hFKBP25 released from ribosomal pellet with 0.5 M NaCl.

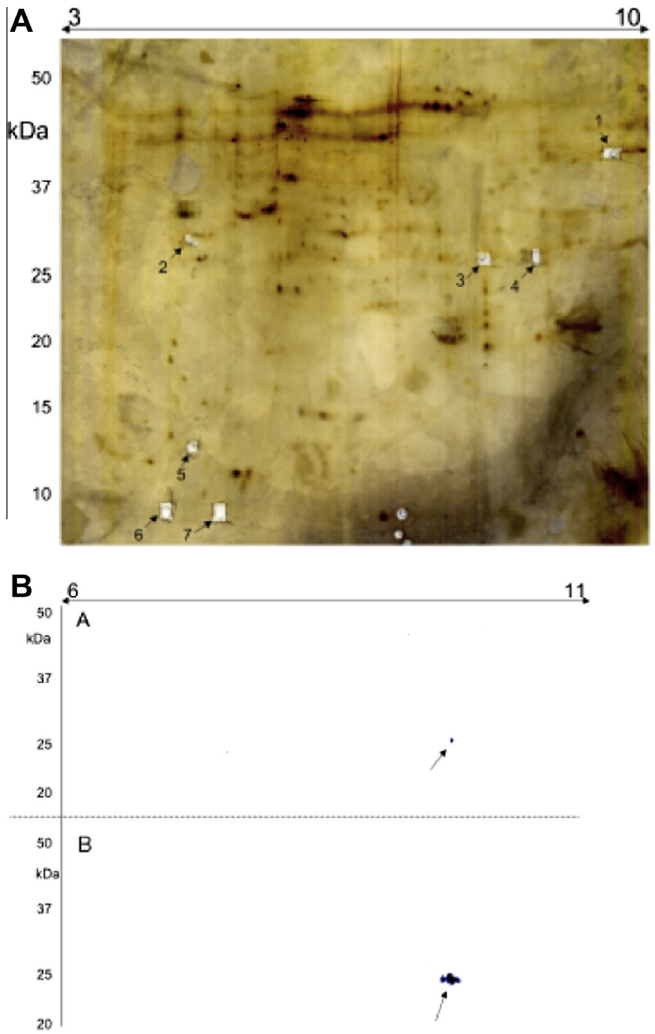

Fig. 4. (A) 2D image of nuclear proteins from HeLa bound to $(dT)_{12}$ affinity gel (3–10); (B) Immunoblots with antiFKBP25 of 2D gels (6–11) of HeLa proteins eluted from $(dT)_{12}$ affinity gels; cytosolic fraction (upper panel) and nuclear fraction (lower panel).

enabled the identification of several proteins associated to spliceosomal components [19,22], namely we found U6 snRNA-associated Sm-like protein (gi:6912488), small nuclear ribonucleoprotein F (gi:4507131), eukaryotic translation initiation factor (gi:4503507) and splicing factor 3B subunit 4 (gi:5032069). The presence of hFKBP25 in the above proteome, and that retained on the affinity beads equilibrated with the cytosolic proteins (the right side of Fig. 1), was established with immunoblotting using antiFKBP25 (Fig. 4B). In Fig. Fs5 (Supplementary data) are shown several profiles of intrinsic PPIase activity of proteins eluted from $(dT)_{12}$ affinity beads (curve A, blue) and its inhibition with cyclosporin A alone (CsA at 15 μM, curve B, green) and after addition of rapamycin at 6 μM and CsA at 15 μM (curve D, violet). The latter curve is similar to the uncatalyzed cis/trans isomerisation of the model peptide Suc-ALPF-pnA (curve C, orange), which indicates that all the PPIase activity was fully inhibited. CsA could only partially inhibit the intrinsic PPIase activity of some cyclophilins associated to spliceosomal components and only the added rapamycin fully inhibited the activity.

4. Discussion

High expression level of the FKBP25 could indicate that this immunophilin would have multiple intracellular targets. Functional interactions between FKBP25 and its diverse targets are consistent with certain sequence-structure related attributes of the immunophilin. For example, the FKBP25s have the high pIs, low hydrophobicity indexes and their fold is made up from several peculiar spatial features present in both domains, namely a basic tilted helix bundle in its N-terminal domain [2] that is linked via a flexible acidic segment to the C-terminus PPIase domain, which has a highly charged bulge [1,3,4]. The latter domain via its cis/trans isomerization potential of X-Pro epitope (PPIase activity) might control spatial positioning of interacting components of a macromolecular structure (conformational switch) as it has been shown for the recruitment and binding of translational GTPase to the ribosomes [23]. Some of the intracellular macromolecular entities, namely 1° nucleosomes; 2° spliceosomal complexes and 3° polyribosomes carry a large percentage of negatively charged segments of DNAs and RNAs, respectively. A relatively strong association of the mammalian FKBP25 with negatively charged biopolymers such as diverse RNAs, DNAs and heparin [4] suggests that one of its functional feature is adapting some proteins to segments of DNAs or RNAs that form large functional entities. Gain of maximal conformational adequacy of some proteins permitting them to fit into the large macromolecular entities could be assisted by conformation-restructuring capacity of the two-domain FKBP25. For example, the spliceosomal complexes contain multiple members of the cyclophilin family of proteins [19,22], which belong to PPIase superfamily of proteins [24].

As the polyribosomal particles treated with rapamycin released only some residual amount of the endogenous FKBP25 indicating that protein/protein interactions between PPIase cavity and some target proteins in the polyribosomes are weak in comparison to charge/charge interactions of the RNA backbone with the surface-exposed positively-charged patches of the immunophilin. This may suggest however that *in vivo* FKBP25 is associated with some proteins associated with polyribosomes through interactions independent of its ribonucleotide binding capacity. One can only speculate that such binding might involve the PPIase cavity from which a natural ligand is dislodged by the immunosuppressive drug. One of the components immuno-precipitated with antiFKBP25 was the S4 ribosomal (scar) protein, which in prokaryotes is a part of 30S subunit of the ribosome (2RWI.pdb) [25]. One might wonder whether FKBP25 *in vivo* could bind to the mammalian ortholog of S4 via its PPIase cavity.

It has been shown that FKBP25 is associated to RNA-granules [26], binds to diverse proteins and biopolymers [4]. Our results suggest that the cytosolic hFKBP25 is RNA-binding protein, which functions in the direct vicinity of the polyribosomes [27]. In the nuclear compartment, FKBP25 is a chaperone of some spliceosomal

Table 1
Proteins identified with MS/MS (tandem mass spectroscopy) from mono- and 2D-dimensional gels of proteins immunoprecipitated with polyclonal antiFKBP25 antibodies in nuclear extract from U937 cells.

Spot	Protein ID Mascot score if detected from MS spectrum (SwissProt restricted in human taxonomy)	# Peptides identified by MS/MS (Mascot scoring)
1	Histone H2A type 1-B/E (H2A1B_HUMAN) Histone H3.3C (H3C_HUMAN) – score 26/4 fragments – 24% sequence coverage Histone H4 (H4_HUMAN) – score 101/9 fragments – 60% sequence coverage Histone H2B type 1-A (H2A1A_HUMAN)	1 (53) 3 (23–76) 5 (38–78) 1 (53)
2	Histone H4 (H4_HUMAN) – score 44/5 fragments – 45% sequence coverage Histone H3.1t (H31T_HUMAN) Histone H2B type 1-A (H2B1A_HUMAN) Histone H2A type 1-B/E (H2A1B_HUMAN) – score 39/5 fragments – 23% sequence coverage	4 (19–58) 2 (26–68) 1 (41) 3 (24–54)
3	Histone H2B type 1-A (H2B1A_HUMAN) Histone H2A type 1-B/E (H2A1B_HUMAN)	1 (42) 1 (57)
4	Histone H2A type 1-B/E (H2A1B_HUMAN) Histone H2B type 1-A (H2B1A_HUMAN)	2 (58–72) 1 (40)
5	Histone H2B type 1-A (H2B1A_HUMAN)	1 (44)
6	Serine/arginine-rich splicing factor 3 (SRSF3_HUMAN) Histone H1.1 (H11_HUMAN) 60S ribosomal protein L13a (RL13A_HUMAN)	1 (20) 1 (18) 1 (36)
7	60S ribosomal protein L7 (RL7_HUMAN)	1 (46)

components. As the FKBP25 co-precipitate with the nucleosomal proteins, thus it may be a crucial component of some steps during chromatin remodeling, DNA repairs or gene transcription [28–30]. However, rapamycin bound to FKBP25 causes some structural rigidity of 80s loop of the immunophilin, which could perturb its intrinsic chaperone activity, and thus may influence some vital cellular processes such as chromatin remodeling (nucleosome), transport and splicing of pre-mRNAs (spliceosome) and the assembly of the ribosome.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.06.105.

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