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RNA Editing Underlies Temperature Adaptation in K⁺ Channels from Polar Octopuses

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Abstract

To operate in the extreme cold, ion channels from psychrophiles must have evolved structural changes to compensate for their thermal environment. A reasonable assumption would be that the underlying adaptations lie within the encoding genes. Here we show that delayed rectifier K⁺ channel genes from an Antarctic and a tropical octopus encode channels that differ at only four positions and display very similar behavior when expressed in *Xenopus* oocytes. However, the transcribed mRNAs are extensively edited, creating functional diversity. One editing site, which recodes an isoleucine to a valine in the channel's pore, greatly accelerates gating kinetics by destabilizing the open state. This site is extensively edited in both Antarctic and Arctic species, but mostly unedited in tropical species. Thus A-to-I RNA editing can respond to the physical environment.

Action potentials, during which the electrical potential across a cell membrane rapidly rises and falls, are the nervous system's basic unit of communication. In 1949 Hodgkin and Katz, using the squid giant axon, showed that the action potential's falling phase has steeper temperature dependence than the rising phase and that the membrane's return to the resting potential is particularly sensitive to temperature (1). Accordingly, they hypothesized that ectotherms would be forced to regulate the waveform of their action potentials to accommodate the thermal environment. As it emerged that the rising phase was due to voltage-dependent Na⁺ channels, and the falling phase to voltage-dependent K⁺ channels (2) (3), the early observations suggested that potassium channel gating is more temperature sensitive than sodium channel gating. This assertion has since been demonstrated (4). These observations also suggested that the closing kinetics of potassium channels, which determine the rate of return to rest, should be especially temperature sensitive, and this has also been shown (5). If K⁺ channel kinetics did not adapt to temperature, the cold would make action

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Supporting Online Material

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potentials disproportionately broad and severely limit repetitive firing. Accordingly, potassium channel kinetics should be a prime target for regulation in organisms adapted to the extreme cold. This study shows that they are, but by an unsuspected mechanism.

To identify mechanisms of cold adaptation, we compared potassium channel orthologs from a tropical and an Antarctic octopus. Despite the enormous body of work on squid axons, we chose octopus as a comparative model because individuals generally have small home ranges, and species inhabit widely different thermal environments, from the poles to the equator. The Antarctic octopus was a *Pareledone* sp., which was collected from McMurdo Station (6), where the waters, at -1.8°C , are in equilibrium with sea ice, and have been so for the last 28 to 38 million years (7). For comparison we used *Octopus vulgaris* which we collected from a Puerto Rican reef at 30°C . Over the year, the temperature at this location fluctuates between $\sim 25^{\circ}\text{C}$ and 35°C . We sequenced the ortholog of the squid delayed rectifier from each species (8).

Based on conventional natural selection, we hypothesized that the channels' genes would have evolved mutations to help tune them to their respective environments. Surprisingly, the primary sequences encoded by the two genes were virtually identical, differing at only four positions (fig. S1). They also shared 95% identity with the widely studied *Loligo* ortholog, indicating that the basic genetic template for the delayed rectifier is remarkably similar in coleoid cephalopods. To test whether the four differences affected function, each channel was expressed in *Xenopus* oocytes and characterized electrophysiologically at 2, 15 and 25°C . Experiments focused on opening and closing kinetics, which are the most temperature sensitive properties, but also on the voltage dependence of opening and the rate of inactivation. Functionally, the two channels were virtually identical (Fig. 1A and table S1). As with the squid delayed rectifier, they opened rapidly in response to depolarization and exhibited a steep voltage-dependence between -20 mV and 20 mV . Upon repolarization, they closed quickly, following a simple exponential time course. When held at a constant depolarizing potential, both channels inactivated slowly, over the course of several seconds. There were some subtle differences. Antarctic channels opened slightly faster (Fig. 1A), but this difference was only significant at potentials near the threshold for activation. The steepness factor (Z) of their steady-state voltage dependence was also slightly smaller (table S1). Overall though, in spite of their drastically different environments, the genomically encoded channels displayed essentially the same behavior. At their respective native temperatures, Antarctic channels would open about 14 times slower, and close about 60 times slower, than tropical channels (see Q_{10} 's in table S1). Either the Antarctic octopus barely compensates for the cold, or post-transcriptional mechanisms are important.

Adenosine deamination, the most common form of RNA editing, is carried out by a family of enzymes known as ADARs (Adenosine Deaminases that Act on RNA). ADARs convert adenosine (A) to inosine (I) (9). Because inosine is read as guanosine by the translational machinery, codons in mRNAs can change (10). A to I editing is common in the nervous system, having been identified in mRNAs encoding ion channels and receptors (11-13). Extensive editing by this mechanism has been described in squid, where it modifies diverse mRNAs, including the squid delayed rectifier K^{+} channel (14, 15). We compared cDNA sequences to check whether the octopus channels were edited as well. Our preliminary

cloning results showed site specific A/G variation in the electropherograms of direct sequences, a hallmark of A-to-I RNA editing. To investigate further, we cloned and sequenced 50 individual cDNAs from the stellate ganglia of each species and then looked for A or G variation that was not present in the gene sequence. Each species showed extensive editing. For the Antarctic octopus, there were 18 editing sites, nine of which caused amino acid changes (fig. S1). For the tropical octopus, there were 15 editing sites, ten of which caused amino acid changes. Of the 12 non-silent sites, five were species specific and four of the shared sites were edited to significantly different extents (Fig. 2A). Thus in these channels greater species diversity is generated by RNA editing than by gene mutations.

Do the RNA edits alter channel function? Four editing sites were selected for further study because they were edited exclusively, or to a much greater extent, in one species or the other; N105G and I321V were considered candidates for cold adaptation, while N40S and S54G were considered candidates for warm adaptation. N105G is only found in the Antarctic channel and N40S is only found in the tropical channel. The other two sites showed greater than 50% differences in editing, with I321V highly edited in the Antarctic channel and S54G highly edited in the tropical channel (Fig 2A). I321V lies in the fifth transmembrane span, (S5) within the pore domain (Fig. 2B) (16). The rest of the sites are located in the T1 domain (Fig. 2B), a region important for tetramerization (17, 18). At the genomic level, all of these positions except S54G are nearly invariant among Kv1 channels, suggesting that they are functionally important.

To characterize their effects, each of the four edits were singly introduced into the unedited channel background (genomic) and studied at 15 and 25°C. Three of the four editing sites produced clear functional changes to a number of channel properties (table S1). The tropical edits, (N40S and S54G), had similar effects: both slowed channel opening by about 50%, and both increased the rate of inactivation, by 30% for N40S and by 60% for S54G (fig. S2). These effects on gating were somewhat surprising, given that both sites are in the cytoplasmic tetramerization domain, thought to be physically separate from the membrane-bound voltage sensor and pore (16, 19). However, others have reported that the T1 domain influences function (20, 21). The Antarctic edits also altered function. N105G caused subtle changes in activation and deactivation kinetics. The most pronounced effect of all, however, was due to I321V, which more than doubled the rate of closure (Fig. 1B and C) and produced a positive shift in the voltage dependence of opening (table S1). This effect was dominant, speeding closing when combined with other editing sites that mimicked natural editing patterns (Supporting Online Material, table S1 and fig. S3).

Since I321V was highly edited in the Antarctic species and dramatically accelerated deactivation kinetics, which underlie the action potential's afterhyperpolarization, it was a good candidate for cold adaptation. This position lies in the S5 helix, an interface between the voltage sensor and the ion conduction pathway (16, 22, 23). At negative voltages, the voltage sensor is thought to force the gate shut via contact between the S4-S5 linker and the S6 helix. At positive potentials, the voltage sensor moves away, allowing the channel to open (22, 24, 25). The open state is not stable, however, and channels flicker between open and closed conformations (26, 27). Because I321V causes the channel to close faster and

also shifts its voltage sensitivity to more positive potentials, we hypothesized that it destabilizes the open state. This possibility was examined directly by recording single channel events. Representative records for both unedited and I321V Antarctic channels show that I321V channels close more frequently (Fig. 3A and C). To quantify this phenotype, recordings were idealized using an algorithm and the durations of open and closed events were determined (28). The average open duration for the unedited Antarctic channels was twice that of I321V channels. The durations of closed events did not change, suggesting that I321V selectively affects the open to closed state transition. This idea was reinforced by a simple 5 state model (Fig. 3E and F): by doubling the backwards rate constant for the final transition, both the macroscopic difference in closing kinetics (Fig. 3E and F), and the single channel difference in open duration (Fig. 3G and H), could be recapitulated. In agreement with experimental data, the model predicts nearly identical activation kinetics and open probability for the two channels (Fig 3E and F). Thus it appears that once open, I321V channels are poised to close rapidly, but the opening kinetics and other properties are preserved. In an axon, we predict that the major effect of I321V channels would be to accelerate the after hyperpolarization, and thus shorten the refractory period, increasing repetitive firing rates.

If editing of codon I321 is indeed a cold adaptation, then we might expect other cold adapted octopuses to make the same edit. To test this idea, we collected two Arctic species from benthic trawls northwest of the Svalbard archipelago, where water temperatures at $\sim 0^{\circ}\text{C}$ were similar to those in Antarctica. We also collected two more tropical species, one from Puerto Rico and one from a desert lagoon in Baja California, and two temperate species from California (Fig. 4A). Editing levels at I321V, as determined by a primer extension assay, correlated very well with the environmental temperature where the species were captured (Fig. 4B and C). We also quantified all editing sites in the six new species using direct sequencing. Although many of the other editing sites were edited differentially among the eight species, I321V correlated most closely with temperature (table S2).

In Coleoid Cephalopods, and in other higher metazoans, A-to-I RNA editing adds a layer of complexity to the proteome. A clear advantage to this strategy is that it allows options: different isoforms can be expressed in response to different conditions. Exactly how organisms exercise these options is largely unknown. *Drosophila* and rodents use editing to fine tune protein function temporally, over the course of development (29, 30) and spatially, in different brain regions (12). Here we present evidence that RNA editing can respond to an external pressure: temperature. While still maintaining the basic K^+ channel plan, octopus can make fast closing versions, and the extent of their expression can be graded. A basic question that remains is whether octopuses use editing for rapid acclimation or longterm adaptation. For each possibility, the biochemical mechanisms that impart temperature sensitivity to the editing process would be different. Others have shown that the RNA structures that drive editing evolve and generate species specific patterns, suggesting a plausible mechanism for adaptation (31). Acclimation could arise from temperature sensitive RNA structures, or temperature-dependent expression of other factors that control ADAR's access to specific editing sites.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and Notes

1. Hodgkin AL, Katz B. J Physiol. Aug.1949 109:240. [PubMed: 15394322]
2. Hodgkin AL, Huxley AF. J Physiol. Apr.1952 116:449. [PubMed: 14946713]
3. Armstrong CM, Bezanilla F, Rojas E. J Gen Physiol. Oct.1973 62:375. [PubMed: 4755846]
4. Kukita F. J Membr Biol. 1982; 68:151. [PubMed: 6286975]
5. Rodriguez BM, Sigg D, Bezanilla F. J Gen Physiol. Aug.1998 112:223. [PubMed: 9689029]
6. Materials and methods are available as supporting material on *Science* Online
7. Katz ME, et al. Science. May 27.332:1076. [PubMed: 21617074]
8. Rosenthal JJ, Vickery RG, Gilly WF. J Gen Physiol. Sep.1996 108:207. [PubMed: 8882864]
9. Bass BL, Weintraub H. Cell. Dec 23.1988 55:1089. [PubMed: 3203381]
10. Basilio C, Wahba AJ, Lengyel P, Speyer JF, Ochoa S. Proc Natl Acad Sci US A. Apr 15.1962 48:613.
11. Bhalla T, Rosenthal JJ, Holmgren M, Reenan R. Nat Struct Mol Biol. Oct.2004 11:950. [PubMed: 15361858]
12. Burns CM, et al. Nature. May 15.1997 387:303. [PubMed: 9153397]
13. Melcher T, Maas S, Higuchi M, Keller W, Seeburg PH. J Biol Chem. Apr 14.1995 270:8566. [PubMed: 7721757]
14. Patton DE, Silva T, Bezanilla F. Neuron. Sep.1997 19:711. [PubMed: 9331360]
15. Rosenthal JJ, Bezanilla F. Neuron. May 30.2002 34:743. [PubMed: 12062021]
16. Long SB, Campbell EB, Mackinnon R. Science. Aug 5.2005 309:897. [PubMed: 16002581]
17. Kreuzsch A, Pfaffinger PJ, Stevens CF, Choe S. Nature. Apr 30.1998 392:945. [PubMed: 9582078]
18. Li M, Jan YN, Jan LY. Science. Aug 28.1992 257:1225. [PubMed: 1519059]
19. Kobertz WR, Williams C, Miller C. Biochemistry. Aug 29.2000 39:10347. [PubMed: 10956024]
20. Cushman SJ, et al. Nat Struct Biol. May.2000 7:403. [PubMed: 10802739]
21. Minor DL, et al. Cell. Sep 1.2000 102:657. [PubMed: 11007484]
22. Lu Z, Klem AM, Ramu Y. J Gen Physiol. Nov.2002 120:663. [PubMed: 12407078]
23. Soler-Llavina GJ, Chang TH, Swartz KJ. Neuron. Nov 22.2006 52:623. [PubMed: 17114047]
24. Long SB, Tao X, Campbell EB, MacKinnon R. Nature. Nov 15.2007 450:376. [PubMed: 18004376]
25. Pathak MM, et al. Neuron. Oct 4.2007 56:124. [PubMed: 17920020]
26. Conti F, Neher E. Nature. May 15.1980 285:140. [PubMed: 6246440]
27. Llano I, Webb CK, Bezanilla F. J Gen Physiol. Aug.1988 92:179. [PubMed: 3171538]
28. Qin F, Li L. Biophys J. Sep.2004 87:1657. [PubMed: 15345545]

29. Graveley BR, et al. *Nature*. Mar 24.2011 471:473. [PubMed: 21179090]
30. Lomeli H, et al. *Science*. Dec 9.1994 266:1709. [PubMed: 7992055]
31. Reenan RA. *Nature*. Mar 17.2005 434:410.
32. Liman ER, Tytgat J, Hess P. *Neuron*. Nov.1992 9:861. [PubMed: 1419000]
33. Taglialatela M, Toro L, Stefani E. *Biophys J*. Jan.1992 61:78. [PubMed: 1311612]
34. Milescu LS, Akk G, Sachs F. *Biophys J*. Apr.2005 88:2494. [PubMed: 15681642]
35. Roberson LM, Rosenthal JJ. *RNA*. Oct.2006 12:1907. [PubMed: 16957279]

Fig 1.

RNA editing, but not gene level differences, change channel function. A, Current traces for Antarctic and tropical K_v1 genomic channels in response to a voltage step from -80 mV to +60 mV. Traces have been scaled in order to show the near identity in opening and closing kinetics. B, Representative current traces focusing on closing kinetics for genomic Antarctic and I321V edited channels. Currents were activated by a stimulus to +50 mV, but only their decay following a return to -80 mV is shown. C, Channel closing kinetics over a range of repolarization voltages following an activating step to +50 mV. Error bars = s.e.m; $n = 16$ for genomic and 9 for I321V. ♦ = genomic Antarctic and ■ = I321V edited. All data was recorded from voltage clamped *Xenopus* oocytes injected with cRNA for the appropriate construct and the temperature was maintained at 15°C.

Fig 2. mRNAs encoding octopus K_v1 channels are extensively edited. A, Editing percentages for the 12 non-silent sites found in the Antarctic and tropical octopus K_v1 channels calculated by sequencing 50 individual cDNA clones for each channel. B, Octopus editing sites occur in different functional domains. Homologous positions to those altered by RNA editing in octopus K_v1 channels, shown in red, have been mapped on the $K_v1.2$ crystal structure(23). One full subunit of the tetramer is shown (blue); for the pore region, all four subunits are shown, each in a different color.

Fig 3.

The editing site I321V speeds channel closing kinetics by destabilizing the open state. Current traces from cell attached patches containing single Antarctic genomic (A) and I321V (C) Kv1 channels. Channels opened in response to a voltage step from -80 mV to +60 mV. I321V channels close more frequently. Overlapping red lines (insets) show idealizations of traces as a series of open and closed events. B and D, Duration distributions of open events for genomic Antarctic and I321V channels. The average duration of open events in the genomic channel (3.42 ms) was about twice that of I321V channels (1.72 ms). E and F, Simple 5 state models for genomic Antarctic and I321V channel gating and simulations generated from the models. Doubling the backwards rate constant for the final transition recapitulates both the macroscopic difference in closing kinetics and the single channel behavior (G and H) between genomic and I321V channels. Rate constants were assumed to depend exponentially on voltage: $k = k_0 e^{(zFV/RT)}$ where F is the Faraday's

constant, V is voltage, R is the universal gas constant, and T is the temperature. For the above models: $k_{1f} = 700$, $z = 0.3$; $k_{1b} = 50$, $z = 1.6$; $k_{2f} = 1200$, $z = 1.8$; $k_{2b} = 25$, $z = 1.1$; $k_{3f} = 600$, $z = 0.8$; $k_{3b} = 150$, $z = 1.5$; $k_{4f} = 3000$, $z = 0.02$; k_{4b} variable, $z = 0.2$. A stochastic simulation of 1000 channels pulsed from -80mV to +60mV and back to -80mV showed faster closing with the I321V model. The maximum open probability was similar: 0.59 vs. 0.55 for genomic and I321V, respectively. Closing rates (single exponential fit, overlapping red line) were similar to those obtained from room temperature experimental data: 625 and 1250 (s^{-1}) simulated kinetics vs. 610 and 1423 (s^{-1}) experimental kinetics for genomic and I321V, respectively. For the single channel simulations, the average open duration using the I321V model was half that for the genomic model.

Fig 4.
The extent of editing at I321V correlates with the water temperature where octopus species were captured. A, Collection sites for eight octopus species and the water temperatures and habitats at the time of capture. B, representative poison primer extension assay showing the amount of I321V editing among the eight species. D, I321V editing percentages for the eight species, based on poison primer extension assays, versus water temperature at capture site. Error bars = SEM; $n = 4$.