Gender-bending alligators use ESR1

It's a boy! Or is it? We live in an era of blurred gender lines, but for many reptiles this has always been a hot topic. In crocodilian as well as several turtle species, cranking up the thermostat a few degrees during a critical developmental window, called the thermo-sensitive period, will turn a whole clutch of eggs into males. Hormones also contribute to sex determination in these species, and exposure to female hormones – estrogens – can feminize alligators developing at a male-producing temperature. But while there is more than a decade of research on estrogen-mediated sex reversal, the mechanisms behind this phenomenon remain largely elusive. Knowing that estrogens can communicate with cells using either of two receptors (ESR1 or ESR2), a team of scientists based in South Carolina, at the lab of Louis Guillette Jr, wanted to know which of these two receptors is responsible for changing baby boy alligators into baby girls.

Guillette's team, led by Satomi Kohno, began by identifying two estrogen-like chemicals to differentially stimulate the estrogen receptors of the American alligator. The first chemical interacted only with ESR1 and the second chemical preferred ESR2. Armed with the tools needed to solve the sex-shifting conundrum, Kohno set out to raid alligator nests and collect freshly laid eggs for his gender-bending experiments. Back at the lab, he divided the eggs into four groups and left the first group to develop at a temperature that would produce female alligators. He put the other three groups at a warmer male-producing temperature, but he applied the ESR1-stimulating chemical to one group, to another he applied the ESR2-stimulating chemical and the last clutch received no chemicals. Kohno wanted to know which of the estrogen-like chemicals would feminize the developing gators. After allowing enough time for the gonads of the alligator embryos to develop, the team cracked the eggs, and the case!

Kohno compared the little gators from the egg groups given the ESR1- and ESR2-stimulating chemicals to see whether their developing gonads more closely resembled ovaries or testes. They found that eggs given the ESR1-stimulating chemical and incubated at a male temperature produced alligators with gonads that more closely resembled the appearance and gene expression patterns of ovaries, like those seen in the alligators that developed at the female temperature. Conversely, they found that the eggs given the ESR2-stimulating chemical shared the gonadal characteristics of the chemical-free male-producing group. This means that the sex reversal seen in male alligators exposed to estrogens during the thermo-sensitive period is mediated through ESR1, not ESR2. The switcheroo is through one, not two!

10.1242/jeb.112540

Kohno, S., Bernhard, M. C., Katsu, Y., Zhu, J., Bryan, T. A., Doheny, B. M., Iguchi, T. and Guillette, L. J., Jr (2015). Estrogen receptor 1 (ESR1; ERα), not ESR2 (ERβ), modulates estrogen-induced sex reversal in the American alligator, a species with temperature-dependent sex determination. *Endocrinology* **156**, 1887-1899.

Sarah Alderman
University of Guelph
alderman@uoguelph.ca

Begrudging the bat

Man has long envied the august lifestyle of the soaring bat. As our closest living relative with the ability to fly, the bat enjoys all the perks of placental mammal-hood (i.e. a pleasantly warm internal body temperature and the convenience of internal reproduction), without being confined to the wearisome two-dimensional reality of the common surface dweller.

Even other creatures of the sky have good reason to be jealous of bats. While bird and insect wings are built primarily of stiff, dead tissue (keratin and chitin, respectively), the bat wing is a flexible living membrane. The skin that makes up the bat wing, called the patagium, contains sensory neurons and muscles that finely control the shape, area and camber of the wing membrane. These features endow the bat with unsurpassed aerial maneuverability and panache.

As bats are the only flying creatures with wings made of skin, an interesting question is how peripheral sensory neurons in the bat's wing are specialized to sense mechanical forces during flight. A recent collaborative study from the laboratories of Ellen Lumpkin and Cynthia Moss, at Columbia University, USA, and the University of Maryland, USA, has investigated this question by describing the identity and organization of mechanoreceptor neurons that innervate the wing of the big brown bat, *Eptesicus fuscus*.

Kara Marshall and colleagues first used fluorescent dye-fill techniques to trace the axons of wing mechanoreceptor neurons back into the spinal cord. They were surprised to discover that many of these neurons arose from lower regions of the spinal cord, which do not typically innervate forelimbs in other mammals. In contrast, they found that wing motor neurons exhibited a similar organization to the mouse spinal cord. This means that some sections of the bat spinal cord contain sensory and motor neurons that innervate distinct regions of the body, a unique arrangement that could have interesting implications for central sensorimotor processing in the spinal cord and brain.

The team next examined the occurrence and distribution of sensory receptors on the bat wing. They identified many of the same mechanoreceptor neurons found in mouse and human hairy skin, with some important distinctions. For example, they discovered that hair follicles on the bat wing are often innervated by two distinct sensory neuron classes: lanceolate endings, which detect hair movement, and Merkel cells, which respond to sustained indentation of the skin. This arrangement may allow the bat to monitor airflow across the wing during flight, via the lanceolate endings, while maintaining the ability to sense and manipulate objects with the forelimbs, using Merkel cell complexes.

Finally, the authors measured the activity of single neurons in somatosensory cortex while they mechanically stimulated the wing. They found that cortical neurons typically responded to the onset of touch stimuli, whether brief air puffs or sustained poking, by firing sparse bursts of action potentials. These results are consistent with cortical recordings in other mammalian species, indicating that tactile signals from the bat wing may be encoded much like touch signals from the primate hand or rodent paw.

Overall, this study reveals that several of the basic components of mammalian somatosensation, in particular the peripheral mechanosensory neurons in the wing membrane, are uniquely specialized to empower the bat's flamboyant flight style. In parallel with recent advances in the genetic identification and targeting of mechanoreceptors in hairy skin of mice, studies in the bat can show how similar sensory structures may be specialized to serve widely divergent functions. Unlocking the secrets of the patagium may also help to allay the plague of bat envy that has infected humankind for generations.

10.1242/jeb.112524

Marshall, K. L., Chadha, M., deSouza, L. A., Sterbing-D'Angelo, S. J., Moss, C. F. and Lumpkin, E. A. (2015). Somatosensory substrates of flight control in bats. *Cell Rep.* **11**, 851-858.

John Tuthill
Harvard Medical School
John_tuthill@hms.harvard.edu

Slime mold sticks to slink along

Slime mold: it's repulsive and slimy, isn't actually a fungus and can't possibly be of any use. And yet, slime molds are serving as excellent model systems for one of the most ubiquitous and important cellular phenomena, cell migration. Cell migration is critical for wound healing, embryonic development and the immune response, among other processes – and while the molecular mechanisms driving cell migration have been extensively studied, the physical mechanics of cell migration remain largely a mystery. So, how could slime molds help us to understand cell migration? For one, slime molds are very good at moving around. A plasmodial slime mold, like the yellow *Physarum*, is a single huge multinucleate cell that rhythmically shuttles its cytoplasm through its own tubular network. Smaller slime mold amoebae also exhibit periodic movement of their cytoplasm, which coincides with a tremendous increase in cell migration speed. Given the motility of slime mold amoeba and slime mold's large cell sizes, they make an excellent system for studying the mechanics of cell migration.

Owen Lewis of the University of Utah, USA, and colleagues at the University of California in San Diego and Davis took advantage of the benefits of working with slime mold to examine how cytoplasmic flow, cytoskeletal contraction and substrate adhesion contribute to cell locomotion. The researchers took a two-pronged approach to their study, coupling measurements of cytoplasmic flow, traction and substrate displacement taken from real slime mold amoeboid cells with a computational approach where they incorporated those data into a model of a slime mold cell that included additional parameters for flow, cytoskeletal contraction, and adhesion.

The slime mold's movement was characterized by a traveling wave of cytoplasmic flow and a traveling wave of contraction. These waves had equal periods, but a phase lag of about one-third of a cycle. This produces a cycle of traveling contracting and expanding regions, moving from anterior to posterior. Earlier studies had suggested that this pattern of flow alone could produce forward movement – but Lewis and colleagues' data showed that the observed flow patterns in the slime mold cells would actually tend to produce backward movement without other influences.

With this apparent paradox in hand, the researchers used their computational model to see whether one of the other parameters they modeled was critical for forward motion. When they varied the adhesion parameters, they found that not only is hydrodynamic flow essential for locomotion but also the transmission of the flow stress to the substrate via adhesion was necessary. The phase of adhesive forces relative to flow stress was key – flow and adhesion must be strictly coordinated if the cell is to move anywhere quickly.

Lewis and colleagues were also able to test several other hypotheses about amoeba motility by using their integrated experimental dataset and computational model. They showed not only that slime mold movement is a precisely coordinated dance of cellular flow and adhesion but

also – and perhaps more importantly – the utility of using this slimy system for understanding cell migration.

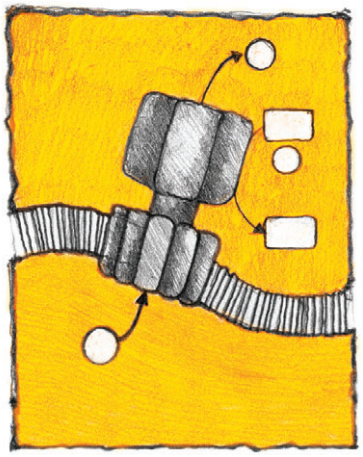
10.1242/jeb.112516

Lewis, O. L., Zhang, S., Guy, R. D. and del Álamo, J. C. (2015). Coordination of contractility, adhesion and flow in migrating Physarum amoebae. *J. R. Soc. Interface* **12**, 20141359.

Kara Feilich
Harvard University
kfeilich@fas.harvard.edu

Evolution of insects' renal control system

RENAL FUNCTION



The Malpighian (renal) tubules of insects are functionally analogous to mammalian kidneys, as they are involved in the homeostatic maintenance of the insects' fluid balance by controlling the volume and ion/solute composition of the urine they produce. The production of urine (diuresis) is under the control of different neuropeptide/receptor systems whose evolutionary origin and functional relation to the renal tubule's architecture is largely unexplored. In a study published recently in *Nature Communications*, a team of scientists led by Julian Dow from the University of Glasgow, UK, examined the origin of insect renal function using an exceptional approach based on fluorescent neuropeptide analogues to localize neuropeptide receptors in renal cells.

Renal tubules of insects have been extensively studied in flies and mosquitoes. They are formed by a single-layered epithelium which typically consists of two cell types, so-called principal and secondary (or stellate) cells. According to the classical two-cell-type model, diuresis is driven by a V-type ATPase that resides in the apical membranes of the principal cells. This pump powers the exchange of potassium ions against protons so that net potassium ions that are secreted into the lumen are passively followed by water. Several diuretic and antidiuretic hormones control fluid secretion, including the highly conserved diuretic neuropeptides kinin, capa and DH31. While the last two act on the principal cells stimulating V-type ATPase activity, kinin increases diuresis by stimulating the transcellular chloride transport in secondary cells, which facilitates potassium ion secretion. But does this model of fluid secretion account for insects other than flies and mosquitoes and when was this system established during the evolution of insects?

To answer these questions, Dow's team designed a set of nifty experiments that allowed them to test neuropeptide function along with their sites of action. For this purpose, they synthesized the kinin, capa and DH31 neuropeptides and coupled them to a red fluorophore, hoping that the artificial neuropeptides would work like the natural ones and stimulate diuresis in isolated renal tubules. They further reasoned that if the neuropeptides were functional, they should be able to use them to identify principal and secondary cells by fluorescence microscopy after they had bound to the corresponding receptors in the basal membranes of these cells.

Testing their concept in the well-characterized model fly *Drosophila melanogaster*, the team found that the neuropeptides were functional, binding to the corresponding receptor in the correct target cell. Next, they traced the evolutionary origin of the two-cell-type model by probing renal tubules from various strategically selected insect species representing different phylogenetic orders. By comparing these data with genomic (presence/absence of neuropeptide/receptor genes) and functional data (neuropeptide effects on diuresis), they constructed a clear picture on the structure–function relationship of the hormonal system regulating diuresis in the insect's renal tubules.

It turned out that secondary cells are much more widespread than previously believed, because they could detect kinin labelling in secondary cells of most of the more advanced holometabolous insects. In the more primitive hemimetabolous insects, however, kinin bound uniformly to the renal tubule, with no evidence for secondary cells, suggesting that the kinin signalling pathway evolved before the radiation of insects and that holometabolous insects have evolved kinin-responding secondary cells. A more surprising finding was that diuretic control is organized differently in the largest insect order, the beetles, as these holometabolous insects obviously have lost the kinin signalling pathway secondarily, and only a subset of their renal cells respond to capa and DH31 signals.

Dow and his team have convincingly demonstrated that the two-cell-type model describing renal function and control accounts for most advanced insects, except for beetles, and that it may have evolved from a single cell-type renal system already capable of responding to kinin signals.

10.1242/jeb.112532

Halberg, K. A., Terhzaz, S., Cabrero, P., Davies, S. A. and Dow, J. A. T. (2015). Tracing the evolutionary origins of insect renal function. *Nature Commun.* **6**, 6800.

Hans Merzendorfer
University of Osnabrueck
merzendorfer@chemie-bio.uni-siegen.de