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. Copyright © 2005 John Wiley & Sons, Ltd.

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 Bruce, 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 Bruce, 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; Rul ten 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 FKBP s 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 (Devashhayam 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 it appears that they do not 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 (Rbt) 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 S. 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), S. 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), S. 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*, *S. 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       | PPlase | Uniprot Acc. # | kDa | Signal Seq. | Predicted Localisation | Domain Architecture |
|---------|--------|----------------|-----|-------------|------------------------|---------------------|
| Cyp-A   | P62937 | 18.0           | —   | Cytoplasmic | PPlase Only            |                     |
| PPI1 (CGI-124) | Q9Y3C6 | 18.2           | ~   | Cytoplasmic | PPlase Only            |                     |
| PPI3   | Q9BXZ1 | 18.6           | ~   | Cytoplasmic | PPlase Only            |                     |
| USA-Cyp (Cyp-H) | O43447 | 19.2           | —   | Cytoplasmic | PPlase Only            |                     |
| Cyp-F (D) | P30405 | 22.0           | —   | Mitochondrial | PPlase Only          |                     |
| Cyp-B   | Q9BVS5 | 22.7           | N-term | ER | PPlase Only            |                     |
| Cyp-C   | P84777 | 22.8           | N-term | ER | PPlase Only            |                     |
| "Cyp29" | P49069 | 28.9           | ~   | Nuclear    | N-term PPlase Only    |                     |
| Cyp33 (E) | Q9UNP9 | 33.4           | —   | Cytoplasmic | N-term RRM, C-term PPlase |                     |
| Cyp40 (D) | Q08752 | 40.8           | —   | Cytoplasmic | N-term PPlase, C-term TPR (3x) motifs |                     |
| SDCCAG10 | Q6UX04 | 53.8           | —   | Nuclear    | N-term PPlase, positively charged C-term |                     |
| "Cyp57" | Q8WUA2 | 57.2           | ~   | Nuclear    | N-term PPlase, Central RRM |                     |
| Cyp60   | Q13356 | 58.8           | ~   | Nuclear    | N-term U-box, C-term PPlase |                     |
| HALS39  | Q9BP73 | 73.6           | —   | Cytoplasmic | N-term WD40 (x3) motifs, C-term PPlase |                     |
| Cyp88 (CARS/G) | Q13427 | 88.6           | —   | Nuclear    | N-term PPlase, C-term RS domain |                     |
| "Cyp147" | P30414 | 165.7          | —   | Nuclear    | N-term PPlase, C-term RS domain containing a Moca motif |                     |
| RunBP1  | P47979 | 338.2          | —   | Nuclear    | N-term TPR, central Zn-fingers, central/C-term RBI domains, C-term PPlase |                     |
| FKB12.6 | P68106 | 11.8           | —   | Cytoplasmic | FKB (x1) Only         |                     |
| FKB12   | P62942 | 12.0           | —   | Cytoplasmic | FKB (x1) Only         |                     |
| FKB13   | P26885 | 15.6           | N-term | Mitochondrial | FKB (x1) Only   |                     |
| FKB19   | Q9NYL4 | 22.2           | N-term | ER | FKB (x1) Only         |                     |
| FKB22   | Q9NVM8 | 24.3           | N-term | ER | FKB (x1) Only         |                     |
| FKB23   | Q9Y680 | 30.0           | —   | ER         | N-term FKB (x1), C-term EF-Hand motif |                     |
| FKB25   | Q00688 | 25.2           | —   | Cytoplasmic | C-term FKB (x1) Only |                     |
| FKB36   | Q73344 | 37.2           | ~   | Cytoplasmic | N-term FKB (x1), C-term TPR (x2) motifs |                     |
| FKB38   | Q14318 | 38.4           | —   | Cytoplasmic | N-term FKB (x1), C-term TPR (x2) motifs |                     |
| FKB51   | Q13451 | 51.2           | —   | Cytoplasmic | N-term FKB (x2), C-term TPR (x2) motifs |                     |
| FKB52   | Q07900 | 51.6           | —   | Cytoplasmic | N-term FKB (x2), C-term TPR (x3) motifs |                     |
| FKB56   | Q93032 | 57.2           | ~   | Nuclear    | N-term FKB (x4), C-term EF-Hand motif |                     |
| FKB65   | Q9YE43 | 64.7           | N-term | ER | N-term FKB (x4), C-term EF-Hand motif |                     |
| Pin1    | Q13526 | 9.0            | —   | Nuclear    | N-term WW domain, C-term Rotamase |                     |
| Parl4   | 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...
| B            | PPlase | Uniprot Acc. # | kDa | Signal Seq. | Predicted Localisation | Domain Architecture |
|------------|--------|----------------|-----|-------------|------------------------|---------------------|
| CG7768     | Q9VUD6 | 17.8           |      | Cytoplasmic | PPlase Only             |                     |
| CG1177     | Q8MK9l | 17.8           |      | Cytoplasmic | PPlase Only             |                     |
| Cyp1       | P25007 | 17.9           |      | Cytoplasmic | PPlase Only             |                     |
| CG13892    | Q9W0Q2 | 19.5           |      | Cytoplasmic | PPlase Only             |                     |
| CG17266    | Q9V9R9 | 20.2           |      | Cytoplasmic | PPlase Only             |                     |
| CG2852     | Q9W227 | 22.2           |      | N-term ER   | PPlase Only             |                     |
| NinaA      | P15425 | 26.4           |      | N-term      | PPlase Only             |                     |
| Cyp33      | Q9V3G3 | 33.3           |      | Cytoplasmic | N-term RRM, C-term PPlase|                     |
| CG8336     | Q8V72l | 43.1           |      | Cytoplasmic | N-term PPlase, C-term TPR (2×) motifs|                     |
| CG10907    | Q9VT7 | 56.6           |      | Nuclear     | N-term PPlase, C-term RS domain|                     |
| CG7747     | Q9V7M9 | 59.0           |      | Nuclear     | N-term U-box, C-term PPlase|                     |
| CG3511     | Q96Q8 | 71.8           |      | Cytoplasmic | N-term WD40 motif, C-term PPlase|                     |
| CG5808     | Q9XYZ6 | 75.5           |      | Nuclear     | N-term PPlase, central RRM, C-term RS domain|                     |
| Moca-Cyp   | Q8SE5 | 112.3          |      | Nuclear     | N-term PPlase C-term RS domain containing a Moca motif|                     |
| Cyclophilins |       |                |      |             |                        |                     |
| Cyp1       | P52009 | 20.7           |      | Mitochondrial | PPlase Only |                     |
| Cyp2       | P52010 | 18.5           |      | Cytoplasmic | PPlase Only             |                     |
| Cyp3       | P52011 | 18.6           |      | Cytoplasmic | PPlase Only             |                     |
| Cyp4       | P52012 | 58.3           |      | Cytoplasmic | N-term U-box, C-term PPlase|                     |
| Cyp5       | P52013 | 22.4           |      | N-term ER   | PPlase Only             |                     |
| Cyp6       | P52014 | 21.9           |      | N-term ER   | PPlase Only             |                     |
| Cyp7       | P52015 | 18.4           |      | Cytoplasmic | PPlase Only             |                     |
| Cyp8       | P52016 | 53.6           |      | Nuclear     | N-term PPlase C-term RS domain containing a Moca motif|                     |
| Cyp9       | Q9637 | 35.8           |      | Nuclear     | N-term PPlase C-term RS domain containing a Moca motif|                     |
| Cyp10      | P52017 | 18.0           |      | Cytoplasmic | PPlase Only             |                     |
| Cyp11      | P52018 | 30.2           |      | Cytoplasmic | PPlase Only             |                     |
| Cyp12      | Q18444 | 18.5           |      | Cytoplasmic | PPlase Only             |                     |
| Cyp13      | Q9U256 | 36.4           |      | Cytoplasmic | N-term RRM, C-term PPlase|                     |
| Cyp14      | O18160 | 50.4           |      | Nuclear     | N-term PPlase, C-term RRM|                     |
| Cyp15      | Q9U1JQ | 70.8           |      | Cytoplasmic | N-term WD40 motifs, C-term PPlase|                     |
| Cyp16      | Q9KX7 | 25.2           |      | Cytoplasmic | PPlase Only             |                     |
| Cyp17      | O1B80 | 57.7           |      | Nuclear     | Positively charged N-term, C-term PPlase|                     |
| Fkb1       | Q20107 | 15.5           |      | N-term      | Cytoplasmic | FKB (1×) Only             |                     |
| Fkb2       | Q9U2Q8 | 11.6           |      | Cytoplasmic | FKB (1×) Only             |                     |
| Fkb3       | O16309 | 29.1           |      | N-term ER   | FKB (2×) Only             |                     |
| Fkb4       | Q23338 | 29.3           |      | N-term ER   | FKB (2×) Only             |                     |
| Fkb5a      | P91180 | 29.9           |      | N-term ER   | FKB (2×) Only             |                     |
| Fkb6       | O4514 | 48.1           |      | Cytoplasmic | N-term FKB (2×), C-term TPR (3×) motifs|                     |
| Fkb7       | O61826 | 36.2           |      | N-term ER   | N-term FKB (1×), C-term EF-hand motif|                     |
| Fkb8       | Q8H4G | 32.4           |      | Cytoplasmic | FKB (2×) Only             |                     |
| Pin1       | Q9NA92 | 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     | PPlase | Uniprot Acc. # | kDa  | Signal Seq. | Predicted Localisation | Domain Architecture  |
|-------|--------|----------------|------|-------------|------------------------|----------------------|
| Cyp1  | P87051 | 17.4           | ~    | Cytoplasmic | PPlase only            |
| Cyp2  | P18253 | 16.9           | ~    | Cytoplasmic | PPlase only            |
| Cyp3  | O74729 | 18.9           | ~    | ER          | PPlase only            |
| Cyp4  | O94273 | 22.2           | N-term | Cytoplasmic | N-term, PPlase, C-term, TPR(×3) |
| Cyp5  | Q11004 | 40.2           | ~    | Nuclear     | N-term, PPlase, C-term, positively charged |
| Cyp6  | Q9UUE4 | 50.8           |      | Nuclear     | N-term, U-Box, C-term, PPlase |
| Cyp7  | O42941 | 52.2           | ~    | Nuclear     | N-term, WD40(×3), C-term, PPlase |
| Cyp8  | Q09928 | 53.6           | ~    | Nuclear     | N-term, PPlase, C-term, positively charged |
| Cyp9  | O74942 | 69.0           | ~    | Nuclear     | N-term, WW domain, C-term, Rotamase |
| B     | PPlase | Uniprot Acc. # | kDa  | Signal Seq. | Predicted Localisation | Domain Architecture  |
| Cpr1  | P14832 | 17.4           | ~    | Cytoplasmic | PPlase Only            |
| Cpr2  | P23285 | 22.8           | N-term | ER          | PPlase Only            |
| Cpr3  | P52719 | 19.9           | ~    | Mitochondrial | PPlase Only            |
| Cpr4  | P52334 | 35.8           | N-term | ER          | Central PPlase Only    |
| Cpr5  | P53176 | 25.3           | N-term | ER          | PPlase Only            |
| Cpr6  | P53691 | 42.1           |      | Cytoplasmic | N-term, PPlase, C-term, TPR(3×) motifs |
| Cpr7  | P47103 | 45.1           | ~    | Cytoplasmic | N-term, PPlase, C-term, TPR (3×) motifs |
| Cpr8  | P53728 | 34.9           | ~    | Membrane    | Central PPlase         |
| Cwc27 | Q27270 | 35.0           | N-term | Nuclear     | Divergent N-term PPlase Only |
| 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  | Q66205 | 43.9           | ~    | Nuclear     | Negatively charged N-term, C-term FKBP (1×) |
| Parv  | E51    | 21.7           | ~    | Nuclear     | N-term WW domain, C-term Rotamase |

The other group is 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., 2002), 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).
Comparison of peptidyl-prolyl cis/trans Isomerase Repertoires

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).

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 | Cyclophilins | FKBPs | Parvulins | Total |
|--------------|-------|--------------|-------|-----------|-------|
| *H. sapiens* | 24-40,000<sup>a</sup> | 17 | 13 | 2 | 32 |
| *C. elegans* | 18,424<sup>b</sup> | 17 | 8 | 2 | 27 |
| *D. melanogaster* | 13,601<sup>c</sup> | 14 | 7 | 3 | 24 |
| *S. cerevisiae* | 5,885<sup>d</sup> | 8 | 4 | 1 | 13 |
| *Sz. pombe* | 4,824<sup>e</sup> | 9 | 3 | 1 | 13 |

<sup>a</sup> Lander et al. (2001); <sup>b</sup> Rubin et al. (2000); <sup>c</sup> Goffeau et al. (1996); <sup>d</sup> 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
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 (Skrzynz et al., 2001), which gives them a role within a broad range of signal transduction pathways (Skrzynz 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-protease 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 polycistrionic 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 A5. 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, ScCpr6 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 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 (Alkhathib 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 FKBP s 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 receptor (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 has an additional TPR-containing nuclear FKBP, DmShuttleDown (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-Kriegl 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 proposed a 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 reticulum (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 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), a regulatory role in transcription (Albert et al., 1999; Wen and Shatkin, 1999), a role in the cell cycle G2–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 Ca2+-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.,
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. Cross-talk 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-dependent kinase (a G2/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 S. 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 FKBP s is low by comparison. It therefore appears that the cyclophilins and parvulins have evolved to perform conserved functions, while the FKBP s 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 these 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 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 compatrions 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 FKBP s 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 FKBP s 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 hPar14, with the higher eukaryotes sharing an additional parvulin in common related to hPar14. The hPar14 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 hPar14. 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. S. 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 FKBP, however, reside predominantly within the endoplasmic reticulum and cytoplasm. Nuclear FKBP 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. S. 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 FKBP 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 FKBP are evolving, in most cases, to meet the more individual needs for protein chaperoning. The presence of nuclear FKBP within the two yeasts and D. melanogaster could be examples of functions initially performed by the FKBP that have since evolved to be filled by cyclophilins within C. elegans and humans. The lack of an S. 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.

Acknowledgements

Trevor Pemberton was in receipt of a BBSRC Studentship at the time of this research. The authors would like to thank Professor Andrew Smith (University of Sussex) and Dr. Peter Klappa (University of Kent) for their help in the preparation of this manuscript.

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