Title: FKBPs in bacterial infections

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ABSTRACT

BACKGROUND: FK506-binding proteins (FKBPs) contain a domain with peptidyl-prolyl-*cis/trans*-isomerase (PPIase) activity and bind the immunosuppressive drugs FK506 and rapamycin. FKBPs belong to the immunophilin family and are found in eukaryotes and bacteria.

SCOPE OF REVIEW: In this review we describe two major groups of bacterial virulence-associated FKBPs, the trigger factor and Mip-like PPIases. Moreover, we discuss the contribution of host FKBPs in bacterial infection processes.

MAJOR CONCLUSIONS: Since PPIases are regarded as alternative antiinfective drug targets we highlight current research strategies utilizing pipecolinic acid and cycloheximide derivatives as well as substrate based inhibitors.

GENERAL SIGNIFICANCE: The current research strategies suggest a beneficial synergism of drug development and basic research.

1. Introduction

Other than their eukaryotic homologs, bacterial peptidyl-prolyl-*cis/trans*-isomerases (PPIases) are mainly analyzed under the perspective of protein secretion, and in the case of Gram-negative bacteria also in the context of outer membrane protein assembly (Behrens-Kneip, 2010; Schiene-Fischer et al., 2011; Unal and Steinert, 2014). As chaperones and foldases PPIases are involved in diverse protein-protein interactions that are short lived, difficult to entangle, and pleiotropic in their biological impact on bacterial physiology. Hence, it is not surprising that PPIases also very often correlate with virulence in pathogenic bacteria (Alonzo et al., 2009; Behrens-Kneip, 2010; Moro et al., 1995; Obi et al., 2011). This has become even more evident since the identification of macrophage infectivity potentiator (Mip) of *Legionella pneumophila*, as the first virulence-associated PPIase, and has opened the way to the analysis of bacterial FKBPs under the aspect infections with implications in alternative drug design strategies (Cianciotto et al., 1989; Fischer et al., 1992, Ünal et al., 2011; Ünal and Steinert, 2014).

Among bacterial FKBPs two major groups of homologs are reported to be involved in infectious processes in many pathogens: trigger factor (TF) and Mip-like FKBPs. While TF rather acts in an accessory way by most probably influencing the maturation of other virulence factors, Mip-like PPIases can act as accessors or as in the case of the Mip-protein of *L.pneumophila* directly, hence, as virulence factors. Besides that, the contribution of single PPIases to infection processes is very species specific and the phenotypes they are related to are very diverse.

2. Bacterial virulence-associated FKBPs

2.1. Trigger Factor

Trigger factor can be considered as an essential FBKP in bacterial kingdom, since even *Mycoplasma genitalium*, the free-living bacterium with the smallest genome, possesses

TF as its sole PPIase (Bang et al., 2000). Although its deletion is not deleterious under laboratuary conditions, a double knockout of *tig*, and *dnaK*, encoding for TF and the major cytoplasmic chaperone, respectively, results in synthetic lethality above 30 °C in *E. coli* (Deuerling et al., 1999; Teter et al., 1999). In TF the central FKBP domain is flanked by an N-terminal ribosome binding domain and a C-terminal domain with chaperone activity (Merz et al., 2006). The N-terminal domain facilitates the association with ribosomes in a 1:1 ratio. Here TF gets into contact with newly forming proteins mainly via hydrophobic interactions by the involvement of all three domains, and remains attached to the polypeptides as the elongation proceeds even after leaving the ribosome. Hence, multiple TF proteins assist during maturation of newly translated proteins (Hoffmann et al., 2010; Kramer et al., 2004; Lakshmipathy et al., 2010; Saio et al., 2014). Since, during translation nascent polypeptides exit the ribosomes in an unfolded state with isomerization prone peptidyl-prolyl bonds, the participation of TF has initially been considered as the ultimate proof for an *in vivo* PPIase function. But although even the binding of the PPIase domain to nascent peptides could be demonstrated, this property was not dependent on the enzymatic activity, and furthermore, needed assistance by the N- and C-terminal domains (Kramer et al., 2004; Lakshmipathy et al., 2007).

Virulence association of is TF until now only reported in Gram-positive pathogens. In the dental pathogen *Streptococcus mutans* causing dental caries, deletion of the TF homolog RopA influenced the differential expression of about 33 proteins. Among the 22 up-regulated proteins were the general chaperones DnaK, GroEL and GroES most probably in order to compensate for the loss of the chaperoning activity of TF. Interestingly, several glycosyltransferase genes were downregulated that participate in the production of glucans, and mediate cell-cell and cell-surface adhesion. Hence, in the presence of glucose as the only carbon source *in vitro* biofilm formation decreased in the RopA-negative mutant. Besides this,

further virulence associated properties like genetic competence, acid and peroxide tolerance were negatively affected by the deletion of *ropA* (Wen et al., 2005).

Pleiotropic effects on virulence of TF were also observed in the animal pathogen *S. suis*, and the intestinal human pathogen *L. monocytogenes*. In *S. suis* several virulence phenotypes including adherence to host cells, hemolytic activity, resistance to several stress factors and virulence in the CD1 mice infection model were affected negatively (Wu et al., 2011). In *L. monocytogenes* in the absence of TF the bacteria forfeited their tolerance towards heat and ethanol as well as persisted to a lesser degree in the organs of infected mice. However, the molecular efectors that influence these phenotypes and depend on TF activity are not known. Furthermore, the extent of contribution of the PPIase activity of TF still needs additional evaluation (Bigot et al., 2006).

The clearest relationship between the activity of a virulence factor and the PPIase action of a TF could be shown in *S. pyogenes*. Streptococcal pyogenic exotoxin B (SpeB), a secreted cysteine proteinase, has diverse implications in the extracellular virulence of this pathogen. Its secretion and processing is dependent on a functional RopA as it was shown by deleting the PPIase domain or site directed PPIase-deficient mutants of RopA (Lyon and Caparon, 2003; Lyon et al., 1998). In accordance with this, RopA interacts with SpeB via a proline residue at position 78 of SpeB, which is thought to keep the preproprotease in a secretion-competent conformation until it reaches the general secretory pathway (Lyon and Caparon, 2003).

2.2. Mip-like PPIases of bacterial pathogens

The much more thoroughly analyzed group of virulence associated bacterial FKBPs comprises Mip and Mip-like PPIases. The name giving representative of this group of FKBPs was initially identified as the first virulence factor of *L. pneumophila* that affects the replication of this intracellular pathogen in macrophages, and was soon characterized as a

PPIase (Cianciotto and Fields, 1992; Cianciotto et al., 1989; Fischer et al., 1992). Mip-like PPIases are generally spoken virulence-associated, secreted and typically outer membrane localized FKBPs of many Gram-negative bacteria.

Many reports on Mip-like PPIases are restricted to the identification of the protein or the gene in a pathogen, and very often limited to the description of some phenotypes observed in isogenic gene deletion mutants. Usually, the molecular mechanisms that connect a Mip-like PPIase to the certain phenotypes remain unsolved. Nevertheless, the exact role of this protein family during infection is now successively being unraveled beginning with *L. pneumophila, Burkholderia pseudomallei*, and some other pathogens.

The Mip-like PPIase of the obligate intracellular pathogen *Chlamydia trachomatis* is a lipoprotein that is located on elementary bodies (Lundemose et al., 1993a; Neff et al., 2007). Inhibiting its PPIase activity with FK506 results in irregularities during inclusion body formation inside the host cell together with reduced infectivity (Lundemose et al., 1993b). Surface-exposed Mip-like proteins were also found in the important human pathogens *Neisseria gonorrhoeae* and *N. meningitidis* where they mediate persistence in macrophages or improve bacterial survival in the blood, respectively (Echenique-Rivera et al., 2011; Leuzzi et al., 2005). In the caries-associated periodontal pathogen *Aggregatibacter actinomycetemcomitans* a Mip-like protein was found to be up-regulated in primary isolates. Also, deletion of the gene caused significant reduction in the efficiency of the bacteria to invade HeLa cells (Maeda et al., 2010).

In the plant pathogen *Xanthomonas campestris* that causes black rot on all cultivated brassicas a Mip-like PPIase was found to be in the periplasm (Mansfield et al., 2012; Zang et al., 2007). It was shown to influence exopolysaccharide production, an important colonization factor. In addition, the mutant elicited smaller lesions on infected leaves, probably caused by the inefficient transport of the type II secreted extracellular protease PrtA (Meng et al., 2011).

Also, protozoan parasites like *T. cruzi* and *L. infantum* were shown to possess Mip-like PPIases that are secreted into the culture supernatant (Debroy et al., 2006; Moro et al., 1995). In *T. cruzi*, Mip-like protein facilitates invasion of mammalian epithelial cells (Moro et al., 1995). Interestingly, the degree of structural similarity between TcMip and Mip protein of *L. pneumophila* enables a partial compensation of the invasion deficiency of Mip-deficient *T cruzi* by recombinant *Legionella*-Mip protein (Pereira et al., 2002).

2.2.1. Mip of *Legionella pneumophila*

Mip was the first genetically identified virulence factor of *L. pneumophila* shown to be relevant for intracellular infection. Deletion of the genetic locus harboring *mip* caused reduced intracellular replication rates in human alveolar macrophages and protozoa, leading to the name **m**acrophage **i**nfectivitiy **p**otentiator (Cianciotto and Fields, 1992; Cianciotto et al., 1989; Engleberg et al., 1989). The subsequent finding that Mip is a FKBP-type PPIase was novel within this protein family (Fischer et al., 1992).

Mip is a 24 kDa basic protein which exists as a homodimer on the outer membrane of the bacteria (Helbig et al., 2001; Schmidt et al., 1994). Besides an N-terminal signal sequence that is cleaved off during transport across the cytoplasmic membrane, Mip reveals three structurally and functionally different regions: an N-terminal domain consisting of two α-helices, a long connecting α-helix in the center and a C-terminal FKBP domain (Ceymann et al., 2008; Horstmann et al., 2006; Riboldi-Tunnicliffe et al., 2001). The globular N-terminal domain facilitates the dimerization via a methionine knot. This dimerization mechanism via the mirror image like interaction of hydrophobic amino acid pairs is typical for dimeric bacterial PPIases, and is repeated as a Leu-Val knot in FkpA of *E. coli* and a Val-Leu knot in FKBP22 of *Shewanella* sp. SIB1 (Budiman et al., 2009).

The connecting α-helix between the N- and C-termini is of unknown function with probable important roles regarding protein interactions and virulence. In NMR studies this helix revealed remarkable structural flexibility while the helical structure is sustained (Horstmann et al., 2006). Thus, it is possible that this helix confers Mip a certain flexibility that allows binding different substrates as a dimer. The C-terminal domain has the typical globular β-fold structure of FKBPs (Riboldi-Tunnicliffe et al., 2001).

The contribution of the PPIase activity to infection processes is still not proven to its full extent. When the highly conserved residues Asp142 and Tyr185 were exchanged by a leucine and by an alanine residue, respectively, in complementation studies no substantial difference to the wild type bacteria could be observed. In contrast, PPIase activities of the purified recombinant proteins were dramatically dimished in *in vitro* peptide folding assays (5.3 % for Asp142Leu and 0.6 % Tyr185Ala) (Wintermeyer et al., 1995). Hence, either additional properties other than PPIase activity are important for Mip action during intracellular infection, or the residual enzymatic activity of the mutant proteins still allowed exerting PPIase dependent phenotypes.

Furtheron, a variant, which was generated by truncating Mip in the middle of the long connecting α-helix yielding a monomeric C-terminal variant containing the FKBP domain, and a variant containing the N-terminal dimerization domain were enzymatically tested. While the monomer retained its enzymatic activity in peptide folding assays, in protein folding experiments with denatured RCM-T_1 as substrate, a significant loss in activity was observed (Köhler et al., 2003). Interestingly, in cell culture and guinea pig infections complementing the mutant with monomeric Mip reverted the phenotype only to a limited degree. Moreover, the Tyr185Ala mutant with 1-2 % PPIase activity also failed to complement the phenotype back to wild type levels. Hence, infection studies suggest that for complete Mip action *in vivo*, the dimeric nature of the protein is substantial, and that the

PPIase and the N-terminal accessory chaperone activity, which is not yet in detail described, need to act in a concerted fashion (Köhler et al., 2003).

Mip promotes systemic infection as demonstrated in guinea pigs where Mip-defficient bacteria cannot disseminate within the lung tissue and to the spleen (Cianciotto et al., 1990; Wagner et al., 2007). This is indicative of a role of Mip in the extracellular virulence of *L. pneumophila*. Since within the lung the bacteria are mainly confronted with epithelial cells and the components of the extracellular matrix (ECM), single ECM components were tested for Mip-binding. Collagens were found to be the main target for Mip. Among the potential collagens Mip primarily binds to collagen IV (Wagner et al., 2007). This facilitates the interaction with lung epithelial cells and promotes the *in vitro* transmigration of the bacteria across an epithelial cell barrier. The involvement of the PPIase active site could be shown by rapamycin inhibition, and the less pronounced binding of the Asp142Leu and Tyr185Ala substitution mutantsto collagen IV (Wagner et al., 2007).

In human collagen IV Mip targets a specific sequence within the globular non-collagenous NC1 domain of the α1 isomer of collagen IV (Uenal et al., 2011). According to the published crystal structure of the NC1 domain (pdb 1LI1) this 13-mer peptide (IPPCPSGWSSLWI, named P290) is completely localized on the surface. Competitive binding to immobilized collagen IV, co-precipitation studies using rapamycin as inhibitor, and molecular docking models generated according to NMR measurements of the complex of the C-terminal Mip fragment with P290 in solution confirmed that P290 binds to the PPIase active domain of Mip. Computer models generated from NMR measurements and simulations of the Mip dimer-NC1 complex over 40 ns further suggest that Mip rather acts like an adhesin binding its receptor making it a moonlighting protein (Ünal et al., 2011).

2.2.2. Mip-like PPIase of *Burkholderia pseudomallei* (BpML1)

The genome of *B. pseudomallei* encodes for at least two FKBPs, BpML1 and BPSL0918. Although BPSL0918 lacks any measurable PPIase activity due to mutation of several key residues in the enzymatic cavity, its deletion has severe effects on the intracellular replication capacity of the bacteria. It is suggested that BPSL0918 functions as a chaperone with probable impact on outer membrane integrity and stress tolerance with implications on virulence (Norville et al., 2011a, 2011b).

In contrast, BpML1 has PPIase activity, and is the second most thoroughly analyzed Mip-like PPIase. Other than Mip, BpML1 does not have a long α-helix and the N-terminal dimerization domain. Also, localization to the outer membrane or secretion is not reported. BpML1 is connected to a multitude of virulence-related phenotypes, including resistance to low pH, survival in macrophage-like and epithelial cells, swarming due to flagellation and extracellular protease activity. Additionally, a BpML1-deficient mutant was significantly attenuated in a mouse infection model (Norville et al., 2011a). Whether BpML1 directly participates in the outcome of the infection or rather as a mediator via other virulence factors needs further evaluation.

The crystal structure of BpML1 revealed high analogy to the FKBP-domain of *Legionella*-Mip. Due to the high structural resolution of 0.91 Å the authors were able to observe pronounced flexibility of the crucial active side residues Asp44 and Tyr89 (corresponding to Asp142 and Tyr185 in Mip, respectively). This structural flexibility could provide BpML1 some functional flexibility regarding its substrate spectrum. Interestingly, in the crystal structure the purification tag of one protein molecule underwent close interaction with the active site of the next protein molecule. As in case of the P290-Mip complex, the hydrophobic pocket was not occupied by the proline within this tag, but by a preceding valine residue in a manner that is not usual for known peptide-PPIase interactions (Norville et al., 2011c).

3. Host FKBPs in bacterial infection processes

Host PPIases, especially cyclophilins, are very well known to be part of viral infections (reviewed in Frausto et al., 2013; Liu et al., 2013). Furtheron, the connection between several eukaryotic PPIases like FKBP12 and immunomodulation was one of the key discoveries regarding this protein family (Fischer et al., 1989; Harding et al., 1989; Siekierka et al., 1989; Takahashi et al., 1989). Nevertheless, the role of host PPIases during bacterial infections has not attracted much attention. Accordingly, reports on this topic are rather limited. Most of the findings indicate that, as it is the case for viral infections, cyclophilins play a more dominant role in bacterial infections as mediators or targets of bacterial effectors (reviewed in Ünal and Steinert, 2014), and only few examples of host FKBPs are described in the context of bacterial infections.

In a meta-analysis study, where 17 microarray studies on plant-pathogen interactions were screened for the changes in the expression profiles of PPIases of *Arabidopsis thaliana*, *At*FKBP65 had the most pronounced over-expression rate after pathogen challenge (Pogorelko et al., 2014). *At*FKBP65 together with the cyclophilins *At*Cyp19 and *At*Cyp57 was shown to be expressed at the site of infection, and promote callose accumulation forming a physical barrier against the invaders. While deletion of the genes resulted in higher susceptibility towards *Pseudomonas syringae*, transgenic expression of the same genes in tobacco plants provided increased resistance (Pogorelko et al., 2014). How *At*FKBP65 and other cyclophilins exert their immunomodulatory actions is not known in detail. But in case of *At*FKBP65 a direct involvement in gene expression can be speculated, since this modular PPIase is found in the plant cell nucleus, and has tetratricopeptide and calmodulin-binding motifs that mediate protein-protein interactions linked to Ca^{2+}-signalling (He et al., 2004; Pogorelko et al., 2014).

A very interesting example of host PPIase contribution has been described for the translocation of bacterial binary toxins that consist of a receptor subunit and an enzymatically active subunit exerting toxic activity. The receptor subunit mediates binding and the subsequent endocytic uptake of the binary toxin complex into the host cells. Upon acidification of the endosome, the receptor domain changes its conformation, and forms a translocation pore for the enzymatically active subunit. This translocated subunit exerts the actual toxic activity by modifying host proteins. In case of clostridial binary toxins, like the C2 toxin of *C. botulinum*, iota toxin of *C. perfringens* and CDT of *C. difficile*, this modification is ADP-ribosylation of host G-actin that results in actin depolymerization and cytotoxicity (Aktories et al., 1986; Barth et al., 2004). Initially, a protective effect of CsA against intoxication binary toxins was observed (Dmochewitz et al., 2011). This, in the following, led to the identification human CypA and its multidomain homolog Cyp40 as interaction partners of these toxins which also facilitate their translocation from acidified endosomes into the host cell cytosol (Ernst et al., 2014; Kaiser et al., 2011). In a subsequent study, by using the toxic C2I subunit of botulinum toxin in co-precipitation experiments, FKBP51 was identified as another PPIase capable of binding binary toxins and promoting their translocation into the cytosol. This action was sensitive to FK506 suggesting that the PPIase domain is involved in the interaction of both proteins. Interestingly, FKBP51 was able to bind denatured C2I more efficiently, which, in addition, hints at a foldase action of this FKBP on a bacterial effector. While FK506 was able to delay the intoxification of the cells by binary toxins, it could not protect the cells against the large Rho-glucoslysating toxin of *C. difficile*, again indicating that the PPIase involvement is specific to binary toxins (Kaiser et al., 2012). As the authors of the study suggest, it still remains to be solved whether host PPIases facilitate the translocation of bacterial binary toxins via their enzymatic activity, or rather their chaperone-like interaction. Nevertheless, the observed pharmacological effects of

known PPIase inhibitors encourage the search for non-immunosuppresive drugs that can be applied in toxin-associated diseases (Ernst et al., 2014; Kaiser et al., 2012).

4. Targeting Mip-like PPIases

In the case of most Mip-like PPIases understanding their exact modes of action during infection still needs extensive research. Nevertheless, their noticeable association with pathogenic microorganisms predestines this distinct group of virulence factors as an attractive drug target. One obstacle that may complicate the design of inhibitors of Mip-like PPIases is the high degree of sequence and structural similarity among bacterial and host FKBPs that may result in contra-productive immunomodulatory side effects. Hence, high discriminatory specificity towards Mip-like PPIases should be a major goal. One advantage in this respect is that FKBP12 alone, and in complex with FK506 as well as several of its derivatives are structurally well characterized (Babine and Bender, 1997; Van Duyne et al., 1991). Furthermore, crystallographic and NMR-data of Mip, and BpML1 enable the design of specific inhibitors on the basis of distinguishing structural features (Ceymann et al., 2008; Norville et al., 2011c; Riboldi-Tunnicliffe et al., 2001). Hence, the dilemma with structural conservation among FKBPs can be overcome by the integration of information derived from structural data. Another advantage is that research on non-immunostimulatory derivatives of FK506 and rapamycin has lead to the design of a plethora of alternative inhibitory structures (reviewed in Ünal and Steinert, 2014) that have the potential to serve as lead compounds specific to one or many bacterial FKBPs. Also where possible, characterization of PPIase-protein interactions may allow the discovery of novel and specific inhibitors. Accordingly, research of the past years has revealed derivatives of pipecolinic acid, and cycloheximide as well as substrate based inhibitors as three types of potential lead structures for inhibitors of Mip-like PPIases (Fig. 1).

4.1. Pipecolinic acid derivatives

The natural inhibitors of FKBPs, the macrolides FK506 and rapamycin, interact with the enzymatically active hydrophobic cavity of these via a “binding domain”, which contains a pipecolinyl ring that mimics a peptidyl-prolyl bond (Van Duyne et al., 1991, 1993). The remaining parts of the macrolides stick out, and are solvent exposed. This creates a unique “effector domain” that enables FKBP12 to bind to calcineurin or mTOR (mammalian target of rapamycin) when it is in complex with FK506 or rapamycin, respectively, leading to the immuno-modulatory side effects of these drugs (Babine and Bender, 1997; Chiu et al., 1994; Liu et al., 1991; Sabers et al., 1995). The bi-functional nature of FK506 easily allowed the design of drugs with only PPIase inhibitory properties. As a result the core of the binding domain of FK506 and rapamycin consisting of the pipecolinyl ring and the keto amide group, which interact with the hydrophobic pocket of FKBPs, were kept as the scaffold for acyclic derivatives (Fig. 1A).

Recently, small molecule inhibitors based on this core inhibitory structure were tested on Mip and BpML1 (Begley et al., 2014; Juli et al., 2011). In order to find novel inhibitors of Mip, two previously reported pipecoline ring containing inhibitors A and B with $K_{i\ (app)}$ of 10 nM and 0.23 μM, respectively, were used as lead structures. While structure A contained the keto amide moiety, in structure B this group was replaced by a sulfonamide anchor (Holt et al., 1993, 1994). Molecular docking studies revealed that structure A fitted less well to Mip than to the human homolog FKBP12. The *(S)*-enantiomer of structure B containing the less bulky sulfonamide fitted much better into the hydrophobic pocket of Mip (Fig. 1A) (Juli et al., 2011).

Among several derivatives of each compound, none of structure A inhibited Mip in line with the predictions of the molecular docking analyses. In contrast, structure B inhibited Mip at low micromolar concentrations. Here the stereochemistry seemed to influence the

activity, since a pure *(S)*-enantiomer of structure B (S-4c) had a slightly better inhibitory activity towards Mip than its racemic mixture 4c (IC_{50} 6 μM vs. 9 μM). Also, substituting the extended aromatic group, phenyl sulfonamide, by benzyl sulfonamide reduced the inhibitory potential about 4 times. Interestingly, treating U397 macrophage-like cells that were infected with *L. pneumophila* with 50 μM S-4c had no detrimental effect on the infection. The authors concluded from this that properties other than the PPIase activity of the Mip protein determined its virulence characteristics (Juli et al., 2011).

In a very recent study further similar pipecolic acid derivatives with sulfonamide anchors replacing the ketoamide functionality were tested against BpML1 and K_I values between 10 μM and 0.17 μM were achieved while again a stereospecificity could be observed. All but one of the tested substances were also able to prevent *B. mallei*-induced cytotoxicity in J744A.1 macrophage cells. However, here again no strict correlation to the PPIase inhibitory activity could be drawn (Begley et al., 2014).

4.2. Cycloheximide derivatives

Cycloheximide, a known inhibitor of eukaryotic protein biosynthesis, was identified by screening a compound library containing structurally diverse secondary metabolites for novel FKBP12 inhibitors (Christner et al., 1999). The authors generated derivatives of this new lead structure with an IC_{50} of 3.6 μM in order to reduce its cytotoxic properties. As a result cycloheximide-*N*-ethylethanoate (Fig. 1B) was identified as the most promising candidate since it was about 200 times less cytotoxic without losing its inhibitory capacity (IC_{50} 4.4 μM). The same derivative was later on reported to be a potent inhibitor of the Mip-like PPIase BpML1 of *B. pseudomallei* (Norville et al., 2011c). NMR measurements showed that the inhibitor interacted with BpML1 in a similar way as it interacts with a probable protein substrate. Although the inhibitor was not tested in infection studies, this study points out the value of existing structures valuable lead structures for new targets.

4.3. Substrate based peptide inhibitors

Substrate based inhibitors require the identification of *in vivo* interaction partners of a particular FKBP, which can be a challenging task. But, in return this holds the potential of designing very specific inhibitors that ideally target crucial virulence-related interactions. Currently, with P290 there is only one example of this approach in regard to bacterial virulence-associated PPIases. The peptide-protein interaction of the 13-mer peptide P290 (IPPCPSGWSSLWI) that was identified as the Mip binding sequence within human collagen IV was evaluated by NMR measurements (Ünal et al., 2011). Molecular docking studies derived form these measurements suggest that P290 interacts over its full length with Mip, and that the catalytic cleft is occupied by the central tryptophan and proline residues. The reamining of P290 forms a hairpin-like structure, and aligns along both sides of the topological outside of the catalytically active, hydrophobic pocket (Fig. 1C). This conformation is stabilized by several intermolecular hydrogen bonds between P290 and Mip along the interaction surface. The specificity of this interaction was confirmed by further docking studies, where a possible complex between P290 and human FKBP12 was modelled. The average B-factor, reflecting the degree of stability of protein complexes by measuring the flexibility of each amino acid residue, was calculated as 59.08 or 200 for P290 in complex with Mip or FKBP12, respectively. In the P290-Mip complex the residues Pro131 and Leu137 were the most stable positions with B-factors ranging around 30 (Ünal et al., 2011).

In order to improve binding of P290 to Mip, single amino acid substitutions, cyclization of P290, and using D-amino acids for peptide synthesis were chosen as possible strategies. Interestingly, Mip bound none of the P290-variants composed of D-amino acids indicating that there is stereoselectivity. As feasible positions for amino acid variations Cys4 and Trp8 were picked. Cys4 occupies the discriminating amino acid position P_1 with respect to the central Pro5, which is the proline residue closest to the enzymatically active site. Trp8

was chosen because it mainly interacts with the enzymatic cleft. Among the tested P290 variants the ones containing either a Cys4Leu, or a Trp8Phe substitution showed improved binding capacity with 2 and 1.8-fold better Mip binding, respectively. Basing on this, new variants were screened by molecular docking simulations, and interestingly, revealed that the stability of the complex is even higher when Pro5 instead of Cys4 is replaced by a leucine residue (Ünal et al., 2011).

Furtheron, cyclization of P290 by replacing the terminal isoleucine residues with cysteines achieved a 2-fold improvement. Whereas a shorter cyclic version containing only the core residues 5PSGW8 flanked by two cysteines, bound Mip 95 % less efficiently than P290. All these results helped to postulate that an optimized P290 variant should be cyclic, approximately 13 amino acids long, and optimized at positions 5 and 8. Accordingly, a new cyclic variant of P290 (cP290, CPPC**L**SG**F**SSLWC) was produced, and tested in a molecular docking simulation and in *in vitro* bacterial transmigration assays. The calculated average B-factor of cP290 was improved by 5 %, and its solvent exposed surface was reduced by 7 % indicating that its interaction with Mip is stronger. Also, cP290 exhibited a higher prohibitory effect on bacterial transmigration across an epithelial cell barrier.

5. Conclusions

The objective of this review was to summarize our current understanding of FKBPs during bacterial infections. Although much has been learned about the importance of FKBPs during a plethora of biological processes, the exact mechanisms responsible for the observed phenotypes remain largely elusive. Since FKBPs are regarded as alternative antiinfective drug targets and several inhibitory lead structures are being developed, this might result in a beneficial synergism of drug development and basic research in the future.

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References

Aktories, K., Bärmann, M., Ohishi, I., Tsuyama, S., Jakobs, K.H., and Habermann, E. (1986). Botulinum C2 toxin ADP-ribosylates actin. Nature *322*, 390–392.

Alonzo, F., 3rd, Port, G.C., Cao, M., and Freitag, N.E. (2009). The posttranslocation chaperone PrsA2 contributes to multiple facets of *Listeria monocytogenes* pathogenesis. Infect. Immun. *77*, 2612–2623.

Babine, R.E., and Bender, S.L. (1997). Molecular Recognition of Proteinminus signLigand Complexes: Applications to Drug Design. Chem. Rev. *97*, 1359–1472.

Bang, H., Pecht, A., Raddatz, G., Scior, T., Solbach, W., Brune, K., and Pahl, A. (2000). Prolyl isomerases in a minimal cell. Catalysis of protein folding by trigger factor from *Mycoplasma genitalium*. Eur. J. Biochem. FEBS *267*, 3270–3280.

Barth, H., Aktories, K., Popoff, M.R., and Stiles, B.G. (2004). Binary bacterial toxins: biochemistry, biology, and applications of common *Clostridium* and *Bacillus proteins*. Microbiol. Mol. Biol. Rev. MMBR *68*, 373–402, table of contents.

Begley, D.W., Fox, D., Jenner, D., Juli, C., Pierce, P.G., Abendroth, J., Muruthi, M., Safford, K., Anderson, V., Atkins, K., et al. (2014). A structural biology approach enables the development of antimicrobials targeting bacterial immunophilins. Antimicrob. Agents Chemother. *58*, 1458–1467.

Behrens-Kneip, S. (2010). The role of SurA factor in outer membrane protein transport and virulence. Int. J. Med. Microbiol. IJMM *300*, 421–428.

Bigot, A., Botton, E., Dubail, I., and Charbit, A. (2006). A homolog of *Bacillus subtilis* trigger factor in *Listeria monocytogenes* is involved in stress tolerance and bacterial virulence. Appl. Environ. Microbiol. *72*, 6623–6631.

Budiman, C., Bando, K., Angkawidjaja, C., Koga, Y., Takano, K., and Kanaya, S. (2009). Engineering of monomeric FK506-binding protein 22 with peptidyl prolyl cis-trans isomerase. Importance of a V-shaped dimeric structure for binding to protein substrate. FEBS J. *276*, 4091–4101.

Ceymann, A., Horstmann, M., Ehses, P., Schweimer, K., Paschke, A.-K., Steinert, M., and Faber, C. (2008). Solution structure of the *Legionella pneumophila* Mip-rapamycin complex. BMC Struct. Biol. *8*, 17.

Chiu, M.I., Katz, H., and Berlin, V. (1994). RAPT1, a mammalian homolog of yeast Tor, interacts with the FKBP12/rapamycin complex. Proc. Natl. Acad. Sci. U. S. A. *91*, 12574–12578.

Christner, C., Wyrwa, R., Marsch, S., Küllertz, G., Thiericke, R., Grabley, S., Schumann, D., and Fischer, G. (1999). Synthesis and cytotoxic evaluation of cycloheximide derivatives as potential inhibitors of FKBP12 with neuroregenerative properties. J. Med. Chem. *42*, 3615–3622.

Cianciotto, N.P., and Fields, B.S. (1992). *Legionella pneumophila mip* gene potentiates intracellular infection of protozoa and human macrophages. Proc. Natl. Acad. Sci. U. S. A. *89*, 5188–5191.

Cianciotto, N.P., Eisenstein, B.I., Mody, C.H., Toews, G.B., and Engleberg, N.C. (1989). A *Legionella pneumophila* gene encoding a species-specific surface protein potentiates initiation of intracellular infection. Infect. Immun. *57*, 1255–1262.

Cianciotto, N.P., Eisenstein, B.I., Mody, C.H., and Engleberg, N.C. (1990). A mutation in the mip gene results in an attenuation of *Legionella pneumophila* virulence. J. Infect. Dis. *162*, 121–126.

Debroy, S., Aragon, V., Kurtz, S., and Cianciotto, N.P. (2006). *Legionella pneumophila* Mip, a surface-exposed peptidylproline cis-trans-isomerase, promotes the presence of phospholipase C-like activity in culture supernatants. Infect. Immun. *74*, 5152–5160.

Deuerling, E., Schulze-Specking, A., Tomoyasu, T., Mogk, A., and Bukau, B. (1999). Trigger factor and DnaK cooperate in folding of newly synthesized proteins. Nature *400*, 693–696.

Dmochewitz, L., Lillich, M., Kaiser, E., Jennings, L.D., Lang, A.E., Buchner, J., Fischer, G., Aktories, K., Collier, R.J., and Barth, H. (2011). Role of CypA and Hsp90 in membrane translocation mediated by anthrax protective antigen. Cell. Microbiol. *13*, 359–373.

Van Duyne, G.D., Standaert, R.F., Karplus, P.A., Schreiber, S.L., and Clardy, J. (1991). Atomic structure of FKBP-FK506, an immunophilin-immunosuppressant complex. Science *252*, 839–842.

Van Duyne, G.D., Standaert, R.F., Karplus, P.A., Schreiber, S.L., and Clardy, J. (1993). Atomic structures of the human immunophilin FKBP-12 complexes with FK506 and rapamycin. J. Mol. Biol. *229*, 105–124.

Echenique-Rivera, H., Muzzi, A., Del Tordello, E., Seib, K.L., Francois, P., Rappuoli, R., Pizza, M., and Serruto, D. (2011). Transcriptome analysis of *Neisseria meningitidis* in human whole blood and mutagenesis studies identify virulence factors involved in blood survival. PLoS Pathog. *7*, e1002027.

Engleberg, N.C., Carter, C., Weber, D.R., Cianciotto, N.P., and Eisenstein, B.I. (1989). DNA sequence of *mip*, a *Legionella pneumophila* gene associated with macrophage infectivity. Infect. Immun. *57*, 1263–1270.

Ernst, K., Langer, S., Kaiser, E., Osseforth, C., Michaelis, J., Popoff, M.R., Schwan, C., Aktories, K., Kahlert, V., Malesevic, M., et al. (2014). Cyclophilin-Facilitated Membrane Translocation as Pharmacological Target to Prevent Intoxication of Mammalian Cells by Binary Clostridial Actin ADP-Ribosylated Toxins. J. Mol. Biol.

Fischer, G., Wittmann-Liebold, B., Lang, K., Kiefhaber, T., and Schmid, F.X. (1989). Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. Nature *337*, 476–478.

Fischer, G., Bang, H., Ludwig, B., Mann, K., and Hacker, J. (1992). Mip protein of *Legionella pneumophila* exhibits peptidyl-prolyl-cis/trans isomerase (PPIase) activity. Mol. Microbiol. *6*, 1375–1383.

Frausto, S.D., Lee, E., and Tang, H. (2013). Cyclophilins as modulators of viral replication. Viruses *5*, 1684–1701.

Harding, M.W., Galat, A., Uehling, D.E., and Schreiber, S.L. (1989). A receptor for the immunosuppressant FK506 is a cis-trans peptidyl-prolyl isomerase. Nature *341*, 758–760.

He, Z., Li, L., and Luan, S. (2004). Immunophilins and parvulins. Superfamily of peptidyl prolyl isomerases in Arabidopsis. Plant Physiol. *134*, 1248–1267.

Helbig, J.H., Lück, P.C., Steinert, M., Jacobs, E., and Witt, M. (2001). Immunolocalization of the Mip protein of intracellularly and extracellularly grown *Legionella pneumophila*. Lett. Appl. Microbiol. *32*, 83–88.

Hoffmann, A., Bukau, B., and Kramer, G. (2010). Structure and function of the molecular chaperone Trigger Factor. Biochim. Biophys. Acta *1803*, 650–661.

Holt, D.A., Luengo, J.I., Yamashita, D.S., Oh, H.J., Konialian, A.L., Yen, H.K., Rozamus, L.W., Brandt, M., and Bossard, M.J. (1993). Design, synthesis, and kinetic evaluation of high-affinity FKBP ligands and the X-ray crystal structures of their complexes with FKBP12. J Am Chem Soc *115*, 9925–9938.

Holt, D.A., Konialian-Beck, A.L., Oh, H.-J., Yen, H.-K., Rozamus, L.W., Krog, A.J., Erhard, K.F., Ortiz, E., Levy, M.A., Brandt, M., et al. (1994). Structure-activity studies of synthetic FKBP ligands as peptidyl-prolyl isomerase inhibitors. Bioorg. Med. Chem. Lett. *4*, 315–320.

Horstmann, M., Ehses, P., Schweimer, K., Steinert, M., Kamphausen, T., Fischer, G., Hacker, J., Rösch, P., and Faber, C. (2006). Domain motions of the Mip protein from *Legionella pneumophila*. Biochemistry (Mosc.) *45*, 12303–12311.

Juli, C., Sippel, M., Jäger, J., Thiele, A., Weiwad, M., Schweimer, K., Rösch, P., Steinert, M., Sotriffer, C.A., and Holzgrabe, U. (2011). Pipecolic acid derivatives as small-molecule inhibitors of the *Legionella* MIP protein. J. Med. Chem. *54*, 277–283.

Kaiser, E., Kroll, C., Ernst, K., Schwan, C., Popoff, M., Fischer, G., Buchner, J., Aktories, K., and Barth, H. (2011). Membrane translocation of binary actin-ADP-ribosylating toxins from *Clostridium difficile* and *Clostridium perfringens* is facilitated by cyclophilin A and Hsp90. Infect. Immun. *79*, 3913–3921.

Kaiser, E., Böhm, N., Ernst, K., Langer, S., Schwan, C., Aktories, K., Popoff, M., Fischer, G., and Barth, H. (2012). FK506-binding protein 51 interacts with *Clostridium botulinum* C2 toxin and FK506 inhibits membrane translocation of the toxin in mammalian cells. Cell. Microbiol. *14*, 1193–1205.

Köhler, R., Fanghänel, J., König, B., Lüneberg, E., Frosch, M., Rahfeld, J.-U., Hilgenfeld, R., Fischer, G., Hacker, J., and Steinert, M. (2003). Biochemical and functional analyses of the Mip protein: influence of the N-terminal half and of peptidylprolyl isomerase activity on the virulence of *Legionella pneumophila*. Infect. Immun. *71*, 4389–4397.

Kramer, G., Patzelt, H., Rauch, T., Kurz, T.A., Vorderwülbecke, S., Bukau, B., and Deuerling, E. (2004). Trigger factor peptidyl-prolyl cis/trans isomerase activity is not essential for the folding of cytosolic proteins in *Escherichia coli*. J. Biol. Chem. *279*, 14165–14170.

Lakshmipathy, S.K., Tomic, S., Kaiser, C.M., Chang, H.-C., Genevaux, P., Georgopoulos, C., Barral, J.M., Johnson, A.E., Hartl, F.U., and Etchells, S.A. (2007). Identification of nascent chain interaction sites on trigger factor. J. Biol. Chem. *282*, 12186–12193.

Lakshmipathy, S.K., Gupta, R., Pinkert, S., Etchells, S.A., and Hartl, F.U. (2010). Versatility of trigger factor interactions with ribosome-nascent chain complexes. J. Biol. Chem. *285*, 27911–27923.

Leuzzi, R., Serino, L., Scarselli, M., Savino, S., Fontana, M.R., Monaci, E., Taddei, A., Fischer, G., Rappuoli, R., and Pizza, M. (2005). Ng-MIP, a surface-exposed lipoprotein of *Neisseria gonorrhoeae*, has a peptidyl-prolyl cis/trans isomerase (PPIase) activity and is involved in persistence in macrophages. Mol. Microbiol. *58*, 669–681.

Liu, J., Farmer, J.D., Jr, Lane, W.S., Friedman, J., Weissman, I., and Schreiber, S.L. (1991). Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell *66*, 807–815.

Liu, X., Zhao, Z., and Liu, W. (2013). Insights into the roles of cyclophilin A during influenza virus infection. Viruses *5*, 182–191.

Lundemose, A.G., Rouch, D.A., Penn, C.W., and Pearce, J.H. (1993a). The *Chlamydia trachomatis* Mip-like protein is a lipoprotein. J. Bacteriol. *175*, 3669–3671.

Lundemose, A.G., Kay, J.E., and Pearce, J.H. (1993b). *Chlamydia trachomatis* Mip-like protein has peptidyl-prolyl cis/trans isomerase activity that is inhibited by FK506 and rapamycin and is implicated in initiation of chlamydial infection. Mol. Microbiol. *7*, 777–783.

Lyon, W.R., and Caparon, M.G. (2003). Trigger factor-mediated prolyl isomerization influences maturation of the *Streptococcus pyogenes* cysteine protease. J. Bacteriol. *185*, 3661–3667.

Lyon, W.R., Gibson, C.M., and Caparon, M.G. (1998). A role for Trigger Factor and an Rgg-like regulator in the transcription, secretion and processing of the cysteine proteinase of *Streptococcus pyogenes*. EMBO J. *17*, 6263–6275.

Maeda, T., Maeda, H., Yamabe, K., Mineshiba, J., Tanimoto, I., Yamamoto, T., Naruishi, K., Kokeguchi, S., and Takashiba, S. (2010). Highly expressed genes in a rough-colony-forming phenotype of *Aggregatibacter actinomycetemcomitans*: implication of a mip-like gene for the invasion of host tissue. FEMS Immunol. Med. Microbiol. *58*, 226–236.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S.V., Machado, M.A., et al. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. Mol. Plant Pathol. *13*, 614–629.

Meng, Q.-L., Tang, D.-J., Fan, Y.-Y., Li, Z.-J., Zhang, H., He, Y.-Q., Jiang, B.-L., Lu, G.-T., and Tang, J.-L. (2011). Effect of interactions between Mip and PrtA on the full extracellular protease activity of *Xanthomonas campestris* pathovar campestris. FEMS Microbiol. Lett. *323*, 180–187.

Merz, F., Hoffmann, A., Rutkowska, A., Zachmann-Brand, B., Bukau, B., and Deuerling, E. (2006). The C-terminal domain of *Escherichia coli* trigger factor represents the central module of its chaperone activity. J. Biol. Chem. *281*, 31963–31971.

Moro, A., Ruiz-Cabello, F., Fernández-Cano, A., Stock, R.P., and González, A. (1995). Secretion by *Trypanosoma cruzi* of a peptidyl-prolyl cis-trans isomerase involved in cell infection. EMBO J. *14*, 2483–2490.

Neff, L., Daher, S., Muzzin, P., Spenato, U., Gülaçar, F., Gabay, C., and Bas, S. (2007). Molecular characterization and subcellular localization of macrophage infectivity potentiator, a *Chlamydia trachomatis* lipoprotein. J. Bacteriol. *189*, 4739–4748.

Norville, I.H., Harmer, N.J., Harding, S.V., Fischer, G., Keith, K.E., Brown, K.A., Sarkar-Tyson, M., and Titball, R.W. (2011a). A *Burkholderia pseudomallei* macrophage infectivity potentiator-like protein has rapamycin-inhibitable peptidylprolyl isomerase activity and pleiotropic effects on virulence. Infect. Immun. *79*, 4299–4307.

Norville, I.H., Breitbach, K., Eske-Pogodda, K., Harmer, N.J., Sarkar-Tyson, M., Titball, R.W., and Steinmetz, I. (2011b). A novel FK-506-binding-like protein that lacks peptidyl-prolyl isomerase activity is involved in intracellular infection and in vivo virulence of *Burkholderia pseudomallei*. Microbiol. Read. Engl. *157*, 2629–2638.

Norville, I.H., O'Shea, K., Sarkar-Tyson, M., Zheng, S., Titball, R.W., Varani, G., and Harmer, N.J. (2011c). The structure of a *Burkholderia pseudomallei* immunophilin-inhibitor complex reveals new approaches to antimicrobial development. Biochem. J. *437*, 413–422.

Obi, I.R., Nordfelth, R., and Francis, M.S. (2011). Varying dependency of periplasmic peptidylprolyl cis-trans isomerases in promoting *Yersinia pseudotuberculosis* stress tolerance and pathogenicity. Biochem. J. *439*, 321–332.

Pereira, P.J.B., Vega, M.C., González-Rey, E., Fernández-Carazo, R., Macedo-Ribeiro, S., Gomis-Rüth, F.X., González, A., and Coll, M. (2002). *Trypanosoma cruzi* macrophage infectivity potentiator has a rotamase core and a highly exposed alpha-helix. EMBO Rep. *3*, 88–94.

Pogorelko, G.V., Mokryakova, M., Fursova, O.V., Abdeeva, I., Piruzian, E.S., and Bruskin, S.A. (2014). Characterization of three *Arabidopsis thaliana* immunophilin genes involved in the plant defense response against Pseudomonas syringae. Gene *538*, 12–22.

Riboldi-Tunnicliffe, A., König, B., Jessen, S., Weiss, M.S., Rahfeld, J., Hacker, J., Fischer, G., and Hilgenfeld, R. (2001). Crystal structure of Mip, a prolylisomerase from *Legionella pneumophila*. Nat. Struct. Biol. *8*, 779–783.

Sabers, C.J., Martin, M.M., Brunn, G.J., Williams, J.M., Dumont, F.J., Wiederrecht, G., and Abraham, R.T. (1995). Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells. J. Biol. Chem. *270*, 815–822.

Saio, T., Guan, X., Rossi, P., Economou, A., and Kalodimos, C.G. (2014). Structural basis for protein antiaggregation activity of the trigger factor chaperone. Science *344*, 1250494.

Schiene-Fischer, C., Aumüller, T., and Fischer, G. (2011). Peptide Bond cis/trans Isomerases: A Biocatalysis Perspective of Conformational Dynamics in Proteins. Top. Curr. Chem.

Schmidt, B., Rahfeld, J., Schierhorn, A., Ludwig, B., Hacker, J., and Fischer, G. (1994). A homodimer represents an active species of the peptidyl-prolyl cis/trans isomerase FKBP25mem from *Legionella pneumophila*. FEBS Lett. *352*, 185–190.

Siekierka, J.J., Hung, S.H., Poe, M., Lin, C.S., and Sigal, N.H. (1989). A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. Nature *341*, 755–757.

Takahashi, N., Hayano, T., and Suzuki, M. (1989). Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. Nature *337*, 473–475.

Teter, S.A., Houry, W.A., Ang, D., Tradler, T., Rockabrand, D., Fischer, G., Blum, P., Georgopoulos, C., and Hartl, F.U. (1999). Polypeptide flux through bacterial Hsp70: DnaK cooperates with trigger factor in chaperoning nascent chains. Cell *97*, 755–765.

Uenal, C., Schwedhelm, K.F., Thiele, A., Weiwad, M., Schweimer, K., Frese, F., Fischer, G., Hacker, J., Faber, C., and Steinert, M. (2011). Collagen IV-derived peptide binds hydrophobic cavity of *Legionella pneumophila* Mip and interferes with bacterial epithelial transmigration. Cell. Microbiol. *13*, 1558–1572.

Unal, C.M., and Steinert, M. (2014). Microbial Peptidyl-Prolyl cis/trans Isomerases (PPIases): Virulence Factors and Potential Alternative Drug Targets. Microbiol. Mol. Biol. Rev. MMBR *78*, 544–571.

Ünal, C., Schwedhelm, K.F., Thiele, A., Weiwad, M., Schweimer, K., Frese, F., Fischer, G., Hacker, J., Faber, C., and Steinert, M. (2011). Collagen IV-derived peptide binds hydrophobic cavity of *Legionella pneumophila* Mip and interferes with bacterial epithelial transmigration. Cell. Microbiol. *13*, 1558–1572.

Wagner, C., Khan, A.S., Kamphausen, T., Schmausser, B., Unal, C., Lorenz, U., Fischer, G., Hacker, J., and Steinert, M. (2007). Collagen binding protein Mip enables *Legionella pneumophila* to transmigrate through a barrier of NCI-H292 lung epithelial cells and extracellular matrix. Cell. Microbiol. *9*, 450–462.

Wen, Z.T., Suntharaligham, P., Cvitkovitch, D.G., and Burne, R.A. (2005). Trigger factor in *Streptococcus mutans* is involved in stress tolerance, competence development, and biofilm formation. Infect. Immun. *73*, 219–225.

Wintermeyer, E., Ludwig, B., Steinert, M., Schmidt, B., Fischer, G., and Hacker, J. (1995). Influence of site specifically altered Mip proteins on intracellular survival of *Legionella pneumophila* in eukaryotic cells. Infect. Immun. *63*, 4576–4583.

Wu, T., Zhao, Z., Zhang, L., Ma, H., Lu, K., Ren, W., Liu, Z., Chang, H., Bei, W., Qiu, Y., et al. (2011). Trigger factor of *Streptococcus suis* is involved in stress tolerance and virulence. Microb. Pathog. *51*, 69–76.

Zang, N., Tang, D.-J., Wei, M.-L., He, Y.-Q., Chen, B., Feng, J.-X., Xu, J., Gan, Y.-Q., Jiang, B.-L., and Tang, J.-L. (2007). Requirement of a mip-like gene for virulence in the phytopathogenic bacterium *Xanthomonas campestris* pv. campestris. Mol. Plant-Microbe Interact. MPMI *20*, 21–30.

Figure Legends

Figure 1: Potential lead structures for future inhibitors of bacterial FKBPs. (A) The natural macrolide FK506 interacts with FKBP via its "binding domain", here highlighted in red, that contains a pipecoline ring mimicking proline. In pipecolinic acid containing derivatives as in substance B this binding domain is optimized in order to yield non-immunostimulatory inhibitors (Juli *et al.,* 2011). **(B)** Cycloheximide, the inhibitor of eukaryotic translation, was improved for FKBP inhibition and reduced cytotoxicity by derivatization with an ethylethanoate moiety. **(C)** P290 is the first substrate based peptide inhibitor of the FKBP Mip of *L. pneumophila*. Shown is the crystal structure of the hexameric NC1-domain of human collagen IV that is a Mip substrate (pdb number 1LI1). The left half consisting of three monomers is given as a surface model, while the right half is depicted as ribbons. P290 in each monomer is highlighted in red. It was shown by NMR, and molecular docking studies that P290, when in compex with Mip, binds to the hydrophobic cavity that exerts the PPIase activity, and forms a hairpin-like structure.