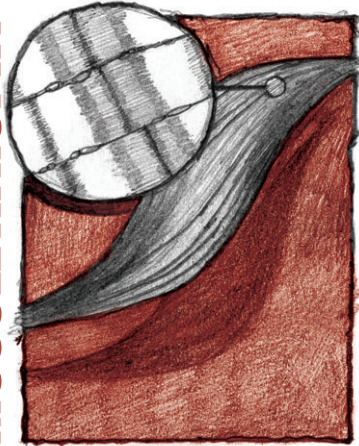


MUSCLE ATROPHY



MYONUCLEI ARE NOT LOST DURING MUSCLE ATROPHY

We have all experienced the fact that skeletal muscles can change size. When we go to the gym and lift weights, our muscles get bigger. However, when we are lazy and stop working out, our muscles decrease in size. Such muscle shrinkage is termed disuse atrophy and, currently, the only way to build the muscle up again is to increase physical activity and nerve-evoked contractile activity. Muscles also deteriorate with numerous neuromuscular disorders that medical research is endeavoring to cure.

A change in muscle size normally results from an alteration of the size of the individual muscle cells (otherwise known as muscle fibers), which contain numerous nuclei (called myonuclei). The classical belief is that muscle cell size is closely correlated to the number of myonuclei, and that a reduction of muscle fiber size is accompanied by a regulated reduction (i.e. apoptosis) of myonuclei. Thus, a loss of nuclei during disuse atrophy implies that the recovery of muscle strength requires the nuclei to be replenished by muscle satellite cells (the muscle's stem cells). Further, this notion dictates that potential intervention and treatment therapies for neuromuscular disorders should involve activation of the satellite cells or interference of the apoptotic pathways. However, it is likely that the classical view of muscle disuse atrophy needs to be revised, given a recent publication by Jo Bruusgaard and Kristian Gundersen in *The Journal of Clinical Investigation*.

The team from the University of Oslo, Norway, decided to examine the phenomenon of muscle disuse atrophy using an *in vivo* time-lapse microscopy technique. First, the team stained myonuclei of both slow- and fast-twitch leg muscle fibers in live mice by transfecting the mice with a plasmid encoding green fluorescent

protein (GFP) that localized to muscle nuclei. Subsequently, they inactivated the leg muscles of the mice by denervating the muscle by severing the sciatic nerve, blocking nerve impulses to the muscle with the voltage-gated sodium channel blocker tetrodotoxin, or mechanically unloading the muscle. Following these procedures, the team monitored changes in fiber size and number of myonuclei in the same muscle segment over several days and weeks.

Astonishingly, despite a greater than 50% reduction in the cross-sectional area of the muscle fibers following the inactivation techniques, the team did not observe any loss of myonuclei. Even though the muscle fibers were inactivated, by denervation, nerve impulse block or mechanical unloading, the number of myonuclei in individual muscle fibres did not decrease. Thus the team discovered that, in contrast with the classical belief, muscle nuclei are not lost during disuse. Indeed, the team observed high numbers of nuclei undergoing regulated reduction by apoptosis in inactive muscles, but the apoptosis was confined to the nuclei in satellite cells and surrounding stromal cells, not those situated inside the muscle fiber.

The team argues that their novel findings indicate that future therapeutic regimes for the treatment of muscle atrophy should focus on intracellular regulatory mechanisms related to protein degradation and synthesis, and not on the regeneration of myonuclei from stem cells. Nevertheless, the team cautions that their findings do not exclude the possibility that myonuclear apoptosis occurs after longer periods of disuse.

10.1242/jeb.010991

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TO SINK OR SWIM?

When the man on the street thinks of a sturgeon, he probably thinks of caviar. When a biologist considers this fish, he or she is likely to be struck by some character that can, crudely, be related to its 'primitive' or 'not quite finished evolving' state – that is, if you think of a goldfish as the 'target' of evolution. From the ridges of bony scales along the body to the tip of their asymmetrical tails, sturgeons appear stuck in the evolutionary past. Further, sturgeons possess 'almost' swim bladders, so controlling their buoyancy and depth in the water column presents some fascinating challenges. While more derived fish (such as goldfish) are capable of dynamically controlling their buoyancy by releasing or absorbing gas into their swim bladders, sturgeon have to suck in or blow out air to influence their overall body density. This has a number of potential implications, some of which were postulated over 40 years ago by R. McNeil Alexander. However, these are only now being measured by a team of Japanese and Chinese researchers.

Yoshi Watanabe and colleagues attached data loggers recording the sturgeon's speed (*via* a little propeller), lateral acceleration (giving tailbeat frequency), pitch and depth. On one fish, a camera was also added to see whether the fish sat on the bottom. After being carried around by the fish for a while, these electronic packages were severed by a time-scheduled release mechanism. The packages floated to the surface, and transmitted a VHF radio signal to allow recovery.

The remarkable finding from these measurements is that there appear to be two distinct behavioural strategies, both of which may be influenced by the retention of the old fashioned swim bladder system. Of the seven fish (203 h of data) measured, four fish swam near the water surface, continuously beating their tails, swimming

successively upward and downward, and occasionally popping up to the surface (rising with a body angle up to 80 deg.) for a couple of seconds, probably taking a gulp of air. Given that the tailbeat frequency for a given speed during ascent and descent was broadly similar, it presumably took as much power to swim up as to swim down – so these fish appeared to be near neutral buoyancy.

In contrast, the remaining three sturgeons adopted a quite different strategy. For long periods, these fish remained quite still, sitting on the bottom of the river. Every 15–20 min, these fish swam upwards, sometimes to the same level as the more active fish, but then glided back down with a nose-up inclination and without active swimming.

Both of these behaviours are consistent with the limitations of a primitive air sac system. The actively swimming group probably maintained the flotation benefits of the airsac through regular trips to the water surface, and gently swam up and down past a position of neutral buoyancy. Apart from the occasional forays to the surface, the active swimmers behaved as conventional midwater fish. The bottom-dwelling group probably had little gas remaining in their air sacs, and always remained denser than water, effectively adopting the low-energy sit-and-wait strategy of flatfish and skates.

The motivation behind choosing which strategy to adopt – even whether sturgeons can easily move from one strategy to the other – is unclear at this stage. But, while sturgeons may be lagging behind goldfish in some respects, this study shows there is more than one way of surviving despite an outdated airsac design.

10.1242/jeb.011007

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CALCIUM: A KEY PLAYER IN RAPID COLD-HARDENING

Some insects are capable of dramatically adjusting their cold tolerance over an extremely short period of time. Early studies in fruit flies showed that a brief pre-treatment of an hour or two to a sub-lethal temperature allowed these individuals to survive what would have otherwise been a lethal cold exposure. The result of this work was the discovery of a groundbreaking physiological response, termed 'rapid cold-hardening'. Later work showed that isolated cell and tissue samples also possessed the capacity for rapid cold-hardening and discounted the role of the central nervous system as a major regulator of the speedy response. Subsequently, many researchers have sought to explain the mechanisms involved in this swift alteration of lethal low temperatures. Are there common cellular stress pathways responsible for rapid cold-hardening? What are the cellular triggers responsible for generating this response?

Nicholas Teets and co-workers from David Denlinger's laboratory at Ohio State University and Rick Lee's research group at Miami University teamed up to tackle some of these challenging questions. For this work, the team used larvae of a midge, *Belgica antarctica*, collected from Antarctica during a summer field season and shipped back to the lab at Miami University. Next, the team performed a series of whole animal, *in vivo* and *in vitro* experiments to elucidate several questions related to the rapid cold-hardening (RCH) response. Specifically, the team asked whether RCH occurs *in vitro* at the cellular and tissue levels and whether calcium is necessary to generate this response.

First, the team reconfirmed that *B. antarctica* produces a typical RCH response: 1 h at -5°C increased the insects' survival from less than 10% after 24 h at -20°C to 70%, while survival at -15°C

increased from ~55% to ~85%. Second, the isolated tissues from the fat body, midgut and Malpighian tubules retained the RCH response and cell survival improved under potentially lethal conditions. Using viability assays that discriminately stain damaged and normally functioning cells, Teets and his co-workers showed that cold sensing and RCH occur at the cellular level in this insect.

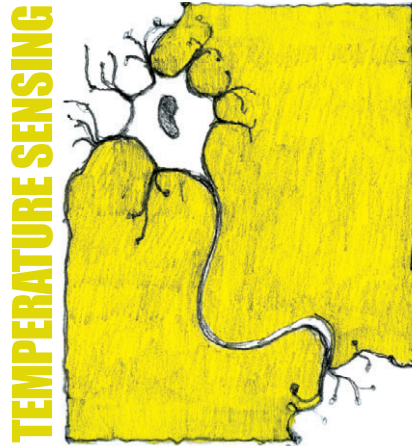
Next, the team demonstrated that calcium played an important role in the RCH responses. When calcium was blocked the RCH response was significantly suppressed. Finally, a calmodulin inhibitor reduced cell survival during cold treatments, further supporting the functional importance of calcium as a secondary messenger in the RCH response.

Calcium has already been demonstrated to be an important cellular messenger in cold stress responses over long time scales in other organisms, as well as functioning in a number of important downstream pathways including the regulation of gene expression and subsequent protein synthesis. The results of this study extend this information into insects and clearly show that calcium plays an important role in RCH of insects. Furthermore, it shows that calcium probably acts as a key first step in a line of complex responses. With the results of this neat study the precise cellular responses and how these are fine-tuned to meet the animal's needs are beginning to be mapped out in detail. These results have significant implications for understanding how insects can rapidly adjust thermal tolerances in their ever-changing environment.

10.1242/jeb.011015

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NEURONAL CONTROL OF CELLULAR HEAT SHOCK RESPONSE IN NEMATODES

Temperature is one of the most pervasive factors that affect biological systems. Thus, it is not surprising that almost all organisms have evolved mechanisms to sense and respond to changes in environmental temperature. Indeed, the ability to seek out optimal temperatures for growth and reproduction is a common trait among ectothermic organisms. For most biological processes there is a narrow range of optimal temperatures outside of which efficiency steeply declines. Temperatures only a few degrees outside the normal range for a species may lead to direct damage of cellular machinery. Almost all cells possess the intrinsic ability to elicit a heat shock response (HSR) to protect their proteins and other macromolecules from heat stress. This response includes the production of a highly conserved group of molecular chaperones called the heat shock proteins. However, the mechanisms that regulate and integrate a cellular HSR into a HSR at the tissue and organismal level have received little attention. Veena Prahlad and colleagues at Northwestern University set out to investigate the role of temperature sensing by thermosensory AFD neurons in regulating the cellular HSR in the nematode worm *C. elegans*.

To investigate the role of the AFD neurons in the cellular HSR, they raised wild-type and mutant worms that lacked functional AFD neurons at 20°C and then exposed them to a transient heat shock of 30 or 34°C for 15 min. After allowing the worms to recover for 2 h, they measured the total amount of mRNA for a major heat shock protein (cytoplasmic hsp70) in the whole worms. They also investigated the location of hsp70 expression within a worm using a green fluorescent protein reporter.

The team found that mutant worms lacking functional AFD neurons had significantly

reduced levels of hsp70 mRNA compared with the wild-type worms following the heat shock regimen. All mutant worms' cells produced less hsp70 mRNA in the absence of AFD thermosensing, despite retaining the HSR cellular machinery. The attenuated HSR in these worms also led to a lower tolerance of heat stress. Further, the team illustrated that this affect was temperature specific by eliciting a full-scale HSR in mutant worms that lacked functional AFD neurons by exposing them to the heavy metal cadmium, which is known to induce a HSR in the cells of a variety of organisms.

Prahlad and the team also explored whether the worm's metabolic state influences the HSR by repeating their experiments in either the presence or the absence of dauer hormone. High amounts of dauer hormone are produced when population densities are high and food becomes limited. The hormone signals larvae to enter a state of metabolic dormancy to await more favourable conditions. When exposed to heat shock in the presence of dauer hormone, the wild-type worms responded with an attenuated HSR. However, the worms that lacked functional AFD neurons produced a HSR that exceeded that of control wild-type worms. Based on this unexpected result, the team suggest that information on the nematode's metabolic state is integral to the regulation of the organismal HSR, and that thermal and metabolic inputs are responsible for mutually down regulating the HSR.

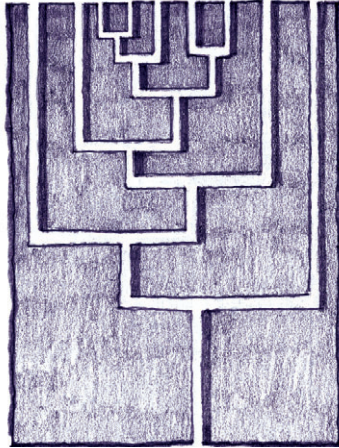
Prahlad and colleagues have illustrated that the cellular HSR is not regulated within each cell of *C. elegans* in response to temperature stress, but is instead regulated by the thermosensory AFD neurons. Their results are consistent with a model that integrates metabolic and thermosensory signals to generate an organismal-level HSR. The authors suggest that the AFD neurons must be communicating with individual cells in the nematode's body via an unidentified extracellular signalling molecule because these neurons do not directly innervate the cells that respond to heat stress with a HSR.

10.1242/jeb.011023

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FEEDING



LIZARDS INCAPACITATE ANTS WITH MUCUS

Ants don't make very good meals. They're tiny, not all that nutritious and many have evolved an array of morphological, chemical and behavioral adaptations to make predators pay. Horned lizards (*Phrynosoma* spp.) are unique in that their diet consists almost entirely of the nastiest ants imaginable; Harvester ants (*Pogonomyrmex* spp.). Native to the American southwest, these ants have powerful mandibles, a stinger tipped with potent venom, and a propensity to swarm all over attacking predators. However, horned lizards suck down these ants whole by the hundreds. How can these lizards gulp down intact (and almost certainly very agitated) venomous ants without incurring painful

bites and stings all over the insides of their mouths? Wade Sherbrooke and Kurt Schwenk from the University of Connecticut recently addressed this question in *The Journal of Experimental Zoology* using a combination of high-speed videography, anatomy, and the natural historian's classic tool, stomach content analysis.

First, the team brought horned lizards into the lab and filmed them gulping down their prey. Usually, lizards will snag prey with their tongues, crush them with their teeth, then slowly move the prey through the mouth to the back of the throat before swallowing. Each eating phase has clear time boundaries in most lizards. Horned lizards have evolved a different feeding strategy. They grab prey items with their tongue, then in one extremely fast motion (~30 ms) roll the prey directly into their esophagus. This kinematic pattern is unique among lizards studied to date.

Next, Sherbrooke and Schwenk wanted to see what prey items look like after being snapped up by horned lizards. To do this, the team examined ants in the stomachs of freshly road killed animals. They found each individual ant to be wrapped up in a compact ball of mucus; the ants' mandibles, stingers and limbs were totally immobilized. This observation suggests that horned lizards actually roll their prey up in mucus as they suck them down.

Finally, the team went looking for mucus-producing cells in the mouths of captured horned lizards. They found 'thick carpets' of highly folded mucus-producing structures lining the floor of the animals' mouths. Furthermore, the authors could find no evidence that other lizards have the same degree of surface area devoted to mucus secretion. Compared with other lizard species, horned lizards clearly have a huge advantage when it comes to mucus production.

Sherbrooke and Schwenk provide several lines of evidence for a unique use of mucus by a predator. The team provides strong circumstantial evidence for a mucus prey-binding system that no other vertebrate has. We don't usually think of mucus as useful, but the work of Sherbrooke and Schwenk reminds us that it's actually pretty handy stuff. In fact, it can be totally crucial in an animal's life. More importantly, this work should serve to remind us that animals can evolve to use almost anything as a weapon – even something as innocuous as mucus.

10.1242/jeb.011031

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