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Alternative translation initiation further increases the molecular and functional diversity of ion channels

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The diversity of ion channels is attributed to the different subunit genes expressed in a given cell, to alternative splicing, to hetero-multimerization and to post-translational modifications. Another mechanism, called alternative translation initiation (ATI), was recently shown to further increase the molecular and functional complexity of K^+ channels (Thomas *et al.* 2008; and see Simkin *et al.* 2008). The genes produce two or more versions of the encoded proteins from a single mRNA, and the shorter version, initiated from a downstream in-frame start codon, lacks the N-terminal fragment of the full-length isoform version. ATI is thought to occur when the ribosome skips the first translation initiation site because of a non-optimal sequence (Kozak sequence) near the initiation site and moves down to the next AUG codon to initiate translation of the protein (Fig. 1).

K_{2P} channel subunits (15 genes in human), including TREK-1 ($K_{2P}2.1$) and TREK-2 ($K_{2P}10.1$), are made of four transmembrane segments, two P domains in tandem, an extended M1P1 extracellular loop and intracellular N- and C-termini (Honoré, 2007). These channels are widely expressed and present in neuronal as well as non-neuronal cells. A functional channel is a dimer of K_{2P} subunits with possible hetero-multimerization. Cell specific alternative splicing produces different isoforms with shorter amino terminal domains (by 15 or 4 amino acids as demonstrated for TREK-1 and TREK-2), with identical functional properties (Gu *et al.* 2002; Xian Tao *et al.* 2006). TREK-1 and TREK-2 share functional properties with activation by both physical and chemical stimuli including membrane stretch, heat, polyunsaturated fatty acids, volatile and gaseous anaesthetics, and intracellular acidosis, but are inhibited by both PKA- and PKC-mediated phosphorylation (Honoré, 2007). Once activated, these channels behave as typical background or leak K^+ channels, thus affecting the resting conductance as well as the action potential duration.

ATI produces two isoforms of TREK-1, the full length and a shorter form lacking the first 56 amino acids of the amino terminal domain (Thomas *et al.* 2008). The smaller and larger TREK-1 isoforms are differentially expressed throughout the brain and during development. The shorter isoform yields smaller currents besides normal suface expression and surprisingly is permeable not only to K^+ but also to Na^+ under physiological conditions (Thomas *et al.* 2008). Indeed, expression of the shorter isoforms produces depolarization in transfected rat hippocampal neurons. By contrast, the longer isoform selectively passes K^+ thus producing cell hyperpolarization.

TREK-1 and TREK-2 exhibit small (41 pS and 52 pS) and large (132 pS and 220 pS) conductance phenotypes when either expressed in transfected mammalian cells or recorded from native cells including cardiac myocytes (TREK-1) or cerebellar granule neurons (TREK-2) (Xian Tao *et al.* 2006; Kang *et al.* 2007). No switching between the small and the large conductance channel has ever been observed in patches containing a single channel, thus indicating that this phenomenon is not related to different gating modes resulting in subconductance states. Moreover, clustering of small conductance channels that would yield the large conductance is also unlikely as the open channel noise is higher for the small conductance. Both channel types have similar gating and regulation properties (Kang *et al.* 2007).

In this issue of *The Journal of Physiology*, an article by Simkin *et al.* (2008) demonstrates that ATI produces TREK-2 isoforms of different size from three

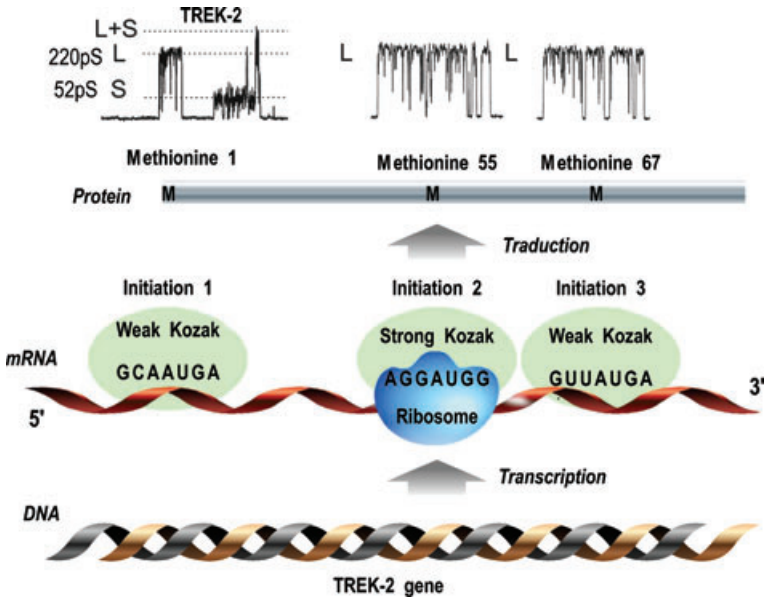

Figure 1. Alternative translation initiation of TREK-2
Usually, the start codon is flanked by a Kozak consensus sequence with the third nucleotide base (–3) upstream of AUG being a purine (A or G) and a guanine right after the AUG (+ 4) (initiation site 2). In this case, translation initiation starts at the first cap-proximal AUG, but if the first AUG is within a weak Kozak consensus sequence as shown for TREK-2, the ribosome might read through and continue scanning the mRNA until it finds the next AUG to initiate translation of a shorter isoform (54 kDa instead of 60 kDa). Other yet unknown factors and/or sequences near the start codon may also regulate ATI. The short TREK-2 isoforms (initiated at methionine 55 or 67) are characterized by a larger single channel conductance.

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potential translation initiation sites within the N terminus (isoforms shorter by 54 or 66 amino acids) (Fig. 1). The longer TREK-2 isoform produces both the small and large conductance channels, while the two shorter forms yield only the large conductance channel. Importantly, in sharp contrast to TREK-1, the shorter isoforms of TREK-2 remain highly K^+ selective with little or no increase in Na^+ permeability.

How might the N-terminus control the unitary conductance of TREK-2? Secondary structure prediction suggests that the distal end of the N terminal domain contains an α helical region. Deletion experiments indicate that the largest change in the conductance level occurs after removal of this region (55 amino acids). This region may interact with other regions of the channel, including the carboxy terminal domain that directly affects the pore properties. Alternatively, this region may interact with phospholipids of the membrane, thus stabilizing the channel in a specific conformation influencing the single channel conductance.

These recent findings demonstrate that ATI plays a major role in the control of K_{2P} channel diversity by modulating single channel conductance and/or selectivity. This mechanism is thus expected to have a major influence on cell electrogenesis.

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