Tightening the handle on malaria

Different methods have contributed to a better understanding of the malaria parasite, but improvements are still needed to uncover basic *Plasmodium* biology.

Thursday, 25 April 2019, is World Malaria Day. Disease statistics issued by the World Health Organization for 2016 are still grim and highlight the seriousness of the disease: 216 million cases worldwide result in around half a million deaths per year. A day dedicated to raising awareness of the disease is a good opportunity to ask how far malaria research has come and which methods are needed for further breakthroughs.

Much progress has been made in recent years toward visualizing the different stages in *Plasmodium*'s life cycle. Various imaging techniques, such as wide-field, confocal and spinning-disk intravital microscopy, and super-resolution microscopy, have allowed researchers to follow the parasites. After transmission by a mosquito, plasmodia migrate out of the connective tissue of the skin into the liver, are released into the bloodstream, invade red blood cells and finally rupture the infected erythrocytes (reviewed in *Nat. Rev. Microbiol.* **15**, 37–54; 2017).

One challenge for live-cell imaging methods is that the parasite is small and very fragile and cannot withstand repeated exposure to light. It will be interesting to see whether more gentle imaging techniques such as lattice light-sheet microscopy can follow the parasite in even more detail.

In 2018, cryo-electron microscopy enabled scientists to generate a near-atomic-resolution structure of the *Plasmodium falciparum* translocon, a complex needed to transport effector proteins from the parasite vacuole into the host erythrocyte. For the first time, researchers, led by Pascal Egea and Z. Hong Zhou from UCLA, solved the unusual shape of a heptamer channel that spans the vacuolar membrane tethered to a spiral-shaped hexamer that helps reset the translocon for the next infection cycle (*Nature* **561**, 70–75; 2018).

Such a detailed look at essential proteins will be invaluable for understanding the parasite's interaction with the host cell. In conjunction with these targeted studies, genome-wide functional genomic screens have provided a global view on essential *Plasmodium* genes.

A study led by John Adams at the University of South Florida using saturation-scale mutagenesis on the *P. falciparum* genome revealed that of 5,399 disrupted genes, 2,680 are essential for the blood stage (*Science* **360**, eaap7847; 2018). Oliver Bilker, from the Wellcome Trust Sanger Institute, measured competitive growth rates in mice with ~2,578 barcoded *Plasmodium berghei* knockout mutants and also saw that over 60% of genes are needed for optimal growth in the blood stage (*Cell* **170**, 260–272; 2017).

Other genome-wide screens focused on genomic changes in parasites resistant to common malaria drugs. Work led by Elizabeth Winzeler at UCSD mapped the drugable *P. falciparum* genome and discovered many new candidates that contribute to antimalarial resistance (*Science* **359**, 191–199; 2018). Recently, this team also reported a treasure trove of new antimalarials from which new medicines could be developed that target the liver stages and could act as prophylactic and curative medicines (*Science* **362**, eaat9446; 2018).

The results of these large-scale screens require targeted follow-up, and methods to home in on the function of a particular gene have flourished recently. Despite the AT-richness of the *Plasmodium* genome, the CRISPR–Cas9 system is being used with increasing success to introduce mutations (*Genome Med.* **6**, 63; 2014), tag endogenous genes (*Parasit. Vectors* **10**, 595; 2017) and, very recently, manipulate gene expression via epigenome editing (*Proc. Natl. Acad. Sci. USA* **116**, 255–260; 2019), though this remains to be tested on a wider range of genes.

Another method to study protein function is selection-linked integration, developed in the lab of Tobias Spielmann. Parasites with the desired integration can be selected, and the mislocalization of targeted endogenous proteins, known as knock-sideways, then allows their functional analysis (*Nat. Methods* **14**, 450–456; 2017).

In an alternative approach to isolate particular genes, Stefan Kappe and colleagues at the Seattle Biomedical Research Institute successfully used human-liver-chimeric mice for genetic crosses of *P. falciparum* (*Nat. Methods* **12**, 631–633; 2015). This type of work had previously required splenectomized chimpanzees that can no longer be used for this purpose. Crossing drug-resistant parasites with drug-sensitive strains provides a powerful way to identify the genes that control resistance, and should reveal ways to combat it.

A gene that has been identified by many groups as conferring resistance to the current first-line drug artemisinin is kelch 13 (*K13*). Mutations in this gene appear to enhance the parasite's ability to survive cellular stress resulting from artemisinin action, yet the precise function of *K13* remains to be discovered. Analyses of the pathways to which *K13* contributes and, importantly, its interaction partners are vital topics of investigation. This seems particularly important in light of the fact that the genetic background of the parasite influences the effect of the *K13* mutations.

We are only at the beginning of understanding how *Plasmodium* regulates its interaction with the environment. How does gene transcription change during a parasite's life cycle? The Malaria Cell Atlas, single-cell transcriptomic data from human and rodent parasites across the different stages of infection, will be a valuable resource. But more needs to be understood also about post-transcriptional regulation. What roles do the metabolome and proteome play in the parasite's life cycle? How are host–parasite interactions in the liver stage different from those in the blood stage? How does the parasite suppress innate immunity, and how does it avoid sterilizing immunity in the host?

Some *Plasmodium* species face even more basic barriers. *Plasmodium vivax*, for example, which is as widespread among humans as *P. falciparum* but not as deadly, cannot be cultured long term in vitro. It has an interesting epidemiology with a long dormant liver stage. Being able to culture this parasite and discover the molecular cues that trigger dormancy and activation will be a breakthrough for the field.

While the sequence homology between *P. falciparum* and *P. vivax* genomes is limited and not all essential genes are the same, it will nonetheless be very illuminating to compare their mechanisms of infection and drug evasion.

The theme of this year's World Malaria Day is "End Malaria for Good"; we encourage our readers to check out more information and consider whether their expertise could lend itself to collaborations. And we prompt all the skydivers, runners and chefs among our readers to think about how they can support the malaria-elimination agenda. ❐

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