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Evolutionary variations in HLH domain modulates the fast inactivation phase in calcium selective TRP channels.

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Graphical Abstract

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Abstract.

TRPV5 and TRPV6 are highly calcium-selective channels from the Transient Receptor Potential (TRP) family[1]. These channels are considered gatekeepers of epithelial calcium transport, essential for calcium homeostasis[1]. At negative potentials, the channels exhibit a two-phase calcium-dependent inactivation where the slow component is shared and determined by the binding of Ca^{2+}-Calmodulin complex to the C-terminal region of the channel [(2,3)]. In contrast, the rapid phase of inactivation is independent of the calcium-Calmodulin complex and allows differentiating both channels from a functional point of view; while TRPV6 shows a very robust inactivation, at the same calcium concentrations, the inactivation of TRPV5 conductance is modest[4]. The intracellular loop S2-S3[5] and residues downstream the transmembrane segment S6[6] has been associated to the differences observed in the kinetics of the rapid phase of inactivation. However, the exact location of the putative calcium-binding site and the molecular mechanism governing this process

	are not known. A thorough phylogenetic reconstruction in vertebrates suggest that the genes encoding for these channels duplicates more than once during evolution, naturally introducing the same set of mutations within a HLH domain located at the N terminal region. Further sequence analysis unveiled that the HLH domain acts as a fingerprinting in both channels. Molecular dynamics simulations, allowed us to identify a putative calcium-binding site that put together three different portions of the folded channel. By means of site-directed mutagenesis and patch clamp electrophysiology we reversed the phenotype of inactivation in these channels, confirming that the HLH sequence serves as modulator for the calcium-induced inactivation process. We conclude that subtle evolutionary-related variations within the binding region affect the phenotype of the fast inactivation phase.

Introduction *(optional)*

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Materials and Methods *(optional)*

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Results and Discussion *(optional)*

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Conclusions *(optional)*

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References *(mandatory)*

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