

the same variability in the freely feeding sea slugs?

Carrying out experiments in feeding *Aplysia*, the team made recordings from the nerves and radula muscles. They thought that control mechanisms from the rest of the nervous system in an intact animal would kick in and remove some of the variation – not so. The results were always the same: even when *Aplysia* were feeding on seaweed that was controlled for shape and size, the feeding rhythm was still variable. The team think most of the variability comes from the central pattern generator, and next want to discover the mechanism that generates the variability.

It would seem that *Aplysia* operate a far from perfect eating system, but Horn suggests that it is this variability that allows the feeding mechanism to function effectively over a very wide range of circumstances, tolerating disturbances and hiccups; a less variable system could fail to cope with problem situations. While this can cause *Aplysia* to mess up sometimes, missing its mouth or inadvertently spitting out half chewed pieces, in the end this could be the best strategy to ensure that the hungry mollusc gets the most from a meal, while using the least amount of energy per mouthful.

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**Horn, C. C., Zhurov, Y., Orekhova, I. V., Proekt, A., Kupfermann, I., Weiss, K. R. and Brezina, V.** (2004). Cycle-to-cycle variability of neuromuscular activity in *Aplysia* feeding behaviour. *J. Neurophysiol.* **92**, 157-180.

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## NEW ATPASE FOUND IN ATLANTIC STINGRAY

An exciting new discovery has been made in the Atlantic stingray that has implications for ion regulation in elasmobranchs. In mammals, an active transport protein (HK $\alpha$ 1) is present in the stomach and kidney that mediates proton (H<sup>+</sup> or acid) secretion in exchange for potassium ions (K<sup>+</sup>) and functions in digestion, acid–base balance and ion regulation. Like mammals, elasmobranchs (sharks, skates and rays) are also believed to secrete acid into their stomachs, but unlike mammals, they primarily use their gills for acid excretion and ion regulation instead of their kidneys. Keith Choe, working with David Evans and his team, set out to determine whether an HK $\alpha$ 1-like protein exists in the Atlantic stingray and to determine if it is expressed in the gill.

The group searched for HK $\alpha$ 1 in the Atlantic stingray gill and stomach by using an antibody made against pig HK $\alpha$ 1. With this antibody, the group identified HK $\alpha$ 1 in gill cells rich in Na<sup>+</sup>/K<sup>+</sup> ATPase. These cells are important in acid secretion and ion absorption in fish. HK $\alpha$ 1 immunoreactivity was not found in cells that stained for V-type H<sup>+</sup>-ATPase; cells that are thought to be important in base secretion in fish. Knowing that HK $\alpha$ 1 is found in mammalian gastric glands, the team also found HK $\alpha$ 1 immunoreactivity in the stomach of the stingray. Having found that the stingray produces HK $\alpha$ 1, the next step was to determine whether its expression is regulated during freshwater exposure or ion regulation.

Characterising stingray HK $\alpha$ 1 on a molecular level, Evan's team found that it is highly similar to rat HK $\alpha$ 1, and that it is expressed in the stingray's stomach and gill but, surprisingly, not in the kidney. Using

quantitative, real-time PCR, the team determined that the expression of gill HK $\alpha$ 1 does not change with elevated environmental CO<sub>2</sub> but is higher in fish acclimated to freshwater than those acclimated to seawater suggesting that it plays a role in freshwater acclimation.

That the expression of HK $\alpha$ 1 in the gill is unaffected by the respiratory acidosis caused by high environmental CO<sub>2</sub> suggests that this gene is not involved in acid–base balance. However, this finding does not exclude the involvement of pre-existing gill HK $\alpha$ 1 proteins in response to acid–base disturbance. Importantly, HK $\alpha$ 1 is upregulated in freshwater-acclimated stingrays, suggesting that it may act as a mechanism to increase active K<sup>+</sup> uptake across the gill when environmental K<sup>+</sup> levels are low, possibly making HK $\alpha$ 1 vital for the physiological fitness of this freshwater organism.

This study is the first to provide direct evidence for the presence of HK $\alpha$ 1 in any fish. From an evolutionary perspective, stomach acid secretion first appeared in elasmobranchs, which suggests that the Atlantic stingray may be one of the oldest living organisms to have HK $\alpha$ 1.

10.1242/jeb.01301

**Choe, K. P., Verlander, J. W., Wingo, C. S., and Evans, D. H.** (2004). A putative H<sup>+</sup>/K<sup>+</sup> ATPase in the Atlantic stingray, *Dasyatis sabina*: primary sequence and expression in gills. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **286**, R1152-R1157. doi:10.1152/ajpregu.00513.2003

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