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# Relationship between the Subcellular Localization and Structures of Catalytic Domains of FKBP-Type PPIases[1]

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**The *Schizosaccharomyces pombe* gene, *fkp39*$^+$, encoding a homolog of FKBP(FK506 binding protein)-type peptidyl prolyl *cis-trans* isomerase (PPIase), was isolated and the primary structure was determined. This gene product (SpFkbp39p) showed PPIase enzymatic activity in a chymotrypsin-dependent enzyme assay involving recombinant SpFkbp39p. Comparison of the primary structures of the catalytic domains of FKBPs, including SpFkbp39p, revealed that FKBPs could be classified into four groups. This categorization corresponding to the known subcellular localization of the FKBPs, makes the prediction of the subcellular localization of FKBPs based on their primary structures feasible. SpFkbp39p was considered to be a member of the nuclear-type FKBP group from this relationship beween primary structure and subcellular localization. An immunofluorescence assay against HA-epitope-tagged SpFkbp39p revealed that SpFkbp39p is localized to the nucleus, as predicted. Residues conserved in a "group-specific" manner in the catalytic domain were mapped to their corresponding three-dimensional positions; these "group-specific" residues were located in close proximity in distinct regions mostly on the protein surface, which implies the presence of "group-specific" regulatory functional regions. We also found that nuclear-type FKBPs, including SpFkbp39p, have two highly conserved domains other than catalytic ones, with further basic and acidic charged regions, especially in the case of nuclear-type FKBPs. This is the first report indicating that there is a rule for the relationship between the subcellular localization and structure of the catalytic domain of a FKBP.**

**Key words: FKBP, localization, nucleus, peptidyl prolyl isomerase, structure.**

Conformational changes of proteins frequently affect protein-protein interactions, which play crucial roles in controlling the qualitative state of protein function. The peptidyl prolyl *cis-trans* isomerase (PPIase) may induce a conformational change in proteins *in vivo* because it catalyzes the interconversion of the *cis-* and *trans*-isomers of peptidyl-prolyl bonds in peptide and protein substrates *in vitro* (*1, 2*). PPIases are present in both prokaryotes and eukaryotes, and the primary structures of their catalytic domains are highly conserved (*3*). PPIases are thought to be involved in protein folding (*4*) because they accelerate the refolding of denatured proteins *in vitro* (*5-7*). On the other hand, the interaction of PPIases with specific molecules suggests that PPIases may have other functions. For example, FK506 binding proteins (FKBPs), submembers of the PPIase family, have been shown to interact with various factors [*e.g.* FKBP12 interacts with TGF$\beta$ type I receptor (*8*) and transcription factor YY1 (*9*), FKBP52 (also known as p59, FKBP59, HSP56, or HBI) with steroid receptors (*10-12*), and FKBP25 with casein kinase II and nucleolin (*13*)]. Interaction between FKBPs and specific target molecules affects the functional state of their targets in several cases (*9, 14*). Recent genetic analysis involving multi-cellular organisms revealed that FKBPs are involved in biological phenomena such as development (*15-17*).

PPIases can be classified into three subfamilies on the basis of differences in sensitivity to potent inhibitors, *i.e.* the FKBP, cyclophilin (CyP), and parvulin families. FK-506 and cyclosporin A selectively bind FKBPs and CyPs, respectively, and inhibit PPIase activities (*18-21*), whereas the parvulin family is insensitive to these drugs (*22*). The primary structures of the catalytic domains are similar to each other within a subfamily but differ among the three

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Abbreviations: Sp, *Schizosaccharomyces pombe*; Sc, *Saccharomyces cerevisiae*; Dm, *Drosophila melanogaster*; Sf, *Spodoptera frugiperda*; Hs, *Homo sapiens*; Bt, *Bos taurus*; Mm, *Mus musculus*; Rn, *Rattus norvegicus*; At, *Arabidopsis thaliana*; Ta, *Triticum aestivum*; Nc, *Neurospora crassa*; Ce, *Caenorhabditis elegans*; Ca, *Candida albicans*; *E. coli*, *Escherichia coli*; TGF$\beta$, transforming growth factor $\beta$; SDS, sodium dodesyl sulfate.

subfamilies. Moreover, comparison of the tertiary structure of each protein revealed that similar residues among primary structures in each subfamily are also limited by a three-dimensional structure (*23*). In eukaryotic cells, PPIases are localized in various cellular compartments (*21*). In *Saccharomyces cerevisiae*, there are four FKBPs (*24-29*), eight cyclophilins (*29-36*), and one parvulin (*22, 37*), and three PPIases are localized in the cytoplasm (Fpr1, Cpr1, 6), four in the ER (Fpr2, Cpr2, 4, 5), one in the mitochondria (Cpr3), and two in the nucleus (Fpr3, Ess1) (*22, 29*), suggesting that functional targets of PPIase might be diverse.

To elucidate the functional roles of PPIase in each cellular compartment, but particularly in the nucleus, we isolated a nuclear-type PPIase from *Schizosaccharomyces pombe*, a favorable model organism for analyzing biological function both biochemically and genetically. Clues for understanding the universal molecular mechanisms in mono- and multi-cellular organisms may be obtained from *S. pombe* because the molecular mechanisms of several biological phenomena in *S. pombe* are more analogous to those in multicellular organisms than those in *S. cerevisiae* in several aspects (*38, 39*). The evolutionary distances between *S. pombe*, *S. cerevisiae*, and multicellular organisms also make it useful to identify potent functional motifs by comparing the structures of their homologues.

We now report the primary structure of a novel gene, Sp*fkp39*$^+$, which was isolated as a FKBP-type PPIase from *S. pombe*. Comparative studies on the primary structures of FKBPs indicated that FKBPs can be classified into four groups on the basis of differences in the primary structures of their catalytic domains. This may allow one to predict the subcellular localizations of FKBPs from the primary structure of not localization signals, but of their catalytic domains. We also determined several biochemical characteristics of SpFkbp39p by revealing its nuclear localization as well as by identifying several novel putative structural motifs, which are specific for the nuclear-type FKBP group.

## MATERIALS AND METHODS

*Isolation of a S. pombe DNA Fragment Containing fkp39*$^+$ *by PCR Using Degenerate Oligonucleotide Primers*—General methods for DNA manipulation were performed as described (*40*). Two degenerate oligonucleotide PCR primers (5′-SAR GTI ATH MRI GSI TGG-3′ and 5′-ARY TCI ACI TCR AAI RYI ARI GT-3′. I, inosine; S, cytosine or guanine; R, adenine or guanine; H, adenine, thymine, or cytosine; M, adenine or cytosine; W, adenine or thymine; Y, cytosine or thymine) designed from the sequences, E/Q-V-I-K/R-A/G-W and T-L-V/T-F-E/D-V-E-L, of the catalytic domains conserved among FKBPs, were used to amplify DNA fragments from *S. pombe* genomic DNA by Polymerase Chain Reaction (PCR). PCR amplification was performed using *Taq* DNA polymerase (Boehringer Mannheim), the manufacturer's buffers and a GeneAmp PCR System 9600 (Perkin Elmer). Each PCR protocol cycle comprised for 30 s at 95°C, 2 min at 40°C, and 1 min at 72°C. The resulting PCR products were subcloned into pBluescriptIISK(-) (Stratagene) at the *Sma*I site, and the nucleotide sequences were determined using Dye Terminator Cycle Sequencing Ready Reaction DNA sequencing kits (Perkin Elmer) and a DNA sequencer ABI377 (Perkin Elmer), according to the manufacturers' instructions.

*Isolation of S. pombe Genomic DNA Fragments Containing Full-Length fkp39*$^+$—To isolate the full-length open reading frame, PCR-amplified DNA fragments were used to screen the *S. pombe* genomic library (provided by Dr. A. Ishihama) by plaque hybridization, using the Gene Images random prime labeling and detection system (Amersham), according to the manufacturer's instructions. The three independent positive clones isolated were digested with *Apa*I and *Xho*I, and then subcloned into pBluescriptIISK(-) (Stratagene) all at the same sites. The resultant plasmid was named pBS-Sp*fkp39*$^+$. The nucleotide sequences of these clones were determined on both strands, as described above. This nucleotide sequence has been submitted to the GenBank™ under accession number AF017990.

*Construction of Plasmids Which Express the Fkbp39p Protein*—*Bam*HI and *Nde*I sites were engineered at the translation initiation site and *Bam*HI site at the translation stop codon of SpFkbp39p by PCR. Two primers, 5′-GCG GAT CCA TAT GTC TCT TCC AAT TGC TG-3′, which has *Bam*HI and *Nde*I sites, and 5′-CCG GAT CCT TAG TGA ACG CGA ACA AGC TTG ACT TC-3′, which has a *Bam*HI site, were used to amplify the fragment from the pBS-Sp*fkp39*$^+$ plasmid. This fragment was subcloned into pBluescriptIISK(-) (Stratagene) at *Bam*HI sites, as described above. The resultant vector was named pBS-Fkbp39p. pBS-Fkbp39p was digested at *Nde*I and *Bam*HI sites, and then subcloned into 6His-pET11d (*41*) and pREP1HA2 (T. Kuzuhara and M. Horikoshi, unpublished results), a pREP1 (*42*)-based *S. pombe* vector which expresses proteins with two copies of the influenza virus hemagglutinin (HA) sequence (YPYDVPDYA) appended to its $NH_2$-termini. The resultant plasmids were named pET11d-6His-Fkbp39p and pREP1-HA-Fkbp39p, respectively.

*Expression and Purification of the Recombinant SpFkbp39p Protein*—The recombinant SpFkbp39p protein was expressed in *Escherichia coli* with an amino-terminal histidine tag to facilitate purification *via* $Ni^{2+}$ affinity chromatography. pET11d-6His-SpFkbp39p was used to transform *E. coli* BL21(DE3). Cells (160 ml) were grown to an $OD_{600}$ of 0.8 at 27°C, induced with 0.4 mM isopropyl β-D-thiogalactopyranoside, harvested, and then disrupted by sonication (Branson) in buffer containing 20 mM Tris, pH 7.9 (4°C), 500 mM NaCl, 10% glycerol, and 10 mM β-mercaptoethanol. The cleared lysate was applied to ProBond resin (Invitrogen), and SpFkbp39p was eluted and further purified by POROS 20S ion-exchange chromatography, using a BIO-CAD system (PerSeptive). The purified SpFkbp39p protein was determined by gel electrophoresis to be about 90% pure, as estimated from CBB staining (Fig. 2A). Proteins were determined by the Bradford assay (Bio-Rad) with BSA as a standard.

[$^3H$]*Dihydro-FK506 Binding Assay*—Binding of [$^3$H]-dihydro-FK506 (NEN) was determined using the LH-20 assay (*25*) with 12 nM [$^3$H]dihydro-FK506 and 0.1 μM SpFkbp39p in 20 mM Tris, pH 7.5, 200 mM NaCl. Bound [$^3$H]dihydro-FK506 was separated from free [$^3$H]dihydro-FK506 by chromatography on individual Sephadex LH-20 columns (Pharmacia). Samples were fractionated and counted with an LS-6000LL scintillation counter (Beckman Instruments).

*Peptidyl-Prolyl Cis/Trans-Isomerization Activity of Recombinant FKBPs*—PPIase activity was assayed essentially as described (*1, 43*). The assay used measures the '*cis*' to '*trans*' isomerization of the leucine-proline peptide bond in the peptide, *N*-succinyl-Ala-Leu-Pro-Phe-*p*-nitroanilide (Bachem). The release of *p*-nitroanilide is quantitated spectrophotometrically at 390 nm. Reaction mixtures (1 ml) were made at 0°C and contained 0.17 mM substrate, 50 mM Hepes-Na (pH 8.0), 100 mM NaCl, FKBPs, and 10 $\mu$M FK506 (Fujisawa Pharmaceutical). Immediately before starting the assay, 10 $\mu$l of a chymotrypsin solution (10 mg/ml in 0.001 M HCl; final concentration, 1 mg/ml) was added. After mixing, the increase in absorbance at 390 nm was measured with a Beckman DU680 spectrophotometer at 1 s intervals (Fig. 3). The first order rate constant, $k$ ($s^{-1}$), was calculated from the slope of the resulting line (Table I).

*Determination of the Subcellular Localization of SpFkbp39p*—The plasmids pREP1-HA-Fkbp39p, and pREP1-HA-TBP (T. Yamamoto and M. Horikoshi, unpublished results), and mock plasmid pREP1 were transformed into *S. pombe* strain JY741 (*h⁻ ade6-M216 leu1 ura4-D18*), using general methods. Staining of these yeast cells by indirect immunofluorescence was performed essentially as described (*44*) but with the following modification. Cells were cultured in 4 ml of adenine and uracil complemented MM medium (MMAU) to $10^6$ cells/ml. Cell walls were removed by incubation with 20 $\mu$g/ml Zymolyase-20T (Wako) at 30°C for 30 min. Primary antibodies [anti-HA mouse IgG (KODAC)] were incubated with the fixed cells at room temperature for over 12 h. Secondary antibodies [FITC-labeled anti-mouse IgG rabbit IgG (Wako)] were applied for over 12 h. Cells were stained with Hoechst 33342 (SIGMA), examined microscopically and then photographed (Fuji color slides).

*Method of Computer Analysis*—CLUSTAL W (*46*) was used for the alignment of amino acid sequences and NJPLOT for the determination of the phylogenetic tree. The three-dimensional structure of HsFKBP12 was drawn and analyzed with RasMac (ver. 2.5). Other analyses were performed with GENETYX-MAC (ver. 9.0) (Software Development).

## RESULTS AND DISCUSSION

*Isolation of the fkp39⁺ Gene*—To isolate novel *S. pombe* nuclear-type PPIase genes, PCR was carried out with degenerate oligonucleotide primers based on the conserved catalytic domains of FKBPs. The amplified *S. pombe* genomic DNA fragments were isolated and their nucleotide sequences were determined. The deduced amino acid sequence encoded a fragment [arrow under the nucleotide sequence (1525-1665) in Fig. 1] showing high similarity (over 40% identity) with the catalytic domains of other FKBPs. To obtain the full-length clone, we screened a *S. pombe* genomic library, using this PCR fragment as a probe. Three independent positive clones were isolated and their nucleotide sequences were determined.

An open reading frame (ORF) is located from 601 (start codon: 601-603) to 1685 (stop codon: 1686-1688) (Fig. 1). An in-frame stop codon is located six bases upstream (positions 595-597, underlined in Fig. 1) of the putative translation initiation site. There are no consensus sequences for donor, acceptor or branch sites for splicing in this region (*46*). The deduced amino acid sequence of this ORF is most like that of *S. cerevisiae* FKBP-type PPIase, Fpr3 (45% identity) (*27, 28*), consisting of 361 amino acids (39,301 Da) with the FKBP catalytic domain located at the C terminal region (267-361, double underlined in Fig. 1).

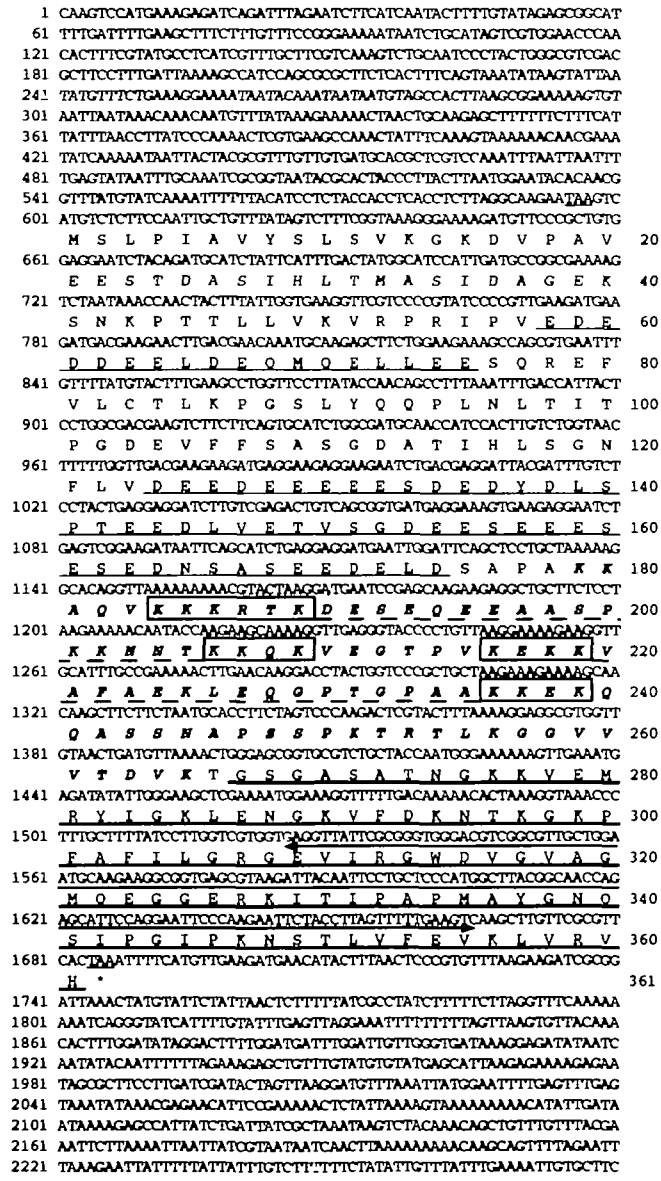


Fig. 1. **Nucleotide and deduced amino acid sequences of the cloned Sp*fkp39⁺* gene.** The numbers of the nucleotide sequence from the 5′-terminal are shown on the left, and those of the amino acid sequence from the N-terminal on the right. The arrow at nucleotide positions 1525-1665 indicates the position of the DNA fragment we first obtained on PCR, using degenerate oligonucleotide primers. The in-frame nonsense codons (TAA) at nucleotide positions 595-597 and 1684-1686 are underlined. The C-terminal region homologous to the catalytic domains of FKBPs is double underlined (at amino acid positions 267-361). One basic (at amino acid positions 179-265) and two acidic (positions 58-75 and 124-174) regions are shown underlined and in bold letters, respectively. The consensus bipartite nuclear targeting sequences within the basic region are boxed (basic stretch) and dotted-lined (linker) (at amino acid positions 184-209 and 215-239).

Two putative nuclear targeting sequences consisting of bipartite short stretches of basic amino acids with non-conserved linker regions (*47*) are located at positions 184-209 and 215-239 [boxed (basic stretch) and dotted-lined (linker) in Fig. 1], suggesting nuclear localization. Based on the results mentioned above taken together, we conclude that this isolated gene encodes a putative nuclear-type FKBP, named SpFkbp39p, which is most likely the *S. pombe* homolog of *S. cerevisiae* Fpr3.

*SpFkbp39p Binds to FK506 and Has PPIase Catalytic Activity*—The primary structure of the C-terminal region of SpFkbp39p is similar to those found in the catalytic domains of FKBPs. FKBP-type PPIases interact with FK506 and their PPIase enzymatic activities are inhibited by these interactions. To determine if SpFkbp39p has biochemical characteristics of FKBP-type PPIases, we prepared the bacterial-expressed SpFkbp39p protein. A plasmid, pET11d-6His-Fkbp39p, which expresses His-tagged SpFkbp39p in *E. coli*, was constructed, and recombinant His-tagged SpFkbp39p (His-SpFkbp39p) was purified by Ni-agarose chromatography as described under "MATERIALS AND METHODS."

We first observed the interaction between SpFkbp39p and FK506. The purified recombinant His-SpFkbp39p bound [$^3$H]dihydro-FK506, which was detected with the LH-20 assay (*25*) (Fig. 2B). Next, we measured the PPIase catalytic activity of SpFkbp39p as enhancement of the rate of chymotrypsin cleavage of a synthetic Pro-containing peptide substrate in a coupled spectrophotometric assay (*1, 43*). This assay demonstrated that the His-SpFkbp39p protein has comparable PPIase activity to HsFKBP12 (control), and that this enzymatic activity is also almost perfectly inhibited by FK506 (Fig. 3). The specific activities ($k_c/K_m$) of these molecules in reactions are summarized in Table I, which shows that $k_c/K_m$ of the control enzyme, HsFKBP12, is similar to the previous data (*1, 43*), and that $k_c/K_m$ of SpFkbp39p is almost half that of HsFKBP12. Taken together, the results obtained in these biochemical assays indicate that SpFkbp39p exhibits FKBP-type PPIase activity *in vitro*.

*Classification of FKBPs as to the Primary Structures of Catalytic Domains*—Distinct FKBPs are localized to different cellular compartments (*21*). It is speculated that differences in the primary structures of catalytic domains reflect differences in the subcellular localization of FKBPs because the substrates and/or interactors of FKBPs might differ in each cellular compartment. Therefore, we compared the primary structure of SpFkbp39p with eukaryotic FKBPs in order to elucidate the relationship between the primary structure and subcellular localization of FKBPs.

Phylogenetic tree analysis based on the primary structures of the catalytic domains of known FKBPs (Fig. 4) indicated that 40 FKBPs can be classified mainly into four groups (groups A to D), only four FKBPs being excluded. Surprisingly, this classification corresponds to categorization as to the subcellular localization of each group; localized in the nucleus (group A), cytoplasm (group B), nucleus or nucleus and cytoplasm (group C), and ER (group D). This observation may make prediction of the subcellular localization of FKBPs from the primary sequences of their catalytic domains feasible.

*Detailed Analysis of Amino Acid Residues of Catalytic Domains*—FKBPs are classified into four groups according to their primary structures, as shown in Fig. 4. To determine whether or not this classification of FKBPs reflects differences in amino acid residues among the protein groups, we mapped the "conserved" and "group-specific" residues by alignment of the primary structures of FKBPs (Fig. 5A). We defined the amino acid residues with over 90% identity and 100% similarity in all groups as "conserved" residues (blue- and red-, and cyan-shadowed, respectively, in Fig. 5A), and the amino acid residues which

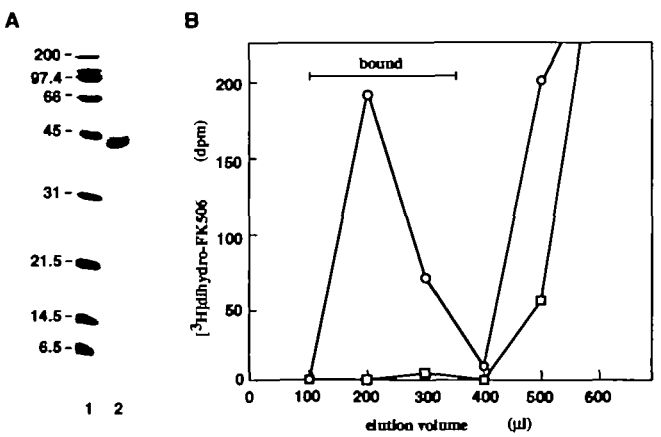



Fig. 2. **Interaction between SpFkbp39p and FK506.** A: Purified recombinant SpFkbp39p protein. Lane 1, protein standard (kDa); lane 2, purified SpFkbp39p. The recombinant SpFkbp39p protein (1 μg) was resolved on a 12.5% SDS-polyacrylamide gel. The gel was stained with Coomassie Blue. B: Binding of [$^3$H]dihydro-FK506 to SpFkbp39p. [$^3$H]Dihydro-FK506 was incubated in the presence (circle) or absence (square) of SpFkbp39p, and then fractionated by LH-20 gel filtration chromatography. SpFkbp39p-bound [$^3$H]dihydro-FK506 was eluted in the void volume.

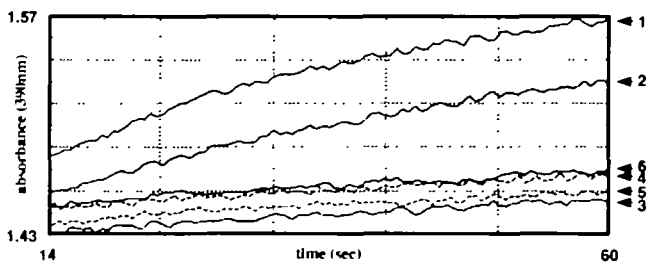



Fig. 3. **Peptidyl-prolyl *cis-trans* isomerase activity of SpFkbp39p and its inhibition by FK506.** The absorbance at time *t* was plotted against time. Line 1, SpFkbp39p (52 nM); line 2, HsFKBP12 (17 nM); line 3, enzyme (−); line 4, SpFkbp39p + FK506 (10 μM); line 5, HsFKBP12 + FK506; line 6, enzyme (−) + FK506.

TABLE I. **Determination of the turnover numbers ($k_c$) and specific activities ($k_c/K_m$) of FKBPs at 0°C.** The first order rate constant, $k$ ($s^{-1}$), is calculated from the slope of the resulting line in Fig. 3. $k_c = k - k$[non-enzyme], $k_c/K_m = k_c/$[enzyme].

| | FK506 (10 μM) | $k_c$ ($10^{-2}$ $s^{-1}$) | $k_c/K_m$ ($10^6$ $s^{-1}\cdot M^{-1}$) |
|---|---|---|---|
| SpFkbp39p (52 nM) | − | 1.8±0.17 | 0.34±0.032 |
| | + | 0.010±0.009 | 0.004±0.0036 |
| HsFKBP12 (17 nM) | − | 1.3±0.27 | 0.76±0.158 |
| | + | <0.001 | <0.001 |
| Enzyme (−) | − | 0 | − |
| | + | 0.017±0.009 | − |

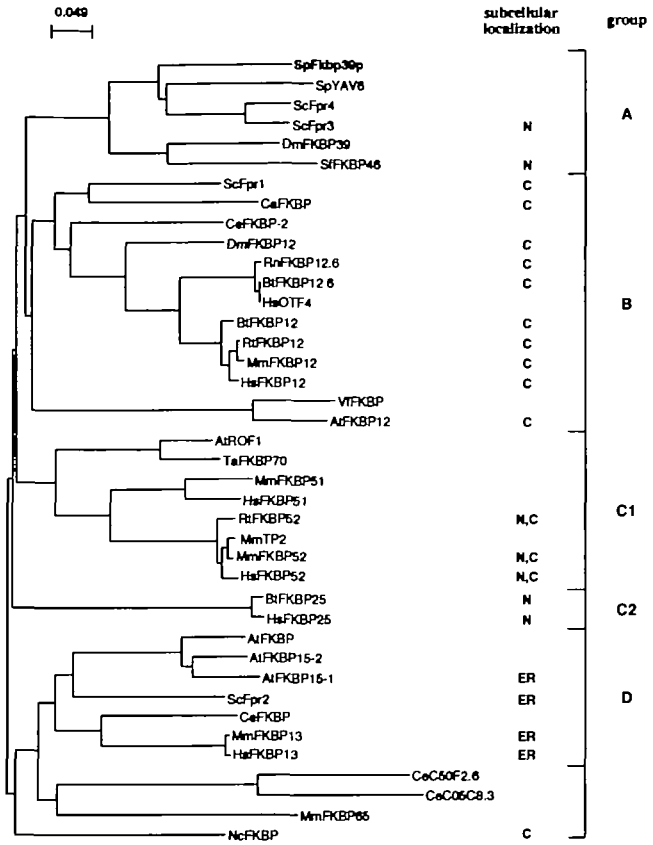



Fig. 4. **Phylogenetic tree analysis of the PPIase catalytic domains of eukaryotic FKBPs.** This tree was analyzed using the CLUSTAL W and NJPLOT programs (*45*) with the primary sequences of PPIase catalytic domains. The symbols, N (nucleus), C (cytoplasm), and ER (endoplasmic reticulum), represent the subcellular localization of previously determined FKBPs.

are completely identical and similar within one, two or three group(s) other than "conserved" residues as "group-specific" residues (green-shadowed in Fig. 5B). The alignment showed that both "conserved" and "group-specific" residues are not closely located in a part but rather located in the catalytic domains in a non-consecutive manner. However, it is possible that these residues might form functional regions and thus be located together at the three-dimensional level.

To define the presence of the putative functional domains discussed above, we mapped these "conserved" and "group-specific" residues to the corresponding positions in the three-dimensional structure of human FKBP12 (HsFKBP12), the tertiary structure of which has been determined (*48, 49*) (Fig. 5, B and C). The similarity of the primary structures and tertiary structures [FKBP12 (group B) (*48, 49*), FKBP52 (group C2) (*50*), and FKBP25 (group C1) (J. Liang, D.T. Hung, S.L. Schreiber, and J. Clardy, unpublished results shown in PDB database)] of the catalytic domains of FKBPs suggests that the backbone structures of the catalytic domains of most FKBPs should be similar as well. As a result, the "conserved" residues (blue-, red-, or cyan-shadowed in Fig. 5) are located in proximity (Fig. 5B) and some form a hydrophobic pocket (*48, 49*), which is required for FKBP-immunoretardant interactions and basal PPIase activity. Interestingly, "group-specific" residues (green-shadowed in Fig. 5) are also located together in several regions and sites which have not been described (circled in Fig. 5, B and C), although these residues are located in a non-consecutive manner in the primary structure. Because these regions and sites consist of "group-specific" residues which exist in FKBPs in a "group-specific" manner, they might be involved in "group-specific" functions, for which our classification of FKBPs by means of a phylogenetic tree based on the primary structures of catalytic domains can be appropriate.

Several FKBPs interact with specific molecules (*9-11, 13, 14*). It would be of interest to determine whether or not "group-specific" regions, as shown above, are required for interactions between FKBPs and known interactors. If so, this will lead to elucidation of the "group-specific" function of FKBPs.

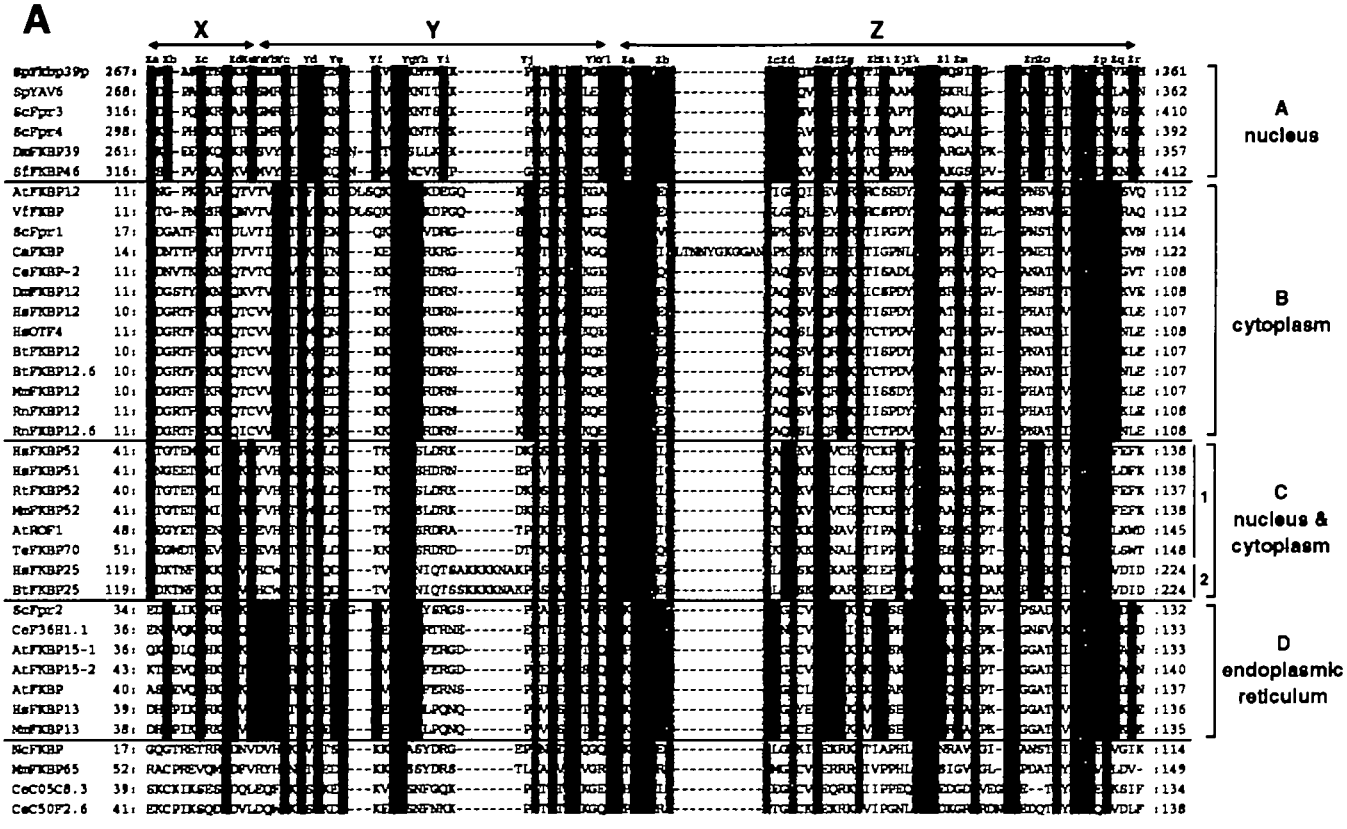
*Nuclear Localization of SpFkbp39p*—We proposed the presence of four FKBP groups and "group-specific" regions. From this proposal, SpFkbp39p is predicted to be localized to the nucleus. This provides experimental support of our hypothesis regarding the relationship between the primary structures of the catalytic domains of FKBPs and their subcellular localization. We analyzed the subcellular localization of SpFkbp39p, using the fluorescence antibody technique to elucidate localization in cells. We expressed HA-tagged SpFkbp39p and TBP (TATA box-binding protein) in *S. pombe* cells, and an anti-HA antibody was used for detection.

This analysis revealed that the nuclei (stained with Hoechst 33342) of the resultant cells carrying pREP1-HA-SpFkbp39p were stained with FITC, similar to the control cells carrying pREP1-HA-TBP (Fig. 6), which indicates that SpFkbp39p is localized to the nucleus. This observation supports the validity of our prediction regarding the subcellular localization of the FKBP family. Further investigations are expected to define rules for the subcellular localization of FKBPs.

*Characteristics of Group A FKBPs*—The newly isolated SpFkbp39p is classified into nuclear-type group A with five other known FKBPs, and SpFkbp39p is localized to the nucleus (Fig. 6). To identify conserved structural motifs other than the catalytic domain which might act as a functional domain, we compared the full-length primary structures of group A FKBP members with members of other groups of FKBPs. Interestingly, group A proteins have long $NH_2$-terminal regions adjacent to the catalytic domains (Fig. 7B). Alignment of these regions of group A proteins demonstrated that they have two novel conserved domain structures specific for group A FKBPs which are named conserved domains I (15-41% identity and 41-70% similarity) and II (30-55% identity and 50-70% similarity), as shown by the colored-box in Fig. 7A. They also contain acidic (in SpFkbp39p, underlined in Fig. 1, and red-lined above in Fig. 7A) and basic (bold in Fig. 1, and blue-lined above in Fig. 7A) regions (*26, 27, 51, 52*), which are also rich in both Ser and Thr residues (Fig. 7A). The orders of each domain and region are the same (Fig. 7B), suggesting that not only the primary structure but also the position of each domain and region might be significant as to the functional roles of group A proteins. This domain structure is not present in other groups [ScFpr1 (group B), HsFKBP52 (group C1), HsFKBP25 (group C2), and ScFpr2 (group D)] (Fig. 7B), and strongly suggests that group A

FKBPs form a functional subfamily. The primary structures of yeast and insect group A FKBPs differ in the $NH_2$-terminal regions. Therefore, we designated this a "species-specific" region (Fig. 7B), suggesting that there is functional diversity among group members.

*Conclusion and Perspective*—In conclusion, we isolated a novel *S. pombe* gene product, SpFkbp39p, which forms a nuclear-type subfamily with several FKBPs. This subfamily is classified according to differences in the primary structures of FKBP catalytic domains. SpFkbp39p has



biochemical properties similar to those of other FKBPs. Moreover, we identified novel domain structures conserved in the N-terminal regions found only among nuclear-type FKBPs. These structural motifs include both negatively- and positively-charged regions which might interact with positively-charged proteins in the nucleus, such as histones, and acidic molecules, such as DNA, respectively.

Fig. 5. **Detailed analysis of amino acid residues of the catalytic domains.** A: Alignment of the amino acid sequences of the PPIase catalytic domains of FKBPs including SpFkbp39p. The alignment was determined using the CLUSTAL W program (*45*). "Conserved" residues are blue-shadowed (conserved over 90% identity) and cyan-shadowed (100% similarity). D37 (HsFKBP12) residue essential for full PPIase activity (*51*) are red-shadowed. "Group-specific" residues, which are identical and similar within one, two or three group(s), are green-shadowed. The three regions whose "group-specific" residues are closely located at the three-dimensional level are named regions X, Y, and Z, respectively (indicated by arrows). Letters above the alignment show the positions of amino acid residues in the three-dimensional structure of HsFKBP12, as discussed in B and C. B: "Conserved" and "group-specific" residues mapped on the corresponding positions in the three-dimensional structure of HsFKBP12. The amino acids denoted by colors and letters correspond to those in A. The "group-specific" region and sites are indicated by circles. The accession number of HsFKBP12 in the PDB database is 1FKF. C: "Conserved" and "group-specific" residues viewed from another angle. The amino acids denoted by colors and letters correspond to those in A and B. The "group-specific" region and sites are indicated by the circle.

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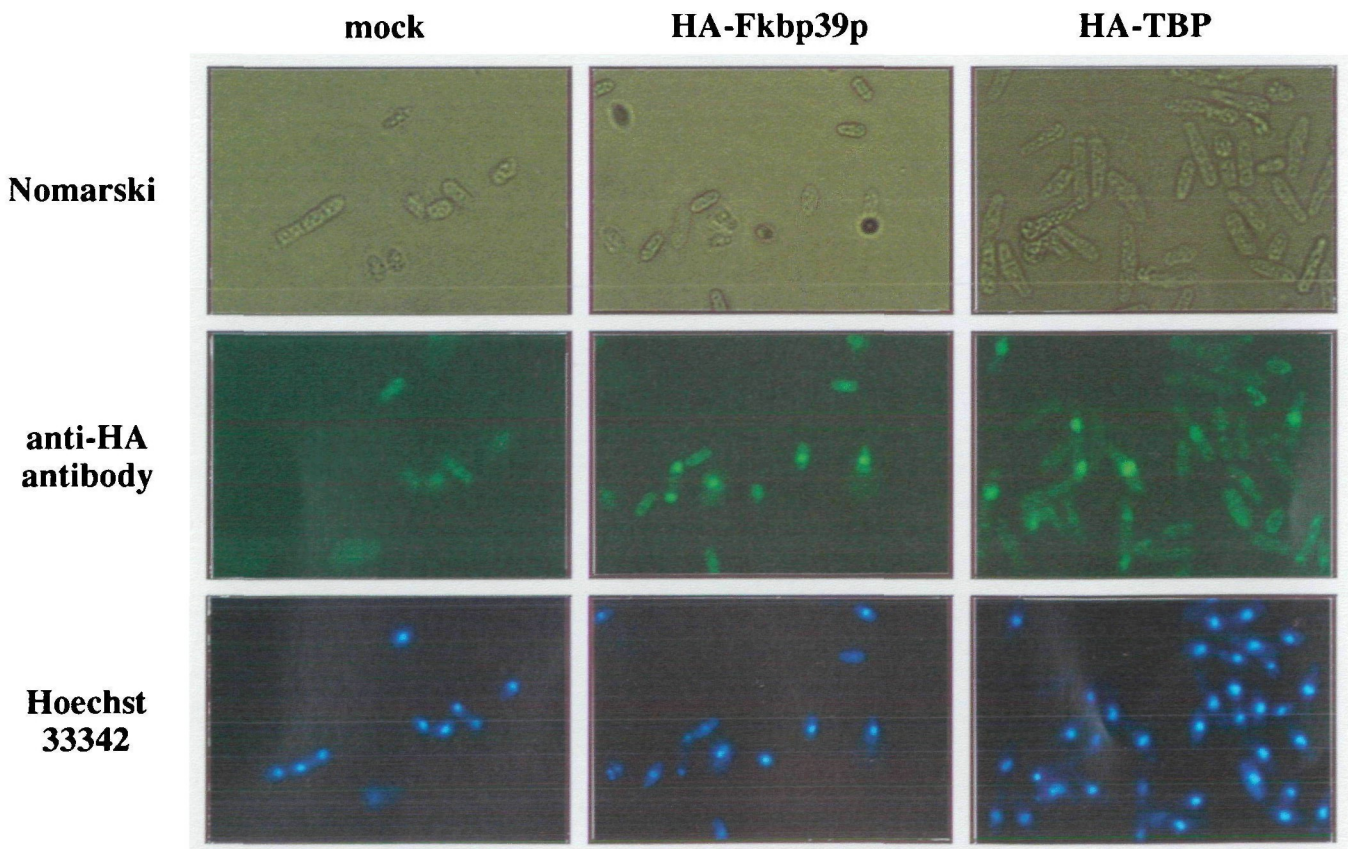


Fig. 6. **Nuclear localization of HA-SpFkbp39p.** Strains carrying the pREP1, pREP1-HA-Fkbp39p, and pREP1-HA-TBP plasmids were stained with anti-HA antibodies. Photographs of cells showing Nomarski (upper), anti-HA antibody immunofluorescence (middle), and Hoechst 33342 fluorescence of DNA (lower).

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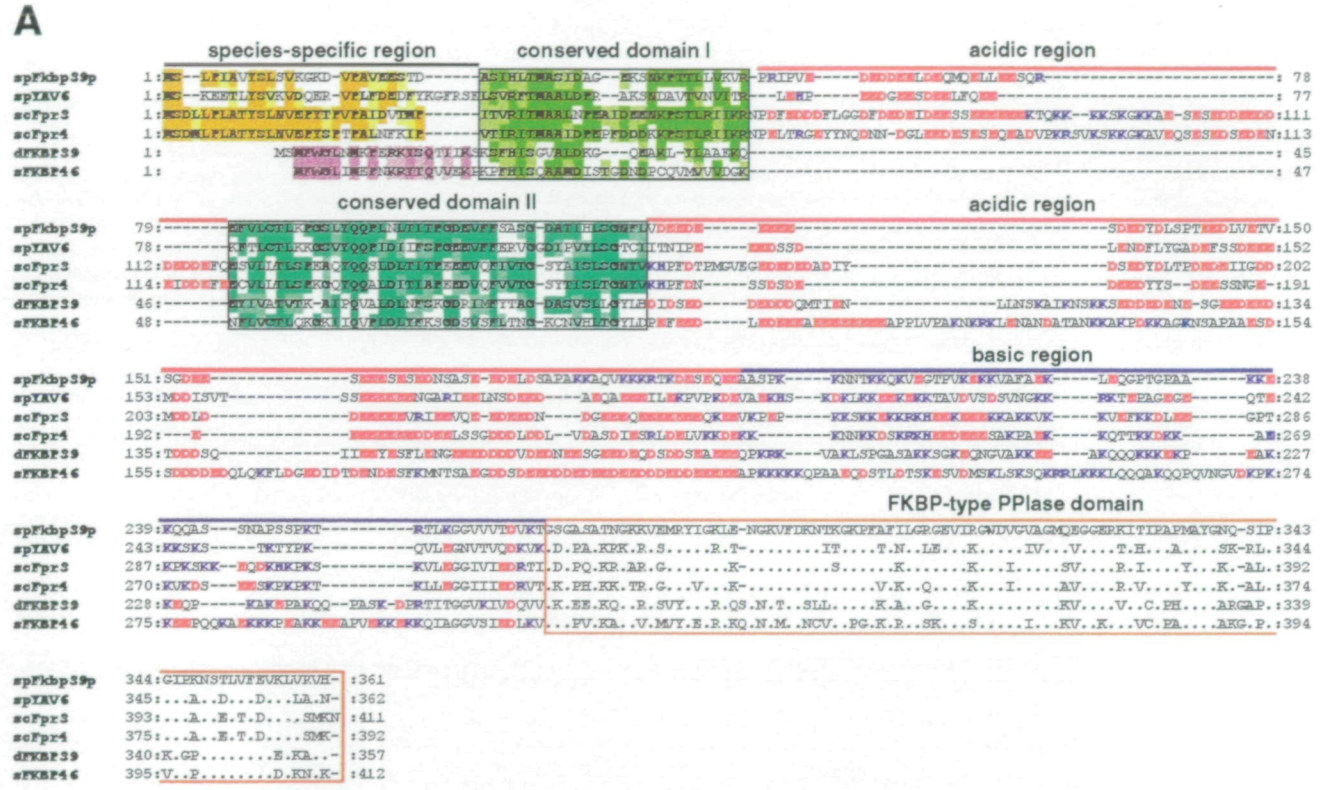


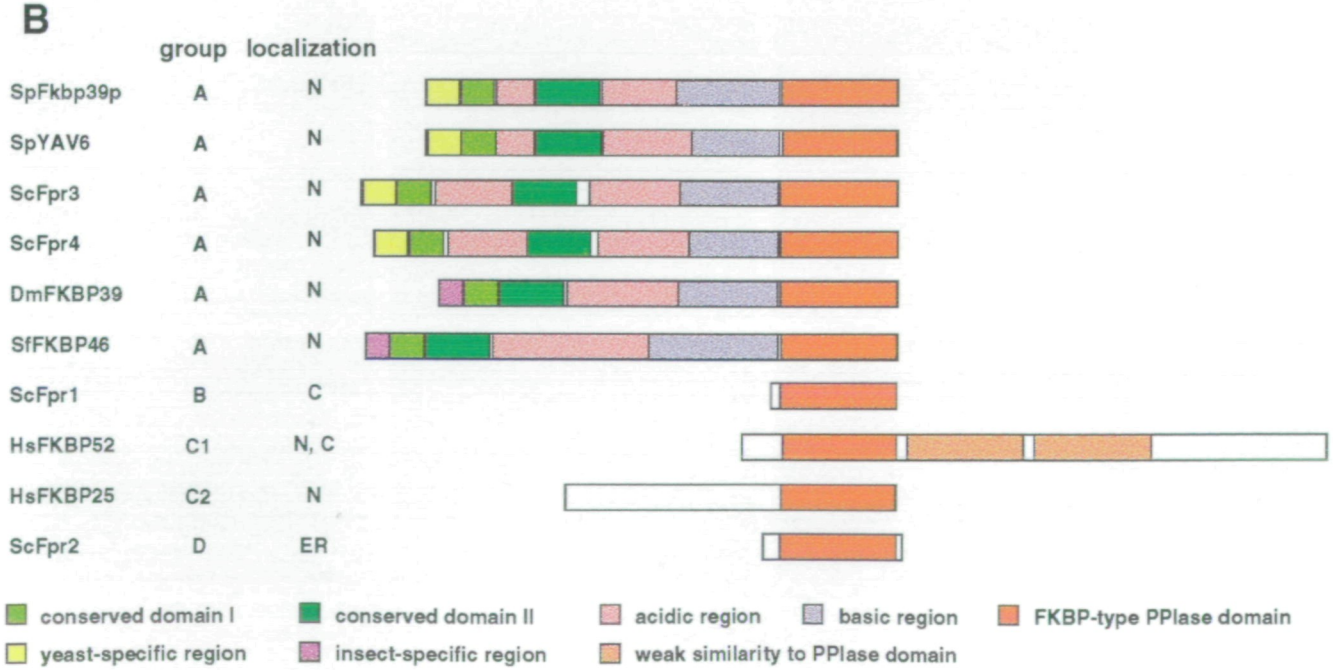

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Fig. 7. **Characteristic domain structure of group A FKBPs.** A: Alignment of the primary structures of group A FKBPs including SpFkbp39p. The alignment was carried out using the CLUSTAL W program (*45*). Conserved domains I and II, and the PPIase catalytic domain are boxed, respectively. In conserved regions I and II, identical and similar amino acids are dark- and pale-shadowed, respectively, and in the PPIase domain, the amino acids identical to those in SpFkbp39p are denoted by dots. The acidic and basic regions are red and blue lined above, respectively. The acidic (D, E) and basic (K, R, and H) residues in charged regions are in red and blue. The species-specific region in which the primary structures of yeast and insect (yellow and purple colored, respectively) group A FKBPs diverge is present in the most N-terminal region, and identical and similar amino acids within either yeasts or insects are dark- and pale-shadowed, respectively. B: Summary of the domains and regions which are present in FKBPs. Group A FKBPs have conserved domains I (indicated in pale green) and II (dark green), acidic (red) and basic (blue) regions, and the PPIase domain (orange). At the N-terminal region, there are "species-specific" regions (indicated in yellow and purple in yeasts and insects FKBPs, respectively) in which the primary structures of yeasts and insects group A FKBPs diverge. These characteristic domains are not present in group B, C, or D. HsFKBP52 (group B2) has two domains exhibiting some similarity to the FKBP-type PPIase catalytic domain (indicated in pale orange) in addition to the functional catalytic domain.

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