

INSIDE JEB

Rh protein is key for crab waste disposal



A Japanese blue crab (*Portunus trituberculatus*) after it has moulted. Photo credit: Lingjun Si.

The seabed is far from pristine and can accumulate toxic ammonia in the debris and decaying remains that accumulate there, threatening other dwellers. ‘Ammonia is a major environmental limitation for crabs’, says Luqing Pan from the Ocean University of China. In addition, crabs and other marine species naturally produce and excrete the gas as waste and he details several mechanisms that allow the resourceful crustaceans to excrete it in polluted environments. However, it was not clear whether crabs also carry a specialised class of proteins, known as Rhesus-like glycoproteins (Rh proteins), which pump ammonia out of tissues in mammals, some insects and

crustaceans. Intrigued by the possibility that the Japanese blue crab (*Portunus trituberculatus*) may depend on the protein to clear their bodies of ammonia, Pan and Lingjun Si began searching for one of the elusive Rh protein genes in the crab’s DNA.

Collecting gills from the crabs, the duo successfully identified a 1855 base pair gene that codes for a protein with all of the essential amino acid characteristics of a Rh protein. And when they searched for evidence of mRNA produced by the gene in a variety of tissues, they found it was mostly produced in the gills, which are the main site of ammonia excretion in

many aquatic species. But the duo needed evidence that Rh protein was actually produced from the mRNA in the gill and was responsible for ammonia excretion.

Injecting small fragments of RNA into the crustaceans to disrupt production of the protein, the team noticed that the crabs switched to producing other proteins that could contribute to ammonia excretion. And, when they measured how fast the animals were expelling ammonia from their bodies, the rate fell dramatically in the animals that had lost use of their Rh gene, while ammonia accumulated in their haemolymph.

Having convinced themselves that the crabs produce the Rh protein, Si and Pan teamed up with Hongdan Wang and Xin Zhang to monitor the responses of several genes that are involved in ammonia excretion in real time to better understand the role of the Rh protein, in combination with other pump proteins, in ammonia waste disposal and they suspect that it is critical. ‘[It] may be the basis of the crab’s ability to inhabit benthic water with high ammonia levels’, says Si.

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