Matrin CYP, an SR-rich Cyclophilin That Associates with the Nuclear Matrix and Splicing Factors*

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We report the identification and cloning of a nuclear matrix protein termed matrin cyclophilin or matrin CYP. The derived sequence of matrin cyp encodes a protein of 752 amino acids with a predicted mass of 88 kDa. A 172-residue stretch at the amino terminus shows high identity with the ubiquitous family of cyclophilins. Clustered throughout the carboxyl half of the protein are a series of serine-arginine (SR) repeats that are a characteristic feature of many RNA splicing factors. Antibodies raised against matrin CYP recognize a 106-kDa antigen that is detected in isolated nuclei and quantitatively subfractionates in the nuclear matrix. Laser scanning confocal microscopy localizes most of the anti-matrin CYP-specific antigen within the nucleus in a pattern of large bright speckles that co-localize with splicing factors and diffuse nucleoplasmic staining. A strikingly similar pattern of staining is observed in cells extracted for in situ nuclear matrices. A fusion protein containing the cyclophilin domain of matrin CYP exhibits cyclosporin A (CsA)-sensitive, peptidylprolyl cis-trans-isomerase (PPIase) activity, and covalently associated with nuclear speckles. Therefore, we conclude that matrin CYP is an SR-rich cyclophilin that associates with the nuclear matrix.

Several major proteins of the internal nuclear matrix have been identified by immunological methods and termed the nuclear matrices (14). The nuclear matrices include previously characterized proteins such as human RNP-A, human RNP B (15), the nucleolar protein, B23/numatrin (14, 16), the hyperphosphorylated form of RNA pol II LS (17, 18), numerous SR-related proteins (19), and a 125-kDa acidic protein, termed matrin 3 (20). A recent study has confirmed the RNP nature of many of the major nuclear matrix proteins (21).

Here we present results on the isolation of a cDNA that encodes an 88-kDa protein with a cyclophilin domain at the amino terminus and a series of SR repeats throughout the carboxyl half of the protein. This protein, termed matrin cyclophilin or matrin CYP, is enriched in the nuclear matrix, co-localizes with splicing factors at nuclear speckles, and undergoes dynamic rearrangement during mitosis. Matrin CYP fusion protein expresses a cyclosporin A (CsA)-sensitive peptidylprolyl cis-trans-isomerase (PPIase) activity that is characteristic of cyclophilins. We further measure, for the first time, PPIase activity in isolated cell nuclei and demonstrate the quantitative recovery of total nuclear CsA-sensitive activity in the nuclear matrix.

EXPERIMENTAL PROCEDURES

Screening of a Rat Insuloma Library—A random primer digoxigenin-11 dUTP-labeled rat liver partial length cDNA probe was used to screen a pCD-X rat insuloma library (22) via colony hybridization. The probe was detected with the Genius chemiluminescence kit (Boehringer Mannheim). 250,000 colonies were screened and two positives obtained from the quaternary screening. Both positives contained similar sized inserts, and their restriction maps were indistinguishable.

Sequence Analysis of Matrin cyp cDNA—Twelve fragments encompassing the entire matrin CYP full-length cDNA were subcloned into pGEM-3Z (Promega) and sequenced. Sequencing was performed by the dideoxy method using [³²P]dATP and the Sequenase 2.0 kit (U. S. Biochemical Corp.) (23). Additional sequencing was performed over ambiguous regions using synthesized oligo primers (Integrated DNA Technologies). Each fragment was sequenced in the forward and reverse directions, and overlapping sequence was obtained for parts of the cDNA. The cDNA sequence was assembled and analyzed using the GCG computer package. The EMBL, Swiss-protein, and National Institutes of Health nucleotide and amino acid data bases were searched for homology to the matrin cyp cDNA using FASTA, BLAST, and BLITZ programs.

Construction, Expression, and Purification of GST/Matrin CYP-CT and GST/Matrin CYP: NT Fusion Proteins—for GST/matrin CYP-CT a 389-bp region from the matrin CYP cDNA corresponding to nucleotides 1930–2319 was amplified (GeneAmp, Perkin-Elmer) using the following primers with engineered restriction sites: 5’-GATGGATC-3’ and 5’-GATCCGCG-3’. The PCR product was digested and cloned into pGEX-2T (24). Additional sequencing was performed over ambiguous regions using synthesized oligo primers (Integrated DNA Technologies). Each fragment was sequenced in the forward and reverse directions, and overlapping sequence was obtained for parts of the cDNA. The cDNA sequence was assembled and analyzed using the GCG computer package. The EMBL, Swiss-protein, and National Institutes of Health nucleotide and amino acid data bases were searched for homology to the matrin cyp cDNA using FASTA, BLAST, and BLITZ programs.

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§ The abbreviations used are: RNP, ribonucleoprotein; snRNP, small nuclear ribonucleoprotein; CYP, cyclophilin; CsA, cyclosporin A; CTD, carboxyl-terminal domain of RNA polymerase II large subunit; DTT, dithiothreitol; GST, glutathione S-transferase; NK-TR, natural killer tumor recognition molecule; NLS, nuclear localization signal; PBS, phosphate-buffered saline; PPIase, peptidylprolyl cis-trans-isomerase; pol II, polymerase II; bp, base pair(s); AAPF, Ala-Ala-Pro-Phe; DAPI, 4,6-diamidino-2-phenylindole.

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CAGCCAAGACGATAGCTTCCACAG and 3'-GGAGAATTTCGGAGGGCGTTGTTTAAGTAGAT (Integrated DNA Technologies). A 536-bp region from the matrin cyp cDNA corresponding to nucleotides 1-536 was amplified using the following primers: 5'-CACTGGATCCTACGAGCTC and 3'-GAGAATTTCGGAGGGCGTTGTTTAAGTAGAT. The PCR fragments were digested with EcoRI and BamHI and subcloned into pGE-knt (24). The open reading frame and sequence of the pGE-knt clones were verified by DNA sequencing. Each fusion was transformed into JM101 and a single recombinant colony transferred to 1 liter of LB media. The fusion protein was purified by a previously described procedure (25) except that the cells were induced with isopropyl β-D-thiogalactopyranoside, lyzed in a French press (p.s.i. = 18,000), and the fusion proteins were purified over a 1-ml glutathione-agarose bead column (Sigma). The GST/matin CYP-CT fusion protein was cleaved with thrombin (Sigma) while still associated with the fusion protein fractions were run on a 10% SDS-polyacrylamide gel and immunoblotted as described previously (27). Antibodies were further purified by passage over a 1-ml affinity column of matrin CYP-CT linked to StreptHARPORE 4B (Amersham Pharmacia Biotech). The antigen preparation showed no detectable cross-reactivity with the GST portion of the fusion protein.

Subcellular Fractionation and Nuclear Matrix Isolation from Rat Liver Cells—Livers were extracted from adult male Sprague-Dawley rats and processed for rat liver matrices using exogenously added DNase I (28). The supernatants of the washes from the extraction procedure were saved for later use. Additionally, the first post-nuclear supernatant was retained for separation into various cytoplasmic subfractions. A 10,000 × g centrifugation separated a crude mitochondrial pellet and post-mitochondrial supernatant. Next, centrifugation of the post-mitochondrial supernatant at 100,000 × g resulted in a microsomal pellet and cytosol supernatant. Protein fractions were quantified by the BCA protein detection kit (Pierce) and separated by 4-week intervals. Isolation of IgY from egg yolks was performed as described previously (27). Antibodies were further purified by passage over a 1-ml affinity column of matrin CYP-CT linked to Sepharose 4B (Amersham Pharmacia Biotech). The antigen preparation showed no detectable cross-reactivity with the GST portion of the fusion protein.

Phosphatase Treatment—Rat liver nuclear matrices were pelleted to the bottom of a microcentrifuge tube and treated with potato acid phosphatase (Sigma) at a concentration of 0.4 units of phosphatase per g of matrin CYP-CT in incomplete Freund’s adjuvant was given, and the eggs produced by this chicken were immediately retrieved. A total of three booster shots were separated by 4-week intervals. Isolation of IgY from egg yolks was performed as described previously (27). Antibodies were further purified by passage over a 1-ml affinity column of matrin CYP-CT linked to Sepharose 4B (Amersham Pharmacia Biotech). The antigen preparation showed no detectable cross-reactivity with the GST portion of the fusion protein.

Immunoblot Analysis—Protein samples run on an SDS-polyacrylamide gel using standard procedures and stained with Coomassie Blue or processed for immunoblot analysis (described below). The fusion proteins were quantified using the BCA protein detection kit (Pierce). The indicators for the fusion protein were quantified by the BCA protein detection kit (Pierce) and separated by 4-week intervals. Isolation of IgY from egg yolks was performed as described previously (27). Antibodies were further purified by passage over a 1-ml affinity column of matrin CYP-CT linked to Sepharose 4B (Amersham Pharmacia Biotech). The antigen preparation showed no detectable cross-reactivity with the GST portion of the fusion protein.

RESULTS

Molecular Cloning and Sequence Analysis of the Matrin CYP cDNA—Chicken polyclonal antibodies raised against a nuclear matrix protein of approximately 106 kDa were used to isolate a rat liver cDNA from a lambda gt11 expression library. The expressed fusion protein from this clone reacted with the anti-sera, and when sequenced appeared to constitute only the 3' portion of a full-length transcript (data not shown).

By using the partial length cDNA as a probe, a 3971-bp full-length cDNA was isolated from rat liver nuclear matrix cDNA library. Sequence analysis of the cDNA, referred to as matrin CYP, revealed that it contains the partial length cDNA sequence as well as a continuous open reading frame of 2256 bp (Fig. 1B). The ATG at position +1 is a likely candidate for the start codon since the nucleotides around this ATG show near identity to the “Kozak” ribosome binding consensus sequence, and five
codons upstream of this ATG is a termination codon (35). The 3'-untranslated region contains two consensus polyadenylation signals (e.g. AAUAAA). The consensus polyadenylation signal located at nucleotide 3557 appears to be utilized by the full-length rat insuloma cDNA.

The open reading frame of matrin cyp encodes a polypeptide of 752 amino acids. The derived amino acid sequence has a predicted molecular mass of 88,072 daltons and an isoelectric point of 11.05. One intriguing aspect of this amino acid sequence is the preponderance of charged and serine residues as follows: arginine and lysine residues account for 29%, aspartate and glutamate for 16%, and serines for 15% of the total number of residues of matrin CYP (Fig. 1B).

**FIG. 1.** The nucleotide and derived amino acid sequence of matrin CYP. A, a schematic representation of the derived amino acid sequence of matrin CYP indicating the cyclophilin domain (open box), the acidic serine domain (solid box), and the SR repeat regions (stippled boxes). The predicted molecular mass of matrin CYP is 88 kDa, and the predicted isoelectric point is 11.05. B, the nucleotide sequence of the full-length matrin cyp cDNA with the predicted amino acid sequence below. The putative polyadenylation signals are single underlined in the 3'-untranslated region. The cyclophilin domain is single underlined, the acidic serine region is dashed underlined, and the SR repeats are double underlined.
Matrin CYP Is an SR-rich Cyclophilin

Searching computer data bases for similarities to the nucleotide and predicted amino acid sequences identified two regions within the amino acid sequence that show striking similarity to several previously cloned and characterized proteins (Fig. 1A). The first of these domains is located between residues 6 and 178 (Fig. 1B). This region shows a high level of identity with several distinct cyclophilin proteins. Cyclophilin domains have been demonstrated to function as both a proline isomerase and a molecular chaperone (36, 37).

The second domain is composed of a series of SR repeats (Fig. 1B, 29 repeats are marked by bold underline) that cover much of the carboxyl half of the protein. SR-rich regions have been found in several protein factors that are associated with pre-mRNA splicing (38, 39).

Adjacent to the cyclophilin domain (residue 193) is a stretch of 30 amino acids containing 19 serines and 8 aspartate or glutamate residues (Fig. 1B, dashed underline). This acidic serine region is immediately followed by another stretch of 30 amino acids containing 77% basic residues. Various nuclear localization signal (NLS) binding proteins contain similar domains (40, 41).

Consistent with a nuclear localization of matrin CYP, there are numerous NLS motifs in the amino acid sequence. Three putative NLS motifs are particularly rich in SR repeats (amino acids 342–354, 456–469, and 512–567) and resemble the SR repeat regions of the SuWA and Tra Drosophila splicing proteins that have been demonstrated to target cytoplasmic proteins to the nucleus (42). In addition to the SR-rich regions, matrin CYP contains nine classical NLS motifs as follows: six different bipartite-type NLS (starting at positions 226, 233, 338, 437, 452, 608) and three different SV40 large T antigen-type NLS at positions 226, 243, and 619 (43).

Immunological Analysis of Matrin CYP—A fusion protein comprising the carboxyl-terminal residues (644–752) of the full-length matrin cyp cDNA (GST/matrin CYP-CT) was expressed, and polyclonal antibodies were raised against this peptide in chickens (see “Experimental Procedures”). This region of matrin CYP was chosen because it did not contain clusters of SR repeats nor significant similarity with other reported proteins.

Rat liver whole cell extracts were subfractionated into cytoplasmic and nuclear fractions and then probed with the purified anti-matrin CYP-CT antibody on immunoblots. The antibody did not stain protein bands in the cytoplasmic fractions but recognized a diffuse band at about 106 kDa in the purified nuclear fraction (Fig. 2, lane 7). Over 90% of the 106-kDa protein was recovered in the nuclear matrix fraction based on a total nuclear protein recovery of 29% in the nuclear matrix and an approximately 3-fold increase in signal from the purified nuclei to the nuclear matrix fractions (Fig. 2, compare lanes 7 and 11). Correspondingly, no anti-matrin CYP-CT signal was detected in the soluble protein fractions obtained from the nuclease digestion, high salt, or detergent extractions used to prepare the nuclear matrices (Fig. 2, lanes 8–10). Similar protein blots stained with anti-lamin A/C and anti-matrin 3 antibodies, both nuclear matrix-associated proteins, resulted in levels of nuclear matrix enrichment and recovery similar to those observed for matrin CYP (data not shown).

The specificity of the anti-matrin CYP-CT antibody for the 106-kDa antigen was demonstrated by preincubating the antibody with an equal molar concentration of the matrin CYP-CT fusion protein and then probing rat liver nuclear matrix proteins with this mixture. This resulted in the complete inhibition of binding of the anti-matrin CYP-CT antibody to the 106-kDa antigen (Fig. 2, lane 13), whereas a corresponding mock treatment (Fig. 2, lane 12) had no effect.

The difference observed between the apparent molecular mass of 106 kDa and the predicted molecular mass of 88 kDa based on cDNA sequencing is consistent with the highly charged nature of matrin CYP and previous observations that numerous SR proteins exhibit apparent molecular masses on SDS-polyacrylamide gel electrophoresis that are significantly higher than predicted from amino acid sequences (38, 44–46). Moreover, sequence analysis revealed 46 known phosphorylation motifs in matrin CYP including 19 casein kinase II and 14 protein kinase C sites. Indeed, a phosphatase-mediated shift in the migration of matrin CYP from 106 to 98 kDa (Fig. 3, lane 1) indicates that a significant portion of the higher apparent molecular weight exhibited by matrin CYP is due to a phosphorylated state of this protein in the cell nucleus.

Matrin CYP Is Localized to Nuclear Speckles during Interphase—Immunofluorescence analyses using anti-matrin CYP-CT confirmed the immunoblot results indicating that this
antibody is highly specific for a nuclear antigen. Double staining immunofluorescence of primary cultures of rat parenchymal cells with anti-lamin A/C and anti-matrin CYP-CT was imaged by laser scanning confocal microscopy and revealed that anti-matrin CYP-CT decorated intranuclear structures consisting predominantly of large irregularly shaped foci that do not correspond to the nucleoli (Fig. 4, A–C). Similar structures were stained by anti-matrin CYP-CT using a wide variety of fixation protocols (see “Experimental Procedures”) or in other cell lines such as mouse 3T3 fibroblasts or human HeLa cells (data not shown). In addition to the bright foci, a less intense, diffuse staining was detected throughout the nucleoplasm (Fig. 4A). A very low intensity background staining was also consistently observed throughout the cytoplasm.

The specificity of anti-matrin CYP for these various structures was tested by preincubating this antibody with different levels of matrin CYP-CT fusion protein and performing immunofluorescence with these mixtures. By using this competitor peptide approach, the very intense staining of the speckled sites was virtually abolished at an antibody:matrin CYP fusion protein molar ratio of 0.3, whereas the diffuse nucleoplasmic and cytoplasmic staining was not affected (Fig. 5). We conclude that the matrin CYP antibody is at least predominantly decorating matrin CYP at the speckled sites. These findings are consistent with the immunoblot results (Fig. 2) that did not detect matrin CYP antigens in the cytoplasmic fractions from rat liver cells.

Rat parenchymal cells in primary culture were extracted for in situ nuclear matrices (see “Experimental Procedures”) and then double-stained as above. The results show that, like those observed with immunoblot analysis, the anti-matrin CYP-CT-specific antigens are tightly associated with the nuclear matrix (Fig. 4, G–I). Moreover, this association is maintained in a similar distribution of bright foci and less intense nucleoplasmic staining characteristically observed for fixed cells (Fig. 4, G–I).
Matrin CYP Redistributes during Mitosis and Associates with Other Non-snRNP SR Proteins—Mouse 3T3 cells were synchronized by serum deprivation and examined at times when mitotic figures are maximal (see “Experimental Procedures”). At the onset of mitosis, matrin CYP is remodeled from its characteristic speckle pattern to a more diffuse pattern that is distributed between the condensing chromosomes (Fig. 6A). In late prophase (as the nuclear envelope breaks down) the diffuse staining of matrin CYP extends into the cytoplasm (Fig. 6C). At prometaphase, metaphase, and anaphase (when the chromosomes are fully condensed) the diffuse staining of anti-matrin CYP-CT distributes uniformly throughout the mitotic cytoplasm along with a limited number of small punctate structures (Fig. 6, E, G, and I). The characteristic exclusion of matrin CYP from the chromosomes is maintained throughout mitosis. During telophase, matrin CYP undergoes a significant rearrangement as the diffuse staining becomes less pronounced and many round bright foci are observed scattered throughout the cytoplasm (Fig. 6K).

Similar nuclear redistributions during mitosis have been previously reported for several antibodies specific for non-snRNP SR splicing factors (80). Consistent with these results, we found in late telophase cells a nearly identical localization of anti-matrin CYP-CT and the monoclonal antibody B1C8 (Fig. 7, A and B), which recognizes an SR protein of 160 kDa (33). An antibody specific for a small constellation of non-snRNP, SR proteins, NM-4 (19), also showed a very similar distribution as anti-matrin CYP-CT in late telophase cells (data not shown). In contrast, snRNP proteins, as detected by Y12, localized predominantly to the interior of the nucleus during late telophase and showed no enrichment in the cytoplasmic foci stained by anti-matrin CYP-CT (Fig. 7, D and E). Identical results were obtained using the U1 70-kDa specific antibodies (data not shown).

By using two alternative procedures, we next examined whether matrin CYP fractionates with other SR proteins. Matrin CYP was enriched from 5- to 10-fold on a protein basis in the SR protein fraction of Blencowe et al. (19) compared with the initial HeLa nuclear extract (Fig. 8, lanes 1 and 2). In contrast, matrin CYP was not detected in the classical SR protein preparation of Zahler et al. (50) and was, therefore, highly depleted in this fraction compared with the nuclear extract (Fig. 8, lanes 1 and 3). These results support previous observations (19) that numerous SR-related proteins (including several nuclear matrix proteins) are not present in the final preparations of Zahler et al. (50).

Matrin CYP Is a Functional PPIase—A fusion protein containing the cyclophilin domain of matrin CYP linked to the carboxyl tail of glutathione S-transferase (GST/matrin CYP-NT, see Fig. 1) was expressed in Escherichia coli and purified to over 90% purity from a glutathione bead column (Fig. 9A). GST/matrin CYP-NT was capable of catalyzing the conversion of the cis-proline tetrapeptide substrate, AAPP, to the transform in an in vitro assay (Fig. 9B, line a). This activity was well above the spontaneous background observed when no exogenous proteins were added to the assay (Fig. 9B, line g). The isomerase activity is a function of the matrin CYP-NT domain since the GST portion was not capable of acting as a PPIase alone (Fig. 9B, line f). The homologous NK-TR cyclophilin (26) also exhibited PPIase activity, albeit at a slightly lower level than matrin CYP (Fig. 9B, line b). The calculated $k_{\text{cat}}/K_m$ for GST/matrin CYP-NT is 16-fold lower than those reported for human CYP A, but within the range reported for other cyclophilins using similar tetrapeptide substrates (Table I).

The PPIase activity of this fusion protein was completely abolished by preincubation with CsA (Fig. 9B, line e) as was its

Since the predicted amino acid sequence of matrin CYP contains clusters of SR repeats and the nucleoplasmic foci stained by anti-matrin CYP-CT were reminiscent of splicing factor rich nuclear “speckles” observed in other studies (32, 33, 47–49), double labeling immunofluorescence microscopy was performed with anti-splicing factor specific antibodies and anti-matrin CYP-CT. Indeed, antibodies specific for the 70-kDa subunit of the U1 snRNP co-localizes with anti-matrin CYP-CT in rat parenchymal cells (Fig. 4, D–F). Similar results were obtained using other splicing factors (e.g. Y12) or different mammalian cell lines, e.g. mouse 3T3 fibroblasts or human HeLa cells (data not shown).
Matrin CYP had an inhibitory sensitivity to CsA (IC50) of 220 nM that is within the range of IC50 values reported for other cyclophilins (Table I). Obtaining the direct binding constants for CsA and GST/matrín CYP-NT was not feasible for the same reasons as previously discussed for the NK-TR cyclophilin (26).

Functional Nuclear Cyclophilins Associate with the Nuclear Matrix—Since matrin CYP is quantitatively recovered in the nuclear matrix, we next investigated whether CsA-inhibited PPIase activity is present within endogenous rat liver nuclear matrix proteins. Rat liver nuclear and nuclear matrix proteins were incubated in 9M urea and 50 mM DTT that resulted in the nearly complete (>90%) solubilization of proteins from these fractions (Fig. 10A, lanes 1 and 3). Most of the protein, including matrin CYP, remained in solution after the urea and DTT were diluted 10,000-fold by dialysis (Fig. 10, A and B, lane 5). The relatively harsh procedure of urea and DTT treatment used to solubilize rat liver nuclear and nuclear matrix proteins had little inhibitory effect on the PPIase activity of similarly treated GST/matrín CYP-NT (>90% recovery of total enzyme activity).

The in vitro PPIase revealed a 2.2-fold higher specific activity in the nuclear matrix proteins compared with the nuclear proteins (Table II). This corresponded to a recovery of 63% of the total nuclear PPIase activity in the nuclear matrix fraction. Moreover, the CsA-sensitive PPIase activity was quantitatively associated with the nuclear matrix fraction (Table II) with a GST/NK-TR counterpart ((Fig. 9B, line d). Matrin CYP and GST/NK-TR cyclophilins were expressed in E. coli and purified to near homogeneity. Cell lysates or purified fusion proteins were run on a 10% SDS-polyacrylamide gel and stained with Coomassie Blue. Lane 1, GST/matrín CYP-NT uninduced; lane 2, GST/matrín CYP-NT induced; lane 3, GST/matrín CYP-NT purified; lane 4, GST/NK-TR uninduced; lane 5, GST/NK-TR induced; lane 6, GST/NK-TR purified. B, GST/matrín CYP-NT, GST/NK-TR, and GST alone were tested for in vitro PPIase activity in a chymotrypsincoupled assay using the tetrapeptide substrate N-succinyl-AAPF-p-nitroanilide (34). The graph indicates PPIase activity as an increase in absorbance. Line a, 145 nM GST/matrín CYP-NT; line b, 145 nM GST/NK-TR; line c, 36 nM GST/matrín CYP-NT; line d, 100 nM GST/NK-TR and 10 μM CsA; line e, 100 nM GST/matrín CYP-NT and 10 μM CsA; line f, 145 nM GST; line g, no protein added.
TABLE I

PPIase activity associated with cyclophilin proteins

| Protein | $k_{cat}/K_m$ | $IC_{50}$ |
|---------|-------------|-----------|
| NK-TR   | $7.4 \times 10^6$ | 770 |
| Matrin  | $1.0 \times 10^6$ | 220 |
| CYP     | $1.9 \times 10^5$ | 300 |
| CYP-40   | $6.3 \times 10^4$ | 84 |
| Human   | $1.5-1.7 \times 10^3$ | 6.6-25 |

* Rin fret et al. (26).
* Ki effer et al. (54).
* Price et al. (55).

Fig. 10. Solubilization of total nuclear matrix proteins includes matrin CYP. The majority (>80%) of nuclear matrix proteins, including matrin CYP, remain in the supernatant after solubilization and dialysis. A, rat liver nuclear matrix proteins were solubilized in 9 M urea, 50 mM DTT and dialyzed as described under "Experimental Procedures." Equal volumes were loaded in lanes 1–5 as follows: lane 1, untreated rat liver nuclear matrix (10 µg); lane 2, pellet from urea/DTT-solubilized rat liver nuclear matrix; lane 3, supernatant from urea/DTT-solubilized nuclear matrix; lane 4, pellet from dialyzed and solubilized nuclear matrix; lane 5, supernatant from dialyzed and solubilized nuclear matrix. The gel was stained with Coomassie Blue. B, immunoblot stained with anti-matrin CYP-CT of a gel similar to A. Total nuclear proteins were solubilized similarly to nuclear matrix proteins (data not shown). Size markers are indicated in kDa.

4.4-fold increase in specific activity compared with the corresponding total nuclear CsA-sensitive activity. In contrast, CsA-resistant activity was not significantly enriched (1.1-fold) on the nuclear matrix compared with the total nuclear activity (Table II).

DISCUSSION

The Cyclophilin Domain of Matrin CYP—Matrin CYP belongs to a highly conserved and large class of proteins termed cyclophilins that function as peptidylprolyl-isomerases (PPIases) to catalyze the conversion of cis-proline to trans-proline in a polypeptide chain. Cyclophilins bind the immunosuppressive drug cyclosporin A that inhibits the associated PPIase activity (51, 52). They are ubiquitous throughout the phylogenetic scale (53) and are located in a wide variety of subcellular compartiments (37, 54–63) where they have been proposed to function in protein folding as well as a chaperone for protein targeting and macromolecular assembly (26, 36, 37, 52, 55, 57, 59, 64–69). Initial studies indicate a wide range of functional roles for cyclophilins such as involvement in T-cell activation (70), natural (tumor) killer cell function (71), protein secretion (59, 69), photoreceptor cell function via formation of active forms of rhodopsin 1 and 2 proteins (64, 67), steroid receptor function (68), myeloid cell differentiation (72), tumor cell growth (73), and formation of infectious human immunodeficiency virus-I virions through interactions with the Gag polyprotein (74, 75).

Although matrin CYP is the first cyclophilin demonstrated to be predominantly located within the nucleus, an FK506-binding protein PPIase was previously found in the nucleolus (76), and a parvulin PPIase has been localized to the nuclear speckles (77). Neither of these PPIase activities exhibit sensitivity to CsA. Nestel et al. (78) and Bourquin et al. (79), using the yeast two-hybrid approach, independently identified and sequenced the human homolog of the rat matrin cyp (GenBank™ accession numbers U40763 and X99717, respectively) that is 93% identical in amino acid sequence. Complementing our findings, transient transfection experiments revealed that the overexpressed human matrin CYP protein co-localized at splicing factor-rich nuclear speckles and exhibited a possible nuclear matrix association (79). Matrin CYP also shows significant homology to the cyclophilin containing protein termed NK-TR (GenBank™ accession numbers LO4288 and LO4289, Ref. 71). Although originally described as a plasma membrane component, NK-TR may also be a nuclear component involved in myeloid cell maturation (72).

Consistent with the localization of matrin CYP in the cell nucleus and its enrichment in the nuclear matrix, we measured significant levels of PPIase activity associated with isolated rat liver nuclei (Table II). The nearly complete recovery of matrin CYP and its characteristic CsA-sensitive PPIase activity in the nuclear matrix fraction (Table II) indicates that a part (if not all) of the CsA-sensitive PPIase activity in the nucleus and nuclear matrix is contributed by the matrin CYP protein. This suggests an involvement of higher order structure in the important but poorly understood events of protein folding, targeting, and macromolecular assembly at discrete sites of nuclear function (e.g. DNA replication, transcription, and/or RNA splicing).

Possible Functions of Matrin CYP—Further studies are needed to determine the properties of matrin CYP compared with other SR proteins (39). The SR repeats of matrin CYP, for example, may potentiate specific interactions with other SR proteins (81–83). Enrichment of matrin CYP in a nuclear matrix-associated SR protein fraction (Fig. 8 and Ref. 14) suggests a possible role of matrin CYP in the higher order assembly of SR proteins in the cell nucleus. One property associated with SR proteins is the specific phosphorylation of serine residues present in the serine-arginine dipeptide repeats (39). Misteli and Spector (84) have recently presented a model outlining a central role of protein phosphorylation/dephosphorylation in the spatial and temporal coordination of transcription and pre-mRNA splicing. For example, phosphorylation of SC-35 and other splicing factors by the cyclin-independent mitotic kinase SRPK-1 has been linked to the disruption of nuclear speckles observed at the onset of mitosis (85). Similarly, overexpression of the clk kinase resulted in the redistribution of splicing factors from speckles to a diffuse pattern (86). Clk kinase binds to a number of SR proteins and directs (at least in part) their phosphorylation states (86). Likewise, matrin CYP has recently been identified as a specific clk kinase binding protein via the yeast two-hybrid approach (78). Clk kinase is,
therefore, a prime candidate for being involved in matrin CYP phosphorylation and the regulation of matrin CYP function such as its associated PPIase activity.

Evidence suggests that splicing factors undergo a dynamic redistribution after participating in pre-mRNA splicing. This has been demonstrated by the cycling of snRNP and non-snRNP splicing factors to and from nuclear speckles (89) and, more recently, by direct observations in living cells (88). We propose that matrin CYP may act as a molecular chaperone that is involved in the dynamic regulation of the nuclear speckle domains. Through its cyclophilin domain and associated PPIase activity, matrin CYP may hold SR proteins in a state from which they cannot associate with each other. This could prevent undesirable aggregation of splicing factors and would be an efficient means to keep a warehouse of splicing factors ready for the variable expression needs of the cell.

In addition, the proline isomerase of matrin CYP may assist proteins in nuclear speckles to properly fold and associate into appropriate macromolecular assemblies.

The dynamic movement of speckle-associated proteins is also closely coupled to RNA polymerase II transcription (88). In this regard, a hyperphosphorylated form of the large subunit of pol II (pol IIo) is associated with pre-mRNA splicing, and a subset of SR proteins may mediate this association through interactions with the highly phosphorylated carboxyl-terminal domain (CTD) of pol IIo (17, 18, 90–92). Recently, a yeast two-hybrid screen has identified the human homolog of matrin CYP as strongly interacting with the CTD of pol II and, thus, a member of this CTD-binding protein family (79, 92). This interaction implicates matrin CYP as a possible regulator in the coordination of transcription and pre-mRNA splicing perhaps through its cyclophilin domain. Indeed, the extraordinarily high proline content of the CTD (2 prolines per heptapeptide repeat or 30% of total amino acids) makes it an obvious target for interaction with the CTD of pol IIo.

Interaction of matrin CYP with the CTD of pol II and/or other CTD-binding proteins may, in turn, be regulated by specific phosphorylation events mediated by cdk kinase and other protein kinases such as SAPK-1. In this regard, Bourquin et al. (79) demonstrated that human matrin CYP requires the SR repeat motifs (known phosphorylation target sites of the cdk kinase, see Ref. 86) to interact with the CTD in vitro. In contrast, all other characterized CTD-binding proteins interact with the CTD at other regions on the proteins (90, 92, 93). The nuclear matrix association of pol IIo (17, 18), matrin CYP (Ref. 79 and this study), and a recently characterized CTD-binding protein termed SCF89 (94) together imply an important role of nuclear architecture for coupling transcription and RNA splicing in the cell nucleus (87).

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