remarkable similarities in the mechanisms elicited upon infection of cells by both viruses, indicating that LTβR may operate similarly against both viruses.

Identifying the underlying molecular and cellular mechanisms of host response to MCMV is not only of scientific interest but also of medical relevance. High type I interferon levels are deleterious in autoimmune diseases. Therefore, manipulation of the LTβR pathway has been pursued as a possible treatment. Inhibition of the LT pathway stops the development of collagenase-induced arthritis and protects adult animals against experimental autoimmune encephalomyelitis (EAE) (Ware, 2005). The study of Schneider et al. (2008) indicates that regulation of the type I IFN through LT signaling may also represent an alternative therapeutic strategy in cases of severe HCMV infection.

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Deploying Parasite Profilin on a Mission of Invasion and Danger

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Interleukin-12 (IL-12) is important in inducing Th1 responses during infection with microbial pathogens such as the protozoan *Toxoplasma gondii*. In this issue of *Cell Host & Microbe*, Plattner and colleagues describe an engineered *Toxoplasma* strain that lacks profilin, an actin-binding molecule previously implicated in Toll-like receptor-11-dependent IL-12 induction and now shown to be important in parasite motility and host cell invasion.

The phylum Apicomplexa is composed of unicellular eukaryotes that live as intracellular parasites. Many cause acute disease and death in humans as well as impacting the livestock industry throughout the world. However, one such apicomplexan, *Toxoplasma gondii*, while extremely common (between 20% and 80% of humans worldwide harbor the parasite), normally causes asymptomatic infection. Yet, true to form for an opportunistic pathogen, *Toxoplasma* can cause serious trouble in hosts with an impaired immune system. In the laboratory, *Toxoplasma* has become a favorite model pathogen of cell biologists and immunologists alike. Cell biologists have come far in unraveling the molecular machinery the parasite uses to invade and live within the host cell. This effort has been tremendously helped by sequencing of the *Toxoplasma* genome, and the development of a sophisticated toolbox of forward and reverse genetic techniques. Immunologists know *T. gondii* as a prototypic Th1-inducing pathogen, at the center of which is the parasite's ability to induce the cytokine interleukin-12 (IL-12). In this issue of *Cell Host & Microbe*, Plattner and colleagues provide a striking example of how tools of molecular biology can be skillfully wielded to unite immunology and cell biology, providing insight into a parasite molecule that seems to be prominent in the infection biology of *T. gondii* (Figure 1).

The molecule in question is a *Toxoplasma* profilin-like protein designated TgPRF. Profilins are most well known for their function in actin filament polymerization across eukaryotes, and they are important in cytoskeleton assembly and myosin-based motility. Using a biochemical fractionation approach, Alan Sher and colleagues recently identified TgPRF as a parasite molecule with IL-12-inducing activity on mouse splenic dendritic cells (DC) (Yarovinsky et al., 2005). In the same study, TgPRF was found to activate DC through mouse Toll-like receptor (TLR)-11. As such, TgPRF was identified as a protozoan "danger" molecule, alerting the host innate immune system to the presence of infection. Although TLR11 was originally characterized for its involvement in responses to uropathogenic *E. coli* (Zhang et al., 2004), TgPRF was, and still is, the only biochemically characterized ligand of mouse TLR11. Importantly, TLR11 knockout mice have decreased IL-12 responses and increased susceptibility during *T. gondii* infection. Nevertheless, the importance of the TgPRF/TLR11 interaction compared to other presumed parasite TLR ligands was uncertain because mice deficient in MyD88, a central adaptor in most TLR signaling, were much more susceptible to infection than $TLR11^{-/-}$ animals. In the present study, the groups of Sher and Dominique Soldati-Favre have collaborated to rigorously test the importance of TgPRF by producing a parasite whose profilin gene has been genetically deleted. For the first time, this has now allowed direct assessment of TgPRF in the immune response to *Toxoplasma* and the life cycle of the parasite.

Host cell invasion by *Toxoplasma* is well known to depend upon parasite actin-based motility (Dobrowolski and Sibley, 1996). The present investigators therefore began with the assumption that straightforward deletion of the profilin gene by homologous recombination would likely be lethal for the parasite (*Toxoplasma* is haploid). Accordingly, the investigators set about engineering a conditional knockout of TgPRF. This elegant but technically challenging approach was pioneered by Soldati-Favre and coworkers several years ago to examine the role of parasite myosin A in invasion (Meissner et al., 2002). For the present study, the strategy involved introducing an ectopic TgPRF gene whose expression was under the control of a tetracycline regulatable promoter, followed by genetic deletion of the parasite's endogenous profilin gene. Now expression of TgPRF could be switched off at will by addition of anhydrotetracycline to the growth medium or drinking water of infected mice (Plattner et al., 2008).

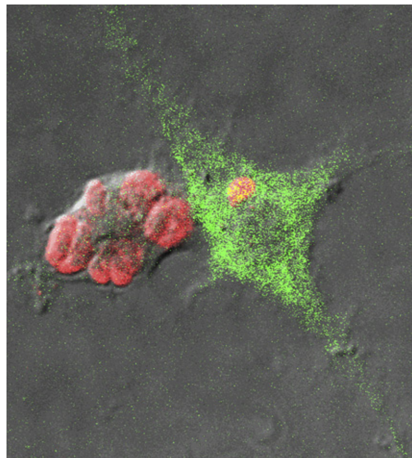

Figure 1. The Double Life of a Parasite Profilin
A host cell infected with *Toxoplasma* (red) adjacent to a cell producing IL-12 (green). *Toxoplasma* uses profilin to invade cells, and host cells respond to the same molecule through TLR11-dependent IL-12 production.

As predicted, profilin-negative parasites were unable to complete their life cycle during in vitro infection and were also avirulent in vivo. When parasites were reconstituted with constitutively expressed TgPRF, in vitro infectivity and in vivo virulence were restored. Invasion, which requires an actin-based cytoskeleton-associated motor called the glideosome, was defective in the absence of TgPRF. However, profilin was dispensable for intracellular growth of the parasite, a result that is consistent with previous studies using pharmacological ablation of actin filaments. The actin-based glideosome has been implicated in not only how parasites enter cells but also how they exit. *Toxoplasma* egress can occur through two mechanisms. One involves straightforward membrane rupture as the cell becomes overburdened with intracellular parasites. As might be predicted, this did not require profilin. The second method, dubbed "induced egress," requires parasite motility. This type of egress can be induced by calcium ionophores such as A23817, but only, as it turns out, if the parasites express TgPRF.

It seems likely that TgPRF functions as a true profilin, promoting actin filament assembly and parasite motility, but some caution is warranted with this interpretation. First, apicomplexan actin filaments have so far proven impossible to visualize. Evidence suggests that this is because apicomplexans such as *Toxoplasma* produce only short and unstable actin filaments (Sahoo et al., 2006). As a result, it is not possible to directly examine actin structure in the presence and absence of TgPRF. Second, the activities of two drugs that stabilize actin filaments (measured by appearance of pelletable actin) are unaffected by knockout of TgPRF (Plattner et al., 2008). Although the investigators of the present study show that TgPRF and other apicomplexan profilins are active in conventional actin polymerization assays, it remains to be shown that parasite profilins display similar activity on native parasite actin molecules.

The second aspect of this study concerns the profilin/TLR11 interaction and its role in IL-12 induction. As would be predicted based upon earlier studies (Yarovinsky et al., 2005), parasites that do not express TgPRF are defective in IL-12-inducing activity on mouse splenic DC. Furthermore, infection of mice with TgPRF-negative parasites fails to elicit serum IL-12. However, the in vivo result might be a trivial consequence of the mutant's inability to generate a sustained infection. To dissociate the cell biological from the immunological phenotype, the authors came up with a very clever trick: they transfected the mutant with the PRF gene of the related malaria parasite *Plasmodium falciparum*. This fully restored invasion and infectivity but, importantly, failed to elicit IL-12 in cultured DC or in mice. The result suggests that lack of IL-12 induction in vivo by TgPRF-negative parasites is a direct result of failure to express TgPRF rather than being the result of defects in invasion.

Both $MyD88^{-/-}$ and $IL\text{-}12^{-/-}$ animals are unable to survive acute *Toxoplasma* infection (Scanga et al., 2002). In contrast, mice survive infection without TLR11, although they are clearly more susceptible, as measured by cyst number in the brain during chronic infection (Yarovinsky et al., 2005). Given the apparent lack of IL-12 induction in the absence of parasite TgPRF or host TLR11, why are $TLR11^{-/-}$ mice so resistant to infection? First, there

are very possibly TLR11/TgPRF-independent IL-12 sources not apparent in serum or in splenic DC cultures that were employed in these studies. Indeed, evidence indicates that *T. gondii* glycosylphosphoinositols possess TLR2- and TLR4-activating properties (Debierre-Grockiego et al., 2007). It also seems possible that MyD88-independent pathways leading to IL-12 could contribute to resistance in the absence of TLR11 (Kim et al., 2006).

Finally, why hasn't the immunostimulatory property of *T. gondii* profilin been selected against through evolutionary pressure? For many pathogen-associated molecular patterns, the answer seems to be that the innate immune system favors recognition of molecules essential for microbial survival (TLR5 recognition of bacterial flagellin would be one example [Smith et al., 2003]). This does not seem to be the case for TgPRF, since expression of *Plasmodium* profilin enables invasion, but not TLR11-dependent IL-12 induction. This raises the possibility that optimal survival of *T. gondii* in the mouse host is promoted by recognition of TgPRF and subsequent IL-12 induction. In this scenario, absence of IL-12 induction would lead to uncontrolled parasite growth, quickly leading to host and consequently parasite demise. That would seem to be at odds with the present study, which shows no difference in virulence between TgPRF-positive *Toxoplasma* and TgPRF-negative parasites expressing *P. falciparum* profilin. However, the strain of *Toxoplasma* engineered in these studies is a member of the type I class of parasites, which are known for extremely high virulence in mice (one parasite is sufficient to kill the host in this species). It will be most valuable to construct TgPRF knockout parasites that express *Plasmodium* PRF using *T. gondii* strains with lower virulence. This will allow an assessment of TgPRF IL-12-inducing activity in a situation where stable long-term infection is the normal outcome.

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New Pieces for the Malaria Liver Stage Puzzle: Where Will They Fit?

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Malaria starts with the infection of the liver by *Plasmodium* parasites. Although extensive analysis of *Plasmodium* has revealed the expression patterns of this parasite in every stage of its life cycle, the liver stage remained unexplored. Recently, Tarun et al. have published the first complete transcriptome and proteome analysis of this intriguing parasite stage, providing a list of potential candidate target genes for antimalarial vaccines and drugs.

Malaria is a devastating disease that kills more than one million children per year, mainly in sub-Saharan Africa. *Plasmodium* spp., the causative agents of malaria, are parasites that are transmitted by the bite of an infected *Anopheles* mosquito. During a blood meal, mosquitoes inject *Plasmodium* sporozoites under the skin of the host, from where they migrate into the circulation and then into the liver.

The *Plasmodium* liver stage begins upon parasite infection of hepatocytes. During a period lasting between 2 and 16 days depending on the species, the parasite replicates and undergoes further development, ultimately giving rise to merozoites. Upon release into the blood, merozoites infect erythrocytes, causing the fever and the pathologies associated with malaria. Notably, the liver stage is essential for the parasite's amplification; as *Plasmodium* parasites are introduced into its host as a small number of