By contrast, in mice genetically engineered to lack the enzyme that normally degrades retinoic acid, the resulting increase in retinoic acid signalling led to the development of more projections between the medial thalamus and the mPFC. It also increased expression of *Rorb*, a gene that is expressed by a population of cells in layer 4 of the cortex that receive inputs from the thalamus and that are present in the PFC of humans and other primates, but mainly absent in the mouse PFC[9].

It is still unclear how the reciprocal connections between the mPFC and medial thalamus contribute to human brain function, but they could be involved in coordinating computations in the cortex, facilitating cognition and flexible decision-making. Shibata and colleagues' discovery of mechanisms that control the development of the medial thalamus–mPFC pathway might lay the foundation for detailed investigations of the development and function of these connections in non-primate model organisms.

In their second article[3], Shibata *et al.* focused on *CBLN2*, a gene that is enriched in the PFC and that encodes the synapse-organizing protein cerebellin 2. *CBLN2* is expressed by more types of PFC neuron in humans than in macaques or mice. Building on their observation that *CBLN2* expression is increased by retinoic acid signalling, the authors examined putative CREs near this gene, and discovered one such DNA sequence that promotes gene expression — called an enhancer — and that is active during early PFC development. This enhancer contains several sites to which retinoic acid receptors can bind, leading to increased *CBLN2* expression in response to retinoic acid (Fig. 1b).

The authors found that the enhancer also contains several sites to which a protein called SOX5 can bind to suppress gene expression. Comparisons of the enhancer sequence in different species revealed that two deletions that probably occurred between about 7 million and 12 million years ago removed some of the SOX5 binding sites from the genome of the common ancestor of humans and chimpanzees. In cultured cells, the human and chimpanzee *CBLN2* enhancers were not suppressed by SOX5, whereas the gorilla and macaque versions of the enhancer were moderately suppressed, and the mouse version, which has more SOX5 binding sites than the primate versions, was most strongly suppressed.

The authors then generated mice in which the *Cbln2* enhancer was replaced by the human version to determine which features of the brain were specifically related to this single CRE change. Remarkably, compared with unmodified animals, mice with the human enhancer had more neuronal structures called dendritic spines (Fig. 1b), which are involved in synapse formation, as well as higher expression of *Cbln2* during PFC development and more synaptic structures in the PFC both during development and in adulthood. These results suggest that the symphony of human PFC evolution involves a coordinated increase in the expression of an ensemble of genes through retinoic acid receptor signalling that acts in *trans*, and a further 'crescendo' of increased expression of at least one response gene, *CBLN2*, through the loss of SOX5 binding sites in an enhancer element acting in *cis*.

These studies leave several questions unanswered. First, what is the source of the relatively high levels of retinoic acid in the primate brain? The authors analysed the expression of several retinoic acid-synthesizing enzymes, but, given that these enzymes are expressed in various types of neuronal and non-neuronal brain cell and in the meningeal layers that envelop the brain, it is difficult to narrow down which cell type or types and evolutionary changes are responsible.

Second, at which points during primate evolution did these changes in *cis*- and *trans*-acting regulators occur? Shibata and colleagues found a graded increase in retinoic acid signalling from mouse to macaque to human[2], as well as a previously unrecognized area of this signalling in the inferior temporal cortex (another area of the cortex that is important for high-level cognition) in humans, but not in mice or macaques. However, whether these differences are truly human-specific and are absent from our closest living relatives, chimpanzees, remains untested. Similarly, although the CRE changes near the *CBLN2* gene are shared by humans and chimpanzees, other molecular and genetic changes that have arisen exclusively in humans, concomitant with the evolution of key traits such as language and syntactical grammar, remain to be investigated.

More broadly, these studies provide an intriguing example of *cis*- and *trans*-acting mechanisms that act in concert to change key properties of the brain. Only further studies of primate brain evolution can reveal whether this symphony of mechanisms is the exception or the rule in the evolution of other structural and behavioural specializations of the human brain.

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Condensed-matter physics

Multiple phases in untwisted trilayer graphene

Thiti Taychatanapat

Superconductivity and magnetism have been observed in layered graphene in which the sheets are twisted with respect to each other. But a simpler, more stable graphene system also exhibits these phases. **See p.429 & p.438**

Electrons typically propagate without interacting in graphene, a single layer of carbon atoms arranged in a honeycomb lattice. But when three sheets of graphene are stacked on top of one another so that their lattices are aligned but offset — forming rhombohedral trilayer graphene — an electric field can be used to induce interactions between the electrons. In two papers in *Nature* (pages 429 and 438), Zhou *et al.* report that these interactions give rise to ferromagnetism[1], the type of magnetism found in iron magnets, and superconductivity[2] (zero electrical resistance). These states have already been observed in other trilayer configurations, in which the graphene sheets are slightly rotated out of alignment with each other or with a substrate, but rhombohedral trilayer graphene is more structurally stable than these materials.

The crystal structure of rhombohedral

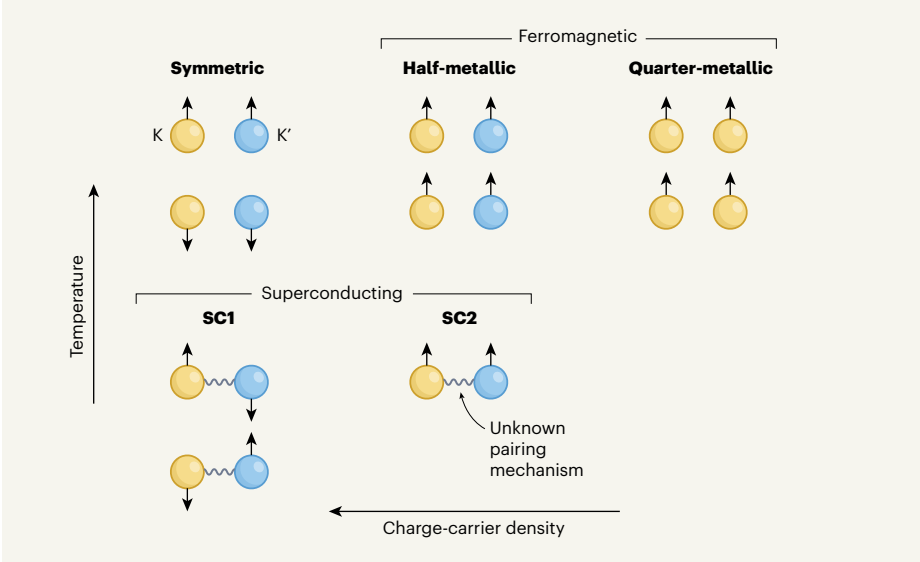

Figure 1 | Phases in rhombohedral trilayer graphene. Zhou *et al.*[1,2] studied rhombohedral trilayer graphene, in which three sheets of graphene are layered such that their lattices are offset (not shown). When the density of charge carriers (electrons and their positive counterparts) is high, this material exhibits a symmetric phase, in which electrons are equally likely to have their spins (angular momenta) pointing up or down, and energy minima known as valley degrees of freedom take values K (yellow) and K′ (blue), which are equally probable. At intermediate carrier density, the spin directions align and the system becomes a half-metallic ferromagnet, characterized by the type of magnetism seen in iron, but the valley variables are distributed evenly between K and K′. At low carrier density, there is a quarter-metallic phase, in which all the spins point in the same direction and valley degrees of freedom take only one value. At very low temperatures, superconducting phases (SC1 and SC2) emerge from the symmetric and half-metallic phases, respectively. These phases require a pairing between two electrons, of a form that is not yet known.

trilayer graphene leads to a particular relationship between the kinetic energy and momentum of each electron, known as the material's band structure. Applying an electric field modifies this band structure such that a large number of electrons with different values of momentum can populate a narrow range of energies[3], and this strengthens the interaction between electrons.

Zhou *et al.* discovered that their layered graphene exhibited multiple electronic ground states by measuring its electrical resistance and its electronic compressibility, which is the change in the energy of a system when an extra electron is added. Typically, the compressibility is positive because the energy of the system increases with each electron added, much as the level of water in a cup rises as more is poured in. However, when the authors varied the electric field and the electron density, they detected multiple regions of positive compressibility separated by boundaries of negative compressibility, which usually indicates a phase transition between different electronic ground states[4]. Imagine that our cup can expand if the water level reaches a certain height — immediately after the expansion, the water level will go down, even as more water is added.

To understand more about these phases, Zhou *et al.* studied the system's response to in-plane and out-of-plane magnetic fields. An electron has an intrinsic magnetic moment, which points either up or down depending on its angular momentum (also known as spin). When electrons do not interact, it is energetically favourable to have equal numbers with spin up and spin down, resulting in no net magnetic moment. Electrons can also be characterized by two minima in their band structure, known as their valley degrees of freedom, taking values K and K′. Together, these four states — (up, K), (up, K′), (down, K) and (down, K′) — define the isospin of an electron.

"Rhombohedral trilayer graphene is easier to obtain than twisted trilayer graphene because it exists in nature."

When the density of charge carriers (electrons and their positive counterparts) is high, all four isospin states are equally represented, yielding a symmetric phase with no net magnetic moment (Fig. 1). But in rhombohedral trilayer graphene, Zhou *et al.*[1] found two distinct ferromagnetic phases at intermediate and low carrier densities. At the intermediate density, they observed a half-metallic phase, in which all the electron spins were pointing the same way, but the valley variables were evenly distributed between K and K′. At the low density, both spins and valleys took a single value, and the authors dubbed this a quarter-metallic state, referring to the spin–valley combination taking one state of four possibilities. These phases differ from those in most conventional ferromagnets, in which both types of spin are present, but in different proportions.

Two distinct superconducting phases also emerge in Zhou and co-workers' system: one (SC1) from the symmetric phase below a transition temperature of 100 millikelvin (mK) and one (SC2) from the half-metallic phase below a transition temperature of 20 mK. Superconductivity relies on a pairing mechanism that allows two electrons to overcome the repulsion arising from their like charges. In a conventional superconductor, this pairing occurs between electrons with opposite spins through vibrations of the crystal lattice that hosts the electrons. A magnetic field can therefore destroy superconductivity by aligning the electron spins, and breaking the pairing.

Zhou *et al.*[2] applied an in-plane magnetic field, which couples to the electron spins, and observed that the superconductivity in SC1 disappeared at a magnetic field strength of 300 millitesla (mT). This is commensurate with the magnetic field strength required to align spins in a conventional superconductor, known as the Pauli paramagnetic limit.

However, the authors found that the superconductivity in SC2 persisted to much larger values of magnetic field strength — more than 1 T — violating the Pauli paramagnetic limit by at least a factor of ten. This large field strength, together with the fact that all the spins usually point the same way in SC2, suggested a pairing between the electrons with aligned spins.

Although SC1 and SC2 behave differently under a magnetic field, they share a few common features. For example, they are both characterized by an annular Fermi surface, which describes the momenta of electrons at a given energy. Both states also occur at parameters that are close to those at which phase transitions to more ordered states take place. On the strength of this evidence, Zhou *et al.* hypothesized that fluctuations in the ordered states, such as spin, might mediate a pairing mechanism, as proposed in other systems[5].

Superconductivity and magnetism have been observed previously in moiré graphene systems, in which there is rotational misalignment between layers. For example, ferromagnetism[6] and signatures of superconductivity[7] have been reported in moiré rhombohedral trilayer graphene, and the Pauli limit has also been found to be violated in another type of twisted trilayer graphene[8]. But the lattice structure of moiré systems is more complicated than that of rhombohedral trilayer graphene, making it more difficult to study. Rhombohedral trilayer graphene is also easier to obtain because it exists in nature, whereas moiré systems need to be engineered.

Zhou *et al*. measured the electronic compressibility in moiré rhombohedral trilayer graphene and found it largely unchanged compared with the untwisted material, with the exception of extra energy gaps in the band structure introduced by the twist. The similarity between the behaviour of the ferromagnetic and superconducting states in rhombohedral trilayer graphene and moiré graphene systems might hint at a close connection between the mechanisms underlying these states.

Further studies in rhombohedral graphene will be required to confirm and determine the origins of these phases. For example, measuring a type of particle scattering known as Andreev reflection[9] will provide further evidence that the spins are fully aligned in the quarter- and half-metallic phases even in the absence of a magnetic field. The simple lattice structure and reproducibility of rhombohedral trilayer graphene could help theoretical and experimental studies aimed at revealing the nature of the phases identified by Zhou and colleagues — and might offer insights into other moiré graphene systems.

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Drug discovery

A step towards dengue therapeutics

Scott B. Biering & Eva Harris

Finding a treatment for dengue, the most prevalent mosquito-borne viral disease in humans, has been difficult. A compound called JNJ-A07 displays promising activity against dengue virus in mouse models of infection. **See p.504**

Despite immense effort spent in pursuit of a therapeutic for dengue, no treatment is currently available. An effective drug against dengue virus (DENV) is urgently needed, because this mosquito-transmitted virus represents a tremendous public-health burden, with about 3.9 billion people at risk of infection (see go.nature.com/3fbf9xz) and an estimated 51 million cases of dengue annually worldwide[1]. Several challenges have precluded the development of such a therapeutic: it must be administered orally, must rapidly lower the amount of virus in the bloodstream to stem progression to severe dengue, and must be similarly potent against all four main types of DENV (dubbed serotypes DENV-1 to DENV-4), all while exhibiting an acceptable safety profile. Excitingly, on page 504, Kaptein *et al*.[2] describe a compound that targets the dengue virus protein NS4B and ticks all of these boxes.

The lead candidate compound identified by Kaptein and colleagues, JNJ-A07, emerged from a set of 2,000 related molecules that were generated by making chemical modifications to a compound already selected for its ability to inhibit DENV-2-mediated cell death[3]. The authors found that JNJ-A07 was highly effective against all tested genetic variants of the four serotypes, including 21 different virus strains isolated from infected individuals. The effects of JNJ-A07 were tested in various cell types, including mosquito cells and human dendritic cells — a type of immune cell thought to be a key target of DENV infection in humans. Notably, this compound was not effective against other viruses, including other closely related members of the *Flavivirus* genus, suggesting that it is highly specific for DENV.

Because DENV is an RNA virus that evolves rapidly to escape antiviral pressures such as drugs, the authors investigated the ability of DENV-2 to escape the antiviral activity of JNJ-A07 in cell culture. Mutant virus strains that could escape the antiviral effects of JNJ-A07 were not detected until about 15 weeks after infection, and complete resistance of DENV-2 emerged only after about 40 weeks of continuous exposure to the compound. Resistance was correlated with mutations in the virus's RNA that caused substitutions of three amino-acid residues in the NS4B protein, suggesting that this protein is the target of the compound. The length of time taken to develop resistance and the requirement for multiple mutations suggest that the virus would have to overcome a high barrier to become resistant to JNJ-A07.

Crucially, the resistance mutations that enabled DENV-2 to escape the antiviral activity of JNJ-A07 in human cells also rendered the virus unable to replicate in mosquito cells. This indicates that, even if it did develop resistance to JNJ-A07 in individuals treated for dengue, the mutant virus would be at a 'dead end', given that it relies on mosquitoes for transmission to its next human host. Such a possibility is intriguing, and requires follow-up investigations in animal models.

The NS4B protein spans the membrane of an organelle in the host cell called the endoplasmic reticulum, and is crucial for the replication of DENV[4]. The protein interacts directly with another viral protein, NS3, which catalyses reactions involving proteins and nucleic acids[4]. The authors found that JNJ-A07 blocks the formation of the NS3–NS4B complex (Fig. 1). This mechanistic detail is valuable, but further structural studies demonstrating the direct interaction between JNJ-A07 and NS4B would provide insights into how it inhibits viral replication and could inform the design of other NS4B inhibitors. Further work should evaluate the ability of JNJ-A07 to interfere with other known interactions of NS4B (including those with the viral proteins NS1, NS2B and NS4A), and with other functions of NS4B, such as its ability to form dimers and to modulate the host's immune response[4].

Dengue virus does not normally replicate easily in mice. Therefore, the authors investigated the effects of JNJ-A07 in DENV-infected mice that had been genetically engineered to lack a set of proteins involved in interferon signalling, which is involved in the immune response to viruses. Kaptein and colleagues infected these immunocompromised mice with lethal or sublethal amounts of DENV-2, and found that JNJ-A07 was highly effective as an antiviral drug. It rapidly decreased virus levels in the bloodstream, reduced the production of inflammatory molecular signals called cytokines (which are thought to contribute to severe dengue disease) and prevented mice from dying of the infection. Furthermore, the efficacy of JNJ-A07 was observed when it was given either therapeutically (four to five days after DENV infection) or preventively.

The efficacy of this compound in mouse models warrants moving JNJ-A07 forward into investigations of its safety profile, how it moves around the body, its potency against all four serotypes in animals, studies in non-human primates and, eventually, if warranted, clinical trials in humans. It will also be crucial to assess the efficacy of JNJ-A07 in