52 million years ago). Bat shoulder skeletons indicate that, by this time, the animals could fly. Whether they could echolocate is a topic of ongoing discussion[5].

Did the immediate ancestor of bats echolocate? Wings and flight capacity are common to all of the more than 1,400 living species of bat; however, in addition to the fact that not all bats echolocate, variations in echolocation behaviour contribute to bat diversity. Furthermore, Sulser and colleagues suggest that the wall-less Rosenthal's canals of Yangochiroptera are derived through evolutionary transformations from walled canals characteristic of Yinpterochiroptera. This interpretation supports the Yinpterochiroptera and Yangochiroptera classification and the view that echolocation is ancestral to bats (and lost in some Yinpterochiroptera).

Echolocation underlies the diversification of bats in both Yinpterochiroptera and Yangochiroptera. Interestingly, although yinpterochiropterans occur only in the Old World, yangochiropterans have flourished almost everywhere there are bats. There are astonishing evolutionary convergences that have driven echolocation in yinpterochiropterans and yangochiropterans.

In either proposed classification scheme (Megachiroptera and Microchiroptera or Yinpterochiroptera and Yangochiroptera), echolocation looms as a notable feature. Most species of the pteropodid family of bats do not echolocate, and the few that do use tongue clicks as echolocation signals, rather than chirps produced in the larynx (laryngeal echolocation is the echolocation mode of most bats). Sulser and colleagues' findings support the view[3] that pteropodid bats are yinpterochiropterans that lost their capacity for laryngeal echolocation during the course of evolution. A study[6] reporting patterns of growth of the ear structure known as the cochlea provided evidence that pteropodids lost their capacity for laryngeal echolocation. Another investigation[7] of bat embryological development proposed that laryngeal-mediated echolocation evolved independently in Yangochiroptera and Yinpterochiroptera. The samples from that study lacked species of three families of Yinpterochiroptera (Craseonycteridae, Rhinopomatidae and Megadermatidae) that were part of the samples assessed by Sulser and colleagues. Sulser *et al.* have used a combination of a large sample size and the presentation of previously unreported neuroanatomy to sharpen our view of the family tree of bats.

Arguably, flight combined with echolocation gave the ancestors of bats a competitive advantage over other animals, such as nocturnal birds — namely, access to nocturnal flying insects as food. Bats in both yinpterochiropteran and yangochiropteran suborders show an astonishing variation in their echolocation tactics, from the signals used to the specializations for broadcasting signals and receiving echoes, and the patterns of sound emissions.

Some bats produce very strong signals (to maximize range), whereas others generate quiet signals. Outgoing pulses are much stronger than returning echoes, so most echolocators avoid deafening themselves by separating pulse and echo in time. Simply put, they cannot broadcast and receive simultaneously.

However, using a phenomenon called a Doppler shift to separate pulse and echo in frequency allows some bats to simultaneously broadcast and receive. Some species in both suborders also use Doppler shifts to detect the flutter of the wings of a flying insect. In bats, flutter detection is much more prevalent among yinpterochiropterans (3 families and approximately 210 species) than among yangochiropterans (one family, and approximately 3 out of 12 species)[3]. The success of this approach in detecting flying insects by echolocation is central to the diversity of yinpterochiropterans. Sulser and colleagues have provided insights that also give us a fresh perspective on how bats diversified (their adaptive radiation).

Using neuroanatomical data, the authors provide robust support for the molecularly based classification of Yinpterochiroptera and Yangochiroptera, and have opened up new avenues for bat research. These extend from understanding the details of how bats use echolocation, to investigating the community structure of groups of bats (bat assemblages). Echolocation continues to be a gift that keeps on giving.

M. Brock Fenton is in the Department of Biology, Western University, London, Ontario N6A 5B7, Canada.
e-mail: bfenton@uwo.ca

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Virology

The human and mosquito receptors for alphaviruses

Caroline K. Martin & Margaret Kielian

Alphaviruses are transmitted by mosquitoes to many species, and can be fatal to humans. The identification of virus receptors that are evolutionarily conserved between mosquitoes and humans might explain the wide range of viral hosts. **See p.475**

The group of viruses called alphaviruses can cause severe disease, including inflammation of the brain (encephalitis) and of joints (arthritis). Despite the high disease potential of these viruses, which can cause fatal illness, there are currently no licensed vaccines or antiviral therapeutics available to tackle human alphavirus infections. On page 475, Clark *et al.*[1] now pinpoint key targets that enable several of these viruses to infect cells (Fig. 1).

In general, alphaviruses can infect many different types of animal host, including mosquitoes, which act as a vector to transmit the viruses to humans, birds, horses and other vertebrates. To successfully infect a host cell, an alphavirus — which is surrounded by a membrane envelope — needs to deliver its genome through the cell's membrane into the cytoplasm. This pathway involves the virus binding to specific proteins on the cell surface called virus receptors, which are key determinants of infection of hosts and tissues.

After an alphavirus binds to a receptor, the virus is internalized into the cell inside a membrane-bound structure called a vesicle. As the vesicle and its alphavirus cargo are transported, the vesicle becomes increasingly acidic, which triggers the fusion of the virus's membrane envelope with the vesicle membrane[2]. This releases the viral genome into the cytoplasm. These stages of alphavirus entry have been extensively studied using Semliki Forest virus (SFV) as a model system, and many other alphaviruses have been shown to have similar entry properties. However, the specific cell-entry receptors for SFV and for eastern equine encephalitis virus (EEEV), an alphavirus associated with lethal human disease, have remained elusive until now.

The arduous process of identifying receptors for a virus of interest has been revolutionized by the introduction of methods that

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use small interfering RNAs or a gene-editing technique called CRISPR–Cas9 to screen candidates. These approaches enable thousands of cellular protein candidates to be tested in parallel, and can identify host factors that promote or inhibit viral infection.

To qualify as a virus receptor[3], a candidate protein needs to be not only essential for infection, but also expressed on the membrane of susceptible cells, to bind directly and specifically to the virus and to promote genome entry by, for example, mediating virus internalization. To complete the picture, the interaction of a virus-receptor candidate (and virus infection) should be blocked by anti-receptor antibodies, by soluble forms of the receptor (which bind to the virus and hinder its interaction with the full-length receptor) and by mutations that disrupt the receptor's virus-binding region, or by some combination of these. Genetic screens using cells from fruit flies or humans led to the current list of known alphavirus receptors. This includes NRAMP2 for Sindbis virus[4] (SINV), MXRA8 for chikungunya (CHIKV), Ross River, Mayaro and O'nyong nyong viruses[5] and LDLRAD3 for Venezuelan equine encephalitis virus[6] (VEEV).

Clark and colleagues used a CRISPR–Cas9 approach to investigate candidate alphavirus receptors, and showed that a human cell line lacking the protein VLDLR and its close relative ApoER2 was resistant to infection by SFV, EEEV and SINV. Artificial expression of either receptor allowed these viruses to enter and infect the cells, albeit with varying efficiencies. Remarkably, virus infection of this cell line was increased by the expression of VLDLR not only from species such as humans and horses, but even from those as evolutionarily distant as mosquitoes and nematode worms. This supports a role for these proteins as receptors in both vertebrate hosts and the mosquito vector. SFV is more closely related to CHIKV than it is to either EEEV or SINV[7]. However, interestingly, Clark *et al.* show that SFV cannot use CHIKV's receptor MXRA8 for entry, nor can CHIKV enter cells using VLDLR or ApoER2.

But how do VLDLR and ApoER2 promote infection by SFV, EEEV and SINV? A clue might come from the receptors' function in uninfected cells. VLDLR and ApoER2, as well as the VEEV receptor LDLRAD3, belong to the low-density lipoprotein (LDL) receptor family[8], a group of related cellular membrane proteins that recognize lipoproteins and transport them into the cell using the same vesicle pathway as that described for alphaviruses. Although these receptors differ in their tissue distribution and in their lipoprotein cargo, they share the same fundamental building blocks (Fig. 1a): an extracellular ligand-binding domain (LBD), a transmembrane domain and an intracellular 'tail' that interacts with the cellular transport machinery[8].

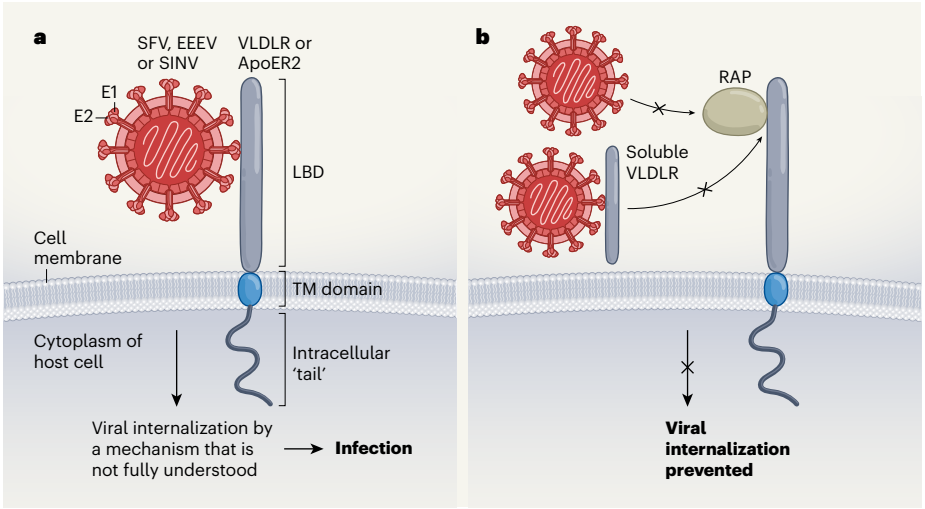

Figure 1 | Evolutionarily conserved receptors enable alphaviruses to enter cells. Alphaviruses infect a wide range of host cells, and can cause fatal human disease. **a**, Clark *et al.*[1] report that the receptor protein VLDLR (and a related protein ApoER2), which is found in organisms as diverse as humans and mosquitoes, enables the alphaviruses Semliki Forest virus (SFV), eastern equine encephalitis virus (EEEV) and Sindbis virus (SINV) to enter human cells. This type of receptor has a ligand-binding domain (LBD), a transmembrane (TM) domain and an intracellular 'tail' region. Clark and colleagues demonstrate that the viral proteins E1 and E2 bind directly to the LBD of the receptor. This binding is required for virus entry, although how the internalization process occurs remains to be fully described. **b**, In addition to supporting alphavirus infection, VLDLR exhibits other hallmarks of being a true virus receptor. For example, Clark *et al.* show that a VLDLR-binding protein called RAP or a soluble version of VLDLR (a truncated version containing only the LBD) prevent virus entry.

Viruses have evolved different strategies to exploit the LDL receptor family. Hepatitis B virus, for example, coats itself with cellular lipoproteins to activate binding and uptake by the LDL receptor[9]. Clark *et al.* present evidence that SFV, EEEV and SINV use a strategy that does not rely on lipoproteins. Instead, the complex of proteins E2 and E1 on the viral membrane seems to dock directly with the LBD of VLDLR and ApoER2. This model is supported by structural analysis[10,11] of VEEV bound to its receptor LDLRAD3, which shows direct contact of the receptor with E2 and E1. Future structural studies of SFV, EEEV and SINV with their receptors, together with analysis of mutated versions of the LBDs, could clarify the specific interactions involved and enable their comparison with those of other alphaviruses and their receptors.

It is unclear how binding to VLDLR or ApoER2 mediates virus internalization, and whether this process is similar for other alphaviruses and receptors. In the case of LDLRAD3 and VEEV, an extracellular LBD attached to the cellular membrane by a lipid anchor is sufficient to mediate infection, and neither the receptor's transmembrane domain nor the intracellular tail is required[5]. Given that the intracellular tail contains a motif (protein region) that is key for internalization, this suggests that VEEV-bound LDLRAD3 can trigger uptake through an alternative mechanism that does not depend on this motif. Perhaps a VEEV particle binding to several LDLRAD3 receptors causes sufficient receptor clustering to induce internalization, or mediates the recruitment of another type of receptor.

So far, although all the known alphavirus receptors promote virus uptake, none has yet been demonstrated to be internalized along with the virus. That means that interesting questions remain regarding the events that occur after the interaction between each virus and its receptor. Gaining more in-depth mechanistic and structural understanding of this process might aid the development of targeted therapeutics for alphaviruses, and potentially for other virus groups that use members of the LDL receptor family for cellular entry. For example, SFV infection usually kills newborn mice after three days, but Clark *et al.* show that blocking virus–receptor binding by adding a soluble form of VLDLR significantly improves survival time compared with that of untreated control mice.

The authors report that the addition of receptor-associated protein (RAP), a binding partner for many LDL family receptors, inhibits infection by SFV, EEEV and SINV in cells grown *in vitro* (Fig. 1b). Human RAP binds to VLDLR and ApoER2 from different species, including human, horse and mosquito. This suggests that RAP, or a small-molecule drug that binds to the same receptor site as RAP, might prevent alphavirus infection in various mammalian hosts. Treatment with a fragment of RAP protects mice from lethal infection with Rift Valley fever virus, a bunyavirus that uses a member of the LDL receptor family for entry[12]. RAP or a general LDL receptor-inhibiting drug might thus have the potential to be developed as an antiviral against several virus groups.

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Clark and colleagues' work identifying VLDLR and ApoER2 as alphavirus receptors also demonstrates that the receptors' function is evolutionarily conserved for the human, mosquito and horse versions of these proteins. Thus, the authors not only provide information on important receptors for SFV and EEEV, but also offer a possible explanation for how these alphaviruses infect such a wide range of hosts.

Caroline K. Martin and **Margaret Kielian** are in the Department of Cell Biology, Albert Einstein College of Medicine, New York, New York 10461, USA.
e-mails: caroline.martin@einsteinmed.edu; margaret.kielian@einsteinmed.edu

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Astronomy

The glowing dusty heart of a hidden quasar

Robert Antonucci

The torus of dust surrounding a quasar — a very luminous supermassive black hole that accretes matter from its surroundings — has now been captured with high-resolution infrared imaging. **See p.403**

An active galactic nucleus is a relatively tiny region at the centre of some galaxies that has abnormally high luminosity. Quasars are the most powerful active galactic nuclei. On page 403, Gámez Rosas *et al.*[1] report very sharp and sensitive imaging of a nearby active galactic nucleus, showing a glowing doughnut-shaped object surrounding the central black hole.

Such 'dusty tori' are generic to supermassive black holes that are accreting matter at rates high enough to produce conspicuous amounts of light (Fig. 1). They are essential to the widely accepted unified model for active galactic nuclei, which itself derives from optical polarimetry measurements. The dust in the torus must re-radiate the optical-light energy it absorbs from the quasar in the infrared, and this infrared emission is well studied spectrally. But the obscuring torus is very compact and it has never been imaged satisfactorily. Now, Gámez Rosas and colleagues have taken the first crude picture of the torus glow.

The story of the unified model began around 1980 when I was in graduate school at the University of California, Santa Cruz. I was keen to work on active galactic nuclei, but my adviser Joe Miller described the field as mere 'stamp collecting' because of the wide variety of inexplicable behaviours seen among this seemingly highly heterogeneous group.

These behaviours include central luminosities up to thousands of times that of entire galaxies from regions no larger than the Solar System; apparent faster-than-light motion in radio images; and pairs of synchrotron-radiation-emitting clouds that span hundreds of thousands of parsecs (1 parsec is 3.08 light years) and can contain 10^{54} joules of energy or even more. We know today that these beacons lie at the centres of galaxies, and they are all ultimately powered by the gravitational potential energy of matter falling towards supermassive black holes with masses millions to billions of times that of the Sun.

Most of the optical–ultraviolet spectra for active galactic nuclei fall into two classes. In the spectra for type 1 objects, one sees a powerful variable continuum, which most people assume is thermal radiation from hot accreting gas, although there is no successful predictive model for the actual accretion and radiation process[2]. The inner region of quasars also hosts a site comprising many dense, rapidly moving gas clouds, known as the broad emission line region. This continuum and these fast-moving clouds are signatures of the black hole. Well outside these features lies the narrow-line region, consisting of rarefied ionized gas, on scales of 10 to 1,000 parsecs. For type 2 objects, only the narrow-line region is seen.

Miller had built a unique instrument that sorted photons according to their polarization as well as their wavelength, in the hope that clues resided in that neglected aspect of quasar radiation. This really paid off: we used the instrument to isolate traces of light polarized by scattering near the heart of active galactic nuclei. We discovered that there is an extremely convenient natural periscopic mirror in many of the type 2 objects. This mirror allowed us to view a nucleus from a direction roughly perpendicular to our actual line of sight from Earth.

The polarized light spectra for the type 2 objects showed exactly the black-hole-related components seen previously only in the type 1 objects. Thus, we knew for certain that the type 2 objects possess the components relating to black holes, and that those components would be seen directly only along the axis of the active galactic nucleus. Our inference was that the other equatorial directions must be blocked by a torus. In fact, if astronomers are distributed randomly in the Universe, around half of them must classify our type 2 objects as type 1! As for the narrow region, it's too large to be obscured by the torus, and, in retrospect, this had hinted at black-hole activity in the nuclei even in the type 2 objects.

Many observations, especially those of radio-emitting jets of plasma, indicate that active galactic nuclei are approximately axisymmetric objects, and reveal their axes as projected on the plane of the sky. Given the polarization angles of the scattered light, we know that when the scattered photons first stream out of the nuclear region, they do so roughly along the jet direction. Apparently, those emitted near the equatorial plane are blocked: there must be some opaque structure that acts like an equatorial torus in its shadowing properties. We often refer to it as the active galactic nucleus torus, but this is shorthand for the shadowing geometry and doesn't provide any detail about the actual structure beyond its shadowing properties.

Emitted photons would spray out of this nuclear configuration in a broad bicone, which should be seen in polarization images, and also in the lines corresponding to highly excited gas in the narrow-line region. Although a range of morphologies has been seen, the overall picture has been amply verified by many astronomers[3–5].

All this work was the opposite of clever. The key inferences are deductive and thus very robust. It's also very natural for an accreting object to accumulate material in a plane set by its net angular momentum[6]. But this rotating equatorial matter is more than a passive structure casting a shadow: excellent early theory papers showed that cloud–cloud collisions

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