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


## SQUIRRELS REVEAL BITS OF HIBERNOME

Mammalian hibernators are unique in their ability to cope with freezing temperatures. Some mammals can lower their body temperatures to as low as –2.9°C for several weeks. In the golden-mantle ground squirrel, *Spermophilus lateralis*, this period of low body temperature, called torpor, is interrupted by arousals during which body temperature rises as much as 30°C in a matter of hours. Squirrels cannot survive frosty winters without these arousals. But hibernation researchers know very little about the proteins expressed during arousals that allow squirrels to re-enter torpor after arousals. To investigate the physiology of hibernation, Elaine Epperson and colleagues from the University of Colorado turned to the differential expression of hundreds of proteins, the proteome, or the squirrels' 'hibernome'.

Epperson's team separated liver proteins by isoelectric point and by molecular mass. They obtained about 900 spots and estimate that these only represent 3–5% of the squirrels' liver proteins. When they compared summer squirrels with winter squirrels that had completed arousal and were re-entering torpor, they found differences in 84 spots. The team concluded that these proteins were being differentially expressed during arousal in winter squirrels. But here the team encountered a problem: how to identify these spots without a squirrel genome sequence. The team managed to identify 68 of the 84 spots by matching short stretches of amino acids found in the squirrels to a mammalian database that included the rat and mouse genomes, which share many homologous proteins with the distantly related squirrels.

What insight does this natural history perspective of proteomic changes during hibernation offer? At first sight, it confirms what is already known. For example, some of the 68 proteins that the team found were being upregulated during arousal are involved in protein degradation and synthesis. The team also found that several glycogen-producing proteins were upregulated, confirming that glycogen stores are used during arousal and have to be regenerated. Hibernating winter squirrels are known to rely heavily on lipids as a fuel source. Typical for animals relying on lipids as metabolic fuel, the team observed an increase in expression of a fatty-acid-binding protein and a key ketone body-forming enzyme.

Epperson's proteomic approach also reveals interesting novel findings concerning differential expression among members of a protein family. For example, all but one of several enzymes involved in the detoxification of aldehydes resulting from lipid peroxidation were upregulated in winter compared with summer squirrels, indicating that there is an increase in lipid peroxidation during torpor despite fasting. Interestingly, the only enzyme that was downregulated prefers dietary substrates that occur in low abundance during fasting. Members of an esterase protein family, enzymes that hydrolyze fatty acid esters, were either up- or down-regulated in winter squirrels depending on their affinity for fatty acyl esters, indicating subtle and important metabolic differences between summer and winter squirrels. Thus, a proteomic approach can differentiate among several members of a protein family and reveal a great number of differentially expressed proteins.

The extent to which squirrels change the expression of enzymes that regulate major metabolic pathways during arousals probably came as a surprise to the authors. Having found so many interesting alterations in protein expression in just 3% of the squirrels' liver proteins, they are now eager to tap into the vast remaining pool of differentially expressed proteins.

10.1242/jeb.01390

**Epperson, E., Dahl, T. A. and Martin, S. L.** (2004). Quantitative analysis of liver protein expression during hibernation in the golden-mantle ground squirrel. *Mol. Cell. Proteomics* **3**, 920-933.

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## ANOXIA NOT A REAL HEARTBREAKER

While the mammalian heart cannot function without oxygen, this is not true of all vertebrates. Several vertebrate species can survive anoxia, the complete absence of oxygen, for days or even months. These anoxia-tolerant animals include freshwater turtles in the genera *Chrysemys* and *Trachemys*, and the crucian carp *Carassius carassius*. The turtles and fish, however, do not use all of the same adaptations to survive without oxygen. Turtles survive without oxygen by decreasing metabolism by up to 90%, subsisting on the reduced energy provided by anaerobic respiration. The anoxic turtle is essentially comatose, with energy savings provided by greatly reduced brain function and suppressed cardiac output. In contrast to the sluggish turtles, the crucian carp continues to be active for months at a time despite having no access to oxygen, compensating by increasing anaerobic metabolism. The problem is that anaerobic respiration results in the accumulation of toxic waste products (primarily lactic acid) in the fish's tissues. To avoid self-pollution, this lactate is converted to ethanol and excreted across the gills. So to survive in oxygen-less environments, the fish needs a transport system, which depends on a beating heart and functioning circulatory system. But part of the anoxic turtle's energy saving comes from dramatically suppressing its cardiac activity, which results in lowered circulation and corresponding loss of an effective transport system. Goran Nilsson and his group at the University of Oslo wondered if the active carp maintained its cardiac function and nervous control of its heart rate despite the lack of oxygen.

To find out, Nilsson and his team examined cardiac function in the crucian carp over a period of 5 days of anoxia at 8±1°C. In a paper recently published in *Science*, they report that following an initial 24 h adjustment period of increased stroke volume and cardiac output, the carp's cardiac function returned to normal levels. The team also found that blocking inhibitory heart receptors increased heart rate and cardiac output, while blocking excitatory heart receptors decreased cardiac function. This suggests that, in direct contrast to the turtle, nervous system control of heart rate remains intact in the crucian carp. In fact, the only cardiovascular functions that seemed to have changed over 2–5 days of anoxia were the fish's peripheral vascular resistance and blood pressure in the ventral aorta, which continued to be depressed, indicating that the fish's blood vessels had dilated. This vasodilation allows the carp to transport the glucose required for anaerobic respiration to metabolically active tissues while also enabling the removal of toxic waste products. Nilsson and his team suspect that the heart's ability to continue beating, coupled with an effective vascular transport system, allows the fish to remain active even when oxygen levels plummet.

Amazingly, then, some vertebrate hearts can function without oxygen! It would be of interest to investigate in more detail the biochemical and molecular bases of continued cardiac function during anoxia. As an alternative to the rapidly dying mammalian models, this could reveal new treatments in the face of mammalian heart failure.

10.1242/jeb.01389

**Stecyk, J. A. W., Stenslokken, K.-O., Farrell, A. P. and Nilsson, G. E.** (2004). Maintained cardiac pumping in anoxic crucian carp. *Science* **306**, 77.

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## THE MYSTERIOUS LITTLE FATTY FIN

Sitting on the back of many fishes, in between the dorsal fin and the tail, is an enigmatic little fatty flap of skin called the adipose fin. It looks a bit like an extra dorsal fin, and though it's present in eight large groups of fishes, no one knows why it's there. It might help prevent flow from wrapping over the top of the fish; it might help counteract forces from the anal fin, which is in about the same place, but on the ventral side; it might be a flow sensor; or it might not do anything, persisting due to developmental constraints. Whatever the fin's function, most fisheries scientists think it's not terribly important, because they regularly snip it off to mark millions of hatchery fish released into the wild each year.

Thomas Reimchen and Nicola Temple at the University of Victoria in Canada devised a simple test to find out how important the adipose fin really is. They swam steelhead trout at speeds between about one and three body lengths per second, measured the tail beat frequency and amplitude, then clipped the adipose fin off and made the same measurements again. They expected that the standard fisheries wisdom would be right, and they'd see no difference between the clipped and unclipped fish.

But, in fact, the fish with clipped fins tended to use a higher tail beat amplitude at all swimming speeds. It wasn't a lot higher – only about 8% on average – but it was usually a significant difference, except in the smallest fish. Reimchen and Temple worried, though, that the effect might not represent any intrinsic function of the adipose fin, but just the trauma of having a fin snipped off. So they tested another batch of fish in which they made a scratch along the base of the adipose fin, without

actually cutting the fin off. The scratched fish swam the same as the unscratched ones, eliminating the trauma as a possible cause of the increase in tail beat amplitude.

Why would snipping off the adipose fin lead to a higher amplitude? Reimchen and Temple can only speculate, but they raise some important questions. Perhaps the fin generates some thrust on its own, or makes vortices that increase the thrust of the tail fin. Without the extra thrust, trout would have to compensate by swimming harder. Or the adipose fin might help the fish swim more efficiently by sensing vortices upstream of the tail fin. Trout might counteract the lower efficiency after their adipose fin is clipped by using a higher tail beat amplitude. The suggestion that the adipose fin functions as a flow sensor seems plausible, since Reimchen found some small nerves running to the base of the fin.

Whatever the mechanism, it appears that trout with clipped adipose fins must swim harder. It would be useful to compare the oxygen consumption of clipped and unclipped fish, to verify that swimming without an adipose fin is truly more difficult. But Reimchen and Temple's results should give fisheries scientists pause for thought, because they could have serious consequences for the millions of fish with clipped adipose fins released from hatcheries each year.

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**Reimchen, T. E. and Temple, N. F.** (2004). Hydrodynamic and phylogenetic aspects of the adipose fin in fishes. *Can. J. Zool.* **82**, 910-916 (doi: 10.1139/Z04-069).

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## PERITROPHIC ENVELOPE IS CANNON FODDER

Topologically speaking, insects are ring donuts. The gut is therefore an important point of contact between insects and the plants they eat, and is where much of the cloak-and-dagger intrigue between plants and insects is played out. One devious plant strategy is to foment the formation of free radicals inside its adversary. Free radicals lead to a host of ills *via* the damage they inflict on diverse classes of molecules. Some of the most reactive – hydroxyl and alkoxyl radicals – are formed when common constituents of gut fluid occur together with a suitable catalyst, such as ferrous iron ions. This chemical potential has not gone unnoticed by plants. Many contain enough iron in their tissues to trigger free radical formation in insects' guts, attacking their foes from the inside out. What's an insect on an iron-rich diet to do?

Raymond Barbehenn and Jasmine Stannard recently tested the hypothesis that insects protect themselves from free radicals by placing the peritrophic envelope in the line of fire. The envelope surrounds the gut contents like a leaky glove. It is secreted by cells at the foregut–midgut junction, is carried along by movement of food in the gut tract, and eventually is excreted. Orthodox thinking says that the peritrophic envelope protects gut cells from abrasion while still letting through digested nutrients. Could it also protect insects from an onslaught of free radicals?

To find out, the team first fed *Malacosoma disstria* caterpillars artificial diets containing low, medium, or high amounts of iron, and then removed the caterpillars' peritrophic envelopes and analyzed them for iron content. They found, as suspected, that envelopes scavenge iron. The next step was to test whether the peritrophic envelope protects midgut tissue from oxidative damage. The researchers spiked diets with radical-producing compounds (tannins) and different levels of iron, and then measured damage to proteins in midgut tissue. The level of iron made no difference; midgut tissues were protected even from high-iron diets. Finally, the team examined whether the peritrophic envelope itself takes the fall, by feeding caterpillars tannin or no-tannin diets, both containing iron. They found that envelope proteins from caterpillars eating tannins sustained about twice the oxidative damage as those in caterpillars fed no-tannin diets. Barbehenn and Stannard's findings suggest that the peritrophic envelope both prevents iron from reaching midgut tissues and absorbs the free radicals catalyzed by iron. The disposable envelope thus takes the oxidative fall for the more permanent and highly active midgut epithelium.

A curmudgeon could object that these experiments do not definitively rule out a primary role for other systems that protect from free radicals. Barbehenn and Stannard pre-empt such criticisms with an elegant peritrophic envelope disruption experiment. They fed caterpillars a diet containing both tannin and Calcoflour, which disrupts peritrophic envelope structure. Caterpillars receiving this experimental diet had two- to three-fold higher levels of oxidative damage in their midgut tissue than caterpillars fed control diets. The peritrophic envelope indeed appears to be a primary system protecting midgut tissues from free radical damage.

The simple view of peritrophic envelope function – that it protects the midgut from abrasion – is increasingly dated. An emerging multifunctional view emphasizes the envelope's multiple roles in digestion and its ability to alter chemical conditions in the gut to the insect's advantage. In the baroque and interlocking battle between insects and plants, the peritrophic envelope now appears to be a central player.

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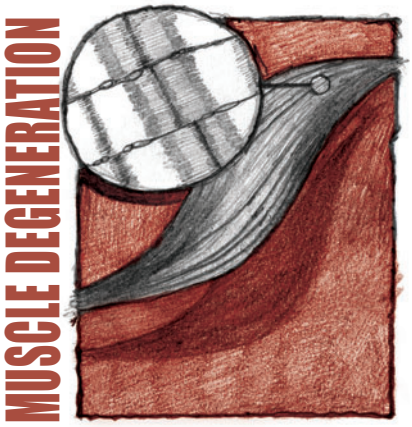


## MUSCULAR DYSTROPHIES: A NOVEL PLAYER

Muscular dystrophies are inherited disorders leading to progressive muscle destruction. Duchenne's disease and another type of muscular dystrophy result from genetic defects that affect the dystrophin-glycoprotein complex (DGC). The DGC is a network of proteins that contacts the muscle cell's outer membrane and transmits the muscle's contractile force to the extracellular matrix. The DGC also appears to play an important role in cell signalling. However, the precise contribution of the DGC to muscular degeneration remains elusive. A novel gene discovered by Steven McIntire's team at the University of California in San Francisco may provide a key towards the understanding of muscular dystrophy.

Neurotransmitters are chemicals that transmit information across the synapse, a specialized junction between two nerve cells or a nerve and a muscle cell. A nerve cell normally induces muscle contraction by releasing the neurotransmitter acetylcholine into the synaptic gap between the nerve cell and a muscle cell. This chemical stimulus was thought to be switched off by an enzyme that breaks down acetylcholine. Most other neurotransmitters are removed from the synapse by specific transporters. However, enzymatic breakdown of acetylcholine was considered to be so effective that nobody seriously considered the existence of a specific transporter for its clearance. McIntire's team now provides exciting evidence suggesting that a transporter removes acetylcholine from the neuromuscular synapse.

The roundworm, *Caenorhabditis elegans*, is a useful genetic model for human muscular dystrophies because disruption of *DGC* genes causes easily observable uncoordinated movements in the worms. The US team identified 12 mutant worms showing locomotory defects, with seven of these showing disruptions of known *DGC* genes. However, five other mutants exhibited mutations in a previously unidentified gene, *SNF-6*. After cloning and sequencing *SNF-6*, the team found that it is strikingly similar to mammalian genes for neurotransmitter transporters. Gene expression studies suggested that the SNF-6 protein might transport acetylcholine and clear it away from the synaptic gap. The team tested whether the newly discovered protein transports acetylcholine by measuring uptake of radiolabelled acetylcholine by mammalian cells expressing *SNF-6*. They observed a specific uptake of acetylcholine, which was saturable and dependent on the presence of sodium, indicating that the SNF-6 protein is indeed a sodium-dependent acetylcholine transporter.

McIntire and his colleagues suspected that the DGC mutants' uncoordinated locomotion might be due to loss of the SNF-6 transporter, resulting in elevated concentrations of acetylcholine at the neuromuscular synapse. The team showed that components of the DGC preserve the SNF-6 transporter at the synapse. Furthermore, *DGC* mutants have no SNF-6 at their neuromuscular junctions. These findings suggest that loss of *DGC* function leads to muscle degeneration because the SNF-6 acetylcholine transporter disappears. McIntire's team confirmed that when acetylcholine transporter function is disrupted, muscle degeneration typical of muscular dystrophy results. Thus, insufficient clearing of acetylcholine from the neuromuscular synapse may contribute to the pathogenesis of this disease.

Since basic muscle components are conserved in roundworms and humans, identification of an acetylcholine transporter orthologue in mammals may only be a question of time. Doubtless, its discovery would expedite development of new therapeutics to treat muscular dystrophy.

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