that *galloprovincialis* had left the Pacific for the warmer Mediterranean a mere 3.5 million years ago, but what adaptations had the mussel made during its brief European sojourn that allowed it to thrive and drive *trossulus* out? Peter Fields, returning to Somero's lab for a brief sabbatical period, decided to take up the challenge by investigating one of the mussel's key metabolic enzymes: cytosolic malate dehydrogenase (p. 656).

According to Fields, malate dehydrogenase is a well-characterised, and essential, component of several metabolic pathways, but the enzyme is also temperature sensitive, making it an ideal candidate for adaptation to warmer conditions. However, sequencing the gene from mussels proved trickier than Fields had hoped. Little is known about mollusc genomes, so designing the DNA primers essential for the sequencing process proved challenging. Fortunately Somero's colleague, Andy Gracey, was on hand to guide Fields through the complex sequence databases needed to design the primers, before Fields ran the sequencing reactions on both blue mussels and another California local, *Mytilus californianus*, that lives in the mild waters where *galloprovincialis*'s and *trossulus*'s territories overlap.

Having sequenced the three genes, Fields realised that one position in the gene lit up as a mutation hot spot; all three species had completely different amino acids at position 114, the hinge of a loop region essential for the enzyme's catalytic activity. Fields was astonished. He explains that he'd investigated related enzymes in cold adapted species and never seen mutations at this location before. What effect would this mutation have on the enzyme's function?

Instead of extracting the enzyme directly from each of the three mussels, Fields' student, Emily Rudomin, expressed all three proteins in bacteria, allowing her to produce larger quantities of each enzyme than she could extract from the mussels. Measuring each enzyme's kinetics, Fields realised that each of the enzymes were well adapted to the water temperature where the mussels lived. He explains that enzymes that function well in warm conditions have low substrate turnover rates and bind their substrate more tightly than enzymes adapted to cold conditions, which process their substrate faster and bind substrate more loosely. Sure enough, the warm adapted *galloprovincialis*' malate dehydrogenase behaved like a warm adapted enzyme, while the cold adapted *trossulus* enzyme functioned best at low temperatures. *Mytilus californianus*, found on the beach outside Somero's Pacific Grove lab, was optimised for a mild, intermediate temperature.

The mutated amino acid located in the hinge region had dramatically affected the enzyme's function, and is one of a raft of adaptations giving the impostor, *Mytilus galloprovincialis*, the upper hand over *Mytilus trossulus* in hot water.

10.1242/jeb.02107

Fields, P. A., Rudomin, E. L. and Somero, G. N. (2006). Temperature sensitivities of cytosolic malate dehydrogenases from native and invasive species of marine mussels (genus *Mytilus*): sequence-function linkages and correlations with biogeographic distribution. *J. Exp. Biol.* **209**, 656-667.

TUNICATES SET TREND FOR POTASSIUM CHANNEL

Ion channels are proteins that form miniscule pores in nerve cell membranes, and when open they generate currents across the membrane to propagate nerve signals. Andrew Spencer from the University of Alberta, Canada, explains that one class of ion channels, the potassium channels, play a major role in shaping the action potentials in many tissues, including heart, and as a consequence have been subject to strong natural selection resulting in a wide variety of potassium channels, each finely tuned to a particular function. One family of potassium ion channels found in vertebrate hearts, the Kv4 voltage-gated potassium channels, comprises three individual members (Kv4.1, Kv4.2 and Kv4.3) that evolved from one of our ancestor's early Kv4 channels. However, the hearts of simpler invertebrates only carry a single Kv4 channel, like our ancestors. Knowing that sea squirts (tunicates) are our most ancient living relatives, Vicenta Salvador-Recatalà and Andrew Spencer wondered whether our tunicate ancestors are more like invertebrates, with only one Kv4 channel, or whether the *Kv4* gene had been duplicated early enough in evolutionary history for tunicates to carry multiple Kv4 ion channels. Salvador-Recatalà decided to investigate how many copies of the gene the tunicate *Ciona interstinalis* possesses, and whether the ion channel plays a role in the tunicate's simple heart (p. 731).

Searching through the *C. intestinalis* genome, Salvador-Recatalà, Warren Gallin and Spencer identified a single *Kv4* gene; the vertebrate channel must have become duplicated after tunicates diverged from our family tree. Salvador-Recatalà also knew that an accessory protein, KChIP, modulates the function of modern Kv4 channels, and wondered whether our tunicate predecessors still carry this gene? Trawling through the genome again, the team was delighted to discover and clone the first non-vertebrate *KChIP* subunit gene.

Curious to know how the tunicate's potassium channel functions, Salvador-Recatalà, Peter Ruben and Jennifer Abbruzzese began investigating the ion channel's electrical properties by injecting *Xenopus* egg cells with different combinations of Kv4 and KChIP RNA. Knowing that the cells would use the RNA to produce the ion channel and modulating protein, the team monitored the electrical properties of the modified cells and found that when they introduced the channel alone, the egg cells generated a significant potassium current. However, when the team generated both Kv4 and KChIP proteins in the cells, they saw a dramatic shift in the Kv4 channel's electrical properties; KChIP increased the strength of the egg cell's potassium current as well as prolonging the current's duration, suggesting that the ancient channel contributes to tunicate heart function. Spencer suspects that KChIP probably aids insertion of the ion channel in the cell's membrane, increasing the number of ion channels in the membrane, and the potassium current in turn.

Probing the ion channel's function further, the team removed the first 32 amino acids of the Kv4 protein, which interact with KChIP in vertebrates, to see whether this affected the tunicate channel's function; KChIP no longer modulated the truncated ion channel's function, suggesting that KChIP interacts with tunicate Kv4 through the channel's N terminus, just like modern KChIP. So tunicates invented the blueprint for Kv4 ion channel modulation while vertebrates have refined ion channel function further by expanding their repertoire of *Kv4* genes.

10.1242/jeb.02109

Salvador-Recatalà, V., Gallin, W. J., Abbruzzese, J., Ruben, P. C. and Spencer, A. N. (2006). A potassium channel (Kv4) cloned from the heart of the tunicate *Ciona intestinalis* and its modulation by a KChIP subunit. *J. Exp. Biol.* **209**, 731-747.