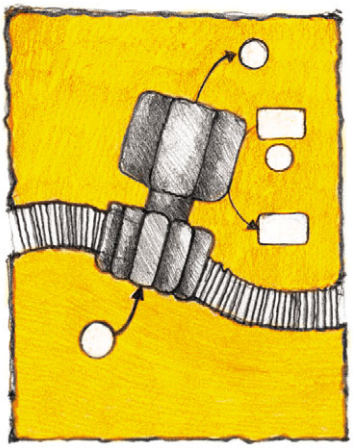
PROTEIN STRUCTURE



OLD PORE - NEW EDITION

Crystallizing membrane proteins is no easy task. Rod MacKinnon and his co-workers, however, are true master craftsmen in such bold ventures. They were the first to report the crystal structure of a bacterial potassium channel, a pioneering accomplishment for which MacKinnon was awarded the Nobel prize. Although this and subsequent crystal structures of prokaryotic channels provided first insights into how potassium channels work, many questions remained. In a recent pair of *Science* papers, MacKinnon and his colleagues present the first crystal structure of a mammalian voltage-dependent potassium channel, Kv1.2, and substantially refine the current model of how these channels open and close.

In nerve cells, voltage-dependent potassium channels are involved in the generation of electrical impulses. They are membrane proteins composed of four α-subunits. Each single α-subunit contains six membrane-spanning α-helices (S1–6), two of which form a central pore (S5, S6) through which potassium ions selectively pass. In addition, regulatory β-subunits are attached to each α-subunit from the cytoplasmic side. In order to react to changes in the membrane potential, voltage-dependent potassium channels are equipped with positively charged sensors lying within the membrane. Most current models propose that these voltage sensors move in response to an altered electric field across the membrane and thus perform mechanical work that influences channel conductivity.

Precisely how the sensors move has been investigated intensively, but the data from different experimental approaches have not yielded a uniform picture. Even the available crystal structures of potassium channels could not elucidate the matter, because the region of the voltage sensor was barely resolved. Previous attempts by the MacKinnon lab to stabilize this region using antibody fragments did not reveal satisfying results because the voltage sensors, although clearer, were found in artificial positions, evidently contradicting solid electrophysiological data.

To stabilize the sensors without the use of antibodies, MacKinnon and his colleagues grew the crystal of the Kv1.2 channel in complex with the β-subunits in a mixture of lipids and detergents. The crystal allowed the team to determine the channel structure in its open position at a resolution of 2.9 angstroms, which is sufficient to deduce an atomic model of the protein. To the gratification of many, the new structure confirmed most expectations. In particular, the voltage sensors were now largely visible in their native conformation. As previously suggested by the authors, the sensor unit appears to be a highly mobile 'paddle' formed by helices S3 and S4 that protrudes partly into the extracellular space. This may explain why Kv1.2 is susceptible to certain spider venoms that are known to poison the voltage sensor from the outside. Voltage-dependent movements of the sensor are coupled to pore opening and closing by a transverse linker-helix between S4 and S5. Its position determines the pore diameter by pushing or pulling the inner pore helix S6. Interestingly, in the open conformation, the sensor's charge-carrying S4 helix is partly exposed to lipids that presumably fill the space between pore- and sensor-helices. However, some of the S4 charges are also shielded from the low dielectric lipid environment by helices S1 and S2, which may be an essential requirement for sensor functioning.

MacKinnon's new potassium channel structure has important implications for our understanding of voltage-sensing and electromechanical coupling. But a static snapshot of the channel's open conformation cannot demonstrate to what extent the voltage sensor moves within the membrane. Therefore, we impatiently await the MacKinnon lab's next snapshot showing the channel in its closed conformation.

10.1242/jeb.01994

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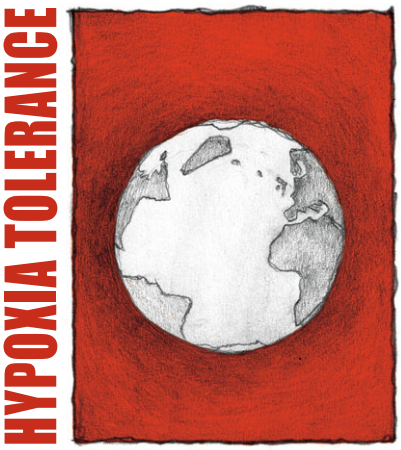
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB



FETAL LLAMAS GIVE HYPOXIA THE COLD SHOULDER

While the mammalian brain is considered to be highly sensitive to low oxygen (hypoxia), not all animals at all life stages are equally vulnerable to hypoxic stress. In fact, some mammals are highly adapted to low oxygen conditions. Diving marine mammals and high altitude species, for example, share such similar traits as high hemoglobin and myoglobin concentrations that increase the body's oxygen carrying capacity. Fetal mammals are also adapted to low oxygen conditions; fetal hemoglobins in general bind oxygen more strongly than adult hemoglobin, and the fetal brain at the cellular level withstands hypoxia better than the adult brain.

Compared with lowland species, fetal life for high-altitude animals like llamas is particularly challenging. Fetal llamas exhibit several adaptations to deal with the double blow of a low oxygen environment *in utero* and their mother's low oxygen montane environment. These include hemoglobin that can strip oxygen from the maternal circulation, lower cardiac output and organ perfusion, and increased oxygen extraction efficiency.

This led Roberto Reyes' group at the Universidad de Chile at Santiago and their collaborators to question if a fetal llama can decrease its brain's energy demands as oxygen supply decreases. This hypometabolism is a well-studied phenomenon in true facultative anaerobes such as the freshwater turtle *Trachemys* and the Crucian carp, which can withstand days to months without oxygen. By decreasing energy demand to match reduced energy supply, facultative anaerobes ensure that no energy imbalance occurs and their brain survives, albeit at a reduced activity level.

To see if fetal llamas show hypometabolism when their mother's arterial oxygen levels plummet, Reyes' group looked for decreased fetal llama brain temperature, Na^+ and K^+ channel density, and Na^+/K^+-ATPase activity in the fetal brain. The team induced fetal hypoxemia (low blood oxygen) for 24 h by reducing the inspired oxygen fraction of pregnant llama mothers to ~12 mmHg. They succeeded in lowering maternal arterial oxygen from an average of 92.5 to 66.4 torr and fetal blood oxygen from ~18 torr to 12 torr, with no change in arterial CO_2, pH or heart rate. During the 24 h hypoxemia, the investigators found that fetal brain temperatures declined an average of 0.56°C, with no change in core body temperature. This temperature decrease was accompanied by a 51% decrease in activity of the Na^+/K^+ pump and a 44% decrease in the protein content of the voltage-gated Na^+ channel NaV1.1 in the fetal llamas' brains, all tell-tale signs that the llamas had depressed their brains' metabolism. Interestingly, when the team examined the fetal brains for degradation of poly ADP-ribose polymerase (PARP), an indicator of cell death, they found no evidence for increased PARP proteolysis. Apparently, fetal llama brains can cope with 24 h hypoxemia without suffering increased cell death.

The fetal llama brain, then, is evidently able to utilize some of the same mechanisms as well-studied facultative anaerobes to decrease brain metabolism in times of energy crisis. By decreasing temperature, ion leak and ion pumping, llama babies reduce the energy requirements of their brains and can give hypoxia the proverbial 'cold shoulder'!

10.1242/jeb.01995

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PTEROSAURS PTAKE OFF

A strange little bone called the pteroid protruded from the wrists of pterosaurs, the flying reptiles of the Mesozoic. It was sort of like a thumb, but extended from the base of the wrist and pointed in the opposite direction – towards the body. Or at least that's what most palaeontologists believed, because that was the orientation it always took in fossilised animals. Now, Matthew Wilkinson, David Unwin and Charles Ellington argue that it pointed straight forward – and may have been instrumental in allowing the largest pterosaurs to fly at all.

The main part of the pterosaur wing was a thin membrane stretching between the fore- and hindlimbs and supported in front by the arm with a hugely elongated fourth finger. In front of the arm, though, was a smaller forewing called the propatagium, supported by the pteroid bone. If the pteroid pointed forward, the propatagium would have formed a broad leading edge flap, with potentially dramatic aerodynamic consequences, Wilkinson thought.

First, the researchers examined fossilised pterosaurs to make sure that it was possible for the pteroid bone to swing into a forward-pointing orientation. Facets in the joint where the small bone articulated made it clear that it could swing from pointing towards the body to pointing forwards, furling and extending the forewing.

Then they built three models of the wing with different pteroid positions – fully extended forward, partially furled, and completely absent – and covered them with ripstop nylon to approximate the wing membrane. When they tested the wing models in the University of Cambridge wind tunnel, they found that the extended

pteriod conferred a dramatic advantage. The broad forewing increased maximum lift forces by about 60% and, when the wing was nearly parallel with the flow, lift forces jumped from five times the drag force with the forewing furled to about 18 times the drag with forewing extended. As the wing angled up to become more perpendicular to the flow, the pteroid continued to help – it prevented the wing from stalling out, which would cause the lift to drop to zero. Surprisingly, the forewing only seemed to help when it was fully extended; the model with the partially furled forewing performed no better than the model with no forewing at all.

In many ways, the forward-pointing pteroid and broad forewing increased the pterosaur wing's performance in the same way that a leading edge flap augments an airplane wing. So the results weren't unexpected – although the amount the forewing helped was impressive. But some researchers had previously rejected the idea of a forward-pointing pteroid, arguing that the puny bone couldn't have supported the stresses that a broad forewing would have imposed on it. Wilkinson waves this objection away, noting that the tip of the fourth digit – which supports the main part of the wing – is as slender as the pteroid and must have supported much higher forces.

The pteroid's remarkable aerodynamic performance sheds light on how the largest pterosaurs took off. These giants had wingspans exceeding 10 m – as big as a small plane. But without propellers or jet engines, how did they take off? Some clearly jumped off cliffs. But other specimens have been found with fossilised footprints, suggesting that they took off from flat ground. Wilkinson's results suggest that giant pterosaurs might have merely needed to spread their wings while facing into a moderate breeze to take off.

10.1242/jeb.01997

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WARM HEARTS PREPARE RATS FOR BEING SHORT OF BREATH

Organisms that have acclimated to a particular stressor often find that they can cope better with another stressor that they were not exposed to during their acclimation, a phenomenon known as cross-tolerance. For example, when rats acclimate to high temperatures, their hearts step up their rate of ATP production through anaerobic glycolytic pathways, which suggests that the warm-acclimated heart is also able to cope better with stress caused by oxygen depletion. Since oxygen depletion is a leading cause of heart injury, it would be intriguing to find a molecular switch that confers cross-tolerance to reduced oxygen levels in the heat-acclimated mammalian heart. To find one such potential switch, Maloyan and colleagues from The Hebrew University in Jerusalem examined heat-acclimated rat hearts, focusing on the activation of hypoxia-inducible factor 1 (HIF-1), a transcription factor of genes that are expressed during oxygen depletion.

HIF-1 consists of two subunits, α and β, which together form the active transcription factor that binds to DNA. Unlike HIF-1β, which is continually present, HIF-1α levels increase in response to low oxygen levels. To evaluate the chronic response of HIF-1α to heat, Maloyan and colleagues acclimated 3-week-old rats to either 24°C or 34°C for 30 days. They used immunoblot assays to determine that rats acclimated to 34°C had higher HIF-1α levels in their hearts than those acclimated to 24°C. To measure the acute response of HIF-1α to heat, they exposed acclimated animals to 41°C for 2 h. They found that HIF-1α levels increased in response to an acute heat shock in non-acclimated but not in heat-acclimated rats. But they didn't see an increase in rats' HIF-1α mRNA levels following heat acclimation, suggesting that changes in translation or degradation are responsible for the higher HIF-1α levels.

HIF-1α is only active if it associates with the β-subunit to create the HIF-1 dimer, so the authors set out to prove that heat stress results in the formation of HIF-1 dimers. They used an anti-HIF-1β antibody that binds to the β-subunit of the protein; when they analysed the resulting antibody–protein complex, they found that the α-subunits were also bound to the complex, which meant that they had associated with the β-subunits. Using this technique, the team showed that chronic as well as acute heat stress increases the dimerization of the protein. They further showed that these HIF-1 dimers are active when present at high levels and bind to a HIF-1 DNA-binding element, which results in the activation of many of the HIF-1 target genes. For example, the team presented evidence that these higher HIF-1 levels stimulate the expression of erythropoietin, a protein that activates the production of red blood cells, which helps the animals cope with lower oxygen levels.

Does this increase in HIF-1 due to heat acclimation translate into greater protection of the heart from the damaging effects of low oxygen levels? The authors evaluated the rats' cross-tolerance to reduced oxygen levels by lowering the perfusion rate of isolated rat hearts by 75%. Sure enough, they found evidence for cross-tolerance; they saw that heat-acclimated rat hearts had smaller patches of injured heart tissue than non-acclimated hearts. They also showed that, like heat acclimation, acute oxygen depletion activates an increase in HIF-1 levels, with similar consequences. This may be the best evidence yet that a warm heart helps animals survive shortness of breath.

10.1242/jeb.01996

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ON FABACEAE, NO ONE CAN HEAR YOU SCREAM

One of the more gruesome Hollywood sub-genres is the alien-parasite thriller, the signature moment of which is undoubtedly the 'birth' scene from *Alien* (1979). What the public doesn't suspect is how disturbingly real these scenarios can be, at least for insects. All herbivorous insect species are parasitized by one or more species of parasitoids, usually flies or wasps. Female parasitoids lay eggs in, on or near the intended host, and the young parasitoid, if it establishes itself successfully, eats host tissues and may ultimately consume it from the inside out. The sight of *Cotesia* wasp larvae emerging from barely living *Manduca* caterpillars is enough to make any horror director smile.

Parasitism poses a conundrum, because insects are not always defenceless – for example, *Drosophila* possess an immune system that sometimes encapsulates (and thereby kills) parasitoid eggs deposited in its blood. Why then don't all insects resist such an important and persistent source of mortality? One intriguing possibility is that maintaining a vigilant, effective immune system imposes costs that may not always be worth paying.

Gwynn and colleagues studied this problem in the pea aphid (*Acyrthosiphon pisum*), a pest on legumes (Fabaceae). Pea aphids host a number of wasp parasitoids, among them *Aphidius ervi*. When *A. ervi* oviposits into an aphid, the egg doesn't always hatch, but not because aphids possess a *Drosophila*-style encapsulation response. Rather, the wasp egg simply fails to develop and ends up breaking down. Why? Current thinking implicates endosymbiotic bacteria. Pea aphids often carry bacteria known as PASS(R) and PABS(T), and experimental infection of aphids with these bacteria confers increased resistance to parasitoids. Why then don't all aphids carry PASS(R) and PABS(T)? Perhaps the resistance they confer is offset by other fitness costs.

The team present a direct test of this idea. They collected aphids from the wild and established clonal lineages on bean plants. The authors first confined aphids from each clone for six hours on bean plants together with mated female *A. ervi*. To determine the clones' resistance to *A. ervi*, the authors counted aphid survivors and 'mummies' (containing wasps) 10 days later. To determine each clone's fecundity, they placed aphids from each clone on bean plants and left them unmolested for several days, after which they counted the aphid offspring. Resistance and fecundity showed a clear inverse relationship – clones with the highest number of offspring had low resistance to *A. ervi*.

What mechanism underlies this trade-off? Gwynn and coworkers suspect, but did not show, that it's mediated by PASS(R) and PABS(T) bacteria. Such a role would be consistent with prior work showing that experimentally induced infection with these bacteria boosts resistance to parasitoids and with other recent work showing that PASS(R) and PABS(T) infections lower aphid fecundity and longevity. Indeed, PASS(R) and PABS(T) are known to reduce intra-aphid population sizes of obligate endosymbiotic *Buchnera aphidicola* bacteria, which likely provide nutrients to their aphid hosts. Gwynn et al. speculate that the resistance-fecundity trade-off is mediated *via* bacterial interactions – that PASS(R) and PABS(T) provide resistance at the cost of obtaining fewer offspring-destined nutrients from *Buchnera*. More broadly, the authors suggest that variation in resistance stems from temporal and spatial variation in selection on survival and fecundity. Pity the aphid, whose fate appears increasingly determined by competing alien genomes.

10.1242/jeb.01998

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