Microbiology

One gene to rule them all in a chronic brain infection

Eva-Maria Frickel

A gene has been found that controls the conversion of the parasite *Toxoplasma gondii* into a form that chronically infects the human brain. The discovery could aid the design of therapies to eliminate this currently untreatable infection.

It is estimated that around one-third of the global human population[1] is infected with the single-celled organism *Toxoplasma gondii*, a parasite that can be ingested in food or picked up from activities such as gardening[2]. The parasite needs to differentiate into a chronic-stage form to establish a permanent infection in brain and muscle tissue, but how this parasite conversion occurs has been a mystery. Writing in *Cell*, Waldman *et al.*[3] report the identification of a gene that encodes a master regulator of this differentiation event.

Toxoplasma gondii can infect any warm-blooded animal. Human infection can occur through eating undercooked meat from infected livestock or by ingesting contaminated food or water. Within a couple of weeks of entering its host, *T. gondii* is converted from a form called an acute-stage tachyzoite into a bradyzoite, which establishes a chronic infection (Fig. 1). A bradyzoite forms a cyst that resides in host cells and is surrounded by a thick wall of proteins and sugars. The wall is a formidable barrier that makes the cyst inaccessible and thwarts its elimination by drugs or the host's immune system.

Although *T. gondii* infection is widespread in human populations, it is often harmless, being in the relatively quiescent state of bradyzoites that have not reverted to the activated tachyzoite form associated with disease. However, *T. gondii* infection can be life-threatening for unborn fetuses or for people whose immune systems are compromised. Moreover, in the United States, 2% of *T. gondii* infections result in sight problems or blindness owing to ocular damage caused by treatment-resistant parasites[4].

To uncover the signal that controls the formation of bradyzoites, Waldman and colleagues engineered *T. gondii* to express a green fluorescent protein if such cysts formed. Monitoring the fluorescent protein using microscopy and cell-sorting technologies offered a way of assessing whether the parasite had differentiated into the form associated with chronic infection. Exposure to stress-inducing treatment in culture conditions, such as an alkaline pH, made the parasite differentiate into bradyzoites. The authors used the gene-editing tool CRISPR to disrupt selected genes to assess whether any of them affected differentiation. The results were stunning and clear. The disruption of only one targeted gene — which the authors call bradyzoite-formation deficient 1 (*BFD1*) — prevented the formation of bradyzoites.

"The parasite needs to differentiate into a chronic-stage form to establish a permanent infection."

BFD1 encodes a transcription-factor protein belonging to a family known as Myb-domain-containing proteins. Waldman *et al.* demonstrate that the Myb domain of BFD1 protein drives *T. gondii* differentiation. This is particularly intriguing because another Myb-domain-containing protein controls chronic-stage cyst formation in the parasite *Giardia lambia*[5]. Moreover, a related member of the Myb-domain-containing protein family enables *Plasmodium* parasites to develop in red blood cells[6]. In addition to BFD1, *T. gondii* encodes 13 other Myb-domain-containing proteins. Identifying their functions and determining whether any of them aid the infection process should be a priority.

Waldman and co-workers report that *T. gondii* lacking BFD1 fail to establish a chronic infection in mice. When investigating the regulation of BFD1 expression, the authors made the counter-intuitive discovery that the messenger RNA that encodes BFD1 was expressed at a similar level during both the acute and chronic stages of infection. The presence of BFD1 is sufficient to drive parasite differentiation into a bradyzoite, and the mRNA encoding BFD1 is preferentially translated into protein during the chronic stage of infection. Leveraging this finding, Waldman *et al.* engineered *T. gondii* to express a form of BFD1 that is unstable unless a specific compound is also given. Consistent with the authors' model, the compound-mediated stabilization of BFD1 caused the parasite to form a bradyzoite.

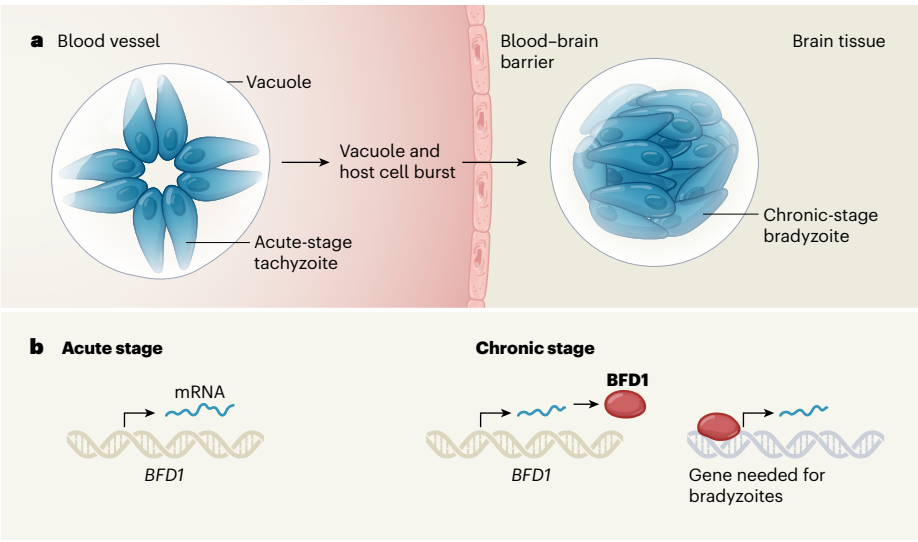

Figure 1 | How *Toxoplasma gondii* parasites differentiate to cause a chronic infection. a, *Toxoplasma gondii* infects humans, and can be life-threatening. During the initial stages of infection, the parasite exists in the bloodstream in a form called an acute-stage tachyzoite, which is in a vacuole. It is taken up by a host cell (not shown) and the cell and vacuole subsequently burst. The parasite enters the brain and gives rise to a chronic infection. Such infection occurs when the parasite differentiates into a form called a chronic-stage bradyzoite. **b**, Waldman *et al.*[3] report that the gene *BDF1* is required for this differentiation step. In both the acute and chronic stages of infection, this gene is transcribed into messenger RNA. However, the encoded protein BFD1 is preferentially made during chronic infection. BFD1 is a transcription-factor protein that can drive the expression of genes needed for the formation of bradyzoites.

This discovery raises the question of how the translation of the mRNA that encodes BFD1 is regulated, possibly in response to stress, to trigger chronic infection.

As expected, the authors observed that *T. gondii* parasites differentiated into bradyzoites after several rounds of replication in host cells *in vitro* under stressful conditions (*in vivo* stress arises, in part, from the host's mounting immune response). This process was not synchronous across all parasites being cultured or even for those in one host cell. The researchers therefore used single-cell RNA profiling of wild-type and BFD1-deficient parasites to assess gene-expression profiles associated with the differentiation event. They also investigated the regions of the parasite genome to which BFD1 binds. Gratifyingly, as expected for a transcription factor, BFD1 bound to gene regions called transcription start sites, and, in particular, to those in a large set of genes that the authors had identified as being expressed at higher than normal levels during differentiation.

Many questions remain unanswered regarding BFD1's regulation of differentiation, and how it might act upstream of a group of previously identified transcription factors called ApiAP2s, which are important, but not sufficient, for differentiation[7]. Considering that BFD1 is probably regulated by translational control, approaches that determine the RNA content of single cells might not be enough to identify the full cohort of factors driving differentiation. Another way to investigate translational control is to profile RNAs bound to the translational machinery of the ribosome complex. This method has already been used for *T. gondii*[8,9], and should be enlisted to study bradyzoites.

Bradyzoites can now be maintained in host cells grown *in vitro* without adversely affecting the host cells, opening many vistas for future experiments. Particularly exciting is the possibility of analysing bradyzoites during brain infection by using an approach that harnesses stem-cell technologies, such as those that produce neuronal stem cells. CRISPR provides a way of testing the role of host genes, and this method can also target *T. gondii* both *in vitro* and *in vivo*[10–12]. The availability of these tools sets the stage for new discoveries about the interplay between the parasite, host and immune system throughout the acute and chronic stages of infection. The development of artificial-intelligence methods that enable computer-driven assessments of complex and subtle differences in images of *T. gondii* offers another way of assessing the infection process[13].

Given that bradyzoites are the most relevant and challenging stage of the *T. gondii* life cycle to tackle for the treatment of the human disease, targeting BFD1 shows real potential for making progress in the development of drugs or vaccines. The discovery of one gene that can rule them all moves us closer to solving the riddle of this chronic infection.

Eva-Maria Frickel is at the Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Birmingham B15 2TT, UK, and at the Francis Crick Institute, London, UK.
e-mail: e.frickel@bham.ac.uk

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Structural biology

A self-activating orphan receptor

Brian Krumm & Bryan L. Roth

The first 3D structure of a full-length G-protein-coupled receptor whose natural activator is unknown has been determined, providing insights into an unusual mode of activation and a basis for discovering therapeutics. **See p.152**

G-protein-coupled receptors are the largest class of membrane protein in the human genome, and represent the most abundant pharmaceutical targets. More than 800 such receptors are known in humans, of which perhaps 100 are orphan receptors — those for which the naturally occurring (endogenous) ligand molecules that bind to and activate them have yet to be identified[1,2]. This lack of understanding of orphan G-protein-coupled receptors (oGPCRs) impedes our ability to exploit their potential as therapeutic targets. On page 152, Lin *et al.*[3] close this gap in knowledge by reporting the first 3D structure of a full-length oGPCR, GPR52, in multiple states.

GPR52 is a potential drug target for treating several neuropsychiatric disorders, including Huntington's disease and schizophrenia. When activated, it selectively binds to the G_s family of G proteins inside cells, and thereby stimulates the production of cyclic AMP (cAMP) signalling molecules, which regulate various cellular processes. Efforts to find drugs that target GPR52 would benefit from a greater knowledge of how the receptor couples to G_s and its activation process.

Lin *et al.* began their investigation of the structural basis for GPR52 activation using X-ray crystallography. In their initial studies, the authors used a variety of strategies, including extensive protein engineering, to both stabilize the receptor and enable its production in sufficient quantities to produce high-resolution crystal structures. The

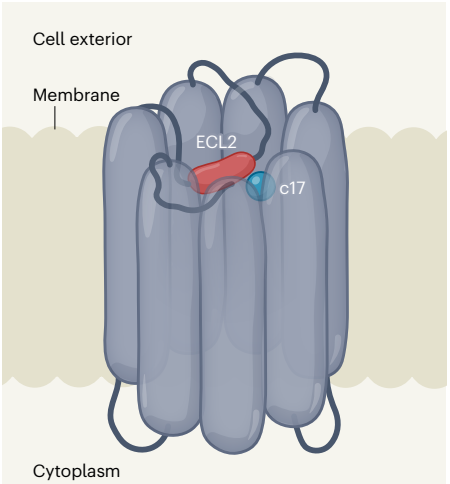

Figure 1 | Binding sites in the receptor GPR52. Lin *et al.*[3] report structures of the membrane receptor GPR52, a potential drug target for which the putative naturally occurring agonist — the ligand molecule that activates the receptor — is unknown. The authors find that a region of GPR52 known as extracellular loop 2 (ECL2) binds to a site in the receptor that is analogous to the agonist-binding site in other receptors from the same family. ECL2 seems to activate the receptor, removing the need for an external agonist. The authors also find that the synthetic molecule c17, which activates GPR52, binds to a different region next to the site bound by ECL2, and might therefore be an allosteric modulator (a compound that potentiates the activity of the receptor but does not bind at the agonist-binding site).