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Genome-wide analysis of genes encoding FK506-binding proteins in rice

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Abstract The FK506-binding proteins (FKBPs) are a class of peptidyl-prolyl *cis*/*trans* isomerase enzymes, some of which can also operate as molecular chaperones. FKBPs comprise a large ubiquitous family, found in virtually every part of the cell and involved in diverse processes from protein folding to stress response. Higher plant genomes typically encode about 20 FKBPs, half of these found in the chloroplast thylakoid lumen. Several FKBPs in plants are regulators of hormone signalling pathways, with important roles in seed germination, plant growth and stress response. Some FKBP isoforms exists as homologous duplicates operating in finely tuned mechanisms to cope with abiotic stress. In order to understand the roles of the plant FKBPs, especially in view of the warming environment, we have identified and analysed the gene families encoding these proteins in rice using computational approaches. The work has led to identification of all FKBPs from the rice genome, including novel high molecular weight forms. The rice FKBP family appears to have evolved by duplications of FKBP genes, which may be a strategy for increased stress tolerance.

Keywords FKBPs · PPIases · Chloroplast · Chaperone

Abbreviations

FKBP	FK506- and rapamycin-binding protein
FKBd	FK506- and rapamycin-binding domain
CYP	Cyclophilin
PPIase	Peptidyl prolyl *cis*/*trans* isomerase
TPR	Tetratricopeptide repeat
CaMBd	Calmodulin-binding domain

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Introduction

The FK506-binding proteins (FKBPs) join the cyclophilins (CYPs) under the umbrella term 'immunophilin' due to their discovery as cellular receptors for immunosuppressant drug ligands FK506 and rapamycin (Siekierka et al. 1989; Harding et al. 1989; Bierer et al. 1990). The FKBP-ligand complexes interrupt signal transduction pathways, leading to T-cell suppression (FK506) and T-cell cycle arrest (rapamycin) (reviewed in Kay 1996). Both types of immunophilin catalyse rotation of the peptide bond preceding proline residues between *cis* and *trans* configurations, earning them the title 'peptidyl-prolyl *cis*/*trans* isomerase' (PPIase) (Fischer et al. 1989; Harding et al. 1989; Takahashi et al. 1989) that also describes a third family, the parvulins (Rahfeld et al. 1994). FKBPs are ubiquitous and, in most organisms, comprise protein families distributed throughout the cell (Galat 2000). Four FKBPs exist in *Saccharomyces cerevisiae* (Dolinski et al. 1997), while 15 have been detected in humans (Rulten et al. 2006).

FKBPs are defined by the presence of at least one 'FK506-binding domain' (FKBd), a conserved sequence of approximately 110 amino acids (Galat 2000; Somarelli and Herrera 2007). Structural modelling of FKBPs from organisms separated by large evolutionarily distances, e.g., mammals and plants, has demonstrated that the FKBd forms a highly conserved tertiary structure (Somarelli et al. 2008) that provides the active site for substrate isomerisation (PPIase

activity) (Siekierka et al. 1990; Heitman et al. 1991). Despite strict FKBd conservation, numerous FKBPs demonstrate low or no PPIase activity (Goodyear et al. 1997; Carol et al. 2001; Kamphausen et al. 2002; Sinars et al. 2003; Lima et al. 2006) and it is becoming increasingly clear that FKBPs operate primarily as chaperones, with the FKBd providing a binding site for protein interaction (Wilson et al. 1995; Galigniana et al. 2001; Denny et al. 2005; Riggs et al. 2007; Galat 2008).

Significant size variation exists among members of the FKBP family, ranging from single domain (SD) isoforms comprising a single FKBd, to large (>100 kDa) complex multi-domain (MD) proteins (Galat 2003; Rulten et al. 2006). The most extensively characterised SD isoform is the mammalian FKBP12 which, in addition to its involvement in drug-mediated immunosuppression (Bierer et al. 1990), has a native role involving interaction with ryanodine receptors (RyR) in the release of Ca^{2+} ions in contracting muscle (Timerman et al. 1993), and with a transforming growth factor β (TGFβ) receptor in cellular signal transduction (Wang et al. 1994). In both cases, FKBP12 plays a regulatory role to prevent leaky signalling. Additional domains in the MD FKBPs are often tetratricopeptide repeat (TPR) regions, which are degenerative, helix-forming motifs that are sites of protein interactions (Goebl and Yanagida 1991), or additional FKBds (Galat 2003). Numerous MD FKBPs possess calcium- or calmodulin (CaM)-binding domains (CaMBd) that confer sensitivity to Ca^{2+} signalling. Binding of the Ca^{2+}–CaM complex to mammalian FKBP38 activates its PPIase- and chaperone activities and facilitates its regulation of apoptosis (Edlich et al. 2005). Another well-known MD FKBP in mammals, FKBP52, possesses two successive FKBds, a TPR region and a CaMBd (Callebaut et al. 1992; Radanyi et al. 1994). The TPRs facilitate binding between FKBP52 and the heat shock protein chaperone HSP90 (Silverstein et al. 1999), which escorts transcription regulators such as the glucocorticoid steroid receptor (Tai et al. 1992) and the p53 transcription factor (Galigniana et al. 2004) to the nucleus. The first (N-terminal) PPIase domain of FKBP52 binds to the dynein cellular motor complex (Galigniana et al. 2001), improving the efficiency of nuclear transportation of the attached multiprotein heterocomplex through retrograde movement along cytoplasmic microtubules (reviewed in Pratt et al. 2004). A second mammalian MD isoform, FKBP51, shares sequence and structural homology with FKBP52 (Sinars et al. 2003; Wu et al. 2004) and binds with HSP90 complexes, although shows reduced interaction with dynein (Wochnik et al. 2005). FKBP51 is thought to compete with FKBP52 for HSP90-binding to regulate hormone-dependant recruitment of transcription factors to the nucleus (Davies et al. 2005).

The plant genome encodes the largest FKBP gene family described so far, with 23 identified in both *Arabidopsis thaliana* (He et al. 2004) and rice (Nigam et al. 2008), including both SD and MD isoforms. FKBPs have been isolated from the cytosol (Xu et al. 1998), nucleus (Carol et al. 2001) and ER (Luan et al. 1996) of plants, and may also occur in the mitochondrion (Breiman et al. 1992), however, the majority of isoforms in plants is targeted to the chloroplast thylakoid (Peltier et al. 2002; Schubert et al. 2002; Friso et al. 2004). Like its mammalian counterpart, the archetypal FKBP12 is also the smallest isoform in plants (Luan et al. 1994). In maize and *Chlamydomonas reinhardtii* FKBP12 binds rapamycin (Agredano-Moreno et al. 2007; Crespo et al. 2005), forming a complex that inhibits the 'target of rapamycin' (TOR) kinase, a powerful regulator of plant germination and development (Mahfouz et al. 2006). Arabidopsis, on the other hand, is rapamycin-insensitive (Menand et al. 2002). The Arabidopsis FKBP12 interacts with a nuclear protein involved in mRNA splicing, although the physiological relevance of this interaction is unknown (Faure et al. 1998a; Vespa et al. 2004). SD FKBPs localised to the ER of broad bean, rice and Arabidopsis were upregulated after heat treatment, with a likely role in protein folding (Luan et al. 1996), while recently a nuclear SD FKBP was identified as a heat-inducible molecular chaperone (Nigam et al. 2008).

The importance of some MD FKBPs to plant development was revealed in gene knockout plants. The Arabidopsis FKBP72 (PAS1), exhibiting marginal PPIase activity, contains triplicate FKBds, a TPR and a CaMBd (Carol et al. 2001). PAS1 regulates cell proliferation by targeting a transcription factor from the cytosol to the nucleus (Smyczynski et al. 2006), and *PAS1* silencing caused uncoordinated cell division in leaves and meristems, leading to thick hypocotyls and undeveloped cotyledons in mutants called PASTICCINO (Vittorioso et al. 1998; Faure et al. 1998b). Stunted growth and helical rotation in another Arabidopsis mutant, separately called ULTRACURVATA (Perez-Perez et al. 2004) and TWISTED DWARF (Kamphausen et al. 2002), was the result of *FKBP42* knockout. AtFKBP42 (UCU2/TWD1) contains a single inactive PPIase domain, TPR region, CaMBd and a C-terminal membrane anchor (Kamphausen et al. 2002), and chaperones two distinct ATP-binding cassette (ABC) transporters at distinct cellular membranes (Geisler et al. 2003, 2004) to regulate efflux of auxin (Bouchard et al. 2006; Bailly et al. 2008).

Certain other MD isoforms in plants operate primarily as chaperones involved in abiotic stress tolerance. In Arabidopsis, closely related FKBP62 (ROF1) and FKBP65 (ROF2) are orthologues of mammalian FKBP51/FKB52, possessing triplicate FKBPds, a TPR region and a CaMBd (Vucich and Gasser 1996; Aviezer-Hagai et al. 2007). ROF1 is constitutively expressed, and interacts via TPRs with HSP90 in Arabidopsis (Aviezer-Hagai et al. 2007),

chaperoning the HSP90 complex to the nucleus following heat treatment, probably to induce expression of thermotolerance factors (Meiri and Breiman 2009). ROF2 expression occurs solely after heat stress (Aviezer-Hagai et al. 2007). Despite strong homology with ROF1, ROF2 was unable to bind Arabidopsis HSP90 (Meiri and Breiman 2009). An orthologous FKBP pair in wheat demonstrates similar constitutive (wFKBP73) and heat induced (wFKBP77) expression (Kurek et al. 1999), although both wheat isoforms interact with HSP90 through TPRs (Reddy et al. 1998). With chaperone capabilities independent of its PPIase activity (Kurek et al. 2002), wFKBP73 has been implicated in vacuole formation and seed maturation, and nuclear wFKBP77 in stress response (Dwivedi et al. 2003). Analogous MD FKBP pairs operate in rice (Magiri et al. 2008) and possibly maize (Hueros et al. 1998). It is not known whether these plant MD FKBPs utilise native dynein for cellular transport, although the capacity of wFKBP73 and wFKBP77 to bind to rabbit dynein has been demonstrated (Harrell et al. 2002).

Around half of the FKBPs in plants reside in the chloroplast thylakoid, directed there by cleaved N-terminal transit peptides in the precursor proteins (He et al. 2004), although the majority of lumenal isoforms remain uncharacterised. The most abundant FKBP in plants, the FKBP13 (Luan et al. 1994) is the only lumenal FKBP with PPIase activity (Shapiguzov et al. 2006; Edvardsson et al. 2007). In Arabidopsis, interaction between precursor FKBP13 and Rieske, the iron-sulphur subunit of cytochrome b_6f complex, is reported to regulate membrane accumulation (Gupta et al. 2002). AtFKBP13 catalysis is regulated by redox (Gopalan et al. 2004, 2006) in a mechanism thought to restrict PPIase activity to the oxidising lumen (Buchanan and Luan 2005). Catalytic inactivity of the remaining lumenal FKBPs has prompted suggestions that these isoforms are chaperones that have evolved specificities for individual protein substrates (Romano et al. 2005; Shapiguzov et al. 2006). In support of this, AtFKBP20-2 has shown to chaperone photosystem II (PSII) supercomplex formation (Lima et al. 2006), although further details of lumenal FKBP functionality await elucidation.

Over the last 10 years, several valuable functional characterisations of individual plant FKBPs have been carried out, but it has been identification and analyses of all FKBPs in the Arabidopsis genome (He et al. 2004; Romano et al. 2005) that first revealed the true size and character of the FKBP family in higher plants. As such, we have taken advantage of the sequenced genome of *Oryza sativa* (rice) and selected bioinformatics applications to perform a molecular characterisation of the entire FKBP family of rice, a worthy target of study in its own right, as the most widely cultivated plant worldwide, but also because it provides a model genome for the molecular study of other important cereal species. Our analysis has revealed the largest FKBP family of any organism so far reported, including some isoforms that are completely novel. The rice FKBP family has been shaped by gene duplications, with some duplicates performing coordinated roles in plant stress tolerance. In this report we describe several FKBPs in rice that appear to be important regulators of plant development, while other isoforms may be involved in stress response. Rising temperatures pose an immediate and global challenge to cereal production, while the worldwide demand for carbohydrates increases. As potential development-regulators and potentiators of stress tolerance, the FKBP genes in rice and other cereals may offer target genes for engineering adaptable crop species.

Materials and methods

Retrieval of *FKBP* gene sequences

Several approaches were used to identify the FKBP genes from rice. Searches of the *Oryza sativa* (Japonica cultivar-group) genome were conducted using the tBLASTn function in the NCBI database (http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid=4530) and the amino acid sequence translated from coding sequence of the archetypal *FKBP12* in *Arabidopsis thaliana* (GenBank acc. U96924, Xu et al. 1998; Faure et al. 1998a) as query. Further, the NCBI GenBank Database 'Gene' section was probed using 'fkbp *Oryza sativa*' as the search term, and the amino acid sequence corresponding to each hit was verified as a bona-fide FKBP by identification of FKBds through sequence alignment with *A. thaliana* FKBP12. Finally, the translated sequence of each *FKBP* in *A. thaliana* (He et al. 2004) was used as query in separate BLAST searches of the genome of *O. sativa* japonica cultivar-group (rice pseudomolecule Osa1, genomic release 6) in the Rice Genome Annotation Project database (http://rice.plantbiology.msu.edu/) and the top-scoring sequences were investigated for FKBds.

Genomic locations of rice *FKBP* genes were identified through the BLAST function in the Map Viewer application of NCBI (http://www.ncbi.nlm.nih.gov/mapview/). *FKBP* orthologues in sorghum (*Sorghum bicolour*) were identified by BLAST searching its genome in the Gramene database (http://www.gramene.org/multi/blastview) using sequences translated from rice *FKBP* coding sequences and those in maize (*Zea mays* L.) were identified similarly from Maize sequence (http://www.maizesequence.org/index.html). *FKBP* genes were also identified from the cyanobacteria *Synechocystis* sp. PCC 6803, *Synechococcus* sp. WH 8102 and *Prochlorococcus marinus* str. MIT9313, the green

algae *Chlamydomonas reinhardtii* and *Ostreococcus lucimarinus*, and the moss *Physcomitrella patens*, by searching these genomes in the 'genomic BLAST' database at NCBI (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi) using the *A. thaliana* FKBP12 amino acid sequence and validating each putative FKBP manually as above.

EST retrieval and coding sequence construction

Expressed sequence tags (ESTs) corresponding to the *FKBP* genes in rice, sorghum and maize were isolated from by nucleotide BLAST searches of the NCBI EST database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and by specifying the target organism (e.g. *Z. mays*) from the 'Organism' menu, using the Arabidopsis and rice FKBP amino acid sequences as queries. Suggested coding sequences were automatically provided by the various genome annotation databases described above, and were verified by alignment with corresponding ESTs. Each putative coding sequence was manually aligned with the corresponding genomic gene sequence to verify exon/intron junction sites.

Sequence analysis

All DNA and amino acid sequence alignments were carried out in BioEdit v7.0 (available at http://www.mbio.ncsu.edu/BioEdit/bioedit.html) using the ClustalW function. Sequence identity calculations were performed in BioEdit using the pairwise GLOBAL alignment function. Phylogenetic trees were constructed in TreeView v1.6.6 (available at http://taxonomy.zoology.gla.ac.uk/rod/treeview.html) from the alignment data. The molecular weights and isoelectric points (pI) of putative FKBPs were predicted using the Compute pI/MW Tool at the Expert Protein Analysis System (ExPAsy) site (http://au.expasy.org/tools/pi_tool.html).

Domain identification

Conserved FKBP, TPR and CaM-binding domains were identified in putative MD FKBPs using the Conserved Domain Database (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and verified by manual alignment with those in corresponding Arabidopsis FKBPs (He et al. 2004).

Signal peptide identification

Signal and target peptide predictions and estimations of peptide proteolysis sites were performed in ChloroP, SignalP and TargetP servers (http://www.cbs.dtu.dk/services/). Thylakoid target peptides were verified by plotting the hydropathy of the N-terminal region of the proteins by Kyte-Doolittle analysis in BioEdit, taking advantage of the characteristic hydrophobicity of the h-domain of target peptides (von Heijne 1986) In some cases, additional confirmation of target peptides and cleavage sites was achieved through manual alignments with Arabidopsis FKBPs (He et al. 2004).

Tertiary structure analysis

Putative amino acid sequences of various FKBPs were submitted to the 3D Jigsaw Comparative Modelling database http://bmm.cancerresearchuk.org/~3djigsaw/) (Bates et al. 2001; for tertiary structure predictions. Three dimensional models of other proteins were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). All protein models were viewed and edited using Deepview/Swiss PDB Viewer v4.0 software (http://spdbv.vital-it.ch/).

Microarray analysis

Rice probes corresponding to selected rice *FKBP* genes, as stated in text, were retrieved from RiceChip.Org (http://www.ricechip.org/) using the gene locus identifiers stated in Table 2. Analyses of expression profiles were performed in silico using the Genevestigator microarray analysis tool (https://www.genevestigator.com/gv/index.jsp).

Phylogenetic tree construction

Individual FKBd amino acid sequences were aligned and back-translated to the corresponding DNA sequence. The resulting alignment was used to create a phylogenetic tree in Mega v4 (available at http://www.megasoftware.net/) using the Neighbour-Joining method, p-distance model, bootstrapping set at 1,000 replications. Bootstrap consensus tree is shown in Fig. 4.

Results

FKBPs form a conserved multigene family in rice

In an effort to gauge the level of conservation among the FKBP families in higher plants, we scanned the sequenced genome of *Oryza sativa* (rice) for these genes using multiple techniques described above. Our analysis yielded 29 distinct loci encoding putative FKBPs containing the characteristic FKBd. This exceeds the number (23) identified previously in rice (Nigam et al. 2008) and in Arabidopsis (He et al. 2004) mainly due to our identification of additional chloroplastic isoforms and MD isoforms, respectively (Fig. 1; Table 1; detailed below). We have also adopted, and recommend, a system of nomenclature

whereby the plant FKBPs are labelled according to their orthology with reported isoforms in Arabidopsis, rather than solely based on their estimated molecular weights. For example, the putative FKBP from rice that shares highest amino acid identity (58%) with AtFKBP16-3 is denoted here as 'OsFKBP16-3', despite its predicted molecular weight (15 kDa) (Table 2). In some cases, similarities in domain structures or pI have also been considered.

Cytosolic FKBPs

OsFKBP12 comprises a single FKBd of 112 amino acids (Fig. 1) that exhibits 42% identity with human FKBP12 (GenBank acc. CAA39272) and slightly higher identity (45%) with the first FKBd of the MD human FKBP52 (GenBank acc. NP_002005) (data not shown). As shown in Fig. 2, OsFKBP12 conserves three of the five residues with strongest influence over catalytic activity in mammalian FKBP12 (DeCenzo et al. 1996; Tradler et al. 1997), as well as a cysteine pair that is unique to the plant FKBP12 isoforms and was vital for interaction with CaN in vitro (Xu et al. 1998). The motif 'QGS' appearing in OsFKBP12 (position 52, Fig. 2) also occurs in maize FKBP12, where it was shown to govern rapamycin-FKBP12-binding (Agredano-Moreno et al. 2007), while the divergent 'KGA' motif in AtFKBP12 (Fig. 2) was responsible for rapamycin-insensitivity in Arabidopsis (Mahfouz et al. 2006; Agredano-Moreno et al. 2007).

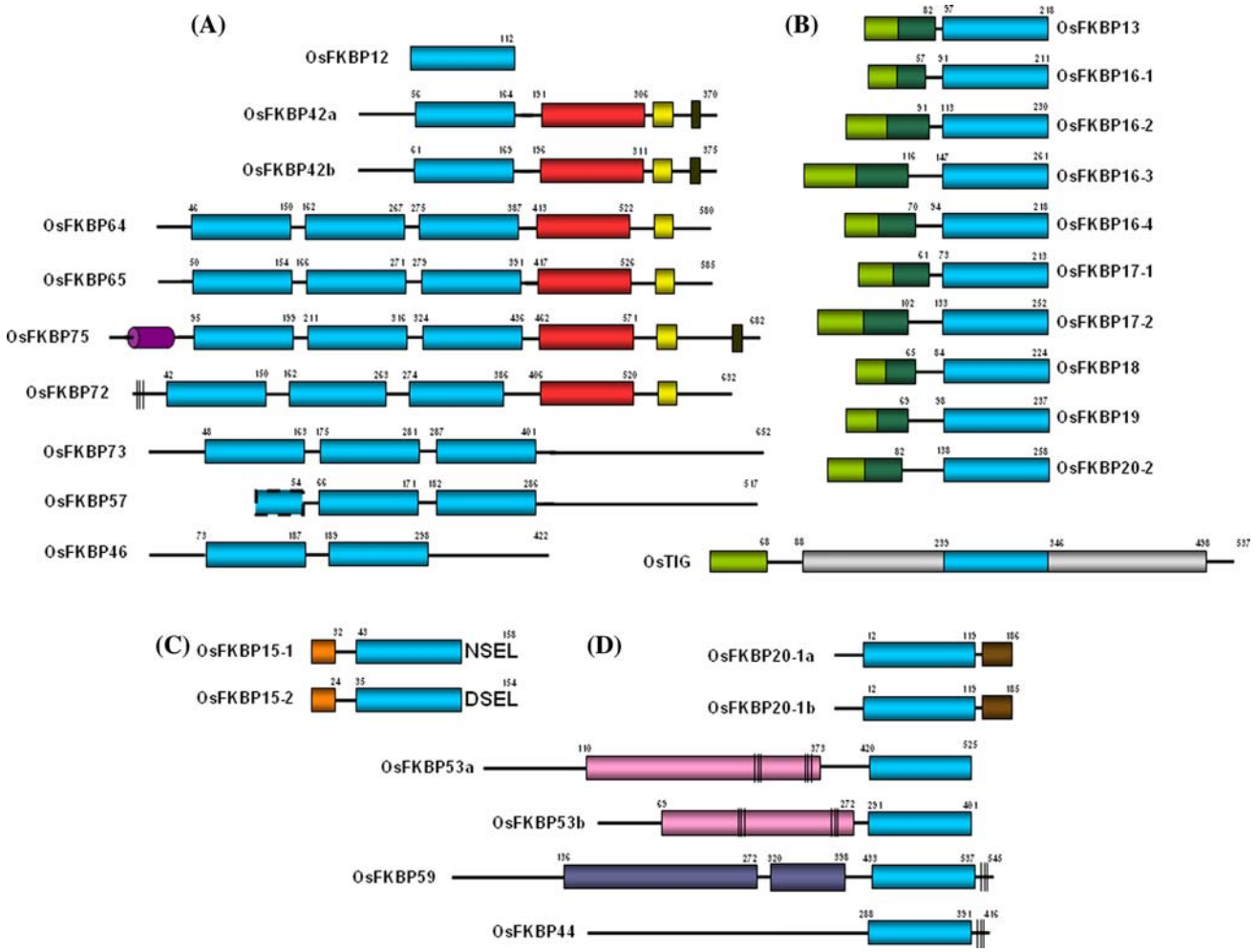

Fig. 1 Schematic representation of the predicted domain structures and subcellular locations of putative FKBP family members in rice. **a** Cytosolic; **b** Chloroplast-localised; **c** ER-localised; **d** Nucleus-localised. Lengths of proteins and domains stated as number of amino acids. *Blue boxes*: FKBds; *blue boxes* with *dashed outlines*: truncated FKBP domains; *brown boxes*: nuclear localisation signals; *light green*: chloroplast signal peptide; *dark green*: thylakoid target peptide; *orange*: ER localisation signals, C-terminal ER retention motifs indicated as written; *black boxes*: membrane anchor sequences; *Pink boxes pink*: lysine/arginine-rich regions; *purple*: regions of high concentrations of charged residues; *red boxes*: TPR domains; *yellow boxes*: calmodulin-binding domains; *grey boxes*: trigger factor domains; vertical *black lines*: lysine motifs; *purple cylinder* in OsFKBP75: Gly-rich repeat

Table 1 Predicted subcellular locations of FKBPs in rice and *A. thaliana*

Subcellular location	Rice[a]	Rice[b]	Arabdopsis[c]
Cytosol	9	10	4
Nucleus	7	6	5
Chloroplast	11	4	12
ER	2	0	2
Mitochondria	0	2	0
Extracellular matrix	0	1	0
Total	29	23	23

[a] This work

[b] Nigam et al. (2008)

[c] He et al. (2004)

Two rice genes (*OsFKBP42a* and *OsFKBP42b*) encoding orthologues of *AtFKBP42* are found on chromosomes 11 and 12 in regions that are known to be duplicates in rice (Wu et al. 1998). Their putative products, OsFKBP42a and OsFKBP42b are 90% identical and 96% similar (data not shown), both exhibiting domain structures analogous to that of AtFKBP42, including single N-terminal FKBDs (Fig. 1) with 76–78% identity to the AtFKBP42 FKBPd, followed by TPR repeats that both conserve the five residues implicated in HSP90-binding (Aviezer-Hagai et al. 2007), and a CaMBd. Finally, C-terminal membrane anchors in the OsFKBP42 isoforms (Fig. 1) are virtually identical in amino acid sequence to that of AtFKBP42, which anchors this FKBP to the tonoplast and plasma membranes in Arabidopsis (Kamphausen et al. 2002; Scheidt et al. 2007). Only two catalytic residues, Tyr26 and Asp37, are conserved in OsFKBP42 FKBDs, also the case for the PPIase-inactive FKBd of AtFKBP42 (Kamphausen et al. 2002) that instead provides the site of P-glycoprotein (PGP) interaction (Geisler et al. 2004). In the FKBd of AtFKBP42, Cys70 and Leu72 were shown to significantly influence AtFKBP42 interaction with PGP1 (Geisler et al. 2004). Both residues are conserved in OsFKBP42b, but OsFKBP42a has a conservative Leu72Val substitution. Both OsFKBP42 paralogues have ESTs in the database, indicating both are expressed, and micro-array data show distinct spatial expression profiles for each, with OsFKBP42a (probe ID Os.52139.1.S1_at) expressed at highest levels in crown tissue, while OsFKBP42b (probe ID Os.17539.1.S1_at) is expressed more highly in roots (not shown).

The rice genes *OsFKBP64* and *OsFKBP65* have 73% identical CDS and are most similar to *AtFKBP62,* and encode the putative MD FKBPs OsFKBP64 and OsFKBP65 (Table 2), which conserve identical domain structures to AtFKBP62 and AtFKBP65; triplicate FKBDs followed by a TPR region and a CaMBd (Fig. 1). These results are consistent with a previous report (Magiri et al. 2006) that rFKBP64 (OsFKBP64) and rFKBP65 (OsFKBP65) are orthologues of the MD FKBP pairs in Arabidopsis (ROF1 and ROF2; Aviezer-Hagai et al. 2007) and wheat (wFKBP73 and wFKBP77; Kurek et al. 1999). This rice FKBP pair is likely to have PPIase capabilities, with activity probably limited to the first FKBDs, as these conserve all five catalytic residues (Fig. 3). Subsequent FKBDs include two key residues, and one key residue, respectively. The five HSP90-binding residues appear in the TPR regions of OsFKBP64 and OsFKBP65 (not shown), suggesting the probability of analogous interaction with OsHSP90-client complexes.

Another putative *FKBP* in rice encoding a protein with 58% identity to *both* AtFKBP62 and AtFKBP65 is designated here as OsFKBP75 from its predicted molecular weight (Table 2), and is identical to 'rFKBP75'described by Magiri et al. (2006). It exhibits the same domain structure of OsFKBP62s, but also a distinctive N-terminal '$(Xaa\text{-}Gly\text{-}Gly)_{12}$' repeat motif, where Xaa is Lys, Met or Phe (Fig. 1). Unique among FKBPs, this region was predicted to implicate targeting to the endoplasmic reticulum (ER) in rice (Magiri et al. 2006), although we noted similarity to motifs occurring in certain cytosolic, chloroplastic and mitochondrial folding chaperones (discussed below). We detected a previously unreported hydrophobic domain of approximately 40 residues at the C-terminus of OsFKBP75 (Fig. 1) with a hydrophobicity profile virtually identical to that of a predicted membrane anchor at the C-terminus of AtFKBP72 (He et al. 2004) (results not shown). Aside from unique N- and C-termini, OsFKBP75 displays striking sequence homology with OsFKBP64/65 (around 70% identical), including identical conservation of key catalytic residues in the three FKBDs (Fig. 3).

OsFKBP72 is another MD isoform that includes three FKBDs, although these are dissimilar to other MD FKBPs of rice (Fig. 4) and probably catalytically inactive (Fig. 3). Like the orthologous AtFKBP72 (PAS1), OsFKBP72 includes a TPR region and CaMBd (Fig. 1), although it lacks a homologous C-terminal hydrophobic motif that was identified in OsFKBP75 (above). The 130 residue sequence at the C-terminus of AtFKBP72 (PAS1) was required for binding to the FAN transcription factor and regulating nuclear recruitment of the complex (Smyczynski et al. 2006). Although lacking homology in the extreme C-terminus, OsFKBP72 and AtFKBP72 share sequence identity in the remaining C-terminal domain, which includes the CaMBd (Carol et al. 2001). *OsFKBP72* encodes a Lys-rich N-terminus analogous to AtFKBP72, likely providing a nuclear localisation signal (NLS) for these proteins.

Three FKBd-encoding loci were identified within approximately 180 kb on rice chromosome 1 (Supplementary data Fig. S1), and several transposable elements

Table 2 Properties of putative FKBPs identified from *O. sativa* genome

O. sativa FKBP name	*O. sativa* FKBP locus ID	Arabidopsis homologue (ID/Sim)[a]	ESTs[b]	Subcellular location[c]	Putative length[d]	Molecular weight (kDa)[e]	pI[f]	Comments
OsFKBP12	Os02g52290	AtFKBP12 (74/86)	208	Cytosol	112	12.1	7.8	
OsFKBP13	**Os06g45340**	**AtFKBP13 (52/63)**	**27**	**Lumen**[1]	**218/136**	**22/14.2**	**5.8**	
OsFKBP15-1	Os01g68710	AtFKBP15-1 (69/79)	71	ER	158/127	16.8/13.6	8.8	
OsFKBP15-2	Os09g32526	AtFKBP15-1 (72/81)	47	ER	154/131	16.3/14.1	5.0	
OsFKBP16-1	**Os02g10590**	**AtFKBP16-1 (54/66)**	**5**	**Lumen**[2]	**211/136**	**22.8/17.0**	**6.0**	
OsFKBP16-2	**Os02g51570**	**AtFKBP16-2 (54/65)**	**33**	**Membrane/lumen**[3,4]	**230/139**	**23.4/14.9**	**8.9**	
OsFKBP16-3	**Os08g42850**	**AtFKBP16-3 (58/69)**	**38**	**Lumen**[1,3]	**261/145**	**27.1/15.4**	**5.0**	
OsFKBP16-4	**Os07g09040**	**AtFKBP16-4 (64/75)**	**8**	**Membrane/lumen**[3,4]	**218/148**	**22.7/15.8**	**9.6**	
OsFKBP17-1	**Os02g07220**	**AtFKBP17-1 (57/70)**	**11**	**Lumen**	**213/158**	**22.2/16.4**	**5.6**	
OsFKBP17-2	**Os03g50080**	**AtFKBP17-2 (58/74)**	**18**	**Lumen**	**252/150**	**26.6/16.1**	**4.9**	AtFKBP17-2: called AtFKBP26[7]
OsFKBP18	**Os02g02550**	**AtFKBP18 (57/67)**	**10**	**Lumen**[1,2]	**242/159**	**25.8/17.3**	**8.0**	
OsFKBP19	**Os07g04160**	**AtFKBP19 (63/73)**	**20**	**Lumen**[1,3]	**237/168**	**25.9/18.7**	**5.6**	
OsFKBP20-1a	Os05g38370	AtFKBP20-1 (77/90)	42	Nucleus	186	19.9	6.8	
OsFKBP20-1b	Os01g62610	AtFKBP20-1 (75/88)	7	Nucleus	185	20.1	5.9	
OsFKBP20-2	**Os07g30800**	**AtFKBP20-2 (59/73)**	**16**	**Lumen**[1]	**258/176**	**28/19.9**	**8.8**	
OsFKBP42a	Os12g05090	AtFKBP42 (66/81)	15	Cytosol	370	42.2	5.7	
OsFKBP42b	Os11g05090	AtFKBP42 (66/80)	54	Cytosol	375	42.4	6.0	
OsFKBP53a	Os04g36890	AtFKBP53 (40/57)	60	Nucleus	525	57.2	6.0	Lys-rich, acidic residues
OsFKBP53b	Os09g12270	AtFKBP53 (33/50)	6	Nucleus	401	44.1	5.7	Lys-rich, acidic residues
OsFKBP59	Os09g01650	AtFKBP53 (27/42)	5	Nucleus	531	58.8	5.1	
OsFKBP44	Os09g01670	AtFKBP53 (26/41)	0	Nucleus	416	44.3	5.2	
OsFKBP64	Os08g41390.2	AtFKBP62 (75/85)	238	Cytosol	591	64.1	5.2	Called rFKBP64[5]
		AtFKBP65 (71/84)						
OsFKBP65	Os04g28420	AtFKBP62 (70/85)	45	Cytosol	585	65.6	5.4	Called rFKBP65[5]
		AtFKBP65 (69/84)						
OsFKBP75	Os02g28980	AtFKBP62 (58/70)	31	Cytosol (ER?[5])	682	74.8	5.2	Called rFKBP75[5]
		AtFKBP65 (58/70)						cpn60-like repeat, membrane anchor
OsFKBP73	Os01g38229.2	AtFKBP65 (35/63)	82	Cytosol	652	72.9	4.9	3 PPIase domains
OsFKBP57	Os01g38180	AtFKBP65 (32/48)	6	Cytosol	517	57.4	5.4	2.5 PPIase domains
OsFKBP46	Os01g38359	AtFKBP65 (25/37)	35	Cytosol	422	45.6	5.2	2 PPIase domains
OsFKBP72	Os03g25140	AtFKBP72 (65/77)	54	Cytosol/nucleus[6]	632	70.4	5.4	
OsTIG	Os06g20320	AtTrigger Factor (52/52)	29	Stroma	537/466	59.9/52.6	4.8	

Putative lumenal isoforms shown are shown in bold

References: [1] Schubert et al. (2002); [2] Goulas et al. (2006) (see Supplementary Material therin); [3] Peltier et al. (2002); [4] Friso et al. (2004); [5] Magiri et al. (2006); [6] Carol et al. (2001); [7] Galat (2003). All other locations not determined (see He et al. 2004)

[a] Homologies with corresponding FKBPs in *A. thaliana* calculated from GLOBAL pairwise alignments of amino acid sequences translated from coding sequences. Amino acid sequence identity and similarity scores are given

[b] The number of distinct ESTs for each rice FKBP in the NCBI *O. sativa* EST database

[c] Subcellular location on predicted localisation of orthologous FKBPs in *A. thaliana*; see references below

[d] Stated as number of amino acids; in some cases the lengths of both precursor and mature domains are given

[e] Predicted as described in "Methods"; in some cases the MW of both precursor and mature protein are given

[f] Predicted as described in "Methods"

were also detected in this region. The putative amino acid sequences of each of these FKBPs exhibit 25–35% identity to AtFKBP65 (Table 2) and AtFKBP62 (data not shown) and are unlikely to be true orthologues due to absence of recognisable TPRs and CaMBds in the rice isoforms. Accordingly, these have been labelled after their molecular weights: OsFKBP73 (LOC_Os01g38229.2), OsFKBP57 (LOC_Os01g38180.1) and OsFKBP46 (LOC_Os01g383

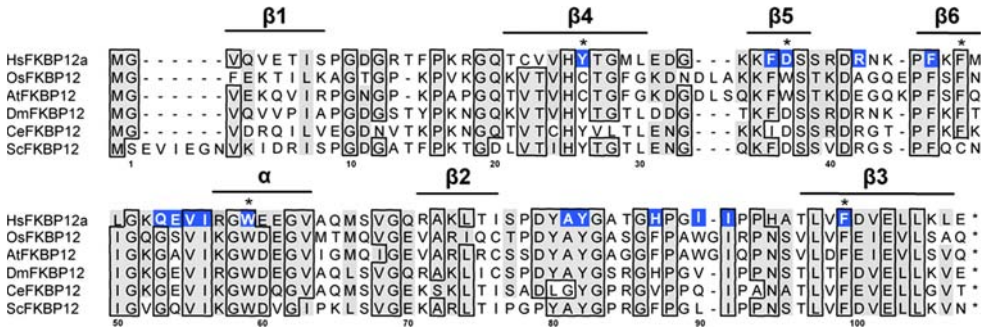

Fig. 2 Amino acid alignment of FKBP12 isoforms from different species. *Homo sapiens* (Hs; Genbank protein accession number AAI19733), *Arabidopsis thaliana* (At; U96924), *Drosophila melanogaster* (Dm; AAF57582), *Caenorhabditis elegans* (Ce; CAA22330) and *Saccharomyces cerevisiae* (Sc; AAA03564), aligned with *Oryza sativa* (Os). Alignment positions numbered according to human FKBP12 sequence (Standaert et al. 1990). Asterisks above residues denote residue that are important for PPIase activity in mammalian FKBP12 (DeCenzo et al. 1996; Tradler et al. 1997). Residues highlighted blue are involved in FK506 and/or rapamycin binding in mammalian FKBP12 (Van Duyne et al. 1993)

59.1) (Table 2; Fig. 1). ESTs indicate that all three loci are expressed, and no subcellular localisation motifs were detected, indicating they may be cytosolic. Three successive FKBds occur in OsFKBP73 and two in OsFKBP46, while OsFKBP57 possesses an incomplete N-terminal FKBd that begins at the region forming the alpha helix in the archetypal FKBd tertiary structure (Van Duyne et al. 1993) (residue 56 in hFKBP12, Fig. 2), followed by two complete FKBds. Variability in key residues (Fig. 3) indicates that PPIase activity may not be expected from any of these FKBds. Close inspection of the DNA sequence upstream of the putative ATG start codon of *OsFKBP57* revealed significant identity to the 5′ region of *OsFKBP73* CDS, suggesting that these two genes may be duplicates, and the start codon used may have relocated downstream in *OsFKBP57* following duplication. The amino acid sequences of N-terminal FKBds in OsFKBP73 and OsFKBP46 and the first complete FKBd in OsFKBP57 are ~60% identical, causing these domains to cluster together in phylogenetic analysis (Fig. 4). The C-terminal 50 residues of OsFKBP73 and OsFKBP46 also show 50% identity. These results strongly suggest that these three genes have arisen through regional duplications on rice chromosome 1.

Nuclear FKBPs

Two loci in rice encode proteins (OsFKBP20-1a and OsFKBP20-1b) with 85% identity, suggesting a recent common ancestor gene, which appear to be orthologous to the nuclear AtFKBP20-1 (He et al. 2004), while AtFKBP15-3 (similar to AtFKBP20-1 except its NLS is N-terminal) does not have a rice orthologue. Both OsFKBP20-1a and OsFKBP20-1b exhibit a C-terminal, Lys-rich putative NLS and a single FKBd. Conservation of crucial residues may infer PPIase activity for the OsFKBP20-1 isoforms, of which OsFKBP20-1a (OsFKBP20) is a proven nuclear chaperone (Nigam et al. 2008). Micro-arrays show that OsFKBP20-1a (Os.11124.1.S1_at) is expressed at high levels in all rice tissues, while OsFKBP20-1b (Os.54469.1.S1_at) expression is restricted to the seed, mirroring the expression profiles of AtFKBP20-1 (251799_AT) and AtFKBP15-3 (250772_AT), respectively (data not shown).

Four loci in rice code for putative orthologues of the nucleus-localised AtFKBP53 (Table 2). Of these, OsFKBP53a and OsFKBP53b possess a single C-terminal FKBd and multiple Lys-rich domains in N-terminal regions (Fig. 1), as noted in AtFKBP53 (He et al. 2004) and in nuclear FKBPs from *Spodoptera frugiperda* (Alnemri et al. 1994), *Saccharomyces cerevisiae* (Dolinski et al. 1997) and *Neurospora crassa* (Pinto et al. 2008), strongly suggesting nuclear localisation for the rice proteins. Both OsFKBP53 isoforms include high concentrations of acidic residues (Asp and Glu) that were predicted to facilitate DNA binding for AtFKBP53 (He et al. 2004). Although this remains to be demonstrated in plants, homologous acidic domains in nuclear FKBPs facilitate DNA- and histone-binding in *S. cerevisiae* (Fpr4: GenBank acc. EDN59346) (Kuzuhara and Horikoshi 2004; Xiao et al. 2006) and RNA interaction in *Bombix mori* (FKBP45: GenBank acc. AAY86706) (Somarelli et al. 2008). OsFKBP53a may be an operational PPIase, as it conserves all five catalytic residues (Fig. 3), while OsFKBP53b has only three. Both OsFKBP53 isoforms are highly expressed in rice roots. While OsFKBP53b (probe ID OsAffx.17661.1.S1_at) is also expressed at a considerable level in anthers, expression of OsFKBP53a (probe ID Os.16231.1.S2_at), which has ten times the number of ESTs in the database, was distinctly absent from anther tissue (not shown).

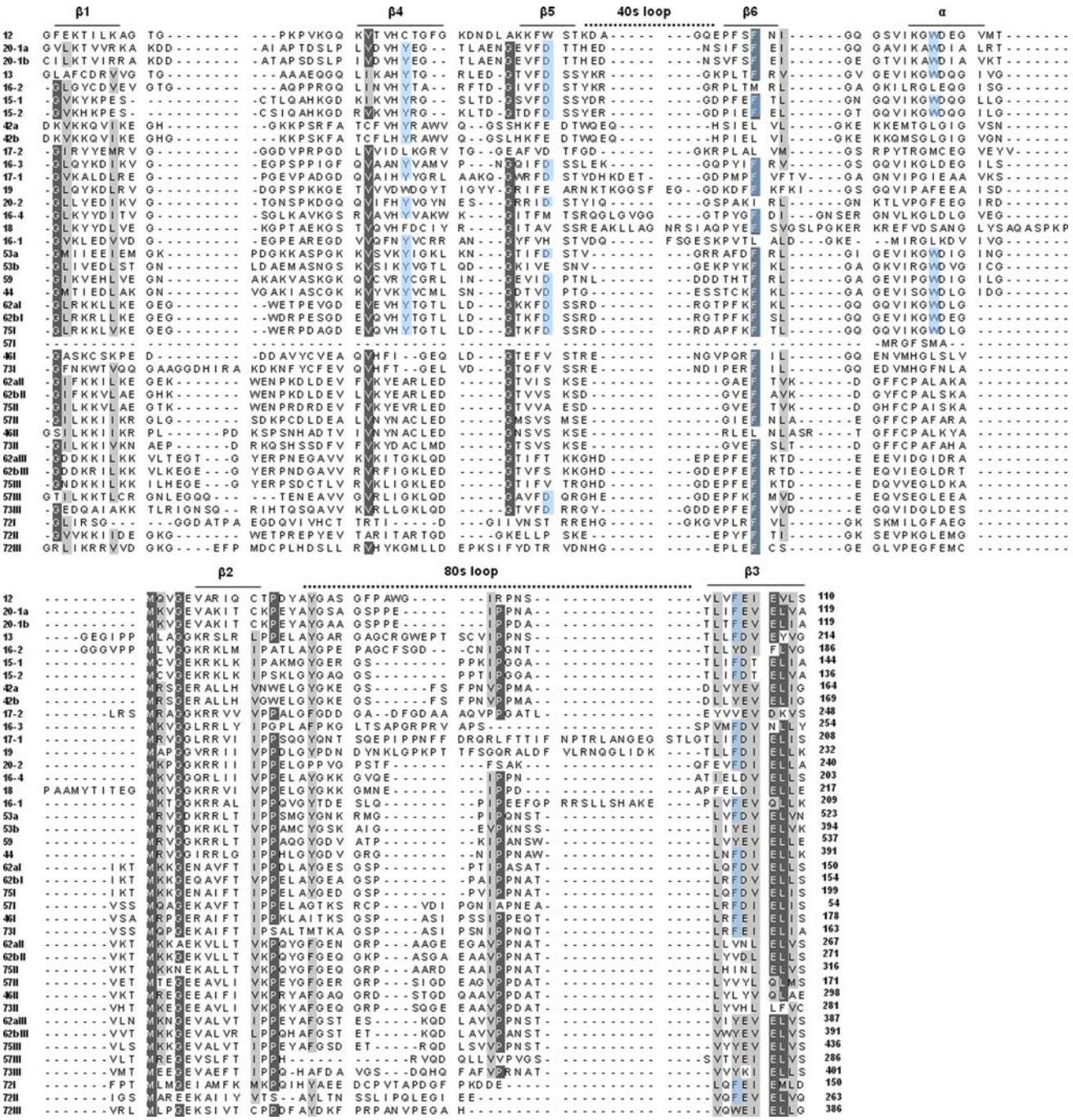

Fig. 3 Alignment of the putative amino acid sequences of FKBds predicted from all *O. sativa* FKBP genes. Identical residues shown as white on dark grey background, similar residues shaded light grey, dashes indicate gaps. Residues shaded blue are identical to those with major influence over PPIase activity (DeCenzo et al. 1996; Tradler et al. 1997). Predicted alpha helix and beta sheet tertiary structures are indicated, as per predicted 3D model of OsFKBP12 (generated by 3D JIGSAW). Protein-binding 40s and 80s loops are labelled. Number at end of sequence indicates position of C-terminus of FKBd in total protein sequence

The remaining AtFKBP53-like isoforms from rice have been designated OsFKBP59 and OsFKBP44 based on estimated molecular weights, as they exhibit relatively low identities with AtFKBP53 (Table 2) and, although both conserve the C-terminal FKBds (Fig. 1) that include four (OsFKBP59) and five (OsFKBP44) catalytic residues, they lack the highly acid regions found in AtFKBP53. The close proximity of *OsFKBP59* and *OsFKBP44* on chromosome 9 (Supplementary data Fig. S1) and major sections of amino acid identity (not shown) indicate these genes also

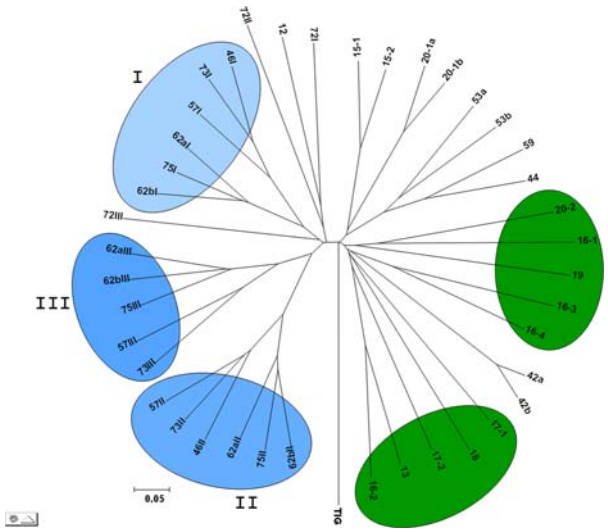

Fig. 4 Phylogenetic relationship of individual FKBds of rice FKBPs. 'I' indicates N-terminal FKBds, 'II' and 'III' indicate subsequence domains. Green shading shows domains from lumenal isoforms. Method of tree construction is detailed in "Materials and methods" section

probably arose from a regional duplication. No ESTs were identified for *OsFKBP44*, suggesting a possible pseudogene. OsFKBP59 and OsFKBP44 have been previously predicted to be cytoplasmic and mitochondrial, respectively (Nigam et al. 2008); however, our predictions indicate nuclear localisation for both. Characteristic 'KRAKR' and 'KKARK' motifs at extreme C-termini of OsFKBP59 and OsFKBP44 respectively, provide NLS for numerous eukaryotic nuclear proteins (Aster et al. 1997; Imamura et al. 2001; Santiago et al. 2009) and may be translocation domains for these rice FKBPs.

FKBPs in the ER

Two genes in rice encode putative FKBPs that show highest amino acid sequence identity with AtFKBP15-1 and lower with AtFKBP15-2, the two ER-localised isoforms of Arabidopsis (Luan et al. 1996). We designated the rice isoforms as OsFKBP15-1 and OsFKBP15-2 based on their estimated pI values (Table 2), which reflect those of AtFKBP15-1 and AtFKBP15-2, respectively (He et al. 2004). OsFKBP15-1 and OsFKBP15-2 share 70% amino acid identity, with heterogeneity occurring mainly at the C-terminus, which contains an acidic region in OsFKBP15-2 that is missing from OsFKBP15-1 (not shown). Both rice *FKBP15* genes encode all five PPIase residues, and possess C-terminal ER-retention signals (Fig. 1) analogous to those in the Arabidopsis orthologues (He et al. 2004). It is interesting to note a lack of any MD FKBP isoforms from the rice ER, in contrast to the mammalian ER that contains at least four calcium-regulated MD FKBP chaperones (Rulten et al. 2006).

FKBPs in the chloroplast

We identified ten genes encoding putative lumenal FKBPs in the rice genome (Table 2). Eleven lumenal FKBPs have been described in Arabidopsis (He et al. 2004), of which only *AtFKBP17-3*, most likely a duplicate of *AtFKBP17-2* (P. Gollan, unpublished), has no rice orthologue. All lumenal FKBPs in rice are SD isoforms, comprising chloroplast and thylakoid targeting signal peptides at the N-terminus of a single FKBd. Complete conservation of the central twin-arginine motif in the target peptides supports their predicted thylakoid localisation and also indicates that they most likely enter the thylakoid via the 'twin-arginine translocation' (Tat) pathway, as proven for AtFKBP13 (Gupta et al. 2002). This is significant because Tat transports *folded* proteins across the thylakoid membrane, independently of nucleotide triphosphates and protein cofactors (reviewed in Cline and Theg 2007). Most notably, the twin-Arg motif is conserved in OsFKBP16-2, unlike the 'Lys-Arg' in AtFKBP16-2 (He et al. 2004), a motif associated with less efficient Tat-mediated transport of the photosynthetic Rieske iron-sulphur protein (Molik et al. 2001). The twin-Arg in the transit peptide is preceded by a polar 'n-domain' and followed by a predominantly hydrophobic 'h-domain', terminating in the thylakoid processing peptidase (TPP) cleavage site, generally 'Ala-Xaa-Ala'. In the absence of experimental data, exclusive Tat transport cannot be claimed for the lumenal FKBPs, due to homologies between Tat peptide substrates and those of the 'secretory' (Sec) method of thylakoid entry, which acts on *unfolded* proteins (Cline and Theg 2007). Further, the ability of some proteins to employ both methods has been demonstrated (Tullman-Ercek et al. 2007; Koussevitzky et al. 2008).

OsFKBP13 may be the sole FKBP contributor to lumenal PPIase activity, as it is the only isoform to conserve all five catalytic residues. This is in line with previous results that show AtFKBP13, which shares 51% amino acid identity with OsFKBP13, to be the only active FKBP in the Arabidopsis thylakoid (Shapiguzov et al. 2006; Edvardsson et al. 2007). OsFKBP13 includes four Cys residues that also occur in the Arabidopsis orthologue, where they govern enzyme activity through disulphide formation (Gopalan et al. 2004, 2006). We detected the 'Trp-Glu-Pro-Thr' motif occurring between the C-terminal Cys residues of OsFKBP13, while such a motif is not found at the corresponding site in AtFKBP13, although it does appear in the maize and sorghum orthologues (P. Gollan, unpublished). Four Cys residues were found in OsFKBP16-2 at positions virtually identical to OsFKPB13. Our analysis of

both proteins predicts the formation of N- and C-terminal disulphide bonds under oxidising conditions in each case (results not shown; see "Methods" for disulphide prediction). Two Cys residues occurring in OsFKBP16-3, located between the putative thylakoid target peptide and the FKBd (not shown), were also predicted to be disulphide-forming. Another Cys pair occurring in at the extreme C-terminus of OsFKBP20-2 is conserved in AtFKBP20-2, and was thought to confer redox-sensitivity to that FKBP (Lima et al. 2006).

In comparison to their non-lumenal counterparts, the PPIase domains of the lumenal FKBPs exhibit a high degree of sequence variability (Fig. 3). Unique sequence insertions occur in several lumenal isoforms and are localised to regions of the protein that form loops between conserved secondary structures of the active site. In several non-lumenal FKBPs the loop regions, particularly the '40s' and '80s' loops, have shown to provide interaction sites for specific protein partners (Griffith et al. 1995; Ivery and Weiler 1997; Samso et al. 2006; Riggs et al. 2007; Galat 2008), suggesting that variability in the lumenal FKBPs may infer diversity in protein interactors.

The trigger factor

Presence of an FKBd technically confers the title of FKBP to the Trigger Factor (TIG). TIG is found in bacteria and plant chloroplast stroma, but absent from other eukaryotes, and its limited sequence identity with archetypal FKBP distances it phylogenetically from other FKBPs (Stoller et al. 1995; Romano et al. 2005). A single *TIG* gene was found in rice, coding for a protein that is 52% identical to the Arabidopsis counterpart (Table 2) and includes a chloroplast signal peptide and a single FKBd flanked by 'trigger factor' domains (Fig. 1). A role for plant TIG remains undefined, but prokaryotic TIG is known to be a highly active PPIase (Stoller et al. 1995) that associates with ribosomes, where it probably operates as a chaperone for nascent peptides (Merz et al. 2008). Although its role remains unexplored, chloroplastic TIG may chaperone newly synthesised proteins in the chloroplast stroma.

FKBP evolution in the rice GENOME

The FKBP multigene family is thought to have emerged through domain duplication and exon shuffling events early in eukaryotic evolution (Pahl and Keller 1994; Patterson et al. 2002; Galat 2004). To explore the nature of putative *FKBP* multiplication in rice, we isolated the individual FKBds for phylogenetic comparison. Figure 4 shows that analogous binding domains from some MD isoforms, for example the domain Is, IIs and IIIs, populate single branches of the phylogenetic tree. This strongly suggests duplication of entire MD genes rather than successive duplications of a single FKBd. This analysis further confirms paralogy between OsFKBP62a and OsFKBP62b, and suggests that the unique OsFKBP75 is the result of a third duplication in this group. Other clear MD paralogues are the putative regional duplicates OsFKBP46, OsFFBP57 and OsFKBP73, which may have also arisen from the OsFKBP62 group. The FKBds of the PAS1 orthologue OsFKBP72 occur as relative outliers, suggesting a unique origin for this gene. The OsFKBP42-type FKBd also appears to be unique in rice, aside from *OsFKBP42* duplicates that arose in rice from chromosomal duplication, as previously stated. The Arabidopsis orthologues of OsFKBP72 (PAS1) and OsFKBP42 (TWD1) are also phylogenetic outliers (Geisler and Bailly 2007). These results indicate FKBP72 and FKBP42, which are vital for plant development, may have arisen earlier in plant evolution than other MD duplicates. Indeed, PAS1 and TWD1 orthologues are unique in the moss *Physcomitrella patens* and are >50% identical to their Arabidopsis counterparts (results not shown), indicating an ancient and conserved role for these FKBPs. FKBd homology among the putative nuclear isoforms OsFKBP53a, OsFKBP53b, OsFKBP44 and OsFKBP59 indicates they may have arisen from a common ancestor FKBP, while the duplicates OsFKBP20-1a and OsFKBP20-1b are evolutionarily distant from this group, perhaps suggesting distinct roles for the PPIase domains of high and low molecular weight FKBPs in the nucleus (Fig. 4).

The PPIase domains of the lumenal FKBPs congregate at approximately the same region of the tree, but the branching between most individuals occurs closer to the root than seen in non-lumenal paralogous FKBds, indicative of earlier duplication and neofunctionalisation among lumenal isoforms. The occurrence of a single 'lumenal' (periplasmic) FKBP in cyanobacteria (P. Gollan, unpublished) and over ten chloroplastic FKBPs in algae (Vallon 2005; P. Gollan, unpublished) pinpoints the expansion of this FKBP subfamily to early evolution of photosynthetic eukaryotes. One exception is the pair OsFKBP13 and OsFKBP16-2 that is over 55% identical in the mature sequences (not shown) and may have arisen from a more recent duplication.

Discussion

In eukaryotes, the FKBPs form large and diverse protein families that arose through duplication of an original PPIase domain and have been subsequently shaped by gene duplications and exon shuffling (Pahl and Keller 1994; Patterson et al. 2002; Galat 2004; Somarelli et al. 2008). Although historically linked to immunosuppression and

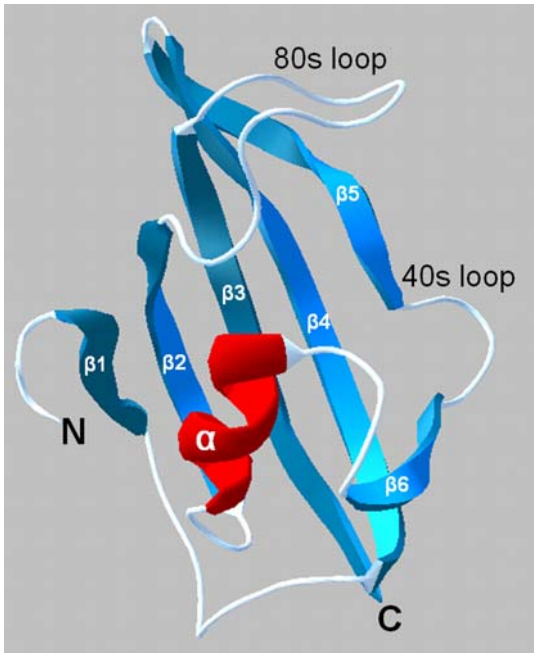

Fig. 5 Predicted three-dimensional tertiary structure of OsFKBP12. Prediction based on homology with proteins of known structures, and produced by 3D JIGSAW (Bates et al. 2001). Beta-sheet regions are shown in *blue* and numbered corresponding to Fig. 2; alpha helices shown in *red*. Image edited using Deepview/Swiss PDB Viewer v4.0 software

proline bond rotation, the physiological importance of FKBPs far surpasses FK506-binding and general protein folding, extending to cellular processes including signal transduction, DNA transcription, protein trafficking, apoptosis and fertility (reviewed in Kang et al. 2008). In plants, FKBPs have proven to be vital for regulating normal growth and development, and for coping with stress conditions, both fundamental considerations for modern cereal farming. Our analysis of the sequenced *Oryza sativa* genome has revealed the largest FKBP family so far reported. We identified 29 *FKBP* genes in rice, including several previously unreported MD isoforms, and have performed sequence- and bioinformatics-based characterisations of these genes and their proteins. A prominent theme throughout our analysis was the frequency of FKBP gene multiplications in rice, with at least 18 FKBPs occurring as duplicates or triplicates. The persistence of newly created *FKBP* genes in the rice genome is a testament to the evolutionary advantages provided to rice by the duplicates which, in many cases, appears to lie in the adoption of individual expression profiles of the paralogues.

The classic scenario of differential expression in duplicate FKBPs in plants is that of OsFKBP64/OsFKBP65, and of their orthologues in wheat and Arabidopsis. OsFKBP64 is expressed constitutively and upregulated by heat treatment, while it's duplicate is detected only under stress conditions (Magiri et al. 2006). Recent results of Breiman and co-workers indicate the constitutively expressed member of this pair is involved in genetically inducing prolonged plant thermotolerance, while the heat-induced member may operate as a negative regulator of this process (Meiri and Breiman 2009). Although awaiting further resolution, this scenario may have some analogy with the mammalian orthologues, where FKBP51 and FKBP52 operate to antagonise and potentiate, respectively, the nuclear transport of glucocorticoid (Davies et al. 2005).

A majority of the cytosolic FKBP isoforms in rice possess TPR regions that conserve the residues involved in binding to HSP90, also the case in Arabidopsis (Aviezer-Hagai et al. 2007). As such, these rice FKBPs are likely to regulate the activity of HSP90-client complexes and may operate to link the multi-functional HSP90 to FKBP-client proteins bound at the FKBd, such as dynein or membrane transporters, as has been demonstrated. In this case, differential MD FKBP functionality may be achieved through distinct specificities of the N-terminal FKBd(s) and/or distinct expression profiles of homologous isoforms. It will be interesting to investigate the expression profiles of the novel rice genes *OsFKBP46* and *OsFKBP73*, particularly under stress conditions, as these exhibit some orthology to ROF, but appear to lack HSP90 and CaM-binding capacity, presenting the potential for new FKBP functionality in rice cytosol.

There may be some correlation between FKBP duplication and the involvement of duplicates in plant stress response. Knockout of the ROF duplicates showed no severe effect on the growth and development of mutant Arabidopsis under normal conditions (Meiri and Breiman 2009), nor did silenced expression of *AtFKBP13*, another putative duplicate, effect plant viability (Gupta et al. 2002). Further, the ER-localised FKBP15 isoforms and the nuclear FKBP20-1s, also duplicates, have been shown to respond to heat stress (Luan et al. 1996; Nigam et al. 2008). This invites speculation that homologous FKBP pairs operate in cooperative, stress-specific roles in plants, in contrast to developmental roles played by unique isoforms such as PAS1 and TWD1. The occurrence of two *FKBP42* genes in rice may appear to contradict this theory. This pair is the result of segmental chromosome duplication (Wu et al. 1998) and both isoforms possess the domain characteristics required for TWD1 interaction with ABC transporters (Geisler et al. 2003, 2004), conceivably introducing the potential for over-efflux of auxin in rice. Rice may have avoided this in the evolution of distinct spatial expression of individual isoforms in crowns or roots; tissues that were severely distorted in the *AtFKBP42* knockout mutants (Perez-Perez et al. 2004).

Another unique isoform, OsFKBP75 is likely to have originated from an *OsFKBP62*-like ancestor gene, and may

have acquired divergent functionality though acquisition of a distinctive hydrophobic glycine-repeat region. Previously predicted to indicate ER-targeting (Magiri et al. 2006), we recognised homology with the distinctive 'Gly-Gly-Met' repeat motif at the C-termini of cpn60-type chaperonins from prokaryotes, chloroplasts and mitochondria (reviewed in Hartl and Hayer-Hartl 2002). This motif creates a hydrophobic interaction domain in *E. coli* cpn60 (GroEL) that stabilises folding intermediates (Jewett et al. 2004). Such a mechanism is conceivably relevant to a putative chaperone and active PPIase. A potential membrane anchor in OsFKBP75 is identical to that in PAS1, and is absent from the rice orthologue OsFKBP72. It is not clear whether the hydrophobic motif of PAS1 plays a role in binding to the transcription factor partner, or whether this occurs at the CaMBd (Smyczynski et al. 2006). OsFKBP75 appears to be a worthwhile candidate for functional investigation in rice.

The sheer population of FKBPs in the chloroplast thylakoid may be interpreted to infer distinct and important roles for the members of this subfamily. Mounting experimental evidence indicates that such roles are more likely to involve substrate interactions than folding catalysis (Lima et al. 2006; Shapiguzov et al. 2006; Edvardsson et al. 2007). We have shown high degrees of heterogeneity in the lumenal FKBds, particularly in loop regions that participate in FKBP-substrate interactions, which is consistent with their operation in binding with specific partner proteins. The only lumenal FKBPs so far characterised have shown to be involved in regulating photosynthetic membrane assembly (Gupta et al. 2002; Lima et al. 2006), inviting the possibility that the lumenal FKBPs operate as chaperones for membrane proteins. As potential regulators of photosynthetic membrane assembly, lumenal FKBPs may be important for both development; in chloroplast biogenesis, and for stress response; in photosystem maintenance and turnover. Recent identification of lumenal isoforms in wheat etioplasts, alongside unassembled photosynthetic subunits (Blomqvist et al. 2008) is in line with a role in thylakoid maturation, while the redox sensitivity of some isoforms (Marchand et al. 2004; Gopalan et al. 2006; Lima et al. 2006; Michelet et al. 2008) may facilitate stress-responsive regulation.

Concluding remarks

The structurally conserved FKBd provides the interaction domain for client proteins in a number of FKBPs, although the relevance of PPIase activity to protein interactions has been seldom demonstrated, pointing to chaperone activity rather than protein folding to justify the emergence of large *FKBP* multigene families. Conceivably, an archetypal FKBP-type PPIase with its conserved hydrophobic core may have had the capacity to interact with a broad range of proline-containing substrates, while duplications of this domain provided opportunities for substrate specialisation through sequence mutations, particularly in the interactive 40s and 80s loops of the FKBd (Fig. 5). The FKBP multigene family in rice has been shaped by gene duplications, many of which are so far unique to rice. In some cases, FKBP duplications may have occurred in rice as a mechanism for fine-tuning signal transductions in response to stress conditions, and these genes present potential targets for improving stress tolerance in rice. Other rice FKBPs may be important regulators of plant development, and may provide opportunities to enhance and manipulate development processes like seed dormancy and sprouting.

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