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Invited Review

Peptidyl-prolyl cis–trans isomerases (immunophilins) and their roles in parasite biochemistry, host–parasite interaction and antiparasitic drug action

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Abstract

Immunophilin is the collective name given to the cyclophilin and FK506-binding protein families. As the name suggests, these include the major binding proteins of certain immunosuppressive drugs: cyclophilins for the cyclic peptide cyclosporin A and FK506-binding proteins for the macrolactones FK506 and rapamycin. Both families, although dissimilar in sequence, possess peptidyl-prolyl *cis–trans* isomerase activity in vitro and can play roles in protein folding and transport, RNA splicing and the regulation of multi-protein complexes in cells. In addition to enzymic activity, many immunophilins act as molecular chaperones. This property may be conferred by the isomerase domain and/or by additional domains. Recent years have seen a great increase in the number of known immunophilin genes in parasitic protozoa and helminths and in many cases their products have been characterised biochemically and their temporal and spatial expression patterns have been examined. Some of these genes represent novel types: one example is a *Toxoplasma gondii* gene encoding a protein with both cyclophilin and FK506-binding protein domains. Likely roles in protein folding and oligomerisation, RNA splicing and sexual differentiation have been suggested for parasite immunophilins. In addition, unexpected roles in parasite virulence (Mip FK506-binding protein of *Trypanosoma cruzi*) and host immunomodulation (e.g. 18-kDa cyclophilin of *T. gondii*) have been established. Furthermore, in view of the potent antiparasitic activities of cyclosporins, macrolactones and non-immunosuppressive derivatives of these compounds, immunophilins may mediate drug action and/or may themselves represent potential drug targets. Investigation of the mechanisms of action of these agents may lead to the design of potent and selective antimalarial and other antiparasitic drugs. This review discusses the properties of immunophilins in parasites and the 'animal model' *Caenorhabditis elegans* and relates these to our understanding of the roles of these proteins in cellular biochemistry, host–parasite interaction and the antiparasitic mechanisms of the drugs that bind to them.

Keywords: Protein folding; Cyclophilin; FKBP; Cyclosporin; FK506; Rapamycin

1. Introduction

The cyclophilin (CYP) and FK506-binding protein (FKBP) families, although unrelated in sequence, are often considered together because of their shared enzymic activities. Both cyclophilins and FKBPs, along with a smaller protein class, the parvulins, exhibit peptidyl-prolyl *cis–trans* isomerase (PPIase: EC 5.2.1.8) activity that plays a vital role in protein folding (Fischer and Aumüller, 2003). Although the peptide bonds of nascent polypeptides emerge from the ribosome in the *trans*-conformation, and the majority retains that energetically favoured state in fully folded proteins, there is a significant minority (~5–7% of the proteins with structures solved) of peptidyl-prolyl (Xaa-Pro) bonds that switch to the *cis*-conformation during folding, transport and assembly. The *cis–trans* isomerisation of Xaa-Pro bonds is one of the rate-limiting steps of protein folding. However, the influence of cyclophilins and FKBPs on the conformations, locations, oligomeric states and activities of various proteins in cells cannot be explained by PPIase activity alone. At least some cyclophilins and FKBPs can act as molecular chaperones in an analogous manner to certain members of stress protein families. The chaperone activity can be measured separately

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in vitro, for example via aggregation assays using model substrates, and may or may not be dependent on the presence of a functioning PPIase domain. An additional property common to most eukaryotic and prokaryotic members of each family is their interaction with certain immunosuppressive drugs: the cyclic undecapeptide cyclosporin A (CsA) binds to cyclophilins and the macrolactones FK506 and rapamycin to FKBPs. For this reason, cyclophilins and FKBPs are known collectively as the immunophilins. Binding of any of these immunosuppressants inhibits the PPIase activity of its respective partner. Although this inhibition may have physiological consequences, it does not represent the mechanism of immunosuppressive action as such. In T-lymphocyte suppression, CsA-cyclophilin or FK506-FKBP12 complexes form composite surfaces that strongly inhibit the protein phosphatase calcineurin, a crucial component of a Ca^{2+}-dependent signalling pathway (Matsuda and Koyasu, 2000). Calcineurin is also the relevant target in fungi, where inhibition of this phosphatase prevents recovery from pheromone-induced cell-cycle arrest (*Saccharomyces cerevisiae*) or growth at elevated temperatures relevant to virulence (*Cryptococcus neoformans*) (Wang and Heitman, 2005). In the case of rapamycin-FKBP, the relevant target is not calcineurin but the protein kinase TOR (target of rapamycin), and the downstream blockade is not on T-cell activation (G_0–G_1 transition) but on proliferation (G_1–S). Therefore immunophilins are not only involved in the folding, trafficking and activity of a range of cellular proteins, but also mediate the effects of certain pharmacologically active small molecules. Aside from their roles in cellular biochemistry, immunophilins of parasites are particularly interesting for two additional reasons. First, there is evidence that some are involved in the pathogenesis of infections caused by protozoa and other microorganisms (Hacker and Fischer, 1993). Second, CsA, FK506, rapamycin and more excitingly, non-immunosuppressive analogues of these compounds, have strong inhibitory effects on certain parasites in culture and in animal models of infection (Bell et al., 1996). This review considers the properties of the well-characterised immunophilin genes and their products, focussing on their roles in host–parasite interaction and the antiparasitic actions of certain drugs. The emphasis is on work published in the last 10 years: for a fuller discussion of earlier work, see the reviews by Page et al. (1995b); Bell et al. (1996).

2. Cyclophilins

2.1. Genes and transcription

It is common that a given genome contains more than one cyclophilin gene—eight in *S. cerevisiae* and at least 16 in humans (Galat, 2003)—and this may also be the case for most parasites. In this section we shall confine ourselves to those genes that are expressed and for which data of some functional relevance, e.g. PPIase or chaperone activity, cyclosporin binding, or specific distribution, have been obtained. Browsing of annotated genes in parasite genome databases reveals more putative cyclophilin or cyclophilin-like genes and more detailed data mining using conserved cyclophilin sequences can expose even more. We shall refrain from speculating on sequences that may or may not encode actual cyclophilins, except where it is likely to be informative, e.g. where a clear orthologue of a well-characterised gene in one parasite species is found in the genome of a closely related species.

The properties of the protozoal cyclophilins for which there are firm, published expression and/or functional data are shown in Table 1. The nomenclature we shall use for protozoal cyclophilins is similar to the convention of Galat (2003): cyclophilin is abbreviated to CYP and a species-specific prefix and, especially if there is more than one type in one organism, a suffix representing the approximate molecular mass in kilo Dalton of the mature protein (if known), are added, e.g. hCYP18 (human 18-kDa cyclophilin, hCyPA), PfCYP19A (one of two *Plasmodium falciparum* cyclophilins of 19-kDa). In general, the known cyclophilins of protozoal parasites are closely related in sequence to each other and to hCYP18, the first cyclophilin to be discovered and the one with which other cyclophilins are usually compared. The residues known from three-dimensional structures to make close contact with CsA, especially the crucial tryptophan (position 121 in hCYP18), are for the most part well conserved in the protozoal cyclophilins (Fig. 1). The appearance of the residues known to contact calcineurin in the presence of CsA is less consistent.

Three cyclophilin genes have been identified in *P. falciparum: Pfcyp19B* (formerly *PfCyP, PfCyP22:* Hirtzlin et al., 1995), *Pfcyp24* (formerly *PFCyP*: Reddy, 1995) and *Pfcyp19A* (formerly *PfCyP19:* Berriman and Fairlamb, 1998). These genes are located on chromosomes 11, 8 and 3, respectively. Aside from the N-terminal extensions of PfCYP19B and PfCYP24, the major difference between the *P. falciparum* cyclophilins and hCYP18 sequences lies in 'insertions' of four to six amino acids situated around position 43–44 (hCYP18 numbering; Fig. 1), which lies in the linker region between helix α1 and strand β3 (Dornan et al., 2003). This corresponds to a region of substantial diversity among cyclophilins in general (Galat, 1999). All three *P. falciparum* cyclophilins have orthologues encoded in the *Plasmodium yoelii* and *Plasmodium berghei* genomes (Carlton et al., 2002; Hall et al., 2005), PfCYP19A and PfCYP19B in the *Plasmodium chabaudi* genome (Hall et al., 2005) and PfCYP19A in *Plasmodium vivax* (Cui et al., 2005). Of these non-falciparum cyclophilins, only the *P. berghei* PfCYP19A orthologue has been characterised (Nunes, J., 2003. Cyclophilins and the antimalarial activity of cyclosporin A. PhD thesis. University of Dundee).

All three *P. falciparum* cyclophilins are expressed at the mRNA level in erythrocytic parasites (Hirtzlin et al., 1995; Reddy, 1995; Bozdech et al., 2003; Le Roch et al., 2003): interestingly, *Pfcyp24* mRNA is highest in the immature (ring) stages, *Pfcyp19A* mRNA in the middle of the erythrocytic cycle and *Pfcyp19B* mRNA in the more mature (late trophozoite/schizont) stages. If these transcript levels are reflected in protein levels (which has been confirmed for PfCYP19B: