effect completely dominates, and electron motion between layers is suppressed by a kinematic barrier[5].

However, at very low rotation angles, a moiré pattern is produced by the misaligned lattices (Fig. 1b); the unit cell is greatly enlarged and so the effects of this come into play[6,7]. The misalignment of the band structure essentially disappears, and theory predicts that the low-energy electronic states are completely reconstructed[7]. Coupling between electrons in the different layers becomes strong, and new narrow bands emerge at certain 'magic' rotation angles below 1.05° when the bilayer system is close to charge neutrality. Electrons in these narrow bands are found mainly in regions of the moiré pattern in which the atoms are stacked directly above each other (the light regions in Fig. 1b). In these circumstances, the bilayer can be thought of as a synthetic, triangular lattice of weakly coupled quantum dots (tiny semiconductor particles that bind electronic states) with a residual tunnelling of electrons between them[6].

Cao *et al.* fabricated twisted bilayer graphene so that the sheets are rotated at magic angles, and accumulated or depleted charge carriers in the system to study how the charge-transport properties of the system depend on the filling of the energy bands. The authors observed[2] strong insulating behaviour when each unit cell of the synthetic lattice contained four charge carriers, a density that corresponds to complete filling of the bands. Intriguingly, they also find evidence for additional insulating states at lower densities in which the number of carriers per unit cell is an integer, but for which the narrow energy bands of the system are fractionally occupied. This suggests that the additional states are Mott insulating states, in which free motion of the carriers is prevented by their mutual repulsion, producing gridlock on the lattice. Mott insulators are a strongly correlated, non-conducting form of matter.

Even more intriguing is what happens when charge carriers are added to the Mott-insulator states associated with half-full unit cells of the synthetic lattice. The authors observe[1] that the system enters a state that has zero electrical resistance below a critical temperature of approximately 1.7 kelvin, in a phase change known as a Berezinskii–Kosterlitz–Thouless transition, thus forming a 2D superconductor. This transition temperature is remarkably high, given the very low carrier density achieved in these measurements (10^{11} charge carriers per square centimetre). The high transition temperature and the apparent connection to correlated insulating states invites comparison of this superconducting state to that of a family of 'unconventional' superconductors[8], which also have a close relationship with other strongly correlated electronic ground states. Twisted bilayer graphene might therefore be a useful experimental system in which to investigate the mechanism of unconventional superconductivity.

In the meantime, Cao and colleagues' discoveries will stimulate a wave of activity as scientists seek to unwind the microscopic basis for the reported striking phenomena. The findings also demonstrate the promise of using twisted bilayer graphene as a flexible and tunable platform in which correlated electronic phenomena can be readily observed, and possibly even engineered and exploited[9]. ■

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STRUCTURAL BIOLOGY

Two-pore channels open up

Two-pore channels span the membranes of acidic organelles inside cells. A structural and functional analysis reveals secrets about how these channels open to allow ions to pass across the membrane. SEE LETTER P.130

SANDIP PATEL

Two-pore channels (TPCs) are an ancient family of ion channels that are unusual because they are found, not at the cell surface, but spanning the membranes of acidic organelles such as endosomes and lysosomes. These organelles mediate biomolecule transport and breakdown, and serve as stores of calcium ions[1] (Ca^{2+}). TPCs are key for several organellar functions — releasing Ca^{2+} into the cytoplasm to control trafficking of material such as receptor proteins and viruses, for instance, and stabilizing junctions with other organelles[1,2]. They are increasingly being associated with disorders such as Parkinson's disease, and are therefore emerging as potential therapeutic targets[1]. Detailed structural information is scant, but advances in cryo-electron microscopy are revolutionizing our ability to study ion channels. On page 130, She *et al.*[3] use this technique to provide the first detailed view of an animal TPC.

Previous work[4,5] has reported the atomic structure of a plant TPC. This consists of two subunits, each containing two similar transmembrane domains (6-TMI and 6-TMII) connected by a large cytoplasmic linker. 6-TMI and 6-TMII are in turn each made up of six membrane-spanning regions, dubbed S1–S6. The pore through which ions flow is formed by S5 and S6 from each transmembrane domain in each subunit.

She *et al.* resolved the structure of mouse TPC1. Their results revealed that the overall folding of this channel is, as expected, like that of plant TPC. Nonetheless, there is a surprising degree of structural conservation between the linkers, given that animal and plant TPCs have very different amino-acid sequences in this region.

There are some structural differences, however. In plant TPC, the linker binds Ca^{2+} to help open the channel[4,5]. But Ca^{2+} binding by mouse TPC1 is unlikely, because amino acids essential for this interaction are missing. And the authors show that the carboxy-terminal domain of mouse TPC1, which is longer than the equivalent domain in plant TPC, forms a horseshoe-shaped arrangement of four helices that makes direct contact with the linker. This animal-specific feature probably serves to fine-tune channel activity.

Activation of animal TPCs is complex and multifaceted. These channels were originally identified[6,7] as the targets for a messenger molecule called NAADP, which releases Ca^{2+} from acidic organelles[8]. Subsequent work revealed[9] that TPCs are also activated by the lipid $PI(3,5)P_2$. In addition, TPC1 is regulated by changes in voltage across the organelle membrane[10,11]. She *et al.* demonstrated that both $PI(3,5)P_2$ and voltage changes are required to open TPC1; neither alone is sufficient (they did not examine NAADP). The authors then resolved structures of TPC1 in both the absence and presence of $PI(3,5)P_2$, giving insight into the structural transitions that occur during channel opening. This analysis produced two key findings.

First, the group pinpointed the $PI(3,5)P_2$ binding site, which lies in 6-TMI (Fig. 1). Mutation of any one of several amino-acid residues in the network that forms this binding site can prevent TPC1 activation by $PI(3,5)P_2$. Interestingly, two of these residues — arginines in a short linker between S4 and S5 — are also required[12] for channel activation by NAADP. This suggests that $PI(3,5)P_2$ probably acts as a cofactor for NAADP action. Comparison of the free and $PI(3,5)P_2$-bound forms of TPC1 revealed that a single lysine residue in S6 transmits conformational changes to the pore in response to $PI(3,5)P_2$ binding, thus directing the first stage of channel opening.

Second, the authors found that changes in voltage are sensed by arginine residues in 6-TMII (Fig. 1). Both 6-TMI and 6-TMII contain sequences in S1–S4 that are reminiscent of voltage sensors in other channels, but only 6-TMII has a specific helix in S4 that is required for voltage gating. The 6-TMII voltage sensor is in an upward, 'activated' form in both structures obtained by the authors — in this form, it can probably transmit changes to the pore, to which it is adjacent, completing opening of the channel.

She and colleagues' work on TPCs from animals, together with analyses[4,5] of plant TPCs, indicate that both 6-TMI and 6-TMII cooperate to open the channel. 6-TMII is a target for voltage changes in both proteins. By contrast, 6-TMI is targeted directly by $PI(3,5)P_2$ in animal TPC1, and indirectly by Ca^{2+} in plant TPC. This is a prime example of how evolutionarily distant proteins have adapted to conserve a core function.

Which ions pass through animal TPCs once they open? Much research suggests that these channels are non-selective, like plant TPC, but some work indicates that they are selective for sodium ions[1,9] (Na^+). She *et al.* found that TPC1 was about 70 times more permeable to Na^+ than to potassium ions (K^+). Their structures reveal that the narrowest part of the pore through which ions are filtered is shaped like an oblong 'coin slot', constricted by specific asparagine residues. The authors provide evidence that these residues allow the small Na^+ ions through, but not the larger K^+ ions. This sieve effect is unlikely to explain the authors' data indicating that TPC1 apparently selects for Na^+ over Ca^{2+}, because these ions are about the same size. However, the electrophysiological experiments used by the researchers to determine ion selectivity were performed under very different conditions from those in live cells, where the permeability of TPCs to Ca^{2+} is readily demonstrable[13].

In sum, She and colleagues' structures provide major insight into how TPCs work. They join recently reported structures[14–16] for a related family of ion channels, the TRP mucolipins (TRPMLs). Like TPCs, TRPMLs reside in acidic organelles, are activated by $PI(3,5)P_2$ and release Ca^{2+} to control cellular functions such as gene transcription[17]. The $PI(3,5)P_2$ binding site in TRPMLs is probably in the protein's amino-terminal region[16,17] and is thus very different from that in TPCs, although it has yet to be directly observed.

These rapid advances in the structural biology of organellar ion channels will aid future attempts to rationally design drugs that modulate ion flux through the channels. This is

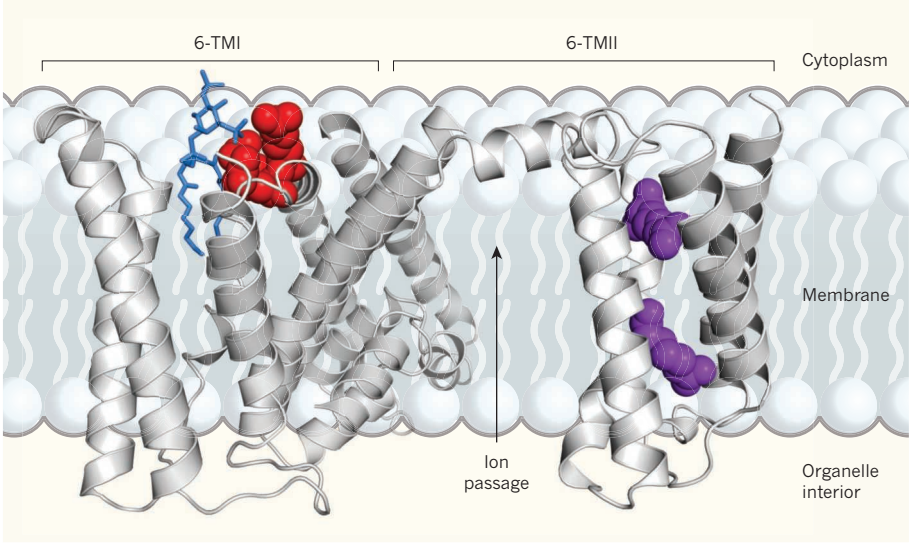

Figure 1 | Structure of mouse TPC1. She *et al.*[3] have resolved the structure of the channel protein TPC1, which is found at organelle membranes. TPC1 has two subunits, each of which contains two transmembrane domains (6-TMI and 6-TMII), connected by a linker. Square brackets indicate the top of the helices that make up 6-TMI and 6-TMII. Here, only one subunit is depicted. The authors found that channel activation requires the lipid $PI(3,5)P_2$ (blue), which binds to arginine amino-acid residues (red) in 6-TMI, and voltage sensing through arginine residues (purple) in 6-TMII. Activation results in the flow of ions through the central pore region into the cytoplasm.

50 Years Ago

The University of Loughborough is already receiving encouraging response to its announcement last week of a new type of vacation course. Three weeks of courses are being arranged in July for technologists, scientists, managers, teachers and — here lies the novelty — for their spouses. Provision is being made for children so that families can be together while parents catch up on some mid-career training … Twenty-four technical courses during the three weeks will cover such subjects as optics, ultrasonics … statistics and management … Cultural courses for spouses … will cover industrial archaeology, music, drama and new techniques of food production … Accommodation is provided on the campus for families at reasonable rates … While hoping to provide all the facilities of a holiday camp, the university believes that its vacation courses will be more valuable than the description "intellectual Butlins" implies.

From *Nature* 6 April 1968

100 Years Ago

The recent development of aviation has provided a means of observing clouds which is much superior to any hitherto known. A modern aeroplane can reach the clouds in a very short time, and in many cases get above them. Observations of temperature can easily be obtained, and probably humidity observations would present no great difficulties. The "bumps" experienced also give some information as to the nature of the disturbance causing the formation of the clouds. It is well known that the two most important processes which cause clouds to form are (1) the mixture of layers of air of high humidity and different potential temperature, (2) adiabatic expansion due to upward movement.

From *Nature* 4 April 1918

pertinent as the number of diseases found to be associated with channel abnormalities grows. Mutations in TRPML1 cause a lysosomal storage disorder affecting children, and TPCs have been implicated in fatty liver disease, Ebola infection and several neurodegenerative disorders[17,18]. In this context, a human TPC structure would be most welcome.

Another challenge is to resolve the structure of TPC2. This protein is regulated by NAADP and $PI(3,5)P_2$, but not by changes in voltage — begging the question of how conformational changes in one TM domain are transmitted to the other to allow channel opening. No doubt, TPCs will reveal further secrets through forthcoming structures. ■

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MICROBIOLOGY

Bacterial persister cells tackled

Chronic infections can be hard to treat because slow-growing bacteria known as persister cells are usually unharmed by antibiotics. The identification of molecules that target such cells might provide a solution. SEE LETTER P.103

JULIAN G. HURDLE & ADITI DESHPANDE

The use of antibiotics to treat an infection can be unsuccessful when bacteria evade such drugs through genetic changes that endow them with antibiotic resistance. Pathogenic bacteria can also avoid antibiotic-mediated destruction through another route: some bacterial cells enter a metabolically inactive or dormant state to become persister cells, which grow slowly or not at all. Most antibiotics were discovered in experiments that tested the ability of compounds to inhibit bacterial growth, and they are therefore often ineffective for treating non-growing persister cells[1]. On page 103, Kim *et al.*[2] now report the identification of small molecules that can kill persister cells.

Persister cells are the source of many of the recurrent bacterial infections that affect people, for example those associated with implanted medical devices, such as the heart infection endocarditis, and also lung infections that can arise in cystic fibrosis[1,3]. Curing such chronic infections can require surgery, which places an added health burden on patients.

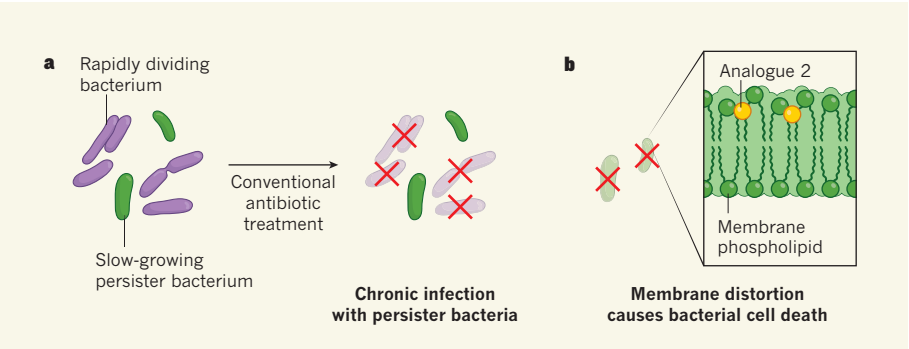

Figure 1 | A retinoid compound destroys bacterial persister cells. **a**, Bacterial populations commonly consist of rapidly dividing cells and slow-growing cells that are known as persister cells. When bacteria are treated with conventional antibiotics, the rapidly dividing cells are destroyed. However, the persister cells can remain, giving rise to chronic infection. **b**, Kim *et al.*[2] report a retinoid compound called analogue 2 that was optimized for targeting persister bacteria. In a mouse model of bacterial infection, the authors found that analogue 2 could kill persister cells. Electron microscopy and computer modelling revealed that analogue 2 probably binds to phospholipid molecules in the bacterial membrane, resulting in membrane distortion that might help to kill the bacteria.

And any necessary extended periods of treatment with antibiotics will increase the probability that bacteria evolve resistance. The development of treatments for killing persister cells is therefore urgently needed[4], especially to target persisters that arise in infections with strains of the 'superbug' bacterium methicillin-resistant *Staphylococcus aureus* (MRSA), which is resistant to several common antibiotics. Infection with MRSA is associated with illness and death, particularly among people with invasive infections[5].

Kim and colleagues decided to search for molecules that could offer protection from MRSA infection, using the roundworm *Caenorhabditis elegans* as a model system. Taking a high-throughput approach, the authors tested the ability of around 82,000 small synthetic molecules to protect worms from death mediated by MRSA infection. Of the 185 compounds that conferred protection, the authors focused on two molecules called CD437 and CD1530, both of which kill MRSA cells rapidly and can also target *Enterococcus faecium*, a bacterium that is linked to endocarditis. Unfortunately, these compounds had no effect against Gram-negative bacteria — a group that includes *Escherichia coli* — for which new therapeutic options are desperately needed because they, too, can form antibiotic-resistant superbugs.

CD437 and CD1530 belong to a class of molecule known as the retinoids, which are structurally similar to vitamin A. Since the 1960s, retinoids have been developed to treat various conditions, including acne[6]. Subsequent synthetic modification of the retinoids has therefore generated derivatives that are often present in chemical libraries used in drug discovery.

The authors concluded that prompt killing of MRSA cells occurred when the two retinoid molecules distorted the structure of the bacterial membrane's lipid bilayer. Kim and colleagues then carried out electron-microscopy studies, which revealed that the retinoid treatment caused curvature and folding of the bacterial membrane but did not result in membrane destruction.

The bacterial membrane is a permeability