Novel CRISPR method identifies key genes for Toxoplasma survival

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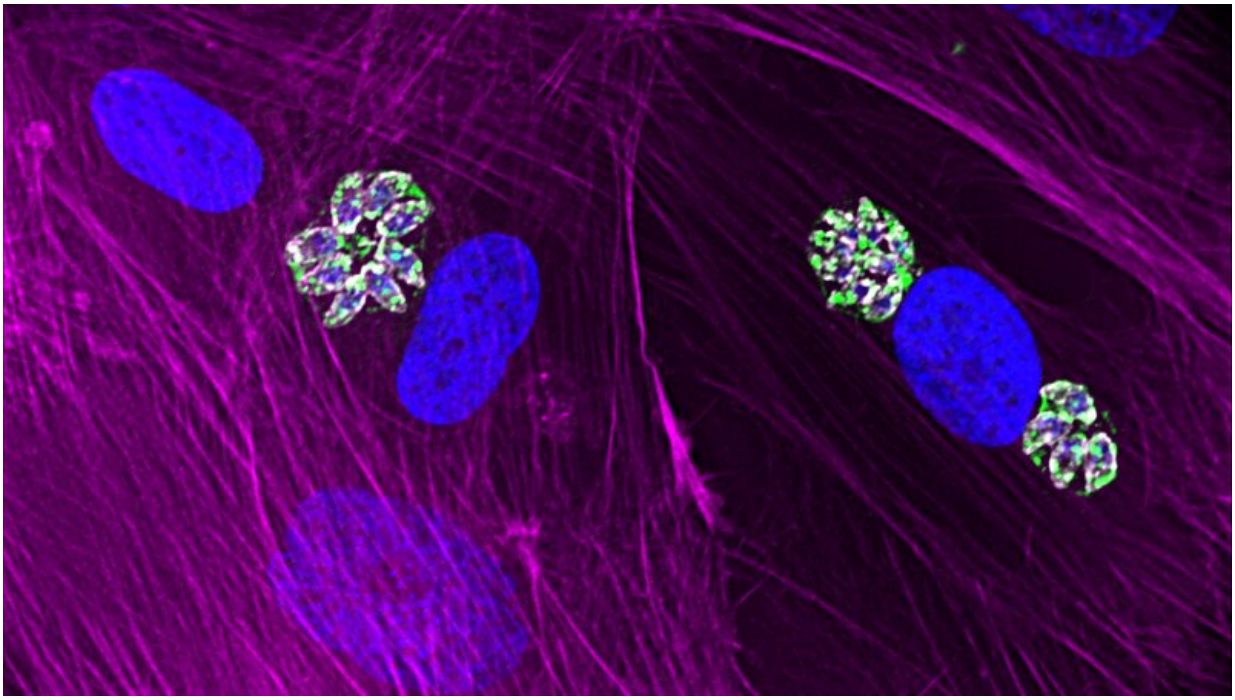

Human cells (with the cytoskeleton shown in purple) infected with Toxoplasma gondii (white). Credit: Simon Butterworth

Using a new approach to genetic screening with CRISPR, researchers at the Crick have identified key genes for the survival of the parasite *Toxoplasma gondii* in mice.

Their new method, published in *Nature Communications*, provides a

flexible new approach that could expand the use of CRISPR screening. This method also allows testing hundreds of parasites' genes in mice simultaneously, substantially reducing the number of research animals used.

Versatile library generation for CRISPR screening

CRISPR screening is an extremely powerful way to identify gene function. Using guide RNAs (known as gRNA), specific genes are targeted and "knocked out." This elimination helps determine which genes are essential for the cell to survive. However, these screenings are often performed genome-wide and this scale can limit the types of experiments one can do.

To counter the limitations of genome-wide screening, the team developed a new method to rapidly generate and analyze custom CRISPR libraries of varying size and composition using a single-step cloning method. This research was led by the Signalling in Apicomplexan Parasites Lab, working with members of the Bioinformatics and High Throughput Screening facilities.

"This method of creating and analyzing gRNA pools that target only a subset of genes has allowed us to perform CRISPR screens much more rapidly and move them to the in vivo infection model," says Crick group leader Moritz Treeck, senior author of the paper.

"gRNAs targeting genes of interest are selected from an array, then cloned and assembled into pooled CRISPR libraries. This custom CRISPR library is then transfected into the parasite to generate a pool of mutants that is then grown in mice, or in cell culture. By sequencing input and output mutant pools we are able to identify those genes important for growth in any selection condition."

Novel genes

Toxoplasma gondii is a parasite that affects around a third of the human population. While in a healthy individual there are few symptoms associated with infection, the parasite is a serious threat to AIDS patients, child development in the uterus and virulent strains causing ocular disease are prevalent in South America. Previously, genome-wide genetic screening has been used in vitro to identify the genes that are required for growth in cell culture, but screening methods to identify those genes important to regulate the host's immune response and ensure survival in vivo, known as "virulence factors," have not been available.

"By selecting a subset of genes to target, we didn't run the risk of overwhelming the host before the parasite was able to propagate," says Joanna Young, postdoc at the Crick and co-lead author of the paper. "We infected mice with pools of Toxoplasma mutants we predicted to play a role in the interaction with the host. This revealed new virulence factors as well as confirming those that were known from previous work."

Interestingly, the team also found that some strains that are unable to survive on their own, were successful when co-infected in the mixed pool.

"For example, the MYR1 gene is required for the functions of many of *Toxoplasma*'s secreted proteins, and without it the parasite cannot mount an infection," explains Joanna. "But we were surprised to find that the MYR1 knockouts were still present and able to survive in mice when they are infected together with other mutants. Now we need to find out how this happens!"

Next steps

The next stage is already underway. The team will be collaborating with the Advanced Sequencing facility to identify individual CRISPR mutants and the whole RNA of the infected host cell to identify virulence factors that specifically interfere with the response of the host.

More information: Joanna Young et al. A CRISPR platform for targeted in vivo screens identifies Toxoplasma gondii virulence factors in mice, *Nature Communications* (2019). DOI: 10.1038/s41467-019-11855-w

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