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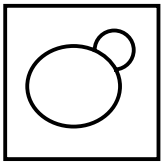

Research Article

Identification and comparative analysis of the peptidyl-prolyl *cis/trans* isomerase repertoires of *H. sapiens, D. melanogaster, C. elegans, S. cerevisiae and Sz. pombe*

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Abstract

The peptidyl-prolyl *cis/trans* isomerase (PPIase) class of proteins comprises three member families that are found throughout nature and are present in all the major compartments of the cell. Their numbers appear to be linked to the number of genes in their respective genomes, although we have found the human repertoire to be smaller than expected due to a reduced cyclophilin repertoire. We show here that whilst the members of the cyclophilin family (which are predominantly found in the nucleus and cytoplasm) and the parvulin family (which are predominantly nuclear) are largely conserved between different repertoires, the FKBPs (which are predominantly found in the cytoplasm and endoplasmic reticulum) are not. It therefore appears that the cyclophilins and parvulins have evolved to perform conserved functions, while the FKBPs have evolved to fill ever-changing niches within the constantly evolving organisms. Many orthologous subgroups within the different PPIase families appear to have evolved from a distinct common ancestor, whereas others, such as the mitochondrial cyclophilins, appear to have evolved independently of one another. We have also identified a novel parvulin within *Drosophila melanogaster* that is unique to the fruit fly, indicating a recent evolutionary emergence. Interestingly, the fission yeast repertoire, which contains no unique cyclophilins and parvulins, shares no PPIases solely with the budding yeast but it does share a majority with the higher eukaryotes in this study, unlike the budding yeast. It therefore appears that, in comparison with *Schizosaccharomyces pombe, Saccharomyces cerevisiae* is a poor representation of the higher eukaryotes for the study of PPIases.

Keywords: **peptidyl-prolyl *cis/trans* isomerase; cyclophilin; FKBP; parvulin**

Introduction

The peptidyl-prolyl *cis/trans* isomerase (PPIase) class of protein is comprised of three known protein families, the cyclophilins (cyclosporin A binding proteins), FKBPs (FK506-binding proteins) and parvulins. These structurally distinct families are linked by their shared ability to catalyse the bond preceding a proline residue between its *cis* and *trans* forms. While they are all believed to employ the 'twisted amide' mechanism of catalysis, as is seen with prolyl isomerization in water, the cyclophilins (Eisenmesser *et al*., 2002; Hur and Bruice, 2002) and parvulins (Ranganathan *et al*., 1997) achieve this through the use of near attack conformers, whereas the FKBPs use hydrophobic distortion (Harrison and Stein, 1992; Hur and Bruice, 2002). They are found widely distributed in eukaryotes, prokaryotes and archaea (Galat, 1993, 1999; Galat and Metcalfe, 1995; He *et al*., 2004; Ivery, 2000; Maruyama and Furuani, 2000; Rulten *et al*., 1999), implying that their function is

required in cellular processes from bacteria to man, and in all the major compartments of the cell (Bose *et al*., 1994; Halestrap and Davidson, 1990; Handschumacher *et al*., 1984; Jin and Burakoff, 1993; Lu *et al*., 1996; Nigam *et al*., 1993; Siekierka *et al*., 1989; Uchida *et al*., 1999; Wang *et al*., 1996).

All of the cyclophilins and FKBPs in the budding yeast *Saccharomyces cerevisiae* have been individually and collectively knocked out with no effect on cell viability (Dolinski *et al*., 1997a; Hemenway and Heitman, 1993). Only Ess1, the *S. cerevisiae* orthologue of the human parvulin Pin1, has been shown to be essential within *S. cerevisiae* (Hanes *et al*., 1989), as has the Pin1 orthologue in the pathogenic yeast *Candida albicans* (Devasahayam *et al*., 2002). However, the Pin1 orthologues in their fellow yeast *Schizosaccharomyces pombe* (Huang *et al*., 2001), the fruit fly *Drosophila melanogaster* (Maleszka *et al*., 1996) and *Cryptococcus neoformans* (Ren *et al*., 2005) have been shown to be non-essential, indicating that the essential function of Pin1 orthologues is limited to certain organisms or that redundancy mechanisms are present in these other organisms. In the eubacterium *Bacillus subtilis*, the two cytosolic PPIases, PpiB and trigger factor, have been shown to be necessary for cell viability under starvation conditions (Gothel *et al*., 1998) but they appear not to possess an essential function within a cell under normal growth conditions. In mammals, an FKBP12 knock-out mouse showed normal skeletal muscle but suffered from severe cardiomyopathy and ventricular septal defects that mimic a human congenital heart disorder (Shou *et al*., 1998), an effect assigned to its modulation of calcium release activity of both skeletal and cardiac ryanodine receptors. Recently a mutation of the *D. melanogaster* cyclophilin CG3511, which severely truncates the protein, has been shown to confer a synthetic lethal phenotype on cells that lack the retinoblastoma (Rbf) protein (Edgar *et al*., 2005). Despite the high conservation of the PPIases throughout the eukaryotes and prokaryotes, it appears that they do not possess an essential function within many cells under normal growth conditions but may become essential in the absence of other cellular factors.

Much of the research on the PPIases has been on individual proteins spread throughout many different organisms. Some recent reviews have considered different families of PPIases individually (Galat, 1999, 2004; Patterson *et al*., 2002) but none have considered PPIases repertoires on a whole. We report here the comparative analysis of the PPIase repertoires of the mammal *Homo sapiens*, the fruit fly *D. melanogaster*, the nematode *Caenorhabditis elegans* and the two yeasts *Sz. pombe* and *S. cerevisiae*. By comparing these five diverse repertoires we hope to identify key conserved PPIases that are found within all their repertoires as well as those that are specific to each. By comparing the identified functions of each PPIase and its orthologues, we hope to understand better their functions within the cell and to identify those that function within a broad range of eukaryotes from those that are specific to multicellular eukaryotes.

Materials and methods

BLAST searching

The identification of putative PPIases and their orthology between repertoires was performed using both BLASTP (protein vs. protein) and TBLASTN (protein vs. DNA sequence) searches of the complete annotated genome sequences of *H. sapiens* (Lander *et al*., 2001), *D. melanogaster* (Adams, 2000), *C. elegans* (The *C. elegans* Genome Consortium, 1998), *Sz. pombe* (Wood *et al*., 2002) and *S. cerevisiae* (Goffeau *et al*., 1996; Wood *et al*., 2001) maintained by either the National Center for Biotechnology Information (NCBI) (Altschul *et al*., 1997) (**http://www.ncbi.nlm.nih.gov/BLAST/**) or The Institute for Genomic Research (TIGR) (Gish *et al*., 1990) (**http://tigrblast.tigr.org/tgi/**).

Identification of the PPIase repertoires

The peptidyl-prolyl *cis/trans* isomerase (PPIase) repertoires present in the complete annotated genome sequences of *H. sapiens* (Lander *et al*., 2001), *D. melanogaster* (Adams, 2000), *C. elegans* (The *C. elegans* Genome Consortium, 1998), *Sz. pombe* (Wood *et al*., 2002) and *S. cerevisiae* (Goffeau *et al*., 1996; Wood *et al*., 2001) were identified using the protein sequences of human cyclophilin A (hCypA; Accession No. P05092), human FKBP12 (hFKBP12; P20071) and human Pin1 (hPin1; Q13526) as probes in BLASTP and TBLASTN searches of their sequences. Proteins were selected based upon the level of homology, both in regard to actual sequence homology and/or the presence of characteristic motifs, their PPIase

catalytic domain exhibited towards that of their probes sequence.

Protein sequence analysis

The identification of putative domains within the identified PPIases was performed using two NCBI search engines, the CDD (Conserved Domain Database) world-wide web-based BLAST server (Altschul *et al*., 1997) (**http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi**) and their CDART (Geer *et al*., 2002) (Conserved Domain Architecture Retrieval Tool; **http://www.ncbi.nlm.nih.gov/Structure/lexington/lexington.cgi**) analysis program.

The predicted localization of the PPIases and the identification of sequence motifs that support this were identified using the PSORT world-wide web-based search program (Horton and Nakai, 1996, 1997) located on the National Institute for Basic Biology (NIBB) server (**http://psort.nibb.ac.jp/**). The theoretical molecular weights of the predicted proteins were calculated using the calculation tool on the ExPaSy server (**http://www.expasy.ch/tools/pi_tool.html**).

Comparative sequence analysis

Alignments of each family of PPIases were produced using version 1.81 of the ClustalX program (Thompson *et al*., 1997) downloaded from **http://inn-prot.weizmann.ac.il/software/ClustalX.html**. This program performs a pairwise alignment of the sequences prior to the construction of a dendrogram, which describes the approximate groupings of the sequences by similarity, with the final alignment carried out using this dendrogram as a guide. The dendrogram was visualized using TreeView version 1.6.6 (Dr R Page; University of Glamorgan) downloaded from **http://taxonomy.zoology.gla.ac.uk/rod/rod.html** from the files generated by the ClustalX alignment. The scales of the different dendrograms are not cross-comparable.

Identification of orthologues

PPIases were considered to be orthologues if they fulfilled three criteria. First, they should be of approximately the same size and possess the same domain architecture. Second, in BLAST searches they should identify each other ahead of all other PPIases within their respective genomes. This is because they should, in theory, share a more recent common ancestor than they do with the other PPIases. Sequence variation, resulting from the distinct divergent evolution of each protein, should therefore be less between two orthologues than with other PPIases. Third, they should have the same intracellular location and function. This latter criterion is, however, reliant upon prior research, which is not applicable to all PPIases. In these cases, so long as the first two criteria were met, the proteins were deemed to be putative orthologues.

Three methods were employed to identify the orthology between the repertoires and answer the above criteria. First, the individual PPIases were used as probes in BLAST searches of the other species' genomes. Second, the sequences for all the member proteins from all of the comparative organisms of each of the three different PPIase families (cyclophilins, FKBPs and parvulins) were subjected to global sequence comparison by family, using the ClustalX program (Thompson *et al*., 1997) for the purpose of creating a dendrogram. This analysis creates a putative model for how the individual subgroups of each PPIase family may have diverged from one another, based on relationships between their individual sequences, which allow us to infer a putative model for their evolution in the species compared here. As each orthology group should share a more recent common ancestor with themselves than with the other PPIases, they should group together within the dendrogram, ideally as an individual branch with a distinct common ancestor. Third, literature analysis looking for prior publications on the individual PPIases was performed, which in some cases has allowed putative function(s) to be assigned to orthology groups.

Results

The identified PPIase repertoires of *H. sapiens, D. melanogaster, C. elegans, Sz. pombe* and *S. cerevisiae* can be found in Tables 1 and 2. Table 3 shows a comparison of the number of members of each PPIase family found within the different species. The repertoire orthology of the cyclophilins, FKBPs and parvulins as identified by BLAST analysis can be found in Table 4.

Table 1. Peptidyl-prolyl *cis/trans* isomerases identified by BLAST searching of the NCBI database of the complete genome and proteome of the multicellular organisms (A) *H. sapiens* (Lander *et al*., 2001), (B) *D. melanogaster* (Adams, 2000) and (C) *C. elegans* (The C. elegans Genome Consortium, 1998). Localization was predicted using the PSORT II server, molecular weight was predicted using the ExPaSy server and domains were identified using the CCD BLAST program on the NCBI server apart from the *Moca* domain, which was included for those cyclophilins identified as possessing one by Cavarec *et al.* (2002)

A	PPIase	Uniprot Acc. #	kDa	Signal Seq.	Predicted Localisation	Domain Architecture
Cyclophilins	Cyp-A	P62937	18.0	—	Cytoplasmic	PPIase Only
	PPIL1 (CGI-124)	Q9Y3C6	18.2	~	Cytoplasmic	PPIase Only
	PPIL3	Q9BXZ1	18.6	~	Cytoplasmic	PPIase Only
	USA-CyP (Cyp-H)	O43447	19.2	—	Cytoplasmic	PPIase Only
	Cyp-F (D)	P30405	22.0	—	Mitochondrial	PPIase Only
	Cyp-B	Q9BVK5	22.7	N-term	ER	PPIase Only
	Cyp-C	P45877	22.8	N-term	ER	PPIase Only
	"Cyp29"	P49069	28.9	~	Nuclear	N-term PPIase Only
	Cyp33 (E)	Q9UNP9	33.4	—	Cytoplasmic	N-term RRM, C-term PPIase
	Cyp40 (D)	Q08752	40.8	—	Cytoplasmic	N-term PPIase, C-term TPR (3x) motifs
	SDCCAG10	Q6UX04	53.8	—	Nuclear	N-term PPIase, positively charged C-term
	"Cyp57"	Q8WUA2	57.2	~	Nuclear	N-term PPIase, Central RRM
	Cyp60	Q13356	58.8	~	Nuclear	N-term U-box, C-term PPIase
	HAL539	Q96BP3	73.6	—	Cytoplasmic	N-term WD40 (×3) motifs, C-term PPIase
	Cyp88 (CARS/G)	Q13427	88.6	—	Nuclear	N-term PPIase, C-term RS domain
	NK-Cyp (158/SR)	P30414	165.7	—	Nuclear	N-term PPIase C-term RS domain containing a *Moca* motif
	RanBP2	P49792	358.2	—	Nuclear	N-term TPR, central Zn-fingers, central/C-term RB1 domains, C-term PPIase
FKBPs	FKBP12.6	P68106	11.8	—	Cytoplasmic	FKBP (×1) Only
	FKBP12	P62942	12.0	—	Cytoplasmic	FKBP (×1) Only
	FKBP13	P26885	15.6	N-term	Mitochondrial	FKBP (×1) Only
	FKBP19	Q9NYL4	22.2	N-term	ER	FKBP (×1) Only
	FKBP22	Q9NWM8	24.3	N-term	ER	FKBP (×1) Only
	FKBP23	Q9Y680	30.0	—	ER	N-term FKBP (×1), C-term EF-Hand motif
	FKBP25	Q00688	25.2	—	Cytoplasmic	C-term FKBP (×1) Only
	FKBP36	O75344	37.2	~	Cytoplasmic	N-term FKBP (×1), C-term TPR (×2) motifs
	FKBP38	Q14318	38.4	—	Cytoplasmic	N-term FKBP (×1), C-term TPR (×2) motifs
	FKBP51	Q13451	51.2	—	Cytoplasmic	N-term FKBP (×2), C-term TPR (×2) motifs
	FKBP52	Q02790	51.6	—	Cytoplasmic	N-term FKBP (×2), C-term TPR (×3) motifs
	FKBP60	O95302	57.2	~	ER	N-term FKBP (×4), C-term EF-Hand motif
	FKBP65	Q96AY3	64.7	N-term	ER	N-term FKBP (×4), C-term EF-Hand motif
Parvs	Pin1	Q13526	9.0	—	Nuclear	N-term WW domain, C-term Rotamase
	Par14	Q9Y237	13.9	—	Cytoplasmic	Rotamase Only

Figure 1 shows the dendrograms generated for the cyclophilins, FKBPs and parvulins.

Cyclophilin orthology

Table 4D shows that *D. melanogaster* and *H. sapiens* share the greatest number of orthologues, closely followed by their orthology to *C. elegans*. Of the two yeasts, *Sz. pombe* shares its entire repertoire of nine in common with *D. melanogaster* and *H. sapiens*, compared to only three shared by *S. cerevisiae*, which has a potential fourth in common (ScCwc27) that is discussed below. *Sz. pombe* is therefore the only organism in this comparison that has no unique cyclophilins within its repertoire and, interestingly, its repertoire shows less orthology to that of its fellow yeast *S. cerevisiae* than to the higher eukaryotes.

Table 4A shows that there are only two cyclophilin groups found in all five of the organisms. One group are the cyclophilin A orthologues, a ubiquitous group of cyclophilins that have been identified within the cytoplasm (Handschumacher *et al*., 1984; Harding *et al*., 1986; Huh *et al*., 2003), although a recent report has found ScCpr1

Table 1. Continued

B	PPIase	Uniprot Acc. #	kDa	Signal Seq.	Predicted Localisation	Domain Architecture
Cyclophilins	CG7768	Q9VUD6	17.8	—	Cytoplasmic	PPIase Only
	CG11777	Q8MKJ6	17.8	—	Cytoplasmic	PPIase Only
	Cyp1	P25007	17.9	~	Cytoplasmic	PPIase Only
	CG13892	Q9W0Q2	19.5	~	Cytoplasmic	PPIase Only
	CG17266	Q9V9B9	20.2	—	Cytoplasmic	PPIase Only
	CG2852	Q9W227	22.2	N-term	ER	PPIase Only
	NinaA	P15425	26.4	N-term	ER	PPIase Only
	Cyp-33	Q9V3G3	33.3	~	Cytoplasmic	N-term RRM, C-term PPIase
	CG8336	Q9VT21	43.1	~	Cytoplasmic	N-term PPIase, C-term TPR (2×) motifs
	CG10907	Q9VTN7	56.6	—	Nuclear	N-term PPIase, C-term RS domain
	CG7747	Q9V7M9	59.0	—	Nuclear	N-term U-box, C-term PPIase
	CG3511	Q960Q8	71.8	~	Cytoplasmic	N-term WD40 motif, C-term PPIase
	CG5808	Q9XYZ6	75.5	~	Nuclear	N-term PPIase, central RRM, C-term RS domain
	Moca-Cyp	Q8ISE5	112.5	—	Nuclear	N-term PPIase C-term RS domain containing a *Moca* motif
FKBPs	FKBP12	P48375	11.6	~	Cytoplasmic	FKBP (1×) Only
	CG14715	Q9VGK3	14.8	N-term	ER	FKBP (1×) Only
	FKBP13	Q8MLW1	25.7	—	ER	Central FKBP (1×), C-term EF-Hand motif
	FKBP39	P54397	39.3	—	Nuclear	Positively charged N-term, C-term FKBP (1×)
	CG5482	Q9V8K4	44.9	—	Cytoplasmic	N-term FKBP (1×), C-term TPR (2×) motifs
	FKBP59	Q9VL78	48.8	~	Cytoplasmic	N-term FKBP (2×), C-term TPR (2×) motifs
	shutdown	Q9W1I9	51.8	~	Nuclear	Central FKBP (1×), C-term TPR (1×) motif
Parvulins	Dodo	P54353	18.4	—	Nuclear	N-term WW domain, C-term Rotamase
	CG11858	Q9VBU4	13.9	~	Cytoplasmic	Rotamase Only
	CG32845	Q8IRJ5	44.3	~	Nuclear	Central Rotamase Only

C	PPIase	Uniprot Acc. #	kDa	Signal Seq.	Predicted Localisation	Domain Architecture
Cyclophilins	Cyp1	P52009	20.7	~	Mitochondrial	PPIase Only
	Cyp2	P52010	18.5	~	Cytoplasmic	PPIase Only
	Cyp3	P52011	18.6	~	Cytoplasmic	PPIase Only
	Cyp4	P52012	58.5	—	Cytoplasmic	N-term U-box, C-term PPIase
	Cyp5	P52013	22.4	N-term	ER	PPIase Only
	Cyp6	P52014	21.9	N-term	ER	PPIase Only
	Cyp7	P52015	18.4	~	Cytoplasmic	PPIase Only
	Cyp8	P52016	53.6	—	Nuclear	N-term PPIase C-term RS domain containing a *Moca* motif
	Cyp9	Q09637	35.8	~	Nuclear	N-term PPIase C-term RS domain containing a *Moca* motif
	Cyp10	P52017	18.0	—	Cytoplasmic	PPIase Only
	Cyp11	P52018	20.2	~	Cytoplasmic	PPIase Only
	Cyp12	Q18445	18.5	~	Cytoplasmic	PPIase Only
	Cyp13	Q9U2S6	36.4	—	Cytoplasmic	N-term RRM, C-term PPIase
	Cyp14	O18161	50.4	—	Nuclear	N-term PPIase, C-term RRM
	Cyp15	Q9U1Q3	70.8	—	Cytoplasmic	N-term WD40 motifs, C-term PPIase
	Cyp16	Q9XX17	25.2	~	Cytoplasmic	PPIase Only
	Cyp17	O01880	57.7	~	Nuclear	Positively charged N-term, C-term PPIase
FKBPs	Fkb1	Q20107	15.5	N-term	Cytoplasmic	FKBP (1×) Only
	Fkb2	Q9U2Q8	11.6	~	Cytoplasmic	FKBP (1×) Only
	Fkb3	O16309	29.1	N-term	ER	FKBP (2×) Only
	Fkb4	Q23338	29.3	N-term	ER	FKBP (2×) Only
	Fkb5a	P91180	29.9	N-term	ER	FKBP (2×) Only
	Fkb6	O45418	48.1	~	Cytoplasmic	N-term FKBP (2×), C-term TPR (3×) motifs
	Fkb7	O61826	36.2	N-term	ER	N-term FKBP (1×), C-term EF-hand motif
	Fkb8	Q8I4L5	32.4	~	Cytoplasmic	FKBP (2×) Only
Parvs	Pin1	Q9N492	19.2	—	Nuclear	N-term WW domain, C-term Rotamase
	Pin2	Q9NAF9	13.3	—	Nuclear	Rotamase Only

Table 2. Peptidyl-prolyl *cis*/*trans* isomerases identified by BLAST searching of the NCBI database of the complete genome and proteome of the unicellular organisms (A) *Sz. pombe* (Wood *et al*., 2002) and (B) *S. cerevisiae* (Goffeau *et al*., 1996). Localization was predicted using the PSORT II server, molecular weight was predicted using the ExPaSy server and domains were identified using the CCD BLAST program on the NCBI server

A	PPIase	Uniprot Acc. #	kDa	Signal Seq.	Predicted Localisation	Domain Architecture
Cyclophilins	Cyp1	P87051	17.4	—	Cytoplasmic	PPIase only
	Cyp2	P18253	16.9	~	Cytoplasmic	PPIase only
	Cyp3	O74729	18.9	~	Cytoplasmic	PPIase only
	Cyp4	O94273	22.2	N-term	ER	PPIase only
	Cyp5	Q11004	40.2	—	Cytoplasmic	N-term. PPIase, C-term. TPR(×3)
	Cyp6	Q9UUE4	50.8	—	Nuclear	N-term. PPIase, C-term. RRM
	Cyp7	O42941	52.2	—	Nuclear	N-term. PPIase, C-term. positively charged
	Cyp8	Q09928	53.6	~	Nuclear	N-term. U-Box, C-term. PPIase
	Cyp9	O74942	69.0	~	Cytoplasmic	N-term. WD40(×3), C-term. PPIase
FKBPs	FKBP12	O42993	12.0	—	Cytoplasmic	FKBP only
	FKBP39	O74191	39.3	—	Nuclear	N-term. Positively charged, C-term. FKBP
	FKBP39a	Q10175	40.5	—	Cytoplasmic	N-term. Positively charged, C-term. FKBP
Parv	Pin1	O74448	19.8	~	Nuclear	N-term. WW domain, C-term. Rotamase

B	PPIase	Uniprot Acc. #	kDa	Signal Seq.	Predicted Localisation	Domain Architecture
Cyclophilins	Cpr1	P14832	17.4	~	Cytoplasmic	PPIase Only
	Cpr2	P23285	22.8	N-term	ER	PPIase Only
	Cpr3	P25719	19.9	—	Mitochondrial	PPIase Only
	Cpr4	P25334	35.8	N-term	ER	Central PPIase Only
	Cpr5	P35176	25.3	N-term	ER	PPIase Only
	Cpr6	P53691	42.1	—	Cytoplasmic	N-term PPIase, C-term TPR(3×) motifs
	Cpr7	P47103	45.1	—	Cytoplasmic	N-term PPIase, C-term TPR (3×) motifs
	Cpr8	P53728	34.9	—	Membrane	Central PPIase
	Cwc27	*Q02770*	*35.0*	~	*Nuclear*	*Divergent N-term PPIase Only*
FKBPs	Fpr1	P20081	12.2	~	Cytoplasmic	FKBP (1×) Only
	Fpr2	P32472	14.5	N-term	ER	FKBP (1×) Only
	Fpr3	P38911	46.6	—	Nuclear	Negatively charged N-term, C-term FKBP (1×)
	Fpr4	Q06205	43.9	—	Nuclear	Negatively charged N-term, C-term FKBP (1×)
Parv	ESS1	P22696	21.7	—	Nuclear	N-term WW domain. C-term Rotamase

to be nuclear (Arevalo-Rodriguez and Heitman, 2005), which is contrary to that found by Huh *et al*. (Huh *et al*., 2003). Members of this group have been reported to function in protein folding (Davis *et al*., 1989; Kern *et al*., 1995; Reader *et al*., 2001; Steinmann *et al*., 1991), protein activity regulation (Ansari *et al*., 2002; Brazin *et al*., 2002; Yurchenko *et al*., 2005), transcriptional regulation (Ansari *et al*., 2002; Arevalo-Rodriguez *et al*., 2000; Pijnappel *et al*., 2001), receptor signalling pathways (Allain *et al*., 1994; Bukrinsky, 2002; Syed, 2003; Huang *et al*., 2002; Nagata *et al*., 2000; Pushkarsky *et al*., 2001; Rycyzyn *et al*., 2000; Yurchenko *et al*., 2001, 2002), apoptosis (Cande *et al*., 2004), the cellular oxidative stress response (Jin *et al*., 2000; Lee *et al*., 2001), a vesicular import pathway (Brown *et al*., 2001) and in the control of both the meiotic (Arevalo-Rodriguez and Heitman, 2005) and mitotic cell cycles in *S. cerevisiae* (Fujimori *et al*., 2001).

The other group are the cyclophilin B orthologues, a group identified by their targeting to the endoplasmic reticulum (ER) (Frigerio and Pelham, 1993; Kumar *et al*., 2002; Price *et al*., 1991) courtesy of their N-terminal signal peptide. They function within the secretory pathway, where they have been reported to be involved in the chaperoning of plasma membrane proteins (Horibe *et al*.,

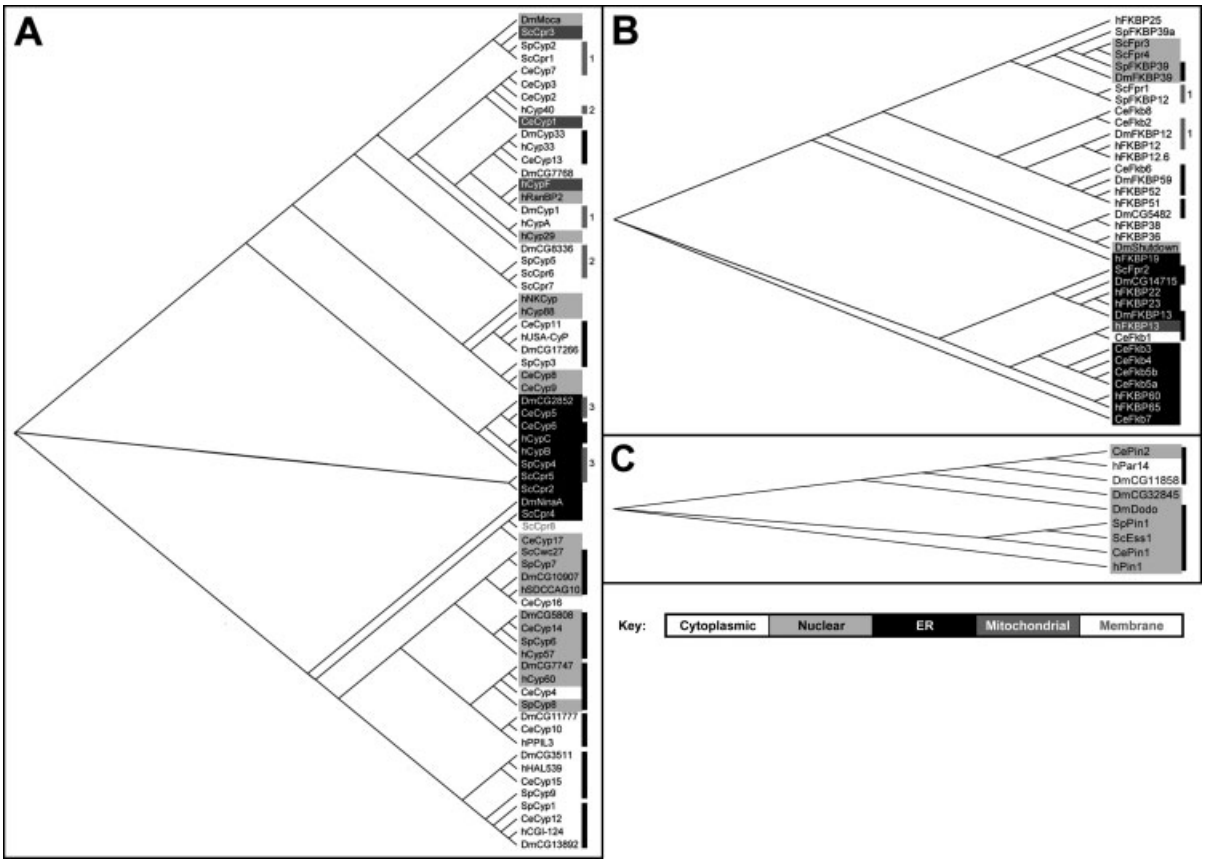

Figure 1. Dendrograms depicting the predicted history of divergence of (A) the cyclophilins, (B) the FKBPs and (C) the parvulins of *Sz. pombe* (Sp), *S. cerevisiae* (Sc), *D. melanogaster* (Dm), *C. elegans* (Ce) and *H. sapiens* (h), based upon a comparison of their protein sequences by the ClustalX program (Thompson *et al.*, 1997) with the dendrogram generated using TreeView version 1.6.6 (Dr R Page; University of Glamorgan). Background and text colours identify their PSORT predicted localization. Bars up the right hand side indicate groups identified as orthologous by BLAST analysis (Table 4). Black bars indicate a group where all members are found at the same point in the dendrogram. Groups with members in more than one location are shown by grey bars and identified by the numbers to their right (key: (A) CypA, group 1; CypB, group 2; Cyp40, group 3; (B) FKBP12, group 1)

2002; Klappa *et al*., 1995; Meunier *et al*., 2002; Price *et al*., 1991, 1994; Zhang and Herscovitz, 2003) and also have proposed functions in receptor signalling pathways (Allain *et al*., 1994; Bukrinsky, 2002; Nagata *et al*., 2000; Obata *et al*., 2005; Rycyzyn and Clevenger, 2002; Rycyzyn *et al*., 2000; Yurchenko *et al*., 2001).

Only two other groups have an *S. cerevisiae* member but both lack an apparent *C. elegans* orthologue (Table 4A). The cyclophilin 40 orthologues are a group of heat shock-inducible (Lebeau *et al*., 1999; Mark *et al*., 2001; Mayr, 2000; Weisman *et al*., 1996), predominantly nuclear (Huh *et al*., 2003; Lebeau *et al*., 1999; Mark *et al*., 2001) cyclophilins that interact with the C-terminal MEEVD pentapeptide of heat shock protein 90 (Hsp90) (Ward *et al*., 2002) and have been reported to function within the Hsp90 complex (Davies *et al*., 2005; Duina *et al*., 1998; Sykes *et al*., 1993), potentially regulating its ATPase activity (Prodromou *et al*., 1999) during its functions in cellular signalling pathways that regulate transcription (Pratt and Toft, 1997; Sanchez and Ning, 1996; Ward *et al*., 2001; Warth *et al*., 1997), the cellular heat shock response (Bharadwaj *et al*., 1999) and also in maintaining the cell cycle protein kinases Mik1, Wee1 and Swe1 (Goes and Martin, 2001). Interestingly, the Hsp90 complex is

present within *C. elegans* (Birnby *et al*., 2000), making the absence of an associated cyclophilin surprising.

The second group contains both ScCwc27, which has not formed part of previous research on the *S. cerevisiae* repertoire due to the presence of a very degenerate PPIase domain, and hSDCCAG10, which was formerly called hNY-CO-10 until its re-annotation to include a complete cyclophilin-like catalytic domain. They share a region rich in S/K-R/E residues that is similar to those observed in hnRNP-binding proteins (Romano *et al*., 2004; Weighardt *et al*., 1999) (data not shown) and both

Table 3. The numbers of the three different families that make up the peptidyl-prolyl *cis/trans* isomerase repertoires of *H. sapiens, D. melanogaster, C. elegans, S. cerevisiae* and *Sz. pombe*

Organism	Genes	Cyclo-philins	FKBPs	Par-vulins	Total
H. sapiens	24-40.000[a]	17	13	2	32
C. elegans	18.424[b]	17	8	2	27
D. melanogaster	13.601[b]	14	7	3	24
S. cerevisiae	5.885[c]	8	4	1	13
Sz. pombe	4.824[d]	9	3	1	13

[a] Lander *et al.* (2001); [b] Rubin *et al.* (2000); [c] Goffeau *et al.* (1996); [d] Wood *et al.* (2002).

Table 4. Orthology between the (A) cyclophilin, (B) FKBP and (C) parvulin repertoires of *S. cerevisiae, Sz. pombe, D. melanogaster, C. elegans* and *H. sapiens*, identified by BLAST searching of the NCBI database of their complete genomes and proteomes (Adams, 2000; The C. elegans Genome Consortium, 1998; Goffeau *et al*., 1996; Lander *et al*., 2001; Wood *et al*., 2002, respectively). PPIases are ordered by increasing size and PSORT predicted localization with any secondary domains or domain architecture shown down the left hand side (ND = none detected; ± = charged region). (D) The number of orthologues shared between the different repertoires. Cyclophilins (top) and FKBPs (bottom) are shown on the right with the parvulins shown on the left. *S. cerevisiae* numbers outside of brackets are exclusive of, and numbers within brackets are inclusive of, ScCwc27

A

	S. cerevisiae	Sz. pombe	D. melanogaster	C. elegans	H. Sapiens
ND	Cpr1	Cyp2	Cyp1 CG7768	Cyp7 Cyp2 Cyp3	Cyp-A
		Cyp1	CG13892	Cyp12	CGI-124 (PPIL1)
			CG11777	Cyp10	PPIL3
		Cyp3	CG17266	Cyp11	USA-CyP (Cyp-H)
				Cyp16	
RRM			Cyp33	Cyp13	Cyp33 (E)
TPR	Cpr6 Cpr7	Cyp5	CG8336		Cyp40 (D)
WD40		Cyp9	CG3511	Cyp15	HAL539
ND					"Cyp29"
RRM		Cyp6	CG5808	Cyp14	"Cyp57"
+/-	Cwc27	Cyp7	CG10907		SDCCAG10
ND				Cyp17	
U-box		Cyp8	CG7747	Cyp4	Cyp60
RS					Cyp88 (CARS/G)
					NK-Cyp (158/SR)
RS & MOCA			Moca-Cyp		
				Cyp8	
				Cyp9	
TPR, RB1 & ZF					RanBP2
ND	Cpr5	Cyp4	CG2852	Cyp5	Cyp-B
				Cyp6	Cyp-C
	Cpr2				
			NinaA		
	Cpr4				
ND					Cyp-F (D)
				Cyp1	
	Cpr3				
ND	Cpr8				

B

	S. cerevisiae	Sz. pombe	D. melanogaster	C. elegans	H. Sapiens
1xFKBP	Fpr1	FKBP12	FKBP12	Fkb2	FKBP12
					FKBP12.6
					FKBP25
2xFKBP				Fkb8	
1xFKBP & 1xTPR					FKBP36
					FKBP38
1xFKBP & +/-		FKBP39a			
2xFKBP & 1xTPR			CG5482		FKBP51
			FKBP59	Fkb6	FKBP52
1xFKBP & +/-	Fpr3				
	Fpr4				
		FKBP39	FKBP39		
1xFKBP & 1xTPR			shutdown		
	Fpr2		CG14715		
1xFKBP					FKBP19
					FKBP22
					FKBP23
1xFKBP & 1xEF				Fkb7	
2xFKBP				Fkb3	
				Fkb4	
				Fkb5	
4xFKBP & 1xEF					FKBP60
					FKBP65
1xFKBP			FKBP13	Fkb1	FKBP13

C

	S. cerevisiae	Sz. pombe	D. melanogaster	C. elegans	H. Sapiens
ND			CG11858	Pin2	Par14
WW	ESS1	Pin1	Dodo	Pin1	Pin1
ND			CG32845		

Key: Cytoplasmic | Nuclear | ER | Mitochondrial | Membrane

D

S. cerevisiae	Sz. pombe	C. elegans	D. melanogaster	H. sapiens
1	1	2	2	
1	1	2		
1	1			
1				

Species	H. sapiens	D. melanogaster	C. elegans	Sz. pombe	S. cerevisiae
H. sapiens		11	10	9	3(4)
D. melanogaster	4		10	9	3(4)
C. elegans	3	3		7	2
Sz. pombe	1	2	1		3(4)
S. cerevisiae	1	2	1	1	

SpCyp7 and ScCwc27 have been reported to be components of their respective Cdc5 complexes (Ohi *et al*., 2002), but their function within this complex remains unknown. The apparent absence of a *C. elegans* orthologue cannot be explained by an absence of the Cdc5 complex, which has been reported as present within the nematode (Ohi *et al*., 1998).

There are five groups that lack only an *S. cerevisiae* member (Table 4A), highlighting the large difference between the two yeast repertoires. Three groups are predicted to be cytoplasmic, of which one group has hCGI-124 as a member. hCGI-124 has been reported to be highly expressed in the heart and adult brain (Ozaki *et al*., 1996) and members of this group have been identified as orthologues of *Dictyostelium discoideum* CypE (Skruzny *et al*., 2001), which gives them a role within a broad range of signal transduction pathways (Skruzny *et al*., 2001).

In the second group, hUSA-CyP (also called hCyp20) has been previously reported to be an orthologue of SpCyp3 (Pemberton *et al*., 2003) and to associate with two components of the pre-mRNA spliceosome (Horowitz *et al*., 1997), where it is required for the second stage of pre-mRNA splicing (Horowitz *et al*., 2002). Both have been found to be predominantly nuclear (Pemberton *et al*., 2003; Teigelkamp *et al*., 1998), contrary to the PSORT prediction. The absence of nuclear localization sequences within their sequence (Pemberton *et al*., 2003) would imply that functional interactions lead to their translocation to the nucleus. The role in pre-mRNA splicing would explain the absence of an *S. cerevisiae* orthologue, as the budding yeast performs very limited mRNA splicing in comparison with the other organisms.

The third cytoplasmic group has the human cyclophilin HAL539 as a member. Its members possess WD40 motifs in their N-terminal region, which are found in all eukaryotes, but not in prokaryotes, in a large variety of proteins that share no obvious commonality in their functions (Neer *et al*., 1994). Recently, the severe truncation of DmCG3511 has been reported to cause synthetic lethality to cells lacking the Rbf protein (Edgar *et al*., 2005), an orthologue of the human retinoblastoma protein that is linked to many human cancers, but it remains unknown by what mechanism this lethality is caused.

The remaining two orthology groups that lack an *S. cerevisiae* member are predicted to be nuclear (Table 4A). The group containing SpCyp6 and CeCyp14 all possess an RNA recognition motif (RRM), which is found in metazoan protein factors involved in constitutive pre-mRNA splicing and alternative splicing regulation (Birney *et al*., 1993), and appear to be RNA-interacting cyclophilins linked to cell morphogenesis, cortical organization and nuclear reorganization (Krzywicka *et al*., 2001).

The second is a predominantly nuclear group that contains hCyp60. Its members possess a U-Box motif, which is reported to be a modified RING-finger motif involved in protein–protein interactions that has been primarily identified in proteins involved in the ubiquitin–proteasome system (Pringa *et al*., 2001). Expressed in the thymus, pancreas, testis and kidney, hCyp60 is a nuclear cyclophilin that interacts with the well-characterized leech serine-proteinase inhibitor elgin-c (Wang *et al*., 1996). CeCyp4 has been found to be important in larval muscle development (Page and Winter, 1998) in the nematode, and interestingly is predicted by PSORT to be cytoplasmic, rather than nuclear like the rest of this group, which is also contrary to the reported localization of hCyp60 (Wang *et al*., 1996).

The three remaining groups all lack a yeast member, containing members solely from the higher eukaryotes (Table 4A), indicating that their role is required solely within the additional pathways found within these multicellular organisms. Two of these groups are cytoplasmic, of which one contains hPPIL3 (Zhou *et al*., 2001) and CeCyp10 (Page *et al*., 1996), but as yet no functions have been identified for its members. Members of the second group possess an RRM and include the human RNA-binding cyclophilin hCyp33 (Mi *et al*., 1996), DmCyp33, which has been reported to interact with the trx/MLL protein family which modulate the expression of the HOXC genes (Anderson *et al*., 2002) and CeCyp13, which is found in an essential polycistronic operon (Mazroui *et al*., 1999) but has itself been shown to be non-essential (Zorio and Blumenthal, 1999).

The final group is found within the endoplasmic reticulum and contains only two members; hCypC and CeCyp6 (Table 4A). hCypC is reported to function in the secretory pathway of specific tissues (Friedman *et al*., 1993), namely bone marrow,

ovaries, testis and kidney (Friedman *et al*., 1994), and CeCyp6 was reported to localize exclusively to the nematode's gut (Picken *et al*., 2002).

The dendrogram shown in Figure 1A backs up many of the BLAST-identified orthology groupings detailed above. These groups are found clustered on the same distinct branch of the dendrogram leading back to their distinct common ancestor. Many of these branches show their members segregating in agreement with the divergence of the compared species (*Sz. pombe* and *S. cerevisiae*, followed by *C. elegans*, then *D. melanogaster* and finally *H. sapiens*). The groups with hCGI-124, hSDCCAG10 and hCyp60 would be good examples of this (Figure 1A, lower branch). The group containing human PPIL3 appears evolutionarily linked to the hCyp60 group and the group containing hHAL539 appears evolutionarily linked to the hCGI-124 group. The latter shows SpCyp9 separating from the other members of its group prior to their split with the hCGI-124 group. The others all segregate in order of species divergence from a distinct common ancestor, implying that SpCyp9 may be more distantly related to this group.

In the upper branch of the dendrogram (Figure 1A) only the hCyp33 group is found to diverge from a distinct common ancestor. The position of their branch within the dendrogram indicates that they are linked with the evolution of the cyclophilin As. The latter are seen to appear within the same branch of the dendrogram but without a common ancestor that is distinct only to them. They do, however, follow the same order of divergence as the species they are a part of, with both SpCyp2 and ScCpr1 diverging first, followed by CeCyp7 and then finally DmCyp1 and hCypA.

As was seen with the hHAL539 group, the hUSA-CyP group shows SpCyp3 to diverge away from the others prior to the distinct common ancestor from which all the others diverged (Figure 1A). Given the lack of a secondary functional domain to increase the certainty of SpCyp3's orthology to the other members of this group, the different position of SpCyp3 within the dendrogram may potentially indicate that it is not a true member of this orthology group, with it fulfilling a different function that has led to its reduced sequence conservation with the other members of its orthology group.

Another group that appear to have evolved without a distinct common ancestor are the heat shock protein 90 (Hsp90)-associated cyclophilins. hCyp40 is found on a branch amongst the *C. elegans* cyclophilin A-related proteins (Figure 1A). hCyp40 does possess a divergent loop located to one side of the active site which is present in both its cyclophilin 40 orthologues (data not shown) and, more importantly in the context of this observation, in CeCyp3 (Dornan *et al*., 1999). This could in part explain this observation, but the absence of the TPR domain within CeCyp3 still makes the answer to this linkage elusive. Unsurprisingly, both the yeast cyclophilin 40 proteins (SpCyp5 and ScCpr6) are found on the same branch. The *D. melanogaster* cyclophilin 40 (DmCG8336) is found on its own as part of a branch that links it to hCyp40 and which, if traced back, can also be remotely linked to the yeast proteins (Figure 1A). The published research is the only confirmation that SpCyp5, SpCpr6 and hCyp40 are likely to show functional orthology, with DmCD8336 remaining uncharacterized.

Finally, we have the ER-located cyclophilins, which are seen to appear from all three initial branches of the dendrogram (Figure 1A), although all putative cyclophilin B and C orthologues are found diverging from the upper branch, with the exception of the *S. cerevisiae* cyclophilin B orthologue ScCpr5 (central branch). In the upper branch, SpCyp4 is seen to diverge away from the others first, followed by hCypB before CeCyp5 and DmCG2852 diverge. The latter divergence is different from that of the respective species, implying that sequence variation within the *C. elegans* and *D. melanogaster* cyclophilins may have been influenced for similar reasons that are distinct from that of humans. ScCpr5 appears on a branch with only one other distinct protein, ScCpr2, and as this branch appears distinct from all others, it implies that these two cyclophilins evolved independently from a distinct common ancestor within *S. cerevisiae*. The cyclophilin C group appear to have evolved independently of each other, with both being seen to diverge from their respective cyclophilin B orthologue (Figure 1A, upper branch).

Besides the orthology groups identified above by both BLAST and sequence analysis, there remain individual cyclophilins found within the individual repertoires of the organisms in this comparison. The dendrogram (Figure 1A) can in some cases help to shed light on their potential role within the cell through linkage with other groups of known function. An example of this would be that both

CeCyp2 and CeCyp3, shown to have no identifiable orthologues by BLAST analysis (Table 4A), are linked by a distinct common ancestor to their cyclophilin A orthologue, CeCyp7. BLAST analysis did show that they have a high similarity to the cyclophilin As, indicating that these are likely to be additional cyclophilin A-like cyclophilins functioning within the nematode. DmCG7768 is found in the same branch as the hCyp33 group (Figure 1A, upper branch), sharing a distinct common ancestor with them. Although it lacks the requisite domains to be a member of this group, looking similar in size and structure to the cyclophilin As, this may indicate its evolution came about by a gene duplication of the DmCyp33 gene, either prior to it gaining the N-terminal RRM domain or in such a fashion that the RRM was lost. hCyp29 appears closely linked to hCypA, both sharing a distinct common ancestor, but has since evolved to be a larger protein with a putative function within the nucleus. Human RanBP2 is also seen as part of the cyclophilin A and 33 branch (Figure 1A, upper branch), with it appearing evolutionarily linked to the cyclophilin 33s, although its size and multidomain structure have led to its localization to the cytoplasmic periphery of the nuclear pore complex (Wu *et al*., 1995), putatively as a SUMO1 E3 ligase (Pichler *et al*., 2002). ScCpr7 is a second TPR-containing cyclophilin distinct to *S. cerevisiae* that is found on the same branch as its cyclophilin 40 orthologue ScCpr6 (Figure 1A), with it also reported to interact with Hsp90 (Marsh *et al*., 1998; Mayr, 2000; Tesic *et al*., 2003). It appears from this dendrogram that *S. cerevisiae* evolved a second cyclophilin 40-like cyclophilin after its divergence away from the other organisms within this study.

Four of the individual cyclophilins on the upper branch of the dendrogram (Figure 1A) appear to possess a common non-cyclophilin domain. All are RS-cyclophilins that possess what has been termed a 'moca' domain in a published characterization study on the *D. melanogaster* cyclophilin, DmMoca (Cavarec *et al*., 2002). CeCyp8 and CeCyp9, along with human NK-Cyp, were also reported in this study to possess this 'moca' domain, but in all cases the proteins show no linkage between species, with only the two *C. elegans* cyclophilins appearing to have a recent, and in their case distinct, common ancestor (Figure 1A). hNK-Cyp functions almost solely on the outer cell membrane of natural killer cells (Alkhatib *et al*., 1997; Anderson *et al*., 1993; Giardina *et al*., 1996) as an important component in the recognition of infected cells (Chambers *et al*., 1994), a process not found in the other compared organisms. The evolution and conservation of this 'moca' domain is therefore elusive when looked at in the context of this dendrogram (Figure 1A) and the function of the proteins that are known to possess it.

Human NK-Cyp also shows linkage to another individual human cyclophilin, hCyp88. Also an RS protein, hCyp88 lacks the 'moca' domain of hNK-Cyp and has been reported to interact with the C-terminal domain of RNA polymerase II (Bourquin *et al*., 1997) and Cdc28 (Nestel *et al*., 1996), where it is believed to function in pre-mRNA splicing after co-localizing with splicing factors into nuclear speckles (Bourquin *et al*., 1997).

The remaining three ER-located cyclophilins are distinct proteins that have evolved, with the exception of ScCpr2, from the lower of the three initial branches (Figure 1A). Two of these individuals are found within *S. cerevisiae*. ScCpr2 is reported as present in the yeast's secretory pathway (Dolinski *et al*., 1997a; Koser *et al*., 1991) and induced by heat stress and tunicamycin (Gothel and Marahiel, 1999). It appears linked to its cyclophilin B orthologue, which could imply that it may have evolved from gene duplication and has since gained an individual role within the secretory pathway. The other *S. cerevisiae* protein, ScCpr4, has been reported to localize to the endoplasmic reticulum (Dolinski *et al*., 1997a), function within the secretory pathway (Gothel and Marahiel, 1999), possess a putative transmembrane domain and to be induced by heat shock and tunicamycin. The remaining ER cyclophilin is *D. melanogaster*'s NinaA (neither inactivation nor after potential A), which is on a branch of its own (Figure 1A). It is expressed solely in the eye (Schneuwly *et al*., 1989) and is reported as required for visual transduction (Shieh *et al*., 1989) as an integral membrane protein functioning within the endoplasmic reticulum, as a chaperone in the secretory pathway (Colley *et al*., 1991; Stamnes *et al*., 1991) that is required for the correct secretion of the Rh1 subset of rhodopsins (Baker *et al*., 1994).

Another individual *S. cerevisiae* cyclophilin, ScCpr8, is found linked with ScCpr4 and also CeCyp17 (Figure 1A), and has been reported to be a membrane-bound protein (Franco *et al*., 1991).

CeCyp16 appears linked to the CeCyp14 RRM-possessing cyclophilin group, with it reported to be expressed within the anterior and posterior distal portions of the intestine in all larval and adult stages except for the dauer stage, where it is observed in both cell bodies and processes of the ventral chord motor neurons but, interestingly, it was absent from the intestine at these times (Ma *et al*., 2002).

The three remaining cyclophilins have a putative mitochondrial localization. hmCypD (also referred to as cyclophilin F) is found in the mitochondrial matrix (Bergsma *et al*., 1991; Connern and Halestrap, 1992; Inoue *et al*., 1993), with reported functions in the mitochondrial protein-folding machinery (Rassow *et al*., 1995) and as part of the mitochondrial permeability transition pore complex (Baines *et al*., 2005; Basso *et al*., 2005; Halestrap *et al*., 2002; He and Lemasters, 2002; Lin and Lechleiter, 2002; Nakagawa *et al*., 2005; Sullivan *et al*., 1999; Waldmeier *et al*., 2002). ScCpr3 has been reported as a mitochondrial cyclophilin required for mitochondrial function under heat stress (Dolinski *et al*., 1997b) and as a protein-folding chaperone within the mitochondria (Davis *et al*., 1992; Gothel and Marahiel, 1999; Matouschek *et al*., 1995). The function of CeCyp1 remains unknown, with no published research on its function at the time of writing. The dendrogram (Figure 1A, upper branch) shows that these mitochondrial cyclophilins share very little in common, with each appearing near its respective cyclophilin A orthologue. This observation is supported by their failure to identify each other during BLAST analysis, in which they identify their respective cyclophilin A orthologues instead. Their function may not, therefore, be conserved within their distinct common location within the cell or, if it is, then this most likely came about through convergent evolution.

FKBP orthology

Table 4D shows that *D. melanogaster* and *H. sapiens* share the greatest number of orthologues, which is closely followed by their orthology to *C. elegans*. All except *D. melanogaster* share just a sole orthologue with the yeasts, with the fruit fly sharing two. This orthology only accounts for at most a quarter of any given higher eukaryotic repertoire or half of a yeast repertoire. It therefore appears that most FKBPs within the repertoires of these organisms are distinct individuals found solely within that repertoire.

Table 4B shows that the only FKBP group to have members in all the compared organisms are those related to hFKBP12. This group has been implicated in transcriptional regulation (Yang *et al*., 1995), as a regulated inhibitor of tumour growth factor (TGF)-β type I signalling (Bryant *et al*., 1999), as well as in the regulation of the cell cycle (Chen *et al*., 1997; Okadome *et al*., 1996; Yao *et al*., 2000) and calcium release channels (Bultynck *et al*., 2001a, 2001b; Cameron *et al*., 1995; Carmody *et al*., 2001; Wagenknecht *et al*., 1997). SpFKBP12 has been reported to be important in the early steps of the sexual development pathway of the fission yeast (Weisman *et al*., 2001), showing that this group appears to have wide-ranging roles.

Only two other groups have a yeast orthologue (Table 4B), with each of the yeasts sharing a single FKBP solely with *D. melanogaster*. SpFKBP39 and DmFKBP39 (Table 4B) are the members of one group, with DmFKBP39 shown to be expressed throughout development (Theopold *et al*., 1995), and SpFKBP39 has been reported as nuclear (Himukai *et al*., 1999). A report has implicated this group of cyclophilins in chromatin remodelling involved in ribosomal DNA silencing through a potential role as a histone chaperone (Kuzuhara and Horikoshi, 2004). The second group contains ScFpr2, which has been identified as resident within the ER (Partaledis and Berlin, 1993), but nothing further is known about this group at the time of writing.

There are only three more identified FKBP orthology groups, two related cytoplasmic groups and the other believed to function within the endoplasmic reticulum, despite the varying predicted localizations of its component members (Table 4B). In the latter group hFKBP13 has been reported as resident within the ER (Jin *et al*., 1991), which is contrary to the PSORT-predicted mitochondrial localization represented in Table 4B, and upregulated in the presence of an increased number of unfolded proteins in the ER (Bush *et al*., 1994) where it has an apparent role in vesicular trafficking (Padilla *et al*., 2003).

One of the cytoplasmic groups is found only in the three higher eukaryotes, with the second only found in *D. melanogaster* and humans

(Table 4B). Both are TPR-possessing FKBPs, with one group having human FKBP52 as a member as well as DmFKBP59, which is reported to be expressed throughout the life-cycle of the fruit fly in the lymph glands, garland cells and oenocyte cells, leading to a proposed function in the exocytic/endocytic pathways that cycle intensively within these tissues (Zaffran, 2000). hFKBP52 (Peattie *et al*., 1992) is closely related to a member of the second group, hFKBP51 (Sanchez, 1990; Wiederrecht *et al*., 1992). It has been shown that both bind competitively to the same site on the Hsp90 complex (Nair *et al*., 1997; Young *et al*., 1998) and that shuffling between the two effects the subcellular localization and transport of steroid receptors (Davies *et al*., 2002, 2005; Riggs *et al*., 2003). The absence of a *C. elegans* orthologue of the hFKBP51 group could potentially indicate a difference in function of the Hsp90-related FKBPs in the nematode.

The remaining FKBPs in the different organisms all appear to be distinct individuals. There are an additional two cytoplasmic TPR containing FKBPs within the human repertoire (Table 4B), hFKBP36 and hFKBP38. hFKBP38 is capable of inhibiting calcineurin in the absence of FK506 (Shirane and Nakayama, 2003), unlike the other FKBPs, suggesting that it functions as a natural inhibitor of the protease, like the previously identified calcineurin inhibitor CAIN (Lai *et al*., 1998). Its ability to anchor Bcl-2 and Bcl-x(L) to the mitochondria has implicated it in the regulation of apoptosis (Shirane and Nakayama, 2003) and a role in homologous chromosome pairing in meiosis has also been reported (Crackower *et al*., 2003). *D. melanogaster* also has an additional TPR-containing nuclear FKBP, DmShutdown (Table 4B), which appears to have an essential function in the regulation of germ cell division (Munn and Steward, 2000). The remaining two distinct nuclear FKBPs are both found in *S. cerevisiae* (Table 4B). ScFpr3 has been identified as nuclear (Benton *et al*., 1994; Manning-Krieg *et al*., 1994; Shan *et al*., 1994), as has ScFpr4 (Davey *et al*., 2000; Dolinski *et al*., 1997b), with both ScFpr3 and ScFpr4 having been shown to suppress defects seen in the absence of the E3 ubiquitin ligase TOM1 (Davey *et al*., 2000). SpFKBP39a and CeFkb8 are the only distinct PSORT-predicted cytoplasmic non-human FKBPs (Table 4B). The former has been shown to be nuclear (Himukai *et al*., 1999) but as yet neither has had any functions identified for it.

The remaining two distinct cytoplasmic FKBPs are all within the human repertoire (Table 4B). hFKBP12.6, which shows 85% similarity to hFKBP12 (Sewell *et al*., 1994), shares its ability to bind to calcium release channels (Lam *et al*., 1995; Timerman *et al*., 1996) and has a proposed role in controlling calcium channel gating through an interaction with cyclic-ADP ribose (Noguchi *et al*., 1997). hFKBP25 has an N-terminal amphipathic DNA binding helix–loop–helix structure (Hung and Schreiber, 1992; Riviere *et al*., 1993) and is found to be predominantly nuclear, contrary to its PSORT-predicted cytoplasmic localization, where it has putative roles in cellular control (Jin and Burakoff, 1993), which is supported by its downregulation following p53 induction (Ahn *et al*., 1999) and transcriptional regulation (Yang *et al*., 2001).

In total there are four distinct *C. elegans* FKBPs and five human FKBPs that are predicted by PSORT to be endoplasmic reticular (Table 4B). Three of the *C. elegans* FKBPs (dao1 = CeFkb3; dao8 = CeFkb4; dao9 = CeFkb7) have been identified in a study on the DAF-2 insulin receptor-like pathway, which is involved in dauer larva formation, as proteins whose expression is controlled by this pathway (Yu and Larsen, 2001), but quite what their function is remains unknown. The final *C. elegans* FKBP is CeFkb7, whose function remains unknown, although the presence of a calcium-binding EF-hand motif in its C-terminal region may indicate that its function is regulated by intracellular calcium levels (Honore and Vorum, 2000).

Besides hFKBP65, which has been reported as localized within the ER lumen of cells only during the growth and development of tissues when it appears to function as a protein chaperone (Patterson *et al*., 2000), the remaining distinct human FKBPs have yet to have functions assigned to them. The presence of a calcium-binding EF-hand motif in its C-terminal region of both hFKBP60 and hFKBP65 may indicate that their function, like that of CeFkb7, is regulated by intracellular calcium levels (Honore and Vorum, 2000).

The dendrogram generated from the FKBP sequences (Figure 1B) only tentatively supports the BLAST-identified orthology groups between the FKBP repertoires. The group containing

SpFKBP39 and DmFKBP39 does appear in the same branch of the dendrogram, which also contains SpFKBP39a, confirming that the two 39 kDa *Sz. pombe* FKBPs are related. This branch also contains the two distinct nuclear *S. cerevisiae* FKBPs, ScFpr3 and ScFpr4, and hFKBP25, which has been reported to be nuclear (Jin and Burakoff, 1993), contrary to its PSORT-predicted localization. All but one of the nuclear FKBPs therefore appears to have evolved from a single common ancestor, with the exception being DmShutdown. The TPR-containing group, which has hFKBP52 as a member, all appear on the same branch of the dendrogram, with a distinct common ancestor shared with hFKBP51, which appears to diverge from hFKBP52 after their common ancestor diverged from the other members of the hFKBP52 group. The other member of the hFKBP51 group appears on a separate branch closely associated with the TPR-containing hFKBP38 and more loosely with hFKBP36 and DmShutdown. The only endoplasmic reticular group containing ScFpr2 and DmCG14715 appears on the same branch diverging from a distinct common ancestor, with hFKBP22, hFKBP23 and DmFKBP13 also found linked on this branch. DmFKBP13 is separated from the rest of its orthology group, which are located on another branch along with all but one of the distinct *C. elegans* endoplasmic reticular FKBPs, the exception being CeFkb7, which appears on a separate branch on its own. Finally we have the FKBP12 group, which are found in two different locations. ScFpr1 and SpFKBP12 are found on the same branch, diverging from a distinct common ancestor, and linked to their other FKBPs, with the exception of ScFpr2. The remaining FKBP12s all diverge from a single branch in the same order as their species diverged, but they share their common ancestor with the hFKBP52 group.

Despite the dendrogram's lack of support for the orthology groups, it does imply that there is more significance in their location within the cell and the domains they possess than in orthologous function. All the cytoplasmic and nuclear FKBPs appear to have evolved from the upper branch of the dendrogram (Figure 1B), with all the endoplasmic reticular FKBPs, with the exception of hFKBP19, appearing to evolve from the lower branch of the dendrogram, although CeFkb7 appears to have evolved independently. Within each of the major branches, the subbranches can be seen to group such that those FKBPs with the same domains present have largely evolved from the same branch or group of linked branches.

Parvulin orthology

Compared with the cyclophilin and FKBP repertoires, the parvulin repertoires are relatively small. All the compared organisms share a single parvulin in common, with a second parvulin only shared between the higher eukaryotes (Table 4D). The sole parvulin they all share in common is that of the hPin1 group identified by BLAST analysis (Table 4C).

hPin1 has been reported to specifically isomerize only phosphorylated serine/threonine–proline bonds (Lu *et al*., 2002), making it likely that all its functions can be linked to a regulatory role with phosphoproteins. Its catalytic activity has been implicated in the restoration of the function of the phosphorylated-neuronal Tau protein (Lu *et al*., 1999; Zhou *et al*., 2000) and in maintaining Bcl2 in a phosphorylated state (Basu *et al*., 2002). It has been reported to have a critical regulatory role in the function of p53 (Wulf *et al*., 2002; Zacchi *et al*., 2002; Zheng *et al*., 2002), a regulatory role in transcription (Albert *et al*., 1999; Wen and Shatkin, 1999), a role in the cell cycle G_2–M progression (Crenshaw *et al*., 1998) and with a potential function also reported in mitosis (Messenger *et al*., 2002). Interestingly, the cytoplasmic component of the transcription factor NF-AT (nuclear factor of activated T-cells), when in a phosphorylated form, interacts with Pin1's WW domain and this interaction stops NF-ATc being dephosphorylated by the Ca^{2+}-dependent protein phosphatase calcineurin (Liu *et al*., 2001), indicating a role in the regulation of NF-AT signalling. The immunosuppressive drugs cyclosporin A and FK506 both cause immunosuppression by this mechanism (Schreiber and Crabtree, 1992), making it appear that Pin1 has a role in the regulation of the immune response.

DmDodo has also been reported as involved in signal transduction (Maleszka *et al*., 1997), protein folding (Maleszka *et al*., 1997) and, more recently, as a MAP kinase signal responder during oogenesis (Hsu *et al*., 2001).

Of the yeast Pin1 orthologues, SpPin1 is believed to be it a positive regulator of the cell cycle control proteins Wee1 and Cdc25 (Huang *et al*.,

2001). ScEss1 is reported as nuclear and is involved in transcription (Kops *et al*., 2002; Morris *et al*., 1999; Wilcox *et al*., 2004; Wu *et al*., 2000, 2003; Xu *et al*., 2003), cell cycle regulation (Huang *et al*., 2001) and is essential for vegetative growth (Hanes *et al*., 1989). Interestingly, ScEss1 has been reported as an essential gene (Hanes *et al*., 1989), whereas SpPin1 (Huang *et al*., 2001), DmDodo (Maleszka *et al*., 1996) and *Cryptococcus neoformans* Ess1 (Ren *et al*., 2005) have been shown to be non-essential. Crosstalk between ScEss1 and ScCpr1 (Fujimori *et al*., 2001), its hCypA orthologue, has been shown to modulate the activity of the Sin3–Rpd3 complex, with excess histone deacetylation causing mitotic arrest in ScEss1 mutants (Arevalo-Rodriguez *et al*., 2000), and CnEss1-null mutants have been reported to be hypersensitive to cyclosporin A (CsA; Ren *et al*., 2005), suggesting a cyclophilin-mediated redundancy mechanism. Disruption of ScEss1 can be complemented by DmDodo (Maleszka *et al*., 1996) and the plant *Digitalis lanata*'s Par13 (Metzner *et al*., 2001), which lacks the WW domain conserved in the other proteins, indicating that a conserved functionality may exist between all Pin1 orthologues that is essential in some but not all organisms under normal growth conditions.

The parvulin orthology group found only within the higher eukaryotes are related to hPar14 (EPVH; Table 4C), which has been reported in two different studies to localize within two different areas of the cell, preferentially within the mitochondrial matrix (Rulten *et al*., 1999) and preferentially within the nucleus (Uchida *et al*., 1999). It has been reported to be part of the preribosomal ribonucleoprotein (pre-rRNP) complexes and as interacting with fibronectin, p160 (Myb-binding), p58 cyclin-dependant kinase (a G_2/M-specific protein kinase) and α- and β-tubulin (Fujiyama *et al*., 2002). The PSORT-predicted cytoplasmic localization of both hPar14 and DmCG11858 is in contrast to the predicted nuclear localization of CePin2. Given the apparent functions of this group, a nuclear localization is more likely, contrary to the reported mitochondrial localization, indicating that the localization events may be due more to interactions with other molecules than sequence motifs.

The only distinct parvulin is in the repertoire of *D. melanogaster*, DmCG32845. No orthologues were identified by BLAST searching of the currently available sequence databases, indicating that it may be unique to the fruit fly. It is just over twice the size of the hPin1 group but only a single parvulin-like rotamase domain is identified within its sequence (data not shown). It has a predicted nuclear localization but it has not been previously reported, making this is a novel identification in this study. Research into its function will be invaluable to see what function this parvulin performs solely within *D. melanogaster*, thus indicating reasons for its absence in the other organisms. The dendrogram for the parvulins (Figure 1C) implies that this novel parvulin is more closely related to the hPar14 group, which also shares its branch with the *D. melanogaster* hPin1 orthologue, DmDodo. It therefore appears that the evolution of the parvulins of *D. melanogaster* is more closely linked than those of the other compared organisms. hPin1 appears to have evolved in a more independent fashion, with the remaining hPin1 orthologues appearing to evolve from a distinct common ancestor, with SpPin1 and ScEss1 unsurprisingly sharing a distinct common ancestor themselves.

Discussion

Of the identified PPIase repertoires, humans unsurprisingly possess the greatest number, although fewer than we would expect, given the trend of PPIase numbers vs. genes seen with the organisms in the Table 3. A repertoire size of ca. 40 would be more in keeping with its genome size. Looking at the component PPIase member numbers that make up their repertoires, humans possess a lower number of cyclophilins than we would expect but its number of FKBPs and parvulins are as expected, indicating that its smaller than expected PPIase repertoire can be accounted for by a smaller cyclophilin family. With two parvulins found in the human and *C. elegans* repertoires and only a single parvulin found in both of the yeasts, *D. melanogaster* is the only known eukaryote to have three parvulins, with its third parvulin appearing to be unique.

We have shown that the repertoires of these organisms have both members with common function and those which appear distinct for any given organism. Interestingly, the cyclophilin repertoire of *Sz. pombe* has no unique members and the two yeasts share no PPIases that are unique to themselves. The proportion of cyclophilins and

parvulins with identified orthologues within the repertoires is high, whereas the proportion seen with the FKBPs is low by comparison. It therefore appears that the cyclophilins and parvulins have evolved to perform conserved functions, while the FKBPs have evolved to fill ever-changing niches within these constantly evolving organisms.

There were a total of 12 distinct cyclophilin orthology groupings identified by BLAST analysis, and confirmed in most cases by sequence analysis, with eight identified domain architectures between them. Only four of theses groups have an *S. cerevisiae* member, whereas nine have members in its fellow yeast *Sz. pombe*, leaving only three that are unique to the repertoires of the multicellular organisms. *S. cerevisiae* lacks members of three cytoplasmic groups, which include those involved in transcriptional regulation, pre-mRNA splicing and signal transduction, and it also lacks members of two nuclear groups believed to be involved in transcriptional regulation as well as cell morphogenesis, cortical organization and nuclear reorganization. Those orthology groups that lack a *Sz. pombe* and *S. cerevisiae* member appear to function in pathways that would not be found in single-celled yeasts, such as the control of cell differentiation, or show differential expression linked to cell type.

Although no unique cyclophilins are present in the *Sz. pombe* repertoire, there are five in that of its fellow yeast *S. cerevisiae*, which equals the number in humans and is only two less than are found in *C. elegans*. *D. melanogaster* has only three, one in each of the cytoplasmic, nuclear and endoplasmic reticular compartments, with two of these appearing to be involved in pathways specific to the fruit fly and one being similar to its cytoplasmic cyclophilin A orthologue. One of the *S. cerevisiae* unique cyclophilins appears to be a second member of the TPR-possessing cyclophilin 40 group only found within this yeast, with another two unique cyclophilins found within its endoplasmic reticulum. The final two unique *S. cerevisiae* cyclophilins have distinct localizations, one to the plasma membrane and the other within the mitochondria. The latter has compatriots in humans and *C. elegans*, although these appear unrelated. No mitochondrial cyclophilin is found in either *Sz. pombe* or *D. melanogaster*. Besides this mitochondrial cyclophilin, *C. elegans* also has three unique cyclophilins in both the nucleus and cytoplasm. Two of the latter appear to be additional cyclophilin A-like cyclophilins, with the third appearing to be involved in cell morphogenesis and cortical organization. The former group has one of unknown function, with the remaining two appearing to be part of a family that includes a unique protein in both *D. melanogaster* and human. These appear to all possess what has been termed a 'moca' domain, but their function remains largely unknown. All of the five extra human cyclophilins, which includes the 'moca'-possessing protein, are located in the nucleus with the exception of the mitochondrial cyclophilin mentioned above. The final cyclophilin is hRanBP2, a large multidomain cyclophilin that functions as part of the nuclear pore complex.

S. cerevisiae appears to require a greater number within the endoplasmic reticulum than any of the other organisms compared, implying that either their function may have been incorporated into the PPIases they possess within that cellular structure, or that their function is not required in these other organisms. The lack of a mitochondrial cyclophilin in *Sz. pombe* is surprising, given its presence in all the others with the exception of *D. melanogaster*. It therefore appears that mitochondria in *Sz. pombe* share a greater similarity with those in *D. melanogaster* in this respect. However, a single *Neurospora crassa* cyclophilin gene has been reported to encode both a cytosolic and a mitochondrial isoform (Tropschug *et al*., 1988), which could possibly explain the absence of a dedicated mitochondrial cyclophilin within *Sz. pombe* and *D. melanogaster*. The isolation of a cyclophilin from within the mitochondria of both these organisms would therefore clarify this.

The FKBP repertoires of the compared organisms show less orthology than was seen with the cyclophilin repertoires, with the yeasts showing approximately 50% orthology with the higher eukaryotes, which themselves show little more than a 20% orthology with each other. A majority of FKBPs therefore appear to be distinct to any given organism, implying that their function is organism-specific in most cases, with the sole FKBP they all share in common being the FKBP12 family. This family appears to have a wide range of functions within cell cycle regulation, calcium release and transcriptional regulation that appear to place the function of the FKBP12 group predominantly within intracellular signalling. Whilst the functions of the additional FKBPs in the two yeasts are

largely unknown, those in the multicellular eukaryotes appear to function either in processes, such as the growth and differentiated development of tissues, or pathways, such as the anchoring of Bcl-proteins to the mitochondria, that are probably not required/present within the yeasts.

Unlike with the cyclophilins, sequence analysis did not support many of the BLAST-identified FKBP orthology groups. Although most groups clustered into the same regions within the dendrogram, many were not able to be traced back to a distinct common ancestor. The main organization of the dendrogram appeared to be based upon their secondary domains and localization rather than by orthologous function.

In contrast to the cyclophilin and FKBP repertoires, the number of parvulins within the compared organisms is small. They all share a single parvulin in common, orthologues of hPin1, with the higher eukaryotes sharing an additional parvulin in common related to hPar14. The hPin1 orthology group appear to function in a wide range of processes from intracellular signalling to the regulation of transcription and the cell cycle, with some appearing essential for cell survival, whilst the hPar14 group also appear to potentially function in some intracellular signalling pathways as well as within the ribosomal processes. The reason for the absence of a hPar14 orthologue within the genomes of both the yeasts cannot easily be explained by their function, making the most likely explanation that, while the yeasts can cope with just a single parvulin, the evolution of the higher eukaryotes has required an additional parvulin to either share the work-load or to fill a particular niche that requires a parvulin of divergent form to that of hPin1. A novel parvulin that identified no orthologues in the presently available sequence databases is present solely within the repertoire of *D. melanogaster* and appears, based on sequence analysis, to be a distant relative of the hPar14 group, whilst evolving to an unknown function that currently appears to be required solely within the fruit fly. Analysis of the function of this parvulin will therefore be of great interest in unlocking why it is present solely within the fruit fly.

Looking at the global localization patterns of the three PPIase families within the compared organisms (Table 4A–C), the cyclophilins appear predominantly within the cytoplasm and nucleus, with only a few present within the endoplasmic reticulum. *Sz. pombe* has just a single cyclophilin within the endoplasmic reticulum, unlike the other compared organisms that have two, with the exception of *S. cerevisiae* which has three. They all share a single ER-resident cyclophilin in common, while the remaining ER cyclophilins in both *S. cerevisiae* and *D. melanogaster* are specific to each of them, with only the additional cyclophilin in human and *C. elegans* appearing orthologous.

The FKBPs, however, reside predominantly within the endoplasmic reticulum and cytoplasm. Nuclear FKBPs are only present within the repertoires of the two yeasts and *D. melanogaster*, making these distinct from those of humans and *C. elegans*, which possess a greater number within the endoplasmic reticulum, although these appear specific to each of them. *Sz. pombe* is unique in lacking an FKBP within the endoplasmic reticulum, unlike *S. cerevisiae*, which has a single one present. This greater localization to the vesicular pathway could explain the lack of orthology observed within the FKBP family of the compared organisms, which we have attributed to their potential evolution to serve in variable niches within the different organisms. As the number of genes in any given organism increases, so do the number of proteins, and with that an increase in the number of proteins requiring chaperoning is to be expected. Given the location of the FKBPs within the protein-folding pathway and the apparent linkage between the number present in any given organism and its number of genes (Table 3), it could be that they are evolving to serve this increased requirement for chaperoning, driven independently within each organism, thus leading to their observed lack of orthology. Taking this into consideration, we would hypothesize that the cyclophilins are evolving to perform specific conserved functions within the different organisms, while the FKBPs are evolving, in most cases, to meet the more individual needs for protein chaperoning. The presence of nuclear FKBPs within the two yeasts and *D. melanogaster* could be examples of functions initially performed by the FKBPs that have since evolved to be filled by cyclophilins within *C. elegans* and humans. The lack of an *Sz. pombe* FKBP within the endoplasmic reticulum and the lack of any compensatory cyclophilins does, however, indicate that that the true reasons behind this may be more complicated

than the simplified hypothesis we have proposed here.

The parvulins appear to be a family of PPIases found solely within the nucleus. They all share a single parvulin in common, with the higher eukaryotes also sharing a second smaller parvulin. Their greater presence in the prokaryotes, where in some cases they are the largest or sole PPIase family present (data not shown), makes it appear that their evolution has been such that the cyclophilins and FKBPs have replaced them in their function or that their functions have evolved such that they do not require them.

This comparison has shown that, while the PPIase repertoire of *S. cerevisiae* has been the subject of a great deal of research to identify their functions within the cell, it is a poor representative of the repertoires of the more complex organisms. In contrast, its fellow yeast *Sz. pombe* appears to be a good model organism for the study of two of the three PPIase families, the cyclophilins and parvulins, with it not appearing to be a good system for the study of the FKBP family. This lack of orthology appears global in the FKBP repertoires, implying that they function in a capacity that is specific to each organism. Thus, *Sz. pombe* represents an excellent single-celled model organism for the study of the functions of the different PPIase families, which can be related to the function of their orthologues within more complex eukaryotes.

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