The super-family of voltage gated ionic channels comprises usual voltage-dependent Na^+, K^+ and Ca^{2+} channels, but also cyclic nucleotide gated (CNG) channels which are not voltage dependent. Voltage dependency in this superfamily of ion channels is caused by the motion of the voltage sensor, identified as a positively charged transmembrane helix, referred to as S4. This voltage sensor is present in all voltage gated Na^+, K^+ and Ca^{2+} channels as well as in CNG channels, and why CNG channels are not voltage dependent although they have a voltage sensor is still an unresolved question. When Phe380, Glu363, Thr355 and Leu356 of the CNGA1 channel from bovine rods are replaced by alanine, mutant channels desensitize and exhibit significant voltage dependence. The mutant channel F380A has a reduced degree of desensitization but a very high degree of voltage dependence. Double mutant channels L356D+F380K and L356C+F380C do not desensitize, but exhibit a voltage dependent gating very similar to what observed in usual voltage gated Na^+, K^+ and Ca^{2+} channels. Therefore, when the hydrophobic bond coupling Phe380 in the upper portion of S6 to Leu356 in the P helix is impaired CNGA1 channels become voltage dependent. It is concluded that the voltage sensor in CNG channels is functional but it is inactivated by the tight hydrophobic coupling between the P-helix and S6, necessary to make the channel to open upon binding to cyclic nucleotide and not to voltage.

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Identification And Analysis Of CNGA3 And CNGB3 From Zebrafish

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Cyclic nucleotide-gated (CNG) channels are a crucial component of the phototransduction cascade in vertebrate photoreceptors. The opening and closure of these channels and consequently the influx of sodium and calcium ions into the photoreceptor outer segment is directed by the intracellular light-dependent cGMP level. Cone CNG channels are heterooligomers consisting of two A3- and two B3-subunits, which are encoded by the CNGA3 and the CNGB3 gene. In both genes mutations have been identified, which can lead to a dysfunction of the CNG channels in cone photoreceptors. In humans this results in the autosomal-recessively inherited disease achromatopsia (color blindness).
In order to characterize CNG channels in zebrafish, which possess four morphologically and physiologically distinct classes of cones, we have identified two homologous candidate genes for CNGA3 and two for CNGB3 by in silico database analyses. All four genes as well as a splice variant of CNGA3-1 have been cloned and were heterologously expressed in HEK293 cells. Subsequently, the zebrafish CNG channels were functionally characterized by calcium imaging and patch-clamp measurements.
The retinal expression of all four genes has been confirmed by RT-PCR. In silico analyses revealed, that the two CNGA3 candidates are located at two different locations in the zebrafish genome and are presumably a result of the whole genome duplication as it is known for several genes in zebrafish. In contrast to that, the two CNGB3 candidates are located in a tandem as a result of an additional gene duplication event. ZfCNGA3-1 and zfCNGA3-2 have 62 % identity with the human CNGA3 protein. ZfCNGB3-1 has 43 % and zfCNGB3-2 has 49 % identity with human CNGB3.

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The Cloning And Characterization Of Two Urochordate Hyperpolarization-activated Cyclic Nucleotide-modulated (HCN) Channels

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We have cloned two novel HCN genes from the urochordate species, *Ciona Intestinalis*, referred to as ciHCNa and ciHCNb, which share ~50% identity with mammalian HCN isoforms between S1 and the end of the CNBD. Based on our previous phyogenetic analysis of primary sequence, ciHCNb is more closely related to the vertebrate isoforms except that it lacks a putative N-glycosylation site near the pore, which is found in vertebrate sequences and ciHCNa. When expressed in *Xenopus* oocytes, both clones produce a slowly-activating current (I_h) in response to hyperpolarizing pulses, which is inhibited by Cs+ and ZD7288. For ciHCNb, I_h has a reversal potential of -32.4mV in 5mM K^+/91 mM Na^+ extracellular solution, which shifts to -1.9mV in 96mM K^+ solution. This suggests that I_h is carried by both Na+ and K+, a characteristic of other known HCN channels. Fitting I_h traces, generated from a -70 mV pulse, with a single exponential function yielded values for τ of 1.15 +/- 0.06s and 1.14 +/- 0.17s for ciHCNa and ciHCNb, respectively. Application of 10mM 8-bromo cAMP in the 96mM K^+ bath solution produced positive shifts in the I_h activation curve for each clone. Boltzmann fits of normalized tail current amplitudes versus test voltage (I_h activation curves) yielded $V_{1/2}$ values in the presence of cAMP of -59.86 +/- 1.62mV and -48.5 +/- 3.70mV for ciHCNa and ciHCNb, respectively. ciHCNb also produces a very large instantaneous current, which is blocked by Cs+ and ZD7288 and proportional in size to I_h. This component more similar in size to that of the sea urchin SPIH channel than to those of the mammalian HCNs. Together, the data suggest that the mammalian HCNs are evolutionarily closer to ciHCNa than to ciHCNb.

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Evolutionary Emergence of Isoform-specific Regulation by N-glycosylation in Hyperpolarization-activated Cyclic Nucleotide-modulated (HCN) Channels

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For certain ion channels in the Kv superfamily, N-glycosylation maintains stability and promotes cell surface expression; however, to our knowledge, none require it to form functional channels. To date, the role of N-glycosylation in Hyperpolarization-activated Cyclic Nucleotide modulated (HCN) channel function has been examined using only the mouse HCN2 isoform, in which, surprisingly, mutation of Asn to Gln at a predicted N-glycosylation site adjacent to the selectivity filter abolished functional expression in HEK cells. Nevertheless, other studies show that sea urchin HCN (spIH) channels are functional in HEK cells despite lacking the Asn-Xaa-Ser/Thr consensus sequon. These data raise three important questions about N-glycosylation: when in HCN evolution did it arise, do all mammalian HCN isoforms require it for function, given that they share a common ancestor with spIH, and does it affect HCN function? Here, we used phylogenetic analysis to show that invertebrates, but not chordate or urochordates, lack this N-glycosylation sequon, suggesting that it arose at a critical juncture in evolutionary time. We also show that individual mammalian HCN isoforms have distinct N-glycosylation requirements: mutation of Asn to Gln at the putative sequon renders mouse HCN2 non-functional, whereas mouse HCN1 is functionally expressed, albeit with reduced current density but minimally altered responses to voltage and cation selectivity. spIH yields robust currents but is not N-glycosylated, consistent with the absence of a predicted sequon. Taken together, these data suggest that N-glycosylation at this site emerged during chordate evolution as a regulatory mechanism that has developed uniquely for individual (uro)chordate HCN isoforms.

Neuronal Systems & Modeling

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Identifying Electrically Active Cells in Neuronal Culture and Tissue using CMOS based Multi-Transistor Arrays (MTAs)

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A unique feature of CMOS based Multi-Transistor Arrays (MTAs) compared to Metal-Electrode Arrays (MEA) is the high density of the sensor pixels over a large sensor array [1-3]. Key parameters for MTAs are a spatial resolution of 7.8µm, a temporal resolution of 6 kHz (full frame readout) and a size of $1mm^2$ (16384 sensors in total).
When using these chips for measuring the electrical activity of neurons in culture or tissue, usually the signal of one neuron is detected on several transistors. We make use of this feature to automatically identify action potentials and individual neurons in recorded data, even if the coupling area of neighboring cells overlap and therefore a sensor transistor records activity of different cells.
In a first step we detect statistically significant data by examining the combined deviation of the signal from its average on the considered transistor and its neighbors in space and time. This results in a map of data points in space and time for each action potential of all electrically active cells. By grouping signals that form cohesive neighborhoods in space and time we can identify action potentials. By examination of the cross correlation between pairs of action potentials it is possible to identify single cells, even if the coupling area of neighboring cells overlap and therefore a sensor transistor records activity of different cells. We show an application of this method to dissociated cultures of hippocampal rat neurons and rabbit retina.
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