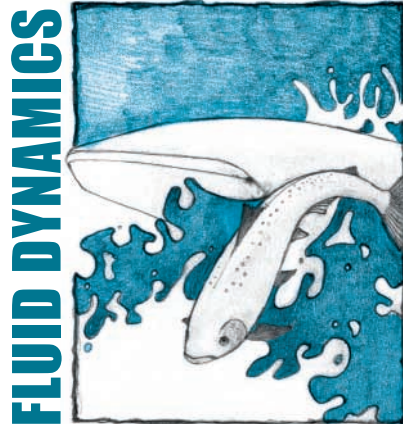


Start/end page	COLOUR	1st Proof:	Issue	MS order	Page total
iv	Page nos.	Press:			

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



HUMPBACKS' BUMPY FLIPPERS

Humpback whales' lumpy flippers don't look like anything a human engineer would design – unlike airplane wings, bumps called tubercles cover the fins' leading edges, giving them an almost serrated appearance. But humpbacks are some of the most agile of the large whales. Maybe their strange flippers actually give them an advantage in maneuvering, one that engineers haven't explored.

After pondering humpback whale flippers for many years, Frank Fish decided to test this idea. Reporting in *Physics of Fluids*, he and his colleagues from the US Naval Academy and Duke University showed that the tubercles do increase hydrodynamic performance – they delay stall, increase lift, and decrease drag.

The group built two model humpback whale flippers, one with a smooth leading edge and one with a wavy edge approximating the usual spacing of tubercles. They tested the scaled-down fins in the Naval Academy's wind tunnel by matching the Reynolds number of a swimming humpback, around 500,000, and measured the steady lift and drag forces on the fins at a variety of angles to the oncoming flow, ranging from -2° to 20° .

The smooth-edged flipper behaved much like a standard airplane wing, although its shape gave it a few advantages over a normal wing. As the angle of attack increased, the lift force increased until the flipper stalled out at around 12° when the lift dropped and the drag increased substantially. This performance is very similar to a standard wing, but the lift did not drop as much as the group expected, possibly because fins are tapered towards the tip. The taper might let different parts

of the fin stall later than others, leading to a more gradual drop off in the lift force.

The really impressive results, however, came from the flipper with tubercles. The fin did not stall until it reached an angle of 16° and produced up to 6% higher lift and as much as 32% lower drag than the smooth fin. Over nearly the entire operating range of angles the bumpy flipper performed better.

Summarizing the performance of the flipper models as an aerodynamic efficiency – the ratio of lift force to drag force – they found that tubercles increased efficiency at almost any angle, and particularly augmented the efficiency at high angles. Maximum efficiency jumped from 22.5 to 23.5.

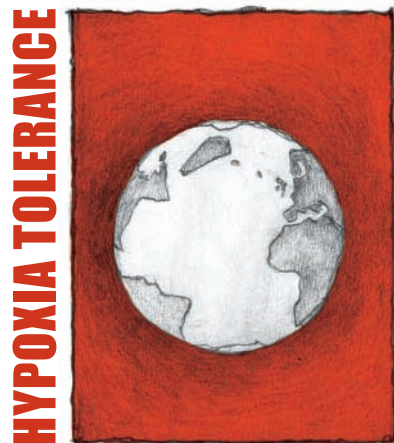
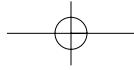
The group hypothesizes that the tubercles function like vortex generators, speeding up the flow in the gaps between bumps. The energized flow stays attached to the flipper better, helping to prevent stall at high angles and increasing the lift force. These increased forces probably contribute to humpbacks' surprising agility.

While the effect of tubercles may not seem particularly huge, remember that engineers have been tinkering with the shape of airplane wings for more than 50 years. At this point, a change in force or efficiency of a few percent or so is considered large. But humpback flippers reveal a simple, passive way to increase lift by 6%, decrease drag by 32%, and delay stall by 40% – pretty astounding results in the engineering world. Simply adding tubercles – bumpy modified knuckles – to the leading edge does the trick.

10.1242/jeb.01249

Miklosovic, D. S., Murray, M. M., Howle, L. E. and Fish, F. E. (2004). Leading-edge tubercles delay stall on humpback whale (*Megaptera novaeangliae*) flippers. *Phys. Fluids* **16**, L39-L42.

Eric Tytell
Harvard University
tytell@oeb.harvard.edu



BLIND AGAINST HYPOXIA STRESS

Living in underground burrows, *Spalax*, the blind subterranean mole rat, is often exposed to extreme fluctuations of oxygen level due to changes in the soil's gas permeability caused by seasonal floods that saturate the soil. As a result of their subterranean life-style, *Spalax* belong to the group of mammals with the greatest degree of hypoxia tolerance. Intrigued by the animal's remarkable hypoxia tolerance, Imad Shams and colleagues from University of Haifa in Israel decided to focused on the expression of two key indicators of hypoxic stress, erythropoietin (*Epo*) and hypoxia-inducible factor 1 α (*HIF-1 α*) to assess the animals' hypoxia tolerance.

HIF1 is a transcription factor that responds rapidly to periods of hypoxia. Under normoxic conditions, one of the HIF1 components, HIF-1 α , is constantly broken down, and so HIF1 is unable to activate hypoxia-induced genes. However, under hypoxic conditions, the HIF-1 α component is stabilised, and HIF-1 can in turn bind to an enhancer element in hypoxia-induced genes, such as the *Epo* gene, and activate its transcription; HIF-1 α levels are said to be post-translationally regulated. *Epo*'s main, but not its only, function is the regulation of levels of red blood cells. Knowing that the kidney is the main site of *Epo* production, Shams and coworkers compared *Epo* mRNA expression levels in the kidneys of white rats and several species of mole rats, *Spalax*, and found that *Epo* expression increased more in the hypoxia tolerant subterranean mole rats than in white rats at low oxygen levels. But when they looked at a time course of *Epo* expression in subterranean mole rat, they were in for a surprise! Although *Epo* levels increased dramatically over the first 24 h of hypoxia, its expression returned close to

normoxic levels after 44 h. Differences between *Epo* expression were not only found between white and mole rats, but also between different species of *Spalax* in extreme hypoxic conditions, with animals from damp hypoxic burrows producing higher levels of *Epo* than animals from well oxygenated burrows. Interestingly, the time course and the fact that the development of erythrocytes takes up to 2 weeks suggests that *Epo* may have other functions than erythropoiesis in setting hypoxia tolerance limits in *Spalax*.

Investigating the levels of *HIF-1 α* , the team found that under normal conditions, hypoxia tolerant blind subterranean mole rats naturally produced twice as much *HIF-1 α* mRNA as hypoxia-sensitive white rats. And when they dropped the oxygen levels to only 3%, blind mole rat's *HIF-1 α* mRNA levels rose dramatically, peaking after 4 h, while the *HIF-1 α* levels of rats exposed to low levels of oxygen didn't change from their normoxic levels. Given that HIF-1 α levels are normally regulated at the protein level, the team was surprised to see that the blind subterranean mole rats seemed able to regulate the levels of *HIF-1 α* mRNA, making them suspect that the *HIF-1 α* gene itself might also be regulated by hypoxia.

Next the team compared the *HIF-1 α* levels of two different species of blind mole rats, from flooded and well-aerated areas, and found that the animals from the wetter and more hypoxic environment had higher levels of *HIF-1 α* than the animals from well-aerated burrows. Shams suggests that 'this pattern of *Epo* and *HIF-1 α* expression is a substantial contribution to the adaptive strategy of hypoxia tolerance in *Spalax*'.

Having identified that *HIF-1 α* and *Epo* are probably major players in the rodents' remarkable hypoxia tolerance, Shams and colleagues are keen to know whether the mRNA and protein levels of *HIF-1 α* respond to fluctuating oxygen levels, given that blind mole rats survive such large fluctuations in oxygen level. hopefully making us less blind towards the promising lessons we can learn from an odd mammal living underground.

10.1242/jeb.01247

Sham I., Avivi, A. and Nevo, E. (2004). Hypoxic stress tolerance of the blind subterranean mole rat: Expression of erythropoietin and hypoxia-inducible factor 1 α . *Proc. Natl. Acad. Sci. USA* **101**, 9698-9703.

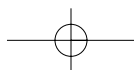
Lars Tomanek
University of California at Davis
ltomanek@ucdavis.edu



COOL SQUIRRELS TURN DOWN THEIR AKT

Neurons are generally viewed as among the most sensitive of all cells when faced with hypoxia (periods of low oxygen) or anoxia (no oxygen), though recent studies have shown a wide variation in the capacity of neurons to tolerate hypoxia. Even the most vulnerable neurons are not defenseless, and the most tolerant can withstand extreme periods of complete anoxia and recover fully, despite oxidant damage when oxygen returns. There is currently an increasing interest in anoxia-tolerant vertebrates as models to identify survival strategies in neurons adapted to survive hypoxia and reperfusion. However, the mixed cellular responses observed in hypoxia-sensitive neurons have generated controversy over which molecular events are essential for cellular protection and which induce cell death. Serine/threonine kinase (Akt), for example, is a central molecule controlling the balance between cell survival and apoptosis, as well as cell proliferation and cell cycle arrest. Akt is considered to promote survival in many cell types, including neurons, where blocking Akt activation increases cell death in response to oxidative stress. Other researchers, however, report that the inhibition of Akt activity delays cell death, and the downregulation of Akt pathways in the nematode *C. elegans* causes it to shift into the energy conserving state known as the dauer larval stage.

John Hallenback and his associates at the National Institute of Neurological Disorders and Stroke were interested in whether Akt pathways in hibernating mammals are up- or downregulated, as hibernation is an evolutionary adaptation to harsh environmental conditions, like the dauer larval stage in *C. elegans*. Also, hibernating mammals often inhabit hypoxic burrows and, in hibernation, reduce blood



Outside JEB

vi

flow to the brain, so are interesting models of neuronal hypoxic survival. In a recent issue of *Brain Research*, the researchers publish data describing Akt activity in hibernating 13-lined ground squirrels.

Wild-caught ground squirrels were placed in a cold chamber in constant darkness at 5°C. Akt levels and activity (phosphorylation) were compared in hibernating animals *versus* control (active, not cold-exposed) and cold-adapted (active, in cold chamber) ground squirrels. While there were no differences between groups in total Akt expression, Akt phosphorylation was significantly decreased in hibernating ground squirrels compared to active and cold-active groups. This reduction in phosphorylation was associated with a corresponding decrease in Akt kinase activity, and occurred in brain and other tissues including muscle, heart and kidney, indicating a generalized response. Akt kinase activity declined by nearly half in the brains of hibernating ground squirrels.

The authors admit that it appears paradoxical for Akt activity to decrease in hibernators, when increased activity is a known critical component of hypoxia survival through its downstream effects that increase survival. They hypothesize that similar survival pathways are at work in the hibernating ground squirrel and the dauer larval *C. elegans*; the downregulation of Akt pathways permits the upregulation of other pro-survival factors such as antioxidants, growth arrest and DNA damage response genes.

As molecular pathways are often highly conserved across the phyla, the use of alternative model systems naturally tolerant to low oxygen and reperfusion stresses can reveal new targets for the potential treatment of stroke and other forms of organ ischemia.

10.1242/jeb.01246

Cai, D., McCarron, R. M., Yu, E. Z., Li, Y. and Hallenbeck, J. (2004). Akt phosphorylation and kinase activity are down-regulated during hibernation in the 13-lined ground squirrel. *Brain. Res.* **1014**, 14-21.

Sarah Milton
Florida Atlantic University
smilton@fau.edu



SICK, COLD OR HUNGRY? TAKE YOUR PICK

Maintaining adequate immune function can be quite a challenge for birds during winter. The cold season demands extra energy for thermoregulation while food supply is often reduced. Such times of environmental stress may suppress energy-demanding immune responses, increasing the risk of disease. But which parts of the immune system are energetically expensive? Is there a trade-off between different arms of the immune system? And do growth, thermoregulation and immune function all draw from the same resources? These questions are interesting not only for physiological ecologists, but also for chicken farmers as they shift towards farming systems that resemble more natural conditions. In contrast, physiological ecologists can also learn how birds deal with several simultaneous environmental stressors from research into the ways chickens cope. Basav Hangalapura and his colleagues at Wageningen University, the Netherlands, investigated how resources are allocated to thermoregulation, growth and various components of the immune system in chickens. They kept cold-stressed chicks on a restricted diet, and simulated a disease by inoculating the birds with a harmless substance: keyhole limpet hemocyanin.

The team used three genetically selected lines of chickens: two with either high or low specific antibody responses and the third, a control line that resembled the original stock. In two experiments, 26-day old chicks from each line were cold-stressed for 2 or 7 days before immunization with hemocyanin at various time points. During the experiments, the chicks were fed a reduced diet, receiving only 80% of the food needed for normal growth. The researchers took blood samples for a variety of immunological

tests at 0, 1, 7, 10 and 28 days after immunization. These tests quantified the responses of different parts of the immune system: the specific antibody response to hemocyanin and cell-mediated immunity as measures of adaptive immunity, and phagocyte activity as an indicator of innate immunity. In addition, the team monitored the growth of the young chickens.

The results showed different effects of cold-stress on various parts of the immune system. Cold-stress did not affect the specific antibody response, but it enhanced cell-mediated immunity, especially when the cold-exposure lasted for 7 days. Testing each group of bird's innate immune response to the cold-stress and time of immunization, the team found that the response is influenced by the time between immunization and application of the cold stress. This led the authors to conclude that innate immunity can be sensitive to environmental stress, depending on its timing, and is less affected by genotype.

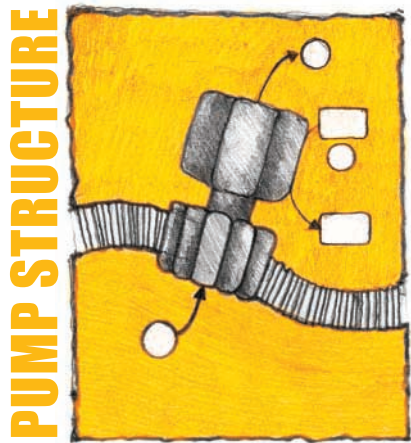
Overall, the findings indicate that thermoregulation, growth and cellular immunity draw on the same energy resource: cold-stressed chicks grew slower than control birds, and fast growth came at the cost of a reduced cellular immune response. But in contrast to expectations, no clear trade-off existed between cellular and humoral immune components.

With hundreds of chickens waiting at their beg and call, Hangalapura and his colleagues now plan to subject their birds to a simulated long winter, instead of a short cold spell. They hope that such a prolonged stress will reveal more clearly the chicken's evolved preference for being cold, hungry or sick. Physiological ecologists can't wait to see the results.

10.1242/jeb.01250

Hangalapura, B. N., Nieuwland, M. G. B., de Vries Reilingh, G., van den Brand, H., Kemp, B. and Parmentier, H. K. (2004). Duration of cold stress modulates overall immunity of chicken lines divergently selected for antibody responses. *Poultry Sci.* **83**, 765-775.

B. Irene Tieleman
University of Missouri - St Louis
tielemani@umsl.edu



CALCIUM PUMP: A CLOSER LOOK

Contractions of cardiac and skeletal muscle cells are triggered by the rapid, voltage-dependent release of Ca^{2+} into the cell's cytoplasm, mainly achieved by emptying the internal Ca^{2+} stores of the sarcoplasmic reticulum. The increase in cytoplasmic Ca^{2+} concentrations is opposed by several outward transport systems that remove Ca^{2+} from the cytoplasm. In order to keep calcium-dependent systems running, muscle cells must maintain tight control of Ca^{2+} homeostasis. The sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA) is one such controller of Ca^{2+} homeostasis. This ATP-driven pump returns a significant portion of the cytoplasmic Ca^{2+} to the sarcoplasmic reticulum. But how exactly does this enzyme fulfill this vital physiological role?

The first few glimpses of this unique ion pump structure were taken in two high-resolution atomic models published a few years ago by Chikashi Toyoshima and colleagues. In a recent *Nature* paper,

Toyoshima and Tatsuaki Mizutani now add new color to the palette of available crystal structures, thereby providing fascinating insights into the enzyme's allosteric mode of action.

SERCA is a member of the P-type ion translocating ATPase superfamily, which form a phosphorylated intermediate during the reaction cycle. SERCA is so far the only pump of this superfamily for which high-resolution structures have been determined and hence it is a structural 'blue print' for other P-type ATPases. The enzyme is an integral membrane protein of about 110 kDa consisting of 10 transmembrane helices and three cytoplasmic domains: the actuator (A), the nucleotide binding domain (N) and the phosphorylation domain (P). The classical model describing the reaction cycle of P-type ATPases is based on the protein assuming two alternate conformations, known as E1 and E2. For SERCA it is thought that Ca^{2+} enters the enzyme in the E1 state *via* two high affinity-binding sites exposed to the cytoplasm. When the pump binds magnesium and ATP, the enzyme becomes auto-phosphorylated and undergoes several conformational changes, first occluding the Ca^{2+} ions and then releasing them from the low affinity-binding sites in the E2 state through a luminal gate. After Ca^{2+} release, the enzyme dephosphorylates and is recycled to its initial state. Although the E1–E2 model does not enjoy unanimous acceptance, many basic features appear to have structural equivalents in the atomic models deduced by Toyoshima and coworkers.

To zoom in on the reaction cycle, Toyoshima and Mizutani grew crystals in the presence of a non-hydrolysable ATP analogue, Mg^{2+} and Ca^{2+} , trapping the

protein in a transition state between the E1 and E2 states just before the phosphoryl transfer. The comparison of this crystal structure with previous ones revealed major allosteric changes within the molecule. Binding the ATP analogue leads to the rearrangement of the cytoplasmic A, N and P domains, which are widely separated in the nucleotide-free state, but form a compact headpiece in the trapped state. The bound nucleotide also seems to bridge the N and P domains and may function as a cleavable cross linker. Moreover, the structure of the P domain itself is altered, also affecting in turn the orientation of the N domain. Collectively, these positional alterations tilt the A domain, locking the cytoplasmic gate by moving one of the transmembrane helices to occlude the bound Ca^{2+} ions. At the same time, structural strain seems to be generated within the molecule, which may open the luminal gate in a later step of the reaction cycle.

Although the pathway of Ca^{2+} across the membrane is still not completely elucidated, a detailed structural framework is now available for integrating other data about the pump's function. By providing a snapshot of the Ca^{2+} pump frozen in one of the reaction cycle's transition states, Toyoshima and his colleagues have once again applied their genius to the fascinating machinations of P-type ATPases in general, and SERCA in particular.

10.1242/jeb.01248

Toyoshima C. and Mizutani T. (2004) Crystal structure of the calcium pump with a bound ATP analogue. *Nature*, doi:10.1038/nature02680.

Hans Merzendorfer,
University of Osnabrueck,
merzendorfer@biologie.uni-
osnabrueck.de