



**A simplified phase diagram of water.** Roman numerals denote different solid phases of water

If there really is a liquid-liquid critical point in water, then the transition from one liquid to the other must be first-order, that is, discontinuous in density. If LDA and HDA are the low-temperature manifestations of these two forms of water, then the transition from LDA to HDA, or vice versa, must be sudden. But is the HDA-LDA transition really sudden, or does the structure relax in a stepwise fashion via a series of intermediate structures? Could the relaxation even be continuous? On page 1320 of this issue, Tulk *et al.* (6) provide experimental evidence for such a continuum. (Of course, if HDA and LDA are not the low-temperature forms of the two purported liquids, then

nearest-neighbor tetrahedron, the hydrogen bonds become increasingly bent or broken as the pressure (and hence density) is increased.

At the temperature at which HDA is stable, the transition from LDA to HDA cannot be induced directly, because the material is invariably in powdered form and hence the density cannot be increased continuously by applying hydrostatic pressure. Instead, Tulk *et al.* attempt to capture amorphous ice in its intermediate states by carefully annealing samples of HDA at different temperatures. After each annealing, they cool the samples to 40 K and measure their x-ray and neutron diffraction patterns. The results seem unambiguous: A whole series of intermediate

there might be some new forms of water out there still to be discovered.)

Another known liquid water phase may provide some clues to the low-temperature behavior of water. In the stable liquid phase just above the melting point, the structure of water undergoes a characteristic and continuous transition from an open network structure at low pressures to a much denser form at high pressure. As the density increases, the tetrahedral arrangement of nearest-neighbor molecules found in ordinary water and hexagonal ice is preserved, as it is in many of the phases of ice. However, beyond the

(and possibly continuous) structures is found, and the diffraction patterns cannot be represented as a linear combination of the diffraction patterns of HDA and LDA.

In other words, the intermediate structures must be distinct from those of both of these amorphous ices. If this interpretation is correct, then HDA and LDA cannot exist as unique entities. Tulk *et al.*'s evidence for a continuous, or quasi-continuous, transition between LDA and HDA (6) makes it more difficult to support a thesis that there is some point in the water phase diagram where two distinct disordered forms of water can coexist.

Of course, one experiment does not resolve all the issues. The liquid-liquid critical point scenario has spawned many theoretical and experimental studies, and it does provide one of the first qualitative frameworks for describing water properties. Nonetheless, alternative scenarios such as that described in (7) can also be quite successful at predicting water properties. What is certain is that experiments like that reported by Tulk *et al.* (6) will provoke ever more incisive techniques for looking at water at the molecular level.

#### References

1. G. S. Kell, *J. Chem. Eng. Data* **20**, 97 (1975).
2. R. J. Speedy, *J. Phys. Chem.* **86**, 982 (1982).
3. F. X. Prielmeier, E. W. Lang, R. J. Speedy, H.-D. Lüdemann, *Phys. Rev. Lett.* **59**, 1128 (1987).
4. O. Mishima *et al.*, *Nature* **310**, 393 (1984).
5. P. H. Poole, F. Sciortino, U. Essmann, H. E. Stanley, *Nature* **360**, 324 (1992).
6. C. A. Tulk *et al.*, *Science* **297**, 1320 (2002).
7. T. M. Truskett, P. G. Debenedetti, S. Sastry, S. Torquato, *J. Chem. Phys.* **111**, 2647 (1999).

#### PERSPECTIVES: NEUROSCIENCE AND EVOLUTION

## Snake Sodium Channels Resist TTX Arrest

Raymond B. Huey and William J. Moody

...there is a constant struggle...between the instinct of the one to escape its enemy and of the other to secure its prey.

—Charles Darwin (*1*, p. 380)

**E**ye of newt is a necessary ingredient in a witch's broth, but skin of newt makes a better defensive toxin. Indeed, skin of the North American newt *Taricha* contains tetrodotoxin (TTX), a potent neurotoxin that paralyzes nerves and muscles by selectively blocking sodium

channels. Armed with their toxic skin, these newts wander around brazenly during the day, safe from attack by hungry predators—safe that is, unless they live in parts of western North America. Here, garter snakes (*Thamnophis sirtalis*) dine readily on newts because they have evolved a marked resistance to TTX (see the figure). On page 1336 of this issue, Geffeney *et al.* (2) explore how these snakes avoid TTX poisoning and implicate voltage-gated sodium channels in muscle as the site of TTX resistance.

Salamanders are easy prey for snakes. Not surprisingly, many salamanders have evolved remarkable defenses (3). Newts of the salamander genus *Taricha* have taken this

predator-prey arms race (4) to an extreme by sequestering TTX in their skin (5). However, newts are not the only organisms to use TTX in self-defense. They are accompanied by a phylogenetic smorgasbord of animals—*Fugu* pufferfish (see the Perspective on page 1283), *Atelopus* frogs, blue-ringed octopus, and *Phallusia* tunicates. But not all predators are deterred by TTX: Humans voluntarily ingest small quantities of TTX when dining on *Fugu* in Japan, and may ingest it involuntarily during voodoo rituals in Haiti (6).

Virtually all snakes die if they mistakenly eat a newt. However, a few populations of garter snakes have evolved a high level of resistance to newt TTX (2, 7). These snakes readily eat newts, although their postprandial crawling speed is temporarily slowed. Resistance to TTX is variable (genetically and phenotypically) within garter snake populations. Importantly, resistant snakes are slower than their more susceptible counterparts, reflecting a negative genetic correlation (trade-off) between resistance and speed (8).

The authors are at the Department of Zoology, University of Washington, Seattle, WA 98195–1800, USA. E-mail: hueyrb@u.washington.edu

The sodium channels of snake neurons and skeletal muscle are implicated in TTX resistance because they are the targets of TTX and differ in their TTX sensitivity. Geffeney and colleagues now test this possibility in their new study (2). To quantify whole-animal resistance, these authors measured the dose of TTX required to reduce a garter snake's crawling speed by 50%. To quantify resistance of muscle to TTX, they excised snake skeletal muscle—thus adapting another preparation, “fillet of a finny snake,” pioneered by the witches of *Macbeth*. Then they measured the effect of TTX on the maximum rate of rise of action potentials, a rough estimate of the sodium current. By applying both assays to individual snakes, the authors found that the sensitivity of muscle action potentials to TTX was positively associated with whole-animal sensitivity—that is, snakes with resistant muscle cells showed a major reduction in crawling speed only at high doses of TTX.

These results strongly imply that sodium channels in snake skeletal muscle are key players in the evolution of TTX resistance, although not necessarily the only players. As Geffeney and co-workers note, variations in the TTX sensitivity of neuronal sodium channels could also influence variations in whole-animal resistance, as well as account for the slow crawling speed of TTX-resistant snakes.

Variations in the TTX sensitivity of the muscle action potentials have yet to be explained. One possibility derives from the observation that mammalian muscle cells contain both TTX-sensitive and TTX-resistant sodium channels (9). If snakes have both



**Fugu for garter snakes.** Some garter snakes dine on newts and are seemingly undeterred by large quantities of the potent neurotoxin TTX in the newt's skin.

types of sodium channels, then the TTX resistance of muscle and of the whole animal could be adjusted by an evolutionary alteration in the ratio of the two channel types in muscle membranes. An intriguing way of achieving this would be through a genetic alteration in the timing of the developmental program (“heterochrony”) for snake skeletal muscle. During mammalian muscle development, TTX-resistant sodium channels predominate early but are mostly replaced by TTX-sensitive forms as the muscle matures and becomes innervated (10). Thus, variations in the TTX resistance of snake muscle could be achieved by arresting the switch between TTX-resistant and TTX-sensitive channel types at different stages of development.

Why is marked TTX resistance found only in certain populations of garter snakes, even though many other garter snake populations inhabit areas where newts also live (2)? This may reflect idiosyncratic (contingent) historical influences. Alternatively, perhaps the snake-newt arms race includes a third trophic level—animals that prey on the snakes themselves. Even though TTX-resistant snakes gain sole access to an untapped food resource, they may suffer an increased risk of predation because they are chronically slow (8, 11). This trade-off may put evolutionary brakes on a further escalation of snake resistance to TTX (4, 8). Moreover, resistance might be favored only where food for snakes is limited and where predators of snakes are rare. Testing this geographic, coevolutionary hypothesis (12) may prove difficult.

The work of Geffeney and colleagues demonstrates the power of integrative biology and will stimulate complementary tests of diverse neurobiological and evolutionary hypotheses. Moreover, the take-home message seems to be that getting a leg up in the predator-prey arms race requires neither arms, nor legs, nor speed.

#### References

1. G. J. Romanes, *Mental Evolution in Animals* (Appleton, New York, 1884), p. 380.
2. S. Geffeney, E. D. Brodie Jr., P. C. Ruben, E. D. Brodie III, *Science* **297**, 1336 (2002).
3. S. J. Arnold, *Copeia* **1982**, 247 (1982).
4. R. Dawkins, J. R. Krebs, *Proc. R. Soc. London Ser. B* **205**, 489 (1979).
5. H. S. Mosher, F. A. Fuhrman, H. D. Buchwald, H. G. Fischer, *Science* **144**, 1100 (1964).
6. C. Benedek, L. Rivier, *Toxicol.* **27**, 473 (1989).
7. E. D. Brodie Jr., B. J. Ridenhour, E. D. Brodie III, *Evolution*, in press.
8. E. D. Brodie III, E. D. Brodie Jr., *Evolution* **53**, 626 (1999).
9. A. L. Goldin, *Annu. Rev. Physiol.* **63**, 871 (2001).
10. R. G. Kallen et al., *Neuron* **4**, 233 (1990).
11. B. C. Jayne, A. F. Bennett, *Evolution* **44**, 1204 (1990).
12. J. N. Thompson, *The Coevolutionary Process* (Univ. of Chicago Press, Chicago, IL, 1994).

#### PERSPECTIVES: APOPTOSIS

## A Cinderella Caspase Takes Center Stage

Sharad Kumar and David L. Vaux

**A**ctivation of proteolytic enzymes called caspases is a key step in the apoptotic program. Caspases exist in latent forms in almost all animal cells and become activated in response to apoptotic

signals such as those induced by cell stress (for example, DNA damage and withdrawal of trophic support). The first caspases to become activated are so-called “initiator caspases.” These caspases have long amino-terminal prodomains containing specific protein-protein interaction motifs. Through these domains the caspases interact with adaptor proteins that recruit them to specific “death complexes” (large multiprotein

complexes that mediate caspase activation). In mammals, these death complexes include the Apaf-1/caspase-9 apoptosome and the FADD/caspase-8 death-inducing signaling complex (DISC). Once the initiator caspases are activated, they process and activate downstream effector caspases, such as caspases 3, 6, and 7 (1). The apoptosome and DISC are thought to account for most caspase-dependent apoptosis. The upstream signaling pathways leading to the assembly of these death complexes are often called the mitochondrial (intrinsic) and death receptor (extrinsic) pathways of apoptosis.

During stress-induced apoptosis, mitochondria release their cytochrome c, which binds to Apaf-1 and promotes apoptosome formation and caspase-9 activation (1). Thus, the most widely held view is that caspase-9

S. Kumar is at the Hanson Institute, Frome Road, Adelaide 5000, Australia. E-mail: sharad.kumar@imvs.sa.gov.au D. L. Vaux is at the Walter and Eliza Hall Institute, Melbourne 3050, Australia.