INSIDE JEB

Hiat proteins help zebrafish excrete toxic ammonia

A 4 day old zebrafish larva. Photo credit: Yihang (Kevin) Pan.

Proteins are everywhere. Contributing to the bodies of every life form on the planet, from single-celled organisms to mighty blue whales, they are fundamental. But proteins decay, and one form of the resulting waste, ammonia, is dangerously toxic. 'To avoid its negative side-effects, ammonia needs to be rapidly excreted from body fluids', says Haonan Zhouyao from University of Manitoba, Canada. Fish excrete the toxin into the water, where it is rapidly diluted for safety, and many depend on a suite of specialised transporter proteins within cell membranes to transport the toxin from tissues through the blood to their gills, where it is ejected. However, Dirk Weihrauch (University of Manitoba) explains that some aquatic creatures do not conform to the established ammonia excretion cascade, employing alternative proteins, such as 'hippocampus-abundant transcript 1' protein (Hiat1) to eject ammonia from their bodies. When Alex Zimmer – working at the University of Ottawa, Canada, with Steve Perry – heard about Sandra Fehsenfeld's (Université du Quebec, Canada) discovery of Hiat1, he wondered whether his favourite fish, zebrafish, may also benefit from the alternative ammonia transporter. The Weihrauch and Perry labs teamed up with Peter Eck's group (University of Manitoba) to find out.

Knowing that zebrafish carry three *Hiat1*-like genes, Zhouyao and Zimmer checked whether the proteins encoded by the genes carried the key features essential for them to function as an ammonia transporter. Analysing the amino acid sequences, Zhouyao was encouraged that the proteins all contained the defining features and could contribute to zebrafish ammonia excretion, but would they in practice?

To answer this question, Zhouyao painstakingly prepared mRNA coding for two of the zebrafish Hiat proteins and injected each into African clawed frog eggs to see whether they would produce the zebrafish Hiat proteins and whether these proteins could transport a close relative of ammonia, methylamine, into the eggs. Impressively, the modified frog eggs did absorb methylamine: the zebrafish Hiat proteins were capable of transporting ammonia. So the team decided to find out where the genes for the potential ammonia transporting proteins were most active in the bodies of developing zebrafish embryos.

Injecting tiny RNA probes that would seek out locations in the zebrafish embryo bodies that were actively translating the *Hiat1* genes into proteins, Thomas Liebenstein, David Richter and Gerrit Begemann (Universität Bayreuth, Germany) discovered that the embryos began producing the proteins in almost all of their tissues 4 days after fertilization. The proteins were turning up everywhere: in the heart, brain, gills and digestive system. One or both of the zebrafish Hiat1 proteins must be doing something important for the fish to be producing them in so many organs, but the team needed categorical proof that the proteins were capable of expelling ammonia from the larvae.

This time, Zhouyao injected two additional forms of RNA into the developing zebrafish, one of which would prevent the larvae from producing the zebrafish Hiat1a protein and another to block production of the Hiat1b protein, and then tracked how much ammonia the larvae were able to expel from their bodies. The embryos that could not produce zebrafish Hiat1b suffered a 30% reduction in their ability to excrete ammonia. And, when Zimmer checked the level of ammonia excretion near to the developing gill, it was significantly reduced. Zebrafish Hiat1b definitely contributes to the larvae's ability to excrete ammonia.

'This study is the first to identify zebrafish Hiat1b as an important contributor to ammonia excretion in larval zebrafish', says Zhouyao, adding that researchers should consider these alternative transporters, which are carried by many other creatures, when investigating the complexities of ammonia excretion in fish.

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