Switching parasite proteins on and off

Boris Striepen

Parasite infections such as malaria threaten the lives of millions of people around the globe. New genetic tools have been developed to evaluate parasite proteins as targets for urgently needed drug and vaccine development.

Diseases caused by infection with protozoan parasites remain a largely unchallenged threat to human health. These infections inflict their worst toll on the part of the world's population with the least income to devote to health and medical care.

Malaria alone claims more than a million lives each year, followed by a long list of scourges that collectively reinforce a vicious cycle of disease and poverty. Why are effective drugs and vaccines for most of these diseases still lacking? One reason is their link to poverty and, as a result, the limited interest of the pharmaceutical industry to invest into what likely will be a poor market. Another substantial hurdle is our limited understanding of our foe, the parasite. A recent evaluation of malaria vaccine trials therefore issued a call for a 'back-to-the-basics' approach asking for more laboratory research before embarking on costly clinical trials[1]. Experimental tools and models are thus urgently needed to critically gauge the potential value of vaccine candidates or potential drug targets. Two studies published in this issue of *Nature Methods*[2,3] report an important advance on this front—a conditional protein expression system for the Apicomplexan parasites *Plasmodium falciparum* (the agent of the most severe form of malaria) and *Toxoplasma gondii* (the cause of encephalitis in AIDS patients and unborn children).

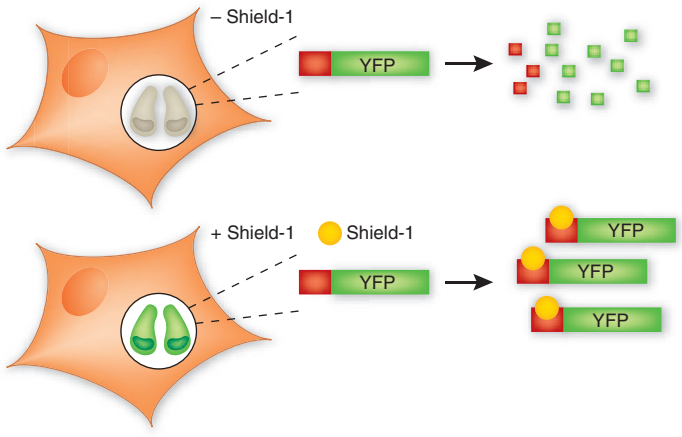

Figure 1 | Conditional gene expression through protein destabilization. A host cell harbors two parasites (light gray) contained within the parasitophorous vacuole (white). The parasites are transfected with a target protein (YFP) fused to an FKBP destabilization domain. Without the stabilizing ligand Shield-1, YFP is destroyed. Upon the addition of Shield-1 YFP is stabilized and is easily detectable.

Boris Striepen is at the Center for Tropical and Emerging Global Diseases, and the Department of Cellular Biology, University of Georgia, 500 D.W. Brooks Drive, Athens, Georgia 30602, USA.
e-mail: striepen@cb.uga.edu

Apicomplexa are an ancient phylum of unicellular parasitic eukaryotes. They are intracellular parasites that develop in a specialized compartment, the parasitophorous vacuole. A long history of coevolution has endowed them with formidable skills to exploit and outwit their hosts. These include mechanisms to invade and manipulate cells, intercept metabolites and evade the immune system[4,5].

Two elements have driven progress toward unraveling the molecular detail of the parasite's arsenal as well as potential chinks in its armor. Researchers now have access to the detailed genomic blueprint of many Apicomplexan species and strains, and a powerful bioinformatics interface allows them to mine this resource[6]. Experimental tools also have been developed to genetically manipulate the parasite.

This advance was led by *T. gondii*, which has been established as the Apicomplexan version of yeast—a facile genetic model organism with a broad range of reverse and forward genetic tools[7]. However, techniques have been quick to move into malaria parasites, overcoming some of the initial challenges. Using these tools, genes and proteins can be tagged with reporters to trace their expression, localization and dynamic behavior. Genes can also be deleted from the genome altogether, and such a knockout experiment can, for example, guide the drug development process by establishing whether a target pathway is essential for parasite survival[8].

However, Apicomplexa are haploid organisms, and essential genes cannot be deleted because the resulting mutants are not viable. The trick to overcome this conundrum has been to engineer systems to conditionally express parasite proteins. In *T. gondii* this can be done using temperature sensitivity or a tetracycline-regulated promoter system; so far these approaches have not found broad use in *P. falciparum*.

The two reports in this issue establish a third route: modulating protein stability using a small molecule. Initially developed for mammalian cells, this method is based on fusion of the target protein to a mutant version of the human rapamycin binding protein FKBP12 (ref. 9). Addition of this destabilization domain (ddFKBP; **Fig. 1**) results in degradation of the target protein. The protein can be spared from this fate by addition of a cell-permeable small molecule ligand of ddFKBP dubbed Shield-1.

This system produces robust regulation of a variety of proteins in both parasites[2,3]. Depending on the configuration, this occurs with remarkable speed: in *T. gondii* a fusion of the destabilization domain and yellow fluorescent protein (ddFKBP-YFP) is upregulated to full level within 90 min upon addition of Shield-1 (ref. 3). Experiments using endogenous parasite proteins suggest that the approach is well suited for the construction of conditional knockout as well as dominant negative mutants. The ddFKBP model does not depend on a specific promoter element (as does the tetracycline system[10]) thus avoiding complications resulting from inappropriate promoter strength or timing.

Another strength of this system is the ability to precisely dose the level of target protein by varying the concentration of Shield-1. On the downside, it requires fusion of the target protein to the 12-kDa ddFKBP. One of the proteins tested here[3], myosinA, serves as an example where such fusion results in loss of function. Also destruction of the protein likely depends on the cytoplasmic proteoasome, which could imply that target proteins have to be cytoplasmic for optimal regulation. Additional work is needed to evaluate the regulation of proteins that are targeted to other cellular locales.

However, Armstrong and Goldberg already demonstrate regulation of falcipain-2 (ref. 2). This protease is used by the malaria parasite to digest red cell hemoglobin and is localized to the food vacuole, a compartment equivalent to the lysosome. This finding suggests that (at least some) parasite secretory proteins can be manipulated using this system. This is important because secretory proteins constitute a large portion of the parasite's host cell invasion and manipulation machinery.

What could be the broader uses for ddFKBP beyond the obvious gene-by-gene approach? Two areas come to mind. ddFKBP could enable work toward a comprehensive catalog of conditional mutants canvassing essentially the entire genome, similar to efforts in other model organisms. Combining ddFKBP with approaches already available[11] might yield conditional insertional mutants and open the door to forward genetic analysis of the rich and unique biology of Apicomplexan parasites.

1. Callaway, E. *Nature* **449**, 266 (2007).
2. Armstrong, C.M. & Goldberg, D.E. *Nat. Methods* **4**, 1007–1009 (2007)
3. Herm-Götz, A. *et al. Nat. Methods* **4**, 1003–1005 (2007).
4. Sibley, L.D. *Science* **304**, 248–253 (2004).
5. Marti, M. *et al. Science* **306**, 1930–1933 (2004).
6. Aurrecoechea, C. *et al. Nucleic Acids Res.* **35** (database issue), D427–D430 (2007).
7. Meissner, M. *et al. Curr. Opin. Microbiol.* **10**, 349–356 (2007).
8. Mazumdar, J. *et al. Proc. Natl. Acad. Sci. USA* **103**, 13192–13197 (2006).
9. Banaszynski, L.A. *et al. Cell* **126**, 995–1004 (2006).
10. Meissner, M. Schluter, D. & Soldati, D. *Science* **298**, 837–840 (2002).
11. Roos, D.S. *et al. Methods* **13**, 112–122 (1997).

Illuminating aggregate heterogeneity in neurodegenerative disease

Walker S Jackson & Susan Lindquist

Luminescent conjugated polymers (LCPs) bind to prion aggregates and emit different fluorescent spectra depending on their binding conformation. As such, they are promising tools for investigating the biophysical basis of prion strains.

The transmissible spongiform encephalopathies, prion diseases, are perhaps the most bizarre and perplexing diseases known. Prions cause the notorious 'mad cow' disease in cattle, scrapie in sheep and chronic wasting disease in deer and elk. In humans they produce a spectrum of disorders known as fatal familial insomnia, Creutzfeldt-Jakob disease and Gerstmann-Sträussler-Scheinker syndrome. Despite years of study, many questions remain concerning how the infectious agent replicates in the brain, how it kills and how it is transmitted within and between species. In this issue of *Nature Methods*, Sigurdson and colleagues from the Aguzzi laboratory bring a powerful new histological imaging probe to bear on the problem[1].

The prion diseases are caused by misfolding of a protein known as PrP, which is the main component of the infectious agent. PrP is present in normal brains in an alpha helix–rich state. But it is capable of adopting an infectious conformation rich in beta sheet structure, which somehow entices the normal protein to adopt the same conformation. This protein-misfolding chain reaction generates new infections material and, in a manner still quite mysterious, wrecks havoc in the brain.

The process can start in several ways. In most cases, it appears to happen spontaneously, thankfully in only about one in a million individuals per year. It occurs with high likelihood in people harboring mutations in PrP that make the protein prone to misfolding. Less commonly (but certainly more newsworthy), it can be initiated by environmental exposure to the proteinaceous infectious agent via ingestion, infected surgical equipment or even blood transfusion. A strong species barrier greatly reduces cross-species transmission, which appears to have protected most, but not all, people who came into contact with infectious material during the epidemic of mad cow disease in Great Britain.

Walker S. Jackson and Susan Lindquist are at the Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, Massachusetts 02142, USA. Susan Lindquist is also at Howard Hughes Medical Institute and the Massachusetts Institute of Technology, Department of Biology, Cambridge, Massachusetts 02142, USA.
e-mail: lindquist_admin@wi.mit.edu