Channels get in an HUFA: RNA editing gets them out of a jam

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The enzymatic conversion of adenosine-to-inosine (A-to-I) in RNA rewrites the informational output of a number of different classes of coding and non-coding RNA transcripts. In this issue, Decher *et al* (2010) reveal a new functional consequence for an ancient RNA editing site in certain animal voltage-gated K⁺ channel (Kv) genes. Abundant highly unsaturated fatty acids (HUFAs), which are essential in mammals for normal brain function, block conduction of Kv channels—an effect that can be nearly obliterated by the recoding of a single amino acid through RNA editing.

The central dogma of molecular biology elaborates a simple scheme for information flow that has had every biology major reciting the mantra, ‘DNA goes to RNA goes to protein’, for 40 years. Enter the rather byzantine process of RNA editing, which alters an RNA copy post-transcriptionally, rewriting archival genomic information. Achieving this subterfuge requires a diversity of mechanisms, among which is modification of mRNA by the adenosine deaminases acting on RNA (ADAR), which perform a simple hydrolytic deamination on adenosine residues converting them to inosine (Farajollahi and Maas, 2010). Numerous targets have been identified in mRNAs encoding proteins involved in rapid electrical and chemical neurotransmission. In these targets, complex and imperfect short dsRNA structures direct ADAR with exquisite specificity to modify only certain coding positions. As inosine is translated as guanosine by the ribosome, such modification can lead to alteration of amino acid coding positions.

One class of targets for ADAR-mediated RNA editing of particular importance are the voltage-gated ion channels (VGICs) genes, where transcripts encoding Na⁺, Ca²⁺ and K⁺ selective channels have all turned up as ADAR substrates in one organism or another. In most cases, the functional consequence of VGIC editing is unknown. However, Decher *et al* (2010) in this issue of *The EMBO Journal* reveal a novel role for environment/metabolism to intersect the biological function of an RNA editing site in the potassium channel, Kv1.1. The focus of their studies involves a particular editing event in the Kv1.1 transcript that converts isoleucine-to-valine (I400V). Intriguingly, editing at this exact position has independently evolved by convergence at the homologous position at least three times to enact the same I-to-V change in Kv1 and Kv2 gene families in chordates, mollusks and arthropods.

Earlier work demonstrated that the I400V recoding event leads to profound changes in the inactivation properties of the mammalian Kv1.1 and *Drosophila Shaker* potassium channels in the presence of a protein inactivation ‘ball’, consisting of the extended N-terminus of the channel itself or an accessory β subunit (Bhalla *et al*, 2004). This cytoplasmic ball ‘snakes’ into the vestibule of an open channel and apparently interacts with the I400 position, thus plugging the pore (Zhou *et al*, 2001). Editing dramatically destabilizes this interaction, leaving a channel energetically eager to escape the inactivated state. Here, Decher *et al* (2010) show that HUFAs also seem to act as soluble inactivation balls, binding to the I400 side chain in Kv1.1 (Figure 1). Moreover, the

Figure 1 Tetrameric voltage-gated potassium channels shown (with one subunit removed) in three interconvertible states; closed (C), open (O) and inactivated (I). Closed and inactivated states are non-conducting states. On depolarization, activation gates convert from a closed configuration (red) to the open ion-conducting state (green). On entering the open state, the channel is in a conformation to allow binding of arachidonic acid (AA, shown in red) and is then converted into the inactivated non-conducting state. The I400V RNA editing site (black ovals) affects the off-rate of AA binding to channels.

I400V alteration likewise disrupts the ability of these lipids to plug the pore.

An earlier study reported that lipids could act on the process of potassium channel inactivation in opposing directions, dependent on lipid identity (Oliver *et al*, 2004). However, this study did not provide mechanistic data. Decher *et al* propose that a lipid molecule like arachidonic acid (AA) can serve as an inactivation ball—an unprecedented claim that warrants some guarded scrutiny. However, the authors support this parsimonious proposal with compelling evidence, as follows: HUFAs act from the cytoplasmic side of the membrane. The HUFA-induced inactivation is competitive with binding of intracellular TEA or Kv β 1.1 binding to the pore vestibule. HUFA binding interferes with closing of the activation gate at negative potentials (i.e. deactivation). Finally, an alanine scan mutagenesis of the pore-lining S6 segments shows disruption of HUFA binding, but only for side chains pointing into the central axis of the pore.

As a Kv channel consists of four S6 segments, it is reasonable to explore the stoichiometry of the effect of the editing mutation. Is it dominant or recessive? This was tested by co-expressing wild type and I400V in various relative amounts. The data suggest a dominant effect in that a single mutant subunit appears to maximally disrupt HUFA binding. This result immediately suggests an important consequence of editing, because Kv1 channels promiscuously form heteromers. Subunits containing the edited residue should therefore have a significant effect on a channel's sensitivity to

endogenous HUFAs. The authors confirm this idea by comparing HUFA effects on two areas of the brain that have either high (thalamus) or low (hippocampus) levels of editing at this site. Exactly as expected, Kv1 currents in the hippocampus were more sensitive to HUFA block than those in thalamic neurons.

Several provocative offshoots of this project deserve to be explored. One thing is the nature of the interaction between inactivation balls, lipids and I400. Why is there such a large effect of the I400V mutation, which subtracts a single methyl group (CH₃) reducing the surface area of the aliphatic side chain by a meager 15%? Moreover, given the clear connections between cellular physiology and signalling through lipid mediators and neuronal channel activity, is RNA editing of this site regulated *in vivo* in response to changes in lipid composition? Another puzzle is how the lipid molecule reaches its binding site in the aqueous vestibule of the pore. Does it diffuse in through the open activation gate, as suggested by authors' Figure 2A, or does it integrate into the lipid bilayer and sneak into the vestibule between the helices lining the permeation pathway? Last, given the repeated appearance of this editing site on the evolutionarily stage, what are the behavioral ramifications of the regulation of channel activities by HUFA and RNA editing?

Conflict of interest

The authors declare that they have no conflict of interest.

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