

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 differentiation⁷. 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 process¹³.

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.

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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

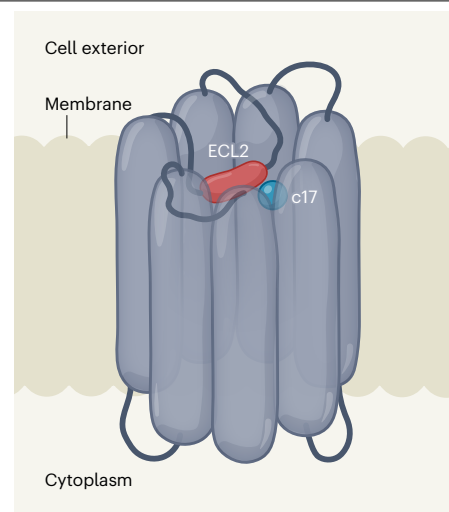


Figure 1 | Binding sites in the receptor GPR52. Lin *et al.*³ report structures of the membrane receptor GPR52, a potential drug target for which the putative naturally occurring agonist – the ligand molecule that activates the receptor – is unknown. The authors find that a region of GPR52 known as extracellular 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).

researchers thus obtained the structures of human GPR52 in the ligand-free (apo) state and in complex with c17, a synthetic molecule that acts as an agonist (that is, it activates the receptor).

Not unexpectedly, GPR52-apo adopts the GPCR architecture that has been seen in many other structures, involving seven transmembrane domains. Surprisingly, a region of the receptor known as extracellular loop 2 (ECL2) folds into what would normally be the binding site for an endogenous ligand (the orthosteric binding site), where it acts as a lid that blocks the entrance to this site (Fig. 1). Lin *et al.* observed that the activity of GPR52 is significantly diminished when ECL2 is mutated or deleted, indicating that the loop is essential for signalling activity in the receptor's native environment. Meanwhile, the crystal structure of the receptor in complex with c17 suggests that this agonist binds to a 'side pocket' that has not been observed in previously reported structures of GPCRs. The authors therefore speculate that c17 acts allosterically – at a site remote from the orthosteric binding site – to potentiate GPR52's activity.

Remarkably, the authors were then able to form a stable complex of GPR52 with a modified G_s protein in the absence of an agonist, and to obtain the structure of the complex using cryo-electron microscopy. The receptor in this complex has the structural hallmarks of previously visualized, active GPCRs captured in complex with G proteins¹. The arrangement of ECL2 in this active-state structure is the same as in the crystal structure of GPR52-apo, implying that ECL2 acts as a 'tethered agonist' under physiological conditions to facilitate signalling pathways in the absence of an endogenous agonist – similarly to the behaviour of some other GPCRs, such as the PAR1 protease-activated receptor⁴.

Most GPCRs have some basal (constitutive) activity wherein they spontaneously couple to their particular G proteins. The constitutive activity of GPR52 is exceptionally high⁵. Indeed, Lin and colleagues find that GPR52's basal activity is so great that the receptor's ability to signal by increasing cAMP levels is only slightly augmented by the addition of c17.

The authors report that this high level of constitutive activity is achieved by at least two structural features that are unusual for GPCRs: the lack of a binding site for sodium ions, and the occupation of an apparent agonist-binding site by the tethered agonist in ECL2. The sodium-binding site of GPCRs is known to be important for damping constitutive activity⁶, and so the observation that a GPCR that lacks such a site has a high level of basal activity is not entirely surprising. By contrast, the discovery of a tethered agonist that helps to maintain GPR52 in the active state in the absence of

an external agonist is truly striking. The new findings raise the intriguing possibility that, for at least some oGPCRs, the incorporation of agonists within the receptor itself obviates the need for external ligands. Indeed, several other oGPCRs that have high constitutive activities⁵ have been identified, along with others that don't have sodium-binding sites⁶.

It should be kept in mind that – as with all structural studies – Lin and colleagues' work has provided only a few snapshots of the receptor structure. Further biochemical and biophysical studies will be essential to work out the details of GPR52's dynamic behaviour under physiological conditions.

Nevertheless, the authors' high-resolution structures should aid the development of drugs that selectively target GPR52, but avoid other potential drug targets – for instance, by enabling computational studies⁷ in which ultra-large libraries of potential ligands are docked into the binding site revealed by the

structures. Moreover, if the approaches used by Lin *et al.* for the structural elucidation of GPR52 are applied to other oGPCRs that have high constitutive activity^{5,6}, they might transform our understanding of oGPCRs and accelerate their therapeutic exploitation.

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Ecology

Biodiversity theory backed by island bird data

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Analysis of a unique global data set reveals how the species diversity of birds is affected by the properties of archipelagos and offers a way to test an influential theory. Has this improved our understanding of island biodiversity patterns? **See p.92**

The thousands of islands in the Aegean Sea between Greece and Turkey have inspired countless myths and works of literature. This region is also where the word archipelago, which means a group of islands, has its roots. Archipelagos and their constituent islands have long been viewed as natural 'laboratories' for developing and testing theories that aim to answer key questions about biodiversity^{1–5}. On page 92, Valente *et al.*⁶ report an impressive analysis of birds on archipelagos worldwide that provides some of these long-awaited answers.

In the 1960s, the biologists R. H. MacArthur and E. O. Wilson proposed the theory of island biogeography^{7,8}, which is commonly used to explain observed patterns of species richness (the number of different species) on islands. This development marked the dawning of a renaissance for biogeography (the study of species distributions over space and time) that advanced this field from a largely descriptive endeavour to a quantitative and predictive science^{1–5}.

The theory of island biogeography was

inspired by two well-established patterns of species diversity. One pattern is that species richness increases if a greater area is sampled. The other pattern is that the species richness of an island is lower the greater the isolation of the island – the farther away the island is from a potential source of species, such as the closest mainland. The theory of island biogeography predicts that the species richness observed on an island is the result of the interplay between three fundamental processes – extinction, colonization (the dispersal and establishment of species from the continental landmass to an island) and speciation (the generation of new species) – and that these processes depend on island area and isolation. This theory has had a wide-reaching influence on researchers in fields including ecology and conservation biology, and has underpinned the emergence of subdisciplines in these fields, such as macroecology and metapopulation biology^{1–5}.

Yet despite a multitude of studies^{3,5} testing the theory of island biogeography, few have sought to use molecular phylogenies to