Hibernating Mammals Pass the Acid Test: Multiple Independent Evolutions of Enhanced Proton Block of Sodium Channels in Acid-Sensing Pain Receptors

Harold Zakon^{a–c}

Departments of ^aNeuroscience and ^bIntegrative Biology, University of Texas, Austin, Tex., and ^cBay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole, Mass., USA

Stephen Jay Gould famously described a *Gedankenexperiment* of rewinding and replaying the ‘tape of life’ to see whether life would inexorably evolve as we know it, or whether chance, instead, would result in a drastically different world. And as biologists have pointed out, examining instances of independent (parallel or convergent) trait evolution is as close as it gets to attaining this goal. We can point to a plethora of traits that have evolved independently: wings, a hydrodynamic body shape in rapid swimmers, spines and wax in desert plants, thumbs in pandas and primates, etc. But in the molecular age, we must push one layer deeper: do identical or similar changes at the molecular level underlie independently derived similarities at the organismal level [Zakon, 2002]? A recent paper on evolutionary change in voltage-gated sodium channels in pain receptors gives a resounding ‘Yes!’

Naked mole rats (*Heterocephalus glaber*) live jam-packed in large subterranean colonies. One consequence of this is that they breathe stale air high in CO₂; this potentially results in acidosis in body tissues as the CO₂ mixes with water, forming carbonic acid, which dissociates into carbon-

ate and protons (H⁺). Naked mole rats have evolved excellent pH regulation to counter this problem [Park, pers. commun.], but exposure to such high levels of carbonic acid on the moist skin, untouched by internal buffering, would potentially burn the eyes and respiratory membranes of animals by activating the free nerve endings of small-diameter trigeminal nerve fibers (this is why carbonated soda pop stings the eyes and tickles the throat). Acid activates a variety of channels (ASIC, TRPV) [Holzer, 2011] that depolarize the trigeminal terminals. Depolarization of the terminals then recruits two voltage-gated sodium channels (Na_v1.7 and Na_v1.8) to trigger action potentials sending a message of ‘pain’ to the CNS. However, as an adaptation to this high CO₂ environment, naked mole rats show no behavioral avoidance of or nociceptor activation by acid [Park et al., 2008; LaVinka and Park, 2012].

Smith et al. [2011] elegantly elucidated a molecular mechanism for this protection. Surprisingly, the acid-sensing channels, the most obvious location for adaptive molecular change, did not differ between naked mole rats and mice. However, the sodium channel Na_v1.7 showed an enhanced

proton block. Sodium channels are blocked by protons that bind to the outer mouth of the channel [Khan et al., 2002]. However, the affinity for protons is so low that this only becomes physiologically relevant to most sodium channels in pathological situations such as ischemia-induced acidosis, unless an animal lives in a cloud of CO₂. In trigeminal terminals, H⁺ acts in opposing directions, simultaneously depolarizing the terminal and blocking the sodium channels. Normally, the depolarization is large and the block is slight, so action potentials are produced, albeit fewer at a low pH than at a pH of 7.0. In the Na_v1.7 channel of the naked mole rat, this balance is altered so that the block of sodium channels is enhanced, further decreasing action potential generation at a low pH. Comparisons of Na_v1.7 sequences of naked mole rats and a few other mammals pinpointed two closely spaced, negatively charged amino acids in the outer pore of the naked mole rat channel at sites of highly conserved positive or neutral amino acids in Na_v1.7 of other mammals. Site-directed molecular tinkering demonstrated that the proton affinity was causally related to the two negatively charged amino acids.

But naked mole rats are not the only mammals with a life history that exposes them to excess CO₂; this is also true of animals that hibernate. With a known sequence for enhanced proton binding in Na_v1.7 channels in hand, Liu et al. [2013] obtained Na_v1.7 sequences from genome databases or by their own cloning efforts from 71 species across a wide range of mammals (monotremes to placentals). Their dataset targeted hibernators and species that do not hibernate. They found sequences with two negative amino acids as observed in naked mole rats in hedgehogs and 3 unrelated families of bats, all of which hibernate, suggesting 5 independent evolutions of the dual amino acid-based proton sensitivity. Hibernating marsupials had a single negative charge at this site, and 2 hibernating lineages (marmots and tenrecs) showed no substitutions. On the other hand, there were no amino acid substitutions in any of the species that do not hibernate. The authors then made three-dimensional models of the sodium channel pore with all the various amino acid substitutions that they encountered and estimated the proton binding affinity for each. All the sequences, even those with only a single

negatively charged amino acid, had enhanced proton binding affinities compared with the conserved sequence from nonhibernators. Finally, an application of tests for rates of evolution (d_N/d_S) indicated that the sites in question had evolved under positive selection. They conclude that enhanced sensitivities for protons occurred in hibernating groups many times and significantly more often than expected by chance.

This raises the question of whether enhanced proton block has independently evolved in the other nociceptive sodium channel, Na_v1.8, in CO₂-exposed mammals. This channel certainly shows evolutionary flexibility: a recent study on the evolution of resistance in the nociceptors of grasshopper mice (*Onychomys torridus*) to the pain caused by the venom of their scorpion prey highlighted Na_v1.8 [Rowe et al., 2013]. Here, again, a molecular signature for resistance was defined. An intriguing question for the future is whether Na_v1.8 has independently evolved similar amino acid substitutions in other mammals that take scorpions.

These studies augment other work on the independent evolution of Na⁺ channel pore sequences wrought by exposure to the

sodium channel blocker tetrodotoxin in the tetrodotoxin-sequestering pufferfishes [Jost et al., 2008] and different species of snakes that prey on tetrodotoxin-protected newts [Feldman et al., 2012]. In many of these examples, candidate amino acid substitutions in focal species jump out from sequence alignments even to the untrained eye as they contrast with the otherwise highly conserved amino acids in the sequences from nonfocal species. This raises a new set of questions. Highly conserved sequences are that way for some functional reason. In the cost-benefit ratio of natural selection, what has a sodium channel, and ultimately the organism, given up in order to alter its properties [Lee et al., 2011; Wu et al., 2013]?

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