GENOME WATCH

A CRISPR outlook for apicomplexans

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This month's Genome Watch discusses how advances in genome editing have contributed to our understanding of apicomplexan parasite biology.

Reverse genetics, an approach that is used for the discovery of gene function by studying the phenotypic effects of genetic modification, is a powerful way to functionally annotate entire genomes. Although this method has been very successful in several model organisms, it has been technically challenging in Apicomplexa, a phylum of obligate intracellular parasites that includes *Plasmodium* spp., which are the causative agents of malaria, and *Toxoplasma gondii*, which is often considered the world's most 'successful' parasite for causing severe opportunistic infections. In addition, because apicomplexans are only distantly related to more genetically tractable eukaryotic organisms, the function of a large portion of their genomes still remains unknown. Genome-wide genetic screens have proven especially difficult and time consuming in *Plasmodium* spp., owing to their extremely high AT content, poor transfection efficiencies and low rates of homologous recombination. By contrast, high transfection rates and relative ease of culture make *T. gondii* much more amenable to genetic manipulation, and, as such, the parasite has long served as a model system for the study of apicomplexan parasites.

Sidik *et al.*[1] carried out a genome-wide CRISPR–Cas9 screen in *T. gondii*[2] to identify essential apicomplexan genes. The authors designed single-guide RNAs (sgRNAs) for all predicted protein-coding genes and identified several putative fitness-conferring genes. To suppress Cas9-mediated toxicity, the authors co-expressed a 'decoy' sgRNA that enabled the parasites to tolerate constitutive Cas9 expression. The authors identified fitness-conferring genes based on the loss of their corresponding sgRNA sequences from the mutant population. Of these identified genes, 200 lacked functional annotation, and as they were conserved within apicomplexans, they referred to them as 'indispensable conserved apicomplexan proteins' (ICAPs). Two proteins that are encoded by ICAPs, including ICAP12, localized to micronemes, and using functional assays, the authors identified a role for ICAP12 in host cell invasion in both *T. gondii* and *P. falciparum*. Confirmation of the essential role of ICAP12 in a different species opens up the exciting possibility of using functional screens in *T. gondii* to infer the functions of orthologous genes in related parasites, such as *Plasmodium* species.

To date, the largest screens in *Plasmodium* spp. have been on specific sets of enzymes or gene sets, each comprising less than 100 genes[3,4]. One of the main impediments to implementing similar CRISPR-based screens in *Plasmodium* spp. is the absence of the non-homologous end joining DNA repair mechanism, necessitating an alternative approach that relies on double homologous recombination while providing a sequence that has enough homology for repair. Using this approach, Gomes *et al.*[3] transfected a 'cocktail' of uniquely barcoded knockout vectors into a pool of *Plasmodium berghei* parasites, and then injected the mutant pool into a single mouse. Growth curves for individual mutants, tracked by barcode sequencing, revealed the relative contribution of each targeted gene to the fitness of the parasite during infection. To validate their approach, the authors targeted 46 protein kinases in *P. berghei* that had been previously knocked out by Tewari *et al.* using a conventional gene-by-gene knockout screen[4]. Gomes *et al.* were able to confirm disruption of most targeted kinases, including a few genes that were not identified in the previous study. More importantly, the authors demonstrated the feasibility of large-scale reverse genetic screens in *P. berghei* for the first time.

The availability of comprehensive genome resources for apicomplexan parasites makes it easy to identify genes that are conserved between different species. Using the results of genome-wide loss-of-function screens in *T. gondii*, researchers can predict the possible functions of orthologous genes in related species and design experiments to test these predictions. In the case of *P. falciparum*, the results of large-scale screens in both *T. gondii* and *P. berghei* can be leveraged to more precisely identify genes that are important in the life cycle of the parasite.

Together, these studies show the promise of large-scale loss-of-function screens in elucidating gene function in closely related species and also demonstrate how screening approaches using either CRISPR–Cas or knockout vectors can be used to increase our understanding of these parasites, with the eventual goal of translating this knowledge into promising targets for vaccine development and drug discovery.

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Competing interests statement
The author declares no competing interests.