Cloning and Sequence Analysis of the Voltage-Gated Muscle Na⁺ Channel from the Poison Dart Frog *Phyllobates aurotaenia*.

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Poison dart frogs of the genus *Phyllobates* secrete lipophilic alkaloid toxins through their skin that were used by Colombian Amerindians to poison the tips of blowdarts. One of the most potent toxins identified is batrachotoxin (BTX) which is an activator of voltage-gated Na⁺ channels. BTX causes sustained opening of these channels by shifting the voltage-dependent activation to more hyperpolarized potentials and by disabling both fast and slow inactivation. It also alters pore conductance and selectivity. Endogenous Na⁺ channels of the poison arrow frog have been proposed to be insensitive to lethal amounts of BTX. In this project we aim to identify what confers BTX insensitivity to Na⁺ channels of the host frog *Phyllobates aurotaenia*, therefore we cloned its skeletal muscle NaV channel. Total RNA from skeletal muscle of *Phyllobates aurotaenia* was isolated and cDNA was obtained with degenerate primers.

The 1819 amino acids sequence shares 72% sequence identity with the rat Na⁺ channel NaV1.4, and 73% with that of the snake *Thamnophis sirtalis*. The TM's are extremely well conserved (87%) with absolute conservation of S4 in all domains. The N-and C-termini as well as the cytoplasmic linkers between domains are more divergent. The D3-D4 linker containing the IFM motif is highly conserved except for Q1348E and K1350P. The DEKA-motif is also absolutely conserved as are the GGGS gating hinge and the QGFS motifs. BTX is thought to bind in the pore region, from the selectivity filter ring to the pore lining S6 TM's. We have identified two S to A mutations flanking the gating-hinge in domains 1 and 3 that may participate in toxin-insensitivity of the *Phyllobates* channel by impairing the binding of BTX. Supported by NIH GM68044(AMC) and GM30376(FB) and by COLCIENCIAS1106-12-13836(LF).