

Inside JEB is a twice monthly feature, which highlights the key developments in the *Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

FROM GOO TO GLUE



Life at the tide's edge is never dull. Some species take refuge in rock pools as the waves recede, while others secure themselves with glue to rocks, for fear of being washed away by the returning tide. Marsh dwelling periwinkles, on the other hand, have a different dilemma. They tether themselves high up on grasses to evade the returning waves, preventing themselves from being blown free with an adhesive gel. Andy Smith is fascinated by the biomechanical properties of the periwinkle's flexible, gel-like glue. He explains that while even the most flexible commercial glues are essentially solid, the periwinkle's adhesive is often 97% water, and the molluscs adhere as well to soggy surfaces as they do to dry. So how do these amazing adhesives work? After all, at first glance, the glue looks like the gooey slime left by roving molluscs! But when Smith took a closer look at the glue's molecular components, he discovered that the glue had one extra, vital ingredient; a protein, which made up 50% of the glue's solid material. Intrigued by the mystery ingredient, Smith decided to discover whether the protein was all it took for molluscs to convert goo to glue (p. 1127).

But first he had to get enough of the tacky adhesive to be able to isolate the tiny amount of protein each periwinkle produced. Scraping the glue from the glass walls of an aquarium, the team redissolved the glue and isolated the protein from the glue's slimy components, ready to test its gelling activity. Dissolving pectin in a 0.1% solution of the protein, Smith was delighted when the mixture quickly turned into a clear lump of gel. And when Jan Pawlicki, Laura Pease and Yuanming Zhang tested the glue's stiffness in a rheometer, they found that it was much stiffer than a gel made from pectin alone. Not only did the protein cause the glue to gel, but it reinforced it too.

However, Smith needed much more gelling protein than the tiny periwinkles could produce, to get to grips with the gluey material. He needed prolific glue producers, so he turned to terrestrial slugs and snails.

Comparing glue and trail goo from both species, the team isolated a 15kDa glue protein from the slug, and three large glue proteins from the snail. And when the team tested the effect of the snail's protein on a selection of polysaccharides, they were surprised; the protein's effects were non-specific, causing almost all of the negatively charged polysaccharides that they tested to gel. However, when they tested uncharged polysaccharides with the gel-forming proteins, they barely gelled at all.

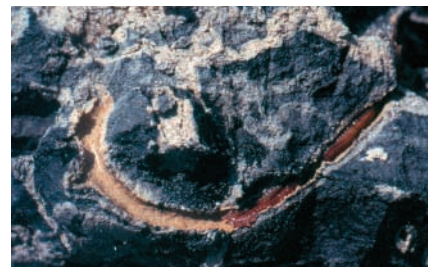
All of which could hold a clue to the protein's function. Smith suspects that the proteins act as electrostatic cross linkers between the charged polysaccharide chains, allowing the gel to form quickly. Smith also noticed that the protein improves the glue solution's wetting properties, allowing it to spread more effectively over a surface before gelling, to get a good grip.

So next time you're clambering around at the seashore, spare a thought for the humble molluscs, which anchor down there, and their amazing adhesive proteins.

10.1242/jeb.00917

Pawlicki, J. M., Pease, L. B., Pierce, C. M., Startz, T. P., Zhang, Y. and Smith, A. M. (2004). The effect of molluscan glue proteins on gel mechanics. *J. Exp. Biol.* **207**, 1127-1135.

ARE WORMS HEAVY METAL FANS?



Sylvain Demuyne is fascinated by the enormous variety of invertebrate life that populates our planet. He always has been, but at the moment he's focusing on just one: the annelid worm *Nereis diversicolor*. Found living in brackish coastal waters, the worm's habitats can be close to ex-industrial sites that are often polluted by heavy metals such as cadmium. Unfortunately cadmium is not an element required by animals; in fact it can compete with other essential metals such as zinc or iron, causing serious metabolic problems for organisms exposed to high doses. Fortunately, some estuarine species have overcome the dangers posed by heavy metals by mopping them up with proteins known as metallothioneins. But

Nereis has no such defences. So just how does our wriggly friend survive on contaminated coasts? Puzzled by this conundrum, Demuyneck and his colleagues have gone some way towards unravelling the mystery of this invertebrate's toxic tolerance (p. 1101).

A major entry route for heavy metals is via the gut, so Demuyneck and his team examined the intestines of cadmium-exposed worms with chromatography to look for proteins that might be binding the element. They found cadmium bound to a protein called MPIO. Demuyneck already knew that MPIO is very similar to a *Nereis* protein called myohemerythrin, which usually binds iron and carries oxygen round the worm's primitive blood system. Could the mysterious protein be involved in decontaminating the heavy metal?

The team began analysing the worms' intestines with an anti-MPIO antibody, and found many MPIO-positive cells in the lining of the gut; an unusual location for a blood-related molecule. Demuyneck's suspicions that this protein might play a role in removing cadmium were confirmed when he found an increase in MPIO protein levels when the worms were exposed to the metal. In addition the MPIO cells changed in morphology, suggesting protein production was being stepped up in response to cadmium poisoning.

Curious to find how levels of the decontaminating protein are regulated, the team looked at the levels of mRNA in *Nereis*'s gut cells and found that mRNA didn't rise in response to cadmium exposure; regulation of the protein must occur at the post-transcriptional level. This could either be achieved by an increase in protein translation from an existing pool of mRNA or by increasing the stability of MPIO, but which mechanism the invertebrate uses is unclear. In addition, the precise function of the protein in these worms remains a mystery. Demuyneck suspects that different forms of the protein perform distinct functions; perhaps one form carries oxygen in the blood, while another removes cadmium.

Digging by hand and losing your boots in the mud of a smelly estuary might not sound like everyone's idea of a great science project, but Demuyneck loves it, and hopes that by trawling through stinking mud for worms, he will eventually work out how these toxins are taken up and how they affect the animals that make their homes there. But while he is confident that the mystery of *Nereis*' toxic tolerance may be a step closer to being solved, Demuyneck adds

a note of caution that the levels and mixtures of pollutants accumulating on our industrial coastlines is becoming worryingly high, not just for fauna, but for humans too.

10.1242/jeb.00918

Demuyneck, S., Bocquet-Muchembled, B., Deloffre, L., Grumiaux, F. and Lepretre, A. (2004). Stimulation by cadmium of myohemerythrin-like cells in the gut of the annelid *Nereis diversicolor*. *J. Exp. Biol.* **207**, 1101-1111.

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WIRING AN ELECTRIC CONVERSATION



Picture by Robert Lewis

Whether we twitter, dance or send out scents, most creatures communicate with a suite of signals only appreciated by their own species. Many creatures have co-opted sound, colour and movement for communication, but mormyrid fish have opted for a relatively unconventional communication mode; they emit weak electric pulses. Bruce Carlson explains that each fish emits its own personalised electric bleep, but when Carlson recorded the fish's electric conversations, he realised that the fish also bleep in one of four distinct calling patterns. When resting, the fish discharge a simple train of low frequency pulses, but when they burst into action, they have the choice of three electric call patterns: scallops, accelerations and rasps. Knowing that the fish's conversational repertoire was limited to four 'calls', Carlson and his advisor Carl Hopkins decided to discover how the fish regulates its electric voice, and found that mormyrids use a single negative feedback loop to control the entire signalling system (p. 1073).

But before he could work out how the circuitry controls the fish's call patterns, Carlson had to trace the circuit's intricate network of neurons. From previous work, it was already known that a region of the brain, known as the command nucleus, directly controls each electrical discharge; there's a 1:1 correlation between an electronic discharge and command nucleus activity. But when he mapped the regions of

the fish's brain that triggered each electric organ discharge Carlson realised that the command nucleus was in turn regulated by both the midbrain precommand nucleus (PCN) and the dorsal posterior nucleus (DP). Carlson was puzzled; why was the command nucleus regulated by two upstream command centres?

Stimulating the PCN with the neurotransmitter glutamate, Carlson recorded the resulting electric discharge, and realised that the PCN was driving call patterns similar to the scallop signal. Repeating the experiments but stimulating the DP, Carlson recorded signals similar to the fish's acceleration call. Each centre controls a specific discharge signal, and they probably work together to produce the third.

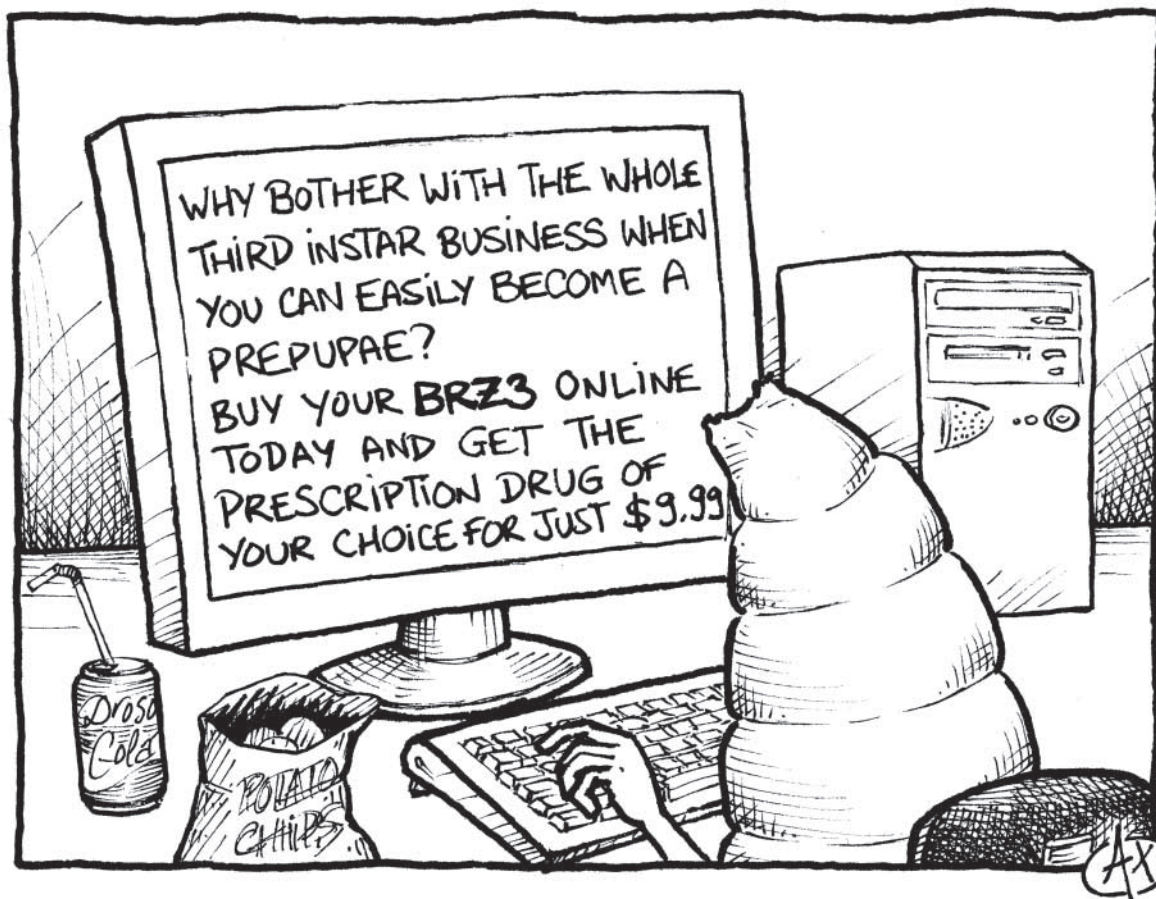
Carlson's neuron mapping had also thrown up an additional neural connection between the command nucleus and the PCN and DP; a feedback loop, that went *via* the ventroposterior nucleus of the torus semicircularis (VP). Carlson wondered why the fish might use a feedback loop from the command nucleus to regulate both driving nuclei. Was it inhibiting the signals from the PCN and DP to maintain the fish's gently repeating resting calls? If the feedback loop was inhibitory, cutting it off should provoke the fish to being uncontrollably discharging. Carlson decided to pharmacologically cut off the fish's feedback loop, and see what signals they began sending.

Blocking the feedback signal from the VP, Carlson measured the electric organ discharge. Sure enough, 'the fish suddenly started bursting like crazy' says Carlson, 'there was a very, very dramatic shift of activity ... it was clear that negative feedback plays a big role' he added. And when he took a closer look at the way the feedback signal interacted with both the DP and PCN, he realised that the signal also sets up differences between the two driving nuclei, resulting in the different calling patterns. 'The negative feedback sets up the entire system' says Carlson 'keeping it regular, but also allows bursts of electrical discharge'.

10.1242/jeb.00916

Carlson, B. A. and Hopkins, C. D. (2004). Central control of electric signaling behavior in the mormyrid *Brienomyrus brachyistius*: segregation of behavior-specific inputs and the role of modifiable recurrent inhibition. *J. Exp. Biol.* **207**, 1073-1084.

FAST DEVELOPERS



Axel Innis is a postdoctoral fellow in Bangalore India

Growing up is never easy, but with discrete developmental stages to be successfully completed, growing up fly-style is far more risky. Fly larvae embark upon a developmental program where they progress through instars, triggered by ecdysteroid hormones and regulated by transcription factors, before pupating. But Lynn Riddiford and her team at the University of Washington discovered that if one of the ecdysteroid hormone regulated transcription factors BR-Z3, known as Broad, was inadvertently activated, the larvae failed to moult, bypassing the usual moulting

program, and progressing directly on to the insect's final right of passage: pupation. Intrigued by this unusual departure from the insect's developmental program, Xiaofeng Zhou began investigating *Drosophila*, where he could activate BR-Z3 and related transcription factors at will. Watching the flies altered developmental progress, he discovered that misexpression of Broad transcription factors blocks the surge of ecdysteroid hormones that insects need to initiate moulting. Instead the insects continued growing until they reached their critical weight, when they begin pupating

despite having missed their final instar (p. 1151).

10.1242/jeb.00915

Zhou, X., Zhou, B., Truman, J. W. and Riddiford, L. M. (2004). Overexpression of *broad*: a new insight into its role in the *Drosophila* prothoracic gland cells. *J. Exp. Biol.* **207**, 1151-1161.

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