FAP48, a New Protein That Forms Specific Complexes with Both Immunophilins FKBP59 and FKBP12

PREVENTION BY THE IMMUNOSUPPRESSANT DRUGS FK506 AND RAPAMYCIN*

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We have identified a human gene encoding a 48-kDa protein that specifically interacts with the peptidyl prolyl isomerase FK506-binding protein 59 (FKBP59) and also with the well known FKBP12. FKBP59 and FKBP12 belong to the large family of immunophilins that bind the macrolide immunosuppressant drugs FK506 and rapamycin. The yeast two-hybrid system was used to isolate target proteins that interact with the immunosuppressant drug binding domain of the rabbit FKBP59. The cDNA for an as yet unidentified protein was isolated and cloned from a Jurkat cell library. The cDNA sequence of 1804 base pairs reveals an open reading frame of 417 amino acids. In vitro experiments suggest a direct interaction between FKBP59 and this new target protein. This specific association seems to be restricted to the FKBP family, since it also occurs both in vivo and in vitro with FKBP12 but not with cyclophilin 40. This novel protein was named FKBP-associated protein (FAP48). The formation of the complexes between FKBP59 or FKBP12 and FAP48 is prevented by FK506 and rapamycin in a dose-dependent manner. These results suggest that FAP48 shares or overlaps the macrolide binding site on FKBP59 as well as on FKBP12 and therefore may represent a natural common ligand of these immunosuppressant drug receptors.

Immunophilins are a family of ubiquitously expressed intracellular receptors for the structurally unrelated immunosuppressant drugs cyclosporin A (CsA, a cyclical undecapeptide), FK506, and rapamycin (two related macrolides). CsA binds to cyclophilins, whereas FK506 and rapamycin bind to distinct receptor proteins termed FK506-binding proteins (FKBPs). Among the growing family of FKBPs the best characterized member is a 12-kDa isoform, FKBP12 (reviewed in Refs. 1–4).

Although cyclophilins and FKBP12 differ markedly in amino acid sequence, both immunophilins display peptidyl-prolyl cis-trans-isomerase (rotamase) activity, which is inhibited by their respective ligands. However, this inhibition does not appear to explain the effects of the immunosuppressants (5). In fact, CsA and FK506 stabilize the binding of their receptors to the calcium-activated serine phosphatase calcineurin (6 and refs. therein). This association leads to the inhibition of the catalytic activity of calcineurin, which is required for the initial Ca2+-dependent step in the activation of T lymphocytes via the T cell receptor. On the other hand, the rapamycin-FKBP12 complex does not inhibit calcineurin activity but interacts with a target protein termed rapamycin and FKBP12 target (RAFT) (7) or FKBP-rapamycin-associated protein (FRAP) (8) and appears to block the T cell response at a later stage (9). In the presence of calcineurin, FKBP12 is tightly associated with calcium release channel proteins, the ryosine receptor, and the inositol, 1,4,5-trisphosphate receptor, which mediate calcium flux (10, 11). These associations do not require FK506 or rapamycin, and indeed these drugs dissociate the calcineurin-FKBP12 channel protein complexes (10–12). These recent findings led to the proposal that FKBP12 may function by anchoring the phosphatase to its appropriate substrates, thus facilitating the cycle of receptor phosphorylation and dephosphorylation, which in turn would modulate Ca2+ flux (12).

Additional immunophilins have been cloned and characterized (reviewed in Refs. 2 and 4), including a 59-kDa protein that was originally discovered as a component of unactivated steroid receptor heterocomplexes (13, 14). Elucidation of the primary structure of the rabbit FKBP59 (15) and its human homologue FKBP52 (16), hydrophobic cluster analysis (17), and biochemical data (18–21) have demonstrated a modular organization of this protein, including individual and functional domains as structural units that can be active independently of each other. The N-terminal domain (amino acids 1–149), defined as FKBP59-I, shares 55% identity with FKBP12 (over a common sequence of 106 amino acids) (15, 16), binds FK506 and rapamycin, and displays peptidyl-prolyl cis-trans-isomerase activity (18). The second domain (amino acids 149–263), FKBP59-II, is able to bind ATP and GTP (22). The C-terminal part of the molecule, covering domains III and IV, includes a calmodulin binding site (amino acids 406–420) (23) and three tetratricopeptide repeat sequences (amino acids 273–389) (24). We have recently reported that the tetratricopeptide repeat sequences of FKBP59 are required for its binding to Hsp90 (25), an abundant (~1%) and widely expressed soluble protein, which is another component of nontransformed steroid receptors. This result has been confirmed for the cyclosporin A-binding protein of 40 kDa (CyP40), which also includes tetratricopeptide repeat sequences (26, 27). Although the exact relationship between FKBP59 and steroid hormone receptors is currently not defined, the association between the two proteins,
via Hsp90, raises the possibility of influence by immunosuppressive drugs on steroid hormone receptor function. The fact that FKBP59 does not interact with calcineurin (28–30) or other cellular proteins already described as interacting with FKBP12 warranted a search for FKBP59 targets. In addition, the relatively strong FK506-FKBP59 binding in vitro suggests that FK506 is mimicking some natural, possibly peptidyl, ligand for FKBP59. We undertook the search for other proteins that interact with FKBP59, since defining the natural ligands and substrates of immunophilins may elucidate the cellular events associated with signal transduction pathways and protein folding. We chose to use the yeast two-hybrid screening system originally described by Fields and Song (31), since it has the advantage of detecting protein-protein interactions in vitro, including those of low affinity (32).

Since we sought to identify proteins that would interact with the immunophilin domain of rabbit FKBP59 and avoid the isolation of Hsp90, the first domain of FKBP59 (FKBP59-I) was used as the bait to isolate different targets in a Jurkat cell cDNA library. This led to the identification and characterization of a gene encoding a novel protein that binds the first domain of FKBP59 as well as the full-length protein. We also present evidence that this protein interacts specifically with FKBP59. We undertook the search for other proteins including those of low affinity (32).

FKBP12 warranted a search for FKBP59 targets. In addition, the relatively strong FK506-FKBP59 binding in vitro, suggests that this protein interacts specifically with FKBP12 as a fusion protein with glutathione S-transferase (GST) (33). The GST-FKBP12 fusion protein was used as the bait to isolate different targets in a Jurkat cell cDNA library using the lithium acetate method (34). Double transformants were plated on synthetic medium lacking histidine, leucine, and adenine. The yeast L40 strain either with pLexA-FKBP59-I or with an extraneous plasmid (pBTM116 vector, previously treated with DNA polymerase (Klenow fragment). The resulting plasmid pLexA-FKBP12 expressed FKBP12 as a fusion protein with the DBD of LexA. For expression of FKBP12 as a fusion protein with glutathione S-transferase (GST), the insert described above was ligated into the Smal restriction site of the pGEX-2T vector (Pharmacia Biotech Inc.).

FKBP59-L1 (amino acids 32–138) was obtained from FKBP59-I cloned in pGEM-7Zf (+) (Promega Corp., Madison, WI) (15, 18) into the EcoRI restriction site of the pBTM116 plasmid to give respectively, pLexA-FKBP59 and pLexA-FKBP59-I. The pGEM-L1 (amino acids 32–138) was obtained from FKBP59-I cloned in pGEM-7Zf (+) (Promega) and sequenced by the manufacturer. The mutations were checked by sequencing the cDNA corresponding to clone 26, inserted into the pGEM-11Zf (+) vector, which was transformed into Escherichia coli. The mutations were determined by dideoxy termination method. The concentration of bound protein as reported (43) using bovine serum albumin as a standard. All experiments described below were performed with 5 μg of immobilized bait proteins. Sepharose-bound GST alone or GST fusion proteins were diluted with Sepharose CL-4B and added to binding buffer (HBD; 20 mM HEPES, KOH, pH 7.8, 75 mM NaCl, 0.1 mM EDTA, 2.5 mM MgCl2, 10% glycerol, and 1 mM dithiothreitol).

Labeled proteins were prepared by transcription and translation in the presence of [35S]methionine in the TNT T7 coupled rabbit reticulocyte lysate system as recommended by the supplier (Promega). The translated lysates were diluted with HBD and used directly in binding assays. Briefly, the diluted sample was divided into aliquots and incubated with 200 μl of 50% (v/v) Sepharose beads overnight at 4°C. The beads were washed five times with 1 ml of HBD, and then bound proteins were solubilized with HBD and Laemml sample buffer. The supernatant was subjected to SDS-polyacrylamide gel electrophoresis, and [35S]-labeled polypeptides were detected by autoradiography and fluoroimaging. The duration of exposure of the gels was 1–4 days using Kodak XAR film. Molecular weight markers were myosin (M r 200,000), rabbit muscle phosphorylase b (M r 97,400), bovine serum albumin (M r 67,000), ovalbumin (M r 45,000), and glutathione S-transferase (M r 28,000) (all from Life Technologies), or myosin (M r 200,000), β-galactosidase (M r 116,250), phosphorylase b (M r 97,400), serum albumin (M r 66,200), and ovalbumin (M r 45,000) (all from Bio-Rad). Where indicated, increasing amounts of immunosuppressant drugs were added from 10 mM stocks in ethanol to the GST-immunophilin-coupled...
RESULTS

Strategy—Because, although ubiquitous, FKBP59 is expressed at an especially high level in T lymphocytes (44), a cDNA library from the Jurkat cell line was used to identify proteins that interact with domain I of FKBP59. Plasmids encoding FKBP59-I fused to LexA-DBD:pLexA-FKBP59-I and the Jurkat cell cDNA library were cotransformed into the yeast L40 strain. Two million yeast double transformants (corresponding to $5 \times 10^5$ independent clones) were screened and selected for histidine prototrophy. Among the 100 colonies isolated as His$^+$, 48 were found to display $\beta$-galactosidase activity. Plasmids were rescued from each of these clones and used for retransformation of the reporter strain in combination with pLexA-FKBP59-I or with an extraneous bait, pLexA-lamin. Seven clones remained positive (clones 2, 4, 20, 24, 26, 29, and 89). From restriction mapping and partial sequence analysis, it appeared that two groups of cDNA, encoding distinct proteins, had been isolated. Clones 2, 4, 24, and 89 correspond to the same sequence and will be described elsewhere. Clone 26, which is redundant with clones 20 and 29, was selected for the present study.

Structure of FAP48—Nested deletions of the cDNA insert from clone 26 were subjected to double-stranded dyeodeoxyribonucleotide sequence analysis, which revealed an open translational reading frame throughout its 1804 bp.

The full-length size of mRNA encoding clone 26 was first determined by Northern blotting of poly(A)$^+$ RNA (5 $\mu$g) from Jurkat cells using the labeled clone 26 as probe. A unique 2-kilobase RNA band was revealed, as shown in Fig. 1. To map the 5'9-end of this mRNA more precisely, a primer extension was performed by using EXA1 as primer (annealing to positions 74–97), and a predominant product of 229 bp was observed (data not shown), in good agreement with the result of the Northern blotting analysis.

To obtain the cDNA containing the remainder of the coding sequence, we performed a 5'-RACE-PCR from clone 26 were subjected to double-stranded dyeodeoxyribonucleotide sequence analysis, which revealed an open translational reading frame throughout its 1804 bp.

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To obtain the cDNA containing the remainder of the coding sequence, we performed a 5'-RACE-PCR (as described under “Materials and Methods”). Of the 27 5'-cDNA-specific clones that were recovered, the 4 longest contained 33 additional base pairs. This 33-base pair sequence contained the remainder of the coding sequence of FAP48. This sequence includes an open reading frame of 1251 nucleotides (nucleotides 94–1344) encoding a protein of 417 amino acids with a predicted molecular mass of 48,135 daltons, a calculated pI of 5.57, and a predicted index of polarity of 46.76%. This sequence contains a 3'-untranslated region the three potential polyadenylation signal sites are underlined.

The nucleotide and derived amino acid sequence of FAP48, +, in-frame termination codon upstream and downstream from the first methionine (box). Arrow, first base of clone 26. In the 3'-untranslated region the three potential polyadenylation signal sites are underlined.
found with four expressed sequence tags, HUM519D05B, HHEA18H, R58066, and H10872, isolated, respectively, from human placenta, adult and fetal heart, and infant brain cDNA libraries, we did not find any other significant homologies with known sequences in these data bases.

The consensus secondary structure prediction indicated a large proportion of an \( \alpha \)-helix and a little \( \beta \)-sheet: 58% \( \alpha \)-helix, 8% \( \beta \)-sheet, and 34% turn/coil. A dot plot gave no indication of internal homologies in FAP48. Despite the high proportion of leucines (16.5%), neither a potential transmembrane helix nor a perfect potential leucine zipper was found (five nonoverlapping sequences present one residue mismatch each, and three of these contain proline residues). The search for protein signatures (Prosite) detected two potential cAMP- and cGMP-dependent protein kinase phosphorylation sites (from residues 265 and 381, respectively) and four potential protein kinase C phosphorylation sites (from residues 81, 114, 215, and 379).

**Interaction of FAP48 with FKBP59 in Vivo and in Vitro: Evidence and Characterization**—We took advantage of the two-hybrid system to further characterize the interaction between FAP48 (encoded by clone 26) and full-length FKBP59. For this purpose the plasmids encoding full-length FKBP59 fused to FKBP59-I and GST-FKBP59-I, and GST-FKBP59-I, and GST-FKBP59-1A (amino acids 32–138) retained a \( M_r \) of 48-kDa \( ^{35}\text{S} \)-labeled polypeptide, consistent with the deduced amino acid sequence of FAP48 (Fig. 4, lanes 2–4), whereas no detectable binding was observed with GST-FKBP59-II or -IV (Fig. 4, lanes 5 and 6) or GST alone (Fig. 4, lane 7). These data further validate the assay and provide additional support for the specificity of the interaction and direct binding of FKBP59 to FAP48 via its first domain.

**FAP48 Interacts with FKBP12 but not With CyP40**—Given the ability of FAP48 to interact with the truncated domain I of FKBP59 (FKBP59-I), corresponding structurally to the core domain of FKBP12, we reasoned that FAP48 could also be a target for FKBP12. To check this hypothesis in vivo, the entire coding sequence of FKBP12 was fused to LexA-DBD and co-transformed with pGAD26 into the yeast L40 strain as described above. The resulting strain was tested for its histidine prototrophy and \( \beta \)-galactosidase activity. As already observed for FKBP59-I and the full-length constructs, the yeast transformant LexA-FKBP12/pGAD26 was able to grow without histidine and displayed \( \beta \)-galactosidase activity (see below), thus providing evidence of a physical interaction between FAP48 and FKBP12 in vivo.

To demonstrate the in vitro binding of FAP48 to FKBP12, the FKBP12 cDNA was expressed as a fusion protein (GST-FKBP12) in E. coli; with this system, high levels of pure protein were readily recovered in a single step (data not shown). As shown in Fig. 5, FAP48, produced by in vitro translation with \( ^{35}\text{S} \)-methionine in the reticulocyte lysate, bound to GST-FKBP12 (lane 1) and again not to GST alone (data not shown).

These experiments carried out in vivo and in vitro demonstrate that, in addition to the FKBP12-like domain of FKBP59, FAP48 is able to bind specifically and directly to FKBP12 itself.

This finding prompted us to examine whether binding to immunophils could be a common feature of FAP48. In fact, although FKBP12 and cyclophilins are two sequence-unrelated families of proteins, they both possess peptidyl-prolyl cis-trans-isomerase activities, which are inhibited by their cognate drugs, and, in addition, several immunophils also interact with common proteins in the absence of their exogenous ligands (6, 45). According to these results, we reasoned that FAP48 could be a natural ligand or substrate of both classes of immunophils. The inability of GST-CyP40 to retain \( ^{35}\text{S} \)-labeled FAP48 (Fig. 5, lane 2) shows that FAP48 is not a common
target of immunosuppressive drug receptors but seems rather peculiar to FKBP.

FK506 and Rapamycin Prevent the Association of FKBP with FAP48 in Vitro and in Vivo—FK506 and rapamycin are two immunosuppressant drugs that bind at the same site on FKBP. We therefore investigated whether these drugs could affect the binding of FAP48 to FKBP in vivo and in vitro.

In vitro, the yeast transformants (pLexA-FKBP59/pGAD26, pLexA-FKBP59-I/pGAD26, and pLexA-FKBP12/pGAD26) were grown overnight in the absence or presence of 1 μM FK506, a concentration that does not affect yeast growth. Cells were collected, and β-galactosidase activity was measured. In these experimental conditions, for the strain expressing FKBP12, the inhibition of β-galactosidase activity was 100% with regard to the reference sample treated only with ethanol (the solvent for the drugs; n = 4), and for the strains expressing FKBP59 and FKBP59-I, the inhibition of β-galactosidase activity observed was 41 and 53% (n = 4), respectively. In complementary experiments, the highest level of inhibition of β-galactosidase activity was determined using increasing concentrations of FK506. As shown in Fig. 6, 50 μM FK506 was sufficient to completely abolish the binding of FAP48 to FKBP59 or to FKBP59-I, whereas FK506 at a concentration of 1 μM, totally prevented the binding of FAP48 to FKBP12.

In in vitro experiments, GSH-Sepharose beads loaded with GST-FKBP59 were incubated with increasing amounts of FK506 or rapamycin prior to (Fig. 7A) or after (Fig. 7B) addition of in vitro translated 35S-labeled FAP48. In both cases, FK506 and rapamycin markedly reduced the FAP48 content of the immunophilin-coupled beads in a dose-dependent manner (Fig. 7, A and B). This inhibitory effect was in fact due to the ligand, since the same amounts of ethanol were added in all cases. The specificity of the effect of the immunosuppressive drugs was confirmed by the fact that increasing concentrations of CsA did not further affect association between the two proteins (Fig. 7C), a result consistent with the failure of FKBP59 to bind CsA, contrary to FK506 and rapamycin. FK506 and rapamycin effectively competed with FAP48 for FKBP59 binding at a concentration of 10 μM but not at 1 μM. Since the concentration of immobilized FKBP59 was −1 μM, this result shows that titration with only a small excess of FK506 or rapamycin equivalents leads to the dissociation of FAP48 from the immunophilin.

Altogether these different experiments confirm the specificity of the interaction and the direct binding of FAP48 to FKBP59 and suggest that FAP48, FK506, and rapamycin share or overlap the same binding region on the FKBP.

**DISCUSSION**

A genetic screen, using the first domain of FKBP59 and a Jurkat cell cDNA library, led us to isolate a cDNA encoding a novel protein of 417 amino acids and an apparent molecular mass of 48 kDa, hence the name FAP48.

**Characterization of FAP48**—The FAP48 sequence contains a high level of hydrophobic residues, namely leucine (16.5%). However, the spacing of the leucines in FAP48 is not characteristic of leucine zipper structures. This feature has already been reported for another target of FKBP12, TOR2, which is homologous to rapamycin and FKBP12 target in the yeast; TOR2 is a 282-kDa protein and includes 13% leucine (46). The high levels of hydrophobic residues found in FAP48 led us to speculate that FAP48 could have membrane affinity and could function as an anchoring protein; however, a search for transmembrane helices revealed no evidence of a transmembrane domain. A search in the Genbank and the SwissProt data bases, using the Blast and Fasta programs revealed no substantial similarities with already known sequences, including the recently identified Hip protein (47) which is a homologue of the human p48 found in the steroid receptor heterocomplexes (48). Although we have not yet performed detailed FAP48 expression studies, expression of its gene appears ubiquitous; a search of the GenBank data base allowed us to find high homologies (>93%) with expressed sequence tags isolated from four different human libraries: placenta, adult and fetal heart, and infant brain.

**Fig. 5. Binding of FAP48 to GST-FKBP12 but not to GST-CyP40.** Binding reactions of FAP48 to GST-FKBP12 (lane 1) and GST-CyP40 (lane 2) were carried out as described in the legend to Fig. 4.

**Fig. 6. Interaction of FAP48 with FKBP59, FKBP59-I, and FKBP12 in the yeast two-hybrid system.** β-Galactosidase activities were quantified by liquid assay in the strains FAP48-FKBP59 (A), FAP48-FKBP59-I (B), and FAP48-FKBP12 (C) grown in the absence or presence of different concentrations of FK506. In each case the reference sample (β-galactosidase activity measured in the extract of the untreated strains) was considered as 100, and for the extracts of the strains treated with the drug, the values obtained were standardized with regard to the reference sample.
we preferentially suggest that a direct interaction between system, recombinant proteins, and those of many reports based on the use of the two-hybrid experiments. Therefore, according to the results obtained here, potential bridging component would also be required in yeast. Nevertheless, the possibility that GST-FAP48 and FKBP59 interact via a bridging cellular protein had to be considered. Reconstitution experiments with purified proteins, as carried out for the Hsp90-FKBP59 interaction, were unsuccessful, whereas FKBP12 has not been reported to bind Hsp90; (ii) the association of FKBP59 and FAP48 required only the highly conserved FKBP12-like core FKBP59-I domain, since the FKBP59 mutant deleted of the N- and C-terminal extremities of the first domain (FKBP59-IΔ) was sufficient to interact with FAP48; it was thus deduced that the binding of FAP48 to FKBP59 does not require the participation of Hsp90; and (iii) as previously reported, in vitro translated, as well as endogenous, FKBP59 behaves as a monomer and does not seem to be associated, in the rabbit reticulocyte lysate, with other cellular proteins; therefore, a direct interaction between FAP48 and FKBP59 remains likely.

Besides the experiments performed in vivo in yeast cells, it may be recalled that we used two strategies in vitro to demonstrate binding of FAP48 to FKBP59 or to FKBP12. First, recombinant FAP48 purified from E. coli was mixed with crude 35S-labeled FKBP59 containing rabbit reticulocyte lysate, and second, recombinant highly purified FKBP59 or FKBP12 produced in E. coli was combined with crude in vitro translated FAP48. Under these circumstances, it is highly improbable that the same bridging protein copurified from E. coli could be associated with both FKBP59 and FKBP12 (given that proteins that bind FKBP59 (14, 23) and proteins that bind FKBP12 (reviewed in Ref. 49) are unrelated) in their complexes with FKBP59, and, for the same reasons, that following translation, FAP48 could be associated with an endogenous rabbit lysate protein, able to interact with both immunophilins. Moreover, it must be mentioned that, given the results obtained in vivo, this potential bridging component would also be required in yeast experiments. Therefore, according to the results obtained here and those of many reports based on the use of the two-hybrid system, recombinant proteins, and in vitro translated products, we preferentially suggest that a direct interaction between FAP48 and FKBP's occurs in vivo and in vitro.

Finally, the lack of a physical interaction between CyP40 and FAP48 provided evidence that FAP48 is not a common target of all immunosuppressant drug receptors but, rather, seems peculiar to FKBP's.

Physiological Relevance of the FAP48-FKBP Interaction—We also demonstrated that FK506 and rapamycin effectively competed with FAP48 for binding with full-length or truncated FKBP59 and FKBP12, suggesting that FAP48 and the drugs share the same or overlapping binding sites on both immunophilins. Alternatively, FAP48 could interact with the immunophilins at an allosteric site that is conformationally altered by immunosuppressant binding. In vivo, titration with FK506 showed that the concentration of FK506 required for the inhibition of FAP48 binding to FKBP12 was significantly lower than the concentration of FK506 required for the inhibition to FKBP59. Although the recombinant immunophilin content in the respective yeast strains and lysates could be different, this finding probably reflects a difference in their FK506 binding affinities, since a dissociation constant of 0.4 nM with respect to FK506 has been reported for FKBP12 (50), whereas values of 40 and 66 nM have been determined for FKBP59 (21, 51).

Physiologically relevant effects of FK506 preventing association between FKBP12 and different target proteins have been observed. These target proteins include the type I receptor of the transforming growth factor β family (52), the ryanodine receptor (10), the inositol-1,4,5-triphosphate receptor (11) YY1, a zinc finger transcription factor (45), and the multidrug resistance pump P-glycoprotein (53). Similarly, efficient disruption of the human immunodeficiency virus type 1 Gag polyprotein Pro5-48, CyPA and CyPB interactions by cyclosporin A has been also reported (54, 55). In the same manner the interaction between FAP48 and FKBP's is disrupted by immunosuppressive drugs, thus providing further evidence for the high specificity and physiological relevance of these associations.

The major finding of this article is the isolation for a group of immunophilins of a new target, which can be considered as the first common target of FKBP59 and FKBP12 to be described. This opens the possibility of asking several questions. In particular, could FAP48 represent an endogenous ligand, the actions of which are mimicked by the various immunosuppressive drugs? Recently it has been reported that in both Jurkat and murine T cells, the intracellular concentration of FKBP is 6–7 μM, with only 3–5% of the FKBP pool bound by FK506 at the IC50 for inhibition of activation (56). In fact, if FKBP's are already associated with intracellular targets such as FAP48, this surprising finding could in part be explained. Also, does FAP48 enhance or inhibit the interactions between immunophilins and other proteins? If so, it is a better signal transduction effector than the drugs? It would be interesting to examine whether overexpression of FAP48 could mediate the same inhibitory effects as FK506 in signaling pathways such as interleukin 2 gene transcription and thus determine how this protein could participate in cellular regulation networks. Given its ability to associate with FKBP59, a steroid hormone recep-
tor component, the function of which is unknown to date, the isolation and further characterization of FAP48 may also prove useful for understanding the immunosuppressant drug-sensitive effects on steroid hormone receptor-induced gene transcription (57–59). Finally, identification of a putative FK506-rapamycin mimetic structure in the FAP48 polypeptide may prove useful for the design of peptide compounds with immunosuppressive drug properties but devoid of toxic effects. Additional studies in our laboratory will focus on these important issues.

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REFERENCES

1. Schrieber, S. L. (1991) Science 251, 283–287
2. Wiede, H. G., and Etkan, F. P. (1994) Perspect. Drug Discov. Des. 2, 57–84
3. Bierer, B. E. (1994) Chem. Immunol. 59, 128–155
4. Galat, A. (1993) Eur. J. Biochem. 216, 689–707
5. Bierer, B. E., Somers, P. K., Wandelless, T. J., Burakoff, S. J., and Schriever, S. L. (1990) Science 250, 556–559
6. Cardenas, M. E., Hemenway, C., Muir, R. S., Ye, R., Fiorentino, D., and Vet, J. E. M. (1994) EMBO J. 13, 5944–5957
7. Sabatini, D. M., Erdjument-Bromage, H., Lui, M., Tempst, P., and Snyder, S. H. (1994) Cell 78, 35–43
8. Brown, E. J., Albers, M. W., Shin, T. B., Ichikawa, K., Keith, C. T., Lane, W. S., and Schriever, S. L. (1994) Nature 369, 756–758
9. Bierer, B. E., Mattila, P. S., Staender, R. F., Herzenberg, L. A., Burakoff, S. J., Crabtree, G., and Schriever, S. L. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9231–9235
10. Jayaraman, T., Brantlett, A.-M., Timmerman, A. P., Fleischer, S., Erdjument-Bromage, H., Tempst, P., and Marks, A. R. (1992) J. Biol. Chem. 267, 9474–9477
11. Cameron, A. M., Steiner, J. P., Sabatini, D. M., Kaplin, A. I., Walensky, L. D., and Snyder, S. H. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1784–1788
12. Cameron, A. M., Steiner, J. P., Roskams, A. J., Ali, S. M., Ronnett, G. V., and Snyder, S. H. (1995) Cell 83, 463–472
13. Tai, P.-K. K., Maeda, Y., Nakao, K., Wakisaka, T., and Faber, L. E. (1986) Biochemistry 25, 5269–5275
14. Renoir, J. M., Radanyi, C., Faber, L. E., and Baulieu E. E. (1990) J. Biol. Chem. 265, 10740–10745
15. Lebeau, M. C., Messier, L., Mercier-Bodard, C., Hoffmann, K., Le Bihan, S., and Go¨ttlinger, H. G. (1994) Nature 372, 303–307
16. Kunz, J., Henriques, R., Schneider, U., Deinter-Reinhard, M., Movva, N. R., and Hall, M. N. (1999) Cell 93, 585–596
17. Hufnfeld, J., Minami, Y., and Hartl, F.-U. (1995) Cell 83, 598–599
18. Prapapanich, V., Chen, S., Nair, S. C., Rimmerman, R. A., and Smith, D. F. (1986) Mol. Endocrinol. 10, 420–431
19. Nogawa, S. H., and Sabatini, D. M. (1995) J. Biol. Chem. 270, 15181–15193
20. Kunz, J., Henriques, R., Schneider, U., Deinter-Reinhard, M., Movva, N. R., and Hall, M. N. (1999) Cell 93, 585–596
21. Tai, P.-K. K., Albers, M. W., Chang, H., Faber, L. E., and Schriever, S. L. (1992) Science 256, 1315–1318
22. Wang, T., Donahoe, P. K., and Zervos, A. S. (1994) Science 265, 674–676
23. Hemenway, C. S., and Heitman, J. (1996) J. Biol. Chem. 271, 18525–18534
24. Luban, J., Bossolt, K. L., Franke, E. K., Kalpana, G. V., and Goff, S. P. (1993) Cell 73, 1067–1078
25. Thal, M., Bukovský, A., Kondo, E., Rosenwirth, B., Welsh, C. T., Sodroski, J., and Gottlinger, H. G. (1994) Nature 372, 363–365
26. Dement, F., Kastner, C., Iaccovone, F., and Fischer, P. (1994) J. Pharmacol. Exp. Ther. 268, 32–41
27. Ying, Y., and Sanchez, E. E. (1993) J. Biol. Chem. 268, 6073–6076
28. Tai, P.-K. K., Albers, M. W., McDonnell, D. P., Chang, H., Schriever, S. L., and Faber, L. E. (1994) J. Biol. Chem. 269, 8842–8847
29. Renoir, J. M., Le Bihan, S., Mercier-Bodard, C., Gold, A., Arjomandii, M., Radanyi, C., and Baulieu, E. E. (1994) J. Steroid Biochem. Mol. Biol. 48, 101–110